**Table of contents**

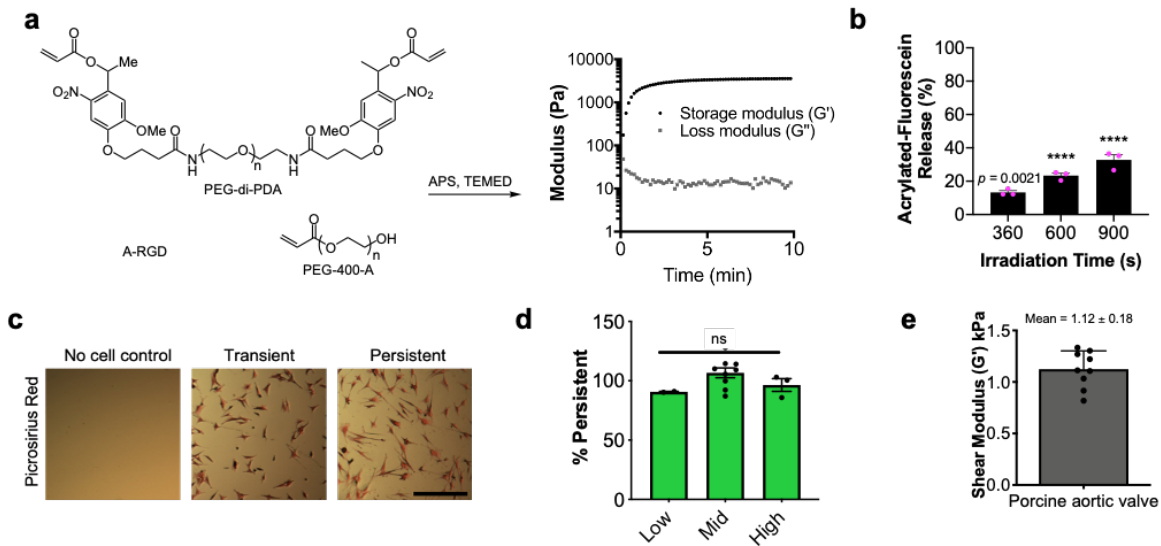**Supplementary Methods**

**Omni-ATAC procedure, modified:** The omni ATAC libraries were prepared following the original omni-ATAC protocol 29 with the following modifications. Cells were grown on hydrogels with each replicate having been grown and processed at different dates. Approximately 50,000 cells per well were used for each transposition reaction, with each reaction taking place while the cells were still attached onto their hydrogel substrate. A 300 µL total volume per 12-well plate well reaction was prepared as follows: 150 µL Tagment DNA Buffer (Illumina Ref 15027866), 7.5 µL Tagment DNA Enzyme 1 (Illumina Ref 15027865), 3 µL digitonin (Promega Ref G944A, diluted 1:1 with water), 30 µL Tween-20, 10.5 µL water, 89 µL PBS, 10 µL S2 Drosophila melanogaster nuclei; incubating for 50 minutes at 37°C. The Drosophila spike-in was added for the first replicate for a total of 500 nuclei (1% of total nuclei), and for the second replicate pair it was added for a total of 2,500 nuclei (5% of total nuclei). A third replicate was produced, but dropped as it did not contain Drosophila spike-in controls, a fourth replicate was produced but was dropped because it did not look similar to the other replicates (technical error). The pre-amplified transposed fragments were extracted from the hydrogel-attached cells with a phenol/chloroform/isoamyl alcohol (25:24:1) DNA precipitation. The post-amplified ATAC-libraries were cleaned-up with DNA Clean and Concentrator-5 Kit (Zymo Research Ref D4014). The qPCR amplification was done using NEBNext Ultra II Q5 Master Mix (NEB Ref M0544S), SYBR Gold (Life Tech Ref S11494), and Nextera DNA CD Indices (Illumina Ref 20015882). The libraries were size-selected to remove DNA fragments greater than 1000 bp with a Sage Science BluePippin. The ATAC-seq libraries were quantified with Qubit HS DNA assay and their fragment size-distributions determined with Agilent HS D5000 ScreenTape.
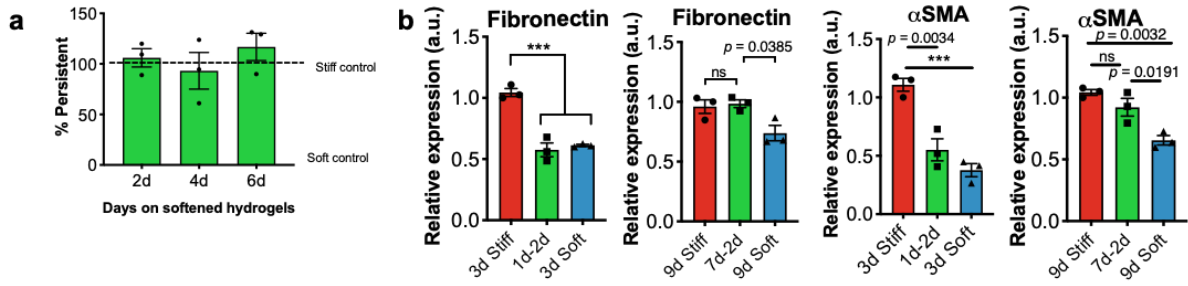
**Sequencing and processing of omni-ATAC libraries**: Libraries were pooled and sequenced as paired-end 2x37 reads with a NextSeq sequencer at the University of Colorado Boulder BioFrontiers Next Generation Sequencing Core. The libraries were adapter- and quality-trimmed with BBTools BBDuk module (version 38.05), aligned to both the pig genome assembly susScr11/Sscrofa11.1 and to the Drosophila melanogaster genome assembly dm6 (by filtering out paired-reads that mapped to both genomes) with HISAT2 87 (version 2.1.0), format converted with SAMtools 88(version 1.8), removed read duplicates using Sambamba 89 (version 0.6.6); and ATAC peaks were called using MACS2 90 (version 2.1.1.20160309) using the same parameters as described here 29 (--nolambda --nomodel --keep-dup all --call-summits). For downstream analysis, we determined 'blacklisted' regions (e.g. genome regions prone to accumulating a high degree of mapping artifacts) in the pig genome assembly susScr11 with a simplified procedure as to how they are systematically annotated and described in https://github.com/Boyle-Lab/Blacklist/blob/master/lists/hg19-blacklist-README.pdf 91 . Specifically, we used publicly available ChIP-seq input and IgG control datasets with SRA accession numbers SRR414976, SRR8815619, SRR8815651, SRR12107014, SRR12107011, ERR3958966, ERR3184744, ERR3154131, and ERR3154134. To obtain FRIP scores, we calculated the fraction of the paired-reads that mapped to a merged bed file of MACS2 narrow peaks from both transient and persistent replicate pairs, filtering out peaks with a MACS2 score of less than 100. To obtain the FPKM normalized values, we used the featureCounts function (version 1.6.1) from the Rsubread R package on merged SAF-formatted files of MACS2 narrow peaks from both transient and persistent replicate pairs. For comparison between 9 day stiff and soft samples, the narrow peaks MACS2 files were inputted into DiffBind (2.14.0) for differential accessibility analysis. The biological replicate variability was used as a blocking factor in the edgeR based analysis, and an adjusted p-value (FDR) of <0.05 was used.

**Normalization of ATAC-seq signal based on Drosophila nuclei spike-ins:** We normalized the ATAC-seq datasets to account for the global reduction in chromatin accessibility in the persistent relative to the transient myofibroblasts. We used a fixed number of Drosophila melanogaster S2 nuclei (1% for 1st replicate, and 5% for 2nd replicate) for both the persistent and transient transposition reactions. We rescaled the datasets according to the fraction of the reads that mapped to the Drosophila melanogaster (dm6) genome. In the 1nd replicate, the fraction of Drosophila reads from the total mapped reads in the transient dataset was 0.99% and in the persistent it was 1.57%, such that the scaling factor for the transient dataset was 1.0 (0.99/0.99), and for the persistent was 1.586 (1.57/0.99). In the 2nd replicate, the fraction of Drosophila reads from the total mapped reads in the transient dataset was 1.354% and in the persistent it was 4.572%, such that the scaling factor for the transient dataset was 1.0 (1.354/1.354), and for the persistent was 3.377 (4.572/1.354).
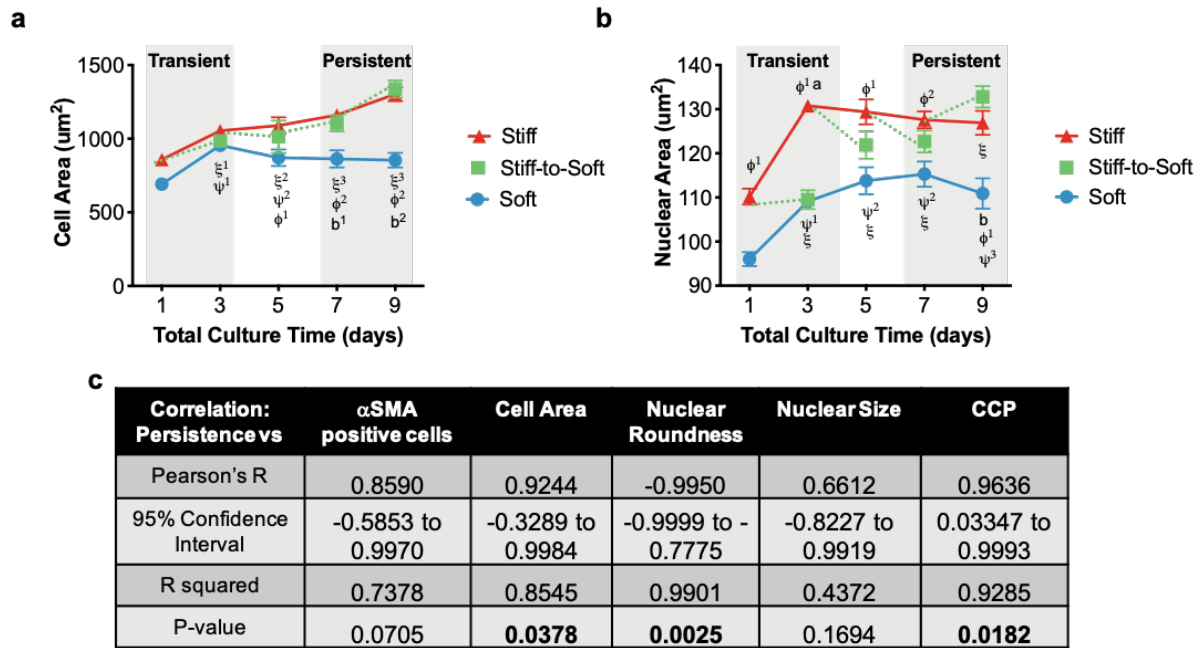
**Differential transcription factor/regulator activity:** To look for putative differential transcription factor (TF) activity from the omni-ATAC datasets from the transiently and persistently activated myofibroblast phenotypes, we used the Transcription Factor Expression Analysis (TFEA) 33. The TF activity was assessed with both the JASPAR2020_CORE_vertebrates_non-redundant motif database 92, and on a curated list of human transcription factor motifs 93. In brief, TFEA estimates enrichment scores on the co-occurrence of TF motifs with ATAC-seq peak summits, then estimates a change in this score across distinct experimental conditions per TF as a surrogate for differential transcription factor activity.
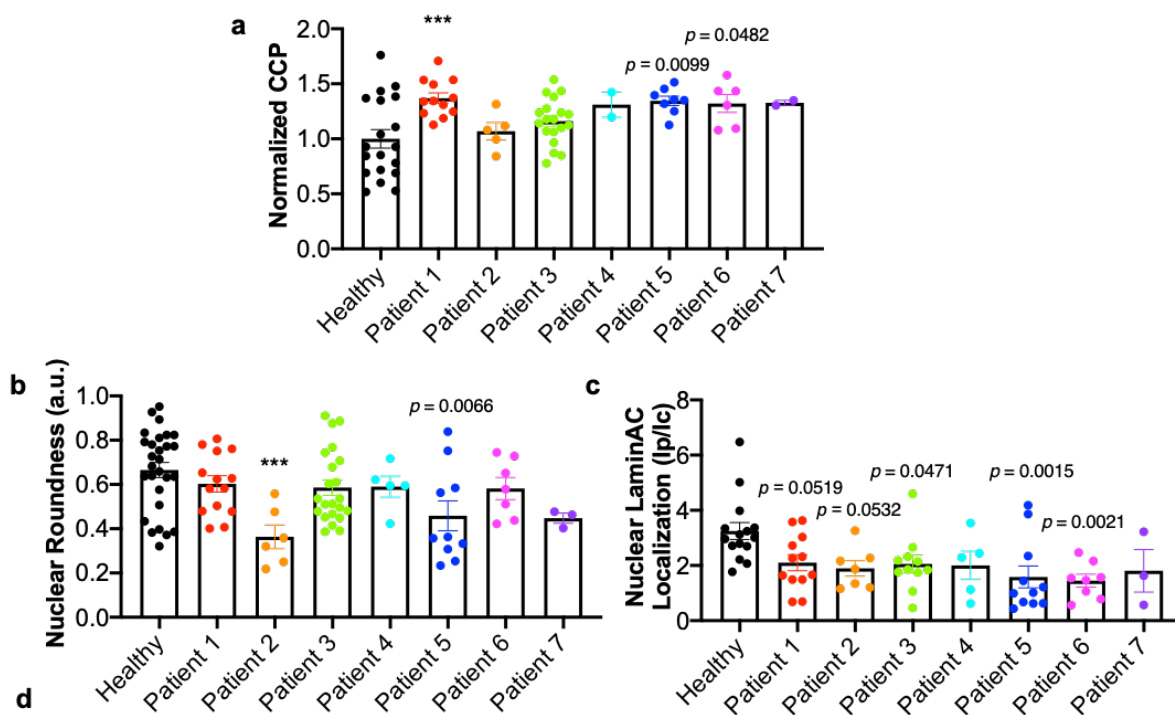
**Supplementary Figure 1:** Phototunable hydrogel characterization. a) Chemical structure of hydrogel components and rheological trace of polymerization. b) Quantification of released hydrogel incorporated acrylated-fluorescein after photo-degradable for 360, 600, and 900 seconds of 10mW/cm2 as a proxy for percent of acrylated-RGD released during degradation. One-way ANOVA with Bonferroni post-hoc applied. n=3 hydrogels. c) Picrosirius red staining of hydrogels cultured with no cells, transient myofibroblasts (1d stiff), or persistent myofibroblasts (7d stiff) which displays no observable collagen deposition. (n = 22 images across 2 hydrogels per condition).  d) Cells cultured on stiff hydrogels for 7 days, followed by cultured on softened hydrogel for 2 days at varying cell seeding densities (7d-2d), indicating persistence is independent of cell density. Low = 10k cells/cm2, Mid= 20k cells/cm2, High = 40k cells/cm2. One-way ANOVA with Bonferroni post-hoc applied. n=hydrogels: low=2, mid=9, high=3. e) Stiffness of porcine aortic valve measured by shear rheology. One-way ANOVA with Bonferroni post-hoc applied. n=9 pig valves. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.

**Supplementary Figure 2:** Characterization of myofibroblast persistence. a) Myofibroblasts cultured on stiff hydrogels remain persistently activated up to 6 days on softened hydrogels. Normalization of myofibroblast activation for 7 days stiff followed by 2, 4, or 6 days on soft in relation to stiff and soft controls, yielding percent irreversibly activated. One-way ANOVA with Bonferroni post-hoc applied. n=3 hydrogels. b) Quantification of western blots for protein expression of EDA fibronectin and $\alpha$SMA in transient and persistent myofibroblasts and corresponding controls. Whole blots can be viewed on Supplementary Fig. 15. One-way ANOVA with Bonferroni's posthoc test applied, n=3 hydrogels. *** p< 0.001. Data reported as mean ± SEM.

**a**



**b**



**c**

| Correlation: Persistence vs | αSMA positive cells | Cell Area | Nuclear Roundness | Nuclear Size | CCP |
|---|---|---|---|---|---|
| Pearson's R | 0.8590 | 0.9244 | -0.9950 | 0.6612 | 0.9636 |
| 95% Confidence Interval | -0.5853 to 0.9970 | -0.3289 to 0.9984 | -0.9999 to -0.7775 | -0.8227 to 0.9919 | 0.03347 to 0.9993 |
| R squared | 0.7378 | 0.8545 | 0.9901 | 0.4372 | 0.9285 |
| P-value | 0.0705 | **0.0378** | **0.0025** | 0.1694 | **0.0182** |

**Supplementary Figure 3:** Phenotypic correlations with myofibroblast persistence. a) Cell area of cells cultured on stiff, soft, or stiff-to-soft hydrogels for 1-9 days. Two-way ANOVA with Bonferroni multiple comparison test applied, n=hydrogels: 1d stiff=6, 1d soft=6, 3d stiff=6, 3d soft=6, 1d2d=5, 5d stiff=9, 5d soft=8, 3d2d=5, 7d stiff=7, 7d soft=6, 5d2d=5, 9d stiff=9, 9d soft=8, 7d2d=7. "b" = Significance of stiff-to-soft versus soft b1 p=0.0171, b2 p<0.001; "$\phi$" = Significance of stiff versus soft $\phi$1 p= 0.006, $\phi$2 p<0.001; "$\xi$" = Significance stiff time point versus stiff day 0. $\xi$1 p=0.0247, $\xi$2 p=0.002, $\xi$3 p<0.001; "$\psi$" = Significance soft time point versus soft day 1 $\psi$1 p=0.0012, $\psi$2 p=0.0281. b) Nuclear area of cells cultured on stiff, soft, or stiff-to-soft hydrogels for 1-9 days. Two-way ANOVA with Bonferroni multiple comparison test applied, n=hydrogels: 1d stiff=6, 1d soft=6, 3d stiff=7, 3d soft=7, 1d2d=6, 5d stiff=13, 5d soft=7, 3d2d=7, 7d stiff=11, 7d soft=8, 5d2d=6, 9d stiff=9, 9d soft=7, 7d2d=6. "a" = Significance of stiff-to-soft versus stiff p<0.001; "b" = Significance of stiff-to-soft versus soft p<0.001; "$\phi$" = Significance of stiff versus soft $\phi$1 p<0.001, $\phi$2 p=0.0017; "$\xi$" = Significance stiff time point versus stiff day 0. p<0.001; "$\psi$" = Significance soft time point versus soft day 1 $\psi$1 p=0.0076, $\psi$2 p<0.001, $\psi$3 p=0.0019. c) Correlation analyses testing cell phenotype correlation to myofibroblast persistence. Data reported as mean ± SEM.

| Patient | SampleID | Sex | Medical History | Surgery |
|---|---|---|---|---|
| Healthy | FR00019495 | M | Unknown | |
| 1 | FR0001995D | M | CAD; HTN; BPH; S/p MI; DM; s/P CABG; High Chol; S/p Stent - Svg - Lad; Smoker, quit; CEA; ETOH, quit. | Heart valve replacement |
| 2 | FR5B339285 | M | Current surgery for aortic insufficiency. History includes hypertension, intermittent claudication, shortness of breath, Ross procedure with aortic valve insufficiency and root dilatation. Smokes 1 pack of cigarettes daily for 6 years and a pipe for 20 years,he has 3 to 4 glasses of wine weekly | Heart valve repair |
| 3 | FR5B339286 | M | Current surgery for aortic insufficiency. History includes hypertension, intermittent claudication, shortness of breath, Ross procedure with aortic valve insufficiency and root dilatation. Smokes 1 pack of cigarettes daily for 6 years and a pipe for 20 years,he has 3 to 4 glasses of wine weekly | Heart valve repair |
| 4 | FR0001E235 | F | Mitral regurgitation, aortic stenosis; Secondary dx includes shortness of breath, skin cancer, vaginal cancer. Smoked 1 pack daily for 46 years. | Heart valve excision |
| 5 | FR0001EA5D | F | Principal diagnosis: aortic insufficiency. History of breast cancer treated with chemo/radiation. Non-smoker. mother has history of sclerotic aortic valve. | Heart valve replacement |
| 6 | FR000243F1 | F | Critical aortic stenosis; glaucoma, has multiple medical sensitivities to medications, heart murmur, obesity, hypercholesterolemia; Non-smoker | Heart valve replacement |
| 7 | FR0002A2A7 | F | Aortic insufficiency. | Heart valve replacement |

**Supplementary Figure 4:** Human patient samples used for analyzing myofibroblasts. a-c) Analysis for CCP (a), nuclear roundness (b), and lamin AC (c) myofibroblasts of healthy and diseased patient samples separated by individual patients. Patients had variable levels of myofibroblasts identified with αSMA positive staining which is reflected in the number of cells analyzed. (a) n=cells: healthy=12, patient 1=5, patient 2=19, patient 3=2, patient 4=8, patient 5=6, patient 6=2, patient 7=19. (b) n=cells: healthy=14, patient 1=6, patient 2=22, patient 3=5, patient 4=10, patient 5=7, patient 6=3, patient 7=28. (c) n=cells: healthy=12, patient 1=7, patient 2=11, patient 3=5, patient 4=11, patient 5=8, patient 6=3, patient 7=15. One-way ANOVA with Bonferroni post-hoc applied against healthy sample. d) De-identified medical information for each patient that was provided by the tissue collection company (Origene). Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.
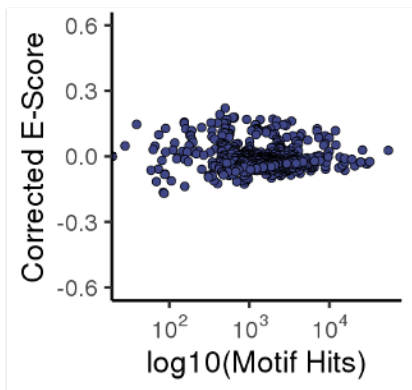
**Supplementary Figure 5:** Comparison of ATAC-seq datasets without and with Drosophila nuclei spike-ins. Changes in open chromatin are genome-wide. a) The FRIP (Fraction of Reads In Peaks) scores between the transient and the persistent myofibroblast datasets expressed as either their absolute value (left), or as relative to the persistent myofibroblasts to more easily observe the reduced FRIP score in transient compared to persistent states (right). b) (Top) Average ATAC-seq peak profile from both the transient and persistent myofibroblast cells. (Bottom) Heatmap from same datasets. ATAC-seq data was normalized by both counts per million mapped reads to account for differences in sequencing depth (left), and by the Drosophila nuclei spike-ins to account for genome-wide changes in ATAC-seq signal (right). c) Average ATAC-seq peak profile from both the transient (gray) and persistent (black) myofibroblast cells. ATAC-seq data was normalized by both counts per million mapped reads to account for differences in sequencing depth, and by the Drosophila nuclei spike-ins to account for genome-wide changes in ATAC-seq signal. Data shown for replicate 2. The peaks were sorted based on descending signal intensity and divided into four equal-sized quartiles, and the average peak profiles were redrawn. The difference between transient and persistent datasets is still evident even between the peaks with highest (1st quartile) or weakest (4th quartile) signal.
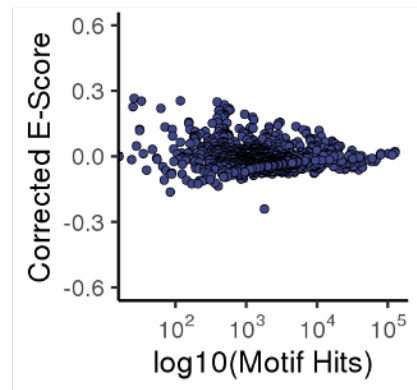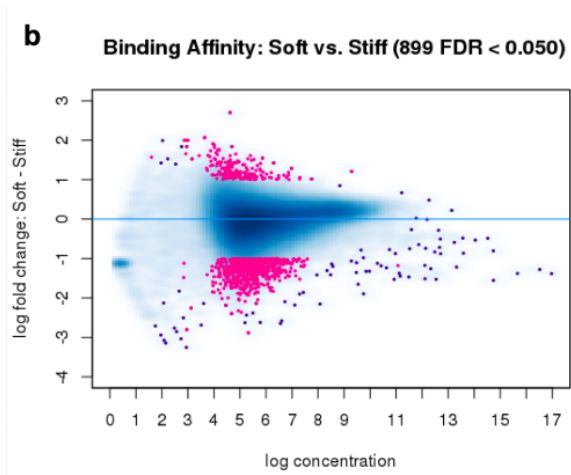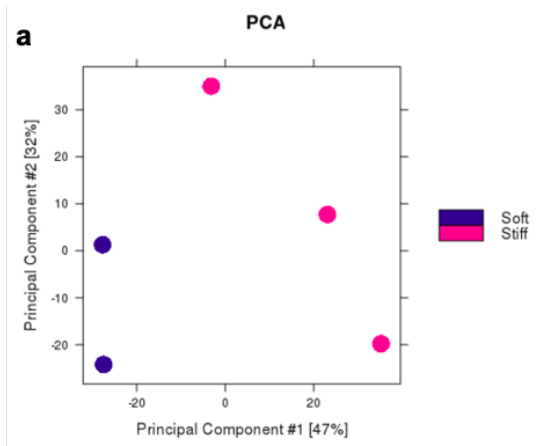
**TFEA MA plot**
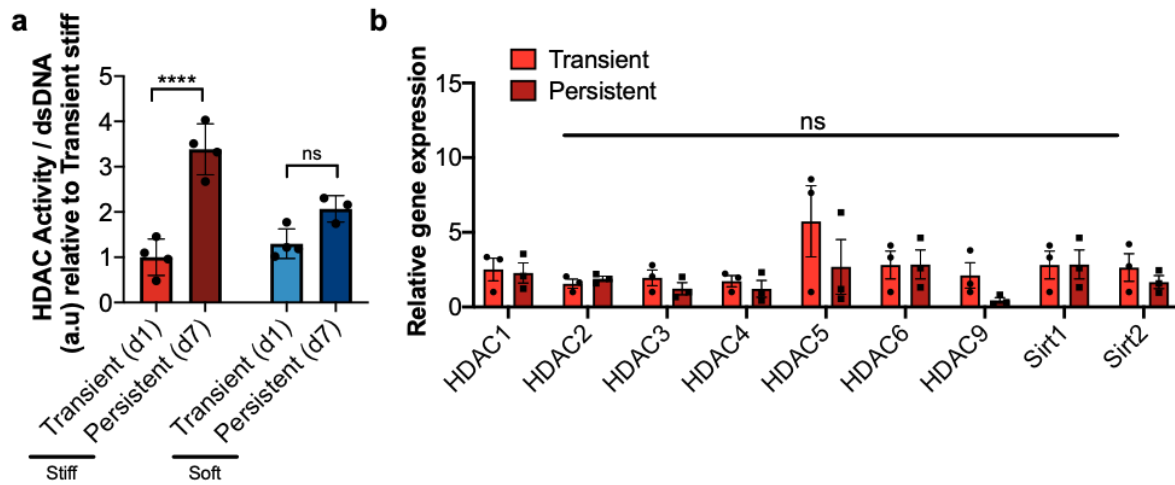JASPAR2020 vertebrate motif DB
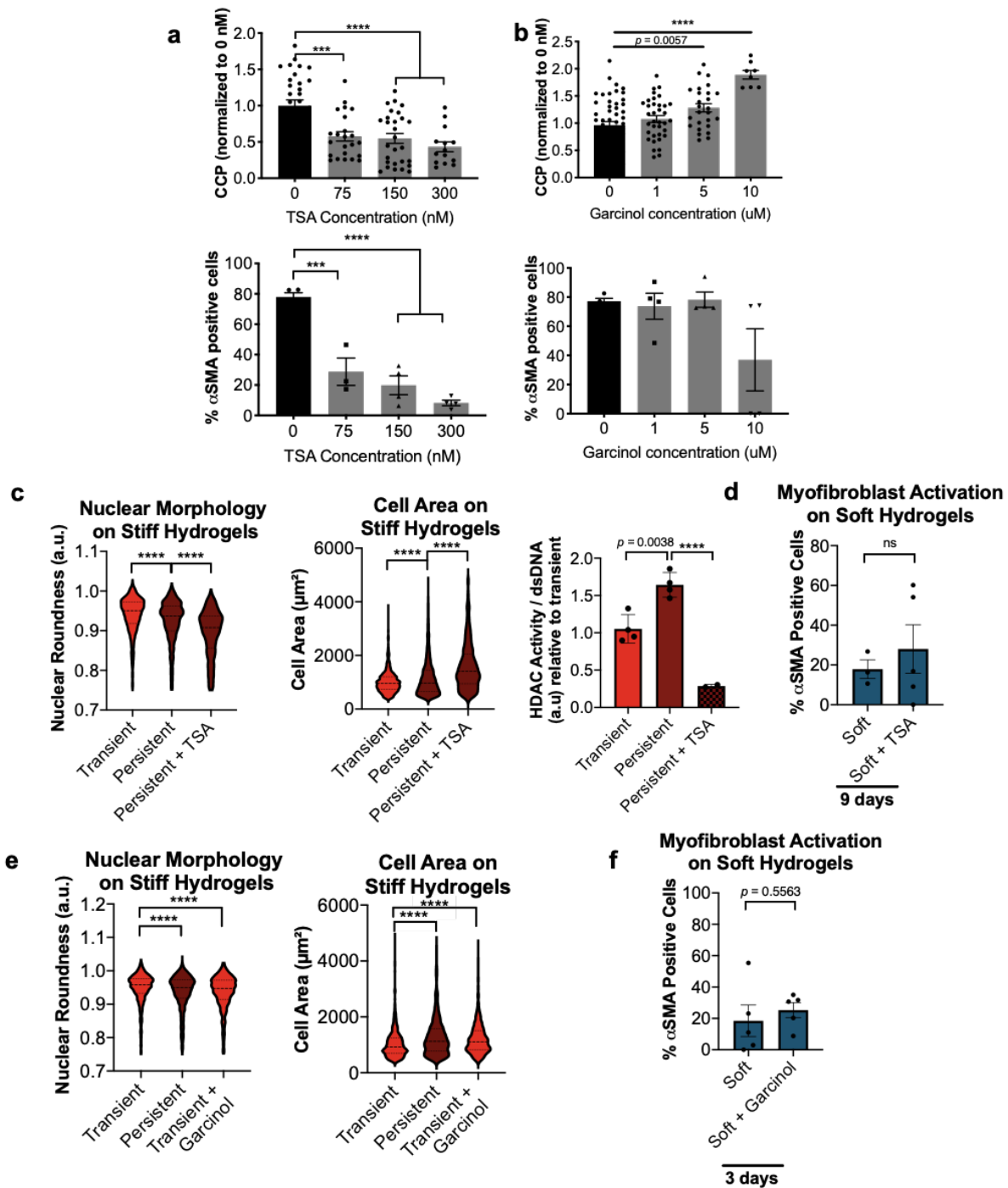
**TFEA MA plot**
best-human motif DB

**Supplementary Figure 6:** No clear chromatin remodeler or transcription factor was identified as helping drive the observed phenotype of the differences in global chromatin accessibility. Transcription Factor Enrichment Analysis 88 MA plots showing the corrected enrichment score (e-score) on the y-axis, and the number of motif instances on the x-axis, using the JASPAR2020 vertebrate non-redundant motif database (left) or a curated human transcription factor motif database 90 (right).

**Supplementary Figure 7:** The chromatin landscape of persistent myofibroblasts and fibroblasts are distinct. ATAC-sequencing analysis of persistent myofibroblasts (9 days stiff) and fibroblasts (9 days soft). a) Principal component analysis (PCA) of persistent myofibroblasts and fibroblasts sequencing samples. b) MA plot of significantly differentially accessible regions (pink) identified using DiffBind between persistent myofibroblasts and fibroblasts (FDR < 0.05, log fold change > abs(1)).
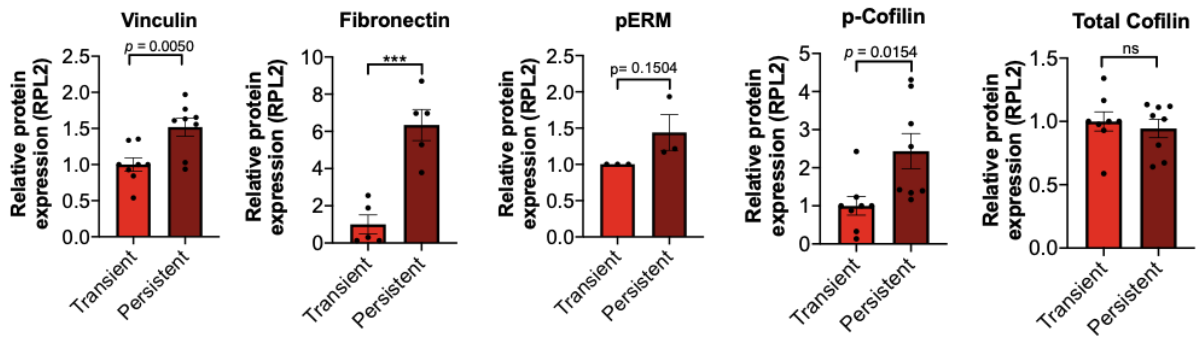
**Supplementary Figure 8:** HDAC activity and expression in transient and persistent myofibroblasts. a) HDAC activity assays for cells cultured on stiff or soft hydrogels for 1 (transient) or 7 (persistent) days and normalized to total cell number using dsDNA. Two-way ANOVA with Bonferroni's posthoc applied. n=hydrogels: stiff transient=4, stiff persistent=4, soft transient=4, soft persistent=3. b) RT-qPCR used to quantify gene expression of individual HDACs in transient (3d stiff) and persistent (9d stiff) myofibroblasts. Primers can be found in Supplementary Table 1. n=3 hydrogels. Two-way ANOVA with Bonferroni's posthoc applied. Significance indicated by ***p < 0.001, and ****p < 0.0001. Data reported as mean ± SEM.
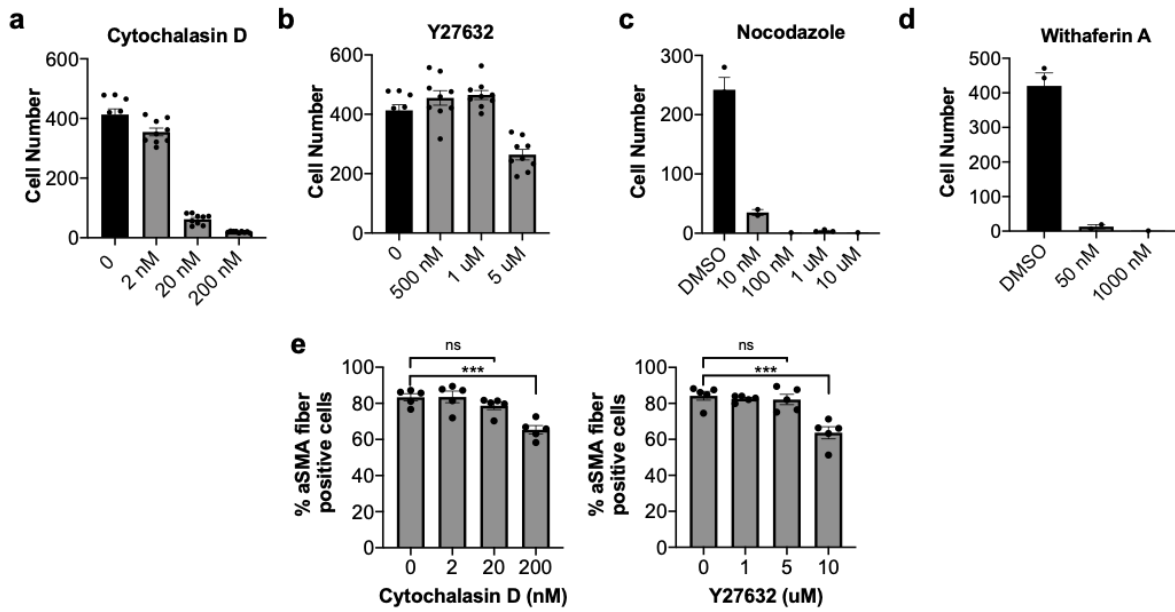
**Supplementary Figure 9:** Effects of Trichostatin A (TSA) and garcinol at various concentrations. a) Cells were cultured on stiff hydrogels for 1 day, followed by 2 days on treatment with 0, 75, 150, or 300 nM of TSA. Appropriate vehicle control was used (DMSO). Cells were evaluated for CCP and myofibroblast activation. One-way ANOVA with Bonferroni's posthoc applied. CCP: n=cells: 0=32, 75=23, 150=28, 300=14. % αSMA positive: n=hydrogels: 0=4, 75=3, 150=4, 300=4. b) Cells were cultured on stiff hydrogels for 1 day, followed by 2 days on treatment with 0, 1, 5, or 10 nM of garcinol. Cells were evaluated for CCP and myofibroblast activation. One-way ANOVA with Bonferroni's posthoc applied. CCP: n=cells: 0=54, 1=36, 5=26, 10=8. % αSMA positive: n=4 hydrogels. c) Nuclear morphology and cell area for transient myofibroblasts (1d stiff, 2d stiff+DMSO), persistent myofibroblasts (7d stiff, 2d stiff+DMSO), and persistent myofibroblasts treated with TSA (7d stiff, 2d stiff+TSA). Nuclear roundness: n=cells: transient=2183, persistent=1723, persistent+TSA=1021. Cell area: n=cells: transient=863, persistent=1257, persistent+TSA=1021. HDAC activity normalized by double stranded DNA (dsDNA) in transient myofibroblasts (1d stiff, 2d stiff + DMSO), persistent myofibroblasts (7d stiff, 2d stiff + DMSO), and TSA-treated persistent myofibroblasts (7d stiff, 2d stiff + TSA). One-way ANOVA with Bonferroni posthoc applied, n=hydrogels: transient=4, persistent=4, persistent+TSA=2. d) Myofibroblast activation of cells cultured on soft hydrogels for 7 days followed by vehicle or TSA treatment for 2 days (7d soft, 2d soft+DMSO or TSA). One-way ANOVA with Bonferroni's posthoc applied. n=hydrogels: Soft=3, Soft+TSA=5. e) Nuclear morphology and cell area for transient myofibroblasts
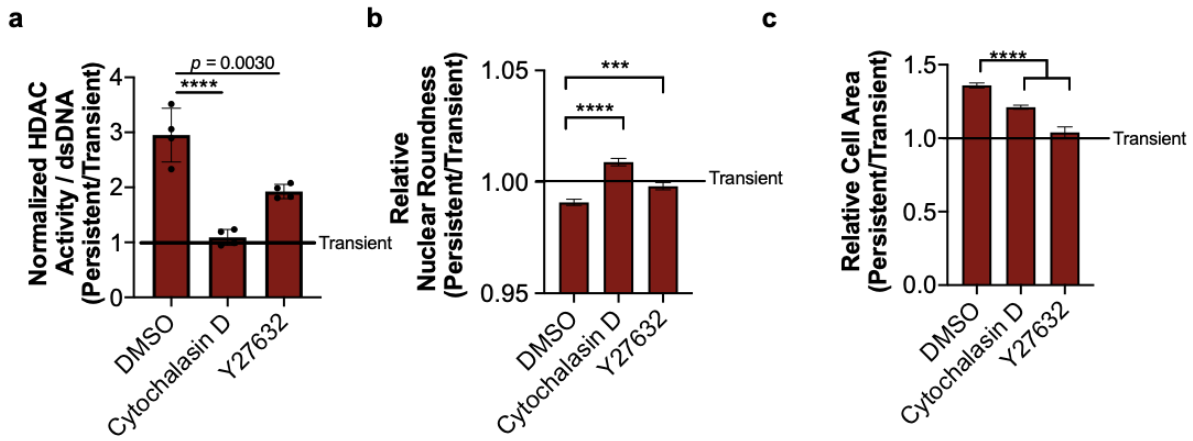
(1d stiff, 2d stiff+DMSO), persistent myofibroblasts (7d stiff, 2d stiff+DMSO), and transient myofibroblasts treated with garcinol (1d stiff, 2d stiff+garcinol). n=cells: transient=1086, persistent=818, transient+garcinol=583. One-way ANOVA with Bonferroni's posthoc applied. f) Myofibroblast activation of cells cultured on soft hydrogels for 1 day followed by vehicle or garcinol treatment for 2 days (1d soft, 2d soft+DMSO or TSA). One-way ANOVA with Bonferroni's posthoc applied. n=5 hydrogels. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.

**Supplementary Figure 10:** Protein expression of cytoskeletal stabilization components and focal adhesions. Protein expression of transient myofibroblasts (1 day stiff) and persistent myofibroblasts (7 day stiff) of vinculin, fibronectin, phosphorylated erzin radixin moesin (pERM), phosphorylated cofilin (p-cofilin), and total cofilin. Expression levels were normalized to RNA polymerase (RNPII). Whole blots can be found in Supplementary Fig. 17. Two-tailed student's t-test applied, vinculin, p-cofilin, total cofilin: n=8 hydrogels. Fibronectin: n=5 hydrogels. pERM: n=3 hydrogels. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.
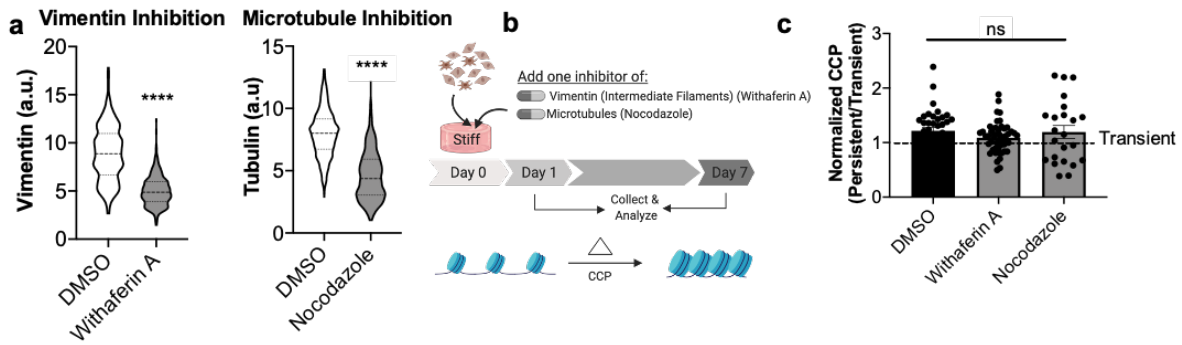
**Supplementary Figure 11:** Effects of cytoskeleton inhibitors at different doses on cell number. a) Quantification of cell number for cells treated with 2, 20, or 200 nM of cytochalasin D on glass for 7 days. n=9 wells. b) Quantification of cell number for cells treated with 0, 500 nM, 1 uM, or 5 uM of Y27632 on glass for 7 days. n=9 wells. c) Quantification of cell number for cells treated with 10 nM, 100 nM, 1 uM, 10 uM of Nocodazole on glass for 7 days. n=wells: DMSO=3, 10=2, 100=1, 1=3, 10=1. d) Quantification of cell number for cells treated with 5 or 1000 nM of Withaferin A on glass for 7 days. n=wells: DMSO=3, 50=2, 1000=1. e) αSMA positive cell quantification for Cytochalasin D and Y27632 dose curve on TCPS. n=9 wells. One-way ANOVA with Bonferroni post-hoc applied. Significance indicated by ***p < 0.001, and ****p < 0.0001. Data reported as mean ± SEM.
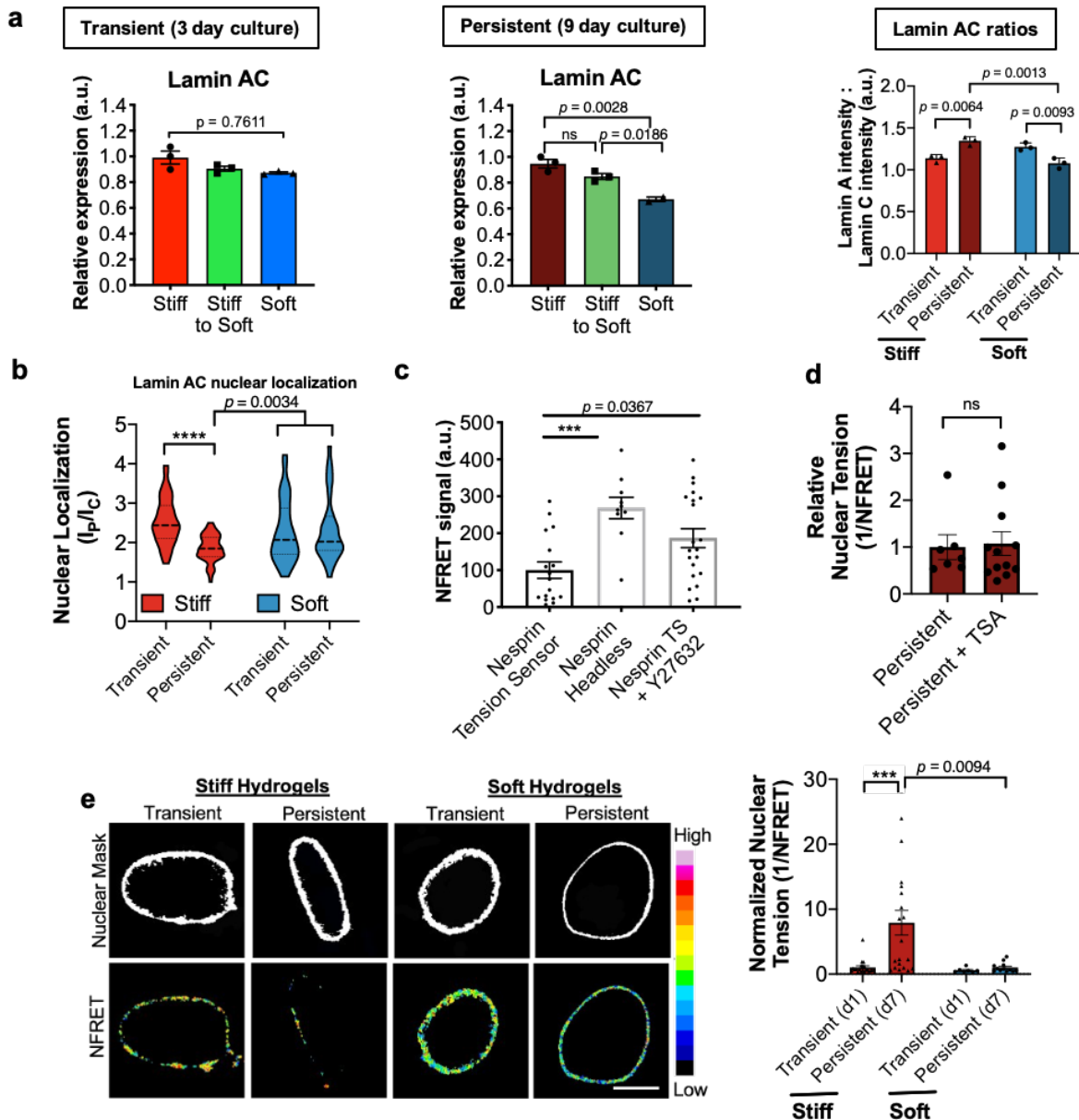
**Supplementary Figure 12:** Effect of actin inhibitors on myofibroblasts cultured for 7 days on stiff hydrogels. a) Persistent (7d stiff) HDAC activity normalized to transient (1d stiff) HDAC activity for cells cultured with vehicle (DMSO), Cytochalasin D, and Y27632. One-way ANOVA with Bonferroni post-hoc applied, n=4 hydrogels. b) Persistent (7d stiff) nuclear roundness normalized to transient (1d stiff) nuclear roundness for cells cultured with vehicle (DMSO), Cytochalasin D, and Y27632. One-way ANOVA with Bonferroni post-hoc applied, n=cells: DMSO=1513, cytochalasin D=674, Y27632=941. c) Persistent (7d stiff) cell area normalized to transient (1d stiff) cell area for cells cultured with vehicle, Cytochalasin D, and Y27632. One-way ANOVA with Bonferroni post-hoc applied, n=cells: DMSO=1391, cytochalasin D=1417, Y27632=239. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.
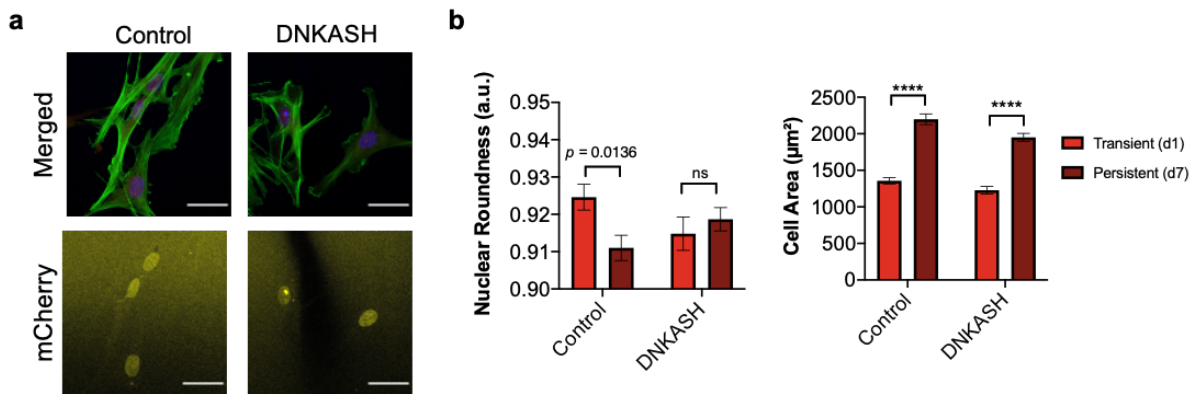
**Supplementary Figure 13:** Intermediate filaments and microtubules do not affect chromatin condensation in persistent myofibroblasts. a) Quantification of cytoskeletal protein expression after inhibitor treatment for 7 days on stiff hydrogels. Two-tailed student's t-test applied. n=cells, Vimentin inhibition: DMSO=378, Witherferin A=308. Microtubule Inhibition: DMSO=627, Nocodazole=630. b) Schematic of cell culture timeline to assess role of cytoskeleton components (intermediate filaments and microtubules) in progressive decrease in chromatin accessibility over time on stiff hydrogels. c) Quantification of CCP of cells on stiff hydrogels with cytoskeletal inhibitors for 7 days (persistent) compared to day 1 (transient) values. The dotted line represents day 1 (transient) values. One-way ANOVA with Bonferroni's posthoc applied, n=cells: DMSO=38, Withaferin A=51, Nocodazole=23. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.
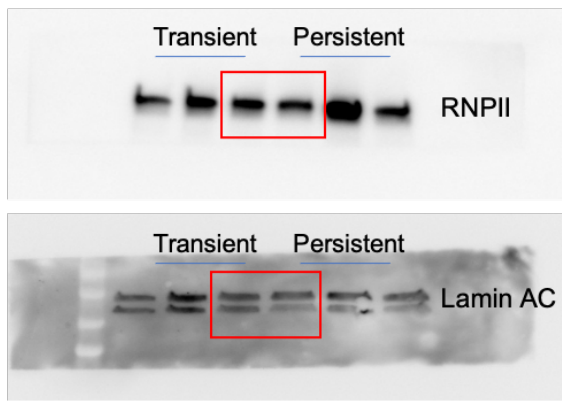
**Supplementary Figure 14:** Persistent myofibroblasts have higher nuclear tension than transient myofibroblasts. a) Protein expression analysis of Lamin AC for cell cultured on stiff, soft, or stiff-to-soft hydrogels for time points defined for transience (1d-2d) and persistence (7d-2d). One-way ANOVA with Bonferroni posthoc test applied, n=3 hydrogels. Lamin A to lamin C ratios for cells cultured on stiff or soft hydrogels for 1 and 7 days. Western blots can be found in Supplementary Fig. 16. Two-way ANOVA with Bonferroni post-hoc applied, n=3 hydrogels. b) Nuclear localization of lamin AC (intensity periphery (Ip)/intensity center (Ic)) of cells cultured on stiff or soft hydrogels for 1 day (transient) or 7 days (persistent). Two-way ANOVA with Bonferroni post-hoc applied, n=cells: stiff transient=40, stiff persistent=63, soft transient=36, soft persistent=31. c) Nesprin tension sensor displays significant differences between nesprin tension sensor (no treatment), or low control conditions (Y27632 & nesprin headless). Normalized fluorescent resonance energy transfer (NFRET) for cells cultured on glass coverslips and transfected with either nesprin tension sensor or nesprin headless. One-way ANOVA with Bonferroni posthoc test applied; n=cells: Nesprin Tension Sensor=17, Nesprin Headless=10, Nesprin TS+Y27632=21. d) Nuclear tension measured using nesprin NFRET sensor in persistent myofibroblasts (7d stiff, 2d stiff+DMSO) and persistent myofibroblasts treated with TSA (150 nmol) (7d stiff, 2d stiff+TSA). Two-tailed student's t-test applied, n=cells: persistent=7, persistent+TSA=12. e) Nuclear tension measured using nesprin NFRET sensor for cells cultured on stiff or soft hydrogels for 1 or 7 days. (n = 120 images across 4 hydrogels per condition). Normalized relative to transient stiff (1d stiff). One-way ANOVA with Bonferroni posthoc applied; n=cells: stiff transient=21, stiff persistent=20, soft transient=7, soft persistent=12. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.
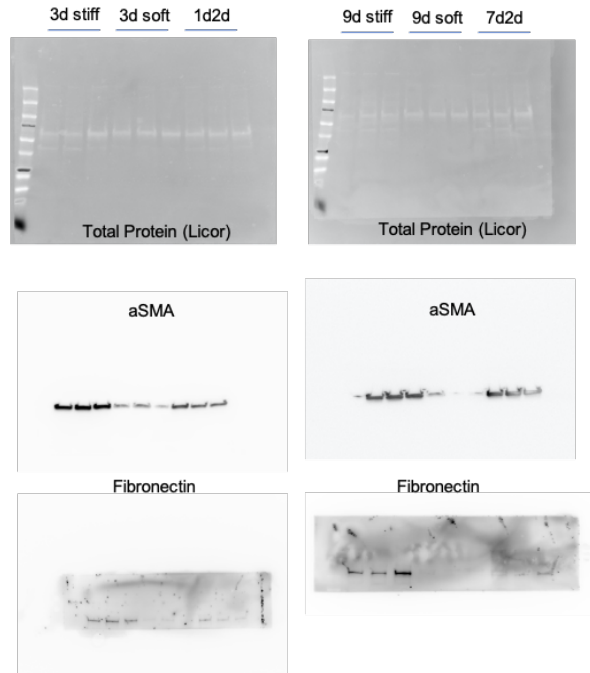
**Supplementary Figure 15:** DNKASH and control cells cultured on stiff hydrogels for 1 or 7 days. a) Confirmation of expression of mCherry and DNKASH. (n = 10 images across three biologically independent experiments). b) Nuclear roundness and cell area of control and DNKASH cells cultured on stiff hydrogels for 1 (transient) and 7 days (persistent). Two-way ANOVA with Bonferroni posthoc applied, n=cells: transient control=215, persistent control=259, transient DNKASH=181, persistent DNKASH=281. Significance indicated by ***$p < 0.001$, and ****$p < 0.0001$. Data reported as mean ± SEM.
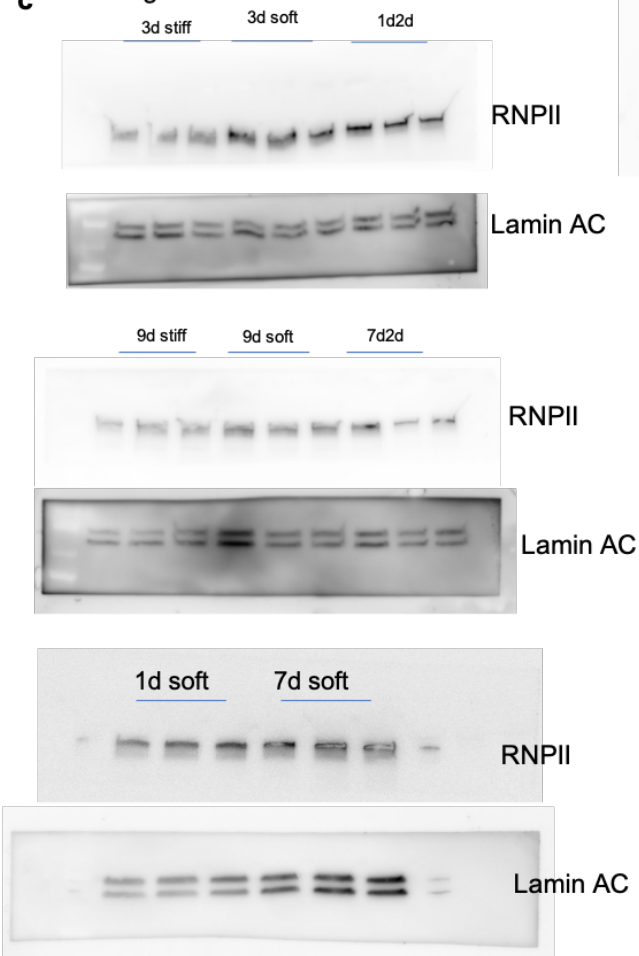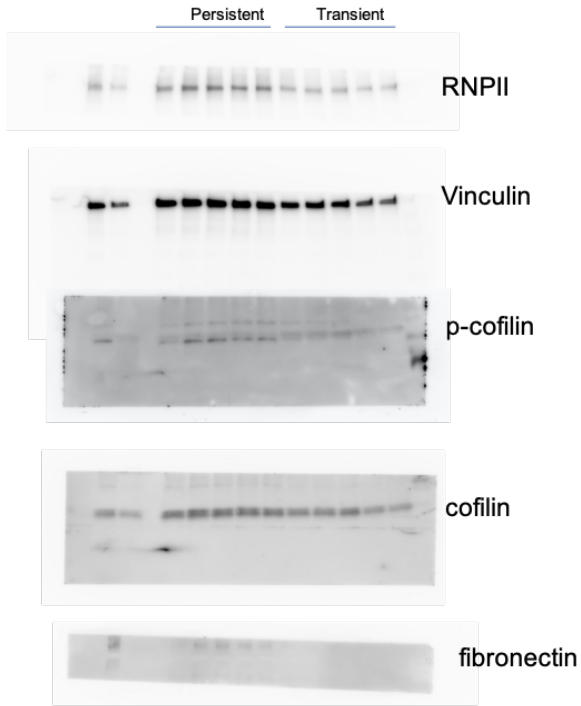
**Supplementary Figure 16:** Entire western blots for Figure 6 (a), Supplementary Figure 2 (b), and Supplementary Figure 14 (c). Cropped images used in figures outlined in red. RNA polymerase II (RNPeII).

SI Fig. 10 – set 1

Persistent    Transient

RNPII

Vinculin

p-cofilin

cofilin

fibronectin

SI Fig. 10 – set 2

RNPII

Vinculin

pERM

p-cofilin

**Supplementary Figure 17:** Entire western blots for Supplementary Figure 10.

**Supplemental Table 1**

| GENE | FORWARD PRIMER (5'-3') | REVERSE PRIMER (5'-3') |
|---|---|---|
| RPL30 | AGATTTCCTCAAGGCTGGGC | GCTGGGGTACAAGCAGACTC |
| HDAC1 | AGTGCGGTCGTCTTACAG | CCTCCCAGCATCAACATA |
| HDAC2 | GGAACAGGAGACTTGAGGGAT | CAGCACCACATTGTAACACGAC |
| HDAC3 | GCTGCTGGACGGATGAGA | CTGGATGGAGCGTGAAGT |
| HDAC4 | TCAAGGCACCCGAGAAGA | ACGACGGAGACAAACAGACAAG |
| HDAC5 | AACTCTGTAGCCATCACAACCA | CCCTCCGCCAACCACTT |
| HDAC6 | CCGCCCGAAGTGTAAAT | AGACCTGCCAGTCATCCC |
| HDAC9 | AGCCCATCTCGCCTTTA | TTGCTGCGGTTGCTGAAT |
| HDAC10 | GCCGTCTACTTCCACCCG | GCACAACTCCCGCCATC |
| SIRT1 | CTTCAGTTGCCGAAACAGTAAGAA | CATCAAGCCGTTTACTAATCTGC |
| SIRT2 | CTTCGCCCTCGCCAAGGAACTCTA | CCGCCACTCGCTCCAGGGTGTCTA |