Supplemental Fig. 2: Inactivation of RANK Motifs 2 and 3 impairs the capacity of RANKL to activate the expression of NFATc1 mRNA

BMMs from WT or $RANK^{KI/KI}$ (KI) mice were seeded on 60 mm tissue culture dishes and were treated with M-CSF (40 ng/ml) plus RANKL (100 ng/ml) for 1, 2, 3, or 4 days. Total RNA from cells was extracted using TRIzol reagent (15596026; Invitrogen, Waltham, MA, US). 3 μg total RNA was reverse-transcribed into cDNA by using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). The resulting cDNA was subjected to real-time quantitative PCR with the PowerUp SYBR Green Master Mix (Invitrogen). Relative expression of the tested genes were normalized to GAPDH mRNA, and calculated by the comparative 2- Δ Δ CT method. PCR Primers: NFATc1: 5'-CCGTTGCTTCCAGAAAATAACA-3' (forward) & 5'-TGTGGGATGTGAACTCGGAA-3' (reverse); GAPDH: 5'-ACATCATCCC TGCATCCACTG-3' (forward) & 5'-TCATTGAGAGCAATGCCAGC-3' (reverse). Data are mean ± S.D. of three independent experiments. *, $p < 0.05$; **, $p < 0.01$.