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Supplemental information

ZMYND8-regulated IRF8 transcription axis is an acute myeloid leukemia dependency

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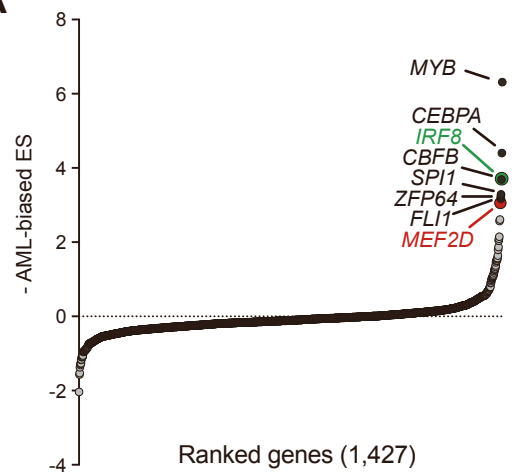
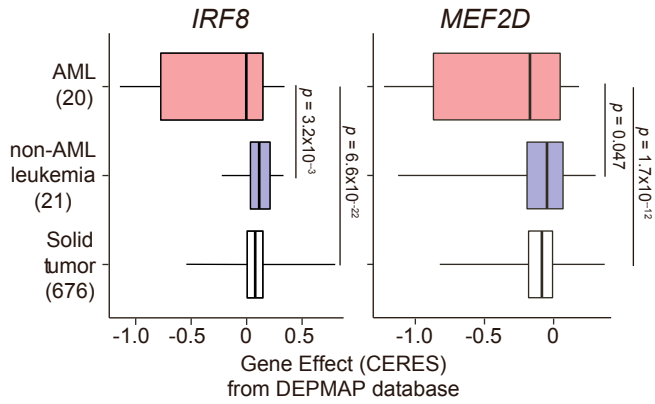
A



B

Cell line	Subtype	Genotype
MOLM-13	AML	*MLL-AF9/FLT3^{ITD}*
MV4;11	AML	*MLL-AF4/FLT3^{ITD}*
NOMO-1	AML	*MLL-AF9/KRAS^{G13D}*
THP-1	AML	*MLL-AF9/NRAS^{G12D}*
OCI-AML3	AML	*DMMT3A^{R882C}/NRAS^{Q61L}/NPM1^{mut}*
ML-2	AML	*MLL-AF6/KRAS^{A146Y}*
SEM	B-ALL	*MLL-AF4/CDKN2A^{83Y}/TP53^{mut}*
HEL	AML	*JAK2^{V617F}*
U937	AML	*CALM-AF10*
SET-2	AML	*JAK2^{V617F}*
JURKAT	T-ALL	*TP53^{mut}/NOTCH^{R1627H}*
REH	B-ALL	*ETV6-RUNX1*
K562	CML	*BCL-ABL*

C



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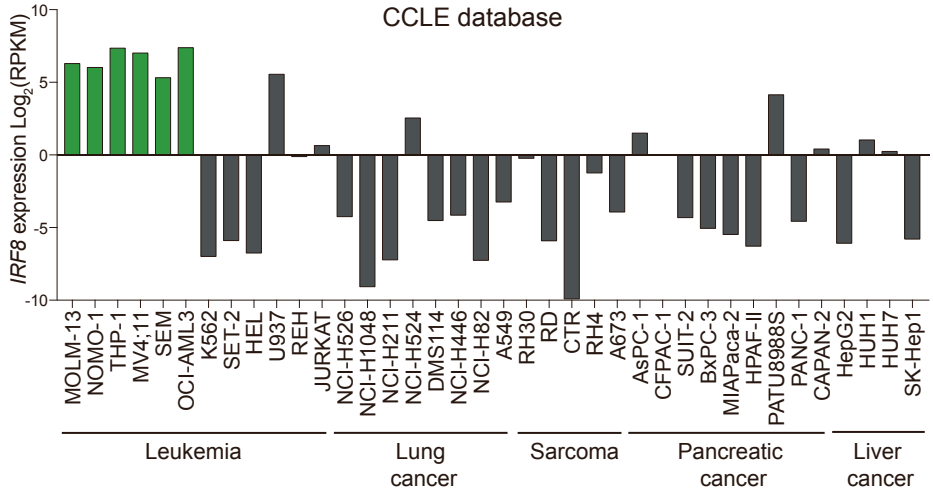

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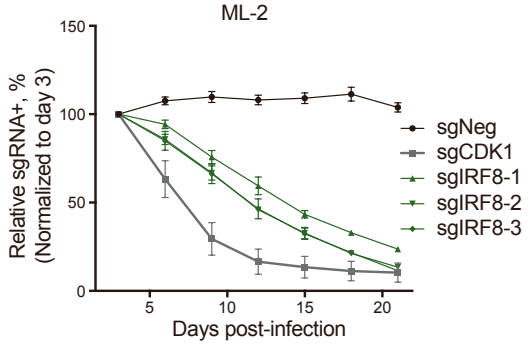

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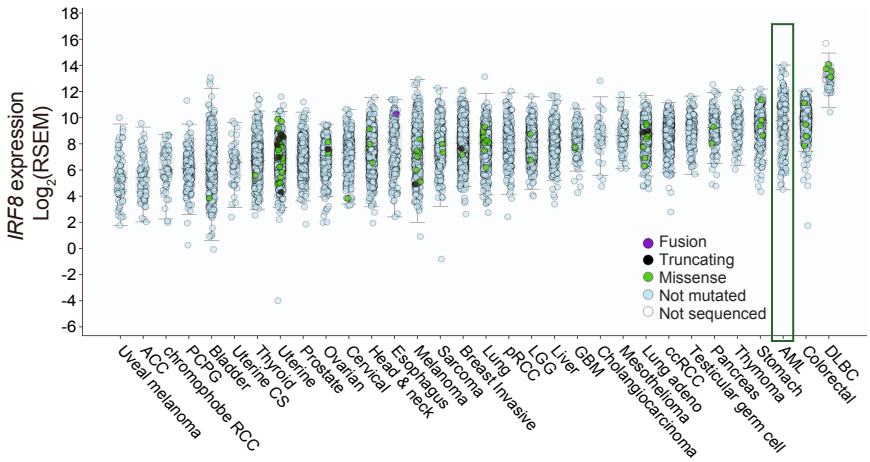

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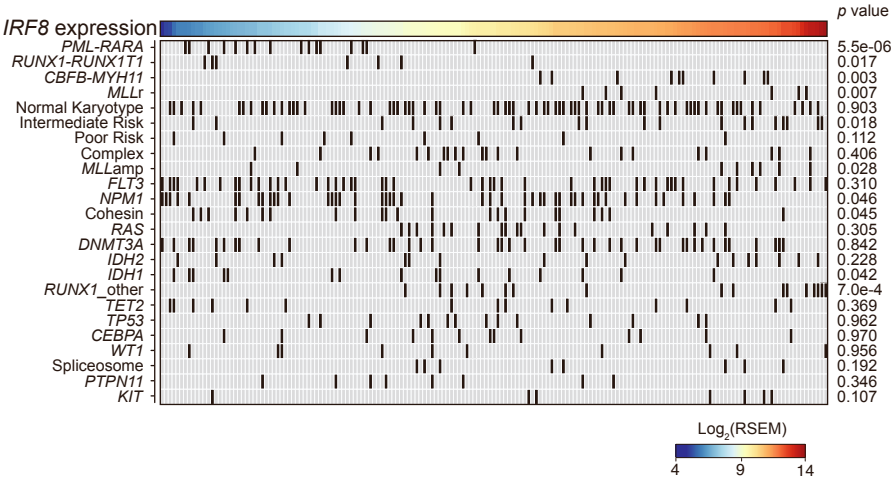

H

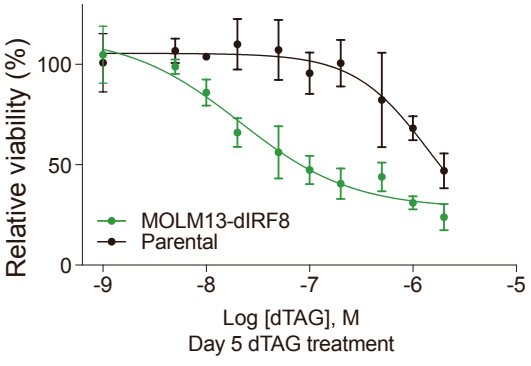

Figure S1. IRF8 is an AML-biased TF dependency. Related to Figure 1.

(A) Rank plot of negative AML-biased ESs of all 1,427 transcription factors. Known AML dependencies labeled in black. **(B)** Known genetic alterations and pathology of 13 leukemia cell lines used in this study. **(C)** IRF8 and MEF2D ESs extracted from DEPMAP dataset (Meyers et al., 2017). Shown is a box and whisker plot of the copy number–adjusted ESs of IRF8 across 20 AML, 21 non-AML leukemia, and 676 solid tumor cell lines. *p* values were calculated by Welch's two-sided *t*-test. CERES, a normalized metric of gene essentiality. **(D)** Competition-based proliferation assay performed in ML-2 cells. Plotted is the relative sgRNA+ population normalized to the day 3 sgRNA+ population over 21 days. sgNeg, negative control; sgCDK1, sgRNA targeting essential kinase *CDK1* as a positive control. Three independent sgRNAs targeting IRF8 are shown. Data points of the line graph represent the average of three independent biological replicates (n = 3). Error bars represent mean ± SEM. **(E)** IRF8 mRNA expression level ($\log_2$ RPKM values from RNA-seq) across cancer cell lines used in this study from CCLE database (Barretina et al., 2012). Cell lines with high IRF8 expression (*IRF8*^{hi}, RPKM > 5, with detectable protein level shown in Figure S3G) are labeled in green. **(F)** IRF8 mRNA expression level ($\log_2$ RESM values from RNA-seq) across 32 cancer types ranked by median expression level from TCGA dataset. Each dot represents *IRF8* expression levels in one patient, and different mutations are labeled with indicated colors. *IRF8* expression in AML is indicated with a green box. Error bars represent mean ± SEM. **(G)** Heatmap displaying patterns of IRF8 expression in 173 primary AML patients. IRF8 expression values from RNA-seq data ($\log_2$ RESM) shown in color gradient and the mutation and cytogenetics profiles for each patient are represented by a single black line. *p* values are indicated (two-tailed Mann–Whitney *U*-test). Data retrieved from the TCGA database. *MLL*amp, MLL amplification. **(H)** Relative viability of MOLM-13-dIRF8 or parental cells measured by CellTiter-Glo assays. Normalized relative luminescence units (RLU) shown after 5 days in culture with 0.05% DMSO (vehicle control) or dTAG-47 at indicated concentrations. Error bars smaller than symbol width are not shown. Data points are the mean ± SEM (n = 3) and 4-parameter dose-response curves.

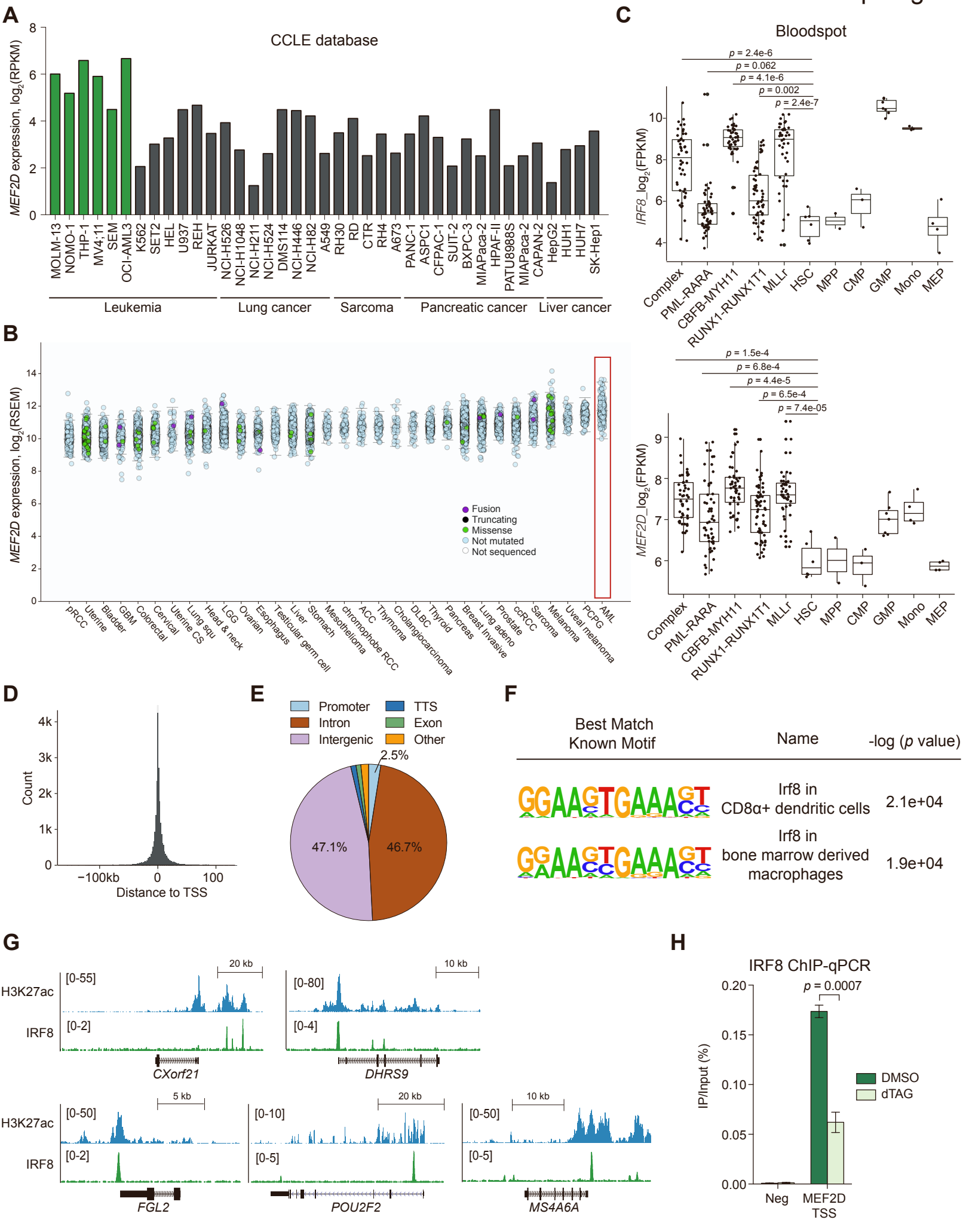
A
CCLE database
MEF2D expression, log2(RPKM)
MOLM-13
NOMO-1
THP-1
MV4;11
SEM
OCI-AML3
K562
SET2
HEL
U937
REH
JURKAT
NCI-H526
NCI-H1048
NCI-H211
NCI-H524
DMS114
NCI-H446
NCI-H82
A549
RH30
RD
CTR
RH4
A673
PANC-1
ASPC1
CFPAC-1
SUIT-2
BXPC-3
MIAPaca-2
HPAF-II
PATU8988S
MIAPaca-2
CAPAN-2
HepG2
HUH1
HUH7
SK-Hep1
Leukemia
Lung cancer
Sarcoma
Pancreatic cancer
Liver cancer
B
MEF2D expression, log2(RSEM)
Fusion
Truncating
Missense
Not mutated
Not sequenced
pRCC
Uterine
Bladder
GBM
Colorectal
Cervical
Uterine CS
Lung squ
Head & neck
LGG
Ovarian
Esophagus
Testicular germ cell
Liver
Stomach
Mesothelioma
chromophobe RCC
ACC
Thymoma
Cholangiocarcinoma
DLBC
Thyroid
Pancreas
Breast Invasive
Lung adeno
Prostate
ccRCC
Sarcoma
Melanoma
Uveal melanoma
PCPG
AML
C
Bloodspot
IRF8_log2(FPKM)
p = 2.4e-6
p = 0.062
p = 4.1e-6
p = 0.002
p = 2.4e-7
Complex
PML-RARA
CBFB-MYH11
RUNX1-RUNX1T1
MLLr
HSC
MPP
CMP
GMP
Mono
MEP
MEF2D_log2(FPKM)
p = 1.5e-4
p = 6.8e-4
p = 4.4e-5
p = 6.5e-4
p = 7.4e-05
D
Count
Distance to TSS
−100kb
0
100
E
Promoter
Intron
Intergenic
TTS
Exon
Other
2.5%
47.1%
46.7%
F
Best Match
Known Motif
Name
-log (p value)
Irf8 in
CD8α+ dendritic cells
2.1e+04
Irf8 in
bone marrow derived
macrophages
1.9e+04
G
H3K27ac
IRF8
20 kb
10 kb
5 kb
CXorf21
DHRS9
FGL2
POU2F2
MS4A6A
H
IRF8 ChIP-qPCR
p = 0.0007
IP/Input (%)
DMSO
dTAG
Neg
MEF2D
TSS

Figure S2. IRF8 is enriched at the *MEF2D* locus and modulates *MEF2D* expression. Related to Figure 2.

(A) *MEF2D* mRNA expression level (log_2 RPKM values from RNA-seq) across cancer cell lines used in this study from the CCLE database. Cell lines with high IRF8 expression (*IRF8*^{hi}, RPKM>5, with detectable protein levels shown in Figure S3G) are labeled in green. **(B)** *MEF2D* mRNA expression level (log_2 RESM values from RNA-seq) across 32 cancer types ranked by median expression level from the TCGA dataset. Each dot represents IRF8 expression levels in one patient, and different mutations are labeled with indicated colors. *MEF2D* expression in AML is labeled with a red box. **(C)** *IRF8* (top) and *MEF2D* (bottom) mRNA expression in human leukemia patients harboring different cytogenetics or purified normal human hematopoietic cells from BloodSpot data collection. *MLL*r, *MLL*-rearrangement; HSC, Hematopoietic stem cell; MPP, Multipotent progenitor; CMP, Common myeloid progenitor; GMP, Granulocyte monocyte progenitor; Mono, Monocyte; MEP, Megakaryocyte-erythroid progenitor. **(D)** Meta-profile of IRF8 occupancy surrounding the *MEF2D* TSS in MOLM-13 cells. TSS, transcription start site. **(E)** Pie chart annotating the location distribution of 23,429 IRF8 peaks in MOLM-13 cells. TTS, transcription termination site. Other, UTR and non-coding RNA regions. **(F)** IRF8 ChIP-seq-derived known motif consensus sequences. Shown are the top 2 motifs with the lowest *p* values. Statistical analysis (*p* value) was calculated using the binomial test. **(G)** Gene tracks of H3K27ac and IRF8 enrichment at the *Cxorf21*, *DHRS9*, *FGL2*, *POU2F2*, and *MS4A6A* loci in MOLM-13 cells. **(H)** ChIP-qPCR with an IRF8 antibody near IRF8-bound MEF2D TSS in MOLM-13 cells treated with DMSO or 500 nM dTAG for 4 hours (n=3). Statistical analysis (*p* value) was performed using an unpaired Student's t-test. All error bars represent mean ± SEM.

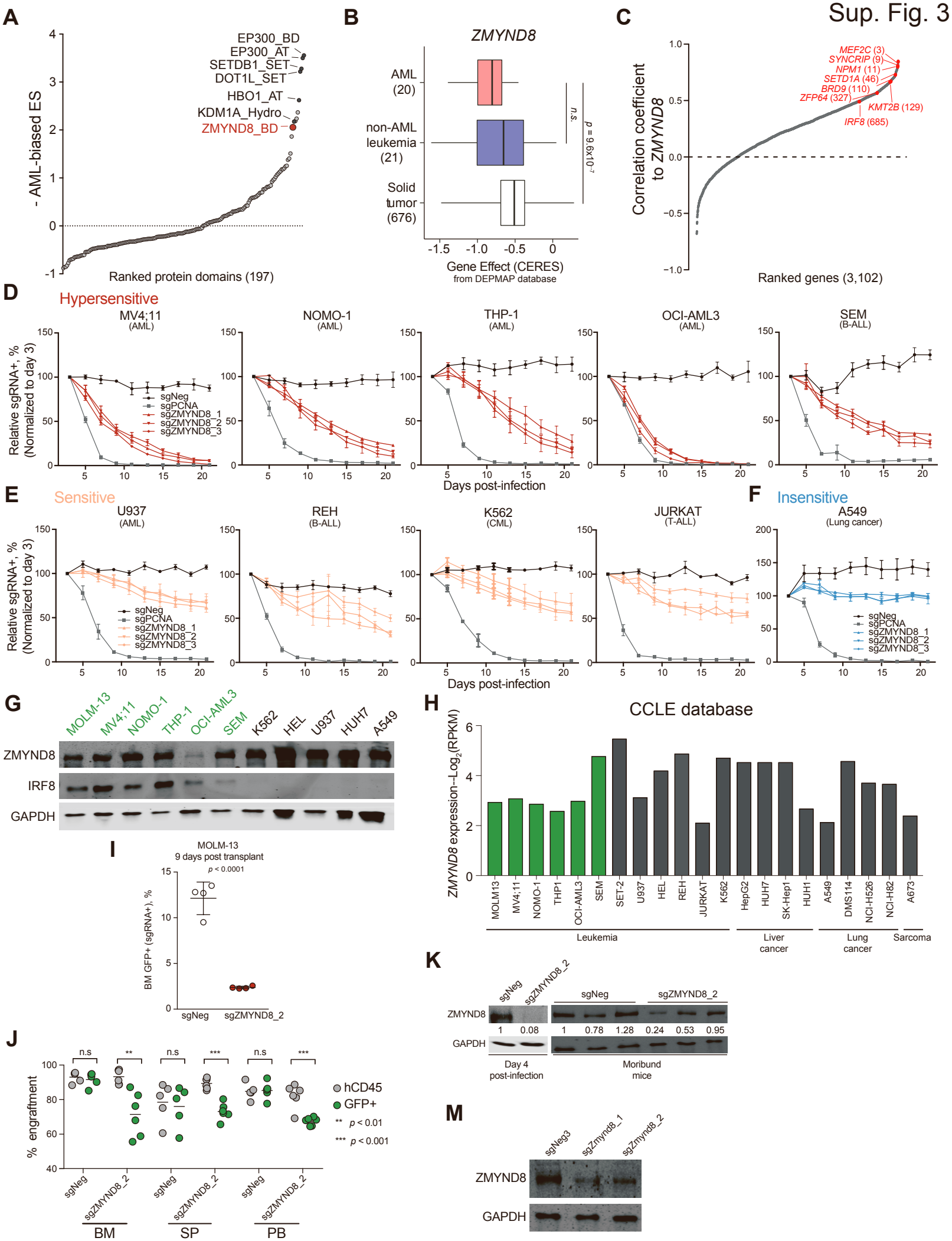
A
- AML-biased ES
EP300_BD
EP300_AT
SETDB1_SET
DOT1L_SET
HBO1_AT
KDM1A_Hydro
ZMYND8_BD
Ranked protein domains (197)
B
ZMYND8
AML (20)
non-AML leukemia (21)
Solid tumor (676)
n.s.
p = 9.6X10-7
Gene Effect (CERES)
from DEPMAP database
C
Correlation coefficient to ZMYND8
MEF2C (3)
SYNCRIP (9)
NPM1 (11)
SETD1A (46)
BRD9 (110)
ZFP64 (327)
KMT2B (129)
IRF8 (685)
Ranked genes (3,102)
D
Hypersensitive
MV4;11 (AML)
NOMO-1 (AML)
THP-1 (AML)
OCI-AML3 (AML)
SEM (B-ALL)
Relative sgRNA+, % (Normalized to day 3)
sgNeg
sgPCNA
sgZMYND8_1
sgZMYND8_2
sgZMYND8_3
Days post-infection
E
Sensitive
U937 (AML)
REH (B-ALL)
K562 (CML)
JURKAT (T-ALL)
F
Insensitive
A549 (Lung cancer)
G
MOLM-13
MV4;11
NOMO-1
THP-1
OCI-AML3
SEM
K562
HEL
U937
HUH7
A549
ZMYND8
IRF8
GAPDH
H
CCLE database
ZMYND8 expression--Log2(RPKM)
MOLM13
MV4;11
NOMO-1
THP1
OCI-AML3
SEM
SET-2
U937
HEL
REH
JURKAT
K562
HepG2
HUH7
SK-Hep1
HUH1
A549
DMS114
NCI-H526
NCI-H82
A673
Leukemia
Liver cancer
Lung cancer
Sarcoma
I
MOLM-13
9 days post transplant
p < 0.0001
BM GFP+ (sgRNA+), %
sgNeg
sgZMYND8_2
J
% engraftment
n.s
**
n.s
***
n.s
***
hCD45
GFP+
** p < 0.01
*** p < 0.001
sgNeg
sgZMYND8_2
BM
SP
PB
K
sgNeg
sgZMYND8_2
ZMYND8
1
0.08
1
0.78
1.28
0.24
0.53
0.95
GAPDH
Day 4 post-infection
Moribund mice
M
sgNeg3
sgZmynd8_1
sgZmynd8_2
ZMYND8
GAPDH

Figure S3. CRISPR screens identify ZMYND8 as an AML-biased dependency. Related to Figure 3.

(A) Rank plot of negative AML-biased ES of 197 domains of chromatin regulators (CRs). Known AML dependencies are labeled in black. **(B)** ZMYND8 ESs extracted from the DEPMAP dataset. Shown is a boxplot of the copy number–adjusted ESs of IRF8 across 20 AML lines, 21 non-AML leukemia lines, and 676 solid tumor cell lines. CERES, a normalized metric of gene essentiality. *p* values were calculated by Welch's two-sided *t*-test. **(C)** Correlated essentiality between *ZMYND8* ES and 3,102 gene ESs from a genome-wide CRISPR screen in leukemia cells (Wang et al., 2017). Pan-essential and non-essential genes are excluded. Remaining gene ESs were ranked by Pearson's correlation coefficient to *ZMYND8* ES. Known AML dependencies are labeled in red. Numerical ranking from the top most highly correlated gene ES with *ZMYND8* ES is indicated in parentheses. **(D-F)** Additional competition-based proliferation assays performed in hypersensitive (D), sensitive (E), and insensitive (F) cell lines as shown in Figure 3D. Three independent sgRNA were used. Data points represent the average of three independent biological replicates (n = 3). **(G)** Immunoblotting of ZMYND8, IRF8, or GAPDH (loading control) in indicated cell lines. A representative experiment of three biological replicates is shown. **(H)** *ZMYND8* mRNA expression level ($\log_2$ RPKM values from RNA-seq) across cancer cell lines from the CCLE database. Cell lines with high IRF8 expression (*IRF8*^{hi}, RPKM>5, with detectable protein levels shown in Figure S3G) are labeled in green. **(I)** Flow-cytometry analysis of GFP+ MOLM-13 cells in BM of recipient mice sacrificed after 9 days post-transplantation. Prior to transplantation, MOLM-13 cells were transduced with indicated sgRNAs and sorted for GFP% after 48 hours. Statistical analysis (*p* value) was performed using unpaired Student's t-test. Plotted are the mean ± SEM (n = 4). **(J)** Flow-cytometry analysis of hCD45/GFP% leukemia in BM, SP and PB of moribund mice (n=5-6). Statistical analysis (*p* value) was performed using unpaired Student's t-test. **(K)** Western blot analysis of MOLM-13 cells with indicated sgRNA prior transplantation, or sorted GFP+ MOLM-13 cells from the BM of moribund mice (n=3). Relative protein expression normalized to sgNeg shown. **(L)** Immunoblotting of ZMYND8 or GAPDH (loading control) in whole-cell lysates prepared from Cas9+ murine BM cells. BM, bone marrow; SP, spleen; PB, peripheral blood. Error bars smaller than symbol width are not shown. Error bars represent mean ± SEM.

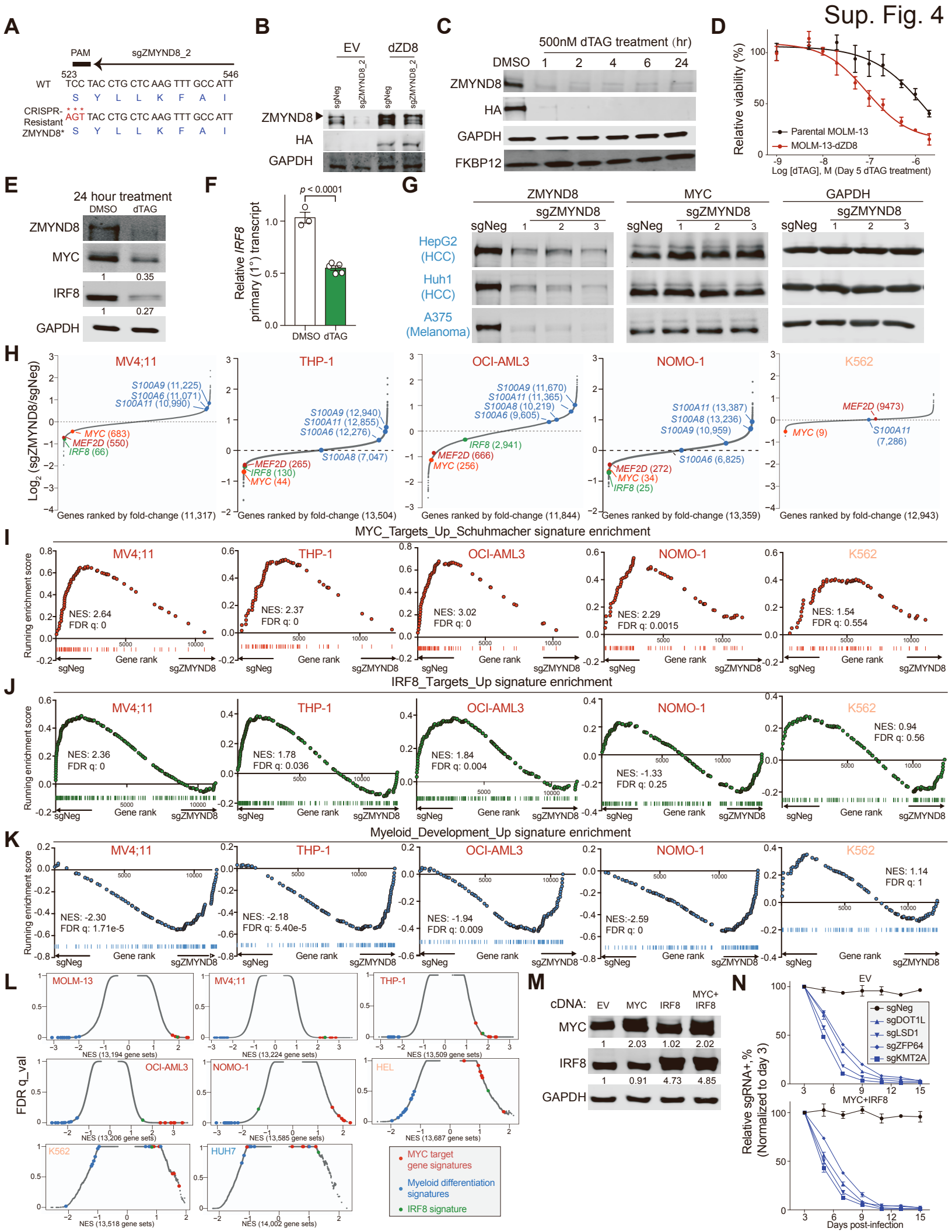
A
PAM
sgZMYND8_2
523
546
WT TCC TAC CTG CTC AAG TTT GCC ATT
S Y L L K F A I
CRISPR-Resistant ZMYND8* AGT TAC CTG CTC AAG TTT GCC ATT
S Y L L K F A I
B
EV
dZD8
sgNeg
sgZMYND8_2
ZMYND8
HA
GAPDH
C
500nM dTAG treatment (hr)
DMSO 1 2 4 6 24
ZMYND8
HA
GAPDH
FKBP12
D
Relative viability (%)
Parental MOLM-13
MOLM-13-dZD8
Log [dTAG], M (Day 5 dTAG treatment)
E
24 hour treatment
DMSO dTAG
ZMYND8
MYC
1 0.35
IRF8
1 0.27
GAPDH
F
p < 0.0001
Relative IRF8 primary (1°) transcript
DMSO dTAG
G
ZMYND8
MYC
GAPDH
sgZMYND8
sgNeg 1 2 3
HepG2 (HCC)
Huh1 (HCC)
A375 (Melanoma)
H
MV4;11
THP-1
OCI-AML3
NOMO-1
K562
Log2 (sgZMYND8/sgNeg)
S100A9 (11,225)
S100A6 (11,071)
S100A11 (10,990)
MYC (683)
MEF2D (550)
IRF8 (66)
Genes ranked by fold-change (11,317)
S100A9 (12,940)
S100A11 (12,855)
S100A6 (12,276)
S100A8 (7,047)
MEF2D (265)
IRF8 (130)
MYC (44)
Genes ranked by fold-change (13,504)
S100A9 (11,670)
S100A11 (11,365)
S100A8 (10,219)
S100A6 (9,605)
IRF8 (2,941)
MEF2D (666)
MYC (256)
Genes ranked by fold-change (11,844)
S100A11 (13,387)
S100A8 (13,236)
S100A9 (10,959)
S100A6 (6,825)
MEF2D (272)
MYC (34)
IRF8 (25)
Genes ranked by fold-change (13,359)
MEF2D (9473)
MYC (9)
S100A11 (7,286)
Genes ranked by fold-change (12,943)
I
MYC_Targets_Up_Schuhmacher signature enrichment
Running enrichment score
NES: 2.64
FDR q: 0
NES: 2.37
FDR q: 0
NES: 3.02
FDR q: 0
NES: 2.29
FDR q: 0.0015
NES: 1.54
FDR q: 0.554
sgNeg
Gene rank
sgZMYND8
J
IRF8_Targets_Up signature enrichment
NES: 2.36
FDR q: 0
NES: 1.78
FDR q: 0.036
NES: 1.84
FDR q: 0.004
NES: -1.33
FDR q: 0.25
NES: 0.94
FDR q: 0.56
K
Myeloid_Development_Up signature enrichment
NES: -2.30
FDR q: 1.71e-5
NES: -2.18
FDR q: 5.40e-5
NES: -1.94
FDR q: 0.009
NES:-2.59
FDR q: 0
NES: 1.14
FDR q: 1
L
MOLM-13
MV4;11
THP-1
OCI-AML3
NOMO-1
HEL
K562
HUH7
FDR q_val
NES (13,194 gene sets)
NES (13,224 gene sets)
NES (13,509 gene sets)
NES (13,206 gene sets)
NES (13,585 gene sets)
NES (13,687 gene sets)
NES (13,518 gene sets)
NES (14,002 gene sets)
MYC target gene signatures
Myeloid differentiation signatures
IRF8 signature
M
cDNA: EV MYC IRF8 MYC+ IRF8
MYC
1 2.03 1.02 2.02
IRF8
1 0.91 4.73 4.85
GAPDH
N
EV
MYC+IRF8
sgNeg
sgDOT1L
sgLSD1
sgZFP64
sgKMT2A
Relative sgRNA+,% (Normalized to day 3)
Days post-infection

Figure S4. ZMYND8 regulates *IRF8* and *MYC* transcription to sustain AML proliferation. Related to Figure 4.

(A) Design of CRISPR-resistant ZMYND8 cDNA. The CRISPR-resistant ZMYND8 cDNA sequence contains the synonymous mutagenized 3' PAM sequence (red). Encoded amino acids are labeled in blue at the bottom of the cDNA sequence. **(B)** Immunoblotting of ZMYND8, HA, or GAPDH (loading control) in whole-cell lysates from MOLM-13 cells expressing indicated cDNA and sgRNA. Arrowhead denotes the ZMYND8 signal. A representative experiment of three biological replicates is shown. **(C)** Immunoblotting of MOLM-13-dZD8 whole cell lysate treated with DMSO or 500 nM dTAG-47 over time. FKBP12 serves to control $FKBP12^{G36V}$ levels; GAPDH serves as a loading control. A representative experiment of three biological replicates is shown. **(D)** Relative viability of MOLM-13-dZD8 or parental cells measured by CellTiter-Glo assays. Normalized relative luminescence units (RLU) are shown after 5 days in culture with 0.05% DMSO or dTAG-47 at indicated concentrations. Plotted are the mean ± SEM (N = 3) and 4-parameter dose-response curves. **(E)** Immunoblotting of ZMYND8, MYC, IRF8, or GAPDH in whole-cell lysates prepared from MOLM13-dZD8 cells treated with DMSO or 500 nM dTAG for 24 hours. Relative protein expression normalized to GAPDH and DMSO counterparts for MYC and IRF8 are shown. **(F)** RT-qPCR of relative *IRF8* nascent transcript level within 4 hours of 500nM dTAG exposure in MOLM13-dZD8 cells (n=3-5). **(G)** immunoblotting in solid tumor lines, related to Figure 4F. **(H)** *RNA*-seq analysis of gene expression changes in additional cell lines 5 days after sgRNA transduction. Complementary to Figure 4A. Note that IRF8 is not significantly expressed in K562 cells. **(I-K)** GSEA analysis of RNA-seq data in Figure S4A. (I), IRF8_Targets_Up (J) or Myeloid_Development_Up (K) signature was used. Normalized enrichment score (NES) and False Discovery Rate (FDR) q value are shown. **(L)** Unbiased GSEA analysis of RNA-seq data in Figure 4A and Figure S4A using all signatures from MSigDB v6.1 (Liberzon et al., 2015). Each gene set is represented as a single dot. MYC target gene signatures and myeloid differentiation signatures are provided in Table S1. **(M)** Immunoblotting of MYC or IRF8 expression in whole-cell lysates from MOLM-13 cells transduced with EV, MYC, IRF8, or MYC+IRF8 cDNA. EV, empty vector. A representative experiment of three biological replicates is shown. Below is the quantification of expression normalized by GAPDH and EV counterparts. **(N)** Competition-based proliferation assays performed in MOLM-13 cells expressing sgRNA-resistant EV or MYC+IRF8, and transduced with indicated sgRNAs.

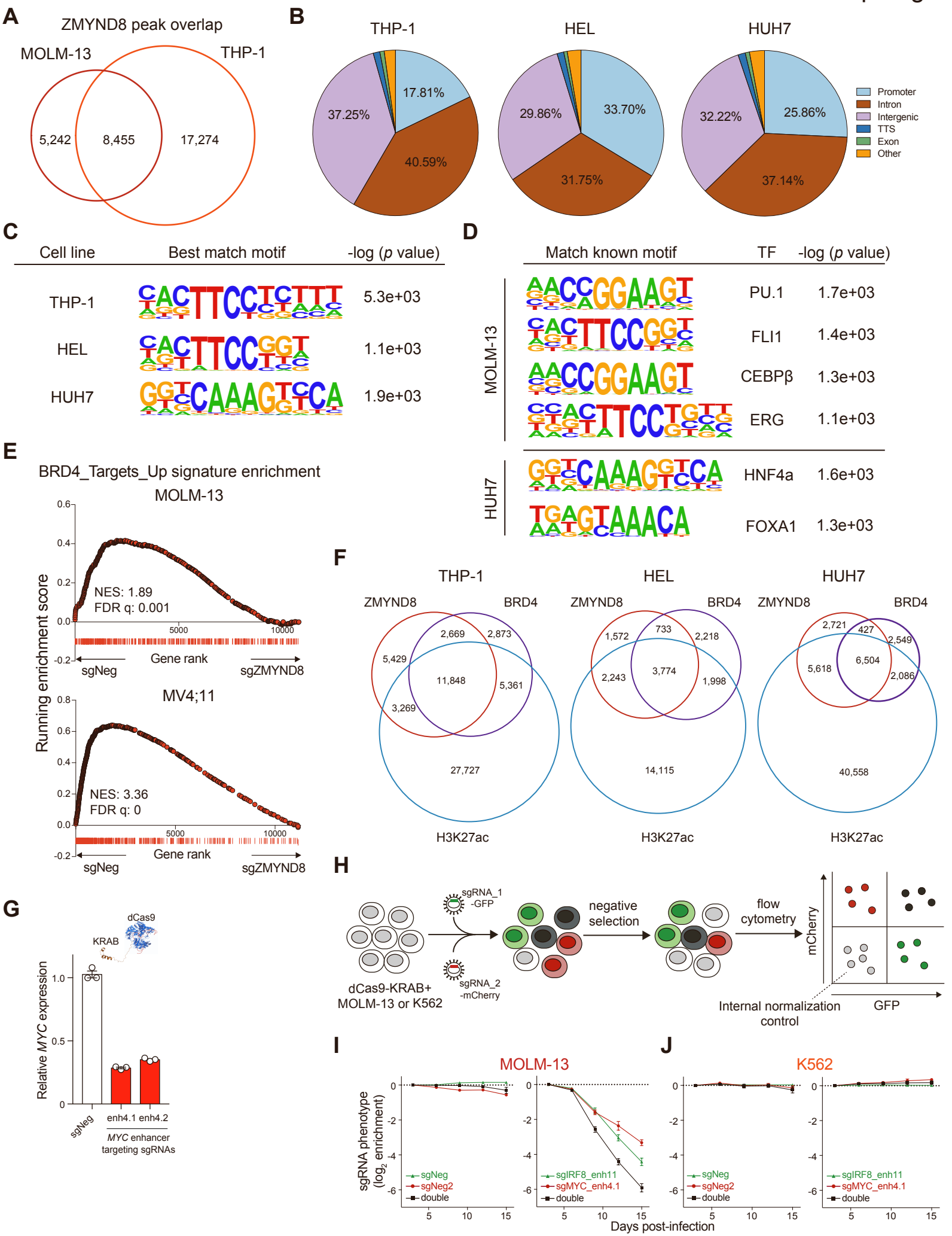
A
ZMYND8 peak overlap
MOLM-13
THP-1
5,242
8,455
17,274
B
THP-1
HEL
HUH7
17.81%
40.59%
37.25%
33.70%
31.75%
29.86%
25.86%
37.14%
32.22%
Promoter
Intron
Intergenic
TTS
Exon
Other
C
Cell line
Best match motif
-log (p value)
THP-1
5.3e+03
HEL
1.1e+03
HUH7
1.9e+03
D
Match known motif
TF
-log (p value)
MOLM-13
PU.1
1.7e+03
FLI1
1.4e+03
CEBPβ
1.3e+03
ERG
1.1e+03
HUH7
HNF4a
1.6e+03
FOXA1
1.3e+03
E
BRD4_Targets_Up signature enrichment
MOLM-13
NES: 1.89
FDR q: 0.001
Running enrichment score
Gene rank
sgNeg
sgZMYND8
MV4;11
NES: 3.36
FDR q: 0
F
THP-1
HEL
HUH7
ZMYND8
BRD4
H3K27ac
5,429
2,669
2,873
11,848
5,361
3,269
27,727
1,572
733
2,218
2,243
3,774
1,998
14,115
2,721
427
2,549
5,618
6,504
2,086
40,558
G
dCas9
KRAB
Relative MYC expression
sgNeg
enh4.1
enh4.2
MYC enhancer targeting sgRNAs
H
sgRNA_1 -GFP
sgRNA_2 -mCherry
dCas9-KRAB+ MOLM-13 or K562
negative selection
flow cytometry
mCherry
GFP
Internal normalization control
I
MOLM-13
J
K562
sgRNA phenotype (log2 enrichment)
sgNeg
sgNeg2
double
sgIRF8_enh11
sgMYC_enh4.1
Days post-infection

Figure S5. Genome-wide binding profiles reveal the co-occupancy of ZMYND8 and BRD4 in active enhancer regions. Related to Figure 5.

(A) Venn diagram of ZMYND8 peak overlap between MOLM-13 and THP-1 cell lines. **(B)** Pie chart annotating the distribution of 13,125 ZMYND8 peaks in indicated cell lines. TTS, transcription termination site. Other, UTR and non-coding RNA regions. **(C)** ZMYND8 CUT&RUN-derived *de novo* motif analysis in indicated cell lines using HOMER. Statistical analysis (*p* value) was calculated using a binomial test. **(D)** ZMYND8 CUT&RUN-derived known motif analysis in MOLM-13 and HUH7 cell lines using HOMER. Shown are known TF motifs. Statistical analysis (*p* value) was calculated using a binomial test. **(E)** GSEA analysis of RNA-seq data in MOLM-13 (top) and MV4;11 (bottom) cells transduced with two independent sgRNAs targeting ZMYND8 or two negative control sgRNAs (sgNeg). The BRD4 signature was defined as the top 500 downregulated genes in AML cells following 24 hours of BET inhibitor (JQ1) treatment. Data derived from GSE63782. Normalized enrichment score (NES) and False Discovery Rate (FDR) q values are shown. **(F)** Venn diagrams of peak overlap between ZMYND8, BRD4, and H3K27ac in indicated cell lines. **(G)** RT-qPCR of *MYC* levels in MOLM-13 cells transduced with sgRNA targeting *MYC* enhancer ME4 (n=3). **(H)** Scheme of dual sgRNA validation assay (Horlbeck et al., 2018). Cells expressing dCas9-KRAB were simultaneously transduced with lentiviral sgRNA vectors containing either GFP or mCherry, which produced a mixed population of uninfected, GFP+, mCherry+ or GFP+/mCherry+ cells. Growth effect of a single or combinatorial sgRNA was assessed by the ratios of these populations measured by the fluorescent signals, with the uninfected cells serving as an internal control for normalization. (**I-J)** Individual validation experiments for sgMYC_enh4.1 and sgIRF8_enh11 pairs in MOLM-13 (I) or K562 (J) cells. sgRNA+ populations were monitored over time with a GFP or mCherry co-expression marker. Green, red, and black lines represent single sgRNA phenotypes of GFP+ vectors, single phenotypes of mCherry+ vectors, and double positive phenotypes, respectively. Plotted are the relative sgRNA+ populations normalized to the day 3 sgRNA+ population over 15 days (n=3-5). Error bars smaller than symbol width are not shown. Error bars represent mean ± SEM.

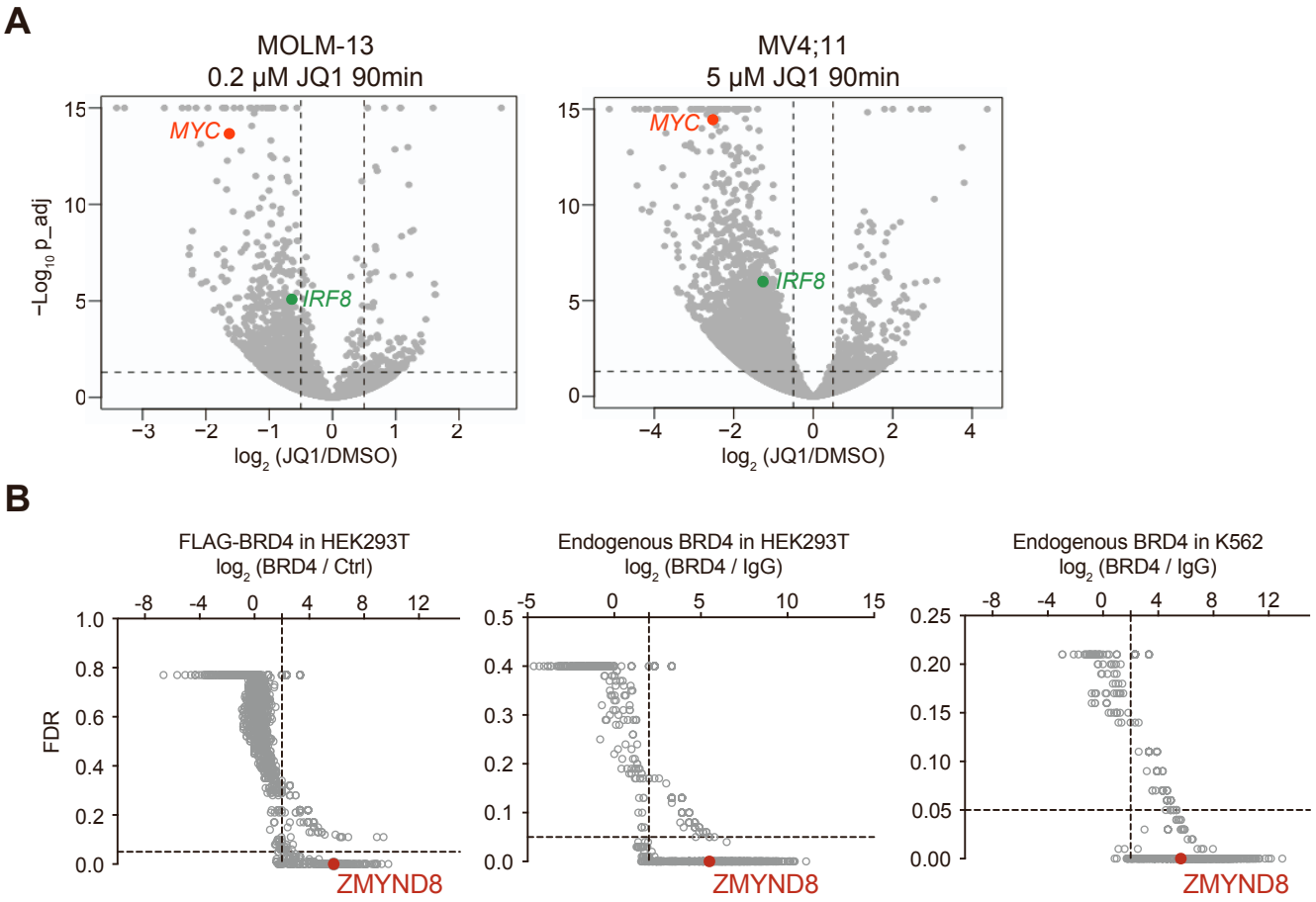
A
MOLM-13
0.2 μM JQ1 90min
MV4;11
5 μM JQ1 90min
MYC
IRF8
−Log10 p_adj
log2 (JQ1/DMSO)
B
FLAG-BRD4 in HEK293T
log2 (BRD4 / Ctrl)
Endogenous BRD4 in HEK293T
log2 (BRD4 / IgG)
Endogenous BRD4 in K562
log2 (BRD4 / IgG)
FDR
ZMYND8

Figure S6. ZMYND8 occupies active elements in AML through binding the ET domain of BRD4. Related to Figure 6.

(A) SLAM-seq analysis of MOLM-13 cells treated with 0.2 µM JQ1, or MV4;11 cells treated with 5 µM JQ1 for 90 minutes. *p* values were calculated using DESeq2. Data were extracted from GSE63782. **(B)** IP-MS analysis performed with nuclear lysates prepared from HEK293T cells transiently expressing FLAG-BRD4 or a streptavidin bead control (left), nuclear lysates prepared from HEK293T cells using anti-BRD4 antibody to enrich endogenous BRD4 (middle), or nuclear lysates prepared from K562 cells using an anti-BRD4 antibody to enrich endogenous BRD4 (right). ZMYND8 peptide enrichment is labeled in red. Data were extracted from Lambert et al., 2018. FDR, False Discovery Rate.

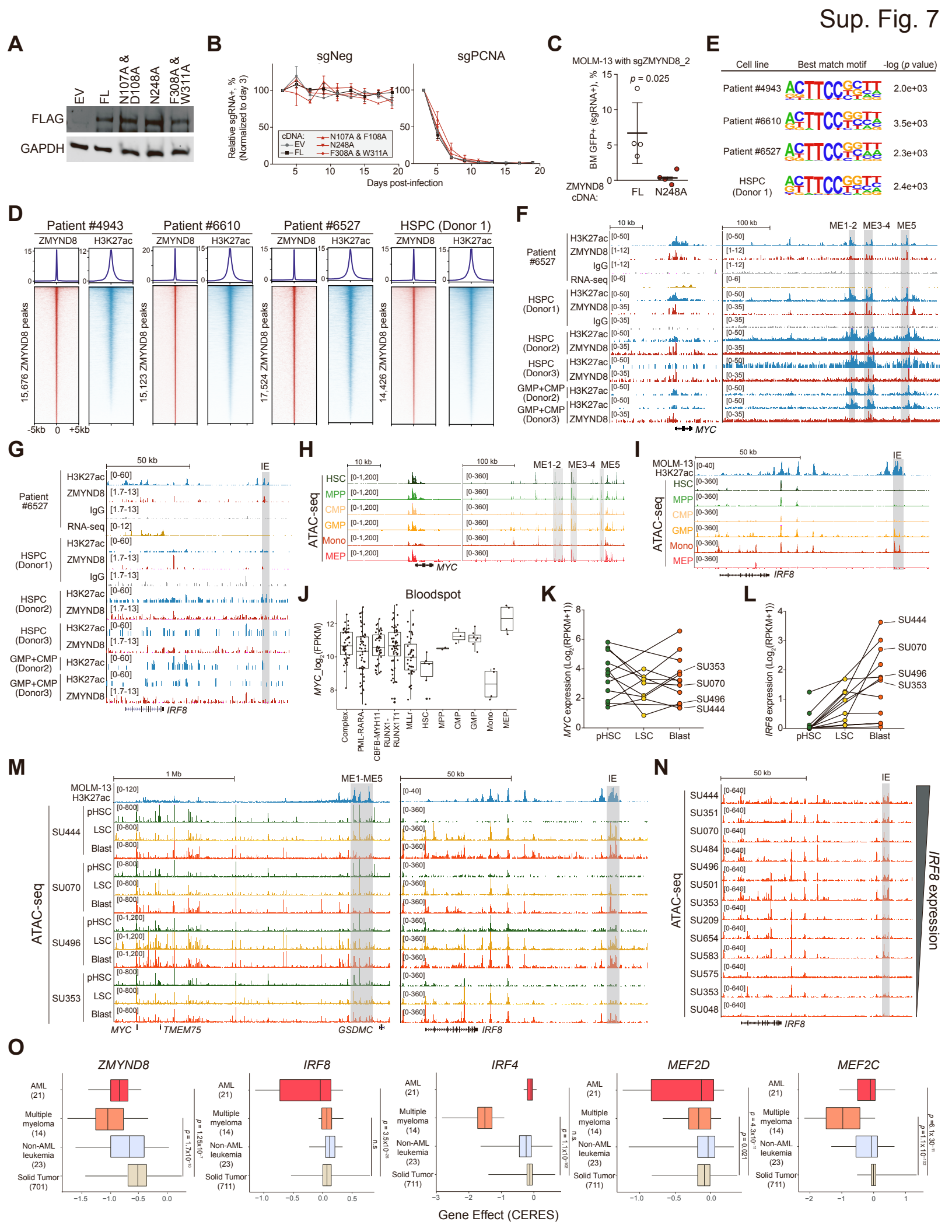
A
EV
FL
N107A & D108A
N248A
F308A & W311A
FLAG
GAPDH
B
sgNeg
sgPCNA
Relative sgRNA+, % (Normalized to day 3)
cDNA: EV FL N107A & F108A N248A F308A & W311A
Days post-infection
C
MOLM-13 with sgZMYND8_2
p = 0.025
BM GFP+ (sgRNA+), %
ZMYND8 cDNA:
FL
N248A
E
Cell line
Best match motif
-log (p value)
Patient #4943
2.0e+03
Patient #6610
3.5e+03
Patient #6527
2.3e+03
HSPC (Donor 1)
2.4e+03
D
Patient #4943
Patient #6610
Patient #6527
HSPC (Donor 1)
ZMYND8
H3K27ac
15,676 ZMYND8 peaks
15,123 ZMYND8 peaks
17,524 ZMYND8 peaks
14,426 ZMYND8 peaks
-5kb 0 +5kb
F
Patient #6527
HSPC (Donor1)
HSPC (Donor2)
HSPC (Donor3)
GMP+CMP (Donor2)
GMP+CMP (Donor3)
H3K27ac
ZMYND8
IgG
RNA-seq
ME1-2
ME3-4
ME5
MYC
G
Patient #6527
HSPC (Donor1)
HSPC (Donor2)
HSPC (Donor3)
GMP+CMP (Donor2)
GMP+CMP (Donor3)
IE
IRF8
H
ATAC-seq
HSC
MPP
CMP
GMP
Mono
MEP
ME1-2
ME3-4
ME5
MYC
I
MOLM-13 H3K27ac
ATAC-seq
IE
IRF8
J
Bloodspot
MYC_log2(FPKM)
Complex
PML-RARA
CBFB-MYH11
RUNX1-RUNX1T1
MLLr
HSC
MPP
CMP
GMP
Mono
MEP
K
MYC expression (Log2(RPKM+1))
pHSC
LSC
Blast
SU353
SU070
SU496
SU444
L
IRF8 expression (Log2(RPKM+1))
pHSC
LSC
Blast
SU444
SU070
SU496
SU353
M
MOLM-13 H3K27ac
ATAC-seq
SU444
SU070
SU496
SU353
pHSC
LSC
Blast
ME1-ME5
IE
MYC
TMEM75
GSDMC
IRF8
N
ATAC-seq
SU444
SU351
SU070
SU484
SU496
SU501
SU353
SU209
SU654
SU583
SU575
SU353
SU048
IE
IRF8
IRF8 expression
O
ZMYND8
IRF8
IRF4
MEF2D
MEF2C
AML (21)
Multiple myeloma (14)
Non-AML leukemia (23)
Solid Tumor (701)
Solid Tumor (711)
p = 1.25x10^-27
p = 1.7x10^-10
p = 5x10^-25
n.s
p = 1.1x10^-102
p = 4.3x10^-11
p = 0.021
p = 6.1x10^-11
p = 1.1x10^-102
Gene Effect (CERES)

Figure S7. ZMYND8 PHD-BD-PWWP reader cassette is required for association with BRD4 on chromatin and leukemia growth. Related to Figure 7.

(A) Immunoblotting for FLAG or GAPDH (loading control) in nuclear lysates prepared from MOLM-13 cells stably expressing EV, FL, or mutated ZMYND8 cDNA, and transduced with sgNeg or sgZMYND8_2. Cells were collected 5 days post-infection. A representative experiment of three biological replicates is shown. **(B)** Competition-based proliferation assays performed in MOLM-13 cells stably expressing the indicated cDNA and sgRNA. Data points represent the average of three independent biological replicates (n = 3). Error bars smaller than symbol width are not shown. Error bars represent mean ± SEM. **(C)** Flow-cytometry analysis of GFP+ leukemia cells in BM of recipient mice sacrificed after 9 days post-transplantation. MOLM-13 cells stably expressing WT or BD-mutated (N248A) ZMYND8 were transduced with indicated sgRNAs. Prior to transplantation, GFP+ cells were sorted after 48 hours post-transduction. Statistical analysis was performed using an unpaired Student's t-test. Plotted are the mean ± SEM (n = 4). BM, bone marrow. **(D)** Meta-profile (top) and density plot (bottom) of ZMYND8 and H3K27ac CUT&RUN peaks at ZMYND8-occupied regions in primary AML blasts or HSPCs. Peaks were ranked by ZMYND8 CUT&RUN tag counts. HSPC, hematopoietic stem and progenitor cell. **(E)** ZMYND8 CUT&RUN-derived *de novo* motif analysis in primary AML blasts or HSPCs using HOMER. Statistical analysis (*p* value) was calculated using the binomial test. **(F-G)** Gene track of H3K27ac and ZMYND8 enrichment in leukemic *MYC* enhancer (F), and *IRF8* (G) regions in AML patient blast #6527, three independent HSPC or two independent GMP+CMP populations. RNA-seq data also shown. **(H-I)** Gene track of ATAC-seq in normal myelogenesis in the *MYC*, leukemic *MYC* enhancer (H), and *IRF8* (I) regions. Data were extracted from GSE75384. HSC, Hematopoietic stem cell; MPP, Multipotent progenitor; CMP, Common myeloid progenitor; GMP, Granulocyte monocyte progenitor; Mono, Monocyte; MEP, Megakaryocyte-erythroid progenitor. **(J)** *MYC* mRNA expression in human leukemia patients harboring different cytogenetics or in purified normal human hematopoietic cells from BloodSpot data collection. *MLL*r, *MLL*-rearrangement. Error bars represent mean ± SEM. **(K-L)** mRNA expression analysis of *MYC* (K) or *IRF8* (L) during leukemogenesis in primary AML samples. pHSC, preleukemic hematopoietic stem cell. LSC, leukemic stem cell. **(M)** Gene track of ATAC-seq during leukemogenesis in four primary AML samples at leukemic *MYC* (left) and *IRF8* (right) enhancer regions. **(N)** Gene track of ATAC-seq in 13 primary AML blasts at IE regions. Data were extracted from GSE75384. **(O)** *ZMYND8*, *IRF8*, *IRF4*, *MEF2D*, and *MEF2C* CERESs from the DEPMAP dataset. Note that ZMYND8 CERES of 10 solid tumor lines were filtered out in DEPMAP due to low screening quality.