

## Supplementary Material

### **Oncogenic MSH6-CXCR4-TGFB1 feedback loop: a novel therapeutic target of photothermal therapy in glioblastoma multiforme**

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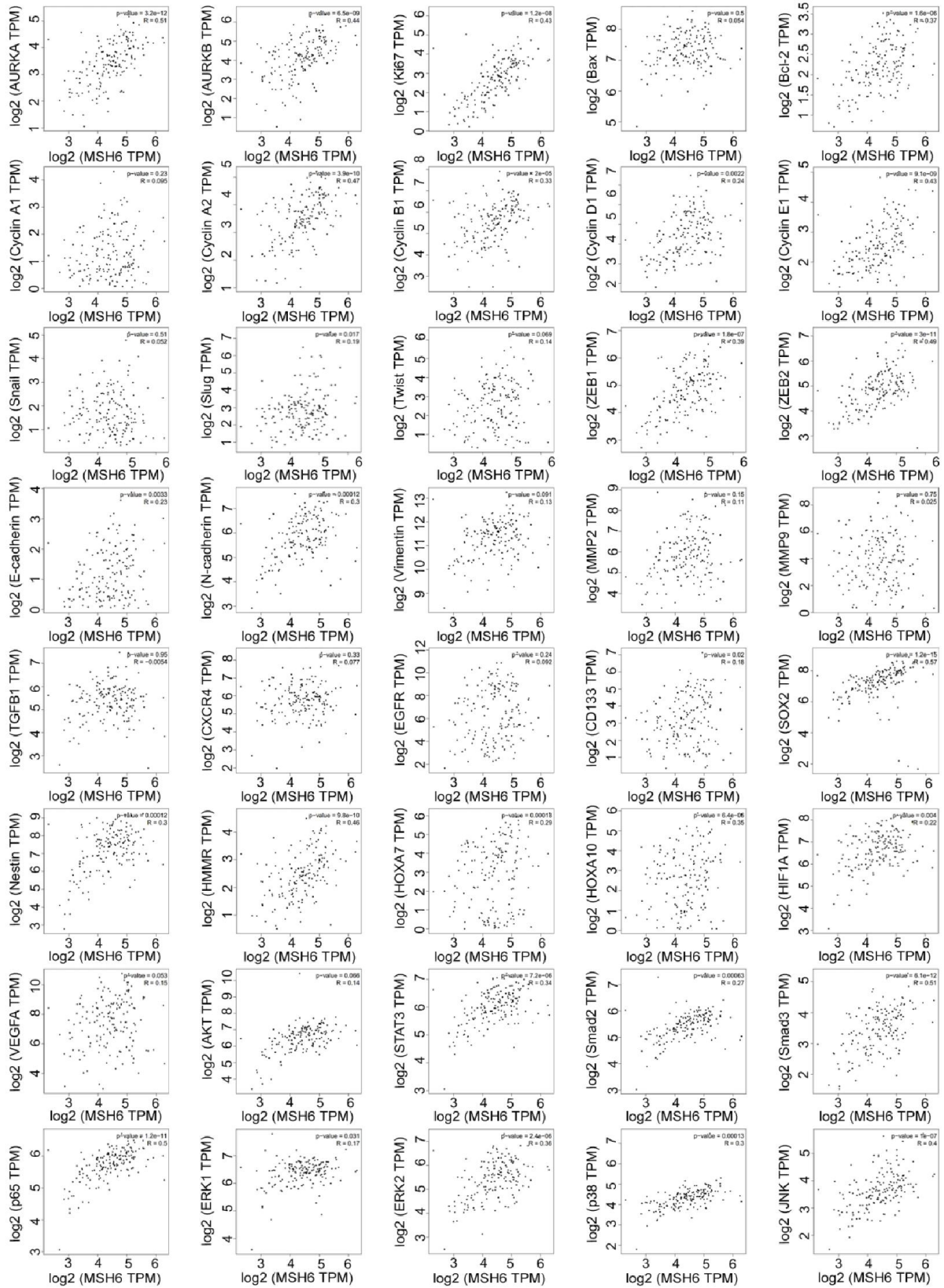
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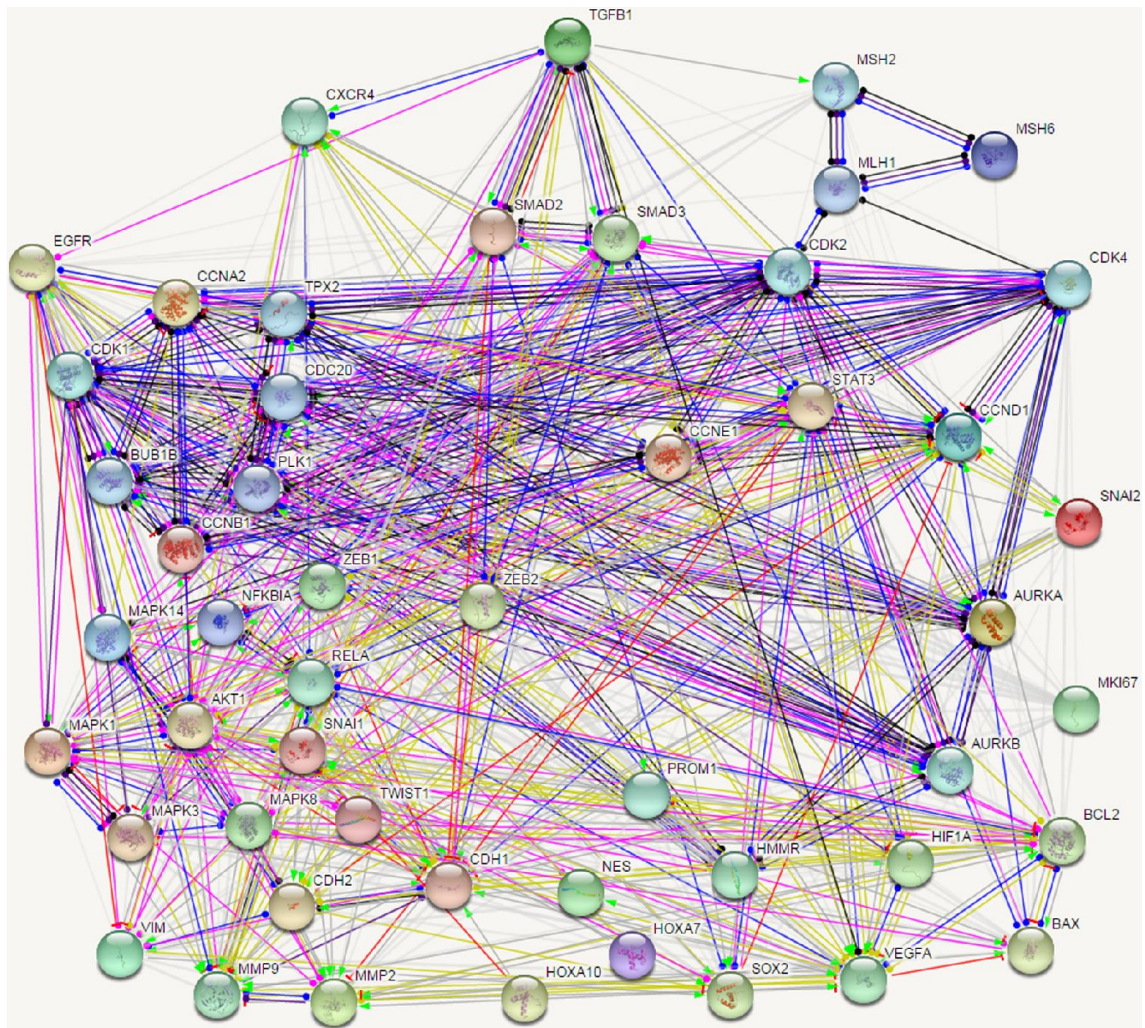
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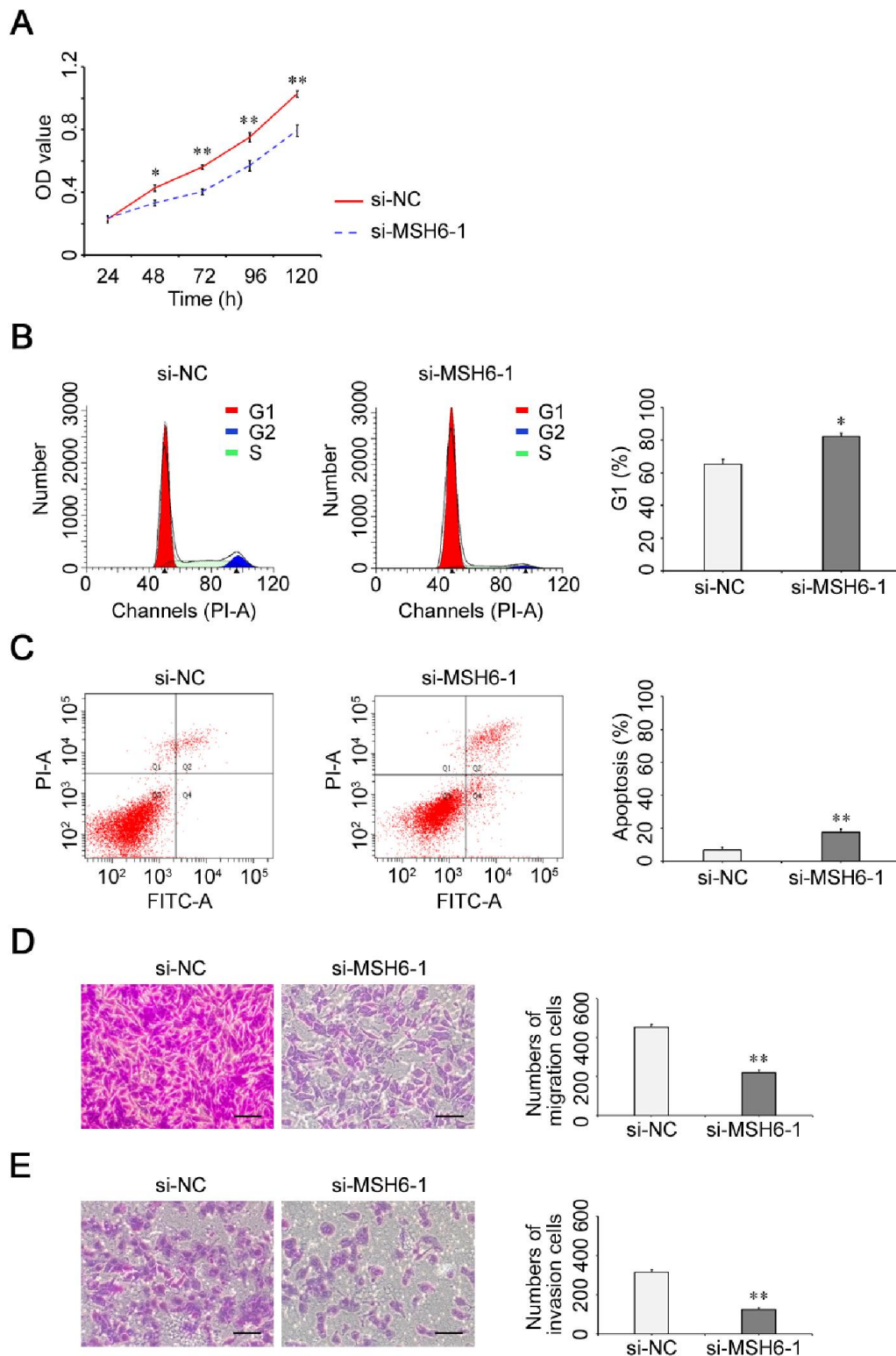


**Figure S1.** Pearson correlation analysis of MSH6 and a series of functional genes (GEPIA).

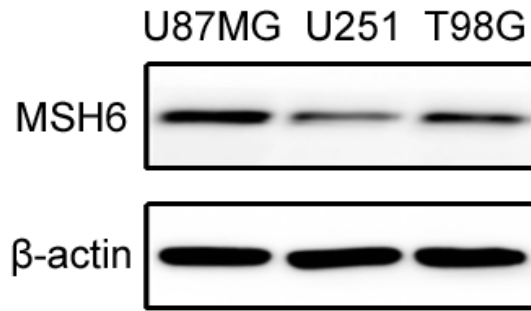


**Figure S2.** Protein-protein interactions of MSH6 and a series of functional genes (STRING).

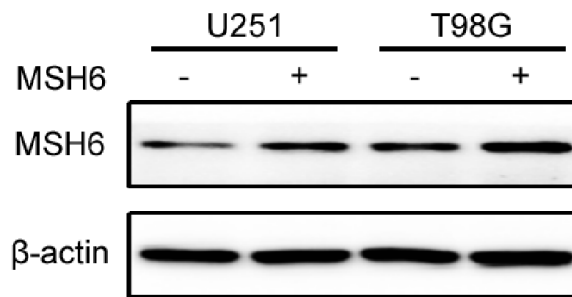




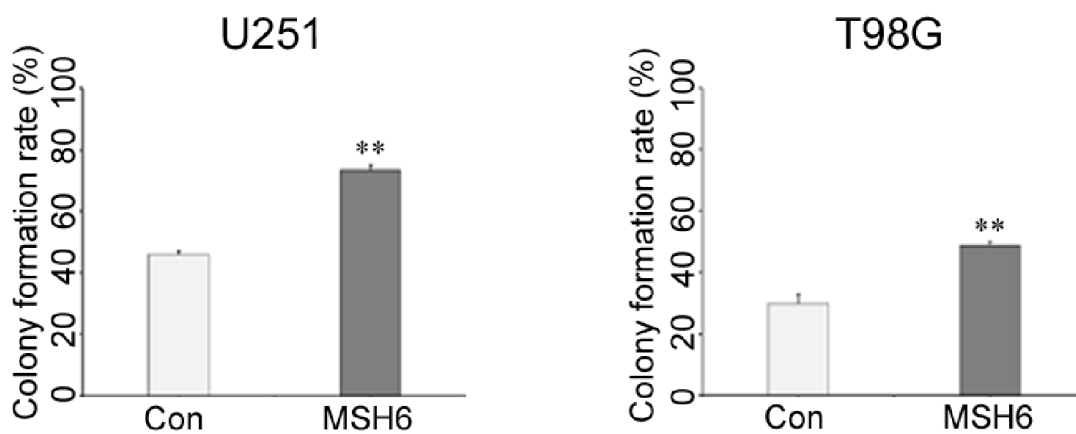
**Figure S3.** (A) Cell viability assays, (B) cell cycle assays, (C) cell apoptosis assays, (D) cell migration assays (scale bar represents 200  $\mu$ m), and (E) cell invasion assays (scale bar represents 200  $\mu$ m) of U87MG cells after silencing MSH6 with si-MSH6-1. Error bars represent the standard deviation, n = 3. \* P < 0.05, \*\* P < 0.01.



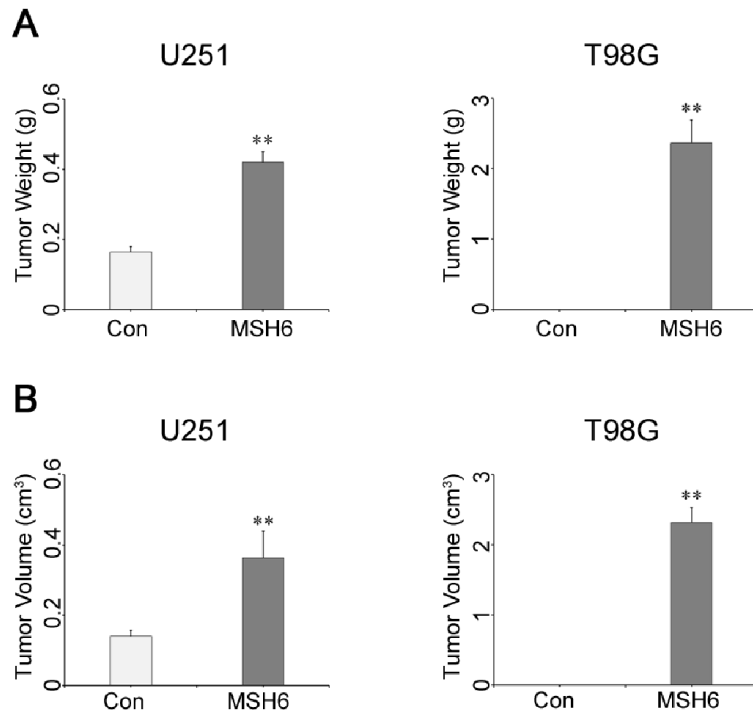
**Figure S4.** The expression of MSH6 in different GBM cell lines.



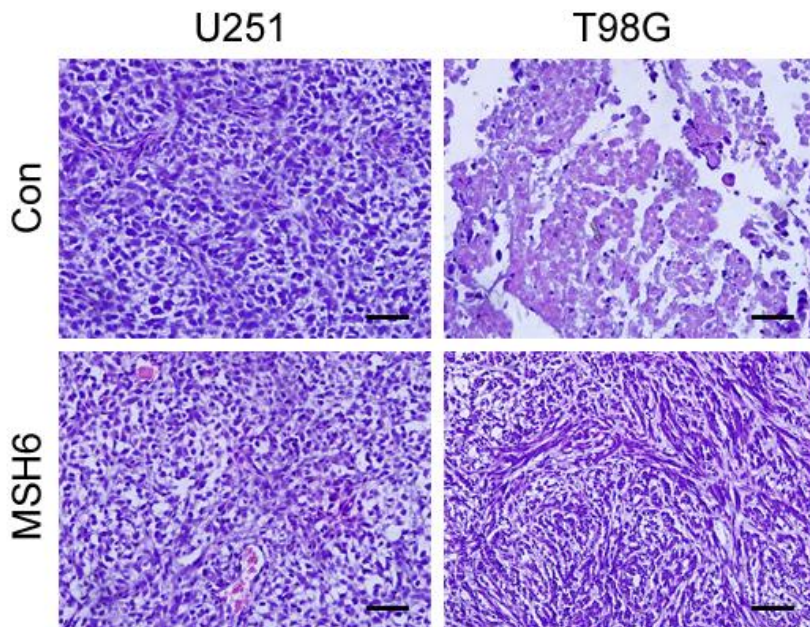
**Figure S5.** The expression of MSH6 after overexpressing MSH6 in U251 and T98G cells.



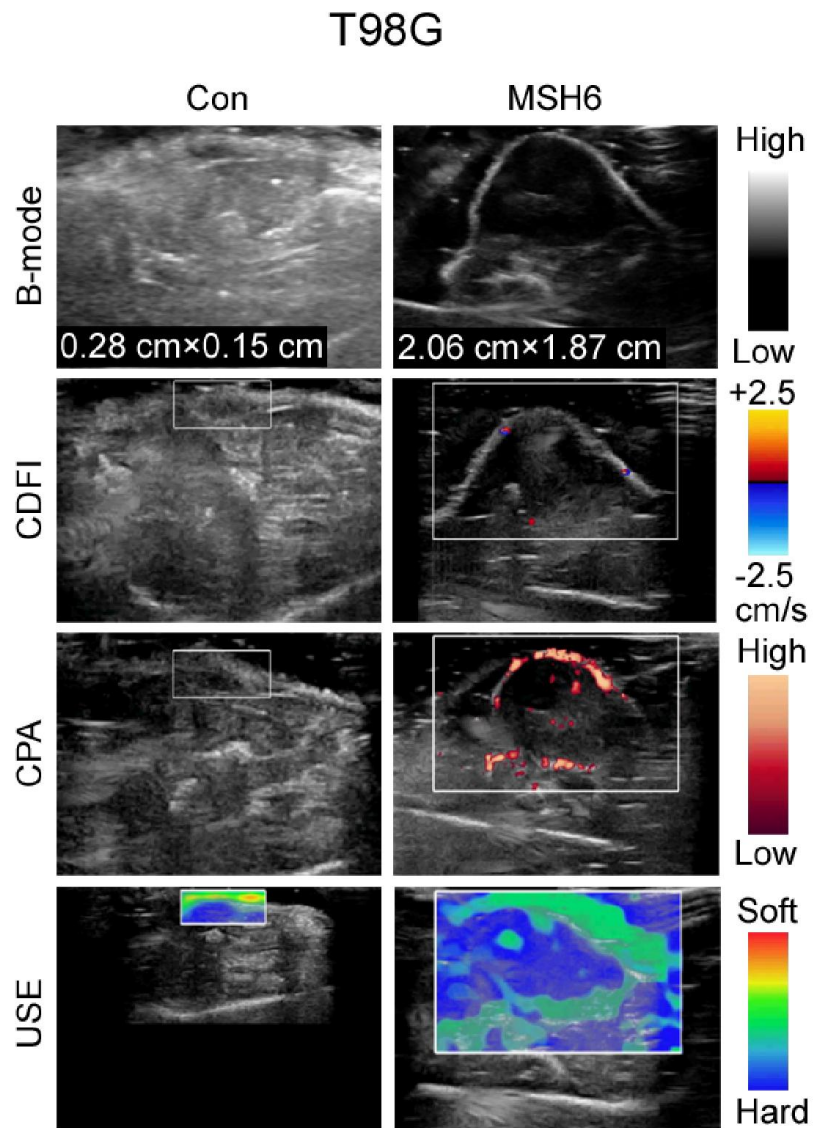
**Figure S6.** Colony formation rate of U251 and T98G cells after overexpressing MSH6. Error bars represent the standard deviation, n = 3. \*\* P < 0.01.



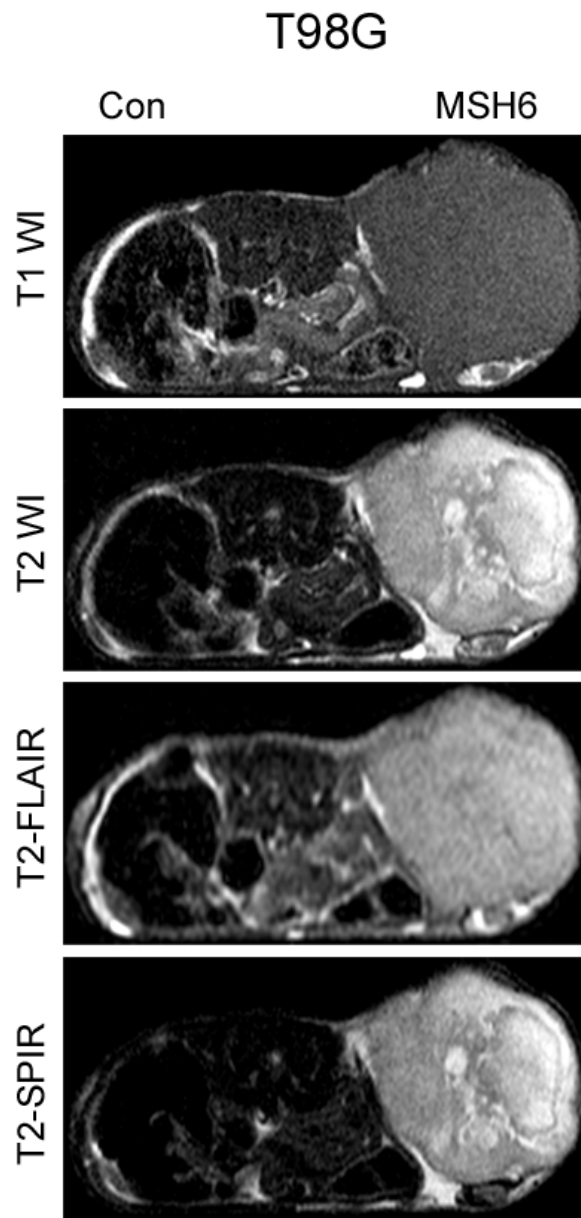
**Figure S7.** (A) The weights and (B) volumes of tumors from U251-Con/MSH6 or T98G-Con/MSH6 tumor-bearing nude mice. Error bars represent the standard deviation, n = 3. \*\* P < 0.01.



**Figure S8.** H&E staining of tumors from U251-Con/MSH6 or T98G-Con/MSH6 tumor-bearing nude mice (scale bar represents 50  $\mu$ m).

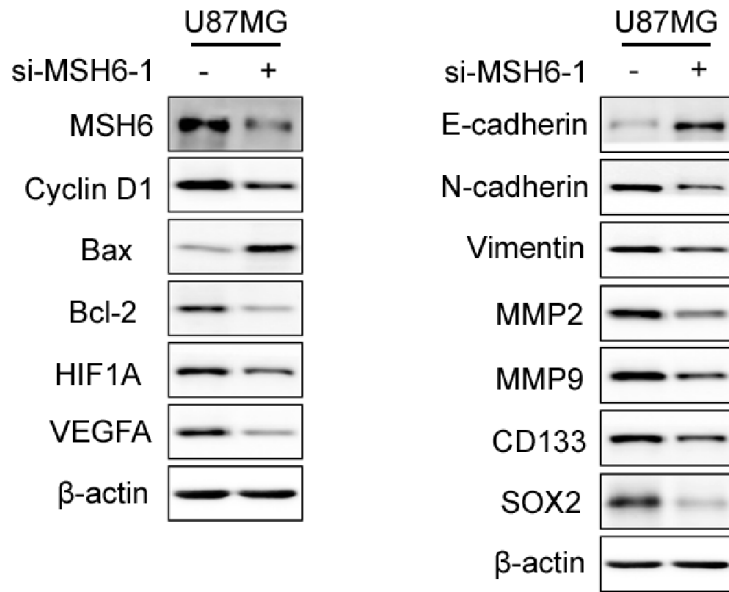


**Figure S9.** USI images of tumors from T98G-Con/MSH6 tumor-bearing nude mice.

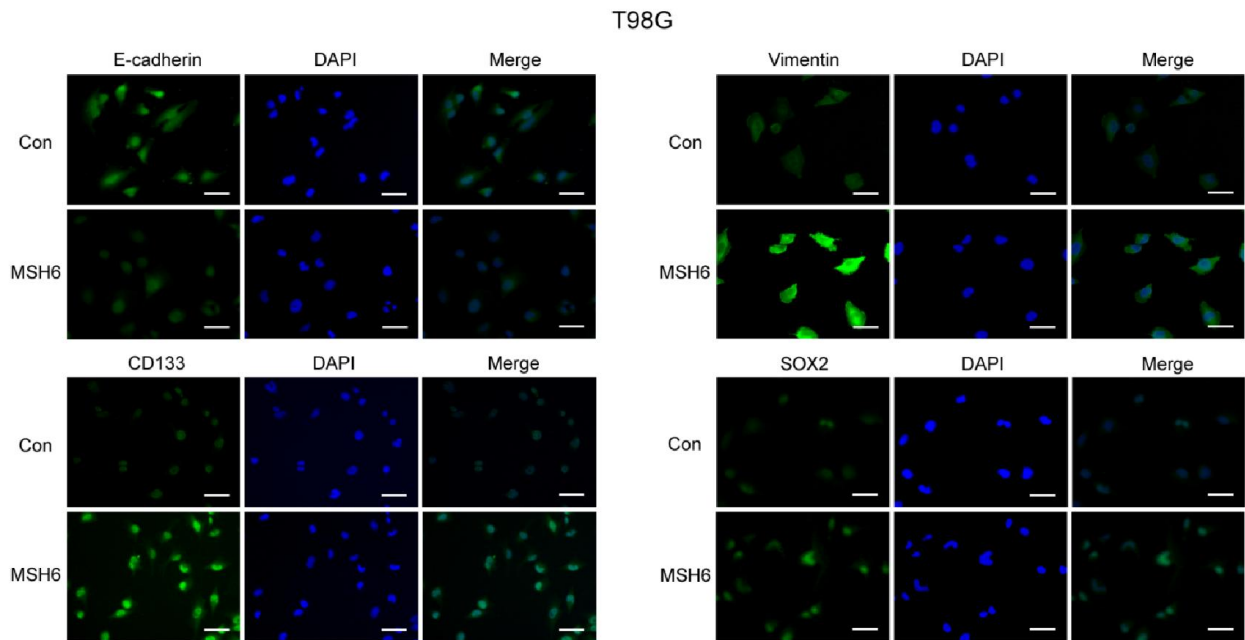


**Figure S10.** MRI images of tumors from T98G-Con/MSH6 tumor-bearing nude mice.

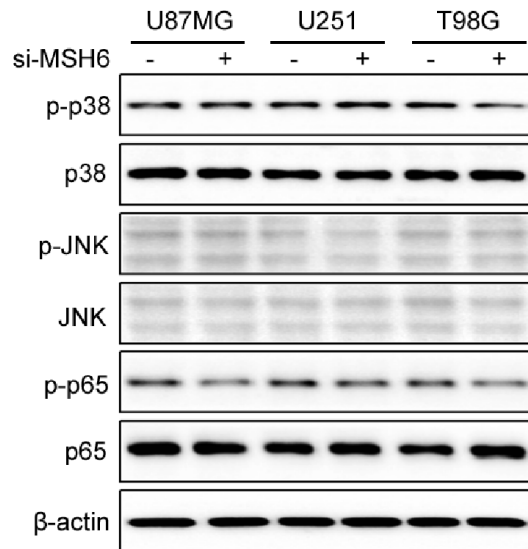




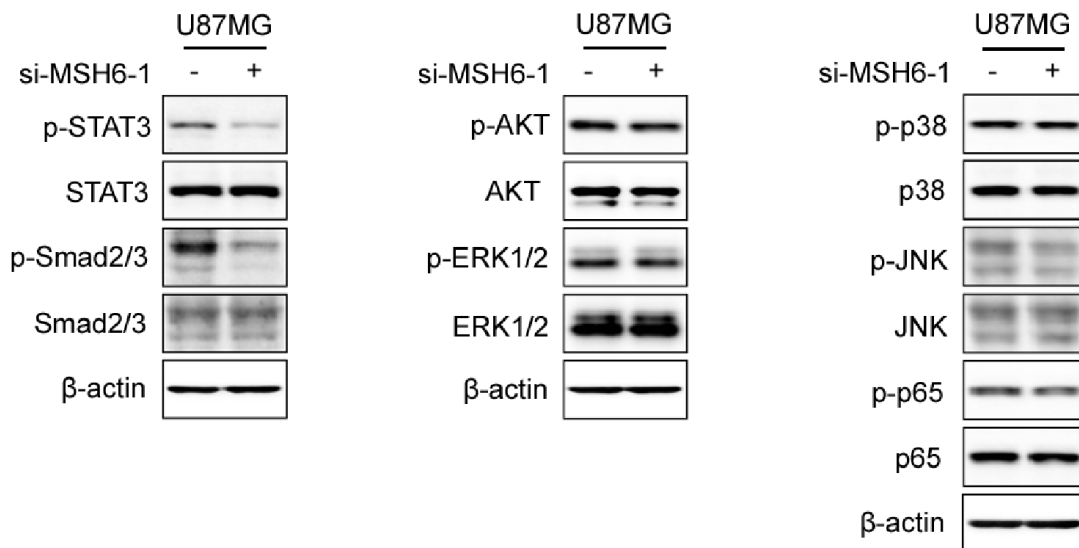
**Figure S11.** The expression of some typical regulatory factors and markers was detected by western blot assays after silencing MSH6 with si-MSH6-1 in U87MG cells.



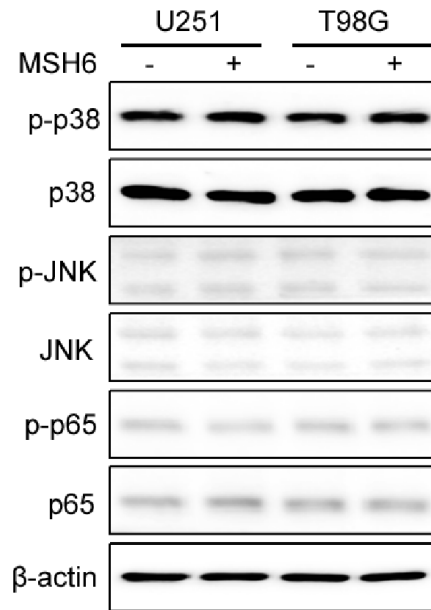
**Figure S12.** E-cadherin, Vimentin, CD133 and SOX2 protein expression and subcellular localization were determined by immunofluorescence assays in T98G cells (scale bar represents 100  $\mu$ m).



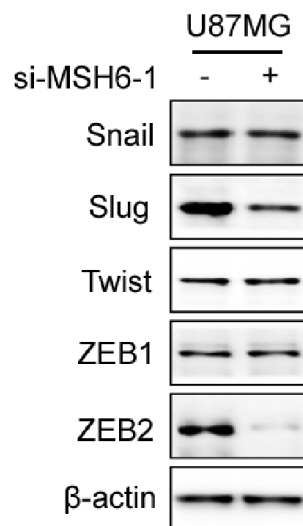
**Figure S13.** The expression of some signaling pathway-related proteins was evaluated by western blot assays after silencing MSH6.



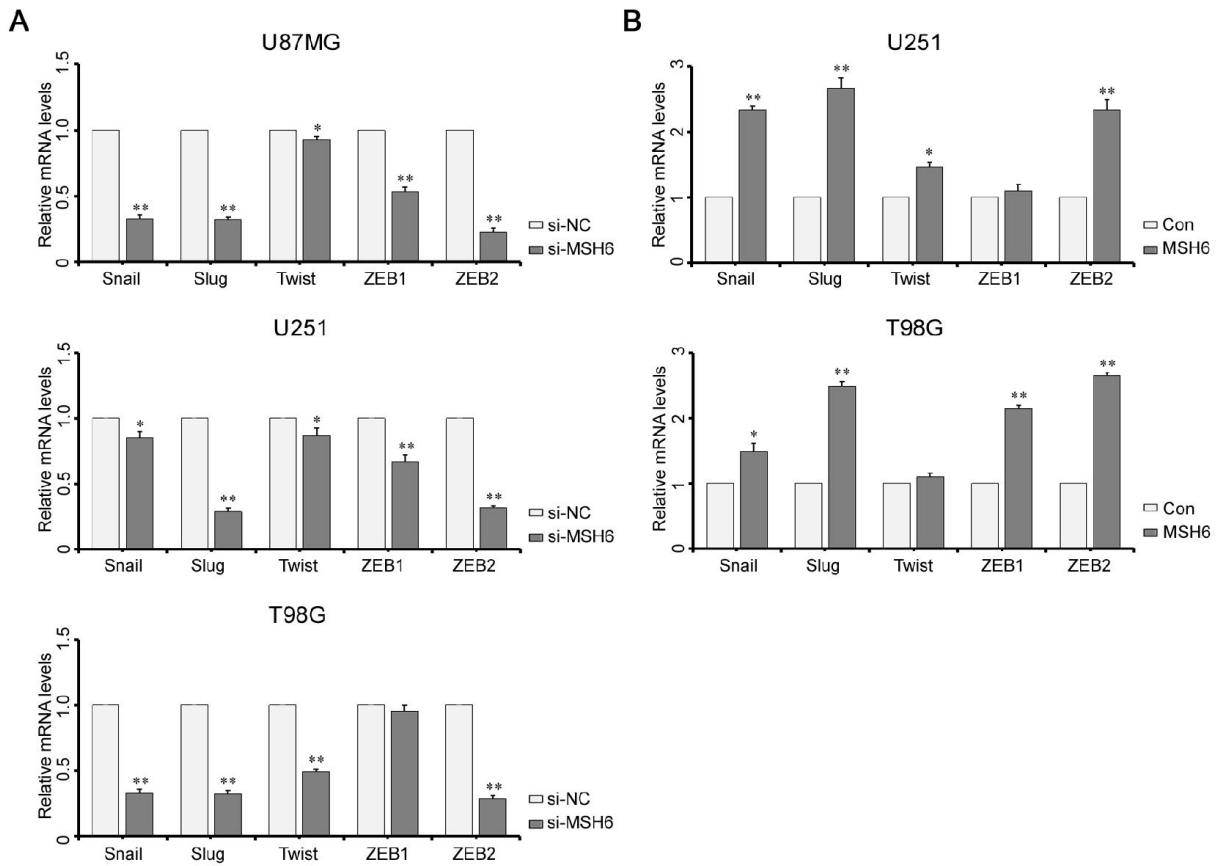
**Figure S14.** The expression of some signaling pathway-related proteins was detected by western blot assays after silencing MSH6 with si-MSH6-1 in U87MG cells.



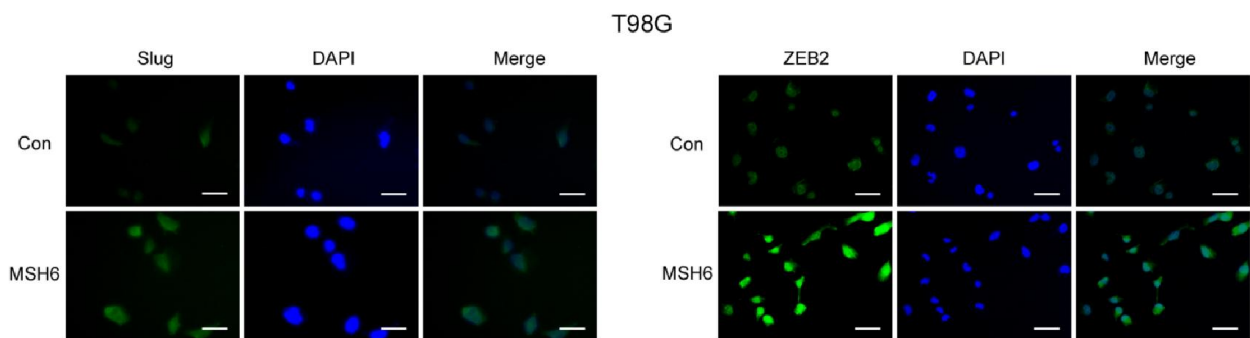
**Figure S15.** The expression of some signaling pathway-related proteins was evaluated by western blot assays after overexpressing MSH6.



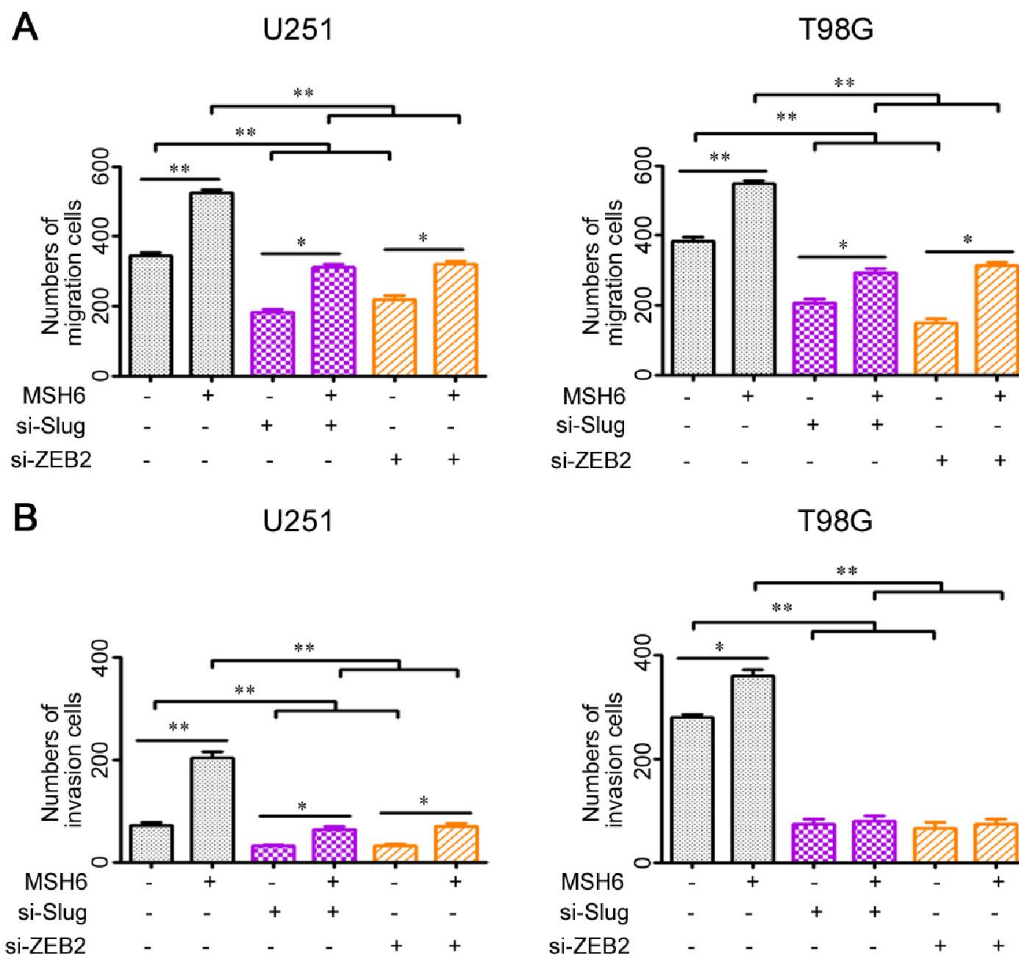
**Figure S16.** The expression of five EMT regulatory factors was detected by western blot assays after silencing MSH6 with si-MSH6-1 in U87MG cells.



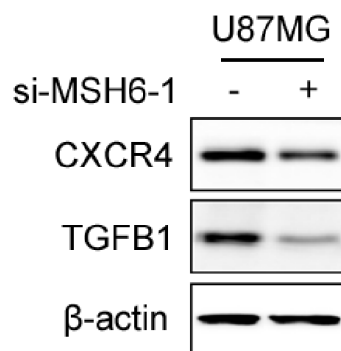
**Figure S17.** (A) qRT-PCR assays of Snail, Slug, Twist, ZEB1 and ZEB2 after silencing MSH6 or (B) overexpressing MSH6. Error bars represent the standard deviation, n = 3. \* P < 0.05, \*\* P < 0.01.



**Figure S18.** Slug and ZEB2 protein expression and subcellular localization were determined by immunofluorescence assays in T98G cells (scale bar represents 100  $\mu$ m).

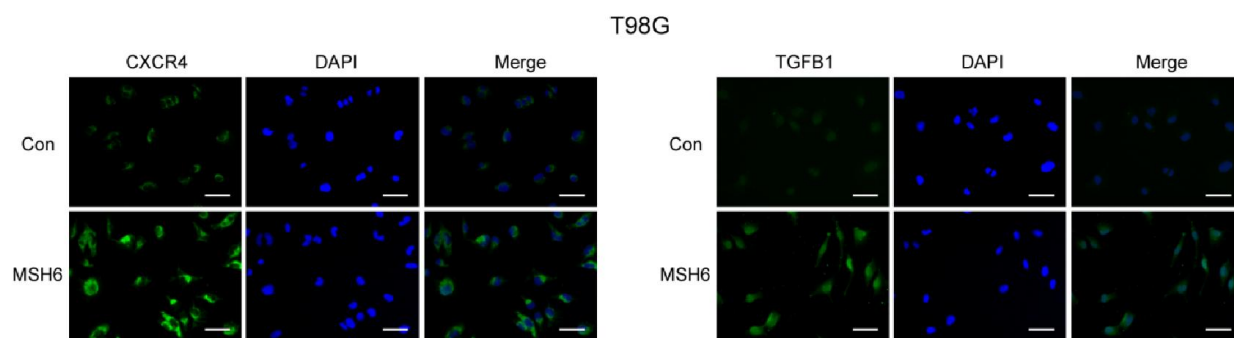


**Figure S19.** (A) The number of migratory or (B) invasive cells after silencing Slug or ZEB2, while simultaneously overexpressing MSH6 in U251 and T98G cells. Error bars represent the standard deviation, n = 3. \* P < 0.05, \*\* P < 0.01.

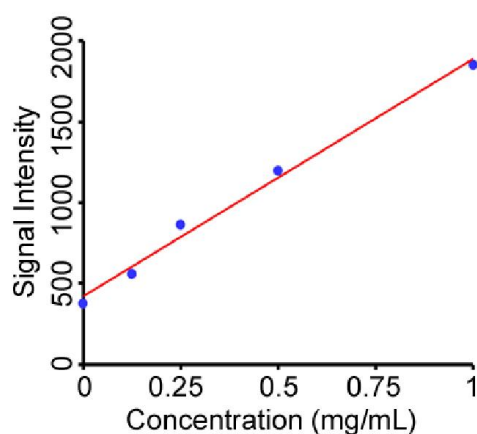


**Figure S20.** CXCR4 and TGFB1 expression was detected by western blot assays after silencing MSH6 with si-MSH6-1 in U87MG cells.

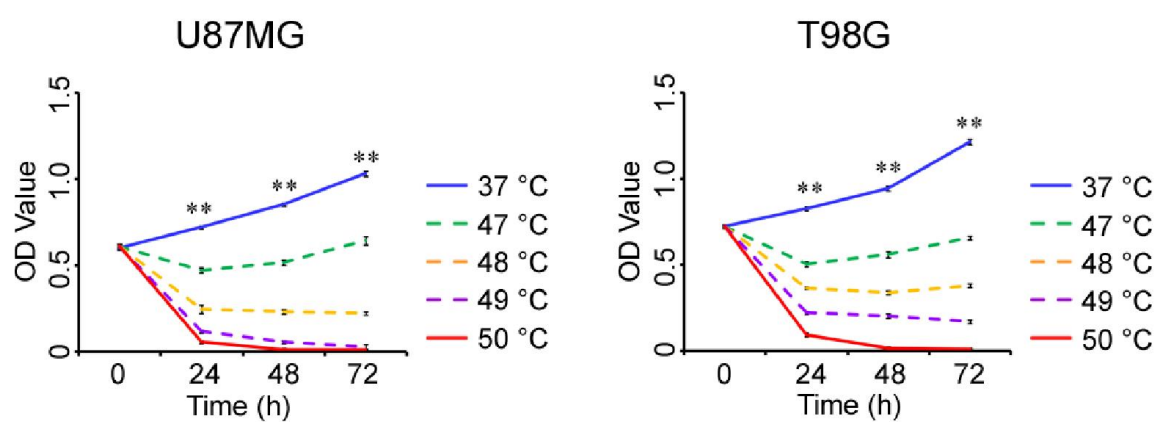




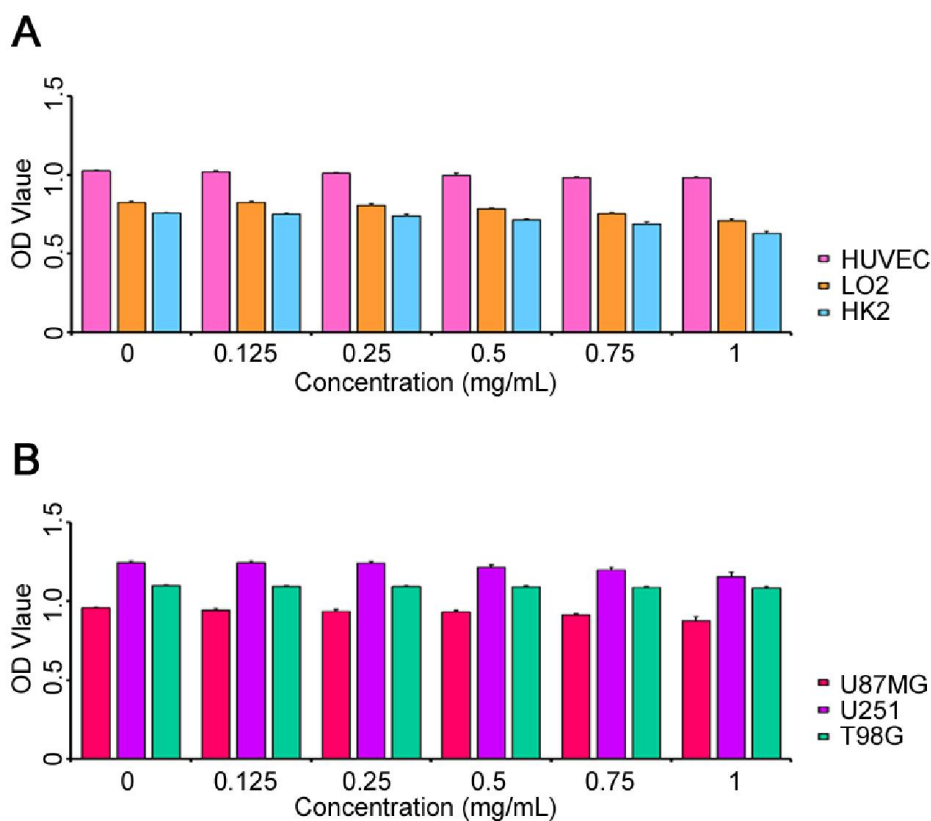
**Figure S21.** CXCR4 and TGFB1 protein expression and subcellular localization were determined by immunofluorescence assays in T98G cells (scale bar represents 100  $\mu\text{m}$ ).



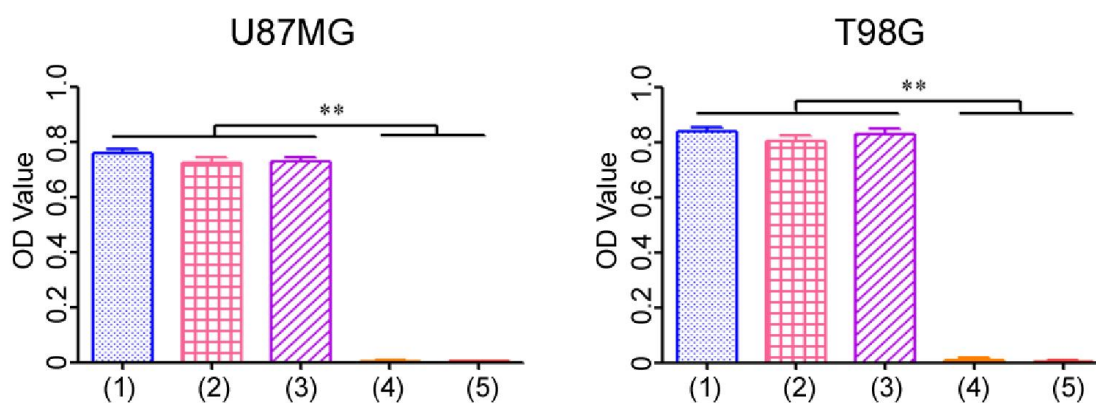
**Figure S22.** Signal intensity of  $\text{Cu}_2(\text{OH})\text{PO}_4@\text{PAA}$  at different concentrations in T1WI.



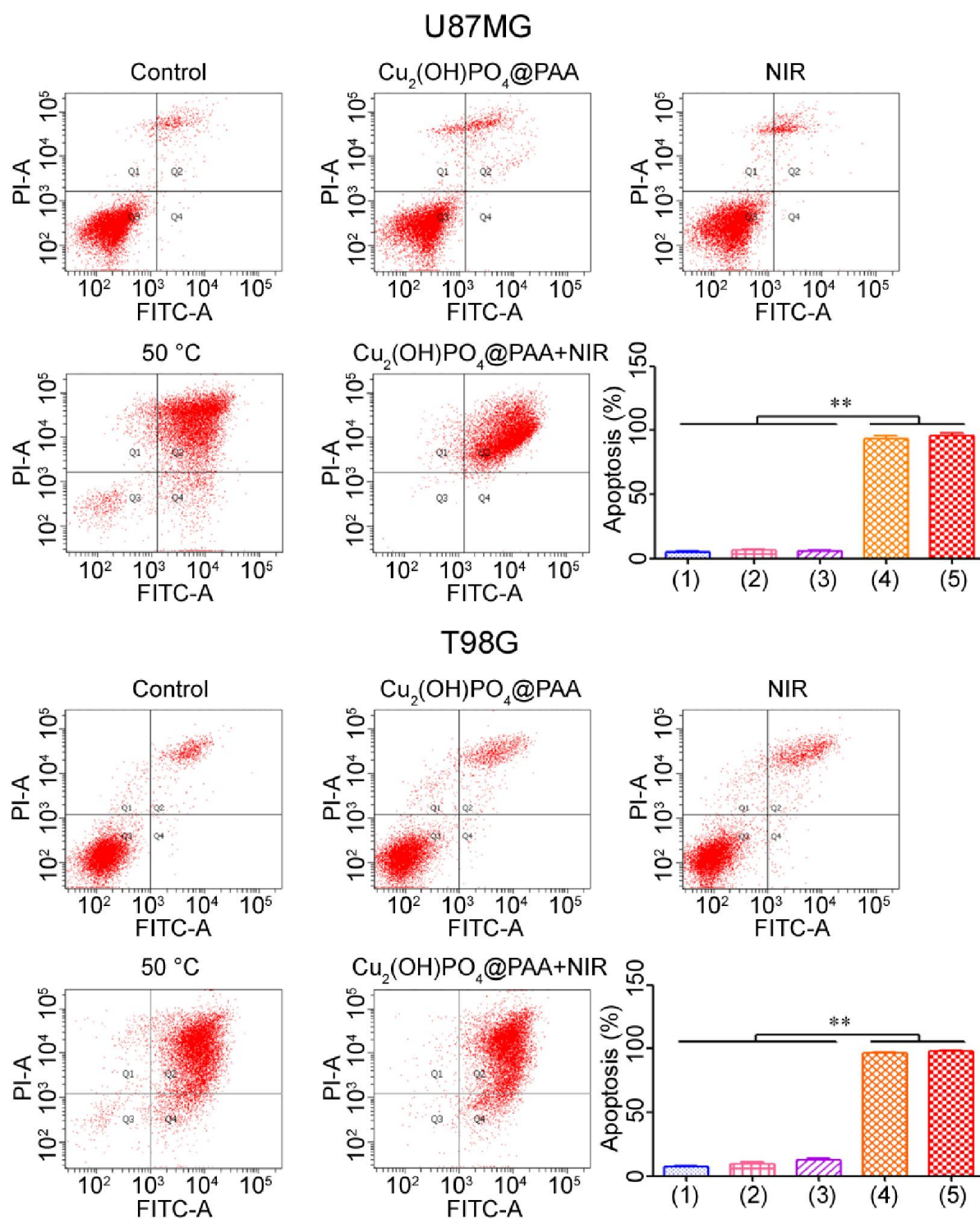
**Figure S23.** Cell viability assays of U87MG and T98G cells after hyperthermia at different temperatures (water bath). Error bars represent the standard deviation,  $n = 3$ . \*\*  $P < 0.01$ .



**Figure S24.** (A) Cell viability assays of HUVECs, LO2 cells and HK2 cells after adding different doses of  $\text{Cu}_2(\text{OH})\text{PO}_4\text{@PAA}$ . (B) Cell viability assays of U87MG, U251 and T98G cells after adding different doses of  $\text{Cu}_2(\text{OH})\text{PO}_4\text{@PAA}$ . Error bars represent the standard deviation,  $n = 3$ .



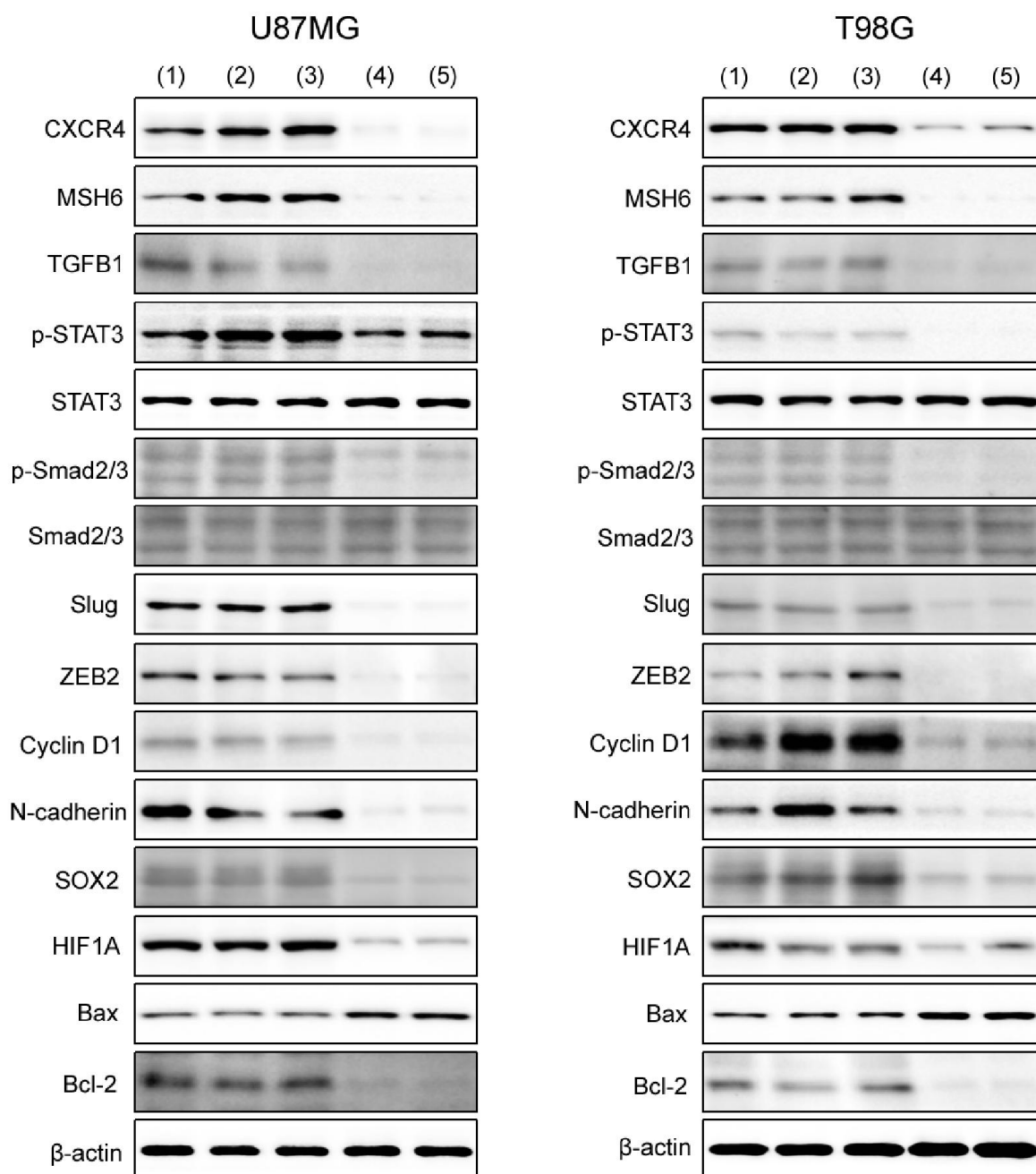
**Figure S25.** Cell viability assays of U87MG and T98G cells after different treatments. Groups: (1) control; (2)  $\text{Cu}_2(\text{OH})\text{PO}_4\text{@PAA}$ ; (3) NIR; (4) 50 °C (water bath); and (5)  $\text{Cu}_2(\text{OH})\text{PO}_4\text{@PAA}$  + NIR. Error bars represent the standard deviation,  $n = 3$ . \*\*  $P < 0.01$ .



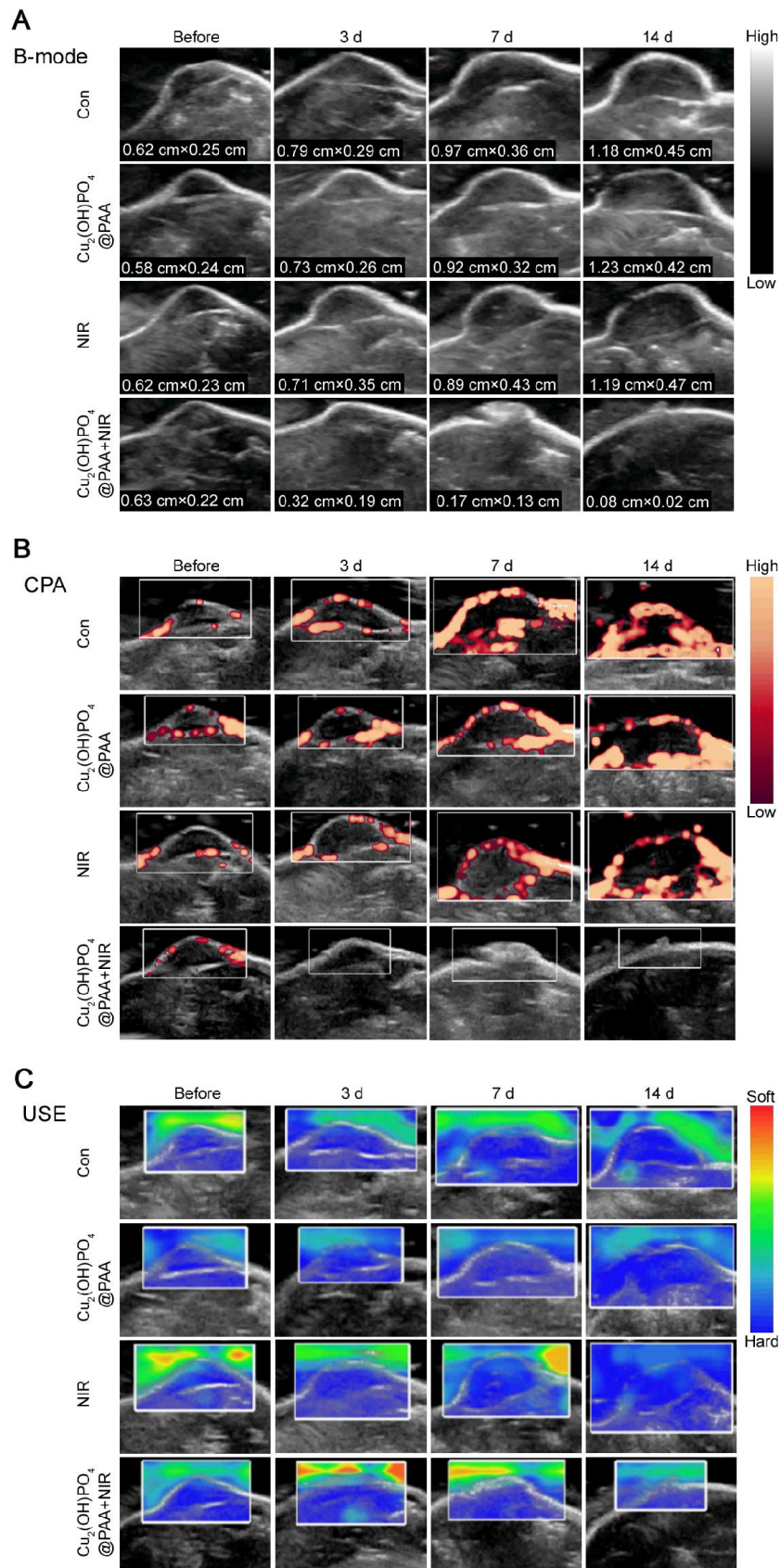
**Figure S26.** Cell apoptosis assays of U87MG and T98G cells after different treatments.

Groups: (1) control; (2)  $\text{Cu}_2(\text{OH})\text{PO}_4@\text{PAA}$ ; (3) NIR; (4) 50 °C (water bath); and (5)

$\text{Cu}_2(\text{OH})\text{PO}_4@\text{PAA} + \text{NIR}$ . Error bars represent the standard deviation,  $n = 3$ . \*\*  $P < 0.01$ .

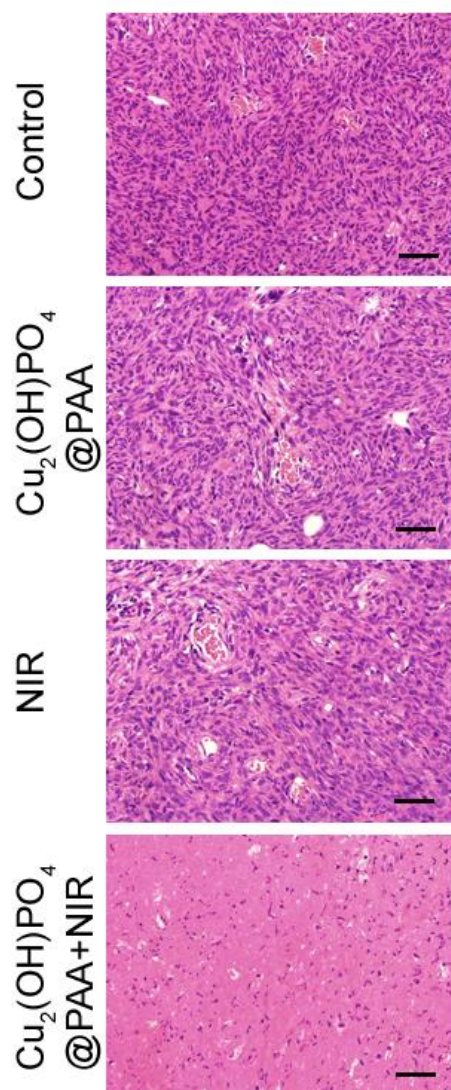


**Figure S27.** The expression of MSH6-related regulatory factors and markers was evaluated by western blot assay after different treatments in U87MG and T98G cells. Groups: (1) control; (2)  $\text{Cu}_2(\text{OH})\text{PO}_4@\text{PAA}$ ; (3) NIR; (4)  $50^\circ\text{C}$  (water bath); and (5)  $\text{Cu}_2(\text{OH})\text{PO}_4@\text{PAA}$  + NIR.

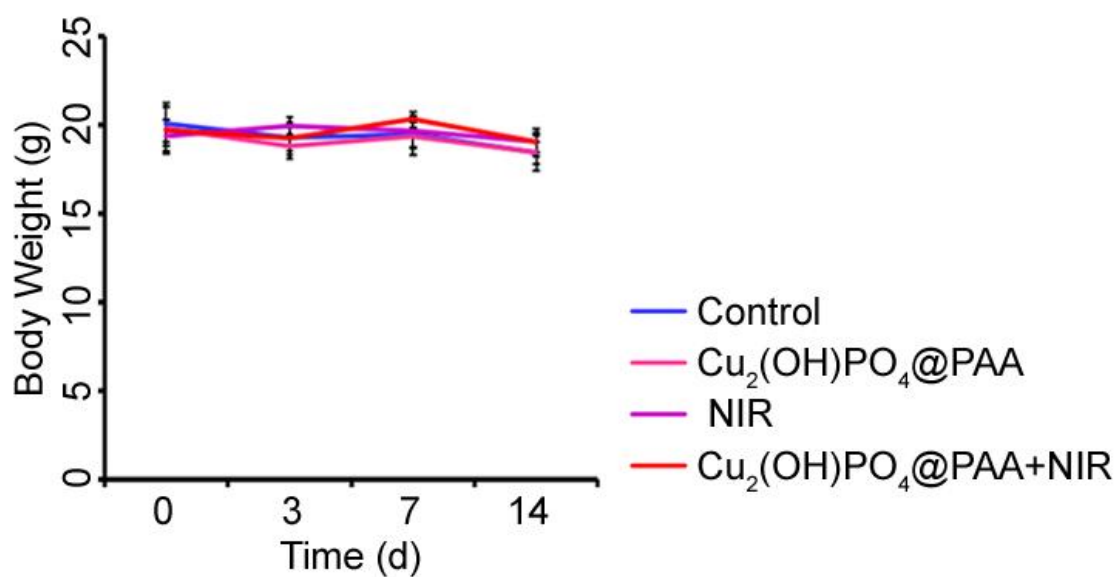


**Figure S28.** (A) B-mode, (B) CPA and (C) USE images of U251 tumor-bearing mice before and 3 days, 7 days and 14 days after different treatments.

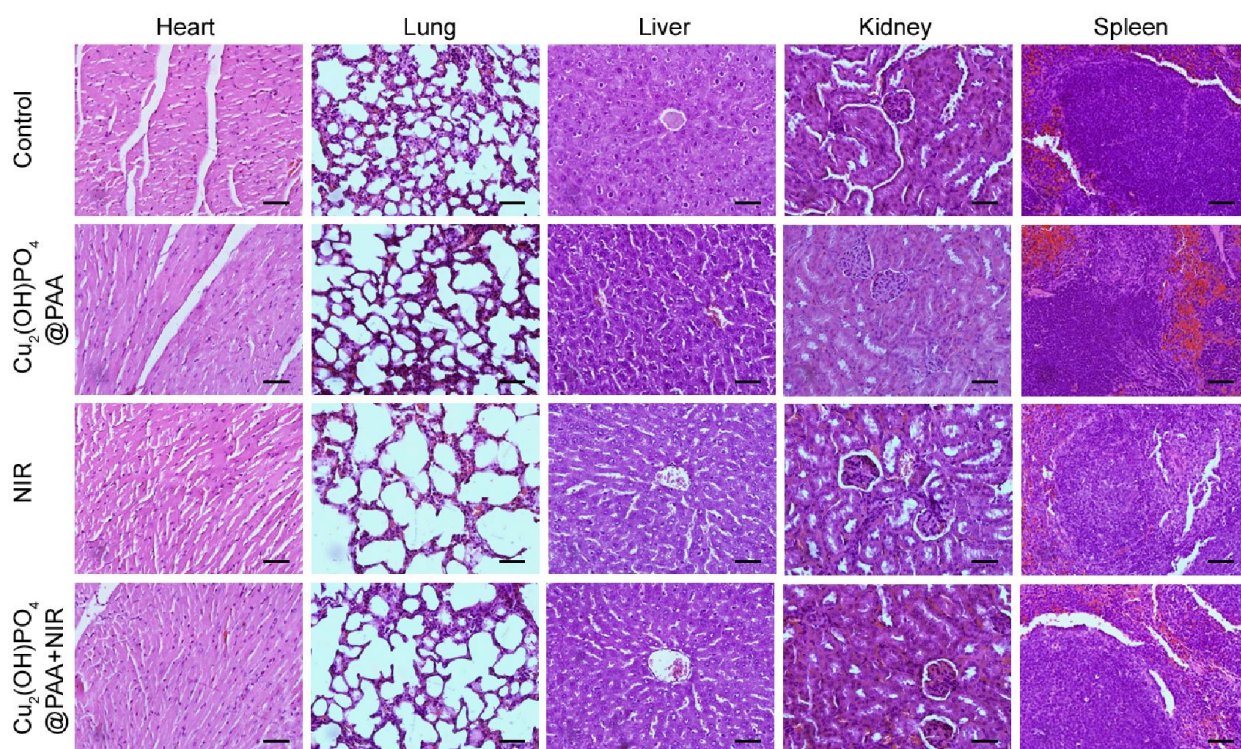




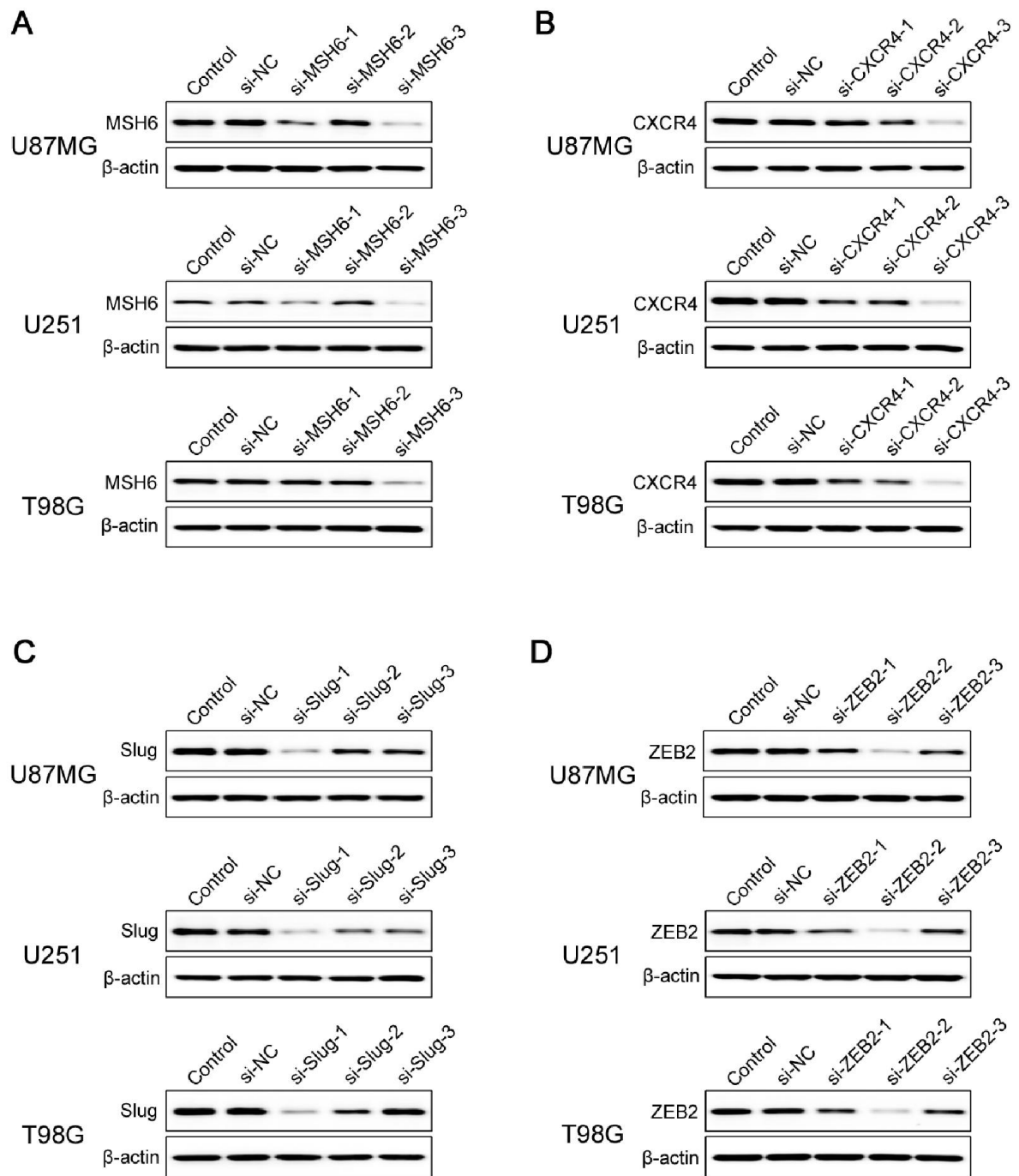
**Figure S29.** H&E staining of tumors from U251 tumor-bearing mice after different treatments (scale bar represents 50  $\mu\text{m}$ ).



**Figure S30.** Body weights of U251 tumor-bearing mice after different treatments.



**Figure S31.** H&E staining of major organs dissected from mice in different treatment groups on the 14th day (scale bar represents 50 μm).



**Figure S32.** (A) The ability of different siRNA sequences to knock down MSH6, (B) CXCR4, (C) Slug and (D) ZEB2 in U87MG, U251 and T98G cells.

**Table S1.** The differences in protein expression between MSH6-altered and MSH6-unaltered GBM tissues.

Gene	Cytoband	MSH6 altered	MSH6 unaltered	p-Value	q-Value
PTGS2	1q31.1	-1.6 ± 0.18	-1.09 ± 0.69	4.63E-08	7.50E-06
WWTR1	3q25.1	-0.33 ± 0.15	-0.04 ± 0.43	2.93E-05	2.37E-03
SRC_PY416	20q12-q13	-0.82 ± 0.28	-0.41 ± 0.67	6.02E-04	0.0196
EIF4EBP1	8p11.23	-1.48 ± 0.39	-2.05 ± 0.27	6.02E-04	0.0196
MSH2	2p21-p16.3	-2.26 ± 0.69	-3.25 ± 0.29	7.28E-04	0.0196
ERBB2_PY124	17q12	-0.05 ± 0.52	0.67 ± 1.07	8.40E-04	0.0196
8					
NOTCH1	9q34.3	-0.68 ± 0.52	0.05 ± 0.43	9.32E-04	0.0196
RPS6	9p22.1	0.75 ± 0.38	0.23 ± 0.55	9.70E-04	0.0196
EGFR_PY1173	7p12	-1.28 ± 0.44	-0.67 ± 1.33	1.19E-03	0.0214
MAPK9	5q35.3	-1.37 ± 0.24	-1.68 ± 0.29	1.51E-03	0.0233
CASP8	2q33.1	-1.02 ± 0.24	-0.71 ± 0.29	1.58E-03	0.0233
CHEK2	22q12.1	-0.65 ± 0.7	-1.52 ± 0.36	2.03E-03	0.0274
ACACA	17q12	2.17 ± 0.36	1.77 ± 0.62	3.95E-03	0.0486
LCK	1p35.2	-1.47 ± 0.34	-1.08 ± 0.41	4.20E-03	0.0486
STAT3_PY705	17q21.31	-1.06 ± 0.53	-0.48 ± 0.52	4.59E-03	0.0495
ANXA1	9q21.13	0.34 ± 1.96	2.46 ± 1.03	5.01E-03	0.0507
ERBB3	12q13.2	-0.41 ± 0.47	0.09 ± 0.76	5.69E-03	0.0512
TGM2	20q11.23	-0.18 ± 0.11	-0.06 ± 0.2	5.75E-03	0.0512
DVL3	3q27.1	-0.57 ± 0.2	-0.78 ± 0.34	6.37E-03	0.0512
CAV1	7q31.2	-0.42 ± 1.21	0.82 ± 1.13	6.70E-03	0.0512

PRKCA_PS657	17q22- q23.2	-0.17 ± 1.21	1.07 ± 0.71	6.76E-03	0.0512
PCNA	20p12.3	-0.96 ± 0.36	-1.33 ± 0.3	6.95E-03	0.0512
AKT3_PT308	1q44	-0.57 ± 0.6	0.04 ± 0.74	7.37E-03	0.0519
MAPK1	22q11.22	0.92 ± 1.31	2.21 ± 0.49	8.52E-03	0.0563
PEA15	1q23.2	-0.11 ± 1.08	0.95 ± 0.62	8.69E-03	0.0563
CDH1	16q22.1	-1.2 ± 0.6	-1.77 ± 0.43	0.0101	0.0614
STK11	19p13.3	0.13 ± 0.33	0.44 ± 0.17	0.0106	0.0614
BCL2	18q21.33	-2.04 ± 0.32	-2.35 ± 0.19	0.0106	0.0614
XRCC1	19q13.31	-1.68 ± 0.3	-1.96 ± 0.13	0.0116	0.0627
MYC	8q24.21	-1.06 ± 0.35	-1.38 ± 0.24	0.0119	0.0627
YAP1	11q22.1	-1.39 ± 0.15	-1.24 ± 0.46	0.0122	0.0627
PRKCA_PS664	17q22- q23.2	-0.91 ± 0.52	-0.43 ± 0.57	0.0126	0.0627
PIK3R1	5q13.1	0.9 ± 0.53	1.39 ± 0.38	0.0128	0.0627
CDKN1B_PT1 98	12p13.1- p12	-1.43 ± 0.14	-1.56 ± 0.2	0.014	0.0669
PRKCA	17q24.2	-0.43 ± 0.9	0.35 ± 0.7	0.0172	0.0779
CHEK1_PS345	11q24.2	-1.85 ± 0.24	-2.06 ± 0.16	0.0173	0.0779
JUN_PS73	1p32-p31	-2.18 ± 0.53	-2.64 ± 0.29	0.0185	0.081
CCNE1	19q12	-0.97 ± 0.83	-1.68 ± 0.39	0.0192	0.0817
IGFBP2	2q35	-0.6 ± 0.96	0.21 ± 1.08	0.0197	0.0817
BIRC2	11q22.2	0.19 ± 0.49	0.6 ± 0.25	0.0214	0.0866
XIAP	Xq25	-3.17 ± 0.96	-3.94 ± 0.2	0.0241	0.0953



PTK2	8q24.3	$0.42 \pm 1.1$	$1.28 \pm 0.73$	0.0275	0.101
PTEN	10q23.31	$0.8 \pm 0.57$	$1.24 \pm 0.39$	0.0282	0.101
IRS1	2q36.3	$-1.03 \pm 0.38$	$-1.33 \pm 0.22$	0.0285	0.101
CTNNA1	5q31.2	$-0.48 \pm 0.31$	$-0.24 \pm 0.32$	0.0285	0.101
ERBB2	17q12	$-0.06 \pm 0.43$	$0.28 \pm 0.6$	0.0293	0.101
ARID1A	1p36.11	$-0.65 \pm 0.39$	$-0.95 \pm 0.24$	0.0294	0.101
BAK1	6p21.31	$-2.32 \pm 0.51$	$-2.71 \pm 0.25$	0.0315	0.102
EEF2	19p13.3	$-1.09 \pm 0.35$	$-1.36 \pm 0.27$	0.0327	0.102
NCOA3	20q13.12	$-1.18 \pm 0.35$	$-1.44 \pm 0.26$	0.0327	0.102
SMAD4	18q21.2	$-2.02 \pm 0.6$	$-2.46 \pm 0.24$	0.0332	0.102
WWTR1_PS89	3q23-q24	$-1.36 \pm 0.36$	$-1.62 \pm 0.2$	0.0334	0.102
AKT3_PS473	1q44	$-0.79 \pm 0.97$	$-0.06 \pm 0.86$	0.0335	0.102
ESR1	6q25.1- q25.2	$-3.36 \pm 1.51$	$-4.48 \pm 0.3$	0.034	0.102
PARK7	1p36.23	$0.92 \pm 0.55$	$1.33 \pm 0.43$	0.0351	0.104
RAD50	5q31.1	$-0.6 \pm 0.31$	$-0.82 \pm 0.3$	0.0382	0.111
CDKN1B	12p13.1	$-0.85 \pm 1.03$	$-1.57 \pm 0.41$	0.0441	0.125

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**Table S2.** The siRNA sequences for the MSH6, CXCR4, Slug and ZEB2 genes.

siRNA	Sense (5' to 3' )
si-NC (random control sequence)	UUCUCCGAACGUGUCACGUTT
si-MSH6-1	GCCAGACACUAAGGAGGAATT
si-MSH6-2	GCAUUUCAUCAGAAACCAATT
si-MSH6-3	CCACAUGGAUGCUCUUAUUTT
si-CXCR4-1	GGGACUAUGACUCCAUGAATT
si-CXCR4-2	CCGACUUCAUCUUUGCCAATT
si-CXCR4-3	CCCUCAAGACCACAGUCAUTT
si-Slug-1	GAAUGUCUCUCCUGCACAATT
si-Slug-2	CCCAUUCUGAUGUAAAGAATT
si-Slug-3	GCGCCCUGAAGAUGCAUAUTT
si-ZEB2-1	GAAGCUACGUACUUUAAUATT
si-ZEB2-2	GGCAAGGCCUUCAAAUAUATT
si-ZEB2-3	GACCACUCCAGGAGUAAUATT

**Table S3.** Primer sequences for the Snail, Slug, Twist, ZEB1, ZEB2 and  $\beta$ -actin genes.

Gene	Primer sequences (5' to 3' )
Snail-F	TTCAACTGCAAATACTGCAACAAG
Snail-R	CAGTGTGGGTCCGGACATG
Slug-F	TGGGCTGGCCAAACATAAG
Slug-R	CCGCAGATCTTGCAAACACA
Twist-F	TGAGCAAGATTCAGACCCTCAA
Twist-R	CCATCCTCCAGACCGAGAAG
ZEB1-F	TCCATGCTTAAGAGCGGTAGCT
ZEB1-R	GTATCTTGTCTTTCATCCTGATTTCCA
ZEB2-F	TTCCTGGGCTACGACCATAACC
ZEB2-R	CAAGCAATTCTCCCTGAAATCC
$\beta$ -actin-F	GGGAAATCGTGCGTGACATT
$\beta$ -actin-R	GGAACCGCTCATTGCCAAT