Science Translational Medicine
AAAS

# Supplementary Materials for

## Spermidine metabolism regulates leukemia stem and progenitor cell function through kat7 expression in patient-derived mouse models

Vincent Rondeau *et al.*

Corresponding author: Courtney L. Jones, courtney.jones@cchmc.org

*Sci. Transl. Med.* **16**, eadn1285 (2024)
DOI: 10.1126/scitranslmed.adn1285

**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

**Supplemental Materials and Methods:**

**Cell culture**

Molm13 (RRID:CVCL_2119) and MV4;11 (RRID:CVCL_0064) cells were maintained in RPMI-1640 media (Gibco; 11875085) with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL of streptomycin (Gibco; 15140122). Patient acute myeloid leukemia (AML) cells and normal bone marrow (NBM) cells were cultured in X-Vivo10 media (Lonza; 04-380Q), supplemented with 20% BIT (StemCell; 09500) and 10nM cytokines [interleukin (IL)-3, IL-6, stem cell factor (SCF), and fms related receptor tyrosine kinase 3 (FLT3) ligand] purchased from stem cell technologies. For arginine depletion experiments, cells were cultured in amino acid free Iscove's modified Dulbecco's medium (IMDM) supplemented with all amino acids except arginine as previously described(*7*). Briefly, all amino acids except arginine were added to amino acid free IMDM at concentration observed in human plasma. AML cells were then incubated in the media for 24 hours before performing colony formation assays.

**Inhibitors/chemicals**

DENSpm was obtained from Tocris (0468). Spermidine (S0266), spermine (S4264), WM-2474 (SML3159), L-Arginine-13C6,15N4 (608033), puromycin (P7255), GC7 (259545), and DFMO (296082) were purchased from Millipore-Sigma. Venetoclax (HY-15531) and WM-3835 (S9805) were obtained from Cederlane Labs. AMXT 1501 was purchased from MedChem Express (HY-124617A). Recombinant human KAT7 was obtained from Abcam (ab268698).

**Cell sorting**

Primary AML specimens were thawed and stained with 1:40 CD45-brilliant blue (BB) 515 antibody (Ab) (BD Biosciences; 564585) (RRID:AB_2732068) to identify the blast population, 1:20 CD19-phycoerythrin (PE) (BD Biosciences; 555413) (RRID:AB_395813) and 1:40 CD3-PE-Cyanine (Cy) 7 (BD Biosciences; 557749) (RRID:AB_396855) Abs to exclude the lymphocyte populations, 0.5µg/mL DAPI to exclude dead cells, and 5µM CellRox deep red (Thermo Fisher Scientific; C10422) to assess reactive oxygen species (ROS) abundance. Cell surface markers staining was performed at 4 degrees Celsius for 20-30 minutes. CellRox was then incubated with cells for 30 minutes at 37 degrees Celsius for 30 minutes. Finally, cells were incubated with DAPI on ice for five minutes before analyzing by flow cytometry. ROS-low leukemia stem cells (LSCs) were identified as the cells with the 20% lowest ROS abundance, as previously described(*27*) DOI: 10.1016/j.xpro.2020.100248. Cells were sorted on a BD FACSAria Fusion.

**Colony forming assays**

Colony forming assays were performed using enriched MethoCult media (StemCell; 04435). Cells were treated for 24 hours with DENSpm, DFMO, AMXT 1501, DFMO + AMXT 1501, or GC7 or transfected with siRNA using the methods described below. Alternatively, WM-2474 or WM-3835 (1 µM) were added directly in Methocult media. Colony numbers were evaluated 1 to 3 weeks later. Methocult media was then diluted using 3 mL of phosphate-buffered saline (PBS), cells were filtered and stained for flow cytometry or morphological analysis as described below.

**Flow cytometric analysis of engraftment and differentiation**

Cells collected from engraftment studies and colony forming assays were stained with CD45-BB515 Ab (BD Biosciences; 564585) (RRID:AB_2732068), CD19-PE Ab (BD Biosciences; 555413) (RRID:AB_395813), CD3-PE-Cy7 Ab (BD Biosciences; 557749) (RRID:AB_396855), CD11b-allophycocyanin (APC)/Cy7 Ab (BD Biosciences; 557754) (RRID:AB_396860), CD33-brilliant violet (BV) 711 Ab (BD Biosciences; 563171) (RRID:AB_2738045), CD15-BV786 Ab (BD Biosciences; 563838) (RRID:AB_2738444) and Live/Dead Far Red (Thermo Fisher; L34974).

Analysis was performed using the BD FACSCelesta and FlowJo. Cell number was determined using the integrated BD High-Throughput Sampler on the BD FACSCelesta.

**Viability assays**

Primary cells and AML cell lines were treated with DENSpm or DFMO + AMXT 1501 for 24 hours. Viability was monitored using DAPI and Annexin V-FITC (BioLegend; 640945) staining or manual cell counting. The cells were analyzed by flow cytometry (BD FACSCelesta).

**Cell cycle analysis**

For cell cycle analysis, ROS-low LSCs, Human bone marrow $CD34^+$ progenitor cells, and AML cell lines were treated with DENSpm (10 μM) or vehicle for 24 hours. Cells were then permeabilized and fixed according to the manufacturer's instructions with FlowX FoxP3/Transcription Factor Fixation & Perm Buffer Kit (R&D systems; FC012) and then stained with Ki-67 AlexaFluor700 Ab (BD Biosciences; 561277) (RRID:AB_10611571) and DAPI. The stained cells were analyzed by flow cytometry (BD FACSCelesta).

**Polyamine assay**

For measurement of total polyamine concentration, AML cell lines were treated with DENSpm (10 μM) or vehicle for 24 hours. Polyamine concentration was determined using Total Polyamine Assay Kit (Sigma-Aldrich; MAK349) according to the manufacturer's instructions.

**Protein synthesis measuring assays**

For protein translation measurement, primary cells (AML or human bone marrow mononuclear) and AML cell lines were treated with DENSpm (10 μM) or vehicle for 24 hours. During the last 30 minutes of treatment, puromycin (10 μg/mL) was added to cells to label nascent peptides. For primary specimens, cells were also incubated with CD34-BV421 Ab (BD; 562577) (RRID:AB_2687922) at this step. Cells were washed once with PBS and then fixed and permeabilized using FlowX FoxP3/Transcription Factor Fixation & Perm Buffer Kit (R&D systems; FC012). Cells were incubated with anti-puromycin Ab (Sigma-Aldrich; MABE343) for 1 hour at room temperature, washed, and then stained with anti-mouse IgG2a BV786 Ab (BD Biosciences; 744534) (RRID:AB_2742307) for 30 min at room temperature. After washing, DAPI was used to counterstain live cells and DNA content by flow cytometry (BD FACSCelesta and BD FACSCanto).

**Immunoblotting**

For protein lysate preparation, cells were incubated on ice with RIPA buffer supplemented with Halt Protease Inhibitor cocktail and EDTA (Thermo Fisher Scientific; 78438) for 1 hour. Lysates were collected after centrifugation at 10000 rpm for 20 min at 4°C. Protein concentration was determined using Pierce BCA protein assay kit (Thermo Scientific; 23227) according to the manufacturer's instructions. Lysates were loaded on 4-15% polyacrylamide gel and transferred to a PDVF membrane using the mini trans-blot transfer system (Bio-Rad). To detect specific antigens, blots were probed with 1:1000-1:500 primary SAT1 Ab (Proteintech; 10708-1-AP) (RRID:AB_2877739), 1:500 Hypusine Ab (Creative Biolabs; PABL-202) (RRID:AB_2877637), 1:1000 KAT7 Ab (Abcam; ab70183) (RRID:AB_1269226), and 1:1000 GAPDH Ab (Santa Cruz Biotechnology; sc-32233) (RRID:AB_627679) on a shaker at 4°C overnight. Blots were then incubated with 1:1000 IRDye 680RD anti-mouse IgG (LICOR; 926-68070) or 1:1000 anti-rabbit IgG (LICOR; 926-68071) Abs for 1 hour at room temperature. Blots were imaged using the LICOR Odyssey Dlx imaging system. Quantification analysis were performed using ImageJ. Uncropped western blots are shown in data file S3.

**Quantitative real-time polymerase chain reaction (qPCR)**
AML cell lines were treated with DENSpm (10 μM) or vehicle for 24 hours. RNA was isolated using RNeasy mini kit (Qiagen; 74014) and cDNA were synthesized using the iScript cDNA Synthesis Kit (Bio-Rad; 1708891) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction was performed using Itaq Universal SYBR (Bio-Rad; 1725122) and the following primers:
SAT1 reverse primer: 5'-TCC AAC CCT CTT CAC TGG AC-3'
SAT1 forward primer: 5'-CCG TGG ATT GGC AAG TTA TT-3'
GAPDH reverse primer: 5'-GTG GGG GAC TGA GTG TGG-3'
GAPDH forward primer: 5'-CAG CAA GAG CAC AAG AGG AA-3'

**siRNA transfection**
Primary AML specimens were electroporated using the Neon transfection system as previously described(*65*) using buffer T for primary cells and buffer R for cell lines at 1600V, 20ms, 3 pulses. SMARTpool siRNAs and non-targeting control siRNA were purchased from Dharmacon (Non-targeting control, D-001810-10-50; eI5FA, L-015739-00-005).

**Protein Transfection**
2 μg of recombinant KAT7 (abcam, ab268698) or beta-gal was transfected into AML cells using the TakaraBio Clontech Labs 3P XFECTA Protein Transfection Reagent following the manufacturer's protocol.

**RNA-sequencing**
Primary AML LSCs and $CD34^+$ bone marrow cells were treated with DENSpm (10 μM) or vehicle for 24 hours. RNA was isolated from 50,000 cells using RNeasy micro kit (Qiagen; 74004). Sample quality was assessed using Agilent Bioanalyzer prior to library preparation and sequencing. RNA sequencing was performed using Illumina Novaseq 6000 in pair end 2x150 pb resulting in ~77-96M reads/sample. Short-read quality control was performed using FastQC v0.11.5. Trim Galore v0.6.6 was used for trimming sequencing adapters and low-quality reads. Subsequently, reads were aligned to GENCODE human reference genome v38(*55*) using STAR v.2.7.9a(*56*). After filtering low-quality alignments (mapq score < 15), we used featureCounts v2.0.1 (57) to quantify the gene expression. Next, genes with less than 10 total counts across comparing samples were filtered. DESeq2 v1.40.1(*58*) R package was used to identify the differentially expressed genes between treatment groups while controlling for the primary sample batch in the design. Gene set enrichment analysis (GSEA)(*59*) was performed using fgsea v1.26.0(*60*) and the curated list of pathways published by Bader lab (http://baderlab.org/GeneSets - updated August 2022)(*61*). All p-values were adjusted using the Benjamini-Hochberg method(*62*).

**Metabolomics**
For steady state metabolomics, primary AML LSCs and $CD34^+$ bone marrow cells were collected in technical replicates of 3 or 4 at 100,000 cells per replicate. Each replicate was washed two times on ice with PBS. All buffer was aspirated from the cell pellet then it was flash frozen in liquid nitrogen vapors, prior to storage at -80°C. Metabolomics analyses were performed at the University of Colorado School of Medicine Metabolomics Core. Cell pellets were treated with ice-cold extraction solution (methanol:acetonitrile:water (5:3:2 v/v)) at $2\times10^6$ cells per mL. After vortexing for 30 min at 4°C, samples were centrifuged at 12,000 g for 10 min at 4°C and supernatants isolated for metabolomics analyses. 10 μL of sample extract was injected onto a

Kinetex C18 1.7 µm, 100 × 2.1 mm (Phenomenex) reversed phase column (Positive ion mode—phase A: water, 0.1% formic acid; B: acetonitrile, 0.1% formic acid; Negative ion mode—phase A: 1 mM NH4OAc 95:5 water: acetonitrile; phase B: 1 mM $NH_4OAc$ 95:5 acetonitrile: water) via an ultra-high pressurechromatographic system (UHPLC—Vanquish, Thermo Fisher). The UHPLC was coupled with a Q Exactive (Thermo Fisher) mass spectrometer run in both polarity modes at 70,000 resolution (at 200 m/z) and metabolites were separated through a 5 min gradient with the phases described above. To quantify metabolite peaks, the raw files (.raw) were converted to mzXML using RawConverter. Metabolites were then assigned using the KEGG database within Maven software.

**Metabolic Flux**

To measure arginine flux, 500,000 primary AML cells or $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) were incubated with 100 µM L-arginine-$^{13}C_6$,$^{14}N_4$ hydrochloride (Sigma-Aldrich) for 6 or 24 hours. Cell pellet preparation, metabolite extraction, and data acquisition were performed exactly as detailed above. Isotopologue peak areas were extracted in Maven and visualized using xyz.

**Proteomics**

Molm13 cells were treated with DENSpm (10 µM) or vehicle for 24 hours. Cells were processed using S-Trap columns as per manufacturer's protocol (Protifi). Briefly, cells were lysed with sodium dodecyl sulfate (SDS( (5%) in tetraethylammonium bromide (TEAB) (50mM, pH 8.5). Lysates were cleared, reduced/alkylated (5mM dithiothreitol (DTT), 10mM iodoacetamide) and digested on column for 2 hours at 47°C with Trypsin/Lys-C (1µg; Promega, Madison, WI) in TEAB (50mM). Following elution, samples were de-salted and reconstituted in 0.1% HCOOH. Liquid chromatography was performed by loading samples on a pre-column (C18 Acclaim PepMap 100, 75µm ID x 2cm, 3µm, 100Å) prior to chromatographic separation with analytical column (C18 Acclaim PepMap RSLC, 75µm ID x 50cm, 3µm, 100Å) over a reversed-phase gradient (120-minute gradient, 5-30% CH3CN in 0.1% HCOOH) at 225nL/min on an EASY-nLC1200 pump. LC was done in-line with a Q-Exactive HF (Thermo Scientific) mass spectrometer operated in positive electrospray ionization (ESI) mode. Parent ion MS1 ion scans were performed at 60,000 fwhm followed by fragment ion MS/MS scans (HCD, 15,000 fwhm) of the 20 most intense parent ions (minimum ion count of 1000 for activation). Dynamic exclusion (within 10 ppm) was set at 5 seconds.

Raw spectrum files (.raw) were converted to .mzML using Proteowizard (v3.0.19311) and sequence database searches were performed using X!Tandem (v2013.06.15.1) and Comet (v2014.02.rev.2) against human RefSeqV104 database (36,113 entries). Search parameters specified a parent ion mass tolerance of 15ppm and an MS/MS fragment ion tolerance of 0.4Da, with up to two missed cleavages. Carbamidomethylation (C) was set as a fixed modification and deamidation (NQ), oxidation (M), acetylation (protein N-term) were set as variable modifications. Search results were validated using the trans-proteomic pipeline (TPP v4.7) platform. Proteins were identified with an iProphet cut-off of 0.9 and at least two unique peptides. Additionally, mzML data files were analyzed using Fragpipe (v20.0) to generate MS1-level quantification of peptide intensities. Raw mass spectrometry files have been submitted to the MassIVE repository (massive.ucsd.edu) under accession MSV000093543.

## Supplemental Figures

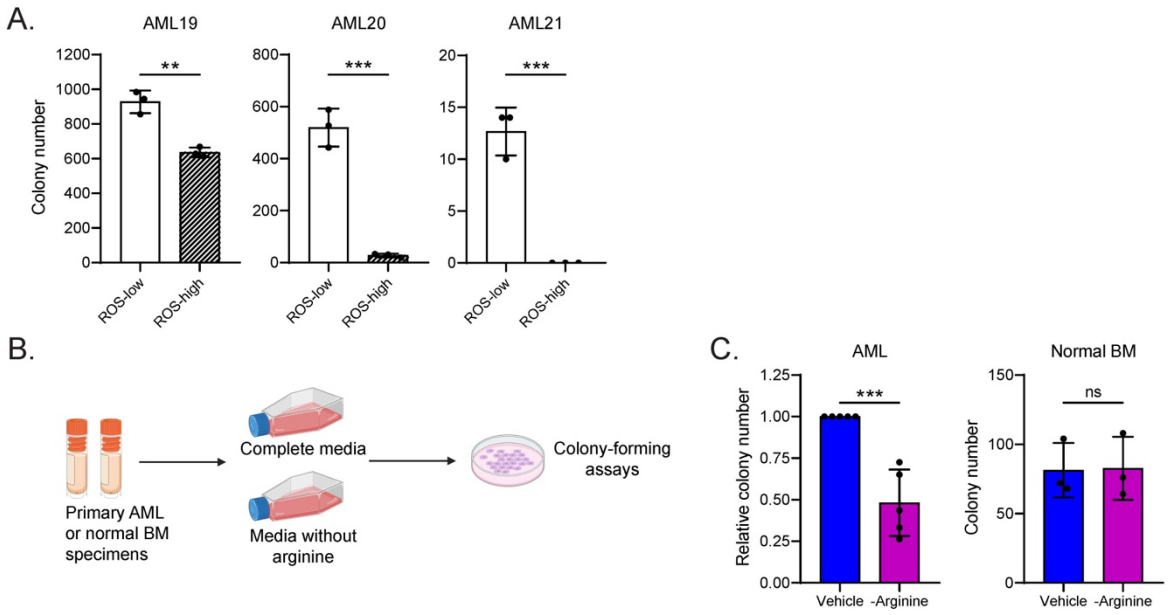


**Fig. S1**. **Arginine depletion targets LSCs. (A)** Colony formation measured in three primary AML specimens (AML19-21) sorted into ROS-low and ROS-high expressing cells. Statistical significance was determined using an unpaired t-test. **(B)** Five primary AML specimens (AML22-26) and one normal BM specimen were cultured in arginine-depleted media for 24 hours. Cells were then seeded in methocult media to assess colony forming ability. Colony numbers were determined after 1-3 weeks. **(C)** Colony forming ability of five AML samples and one CD34-enriched BM sample cultured for 24 hours in a media depleted or not for arginine. Statistical significance was determined using an unpaired t-test. All error bars represent standard deviation. ***P*<0.01, ****P*<0.005; ns: not significant.

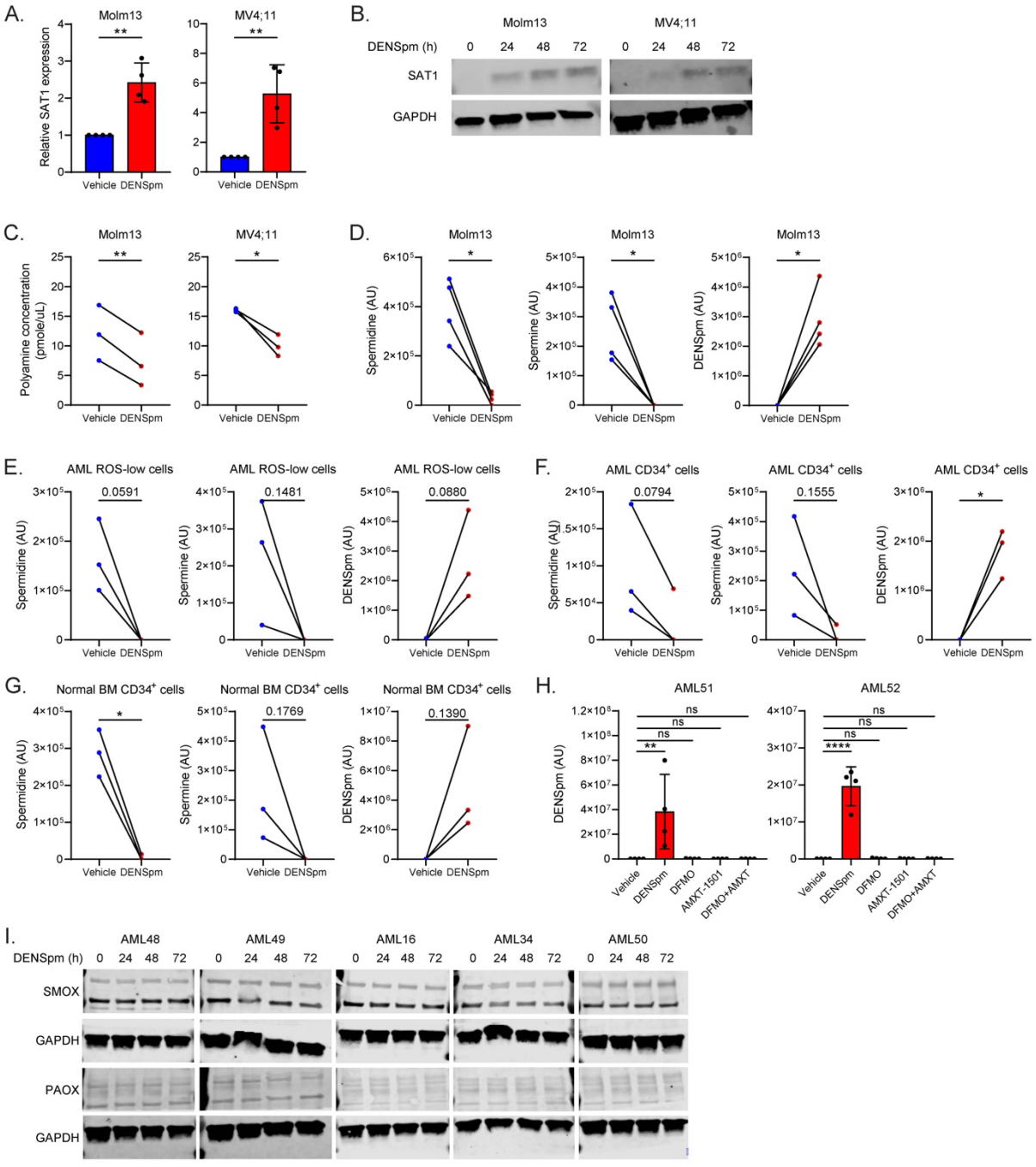


**Fig. S2**. **DENSpm treatment reduces polyamine abundance. (A)** Expression of *SAT1* mRNA determined by qPCR on Molm13 and MV4;11 cells incubated for 24 hours with 10 μM DENSpm or vehicle. Statistical significance was determined using an unpaired t-test. **(B)** Expression of SAT1 determined by Western blot on Molm13 and MV4;11 cells incubated for 0, 24, 48 or 72 hours with 10 μM DENSpm. **(C)** Polyamine concentration in Molm13 and MV4;11 cells incubated for 24 hours with 10 μM DENSpm or vehicle. Statistical significance was determined using a paired t-test. **(D)** Spermidine, spermine, and DENSpm abundance determined by mass spectrometry in Molm13 cells after 24 hours incubation with 10 μM DENSpm. Statistical

significance was determined using a paired t-test. **(E)** Spermidine, spermine, and DENSpm abundance determined by mass spectrometry ROS-low enriched LSCs sorted from three primary AML specimens (AML19-21) after 24 hours incubation with 10 µM DENSpm. Statistical significance was determined using a paired t-test. **(F)** Spermidine, spermine, and DENSpm abundance determined by mass spectrometry $CD34^+$ enriched LSCs sorted from three primary AML specimens (AML19-21) after 24 hours incubation with 10 µM DENSpm. Statistical significance was determined using a paired t-test. **(G)** Spermidine, spermine, and DENSpm abundance determined by mass spectrometry $CD34^+$ enriched HSPCs sorted from three NBM specimens after 24 hours incubation with 10 µM DENSpm. Statistical significance was determined using a paired t-test. **(H)** DENSpm abundance determine by mass spectrometry in AML51 and 52. Statistical significance was determined using two-way ANOVA. **(I)** Expression of SMOX and PAOX determined by Western blot in five primary AML specimens (AML16, 34, 48-50) incubated for 0, 24, 48 or 72 hours with 10 µM DENSpm. * $P<0.05$, ** $P<0.01$, *** $P<0.005$; ns: not significant.

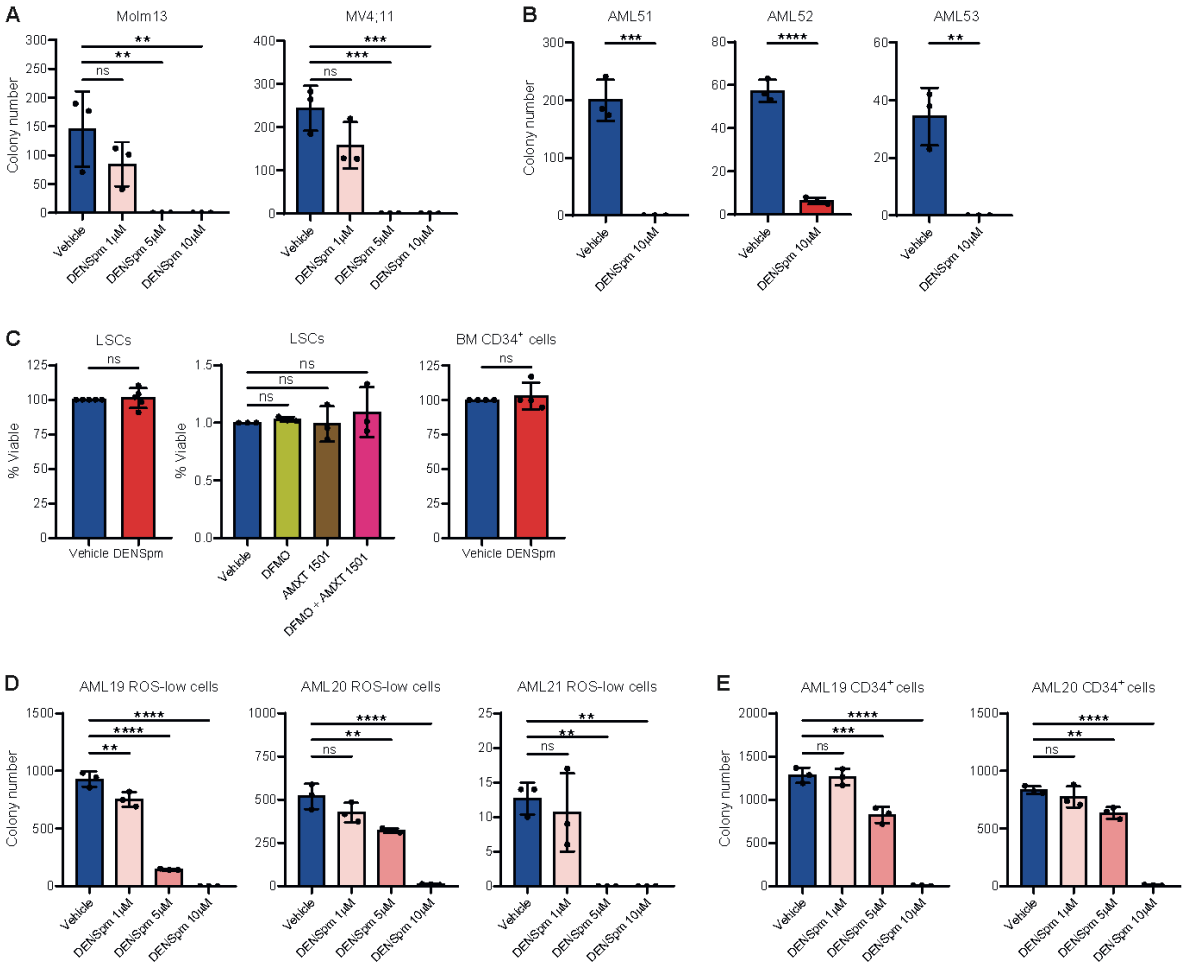


**Fig. S3**. **Polyamine depletion targets AML cells**. **(A)** Colony forming ability of Molm13 and MV4;11 cells after 24 hours incubation with indicated doses of DENSpm or vehicle. Colony numbers were determined after 14 days. Statistical significance was determined using two-way ANOVA. **(B)** Colony forming ability of primary AML specimens (AML51-53) after 24 hours incubation with indicated doses of DENSpm or vehicle. Colony numbers were determined after 14 days. Statistical significance was determined using an unpaired t-test. **(C)** Viability of ROS-low LSCs enriched from five primary AML specimens (AML45, 49, 54-56) and HSPCs enriched using four CD34-enriched bone marrow samples. Cells were incubated for 24 hours with 10 μM DENSpm or vehicle. Viability of three primary AML specimens (AML51-53) upon incubated for 24 hours with 2.5mM DFMO and 400nM AMXT 1501 or vehicle. Significance was determined using an unpaired t-test. **(D)** Colony forming ability of ROS-low enriched LSCs from three primary AML specimens (AML19-21) after 24 hours incubation with indicated doses of DENSpm or vehicle. Colony numbers were determined after 14 days. Statistical significance was determined using two-way ANOVA. **(E)** Colony forming ability of CD34-enriched LSCs from two primary AML specimens (AML19-20) after 24 hours incubation with indicated doses of DENSpm or vehicle. Colony numbers were determined after 14 days. Statistical significance was determined using two-way ANOVA. All error bars represent standard deviation. ***P*<0.01, ****P*<0.005, *****P*<0.001; ns: not significant.

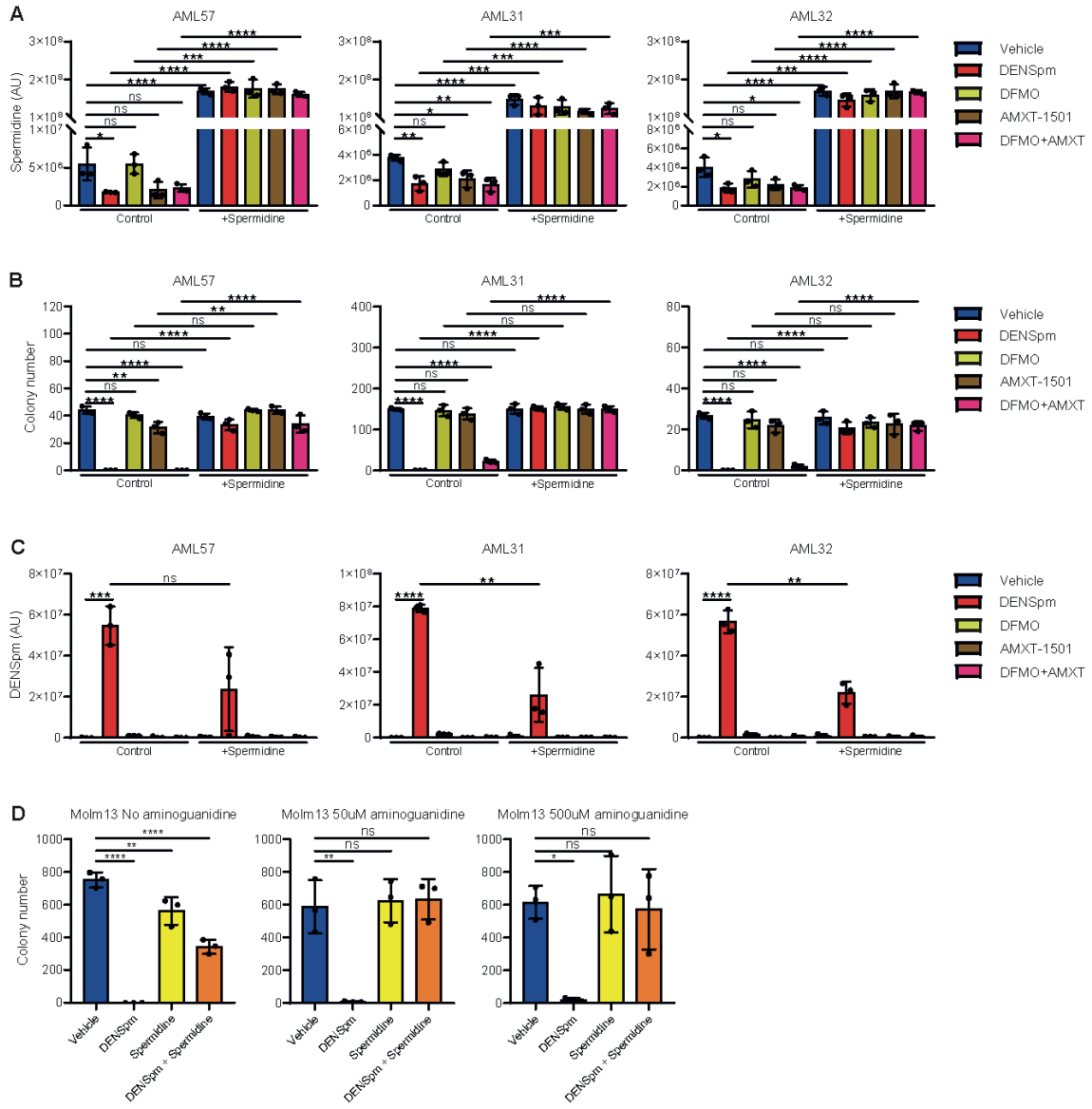


**Fig. S4**. **Exogenous spermidine rescues polyamine depletion**. **(A)** Spermidine abundance in three primary AML specimens (AML31-32, 57) upon a 24-hour treatment with DENSpm, DFMO, AMXT 1501, or DFMO + AMXT1501 with or without the addition of 10 μM spermidine. Statistical significance was determined using two-way ANOVA. **(B)** Colony forming ability of three primary AML specimens (AML31-32, 57) upon a 24-hour treatment with DENSpm, DFMO, AMXT 1501, or DFMO + AMXT1501 with or without the addition of 10 μM spermidine. Statistical significance was determined using two-way ANOVA. **(C)** DENSpm abundance in three primary AML specimens (AML31-32, 57) upon a 24-hour treatment with DENSpm, DFMO, AMXT 1501, or DFMO + AMXT1501 with or without the addition of 10 μM spermidine. Statistical significance was determined using two-way ANOVA. **(D)** Colony forming ability of Molm13 cells upon treatment with DENSpm, spermidine, or DENSpm + spermidine with and without co-treatment with aminoguanidine at indicated concentrations. Statistical significance was determined using two-way ANOVA. All error bars represent standard deviation. **P*<0.05, ***P*<0.01, ****P*<0.005, *****P*<0.001; ns: not significant.

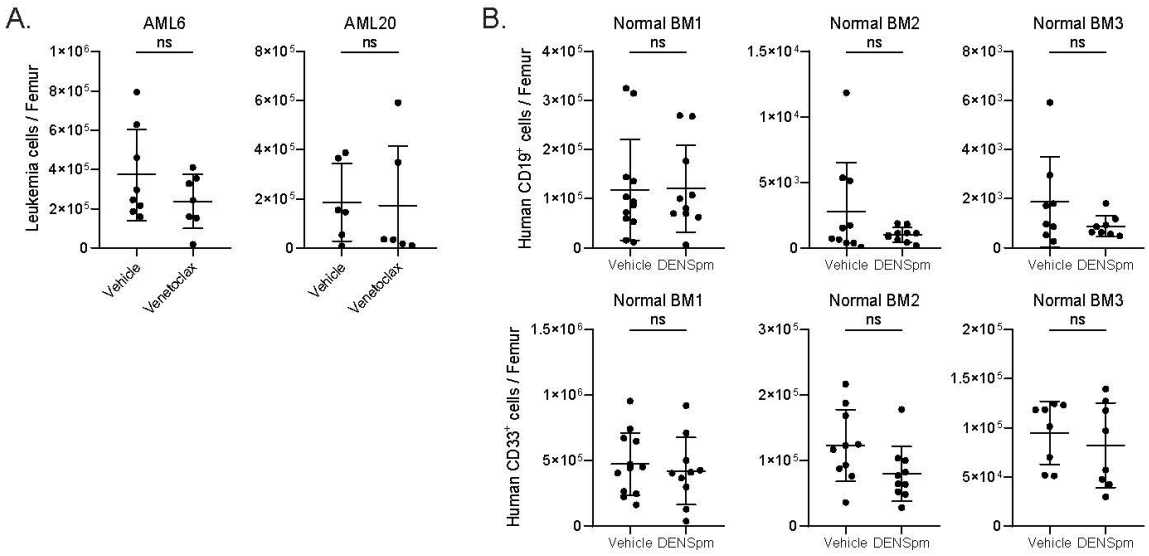


**Fig. S5**. **Polyamine depletion does not alter HSPCs. (A)** Engraftment of two primary AML specimens (AML6, 20) in NSG-SGM3 mice following ex vivo treatment with venetoclax or vehicle. Each point represents a single mouse. Statistical significance was determined using an unpaired t-test (AML6) or an unpaired Mann-Whitney test (AML20). **(B)** Numbers of $CD19^+$ and $CD33^+$ cells in NSG-SGM3 mice following ex vivo treatment with DENSpm or vehicle for three normal BM specimens. Each point represents a single mouse. Statistical significance was determined using an unpaired t-test. All error bars represent standard deviation. ns: not significant.

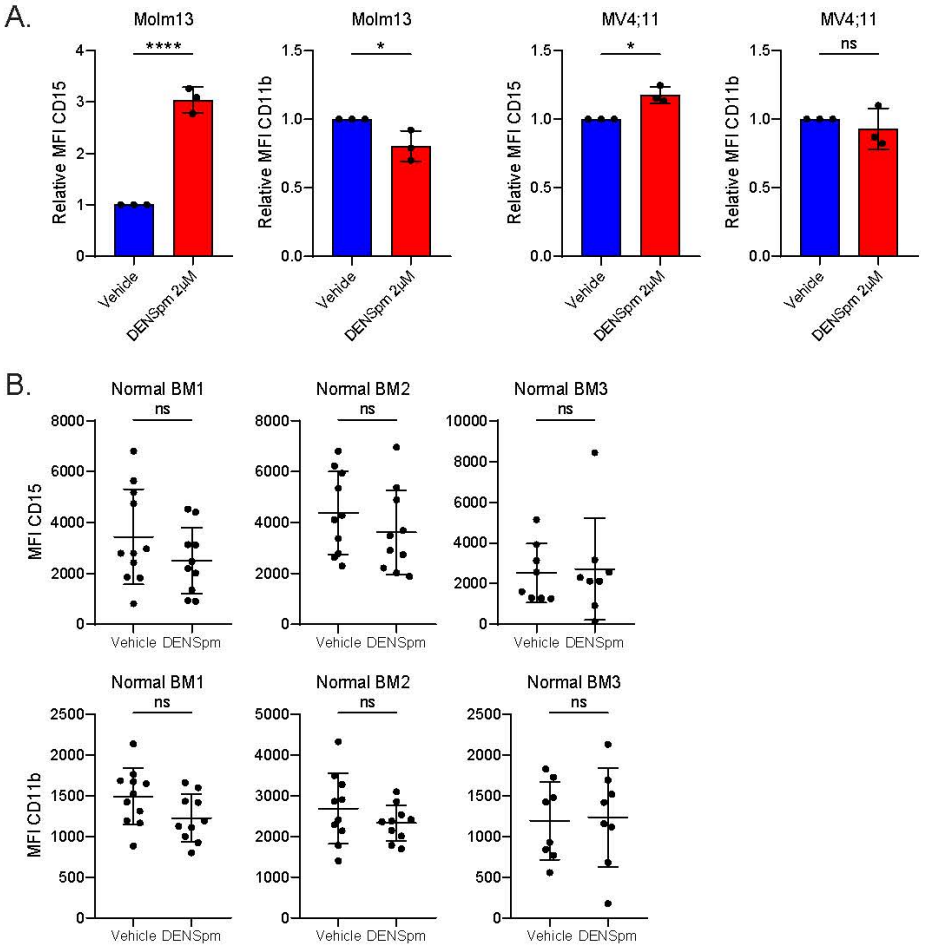


**Fig. S6**. **Polyamine depletion induces differentiation in AML cells but not NBM**. **(A)** Molm13 and MV4;11 cells were incubated for 24 hours with 2 μM DENSpm or vehicle prior colony forming assays. After 14 days, mean fluorescence intensity (MFI) for CD15 and CD11b in colonies was determined by flow cytometry. Statistical significance was determined using an unpaired t-test. **(B)** MFI for CD15 and CD11b on $CD45^+$ human cells in NSG-SGM3 recipient mice following ex vivo treatment with DENSpm or vehicle for three normal BM specimens. Each point represents a single mouse. Statistical significance was determined using an unpaired t-test. All error bars represent standard deviation. *$P$<0.05, ****$P$<0.001; ns: not significant.

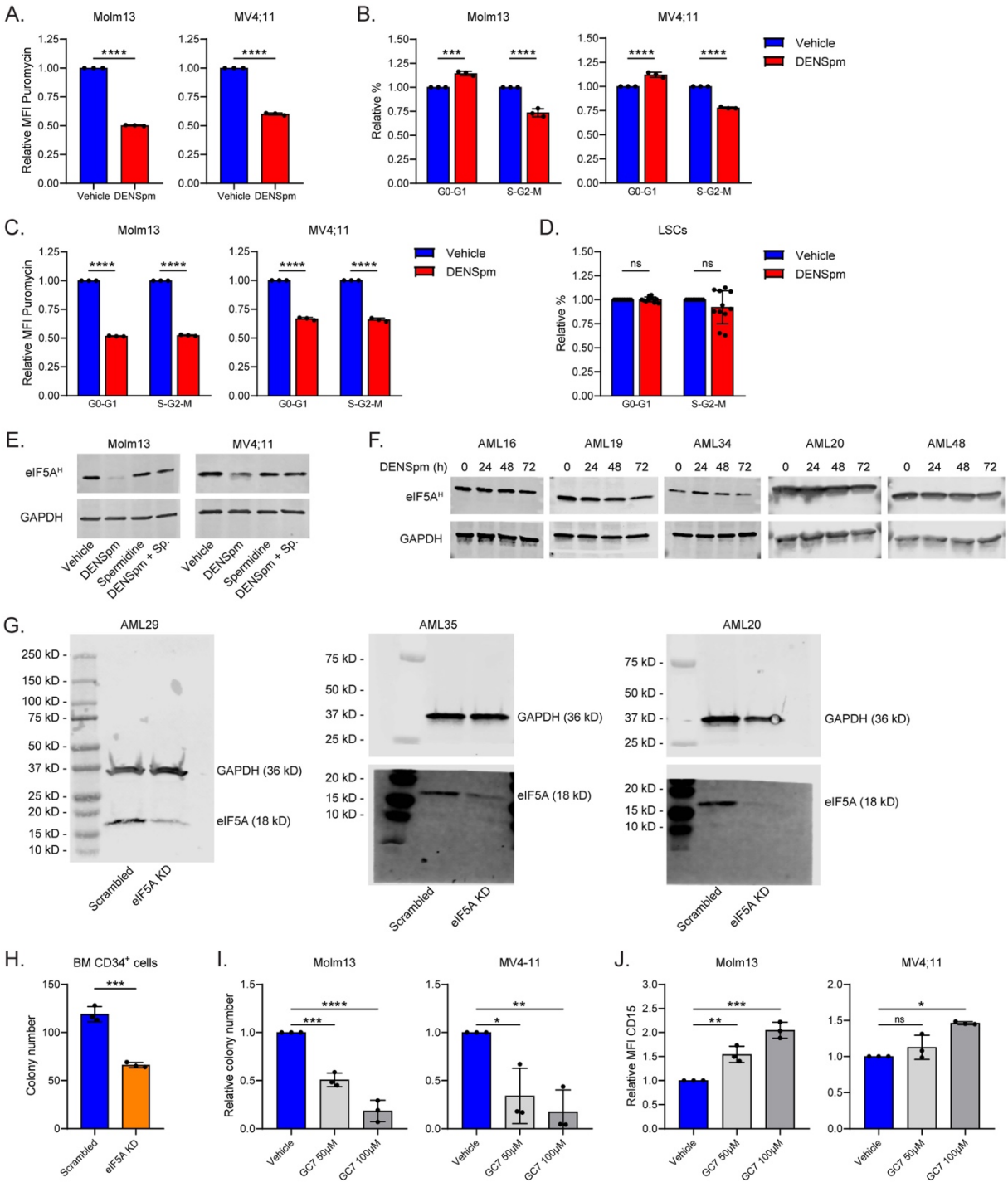


**Fig. S7**. **Protein synthesis reduction upon polyamine depletion is not cell cycle dependent**. **(A)** MFI for puromycin determined by flow cytometry on Molm13 and MV4;11 cells incubated for 24 hours with 10 μM DENSpm or vehicle. Statistical significance was determined using an unpaired t-test. **(B)** Proportions of Molm13 and MV4;11 cells in G0/G1 and S/G2/M phases of the cell cycle after 24 hours incubation with 10 μM DENSpm or vehicle. Statistical significance was determined using an unpaired t-test. **(C)** MFI for puromycin determined by flow cytometry on Molm13 and MV4;11 cells incubated for 24 hours with 10 μM DENSpm or vehicle depending on

their cell cycle status (G0/G1 or S/G2/M). Statistical significance was determined using an unpaired t-test. **(D)** Proportions of $CD34^{+}$ LSCs enriched from eleven primary AML specimens (AML6, 20-21, 34-35, 45, 47, 49, 55-56, 60) in G0/G1 and S/G2/M phases of the cell cycle after 24 hours incubation with 10 µM DENSpm or vehicle. Statistical significance was determined using an unpaired t-test. **(E)** Expression of $eIF5A^{H}$ determined by Western blot on Molm13 and MV4;11 cells incubated for 24 hours with vehicle, 10 µM DENSpm, 10 µM spermidine or their combination. **(F)** Expression of $eIF5A^{H}$ determined by Western blot in primary AML specimens incubated with DENSpm for 24, 48, and 72 hours. **(G)** Expression of eIF5A determined by Western blot in primary AML specimens (AML20, 29, 35) upon transfection with non-targeting scrambled control or eIF5A targeting siRNAs. **(H)** Colony forming capacity of NBM upon transfection with non-targeting scrambled control or eIF5A targeting siRNAs. Statistical significance was determined using an unpaired t-test. **(I)** Colony forming capacity of Molm13 and MV4;11 cells upon treatment with increasing doses of GC7. Statistical significance was determined using two-way ANOVA (Molm13) or an unpaired Mann-Whitney test (MV4;11). **(J)** Molm13 and MV4;11 cells were incubated for 24 hours with increasing doses of GC7 or vehicle prior colony forming assays. After 14 days, mean fluorescence intensity (MFI) for CD15 and CD11b in colonies was determined by flow cytometry. Statistical significance was determined using two-way ANOVA. All error bars represent standard deviation. *$P$<0.05, **$P$<0.01, ***$P$<0.005, ****$P$<0.001; ns: not significant.

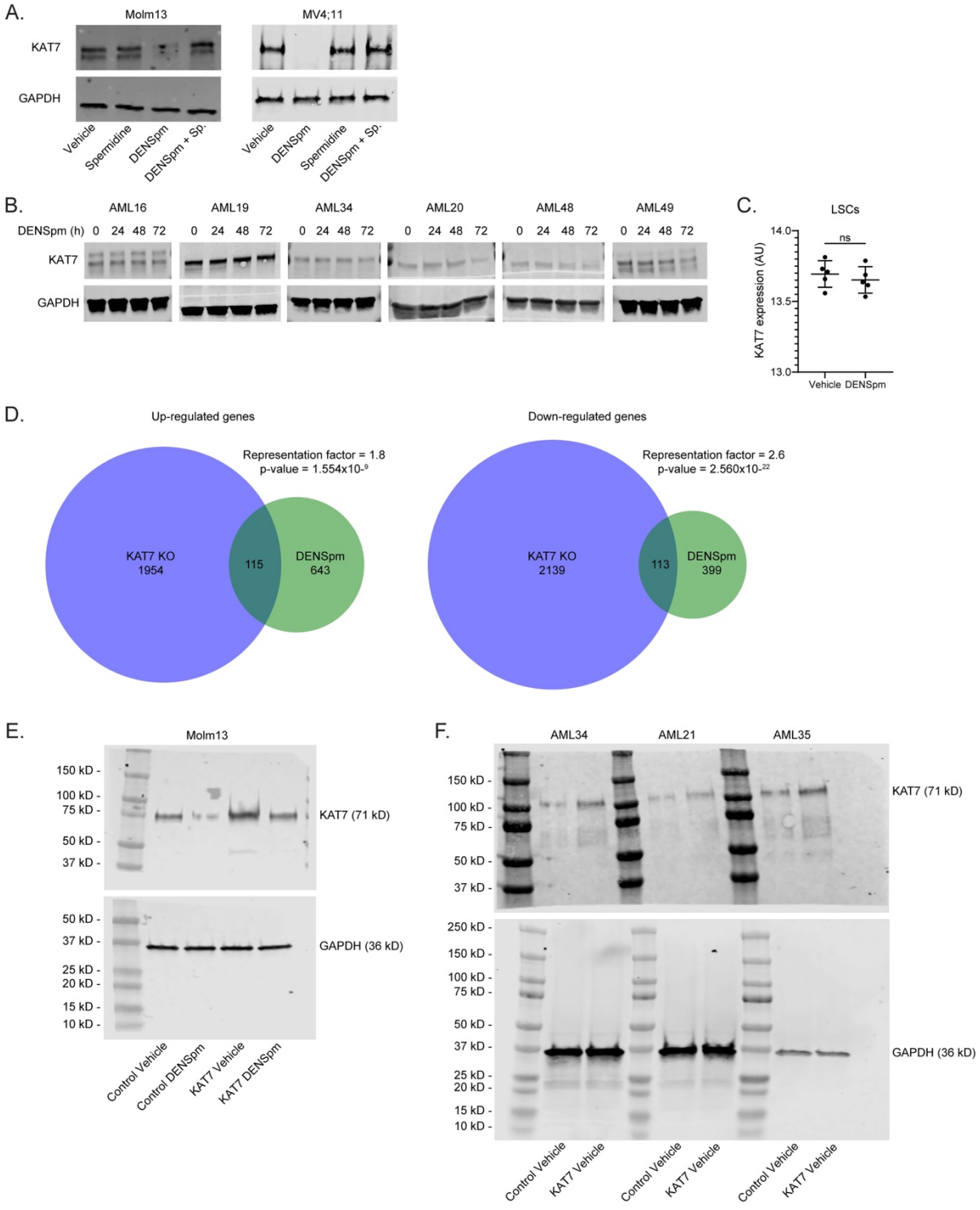


**Fig. S8**. **KAT7 expression upon polyamine depletion. (A)** Expression of KAT7 determined by Western blot on Molm13 and MV4;11 cells incubated for 24 hours with vehicle, 10 μM DENSpm, 10 μM spermidine or their combination. **(B)** Expression of KAT7 determined by Western blot in primary AML specimens incubated for 24, 48, and 72 hours with 10 μM DENSpm. **(C)** Expression

of KAT7 mRNA determined by RNA-seq in ROS-low LSCs enriched from five primary AML specimens (AML28-29, 45-47) incubated for 24 hours with 10 µM DENSpm or vehicle. Statistical significance was determined using an unpaired t-test. AU: arbitrary unit. **(D)** Venn diagram of the differentially expressed genes within DENSpm-treated LSCs and KAT7 knockout AML cells. The overlap for both significantly up- and down- regulated genes is shown. **(E)** Expression of KAT7 determined by Western blot in Molm13 cells incubated for 48 hours with 10 µM DENSpm or vehicle and transfected or not with KAT7 recombinant. **(F)** Expression of KAT7 determined by Western blot in three primary AML specimens (AML21, 34-35) transfected or not with KAT7 recombinant. All error bars represent standard deviation. ns: not significant.

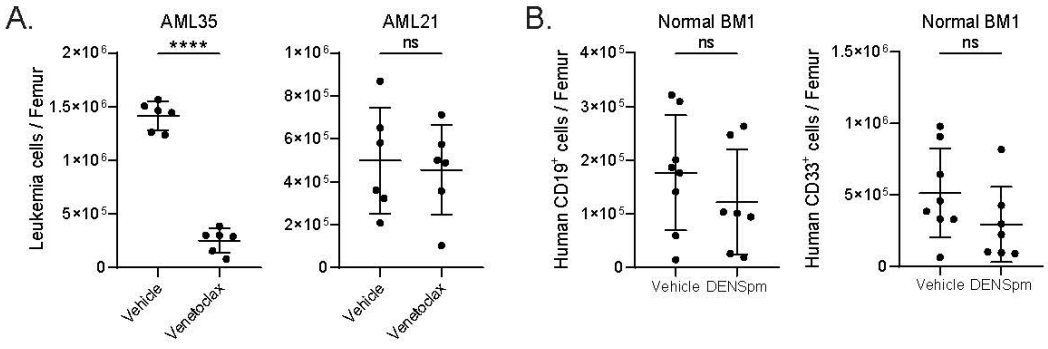


**Fig. S9**. **In vivo polyamine depletion does not alter HSPC differentiation. (A)** Leukemic burden of two primary AML specimens (AML21, 35) in NSG-SGM3 mice following in vivo treatment with venetoclax or vehicle. Each point represents a single mouse. Statistical significance was determined using an unpaired t-test. **(B)** Numbers of $CD19^+$ and $CD33^+$ cells in NSG-SGM3 mice following in vivo treatment with DENSpm or vehicle for one normal BM specimen. Each point represents a single mouse. Statistical significance was determined using an unpaired t-test. All error bars represent standard deviation. ****$P$<0.001; ns: not significant.

**Supplementary Data Files**

Data file S1. Patient sample characteristics

Data file S2. Individual-level data for experiments where n<20

Data file S3. Uncropped western blots