**Supplementary data for:**

Nanovibrational stimulation inhibits osteoclastogenesis and enhances osteogenesis in co-cultures.

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***Supplementary figure 1.*** *Nanovibrational measurement. Vibrational readouts from 24 well plates showing the variance in vibration in 2D and 3D. Values are the mean of 3 readings.*



***Supplementary Figure 2.* *QPCR data for Figure 2g – CD14 culture.*** *Data shows mean and individual data points; n=(d=1–3, r=3), statistics by t-test where \*=p<0.05.*

Chart, radar chart

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***Supplementary figure 3.******Osteoclast response to nanovibrational stimulation in 2D.*** *Ingenuity pathway analysis of metabolite data after 7 days of culture inferred inhibition of NFκB signalling with nanovibration (n=3).*



***Supplementary Figure 4.* *QPCR data for Figure 3h 2D (top) – 2D co-culture.*** *Data shows mean and individual data points; n=(d=1-4,r=3-4), statistics by t-test where \*=p<0.05.*



***Supplementary Figure 5.* *QPCR data for Figure 3h 2D (bottom) – 3D co-culture.*** *Data shows mean and individual data points; n=(d=1-4,r=3-4), statistics by t-test where \*=p<0.05, \*\*=p<0.01 and \*\*\*-p<0.001.*

*![Diagram

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***Supplementary Figure 6.* *Metabolite network analysis implying differential Akt regulation.*** *Untargeted metabolite analysis for 2D and 3D nanovibrational cultures compared to controls at days 14 and 21 of culture all linked to Akt signalling. Akt was predicted to be up-regulated at day 14 and down-regulated at day 21 (n=(d=3,r=4)).* It has been implicated in bone cell differentiation, specifically TGFβ1 (transforming growth factor beta 1) and BMP2 (bone morphogenetic protein 2) stimulated osteogenesis.1, 2 Disruption of Akt1 causes osteoblast apoptosis and decreased activity of the master osteoblast transcriptional regulator runt-related transcription factor 2 (RUNX2); this in turn reduces RANKL expression and prevents osteoclast fusion.3 Inhibition of Akt has also been potentially implicated in reduced osteoclastogenesis. Guanine nucleotide-binding protein subunit α13 (Gα13) negatively regulates osteoclast formation through increased Akt/GSK3β/NFATc1 (glycogen synthase kinase 3 beta / nuclear factor of activated T cells 1) signalling.4 Further, Akt inhibition rescues osteoclast hyper-activation in Gα13 deficient osteoclasts. However, M-CSF and RANKL stimulation of osteoclastogenesis stimulates Akt to mediate survival, proliferation and differentiation of osteoclast progenitors.4, 5 This effect was only demonstrated in the co-culture, and as such the initial implied increase in Akt signalling seen here could therefore be acting to enhance osteogenesis and moderate osteoclastogenesis. However, the metabolomics data represents a correlative association and causation has not been confirmed in this study.

***Supplementary references.***

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2. Suzuki, E. et al. Akt activation is required for TGF-beta1-induced osteoblast differentiation of MC3T3-E1 pre-osteoblasts. *PLoS ONE* **9**, e112566 (2014).

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4. Wu, M. et al. Galpha13 negatively controls osteoclastogenesis through inhibition of the Akt-GSK3beta-NFATc1 signalling pathway. *Nature communications* **8**, 13700 (2017).

5. Matsumoto, T. et al. Regulation of bone resorption and sealing zone formation in osteoclasts occurs through protein kinase B-mediated microtubule stabilization. *J Bone Miner Res* **28**, 1191-1202 (2013).