

GINS2 accelerates hepatocellular carcinoma

Table S1. Primer sequences used for real-time PCR (5' to 3')

Symbol	Primer	Sequence (5'→3')
GINS2	Forward Primer	CCCTGGTTTACCCGTGGAAG
	Reverse Primer	GGGAGCAGCGACATTCT
E2F1	Forward Primer	CATCAGTACCTGGCCGAGAG
	Reverse Primer	CCCGGGATTTCACACCTTT
GAPDH	Forward Primer	TCGACAGTCAGCCGCATCTT
	Reverse Primer	AGGCGCCCAATACGACCAA
β-actin	Forward Primer	AGCGAGCATCCCCAAAGTT
	Reverse Primer	GGGCACGAAGGCTCATCATT

Table S2. Information of antibodies used in present study

Antibody	Catalog	Specificity	Company
E2F1	66515-1-Ig	Mouse monoclonal	Proteintech, China
GINS2	ab197123	Rabbit, polyclonal	Abcam, USA
PI3K	4292	Rabbit, polyclonal	CST, USA
p-PI3K	4228	Rabbit, polyclonal	CST, USA
AKT	9272	Rabbit, polyclonal	CST, USA
p-AKT	9271	Rabbit, polyclonal	CST, USA
mTOR	2972	Rabbit, polyclonal	CST, USA
p-mTOR	2971	Rabbit, polyclonal	CST, USA
CDK2	22060-AP	Rabbit, polyclonal	Proteintech, China
CDK4	11026-1-AP	Rabbit, polyclonal	Proteintech, China
CCND1	60186-1-Ig	Mouse monoclonal	Proteintech, China
VEGF	19003-1-AP	Rabbit, polyclonal	Proteintech, China
Ki-67	ab15580	Rabbit, polyclonal	Abcam, USA
β-actin	ab8227	Rabbit, polyclonal	Abcam, USA

Table S3. Sequence of primers for ChIP assay

GINS2-chip-primer	Sequence (5' to 3')
GINS2-E1-left	GGCTGTCTACCGCCTTCCTATAG
GINS2-E1-right	CAGAGCCTCACGGTCTCCTCG
GINS2-E2-left	CCCCACCCCGGCTCTTGATTAGG
GINS2-E2-right	GGCGTGACCTGGCGCTGTCAG
GINS2-E2-right	GGCGTGACCTGGCGCTGTCAG
GINS2-E3-right	ATTCGGACACTTCAGAGCAGGAG

Table S4. GINS2 expression associated with clinical pathological characteristics (logistic regression)

Characteristics	Total (N)	Odds Ratio (OR)	P value
T stage (T2 & T3 & T4 vs. T1)	368	2.019 (1.336-3.067)	< 0.001
N stage (N1 vs. N0)	256	0.924 (0.109-7.795)	0.937
M stage (M1 vs. M0)	270	0.305 (0.015-2.413)	0.306
Pathologic stage (Stage III & Stage IV vs. Stage I & Stage II)	347	1.739 (1.072-2.850)	0.026
Tumor status (With tumor vs. Tumor free)	352	1.758 (1.149-2.701)	0.010
Histologic grade (G3 & G4 vs. G1 & G2)	366	2.710 (1.750-4.241)	< 0.001
Vascular invasion (Yes vs. No)	315	1.352 (0.849-2.159)	0.204

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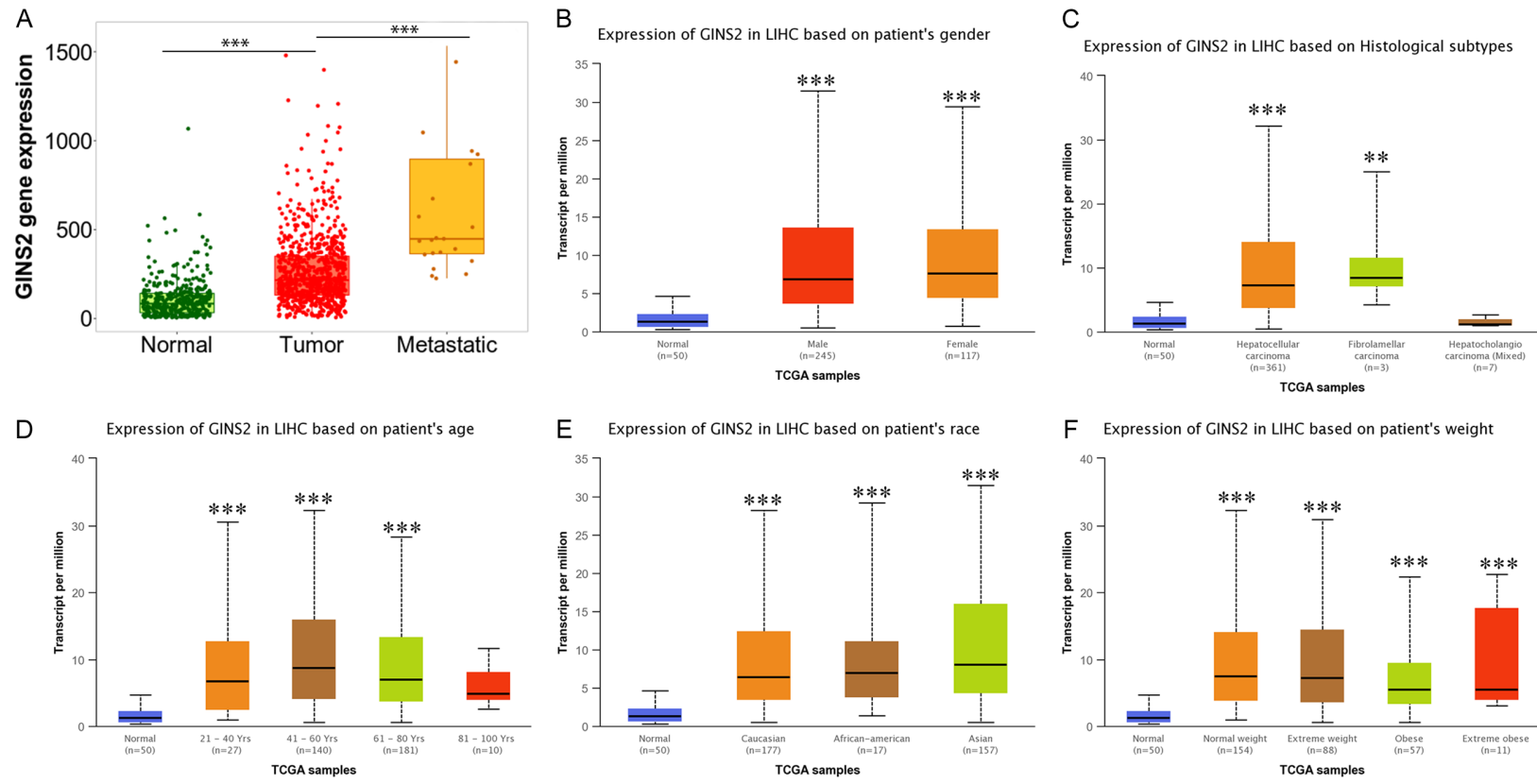


Figure S1. Box plot evaluating GINS2 expression in subgroups of LIHC samples (UALCAN and TNMplot). Upregulated mRNA of GINS2 is independent of patients' metastatic status (A), gender (B), histological subtypes (C), age (D), race (E), and weight (F). The t-test was used to estimate the significance of differences in gene expression levels between groups. *P < 0.05; **P < 0.01; ***P < 0.001.

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Table S5. Univariate and multivariate Cox's regression analysis of factors associated with OS in HCC

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	370				
≤ 60	177	Reference			
> 60	193	1.248 (0.880-1.768)	0.214		
Gender	370				
Male	249	Reference			
Female	121	1.225 (0.860-1.746)	0.260		
Pathologic stage	346				
Stage I & Stage II	256	Reference			
Stage III & Stage IV	90	2.449 (1.689-3.549)	< 0.001	1.527 (0.208-11.194)	0.677
T stage	367				
T1 & T2	274	Reference			
T3 & T4	93	2.540 (1.785-3.613)	< 0.001	1.410 (0.192-10.359)	0.736
N stage	256				
N0	252	Reference			
N1	4	2.004 (0.491-8.181)	0.333		
M stage	270				
M0	266	Reference			
M1	4	4.032 (1.267-12.831)	0.018	1.870 (0.423-8.273)	0.409
Tumor status	351				
Tumor free	201	Reference			
With tumor	150	2.361 (1.620-3.441)	< 0.001	1.960 (1.226-3.133)	0.005
Histologic grade	365				
G1 & G2	232	Reference			
G3 & G4	133	1.120 (0.781-1.606)	0.539		
Vascular invasion	314				
No	206	Reference			
Yes	108	1.348 (0.890-2.042)	0.159		
AFP (ng/ml)	277				
≤ 400	213	Reference			
> 400	64	1.056 (0.646-1.727)	0.827		
GINS2	370				
Low	185	Reference			
High	185	1.692 (1.193-2.399)	0.003	1.653 (1.023-2.671)	0.040

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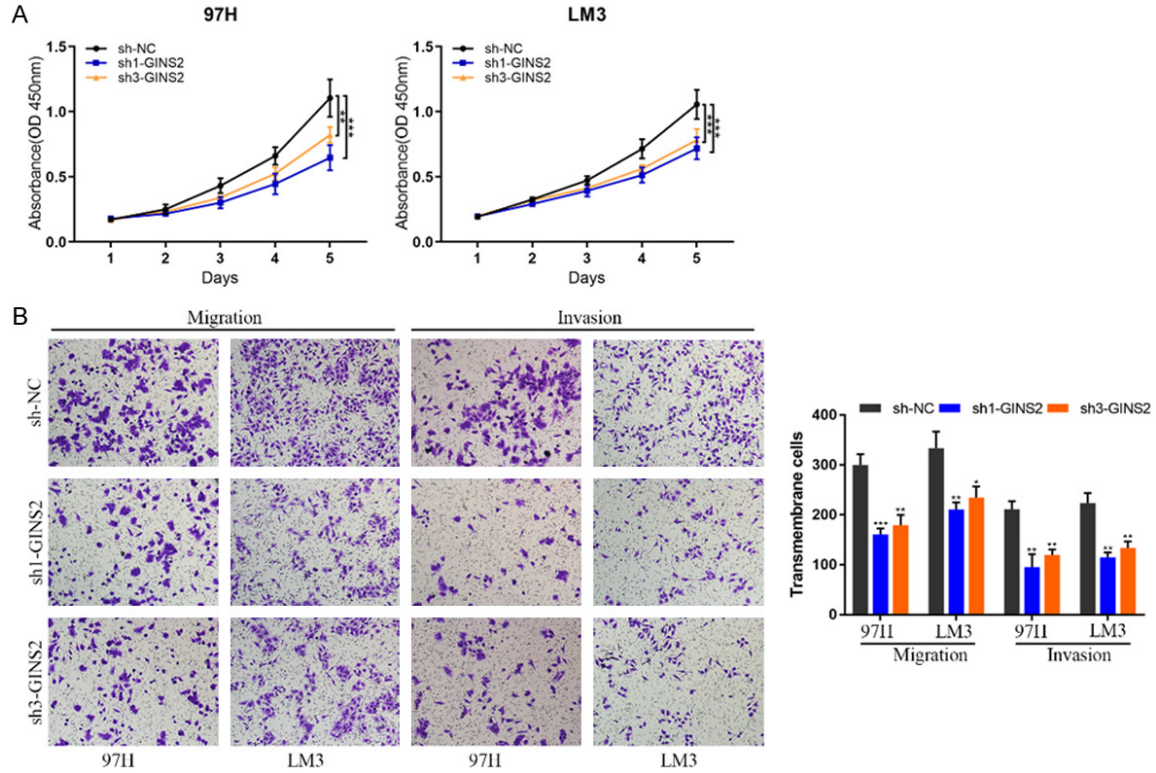


Figure S2. Knockdown of GINS2 suppresses cell proliferation, invasion, and migration of HCC cells *in vitro*. A. CCK-8 assay was performed in 97H and LM3 cells transfected with sh-NC, sh1-GINS2, or sh3-GINS2. B. Invasive or migrated cells were measured by transwell assay with or without matrigel. All data are represented as the means \pm S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

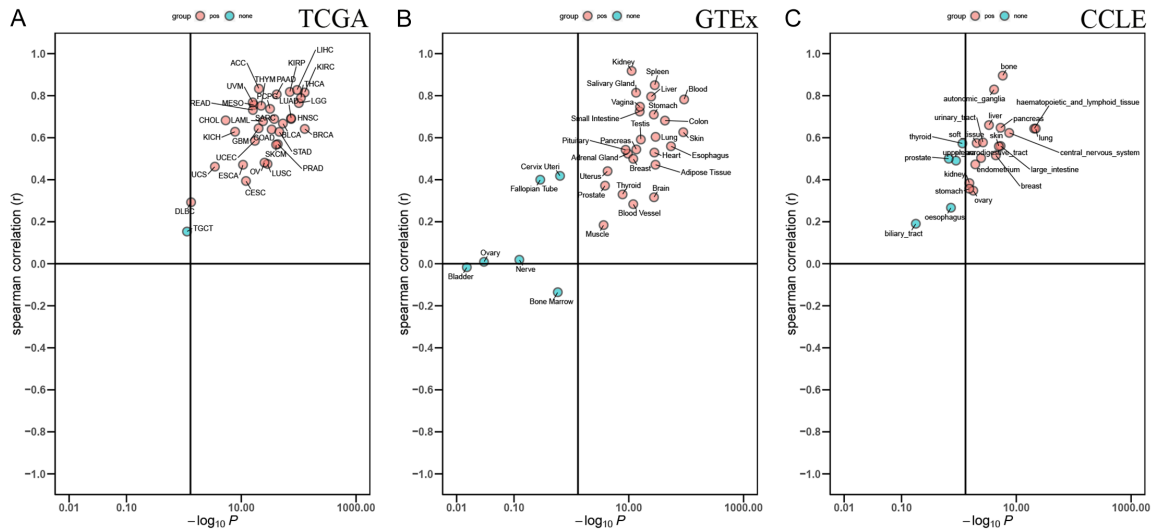


Figure S3. Correlation analysis of E2F1 and GINS2. The expression levels of E2F1 and GINS2 showed a strong positive correlation in the TCGA (A), GTEx (B), and CCLE (C) databases.

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Figure S4. Potential E2F1 binding sequences on the GINS2 promoter regions. A. Three E2F1 binding sequences on the GINS2 promoter regions according to the JASPAR website. B. Mutant sequences used in the dual-luciferase reporter assay.