

Supporting Information:

Bone Formation Promoted by Bone Morphogenetic Protein-2 Plasmid-Loaded Porous Silica Nanoparticles with Involvement of Autophagy

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Table S1. Zeta-potential of PSN-NH₂, PSN-COOH and PPSN, respectively.

	Zeta potential (mV)
PSN-NH ₂	6.2 ± 0.6
PSN-COOH	-9.8 ± 0.5
PPSN	50.3 ± 0.8

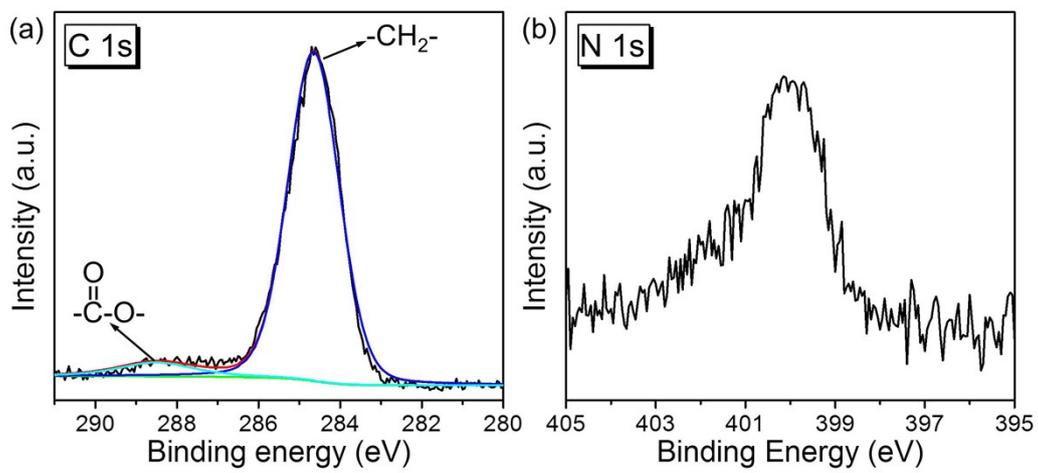


Figure S1. XPS spectra of C 1s in PSN-COOH (a) and N 1s in PPSN (b).

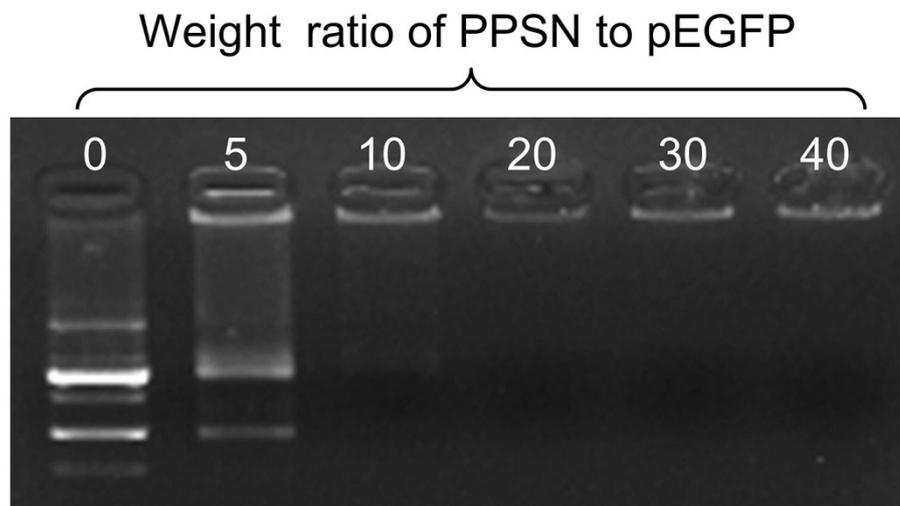


Figure S2. Agarose gel electrophoresis retardation assay. PPSN and pEGFP at various weight ratios of 0:1, 5:1, 10:1, 20:1, 30:1 and 40:1.

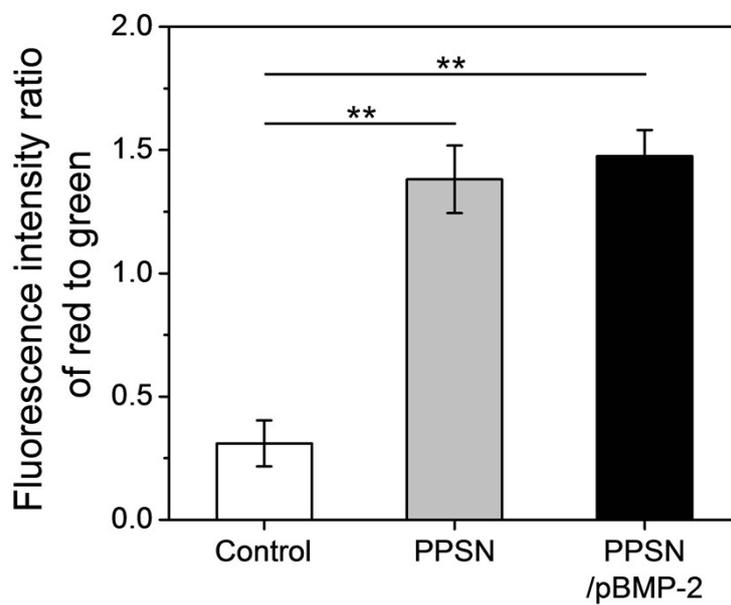


Figure S3. Quantitative analysis for AO staining assay. Data from all three experiments are presented as mean \pm SD. *: $P < 0.05$, **: $P < 0.01$.

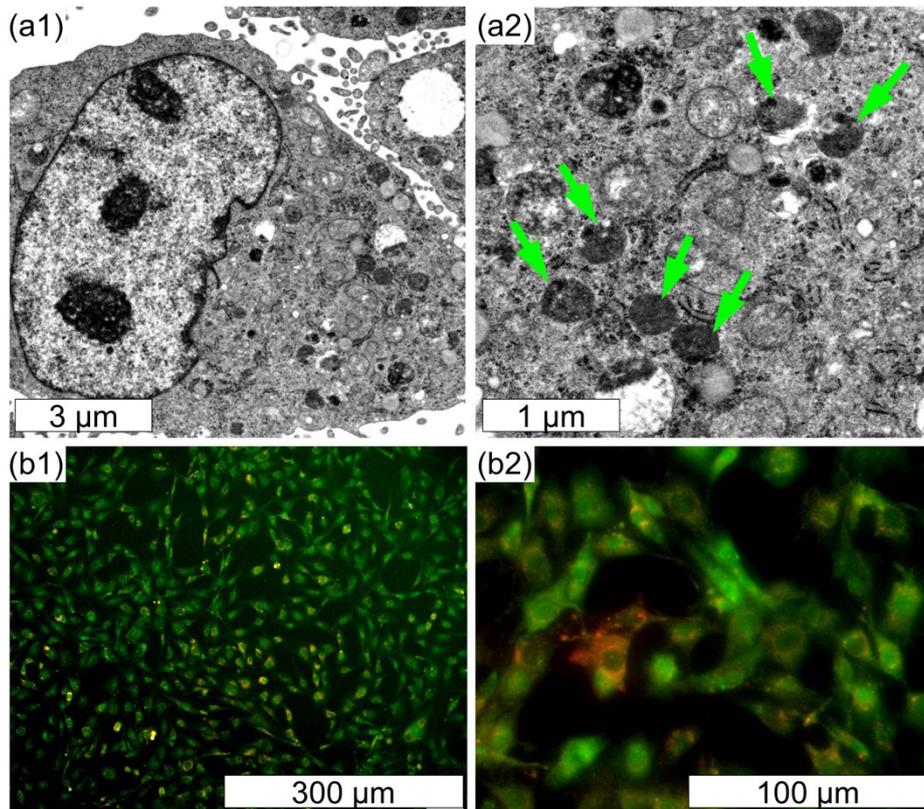


Figure S4. TEM images of MC3T3-E1 cells treated with pBMP-2 at low (a1) and high (a2) magnification, and AO staining of MC3T3-E1 cells treated with pBMP-2 at low (b1) and high (b2) magnification.

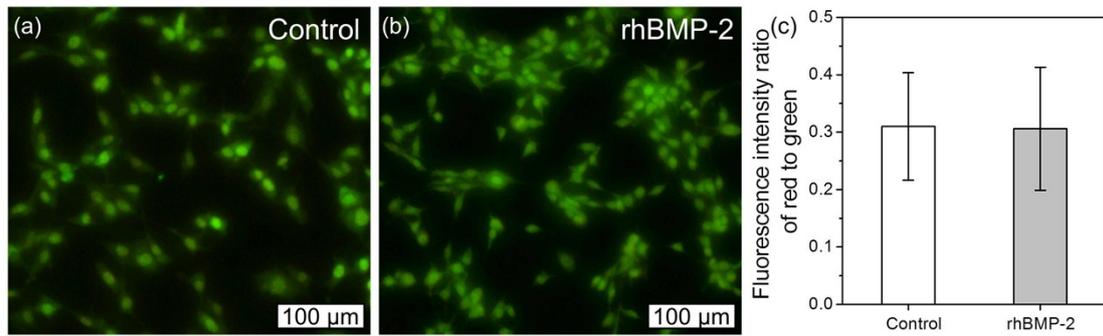


Figure S5. AO staining of MC3T3-E1 cells treated without or with rhBMP-2. Data from all three experiments are presented as mean \pm SD. *: $P < 0.05$, **: $P < 0.01$.

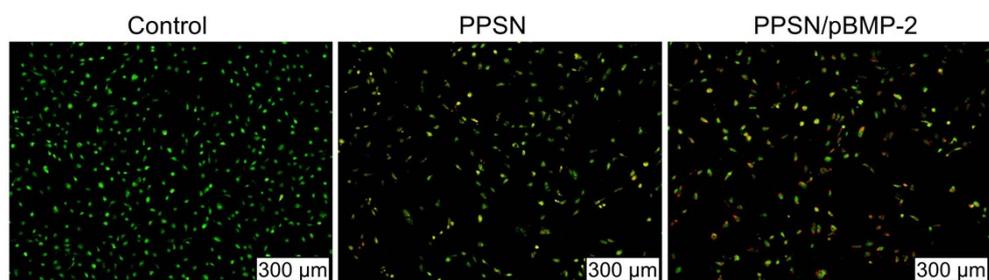


Figure S6. Effects of autophagy inhibitor (3-MA, 3 mM) on AO staining. MC3T3-E1 cells without treatment (control), MC3T3-E1 cells treated with PPSN at 50 $\mu\text{g}/\text{well}$, and MC3T3-E1 cells treated with PPSN/pBMP-2 at weight ratio of 20:1.

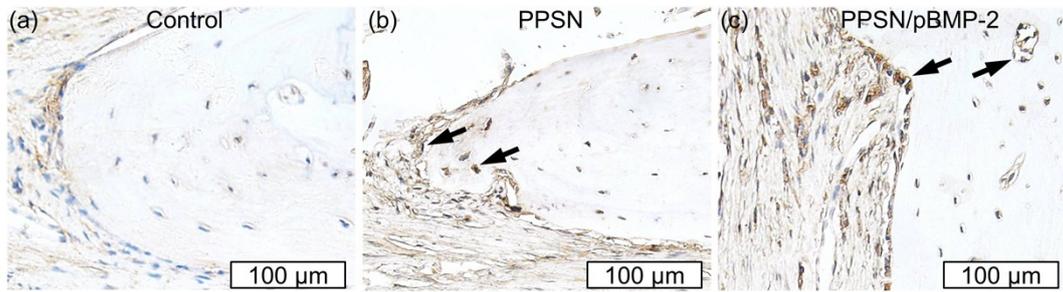


Figure S7. IHC staining of LC3 in the rat calvarial bone defect twelve weeks post-treatment. Control group is rat calvarial bone defect without treatment, PPSN group is treated with 200 μg of PPSN, and PPSN/pBMP-2 group is treated with PPSN/pBMP-2 at weight ratio of 20:1.

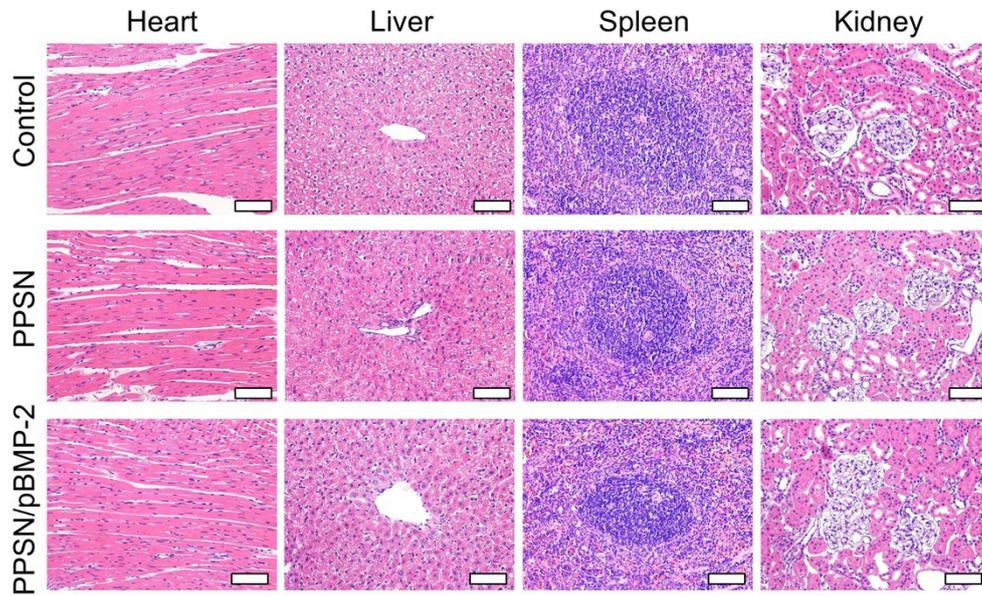


Figure S8. Effects of PPSN or PPSN/pBMP-2 on heart, liver, spleen and kidney by histological evaluation (H&E staining) at the end of 12 weeks after local application of PPSN or PPSN/pBMP-2 at rat calvarial bone defect model *in vivo*. The scale bar is 50 μ m.