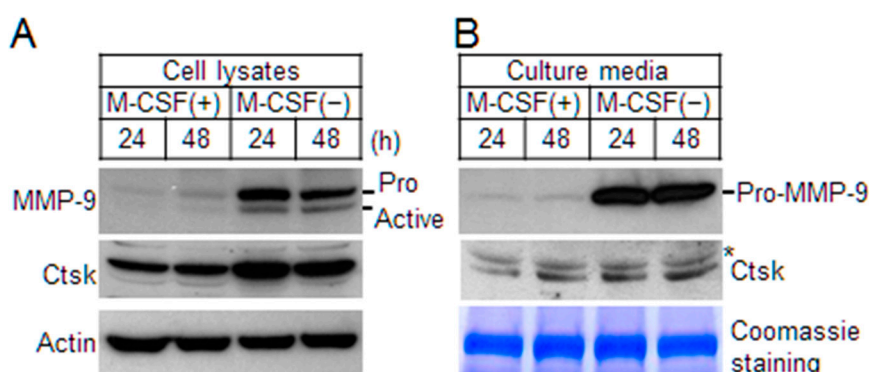
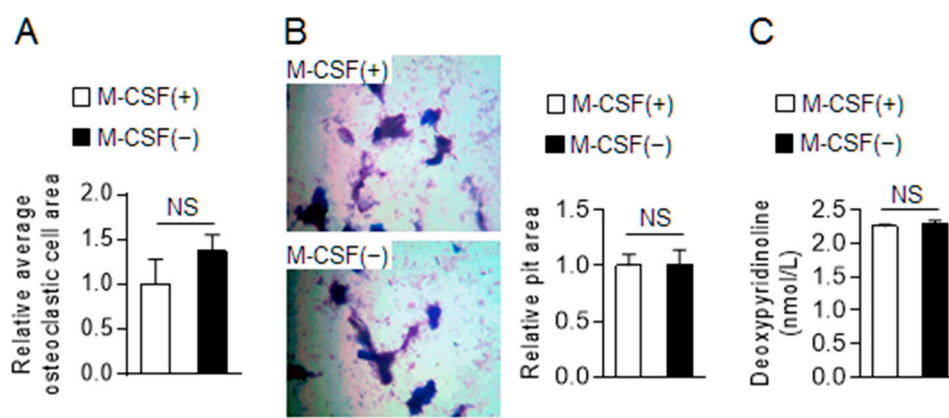


# Supplementary Materials: Synchronized Cell Cycle Arrest Promotes Osteoclast Differentiation

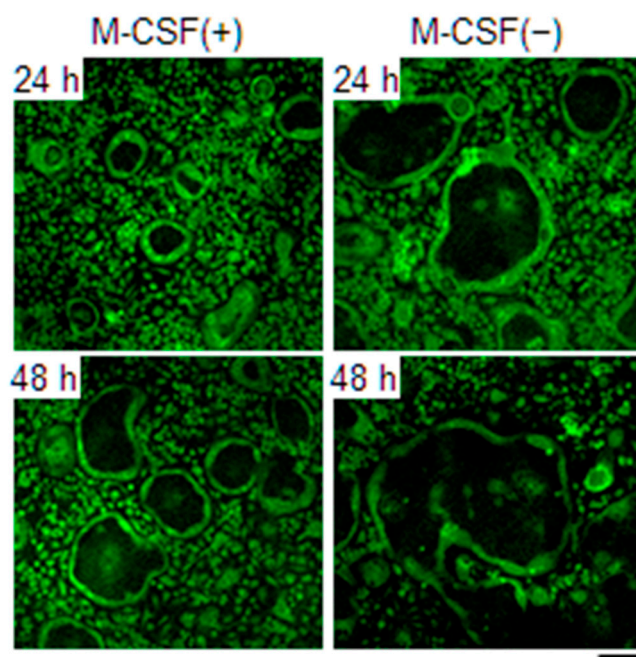
Minsuk Kwon, Jin-Man Kim, Kyunghye Lee, So-Young Park, Hyun-Sook Lim, Taesoo Kim and Daewon Jeong



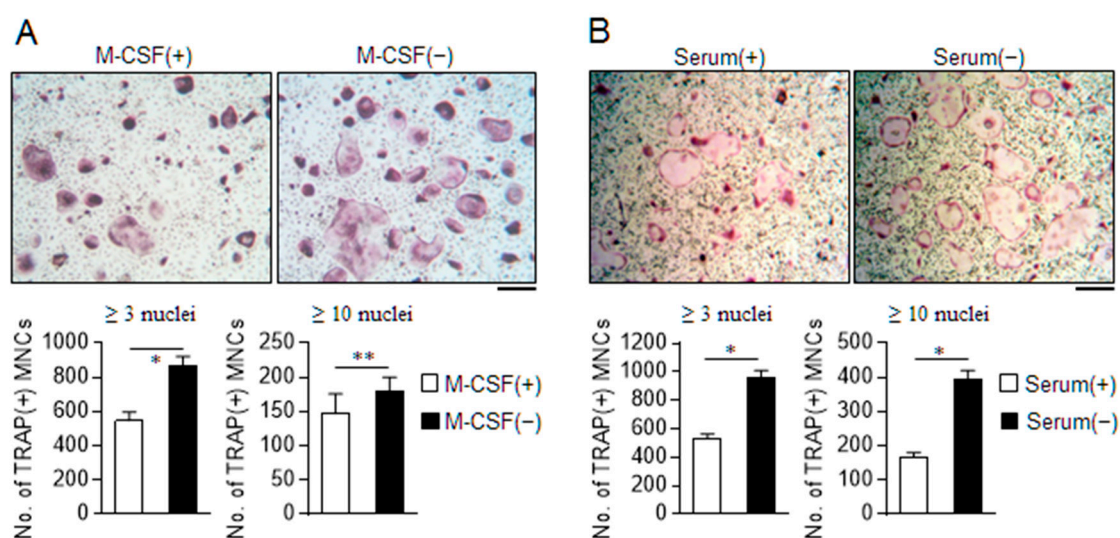
**Figure S1.** The expression level of bone-resorptive proteases during pit formation. Osteoclast precursors with or without M-CSF withdrawal for 12 h were differentiated into osteoclasts in the presence of M-CSF and RANKL for 4 days, after which osteoclasts were further incubated for the indicated times, and cell lysates (A) and culture media (B) were subjected to immunoblotting with specific antibodies to matrix metalloproteinase-9 (MMP-9) and cathepsin K (Ctsk). The actin and coomassie-stained band were used a loading control. \*: non-specific band.



**Figure S2.** Change of osteoclastic cell size in M-CSF-deprived cells and bone pit formation assay. (A) Osteoclast progenitors were cultured in the absence or presence of M-CSF for 12 h. After exposure to M-CSF and RANKL for 4 days to induce osteoclast differentiation, the cells were stained for TRAP and relative average osteoclastic cell area of TRAP(+) MNCs with  $\geq 3$  nuclei was determined by dividing the total cell area by the number of cells; (B) Osteoclast progenitors were seeded on dentine slices (IDS Ltd., Boldon, UK) in the absence or presence of M-CSF for 12 h and differentiated into mature osteoclasts in the presence of M-CSF and RANKL for 4 days followed by a further incubation for 2 days to resorb bone. The slices were stained with 1% toluidine blue and the resorption pit area was analyzed using Image-Pro Plus version 6.0 software (MediaCybernetics, Silver Spring, MD, USA). Scale bar: 50  $\mu$ m; (C) Bone-resorptive end product, deoxypyridinoline (DPD), in culture media as in (B) was determined using MicroVue DPD EIA kit (Quidel, San Diego, CA, USA). NS stands for Not Significant.



**Figure S3.** F-actin staining in osteoclasts. Osteoclast precursors with or without M-CSF withdrawal for 12 h were differentiated into osteoclasts in the presence of M-CSF and RANKL for 4 days, after which osteoclasts were further cultured for the indicated times, fixed, permeabilized, stained with fluorescein isothiocyanate (FITC)-conjugated phalloidin (Invitrogen, Carlsbad, CA, USA), and examined by fluorescence microscopy. Scale bar: 100  $\mu$ m.



**Figure S4.** Increased osteoclast differentiation by cell-cell contact inhibition or serum withdrawal. (A) Osteoclast progenitors ( $2 \times 10^5$  cells per well) were seeded in 48-well plates to reach 100% confluence and cultured in the absence or presence of M-CSF for 12 h, after which osteoclast differentiation was achieved by treating with M-CSF and RANKL for 4 days; (B) Osteoclast progenitors ( $5 \times 10^4$  cells per well in 48-well culture plates) were incubated in culture medium with M-CSF plus 10% FBS [Serum (+)] or 0.5% FBS only [Serum (-)] for 12 h and further differentiated into osteoclasts in the presence of M-CSF and RANKL for 4 days. After cells as in (A) and (B) were stained for TRAP, the number of TRAP(+) MNCs with  $\geq 3$  or  $\geq 10$  nuclei were counted under a light microscope. Scale bar: 200  $\mu$ m. Data are means  $\pm$  SD \*  $p < 0.01$ , \*\*  $p < 0.05$  (Student's *t* test).