ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.,* DOI: 10.1002/adhm.202100315

Stabilization of damaged cartilage with hydrogel-mediated reinforcement and sealing

*Jay M Patel, Claudia Loebel, Kamiel S Saleh, Brian C Wise, Edward D Bonnevie, Liane M. Miller, James L Carey, Jason A Burdick, Robert L Mauck**

WILEY-VCH

Supporting Information

Title: Stabilization of damaged cartilage with hydrogel-mediated reinforcement and sealing

*Jay M Patel, Claudia Loebel, Kamiel S Saleh, Brian C Wise, Edward D Bonnevie, Liane M Miller, James L Carey, Jason A Burdick, Robert L Mauck**

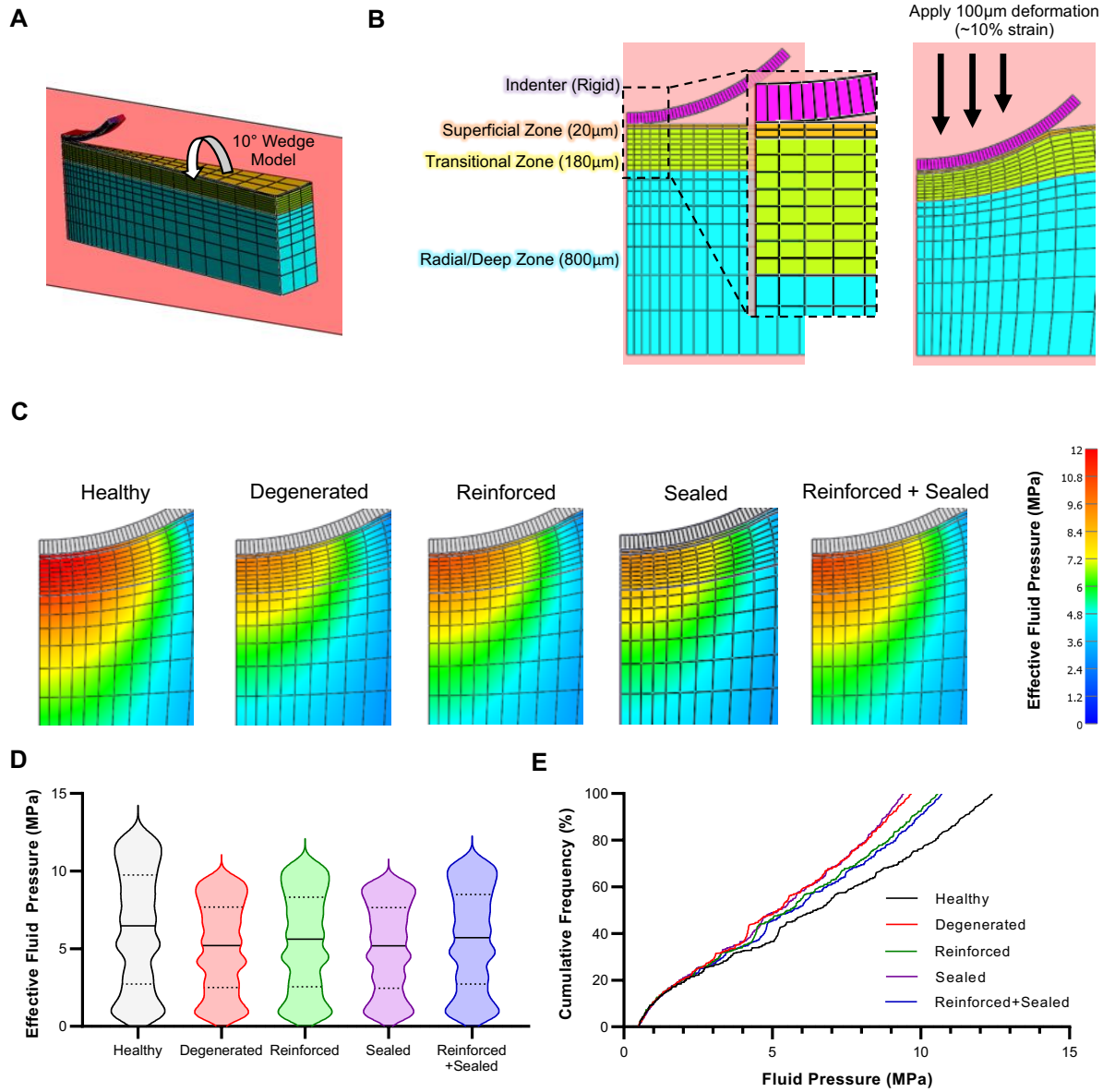

Figure S1. Finite element modeling of stabilized cartilage. A) 3D rendering of cartilage indentation model with 10° wedge. **B)** FE model components, including the rigid indenter, and 3 layers of cartilage: superficial, transitional, and radial/deep zone cartilage. The indenter was displaced to apply 100μm of deformation. **C)** FE simulations of five conditions: healthy with a superficial layer, degenerated defect, a reinforced degenerated defect, a degenerated defect sealed with a superficial layer, and a degenerated defect reinforced and sealed with a superficial layer. Color bar range = 0.0 – 12.0MPa **D)** Distribution of effective fluid pressure from single simulation, represented as violin plot (solid line = median, dashed lines = quartiles) and **E)** cumulative distribution plot of effective fluid pressure in the five conditions.

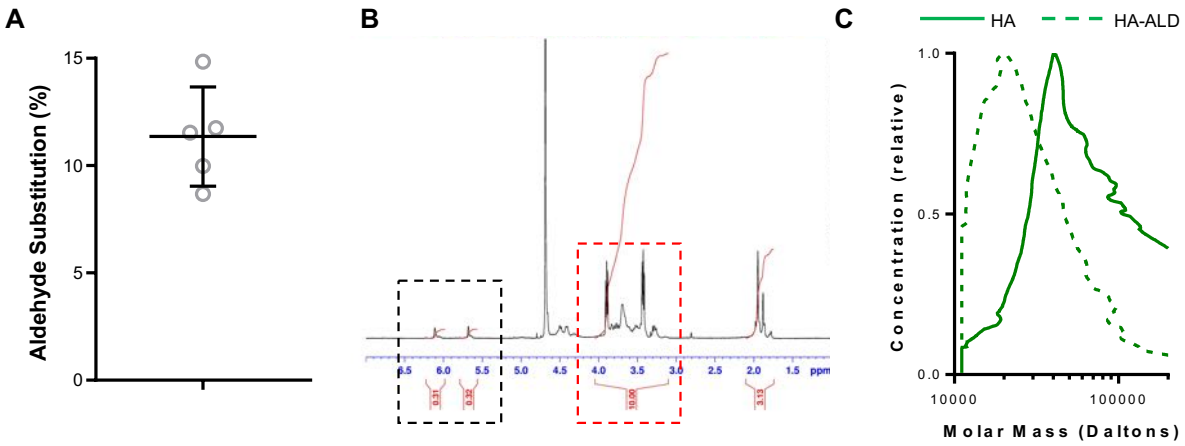

Figure S2. HA modification. A) Aldehyde substitution percentage of five individual samples. Mean ± standard deviation. **B)** Nuclear magnetic resonance of methacrylated hyaluronic acid reveals methacrylation degree of 32%. Methacrylation degree is determined by integration of the vinyl singlets (black dashed box; 1H each) relative to the sugar ring of the hyaluronic acid (red dashed box; 10H) **C)** Molar mass profile (via size exclusion chromatography [SEC-MALS]) shows decrease in molecular weight due to oxidation.

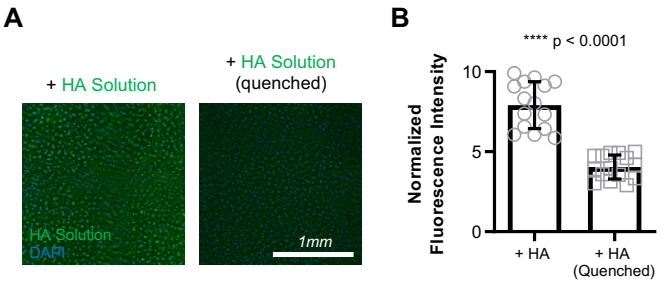

Figure S3. HA therapy attaches to damaged cartilage. A) Adherence of HA solution to cartilage (cartilage cell nuclei visualized with DAPI), observed via fluorescent intensity (fluoresceine; green), is reduced upon aldehyde quenching, prior to HA application to cartilage, with tert-Butyl carbazate. **B)** Normalized fluorescent intensity of HA solution adhered to cartilage, without and with aldehyde quenching. Mean ± standard deviation, n = 15 images per group.

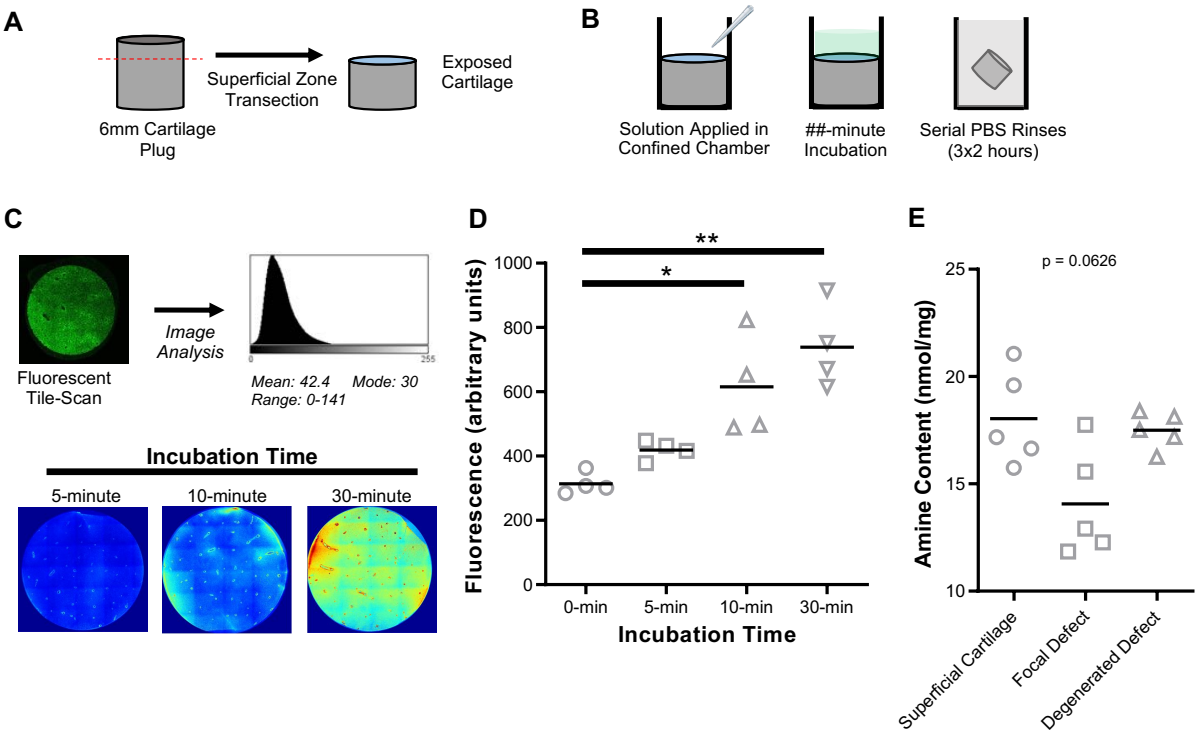

Figure S4. Time-dependence of attachment of aldehyde-substituted HA to cartilage. A) Schematic depicting removal of superficial zone to expose "damaged" cartilage. **B)** Schematic depicting confined application of modified HA to the cartilage defect surface (no light exposure for photocrosslinking), followed by incubation and serial saline rinsing. **C)** Top: Cartilage plugs were imaged with fluorescent tile scans and intensity was quantified. Bottom: Sample intensity profiles on plugs with 5, 10, and 30 minutes of incubation. **D)** Fluorescence (fluoresceine-conjugated HA) versus incubation time. Horizontal line depicts mean, n=4 per group, * and ** represent $p < 0.05$ and 0.01, respectively. **E)** Amine content of cartilage tissue was generally determined, in nmol/mg of tissue, for superficial cartilage, cartilage from a simulated focal defect (non-digested), and cartilage from a simulated degenerated defect (digested). Horizontal line depicts mean, n=5 per group.

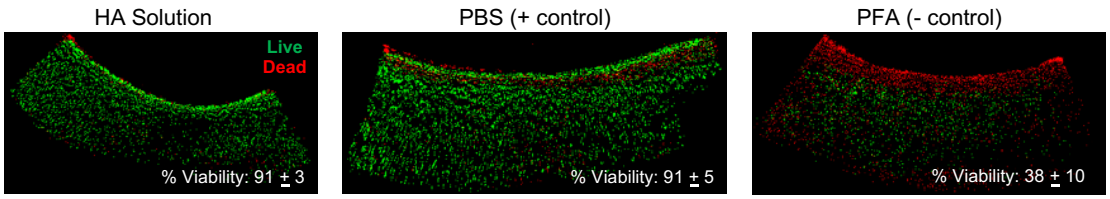

Figure S5. Cyto-compatibility of aldehyde-substituted HA. Cartilage explants incubated with either HA therapy solution (no light exposure), phosphate buffered saline (PBS; positive control), or paraformaldehyde (PFA; negative control) were stained for live (green; Calcein AM) and dead (red; ethidium homodimer-1). Percent viability reported in white text; mean ± standard deviation.

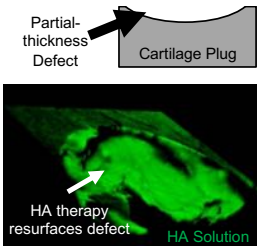

Figure S6. HA therapy resurfaces a simulated partial thickness defect. (Top) Schematic showing partial thickness defect on cartilage plug, which was created with a surgical curette. (Bottom) Localized HA therapy application (5 minutes) and light exposure (320-400nm, ~5mW/cm^2, 15 minutes) allows HA (shown in green) to resurface the cartilage defect, with little to no fluorescence on the surrounding surfaces.

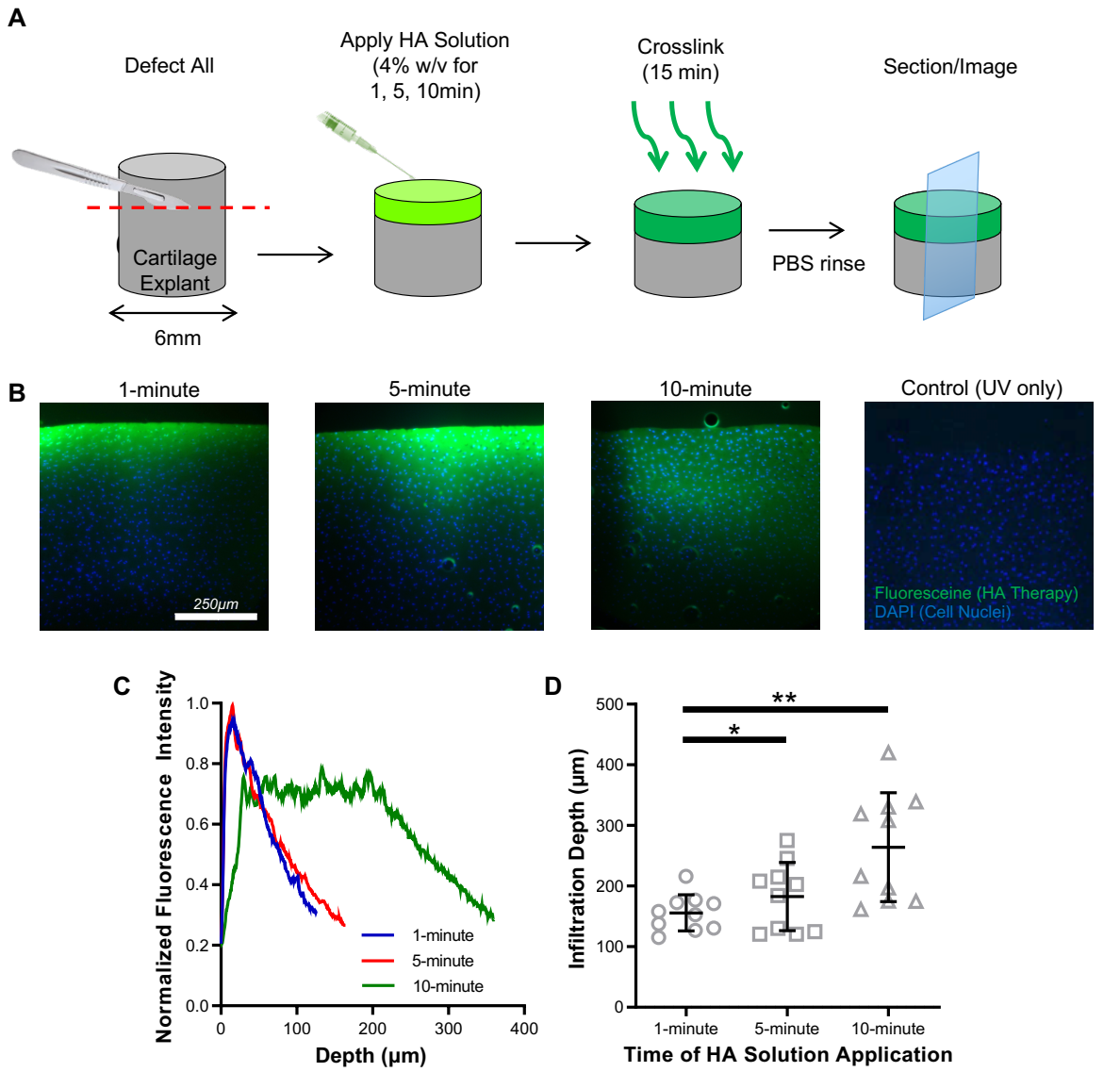

Figure S7. HA solution infiltrates into cartilage prior to crosslinking. A) Cartilage explants are defected by removing superficial zone, subject to HA solution application (4% w/v) for varying amounts of time, crosslinked (320-400nm wavelength, ~5mW/cm^2 for 15 minutes), and sectioned/imaged. **B)** Sample cross-sectional images of HA therapy infiltration into cartilage for 1, 5, and 10 minutes of application. Scale bar = 250μm. Control plug, with UV cross-linking only, also shown. **C)** Fluorescence intensity profile, normalized to maximum intensity, as a function of depth from the cartilage surface. **D)** Infiltration depth with time of HA therapy application. mean ± standard deviation, n=10 per group. * and ** represent $p < 0.05$ and 0.01, respectively.

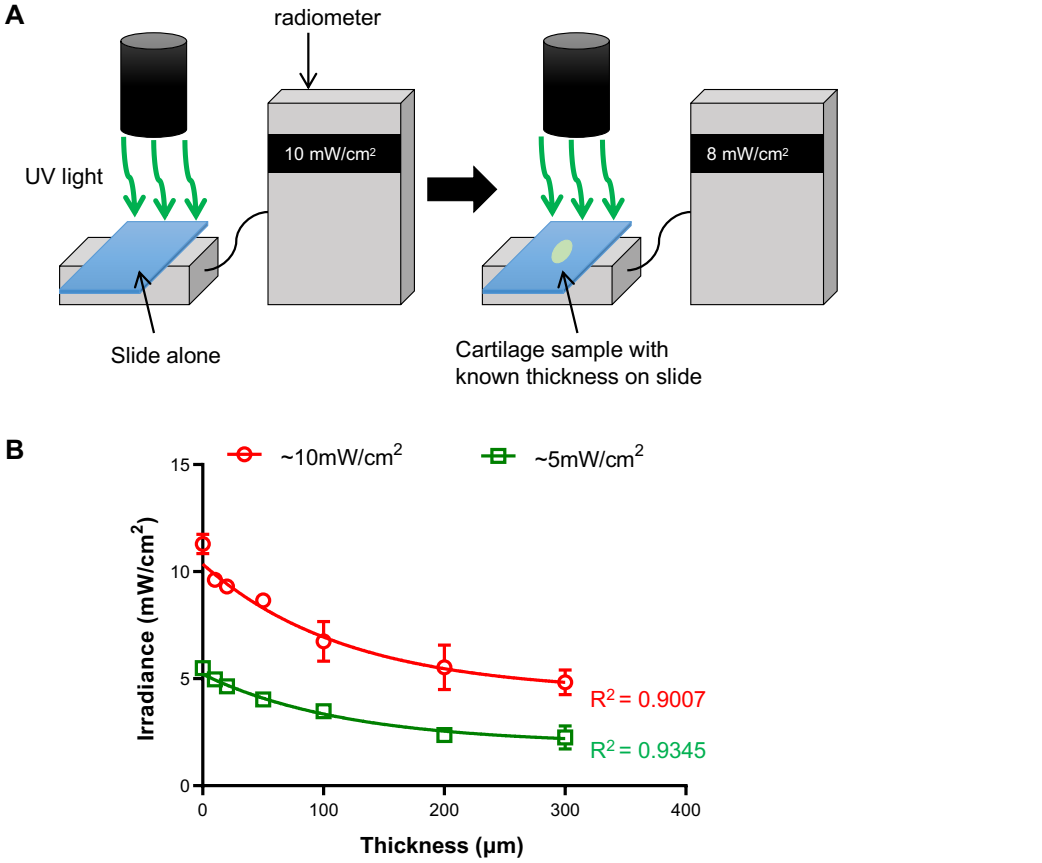

Fig S8. Irradiance through cartilage tissue. A) Ultraviolet (UV) light measured with radiometer, and measured again with varying thickness of cartilage tissue between light source and radiometer. **B)** Irradiance measured cartilage of varying thickness, with starting intensities of UV light of ~5 (green) and ~10 (red) mW/cm^2. mean $\pm$ standard deviation, n=3-10 per thickness per group. One-phase exponential decay regression analysis.

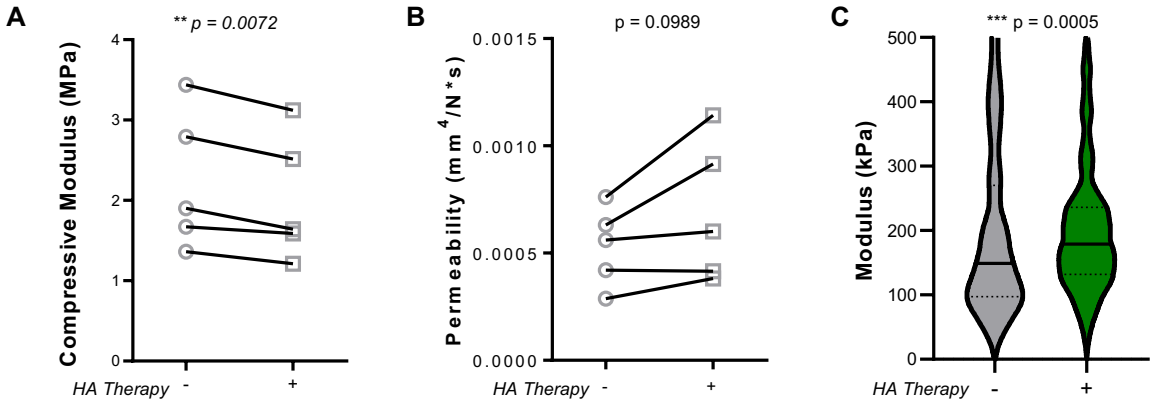

Figure S9. Biomechanics of cartilage with focal defects reinforced with HA therapy. A) Focal defect compressive modulus (MPa) and **B)** permeability ($mm^4/N*s$) of cartilage explants before (-) and after (+) HA solution application (5 minutes) and 15-minute crosslinking (320-400nm, ~$5mW/cm^2$). Matched samples connected by line. mean ± standard deviation, n=5 samples per group. **C)** Micro-scale modulus measured by nanoindentation in focal defect cartilage. n > 400 measurements (n=5 specimens per group).

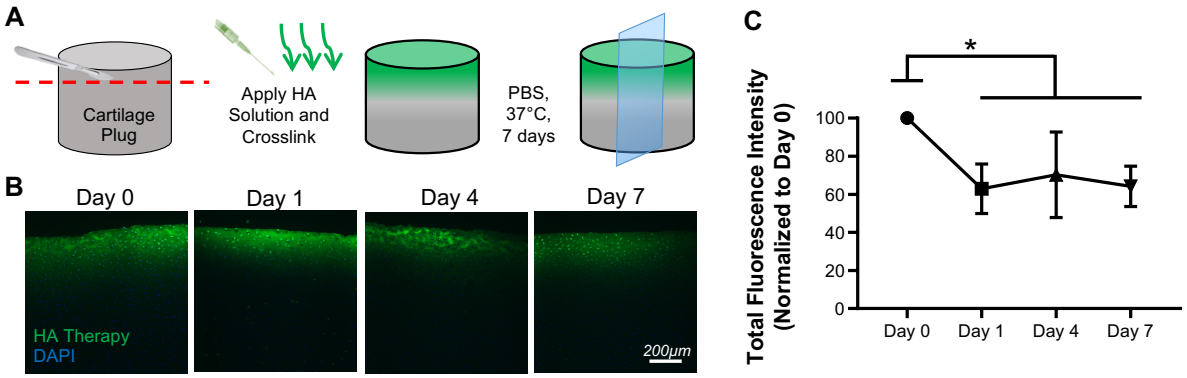

Figure S10. HA therapy retention on *in vitro* cartilage explants. A) Schematic of explant retention study. Cartilage plugs were retrieved from juvenile bovine trochlea, and superficial cartilage was transected. Plugs were then subject to HA application (5 minutes) and crosslinking via light exposure (320-400nm, ~5mW/cm^2, 15 minutes). **B)** Cross-sectional profile of HA therapy (green) retained within cartilage at 1, 4, and 7 days after application and crosslinking. **C)** Total fluorescent intensity of retained HA therapy. mean ± standard deviation, n = 4 per time point, normalized to Day 0. * denotes $p < 0.05$.

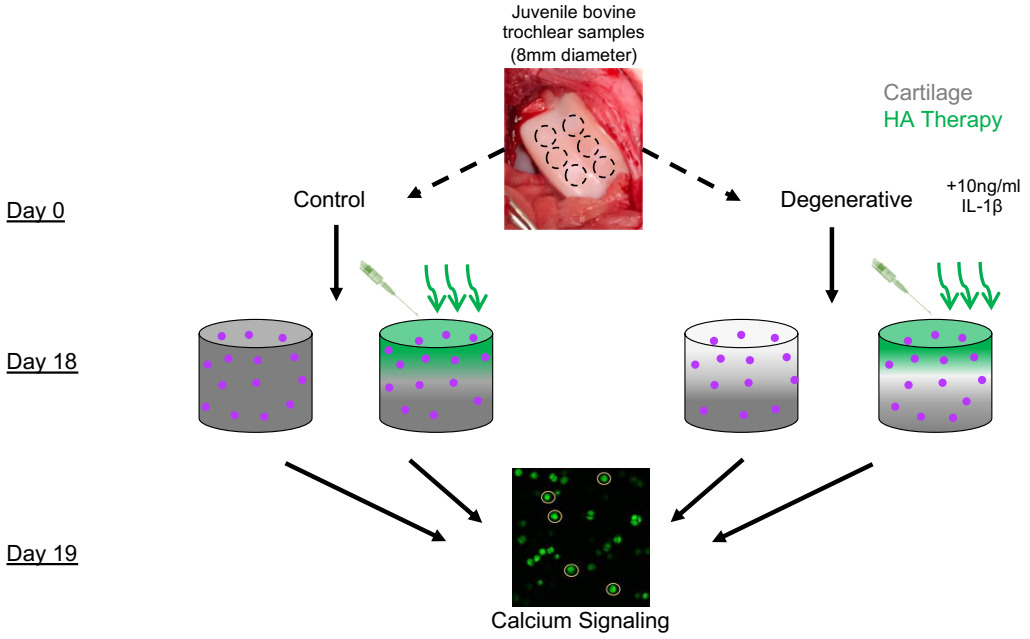

Figure S11. Methods for calcium signaling in cartilage explants. Cartilage explants are retrieved from juvenile bovine trochleas, transected to remove superficial cartilage, and cultured in control medium or degenerative medium (10ng/mL IL-1β) for 18 days, followed by HA solution application (5 minutes) and crosslinking (320-400nm, ~5mW/cm^2, 15 minutes), and testing for calcium signaling activity.

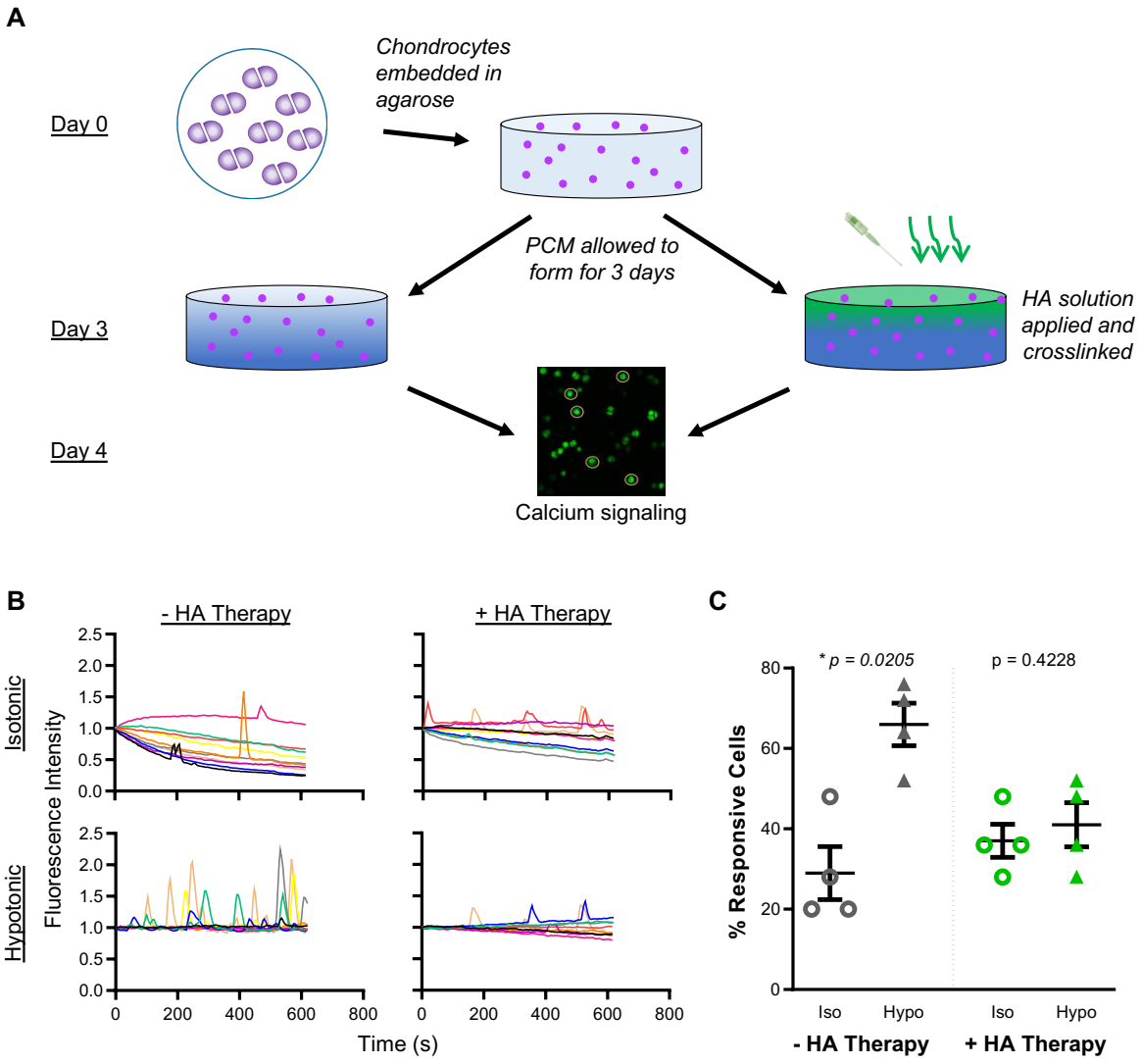

Figure S12. Calcium signaling in agarose constructs. A) Juvenile bovine chondrocytes are embedded in agarose gel, cultured in chondrogenic medium (+TGF-β3) for 3 days, followed by HA therapy (application for 5 minutes and photocrosslinking [320-400nm, ~5mW/cm², 15 minutes]) and testing for calcium signaling activity. **B)** Sample traces of fluorescent intensity of calcium flux in chondrocytes within agarose constructs. Constructs without (- HA Therapy) and with (+ HA Therapy) HA solution application and crosslinking are shown, both in the isotonic and hypotonic states. **C)** % of calcium-responsive chondrocytes in agarose constructs without and with HA Therapy. Isotonic and hypotonic conditions are shown. mean ± standard deviation. n=4 biological replicates, n >100 cells per replicate.

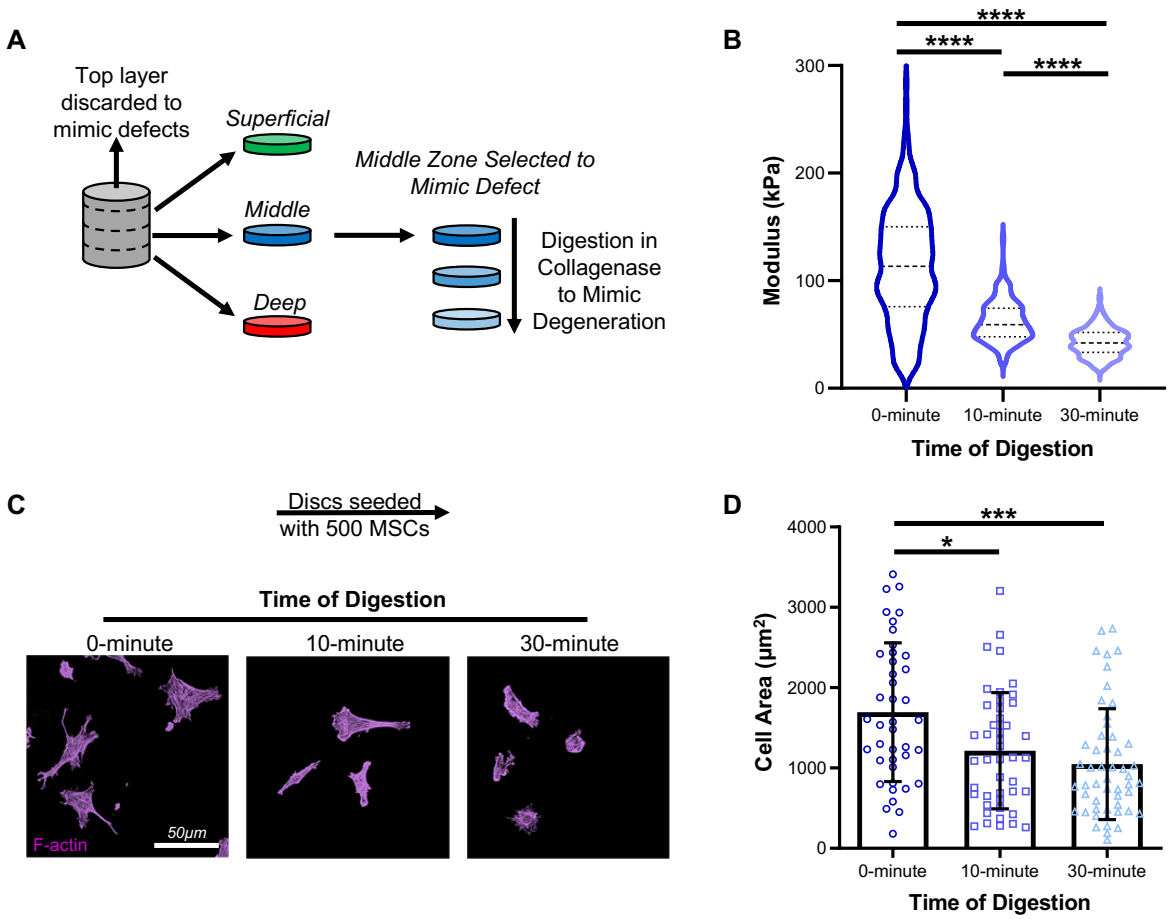

Figure S13. Cell attachment with cartilage degeneration. A) Schematic showing cartilage explants, with middle zone retrieval to mimic defects and digestion in collagenase (type IV, 0.01% w/v) to mimic degeneration. **B)** Nanoindentation modulus (kPa) of cartilage samples with varying times of digestion. Represented as violin plot, with dashed line representing median and dotted lines representing quartiles. n>220 measurements per group, n=3 replicates per group. **** represents p<0.0001. **C)** MSCs seeded on cartilage discs digested to mimic degeneration (0, 10, 30 minutes), and visualized with F-actin (magenta). t = 24 hours. Scale bar = 50μm. **D)** Cell area of MSCs on digested cartilage discs (0, 10, 30 minutes of digestion). mean ± standard deviation, n>40 cells per group, from 3 biological replicates. * and *** represent $p < 0.05$ and 0.001, respectively.

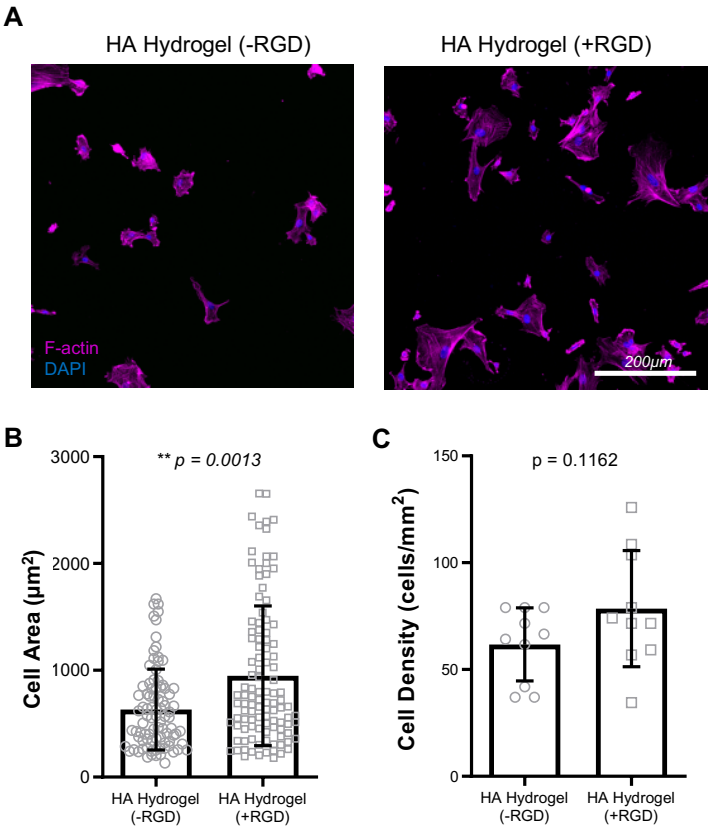

Figure S14. Hydrogels with RGD-peptide display improved cell attachment. A) MSCs cultured on HA hydrogels (MeHA-ALD), without (-) or with (+) RGD peptide. Cells visualized with F-actin (red) and nuclear (DAPI; blue) staining. t = 24 hours. Scale bar = 200μm. **B)** Cell area of MSCs on gels without (-RGD) or with (+RGD) cell-adhesive peptide. mean ± standard deviation, n>85 cells per group. **C)** Cell density (cells/mm^2) of MSCs on –RGD and +RGD gels. mean ± standard deviation, n>10 images per group.

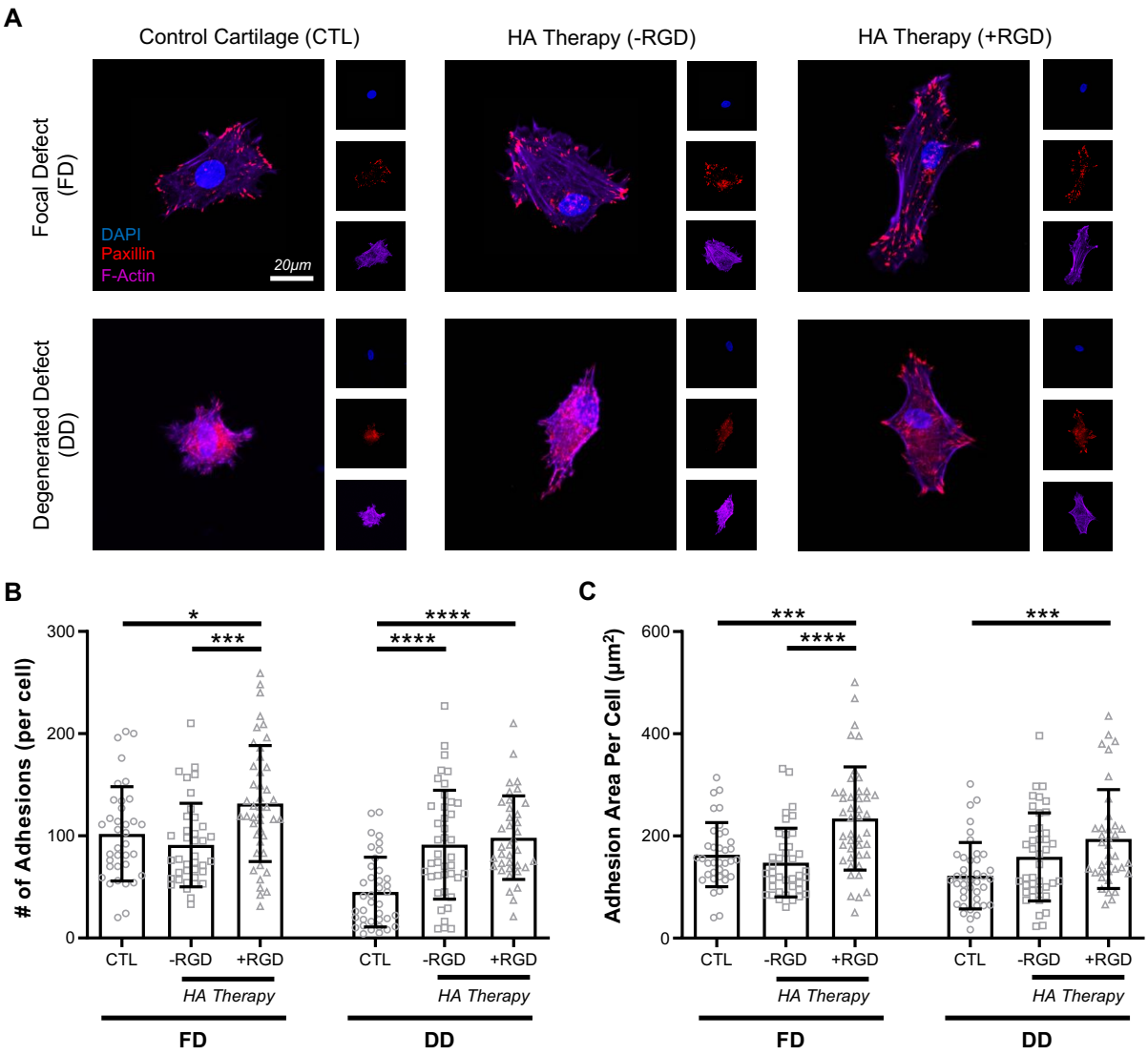

Figure S15. RGD-dependent MSC adhesion. A) MSCs on control cartilage alone (CTL; middle zone), cartilage modified with HA therapy without RGD peptide (-RGD), and cartilage modified with HA therapy (application for 5 minutes and photocrosslinking [320-400nm, ~5mW/cm², 15 minutes]) with RGD peptide (+RGD). Both focal defect (FD; non-digested) and degenerated defect (DD; digested; 0.01% w/v collagenase type IV for 30 minutes) scenarios are presented. Cells stained with F-actin (magenta), paxillin (red), and DAPI (blue). t = 24 hours. Scale bar = 20μm. **B)** Number of adhesions per cell and **C)** adhesion area per cell (μm²) of MSCs on CTL, HA Therapy (-RGD), and HA Therapy (+RGD) cartilage in both FD and DD scenarios. mean ± standard deviation, n>35 cells per group. *, ***, and **** represent $p < 0.05$, 0.001, and 0.0001, respectively.

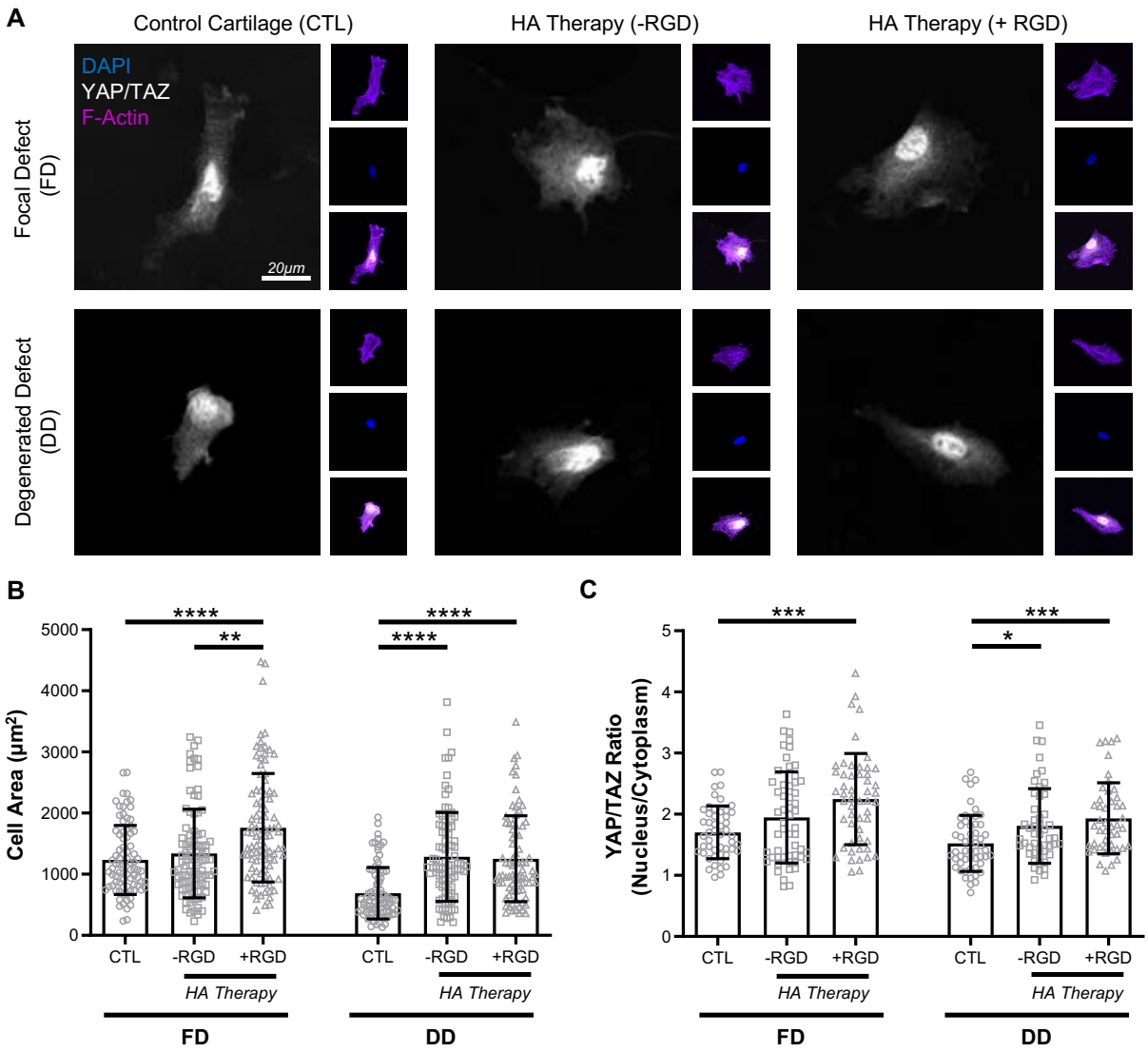

Figure S16. RGD-dependent MSC area and YAP/TAZ. A) MSCs on control cartilage alone (CTL; middle zone), cartilage modified with HA therapy (application for 5 minutes and photocrosslinking [320-400nm, ~5mW/cm², 15 minutes]) without RGD peptide (-RGD), and cartilage modified with HA therapy with RGD peptide (+RGD). Both focal defect (FD; non-digested) and degenerated defect (DD; digested; 0.01% w/v collagenase type IV for 30 minutes) scenarios are presented. Cells stained with F-actin (magenta), YAP/TAZ (white), and DAPI (blue). t = 24 hours. Scale bar = 20μm. **B)** Cell area (n>80 cells per group) and **C)** YAP/TAZ nuclear ratio (n>45 cells per group) of MSCs on CTL, HA Therapy (-RGD), and HA Therapy (+RGD) cartilage in both FD and DD scenarios. mean ± standard deviation, *, **, ***, and **** represent $p < 0.05$, 0.01, 0.001, and 0.0001, respectively.

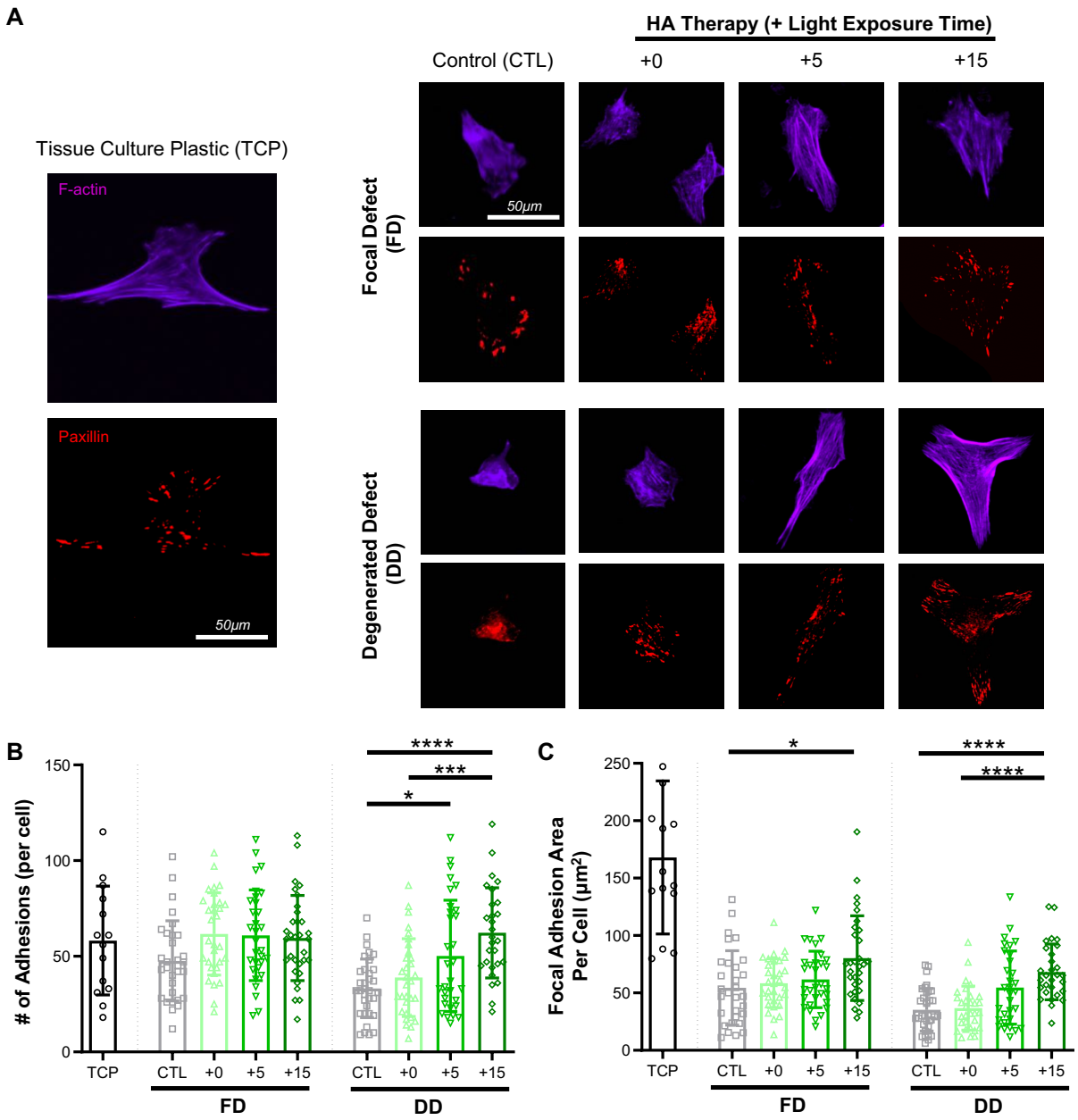

Figure S17. Crosslinking dependent MSC adhesion. A) MSCs on tissue culture plastic (TCP), cartilage alone (CTL; middle zone), and with HA therapy (application for 5 minutes) and light exposure (crosslinking; 320-400nm, ~5mW/cm^2) for 0, 5, or 15 minutes (+0, +5, +15, respectively). Both focal defect (FD; non-digested) and degenerated defect (DD; digested; 0.01% w/v collagenase type IV for 30 minutes) scenarios are presented. Cells stained with F-actin (magenta) and paxillin (red). t = 24 hours. Scale bar = 50μm. **B)** Number of adhesions per cell and **C)** focal adhesion area per cell (μm^2) of MSCs on CTL, +0, +5, +15 cartilage in both FD and DD scenarios. TCP control also shown. mean ± standard deviation, n>30 cells per group (n=14 for TCP). *, ***, and **** represent $p < 0.05$, 0.001, and 0.0001, respectively.

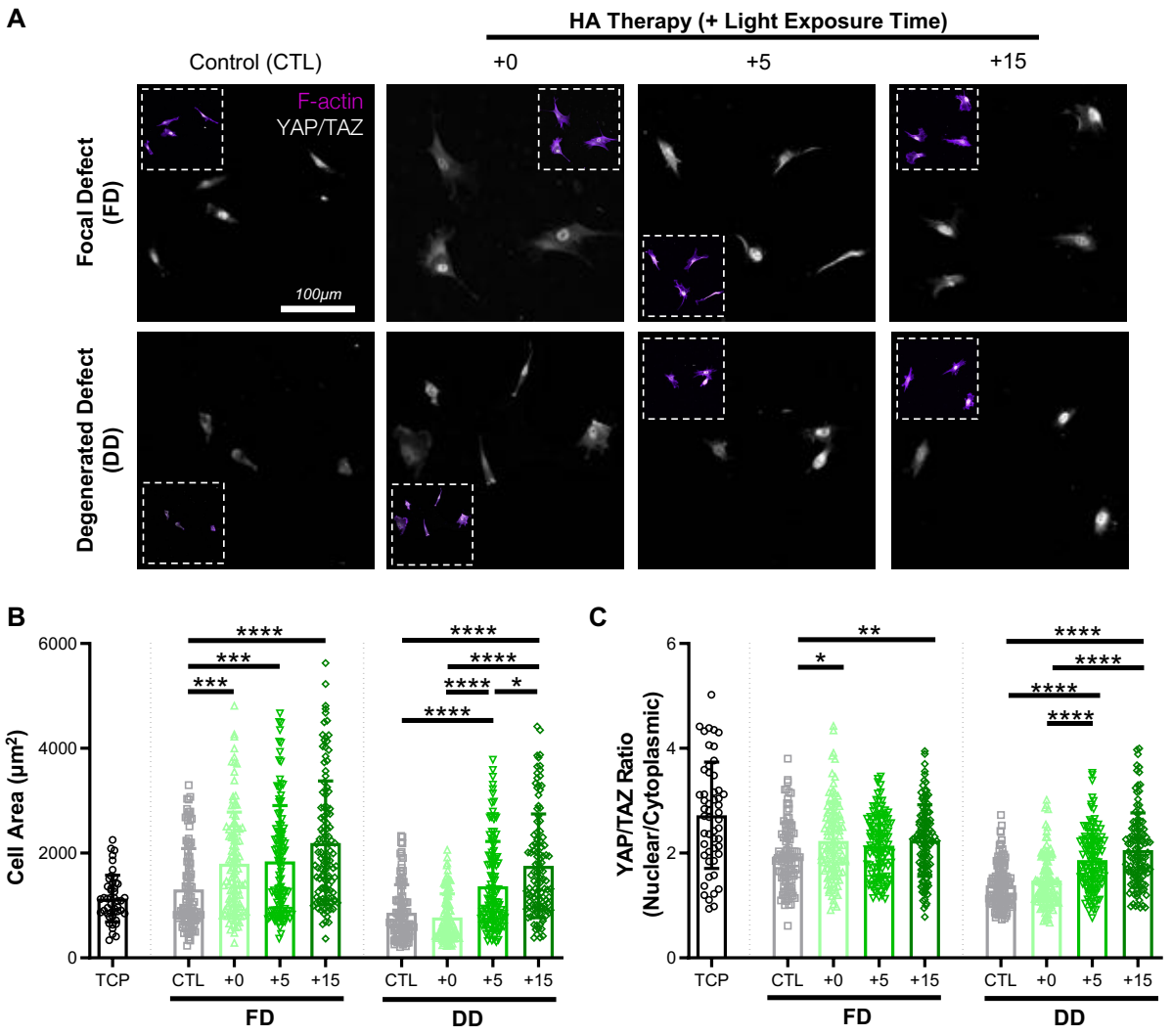

Figure S18. Crosslinking dependent MSC adhesion. A) MSCs on tissue culture plastic (TCP), cartilage alone (CTL; middle zone), and with HA therapy (application for 5 minutes) and light exposure (crosslinking; 320-400nm, ~5mW/cm^2) for 0, 5, or 15 minutes (+0, +5, +15, respectively). Both focal defect (FD; non-digested) and degenerated defect (DD; digested; 0.01% w/v collagenase type IV for 30 minutes) scenarios are presented. Cells stained with F-actin (magenta; inset) and YAP/TAZ (white), YAP/TAZ images shown, with combination as inset. t = 24 hours. Scale bar = 100μm. **B)** Cell area and **C)** YAP/TAZ ratio (nuclear to cytoplasm) of MSCs on CTL, +0, +5, +15 cartilage in both FD and DD scenarios. TCP control also shown. mean ± standard deviation, n>100 cells per group (n=47 for TCP). *, **, ***, and **** represent $p < 0.05$, 0.01, 0.001, and 0.0001, respectively.

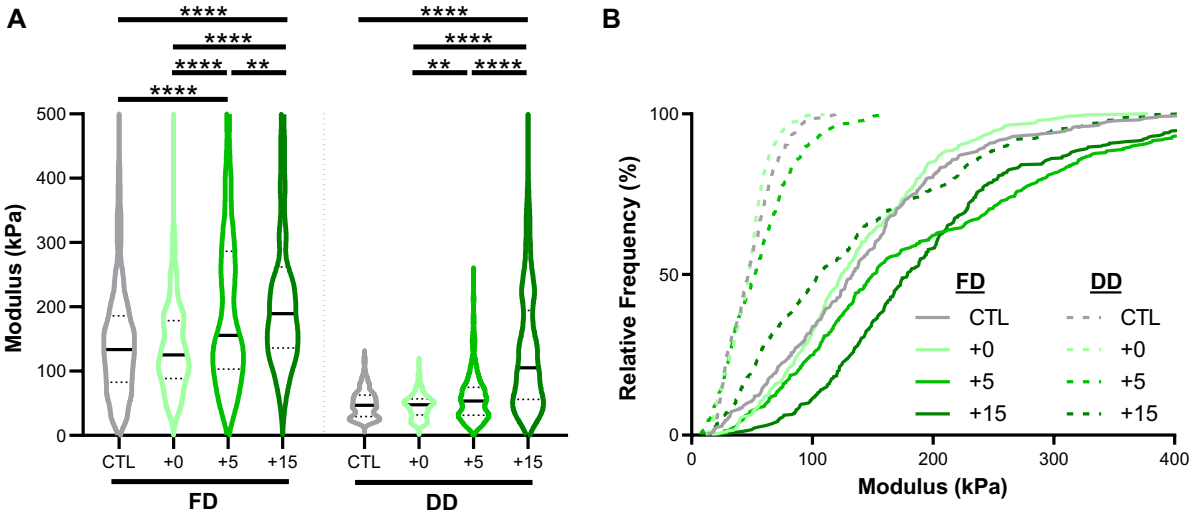

Figure S19. Crosslinking dependent micromechanics. A) Violin plot and **B)** cumulative distribution plot of Hertzian modulus via naonindentation of focal defect (FD) and degenerated defect (DD; digested in 0.01% w/v collagenase type IV for 30 minutes) cartilage, with HA therapy (application for 5 minutes) and light exposure (crosslinking; 320-400nm, ~5mW/cm^2) for 0, 5, or 15 minutes (+0, +5, +15, respectively). n>370 pooled measurements per group (n=5 samples per group). **, ***, and **** represent $p < 0.01$, 0.001, and 0.0001, respectively.

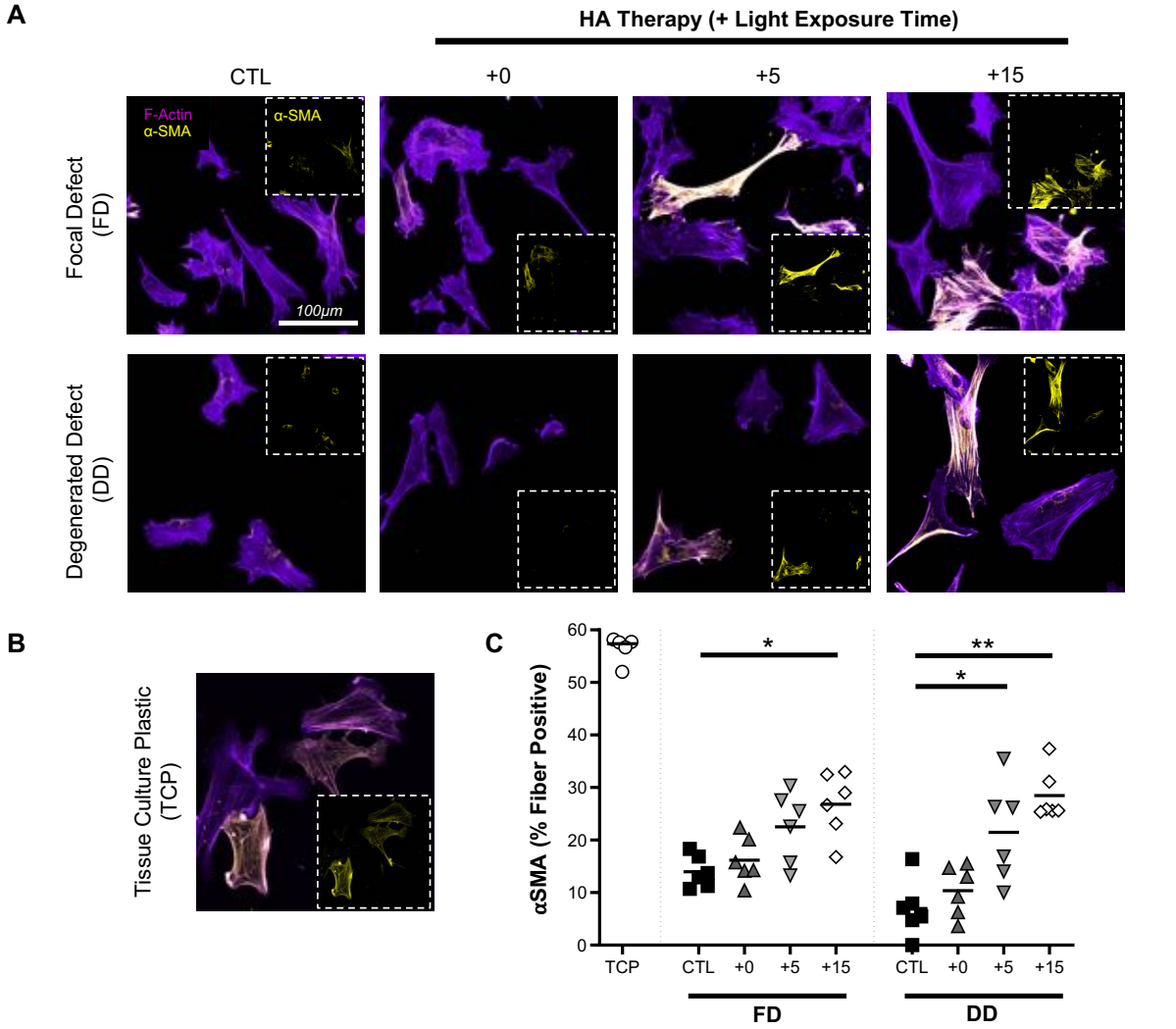

Figure S20. Crosslinking dependent pro-matrix synthesis phenotype. A) MSCs on tissue culture plastic cartilage alone (CTL; middle zone), and with HA therapy (application for 5 minutes) and crosslinking (320-400nm, ~5mW/cm^2) for 0, 5, or 15 minutes (+0, +5, +15, respectively). Both focal defect (FD; non-digested) and degenerated defect (DD; digested; 0.01% w/v collagenase type IV for 30 minutes) scenarios are presented. Cells stained with F-actin (magenta) and α-smooth muscle actin (α-SMA; yellow). Inset shows α-SMA alone. t = 7 days. Scale bar = 100μm. **B)** TCP control also shown. **C)** % of cells positive for α-SMA fibers for CTL, +0, +5, +15 groups, on both FD and DD tissues. Horizontal line depicts mean. n = 6 replicates per group, n>30 cells per replicate. * and ** represent $p < 0.05$ and 0.01, respectively.

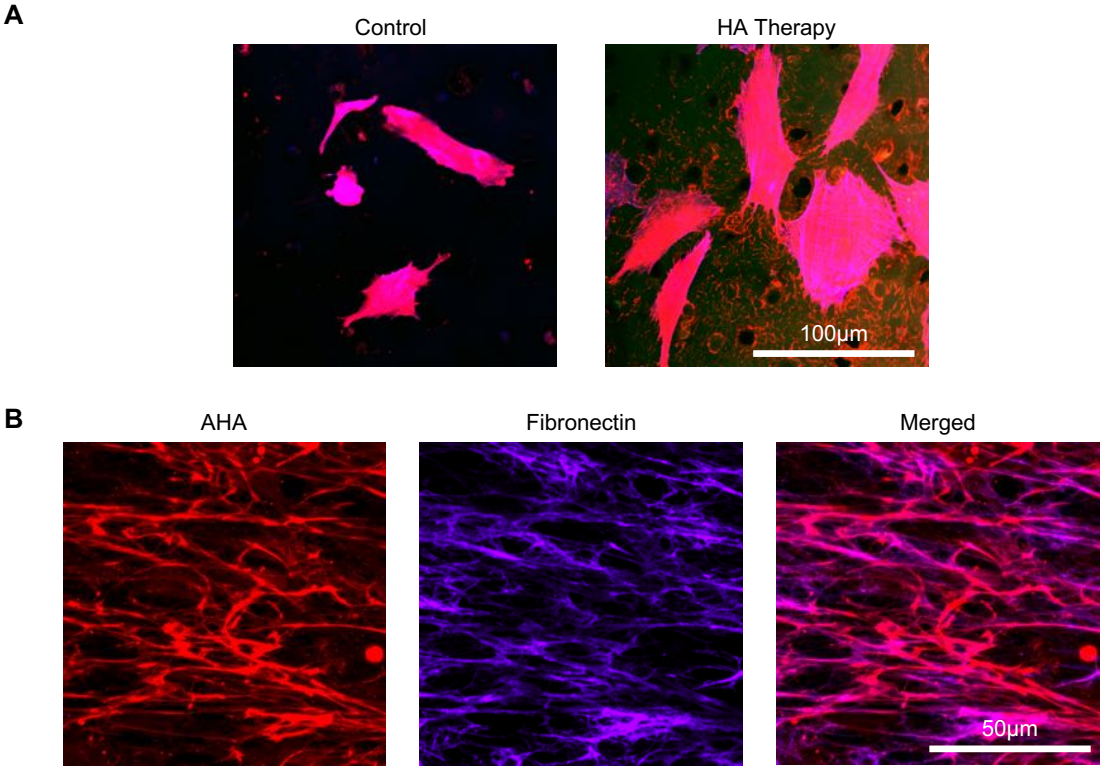

Fig S21. Nascent matrix and fibronectin staining. A) Nascent matrix deposition of single cells, visualized by staining of azidohomoalanine (AHA) of MSCs seeded on cartilage alone (CTL), and with HA therapy (5-min application, 15-minute cross-linking). Cells stained with F-actin (magenta) and DBCO-555 (AHA staining, red). t=7 days. Scale bar = 100µm. **b,** Nascent fibronectin production visualized by co-staining for AHA (red) and fibronectin (magenta). Merged image also shown. t=7days. Scale bar = 50µm.