Supplemental Material

Biomimetic culture system for probing the role of matrix properties in fibroblast activation for studying wound healing and fibrosis

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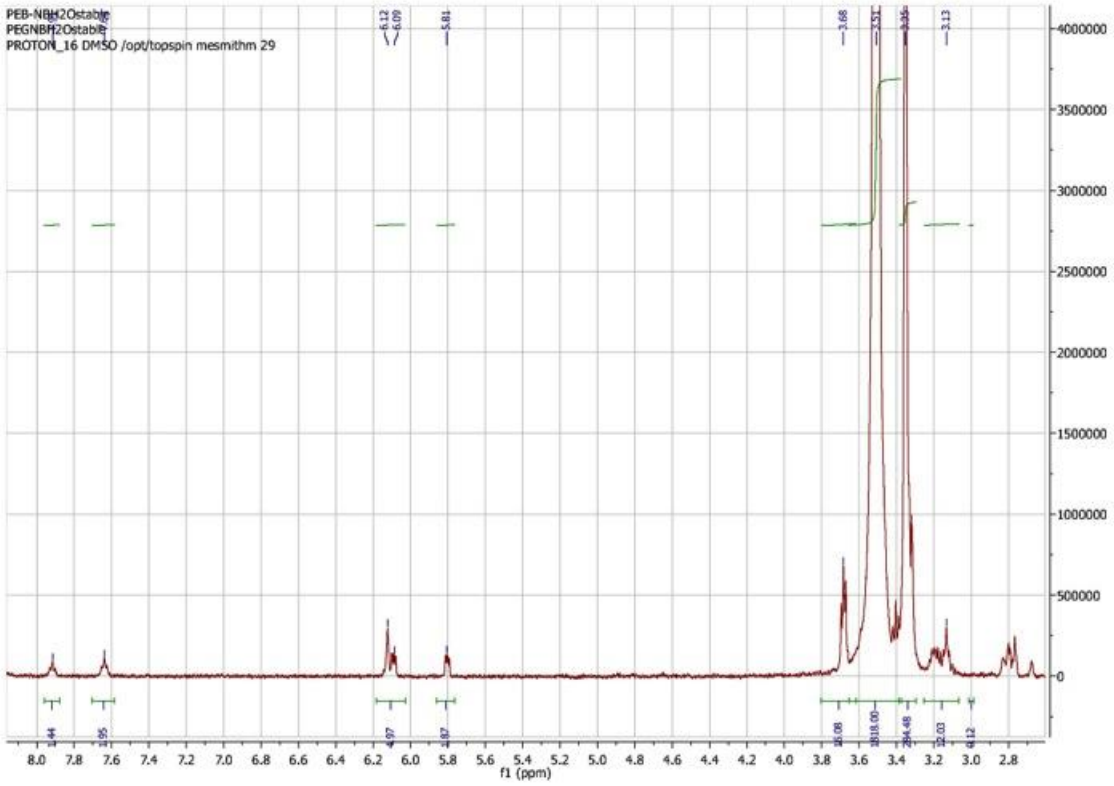

Figure S1. Functionalization of PEG with norbornene end groups was determined using ^{1}H NMR in DMSO (400 mHz): δ 6.15 to 5.7 (m, 16H), δ 3.7 to 3.42 (m, 3636H) and disappearance of the peak at δ 3.12 to 3.02 (t, 16H). Typical reactions resulted in 85% functionality on average.

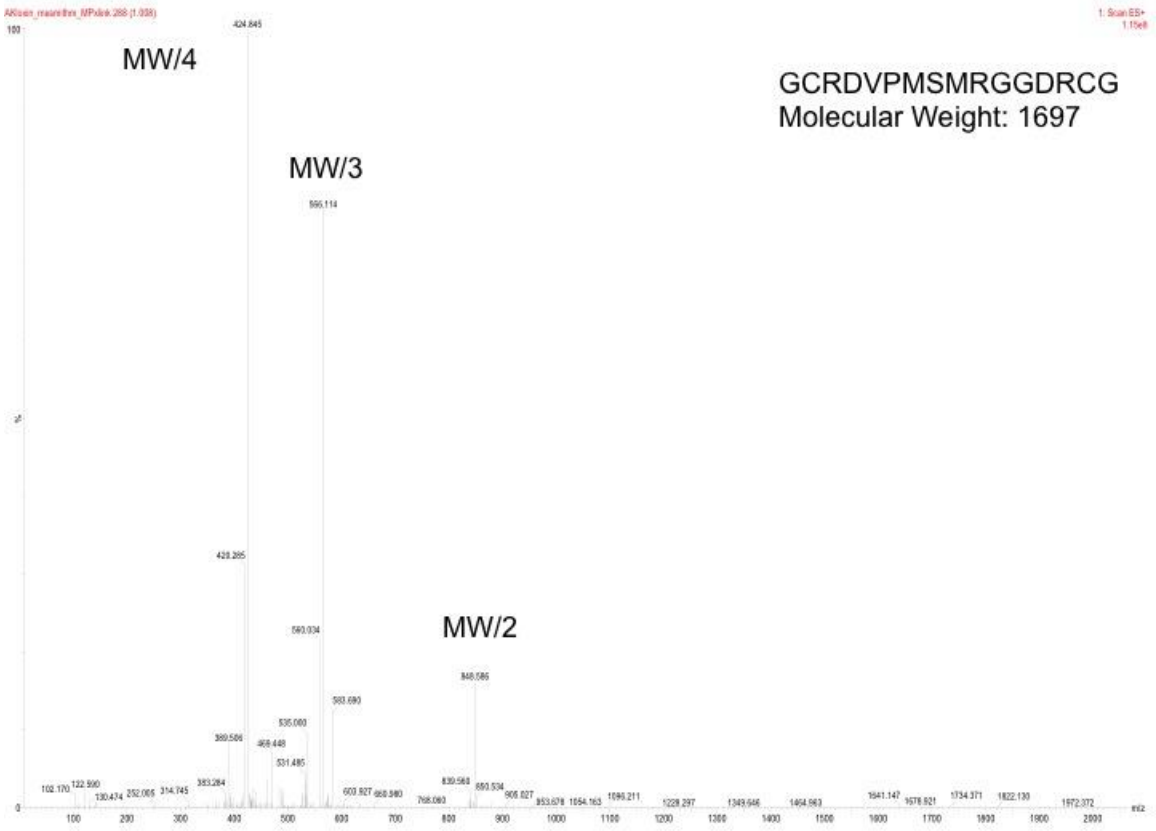

Figure S2: GCRDVPMSMRGGDRCG peptide identity was confirmed using tandem chromatography and electrospray ionization mass spectrometry (Acquity UPLC H-Class/SQD2).

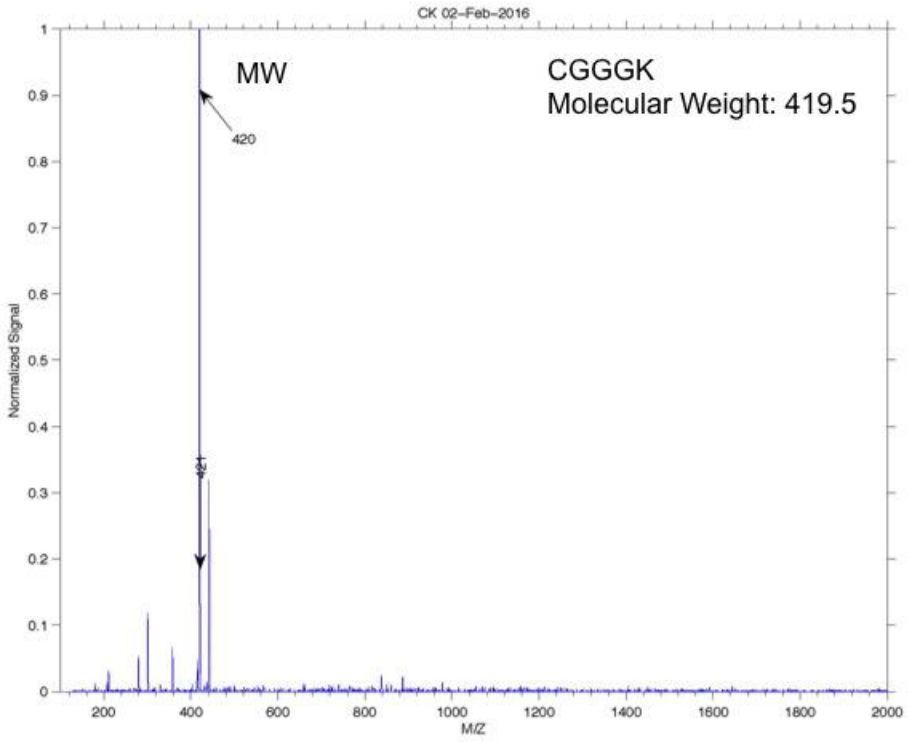

Figure S3: CGGGK peptide identity was confirmed using electrospray ionization mass spectrometry (Shimadzu LCMS 2020).

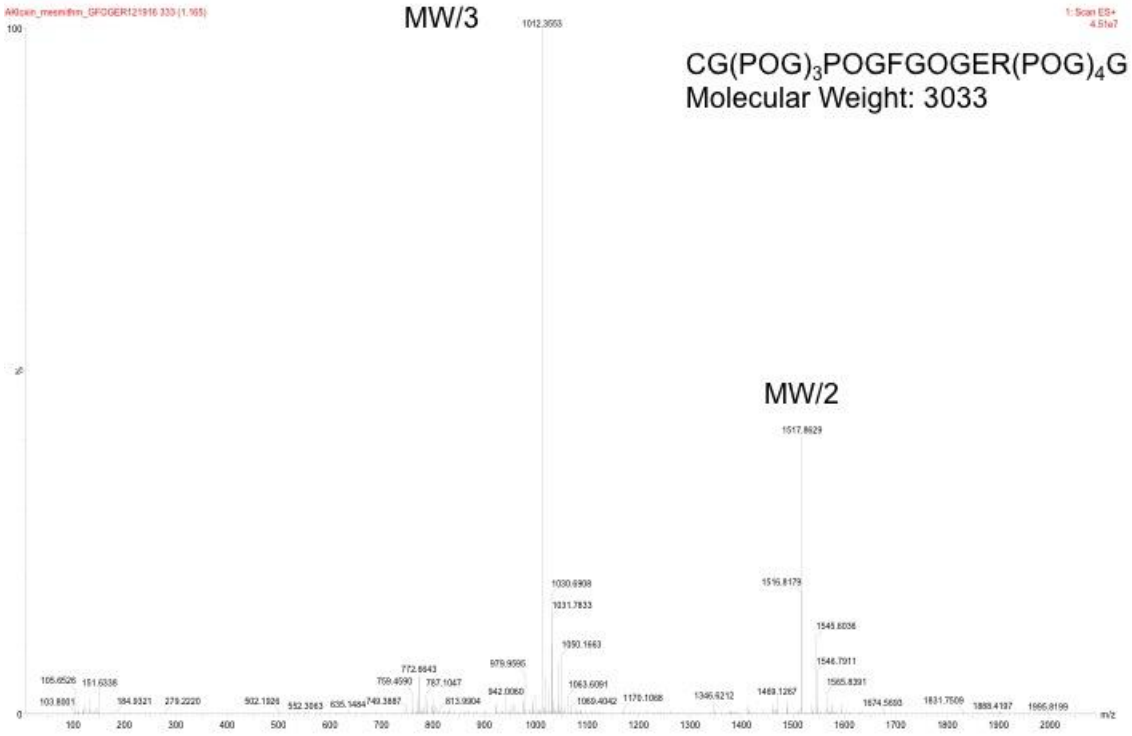

Figure S4: $CG(POG)_3POGFOGER(POG)_4$ peptide identity was confirmed using an Acquity UPLC H-Class/SQD2.

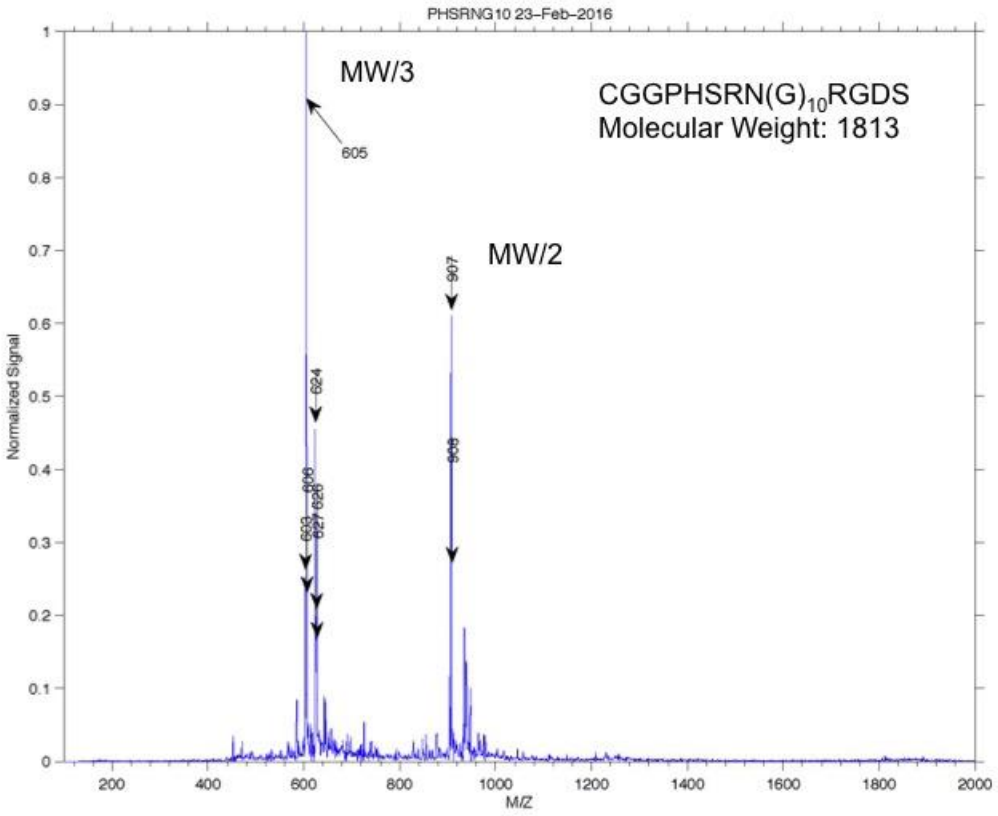

Figure S5: CGGPHSRN$(G)_{10}$RGDS the peptide identity was verified using electrospray ionization mass spectrometry (Shimadzu LCMS 2020).

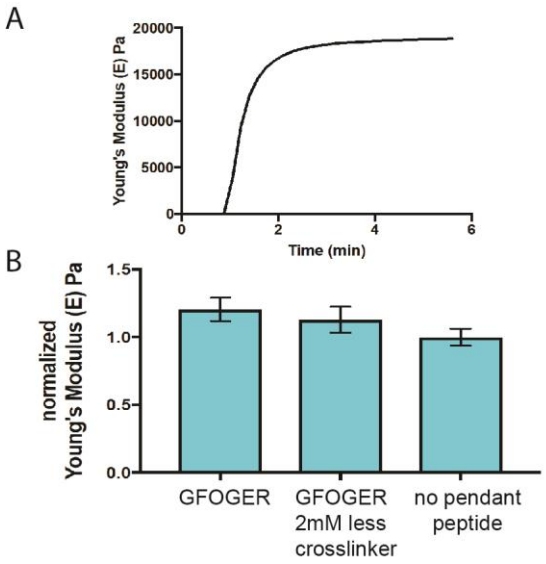

Figure S6: A) Hydrogels rapidly were polymerized using LAP photoinitiator in the presence in roughly 2 minutes (10 mW/cm^2 at 365 nm). **B)** Including the monocysteine, self-assembling GFOGER sequence within the hydrogel increased the modulus, potentially by adding physical crosslinks to the network.[1] Decreasing the amount of crosslinking peptide within GFOGER-containing hydrogels lowered their modulus such that there was no statistical difference (n.s.) between the moduli of the hydrogels with or without different peptides (* $p \leq 0.05$).

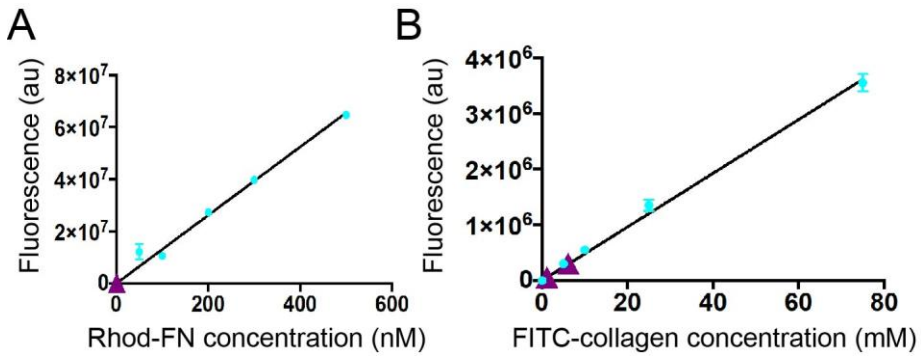

Figure S7: **Assessment of protein incorporation.** To assess the incorporation of protein within or on the hydrogel matrices, the amount of protein lost during wash steps was quantified for **A)** encapsulated fibronectin or **B)** covalently immobilized collagen, respectively. **A)** For fibronectin, hydrogels (n=3) were formed with encapsulated rhodamine-labeled fibronectin (Rhod-FN) at a concentration of 300 nM, and the resulting hydrogels were swollen in PBS overnight. The fluorescence intensity of the resulting wash solution then was measured (purple triangles). The fluorescence intensity of these solutions were compared to solutions of known concentrations of rhodamine-labeled fibronectin in PBS (blue circles, n=3), creating a standard curve that was used to calculate the protein concentration in the wash solution. From this analysis, ~ 15 % of the fibronectin was estimated to be lost upon equilibrium swelling of the hydrogel. The balance of the fibronectin (85% of the 300 nM in the hydrogel precursor solution) was estimated to be retained and entrapped within the hydrogel. **B)** For collagen, sulfo-SANPAH was used to conjugate FITC-labeled collagen (FITC-collagen) to hydrogels (n=3) at three different concentrations: 500 µg/mL, 50 µg/mL, or 5 µg/mL. After the overnight reaction at 4 °C, the hydrogels were washed with PBS. The fluorescence intensity of the wash solution was measured (purple triangles), and the fluorescence intensity of this solution was compared to solutions of known concentrations of FITC-labeled collagen in PBS (blue circles), creating a standard curve that was used to calculate the protein concentration in the wash solution. From this analysis, protein loss of ~ 0.2% (50 µg/mL and 5 µg/mL) and ~ 1.2% (500 µg/mL) was observed upon washing, suggesting that most of the applied protein was conjugated to the hydrogel surface for cell culture.

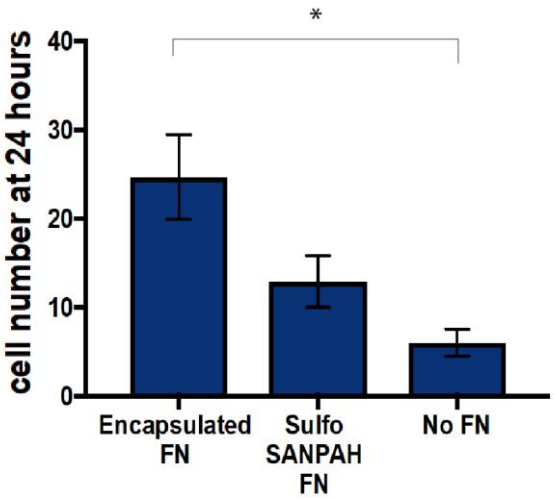

Figure S8: Fibroblasts were seeded on hydrogels containing encapsulated fibronectin (300 nM), Sulfo-SANPAH functionalized fibronectin (300 nM), or no fibronectin (control hydrogel). At 24 hours, cell attachment was assessed by staining the nuclei with hoescht, imaging, and counting the number of cells per multiple fields of view with ImageJ. A statistically significant increase in attached cells as observed for substrates presenting encapsulated fibronectin in comparison to covalently-immobilized fibronectin (* $p < 0.01$).

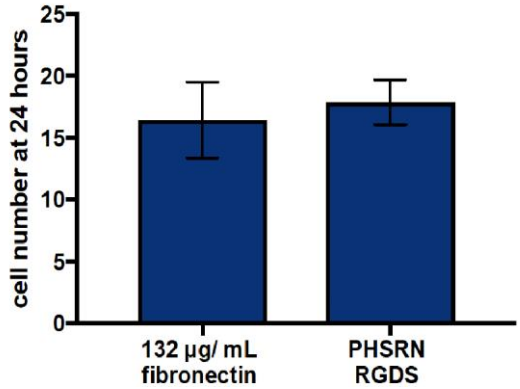

Figure S9: Fibroblasts were cultured on hydrogels containing 300 nM encapsulated fibronectin or 2 mM PHSRN-RGDS. At 24 hours, cell attachment was assessed by staining the nuclei with Hoescht, imaging, and counting the number of cells per multiple fields of view with ImageJ. No significant difference in cell attachment was observed between these substrates.

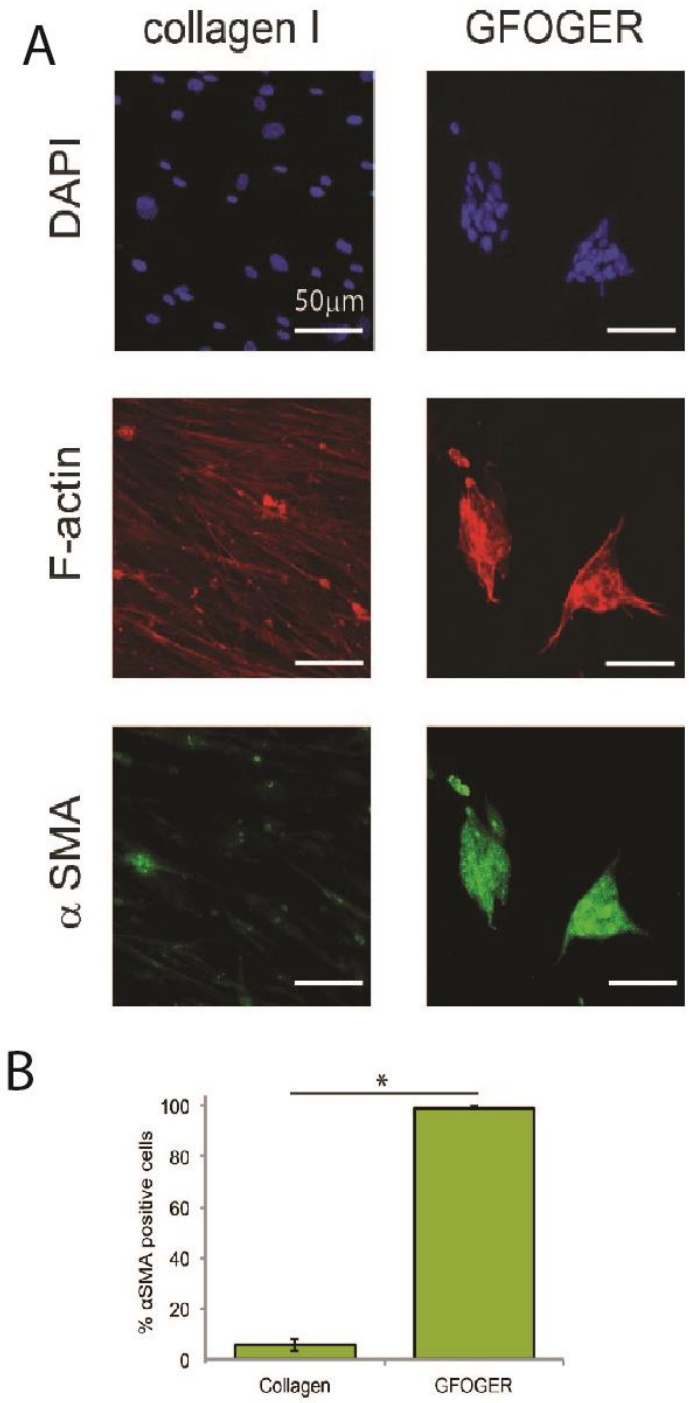

Figure S10: Primary normal human lung fibroblasts (NHLF, Lonza) were cultured on GFOGER- and collagen I-functionalized hydrogels (n=3). Similar cellular responses were observed for NHLF to those observed for CCL151 pulmonary fibroblasts: at 72 hours, **A)** percentage of alpha smooth muscle actin positive cells and **B)** representative images (alpha smooth muscle actin (green), F-actin (red). Specifically, on GFOGER-presenting hydrogels, significantly more cells expressed alpha smooth muscle actin, suggesting increased activation on the peptide-presenting substrates.

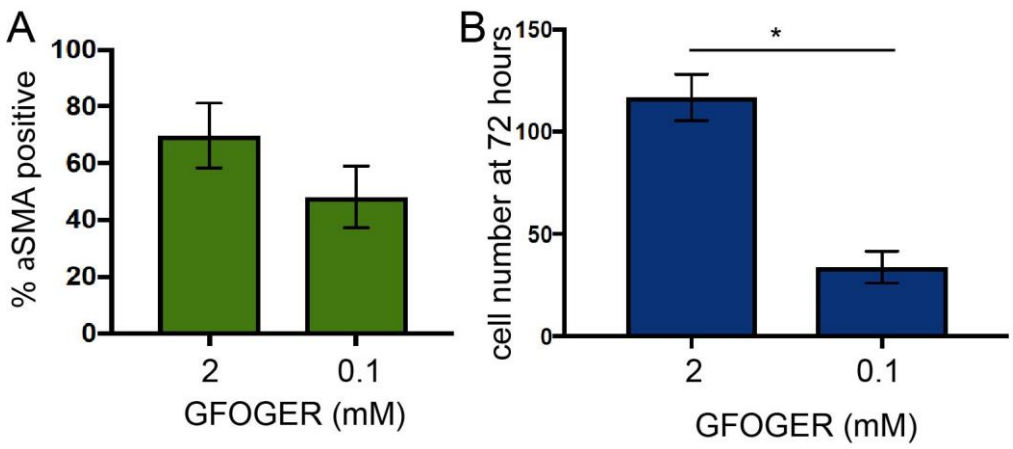

Figure S11: Fibroblasts were cultured on hydrogels containing 2 mM GFOGER or 0.1 mM GFOGER (n=3 for each). At 72 hours, cells were stained for αSMA, F-actin, and nuclei and imaged with confocal microscopy, and the number of αSMA positive cells counted, as done in Figure 6. **A)** The number of αSMA positive cells was observed to decrease with decreased GFOGER but was not statistically different between the two conditions ($p > 0.05$). **B)** However, the cell number was observed to statistically decrease with decreasing peptide concentration (* $p < 0.01$). This result suggests that, while cell attachment was decreased with decreased peptide concentration, even low levels of GFOGER promoted activation of the fibroblasts that were attached to these matrices.