

Supplemental Materials

Molecular Biology of the Cell

Herum et al.

Supplementary Figure Legends

Supplementary Figure 1. (A) Smooth muscle α -actin (SMA) staining (bright nuclei in CFB on PA gels are nuclear staining of EdU from the proliferation assay). (B) Proliferation of cardiac fibroblasts (CFB) cultured on HA and PA gels with stiffness of 3kPa and 8kPa. (C) Confocal cross-section micrographs and quantification of collagen staining intensity on methacrylated hyaluronic acid (HA) gels and PA gels. Student's *t*-test showed no significant difference for proliferation and collagen coating between HA and PA gels.

Supplementary Figure 2. Paracrine signaling model. (A) Proliferation rate of cardiac fibroblast (CFB) cultures alone, or in co-culture with non-stretched cardiomyocytes (CM) or stretched CM. CFB were plated on PA gels with 3kPa stiffness. Two-way ANOVA showed significant effect of culture type*** and Tukey's post hoc test as indicated in figures. N=3-6. (B) Tissue inhibitor of metalloproteinase 1 (TIMP1), chemokine (C-X-C motif) ligand (CXCL) 1, colony stimulating factor (CSF)-1, C-C Motif Chemokine Ligand (CCL) 2, CCL3, CCL4, CXCL2, CXCL12, cluster of differentiation 54 (CD54) and interleukin-1 receptor antagonist (IL-1ra) protein expression in media of cardiac fibroblasts (CFB) co-cultured with non-stretched cardiomyocytes (CM) and stretched CM. N=1. (C) Immunofluorescent staining for smooth muscle α -actin (SMA). (D) Immunofluorescent staining for fibronectin (FN) and (E) quantification of FN staining presented as relative to controls in CFB cultures on 3 and 8kPa gels alone, or in co-culture with non-stretched and stretched CM. One-way ANOVA was used to determine significant effect of culture type. N 3-6.

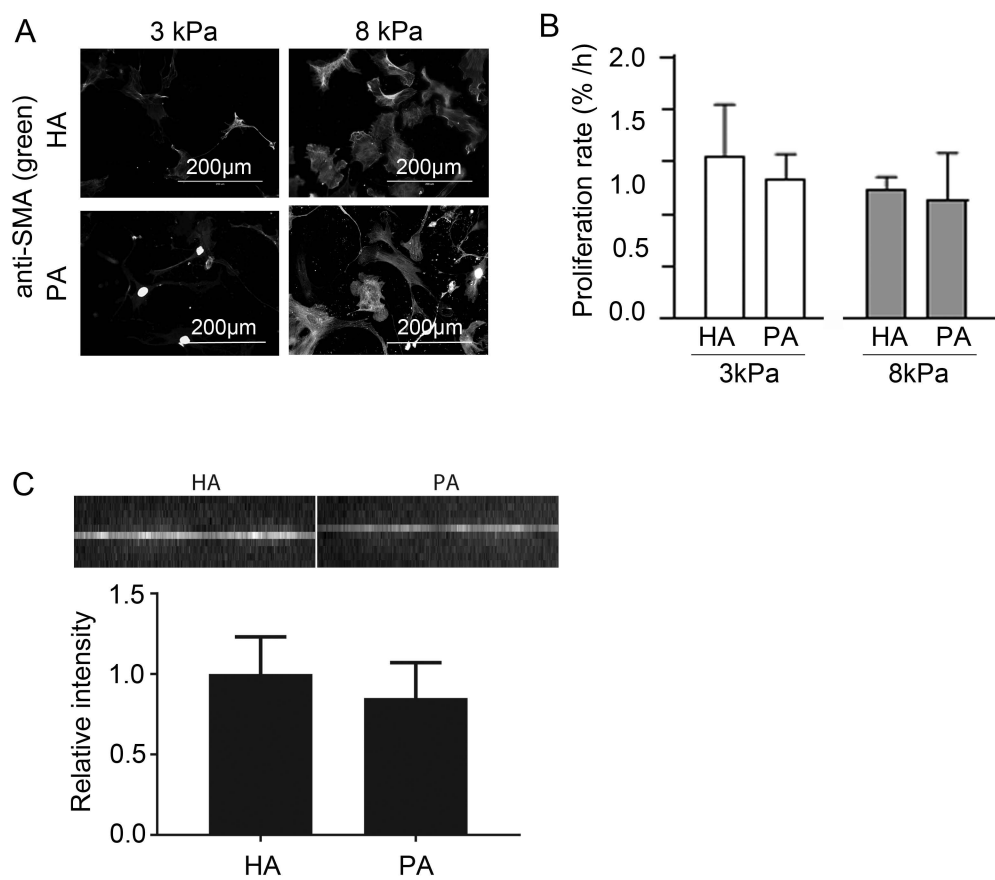
Supplementary Figure 3. Cell area after static stretch. (A) Micrograph of 2 cells before and after static stretch. Color overlay to indicate cell area. (B) Cell area for 8 cardiac fibroblasts before and after static stretch. Each line represents one cell. (C) Cell area of cardiac fibroblasts on 3 and 8kPa PA gel controls (ctrl) and after 24h static stretch.

Supplementary Figure 4. Transforming growth factor β (TGF β) mRNA (A), TGF β activity (B) and fibronectin EDA mRNA (C) and integrin α v (Itg α v; E) mRNA normalized to GAPDH in control (C) or stretched (S) cardiac fibroblasts on 3 and 8kPa PA gels. (D) Immunostaining micrographs and quantification for fibronectin (FN) on 3 and 8kPa PA gels.

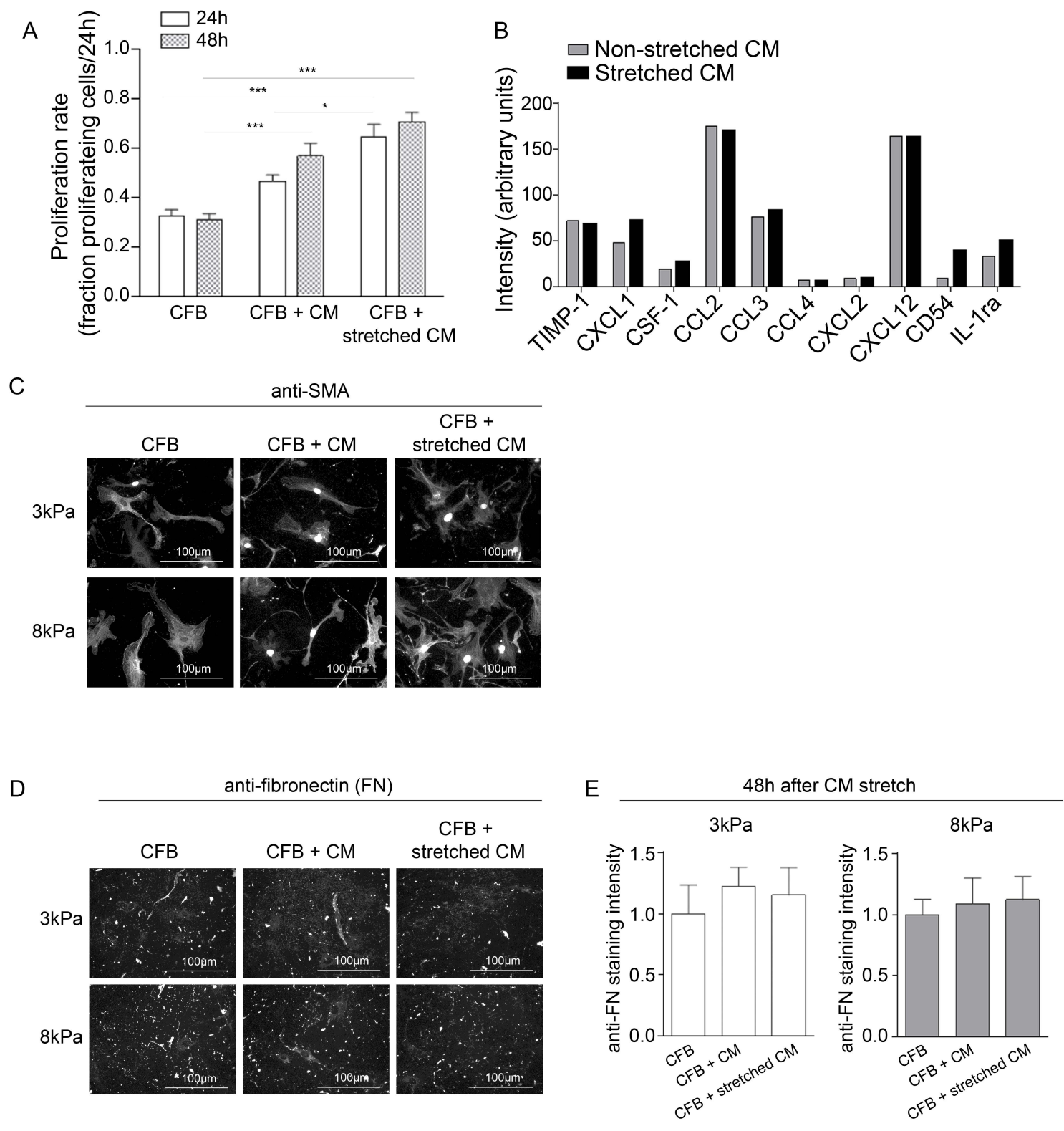
Supplementary Figure 5. UV light exposure of the duration of 3.5min does not cause damage to cardiac fibroblasts. (A) Immunostaining for p53 protein after exposure to UV light in the presence or absence of the photoinitiated cross-linker Irgacure. No primary antibody (No 1^oAB) was used as negative control and 15min UV exposure was used as positive control. (B) Live-dead stain showing nuclei of live cells in blue and dead cells in green.

Supplementary Figure 6. Matrix stiffening model. (A) Immunofluorescent staining for smooth muscle α -actin (SMA; green) and phalloidin staining for F-actin (red). mRNA of transforming

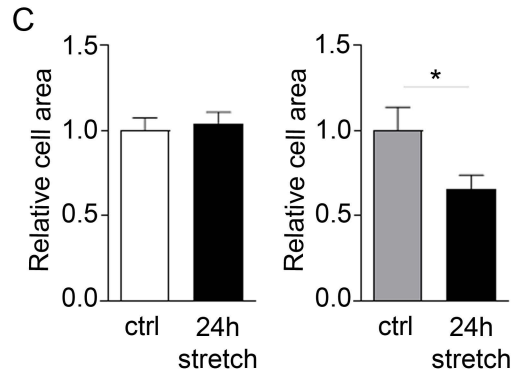
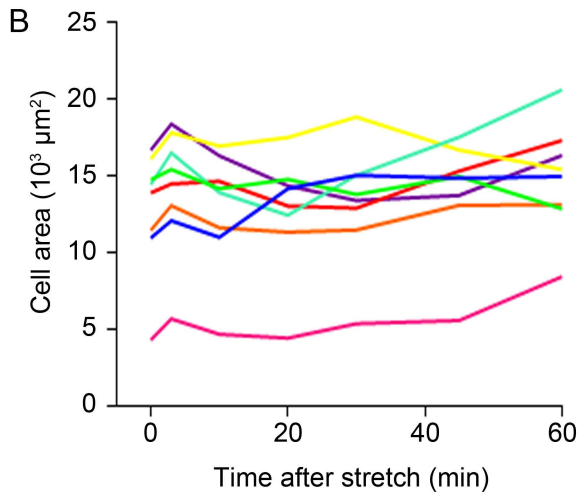
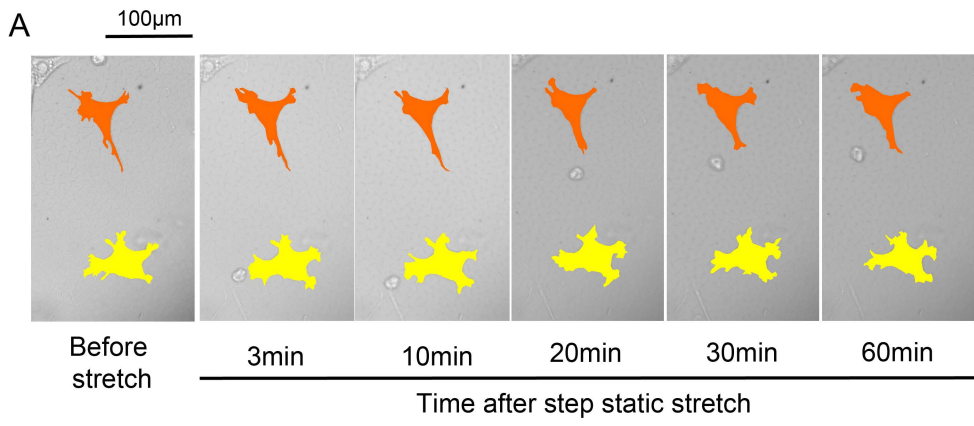
growth factor β (TGF β) and fibronectin extradomain A (EDA) in (B) and tenascin C (TNC), secreted protein acidic and rich in cysteine (SPARC), periostin (POSTN), thrombospondin 1 (THBS1) and metalloproteinase (MMP) 2, bone morphogenetic protein 1 (BMP1), tissue inhibitor of metalloproteinase (TIMP) 1 and 2 in (D) normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA in cardiac fibroblasts on 8kPa gels stiffened to 30kPa. Relative lysyl oxidase (LOX) activity (C) and proliferation (E) of CFB on 8kPa and stiffened gels. Student's *t*-tests were used to determine significant changes. N=4-12.



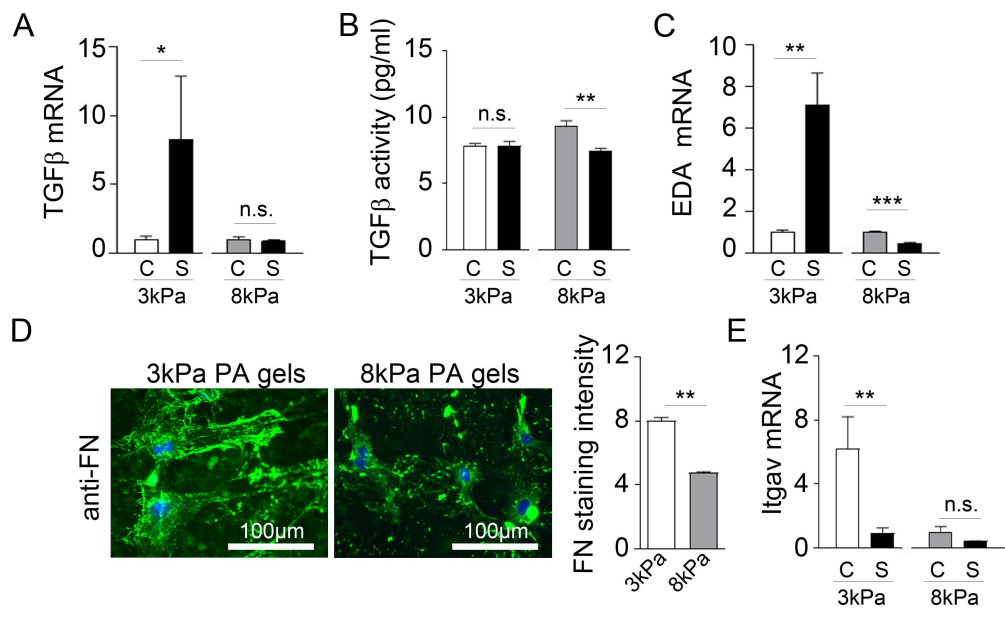
Supplementary Figure 1.



Supplementary Figure 2.

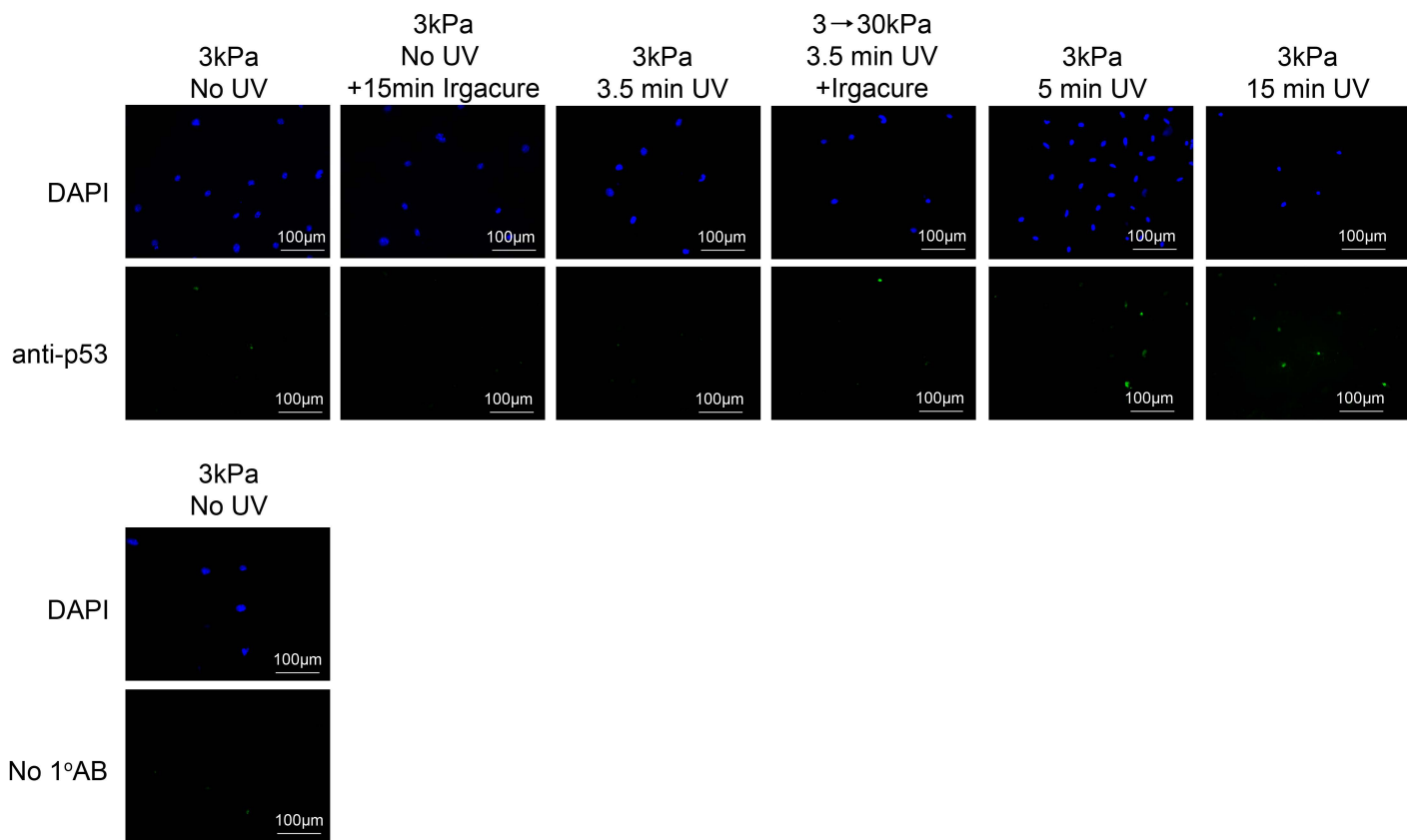


Supplementary Figure 3.

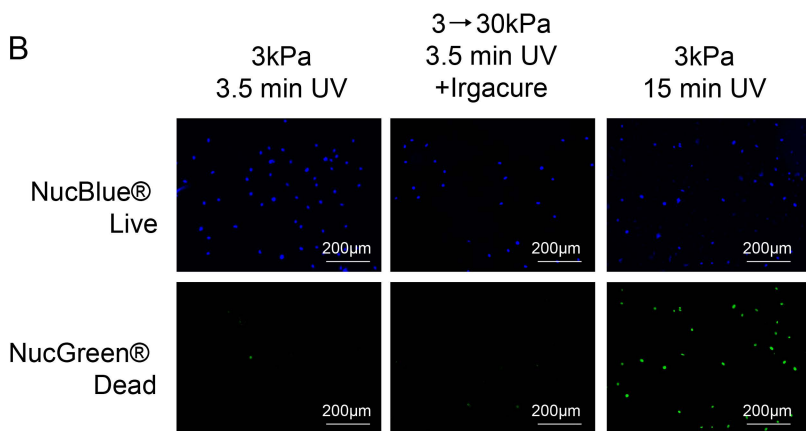


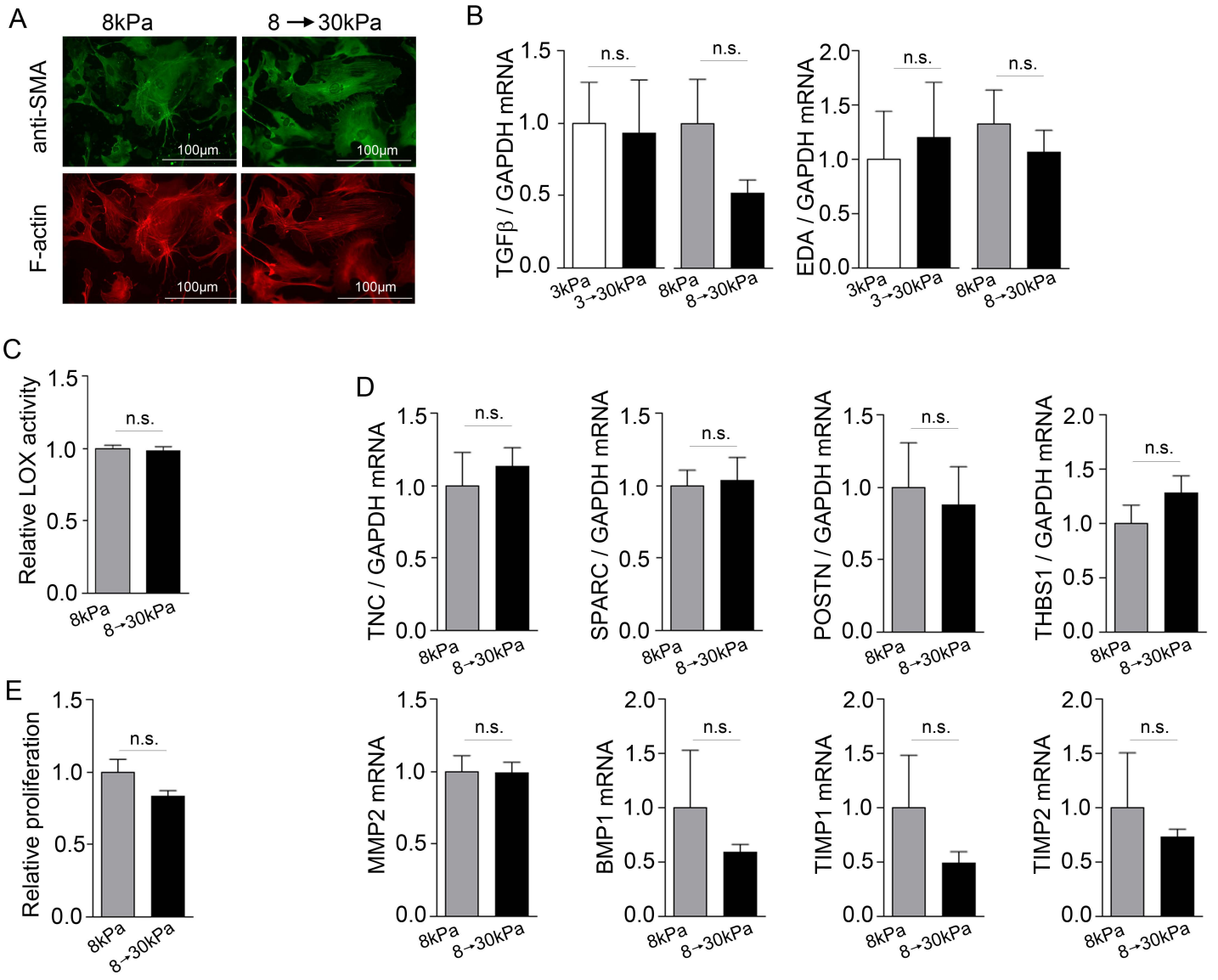
Supplementary Figure 4.

A



B





Supplementary Figure 6.

Supplementary Table 1. List of primer oligo names, gene names and primer oligo sequences.

Oligo Name	Gene name	Oligo Sequence (5' - 3')
EDA-F1	Fibronectin extradomain A	TGGGTGTCACCTGACTGAAC
EDA-R1	Fibronectin extradomain A	AGTCCCTACGAGGACCATCC
COL1A1-F1	Collagen1a1	GCTCCTCTTAGGGGCCACT
COL1A1-R1	Collagen1a1	CCACGTCTCACCATTGGGG
COL1A2-F1	Collagen 1a2	GGTGAGCCTGGTCAAACGG
COL1A2-R1	Collagen 1a2	ACTGTGTCCTTTCACGCCTTT
COL3A1-F1	Collagen 3a1	CTGTAACATGGAAACTGGGGAAA
COL3A1-R1	Collagen 3a1	CCATAGCTGAACTGAAAACCACC
OPN-F1	Osteopontin	AGCAAGAAACTCTTCCAAGCAA
OPN-R1	Osteopontin	GTGAGATTCGTCAGATTCATCCG
FN1-F1	Fibronectin	ATGTGGACCCCTCCTGATAGT
FN1-R1	Fibronectin	GCCCAGTGATTTACAGCAAAGG
POSTN-F1	Periostin	CACGGCATGGTTATTCCCTTCA
POSTN-R1	Periostin	TCAGGACACGGTCAATGACAT
THBS1-F1	Thrombospondin 1	GTGAGGTTTGTCTTTGGAACCA
THBS1-R1	Thrombospondin 1	GTTGTTGTCAAGGGTAAGAAGGA
TNC-F1	Tenascin C	GCATCCGTACCAAAACCATCA
TNC-R1	Tenascin C	AACCCGTAGGGATTAGTGTCG
SPARC-F1	Secreted protein acidic and rich in cysteine	GTGGAAATGGGAGAATTTGAGGA
SPARC-R1	Secreted protein acidic and rich in cysteine	CTCACACACCTTGCCATGTTT
LOX-F1	Lysyl oxidase	TCTTCTGCTGCGTGACAACC
LOX-R1	Lysyl oxidase	GAGAAACCAGCTTGGAACCAG
MMP2-F1	Matrix metalloproteinase 2	CAAGTTCCCCGCGCATGTC
MMP2-R1	Matrix metalloproteinase 2	TTCTGGTCAAGGTCACCTGTC
MMP9-F1	Matrix metalloproteinase 9	CTGGACAGCCAGACACTAAAG
MMP9-R1	Matrix metalloproteinase 9	CTCGCGCAAGTCTTCAGAG
MMP13-F1	Matrix metalloproteinase 13	CTTCTTCTTGTTGAGCTGGACTC
MMP13-R1	Matrix metalloproteinase 13	CTGTGGAGGTCCTGTAGACT
TGFb-F1	Transforming growth factor β	CTCCCGTGGCTTCTAGTGC
TGFb-R1	Transforming growth factor β	GCCTTAGTTTGGACAGGATCTG
CXCL1-F1	Chemokine (C-X-C motif) ligand 1	CTGGGATTCACCTCAAGAACATC
CXCL1-R1	Chemokine (C-X-C motif) ligand 1	CAGGGTCAAGGCAAGCCTC
CD54-F1	Cluster of differentiation 54	GTGATGCTCAGGTATCCATCCA
CD54-R1	Cluster of differentiation 54	CACAGTTCTCAAAGCACAGCG
IL-1ra-F1	Interleukin-1 receptor antagonist	GCTCATTGCTGGGTACTIONTACAA
IL-1ra-R1	Interleukin-1 receptor antagonist	CCAGACTTGGCACAAGACAGG
CSF1-F1	Colony stimulating factor 1	ATGAGCAGGAGTATTGCCAAGG
CSF1-R1	Colony stimulating factor 1	TCCATCCCAATCATGTGGCTA

SMA-F1	Smooth muscle α -actin	GTCCCAGACATCAGGGAGTAA
SMA-R1	Smooth muscle α -actin	TCGGATACTTCAGCGTCAGGA
PDGFb-F1	Platelet-derived growth factor B	CATCCGCTCCTTTGATGATCTT
PDGFb-F2	Platelet-derived growth factor B	GTGCTCGGGTCATGTTCAAGT
TIMP1-F1	Tissue inhibitor of metalloproteinase 1	GCAACTCGGACCTGGTCATAA
TIMP1-R1	Tissue inhibitor of metalloproteinase 1	CGGCCCGTGATGAGAACT
TIMP2-F1	Tissue inhibitor of metalloproteinase 2	TCAGAGCCAAAGCAGTGAGC
TIMP2-R1	Tissue inhibitor of metalloproteinase 2	GCCGTGTAGATAAACTCGATGTC
GAPDH-F1	Glyceraldehyde 3-phosphate dehydrogenase	AGGTCGGTGTGAACGGATTTG
GAPDH-R1	Glyceraldehyde 3-phosphate dehydrogenase	TGTAGACCATGTAGTTGAGGTCA
FGF2-F1	Fibroblast growth factor 2	GCGACCCACACGTCAAATA
FGF2-r1	Fibroblast growth factor 2	TCCCTTGATAGACACAACCTCCTC
BMP1-F1	Bone morphogenetic protein 1	TTGTACGCGAGAACATACAGC
BMP1-R1	Bone morphogenetic protein 1	CTGAGTCGGGTCCTTTGGC