Expanded View Figures

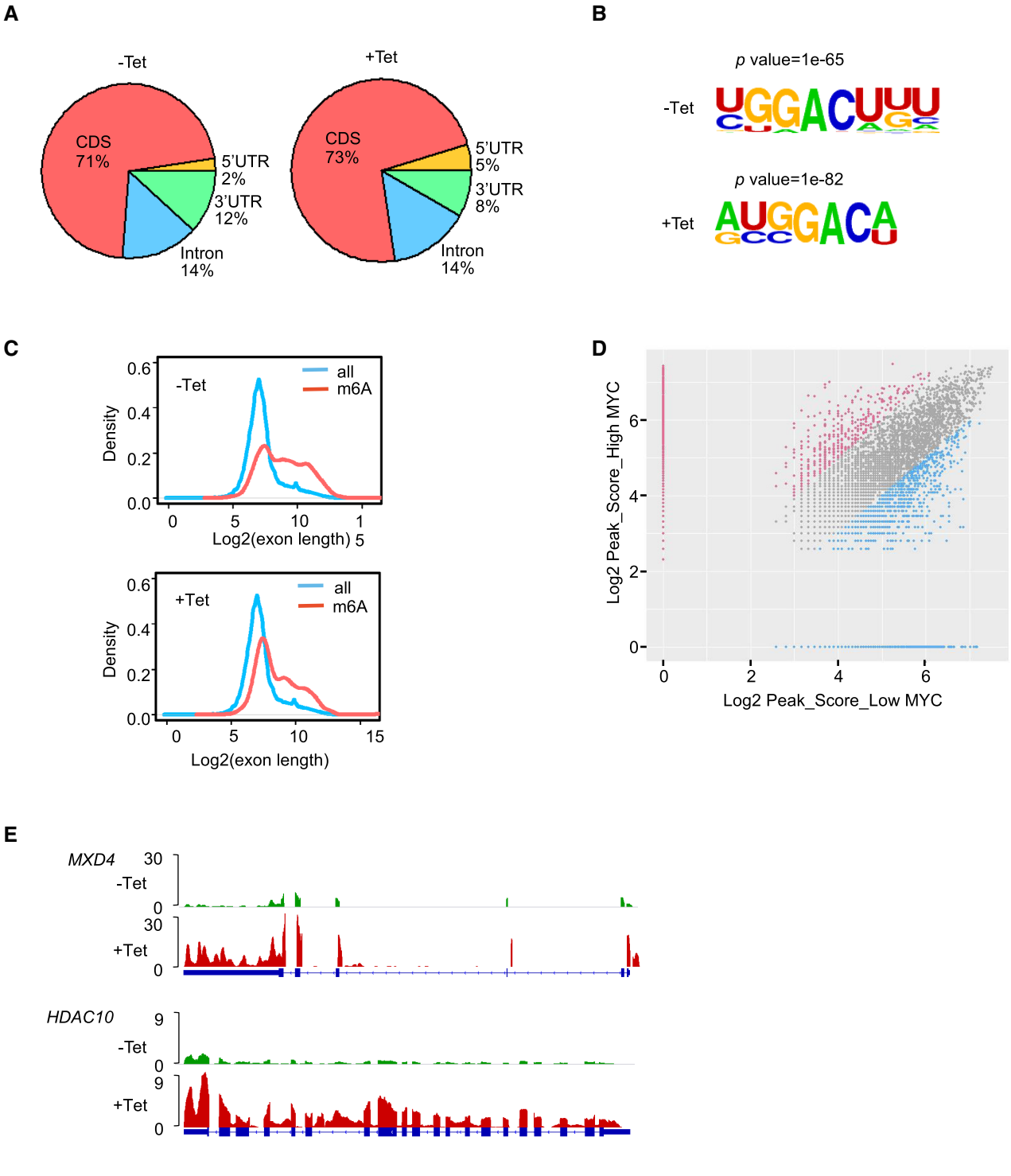

Figure EV1.

Figure EV1. MeRIP analyses show that MYC down-regulates m^{6}A levels of mRNA.

A Pie chart depicting the fraction of m^{6}A peaks in four transcript segments in P493-6 cells treated with Tet for 0 h or 72 h.
B Sequence motif identified within m^{6}A peaks by HOMER database.
C Distribution of m^{6}A sites along the length of mRNA transcripts.
D Scatter plots showing the m^{6}A enrichment in mRNA of high MYC and low MYC samples. m^{6}A-containing mRNA with significantly increased and decreased peak enrichment is highlighted in red and green, respectively (chi-square test, $P < 0.05$).
E IGV graph showing location of m^{6}A peaks on representative genes.

Figure EV2. MYC and m^{6}A down-regulate protein expression but not mRNA expression of SPI1 and PHF12.

A, B Western blot analysis for protein levels of MYC, SPI1, and PHF12 in P493-6 cells (A) or Raji cells (B) that treated with the cycloleucine for 0, 24, 48, and 72 h. HPRT1 and β-actin serve as negative control and loading control, respectively. Data are representative of at least three independent experiments.
C RT–qPCR analysis of the mRNA level of *SPI1* and *PHF12* in P493-6 cells treated with cycloleucine for 0, 24, 48, and 72 h. Data were presented as mean (±SD), $n = 3$ biological replicates.
D RT–qPCR analysis of the m^{6}A levels of MRGs (SPI1 and PHF12) in P493-6 cells treated with cycloleucine for 0 h or 24 h. *HPRT1* serves as negative control. Data are mean (±SD), $n = 3$ biological replicates. ***$P < 0.001$ relative to corresponding 0 h group (Student's *t*-test).
E RT–qPCR analysis of the mRNA level of *SPI1* and *PHF12* in P493-6 cells treated with Tet for 0 h or 24 h. Data were presented as mean (±SD), $n = 3$ biological replicates (Student's *t*-test).
F Western blot analysis for protein levels of MYC and MRGs (SPI1 and PHF12) in P493-6 cells treated with Tet, cycloleucine, or both. HPRT1 and β-actin serve as negative and loading controls, respectively. Data are representative of at least three independent experiments.
G IGV showing the locations of m^{6}A peaks on *CDKN1A* and *CDKN2B*.
H RT–qPCR analysis of the mRNA levels of *CDKN1A* and *CDKN2B* in P493-6 cells treated with Tet for 0 h or 24 h; data were presented as mean (±SD), $n = 3$ biological replicates, *$P < 0.05$ relative to corresponding -Tet group (Student's *t*-test).

Source data are available online for this figure.

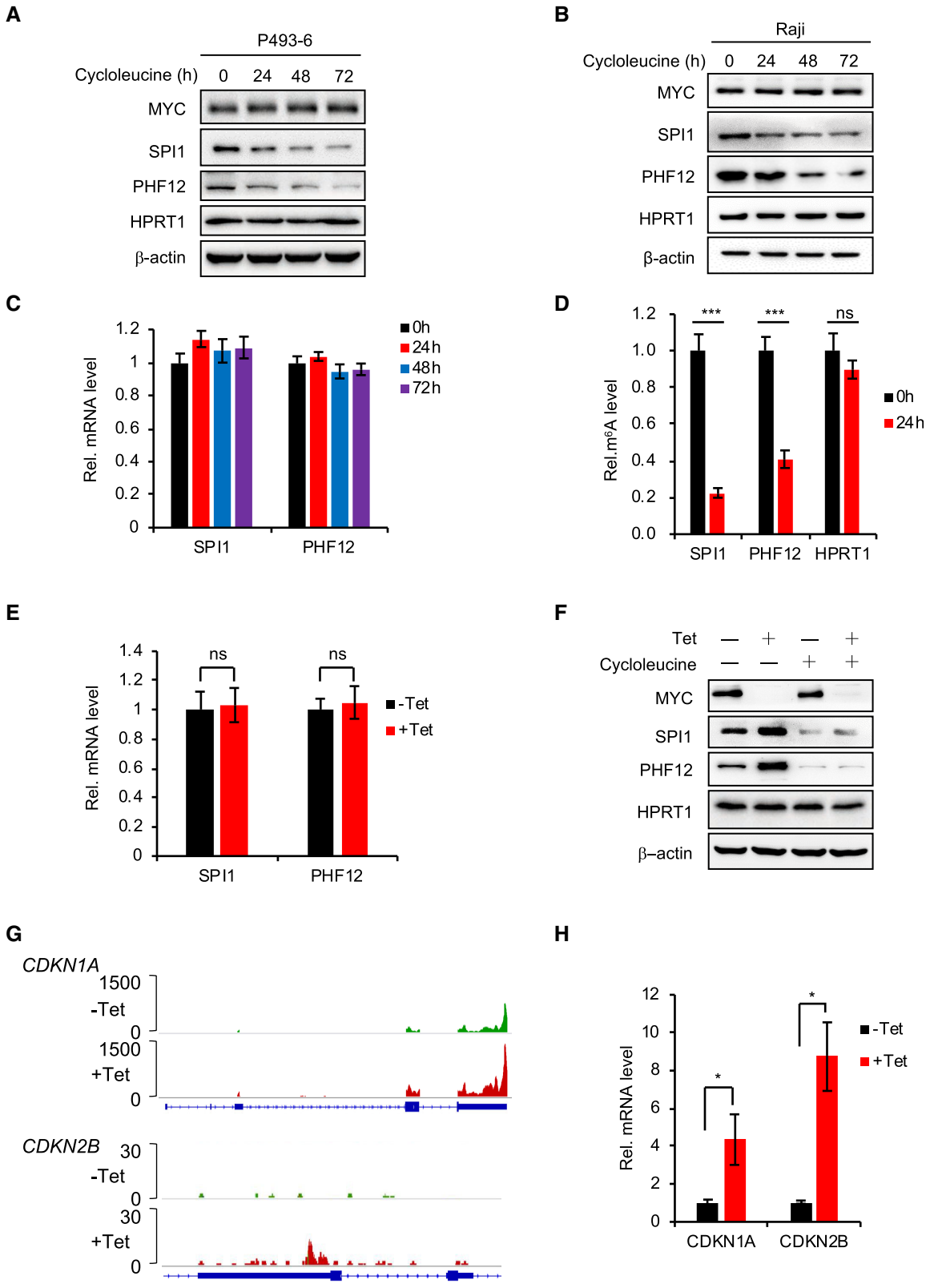

Figure EV2.

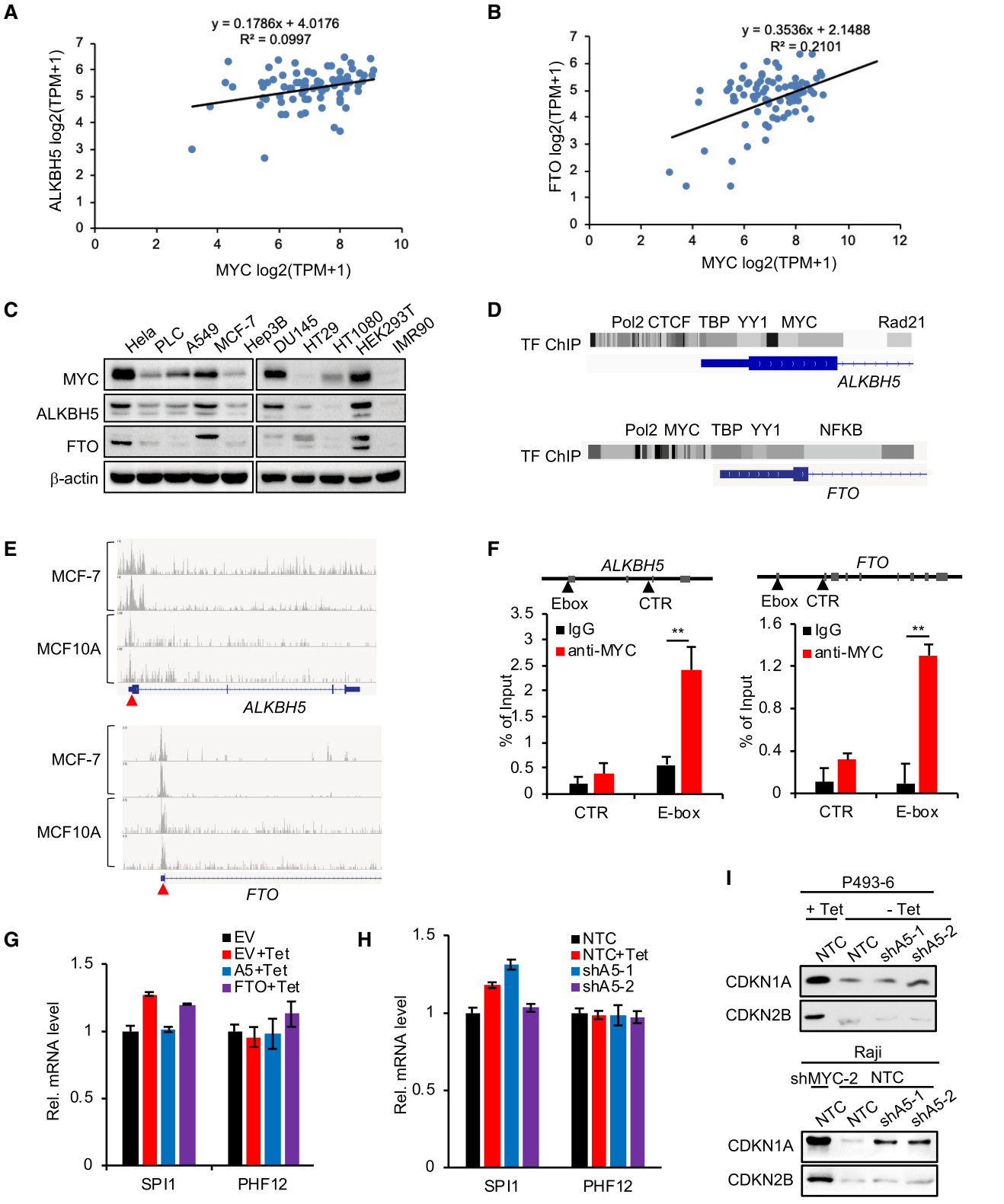
A
y = 0.1786x + 4.0176
R² = 0.0997
ALKBH5 log2(TPM+1)
MYC log2(TPM+1)
B
y = 0.3536x + 2.1488
R² = 0.2101
FTO log2(TPM+1)
MYC log2(TPM+1)
C
Hela PLC A549 MCF-7 Hep3B DU145 HT29 HT1080 HEK293T IMR90
MYC
ALKBH5
FTO
β-actin
D
Pol2 CTCF TBP YY1 MYC Rad21
TF ChIP
ALKBH5
Pol2 MYC TBP YY1 NFKB
TF ChIP
FTO
E
MCF-7
MCF10A
ALKBH5
MCF-7
MCF10A
FTO
F
ALKBH5
Ebox CTR
IgG
anti-MYC
% of Input
CTR E-box
FTO
Ebox CTR
IgG
anti-MYC
% of Input
CTR E-box
G
EV
EV+Tet
A5+Tet
FTO+Tet
Rel. mRNA level
SPI1 PHF12
H
NTC
NTC+Tet
shA5-1
shA5-2
Rel. mRNA level
SPI1 PHF12
I
P493-6
+ Tet - Tet
NTC NTC shA5-1 shA5-2
CDKN1A
CDKN2B
Raji
shMYC-2 NTC
NTC NTC shA5-1 shA5-2
CDKN1A
CDKN2B

Figure EV3.

Figure EV3. MYC regulates demethylases ALKBH5 and FTO, and ALKBH5 inhibits the protein expression of SPI1 and PHF12.

A, B Analyses of the co-expression of MYC and ALKBH5 (A) or MYC and FTO (B) in 78 lymphocyte cell lines from CCLE. Linregress *P* value for (A) is 4.87E-3 and for (B) is 2.44E-5.

C Western blot analysis for protein levels of MYC, ALKBH5, and FTO in ten different human cell lines. β-actin serves as loading controls. Western blot data are representative of at least three independent experiments.

D Analyses of ALKBH5 and FTO promotor in ENCODE transcription factor (TF) ChIP datasets (Data ref: Furey *et al*, 2011; Data ref: Snyder *et al*, 2011).

E IGV graph showing location of MYC-binding peaks on ALKBH5 and FTO from published ChIP-seq datasets (Data ref: Furey *et al*, 2011; Data ref: Snyder *et al*, 2011). Red triangle indicates the binding peak.

F ChIP experiment was performed in P493-6 cells using IgG or anti-MYC antibody. The occupancy of potential E-Box in ALKBH5 and FTO genes by MYC was determined by RT–qPCR. Control (CTR) primer sets were also included. Data were presented as mean (±SD), $n = 3$ biological replicates. $^{**}P < 0.01$ as compared to corresponding IgG group (Student's *t*-test).

G RT–qPCR analysis of the mRNA level of *SPI1* and *PHF12* in P493-6 cells that overexpressed EV, ALKBH5, or FTO and were then treated with Tet or not. RT–qPCR data were presented as mean (±SD), $n = 3$ biological replicates.

H RT–qPCR analysis of the mRNA level of *SPI1* and *PHF12* in P493-6 cells that expressed NTC or *ALKBH5* shRNAs and were then treated with Tet or not. RT–qPCR data were presented as mean (±SD), $n = 3$ biological replicates.

I Western blot analysis for protein levels of CDKN1A and CDKN2B in Fig 2I and J samples that P493-6 cells expressing NTC or ALKBH5 shRNAs and were then treated with or without Tet or in Raji cells expressing NTC or ALKBH5 shRNAs with or without MYC knockdown.

Source data are available online for this figure.

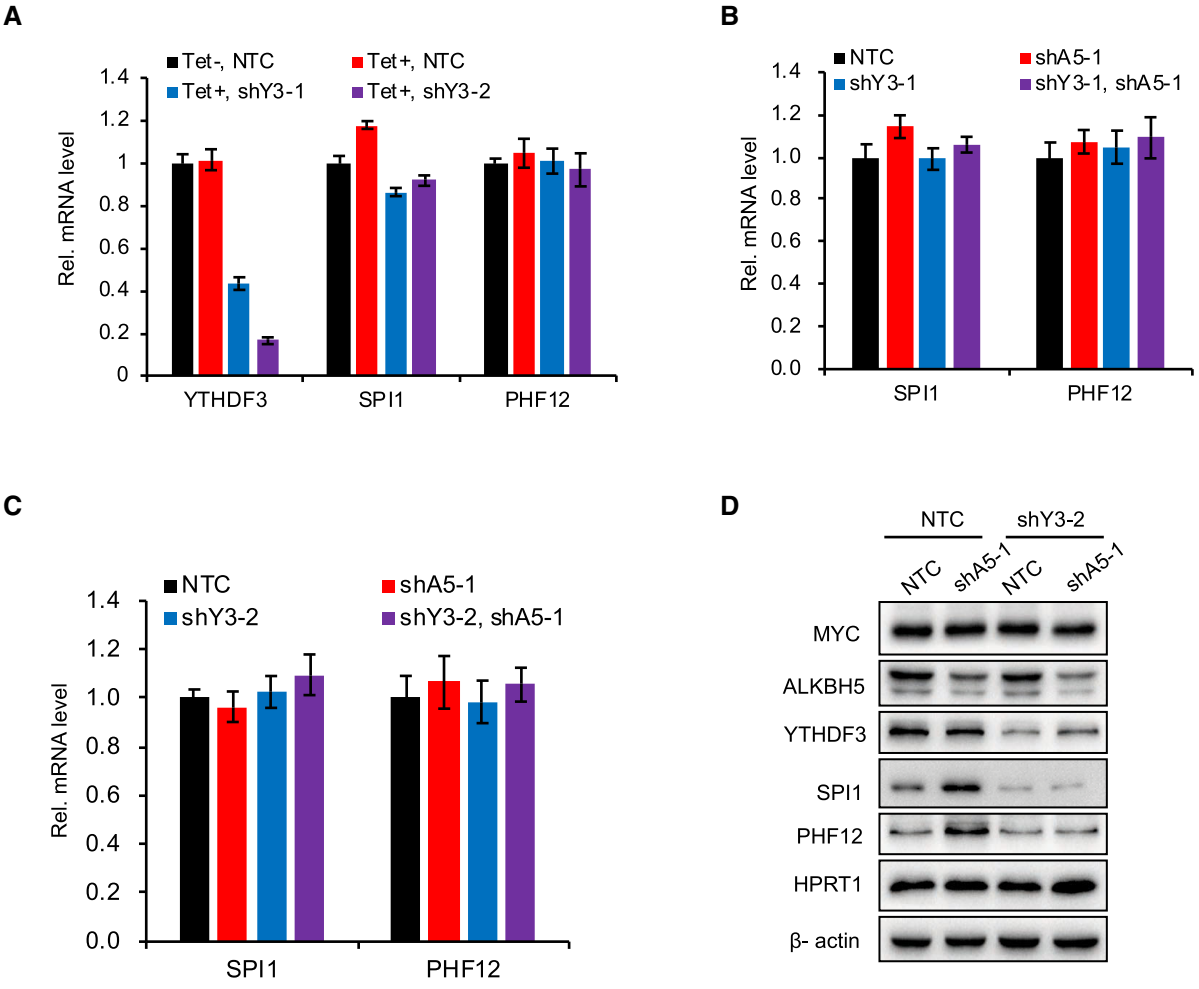

Figure EV4.

Figure EV4. YTHDF3 does not regulate mRNA expression but promotes mRNA translation.

A RT–qPCR analysis of the mRNA level of *SPI1* and *PHF12* in P493-6 cells that expressed NTC or *YTHDF3* shRNAs and were then treated with Tet or not. Data were presented as mean (±SD), $n = 3$ biological replicates.

B, C RT–qPCR assay for mRNA levels of *SPI1* and *PHF12* in P493-6 cells that expressed NTC, *ALKBH5* shRNA, or *YTHDF3* shRNA, or co-expressed *ALKBH5* as well as *YTHDF3* shRNAs. Data were presented as mean (±SD), $n = 3$ biological replicates.

D Western blot analysis for protein levels in P493-6 cells that expressed NTC, or *ALKBH5* shRNA, or *YTHDF3* shRNA. HPRT1 and β-actin serve as negative and loading controls, respectively. Western blot data are representative of at least three independent experiments.

Source data are available online for this figure.

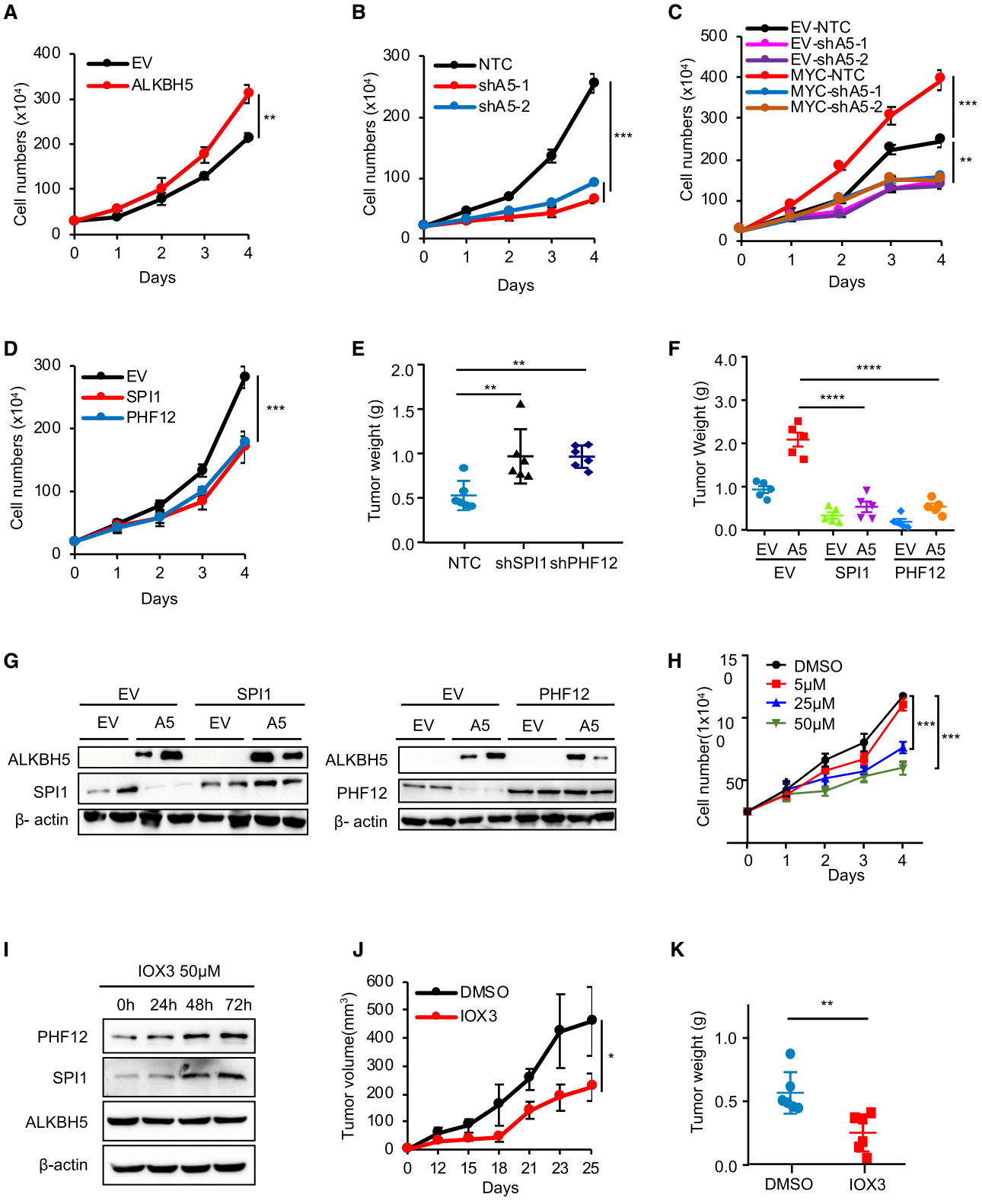

Figure EV5.

Figure EV5. The MYC-ALKBH5-m^{6}A-SPI1/PHF12 axis is critical for cancer progression.

A, B Trypan blue counting was used to analyze growth curves for P493-6 cells overexpressing ALKBH5 (A) and *ALKBH5* shRNAs (B). $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared between indicated groups (mean ± SD, $n = 4$ biological replicates, Student's *t*-test).

C Trypan blue counting was used to analyze growth curves for EV- or MYC-expressing Raji cells that further infected with sh*ALKBH5*. $^{**}P < 0.01$ and $^{***}P < 0.001$ as compared between indicated groups (mean ± SD, $n = 3$ biological replicates, Student's *t*-test).

D Trypan blue counting was used to analyze growth curves for P493-6 cells overexpressing MRGs (SPI1 or PHF12). $^{***}P < 0.001$ as compared between indicated groups (mean ± SD, $n = 3$ biological replicates, Student's *t*-test).

E, F Weight of tumors collected at the end of the experiment in Fig 4G (E) or in Fig 4I (F). Data are presented as mean (±SEM), $n = 5$ for each group. $^{**}P < 0.01$ or $^{****}P < 0.0001$ as compared between indicated groups (Student's *t*-test).

G Western blot analysis for protein levels of ALKBH5, SPI1, and PHF12 from xenograft tumors. β-actin serves as loading controls.

H Cell growth curves for P493-6 cells that treated with different doses of IOX3. $^{***}P < 0.001$ as compared to between indicated groups (mean ± SD, $n = 3$ biological replicates, Student's *t*-test).

I Western blot analysis for protein levels of ALKBH5, SPI1, and PHF12 in P493-6 cells treated with the ALKBH5 inhibitor IOX3 for 0, 24, 48, and 72 h. β-actin serves as loading control. The Western blot data are representative of at least three independent experiments.

J, K P493-6 cells were injected subcutaneously into nude mice ($n = 5$ for each group). Mice were received every other day either IOX3 or DMSO (5% DMSO/H_2O) by intragastric administration from 12 days postinjection (J). Tumor weights were measured at the end of the experiment (day 25) (K). Data are presented as mean (±SEM). $^{*}P < 0.05$ and $^{***}P < 0.001$ as compared between indicated groups (Student's *t*-test).

Source data are available online for this figure.