Supplementary Information

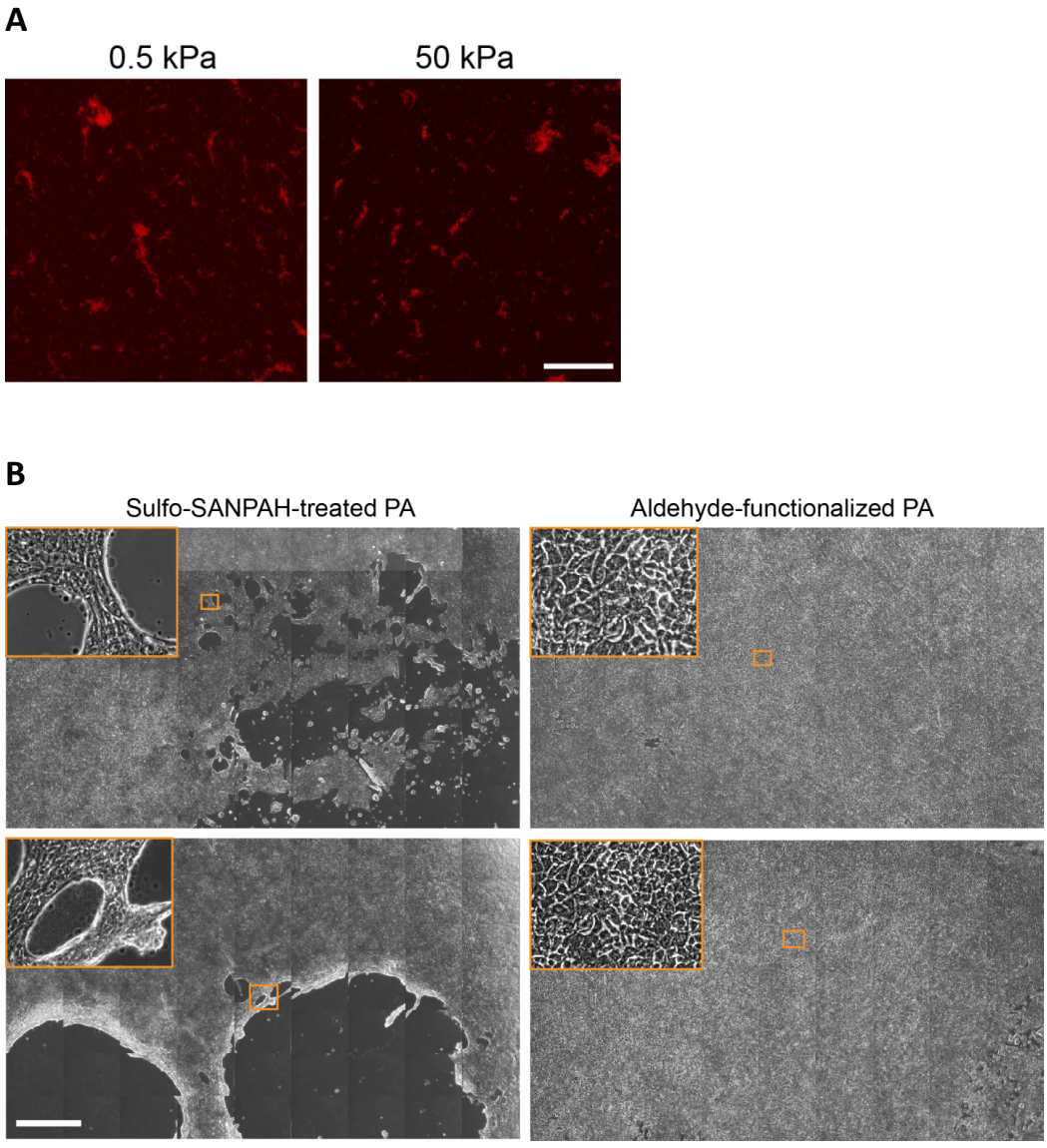

Fig. S1. (A) Inability to form collagen fibers on conventional PA gels crosslinked with sulfo-SANPAH. Confocal microscopy images of fluorescently labelled collagen I on soft (0.5 kPa) and stiff (50 kPa) sulfo-SANPAH-treated conventional PA gels, showing a non-fibrous patchy collagen coating. Scale bar = 100 μm. **(B) More uniform epithelial monolayer**

formation on aldehyde-functionalized PA gels. Phase contrast images of MCF10A cell monolayer on collagen-coated sulfo-SANPAH-treated conventional PA gels (left column) and aldehyde-functionalized PA gels (right column) of low stiffness (~0.2 kPa). Tiled fields of view are combined to show about 80% of the entire monolayer. Broken or discontinuous cell monolayers are observed on sulfo-SANPAH-treated conventional PA gels. In contrast, the aldehyde-functionalized PA gels support more uniform and continuous epithelial cell monolayers. Scale bar = 1 mm.

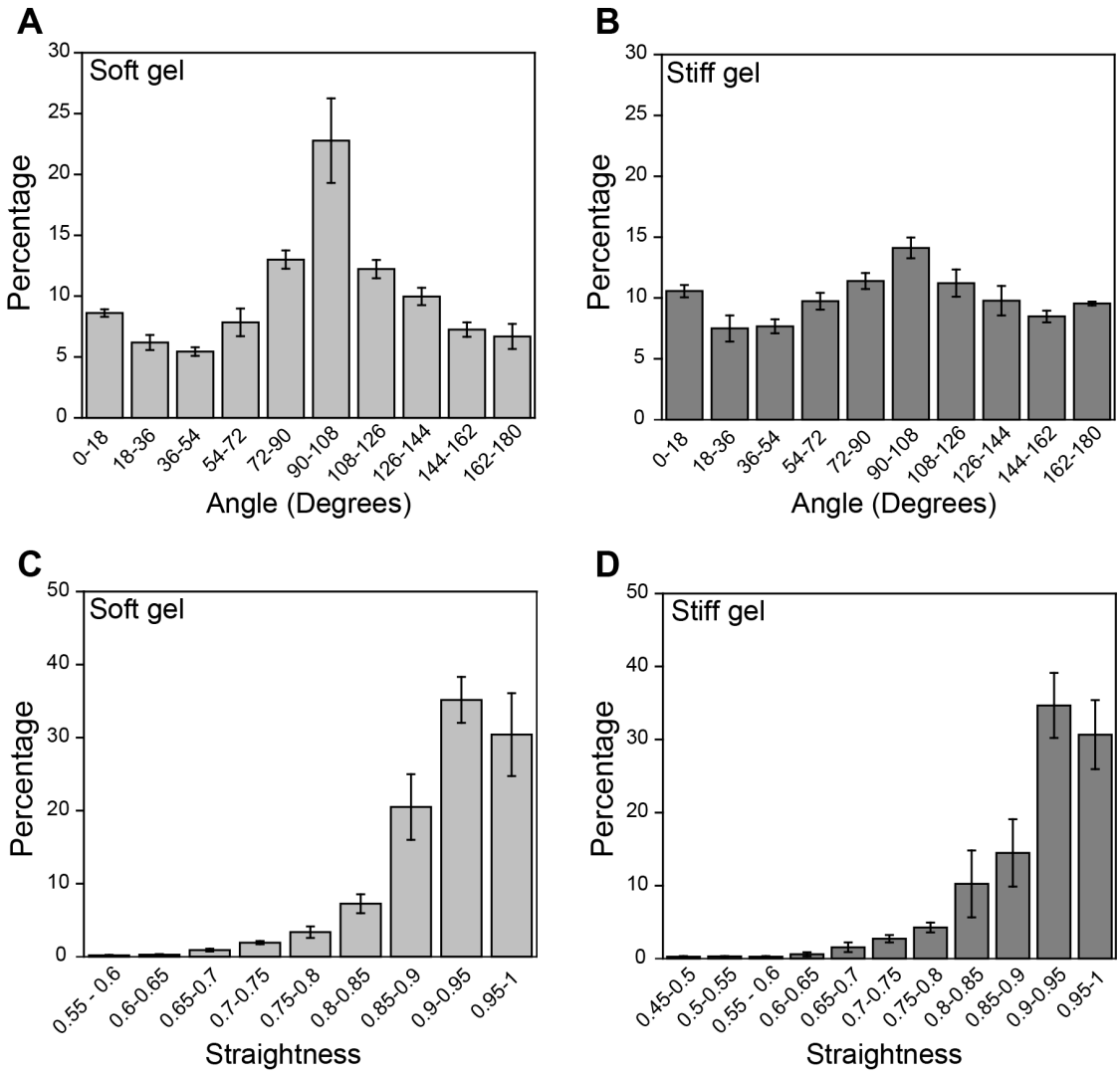

Fig. S2. Anisotropy in collagen fiber alignment and straightness on soft and stiff PAaf gels coated with long collagen fibers. Distribution of collagen fiber angles on (A) soft and (B) stiff gels. Distribution of collagen fiber straightness on (C) soft and (D) stiff gels.

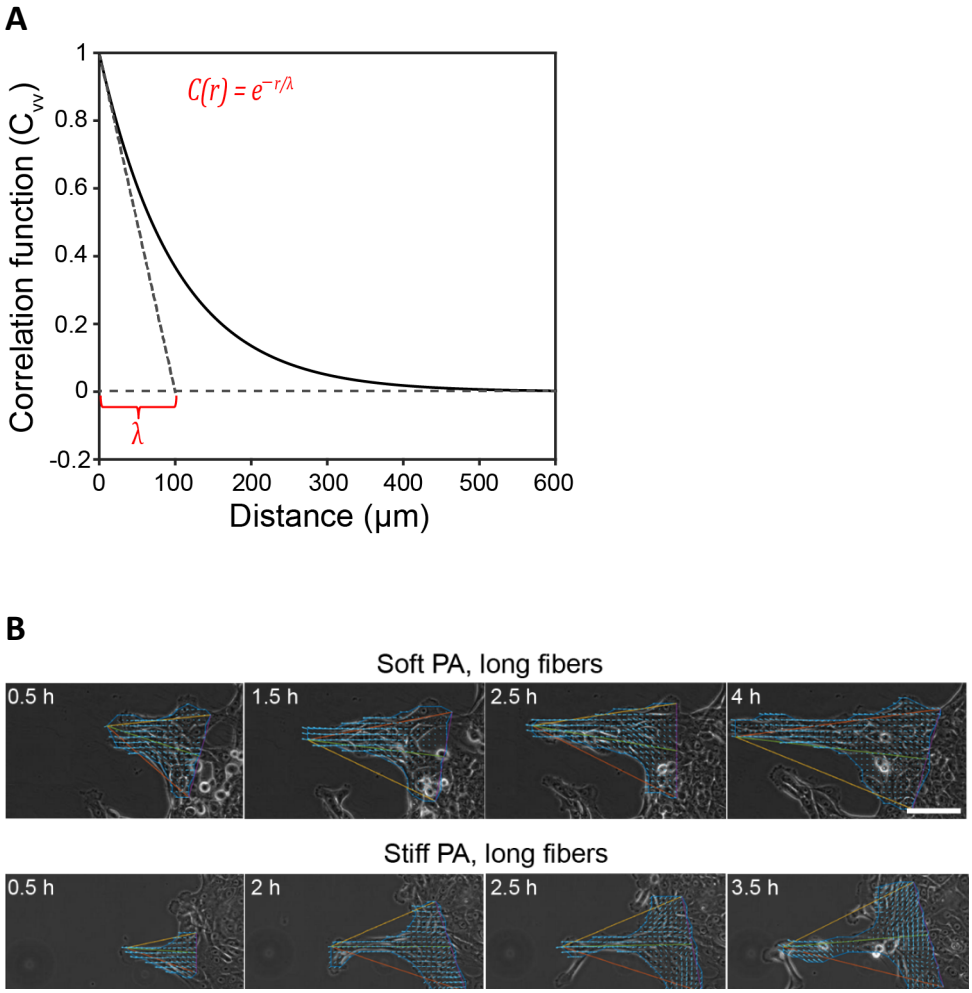

Fig. S3. (A) Correlation length calculation. Fitting correlation function (decreasing exponential) is used to obtain correlation length, λ. **(B) Computing principal direction of multicellular stream ($V_{Principal}$).** Stream and its immediate base are fit into a triangle represented by yellow, red and blue lines, while the green line represents the longest median inside the triangle. Thus, the principal vector for the stream is defined along the longest median. Scale bar = 100 μm.

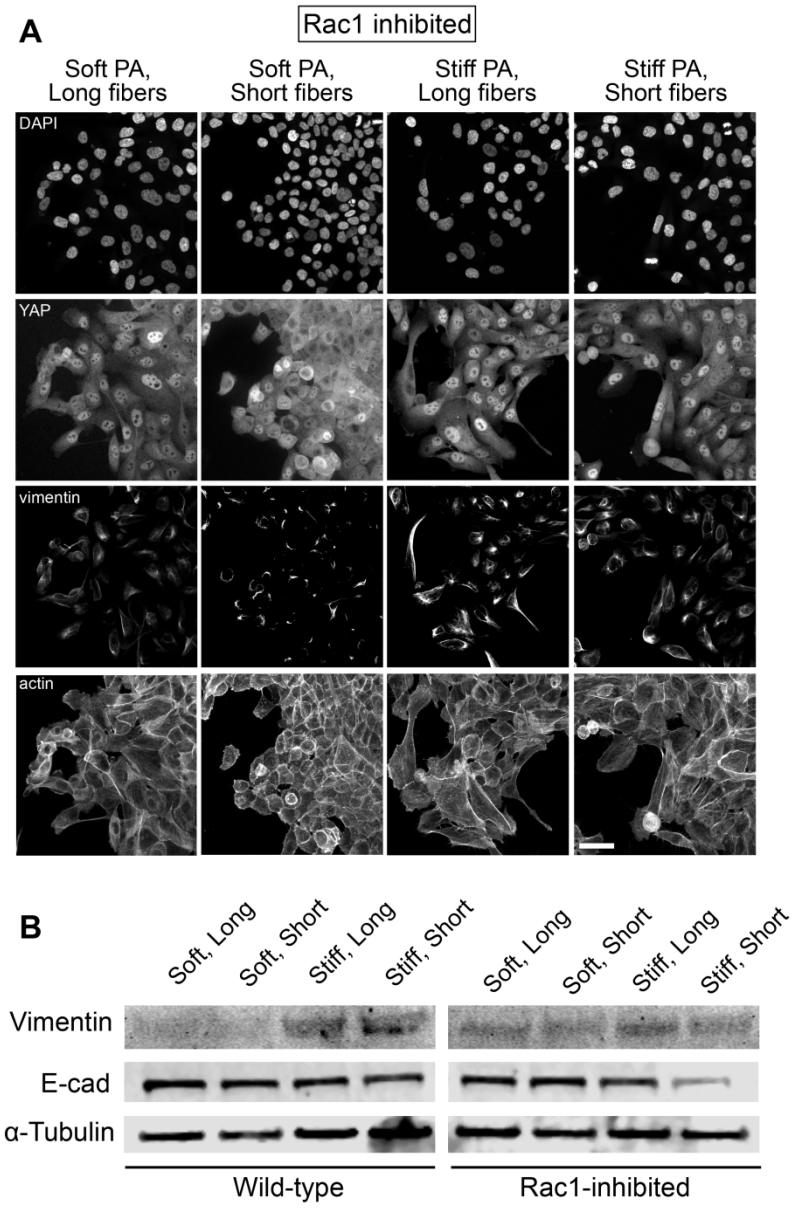

Fig. S4. Split-channel images DAPI, YAP, vimentin and actin in Rac1-inhibted cell monolayers. (A) Immunofluorescence images of DAPI, YAP, vimentin, and F-actin of MCF10A cells treated with Rac1 inhibitory drug. Scale bar = 50 μm. **(B)** Western blot of vimentin, E-cad, and α-Tubulin (control) expression of MCF10A cells shows that vimentin expression increases after Rac inhibition on soft gels of both fiber lengths, but reduces on stiff gels. This indicates that cells on soft gels with long fibers have more epithelial characteristics in wildtype conditions, but Rac inhibition increases their mesenchymal characteristics.

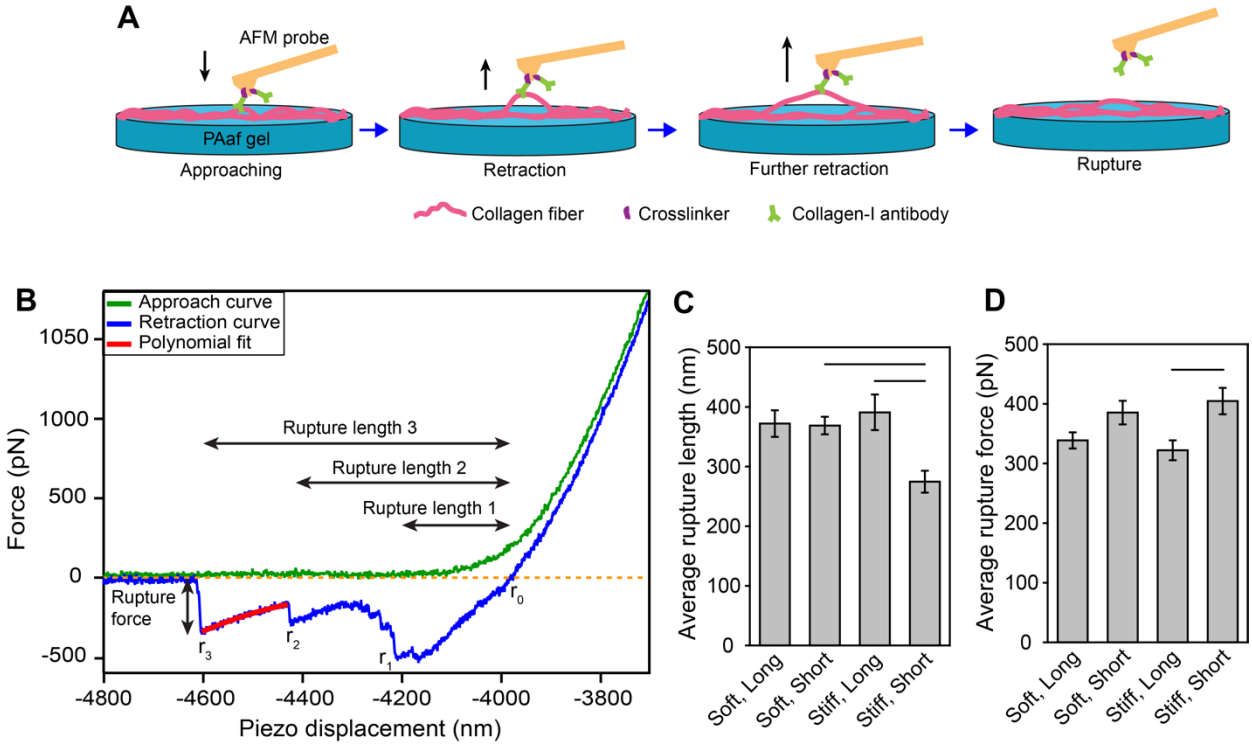

Fig. S5. AFM-based force spectroscopy of collagen tethering to PAaf gels. (A) Schematic illustrating the interaction between a collagen-I antibody-functionalized AFM probe and the collagen-coated PAaf hydrogel in the force-spectroscopy study using AFM. Black arrows represent the direction of motion of AFM cantilever. **(B)** Representative force-distance curve of the collagen antibody-functionalized cantilever approaching (green) and retracting (blue) from a collagen-coated PAaf gel surface. Three ruptures in this retraction curve are denoted by r_1, r_2, and r_3. Rupture lengths are calculated as distance between the surface of the substrate (r_0) and each rupture point. The part of the retraction curve between the two rupture points, r_2 and r_3, called force-extension curve or loading curve, can be fitted by a second-degree polynomial (solid red curve). The slope of this polynomial at extension r_3 is the stiffness of collagen fiber and the corresponding difference between the fitted red curve at rupture point, r_3 and the orange baseline is defined as the rupture force (indicated as a vertical arrow) for the corresponding rupture event. **(C)** Measured average rupture length, and **(D)** average rupture force in each retraction event that occurred on long and short collagen fibers-coated soft and stiff PAaf gels (n = 100). Lines denote significant difference ($p<0.05$) in pairwise comparison.

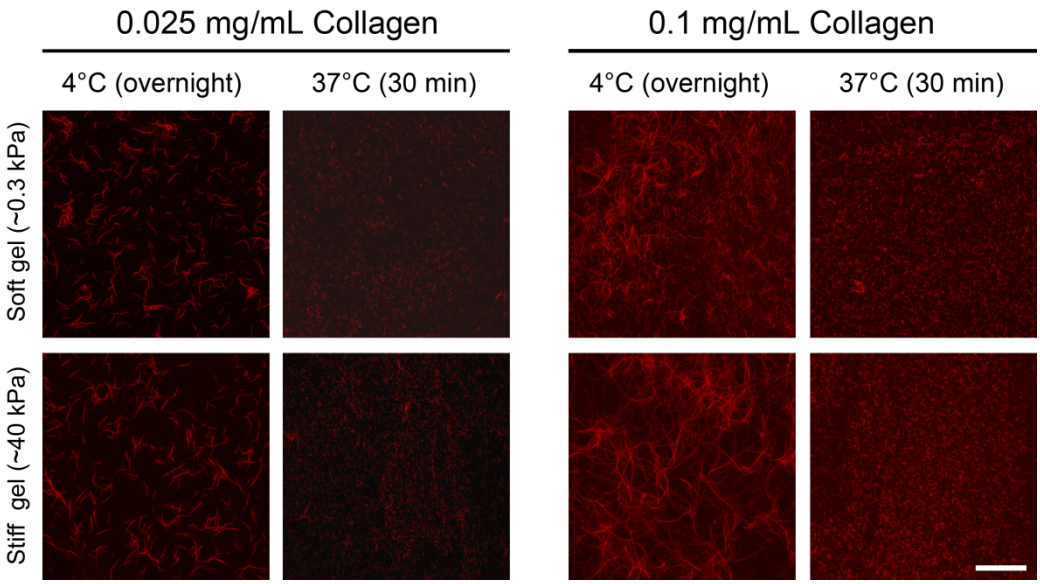

Fig. S6. Representative images of fluorescently labelled collagen type I of two different concentrations on soft and stiff PAaf gels for varying incubation temperatures, showing that the methodology of altering fiber length by varying temperature works for a range of collagen concentrations – 0.025, 0.05, 0.1 mg/ml. Scale bar = 100 μm.

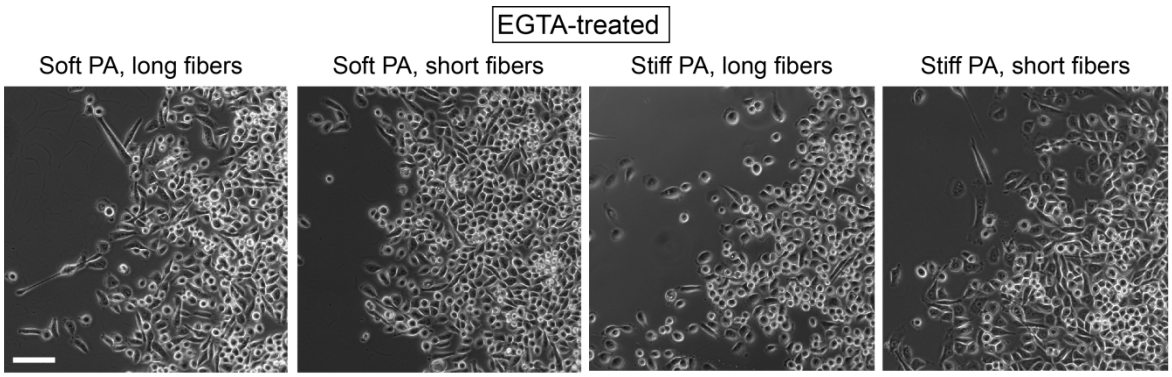

Fig. S7. Phase-contrast images of epithelial monolayer on long and short collagen fibers-coated soft and stiff PAaf gels after 2 hours of treating with EGTA, a calcium chelation agent. These images and our observations on many other samples with EGTA show that abrogation of cell-cell junctions completely eliminates any stream formation. Scale bar = 100 μm.