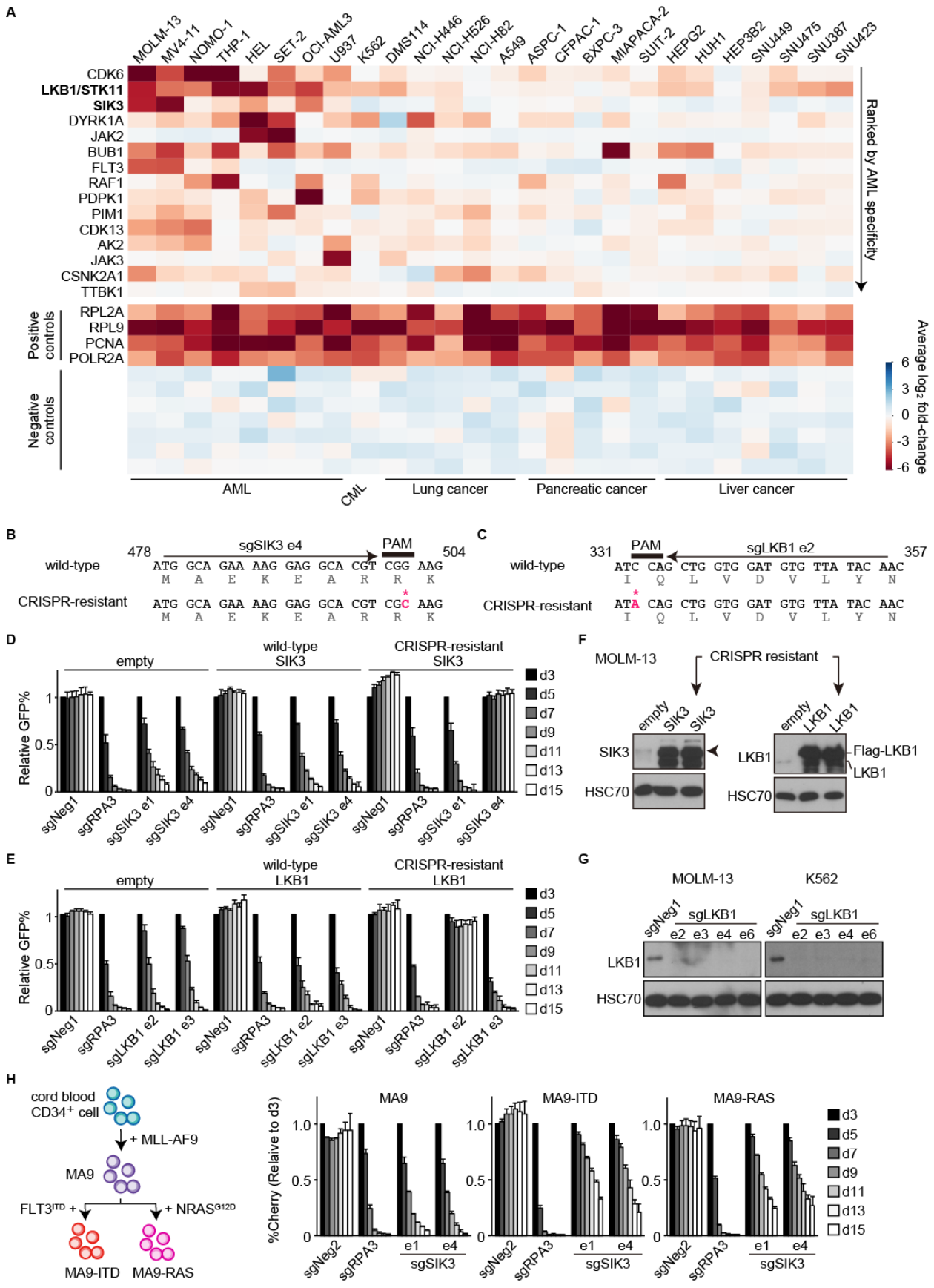

A
MOLM-13
MV4-11
NOMO-1
THP-1
HEL
SET-2
OCI-AML3
U937
K562
DMS114
NCI-H446
NCI-H526
NCI-H82
A549
ASPC-1
CFPAC-1
BXPC-3
MIAPACA-2
SUIT-2
HEPG2
HUH1
HEP3B2
SNU449
SNU475
SNU387
SNU423
CDK6
LKB1/STK11
SIK3
DYRK1A
JAK2
BUB1
FLT3
RAF1
PDPK1
PIM1
CDK13
AK2
JAK3
CSNK2A1
TTBK1
Ranked by AML specificity
Positive controls
RPL2A
RPL9
PCNA
POLR2A
Negative controls
Average log2 fold-change
6
3
0
-3
-6
AML
CML
Lung cancer
Pancreatic cancer
Liver cancer
B
wild-type
478
sgSIK3 e4
PAM
504
ATG GCA GAA AAG GAG GCA CGT CGG AAG
M A E K E A R R K
CRISPR-resistant
ATG GCA GAA AAG GAG GCA CGT CGC AAG
M A E K E A R R K
C
wild-type
331
PAM
sgLKB1 e2
357
ATC CAG CTG GTG GAT GTG TTA TAC AAC
I Q L V D V L Y N
CRISPR-resistant
ATA CAG CTG GTG GAT GTG TTA TAC AAC
I Q L V D V L Y N
D
empty
wild-type SIK3
CRISPR-resistant SIK3
Relative GFP%
d3
d5
d7
d9
d11
d13
d15
sgNeg1
sgRPA3
sgSIK3 e1
sgSIK3 e4
E
empty
wild-type LKB1
CRISPR-resistant LKB1
Relative GFP%
sgNeg1
sgRPA3
sgLKB1 e2
sgLKB1 e3
F
MOLM-13
CRISPR resistant
empty
SIK3
SIK3
SIK3
HSC70
empty
LKB1
LKB1
LKB1
Flag-LKB1
LKB1
HSC70
G
MOLM-13
K562
sgNeg1
sgLKB1
e2 e3 e4 e6
LKB1
HSC70
H
cord blood CD34+ cell
+ MLL-AF9
MA9
FLT3ITD +
+ NRASG12D
MA9-ITD
MA9-RAS
MA9
MA9-ITD
MA9-RAS
%Cherry (Relaive to d3)
sgNeg2
sgRPA3
e1
e4
sgSIK3
d3
d5
d7
d9
d11
d13
d15

Figure S1. Kinase domain-focused CRISPR screen in cancer cell lines identifies LKB1 and SIK3 as AML-biased dependencies. Related to Figure 1.

(A) Summary of pooled kinase domain-focused CRISPR screen in a panel of cancer cell lines. Average $\log_2$ fold-change of the top 15 AML-biased kinase dependencies. Also shown are positive and negative control sgRNAs spiked into the sgRNA library.

(B and C) Design of CRISPR-resistant mutants of SIK3 and LKB1.

(D and E) Competition-based proliferation assays for rescue experiments in MOLM-13 harboring empty vector, wild-type, or CRISPR-resistant SIK3 or LKB1 cDNA. The indicated sgRNA vectors are linked to GFP.

(F) Western blot of SIK3 or LKB1 in MOLM-13 cells harboring the CRISPR-resistant cDNA.

(G) Western blot of LKB1 in MOLM-13 and K562 cells on day 4 after infection with negative control (Neg1) or LKB1 sgRNA.

(H) Schematic diagram of development of MA9 (genetically-engineered human MLL-AF9 AML lines) and its derivatives (left). Competition-based proliferation assays in MA9, MA9-ITD, and MA9-RAS cells infected with control (Neg2 or RPA3) or SIK3 sgRNA.

All bar graphs represent the mean ± SEM (n=3)

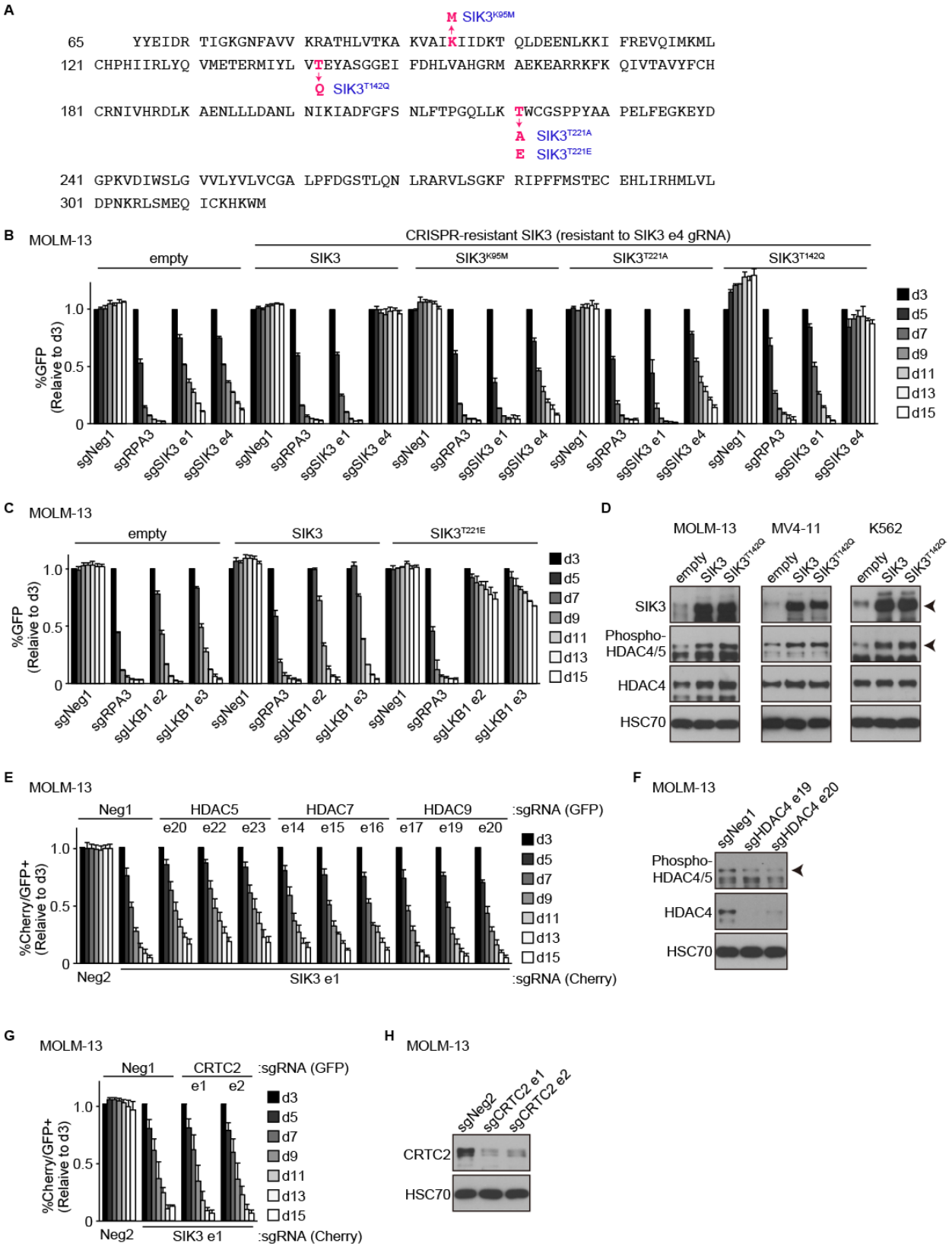


Figure S2. Genetic experiments establish an essential LKB1-SIK3-HDAC4 pathway in

AML. Related to Figure 2.

(A) Amino acid sequence of SIK3 kinase domain and sites of substitutions for making mutant cDNA.

(B and C) Competition-based proliferation assays in MOLM-13 cells harboring empty vector or the indicated SIK3 cDNA, infected with control, SIK3 sgRNA (C) or LKB1 sgRNA (D). This figure shows the individual sgRNA data for the summarized data shown in Figures 2C-D.

(D) Western blot of SIK3, HDAC4, phosphor-HDAC4/5 in MOLM-13, MV4-11 and K562 cells harboring empty vector, SIK3, or $SIK3^{T142Q}$ cDNA.

(E and G) Competition-based proliferation assays in MOLM-13 cells co-infected with GFP-linked sgRNA and mCherry-linked sgRNA. The percentage of mCherry-positive cells in GFP-positive cells is shown. The data set for Neg1-GFP/Neg2-Cherry in (E) is the same in Figure 2I.

(F) Western blot of HDAC4 and phospho-HDAC4/5 in MOLM-13 cells on day 4 after infection with control or HDAC4 sgRNA.

(H) Western blot of CRTC2 in MOLM-13 cells on day 4 after infection with control or CRTC2 sgRNA.

All bar graphs represent the mean ± SEM (n=3)

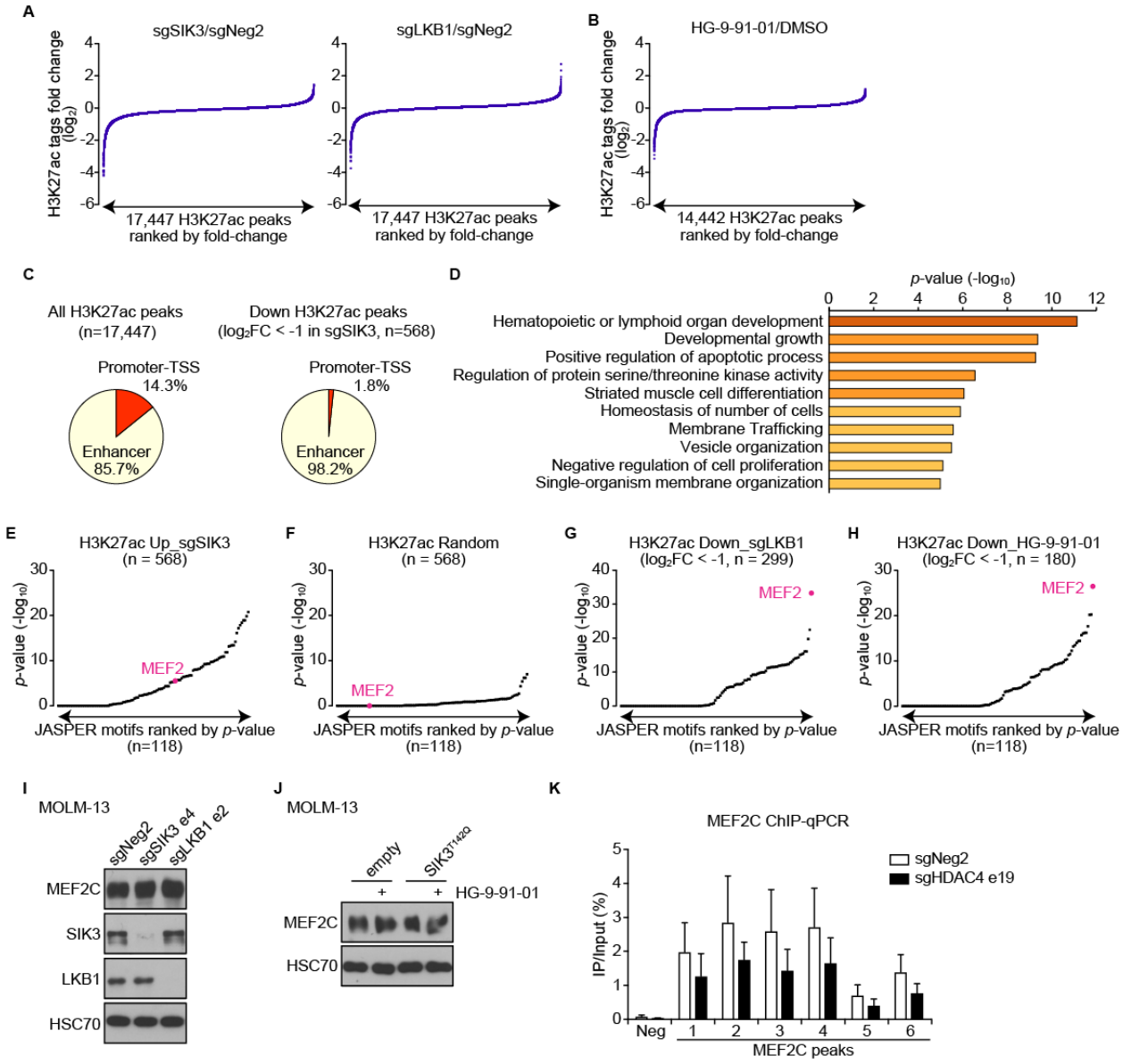


Figure S3. LKB1 and SIK3 are critical to maintain histone acetylation at MEF2C-bound enhancer elements. Related to Figure 3.

(A) Fold-change of H3K27ac tags upon SIK3 or LKB1 knockout in MOLM-13 cells at 17,447 H3K27ac peaks, which have 10 or more tags in control (sgNeg2).

(B) Fold-change of H3K27ac tags upon HG-9-91-01 treatment (100 nM, 2 hr) in MOLM-13 cells at 14,442 H3K27ac peaks, which have 10 or more tags in control (DMSO).

(C) Genomic annotation of all H3K27ac peaks and SIK3-dependent H3K27ac sites in

MOLM-13.

(D) Ontology analysis of genes located near with the decreased H3K27ac in SIK3 knockout MOLM-13 cells.

(E) Transcription factor binding motifs enriched at the sites with increased H3K27ac upon SIK3 knockout in MOLM-13.

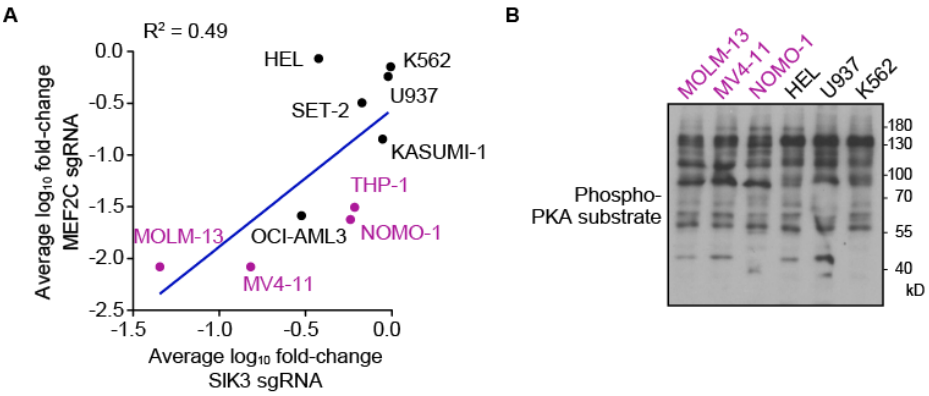
(F) Transcription factor binding motifs enriched in randomly-selected H3K27ac enriched sites in MOLM-13.

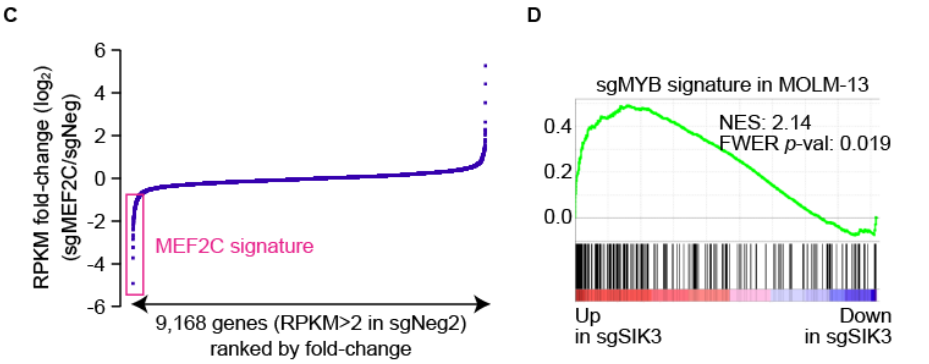
(G and H) Transcription factor binding motifs enriched in the decreased H3K27ac loci upon LKB1 knockout (G) or HG-9-91-01 treatment (100 nM 2 hr) (H) in MOLM-13.

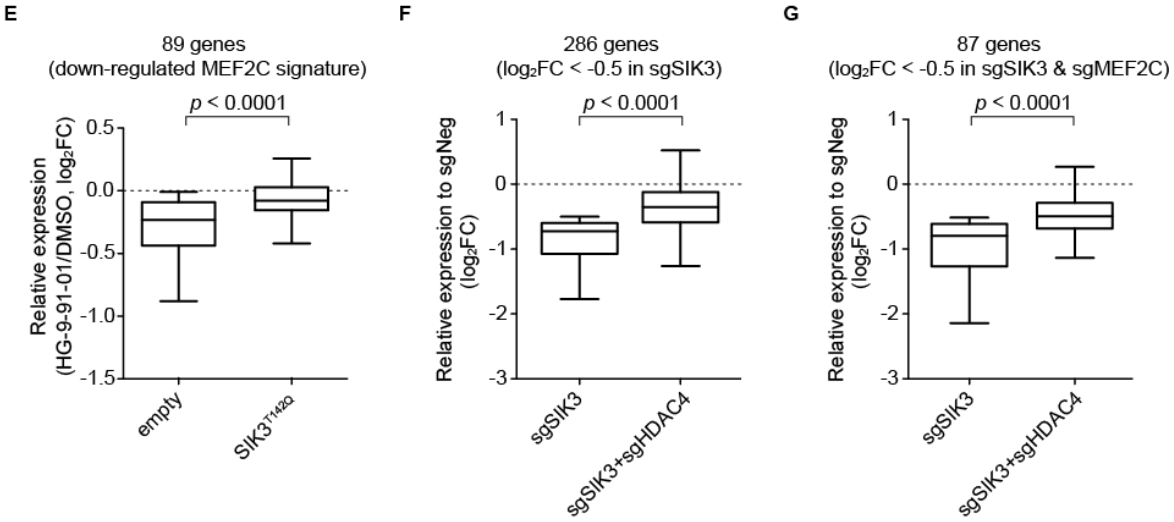
(I) Western blot of the indicated proteins in MOLM-13 cells on day 4 after infection with negative control (Neg2), SIK3 or LKB1 sgRNA.

(J) Western blot of MEF2C in MOLM-13 cells harboring empty vector or $SIK3^{T142Q}$ cDNA, treated with 0.1% DMSO or 100 nM HG-9-91-01 for 2 hrs.

(K) ChIP-qPCR with MEF2C antibody at MEF2C-bound enhancers near the decreased H3K27ac regions in MOLM-13 cells on day 5 after infection with control or HDAC4 sgRNA. Bar graphs represent the mean ± SEM. (n=3)


A
R² = 0.49
HEL
K562
U937
SET-2
KASUMI-1
THP-1
MOLM-13
OCI-AML3
NOMO-1
MV4-11
Average log10 fold-change MEF2C sgRNA
Average log10 fold-change SIK3 sgRNA
B
MOLM-13
MV4-11
NOMO-1
HEL
U937
K562
Phospho-PKA substrate
180
130
100
70
55
40
kD


C
RPKM fold-change (log2) (sgMEF2C/sgNeg)
MEF2C signature
9,168 genes (RPKM>2 in sgNeg2) ranked by fold-change
D
sgMYB signature in MOLM-13
NES: 2.14
FWER p-val: 0.019
Up in sgSIK3
Down in sgSIK3


E
89 genes
(down-regulated MEF2C signature)
p < 0.0001
Relative expression (HG-9-91-01/DMSO, log2FC)
empty
SIK3T142Q
F
286 genes
(log2FC < -0.5 in sgSIK3)
p < 0.0001
Relative expression to sgNeg (log2FC)
sgSIK3
sgSIK3+sgHDAC4
G
87 genes
(log2FC < -0.5 in sgSIK3 & sgMEF2C)
p < 0.0001
Relative expression to sgNeg (log2FC)
sgSIK3
sgSIK3+sgHDAC4

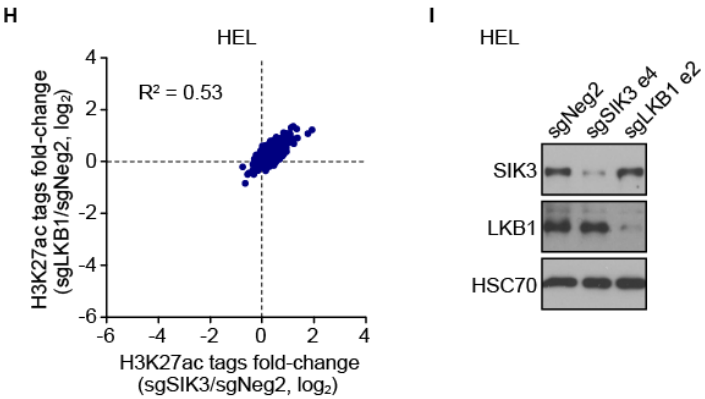

H
HEL
R² = 0.53
H3K27ac tags fold-change (sgLKB1/sgNeg2, log2)
H3K27ac tags fold-change (sgSIK3/sgNeg2, log2)
I
HEL
sgNeg2
sgSIK3 e4
sgLKB1 e2
SIK3
LKB1
HSC70

Figure S4. LKB1, SIK, and MEF2C are linked dependencies in acute myeloid leukemia. Related to Figure 4.

(A) Scatter plot depicting average $\log_{10}$ fold-change of MEF2C or SIK3 sgRNAs for the indicated cell lines in competition-based proliferation assays (arrayed format). The data for SIK3 sgRNAs are the same as in Figure 1B (average of triplicates)..

(B) Western blot of phospho-PKA substrates in leukemia lines.

(C) Fold-change of RPKM of gene expression in MEF2C knockout MOLM-13 cells.

(D) Gene set enrichment analysis (GSEA) plot of the MYB gene signature upon knockout using sgSIK3 compared with control sgNeg2 in MOLM-13 cells. Normalized enrichment score (NES) and family-wise error rate (FWER) *p*-value are shown. MYB gene signatures were prepared as described in Methods section.

(E) Fold-change of the expression of the down-regulated MEF2C signature genes in MOLM-13 cells harboring empty vector or SIK3T^{142Q} treated with DMSO or 100 nM HG-9-91-01 for 2 hrs, is shown in box plot.

(F and G) Fold-change of gene expression in MOLM-13 cells infected with sgSIK3 or co-infected with sgSIK3 and sgHDAC4. The genes that were down-regulated in SIK3 knockout cells (F) or down-regulated both in SIK3 and MEF2C knockout cells (G) are shown in box plot. *p*-value was calculated using unpaired Student's t-test.

(H) Comparison of fold-change of H3K27ac tags upon SIK3 or LKB1 knockout in HEL cells. Each dot represents a single peak of H3K27ac identified in the control (sgNeg2) sample (n=15,146). Cells were harvested on day 5 after sgRNA infection.

(I) Western blot of the indicated proteins in HEL cells on day 4 after infection with negative control (Neg2), SIK3 or LKB1 sgRNA.