Electronic Supplementary Material (ESI) for Biomaterials Science.

Hydrogel mechanics regulate fibroblast DNA methylation and chromatin condensation

Jenna L. Sumey[1], Peyton C. Johnston[2], Abigail M. Harrell[3], Steven R. Caliari[1,2]

[1]Department of Chemical Engineering, [2]Department of Biomedical Engineering, [3]Department of Chemistry, University of Virginia

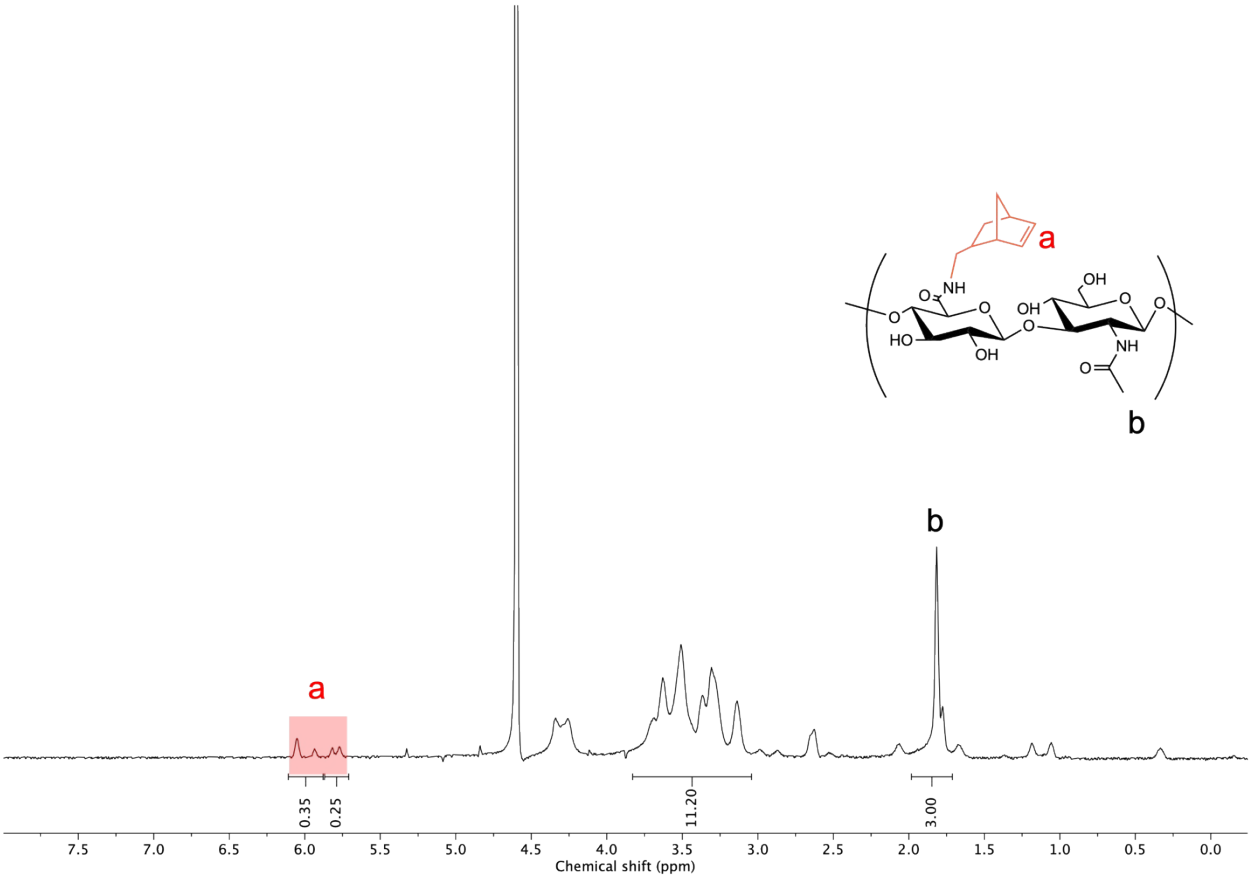

Figure S1. ^{1}H NMR spectrum of norbornene-modified hyaluronic acid (NorHA). The degree of modification was determined to be 30% as indicated by integration of the peaks at δ = 5.75, 6.05, and 6.17 (2H, shaded red) relative to the N-acetyl on HA (3H).

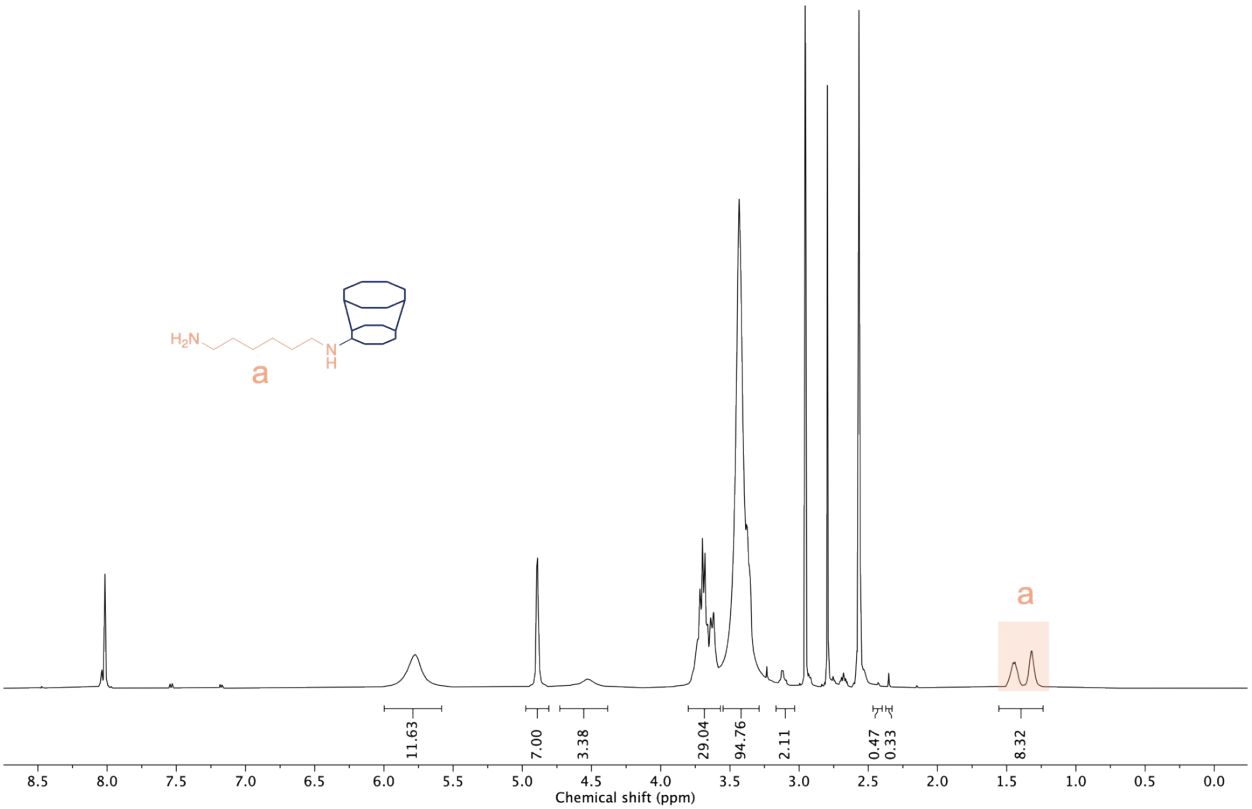

Figure S2. ^{1}H NMR spectrum of β-cyclodextrin hexamethylene diamine (β-CD-HDA). Modification of β-CD with HDA was determined to be 69% as indicated by integration of the peaks at δ = 1.14-1.6 ppm (12H).

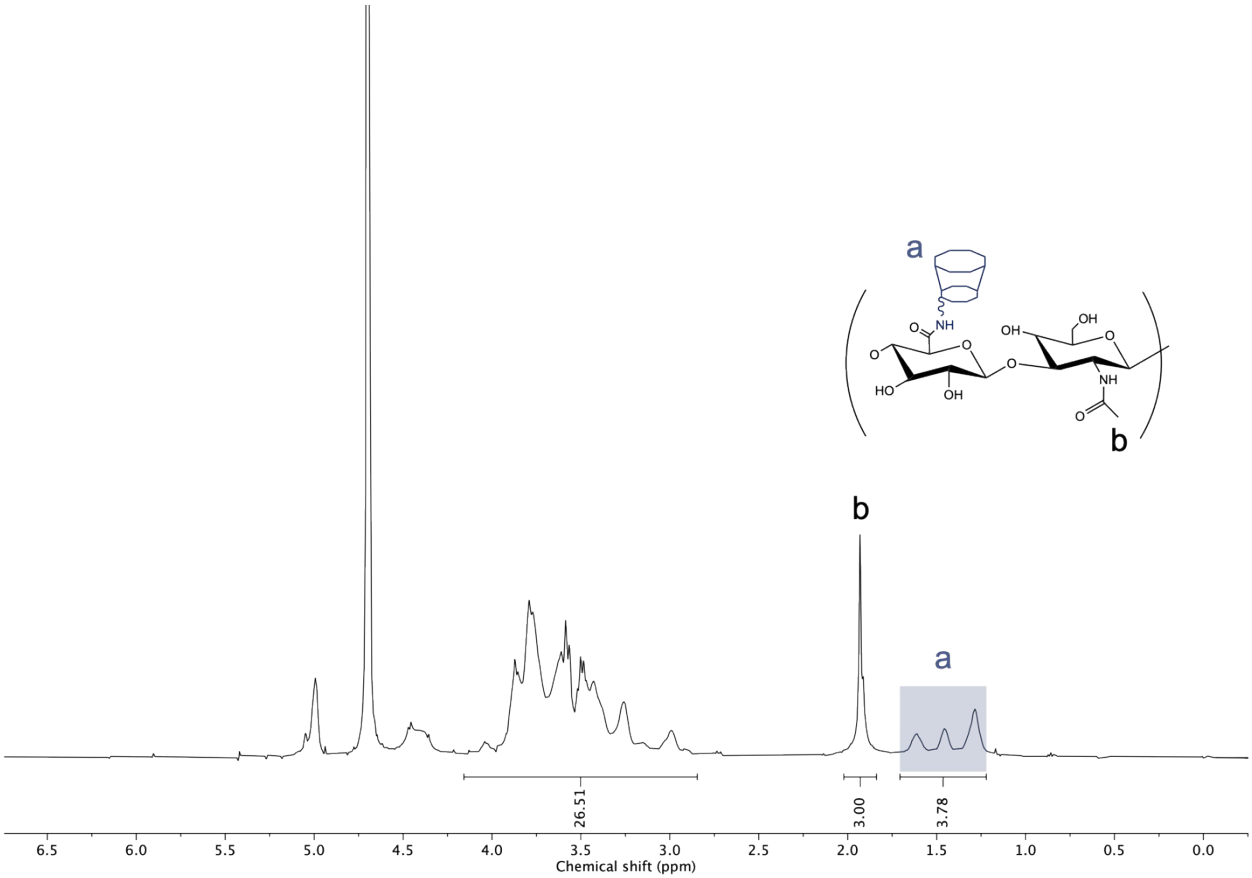

Figure S3. ^{1}H NMR spectrum of β-cyclodextrin-modified hyaluronic acid (CD-HA). The degree of modification was found to be 31% as determined by integration of the peaks at δ = 1.23-1.68 ppm (12H) relative to the N-acetyl on HA (3H).

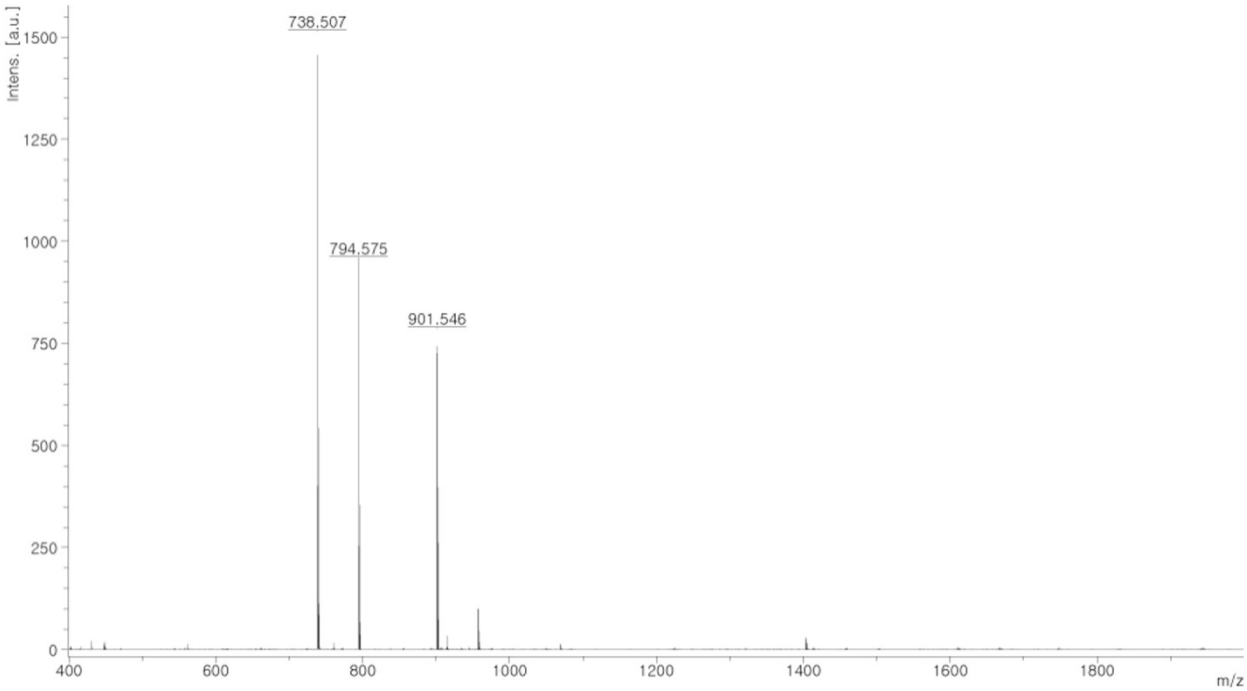

Figure S4. MALDI spectrum of 1-adamantaneacetic acid-KKKCG (adamantane peptide). Expected mass: 738.6 g/mol. Actual mass: 738.5 g/mol.

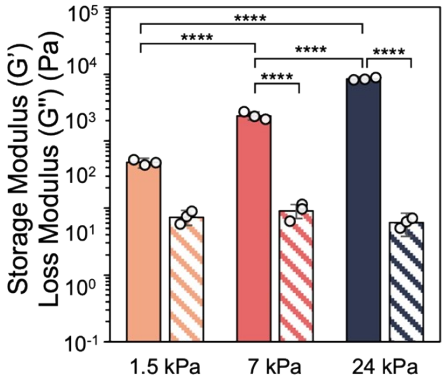

Figure S5. Quantification of the storage (solid bars) and loss (patterned bars) modulus for each hydrogel stiffness. There were no statistically significant differences in loss modulus between the three experimental groups. Data reported as mean ± s.d. $N = 3$ hydrogels per group. **** $P < 0.0001$.

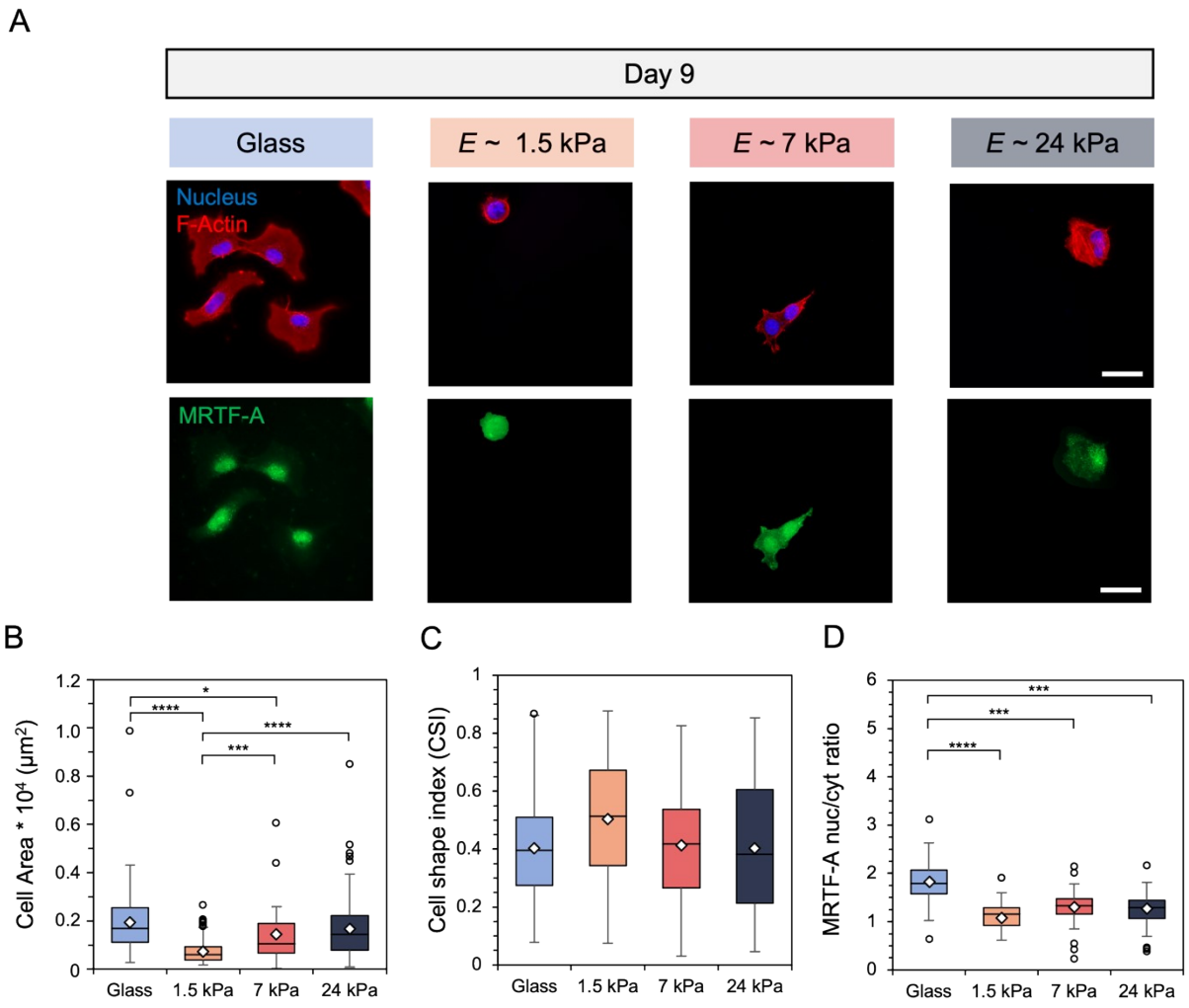

Figure S6. **A**) Representative images of fibroblasts cultured on glass and hydrogels of $E \sim 1.5$, 7, and 24 kPa for 9 days. Scale bars: 50 µm. Fibroblast **B**) spread area (µm^2), **C**) cell shape index, which measures cell circularity, and **D**) nuclear localization of myocardin-related transcription factor-A (MRTF-A) were quantified. $N = 3$ hydrogels per group. ****: $P < 0.0001$, *** $P < 0.0005$, ** $P < 0.01$, * $P < 0.05$.

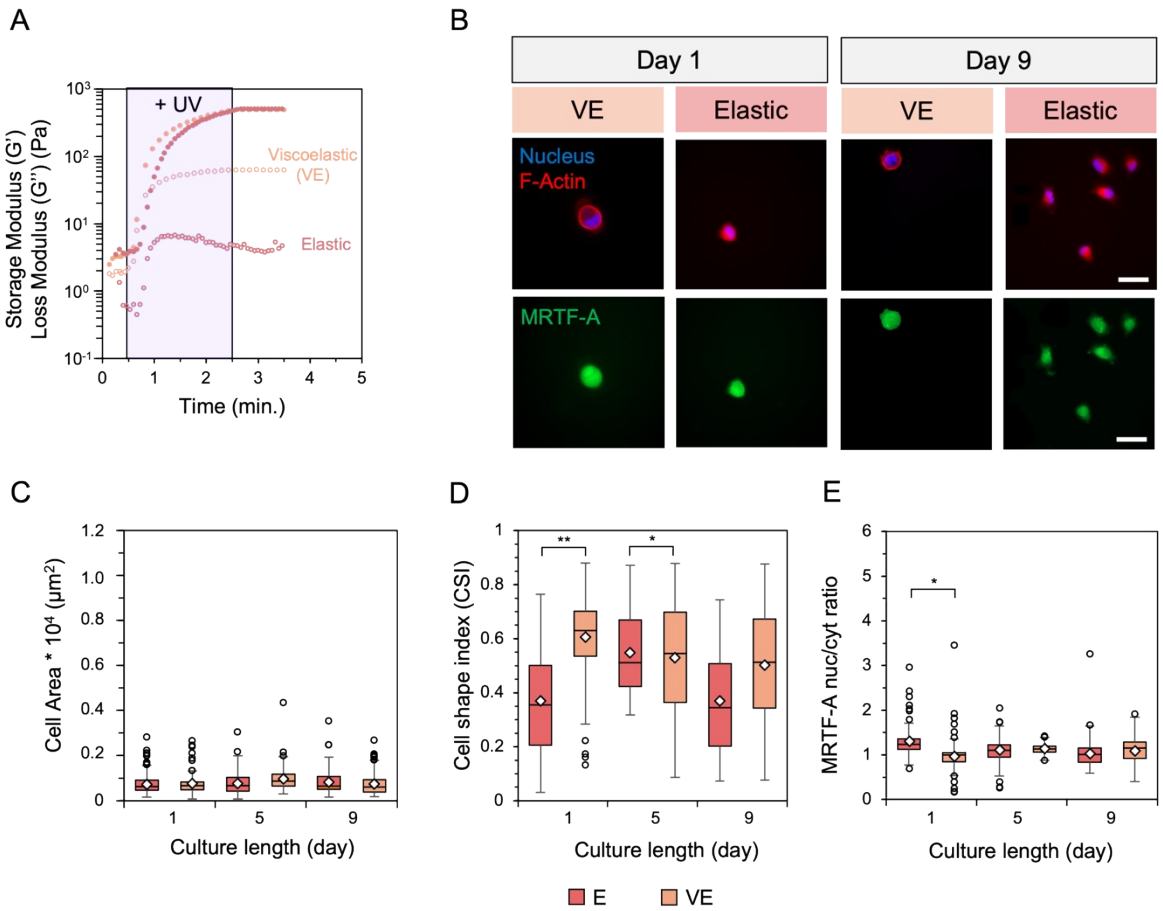

Figure S7. **A**) *In situ* rheology of soft viscoelastic (VE) and soft elastic (E) hydrogels of the same storage modulus (G' ~ 0.5 kPa, closed circles) but different loss moduli (open circles). **B**) Representative images of fibroblasts cultured on hydrogels following 1 (left) and 9 (right) days of culture. Scale bars: 50 μm. Fibroblast **C**) spread area (μm^2), **D**) cell shape index, and **E**) MRTF-A nuclear localization were quantified. $N = 3$ hydrogels per group. ** $P < 0.01$, * $P < 0.05$.

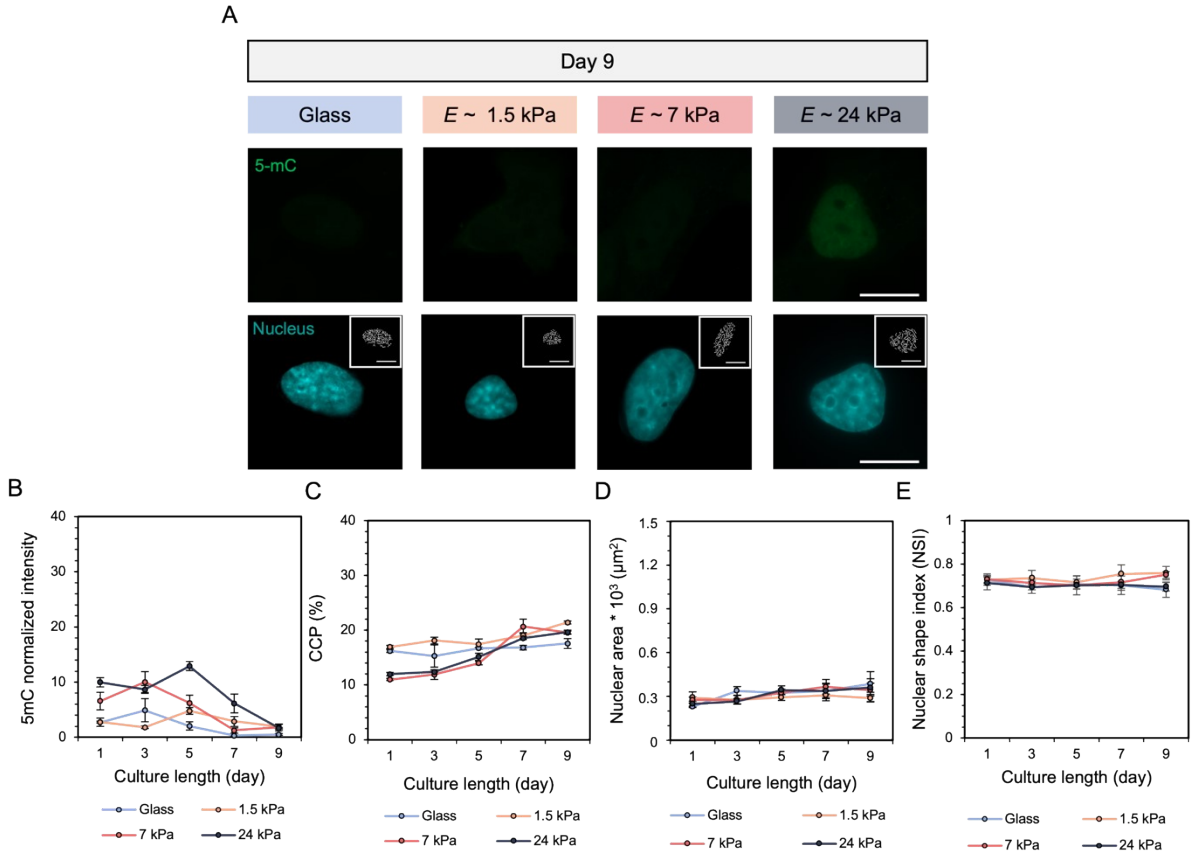

Figure S8. **A**) Representative images of fibroblast global DNA methylation as indicated by 5-mC (green) staining and nuclei (DAPI) on glass and hydrogels following culture for 9 days. Inset images represent pixelated edges within the nuclei, used to quantify chromatin condensation percentage (CCP). Scale bars: 10 µm. Nuclear metrics of **B**) normalized global DNA methylation intensity, **C**) CCP, **D)** nuclear spread area, and **E)** nuclear shape index were quantified as a function of culture length for a total of 9 days. $N = 3$ hydrogels per group. Data reported as total mean ± s.e.m.

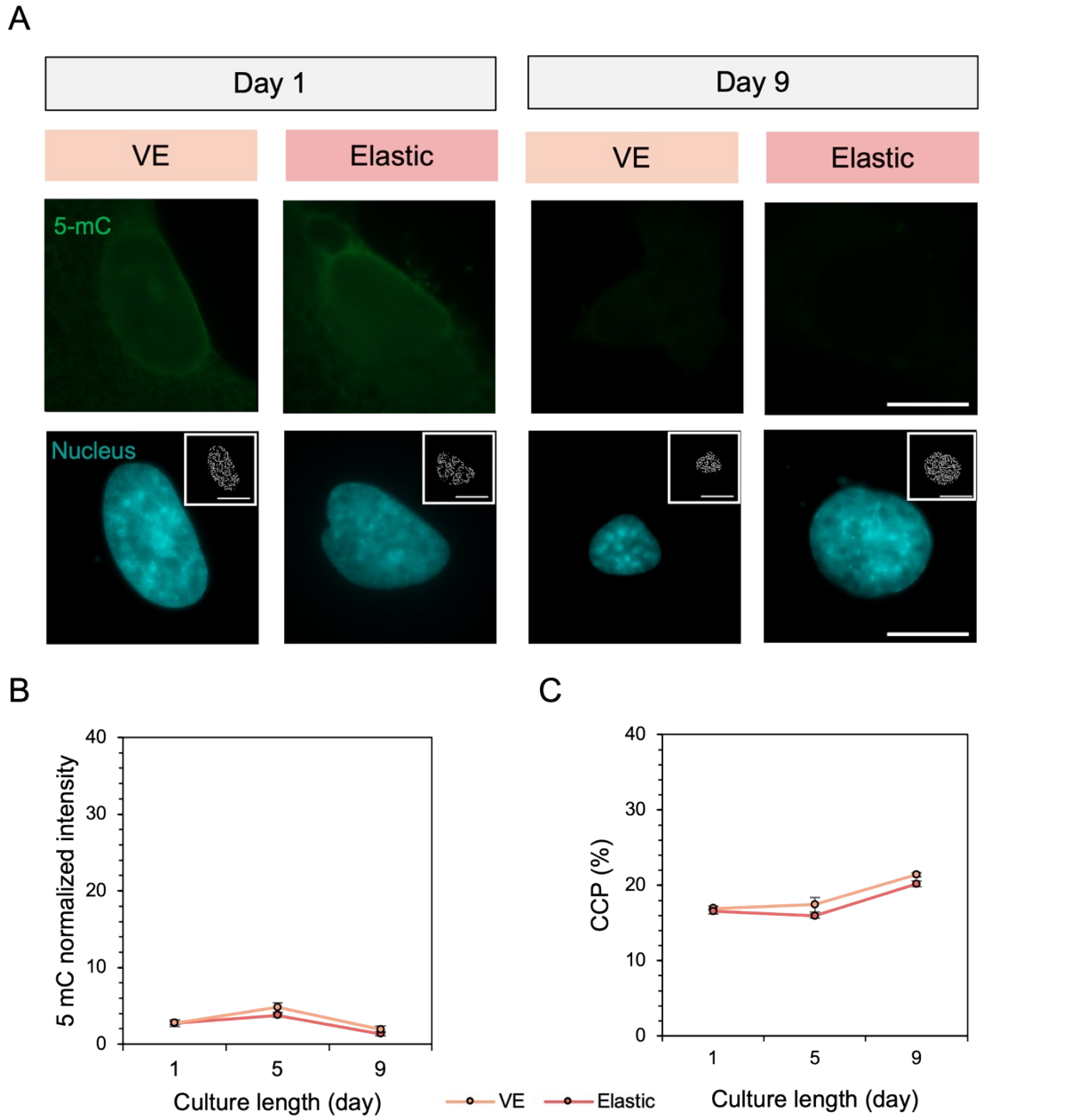

Figure S9. **A**) Representative images of fibroblast global DNA methylation as indicated by 5-mC (green) staining and nuclei (DAPI) on viscoelastic and elastic hydrogels with equivalent $E \sim 1.5$ kPa following 1 (left) and 9 (right) days of culture. Inset images represent pixelated edges within the nuclei, used to quantify chromatin condensation percentage (CCP). Scale bars: 10 μm. Nuclear metrics of **B**) normalized global DNA methylation intensity and **C**) CCP were quantified as a function of culture length for a total of 9 days. $N = 3$ hydrogels per group. Data reported as mean ± s.e.m.

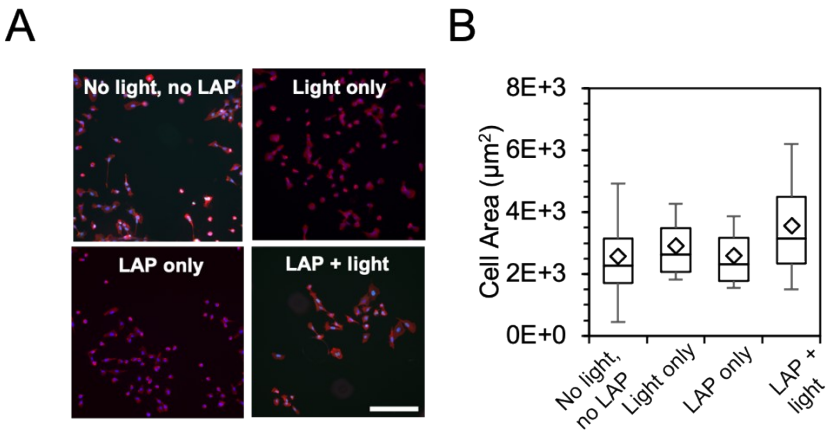

Figure S10. **A**) Fibroblast response to UV (365 nm) light, 1 mM LAP, and LAP + UV light, as well as a no light, no LAP control. Scale bar: 200 μm. **B**) Cell spread area quantification 24 h after light and/or LAP exposure showed no statistically significant differences between experimental groups.

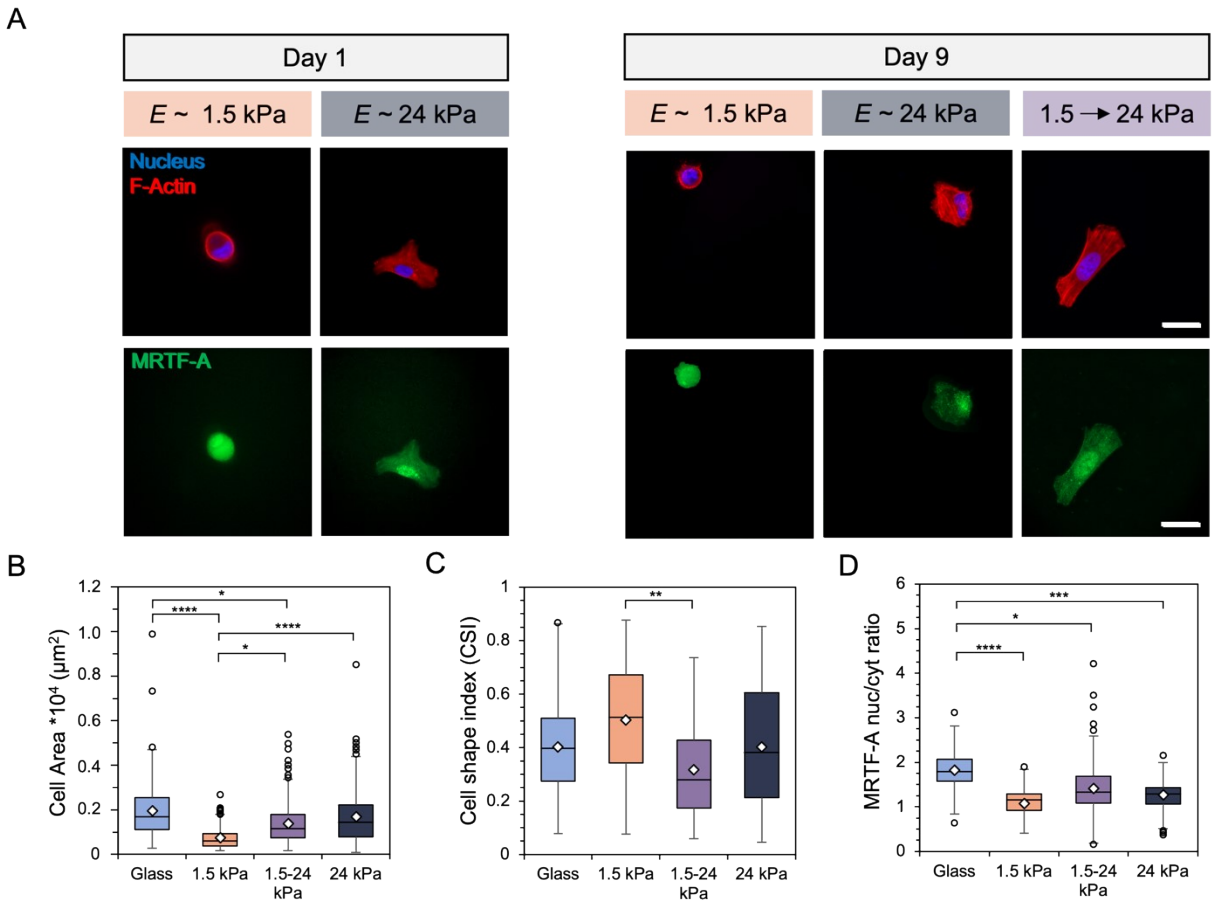

Figure S11. **A**) Representative images of fibroblasts after (left) 1 day of culture on $E \sim 1.5$ or 24 kPa mechanically static hydrogels and (right) after 9 days on either the mechanically static hydrogels or a hydrogel that was stiffened from 1.5 to 24 kPa after 1 day. Scale bars: 50 µm. Fibroblast **B**) spread area (µm^2), **C**) cell shape index, and **D**) MRTF-A nuclear localization were quantified. $N = 3$ hydrogels per group. **** $P < 0.0001$, *** $P < 0.0005$, ** $P < 0.01$, * $P < 0.05$.

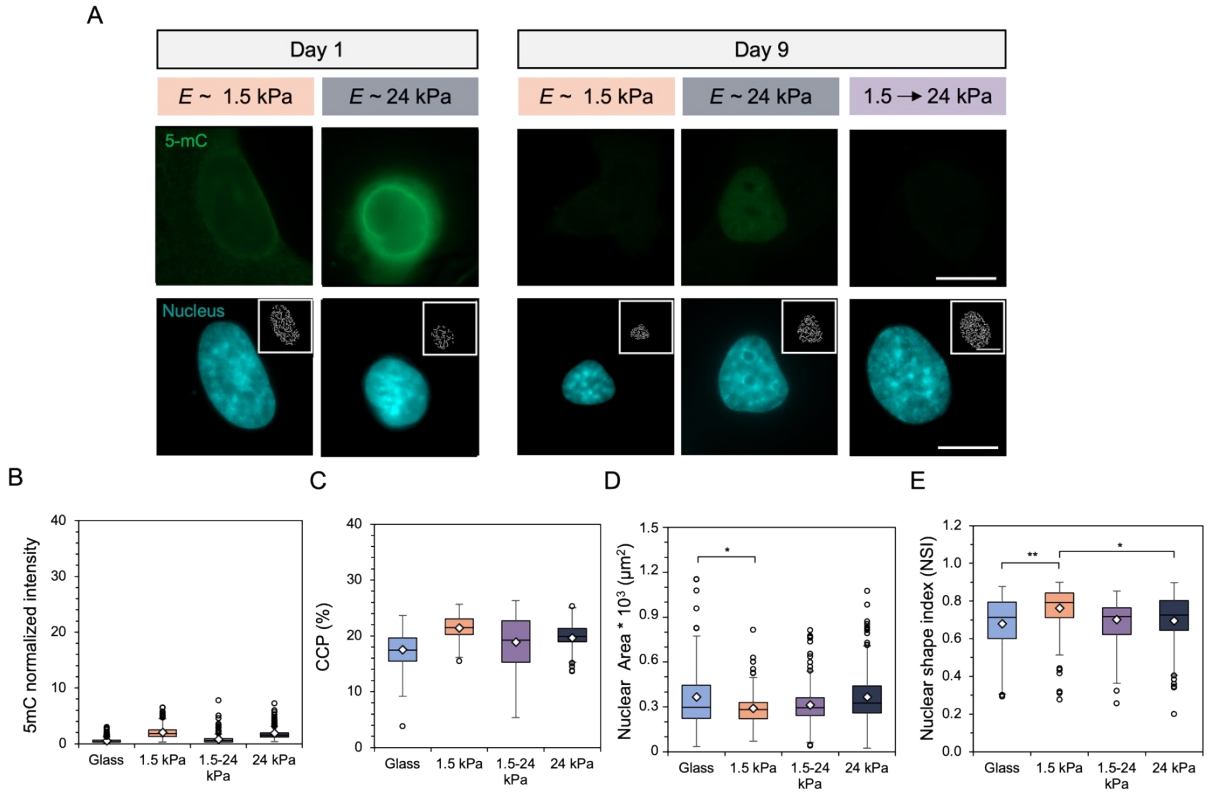

Figure S12. **A**) Representative images of fibroblast global DNA methylation as indicated by 5-mC (green) staining and nuclei (DAPI) after (left) 1 day of culture on E ~ 1.5 or 24 kPa mechanically static hydrogels and (right) after 9 days on either the mechanically static hydrogels or a hydrogel that was stiffened from 1.5 to 24 kPa after 1 day. Inset images represent pixelated edges within the nuclei, used to quantify chromatin condensation percentage (CCP). Scale bars: 10 µm. Nuclear metrics were measured by **B**) global DNA methylation intensity within the nucleus, **C**) the CCP, **D)** nuclear spread area, and **E)** nuclear shape index. $N = 3$ hydrogels per group. ** $P < 0.01$, * $P < 0.05$.

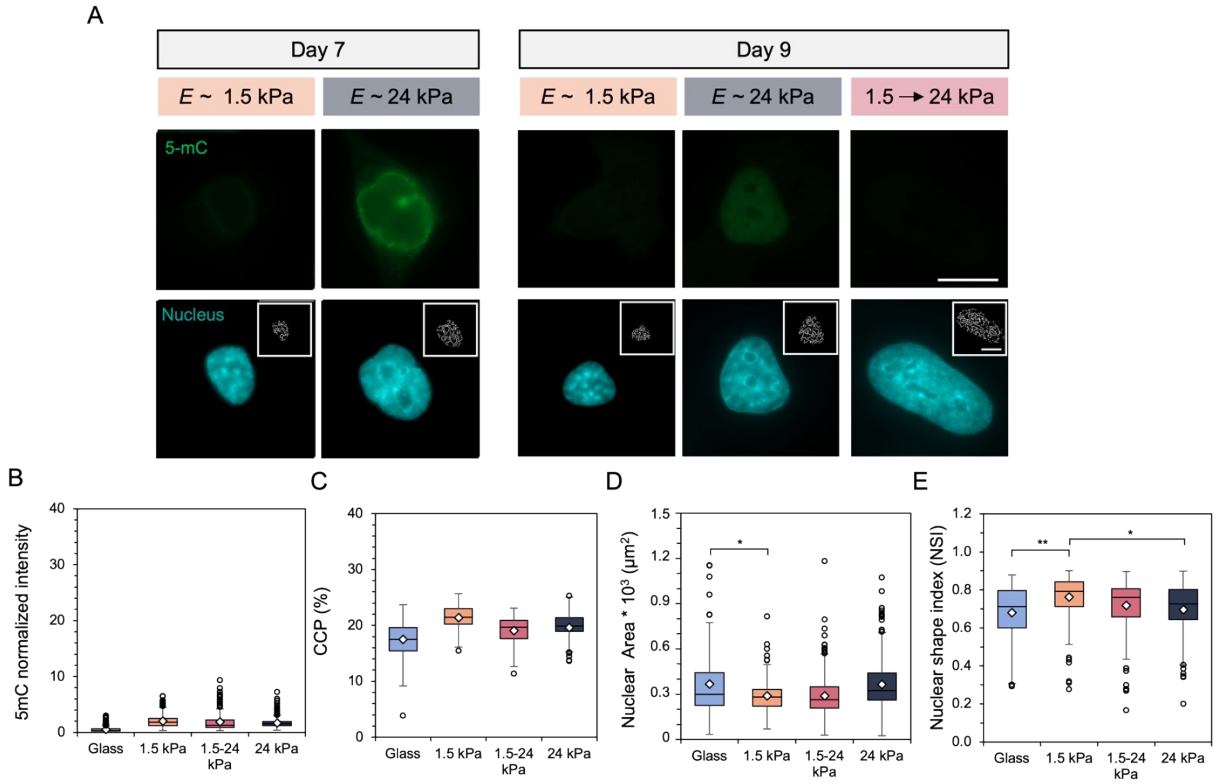

Figure S13. **A**) Representative images of fibroblast global DNA methylation as indicated by 5-mC (green) staining and nuclei (DAPI) after (left) 7 days of culture on $E \sim 1.5$ or 24 kPa mechanically static hydrogels and (right) after 9 days on either the mechanically static hydrogels or a hydrogel that was stiffened from 1.5 to 24 kPa after 7 days. Inset images represent pixelated edges within the nuclei, used to quantify chromatin condensation percentage (CCP). Scale bars: 10 µm. Nuclear metrics were measured by **B**) global DNA methylation intensity within the nucleus, **C**) the CCP, **D)** nuclear spread area, and **E)** nuclear shape index. $N = 3$ hydrogels per group. ** $P < 0.01$, * $P < 0.05$.

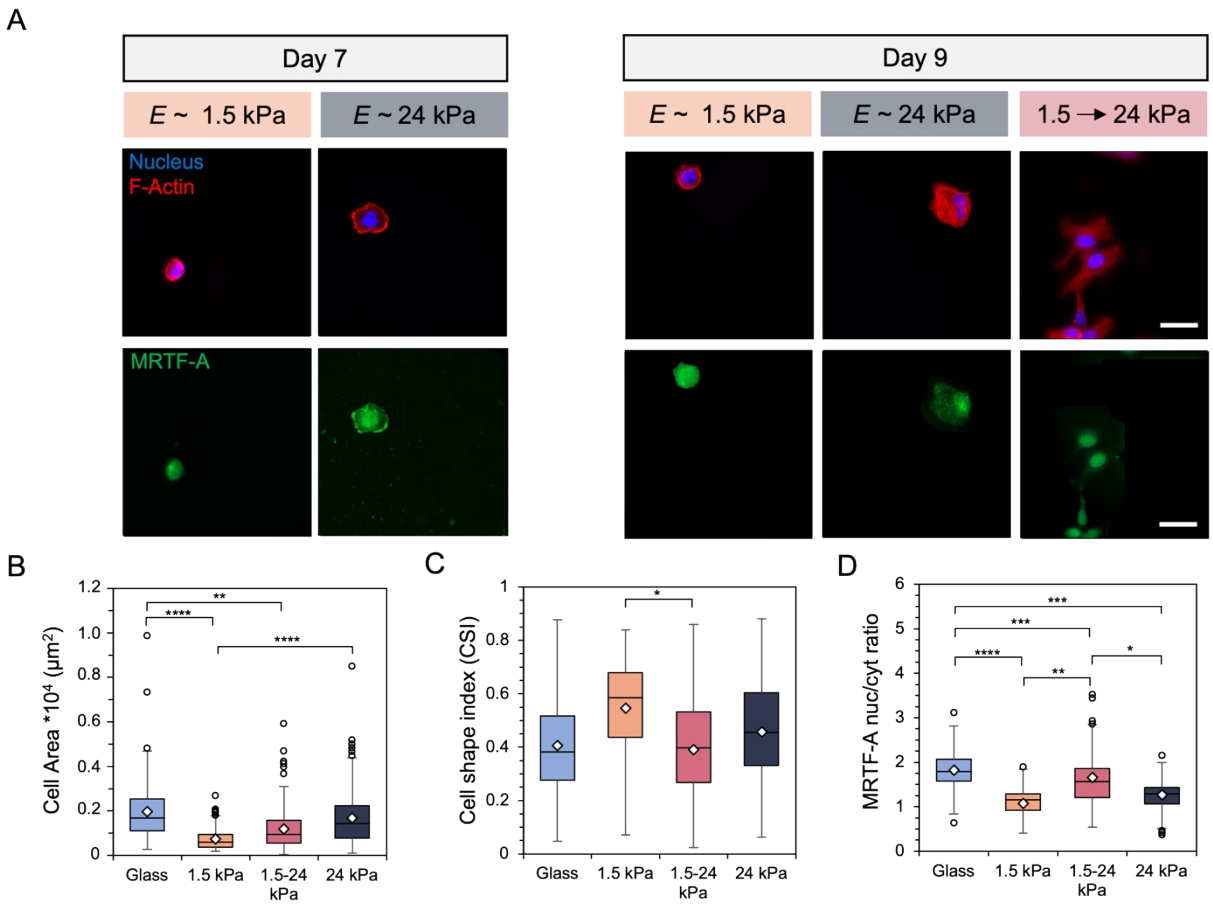

Figure S14. **A**) Representative images of fibroblasts after (left) 7 days of culture on $E \sim 1.5$ or 24 kPa mechanically static hydrogels and (right) after 9 days on either the mechanically static hydrogels or a hydrogel that was stiffened from 1.5 to 24 kPa after 7 days. Scale bars: 50 μm. Fibroblast **B**) spread area (μm^2), **C**) cell shape index, and **D**) MRTF-A nuclear localization were quantified. $N = 3$ hydrogels per group. **** $P < 0.0001$, *** $P < 0.0005$, ** $P < 0.01$, * $P < 0.05$.