

## Supporting Information

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Black Phosphorus Accelerates Bone Regeneration Based on Immunoregulation

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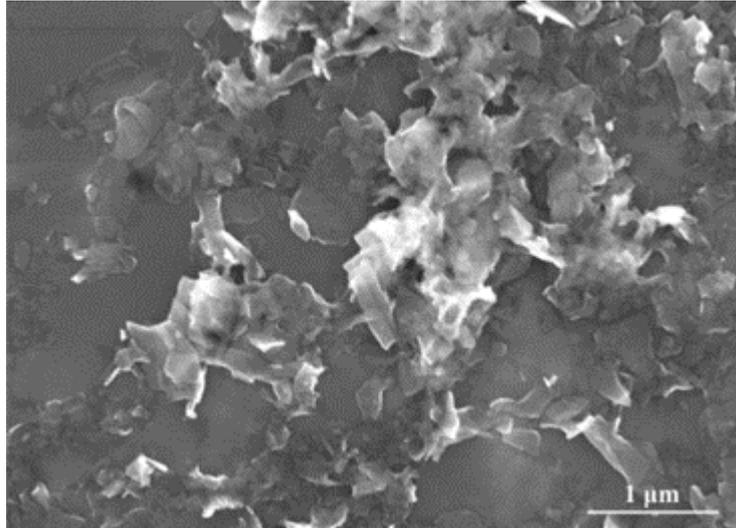
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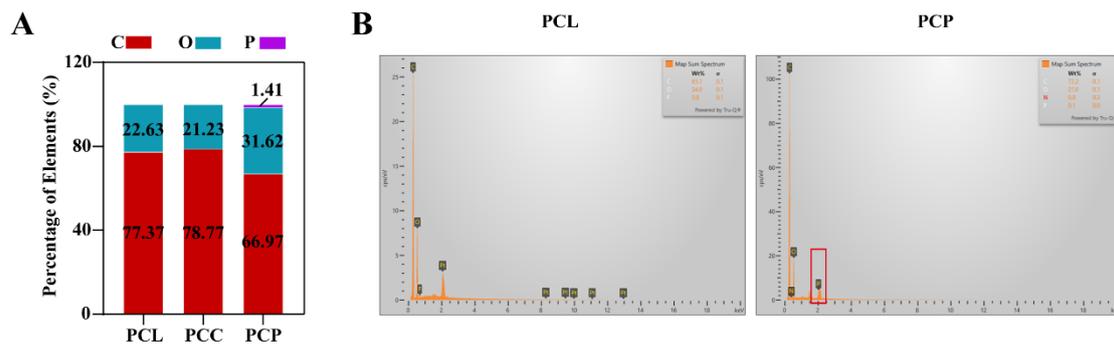
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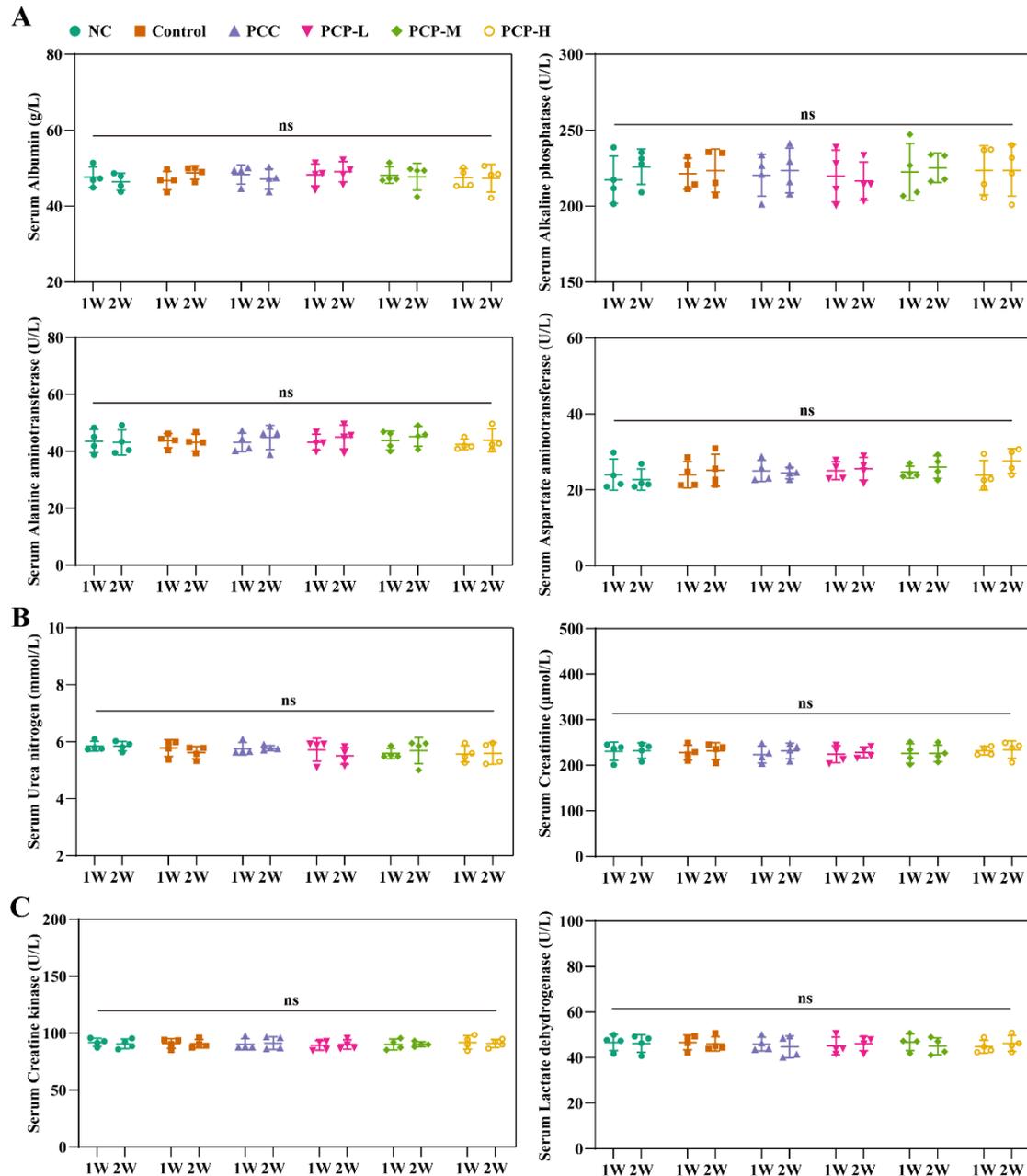
**Keywords:** Black phosphorus; bone regeneration; immunoregulation; RNA sequencing; gene knocking-out



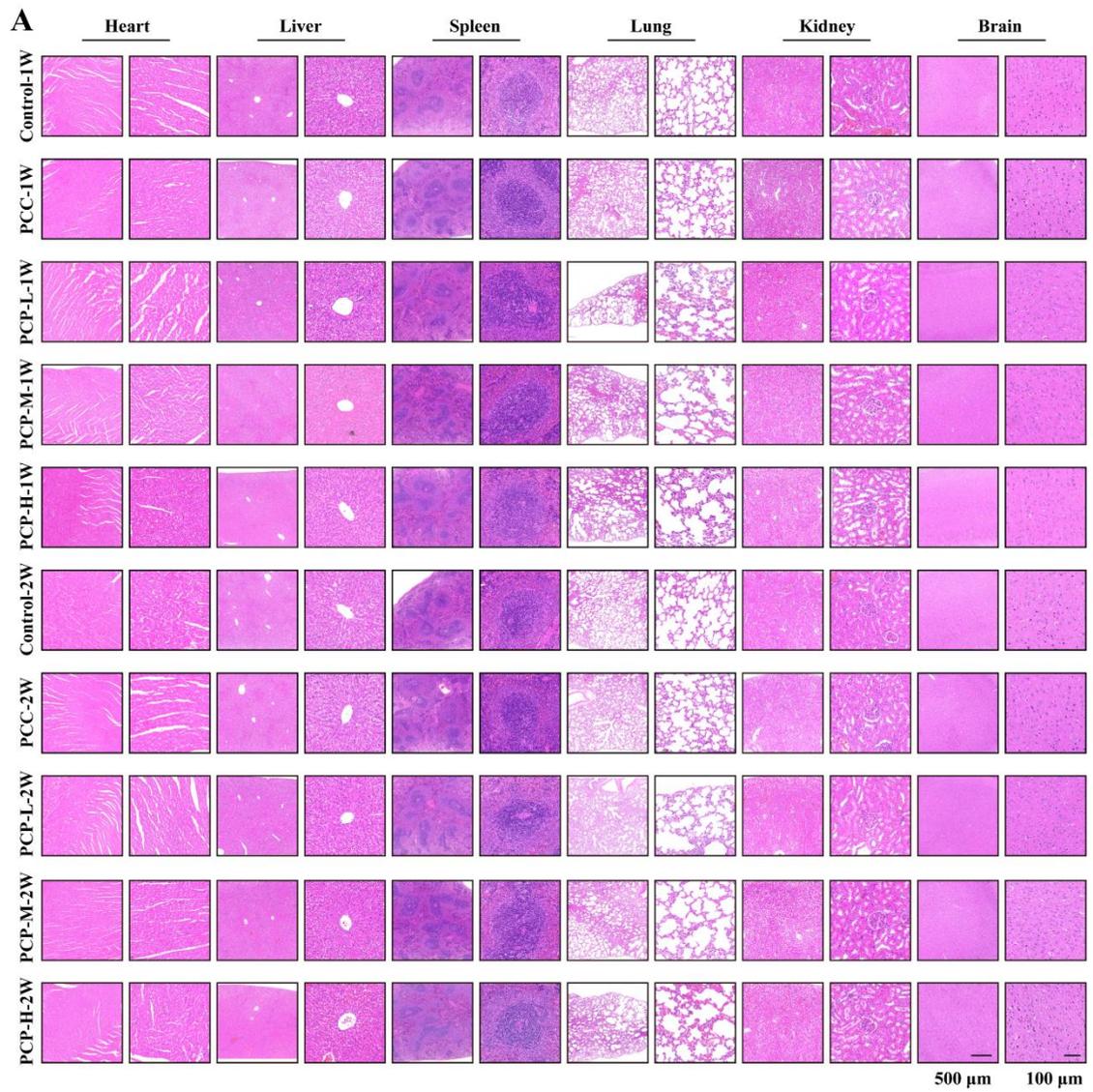
**Figure S1.** Representative SEM images of two-dimensional black phosphorus nanosheets.



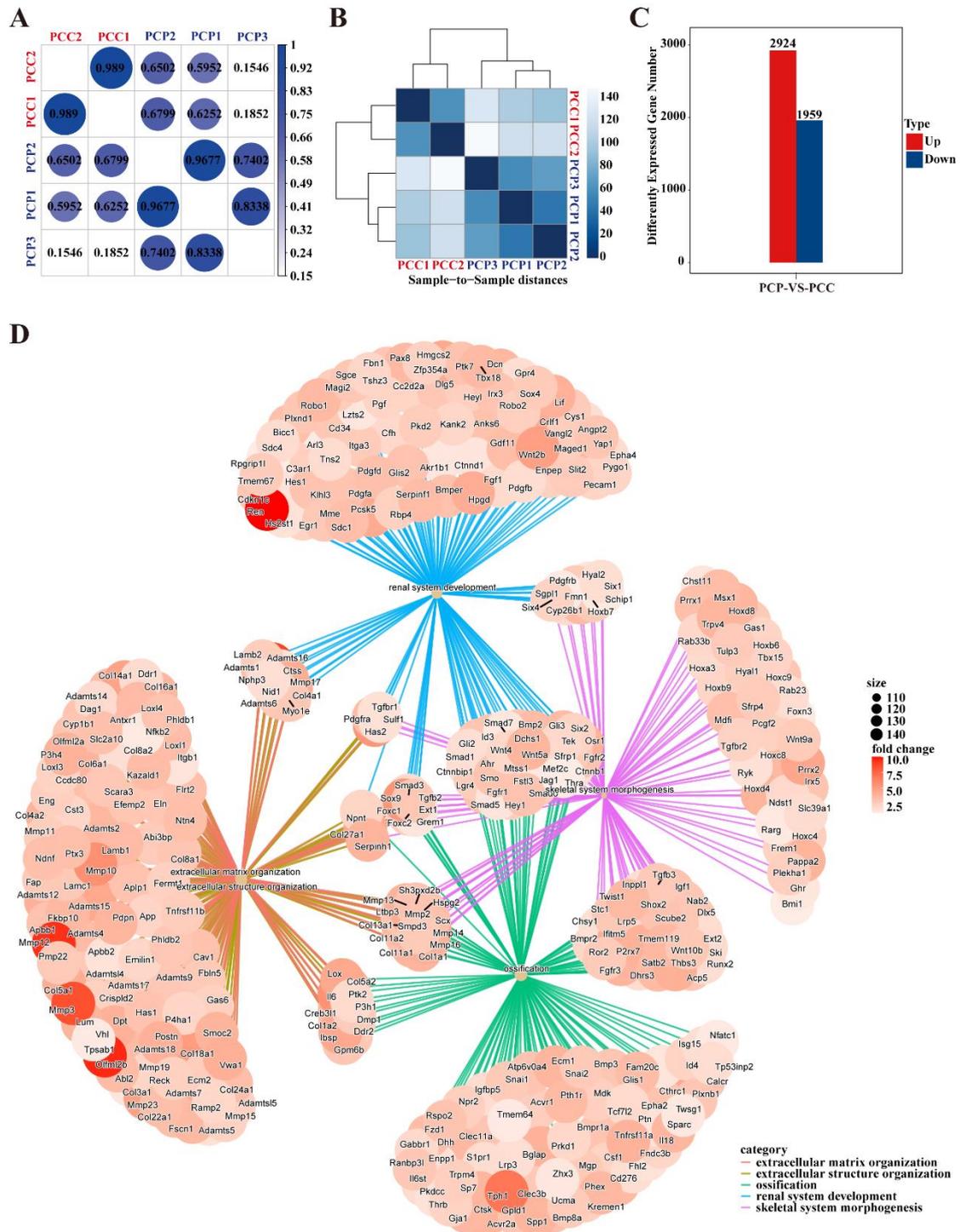
**Figure S2.** Surface energy spectroscopy of PCL and PCP scaffolds.



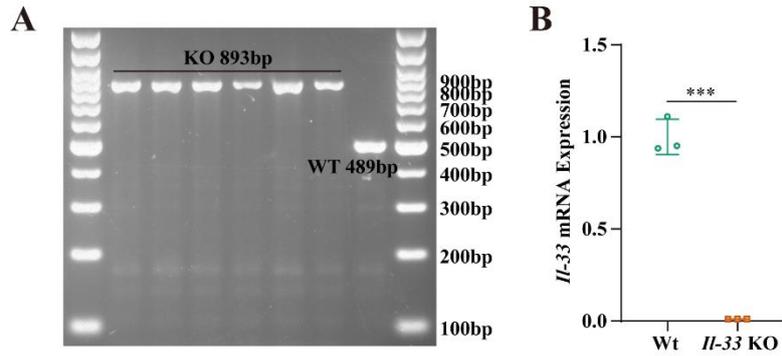
**Figure S3.** Blood biochemical analysis one and two weeks after scaffold implantation in rat femurs. A) Liver function analysis included serum albumin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. B) Renal function analysis included serum urea nitrogen and creatinine. C) Cardiac function analysis includes creatine kinase and lactate dehydrogenase. All data presented as mean  $\pm$  SEM and *P* values were analyzed by two-way ANOVA in A-C. ns, with no significant difference. All data are representative of two to three independent experiments.



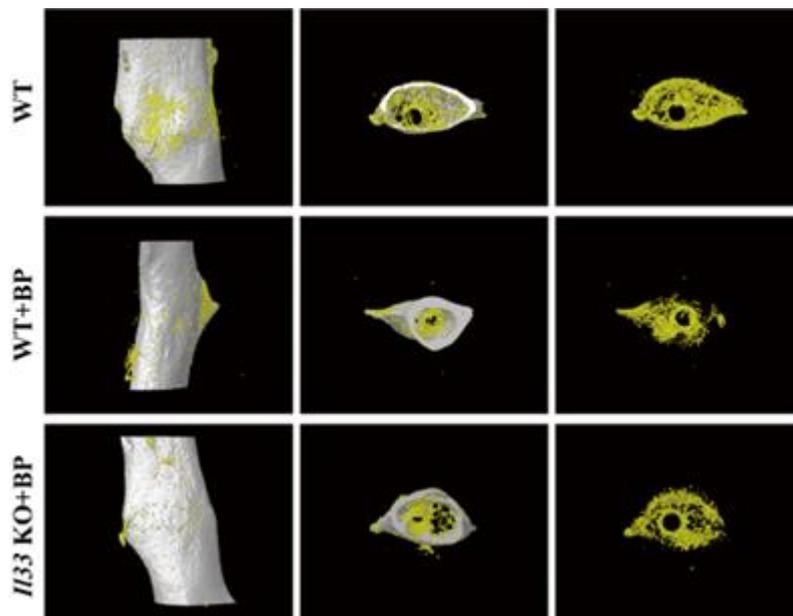
**Figure S4.** HE staining of heart, liver, spleen, lungs, kidneys, and brain tissues one and two weeks after scaffold implantation in rat femurs.



**Figure S5.** Differential gene expression between PCL and PCP groups was analyzed by RNA-seq two weeks after scaffold implantation into the bone defect. **A)** Correlation coefficients analysis of all samples. **B)** sample-to-Sample clustering analysis of all samples. **C)** Number of differentially expressed genes in the PCP group compared to the PCL group. **D)** Brain map analysis shows that differentially expressed genes related to bone regeneration are involved in biological processes.



**Figure S6.** Genetic identification of *Il-33* KO mice and their littermate controls. A) Genotypic gel electropherograms of *Il-33* KO mice and their littermate controls. B) *Il-33* mRNA expression in bone tissue of eight-week-old *Il-33* KO mice and their littermate controls,  $n = 3$ . Data presented as mean  $\pm$  SEM and  $P$  values was analyzed by two-tailed t-tests in B. \*\*\* $P < 0.001$ . All data are representative of two to three independent experiments.



**Figure S7.** Micro-CT images of WT mice, BP treated WT mice, and BP treated *Il-33* KO mice eight weeks after fracture.

**Table S1. Genotyping primers for *Il-33* KO mice**

Primer name	Primer sequence (5' $\rightarrow$ 3')	PCR product size
ex2-Forward	CACTAAGACTACTCAGCCTCAG	

WT-Reverse	CGGTGATGCTGTGAAGTCTG	WT: 489 bp
KO-Reverse	GTGTTCTGCTGGTAGTGGTCG	KO: 893 bp

**Table S2. Primer sequences for real time-PCR**

Gene		Primer sequence (5' - 3')	Species
<i><math>\beta</math>-actin</i>	Forward	GCTGCACCCTTCAACAACCTG	Rat
	Reverse	GTGATCCCGGTCAAAGTGGT	
<i>Il-33</i>	Forward	GCAGCTGCAAGGAAGGGAGAAATCA	Rat
	Reverse	TGGTGAGGCCAGAACGGAGCC	
<i>Alp</i>	Forward	CCCAAGAGACCTTGAAAAATGCCCTGA	Rat
	Reverse	TGTCACTGTGGAGACGCCCATACCAT	
<i>Bmp2</i>	Forward	ATGGTGGCCGGGACCCGCTGT	Rat
	Reverse	CGGGACAAGGGGCGGCCGGA	
<i>Coll</i>	Forward	CCAACGACGTCGAACTTGTTGCTGA	Rat
	Reverse	CAATGTCAAGGAATGGCAGGCGAGA	
<i>Opn</i>	Forward	TTGGCTGAAGCCTGACCCATCTCA	Rat
	Reverse	TCGTCATCATCGTCCATGTGGTCA	
<i><math>\beta</math>-actin</i>	Forward	GGCTGTATTCCCCTCCATCG	Mouse
	Reverse	CCAGTTGGTAACAATGCCATGT	
<i>Il-33</i>	Forward	ATGGGAAGAAGCTGATGGTG	Mouse
	Reverse	CCGAGGACTTTTTGTGAAGG	
<i>Alp</i>	Forward	CCAACCTTTTTGTGCCAGAGA	Mouse
	Reverse	GGCTACATTGGTGTTGAGCTTTT	
<i>Bmp2</i>	Forward	GGGACCCGCTGTCTTCTAGT	Mouse
	Reverse	TCAACTCAAATTCGCTGAGGAC	
<i>Ocn</i>	Forward	CTGACCTCACAGATCCCAAGC	Mouse
	Reverse	TGGTCTGATAGCTCGTCACAAG	
<i>Runx2</i>	Forward	ATGCTTCATTCGCCTCACAAA	Mouse

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	Reverse	GCACTCACTGACTCGGTTGG	
<i>Coll</i>	Forward	GTCCTCTTAGGGGCCACT	Mouse
	Reverse	CCACGTCTCACCATTGGGG	
<i>Tnf-<math>\alpha</math></i>	Forward	GACGTGGAAGTGGCAGAAGAG	Mouse
	Reverse	TTGGTGGTTTGTGAGTGTGAG	
<i>Il-10</i>	Forward	GCTCTTACTGACTGGCATGAG	Mouse
	Reverse	CGCAGCTCTAGGAGCATGTG	
<i>Il-6</i>	Forward	TAGTCCTTCCTACCCCAATTTCC	Mouse
	Reverse	TTGGTCCTTAGCCACTCCTTC	
<i>Igf-1</i>	Forward	CTGGACCAGAGACCCTTTGC	Mouse
	Reverse	GGACGGGGACTTCTGAGTCTT	

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