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Supplemental Information

ALKBH5 Inhibited Cell Proliferation and Sensitized Bladder Cancer Cells to Cisplatin by m6A-CK2α-Mediated Glycolysis

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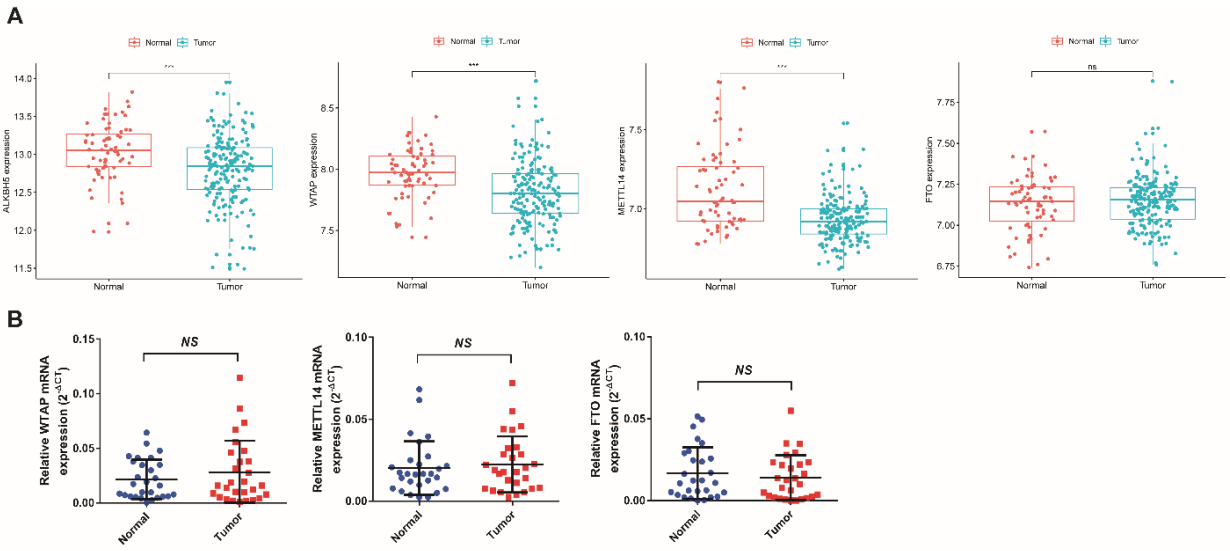
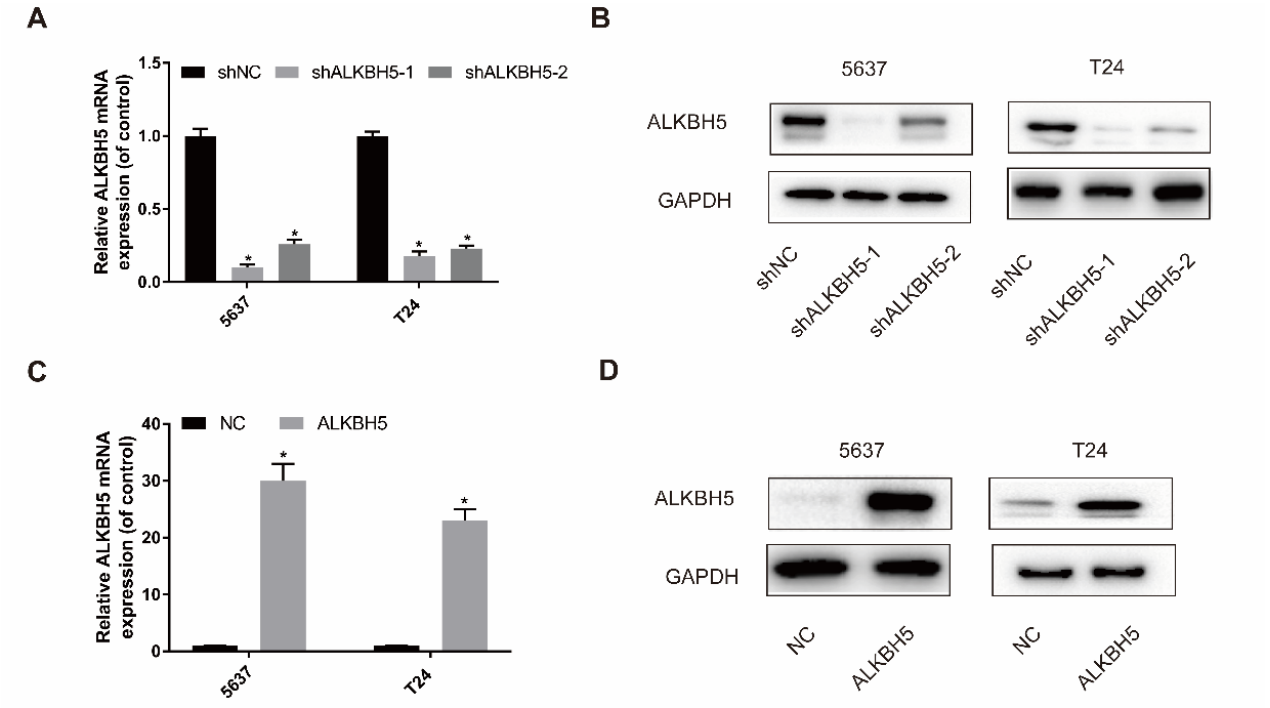
Figure.S1



Supplemental Figure S1 The expression of WTAP, METTL14 and FTO in bladder cancer tissues. (A) GEO database（https://www.ncbi.nlm.nih.gov/geo/, GSE13507）analysis of ALKBH5，WTAP, METTL14, ALKBH5 and FTO in bladder cancer tissues. NS: *P*>0.05, ****P*<0.001. (B) Validation of the mRNA levels of WTAP, METTL14 and FTO in bladder cancer tissues and adjacent normal tissues by qRT-PCR. Date represents the mean ±SD from three independent experiments. NS: *P*>0.05

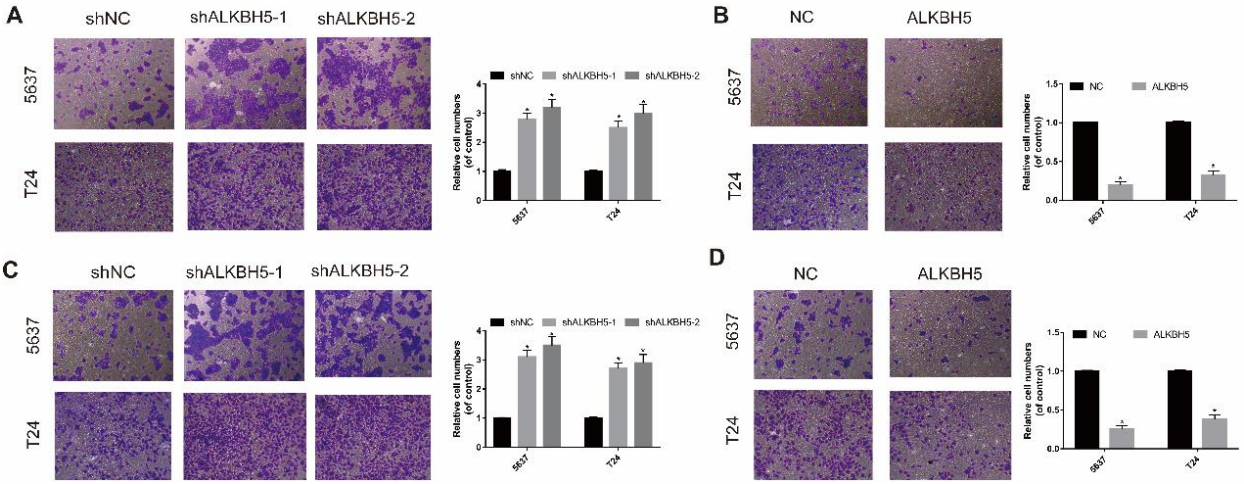
Figure.S2



Supplemental Figure S2 The efficiency of ALKBH5 knockdown and overexpression in bladder cancer cell lines.

(A,B,C,D) Validation of the overexpression and knockdown efficacy of ALKBH5 in T24 and 5637 cell lines by qRT-PCR and western blot. Data represent the mean ±SD from three independent experiments. **P*<0.05

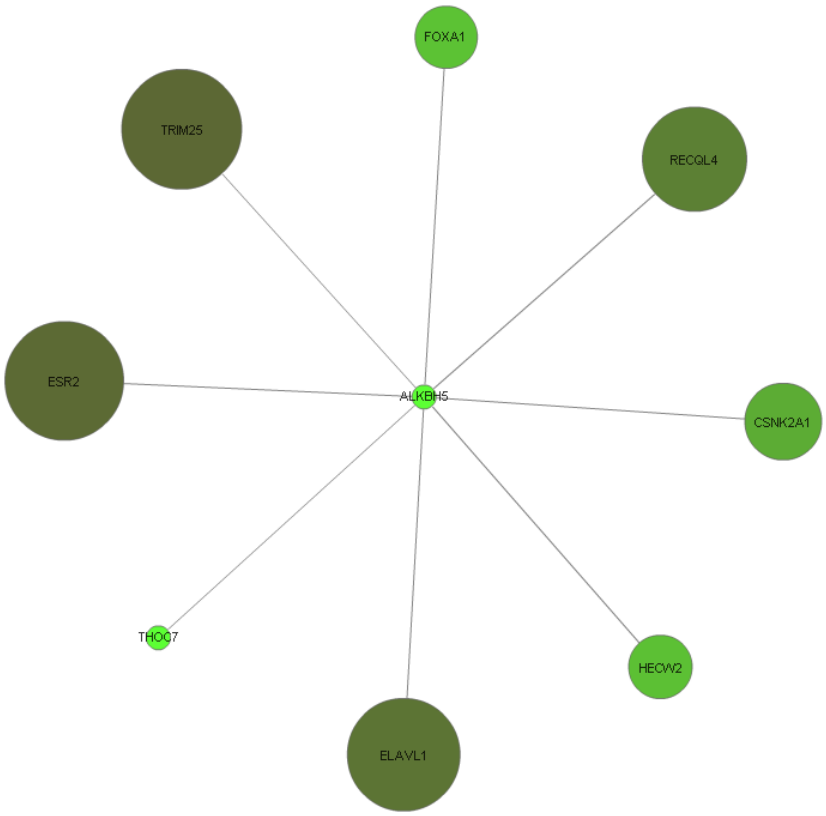
Figure.S3



Supplemental Figure S3 ALKBH5 inhibited bladder cancer cell migration and invasion.

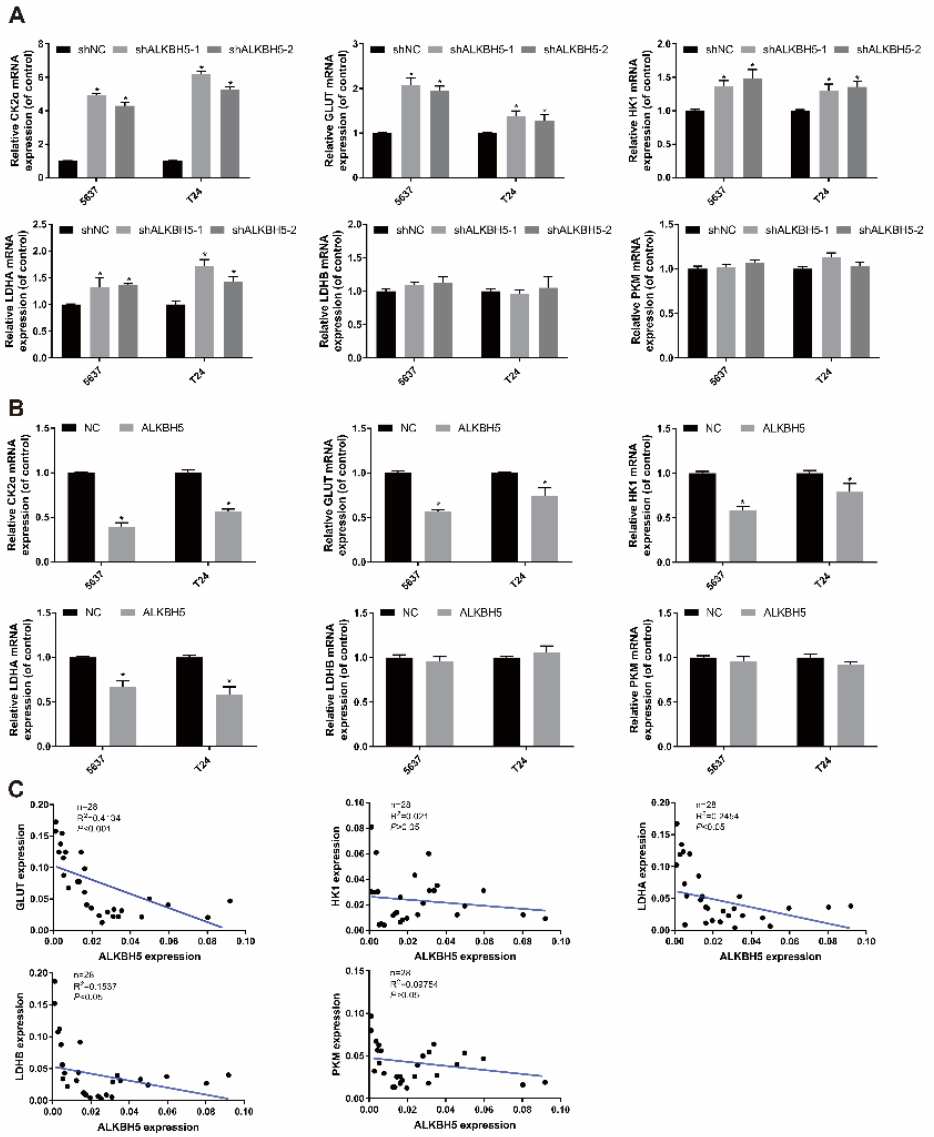
(A,B) Migration and **(C,D)** invasion assay in T24 and 5637 cell lines were measured. The results were expressed as the fold of the crossing cells number per field compared with respective control. Magnification: 100X, Data represent the mean ±SD from three independent experiments. **P*<0.05

Figure.S4



Supplemental Figure S4 TCGA database showed that ALKBH5 regulated CK2α expression.

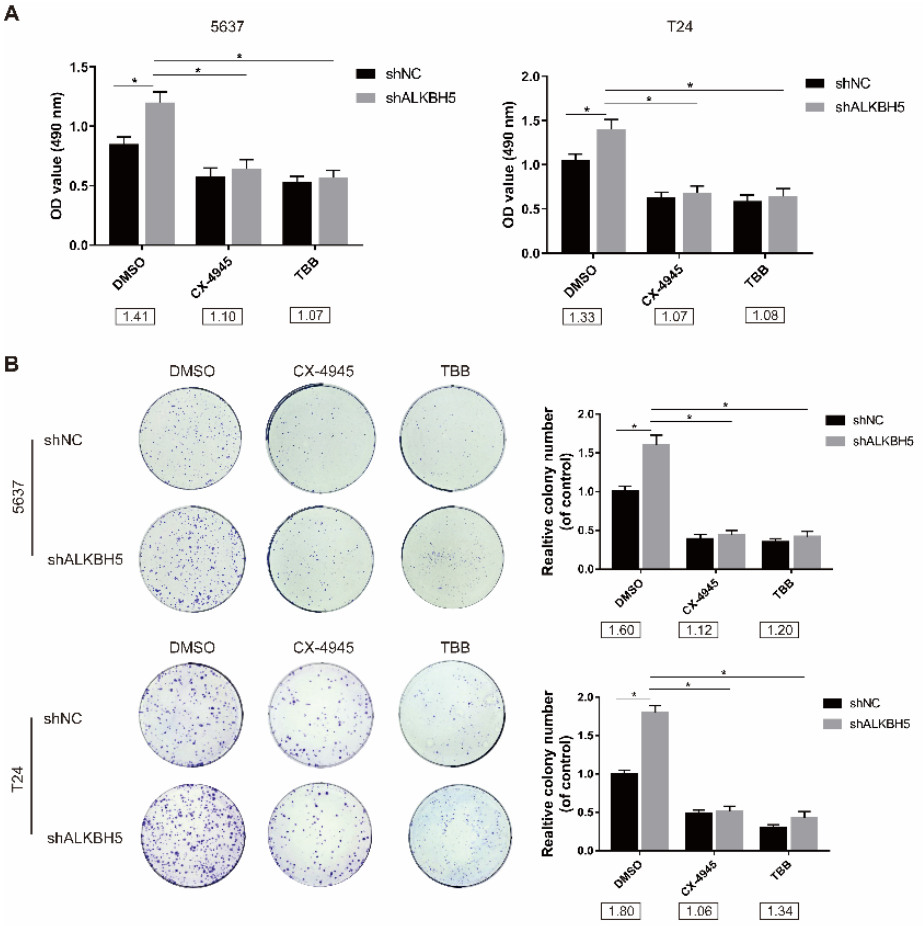
Figure.S5



Supplemental Figure S5 The relationship between ALKBH5 and glycolysis genes in bladder cancer cells and tissues.

(A,B) qRT-PCR analysis of GLUT, HK1, LDHA, LDHB and PKM in 5637 and T24 cells with ALKBH5 knockdown or overexpression. Date represents the mean ±SD from three independent experiments. **P*<0.05 NS: *P*>0.05 **(C)** The relationship between glycolysis related genes and ALKBH5 in patient samples were detected by qRT-PCR. The expression of GLUT, LDHA and LDHB mRNAs were significantly negatively associated with ALKBH5 in bladder cancer tissues. (n=28, *P*<0.05). HK1 and PKM mRNAs were negatively associated with ALKBH5 but not statistically significant. (n=28, *P*>0.05)

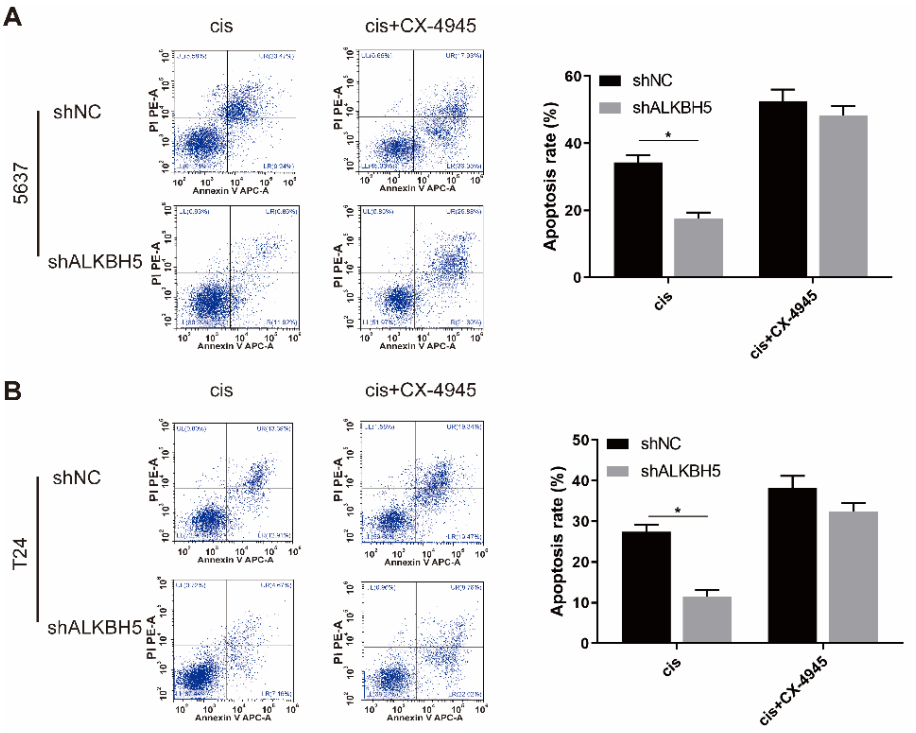
Figure.S6



Supplemental Figure S6 CK2α inhibitors inhibited bladder cancer cell proliferation *in vitro*.

(A,B) CK2α inhibitors (CX-4945 and TBB) decreased the OD value fold (shALKBH5/NC) and the colony fold (shALKBH5/NC) in CCK8 assay and the colony formation assay. Data represent the mean ± SD from three independent experiments. **P*<0.05.

Figure.S7



Supplemental Figure S7 CK2*α* inhibitor increased bladder cancer cell apoptosis rate *in vitro*.

(A,B) CK2α inhibitors CX-4945 increased the percentage of apoptotic cells after treated with cisplatin for 48 hours. The rate of cisplatin-induced apoptosis rescued in knockdown ALKBH5 5637 and T24 cells compared with control cells after treatment with cisplatin. Data represent the mean ± SD from three independent experiments. Student's t-test with two biological independent replicates were used to determine statistical significance; **P*< 0.05

Supplemental Table S1 Oligonucleotide sequences used in this study

Primes		Sequences
ALKBH5	Forward	5′- CTTCCCAAGAAGGTTCGATTGA-3′.
	Reverse	5′- TCAGACTCTCTTAGGCCAGTTAC-3′
CK2α	Forward	5′- CCGAGTTGCTTCCCGATAC-3′
	Reverse	5′- GGGCTGACAAGGTGCTGAT-3′
GLUT	Forward	5′- ACAACCAGACATGGGTCCAC-3′
	Reverse	5′- TAACGAAAAGGCCCACAGAG-3′
HK1	Forward	5′- CACATGGAGTCCGAGGTTTATG-3′
	Reverse	5′- CGTGAATCCCACAGGTAACTTC-3′
LDHA	Forward	5′- ATGGCAACTCTAAAGGATCAGC-3′
	Reverse	5′- CCAACCCCAACAACTGTAATCT-3′
LDHB	Forward	5′- CCTCAGATCGTCAAGTACAGTCC-3′
	Reverse	5′- ATCACGCGGTGTTTGGGTAAT-3′
PKM	Forward	5′- ATGTCGAAGCCCCATAGTGAA-3′
	Reverse	5′- TGGGTGGTGAATCAATGTCCA-3′
WTAP	Forward	5′- TTGTAATGCGACTAGCAACCAA-3′
	Reverse	5′- GCTGGGTCTACCATTGTTGATCT-3′
METTL14	Forward	5′- TTTCTCTGGTGTGGTTCTGG-3′
	Reverse	5′- AAGTCTTAGTCTTCCCAGGATTG-3′
FTO	Forward	5′- CCAGAACCTGAGGAGAGAATGG-3′
	Reverse	5′- CGATGTCTGTGAGGTCAAACGG-3′
β-actin	Forward	5′- AGCGAGCATCCCCCAAAGTT-3′
	Reverse	5′- GGGCACGAAGGCTCATCATT-3′
GAPDH	Forward	5′- CCCAGCCTCAAGATCATCAGCAATG-3′
	Reverse	5′- ATGGACTGTGGTCATGAGTCCTT-3′