

Supplementary Material

# Piezo1 Channels Contribute to the Regulation of Human Atrial Fibroblast Mechanical Properties and Matrix Stiffness Sensing

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**Citation:** Emig, R. Piezo1 Channels Contribute to the Regulation of Human Atrial Fibroblast Mechanical Properties and Matrix Stiffness Sensing. *Cells* **2021**, *10*, 663. <https://doi.org/10.3390/cells10030663>

Academic Editor: Cord Brakebusch, Kate Möller Herum

Received: 25 January 2021

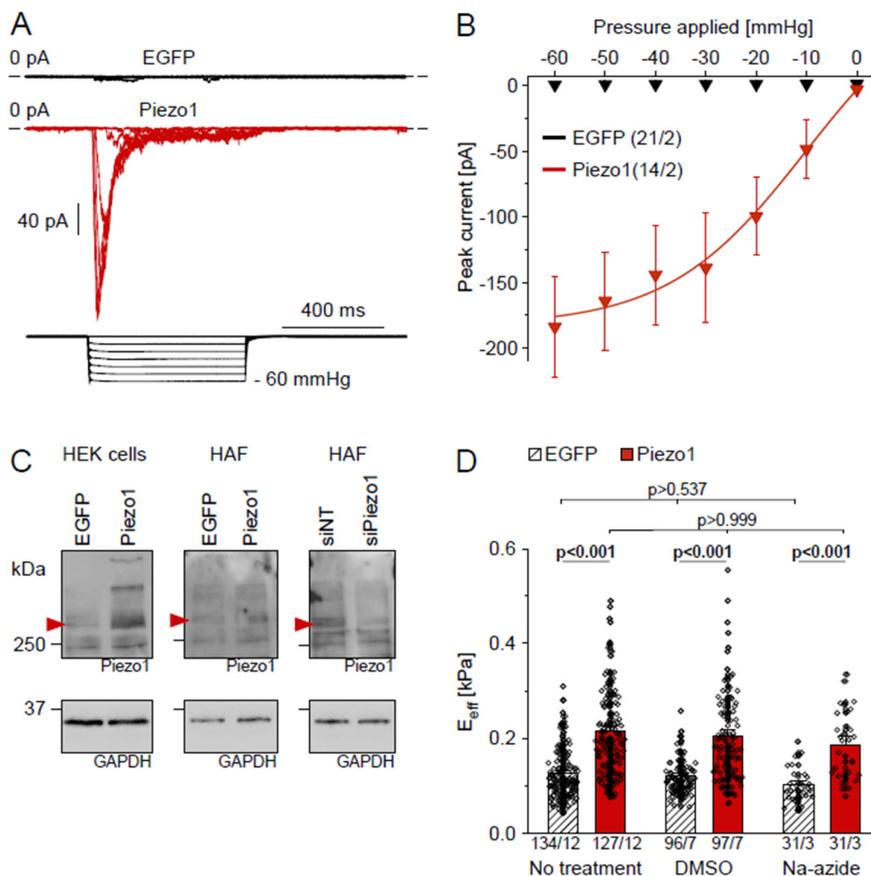
Accepted: 12 March 2021

Published: 16 March 2021

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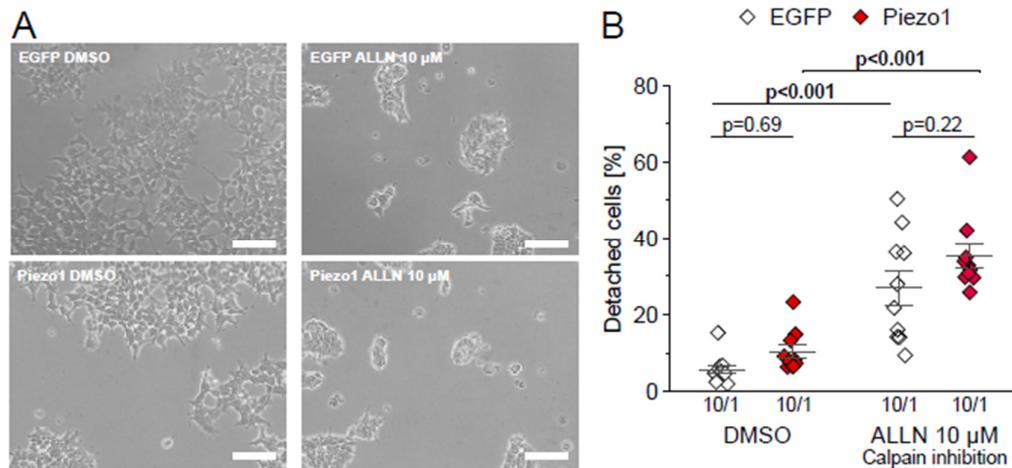


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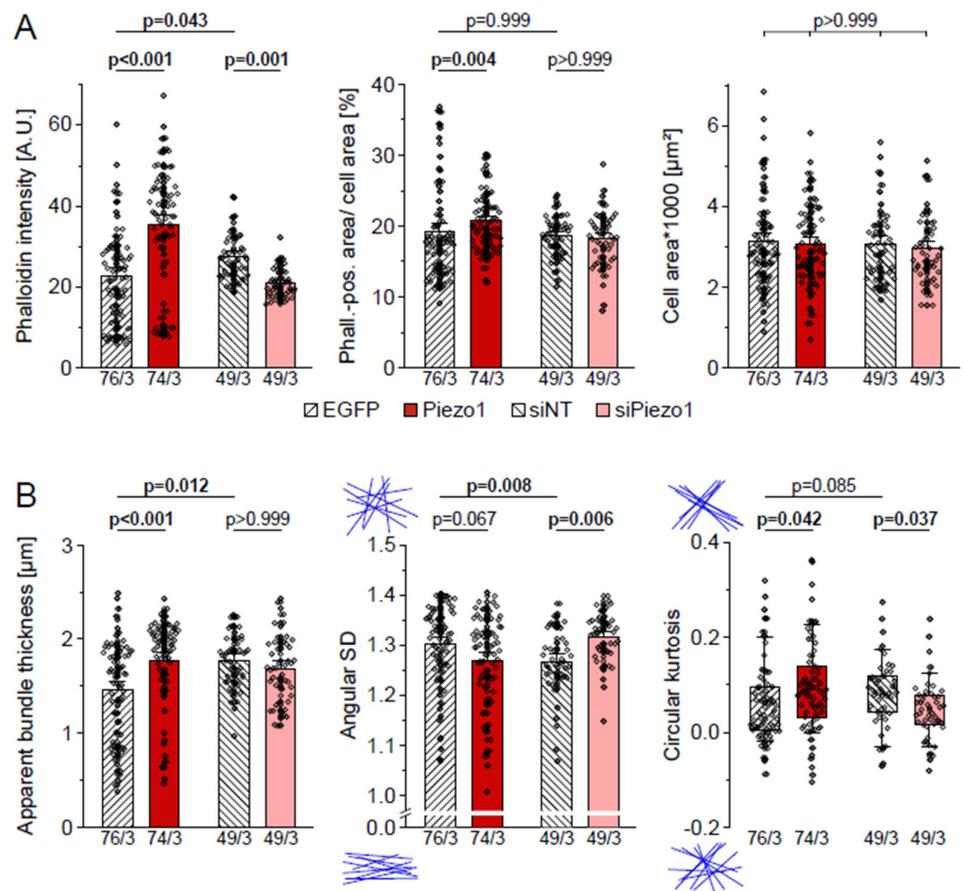


**Figure S1.** Overexpression of Piezo1 results in higher stretch-induced current, protein level and cell stiffness. (A) stretch-activated channel activity was recorded with the patch-clamp technique

in cell-attached mode at  $-80$  mV 3 days post-transfection. Channel activity was elicited by pulses of negative pressure (bottom trace) applied through the patch pipette to stretch the membrane of HEK cells overexpressing EGFP (top trace) or Piezo1 (middle trace). **(B)** corresponding peak currents for all pipette pressures tested (from 0 to  $-60$  mmHg) with EGFP or Piezo1. **(C)** whole cell lysates of HEK cells and HAF were prepared 3 days post-transfection of EGFP, Piezo1, non-targeting siRNA (siNT) or Piezo1-targeting siRNA (siPiezo1), separated by polyacrylamide gel electrophoresis, and immunoblotted for Piezo1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Red arrow heads indicate the expected position of monomeric Piezo1 slightly above 250 kDa. **(D)** Comparison of cell stiffness from all control groups that were either not treated at all, or treated with the solvents of the compounds used in this study (Table 1). DMSO was used at 0.1% (*v/v*) and Na-azide at 0.09% (*v/v*). *n/N* = number of cells/ number of experiments.



**Figure S2.** Calpain-inhibition leads to changes in cell morphology and detachment in HEK cells. **(A)** representative images of cells treated with DMSO or 10  $\mu$ M ALLN for 48 h. **(B)** quantification of cell detachment in response to treatment with DMSO or 10  $\mu$ M ALLN. *n/N* = number of images/ number of experiments.



**Figure S3.** Piezo1-dependent alterations of the stiffness of HAF are the result of differential organization of the actin cytoskeleton. **(A)** average phalloidin intensity, phalloidin-positive area per cell area, and cell area. **(B)** apparent thickness, angular standard deviation (SD) and circular kurtosis of actin bundles. n/N = number of cells/ number of experiments.