Electronic Supplemental Material for

Influence of inherent mechanophenotype on competitive cellular adherence

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Supplementary Text

ECM effects on cells from alternative lineages

Materials and Methods

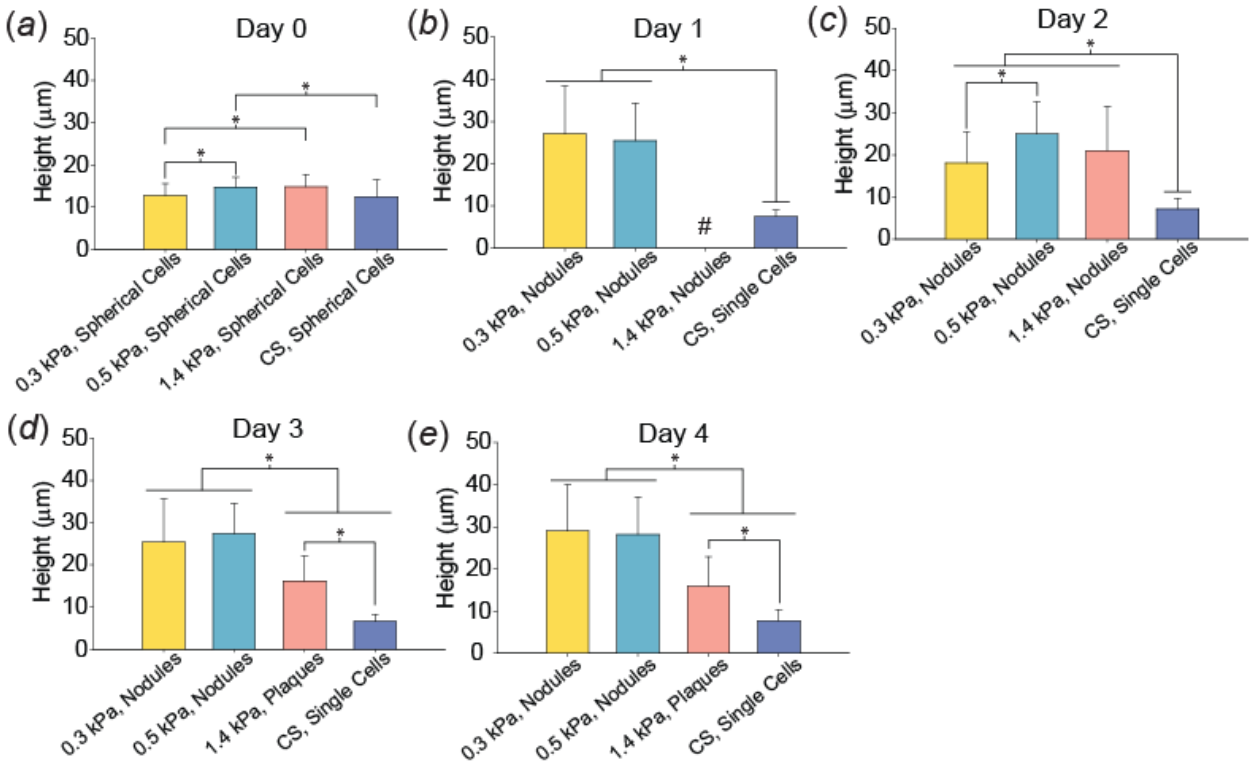
Cell Culture: MG-63 (osteosarcoma, #CRL-1427, ATCC) cells were expanded and maintained in phenol red-free MEM supplemented with 10% FBS, 1% penicillin/streptomycin, 2 mM Glutamax, and 1 mM sodium pyruvate. SH-SY5Y (neuroblastoma, #CRL-2266, ATCC) cells were expanded and maintained in high-glucose DMEM (Hyclone, GE-Healthcare), 10% FBS, 1% penicillin/streptomycin, and 1% Glutamax. Cells were maintained in humidified incubators at 37ºC, 5% CO_2 and passaged at 60-80% confluence using 0.25% trypsin-EDTA (Hyclone, GE Healthcare).

PAAm Gel Fabrication: PAAm gels of three stiffnesses were fabricated using different ratios of acrylamide:bis-acrylamide: 3%:0.06%, 7%:0.03%, and 10%:0.1%. Gels were made to be lower than, equal to, or greater than the stiffness of MG-63 and SH-SY5Y cells (0.3, 2, and 12 kPa). The protocol for gel fabrication is as described in *Methods: Gel Fabrication and Functionalization*. Gels were coated overnight with 20 µg/ml laminin (LN, #354239, BD Biosciences), 100 µg/ml collagen-1 (COL-1, #08-115, Millipore), or 10 µg/ml fibronectin (FN, #33016015, ThermoFisher).

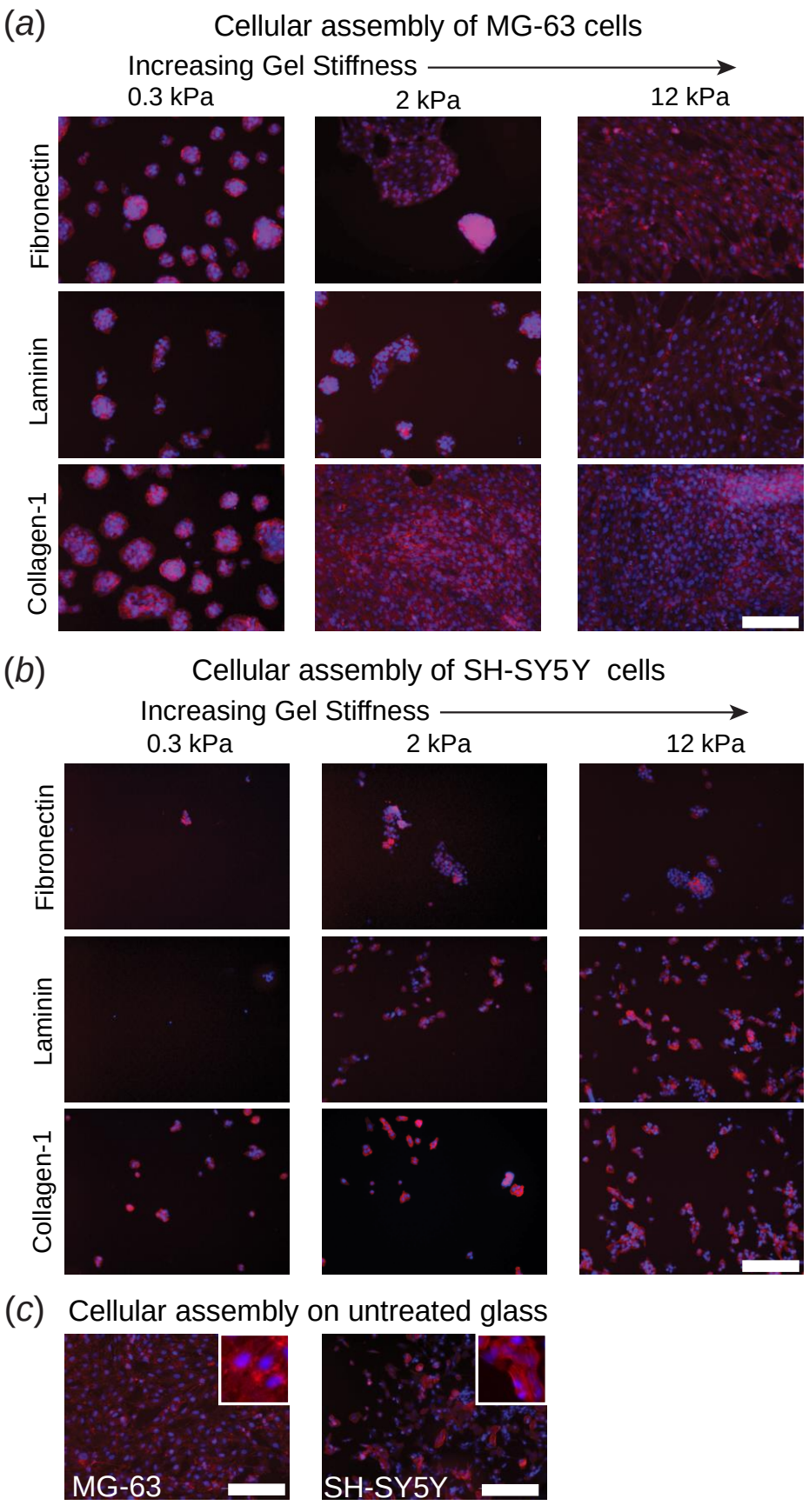
Assessment of Actin Organization and Cellular Assembly: MG-63 and SH-SY5Y cells were seeded and assessed for cellular assemblies as described in *Methods: Assessment of Actin Organization and Cellular Assembly*.

Atomic Force Microscopy: Both MG-63 and SH-SY5Y cells were mechanically characterised as described in *Methods: Atomic Force Microscopy* and *Mechanical Characterization of Cells*. Individual cells were tested on gels and glass CS two days after seeding. Gels were also characterised as described in *Methods: Atomic Force Microscopy* and *Mechanical Characterization of PAAm Gels*.

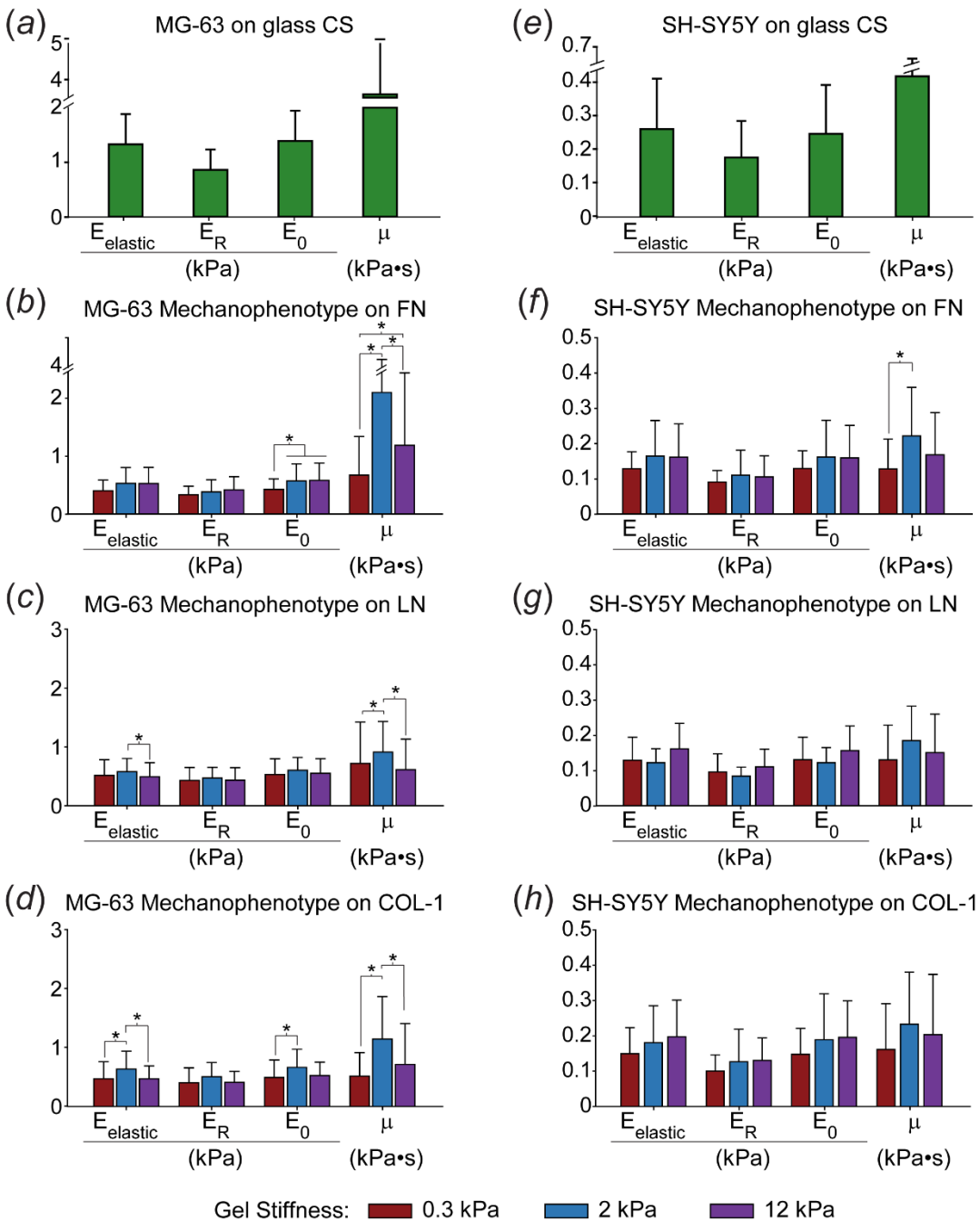
Supplemental Figures



Supplementary Figure 1. Height changes correspond with cellular assembly of non-transfected WI-38 fibroblasts into nodules and plaques. (*a*) Minimal differences in cell heights existed across spherical cells on gels and glass CS on Day 0. In general, the nodules that existed on (*b*) Day 1, (*c*) Day 2, (*d*) Day 3, and (*e*) Day 4 on 0.3 kPa and 0.5 kPa gels were significantly taller than the plaques that existed on 1.4 kPa gels and single cells on glass CS. Heights shown as mean ± s.d., with statistical significance determined using Kruskal-Wallis ANOVA on ranks within each cell line, followed by a Dunn's post-hoc analysis (* $p < 0.05$). #, nodules/plaques did not exist for this condition, only single cells.

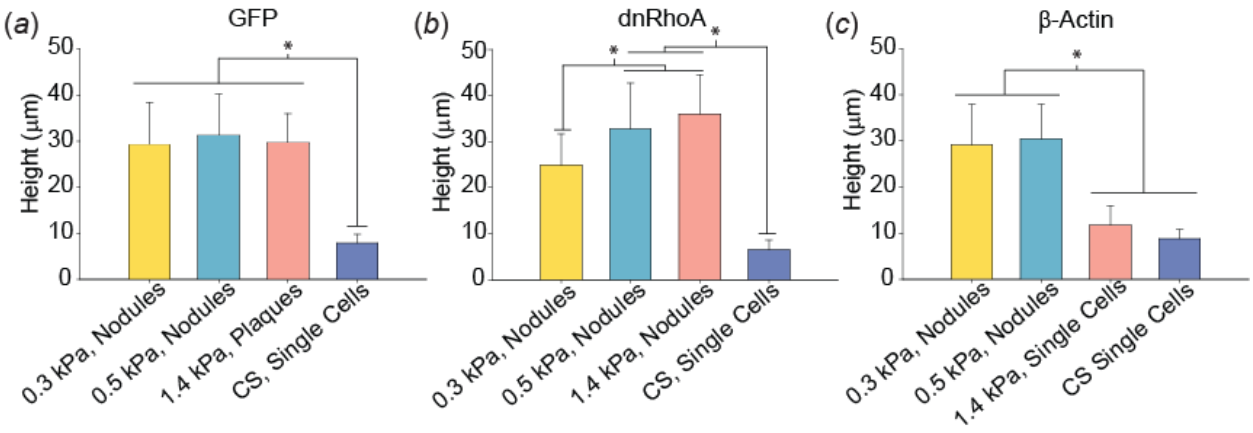

(a)
Cellular assembly of MG-63 cells
Increasing Gel Stiffness
0.3 kPa
2 kPa
12 kPa
Fibronectin
Laminin
Collagen-1
(b)
Cellular assembly of SH-SY5Y cells
Increasing Gel Stiffness
0.3 kPa
2 kPa
12 kPa
Fibronectin
Laminin
Collagen-1
(c)
Cellular assembly on untreated glass
MG-63
SH-SY5Y

Supplementary Figure 2. Effect of substrate stiffness for (*a*) MG-63 cells and (*b*) SH-SY5Y cells on fibronectin, laminin, and collagen-1 functionalized PAAm gels, as shown with DAPI (nuclei: blue) and phalloidin (actin: red) staining. Control cultures were plated on (*c*) untreated, glass coverslips. MG-63 cells, which displayed a mechanophenotype equivalent to the 2 kPa gel, formed more nodules on gels with compliance less than the elastic modulus of the cell. SH-SY5Y cells, with a mechanophenotype equivalent to the softest gels, did not display any organizational transition on the different substrate compliances, possibly because all PA gels were stiffer than the cells. Increased actin organization was visible in spread MG-63 cells compared to cells in nodules. Scale bar: 200 µm. Inset scale: 80 µm x 80 µm.



Supplementary Figure 3. Average mechanical properties of (*a-d*) MG-63 and (*d-f*) SH-SY5Y cells after two days on glass CS (MG-63: n = 20; SH-Y5Y: n = 15) or PAAm gels (MG-63: n = 44-79; SH-Y5Y: n = 22-28) coated with FN, LN, or COL-1. In general, individual cells displayed similar mechanical properties regardless of gel stiffness. Both

MG-63 and SH-SY5Y cells cultured on gels exhibited a more compliant mechanophenotype than cells cultured on much stiffer, untreated glass coverslips. Data shown as mean ± s.d., with statistical significance determined using Kruskal-Wallis ANOVA on ranks for each mechanical parameter within each cell line and ligand coating, followed by a Dunn's post-hoc analysis (* $p < 0.05$). COL-1: collagen-1, LN: laminin, FN: fibronectin.



Supplementary Figure 4. Height changes correspond with cellular assembly of transfected cell types into nodules and plaques for (*a*) GFP, (*b*) dnRhoA, and (*c*) β-Actin cells. Data shown as mean ± s.d., with statistical significance determined using Kruskal-Wallis ANOVA on ranks within each cell line, followed by a Dunn's post-hoc analysis (* $p < 0.05$).

Supplementary Table 1. Number of samples tested corresponding to (a) WI-38 cell mechanics over four days on glass, (b) GFP-, dnRhoA-, and β-Actin-transfected cell mechanics at two days on glass coverslips, and (c) transfected cell mechanics on PAAm gels and glass coverslips (CS) at four days.

a) Sample sizes for WI-38 cells/nodules/plaques tested over four days for each substrate to assess elastic and viscoelastic properties (#, no nodules/plaques present).

Gel Stiffness (kPa)	Day 0	Day 1	Day 2	Day 3	Day 4
0.3	48	15	45	32	53
0.5	56	20	39	56	49
1.4	53	#	40	40	55
CS	57	25	30	43	40

b) Sample sizes for transfected WI-38 cells tested to assess average elastic moduli.

GFP	dnRhoA	β-Actin
61	58	57

c) Sample sizes for GFP-, dnRhoA-, and β-Actin-transfected cells/nodules/plaques tested for each substrate to assess elastic and viscoelastic properties.

Gel Stiffness (kPa)	GFP	dnRhoA	β-Actin
0.3	40	35	10
0.5	41	39	11
1.4	38	39	50
CS	20	7	18