

Supplemental files

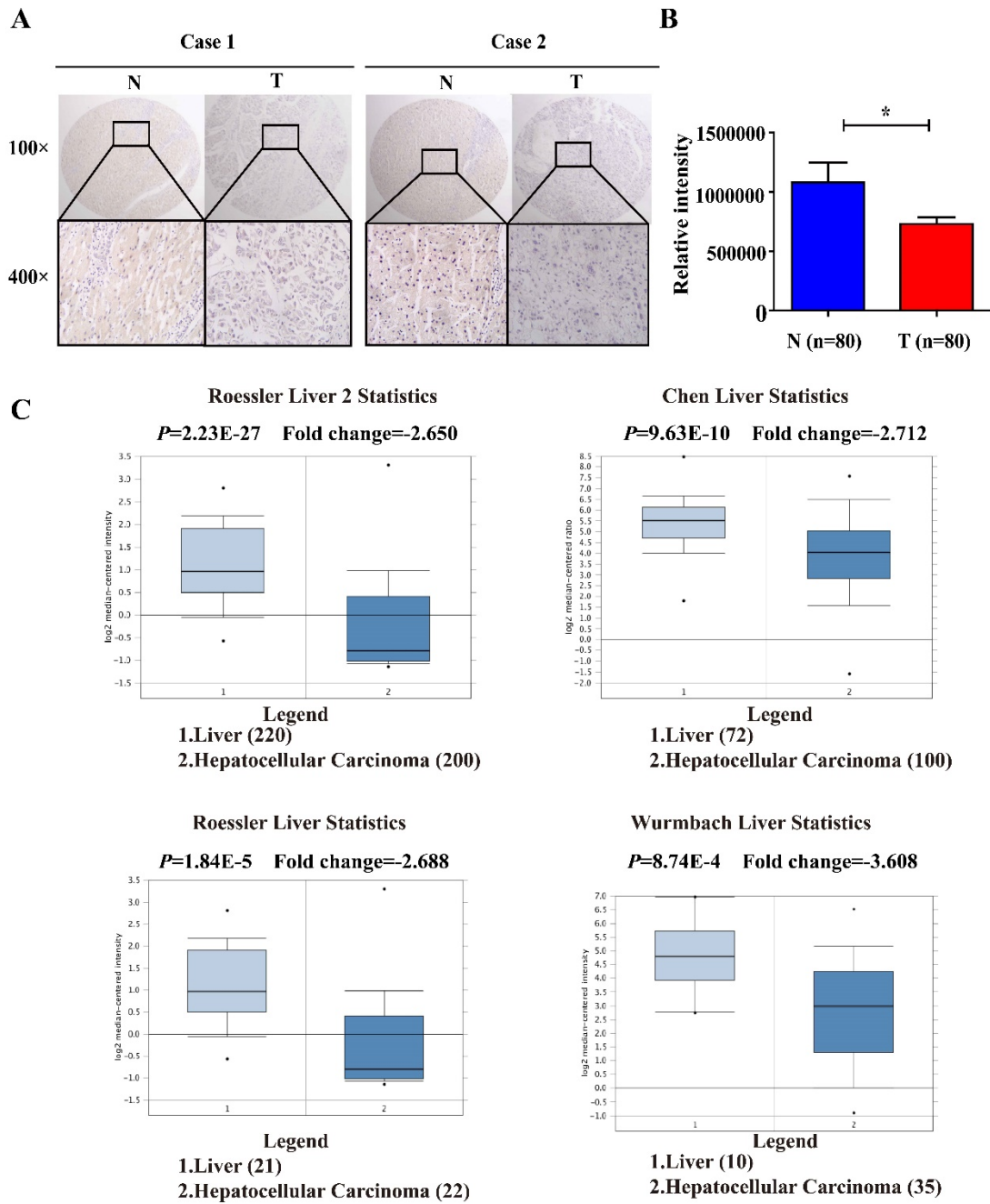


Figure S1. (A) IHC staining of PDK4 in a microarray with 80 paired samples of HCC (magnification: 400×). (B) Integrated optical density (IOD) for PDK4 was obtained for analyses. (C) mRNA level of PDK4 in HCC was significantly decreased in 4 datasets (Roessler Liver Statistics, Roessler Liver 2 Statistics, Wurmbach Liver Statistics, and Chen Liver Statistics).

* $P < 0.05$

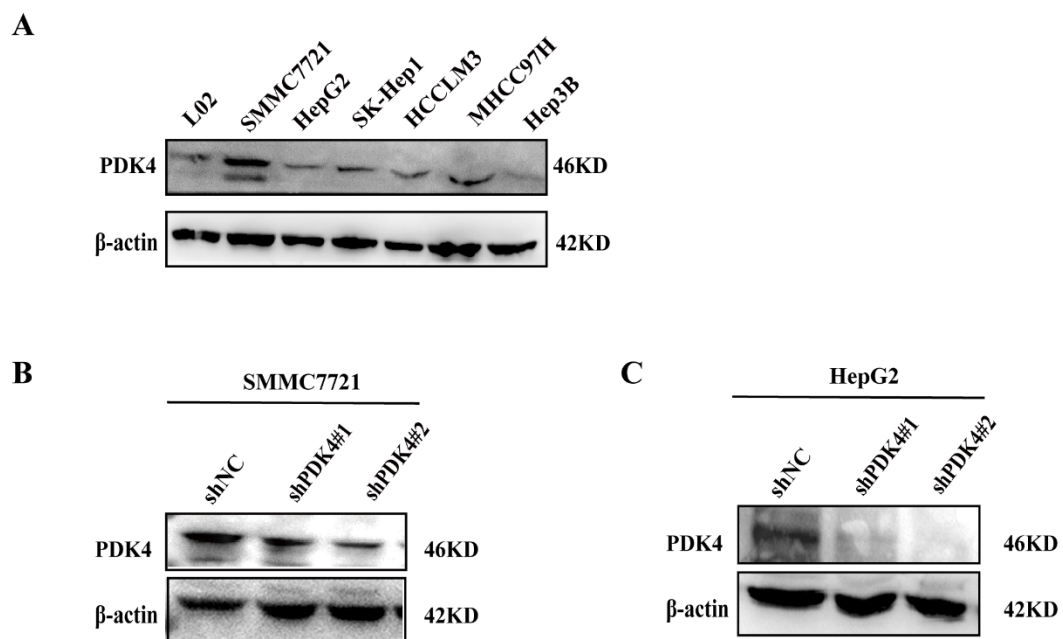


Figure S2. (A) Expression levels of PDK4 were measured in a panel of HCC cell lines and normal hepatic cell. (B, C) Protein levels of PDK4 were detected in HCC cells with knockdown of PDK4.

Glycolysis

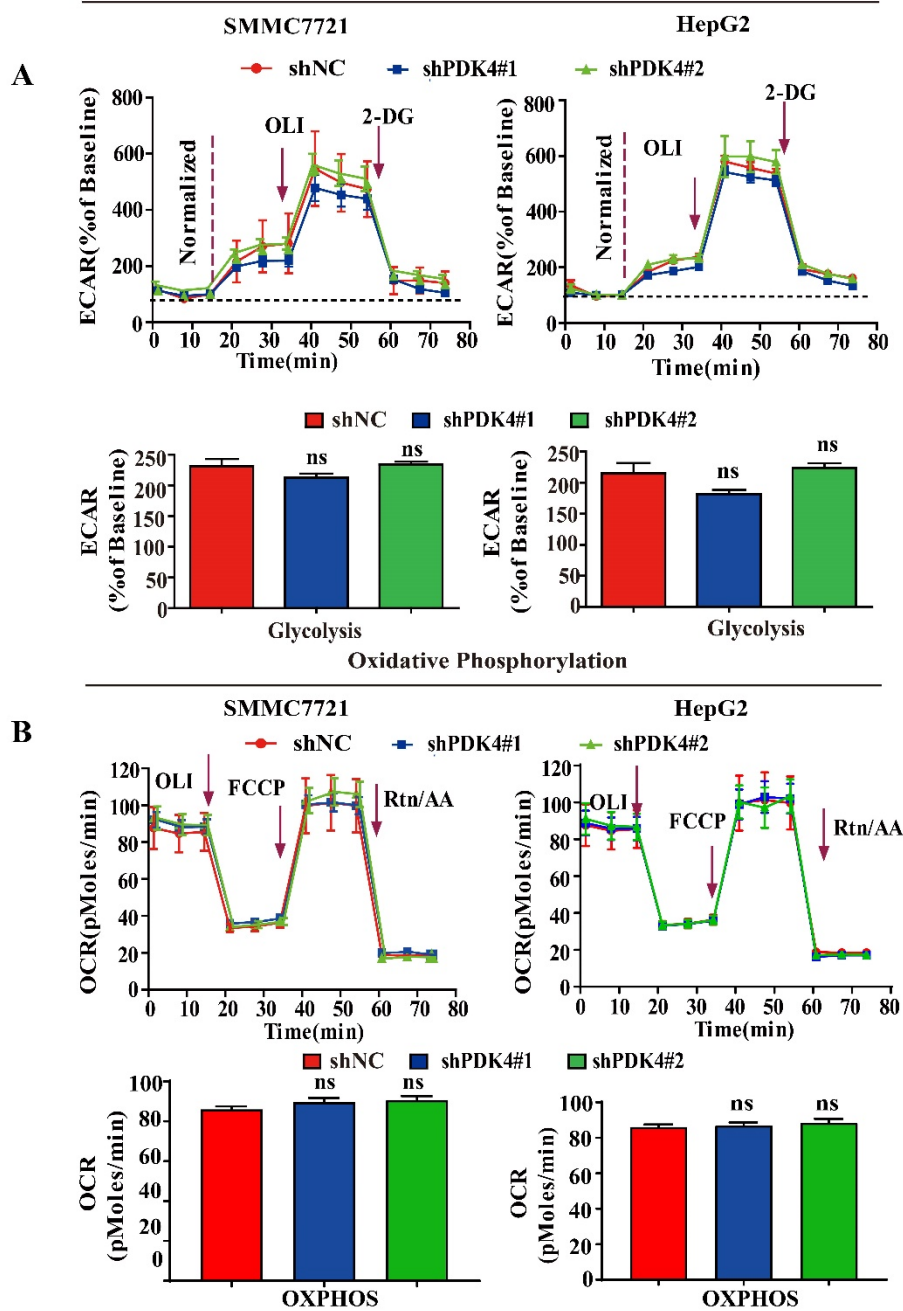


Figure S3. (A, B) The effect of PDK4 on glycolysis and oxidative phosphorylation in control or PDK4 knockdown cells was evaluated by monitoring ECAR and OCR using XF96 Extracellular Flux Analyzer. Basal glycolytic capacity was measured under basal conditions without mitochondrial inhibitor oligomycin (OLI) and 2-Deoxy-D-glucose (2-DG). Basal oxygen consumption rate (OCR) was determined with OLI. NS: not significant.

Table S1. The clinical parameters of HCC samples used for quantitative real-time PCR (n=86), western blotting (n=8), and mRNA microarray (n=14) analyses.

Parameter	Real-time PCR n=86 No. of patients	Western blotting n=8 No. of patients	mRNA microarray n=14 No. of patients
Sex			
male	68	5	9
female	18	3	5
Age			
≤ 50	34	3	10
> 50	52	5	4
TNM			7 [§]
I	57	7	1
II-III	29	1	6
Tumor size			1 [§]
≤ 3cm	32	4	2
>3cm	54	4	11
Tumor number			14 [§]
1	71	8	-
> 1	15	0	-
AFP			
≤ 20 ng/ml	30	3	5
> 20 ng/ml	56	5	9
HBsAg			
negative	12	1	1
positive	74	7	13
Tumor differentiation			14 [§]
I-II	69	7	-
III-IV	17	1	-
Vascular invasion			
no	32	2	10
yes	54	6	4
Child-Pugh stage			14 [§]
A	58	5	-
B	28	3	-
Liver cirrhosis			
no	62	4	7
yes	24	4	7

Abbreviations: TNM: tumor-node-metastasis staging system; AFP: alpha-fetoprotein; HBsAg: hepatitis B surface antigen. §: the number of unknown cases.

Table S2. Relationships between PDK4 expression and clinicopathological features of patients with HCC (n=80)

Parameter	No. of patients	PDK4 (low)	PDK4(high)	<i>P</i> value
Sex				0.066
male	68	47	21	
female	12	5	7	
Age				0.160
≤ 50	40	23	17	
> 50	40	29	11	
TNM				0.452
I	25	15	10	
II	34	21	13	
III-IV	21	16	5	
Tumor size				0.199
≤ 3cm	7	3	4	
>3cm	73	49	24	
Tumor number				0.466
1	62	39	23	
> 1	18	13	5	
AFP				0.679
≤ 20 ng/ml	29	18	11	
> 20 ng/ml	51	34	17	
HBsAg				0.052
negative	21	10	11	
positive	59	42	17	
Tumor differentiation				0.432
I-II	52	33	19	
III-IV	25	16	9	
Vascular invasion				0.652
no	26	16	10	
yes	54	36	18	
Child-Pugh stage				0.929
A	74	48	26	
B	6	4	2	
Liver cirrhosis				0.423
no	6	3	3	
yes	74	49	25	

Abbreviations: TNM: tumor-node-metastasis staging system; AFP: alpha-fetoprotein; HBsAg: hepatitis B surface antigen.

Table S3. Primers used in this study

Primer name	Primer sequences (5'-3')
Primers for quantitative real-time PCR	
PDK4-forward	GGAGCATTTCTCGCGCTACA
PDK4-reverse	ACAGGCAATTCTTGTGCGCAA
β -actin-forward	GCTGTCACCTTCACCGTTCC
β -actin-reverse	GCCTGCTGAGATGGTATTTG
Primers for PDK4-shRNAs	
shPDK4#1	GATCCTATTTATCATCTCCAGAATTAACCTCCTG TCAGATTAATTCTGGAGATGATAAAATATTTTTG
shPDK4#2	GATCCGTATAAAGATGCCTGTACATTCCTGTCAG ATGTACAGGCATCTTTATACTTTTTG
shNC	GATCCTTCTCCGAACGTGTCACGTCTTCCTGTCA GAACGTGACACGTTCCGAGAATTTTTG