

Appendix 1

1. The following primer sequences were used in this study:

TFR1-F	GCTCGGCAAGTAGATGGCGATAAC
TFR1-R	ATTGTCAATGTCCCAAACGTCACC
SLC7A11-F	AGCCTGTTGTGTCCACCATCTCC
SLC7A11-R	GTCAGAGTGATGACGAAGCCAATC
PRKAA2-F	GTGAAGATCGGACACTACGTG
PRKAA2-R	CTGCCACTTTATGGCCTGTTA
18S-F	CAGCCACCCGAGATTGAGCA
18S-R	TAGTAGCGACGGGCGGTGTG
DMT1-F	TGGCTTATCTGGGCTTTGTG
DMT1-R	CACACTGGCTCTGATGGCTA
FPN-F	ACAATACGAAGGATTGACCAGT
FPN-R	ATACCAAGTTCATCCCGAAAT
FTH1-F	GCCGAGAACTGATGAAGCTGC
FTH1-R	GCACACTCCATTGCATTGAGCC
HIF-1 α -F	CCATTAGAAAGCAGTCCGCAAGC
HIF-1 α -R	GTGGTAGTGGTGGCATTAGCAGTAG
HIF-2 α -F	CCAGGGAAAAAGGAACTTGGGT
HIF-2 α -R	GACCCGAAAAGAGGACGGAGA
IRP1-F	AACTGACCACATCTCCCCAG
IRP1-R	ATAGTCTGTGGTGCCTGCTT
IRP2-F	TACGGGTCTTGTGGAAAGCT
IRP2-R	ATCTGTGCGACAAGCAGGAT

2. Target sequences for siRNAs and shRNAs were as follows:

siPRKAA2-1: GUCAUCCUCAUAUUAUCAAAAC

siPRKAA2-2: CAACUUUACCUGGUUGAUAAC

ShPRKAA2:CCGGGTCATCCTCATATTATCAAACCTCGAGGTTTGATAATATGAGGATGACTTTTTTG

3. The following information was provided regarding the primary antibodies used in western blotting assays:

Anti-PRKAA2 antibodies (1/1000; Proteintech, 18167-1-AP, RRID:AB_10695046);

Anti-TFR1 antibodies (1/1000; Abcam, ab214039, RRID:AB_2904534);

Anti-IRP1 antibodies (1/1000; Abcam, ab183721);

Anti-IRP2 antibodies (1/1000; Abclonal, A6382, RRID:AB_2766984);

Anti-HIF-1 α antibodies (1/1000; Abcam, ab179483, RRID:AB_2732807);

Anti-HIF-2 α antibodies (1/1000; Abclonal, A7553, RRID:AB_2768078);

Anti-GAPDH antibodies (1/10000; Abcam, ab181602, RRID:AB_2630358)

1. Detail about the five patients with low AFP level and low PRKAA2 expression

Number	AFP at Diagnosis	Age at diagnosis	PRETEXT	Histology	Tumor Size	Metastasis	Extrahepatic spread	Multifocality	Tumor rupture	Vascular involvement	Caudate involvement
1	696.4 ng/ml	11 months	I	MIX	590 cm3	NO	NO	NO	NO	NO	NO
2	943.5 ng/ml	10 months	I	MIX	448 cm3	NO	NO	NO	NO	NO	NO
3	856.9 ng/ml	31 months	II	Epithelial	643 cm3	NO	NO	NO	NO	NO	NO
4	1010.2 ng/ml	45 months	II	Epithelial	540 cm3	NO	NO	NO	YES	YES	NO
5	690.1 ng/ml	22 months	II-III	NA	252 cm3	NO	NO	NO	NO	NO	NO

2. Specific data of $\Delta\Delta Ct$

Pair Number	$\Delta\Delta Ct = \Delta Ct(\text{normalized-noncancerous}) - \Delta Ct(\text{normalized-Tumor})$	Average $\Delta\Delta Ct$
1	0.187351015	0.750443124
1	0.800498056	
1	1.2634803	
2	1.555538501	1.217900169
2	0.869679343	
2	1.228482663	
3	9.25643847	8.321005907
3	8.459185325	
3	7.247393924	
4	1.157398133	1.356692126
4	1.55598612	
4	1.356692126	
5	0.53221911	0.561389829
5	0.590560547	
5	0.561389829	
6	-1.638684877	-1.316081323
6	-1.292519957	
6	-1.017039135	
7	4.111432552	3.461021244
7	4.708573759	
7	1.56305742	
8	0.471147838	-0.081526378
8	-0.497106906	
8	-0.218620067	
9	10.95563585	8.931872441
9	7.157972881	
9	8.682008593	
10	1.774275047	2.507484573
10	1.763643632	
10	3.984535039	
11	-1.193641773	-0.835490099
11	0.025653702	
11	-1.338482226	
12	0.727220454	0.424500389
12	0.658794265	
12	-0.112513553	
13	-0.008552379	-0.197565551
13	0.141298434	
13	-0.725442709	
14	-2.46922674	-0.474150913
14	0.166230644	
14	0.880543357	
15	0.369754301	-0.096661473
15	0.141497274	
15	-0.801235992	
16	8.014684308	8.811174364
16	9.666180917	
16	8.752657867	
17	6.701586931	6.327822258
17	5.279048674	
17	7.002831169	
18	2.135016937	2.196510297
18	2.207521069	
18	2.246992885	
19	3.477338478	3.297944832
19	3.20062303	
19	3.215872987	
20	2.612746572	2.723212248
20	2.903221421	
20	2.653668751	
21	5.325581803	4.951347183
21	4.565270361	
21	4.963189385	
22	1.741058643	1.802038974
22	2.084580179	
22	1.580478101	
23	2.551942839	3.100305693
23	3.648668547	
23	3.100305693	
24	4.911576917	5.06152224
24	5.211467563	
24	5.06152224	
25	6.16158126	5.820394884
25	5.479208508	
25	5.820394884	
26	4.064519051	3.844358365
26	3.62419768	
26	3.844358365	
27	8.120304956	8.312927469
27	7.891600494	
27	8.926876957	
28	4.077108354	5.483701346
28	6.631571714	
28	5.742423971	
29	2.744173446	2.183797763
29	1.551523796	
29	2.255696047	
30	8.161786668	7.690549381
30	7.725493653	
30	7.184367824	

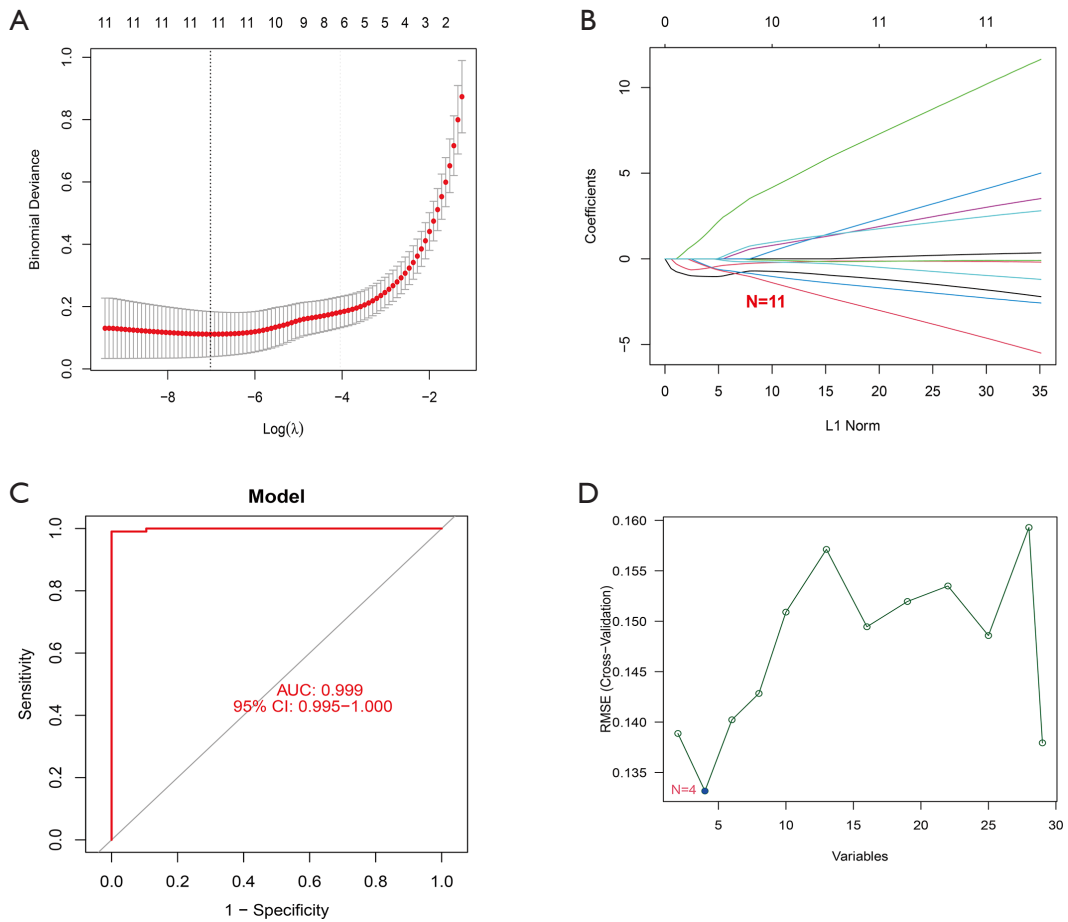


Figure S1 Selection of diagnosis marker candidates for HB by two algorithms. (A) and (B) Eleven gene expression signatures based on ferroptosis-related clusters were selected using the LASSO Cox model. (C) and (D) A visual representation of the SVM-RFE biological marker screening process. HB, hepatoblastoma; LASSO, least absolute shrinkage and selection operator; SVM-RFE, support vector machine recursive feature elimination.

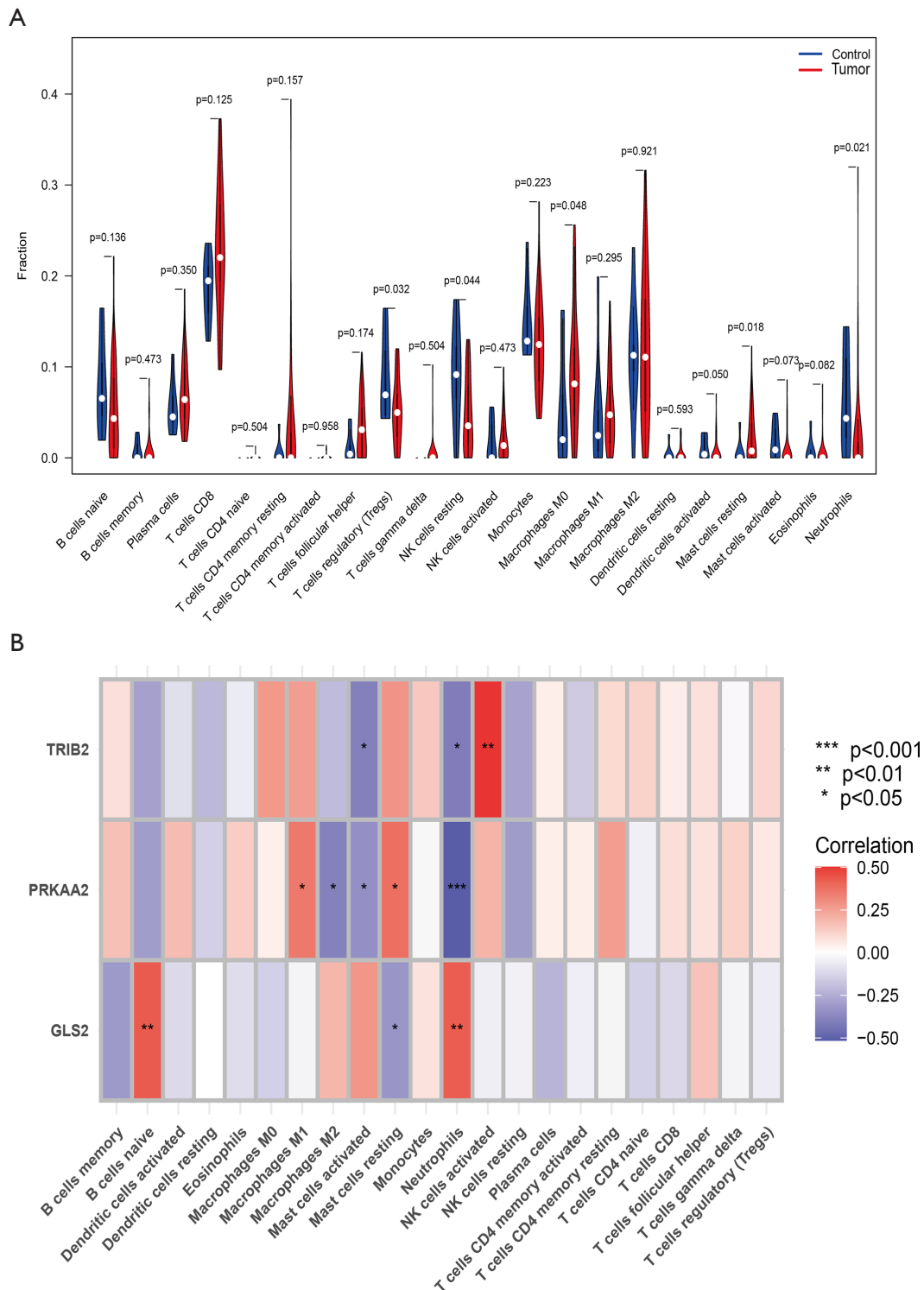


Figure S2 Analysis of immune infiltration level. (A) The differences in immunocytes' structural makeup between HB and noncancerous liver samples. Names of 22 types of infiltrating immune cells are shown in the bottom of the graph. (B) Correlation between 22 immune infiltration cells and three key genes. Gene names are on the left of the graph. *, $P < 0.05$, **, $P < 0.01$, and ***, $P < 0.001$. The gradual colour change from red to blue indicates the changing process from positive correlation to negative correlation. HB, hepatoblastoma; PRKAA2, protein kinase AMP-activated catalytic subunit alpha 2; TRIB2, tribbles homolog 2; GLS2, liver-type glutaminase.

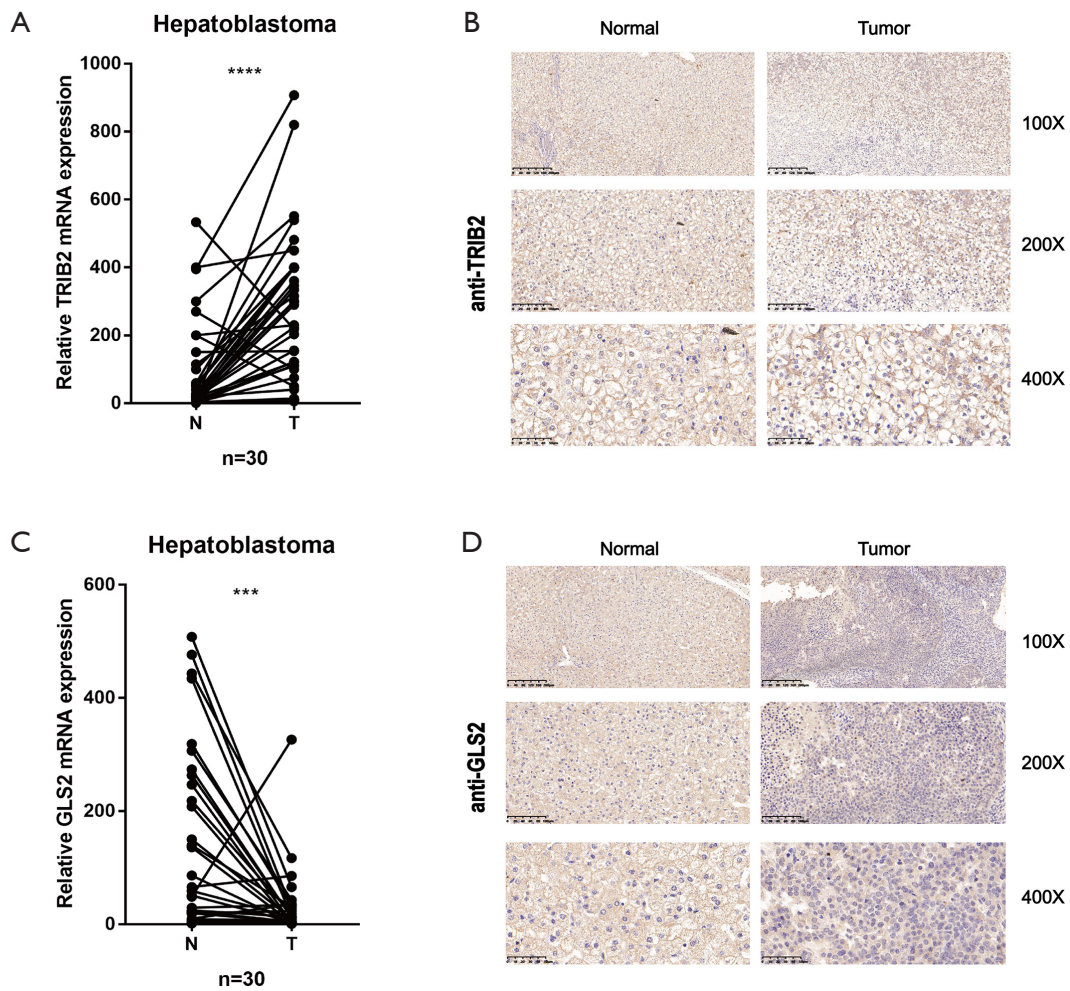


Figure S3 Expression of TRIB2 and GLS2 in paired samples. (A) Expression of TRIB2 mRNA in paired samples (****, $P < 0.0001$ by paired t -test). (B) Analysis of TRIB2 expression in paired samples using immunohistochemistry. Scale bar represents $\times 100$, $\times 200$ and $\times 400$. (C) Expression of GLS2 mRNA in paired samples (***, $P = 0.0004$ by paired t -test). (D) Analysis of GLS2 expression in paired samples using immunohistochemistry. Scale bar represents $\times 100$, $\times 200$ and $\times 400$. TRIB2, tribbles homolog 2; GLS2, liver-type glutaminase.

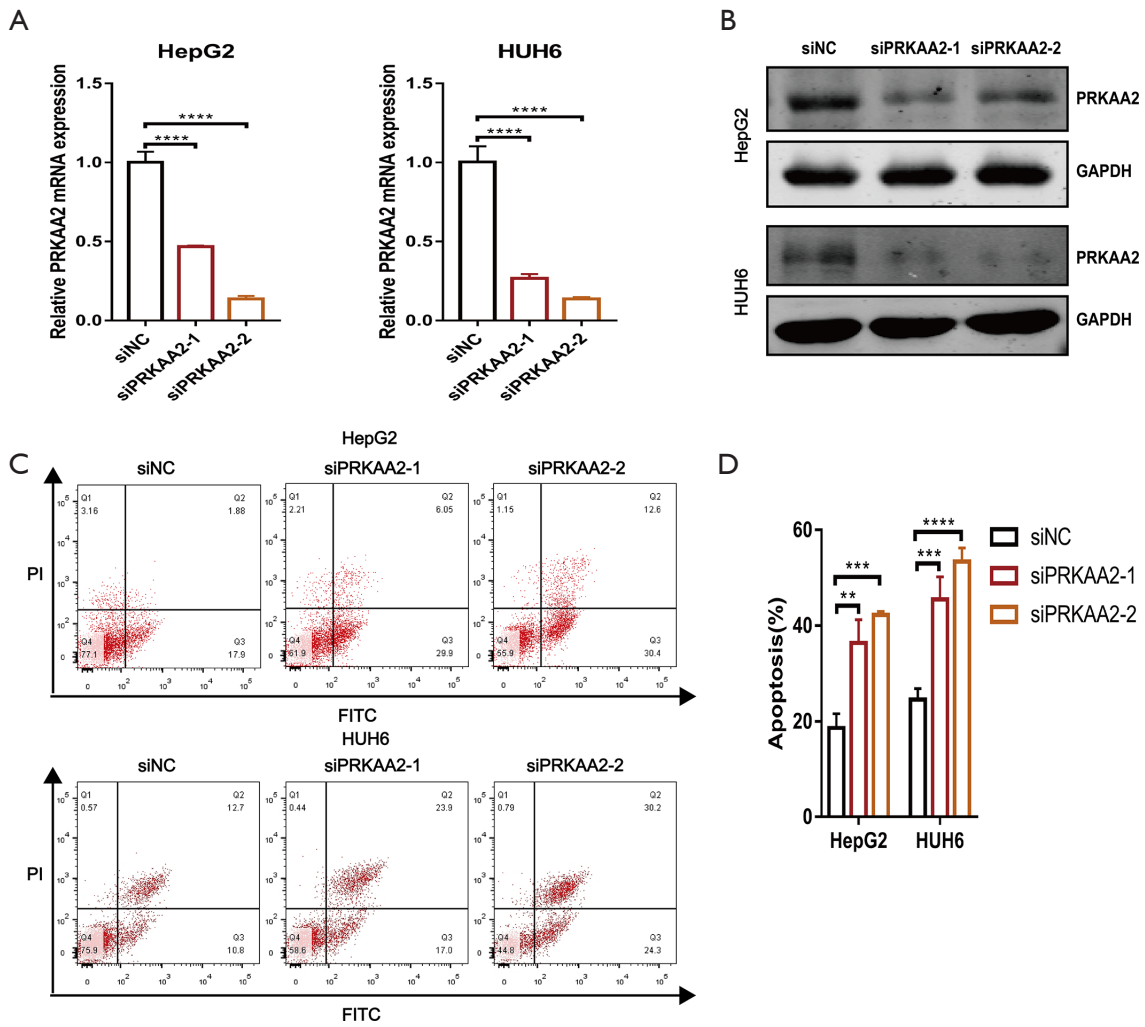


Figure S4 PRKAA2 plays a carcinogenic role in HB. (A) PRKAA2 mRNA expression in siPRKAA2 HB cells compared with the controls was measured. (****, $P < 0.0001$ by t -test). (B) The protein level of PRKAA2 in siPRKAA2 HB cells compared with the controls was measured. (C) and (D) FACS assays were used to measure the apoptosis in HB cells with PRKAA2 knockdown compared with controls. **, $P < 0.01$, ***, $P < 0.001$, and ****, $P < 0.0001$. HB, hepatoblastoma; PRKAA2, protein kinase AMP-activated catalytic subunit alpha 2.

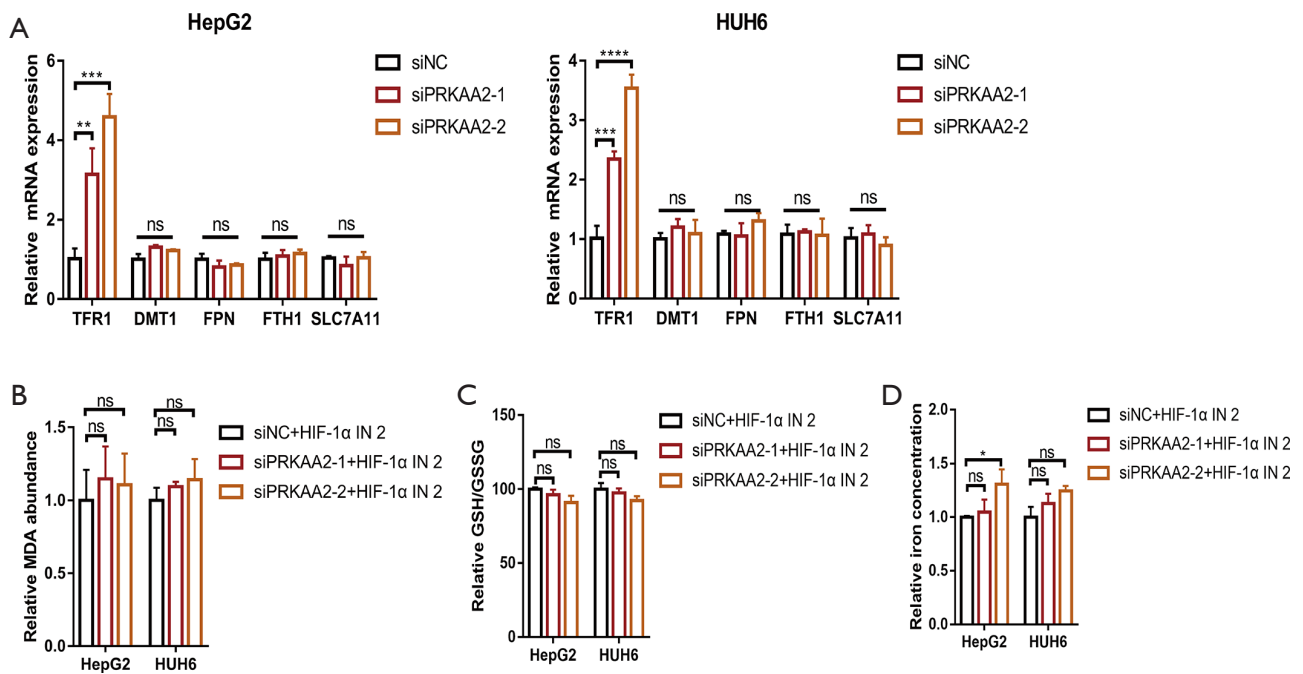


Figure S5 PRKAA2 regulates TFR1 through HIF-1 α . (A) Relative mRNA expression of several genes regulating ferroptosis in siPRKAA2 HB cells compared with the controls were measured. (B-D) The relative MDA concentration (B), GSH/GSSG ratio (C) and iron concentration (D) in HB cells, which were transfected with PRKAA2 siRNA, were detected using lipid peroxidation assay kits, GSH and GSSG assay kits and iron assay kits respectively after treatment with HIF-1 α -IN-2 (1 μ M) for 48 h. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ****, $P < 0.0001$. ns, no significant difference. PRKAA2, protein kinase AMP-activated catalytic subunit alpha 2; TFR1, transferrin receptor 1; HIF-1 α , hypoxia-inducible factor 1 α ; MDA, malondialdehyde; GSH, glutathione; GSSG, glutathione oxidized.