Piezo1 ion channel pore properties are dictated by C-terminal region

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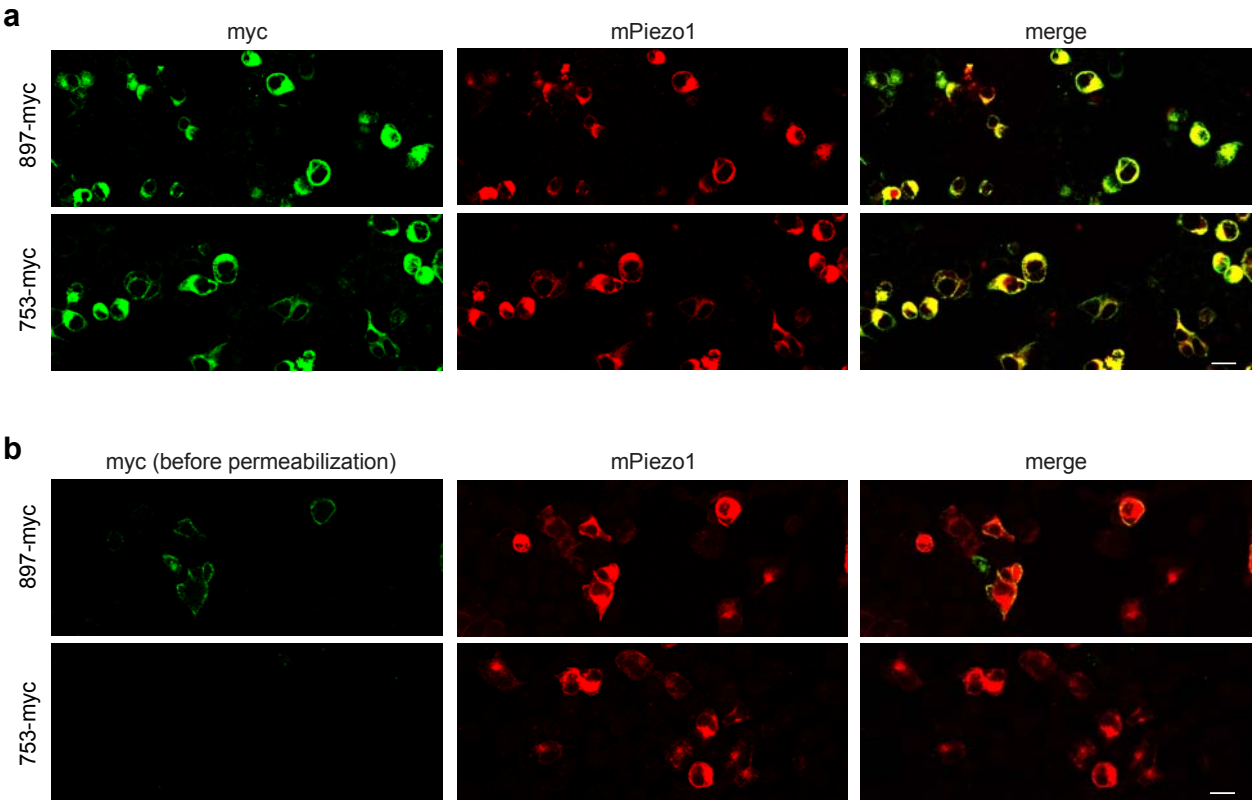
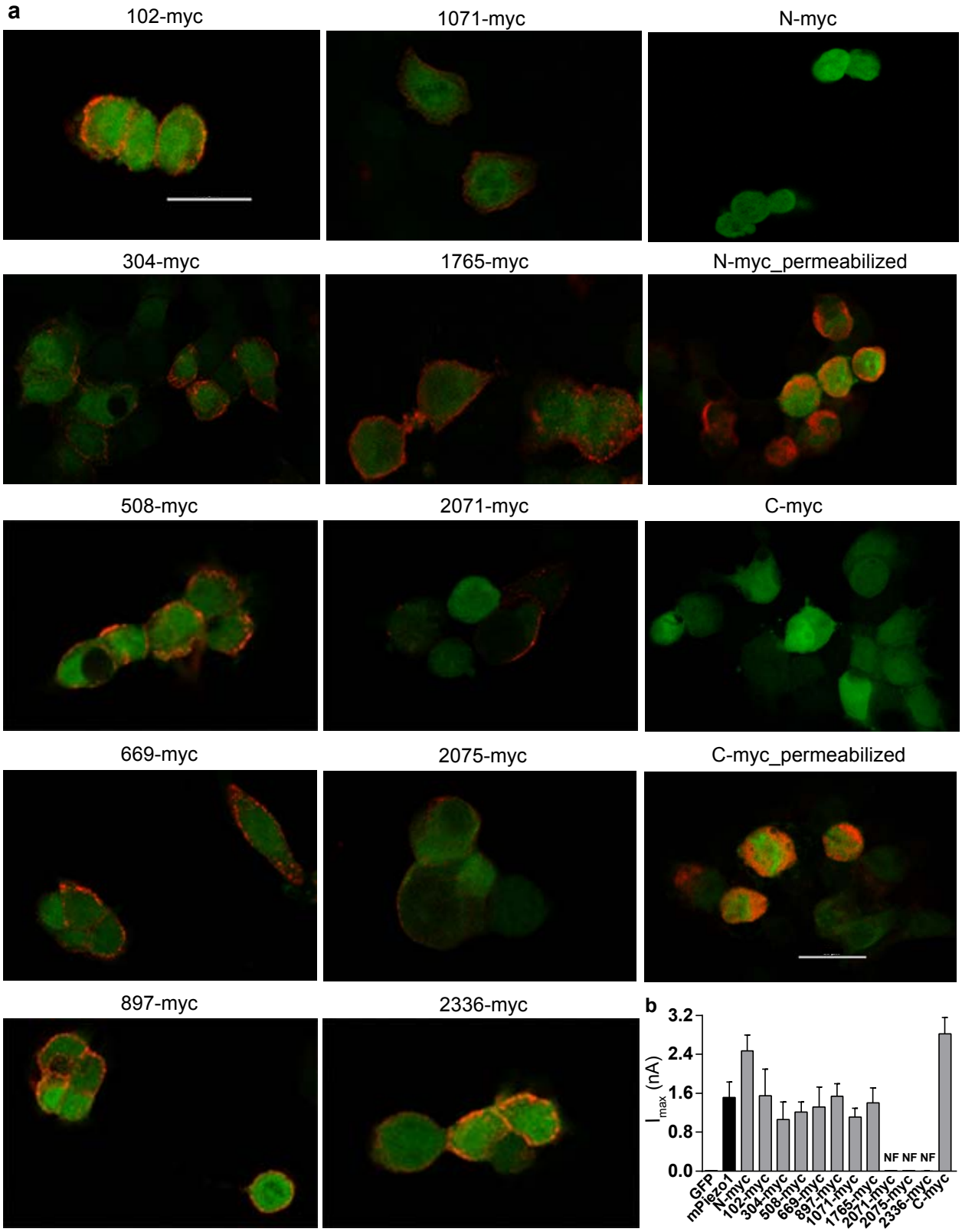
Supplemental Figures and Legends



Supplementary Figure 1. Staining of mPiezo1-myc constructs using mPiezo1 antibody as a control of myc-tag localization experiments. (**a**) Representative images of labeling using a myc antibody (green, left panels) and mPiezo1 antibody (red, middle panels) in mPiezo1-myc transfected cells. Myc tags were inserted at position 897 or 753, as specified. Staining using myc- and mPiezo1-antibodies overlap (right panels), illustrating that the position of myc-tag insertion does not affect their accessibility. (**b**) Same as (**a**) except that myc staining was performed before permeabilization. The level of expression of both mPiezo1-myc constructs evaluated by mPiezo1 labelling is similar illustrating that lack of myc staining of 753-myc constructs under these conditions is not due to lower expression. Scale bar: 20 μm. Note that Myc labelling images in panel (**a**) and (**b**) are the same used in Fig1a, left and right panels, respectively.


a
102-myc
1071-myc
N-myc
304-myc
1765-myc
N-myc_permeabilized
508-myc
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C-myc
669-myc
2075-myc
C-myc_permeabilized
897-myc
2336-myc
b
3.2
2.4
1.6
0.8
0.0
I max (nA)
NF NF NF
GFP
mPiezo1
N-myc
102-myc
304-myc
508-myc
669-myc
897-myc
1071-myc
1765-myc
2071-myc
2075-myc
2336-myc
C-myc

Supplementary Figure 2. Myc staining and functionality data of mPiezo1 myc-tag constructs. (**a**) Representative images of non-permeabilized and permeabilized staining using a myc antibody (red) in mPiezo1-myc iresGFP transfected cells. scale bar 20 μm. Note that extracellular myc detection signal of 2071- and 2075-myc constructs is weaker than for other constructs positively stained by anti-myc without permeabilization. This could reflect impaired membrane trafficking for these two constructs. (**b**) Bar graph representing MA whole-cell I_{max} currents recorded from cells transfected with GFP only, mPiezo1, or mPiezo1 with a myc tag at the following amino acid positions N-terminal, 102, 304, 508, 669, 897, 1071, 1765, 2071, 2075, 2336 and C-terminal (n= 9, 5, 4, 5, 6, 5, 5, 7, 7, 4, 5, 8, 9 and 5, respectively).

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hPiezo1          MEPHVLGAVLYWLLLPCALLAACLLRFSGLSLVYLLFLLLLPWFPGPTRCGLQGHTGRLL 60
hPiezo2          MASEVVCGLIFRLLLPICLAVACAFRYNGLSFVYLIYLLLIPLFSEPTKTTMQGHTGRLL 60
dPiezo           MVFSYACMVLQRIVVPAVLVLAALMRPVGISFVYLLMFFVSPFVPLATRRNFKGSVTAFF 60

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hPiezo2          KSLCFISLSFLLLHIIFHITLVSLEAQHRIAPGYNCSTWEKTFRQIGFESLKGADAGNGI 120
dPiezo           IILLTLSTLVLLGHITLQILAVSLT-----LPIYNCSFSERLLRHIGFVSFIDLQPFAII 115

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hPiezo1         AVYQCVAAHSELLCYFIIILNHMVTASAGSLVLPVLVFLWAMLSIPRPSKRFWMTAIVFT 1737
hPiezo2         AMYNTLVARSEMVCYFVIILNHMVSASMITLLLPILIFLWAMLSVPRPSRRFWMMAIVYT 1972
dPiezo          SSWYALLANTDLICYIVVFINQVVNASLISLPLPIMVFLWGTLSLPRPTKTFWVTLIAYT 1775

mPiezo1         EVMVVTKYLFQFGFFPWNSYVVLRRYENKPYFPPRILGLEKTDSYIKYDLVQLMALFFHR 1797
mPiezo2         EVAIVVKYFFQFGFFPWNKDLEIY--KERPYFPPNIIGVEKKEGYVLYDLIQLLALFFHR 2100
hPiezo1         EIAVVVKYLFQFGFFPWNSHVVLRRYENKPYFPPRILGLEKTDGYIKYDLVQLMALFFHR 1797
hPiezo2         EVAIVVKYFFQFGFFPWNKNVEVN--KDKPYHPPNIIGVEKKEGYVLYDLIQLLALFFHR 2030
dPiezo          QAIVLIKCIFQFKLIWSNYHQLPN----QPLTPAKIFGVENKAHYAIYDLILLLVLFLHR 1831
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mPiezo1          SQLLCYGLWDHEEDRYPKDHCRSSVKDREAKEEPEAKLESQSETGTGHPKEPVLAGTPRD 1857
mPiezo2          SILKCHGLWDEDDIVDSNTDKEGSDDELSLDQGR------------------RGSSDSLK 2142
hPiezo1          SQLLCYGLWDHEEDSPSKEHDKSGEEEQGAEEGP-----------------GVPAATTED 1840
hPiezo2          SILKCHGLWDEDDMTESGMAREESDDELSLGHGR------------------RDSSDSLK 2072
dPiezo           YLLKSQGLWKSGYKDTDNQFTKPTASIDERDDSD-------------------------- 1865

mPiezo1          HIQGKGSIRSKDVIQDPPEDLKPRHTRHISIRFRRRK-ETPGPKGTAVMETEHEEGEGKE 1916
mPiezo2          SINLAASVESVHVTFPEQPAAIRRKRSCSSSQISPRSSFSSNRSKRGSTSTRNSSQKGSS 2202
hPiezo1          HIQVEARVGPTDGTPEPQVELRPRDTRRISLRFRRRKKEGPARKGAAAIEAEDREEEEGE 1900
hPiezo2          SINLAASVESVHVTFPEQQTAVRRKRSGSSSEPSQRSSFSSNRSQRGSTSTRNSSQKGSS 2132
dPiezo           ----------NLSQPDSRQLNDDAAQKLSLQVSQASLPGSPEFSKTGINQLERTKYTSS 1914

mPiezo1          TTERKRPRHTQEKSKFRERMKAAGRRLQSFCVSLAQSFYQPLQRFFHDILHTKYRAATDV 1976
mPiezo2          VLSLK---QKSKRELYMEKLQEHLIKAKAFTIKKTLQIYVPIRQFFYDLIHPDYSAVTDV 2259
hPiezo1          EEKEAPTGREKRPSRSGGRVRAAGRRLQGFCLSLAQGTYRPLRRFFHDILHTKYRAATDV 1960
hPiezo2          VLSIK---QKGKRELYMEKLQEHLIKAKAFTIKKTLEIYVPIKQFFYNLIHPEYSAVTDV 2189
dPiezo           ----------------------------------------LYKFFFSLVHKSR-LATDV 1932

mPiezo1          YALMFLADIVDIIIIIFGFWAFGKHSAATD--IASSLSDDQVPQAFLFMLLVQFGTMVID 2034
mPiezo2          YVLMFLADTVDFIIIVFGFWAFGKHSAAAD--ITSSLSEDQVPGPFLVMVLIQFGTMVVD 2317
hPiezo1          YALMFLADVVDFIIIIFGFWAFGKHSAATD--ITSSLSDDQVPEAFLVMLLIQFSTMVVD 2018
hPiezo2          YVLMFLADTVDFIIIVFGFWAFGKHSAAAD--ITSSLSEDQVPGPFLVMVLIQFGTMVVD 2247
dPiezo           YALMFLCDFVNFFVLLFGFTAFGTQQTESDEGVQTYLAENKVPIPFLIMLLVQFLLIVID 1992

mPiezo1          RALYLRKTVLGKLAFQVVLVVAIHIWMFFILPAVTERMFSQNAVAQLWYFVKCIYFALSA 2094
mPiezo2          RALYLRKTVLGKVIFQVILVFGIHFWMFFILPGVTERKFSQNLVAQLWYFVKCVYFGLSA 2377
hPiezo1          RALYLRKTVLGKLAFQVALVLAIHLWMFFILPAVTERMFNQNVVAQLWYFVKCIYFALSA 2078
hPiezo2          RALYLRKTVLGKVIFQVILVFGIHFWMFFILPGVTERKFSQNLVAQLWYFVKCVYFGLSA 2307
dPiezo           RALYLRKALVNKIIFHFFSVIGIHIWMFFVVPAVTERTFNSLAPPIIFYVIKCFYMLLSS 2052

mPiezo1          YQIRCGYPTRILGNFLTKKYNHLNLFLFQGFRLVPFLVELRAVMDWVWTDTTLSLSNWMC 2154
mPiezo2          YQIRCGYPTRVLGNFLTKSYNYVNLFLFQGFRLVPFLTELRAVMDWVWTDTTLSLSSWIC 2437
hPiezo1          YQIRCGYPTRILGNFLTKKYNHLNLFLFQGFRLVPFLVELRAVMDWVWTDTTLSLSSWMC 2138
hPiezo2          YQIRCGYPTRVLGNFLTKSYNYVNLFLFQGFRLVPFLTELRAVMDWVWTDTTLSLSSWIC 2367
dPiezo           YQIKSGYPKRILGNFFTKGFSMVNMIAFKVYMQIPFLYELRTILDWVCIDSTMTIFDWLK 2112

mPiezo1          VEDIYANIFIIKCSRETEKKYPQPKGQKKKKIVKYGMGGLIILFLIAIIWFPLLFMSLIR 2214
mPiezo2          VEDIYAHIFILKCWRESEKRYPQPRGQKKKKAVKYGMGGMIIVLLICIVWFPLLFMSLIK 2497
hPiezo1          VEDIYANIFIIKCSRETEKKYPQPKGQKKKKIVKYGMGGLIILFLIAIIWFPLLFMSLVR 2198
hPiezo2          VEDIYAHIFILKCWRESEKRYPQPRGQKKKKVVKYGMGGMIIVLLICIVWFPLLFMSLIK 2427
dPiezo           MEDIFSNIYLIRCTRQSETDFPAMRAQKKASLSKLIMGGTIVLLIVICIWGPLCLFALGN 2172

mPiezo1          SVVGVVNQPIDVTVTLKLGGYEPLFTMSAQQPSIVPFTPQAYEELSQQFDPYPLAMQFIS 2274
mPiezo2          SVAGVINQPLDVSVTITLGGYQPIFTMSAQQSQLKVMDNSKYNEFLKSFGPNSGAMQFLE 2557
hPiezo1          SVVGVVNQPIDVTVTLKLGGYEPLFTMSAQQPSIIPFTAQAYEELSRQFDPQPLAMQFIS 2258
hPiezo2          SVAGVINQPLDVSVTITLGGYQPIFTMSAQQSQLKIMDQQSFNKFIQAFSRDTGAMQFLE 2487
dPiezo           -AVGTSNVPFHVSLSIRIGPYDPIYTTNN-YDSIFEINPEMYSQMTNAYIKEKQALTFIA 2230

mPiezo1          QYSPEDIVTAQIEGSSGALWRISPPSRAQMKQELYNGTADITLRFTWNFQRDLAKGGTVE 2334
mPiezo2          NYEREDVTVAELEGNSNSLWTISPPSKQKMIQELTDPNSCFSVVFSWSIQRNMTLGAKAE 2617
hPiezo1          QYSPEDIVTAQIEGSSGALWRISPPSRAQMKRELYNGTADITLRFTWNFQRDLAKGGTVE 2318
hPiezo2          NYEKEDITVAELEGNSNSLWTISPPSKQKMIHELLDPNSSFSVVFSWSIQRNLSLGAKSE 2547
dPiezo           GYDATDVAAVRLAGNSPSLWNIAPPDRQRLLNDLRN-NHTLKARFSYSLTRKAPAKGLKE 2289

mPiezo1          YTNEKHTLELAPNSTARRQLAQLLEGRPDQS------------------VVIPHLFPKYI 2376
mPiezo2          IATDKLSFPLAV--ATRNSIAKMIAGNDTESSNTP--------------VTIEKIYPYYV 2661
hPiezo1          YANEKHMLALAPNSTARRQLASLLEGTSDQS------------------VVIPNLFPKYI 2360
hPiezo2          IATDKLSFPLKN--ITRKNIAKMIAGNSTESSKTP--------------VTIEKIYPYYV 2591
dPiezo           NVGDEHAISLDESFEGRAALIHMLSETHDVEPIHSNGTTNGTTPEVEEVVVIPGMIPKFI 2349

mPiezo1          RAPNGPEANPVKQLQPDEEEDYLGVRIQLRREQVGTGASGEQAGTKASDFLEWWVIELQD 2436
mPiezo2          KAPSDSNSKPIKQLLS--ENNFMNITIILFRDNVTKSNSE------------WWVLNLTG 2707
hPiezo1          RAPNGPEANPVKQLQPNEEADYLGVRIQLRREQ-GAGATG---------FLEWWVIELQE 2410
hPiezo2          KAPSDSNSKPIKQLLS--ENNFMDITIILSRDNTTKYNSE------------WWVLNLTG 2637
dPiezo           KVLNSGDAAVVSVLSP-KHYDYRPLVIKMHRDNETNGLWWEIRDYCN----DTFYNETLS 2404

mPiezo1          CKADCN---LLPMVIFSDKVSPPSLGFLAGYGIVGLYVSIVLVVGKFVRGFFSEISHSIM 2493
mPiezo2          SRIFNQGSQALELVVFNDKVSPPSLGFLAGYGIMGLYASVVLVIGKFVREFFSGISHSIM 2767
hPiezo1          CRTDCN---LLPMVIFSDKVSPPSLGFLAGYGIMGLYVSIVLVIGKFVRGFFSEISHSIM 2467
hPiezo2          NRIYNPNSQALELVVFNDKVSPPSLGFLAGYGIMGLYASVVLVIGKFVREFFSGISHSIM 2697
dPiezo           KFAYSNCTSGIVMYTFNDKKFPSTFSFLTAGGIIGLYTTFVLLASRFMKSFIGGQNRKIM 2464
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mPiezo1         FEELPCVDRILKLCQDIFLVRETRELELEEELYAKLIFLYRSPETMIKWTRERE------ 2547
mPiezo2         FEELPNVDRILKLCTDIFLVRETGELELEEDLYAKLIFLYRSPETMIKWTREKTN----- 2822
hPiezo1         FEELPCVDRILKLCQDIFLVRETRELELEEELYAKLIFLYRSPETMIKWTREKE------ 2521
hPiezo2         FEELPNVDRILKLCTDIFLVRETGELELEEDLYAKLIFLYRSPETMIKWTREKTN----- 2752
dPiezo          FEDLPYVDRVLQLCLDIYLVREALEFALEEDLFAKLLFLYRSPETLIKWTRPKEEYVDDD 2524

mPiezo1         ------------------------
mPiezo2         ------------------------
hPiezo1         ------------------------
hPiezo2         ------------------------
dPiezo          GDTDSIPSRMSVRRPEQLQPQQPQ 2548
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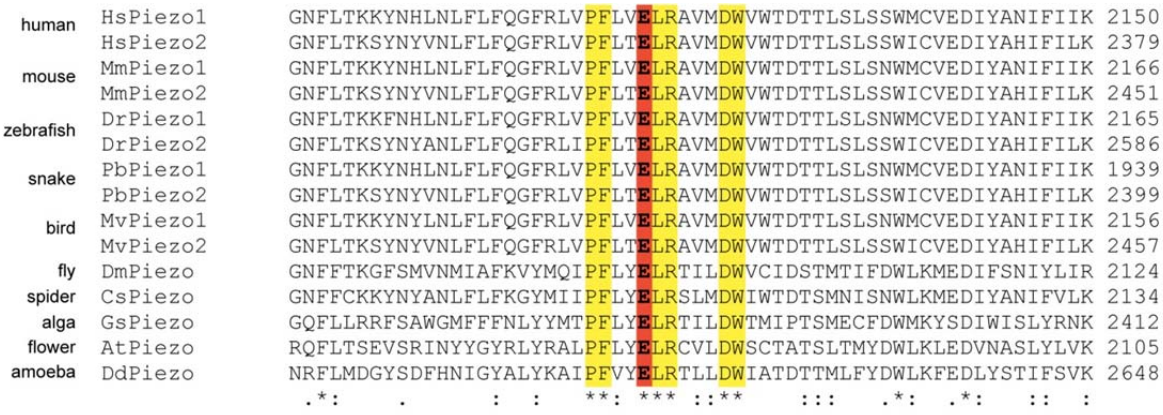
Supplementary Figure 3. Alignment between mouse, human and fly Piezos highlights hydrophobic regions and residues of interest. Multiple sequence alignment between mPiezo1, mPiezo2, hPiezo1, hPiezo2 and dPiezo generated using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Residues highlighted in grey indicate proposed hydrophobic regions in mPiezo1. Residues highlighted in cyan indicate position of myc-tags in extracellular loops. Residues highlighted in pink indicate phosphorylation sites. Residues highlighted in green indicate junction points for the following mPiezo1/dPiezo chimeras; ASRG: $dP^{1\text{-}1316}/mP1^{1316\text{-}2547}$ and $mP1^{1\text{-}1315}/dP^{1317\text{-}2548}$, FLW: $dP^{1\text{-}1752}/mP1^{1715\text{-}2547}$ and $mP1^{1\text{-}1714}/dP^{1753\text{-}2548}$ and TDV: $dP^{1\text{-}1929}/mP1^{1974\text{-}2547}$ and $mP1^{1\text{-}1973}/dP^{1930\text{-}2548}$. Residues highlighted in yellow indicate acidic residues between regions of amino acid numbers 1974-2547 that were mutated to alanine or asparagine.



Supplementary Figure 4. Conservation of a glutamic acid residue involved in ion conduction properties of Piezo channels. Multiple sequence alignment of Piezo proteins from different species using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Alignment of only the region around mouse Piezo1 E2133 (red box) is shown. The yellow boxes highlight conserved residues among species close to mouse Piezo1 E2133. The sequences used for the alignment are:

Homo sapiens: HsPiezo1 (NP_001136336.2) and HsPiezo2 (NP_071351.2)

Mus musculus: MmPiezo1 (ADN28064.1) and MmPiezo2 (NP_001034574.4)

Danio rerio: DrPiezo1 (XP_696355.4) and DrPiezo2 (XP_003198010.2)

Python bivittatus: PbPiezo1 (XP_007431683.1) and PbPiezo2 (XP_007433600.1)

Manacus vitellinus: MvPiezo1 (XP_008925897.1) and MvPiezo2 (XP_008923830.1)

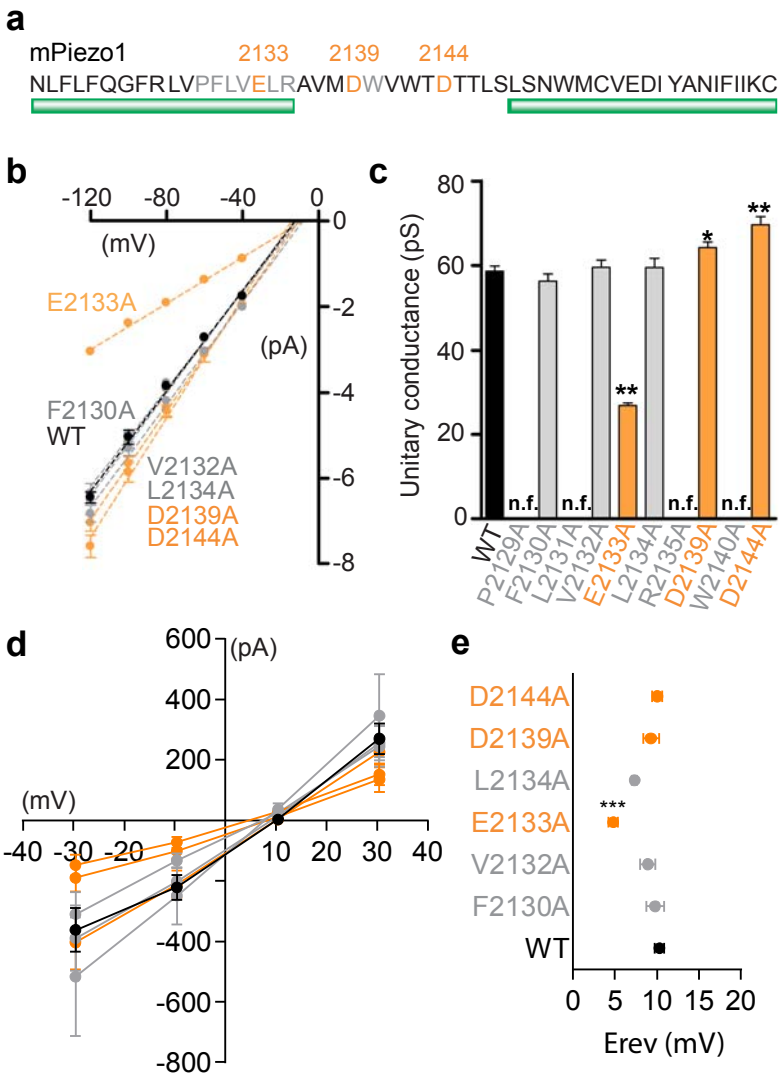
Drosophila melanogaster: DmPiezo (AFB77909.1)

Cupiennius salei: CsPiezo (JAA92956.1)

Galdieria sulphuraria: GsPiezo (XP_005703632.1)

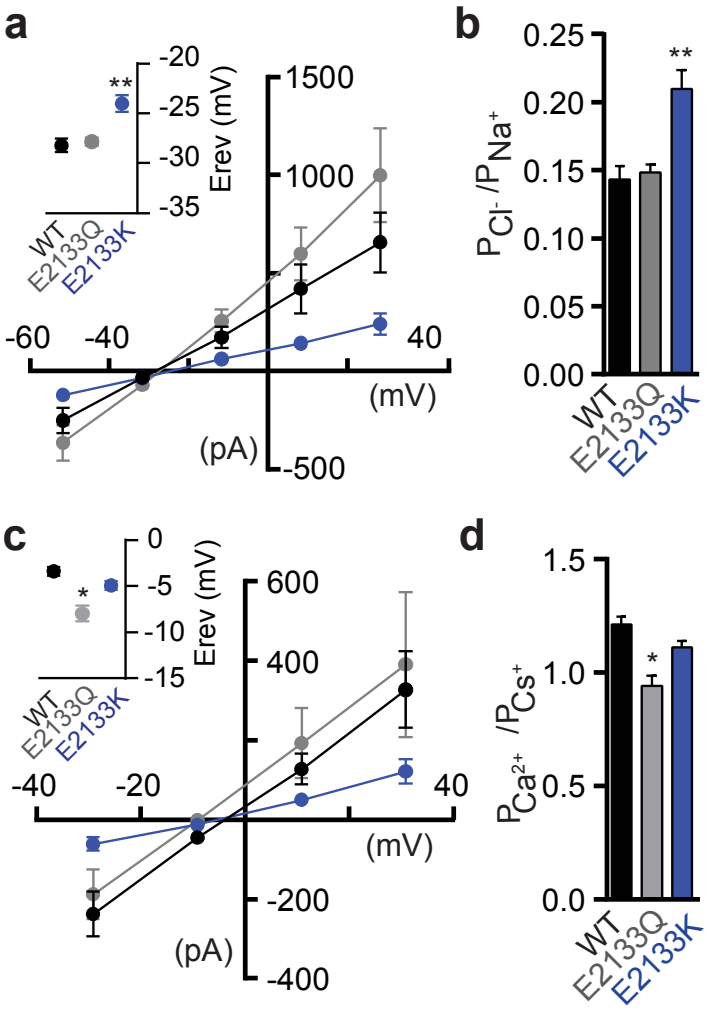
Arabidopsis thaliana: AtPiezo (NP_182327.6)

Dictyostelium discoideum: DdPiezo (Q54S52.1)



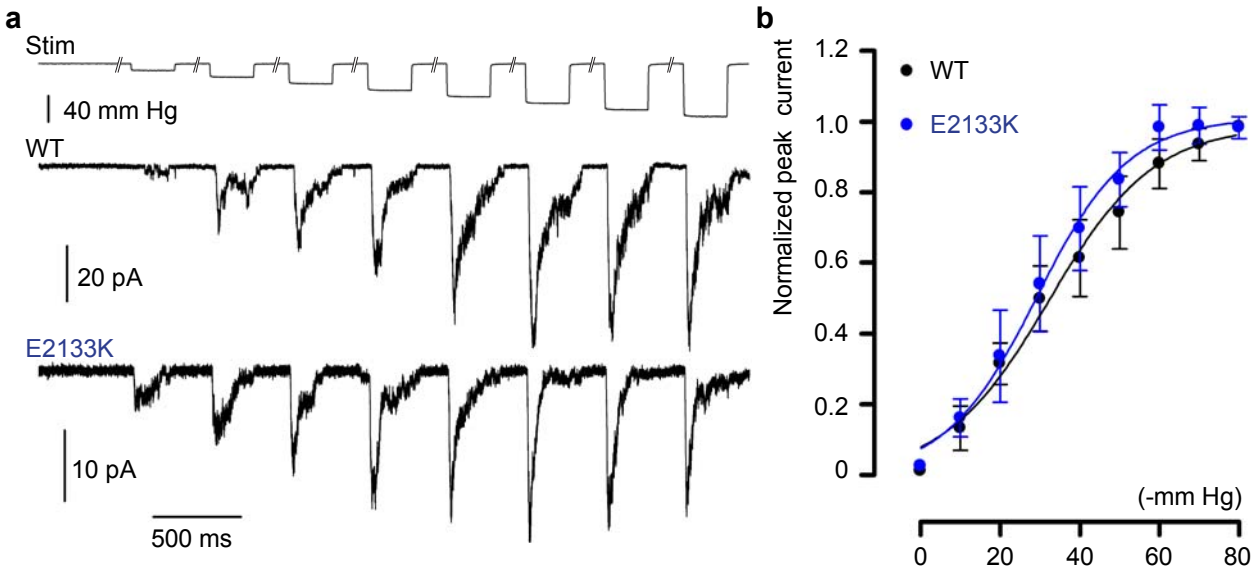
Supplementary Figure 5. Unitary conductance and ion selectivity of alanine single point mutants around E2133. (**a**) mPiezo1 sequence around E2133. Green bars indicate hydrophobic regions. E2133 and other acidic residues are highlighted in orange. Other conserved residues in this region are marked in grey. (**b**) Average I-V relationships of stretch-activated single channels in mPiezo1 WT and F2130A, V2132A, E2133A, L2134A, D2139A and D2144A transfected cells (n =7, 6, 4, 4, 5, 7, and 4, respectively; mean ± s.e.m.). Single-channel amplitude was determined as the amplitude difference in Gaussian fits of full-trace histograms. (**c**) Unitary conductance of stretch-activated channels from mPiezo1 WT and specified single point mutants transfected cells. P2129A, L2131A, R2135A and W2140A mutants are non-

functional. Conductance is calculated from the slope of linear regression line of individual cell single-channel I-V relationships (mean ± s.e.m.; One-way ANOVA with Dunn's comparison relative to WT, **P<0.01, *P<0.05). Panel b and c experiments were done in Na^+-based pipette solution. (**d**) Average I-V relationships of MA currents recorded from mPiezo1 WT, F2130A, V2132A, E2133A, L2134A, D2139A and D2144A expressing cells with 150 mM CsCl based intracellular solution and 100 mM $CaCl_2$ extracellular solution (n = 9, 5, 6, 9, 5, 7 and 7, respectively). (**e**) E_{rev} potential for the indicated mutants corresponding to panel (**d**) experiments WT: 10.3 ± 0.6 mV; F2130A: 9.8 ± 1.0 mV; V2132A: 8.9 ± 0.9 mV; E2133A: 4.8 ± 0.6 mV; L2134A: 7.3 ± 0.3 mV; D2139A: 9.3 ± 0.9 mV; D2144A: 10.0 ± 0.6 mV; mean ± s.e.m.; One-way ANOVA with Dunn's comparison to WT ***P<0.001).

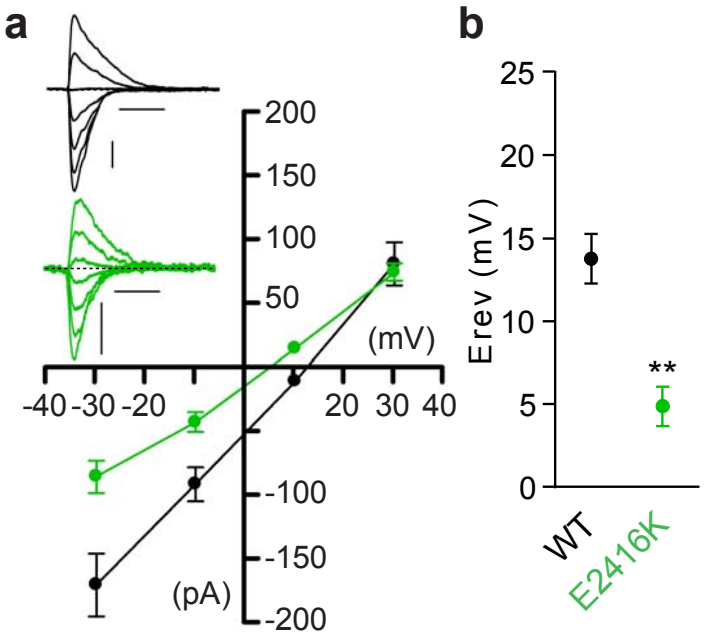


Supplementary Figure 6. Chloride and Calcium permeability for E2133 mutants. (**a**) Average I-V relationships of MA currents recorded from mPiezo1 WT, E2133Q and E2133K expressing cells with 150 mM NaCl based intracellular solution and 30 mM NaCl extracellular solution. Inset: Average reversal potential (mean ± s.e.m.; n = 6, 7 and 6, respectively; One-way ANOVA with Dunn's comparison to WT **P<0.01). (**b**) P_{Cl}/P_{Na} permeability ratios of MA currents from cells transfected with mPiezo1 WT, E2133Q and E2133K (mean ± s.e.m.; n = 6, 7 and 6, respectively; One-way ANOVA with Dunn's comparison to WT **P<0.01). (**c**) Average I-V relationships of MA currents recorded from mPiezo1 WT, E2133Q, and E2133K expressing cells with 149 mM Cs-methanesulfonate, 1 mM CsCl based intracellular solution and 50 mM Ca-Gluconate, 0.5 $CaCl_2$ based extracellular solution. Inset: Average reversal potential (mean ±

s.e.m.; n = 6, 6, and 5, respectively; One-way ANOVA with Dunn's comparison to WT *P<0.05). (**d**) P_{Ca}/P_{Cs} permeability ratios of MA currents from cells transfected with mPiezo1 WT, E2133Q, and E2133K (mean ± s.e.m.; n = 6, 6, and 5, respectively; One-way ANOVA with Dunn's comparison to WT *P<0.05).



Supplementary Figure 7. Pressure sensitivity for WT and E2133K mutant. (**a**) Representative cell-attached stretch activated current traces recorded from HEK293T cells expressing WT or E2133K mPiezo1 at -80 mV with standard pipette solution (see methods). Patches are stimulated with 250-ms negative pressure pulses from 0 to -80 mmHg elicited every 10 seconds (-10 mm Hg increments). (**b**) Average peak current-pressure relationships of stretch activated currents recorded from WT or E2133K mPiezo1 expressing cells as illustrated in (**a**) (n =4 and 5 cells, respectively). Bars indicate mean ± s.e.m. Fits with boltzman equation give pressure for half maximal activation (P_{50}) of 32.3 ± 3.0 and 29.3 ± 2.9 mm Hg for WT and E2133K, respectively.



Supplementary Figure 8. Ion selectivity altered by mPiezo2 mutant. (**a**) Average I-V relationships of MA currents recorded from mPiezo2 WT and E2416K expressing cells with 150 mM CsCl based intracellular solution and 100 mM $CaCl_2$ extracellular solution (n = 6 for each). Inset: typical recording traces for WT (black) and E2416K (green) from -69.6 to +50.4 mV, Δ20 mV. Scale bars: 100 pA, 50 ms. Probe stimulation displacements are 5 and 9 μm, respectively. (**b**) Average reversal potentials from individual cells corresponding to panel (**a**) experiments (mean ± s.e.m.; Mann-Whitney test, **P<0.01).

Supplementary Table 1. Summary of Myc tag analysis used for topology prediction.

Amino acid position for Myc tag	Live labelling*
N-terminal	-
C-terminal	-
102	+
153	-
221	-
304	+
382	-
443	-
508	+
562	-
606	-
669	+
753	-
802	-
897	+
940	-
1000	-
1026	-
1071	+
1126	-
1163	-
1178	-
1204	-
1239	-
1269	-
1299	-
1389	-
1699	-
1765	+
1797	-
1891	-
1892	-
2009	-
2035	-
2041	-
2071	+
2075	+
2086	-
2104	-
2126	-
2143	-
2146	-
2157	-
2173	-
2336	+

*Negative for live labeling does not mean that the tag is intracellular.

Supplementary Table 2. Potential phosphorylated residues in peptide sequences detected by mass spectroscopy

Phosphorylated residue position	Phosphorylation residue	Peptide sequence	Total
351	T	KETPREDEEHELELDHLEPEPQAR	5
396	T	DATQGEMPMTTEPDLDNCTVHVLTSQSPVR	1
397	S	DATQGEMPMTTEPDLDNCTVHVLTSQSPVR	1
399	S	DATQGEMPMTTEPDLDNCTVHVLTSQSPVR	11
738	S	QDAVSEAPLLEHQEEEEVFREDGQSMDGPHQATQVPEGTASK	1
758	S	QDAVSEAPLLEHQEEEEVFREDGQSMDGPHQATQVPEGTASK	1
1385	S	DPQDPSQEPGPDSPGGSSPPR	28
		DPQDPSQEPGPDSPGGSSPPRR	23
		GQLQSKDPQDPSQEPGPDSPGGSSPPR	3
		LQSKDPQDPSQEPGPDSPGGSSPPRRQW	2
		QSKDPQDPSQEPGPDSPGGSSPPRRQW	2
		SKDPQDPSQEPGPDSPGGSSPPRRQW	1
1389	S	DPQDPSQEPGPDSPGGSSPPR	8
		DPQDPSQEPGPDSPGGSSPPRR	7
1390	S	DPQDPSQEPGPDSPGGSSPPR	5
		DPQDPSQEPGPDSPGGSSPPRR	13
		QSKDPQDPSQEPGPDSPGGSSPPRRQW	2
1593	S	DGPSTASSGLGAEEPLSSMTDDTSSPLSTGYNTR	1
1600	S	DGPSTASSGLGAEEPLSSMTDDTSSPLSTGYNTR	4
1604	T	DGPSTASSGLGAEEPLSsMTDDTSSPLSTGYNTR	2
1608	T	NTRSGSEEIVTDAGDLQAGTSLHGSQEL	1
		NTRSGSEEIVTDAGDLQAGTSLHGSQELL	1
1610	S	NTRSGSEEIVTDAGDLQAGTSLHGSQELL	2
		SGSEEIVTDAGDLQAGTSLHGSQELLANAR	2
1612	S	NTRSGSEEIVTDAGDLQAGT	2
		NTRSGSEEIVTDAGDLQAGTSL	4
		NTRSGSEEIVTDAGDLQAGTSLHGSQEL	2
		NTRSGSEEIVTDAGDLQAGTSLHGSQELL	1
		SGSEEIVTDAGDLQAGTSLHGSQELLANAR	9
1617	T	NTRSGSEEIVTDAGDLQAGTSLHGSQELL	1
1626	T	SGSEEIVTDAGDLQAGTSLHGSQELLANAR	1
1627	S	SGSEEIVTDAGDLQAGTSLHGSQELLANAR	6
1631	S	NTRSGSEEIVTDAGDLQAGTSLHGSQELL	4
		NTRSGSEEIVTDAGDLQAGTSLHGSQELLAN	1
		NTRSGSEEIVTDAGDLQAGTSLHGSQELLANARTRM	1
		SGSEEIVTDAGDLQAGTSLHGSQELLANAR	19
1640	T	NTRSGSEEIVTDAGDLQAGTSLHGSQELLANARTRM	1
1644	T	MRTASELLLDR	1
		RTASELLLDR	1
		RTASELLLDRRLHIPELEEAERF	1
1646	S	MRTASELLLDR	5
		RTASELLLDR	4
		RTASELLLDRRLHIPELEEAERF	7
		TASELLLDR	6
		TASELLLDRR	9
1837	S	LESQSETGTGHPK	7

Supplementary Table 3. Pharmacological and biophysical properties of mPiezo1/dPiezo chimeras and mPiezo mutants

	30 μM ruthenium red inhibition at -80 mV (%)	Inactivation time constant at -80mV (tau in ms)	Unitary conductance (pS)	
			Standard pipette solution	Na^+-based pipette solution
WT channels				
mPiezo1	71.4 ± 4.4 (5)	9.9 ± 0.5 (31)	29.1 ± 0.4 (7)	58.6 ± 1.2 (7)
mPiezo2	70.8 ± 1.4 (5)	4.5 ± 0.7 (6)	23.7 ± 0.6 (8)	56.9 ± 1.3 (9)
dPiezo	5.2 ± 6.7 (5)	6.1 ± 0.5 (6)	5.7 ± 0.6 (6)	n.d.
Chimeras				
$mP1^{1-1315}/dP^{1317-2548}$	Non-functional			
$dP^{1-1316}/mP1^{1316-2547}$	64.8 ± 6.7 (5)	15.9 ± 0.8 (30)	23.4 ± 1.0 (5)	n.d.
$mP1^{1-1714}/dP^{1753-2548}$	Non-functional			
$dP^{1-1752}/mP1^{1715-2547}$	76.4 ± 5.3 (6)	25.5 ± 1.3 (5)	27 ± 1 (5)	n.d.
$mP1^{1-1973}/dP^{1930-2548}$	1.3 ± 13 (5)	4.5 ± 0.5 (5)	9.1 ± 0.8 (6)	n.d.
mPiezo1 mutants				
D1975N	74.3 ± 4.8 (4)	7.7 ± 1.2 (9)	31.4 ± 1.1 (4)	n.d.
D1984A	78.0 ± 5.9 (4)	9.7 ± 1.2 (10)	30.2 ± 0.6 (3)	n.d.
D1987A	79.5 ± 2.5 (2)	19.1 ± 1.9 (11)	28.1 ± 0.8 (8)	n.d.
D2006A	59.6 ± 7.7 (3)	11.3 ± 1.7 (9)	28.3 ±0.7 (9)	n.d.
D2013A	56.5 ± 9.5 (4)	5.3 ± 0.4 (3)	28.5 ± 1.8 (6)	n.d.
D2014A	54.1 ± 5.6 (6)	4.2 ± 0.6 (13)	27.8 ± 1.5 (4)	n.d.
D2034N	74.8 ± 7.1 (2)	11.0 ± 1.5 (9)	30.3 ± 0.2 (3)	n.d.
E2070A	64.1 ± 2 (6)	11.7 ± 3.7 (3)	25.4 ± 1.1 (4)	n.d.
P2129A	Non-functional			
F2130A	n.d.	4.3 ± 0.7 (4)	n.d.	56.3 ± 1.7 (6)
L2131A	Non-functional			
V2132A	n.d.	9.6 ± 0.6 (3)	n.d.	59.5 ± 1.7 (4)
E2133A	61.1 ± 11.8 (3)	18.2 ± 2.8 (10)	14.4 ± 0.5 (5)	26.8 ± 0.6 (4)
E2133D	85 ± 2.0 (2)	35.8 ± 1.5 (32)	n.d.	77.5 ± 3.0 (5)
E2133Q	74.6 ± 6.2 (5)	29.5 ± 1.8 (26)	n.d.	30.5 ± 1.3 (5)
E2133K	8.1 ± 7.1 (10)	12.2 ± 0.6 (9)	n.d.	20.0 ± 1.3 (4)
L2134A	n.d.	5.3 ± 1.6 (4)	n.d.	59.4 ± 2.1 (5)
R2135A	Non-functional			
D2139A	57.2 ± 6.7 (4)	7.7 ± 0.8 (12)	32.4 ± 1.5 (4)	64.2 ± 1.2 (7)
W2140A	Non-functional			
D2144A	63.0 ± 7.2 (3)	18.3 ± 1.5 (11)	30.6 ± 1.0 (4)	69.6 ± 1.8 (4)
E2156A	57.0 ± 4.3 (4)	6.4 ± 0.4 (14)	26.5 ± 0.9 (5)	n.d.
D2157A	65.0 ± 6.0 (3)	8.8 ± 0.7 (19)	29.7 ± 1.0 (3)	n.d.
E2170A	69.0 ± 1.0 (2)	11.6 ± 1.3 (3)	27.8 ± 0.1 (3)	n.d.
E2172A	75.3 ± 7.0 (3)	8.4 ± 0.9 (3)	24.3 ±0.3 (4)	n.d.
mPiezo2 mutant				
E2416K	49.6 ± 4.6 (5)	5.7 ± 0.5 (5)	n.d.	32.6 ± 1.0 (6)

Mean ± s.e.m. (n-number); n.d.: not determined