







Α	HD		LD	
TGF-β1	+	-	+	-
αSMA		-	-	
GAPDH		-	-	-

Fig. S1. High cell density promoted myogenic differentiation of bone marrow mesenchymal stem cells. BM-MSCs were seeded at low density (LD: $3x10^3$ cells/cm²) or high density (HD: $30x10^3$ cells/cm²) and 5 days later they were evaluated. (A) Immunostaining for α SMA or CNN1 (green); The nuclei were stained with DAPI (blue); Bar=200µm. (B) WB for myogenic proteins α SMA, CNN1 and MYH11. (*) denotes p<0.05 between HD and LD cells.

Fig. S2. High cell density does not induce differentiation into osteogenic or chondrogenic lineages. RT-PCR for chondrogenic (ACAN, SOX9) or osteogenic (RUNX2, SPARC) genes in low densities or high densities cultures in growth medium. RPL32 served as housekeeping gene. HF-MSCs that were coaxed to differentiate into the chondrogenic (Chondro) or osteogenic (Osteo) lineage using well-established differentiation factors served as positive control.

Fig. S3. High cell density induced α SMA expression. HF-MSCs were seeded at low density (LD: $3x10^3$ cells/cm²) or high density (HD: $30x10^3$ cells/cm²) in presence or absence of TGF- β 1 (10ng/ml) and 5 days later the level of α SMA was measured using western blotting; GAPDH served as loading control.

	Accession number	Gene	Sense sequence (5'-3')
control		Scramble	CAACAAGATGAAGAGCACCAA
siTGFβRII	NM_033664 NM_004257	TGFβRII	GGGATTTATACAGATGTCTGTGTCAGAA
siROCK	NM_004850 NM_005406	ROCK isoforms (ROCK1 and ROCK2)	CAACGGCAAAGAATCTGTTATTACT
siCDH2	NM_001792	Cadherin-2	CAGCCTCCAACTGGTATCTTCATTA

Table S1. Cloning of different siRNAs

Table S2. Primers for (qRT)-PCR

Accession number	Gene	Sense sequence (5'-3')
NM_001792	Cadherin-2	CTGACACTGTGGAGCCTGAT
		GTGGAGCCACTGCCTTCATA
NM_033664	Cadherin-11	CTGGCTCAACATCACTGTCT
		CGGTTGTCTCTGACTGTGAA
NM_001141945	αSMA	GACAGCTACGTGGGTGACGAA
		GATGCCATGTTCTATCGGGTACT
NM_033138.3	CALD1	AGATTGAAAGGCGAAGAGCA
		TTCAAGCCAGCAGTTTCCTT
1	1	

NM 001001522.1	SM22	ATGGCCAACAAGGGTCC
		CTTCAAAGAGGTCAACAG
NM 011577	TGF-β1	CAACACATCAGAGCTCCGAGAA
		AAGGCGAAAGCCCTCAATTT
NM_000994	Ribosomal protein	AGCGTAACTGGCGGAAAC
	L32 (RPL32)	CGTTGTGGACCAGGAACTTC
		TGTGGAGTTTGGTGTCTACTAGTGTGT
NM_001015051	RUNX2	
		GAAATCTGCCATGTGACTGCC
NM 199173	SPARC	ATCHTCCTGTACACIGGCAGIIC
1001_199175	STARC	CTCGGTGTGGGGGGGGGGGGGGC
		GTCTCACTGCCCAACTAC
NM_001135	ACAN	
		GGAACACGATGCCTTTCAC
NM 000346	SOVO	GAGCAGACGCACATCTC
11111_000340	3073	CCTCCCATTCCCCCCA
		CETUUUATTUUUUUA
1	1	