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Supporting Online Material for

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

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**This PDF file includes:**

Materials and Methods
Figs. S1 to S8
Tables S1 and S2
References

## Supporting online material

## Material and Methods

**Cell culture and transient transfection.** Neuro2A cells were grown in Eagle's Minimum Essential Medium containing 4.5 mg.ml$^{-1}$ glucose, 10% fetal bovine serum, 50 units.ml$^{-1}$ penicillin and 50 µg.ml$^{-1}$ streptomycin. C2C12 or Human Embryonic Kidney 293T (HEK293T) cells were grown in Dulbecco's Modified Eagle Medium containing 4.5 mg.ml$^{-1}$ glucose, 10% fetal bovine serum, 50 units.ml$^{-1}$ penicillin and 50 µg.ml$^{-1}$ streptomycin. Cells were plated onto 35 mm dishes or 12-mm round glass coverslips placed in 24-well plates and transfected using lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. For Piezo1 overexpression experiment, 500 to 1000 ng.ml$^{-1}$ of *Piezo1*-IRES-GFP or vector only were transfected and cells were recorded 12-48 hours later. For Piezo2 overexpression experiments, 600 to 1000 ng.ml$^{-1}$ of *mPiezo2* or vector were co-transfected with 300 ng.ml$^{-1}$ *GFP* to identify transfected cells and cells were recorded 12-48 hours later. For siRNA experiment, 20 nM total siRNA and 300 or 500 ng.ml$^{-1}$ GFP to identify transfected cells were co-transfected and cells were used 3 days after transfection. siRNA of Smartpool I directed against *mPiezo1* were purchased from Qiagen (Target sequences: CACCGGCATCTACGTCAAATA (siRNA1), ACCAAGAAATACAACCATCTA (siRNA2), TCGGCGCTTGCTAGAACTTCA (siRNA3) and CGGAATCCTGCTGCTGCTATA (siRNA4)) and used at 5 nM each together in Smartpool I or at 20 nM separately. siRNA4 was toxic at 20 nM, as it caused cell detachment and subsequent death 3 days after transfection. Smartpool II siRNA was a pool of 4 different siRNA purchased from Dharmacon (Target sequences: GAAAGAGATGTCACCGCTA, GCATCAACTTCCATCGCCA, AAAGACAGATGAAGCGCAT, GGCAGGATGCAGTGAGCGA). For Piezo2 siRNA experiment in N2A cells, 600 ng.ml$^{-1}$ of *mPiezo2* and 300 ng.ml$^{-1}$ *GFP* were co-transfected with 20 nM Piezo1 siRNA1 only or together with 20 nM Piezo2 siRNA. Cells were recorded 3 days after transfection. siRNA directed against mPiezo2 was a pool of 4 different siRNA purchased from Dharmacon (Target sequences: GAATGTAATTGGACAGCGA, TCATGAAGGTGCTGGGTAA, GATTATCCATGGAGATTTA, GAAGAAAGGCATGAGGTAA).

**DRG culture and siRNA**

Preparation and culture of mouse dorsal root ganglion neurons (from male C57Bl6 mice) were performed as described previously (*1*) with the following modifications: Growth medium was supplemented with 100 ng/ml nerve growth factor (NGF), 50 ng/ml GDNF, 50 ng/ml BDNF, 50 ng/ml NT-3, 50 ng/ml NT-4. Small interference RNA (siRNA)-mediated knockdown was achieved by nucleofection of siRNA into freshly isolated DRG neurons using the SCN nucleofector kit with the nucleofector device according to the manufacturer's instructions (SCN Basic Neuro program 6; Lonza AG). DRG neurons isolated from one mouse were used per siRNA tested. siRNAs were used at 150 nM-250 nM for TRPA1 (smartpool, Qiagen) and 250 nM for Piezo2 (smartpool, Qiagen), concentrations of scrambled controls (Qiagen) were adjusted accordingly. After nucleofection, neurons were allowed to recover in RPMI medium for 10 min at 37˚C , growth medium (without antibiotics and without AraC) was added and neurons were plated on poly-D-lysine coated coverslips, previously coated with laminin (2 µg.ml$^{-1}$). 2-4hrs after transfection half of the growth medium was exchanged with fresh medium and neurons were grown for 48-72 hours.

**Electrophysiology.** Patch-clamp experiments were performed in standard whole-cell or cell attached recordings using an Axopatch 200B amplifier (Axon Instruments). Patch pipettes had resistance of 2–3 MΩ when filled with an internal solution consisting of (in mM) 133 CsCl, 10 HEPES, 5 EGTA, 1 $CaCl_2$, 1 $MgCl_2$, 4 MgATP, and 0.4 $Na_2GTP$ (pH adjusted to 7.3 with CsOH). The extracellular solution consisted of (in mM) 127 NaCl, 3 KCl, 1 $MgCl_2$, 10 HEPES, 2.5 $CaCl_2$, 10 glucose (pH adjusted to 7.3 with NaOH). NMDG solution consisted of (in mM) 150 NMDG, 10 HEPES (pH 7.5). For ion selectivity experiments, internal solution consisted of (in mM) 150

CsCl, 10 Hepes (pH 7.3 with CsOH), monovalent external solutions consisted of (in mM) 150 NaCl or KCl, 10 HEPES (pH 7.3 with NaOH or KOH) and divalent external solutions consisted of (in mM) 100 $CaCl_2$ or $MgCl_2$, 10 HEPES (pH 7.3 with CsOH). For cell attached recordings, pipette were filled with a solution consisting of (in mM) 130 NaCl, 5 KCl, 10 HEPES, 1 $CaCl_2$, 1 $MgCl_2$, 10 TEA-Cl (pH 7.3 with NaOH) and external solution used to zero the membrane potential consisted of (in mM) 140 KCl, 10 HEPES, 1 $MgCl_2$, 10 glucose (pH 7.3 with KOH). All experiments were done at room temperature. Currents were sampled at 20 kHz and filtered at 2 kHz. Voltages were not corrected for a liquid junction potential excepted for ion selectivity experiments were LJP were calculated using Clampex 10.1 software (Axon Instruments). Leak currents before mechanical stimulations were subtracted off-line from the current traces. 10 mM ruthenium red stock solution was prepared in DMSO; 100 mM gadolinium stock solution, in water.

**Mechanical Stimulation.** For whole-cell recordings mechanical stimulation was achieved using a fire-polished glass pipette (tip diameter 3–4 μm) positioned at an angle of 80° to the cell being recorded. Downward movement of the probe toward the cell was driven by a Clampex controlled piezo-electric crystal microstage (E625 LVPZT Controller/Amplifier; Physik Instrumente). The probe was typically positioned ~2 μm from the cell body. The piezoelectrically driven stimulus intensity used to measure the threshold of MA current activation was defined as the distance traveled beyond that which touched the cell.  The probe had a velocity of 1 μm/ms during the ramp segment of the command for forward motion and the stimulus was applied for 150 ms. To assess the mechanical sensitivity of a cell, a series of mechanical steps in 1 μm increments were applied every 10 s, which allowed full recovery of mechanosensitive currents. Inward MA currents were recorded at a holding potential of -80 mV. For I-V relationship recordings, voltage steps were applied 0.7 s before the mechanical stimulation from a holding potential of -60 mV. For recordings of MA currents in DRG neurons, the inactivation kinetics at a holding potential of -80 mV of traces of currents reaching at least 75 % of the maximal amplitude of current elicited per cell were fitted with mono-exponential equation (or in some case bi-exponential equation for the rapidly-adapting currents, accordingly to previous reports (*2*) and using the fast time constant for analysis, see Fig.4A left panel) giving a value of $\tau_{inac}$ per responsive neuron that we used for analysis.

For cell-attached recordings membrane patches were stimulated with brief negative pressure pulses through the recording electrode using a Clampex controlled pressure clamp HSPC-1 device (ALA-scientific). Otherwise stated, stretch-activated channels were recorded at a holding potential of -80 mV with pressure steps from 0 to -60 mm Hg (-10 mm Hg increments), and 3-10 recording traces were averaged per cell for analysis. Current-pressure relationships were fitted with a Boltzmann equation of the form: $I(P) = [1 + \exp(-(P - P_{50})/s)]^{-1}$, where I is the peak of stretch-activated current at a given pressure, P is the applied patch pressure (in mm Hg), $P_{50}$ is the pressure value that evoked a current value which is 50% of Imax, and s reflects the current sensitivity to pressure.

**Determination of Permeability Ratios.** Reversal potentials for each cell in each solution were determined by interpolation of the respective current-voltage data. In these experiments, I-V relationships were performed from a holding potential of -60 mV with voltage steps ranging from -60 mV to +60 mV (before liquid junction potential correction) in 20 mV increments.

The ratio of permabilities, $P_X/P_{Cs}$, was determined for each test cation X for each cell from the reversal potential of the MA activated whole-cell current when that cation was the major external cation. The Goldman-Hodgkin-Katz (GHK) equation (*3*), simplified for a single permeant cation on each side of the membrane, was employed:

$$E_{rev} = \frac{RT}{zF} \ln \frac{P_X[X]_0}{P_{Cs}[Cs]_i}$$

where RT/zF has the value of 25.5 at 23ºC. For the divalent cations the appropriately modified equation was used:

$$E_{rev} = \frac{RT}{F} \ln \left( \sqrt{\frac{4P_X[X]_o}{P_{Cs}[Cs]_i} + \frac{1}{4}} - \frac{1}{2} \right).$$

Ratio $P_X/P_{Cs}$ were presented for each cation as mean ± SEM.

**Ratiometric calcium imaging.** Intracellular $Ca^{2+}$ imaging experiments were performed by washing cells three times with $Ca^{2+}$ imaging buffer [1× Hanks Balanced Salt Solution (HBSS, 1.3 mM $Ca^{2+}$) supplemented with 10 mM HEPES], then loaded with ratiometric $Ca^{2+}$ indicator dye Fura-2/AM (Molecular Probes) for 30 min at room temperature, according to the manufacturer´s recommendations. Cells were washed three times prior to imaging on an inverted microscope. Fura-2 fluorescence was measured by illuminating the cells with an alternating 340/380 nm light. Fluorescence intensity was measured at 510 nm. The intracellular $Ca^{2+}$ concentration is expressed as the 340/380 ratio.

Ratiometric calcium imaging of cultured DRG neurons was performed essentially as described (*1*). Experiments were conducted at 37˚C 48-72 hrs after plating. Threshold for activation was set at 40 % above the averaged baseline from 5 time points immediately before addition of MO (100 µM). Capsaicin (CAPS, 0.5 µM) was added at the end of each experiment to control for siRNA specificity, neuronal health and responsiveness. All experimental groups to be compared were processed in parallel using the same DRG culture preparation (2 independent preparations were used).

**Generation of Piezo1 antisera.** Custom polyclonal antibodies to the synthetic peptide RQRRERARQERAEQ (aa 1454..1467) were prepared in rabbit by standard methods and affinity purified (Thermo Fisher Scientific, Openbiosystems).

**TRPA1 Live-Labeling and Immunocytochemistry.** TRPA1 live-labeling and immunocytochemistry on HEK 293T cells were performed essentially as described (Schmidt et al., 2009) with the following modifications: For assessment of the specificity of Piezo1 antisera, cells were transfected with a Piezo1-IRES-EGFP construct and used 36 hrs later for immunocytochemistry as outlined below. Piezo1 antisera were used at 1:100 and detected by secondary antibodies conjugated to Alexa Fluor 546 (Invitrogen). For assessment of membrane expression of Piezo1, cells were co-transfected with a murine Trpa1-MYC/His construct and Piezo1 and used for live-labeling 36 hrs after transfection. Surface TRPA1 was labeled by incubating live HEK293T cells with TRPA1 antibodies (1:50) followed by incubation with Alexa Fluor 488 F(ab')2 fragment of goat-anti-rabbit (1:200, Invitrogen). Cells were fixed with 2% paraformaldehyde (PFA) in PBS for 20 min, washed and incubated with an excess of non-labeled goat-anti-rabbit IgG for 1 hour to block binding sites on any remaining unbound TRPA1 antibody. Cells were then permeabilized in PBS containing 0.4% Triton X-100, blocked with normal goat serum (10% serum in PBS), and incubated with primary antibodies against Piezo1 (1:100) and c-MYC (1:100, 9E11, mouse, Santa Cruz Biotechnology), followed by secondary antibodies (Alexa Fluor 568 goat-anti-rabbit IgG and Alexa Fluor 633 goat-anti-mouse IgG; 1:200; Invitrogen).

Immunocytochemistry experiments were imaged using an Olympus (Tokyo, Japan) Fluoview 500 confocal microscope by sequential illumination using the 488 nm line of an argon laser, a

HeNe green 543 nm laser and a HeNe red 633 nm laser. Merge stacked images were created using a 40x and 60x PlanAPO oil-immersion objective, the latter with a zoom of 1,5.

**Real time qPCR**. Dorsal root ganglia were freshly isolated from adult C57BL/6J wildtype mice and snap frozen on dry ice. Total RNA from DRG or siRNA transfected N2A cells (3 days after GFP co-transfection, same conditions then the one used for recordings) was extracted using Trizol treatment. Total RNA from all other tissues were purchased from Zyagen (San Diego). 500 ng total RNA was used to generate 1st strand cDNA using the Quantitect reverse transcription kit (Qiagen). Real time Taqman PCR assays for *mPiezo1* and *mPiezo2* (assay id: Mm01241570_g1 and Mm01262433_m1) were purchased from Applied Biosystems with a FAM reporter dye and a non-fluorescent quencher. Universal TaqMan PCR master mix (20X) without AmpErase UNG (Applied Biosystems) was used. The reaction was run in the ABI 7900HT fast real time system using 1 µl of the cDNA in a 20 µl reaction according to the manufacturer's instructions in triplicate.

Calibrations and normalizations were done using the $2^{-\Delta\Delta CT}$ method (*4*), where $\Delta\Delta C_T$ = (($C_T$ (target gene) -$C_T$ (reference gene)) - ($C_T$ (calibrator) - $C_T$ (reference gene)). For the analysis of mRNA expression in different tissues (Fig. 4C), the target gene was *mPiezo1* or *mPiezo2*, while the reference gene was *GAPDH* and the calibrator was the lung tissue. For the analysis of siRNA induced down-regulation of mRNA expression (Fig. S1A), β-actin was used as the reference gene and Scrambled siRNA transfected N2A cells was the calibrator.

**In situ hybridization and immunohistochemistry**

In situ hybridization and immunohistochemistry were performed as described previously (*1*). In brief, adult male C57BL/6J mice, ages 6–16 weeks, or adult male Sprague Dawley rats, were perfused with 4% PFA and dorsal root ganglia were quickly dissected. Following post-fixation and cryoprotection in 30% sucrose, single DRG were embedded in OCT and sectioned with a cryostat at 10 µm thickness. Four different, 1000 bps cRNA sense and anti-sense probes were generated corresponding to bases-3822-4886; 4837-5849; 5922-7019 and 7102-8171. All probes were in vitro-transcribed and labeled with digoxigenin (Roche Diagnostics). For fluorometric in situ hybridizations, a peroxidase-conjugated anti-digoxygenin-POD antibody (1:500) and tyramide signal amplification (TSA; NEN) were used to detect and visualize the hybridized probes. Immunohistochemistry was performed after in situ hybridization and TSA detection. Chicken anti-NF-200 (1:1000; Abcam) and chicken anti-Peripherin (1:100; Abcam) were used on mouse DRG, while guinea pig anti-TRPV1 (1:1000; Abcam) primary antibodies were used on rat DRG (this antibody did not perform on mouse DRG). Primary antibodies were detected by secondary antibodies conjugated to Alexa Fluor 568. For colorimetric in situ hybridizations, an alkaline phosphatase anti-DIG-AP antibody (Roche; 1:500) was used followed by incubation with NBT/BCIP liquid substrate system (Sigma) for development of the dark purple color. Sections were mounted in Slow fade Gold reagent (Invitrogen) and imaged using a AX70 microscope (Olympus).

Fluorometric in situ hybridizations were used for quantitation and were imaged using an Olympus (Tokyo, Japan) Fluoview 500 confocal microscope by sequential illumination using the 488 nm line of an argon laser and the HeNe green 543 nm laser. Merge stacked images were created using a 20x and a 40x PlanAPO oil-immersion objective. Images for all experimental groups were taken using identical acquisition parameters and raw images were used for analysis with Image J (NIH). Neurons were considered Piezo2-positive if the mean fluorescence intensity (measured in arbitrary units) was higher than the mean background fluorescence plus 4 times the standard deviation measured from at least 10 random unstained cells. Only sections being at least 50 µm apart were considered to avoid double counting neurons. Neurons were considered NF200- or Peripherin-positive if the mean fluorescence intensity (measured in arbitrary units) was higher than the mean background fluorescence plus 3 times the standard deviation measured from at least 10 random unstained cells. Colorimetric in situ hybridizations of Piezo2 gave similar results (26.9 % Piezo2-positive neurons, data not shown), but were not used for quantitation due to variability depending on the length of development with substrate. Only for presentation purposes brightness, contrast and levels of images were adjusted.

**Molecular Cloning of Piezo1**. Primers were designed from cDNA sequence of *mPiezo1* from the NCBI database (NM_001037298). A 7.644 kb fragment was amplified from cDNA libraries generated from Neuro2A total RNA using primers *mPiezo1* fwd (5’ atggagccgcacgtgctg 3’) and *mPiezo1* rev (5’ ctactccctctcacgtgtcca 3’) and cloned into pcDNA3.1(-) (Invitrogen) with NotI and HindIII restriction sites. Fully sequencing this construct revealed insertion of Q at residue 156, and three amino acid changes (R147G, V228I and M1571V) compared to the NCBI sequence.

This vector was further modified to include 3’ AscI and FseI restriction sites and an IRES-GFP PCR fragment from pIRES2-EGFP (Clontech) was then inserted using these sites. The protein sequence of Piezo1 that we cloned from N2A cells is:

MEPHVLGAGLYWLLLPCTLLAASLLRFNALSLVYLLFLLLLPWLPGPSRHSIPGHTGRLLRALLCLSLLFLVAHLAFQICL

HTVPHLDQFLGQNGSLWVKVSQHIGVTRLDLKDIFNTTRLVAPDLGVLLASSLCLGLCGRLTRKAGQSRRTQELQDD

DDDDDDDDEDIDAAPAVGLKGAPALATKRRLWLASRFRVTAHWLLMTSGRTLVIVLLALAGIAHPSAFSSIYLVVFLAIC

TWWSCHFPLSPLGFNTLCVMVSCFGAGHLICLYCYQTPFIQDMLPPGNIWARLFGLKNFVDLPNYSSPNALVLNTKHA

WPIYVSPGILLLLYYTATSLLKLHKSCPSELRKETPREDEEHELELDHLEPEPQARDATQGEMPMTTEPDLDNCTVHVL

TSQSPVRQRPVRPRLAELKEMSPLHGLGHLIMDQSYVCALIAMMVWSIMYHSWLTFVLLLWACLIWTVRSRHQLAML

CSPCILLYGLTLCCLRYVWAMELPELPTTLGPVSLHQLGLEHTRYPCLDLGAMLLYLLTFWLLLRQFVKEKLLKKQKVP

AALLEVTVADTEPTQTQTLLRSLGELVTGIYVKYWIYVCAGMFIVVSFAGRLVVYKIVYMFLFLLCLTLFQVYYTLWRKL

LRVFWWLVVAYTMLVLIAVYTFQFQDFPTYWRNLTGFTDEQLGDLGLEQFSVSELFSSILIPGFFLLACILQLHYFHRPF

MQLTDLEHVPPPGTRHPRWAHRQDAVSEAPLLEHQEEEEVFREDGQSMDGPHQATQVPEGTASKWGLVADRLLDL

AASFSAVLTRIQVFVRRLLELHVFKLVALYTVWVALKEVSVMNLLLVVLWAFALPYPRFRPMASCLSTVWTCIIIVCKML

YQLKIVNPHEYSSNCTEPFPNNTNLQPLEINQSLLYRGPVDPANWFGVRKGYPNLGYIQNHLQILLLLVFEAVVYRRQE

HYRRQHQQAPLPAQAVCADGTRQRLDQDLLSCLKYFINFFFYKFGLEICFLMAVNVIGQRMNFMVILHGCWLVAILTR
RRREAIARLWPNYCLFLTLFLLYQYLLCLGMPPALCIDYPWRWSKAIPMNSALIKWLYLPDFFRAPNSTNLISDFLLLLC
ASQQWQVFSAERTEEWQRMAGINTDHLEPLRGEPNPIPNFIHCRSYLDMLKVAVFRYLFWLVLVVVFVAGATRISIFG
LGYLLACFYLLLFGTTLLQKDTRAQLVLWDCLILYNVTVIISKNMLSLLSCVFVEQMQSNFCWVIQLFSLVCTVKGYYDP
KEMMTRDRDCLLPVEEAGIIWDSICFFFLLLQRRIFLSHYFLHVSADLKATALQASRGFALYNAANLKSINFHRQIEEKS
LAQLKRQMKRIRAKQEKYRQSQASRGQLQSKDPQDPSQEPGPDSPGGSSPPRRQWWRPWLDHATVIHSGDYFLF
ESDSEEEEEALPEDPRPAAQSAFQMAYQAWVTNAQTVLRQRRERARQERAEQLASGGDLNPDVEPVDVPEDEMAG
RSHMMQRVLSTMQFLWVLGQATVDGLTRWLRAFTKHHRTMSDVLCAERYLLTQELLRVGEVRRGVLDQLYVGEDE
ATLSGPVETRDGPSTASSGLGAEEPLSSMTDDTSSPLSTGYNTRSGSEEIVTDAGDLQAGTSLHGSQELLANARTRM
RTASELLLDRRLHIPELEEAERFEAQQGRTLRLLRAGYQCVAAHSELLCYFIIILNHMVTASAASLVLPVLVFLWAMLTIP
RPSKRFWMTAIVFTEVMVVTKYLFQFGFFPWNSYVVLRRYENKPYFPPRILGLEKTDSYIKYDLVQLMALFFHRSQLL
CYGLWDHEEDRYPKDHCRSSVKDREAKEEPEAKLESQSETGTGHPKEPVLAGTPRDHIQGKGSIRSKDVIQDPPEDL
KPRHTRHISIRFRRRKETPGPKGTAVMETEHEEGEGKETTERKRPRHTQEKSKFRERMKAAGRRLQSFCVSLAQSFY
QPLQRFFHDILHTKYRAATDVYALMFLADIVDIIIIIFGFWAFGKHSAATDIASSLSDDQVPQAFLFMLLVQFGTMVIDRAL
YLRKTVLGKLAFQVVLVVAIHIWMFFILPAVTERMFSQNAVAQLWYFVKCIYFALSAYQIRCGYPTRILGNFLTKKYNHL
NLFLFQGFRLVPFLVELRAVMDWVWTDTTLSLSNWMCVEDIYANIFIIKCSRETEKKYPQPKGQKKKIVKYGMGGLIIL
FLIAIIWFPLLFMSLIRSVVGVVNQPIDVTVTLKLGGYEPLFTMSAQQPSIVPFTPQAYEELSQQFDPYPLAMQFISQYSP
EDIVTAQIEGSSGALWRISPPSRAQMKQELYNGTADITLRFTWNFQRDLAKGGTVEYTNEKHTLELAPNSTARRQLAQ
LLEGRPDQSVVIPHLFPKYIRAPNGPEANPVKQLQPDEEEDYLGVRIQLRREQVGTGASGEQAGTKASDFLEWWVIE
LQDCKADCNLLPMVIFSDKVSPPSLGFLAGYGIVGLYVSIVLVVGKFVRGFFSEISHSIMFEELPCVDRILKLCQDIFLVR
ETRELELEEELYAKLIFLYRSPETMIKWTRERE

**Molecular Cloning of Piezo2.** Primers were designed from cDNA sequence of *Piezo2* from the NCBI database (NM_001039485). An 8.469 kb fragment was amplified from cDNA libraries generated from adult C57BL/6J DRG total RNA using primers *mPiezo2* fwd (5'atggcttcggaagtggtgtgc 3') and *mPiezo2* rev (5'tcagtttgtttttctctagtccac 3') and cloned into pCMV-Sport6 (Invitrogen) with KpnI and NotI restriction sites. Sequencing of the cloned *mPiezo2* gene from DRG revealed differences from the NCBI annotation where three regions of amino acid insertions were not correctly assigned as exons (628E, 14 residues at 833 and 56 residues at 1751). The protein sequence of Piezo2 that we cloned from mouse DRG is:

MASEVVCGLIFRLLLPICLAVACAFRYNGLSFVYLIYLLLIPLFSEPTKATMQGHTGRLLQSLCITSLSFLLLHIIFHITLASL
EAQHRITPAYNCSTWEKTFRQIGFESLKGADAGNGIRVFVPDIGMFIASLTIWLVCRTIVKKPDTEEIAQLNSECENEEL
AGGEKMDSEEALIYEEDLDGEEGMEGELEESTKLKILRRFASVASKLKEFIGNMITTAGKVVVTILLGSSGMMLPSLTS
AVYFFVFLGLCTWWSWCRTFDPLLFGCLCVLLAIFTAGHLIGLYLYQFQFFQEAVPPNDYYARLFGIKSVIQTDCASTW
KIIVNPDLSWYHHANPILLLVMYYTLATLIRIWLQEPLVQEEMAKEDEGALDCSSNQNTAERRRSLWYATQYPTDERKL
LSMTQDDYKPSDGLLVTVNGNPVDYHTIHPSLPIENGPAKTDLYTTPQYRWEPSEESSEKKEEEEDKREDSEGEGSQ
EEKRSVRMHAMVAVFQFIMKQSYICALIAMMAWSITYHSWLTFVLLIWSCTLWMIRNRRKYAMISSPFMVVYANLLLVL
QYIWSFELPEIKKVPGFLEKKEPGELASKILFTITFWLLLRQHLTEQKALREKEALLSEVKIGSQELEEKEDEELQDVQV
EGEPTEKEEEEEEEEIKEERHEVKKEEEEEEVEEDDDQDIMKVLGNLVVALFIKYWIYVCGGMFFFVSFEGKIVMYKIIYM
VLFLFCVALYQVHYEWWRKILKYFWMSVVIYTMLVLIFIYTYQFENFPGLWQNMTGLKKEKLEDLGLKQFTVAELFTRI
FIPTSFLLVCILHLHYFHDRFLELTDLKSIPSKEDNTIYSHAKVNGRVYLIINRLAHPEGSLPDLAIMNMTASLDKPEVQKL
AESGEERPEECVKKTEKGEAGKDSDESEEEEDEEEESEEEESSDLRNKWHLVIDRLTVLFLKFLEYFHKLQVFMWWI
LELHIIKIVSSYIIWVTVKEVSLFNYVFLISWAFALPYAKLRRAASSVCTVWTCVIIVCKMLYQLQTIKPENFSVNCSLPNE
NQTNIPLHELNKSLLYSAPVDPTEWVGLRKSSPLLVYLRNNLLMLAILAFEVTVYRHQEYYRGRNNLTAPVSKTIFHDIT
RLHLDDGLINCAKYFVNYFFYKFGLETCFLMSVNVIGQRMDFYAMIHACWLIGVLYRRRRKAIAEVWPKYCCFLACIITF
QYFVCIGIPPAPCRDYPWRFKGAYFNDNIIKWLYFPDFIVRPNPVFLVYDFMLLLCASLQRQIFEDENKAAVRIMAGDN
VEICMNLDAASFSQHNPVPDFIHCRSYLDMSKVIIFSYLFWFVLTIIFITGTTRISIFCMGYLVACFYFLLFGGDLLLKPIKSI
LRYWDWLIAYNVFVITMKNILSIGACGYIGALVRNSCWLIQAFSLACTVKGYQMPEDDSRCKLPSGEAGIIWDSICFAFL
LLQRRVFMSYYFLHVVADIKASQILASRGAELFQATIVKAVKARIEEEKKSMDQLKRQMDRIKARQQKYKKGKERMLSL
TQESGEGQDIQKVSEEDDEREADKQKAKGKKKQWWRPWVDHASMVRSGDYYLFETDSEEEEEEEELKKEDEEPPRK
SAFQFVYQAWITDPKTALRQRRKEKKKLAREEQKERRKGSGDGPVEWEDREDEPVKKKSDGPDNIIKRIFNILKFTWV
LFLATVDSFTTWLNSISREHIDISTVLRIERCMLTREIKKGNVPTRESIHMYYQNHIMNLSRESGLDTIDEHSGAGSRAQ
AAHRMDSLDSRDSISSCYTEATLLISRQSTLDDLDGQDPVPKTSERARPRLRKMFSLDMSSSSADSGSVASSEPTQC
TMLYSRQGTTETIEEVEAEAEEEVVEGLEPELHDAEEKEYAAEYEAGVEEISLTPDEELPQFSTDDCEAPPSYSKAVS
FEHLSFASQDDSGAKNHMVVSPDDSRTDKLESSILPPLTHELTASDLLMSKMFHDDELEESEKFYVDQPRFLLLFYAM
YNTLVARSEMVCYFVIILNHMTSASIITLLLPILIFLWAMLSVPRPSRRFWMMAIVYTEVAIVVKYFFQFGFFPWNKDLEIY
KERPYFPPNIIGVEKKEGYVLYDLIQLLALFFHRSILKCHGLWDEDDIVDSNTDKEGSDDELSLDQGRRGSSDSLKSINL
AASVESVHVTFPEQPAAIRRKRSCSSSQISPRSSFSSNRSKRGSTSTRNSSQKGSSVLSLKQKSKRELYMEKLQEHLI

KAKAFTIKKTLQIYVPIRQFFYDLIHPDYSAVTDVYVLMFLADTVDFIIIVFGFWAFGKHSAAADITSSLSEDQVPGPFLVM
VLIQFGTMVVDRALYLRKTVLGKVIFQVILVFGIHFWMFFILPGVTERKFSQNLVAQLWYFVKCVYFGLSAYQIRCGYPT
RVLGNFLTKSYNYVNLFLFQGFRLVPFLTELRAVMDWVWTDTTLSLSSWICVEDIYAHIFILKCWRESEKRYPQPRGQ
KKKKAVKYGMGGMIIVLLICIVWFPLLFMSLIKSVAGVINQPLDVSVTITLGGYQPIFTMSAQQSQLKVMDNSKYNEFLK
SFGPNSGAMQFLENYEREDVTVAELEGNSNSLWTISPPSKQKMIQELTDPNSCFSVVFSWSIQRNMTLGAKAEIATD
KLSFPLAVATRNSIAKMIAGNDTESSNTPVTIEKIYPYYVKAPSDSNSKPIKQLLSENNFMNITIILFRDNVTKSNSEWWV
LNLTGSRIFNQGSQALELVVFNDKVSPPSLGFLAGYGIMGLYASVVLVIGKFVREFFSGISHSIMFEELPNVDRILKLCT
DIFLVRETGELELEEDLYAKLIFLYRSPETMIKWTREKTN

**Phylogenic analysis.** Accession numbers of Piezo sequences used to make the phylogenic analysis are given with the number of TM domain predicted using TMHMM2 program. For some species, multiple predicted gene sequences were fused to obtain a complete sequence.

Hs Piezo1 (Homo Sapiens): NP_001136336.2, 2521aa (31 TM)

Mm Piezo1 (Mouse musculus): NP_001032375.1, 2546aa (30 TM)

Gg Piezo1 (Gallus gallus): XP_414209.2, 1718aa; XP_423106.2, 217aa (25 TM)

Dr Piezo1 (Danio Rerio): XP_696355.4, 2538aa (29 TM)

Hs Piezo2 (Homo Sapiens): NP_071351.2, 2752aa (35 TM)

Mm Piezo2 (Mus musculus): NP_001034574.3, 2753aa (34 TM)

Gg Piezo2 (Gallus gallus): XP_419138.2, 3080aa (33 TM)

Dr Piezo2 (Danio Rerio):  ; XP_002666625., 2102aa (24 TM)

Ci Piezo (Ciona intestinalis): XP_002122901.1, 1669aa; XP_002128850.1, 591aa (33 TM)

Dm Piezo (Drosophila melanogaster): NP_001036346.3, 2671aa (36 TM)

Ce Piezo (Caenorhabditis elegans): NP_501648.2, 800a; NP_501647.2, 1843 (33 TM)

Dd Piezo (Dictyostelium discoideum): XP_640187, 3080 aa (35 TM)

At Piezo (Arabidopsis thaliana): NP_182327.5, 2440 aa (28 TM)

Os Piezo (Oryza sativa – japonica group): NP_001043105.1, 2196aa (24TM)

Tt Piezo i (Tetrahymena thermophila): XP_976967.1, 4690aa (30 TM)

Tt Piezo ii (Tetrahymena thermophila): XP_001021704.1, 4136aa (29 TM)

Tt Piezo iii (Tetrahymena thermophila): XP_001017682.1, 2636aa (26 TM)

**Data Analysis.** Data in all figures are shown as mean ± SEM. Unless otherwise stated, statistical significance was evaluated using unpaired two-tailed Student's *t*-test for comparing difference between two samples. Unpaired two-tailed Student's *t*-test with Welch correction was used when variances where significantly different. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

# Figure S1

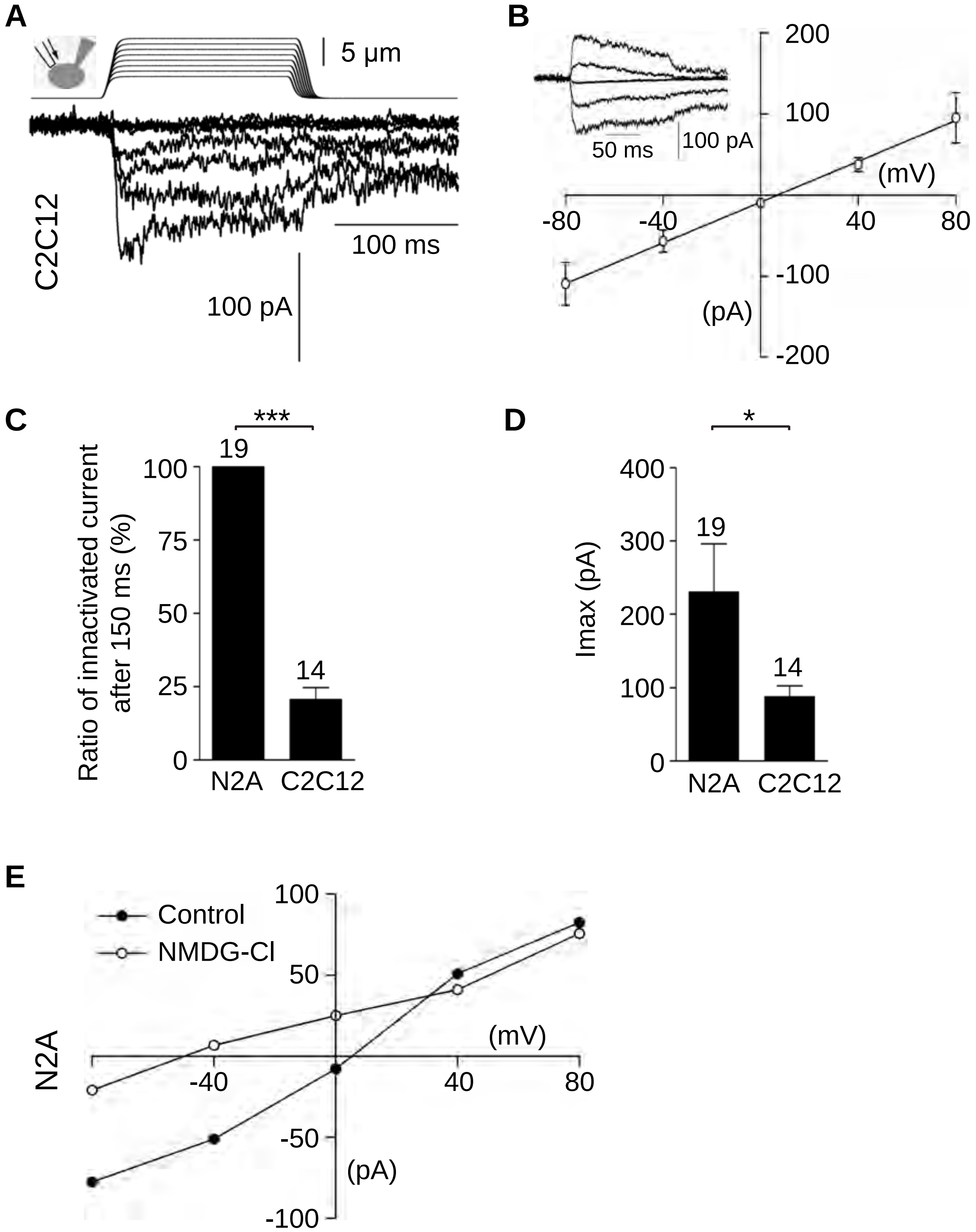

A
5 µm
C2C12
100 ms
100 pA
B
50 ms
100 pA
200
100
-100
-200
(mV)
(pA)
-80
-40
40
80
C
***
19
14
Ratio of innactivated current after 150 ms (%)
100
75
50
25
0
N2A
C2C12
D
*
400
300
200
100
0
Imax (pA)
19
14
N2A
C2C12
E
Control
NMDG-Cl
100
50
-50
-100
(mV)
(pA)
-40
40
80
N2A

**Figure S2**

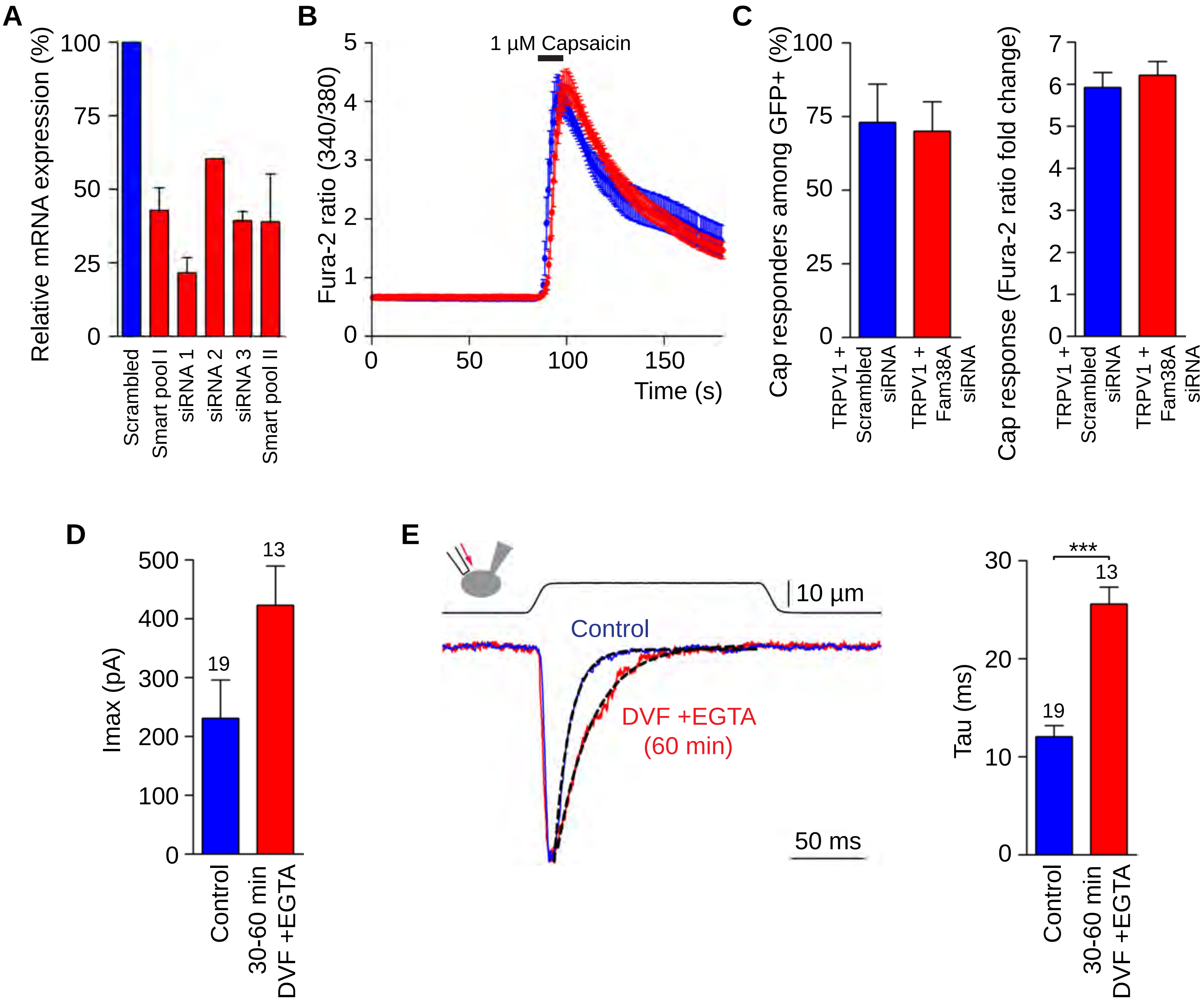
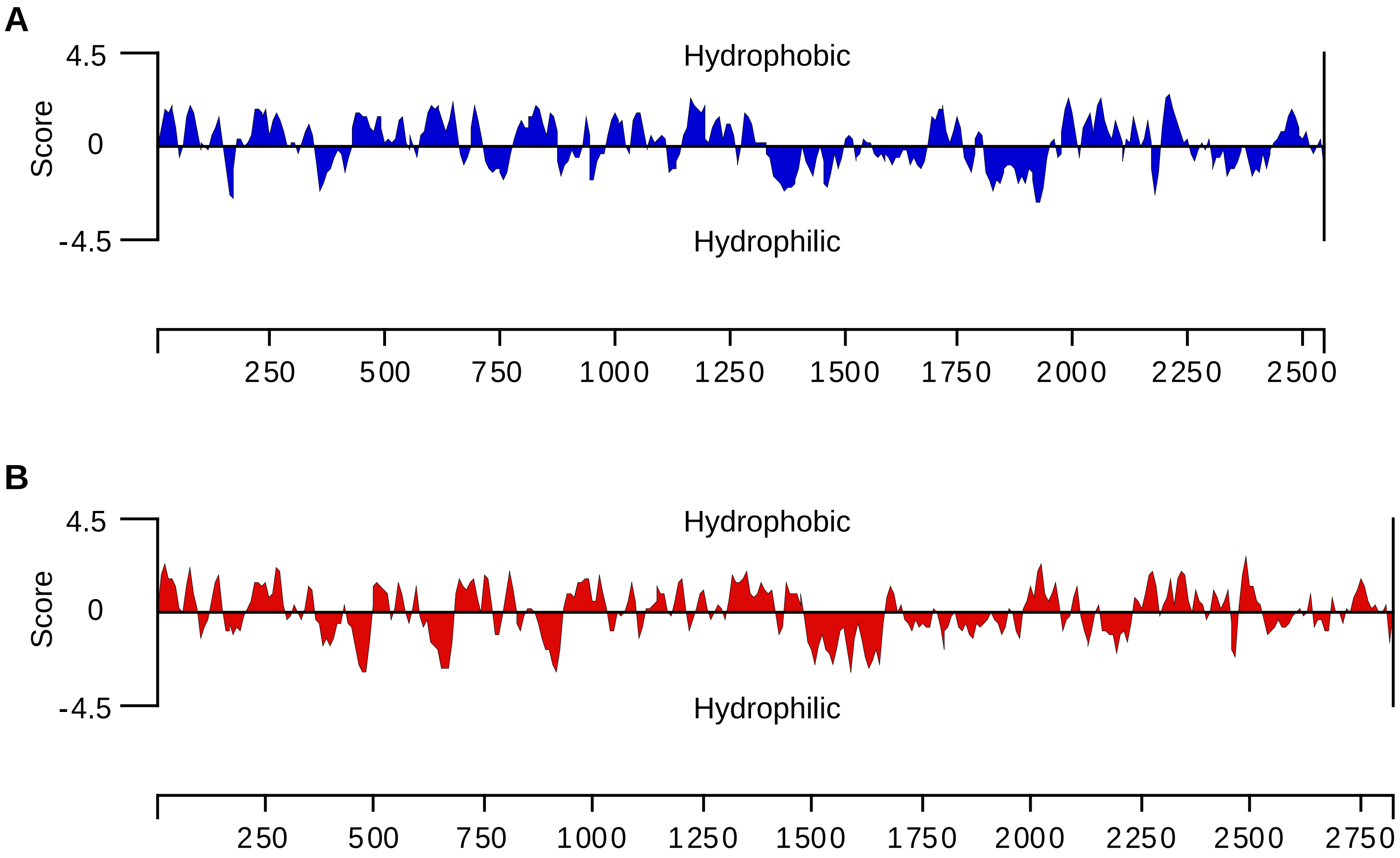
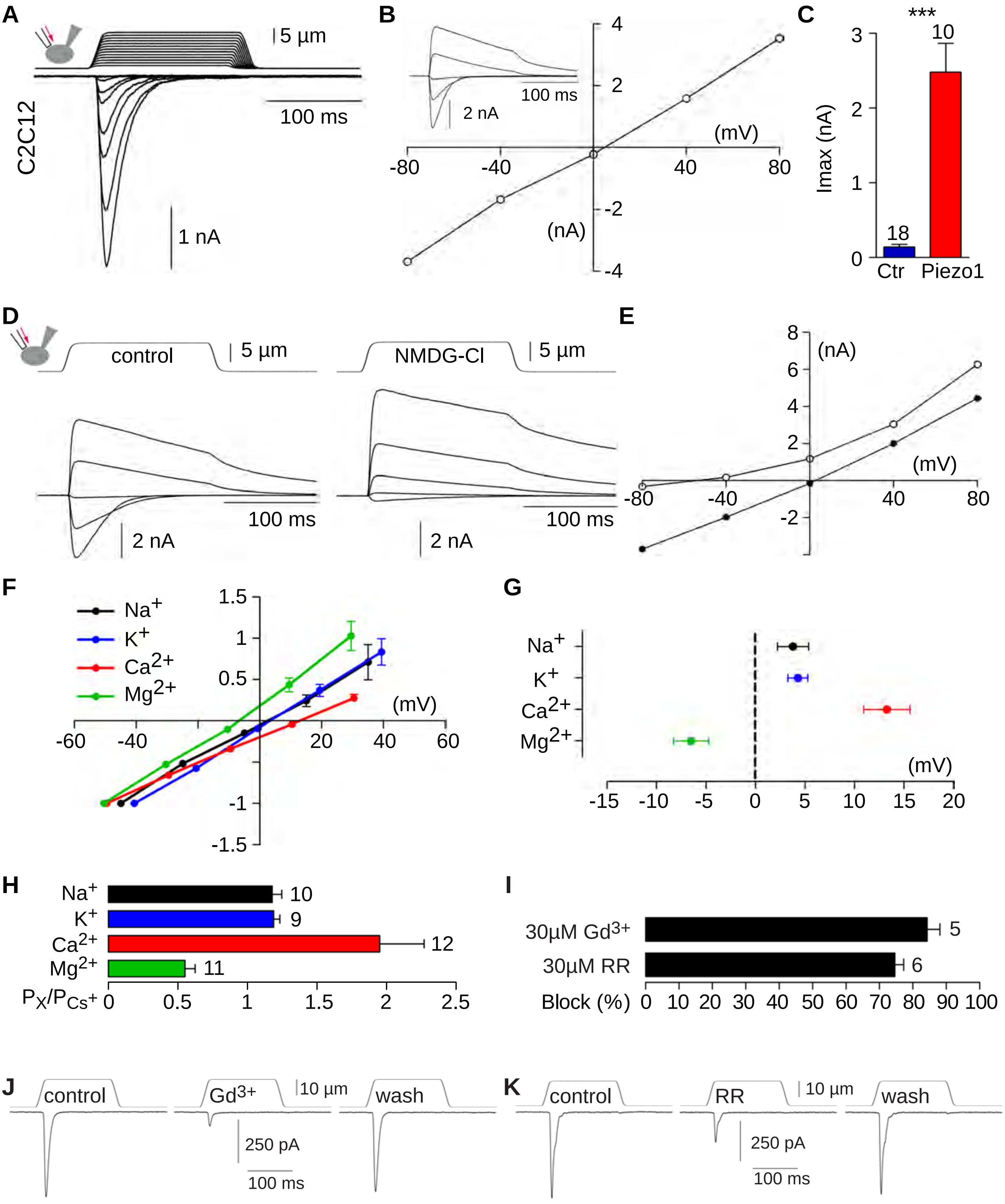

A
Relative mRNA expression (%)
100
75
50
25
0
Scrambled
Smart pool I
siRNA 1
siRNA 2
siRNA 3
Smart pool II
B
Fura-2 ratio (340/380)
5
4
3
2
1
0
1 µM Capsaicin
0
50
100
150
Time (s)
C
Cap responders among GFP+ (%)
100
75
50
25
0
TRPV1 + Scrambled siRNA
TRPV1 + Fam38A siRNA
Cap response (Fura-2 ratio fold change)
7
6
5
4
3
2
1
0
TRPV1 + Scrambled siRNA
TRPV1 + Fam38A siRNA
D
Imax (pA)
500
400
300
200
100
0
19
13
Control
30-60 min DVF +EGTA
E
10 µm
Control
DVF +EGTA (60 min)
50 ms
Tau (ms)
30
20
10
0
***
19
13
Control
30-60 min DVF +EGTA

**Figure S3**


A
4.5
Hydrophobic
Score
0
-4.5
Hydrophilic
250
500
750
1000
1250
1500
1750
2000
2250
2500
B
4.5
Hydrophobic
Score
0
-4.5
Hydrophilic
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750

## Figure S4



A
C2C12
5 µm
100 ms
1 nA
B
100 ms
2 nA
4
2
-2
-4
-80
-40
40
80
(mV)
(nA)
C
***
10
18
3
2
1
0
Imax (nA)
Ctr
Piezo1
D
control
5 µm
NMDG-Cl
5 µm
100 ms
2 nA
100 ms
2 nA
E
8
6
4
2
-2
(nA)
(mV)
-80
-40
40
80
F
Na+
K+
Ca2+
Mg2+
1.5
1
0.5
-1
-1.5
(mV)
-60
-40
20
40
60
G
Na+
K+
Ca2+
Mg2+
(mV)
-15
-10
-5
0
5
10
15
20
H
Na+
K+
Ca2+
Mg2+
10
9
12
11
PX/PCs+
0
0.5
1
1.5
2
2.5
I
30µM Gd3+
30µM RR
5
6
Block (%)
0
10
20
30
40
50
60
70
80
90
100
J
control
Gd3+
10 µm
wash
250 pA
100 ms
K
control
RR
10 µm
wash
250 pA
100 ms

## Figure S5

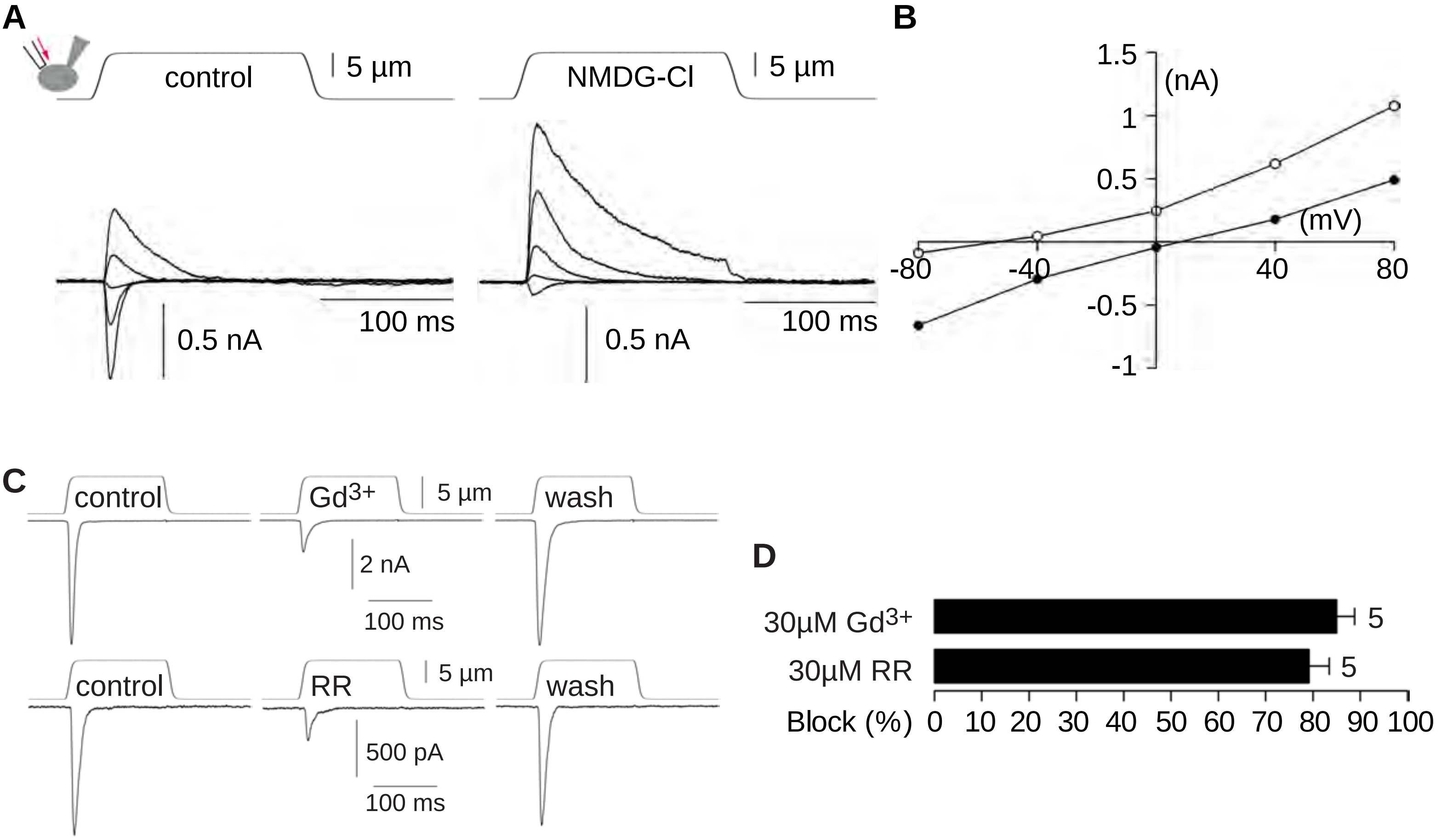
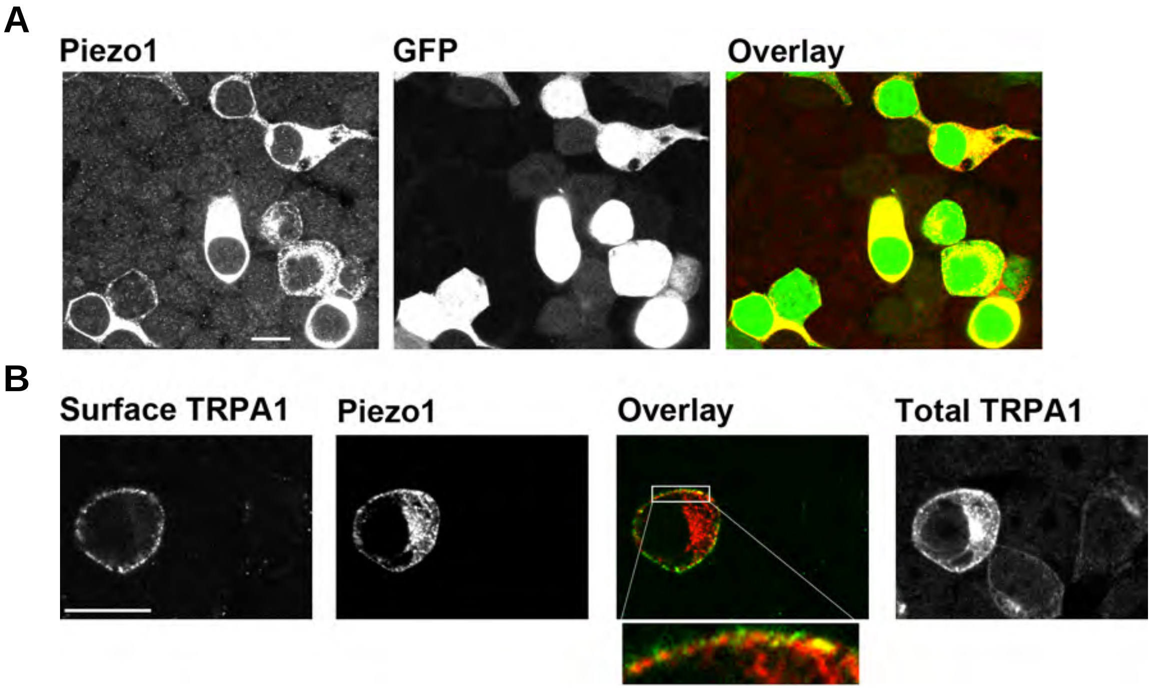
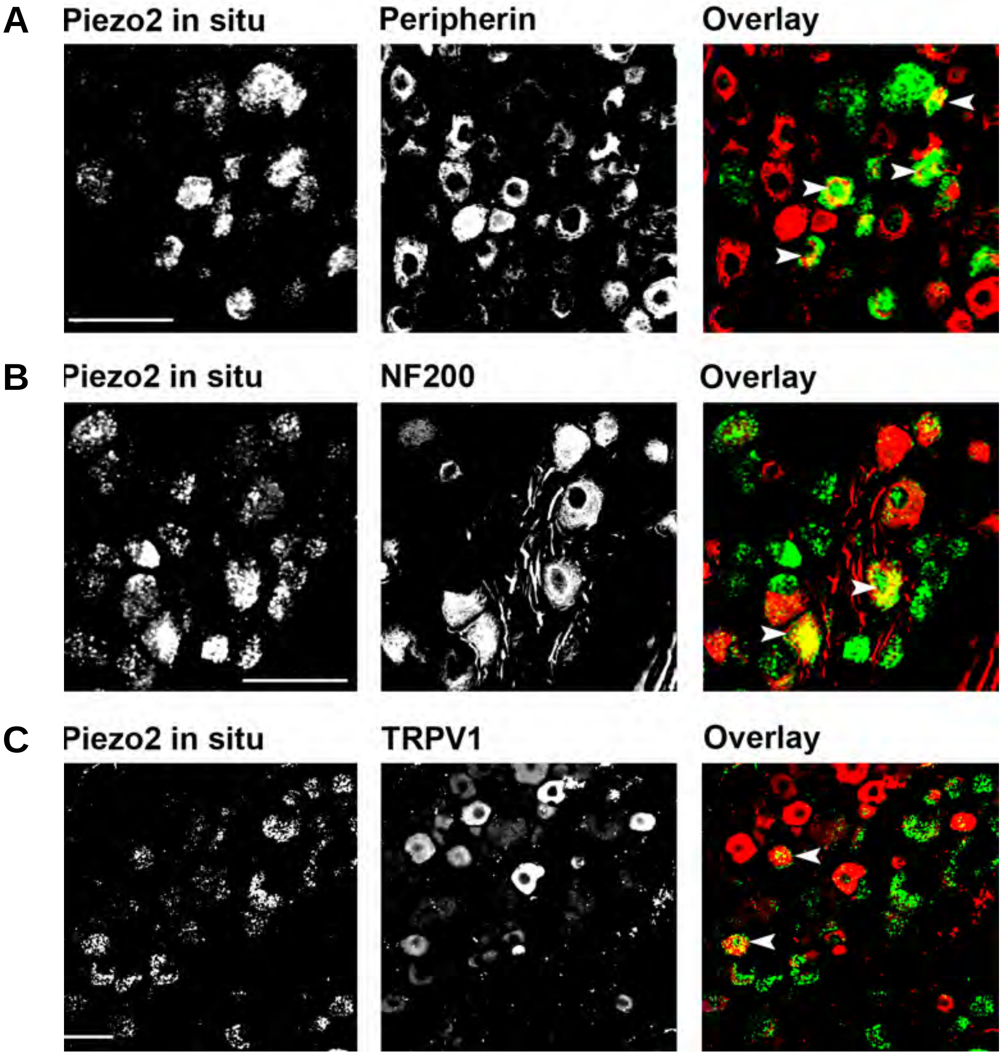

A
control
5 µm
NMDG-Cl
5 µm
0.5 nA
100 ms
0.5 nA
100 ms
B
1.5
(nA)
1
0.5
(mV)
-80
-40
40
80
-0.5
-1
C
control
Gd3+
5 µm
wash
2 nA
100 ms
control
RR
5 µm
wash
500 pA
100 ms
D
30µM Gd3+
5
30µM RR
5
Block (%)
0
10
20
30
40
50
60
70
80
90
100

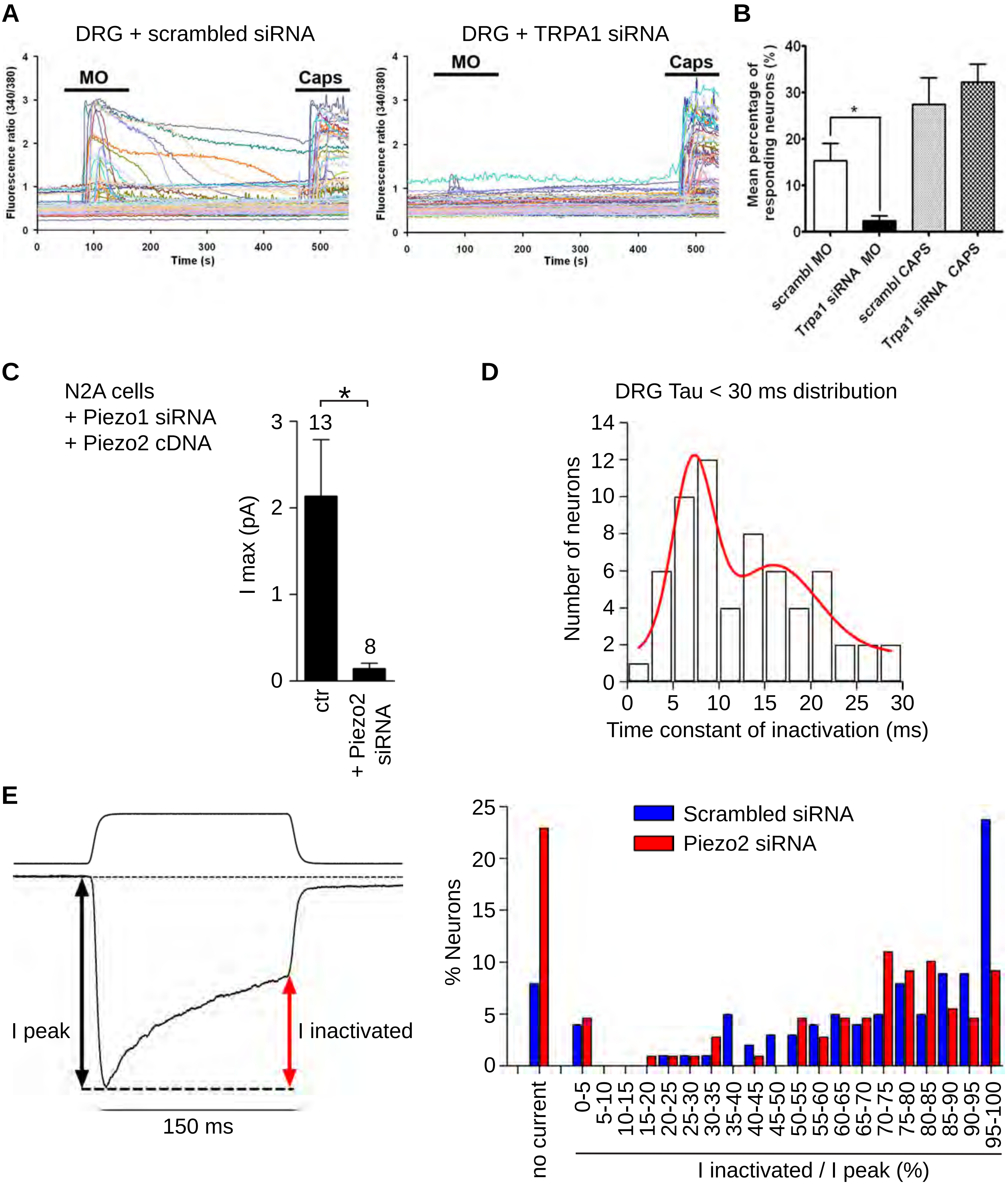
Figure S6


A
Piezo1
GFP
Overlay
B
Surface TRPA1
Piezo1
Overlay
Total TRPA1

# Figure S7


A
Piezo2 in situ
Peripherin
Overlay
B
Piezo2 in situ
NF200
Overlay
C
Piezo2 in situ
TRPV1
Overlay

# Figure S8


A
DRG + scrambled siRNA
MO
Caps
Fluorescence ratio (340/380)
0
1
2
3
4
0
100
200
300
400
500
Time (s)
DRG + TRPA1 siRNA
MO
Caps
Fluorescence ratio (340/380)
Time (s)
B
Mean percentage of responding neurons (%)
0
10
20
30
40
*
scrambl MO
Trpa1 siRNA MO
scrambl CAPS
Trpa1 siRNA CAPS
C
N2A cells
+ Piezo1 siRNA
+ Piezo2 cDNA
I max (pA)
0
1
2
3
*
13
8
ctr
+ Piezo2 siRNA
D
DRG Tau < 30 ms distribution
Number of neurons
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
Time constant of inactivation (ms)
E
I peak
I inactivated
150 ms
% Neurons
0
5
10
15
20
25
Scrambled siRNA
Piezo2 siRNA
no current
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
55-60
60-65
65-70
70-75
75-80
80-85
85-90
90-95
95-100
I inactivated / I peak (%)

## Supporting Figure Legends

### Figure S1. Neuro2A and C2C12 cells display different types of mechanically-activated currents.

(A) Representative traces of mechanically-activated (MA) inward currents expressed in C2C12 cells. The cells are subjected to a series of mechanical steps in 1 µm increments using a stimulation pipette (inset drawing, arrow) in the whole-cell patch configuration at a holding potential of -80 mV. (B) Average current-voltage relationships of MA currents in C2C12 (n = 4) cells. Inset, representative MA currents evoked at holding potentials ranging from -80 to +40 mV (applied 0.7 sec prior to the mechanical step). (C) Ratio of inactivated current at the end of a mechanical step (150 ms duration) relative to the peak current (mean ± SEM, number of cells above the bars) in N2A and C2C12 cells for currents elicited at a holding potential of -80 mV. ***, $P < 0.001$, unpaired t-test. (D) Average maximal amplitude of MA inward currents elicited in N2A and C2C12 cells at a holding potential of -80mV (mean ± SEM, the number of cells tested is shown above the bars). *, $P < 0.05$, unpaired t-test with Welch's correction. (E) MA current-voltage relationships of whole-cell recordings in a N2A cell bathed in control solution (filled symbols) and after perfusion with NMDG-Cl solution (open symbol). Note that inward currents present in control condition are suppressed with NMDG-Cl solution.

### Figure S2. Piezo1 siRNA qPCR and cell viability control, and N2A MA currents after disruption of integrin function.

(A) siRNA-induced down-regulation of Piezo1 mRNA in N2A cells. Transfected and untransfected cells are unsorted and thus these differences are underestimated. (B) Representative ratiometric calcium imaging experiment of capsaicin stimulated N2A cells co-transfected with TRPV1 and GFP, together with either scrambled siRNA or Piezo1 siRNA (mean ± SEM of GFP-positive cell traces). (C) Percentage of GFP-positive cells responding to capsaicin (left panel, mean ± SEM of two experiments) and Fura-2 340/380 ratio fold change of capsaicin responding cells (mean ± SEM). (D) Maximal current amplitude of whole cell MA currents elicited in N2A cells at a holding potential of -80 mV in control conditions or after 30-60 minutes perfusion with a divalent free solution containing 5 mM EGTA. (E) Representative traces of MA currents normalized to peak in control conditions or after 30-60 minutes perfusion with a divalent free solution containing 5 mM EGTA (left panel). Inactivation of currents is fitted with a mono-exponential equation. In the absence of divalent cations, the time constant for inactivation is higher than with control solution (right panel). ***, $P < 0.001$, unpaired t-test.

**Figure S3. Hydrophobicity plot of mouse Piezo1 and Piezo2.**

Hydrophobicity analysis of mouse Piezo1 (A) and mouse Piezo2 (B). The Kyte-Doolittle pattern (19 residues window) shows succession of hydrophobic and hydrophilic regions. 30 and 34 transmembrane domains are predicted by TMHMM2 program analysis, respectively.

**Figure S4. Piezo1-induced MA currents are cationic non-selective currents blocked by gadolinium and ruthenium red.**

(A-C) MA currents of Piezo1-expressing C2C12 cells recorded in the whole-cell configuration. (A) Representative traces of MA inward currents expressed in Piezo1-transfected cells. The cell is subjected to a series of mechanical steps in 1 µm increments using glass probe stimulation and at a holding potential of -80 mV. (B) Representative current-voltage relationships of MA currents expressed in Piezo1-transfected cells. Inset, MA currents evoked at holding potentials ranging from -80 to +40 mV. (C) Average maximal amplitude of MA inward currents elicited at a holding potential of -80 mV in Piezo1-transfected (red) or mock-transfected (blue) cells. Bars represent the mean ± SEM, and the number of cells tested is shown above the bars. ***, $P < 0.001$, unpaired t-test with Welch's correction. (D) Whole-cell MA current traces elicited in a Piezo1 transfected cell bathed in control solution (left panel) or after perfusion with 150 mM NMDG-Cl (right panel) solution. Currents are elicited from -80 mV to +80 mV in 40 mV steps. (E) MA current-voltage relationship from the same cell. Note that inward currents present in control condition (filled symbols) are suppressed with external NMDG-Cl solution (open symbol). (F) Average current-voltage relationship of MA currents elicited in Piezo1 transfected HEK293T cells and recorded with CsCl-based internal solution and 150 mM NaCl-, 150 mM KCl-, 100 mM $CaCl_2$- or 100 mM $MgCl_2$-based extracellular solutions. I-V relationships from individual cells were normalized to the value at -40 mV before liquid junction potentials were corrected. (G) Average of reversal potential values determined for each recording conditions and for individual cells (mean ± SEM). (H) $P_X/P_{Cs}$ ion selectivity ratios of MA currents in Piezo1-expressing cells. (I) Percent block of MA currents in Piezo1-expressing cells by 30 µM gadolinium or ruthenium red. (J-K) Representative current traces of MA currents elicited in Piezo1 transfected cells before, during and after perfusion of 30 µM gadolinium (J) or ruthenium red (K).

**Figure S5. Piezo2-induced MA currents are cationic non-selective.**

(A) Whole-cell MA current traces elicited in a Piezo2 transfected cell bathed in control solution (left panel) and after perfusion with NMDG-Cl (right panel) solution. Currents are elicited at -80, -40, 0, +40 and +80 mV. (B) MA current-voltage relationships from the same cell. Note that inward currents present in control condition (filled symbols) are suppressed with NMDG-Cl solution (open symbol). (C) Representative current traces of MA currents elicited in Piezo2-transfected cells before, during and after perfusion of 30 µM gadolinium (upper panels) or ruthenium red (lower panels). (D) Percent block of MA currents in Piezo2-expressing cells by 30 µM gadolinium and ruthenium red. Bars represent the mean ± SEM, and the number of cells tested is shown above the bars.

**Figure S6. Piezo1 antibodies detect Piezo1 in transfected HEK293T cells.**

(A) Representative images of Piezo1 labeling (red) in Piezo1-IRES-EGFP transfected cells (green). Note, GFP-negative, hence untransfected, cells are devoid of labeling. (B) A proportion of Piezo1 is expressed near or at the plasma membrane of TRPA1 and Piezo1 co-transfected HEK293T cells. Cells were live-labeled with TRPA1 antibodies (green) to delineate the plasma membrane, fixed, permeabilized, and stained for Piezo1 (red) and MYC (total TRPA1). Inset, higher magnification of boxed area in overlay image. Scale bars = 20 µm.

**Figure S7. Piezo2 mRNA is expressed in a subset of DRG neurons.**

To further characterize Piezo2 expression in DRG, we performed combined Piezo2 fluorometric in situ hybridization with immunostaining of known markers of DRG neurons. Mechanosensitivity is a property of the majority of DRG neurons including neurons with unmyelinated C-type fibers and expressing the intermediate filament Peripherin (*5-6*), and neurons with myelinated A-type fibers and expressing Neurofilament 200 (*6-8*).  Most of the former play a role in sensing noxious mechanical and thermal stimuli and many also express the thermoTRP TRPV1 (*6, 9-11*), the latter primarily play a role in mechanosensation (of both noxious and innocuous stimuli) (*6, 8*).

Combined Piezo2 fluorometric in situ hybridization (left panels) with Peripherin (A, middle panel) and Neurofilament 200 (NF200) (B, middle planel) immunostaining in mouse DRG and (C) with TRPV1 (middle panel) immunostaining in rat DRG show that among Piezo2-positive neurons, 60% also expressed Peripherin (n = 204 for Piezo2 and n = 555 for Peripherin of which 23 % express Piezo2, out of 1188 total neurons); 28%, NF200 (n = 277 for Piezo2, and n = 368 for NF200 of which 19 % express Piezo2, out of 1203 total neurons), and 24%, TRPV1 (n = 233 for Piezo2 and n = 394 for TRPV1 of which 15 % express Piezo2, out of 975 total neurons). Arrowheads show examples of neurons expressing Piezo2 and respective markers (A-C, right panels). Scale bars = 50 μm.

**Figure S8. DRG and Piezo2 siRNA control experiments and comparison of MA current inactivation of DRG neurons.**

(A-B) siRNA-mediated knockdown of TRPA1 in cultured DRG neurons. (A) Representative traces of ratiometric calcium-imaging on cultured DRG neurons transfected with scrambled siRNA control (n = 415 neurons, left panel) and TRPA1 siRNA (n = 467 neurons, right panel). (B) Average percentage of neurons responding to mustard oil (MO, agonist of TRPA1 channels) and capsaicin (CAPS, agonist of TRPV1 channels) from 2 independent transfections were assayed 48 to 72 hours after transfection. While the percentage of responders to MO (100 μM) is significantly reduced upon siRNA treatment (scrambled: 15.34 ± 3.69%; siRNA: 2.48 ± 0.96%; P = 0.0286, Mann Whitney test), responses to CAPS (0.5 μM) are unaffected by TRPA1 siRNA treatment (scrambled: 27.43 ± 5.81%; siRNA: 32.28 ± 3.84%; ns). Bars represent the mean ± SEM. (C) Average maximal amplitude of Piezo2-induced MA currents in the presence or absence of Piezo2 siRNA. N2A cells transfected with Piezo1 siRNA to suppress endogenous MA currents were co-transfected with Piezo2 cDNA or Piezo2 cDNA + Piezo2 siRNA. (D)

Histogram of time-constant of inactivation of MA currents recorded in scrambled siRNA transfected DRG neurons. Numbers of neurons expressing MA currents with time constant of inactivation ≤ 30 ms were plotted using a bin of 2.5 ms (the inactivation kinetic of currents with >30 ms time constant is too slow to be accurately fitted over 150 ms). Fit with double Gaussian equation shows two peaks centered at 7.2 ± 0.5 ms and 16.0 ± 2.1 ms, respectively. (E) Example trace of MA current showing comparison between the amount of current inactivated after 150 ms of mechanical stimulation (I inactivated, red double arrow) and the amount of current at the peak (I peak, black double arrow) (left panel). Percentage of DRG neurons with different degrees of current inactivation at 150 ms (I inactivated/ I peak in 5% increments where 100% is completely inactivated) are compared between scrambled or Piezo2 siRNA transfected conditions (right panel).

## Table S1

### List of candidate genes tested in N2A cells using siRNA.

| № : | siRNA : | № : | siRNA : |
|---|---|---|---|
| 1 | Scrambled | 41 | Sfxn5 |
| 2 | 2400010G15Rik | 42 | Slc16a6 |
| 3 | 2410015B03Rik | 43 | Slc47a1 |
| 4 | 4833424O15Rik | 44 | Slc7a3 |
| 5 | Tmem129 | 45 | Slc7a7 |
| 6 | 1500016O10Rik | 46 | Slc8a1 |
| 7 | 2810432L12Rik | 47 | TM6sf1 |
| 8 | 3632451O06Rik | 48 | Tmc6 |
| 9 | 4930500O05Rik | 49 | Tmem108 |
| 10 | 9330182L06Rik | 50 | Tmem136 |
| 11 | A830020B06Rik | 51 | Tmem161a |
| 12 | Accn2 | 52 | Tmem164 |
| 13 | AI848100 | 53 | Tmem16f |
| 14 | B430119L13Rik | 54 | TMEM176a |
| 15 | BC042720 | 55 | TMEM176b |
| 16 | BC062109 | 56 | Tmem181 |
| 17 | Chrna3 | 57 | Tmem20 |
| 18 | Creld1 | 58 | Tmem41a |
| 19 | D630045J12Rik | 59 | Tmem54 |
| 20 | Emb | 60 | Tmem56 |
| 21 | Gpr173 | 61 | Tmem74 |
| 22 | Grik5 | 62 | Tmhs |
| 23 | Htr3a | 63 | Tmtc1 |
| 24 | Htr3b | 64 | Tmtc2 |
| 25 | Josd2 | 65 | TRPM2 |
| 26 | Leprotl1 | 66 | TRPML3 |
| 27 | Lphn1 | 67 | TRPV2 |
| 28 | Mcam | 68 | Tspan13 |
| 29 | Mfap3 | 69 | Tspan18 |
| 30 | Npal2 | 70 | Tspan2 |
| 31 | Npal3 | 71 | Tspan33 |
| 32 | Nrsn1-Vmp | 72 | Xkr6 |
| 33 | P2X3 | 73 | Fam38a |
| 34 | Pcdh1 |  |  |
| 35 | Pcnxl2 |  |  |
| 36 | PKD1L2 |  |  |
| 37 | Prrt3 |  |  |
| 38 | Punc |  |  |
| 39 | Reep2 |  |  |
| 40 | Rom1 |  |  |

The siRNA are in the same order as those in Figure 2A.

# Table S2

## Properties of mechanically activated currents elicited in different cell lines and with various transfection conditions.

| | | Whole-cell mechanical stimulation | | | Cell-attached stretch | |
|---|---|---|---|---|---|---|
| | | Threshold (µm) | Tau inac (ms) | Imax (-80mV) (pA) | P50 (mm Hg) | Imax (-80mV) (pA) |
| **N2A** | control | 5.4 ± 0.3<br>(n = 71) | 12.3 ± 0.6<br>(n = 74) | -194.3 ± 34.7<br>(n = 89) | -28.0 ± 1.8<br>(n = 21) | -8.1 ± 2.0<br>(n = 28) |
| | *Piezo1* siRNA | n.d. | n.d. | -13.8 ± 3.2<br>(n = 67) | n.d. | -1.3 ± 0.5<br>(n = 27) |
| | *Piezo1* cDNA | 3.7 ± 0.7<br>(n = 16) | 15.3 ± 1.5<br>(n = 16) | -3568 ± 567.6<br>(n =16) | -28.1 ± 2.8<br>(n = 13) | -68.6 ± 7.3<br>(n = 13) |
| **C2C12** | control | 3.3 ± 0.4<br>(n = 23) | 129.8 ± 55.4<br>(n = 21) | -101.5 ± 19.7<br>(n = 25) | n.d. | n.d. |
| | *Piezo1* cDNA | 3.0 ± 0.3<br>(n = 10) | 15.6 ± 0.9<br>(n = 10) | -2482 ± 384.4<br>(n = 10) | n.d. | n.d. |
| **HEK293T** | control | 6.0 ± 0.9<br>(n = 9) | 88.7 ± 26.0<br>(n = 7) | -88.5 ± 19.7<br>(n = 10) | n.d. | -1.1 ± 0.2<br>(n = 18) |
| | *Piezo1* cDNA | 2.6 ± 0.7<br>(n = 10) | 16.5 ± 1.4<br>(n = 10) | -3696 ± 641.1<br>(n = 10) | -31.2 ± 3.5<br>(n = 11) | -43.0 ± 12.8<br>(n = 17) |

Values are given as mean ± SEM.
N number are specified for each experiment.
n.d. : not determined.

## Supporting References

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11. K. Kobayashi *et al.*, *J Comp Neurol* **493**, 596 (Dec 26, 2005).