Figure S1. SEPT6 protein expression levels in another 16 paired HCC and corresponding adjacent non-tumor tissue samples were assessed via western blotting. (A, C and D) SEPT6 protein expression levels were increased in 12 HCC tissues compared with the adjacent non-tumor tissues. (B) SEPT6 protein expression levels were decreased in 4 HCC tissues compared with the adjacent non-tumor tissues. **P<0.01 and ***P<0.001 vs. corresponding adjacent non-tumor tissue samples. SEPT6, septin 6; HCC, hepatocellular carcinoma; N, adjacent non-tumor tissues; C, HCC tissues.

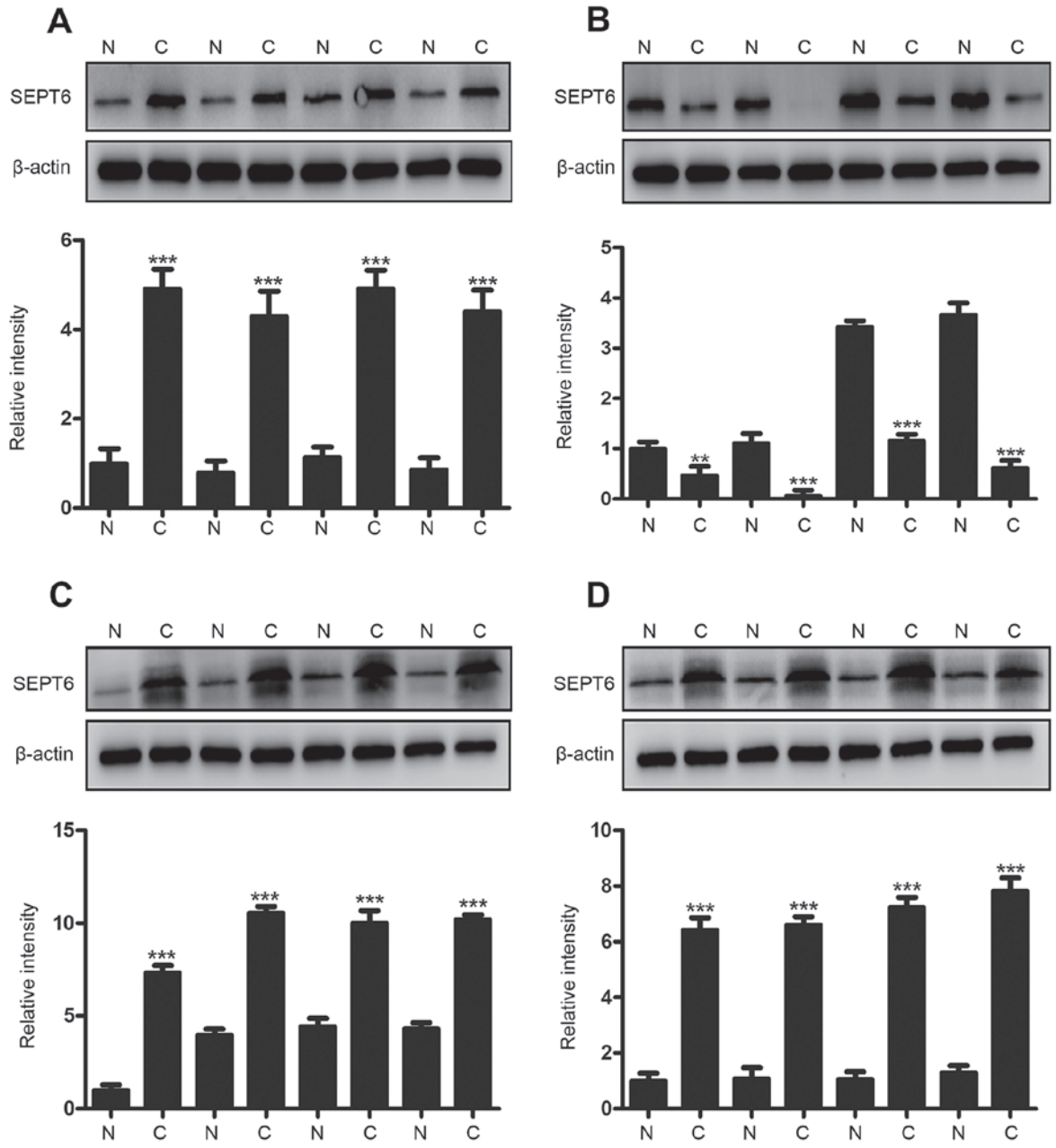

Figure S2. Transfection efficiencies of SEPT6 knockdown and overexpression. Transfection efficiencies of sh-SEPT61/2 in HCC-LM3 cells and SEPT6 in Hep3B cells were determined via (A) reverse transcription-quantitative PCR and (B) western blotting. At 48 h post-transfection, cells were treated with G418 (400 μg/ml) for 2 weeks for stable cell selection. **P<0.01 and ***P<0.001 vs. shcontrol or Vector. SEPT6, septin 6; sh, short hairpin RNA.

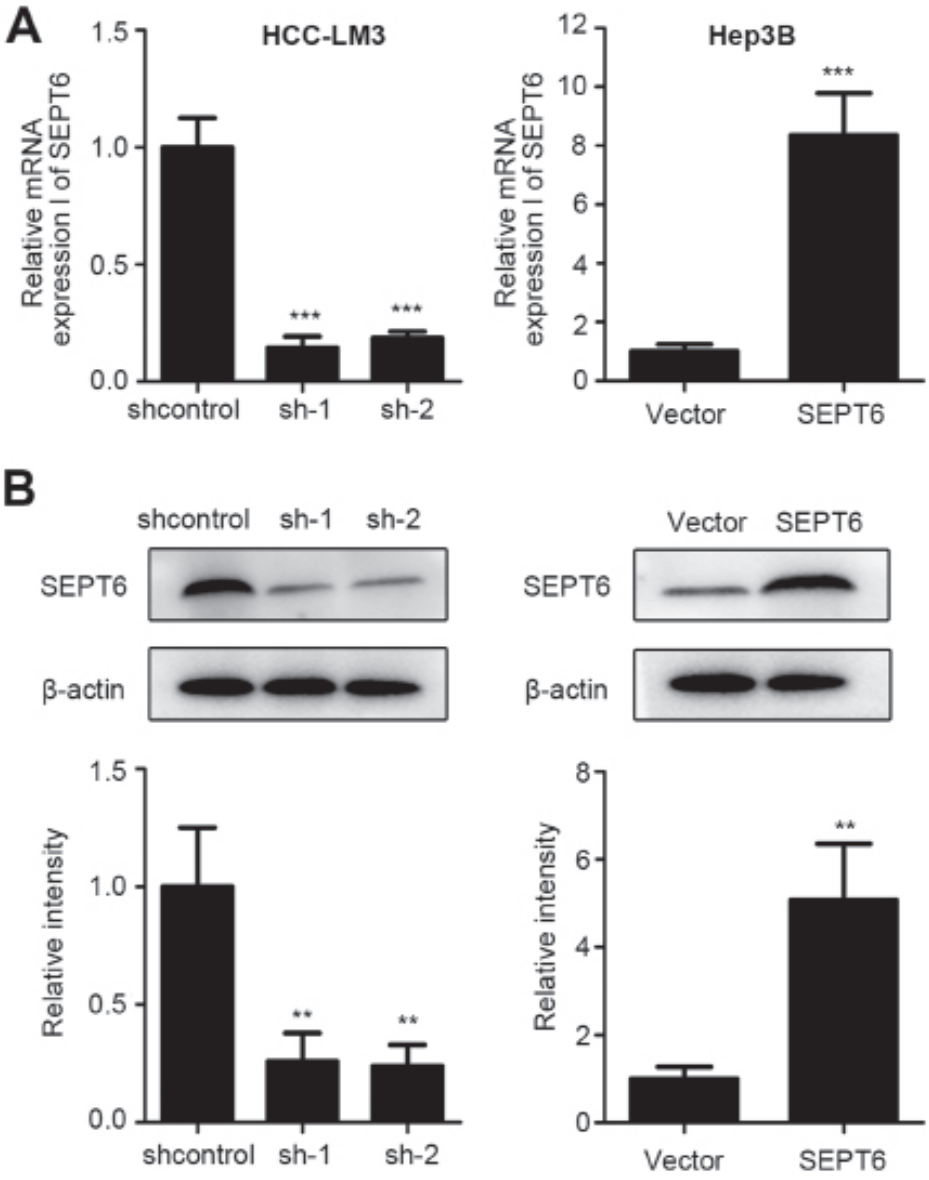

Figure S3. Transfection efficiency of YAP knockdown and overexpression. MHCC-97H cells were transfected with YAP or vector. Huh7 cells were transfected with sh-YAP or sh-control. At 48 h post-transfection, cells were treated with G418 (400 μg/ml) for 2 weeks for stable cell selection. Transfection efficiencies were determined via (A) reverse transcription-quantitative PCR and (B) western blotting. **P<0.01 and ***P<0.001 vs. Vector or shcontrol. YAP, yes-associated protein; sh, short hairpin RNA.

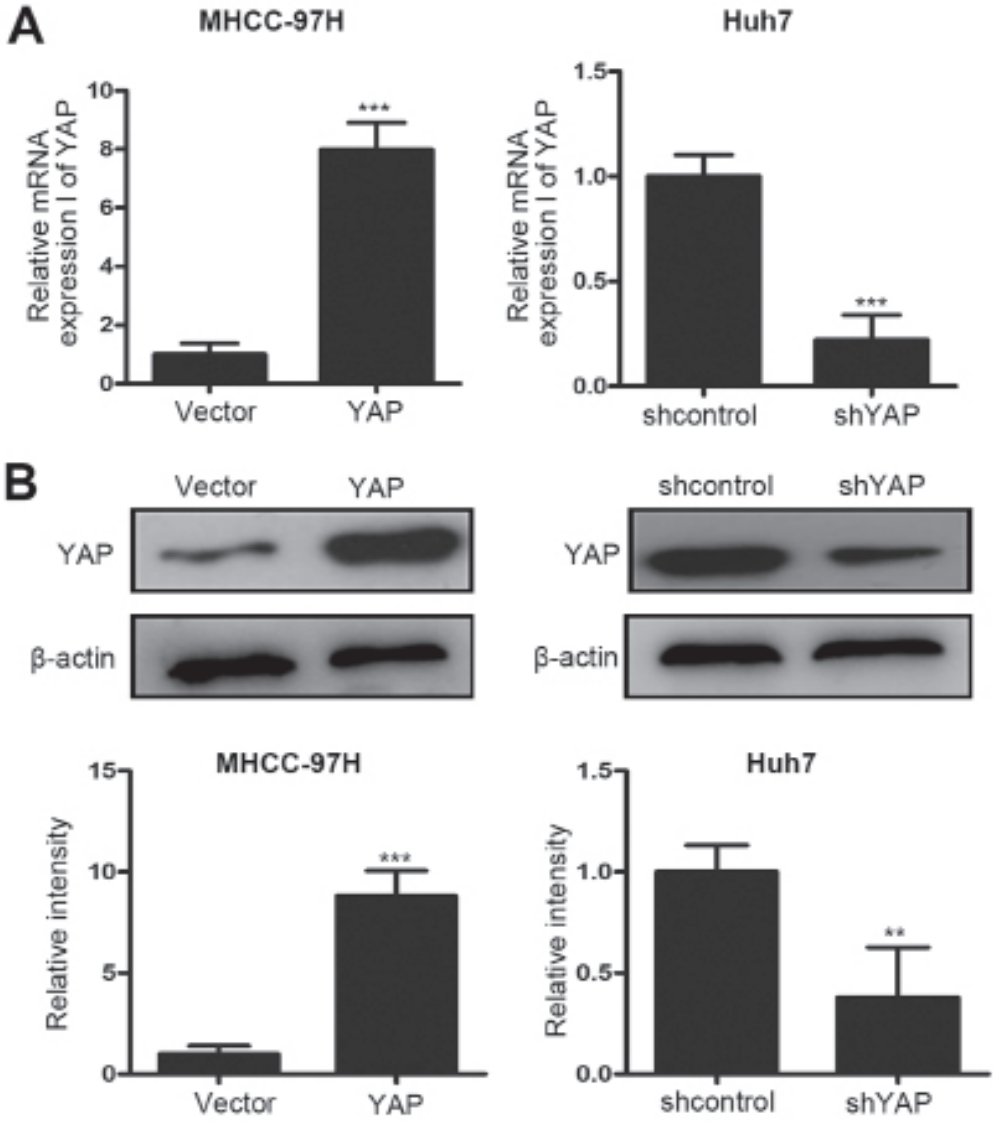

Figure S4. SEPT6 regulates cyclin D1 and MMP2 expression via YAP, and YAP also independently regulates cyclin D1 and MMP2 expression. Cyclin D1 and MMP2 (A) mRNA and (B) protein expression levels were determined via reverse transcription-quantitative PCR and western blotting. ***P<0.001 vs. Vector; ###P<0.001 vs. SEPT6. SEPT6, septin 6; MMP, matrix metallopeptidase; YAP, yes-associated protein; sh, short hairpin RNA

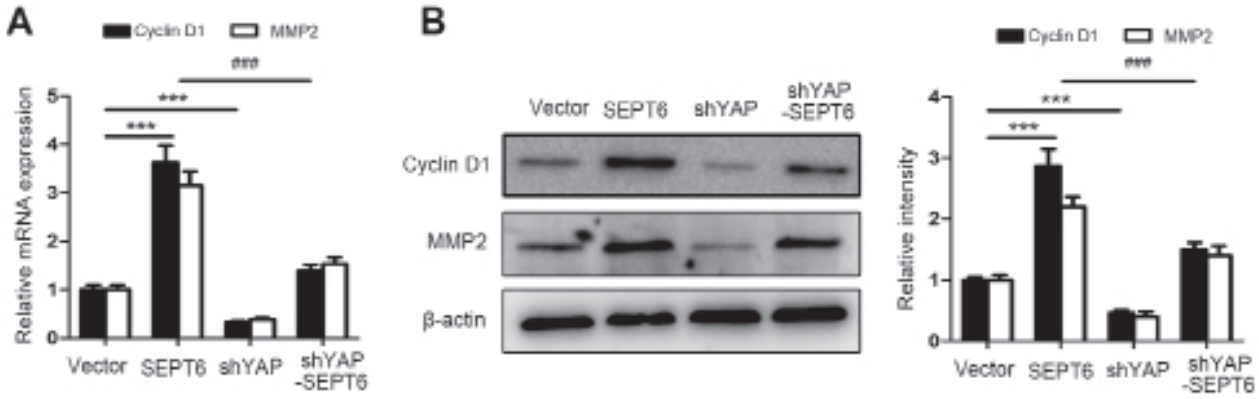

Table SI. Sequences of primers used for quantitative PCR and shRNAs.

Primer/vector	Sequence (5'→3')
β-actin	F: CATGTACGTTGCTATCCAGGC
	R: CTCCTTAATGTCACGCACGAT
SEPT6	F: TCCAAGAGAGCAACGTGAGG
	R: AATTCCACGATAGGCTTGTAGC
Cyclin D1	F: GCTGCGAAGTGGAAACCATC
	R: CCTCCTTCTGCACACATTTGAA
Cyclin E1	F: ACTCAACGTGCAAGCCTCG
	R: GCTCAAGAAAGTGCTGATCCC
MMP2	F: TACAGGATCATTGGCTACACACC
	R: GGTCACATCGCTCCAGACT
MMP9	F: TGTACCGCTATGGTTACACTCG
	R: GGCAGGGACAGTTGCTTCT
YAP	F: TAGCCCTGCGTAGCCAGTTA
	R: TCATGCTTAGTCCACTGTCTGT
shSEPT6-1	GCAGCACAGAAGAACUGAA
shSEPT6-2	GACCUAGUGACUAUGAAGA
shYAP	GGAATTGAGAACAATGACGAC
Control	UUCUCCGAACGUGUCACG

SEPT6, septin 6; MMP, matrix metallopeptidase; sh, short hairpin RNA; YAP, yes-associated protein; F, forward; R, reverse.

Table SII. Antibodies used for western blotting.

Antibody	Dilution	Cat. no.	Supplier details
SEPT6	1:400	12805-1-AP	ProteinTech Group, Inc.
Cyclin D1	1:2,000	60186-1-Ig	ProteinTech Group, Inc.
Cyclin E1	1:1,000	11554-1-AP	ProteinTech Group, Inc.
MMP2	1:1,000	4022	Cell Signaling Technology, Inc.
MMP9	1:300	BA0573	Boster Biological Technology Co., Ltd.
LATS1	1:1,000	9153	Cell Signaling Technology, Inc.
YAP	1:1,000	14074	Cell Signaling Technology, Inc.
p-LATS1	1:1,000	9157	Cell Signaling Technology, Inc.
p-YAP	1:1,000	13008	Cell Signaling Technology, Inc.
LATS2	1:1,000	5888	Cell Signaling Technology, Inc.
Lamin B1	1:5,000	66095-1-Ig	ProteinTech Group, Inc.
β-actin	1:2,000	BM0627	Boster Biological Technology Co., Ltd.
Anti-Rabbit IgG	1:1,000	A0208	Beyotime Institute of Biotechnology
Anti-mouse IgG	1:1,000	A0216	Beyotime Institute of Biotechnology

SEPT6, septin 6; MMP, matrix metallopeptidase; LATS, large tumor suppressor kinase; YAP, yes-associated protein; p, phosphorylated.