Supplementary Table 1

Tumor parameters of the Lgr5 high-expression and low-expression groups.

parameters	Lgr5 expression		total	P value
	low(n=50)	high(n=50)		
Age(y)				0.2298
≥60	23	29	52	
<60	27	21	48	
Gender				0.2348
Male	41	36	77	
Female	9	14	23	
Tumor size(cm)				0.0093*
≥5	18	31	49	
<5	32	19	51	
Tumor number				0.0253*
Solitary	35	24	59	
Multiple	15	26	41	
HBV infection				0.1371
Yes	46	41	87	
No	4	9	13	
BCLC stage				0.0005*
A	43	27	70	
B+C	7	23	30	
Serum AFP (ng/mL)				0.4099
≥400	21	17	38	
<400	29	33	62	
Liver cirrhosis				0.3004
Yes	29	34	63	
No	21	16	37	
PVTT				0.0007*
Yes	6	21	27	
No	44	29	73	

* Significant results ($P < 0.05$) are given in bold.

Abbreviation: BCLC stage, Barcelona Clinic Liver Cancer stage; PVTT, portal vein tumor thrombus.

Supplementary Table 2

Tumor parameters of the PDCD5 high-expression and low-expression groups.

parameters	PDCD5 expression		total	P value
	low(n=50)	high(n=50)		
Age(y)				0.6889
≥60	29	25	52	
<60	21	25	48	
Gender				0.2348
Male	38	39	77	
Female	12	11	23	
Tumor size(cm)				0.0278*
≥5	30	19	49	
<5	20	31	51	
Tumor number				0.2033
Solitary	29	30	59	
Multiple	21	20	41	
HBV infection				0.3724
Yes	45	42	87	
No	5	8	13	
BCLC stage				0.0088*
A	29	41	70	
B+C	21	9	30	
Serum AFP (ng/mL)				0.0993
≥400	15	23	38	
<400	35	27	62	
Liver cirrhosis				0.5344
Yes	33	30	63	
No	17	20	37	
PVTT				0.0132*
Yes	19	8	27	
No	31	42	73	

* Significant results ($P < 0.05$) are given in bold.

Abbreviation: BCLC stage, Barcelona Clinic Liver Cancer stage; PVTT, portal vein tumor thrombus.

Supplementary Table 3

Univariate and multivariate analysis with a Cox proportional hazard regression model for overall survival.

variable	Univariate Analysis			Multivariate Analysis		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**
Age	0.925	0.604-1.418	0.722	-	-	-
Gender	0.693	0.424-1.135	0.145	-	-	-
Tumor size	1.480	0.962-2.279	0.075	-	-	-
Tumor number	1.251	0.810-1.933	0.313	-	-	-
HBV infection	0.830	0.450-1.529	0.549	-	-	-
Serum AFP	0.920	0.601-1.408	0.700	-	-	-
Liver cirrhosis	1.164	0.750-1.807	0.498	-	-	-
PVTT	1.973	1.203-3.229	0.007*	0.342	0.099-1.180	0.090
BCLC stage	2.244	1.388-3.627	0.001*	4.439	1.296-15.207	0.018*
Lgr5 expression	2.053	1.323-3.187	0.001*	1.733	1.094-2.746	0.019*
PDCD5 expression	0.490	0.315-0.762	0.002*	0.600	0.378-0.952	0.030*

* Significant results ($P < 0.05$) are given in bold.

Abbreviations: HR, hazard risk ratio; CI, confidence interval

Supplementary Table 4

List of the antibodies used in this study.

Antibody name	Source	dilutions
Lgr5	Abcam (ab75850)	WB:1/1000;
Lgr5	Abcam (ab75732)	ICH:1/30
PDCD5	Abcam(ab83958)	WB:1/1000;
GAPDH	Proteintech (60004-1-Ig)	WB:1/5000
anti-Flag	Sigma-Aldrich (F1804)	WB:1/1000;
anti-C-Myc	Abcam (ab32072)	WB:1/1000;
E-cadherin	Abcam (ab1416)	WB:1/50
N-cadherin	Abcam (ab18203)	WB:1/600
Vimentin	Abcam (ab92547)	WB:1/2000
Bax	Abcam (ab32503)	WB:1/2000
Bcl2	Abcam (ab32124)	WB:1/1000
Cleaved PARP1	Abcam (ab32138)	WB:1/2000
PARP1	Abcam (ab32065)	WB:1/500
GST	Abcam (ab19256)	WB:1/2000
His	Abcam (ab18184)	WB:1/2000
P53	Abcam(ab1101)	WB:1/1000;
Ki67	ABcam(ab15580)	ICH:1/100

Supplementary Table 5

List of the primers used in this study.

Target gene	Sequence
LGR5	F: CGGGAAACGCTCTGACATACAT
	R: TGAAACAGCTTGGGGGCACATA
PDCD5	F: AAAGCACAGGGAAGCAGAAA
	R: TTGTCCATATCTTGCCATCTG
GAPDH	F: ACGGATTTGGTCGTATTGGGC
	R: CTCGCTCCTGGAAGATGGTGAT
E-cadherin	F: CGAGAGCTACACGTTCACGG
	R: GGGTGTCGAGGGAAAAATAGG
N-cadherin	F: AGCTCCATTCCGACTTAGACA
	R: CAGCCTGAGCACGAAGAGTG
Vimentin	F: AGGCAAAGCAGGAGTCCACTGA

Supplementary Table 6

List of the shRNA and cDNA sequences used in this study.

Name	Sequence
shRNA-Lgr5 #1	CCTAGAGACTTTAGATTTA
shRNA-Lgr5 #2	GACAATGCGTTAACAGAAA
shRNA-Lgr5 #3	CCTCACAAATAACTGAATT
Lgr5	ATGGACACCTCCCGGCTCGGTGTGCTCCTGTCCTTGCCTGTGCTGC TGCAGCTGGCGACCGGGGGCAGCTCTCCCAGGTCTGGTGTGTTGC TGAGGGGCTGCCCCACACACTGTCATTGCGAGCCCGACGGCAGGA TGTTGCTCAGGGTGGACTGCTCCGACCTGGGGCTCTCGGAGCTGC CTTCCAACCTCAGCGTCTTCACCTCCTACCTAGACCTCAGTATGAA CAACATCAGTCAGCTGCTCCCGAATCCCCTGCCCAGTCTCCACTTC CTGGAGGAGTTACGTCTTGCGGGAAACGCTCTGACATACATTCCC AAGGGAGCATTCACTGGCCTTTACAGTCTTAAAGTTCTTATGCTGC AGAATAATCAGCTAAGACACGTACCCACAGAAGCTTTGCAGAATT TGCGAAGCCTTCAATCCCTGCGTCTGGATGCTAACCACATCAGCTA TGTGCCCCCAAGCTGTTTCAGTGGCCTGCATTCCCTGAGGCACCTG TGGCTGGATGACAATGCGTTAACAGAAATCCCCGTCCAGGCTTTT AGAAGTTTATCGGCATTGCAAGCCATGACCTTGGCCCTGAACAAA ATACACCACATACCAGACTATGCCTTTGGAAACCTCTCCAGCTGG GTAGTTCTACATCTCCATAACAATAGAATCCACTCCCTGGGAAAG AAATGCTTTGATGGGCTCCACAGCCTAGAGACTTTAGATTTAAATT ACAATAACCTTGATGAATTCCCCACTGCAATTAGGACACTCTCCA ACCTTAAAGAACTAGGATTTCATAGCAACAATATCAGGTCGATAC CTGAGAAAGCATTTGTAGGCAACCCTTCTCTTATTACAATACATTT CTATGACAATCCCATCCAATTTGTTGGGAGATCTGCTTTTCAACAT TTACCTGAACTAAGAACACTGACTCTGAATGGTGCCTCACAAATA ACTGAATTTCCTGATTTAACTGGAACTGCAAACCTGGAGAGTCTG ACTTTAACTGGAGCACAGATCTCATCTCTTCCTCAAACCGTCTGCA

	ATCAGTTACCTAATCTCCAAGTGCTAGATCTGTCTTACAACCTATT AGAAGATTTACCCAGTTTTTCAGTCTGCCAAAAGCTTCAGAAAATT GACCTAAGACATAATGAAATCTACGAAATTAAAGTTGACACTTTC CAGCAGTTGCTTAGCCTCCGATCGCTGAATTTGGCTTGGAACAAA ATTGCTATTATTCACCCCAATGCATTTTCCACTTTGCCATCCCTAAT AAAGCTGGACCTATCGTCCAACCTCCTGTCGTCTTTTCCTATAACT GGGTTACATGGTTTAACTCACTTAAAATTAACAGGAAATCATGCC TTACAGAGCTTGATATCATCTGAAAACTTTCCAGAACTCAAGGTTA TAGAAATGCCTTATGCTTACCAGTGCTGTGCATTTGGAGTGTGTGA GAATGCCTATAAGATTTCTAATCAATGGAATAAAGGTGACAACAG CAGTATGGACGACCTTCATAAGAAAGATGCTGGAATGTTTCAGGC TCAAGATGAACGTGACCTTGAAGATTTCCTGCTTGACTTTGAGGA AGACCTGAAAGCCCTTCATTCAGTGCAGTGTTCACCTTCCCCAGGC CCCTTCAAACCCTGTGAACACCTGCTTGATGGCTGGCTGATCAGA ATTGGAGTGTGGACCATAGCAGTTCTGGCACTTACTTGTAATGCTT TGGTGACTTCAACAGTTTTCAGATCCCCTCTGTACATTTCCCCCAT TAAACTGTTAATTGGGGTCATCGCAGCAGTGAACATGCTCACGGG AGTCTCCAGTGCCGTGCTGGCTGGTGTGGATGCGTTCACTTTTGGC AGCTTTGCACGACATGGTGCCTGGTGGGAGAATGGGGTTGGTTGC CATGTCATTGGTTTTTTGTCCATTTTTGCTTCAGAATCATCTGTTTT CCTGCTTACTCTGGCAGCCCTGGAGCGTGGGTTCTCTGTGAAATAT TCTGCAAAATTTGAAACGAAAGCTCCATTTTCTAGCCTGAAAGTA ATCATTTTGCTCTGTGCCCTGCTGGCCTTGACCATGGCCGCAGTTC CCCTGCTGGGTGGCAGCAAGTATGGCGCCTCCCCTCTCTGCCTGCC TTTGCCTTTTGGGGAGCCCAGCACCATGGGCTACATGGTCGCTCTC ATCTTGCTCAATTCCCTTTGCTTCCTCATGATGACCATTGCCTACAC CAAGCTCTACTGCAATTTGGACAAGGGAGACCTGGAGAATATTTG GGACTGCTCTATGGTAAAACACATTGCCCTGTTGCTCTTCACCAAC TGCATCCTAAACTGCCCTGTGGCTTTCTTGTCCTTCTCCTCTTTAAT AAACCTTACATTTATCAGTCCTGAAGTAATTAAGTTTATCCTTCTG GTGGTAGTCCCACTTCCTGCATGTCTCAATCCCCTTCTCTACATCTT GTTCAATCCTCACTTTAAGGAGGATCTGGTGAGCCTGAGAAAGCA AACCTACGTCTGGACAAGATCAAAACACCCAAGCTTGATGTCAAT TAACTCTGATGATGTCGAAAAACAGTCCTGTGACTCAACTCAAGC CTTGGTAACCTTTACCAGCTCCAGCATCACTTATGACCTGCCTCCC AGTTCCGTGCCATCACCAGCTTATCCAGTGACTGAGAGCTGCCATC TTTCCTCTGTGGCATTTGTCCCATGTCTCTAA

$Lgr5^{\Delta 22\text{-}561}$	ATGGACACCTCCCGGCTCGGTGTGCTCCTGTCCTTGCCTGTGCTGC TGCAGCTGGCGACCGGGGGCAGCTCTCCCAGGTCTGGTGTGTTGC TGAGGGGCTGCCCCACACACTGTCATTGCGAGCCCGACGGCAGGA TGTTGCTCAGGGTGGACTGCTCCGACCTGGGGCTCTCGGAGCTGC CTTCCAACCTCAGCGTCTTCACCTCCTACCTAGACCTCAGTATGAA CAACATCAGTCAGCTGCTCCCGAATCCCCTGCCCAGTCTCCGCTTC CTGGAGGAGTTACGTCTTGCGGGAAACGCTCTGACATACATTCCC AAGGGAGCATTCACTGGCCTTTACAGTCTTAAAGTTCTTATGCTGC AGAATAATCAGCTAAGACACGTACCCACAGAAGCTCTGCAGAATT TGCGAAGCCTTCAATCCCTGCGTCTGGATGCTAACCACATCAGCTA TGTGCCCCCAAGCTGTTTCAGTGGCCTGCATTCCCTGAGGCACCTG TGGCTGGATGACAATGCGTTAACAGAAATCCCCGTCCAGGCTTTT AGAAGTTTATCGGCATTGCAAGCCATGACCTTGGCCCTGAACAAA ATACACCACATACCAGACTATGCCTTTGGAAACCTCTCCAGCTTGG TAGTTCTACATCTCCATAACAATAGAATCCACTCCCTGGGAAAGA AATGCTTTGATGGGCTCCACAGCCTAGAGACTTTAGATTTAAATTA CAATAACCTTGATGAATTCCCCACTGCAATTAGGACACTCTCCAAC CTTAAAGAACTAGGATTTCATAGCAACAATATCAGGTCGATACCT GAGAAAGCATTTGTAGGCAACCCTTCTCTTATTACAATACATTTCT ATGACAATCCCATCCAGTTTGTTGGGAGATCTGCTTTTCAACATTT ACCTGAACTAAGAACACTGACTCTGAATGGTGCCTCACAAATAAC TGAATTTCCTGATTTAACTGGAACTGCAAACCTGGAGAGTCTGACT TTAACTGGAGCACAGATCTCATCTCTTCCTCAAACCGTCTGCAATC AGTTACCTAATCTCCAAGTGCTAGATCTGTCTTACAACCTATTAGA AGATTTACCCAGTTTTTCAGTCTGCCAAAAGCTTCAGAAAATTGAC CTAAGACATAATGAAATCTACGAAATTAAAGTTGACACTTTCCAG CAGTTGCTTAGCCTCCGATCGCTGAATTTGGCTTGGAACAAAATTG CTATTATTCACCCCAATGCATTTTCCACTTTGCCATCCCTAATAAA GCTGGACCTATCGTCCAACCTCCTGTCGTCTTTTCCTATAACTGGG TTACATGGTTTAACTCACTTAAAATTAACAGGAAATCATGCCTTAC AGAGCTTGATATCATCTGAAAACTTTCCAGAACTCAAGGTTATAG AAATGCCTTATGCTTACCAGTGCTGTGCATTTGGAGTGTGTGAGAA TGCCTATAAGATTTCTAATCAATGGAATAAAGGTGACAACAGCAG TATGGACGACCTTCATAAGAAAGATGCTGGAATGTTTCAGGCTCA AGATGAACGTGACCTTGAAGATTTCCTGCTTGACTTTGAGGAAGA CCTGAAAGCCCTTCATTCAGTGCAGTGTTCACCTTCCCCAGGCCCC TTCAAACCCTGTGAACACCTGCTTGATGGCTGGCTGATCAGA

$Lgr5^{\Delta 56}$ 2-823	ATTGGAGTGTGGACCATAGCAGTTCTGGCACTTACTTGTAATGCTT TGGTGACTTCAACAGTTTTCAGATCCCCTCTGTACATTTCCCCCAT TAAACTGTTAATTGGGGTCATCGCAGCAGTGAACATGCTCACGGG AGTCTCCAGTGCCGTGCTGGCTGGTGTGGATGCGTTCACTTTTGGC AGCTTTGCACGACATGGTGCCTGGTGGGAGAATGGGGTTGGTTGC CATGTCATTGGTTTTTTGTCCATTTTTGCTTCAGAATCATCTGTTTT CCTGCTTACTCTGGCAGCCCTGGAGCGTGGGTTCTCTGTGAAATAT TCTGCAAAATTTGAAACGAAAGCTCCATTTTCTAGCCTGAAAGTA ATCATTTTGCTCTGTGCCCTGCTGGCCTTGACCATGGCCGCAGTTC CCCTGCTGGGTGGCAGCAAGTATGGCGCCTCCCCTCTCTGCCTGCC TTTGCCTTTTGGGGAGCCCAGCACCATGGGCTACATGGTCGCTCTC ATCTTGCTCAATTCCCTTTGCTTCCTCATGATGACCATTGCCTACAC CAAGCTCTACTGCAATTTGGACAAGGGAGACCTGGAGAATATTTG GGACTGCTCTATGGTAAAACACATTGCCCTGTTGCTCTTCACCAAC TGCATCCTAAACTGCCCTGTGGCTTTCTTGTCCTTCTCCTCTTTAAT AAACCTTACATTTATCAGTCCTGAAGTAATTAAGTTTATCCTTCTG GTGGTAGTCCCACTTCCTGCATGTCTCAATCCCCTTCTCTACATCTT GTTC
$Lgr5^{\Delta 62}$ 4-907	AATCCTCACTTTAAGGAGGATCTGGTGAGCCTGAGAAAGCAAACC TACGTCTGGACAAGATCAAAACACCCAAGCTTGATGTCAATTAAC TCTGATGATGTCGAAAAACAGTCCTGTGACTCAACTCAAGCCTTG GTAACCTTTACCAGCTCCAGCATCACTTATGACCTGCCTCCCAGTT CCGTGCCATCACCAGCTTATCCAGTGACTGAGAGCTGCCATCTTTC CTCTGTGGCATTTGTCCCATGTCTC
PDCD5	ATGGCGGACGAGGAGCTTGAGGCGCTGAGGAGACAGAGGCTGGC CGAGCTGCAGGCCAAACACGGGGATCCTGGTGATGCGGCCCAACA GGAAGCAAAGCACAGGGAAGCAGAAATGAGAAACAGTATCTTAG CCCAAGTTCTGGATCAGTCGGCCCGGGCCAGGTTAAGTAACTTAG CACTTGTAAAGCCTGAAAAAACTAAAGCAGTAGAGAATTACCTTA TACAGATGGCAAGATATGGACAACTAAGTGAGAAGGTATCAGAA CAAGGTTTAATAGAAATCCTTAAAAAAGTAAGCCAACAAACAGA AAAGACAACAACAGTGAAATTCAACAGAAGAAAAGTAATGGACT CTGATGAAGATGACGATTATTGA

S-Figure legends

Supplementary Figure 1. A. CCK8 cell proliferation assay in SK-Hep1 and HepG2 cells transfected with Lgr5. **B.** The cell viability of HCC cell lines and the L02 cell line treated with Dox (1 μg/ml) for 48 hours was detected by CCK8. **C.** HCC cell lines and a normal liver cell line (L02) were treated with different concentrations of Dox for 24 hours, and their expression of PDCD5 was detected by RT-qPCR. **D.** HCC cell lines and a normal liver cell line (L02) were treated with Dox (1 μg/ml) and their expression of PDCD5 was detected by qPCR once a day for 3 days. **E.** Western blotting assay was used to analyze the expression of Lgr5 and PDCD5 in HCC cell lines and a normal liver cell line (L02) treated with Dox (1 μg/ml) once a day for 6 days (the ratio of molecule/GAPDH was indicated below). *p<0.05, **p<0.01, ***p<0.001, t-test.

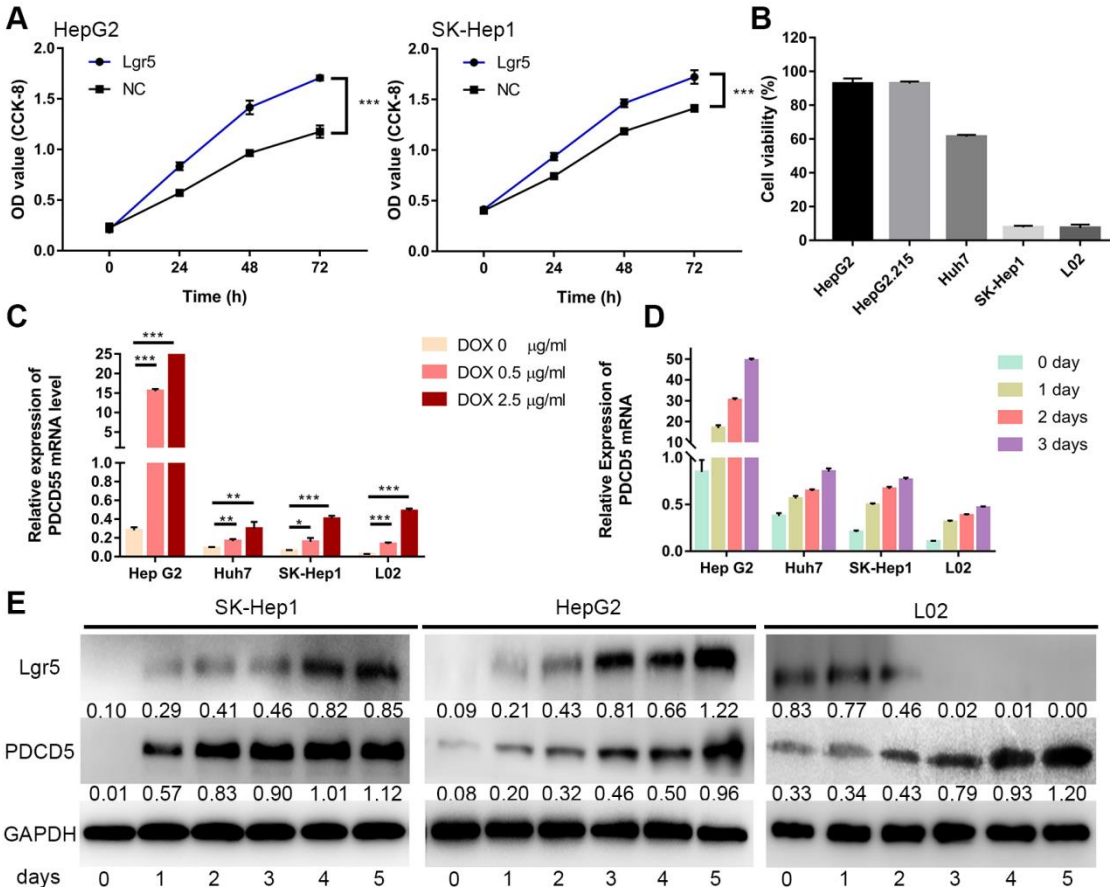
Supplementary Figure 2. A. The relative mRNA levels of PDCD5 between tumor and adjacent nontumor tissues from 100 patients with HCC were analyzed by RT-qPCR. **B.** The protein levels of Lgr5 in 4 paired HCC specimens were detected by western blotting (the ratio of molecule/GAPDH was indicated below). **C.** Representative IHC staining images of PDCD5 in HCC tissue and adjacent nontumor tissue. Scale bars: 50 μm. **D.** Semiquantitative data showed that PDCD5 expression in HCC tumor tissue compared with adjacent tissue. **e.** Kaplan-Meier survival analysis demonstrated the correlation between PDCD5 and overall survival. *p<0.05, **p<0.01, ***p<0.001, t-test.

Supplementary Figure 3. A. Schematic diagram showed the Flag-tagged full-length and deletion mutants of Lgr5. **B.** GST-PDCD5 and His-Lgr5 expressed in the *E. coli* system were detected by western blotting with an anti-GST antibody. **C.** GST-PDCD5 and His-Lgr5 expressed in the *E. coli* system were detected by western blotting with an anti-His antibody.

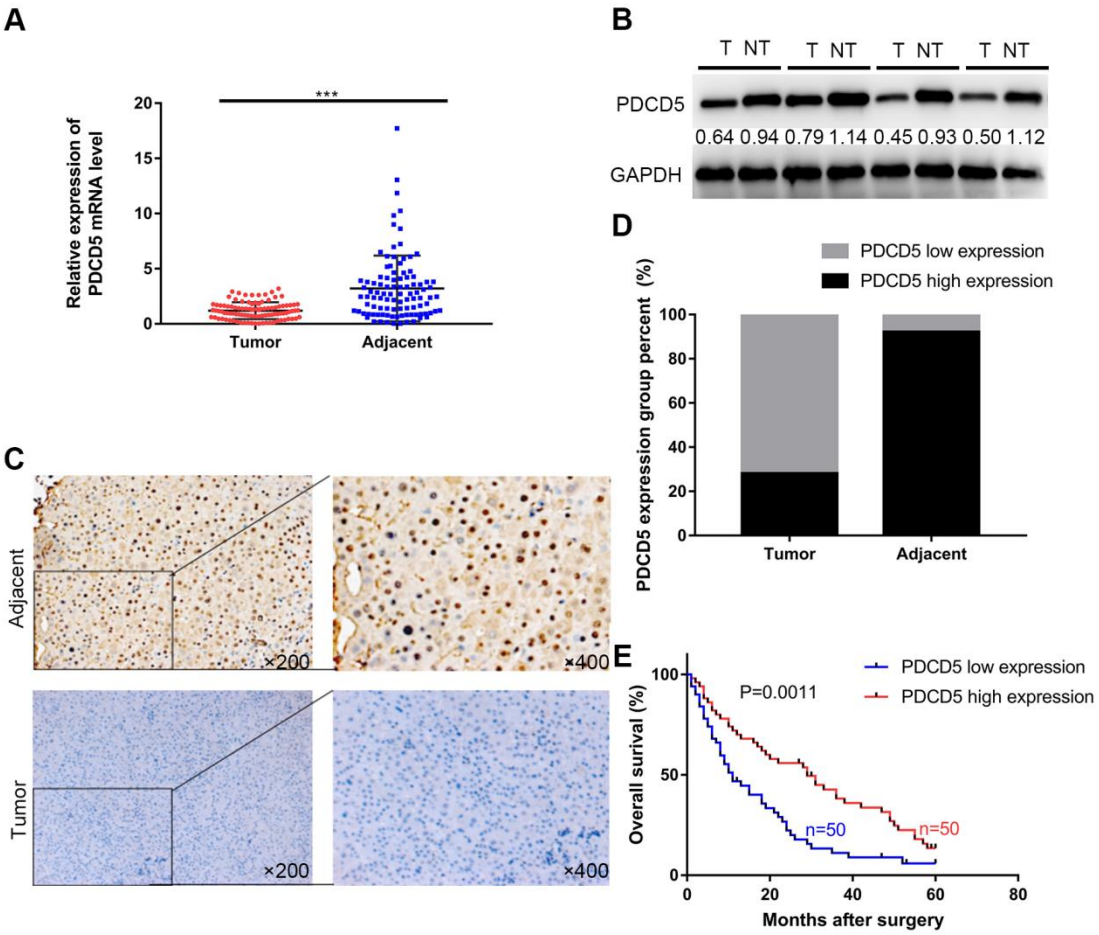
Supplementary Figure 4. A, B. SK-Hep1 and HepG2 cells were transfected with overexpression vectors for Lgr5 and PDCD5. The mRNA levels of Lgr5 and PDCD5 were analyzed by RT-qPCR and western blotting. **C.** The protein levels of Lgr5 and PDCD5 in HCC cells transfected with Lgr5 and PDCD5 were analyzed by western blotting. **D, E.** HepG2 cells were transfected with shRNA plasmids against Lgr5 and nonspecific shRNA as a control. The mRNA levels and protein expression of Lgr5 and PDCD5 were analyzed by RT-qPCR and western blotting. *p<0.05, **p<0.01, ***p<0.001, t-test.

Supplementary Figure 5. A. SK-Hep1 cells were transfected with PDCD5. Flow cytometric analysis was used to detect apoptosis in cells treated with Dox (0 μg/ml or 1 μg/ml). **B.** The cells previously described were analyzed by western blotting with antibodies against apoptosis-related proteins. *p<0.05, **p<0.01, ***p<0.001, t-test.

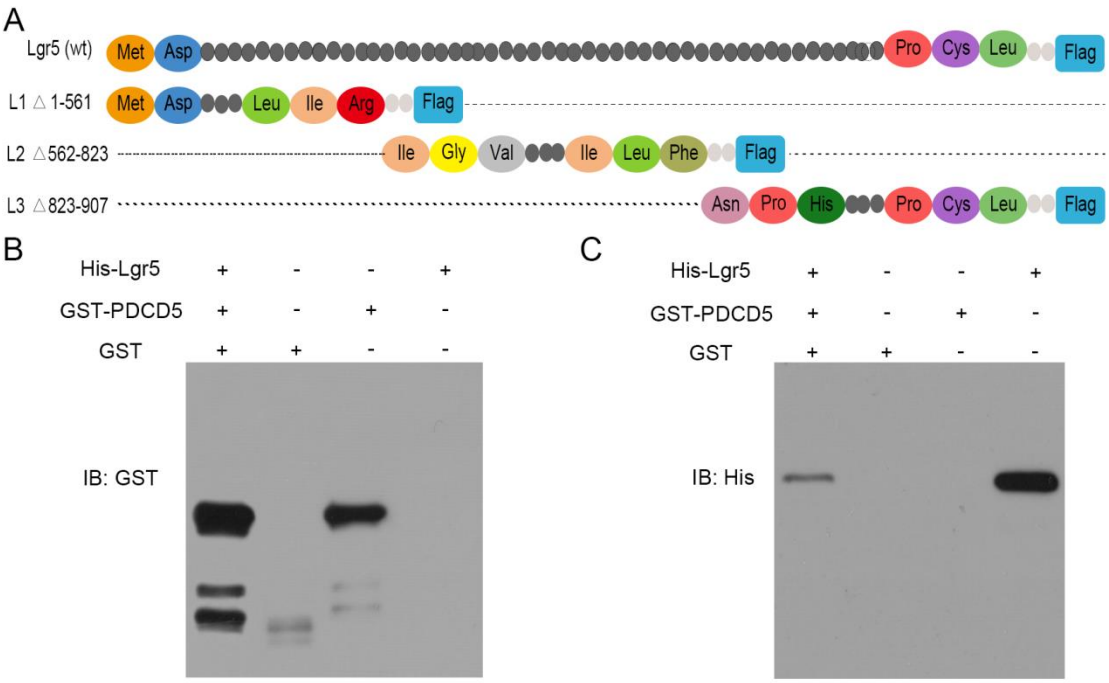
Supplementary Figure 1



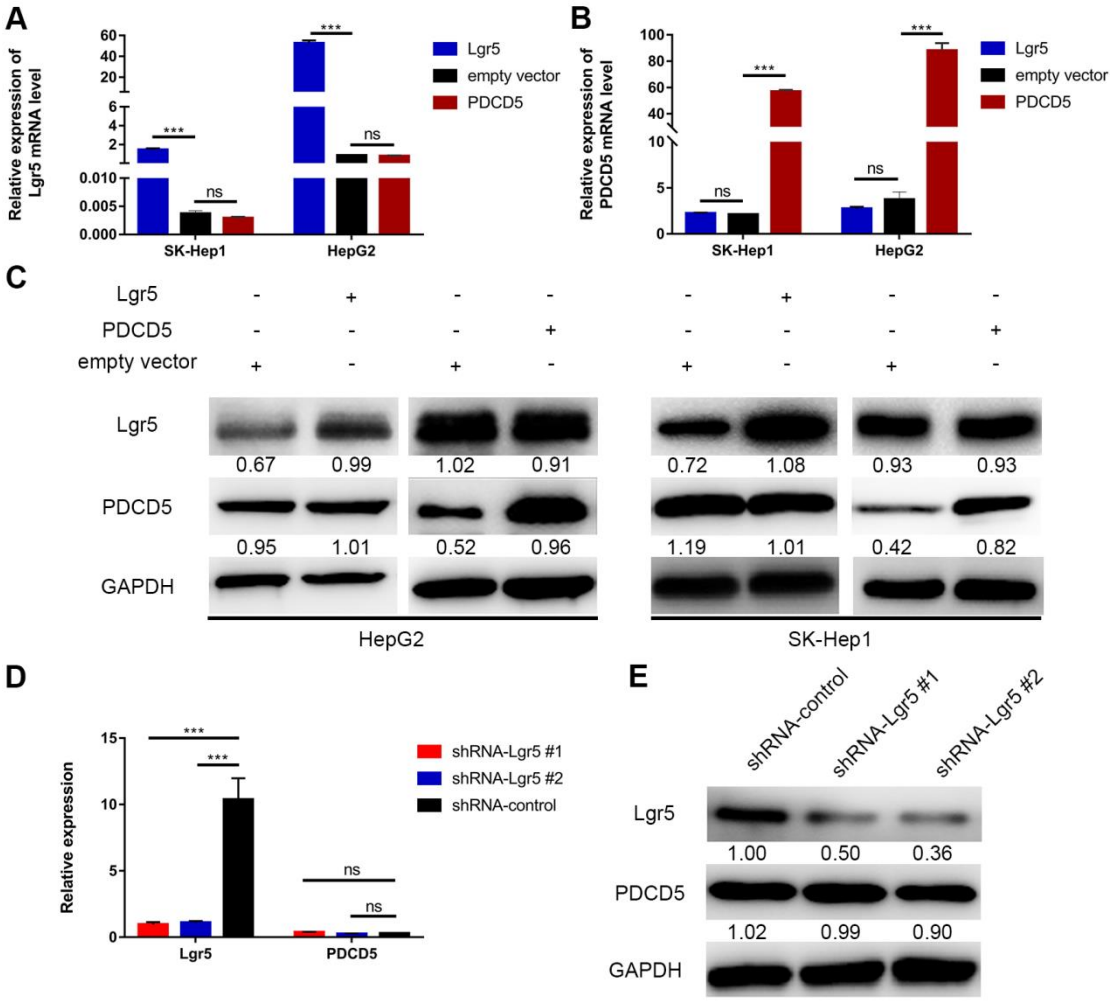
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5