

## Supplementary Materials for

### **Osteoprotegerin reduces osteoclast resorption activity without affecting osteogenesis on nanoparticulate mineralized collagen scaffolds**

Xiaoyan Ren, Qi Zhou, David Foulad, Aleczandria S. Tiffany, Marley J. Dewey, David Bischoff, Timothy A. Miller, Russell R. Reid, Tong-Chuan He, Dean T. Yamaguchi, Brendan A. C. Harley, Justine C. Lee\*

\*Corresponding author. Email: justine@ucla.edu

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Supplementary Materials and Methods

Table S1. Primer sequences.

Fig. S1. Osteoclasts migrate into Col-GAG and MC-GAG scaffolds in the presence of control hMSCs in direct cocultures.

## **Supplementary Materials and Methods**

### **Anti-TRAP Immunofluorescence**

Scaffolds were fixed in 10% formalin, paraffin-embedded, and sectioned at 5 microns. Following deparaffinization, the sections were treated with 0.5% Triton X-100, blocked with 10% serum for 1 hour, and subjected to heat-induced antigen retrieval using 10 mM sodium citrate buffer. Sections were then incubated in anti-TRAP (Thermo Fisher, Eugene, OR) overnight in 4 °C. After washing, sections were incubated in anti-mouse IgG Alexa Fluor Plus 555 (Thermo Fisher, Eugene, OR). Coverslips were mounted with Prolong Gold Antifade Reagent with Dapi (Cell Signaling Technologies, Danvers, MA). Images were captured with Zeiss Axio Observer 3 inverted microscope with ZEN 2.3 Pro software (Zeiss, Oberkochen, Germany).

**Table S1. Primer sequences.**

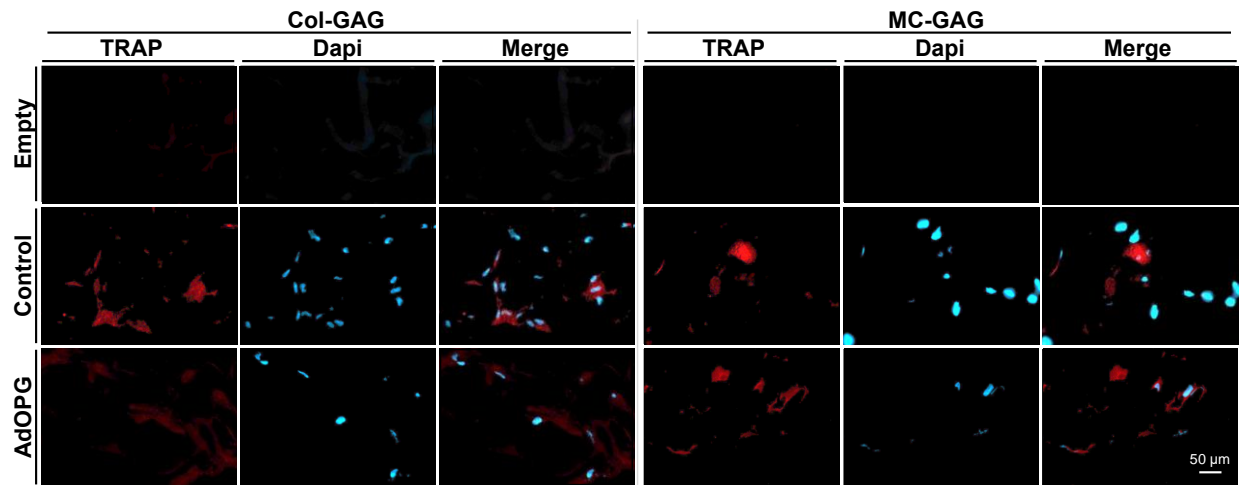
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<b>Genes</b>	<b>Oligonucleotide sequence</b>
18S sense	5'-TAGAGTGTTCAAAGCAGGCCCG-3'
18S antisense	5'-TCCCTCTTAATCATGGCCTCAG-3'
OPN sense	5'- GCTCTAGAATGAGAATTGCACTG-3'
OPN antisense	5'- GTCAATGGAGTCCTGGCTGT-3'
Runx2 sense	5'-TCGGAGAGGTACCAGATGGG-3'
Runx2 antisense	5'-AACTCTTGCCTCGTCCACTC-3'
OPG sense	5'-AGTACGTCAAGCAGGAGTGC-3'
OPG antisense	5'-GCTTGCACCACTCCAAATCC-3
RANKL sense	5'-ACACGCGTATTTACCTGCCA-3'
RANKL antisense	5'-GACACCCTCTCCACTTCAGC-3'

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OPN, Osteopontin; Runx2, runt-related transcription factor 2; OPG, Osteoprotegerin; RANKL, Receptor activator of nuclear factor kappa-B ligand

## Supplementary Figure 1.



**Fig. S1. Osteoclasts migrate into Col-GAG and MC-GAG scaffolds in the presence of control hMSCs in direct cocultures.** Direct co-cultures of osteoclasts with empty scaffold (Empty), control hMSCs (control), or AdOPG-transduced hMSCs (AdOPG) on Col-GAG or MC-GAG for 14 days were fixed in formalin, embedded, sectioned at 5 microns, and stained with anti-TRAP and anti-mouse IgG AlexaFluor Plus 555. Scale bar marked.