

Supplementary figure legends

Figure S1.

(A) The expression of ING5 protein in hepatocellular carcinoma (HCC) tissue (patient ID: 2766) and normal liver tissue (patient ID: 3402) was obtained from the HPA database (<https://www.proteinatlas.org/>). (B) The mRNA and protein expression of ING5 in normal and HCC patients was assessed by TCGA-LIHC and TCGA-CPTAC data obtained from the UALCAN and GEO (GSE102079 and GSE76427) databases. (C) KM analyses of OS, PFS, RFS and DSS of HCC patients based on ING5 expression were performed using KM Plotter (<http://kmplot.com/>). ING5 expression in HepG2 and PLC/PRF/5 cells was validated by western blot (D) and qRT-PCR (E) (n=3). All data are presented as mean \pm SD, Student's t test was used for significance tests, *P < 0.05, ***P < 0.001 vs. the vector group. Scale bar=30 μ m. HR, hazard ratio. OS, overall survival. PFS, progression-free survival. RFS, relapse-free survival. DSS, disease-specific survival.

Figure S2.

Cell viability (A), apoptosis (B) (n=3), migration (C) (n=5), and invasion (D) (n=3) were analyzed in HepG2 and PLC/PRF/5 cells after ING5 silencing or/and treatment with 5 μ M UA (*P < 0.05, **P < 0.01, ***P < 0.001. Scale bar =50 μ m).

Figure S3.

(A) HepG2-SR and parental cells were treated with sorafenib for 24 h, after which cell viability was analyzed by CCK-8 assays. (B) HepG2-SR cells were treated with UA for 24 h, after which cell viability was analyzed by CCK-8 assays. (C) The transfected cells were validated using western blotting. (D) HepG2 cells were exposed to culture medium containing 2% and 12% FBS for 36 h, respectively. HepG2 cells were transfected with ING5 shRNA, SRF or YY1 siRNA following exposure to medium containing 12% FBS for 36 h. HepG2 cells were transfected with ING5 overexpression plasmid following exposure to medium containing 2% FBS for 36 h. Colony formation assays were performed for assessing the clonogenicity of HepG2 cells (n=3, *P < 0.05, **P < 0.01). (E) ChIP assays with anti-IgG and anti-histone H3 antibodies were used as negative and positive controls, respectively.

Figure S4.

Ultrasonography was performed to visualize the livers of spontaneous HCC in mice at 22, 24 and 26 weeks of age.

Figure S1

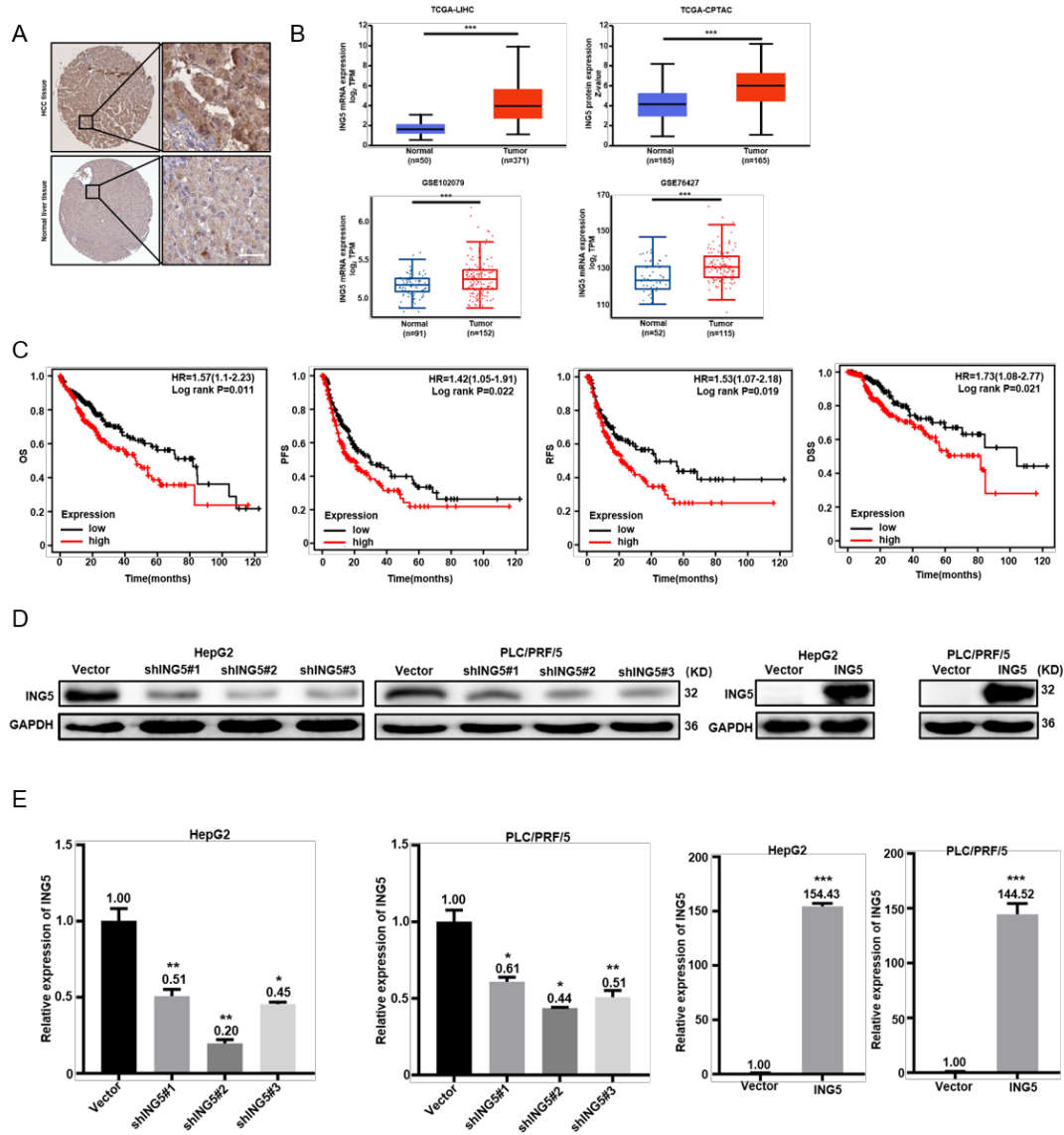


Figure S2

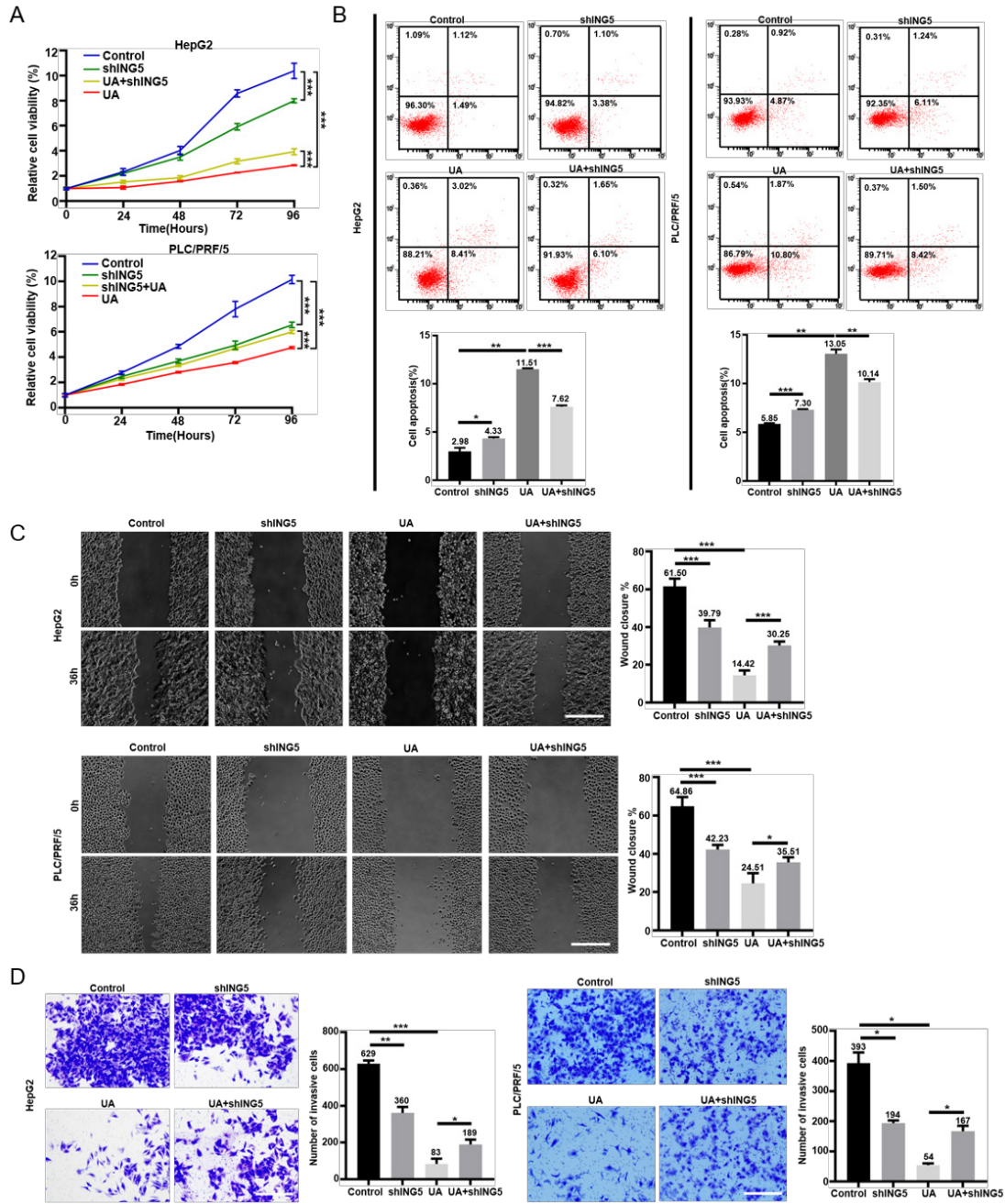


Figure S3

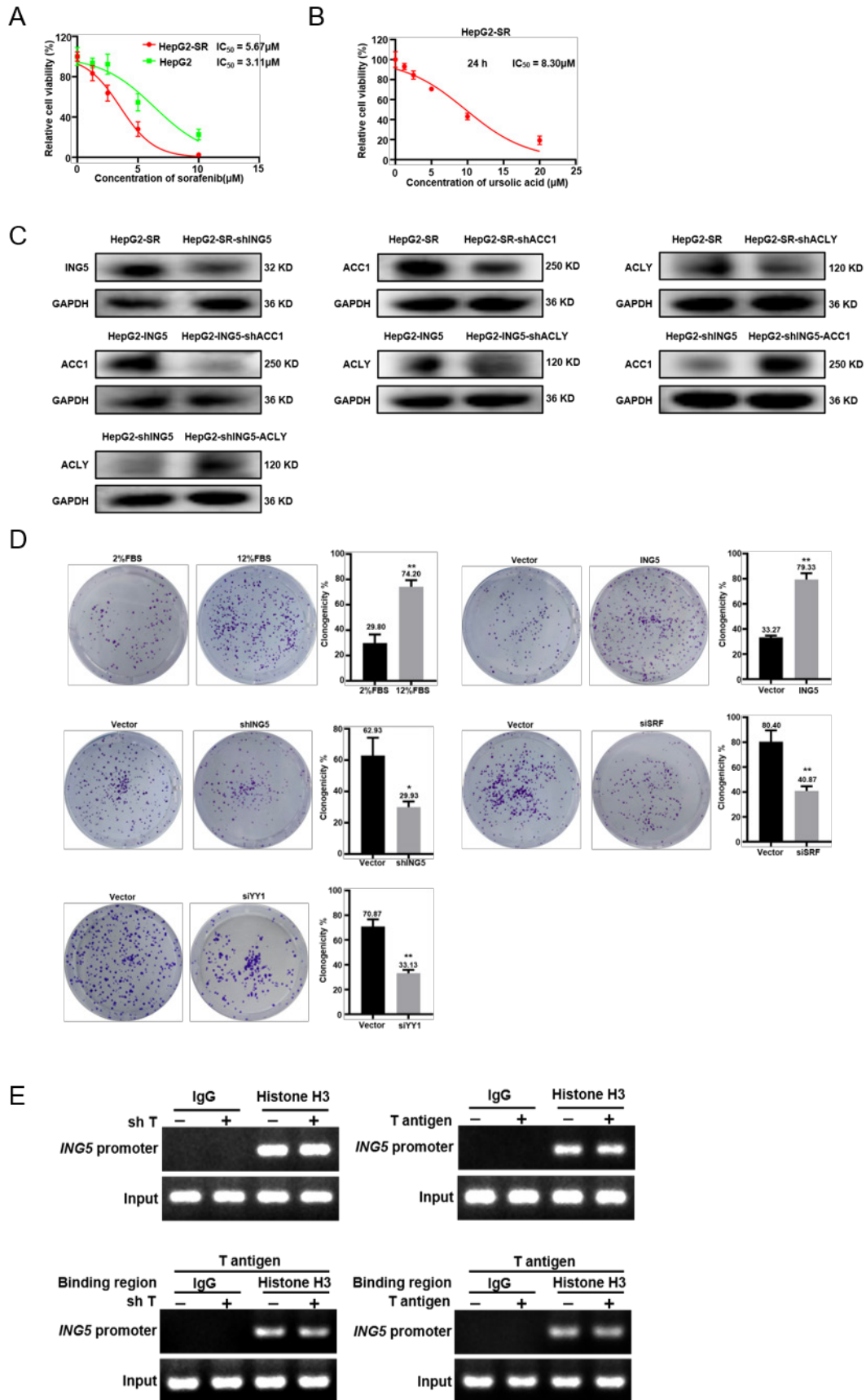
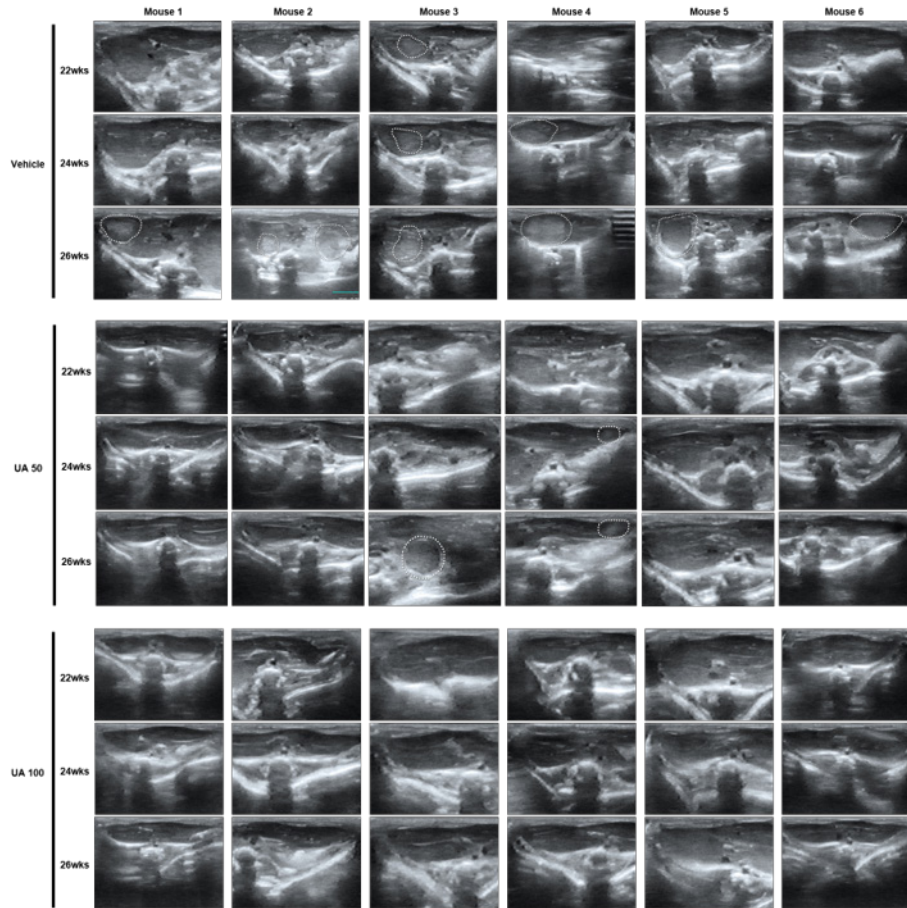


Figure S4



Supplementary tables

Table S1. Primary antibodies used in this study.

Antibody	Manufacturer	Catalog Number	Application
PCNA	Proteintech	60097-1-Ig	1:5000 for WB
Bax	Proteintech	50599-2-Ig	1:2000 for WB
Bcl-2	Proteintech	26593-1-AP	1:1000 for WB
E-cadherin	Bioss	bs-1519R	1:500 for WB
N-cadherin	Bioss	bs-1172R	1:500 for WB
MMP-9	Santa Cruz	sc-21733	1:100 for WB
Cyclin B1	Santa Cruz	sc-245	1:100 for WB
GAPDH	Proteintech	60004-1-Ig	1:20000 for WB
ING5	Proteintech	10665-1-AP	1:500 for WB
			1: 20 for Co-IP
PI3K	Proteintech	60225-1-Ig	1:500 for WB
			1: 20 for Co-IP
p-PI3K	Cell Signaling Technology	17366S	1:1000 for WB
Akt	Proteintech	10176-2-AP	1:2000 for WB
			1: 20 for Co-IP
p-Akt	Proteintech	28731-1-AP	1:1000 for WB
SRF	Cell Signaling Technology	5147S	1:1000 for WB
			1:50 for Co-IP
			1:100 for ChIP
YY1	Cell Signaling Technology	63227S	1:1000 for WB
			1:50 for Co-IP
			1:100 for ChIP

ACC1	Proteintech	21923-1-AP	1:1000 for WB
ACLY	Proteintech	15421-1-AP	1:1000 for WB
MOGAT2	Proteintech	21286-1-AP	1:500 for WB
SV40 T Antigen	Merck Calbiochem	PAb416	1:100 for WB 1:20 for Co-IP 1:50 for ChIP
Ubiquitin	Proteintech	10201-2-AP	1:1000 for WB
Ki-67	Cell Signaling Technology	12202S	1:300 for IHC

Table S2. Primer sequences used in this study.

Target		Sequence (5'-3')
Human ING5	Forward	GAGGACATCAGAGGAAGACACAC
	Reverse	CACTCAATTGGACAGTCTGGATT
Mouse ING5	Forward	AGACTTGATGCTGACCTGGC
	Reverse	TTTCAAGCTCCGTGCTCCA
Human SRF	Forward	CACAACAGACCAGAGAATGAGTG
	Reverse	GTAGAGGTGCTAGGTGCTGTTTG
Mouse SRF	Forward	CGTCACCCTGGACAGACG
	Reverse	TCCTTCGGCCAGTCAGTGT
Human YY1	Forward	ACGGCTTCGAGGATCAGATTC
	Reverse	TGACCAGCGTTTGTTC AATGT
Mouse YY1	Forward	GCCCCTTCGACGGTTGTAAT
	Reverse	CACTCCTGAAGAGGCTTCCC
Human GAPDH	Forward	CAATGACCCCTTCATTGACC
	Reverse	TGGAAGATGGTGATGGGATT
Mouse GAPDH	Forward	GGGTCCCAGCTTAGGTTTCAT
	Reverse	TACGGCCAAATCCGTT CACA

Table S3. Tumor incidence in Alb/JCPyV T antigen transgenic mice across different weeks of age.

Sex	Age (in weeks)	Total incidence
Male	24	100% (8/8)
	20	87.5% (7/8)
	16	50% (4/8)
	12	12.5% (1/8)
Female	32	100% (8/8)
	28	62.5% (5/8)
	24	50% (4/8)
	20	25% (2/8)
	16	0 (0/8)

Table S4. The clinicopathological significance of ING5 mRNA expression in hepatocellular carcinoma patients.

Characteristic	Low expression	High expression	P-value
Age, n (%)			0.088
<=60	80 (21.4%)	97 (26%)	
>60	107 (28.7%)	89 (23.9%)	
Gender, n (%)			0.658
Female	58 (15.5%)	63 (16.8%)	
Male	129 (34.5%)	124 (33.2%)	
Race, n (%)			0.048
Asian	67 (18.5%)	93 (25.7%)	
Black or African			
American	8 (2.2%)	9 (2.5%)	
White	102 (28.2%)	83 (22.9%)	
Weight, n (%)			< 0.001
<=70	76 (22%)	108 (31.2%)	
>70	97 (28%)	65 (18.8%)	
T stage, n (%)			0.456
T1	97 (26.1%)	86 (23.2%)	
T2	46 (12.4%)	49 (13.2%)	
T3	34 (9.2%)	46 (12.4%)	
T4	7 (1.9%)	6 (1.6%)	
N stage, n (%)			1.000
N0	117 (45.3%)	137 (53.1%)	
N1	2 (0.8%)	2 (0.8%)	

M stage, n (%)			0.358
M0	129 (47.4%)	139 (51.1%)	
M1	3 (1.1%)	1 (0.4%)	
Pathologic stage, n (%)			0.293
Stage I	91 (26%)	82 (23.4%)	
Stage II	42 (12%)	45 (12.9%)	
Stage III	37 (10.6%)	48 (13.7%)	
Stage IV	4 (1.1%)	1 (0.3%)	
Tumor status, n (%)			0.005
Tumor free	115 (32.4%)	87 (24.5%)	
With tumor	63 (17.7%)	90 (25.4%)	
Residual tumor, n (%)			0.714
R0	162 (47%)	165 (47.8%)	
R1	9 (2.6%)	8 (2.3%)	
R2	1 (0.3%)	0 (0%)	
Histologic grade, n (%)			< 0.001
G1	32 (8.7%)	23 (6.2%)	
G2	103 (27.9%)	75 (20.3%)	
G3	46 (12.5%)	78 (21.1%)	
G4	3 (0.8%)	9 (2.4%)	
AFP (ng/ml), n (%)			< 0.001
≤400	132 (47.1%)	83 (29.6%)	
>400	14 (5%)	51 (18.2%)	
Child-Pugh grade, n (%)			0.811
A	119 (49.4%)	100 (41.5%)	
B	11 (4.6%)	10 (4.1%)	

C	0 (0%)	1 (0.4%)	
Vascular invasion, n (%)			0.035
No	118 (37.1%)	90 (28.3%)	
Yes	48 (15.1%)	62 (19.5%)	

AFP, alpha-fetoprotein.

Table S5. The prognostic significance of ING5 mRNA expression in hepatocellular carcinoma patients

Clinicopathological features	Overall survival		Progression-free survival		Relapse-free survival	
	HR	P-value	HR	P-value	HR	P-value
	(CI 95%)		(CI 95%)		(CI 95%)	
Total	1.57 (1.1-2.23)	0.0112	1.42 (1.05-1.91)	0.0223	1.53 (1.07-2.18)	0.0187
Stage						
1	1.79(0.98-3.29)	0.0554	1.39(0.83-2.36)	0.212	1.52(0.86-2.69)	0.1457
1-2	1.69(1.05-2.74)	0.303	1.3(0.88-1.9)	0.1824	1.47(0.94-2.29)	0.0883
2	2.49(1.09-5.68)	0.246	2.3(1.09-4.88)	0.0253	2.36(1.01-5.55)	0.0422
2-3	2.2(1.33-3.63)	0.0017	2.13(1.28-3.54)	0.0028	1.91(1.16-1.31)	0.0096
3	2.16(1.15-4.08)	0.0149	2.4(1.24-4.63)	0.0075	1.89(0.94-3.81)	0.0701
3-4	2.07(1.14-3.75)	0.0141	2.14(1.15-3.96)	0.0139	1.89(0.94-3.81)	0.0701
4	—	—	—	—	—	—
Grade						
1	2.54(0.96-6.75)	0.0534	3.22(1.41-3.73)	0.0036	3.12(1.1-8.87)	0.0249
2	1.3(0.77-2.18)	0.323	1.23(0.79-1.89)	0.3579	1.39(0.82-2.36)	0.2183
3	2.65(1.27-5.54)	0.0072	1.61(0.89-2.94)	0.1137	1.35(0.76-2.37)	0.3019
4	—	—	—	—	—	—
AJCC_T						
1	1.65(0.93-2.95)	0.0865	1.34(0.8-2.23)	0.2653	1.5(0.86-2.61)	0.1514
2	2.3(1.09-4.87)	0.0247	2.23(1.13-4.4)	0.0184	2.36(1.07-5.25)	0.0297
3	2.88(1.32-6.29)	0.0058	1.94(0.98-3.84)	0.0521	1.56(0.77-3.17)	0.2112
4	—	—	—	—	—	—
Vascular invasion						

none	1.51(0.86-2.64)	0.148	1.21(0.77-1.89)	0.4035	1.3(0.8-2.11)	0.2933
micro	1.53(0.71-3.28)	0.2725	2.09(0.93-4.67)	0.0665	2.29(1.08-4.85)	0.0263
macro	—	—	—	—	—	—
Gender						
male	1.83(1.17-2.87)	0.0071	1.7(1.18-2.46)	0.0042	1.61(1.04-2.47)	0.0294
female	1.7(0.91-3.17)	0.0894	0.74(0.44-1.25)	0.2636	1.47(0.79-2.72)	0.2223
Race						
white	1.88(1.183-2.98)	0.0067	1.44(0.97-2.16)	0.0721	1.7(1.05-2.77)	0.0303
black or african american	—	—	—	—	—	—
asian	1.85(1.02-3.37)	0.0404	1.71(1.02-2.85)	0.0393	1.62(0.94-2.8)	0.0793
Sorafenib treatment						
treated	2.54(0.79-8.2)	0.107	1.8(0.79-4.08)	0.1558	0.69(0.28-1.67)	0.4065
Alcohol consumption						
yes	3.8(1.92-7.53)	4.6e-5	2.18(1.16-4.08)	0.0137	2.16(1.01-4.63)	0.0422
none	1.5(0.94-2.4)	0.0885	1.45(0.96-2.2)	0.0728	1.62(1.02-2.56)	0.0374
Hepatitis virus						
yes	1.48(0.77-2.86)	0.2383	1.6(1-2.57)	0.0501	1.54(0.92-2.6)	0.0997
none	2.22(1.41-3.51)	0.0005	1.8(1.11-2.9)	0.0158	2.26(1.26-4.07)	0.0054

HR, hazard ratio. CI, confidence interval.