Supplemental Information (SI)

M^{6}A demethylase ALKBH5 selectively promotes tumorigenesis and cancer stem cell self-renewal in acute myeloid leukemia

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I. Supplemental Figures

II. Supplemental Table

SUPPLEMENTAL FIGURES

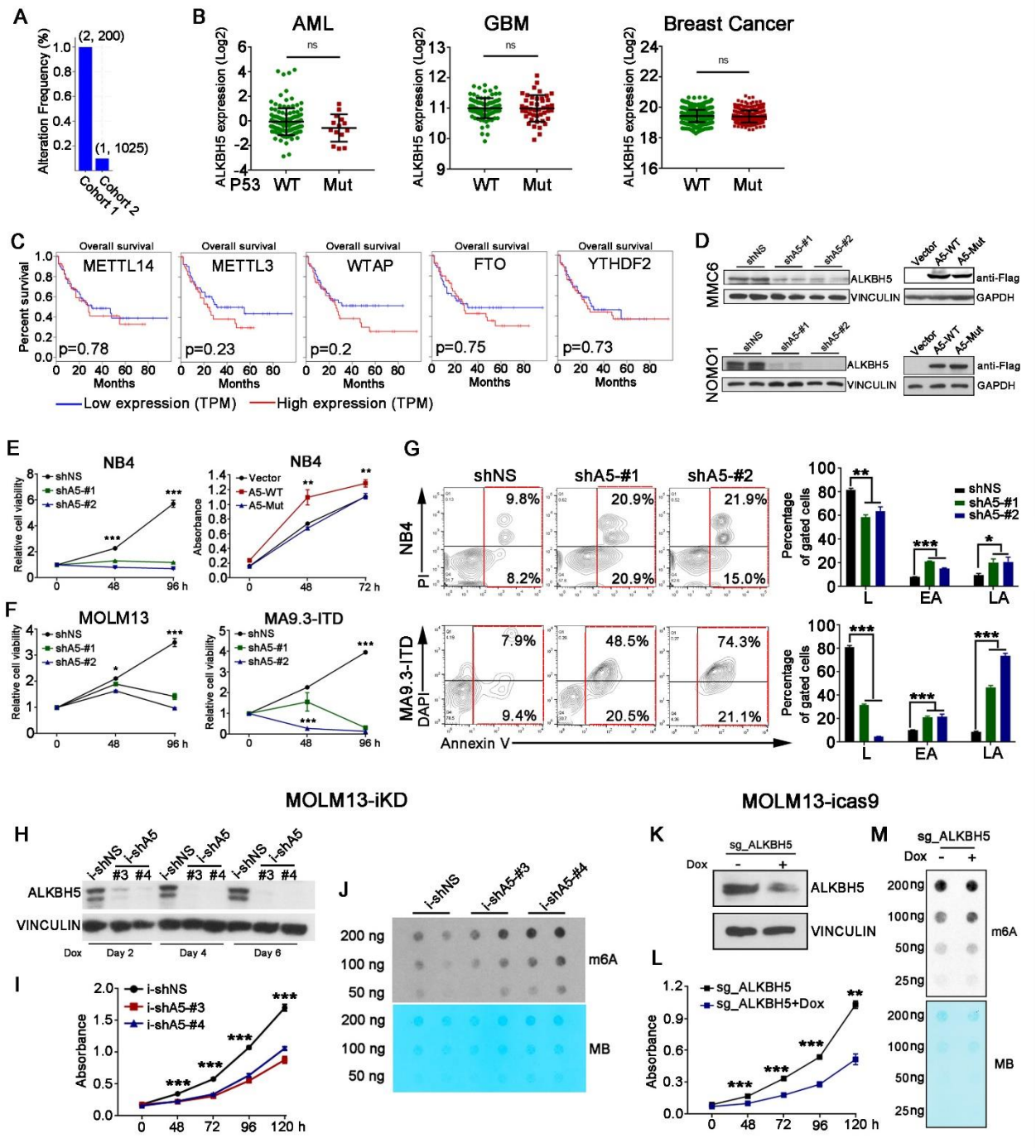


Figure S1 (related to Figure 1). The expression patterns of *ALKBH5* in human AML and effects of *ALKBH5* expression manipulation on the growth/proliferation of human AML cells.

(A) The frequency of *ALKBH5* deletion (only deep depletion was considered, as shallow depletion might not be accurate, according to the databases' instruction) in two human AML cohort datasets: the TCGA AML cohort (n=200; cohort 1) and the TARGET AML cohort (n=1,025; cohort 2).

(B) Comparison of *ALKBH5* expression levels between TP53-wild-type (WT) and -mutant (Mut) patients in AML, Glioblastoma (GBM) and Breast cancer datasets. The expression values of *ALKBH5* of the individual samples were log2-transformed and then normalized.

(**C**) Kaplan-Meier survival analysis of correlation of expression levels of a set of m^6A machinery genes with overall survival (OS) in AML patients based on the TCGA-AML dataset (n=106). The p values were detected by the log-rank test.

(D) Western blotting confirmation of knockdown and forced expression of ALKBH5 by lentiviral constructs in MMC6 (MONOMAC-6) (P53 Mutant) and NOMO1 (P53 Mutant) cells. shNS, pLKO.1-shNS; shA5-#1, pLKO.1-shALKBH5-#1; shA5-#2, pLKO.1-shALKBH5-#2; Vector, empty pCDH vector; A5-WT, pCDH-ALKBH5-WT; A5-Mut, pCDH-ALKBH5-Mut (a mutant that disrupts its m^6A demethylase activity) . VINCULIN or GAPDH was used as a loading control.

(**E**) Effects of *ALKBH5* knockdown (left panel) or forced expression (right panel) on the growth/proliferation of human NB4 (P53 mutant) AML cells.

(**F**) Effects of *ALKBH5* knockdown on the growth/proliferation of human MA9.3-ITD (P53 wild-type) and MOLM13 AML cells (P53 wild-type).

(**G**) Effects of *ALKBH5* knockdown on apoptosis in NB4 and MA9.3-ITD AML cells. A representative flow cytometry analysis was shown (left panel), and data from three independent experiments was analyzed and compared (right panel).

(**H**) Western blot detection of ALKBH5 protein level in Doxycycline induced MOLM13 cells.

(**I**) Effects of inducible *ALKBH5* knockdown on MOLM13 cell growth/proliferation.

(**J**) m^6A dot-blot analysis in inducible *ALKBH5* knockdown MOLM13 cells.

(**K**) Western blot detection of ALKBH5 protein level in sg_ALKBH5 infected MOLM13 cells with or without Doxycycline. VINCULIN was used as a loading control.

(**L**) The effect of inducible *ALKBH5* knockout on MOLM13 cells growth/proliferation.

(**M**) m^6A dot-blot analysis in inducible *ALKBH5* knockout MOLM13 cells.

Mean±SD are shown for Figures S1B, E, G (right panel), I and L. *p < 0.05; **p < 0.01; ***p < 0.001; ns, no significance; t-test

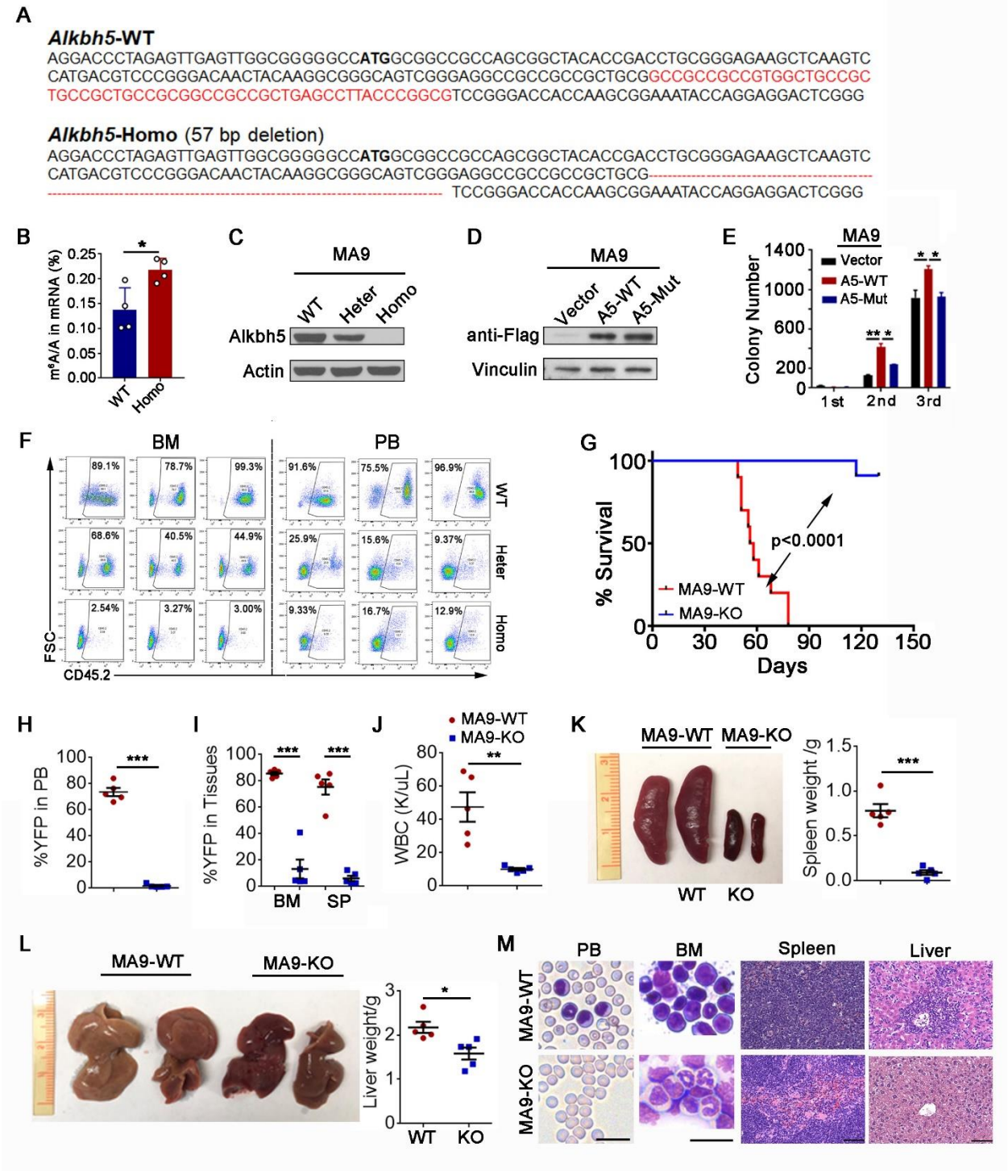

A
Alkbh5-WT
AGGACCCTAGAGTTGAGTTGGCGGGGGCCATGGCGGCCGCCAGCGGCTACACCGACCTGCGGGAGAAGCTCAAGTC
CATGACGTCCCGGGACAACTACAAGGCGGGCAGTCGGGAGGCCGCCGCCGCTGCGGCCGCCGCCGTGGCTGCCGC
TGCCGCTGCCGCGGCCGCCGCTGAGCCTTACCCGGCGTCCGGGACCACCAAGCGGAAATACCAGGAGGACTCGGG
Alkbh5-Homo (57 bp deletion)
AGGACCCTAGAGTTGAGTTGGCGGGGGCCATGGCGGCCGCCAGCGGCTACACCGACCTGCGGGAGAAGCTCAAGTC
CATGACGTCCCGGGACAACTACAAGGCGGGCAGTCGGGAGGCCGCCGCCGCTGCG----------
---------- TCCGGGACCACCAAGCGGAAATACCAGGAGGACTCGGG
B
m⁶A/A in mRNA (%)
0.25
0.20
0.15
0.10
0.05
0.00
*
WT
Homo
C
MA9
WT
Heter
Homo
Alkbh5
Actin
D
MA9
Vector
A5-WT
A5-Mut
anti-Flag
Vinculin
E
MA9
Vector
A5-WT
A5-Mut
Colony Number
1400
1000
600
200
0
1st
2nd
3rd
F
BM
PB
89.1%
78.7%
99.3%
91.6%
75.5%
96.9%
WT
68.6%
40.5%
44.9%
25.9%
15.6%
9.37%
Heter
2.54%
3.27%
3.00%
9.33%
16.7%
12.9%
Homo
FSC
CD45.2
G
% Survival
100
50
0
p<0.0001
MA9-WT
MA9-KO
0
30
60
90
120
150
Days
H
%YFP in PB
100
80
60
40
20
0
***
I
%YFP in Tissues
100
80
60
40
20
0
***
***
BM
SP
J
MA9-WT
MA9-KO
WBC (K/uL)
80
60
40
20
0
**
K
MA9-WT
MA9-KO
WT
KO
Spleen weight /g
1.5
1.0
0.5
0.0
***
L
MA9-WT
MA9-KO
Liver weight/g
3
2
1
0
*
WT
KO
M
PB
BM
Spleen
Liver
MA9-WT
MA9-KO

Figure S2 (related to Figure 2). Role of Alkbh5 in MLL-AF9 mediated leukemogenesis.

(A) Sanger sequencing of the deletion in *Alkbh5* genomic locus induced by CRISPR-Cas9.

(B) LC-MS/MS of mRNA m^6A abundance in bone marrow (BM) cells collected from *Alkbh5* wild-type (WT) and homozygous knockout (Homo) mice (n=4 for each group).

(**C**-**D**) The efficiency of knockout of Alkbh5 (**C**) and overexpression of ALKBH5 wild-type (A5-WT) or mutant (A5-Mut) (**D**) in the colony-formation assays (CFA) were confirmed by Western blots. Actin or Vinculin was used as a loading control.

(E) *Alkbh5* wild-type BM lin^- cells were virally co-transduced with MLL-AF9 (MA9) together with an empty vector (Vector), wild-type ALKBH5 (A5-WT) or mutant (A5-Mut) and then conducted colony-forming/replating assays. Colony forming cell counts at each round of plating are shown (n=3 technical replicates per sample).

(**F**) Three mice from each primary bone marrow transplantation (BMT) group were euthanized at the same time (day 61 post-BMT) for AML progression analysis. These are the original flow plots for the peripheral blood (PB) and BM engraftment analysis shown in Figure 2H-I.

(**G**-**M**) Effects of *Alkbh5* knockout on MA9-induced primary leukemogenesis. BM lin^- cells from *Alkbh5* wild-type or homozygous knockout mice were virally transduced with MA9-YFP retrovirus, (namely MA9-WT or MA9-KO), and then transplanted into primary recipient mice. (**G**) Survive curves of the recipients (MA9-WT, n=10; MA9-KO, n=11). (**H**-**M**) Five mice from each transplant group were euthanized at the same time (day 42 post-BMT), and the engraftment (percentage of YFP^+ cells) in PB (**H**) and in BM and spleen **(I)**, WBC counts (**J**), and the size and weight of spleen (**K**) and liver (**L**) were analyzed. The mean ± SD values were shown. (**M**) Representative images of Wright-Giemsa staining of PB and BM, and hematoxylin and eosin (H&E) staining of spleen and liver of the recipient mice. Bar = 20 μm (for PB and BM) or 50 μm (for spleen and liver).

*p <0.05; **p <0.01; ***p < 0.001; t-test (for Figures S2B, E, H-L; Mean±SD are shown) or log-rank test (for Figure S2G).

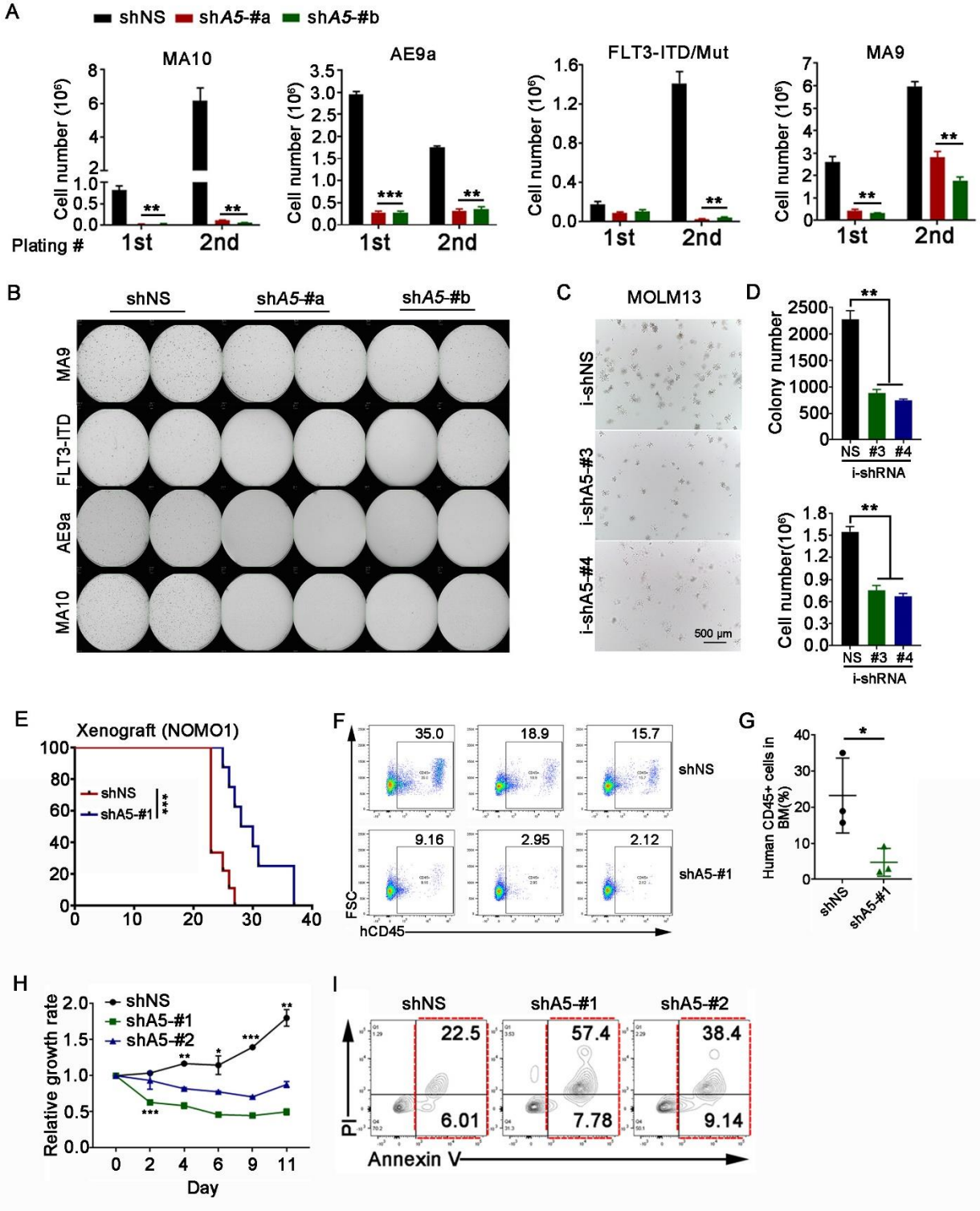

A
shNS
shA5-#a
shA5-#b
MA10
AE9a
FLT3-ITD/Mut
MA9
Cell number (10⁶)
Plating #
1st
2nd
B
shNS
shA5-#a
shA5-#b
MA9
FLT3-ITD
AE9a
MA10
C
MOLM13
i-shNS
i-shA5-#3
i-shA5-#4
500 μm
D
Colony number
NS #3 #4
i-shRNA
Cell number(10⁶)
E
Xenograft (NOMO1)
shNS
shA5-#1
F
35.0
18.9
15.7
9.16
2.95
2.12
FSC
hCD45
G
Human CD45+ cells in BM(%)
H
Relative growth rate
shNS
shA5-#1
shA5-#2
Day
I
22.5
57.4
38.4
6.01
7.78
9.14
PI
Annexin V

Figure S3 (related to Figure 3). Knockdown of *ALKBH5* affects the maintenance of human and murine AML.

(**A-B**) Effects of *Alkbh5* knockdown on the colony-forming/replating ability of mouse BM leukemia cells. Cell numbers per dish of each round of colony formation (**A**) and the representative images of whole dishes scanning (**B**) are shown.

(**C-D**) Effects of *ALKBH5* inducible knockdown on colony-forming ability of human MOLM13 (TP53 wild-type) AML cells. Representative images of the colonies ten days after the plating (**C**) and colony numbers and cell numbers per dish (**D**) are shown.

(**E-G**) Effect of *ALKBH5* knockdown on the maintenance and progression of human AML *in vivo*. NSGS mice were xeno-transplanted with NOMO1 cells that were lentivirally transduced with shNS (mouse number: n=9) or shALKBH5 (shA5-#1) (n=8), and AML cell engraftment and AML progression were monitored. Kaplan–Meier survival curves of the two groups of recipient mice (**E**), as well as representative flow analysis of AML cell engraftment (% of human $CD45^+$ cells in the BM) (**F**) and the statistic summaries of the engraftment data (**G**) of moribund recipient mice, were shown.

(**H-I**) Effects of *ALKBH5* knockdown by shRNAs (shA5-#1 and shA5-#2) on cell growth/proliferation (**H**) and apoptosis (**I**) of human primary AML cells (from Patient #3).

*p < 0.05; **p <0.01; ***p < 0.001; t-test (for Figures S3A, D, G-H; Mean±SD are shown) or log-rank test (for Figure S3E).

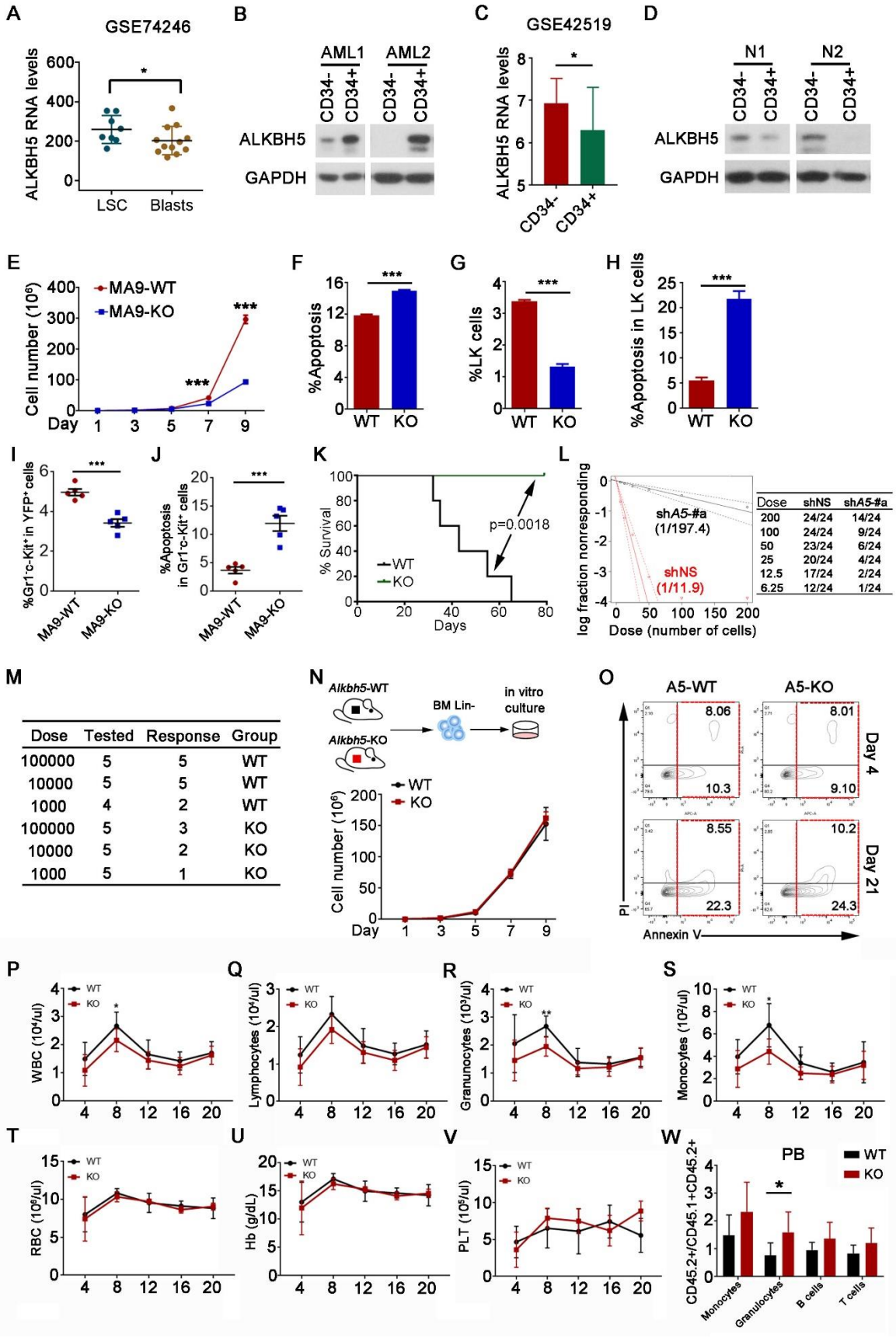

A
GSE74246
ALKBH5 RNA levels
LSC
Blasts
B
AML1
AML2
CD34-
CD34+
ALKBH5
GAPDH
C
GSE42519
ALKBH5 RNA levels
CD34-
CD34+
D
N1
N2
CD34-
CD34+
ALKBH5
GAPDH
E
MA9-WT
MA9-KO
Cell number (10⁶)
Day
F
%Apoptosis
WT
KO
G
%LK cells
WT
KO
H
%Apoptosis in LK cells
WT
KO
I
%Gr1⁻c-Kit⁺ in YFP⁺ cells
MA9-WT
MA9-KO
J
%Apoptosis in Gr1⁻c-Kit⁺ cells
MA9-WT
MA9-KO
K
% Survival
p=0.0018
WT
KO
Days
L
log fraction nonresponding
shA5-#a (1/197.4)
shNS (1/11.9)
Dose (number of cells)
Dose shNS shA5-#a
200 24/24 14/24
100 24/24 9/24
50 23/24 6/24
25 20/24 4/24
12.5 17/24 2/24
6.25 12/24 1/24
M
Dose Tested Response Group
100000 5 5 WT
10000 5 5 WT
1000 4 2 WT
100000 5 3 KO
10000 5 2 KO
1000 5 1 KO
N
Alkbh5-WT
Alkbh5-KO
BM Lin-
in vitro culture
WT
KO
Cell number (10⁶)
Day
O
A5-WT
A5-KO
8.06
8.01
10.3
9.10
8.55
10.2
22.3
24.3
Day 4
Day 21
PI
Annexin V
P
WBC (10⁴/ul)
Q
Lymphocytes (10⁴/ul)
R
Granunocytes (10³/ul)
S
Monocytes (10²/ul)
T
RBC (10⁶/ul)
U
Hb (g/dL)
V
PLT (10⁵/ul)
W
PB
WT
KO
CD45.2+/CD45.1+CD45.2+
Monocytes
Granulocytes
B cells
T cells

Figure S4 (related to Figure 4). Effects of *Alkbh5* knockout on the self-renewal/maintenance of leukemia stem/initiating cells (LSCs/LICs) and normal hematopoiesis.

(**A**) Comparison of *ALKBH5* expression levels between human primary leukemia stem cells (LSCs) and bunk AML cells (blasts), based on the GSE74246 dataset.

(**B**) Western blots showing ALKBH5 protein levels in $CD34^-$ and $CD34^+$ cell compartments isolated from leukemic BM cells of two primary AML patients. GAPDH was used as a loading control.

(**C**) Comparison of *ALKBH5* expression levels between human $CD34^-$ and $CD34^+$ cell compartments isolated from healthy controls (n=22), based on the GSE42519 dataset.

(**D**) Western blots showing ALKBH5 protein levels in $CD34^-$ and $CD34^+$ cell compartments isolated from two healthy donors (N1 and N2). GAPDH was used as a loading control.

(**E**-**H**) Effects of *Alkbh5* knockout on the proliferation/growth and apoptosis of MA9-transformed BM hematopoietic stem/progenitor cells (HSPCs). BM lin^- cells from *Alkbh5* wild-type (WT) or homozygous knockout (KO) mice were transduced with MA9-YFP retrovirus and then cultured *in vitro*. (**E**) Proliferation assays of the transduced cells. (**F**) The percentage of apoptotic cells. (**G**) The percentage of $lin^-c\text{-}kit^+$ (LK) cells. (**H**) The percentage of apoptotic LK cells. F-H were detected by flow cytometry.

(**I-J**) Comparison of $Gr\text{-}1^-c\text{-}Kit^+$ cell percentages in YFP^+ BM cells (**I**) and the apoptotic cell percentages in $Gr\text{-}1^-c\text{-}Kit^+$ cells (**J**) between primary mouse MA9 AML cells with *Alkbh5* wild-type (MA9-WT) or homozygous knockout (MA9-KO). Primary leukemic BM cells were collected from recipients shown in Figure 4A.

(K) Kaplan–Meier survival curves of secondary recipients (n=5 for each group) transplanted with leukemic BM cells collected from primary leukemic recipients shown in Figure 2F. The p value was detected by the log-rank test.

(**L**) *In vitro* limiting dilution assays (LDAs). Mouse MA9 cells were transduced with an *Alkbh5* shRNA (sh*A5*-#a) or control shRNA (shNS) and then seeded into 48-well plates at different doses with 24 replicates per dose. Colony forming cell counts were checked 7 days post seeding. (Left panel) Logarithmic plot showing the percentage of non-responding wells plated at different doses. Non-responding wells: wells not showing MA9 clones 7 days post plating. (Right panel) Table showing the number of wells containing colony forming cells. The estimated LSC/LIC frequency is calculated by ELDA and shown on the plot.

(**M**) Information of the response rates in the *in vivo* limiting dilution assays. Dose, number of donor cells; tested, total number of mice used in the assay; response, mice developed leukemia within 6 weeks post 2^{nd} BMT.

(**N**-**O**) Effects of *Alkbh5* knockout on the growth/proliferation (**N**) and apoptosis (**O**) of normal mouse HSPCs. BM lin^- cells (HSPCs) were isolated from *Alkbh5* wild-type (WT) and homozygous knockout (KO) mice and cultured *in vitro* for the analysis.

(**P**-**W**) *In vivo* competition assays. (**P**-**V**) CBC counts of recipients PB every 4 weeks. (**W**) Ratio of $CD45.2^+/CD45.1^+CD45.2^+$ in the differentiated cell compartments in the PB of recipients.

Mean±SD are shown for Figures S4A, C, E-J, N, P-W, *p <0.05; ***p < 0.001; t-test.

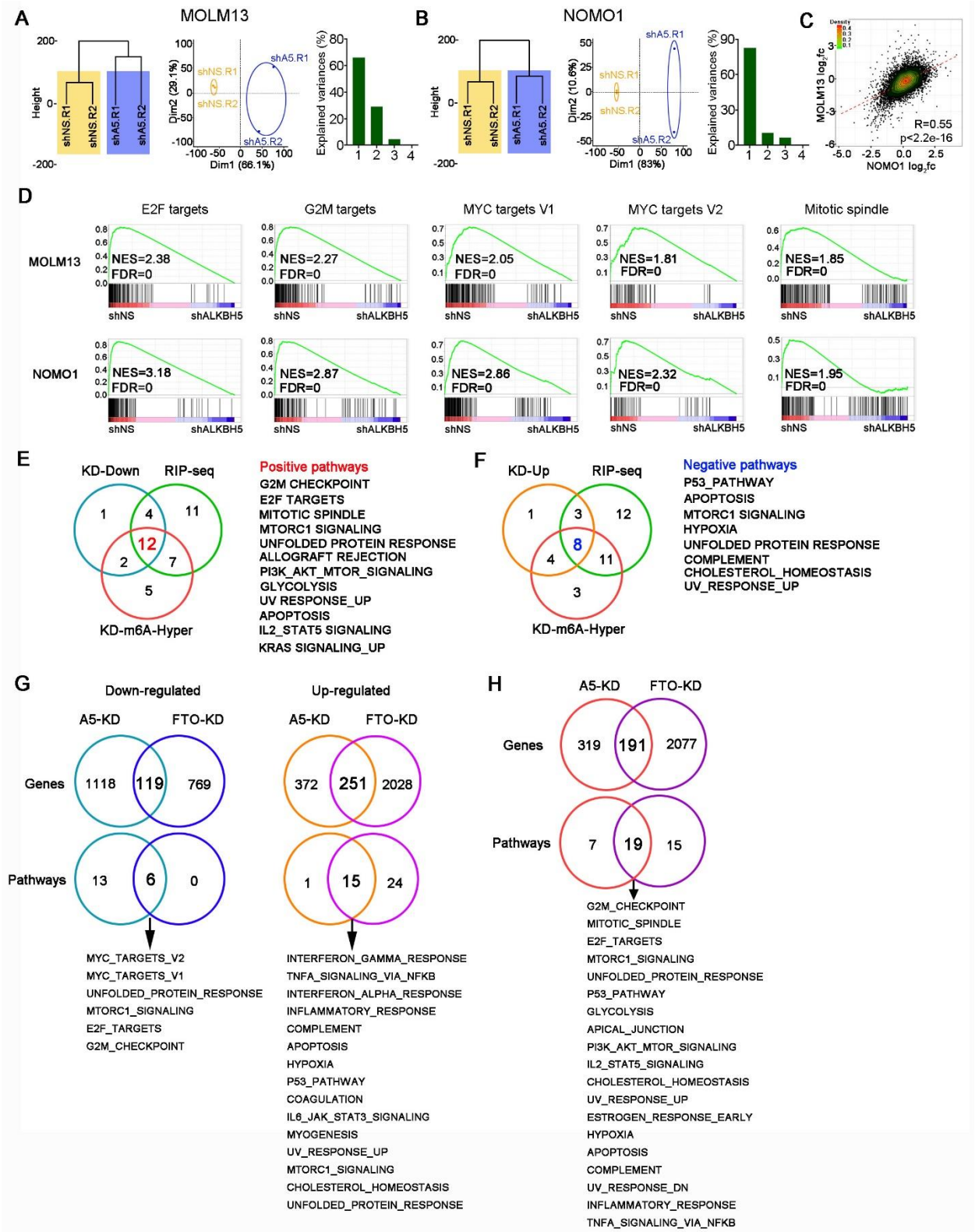

A
MOLM13
Height
shNS.R1
shNS.R2
shA5.R1
shA5.R2
Dim2 (29.1%)
Dim1 (66.1%)
Explained variances (%)
B
NOMO1
Dim2 (10.6%)
Dim1 (83%)
C
MOLM13 log2fc
NOMO1 log2fc
R=0.55
p<2.2e-16
D
E2F targets
G2M targets
MYC targets V1
MYC targets V2
Mitotic spindle
MOLM13
NOMO1
NES=2.38 FDR=0
NES=2.27 FDR=0
NES=2.05 FDR=0
NES=1.81 FDR=0
NES=1.85 FDR=0
NES=3.18 FDR=0
NES=2.87 FDR=0
NES=2.86 FDR=0
NES=2.32 FDR=0
NES=1.95 FDR=0
shNS
shALKBH5
E
KD-Down
RIP-seq
KD-m6A-Hyper
1
4
11
12
2
7
5
Positive pathways
G2M CHECKPOINT
E2F TARGETS
MITOTIC SPINDLE
MTORC1 SIGNALING
UNFOLDED PROTEIN RESPONSE
ALLOGRAFT REJECTION
PI3K_AKT_MTOR_SIGNALING
GLYCOLYSIS
UV RESPONSE_UP
APOPTOSIS
IL2_STAT5 SIGNALING
KRAS SIGNALING_UP
F
KD-Up
RIP-seq
KD-m6A-Hyper
1
3
12
8
4
11
3
Negative pathways
P53_PATHWAY
APOPTOSIS
MTORC1 SIGNALING
HYPOXIA
UNFOLDED PROTEIN RESPONSE
COMPLEMENT
CHOLESTEROL_HOMEOSTASIS
UV_RESPONSE_UP
G
Down-regulated
Up-regulated
A5-KD
FTO-KD
Genes
Pathways
1118
119
769
13
6
0
372
251
2028
1
15
24
MYC_TARGETS_V2
MYC_TARGETS_V1
UNFOLDED_PROTEIN_RESPONSE
MTORC1_SIGNALING
E2F_TARGETS
G2M_CHECKPOINT
INTERFERON_GAMMA_RESPONSE
TNFA_SIGNALING_VIA_NFKB
INTERFERON_ALPHA_RESPONSE
INFLAMMATORY_RESPONSE
COMPLEMENT
APOPTOSIS
HYPOXIA
P53_PATHWAY
COAGULATION
IL6_JAK_STAT3_SIGNALING
MYOGENESIS
UV_RESPONSE_UP
MTORC1_SIGNALING
CHOLESTEROL_HOMEOSTASIS
UNFOLDED_PROTEIN_RESPONSE
H
A5-KD
FTO-KD
Genes
Pathways
319
191
2077
7
19
15
G2M_CHECKPOINT
MITOTIC_SPINDLE
E2F_TARGETS
MTORC1_SIGNALING
UNFOLDED_PROTEIN_RESPONSE
P53_PATHWAY
GLYCOLYSIS
APICAL_JUNCTION
PI3K_AKT_MTOR_SIGNALING
IL2_STAT5_SIGNALING
CHOLESTEROL_HOMEOSTASIS
UV_RESPONSE_UP
ESTROGEN_RESPONSE_EARLY
HYPOXIA
APOPTOSIS
COMPLEMENT
UV_RESPONSE_DN
INFLAMMATORY_RESPONSE
TNFA_SIGNALING_VIA_NFKB

Figure S5 (related to Figure 5). Transcriptome-wide identification of ALKBH5 targets.

(**A-B**) Hierarchical clustering and principal component analysis (PCA) of control (shNS.R1 and shNS.R2) and *ALKBH5* knockdown (shA5.R1 and shA5.R2) MOLM13 (**A**) and NOMO1 (**B**) AML cells based on the RNA-seq data.

(**C**) Scatterplots of expression fold changes of genes upon *ALKBH5* knockdown in MOLM13 and NOMO1 cells based on the RNA-seq data.

(**D**) Gene set enrichment analysis (GSEA) in MOLM13 and NOMO1 cells. Representative gene sets (pathways) that are significantly enriched (FDR<0.001) with genes downregulated in AML cells upon *ALKBH5* knockdown were shown. NES, normalized enrichment score; FDR, false discovery rate.

(**E-F**) Venn diagram of pathways that were positively (**E**) or negatively (**F**) regulated by ALKBH5 in human AML cells. KD-Down/Up: pathways of genes down/up-regulated in *ALKBH5* knockdown RNA-seq samples. RIP-seq, pathways of genes whose transcripts significantly enriched with ALKBH5 in Flag-IP samples. KD-m^{6}A-Hyper: pathways of genes with increased m^{6}A abundance in *ALKBH5* knockdown samples. Only pathways with FDR<0.01 were used for the overlapping analysis.

(**G**) Comparison of down- or up-regulated genes (RPKM>1, fold change>1.5) and pathways (FDR<0.01) caused by *FTO* knockdown (based on the RNA-seq data from (Huang et al., 2019)) with those caused by *ALKBH5* knockdown (based on the RNA-seq data herein).

(**H**) Comparison of m^{6}A hypermethylated genes (p<0.05) and pathways (FDR<0.01) caused by *FTO* knockdown (based on the m^{6}A-seq data from (Su et al., 2018)) with those caused by *ALKBH5* knockdown (based on the m^{6}A-seq data herein).

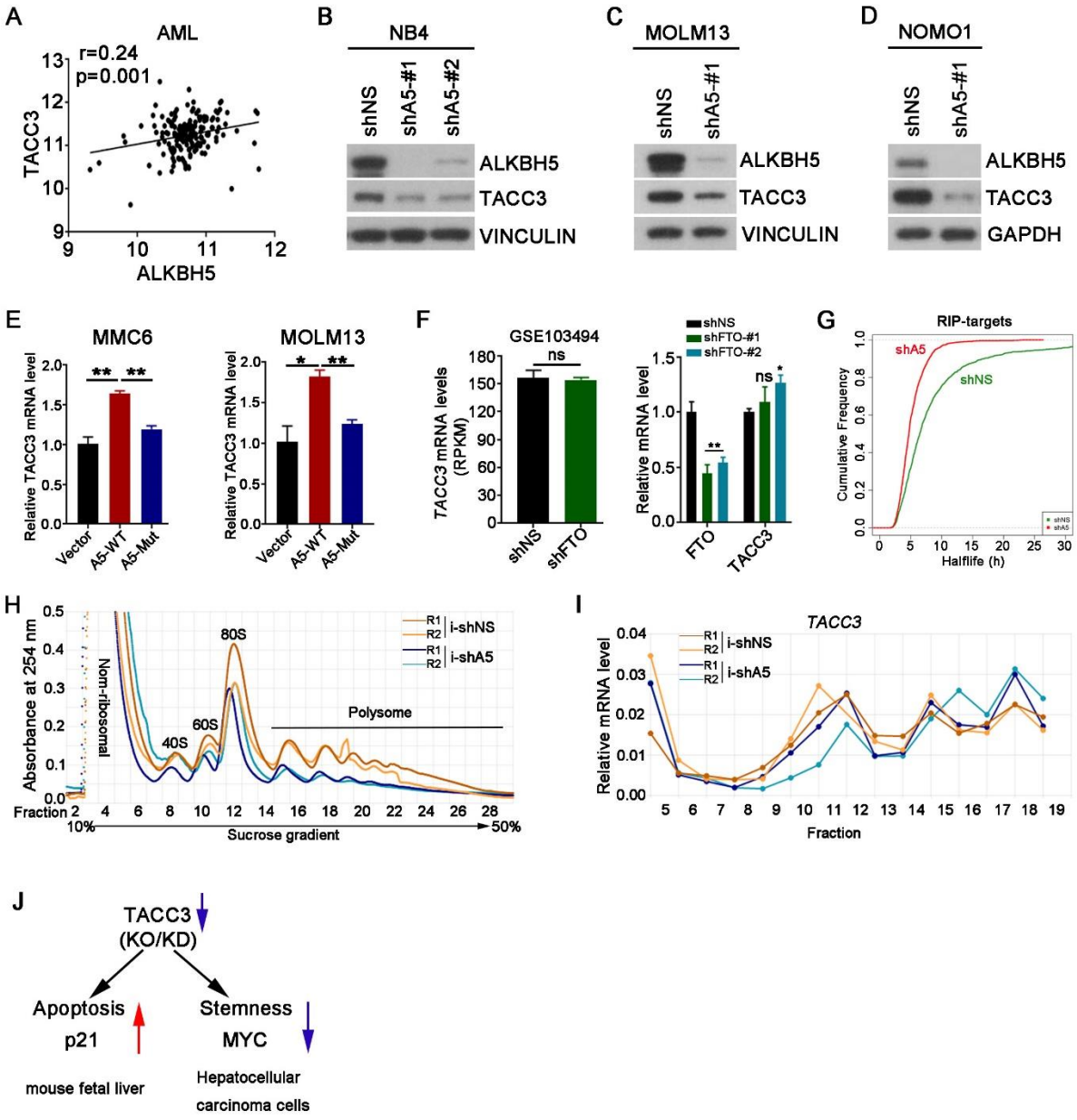
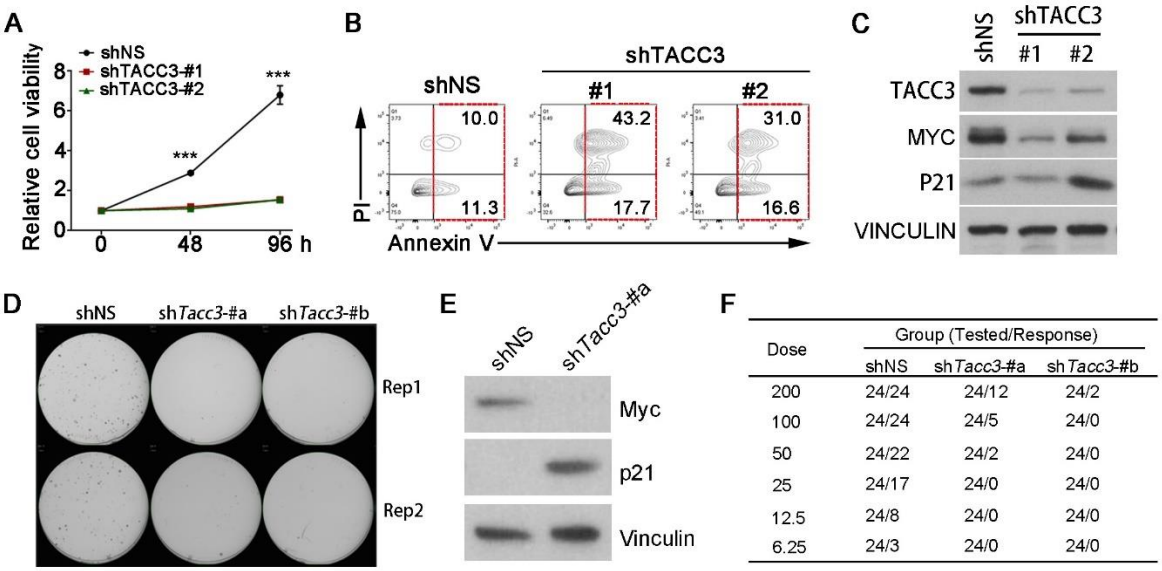


Figure S6 (related to Figure 6). ALKBH5 regulates *TACC3* expression via affecting its mRNA stability

(A) The correlation of *TACC3* with *ALKBH5* in expression across human AML samples in the TCGA-AML dataset (n=177). Pearson correlation analysis was conducted. r, correlation coefficient.

(**B-D**) Western blots of ALKBH5 and TACC3 in NB4 (**B**), MOLM13 (**C**) and NOMO1 (**D**) cells transduced with shNS or *ALKBH5* shRNAs. VINCULIN or GAPDH was used as a loading control.
(**E**) qPCR detection of *TACC3* expression changes in ALKBH5 wild-type (A5-WT)- or ALKBH5-mutant (A5-Mut)-overexpressing AML cells, relative to their controls.
(**F**) Effect of *FTO* knockdown on *TACC3* RNA level in AML cells. (Left panel) *TACC3* mRNA level in the control (shNS) or *FTO* knockdown (shFTO) NB4 cells based on the RNA-seq dataset (GSE103494). (Right panel) qPCR detection of *FTO* and *TACC3* mRNA levels in MMC6 cells transduced with shNS or FTO shRNAs (shFTO-#1 and shFTO-#2).
(**G**) Cumulative distribution of RNA transcript stability changes of ALKBH5 RIP targets between shNS- and shA5-#1-transduced NOMO1 cells.
(**H-I**) Polysome profiling assays. (**H**) Absorbance of different fractions of NOMO1 cell lysates. (**I**) Total RNAs in different fractions of ribosomes were extracted and subjected to qPCR analysis, *TACC3* mRNA level was normalized to *GAPDH* and input.
(**J**) Summary of previous reports about the effects of TACC3 on expression of p21 and MYC. *Tacc3* knockout caused apoptosis in mouse fetal liver with increased p21 expression (Piekorz et al., 2002; Schneider et al., 2008; Suhail et al., 2015). *TACC3* knockdown suppressed cell proliferation and stem-like phenotype of hepatocellular carcinoma cells *in vitro* with decreased MYC expression (Zhou et al., 2015).
Mean±SD are shown for Figures S6E-F, *p<0.05; **p <0.01; ns, no significance; t-test.



Dose	Group (Tested/Response)		
	shNS	sh*Tacc3*-#a	sh*Tacc3*-#b
200	24/24	24/12	24/2
100	24/24	24/5	24/0
50	24/22	24/2	24/0
25	24/17	24/0	24/0
12.5	24/8	24/0	24/0
6.25	24/3	24/0	24/0

Figure S7 (related to Figure 7). *TACC3* is a functionally important target of ALKBH5 in AML.

(A) Effect of *TACC3* knockdown by shRNAs (shTACC3-#1 and shTACC3-#2) on the viability/proliferation of MOLM13 cells.

(**B**) Percentage of apoptotic cells in MOLM13 cells transduced with shNS or individual *TACC3* shRNAs.

(**C**) Western blots of TACC3, MYC and P21 in MOLM13 cells transduced with shNS or individual *TACC3* shRNAs**.** VINCULIN was used as a loading control.

(**D**) Whole dish scanning pictures of mouse MA9 AML cells transduced with shNS or individual *Tacc3* shRNAs (sh*Tacc3*-#a and sh*Tacc3*-#b) in colony forming/replating assays (Figure 7G).

(**E**) Western blots of Myc and p21 in mouse MA9 AML cells transduced with shNS or *Tacc3* shRNA (sh*Tacc3*-#a). Vinculin was used as a loading control.

(**F**) Table for the *in vitro* limiting dilution assays (LDAs) shown in Figure 7H. Dose, number of cells seeded into each well. Tested, total replicate wells plated. Response, wells contain colony forming cells.

Mean±SD are shown for Figure S7A, ***p < 0.001; t-test.

Supplemental Tables

Table S1 (Related to Figure 5). List of the 18 highly confident target genes of ALKBH5 in AML as detected by transcriptome-wide RNA-seq, RIP-seq and m^{6}A-seq.

Correlation with ALKBH5 in expression	Gene	Enriched pathways (Hallmark gene set)	Correlation with patient survival in the TCGA AML cohort	Fold change			
				RNA-seq MOLM13	RNA-seq NOMO1	RIP-seq	m^{6}A-seq
Positive	*ADNP*	N/A	ns	0.63	0.61	2.18	1.88
	CHAF1A	G2M Checkpoint	ns	0.37	0.44	4.80	2.27
	CENPF	G2M Checkpoint Mitotic Spindle	ns	0.05	0.15	2.39	3.53
	HNRNPF	N/A	ns	0.62	0.59	2.02	1.40
	MCM7	E2F Targets; MYC Targets V1	ns	0.19	0.27	2.72	2.49
	MSH6	N/A	ns	0.23	0.54	3.61	3.97
	PHACTR3	N/A	ns	0.36	0.48	2.73	3.89
	PRKDC	E2F Targets	ns	0.26	0.30	4.21	1.82
	SLC43A3	N/A	ns	0.62	0.63	4.07	2.00
	TACC3	E2F Targets; G2M Checkpoint	Poor; p=0.0094	0.38	0.46	3.56	2.38
	USP10	N/A	ns	0.51	0.49	3.67	2.36
	WHSC1	N/A	ns	0.17	0.43	7.23	2.08
Negative	*BCL2A1*	Apoptosis	Poor; p=0.0032	3.04	2.10	3.00	1.91
	CLCN7	N/A	ns	1.86	1.93	6.08	1.45
	DMXL2	N/A	ns	1.52	2.12	11.56	2.57
	KIAA0930	N/A	ns	1.56	1.91	2.14	1.54
	SAT2	N/A	ns	3.42	2.67	2.25	2.75
	TFEB	N/A	Poor; p=0.0024	1.58	2.08	2.74	1.35

N/A, not enriched in Hallmark gene sets. p, p value; ns, not significant ($p<0.05$). Poor, significantly shorter overall survival time of AML patients with expression levels of a given gene higher than 50% of the cohort (median). Fold change: RNA-seq and m^{6}A-seq, shA5/shNS; RIP-seq, IP/input.

Table S2 (Related to Star Method). List of oligonucleotides

Name	**Sequence**	
Alkbh5-F	CGGCCTCAGGACATTAAGGA	qPCR primers for mouse genes
Alkbh5-R	TCGCGGTGCATCTAATCTTG	
Tacc3-F	TGACCAATAAGCGTGAGGC	
Tacc3-R	CCATTTGAACCACAGGTGC	
ADNP-F	CCCAGATTGAGTGGCAGAATAG	qPCR primers for human genes
ADNP-R	AGGGAACCTGGCACTTAGG	
ALKBH5-F	TTTCTCTGGTGTGGTTCTGG	
ALKBH5-R	AAGTCTTAGTCTTCCCAGGATTG	
BCL2A1-F	CTCAAGACTTTGCTCTCCACC	
BCL2A1-R	TGGTATCTGTAGGACGCACTG	
CENPF-F	GCTCAACACAGGAGGAAGTG	
CENPF-R	CGTTCTTTCAGTTTCTCTGCG	
CHAF1A-F	AACTTGTCAACGGGAAGGG	
CHAF1A-R	CGAGTCCTCTGTCAAATCAATG	
CLCN7-F	CCCTGTGCTGTGATGTTGAG	
CLCN7-R	ATTCCACGCTCTGAGGCTC	
DMXL2-F	CAAACCAACAGTAAGGATGGAG	
DMXL2-R	GAGAAACAGGCAGTGAAGGAG	
GAPDH-F	AATCCCATCACCATCTTCCAG	
GAPDH-R	AAATGAGCCCCAGCCTTC	
HNRNPF-F	ACTTCTTCTCTCCTCTCAACCC	
HNRNPF-R	AACTCAACATCTGCTTCACCC	
KIAA0930-F	AACTTCAGCACCCGAGTCAG	
KIAA0930-R	AGGGCAAAGCCATCAAAC	
MCM7-F	TGAACTCGGGAAGAAGCAG	
MCM7-R	TCGTCCAGGTCCACATACAG	
MSH6-F	GGATTGGTAGGAACCGTTAC	
MSH6-R	TCGTTTACAGCCCTTCTTG	
WHSC1-F	CGTTCTGTAAGGAGCACCAG	
WHSC1-R	GCTATTTGCCCTCTGTGACTC	
PHACTR3-F	CACTTCCTTCCTTCTTTGGTG	
PHACTR3-R	CCGTCCTCCATTACACTGC	
SAT2-F	TTCAAGGAGAGGCAACGAG	
SAT2-R	GGGAAGGAGAAACTCAAGACAG	
SLC43A3-F	CTTCCACCACCTTTGAGGAC	
SLC43A3-R	CTGCTTGGCAACTGAGATTC	
TACC3-F	AAGAAGTGGCTGCAGGCC	
TACC3-R	TGGGGGTGCCCTTTTGC	
TFEB-F	CCAAGAAGGATCTGGACCTC	

TFEB-R	GGACATGGTGGACAGAAGTG	
USP10-F	TTTGGTCAGTCCTGCTTCC	
USP10-R	ATGCTTTCCGTGGTGTGC	
28S rRNA-F	TGTCGGCTCTTCCTATCATTGT	
28S rRNA-R	ACCCAGCTCACGTTCCCTATTA	
ACTB-F	CACTCTTCCAGCCTTCCTTC	
ACTB-R	GTACAGGTCTTTGCGGATGT	
sgALKBH5-sense	CACCGCGTGGACGAGATCCCCGAGT	gRNA
sgALKBH5-antisense	AAACACTCGGGGATCTCGTCCACGC	