SUPPLEMENTARY APPENDIX

Arrayed molecular barcoding identifies TNFSF13 as a positive regulator of acute myeloid leukemia-initiating cells

Marion Chapellier,[1] Pablo Peña-Martínez,[1] Ramprasad Ramakrishnan,[1] Mia Eriksson,[1] Mehrnaz Safaee Talkhoncheh,[2] Christina Orsmark-Pietras,[1] Henrik Lilljebjörn[1], Carl Högberg,[1] Anna Hagström-Andersson,[1] Thoas Fioretos,[1] Jonas Larsson,[2] and Marcus Järås[1]

[1]Department of Clinical Genetics and [2]Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Lund, Sweden

 doi:10.3324/haematol.2018.192062

Received: February 27, 2018.

Accepted: February 21, 2019.

Pre-published: February 28, 2019.

Correspondence: *MARCUS JÄRÅS* - marcus.jaras@med.lu.se

Supplementary information for:

Arrayed molecular barcoding identifies TNFSF13 as a positive regulator of acute myeloid leukemia-initiating cells

Marion Chapellier[1], Pablo Peña-Martínez[1], Ramprasad Ramakrishnan[1], Mia Eriksson[1], Mehrnaz Safaee Talkhoncheh[2], Christina Orsmark-Pietras[1], Henrik Lilljebjörn[1], Carl Högberg[1], Anna Hagström-Andersson[1], Thoas Fioretos[1], Jonas Larsson[2], and Marcus Järås[1,*]

AFFILIATIONS
[1]Department of Clinical Genetics, Lund University, 22184 Lund, Sweden; [2]Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, 221 84 Lund, Sweden.

[*]Corresponding author: Marcus Järås, Department of Clinical Genetics, Klinikgatan 28, SE-22184 Lund, Sweden; telephone number: +46 46 222 6996; e-mail: marcus.jaras@med.lu.se

Supplementary Methods

Murine leukemia model
c-Kit$^+$ leukemia cells were isolated by crushing the femurs of leukemic mice, followed by red blood cell lysis using NH_4Cl solution (StemCell technologies, Vancouver, Canada) and c-Kit$^+$ selection using CD117 MicroBeads in MACS® Cell Separation Columns according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany).

Ex vivo cytokine screening using barcoded leukemia cells
Freshly isolated c-Kit$^+$dsRed$^+$ leukemia cells were transduced with the barcoded lentiviral vectors using spinoculation at 650g, and after 24 hours of stimulation with 50 ng/mL of murine stem cell factor (mSCF, Peprotech), the cells were washed, seeded into 96-well plates (50,000 cells per well), and cultured in serum-free expansion medium (SFEM; Stemspan, Stem Cell Technologies) containing 1% penicillin/streptomycin supplemented with one cytokine (100 ng/mL) per well (114 cytokines; purchased from Prospec, East Brunswick, NJ, USA or Peprotech, Rocky Hill, NJ, USA and R&D Systems, Minneapolis, MN, USA; listed in Supplementary Table 2).

Sequencing of barcodes and bioinformatics analysis
After amplifying the barcodes using using pLKO.1-specific primers followed by DNA purification using magnetic beads (Agentcourt AMPure XP, Beckman Coulter), a second PCR was performed to add sequencing adapters (Nextera XT Index Kit v2, Illumina) to the amplicons. DNA concentrations were determined using the Qubit dsDNA Assay Kit (Invitrogen) after magnetic beads purification. The samples were pooled prior to sequencing in a NextSeq 500 Desktop Sequencer (Illumina) using the NextSeq 500/550 Mid Output v2 Kit, 150 cycles (Illumina), according to the manufacturer's instructions. The fastq files were aligned using Bowtie 2[1] to a custom reference genome containing the 11 barcode sequences.

Retroviral MLL-AF9 expression in c-Kit$^+$ bone marrow cells and transplantation into sublethally irradiated mice
c-Kit$^+$ bone marrow cells were pre-stimulated for 2 days in SFEM (Stemspan, StemCell Technologies) containing 1% penicillin/streptomycin and supplemented with murine interleukin-3 (mIL3; 20 ng/mL), mSCF (50 ng/mL), and human interleukin-6 (hIL6; 20 ng/mL). After pre-incubation, cells were mixed (1:1 volume) with the viral supernatant and seeded in RetroNectin-coated plates according to the manufacturer's instructions (Takara Bio Inc, Kusatsu City, Japan). The plates were spinoculated for 1 hours at 32°C and 650g.

Flow cytometric analysis and cell sorting
The flow cytometric analyses were performed using a FACS Canto II (BD Biosciences, San Jose, CA, USA) or a FACS LSRFortessa (BD Biosciences), and cell sorting was performed using a FACS Aria II (BD Biosciences). For sorting of LSK cells, c-Kit-enriched bone marrow cells were stained with c-Kit-APC, Sca1-PacBlue, and Linage-cocktail-PE/Cy5 monoclonal antibodies (mAbs) (all from BioLegend, San Diego, CA, USA). For the HSPC analysis, murine bone marrow cells were stained with Linage-cocktail-AF700, Sca1-BV421, c-Kit-BV510, CD16/32-PE/Cy7, Flt3-PE (all from BioLegend), and CD34-FITC (or CD34-AF647 when used in combination

with TNFRSF17-FITC) mAbs (BD Biosciences) following red blood cell lysis with NH_4Cl solution (StemCell Technologies, Vancouver, Canada). For lineage distribution analysis of bone marrow and peripheral blood, murine cells were stained with CD11b-BV510, CD3-PE, B220-PE/Cy7, and Ter119-PE/Cy5 mAbs (BioLegend) and a Gr1-APC/Cy7 mAb (BD Biosciences), following lysis of red blood cells. For plasma cell purification, $CD138^+$ murine bone marrow cells were enriched using magnetic beads (Miltenyi biotech) and stained using an anti-mouse CD138-BV421 antibody (Biolegend) prior to flow cytometric cell sorting. The expression of murine TNFRSF13B and TNFRSF17 was evaluated with anti-mouse TNFRSF17-FITC (rat IgG1 mAb) and TNFRSF13B-APC (rat IgG2a mAb). The expression of human TNFRSF13B and TNFRSF17 was evaluated with anti-human TNFRSF17-PE (goat IgG polyclonal antibody) and TNFRSF13B-APC (mouse IgG1 mAb) (R&D Systems). The expression of human CD138 (SYNDECAN-1) was detected with an anti-human CD138-APC mAb (mouse IgG1, Biolegend). TNFSF13 binding to the cell surface was evaluated using an anti-TNFSF13-PE antibody (armenian hamster mAb, Biolegend) after 5 minutes stimulation of Mono-Mac-6 cells with human TNFSF13 at 100 ng/mL.

Leukemia cell cultures

Freshly isolated $c\text{-}Kit^+$ leukemia cells were plated in SFEM (Stemspan, StemCell Technologies) supplemented with 1% penicillin/streptomycin. Except for the titration experiment, murine TNFSF13 (Peprotech, lot100329, endotoxin level <0.1 ng/μg), TNFSF13B (Peprotech) and murine IL9 (Prospec) were added to the culture medium at a concentration of 100 ng/mL, and mSCF was added at a concentration of 10 ng/mL. For cell proliferation assays, cell counts were performed using CountBright™ absolute counting beads according to the manufacturer's instructions (BD Biosciences). All human cell lines were obtained from DSMZ; Mono-Mac-6, THP-1, KG-1, Lama-84, K562, EOL-1, and NB-4 were cultured in RPMI with 10% fetal bovine serum (FBS), and OCI-AML1 and OCI-AML3 were cultured in MEM alpha with 20% FBS. For OCI-AML1, the medium was supplemented with 10 ng/mL of GM-CSF. Upon stimulation with human TNFSF13 or TNFSF13B (100 ng/mL; Peprotech, endotoxin level <0.1 ng/μg), cells were cultured in SFEM (Stemspan, StemCell Technologies) supplemented with 1% penicillin/streptomycin, and SCF (10 ng/mL, Peprotech) when specified. To inhibit TNFSF13 binding to Heparan Sulfate Proteoglycans (HSPG) on the cell surface, Mono-Mac-6 cells were treated for 5 min with human TNFSF13 at 100 ng/mL in the presence of 4 IU/mL Heparin (LEO Pharma). To inhibit NF-κB phosphorylation, the Mono-Mac-6 cells were pre-incubated for 10 minutes with 1 or 3 μM of the IκB kinase (IKK) inhibitor TPCA1[2] (Sigma-Aldrich) or with 3 or 5 μM of IkK-16[3] (Sigma-Aldrich). To block TNFRSF17, an anti-TNFRSF17 (Rat mAb IgG2a, R&D systems) antibody was used.

Phospho-flow cytometric analysis

For phospho-flow cytometric analysis, human leukemia cells were stimulated with TNFSF13 (100 ng/mL) for 1 hour and then fixed and permeabilized as described for the cell cycle analysis. A mAb specific for the phosphorylated form of the intracellular protein NF-κB (PE/Cy7) was used (anti-NF-κB p65 (pS529) mouse mAb, BD Biosciences).

ELISA

The concentrations of murine TNFSF13 and TNFSF13B were determined using enzyme-linked immunosorbent assay (ELISA) kits (Cloud Clone Corp and R&D systems, respectively) according to the manufacturer's instructions. Plasma samples for the ELISA were prepared by centrifuging blood samples at 10 000 x *g* and collecting the supernatant. Bone marrow supernatants were prepared by flushing the femurs with 100 µL PBS, followed by centrifugation and harvesting of the supernatant as described for the plasma samples. Both healthy control and leukemic mice were sublethally irradiated 2 weeks prior to sample collection. Cell culture supernatants were collected after 72 hours from normal and leukemic c-Kit$^+$ cells in the serum-free condition supplemented with mIL3 (20 ng/mL), mSCF (50 ng/mL), and hIL6 (20 ng/mL). An Infinite F50 absorbance reader (Tecan, Männedorf, Switzerland) instrument was used for the analysis.

Cell cycle analysis

To determine the cell cycle status of cells stimulated with TNFSF13, the cells were harvested after 72 hours of culture, fixed in 1.6% paraformaldehyde for 10 minutes, and permeabilized, after being washed, with 1 mL chilled ethanol. Subsequently, the cells were washed twice in phosphate-buffered saline (PBS) containing 2% FBS and stained with a Ki67-APC antibody (Miltenyi Biotec) and DAPI (Biolegend) before analysis by flow cytometry.

Apoptosis analysis

For the apoptosis analysis, leukemia cells were cultured for 72 hours prior to harvesting, washed in 1x binding buffer (BD Biosciences), and stained with APC-Annexin V (BioLegend) and 7-AAD (BD Biosciences) for 20 minutes at room temperature. The percentages of Annexin V$^+$ and 7-AAD$^+$ cells were determined by flow cytometric analysis.

RNA extraction and TNFSF13 expression analysis

RNA was extracted from cultured control and leukemic c-Kit$^+$ cells and from leukemia cell lines using the RNAeasy Extraction Kit (Qiagen), followed by cDNA synthesis using standard protocols. Real-time PCR (RT-PCR) was performed using TaqMan probes for mouse *Tnfsf13*, *Tnfrsf17, Gapdh* and human *TNFRSF17* and *18s* (Life Technologies) in an ABI Prism 7500 Real-Time PCR instrument (Applied Biosystems) and ddCT values were calculated as previously described.[4] Data were normalized using *Gapdh* as an endogenous control.

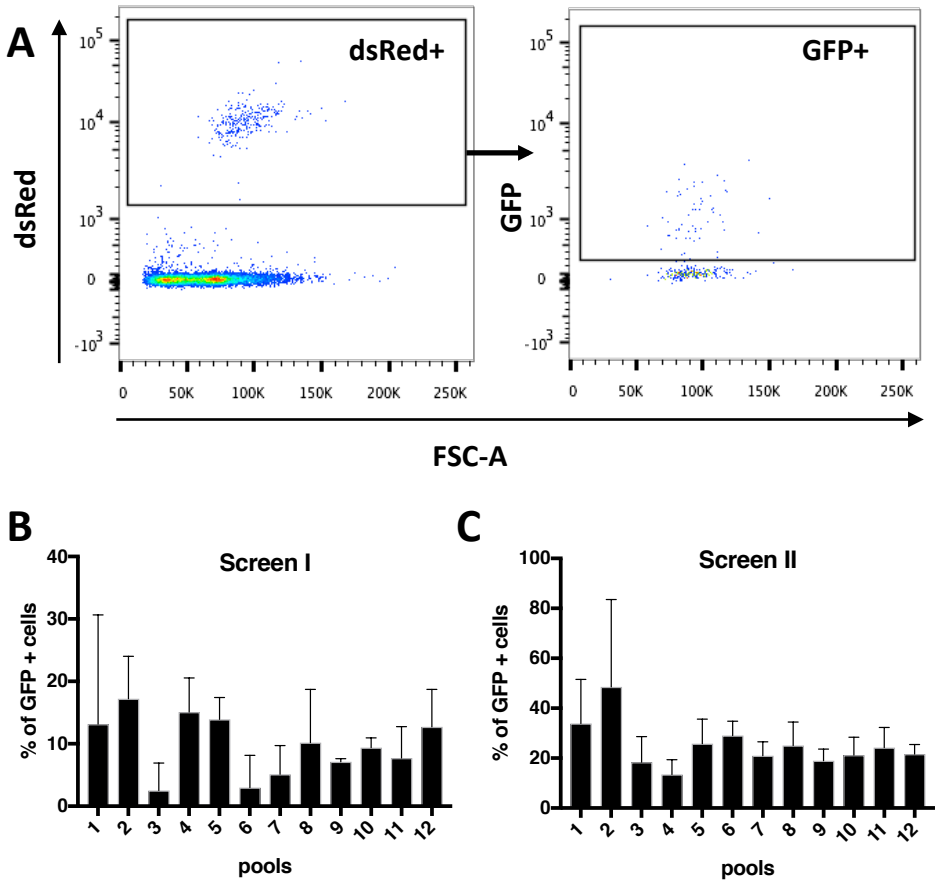
Library preparation and RNA sequencing analysis

RNA sequencing was performed on Mono-Mac-6 cells stimulated with TNFSF13 for 24 hours. RNA was extracted using the Arcturus Picopure Kit (Applied Biosystems), and the RNA quality was validated using a 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). RNA libraries were prepared using the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA, USA), and sequencing was performed on a NextSeq 500 Desktop Sequencer (Illumina) using the NextSeq 500/550 Mid Output v2 Kit, 150 cycles (Illumina). The sequenced reads were aligned to the hg19 reference human genome using TopHat 2.0.13.[5] Statistical analysis, analysis of differential gene expression, and visualization of the RNA sequencing data were performed using Qlucore Omics Explorer 3.0 (Qlucore, Lund, Sweden). Gene set enrichment analysis (GSEA)[6] was performed with pre-ranked gene lists, based on the

logarithms of the *p* values and the sign of the fold-change, following comparison between gene expression levels in TNFSF13-treated versus non-treated cells (control) by *t*-test. The raw data and normalized gene expression data are available in the Gene Expression Omnibus database under accession number GSE104425.

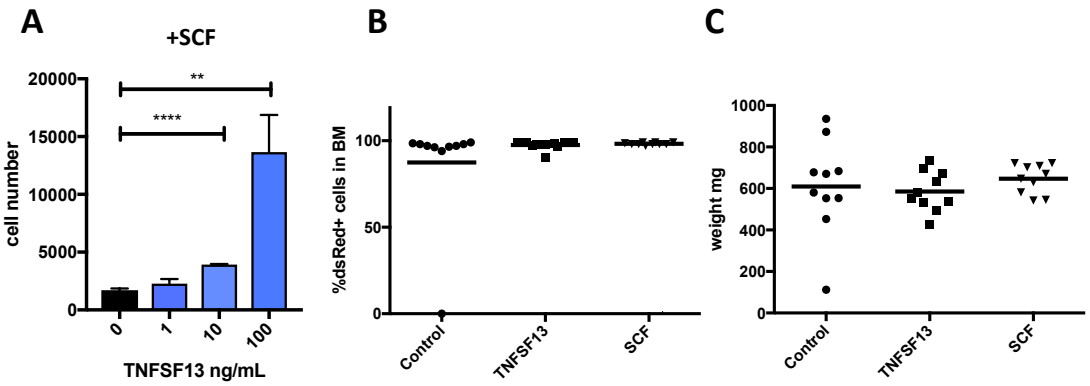
Supplementary Figures

Supplementary Figure S1



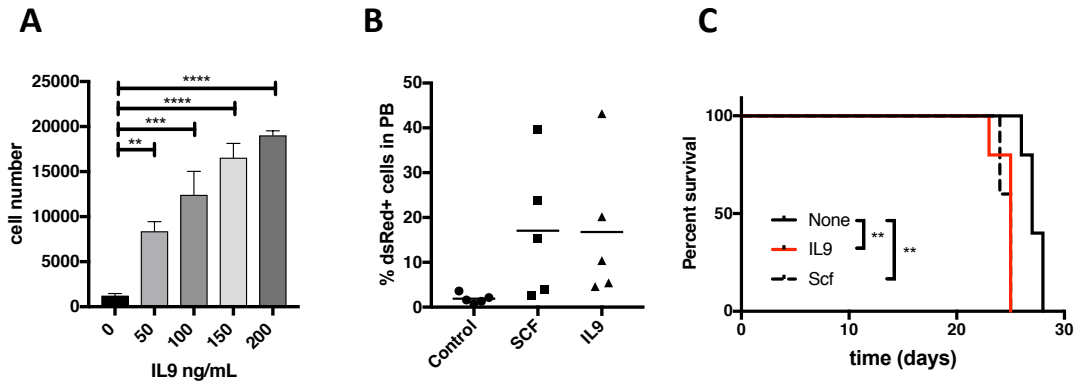
Supplementary Figure S1. Percentage of barcoded cells within leukemic cells *in vivo*. (*A*) Contour plots showing GFP expression that marks barcoded cells within leukemic ($dsRed^+$) bone marrow cells. **(*B-C*)** Percentage of GFP expressing bone marrow cells within $dsRed^+$ cells for each pool (three mice per pool), analyzed at the end of screen I (*B*) and II (*C*). Values are means ± SD.

Supplementary Figure S2



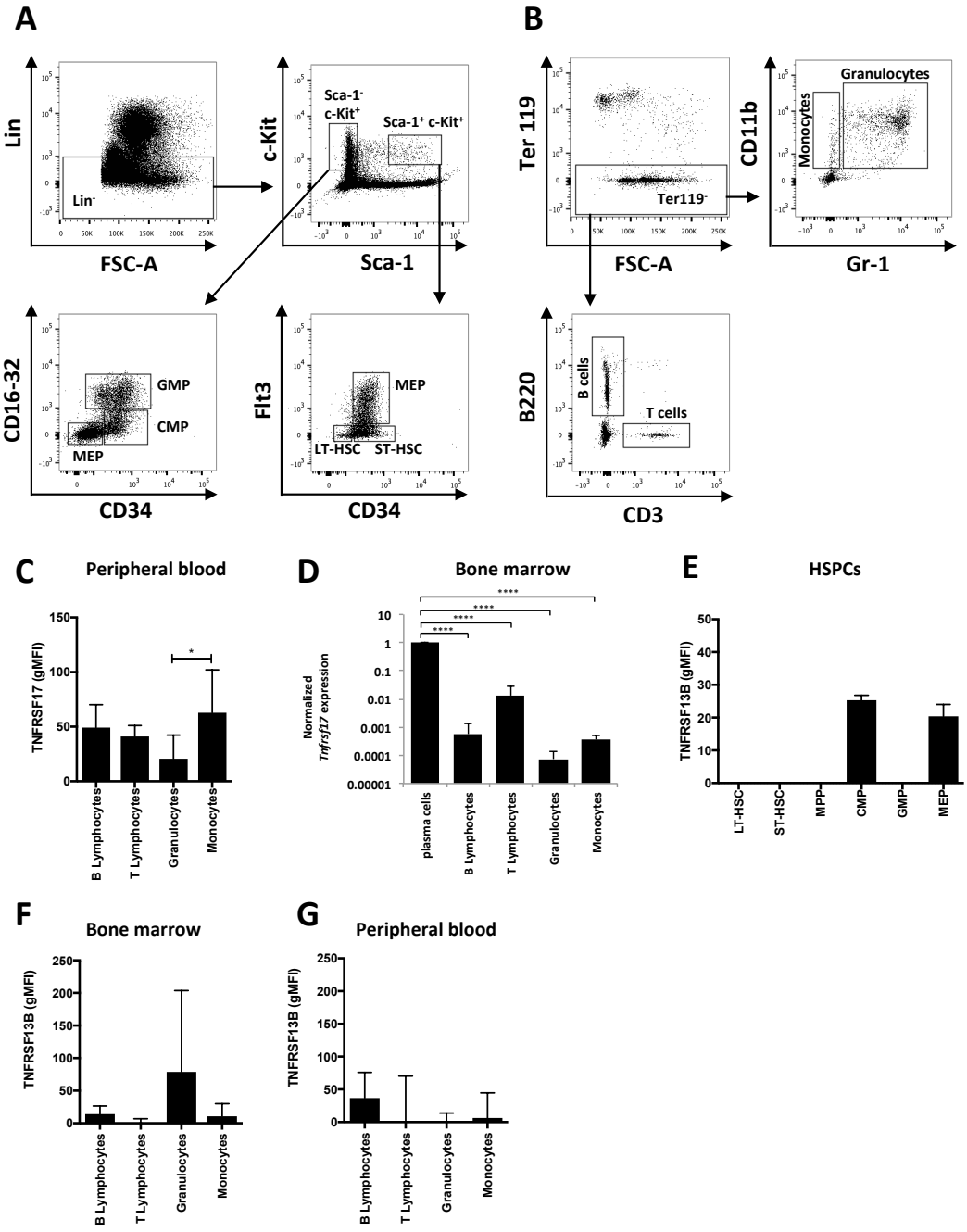
Supplementary Figure S2. Leukemia burden and spleen weight in mice injected with leukemia cells stimulated with TNFSF13. (***A***) Output cell number following dose titration with TNFSF13 for 3 days from a total of 10,000 seeded c-Kit^{+} *MLL-AF9* leukemia cells in media supplemented with 10 ng/mL of SCF (n=3). (***B-C***) 10,000 c-Kit^{+} *MLL-AF9* AML cells were cultured *ex vivo* with SCF (10 ng/mL), TNFSF13 (100 ng/mL) or no cytokine (Control) for 3 days, then all stimulated cells were transplanted into sublethally irradiated mice (10 mice per group, correspond to experiment presented in Figure 2E of the main manuscript). (***B***) Percentage of dsRed^{+} cells detected in the bone marrow (BM) of the mice at the time of sacrifice. (***C***) Spleen weight at the time of sacrifice for each group. Horizontal lines show the mean values for each group. Values are means ± SD; ****P<0.0001, and **P<0.01.

Supplementary Figure S3



Supplementary Figure S3. IL9 promotes leukemia-initiating cells *ex vivo*
(***A)*** Output cell number following dose titration with IL9 for 3 days from a total of 10,000 seeded c-Kit$^+$ *MLL-AF9* leukemia cells (n=3). (***B-C***) A total of 10,000 c-Kit$^+$ *MLL-AF9* AML cells were cultured *ex vivo* with IL9 (100 ng/mL), SCF (10 ng/mL), or no cytokine (Control) for 3 days, then all stimulated cells were transplanted into sublethally irradiated mice (5 mice per group). **(*B*)** Percentage of leukemic (dsRed$^+$) cells in the peripheral blood (PB) 14 days after transplantation. Horizontal lines show the mean values for each group. **(*C*)** Kaplan-Meier curves showing the survival of the mice. Values are means ± SD; ****P<0.0001, ***P<0.001, and **P<0.01.

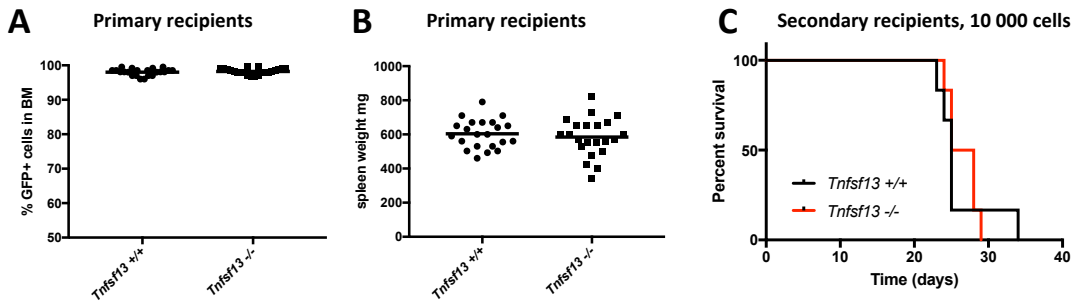
Supplementary Figure S4



Supplementary Figure S4. Expression of TNFRSF17 and TNFRSF13B on HSPCs and mature cell lineages. (*A*) FACS-based gating of hematopoietic stem and progenitor cells (HSPCs) from murine bone marrow. The arrows show how the gating was done. **(*B*)** Flow cytometric gating strategy used to analyze lineage distribution of murine bone marrow and blood cells. The arrows show how the gating was done. **(*C*)** Expression of TNFRSF17 shown as geometric mean fluorescent intensity (gMFI) after subtracting the signal using matching isotype control antibodies within lineage populations of peripheral blood (n=7). **(*D*)** *Tnfrsf17* mRNA expression within lineage populations of bone marrow cells determined by real-time PCR and normalized to expression levels in plasma ($CD138^+$) cells (n=3). **(*E-F*)** TNFRSF13B expression shown as gMFI after subtracting the signal using matching isotype control antibodies within the **(*E*)** Hematopoietic stem and progenitor cell (HSPC) compartment, **(*F*)** hematopoietic lineages of bone marrow cells, and **(*G*)** hematopoietic lineages of peripheral blood cells (n=5). LT-HSC: Long-Term Hematopoietic Stem cells; ST-HSC: Short-Term Hematopoietic Stem cells; MPP: Multipotent progenitors; CMP: Common myeloid progenitors; GMP: Granulocyte-

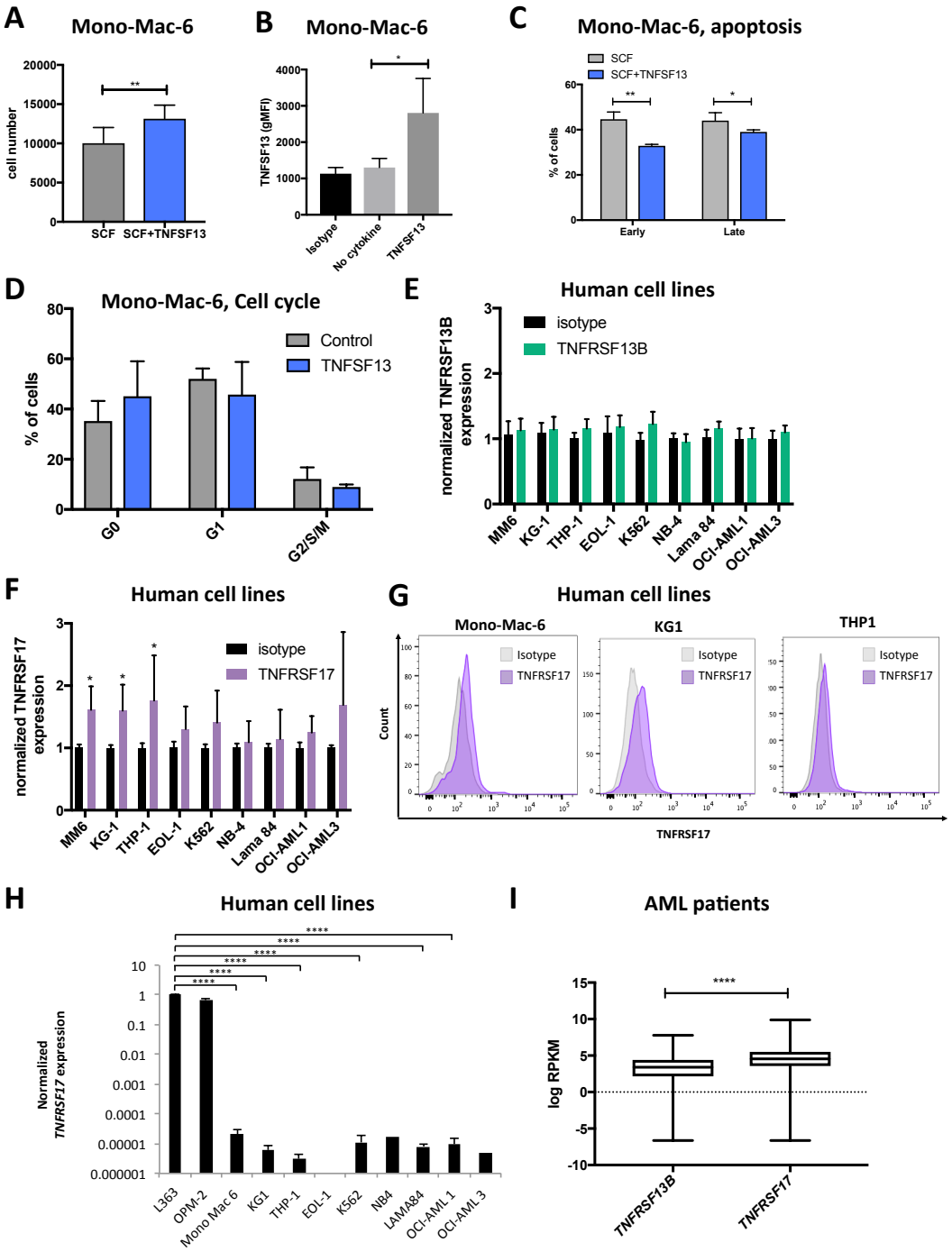
macrophage progenitors; MEP: Megakaryocyte-erythroid progenitors. Values are means ± SD, ****P<0.0001, *P<0.05

Supplementary Figure S5



Supplementary Figure S5. Leukemia burden and spleen weight in primary recipients at the time of sacrifice and survival of secondary recipients. (***A-B***) 250,000 c-Kit^{+} bone marrow (BM) cells from *Tnfsf13*$^{-/-}$ mice were transduced with the MIG-*MLL-AF9* retroviral vector and transplanted into sublethally irradiated *Tnfsf13*$^{+/+}$ or *Tnfsf13*$^{-/-}$ recipient mice (22 mice per group). Data shown is pooled from four independent experiments. **(*A*)** Percentage of leukemic (GFP^{+}) cells in the bone marrow of *Tnfsf13*$^{+/+}$ and *Tnfsf13*$^{-/-}$ primary recipient mice at the time of sacrifice. (***B***) Spleen weight of primary recipient mice at the time of sacrifice. Horizontal lines show the mean values within groups. (***C***) 10,000 leukemia cells harvested from spleens of primary leukemic *Tnfsf13*$^{+/+}$ or *Tnfsf13*$^{-/-}$ recipient mice were transplanted into corresponding *Tnfsf13*$^{+/+}$ and *Tnfsf13*$^{-/-}$ recipient mice (6 mice per group; pooled data from two donors per group). Kaplan-Meier curves show the survival of the mice**.**

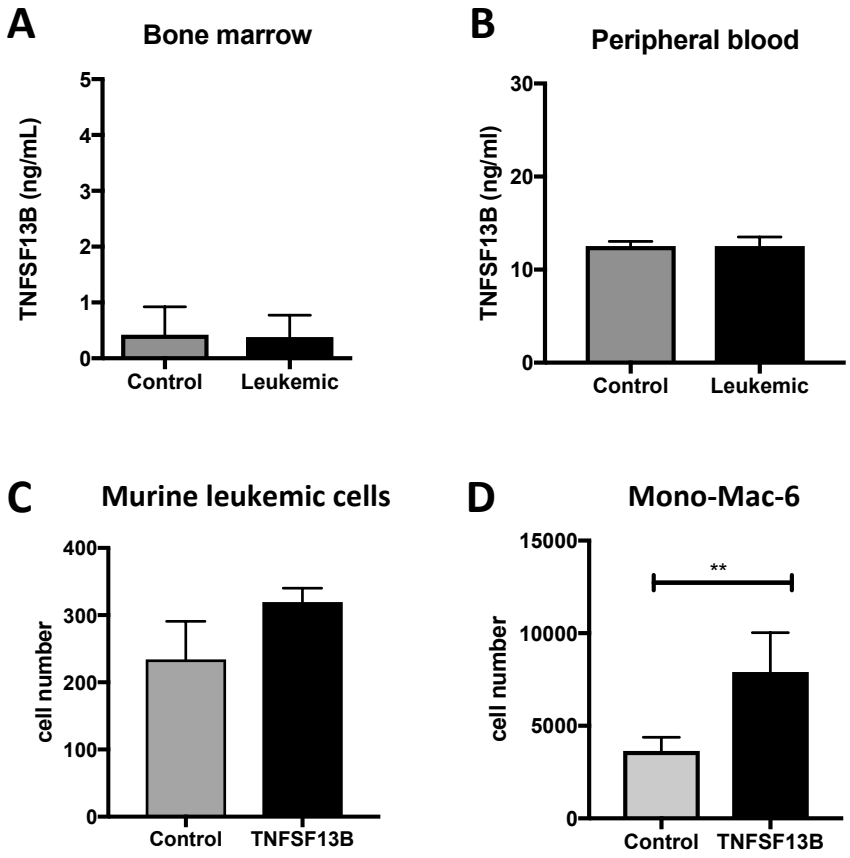
Supplementary Figure S6



Supplementary Figure S6. TNFSF13 promotes growth and survival of human AML cells; TNFRSF17 is more highly expressed than TNFRSF13B. (*A*) Output cell number of Mono-Mac-6 cells cultured under serum-free conditions supplemented with SCF (100 ng/mL) for 3 days +/- TNFSF13. A total of 1,000 cells were seeded per well (n=3). (***B***) Detection of TNFSF13 on cells stimulated for 5 minutes with TNFSF13, shown as geometric mean fluorescent intensity (gMFI). **(*C*)** Percentage of early (Annexin-V$^+$7AAD$^-$) and late (Annexin-V$^+$7AAD$^+$) apoptotic Mono-Mac-6 cells following 3 days of stimulation with TNFSF13 under serum-free conditions supplemented with SCF (n=3). **(*D*)** Cell cycle analysis of Mono-Mac-6 cells cultured under serum free condition +/- TNFSF13 for 3 days. **(*E*)** TNFRSF13B expression on a panel of human myeloid leukemic cell lines shown as gMFI normalized to the isotype control. (***F***) TNFRSF17 expression on a panel of human myeloid

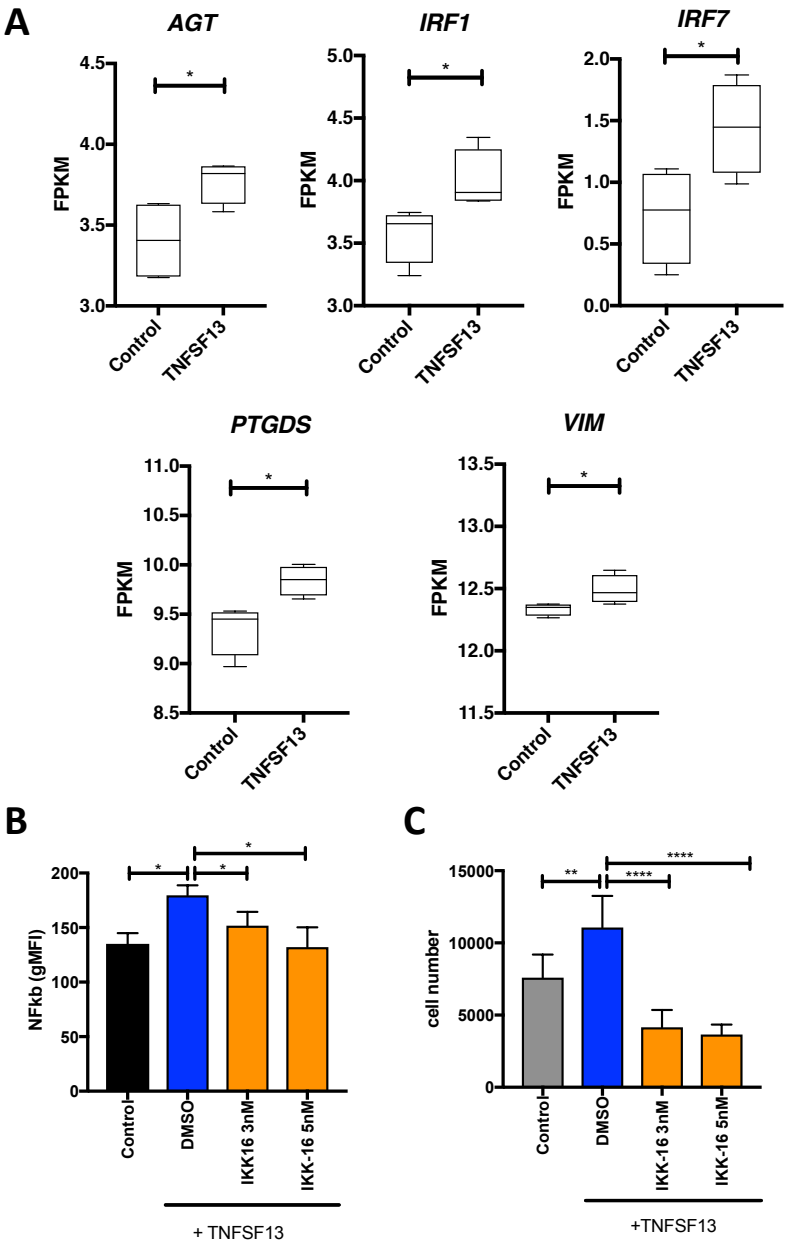
leukemic cell lines presented as gMFI normalized to the isotype control. **(*G*)** Flow cytometric analysis showing TNFRSF17 (purple) gMFI expression on human cell lines Mono-Mac-6, KG-1 and THP-1. Isotype control is shown in light grey. (***H)*** *TNFRSF17* mRNA expression on a panel of human myeloid leukemic cell lines determined by real-time PCR normalized to the reference myeloma cell line L363 (n=3 per group). The myeloma cell line OPM-2 was also included as a reference. (***I***) RPKM (reads per kilobase per million mapped reads) values for *TNFRSF13B* and *TNFRSF17* in human AML patient samples (n=173) from the TCGA database.[7] Data presented as box and whiskers diagrams for the logarithm of the RPKM values, the line indicates median, box limits are first and third quartiles and bars indicate maximum and minimum values. (***A-F, H)*** Graphs show average ± standard deviations (n=3). ****P<0.0001, **$P<0.01$, *$P<0$.05. MM6: Mono-Mac-6. TNFSF13 was used at 100 ng/mL.

Supplementary Figure S7



Supplementary Figure S7. TNFSF13B levels in mice and stimulation of AML cells with TNFSF13B. ELISA quantification of TNFSF13B in **(*A*)** bone marrow samples from healthy and leukemic mice (4 mice per group), and **(*B*)** blood plasma samples from healthy and leukemic mice (4 mice per group). (***C***) Output cell number from a total of 10,000 seeded c-Kit^{+} *MLL-AF9* murine leukemia cells stimulated with TNFSF13B for 3 days (n=3). **(*D*)** Output cell number of human Mono-Mac-6 cells cultured under serum-free conditions for 3 days +/- TNFSF13B. A total of 1,000 cells were seeded per well (n=3). Graphs show average ± standard deviations. ***P<0.01*

Supplementary Figure S8



Supplementary Figure S8. TNFSF13 promotes AML cells by activating the NF-κB pathway. (*A*) FPKM (fragments per kilobase per million mapped reads) values for NF-κB target genes in TNFSF13 stimulated Mono-Mac-6 cells from RNA-seq data. Data presented as box and whiskers diagrams for the FPKM values, the line indicates median, box limits are first and third quartiles and bars indicate maximum and minimum values. **(*B-C*)** Mono-Mac-6 cells were treated with the IKK-16 inhibitor at 3 or 5 μM during TNFSF13 stimulation and analyzed for (***B***) pNF-κB expression after subtracting the signal using matching isotype control antibodies and **(*C*)** output cell number after 3 days. Graphs show average ± standard deviations. ****$P<0.0001$, **$P<0.01$, and *$P<0.05$.

Supplementary Tables

Supplementary Table 1. Molecular barcodes cloned into the pLKO.1 vector.

Name	Sequence 5' -> 3'
Barcode 1	GGGGCTCACTATCTAACCAATCACGCCAGGCGCCGAGGCCTGTTT
Barcode 2	AGCAAGAGTTTTACGGGGGCGACTTGCGCGGAATGTATGAACGA
Barcode 3	TCTAGTAAAGTGGGGGTGGGGACTGTCCGAGCTGGCCGAACGCGC
Barcode 4	AAGGTCGGTCTGAAAGGAATCGGCGGATGCATTGTCATAGCAGAG
Barcode 5	GCAGGTACCGGATTACCAAGTTGTCCTTGACAAATAGAGTATGAA
Barcode 6	GAGGTCCCCAGGGGGAAGTTATGTCAAAGAGAACACAAGAACGGT
Barcode 7	AGACCGGTCAAGCACTAGTAGAGGAGAGAAAACAGATTGAAG
Barcode 8	GATATGGGTGTGAGGGGGTCGCGGGCAAGTGGCGACAAAGTTGCC
Barcode 9	TACAGATCCTCCGGAGGGGCATTGGTGCACCATGGGTTGGCGTGG
Barcode 10	GGGTCAGGTGGCAGACCTTTGGTCGTATACATGGTTGTGACGCTT
Barcode 11	GGGAATGGGACTGATAATGTCATGTTGTGATGCGGAGTATGTGCA

Supplementary Table 2. Cytokine ranking based on screening results.

Cytokine	Company	Screen 1 ± SD	Screen 2 ± SD	Average of the 2 screens
SCF	Prospec	6.32 ± 2.09	2.58 ± 0.85	4.45
SCF	Prospec	5.53 ± 0.43	3.34 ± 0.55	4.44
SCF	Prospec	7.18 ± 5.73	1.61 ± 1.03	4.39
SCF	Prospec	5.11 ± 4.56	3.19 ± 0.65	4.15
SCF	Prospec	5.77 ± 3.08	2.02 ± 0.58	3.89
SCF	Prospec	6.55 ± 0.74	0.90 ± 0.31	3.73
SCF	Prospec	4.83 ± 2.55	1.77 ± 1.25	3.30
TNFSF13	PeproTech	3.53 ± 2.94	2.66 ± 2.72	3.10
SCF	Prospec	4.35 ± 3.72	1.77 ± 0.55	3.06
SCF	Prospec	3.47 ± 2.37	2.48 ± 0.42	2.97
CXCL5	Prospec	4.04 ± 3.18	1.31 ± 0.96	2.67
SCF	Prospec	2.22 ± 0.74	3.08 ± 0.34	2.65
SCF	Prospec	3.06 ± 2.04	1.44 ± 0.93	2.25
IL6	Prospec	2.60 ± 0.45	1.71 ± 0.78	2.16
CCL4	Prospec	1.23 ± 0.64	2.96 ± 3.36	2.09
mCSF	PeproTech	2.79 ± 1.33	1.30 ± 0.64	2.04
IL22	Prospec	0.55 ± 0.37	3.40 ± 1.74	1.97
IL9	Prospec	1.92 ± 1.23	1.63 ± 0.74	1.78
LIF	PeproTech	0.42 ± 0.18	3.03 ± 3.68	1.72
IL1a	Prospec	1.08 ± 1.62	2.11 ± 1.68	1.60
CT1	Prospec	2.18 ± 0.62	0.99 ± 1.02	1.59
LIGHT	Prospec	0.17 ± 0.15	2.59 ± 3.86	1.38
sCD40-L	Prospec	2.55 ± 1.38	0.10 ± 0.07	1.32
TPO	Prospec	2.20 ± 0.97	0.32 ± 0.30	1.26
PDGFAA	Prospec	0.21 ± 0.10	2.24 ± 0.96	1.23
Leptin	Prospec	0.51 ± 0.54	1.90 ± 1.76	1.21
OSM	Prospec	1.34 ± 1.43	1.05 ± 0.38	1.19
GDF-5	Prospec	0.02 ± 0.02	2.34 ± 1.36	1.18
RELMβ	Prospec	1.33 ± 0.29	0.91 ± 0.97	1.12
Neuropoietin	Prospec	0.96 ± 0.63	1.25 ± 1.99	1.11
CCL5	Prospec	0.81 ± 0.58	1.39 ± 1.89	1.10
IL27	Prospec	1.73 ± 1.90	0.40 ± 0.40	1.06
IL20	Prospec	1.92 ± 3.15	0.21 ± 0.19	1.06
CCL9/CCL10	Prospec	0.09 ± 0.11	1.96 ± 1.99	1.02
VEGF	Prospec	0.54 ± 0.57	1.51 ± 2.11	1.02
WNT3a	R&D Systems	1.69 ± 2.78	0.30 ± 0.26	0.99
CXCL12	Prospec	0.73 ± 0.30	1.16 ± 1.38	0.95
FGF18	Prospec	0.48 ± 0.41	1.39 ± 0.81	0.93
CXCL4	Prospec	0.69 ± 0.63	1.15 ± 1.36	0.92
IL10	Prospec	1.37 ± 0.94	0.46 ± 0.67	0.91
Acrp30	Prospec	0.49 ± 0.40	1.25 ± 1.59	0.87
IL5	Prospec	0.84 ± 0.81	0.86 ± 0.79	0.85
CXCL3	Prospec	0.56 ± 0.27	1.10 ± 0.94	0.83
CCL7	Prospec	0.53 ± 0.02	1.09 ± 1.71	0.81
CCL20	Prospec	0.54 ± 0.59	1.06 ± 1.17	0.80
IL15	Prospec	0.65 ± 0.26	0.90 ± 0.92	0.78
IL27b	Prospec	1.32 ± 1.18	0.23 ± 0.21	0.78
CCL3	Prospec	1.31 ± 0.60	0.23 ± 0.26	0.77
CCL22	Prospec	0.29 ± 0.29	1.20 ± 1.09	0.74
IL17	Prospec	0.27 ± 0.38	1.22 ± 0.70	0.74
Flt3Ligand	Prospec	0.84 ± 0.54	0.57 ± 0.73	0.71
IL2	Prospec	0.66 ± 0.32	0.73 ± 0.69	0.70
gAcrp30	Prospec	0.30 ± 0.14	1.07 ± 0.99	0.68
IL28B	Prospec	1.12 ± 1.10	0.24 ± 0.02	0.68
IL1ra	Prospec	0.95 ± 0.54	0.41 ± 0.22	0.68
CCL19	Prospec	1.06 ± 1.15	0.28 ± 0.08	0.67
IL1β	Prospec	0.69 ± 0.49	0.63 ± 0.51	0.66
IGF1	Prospec	0.37 ± 0.13	0.95 ± 0.86	0.66
FGF8	Prospec	0.51 ± 0.64	0.80 ± 1.23	0.66
CXCL1	Prospec	0.20 ± 0.10	1.10 ± 0.36	0.65
IL36A	Prospec	1.03 ± 0.84	0.26 ± 0.29	0.65
FGF7	Prospec	0.60 ± 0.51	0.69 ± 0.61	0.64

Supplementary Table 2 (continued)

Cytokine	Company	Average screen 1 ± SD	Average screen 2 ± SD	Average
CXCL10	Prospec	0.24 ± 0.35	1.03 ± 1.41	0.64
IL36B	Prospec	0.35 ± 0.38	0.87 ± 0.70	0.61
FGF15	Prospec	0.69 ± 0.37	0.47 ± 0.57	0.58
CXCL14	Prospec	0.66 ± 0.19	0.50 ± 0.86	0.58
FGF10	Prospec	0.29 ± 0.12	0.86 ± 0.68	0.57
CXCL2	Prospec	0.73 ± 0.63	0.40 ± 0.17	0.56
RELMγ	Prospec	0.47 ± 0.19	0.64 ± 0.86	0.56
IL33	Prospec	0.34 ± 0.47	0.78 ± 0.53	0.56
Activin-A	Prospec	0.96 ± 0.66	0.13 ± 0.12	0.55
VEGF	Prospec	1.01 ± 0.15	0.09 ± 0.08	0.55
IL7	Prospec	0.55 ± 0.55	0.52 ± 0.63	0.54
BD2	Prospec	0.79 ± 0.48	0.28 ± 0.29	0.54
FGF9	Prospec	0.58 ± 0.70	0.44 ± 0.26	0.51
IL17E	Prospec	0.79 ± 0.62	0.23 ± 0.06	0.51
SF-20	PeproTech	0.46 ± 0.31	0.55 ± 0.62	0.51
LGALS9	Prospec	0.53 ± 0.17	0.48 ± 0.37	0.50
BD1	Prospec	0.79 ± 0.52	0.20 ± 0.19	0.49
CXCL16	Prospec	0.30 ± 0.23	0.69 ± 0.46	0.49
RELMα	Prospec	0.83 ± 0.60	0.15 ± 0.07	0.49
Prolactin	Prospec	0.47 ± 0.50	0.51 ± 0.43	0.49
gCSF	PeproTech	0.28 ± 0.06	0.69 ± 0.43	0.49
IL21	Prospec	0.41 ± 0.35	0.53 ± 0.40	0.47
sRANKL	Prospec	0.70 ± 0.30	0.24 ± 0.03	0.47
CCL28	Prospec	0.19 ± 0.04	0.73 ± 0.79	0.46
gmCSF	PeproTech	0.84 ± 1.08	0.08 ± 0.15	0.46
CCL17	Prospec	0.19 ± 0.24	0.73 ± 0.51	0.46
CCL12	Prospec	0.41 ± 0.41	0.47 ± 0.46	0.44
FGF1	Prospec	0.48 ± 0.36	0.38 ± 0.33	0.43
CCL24	Prospec	0.30 ± 0.10	0.55 ± 0.47	0.42
IL11	Prospec	0.15 ± 0.13	0.69 ± 0.87	0.42
IL4	Prospec	0.46 ± 0.53	0.37 ± 0.32	0.42
TRAIL	PeproTech	0.41 ± 0.68	0.42 ± 0.39	0.42
IFNβ	Prospec	0.57 ± 0.39	0.26 ± 0.14	0.41
CCL21	Prospec	0.36 ± 0.13	0.45 ± 0.78	0.41
Endoglin	Prospec	0.16 ± 0.12	0.60 ± 1.03	0.38
CXCL12	Prospec	0.10 ± 0.07	0.63 ± 0.28	0.37
Visfatin	Prospec	0.31 ± 0.21	0.38 ± 0.60	0.34
LGALS1	Prospec	0.51 ± 0.19	0.17 ± 0.30	0.34
PDGFBB	Prospec	0.20 ± 0.11	0.47 ± 0.28	0.34
CCL2	Prospec	0.45 ± 0.17	0.23 ± 0.40	0.34
SHH	Prospec	0.43 ± 0.28	0.23 ± 0.24	0.33
TGF-β3	Prospec	0.60 ± 0.35	0.07 ± 0.08	0.33
CCL11	Prospec	0.09 ± 0.01	0.56 ± 0.76	0.32
BD3	Prospec	0.15 ± 0.06	0.50 ± 0.27	0.32
TNFα	Prospec	0.30 ± 0.14	0.31 ± 0.27	0.31
CCL8	Prospec	0.30 ± 0.29	0.31 ± 0.27	0.30
IL16	Prospec	0.23 ± 0.15	0.35 ± 0.23	0.29
CCL6	Prospec	0.27 ± 0.43	0.31 ± 0.45	0.29
Resistin	Prospec	0.34 ± 0.48	0.22 ± 0.18	0.28
IL12	Prospec	0.50 ± 0.49	0.00 ± 0.00	0.25
CXCL9	Prospec	0.03 ± 0.04	0.44 ± 0.55	0.24
HGF	Prospec	0.11 ± 0.13	0.36 ± 0.49	0.24
IL13	Prospec	0.23 ± 0.24	0.24 ± 0.15	0.24
NOG	Prospec	0.28 ± 0.49	0.15 ± 0.10	0.22
FGF2	Prospec	0.33 ± 0.19	0.10 ± 0.14	0.21
CCL25	Prospec	0.19 ± 0.07	0.23 ± 0.13	0.21
BTC	Prospec	0.37 ± 0.56	0.00 ± 0.00	0.18
BMP4	PeproTech	0.07 ± 0.09	0.13 ± 0.20	0.10
EGF	Prospec	0.02 ± 0.04	0.17 ± 0.15	0.10
IL3	Prospec	0.03 ± 0.02	0.14 ± 0.16	0.08
IFNγ	Prospec	0.04 ± 0.01	0.13 ± 0.10	0.08
FGF21	Prospec	0.09 ± 0.10	0.05 ± 0.08	0.07

Presented is a ranked list of cytokines based on the screening results. The sources of the cytokines are included. Shown is fold-change *in vivo* versus input of barcoded cell populations in screen 1 and 2 ± standard deviation (SD; n=3), and average of the two screens.

Supplementary Table 3. pLKO.1 specific primers used to amplify the regions containing the barcodes.

pLKO.1 primers 5'->3'	
Forward	**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CAAGCCTGTTACAGAGATAATTGGA
Reverse	**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GGATGAATACTGCCATTTGTCTCG

Overhangs for amplification with Nextera XT primers are indicated in **bold type**.

Supplementary Table 4. Distribution of hematopoietic populations in *Tnfsf13*$^{+/+}$ and *Tnfsf13*$^{-/-}$ mice.

		Tnfsf13 +/+	*Tnfsf13 -/-*	
Peripheral blood	**lineage distribution (percentage of WBC)**			
	Granulocytes	3.77 ± 0.4	4.78 ± 0.6	
	Monocytes	4.25 ± 0.2	4.29 ± 0.4	
	T-lymphocytes	34.56 ± 1.6	24.69 ± 3.9 *	
	B-lymphocytes	53.56 ± 1.3	56.26 ± 5.09	
	White Blood Cells 10^9/l (± SD)	12.43 ± 2.4	14.27 ± 2.3	
	Red Blood Cells 10^12/l (± SD)	10.42 ± 0.3	11.13 ± 0.52	
	Platelets 10^9/l (± SD)	498.25 ± 170	544.75 ± 184	
Bone marrow	**hematopoietic stem and progenitor cells (percentage of viable cells)**			
	LT-HSC	0.0068 ± 0.0007	0.0066 ± 0.0004	
	ST-HSC	0.053 ± 0.003	0.048 ± 0.005	
	MPP	0.08 ± 0.004	0.07 ± 0.006	
	GMP	0.82 ± 0.05	0.66 ± 0.03 *	
	CMP	0.38 ± 0.02	0.4 ± 0.03	
	MEP	1.32 ± 0.08	1.19 ± 0.09	
	lineage distribution (percentage of WBC)			
	Granulocytes	46.47 ± 1.4	42.68 ± 2.12	*P=0.145*
	Monocytes	4.79 ± 0.3	4.04 ± 0.2 *	
	T-lymphocytes	5.587 ± 0.5	4.31 ± 0.6 *	
	B-lymphocytes	36.05 ± 1.8	41.75 ± 1.8 *	

Shown is average percentage ± standard deviation. Bone marrow; 14 *Tnfsf13*$^{+/+}$ and 13 *Tnfsf13*$^{-/-}$ mice. Peripheral blood: 7 mice per group, Blood cell count: 4 mice per group. LT-HSC: Long-Term Hematopoietic Stem cells; ST-HSC: Short-Term Hematopoietic Stem cells; MPP: Multipotent progenitors; CMP: Common myeloid progenitors; GMP: Granulocyte-macrophage progenitors; MEP: Megakaryocyte-erythroid progenitors; WBC: White blood cells. *P<0.05.

Supplementary Table 5. Expression of *TNFRSF13B* and *TNFRSF17* in genetic subgroups of AML.

		TNFRSF13B			*TNFRSF17*		
	Number of patients	p-value	q-value	FC	p-value	q-value	FC
Fusions and partial tandem duplication (PTD)							
RUNX1-RUNX1T1	7	0.053	0.12	0.30	0.70	0.70	0.83
CBFB-MYH11	10	0.33	0.39	0.61	0.47	0.47	0.75
PML-RARA	16	0.54	0.63	0.77	0.17	0.30	0.65
BCR-ABL1	3	0.95	0.95	0.95	0.13	0.31	0.35
MLL-partner	8	0.62	0.83	1.33	0.83	0.83	0.91
MLLT10-partner	6	0.94	0.94	1.05	0.57	0.67	1.33
MLL-PTD	9	0.16	0.56	2.15	0.38	0.67	1.43
GPR128-TFG	3	0.77	0.89	0.76	0.37	0.89	0.53
NUP98-NSD1	3	0.75	0.92	0.75	0.31	0.92	0.49
Other in -frame fusions	10	0.42	0.65	1.52	0.73	0.85	0.87
genes mutated *							
FLT3	49	0.0010	0.0036	0.42	0.00016	0.0011	0.47
NPM1	47	0.059	0.14	0.60	0.33	0.39	0.82
DNMT3A	43	0.56	0.67	0.85	0.67	0.67	0.91
IDH2	17	0.18	0.43	1.72	0.45	0.53	1.26
IDH1	16	0.12	0.41	0.52	0.52	0.63	1.23
TET2	15	0.66	0.91	1.21	0.28	0.91	1.42
RUNX1	**17**	**0.025**	**0.089**	**2.48**	**0.0016**	**0.011**	**2.62**
TP53	**14**	**0.0043**	**0.015**	**3.52**	**0.00042**	**0.0030**	**3.21**
NRAS	12	0.59	0.69	0.77	0.39	0.69	1.37
CEBPA	13	0.42	0.61	0.69	0.23	0.53	0.66
Others, with non of the above genes mutated	43	0.72	0.86	1.10	0.86	0.86	0.96
Others, with non of the above genes mutated and non of the above fusions	8	0.46	0.52	1.54	0.10	0.23	2.05
Molecular risk classification							
Good	33	0.045	0.081	0.54	0.10	0.12	0.68
Intermediate	92	0.46	0.47	1.20	0.20	0.35	1.26
Poor	45	0.28	0.49	1.35	0.69	0.69	1.09
Total	173						

FC: fold-change (in specific genetic subgroups relative to all others). *The 10 most frequently mutated genes in AML according to the TCGA database.

Supplementary Table 6. Genes included in the gene sets enriched in the TNFSF13 signature of Mono-Mac-6 cells. The TNF receptor binding gene set was from molecular signatures database (MsigDB), and the NF-κB gene set has been described previously.[8]

TNF receptor binding	NfkB
CD70	AGT
FADD	ARFRP1
TNFSF9	CD74
TRAF2	GSTP1
TRAF6	HMOX1
TNFSF13B	IRF1
TRAF4	IRF7
TRAP1	JUNB
TRADD	NFKBIA
BRE	PLAU
TNFSF13	PTGDS
TRAF3	STAT5A
TNF	TAP1
TNFSF14	TRAF2
TRAF1	VIM
TNFSF10	BAX
CASP8	SOD2
ERAP1	GADD45B
STAT1	CCL3
TRIM37	IER3
TNFSF4	S100A6
	LGALS3
	FTH1
	TP53
	CCL5
	CCL2
	PIM1
	IL1RN
	RELB
	BMP2
	FCER2
	TNF
	IL15RA
	BCL2L1
	NQO1
	AMH
	CD83
	G6PD
	TERT
	CD48
	IL1B
	TAPBP
	WT1
	PLCD1
	MYC
	NFKB2
	ICAM1
	CASP4
	IL8
	TRAF1
	SLC2A5
	PTGS2
	NFKB1
	F3
	IRF2
	PTAFR
	CD44
	TGM2
	CSF1
	REL
	PTX3
	IRF4
	TPMT
	FN1
	TNFAIP3
	PLA2G4A
	CFLAR
	AGER
	BCL2
	BIRC2
	MYB

Supplementary References

1. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods 2012;9(4):357–359.

2. Podolin PL, Callahan JF, Bolognese BJ, et al. Attenuation of murine collagen-induced arthritis by a novel, potent, selective small molecule inhibitor of IkappaB Kinase 2, TPCA-1 (2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide), occurs via reduction of proinflammatory cytokines and antigen-induced T cell Proliferation. J Pharmacol Exp Ther 2005;312(1):373–381.

3. Waelchli R, Bollbuck B, Bruns C, et al. Design and preparation of 2-benzamido-pyrimidines as inhibitors of IKK. Bioorg Med Chem Lett 2006;16(1):108–112.

4. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008;3(6):1101–1108.

5. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 2013;14(4):R36.

6. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102(43):15545–15550.

7. Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368(22):2059–2074.

8. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999;18(49):6853–6866.