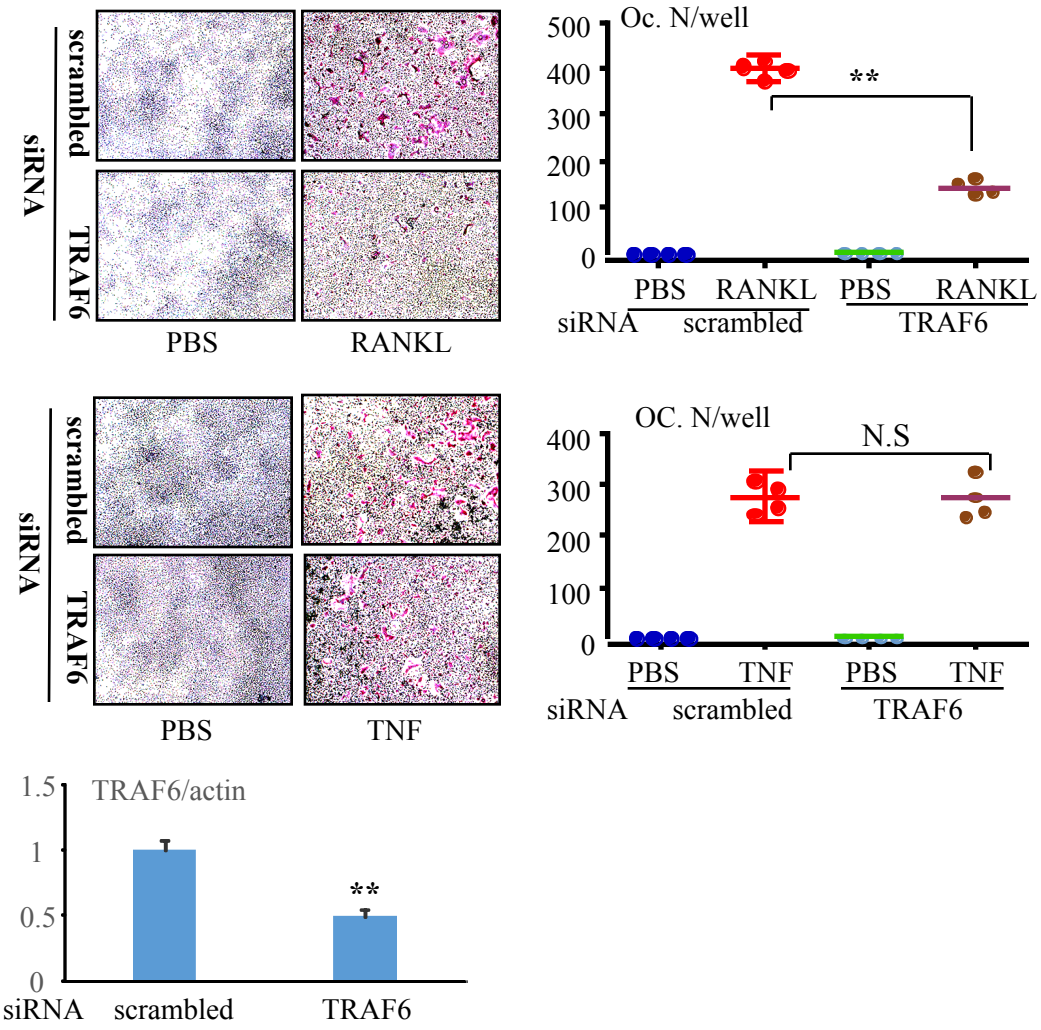
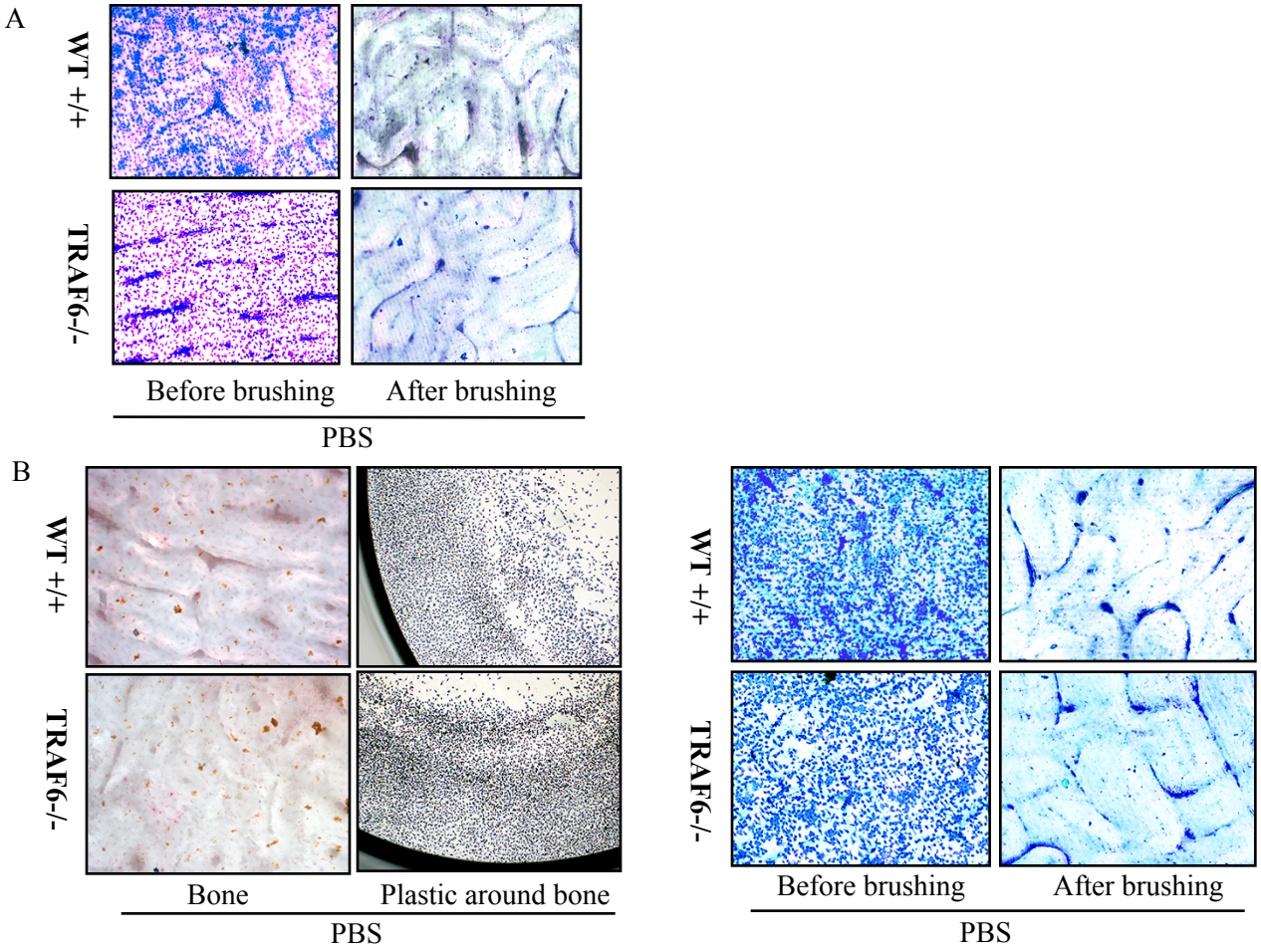
Supplemental Fig. 1.



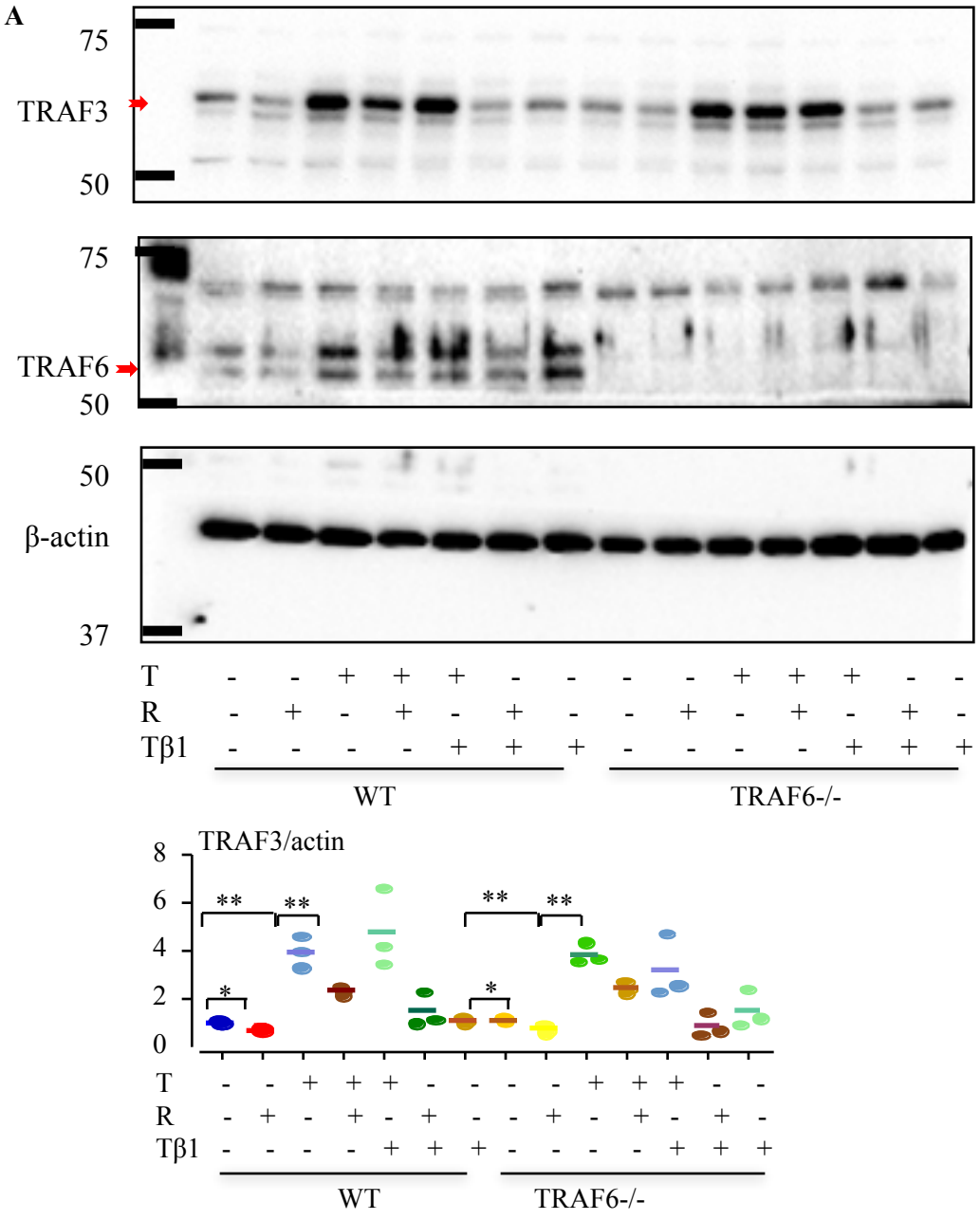
Supplemental Fig. 1. TRAF6 siRNA inhibits RANKL-, but not TNF-induced OC formation. 1-m-old WT mouse BM cells were cultured with M-CSF for 2 days to recruit macrophages, which were then transfected with scrambled or TRAF6 siRNA for 1 day followed by PBS and RANKL treatment for 2 additional days (upper panel) or PBS and TNF for 3 additional days (middle panel). The cells were stained for TRAP activity to count OC numbers. Total RNA was extracted from scrambled- or TRAF6 siRNA-transfected cells for 48 hrs and TRAF6 mRNA expression was tested by real-time PCR (lower panel). *p<0.05 and **p<0.01 vs. scrambled siRNA.

Supplemental Fig 2.



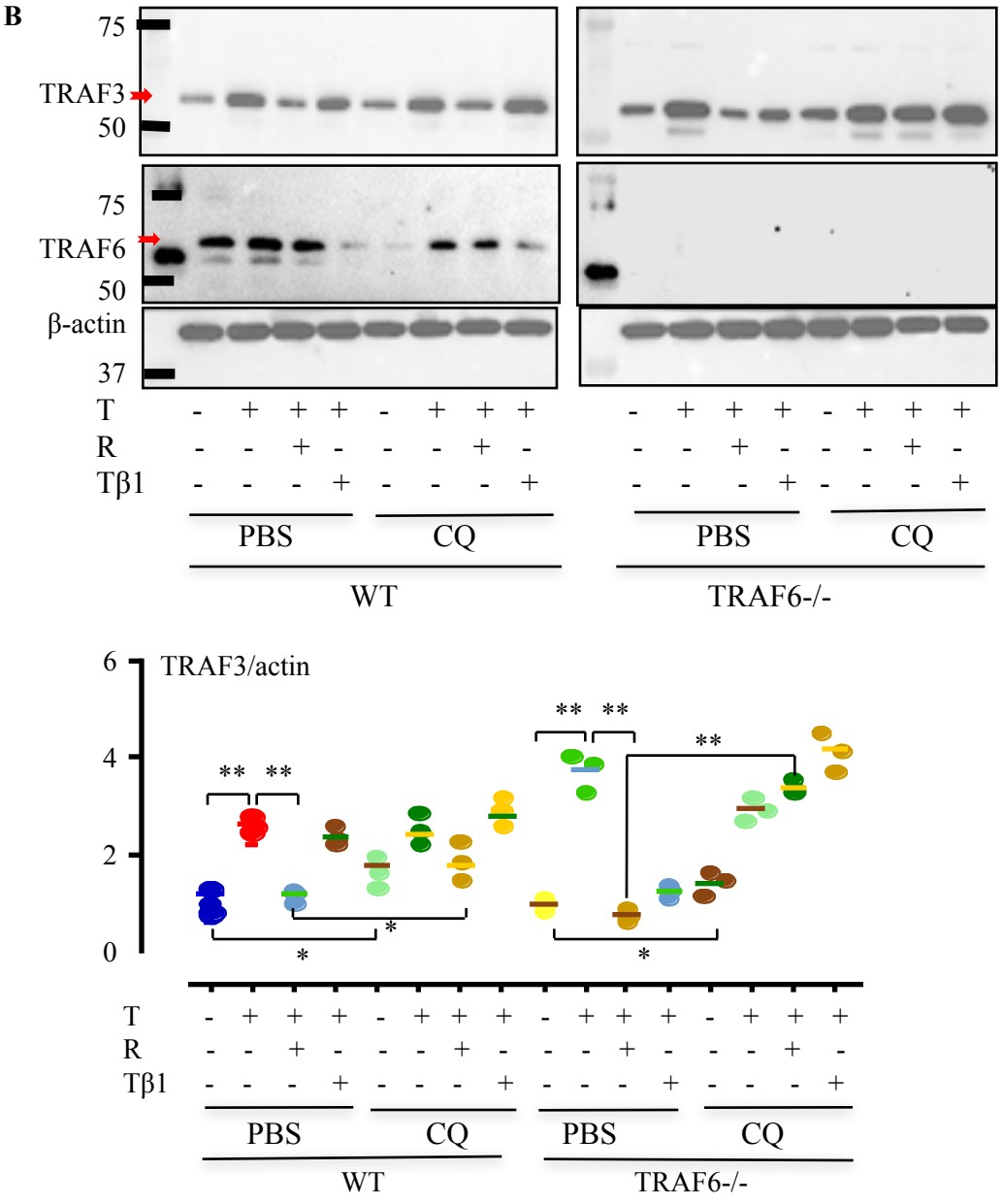
Supplemental Fig. 2. Spleen cells treated with M-CSF alone do not undergo OC differentiation or resorb bone. (A) WT and TRAF6-/- spleen cells were cultured on bone slices in the presence of M-CSF and treated with PBS, as a control for Fig.1C. Toluidine blue staining shows the cells on bone slices before brushing and no resorption pits after brushing. **(B)** WT and TRAF6-/- mouse spleen cells were cultured on bone slices in 96-well plates in the presence of M-CSF, as in Fig.2, but treated with PBS. TRAP staining of bone slices and the plastic around bone slices (left panel); Toluidine blue staining of the bone slices to show the cells before brushing and no resorption pits after brushing (right panel).

Supplemental Fig.3A.



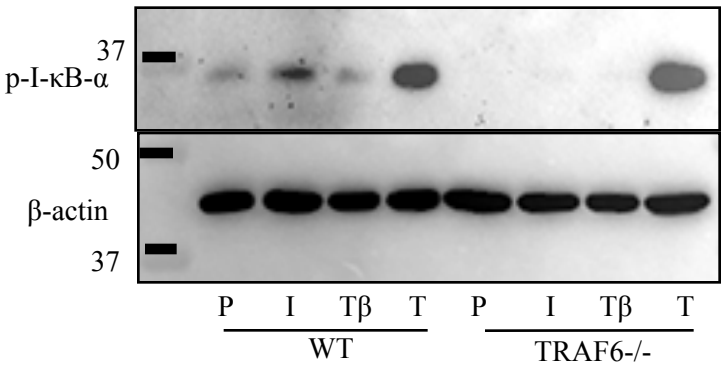
Supplemental Fig. 3. (A) Full images of representative Western blots from Fig.4A and densitometry data from 3 repeats. *p<0.05 and **p<0.01.

Supplemental Fig. 3B.



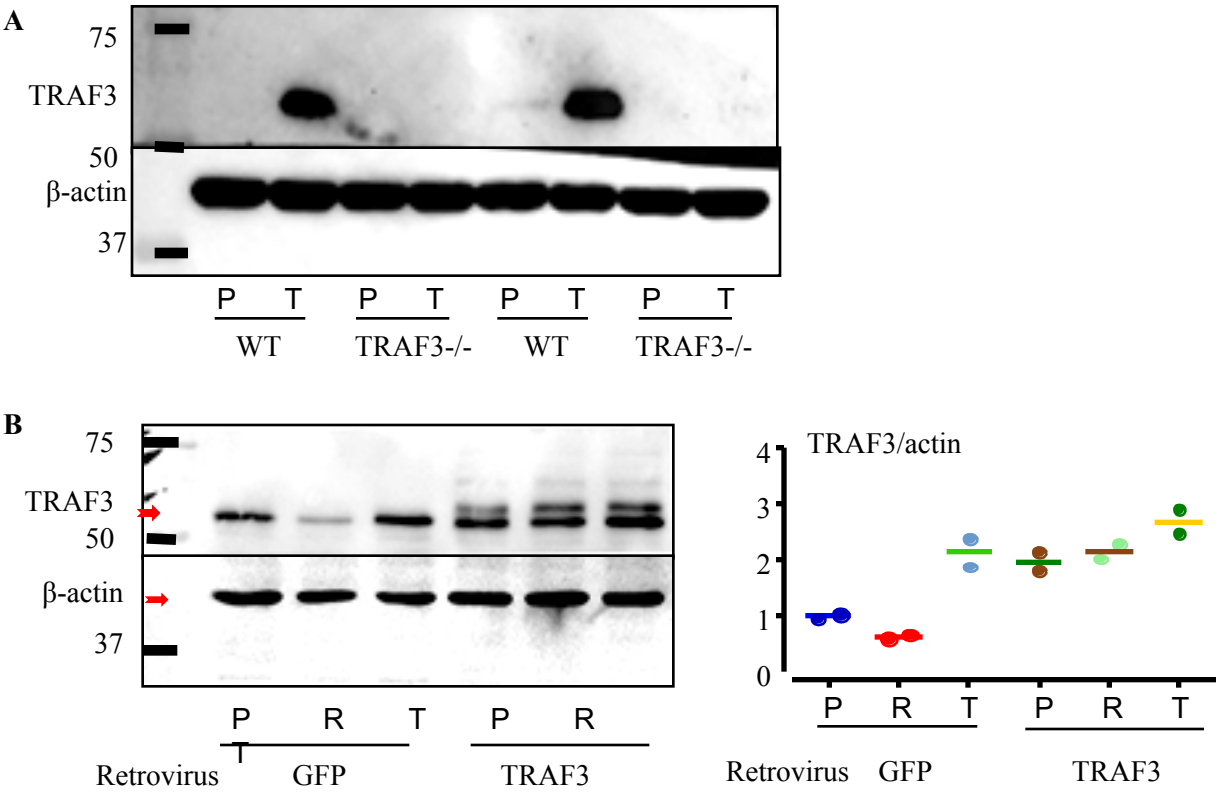
Supplemental Fig. 3. (B) Full images of representative Western blots from Fig.4B and densitometry data from 3 repeats. *p<0.05 and **p<0.01.

Supplemental Fig. 4.



Supplemental Fig. 4. TRAF6 is required for IL-1β- but not TNF-induced I-κB-α phosphorylation. 8-d-old WT and TRAF6-/- mouse spleen cells were cultured with M-CSF for 3 d. The cells were treated with culture medium for 4 hours without FBS&M-CSF followed by treatment with PBS (P), IL-1β (I, 10 ng/ml), TGFβ1 (Tβ, 2 ng/ml) or TNF (T, 20 ng/ml) for 30 minutes. Cell lysates were used to test phosphorylated I-κB-α and β-actin.

Supplemental Fig. 5.



Supplemental Fig. 5. (A) Full images of Western blots from Fig.6A right lower panel. **(B)** Full images of representative Western blots from Fig.6B right lower panel and densitometry data of 2 repeats.