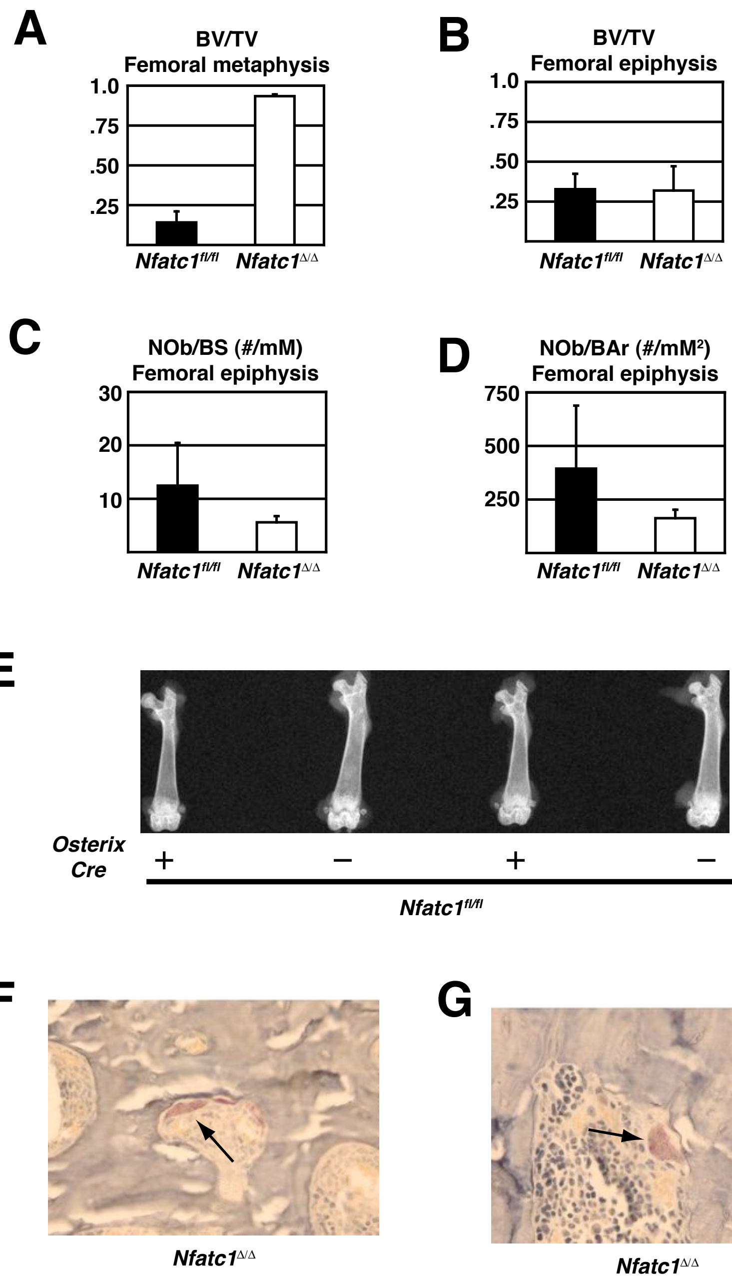
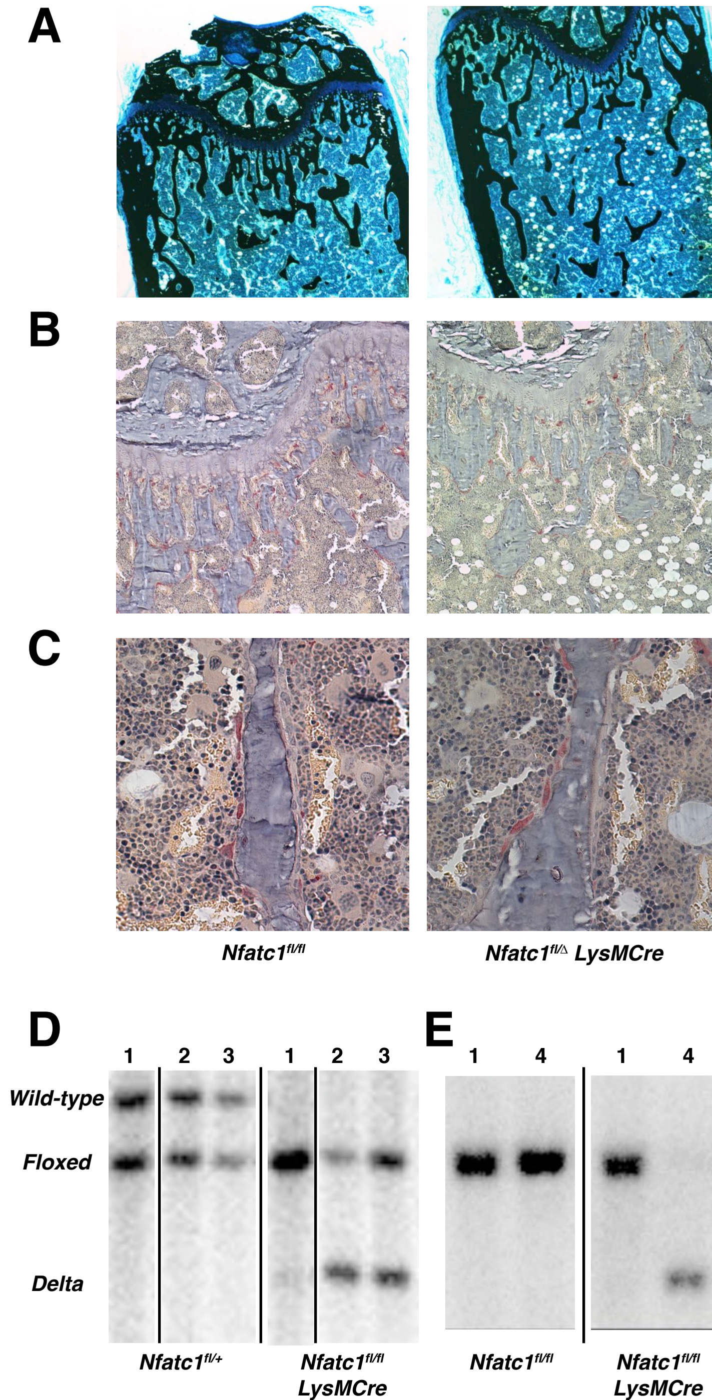


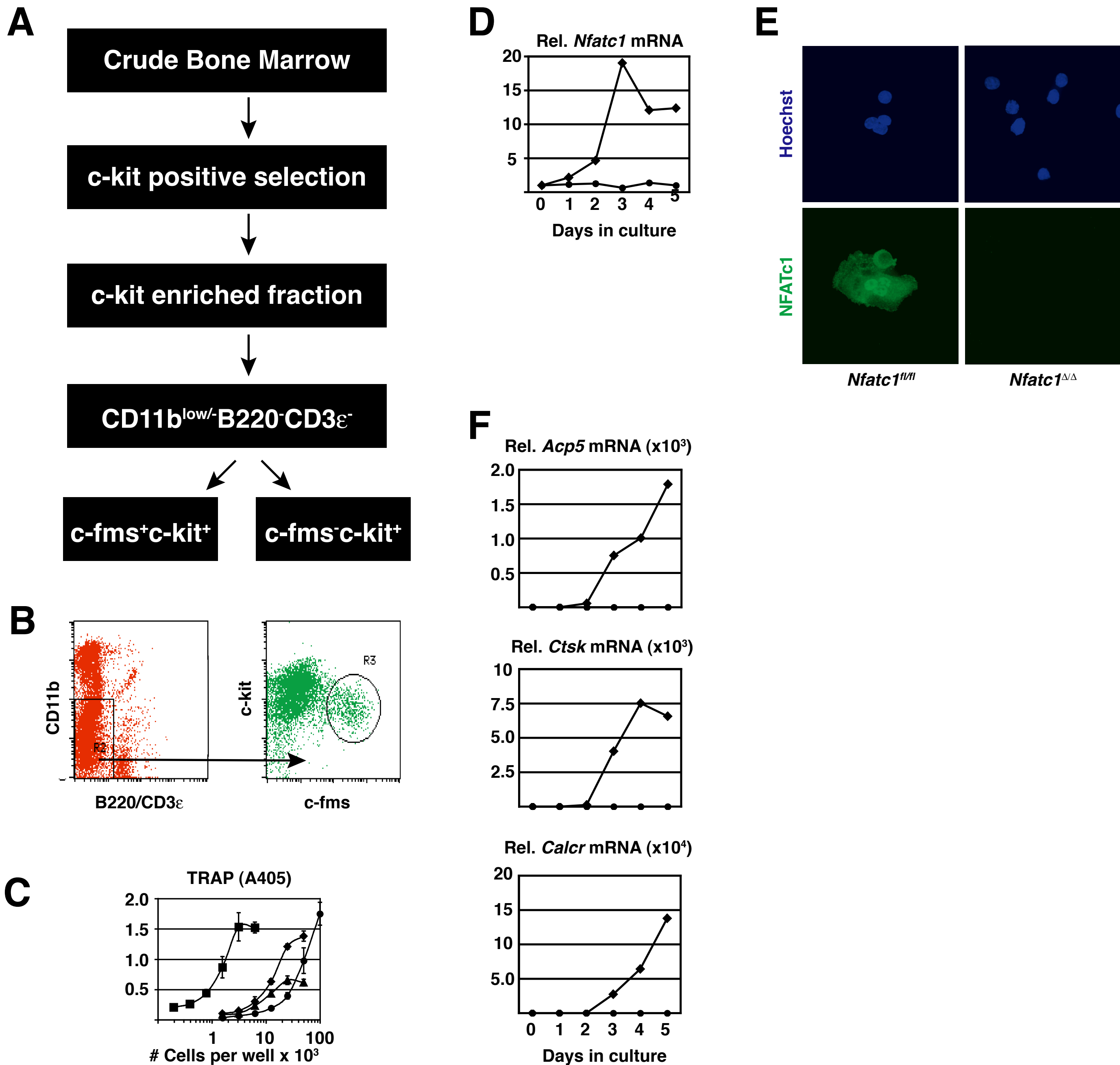
Supplemental Figure 1

Supplemental analysis *of Nfatc1* targeted mice. BV/TV ratio in the femoral (**A**) metaphysis and (**B**) epiphysis of 5 month old, female *Nfatc1*$^{fl/fl}$ (black bars) and *Nfatc1*$^{\Delta/\Delta}$ *(*white bars) mice (n=4/genotype), (A) $p<1x10^{-6}$, (B) $p>0.05$. (**C and D**) Histomorphometric enumeration of osteoblasts within the femoral epiphysis of 5 month old, female *Nfatc1*$^{fl/fl}$ (black bars) and *Nfatc1*$^{\Delta/\Delta}$ (white bars) mice (n=4/genotype), (C) $p>0.05$, (D) $p>0.05$. (**E**) Anteroposterior digital radiograph of the femurs of *Nfatc1*$^{fl/fl}$ and *Nfatc1*$^{fl/fl}$*, Osterix-Cre* mice (n=2/genotype). (**F and G**) Examples (original magnification, x400) of rare TRAP positive osteoclasts (black arrows) at the (**F**) femoral epiphysis and (**G**) base of the femoral metaphysis in 5 month old, female *Nfatc1*$^{\Delta/\Delta}$ mice.

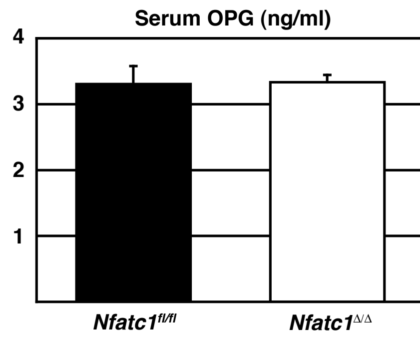


Supplemental Figure 2

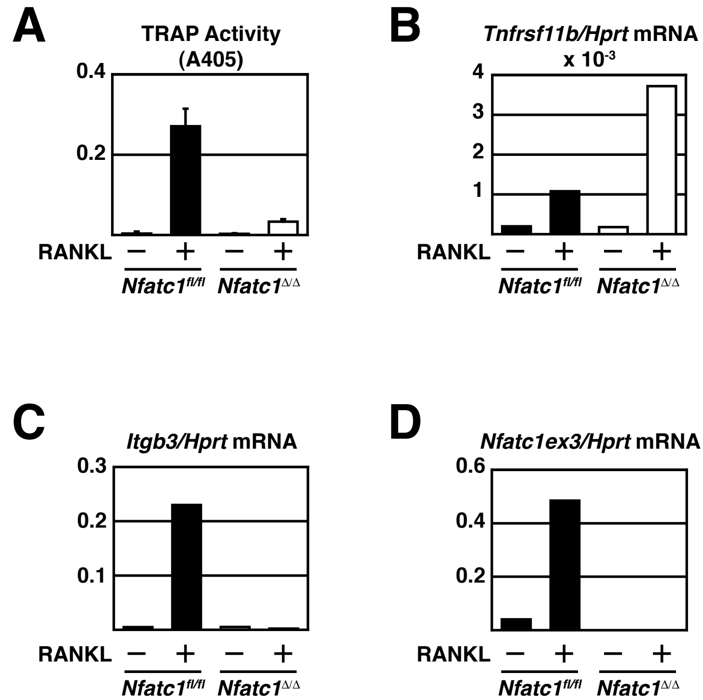
Analysis of *Nfatc1* conditional knockout mice crossed onto the *LysM-Cre* background. (**A**) Von Kossa (original magnification, x100) and (**B**) low (original magnification, x100) and (**C**) high (original magnification, x400) magnification TRAP stains of the distal femurs of 4 month old *Nfatc1*$^{fl/fl}$ and *Nfatc1*$^{fl/\Delta}$*, LysMCre* mice. Histology images in (A-C) are representative of at least 4 femurs analyzed per genotype. (**D and E**) Southern blot analysis of genomic DNA from tail (1), M-CSF primed BM cells incubated with M-CSF (2) or M-CSF and RANKL (3), or concanavalin A elicited peritoneal macrophages (4). These results are representative of at least 3 mice per genotype. Lanes in (D) were cropped from the same gel. Lanes in (E) were cropped from the same gel.



Supplemental Figure 3
Isolation of BMOcPs. (**A**) Flow chart for the purification of CD11b$^{low/-}$B220^{-}CD3ε^{-}c-kit^{+}c-fms^{+} BMOcPs. (**B**) FACS plot of c-kit enriched cells isolated from the BM of C57/BL6 mice. BMOcPs reside in the CD11b$^{low/-}$B220^{-}CD3ε^{-}(R2) and c-kit^{+}c-fms^{+} (R3) fraction. (**C**) TRAP assay of crude BM (circles), c-kit enriched (diamonds), CD11b$^{low/-}$B220^{-}CD3ε^{-}c-kit^{+}c-fms^{-} (triangles) and CD11b$^{low/-}$B220^{-}CD3ε^{-}c-kit^{+}c-fms^{+} (squares) cells incubated at the indicated starting concentrations in 96-well plates with M-CSF and RANKL. The data are the average+/-SD for triplicate wells and are representative of 2 similar experiments. (**D**) qRT-PCR analysis for the expression of the *Nfatc1* mRNA (primer set *Nfatc1ex3*) in C57/Bl6 BMOcPs cultured with M-CSF (circles), or M-CSF and RANKL (diamonds), for 0-5 days. (**E**) Immunofluorescence for NFATc1 in *Nfatc1*$^{fl/fl}$ and *Nfatc1*$^{\Delta/\Delta}$ BMOcPs stimulated for 3 days with M-CSF and RANKL (original magnification, x400). Image is representative of 4 similar microscopic fields. Only weak staining was observed in *Nfatc1*$^{fl/fl}$ BMOcPs cultured with M-CSF alone (A.O.A., unpublished observation). (**F**) qRT-PCR analysis for the expression of osteoclast differentiation markers in C57/Bl6 BMOcPs cultured with M-CSF (circles), or M-CSF and RANKL (diamonds), for 0-5 days. Expression in (D) and (F) are relative to day 0 (mRNA purified from freshly isolated WT BMOcPs).

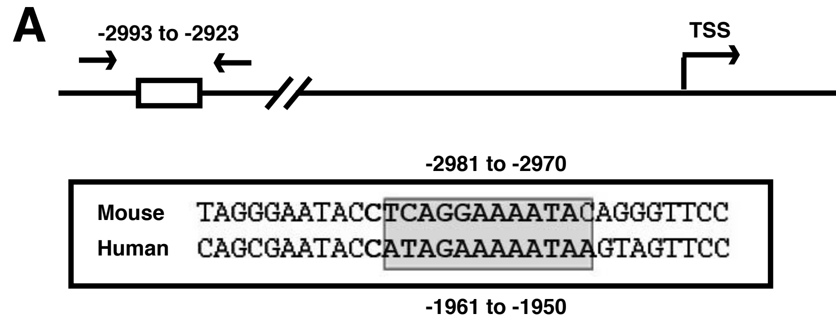


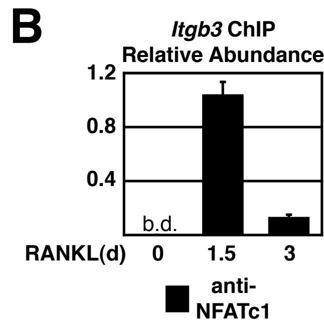
Supplemental Figure 4
Serum OPG levels in *Nfatc1*$^{\Delta/\Delta}$ mice. OPG was measured in the serum of adult *Nfatc1*$^{fl/fl}$ (n=3) and *Nfatc1*$^{\Delta/\Delta}$ (n=4) mice, $p > 0.5$.

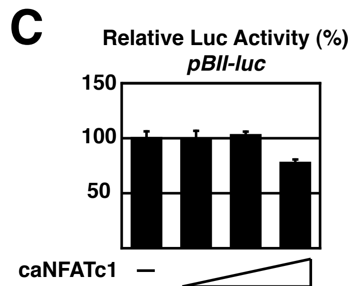


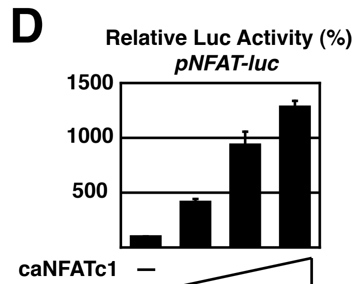
Supplemental Figure 5

RANKL induces *Tnfrsf11b* expression in splenocytes in the absence of NFATc1. (**A**) TRAP activity in the supernatants of *Nfatc1*$^{fl/fl}$ and *Nfatc1*$^{\Delta/\Delta}$ MCSF primed splenocytes incubated with M-CSF or MCSF and RANKL for 4 days. The data are the average+/-SD for triplicate wells. qRT-PCR analysis for (**B**) *Tnfrsf11b*, (**C**) *Itgb3* and (**D**) *Nfatc1* (primer set *Nfatc1ex3*) mRNA in *Nfatc1*$^{fl/fl}$ and *Nfatc1*$^{\Delta/\Delta}$ splenocytes incubated with M-CSF or MCSF and RANKL for 4 days. These data are representative of two experiments.









Supplemental Figure 6

Supplemental data for NFATc1 ChIP and promoter luciferase assays. (**A**) Graphical representation of the mouse *Tnfrsf11b* promoter. The approximate position of the primers used to interrogate NFATc1 ChIP samples, which flank an evolutionarily conserved NFAT binding site (small white box), are shown. Displayed within the large white box is an alignment of a conserved sequence within the mouse *Tnfrsf11b* and human *TNFRSF11B* promoters. A conserved NFATc1 binding site within this sequence is highlighted (shaded box). (**B**) NFATc1 ChIP of osteoclast precursors incubated with RANKL for 0, 1.5 or 3 days. Immunoprecipitated chromatin was analyzed by qRT-PCR for *Itgb3* promoter DNA, which was normalized to input. (b.d., below detection). The immunoprecipitated chromatin sample analyzed here is the same as that in Figure 5H. (**C and D**) Relative luciferase activity of 293T cells transfected with (C) *pBII-luc* or (D) *pNFAT-luc* and increasing amounts of *pMSCV-caNfatc1* (0, 8, 40 and 200 ng/transfection). Data are the average+SD of transfections performed in triplicate.