Manganese superoxide dismutase is required to maintain osteoclast differentiation and function under static force

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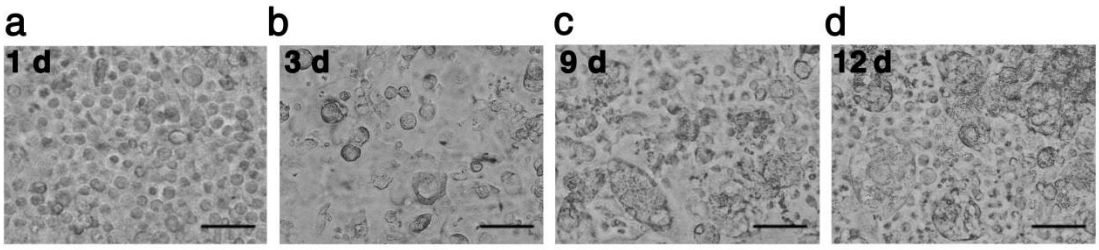
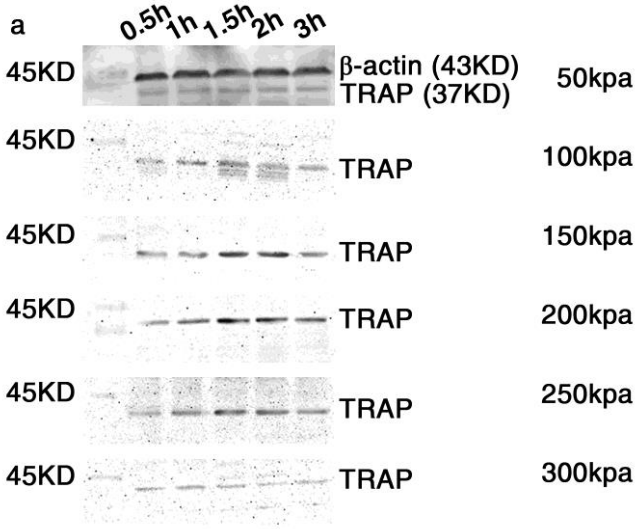
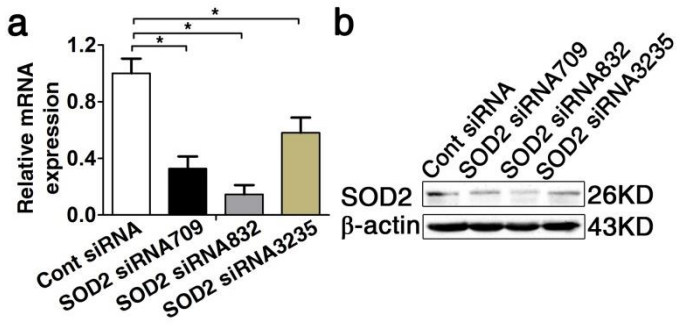


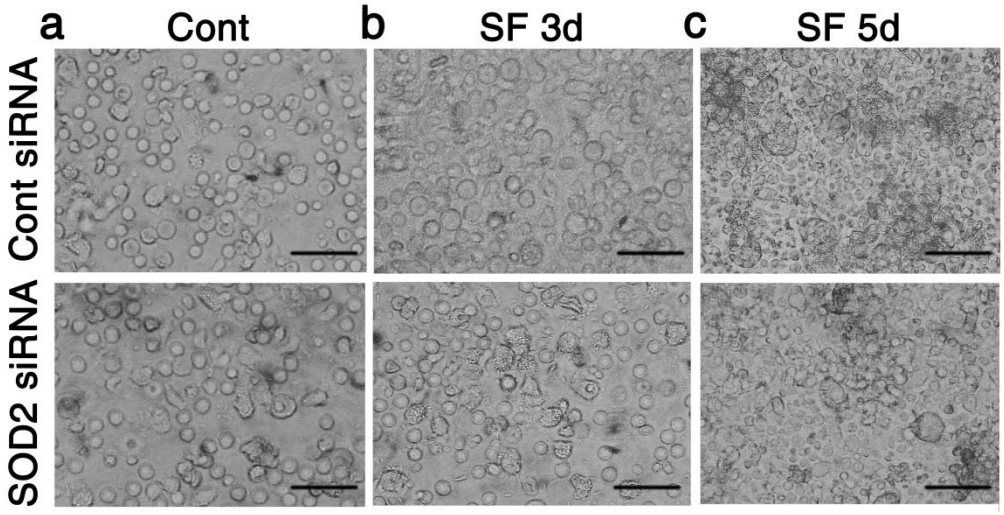
Supplementary Figure S1. Cell morphological changes of differentiation into OCs. Cell morphology of HMNCs changed significantly after the first 3 d of OC induction. At day 5, monocytes began fusing, enlarged and showed 2 to 3 cell nuclei, increasing to 3 to 7 nuclei on day 9. After 12 d of induction, numerous osteoclasts appeared with various morphologies and varying numbers of nuclei ranging from 3 to 20. Some of the cells even had slender pseudopods. magnification ×200.



Supplementary Figure S2. Expression of TRAP for six force levels with five different force duration times evaluated via western blotting. Full-length blots of Western blot, the protein lysates were separated by 12% SDS-PAGE and electrophoretically transferred to a PVDF membrane (Millipore).



Supplementary Figure S3. Transfection efficiency of SOD2 in RAW264.7. (A, B), Efficiency of RNA interference was verified by RT-PCR and western blotting analysis. Si-709, si-832 and si-3235 represent the three different siRNA pairs used for SOD2 transfection. Among these, Si-832 performed most effectively and was used in all subsequent experiments. Cont siRNA, transfected negative control siRNA; SOD2 siRNA, knockdown SOD2 expression.



Supplementary Figure S4. Cell morphology of SOD2 down-regulated RAW264.7 subjected to static force loading. After down-regulating the expression level of SOD2, static force (SF) of 150 kpa for 1.5 h was exerted on RAW264.7. Cell morphologies were not significantly different between these cells and the control group during the first 3 days, but the total number of OCs was significantly reduced in the Si-SOD2 group after being induced for 5 days. magnification ×200. Cont siRNA, transfected negative control siRNA; SOD2 siRNA, knockdown SOD2 expression; SF, static force.