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

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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_{4}Cl 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 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 NH₄Cl 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 TNFRSF17 and TNFRSF13B 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-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 IL-9 (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 TPCA1² (Sigma-Aldrich) or with 3 or 5 µM of IkK-16³ (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, Männendorf, 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 RNeasy 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

(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 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

**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. 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-Monocyte.
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. TNFSF13 promotes growth and survival of human AML cells; TNFRSF17 is more highly expressed than TNFRSF13. (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 TNF, 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 cell lines.
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. 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 | GGGGCTCCTATCTAACCAATCACGCCAGGCGAGGCCTGTTT |
| Barcode 2 | AGCAAGAGTTTTACGGGGGCAGCTTGCGCAATGTATGACGA |
| Barcode 3 | TCTAGTAAAGTGAGGCGGACTTGCCAGCCTGCGCGAGC |
| Barcode 4 | AAGGGCCGGTACTGAAAGTTATACGGAAGCATCCTGAGAGATAAG |
| Barcode 5 | GCAGTACCAGTTAATCTTGCCTTGACAATAGTAGATGAA |
| Barcode 6 | GAGGTCGCAGGGGAAAGTTATGTCAAGAGAAACACAGCGTG |
| Barcode 7 | AGACCCGTTCAAGCCTTAGAGGAGAGAAGAAGAAGATTGAA |
| Barcode 8 | GATATGGGCTGAGGGTGTCGCGGCAAGGGATGAGCGACAAAGATGGCC |
| Barcode 9 | TACAGATCCTCCGGAGGCGATGACGGTTGTGGCGCTGG |
| Barcode 10 | GGTCAGGTGCCAGACCTTGTGTGTAGATGACGCTT |
| Barcode 11 | GGAATGGGAGACTGATAATGTCATTGTGTATGAGGAGTATGCTGCA |
| 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                    |
| PDGF-AA   | Prospec     | 0.21 ± 0.10   | 2.24 ± 0.96   | 1.23                    |
| Leptin    | Prospec     | 0.51 ± 0.54   | 1.90 ± 1.76   | 1.21                    |
| GMS       | Prospec     | 1.34 ± 1.43   | 1.05 ± 0.38   | 1.19                    |
| GDF-5     | Prospec     | 0.02 ± 0.02   | 2.34 ± 1.36   | 1.18                    |
| RELMα     | Prospec     | 1.33 ± 0.29   | 0.91 ± 0.97   | 1.12                    |
| Neuropeitin| 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                    |
| CCL5/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                    |
| IL18      | 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 ± 1.43            | 0.64    |
| IL6B       | 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    |
| FGF150     | Prospec   | 0.29 ± 0.12            | 0.86 ± 0.68            | 0.57    |
| CCL12      | Prospec   | 0.73 ± 0.63            | 0.40 ± 0.17            | 0.56    |
| RELMα      | 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    |
| BDL2       | 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    |
| IL11       | Prospec   | 0.79 ± 0.52            | 0.20 ± 0.19            | 0.49    |
| CCL16      | 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    |
| IL23       | Prospec   | 0.41 ± 0.35            | 0.53 ± 0.40            | 0.47    |
| ST2NL      | Prospec   | 0.70 ± 0.30            | 0.24 ± 0.03            | 0.47    |
| CCL28      | Prospec   | 0.19 ± 0.04            | 0.73 ± 0.79            | 0.46    |
| gmscF      | 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    |
| LGALS5     | Prospec   | 0.51 ± 0.19            | 0.17 ± 0.30            | 0.34    |
| PDGFβB     | 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    |
| BDT20      | 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    |
| IL3        | Prospec   | 0.23 ± 0.24            | 0.24 ± 0.15            | 0.24    |
| NOG        | Prospec   | 0.28 ± 0.49            | 0.15 ± 0.10            | 0.22    |
| FGF21      | 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   | 0.06 ± 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.

| Primers | 5' - 3' |
|---------|---------|
| **Forward** | GTCTCGTGGGCTCGGAGATGTGTATAAGAGACACAGCAAGCCTGTTCAGAGATACAGAATTGGA |
| **Reverse**  | TCGTCGGACGCCGTCAGATGTGTATAAGAGACAGGGATGAATACCTGCCATTTGTTCTCG |

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.

| Fusions and partial tandem duplication (PTD) | Number of patients | *TNFRSF13B* p-value | *TNFRSF13B* q-value | FC | *TNFRSF17* p-value | *TNFRSF17* q-value | FC |
|--------------------------------------------|--------------------|---------------------|---------------------|----|---------------------|---------------------|----|
| 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                | 0.86 |
| 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.

| TNF receptor binding | NFκB |
|----------------------|-------|
| CD79                | AGT   |
| FAA2                | MMFP1 |
| TNFSF9              | IC24   |
| TRAF2               | GSTP1 |
| TRAF6               | HM00X1 |
| TNFSF13B            | H9F1  |
| TRAF4               | H9F7  |
| TRAF1               | JUNB  |
| TRADD               | NFκB4A|
| BRE                 | PLCD3 |
| TNFSF13             | PTGS2 |
| TRAF3               | STAT5A|
| TNF                 | TAP1  |
| TNFSF14             | TRAF2 |
| TRAF1               | JUN   |
| TNFSF10             | BAK   |
| CASP8               | SOD2  |
| TRAP1               | DAO4S68|
| STAT1               | CCL2  |
| TRAK2               | IRS3  |
| TNFSF4              | SLQA46 |
| LOX5L3              |        |
| FTH1                |        |
| TP53                |        |
| CCL5                |        |
| CCL2                |        |
| PABP2               |        |
| IL1RN               |        |
| RELB                |        |
| BMP2                |        |
| FCER2               |        |
| TNF                 |        |
| IL15RA              |        |
| BCL2L1              |        |
| RAG1                |        |
| AMP1                |        |
| CD83                |        |
| G6PD                |        |
| IKBG                |        |
| CO4B                |        |
| IL1B                |        |
| TAP4BP              |        |
| NYF1                |        |
| PLCG1               |        |
| MYC                 |        |
| NFκB2               |        |
| ICAM1               |        |
| CASP4               |        |
| IL8                 |        |
| TRAF1               |        |
| SLC25A              |        |
| PTGS2               |        |
| NFκB1               |        |
| F3                  |        |
| HRF2                |        |
| PTAR8               |        |
| CO4A                |        |
| TGFβ2               |        |
| C5F1                |        |
| REL                 |        |
| PTX3                |        |
| IRF4                |        |
| TPR4T               |        |
| TNF1                |        |
| TNFAIP3             |        |
| PLA2G4A             |        |
| CCL4F               |        |
| RAD51               |        |
| BCL2                |        |
| BIRC2               |        |
| MYB                 |        |
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