Supplemental Fig. 3: Effects of the ectopic expression of CA-NFATc1 in WT or RANK$^{KI/KI}$ BMMs on osteoclastogenesis in the absence or presence of RANKL

WT or RANK$^{KI/KI}$ (KI) BMMs were plated in 60-mm untreated tissue culture dishes, and infected with retrovirus expressing GFP or the constitutively active form of NFATc1 (CA-NFATc1). 36 hours later, the supernatant was removed and cells were maintained with α-10 medium containing 200 ng/ml M-CSF for 2 days. Then, cells were lifted by 0.02% EDTA for further analyses. (*A*) A portion of cells were lysed for the analysis of NFATc1 expression by Western blot. (*B*) Remaining cells were seeded in 24-well treated plates (5×10^4 cells/well) and treated with M-CSF (40 ng/ml) alone or M-CSF (40 ng/ml) plus RANKL (25, 50, or 100 ng/ml) for 4 days. TRAP staining was performed and images of the whole wells of the osteoclastogenesis assays are shown. (*C*) A high power view of a representative area in each well in *B* is shown. Scale bar = 200 μm. Quantification of osteoclastogenesis is shown right below the images. The number of multinucleated TRAP-positive cells (>3 nuclei) of five representative view areas in each well (40× magnification) was obtained. Data are mean ± S.D. of three independent experiments. *, $p < 0.05$; **, $p < 0.01$; NS, not significant.

For the preparation of retrovirus, the retroviral vector encoding CA-NFATc1 (#11102; Addgene, Cambridge, MA, US) or GFP, together with TurboFect Transfection Reagent (00700372; Thermo Scientific) was transfected into 293GPG packing cells. Then the viral supernatants were harvested three successive 24 hours and filtered for use. The virus infection was carried out using a mixture composed of 3 ml viral supernatant and 1ml α-20 medium (α-MEM with 20% heat-inactivated fetal bovine serum) plus 200 ng/ml M-CSF.

Supplemental figure 3