

Figure S1. *ALKBH5* and *METTL3* mRNA expression in patients

(A) qPCR analysis of *ALKBH5* mRNA expression in primary tumors and lymph node (LN) metastases in the same patient (n = 16 pairs); (B) Overall survival of patients with epithelial ovarian cancer (EOC) relative to *ALKBH5* expression in The Cancer Genome Atlas (TCGA) (n = 1657). (C) Progression-free survival of patients with advanced EOC relative to *ALKBH5* expression in TCGA (n = 1436). ns denotes not significant.

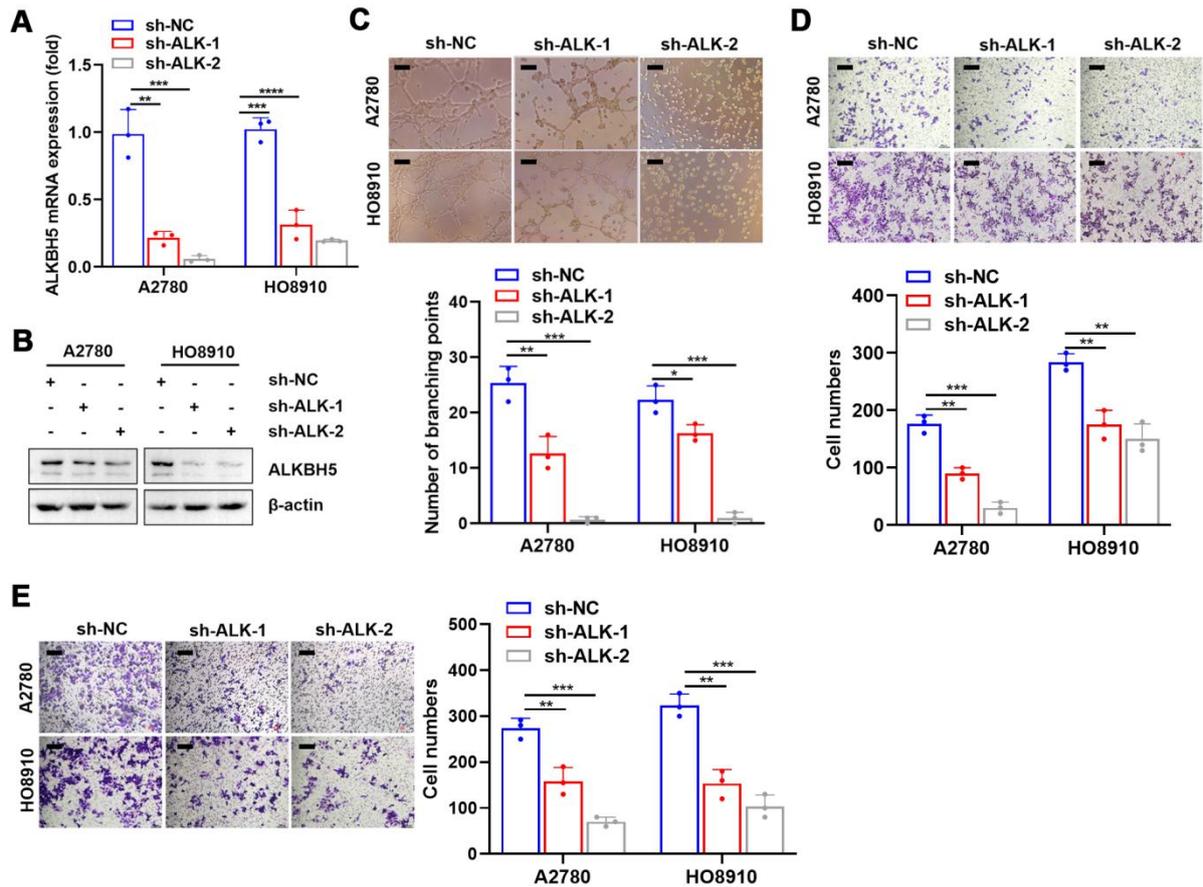


Figure S2. ALKBH5 silencing inhibits lymph node (LN) metastasis *in vitro*

(A and B) qPCR (A) and western blot (B) analysis of the knockdown efficiency of ALKBH5 in A2780 and HO8910 cells. (C) Representative images (upper panels) and histogram (lower panels) of Matrigel tube formation assay with human lymphatic endothelial cells (HLECs). HLECs were cultured in a conditioned medium derived from ovarian cancer cells treated as indicated. (D and E) Representative images (upper panels) and histogram (lower panels) of migration (D) and invasion (E) assays of A2780 and HO8910 cells; all *in vitro* experiments were performed in at least three biological replicates. Error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. ** $P < 0.01$, *** $P < 0.001$.

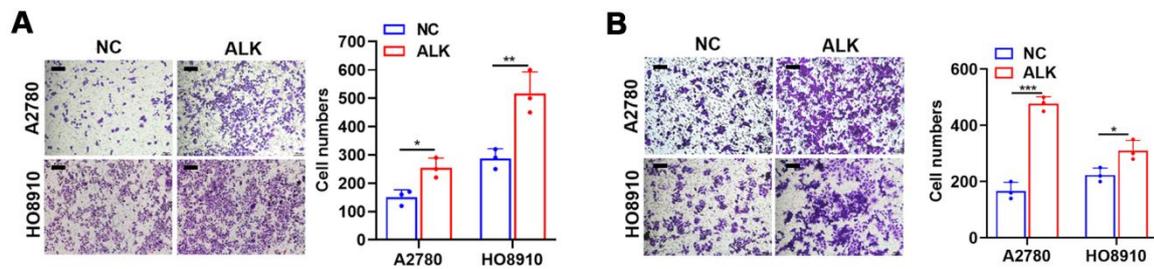


Figure S3. ALKBH5 overexpression promotes tumor cell migration and invasion *in vitro*

(A and B) Representative images (left panels) and histogram (right panels) of (A) migration and (B) invasion assay of A2780 and HO8910 cells. All *in vitro* experiments were performed in at least three biological replicates. Error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

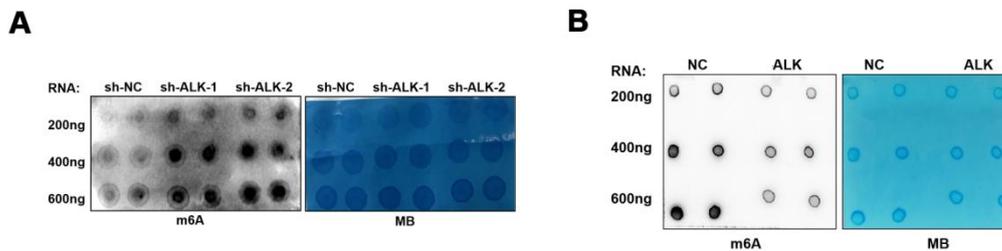


Figure S4. ALKBH5 abrogates m6A modifications in ovarian cancer cells

(A) m6A dot-blot assay showed that ALKBH5 downregulation significantly increased the m6A modification level in A2780 cells; (B) m6A dot-blot assay showed that ALKBH5 overexpression significantly reduced the m6A modification level in A2780 cells.

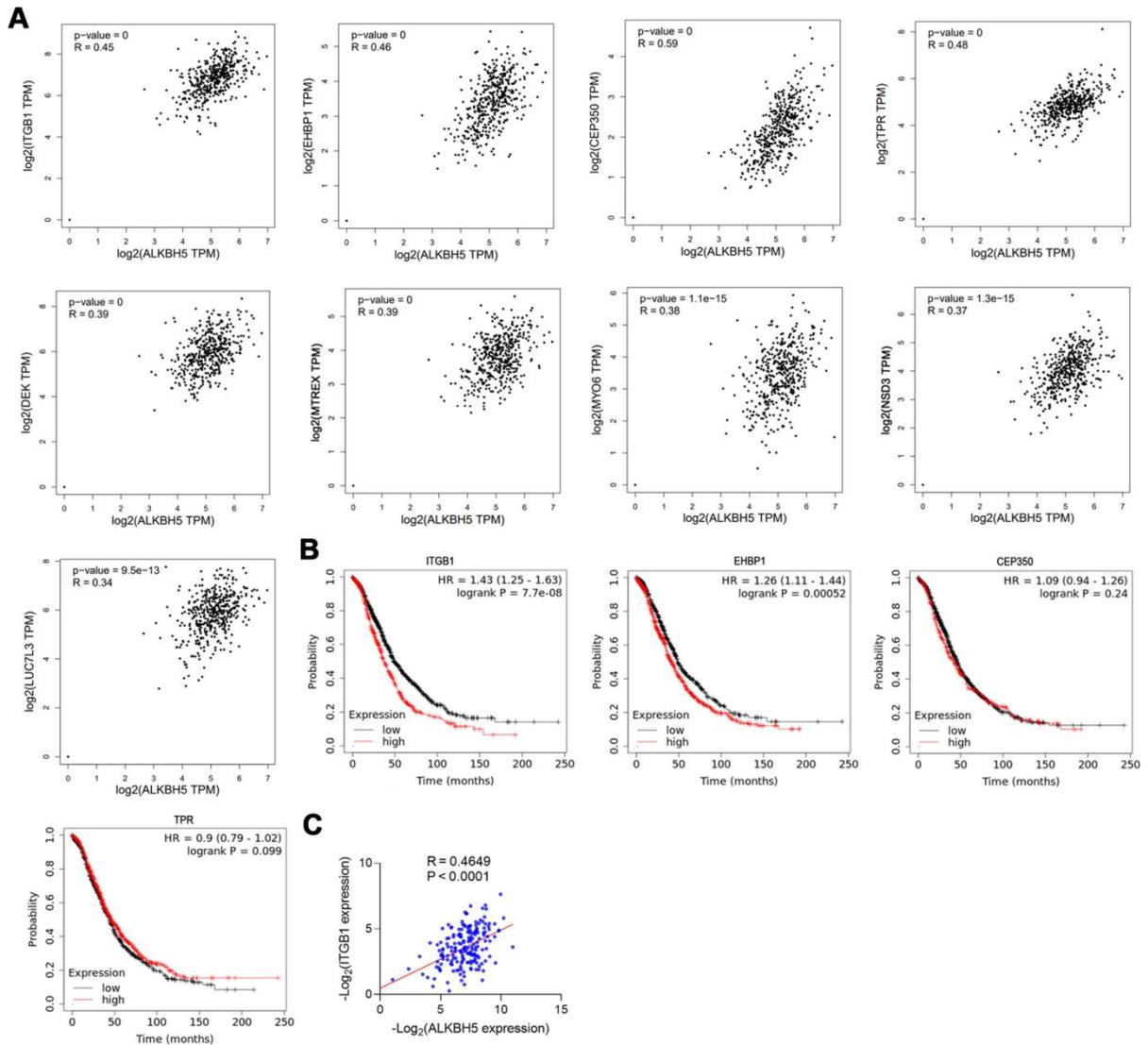


Figure S5. Expression of genes downstream of ALKBH5 in patients and patient survival

(A) Correlation analyses of ALKBH5 and expression of nine target genes in The Cancer Genome Atlas (TCGA) database. (B) Overall survival of patients with ovarian cancer in TCGA data according to the expression of the nine target genes. (C) qPCR analysis of the expression of four genes after the upregulation of ALKBH5 in A2780 cells. (D) Correlation analyses of ALKBH5 and ITGB1 expression in our data (n = 192 cases).

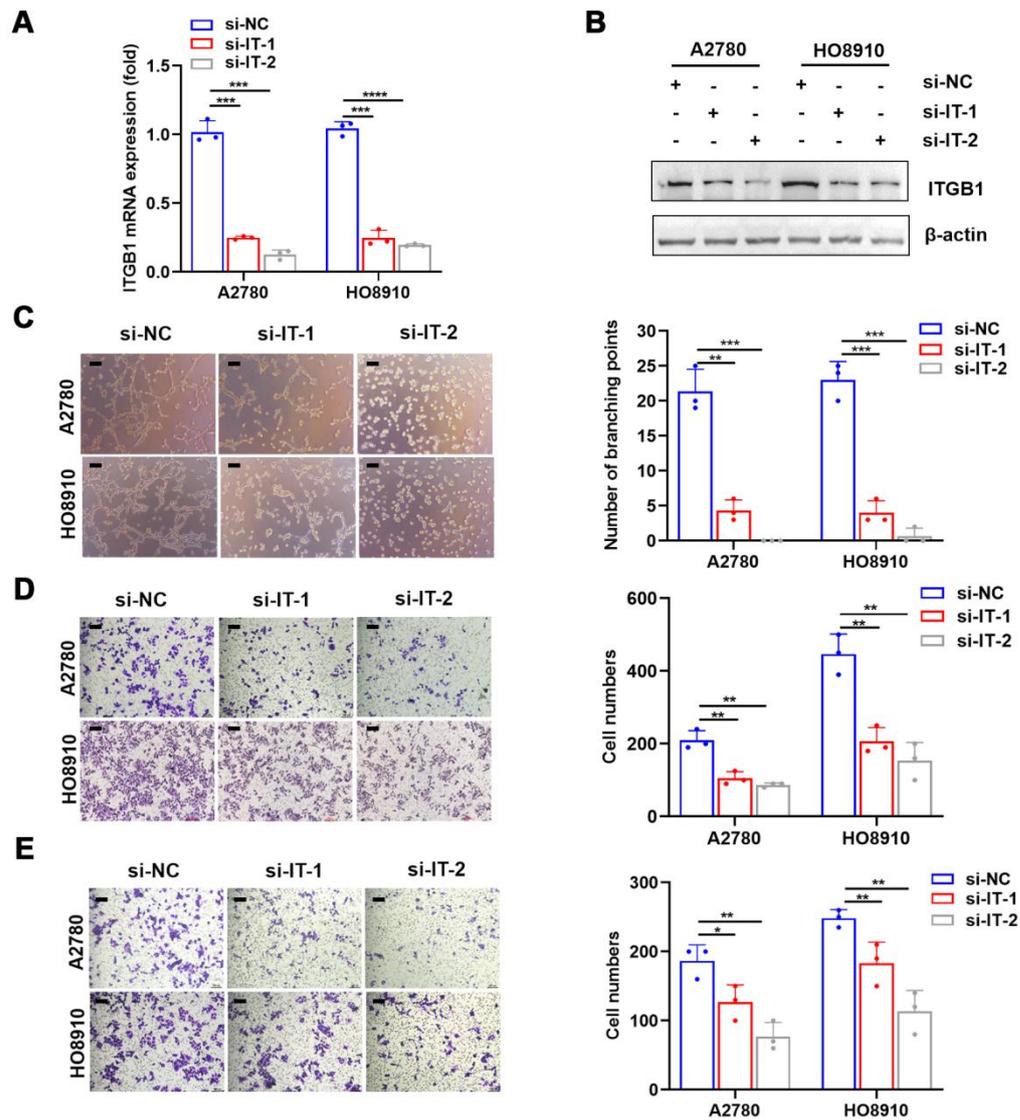


Figure S6. ITGB1 silencing inhibits lymph node (LN) metastasis *in vitro*

(A and B) qPCR and western blot (B) analysis of the transfection efficiency of downregulated-ITGB1 siRNA in A2780 and HO8910 cells. (C) Representative images (left panels) and histogram (right panels) of Matrigel tube formation assay with human lymphatic endothelial cells (HLECs). HLECs were cultured in a conditioned medium derived from ovarian cancer cells treated as indicated. (D and E) Representative images (left panels) and histogram (right panels) of migration (D) and invasion (E) assay with A2780 and HO8910 cells. All *in vitro* experiments were performed in at least three biological replicates. The error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

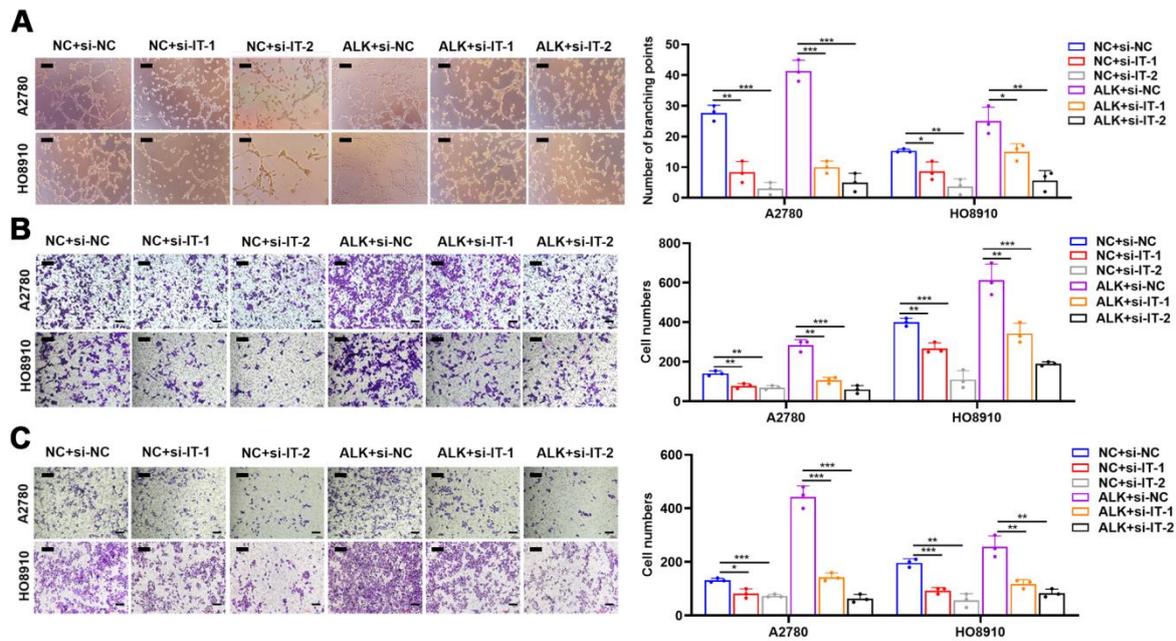


Figure S7. ITGB1 silencing rescues the ALKBH5 overexpression-induced lymph node (LN) metastasis *in vitro*

(A) ITGB1 silencing rescues ALKBH5 overexpression-induced lymphatic tube formation. (B and C) Specific siRNAs that knock down ITGB1 expression in EOC cells with ALKBH5 overexpression significantly suppressed migration (B) and invasion (C). All *in vitro* experiments were performed in at least three biological replicates. Error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

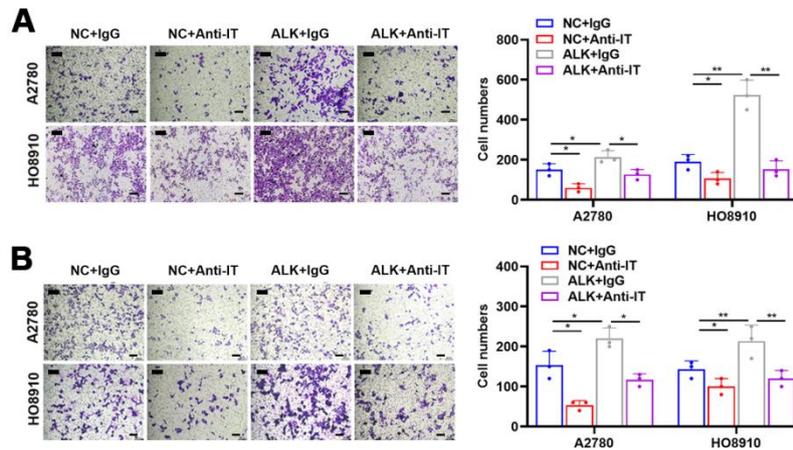


Figure S8. Blocking of ITGB1 rescues the ALKBH5 overexpression-induced tumor cell migration and invasion *in vitro*

(A) Representative images (left panels) and histogram (right panels) of the migration assay with A2780 and HO8910 cells. (B) Representative images (upper panels) and histogram (lower panels) of invasion assay with A2780 and HO8910 cells; all *in vitro* experiments were performed in at least three biological replicates. The error bars indicate the SD of the mean. Scale bars: 100 μm . Statistical significance was assessed using a two-tailed Student's *t* test. * $P < 0.05$, ** $P < 0.01$.

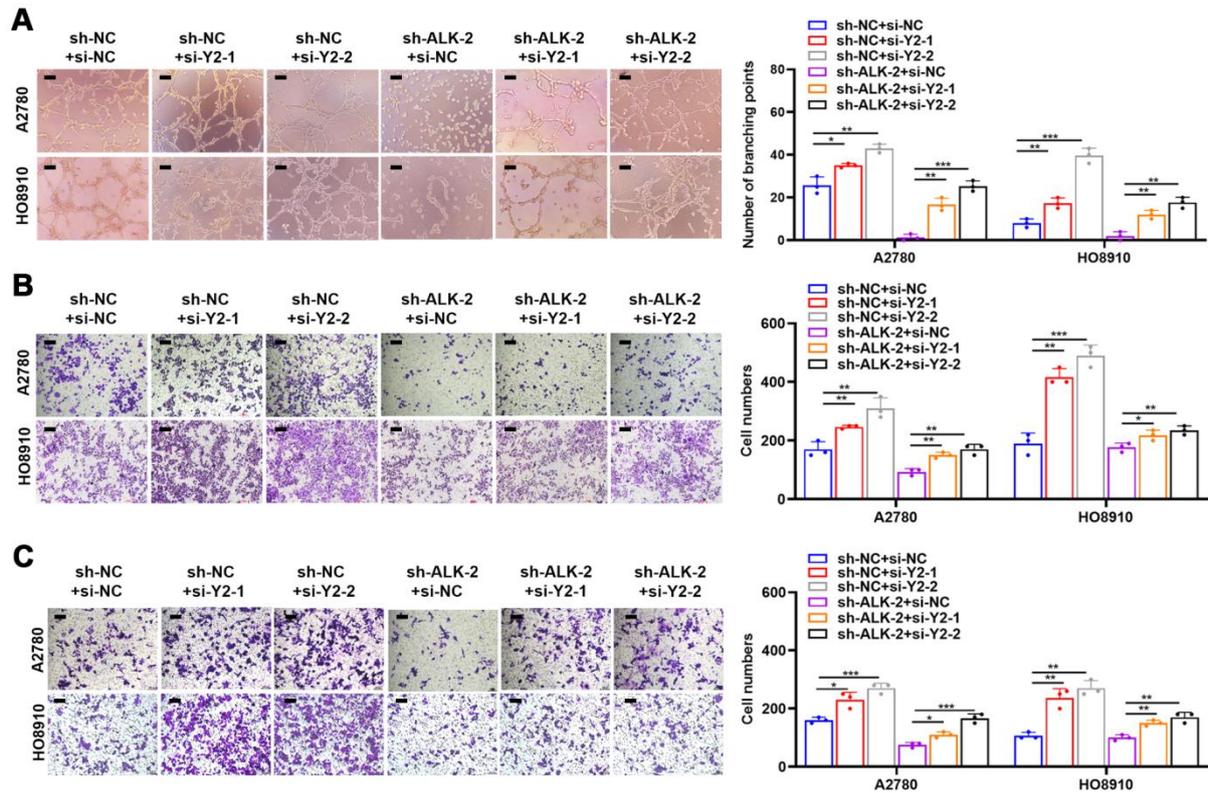


Figure S9. Knockdown of YTHDF2 partially rescues the LN metastasis caused by ALKBH5 downregulation *in vitro*

(A) Representative images (left panels) and histogram (right panels) of Matrigel tube formation assay with human lymphatic endothelial cells (HLECs). HLECs were cultured in a conditioned medium derived from ovarian cancer cells treated as indicated. (B and C) Representative images (left panels) and histogram (right panels) of migration (B) and invasion (C) assay with A2780 and HO8910 cells; all *in vitro* experiments were performed in at least three biological replicates. Error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

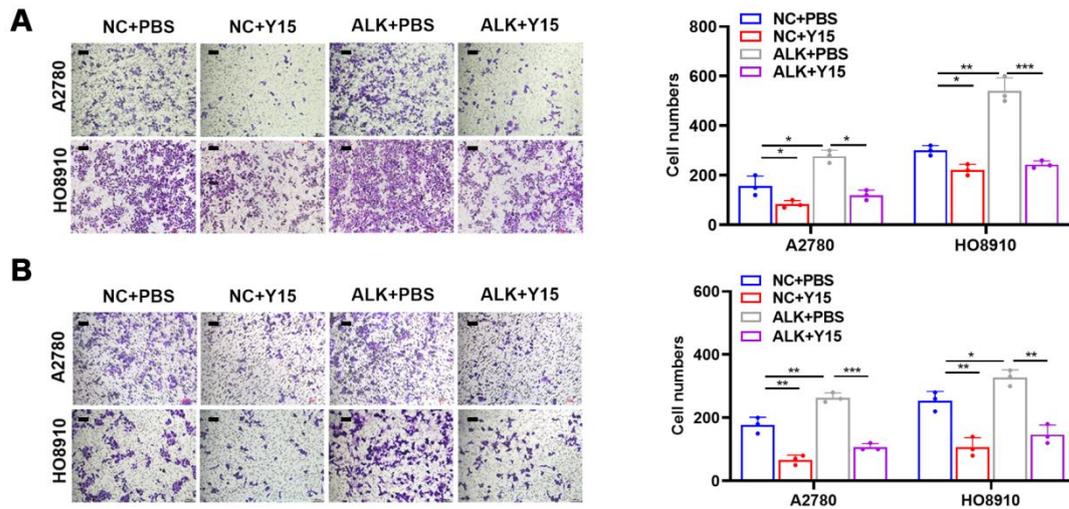


Figure S10. Y15, a p-FAK (Tyr397) inhibitor, blocks ALKBH5 expression during tumor cell migration and invasion

(A and B) Representative images (left panels) and histogram (right panels) of migration (A) and invasion (B) assay with A2780 and HO8910 cells; all *in vitro* experiments were performed in at least three biological replicates. Error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

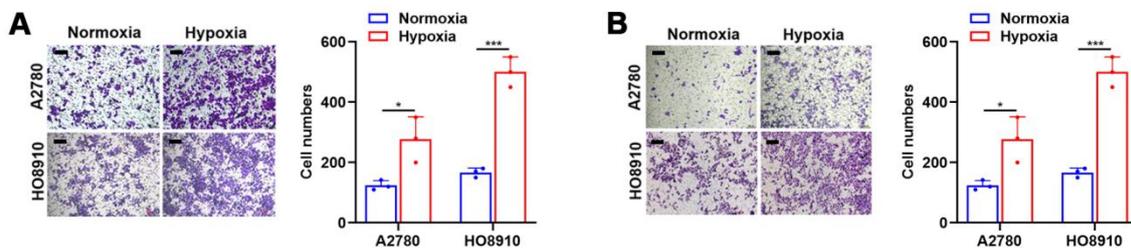


Figure S11. Hypoxia promotes tumor cell migration and invasion *in vitro*

(A and B) Representative images (left panels) and histogram (right panels) of migration (A) and invasion (B) assay with A2780 and HO8910 cells; all *in vitro* experiments were performed in at least three biological replicates. Error bars indicate the SD of the mean. Scale bars: 100 μ m. Statistical significance was assessed using a two-tailed Student's *t* test. * $P < 0.05$, *** $P < 0.001$.

Table S1: The clinical characteristics of 192 EOC patients and the descriptive analysis of ALKBH5 mRNA expression

Characteristic	All	ALKBH5 expression		P value
		High-expression	Low-expression	
Total	192			
Age at surgery				0.134
< 60	122	56	66	
≥60	70	40	30	
Histology type				0.082
Serous	135	62	73	
Other	57	34	23	
Grade				0.008*
G1+G2	61	22	39	
G3+G4	131	74	57	
FIGO Stage				0.035*
I+II	33	11	22	
III+IV	159	85	74	
Tumor size				0.191
< 10cm	85	38	47	
≥10cm	107	58	49	
Lymph node metastasis				0.004*
Positive	96	58	38	
Negative	96	38	58	
Peritoneal cytology				0.559
Positive	110	57	53	
Negative	82	39	43	
Complications				0.209
With diabetes	39	23	16	
Without diabetes	153	73	80	

Table S2: Primers used for qPCR assay

Gene	Forward (5'-3')	Reverse (5'-3')
ALKBH5	CGGCGAAGGCTACACTTACG	CCACCAGCTTTTGGATCACCA
FTO	AACACCAGGCTCTTTACGGTC	TGTCCGTTGTAGGATGAACCC
METTL3	TTGTCTCCAACCTTCCGTAGT	CCAGATCAGAGAGGTGGTGTAG
METTL14	GAGTGTGTTTACGAAAATGGGGT	CCGTCTGTGCTACGCTTCA
WTAP	CTTCCAAGAAGGTTTCGATTGA	TCAGACTCTCTTAGGCCAGTTAC
ZC3H13	TCTGATAGCACATCCCGAAGA	CAGCCAGTTACGGCACTGT
ITGB1	CAAGAGAGCTGAAGACTATCCCA	TGAAGTCCGAAGTAATCCTCCT
YTHDF2	AGCCCCACTTCTACCAGATG	TGAGAACTGTTATTTCCCCATGC
HIF-1α	ATCCATGTGACCATGAGGAAATG	TCGGCTAGTTAGGGTACACTTC
β-actin	GCTGTGCTATCCCTGTACGC	TGCCTCAGGGCAGCGGAACC
Primers used for MeRIP-qPCR		
ITGB1(chr 10: 32900318-32900497)	CCATGATTCAGAGAGCCCAA	GTCTTACTTTGAGTTAGTGCCAT
Primers used for ChIP-qPCR		
ALKBH5	CTTAGCCTTGCGCCCGTTC	AAACTTCTCAGACTGCGGGAC

Table S3: Antibodies used in this study.

Antibodies	Antibodies	Concentration
Anti-ALKBH5	16837-1-AP, Proteintech	1:1000 (WB), 1:400 (IHC and IF), 5 µg for RIP
Anti-ITGB1	ab179471, Abcame	1:2000 (WB), 1:1000 (IHC)
Anti-ITGB1	sc-13590L, Santa Cruz	5 µg for co-IP
Anti-LYVE-1	Ab219556, Abcam	1:1000 (IHC and IF)
Anti-YTHDF2	24744-1-AP, Proteintech	1:1000 (WB), 4 µg for RIP
Anti-FAK	3285T, CST	1:1000 (WB)
Anti-p-FAK(Tyr397)	8556T, CST	1:1000 (WB)
Anti-p-FAK(Tyr397)	AF3398, Affinity	1:200 (IHC)
Anti-Src	2109S, CST	1:1000 (WB)
Anti-p-Src (Tyr416)	6943S, CST	1:1000 (WB)
Anti-HIF1α	20960-1-AP, Proteintech	1:1000 (WB), 1:400 (IHC), 4 µg for ChIP
Anti-Podoplanin	Ab256561, Abcame	1:400 (IF)
Anti-m6A	ab232905, Abcam	1:400 (dot blot)
Anti-m6A	ab208577, Abcam	10 µg for MeRIP
Anti-β-actin	GB15003, Servicebio	1:1000 (WB)

Table S4. The target sequences of genes

gene	target sequences (5'-3')
sh-ALKBH5-1	UCAGAUCGCCUGUCAGGAATT
sh-ALKBH5-2	GGAUAUGCUGCUGAUGAA ATT
si-ITGB1-1	AUGGGACACGGGUGAAAAUTT
si-ITGB1-2	GCUCAGUCUUACUAAUAAATT
si-YTHDF2-1	TTGGCTATGGGAACGTCTT
si-YTHDF2-2	CAAGGAAACAAAGTGCAAA

Table S5: The RNA probe sequences for RNA-pulldown

Probe	Position	Sequences(5'-3')
m6A-1 probe	Chr10: 32900384	AUCGGAUGUCUUG(m6A)CUCUGAUGUAUUUUUAUCAG-biotin
G-1 probe		AUCGGAUGUCUUGGCUCUGAUGUAUUUUUAUCAG-biotin
A-1 probe		AUCGGAUGUCUUGACUCUGAUGUAUUUUUAUCAG-biotin
m6A-2 probe	Chr10: 32900460	GUGCCUUUAGUUUUA(m6A)CAGUUCACUUUUUACAG-biotin
G-2 probe		GUGCCUUUAGUUUUA GCAGUUCACUUUUUACAG-biotin
A-2 probe		GUGCCUUUAGUUUUA ACAGUUCACUUUUUACAG-biotin

Table S6: Detailed RNA seqTences of dual-luciferase reporter

LTciferase reporter	Position	DNA seqTences
ITGB1 wt-1	Chr10: 32900384	AAAUACUAAAUCGGAUGUCUUG <u>A</u> CUCUGAUGUAUU UUAUCAGGUUGUGUGCAUGAAAUUUUUAUAGAUUAA AGAAGUUGAGGAAAAGCA
ITGB1 mut-1	Chr10: 32900384	AAAUACUAAAUCGGAUGUCUUG <u>G</u> CUCUGAUGUAUU UUAUCAGGUUGUGUGCAUGAAAUUUUUAUAGAUUAA AGAAGUUGAGGAAAAGCA
ITGB1 wt-2	Chr10: 32900460	ACUAGUCACAUUCUUGUUUUAAAGUGCCUUUAGUUUU <u>A</u> <u>A</u> CAGUUCACUUUUACAGUGCUAUUUACUGAAGUU AUUUUUUUUUAAUUGCCUA
ITGB1 Mut-2	Chr10: 32900460	ACUAGUCACAUUCUUGUUUUAAAGUGCCUUUAGUUUU <u>A</u> <u>G</u> CAGUUCACUUUUACAGUGCUAUUUACUGAAGUU AUUUUUUUUUAAUUGCCUA