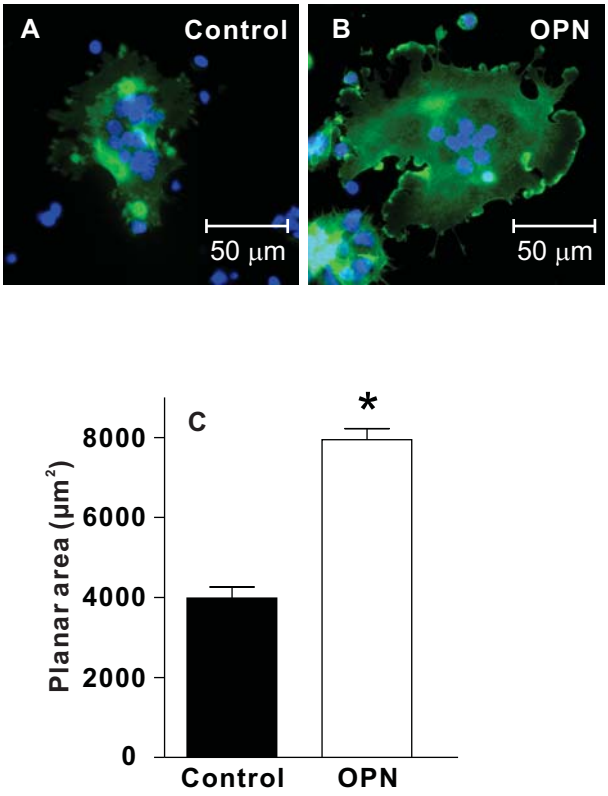
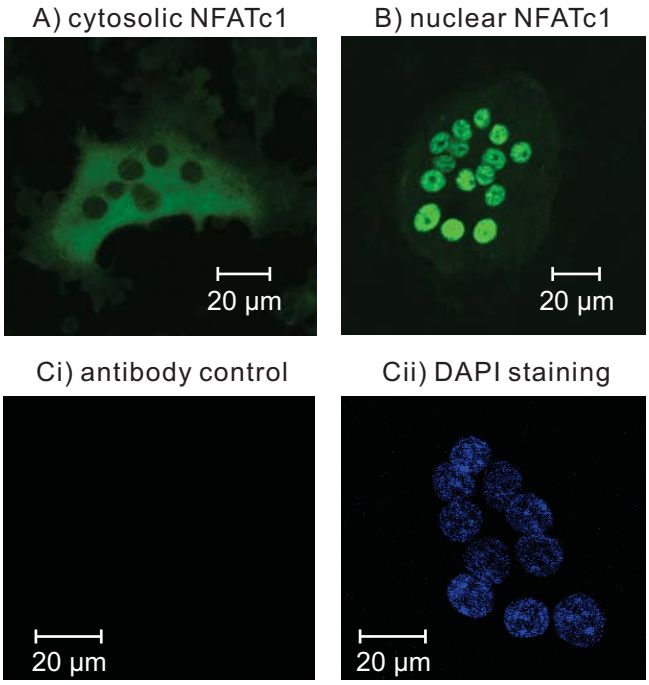
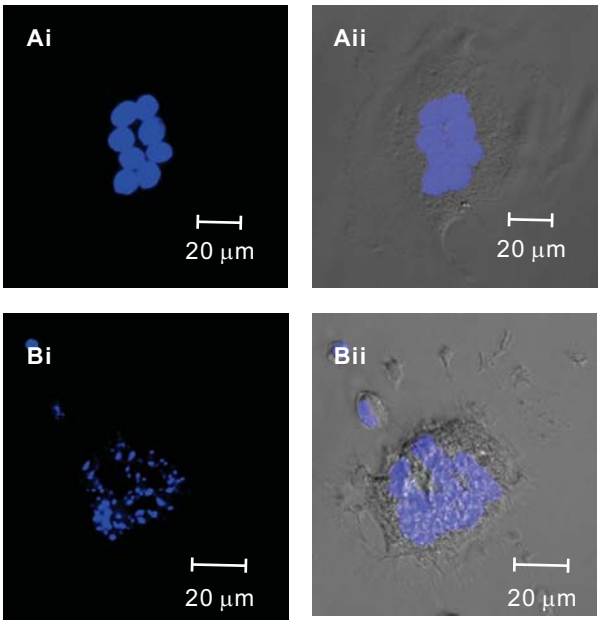


SUPPLEMENTAL FIGURE S1. **Osteopontin enhances osteoclast spreading.** Glass coverslips were uncoated (Control) or coated with recombinant osteopontin (OPN). Neonatal rat osteoclasts were plated on these substrates in supplemented M199 buffered with HEPES (25 mM) and HCO_3^- (26 mM). After 1 hour incubation at 37°C in 5% CO_2, preparations were washed to remove nonadherent cells. Cells were then incubated for an additional 1 hour and fixed. Filamentous actin was labeled with fluorescently tagged phalloidin (green), nuclei labeled with DAPI (blue) and osteoclasts were observed by fluorescence microscopy. *A*) Representative micrograph of osteoclast on uncoated coverslip. *B*) Micrograph of osteoclast on OPN shows enhanced spreading. *C*) Cell spreading was quantified by measuring the planar area. Data are means ± S.E., $n = 3$ independent experiments, each performed using triplicate coverslips with 5 randomly selected osteoclasts analysed per coverslip. * $p < 0.05$ compared to Control.



SUPPLEMENTAL FIGURE S2. **Nuclear localization of NFATc1 in osteoclasts.** Rabbit osteoclasts were plated on glass coverslips in supplemented M199 buffered with HEPES (25 mM) and HCO_3^- (26 mM). After 1 hour incubation at 37°C in 5% CO_2, preparations were washed to remove nonadherent cells. Cells were then incubated for an additional 2 hours and then fixed. Coverslips were processed for NFATc1 immunofluorescence as described under EXPERIMENTAL PROCEDURES and observed using confocal microscopy. *A*) Example of a multinucleated osteoclast exhibiting cytoplasmic localization of NFATc1 (green). *B*) Example of a different osteoclast with NFATc1 localized predominantly in the nuclei. *C*) Negligible staining was observed in control preparations when primary antibody was omitted (*Ci*), with nuclei in the same osteoclast revealed by staining with DAPI (blue, *Cii*).



SUPPLEMENTAL FIGURE S3. **Detection of apoptosis in osteoclasts.** Rat osteoclasts were plated on coated or uncoated glass-bottomed culture dishes in supplemented M199 buffered with HEPES (25 mM) and HCO_3^- (26 mM). After 1 hour incubation at 37°C in 5% CO_2, preparations were washed to remove nonadherent cells. After 6 h, cells were stained with the DNA dye Hoechst 33342 (5 μg/ml, for at least 10 minutes). Left panels show fluorescence images with nuclei stained blue. Right panels show merged fluorescence and brightfield images of the same cell. *A*) Representative healthy osteoclast with characteristic lamellipodia and normal nuclear morphology. *B*) Representative apoptotic osteoclast with condensed and fragmented nuclei.