Primary osteoblast preparation
Mouse primary osteoblasts (POBs) were prepared from the calvaria of newborn C57B/L6 mice by digestion with 0.1% collagenase (Wako) and 0.2% dispase (Roche).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)
Total RNA was recovered with Isogen reagent (Nippongene, Tokyo, Japan), and reverse transcription was performed with ReverScript IV (Wako), according to manufacturer's protocol. Semi-quantitative PCR was performed with Titanium PCR kit (Clontech, Palo Alto, CA). Each primers used were showed in Table S1A. Densitometric values for each band were quantified and normalized using the Image J.

Real-time PCR
c-Fos, NFATc1, cathepsin K, TRAP, RANKL, and PIAS3 transcripts were quantitated on ABI PRISM 7900 (Applied Biosystems, Lincoln, CA) using SYBR Green and were normalized to β-actin transcripts. Each primers used were showed in Table S1B.

Cell proliferation assay
In order to determine cell proliferation, a WST-1 assay (TAKARA, Ohtsu, Japan) was performed. Mock- and PIAS3-transduced RAW264.7 cells were plated at 5 × 104/96-well culture plate and were cultured for 24 h. The cultured cells were incubated with 10 μl of WST-1 solution for additional 3 h. After incubation, the absorbance was measured at 440 nm using a microplate reader.

DNA fragmentation assay
DNA fragmentation assay was performed with the Cell Death Detection ELISA kit (Roche), according to manufacturer's instructions. RAW264.7 cells transduced with mock or PIAS3 were plated at 5 × 104/96-well culture plate and were cultured for 72 h in the presence of RANKL (50ng/ml). Absorbance was read at 405 nm using a microplate reader.

Bone resorption assay using Osteologic disc
RAW264.7 cells transduced with mock or PIAS3, were seeded on Osteologic disc (BD Biosciences, Bedford, MA) at 1 × 104 cells/well (24 well plate) with 50 ng/ml RANKL. After 5 days, the Osteologic discs were treated with 5% NaCl for 5 min at room temperature. After they had been washed with distilled water, the discs were dried and photographed.

RNA interference experiments
Small interference RNA (siRNA) for mouse PIAS3 (L-045382-00-0005) and non-targeting siRNA (negative control) were obtained from Dharmacon (Lafayette, CO). siRNA of negative control or PIAS3 were transfected into RAW264.7 cells, BMMs and POBs using Lipofectamine RNAiMAX, according to the manufacturer's protocol.