SUPPLEMENTARY INFORMATION

Substrate Rigidity Controls Activation and Durotaxis in Pancreatic Stellate Cells

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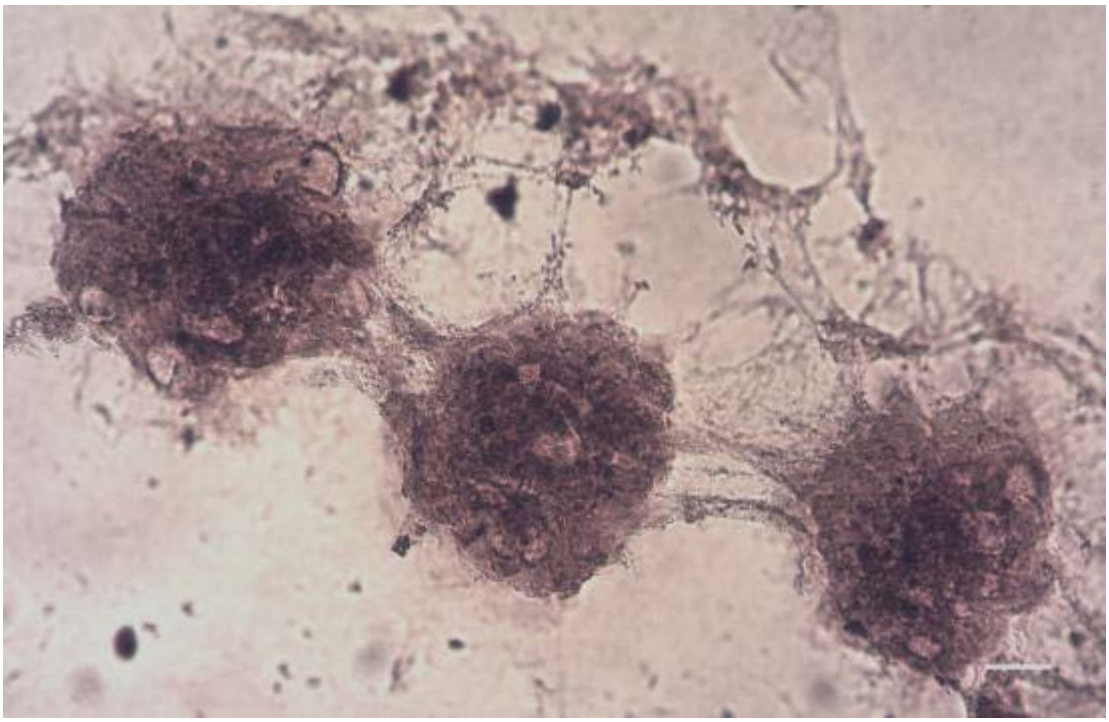
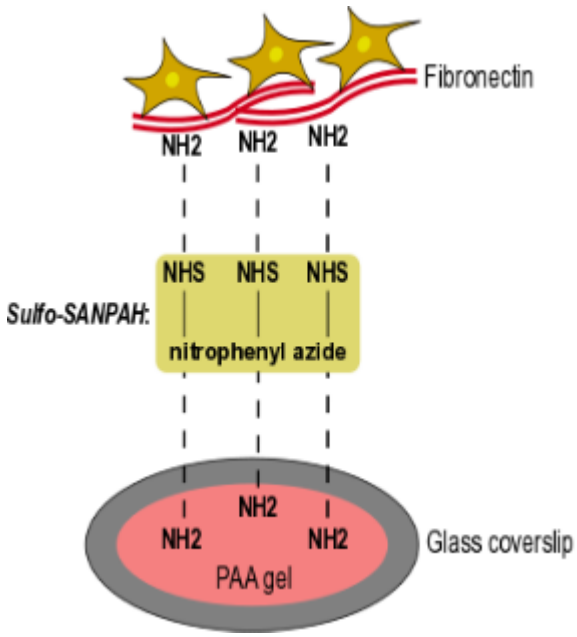
Supplementary Figure S1



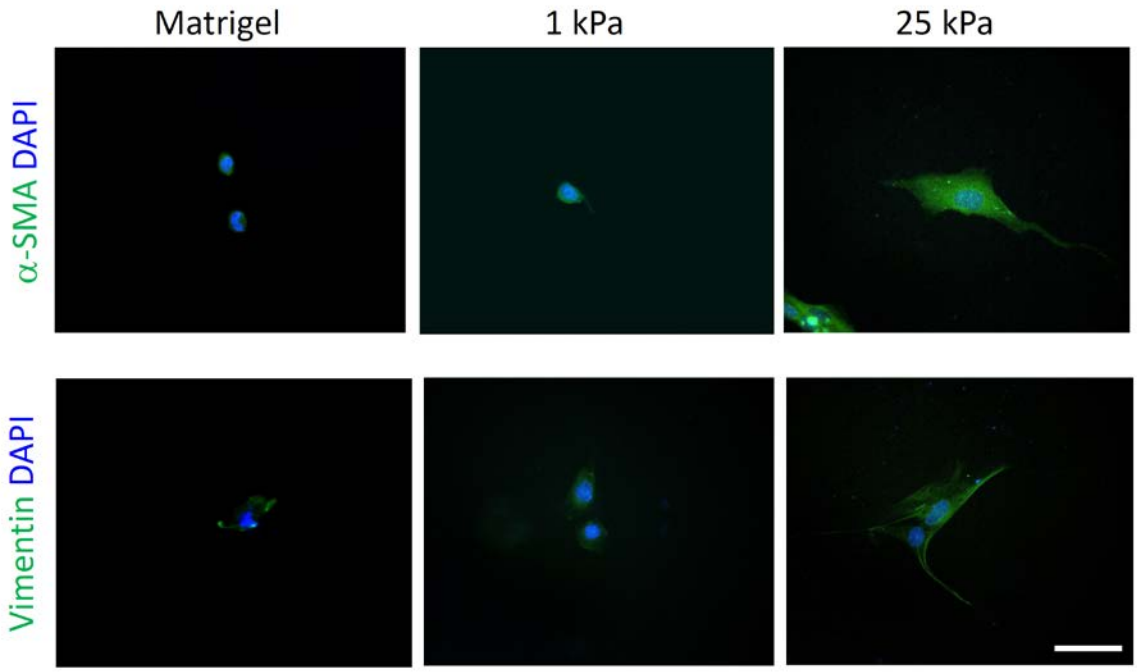
Supplementary Figure 1: Bright field images of Oil Red O stained PSCs cultured on Matrigel for 6 days and showing the clusters connected by a filamentous network characteristic of PSCs quiescence. Scale bar 50 μm

Supplementary Figure S2



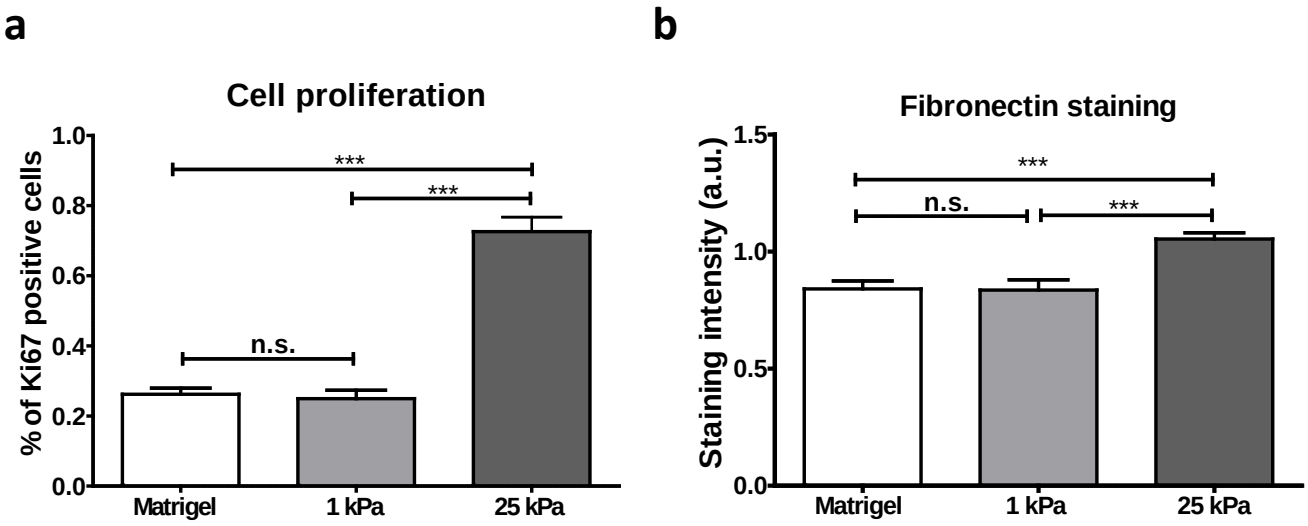
Supplementary Figure 2: Schematic representation of surface preparation for cell attachment on soft and stiff PAA gels**.** Crosslinking of PAA hydrogels with fibronectin through the use of sulfo-SANPAH. sulfo-SANPAH is a heterobifunctional crosslinker containing an amine-reactive NHS and a photoactivatable nitrophenyl azide. Upon UV exposure, this nitrophenyl azide forms a nitrene group that binds to NH_2 groups within the hydrogel, leaving a NHS group free at the gel surface to allow binding of fibronectin.

Supplementary Figure S3



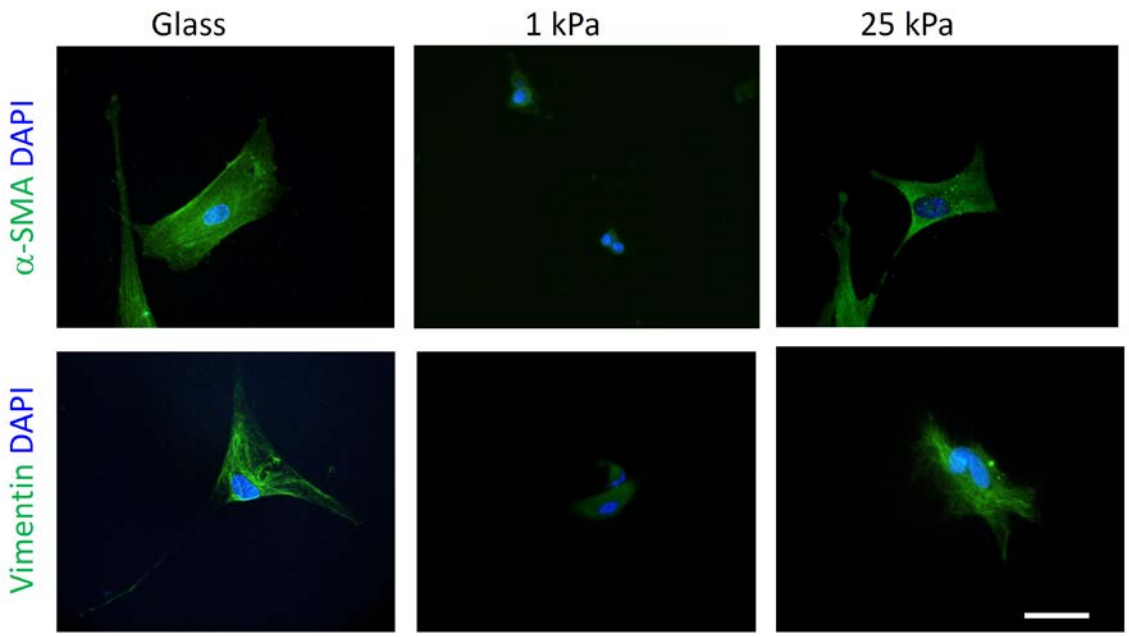
<u>Supplementary Figure 3</u>: Immunofluorescent images of aSMA and vimentin of PSCs seeded on matrices represented in Fig. 1a. Scale bar 50 μm. Quantification in Fig 1e,f.

Supplementary Figure S4



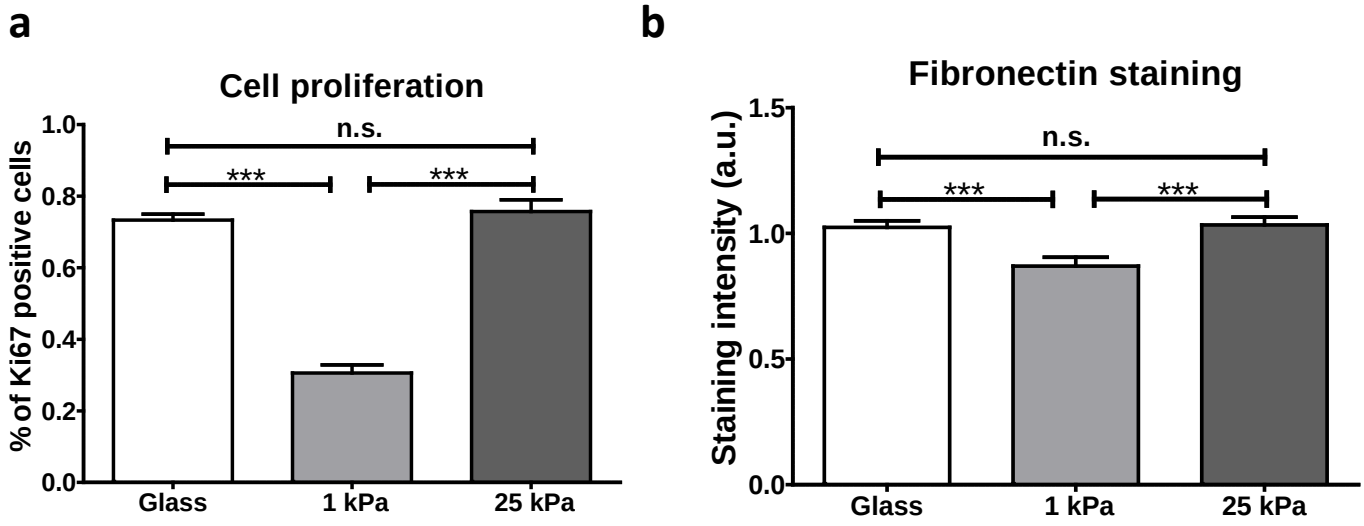
Supplementary Figure 4: Mechanical activation of PSCs by stiff substrates increases cell proliferation and fibronectin production. (a, b) Quantification of ki67 positive cells and fibronectin immunofluorescent staining as markers of cell proliferation and ECM production, respectively. Histogram bars represent mean ± SEM. Representative of 3 independent experiments. (Anova and Tukey posthoc test) *** $p<0.001$.

Supplementary Figure S5



Supplementary Figure 5: Immunofluorescent images of αSMA and vimentin of PSCs seeded on matrices represented in Fig. 2a. Scale bar 50 μm. Quantification in Fig 2 f,g.

Supplementary Figure S6



<u>Supplementary Figure 6</u>: Mechanical deactivation of PSCs by soft substrates decreases cell proliferation and fibronectin production. (a, b) Quantification of ki67 positive cells and fibronectin immunofluorescent staining as markers of cell proliferation and ECM production, respectively. Histogram bars represent mean ± SEM. Representative of 3 independent experiments. (Anova and Tukey posthoc test) *** $p<0.001$.

Supplementary Table S1: Reagent proportions required to obtain specific PAA hydrogel rigidities.

Stiffness (kPa)	Total volume (µl)	PBS (µl)	APS (µl)	TEMED (µl)	acrylamide/bisacrylamide (29:1) 40% vol (µl)
1.3	500	461.6	2.5	1	34.9
25.5	500	371.2	2.5	1	125.3

Supplementary Video S1

This video represents the durotactic movement (towards the stiff region) of one pancreatic stellate cell in the boundary region between a soft (right side) to a rigid substrate (left). The total video represents 6 h in real time.

Supplementary Video S2

This video represents the random undirected movement of one pancreatic stellate cell (siRNA FAK) in the boundary region between a soft (right side) to a rigid substrate (left). The total video represents 6 h in real time.

Supplementary Video S3

This video represents the random undirected movement of one pancreatic stellate cell (treated with blebbistatin) in the boundary region between a soft (right side) to a rigid substrate (left). The total video represents 6 h in real time.