Supplementary Materials

Punicalagin ameliorates wear-particle-induced inflammatory bone destruction by bi-directional regulation of osteoblastic formation and osteoclastic resorption

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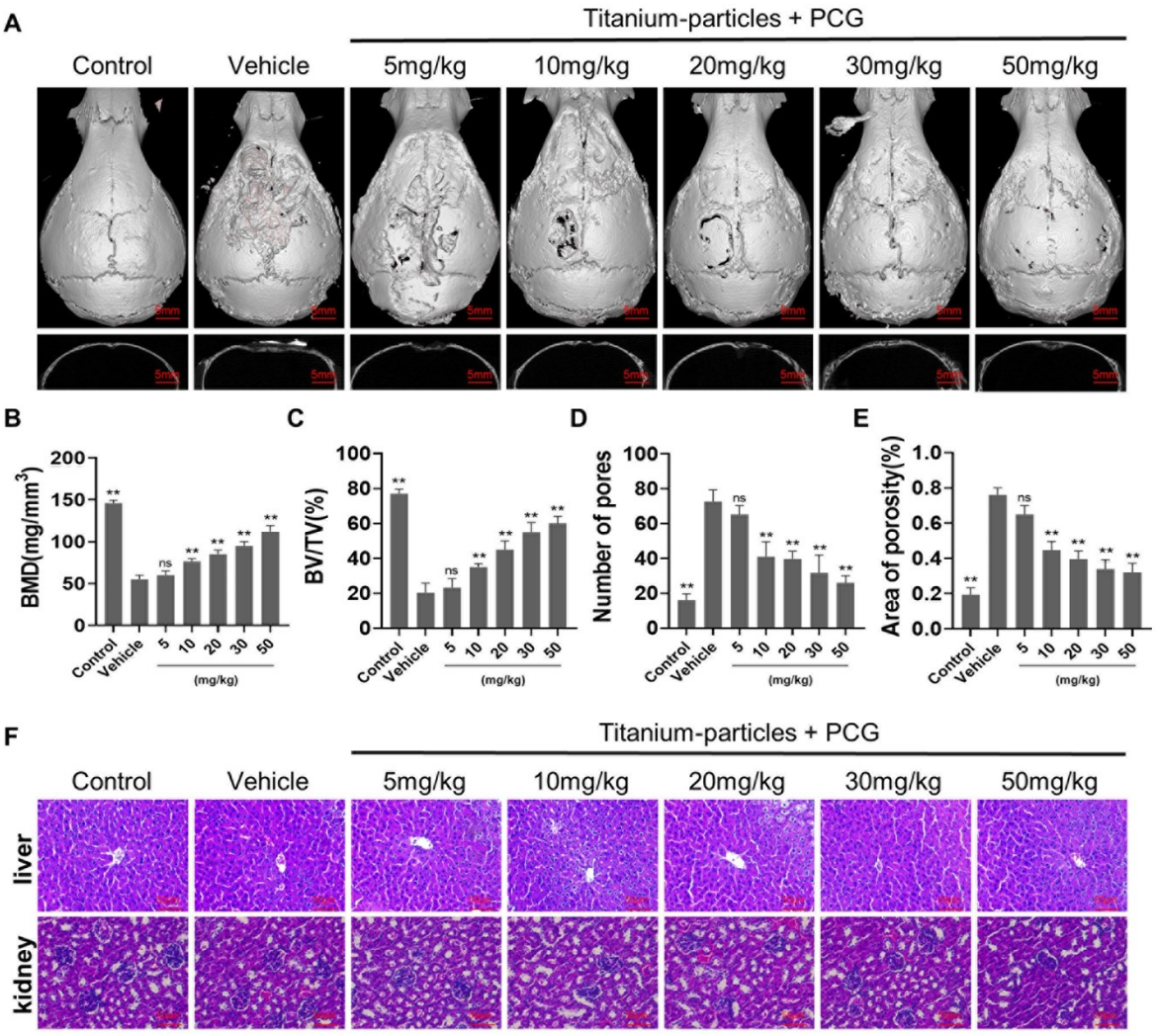

Fig. S1. PCG treatment attenuated Ti particle-induced osteolysis in a dose-dependent manner in vivo. (A) 3D & 2D rebuilt images, Scale bar, 5 mm (B) Quantitative analyses of BMD (mg/mm3). (C) Quantitative analyses of BV/TV (%). (D) Number of pores. (E) Area of porosity (%). (F) H&E staining images of liver and kidney collected from the control group, vehicle group and titanium particles + PCG group mice with different treated concentration of PCG (5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg and 50 mg/kg), Scale bar, 50μm. All PCG treated groups were treated with 30 mg titanium particles. n=5. All data were expressed as the mean ± SD, **$p < 0.01$, ns: no significance, compared with the vehicle group.

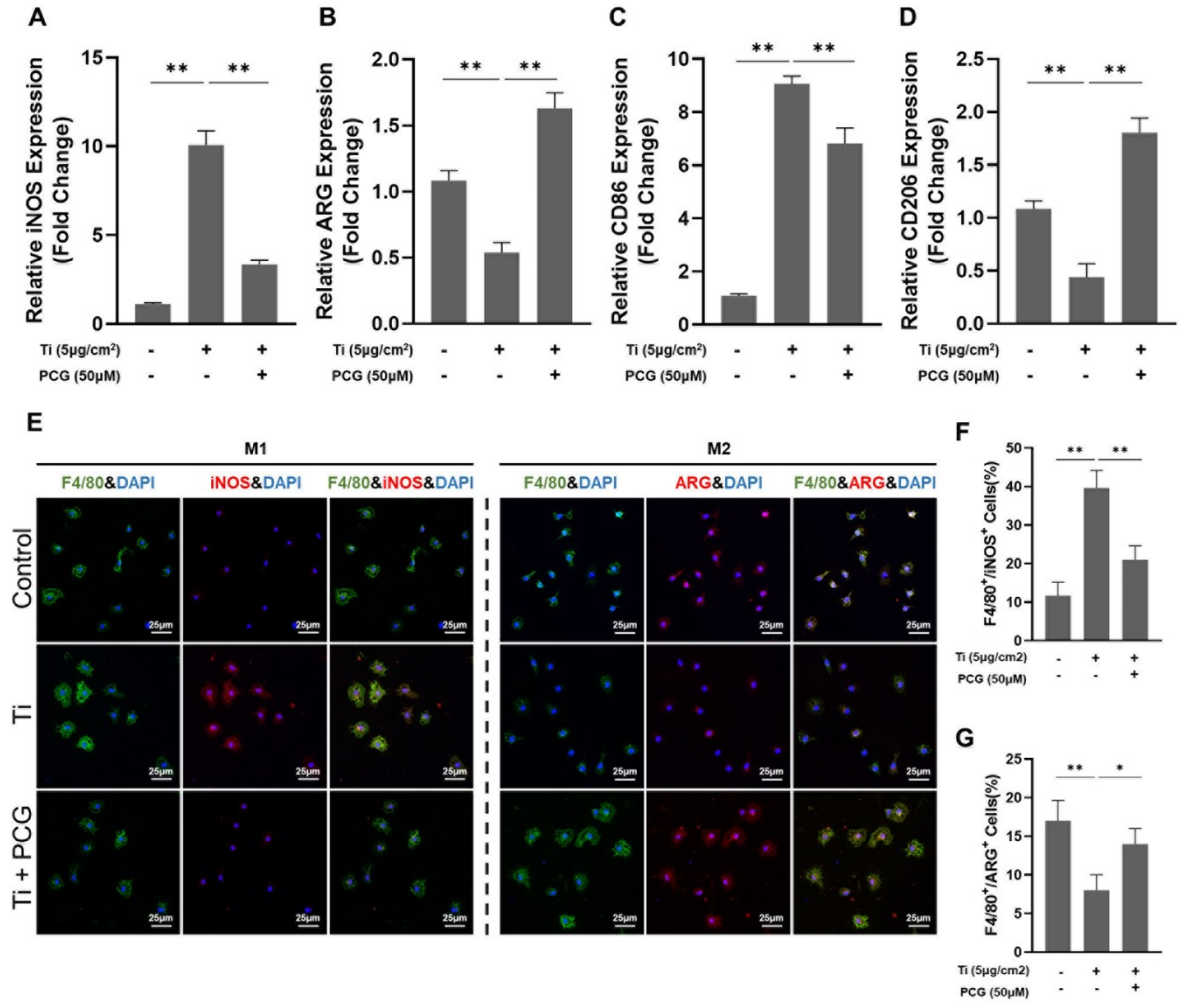

Fig. S2. PCG treatment reduced inflammatory reaction via reversing Ti particle induced M1 macrophage polarization in vitro. (A-D) The mRNA expression levels of M1 (iNOS, CD86) and M2 (ARG, CD206) macrophage phenotype. n=3. (E) Representative images of the Ti induced macrophages after coimmunostaining: red (M1 marker: iNOS and M2 marker: Arg-1), green (F4/80), and blue (nuclei). (F, G) Percentage of double-positive macrophages (n = 6 per group). The concentration of titanium particles was 5μg/cm^2. The concentration of PCG was 50 μM. Scale bar, 25 μm. All data were expressed as the mean ± SD, *p<0.05, **p < 0.01, compared with the Ti group.

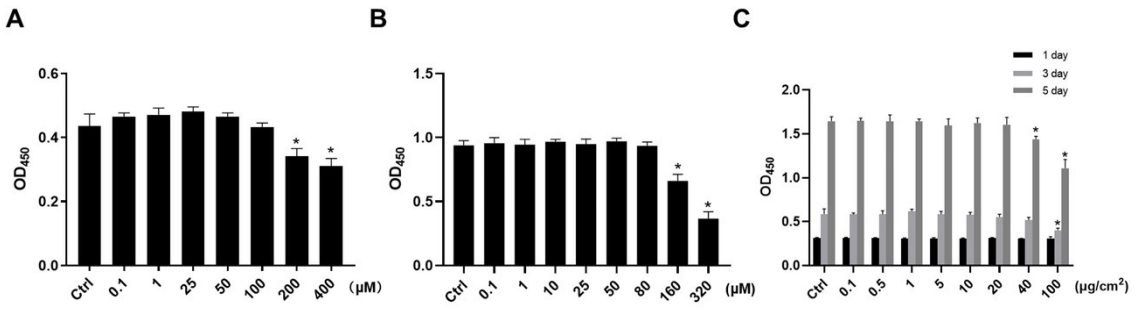

Fig. S3. Cell viability assay analysis. (A) Cell viability after 3 days of PCG stimulation in MC3T3-E1 cells. Cell viabilities were measured by CCK-8 assays. *p<0.05, compared with control group. (B) Cell viability after 3 days of PCG stimulation in RAW264.7 cells. Cell viabilities were assessed by CCK-8 assays. *p<0.05, compared with control group. (C) Cell viability after 1, 3, and 5 days of titanium particles stimulation. Cell viabilities were assessed by CCK-8 assays. *p<0.05, compared with control group separately in 1, 3, and 5 day. n=3. All data were expressed as the mean ± SD.

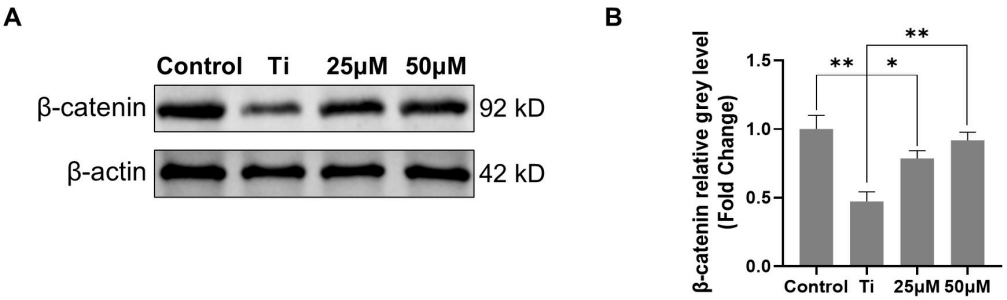

Fig. S4. PCG treatment rescued the Ti particle-induced inhibition of β-catenin in MC3T3-E1 cells. (A) Western blot analysis of expression levels of β-catenin. (B)The relative grey levels of β-catenin. The protein grayscale value was analyzed by ImageJ software. The concentration of titanium particles was 5μg/cm^2. Both PCG-treated groups contained 5μg/cm^2 titanium particles. n=3. All data were expressed as the mean± SD, *$p < 0.05$, **$p < 0.01$, compared with the Ti group.

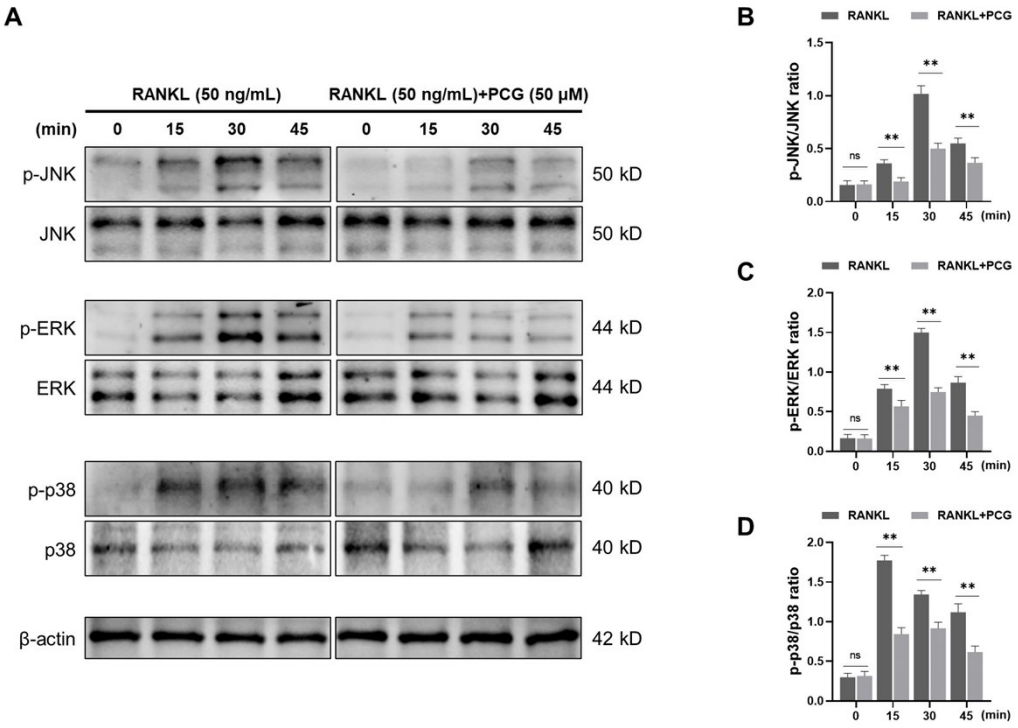

Fig. S5. PCG treatment inhibited osteoclastogensis via MAPK signaling pathway. (A) Western blot analysis of expression levels of JNK, ERK, p38 and phosphorylation of JNK, ERK, p38. (B-D) The corresponding relative grey level ratio of p-JNK/ JNK, p-ERK/ ERK, p-p38/ p38. The protein grayscale value and ratio were analyzed by ImageJ software. n=3. All data were expressed as the mean± SD, $^{**}p < 0.01$, ns: no significance, compared with RANKL group in different time, separately.