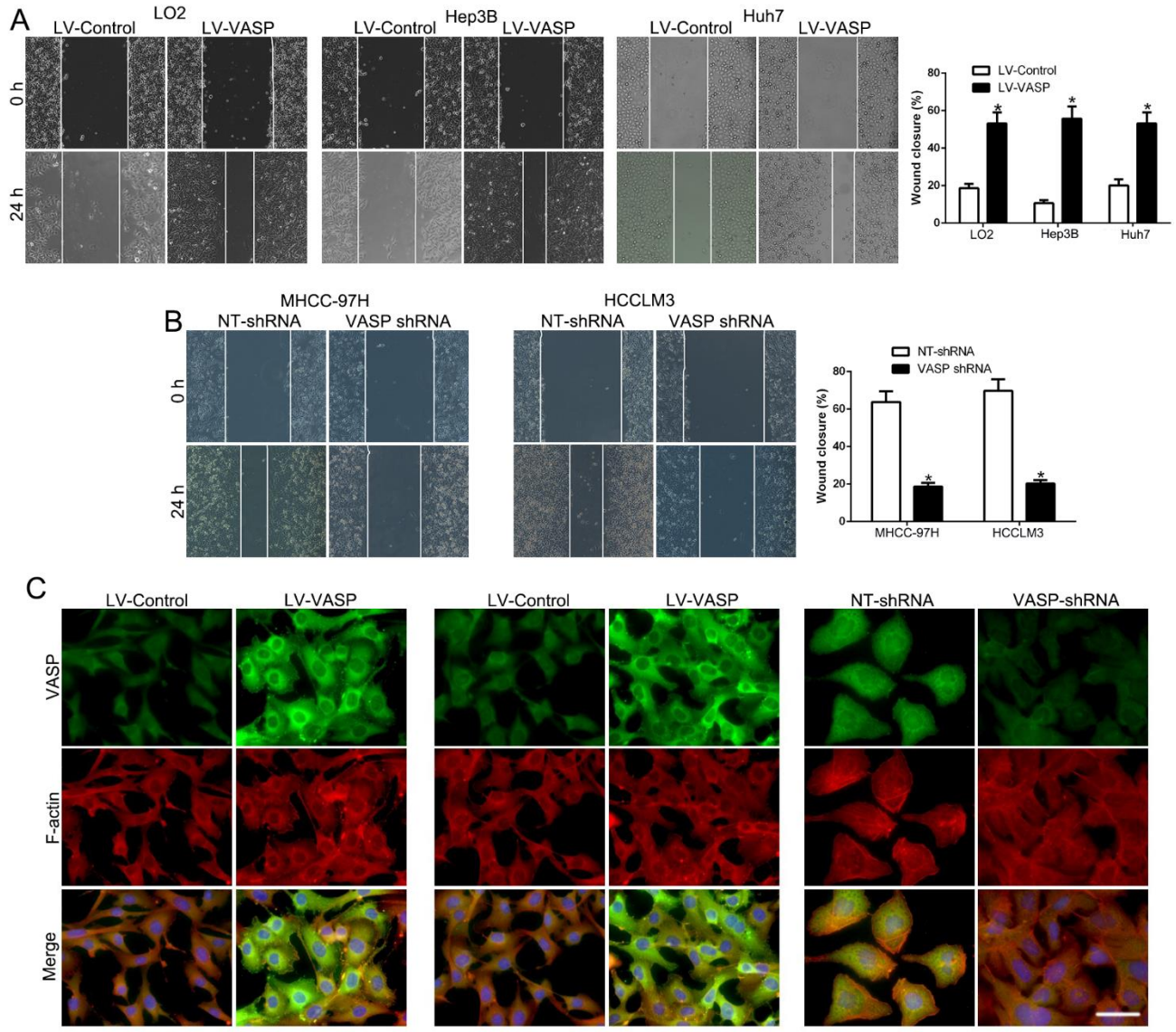
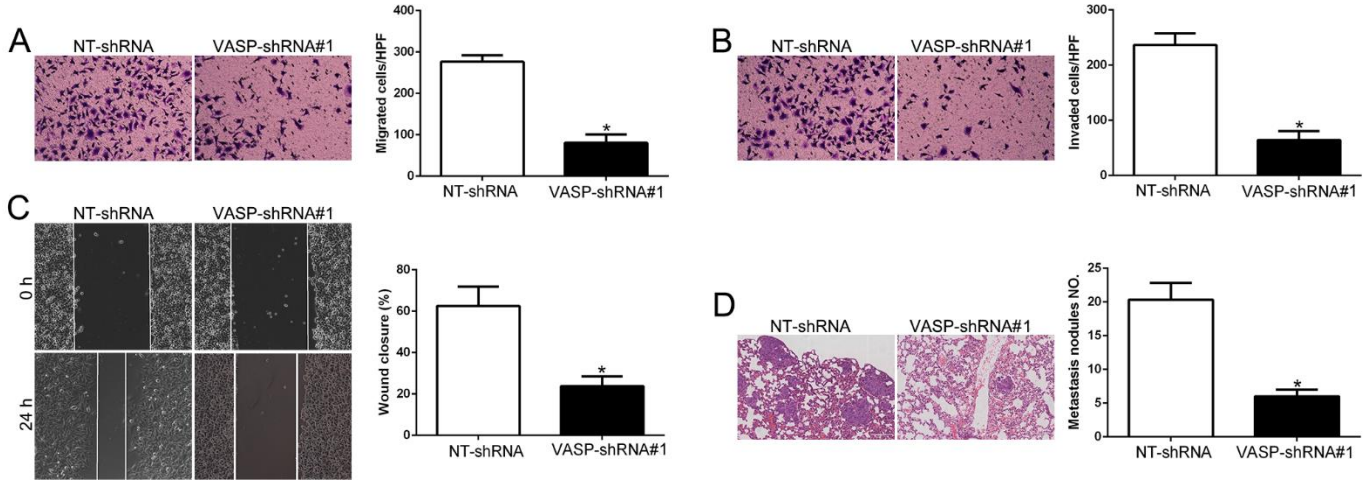
Supplementary Figure 1



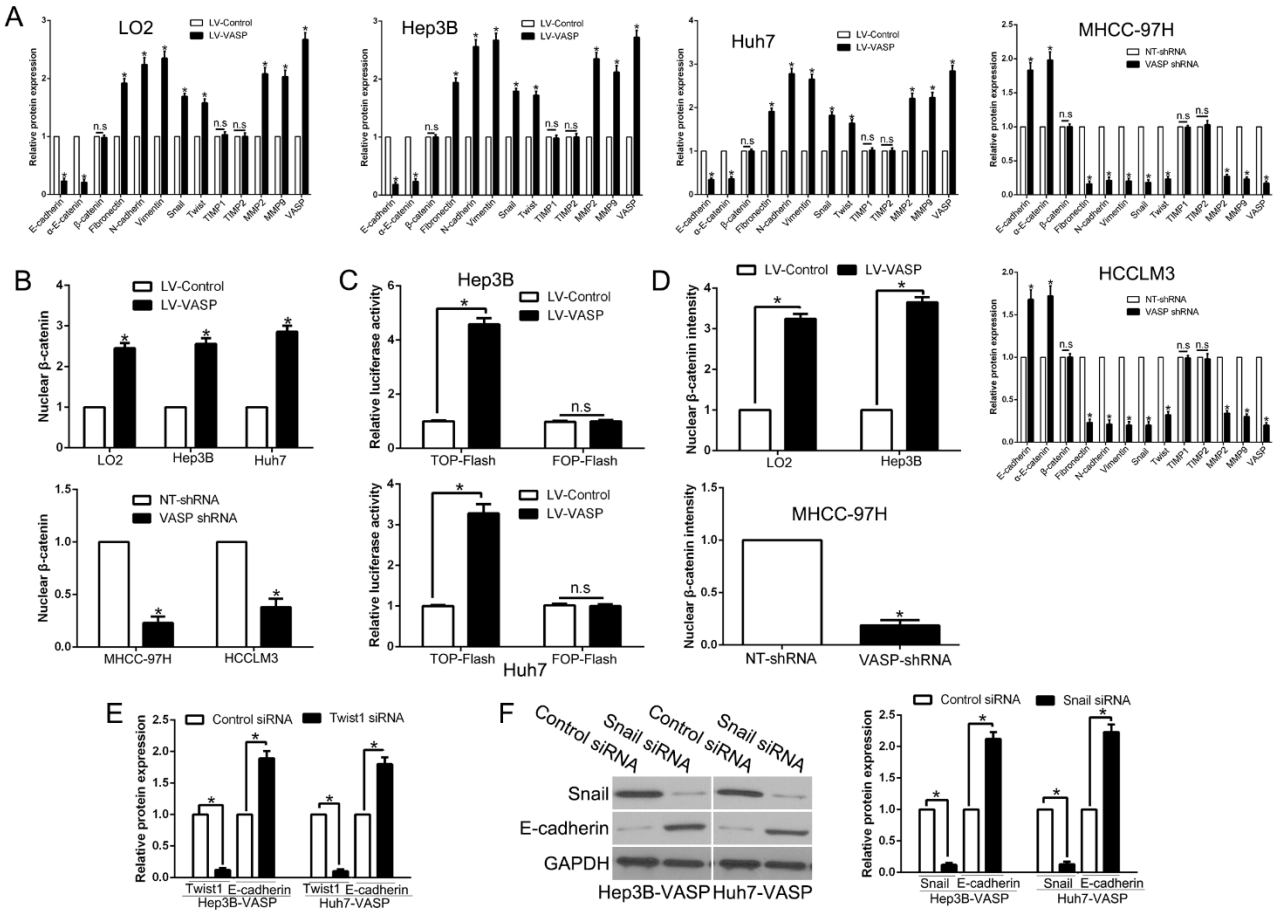
Supplementary Figure 1. **VASP promotes HCC cell migration. (A)** Wound-healing assays comparing the migration of cells transfected with VASP for 24 h. **(B)** Wound-healing assays comparing the migration of cells transfected with VASP shRNA for 24 h. **(C)** Confocal images of LO2 and Hep3B (left) transfected with LV-VASP and MHCC-97H (right) cells transfected with VASP shRNA stained for F-actin (red) and 4′,6-diamidino-2-phenylindole (DAPI; blue) (magnification, 400×). The merged images showing yellow immunofluorescence indicated colocalization.

Supplementary Figure 2



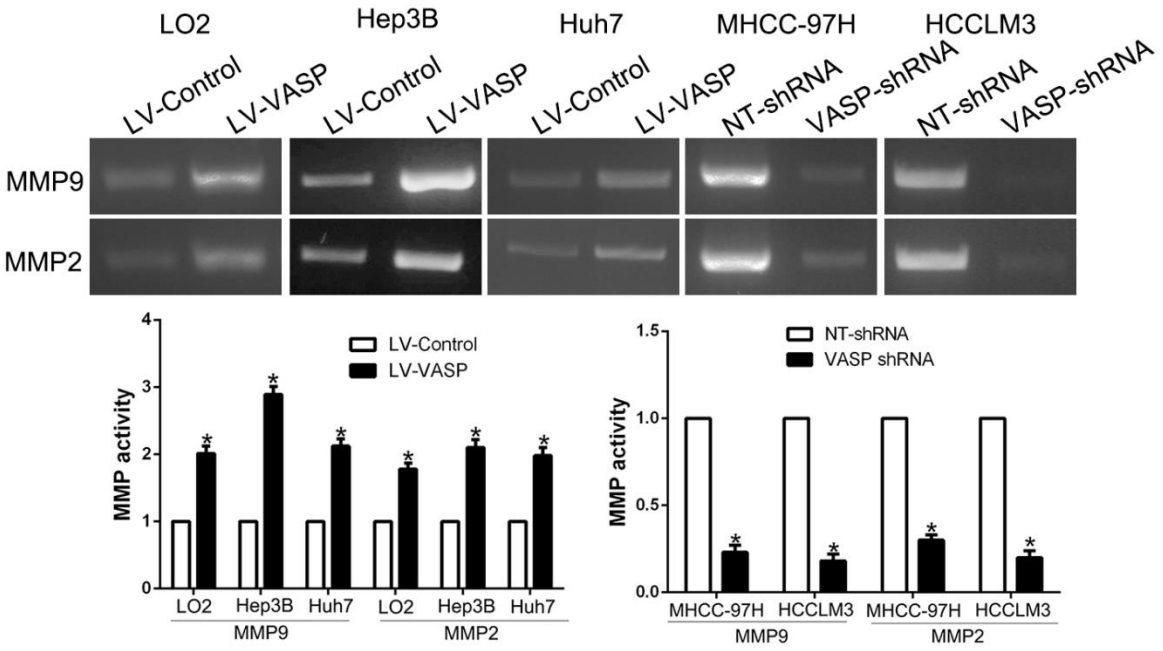
Supplementary Figure 2. VASP knockdown inhibits HCC cell migration and invasion. Transwell assays was used to show that VASP knockdown inhibits HCCLM3 cell migration **(A)** and invasion **(B)**. **(C)** Wound healing assays showed that VASP knockdown suppresses HCCLM3 cell migration. **(D)** Representative hematoxylin and eosin (H&E) images of metastatic nodules from the mouse lung tissue sections of the HCCLM3 NT-shRNA group (left) and HCCLM3 VASP-shRNA#1 group (right). *P<0.05.

Supplementary Figure 3



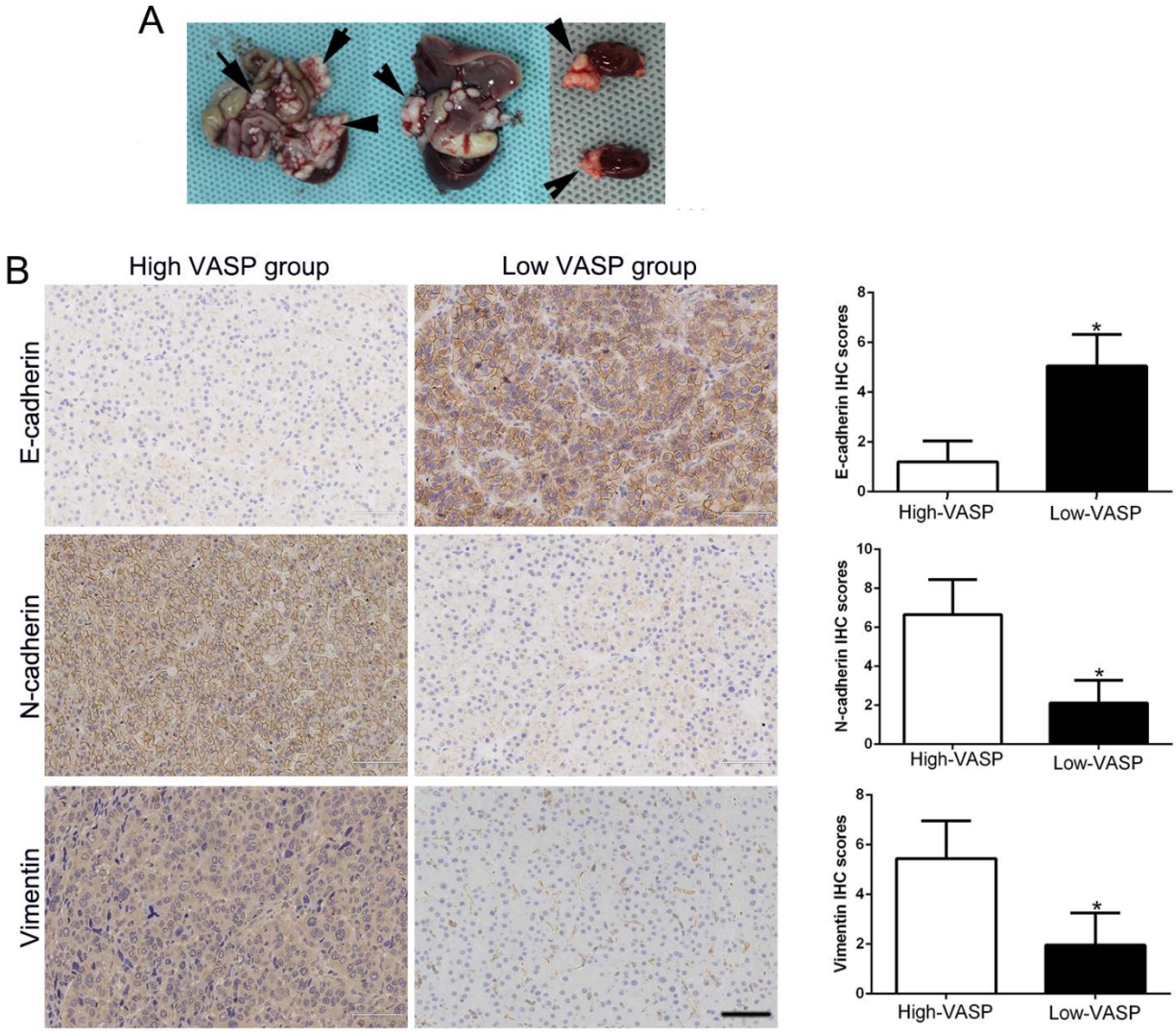
Supplementary Figure 3. VASP induces EMT and β-catenin nuclear translocation. (A) Quantitative analysis of the expression of EMT-associated markers after VASP alteration. **(B)** Quantitative analysis of levels of β-catenin in nuclear and cytosolic fraction detected by Western blotting. **(C)** Top/Fop flash assays detected the β-catenin transcription activity. **(D)** Quantitative analysis of levels of β-catenin in nuclear and cytosolic fraction detected by IF. **(E)** Quantitative analysis of levels in Hep3B LV-VASP or Huh7 LV-VASP cells transfected with siRNA targeting Twist1 (Twist1 siRNA) or scrambled siRNA (Control siRNA). **(F)** Immunoblots were performed to detect expression of indicated proteins in Hep3B LV-VASP or Huh7 LV-VASP cells transfected with siRNA targeting Snail (Snail siRNA) or scrambled siRNA (Control siRNA).

Supplementary Figure 4



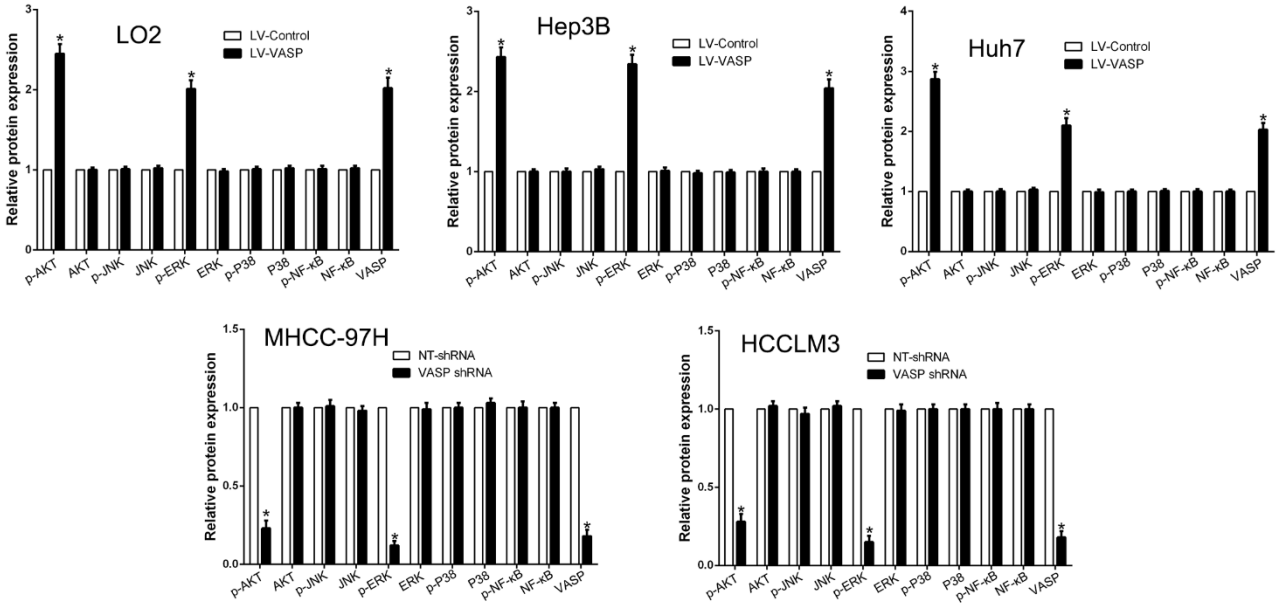
Supplementary Figure 4. Zymography detects activities of MMP2 and MMP9. The bottom histogram shows the average optical density from three repeated experiments.

Supplementary Figure 5



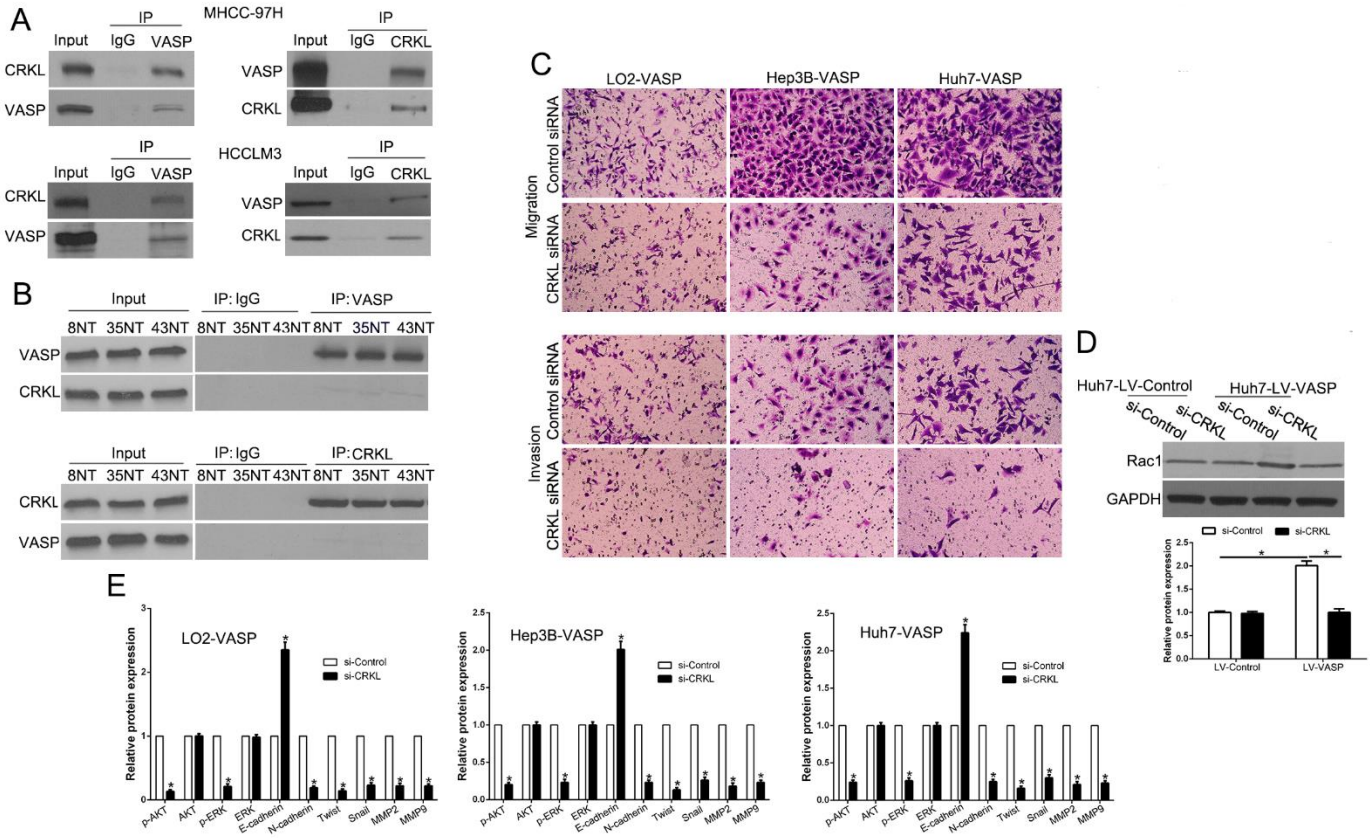
Supplementary Figure 5. VASP promotes metastasis of HCC cells in vivo and positively correlates with EMT markers in HCC tissues. (A) Representative images of metastatic tumors of liver, kidney, and mesentery are shown. **(B)** Immunohistochemical analysis of E-cadherin, N-cadherin, and Vimentin in HCC samples. In cases of low VASP expression (right), there is strong E-cadherin and no detectable N-cadherin and Vimentin protein expression in the same tissue section. In contrast, in the case of high VASP expression (left), there is no detectable E-cadherin and strong N-cadherin and Vimentin protein expression. Values are depicted as Mean ± SEM; *P < 0.05 by t-test.

Supplementary Figure 6



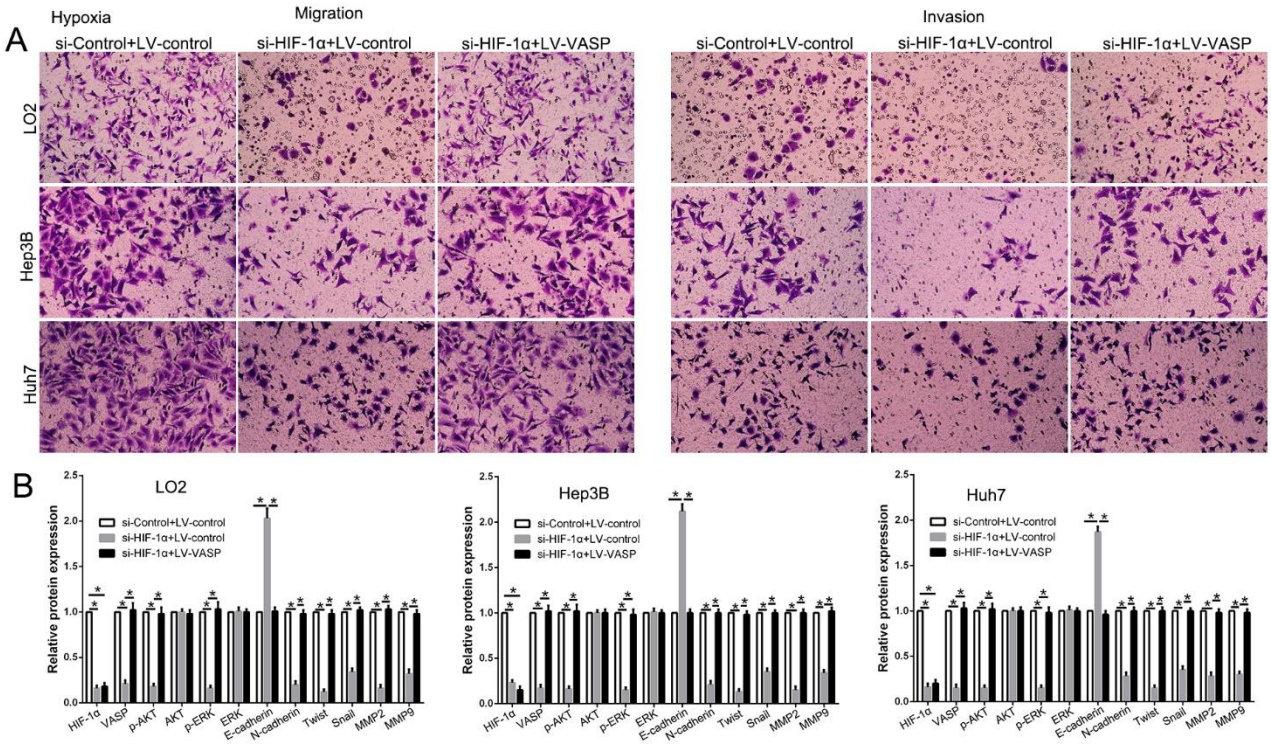
Supplementary Figure 6. Western blotting detects estimated amounts of VASP in AKT, ERK, JNK, MAPK, and NF-κB pathways in indicated cells

Supplementary Figure 7



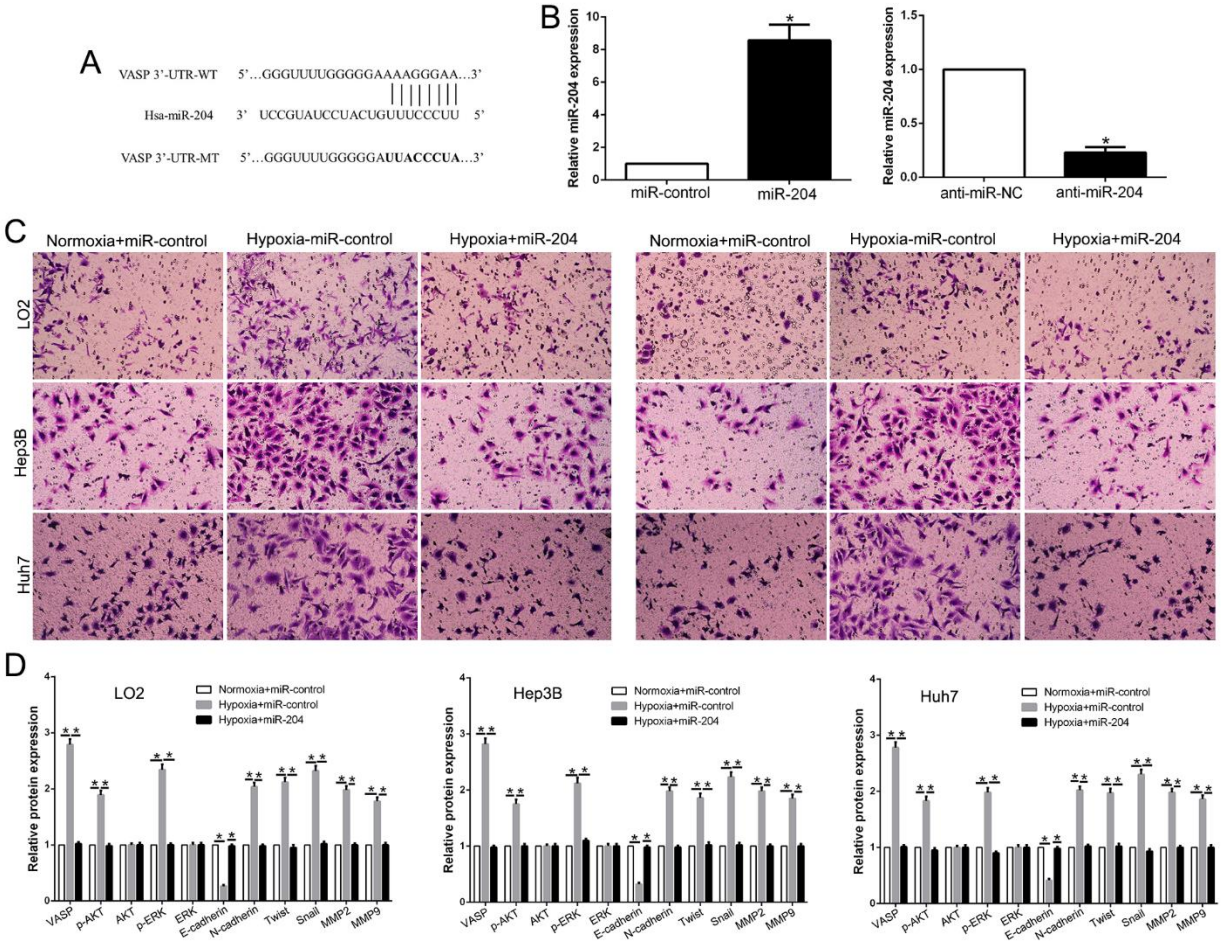
Supplementary Figure 7. CRKL dynamically interacts with VASP and mediates its functional effects. **(A)** Co-IP analysis of the interaction between VASP protein and CRKL protein in HCC cells. **(B)** Co-IP analysis of the interaction between VASP protein and CRKL protein in adjacent non-tumor tissues. **(C)** Transwell assay for migration and invasion was performed in the cells overexpressing VASP and corresponding cells in control group which were transfected with CRKL siRNA or control siRNA. The assay was carried out 48 h after transfection. **(D)** Western blotting detected Rac1 expression in the cells overexpressing VASP. The cells in the control group were transfected with CRKL siRNA or control siRNA and the analysis was performed 48 h after transfection. **(E)** Quantitative analysis of the protein in cells overexpressing VASP and corresponding cells in the control group transfected with CRKL siRNA or control siRNA. Cells were subjected to Western blotting for EMT markers 48 h after transfection

Supplementary Figure 8



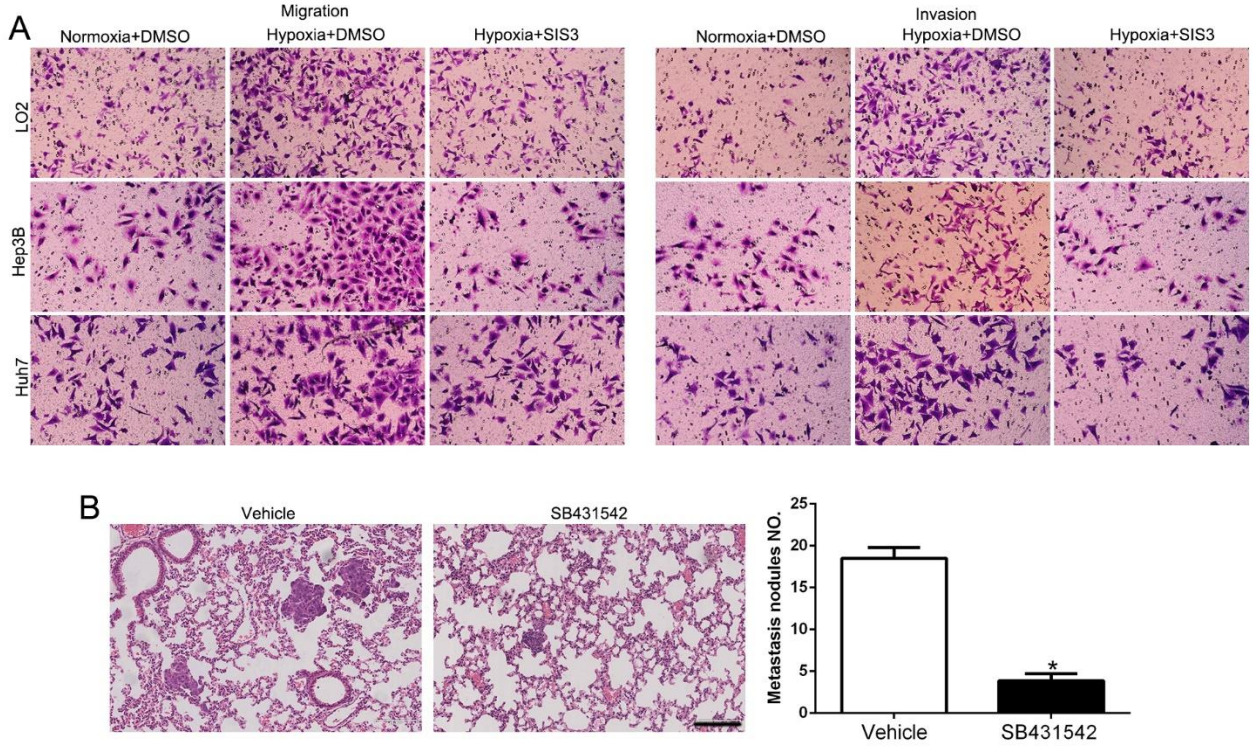
Supplementary Figure 8. HIF-1α mediates hypoxia-induced VASP overexpression on EMT. **(A)** HIF-1α siRNA was transfected into LO2, Hep3B, and Huh7 cells to knock down HIF-1α in hypoxic condition. The Transwell assays for migration and invasion (A) were performed and **(B)** quantitative analysis of the EMT protein markers detected by Western blotting.

Supplementary Figure 9



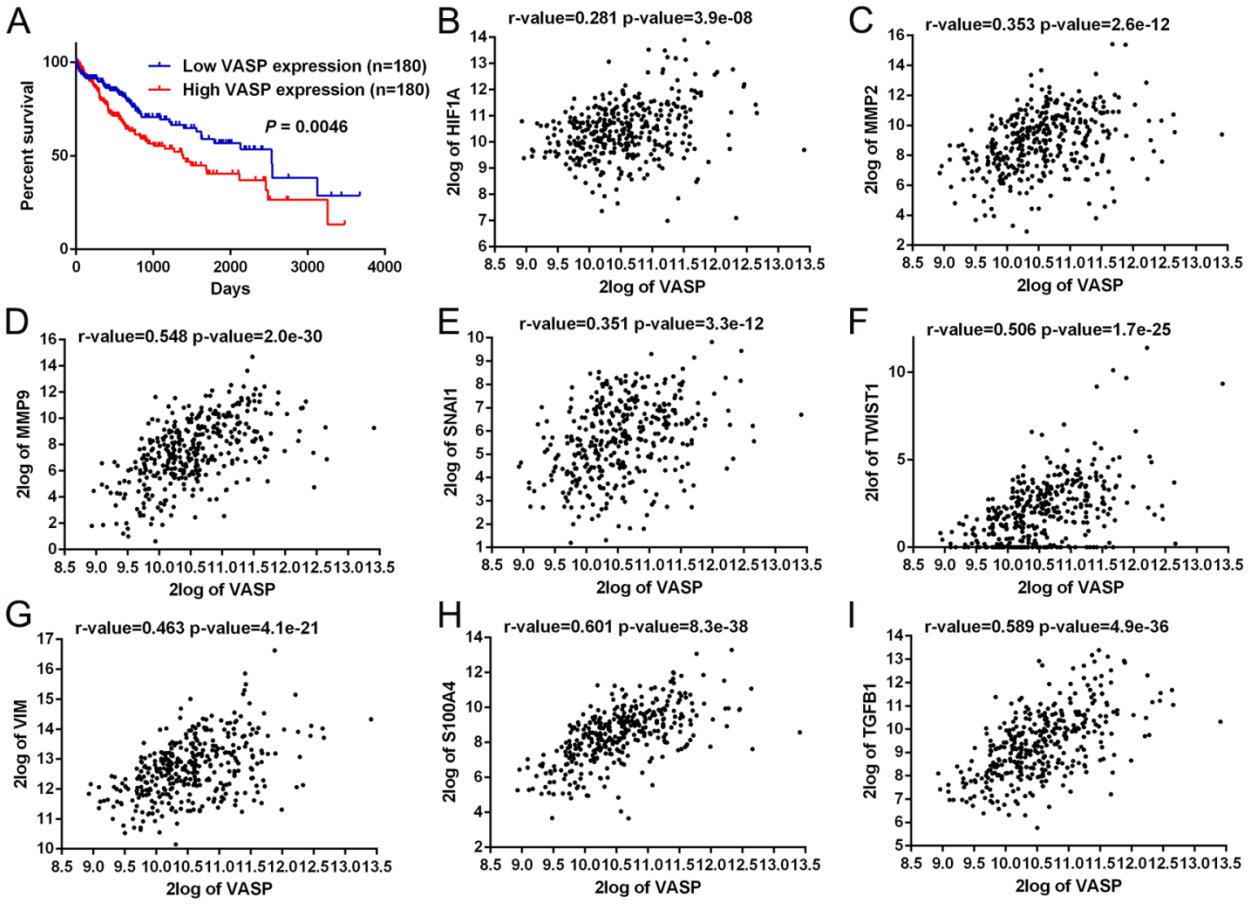
Supplementary Figure 9. Restoration of miR-204 reversed the hypoxia-induced effects on HCC cells. (A) MiR-204 and its putative binding sequence in the 3'-UTR of VASP. **(B)** qRT-PCR was performed to confirm the overexpression or knockdown of miR-204. *, $P<0.05$ by t-test. **(C-D)** LO2, Hep3B, and Huh7 cells cultured in normoxic or hypoxic condition were transduced with miR-204 vector and were subjected to the Transwell assay for migration and invasion (C) and (D) quantitative analysis of the EMT protein markers detected by Western blotting.

Supplementary Figure 10



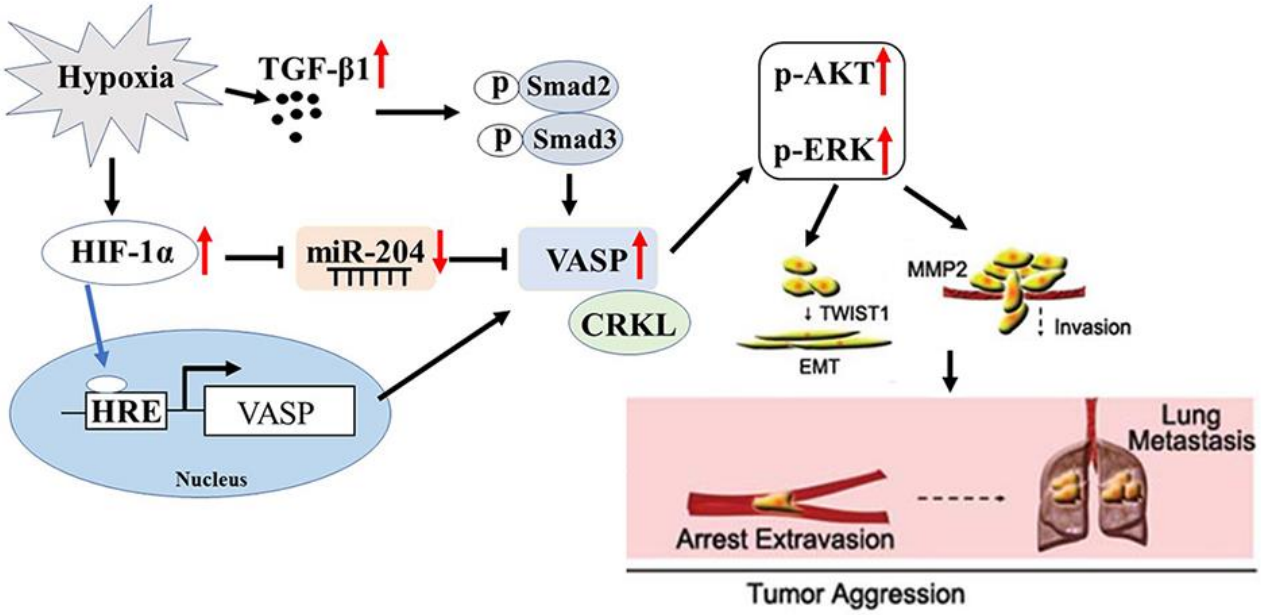
Supplementary Figure 10. The p-Smad3 inhibitor abolished the effects of hypoxia on HCC cells. (A) LO2, Hep3B, and Huh7 cells cultured under the normoxic or hypoxic condition were treated with p-Smad3 inhibitor, SIS3, and were subjected to the Transwell for migration and invasion. **(B)** p-Smad3 inhibitor, SB431542, significantly inhibited the numbers of lung metastases.

Supplementary Figure 11



Supplementary Figure 11. The publicly available database showed the relationship between VASP and EMT associated factors. (A) The publicly available database compiled in The Human Protein Atlas showed that high expression of VASP had a worse overall survival. **(B-I)** Data from R2: Genomics Analysis and Visualization Platform database showed that VASP expression has a positive correlation with the expression of different proteins.

Supplementary Figure 12



Supplementary Figure 12. Working model for the oncogenic function of VASP and its upstream and downstream pathways. VASP promotes the metastasis of HCC cells by activating AKT and ERK pathways. Hypoxia/HIF-1α signaling upregulates VASP expression in HCC cells by multiple mechanisms.

Supplementary table 1. Clinical correlation of VASP expression in HCC (n = 126).

Clinical parameters	Cases (n)	Expression level		*P* value
		$VASP^{high}$ (n=65)	$VASP^{low}$ (n=61)	（* $p<0.05$）
Age(years)				
< 60 years	74	38	36	0.950
≥60 years	52	27	25	
Gender				
Male	101	51	50	0.622
Female	25	14	11	
Tumor size (cm)				0.486
＜5cm	66	36	30	
≥5cm	60	29	31	
Tumor number				**0.006***
solitary	106	49	57	
multiple	20	16	4	
Edmondson				0.065
I+II	32	12	20	
III+IV	94	53	41	
TNM stage				**0.004***
I+II	95	42	53	
III+IV	31	23	8	
Venous infiltration				**0.004***
Present	26	20	6	
Absent	100	45	55	
AFP				0.930
＜400ng/ml	48	25	23	
≥400ng/ml	78	40	38	
HBsAg				0.623
positive	114	58	56	
negative	12	7	5	

HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis. *Statistically significant.

Supplementary table 2. Cox proportional hazard regression analyses for 5-year survival.

Variables	Univariate analysis			Multivariate analysis		
	HR	**95% CI**	***P***	**HR**	**95% CI**	***P***
Age	0.845	0.352-1.869	0.658	1.128	0.706-1.759	0.632
Gender	0.678	0.308-1.462	0.316	1.013	0.548-1.863	0.972
TNM stage	2.565	1.413-4.687	**0.002***	3.347	1.479-7.528	**0.004***
NO. of tumor nodule	9.906	3.235-20.328	**0.000**	9.215	2.806-25.462	**0.000**
Venous infiltration	2.546	1.114-5.526	**0.017**	0.925	0.385-2.987	0.915
VASP upregulation	5.203	2.310-11.403	**0.000**	4.958	1.945-12.654	**0.001**

HR, hazard ratio; CI, confidence interval; * statistically significant.

Supplementary Table 3. The sequences of walking primers of HIF-1α combines the promoter of VASP.

Primer	Sequence
HRE1F	TGGTGAAATCCCATCTCTACTA
HRE1R	TGTAGTCTCTCGCTGTCACC
HRE2F	TTAGTCTACCCATTCTCCCA
HRE2R	ATGTTGTCCTGACCTCCTTT
HRE3F	CCCAGGTAAAGGAGGTCAGG
HRE3R	GGAAAGCCACCAAGGTCTG
HRE4F	GCGCGACCAAATCAGTGA
HRE4R	CGGAGGACAGCAGGATCA
HRE5F	TTACACACCAAAGAAGCCG
HRE5R	AAGGTGAGGAGGAAGTGGG
HRE6F	GCGGTGTTCCGAAGATGG
HRE6R	CTTCCTAGTTCGCTCCCAC
HRE7F	GAACCTCTCATCAGACCGCC
HRE7R	ATGGCTGCTCGGCGGGCGGG

Supplementary Table 4. Significantly deregulated miRNAs (> 2fold) comparing Control-MHCC-97L cells and Hypoxia-treated MHCC-97L cells by miRNA array.

ProbeSet	miRNAs	Normalized expression		Fold change (FC)	Log FC
		Hypoxia	Nomoxia		
20525587	hsa-miR-6813-5p	5.056	2.135	7.573	2.921
20525591	hsa-miR-6815-5p	4.241	1.931	4.958	2.310
20519413	hsa-miR-4638-5p	4.436	2.288	4.433	2.148
20525731	hsa-miR-6885-5p	3.274	1.228	4.129	2.046
20501202	hsa-miR-362-3p	4.214	2.246	3.912	1.968
20525392	hsa-miR-6721-5p	4.087	2.194	3.713	1.893
20506818	hsa-miR-1290	5.798	3.937	3.630	1.860
20517817	hsa-miR-3610	3.149	1.299	3.606	1.850
20518785	hsa-miR-4417	4.659	2.888	3.413	1.771
20500483	hsa-miR-221-5p	4.482	2.784	3.245	1.698
20501177	hsa-miR-99b-3p	0.971	2.663	-3.232	-1.692
20504326	hsa-miR-590-5p	3.123	1.447	3.196	1.676
20525440	hsa-miR-6739-5p	5.918	7.576	-3.157	-1.659
20518455	hsa-miR-3927-3p	2.394	4.04	-3.129	-1.646
20525563	hsa-miR-6801-5p	2.668	1.033	3.107	1.636
20501278	hsa-miR-328-3p	1.153	2.785	-3.099	-1.632
20536811	hsa-mir-4531	2.246	0.627	3.071	1.619
20500197	hsa-miR-16-2-3p	4.240	2.675	2.959	1.565
20525502	hsa-miR-6770-3p	2.129	0.598	2.889	1.531
20525739	hsa-miR-6889-5p	5.533	4.016	2.861	1.517
20515536	hsa-miR-378b	4.987	3.474	2.854	1.513
20519609	hsa-miR-4750-5p	6.453	4.952	2.830	1.501
20506847	hsa-miR-1254	3.770	2.308	2.755	1.462
20519488	hsa-miR-4684-3p	2.828	1.376	2.736	1.452
20526182	hsa-miR-7112-5p	4.982	3.544	2.710	1.438
20504564	hsa-miR-1296-5p	2.358	3.792	-2.702	-1.434
20500443	hsa-miR-34a-3p	2.150	0.728	2.679	1.421
20525695	hsa-miR-6867-5p	3.094	4.495	-2.641	-1.401
20525479	hsa-miR-6759-5p	2.668	1.273	2.631	1.396
20529134	hsa-miR-6516-5p	2.357	0.964	2.626	1.393
20518870	hsa-miR-4479	2.353	0.967	2.612	1.385
20515632	hsa-miR-3192-5p	1.986	0.617	2.583	1.369
20518825	hsa-miR-4448	4.308	2.943	2.577	1.365
20518838	hsa-miR-4458	4.000	2.641	2.565	1.359
20517838	hsa-miR-3622b-5p	2.738	1.387	2.550	1.351
20518926	hsa-miR-4526	4.348	3.006	2.535	1.342
20525434	hsa-miR-6736-5p	2.961	4.293	-2.518	-1.332
20503883	hsa-miR-503-3p	1.674	0.346	2.510	1.328
20525492	hsa-miR-6765-3p	3.421	2.095	2.507	1.326
20520218	hsa-miR-5010-5p	2.782	1.465	2.490	1.316

20504186	hsa-miR-455-5p	2.023	0.746	2.424	1.278
20506835	hsa-miR-1244	3.693	4.969	-2.422	-1.276
20505790	hsa-miR-885-3p	5.331	4.062	2.410	1.269
20519472	hsa-miR-4672	3.015	1.754	2.397	1.261
20525706	hsa-miR-6872-3p	1.743	0.490	2.384	1.253
20522012	hsa-miR-5681a	1.223	2.476	-2.382	-1.252
20515610	hsa-miR-3180-3p	6.872	5.622	2.378	1.250
20517706	hsa-miR-4257	3.911	2.672	2.359	1.238
20525446	hsa-miR-6742-5p	2.696	1.469	2.341	1.227
20504407	hsa-miR-652-5p	2.807	4.013	-2.308	-1.206
20525555	hsa-miR-6797-5p	4.863	3.660	2.303	1.203
20517836	hsa-miR-3622a-5p	5.723	4.525	2.294	1.198
20500142	hsa-miR-21-3p	3.660	2.467	2.287	1.194
20520329	hsa-miR-5090	1.400	2.594	-2.287	-1.193
20518425	hsa-miR-3180	6.964	5.776	2.278	1.188
20502446	hsa-miR-451a	4.042	5.211	-2.249	-1.169
20500155	hsa-miR-26b-3p	1.603	0.435	2.248	1.169
20506862	hsa-miR-1266-5p	1.197	2.362	-2.243	-1.165
20520574	hsa-miR-5194	1.186	2.351	-2.242	-1.165
20505608	hsa-miR-675-5p	4.701	3.544	2.230	1.157
20503879	hsa-miR-502-5p	1.601	0.450	2.220	1.151
20500720	hsa-miR-23b-5p	2.913	4.050	-2.200	-1.138
20509235	hsa-miR-1914-3p	4.715	3.578	2.200	1.137
20529784	hsa-miR-8074	2.416	3.544	-2.185	-1.128
20518895	hsa-miR-4499	2.603	3.727	-2.180	-1.124
20518850	hsa-miR-4465	1.957	0.840	2.169	1.117
20521825	hsa-miR-5585-3p	1.202	2.314	-2.161	-1.112
20504581	hsa-miR-769-3p	2.215	1.113	2.146	1.102
20521811	hsa-miR-664b-3p	5.075	3.975	2.143	1.100
20534752	hsa-mir-155	0.820	1.916	-2.138	-1.097
20517696	hsa-miR-4314	1.710	0.631	2.112	1.079
20515635	hsa-miR-3194-5p	1.591	0.520	2.101	1.071
20517709	hsa-miR-4260	1.899	0.830	2.099	1.069
20519554	hsa-miR-4721	7.669	6.614	2.078	1.055
20519457	hsa-miR-4665-5p	7.288	6.234	2.077	1.054
20518852	hsa-miR-4467	8.675	7.622	2.075	1.053
20500186	hsa-miR-101-3p	2.738	1.692	2.065	1.046
20506880	hsa-miR-1278	2.244	1.200	2.062	1.044
20500170	hsa-miR-92a-1-5p	3.200	2.160	2.057	1.040
20515578	hsa-miR-3157-3p	0.673	1.713	-2.056	-1.040
20501228	hsa-miR-370-3p	2.839	1.806	2.046	1.033
20525679	hsa-miR-6859-5p	1.196	2.225	-2.040	-1.028
20529788	hsa-miR-8078	2.139	1.114	2.035	1.025
20500460	hsa-miR-204-5p	1.050	2.072	-2.031	-1.022
20519695	hsa-miR-4797-5p	3.460	4.482	-2.030	-1.022

20537880	hsa-mir-7162	0.532	1.550	-2.026	-1.019
20517745	hsa-miR-4286	6.832	5.816	2.022	1.016
20522034	hsa-miR-5699-5p	1.259	2.273	-2.020	-1.015
20504310	hsa-miR-582-3p	4.135	3.122	2.018	1.013

Supplementary Table 5. The sequences of walking primers of p-Smad3 combines the promoter of VASP.

Primer	Sequence
Site1F	GGGTGACAGCGAGAGACTAC
Site1R	AGGCCAAGCTTTGACATTAA
Site2F	TTCTGGGGGCTCAGGCAA
Site2R	GCTTCCGCAGCGTGTCT

Supplementary Table 6. Antibodies used in this study

Antigens	Manufacturers	Application
VASP	610447, BD transduction Laboratory, San Jose, CA	1:500 for IF, 1:5000 for WB (46kDa), 1:300 for IHC
GAPDH	AM4300, Invitrogen, Carlsbad, CA, USA	1:10000 for WB
E-cadherin	#14472, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB, 1:400 for IF, 1:300 for IHC
N-cadherin	#13116, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB, 1:400 for IF, 1:300 for IHC
α-E-catenin	#3236, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB

β-catenin	#8480, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB, 1:400 for IF
Fibronectin	Ab2413, Cambridge, MA, USA	1:1000 for WB
Vimentin	#5741, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB, 1:300 for IHC
Snail	#3879, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
Twist1	#46702, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
TIMP1	#8946, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
TIMP2	#5738, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
MMP2	#13132, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
MMP9	#13667, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB, 1:300 for IHC
Histone H3	Ab4729, Cambridge, MA, USA	1:3000 for WB
CRKL	Ab15179, Cambridge, MA, USA	1:1000 for WB, 1:400 for IF
HIF-1α	#36169, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
HIF-2α	#7096, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
AKT	#9272, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-AKT	#4060, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
ERK	#4695, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-ERK	#4370, Cell Signaling Technology,	1:1000 for WB

	Beverly, MA, USA	
JNK	#9252, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-JNK	#9255, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
P38	#8690, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-P38	#4511, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
NF-κB	#8242, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-NF-κB	#3033, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-SMAD2	#18338, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
p-SMAD3	#9520, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
Rac1	#4651, Cell Signaling Technology, Beverly, MA, USA	1:1000 for WB
F-actin	Ab205, Cambridge, MA, USA	1:400 for IF

WB, western blotting; IHC, immunochemistry; IF, immunofluorescence; IP, immunoprepiation