Cellular and Molecular Biophysics

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Mechano-activated channels: PIEZO

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Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels

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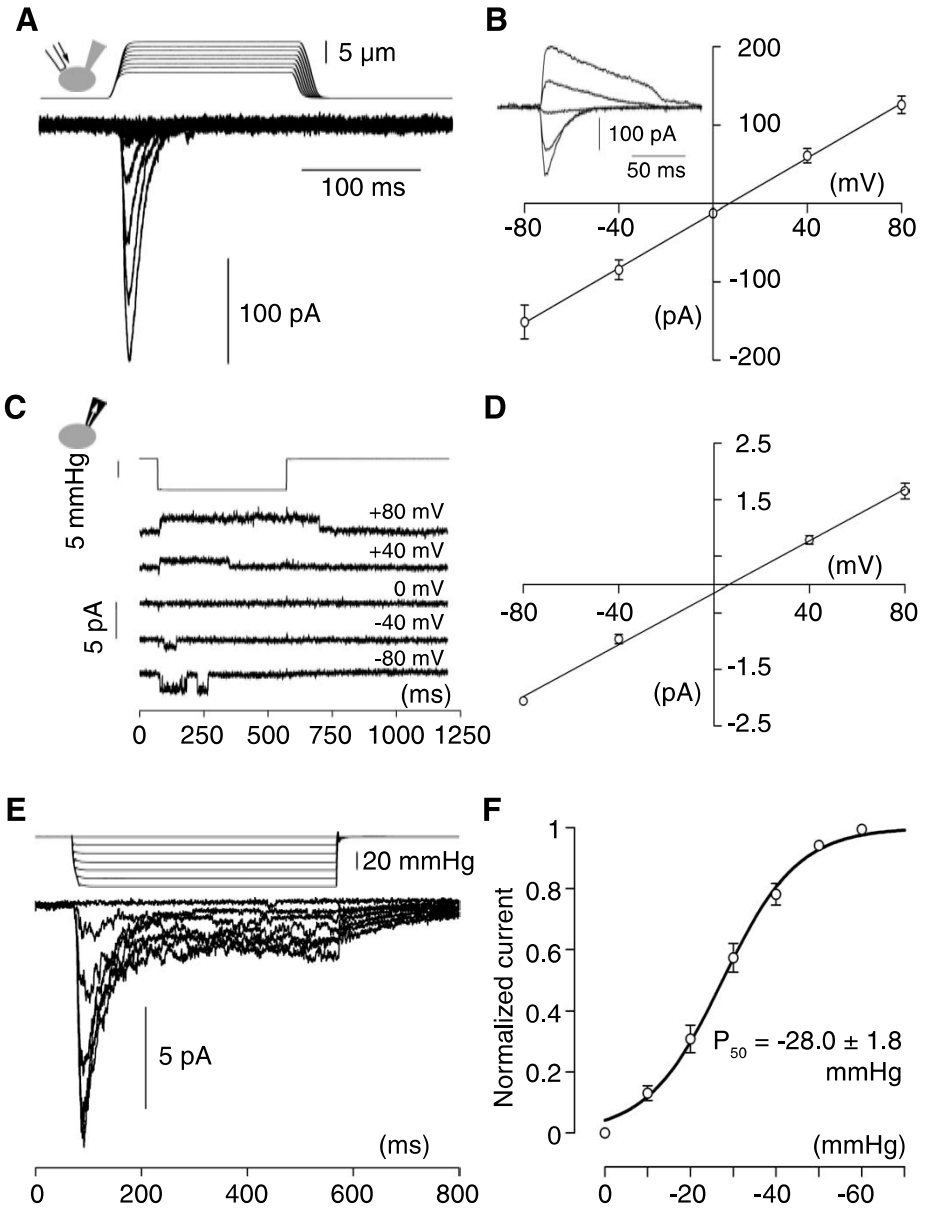
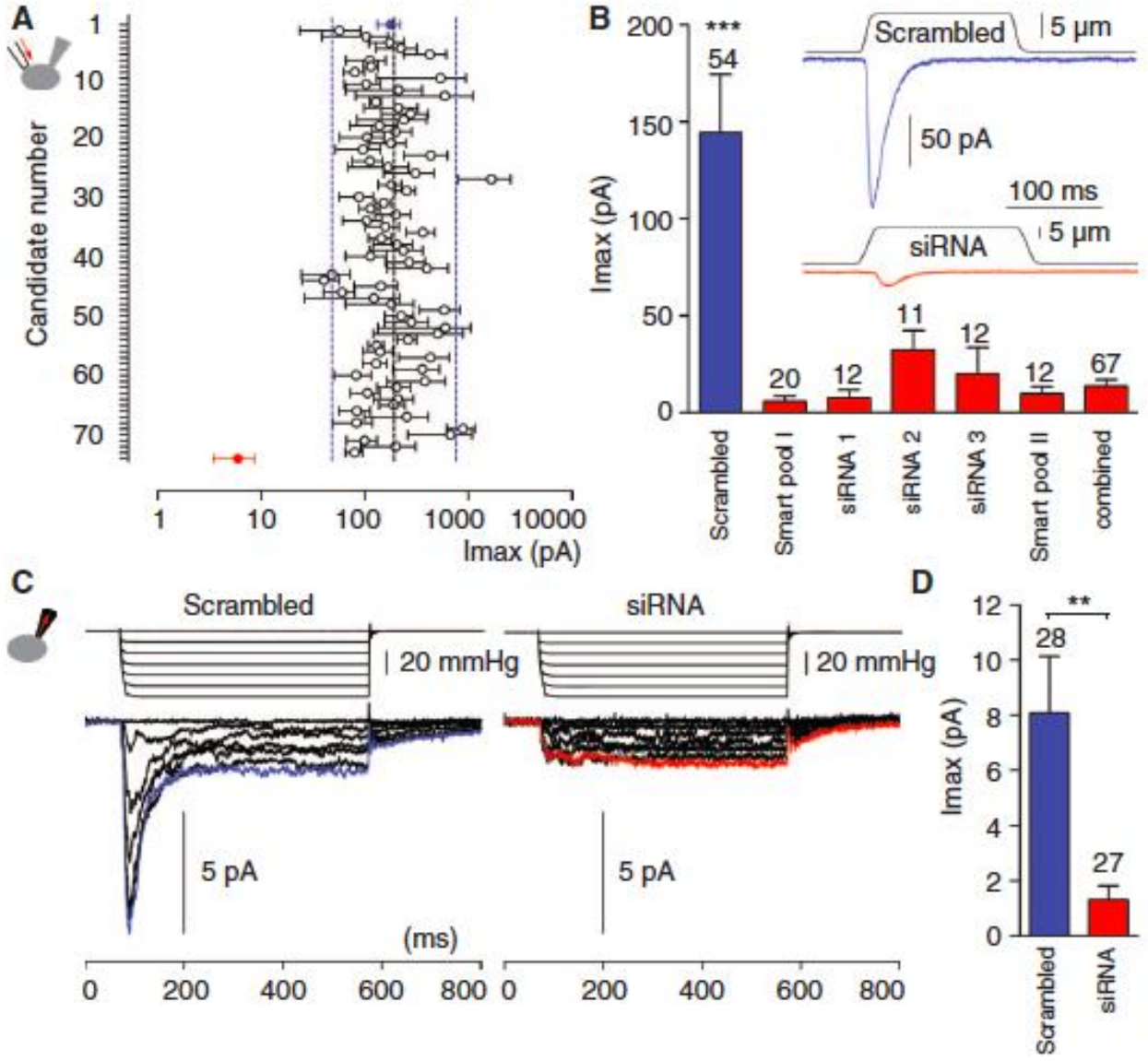
The authors used the mouse neuroblastoma cell line Neuro2A (N2A), which expresses endogenous rapidly-adapting mechanosensitive channels. Two protocols were used to evoke mechanosensitive currents — membrane touch and membrane stretch.

Fig. 1. MA currents in N2A cells. (**A**) Representative traces of MA inward currents expressed in N2A cells. Cells were subjected to a series of mechanical steps of 1-μm movements of a stimulation pipette (inset illustration, arrow) in the whole-cell patch configuration at a holding potential of −80 mV. (**B**) Average current-voltage relationships of MA currents in N2A cells (n = 11 cells). (Inset) Representative MA currents evoked at holding potentials ranging from −80 to +40 mV (applied 0.7 s before the mechanical step). (**C**) Single-channel currents (cell attached patch configuration) induced by means of negative pressure with a pipette (inset illustration, arrow) at holding potentials ranging from −80 mV to +80 mV in a N2A cell. (**D**) Average current-voltage relationships of stretch-activated single channels in N2A cells (n = 4 cells, mean ± SEM). Single-channel conductance was calculated from the slope of the linear regression line of each cell, giving γ = 22.9 ± 1.4 pS (mean ± SEM). Single-channel amplitude was determined as the amplitude difference in Gaussian fits of full-trace histograms. (**E**) Representative currents (averaged traces) induced by means of negative pipette pressure (0 to −60 mmHg, Δ 10 mmHg) in a N2A cell. (**F**) Normalized current-pressure relationship of stretch-activated currents at −80 mV fitted with a Boltzmann equation (n = 21 cells). P_{50} is the average value of P_{50} values from individual cells.



- To generate a list of candidate MA ion channels in N2A, we searched for transcripts that are enriched in N2A cells using Affymetrix microarrays. We selected proteins predicted to span the membrane at least two times (a characteristic shared by all ion channels). We prioritized this list by picking either known cation channels or proteins with unknown function. We tested each candidate (table S1) using small interfering RNA (siRNA) knockdown in N2A cells, measuring MA currents during piezo-driven pressure stimulation in the whole-cell mode.
- Knockdown of Fam38 (Family with sequence similarity 38) caused a pronounced decrease of MA currents



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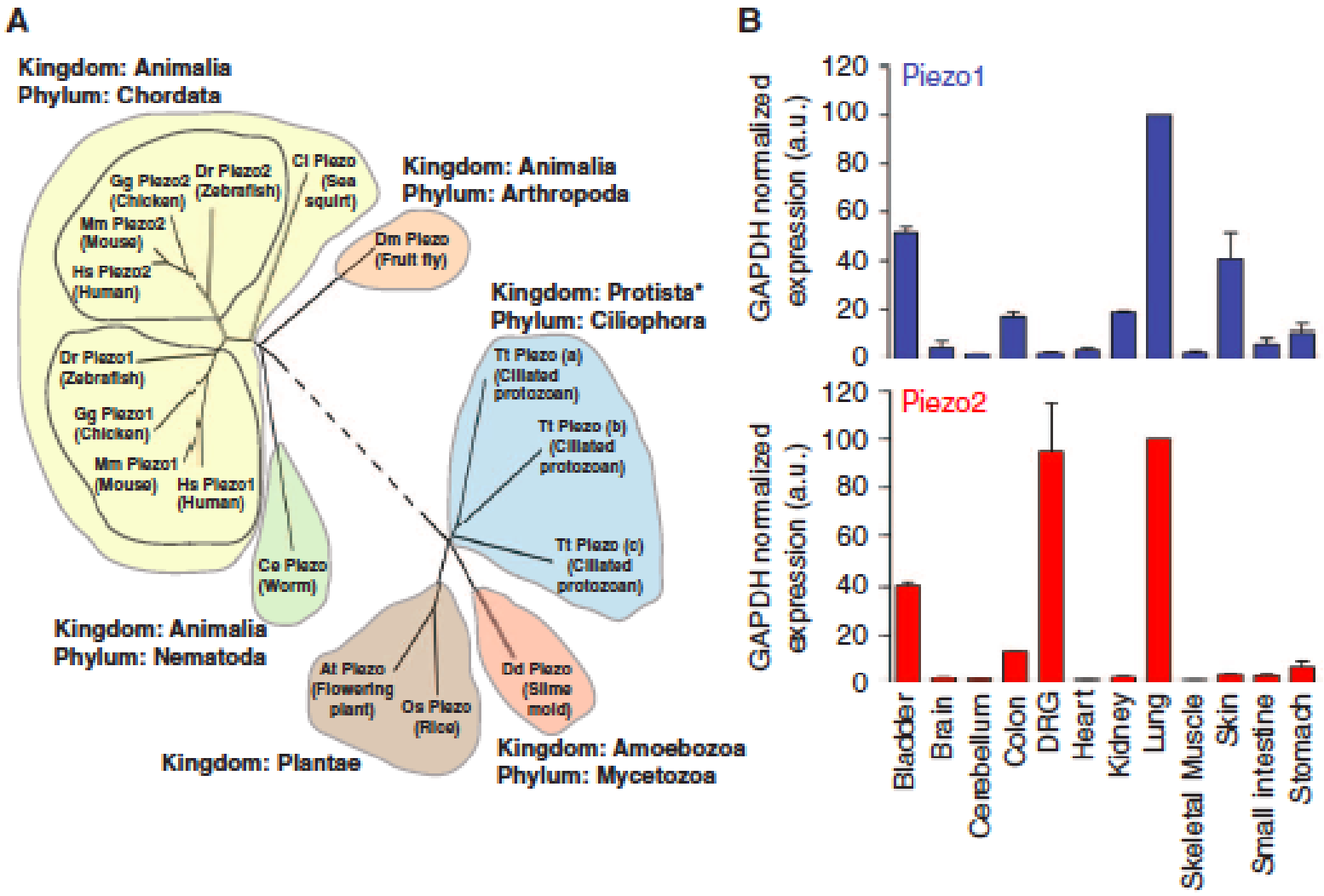
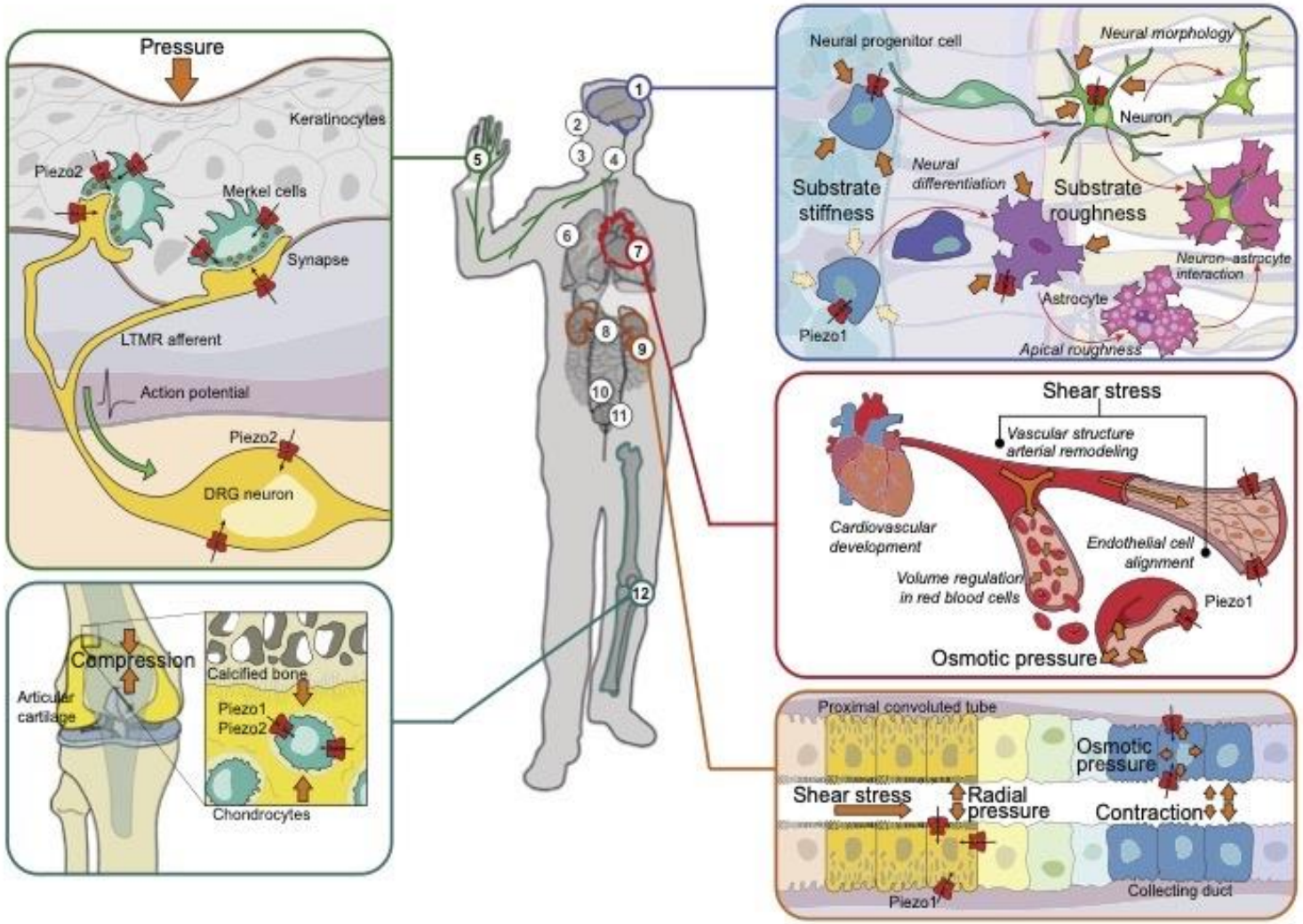
Given that Fam38A encodes a protein required for the expression of ion channels activated by pressure, we named this gene Piezo1, from the Greek *"πιέση" (piesi), meaning pressure.*

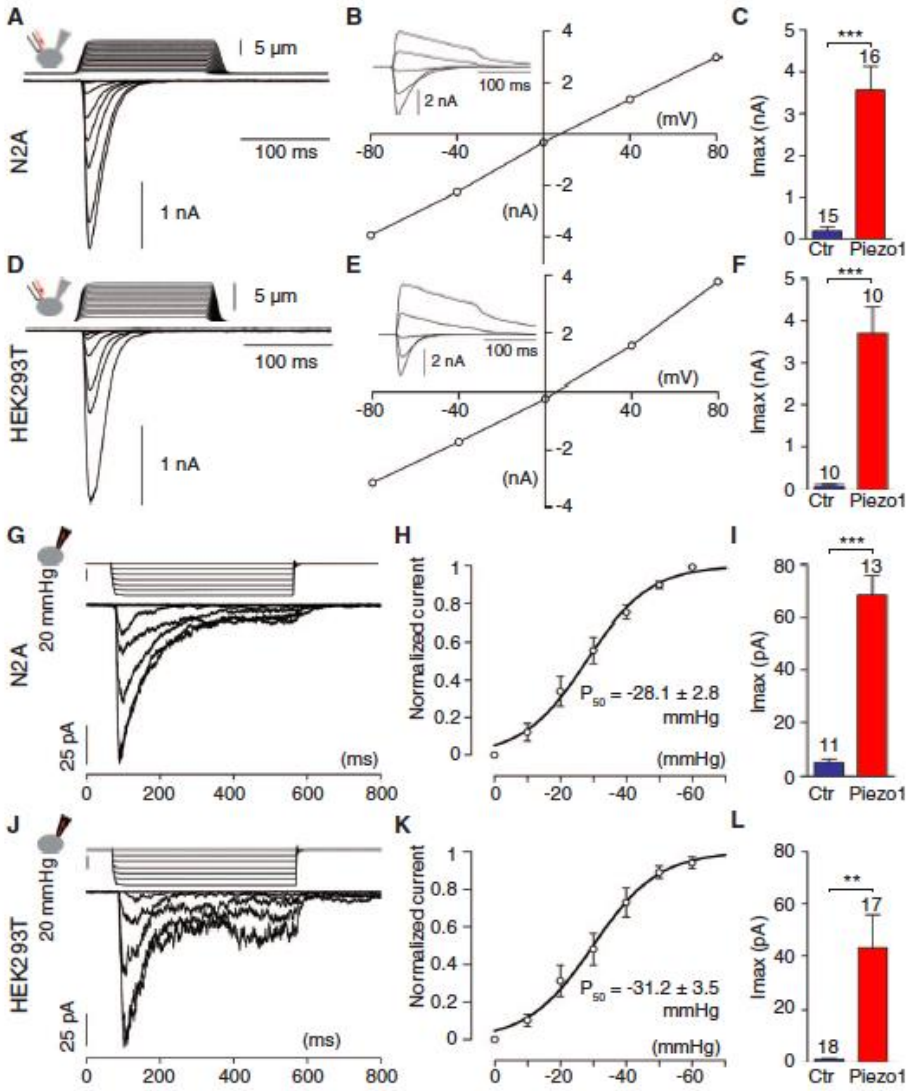
- Many animal, plant, and other eukaryotic species contain a single Piezo. Vertebrates have two members, Piezo1 (Fam38A) and Piezo2 (Fam38B)



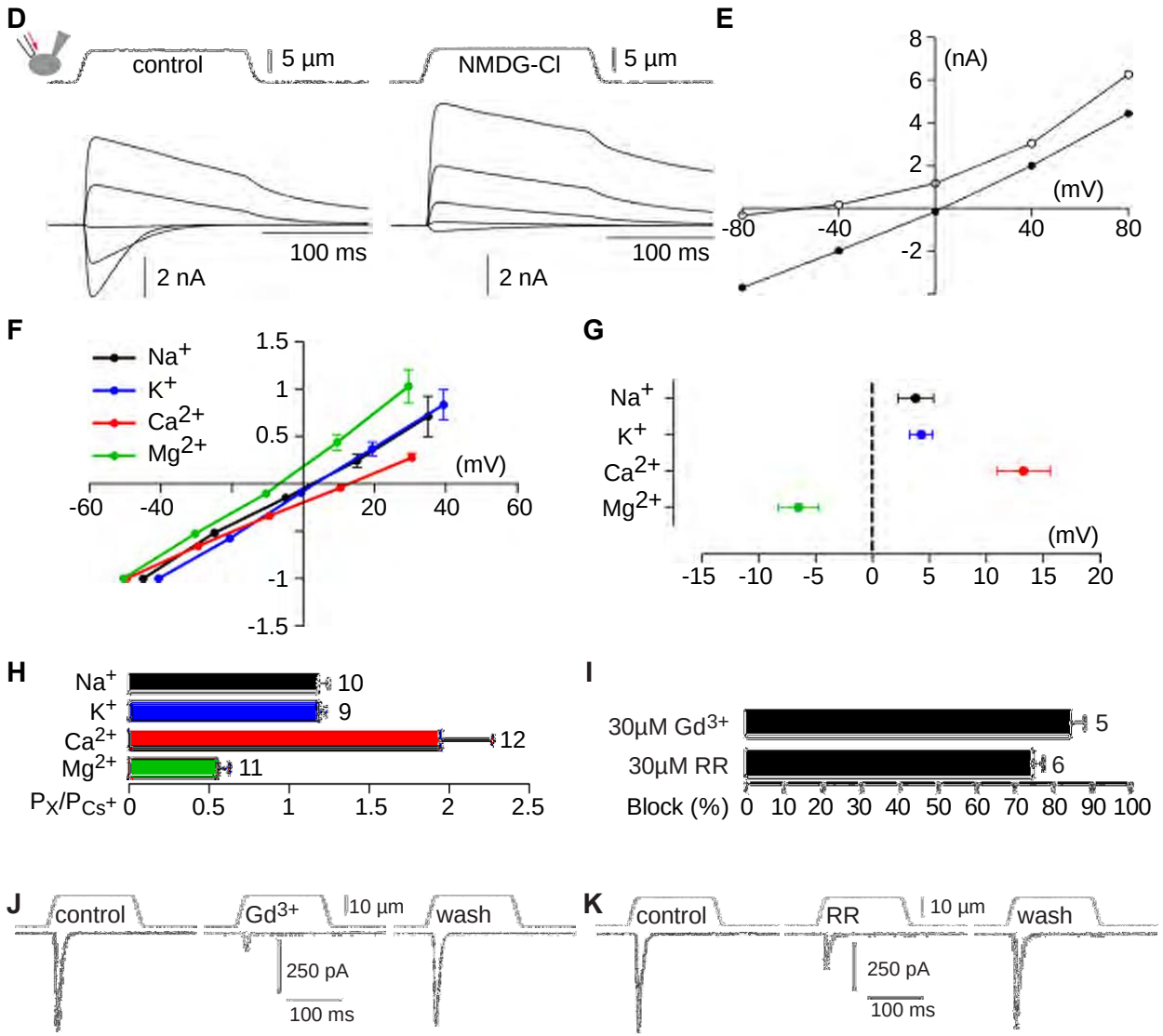

Pressure
Keratinocytes
Piezo2
Merkel cells
Synapse
LTMR afferent
Action potential
Piezo2
DRG neuron
Compression
Articular cartilage
Calcified bone
Piezo1
Piezo2
Chondrocytes
1
2
3
4
5
6
7
8
9
10
11
12
Neural progenitor cell
Neural morphology
Neuron
Neural differentiation
Substrate stiffness
Substrate roughness
Neuron–astrocyte interaction
Astrocyte
Piezo1
Apical roughness
Shear stress
Vascular structure arterial remodeling
Cardiovascular development
Endothelial cell alignment
Volume regulation in red blood cells
Piezo1
Osmotic pressure
Proximal convoluted tube
Osmotic pressure
Shear stress
Radial pressure
Contraction
Collecting duct
Piezo1

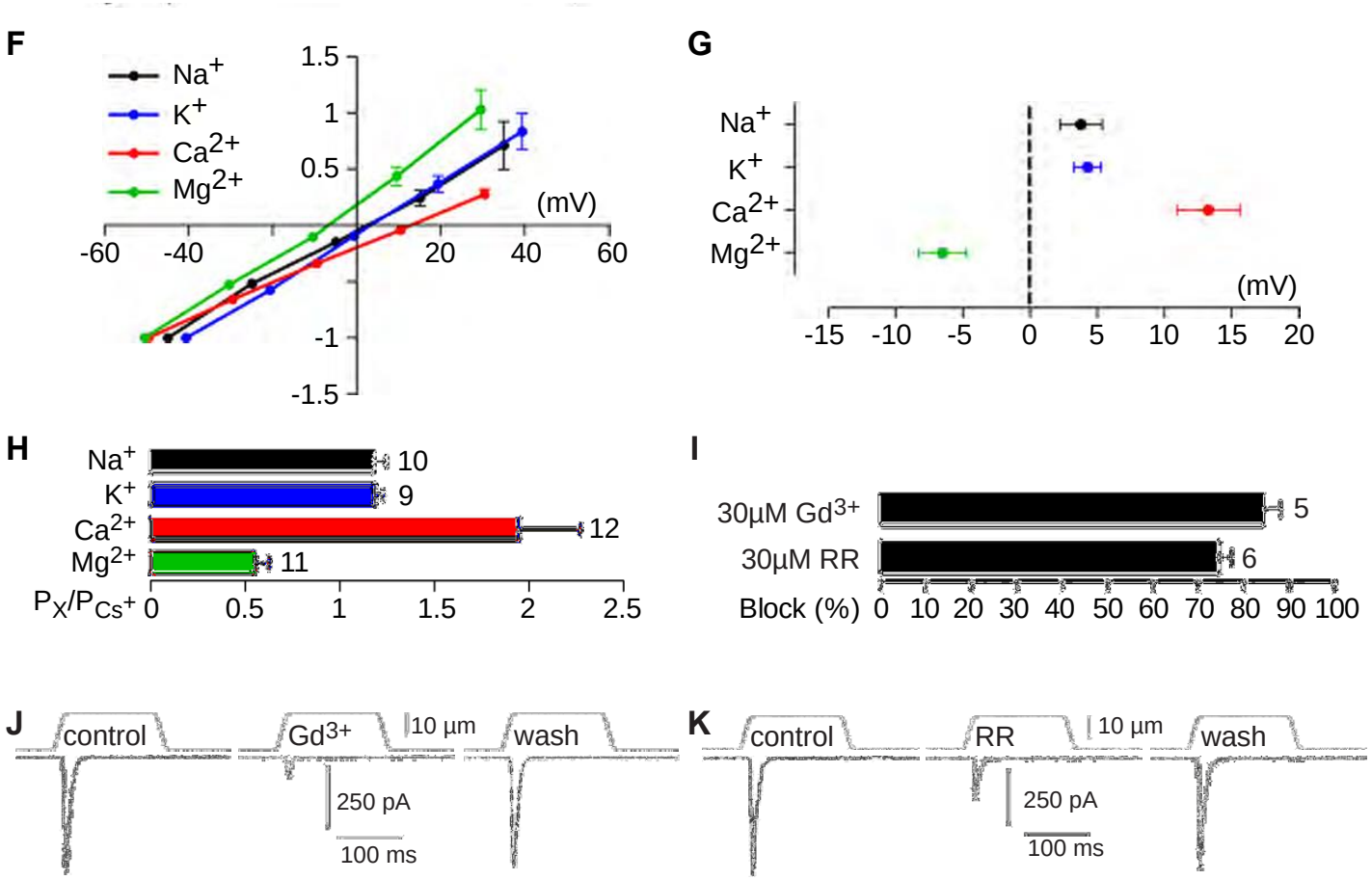
Piezo1 induces MA currents in various cell types

- We cloned full-length Piezo1 from N2A cells into the pIRES2–enhanced green fluorescent protein (EGFP) vector. We recorded MA currents from GFP-positive cells in the whole cell mode 12 to 48 hours after transfection. Piezo1 but not mock-transfected cells showed large MA currents in N2A, human embryonic kidney (HEK) 293 T
- The threshold of activation and the time constant for inactivation of MA currents elicited in Piezo1-overexpressing cells was similar in all three cell lines tested



- We characterized the ionic selectivity of MA currents in cells overexpressing Piezo1. Substituting the nonpermeant cation NMDG (N -methyl-d-glucamine) in the extracellular bathing solution suppressed inward MA currents, demonstrating that this channel conducts cations

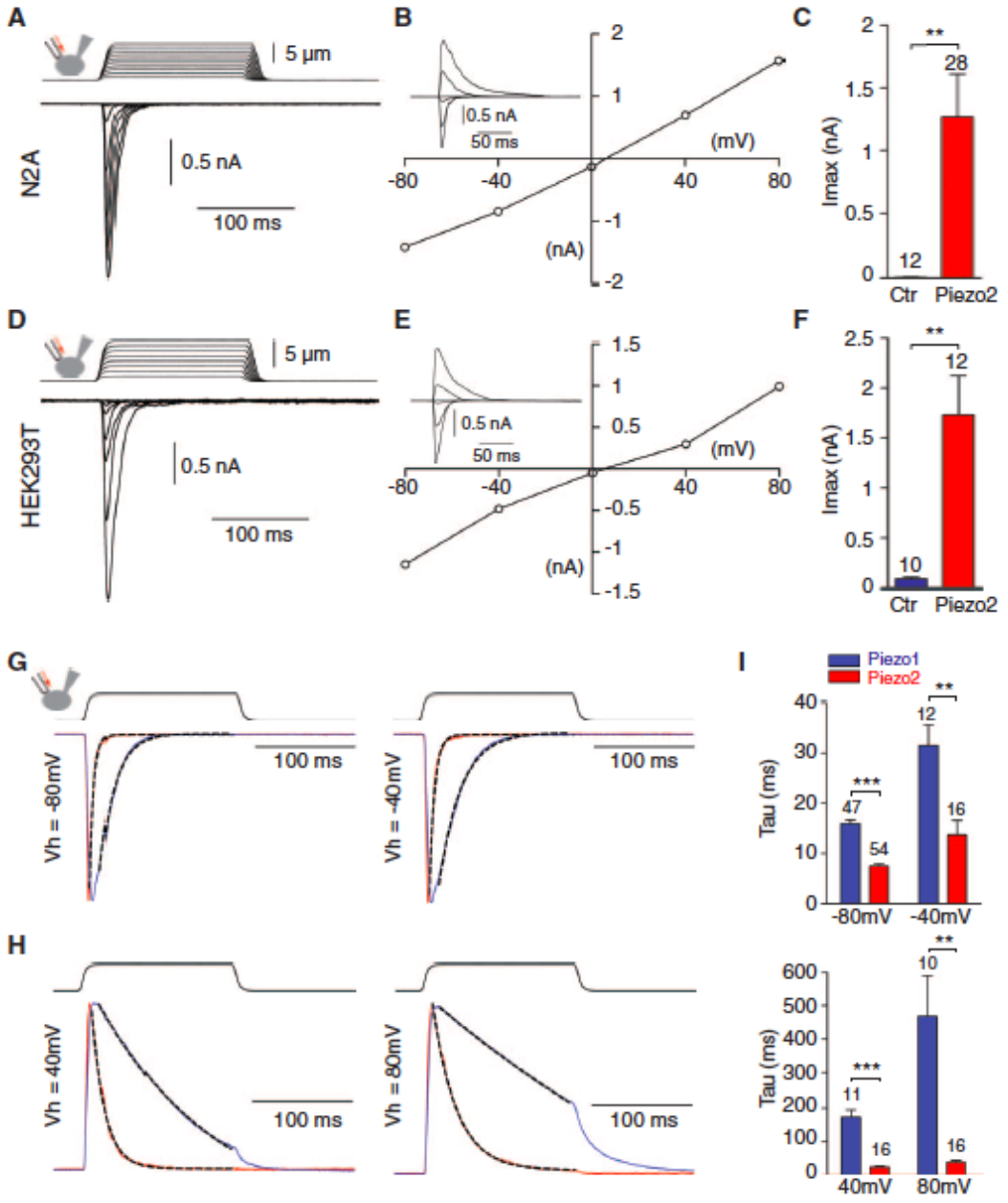




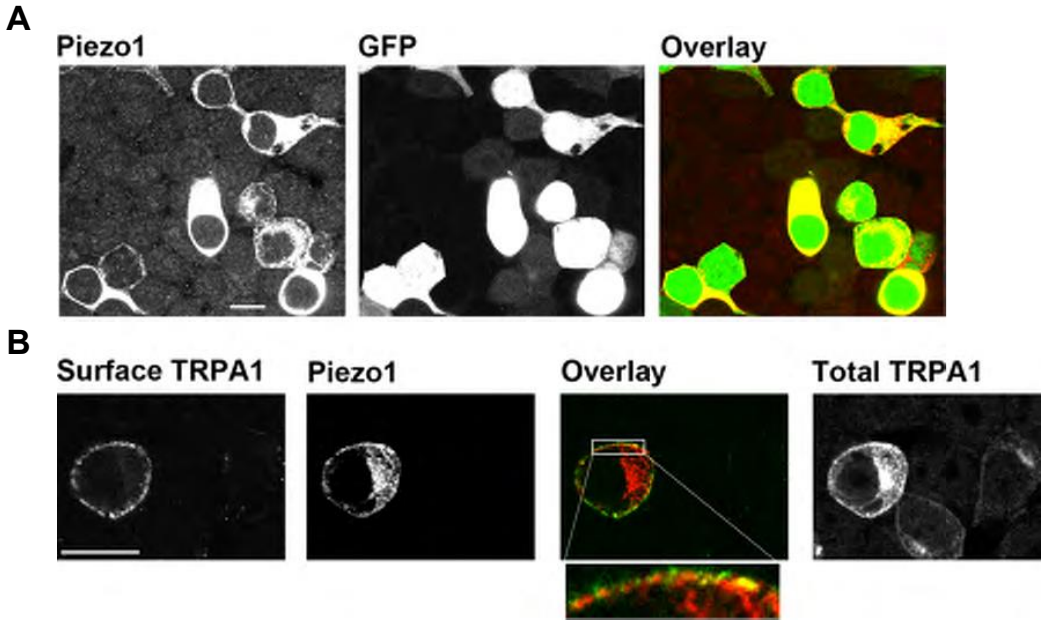
We further examined ionic selectivity by recording with CsCl-only internal solutions and various cations in the bath. Na+, K+, Ca2+ and Mg2+ all permeated, with a slight preference for Ca2+. Moreover, 30 µM of ruthenium red and gadolinium, which are known blockers of many cationic MA current, blocked 74.6 T 2.5% (n = 6 cells) and 84.3 T 3.8% (n = 5 cells) of Piezo1 induced MA current, respectively (fig. S4, I to K).

MA currents in cells overexpressing Piezo2.

- We cloned full-length Piezo2 from DRG neurons. N2A and HEK293T cells transfected with Piezo2 and gene-encoding GFP showed large MAcurrents (Fig. 5, A to F).
- The N2A cells were also cotransfected with Piezo1 siRNA to suppress endogenous MA currents.
- The kinetics of inactivation of Piezo2-dependent MA currents were faster than Piezo1-dependent MA currents, both for inward (Fig. 5G) and outward (Fig. 5H) currents, and at all holding potentials tested (Fig. 5I). Therefore, Piezo1 and Piezo2 confer distinct channel properties



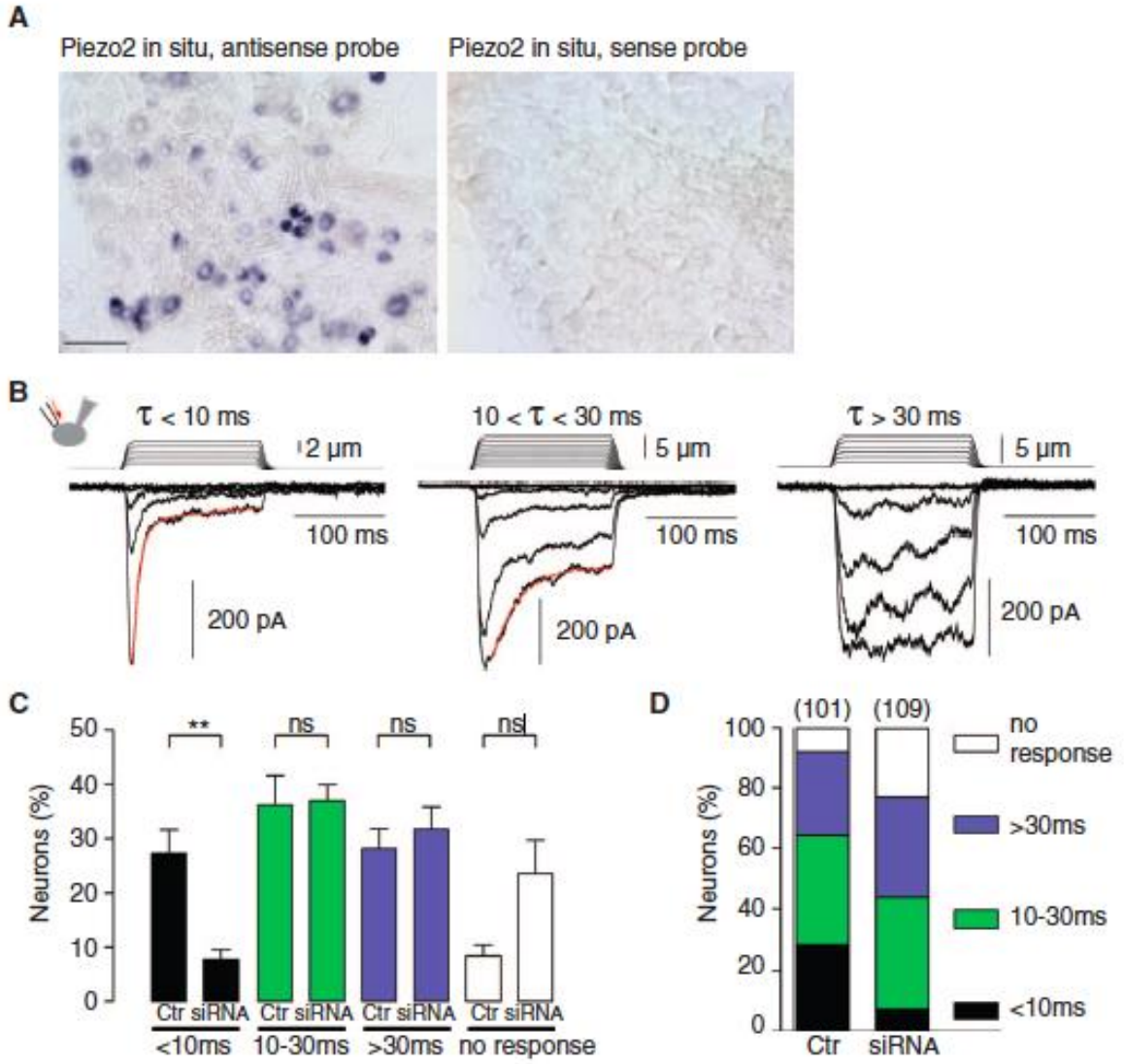
Piezo1 is detected at the plasma membrane



- In cells transfected with Piezo1 and TRPA1—an ion channel known to be expressed at the plasma membrane—we observed some overlap of Piezo1 staining with that of TRPA1 on the cell surface, although most Piezo1 and TRPA1was present inside the cell (fig. S6B). Thus, Piezo1 protein can be localized at or near the plasma membrane

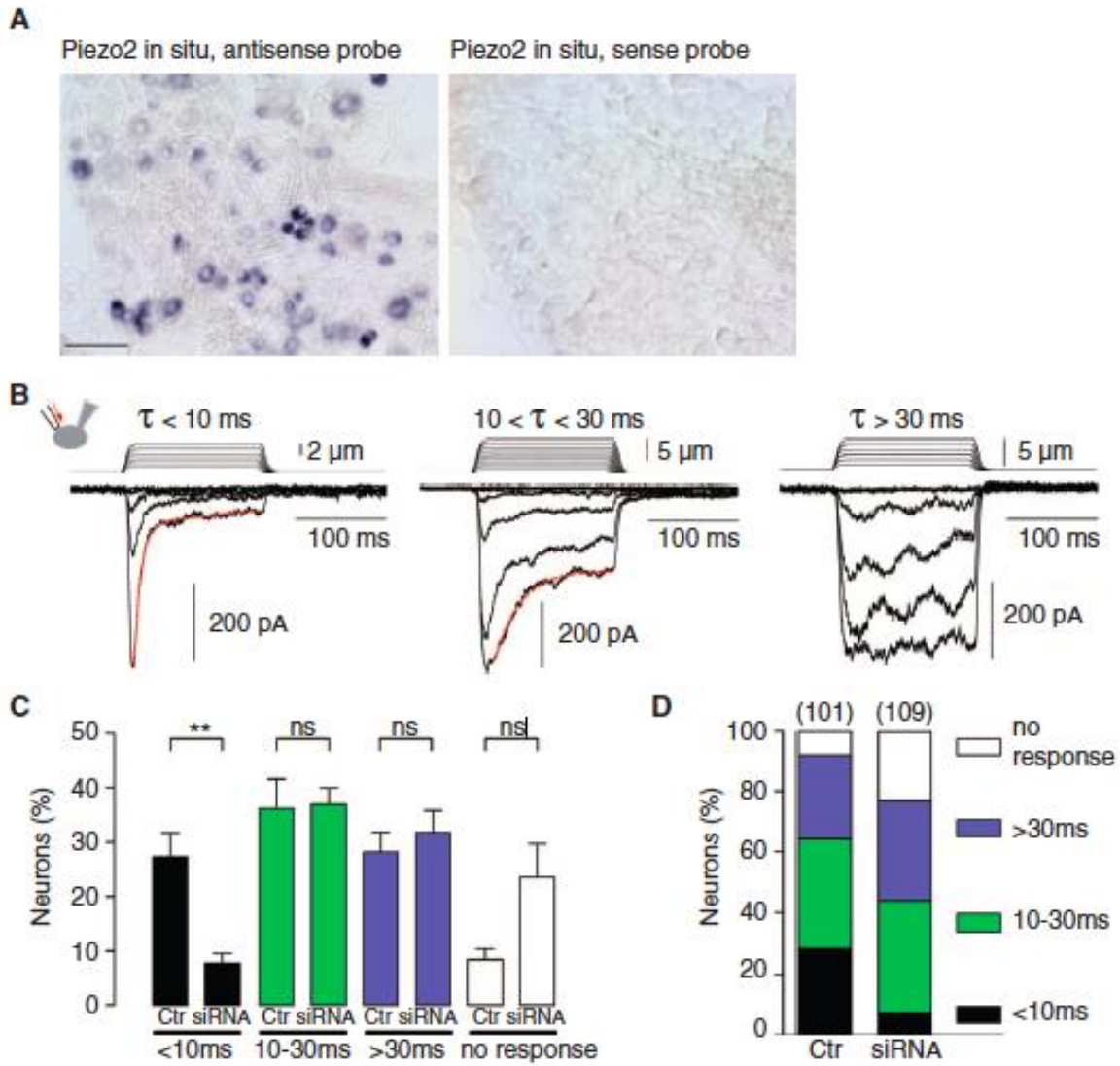
Requirement of Piezo2 for rapidly adapting MA currents in DRG neurons

- To characterize Piezo2 expression within the heterogeneous population of neurons and glial cells of the DRGs, we performed in situ hybridization on adult mouse DRG sections (Fig. 6A)
- We observed Piezo2 mRNA expression in 20% of DRG neurons



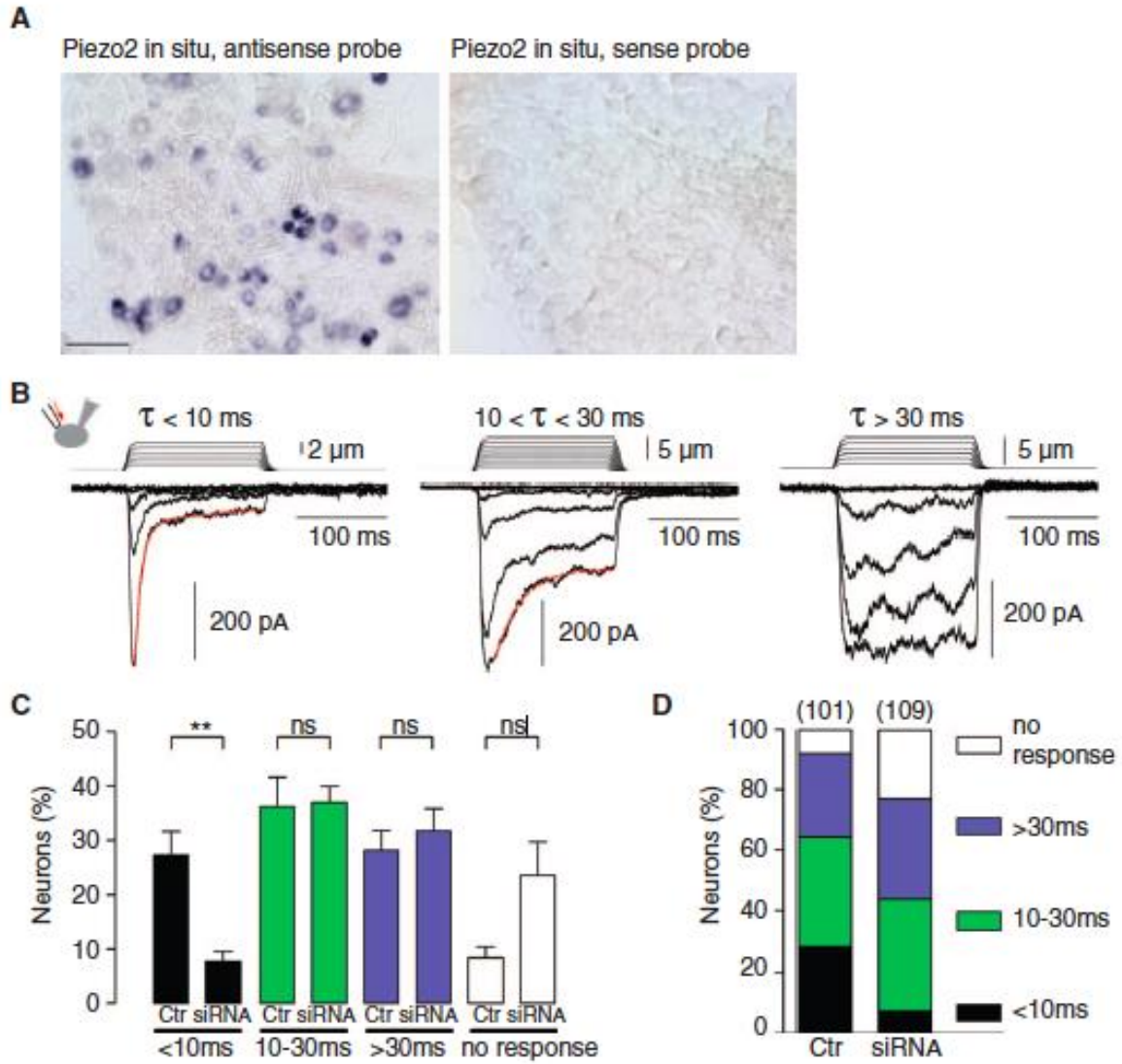
Requirement of Piezo2 for rapidly adapting MA currents in DRG neurons

We recorded whole-cell MA currents from DRG neurons transfected with GFP and either scrambled or Piezo2 siRNA (n = 101 neurons for scrambled and n = 109 neurons for Piezo2 siRNA). We grouped the recorded MA currents according to their inactivation kinetics (Fig. 6B). We defined four different classes of neurons on the basis of t inac distribution in scrambled siRNA transfected cells



Requirement of Piezo2 for rapidly adapting MA currents in DRG neurons

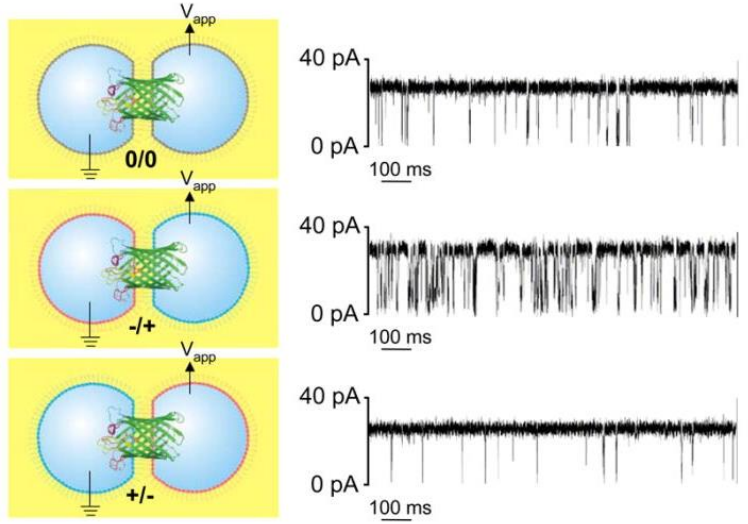
- The proportion of neurons expressing MA currents with t inact < 10ms was specifically and significantly reduced in neurons transfected with Piezo2 siRNA as compared with that of neurons transfected with scrambled siRNA (Fig. 6C).
- 28.7% of scrambled siRNA-transfected neurons had tinac < 10 ms, compared with 7.3% in Piezo2 siRNA-transfected neurons (Fig. 6D)

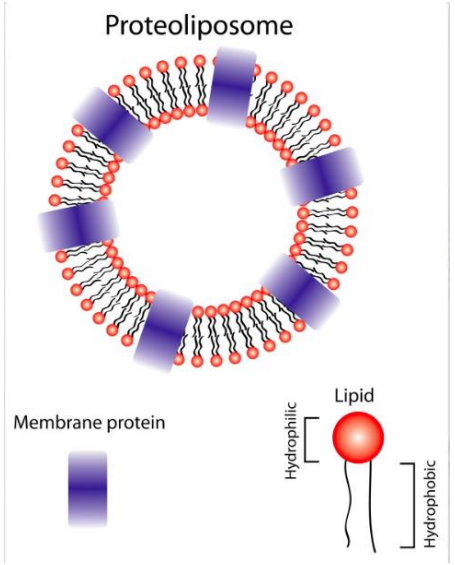


MmPiezo1 reconstitution in lipid bilayers

to assess whether Piezo proteins were sufficient to recapitulate the channel properties recorded from Piezo-overexpressing cells, we reconstituted purified MmPiezo1 proteins into lipid bilayers in two distinct configurations: droplet interface lipid bilayers (DIBs) assembled from two monolayers (Fig. 5a–e and l–q) and proteoliposomes (Fig. 5f–h). In the first configuration, MmPiezo1 was reconstituted into asymmetric bilayers that mimic the cellular environment: the extracellular-facing lipid monolayer is predominantly neutral whereas the intracellular-facing leaflet is negatively charged. In contrast, the lipid composition of the bilayer in the second configuration is uniform.

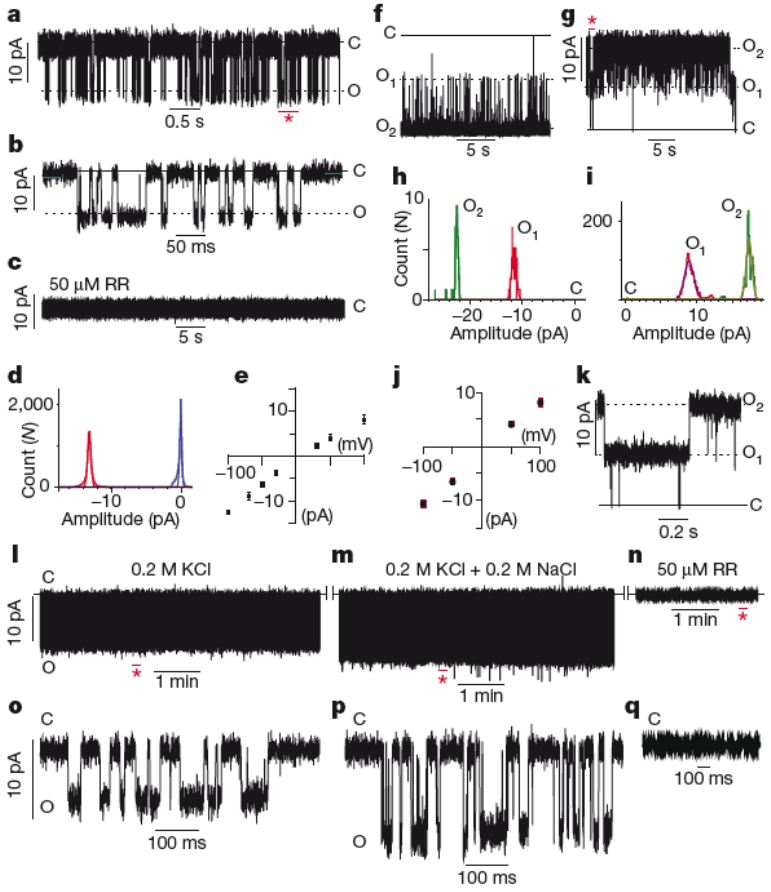
DIB





MmPiezo1 reconstitution in lipid bilayers

- In the DIBs setting, representative segments from a 6-min recording obtained at -100mV show brief, discrete channel openings (Fig. 5a, b) blocked by addition of 50 µM ruthenium red to the neutral facing compartment (Fig. 5c)
- The asymmetric accessibility of ruthenium red block of reconstituted channels agrees with the data obtained from MmPiezo1-overexpressing HEK293T cells (Fig. 2 and), thereby establishing the fidelity of the assays and validating MmPiezo1 protein as an authentic ion channel



ARTICLE

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Architecture of the mammalian mechanosensitive Piezo1 channel

Jingpeng Ge[1,2]*, Wanqiu Li[2]*, Qiancheng Zhao[1,3]*, Ningning Li[2]*, Maofei Chen[1,2], Peng Zhi[3], Ruochong Li[1,2], Ning Gao[2], Bailong Xiao[1,3,4] & Maojun Yang[1,2]

ARTICLE

doi:10.1038/nature25743

Structure and mechanogating mechanism of the Piezo1 channel

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ARTICLE

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Structure of the mechanically activated ion channel Piezo1

Kei Saotome[1,2], Swetha E. Murthy[1], Jennifer M. Kefauver[1,2], Tess Whitwam[1], Ardem Patapoutian[1] & Andrew B. Ward[2]

ARTICLE

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Architecture of the mammalian mechanosensitive Piezo1 channel

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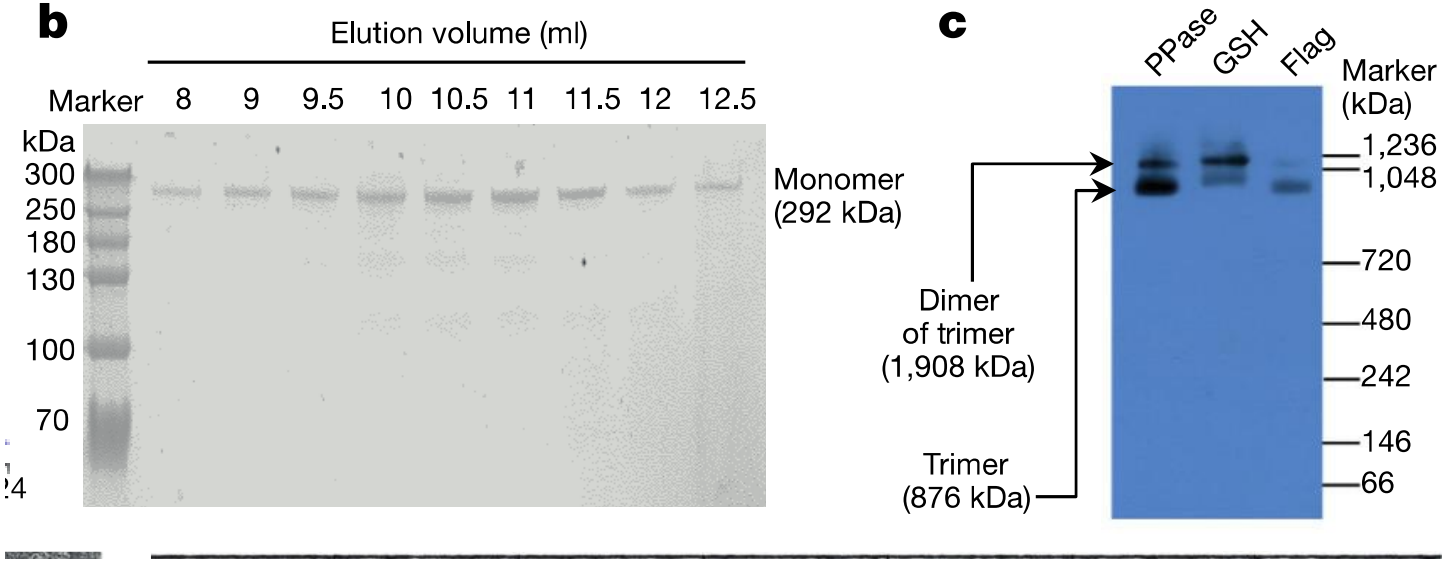
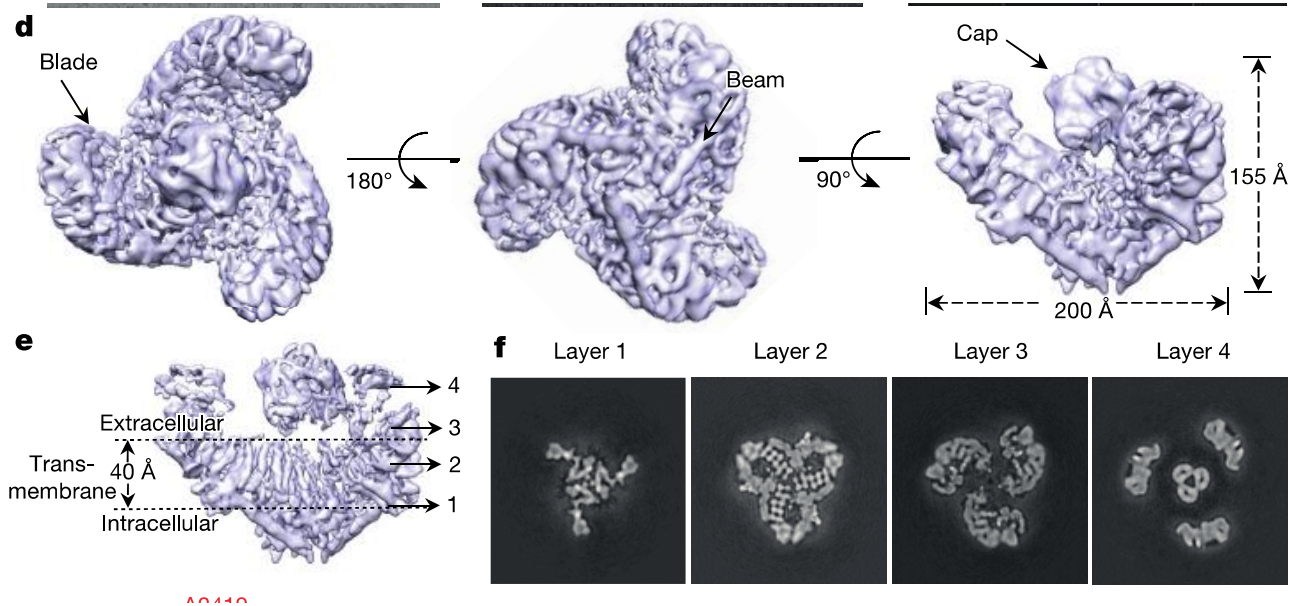
Here we determine the cryo-electron microscopy structure of the full-length (2,547 amino acids) mouse Piezo1 (Piezo1) at a resolution of 4.8 A˚ .

Piezo1 forms a trimeric propeller-like structure (about 900 kilodalton), with the extracellular domains resembling three distal blades and a central cap.

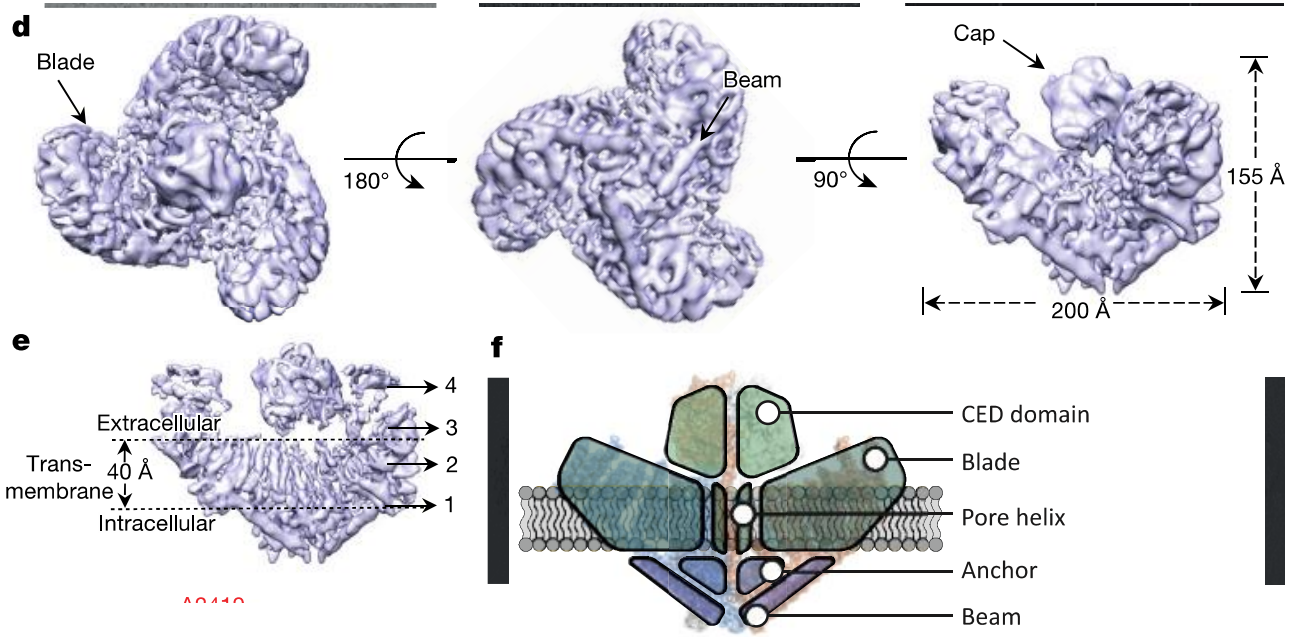
As a further confirmation, Flag-tagged Piezo1 displayed a major band at about 900 kDa on native gels (Fig. 1c). Thus, our data suggest that the major oligomeric state of the purified Piezo1 is trimeric.



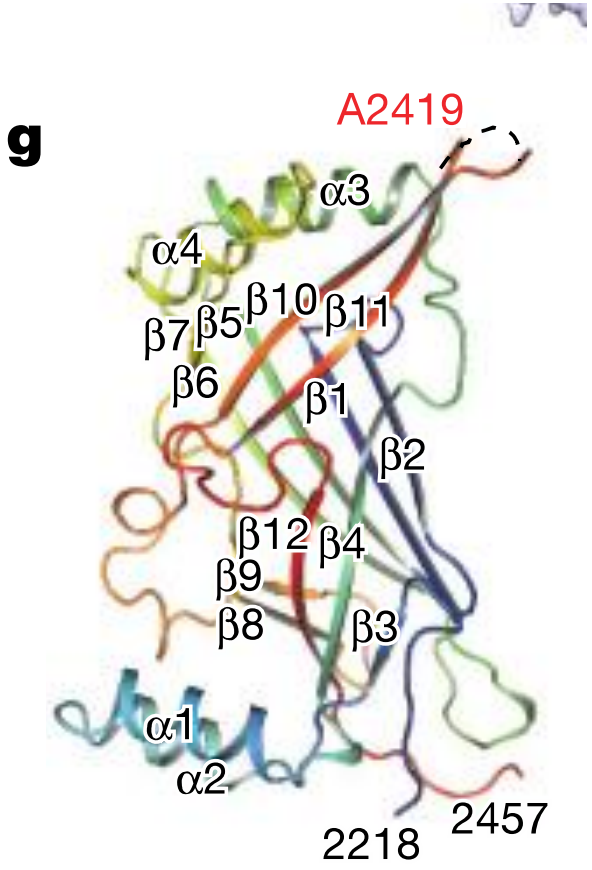
The density map revealed that Piezo1 formed a three-blade, propeller-shaped architecture, with distinct regions resembling the typical structural components of a propeller, including three blades and a central cap. Viewed from the top, the diameter and the axial height of the structure are 200 Å and 155 Å, respectively (Fig. 2d).



A single central cap sits above the surface of the transmembrane core with a gap in between (Fig. 2e). Furthermore, a tightly packed region, likely to be a compact soluble domain, is located on the opposite side of the cap, right below the transmembrane region (Fig. 2e). The anchor may contain the highly conserved PF(X2)E(X6)W motif found in Piezos in all species.



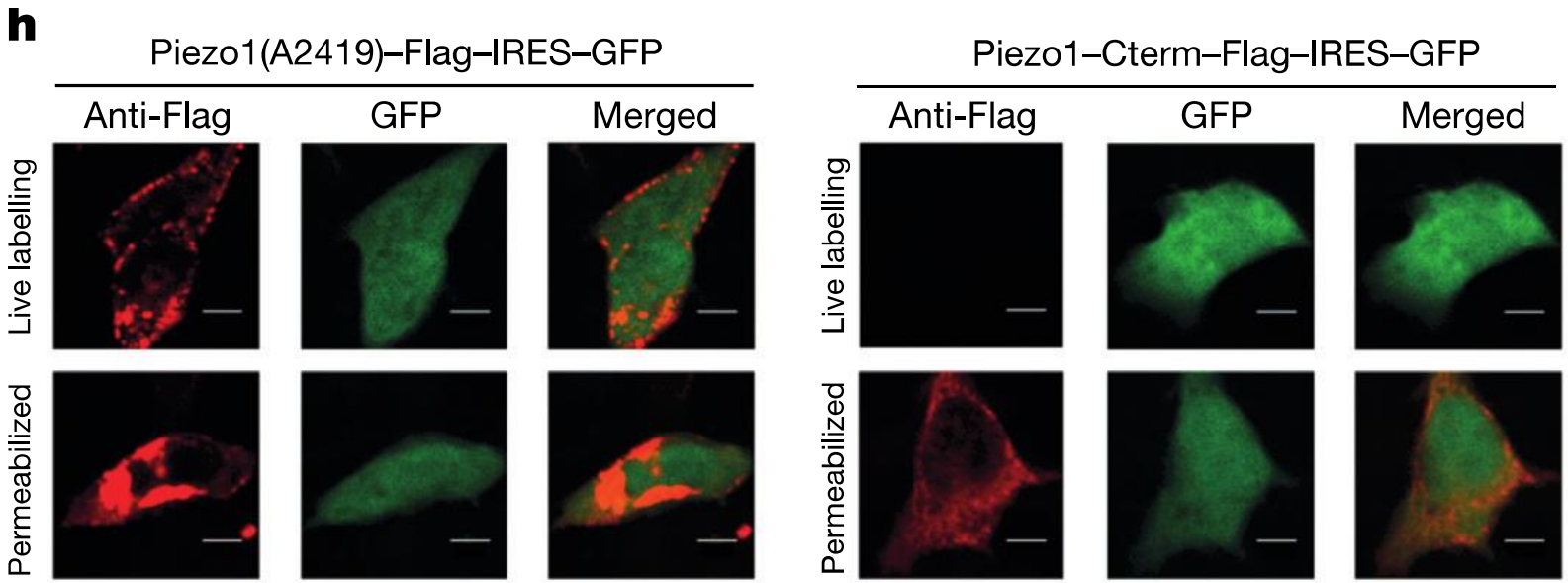
The Cap is constitued by residues from 2210 to 2457 (termed the C-terminal extracellular domain, CED) that form a large extracellular loop followed by the last transmembrane segment at the C terminus.



CRISTAL STRUCTURE OF CED:

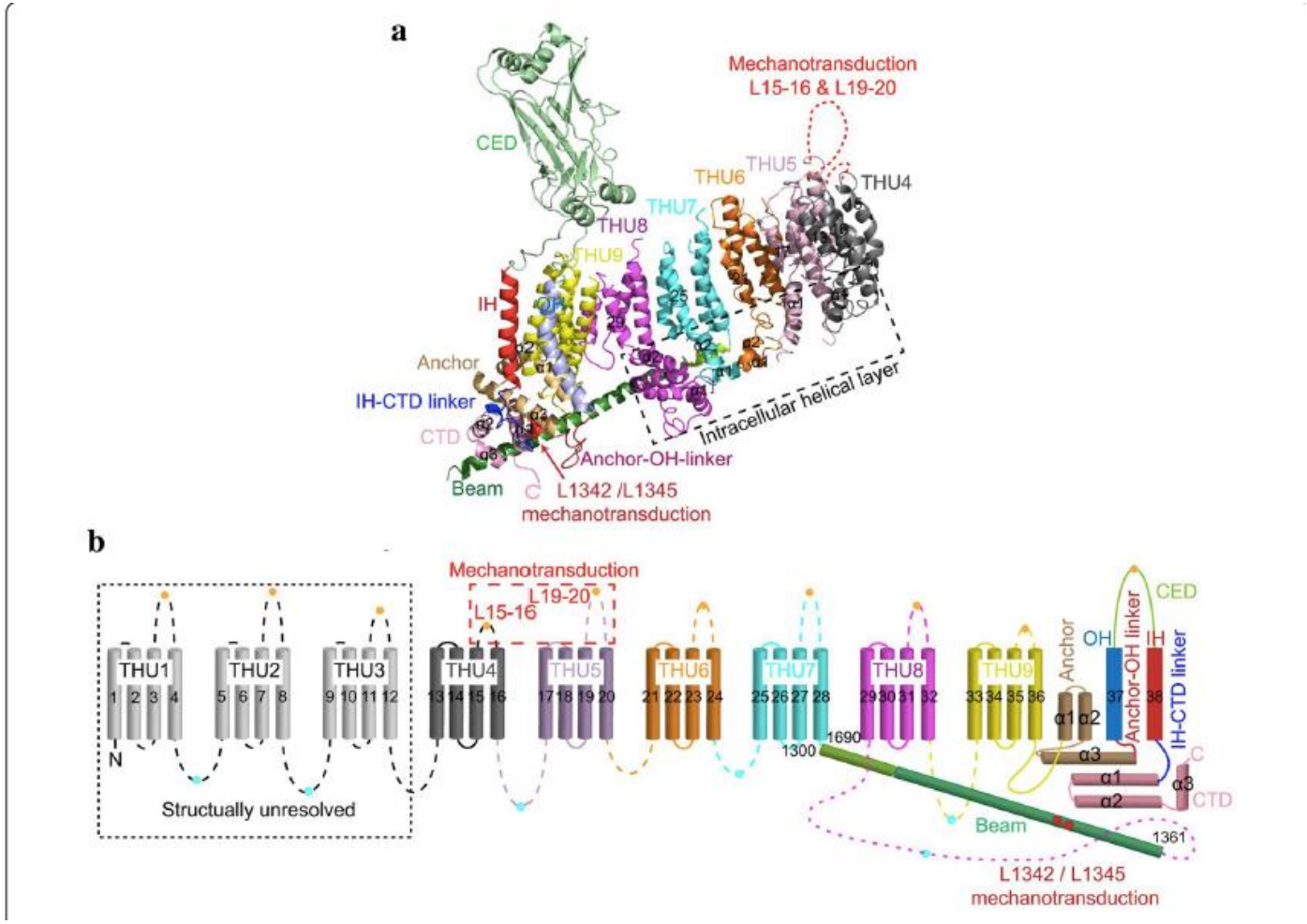
The amino (N) and C termini of the CED are on the same side and close to each other (Fig. 2g), consistent with the topological prediction that the CED is located between the last two transmembrane segments **in the C-terminal region of Piezo1.**

In the 3D structure, the CAP is formed by a CED trimer, further supporting the conclusion that the full-length Piezo1 forms a homotrimer.

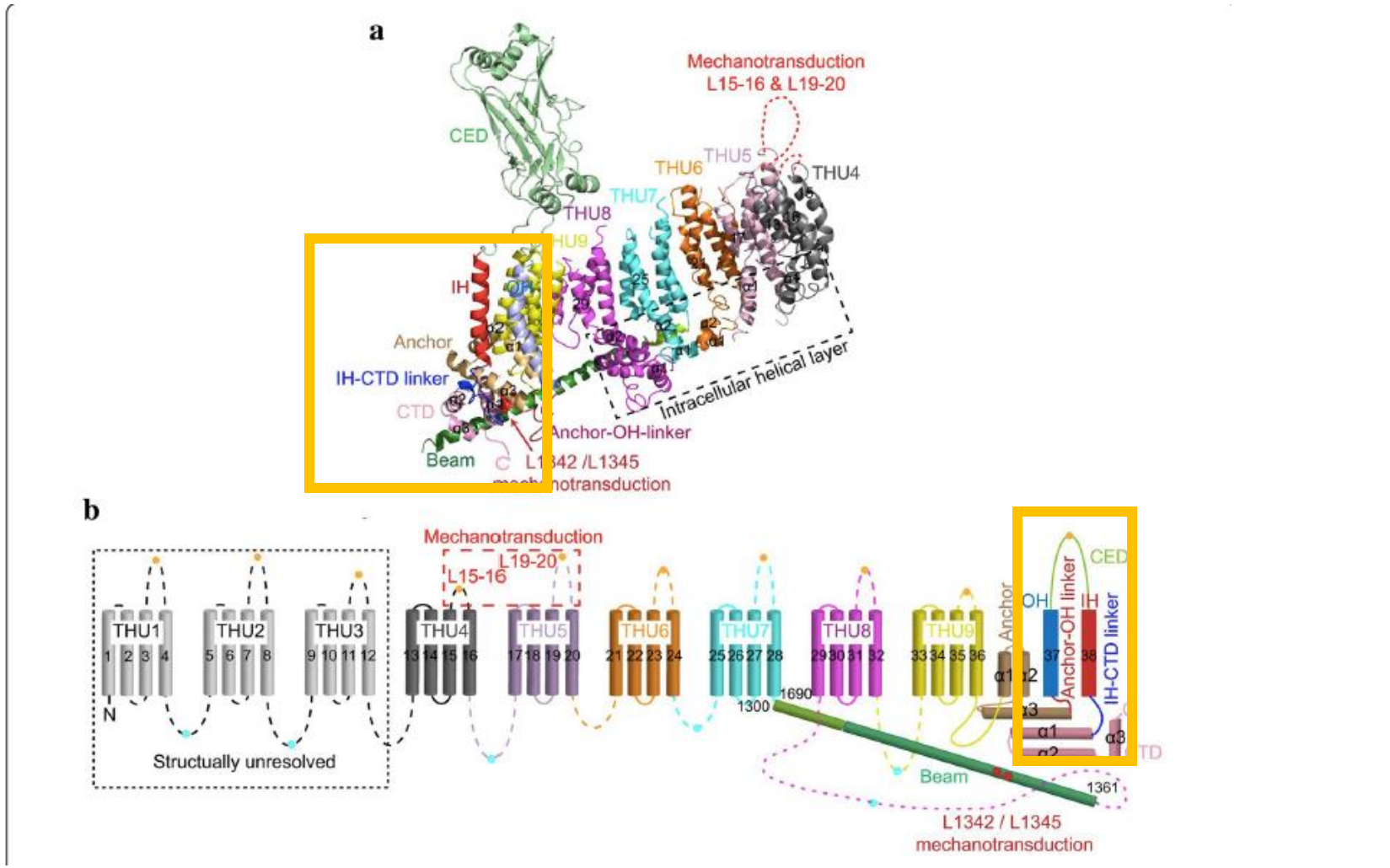


To further confirm the topological location of the CED and the C terminus of Piezo1, we performed immunolabelling of live HEK293T cells expressing Piezo1 with a Flag tag fused either in a flexible loop of the CED (after A2419) or at the C terminus of Piezo1. Using confocal microscopy, we found that the Flag tag could be labelled on the plasma membrane of live cells only when inserted in the CED and not at the C terminus (Fig. 2h). **These data demonstrate that the CED is an extracellular domain, whereas the C terminus is intracellular, consistent with a recent report**

Piezo channels are predicted to possess an unusually large number of TM regions, ranging from 10 to 40. Zhao et al. recently produced high-resolution structures of mouse Piezo1 (mPiezo1), revealing a unique 38-TM topology in each subunit (Fig. 2a, b).

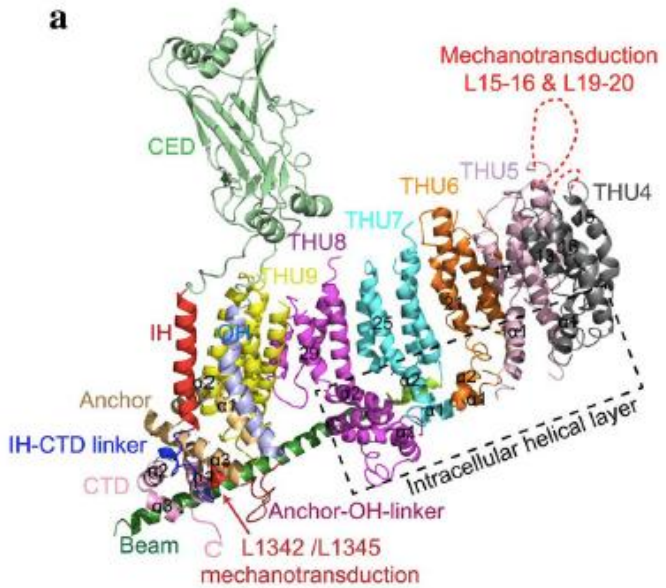


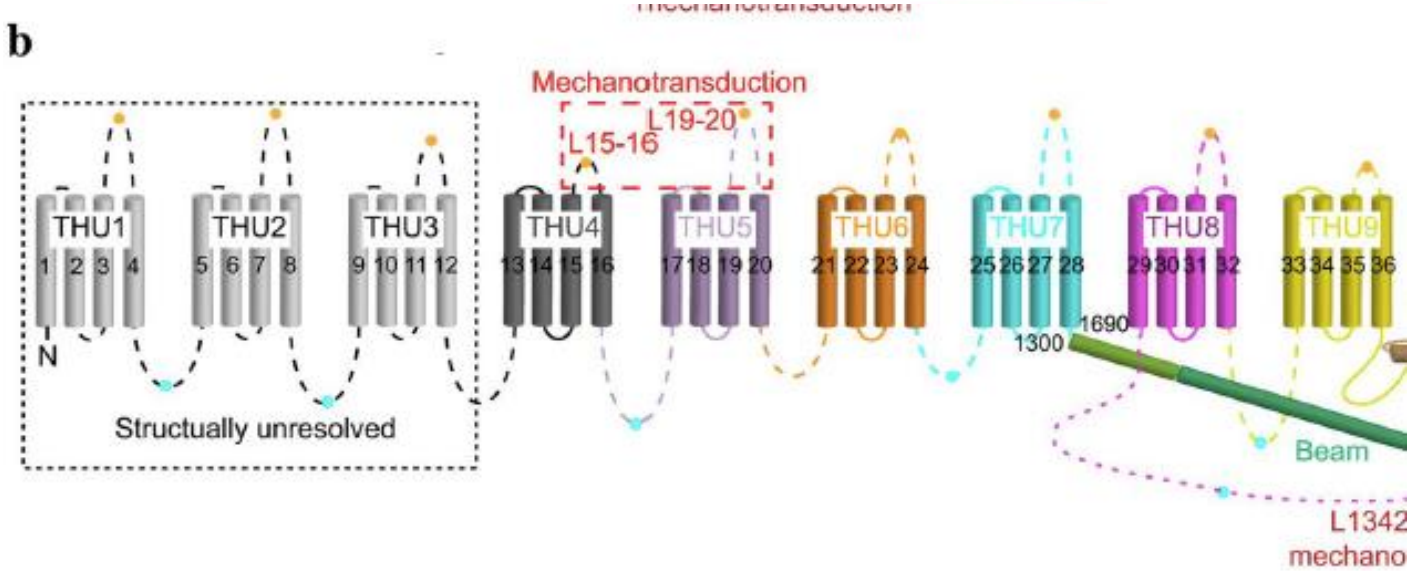
The two TM regions (TM37 and TM38) closest to the center of the protein are designated as the inner helix (IH) and outer helix (OH), respectively, and enclose the transmembrane pore of the central pore module.



The other 36 TM regions (TM1-36) form a **curved blade-like** structure with nine repetitive folds containing 4 TM regions each, named transmembrane helical units (THUs).

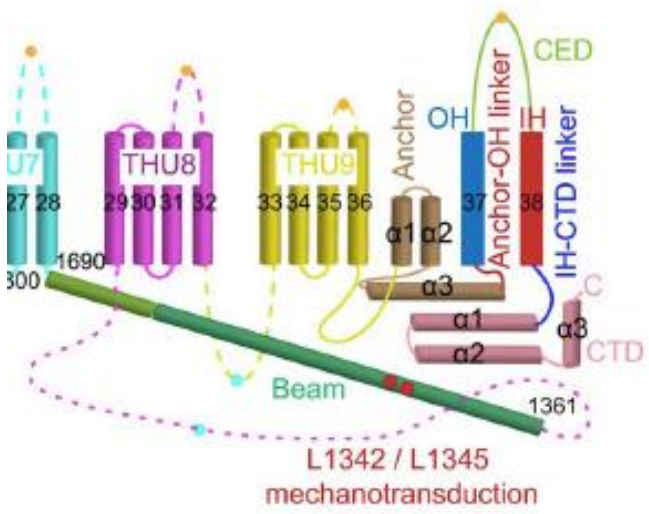
important feature of the blades is the L-shaped helical structures formed by TM13, TM17, TM21, TM25 and TM29. Both identifiable structural features appear to be ideal for not only for mechanosensation but also for the induction of local membrane curvature.

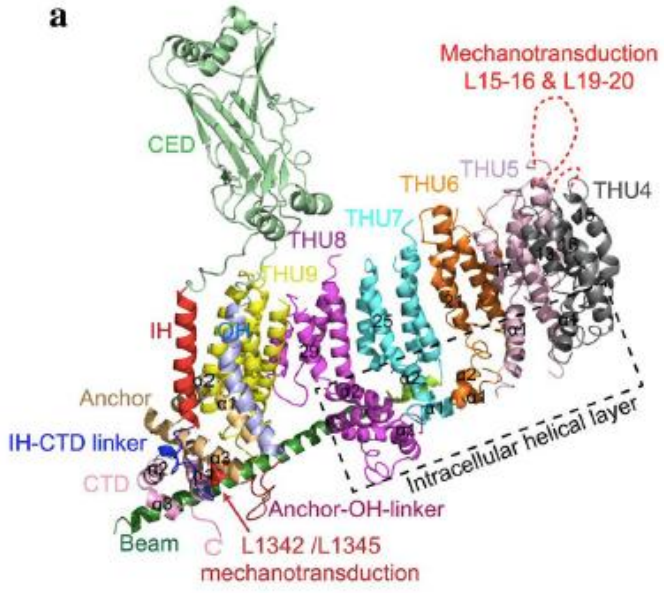




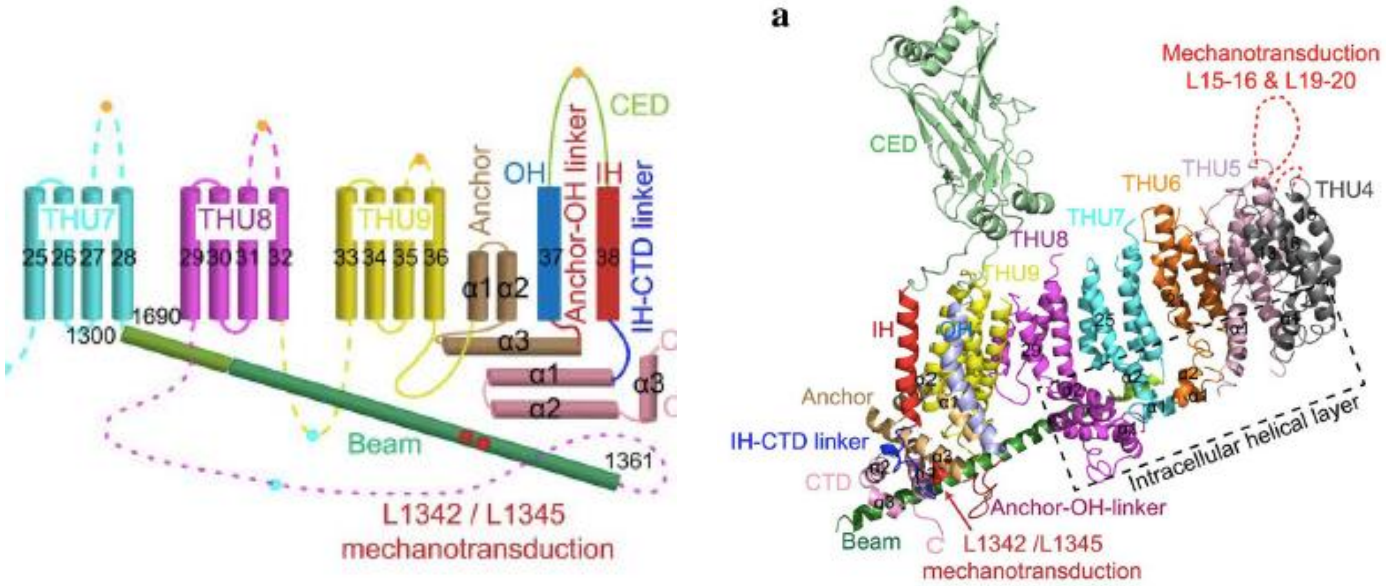
A hairpin structure, referred to as the **anchor**, connects the OH-IH pair to the C-terminal domain (CTD) plane. The anchor is made up of three helices (α1, α2, and α3). Helices α1 and α2 were found to organize into an inverted V-shaped structure, which maintains the integrity of the ion-conducting pore.

A few mutations in Piezo1 at locations including KKKK (2182-K2185), T2143, T2142 (T2127 in human Piezo1), R2514, E2523, and E2522, which are located in α3 in the anchor, have been reported to cause severe disease

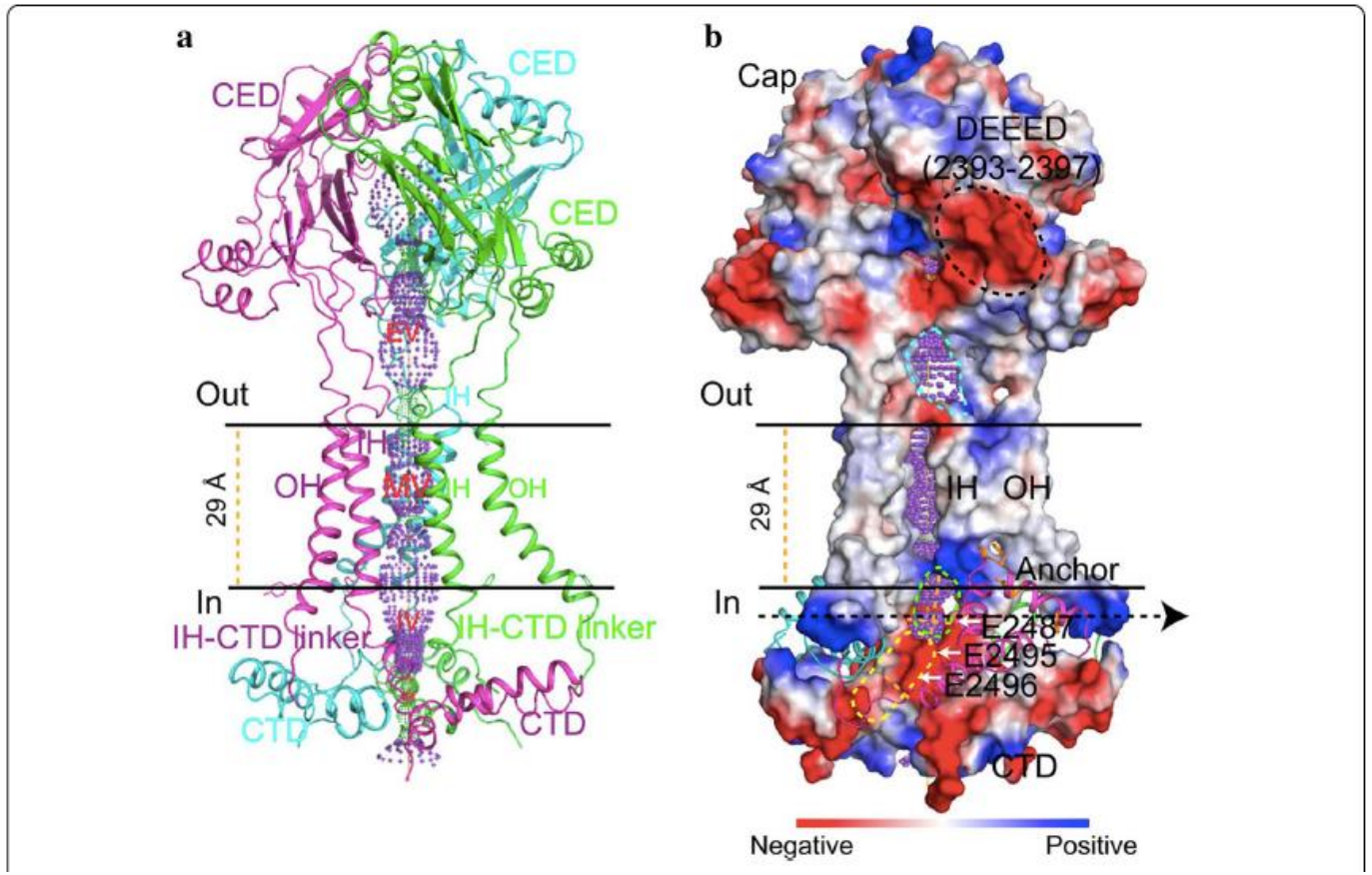




On the intracellular surface, Piezo1 contains three beamlike structures 90 nm in length that are organized at a 30° angle relative to the membrane plane. Residues H1300-S1362 form the **beam structure**. The large intracellular THU7-8 loop might provide the beam with the structural basis for mechanical transmission. Functionally, the three long intracellular beams not only support the whole TM skeleton but also physically bridge the distal THUs to the central ion-conducting pore. When residues 1280 to 1360 (which form this beam structure) were deleted, the resulting mutant protein was absent, suggesting the structural importance of the beam

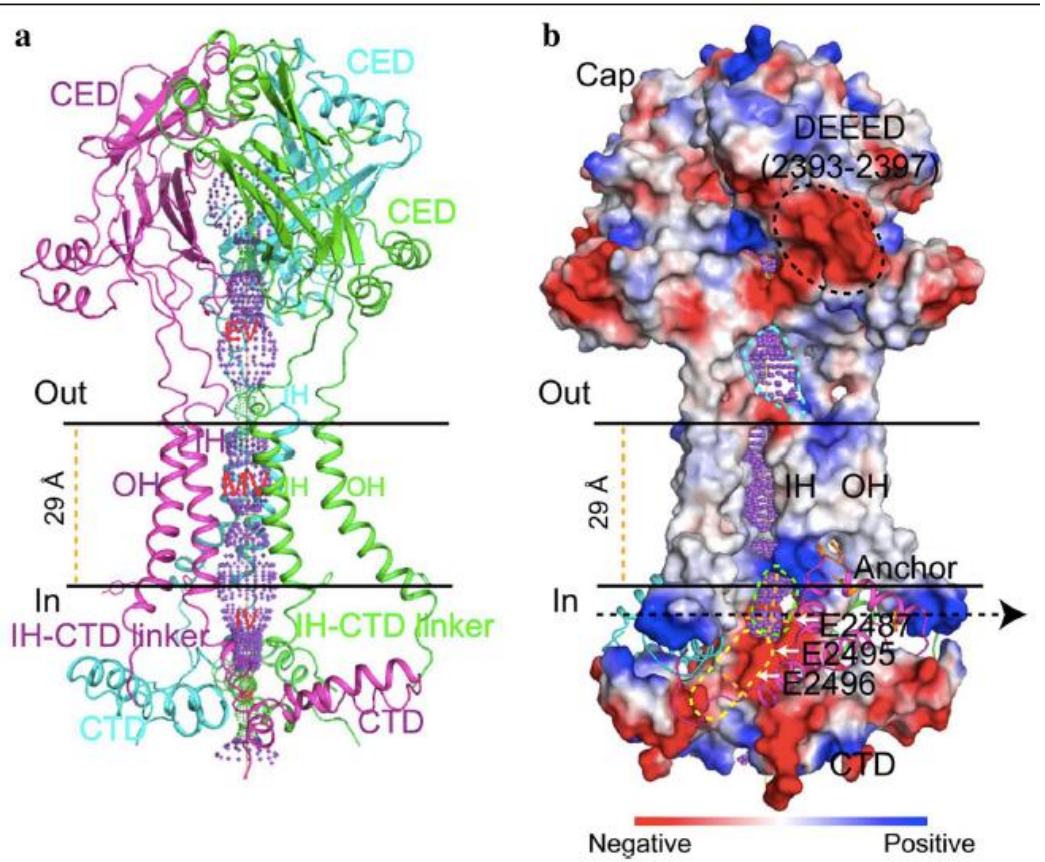


The centre of the Piezo1 channel within the membrane consists of **six transmembrane** helices in a triangular arrangement. Three IHs (1 IH/subunit), are located at the innermost position and seem to line a central pore. Three OHs (1 OH/subunit), extended from the N termini of the CEDs, further enclose the three IHs.



The continuous central channel consists of three parts, an EV within the cap region, a transmembrane vestibule (MV) within the membrane, and an intracellular vestibule (IV) underneath the membrane. Importantly, DEEED (2393–2397), a patch of negatively charged residues residing in the opening of the extracellular “cap” structure is required to ensure efficient ion conduction and determine the selection of cations over anions.

Additionally, two critical acidic residues, E2495 and E2496, located at the CTD-constituted IV, may be responsible for divalent calcium ion selectivity, unitary conductance and pore blockage.



ARTICLE

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Structure and mechanogating of the mammalian tactile channel PIEZO2

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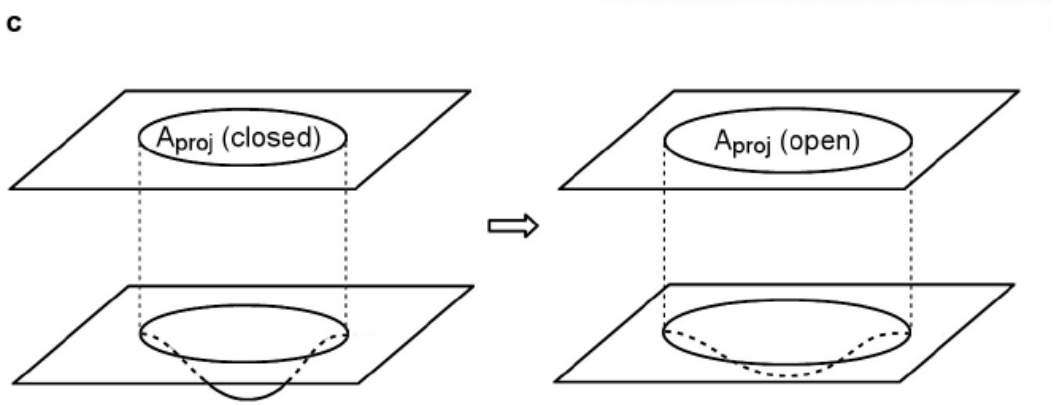
Piezo2 channel is very similar to that of Piezo1 in that it forms a three-bladed, propeller-like homotrimeric structure comprising a central ion-conducting pore module and three peripheral blades with 38 TMs.

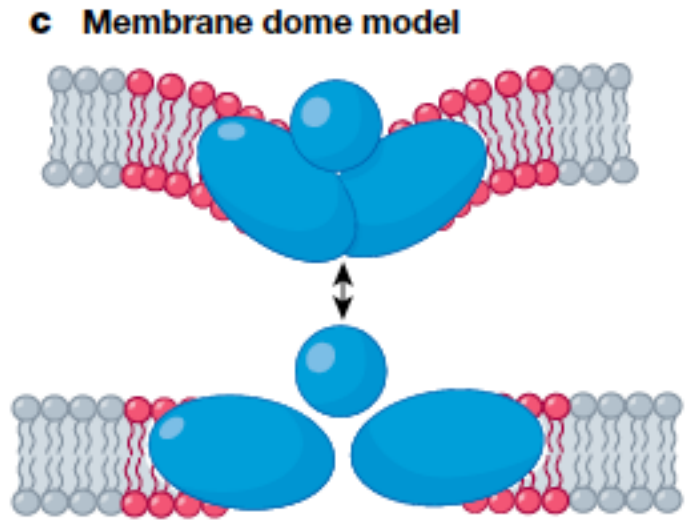
MEMBRANE DOME KINETIC MODEL

The arms of PIEZO, composed of trasmembrane helices, are inside the membrane and FORCE the membrane to curve (suggesting a preferential localization in membrane domains of similar curvature.

The structure of Piezo1 leads us to propose a membrane dome mechanism for the origins of its mechanosensitive gating. In this mechanism a dome of membrane, created by Piezo's shape in its closed conformation, undergoes relative flattening upon channel opening. This mechanism does not require the application of a force pressing onto the dome (i.e. a force component normal to the plane of the membrane): lateral membrane tension alone will favor the flatter (Guo and McKinnon 2017)



Guo & McKinonn, ELife 2017

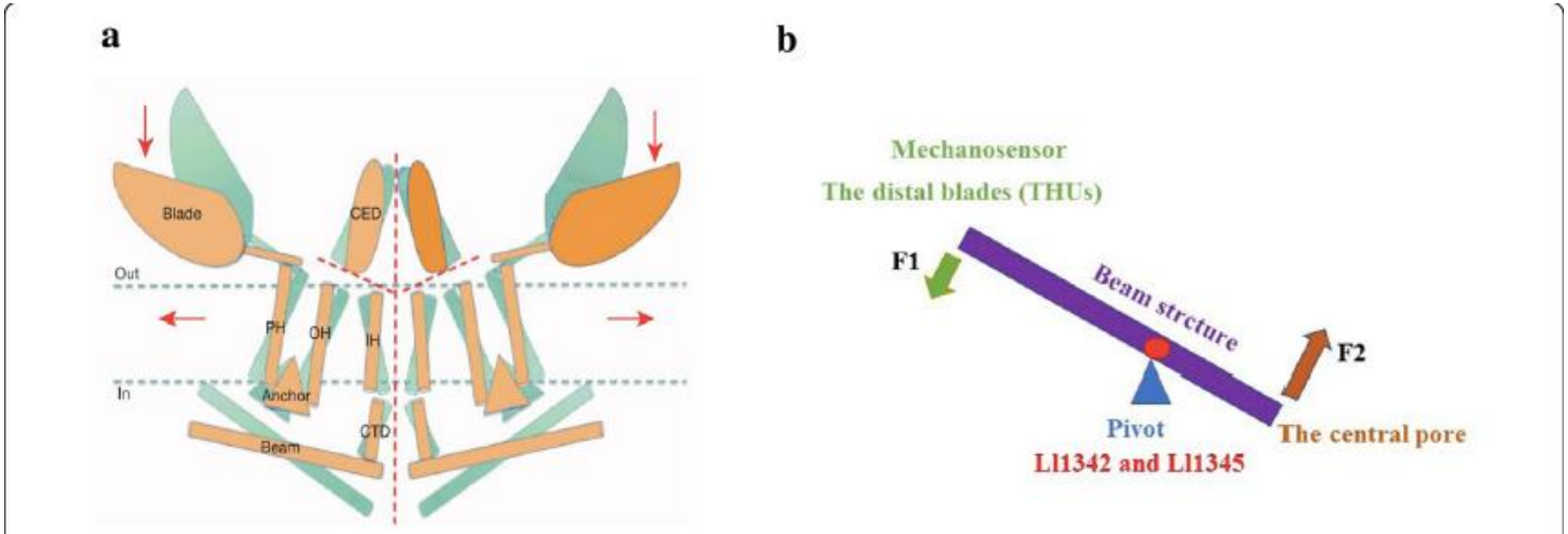


Kefauver et al., Nature 2020

LEVER-LIKE MECHANOTRANSDUCTION MECHANISM

In the mPiezo1 channel, the curved blades composed of THUs can act as a mechanosensor, while the beam structure, with the residues Ll1342 and Ll1345 acting as a pivot, can act as a lever-like apparatus.

Coupling the distal blades and central pore through the lever-like apparatus converts mechanical force into a force used for cation conduction. Because the pivot of the lever is positioned closer to the central pore than to the distal blades, the input force is effectively amplified through the lever-like apparatus. Additionally, a large conformational change in the distal blades is converted into a relatively slight opening of the central pore, allowing cation-selective permeation.



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Thank you