SUPPLEMENTARY INFORMATION for

Immune stress suppresses innate immune signaling in preleukemic precursor B cells to provoke leukemia in predisposed mice

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SUPPLEMENTARY FIGURES:



Legend:

D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales
D_0_Bacteria;D_1_Tenericutes;D_2_Mollicutes;D_3_Anaeroplasmatales
D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales
D_0_Bacteria;D_1_Deferribacteres;D_2_Deferribacteres;D_3_Deferribacterales
$D_0_Bacteria; D_1_Proteobacteria; D_2_Delta proteobacteria; D_3_Desulfovibrionales$
$D_0_Bacteria; D_1_Proteobacteria; D_2_Gamma proteobacteria; D_3_Beta proteobacteriales$
$D_0_Bacteria; D_1_Cyanobacteria; D_2_Melainabacteria; D_3_Gastranaerophilales$
$D_0_Bacteria; D_1_Patescibacteria; D_2_Saccharimonadia; D_3_Saccharimonadales$
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales
$D_0_Bacteria; D_1_Verru comicrobia; D_2_Verru comicrobiae; D_3_Verru comicrobiales$
$D_0_Bacteria; D_1_Actinobacteria; D_2_Actinobacteria; D_3_Bifidobacteriales$
$D_0_Bacteria; D_1_Proteobacteria; D_2_Alphaproteobacteria; D_3_Rhodospirillales$
$D_0_Bacteria; D_1_Proteobacteria; D_2_Gamma proteobacteria; D_3_Enterobacteriales$
$D_0_Bacteria; D_1_Actinobacteria; D_2_Coriobacteriia; D_3_Coriobacteriales$
$D_0_Bacteria; D_1_Proteobacteria; D_2_Gamma proteobacteria; D_3_Xanthomonadales$
D_0Bacteria;::
$D_0_Bacteria; D_1_Proteobacteria; D_2_Alphaproteobacteria; D_3_Rickettsiales$
D_0_Bacteria;D_1_Tenericutes;D_2_Mollicutes;D_3_Mollicutes RF39
D_0_Bacteria;D_1Cyanobacteria;D_2Oxyphotobacteria;D_3Chloroplast
Unassigned:::
D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Bacillales
$D_0_Bacteria; D_1_Proteobacteria; D_2_Gamma proteobacteria; D_3_Pseudomonadales$
D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Micrococcales
$D_0_Bacteria; D_1_Proteobacteria; D_2_Alphaproteobacteria; D_3_Rhizobiales$
$D_0_Bacteria; D_1_Actinobacteria; D_2_Actinobacteria; D_3_Corynebacteriales$
$D_0_Bacteria; D_1_Actinobacteria; D_2_Actinobacteria; D_3_Propionibacteriales$
$D_0_Bacteria; D_1_Fusobacteria; D_2_Fusobacteria; D_3_Fusobacteriales$
$D_0_Bacteria; D_1_Bacteroidetes; D_2_Bacteroidia; D_3_Chitinophagales$
$D_0_Bacteria; D_1_Acidobacteria; D_2_Acidobacteriia; D_3_Acidobacteriales$
$D_0_Bacteria; D_1_Verru comicrobia; D_2_Verru comicrobiae; D_3_Chthonio bacterales$
D_0_Bacteria;D_1_Deinococcus-Thermus;D_2_Deinococci;D_3_Deinococcales
D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Subgroup 2
$D_0_Bacteria; D_1_Planctomycetes; D_2_Phycisphaerae; D_3_Tepidisphaerales$

	D_0_Bacteria;D_1_Synergistetes;D_2_Synergistia;D_3_Synergistales
	D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_D8A-2
	D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Flavobacteriales
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Aeromonadales
	D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Frankiales
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Oceanospirillales
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Gammaproteobacteria Incertae Sedis
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Cellvibrionales
	D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Pedosphaerales
	$D_0_Bacteria; D_1_Proteobacteria; D_2_Alphaproteobacteria; D_3_Sphingomonadales$
	$D_0_Bacteria; D_1_Coprothermobacteraeota; D_2_Coprothermobacteria; D_3_Coprothermobacterales$
	D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Caulobacterales
	D_0_Bacteria;D_1_Deinococcus-Thermus;D_2_Deinococci;D_3_Thermales
	D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Methylacidiphilales
	D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Acidithiobacillales
	D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Micropepsales
	D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Azospirillales
	D_0_Bacteria;D_1_Planctomycetes;D_2_Planctomycetacia;D_3_Pirellulales
	D_0_Bacteria;D_1_Chloroflexi;D_2_Ktedonobacteria;D_3_Ktedonobacterales
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	D_0_Bacteria;D_1_Chloroflexi;D_2_Chloroflexia;D_3_Chloroflexales
	D_0_Bacteria;D_1_Chloroflexi;D_2_Anaerolineae;D_3_Caldilineales
	D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Actinomarinales
	D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;
	D_0Bacteria;D_1Acidobacteria;D_2Blastocatellia (Subgroup 4);D_3Blastocatellales
	D_0_Bacteria;D_1_Acidobacteria;D_2_Acidobacteriia;D_3_Solibacterales
	D_0_Bacteria;D_1WPS-2;D_2Burkholderiales bacterium Beta_02;D_3Burkholderiales bacterium Beta_02
	$D_0_Bacteria; D_1_Proteobacteria; D_2_Gamma proteobacteria; D_3_Thiotrichales$
	D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Reyranellales
	D_0_Bacteria;D_1Cyanobacteria;D_2Oxyphotobacteria;D_3Nostocales
	D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Sphingobacteriales
	D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Cytophagales
	D_0_Bacteria;D_1Actinobacteria;D_2Actinobacteria;D_3_Pseudonocardiales
	D_0_Bacteria;D_1_Actinobacteria;D_2_Acidimicrobiia;D_3_Microtrichales
	D_0_Bacteria;D_1_Acidobacteria;D_2_Subgroup 6;D_3_Acidobacteria bacterium SCN 69-37

Supplementary Fig. S1: Gut microbiome analysis of *Sca1-BCR-ABLp190, Sca1-Lmo2, Pax5*^{+/-} and WT mice housed in a pathogen-free facility. Taxa Bar plot of the Microbial V4-16S signatures (V4-ASVs) grouped by mouse genotype (*Sca1-BCR-ABLp190* n=15, *Sca1-Lmo2* n=15, *Pax5*^{+/-} n=10 and WT n=12) using 4 taxonomic levels. The legend lists the categorical Taxon orderer from more (top) to less (bottom) frequency.



Supplementary Fig. S2: Leukemia incidence of *Sca1-BCR-ABLp190*, *Sca1-Lmo2*, and *Sca1-ETV6-RUNX1* mice with and without gut microbiota deprivation. All the mice were housed in a pathogen-free facility and some of the *Sca1-BCR-ABLp190*, *Sca1-Lmo2*, and *Sca1-ETV6-RUNX1* mice were treated with a cocktail of antibiotics (Abx) for 8 weeks to induce the alteration of the gut microbiome. No significant differences were found using Fisher's exact test. In all, none of the microbiome-deprived *Sca1-ETV6-RUNX1* mice (n=6) developed B-ALL; 30.77% (4/13) of *Sca1-BCR-ABLp190* treated with an antibiotic developed B-ALL and 83.33% (16/24) of the microbiome-deprived *Sca1-Lmo2* developed T-ALL. *Sca1-ETV6-RUNX1* mice need exposure to infection to develop B-ALL, for this reason, none of the mice developed B-ALL under these housing conditions. Source data are provided as a Source Data file.



Supplementary Fig. S3: Flow cytometric analysis of hematopoietic subsets in diseased *Pax5*^{+/-} early-exposure mice. Representative plots of cell subsets from the thymus, spleen, bone marrow (BM), peripheral blood (PB), and lymph nodes (LN) are shown from a diseased *Pax5*^{+/-} early-exposure mouse. A total of 7 diseased mice were analyzed by flow cytometry (age: 9-17 months). FACS analysis revealed a cell surface phenotype CD19^{+/-}B220⁺IgM⁻ for tumor cells that extended through BM, PB, spleen, and LN.



Supplementary Fig. S4: B-ALL in *Pax5*^{+/-} **mice early exposed to common infections.** Haematoxylin and eosin staining of a *Pax5*^{+/-} (X005) mouse housed in SPF conditions and a tumour-bearing *Pax5*^{+/-} early-exposure mouse (R324) showing infiltrating blast cells in spleen, liver, lymph node, and intestine. Loss of normal architecture resulting with cells morphologically resembling lymphoblasts can be shown (n=7). Tissues from a control littermate wild type mouse are shown for reference. Magnification and the corresponding scale bar are indicated in each case. Yellow arrows highlight some of the leukemic infiltrating cells within the tumor. Asterisks highlight leukemic cells in mitosis.



Supplementary Fig. S5: Analysis of BCR clonality of leukemia arising in a *Pax5*^{+/-} **mouse from the early-exposure group.** PCR analysis of BCR gene rearrangements in the bone marrow and lymph nodes of a diseased mouse. Infiltrated tissues show increased clonality within their immunoglobulin repertoire (red square) (n=2). Sorted CD19⁺ splenic B cells (B cells) of healthy mice serve as a control for polyclonal BCR rearrangements (indicated by numbers, 1-4) and CD8⁺CD4⁺ T cells from the thymus of healthy mice served as a negative control. Source data are provided as a Source Data file.



Supplementary Fig. S6: Peripheral B-cell decrease in *Pax5*^{+/-} mice. Percentage of peripheral blood B-cells (B220⁺IgM^{+/-}) at different time points in *Pax5*^{+/-} early-exposure mice (n=19-28) compared to WT early-exposure mice (n=19-21), *Pax5*^{+/-} delay-exposure mice (n=25-34) and WT delay-exposure mice (n=18-23) analyzed by flow cytometry. Error bars represent the standard deviation. Mann Whitney p-values are indicated in each case. (*** for p <0.0001). Source data are provided as a Source Data file.

	DNA sequencing project number	ID	mouse age at disease	CD19 phenoty pe	JAK3 Mutation in mouse	JAK3 Human homolog	Pax5 mutation of WT allele	Pax5 Human homolog	Other mutations
1	123BM	R324	10,4 months	CD19-		-	-	-	
2	141BM	R330	12,03 months	CD19+		-	P80R	P80R	Kras Q61H
3	157BM	T830	9 months	CD19low		-	P80R	P80R	Jak1 F837V
4	158T	R017	17,23 months	CD19-		-	-		Nras G13D



Supplementary Fig. S7: Secondary mutations arising in tumor-bearing *Pax5*^{+/-} early-exposure mice. Whole exome sequencing (WES) of 4 *Pax5*^{+/-} early-exposure tumors and corresponding germline was performed on a HiSeq 2500 (Illumina) platform. *Pax5*^{+/-} early-exposure tumor DNA was derived from leukemic bone marrow, while tail DNA of the respective mouse was used as reference germline material. Likewise, *Pax5*^{+/-} leukemias from the delayed group, *Pax5*^{+/-} early-exposure tumors showed recurrent mutations affecting the remaining WT *Pax5* allele, with Pax5 p.P80R occurring in 2 out of 4 analyzed leukemias. All the variants found by WES were confirmed by Sanger sequencing.





Supplementary Fig. S8: RT2 Profiler PCR Array Mouse Toll-Like Receptor Signaling Pathway. a) Sorted proB cells (B220^{low} IgM⁻) obtained from WT (n= 4), healthy $Pax5^{+/-}$ (n=4), and leukemic $Pax5^{+/-}$ mice (n= 2) exposed to different environmental contexts (SPF, specific-pathogen facility and CF, conventional facility) were assessed by using Toll-like receptor signalling pathway PCR array. **b**) Sorted proB cells (B220^{low} IgM⁻) obtained from WT (n= 4) and healthy *Sca1-ETV6-RUNX1* mice (n=4) exposed to different environmental contexts (SPF and CF facilities) were assessed by using the same Toll-like receptor signalling pathway PCR array. Data were represented as fold change (2- $\Delta\Delta$ Ct) using the comparative CT Method. The p values are calculated based on a Student's ttest of the replicate 2⁽⁻) Delta CT) values for each gene. Source data are provided as a Source Data file.



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Supplementary Fig. S9: Pax5^{+/-}Myd88^{+/-} mice develop B-ALL without infection exposure. a) B-ALL-specific survival of *Pax5^{+/-}Myd88^{+/-}* mice (blue line, n=8), Pax5+/- mice (red line, n=26), and control wild type (WT) (black line, n=24) mice, all of them housed in an SPF facility (without exposure to common infections). Log-rank (Mantel-Cox) test p-value<0.0001 when comparing Pax5^{+/-} *Myd88*^{+/-} and *Pax5*^{+/-} mice. Source data are provided as a Source Data file. **b**) Haematoxylin and eosin staining of a tumour-bearing Pax5^{+/-}Myd88^{+/-} mouse unexposed to common infections showing infiltrating blast cells in the spleen. lymph nodes, Peyer's patches, liver, and kidney and compared with a healthy $Myd88^{+/-}$ mouse. Loss of normal architecture can be seen due to the infiltrating cells morphologically resembling lymphoblast. Magnification and the corresponding scale bar are indicated in each case. c) Flow cytometry representative illustration of the percentage of B cells (CD19⁺B220⁺ and B220⁺lgM^{+/-} subsets) in PB, BM, spleen and LN from a diseased Pax5^{+/-} $Myd88^{+/-}$ mouse compared to an age-matched healthy $Pax5^{+/-}Myd88^{+/-}$ mouse, and a healthy *Mvd88*^{+/-} mouse.



Supplementary Fig. S10: B-ALL in *Pax5*^{+/-};*Myd88*^{+/-} mice. Flow cytometric analysis of hematopoietic subsets in diseased $Pax5^{+/-}$;*Myd88*^{+/-} mice (X012) a healthy *Myd88*^{+/-};*Pax5*^{+/-} mouse (M706) and a healthy *Myd88*^{+/-}mouse (X650). FACS analysis revealed the expected CD19^{+/-}B220⁺IgM⁻cKit^{+/-}CD25^{+/-} cell surface phenotype for B-cell blasts. Representative plots of cell subsets from the peripheral, bone marrow, spleen, and lymph nodes show an accumulation of blast B-cells in leukemic $Pax5^{+/-};Myd88^{+/-}$ mice compared to age-matched healthy $Pax5^{+/-};Myd88^{+/-}$ (M706) mouse.



Supplementary Fig. S11: B-ALL infiltration in non-hematological tissues. Haematoxylin and eosin staining of WT mice and tumour-bearing $Pax5^{+/-}$;*Myd88*^{+/-} mice (V905 and X012) showing infiltrating blast cells in the liver, lung, kidney, and uterus. Loss of normal architecture due to the accumulation of cells morphologically resembling lymphoblast can be seen in both diseased $Pax5^{+/-}$;*Myd88*^{+/-} mice. Magnification and the corresponding scale bar are indicated in each case.



Supplementary Fig. S12: Analysis of BCR clonality of leukemias arising in *Pax5*^{+/-};*Myd88*^{+/-} mice. PCR analysis of BCR gene rearrangements in the bone marrow of diseased mice. Sorted CD19⁺ splenic B cells (B-cells) of healthy mice serve as a control for polyclonal BCR rearrangements. CD8⁺CD4⁺ T cells from the thymus of healthy mice served as a negative control. Bone marrow leukemic cells show increased clonality within their BCR repertoire (indicated by the code number of each mouse analyzed). Source data are provided as a Source Data file.

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Parts - Myd86 (B- WT proB and	L ALL) R=0.023 =0.39 ALL PreB MRC1 SPARC ADCV MCC1 SPARC ADCV MCC1 SPARC ADCV GMACA MIXC MIX	mannose receptor, Cl secreted acidic cysteli adenylate cyclase 6 mixed lineage kinase complement compon myosin light polypeg complement compon myosin ligh cysteline and tyrosine GTPase, link cyclass cyclin dependent kina annexin A4 myosin, light polypeg ATPase, class V, type 1	type 1 ne rich glycoj domain-like tide 3, regula en f factor h -rich protein member 4 IF (PP2C dom viral oncoge protein 1 ain (PTPRF in th coiled-coil th coiled-coile th coiled-coi	protein atory 1 nain contain ne homolog iteracting) domains an	ing) B (ras related ad ankyrin rej	d)	PTPLA SLC2AS RUPY3 ANKRD23 ANKRD23 POK4 RA114 NEDD4 CTGF RHOB CTGF RHOB CTGF RHOB CTGF CRHOR CTGF C CTGF C C C C C C C C C C C C C C C C C C C	protein t solute ca RUN and I ankyrin r reundabo pyruvate retinoic a neural pyruvate thymocyr cysteline i UDP-Galt UDP-Galt Cysteline (GTPase, I) GTPase, I) GTPase, I)	rrosine pho rrier family i PVE domai peat doma peat doma ut homolog dehydroger cursor cell kinase, mus cos facto kinase, mus e selection- kinase,	sphatase-lik 2 (facilitated in containing in containing in containing assekinase, 1 14 expressed, c wwth factor mily, member 1 1 receptors scile associated h motan L4- ga 1 domain co member 6	e (proline ins glucose trar g 3 ila) issenzyme 4 development r 8 uperfamity, m p regulator 1 lactosyltrans ntaining 2	stead of cata ssporter), r i ally down- group box 1 (chordin i 1 (chordin i	talytic argini nember 5 regulated 4 s ške) ypeptide 1	ie), member
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Part5* Myd86* (B- WT proB and	L ALL) R=0.023 =0.39 ALL) PreB MRC1 SPARC ACV5 MIKL MY19 GFH MY018 CYWR1 GMAP4 PPM15 EMP1 TRIO UACA MXRA MYRA ATP10A MYRA ATP10A MYRA MSP01 PLOF PRES CIBP2 PRCH LOX LISRA1 LAMB2	mannose receptor, Cl secreted acidic cysteli adenylate cysteli adenylate cysteli mixed lineage kinase, mycain, light polypeg Complement compon mycain ill cysteline and tyroaine of Prase, IMAP famfy 1 protein phosphatase of Prase, IMAP famfy 1 protein phosphatase orgaterol binding pro cystenol binding pro cystenol binding pro cystenol binding pro cystenol binding pro cystenol binding pro cysteni statistica and statistica mycain, light polypeg Al'Prase, closs V, type 1 phasminogen activato Cereminal binding pr protein kinase C, eta 1 yydy okdase	type 1 he rich glycoj domain-like tide 9, regula ent factor h -rich protein 1 ain (PTPR in 1 for 2000 and 1 th colled-coil tide kinase tide kinase tide kinase tida (chaperonin) r, urokinase otein 2 otein 2	protein atory 1 nain contain ne homolog teracting) domains an	ing) B (ras related id ankyrin rep	d)	PTPLA SLC2AS: RUFY3 ANKRD28 ROGO4 PDK4 RA114 NEDD4 CTGF RHO8 ICA1 TNFRSTIE PKM2 CRM1 B4GALT1 SPKED2 CRY2 CRY2 CRY2 CRY2 CRY2 CRY2 CRY2 CRY	protein ti solute ca RUN and a ankyrin r roundab pyruvate retinoic a neural pyruvate thymocyt cysteine t UDP-Galt Sprouty-r crystallin GTPase, I NAD kina sema don solute ca solute ca solute ca solute ca	rrosine pho rrier family : PYVE domai peet doma peet doma uut homolog dehydroge cursor cell og gene far sutoantigen crosis factor kinase, mus e selection- rich transm eta GicNAc elatad, EVM zeta MAP family se nain, transm rrier family i rrier family i rrier family i rrier family i rrier family i	sphatase-lik 2 (facilitated in containing in 28 g 4 (Drosoph nase kinase, 14 expressed, c wwth factor mily, member 1 cscl associated h membrane BM beta 1,4-ga 1 1 domain co membrane do 38, member 9 (sodium/h 9 (sodium/h 9 (sodium/h 9 (sodium/h 9 (sodium/h 9 (sodium/h 9 (sodium/h 9 (sodium/h	e (proline insi i glucose trar 2 3 iila) isseenzyme 4 Jevelopment or 8 uperfamily, m nigh mobility P regulator 1 lactosyltrans intaining 2 omain (TM), a 1 ydrogen exch protein on and contr	stead of catal ssporter), r is and y down- is and cytopl: and cytopl and cytopl rol gene 1	talytic argini nember 5 regulated 4 s kie) ypeptide 1 asmic domain asmic domain	ne), member n, (semaphor fator 2
Part5-* Myd88* (B- WT proB and	L ALL) R=0.023 =0.39 ALL PreB MRCL SPARC ADCY MCL SPARC ADCY GINAP4 MY018 CYR1 GINAP4 PPM1F RAUB EMP1 TRO UACA OSBPL3 OK6 ANXA4 MYLA ATP10A HSPD1 PLAU CTB92 PRCH LOXE LIJRA1 LIJRA2 SOG52 CGS92	mannose receptor, C1 secreted acidic cyclas denylate cyclase 6 mixed lineage kinase. Complement compon mycsin. Bidt polypeg complement compon mycsin B Cytaten and tyrosine GPase, INAP family i protein phosphatae. vrai simian leukemia protein phosphatae. vrai simian leukemia protein phosphatae. Vrai simian leukemia anneain A4 mycsin. Bidt polypeg Jasminogen activato C-termina binding protein kinase C, eta Juyd oxidase Interteskin 13 recepti laminin, beta 2 suppressor of cyclobil	type 1 ne rich glycor domain-like tide 9, regula ent Tactor h rich protein ain (PTPEF in lain (P	protein atory 1 nain contain ne homolog terracting) domains an	ing) B (ras relates d ankyrin rej	d)	PTPLA SLC2A5 RUFY3 ANKED23 POB64 RA114 NEDD4 CTGF RHOB CA1 RA114 NEDD4 CTGF RHOB CA1 CTGF C C CTGF C C CTGF C C C C C C C C C C C C C C C C C C C	protein ti solute ca RUN and antyrin r roundabe pyruvate retinoica neural pyruvate connectiv ras homo isket cell a 5 tumor ne pyruvate thymocyty cysteine e UDP-Galti Sprouty-r crystallin GTPase, I Sprouty-r crystallin GTPase, I Solute ca solute ca solute ca solute ca solute ca solute ca solute ca solute ca solute ca solute ca	prosine pho rrier family i PVH domai epeat doma speat doma sut hormolog dehydroger cursor cell dehydroger kin sug crosis factor kin sug rosis factor kin sug eta GicrAac elated, EVH zeta anain, transiv rrier family i membrane in 1 recepto oliferation, elin A33 (tra	sphatase-lik 2 (facilitated 3 a (Drosoph mase kinase, 1 14 expressed, c wwth factor mily, member base base sociate BM beta 1,4, g 1 domain co membrane BM beta 1,4, g 1 domain co membrane S sociate BM beta 1,4, g 1 domain co membrane S 9 (sodium/h) protein 2 r accessory differentiati ansmembrane	e (proline ins glucose trar s 3 lia) issenzyme 4 development r 8 uperfamity, m 9 regulator 1 lactosyltrans ntaining 2 omain (TM), 1 1 ydrogen excl portein on and contr e)	stead of cat ssporter), r ally down- inember 1b 1 (chordin i dersse, pol- dersse, pol- and cytopli hanger), m rol gene 1	talytic argini nember 5 regulated 4 ke) ypeptide 1 asmic domain ember 3 regu	n; (semaphori
Parts* Myd86* (B- WT proB and	L ALL) Re0.023 =0.39 ALL PreB MRC1 SPARC ADD SPARC ADD MY19 CTWR1 MY19 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR1 CTWR2 CTWR1 CTWR1 CTWR2 CTTWR2 C	mannose receptor, Cl secreted acidic cystei adenylate cyclase 6 mixed lineage kinase - complement compon myosin. Bith polypeg complement compon myosin. Bith polypeg protein phosphatase v-ral similan leukemia gibtielial membrane ; fripfe functional dom gibtielial membrane ; tripfe functional dom gycsten dependent kin annexin. A4 myosin, Ligh polypeg ATPase, clus V, type 1 heat shock protein 1 jasminogen activato C-terminal binding rotein kinase C, eta Jyyd oddase interieukin 31 recepto lamina, beta 2 suppressor of cyclokin cappase 12 growth arrest specific collagen, type V, alph	type 1 domain-like tide 9. regula ent factor h -rich protein member 4 IF (PP2C domain viral o ncoge protein 1 ain (PTP8F in the oiled-coil tein-like 3 ase 6 tide kinase tide	protein atory 1 nain contain ne homolog teracting) I domains an	ing) B (ras related id ankyrin rep	d)	PTPLA SLC2AS RUFY3 ANKRD28 ROBO4 PDK4 RAD14 NEDD4 CTGF RHOB ICA1 NERD5 CRM1 BAGALT1 TOX CRM1 BAGALT1 SPRED2 CRV2 GIMAP6 NADK SLC3A3A1 SLC3A3A2 SLC3A3A1 SLC3A3C SLC3A3A1 SLC3A3C SLC3A3A1 SLC3A3C SLC3	protein ti solute ca RUN and ankyrin r. roundabs pyruvate retinoic a neural pr connectiv ras homo isket cell a 5 tumor ne pyruvate thymocyr crystallin GTrase, I NAD kina sema don solute ca solute	prosine pho rrier family : FVK domai geat doman ut homologi dehydroges cid induced ecursor cell tog gene far uso antigen crosis facto kinase, mus e selection- kin transm esta GichAc elated, EVH , zeta esta GichAc elated, EVH se anin, transm seta GichAc elated, EVH se anin, transm rier family i membrane in 1 recepto oliferation, zie in A33 (tra e binding pr t gene 6 (1)	sphatase-lik 2 (facilitated in containing 4 (Drosoft) 1 at gapressed, c wwth factor mity, member 1 at consolid 1 domain co membrane BM beta 1.4 ga 1 domain co membrane do 38, member 9 (sodium/h, protein 2 of scacesson differentiati ansmembrane rotein 2 1 doncogene	e (proline ins glucose trar s glucose trar s glucose trar s glucose trar isoenayme 4 development r 8 uperfamily, m high mobility P regulator 7 main main (TM), a tactosyltrans intaining 2 omain (TM), a tactosyltrans intainintain intain intain inta	stead of cat sporter), rf ally down- nember 1b 1 (chordin in derase, poh and cytopal hanger), m: rol gene 1	talytic arginin nember 5 regulated 4 (((((((((() () () () ()	n, (semaphor liator 2
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C KOHLMANN_UP (BCR-ABL ALL)

0.5 0.4 0.3	FDR=0.048 ES=0.59
•2+, •1 •0 • Pax5 +/	Myd88** (B-ALL)
	WT proB and PreB

Core enrichment genes

	MYO1B	myosin IB
	ARHGEF17	Rho guanine nucleotide exchange factor (GEF) 17
	APP	amyloid beta (A4) precursor protein
	RAI14	retinoic acid induced 14
	LGMN	legumain
	PXDN	peroxidasin homolog (Drosophila)
	EMP2	epithelial membrane protein 2

LINKA_UP (ETV6-RUNX1 ALL) d



	PLCH1	phospholipase C, eta 1					
	GRB10	growth factor receptor bound protein 10					
	TNS1	tensin 1					
	EGFL7	EGF-like domain 7					
	RGL1	ral guanine nucleotide dissociation stimulator,-like 1					
	FLT3	FMS-like tyrosine kinase 3					
	TGM2	transglutaminase 2, C polypeptide					
	TFPI	tissue factor pathway inhibitor					
	ARHGEF17	Rho guanine nucleotide exchange factor (GEF) 17					
	VPREB1	pre-B lymphocyte gene 1					
Core	TSPAN7	tetraspanin 7					
COIC	GUCY1A3	guanylate cyclase 1, soluble, alpha 3					
enrichment	MME	membrane metallo endopeptidase					
ennennenne	ANGPTL4	angiopoietin-like 4					
genes	MYH10	myosin, heavy polypeptide 10, non-muscle					
80.000	SOCS2	suppressor of cytokine signaling 2					
	ODZ4	odd Oz/ten-m homolog 4 (Drosophila)					
	MYO18B	myosin XVIIIb					
	SMAD1	MAD homolog 1 (Drosophila)					
	FAM69B	family with sequence similarity 69, member B					
	DYRK3	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3					
	DPEP1	dipeptidase 1 (renal)					
	GTF2IRD1	general transcription factor II I repeat domain-containing 1					
	TMED6	transmembrane emp24 protein transport domain containing 6					
	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a					
	CLEC14A	C-type lectin domain family 14, member a					
	TNFRSF21	tumor necrosis factor receptor superfamily, member 21					
	LALBA	lactalbumin, alpha					
	PXDN	peroxidasin homolog (Drosophila)					
	DBN1	drebrin 1					
	PLXNB1	plexin B1					
	FSCN1	fascin homolog 1, actin bundling protein (Strongylocentrotus purpuratus)					
	SHISA2	shisa homolog 2 (Xenopus laevis)					
	NPR1	natriuretic peptide receptor 1					
	ECE2	endothelin converting enzyme 2					
	PCDHB11	protocadherin beta 11					

Supplementary Fig. S13: Gene expression analysis of leukemic *Pax5*^{+/-} ;*Myd88*^{+/-} mice. a) Unsupervised heatmap showing the differentially expressed genes (n=2463) between bone marrow leukemic cells from *Pax5*^{+/-};*Myd88*^{+/-} mice (n=6) and proB and preB cells (bone marrow B220^{low} IgM⁺cells) from control wild type (WT) mice (n=4). The significance analysis of microarrays was defined by a p-value<0.05 and fold change: FC<-2 or FC>2. **b-d)** GSEA showing that leukemic Pax5^{+/-};Myd88^{+/-} cells from diseased mice present similar profiles for genesets previously identified in human BCR-ABL+ and ETV6-RUNX1+ B-ALL samples¹⁻³. The list of genes in the core enrichment is indicated in each case.







HALLMARK_MYC_TARGETS_V1 -



2.00

1.75

NES



1.25

1.50











GOBP_SEMAPHORIN_PLEXIN_SIGNALING_PATHWAY -

GOBP RIBOSOMAL LARGE SUBUNIT BIOGENESIS -

GOBP_EXTERNAL_ENCAPSULATING_STRUCTURE_ORGANIZATION -

GOBP_EXTRACELLULAR_MATRIX_ASSEMBLY -

GOBP_CELLULAR_RESPONSE_TO_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_STIMULUS -

GOBP_NEUROMUSCULAR_JUNCTION_DEVELOPMENT -

GOBP_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_SIGNALING_PATHWAY -

GOBP_RIBOSOMAL_LARGE_SUBUNIT_ASSEMBLY -GOBP_CALCIUM_MEDIATED_SIGNALING_USING_INTRACELLULAR_CALCIUM_SOURCE -

GOBP_FIBRINOLYSIS -

GOBP_NEGATIVE_CHEMOTAXIS -

GOBP_LEUKOCYTE_TETHERING_OR_ROLLING -

GOBP_CORONARY_VASCULATURE_MORPHOGENESIS -

GOBP_ALDEHYDE_BIOSYNTHETIC_PROCESS -

GOBP_MATURATION_OF_SSU_RRNA_FROM_TRICISTRONIC_RRNA_TRANSCRIPT_SSU_RRNA_5_8S_RRNA_LSU_RRNA -

GOBP_POSITIVE_REGULATION_OF_RECEPTOR_INTERNALIZATION -

GOBP_REGULATION_OF_METALLOPEPTIDASE_ACTIVITY -

GOBP_NEGATIVE_REGULATION_OF_COAGULATION -

GOBP_CELL_SUBSTRATE_JUNCTION_ORGANIZATION -

GOBP_MAINTENANCE_OF_BLOOD_BRAIN_BARRIER -

GOBP_REGULATION_OF_THE_FORCE_OF_HEART_CONTRACTION -

GOBP_LACTATION -

GOBP_TRABECULA_FORMATION -

GOBP_PSEUDOURIDINE_SYNTHESIS -

GOBP_RIBOSOME_BIOGENESIS -

GOBP_REGULATION_OF_ENDOTHELIAL_CELL_DEVELOPMENT -

GOBP_MATURATION_OF_SSU_RRNA -

GOBP_COMPLEMENT_ACTIVATION -

GOBP_HEMATOPOIETIC_STEM_CELL_HOMEOSTASIS -

GOBP_HUMORAL_IMMUNE_RESPONSE_MEDIATED_BY_CIRCULATING_IMMUNOGLOBULIN -

GOBP_FEMALE_SEX_DIFFERENTIATION -

GOBP_CELL_JUNCTION_DISASSEMBLY -

GOBP_REGULATION_OF_CARDIAC_CONDUCTION -

GOBP REGULATION OF ACROSOME REACTION -

GOBP_REGULATION_OF_ENDOTHELIAL_CELL_CHEMOTAXIS -

GOBP_POSITIVE_REGULATION_OF_RHO_PROTEIN_SIGNAL_TRANSDUCTION -

GOBP_NEGATIVE_REGULATION_OF_CALCIUM_MEDIATED_SIGNALING -

GOBP HOMOPHILIC CELL ADHESION VIA PLASMA MEMBRANE ADHESION MOLECULES -

GOBP_NEGATIVE_REGULATION_OF_AXON_EXTENSION_INVOLVED_IN_AXON_GUIDANCE -

GOBP_VASCULAR_ENDOTHELIAL_GROWTH_FACTOR_RECEPTOR_SIGNALING_PATHWAY -

GOBP_RRNA_PROCESSING -GOBP_HIGH_DENSITY_LIPOPROTEIN_PARTICLE_CLEARANCE -

GOBP_BASEMENT_MEMBRANE_ORGANIZATION -

GOBP_RENAL_SYSTEM_VASCULATURE_DEVELOPMENT -

GOBP_DETECTION_OF_MECHANICAL_STIMULUS -

GOBP_POSITIVE_REGULATION_OF_BONE_RESORPTION -

GOBP_CALCIUM_DEPENDENT_CELL_CELL_ADHESION_VIA_PLASMA_MEMBRANE_CELL_ADHESION_MOLECULES -GOBP_ACUTE_PHASE_RESPONSE -

GOBP_REGULATION_OF_EXTRACELLULAR_MATRIX_ORGANIZATION -

GOBP_IMP_METABOLIC_PROCESS -

GOBP_APOPTOTIC_CELL_CLEARANCE -

GOBP_NEURON_PROJECTION_EXTENSION_INVOLVED_IN_NEURON_PROJECTION_GUIDANCE -

GOBP_RESPONSE_TO_INTERFERON_ALPHA -

GOBP_REGULATION_OF_LEUKOCYTE_TETHERING_OR_ROLLING -

GOBP_ANIMAL_ORGAN_REGENERATION -

GOBP_CELLULAR_RESPONSE_TO_DSRNA -

GOBP POSITIVE REGULATION OF RECEPTOR MEDIATED ENDOCYTOSIS -

GOBP_ATRIAL_CARDIAC_MUSCLE_CELL_TO_AV_NODE_CELL_SIGNALING -

GOBP_CORTICOSTEROID_HORMONE_SECRETION -

GOBP_CELL_ADHESION_MOLECULE_PRODUCTION -

GOBP_PTERIDINE_CONTAINING_COMPOUND_METABOLIC_PROCESS -

GOBP_C21_STEROID_HORMONE_BIOSYNTHETIC_PROCESS -

GOBP_NEGATIVE_REGULATION_OF_PROTEIN_MATURATION -

GOBP_ONE_CARBON_METABOLIC_PROCESS -

GOBP_NEGATIVE_REGULATION_OF_RESPONSE_TO_WOUNDING -

GOBP_TISSUE_REMODELING -

GOBP_REGULATION_OF_CELL_SUBSTRATE_JUNCTION_ORGANIZATION -GOBP_NEGATIVE_REGULATION_OF_WOUND_HEALING -

GOBP OVULATION CYCLE PROCESS -

GOBP_POSITIVE_REGULATION_OF_VASCULAR_PERMEABILITY -

GOBP_PEPTIDE_CROSS_LINKING -

GOBP_POSITIVE_REGULATION_OF_CHEMOKINE_PRODUCTION -

GOBP_AXON_EXTENSION -

GOBP_OLIGOSACCHARIDE_BIOSYNTHETIC_PROCESS -GOBP_SULFUR_COMPOUND_CATABOLIC_PROCESS -

GOBP_REGULATION_OF_BODY_FLUID_LEVELS -

GOBP_EMBRYO_IMPLANTATION -

GOBP_C21_STEROID_HORMONE_METABOLIC_PROCESS -

GOBP_RRNA_METABOLIC_PROCESS -

GOBP_ADHERENS_JUNCTION_ORGANIZATION -

GOBP_COMPLEMENT_ACTIVATION_CLASSICAL_PATHWAY -

GOBP_SEMAPHORIN_PLEXIN_SIGNALING_PATHWAY_INVOLVED_IN_NEURON_PROJECTION_GUIDANCE -

GOBP_POSITIVE_REGULATION_OF_MACROPHAGE_DERIVED_FOAM_CELL_DIFFERENTIATION -

GOBP_VOCALIZATION_BEHAVIOR -

GOBP_REGULATION_OF_EXTENT_OF_CELL_GROWTH -

GOBP_DEVELOPMENT_OF_PRIMARY_FEMALE_SEXUAL_CHARACTERISTICS -

GOBP_REGULATION_OF_FIBRINOLYSIS -

GOBP_SYMPATHETIC_NERVOUS_SYSTEM_DEVELOPMENT - •

GOBP_ENDODERMAL_CELL_DIFFERENTIATION -

1.6 1.7 1.8 1.9 NES

FDR.q.val

0.225

0.200

0.175

0.150

0.125

100

200

300

SIZE

Genese

Supplementary Fig. S14: Gene expression enrichment analysis of leukemic Pax5^{+/-};Myd88^{+/-} mice. Gene Set Enrichment Analyses were performed using the gene sets from hallmark collection database a), canonical pathways gene sets derived from the KEGG pathway database b), and gene sets derived from the GO Biological Process Ontology c) from MSigDB⁴⁻⁶. This analysis identifies significant enrichment of several pathways in Myd88^{+/-}Pax5^{+/-} (B-ALL) plotted on the y-axis in comparison to proB and preB cells (bone marrow B220^{low} IgM⁺cells) from control wild-type (WT) mice. On the x-axis, the NES (Normalized Enrichment Score) value is represented for each gene set. The corresponding FDR (False Discovery Rate) value is represented in a blue color scale and the dot size depicts the gene count. Source data are provided as a Source Data file.



b







NES



Geneset

Supplementary Fig. S15: Gene expression analysis of leukemic Pax5^{+/-} ;Myd88^{+/-} mice compared with leukemic Pax5^{+/-} mice. a) Unsupervised heatmap showing the differentially expressed genes (n=1385) between leukemic cells from Pax5^{+/-};Myd88^{+/-} mice (n=6) and leukemic cells from Pax5^{+/-} mice (n=6). The significance analysis of microarrays was defined by a pvalue<0.05 and fold change: FC<-2 or FC>2. b) GSEA showing that leukemic *Pax5*^{+/-} cells from diseased mice are upregulated in signatures for human B-ALL **c-d)** Enrichment analysis of leukemic cells from Pax5^{+/-};Myd88^{+/-} mice compared with leukemic Pax5^{+/-} mice. Gene Set Enrichment Analyses were performed using the gene sets from the hallmark collection database c), canonical pathways gene sets derived from the KEGG pathway database d) and gene sets derived from the GO Biological Process Ontology e) from MSigDB⁴⁻⁶. This analysis identifies enriched pathways in Myd88^{+/-}Pax5^{+/-} (B-ALL) plotted on the y-axis. On the x-axis, the NES (Normalized Enrichment Score) value is represented for each gene set. The corresponding FDR (False Discovery Rate) value is represented in a blue color scale and the dot size depicts the gene count. Source data are provided as a Source Data file.



Supplementary Fig. S16: Whole Genome Sequencing in leukemic *Pax5*^{+/-};*Myd88*^{+/-} mice. Oncoprint of genes with somatic mutations found in 8 leukemia samples from *Pax5*^{+/-};*Myd88*^{+/-} mice. Somatic alterations are clustered by gene. Tumor DNA was derived from whole leukemic bone marrow or lymph nodes, while tail DNA of the respective mouse was used as reference germline material.





Supplementary Fig. S17: Visualization of somatic mutation in leukemic *Pax5*^{+/-};*Myd88*^{+/-} mice. Genome browser visualization of tumor DNA (T) and germline DNA (G) from tumor-bearing *Pax5*^{+/-};*Myd88*^{+/-} mice **a)** Somatic *Pax5* gene deletions identified by WGS in leukemic *Pax5*^{+/-};*Myd88*^{+/-} mice (V905 and W182). **b)** Somatic *Sh2b3* gene deletions identified by WGS in leukemic *Pax5*^{+/-};*Myd88*^{+/-} mice (X012 and X421). **c)** Validation of the germline *Pax5* exon 2 deletion by WGS from all the *Pax5*^{+/-};*Myd88*^{+/-} mice analysed by WGS. In the *Pax5*^{+/-} mice the exon 2 of the *Pax5* gene (which is indispensable for DNA binding) was replaced by the Escherichia coli lac Z and neomycin resistance genes. WGS: whole-genome sequencing.



Supplementary Fig. S18: *Myd88*^{+/-};*Pax5*^{+/-} mice present similar myeloid cells levels than *Pax5*^{+/-} mice. Percentage of myeloid cells in the peripheral blood of Myd88+/-; Pax5+/- (n=23) and Myd88+/- (n=23) mice compared with age-matched Pax5+/- (n=49) and WT (n=55) mice. Error bars represent the mean and SD of each group. For significant differences, p-values corresponding to unpaired t-test (two-tailed) are shown. Source data are provided as a Source Data file.



Supplementary Fig. S19: Gene expression analysis of healthy proB cells from WT, *Pax5*^{+/-}, and *Pax5*^{+/-};*Myd88*^{+/-}mice. a) Unsupervised heatmap showing the differentially expressed genes (n=265) between healthy proB cells from $Pax5^{+/-}$ mice (n=4) and WT mice (n=4). b) Unsupervised heatmap showing the differentially expressed genes (n=237) between healthy proB cells from $Pax5^{+/-}$;*Myd88*^{+/-} mice (n=4) and $Pax5^{+/-}$ mice (n=4). c) Unsupervised heatmap showing the differentially expressed genes (n=327) between healthy proB cells from $Pax5^{+/-}$;*Myd88*^{+/-} mice (n=4) and $Pax5^{+/-}$ mice (n=4). The significance analysis of microarrays was defined by a p-value<0.05 and fold change: FC<-2 or FC>2. d) Venn diagram showing the overlapped genes of the differentially expressed genes between the three groups analyzed.





1.5

1.7 NES

1.3

1.9



b



NES

Supplementary Fig. S20: Gene expression enrichment analysis of healthy proB cells from Pax5^{+/-};Myd88^{+/-} and compared with Pax5^{+/-} proB cells. Gene Set Enrichment Analyses were performed using the gene sets from hallmark collection database a), canonical pathways gene sets derived from the KEGG pathway database b) and gene sets derived from the GO Biological Process Ontology c) from MSigDB⁴⁻⁶. This analysis identifies significant enrichment of several pathways in Myd88^{+/-}Pax5^{+/-} healthy pro B cells plotted on the y-axis. On the x-axis, the NES (Normalized Enrichment Score) value is represented for each gene set. The corresponding FDR (False Discovery Rate) value is represented in a blue color scale and the dot size depicts the gene count. Source data are provided as a Source Data file.









Supplementary Fig. S21: Impact of *Myd88* **downregulation in gene expression of** *Pax5***^{+/-} healthy proB cells. a) Gene set enrichment analyses showing the enriched murine B cell developmental stages, plotted on the y-axis, in** *Pax5***^{+/-};***Myd88***^{+/-} proB cells from healthy mice. 16 different developmental stages were assessed and gene sets were extracted from Green et al.⁷ b) Gene-sets of Pax5-regulated genes, plotted on the y-axis, were more enriched in** *Pax5***^{+/-};***Myd88***^{+/-} proB cells that in** *Pax5***^{+/-} healthy proB cells. Genesets were extracted from Revilla et al.⁸ and Schebesta et al.⁹. On the x-axis, the NES (Normalized Enrichment Score) value is represented for each gene set. The corresponding FDR (False Discovery Rate) value is represented in a blue color scale and the dot size depicts the gene count. (BLP: B-cell-biased lymphoid progenitors; ALP: all-lymphoid progenitors; MATB: mature B cells). Source data are provided as a Source Data file.**





Supplementary Fig. S22: Inflammatory cytokines levels in *Pax5^{+/-}*, *Myd88^{+/-};Pax5^{+/+}* and *Myd88^{+/-};Pax5^{+/-}* mice. IL-2, IL-4, IL-6, IL-10, IL-17a, IFN γ and TNF α serum levels in WT, *Pax5^{+/-}*, *Myd88^{+/-};Pax5^{+/+}* and *Myd88^{+/-};Pax5^{+/-}* mice. The different cytokines' serum levels were at different ages of the mice. Error bars represent the mean and SD. For significant differences between groups, Mann-Whitney p-values are indicated in each case. When comparing each cytokine using Kruskal-Wallis test, significant p-values are only significant for IL4 (p=0.044) and TNF α (p<0.001). Source data are provided as a Source Data file.



Supplementary Fig. S23: Percentages of CD4+ T (a) and B (b) cells in peripheral blood of $Pax5^{+/-}$ and WT mice treated with poly(I:C) compared to untreated mice. Each point represents the mean of the levels of the different populations in all mice in each group for the different time points (n=31 for $Pax5^{+/-}$ poly(I:C)-treated mice, n=8 for $Pax5^{+/-}$ untreated mice, n=14 for WT poly(I:C) treated mice and n=24 for WT untreated mice). Error bars represent the mean and SD of each group. P-values corresponding to unpaired t-test (two-tailed) are shown. Source data are provided as a Source Data file.



Supplementary Fig. S24: Impact of poly(I:C) treatment on the serum cytokines. The plots showed how poly(I:C) treatment changed the levels of some inflammatory cytokines in the serum of Pax5+/- (n=30) and WT (n=14) mice. TNF-alpha, INF-gamma, MCP1, and IL6 levels showed an increase in the serum of the mice after poly(I:C) treatment. IL12p70 and IL10 did not show changes in their levels. The different cytokines' serum levels were measured before and 3 hours after the administration of the poly(I:C). Error bars represent the mean and SD. For the significant differences, paired t-test p-values are indicated in each case. Source data are provided as a Source Data file.





Supplementary Fig. S25: Inflammatory cytokines levels in Pax5^{+/-} and WT mice treated with poly(I:C) compared with untreated Pax5+/- and WT mice. IL-2, IL-4, IL-6, IL-10, IL-17a, IFN γand TNF $\alpha serum$ levels in WT and Pax5^{+/-} mice treated with poly(I:C) compared with WT and *Pax5*^{+/-} mice non-treated. The different cytokines' serum levels were at different ages of the mice. Error bars represent the mean and SD. For significant differences between groups, Mann-Whitney p-values are indicated in each case. When comparing each cytokine using Kruskal-Wallis test, significant pvalues are only significant for IL2 (p=0.013), IL6 (p=0.001) and TNF α (p<0.001). Source data are provided as a Source Data file.



Supplementary Figure S26. Poly(I:C) treatment does not reduce the preleukemic compartment in the bone marrow of $Pax5^{+/-}$ mice. Absolute numbers of preB and proB cells (B220^{low} IgM⁻) in the bone marrow (BM) of $Pax5^{+/-}$ (n=9) and WT (n=5) poly(I:C)-treated mice compared with age-matched $Pax5^{+/-}$ (n=5) and WT (n=4) mice treated with PBS. Error bars represent the mean and SD of each group. For significant differences, p-values corresponding to unpaired t-test (two-tailed) are shown. Source data are provided as a Source Data file.



Supplementary Figure S27. *Pax5*^{+/-} mice treated with poly(I:C) present similar myeloid cells levels than untreated *Pax5*^{+/-} mice. a) Percentage of myeloid cells (Gr1⁺ Mac1⁺) in the peripheral blood (PB) of *Pax5*^{+/-} (n=9) and WT (n=5) poly(I:C)-treated mice compared with age-matched *Pax5*^{+/-} (n=5) and WT (n=4) mice treated with PBS. Error bars represent the mean and SD of each group. For significant differences, p-values corresponding to unpaired t-test (two-tailed) are shown. b) Percentage of myeloid cells (Gr1⁺ Mac1⁺) in the bone marrow (BM) of *Pax5*^{+/-} (n=9) and WT (n=5) poly(I:C)-treated mice compared with age-matched *Pax5*^{+/-} (n=5) and WT (n=5) poly(I:C)-treated mice compared with age-matched *Pax5*^{+/-} (n=5) and WT (n=4) mice treated with PBS. Error bars represent the mean and SD of each group. For significant differences, p-values corresponding to unpaired t-test (two-tailed) are shown. b) percentage of myeloid cells (Gr1⁺ Mac1⁺) in the bone marrow (BM) of *Pax5*^{+/-} (n=5) and WT (n=5) poly(I:C)-treated mice compared with age-matched *Pax5*^{+/-} (n=5) and WT (n=4) mice treated with PBS. Error bars represent the mean and SD of each group. For significant differences, p-values corresponding to unpaired t-test (two-tailed) are shown. Source data are provided as a Source Data file.







Supplementary Fig. S28: Phenotype characterization of leukemic cells from diseased *Pax5*^{+/-} mice treated with poly(I:C). Flow cytometric analysis of hematopoietic subsets in diseased *Pax5*^{+/-} mice treated with poly(I:C) (**a**), in a healthy *Pax5*^{+/-} mice treated with poly(I:C) (**b**) and in a healthy untreated *Pax5*^{+/-} mice (**c**), all of them exposed to common infections. Representative plots of cell subsets from bone marrow, spleen, lymph nodes and peripheral blood show the accumulation of malignant B cells in leukemic *Pax5*^{+/-} mice compared to an aged healthy *Pax5*^{+/-} mouse treated both with poly(I:C) and to a healthy untreated *Pax5*^{+/-} mouse. Flow cytometric images in panel A are representative of 8 mice analysed that developed leukemia after poly(I:C) treatment. The leukemic cells showed a B220^{low} IgM⁻ CD25^{+/-} CD19^{+/-} cKit^{+/-} phenotype and were detected only in the diseased mice.



Supplementary Fig. S29: B-ALL in *Pax5*^{+/-} **mice treated or untreated with poly(I:C).** Haematoxylin and eosin staining of tumour-bearing *Pax5*^{+/-} mice treated or untreated with poly(I:C) showing infiltrating blast cells in the spleen, lymph nodes, and liver. Loss of normal architecture can be seen due to the infiltrating cells morphologically resembling lymphoblast. Magnification and the corresponding scale bar are indicated in each case.



Supplementary Fig. S30: Gating strategy used in FACs analysis. Figure exemplifying the gating strategy used in all cytometric analysis. For each analysis, a total of at least 50,000 viable cells (PI-; Propidium iodide negative cells) were assessed. Singlets were selected prior gating strategy that is specific for each population. It is shown as an example, bone marrow cells stained with IgM-APC and B220-FITC. The same gating strategy has been used in all FACs analysis presented in Figure 2c, Figure 3c, Figure 4b, Figure 6b, Supplementary Fig. S3, Supplementary Fig. S6, Supplementary Fig. S9c, Supplementary Fig. S10, Supplementary Fig. S18, Supplementary Fig. S23, Supplementary Fig. S27, and Supplemental Figure 28.

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