Figure S1. A, Numb expression in HCC cells. Indicated HCC cell lines were grown in accordance with their specific requirements. Numb expression was detected by western blot in total cell lysate. B, Numb knockdown does not induce apoptosis. Indicated HCC cells were transfected with Numb siRNA. Expression of Numb and cleavage of poly ADP ribose polymerase (PARP) and caspase 3 were detected by western blot. C, Numb knockdown does not induce cell death. Indicated HCC cells were transfected with Numb siRNA, stained with acridine orange and ethidium bromide, and photographed using fluorescence microscopy. Live cells were stained green and dead cells were red/yellow. D, Numb knockdown reduces colony formation of Huh1 cells in soft agarose. The number of colonies was evaluated 4 weeks after the cells were seeded and stained with crystal violet. E, colony number (mean ± SD) for Numb knockdown and control Huh1 cells in panel D was calculated and plotted. F & G, Numb knockdown promotes cell motility in SK-Hep1 cells. Cell motility was assessed by wound healing assay in control and Numb knockdown SK-Hep1 cells at 24h and 48h, and graphed. H & I, Numb knockdown reduces cell motility in Huh1 cells. Assay was performed as above. J. Numb-PRR^{S} overexpression inhibits cell invasion. Huh7 cells were stably transfected with either Numb-PRR^{L} or Numb-PRR^{S} under control of the Tet-on promoter. Endogenous Numb was knocked down with an siRNA that targets the 3'-UTR which is absent in the exogenous Numb sequence. Expression of exogenous Numb was induced by doxycycline. Real-time cell invasion was measured as described in Methods. Overexpression of exogenous Numb and lack of expression of endogenous Numb was examined by western blotting. K. Invasiveness of Huh1 cells was evaluated as above. Cells were transfected with control (Sigma, red trace in upper panel and lane 1 of bottom panel) or two distinct Numb siRNAs: Sigma, siRNA 5422 sequence CAGACUUUGUCUCCUGAUU[dT][dT] (green

trace upper panel and lane 2 lower panel), and 5423 sequence GAUAGUCGUUGGUUCAUCA[dT][dT] (purple trace upper panel and lane 3 lower panel). One-day post-transfection, cells were serum-starved for 16 h before being plated in upper chambers containing serum-free media. The lower chambers contained media with 10% serum (serum was used as attractant), except for the negative control, which was transfected with control siRNA but whose lower chamber was serum-free. Impedance signals from invasive cells passing through the membrane between the chambers were recorded in real time to provide continuous quantitative assessment of invasion (upper panel). The assay was performed in a humidified incubator at 37°C and 5 % CO2. Huh1 cells transfected similarly were grown in a 6-well plate and harvested to examine Numb knockdown by western blot, shown in the lower panel.

Figure S2. A, Rbfox2 knockdown increased, while SRPK2 knockdown decreased, the ratio of Numb PRR^L and PRR^S mRNA in Huh7 cells. Huh7 cells were transfected with control siRNA or siRNAs specific to either Rbfox2 or SRPK2. Total RNA was extracted from the cells, and Numb PRR^L and PRR^S mRNA levels were determined by qRT-PCR. The ratio of the 2 mRNAs was calculated and graphed. B, Hsp90 inhibition or SRPK2 knockdown in Huh7 cells decreased the ratio of Numb PRR^L and PRR^S. Huh7 cells were treated with the Hsp90 inhibitor STA9090 or transfected with SRPK2 siRNA. Ratio of Numb PRR^L and PRR^S was determined as described in (A). C, HEK293A cells were transfected with siRNAs for SRPK2, Rbm6, or Rbfox2. Indicated proteins in cell lysate were detected by western blotting. Numb PRR^L and PRR^S levels were quantified by using ImageJ, and the ratios are presented as shown. D, UOK171 cells were transfected with

siRNAs for SRPK2, Rbfox2, or SF2. Protein expression was similarly evaluated as in (C). E, STA9090 altered Numb splicing similar to SRPK2 knockdown, but Hsp90 inhibition did not affect SRPK2 protein stability. UOK171 cells were treated with 1 mM STA9090 for indicated hours (H), and SRPK2 and Numb proteins in total cell lysate were detected by western blot. F, Hsp90 inhibition did not affect the nuclear localization but changed the intranuclear distribution of phosphorylated SR domain-containing splicing factors. Huh7 cells were treated with STA9090 or the vehicle DMSO, and stained with the monoclonal antibody Mab104 which is specific to phosphorylated SR domain-containing proteins. Cells were photographed with a confocal microscope. G, UOK171 cells were transfected with or w/o Hsp90 siRNA, stained with Hsp90 and SRPK2 antibodies, and photographed with confocal microscopy. DAPI staining identifies nuclei. H, HEK293A cells were transfected with Hsp90 plasmid or siRNA targeting the 3'UTR that is not present in the plasmid. PRR^L and PRR^S mRNA levels were quantitated as in Figure 4A. I, Numb PRR^L-expressing HCC cells are more sensitive to Hsp90 inhibitor than PRR^S expressing cells. Indicated HCC cells were treated with STA9090 at different concentrations for 72 hours. Cell growth was evaluated by MTT assay. J, Expression of Numb PRR isoforms is not correlated with HCC sensitivity to selected chemotherapeutic agents (ADM, adriamycin; 5-FU, 5-fluorouracil; AZD8055, mTOR inhibitor). HCC cells were treated for 72 hrs at indicated concentrations. Cell growth was measured by MTT assay.

Figure S1

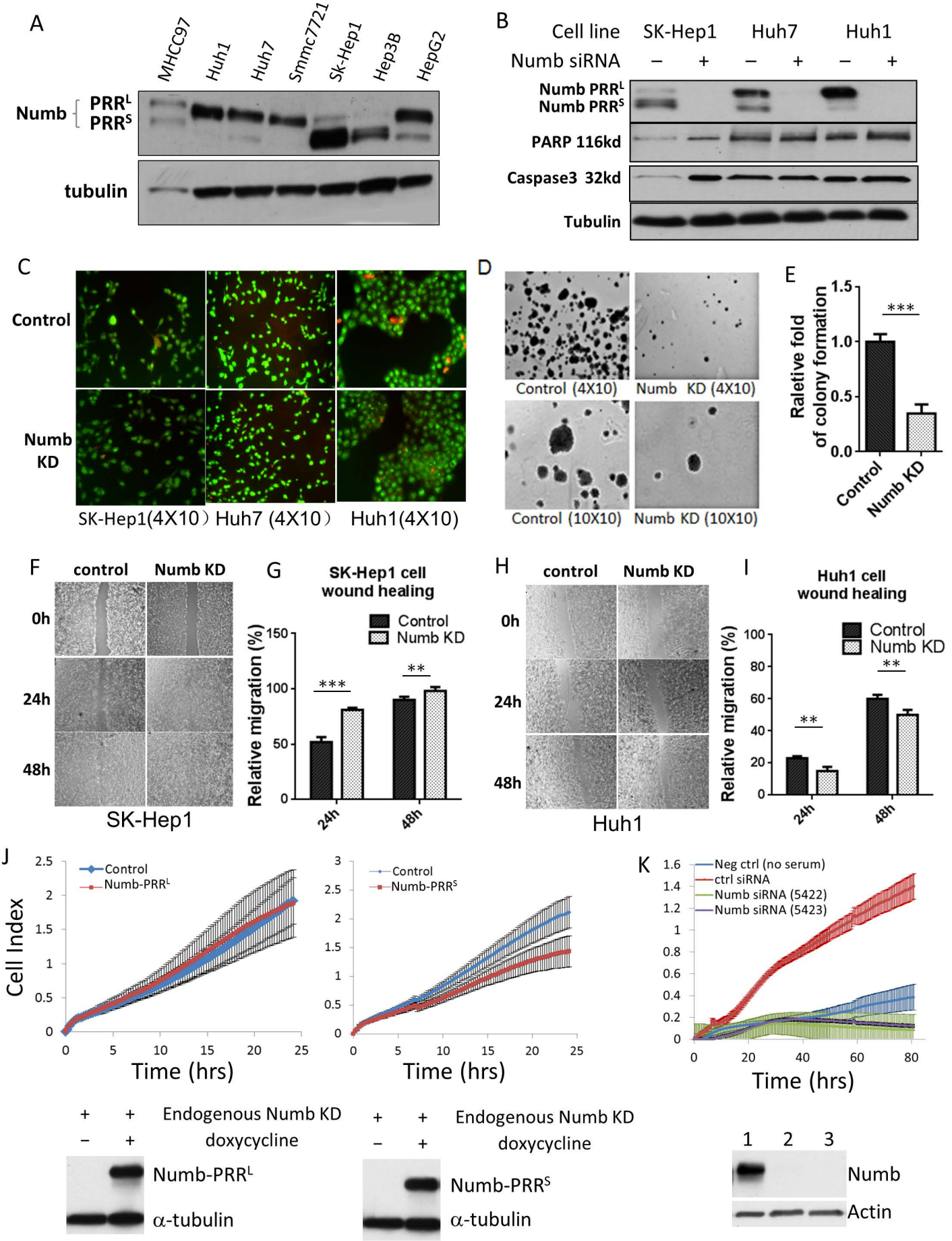
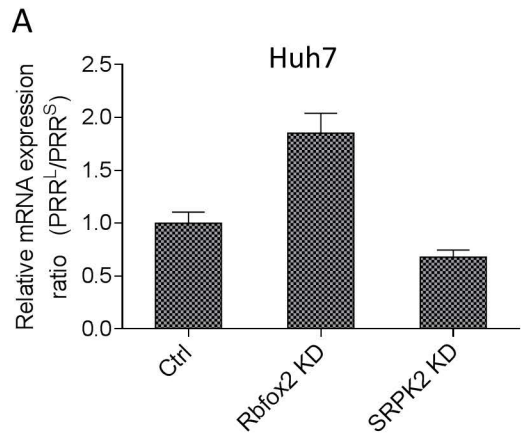
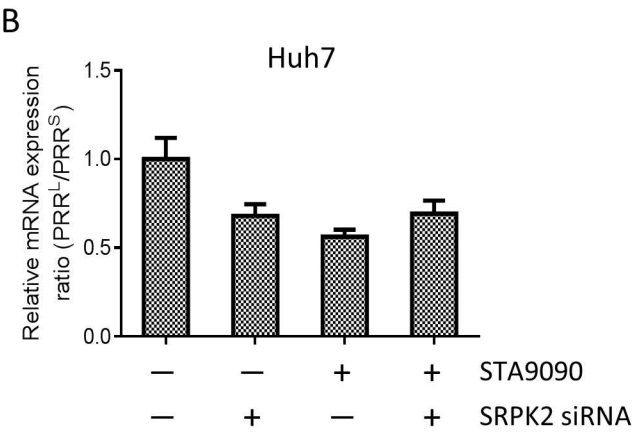
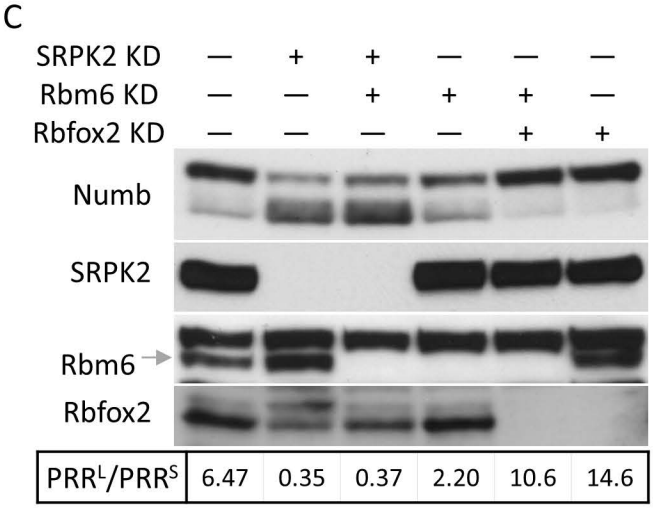

A
MHCC97
Huh1
Huh7
Smmc7721
Sk-Hep1
Hep3B
HepG2
Numb
PRR^L
PRR^S
tubulin
B
Cell line
SK-Hep1
Huh7
Huh1
Numb siRNA
– + – + – +
Numb PRR^L
Numb PRR^S
PARP 116kd
Caspase3 32kd
Tubulin
C
Control
Numb KD
SK-Hep1(4X10）
Huh7 (4X10）
Huh1(4X10)
D
Control (4X10)
Numb KD (4X10)
Control (10X10)
Numb KD (10X10)
E
Raletive fold of colony formation
1.5
1.0
0.5
0.0
***
Control
Numb KD
F
control
Numb KD
0h
24h
48h
SK-Hep1
G
SK-Hep1 cell wound healing
Control
Numb KD
Relative migration (%)
150
100
50
0
***
**
24h
48h
H
control
Numb KD
0h
24h
48h
Huh1
I
Huh1 cell wound healing
Control
Numb KD
Relative migration (%)
100
80
60
40
20
0
**
**
24h
48h
J
Control
Numb-PRR^L
Cell Index
Time (hrs)
Control
Numb-PRR^S
Time (hrs)
K
Neg ctrl (no serum)
ctrl siRNA
Numb siRNA (5422)
Numb siRNA (5423)
Time (hrs)
+ + Endogenous Numb KD
– + doxycycline
Numb-PRR^L
α-tubulin
+ + Endogenous Numb KD
– + doxycycline
Numb-PRR^S
α-tubulin
1 2 3
Numb
Actin

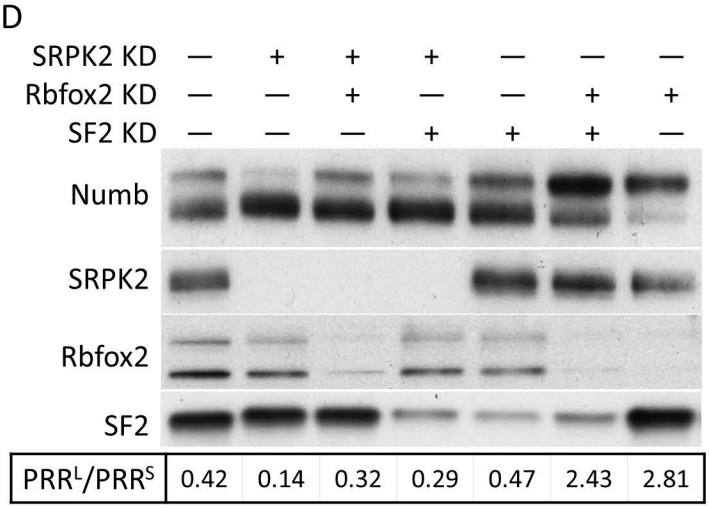
Figure S2



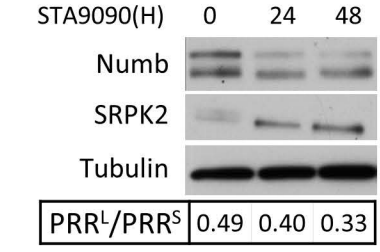




PRR^L/PRR^S	6.47	0.35	0.37	2.20	10.6	14.6



PRR^L/PRR^S	0.42	0.14	0.32	0.29	0.47	2.43	2.81



PRR^L/PRR^S	0.49	0.40	0.33

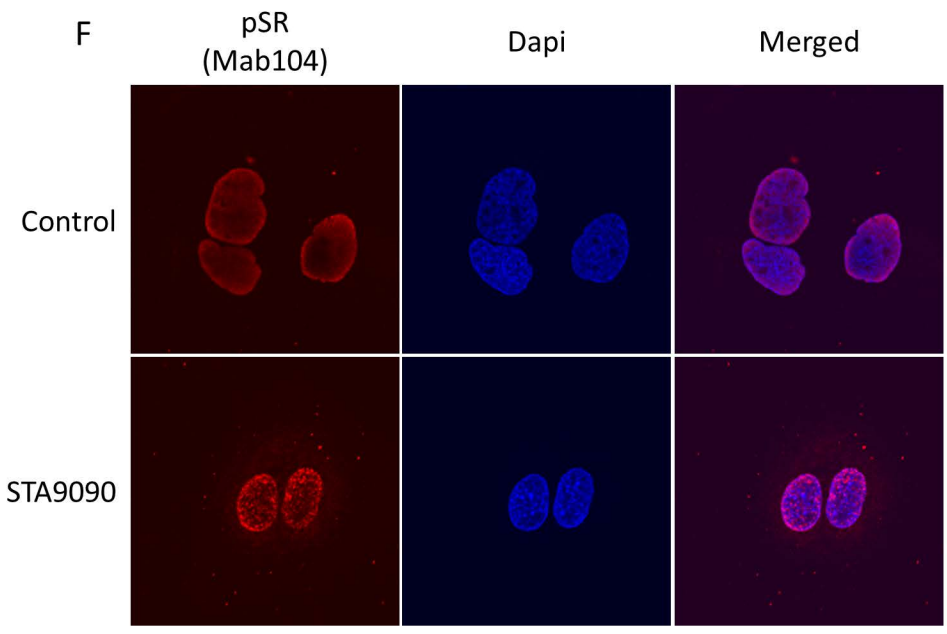
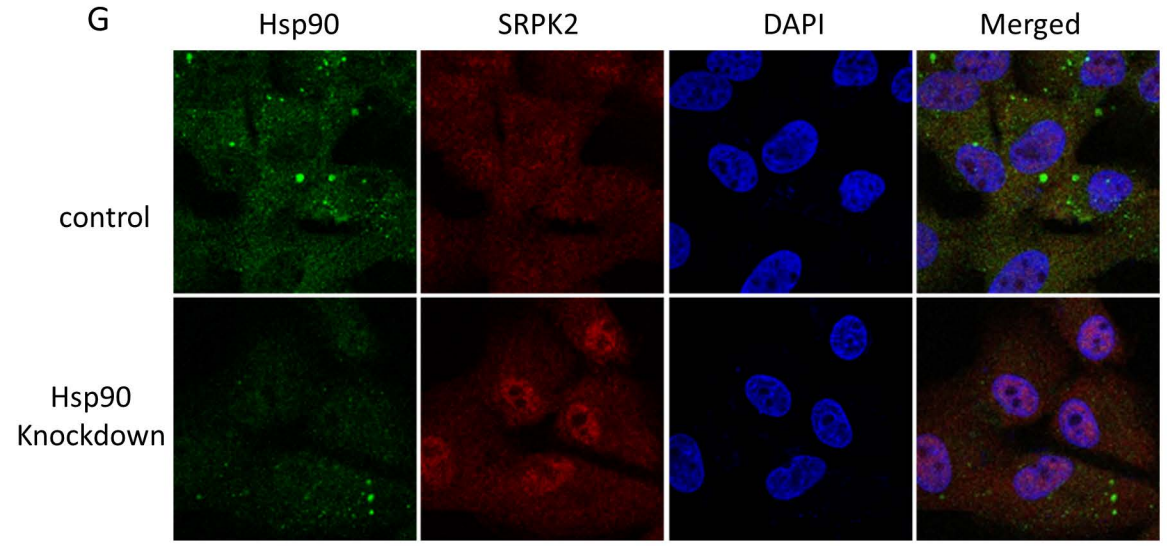
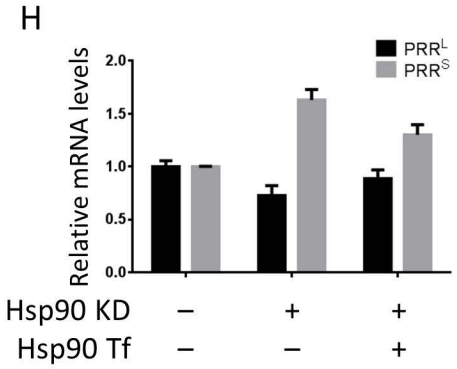
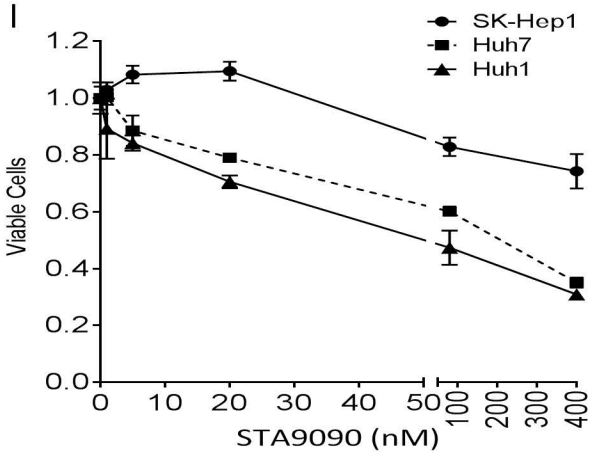


Figure S2 continued







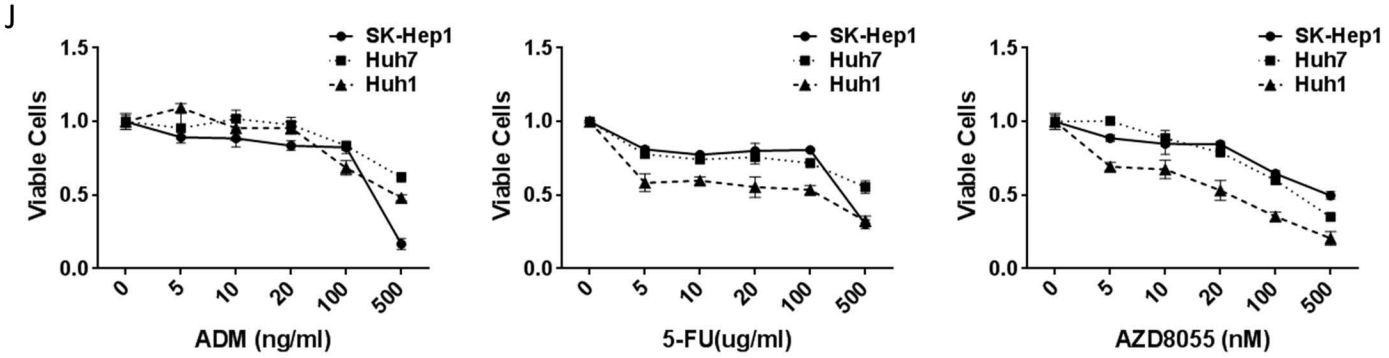


Table S1. Clinical characteristics of HCC patients and correlation with Numb PRR^{L} isoform mRNA expression

Clinical characteristics		Patients (n=23)	Low(n=12)	High(n=11)	P value
Age (y)		23	51.7	54.3	0.4902
Gender	Male	18	9	9	1.0000
	Female	5	3	2	
HBsAg	-	3	0	3	0.0932
	+	20	12	8	
Child-Pugh score	A	20	11	9	0.5901
	B	3	1	2	
AFP (ng/ml)	<=400	9	4	5	0.6802
	>400	14	8	6	
Tumor size(cm)	<=5	15	9	6	0.6641
	>5	8	3	5	
Tumor encapsulation	Complete	10	5	5	1.0000
	None	13	7	6	
Satellite nodule	No	3	2	1	1.0000
	Yes	20	10	10	
BCLC staging	0/A	19	10	9	1.0000
	B/C	4	2	2	
TNM staging	I/II	18	9	9	1.0000
	III/IV	5	3	2	
Recurrence	Yes	10	3	8	**0.0391**
	No	13	9	3	

Table S2. primers for qRT-PCR

Gene name	R/L	Sequence 5’ to 3’
c-myc	R	CACCGAGTCGTAGTCGAGGT
	L	TTTCGGGTAGTGGAAAACCA
Oct4	R	GGTTCTCGATACTGGTTCGC
	L	GTGGAGGAAGCTGACAACAA
UGT2B7	R	CACAGGAAGCTGACAACAA
	L	TTTTGTCCTACCATAAGGGCTTT
ALB	R	TCAGCCATTTCACCATAGGTT
	L	TGCTGATGAGTCAGCTGAAAA

Table S3. Contingency table relating PRR^L level to median overall survival (OS) and time to recurrence (TTR).

PRR^L level	OS（months）	TTR（months）
High	39.7	27.4
Low	54.9	54.9

PRR^L levels in individual tumor specimens were determined by qRT-PCR.