ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Engineered Microenvironmental Cues from Fiber-Reinforced Hydrogel Composites Drive Tenogenesis and Aligned Collagen Deposition

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Supplemental Information

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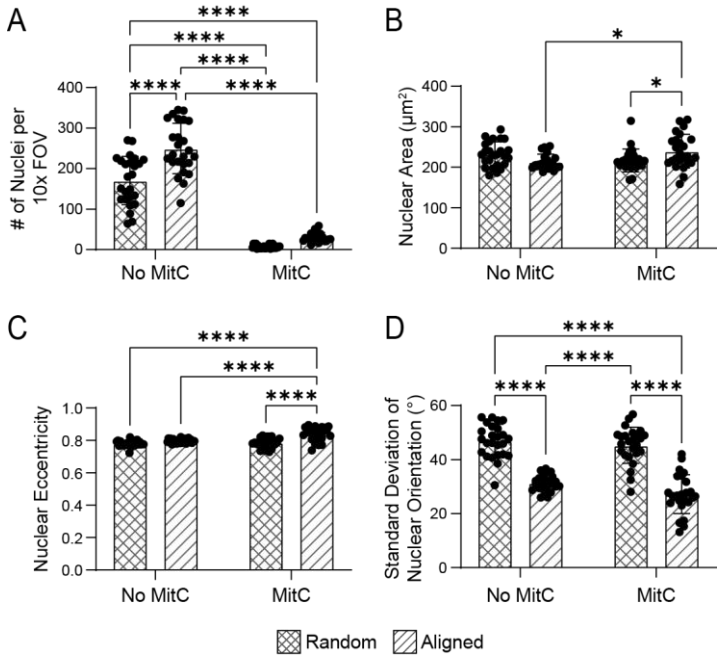


Figure S1 | Blocking cell proliferation minimally affects nuclear morphology or collective orientation. Quantifications of nuclear counts and morphology in TPCs cultured with TGF-β3 for 7 days on random and aligned, fiber-coated coverslips, with and without mitomycin C (MitC) pre-treatment. **A,** Number of nuclei per 10X FOV. **B,** Nuclear area (μm^2). **C,** Nuclear eccentricity. **D,** Standard deviation of orientation (°) of TPC nuclei. All data are reported as mean ± standard deviation, and n = 25 FOVs. Asterisks indicate statistically significant comparisons, with *p<0.05 and ****p<0.0001 by ordinary two-way ANOVA with Tukey's multiple comparisons test.

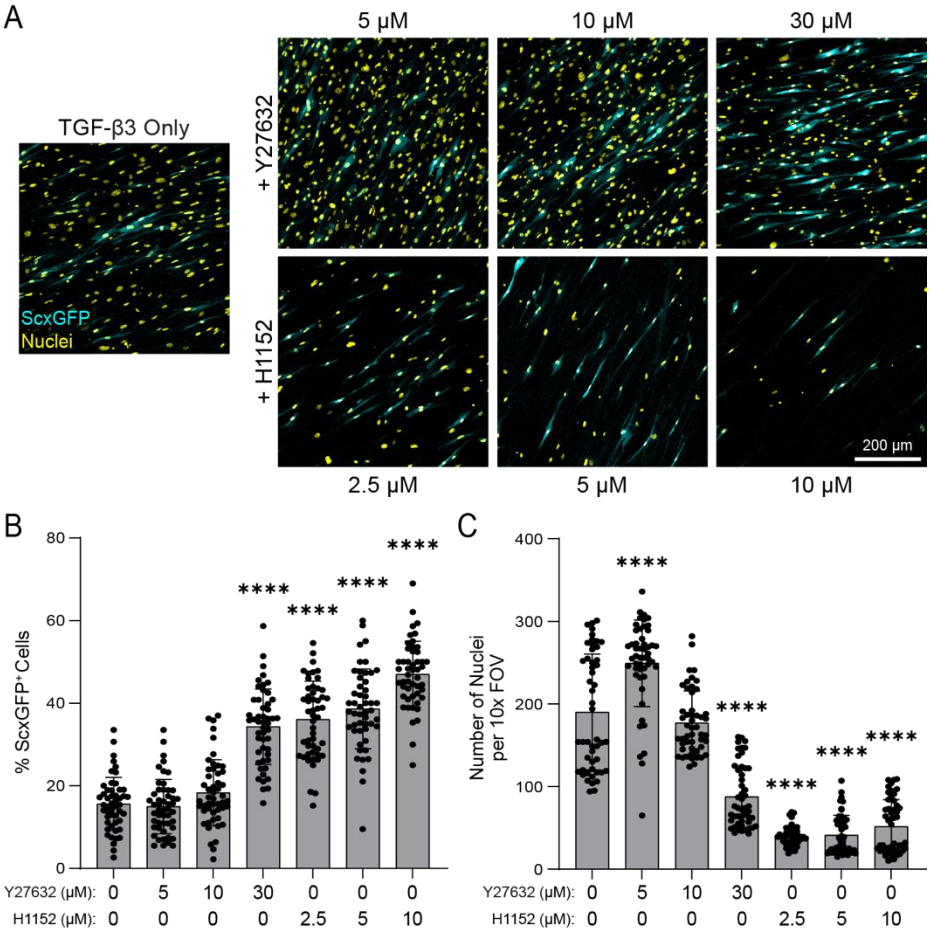


Figure S2 | Dose screens for Rho/ROCK inhibitors Y27632 and H1152. A, Fluorescent images of *ScxGFP* reporter TPCs cultured on aligned, fiber-coated coverslips for 7 days with TGF-β3, TGF-β3 and Y27632 (+Y27632), or TGF-β3 and H1152 (+H1152); inhibitors were tested at a range of doses. Quantification of (**B**) % of TPCs expressing *ScxGFP* and (**C**) number of nuclei per 10x FOV (n = 50 FOVs). All data are reported as mean ± standard deviation. Asterisks indicate statistically significant comparisons, with ****p<0.0001 by ordinary one-way ANOVA with Tukey's multiple comparisons test.

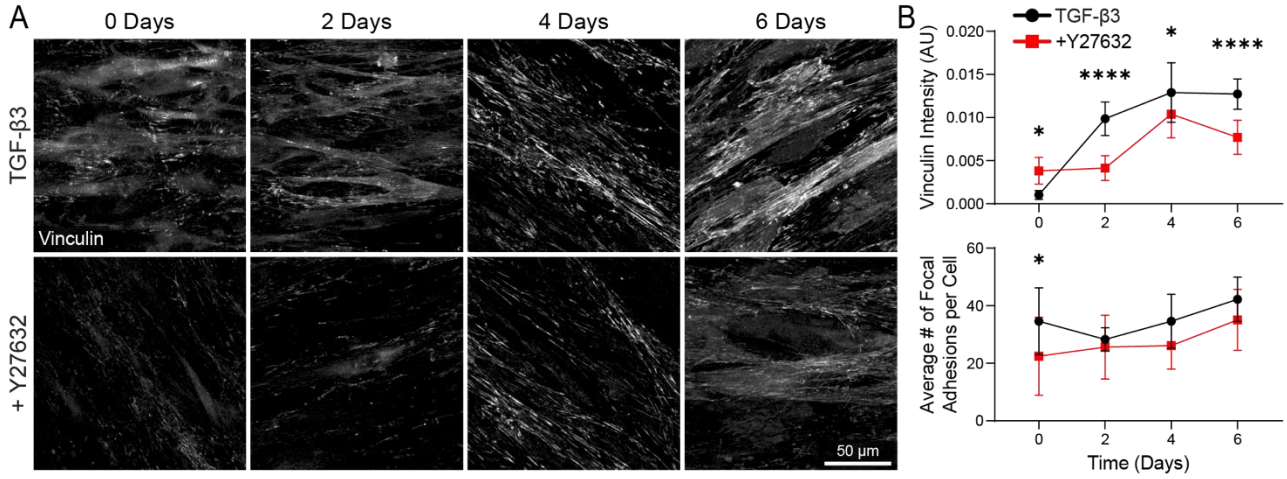


Figure S3 | Vinculin aggregation at focal adhesions during TPC tenogenesis. A, Fluorescent images of TPC cultures on aligned, fiber-coated coverslips with TGF-β3 or TGF-β3 and Y27632 (+Y27632); identical samples were fixed on days 0, 2, 4, and 6. **B,** Quantification of vinculin intensity (AU) and average number of focal adhesions (FA's) per cell (n = 10 FOVs). All data are reported as mean ± standard deviation. Asterisks indicate statistically significant comparisons, with *p<0.05 and ****p<0.0001 by Student's t-test.

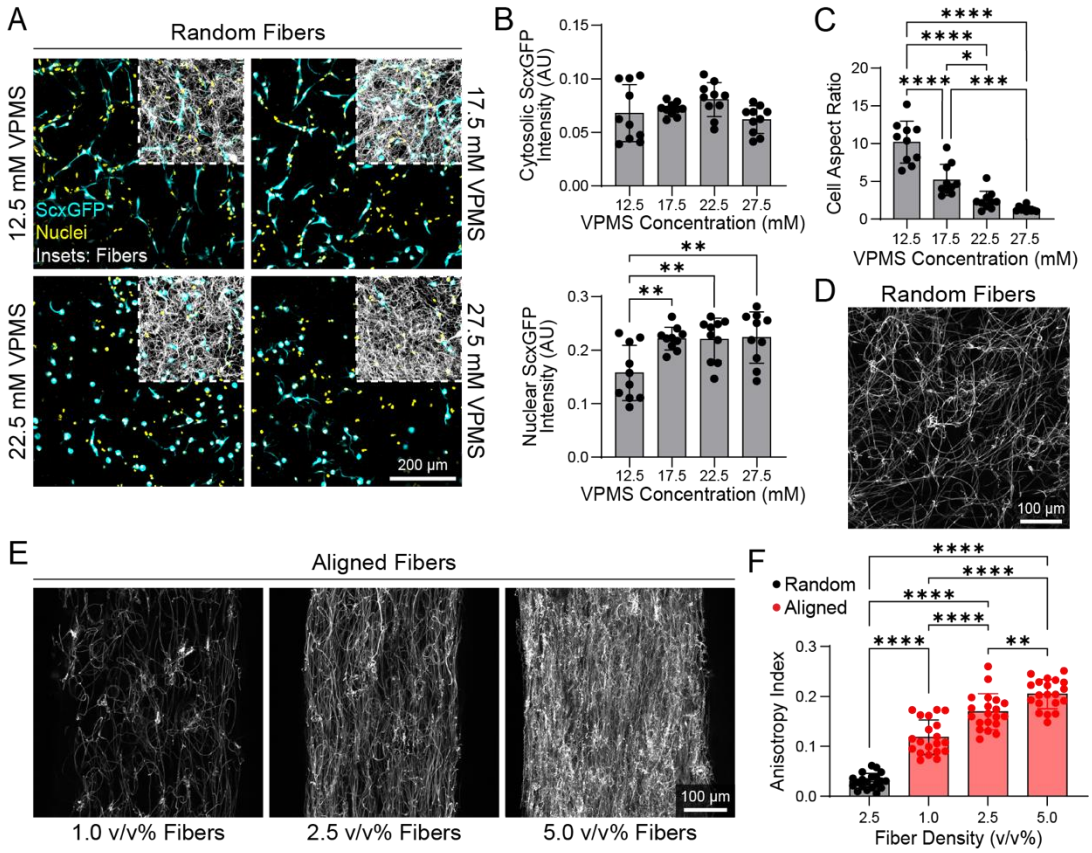


Figure S4 | Screen of bulk crosslink density in FRHCs. A, Fluorescent images of *ScxGFP* reporter TPC cultures with TGF-β3 in FRHCs (2.5 v/v% fibers) with varying crosslink densities for 1 week. Quantification of (**B**) cytosolic and nuclear ScxGFP intensity (AU) and (**C**) cell aspect ratio (n = 10 FOVs). Fluorescent image of (**D**) randomly oriented (2.5 v/v%) and (**E**) aligned fibers at varying fiber densities in FRHCs. **F,** Quantification of fiber anisotropy index. All data are reported as mean ± standard deviation. Asterisks indicate statistically significant comparisons, with *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 by ordinary one-way ANOVA with Tukey's multiple comparisons test.

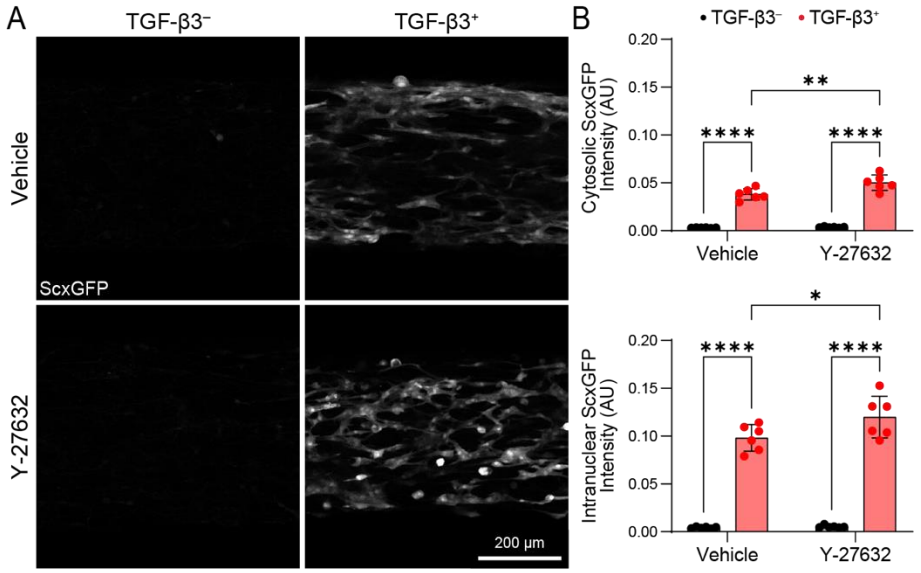


Figure S5 | Rho/ROCK inhibition of TPCs in FRHCs with 30μM Y27632. A, Fluorescent images of *ScxGFP* reporter TPC cultures in flow-aligned FRHCs (2.5 v/v% fibers) with basal media or supplemented with Y27632 (30μM), TGF-β3, or both. Quantification of (**B**) cytosolic and nuclear ScxGFP intensity (AU) (n = 6 FOVs). All data are reported as mean ± standard deviation. Asterisks indicate statistically significant comparisons, with *p<0.05, **p<0.01, and ****p<0.0001 by ordinary two-way ANOVA with Tukey's multiple comparisons test.

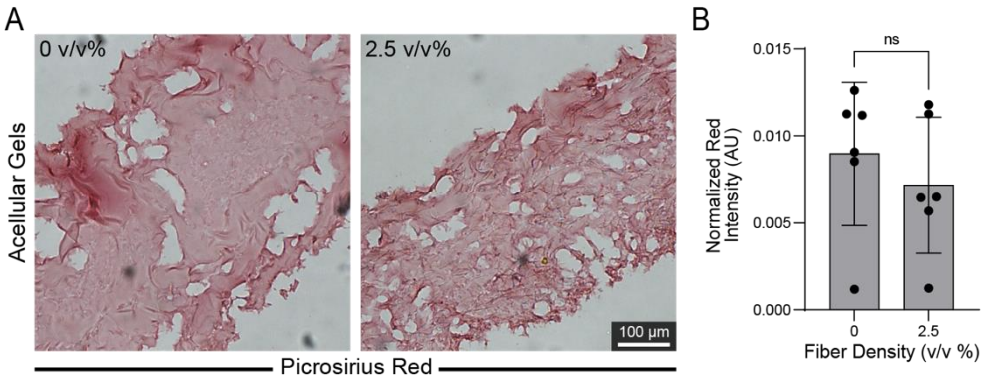
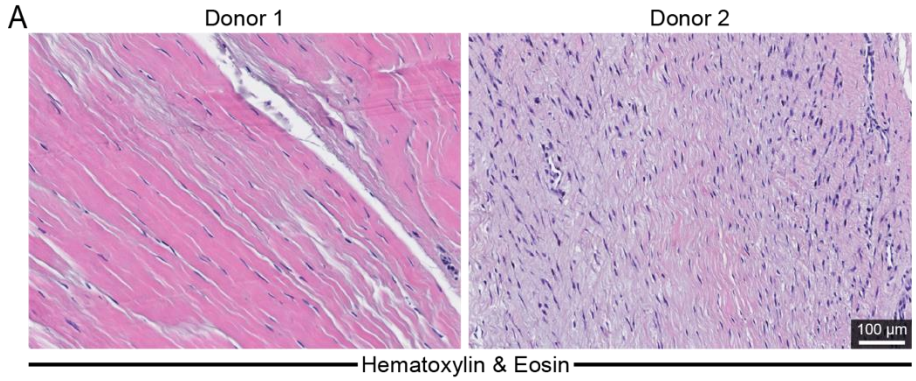


Figure S6 | Fiber density does not impact Picrosirius Red staining in acellular FRHCs. A, Brightfield images of Picrosirius Red-stained frozen sections of random and aligned, acellular FRHCs. **B,** Quantification of normalized red intensity (AU) (n = 6 FOVs). All data are reported as mean ± standard deviation. ns denotes a non-significant comparison by Student's t-test.



B

Bonar Scoring

	Donor 1	Donor 2
Cell Morphology	Grade 0	Grade 3
Collagen Arrangement	Grade 1	Grade 3
Cellularity	Grade 1	Grade 2
Vascularity	Grade 2	Grade 1
Ground Substance	Grade 0	Grade 0

Figure S7 | Bonar tendinopathy scoring for human TDC donors. A, Brightfield images of hematoxylin and eosin-stained paraffin-embedded sections of donor Achilles tendon tissues. **B,** Bonar tendinopathy scoring, where each criteria receives a grade between 0 (least tendinopathic) and 3 (most tendinopathic).

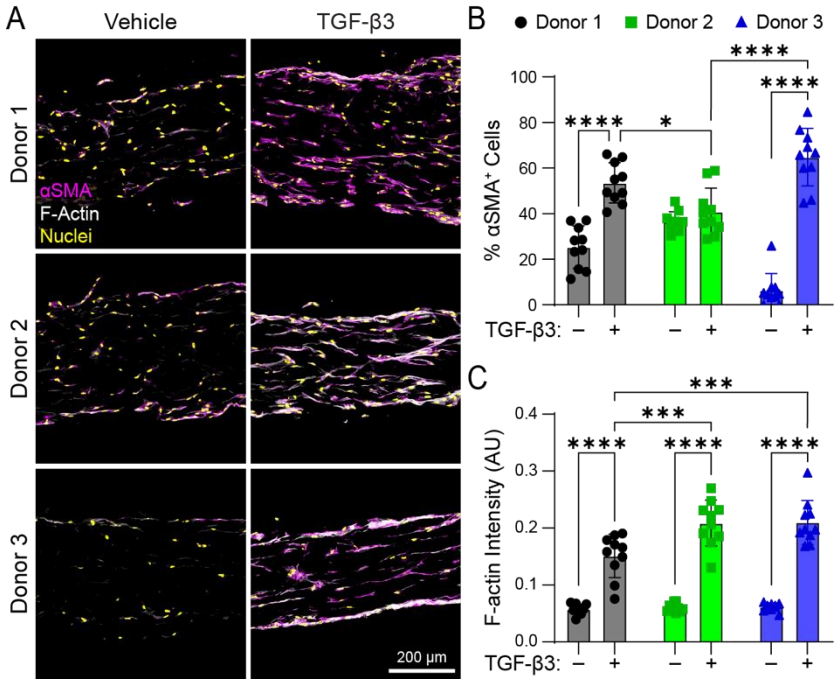


Figure S8 | Myofibroblastic differentiation of human TDCs in FRHCs. A, Fluorescent images of frozen sections of human TDC cultures in flow-aligned FRHCs (2.5 v/v% fibers) with basal media or supplemented TGF-β3. Quantification (**B**) % of TPCs expressing *αSMA* and (**C**) F-actin intensity (AU) (n = 10 FOVs). All data are reported as mean ± standard deviation. Asterisks indicate statistically significant comparisons, with *p<0.05, ***p<0.001, and ****p<0.0001 by ordinary two-way ANOVA with Tukey's multiple comparisons test.