

Supplemental Material

Specific PCR primers were designed as detailed in the main text:

Mouse TRAP: mAcp5 (forward): TTCAGGACGAGAACGGTGTG; (reverse): CTCTCGTGGTGTTCAAGGGTC

Mouse type 1 collagen: mCol1A1 (forward): GTGTGCGATGACGTGCAATG; (reverse): TTGGGTCCCTCGACTCCTAC

Mouse cathepsin K: mCtsK (forward): CTTCCAATACGTGCAGCAGA; (reverse): TTGCATCGATGGACACAGAG

Human PTHrP: hPTHLH (forward): AACACAAAGAACCACCCCGT; (reverse): GTGGAGGTGTCAGACAGGTG

Mouse RPL32: mRPL32 (forward): GCTGCCATCTGTTTTACGGC; (reverse): CGTTGGGATTGGTGACTCTGA

Human RANKL: hTNFSF1 (forward): GGTTGGGCCAAGATCTCCAA; (reverse): TCCGGATCCAGTAAGGAGGG

Mouse RANKL: mTNFSF11(forward): AAAACGCAGGTTTGCAGGAC; (reverse): GTGAGGTGTGCAAATGGCTG

Mouse Opg: mTNFRSF11b (forward): TGAAAGCGTTAACCTGGAGC; (reverse): GAAACCTCAGGGGCTTGGAG

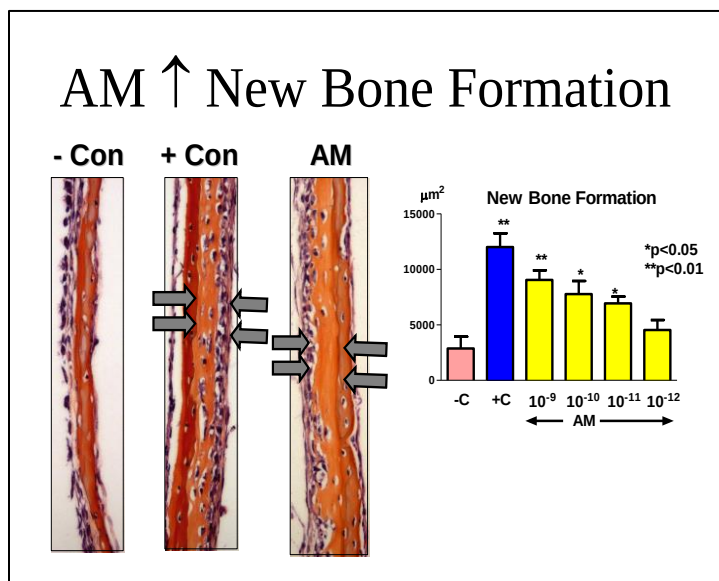


Figure S1: Stimulation of new bone formation by adrenomedullin. AM1-52amide peptide was added to the neonatal calvarial assay for 7d as described by Mohammad et al [31] with n=3. Bones were fixed, decalcified and stained with H&E. New bone area (left) was scored as orange area (between gray arrows). Older bone stains darker red. Positive control is 100nM insulin. New bone area was quantified by computerized histomorphometry and results shown on right.

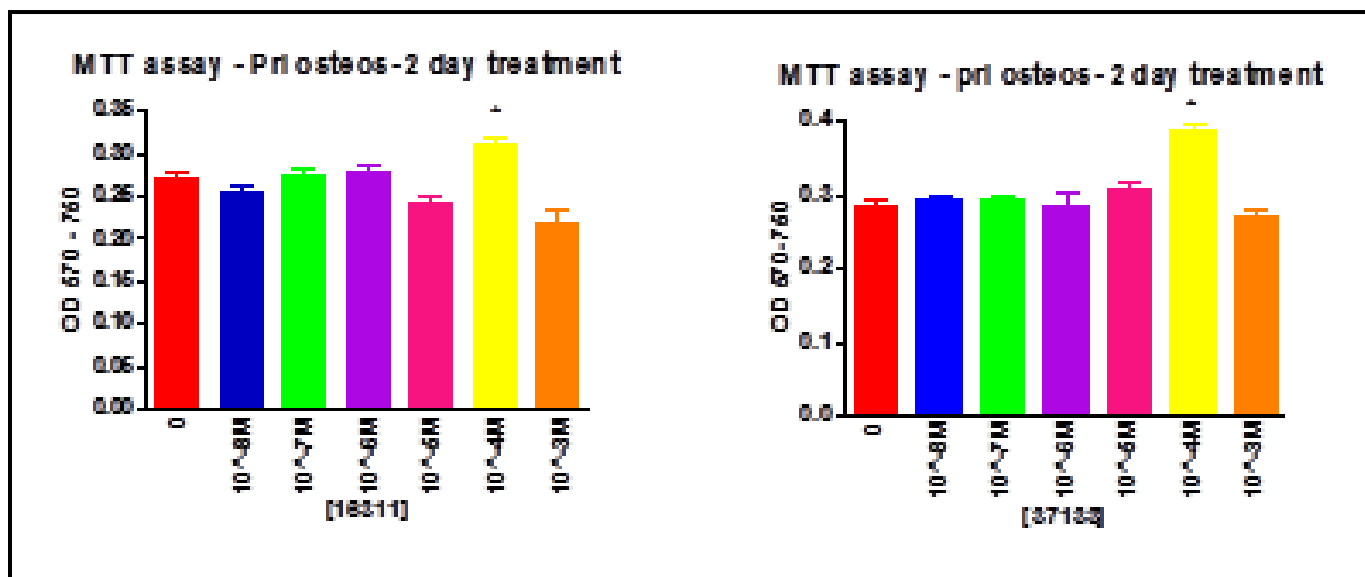


Figure S2: Cellular toxicity of 16311 & 37133. Primary osteoblasts (Pri osteos) treated with 16311 or 37133 for 2d and cells counted by MTT assay. OD measured at 570nm & 750nm for normalization. No toxicity seen with 2d of treatment at up to 1mM. There was slightly increased proliferation at 10⁻⁴M 16311 or 37133 (p<0.05 by one-way ANOVA).

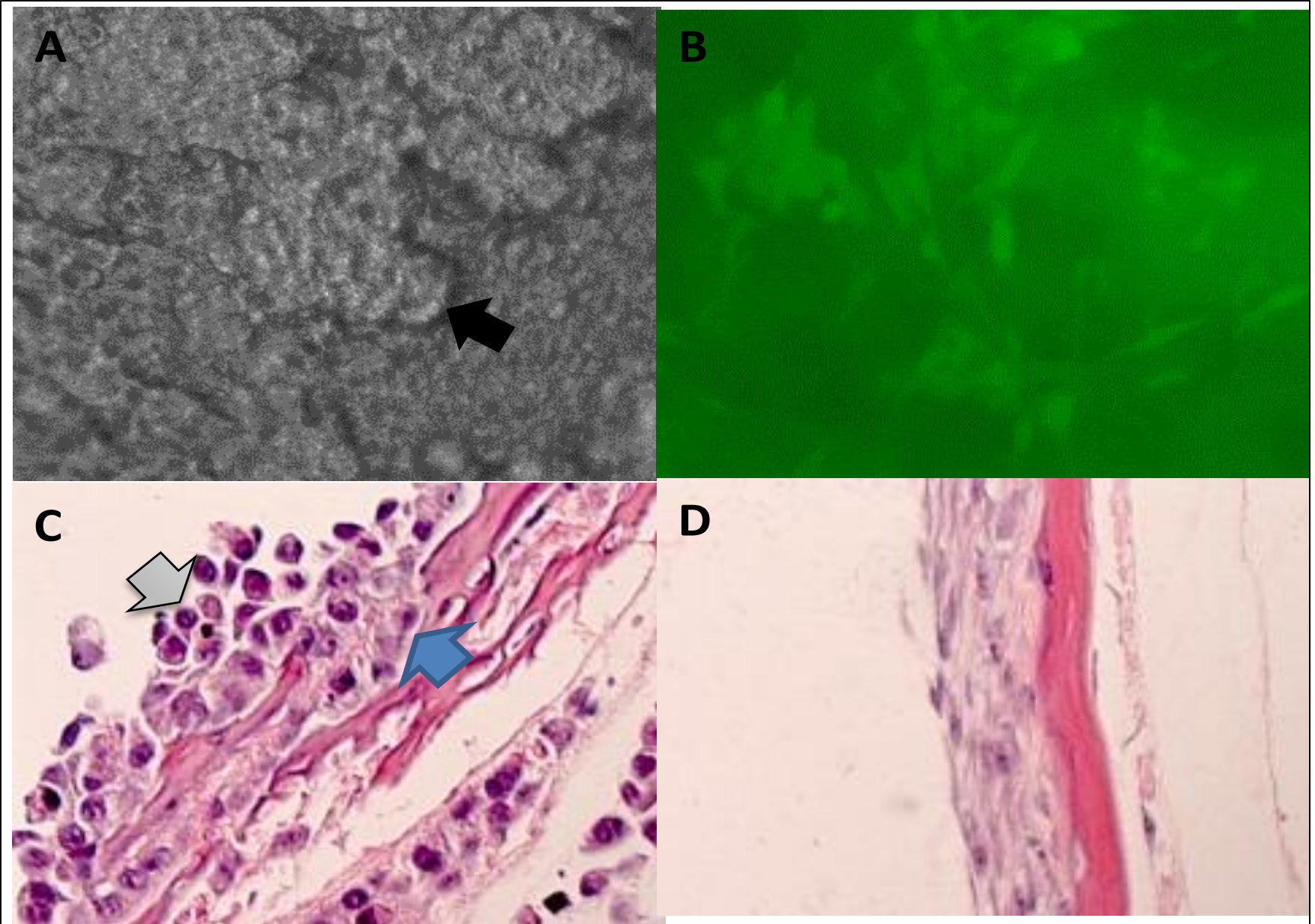


Figure S3: MDA-MB-231 cells grown 7d with mouse calvariae. (A) Phase contrast: lucent lesions with scalloping edges (**arrow**). (B) Fluorescence of GFP cells in clusters. (C) H&E: tumor cells in bone (**gray arrow**) causing osteolysis (**blue arrow**), vs control bone (D).

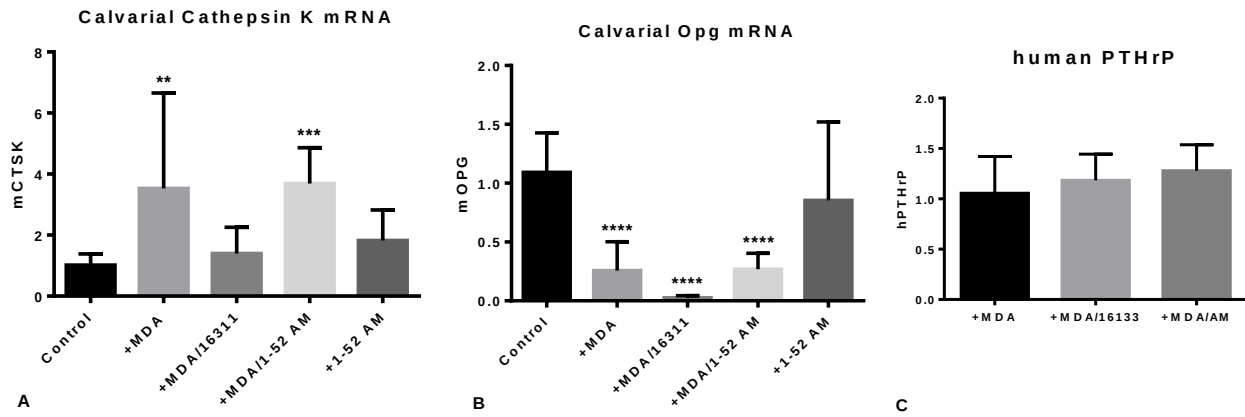


Figure S4: Changes in gene expression in tumor:bone co-cultures. Additional analyses of RNA samples from main Figure 6. **(A)** Osteoclast marker cathepsin K (mCTSK) gave results similar to TRAP in Figure 6C. **(B)** Negative regulator of osteoclast formation, osteoprotegerin (Opg = mTNFRSF11B), which binds and neutralizes RANKL. All groups expressed only low levels of Opg mRNA with C_t values >30 . **(C)** Osteolytic factor PTHrP (hPTHLP) was unchanged in any of the groups. It is expressed by MDA-MB-231 breast cancer cells [25], is increased by TGFbeta in bone [41] and acts on osteoblasts to increase osteolysis by stimulating RANKL expression [42].