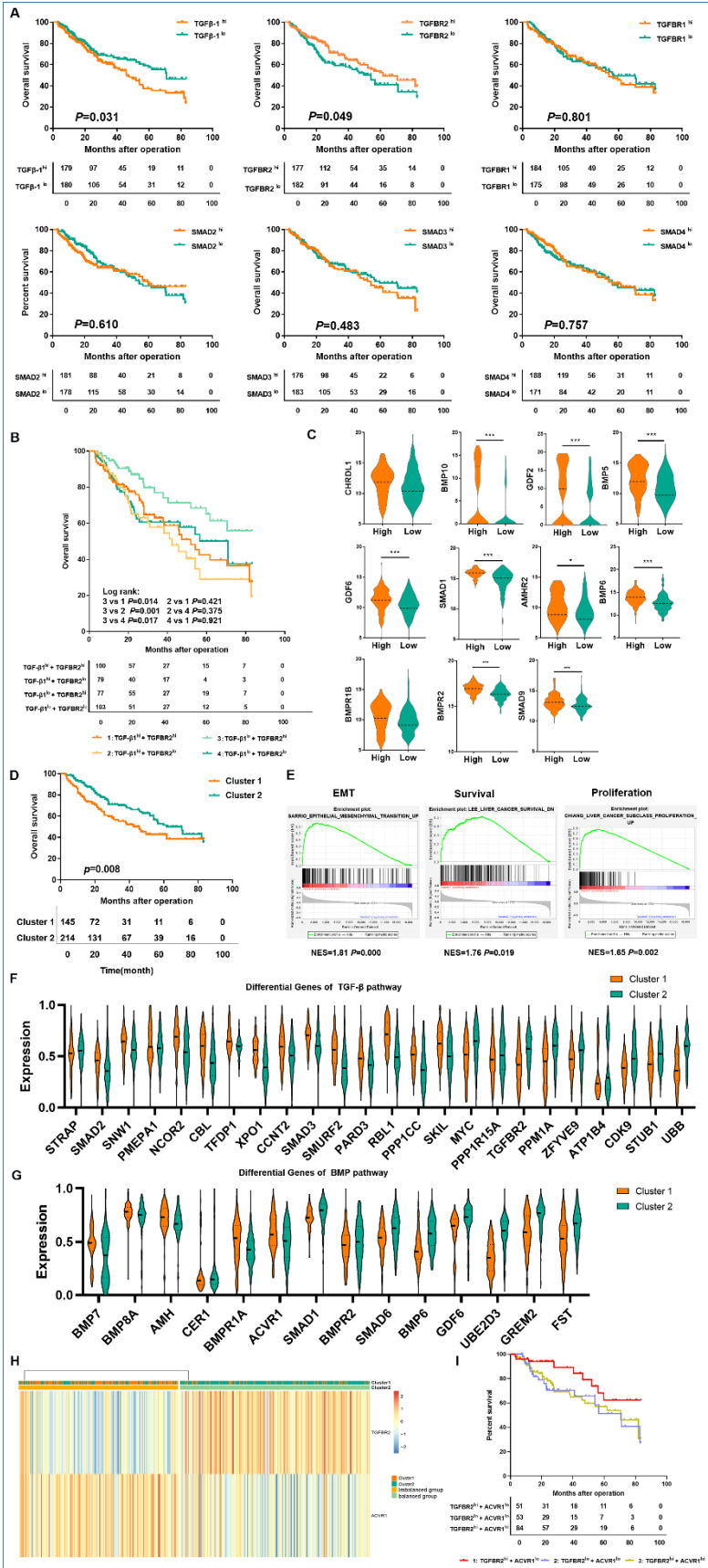


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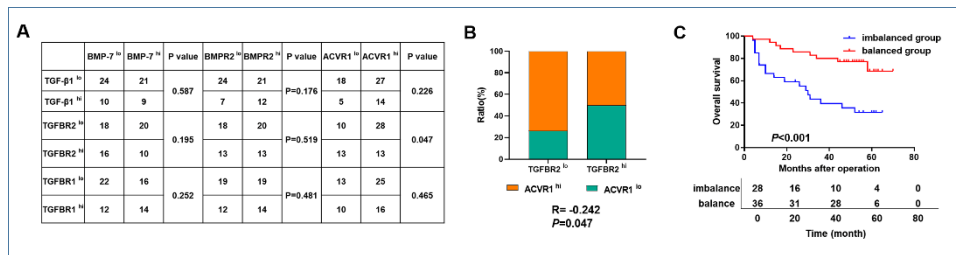
Supplemental Information

**Imbalance of TGF- β 1/BMP-7 pathways induced
by M2-polarized macrophages promotes
hepatocellular carcinoma aggressiveness**

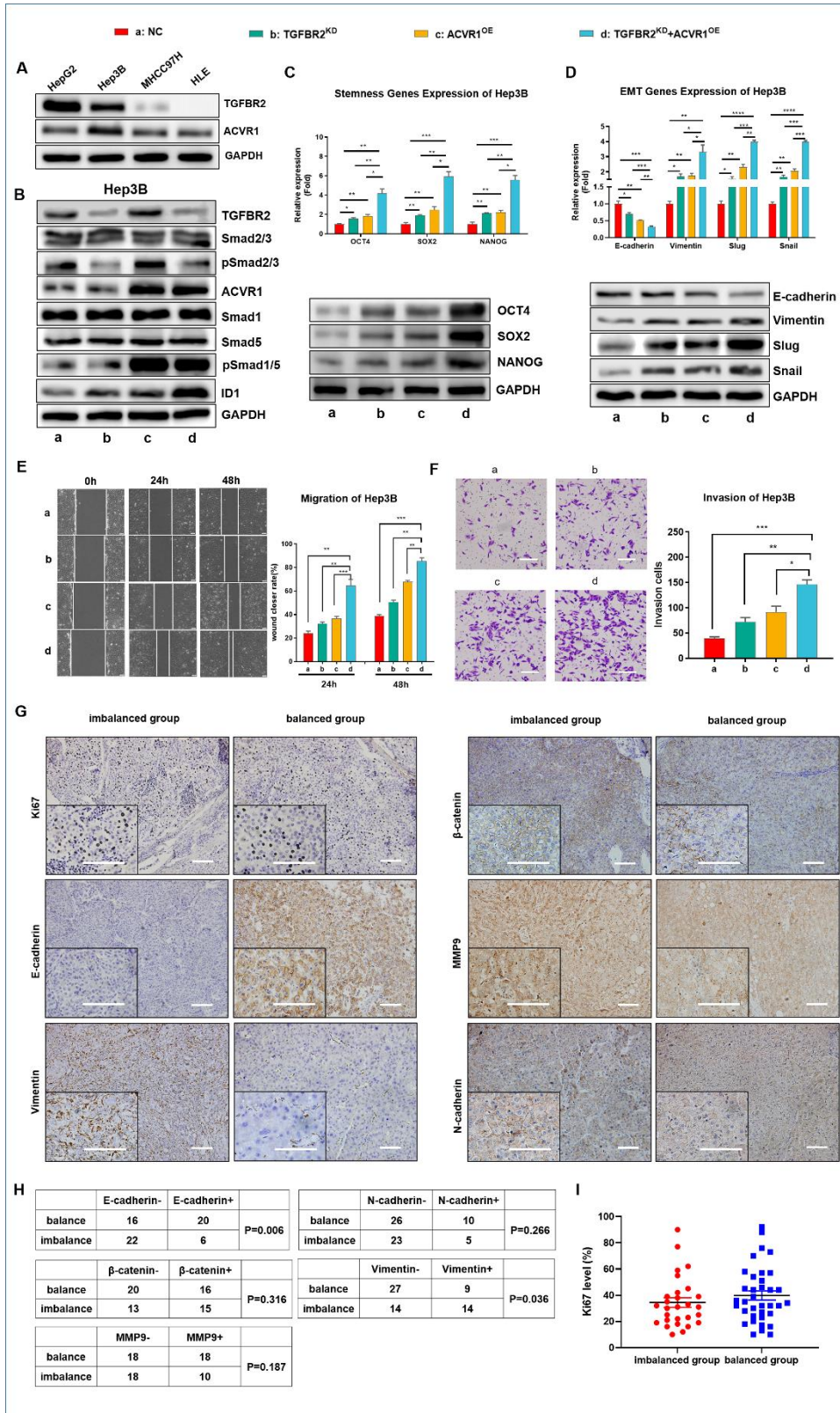
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Supplementary Fig. 1. The imbalance of TGF- β 1/BMP-7 pathways was detected at the mRNA level in HCC and was associated with poor clinical outcomes. (A) Kaplan-Meier analysis of TGF- β 1 pathway components from 359 HCC samples from TCGA cohort patients. (B) Kaplan-Meier analysis of the survival of patients with TGF- β 1^{hi}TGFBR2^{hi} versus TGF- β 1^{hi}TGFBR2^{lo} versus TGF- β 1^{lo}TGFBR2^{hi} versus TGF- β 1^{lo}TGFBR2^{lo} expression in the TCGA cohort. (C) Differential gene expression of BMP pathway components between TGFBR2^{hi} and TGFBR2^{lo} HCC tissues in the TCGA cohort. (D) Kaplan-Meier survival analysis showing the OS of the patients in cluster 1 and cluster 2. (E) GSEA identified differential enrichment of EMT-, liver cancer survival- and proliferation-related gene sets in cluster 1 compared with cluster 2. (F-G) Differentially expressed genes of the TGF- β and BMP pathways in cluster 1 compared with cluster 2. (H) A total of 359 HCC samples from the TCGA were clustered into 2 groups according to the expression of TGFBR2 and ACVR1. (I) The balanced group was separated into TGFBR2^{hi}ACVR1^{lo}, TGFBR2^{lo}ACVR1^{lo} and TGFBR2^{hi}ACVR1^{hi} based on their median data, Kaplan-Meier survival analysis was used to analyze the OS in patients of the three groups.

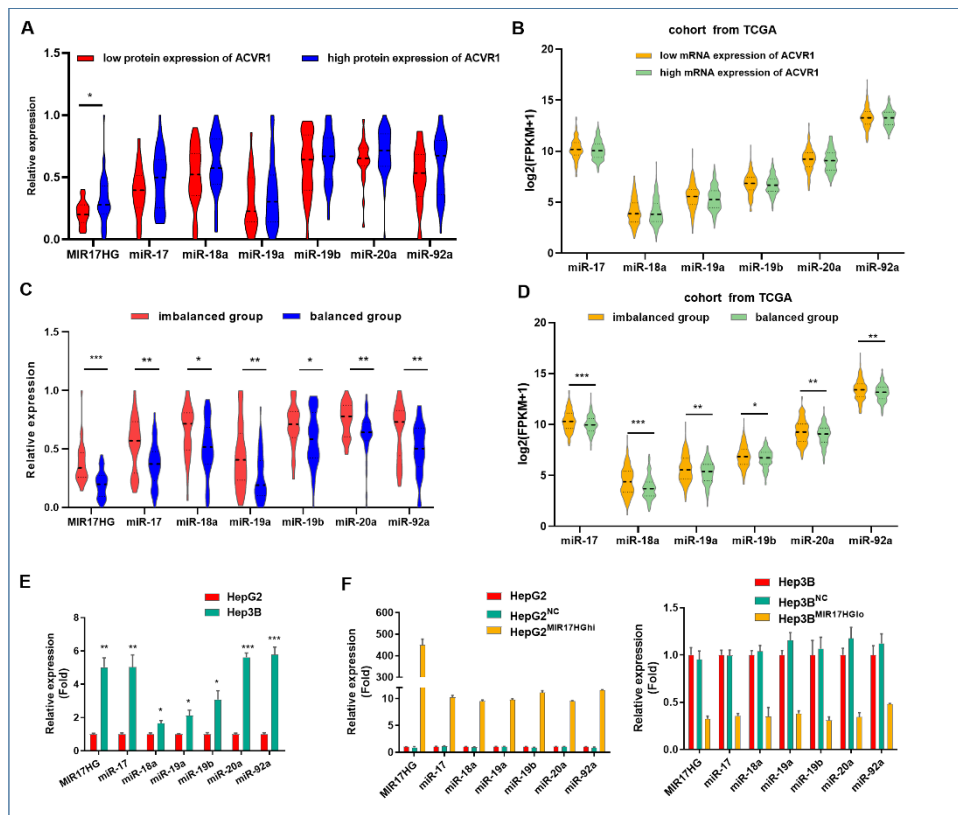


Supplementary Fig. 2. The imbalance of TGF- β 1/BMP-7 pathways was validated at the protein level in HCC and was associated with aggressive pathological features and poor clinical outcomes. (A) Correlations between the components of the TGF- β 1 and BMP-7 pathways. (B) Correlation between TGFBR2 expression and ACVR1 expression in cohort I. (C) Kaplan-Meier survival curves for patients in the imbalanced group and balanced group.



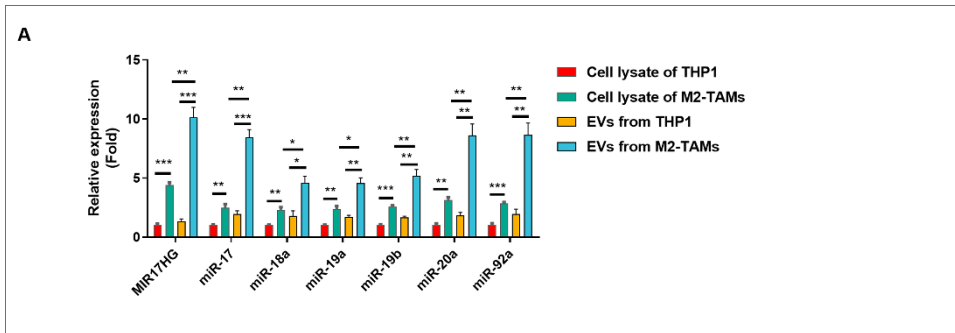
Supplementary Fig. 3. The imbalance of TGF- β 1/BMP-7 pathways dramatically promoted HCC cell invasion by upregulating EMT and stemness via increasing

inhibitor of differentiation (ID1). (A) TGFBR2 and ACVR1 expression at the protein level among HepG2, Hep3B, MHCC97H and HLE cells, as detected by western blot assay. Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase. (B) Western blotting of the levels of TGF- β 1/BMP-7 pathway components in Hep3B cells with separate or simultaneous TGFBR2 knockdown and ACVR1 overexpression. (C) PCR and western blotting of stem cell-related genes, including OCT4, SOX2 and NANOG, in Hep3B cells. (D) PCR and western blotting of EMT-related genes, including E-cadherin, vimentin, slug and snail, in Hep3B cells. (E) Wound healing assay in Hep3B cells at 24 and 48 h. Scale bars, 200 μ m. (F) Transwell assay after 24 h in Hep3B cells. Scale bars, 100 μ m. (G) Tumor proliferation- and invasion-related biomarkers, including Ki67, E-cadherin, N-cadherin, β -catenin, vimentin and MMP9, in the imbalanced group and balanced group. Scale bars, 50 μ m. (H) The chi-square test was used to analyze the correlation between EMT biomarkers and imbalance of the TGF β 1/BMP-7 pathways. (I) The distribution of Ki67 expression in the imbalanced and balanced groups was analyzed by Student's t test. For all panels, *P < 0.05, **P < 0.01, ***P < 0.001.

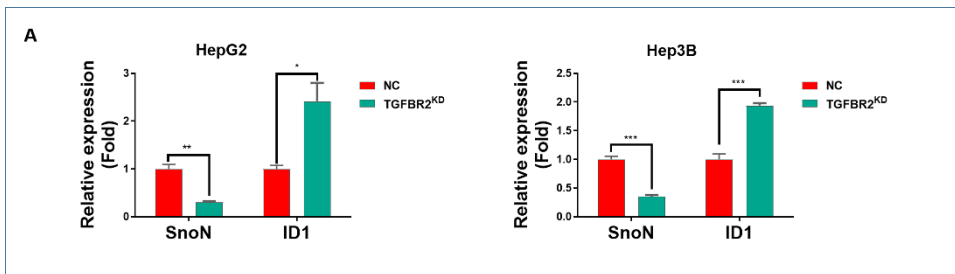


Supplementary Fig. 4. MiR-17-92 cluster promoted the imbalance of TGF- β 1/BMP-7 pathways by interfering with TGFBR2 mRNA expression and enhancing ACVR1 protein expression via Smurf1 silencing. (A) MIR17HG and miR-17-92 cluster in patients in cohort I with high ACVR1 expression and low ACVR1 expression when the

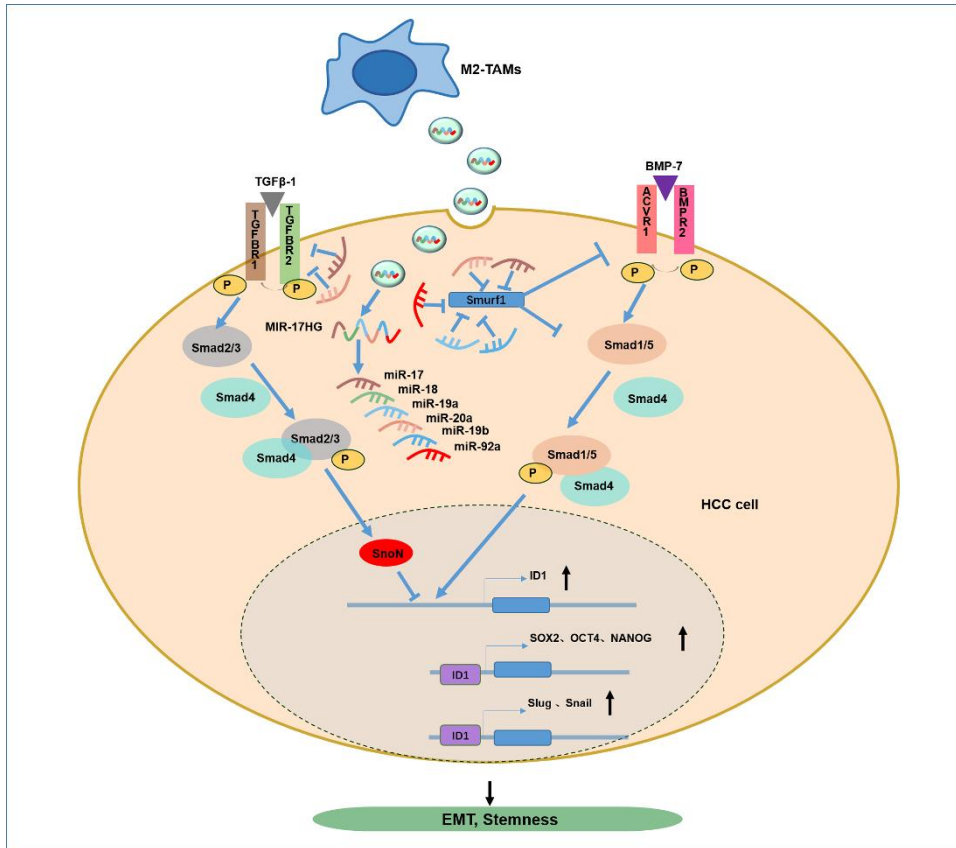
patients were separated by ACVR1 protein level in cohort I and (B) when the patients were separated by ACVR1 mRNA level in the TCGA cohort. (C) Expression of the MIR17HG and miR-17-92 cluster in the imbalanced group and balanced group in cohort I. (D) The levels of miR-17-92 cluster in the imbalanced group and balanced group in the TCGA cohort. *P < 0.05, **P < 0.01, ***P < 0.001. (E) The expression of MIR17HG and miR-17-92 cluster in HepG2 and Hep3B cells was detected by PCR. (F) MIR17HG was overexpressed in HepG2 cells and knocked down in Hep3B cells, and the expression of MIR17HG and miR-17-92 cluster members was detected by PCR. For all panels, *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Fig. 5. M2-TAMs increased the levels of MIR17HG and miR-17-92 cluster in HCC cells via EVs to exacerbate the imbalance of TGF- β 1/BMP-7 pathway. (A) The levels of MIR17HG and miR-17-92 cluster in the cell lysates and EVs of M2-TAMs were detected by PCR.



Supplementary Fig. 6. TGFBR2 KD increased ID1 expression by inhibiting SnoN without affecting the activating step for Smad1/5/8. (A) PCR array of SnoN and ID1 expression in TGFBR2^{KD} HepG2 cells and TGFBR2^{KD} Hep3B cells compared with those NC treated cells. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Fig. 7. The proposed model illustrates the mechanism by which M2-TAMs induce the imbalance of TGF-β1 and BMP-7 pathways in HCC to control HCC progression. In the HCC microenvironment, M2-TAMs transmit EVs containing lncRNA MIR17HG and miR-17-92 cluster to HCC cells; these components target TGFBR2 and Smurf1 in HCC, thus suppressing the TGF-β1 pathway and promoting the BMP-7 pathway. This imbalance upregulates ID1, a direct target gene of BMP-7, and promotes HCC cell EMT and stemness.

Supplementary Table 1. Clinical and pathological features of 359 HCC patients in the imbalanced group and balanced group.

Clinical parameters	imbalanced group	balanced group	<i>P</i> Value
Gender			
Male	95(57.9%)	144(73.8%)	0.002*
Female	69(42.1%)	51(26.2%)	
Age(year)			
≤60	82(50.0%)	86(44.1%)	0.156
>60	82(50.0%)	109(55.9%)	
Cirrhosis			
yes	45(54.9%)	77(59.7%)	0.292
no	37(45.1%)	52(40.3%)	
AFP (ng/ml)			
Abnormal (>20)	67(59.8%)	57(37.7%)	0.000*
Normal (≤20)	45(40.2%)	94(62.3%)	
Histologic grade			
G1	18(11.2%)	35(18.3%)	0.074
G2	72(44.7%)	95(49.7%)	
G3	65(40.4%)	55(28.8%)	
G4	6(3.7%)	6(3.1%)	
Pathologic T-stage			
T1	67(40.9%)	106(54.9%)	0.018*
T2	44(26.8%)	46(23.8%)	
T3/4	53(32.3%)	41(21.2%)	
Pathologic N-stage			
N0	119(72.6%)	125(64.8%)	0.071
N1/Nx	45(27.4%)	68(35.2%)	
Pathologic M-stage			
M0	124(75.6%)	130(66.7%)	0.041*
M1/Mx	40(24.4%)	65(33.3%)	
AJCC Stage			
Stage1	63(41.2%)	103(57.2%)	0.004*
Stage2	38(24.8%)	42(23.3%)	
Stage3/4	52(34.0%)	35(19.4%)	
Vascular invasion			
yes	52(40.3%)	51(29.5%)	0.033*
no	77(59.7%)	122(70.5%)	

Supplementary Table 2. Univariate and multivariate analyses of clinicopathologic parameters associated with OS and DFS.

Supplementary Table 3. Correlations between the imbalance of the TGF-β1/BMP-7 pathways and clinicopathologic parameters.

Supplementary Table 4. Primary antibodies for western blotting and IHC.

Protein	Concentration for WB	Concentration for IHC	Specificity	Company
TGF- β 1	/	10 μ g/ml	Rabbit	Abcam
TGFBR2	2 μ g/ml	/	Goat	R&D Systems
TGFBR2	/	1:200	Mouse	Abcam
TGFBR1	1:200	1:50	Rabbit	Abcam
BMP-7	/	4 μ g/ml	Rabbit	Abcam
BMPR2	1:100	1:200	Mouse	Abcam
ACVR1	/	1:200	Rabbit	Abcam
Ki67		1:500	Mouse	Santa Cruz
E-cadherin		1:100	Mouse	Santa Cruz
N-cadherin		1:200	Mouse	Santa Cruz
Vimentin		1:200	Mouse	Santa Cruz
OCT4	1:1000		Rabbit	Abcam
SOX2	1:1000		Rabbit	Abcam
NANOG	1:1000		Rabbit	Abcam
MMP9		1:200	Mouse	Santa Cruz
β -catenin		1:400	Mouse	Abcam
ACVR1	1:1000	/	Rabbit	Cell Signaling Technology
Smad2/3	1:1000	/	Rabbit	Cell Signaling Technology
pSmad2/3	1:1000	/	Rabbit	Cell Signaling Technology
Smad1	1:1000	/	Rabbit	Cell Signaling Technology
Smad5	1:1000	/	Rabbit	Cell Signaling Technology
pSmad1/5/8	1:1000	/	Rabbit	Cell Signaling Technology
ID1	1:100	1:50	Mouse	Santa Cruz
Smurf1	1:1000	/	Rabbit	Cell Signaling Technology
E-cadherin	1:1000	1:200	Rabbit	Cell Signaling Technology
Vimentin	1:1000	1:200	Rabbit	Cell Signaling Technology
Slug	1:1000	/	Rabbit	Cell Signaling Technology
Snail	1:1000	/	Rabbit	Cell Signaling Technology
GAPDH	1:10000	/	Rabbit	Cell Signaling Technology
CD68	/	1:500	Rabbit	Invitrogen
CD163	/	1:100	Rabbit	ZSGB-BIO
CD66b	/	1:400	Rabbit	Abcam

Supplementary Table 5. Multispectral IF staining.

Position	Antibody	Company	Dilution	Incubation	TSA dyes
1	HepPar-1	ZSGB-BIO	1:500	4°C overnight	690
2	CD163	ZSGB-BIO	1:500	4°C overnight	570
3	CD68	Invitrogen	1:2000	4°C overnight	650
4	TGFBR2	Abcam	1:500	4°C overnight	520
5	ACVR1	Abcam	1:500	4°C overnight	620
6	DAPI	Perkin Elmer Opal 7-color kit	2 drops/ml	5 min	

Supplementary Table 6. Primers used in the study.