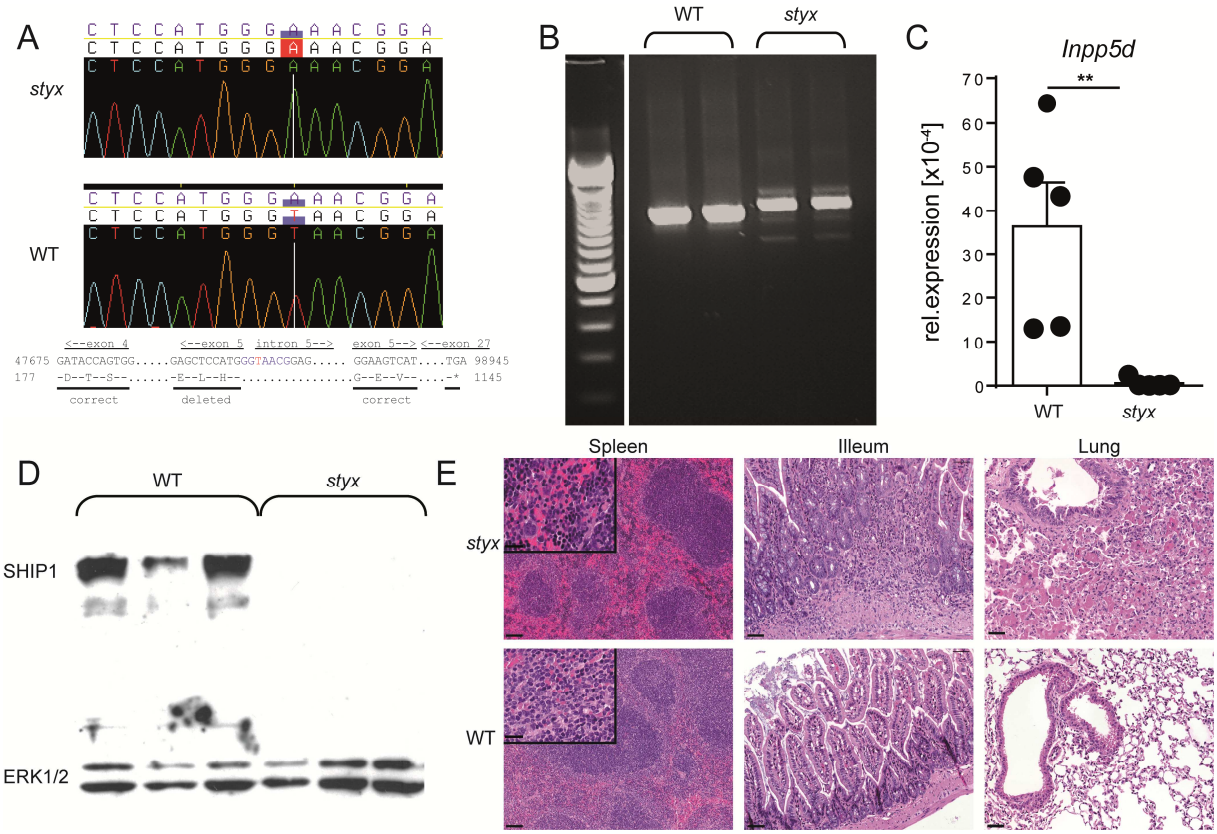
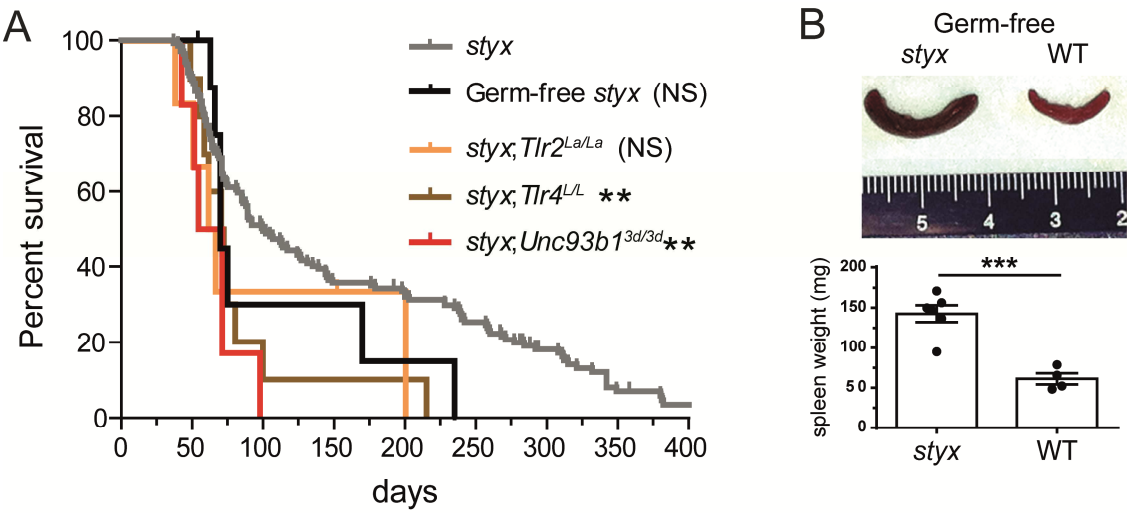
sFigure 1



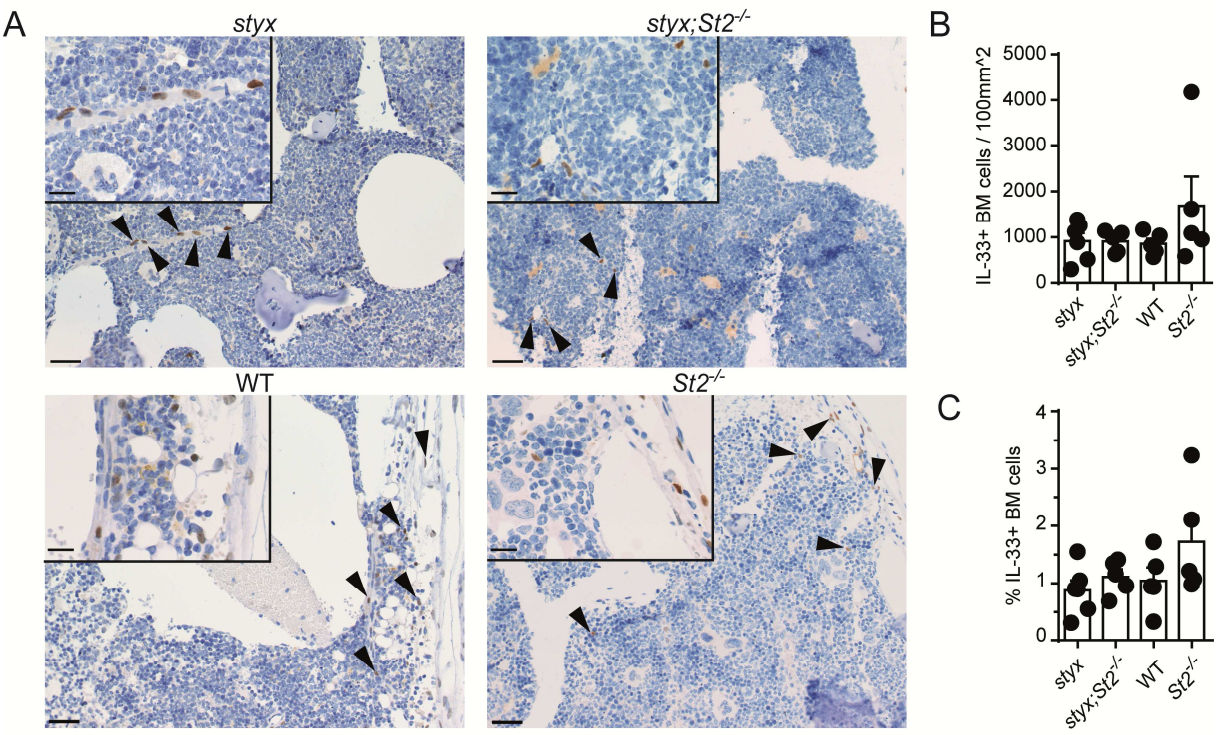
***Styx* mutant mice recapitulate the phenotype of *SHIP*$^{-/-}$ mice.** (A) Analysis of the genomic sequences of a *styx* mutant reveals a T to A transversion in the donor splice site of intron 5 (G**T**AAC→G**A**AAC) of the *Inpp5d* gene at position 49406 in the Genbank genomic region NC_000067. This mutation is predicted to lead to skipping of the 141-nucleotide exon 5. (B) Altered *Inpp5d* cDNA species in *styx* compared with WT splenocytes. (C) Quantitative PCR of *Inpp5d* cDNA from splenocytes. The forward primer used for *Inpp5d* is specific for exon 5, which is skipped in mutant but not WT mice. Data (mean ± SEM) are pooled from two independent experiments, *n*=5 mice per group. (D) SHIP protein is not expressed in *styx* mutants. Western blot analysis of SHIP and ERK1/2 (loading control) in thioglycolate-elicited peritoneal cells of WT and *styx* mice (*n*=3 per group). (E) H&E staining of *styx* and WT spleen (scale bar: overview 100µm; inlay 20µm), ileum (scale bar: 50µm) and lung (scale bar: 50µm) of 12-week-old male mice. Statistics: (C) Standard Student's *t* test. group. $^{**}P < 0.01$.

sFigure 2



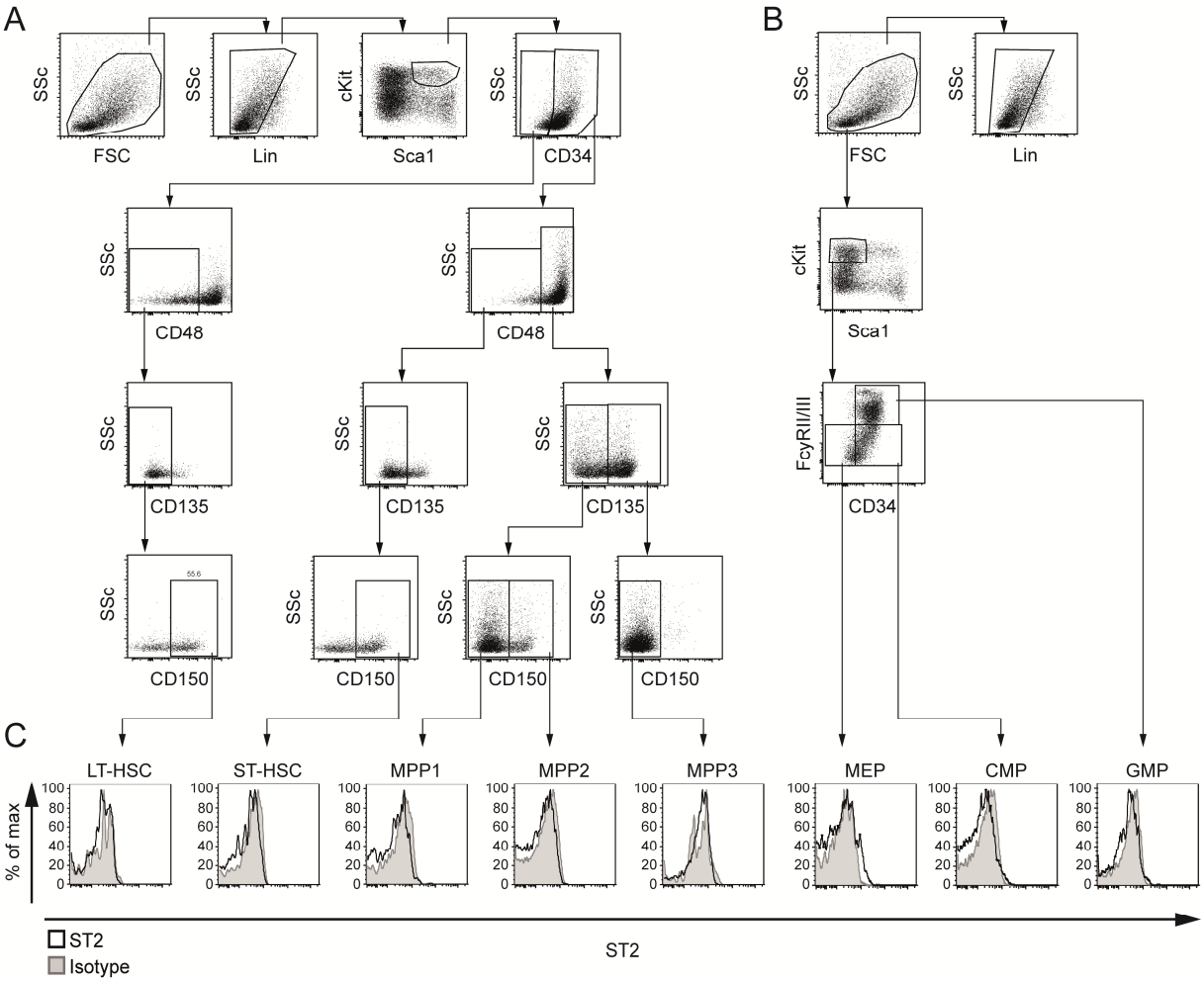
Genetic deletion of TLR signaling or re-derivation into a germ-free environment does not prevent development of MPN-like disease in *styx* mice. (A) Survival curve of SPF (*n*=142) and germ-free (*n*=9) *styx* mice and of SPF *styx* strains deficient for *Tlr2* (*n*=6), *Tlr4* (*n*=10) or *Unc93b1* (*n*=6). (B) Representative spleens of germ-free *styx* and WT mice. Weights of the spleens of these mice are shown in the right panel. Pooled data (mean ± SEM) from 4-6 mice per group. Survival curves for SPF *styx* mice represent the same group of mice as in Figure 1. Statistics: (A) log-rank (Mantel-Cox) test. (B) Standard Student's *t* test. ***P* < 0.01.

sFigure 3



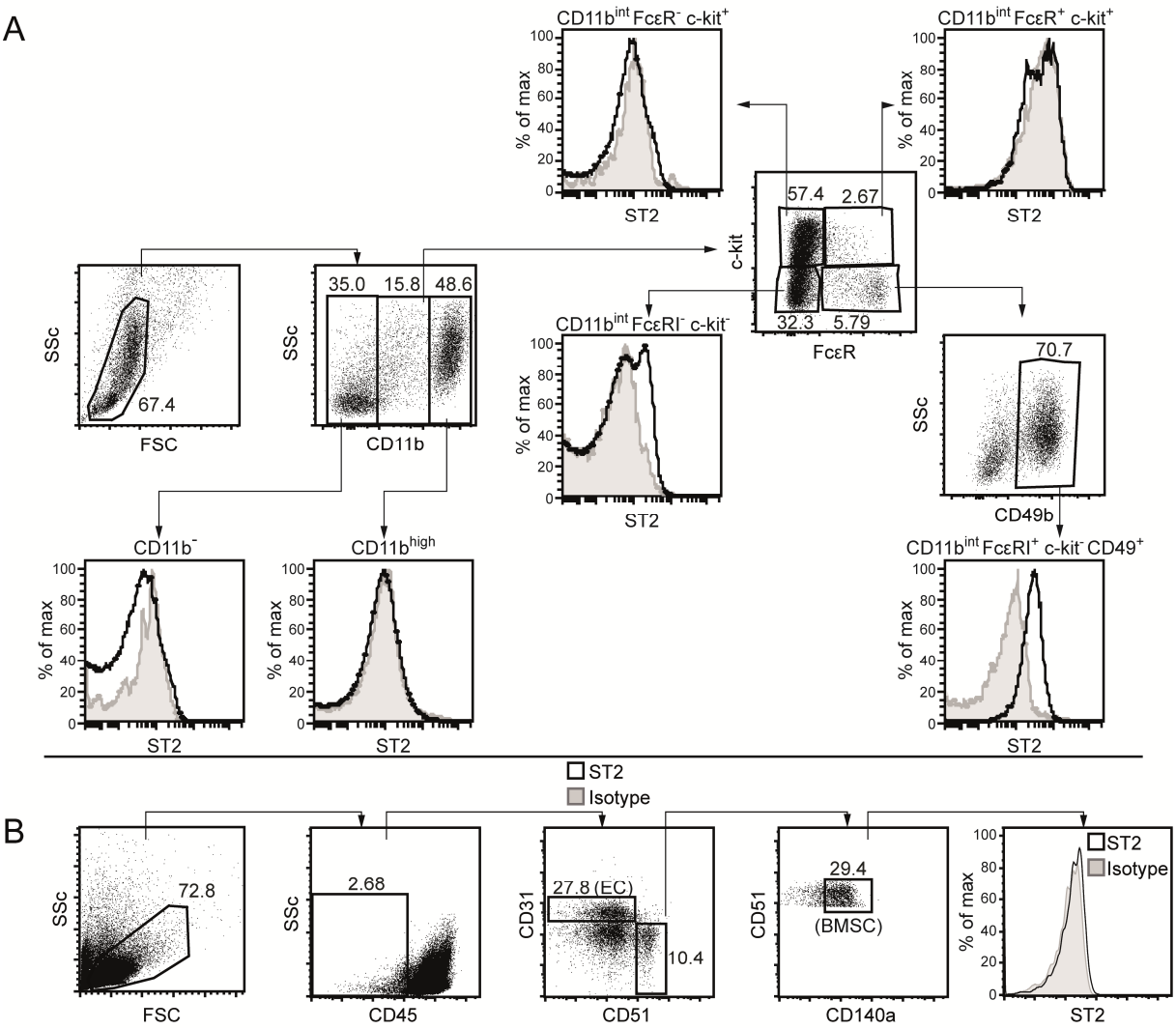
IL-33 expression in mouse BM. (A) Immunohistochemistry for IL-33 on BM sections of the indicated strains. Black arrows indicate IL-33$^+$ nuclei in cells with stromal or endothelial morphology (scale bars: overview: 50 µm; inlay: 20 µm). (B) Amount of IL-33$^+$ nuclei per 100mm^2. (C) Percentage of IL-33$^+$ nuclei. Pooled data (mean ± SEM) of three independent experiments; *n*=5-6 mice per group.

sFigure 4



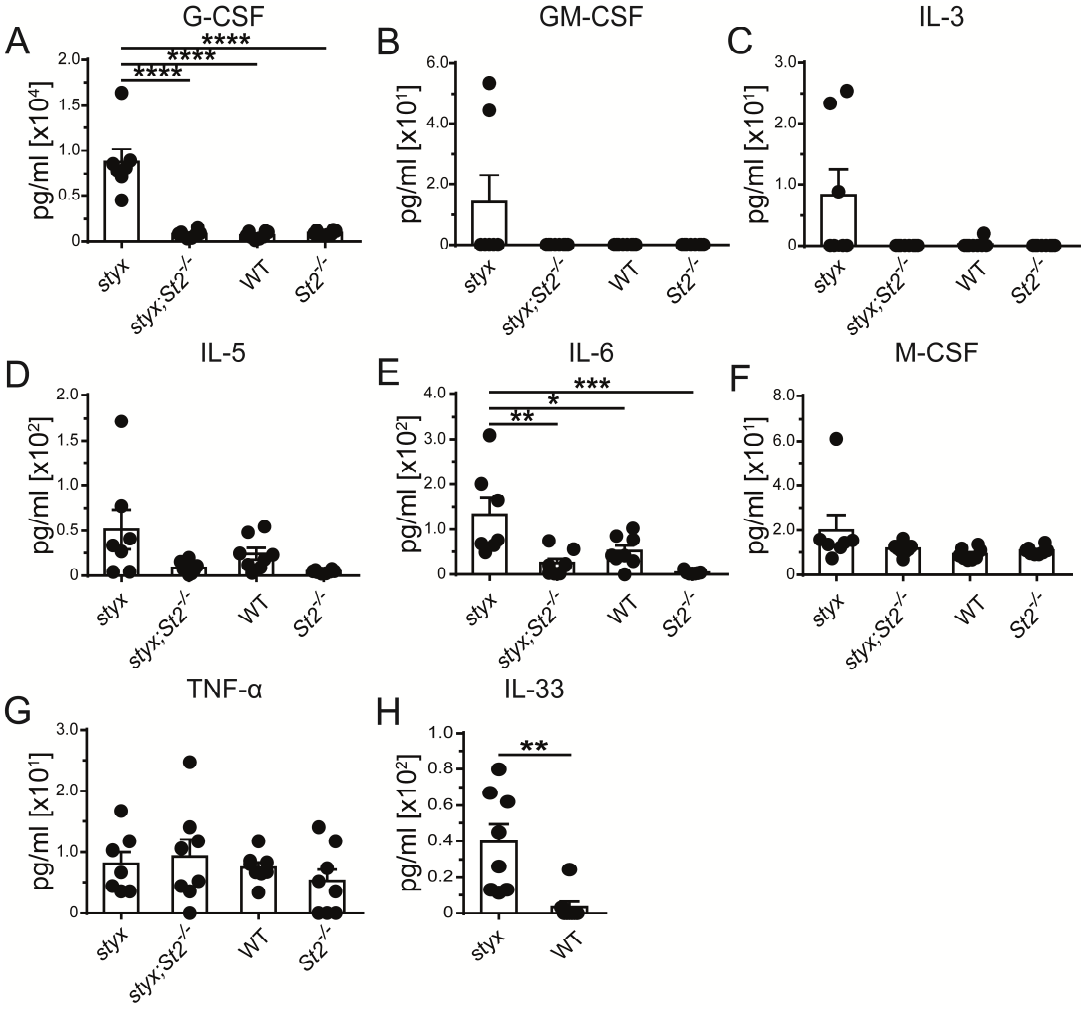
Gating strategy and analysis of ST2 expression for murine HSCs and myeloid progenitors. (A) Gating strategy for long-term (LT-HSC) and short-term (ST-HSC) HSCs, multipotent progenitors (MPP) MPP1, MPP2 and MPP3. (B) Gating strategy for megakaryocyte-erythroid progenitors (MEP), common myeloid progenitors (CMP) and granulocyte-macrophage progenitors (GMP). (C) ST2 expression on LT-HSC, ST-HSC, MPP1, MPP2, MPP3, MEP, CMP and GMP.

sFigure 5



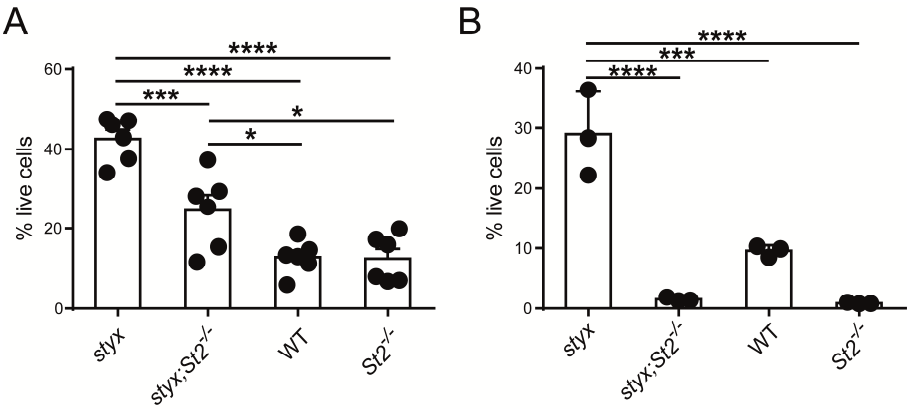
Gating strategy used for the identification and FACS-purification of ST2$^+$ cells from mouse BM. (A) Flow cytometric analysis of BM hematopoietic cells shows that ST2 is mainly expressed on basophils (CD11b$^{int/+}$, FcεRI$^+$, c-kit$^-$, CD49b$^+$) and to some extent on CD11b^{int}, FcεRI$^-$, c-kit$^-$ myeloid cells. (B) Gating strategy for the identification of the non-hematopoietic populations expressing ST2 in the BM. Endothelial cells are specified as CD45$^-$, lin$^-$, CD51$^-$, CD31$^+$ and bone marrow stromal cells (BMSC) are defined as CD45$^-$, lin$^-$, CD31$^-$, CD51$^+$, CD140a$^+$.

sFigure 6



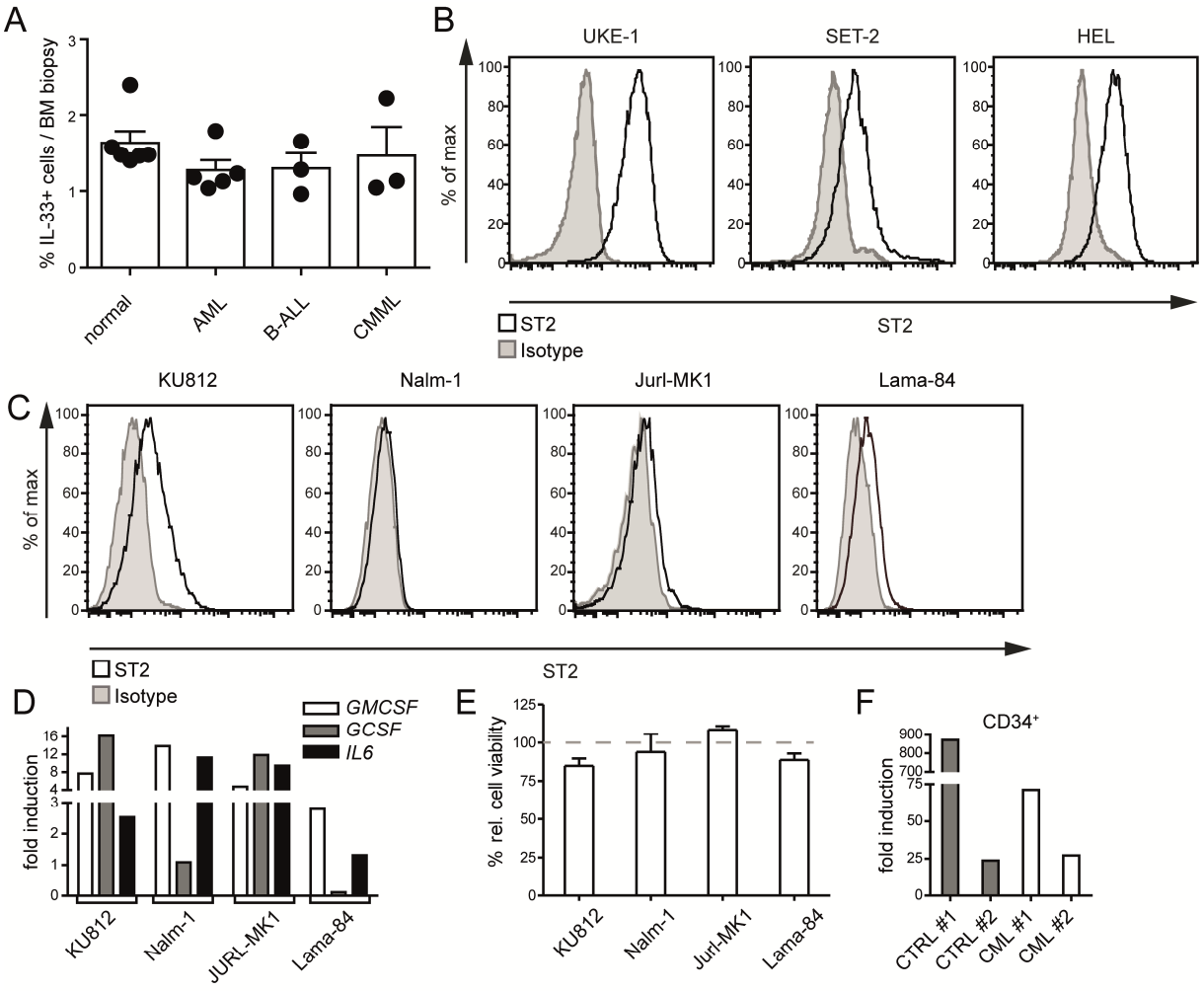
Serum cytokine levels. (A-H) 40-47-day-old mice of the indicated strains were bled and the indicated cytokines were measured in the serum by Multiplexing LASER Bead Technology. Statistics: (A-H) one-way ANOVA with Bonferroni post-test. Pooled data (mean ± SEM) from two independent experiments with 7-8 mice per group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.001$.

sFigure 7



Disruption of IL-33/ST2 signaling prevents the enhanced survival capacity of *Inpp5d*-deficient cells. Frequencies of live (annexin V^- / $DAPI^-$) BM $CD11b^+$ cells (A) 6h after isolation and (B) 48h after isolation and following stimulation with IL-33. Pooled data (mean ± SEM) from (A) two independent experiments and (B) one experiment with 6 or 3 mice per group, respectively. Statistics: (A and B) one-way ANOVA with Bonferroni post-test. *P < 0.05; ***P < 0.001; ****P < 0.001.

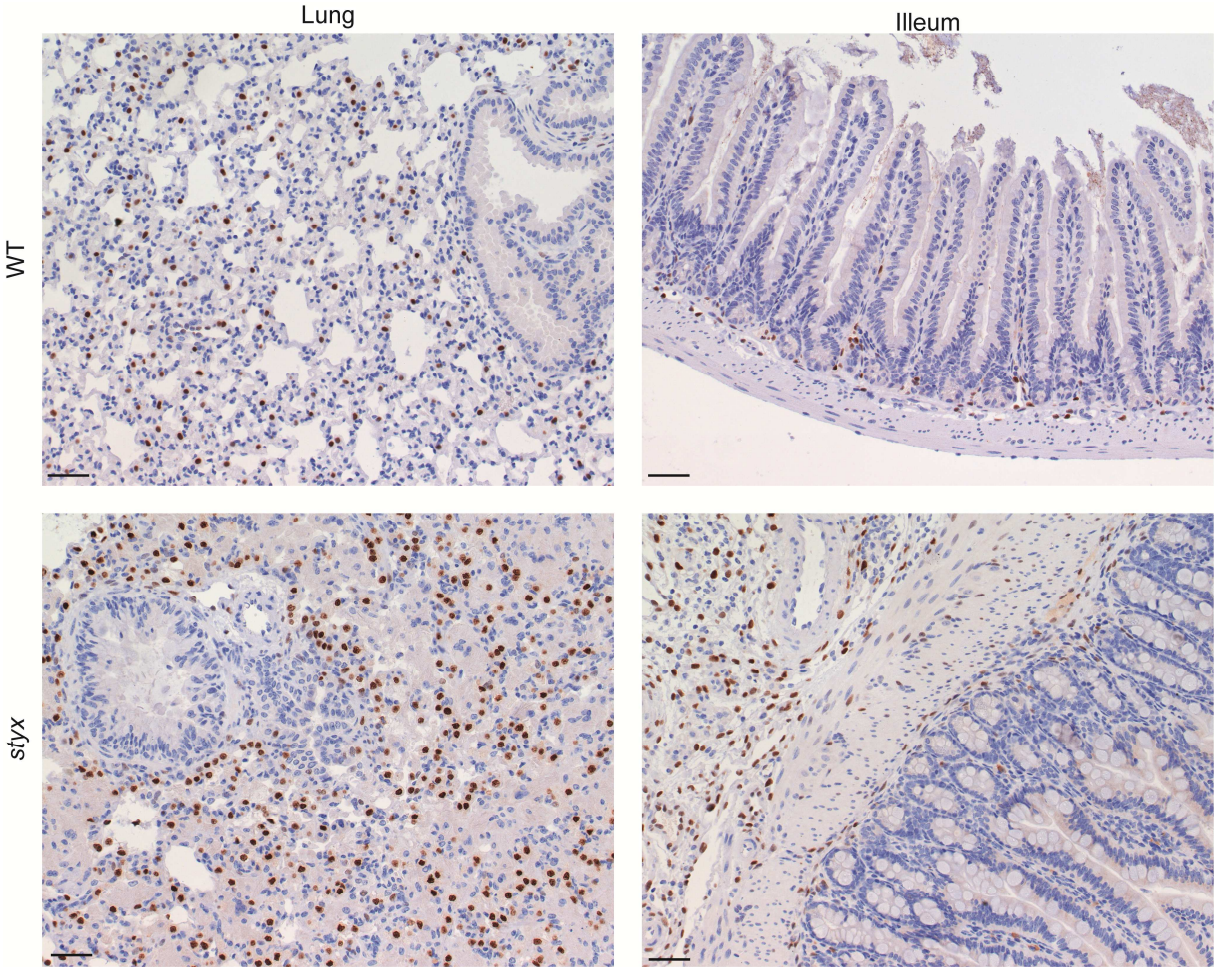
sFigure 8


A
% IL-33+ cells / BM biopsy
normal
AML
B-ALL
CMML
B
UKE-1
SET-2
HEL
% of max
ST2
Isotype
ST2
C
KU812
Nalm-1
Jurl-MK1
Lama-84
% of max
ST2
Isotype
ST2
D
GMCSF
GCSF
IL6
fold induction
KU812
Nalm-1
JURL-MK1
Lama-84
E
% rel. cell viability
KU812
Nalm-1
Jurl-MK1
Lama-84
F
CD34+
fold induction
CTRL #1
CTRL #2
CML #1
CML #2

sFigure 8 (legend)

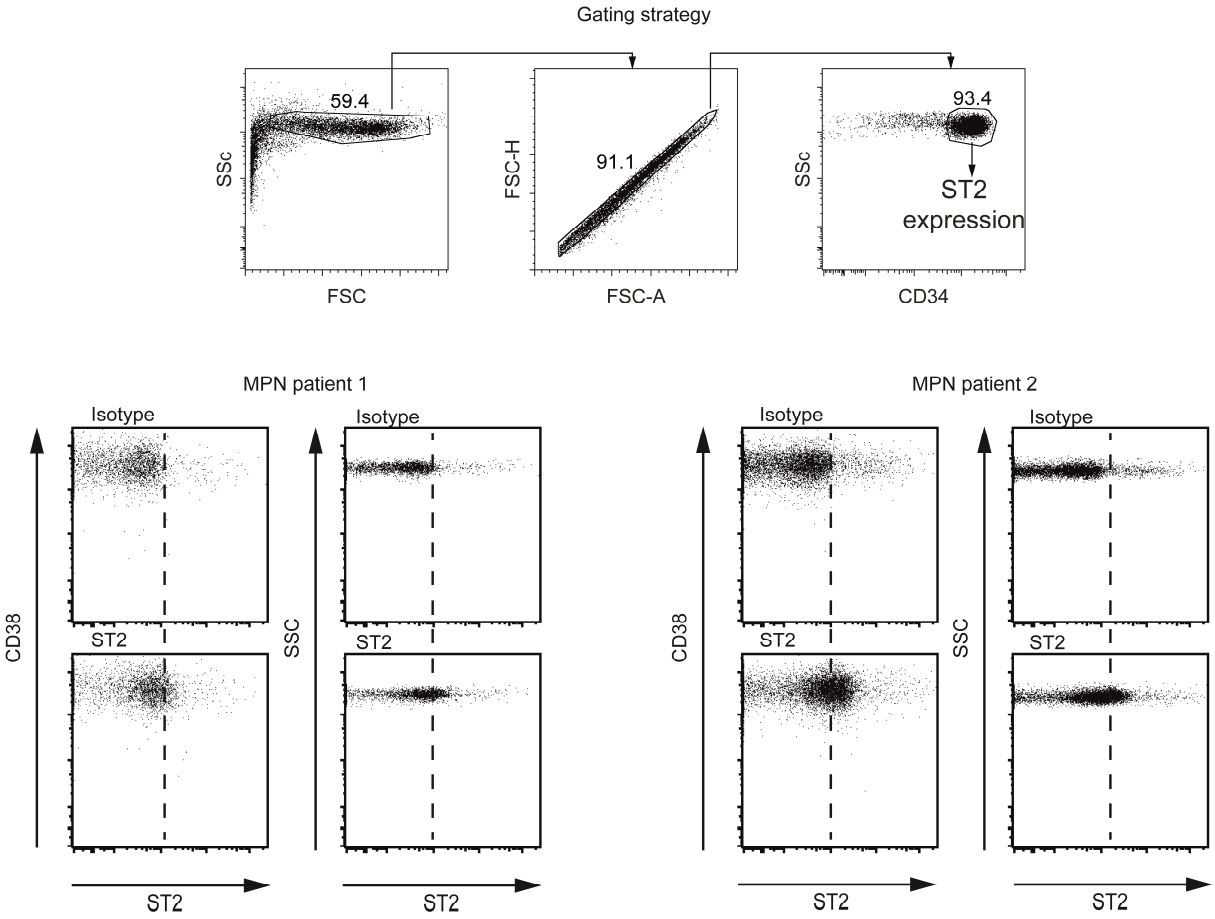
IL-33 and ST2 expression in human cells. (A) Percentages of nuclei positive for IL-33 are similar between control (*n*=6), acute myeloid leukemia (AML; *n*=5), chronic myelo-monocytic leukemia (CMML; *n*=3) and B-acute lymphoblastic leukemia (B-ALL; *n*=3) BM biopsies. Data are mean ± SEM. Control BM biopsies represent the same group of control individuals as in Figure 8C. (B) ST2 expression levels are shown for the indicated *JAK2*-V617F^{+} human cell lines and (C) BCR-ABL1^{+} human CML lines. (D) Indicated BCR-ABL1^{+} human CML cell lines were incubated for 24h ± IL-33 and expression levels were measured for the transcripts of the indicated genes. Expression levels are represented as fold induction and have been normalized to conditions without IL-33. Means from two independent qPCR reactions from pooled biologic triplicates are represented. (E) Indicated BCR-ABL1^{+} human CML cell lines were incubated for 72h ± IL-33 and MTT assay was performed in triplicates. Data have been normalized to conditions without IL-33 and represent mean ± SEM. Experiments were repeated three times. (F) MACS-purified CD34^{-} blood cells from controls or from CML patients were cultured ± IL-33 for 24h and transcription levels of *GMCSF* were measured. Each column represents one individual. Expression levels are represented as fold induction and have been normalized to conditions without IL-33.

sFigure 9



IL-33 expression in mouse tissue. IHC for IL-33 in lung sections and terminal ileum of the indicated strains at 11 weeks of age (scale bar: 50 µm).

sFigure 10



Gating strategy for the analysis of ST2 expression on primary human CD34$^+$ stem/progenitor cells. Flow cytometry dot plots are shown for two representative MPN patients. Expression of CD38 was assessed as a control and not used for histogram representation and data quantification.

Supplemental Table 1 - Overview of the BM chimera experiments

BM chimeras	ST2 on radio-sensitive cells	ST2 on radio-resistant cells	Outcome	Interpretation
styx→WT	+	+	Death Mean survival: 37.5 days	MPN-like disease in chimeras develops from donor styx hematopoietic cells. ST2 expression on both hematopoietic and radio-resistant cells leads to MPN-like disease
styx;St2-/-→ styx;St2-/-	-	-	Survival	MPN-like disease depends on ST2 expression.
styx→St2-/-	+	-	Death Mean survival: 38 days	MPN-like disease can develop independently of ST2 expression on radio-resistant cells
styx;St2-/- →WT	-	+	Death Mean survival: 56 days Delayed MPN-like disease	MPN-like disease can develop independently of ST2 expression on hematopoietic cells. ST2 expression on radio-resistant cells contributes less than ST2 expression on hematopoietic cells.
styx→Il33-/-	+	+	Survival	MPN-like disease depends on IL-33 from radio-resistant cells

Supplemental Table 2 - Antibodies, clones, conjugates and manufacturers

Murine

Specificity	Clone name	Conjugated to	Catalog number	Source
CD135	A2F10	PE	135305	Biolegend
Ly6A/E (Sca-1)	D7	PerCP-Cy5.5	45-5981-80	eBioscience
CD48	HM48-1	Cy7	103423	Biolegend
CD150	TC15-12F12.2	APC	115909	Biolegend
CD117 (ckit)	2B8	APC-Cy7	105825	Biolegend
CD34	RAM34	eFlour 450	48-0341-80	eBioscience
CD127	SB/199	PE	121111	Biolegend
CD16/32 (FcyRII/III)	93	PE-Cy7	101317	Biolegend
CD4	GK1.5	PE	100407	Biolegend
Ly6C	HK1.4	PerCP-Cy5.5	128011	Biolegend
CD11b	M1/70	PE-Cy7	101215	Biolegend
CD8	53-6.7	APC	17-0081-81	eBioscience
CD19	6D5	APC-Cy7	115529	Biolegend
Ly6G	1A8	Pacific Blue	127611	Biolegend
Cd11c	N418	APC/Cy7	117323	Biolegend
FcεRIα	MAR-1	FITC	134305	Biolegend
ST2 (IL-33R)	RMST2-2	PerCP-eFluor® 710	46-9335-80	eBioscience
CD49b	DX5	Pacific Blue	108917	Biolegend
Annexin V		FITC	640905	Biolegend
CD45	104	PE	109807	Biolegend
CD31	390	FITC	11-0311-81	eBioscience
CD140a	APA5	PE	12-1401-81	eBioscience

CD51	RMV-7	Alexa Flour 647	MCA2461A647	Serotec
CD45	104	Alexa Flour 700	109821	Biolegend

Human

Specificity	**Clone name**	**Conjugated to**	**Catalog number**	**Source**
ST2	FAB523P	PE	FAB523P	R&D
CD34	561	APC	343607	Biolegend
CD38	HIT2	PECy7	303515	Biolegend
Annexin V	-	FITC	640905	Biolegend
ST2	B46E	Biotin	101002	MBD Bioproducts
Biotin	-	PE	405203	Biolegend