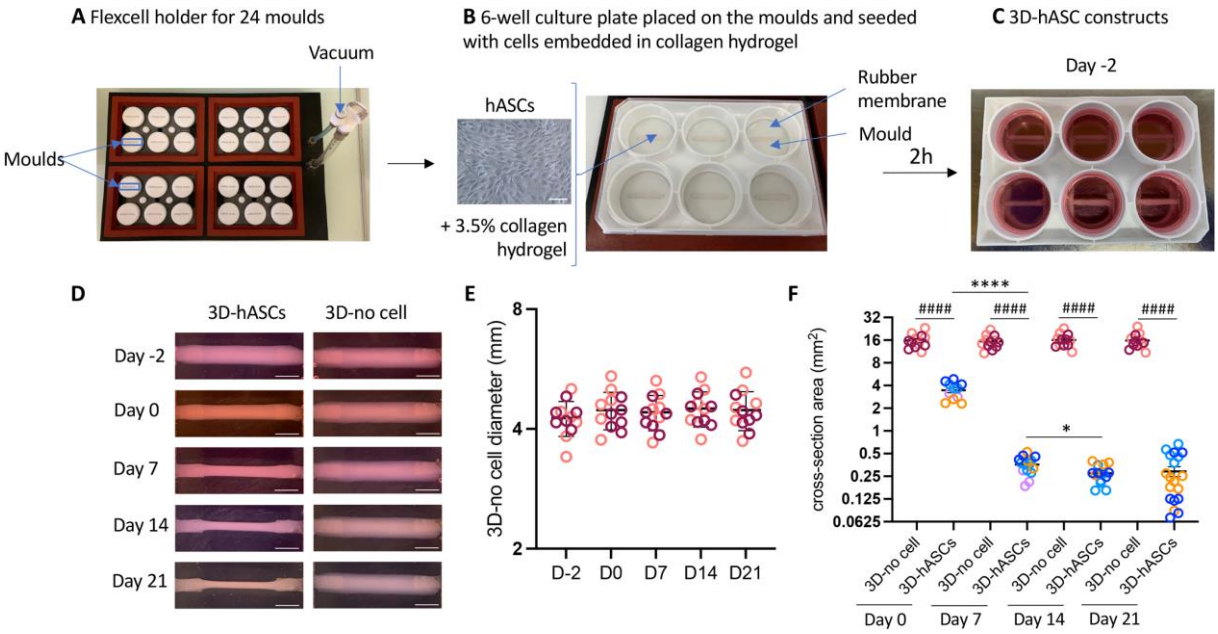

Fig. S1. The Flexcell bioreactor. A) This technology consists of a holder with 24 perforated cylindrical moulds connected to a vacuum pump and controlled by computer. B) Four 6-well plates can be placed on these moulds. The bottom of the wells consists of a rubber membrane with two thick anchor points. When the vacuum is applied, the bottom of the wells takes the cylindrical shape of the moulds. hASCs embedded in 3.5% collagen hydrogel are seeded between the two anchor points and incubated at 37°C, 5% CO_2 for 2 hours. C) At the end of the 2 hours incubation, the vacuum is broken and the plate is removed from the moulds. The plate is left in the incubator at 37°C, 5% CO_2 for 48 hours before experimentation (Day -2 timepoint). D) Pictures of 3D-hASC constructs and 3D-no cell constructs not seeded with cells. Scale bars, 0.5 cm. E) Diameters were measured from constructs at Day 0 (n=12), Day 7 (n=12), Day 14 (n=12) and Day 21 (n=12). Each colour represents a set of experiments. 2 independent experiments were performed with n=6 biological replicates for each experiment. F) Cross-section areas of 3D-no cell and 3D-hASC constructs were calculated from diameters measurements presented Figure 1B: Day 0 (n=11), Day 7 (n=15), Day 14 (n=14) and Day 21 (n=19). Each colour represents a set of experiments. 4 independent experiments were performed with 3<n<8 biological replicates for each experiment. For 3D-hASC constructs, the p-values were obtained using the Mann-Whitney test compared to each following stage. * P<0.05, **** P<0.0001. $^{\#}$ indicates the p-values of cross-section areas of 3D-no cell versus 3D-hASC constructs, $^{\#\#\#\#}$ P <0.0001.

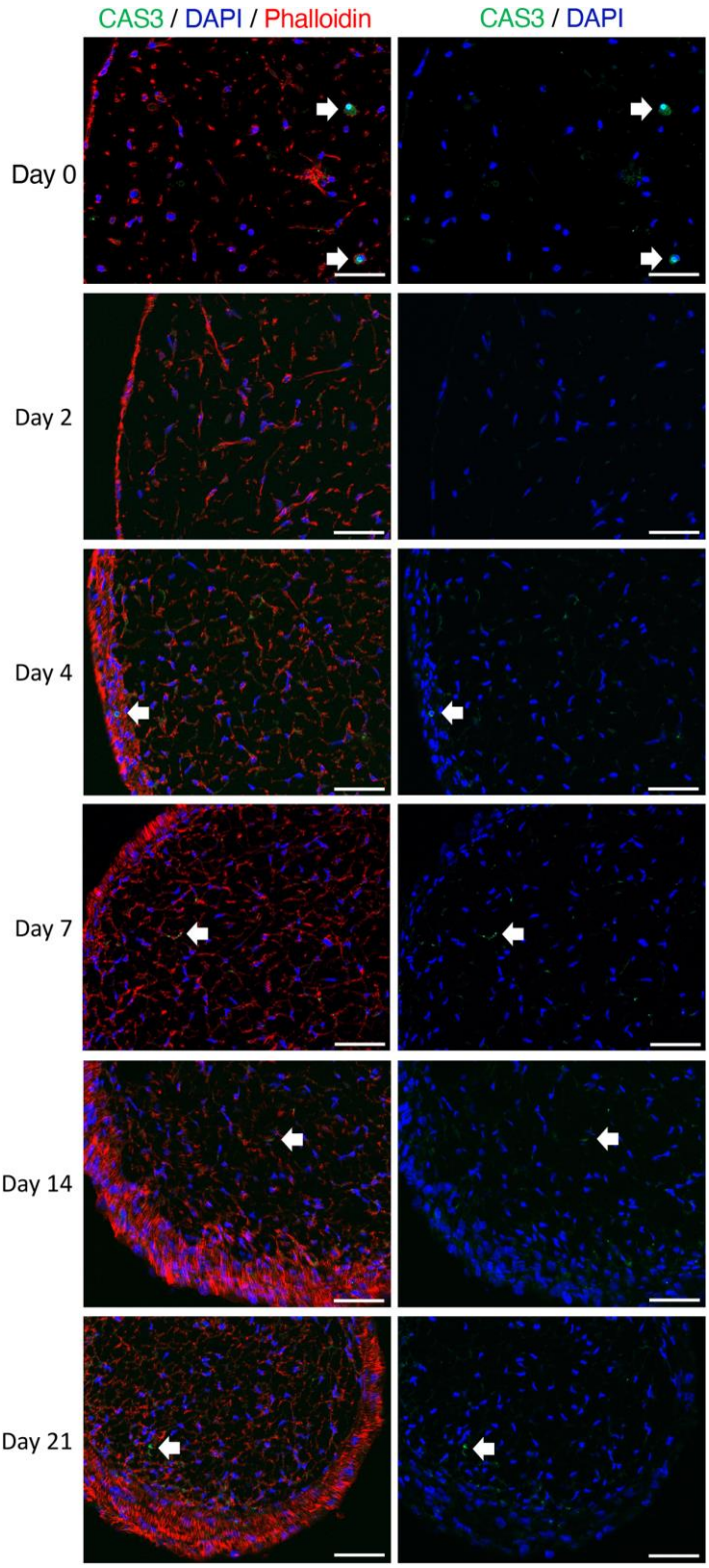

Fig. S2. CASPASE labelling in 3D-hASC constructs over time. A) 12µm transverse sections of 3D-hASC constructs on Day 0 and Day 21 of culture were stained with CAS3 antibody to detect apoptotic cells and DAPI/Phalloidin to visualise cell nuclei and cytoskeletal organisation. Scale bars 50 µm.

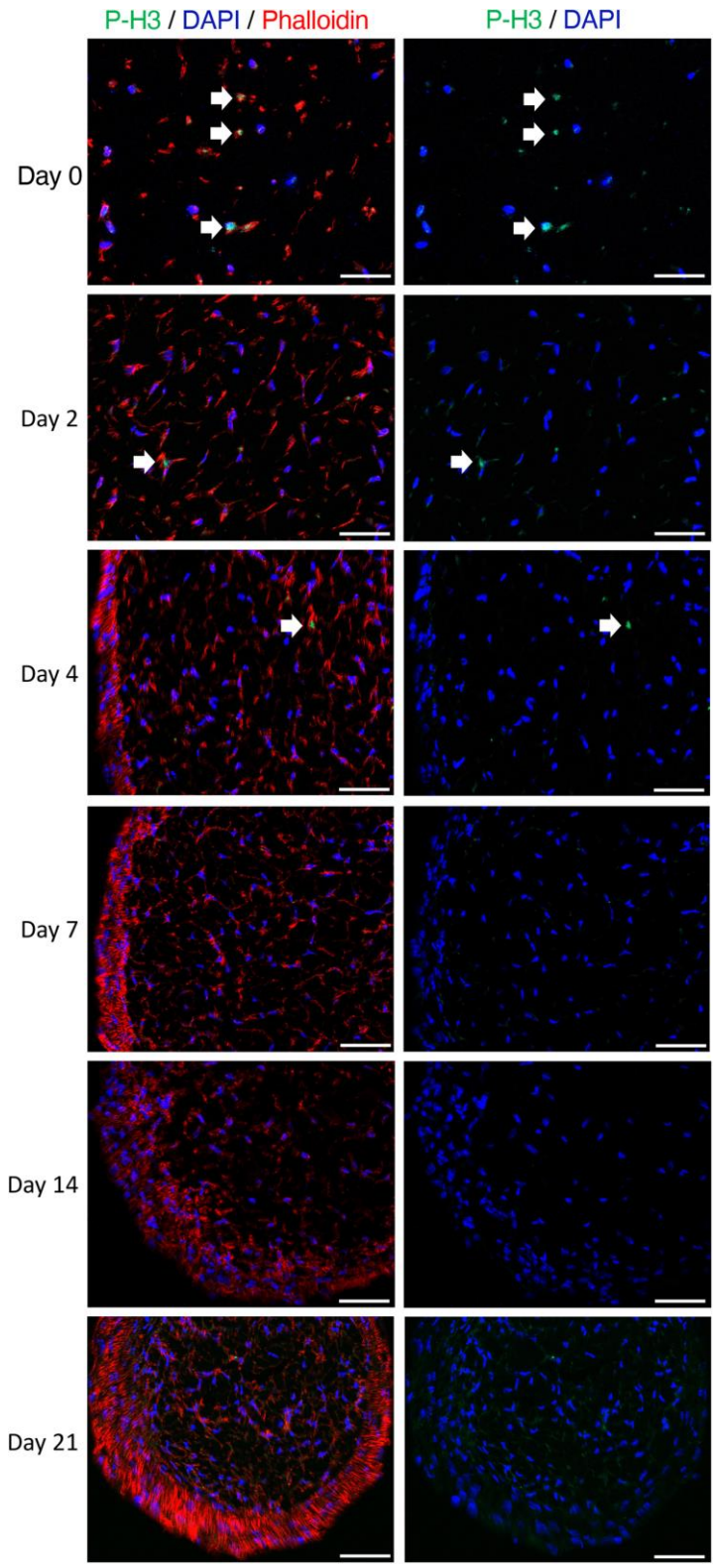

Fig. S3. Phospho-Histone H3 labelling in 3D-hASC constructs over time. A) 12µm transverse sections of 3D-hASC constructs on Day 0 and Day 21 of culture were stained with Phospho-Histone H3 antibody to detect proliferative cells and DAPI/ Phalloidin to visualise cell nuclei and cytoskeletal organisation. Scale bars 50 µm.

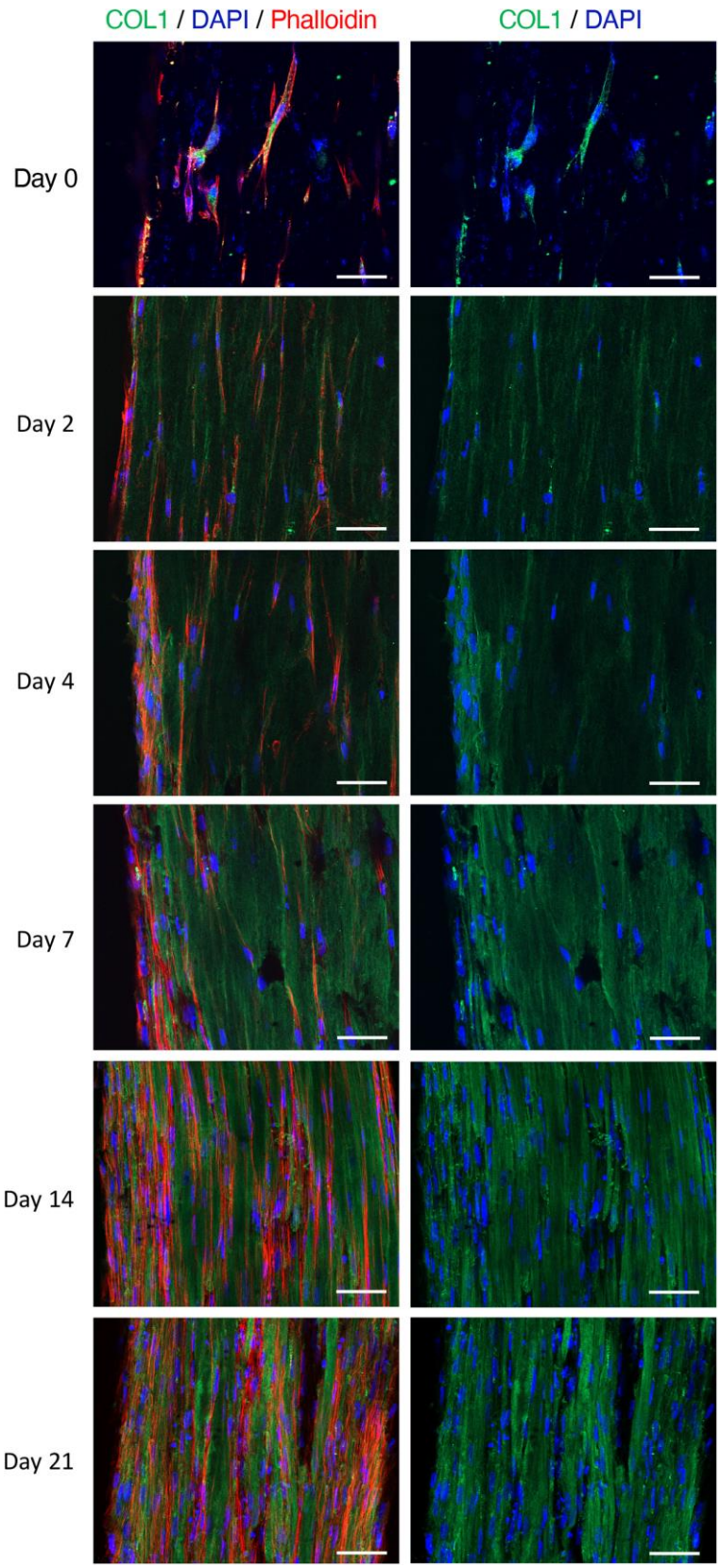

Fig. S4. COL1A1 organisation in 3D-hASCs. 12µm longitudinal sections of tendon constructs were performed on Day 0 and Day 2 and stained with DAPI/Phalloidin to visualise cell shape and COL1A1 antibody.

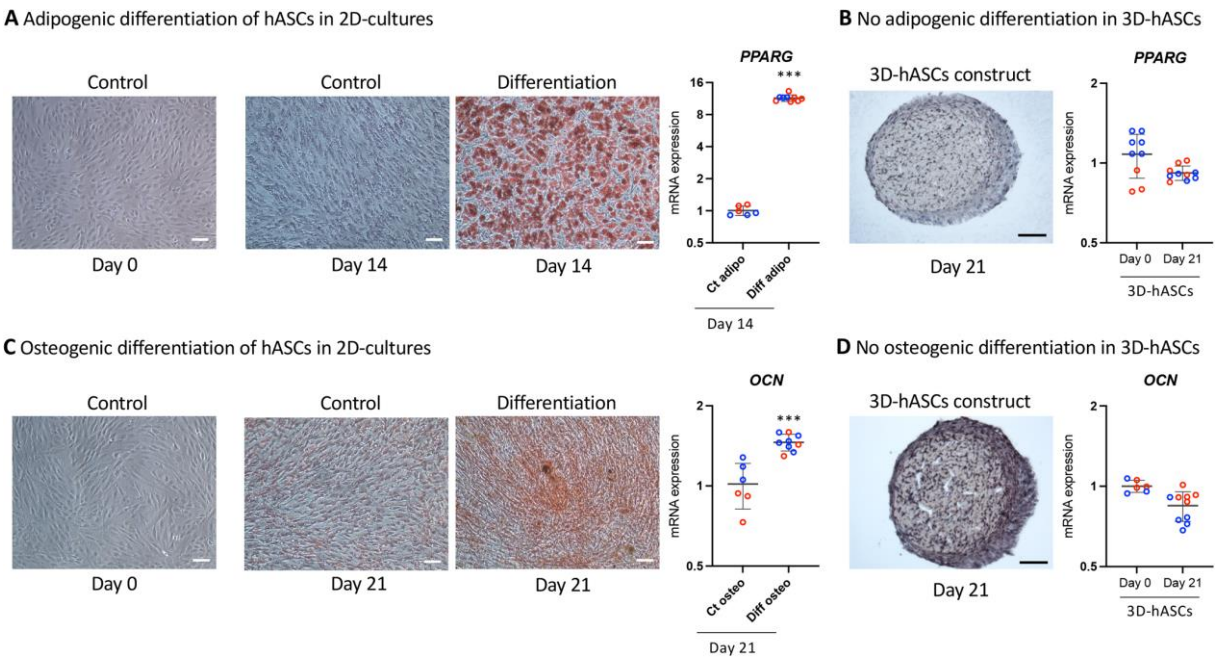

Fig. S5. Adipogenic and Osteogenic differentiation of hASCs. hASCs were subjected to A) Adipogenic differentiation for 13 Days. Control (cultured in basal medium) and differentiated hASCs were stained with Oil Red O and *PPARG* mRNA expression level was analysed by RT-qPCR. Scale bars 100µm. B) 21-Day 3D-hASC constructs were stained with Oil Red O and *PPARG* mRNA expression level was analysed by RT-qPCR. Scale bars 20µm. hASCs were subjected to C) Osteogenic differentiation for 14 Days. Control (cultured in basal medium) and differentiated hASCs were stained with Alizarin Red S and *OCN* mRNA expression level was analysed by RT-qPCR. Scale bars 100µm D) 21-Day 3D-hASC constructs were stained with Alizarin Red S and *OCN* mRNA expression level was analysed by RT-qPCR. Each differentiation experiment was performed 2 independent times with $n \geq 3$ biological replicates. Scale bars 20µm.

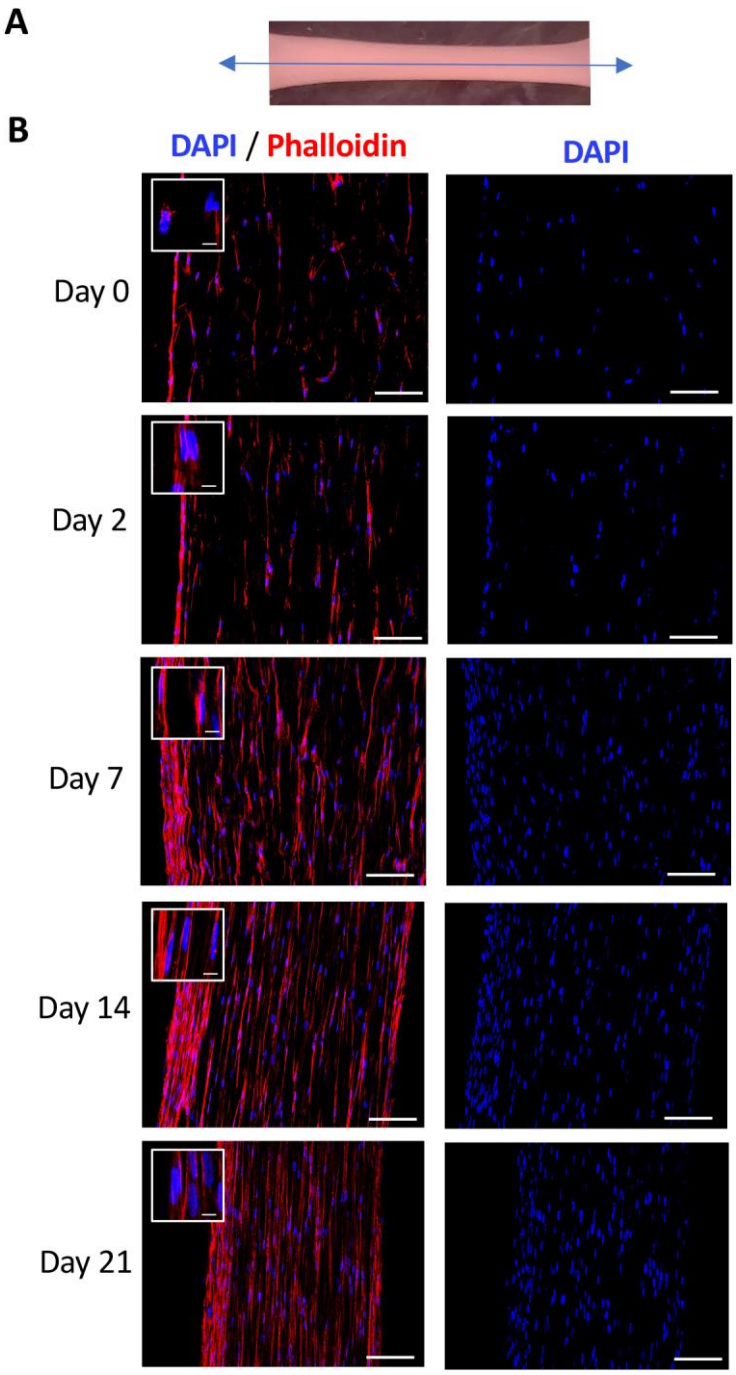

Fig. S6. *COL3A1* mRNA expression in 3D-hASC constructs. A) 3D-hASC constructs at Day 7 were transversally cryo-sectioned. 12 µm sections were hybridised with the DIG-labelled antisense probes for *COL3A1*. Scale bar: 500µm. B) 3D-hASC constructs at Day 0, Day 7, Day 14 and Day 21 were transversally cryo-sectioned. 12 µm sections were hybridised with the DIG-labelled antisense probes for *COL3A1*. Scale bars: 50µm.

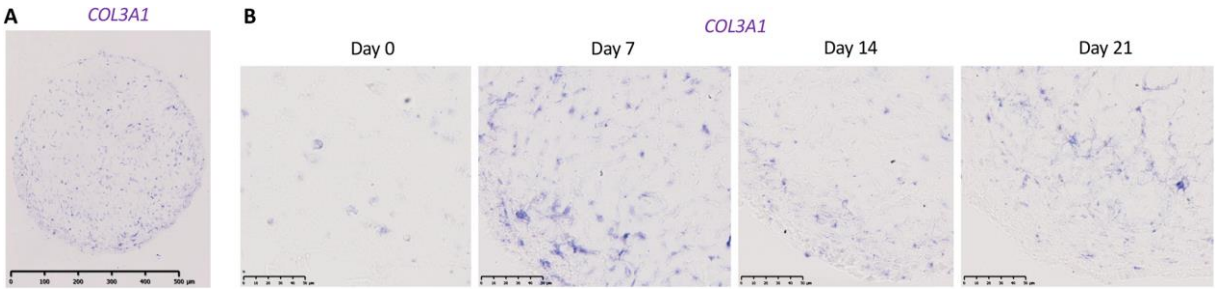

Fig. S7. Cellular and nuclear organisation in 3D engineered 3D-hASCs. A) 3D-hASCs longitudinal section representation. B) Longitudinal sections of 3D-hASC constructs were performed on Day 0, Day 2, Day 4, Day 7, Day 14 and Day 21 3D-constructs and stained with DAPI/Phalloidin to visualise cell shape. Scale bars: 200 µm. White squares represent higher magnification. Scale bars: 100µm.

Table S1.

	RT-qPCR Forward primer	RT-qPCR Reverse primer	Reference
hANXA1	AGTTCTTTGCAAGAAGGTAGAGA	CTGATCCGGGACCACCTTTG	Designed in the lab
hCOL1A1	GATGGCTGCACGAGTCACAC	GTATTCAATCACTGTCTTGCCCC	Designed in the lab
hCOL2A1	TGGCTGACCTGACCTGATGTCC	TGCAGTCTGCCCAGTTCAGGTC	Designed in the lab
h*COL6A3*	AACATCGGCACTTGCCCTTA	ATATCAGCAGCCGCACCATT	Designed in the lab
hCOL14A1	ACTCCGAGGGAAGAGAGCAA	TACATGGGGTGTAGCAGCCA	Designed in the lab
hDPT	TGTCGCTACAGCAAGAGGTG	GTGGTTGTTGCTCCTCGGAT	Designed in the lab
hHIC1	TCCCCAGTCCCAGAAACAGA	CTTTCTGTCCCGTTTGCAGC	Designed in the lab
hMKX	CATCGTCATCAGAACTGAAGGCA	TCTGTTAGCTGCGCTTTCACCC	(Bayer *et al.*, 2014)
hOCN	GGCGCTACCTGTATCAATGG	TCAGCCAACTCGTCACAGTC	Designed in the lab
hPDGFRA	TGTGGGACATTCATTGCGGA	AAGCTGGCAGAGGATTAGGC	Designed in the lab
hPOSTN	GAGGAAGTTGCAAGCCAACA	CACTGAGAACGACCTTCCCT	Designed in the lab
hPPARG	AAGCCCTTCACTACTGTTGACT	CAGGCTCCACTTTGATTG	(Waldner *et al.*, 2018)
hPRRX1	GACCATGACCTCCAGCTACG	TAGCAGGTGACTGACGGAGA	Designed in the lab
hS100A10	GGCTACTTAACAAAGGAGGACC	GAGGCCCGCAATTAGGGAAA	Głowacka et al., 2021
hSCX	CCCAAACAGATCTGCACCTT	CGGTCCTTGCTCAACTTTCT	Designed in the lab
hTHBS2	GACACGCTGGATCTCACCTAC	GAAGCTGTCTATGAGGTCGCA	(Xu *et al.*, 2020)
hTM4SF1	CAGCCCTTGGCTTAGCAGAA	ACTCGGACCATGTGGAGGTA	Designed in the lab
hTNMD	AGCACTTCTGGCCGGAGG	AAGTGTGCTCCATGTCATAGGCT	Designed in the lab
hYWHAZ	CCGCTGGTGATGACAAGAAAGGGAT	AGGGCCAGACCCAGTCTGATAGGA	(Ragni *et al.*, 2013)

Gene name	In situ hybridisation probe Forward primer	In situ hybridisation probe T7-Reverse primer	Reference
hCOL1A1	CTCCCCAGCTGTCTTATGGC	TAATACGACTCACTATAGGGCGC ACCATCATTTCCACGAGC	Designed in the lab
hCOL3A1	CCTACTCGCCCTCCTAATGG	TAATACGACTCACTATAGGGCTC GAAGCCTCTGTGTCCTTT	Designed in the lab
hSCX	GGTCGCTACCTGTACCCTGA	TAATACGACTCACTATAGGGCCT GAGGCAGAAGGTGCAGAT	Designed in the lab
hTHBS2	ACCAGGACAAAGACACGACC	TAATACGACTCACTATAGGGCCC ACGTACATCCGGCTCTTT	Designed in the lab
hTNMD	TGGAAATGGCACTGATGAAA	TAATACGACTCACTATAGGGCCA GCATTGGGTCAAATTCAA	Designed in the lab