Pharmacological evidence for the bone-autonomous contribution of the NFκB/β□catenin axis to breast cancer related osteolysis

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Supplemental figures

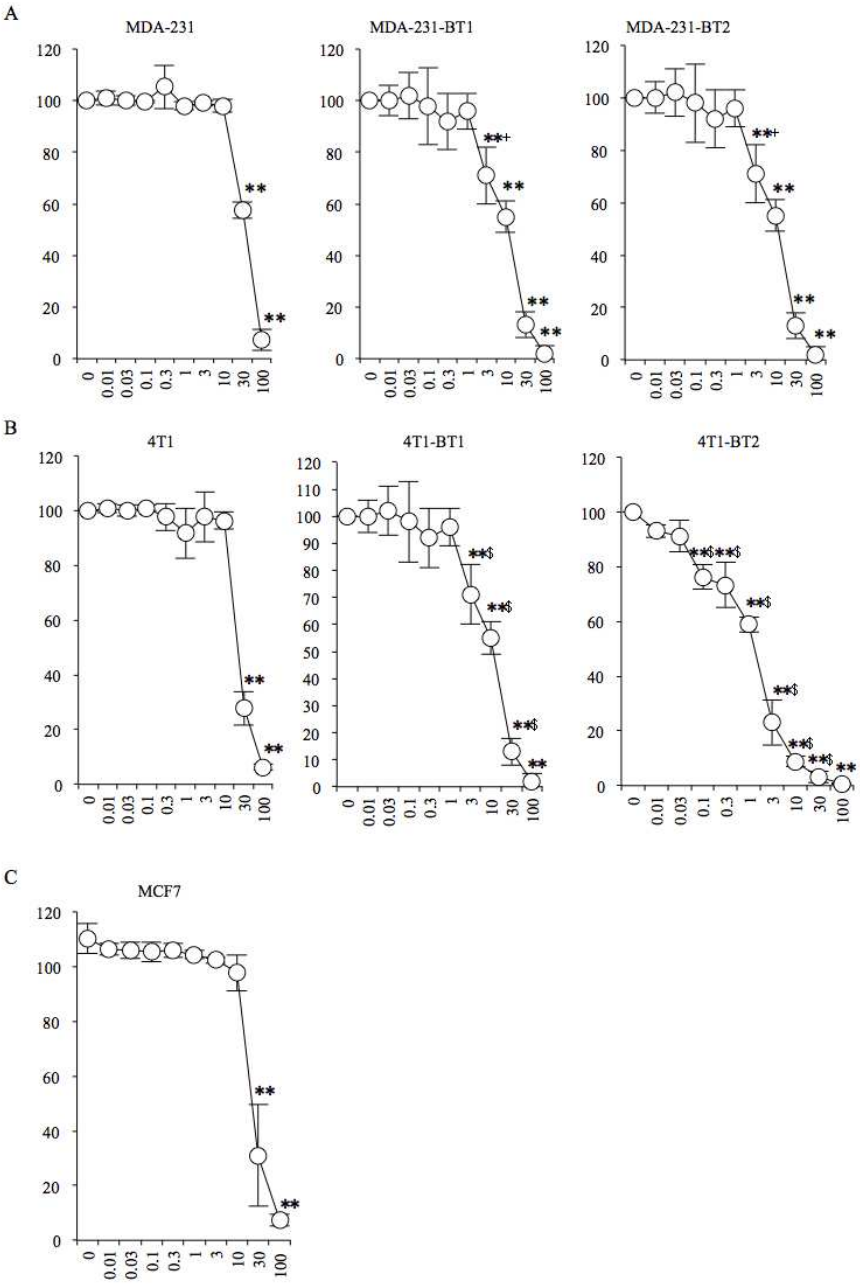

Figure S1. Effects of Parthenolide on the growth of osteotropic breast cancer cells in vitro (related to Table 1). (A) In vitro growth of human parental (left, MDA-231) and osteotropic (middle, MDA-231-BT1; right, MDA-231-BT2) breast cancer cells treated with vehicle (DMSO, 0.01%v/v) or

PTN at the indicated concentrations for 48 hours. (B) In vitro growth of mouse parental (left, 4T1) and osteotropic (middle, 4T1-BT1; right, 4T1-BT2) breast cancer cells treated with vehicle (DMSO, 0.01%v/v) or PTN at the indicated concentrations for 48 hours. (C) In vitro growth of human MCF7 breast cancer cells treated with vehicle (DMSO, 0.01%v/v) or PTN at the indicated concentrations for 48 hours. Cell growth was measured after 48 h of continuous exposure to PTN by AlamarBlue assay. BT denotes osteotropic. Values are mean ± SD; ** $p < 0.01$ from vehicle treated cultures, + $p<0.01$ from correspondent concentration in parental MDA-231 cultures; $ $p<0.01$ from correspondent concentration in parental 4T1 cultures.

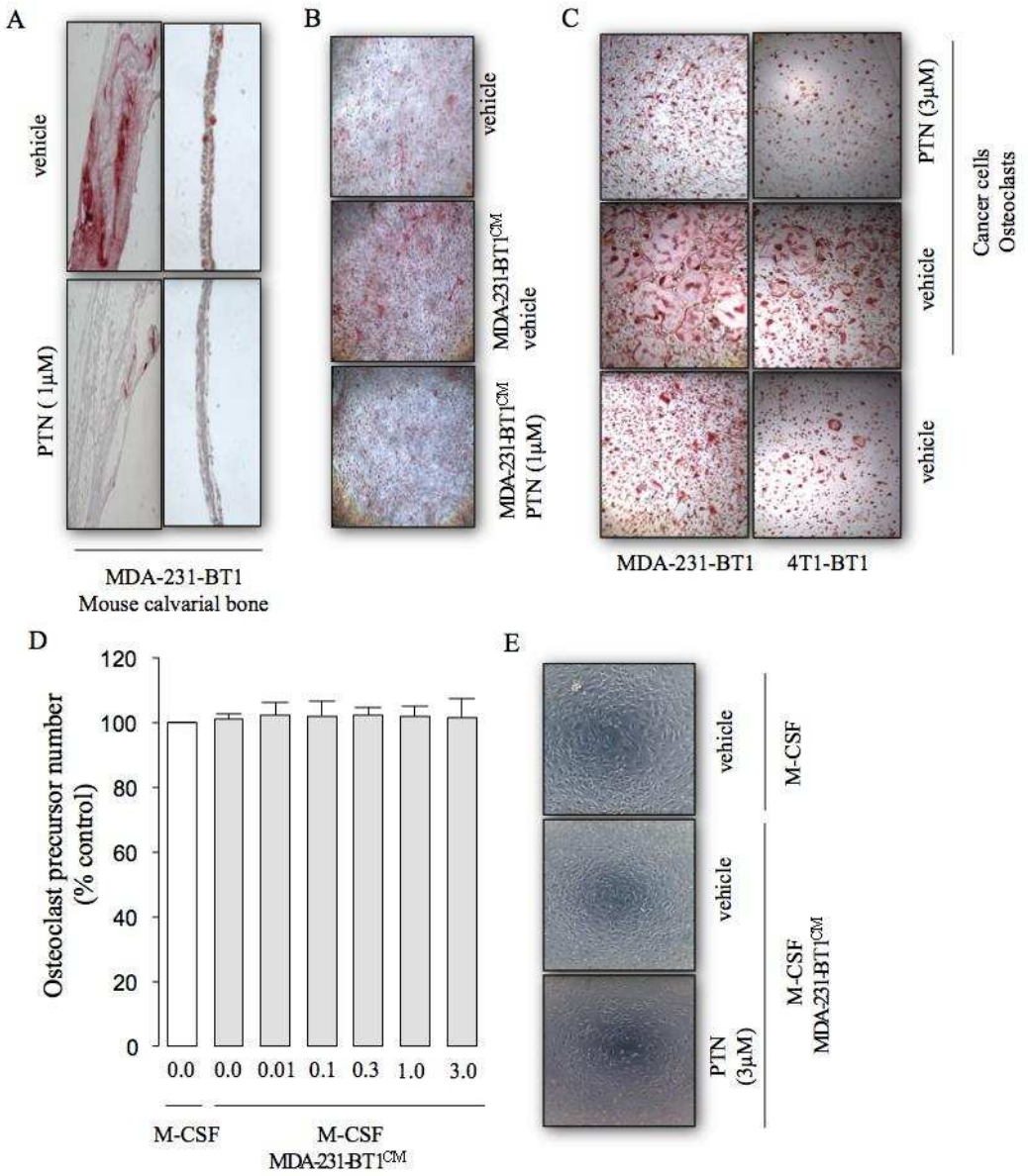

Figure S2. Effects of PTN on osteoclast formation and activity in vitro. (A) Representative photomicrographs of osteoclasts from the experiment described in Fig. 1A (left panel). (B) Representative photomicrographs of osteoclasts from the experiment described in Fig. 1C. (C) Representative photomicrographs of osteoclasts from the experiment described in Fig. 4B. (D) In vitro proliferation of pre-osteoclast cells in mouse bone marrow cultured with M-CSF (25 ng/ml) and conditioned medium (20% v/v) from human MDA-231 breast cancer cells treated with vehicle (0.1% DMSO) or Parthenolide (PTN) at the indicated concentrations for 48 hours. (E) Representative photomicrographs of M-CSF (25ng/ml) generated bone marrow cells from the experiment described in panel D.

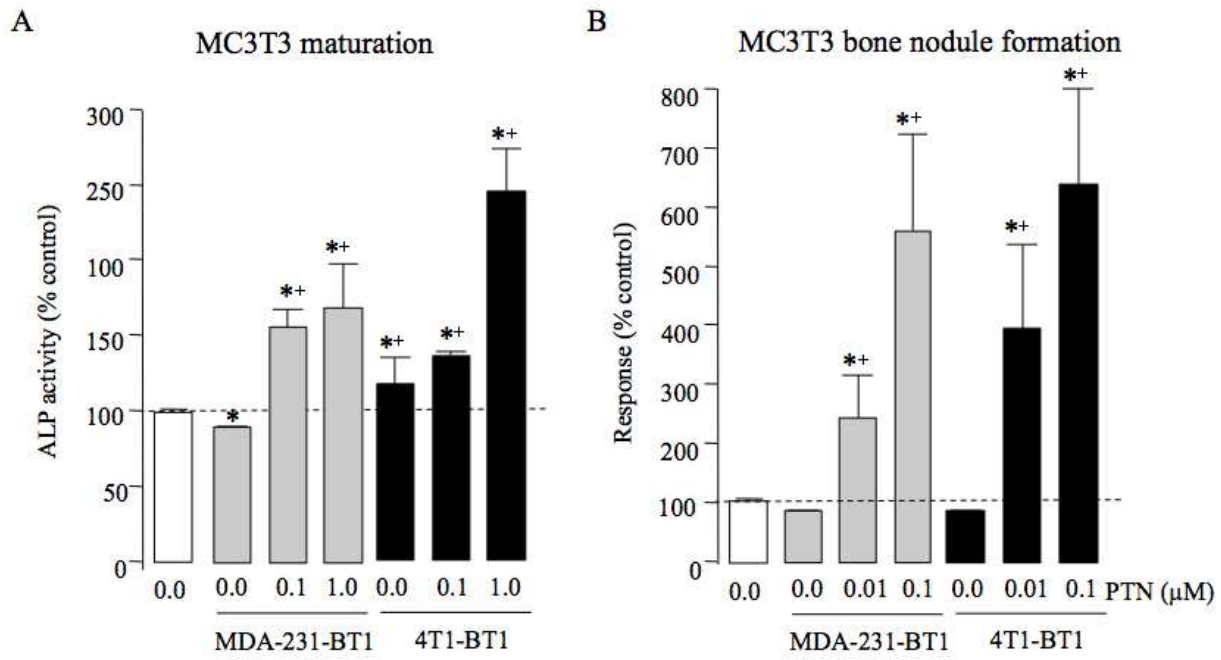

Figure S3. PTN enhances MC3T3 differentiation and bone nodule formation. (A-B) In vitro osteoblast differentiation (A) and bone nodule formation (B) in cultures of mouse osteoblast-like cells MC3T3 exposed to standard or conditioned medium (20%v/v) from human MDA-231-BT1 or mouse 4T1-BT1 cancer cells in the presence or absence of PTN for 7 days. Osteoblast differentiation and bone nodule formation were assessed by Alkaline phosphatase (ALP) and Alazarin Red assays. Values are mean ± SD; * $p<0.05$ from vehicle; + $p<0.05$ from vehicle plus breast cancer cell conditioned medium.

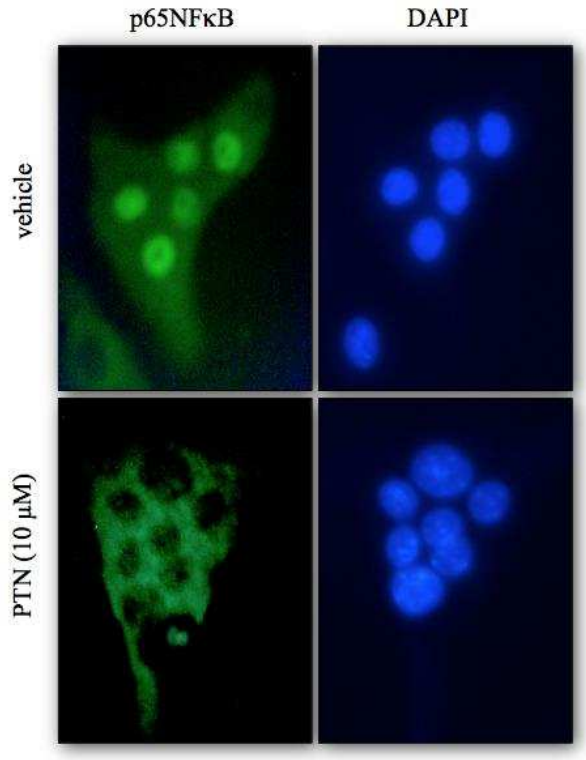

Figure S4. Effect of PTN on RANKL-induced NFκB nuclear translocation in osteoclasts. M-CSF and RANKL-generated osteoclasts were cultured in serum-free medium in the presence of vehicle or Parthenolide (PTN, 10μM) for 1 hour before stimulation with RANKL (100ng/ml) for 25 minutes. Osteoclasts were stained for p65 NFκB (green) and nuclei counterstained with DAPI (blue) as described under materials and methods.

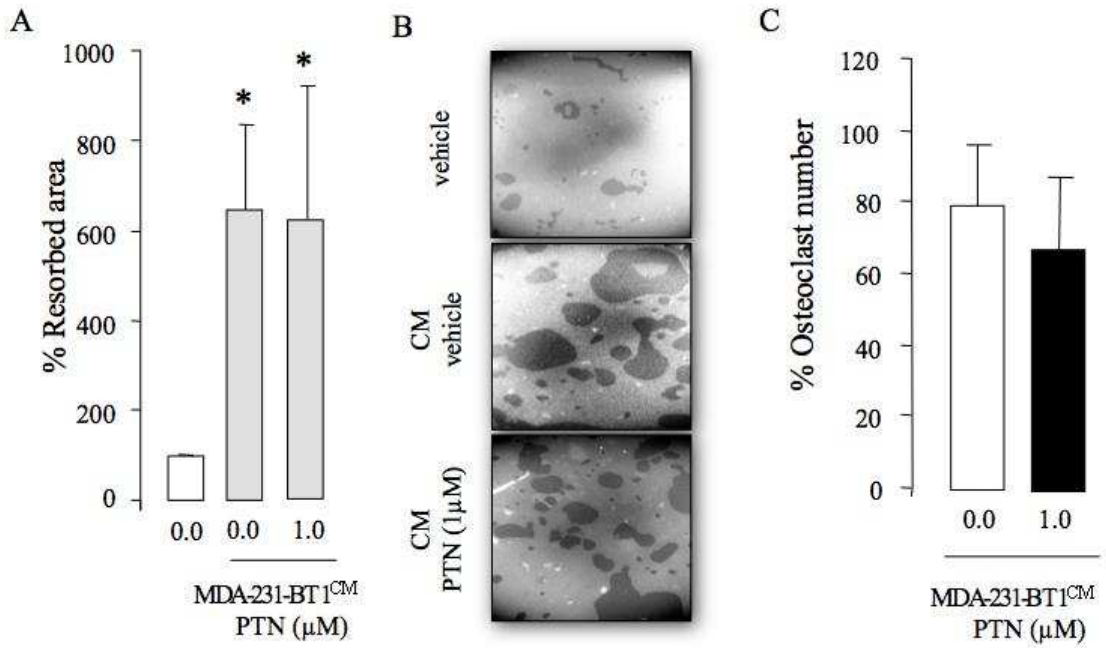

Figure S5. Effects of Parthenolide on osteoclast bone resorption in vitro. (A) In vitro bone resorption in mature osteoclast cultures after exposure to human MDA-231 conditioned medium (20% v/v) in the presence and absence of PTN (1μM) after 72 hours. (B) Representative photomicrographs of resorbed areas from the experiment described in panel A. (C) Total osteoclast number from the experiment described in panels A-B. Values are mean ± SD; * $p<0.05$ from vehicle treated cultures.

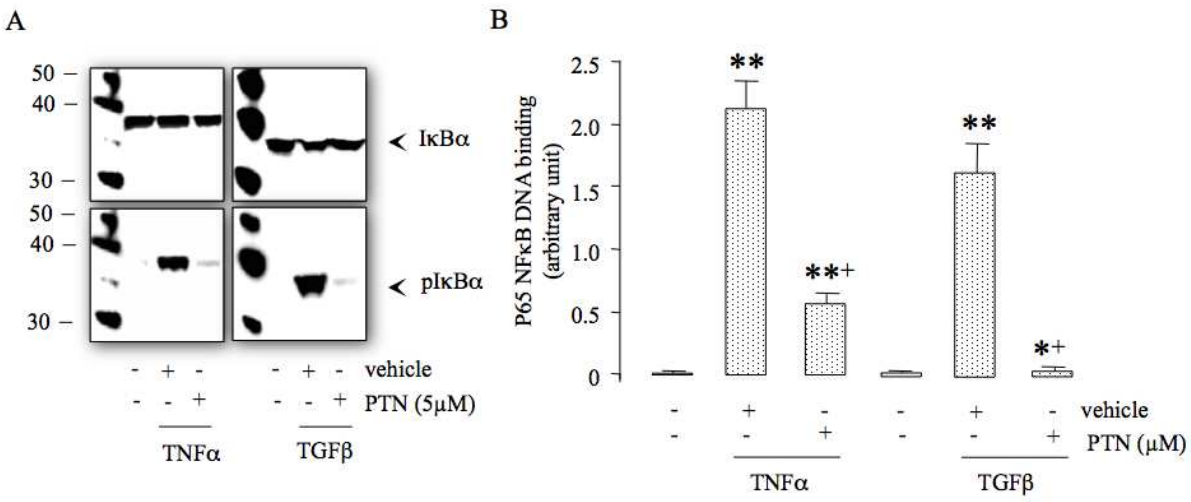

Figure S6. Effects of Parthenolide TNFα– and TGFβ-induced NFκB activation. Western blot of (A) total and phosphorylated IκB and (B) quantification of p65 NFκB – DNA binding in M-CSF (25ng/ml) dependent pre-osteoclasts cultured in MDA-231-BT1 conditioned medium and vehicle (0.01%DMSO) or Parthenolide (PTN, 5μM) for 1 hour and then exposed to human TNFα (10ng/ml) or human TGFβ (10ng/ml) for 10 (A) or 45 minutes (B). Values are mean ± SD; ** $p<0.01$ from vehicle treated cultures; + $p<0.01$ from vehicle, cytokine and breast cancer cells or conditioned medium treated cultures.

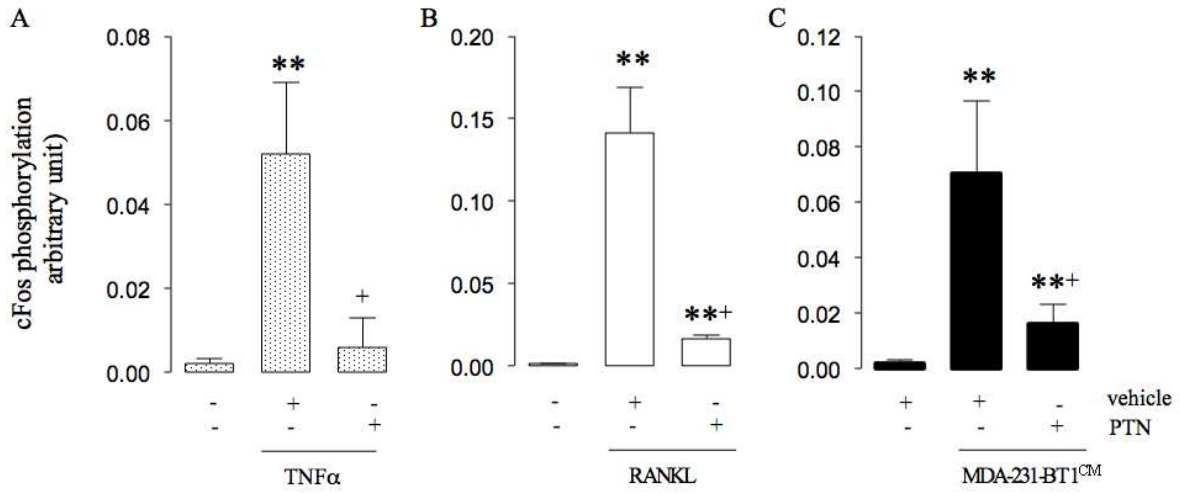

Figure S7. Parthenolide inhibits cFos signaling in pre-osteoclasts. Phosphorylation of cFos in M-CSF dependent pre-osteoclasts exposed to TNFα (A, 10ng/ml), RANKL (B, 100ng/ml) or human MDA-231 conditioned medium (C, 20%v/v) in the presence and absence of Parthenolide (PTN, 5μM). Values are mean ± SD; ** $p < 0.01$ from vehicle treated cultures; + $p<0.01$ from vehicle and cytokine or breast cancer cells or conditioned medium treated cultures.