Supplemental Methods

Plasmids and constructs

Ectopic retroviral vectors containing MLL-AF9-IRES-GFP, MSCV-Cre, and Cre-IRES-MIT were used as previously reported[1]. For shRNA knockdown experiments, the pLKO shRNA library and individual constructs were obtained from the RNAi Consortium at the Broad Institute. A shRNA construct targeting luciferase was used as a control. The pLKO shRNA constructs targeting C/EBPα are listed below. Puromycin was added to culture 48 hours after transduction of target cells, and cells were harvested for mRNA expression analysis after another 48 hours under selection.

Gene	TRCN #	Target Sequence
Luciferase	TRCN0000072256	ACGCTGAGTACTTCGAAATGT
mC/EBPα	TRCN0000321048	TGCGAGCACGAGACGTCTATA
mC/EBPα	TRCN0000273001	AGCCGAGATAAAGCCAAACAA
mC/EBPα	TRCN0000273017	GGACAAGAACAGCAACGAGTA

Mice and leukemia generation

Animals were maintained at the Animal Research Facility at Memorial Sloan Kettering Cancer Center. All animal experiments were approved by the Internal Animal Care and Use Committee. C57BL/6J mice were purchased from Jackson Laboratory and used as donor mice. $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (LSK) cells were FACS-purified, retrovirally transduced with MLL-AF9, and transplanted into syngeneic sublethally irradiated (650 cGy) recipient mice or cultured until transformation for assays at the pre-leukemic stage. Secondary transplantation experiments were performed by FACS-sorting GFP-positive bone marrow-derived leukemia cells from primary transplanted mice and injected into syngeneic sublethally irradiated (650 cGy) recipient mice. $C/EBP\alpha^{fl/fl}$ and $PU.1\ URE^{fl/fl}$ mice were generated as previously described[2,3] and bone marrow cells from these mice were transduced with MLL-AF9 to generate primary leukemias as described above. For PDX experiments, NOD.Cg-*Prkdc*^{scid}Il2rg^{tm1Wjl}/SzJ (NSG) mice were purchased from Jackson

Laboratory, sublethally irradiated (250 cGy), and transplanted with hCD3-depleted bone marrow (Miltenyi Technologies) or peripheral blood mononuclear cells derived from either human patients or from previously transplanted PDX mice, as previously described[4]. Flow cytometric analysis of mononuclear cells from PDX animals was performed using the following antibodies: anti-CD11b (Biolegend, clone M1/70), anti-CD45 (Biolegend, clone HI30), anti-CD33 (Biolegend, clone HIM 3-4), and anti-CD86 (eBioscience, clone IT2.2).

Cell culture

293T cells transiently transfected for virus production were cultured in DMEM supplemented with heat inactivated 10% fetal bovine serum (FBS) and 50 U/mL penicillin/streptomycin (all ThermoFisher Scientific, Waltham, MA). *In vitro* transformed MLL-AF9 $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ bone marrow cells were cultured in IMDM supplemented with heat inactivated 10% fetal bovine serum, 50 U/mL penicillin/streptomycin and murine interleukin-3 (10 ng/mL), interleukin-6 (10 ng/mL) and stem cell factor (SCF) (20 ng/mL, all PeproTech). MLL-AF9 leukemias cells generated from C/EBPα-deficient cells were maintained in IMDM supplemented with 20% FBS, with interleukin-3, interleukin-6, and murine SCF. For colony formation assays, methylcellulose M3434 (Stemcell Technologies) was used.

Cell cycle analysis

Cell cycle analysis was performed by BrdU staining of cells treated *in vitro* for 48 hours with GSK-LSD1. BrdU Flow Kit (BD Biosciences) was used. Briefly, after 48 hours of exposure to GSK-LSD1, cells were exposed to 10 µM BrdU per manufacturer's instructions for 20 min. After this, cells were harvested, permeabilized and stained with anti-BrdU antibody labelled with APC, while leukemic cells were GFP^{+} (harbouring pMSCV-MLL-AF9-IRES-GFP plasmid). For DNA staining SYTOX™ Blue Dead Cell Stain was used. The SYTOX Blue signal was acquired in a linear mode.

***In vitro* and *in vivo* treatment experiments**

MLL-AF9 leukemia cells were treated *in vitro* by culturing cells in IMDM supplemented with 15% FBS, IL-3, IL-6, and mSCF with the addition of vehicle alone or GSK-LSD1 at a concentration of 0.5

μM for 48 hours. Similarly, leukemia cells were treated with the DOT1L inhibitor EPZ4777 for 6 days at a concentration of 1 μM. Colony forming assays were performed according to manufacturer's instructions. Briefly, 500 cells/dish were plated in MC3434 methylcellulose and numbers of colonies were scored after 6 days of incubation. For each arm 3 independent dishes were scored, and colony assays were performed at least in duplicate. GSK-LSD1 was added to MC3434 semisolid medium at day 0 at a concentration of 0.5 μM and colonies were scored six days later. For *in vivo* treatment experiments, GSK-LSD1 was administered via intraperitoneal injections at a dose of 0.5 mg/kg daily. Treatment was initiated only after peripheral blood engraftment of MLL-AF9 leukemia cells was confirmed at a minimum chimerism of 0.1-1% GFP-positive cells for syngeneic murine MLL-AF9 leukemia cells or 12.3% ± 2.7 hCD45-positive cells for xenotransplantation experiments. Mice were treated for 3 days (Figure 1B), 2 weeks (Figure 1C) or 6 weeks (Figure 1G). Cytological staining was performed on cytospin preparations of suspension cells from in vitro culture (Figures 1E, 5E, 6D+F) or from peripheral blood of mice (Figure 1J) using the Deep Quick Stain kit (Patterson Veterinary #J0322).

RNA isolation and qRT-PCR expression analysis

RNA utilized for real-time qRT-PCR was extracted with Trizol according to the manufacturer's instructions (Invitrogen). Purified RNA was reverse-transcribed using Superscript III (Invitrogen) with oligo dT-primed first-strand synthesis according to the manufacturer's protocol. Quantitative real-time PCR (qRT-PCR) was performed on the 7000 ABI Detection System using commercially available Taqman probes according to the manufacturer's instructions (Applied Biosystems). CT values were normalized using the delta CT method to the housekeeping gene beta-actin.

Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq)

LSD1 chromatin immunoprecipitation was performed as previously described[5] using a rabbit polyclonal anti-KDM1/LSD1 antibody (ab17721, Abcam). $1x10^8$ primary MLL-AF9 leukemic cells were double crosslinked with EGS/formaldehyde for ChIP-seq. Transcription factor ChIP-seq

experiments were performed as previously described[6], using a rabbit polyclonal anti-C/EBPα antibody (sc-61, Santa Cruz) and a rabbit polyclonal anti-PU.1 antibody (sc-352, Santa Cruz).

ATAC-seq

ATAC-seq was performed as previously described[7]. For each sample, cell nuclei were prepared from $5x10^4$ cells and incubated with 2.5 µL transposase (Illumina) in a 50 µL reaction for 30 minutes at 37°C. Following purification of transposase-fragmented DNA, the library was amplified by PCR and subjected to high-throughput sequencing on the HiSeq 2000 platform (Illumina). Cells were treated with either vehicle, GSK-LSD1, or IMG-7289 for 48 hours prior to generating the nuclear preparations.

Data analysis and statistical methods

Reads from ChIP-seq and ATAC-seq libraries were trimmed for quality and Illumina adapter sequences using 'trim_galore' and aligned to mouse genome assembly mm9 with bowtie2 using the default parameters. Aligned reads with the same start site and orientation were removed using the Picard tool MarkDuplicates (http://broadinstitute.github.io/picard/). Density profiles were created by extending each read to the average library fragment size for ChIP and 0 bp for ATAC, then computing density using the BEDTools suite (http://bedtools.readthedocs.io). Enriched regions were discovered using MACS (v1.4) and scored against matched input libraries (fold change > 2 and p-value < 1e-5). Dynamic regions between two conditions were scored using MACS with the second ChIP library replacing input, and only sites with an absolute fold change greater than two were scored as a gain/loss. Genomic 'blacklisted' regions (http://mitra.stanford.edu/kundaje/akundaje/release/blacklists/mm9-mouse/mm9-blacklist.bed.gz) were filtered and peaks within 500 bp were merged. All genome browser tracks and read density tables were normalized to a sequencing depth of ten million mapped reads. Motif signatures were obtained using the 'de novo' approach in Homer v4.5 (http://homer.ucsd.edu/homer/index.html) and matching to the default 'known TF' database. Homer was also used for the genome annotation of peaks (http://homer.salk.edu/homer/ngs/annotation.html). To map the GSK-LSD1-induced dynamic

ATAC sites to active enhancers in normal hematopoietic development, enriched regions were intersected with previously annotated H3K27ac peaks (http://compbio.cs.huji.ac.il/blood-chromatin/Data.html)[34]. K-means clustering was applied to the resulting H3K27ac matrix, with the optimal k determined from silhouette analysis. All measurements were taken from distinct samples. *In vitro* and *in vivo* experiments were replicated at least 3 times. Analysis of the data was performed using a non-paired two-sided Student t-test. Analysis of the survival curves was performed using log-rank (Mantel-Cox) test. For *in vitro* and *in vivo* analyses no adjustment for multiple comparisons was performed. In all figures the mean with min. to max. value and standard deviation are shown.

Accession codes

The ATAC-seq and ChIP-seq datas generated and analyzed during the current study have been deposited into the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) with accession code GSE100759. .

Human research participants

Human AML samples were obtained under Institutional Review Board–approved protocols from patients treated at Memorial Sloan Kettering Cancer Center following written informed consent.

References

1. Bernt KM, Zhu N, Sinha AU, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer Cell*. 2011;20(1):66-78.
2. Ye M, Zhang H, Yang H, et al. Hematopoietic Differentiation Is Required for Initiation of Acute Myeloid Leukemia. *Cell Stem Cell*. 2015;17(5):611-623.
3. Will B, Vogler TO, Narayanagari S, et al. Minimal PU.1 reduction induces a preleukemic state and promotes development of acute myeloid leukemia. *Nat Med*. 2015;21(10):1172-1181.
4. Wang K, Sanchez-Martin M, Wang X, et al. Patient-derived xenotransplants can recapitulate the genetic driver landscape of acute leukemias. *Leukemia*. 2017;31(1):151-158.
5. Kerenyi MA, Shao Z, Hsu YJ, et al. Histone demethylase Lsd1 represses hematopoietic stem and progenitor cell signatures during blood cell maturation. *Elife*. 2013;2:e00633.
6. Zhou J, Wu J, Li B, et al. PU.1 is essential for MLL leukemia partially via crosstalk with the MEIS/HOX pathway. *Leukemia*. 2014;28(7):1436-1448.
7. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Curr Protoc Mol Biol*. 2015;109:21 29 21-29.

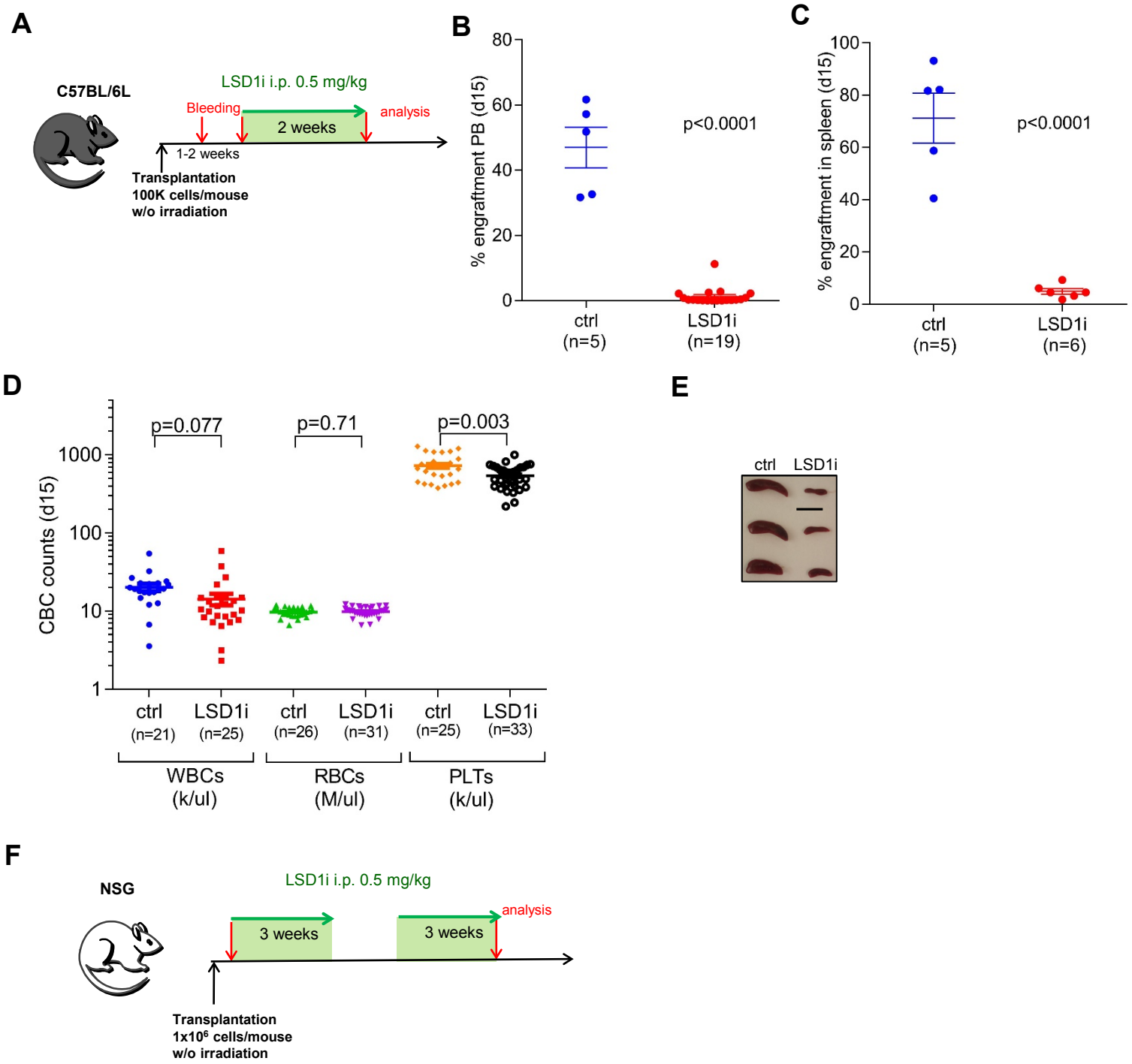
Patient Number	Origin of cells	Cytogenetics	Additional mutations	Immunophenotype
017	PB, AML, first diagnosis; therapy related	t(9;11) MLL-AF9	None, neg. for AML1-ETO-, CEBPa-, FLT3-, NPM1-, KIT-)	75% of blasts: CD13+, CD33+, CD15+, CD64+, CD34+, CD117+, HLA-DR+, CD22+
011	PB, relapsed AML Induction with 7+3, HiDAC, allo transplant, Aza, and MEC	t(11;19) MLL-ENL	t(11q23) in 20% of cells	5% PB blast are positive for Cd11c, 13, 33, 34, 117 and HLA-DR
021	PB, relapsed AML, Induction with 7+3, HiDAC, post allo transplant, MEC, AC220 and HiDAC	FLT3-ITD+	7.5% extra copy of EVI1, der(5) t(3;5)	positive for CD13, 33, 11b, 64, 123, 34, 117, HLA-DR, 25, and 38. Aberrant co-expression of CD33/7. 94% blasts in PB
04	PB, first diagnosis AML	NF1-AK4 fusion	CEBPA D301_K302insKAKQRN, H18fs*138 IKZF1 S55fs*1 Further variants detected: 1. CEBPA exon 1 p.P189_S190delinsSHP (c.564_568delinsCTCGCACC) 2. CEBPA exon 1 p.N307_V308insKAKQRN (c.921_922insAAGGCCAAGCAGCGCAAC)	The blasts have abnormal expression of CD34 (bright to absent), CD33 (variable, dim), CD15 (partial), CD64 (partial), CD13 (variable, bright), CD7, HLADR (variable), MPO (uniform), with normal expression of CD45, CD71, CD117 and CD123, sCD3, cCD3, CD4, CD5, CD11b, CD14, CD15, CD16, CD19, CD56, CD64, cCD79a or surface light chains. CD117 blasts represent 87.6% of WBC. 93.6% blasts in PB. 30% BMA.

Supplementary Table 1. Characteristics of primary human leukemias. Summary table showing available clinical data for leukemia samples that were used for PDX-AML transplantation experiments (MEC = mitoxantrone, etoposide, cytarabine, PB = peripheral blood). MEC: Mitoxantrone, Etoposide, Cytarabine; PB: peripheral blood.

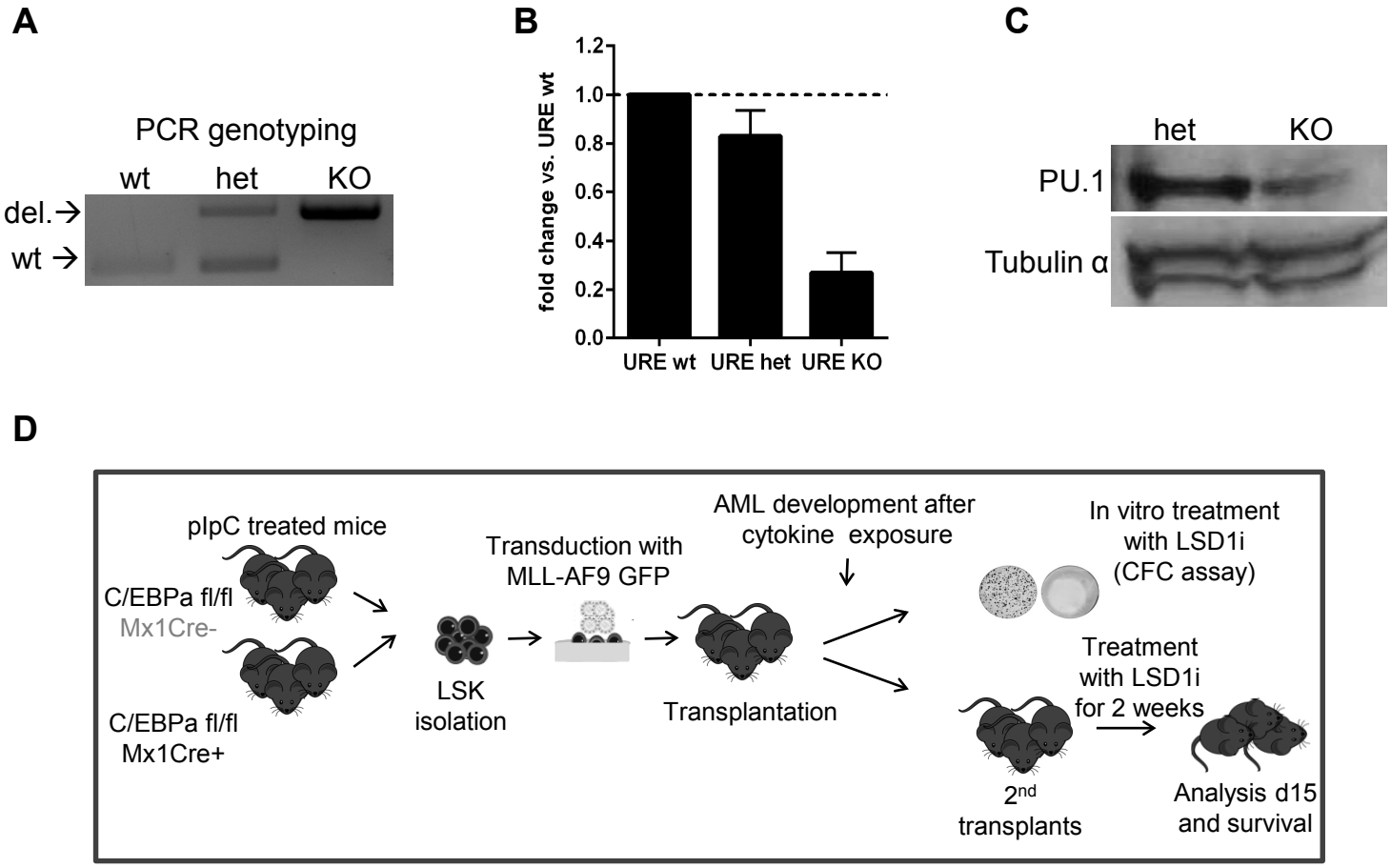
Patient Number	Cytogenetics	Experiment Number	Origin of cells injected	Cells injected for PDX	Time to engraftment (days)	LSD1i treatment duration	Response to LSD1: % positive in LSD1i vs control at end of treatment			
							hCD45	hCD33	hCD11b	hCD86
017	t(9;11) MLL-AF9	#1	patient's PBMCs	n.a.	99	not treated	n/a	n/a	n/a	n/a
		#2	primary tx mouse	$9.5x10^4$	71	3 weeks	25.5 vs. 26.7 (n.s.)	88.6 vs. 74 (n.s.)	35.6 vs. 52.5 (n.s.)	43.2 vs. 3.7 (0.038)
		#3	primary tx mouse	$7x10^5$	64	2 weeks	56.4 vs. 49.4 (n.s.)	57.7 vs. 49.5 (n.s.)	96 vs. 82 (<.000001)	77.7 vs. 40.4 (0.068)
		#4	primary tx mouse	$1x10^6$	21	6 weeks	25.4 vs. 67.7 (0.016)	85.9 vs. 97.2 (0.42)	76.7 vs. 39.5 (0.0033)	70.8 vs. 14.2 (<0.0001)
011	t(11;19) MLL-ENL	#1	patient's PBMCs	$1x10^6$	49	2 weeks	96 vs. 97 (n.s.)	99 vs. 97 (n.s.)	76.4 vs. 48 (n.a.)	98 vs. 96 (n.a.)
		#2	primary tx mouse	$1x10^6$	21	10 days	85.4 vs. 95.3 (n.a.)	23 vs. 36.2 (n.a.)	78.2 vs. 64 (n.a.)	95 vs. 92 (n.a.)
021	FLT3-ITD+ EVI1	#1	primary tx mouse	$3x10^5$	47	2 weeks	86 vs. 84 (n.a.)	87 vs. 53 (n.a.)	53.8 vs. 49.9 (n.a.)	99.5 vs. 96 (n.a.)
04	NF1-AK4 fusion, C/EBPα mut.	#1	patient's PBMCs	$1x10^6$	40	3 weeks	0 vs. 0.17 (0.313)	33.8 vs. 23.5 (0.09)	48.5 vs. 21.4 (0.315)	55.6 vs. 26.4 (0.363)

Supplementary Table 2. PDX-AML transplantation experiments. Summary table showing available transplantation parameters for PDX-AML experiments (n.p. = not performed, n.a. = not applicable (not enough replicates to perform t test), "primary tx mouse"= cells obtained from a mouse directly transplanted with patient cells were used to transplant a secondary mouse which was then treated, "Patient' PBMCs": the corresponding PDX experiment was performed on PDX transplanted with patient-derived PBMCs, "Exp. no.": for some patient-derived cells the *in vivo* experiment was performed more than once (Pat. no. 017→ #2, #3, #4: cells from same primary transplanted PDX were injected in 3 different cohorts of mice – labeled with sequential # – which underwent different treatment schedules, Pat. no. 011 → cells were directly injected into recipient mice in #1, and from these primary tx -untreated- PDX cells were transplanted into further recipients)

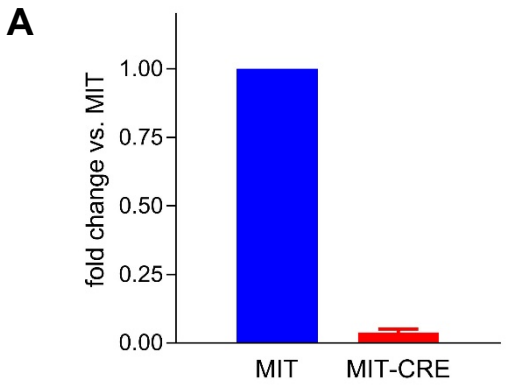
Supplementary Figure 1

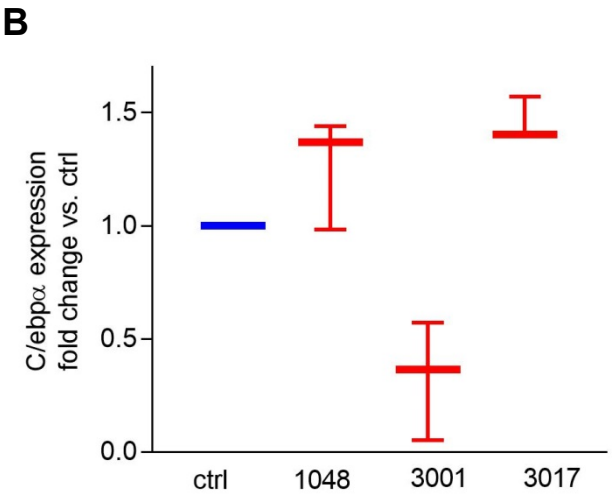


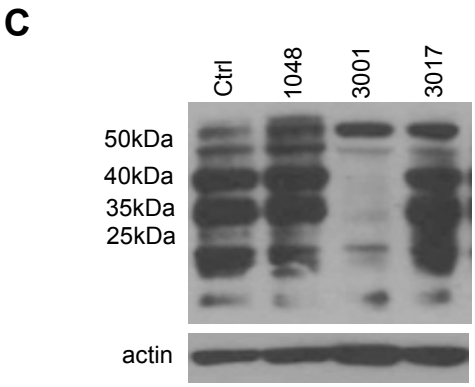
Supplementary Figure 2

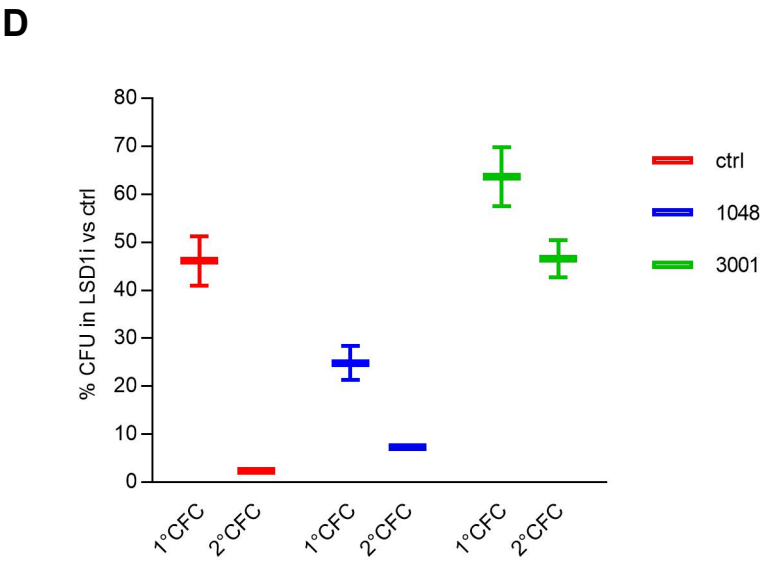


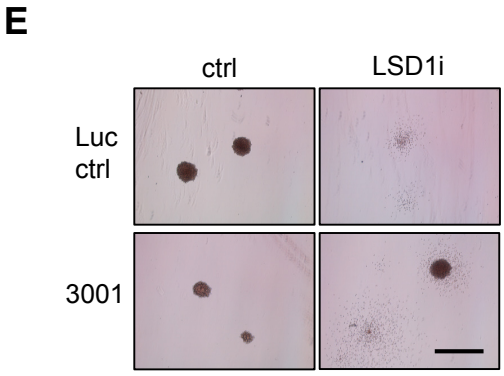
Supplementary Figure 3











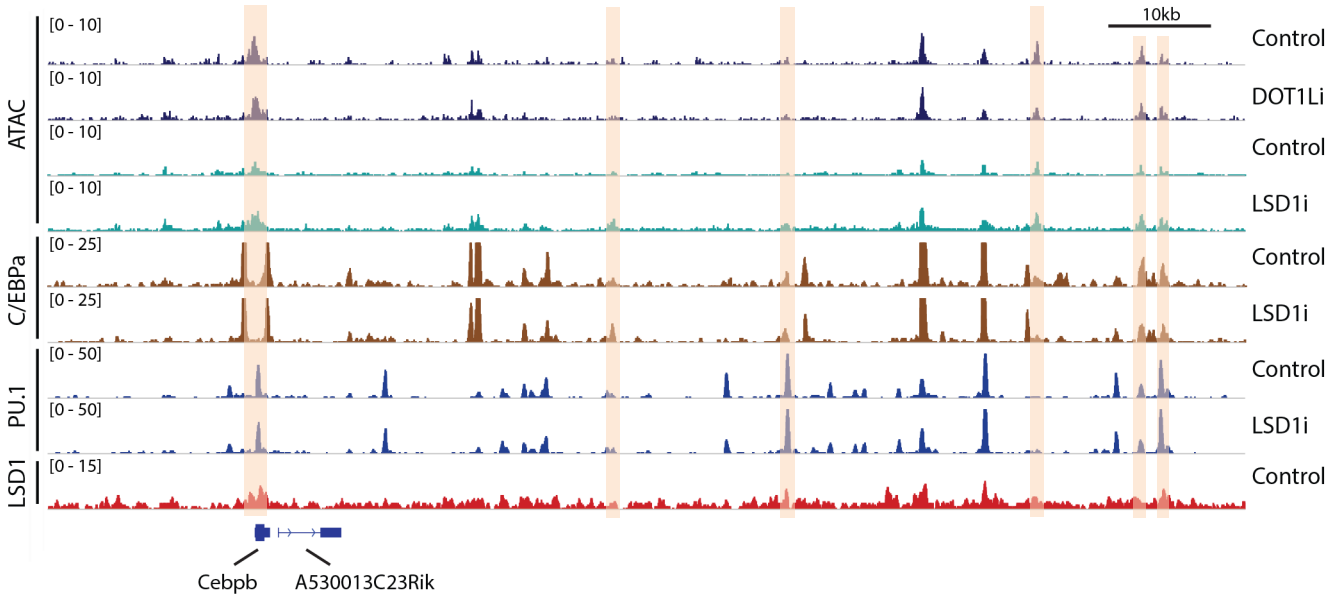
Supplementary Figure 4

A

LSD1i (GSK) vs DMSO de novo motif enrichment

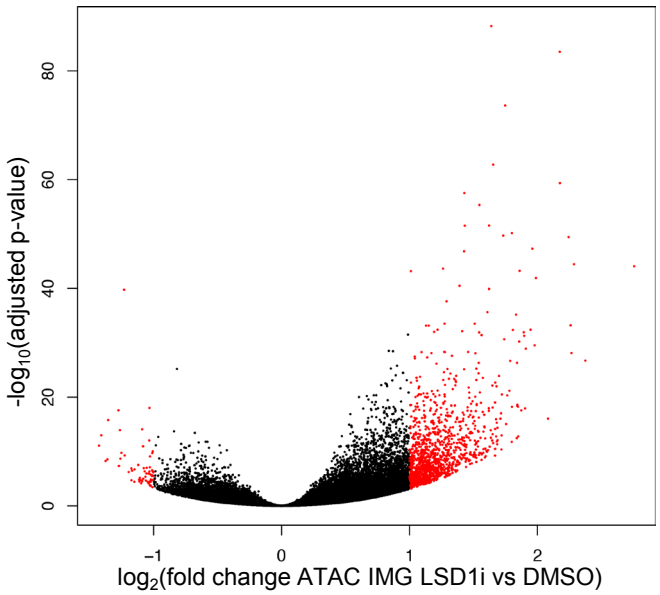
Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details
1		1e-195	-4.510e+02	32.77%	6.86%	123.3bp (148.8bp)	MA0080.3_Spi1/Jaspar(0.965)
2		1e-75	-1.736e+02	11.53%	2.00%	125.1bp (150.7bp)	PU.1-IRF(ETS:IRF)/Bcell-PU.1-ChIP-Seq(GSE21512)/Homer(0.951)
3		1e-73	-1.692e+02	43.12%	22.16%	132.0bp (150.1bp)	RUNX(Runt)/HPC7-Runx1-ChIP-Seq(GSE22178)/Homer(0.954)
4		1e-70	-1.622e+02	30.21%	12.80%	131.6bp (148.8bp)	CEBP(bZIP)/CEBPb-ChIP-Seq(GSE21512)/Homer(0.915)
5		1e-67	-1.548e+02	33.94%	15.80%	128.8bp (149.7bp)	BATF(bZIP)/Th17-BATF-ChIP-Seq(GSE39756)/Homer(0.975)
6		1e-23	-5.366e+01	8.58%	3.15%	139.6bp (148.4bp)	CEBP:AP1(bZIP)/ThioMac-CEBPb-ChIP-Seq(GSE21512)/Homer(0.888)
7		1e-15	-3.683e+01	26.28%	17.77%	140.0bp (152.5bp)	PH0037.1_Hdx/Jaspar(0.763)
8		1e-8	-1.952e+01	19.86%	14.38%	141.6bp (151.8bp)	PB0056.1_Rfxdc2_1/Jaspar(0.735)
9		1e-4	-1.069e+01	0.26%	0.01%	136.6bp (137.0bp)	PB0076.1_Sp4_1/Jaspar(0.694)
10		1e-4	-1.027e+01	0.59%	0.10%	128.4bp (161.2bp)	MA0526.1_USF2/Jaspar(0.739)

B

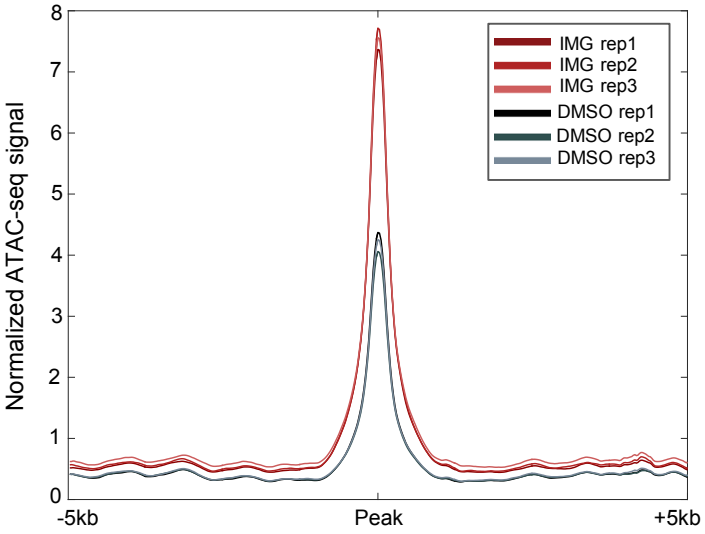


Supplementary Figure 5

A



B



C

LSD1i (IMG) vs DMSO de novo motif enrichment

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details
1		1e-167	-3.857e+02	38.19%	13.19%	550.4bp (276.8bp)	CEBPA/MA0102.3/Jaspar(0.965)
2		1e-154	-3.560e+02	34.39%	11.48%	551.8bp (270.5bp)	PB0058.1_Sfpi1_1/Jaspar(0.953)
3		1e-98	-2.263e+02	3.59%	0.06%	188.6bp (222.1bp)	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer(0.810)
4		1e-92	-2.124e+02	3.08%	0.04%	309.7bp (226.8bp)	MZF1/MA0056.1/Jaspar(0.617)
5		1e-88	-2.030e+02	31.88%	14.08%	725.8bp (278.9bp)	AP-1(bZIP)/ThioMac-PU.1-ChIP-Seq(GSE21512)/Homer(0.977)
6		1e-76	-1.750e+02	3.08%	0.07%	204.0bp (298.4bp)	RORA(var.2)/MA0072.1/Jaspar(0.652)
7		1e-66	-1.526e+02	3.54%	0.16%	232.8bp (292.0bp)	PH0089.1_Isx/Jaspar(0.668)
8		1e-57	-1.321e+02	2.52%	0.07%	198.1bp (259.4bp)	JDP2(var.2)/MA0656.1/Jaspar(0.699)
9		1e-36	-8.515e+01	4.06%	0.62%	1148.7bp (268.0bp)	PB0204.1_Zfp740_2/Jaspar(0.670)
10		1e-20	-4.616e+01	6.37%	2.46%	468.0bp (276.9bp)	POL009.1_DCE_S_II/Jaspar(0.653)