

Electronic Supplementary Material for:

Role of hyaluronan in regulating self-renewal and osteogenic differentiation of mesenchymal stromal cells and pre-osteoblasts

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Table S1. Primer sequences.

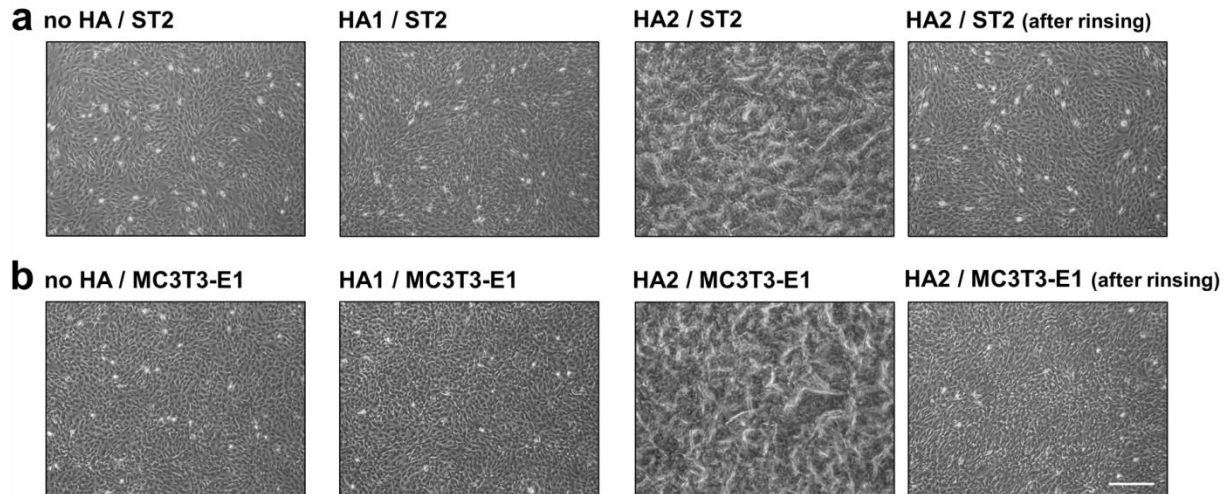


Fig. S1 Morphology of HA-treated ST2 and MC3T3-E1 cells. Images of ST2 (**a**) and MC3T3-E1 (**b**) cells seeded on uncoated (left panels, no HA), HA1- or HA2-coated (middle panels) wells. Cells were plated at 3×10^4 cells/cm² for 24 h before images were taken on a Leica DM IL LED microscope equipped with Leica DFC420 C camera. Both cell types, ST2 and MC3T3-E1, were able to adhere on HA1-coated plates and their morphology did not differ from the morphology of untreated cells seeded on non-coated cell culture plastic (**a** and **b**, compare left with middle panels). In contrast, on HA2-coated plates, cells appeared to adhere solely on the cell culture plastic while HA2 was present in suspension. Images of ST2 and MC3T3-E1 cells plated on HA2-coated wells after rinsing with PBS are shown (**a** and **b**, right panels). Bar, 500 μ m

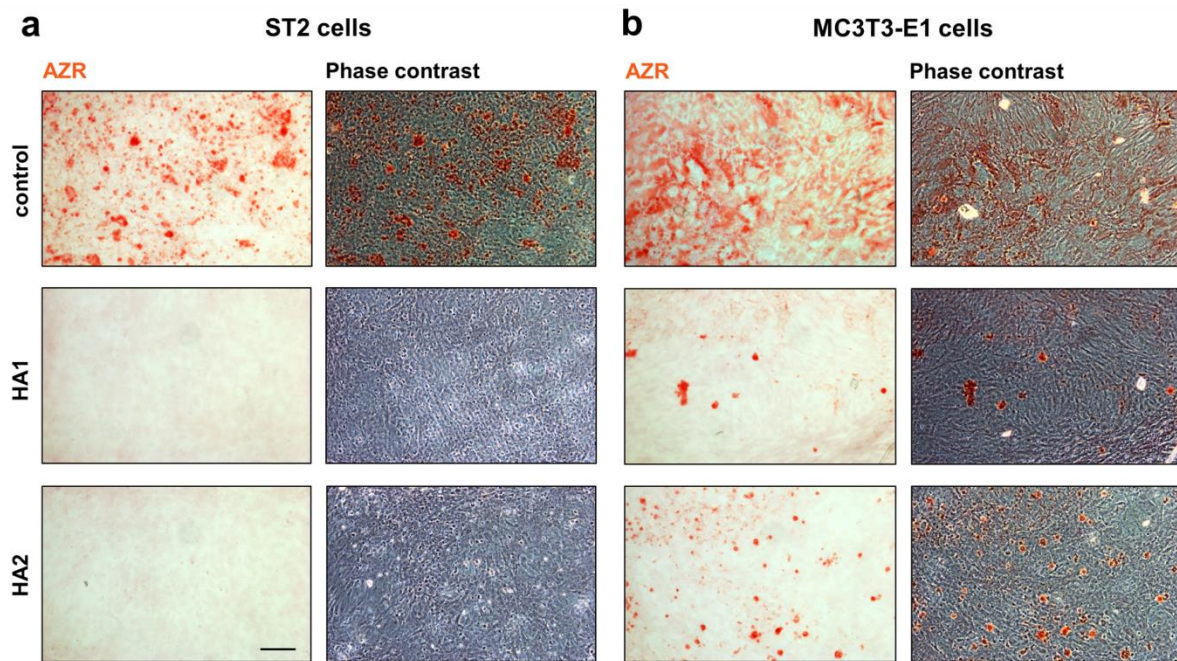


Fig. S2 Mineral deposition capacity of HA-treated ST2 (a) and MC3T3-E1 (b) cells assessed by Alizarin red stain. Cells were seeded on uncoated (control), HA1- or HA2-coated wells of 8-well chamber slides (ThermoFisher Scientific, Reinach, Switzerland) in osteogenic media at a density of 3×10^4 cells/cm². After 21 days, cells were fixed in 96 % ethanol for 15 min and stained with 0.2 % Alizarin Red S (AZR)(Sigma-Aldrich, Basel, Switzerland), pH 6.4 at room temperature for 1 h. Images (including phase contrast) were acquired on an Olympus BX-51. Representative images of the staining in each of the experimental groups are shown. Scale bar, 500 μ m.

Clinical Oral Investigations

Table S1 Primer Sequences

| Gene symbol | Gene bank accession number | Primer pair (fwd/rev) | Amplicon size (bp) |
|--------------------|-----------------------------------|---|---------------------------|
| Bmp2 | NM_007553 | 5' -GGGACCCGCTGTCTTCTAGT-3' 5' -TCAACTCAAATTCGCTGAGGAC-3' | 154 |
| Bmp4 | NM_007554 | 5' -TTCCTGGTAACCGAATGCTGA-3' 5' -CCTGAATCTCGGCGACTTTTT-3' | 114 |
| Bmp7 | NM_007557 | 5' -CCTGTCCATCTTAGGGTTGCC-3' 5' -GGCCTTGTAGGGGTAGGAGA-3' | 151 |
| Tgfb1 | NM_011577 | 5' -CTTCAATACGTCAGACATTCGGG-3' 5' -GTAACGCCAGGAATTGTTGCTA-3' | 142 |
| Fgf1 | NM_010197 | 5' -CCCTGACCGAGAGGTTCAAC-3' 5' -GTCCCTTGTCCCATCCACG-3' | 122 |
| Sox2 | NM_011443 | 5' -CCCACCTACAGCATGTCCTAC-3' 5' -GCCTCGGACTTGACCACAG-3' | 83 |
| Yap1 | NM_001171147 | 5' -TACTGATGCAGGTA CTGCGG-3' 5' -TCAGGGATCTCAAAGGAGGAC-3' | 180 |
| Bmi1 | NM_007552 | 5' -ATCCCCACTTAATGTGTGTCCT-3' 5' -CTTGCTGGTCTCCAAGTAACG-3' | 116 |
| Ctgf | NM_010217 | 5' -GGGCCTCTTCTGCGATTTTC-3' 5' -ATCCAGGCAAGTGCATTGGTA-3' | 151 |
| Ccnd1 | NM_007631.2 | 5' -AGAAGTGCGAAGAGGAGGTC-3' 5' -AGTTCCATTTGCAGCAGCTC-3' | 213 |
| Dkk1 | NM_010051 | 5' -CAGTGCCACCTTGA ACTCAGT-3' 5' -CCGCCCTCATAGAGAACTCC-3' | 129 |
| Apc | NM_007462 | 5' -CCCCGGAGTGAAACTACGC-3' 5' -GGGGACAGGACTGCATTCTC-3' | 97 |
| Gsk3b | NM_019827 | 5' -TGGCAGCAAGGTAACACAG-3' 5' -CGGTTCTTAAATCGCTTGTCTG-3' | 189 |