Supporting Information

Chitosan derived nitrogen-doped carbon dots suppress osteoclastic osteolysis *via* downregulating ROS

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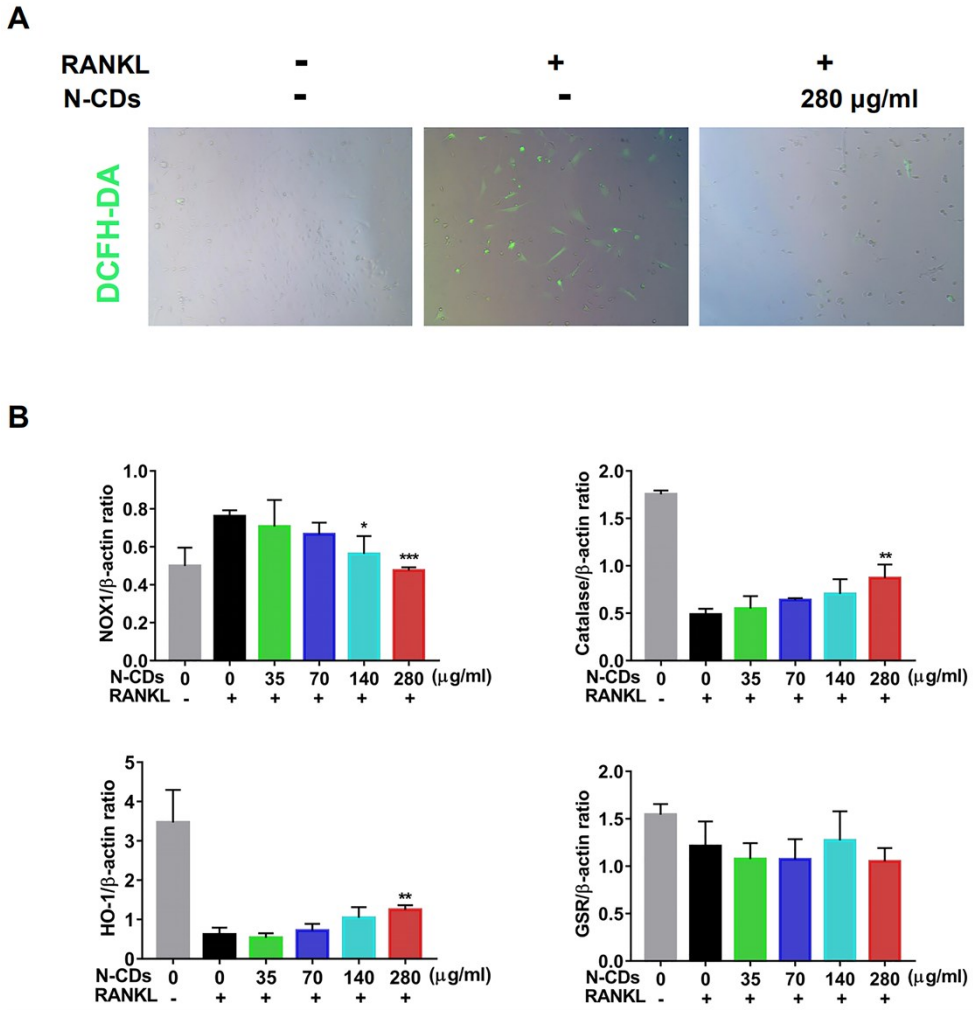
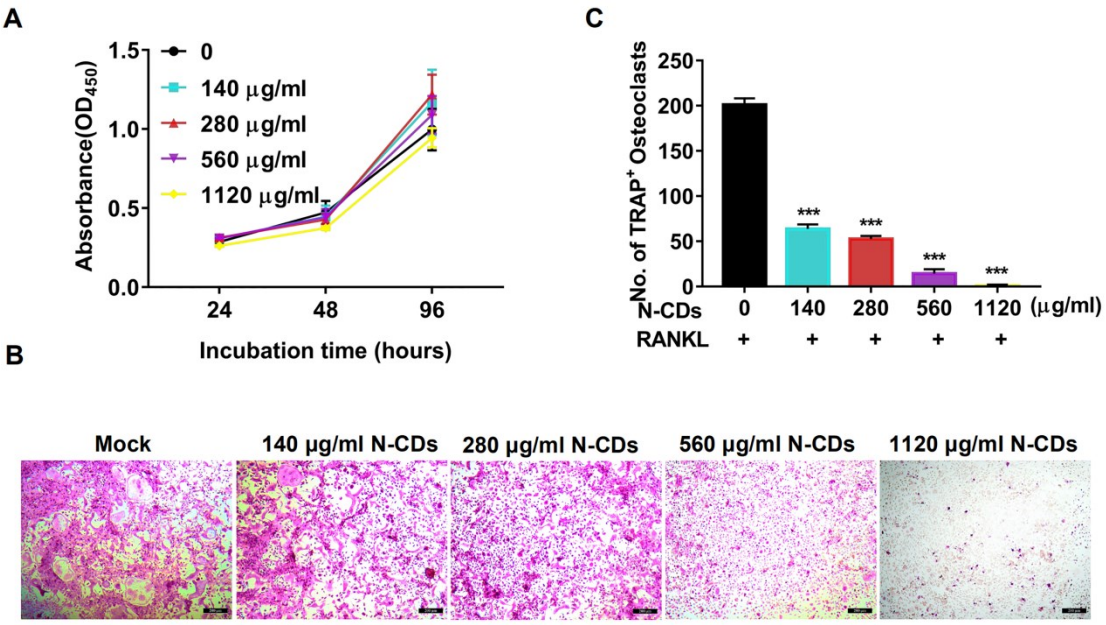
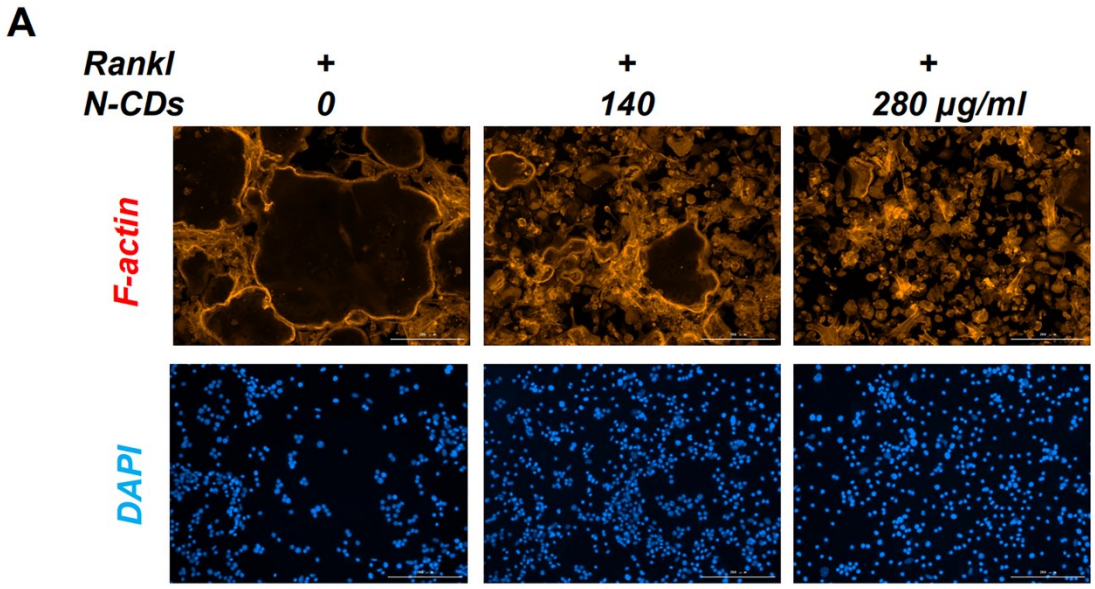


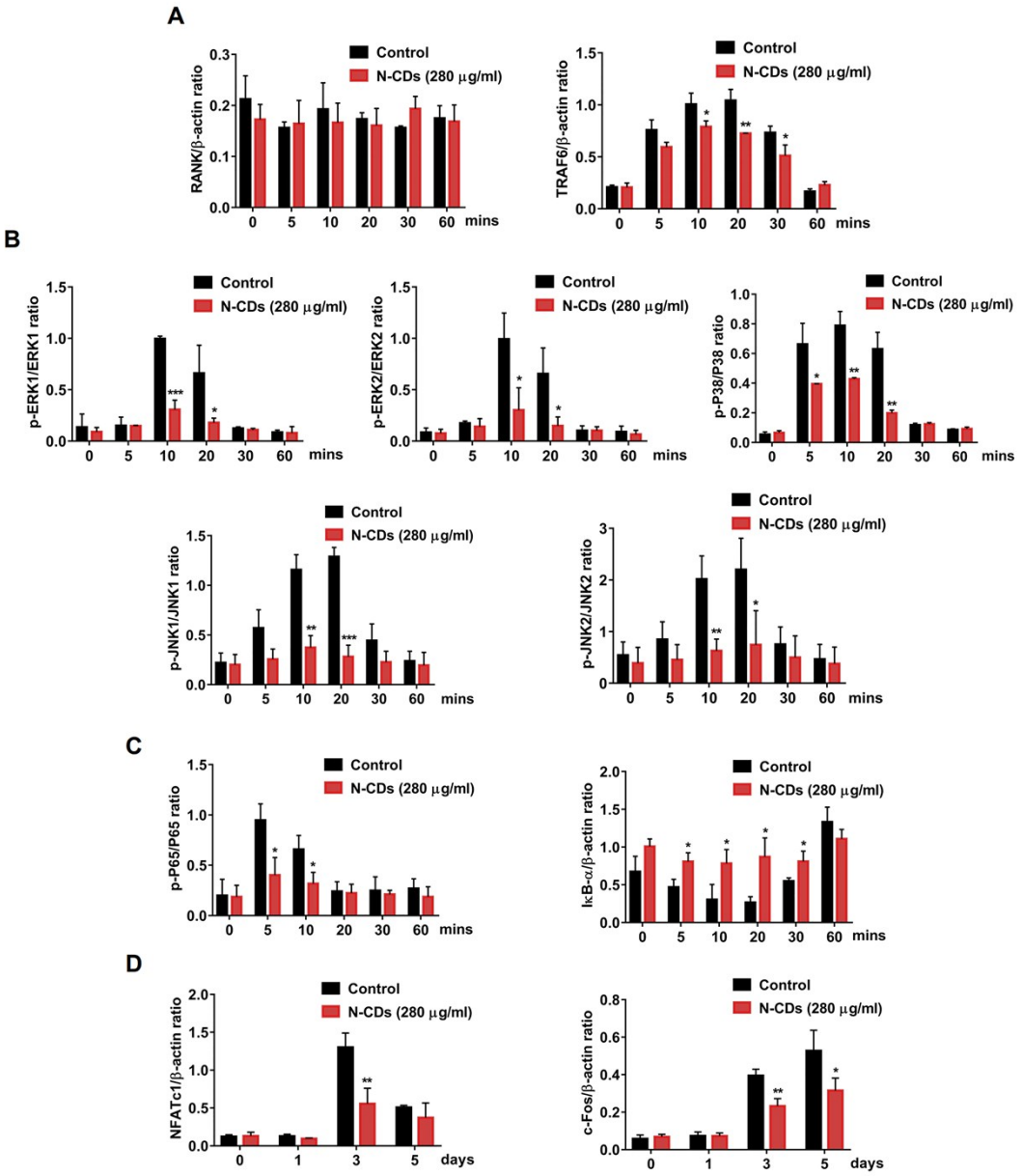
S1. N-CDs downregulated ROS. (A) BMMs were treated with or without N-CDs for 48 hours, and then intracellular reactive oxygen species (ROS) was detected by DCFH-DA. (B) The quantification of the band intensity of NOX1, Catalase, HO-1, and GSR relative to the ratio of β-actin (n=3). All experimental data were expressed as mean ± SD. ***P < 0.001, **P < 0.01, *P < 0.05.



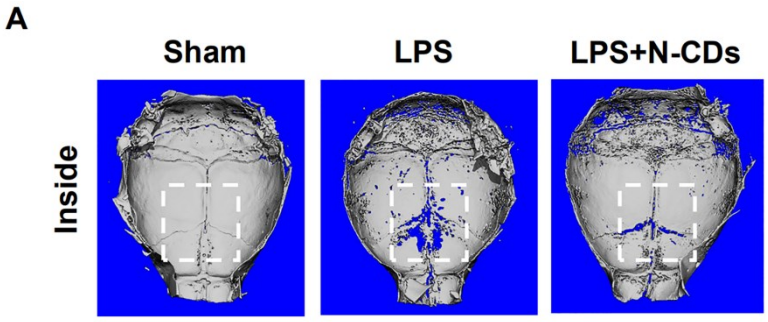
S2. N-CDs inhibited BMMs differentiation. (A) MTS assay was used to detect the cell activity of BMMs after treatment with N-CDs for 24 hours, 48 hours and 96 hours. (B) TRAP staining of BMMs treated with N-CDs, 30 ng/ml M-CSF and 50 ng/ml RANKL for 5 days. (C) Quantitative analysis of TRAP positive multinucleated cells (nucleus > 3). All experimental data were expressed as mean ± SD (n=3). ***P < 0.001, **P < 0.01, *P < 0.05.



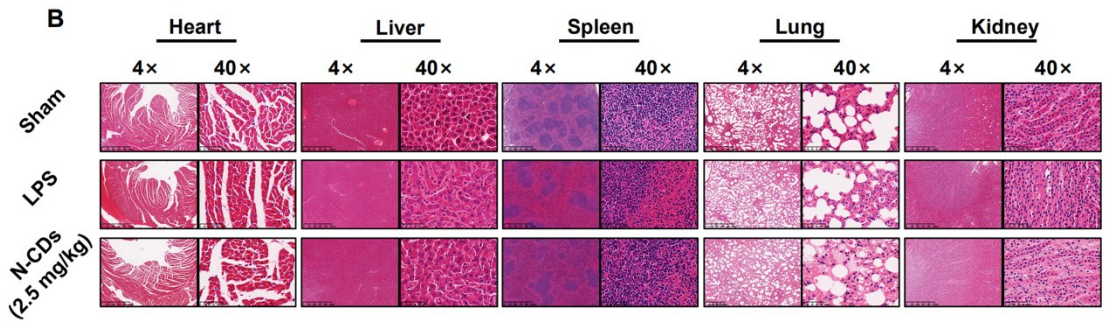
S3. N-CDs suppressed OCs formation. (A) Representative images of F-actin and nuclear staining of OCs.



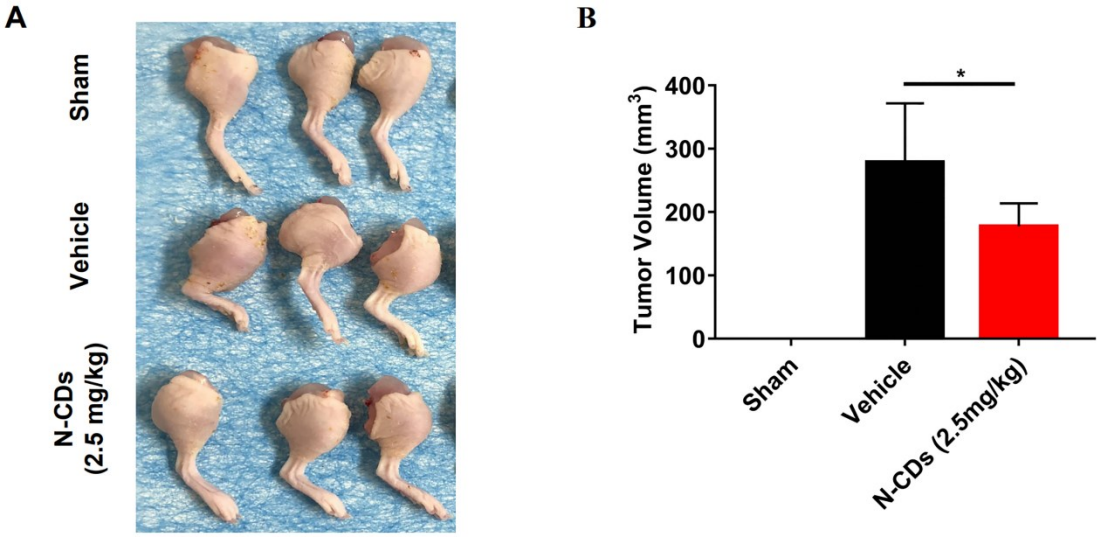
S4. N-CDs suppressed ROS downsteam signaling pathway. (A) The ratio of band intensity of RANK and TRAF6 to β-actin (n=3) was quantitatively analyzed. (B) The ratios of band intensity of p-ERK, p-p38, and p-JNK to ERK, p38 and JNK (n=3), respectively, were quantitatively analyzed. (C) Quantitative analysis of the ratio of band intensity of p-p65 and IκB-α to p65 and β-actin (n=3). (D) The ratio of band intensity of NFATc1 and c-Fos to β-actin (n=3) was quantitatively analyzed. All experimental data were expressed as mean ± SD. ***P < 0.001, **P < 0.01, *P < 0.05.



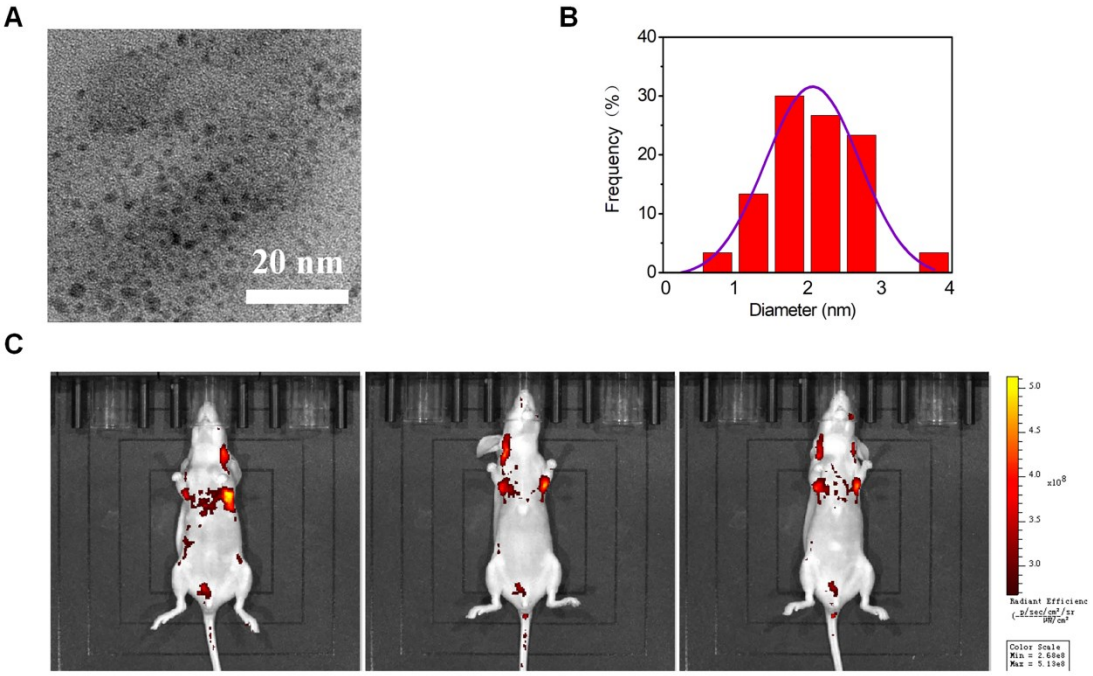
S5. N-CDs prevented calvarial LPS induced osteoclastic bone loss. (A) The representative μCT images of the inside of calvarials of each group were reconstructed.



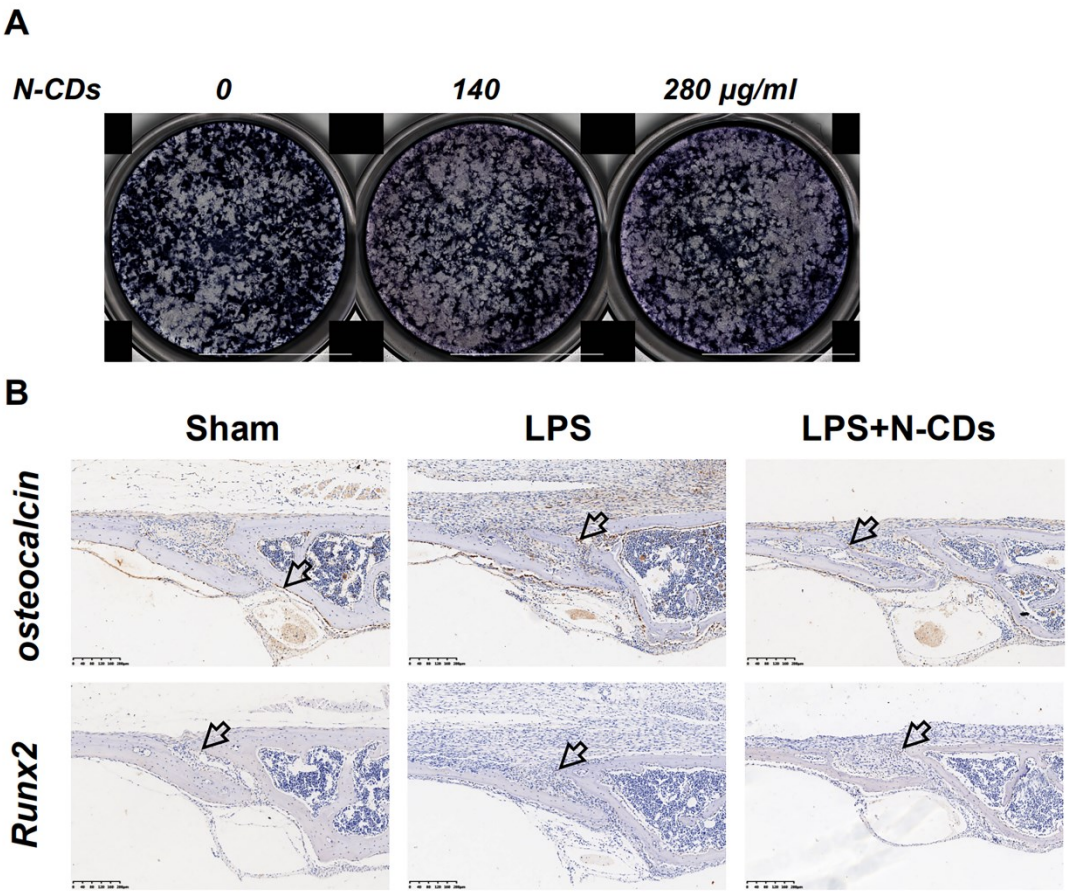
S5. N-CDs prevented calvarial LPS induced osteoclastic bone loss. (B) Representative images of hematoxylin and eosin staining of heart, liver, spleen, lung and kidney.



S6. N-CDs prevented breast cancer induced osteolysis. (A) Representative image of MDA-MB-231 cells injected into bone marrow cavity of tibia of nude mice (n=6). (B) Quantification of tumor volume in each group of nude mice. All experimental data were expressed as mean ± SD. *P < 0.05.



S7. Size and *in vivo* fluorescence characterization of N-CDs. (A) TEM image of the N-CDs. (B) N-CDs with size distribution histogram. (C) *In vivo* fluorescence images of nude mice injected with 100ul N-CDs (2.5 mg/kg) through tail vein. Images are taken every 30 minutes. The color bar represents the fluorescence intensity.



S8. N-CDs had no effect on osteogenesis in vitro and *in vivo*. (A) Representative ALP images of osteoblast culture treated with N-CDs. (B) Representative RUNX2 and osteocalcin images of immunohistochemical staining.