Figure S1

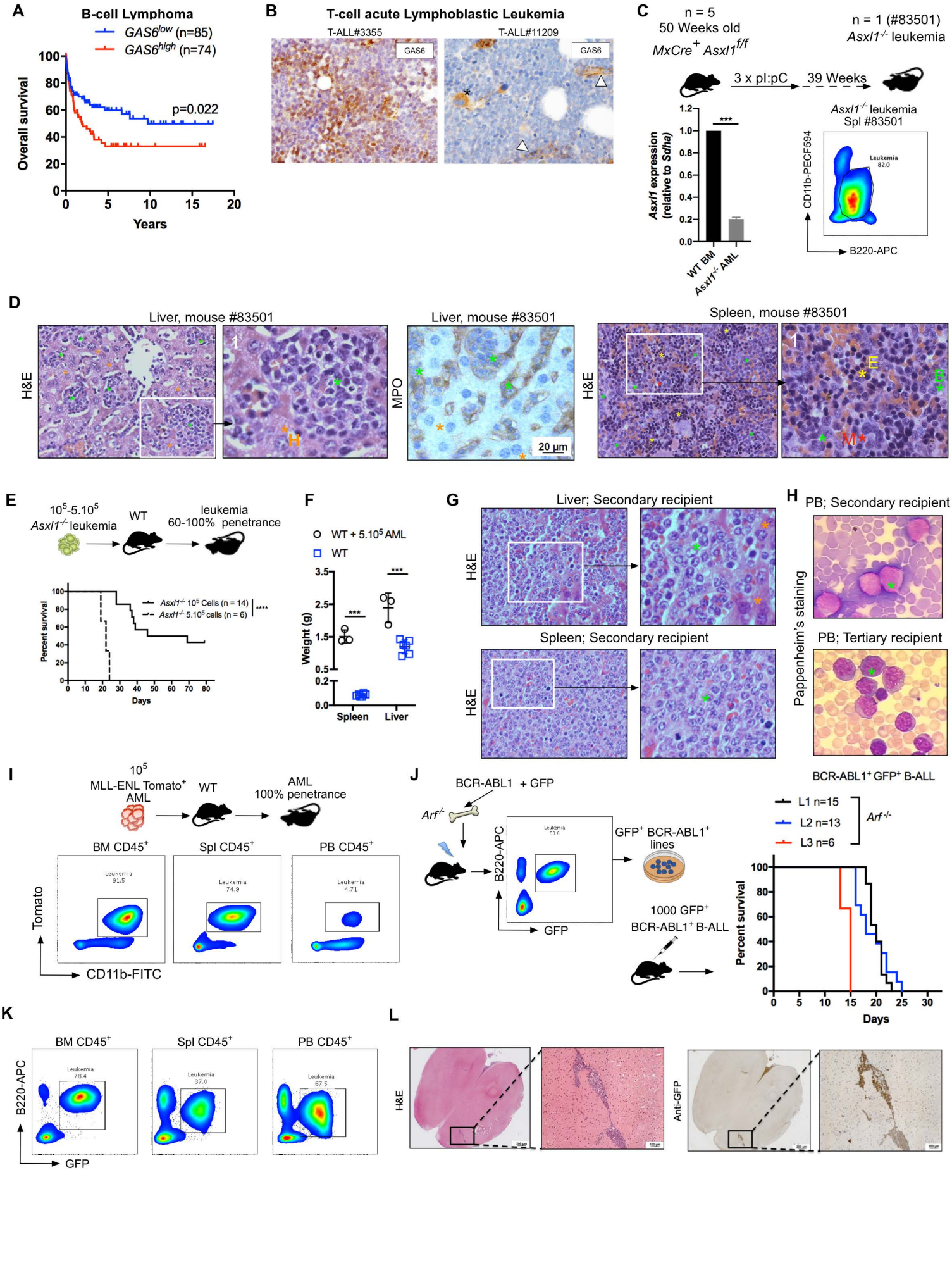
A
B-cell Lymphoma
GAS6low (n=85)
GAS6high (n=74)
Overall survival
p=0.022
Years
B
T-cell acute Lymphoblastic Leukemia
T-ALL#3355
T-ALL#11209
GAS6
C
n = 5
50 Weeks old
MxCre+ Asxl1f/f
3 x pI:pC
39 Weeks
n = 1 (#83501)
Asxl1-/- leukemia
Asxl1 expression (relative to Sdha)
WT BM
Asxl1-/- AML
Asxl1-/- leukemia
Spl #83501
CD11b-PECF594
B220-APC
Leukemia 82.0
D
Liver, mouse #83501
Liver, mouse #83501
Spleen, mouse #83501
H&E
MPO
20 µm
E
10^5-5.10^5 Asxl1-/- leukemia
WT
leukemia 60-100% penetrance
Asxl1-/- 10^5 Cells (n = 14)
Asxl1-/- 5.10^5 cells (n = 6)
Percent survival
Days
F
WT + 5.10^5 AML
WT
Weight (g)
Spleen
Liver
G
Liver; Secondary recipient
Spleen; Secondary recipient
H&E
H
PB; Secondary recipient
PB; Tertiary recipient
Pappenheim's staining
I
10^5 MLL-ENL Tomato+ AML
WT
AML 100% penetrance
BM CD45+
Spl CD45+
PB CD45+
Tomato
CD11b-FITC
J
BCR-ABL1 + GFP
Arf-/-
B220-APC
GFP
GFP+ BCR-ABL1+ lines
1000 GFP+ BCR-ABL1+ B-ALL
BCR-ABL1+ GFP+ B-ALL
L1 n=15
L2 n=13
L3 n=6
Arf-/-
Percent survival
Days
K
BM CD45+
Spl CD45+
PB CD45+
B220-APC
GFP
L
H&E
Anti-GFP

Figure S1. Significance of GAS6 expression in hematological cancers and pre-clinical models used in this study.

(**A**) Prognostic value of *GAS6* expression in B-cell lymphoma (GSE4475 dataset, n=159). Data generated using the KaplanScan mode from the R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl). Survival analysis by log-rank (Mantel–Cox) test.

(**B**) GAS6 expression by IHC on bone marrow trephine biopsies from T-ALL patients at diagnosis. Asterisk marks a megakaryocyte. Stromal cells marked by white pyramid.

(**C**) Schematic view of the generation of an acute leukemia model driven by *Asxl1* loss in hematopoietic cells (detailed description provided in Supplementary Methods). 39 weeks post *Asxl1* ablation, one primary mouse (1/5, #83501) developed a disease characterized by the expansion of cells with significantly reduced *Asxl1* expression as shown by real time PCR and an aberrant $CD11b^{dim}B220^{dim}$ immature surface phenotype. Real-time PCR data are means ± s.e.m. after normalization to a reference gene, *Sdha*.

(D) H&E staining on liver and spleen, as well as myeloperoxidase staining (MPO) on liver sections from mouse #83501 described in C. Green asterisk: Myeloid blasts; Yellow asterisk: Erythroid lineage cells; Red asterisk: Megakaryocyte; Orange asterisk: Hepatocyte.

(E) Spleen cells from the leukemic mouse described in C, were transferred to secondary and tertiary C57BL/6 recipients without prior conditioning. In secondary recipients, 100% disease penetrance was observed when 5.10^5 primary cells are injected (n = 5), and 60-80% if 10^5 cells are injected (n = 14).

(F) Disease-bearing secondary recipients show hepatosplenomegaly (n = 5 WT controls; n = 3 WT mice challenged with $Asxl1^{-/-}$ cells from (C). ***p<0.001, unpaired two-tailed Student's *t*-test.

(G) H&E staining on spleen and liver sections from a secondary recipient mouse. Green asterisk: Myeloid blasts; Orange asterisk: Hepatocyte.

(H) Pappenheim staining of peripheral blood smears from both secondary and tertiary recipient mice, demonstrating the presence of myloblasts with fine cytoplasmic granules.

(I) Injection of 10^5 MLL-ENL $tomato^+$ leukemia cells to non-conditioned C57BL/6 mice propagates leukemia in 100% of the animals as depicted by flow cytometry data showing $CD11b^+$ $tomato^+$ leukemic infiltrate in the bone marrow and spleen of diseased animals. Leukemic blasts are also detected in the peripheral blood albeit at a lower frequency.

(J) Schematic description of the generation of primary GFP^+ $BCR\text{-}ABL1^+$ B-ALL leukemia in an $Arf^{-/-}$ background (detailed description provided in Supplementary Methods). After a short adaptation period on MS5 mesenchymal stromal cells, primary leukemic cells could be expanded and maintained as primary GFP^+ leukemic lines in a stroma-independent fashion. Kaplan-Meier survival analysis of non-irradiated secondary recipient mice transplanted with a 10^3 GFP^+ B-ALL cells from n = 3 independent primary leukemia lines (L1, L2 and L3). Disease penetrance and latency were comparable for all 3 leukemias.

(K) Mice from (I) exhibit full-blown leukemia that can be detected as GFP^+ $B220^+$ blasts by FACS in the bone marrow, spleen and peripheral blood.

(L) H&E and anti-GFP IHC analysis show the presence of GFP^+ leukemic blasts in the brain of leukemic mice.

Figure S2

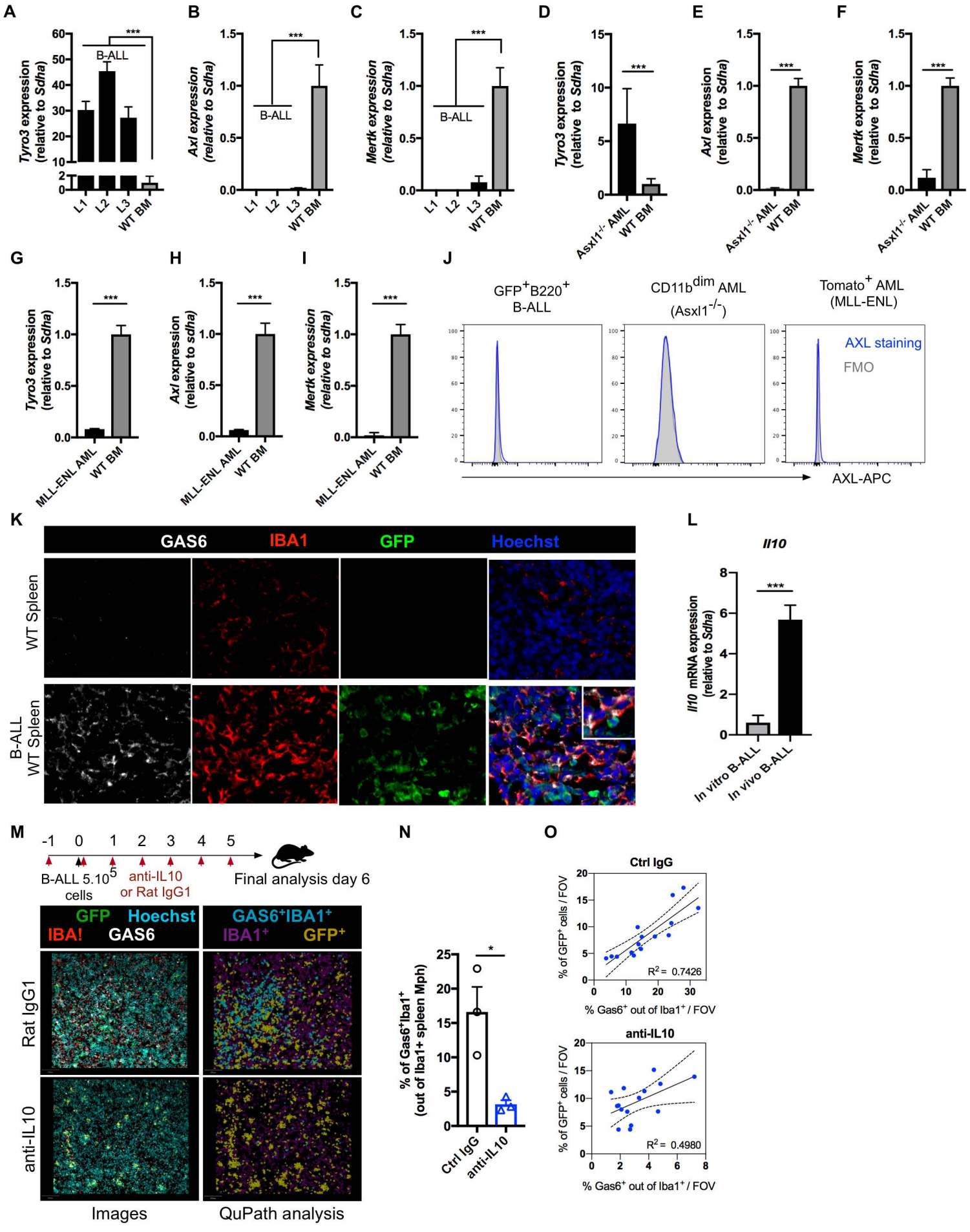
A
B
C
D
E
F
Tyro3 expression (relative to Sdha)
Axl expression (relative to Sdha)
Mertk expression (relative to Sdha)
B-ALL
***
L1
L2
L3
WT BM
Asxl1-/- AML
G
H
I
J
MLL-ENL AML
Axl expression (relative to sdha)
Mertk expression (relative to sdha)
GFP+B220+ B-ALL
CD11bdim AML (Asxl1-/-)
Tomato+ AML (MLL-ENL)
AXL staining
FMO
AXL-APC
K
GAS6
IBA1
GFP
Hoechst
WT Spleen
B-ALL WT Spleen
L
Il10
Il10 mRNA expression (relative to Sdha)
In vitro B-ALL
In vivo B-ALL
M
-1 0 1 2 3 4 5
B-ALL 5.10^5 cells
anti-IL10 or Rat IgG1
Final analysis day 6
GFP Hoechst
IBA! GAS6
GAS6+IBA1+
IBA1+ GFP+
Rat IgG1
anti-IL10
Images
QuPath analysis
N
% of Gas6+Iba1+ (out of Iba1+ spleen Mph)
Ctrl IgG
anti-IL10
O
Ctrl IgG
% of GFP+ cells / FOV
R2 = 0.7426
% Gas6+ out of Iba1+ / FOV
anti-IL10
R2 = 0.4980

Figure S2. Expression pattern of TAM RTKs in murine leukemic cells and GAS6 induction in leukemia associated macrophages.

(**A-I**) TAM (*Tyro3, Axl* and *Mertk*) RTKs expression as evaluated by real-time PCR in L1, L2, and L3 B-ALL (GFP^+ $B220^+$) (**A-C**) as well as FACS purified *Asxl1*$^{-/-}$ AML cells ($CD11b^{dim}B220^{dim}$) (**D-F**) or *MLL-ENL* AML ($Tomato^+$ $CD11b^+$) (**G-I**). In (A-I) WT bone marrow cells were used as a reference (WT BM) and all data are mean ± s.e.m. after normalization to a reference gene, *Sdha*. ***p<0.001, unpaired two-tailed Student's *t*-test.

(**J**) Axl expression as evaluated by flow cytometry in $CD45^+$ leukemic blasts from mice with B-ALL (GFP^+B220^+), *Asxl1*$^{-/-}$ AML ($CD11b^{dim}$) or MLL-ENL AML ($Tomato^+$ $CD11b^+$). Full stain minus one (FMO for Axl) was used as negative reference.

(**K**) Representative immune-fluorescence images assessing the expression of Gas6 (white) in $Iba1^+$ macrophages (red) from WT and B-ALL-bearing (GFP) mice.

(**L**) *Il10* expression as evaluated by real-time PCR in B-ALL cells ($B220^+GFP^+$) expanded *ex-vivo* (n = 4) or FACS purified from leukemia bearing mice (n = 6). Data are mean ± s.e.m. after normalization to a reference gene, *Sdha*. ***p<0.001, unpaired two-tailed Student's *t*-test.

(**M**) Representative immune fluorescence staining with corresponding phenotypic segmentation using QuPath version 0.2.3, depicting Gas6 expression in in $Iba1^+$ macrophages in the spleen of mice that were challenged with B-ALL and treated with either an anti-IL10 (n = 3) or a Rat IgG1 (n = 3) control antibody (300 µg/mouse/day).

(**N**) Depicts the percentage of macrophages that express Gas6 in spleen samples from M. Each data point represents a mean of 5 field of views (FOV) from one independent mouse. *p<0.05, unpaired two-tailed Student's *t*-test.

(**O**) Correlation analysis between the percentage of leukemic blasts per field of view (% GFP^+ cells/ FOV) and the percentage of Gas6-expressing macrophages per field of view (% $Gas6^+$ out of $Iba1^+$/ FOV) in the spleen of mice from M. each dot represents an independent FOV. Five FOVs were analyzed for each mouse.

Figure S3

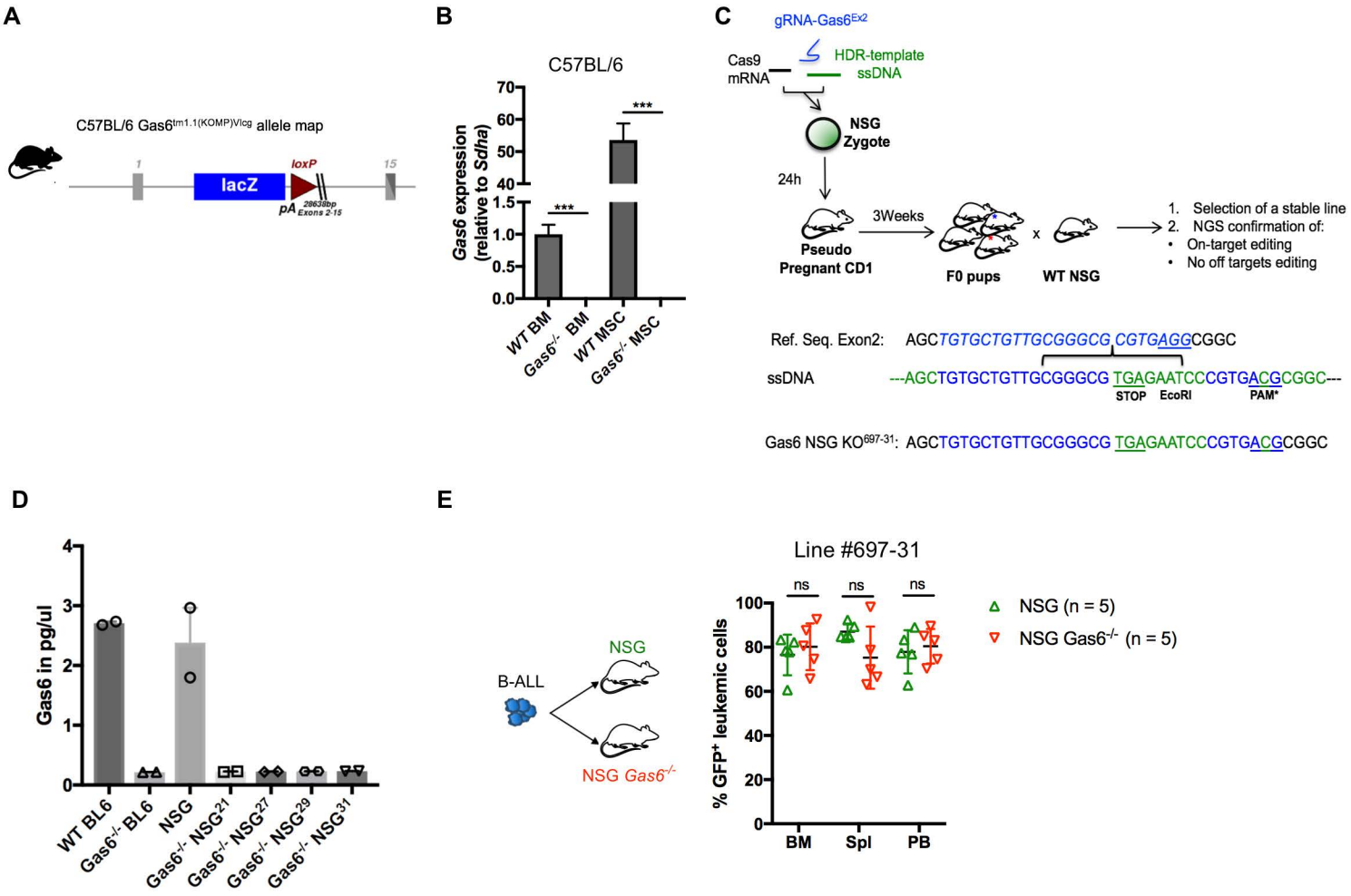
A
C57BL/6 Gas6tm1.1(KOMP)Vlcg allele map
1
lacZ
loxP
15
pA
28638bp
Exons 2-15
B
C57BL/6
Gas6 expression (relative to Sdha)
70
60
50
40
1.5
1.0
0.5
0.0
***
***
WT BM
Gas6-/- BM
WT MSC
Gas6-/- MSC
C
gRNA-Gas6Ex2
Cas9 mRNA
HDR-template ssDNA
NSG Zygote
24h
3Weeks
Pseudo Pregnant CD1
F0 pups
x
WT NSG
1. Selection of a stable line
2. NGS confirmation of:
• On-target editing
• No off targets editing
Ref. Seq. Exon2: AGCTGTGCTGTTGCGGGCG CGTGAGGCGGC
ssDNA ---AGCTGTGCTGTTGCGGGCG TGAGAATCCCGTGACGCGGC---
STOP
EcoRI
PAM*
Gas6 NSG KO697-31: AGCTGTGCTGTTGCGGGCG TGAGAATCCCGTGACGCGGC
D
Gas6 in pg/ul
4
3
2
1
0
WT BL6
Gas6-/- BL6
NSG
Gas6-/- NSG21
Gas6-/- NSG27
Gas6-/- NSG29
Gas6-/- NSG31
E
B-ALL
NSG
NSG Gas6-/-
Line #697-31
% GFP+ leukemic cells
100
80
60
40
20
0
ns
ns
ns
BM
Spl
PB
NSG (n = 5)
NSG Gas6-/- (n = 5)

Figure S3. Generation and characterization of *Gas6*-deficient mouse lines in a C57BL/6 immune-competent and *NOD.Cg-Prkdc^{scid} IL2rgtmWjl/Sz* (NSG) immune-compromised background.

A, C57BL/6 *Gas6* knock out mice were obtained from the Knock Out Mouse Project (KOMP) Repository (https://www.komp.org/index.php). A schematic view of the knockout allele is indicated.

B, *Gas6* expression as evaluated by real-time PCR, both in hematopoietic bone marrow cells (BM) and mesenchymal stromal cells (MSC). ***p<0.001, unpaired two-tailed Student's *t*-test.

C, 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 following a workflow that was previously described by our group (Tirado-Gonzalez et al., 2018). This workflow relies on the use of a donor DNA template and homology directed repair (NSG lack functional non-homologous end-joining repair mechanism). The donor DNA template introduces a stop codon in exon 2 of the *Gas6* gene (to promote non-sense mediated mRNA decay), an EcoR-I site for screening purposes and a silent mutation in the PAM sequence to avoid secondary editing of any productively edited allele. Four independent NSG *Gas6*-deficient lines were generated (697-21, 27, 29 and 31).

D, Gas6 protein was undetectable both in C57BL/6 *Gas6$^{-/-}$* and all four NSG *Gas6$^{-/-}$* lines as shown by ELISA (n = 2 mice per genotype).

E, Validation of data obtained in Fig. 1N, using a different NSG *Gas6$^{-/-}$* line and demonstrating comparable leukemic burden in WT versus *Gas6$^{-/-}$* NSG challenged with B-ALL (NSG n = 5, NSG$^{\#697\text{-}31}$ *Gas6$^{-/-}$* n = 5).

Figure S4

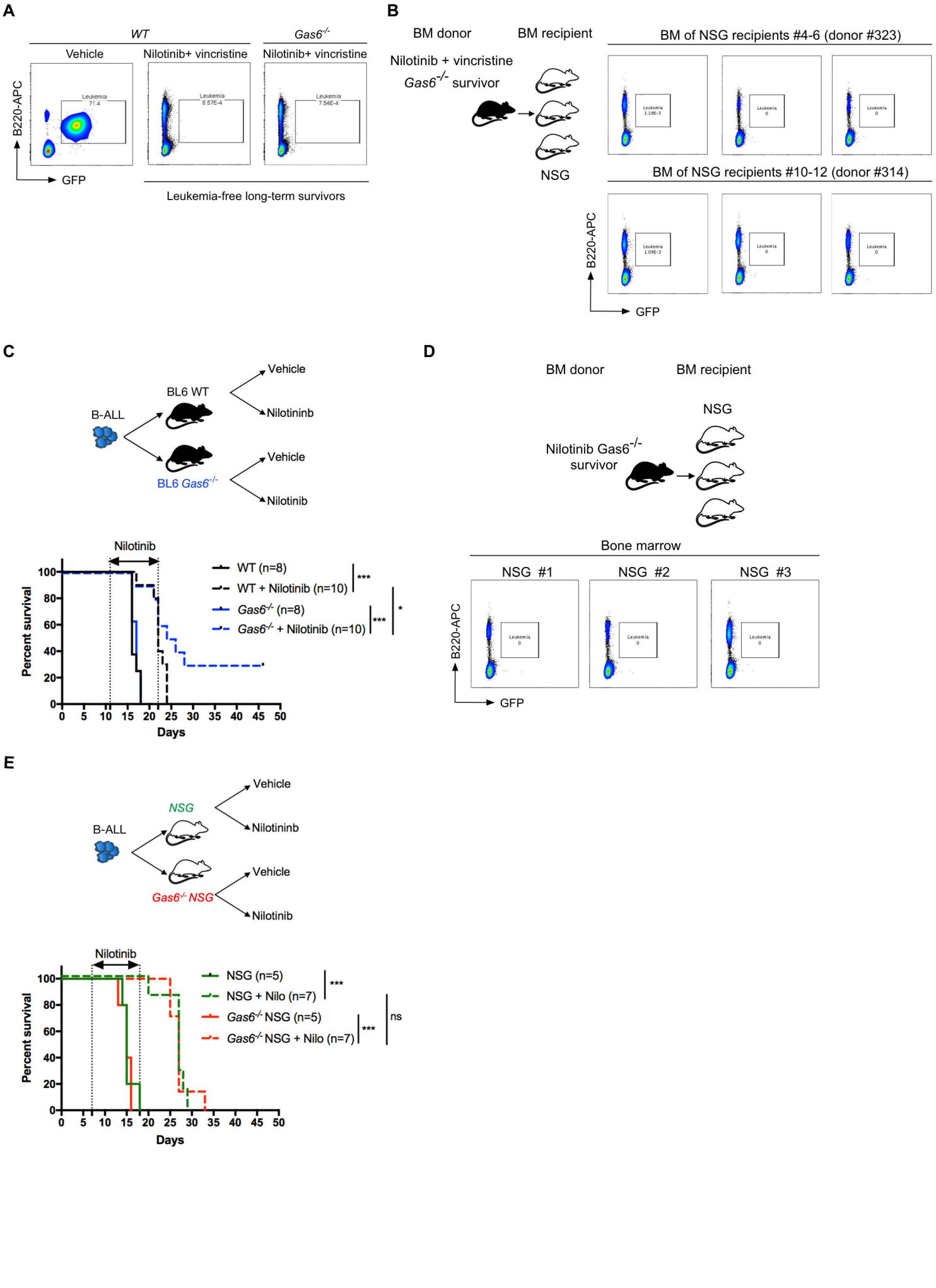
A
WT
Gas6-/-
Vehicle
Nilotinib+ vincristine
Nilotinib+ vincristine
B220-APC
GFP
Leukemia-free long-term survivors
B
BM donor
BM recipient
Nilotinib + vincristine
Gas6-/- survivor
NSG
BM of NSG recipients #4-6 (donor #323)
BM of NSG recipients #10-12 (donor #314)
B220-APC
GFP
C
B-ALL
BL6 WT
BL6 Gas6-/-
Vehicle
Nilotininb
Vehicle
Nilotinib
Nilotinib
Percent survival
Days
WT (n=8)
WT + Nilotinib (n=10)
Gas6-/- (n=8)
Gas6-/- + Nilotinib (n=10)
D
BM donor
BM recipient
NSG
Nilotinib Gas6-/- survivor
Bone marrow
NSG #1
NSG #2
NSG #3
B220-APC
GFP
E
B-ALL
NSG
Gas6-/- NSG
Vehicle
Nilotininb
Vehicle
Nilotinib
Nilotinib
Percent survival
Days
NSG (n=5)
NSG + Nilo (n=7)
Gas6-/- NSG (n=5)
Gas6-/- NSG + Nilo (n=7)
ns

Figure S4. *Gas6* deficiency in the environment synergizes with standard of care treatment to potentiate Ph^+ B-ALL clearance.

(**A**) representative FACS data depicting the leukemic burden ($B220^+$ GFP^+) in the bone marrow of mice from Fig. 1Q.

(**B**) representative FACS data depicting lack of leukemia cells in the bone marrow of NSG mice 30 days after receiving 5.10^6 bone marrow cells isolated from nilotinib and vincristine-treated *Gas6*$^{-/-}$ long-term survivors displayed in Fig. 1Q. In total BM cells from n = 2 donors were each transferred to n = 3 NSG recipients (total n = 6 NSG recipients), none of which developed leukemia.

(**C**) Kaplan–Meier survival analysis of C57BL/6 WT and *Gas6*$^{-/-}$ mice challenged with Ph^+ B-ALL cells (10^3) and treated with either vehicle or nilotinib. *p<0.05, ***p<0.001, log-rank (Mantel–Cox) test.

(**D**) FACS data depicting lack of leukemia cells in the bone marrow of NSG mice 30 days after receiving 5.10^6 bone marrow cells isolated from a nilotinib-treated *Gas6*$^{-/-}$ long-term survivor from C. BM cells from one donor were transferred to n = 3 NSG recipients.

(**E**) same as C, using immune compromised NSG mice. ns, not significant, ***p<0.001, log-rank (Mantel–Cox) test.

In C and E, treatments were initiated and stopped on the days indicated by dotted lines.

Figure S5

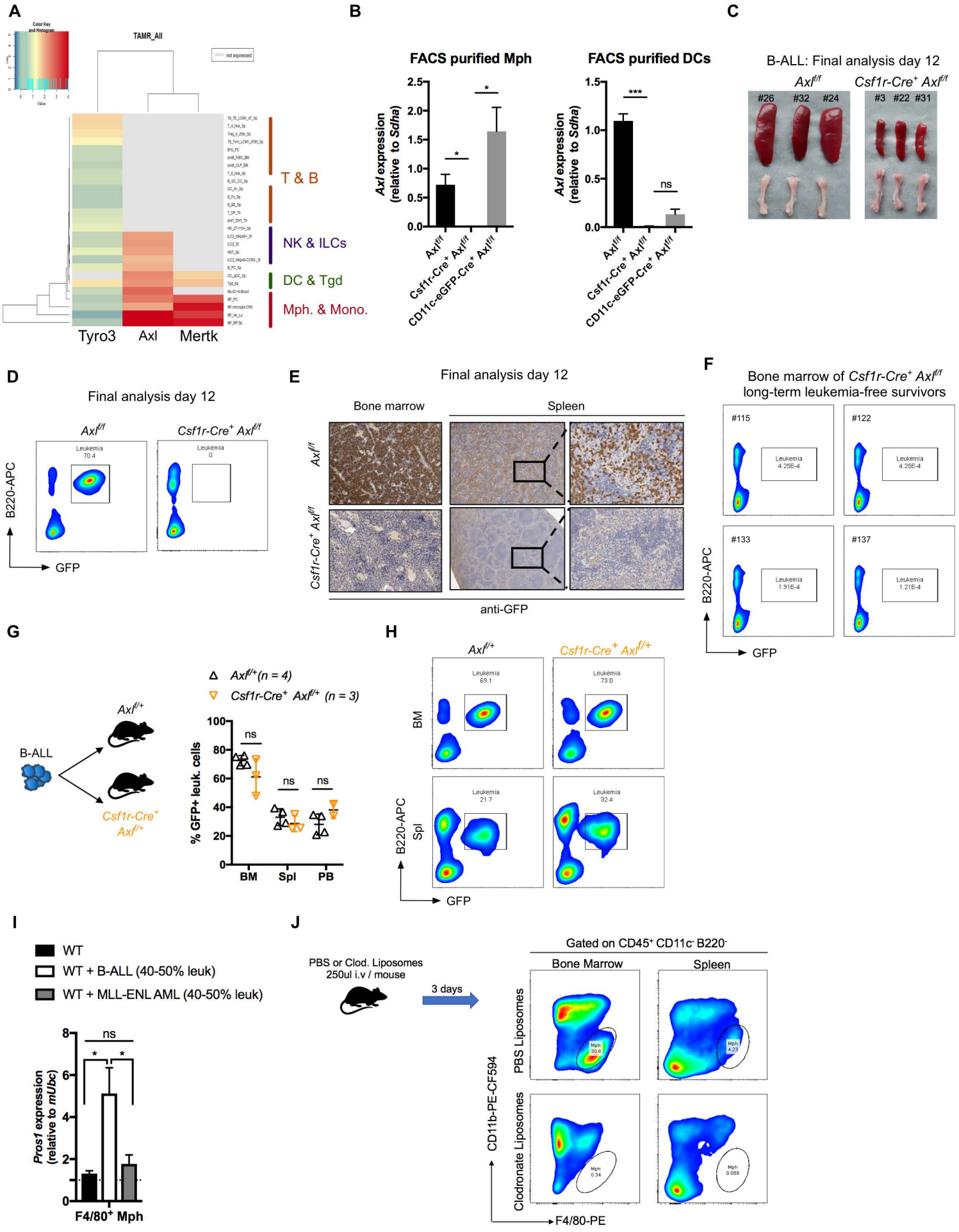
A
TAMR_All
not expressed
T & B
NK & ILCs
DC & Tgd
Mph. & Mono.
Tyro3
Axl
Mertk
B
FACS purified Mph
FACS purified DCs
Axl expression (relative to Sdha)
Axl^f/f
Csf1r-Cre+ Axl^f/f
CD11c-eGFP-Cre+ Axl^f/f
ns
C
B-ALL: Final analysis day 12
Axl^f/f
Csf1r-Cre+ Axl^f/f
#26 #32 #24
#3 #22 #31
D
Final analysis day 12
Axl^f/f
Csf1r-Cre+ Axl^f/f
Leukemia 70.4
Leukemia 0
B220-APC
GFP
E
Final analysis day 12
Bone marrow
Spleen
Axl^f/f
Csf1r-Cre+ Axl^f/f
anti-GFP
F
Bone marrow of Csf1r-Cre+ Axl^f/f long-term leukemia-free survivors
#115
#122
#133
#137
Leukemia 4.25E-4
Leukemia 4.25E-4
Leukemia 1.91E-4
Leukemia 1.21E-4
B220-APC
GFP
G
B-ALL
Axl^f/+
Csf1r-Cre+ Axl^f/+
Axl^f/+ (n = 4)
Csf1r-Cre+ Axl^f/+ (n = 3)
% GFP+ leuk. cells
ns
BM
Spl
PB
H
Axl^f/+
Csf1r-Cre+ Axl^f/+
BM
Spl
Leukemia 69.1
Leukemia 73.0
Leukemia 21.7
Leukemia 32.4
B220-APC
GFP
I
WT
WT + B-ALL (40-50% leuk)
WT + MLL-ENL AML (40-50% leuk)
Pros1 expression (relative to mUbc)
ns
F4/80+ Mph
J
PBS or Clod. Liposomes 250ul i.v / mouse
3 days
Gated on CD45+ CD11c- B220-
Bone Marrow
Spleen
PBS Liposomes
Clodronate Liposomes
CD11b-PE-CF594
F4/80-PE
Mph 30.8
Mph 4.29
Mph 0.34
Mph 0.088

Figure S5. *Axl* deletion in macrophages triggers a potent anti-leukemic immune response against acute leukemia.

(**A**) Heat map of publicly available data from the immunological genome project depicting the expression levels of *Tyro3*, *Axl* and *Mertk* in different immune subsets, in mice. *Axl* displays highest expression in red pulp macrophages (MF_RP.Sp), lung alveolar macrophages (MF_Alv_Lu) and Ly6C$^+$ blood monocytes (Mo.6C+II-Blood).

(**B**) *Axl* expression by real-time PCR in FACS purified macrophages (Mph: CD45$^+$CD11c$^-$ F4/80$^+$CD11b^{low}) and dendritic cells (DCs: CD45$^+$CD11c$^+$MHC-II$^+$) isolated from the spleens of *Axl*$^{f/f}$ (n = 3), *Csf1r-cre*$^+$ *Axl*$^{f/f}$ (n = 3) and *CD11c-eGFP-Cre Axl*$^{f/f}$ (n = 6) mice. Purity of the sorted cells was over 95%. Data are mean ± s.e.m. after normalization to a reference gene, *Sdha*. ns, not significant, *p<0.05, ***p<0.001, unpaired two-tailed Student's *t*-test.

(**C-E**) Representative pictures of spleens and femurs (C), FACS data (D) and GFP IHC staining (E) depicting the B-ALL leukemic burden (on day 12) in the organs of mice of the indicated genotypes from Figure 2B.

(**F**) Representative FACS data showing no detectable leukemia cells in the bone marrow of four independent *Csf1r-Cre*$^+$ *Axl*$^{f/f}$ long-term survivors (#115, #122, #133, #137) initially challenged with B-ALL and depicted in Figure 2C.

(**G-H**) Leukemic burden (G) in the bone marrow (BM), spleen (Spl) and peripheral blood (PB) as well as representative flow-cytometry plots depicting the percentage of leukemic cells (B220$^+$ GFP$^+$) in Control (*Axl*$^{f/+}$, n = 4) and *Csf1r-Cre*-expressing *Axl*$^{f/+}$ mice (*Csf1r-Cre*+ *Axl*$^{f/+}$, n = 3) 12 days after challenged with 10^3 B-ALL cells. ns, not significant, unpaired two-tailed Student's *t*-test.

(**I**) *Pros1* expression as evaluated by real-time PCR in MACS purified F4/80$^+$ macrophages from wild type mice (n = 4) and mice challenged with B-ALL (n = 4) or MLL-ENL AML (n = 4). *p<0.05, unpaired two-tailed Student's *t*-test.

(**J**) Representative FACS data depicting the frequency of macrophages defined as CD45$^+$CD11c$^-$B220$^-$CD11b^{low}F4/80$^+$, in the spleen and bone marrow of mice treated with 250 µl of either PBS-loaded or clodronate-loaded liposomes. Liposomes were administered intra-venously and mice analyzed 3 days post-injection.

Figure S6

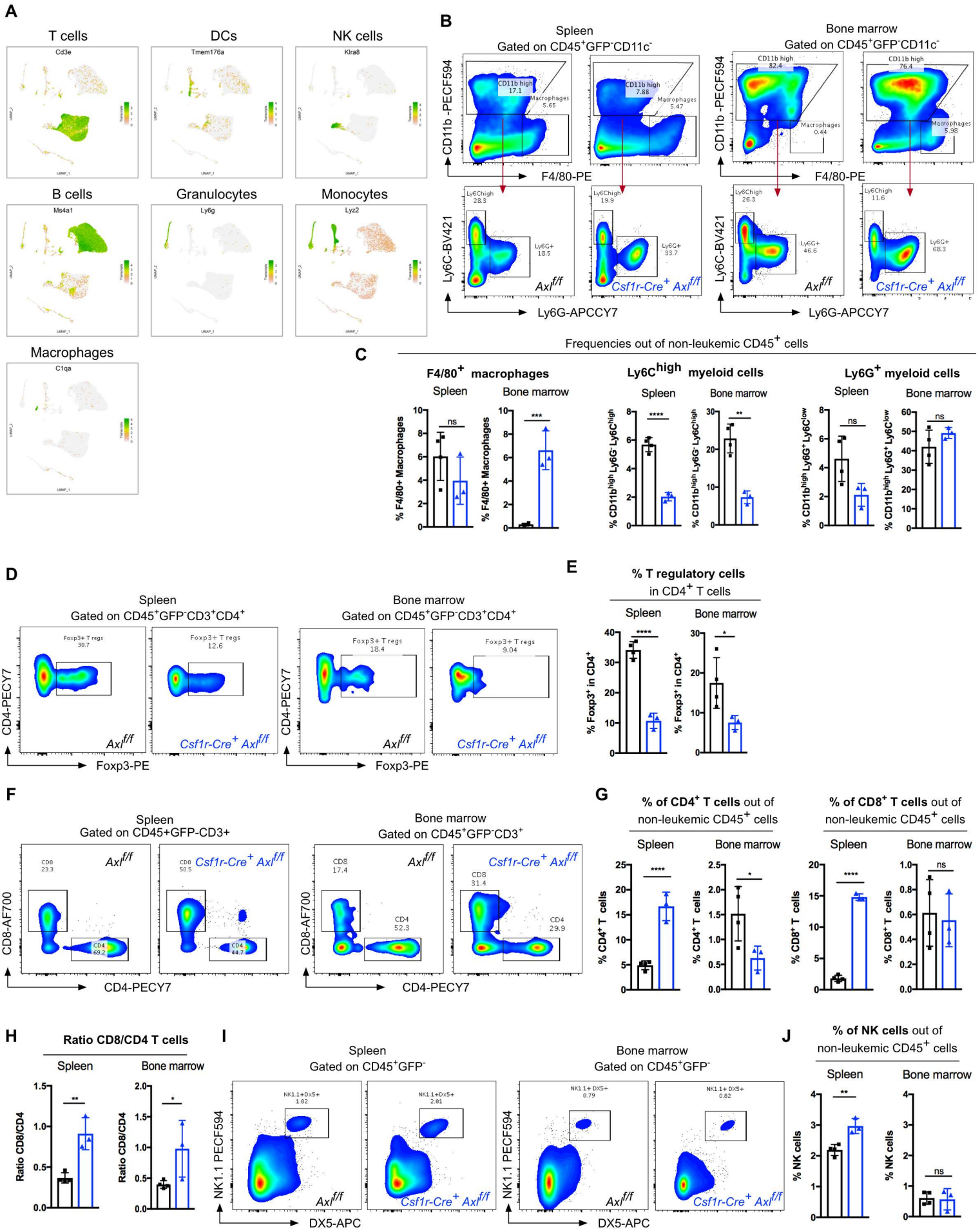
A
T cells
DCs
NK cells
B cells
Granulocytes
Monocytes
Macrophages
B
Spleen
Gated on CD45+GFP-CD11c-
Bone marrow
Gated on CD45+GFP-CD11c-
CD11b -PECF594
F4/80-PE
Ly6C-BV421
Ly6G-APCCY7
Axlf/f
Csf1r-Cre+ Axlf/f
C
Frequencies out of non-leukemic CD45+ cells
F4/80+ macrophages
Ly6Chigh myeloid cells
Ly6G+ myeloid cells
Spleen
Bone marrow
D
Spleen
Gated on CD45+GFP-CD3+CD4+
Bone marrow
Gated on CD45+GFP-CD3+CD4+
CD4-PECY7
Foxp3-PE
E
% T regulatory cells
in CD4+ T cells
F
Spleen
Gated on CD45+GFP-CD3+
Bone marrow
Gated on CD45+GFP-CD3+
CD8-AF700
CD4-PECY7
G
% of CD4+ T cells out of non-leukemic CD45+ cells
% of CD8+ T cells out of non-leukemic CD45+ cells
H
Ratio CD8/CD4 T cells
I
Spleen
Gated on CD45+GFP-
Bone marrow
Gated on CD45+GFP-
NK1.1 PECF594
DX5-APC
J
% of NK cells out of non-leukemic CD45+ cells

Figure S6. *Axl* ablation protects leukemia challenged mice from acquiring an immune suppressive microenvironment.

(**A**) Expression of *Cd3e* (T cells), *Tmem176a* (DCs), Klra8 (NK cells), *Ms4a1* (B cells), *Ly6G* (Granulocytes), *Lyz2* (Monocytes) and *C1qa* (Macrophages) overlaid on the UMAP representation.

(**B-C**) Gating strategy and frequencies of myeloid subsets in the non-leukemic $GFP^{-}CD45^{+}$ fraction of cells from the bone marrow and spleen of $Axl^{f/f}$ (n = 4) and *Csf1r-Cre*+ $Axl^{f/f}$ (n = 3) animals analyzed on day 11 post-challenge with 10^3 B-ALL cells. *Csf1r-Cre*$^{+}$ $Axl^{f/f}$ had no detectable leukemia, while $Axl^{f/f}$ exhibited a mean leukemic burden of 51% in the bone marrow and 30% in spleen (data not shown). Macrophages: $CD11c^{-}CD11b^{low}F4/80^{high}$; $Ly6C^{high}$ Myeloid cells: $CD11c^{-}CD11b^{high}$ $Ly6G^{-}Ly6C^{high}$; $Ly6G^{+}$ Myeloid cells: $CD11c^{-}CD11b^{high}$ $Ly6G^{+}Ly6C^{-}$. ns, not significant, **p<0.01, ***p<0.001, ****p<0.0001, unpaired two-tailed Student's *t*-test.

(**D-E**) Gating strategy and frequency of T regulatory cells ($Foxp3^{+}$) within $CD4^{+}$ T cells (GFP^{-} $CD45^{+}CD3^{+}CD4^{+}$) of mice used in (B-C). *p<0.05, ***p<0.001, ****p<0.0001, unpaired two-tailed Student's *t*-test.

(**F-H**) Gating strategy (F), frequencies of CD4 and CD8 T cells (G) as well as CD8 to CD4 T cell ratios (H) in the spleen and bone marrow of mice described in (B-C). The depicted percentages are out of non-leukemic $GFP^{-}CD45^{+}$ cells. *p<0.05, **p<0.01, ****p<0.0001, unpaired two-tailed Student's *t*-test.

(**I-J**) Gating strategy and frequencies of NK cells ($CD3^{-}NK1.1^{+}DX5^{+}$) in the non-leukemic GFP^{-} $CD45^{+}$ fraction of cells from the bone marrow and spleen of mice described in (B-C). ns, not significant, **p<0.01, unpaired two-tailed Student's *t*-test.

Figure S7

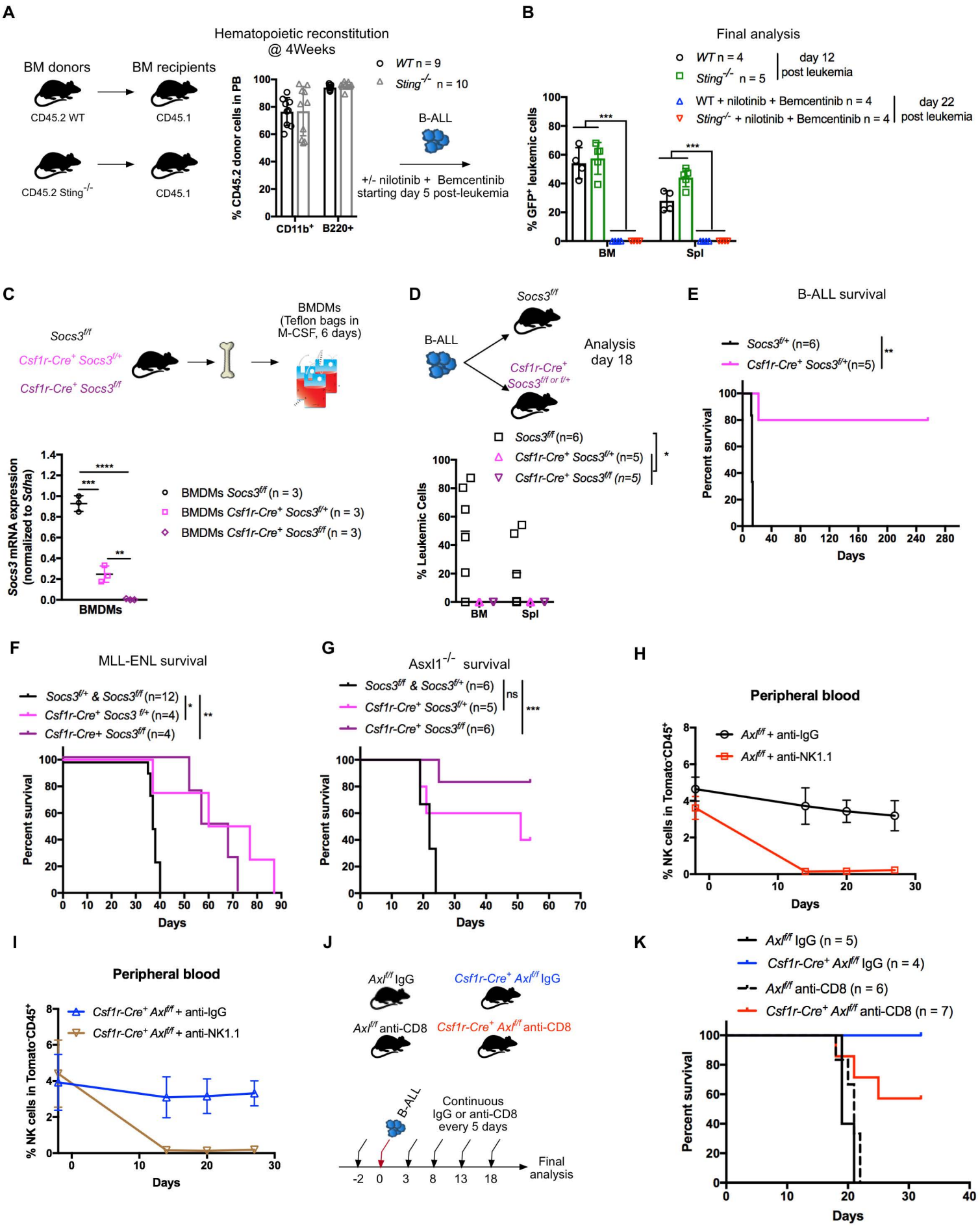
A
Hematopoietic reconstitution
@ 4Weeks
BM donors
BM recipients
CD45.2 WT
CD45.1
CD45.2 Sting-/-
CD45.1
% CD45.2 donor cells in PB
CD11b+
B220+
WT n = 9
Sting-/- n = 10
B-ALL
+/- nilotinib + Bemcentinib
starting day 5 post-leukemia
B
Final analysis
WT n = 4
Sting-/- n = 5
day 12
post leukemia
WT + nilotinib + Bemcentinib n = 4
Sting-/- + nilotinib + Bemcentinib n = 4
day 22
post leukemia
% GFP+ leukemic cells
BM
Spl
C
Socs3f/f
Csf1r-Cre+ Socs3f/+
Csf1r-Cre+ Socs3f/f
BMDMs
(Teflon bags in
M-CSF, 6 days)
Socs3 mRNA expression
(normalized to Sdha)
BMDMs
BMDMs Socs3f/f (n = 3)
BMDMs Csf1r-Cre+ Socs3f/+ (n = 3)
BMDMs Csf1r-Cre+ Socs3f/f (n = 3)
D
Socs3f/f
B-ALL
Csf1r-Cre+
Socs3f/f or f/+
Analysis
day 18
Socs3f/f (n=6)
Csf1r-Cre+ Socs3f/+ (n=5)
Csf1r-Cre+ Socs3f/f (n=5)
% Leukemic Cells
BM
Spl
E
B-ALL survival
Socs3f/+ (n=6)
Csf1r-Cre+ Socs3f/+(n=5)
Percent survival
Days
F
MLL-ENL survival
Socs3f/+ & Socs3f/f (n=12)
Csf1r-Cre+ Socs3 f/+ (n=4)
Csf1r-Cre+ Socs3f/f (n=4)
Percent survival
Days
G
Asxl1-/- survival
Socs3f/f & Socs3f/+ (n=6)
Csf1r-Cre+ Socs3f/+ (n=5)
Csf1r-Cre+ Socs3f/f (n=6)
ns
Percent survival
Days
H
Peripheral blood
Axlf/f + anti-IgG
Axlf/f + anti-NK1.1
% NK cells in Tomato-CD45+
Days
I
Peripheral blood
Csf1r-Cre+ Axlf/f + anti-IgG
Csf1r-Cre+ Axlf/f + anti-NK1.1
% NK cells in Tomato-CD45+
Days
J
Axlf/f IgG
Csf1r-Cre+ Axlf/f IgG
Axlf/f anti-CD8
Csf1r-Cre+ Axlf/f anti-CD8
B-ALL
Continuous
IgG or anti-CD8
every 5 days
Final
analysis
K
Axlf/f IgG (n = 5)
Csf1r-Cre+ Axlf/f IgG (n = 4)
Axlf/f anti-CD8 (n = 6)
Csf1r-Cre+ Axlf/f anti-CD8 (n = 7)
Percent survival
Days

Figure S7. Downstream cellular and molecular mediators of the leukemia protective phenotype imposed by *Axl* deletion in macrophages.

(**A**) WT CD45.1 SJL mice were lethally irradiated and reconstituted with donor bone marrow cells from either CD45.2 WT (n = 9) or CD45.2 *Sting*$^{-/-}$ (n = 10) mice. Percentage of CD45.2 donor cells depicts reconstitution at 4 weeks at which point the mice were challenged with 10^3 B-ALL cells. 5 days later, mice from each genotype were randomized to receive either vehicle or treatment with nilotinib and Bemcentinib as indicated.

(**B**) Leukemic burden in spleen and bone marrow as evaluated by flow cytometry upon final analysis of both vehicle (day 12 post leukemia challenge) and treated (day 22 post leukemia challenge) cohorts. Both WT and *Sting*$^{-/-}$ chimeras effectively cleared leukemia in response to the combination treatment. In (G), two mice (1 *Sting*$^{-/-}$ and 1 WT) were not available for the final analysis due to accidental death unrelated to the experiment. ***p<0.001, unpaired two-tailed Student's *t*-test.

(**C**) *Socs3* expression by real time PCR in bone marrow-derived macrophages (BMDMs) from mice with the indicated genotypes. Each data point represents BMDMs from an independent mouse. **p<0.01, ***p<0.001, ****p<0.0001, unpaired two-tailed Student's *t*-test.

(**D**) Leukemic burden in the bone marrow and spleen of mice of the indicated genotypes 18 days post challenge with 10^3 B-ALL cells. Each dot represents an independent mouse. *p<0.05, unpaired two-tailed Student's *t*-test.

(**E-G**) Kaplan Meier survival analysis of mice of the indicated genotypes challenged with either 10^3 B-ALL cells (E), 10^5 MLL-ENL AML cells (F) or 5.10^5 *Asxl1*$^{-/-}$ AML cells (G). *p<0.05, **p<0.01, ***p<0.001, log-rank (Mantel–Cox) test.

(**H-I**) Frequencies of NK cells ($CD45^+GFP^-CD3^-NK1.1^+DX5^+$) as evaluated by FACS in the experiment described in main Figure 4D.

(**J-K**) Kaplan-Meier survival analysis of mice of the indicated genotypes challenged with 10^3 B-ALL cells and treated with either an anti-CD8 antibody or a Rat IgG2b isotype control every 5 days. Depletion was initiated 2 days before leukemia injection and maintained throughout the experiment. All deceased mice succumbed to *bona fide* GFP^+ $B220^{dim}$ B-ALL (data not shown).

Figure S8

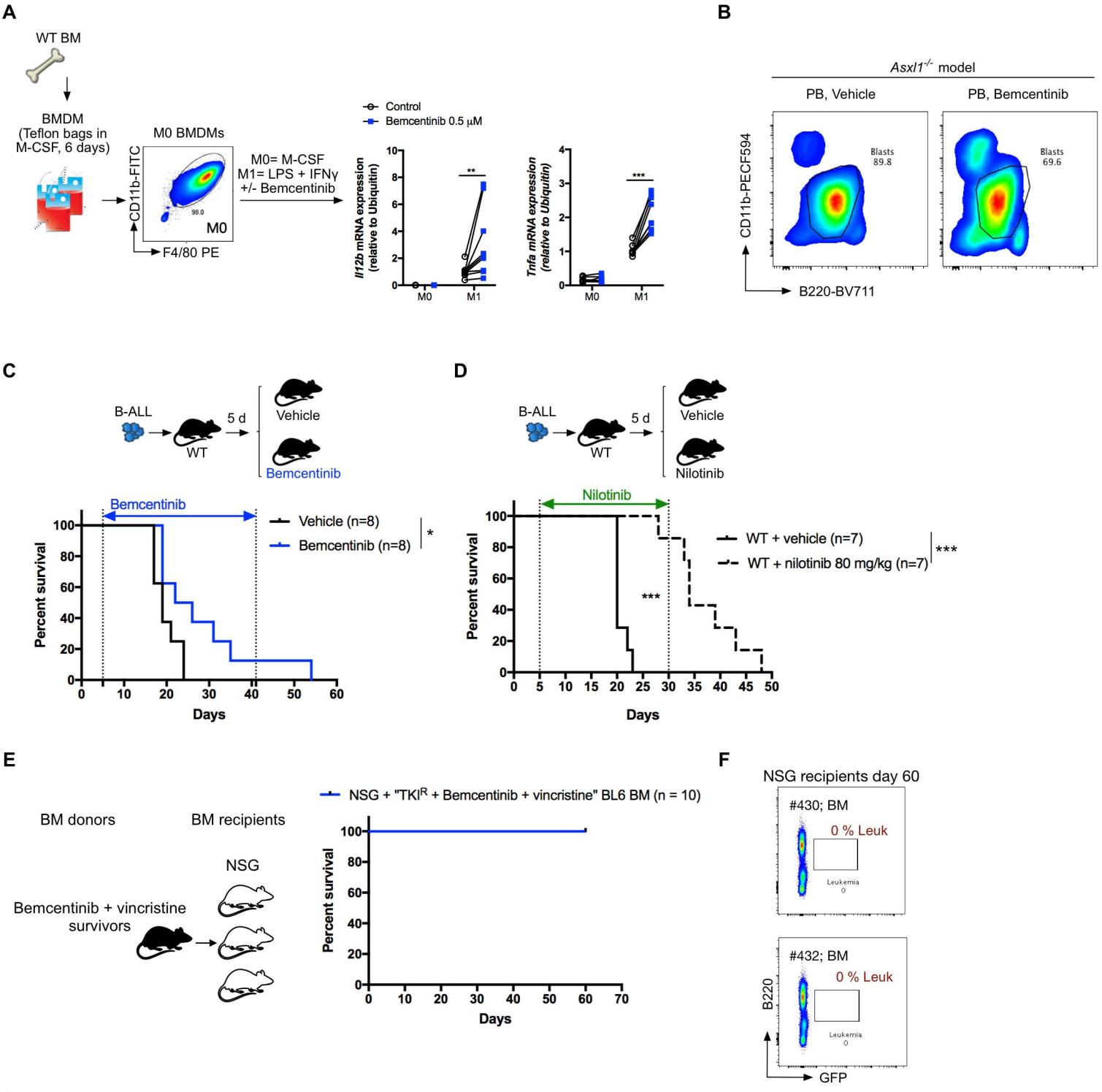
A
WT BM
BMDM
(Teflon bags in
M-CSF, 6 days)
M0 BMDMs
CD11b-FITC
F4/80 PE
M0
98.0
M0= M-CSF
M1= LPS + IFNγ
+/- Bemcentinib
Control
Bemcentinib 0.5 μM
Il12b mRNA expression
(relative to Ubiquitin)
Tnfa mRNA expression
(relative to Ubiquitin)
M0
M1
B
Asxl1-/- model
PB, Vehicle
PB, Bemcentinib
CD11b-PECF594
B220-BV711
Blasts
89.8
Blasts
69.6
C
B-ALL
WT
5 d
Vehicle
Bemcentinib
Bemcentinib
Vehicle (n=8)
Bemcentinib (n=8)
Percent survival
Days
D
B-ALL
WT
5 d
Vehicle
Nilotinib
Nilotinib
WT + vehicle (n=7)
WT + nilotinib 80 mg/kg (n=7)
Percent survival
Days
E
BM donors
BM recipients
NSG
Bemcentinib + vincristine
survivors
NSG + "TKIR + Bemcentinib + vincristine" BL6 BM (n = 10)
Percent survival
Days
F
NSG recipients day 60
#430; BM
0 % Leuk
Leukemia
#432; BM
0 % Leuk
Leukemia
B220
GFP
G
Iba1
GFP
Axl
DAPI
Vehicle
NiloR
VincristineR

Figure S8. Therapeutic efficacy of a clinical grade AXL inhibitor, Bemcentinib.

(**A**) Bone marrow-derived macrophages from WT mice (BMDMs, n = 9 donors) were generated *in vitro* in the presence of M-CSF and either left untreated (M0) or stimulated, for 24h, with IFNγ and LPS to direct M1-like priming (M1), in the presence or absence of 0.5 μM Bemcentinib. *Il12b* and *Tnfα* expression was evaluated using real-time PCR. Data are mean ± s.e.m. after normalization to a reference gene, *Ubc*. Data are pooled from 3 independent experiments. **p<0.01, ***p<0,001, Wilcoxon matched pairs signed rank test.

(**B**) Representative FACS data showing comparable phenotype in terminally ill mice from vehicle and BGB treated mice from Fig. 7A.

(**C**) Kaplan-Meier survival analysis of WT C57BL/6 mice challenged with 10^3 B-ALL cells and randomized on day 5 to vehicle (n = 8) or Bemcentinib (n = 8). All mice succumbed to *bona fide* GFP^+ $B220^+$ leukemia (data not shown). *p<0.05, log-rank (Mantel–Cox) test.

(**D**) Kaplan Meier survival analysis of WT C57BL/6 mice transplanted with 10^3 GFP^+ B-ALL cells and randomized on day 5 to vehicle or nilotinib (80 mg/kg/day) treatment. ***p<0.001, log-rank (Mantel–Cox) test.

(**E**) Kaplan-Meier survival analysis of NSG mice transplanted with 10^7 bone marrow cells isolated from long-term survivors challenged with TKI^R B-ALL and treated with “bemcentinib + vincristine” described in Fig. 7G.

(**F**) Representative FACS plots of bone marrow from two independent NSG mice from E, at final analysis on day 60.

(**G**) Representative images of multiplex immunohistochemistry staining for Axl, Iba1 and GFP in spleen sections from untreated or therapy resistant (nilotinib resistant = $Nilo^R$ GFP^+; vincristine resistant = $Vincristine^R$ GFP^+) B-ALL bearing mice. $IBA1^+$ macrophages (Red), GFP^+ blasts (Green), AXL (White), nuclei (Blue).

For all experiments, Treatments were initiated and stopped on the days indicated by dotted lines.

Sample ID	Diagnosis	Sex	Age	Used in
#10441	Ph+ B-ALL	M	36	Figure 1B
#13693	Ph+ B-ALL	M	30	Figure 1B
#686	B-ALL	M	20	Figure 1B
#3355	T-ALL	F	37	Figure S1B
#11209	T-ALL	M	23	Figure S1B
BSD#1	Healthy	M	42	Figure 1C
BSD#2	Healthy	M	37	Figure 1C
BSD#3	Healthy	F	46	Figure 1C
BSD#4	Healthy	M	32	Figure 1C
BSD#5	Healthy	M	73	Figure 1C
BSD#6	Healthy	F	52	Figure 1C
BSD#7	Healthy	M	35	Figure 1C
BSD#8	Healthy	M	58	Figure 1C
BSD#9	Healthy	F	48	Figure 1C
BSD#10	Healthy	M	24	Figure 1C
BSD#11	Healthy	F	60	Figure 1C
BSD#12	Healthy	F	53	Figure 1C
B-ALL#V	Ph+ B-ALL	ND	adult	Figure 1C
B-ALL#M	Ph+ B-ALL	ND	adult	Figure 1C
#0202	AML	M	81	Figure 1C
#0401	High risk MDS	F	84	Figure 1C

Table S1. Description of the human samples used in this study