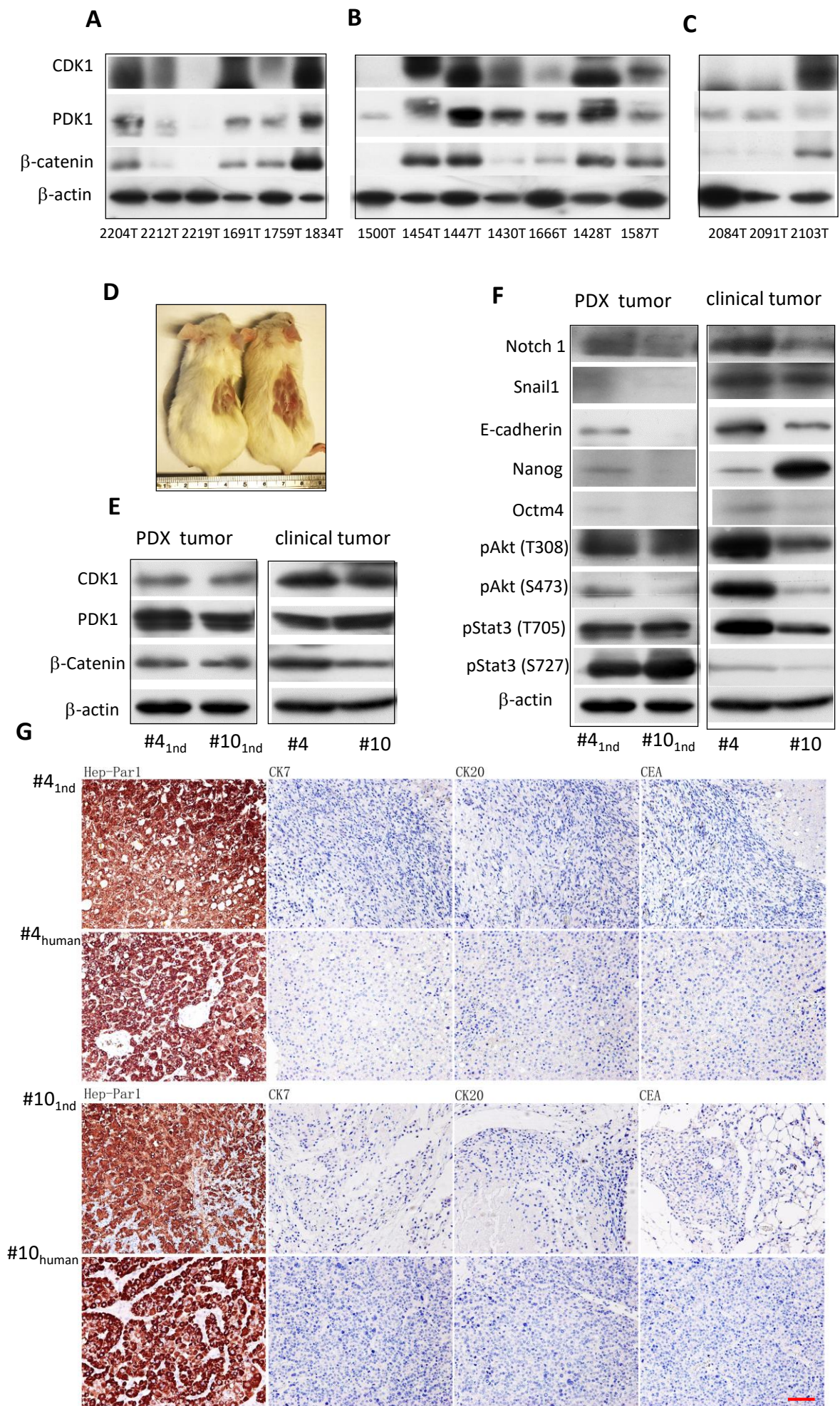
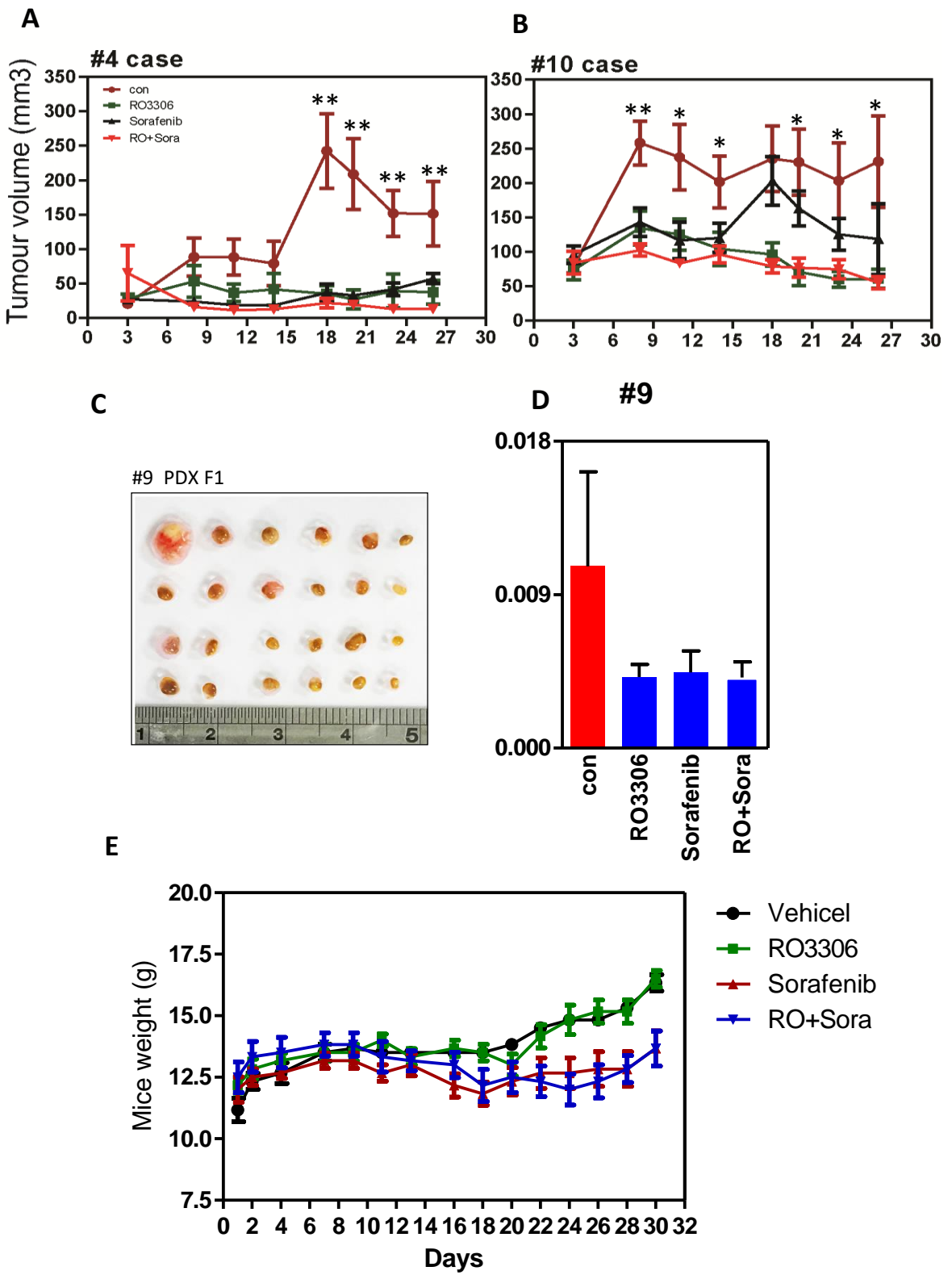


Supplementary figure 1



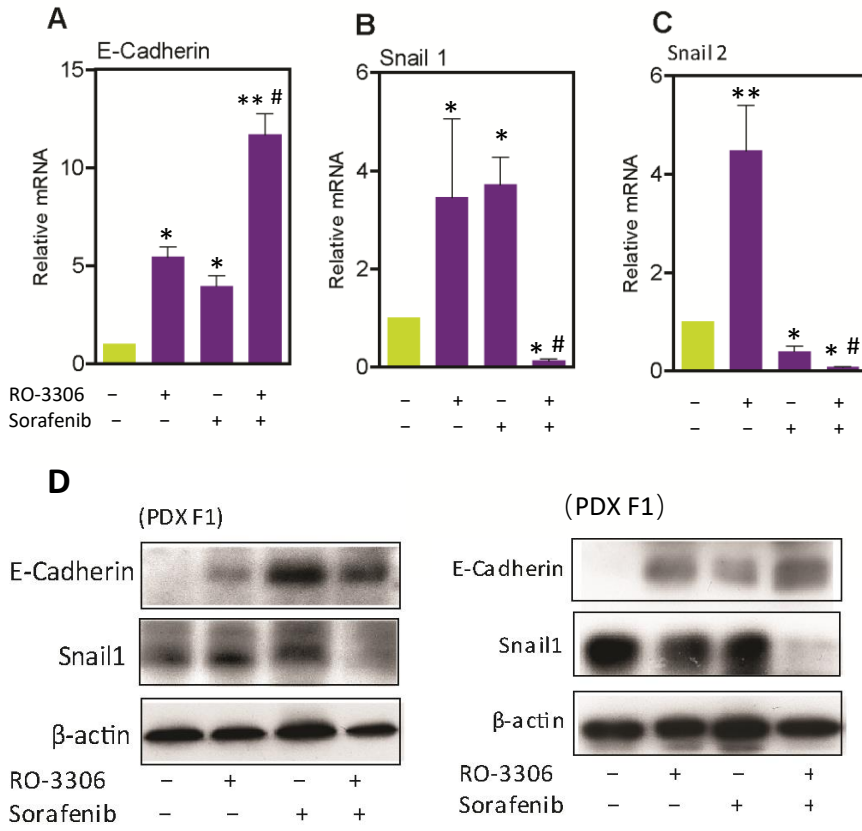
**Supplementary Figure.1 F1 PDX tumor grafts resemble the patient tumors from where they are derived.** A-C, The corresponding expression of CDK1, PDK1 and β-catenin in clinical tumor tissues. D, Image represented the mouse model of F1 PDX tumors. E-F, In comparison of the protein expression levels, PDX tumors highly mimic the paired clinical tumors analysed by western blot. G, Hep Par1, cytokeratin 7 (CK7), cytokeratin 20 (CK20) and CEA staining for F1 PDX tumors and paired clinical tissues. Scale bar represent 100 μm.

Supplementary figure 2



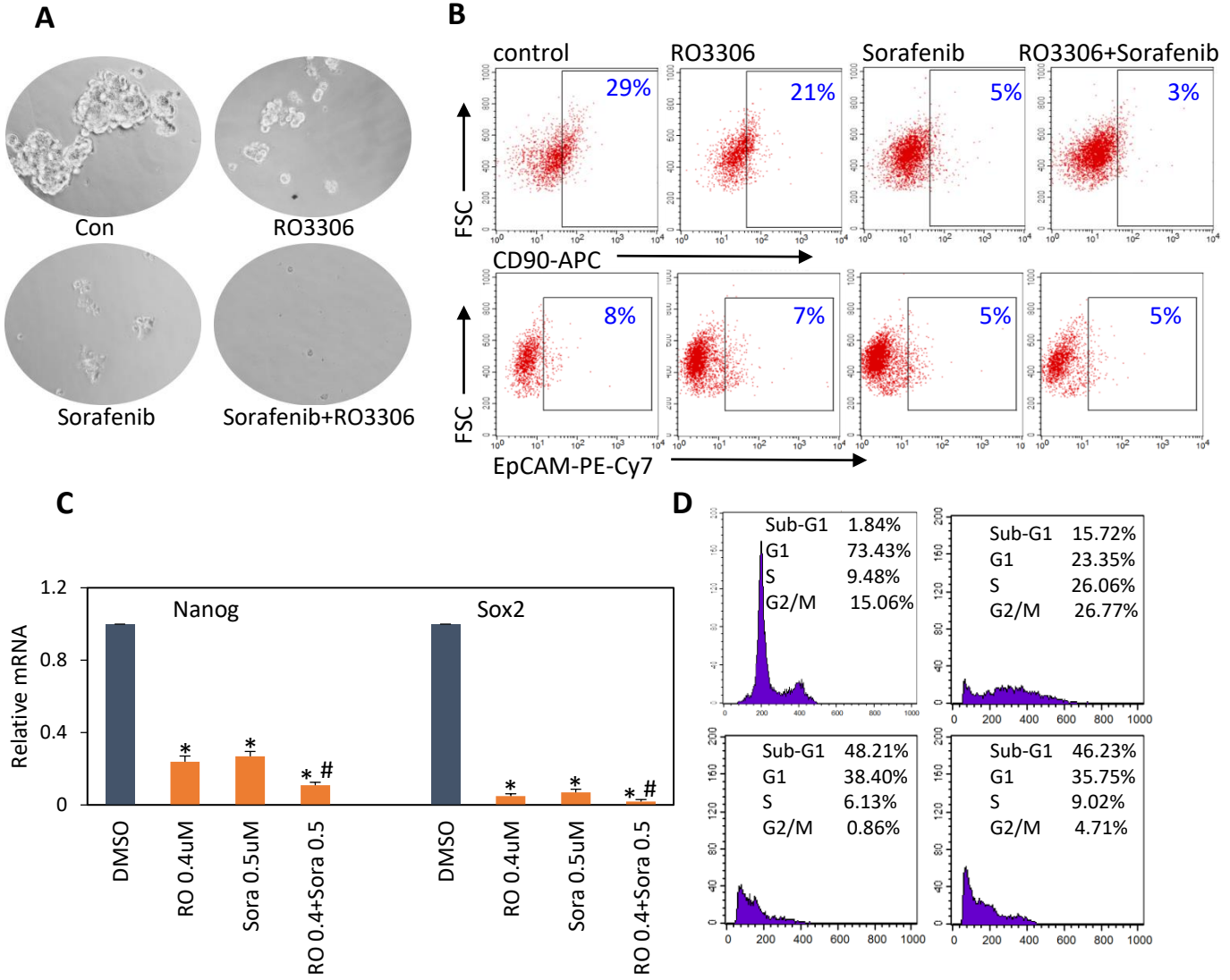
**Supplementary Figure.2 Effectiveness of RO3306 and sorafenib on PDX tumors.** A-B, The growth curves of HCC case #4 and #10 PDX models, respectively. C-D, The no inhibition effect of indicated treatments on HCC case #9 PDX models, due to the low tumor progression process. E, The tumor weight during the treatments from day 1 to day 30 with no obvious body weight change. (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$  compared to control.)

Supplementary figure 3



**Supplementary Figure.3 The combined effect on the EMT transition.** A-C, The synergistic effect of increased E-Cadherin and decreased Snail1 and Snail 2 on F1 PDX models. D, The combined treatment could decrease Snail1 and upregulate E-Cadherin analysed by western blot (left: case #4; right: PDX case #10). (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$  compared to control and #,  $p < 0.05$  compared to single agent treatments.)

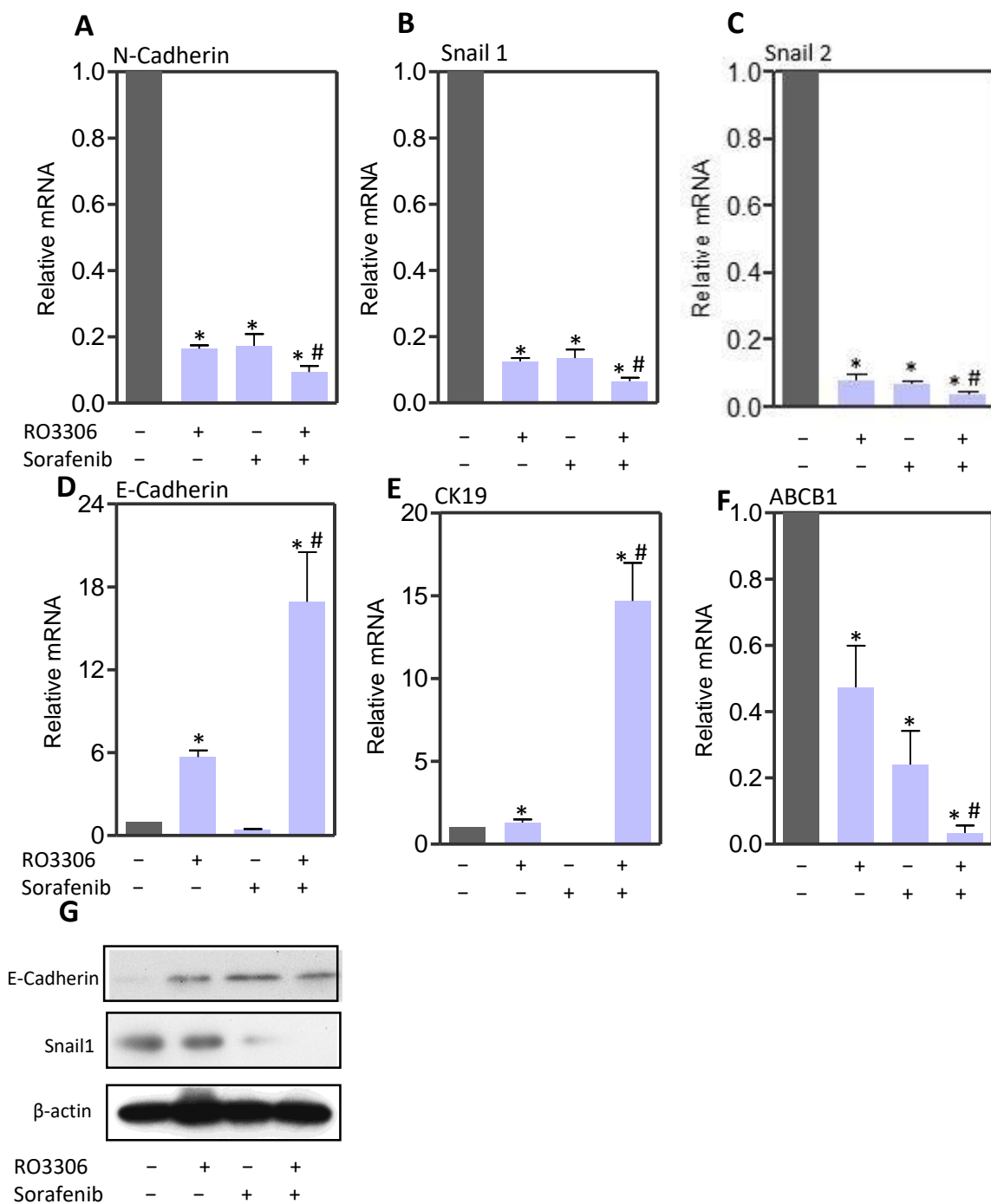
Supplementary figure 4



**Supplementary Figure.4 Anticancer effect of RO3306 and sorafenib combination or alone on 97H sphere cells.** A, The morphology of sphere formation in indicated treatment groups. B, The suppression effect on CD90 and EpCAM CSCs. C, Low dose of RO3306 (4  $\mu$ M) and sorafenib (2.5  $\mu$ M) decreased the stemness-related genes Nanog and Sox2. D, Low dose RO3306 and sorafenib combination for 72h promoted CSCs enter into a Sub-G1 phase. (\*,  $p < 0.01$  compared to control. #,  $p < 0.05$  compared to single agent treatments).

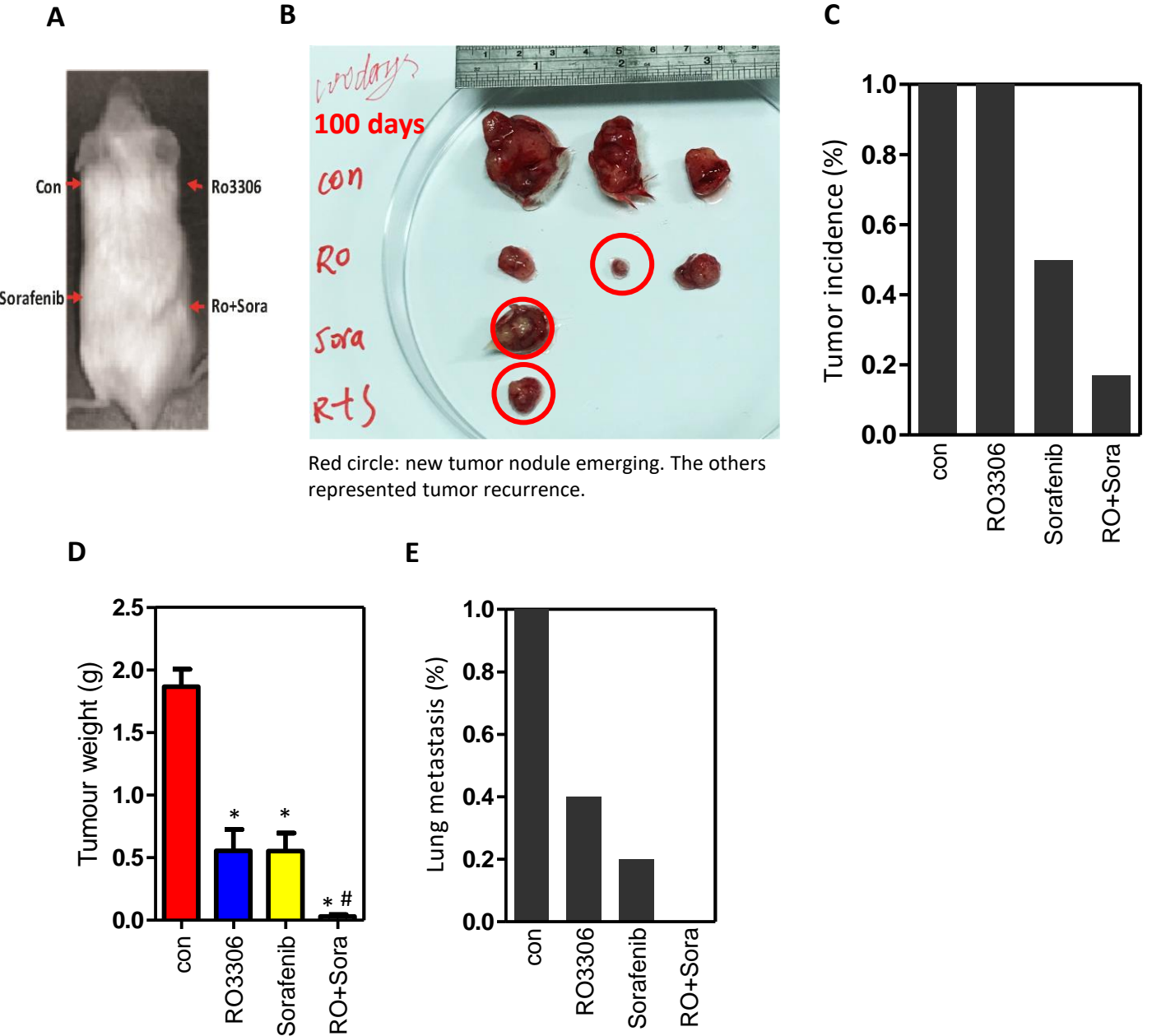


Supplementary figure 5



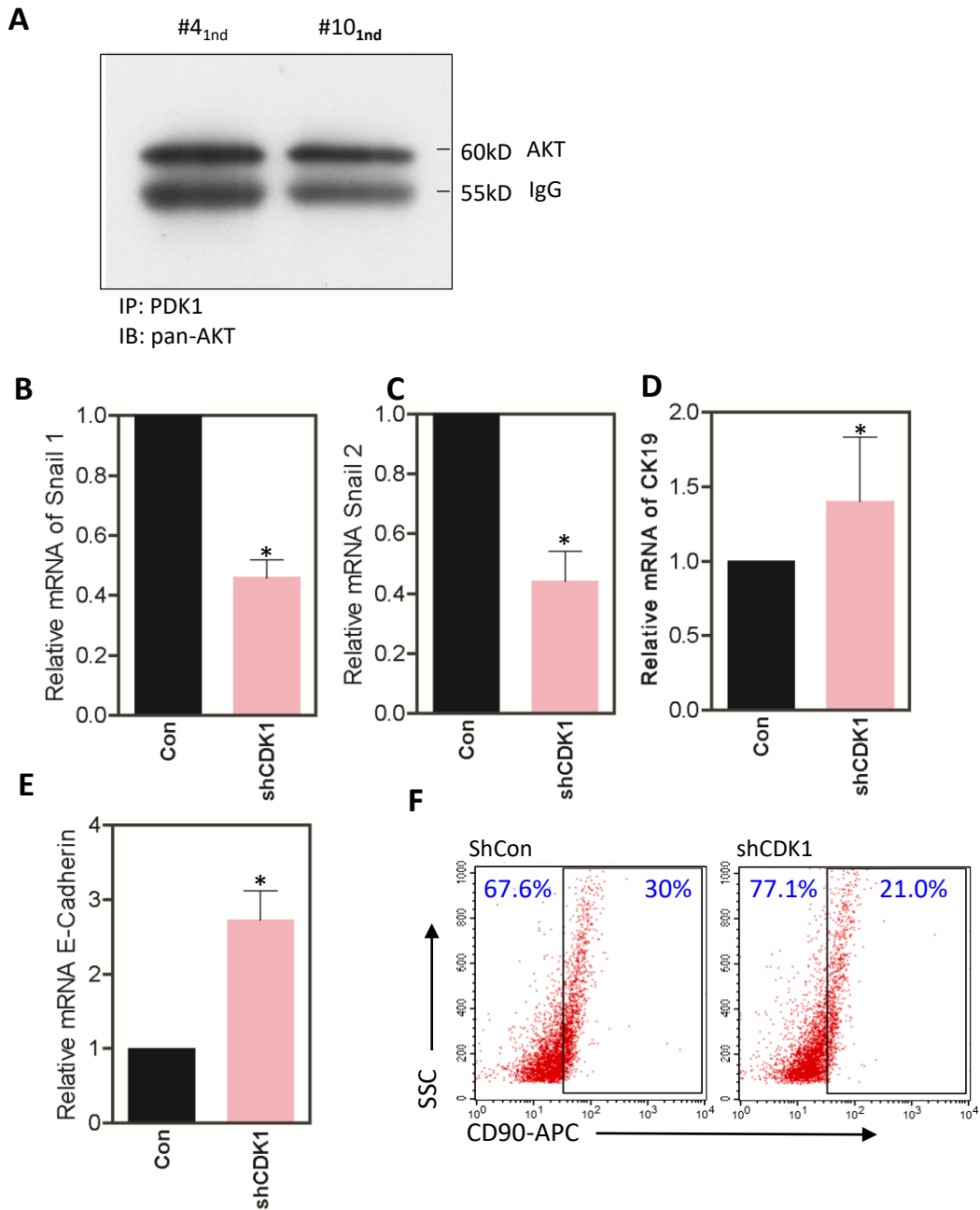
**Supplementary Figure.5 RO3306 and sorafenib alone or combination on the mRNA levels of EMT in 97H sphere cells.** A-E, The synergistic effect of downregulation N-Cadherin, Snail1 and Snail2, whereas the E-Cadherin and CK19 were upregulated. F, The synergistic effect on ABCB1 drugs resistance gene. G, Western blot analysis of E-Cadherin and Snail 1 in indicated groups. (\*,  $p < 0.01$  compared to control. #,  $p < 0.05$  compared to single agent treatments).

Supplementary figure 6



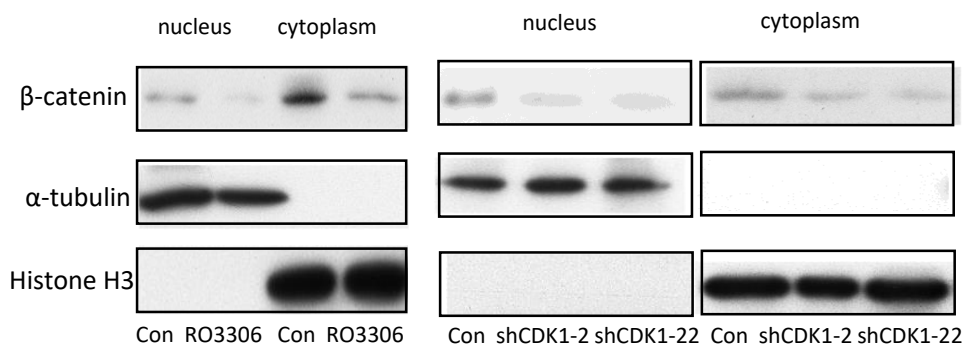
**Supplementary Figure.6 The combined effect on CSCs tumorigenicity after 100 days.** A, The indicated points of the pretreated-CSCs injection. B, Images of recurrence tumor and new emerging tumor after 100 days. C, Statistical comparison of tumor incidence (%) in various pretreat-groups after 100 days without further treatment. D, Tumor weight of 97H CSC-derived orthotopic tumor models in indicated groups. E, The percentage of lung metastasis 100%, 40%, 20% and 0% in four indicated treatments labeled above. (\*,  $p < 0.01$  compared to control. #,  $p < 0.05$  compared to single agent treatments).

Supplementary figure 7



**Supplementary Figure.7 The interaction of PDK1 and AKT, and CDK1 silencing effect on 97H sphere cells.** A, The Co-IP analysis the interaction between PDK1 and AKT. B-E, Knockdown CDK1 reverse EMT process. F, The CD90+ CSCs population of shCDK1 knockdown compared with the scramble group after 24h. (\*,  $p < 0.01$  compared to control).

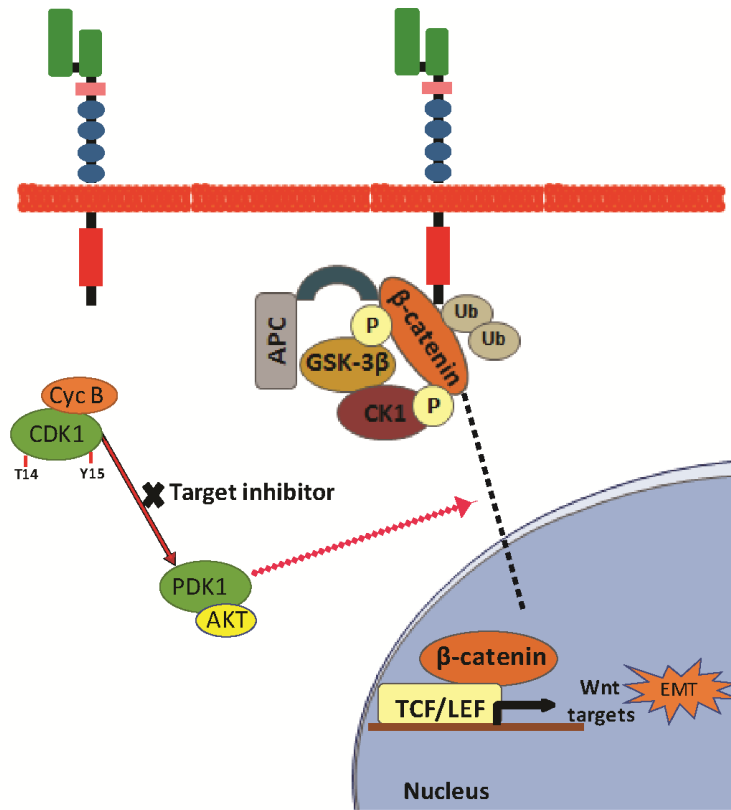
Supplementary figure 8



**Supplementary Figure. 8 The expression level of  $\beta$ -catenin in cytoplasm and nucleus of various groups and CDK1 Knockdown.** A, Level of  $\beta$ -catenin in cytoplasm and nucleus were measured by western blot analysis. Decreased  $\beta$ -catenin was detected both in cytoplasm and nucleus.  $\alpha$ -tubulin and Histone H3 as control loading.



Supplementary figure 9



**Supplementary Figure.9 The CDK1-PDK1-β-Catenin axis.** A schematic diagram illustrates the proposed CDK1-PDK1-β-Catenin associated pathways and its downstream targets EMT relates to HCC progression and metastasis.

**Table S1****Table S1: Primer sequences**

Primer	Sequence (5' to 3') F: forward; R: reverse
NANOG	F: CAAAGGCAAACAACCCACTT R: TCTGCTGGAGGCTGAGGTAT
OCT4	F: CTCACCCTGGGGGTTCTATT R: CTCCAGGTTGCCTCTCACTC
SOX2	F: GCTGCGAACAGTCAGACAGA R: ACCTCCCGTCCAAGGTAGG
CDH1	F: CGACCCAACCCAAGAATCTA R: AGGCTGTGCCTTCTACAGA
CDH2	F: GACAATGCCCTCAAGTGTT R: CCATTAAGCCGAGTGATGGT
CK19	F: TTTGAGACGGAACAGGCTCT R: AATCCACCTCCCACTGACC
SNAIL1	F: CACTATGCCGCGCTCTTTC R: GGTCGTAGGGCTGCTGGAA
SNAIL2	F: GAGCATTTGCAGACAGGTCA R: GCTTCGGAGTGAAGAAATGC
ABC1	F: GCCTGGCAGCTGGAAGACAAATAC R: ATGGCCAAAATCACAAGGGTTAGC
shRNA-Scramble1	AGCGGGATGTGCTTATGCAGGATTCCACGAGTGGAA TCCTGCATAAGCACATCC
shRNA-CDK1-2	AGCGGGTCAGTACATGGATTCTTCACTCGAGAGTGA AGAATCCATGTACTGACC
shRNA-Scramble11	AAAAGGATGTGCTTATGCAGGATTCCACTCGTGGAA TCCTGCATAAGCACATCC
shRNA-CDK1-22	AAAAGGTCAGTACATGGATTCTTCACTCTCGAGTGA AGAATCCATGTACTGACC

Table S2. Correlation between clinicopathological parameters and CDK1 high or low expression

Parameters	Category	Cases (n=39)	CDK1 level		<i>P</i>
			low	high	
Sex	Male	32	19	13	0.139
	Female	7	2	5	
Age	≥60	22	12	10	0.921
	<60	17	9	8	
Tumor UICC7 stage	I-II	30	17	13	0.519
	III-V	9	4	5	
Tumor size	≤ 5 mm	16	9	7	0.802
	> 5 mm	23	12	11	
Tumor nodules (no.)	1-2 nodeuls	31	15	16	0.178
	≥ 3 nodeuls	8	6	2	
Venous infiltration	absent	22	13	9	0.455
	present	17	8	9	
HBsAg	positive	34	18	16	0.768
	negative	5	3	2	
AFP level	low than 20ng/ml	16	9	7	0.802
	high than 20ng/ml	23	12	11	
One year recurrence	no recurrence	29	19	10	0.013*
	recurrence	10	2	8	
Five year recurrence	no recurrence	21	15	6	0.017*
	recurrence	18	6	12	

Table S3. Correlation between clinicopathological parameters and CDK1 and PDK1 related

Parameters	Category	Cases (n=39)	CDK1 and PDK1 related		P
			NO	YES	
Sex	Male	28	15	13	0.790
	Female	5	3	2	
Age	≥60	19	10	9	0.797
	<60	14	8	6	
Tumor UICC7 stage	I-II	25	14	11	0.767
	III-V	8	4	4	
Tumor size	≤ 5 mm	14	9	5	0.335
	> 5 mm	19	9	10	
Tumor nodules (no.)	1-2 nodeuls	31	15	16	0.604
	≥ 3 nodeuls	8	6	2	
Venous infiltration	absent	25	13	12	0.898
	present	8	5	3	
HBsAg	positive	28	16	12	0.478
	negative	5	2	3	
AFP level	low than 20ng/ml	13	9	4	0.172
	high than 20ng/ml	20	9	11	
One year recurrence	no recurrence	24	15	9	0.134
	recurrence	9	3	6	
Five year recurrence	no recurrence	18	13	5	0.025*
	recurrence	15	5	10	

Table S4. The association of clinicopathological parameters, CDK1, PDK1 and CDK1 related PDK1 expression with HCC overall survival and disease-free survival using univariate and multivariate analysis

Clinical parameters	Overall survival						Disease free survival					
	Univariate analysis			multivariate analysis			Univariate analysis			multivariate analysis		
	HR	95%CI	p value	HR	95%CI	p value	HR	95%CI	p value	HR	95%CI	p value
Sex (male vs. female)	3.08	(0.92-10.36)	0.069				1.93	(0.70-5.35)	0.204			
Age (<60 vs. ≥60)	1.37	(0.44-4.24)	0.590				1.58	(0.68-3.67)	0.283			
HBsAg (pres vs. abs)	0.74	(0.16-3.38)	0.698				0.74	(0.24-2.21)	0.586			
AFP level ( ≤20 vs. ≥ 20)	1.39	(0.42-4.63)	0.589				2.07	(0.80-5.32)	0.132			
Tumor UICC7 stage (I,II and III,IV,V)	5.84	(1.84-18.50)	<b>0.003**</b>	4.01	(1.16-13.90)	<b>0.029*</b>	4.33	(1.74-10.758)	0.002**	4.49	(1.50-13.36)	<b>0.007**</b>
Tumor size (<5 vs. ≥5)	2.44	(0.66-9.05)	0.181				1.91	(0.77-4.74)	0.160			
Tumor nodules (no.) (≤1 vs. ≥2)	2.15	(0.68-6.79)	0.191				1.54	(0.65-3.68)	0.328			
Venous infiltration (pres vs. abs)	3.15	(0.95-10.50)	0.061				3.16	(1.29-7.75)	0.012*	3.32	(1.11-9.99)	<b>0.032*</b>
CDK1 (low or high)	4.20	(1.11-15.90)	<b>0.023*</b>	1.99	(0.37-10.69)	0.424	2.08	(0.81-5.38)	0.130			
PDK1 (Low or high)	4.20	(1.11-15.90)	<b>0.035*</b>	2.61	(0.52-13.21)	0.247	2.31	(0.95-5.58)	0.064			
CDK1 related PDK1 (yes or no)	1.04	(0.28-3.86)	0.96				2.91	(1.06-7.96)	0.038**	4.62	(1.47-14.54)	<b>0.009**</b>

Univariate and multivariate analysis by cox proportional hazard regression model was used to analyze the risk factors associated with the survival. HR, hazard ratio; CI, confidence interval. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .