

# Physical mechanism of Piezo1 stiffness sensing

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## Neurons sense environmental stiffness through Piezo1 ①

Rat hippocampal neurons show significantly higher spontaneous activity on soft versus stiff substrates, indicating tissue stiffness as an important parameter governing neuronal maturation (A, B). Knocking down Piezo1 in neurons grown on stiff substrates leads to the increased neuronal activity (C). This suggests that Piezo1 activity delays electrical maturation of neurons in stiffness dependent manner.

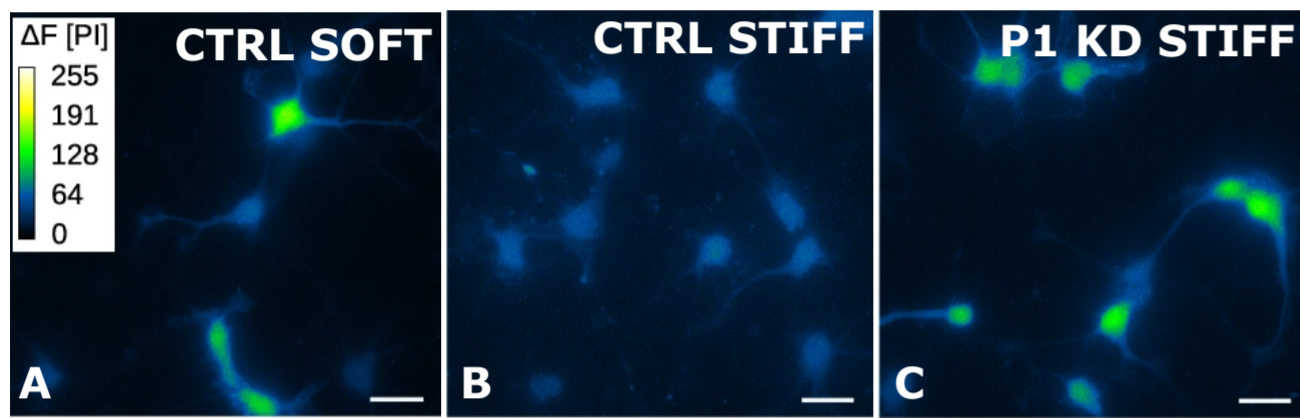
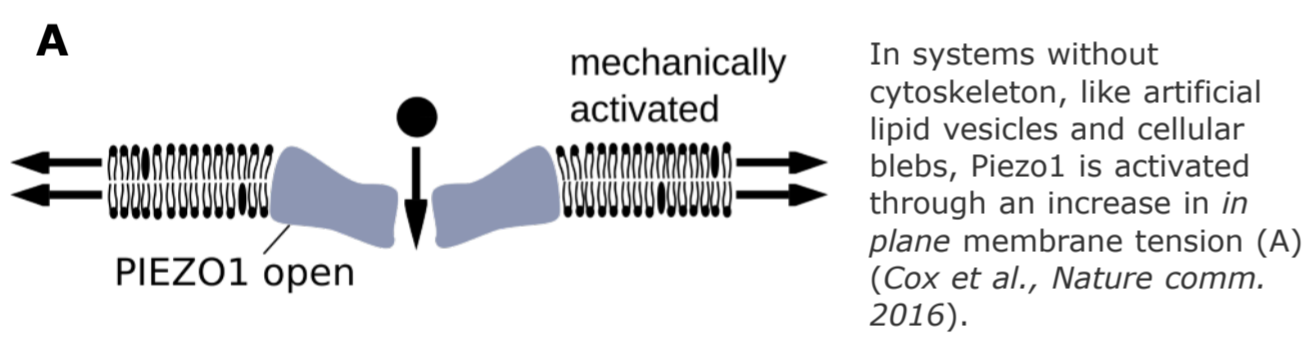


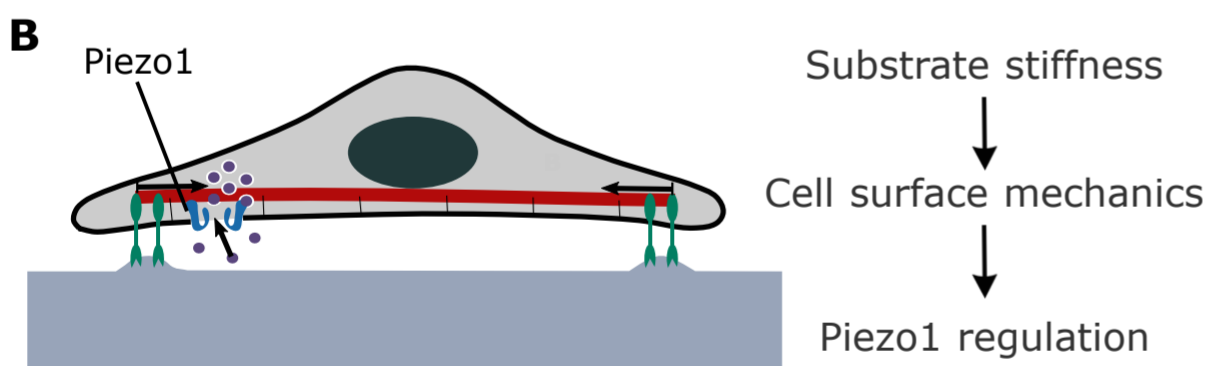
Figure 1 Calcium imaging of Rhod 4 loaded rat hippocampal neurons grown of soft (100 Pa) and stiff (10 000 Pa) laminin coated hydrogels.  $\Delta F$  is a fluorescent intensity change over 40s. Higher  $\Delta F$  indicates higher spontaneous activity of neurons.

Kreysing et al., Nature comm. (2025)

## Linking substrate stiffness to Piezo1 activation ②

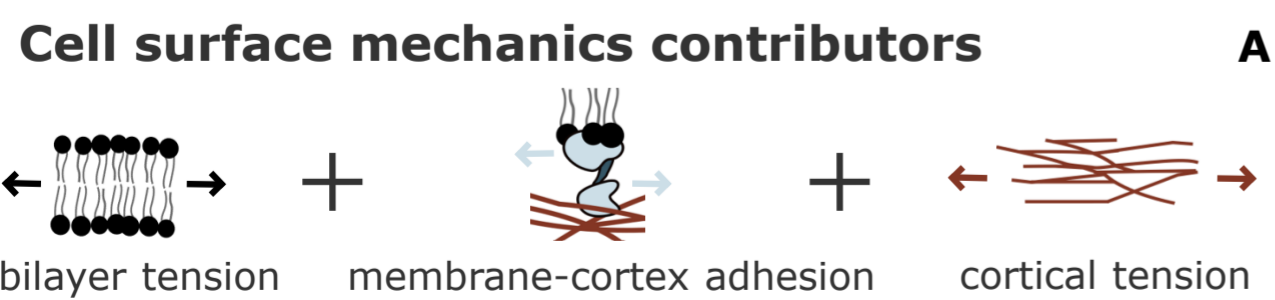


However, whether membrane tension alone is responsible for Piezo1 activation in cells remains unclear. Moreover, if and how membrane tension responds to stiffness changes is still poorly understood. We hypothesise that substrate stiffness alters cell surface mechanics which in turn regulates Piezo1 activation (B).

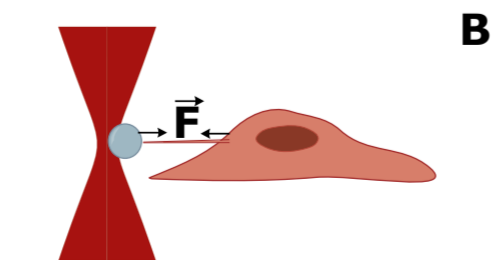


## Probing cell surface response to stiffness changes ③

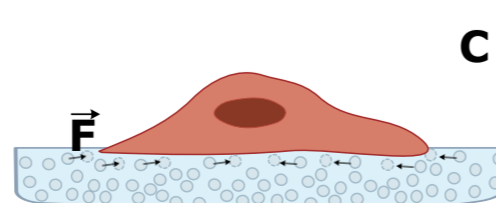
Mechanical properties governing cell force generation and effective membrane tension primarily come from the in plane tension of the lipid bilayer, degree of membrane attachment to the cortex, and actomyosin contractility (A). Assessing how these components respond to substrate stiffness would help clarify how they could contribute to Piezo1 activation.



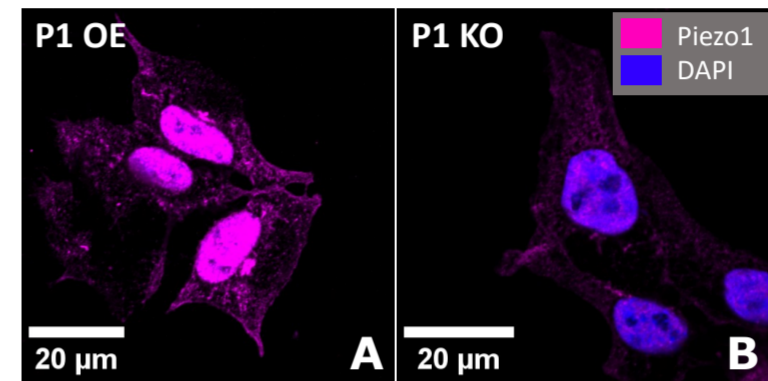
Effective membrane tension of the cell can be quantified by extracting membrane tethers from the cells using optical tweezers. The force that the cells exert on the probe is proportional to the square of membrane tension (B).



Cells use Myosin II-generated traction forces for probing the stiffness of the extracellular matrix. These forces can be quantified by embedding beads in the hydrogel of defined stiffness and tracking their displacement (C).

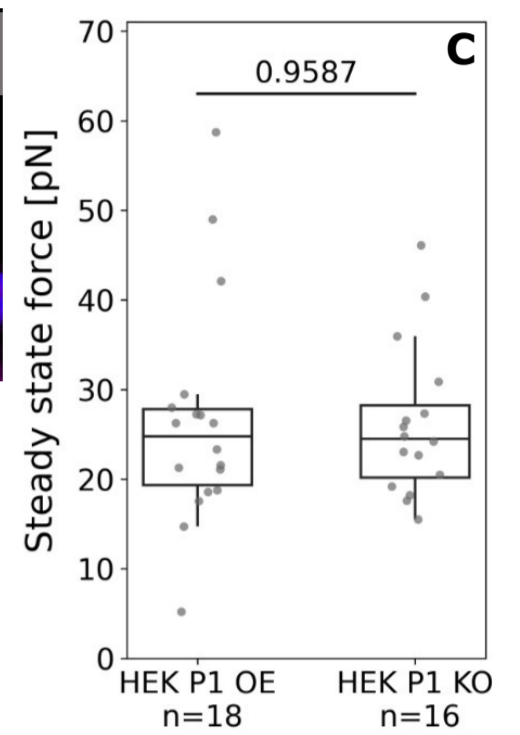


## Piezo1 expression does not affect membrane tension ④

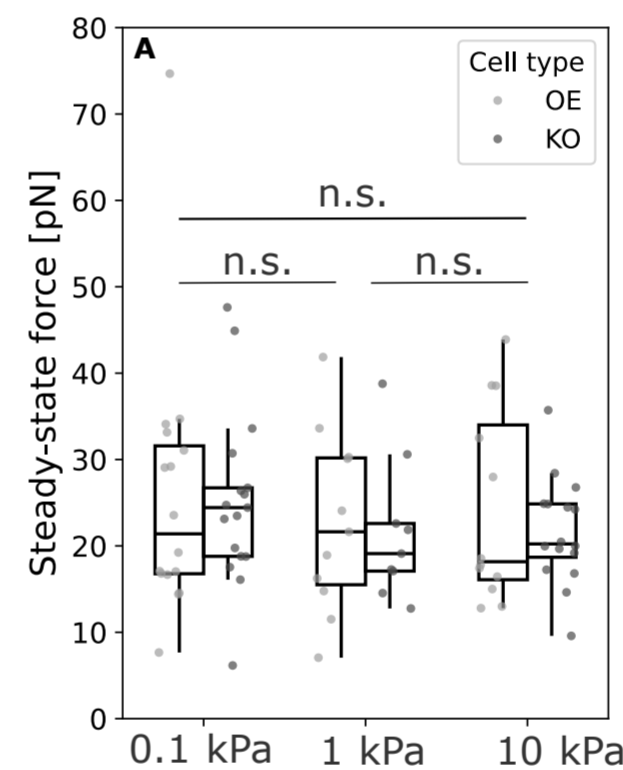


To determine whether Piezo1 expression levels alter the membrane tension we used HEK 293T cell lines (provided by Prof. Boris Martinac) with Piezo1 knock out and overexpression (A, B).

There was no significant difference in tether forces representing effective membrane tension between OE and KO cells measured by optical tweezers (C).



## Membrane tension is not altered by substrate stiffness ⑤

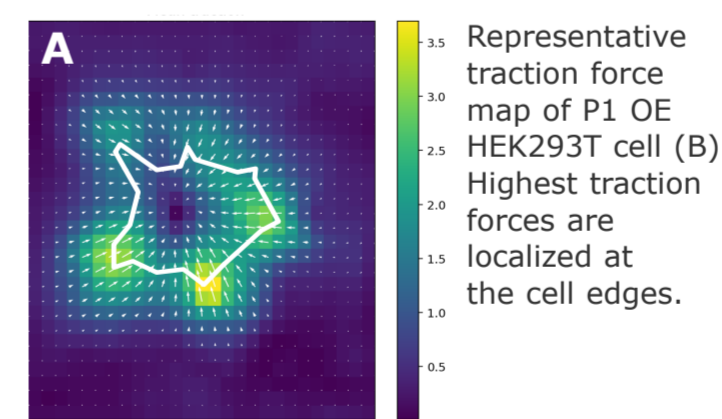


Steady state tether forces remain unchanged in Piezo1 knock out and overexpressed cells across biologically relevant range of hydrogel stiffnesses (0.1 kPa, 1kPa, 10kPa) (A).

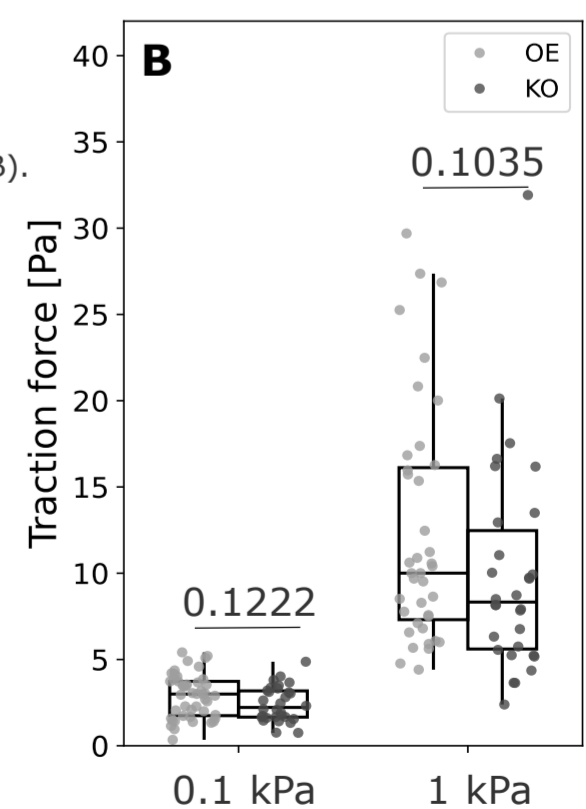
Therefore, neither Piezo1 expression levels or substrate stiffness significantly affect global effective membrane tension.

Taken together, these results suggest that stiffness dependent Piezo1 regulation may involve more localized tension changes at cell-substrate interface or cytoskeletal force transmission.

## Traction forces in Piezo1 OE and KO cells ⑥



Piezo1 knock out cells consistently exerted lower traction forces than overexpressing cells on both 0.1 and 1kPa, although the differences were not statistically significant (A). The difference in KO and OE generated forces is more prominent on 1kPa than 0.1kPa substrates. Additional experiments are required to determine if this reflects a stiffness dependent effect on Piezo1.



## Conclusion ⑦

Piezo1 is a key player in stiffness dependent neuronal maturation, however the mechanical pathway connecting substrate stiffness to the activation of Piezo1 remains unclear.

Effective membrane tension is not affected either by Piezo1 expression or substrate stiffness suggesting that stiffness sensing is not regulated through changes in membrane tension.

Local mechanical processes involving cytoskeletal force transmission may instead play an important role in Piezo1 stiffness sensing.