Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary data

Supplementary materials and methods

Protein	Type	MW(kDa)	Receptor	Supplier
Wnt-3a	human	37.4	Frizzled, LRP5/6	R&D
Wnt-5a	mouse	38.0	Frizzled, Ror2	R&D
Dkk-1	human	25.8	LRP5/6	R&D
GDF-8	mouse	24.8 (homodimer)	ActRIIB	R&D
BMP-2	human	26.0 (homodimer)	BMPR-1A	Dr. M. Ehrbar (UZH)
Dll4	human	55.6	Notch	R&D
Jagged1	human	137	Notch	R&D
Dlk1 (Pref-1)	human	32.0	Notch	Enzo Life Sciences
N-Cadherin	human	89.2 (homodimer)		R&D
Laminin 1	mouse	900		BD Biosciences
CCL2	human	8.7	CCR2	R&D

Table S 1: List of screened proteins

Wnt reporter activity

Thin layers of hydrogel (5% (w/v) with 10% thiol excess) were formed as described. A volume of 50µl of a prepared hydrogel mix was transferred to each well of a 12-well plate (Falcon). A hydrophobic PDMS stamp with spacers was used to produce homogeneous and thin gel layers. The partially cross-linked hydrogel substrate was subsequently functionalized with the respective protein (Wnt3a-VS and Dkk-1-VS) at 50ug/mL in a volume of 5uL under pressure for 90min. The functionalized hydrogel layers were then washed with PBS and UV-sterilized. Subsequently reporter HEK293T reporter cells stably expressing TCF-luciferase were seeded at a density of 150'000 cells per well. As controls TCF-luciferase reporter cells were seeded on hydrogels functionalized with only FNIII(9-10) to promote cell adhesion and incubated with non-functionalized wild type Wnt3a and Dkk-1 (both at 250ng per well corresponding to the relative concentration spotted for immobilization). After a 24-hour incubation, luciferase activity in cell extracts was measured using Luciferase Assay System (Promega).

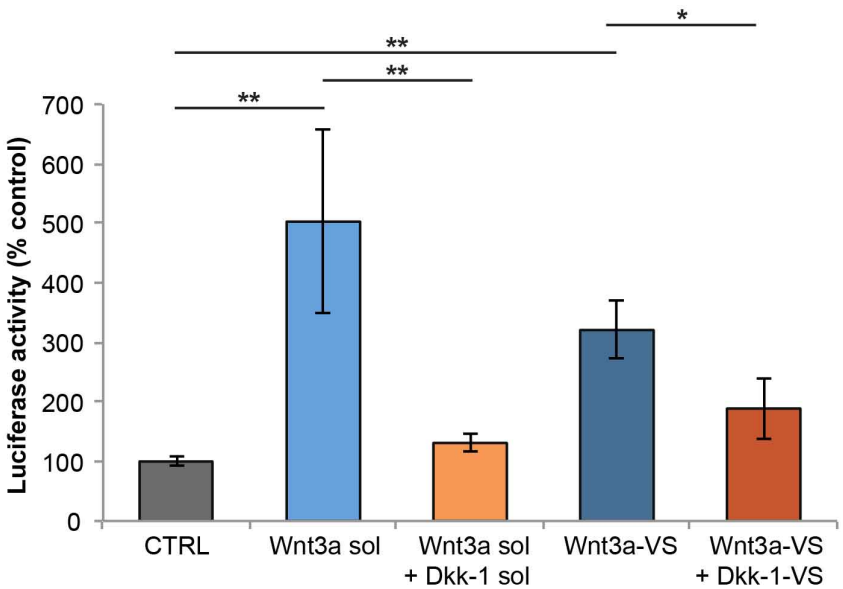

Fig. S 2 Wnt activity assay. Wnt activity of wild type Wnt3a soluble (sol), wild type Dkk-1 soluble (sol) and PEG-linker functionalized Wnt3a (Wnt3a-VS) and Dkk-1 (Dkk-1-VS) was measured with a TCF-luciferase HEK293T reporter cell line.

Real-time quantitative RT-PCR primers

GAPDH	Forward 5'-3'	GGAGCCAAAAGGGTCATCATCT
	Reverse 5'-3'	GCTAAGCAGTTGGTGGTGCAG
PPARγ	Forward 5'-3'	CCTGCATCTCCACCTTATTATTCT
	Reverse 5'-3'	AAACCCTTGCATCCTTCACA
AdipoQ	Forward 5'-3'	AGCCTCTTCAAGAAGGACAAGG
	Reverse 5'-3'	TACACCTGGAGCCAGACTTGG
LPL	Forward 5'-3'	GGCCGCCCTGTACAAGAGA
	Reverse 5'-3'	AACTCCTCCTCCATCCAGTTGA
C/EBPα	Forward 5'-3'	GGTGGACAAGAACAGCAACGA
	Reverse 5'-3'	GCGGTCATTGTCACTGGTCA

Table S 3 List of primers.

Real-time quantitative RT-PCR statistics

For two-group analysis, an unpaired t-test with Welch's correction was used. For all cases, p-values less than 0.05 were considered statistically significant. GraphPad Prism 6.0 software was used for gene expression statistical evaluations.

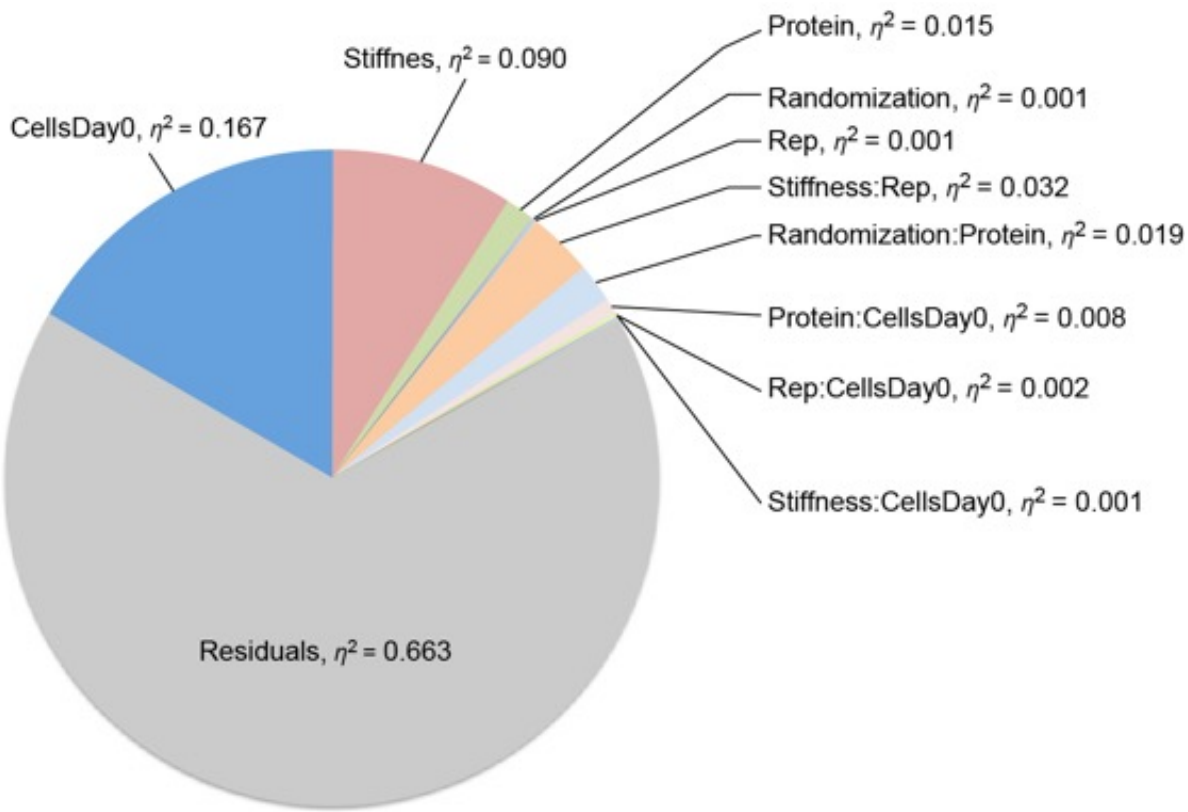

Fig. S 4 Pie chart detailing the effect size of all significant regressors of the multivariate analysis.

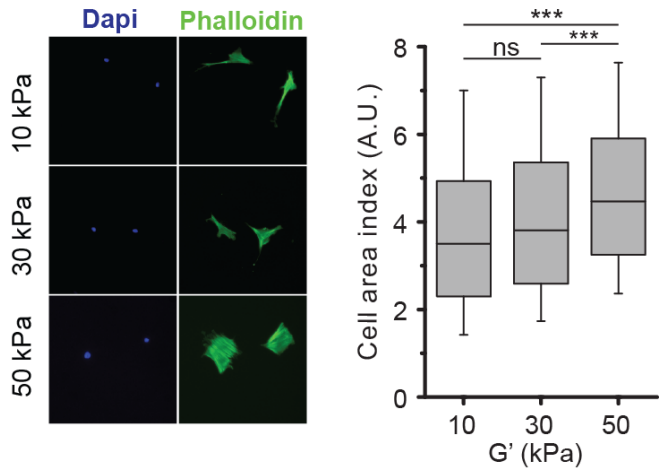

Fig. S 5 Average effect of elastic modulus (G′) on cell surface area within 6h after seeding.

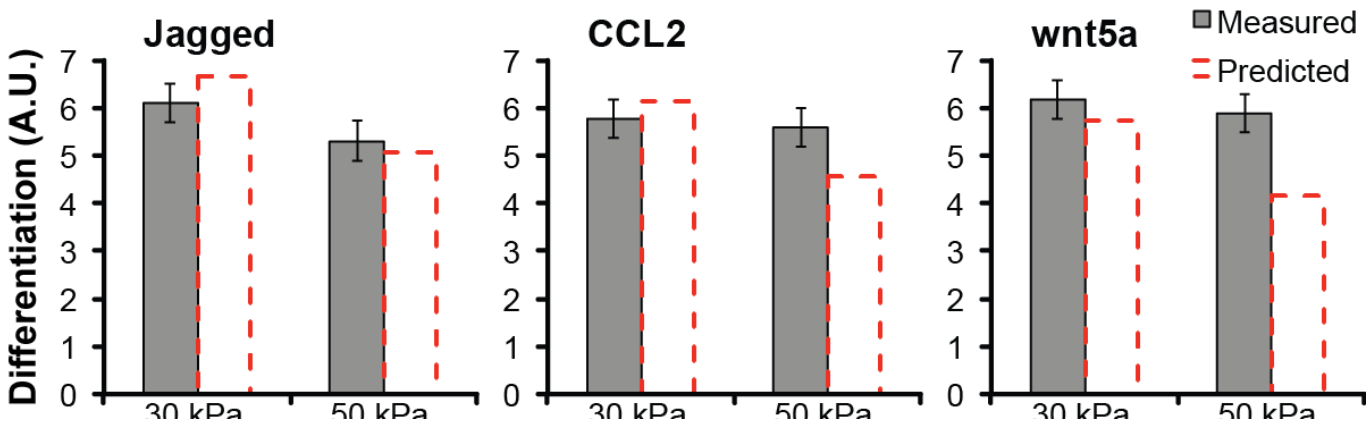

Fig. S 6 Measured versus predicted effects of spotted proteins on hMSc adipogenic differentiation. This illustrate that the

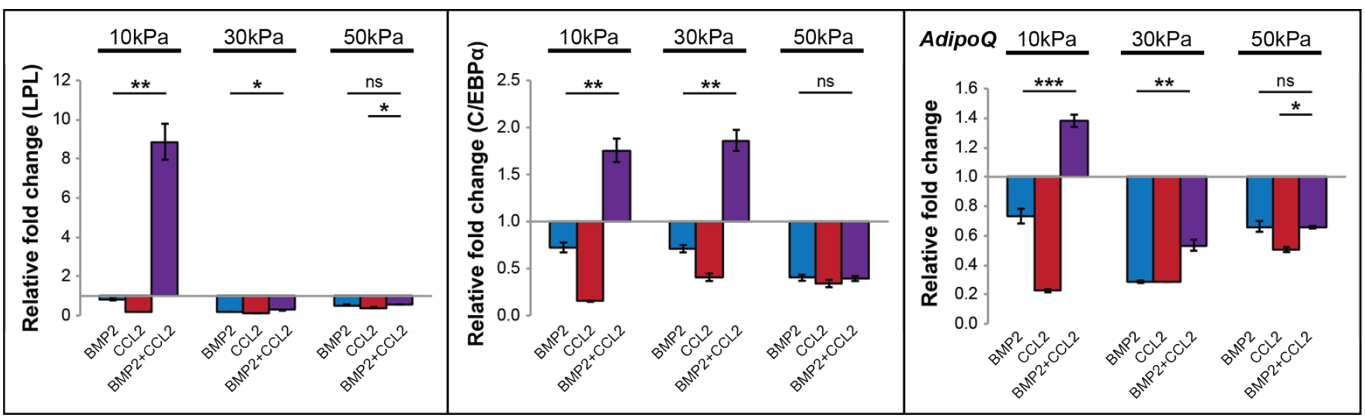

Fig. S 7 Gene expression analysis of four key adipogenesis genes of hMSCs grown under adipogenic differentiation conditions on PEG hydrogels with G′ between 10-50kPa functionaized with BMP2, CCL2 or the combination of BMP2 and CCL2.