The Discovery of Piezo

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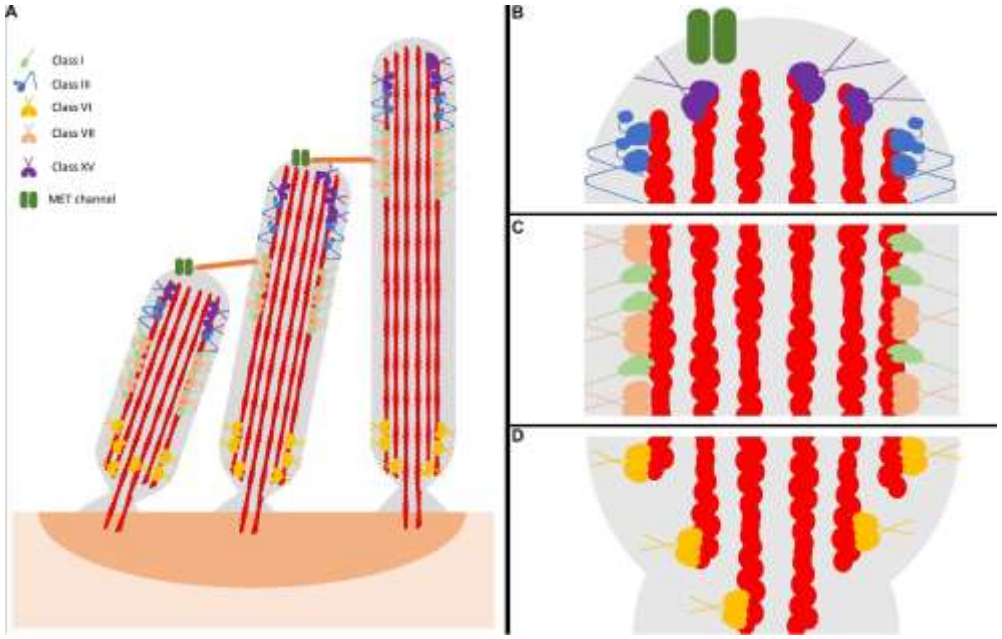
Discovery

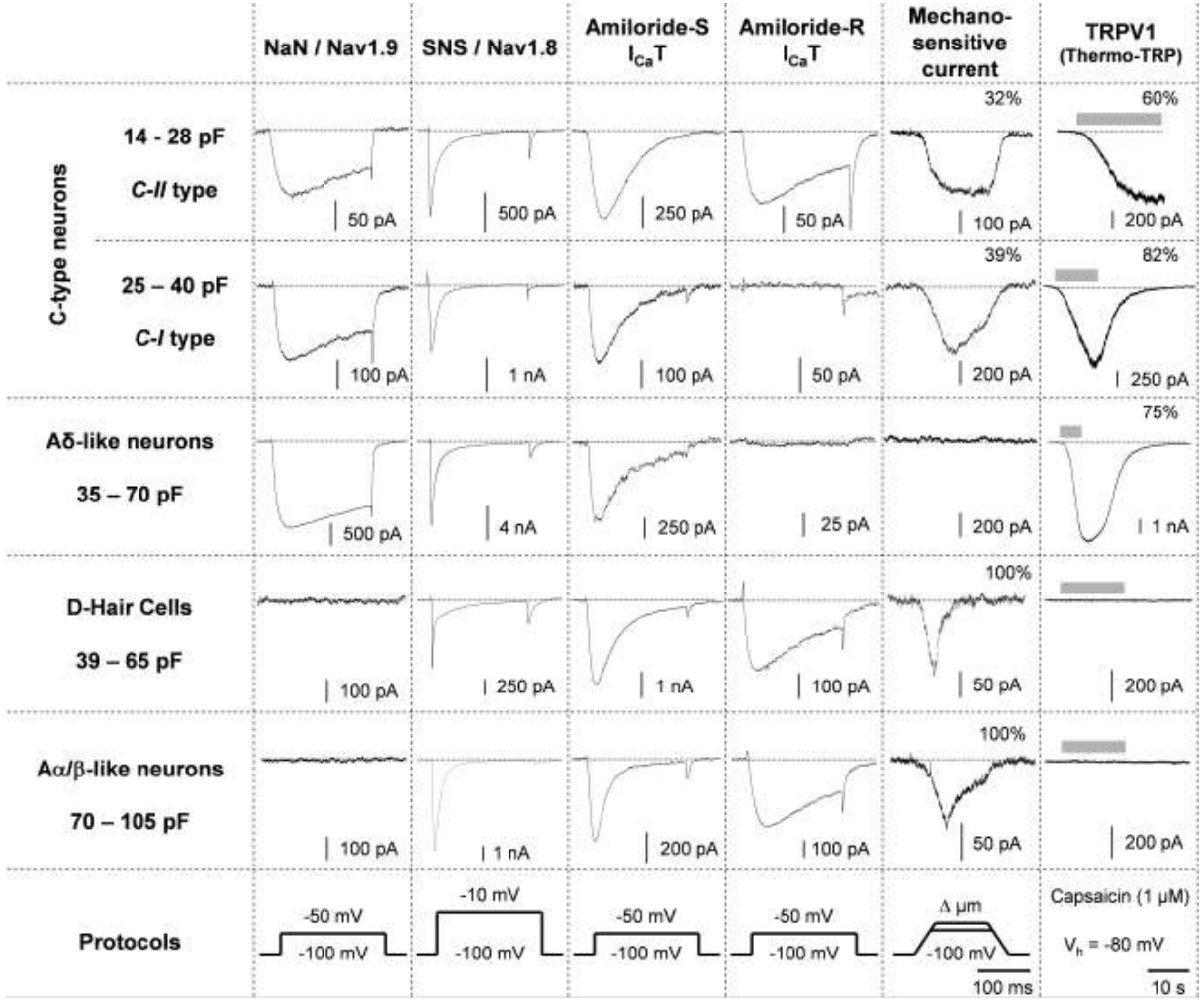
- Mechanically activated (MA) channels can transduce mechanical force into biological signals.
- mechanical force → the open of the channels
- Only few MA channels have been identified.



MET channel
(mechanoelectrical transduction)

Search for the model cells

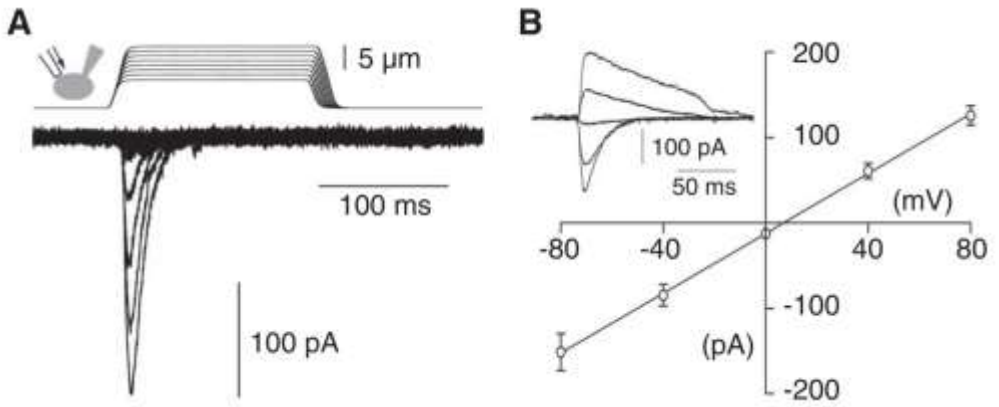
- Search for a cell line that can express MA currents according to previous research.

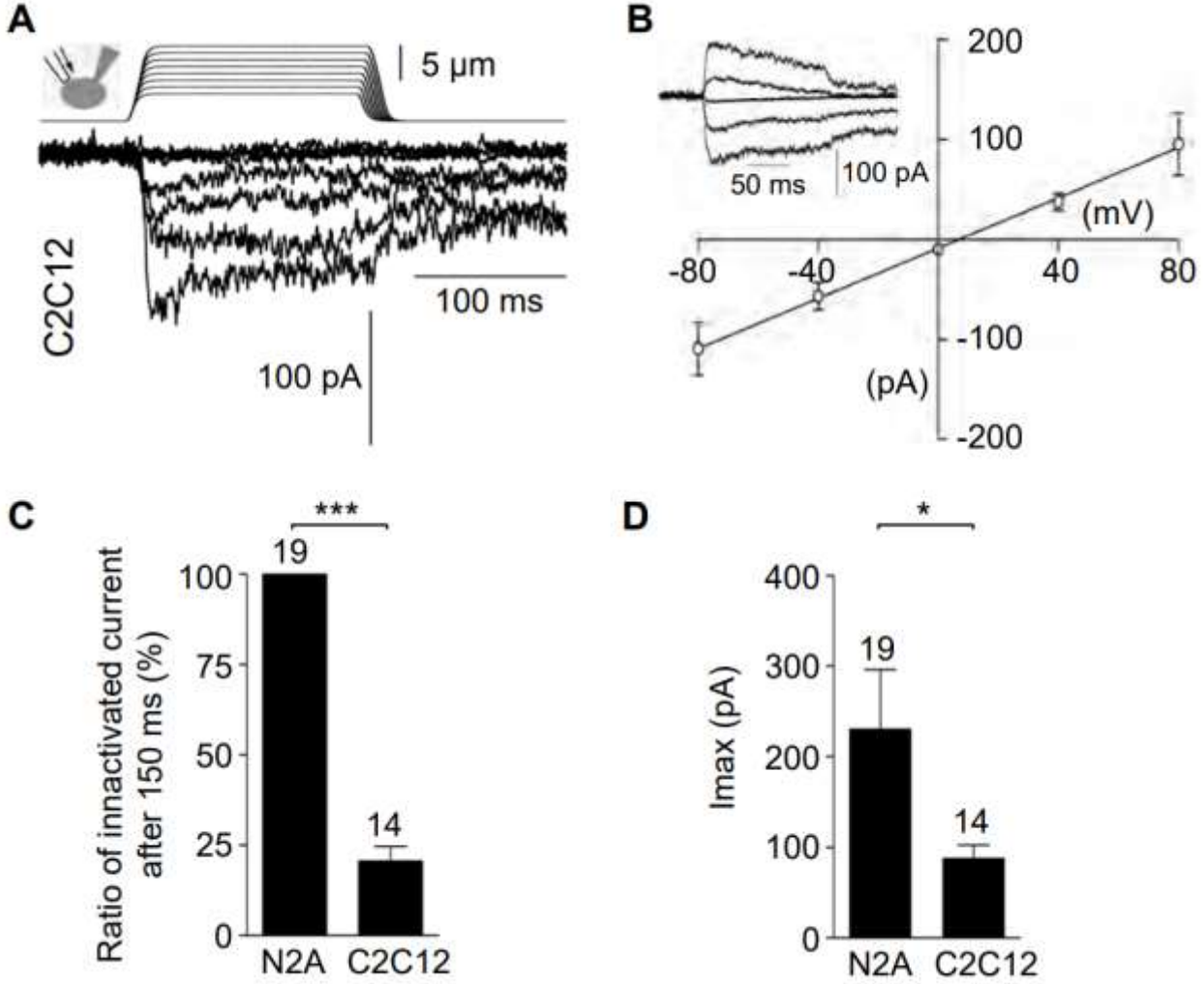


(Coste et al., 2006)

Neuro2A cells express MA currents

- Expressed the most consistent MA currents and showed relatively faster kinetics of adaptation as compared with that of other cell lines, such as C2C12s





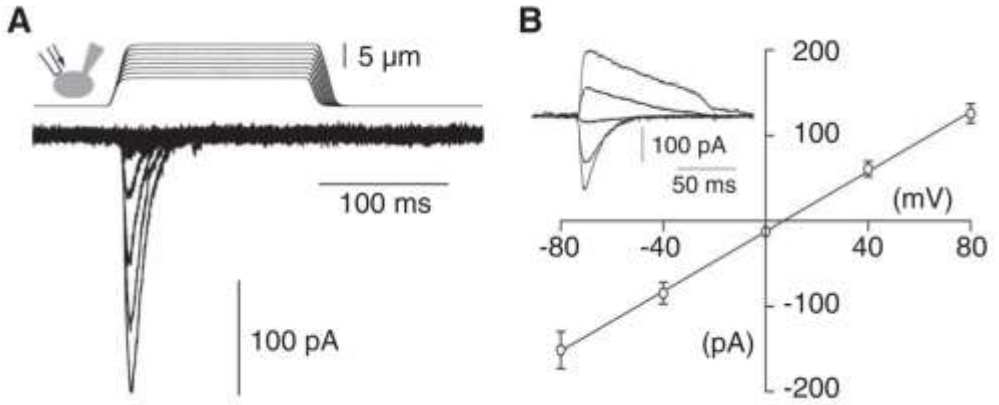
Details in the experiment

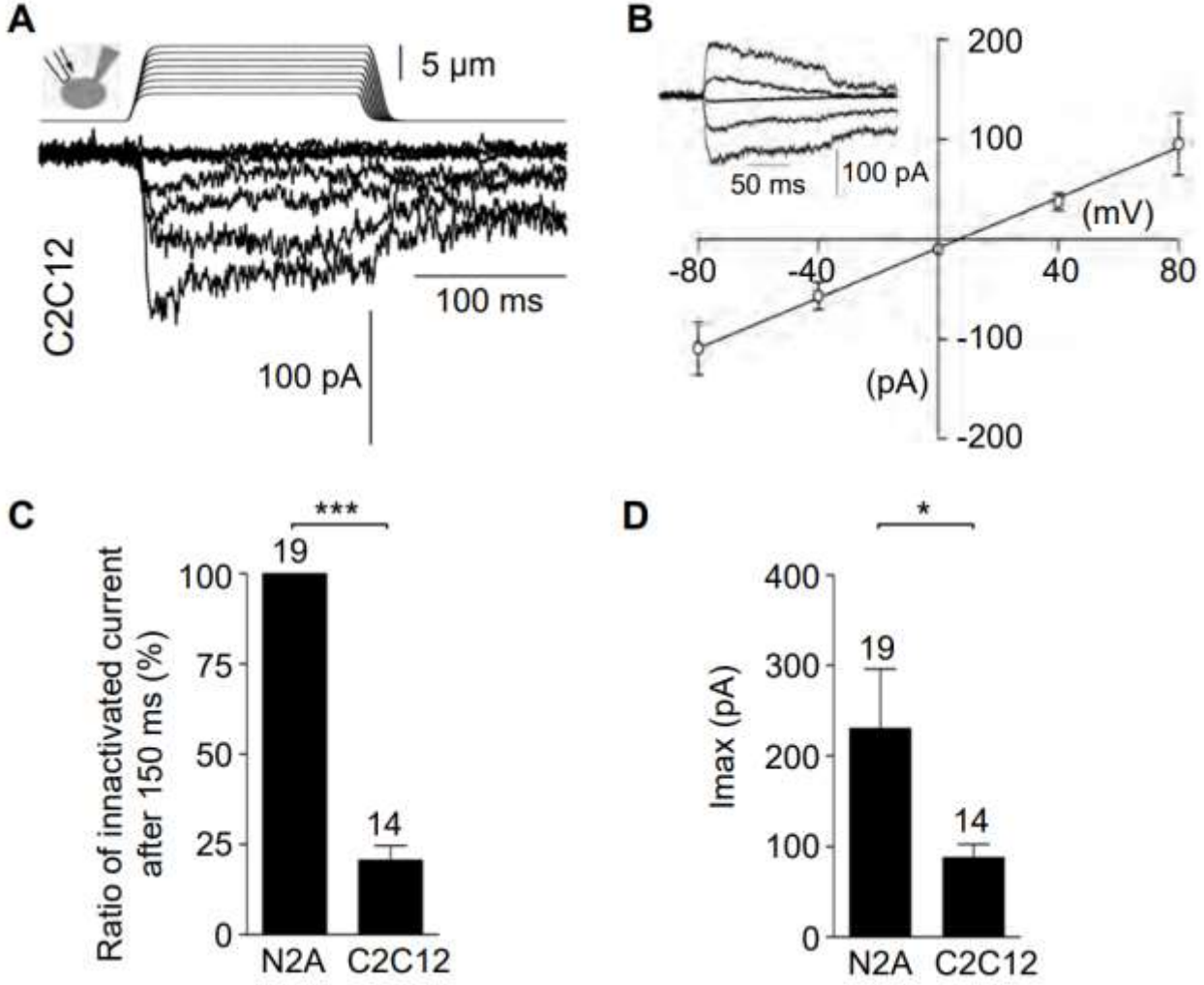
- Holding potential of -80 mV
- The stimulus was applied for 150 ms

- Inward current → -
- Outward current → +

Neuro2A cells express MA currents

- Expressed the most consistent MA currents and showed relatively faster kinetics of adaptation as compared with that of other cell lines, such as C2C12s

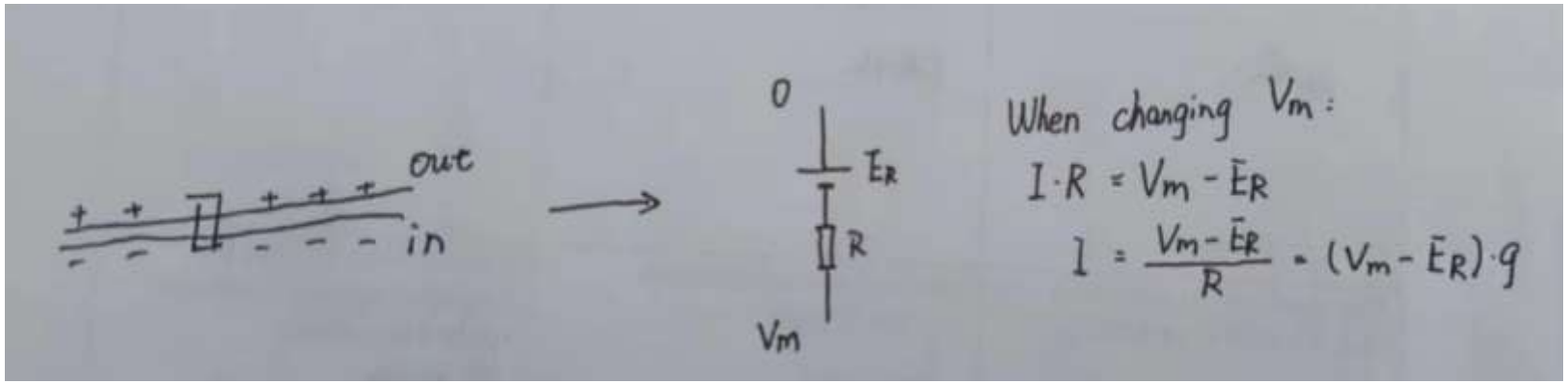


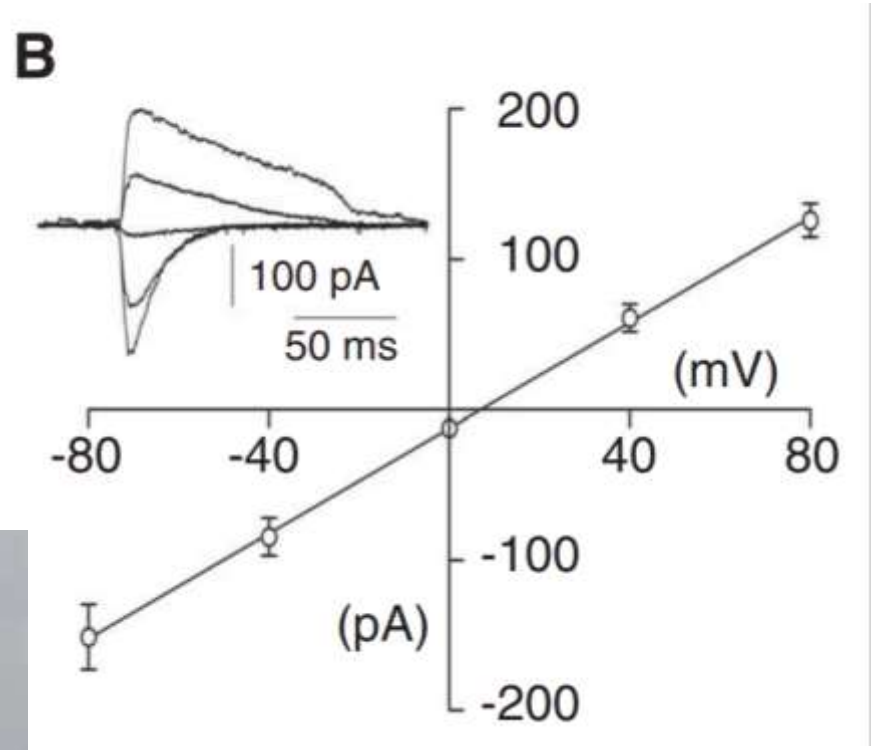


Details in the experiment

- Inward current → -
- Outward current → +

- g - conductance
- E_r - reversal potentials

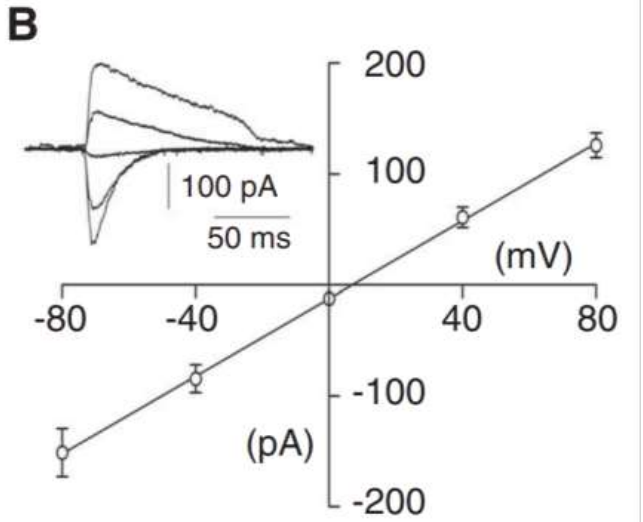


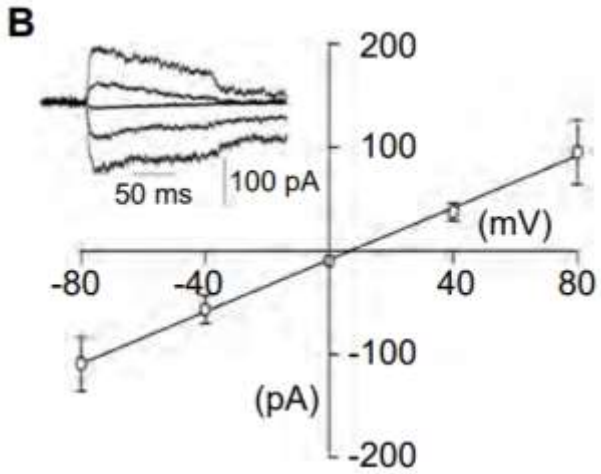


Neuro2A cells express MA currents

- Current-voltage relationships of N2A and C2C12 MA currents were linear between -80 and +80 mV with reversal potentials at +6.6 and +6.7 mV, respectively.

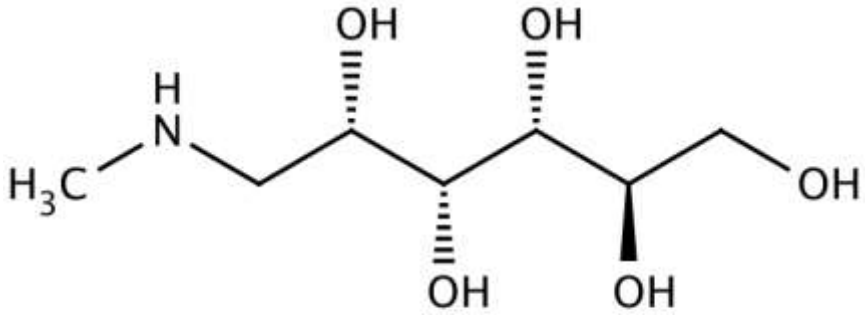
- C2C12 may also have the same channels, but it may be disturbed by other factors.

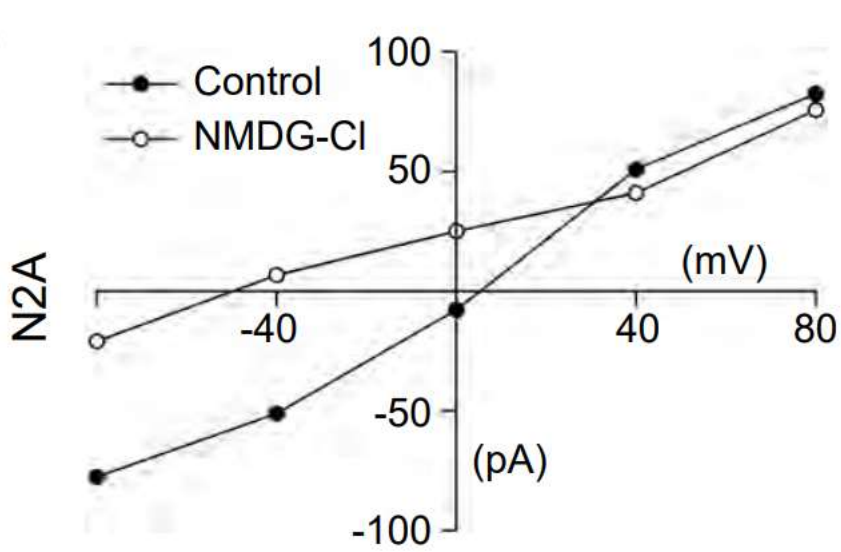




Cationic nonselective permeability

- Inward currents were suppressed with N-methyl-d-glucamine (NMDG)-chloride external solutions

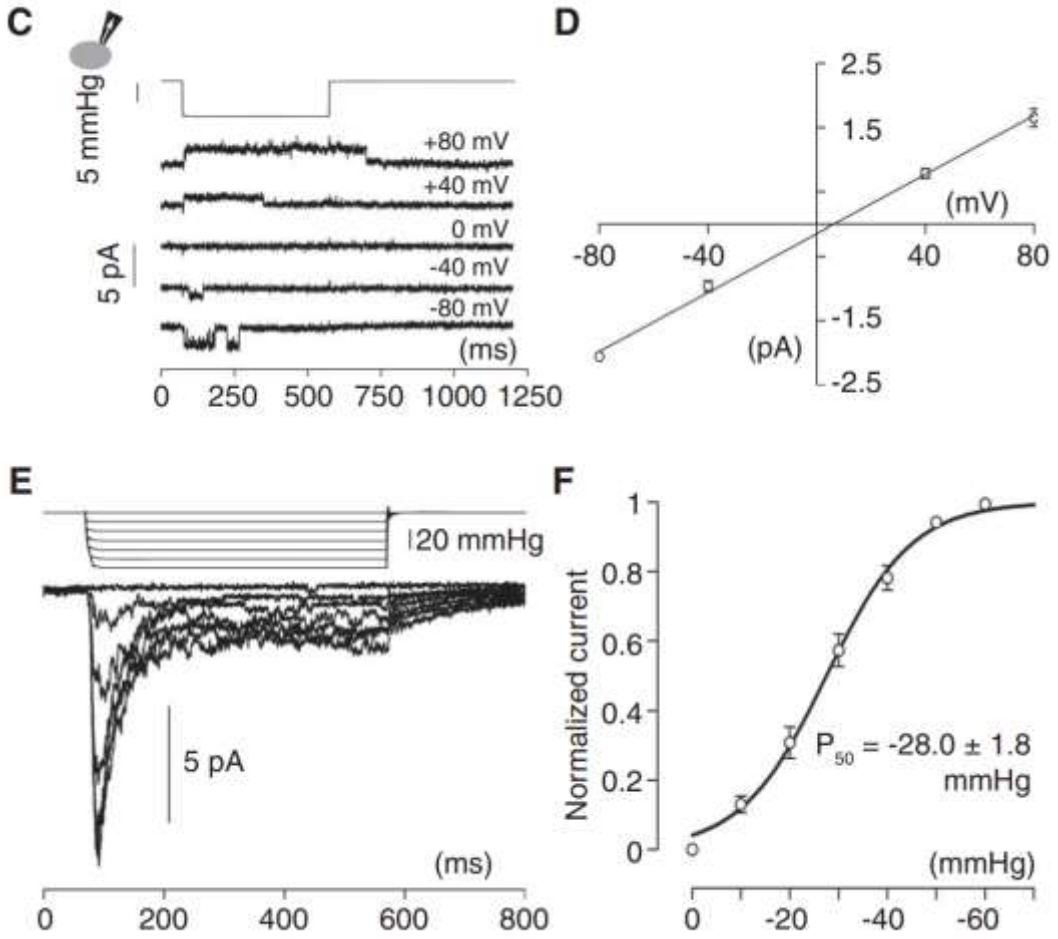




MA currents in response to suction

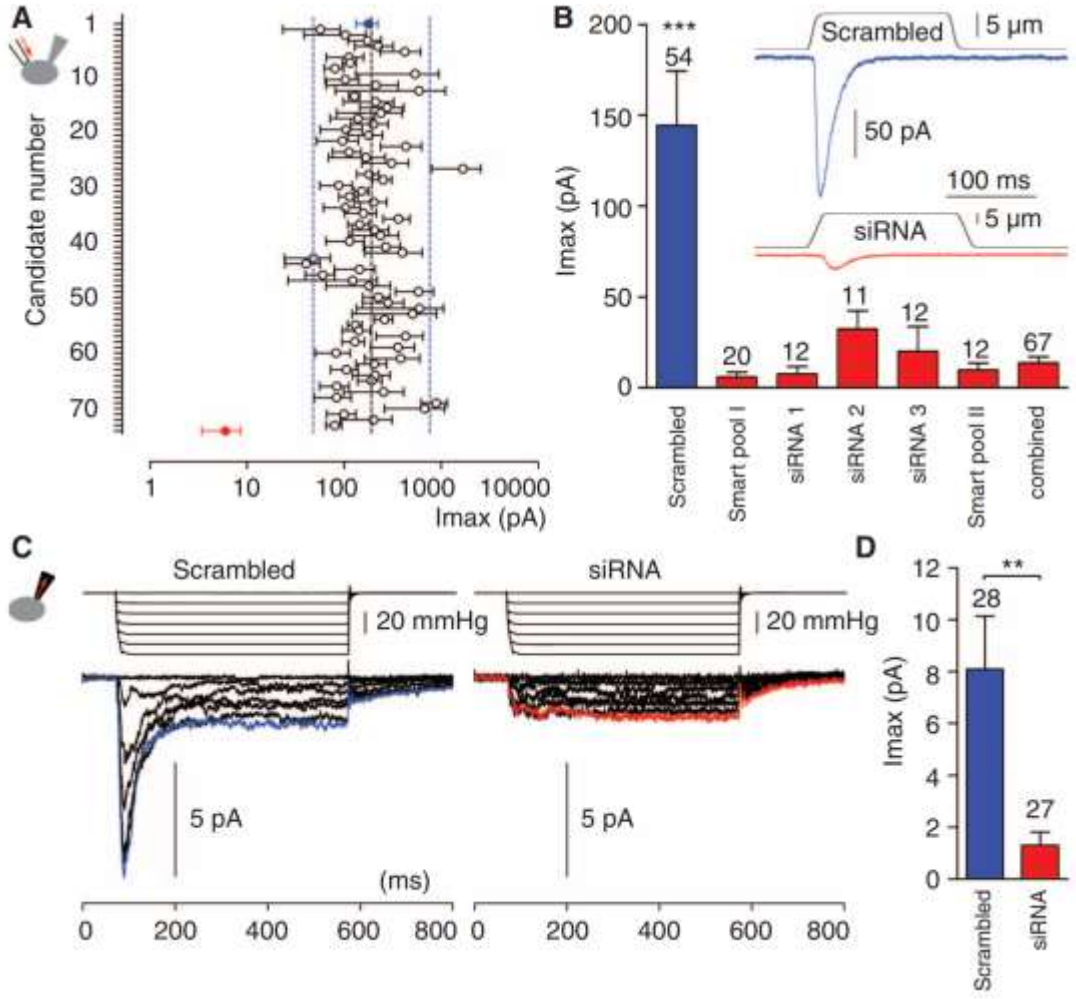
- A single-channel conductance of 22.9 ± 1.4 pS and Erev of +6.2 mV
- Half-maximal activation (P50) of -28.0 ± 1.8 mmHg

- These conductance and P50 values are similar to the properties of reported stretch-activated channels (29.2 ± 0.3 pS & 21.7 ± 2.4 mmHg …)



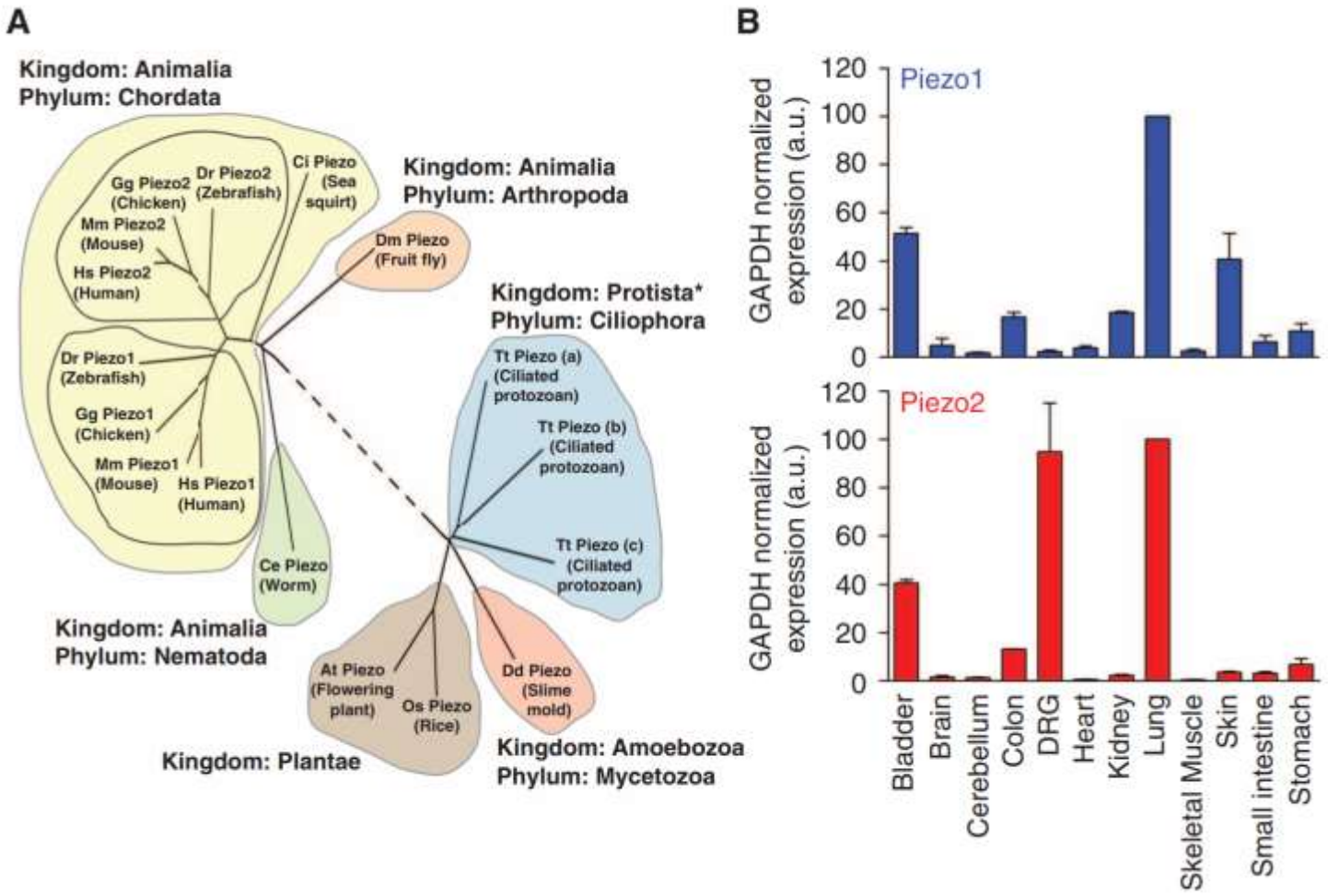
Piezo1 (Fam38A) is required for MA currents of N2A cells

- Use small interfering RNA (siRNA) knockdown in N2A cells



Piezos are large-transmembrane proteins conserved among various species

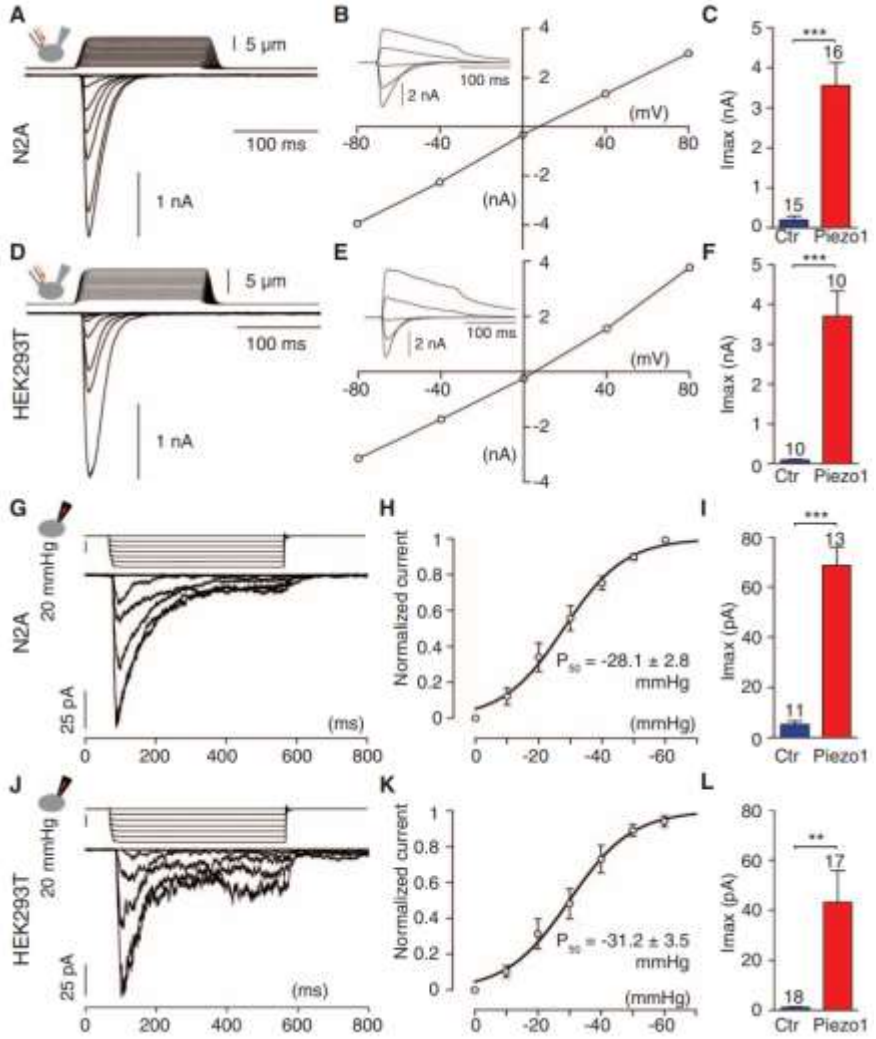
- 2100 to 4700 amino acids
- 24 to 36 predicted transmembrane domains
- GAPDH - loading control



Piezo1 induces MA currents in various cell types

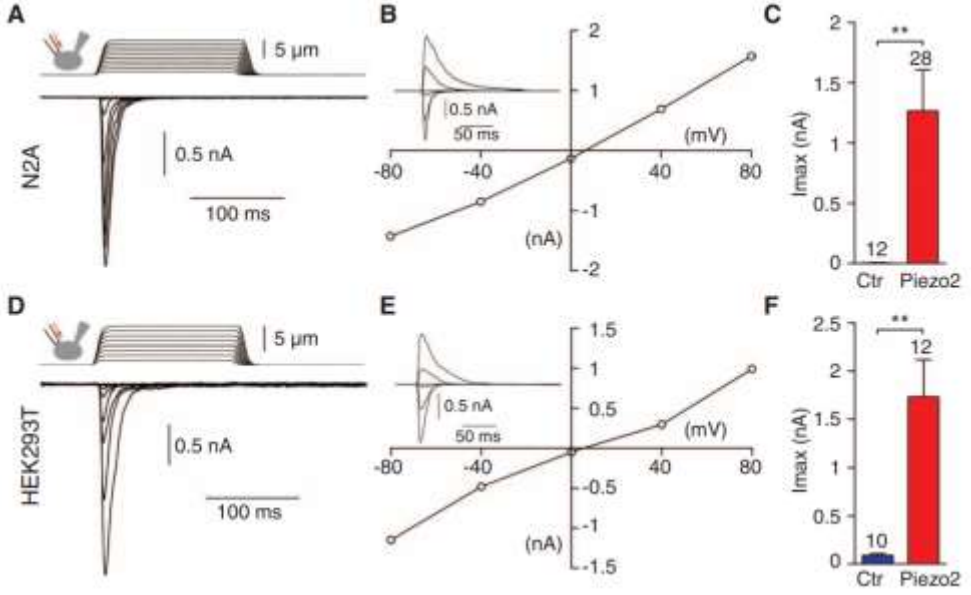
- Repeat all the previous experiments again.

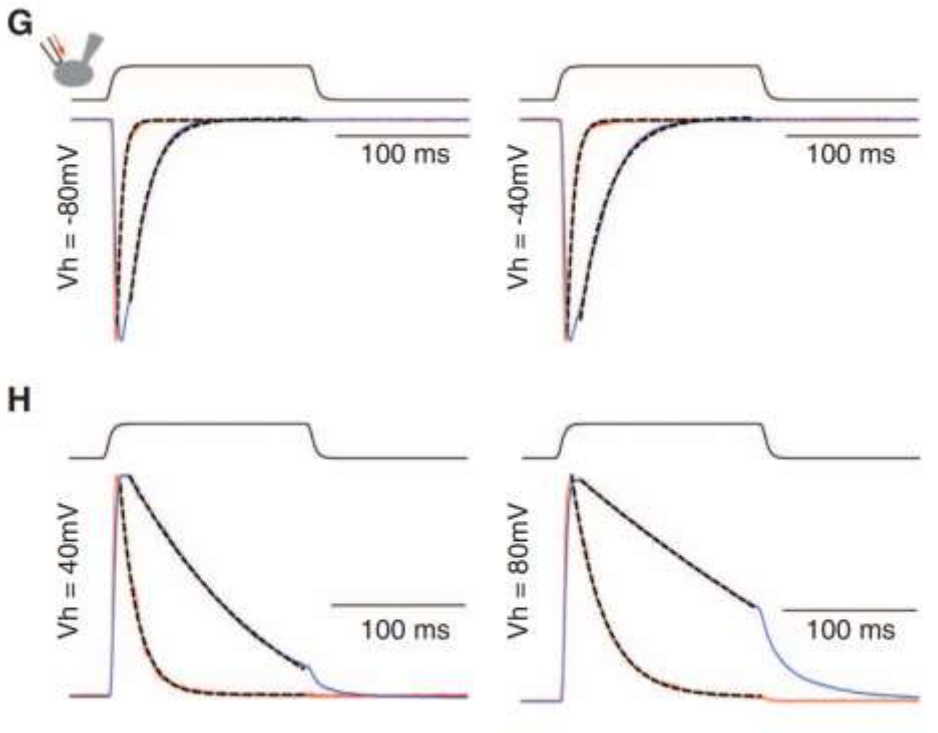
- nonselective permeability and a slight preference for Ca^{2+}

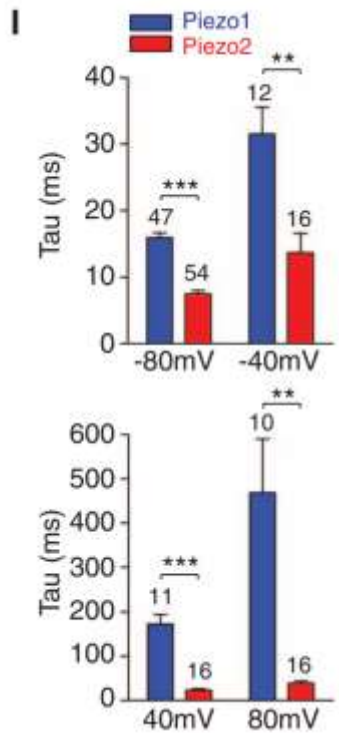


MA currents in cells overexpressing Piezo2

- Again!
- The kinetics of inactivation of Piezo2-dependent MA currents were faster than Piezo1-dependent MA currents

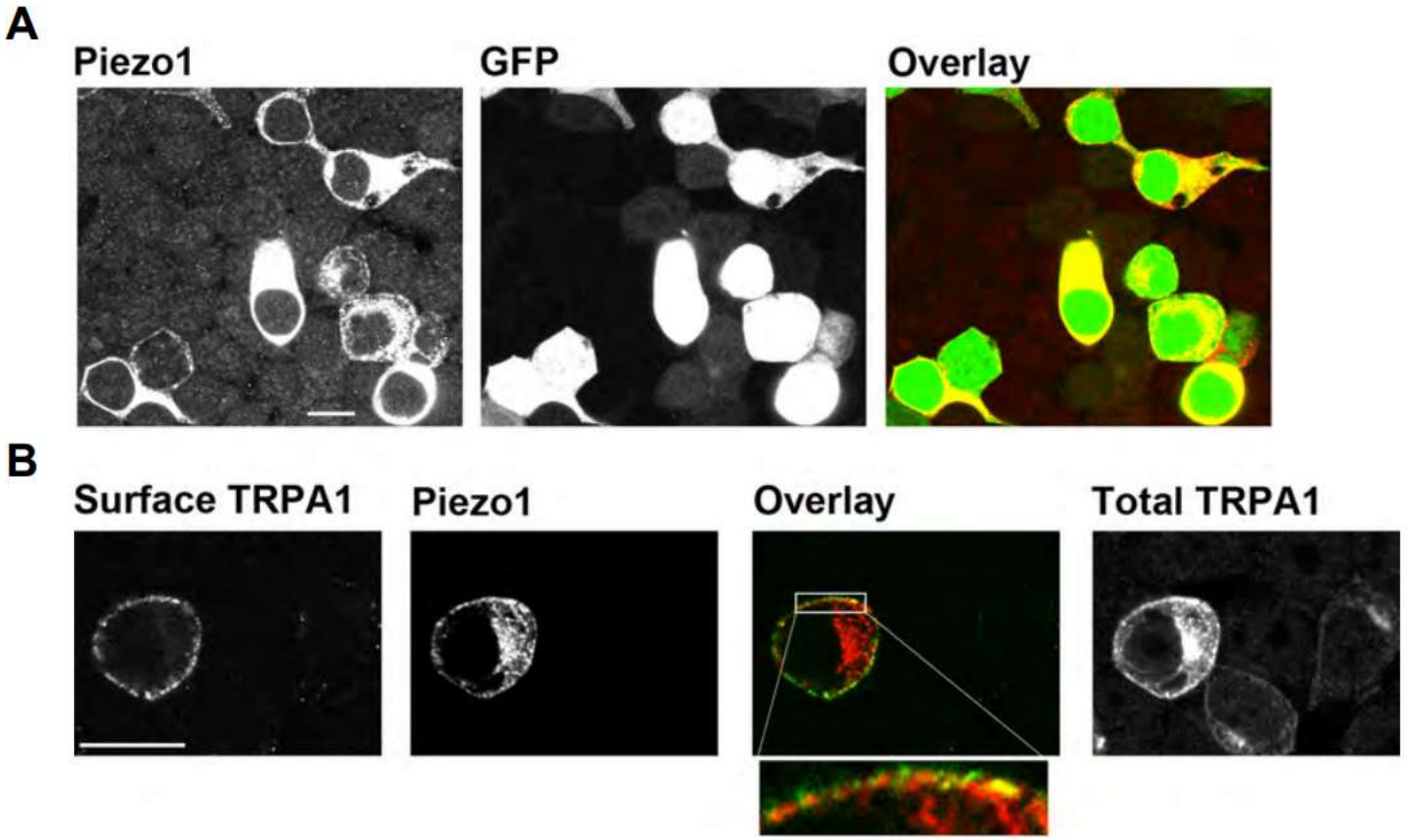






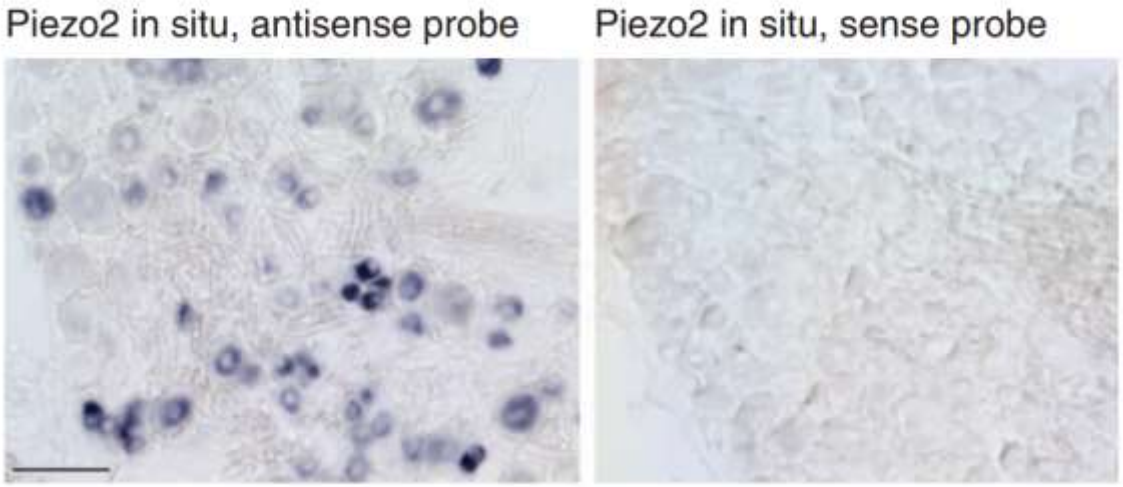
Piezo1 is detected at the plasma membrane

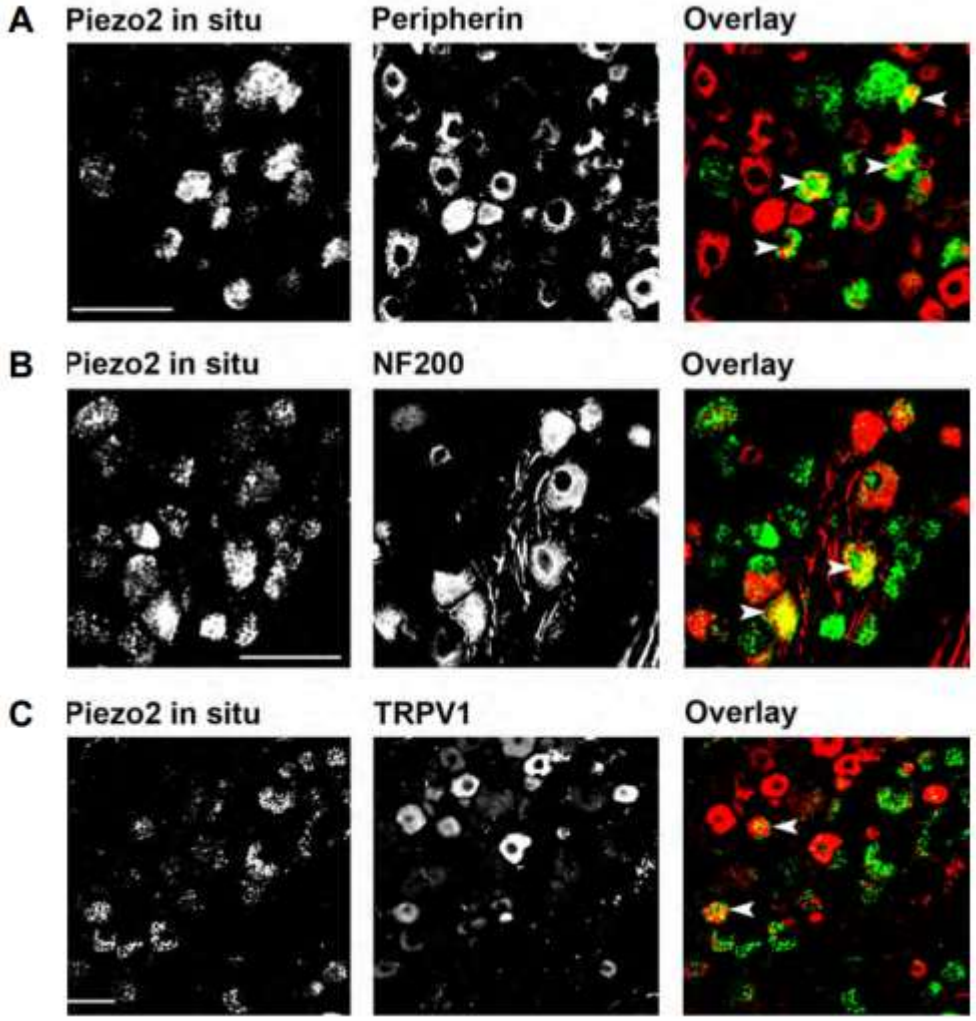
- Locate at or near plasma membrane



Requirement of Piezo2 for rapidly adapting MA currents in DRG neurons

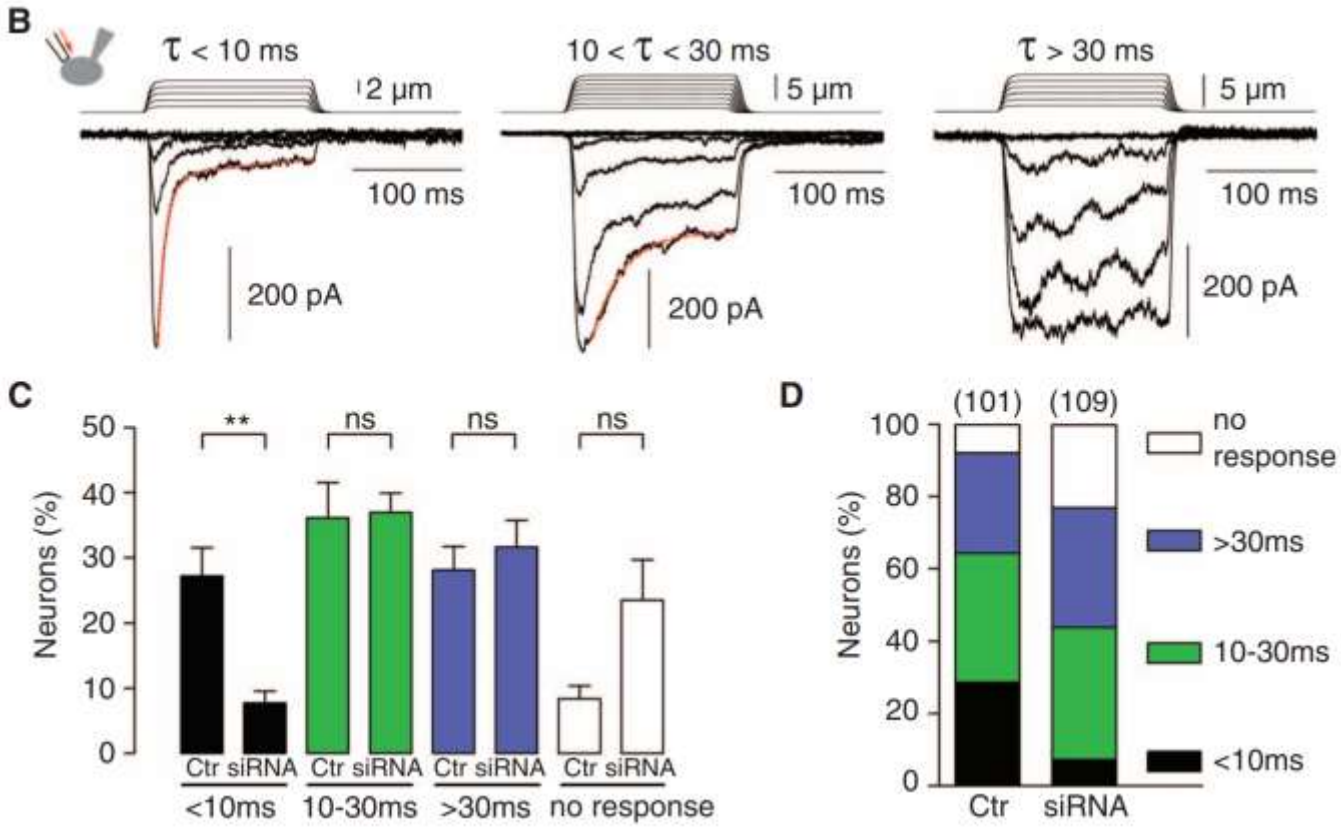
- in situ hybridization
- DRG neurons also expressing peripherin (60%) and neurofilament 200 (28%), which are markers present in mechanosensory neurons



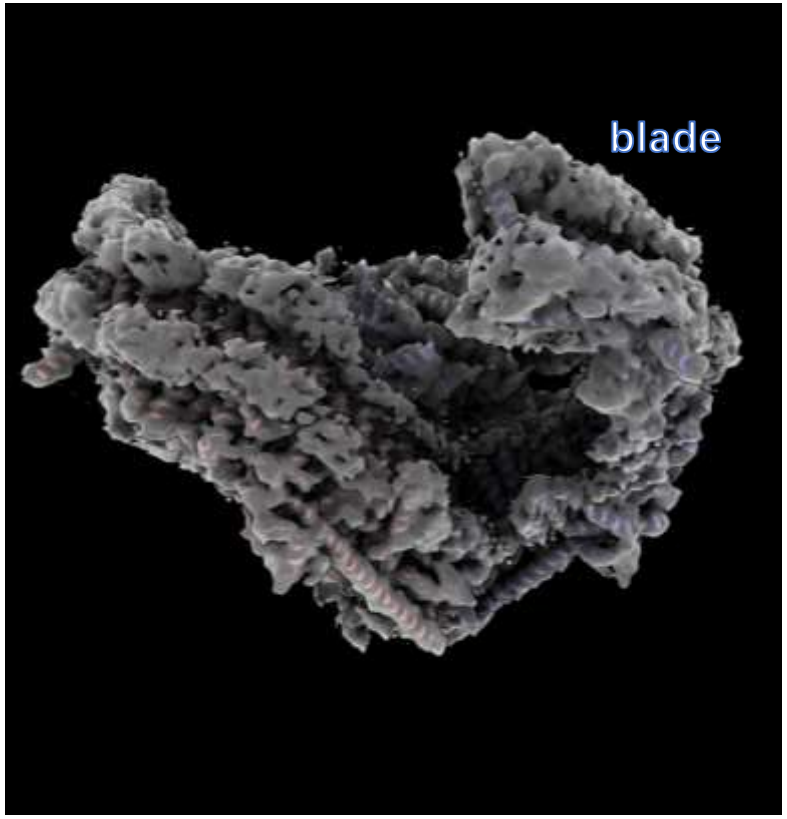


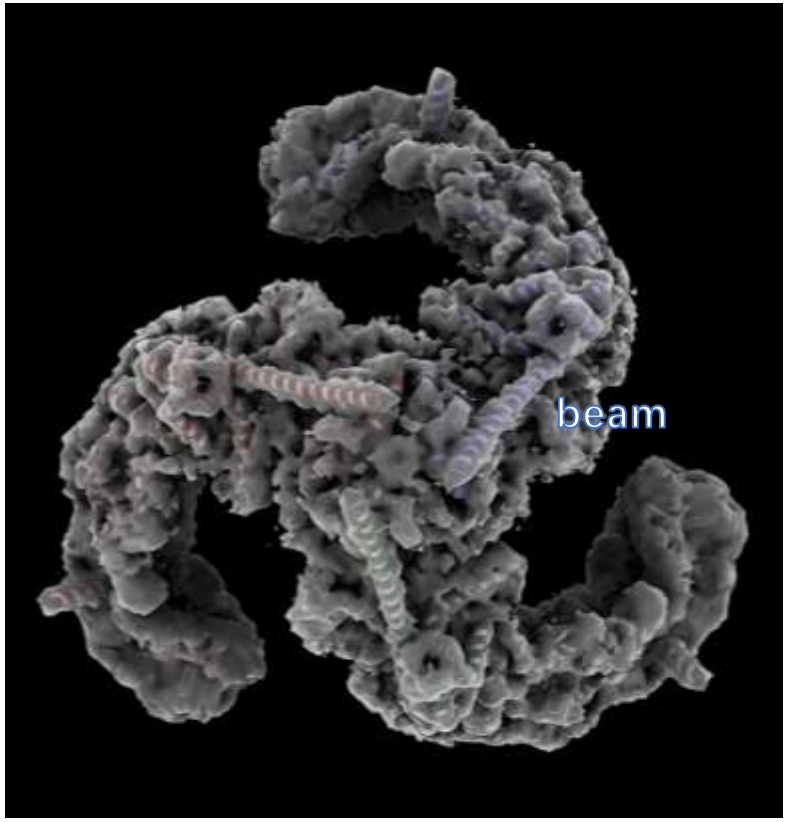
Requirement of Piezo2 for rapidly adapting MA currents in DRG neurons

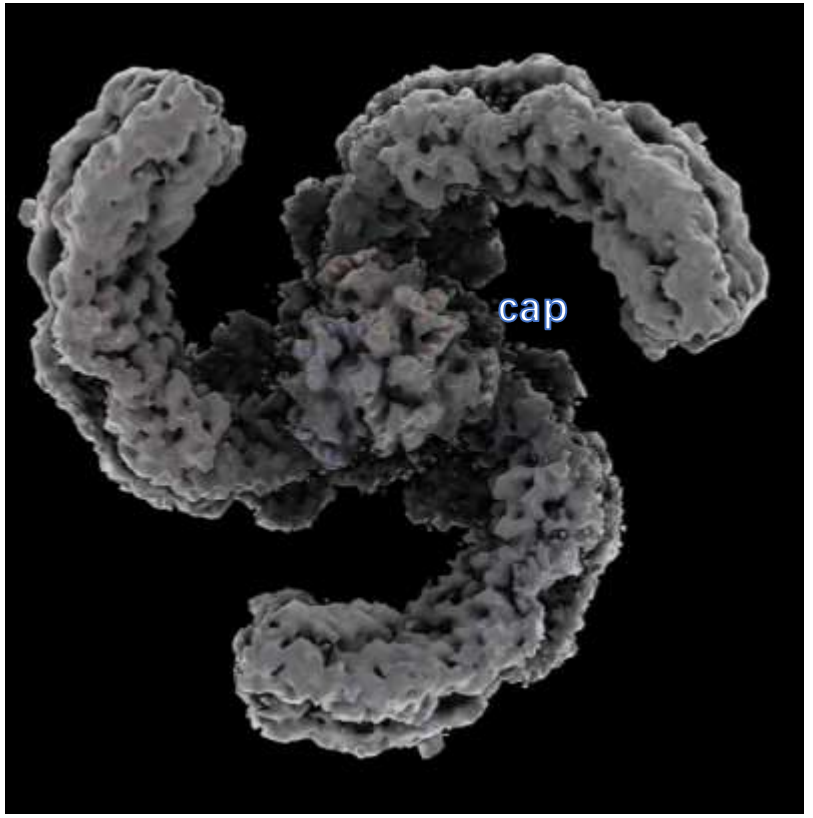
- 4 time-constant range
- Loss of Piezo2 converts rapidly adapting neurons into nonresponders



A little more about structure

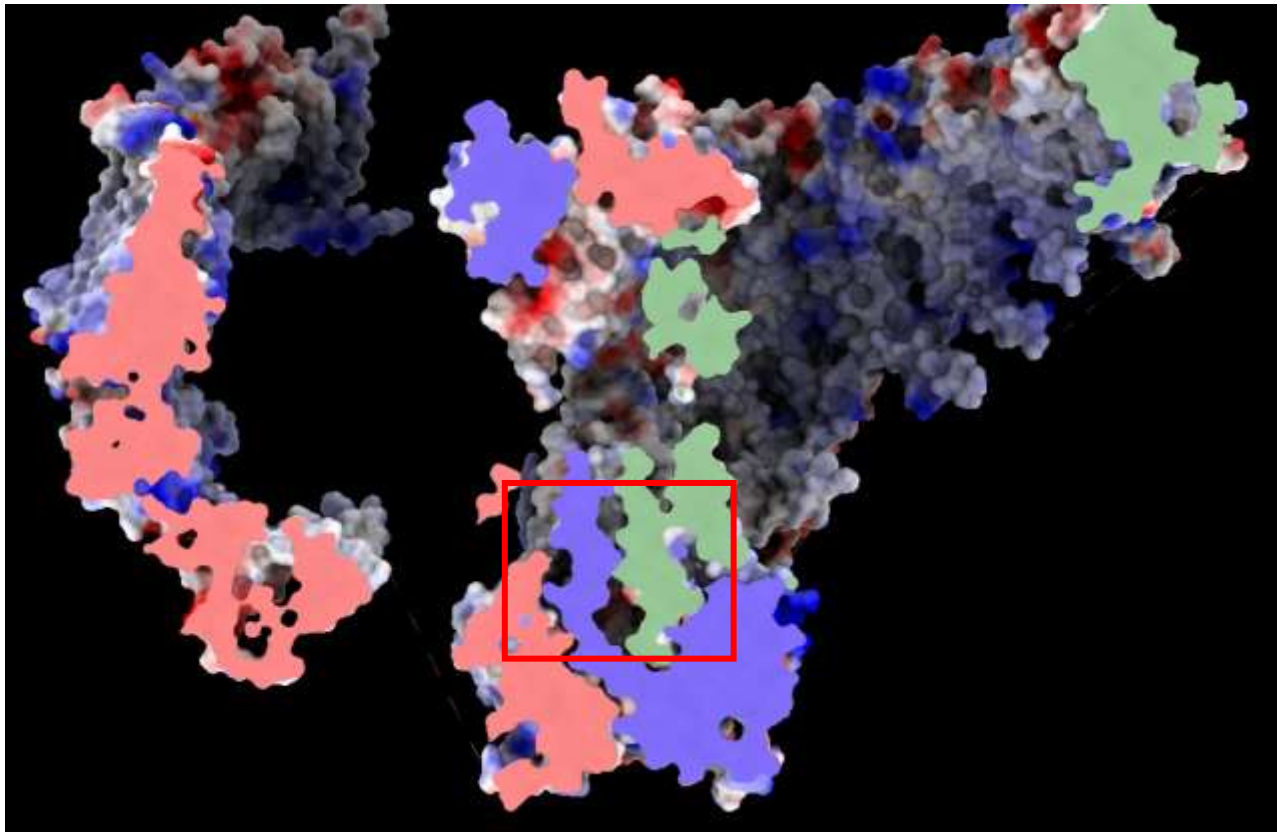


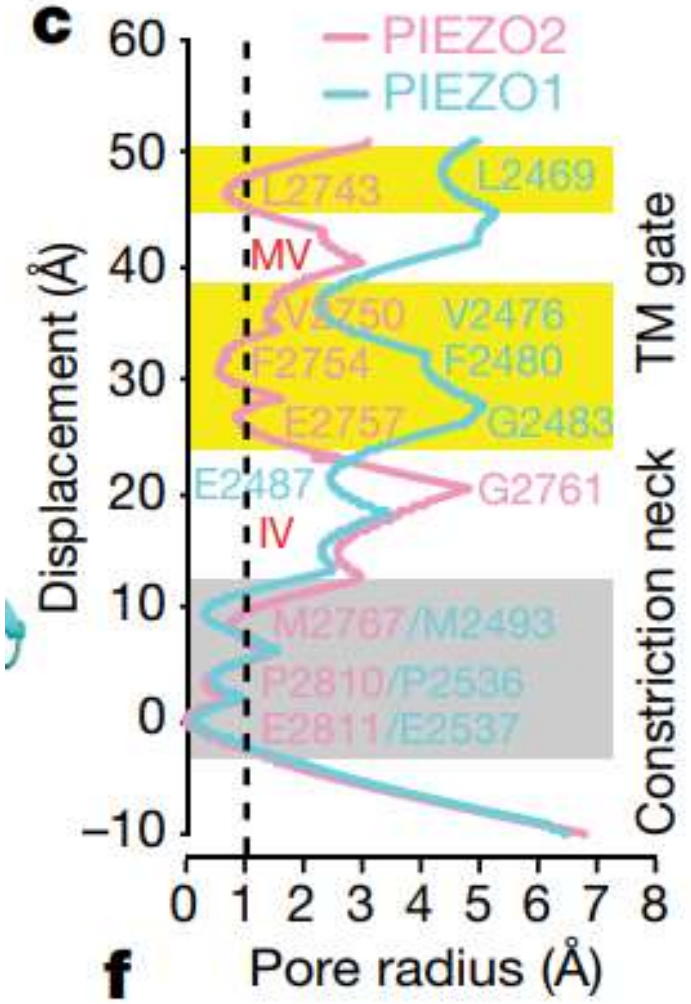




A little more about structure

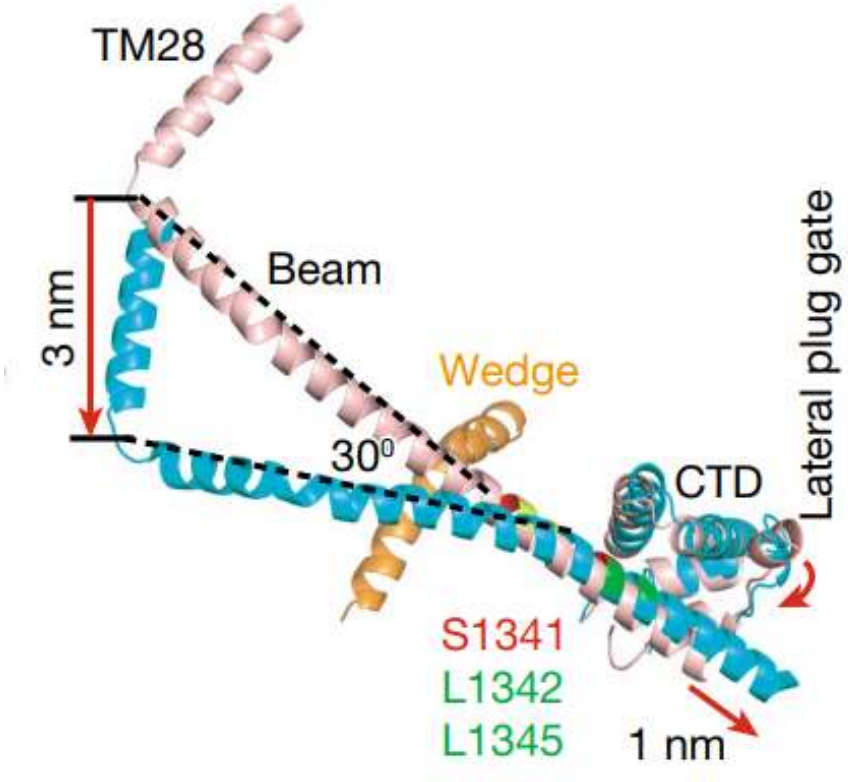
- E2487, E2495 and E2496 in Piezo1
- E2757 in Piezo2

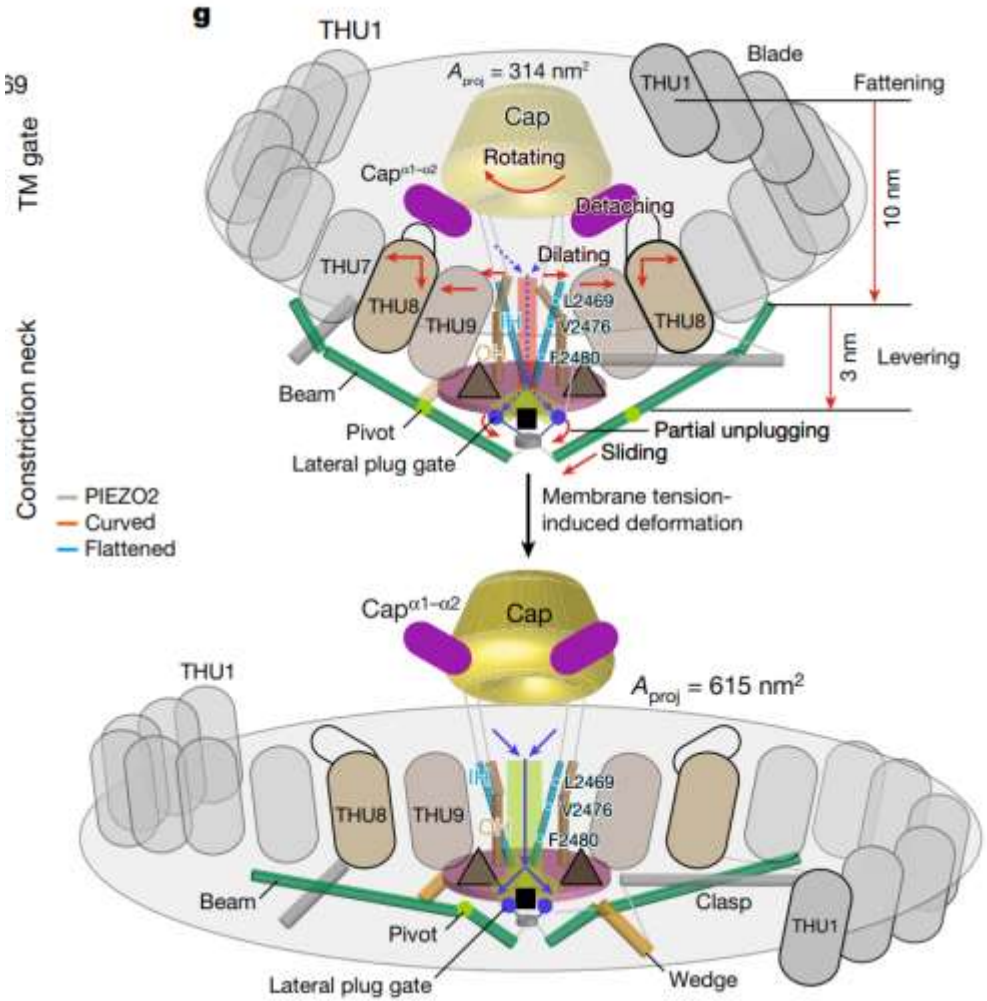




A little more about structure

- S1341 lever-like motion
- L1342 and L1345





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