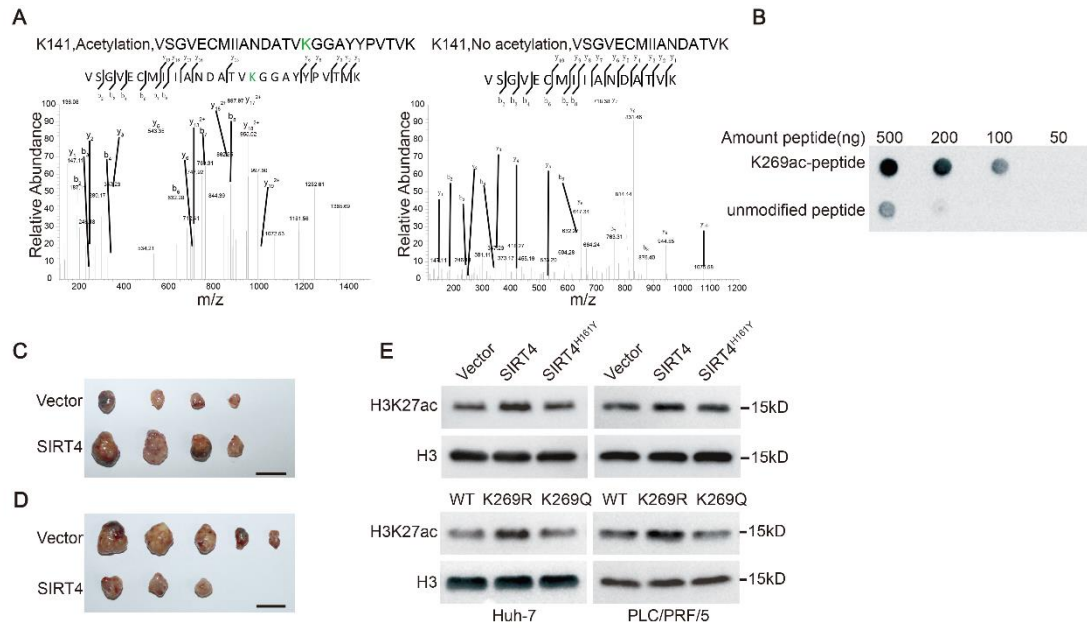


**Figure S1 *CaMKIIδ* promotes HCC TICs and is associated with poor prognosis.**

(A) Western blotting results showing the expression of the indicated molecules in indicated cells overexpressing *CaMKIIδ*. (B and C) Phase contrast micrographs (B) and histograms (C) demonstrating primary and serially passaged spheres formed by the indicated cells after forced expression of *CaMKIIδ*. Data represent mean  $\pm$  SD of 3 independent experiments. \*Two-tailed Student's *t* test. Scale bars = 100 μm. (D) Kaplan-Meier survival analysis for the expression of *CaMKIIδ* mRNA in HCC patients was performed using an online tool (<http://gepia2.cancer-pku.cn/>).



**Figure S2**

(A) Mass spectrometry analysis of the acetylation sites of MCCC2-Flag immunoprecipitated in Figure 3I. (B) The specificity of the antibody against acetyl-K269 of MCCC2 was determined by dot blot assay. (C and D) Photograph of dissected tumors of the tumors formed by  $\alpha 2\delta 1^-$  (C) (n=4) or  $\alpha 2\delta 1^+$  (D) (n=5) PLC/PRF/5 cells overexpressing SIRT4 or empty vector in NOD/SCID mice. (E) Western blotting analysis of the acetylation levels of H3K27 in indicated cells.

**Table S1. Information for Antibodies**

Name.	Vender	Cat No.	Species	Dilution
ABCG2	Abcam	ab108312	Rabbit monoclonal IgG	1:3000
BMI1	Abcam	ab126783	Rabbit monoclonal IgG	1:5000
NANOG	Cell Signaling	4903S	Rabbit monoclonal IgG	1:3000
$\alpha 2\delta 1$	Novus	NB120-2864	Mouse monoclonal IgG	1:3000
$\alpha 2\delta 1$	Sigma	HPA008213	Rabbit monoclonal IgG	1:3000
SIRT4	Sigma	HPA029691	Rabbit polyclonal IgG	1:3000
SIRT3	Proteintech	10099-1-AP	Rabbit polyclonal IgG	1:3000
SIRT5	Abcam	ab259967	Rabbit monoclonal IgG	1:3000
MCCC2	Proteintech	12117-1-AP	Rabbit polyclonal IgG	1:3000
MCCC1	Proteintech	14861-1-AP	Rabbit polyclonal IgG	1:3000
CaMKII $\delta$	Abcam	ab181052	Rabbit monoclonal IgG	1:3000
H3	Beyotime	AH433	Rabbit polyclonal IgG	1:3000
H3K27ac	Abcam	ab4729	Rabbit polyclonal IgG	1:3000
Flag	Cell Signaling	14793S	Rabbit monoclonal IgG	1:5000
HA	Cell Signaling	3724S	Rabbit monoclonal IgG	1:3000
Acetylated-Lysine	Cell Signaling	9441S	Rabbit monoclonal IgG	1:3000
GAPDH	Cell Signaling	2118S	Rabbit monoclonal IgG	1:10000

**Table S2. Sequences for PCR Primers and shRNA/sgRNA constructs**

Name	Primer	Sequences
SIRT4	Forward	5'-CGGGATCCATGAAGATGAGCTTTGCGTTGAC-3'
Wild-type	Reverse	5'-CCGCTCGAGGCATGGGTCTATCAAAGGCAGCAAC-3'
SIRT4-	Forward	5'-CGCCTGACAGAGCTCTACGGATGCATGG-3'
H161Y	Reverse	5'-CTGTCCATGCATCCGTAGAGCTCTGTCAGG-3'
	Forward	5'-gatccCCCGATTGCAATACTGAACATtctcctgtcagaATGTTC
SIRT4		AGTATTGCAATCGGGtttttg-3'
shRNA-1	Reverse	5'-aattcaaaaaCCCGATTGCAATACTGAACATtctgacaggaagAT
		GTTCAGTATTGCAATCGGGg-3'
	Forward	5'-gatccCCGTGCTCGAAAGCCTCCATTtctcctgtcagaAATGG
SIRT4		AGGCTTTCGAGCACGGtttttg-3'
shRNA-2	Reverse	5'-aattcaaaaaCCGTGCTCGAAAGCCTCCATTtctgacaggaagA
		ATGGAGGCTTTCGAGCACGGg-3'
	Forward	5'-gatccGCGAGAAGCGCGATCACATGTTCAAGAGACATG
Scramble		TGATCGCGCTTCTCGtttttg-3'
shRNA	Reverse	5'-aattcaaaaaACGAGAAGCGATCACATGTCTCTTGAACAT
		GTGATCGCGCTTCTCGCg-3'
SIRT3	Forward	5'-CGCGGATCCATGGTGGGGGCCGGCATCAG-3'
Wild-type	Reverse	5'-CCGCTCGAGTTTGTCTGGTCCATCAAGCTTCCCA-3'
MCCC2	Forward	5'-CGGGATCCATGTGGGCCGTCCTGAGGTTA-3'
Wild-type	Reverse	5'-CCGCTCGAGCATCCTGAAGATAACCGAAGT-3'
MCCC1	Forward	5'-CGGGATCCATGAAGTACACAACAGCCACA-3'
Wild-type	Reverse	5'-ATCTTCCGATTCCCTTTTGTCTGATTCTTCCT-3'
MCCC2	Forward	5'-CACCGTTGACAATCTCATAGACCCA-3'
sgRNA	Reverse	5'-AAACTGGGTCTATGAGATTGTCAAC-3'
MCCC2-	Forward	5'-CAATGATGCCACCGTCAGAGGAGGTGCCT-3'
141R	Reverse	5'-AGGCACCTCCTCTGACGGTGGCATCATTG-3'
MCCC2-	Forward	5'-CAATGATGCCACCGTCCAAGGAGGTGCC-3'
141Q	Reverse	5'-GGCACCTCCTTGGACGGTGGCATCATTG-3'
MCCC2-	Forward	5'-GGAGGTGCTGATCTTCATTGCAGAAGGTCT-3'
269R	Reverse	5'-CAGACCTTCTGCAATGAAGATCAGCACCT-3'
MCCC2-	Forward	5'-GGTGCTGATCTTCATTGCAGACAGTCTGGA-3'
269Q	Reverse	5'-TCCAGACTGTCTGCAATGAAGATCAGCACC-3'

**Table S3. Correlation between the clinicopathologic characteristics and expression of SIRT4 protein in hepatocellular carcinoma**

Variable	SIRT4 expression		Chi square value	<i>P</i> value <sup>a</sup>
	Low	High		
Sex				
Male	28	28	0.8634	0.3528
Female	6	3		
Age				
≤60	24	26	1.612	0.2043
>60	10	5		
Hepatic cirrhosis				
Absent	14	8	1.711	0.1909
Present	20	23		
Size				
≤5 cm	21	10	5.659	0.0174*
>5 cm	13	21		
Venous invasion				
Absent	26	19	1.754	0.1854
Present	8	12		
Invasion				
Absent	31	29	0.1285	0.7200
Present	3	2		
Recurrence				
Early <sup>b</sup>	5	14	8.374	0.0038**
Late <sup>c</sup>	29	15		
AFP				
>400 µg/L	6	14	6.330	0.0119*
≤400 µg/L	24	13		

Note: a, Chi-Square Tests.

b, Recurrences occur within 2 years of diagnosis.

c, No recurrences within 2 years of diagnosis.