Supplemental Material and Methods

**Real-time reverse transcriptase-PCR**

Total RNA was extracted from ST2 cells grown for 4 days in the presence or absence of $10^{-8}$ M 1,25-dihydroxyvitamin $D_3$ (Calbiochem), $10^{-7}$ M dexamethasone (SIGMA), and FK506 (Calbiochem and Fujisawa Pharmaceutical). Medium of drug-treated cells was changed every day. First strand cDNA was synthesized from total RNA using Reverse Transcriptase Superscript II (Invitrogen) and oligonucleotide (dT) primers. RANKL (*Tnfsf11*, Assay ID, Mm00441908_m1), OPG (*Tnfrsf11b*, Assay ID, Mm00435452_01), and β-actin (*Actb*, Assay ID, Mm01205647_g1) transcripts were quantified using a primer/probe kit (TaqMan Gene Expression Assays, Applied Biosystems). *Tnfsf11* and *Tnfrsf11b* transcripts were normalized to β-actin (*Actb*) transcripts.

**Primary calvarial osteoblast cultures**

Primary osteoblasts isolated from calvaria of neonatal mice were cultured in α-MEM (GIBCO) containing 10% fetal calf serum (FCS). For ALP activity and staining, differentiation was induced with differentiation medium (5% FCS, 50 mg/ml L-ascorbic acid phosphate magnesium salt n-hydrate (Wako) and 10 mM β-glycerophosphate (Wako) in α-MEM) for 7 days. ALP activity was determined using LabAssay™ ALP kits (Wako), and ALP staining was performed using Alkaline Phosphatase kits (SIGMA-ALDRICH), both according to the manufacturers' protocols. To evaluate mineralized extracellular matrix, medium was supplemented with 50 ng/ml BMP-2 (R&D systems) and differentiation was induced for 10 days. Cells were fixed with 95% ethanol at 4ºC for 20 min, stained with 2% Alizarin Red S (Sigma) for 20 min,

washed three times with water, and then washed once with PBS.

## RANKL-stimulation of NFATc1 and HA-trCot co-overexpressing RAW 264.7 cells

Transfected RAW 264.7 cells were incubated for 6 h after transfection, and cells were incubated another 24 h with or without 50 ng/ml sRANKL in medium containing 0.5% serum. Immunoreacted cells on coverslips were observed using a confocal laser scanning microscope (FV10i; OLYMPUS). Signal intensity was measured using ImageJ software (NIH) to quantify NFATc1 expression levels n=30 cells.