Supplemental Fig. 1. **Characterization of mouse RANKL antibodies, OYC1 and OYC2.**
(A) Binding and potency of 100 ng/mL OYC1 or OYC2 were detected by ELISA with various concentrations of recombinant mouse sRANKL (mRANKL). (B) Inhibitory activity of OYC1 in a TRAP solution assay. RAW264 cells were cultured for 4 days in the presence of 5 nM mRANKL with or without 0.025-1.0 μg/mL OYC1, 1.0 μg/mL OYC2, or 0.1 μg/mL of OPG-Fc. The cells were also cultured in the presence of 5 nM human sRANKL (hRANKL) with 0.2 μg/mL OYC1. The cells were fixed and TRAP activity was measured (5, 6). (C) Inhibitory activity of OYC1 (0.25 μg/mL) in in vitro osteoclastogenesis in RAW264 cells stimulated with 10 nM mRANKL, indicated by TRAP staining. Data are shown as the mean ± SD. a: $p < 0.05$, b: $p < 0.01$ (ANOVA) vs. control.

Supplemental Method

Tartrate resistant acid phosphatase (TRAP) assay

Mouse macrophage RAW264 cells obtained from RIKEN Cell Bank were seeded at 2×10^3/well in a 96-well plate. The cells were cultured in the presence of 5 nM sRANKL with or without 0.025-1 μg/mL OYC1, 1 μg/mL OYC2, or 0.1 μg/mL OPG-Fc for 4 days and then fixed with acetone-ethanol (1:1). For the TRAP solution assay, TRAP solution including 20 nM PNPP and 80 mM sodium tartrate was added to each fixed well and the mixture was incubated for 1 hr at 37°C. The TRAP activity of osteoclasts was measured at 405 nm using a microplate-reader (5, 6). For TRAP staining of cells, after 5 days of culture the cells were fixed with neutral buffered formalin and acetone-ethanol (1:1) and subjected to TRAP staining.

Supplemental Fig. 1