Supplemental Figure 1. Time course of breast cancer factors-induced effects on osteoblast and osteoclast differentiation. Mouse bone marrow cells were grown for 3-15 days with AA (50 nM) without additions or in the presence of MDA-MB-231 CM (10%), fixed at different times and stained for ALP (red), or TRAP (purple).

(A) Representative images of osteoblastic cells in cultures treated with AA only (AA, *left*), or with AA and MDA-MB-231 CM (AA+231, *right*). Scale bar is 20 μm.

(B) Average area covered by ALP-positive cells is significantly reduced in MDA-MB-231 CM-treated cultures at day 6-15. Data are means ± SEM, n = 4 independent experiments, $p < 0.05$.

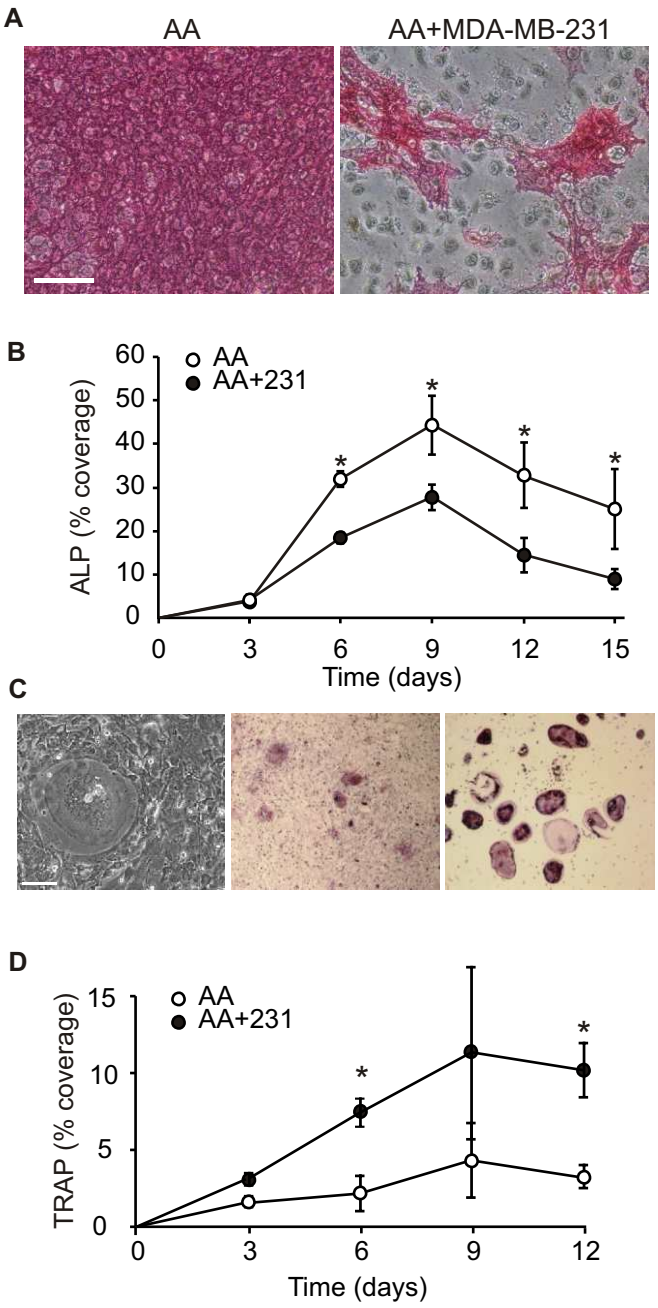
(C) Representative images demonstrating an osteoclast that breached through a layer of osteoblasts (*left*), TRAP-positive condensations, which are likely osteoclasts growing under a monolayer of osteoblasts (*middle*), and multinucleated, TRAP-positive osteoclasts evident after the monolayer of osteoblasts has been lifted (*right*). Scale bar is 20 μm.

(D) Average area covered by TRAP-positive cells is significantly increased in MDA-MB-231 CM-treated cultures at day 9-12. Data are means ± SEM, n = 4 independent experiments, $p < 0.05$.

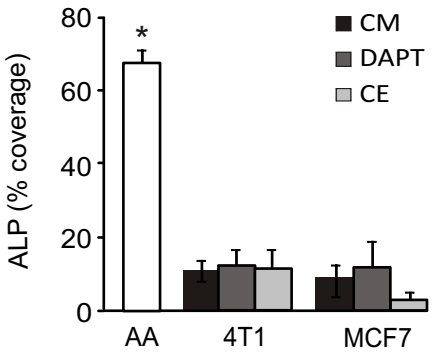
Supplemental Figure 2. Inhibition of γ-secretase does not rescue the anti-osteoblastic phenotype in 4T1 and MCF7 breast cancer cell conditioned medium-treated cultures. Mouse bone marrow cells were grown for 6 days with AA (50 nM) in the absence (AA, open bars) or presence of 4T1 or MCF7 CM (10%, black bars), combined with DAPT (100 nM , dark gray bars), or Compound E (CE, 100 nM; light gray bars). The parallel samples were fixed, stained for ALP and the area covered by ALP-positive cells was assessed. Data are means ± SEM, n = 3-5 independent experiments, asterisk indicates significant difference at $p < 0.05$.

Supplemental Figure 3. TGFβ and Il-11 pathways are not involved in the effects of breast cancer cells on bone cells. Mouse bone marrow cells were grown for 6 days with AA (50 nM) in the absence (AA, open bars) or presence of MDA-MB-231 CM (10%), combined with vehicle (AA+231, black bars), TGFβ neutralizing antibody (anti-TGFβ, 50 nM), or TGFβ type I receptor inhibitor (anti-TβRI, SB431542, 10 μM). The parallel samples were fixed, stained for ALP and the area covered by ALP-positive cells was assessed. Data are means ± SEM, n = 5 independent experiments, different letters indicate significant difference at $p < 0.05$.

Fong et al. Suppl. Figure 1



Fong et al. Suppl. Figure 2



Fong et al. Suppl. Figure 3