BMP4 promotes hepatocellular carcinoma proliferation by autophagy activation through JNK1-mediated Bcl-2 phosphorylation

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Supplementary Information include:
Supplementary Figure S1-S3 and supplementary Table S1-S2.

Figure S1

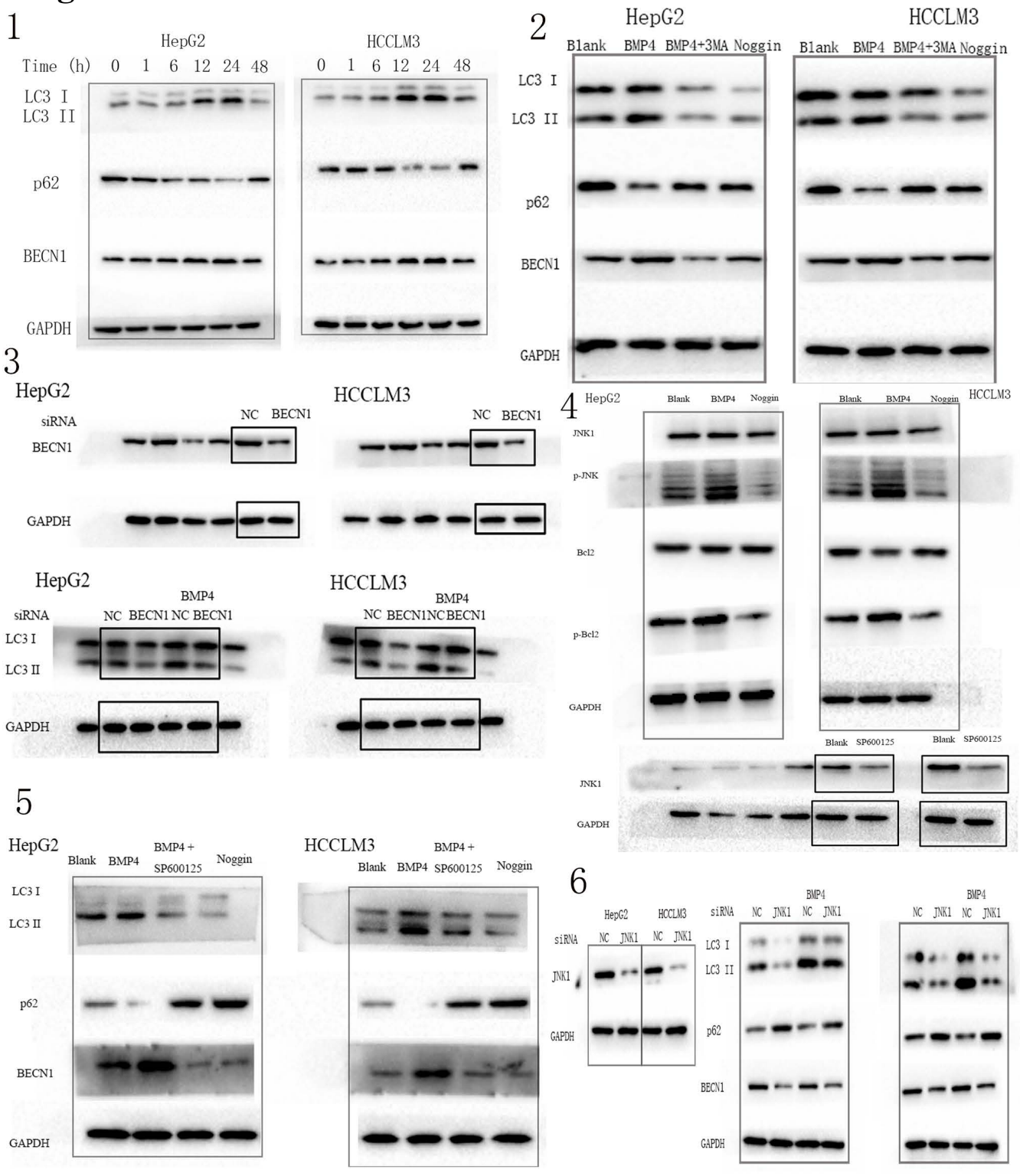

Figure S1: Original blots of Western blot. (1) Original blots of Figure 1. (2) Original blots of Figure 2. (3) Orinigal blots of Figure 3. (4) Orinigal blots of Figure 4. (5) Orinigal blots of Figure 5. (6) Orinigal blots of Figure 6.

Figure S2

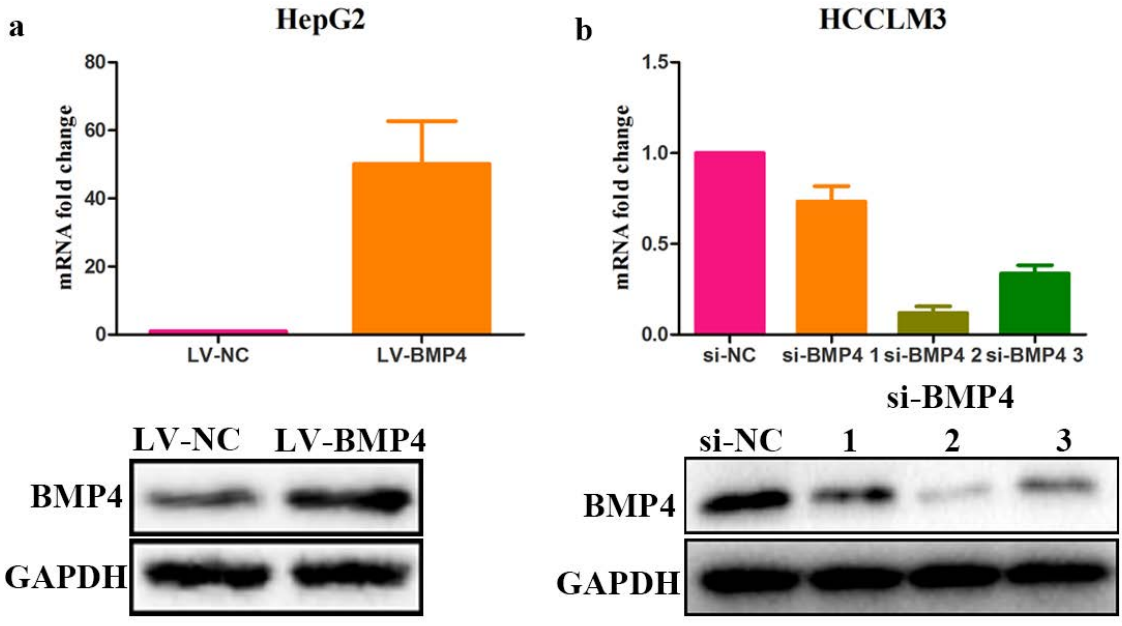

Figure S2: The effeciency of BMP4 expression manipulated by lentivirus. (a)The effeciency of BMP4 overexpression in HepG2 cells was confirmed by qRT-PCR and Western blot. (b)The effeciency of BMP4 knockdown in HCCLM3 cells was confirmed by qRT-PCR and Western blot.

Figure S3

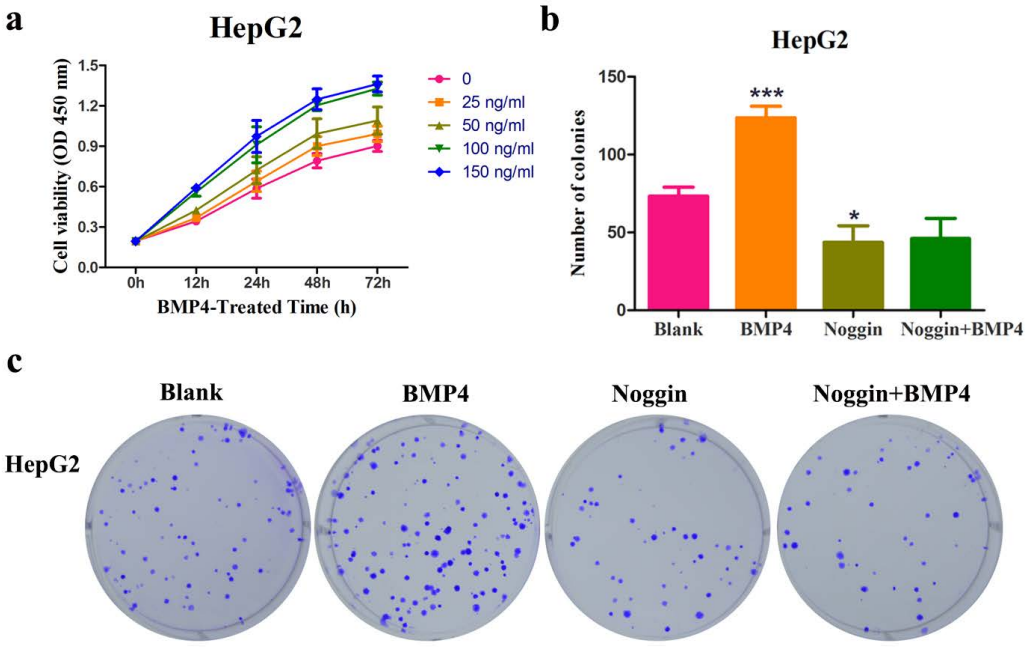

Figure S3: BMP4 promoted HepG2 cells growth: (a) HepG2 cells were treated with various concentrations of human recombinant BMP4 (0, 25, 50, 100 and 150 ng/mL) for different lengths of time to assay the effects on HCC cells proliferation. Cell viability was determined by CCK-8 assays. (b) & (c) Effects of BMP4 or Noggin on long term colony formation in HepG2 cells. The numbers of colonies in the BMP4-treated (100 ng/mL) groups were significantly more than that in the blank groups in HepG2 cells ($p < 0.001$), while Noggin-treated (200 ng/mL) groups displayed significantly less colony numbers than the blank control groups ($p < 0.05$). n=3, one-way ANOVA with post-hoc Tukey's test.

Supplementary Table S1. Information of antibodies for western blot.

Antibody	Working Dilution	Molecular Weight (KDa)	Cat. Number
BMP4	1:5000	47	ab124715
LC3	1:1000	14, 16	#4108
SQSTM1/p62	1:1000	62	ab56416
Beclin1	1:1000	60	ab207612
JNK1	1:1000	48	ab213521
phospho-JNK	1:1000	46, 54	#4668
Bcl-2	1:1000	26	#4223
phospho-Bcl2	1:1000	28	#2827
GAPDH	1:10000	36	ab181602
Goat anti-Mouse IgG	1:5000	—	ab6789
Goat anti-Rabbit IgG	1:5000	—	ab6721

Supplementary Table S2. The sequences of siRNA targeting BMP4.

No.	sequences	titer
BMP4-RNAi1 - 5’	taaGCAGCCAAACTATGGGCTA ctcgagTAGCCCATAGTTTGGCTGCttttttttc	5E+8
BMP4-RNAi1 - 3’	tcgagaaaaaaaaGCAGCCAAACTATGGGCTA ctcgagTAGCCCATAGTTTGGCTGCtta	5E+8
BMP4-RNAi2 - 5’	tgaCCCTGGTCAATTCTGTCAA ctcgagTTGACAGAATTGACCAGGGtctttttttc	4E+8
BMP4-RNAi2 - 3’	tcgagaaaaaaagaCCCTGGTCAATTCTGTCAA ctcgagTTGACAGAATTGACCAGGGtca	4E+8
BMP4-RNAi3 - 5’	tcaCCTCAACTCAACCAACCAT ctcgagATGGTTGGTTGAGTTGAGGtgttttttc	5E+8
BMP4-RNAi3 - 3’	tcgagaaaaaaacaCCTCAACTCAACCAACCAT ctcgagATGGTTGGTTGAGTTGAGGtga	5E+8
BMP4-RNAi4 - 5’	tcaGATCCACAGCACTGGTCTT ctcgagAAGACCAGTGCTGTGGATCtgttttttc	3E+8
BMP4-RNAi4 - 3’	tcgagaaaaaaacaGATCCACAGCACTGGTCTT ctcgagAAGACCAGTGCTGTGGATCtga	3E+8