SUPPLEMENTARY METHODS

Multiplex Immunohistochemistry

For Fig. S8G, the staining procedure was performed on a VENTANA Discovery Ultra instrument. The tissues were deparaffinised and rehydrated using EZ prep solution (Ventana Medical Systems, Tucson, USA) in 3 cycles of 8 min each for a total of 24 min at 69 °C. Antigens were retrieved using CC1 buffer (Ventana Medical Systems, Tucson, USA) for 32 min at 95 °C. Then, the primary antibody was diluted in antibody diluent/block (Akoya Biosciences, Marlborough, USA), added manually to the slides and incubated for 32 min at 36 °C or RT. The matching OmniMap horse radish peroxidase secondary antibody (Ventana Medical Systems, Tucson, USA) was automatically applied for 12 min. Subsequently, the Opal TSA fluorophore (Akoya Biosciences, Marlborough, USA) was diluted in amplification diluent (Akoya Biosciences, Marlborough, USA), added to the sections manually and incubated for 8 min. A denaturation step was performed at 100 °C for 24 min in CC2 buffer (Ventana Medical Systems, Tucson, USA) to remove primary and secondary antibodies. All steps, from primary antibody to stripping, were repeated for each marker until a 3-plex staining was achieved. The sections were then counterstained with DAPI (Merck, Darmstadt, Germany), washed and mounted using Fluoromount-G mounting medium (SouthernBiotech, Birmingham, USA). Primary antibodies used: Axl (AF854, R&D Systems, 1:100), Iba1 (NBP2-19019, NovusBiologicals, 1:100) and GFP (ab6673, abcam, 1:350). Detection was carried out using TSA fluorophores Opal 570 (1:250), Opal 520 (1:100), Opal 570 (1:100) and Opal 650 (1:100), respectively. Multispectral images were obtained using the Vectra 3.0 Automated Imaging System (Akoya Biosciences, Marlborough, USA). Spectral unmixing was performed in inForm (Akoya Biosciences, Marlborough, USA), using a previously built library consisting of single stained tissue slides for each TSA fluorophore.

Generation of *Gas6*-deficient NSG Mice by CRISPR-editing of NSG Zygotes

Immune-compromised *Gas6*-deficient mice were generated by inactivation of the *Gas6* gene using CRISPR-Cas9 editing in *NOD.Cg-Prkdc^scid IL2rgtmWjl/Sz* (NSG) zygotes, as indicated in Supplementary Fig. S3C, using a workflow that was previously described by our group (1). Briefly, gRNA targeting exon 2 of the *Gas6* gene, Cas9 mRNA (5meC, Psi) (Tebu-Bio# L-

6125-100) and a single strand DNA donor template were microinjected in NSG zygotes. The ssDNA template was designed to introduce an in-frame stop codon, an EcoRI restriction site and a mutation in the PAM sequence. The EcoRI site was used to screen edited mice by RFLP (Restriction Fragment Length Polymorphism) assay. All primers used are described in the key resources table. Mutation of the PAM site was used to prevent secondary editing of any productively edited allele. Insertion of the stop codon in an early translated exon expectedly halted translation thereby inducing non-sense mediated mRNA decay (NMD) leading to absence of Gas6 protein, as demonstrated by ELISA, in all four newly generated Gas6 homozygous edited mouse lines (line 697-21, 27, 29 and 31; Supplementary Fig. S3D). Experiments were carried out using NSG $Gas6^{-/-}$ lines #29 and #31 with indistinguishable results (Figure 1N and Supplementary Figure S3E). Editing at predicted off-target sites was excluded by amplicon sequencing as described previously (1). Genome editing of NSG zygotes using CRISPR/Cas9 was approved by the Regierungspräsidium Karlsruhe under animal protocol number G50/15.

Generation of Syngeneic Leukemia Models

The Asxl1 myeloid leukemia model was established by serially transplanting spleen cells from a mouse that developed disease symptoms 39 weeks after inducible ablation of _Asxl1_ selectively in hematopoietic cells. Briefly, aged $MxCre^+ Asxl1^{f/f}$ mice (50 weeks) were treated with polyinosinic-polycytidylic acid (pI:pC) (InvivoGen) every other day at a dose of 20 mg/kg of body weight, and followed for signs of disease by monthly bleeding. Over an additional period of 39 weeks, 4 out of 5 mice remained symptoms free with white blood counts within normal range. One mouse (#83501) however developed disease symptoms with prominent expansion of immature blasts with significantly reduced _Asxl1_ expression as shown by real time PCR and an aberrant CD11b^dim^B220^dim^ surface phenotype (Supplementary Fig. S1C). H&E staining of the spleen and liver, as well as myeloperoxidase staining in the liver, demonstrated massive infiltration of both organs by immature myeloblasts as well as limited signs of extramedullary hematopoiesis in the spleen as shown by prominent erythroid cell clusters and detectable megakaryocytes. However, no mature granulocytes were detected (Supplementary Fig. S1D). Most importantly the disease was serially transplantable, using

spleen cells from the primary mouse, to non-irradiated recipients with a penetrance that was dependent on the cells dose injected (Supplementary Fig. S1E). Non-irradiated recipient mice displayed massive hepatosplenomegaly (Supplementary Fig. S1F) with massive infiltration of spleen and liver by blast cells (Supplementary Fig. S1G) as well as a high number of circulating blasts as evidenced by Pappenheim's staining of peripheral blood smears (Supplementary Fig. S1H). Bio-banked cells from the spleens of the primary and secondary wild type recipients were used for all experiments described in this study. Secondary and tertiary recipients showed comparable hepatosplenomegaly and high number of circulating myeloblasts in the peripheral blood (Supplementary Fig. S1H)

Primary BCR-ABL1-driven B-cell acute lymphoblastic leukemia (Ph$^+$ B-ALL) were generated by isolating untreated bone marrow cells from *Arf$^{-/-}$* mice (The Jackson Laboratories, line #029676) and subsequently subjecting them to *ex-vivo* spin infection (3000 rpm, 33C, 2h) in IMDM media (Gibco) supplemented with 20 ng/ml recombinant mouse IL7 (Peprotech) and 4.5 µg/ml polybrene (Merck Millipore), using a retrovirus expressing both the human BCR-ABL1^{p190} fusion oncoprotein and the green fluorescent protein (GFP) that serves as a reporter for BCR-ABL1 expression (MSCV-BCR-ABL1-IRES-GFP; gift from Dr. Jacques Ghysdael). After transduction these cells were transferred to lethally irradiated (9 Gy) wild type C57BL/6 host recipients. All mice developed full blow precursor B-ALL within 30-60 days (data not shown). Bone marrow cells from these primary mice exhibited high frequency of GFP$^+$B220^{dim} B-ALL blasts. Bone marrow cells from several primary mice were explanted and *in vitro* adapted by culturing on MS5 mesenchymal stromal cells in RPMI containing 15% FBS, 1% penicillin-streptomycin, 1% L-Glutamine and 10mM HEPES. After a short adaptation period on MS5 cells, primary leukemic cells could be expanded and maintained as independent primary GFP$^+$ leukemic lines (L1, L2, L3) in a stroma-independent fashion and serially transplanted in non-irradiated mice as depicted by the Kaplan-Meier survival analysis of recipient mice transplanted with a 10^3 GFP$^+$ B-ALL cells from n = 3 independent primary leukemia lines (L1, L2 and L3) in Supplementary Fig. S1J. For all established B-ALL lines, disease penetrance was 100% after transplantation of as little as 10^3 cells to non-irradiated recipients. Latencies were also comparable for all established leukemia, with a median survival between 12 and 20 days (Supplementary Fig. S1J and data not shown).

KEY RESOURCES

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-mouse CD45-BV786	BD Biosciences	Cat# 564225; RRID:AB_2716861
Anti-mouse CD45.1-FITC	eBioscience	Cat# 11-0453-85; RRID:AB_465059
Anti-mouse CD45.2-PE	eBioscience	Cat# 12-0454-83; RRID:AB_465679
Anti-mouse Gr1-PECY7	BD Biosciences	Cat# 552985; RRID:AB_394535
Anti-mouse CD3-APC-Cy7	BD Biosciences	Cat# 100222; RRID:AB_2242784
Anti-mouse CD8a-AF700	BD Biosciences	Cat# 564983; RRID:AB_2739032
Anti-mouse CD8-PE-Cy7	BD Biosciences	Cat# 25-0081-82; RRID:AB_469584
Anti-mouse CD4-PE-Cy7	eBioscience	Cat# 25-0041-82; RRID:AB_469576
Anti-mouse B220-APC	BD Biosciences	Cat# 553092; RRID:AB_398531
Anti-mouse B220-BV711	BD Biosciences	Cat# 563892; RRID:AB_2738470
Anti-mouse NK1.1-PECF594	BD Biosciences	Cat# 562864; RRID:AB_2737850
Anti-mouse CD11b-FITC	BD Biosciences	Cat# 553310; RRID:AB_394774
Anti-mouse CD11b-PECF594	BD Biosciences	Cat# 562317; RRID:AB_11154422
Anti-mouse CD11c-AF700	BD Biosciences	Cat# 560583; RRID:AB_1727421
Anti-mouse F4/80-PE	BD Biosciences	Cat# 565410; RRID:AB_2687527
Anti-mouse MHC-II-BV650	BD Biosciences	Cat# 563415; RRID:AB_2738192
Anti-mouse Annexin V-APC	BD Biosciences	Cat# 550475
Anti-mouse Axl-APC	eBiosciences	Cat# 17-1084-82 RRID:AB_2734848
Anti-mouse FoxP3-PE	BD Biosciences	Cat# 12-5773-82; RRID:AB_465936
Anti-mouse MHC-II-BV786	BD Biosciences	Cat# 563415; RRID:AB_2738192
Anti-mouse DX5_APC	eBioscience	Cat# 17-5971-81 RRID:AB_469484
Anti-human CD45-PE	BD Biosciences	Cat# 555483; RRID:AB_2649445
Anti-human CD14-APCCY7	BD Biosciences	Cat# 557831; RRID:AB_396889
CD16/32 Fc Block	BD Biosciences	Cat# 553441 RRID:AB_394861
One Comp Beads	eBiosciences	Cat# 01-1111-42
Cytometer Setup and Tracking beads	BD Biosciences	Cat# 641319
Anti-mouse IgG1	BD Biosciences	Cat# 553441; RRID:AB_394861
Anti-human GAS6	Sigma-Aldrich	Cat# HPA008275; RRID:AB_1849497

Anti-human CD10	Novocastra-Leica Biosystems	Cat# ORG-8941
Anti-GFP (IHC)	Abcam	Cat# ab6673; RRID:AB_305643
Anti-Goat IgG (H+L)	Vector Laboratories	Cat# BA-5000; RRID:AB_2336126
Anti-GFP (IF)	Abcam	Cat# ab13970; RRID:AB_300798
Anti-Chicken AF488	Jackson ImmunoResearch Labs	Cat# 703-545-155 RRID:AB_2340375
Anti-mouse Iba1 (Rabbit)	Wako Chemicals	Cat# 019-19741 RRID:AB_839504
Anti-Rabbit AF594	Jackson ImmunoResearch Labs	Cat# 711-586-152 RRID:AB_2340622
Anti-human/mouse Iba1 (Goat)	Abcam	Cat# 48004 RRID:AB_870576
Anti-Goat AF594	Jackson ImmunoResearch Labs	Cat# 705-586-147 RRID:AB_2340434
Anti-human/mouse Phospho_AXL (Y779)	R&D	Cat# AF2228 RRID:AB_2062560
Anti-Rabbit AF647	Jackson ImmunoResearch Labs	Cat# 711-606-152 RRID:AB_2340625
Anti-mouse Axl	R&D	Cat# AF854 RRID:AB_355663
Anti-mouse Gas6	R&D	Cat# ab13970 RRID:AB_300798
Anti-Goat AF647	Jackson ImmunoResearch Labs	Cat# 705-606-147 RRID:AB_2340438
Anti-human Myeloperoxidase	ThermoFisher Scientific	Cat# RB-373-R7; RRID:AB_59600
Anti-mouse CD8a	InVivoMAb	Cat# BE0061; RRID:AB_1125541
Rat IgG2b	InVivoMAb	Cat# BE0090; RRID:AB_1107780
Anti-mouse NK1.1	InVivoMAb	Cat# BE0036, RRID:AB_1107737
Mouse IgG2a	InVivoMAb	Cat# BE0085, RRID:AB_1107771
Anti-mouse PD1	InVivoMAb	Cat# BE0146, RRID:AB_10949053
Rat IgG2a	InVivoMAb	Cat# BE0089, RRID:AB_1107769
Anti-mouse IL12p40	InVivoMAb	Cat# BE0051 RRID:AB_1107698
Anti-mouse TNFa	InVivoMAb	Cat# BE0058 RRID:AB_1107764
Anti-mouse IL10	InVivoMAb	Cat# BE0049 RRID: AB_1107696
Rat IgG1	InVivoMAb	Cat# BE0088 RRID: AB_1107775
Biological Samples		
Bone marrow biopsies	Faculty of Medicine of the Technical University of Munich and the University Hospital Carl Gustav Carus	Table S1
Chemicals, Peptides, and Recombinant Proteins		
Nilotinib	APExBIO	A8232
Bemcentinib	BergenBio, ASA	N/A

Vincristine sulfate	APExBIO	Cat# A1765
M-CSF	ThermoFisher Scientific	Cat# 14-8983-80
INFg	Peprotech	Cat# 315-05
Chlodronate liposomes	Liposoma BV	Cat# C-030
LPS	Sigma-Aldrich	Cat# L4391
polyI:polyC	InVivoGen	Cat# tlrl-pic-5
Tween 80	Sigma-Aldrich	Cat# P4780
Methyl Cellulose 400cp	Sigma-Aldrich	Cat# M0262
Propidium iodide	Sigma-Aldrich	Cat# P4170
Roti®-Histofix 4 %	Carl Roth	Cat# P087.3
0.5 M EDTA PH7.4	Sigma-Aldrich	Cat# ED2SS
penicillin-streptomycin (Gibco)	Thermo Fischer Scientific	Cat# 15140-122
HEPES 1M	Sigma-Aldrich	Cat# H0887
L-Glutamine (Gibco)	Thermo Fischer Scientific	Cat# 25030-024
Fetal Bovine Serum (Gibco)	Thermo Fischer Scientific	Cat# 10270106
Cell Trace Violet	Thermo Fischer Scientific	Cat# C34557
Ficoll-Paque Plus	GE Healthcare	Cat# 17-1440-02
StemSpan^{TM} SFEM	Stem Cell Technologies	Cat# 09650
RPMI 1640 (Gibco)	Thermo Fischer Scientific	Cat# 31870-025
DMEM (Gibco)	Thermo Fischer Scientific	Cat# 21969-035
IMDM (Gibco)	Thermo Fischer Scientific	Cat# 21980-032
Recombinant mouse IL7	Peprotech	Cat# 407-ML
Polybrene	Merck Millipore	Cat# TR-1003-G
Hoechst 33342	Thermo Fischer Scientific	Cat# H3570
PBS	Gibco	Cat# 14190-094
Sucrose	CarlRoth	Cat# 9097.1
OCT	Weckert Labortechnik	Cat# 600001
Triton-X 100	Sigma Aldrich	Cat# X100
BSA	Biomol	Cat# BSA-50
Critical Commercial Assays		
Bond Polymer Refine Detection kit	Leica Biosystems	Cat# DS9800-CN
Bond Intense R Detection	Leica Biosystems	Cat# DS9263
PicoPure™ RNA Isolation Kit	ThermoFisher Scientific	Cat# KIT0204
SuperScript™ VILO™ cDNA Synthesis Kit	ThermoFisher Scientific	Cat# 11754050
Power SYBR™ Green PCR Master Mix	ThermoFisher Scientific	Cat# 4368702
Microvette® 500 Z-Gel	Sarstedt	Cat# 20.1344
Mouse Gas6 DuoSet ELISA	R&D Systems	Cat# DY986
Anti-F4/80 MicroBeads UltraPure, mouse	Miltenyi Biotec	Cat# 130-110-443
CD14-Microbeads, human	Miltenyi Biotec	Cat# 130-050-201
Cytofix/Cytoperm plus	BD Biosciences	Cat# 555028
Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1	10X Genomics	Cat# PN-1000121
Qubit™ dsDNA HS Assaykit	ThermoFisher Scientific	Cat# Q32851
Bioanalyzer High Sensitivity DNA Kit	Agilent	Cat# 5067-4626
NextSeq500/550 high output kit v2.5 (75 cycles)	Illumina	Cat# 20024906
Venor GeM OneStep (Mycoplasma PCR kit)	Minerva biolabs	Cat# 11-8100
AF700 fixable viability dye	BD Biosciences	Cat# 564997
Nunc™ Multidishes with UpCell™	ThermoFisher Scientific	Cat# 174899
Deposited Data		

ImmGen ULI RNASeq data group	Immunological Genome Project (ImmGen)	http://www.immgen.org/Databrowser19/DatabrowserPage.html
Hummel et al. B-cell lymphoma dataset	(2)	GSE4475
AML dataset from The Cancer Genome Atlas (TCGA)	(3)	TCGA-LAML
Single cell RNA sequencing described in Figure 3	This work	European Nucleotide Archive (https://www.ebi.ac.uk/ena/); accession number: PRJEB43830
Experimental Models: Cell Lines		
Mouse: Mesenchymal stromal cells MS-5	DSMZ-German Collection of Microorganisms and Cell Cultures	Cat# ACC-441; RRID:CVCL_2128
Platinum-E (Plat-E) Retroviral Packaging Cell Line	Gift from Jacques Ghysdael; (4)	N/A
Experimental Models: Organisms/Strains		
Mouse: MxCre Asxl1$^{f/f}$	Laboratory of Omar Abdel-Wahab; (5)	N/A
Mouse: Gas6$^{tm1.1(KOMP)Vlcg}$	Knock Out Mouse Project (KOMP) Repository	https://www.komp.org/index.php
Mouse: C57BL/6N	The Jackson Laboratory	Cat# JAX:005304; RRID:IMSR_JAX:005304
Mouse: C57BL/6J	The Jackson Laboratory	Cat# JAX:000664; RRID:IMSR_JAX:000664
Mouse: B6.SJL-*Ptprc^a Pepc^b/BoyJ* (CD45.1)	The Jackson Laboratory	Cat# JAX: 002014; RRID:IMSR_JAX:002014
Mouse: NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG)	The Jackson Laboratory	Cat# JAX:005557; RRID:IMSR_JAX:005557
Mouse: NSG Gas6$^{-/-}$	This paper	N/A
Mouse: Csf1r-Cre Axl$^{f/f}$	Laboratory of Carla Rothlin & Sourav Ghosh; (6)	N/A
Mouse: CD11c-eGFP-Cre Axl$^{f/f}$	Laboratory of Carla Rothlin & Sourav Ghosh; (7)	N/A
Mouse: B6.129S2-Cd8a^{tm1Mak}/J (CD8$^{-/-}$)	The Jackson Laboratory	Cat# JAX:002665 RRID:IMSR_JAX:002665
Mouse: B6;129S4-Socs3^{tm1Ayos}/J	The Jackson Laboratory	Cat# JAX:010944 RRID:IMSR_JAX:010944
Mouse: B6(Cg)-Sting1 $^{tm1.2Camb}$/J (*Sting$^{-/-}$*)	The Jackson Laboratory	Cat# JAX:025805 RRID:IMSR_JAX:025805
Oligonucleotides		
Cas9 mRNA NLS – 5-methyl-C, pseudo-U	Tebu-Bio	Cat# 040L-6125-100
Cas9-mGas6-gRNA1rv AAA CCA CGC GCC CGC AAC AGC ACA C	IDT DNA	N/A
Cas9-mGas6-gRNA1fw CAC CGT GTG CTG TTG CGG GCG CGT G	IDT DNA	N/A
Donor IHRES template: ssDNAmGas6Cas9gRNA1mis CCC GAG TGG GTG TAG GTT GAC CGT ACA CCC TTC TTT CTG CAG CTG TGC TGT TGC GGG CGT GAG AAT TCC GTG ACG CGG CGC AGT TTC TGC GGC CCA GGC AGC GCC GCG CCT ACC AAG TCT TCG AGG AGG	IDT DNA	N/A
Primer: mGas6ex2-RFL-FW TGGCTTCCGAGTCTTCTCAC	IDT DNA	N/A

Primer: mGas6ex2-RFL-RV TGGAGCCCACTCAAGGATAC	IDT DNA	N/A
Primer : Axl FW : CGAGAGGTGACCTTGGAAC	(6)	N/A
Primer : Axl RV : AGATGGTGGAGTGGCTGTC	(6)	N/A
Primer : Sdha FW : AAGTTGAGATTTGCCGATGG	(1)	N/A
Primer : Sdha RV : TGGTTCTGCATCGACTTCTG	(1)	N/A
Taqman probes		
Mouse *Gas6* (Probe ID: Mm00490378_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Pros1* (Probe ID: Mm01343426_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Axl* (Probe ID: Mm00437221_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Mertk* (Probe ID: Mm00434920_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Tyro3* (Probe ID: Mm00444547_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Il12b* (Probe ID: Mm01288989_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Tnfα* (Probe ID: Mm00443258_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Asxl1* (Probe ID: Mm0898215_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Retnla* (Probe ID: Mm00445109_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Chil3* (Probe ID: Mm00657889_mH)	ThermoFisher Scientific	Cat# 4331182
Mouse *Arg1* (Probe ID: Mm00475988_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Il10* (Probe ID: Mm01288386_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Tgfb1* (Probe ID: Mm01178820_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Sdha* (Probe ID: Mm01352366_m1)	ThermoFisher Scientific	Cat# 4331182
Mouse *Ubc* (Probe ID: Mm02525934_g1)	ThermoFisher Scientific	Cat# 4331182
Human *GAS6* (Probe ID: Hs01090305_m1)	ThermoFisher Scientific	Cat# 4331182
Human *GUSB* (Probe ID: Hs00939627_m1)	ThermoFisher Scientific	Cat# 4331182
Recombinant IHRES		
pMIG-p190[BCR-ABL1]-IHRES-GFP	Laboratory of Jacques Ghysdael	N/A
Software and Algorithms		
FACS Diva	BD Biosciences	N/A
FlowJo, v10.4.2	FlowJo, LLC	N/A
GraphPad Prism v7	GraphPad Software	N/A
AxioVision SE64 v4.9	Zeiss	Cat# 410130-0909-000
QuPath v0.2.3	(8)	https://qupath.github.io
R2: Genomics Analysis and Visualization Platform	Department of Oncogenomics, Academic Medical Center (AMC) Amsterdam, the Netherlands.	https://r2.amc.nl
SurvExpress	(9)	http://bioinformatica.mty.itesm.mx:8080/Biomatec/SurvivaX.jsp
Cell Ranger mkfastq version 3.1.0	10X Genomics	N/A
R version 3.6.2		https://www.r-project.org
Seurat package	(10)	https://satijalab.org/seurat/

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