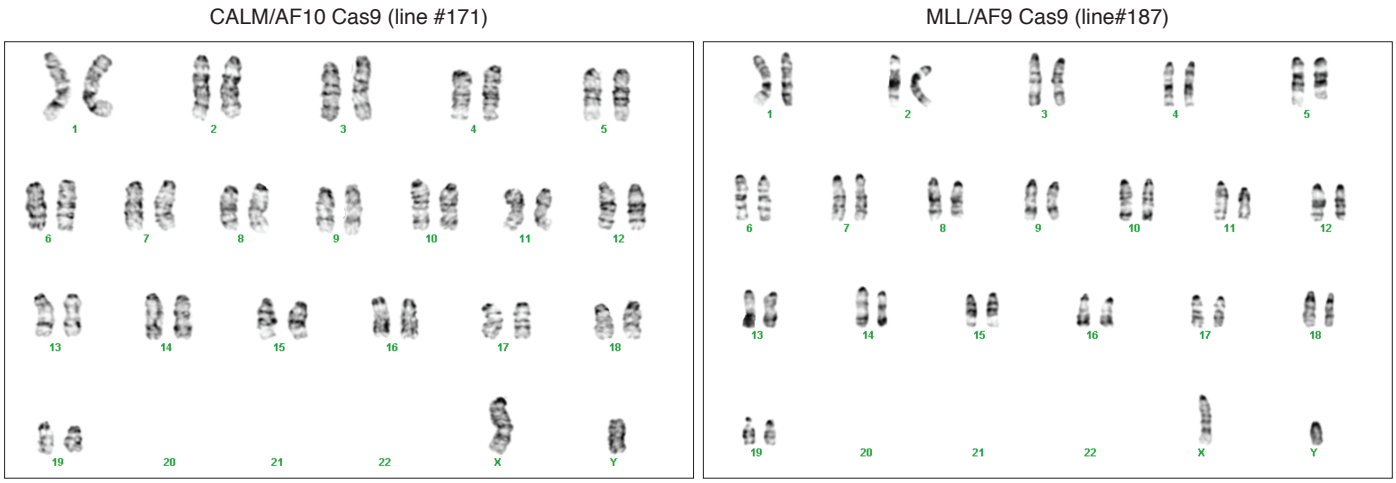
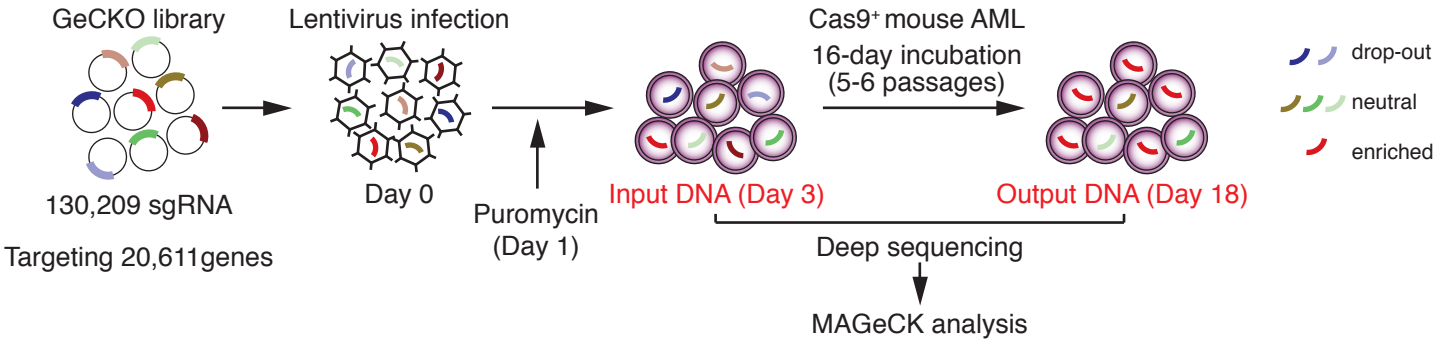
A



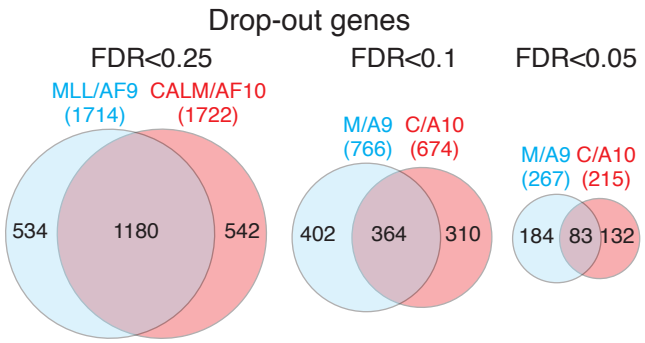
B



C

Sample	Mapped reads	Library sgRNA	Zero counts	Gini Index	Coverage
CALM-AF10_LibraryA_Day3	41,606,471	67,405	773	0.11	x617
CALM-AF10_LibraryA_Day18	40,699,845	67,405	6,006	0.2	x603
CALM-AF10_LibraryB_Day3	42,997,399	62,804	419	0.11	x685
CALM-AF10_LibraryB_Day18	44,087,417	62,804	6,641	0.21	x702
MLL-AF9_LibraryA_Day3	43,433,781	67,405	734	0.1	x644
MLL-AF9_LibraryA_Day18	50,203,365	67,405	6,711	0.21	x745
MLL-AF9_LibraryB_Day3	44,561,317	62,804	1,002	0.11	x710
MLL-AF9_LibraryB_Day18	44,572,208	62,804	5,614	0.2	x710

D



E

MLL/AF9

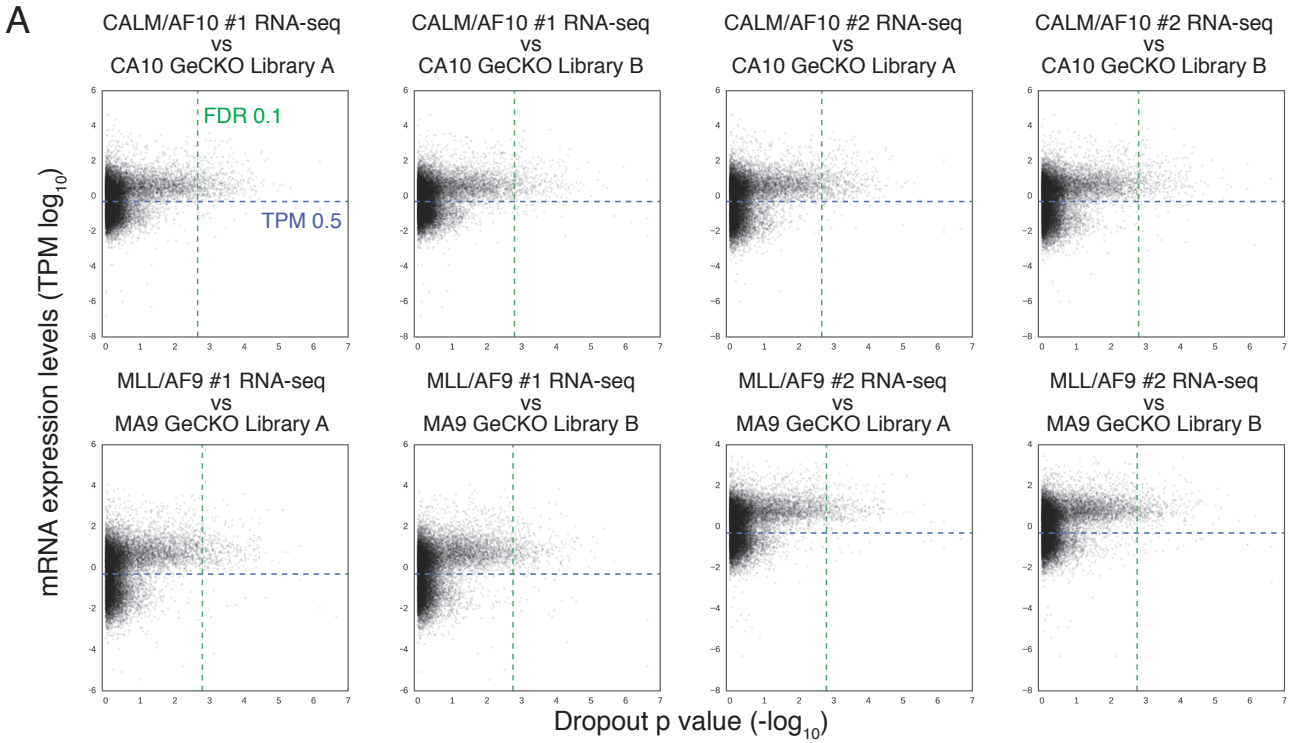
Node name (KEGG pathway)	p value
HSA03010_RIBOSOME	2.44E-17
HSA00970_AMINOACYL_TRNA_BIOSYNTHESIS	3.50E-15
HSA00240_PYRIMIDINE_METABOLISM	9.28E-11
HSA03020_RNA_POLYMERASE	2.78E-10
HSA03050_PROTEASOME	2.66E-08
HSA00190_OXIDATIVE_PHOSPHORYLATION	8.14E-08
HSA00230_PURINE_METABOLISM	1.08E-06

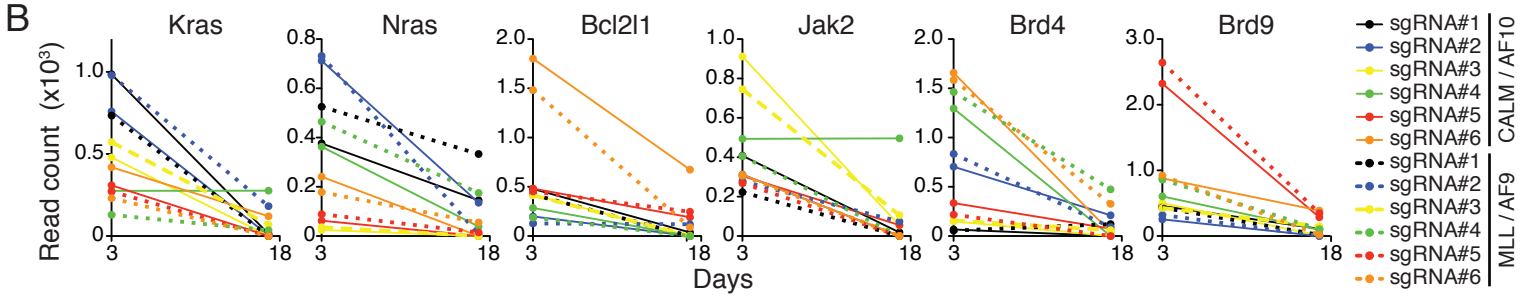
CALM/AF10

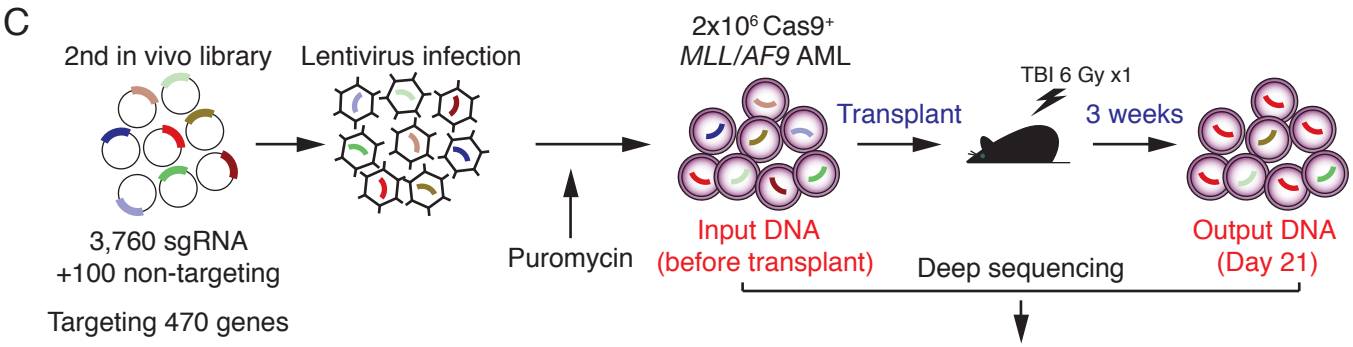
Node name (KEGG pathway)	p value
HSA03010_RIBOSOME	4.69E-21
HSA00190_OXIDATIVE_PHOSPHORYLATION	4.22E-13
HSA00970_AMINOACYL_TRNA_BIOSYNTHESIS	6.79E-12
HSA03050_PROTEASOME	9.19E-09
HSA03020_RNA_POLYMERASE	1.47E-08
HSA00240_PYRIMIDINE_METABOLISM	3.13E-08
HSA04110_CELL_CYCLE	3.39E-06
HSA04120_UBIQUITIN_MEDIATED_PROTEOLYSIS	6.31E-05

Figure S1. Related to Figure 1. CRISPR-Cas9 screens in mouse AML cell lines with normal karyotypes.

(A) Representative results of karyotype analysis. **(B)** Schematic representations of the GeCKO screen. **(C)** Sequencing read counts of each sample. Read mapping and gini index calculations were performed using the MAGeCK count program. **(D)** Venn diagrams show numbers of dropout genes at indicated FDR values. **(E)** Pathway analysis of dropout genes shows enrichment of signatures representing basal cellular machineries.

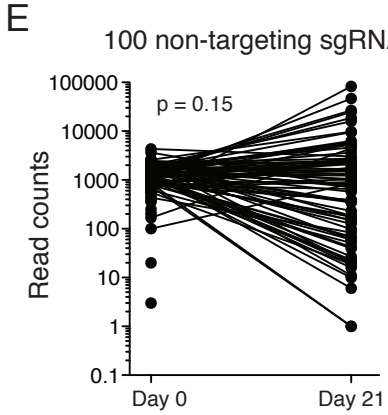






D

Sample	Mapped reads	Library sgRNA	Zero counts	Gini Index	Coverage
MLL-AF9 before transplant	4,966,871	3,860	11	0.05	x1287
MLL-AF9 3wks after transplant	3,235,660	3,860	2,359	0.76	x838



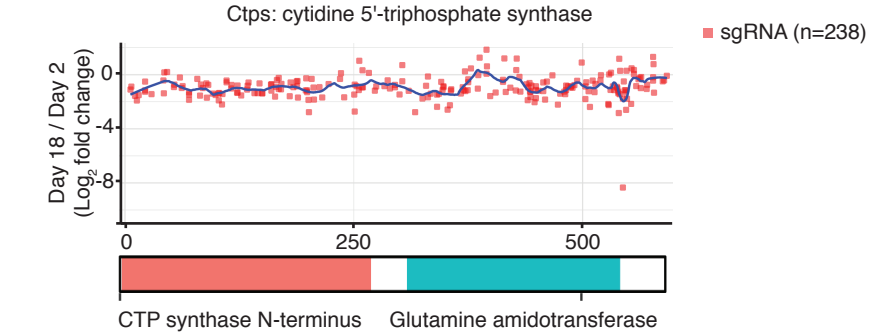


Figure S2. Dropout genes and validation screens: additional information. Related to Figure 1.

(A) RNA-seq experiments were performed using 4 primary mouse AML lines established independently in vivo (CALM/AF10 #1, CALM/AF10 #2, MLL/AF9 #1 and MLL/AF9 #2). mRNA expression levels (y-axis: TPM values) and the dropout p value (x axis: minus $\log_{10}$ values in GeCKO screens) of each gene were plotted. **(B)** Graphs show read counts of individual sgRNAs targeting well-known genes essential for AML, before and after a 16-day incubation. **(C)** Workflow of second screen in vivo. Cas9-expressing MLL/AF9 cells were transduced with a pool of sgRNAs targeting 470 selected genes (8 sgRNA per gene) and 100 non-targeting sgRNAs and then transferred to sub-lethally irradiated recipients. Change in abundance of each sgRNA between the initial and final cell population was analyzed using the MAGeCK program. **(D)** Sequencing results of DNA samples obtained before and after transplant. **(E)** Read counts of each non-targeting sgRNA before and after transplant were plotted. **(F)** Results of CTPS domain-mapping.

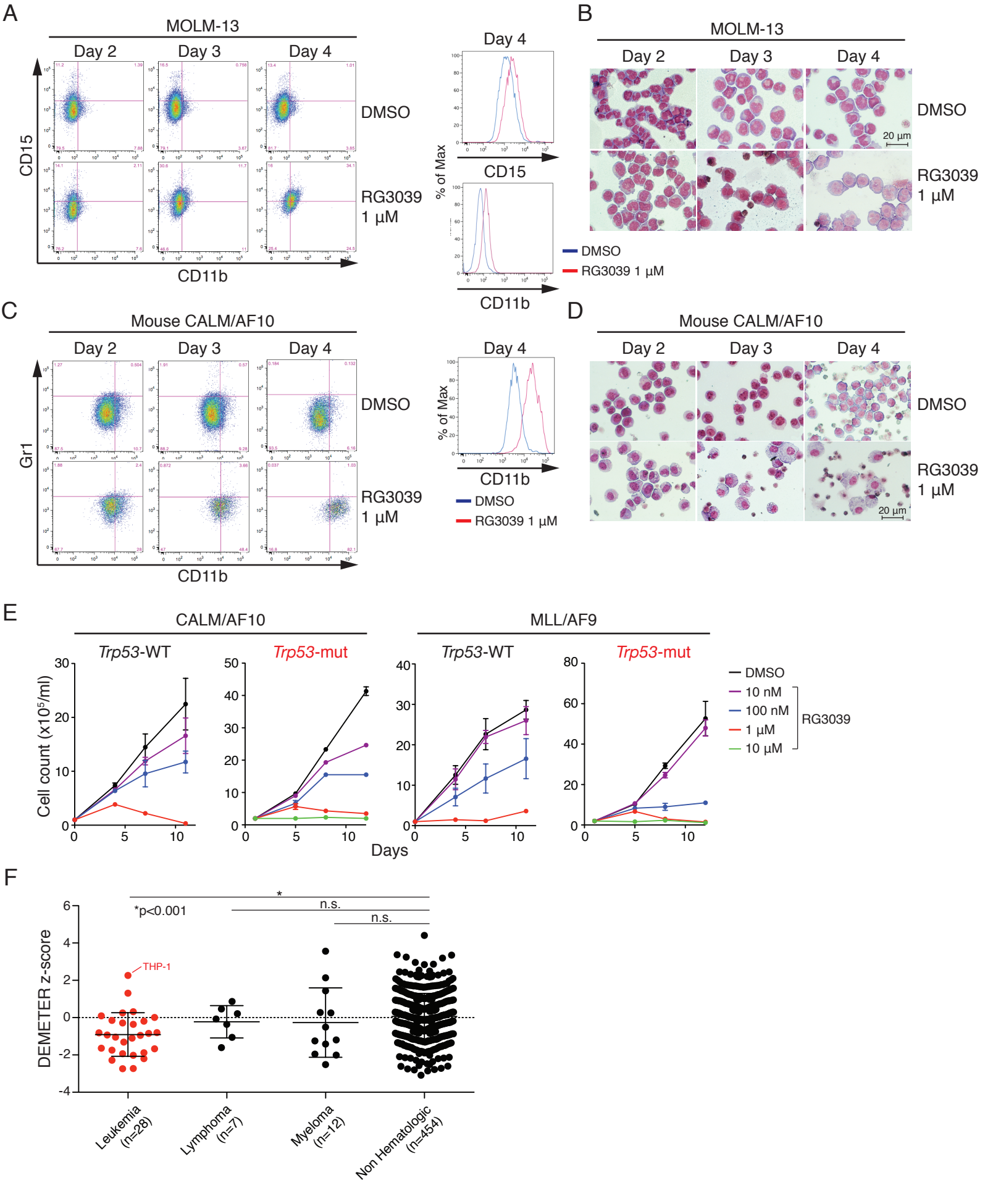
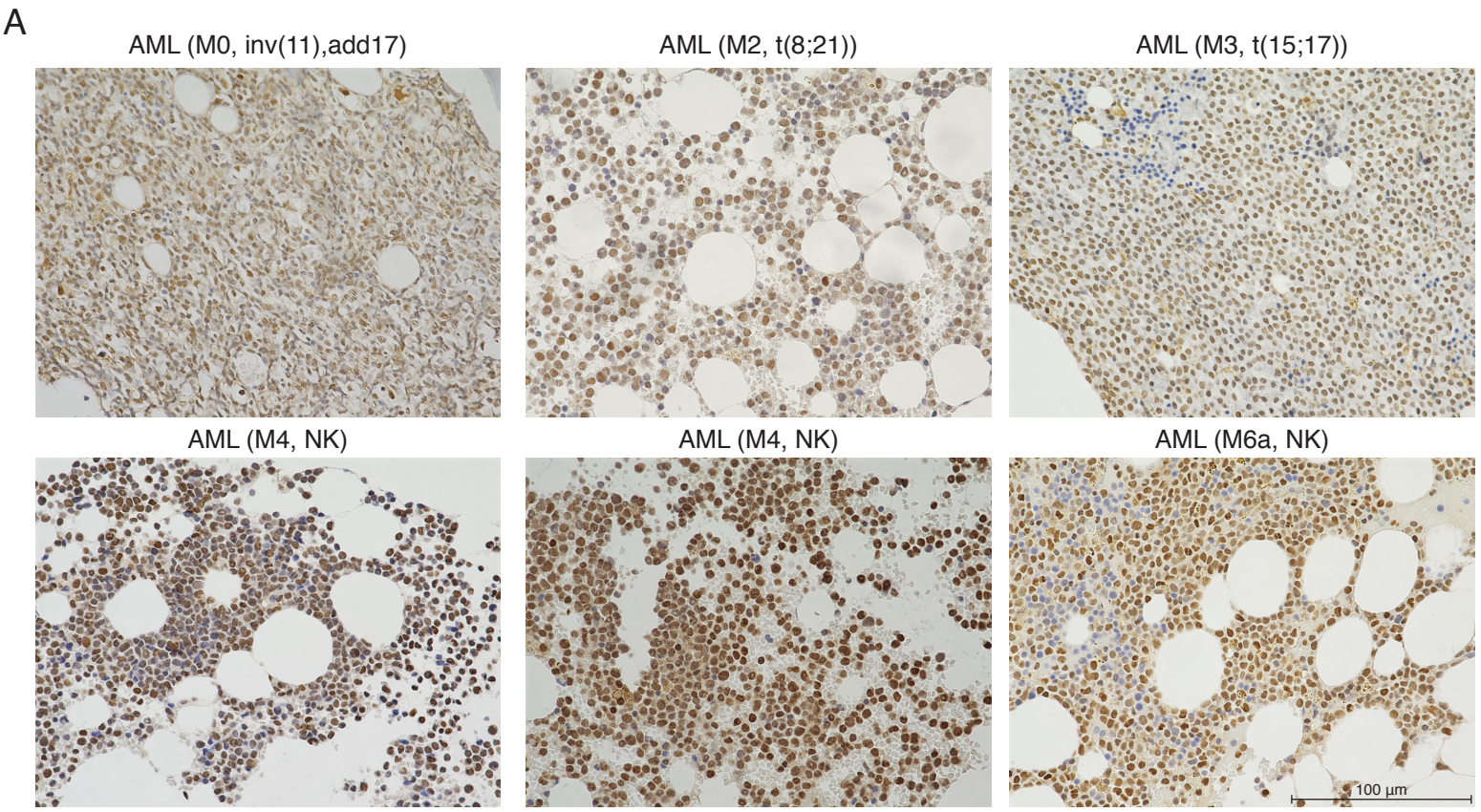
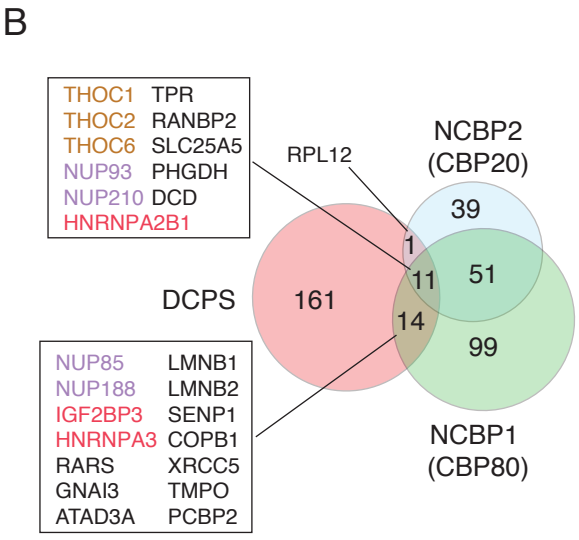
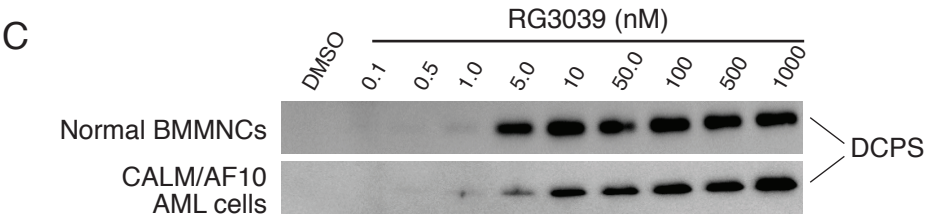
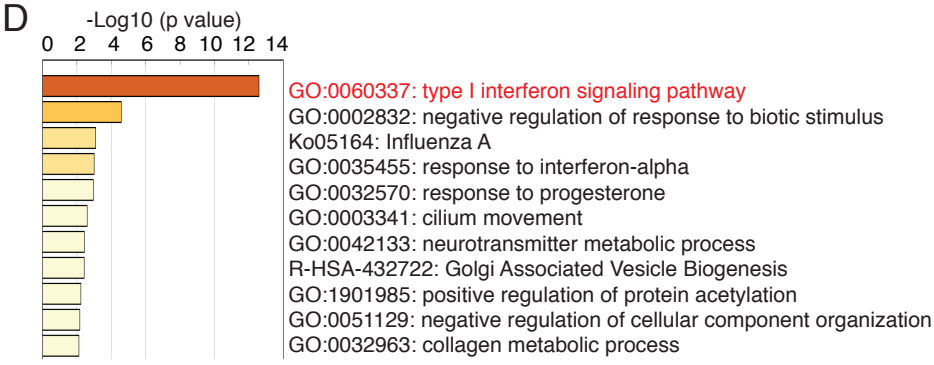

A
MOLM-13
Day 2
Day 3
Day 4
CD15
CD11b
DMSO
RG3039
1 µM
Day 4
% of Max
CD15
CD11b
DMSO
RG3039 1 µM
B
MOLM-13
Day 2
Day 3
Day 4
DMSO
RG3039
1 µM
20 µm
C
Mouse CALM/AF10
Day 2
Day 3
Day 4
Gr1
CD11b
DMSO
RG3039
1 µM
Day 4
% of Max
CD11b
DMSO
RG3039 1 µM
D
Mouse CALM/AF10
Day 2
Day 3
Day 4
DMSO
RG3039
1 µM
20 µm
E
CALM/AF10
MLL/AF9
Trp53-WT
Trp53-mut
Trp53-WT
Trp53-mut
Cell count (x10⁵/ml)
Days
DMSO
10 nM
100 nM
1 µM
10 µM
RG3039
F
*p<0.001
*
n.s.
n.s.
DEMETER z-score
THP-1
Leukemia (n=28)
Lymphoma (n=7)
Myeloma (n=12)
Non Hematologic (n=454)

Figure S3. RG3039 slows proliferation and induces AML cell differentiation via p53-independent mechanisms. Related to Figure 2. (A) Representative FACS plots of MOLM-13 cells treated with either DMSO (vehicle) or RG3039. **(B)** May-Giemsa stain of MOLM-13 cells treated with either DMSO or RG3039. **(C)** Representative FACS plots of mouse CALM/AF10 cells treated with either DMSO or RG3039. **(D)** May-Giemsa stain of CALM/AF10 cells. **(E)** *Trp53*-WT or mutant mouse AML cells were treated with RG3039, and growth curves generated as described in Figure 2I. Data are represented as means ± SD. **(F)** Dot graphs show DCPS dependency of 501 cancer cell lines representing leukemia, lymphoma, multiple myeloma and non-hematologic tumors. Data are shown as means ± SD. The Y-axis represents DEMETER z-score of shRNA targeting DCPS (https://depmap.org/rnai/genedeps?gene=DCPS).


A
AML (M0, inv(11),add17)
AML (M2, t(8;21))
AML (M3, t(15;17))
AML (M4, NK)
AML (M4, NK)
AML (M6a, NK)
100 µm


B
THOC1 TPR
THOC2 RANBP2
THOC6 SLC25A5
NUP93 PHGDH
NUP210 DCD
HNRNPA2B1
RPL12
NCBP2
(CBP20)
39
1
11
51
DCPS
161
14
99
NCBP1
(CBP80)
NUP85 LMNB1
NUP188 LMNB2
IGF2BP3 SENP1
HNRNPA3 COPB1
RARS XRCC5
GNAI3 TMPO
ATAD3A PCBP2


C
RG3039 (nM)
DMSO
0.1
0.5
1.0
5.0
10
50.0
100
500
1000
Normal BMMNCs
CALM/AF10
AML cells
DCPS


D
-Log10 (p value)
0 2 4 6 8 10 12 14
GO:0060337: type I interferon signaling pathway
GO:0002832: negative regulation of response to biotic stimulus
Ko05164: Influenza A
GO:0035455: response to interferon-alpha
GO:0032570: response to progesterone
GO:0003341: cilium movement
GO:0042133: neurotransmitter metabolic process
R-HSA-432722: Golgi Associated Vesicle Biogenesis
GO:1901985: positive regulation of protein acetylation
GO:0051129: negative regulation of cellular component organization
GO:0032963: collagen metabolic process

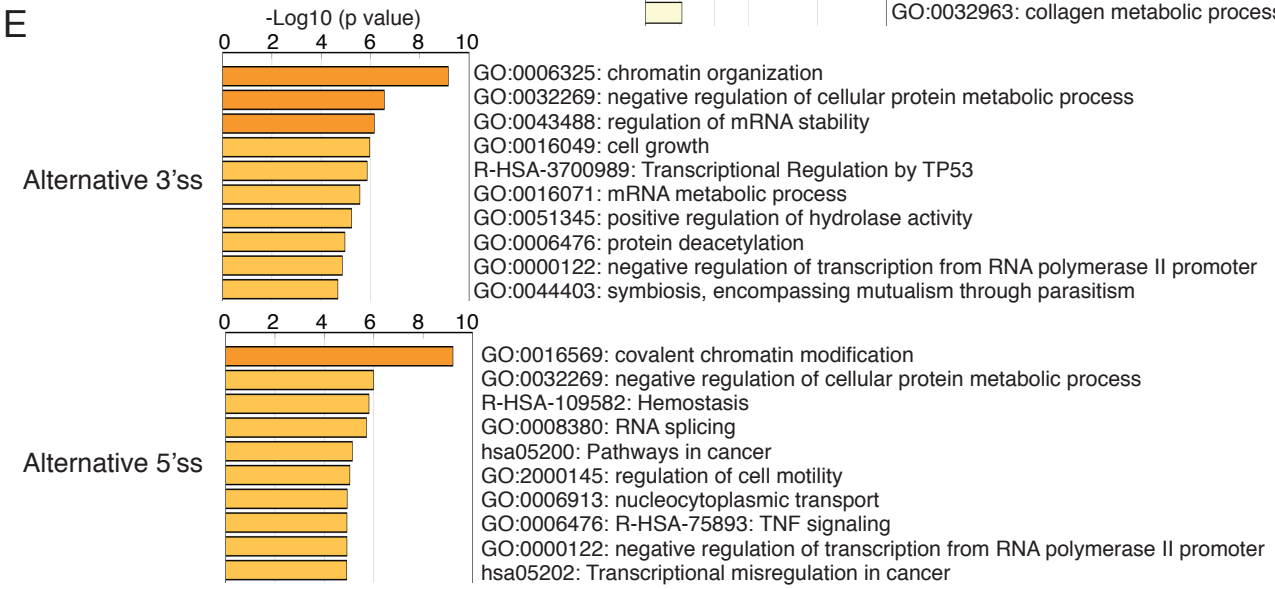

E
-Log10 (p value)
0 2 4 6 8 10
Alternative 3'ss
GO:0006325: chromatin organization
GO:0032269: negative regulation of cellular protein metabolic process
GO:0043488: regulation of mRNA stability
GO:0016049: cell growth
R-HSA-3700989: Transcriptional Regulation by TP53
GO:0016071: mRNA metabolic process
GO:0051345: positive regulation of hydrolase activity
GO:0006476: protein deacetylation
GO:0000122: negative regulation of transcription from RNA polymerase II promoter
GO:0044403: symbiosis, encompassing mutualism through parasitism
0 2 4 6 8 10
Alternative 5'ss
GO:0016569: covalent chromatin modification
GO:0032269: negative regulation of cellular protein metabolic process
R-HSA-109582: Hemostasis
GO:0008380: RNA splicing
hsa05200: Pathways in cancer
GO:2000145: regulation of cell motility
GO:0006913: nucleocytoplasmic transport
GO:0006476: R-HSA-75893: TNF signaling
GO:0000122: negative regulation of transcription from RNA polymerase II promoter
hsa05202: Transcriptional misregulation in cancer

Figure S4. DCPS protein localizes to the nucleus and associates with factors involved in pre-mRNA metabolic pathways. Related to Figures 3 and 4. (A) Immunohistochemistry (IHC) for DCPS protein in BM sections of six human AML cases. Shown are FAB subtype and karyotype in each case. Brown staining: DCPS protein. DCPS is predominantly nuclear in AML cells. NK: normal karyotype. **(B)** CBC, which consists of NCBP1 and NCBP2, and DCPS share some interacting proteins. NCBP1 and NCBP2 binding proteins were described previously (Andersen et al., 2013). **(C)** Affinity between RG3039 and DCPS was assessed by CETSA. **(D)** Results of gene expression signature analysis of RG3039-treated AML cells using the Metascape program (http://metascape.org/). **(E)** Metascape analysis of aberrantly-spliced genes following RG3039 treatment of CALM/AF10 AML cells.

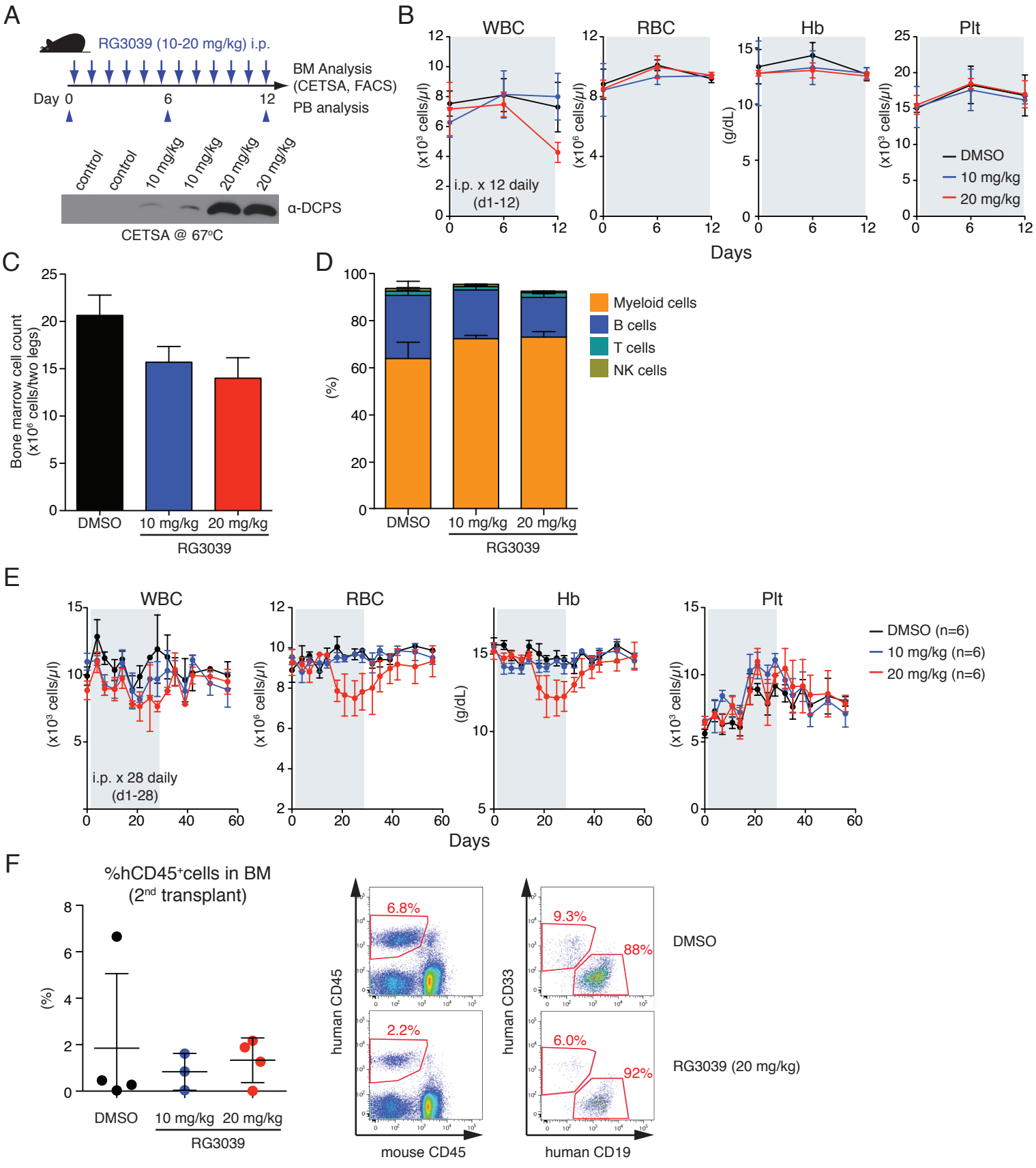
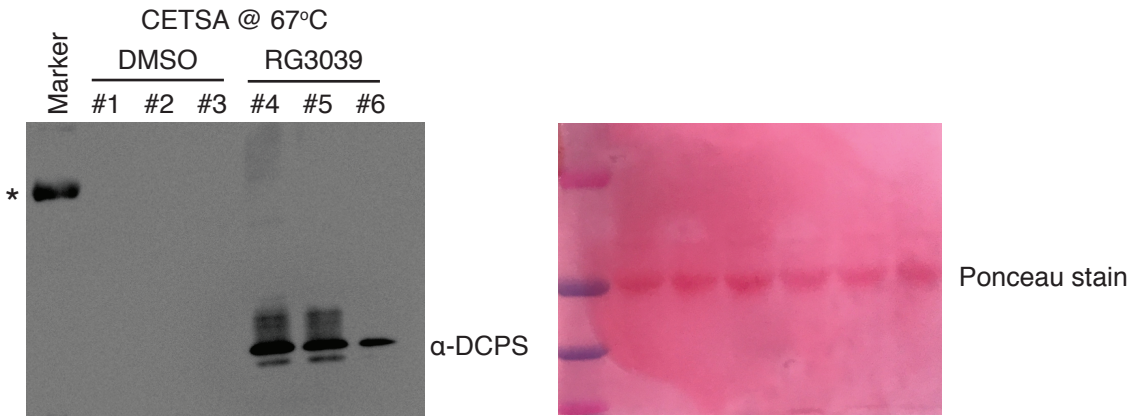

A
RG3039 (10-20 mg/kg) i.p.
BM Analysis
(CETSA, FACS)
PB analysis
Day 0
6
12
control
control
10 mg/kg
10 mg/kg
20 mg/kg
20 mg/kg
α-DCPS
CETSA @ 67°C
B
WBC
RBC
Hb
Plt
(x10³ cells/μl)
(x10⁶ cells/μl)
(g/dL)
i.p. x 12 daily
(d1-12)
DMSO
10 mg/kg
20 mg/kg
Days
C
Bone marrow cell count
(x10⁶ cells/two legs)
DMSO
10 mg/kg
20 mg/kg
RG3039
D
(%)
Myeloid cells
B cells
T cells
NK cells
DMSO
10 mg/kg
20 mg/kg
RG3039
E
WBC
RBC
Hb
Plt
(x10³ cells/μl)
(x10⁶ cells/μl)
(g/dL)
i.p. x 28 daily
(d1-28)
Days
DMSO (n=6)
10 mg/kg (n=6)
20 mg/kg (n=6)
F
%hCD45⁺cells in BM
(2nd transplant)
(%)
DMSO
10 mg/kg
20 mg/kg
RG3039
human CD45
mouse CD45
human CD33
human CD19
6.8%
2.2%
9.3%
88%
6.0%
92%
DMSO
RG3039 (20 mg/kg)

Figure S5. Effects of DCPS inhibition in vivo. Related to Figure 5. (A) DMSO or RG3039 (10 mg/kg or 20 mg/kg) was administered intraperitoneally daily for 12 days, and peripheral blood counts were analyzed before and after treatment (day 6 and day 12). CETSA was performed using BM cells the day after the last RG3039 (or DMSO) injection. **(B)** Peripheral blood counts were analyzed by a hematology analyzer before and after RG3039 treatment. Data are represented as means ± SD. WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; Plt: platelet. **(C)** BM cell counts were measured the day after the last RG3039 (or DMSO) injection. Data are represented as means ± SD. **(D)** Proportions of myeloid, B, T and NK cells in BM mononuclear cells were analyzed by FACS. Data are represented as means ± SD. **(E)** DMSO (vehicle) or RG3039 (10 mg/kg or 20 mg/kg) was administered intraperitoneally daily for 28 days, and peripheral blood counts were analyzed every 5-7 days. Treatment periods are depicted in gray. Data are represented as means ± SD. **(F)** Engraftment of human hematopoietic cells upon a second transplant. The Y-axis shows proportions of $hCD45^+$ cells in BM 6 weeks after the second transplant. Data are represented as means ± SD. Representative BM FACS plots are shown.

A

PDX	DFAM-68555-V1	DFAM-15354-V2	DFAM-71927-V3
WHO classification	Therapy-related AML	AML-MRC	AML-NOS
FAB classification	M5a	N/A	N/A
Karyotype	46,XX,t(9;11)(p22;q23)[3]/47,idem,+8[8]/50,idem,+6,+8,+9,der(9)t(9;11)(p22;q23),+13[cp5] t(9;11)(p22;q23) *MLL-AF9* Hyperdiploid, Trisomy 8	47, XY, +11 Trisomy 11	46,XX,dup(2)(q21q33),t(8;16)(p11;p13),psu,dic(22;1)(p11;p11)[10]/46,XX,dup(1)(q32q42),t(8;16),psu,dic(9;1)(q34;p11)[4]/46,XX,t(8;16),psu dic (19;1)(p13;p11) [4]/46,XX[2] t(8;16)(p11;p13) KAT6A-CREBBP
Molecular details	*FLT3* NM_004119 c.2027A>C p.N676T - in 79.6% of 314 reads Gain of *PIM1* (on 6p), gain of *RAD21* (on 8q), gain of *FLT3* and *PDS5B* (on 13q), gain of *CALR*, *NOTCH3*, *JAK3* and *MEF2B* (on 19p), gain of *CEBPA*, *CNOT3* and *U2AF2* (on 19q), gain of *RUNX1* and *U2AF1* (on 21q).	*DNMT3A* NM_175629 c.1868_1868insT p.Y623fs* - in 4.0% of 375 reads *FLT3* NM_004119 c.2027A>C p.N676T - in 88.7% of 309 reads (*FLT3*-ITD) Gain of *PIM1* (on 6p), gain of *RAD21* (on 8q), gain of *FLT3* and *PDS5B* (on 13q).	*NOTCH1* NM_017617 c.7230_7230insTG p.P2411fs* - in 52.1% of 121 reads Gain of *PIM1* (on 6p) *TP53* mutation (p.V173M)

B



C

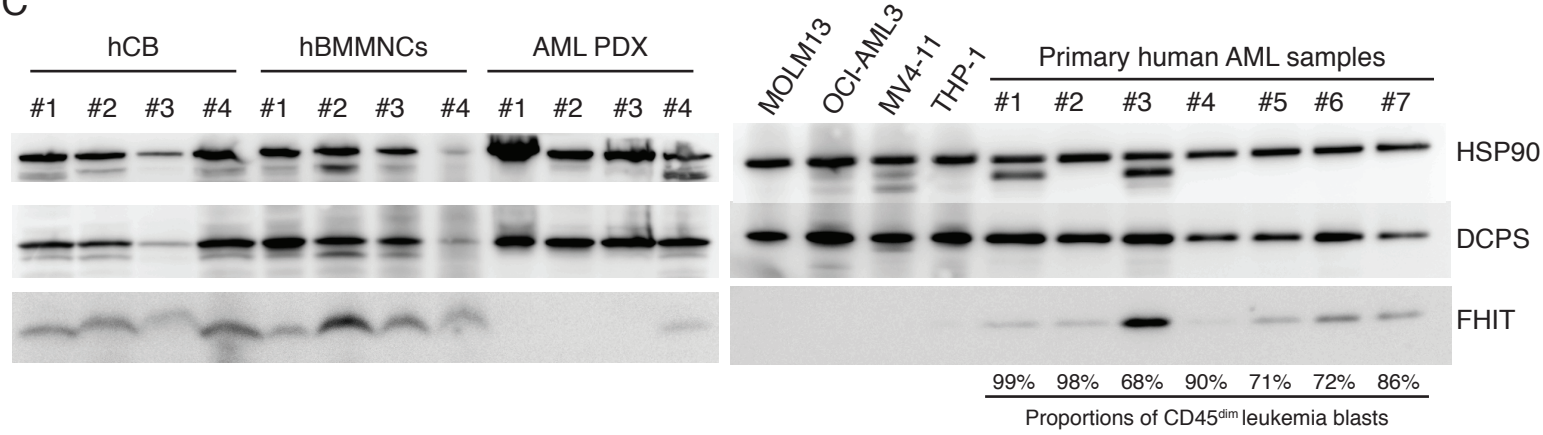


Figure S6. Characteristics of AML PDX lines and Western blot analysis for FHIT. Related to Figure 6. (A) Characteristics of three PDX lines. Information regarding WHO clarification, FAB classification and karyotype was obtained from the ProXe website. Mutational status as assessed by the Rapid Heme Panel (DFCI). **(B)** Representative results of CETSA using human AML cells harvested from BM of DMSO- or RG3039-treated PDX mice. For CETSA analysis AML cells in BM (DFAM-15354) were harvested the day after the last RG3039 (or DMSO) injection. Asterisk denotes non-specific signal. Equal protein loading was validated by Ponceau-S staining. **(C)** Western blot analysis of FHIT using anti-FHIT antibody.