

Supplementary information

ACYP2 functions as an innovative nano-therapeutic target to impede the progression of hepatocellular carcinoma by inhibiting the activity of TERT and the KCNN4/ERK pathway

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Supplementary Figure

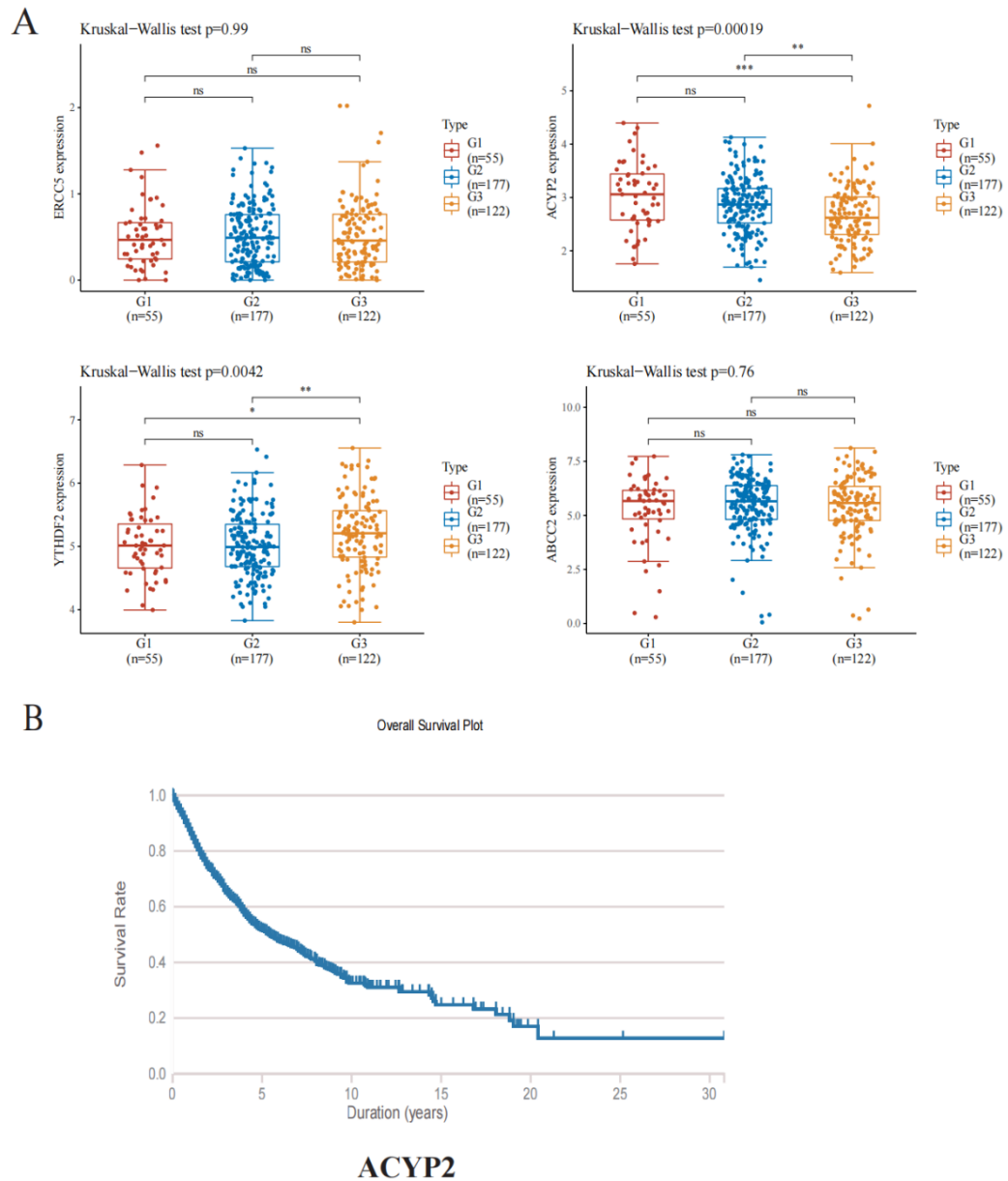


Fig. S1. (A) Expression differences of ERCC5, ABCC2, ACYP2 and YTHDF2 in different G-stage of HCC in TCGA database; **(B)** Correlation analysis of ACYP2 expression with the survival of HCC patients in TCGA database. ns, non-significant, $*p<0.05$, $**p<0.01$, $***p<0.001$

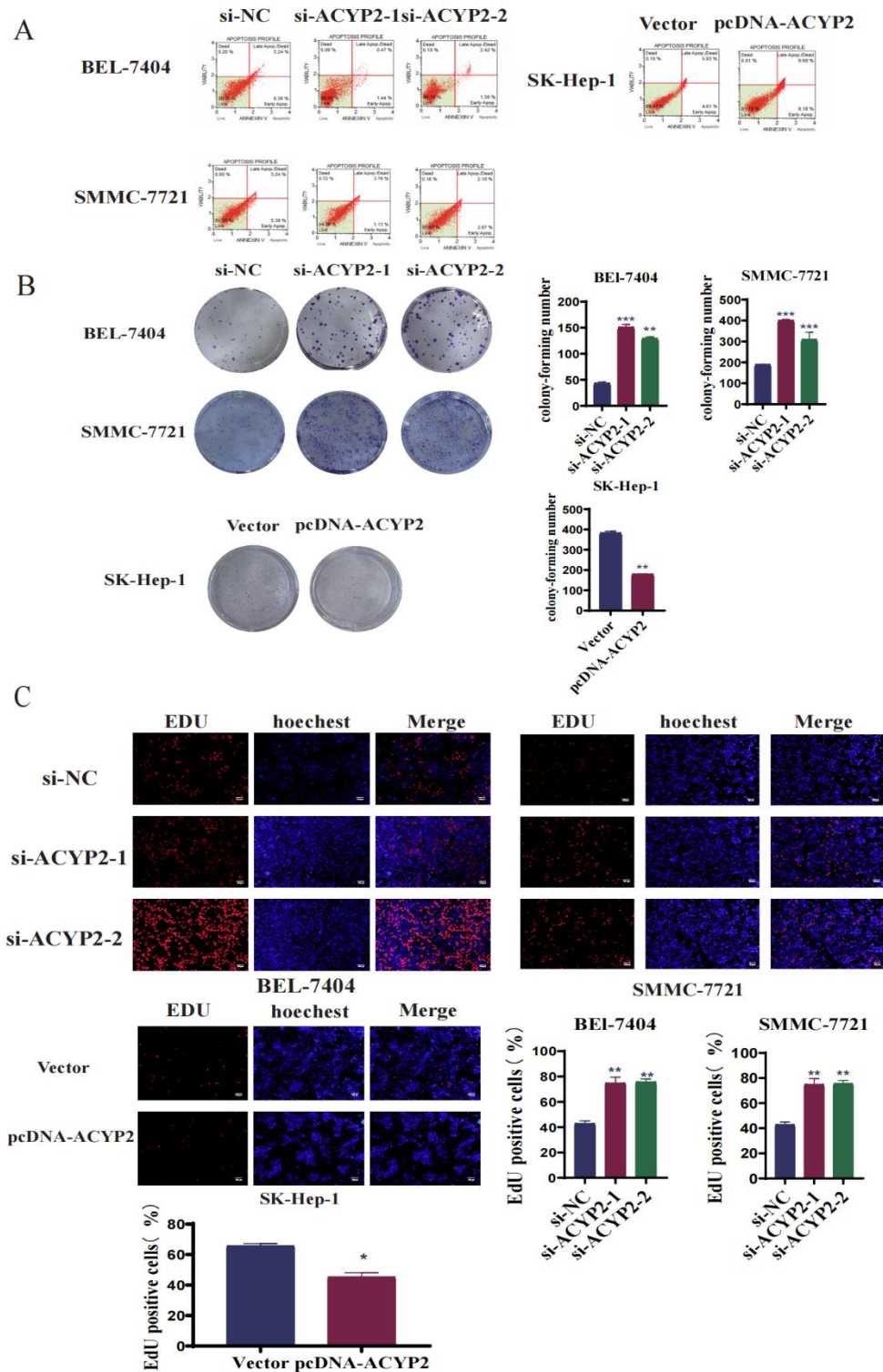


Fig. S2. (A, C) The effects of pcDNA-ACYP2 and si-ACYP2-1, -2 on the proliferation of HCC cells were analyzed by clone formation and EdU assays. **(B)** The apoptotic rates of HCC cells treated with pcDNA-ACYP2 and si-ACYP2-1, -2 were detected by flow cytometry. **Scale bar: 100 μ m; * p <0.05, ** p <0.01, *** p <0.001**

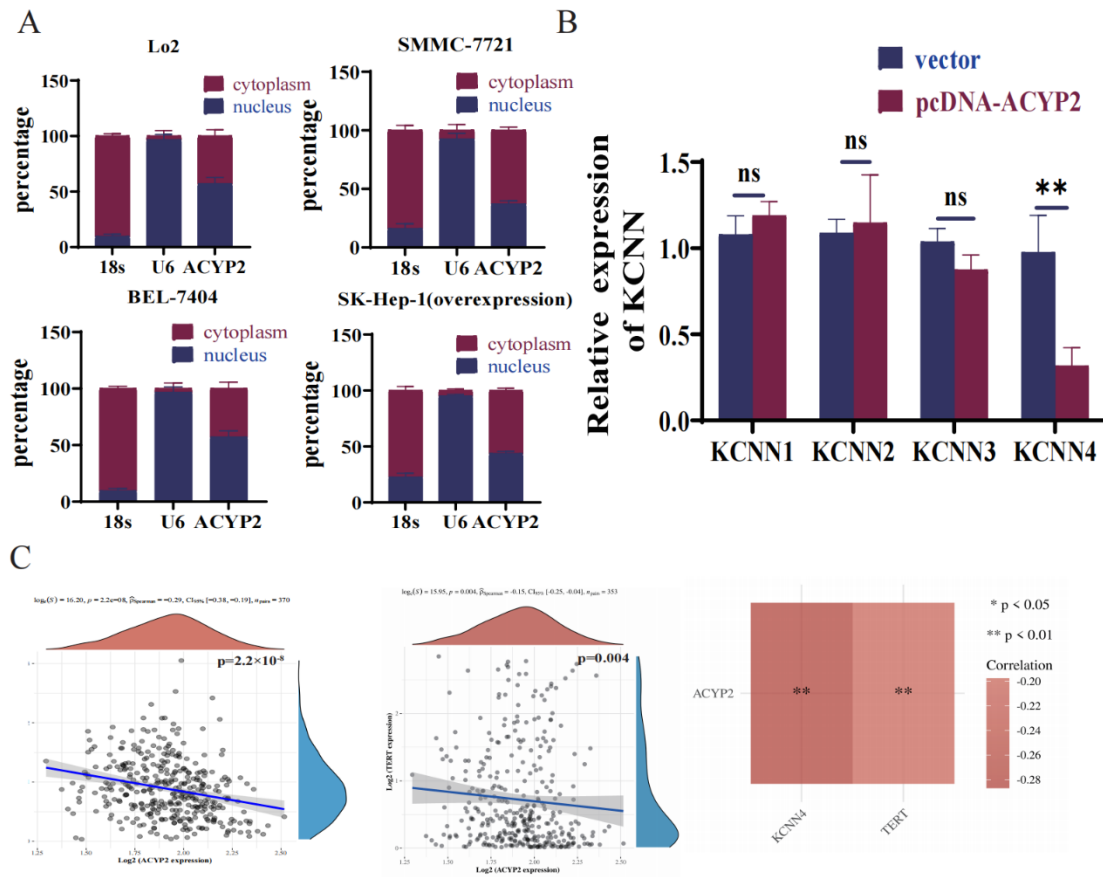


Fig. S3. (A) The cell distribution of ACYP2 in LO2, SMMC-7721, BEL-7404 and SK-Hep-1 cells transfected with pcDNA-ACYP2 was analyzed by nucleoplasm separation assay ; **(B)** The expressions of KCNN1~4 mRNA in pcDNA-ACYP2 treated HCC cells were detected by qRT-PCR; **(C)** The association analyses of ACYP2, KCNN4 and TERT in HCC in the TCGA database. ns, non-significant, ** $p < 0.01$

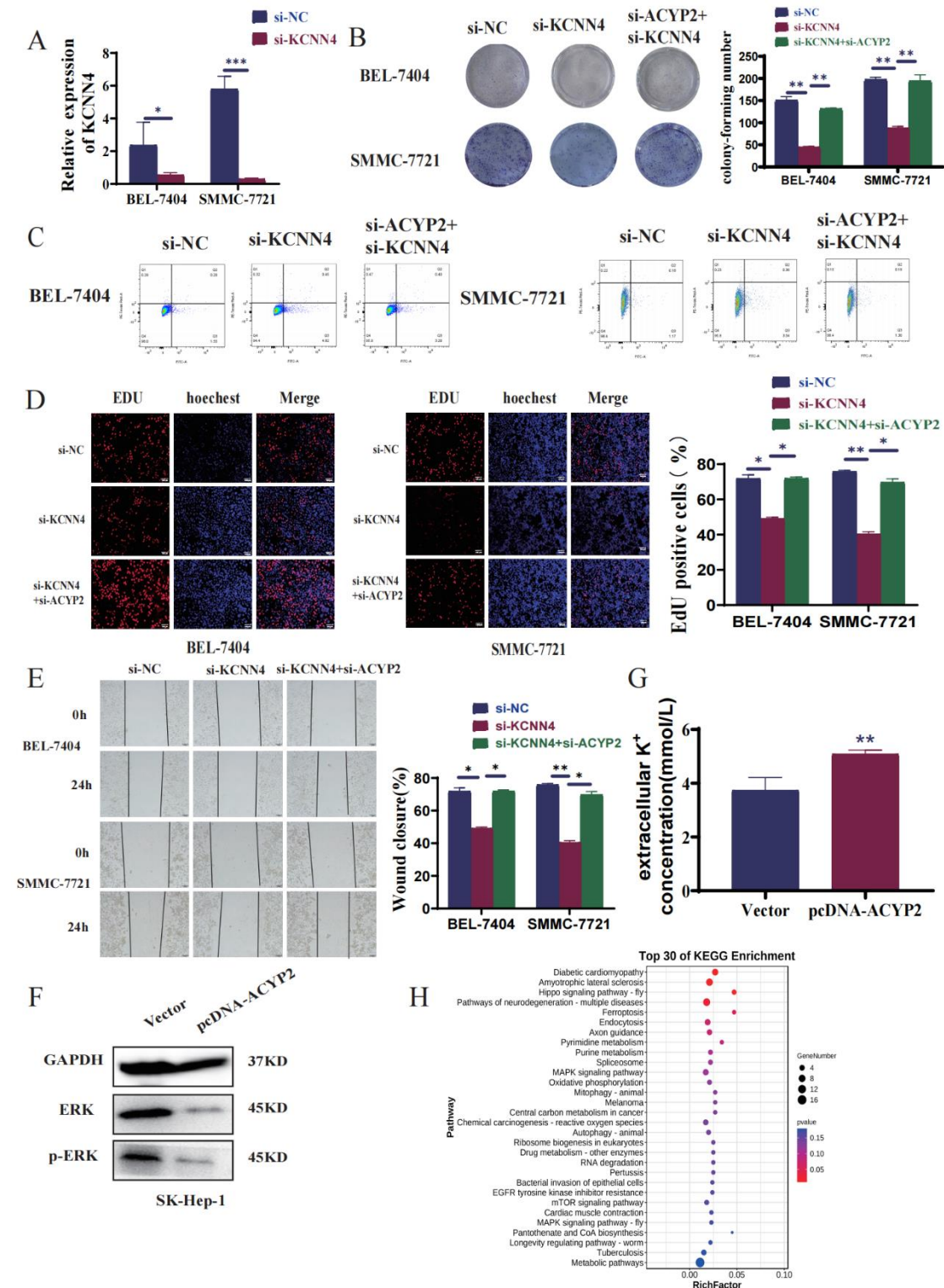


Fig. S4. (A) Transfection efficiency of si-KCNN4 was verified by qRT-PCR; (B, D)

The effects of si-NC, si-KCNN4 and si-ACYP2+si-KCNN4 on HCC cell proliferation

were analyzed by clone formation and EDU assays; (C) The apoptotic rates of si-NC,

si-KCNN4 and si-ACYP2+si-KCNN4 cells treatment groups were detected by flow cytometry. **(E)** The effects of si-NC, si-KCNN4 and si-ACYP2+si-KCNN4 on HCC cell migration were analyzed by wound healing; **(F)** The concentration of extracellular K^+ in pcDNA-ACYP2-treated hepatocellular carcinoma cells by ion chromatography; **(G)** Enrichment analysis of the downstream of ACYP2 in KEGG database; **(H)** The expressions of ERK and p-ERK in pcDNA-ACYP2 transfected HCC cells were analyzed by western blotting. **Scale bar: 100 μ m; * p <0.05, ** p <0.01, *** p <0.001**

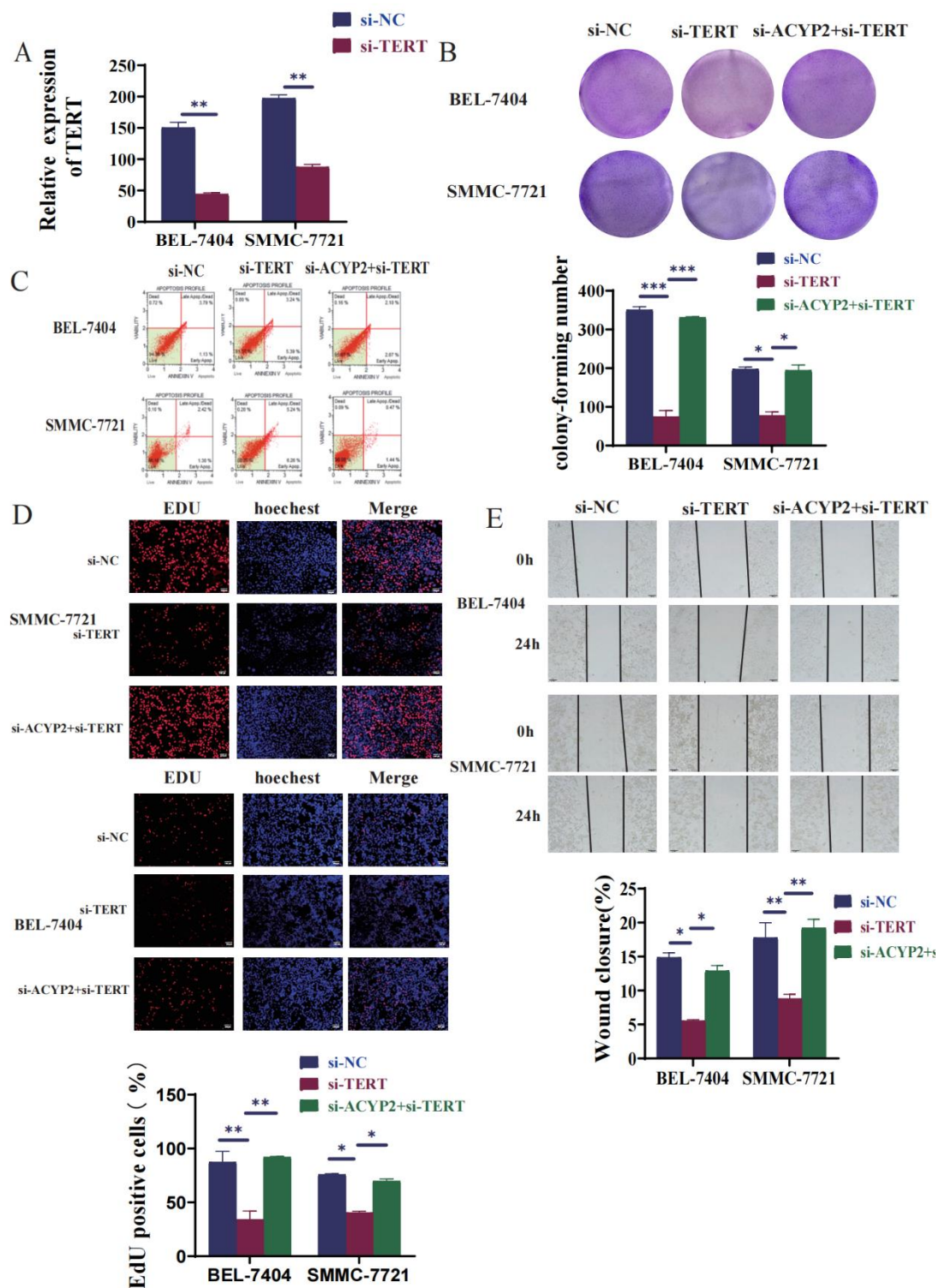


Fig. S5. (A) Transfection efficiency of si-TERT was verified by qRT-PCR; **(B, D)** The effects of si-NC, si-TERT and si-ACYP2+si-TERT on HCC cell proliferation were analyzed by clone formation and EdU assays; **(C)** The apoptotic rates of si-NC, si-

TERT and si-ACYP2+si-TERT cells treatment groups were detected by flow cytometry.

(E) The effects of si-NC, si-TERT and si-ACYP2+si-TERT on HCC cell migration were analyzed by wound healing. **Scale bar: 100 μ m**; * p <0.05, ** p <0.01, *** p <0.001

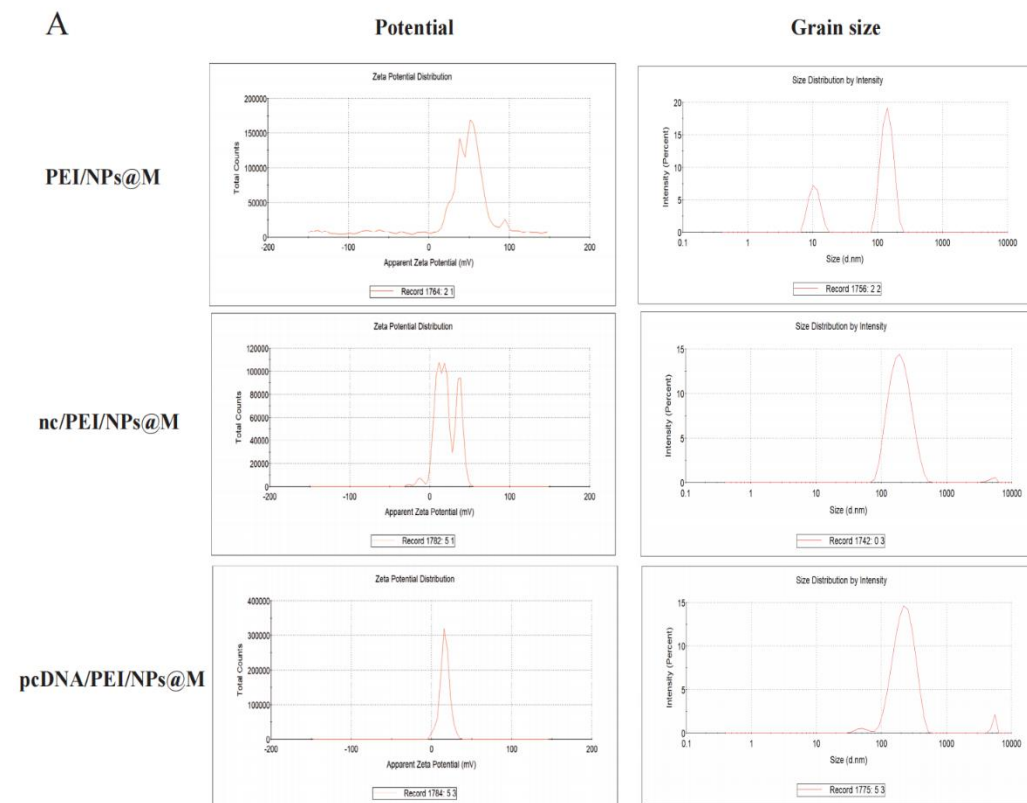


Fig. S6. (A) Based on particle size and zeta potential analysis, the particle size and potential of PEI/NPs@M, nc/PEI/NPs@M and pcDNA/PEI/NPs@M were obtained.

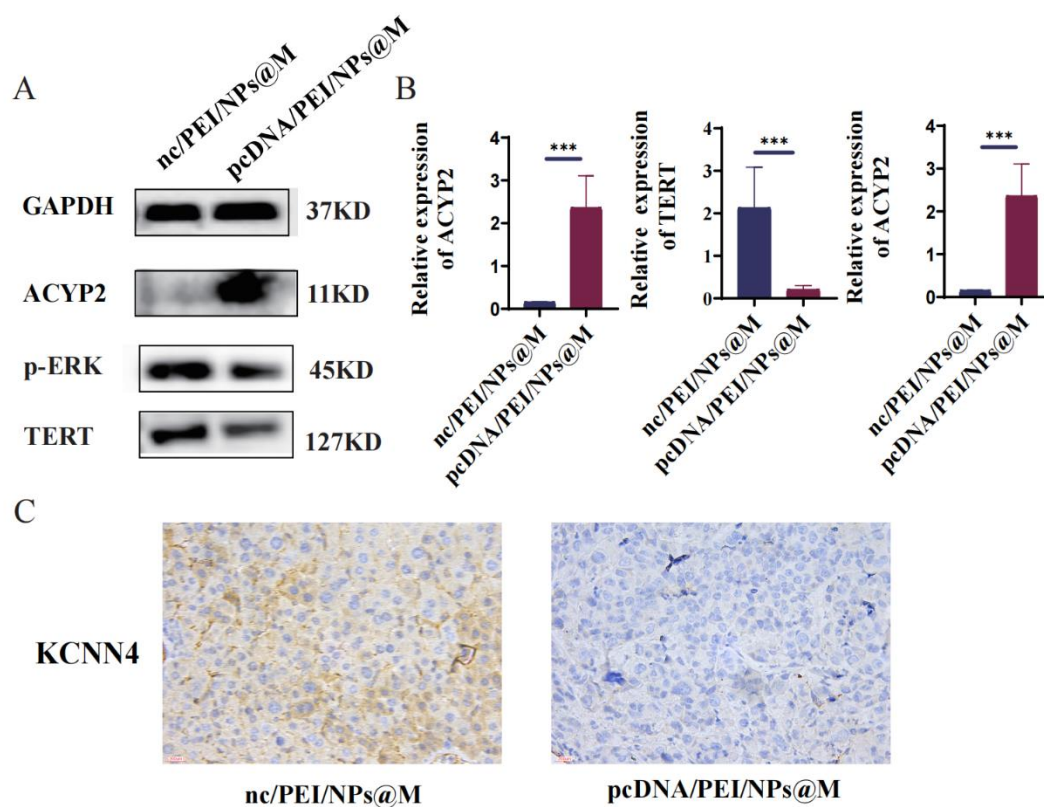


Fig. S7. (A, B) The proteins expression of ACYP2, KCNN4 and TERT and the mRNA expression of ACYP2, p-ERK and TERT in the subcutaneous graft tumor model in nude mice were detected by western blotting and qRT-PCR. **(C)** The expression of KCNN4 in the tumor of nude mice in subcutaneous graft tumor models was detected by IHC staining. **Scale bar: 200 μ m; *** p <0.001**

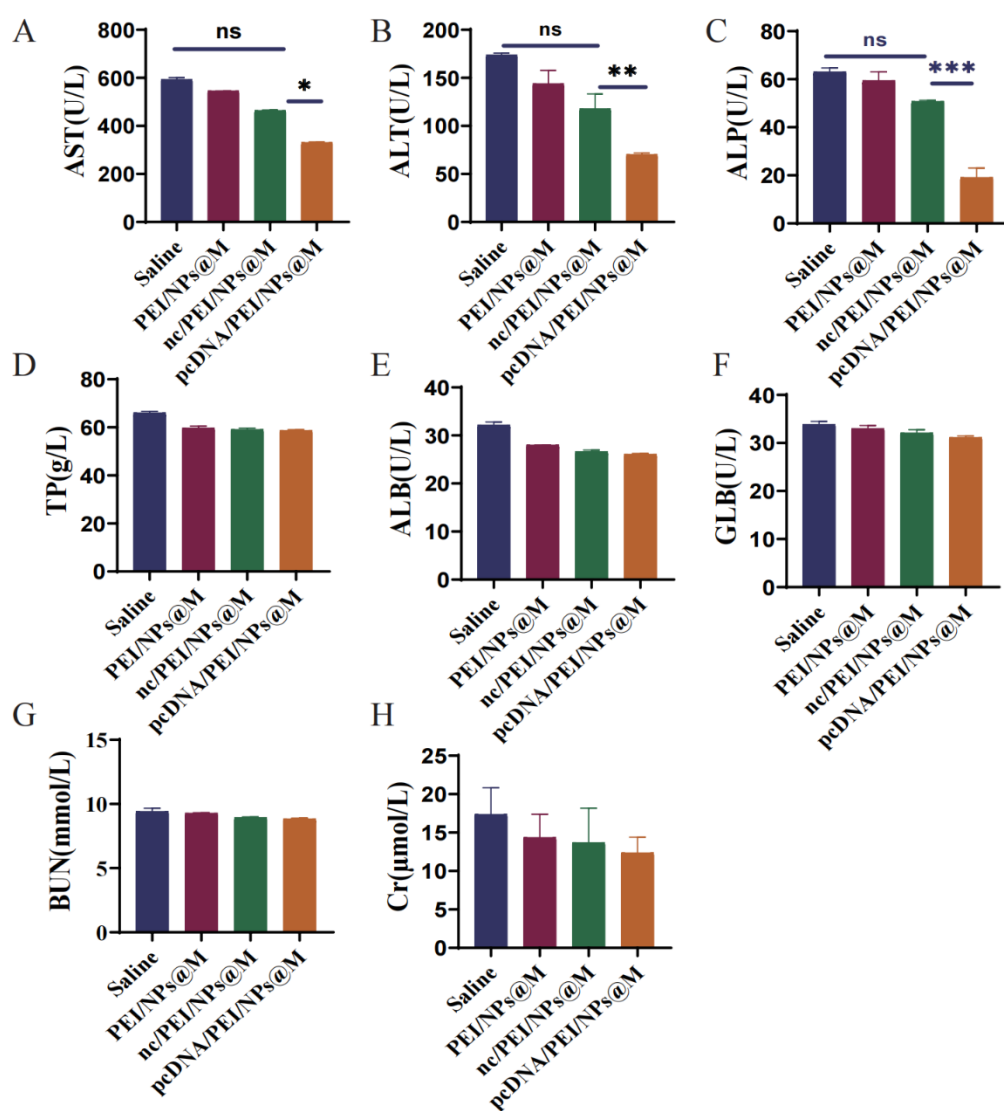


Fig. S8 (A-H) The serum levels of AST, ALT, ALP, Cr, TP, ALB, GLB and BUN were detected in nude mouse eyeball blood of in orthotopic HCC transplantation model.

Table S1. Information on clinicopathological characteristics of HCC cases used in this study.

Variables	ACYP2		n	P value
	High(n=12)	Low(n=40)		
Gender				0.4248
Male	9	25	34	
Female	3	15	18	
Age				0.1576
≤49	4	6	10	
>49	8	34	42	
Hepatocirrhosis				0.1031
Present	2	17	19	
Absent	10	23	33	
Tumor size				0.0343
<5cm	8	13	21	
≥5cm	4	27	31	
Tumor differentiation				0.0183
Well + moderate	7	31	38	
poor	5	9	14	
MVI				0.0099
(-)	9	35	44	
(+)	3	5	8	

Table S2. Sequence information for primers used in this study.

Gene	Sequence (5' -3')
GAPDH	F:AGAAGGCTGGGGCTCATTTG R:AGGGGCCATCCACAGTCTTC
ACYP2	F: CGTGAGCCCCTTCAGTTGTAT R:TCAGAGCTAGACCTCTGGGATTC
KCNN4	F: CTACCTTCAGGAGGCACGAG R: AACGAAACCCTGTGGTGAAG
TERT	F: GCCTTCCACCGTTCATTCTA R: GACTCGCTCCGTTCTCTTC
ABCC2	F:TTAATCATGGAAGGAGCGACACAG R:AGGTCAGGTTCACTCTCGGTTC
YTHDF2	F:CGTCTCGCTTCTGAATAGTATCC R:GATTGGCTCTTGCTCCTCCTG
ERCC5	F;GCGGCAGAGTTGTTTCAGGTTG R:TGTGGACATGGCAGGCTAGTG

Table S3. Sequence information for siRNA vectors used in this study.

siRNA	Sequence (5' -3')
si-ACYP2-1	GCACGAACTTTTCTAATGA
si-ACYP2-2	GGTGAAGAATACCAGCAAA
si-ACYP2-3	CTAAGCTTGAATACTCTAA
si-KCNN4	CATCGGCGCTCTCCAATCAA
si-TERT	GAGCCAGTCTCACCTTCAA