Supporting Information

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SI Text

Basic Theory for the Mechanical Tests Associated with the Study. Cells display varying levels of resistance to deformation (elasticity) and flow (viscosity) in response to an applied force. This dual mechanical behavior, known as viscoelasticity, is dependent on the composition and organization of subcellular structures, particularly the cytoskeleton. Assuming a cell behaves as an elastic material, its resistance to deformation is linearly proportional to the applied stress but inversely proportional to the resulting strain. This resistance to deformation is measured experimentally as the elastic modulus (E_{elastic}). Elastic materials with high elastic moduli are considered stiff because an increase in applied stress results in a negligible increase in their strain.

Compliant elastic materials, though, have low elastic moduli because small increases in applied stress result in substantial deformation. Moreover, elastic materials subjected to a constant stress exhibit a constant strain and recover their original shape completely after the stress is removed. However, cells are viscoelastic materials and exhibit both elastic and viscous properties. Specifically, when a viscoelastic material is kept at a constant strain, the applied stress decreases over time, a phenomenon called stress relaxation. In stress relaxation, the viscoelastic properties of a material can be described by its instantaneous and relaxed moduli. The instantaneous modulus (E_0) is the resistance to deformation measured before the relaxation begins, whereas the relaxed modulus (E_R) is the stiffness of the material at complete equilibrium. The material's apparent viscosity (μ) is determined by the resistance to flow upon the application of a stress. These mechanical properties, which can be extracted from experimental data using appropriate mathematical models, have emerged as biomarkers that are useful for discriminating among the elastic and viscoelastic properties of multiple cell types, including MSCs and differentiated cells.

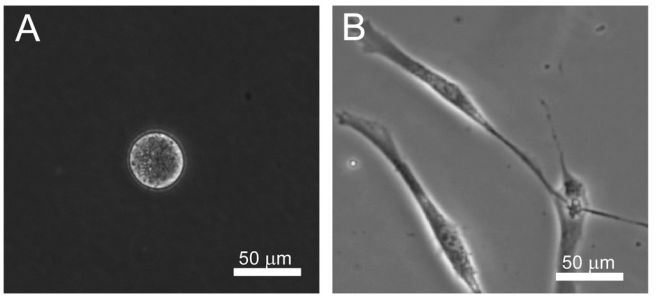

Fig. S1. Morphology of spherical and spread adipose-derived stem cells (ASCs) using phase contrast imaging. P5 ASCs plated for 0.5–1.5 h on a glass substrate exhibit a rounded cell shape (*A*). After 24 h, ASCs spread extensively and exhibited a flattened morphology (*B*). Single indentation and relaxation tests were conducted over the center of the cell or the nucleus, respectively. (Scale bars, 50 μm).

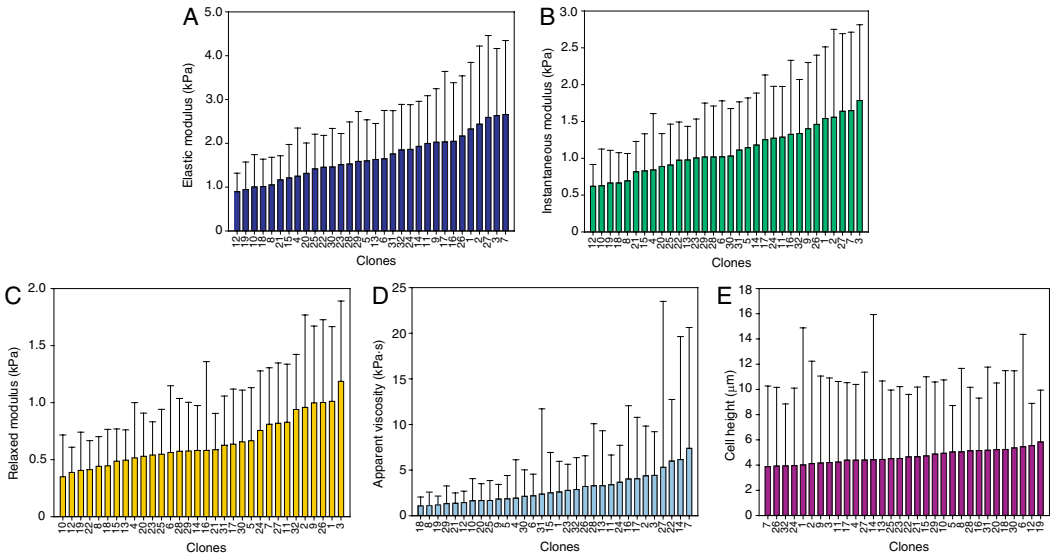

Fig. S2. Mechanical properties of ASC subpopulations for the spread morphology also exhibited heterogeneity. Elastic and viscoelastic properties of 32 ASC clonal populations with spread morphologies were measured by using atomic force microscopy (AFM) indentation and stress relaxation tests, respectively. Within each clonal population, an average of 23 cells was tested via AFM. Measured mechanical properties included elastic modulus (*A*), instantaneous modulus (*B*), relaxed modulus (*C*), apparent viscosity (*D*), and cell height (*E*). As for the spherical morphology, elastic and viscoelastic data were fit well with Hertzian mathematical models ($R^2 = 0.99$ and $R^2 = 0.88$, respectively). Data is presented as geometric mean ± standard deviation.

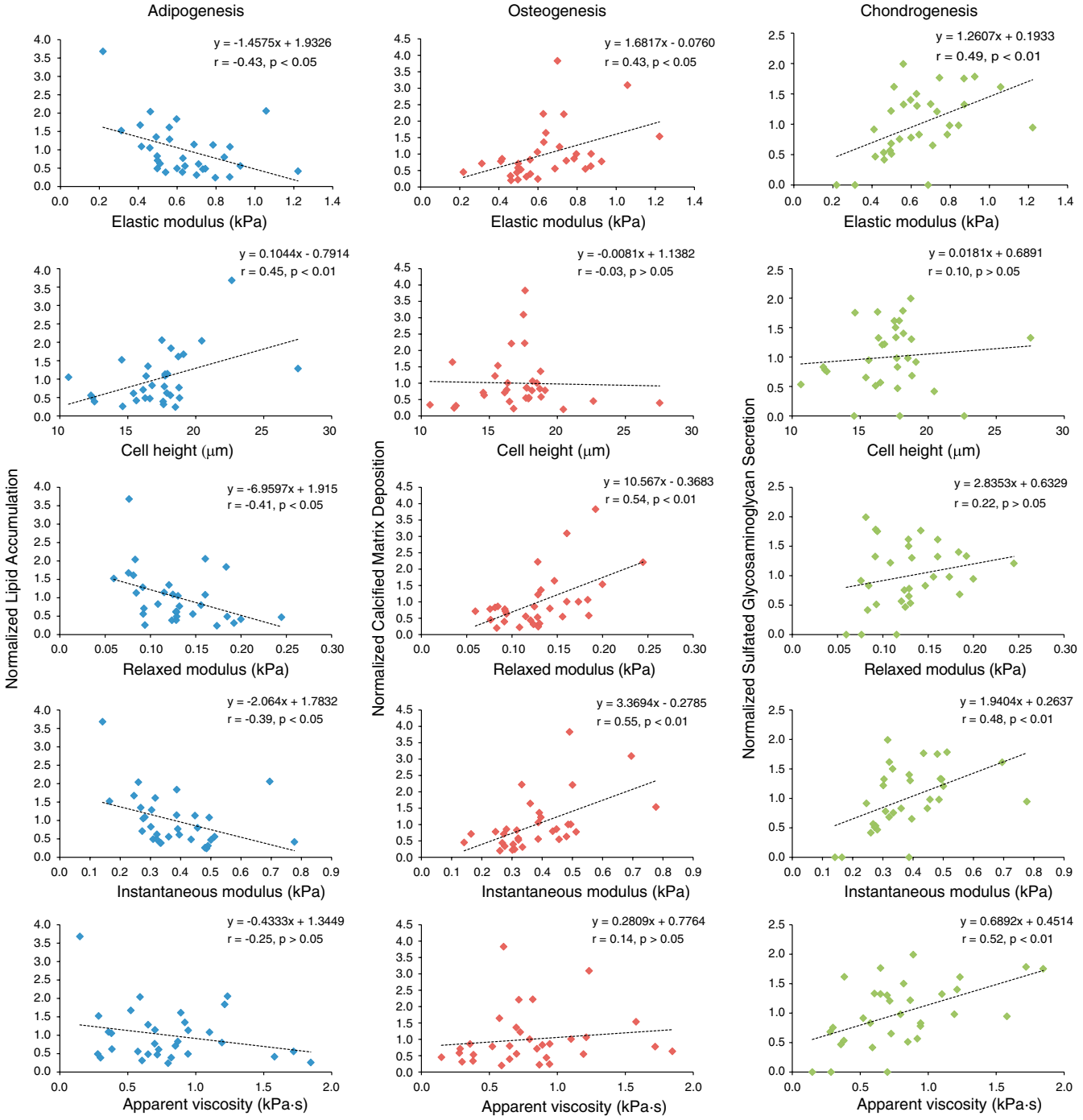

Fig. S3. Cellular mechanical properties correlated with the differentiation potential of ASCs across adipogenic, osteogenic, and chondrogenic lineages. The mechanical properties of 32 ASC clonal populations were characterized via AFM. These data were then correlated with their differentiation potential toward adipogenic (blue dots, left column), osteogenic (red dots, central column), and chondrogenic (green dots, right column) lineages. In all clonal populations, differentiation along the three lineages was assessed via biochemical assays that quantified lipid accumulation, extracellular matrix calcium deposition, and sulfated glycosaminoglycan secretion, respectively. For presentation purposes, biochemical data were normalized to the geometric mean of all clones for each lineage. Pearson's correlation coefficient, *r*, indicated the correlation between each mechanical property and the normalized metabolite production for all clonal populations. Statistical significance was present if $P < 0.05$.

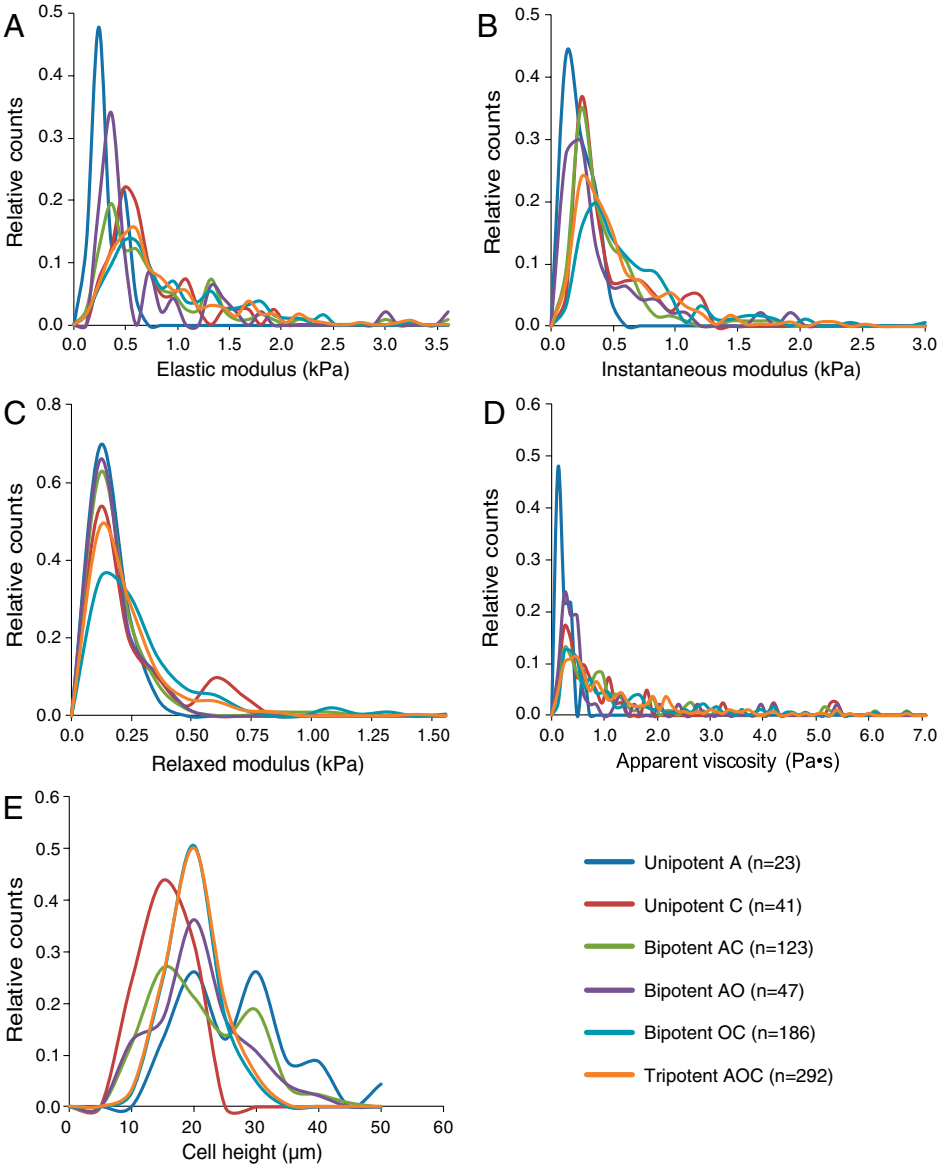

Fig. S4. Mechanical property distributions for ASC clonal populations (spherical morphology) showed that no clear relationship existed with respect to potency. Broadly overlapping distributions were seen for $E_{elastic}$ (*A*), E_0 (*B*), E_R (*C*), μ (*D*), and *Height* (*E*). Distributions were normalized to total cell numbers within each potency group. Note that sample sizes for the different potencies are highly variable, lessening the universal reliability of these distributions. For example, the Unipotent A distribution (dark blue line) includes 23 cells from a single, qualifying clone. The Unipotent C distribution (red line) includes 41 cells from two qualifying clones. Contrast those with the Tripotent AOC distribution (orange line), which includes 292 cells from 14 qualifying clones. See Table S2 for numerical data.

Table S1. Correlations between the mechanical properties of ASCs with spread morphologies and their differentiation potentials

Mechanical property	Lineage	Pearson's r (± 95% CI)	P-value
$E_{elastic}$	Adipogenic	0.17 ± 0.49	0.36
	Osteogenic	−0.13 ± 0.34	0.47
	Chondrogenic	0.01 ± 0.35	0.96
E_0	Adipogenic	0.12 ± 0.34	0.51
	Osteogenic	−0.07 ± 0.34	0.55
	Chondrogenic	0.01 ± 0.35	0.91
E_R	Adipogenic	−0.34 ± 0.31	0.06
	Osteogenic	0.47 ± 0.28	*0.007*
	Chondrogenic	0.05 ± 0.35	0.77
μ	Adipogenic	0.06 ± 0.35	0.73
	Osteogenic	−0.10 ± 0.35	0.60
	Chondrogenic	0.07 ± 0.35	0.72
Height	Adipogenic	−0.07 ± 0.35	0.71
	Osteogenic	−0.11 ± 0.35	0.57
	Chondrogenic	0.07 ± 0.35	0.69

Cellular mechanical properties are indicated by the following abbreviations: $E_{elastic}$ (elastic modulus), E_0 (instantaneous modulus), E_R (relaxed modulus), μ (apparent viscosity), and *Height* (cell height). Error values for Pearson's correlation coefficient *r* represent 95% confidence intervals (95% CI). Correlations were calculated using log-transformed geometric means.

Table S2. Mean and ranges of mechanical properties for spherical clones based on their differentiation potential

			$E_{elastic}$ (kPa)		E_0 (kPa)		E_R (kPa)	
Clone potency	Clones	Cells	Mean	Range	Mean	Range	Mean	Range
Unipotent (A)	1	23	0.2 ± 0.1	0.1–0.6	0.2 ± 0.1	0.1–0.4	0.1 ± 0.1	0.0–0.2
Unipotent (C)	2	41	0.6 ± 0.4	0.2–1.9	0.4 ± 0.3	0.1–1.2	0.2 ± 0.2	0.0–0.7
Bipotent (AC)	5	123	0.6 ± 0.4	0.1–2.7	0.4 ± 0.3	0.1–1.7	0.1 ± 0.1	0.0–1.0
Bipotent (AO)	2	47	0.6 ± 0.7	0.1–3.6	0.3 ± 0.4	0.1–1.8	0.1 ± 0.1	0.0–0.4
Bipotent (OC)	8	186	0.9 ± 0.6	0.1–4.1	0.6 ± 0.5	0.1–3.2	0.2 ± 0.2	0.0–1.4
Tripotent (AOC)	14	292	0.8 ± 0.8	0.1–8.1	0.5 ± 0.5	0.1–3.7	0.2 ± 0.2	0.0–1.3

			μ (kPa·s)		*Cell height* (μm)	
Clone potency	Clones	Cells	Mean	Range	Mean	Range
Unipotent (A)	1	23	0.2 ± 0.1	0.1–0.6	24.4 ± 9.6	10.2–49.2
Unipotent (C)	2	41	1.1 ± 1.6	0.1–7.6	12.9 ± 3.3	7.3–19.7
Bipotent (AC)	5	123	1.1 ± 1.3	0.0–6.7	18.8 ± 8.0	5.9–40.6
Bipotent (AO)	2	47	0.8 ± 1.1	0.1–5.3	18.3 ± 6.8	5.4–37.6
Bipotent (OC)	8	186	1.3 ± 1.5	0.1–9.7	17.2 ± 3.9	8.4–28.4
Tripotent (AOC)	14	292	1.3 ± 1.6	0.0–12.0	17.5 ± 4.4	6.7–32.0

A: Adipogenic, O: Osteogenic, C: Chondrogenic