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Supplemental information

Reprogramming bone progenitor identity and potency through control of collagen density and oxygen tension

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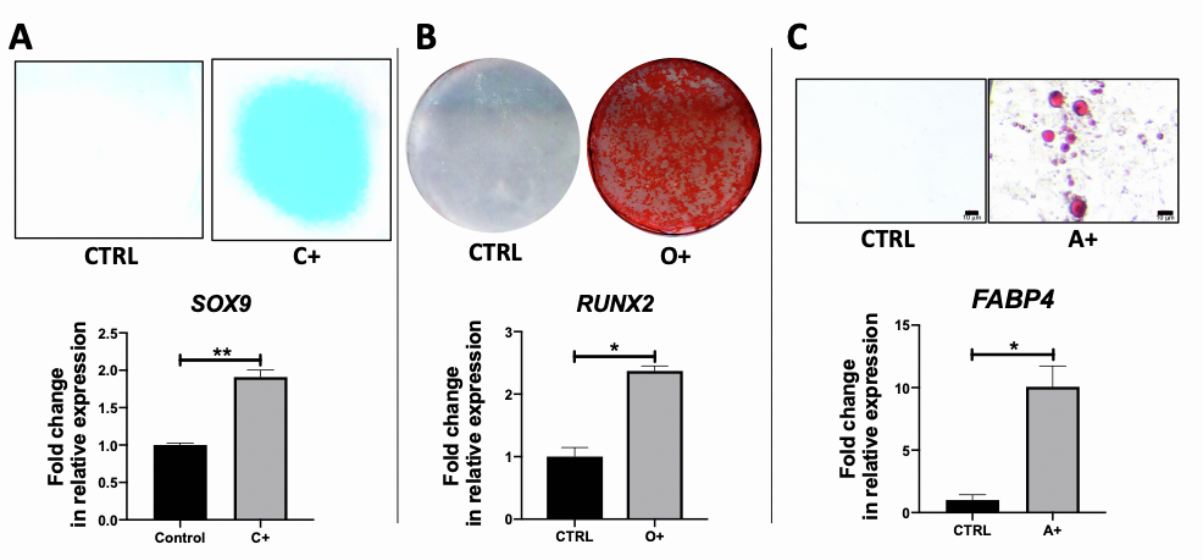

Figure S1 Characterisation of AMSC potency, Related to Figure 1. AMSCs were assessed for their ability to differentiate down the chondrogenic, osteogenic and adipogenic lineages. **A)** Chondrogenic differentiation assessed for GAGs detected using Alcian Blue after 7 days and *SOX9* gene expression analysed by qPCR. **B)** An osteogenic differentiation assay was conducted for 21 days, illustrating positive calcium phosphate staining using Alizarin Red with *RUNX*2 expression analysed by qPCR. **C)** An adipogenic differentiation assay was conducted over 21 days, with the presence of fat droplets analysed using Oil Red O stain and *FABP4* expression relative to control analysed using qPCR (scale bar: 10 μm). (Data are presented as the mean ±S.E.M, Statistical analysis performed using Mann-Whitney test; ***P*<0.01; **P*<0.05; n=3).

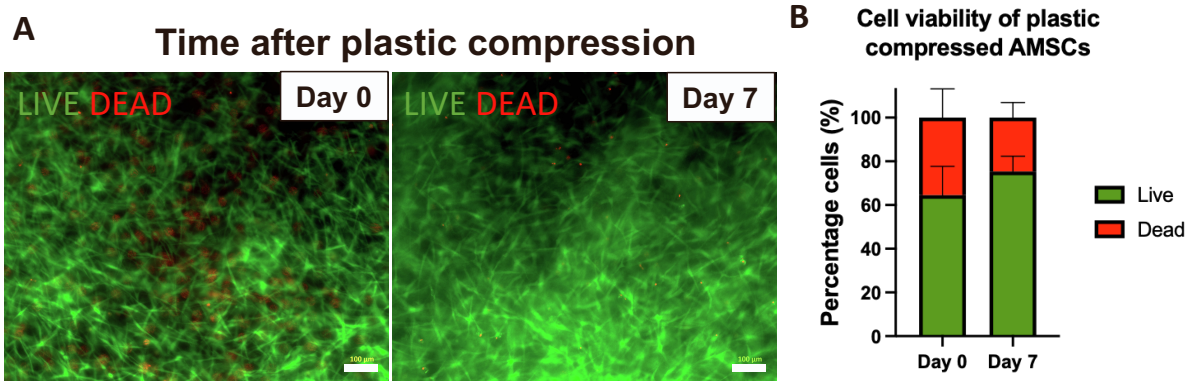

Figure S2 Cell viability of plastic compressed AMSCs, related to Figure 1. AMSCs were seeded in 0.2% collagen type I gels and subjected to plastic compression using RAFT™ absorbers to form a 10% collagen type I gel. A LIVE/DEAD assay was performed to assess cell viability. **A)** Representative images of cells stained with a LIVE/DEAD dye at day 0 and day 7 (Scale bar- 100 μM). **B)** Quantification of percent live and dead cells at day 0 and day 7. An increase in cell viability was observed 7 days after plastic compression.

Table S1 Primer sequences used to conduct gene expression analysis using qPCR, related to STAR Methods.

Gene Name	Forward (5'-3')	Reverse (3'-5')
CD146	GGAAGGTGTGGGTGAAAGAG	GGACATTCAGGGTGCTCAG
CD164	CCTTAGCTTTCTCCCGAACG	TGCTGGGTCGTGTTCTTG
CD73	ACTGGGACATTCGGGTTTTG	CTCTTTGGAAGGTGGATTGC
FABP4	CATACTGGGCCAGGAATTTG	GGACACCCCCTCTAAGGTT
GAPDH	GCTCTCTGCTCCTCCTGTTC	CGACCAAATCCGTTGACTCC
NES	GGCCACGTACAGGACCCTC	CCTCTGGGGTCCTAGGGAAT
PDPN	CTCTGCTCTTCGTTTTGGGA	GAGTCACCACATCATCTTCGG
PRX1	CGAGAGTGCAGGTGTGGTTT	GAGCAGGACGAGGTACGAT
RUNX2	CGCATTCCTCATCCCAGTAT	GCCTGGGGTCTGTAATCTGA
SOX9	TGGAGACTTCTGAACGAGAGC	CGTTCTTCACCGACTTCCTC
TAZ	GCTACACTCCCACTTCTTCAG	CGCCATCTCCTTTCTCTCTTC
YAP	CCCTCGTTTTGCCATGAACC	TGTTGCTGCTGGTTGGAGTT