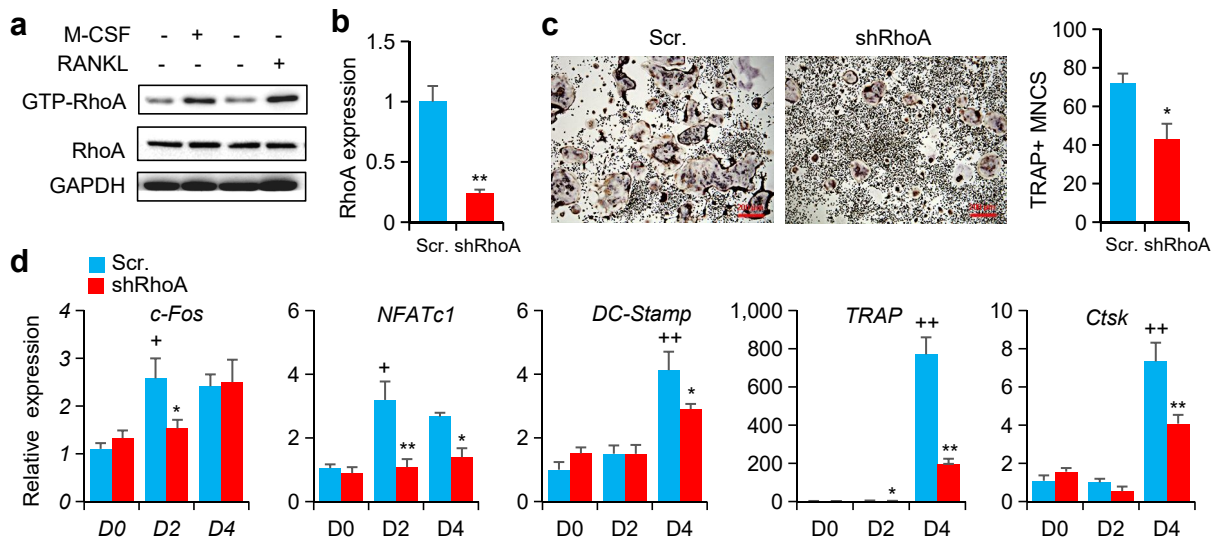
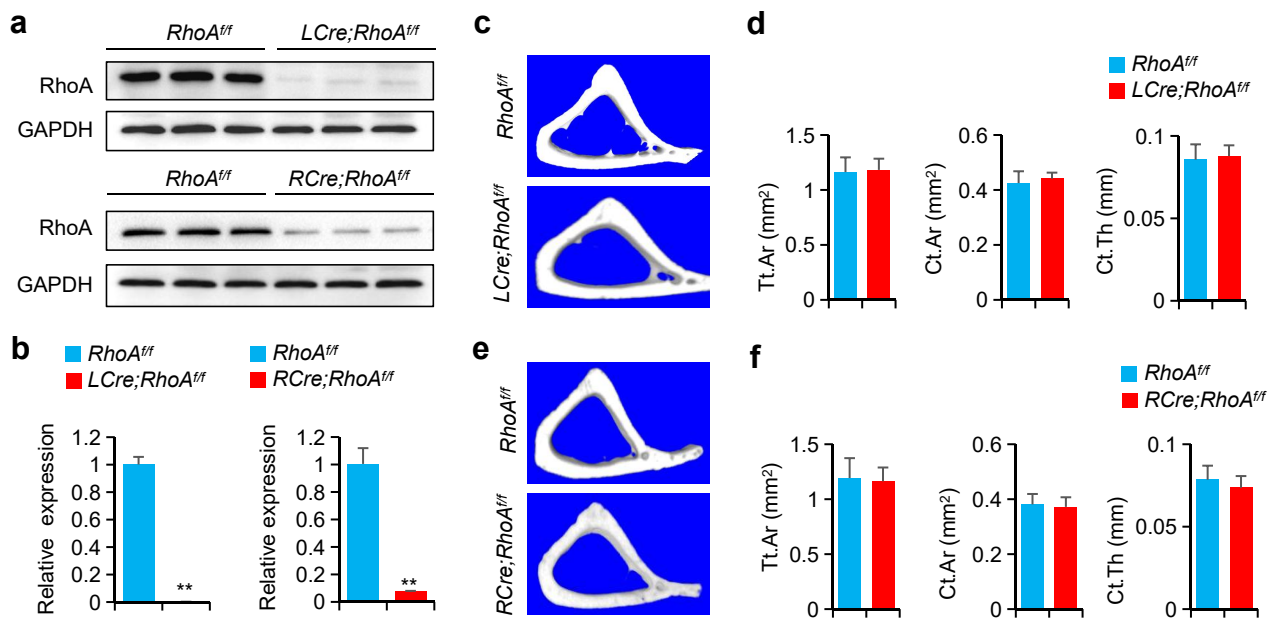




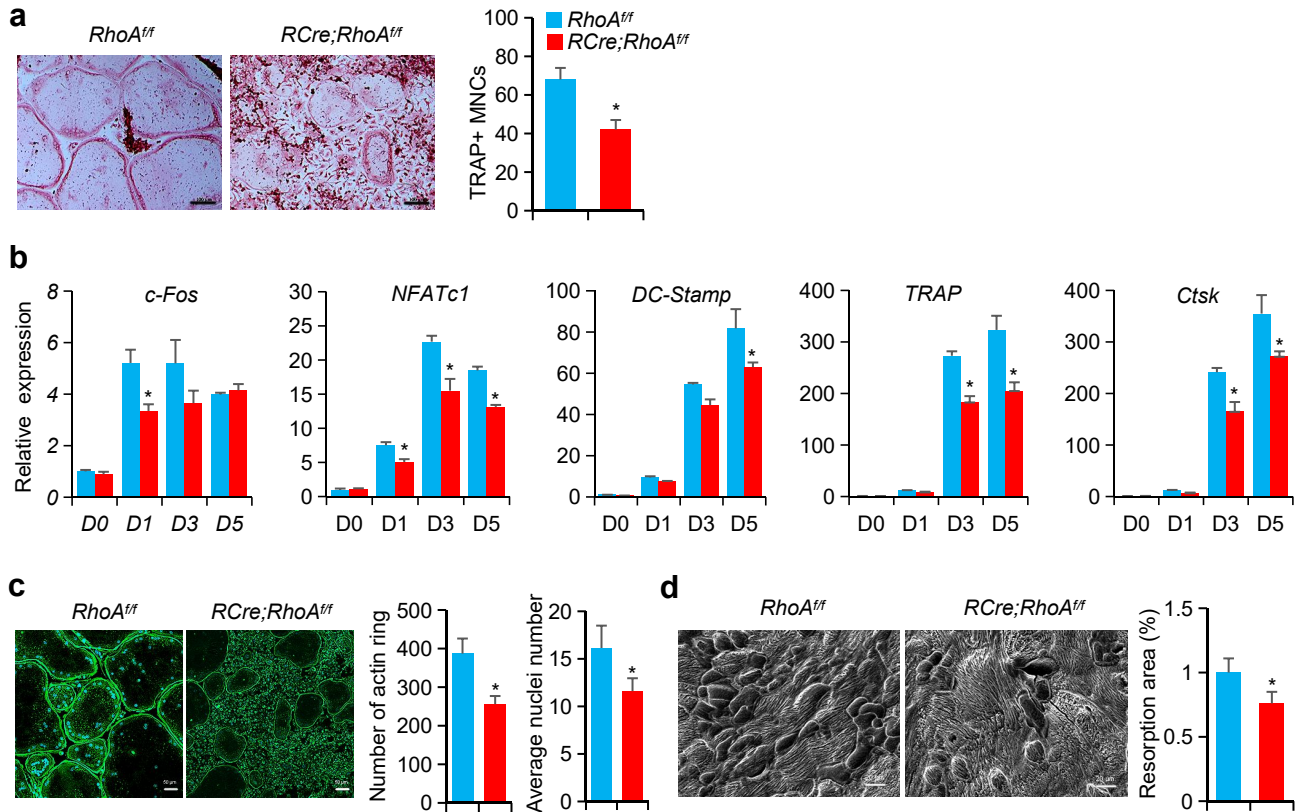
### Supplementary Figure 1. Knock down of RhoA inhibits osteoclastogenesis in RAW264.7 cells.

(a) The effect of RANKL and M-CSF stimulation on RhoA activity in pre-osteoclasts. (b) The qRT-PCR analysis following scrambling small hairpin RNA (shScr.) and RhoA shRNA (shRhoA) knockdown,  $n = 3$ . (c) Representative TRAP staining images of shRhoA infected RAW264.7 cells treated by RANKL for 4 days,  $n = 6$ , Scale bars, 200  $\mu\text{m}$ . (d) The expression levels of the osteoclast-specific gene in shRhoA infected RAW264.7 cells for indicated times carried out with RT-qPCR,  $n = 3$ . Mean  $\pm$  s.d., +,\* $P < 0.05$ , ++,\*\* $P < 0.01$ , Student's t-test or ANOVA analysis.



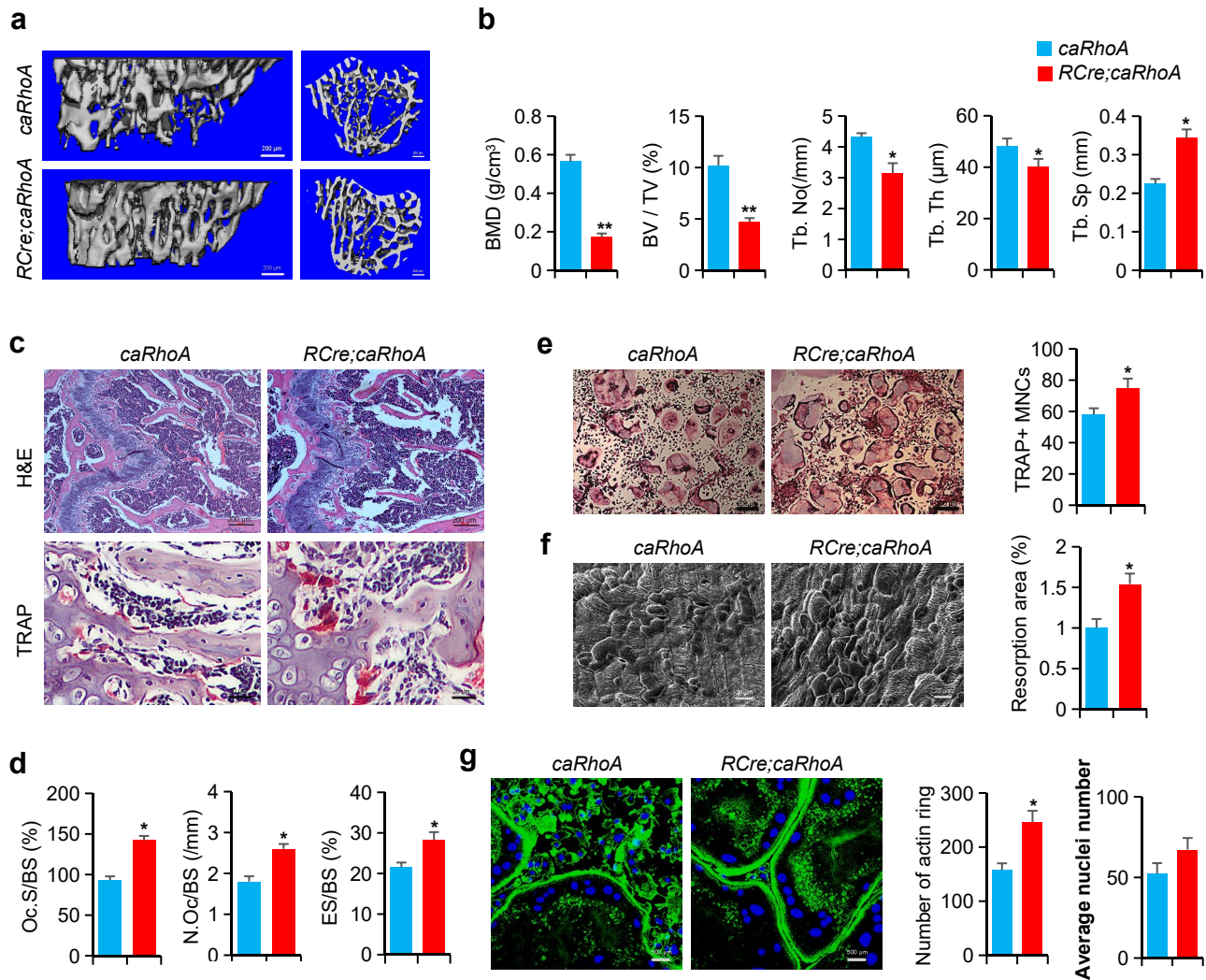
### Supplementary Figure 2. Micro-CT measurement of cortical bone.

(a) The western blotting of RhoA in BMMs from *LCre;RhoA<sup>fl/fl</sup>* and *RCre;RhoA<sup>fl/fl</sup>* mice. (b) The qRT-PCR of RhoA in BMMs from *LCre;RhoA<sup>fl/fl</sup>* and *RCre;RhoA<sup>fl/fl</sup>* mice. (c, e) 3D Micro-CT reconstructions of the tibial midshaft region. (d, f) Micro-CT measurement of cortical bone structural parameters from the midshaft region. n=6, Tt.Ar, total cross-sectional area; Ct.Ar, cortical bone area; Ct.Th, cortical thickness. Mean  $\pm$  s.d., \*\* $P < 0.01$ , Student's t-test.



### Supplementary Figure 3. Loss of *RhoA* in osteoclast inhibited osteoclastogenesis.

(a) Representative TRAP staining images of BMMs from *RhoA<sup>f/f</sup>* and *RCre;RhoA<sup>f/f</sup>* mice cultured with M-CSF and RANKL. The osteoclast numbers were counted (right).  $n=6$ , Scale bars, 100  $\mu\text{m}$ . (b) Expression of osteoclast-specific genes in *RhoA<sup>f/f</sup>* and *RCre;RhoA<sup>f/f</sup>* mice osteoclasts,  $n=6$ . (c) Representative Phalloidin staining images of BMMs, and the quantification of F-actin ring per well and nuclei per osteoclast.  $n=6$ , Scale bars, 50  $\mu\text{m}$ . (d) SEM analysis and quantification of bone resorption area of bone slides,  $n=6$ , Scale bars, 20  $\mu\text{m}$ . Mean  $\pm$  s.d., \* $P < 0.05$ , Student's t-test or ANOVA analysis.



**Supplementary Figure 4. *RhoA* gain-of-function in the early stage of osteoclast differentiation enhanced osteoclast activity and decreases bone mass.**

(a) Representative Micro-CT images of 3-month-old *caRhoA* and *RCre;caRhoA* mice tibias. (b) The parameters of tibias growth plate trabecular bone, n=6. (c) Representative H&E and TRAP staining images of mice femurs. Scale bars, 200  $\mu$ m (H&E) and 20  $\mu$ m (TRAP). (d) The parameters of femur osteoclasts, n=6. (e) Representative TRAP staining images of BMMs from *caRhoA* and *RCre;caRhoA* mice. Scale bars, 200  $\mu$ m. (f) SEM analysis and quantification of bone resorption area of bone slides, n=6, Scale bars, 20  $\mu$ m. (g) Representative Phalloidin staining images and quantification of F-actin ring per well and nuclei per osteoclast, n=3, Scale bars, 500  $\mu$ m. Mean $\pm$ s.d., \* $P$ < 0.05, Student's t-test.