Supporting Information for

Piezo1-induced durotaxis of pancreatic stellate cells depends on TRPC1 and TRPV4 channels

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Numerics References

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Raw data file

Supporting Text

Supporting methods for Fig. S3

Calcium imaging: Live cell calcium imaging was performed on quiescent PSCs cultured on the Matrigel-coated plates using Calcium 6-QF dye (Molecular Devices) as previously described(1, 2). Cells were imaged in HBSS buffer with 2 mM Ca^{2+}. Imaging was performed using a Zeiss Axio observer Z1 with a 20× objective at room temperature, and the intensity of Calcium 6-QF over time was analyzed using MetaMorph image processing and analysis software (Molecular Devices). Faintly and highly fluorescent-loaded cells were excluded from the analysis. The chemicals used in calcium imaging experiments included Yoda1 (Tocris, catalog 5586).

Immunostaining: Mouse PSCs were fixed with 4% paraformaldehyde for 10 minutes at room temperature and then treated with 0.1% Triton X-100 (2). Cells were immunostained with a chicken anti-GFAP antibody (Abcam, 4674) or rabbit anti-Cre antibody (Novus Biological, NB1000-56133) at 2°C–8°C. Secondary antibodies included DyLight 488–conjugated anti–chicken IgG (Jackson ImmunoResearch, 703-546-155), or CY 3-conjugated anti–rabbit IgG (Jackson ImmunoResearch), used for 1 hour at room temperature. All images were captured with a Zeiss Axio observer Z1 with a 20× objective.

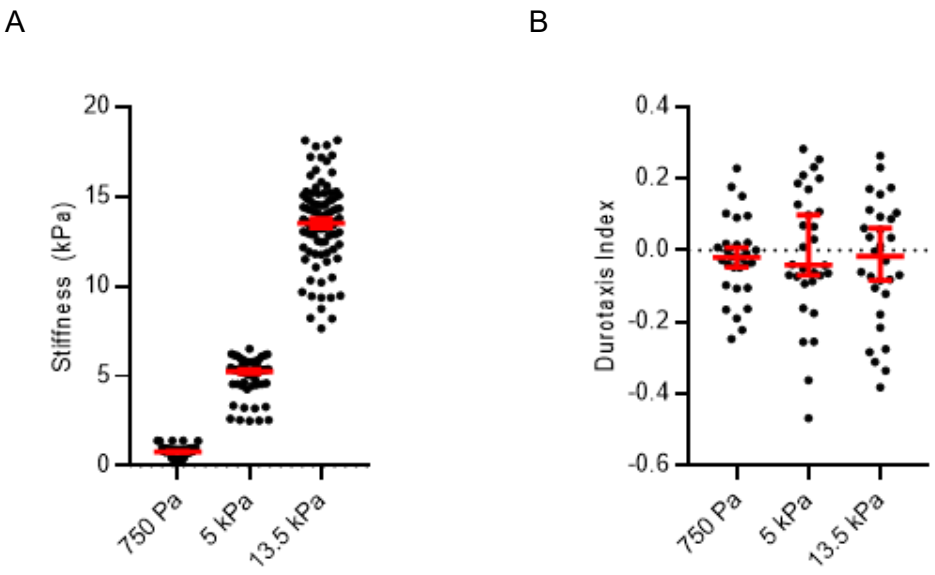

Fig. S1. PSCs on hydrogels with constant stiffness migrate non-directionally

A) Scatter plot shows homogeneous gel stiffnesses measured from n≥10 points of N=5 gels. **B)** Scatter plot indicates durotaxis indices of PSCs derived from the trajectories in Fig. 1B. (n=30 PSCs in N=3 independent experiments). Data in (A) is mean ± SEM and in (B) median ± 95% CI. Statistical test in (B) is one-sample t-test.

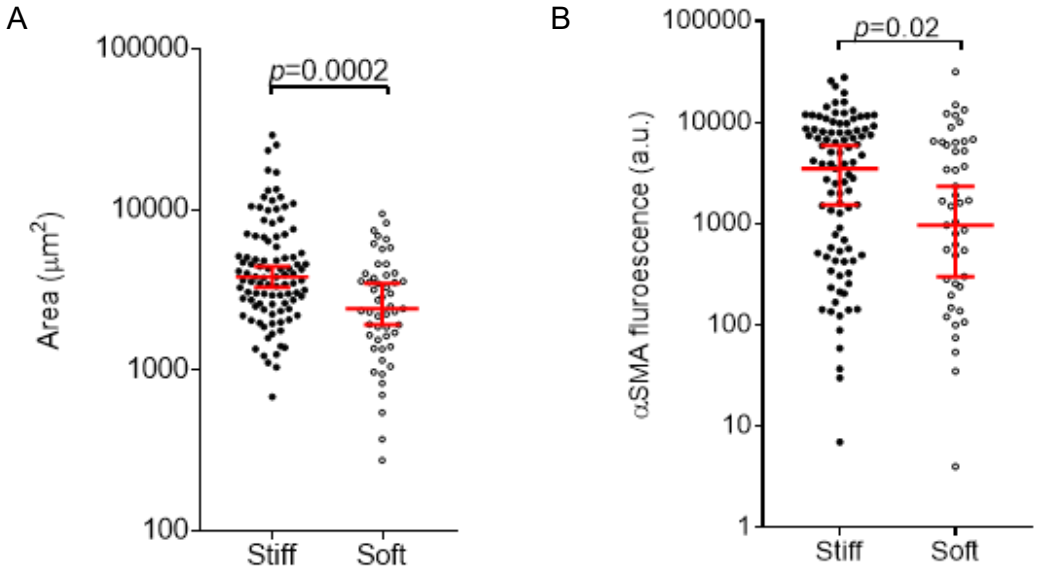

Fig. S2. PSC immunostaining reveals higher cell area and αSMA intensity on a stiffer substrate

A) Scatter plot shows cellular areas derived from αSMA-stained PSCs depicted in Fig. 2E. **B)** Scatter plot of αSMA fluorescence intensity of PSCs on the stiff and soft parts of the gradient hydrogel. n cells measured / N experiments ≥ 51/4. Data in (A) and (B) are median ± 95% CI. Statistical test are Mann-Whitney U-tests.

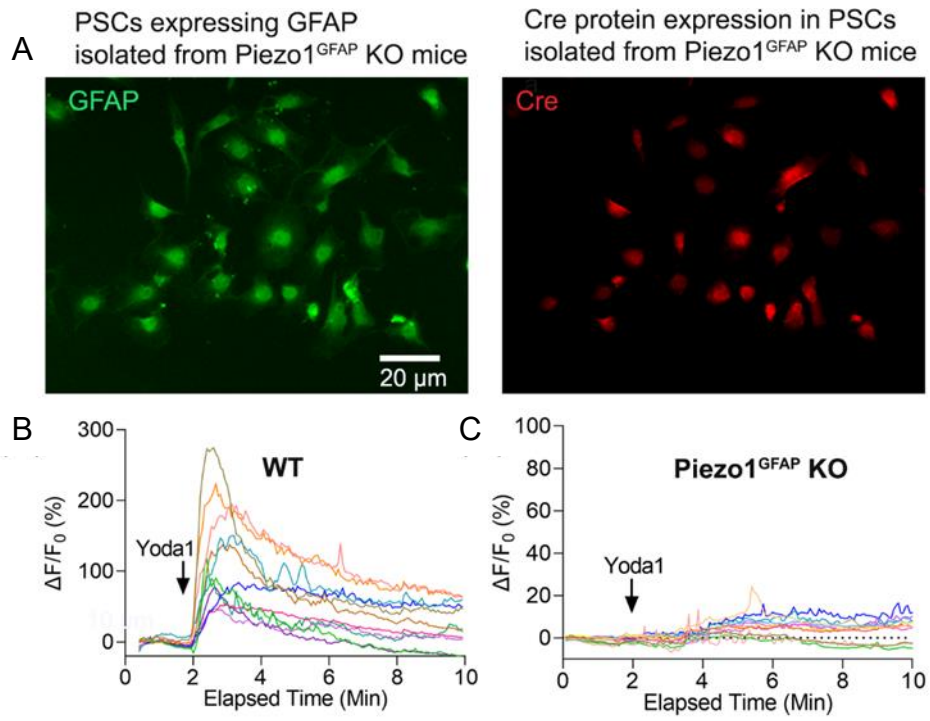

Fig. S3. Piezo1 deletion in GFAP expressing mouse PSCs

A) Images showing PSCs isolated from mouse line B6. Cg-Tg (GFAP- cre/ERT2; *Piezo1$^{fl/f}$*) after tamoxifen injection (referred to as Piezo1^{GFAP} KO mice) cultured for 3 days in a Matrigel-coated plate as described previously (1), expressed both stellate cell marker GFAP (green) and Cre protein (red). Scale bar: 20 µm. **B)** and **C)** Piezo1 agonist Yoda1 (5 µM) induces an elevation of the intracellular Ca^{2+} concentration in PSCs isolated from wild type but not from Piezo1^{GFAP} KO mice, confirming that successful deletion of Piezo1 in GFAP expressing PSCs, n=12 cells. Each trace represents Yoda1-induced single cell relative fluorescence intensity ($\Delta F/F_0$) of the calcium 6-QF dye over time. ΔF is the change in fluorescence intensity ($F-F_0$), and F_0 is the basal fluorescence intensity before Yoda1 application.

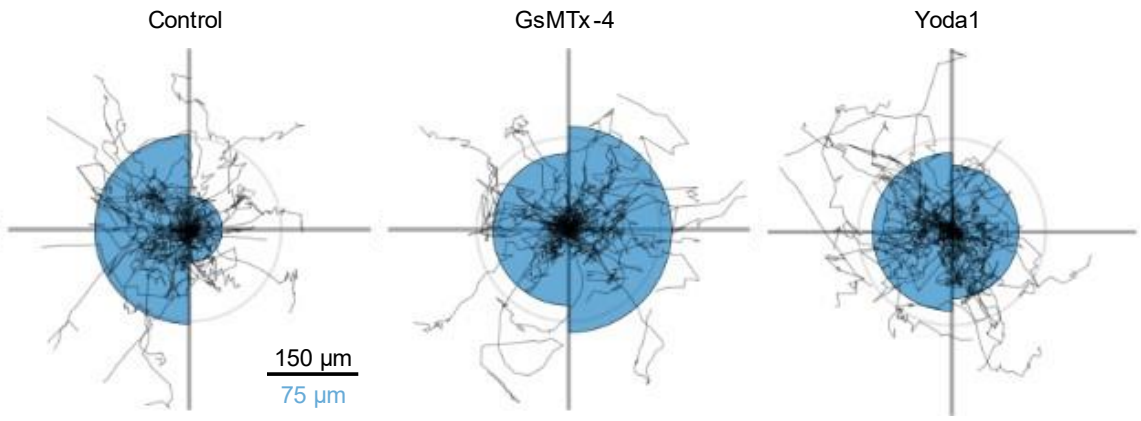

Fig. S4. Durotaxis polar plots of PSCs with pharmacological Piezo1 modulation

The durotaxis plot data aims to support Fig. 3C. Durotaxis polar plots depict individual PSC trajectories over 24 h (black lines). The radius of the blue half circles is proportional to the mean cellular displacement into the directions 0° and 180°, respectively. Radial lines indicate 0°, 90°, 180°, 270°.

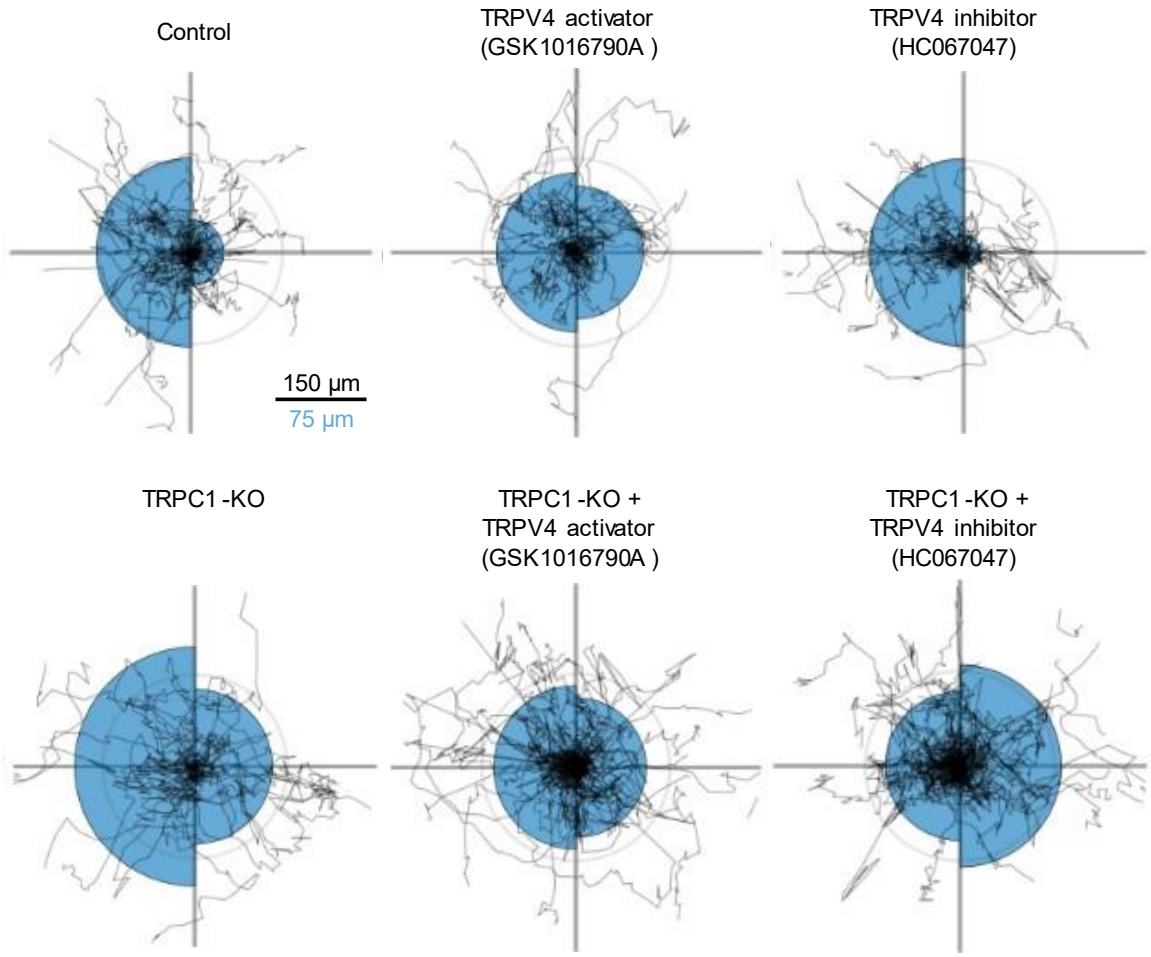

Fig. S5. Durotaxis polar plots of PSCs with TRPV4 and TRPC1 modulation

The durotaxis plot data aims to support Fig. 5A. Durotaxis polar plots depict individual PSC trajectories over 24 h (black lines). The radius of the blue half circles is proportional to the mean cellular displacement into the directions 0° and 180°, respectively. Radial lines indicate 0°, 90°, 180°, 270°.

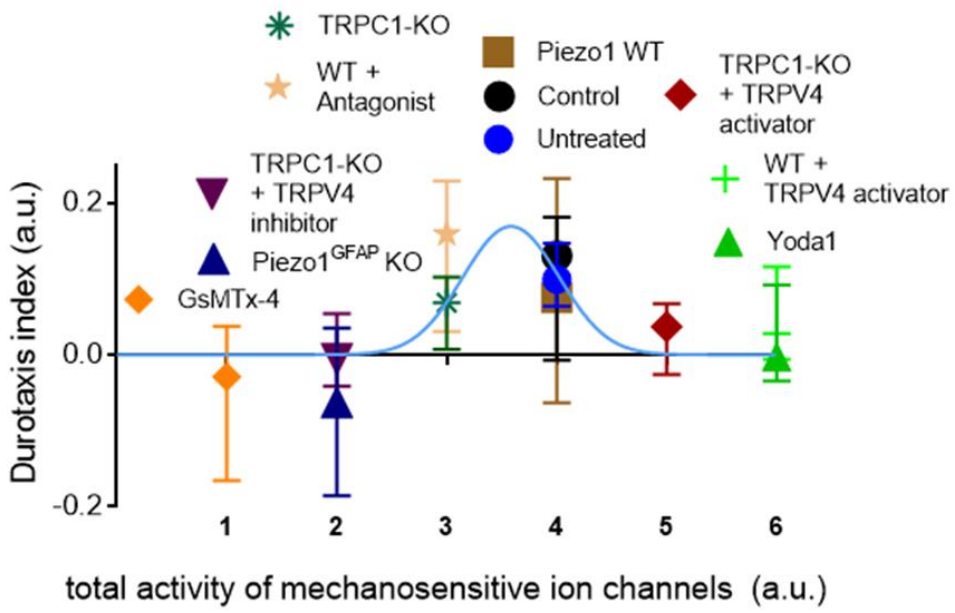

Fig. S6. Durotaxis requires intermediate activity of mechanosensitive ion channels

The depicted data shows details regarding Fig. 5C. Scatter plot shows the durotaxis index as a function of the total ion channel activity, derived from Fig. 5A and B. Inhibitor and activator refer to TRPV4 modulation with HC-0606006 and GSK101489, respectively. The blue line corresponds to a gaussian curve fit from Fig. 5c, indicating the bell-shaped relationship between channel activity and durotaxis. Data points are median ± 95% CI.

Supporting Tables

Table S1. Acrylamide mixture used for hydrogels with constant stiffness

Acrylamide (%)	Bisacrylamide (%)	Hydroxyacryl-amide (%)	Volume Acrylamide mix (µl)	Volume PBS (µl)	Stiffness
2,5	0,07	0,8	53	447	750 Pa
3,54	0,1	1,15	75	425	5 kPa
7,08	0,2	2,3	150	350	13.5 kPa

Supporting References

1. S. M. Swain, J. M. J. Romac, S. R. Vigna, R. A. Liddle, Piezo1-mediated stellate cell activation causes pressure-induced pancreatic fibrosis in mice. *JCI Insight* **7** (2022).
2. S. M. Swain, R. A. Liddle, Piezo1 acts upstream of TRPV4 to induce pathological changes in endothelial cells due to shear stress. *Journal of Biological Chemistry* **296** (2021).