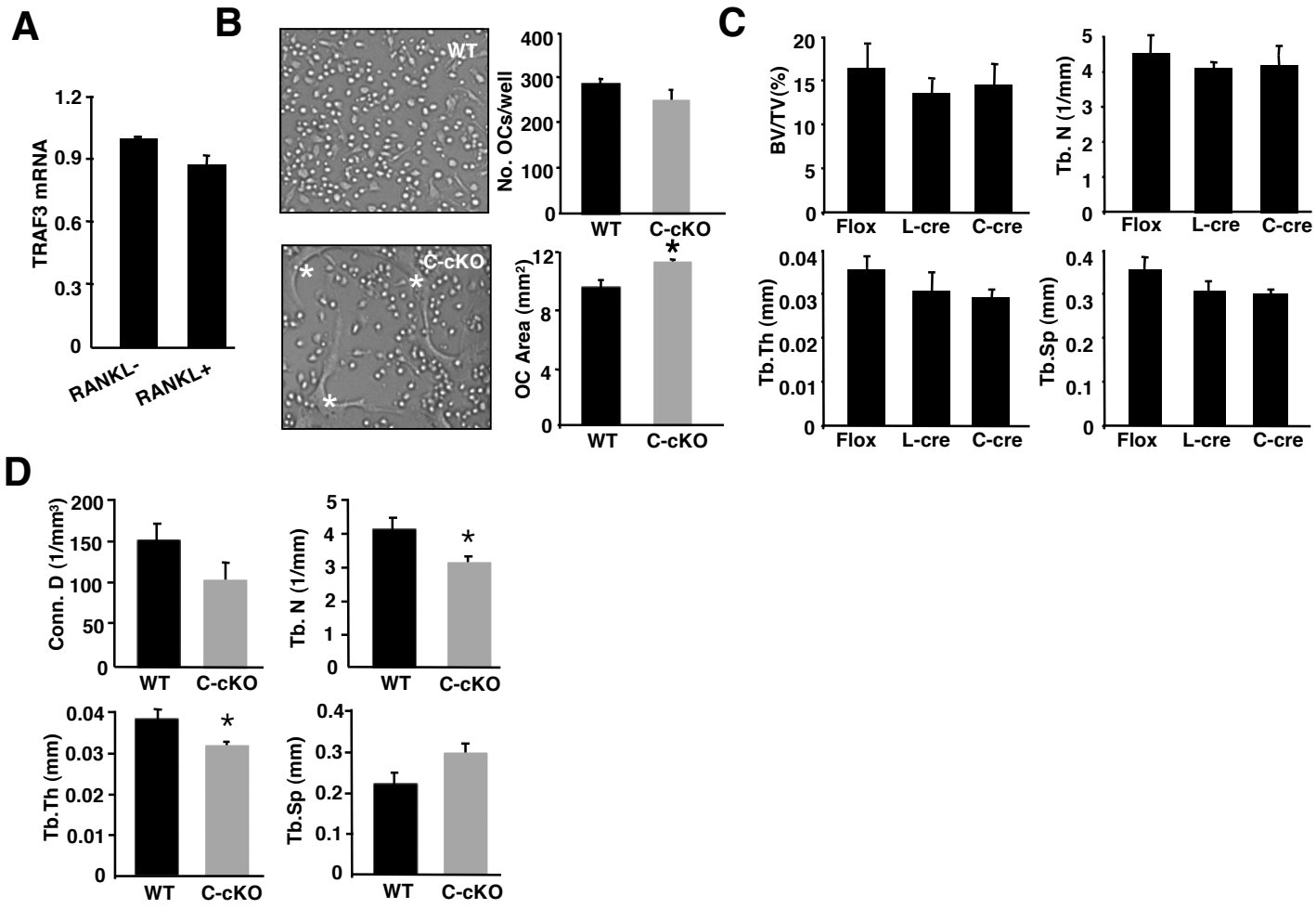

Figure S1. TRAF3 limits RANKL-induced osteoclastogenesis. (A) TRAF3 mRNA levels in WT OCPs treated with or without RANKL normalized to the β-actin mRNA levels. Magnification:x10. (B) OCPs from WT and *Traf3* C-cKO mice cultured with RANKL at Day3 (left panel) and OC numbers and areas counted at Day5. The centers of OCs are indicated (*). (C) μCT data from tibiae of five 2-m-old *Traf3* flox/flox (Flox), three *Lysozyme* Mcre (LyzK-Cre), or four *Cathepsin Kcre* (CatK-Cre) mice. Conn.D: connectivity density; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation. (D) μCT data from tibiae of 2-m-old WT or TRAF3 C-cKO mice. Conn.D: connectivity density; Tb.N: trabecular number; Tb.Th: trabecular thickness; Tb.Sp: trabecular separation. Values are means+SEM of 5 C-cKO mice and 6 WT mice. * $p<0.05$.

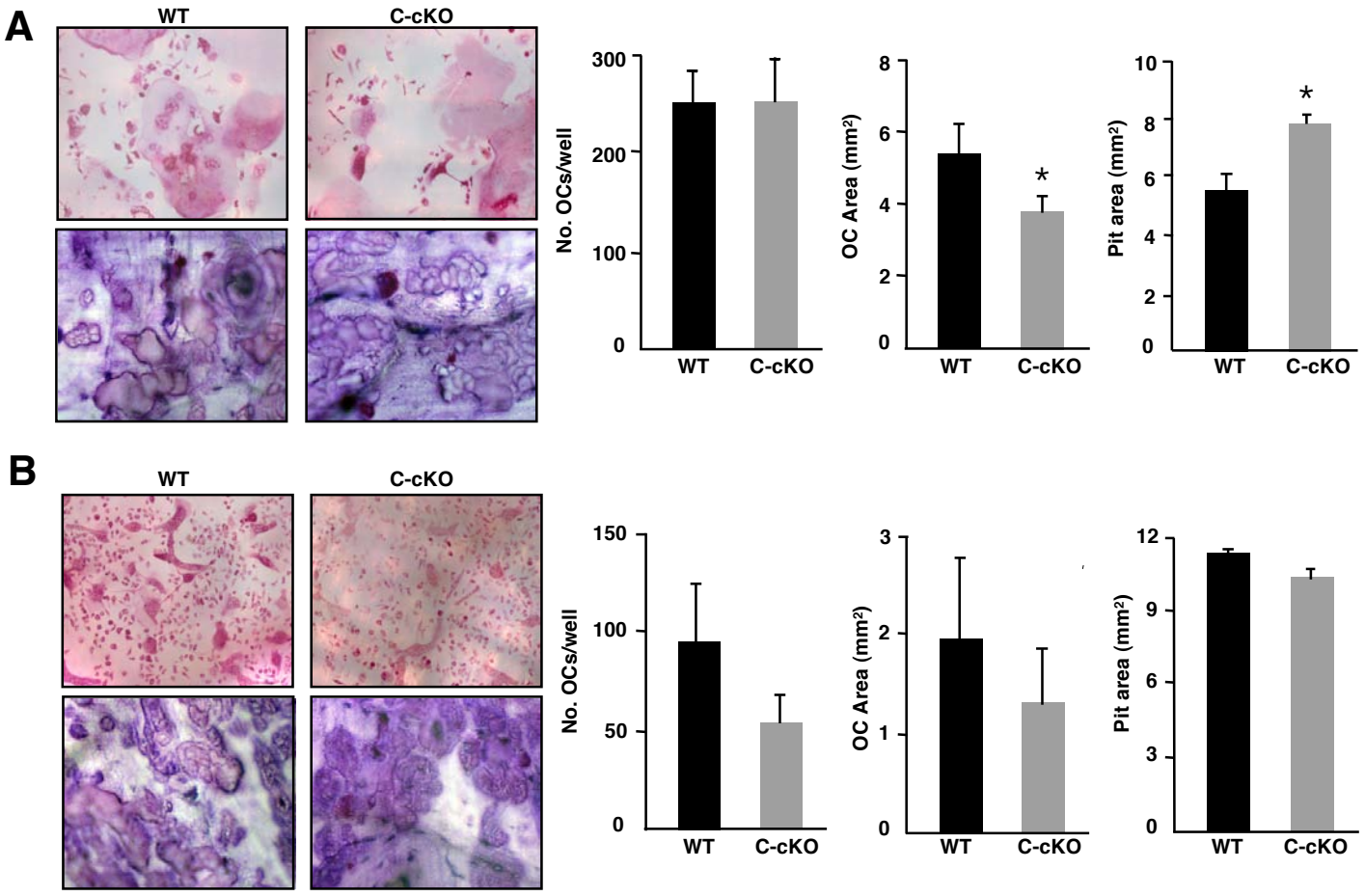

Figure S2. *Traf3* C-cKO cells have increased bone resorbing ability compared to WT cells. *Traf3* C-cKO and WT BM cells cultured with RANKL for 9d on bone slices in 96-well plates (A) or cultured with RANKL for 5d in 6-well plates. Then cells were re-suspended with 0.25%Trypsin/EDTA, replated onto bone slices in 96-well plates and cultured for another 4d (B). TRAP (upper panels, magnification x10) and toluidine blue (lower panels, magnification x20 in (A) and (B), respectively)., OC numbers and areas and resorption pit areas were counted. Values are means+SEM of 4 wells. *p<0.05 WT vs C-cKO.

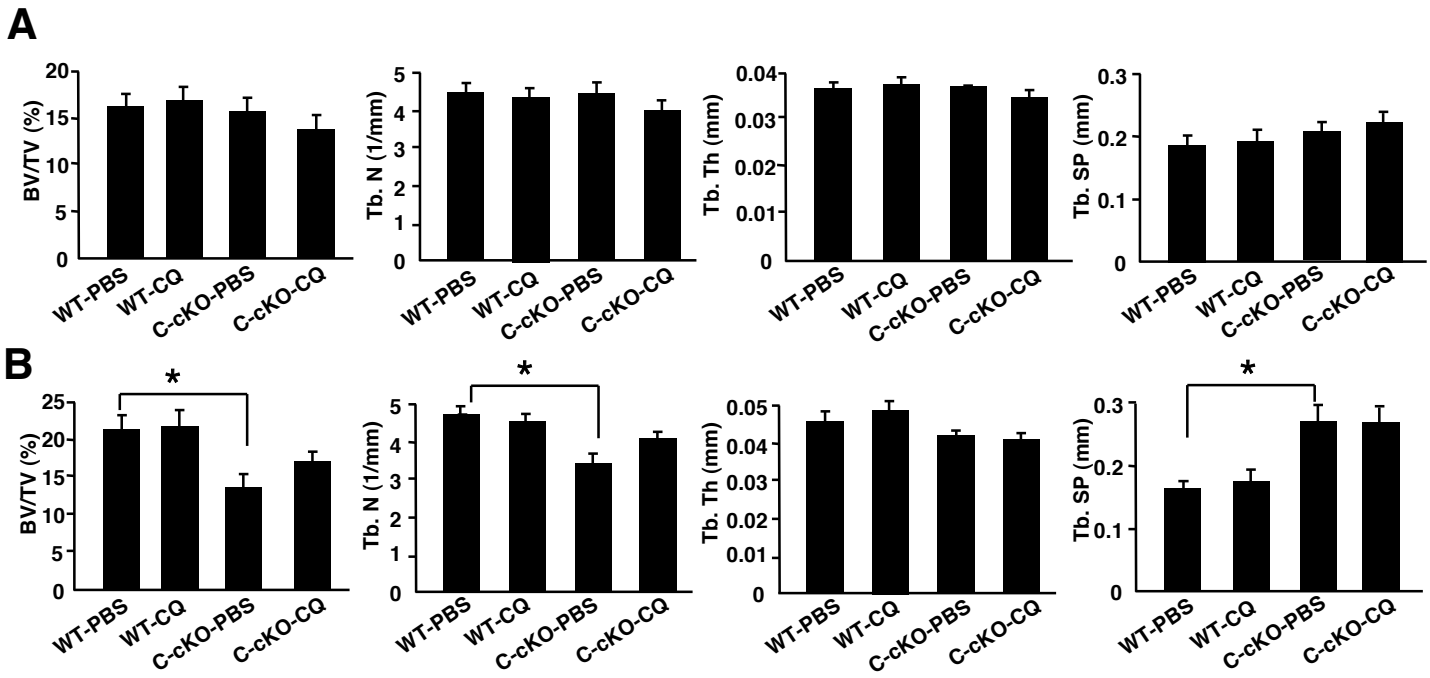

Figure S3. CQ has no effect on basal bone mass. (A) 3-wk- or (B) 3-month-old *Traf3* C-cKO and WT mice were treated with PBS or CQ (50mg/kg, 1/d) for 28d. Tibiae were fixed and bone volume and architectural parameters were assessed using μCT (7-9 mice/group). **p*<0.05 WT vs C-cKO

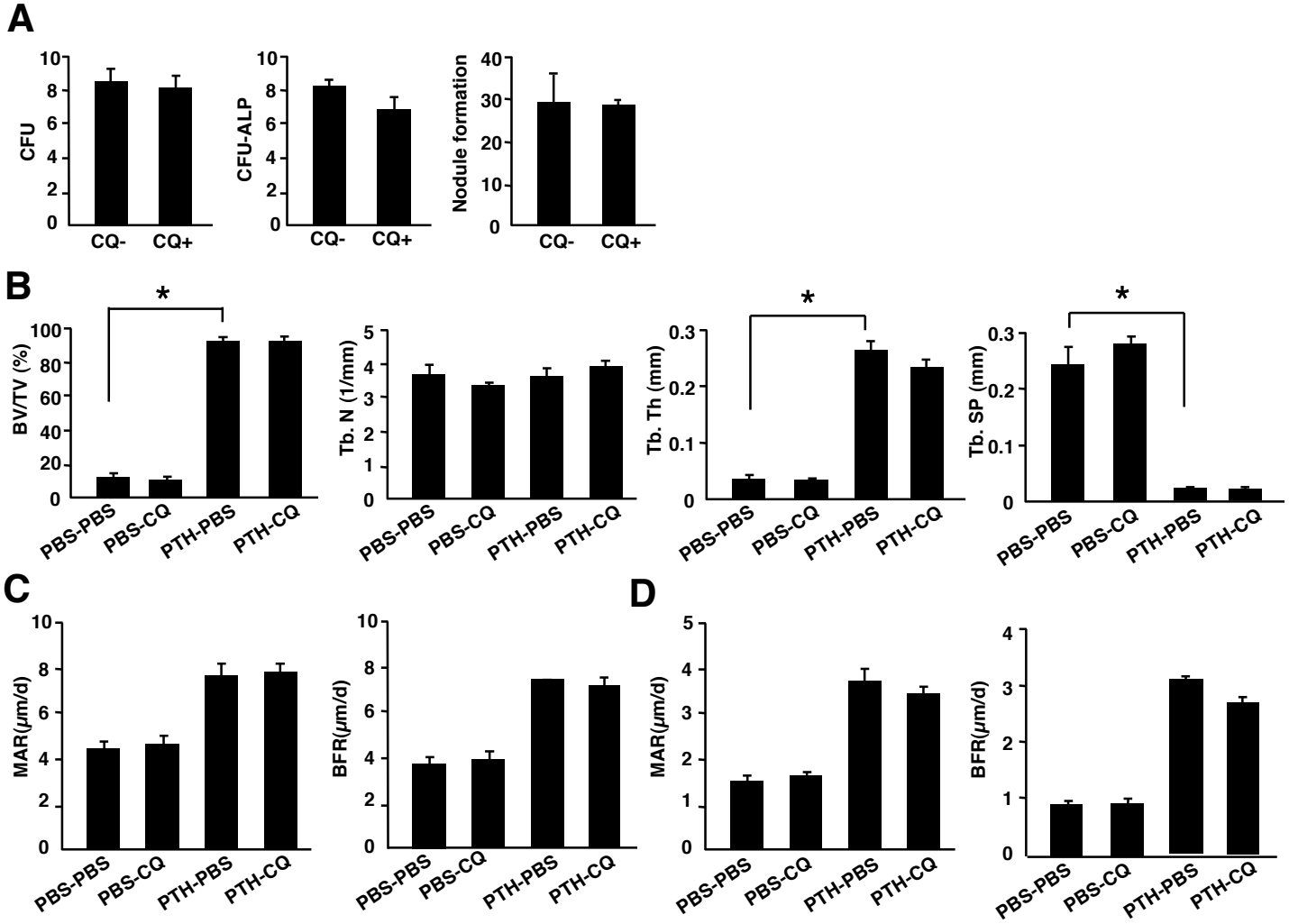

Figure S4. CQ has no effects on osteoblastic cells *in vitro* or on bone formation *in vivo*. (A) Colony forming unit-fibroblasts (CFU-F), ALP+ colonies (CFU-ALP), and mineralized nodule formation in WT bone marrow cell cultures. Values are means±SEM of 3 dishes. (B) Six-wk-old female WT mice (7-8/group) were injected with CQ 50mg/kg (1x/d) and hPTH 2μg/mouse 3x/d for 14d. μCT data for bone volume and structural parameters in tibiae, and mineral apposition and bone formation rates in cortical (C) and trabecular bone (D) were analyzed.

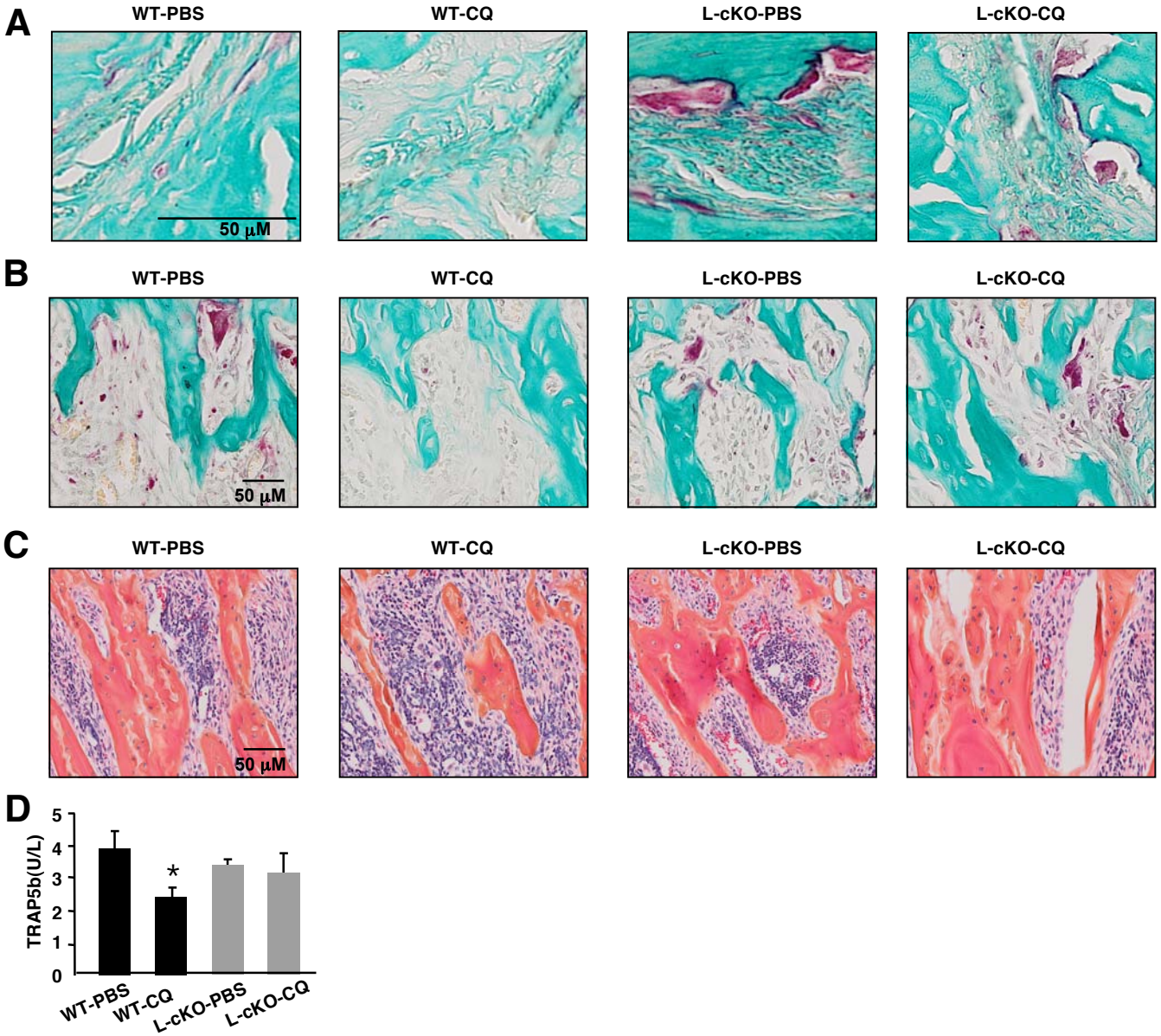

Figure S5. CQ prevents PTH-induced OC formation and marrow fibrosis in WT, but not in *Traf3* L-cKO mice. (A-C) 10-12-week old male WT or TRAF3 L-cKO mice were treated with CQ (50mg/kg daily i.p. for 10d). hPTH(1-34) (10μg/mouse) was injected into the s.c. tissue overlying the calvaria 4x/d for 3d beginning on day 7. The mice were sacrificed 16h after the last PTH injection. Sections of tibiae were stained for TRAP activity (A-B) or with H&E (C). (D) TRAP5b levels in sera from the mice were assessed by ELISA.

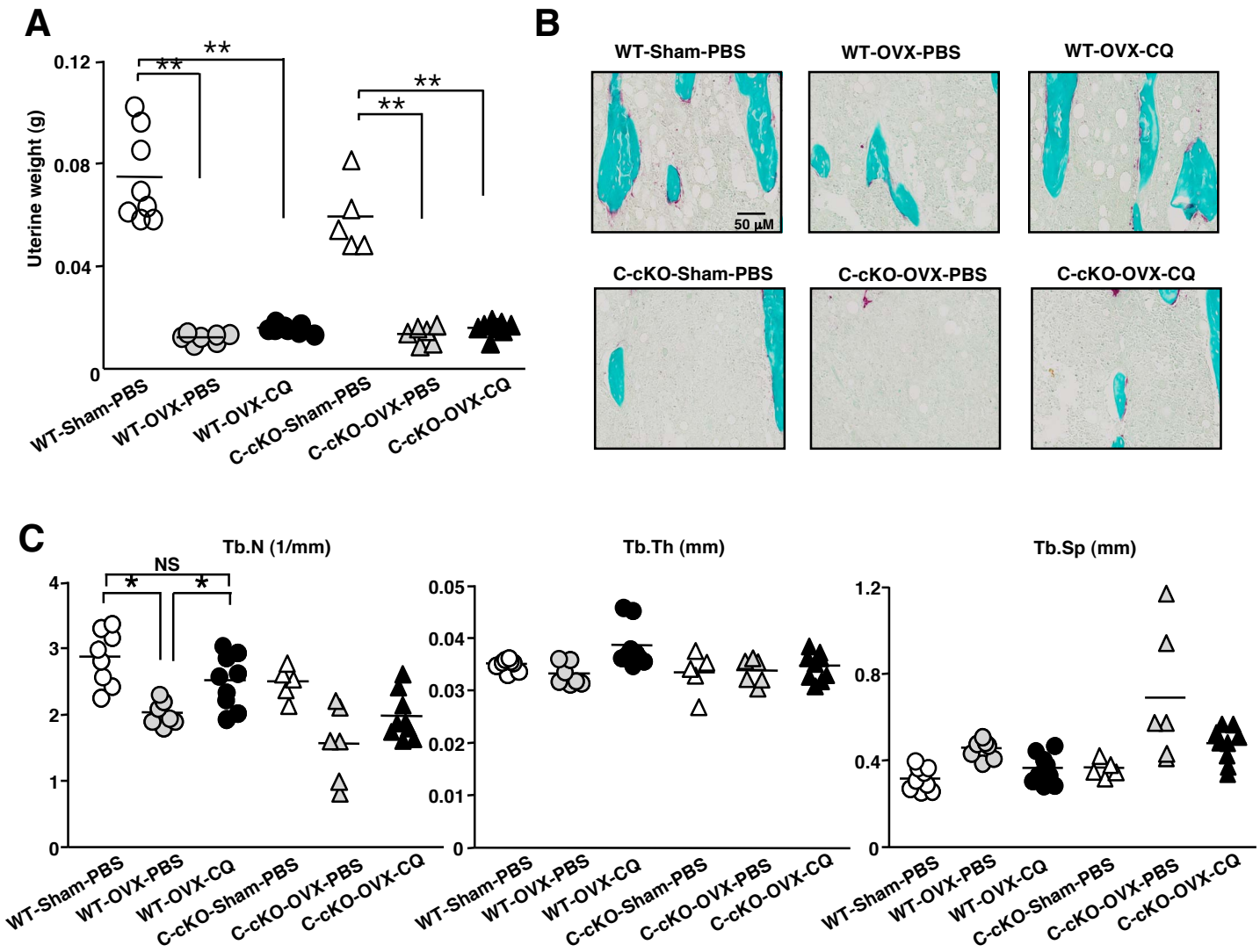

Figure S6. Chloroquine prevents OVX-induced bone loss in WT but not *Traf3* C-cKO mice. 8-9 wk old WT and *Traf3* C-cKO mice were subjected to ovariectomy (OVX) or sham surgery, followed by PBS or CQ (50mg/kg daily i.p) treatment for 28d. (A) uterine weight. (B) Representative TRAP-stained tibial sections. (C) μCT bone microarchitecture parameters. Values are means+SEM of 5~9 mice for each group. *p<0.05, **p<0.01, NS = no significant difference.

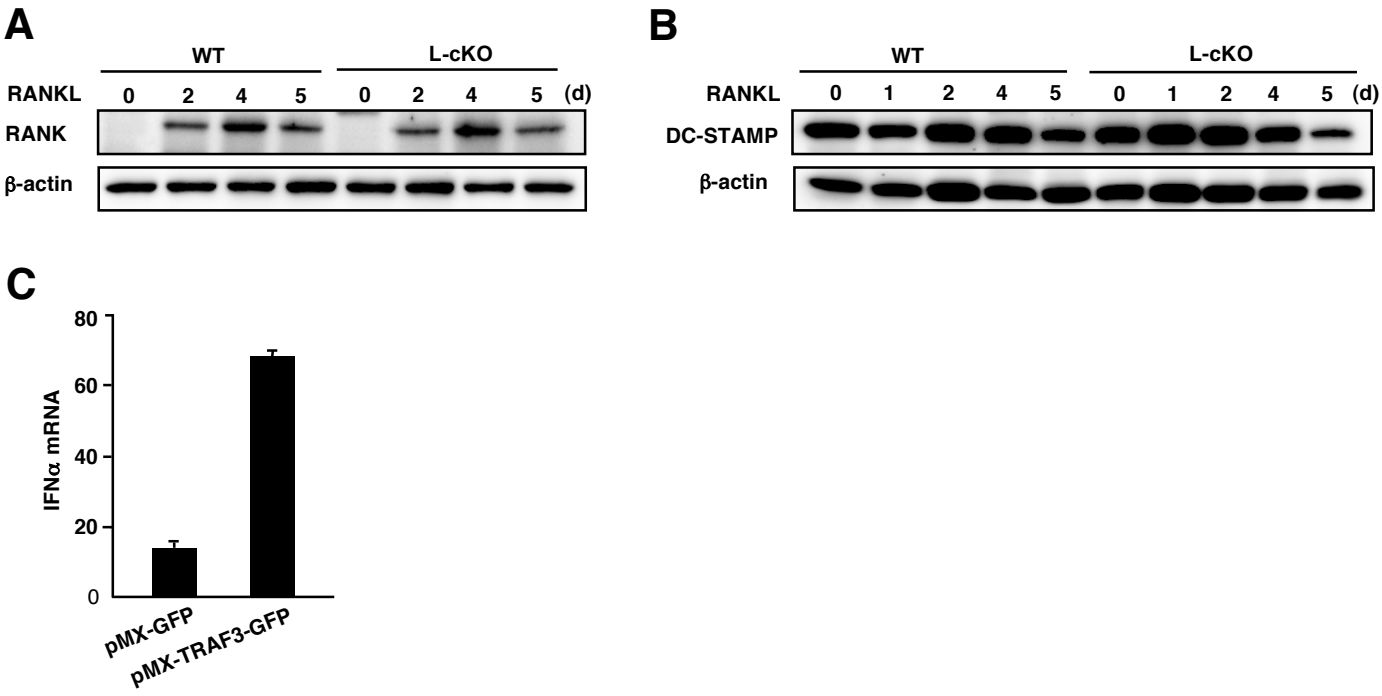

Figure S7. *Traf3* L-cKO cells express comparable levels of RANK and DC-STAMP and similar to WT cells. *Traf3* L-cKO and WT BM cells were cultured with M-CSF for 2d and stimulated with RANKL for various times. (A-B) RANK and DC-STAMP expression levels were assessed by WB. (C) WT OCPs were infected with GFP or TRAF3-IRES-GFP (TRAF3-GFP) retrovirus. IFNα mRNA levels were examined by RT-PCR.

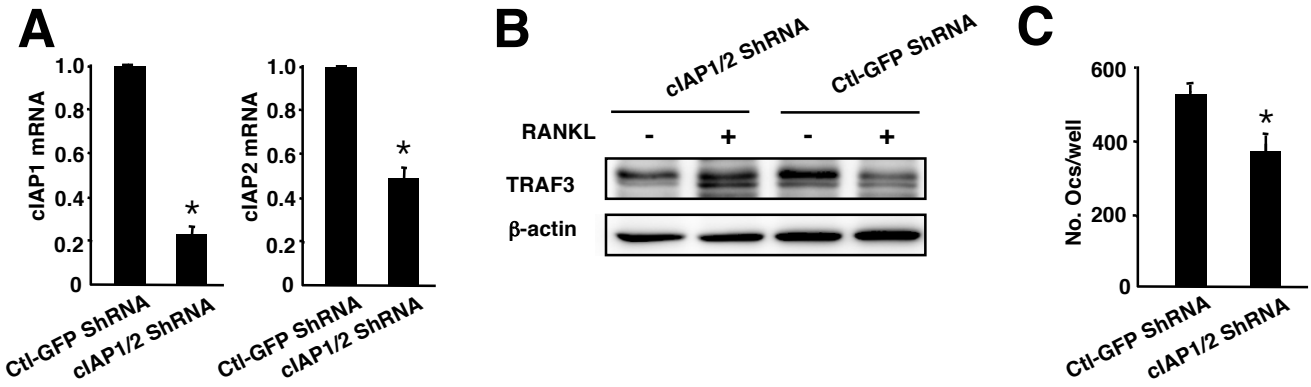

Figure S8. RANKL-induced TRAF3 degradation is impaired in cIAP1/2 knockdown cells. WT OCPs were infected with control GFP (Ctl-GFP), or cIAP1 and cIAP2 ShRNA lentivirus for 2d. (A) cIAP1 and cIAP2 mRNA levels were detected by RT-PCR. (B) Infected cells were treated with RANKL for 8h and TRAF3 protein expression was determined by WB. (C) Infected cells were cultured with RANKL for 5d in OC formation assays and OC numbers were counted. Representative results from two independent experiments. Values are means+SEM of 4 wells. *p<0.05 vs control GFP (Ctl-GFP).

Supplemental Figure 8 Xiu et al