

Supplementary Figure 1

mPiezo1 : EPHVFLGAGLYWLLLECTFLAASLLRNALSLVTLFLLLLMLPGSPRHSIPCHTGRLLRALCLSLLEFVALAFQCLHTVPHLDQFLGQNGSLWV : 99
mPiezo2 : ASEVVCGLIFRLLLIICLAVACAFRYNGLSFVYIYLLLIILFSEPTKATMOCHTGRLLQSCTLSLSEFLLLIIFHTLASLEAQHRTTPAYNCSTWE : 100
dPiezo : VFSYACMVLQRIVVAVLVLAALRPVGISFVYILMFFVSFVPLATRRNFKS VTAFFIITLTLSTLVVLGTTLQILAVSLT-----LPTYNCSFSE : 95

mPiezo1 : KVSQHTGVTRLDLKDIFNTTRLVAIDLGVLLASLCLGLCGRLTRKAGQSRRTQELQDDDDDDDDDEDIDAAP-----AVGLKGAPALATKRRL : 189
mPiezo2 : KTRFQIGFESLKGADAGNIRVFDIIGMFIASLTINLVCRITVVKPDTTEIAQLNSECENEELAGGKMDSEALIYEEDLDGEEGMEGELEESTKLKI : 200
dPiezo : RLLRHIGFVSFDLQFPFAIIEWLVLEVLVFATSLGSLYLVKRVASQVFGAEQLENQVVDGQAEANAQTSQPSAADANGGDVQOATVTTPLQQQQQLRK : 195

mPiezo1 : W-----IASRFRVTAHWLLMSTGRTLVIVLALAGIAHPSAFSSIMLVVFAICMWSCHFPPLSPGENTFCVMVSCFGAGLILCLYCVOTPFIDMLPP : 284
mPiezo2 : LRRFASVASKLKEFFIGNMITTAGKVVTIILGSSGMPLSLTSAVFFVFLGLCMTWSWCRTEFDPLIGGICVLLAIFTAGLILGLYLQVQFFQEA VPP : 300
dPiezo : R-----VSMISQIHIEFGLVKISPLFCLATFFAAVLRPSVGGGFLLEILSGMVAATCQTLQRGHALLLRCVMVVLVLSLSIVSOTPFMWSHLNH : 289

mPiezo1 : GNIFARLECLKNEVDLPNYSNPALVLTAKHAWPIYVSGIILLIYATATSLKHLKSCPSSELRKETPREDE---EHELELDHLEPEPQARDATQG--- : 377
mPiezo2 : NDYARLEFCIKSVIQT-DCASTWKIIVPDLISWYHHAQLLLVMVYTLATLIRIWLQEP-LVQEMAKEDEGALDCSSNQTAERRRSLWYATQYPTDE : 398
dPiezo : TITLARIITCLEPIESYCSPIRIVFLYINKLSLDSYLNFAFFAFALAITTKHLIKPRLVRQSTRKARTPOLESQSSVAPSVTQR----- : 377

mPiezo1 : --EMPMTTEPDLNCTVHVLTQSQSPVRQRPVPRVIAELK-----EMSPHLGHLMD : 428
mPiezo2 : RKLLSMTQDDYKPSDGLLVTVNGPNVDYHTIHPSTPIENGPACTDYTTPOYRWEPEEESSEKKEEEDKRESEGEQSEKRSVRMHAMVAVFQFMK : 498
dPiezo : -----GNDNQLESMEQRSEQENTTTIINDQIS-----YGFVSVGGFIYQ : 416

mPiezo1 : QSYVICALAMVWSLTYHWSLTFVLLWACLITVRSRHLALMLCSPCILLVGLTCCCLRVVWAMEPELPTTLGPVSL-----HQLGLEHTRYPC : 520
mPiezo2 : QSYVICALAMVWSLTYHWSLTFVLLIWSCTLMTNRNRKYAMISPPFMVYANLVLVLOIIVSFEPEIKKVPGFLEK-----KEPG----- : 581
dPiezo : NSYIFTNITMVAWSIYHWSLTFVLLSANVLTMTNQRKAMRSPFVIVAEALIAQMIYGMDDNNEELPTSVP TAGINLQOIGFERPIENQMRPCV : 516

mPiezo1 : DYGAMLLYLLTFLLLRQFVKKLLKKQKVPAALEVTVADT-----EPTQT-----QTLLRSLG : 575
mPiezo2 : EYASKLFTTITFLLLRQHLTEQKALREKE-ALLSEVKIGSQLEEKDEDELQDVQVEGEPTEKEEEEEEEIKERHEVKEEEEEVEEDDDQIMKVLG : 680
dPiezo : PUIVKTAFVLMVTSRQFFKQRDRRRDSTLADFIAPLQITVSGAGSSYLINDGKKT-----SKFLKKA : 582

mPiezo1 : ELVTGIYKYNIYVCAGMFIIVVSFAER-LVVYKIVYVLEFLCLCLTFVYYTLRLLRVSNLWVAVMVLVAVVTFQODPTYRNLTGFTDEQLG : 674
mPiezo2 : NLVVALFIKYNIVCGGMFFVVSFECK-IVMYKIVYVLEFLCVVALYVHYEYWRKILKYSMSVVIWDLVLEFIVTYQENPGLQNTGLKKEKLE : 779
dPiezo : DVIKNLLVRLNWLVLVIFLCAITCENMTGFRVYALEFPELLVFSSSKAVIMYGMDFLFIYASITLITTYQDKDQTYSDYLVNSATLQK : 682

mPiezo1 : DLGFEQFSVSESSILLIGFLLACILQLHYEIRPQMLTDLEHVP-----PPGTR-----HPRWAHRQD : 735
mPiezo2 : DLGKQFTVABEITRIFPTSFLLVCLLHDFHDFDRLELTDLKSIPSKEDNTIYSHAKVNGRVYLIINRLAHPEGSLPDLAIMNMTASLDKPEVQKLA : 879
dPiezo : DLGKRYQTKDLHLVSPITIVILTIVQHYFKRGLIASLQQOPLAG-----GSAQKQKTE : 739

mPiezo1 : AVSEA--LLEHQEEEVFREDGQMDGPHQATQVPDGTAS---KNGVADRLLDLAASFSAVLTRIQVFRRLLELVEFLALYTVVWALKEVSMN : 829
mPiezo2 : SGEEREEECVKKTEKGEAGKSDSEEEEDDEEESSESSDLRNKWHVIDRLTVLFLKELEYFHKLVQFMWVLELHITITISSYIIWVTVKVSLFN : 979
dPiezo : TTALEAPSKRRRSAGSLRKSQSPAEAAPGATTDFTSVR-----DLYRISFRKIKNKSEYIFKNEKDVFRWRELEHIMAYYIAAFVCSVSECVLH : 833

mPiezo1 : LLLVVLWAFALP-YPRFRPMASCLSTVVTCTIIVCKMLYOLKIVNPHEYSNTEPFPPNNTLQPLEINQSLLYRGPVDPANWFGVRRKGYPNLGYIQNHL : 928
mPiezo2 : YVFLISWAFALP-YAKLRRASVCTVVTCTIIVCKMLYOLQTKIPENFSVNSLPEENQNTIPLHELKSLLYSAPVDPTEWGLRKSPLLVYLRNHL : 1078
dPiezo : IIFVFGCVLGATSRKAVQVVISRLISFIVTVLVSQIYQIEYLSHSQHNVVSD-NRTANNAEWIGLTKADKVTG-----GLMSLLRTYI : 918

mPiezo1 : QILLLLVFEAVVYRREHYRQH-QQAPLPAQAVCADGTQRLLDQDILSCLKVFIFEFYKFCLEICFIMANVIGOMNFMVILHGCIVAVITRRERE : 1027
mPiezo2 : LMLAALAEFVYRHHQYRGRN-NLTAPVSTIIFHDITRLHLDGGLINCAKVFVYEFYKFCLEICFIMSNVIGOMDFYAMIHACILGVYRNRK : 1177
dPiezo : IYMVIVTMHAVISLRLOMVKI GALNAPPTKLLFPNITRADAEKLVGLVKKLLFGFYKFCLEISLIALSTIYQDIAVAVVYALNVLVLLRRLS : 1017

mPiezo1 : AIFRLPNYCLLETLLELYQYLLCLQDPEALCIDYPRWSKAI PMNSALIKLYLDFFRAPNSTNISDELLLCASQWQVSAERTEEWORMAGINT : 1127
mPiezo2 : AIFEVPKYCCCLACLIITQYFVCLIDPEAPORDYPRFKGAY-FNDNLIKLYEFDIVRPNPVFVYDEMLLCASLQRIEENKAAVRIMAGNV : 1276
dPiezo : QCAKIGVQAFSAISILTIQIVLVLPSSCLVEPPDEGPFG---EGIQRFAMLGALHFNHPKLIFFIVLILNRKSIICLQRYASNDYPPGGS : 1114

mPiezo1 : DHLEPLRGE-----PNPIPNTHCR-SLLMLAVAVRYFLVLDVVVAVAGATRISIFGLCTLACVYLLFPTLLQKDTRAQLVLDCHILLYNVT : 1219
mPiezo2 : EICMNLDAASF---SOHNPVDSIHR-SLLMSVLIIFSLFVFTIIEITTRISIFCMGYLVACVYPLFGDLLLLKPKSILRYDNIYAYNVF : 1372
dPiezo : NRSVIADIAQLGRVPDNPTHDCSYIRNSLILKNGVLCGFYFTAVVLAETNIADLLAYLIGAFIFLWQSDFYLRPHITIFRKLAFNVA : 1214

mPiezo1 : VVISNMLSLLSVFVEQMSNFCVIQLFSLVCIKVGYYDPKEMMTRDRCLLE-----VEEAGIINDSICFELLORNIELSHYELVSAADKATA : 1313
mPiezo2 : VITMNILSIGAGYIGALVRNSCLIQAFSLACTVKGYQMP-----DSDRCKLE-----SGEAGIINDSICFELLORVMSYELVVAADKASQ : 1462
dPiezo : NULLTSFQMGALFMTQTKDCCLVHMLGITCISNVLTQIQLPEEAELALKGECPKITHQVVLLWDTICFARITFQILKSHYECIITTRKANN : 1314

mPiezo1 : LQASRCFALYNAANLISINFHRQIEKSLAQKQKRRRAKQRYRQSQ-----ASRQQLQSKDPQDPSQEPGDPSPGSSPP---RRQWRPWLDFATV : 1406
mPiezo2 : ILASRCALFQATIVAVKARIEEKKSMDQLKQDRIKARQKYGKGERMLSITQESGEGQDIQKVSEEDDERADKQKAKGKKQWRPVDWASM : 1562
dPiezo : ILASRCADILIESLRHQIAHRHDHAKVHLKIKKRRRATQK-----MLRPLDKQTHFDEHGYPLPAPTVRRRKIEKLEPHA : 1394

mPiezo1 : IHSQVFLFSDSEEEFE--ALPDRPAAQ--SAFQMAVAVNAQTVLQRORR---ARQERAQLASGGDLNPDVEPVDVPE--EDEMAGRSHMMQ : 1496
mPiezo2 : VRSQVYLFSDSEEEFEELKQDEEPPRK--SAFOFVYAWLIDPKTALRQRKREKKLAREEQKRRKSGDGPVWEDREDEPVKKSQDGNIIK : 1660
dPiezo : TRASQVYMFEMDKFEDLIDHIDFLEEENITESEMRKRRKLYDKSKDAPTGFPPSTSGKISKQDAATASSASAPATRDVGLPVI PPPSTGLG : 1494

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mPiezo1 : RVLSTMQFLNVLGQATVGLTRWLRFTKHHRTMSDVLCAEYRLLQELLRVGVEVR-RGVLDQLYVG-----EDEATLSG-----PV : 1572
mPiezo2 : RIFNILKFTWVLFVATVDSFTWLNSISREHIDISTVLRIRCMCLREIKKGNVPT-RESIHMYQNHHMNLRSGLDTIDEHSGAGSRAQAHRMDSL : 1759
dPiezo : REQTSKETS DSKSKMEVDSGEVTAKSDDEDFTNPILRLDGLVLTIRLNRFSRNYRFVNRILAGEKTKLKESSSLNRLGLSAAAMFHLKSNLESD : 1594

mPiezo1 : ETRDGP-----TASSGLGAE-----LSMDDTSSPLSTG-----YNTRSGSEEIVTDAGDLQAGTSLHGSQEL : 1634
mPiezo2 : DSRDSISCSCTEATLLISROSTLDDLDGDDVPKTSERARPRLRKMFSLDMSSSSADSGSVASSEPTQCTMLYSRQGTTEIEEVEAEAEVEVLEPE : 1859
dPiezo : ESEPPASSSTPRR----VVIAPPNATEHSD-----TSTTLTNTNTTTTPLSPPELQPLQPNITSTPQQOQHIRAAEETIELVDT : 1672

mPiezo1 : LANAR-----TRMRTASELLDRRL : 1654
mPiezo2 : LHDAAEKEYAAEYAGVEEISLTPDEELPQFSTDDCEAPPSYKAVSFEHLSFASQDSDGAKNHMVSPDSDRTDKLESSILPPLTHELTASDMLLSKMF : 1959
dPiezo : VDGVA-----HRKQCSINSSPPAKGA : 1692

mPiezo1 : HIPELEAERFEAQGRTRLRLRAGYQCVAFHSELLCYFIIILNHMVTAASAIVLEVLVFLVAMTIPRESKRFFMTAVFLEVMVVTLYLQEGFFPW : 1754
mPiezo2 : HDDELESSEKFXVDQPRFLLLFYAMNTLVRSMEVCFVILILNHMFSASITITLLELILIFLITAMSVPRSRREMMATVYVEVAIVVYFQEGFFPW : 2059
dPiezo : GEFNLEENFAQRDHHIIVEVLISWYALLNTDLCIVVVFIVQVNASLISPLELHVVEITGTSLPRETKTEFVTLIAYVQAVLITLCIQEKLWIS : 1792

mPiezo1 : NSYVLRRYENKCYFPPRLGPKTDSYIKYDVOIMAEFHSQQLCYGLDHEEDRYPKDHCSSVKDREAKEEPEAKLEQSETGTGHPKEPVLAGT : 1854
mPiezo2 : N--KDEIYKERBYEPNLIQVKKKGVLDYDIIQDLAEFHSSTDKCHGLDDEDDIVDSNTDKESDDELSLDQGRGSDSLKSNLAASVESVHTVF : 2157
dPiezo : N---YQLPNEQLLFAKFCVONKAHAIYDIIQLVLELHRYLQKSGELKSG--YKDTDNQFTKPTASIDERDSDNLQOPDSRQLN----- : 1876

mPiezo1 : PRDHIQKGSIRSKDVIQDPPDLKPRHTRHISIRFRRKETPGKGTAVMTEHEEGEKETTERKRPRHTQEKSKFERMKAAGRRLQSFVSLAQSF : 1954
mPiezo2 : P---EQPAAIRRRKSCSSSQISPRSSFSSNRKSGTSTRNSQKGSVLSLKQK-----SKRELIMEKLOEHLIKAKAFTIKTKLQI : 2237
dPiezo : -----DDAAQKLSLQVSQASLPGSEPKGTGINQLERTKYTSS----- : 1914

mPiezo1 : YQPLQRFHDIILTKYRAADVYALMELADIVDIIILLEGVAFCKHSAAT--IASSDSDQVQAFLELLEVQEGTMVDRALYLRKTVLGLAQQV : 2052
mPiezo2 : YVPIRQFEYDLIHPDYSADVYVLMELADIVDFILLVEGVAFCVHSAAD--ITSSSEDQVGGPELVVLELQEGTMVDRALYLRKTVLGLVQVI : 2335
dPiezo : ---LYKFEFSLVSKSR-LADVYALMELCPVNEFVLEGETAFCTQTESLEGVQTYAENKVPPELLELVQELLYVDRALYLRKLVNKLIIHFF : 2010

mPiezo1 : LVVALHIIMFFILAVTERMSQNAVAQLWVFKCIYFALSAYQRCGYETRILGNELTKYNHLLFLQGFRLVPELVLEAVMDRVTDTLSSLN : 2152
mPiezo2 : LVFGLHIMFFILGVTERRKSNLVAQLWVFKCVYFGLSAYQRCGYETRILGNELTKSNYVNLFLQGFRLVPELVLEAVMDRVTDTLSSLN : 2435
dPiezo : SVLGLHIMFFVAVTERMNSLAPPILFVILKCVMLLSYQKSKGYKRIILGNELTKGFMVMIARVYVQIPPELVLELITLDWVCISSMTIFD : 2110

mPiezo1 : MCVEDIYANFIIKSTETPKKYQPKGOKKIKIVYGGGLILFLIAILFPILEMSITRSVVQVYQIDVTVTLKLGVELEFMSAQQPSIVPPT : 2252
mPiezo2 : ICVEDIYAHFIIKSTETPKRYQPRGOKKAVYGGGLIVLLICIVFPILEMSITKVAQVYQILDVTVTLKLGVELEFMSAQQSQLKVM : 2535
dPiezo : LKMEDIYSNLYILRTQSTDFAMRAQKASLALTMGGTIVLLIVICIVGPICLFAGN-AVQTSVVFHVSLSIRIPIIDIIYTNN-YDSIFEIN : 2208

mPiezo1 : PQAEEELSQQFDPYPLMQEISQSPEDIVTAQIESSGAHTRISPE SRAQKQELYNGTADITLRETWNFQDLAKGGTVYTNKHTLELAPNSTARR : 2352
mPiezo2 : NSKNEFLKSGFPGNSGMOLENEREVTVAELEGNNSHWISPE SKQKMIQELTDPNSCFVVSWSIQNMELGAKALATDKLSFPVAVAT--RN : 2633
dPiezo : PEMSQMTNAYIKEKOLTIAGDATVAAVRLACNSPSTINAPEDRQRLINDRN-NHTLKARISYSLTKAPAKGLKRVNGDEHAISDESFEGLA : 2307

mPiezo1 : QLAQLEGRPDQS-----VPHLEPKYIRAPNGPEANPVKQIQPDEEDYLGVRLQLRQEQVGTGASGEQAGTKASDFLEWVIEL : 2434
mPiezo2 : SIAKMIAGNDTESSNTP-----VTHEKIYBYVVKAPSDSNKPIKQLLS--ENNFMNITLILFDNVTKSNS-----EWWWLNL : 2705
dPiezo : ALIHLSETHDVEPIHNGTNGTTPVEEVVVPGMTPKFIKVLNSGDAAVVSVSP-KHYDYRPLVKMHQDNETNGLWWEIRDYCN---DTFFNET : 2402

mPiezo1 : QDCKADCN---LLPMVITSDKVSPSLGTAGYGVGLYVSIIVLVGKVRGPFSEISHSIMFELCVDRIIKLQODIPLVRETRLELEEDYAKLIE : 2531
mPiezo2 : TGSRIFNQGSQALELVVNDKVSPSLGTAGYGVGLYASVIVLVGKVRGPFSGISHSIMFELPNVDRIIKLQODIPLVRETRLELEEDYAKLIE : 2805
dPiezo : LSKFAYSNCTSGIVMYTNDKVFSTFSITAGSILGLYITPVLASRDKSKSIGGQNRKIMFEDLBYVDRVQLCLDIYVREALEFALEEDIFAKLIE : 2502

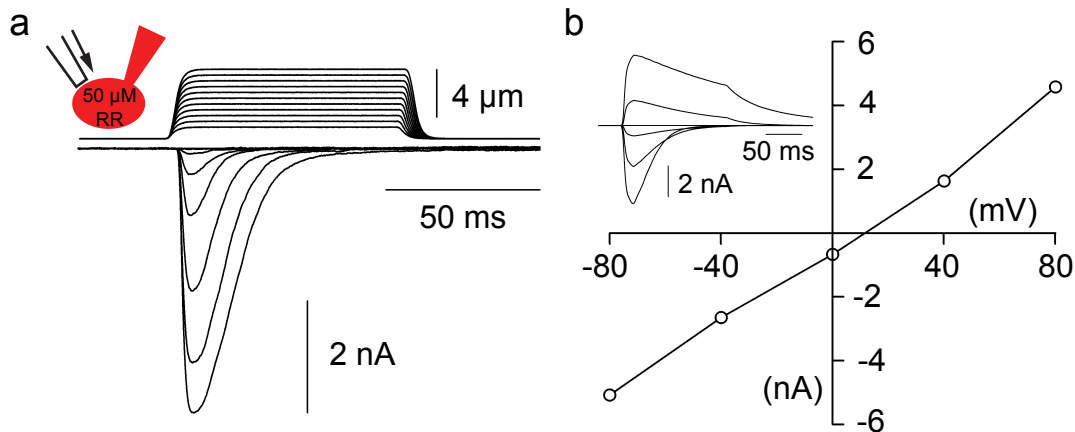
mPiezo1 : LYRSPETIKWTRERE----- : 2547
mPiezo2 : LYRSPETIKWTRKTN----- : 2822
dPiezo : LYRSPETIKWTRKPEEYVDDGDTSIPSRMSVRRPEQLQPQQPQ : 2548

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Supplementary Figure 1. Conservation between mouse and drosophila Piezos.

Alignment of mpiezo1, mpiezo2 and dpiezo proteins. Conserved identical residues are highlighted in gray. dpiezo is 24% identical to both mpiezo1 and mpiezo2. Alignment was done with ClustalW and further analyzed using Genedoc program.

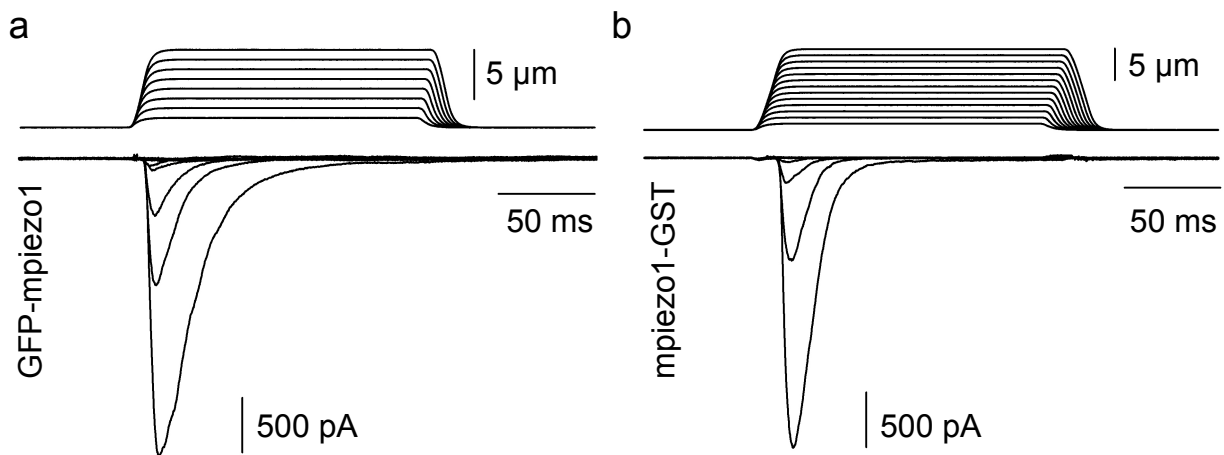
Supplementary Figure 2



Supplementary Figure 2. Cytoplasmically applied RR does not affect mpiezo1 induced MA currents.

(a) Representative traces of whole cell MA inward currents in mpiezo1-transfected HEK293T cells at a holding potential of -80 mV. 50 μ M RR is included in the recording electrode. (b) Current-voltage relationship of MA currents expressed in mpiezo1-transfected HEK293T cells when 50 μ M RR is included in the recording electrode. (Inset) MA currents evoked at holding potentials ranging from -80 to +80 mV. Cells were perfused for at least 5 minutes prior to recordings.

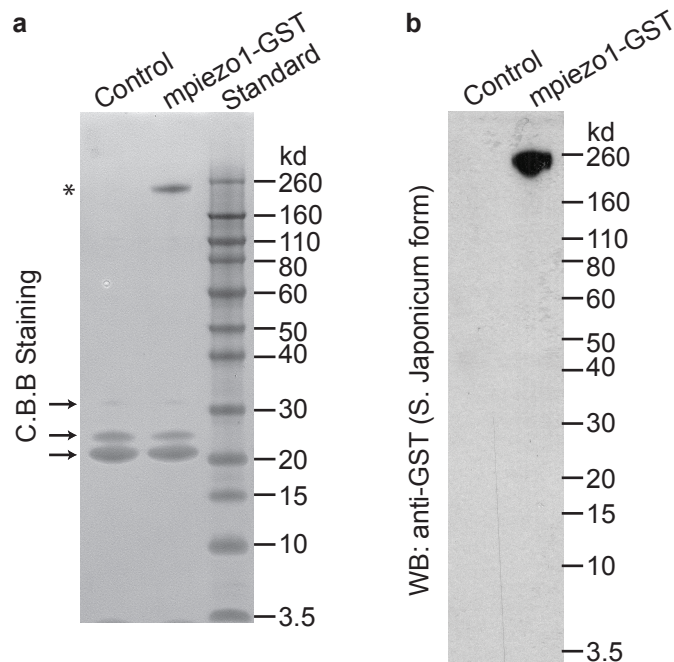
Supplementary Figure 3



Supplementary Figure 3. GFP-mpiezo1 and mpiezo1-GST overexpression induce MA currents in HEK293T cells.

Representative traces of MA inward currents in GFP-mpiezo1- (a) and mpiezo1-GST- (b) transfected HEK293T cells recorded at a holding potential of -80 mV.

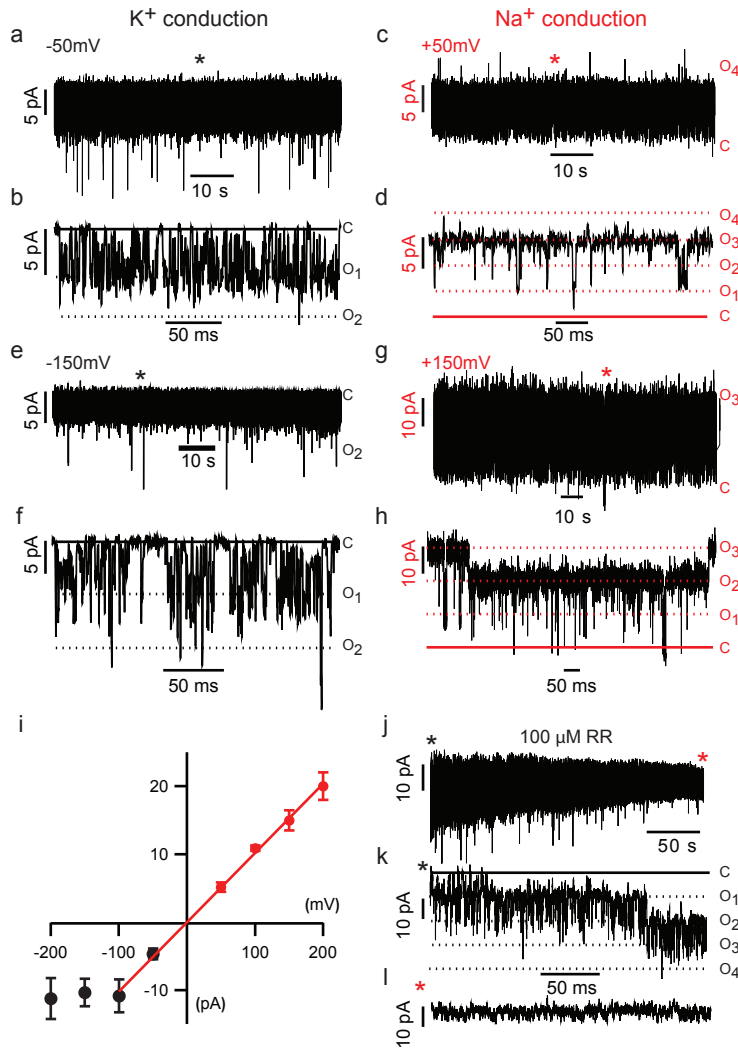
Supplementary Figure 4



Supplementary Figure 4. Characterization of samples purified from HEK293T cells without transfection (control) or transfected with mpiezo1-GST (mpiezo1-GST) on denaturing gel.

Control and mpiezo1-GST samples were separated on a 4-12% Bis-Tris denaturing gel and visualized with Coomassie blue staining (a) or western blotting with an anti-GST (*S. japonicum* form) antibody (b). The Novex sharp pre-stained protein standards were used for indicating the molecular weights of proteins on the gel (a). A protein band at the position of the 260 kDa protein marker specifically present in the mpiezo1-GST sample correspond to mpiezo1-GST proteins, as confirmed by western blotting with the anti-GST antibody (Supplementary Fig. 4b) or an antibody specifically against piezo1 (Fig. 4). The three lower bands indicated by arrows correspond to endogenous glutathione S-transferase pi1 (GSTP1), glutathione S-transferase mu 3 (GSTM3), and carbonyl reductase 1 (CBR1), as identified by mass spectrometry of the gel slices containing the respective bands (data not shown) or the whole purified control and mpiezo1-GST samples (Supplementary Table 2). These proteins were pulled down by the glutathione beads. It should be noted that the anti-GST (*S. japonicum* form) antibody does not recognize the endogenous human GST isoforms.

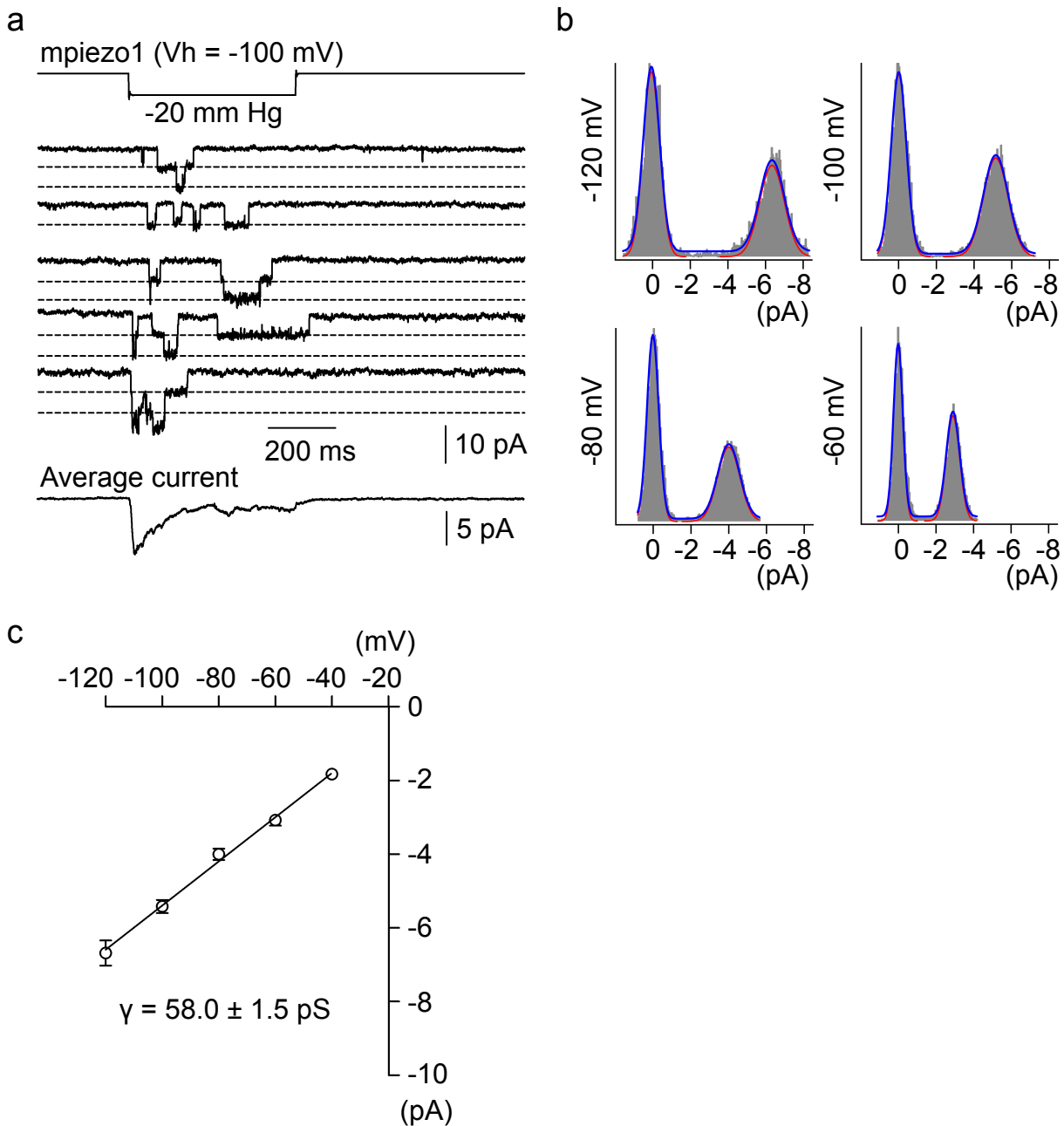
Supplementary Figure 5



Supplementary Figure 5. *mpiezo1* conduction under bi-ionic condition.

In the bi-ionic condition where the concentration of K^+ in the cytoplasm is 200 mM and the extracellular concentration of Na^+ is 200 mM, positive inward currents are carried by K^+ and negative outward currents are carried by Na^+ . For comparison, ~ 2 min. recordings of inward (a, e) and outward (c, g) currents from a single experiment are shown at ± 50 and ± 150 mV. Segments indicated by an asterisk are displayed at higher time resolution (~ 250 to 500 ms) below the long recording to highlight few defined transitions between closed and open (b, f and d, h). At $V=50$ mV and $V=-50$ mV, the single channel inward K^+ current is 4.7 ± 0.8 pA ($g=100 \pm 10$ pS) (b) and the outward Na^+ current is 5.2 ± 0.7 pA ($g=90 \pm 10$ pS) (d). The currents differ in that at -50 mV only 1 channel is carrying K^+ current while at $+50$ mV an average of 3 channels are passing Na^+ . The inward permeation of K^+ appears transient assessed from the frequent short incomplete closures (b). This contrasts to the long uninterrupted outward Na^+ currents observed at -50 mV. When the potential difference is increased to 150 mV (± 150 mV) the single channel inward K^+ current increases to only 10 ± 2 pA ($g=70 \pm 10$ pS) (b) whereas the outward Na^+ current increases to 15 ± 2 pA ($g=100 \pm 10$ pS) (d). At $+150$ mV, the Na^+ outward flux is stable as evidenced by long open times (g, h); at -150 mV, the transitions between open and closed states are faster than the time resolution of the recording and, accordingly, only a fraction of the single channel conductance is captured (e, f). This factor contributes significantly to the apparent saturation of K^+ inward current. A summary of the current-voltage relation for the channel under these bi-ionic conditions extracted from 204,088 events distributed through three experiments is presented in panel i. A fit of the data with a linear function shows that the single channel current is ohmic between -100 and 200 mV with a slope conductance of 102 ± 2 pS. The current reversed direction at 0.0 ± 0.3 mV indicating that the channel does not select between K^+ and Na^+ . To verify channel orientation, the experiment was terminated by the addition of RR ($100 \mu M$) to the bath (j, k, l). After an incubation time of 5 min at a resting potential of 0 mV, the membrane was hyperpolarized to -150 mV (j). A time expansion of the record at the pulse onset (k, black asterisk) shows that at least three channels were open and that these become progressively blocked. A time expansion of the record after ~ 4 minutes at -150 mV (l, red asterisk) indicates that the three channels are still active and that the single channel conductance has decreased to below measurable resolution.

Supplementary Figure 6



Supplementary Figure 6. Conductance characterization of mpiezo1-induced stretch-activated channels with divalent-free pipette solution.

(a) Representative stretch-activated channel openings elicited at -100 mV in mpiezo1-transfected cells in cell-attached configuration. The recording pipette was filled with a 150 mM NaCl solution. Bottom traces represent average of 40 individual recording traces. (b) All-point histograms of single channel opening events (average of 10 individual events) at holding potentials ranging from -120 to -60 mV. (c) Average current-voltage relationships of stretch activated single channels ($n = 7$ cells, respectively; mean \pm s.e.m.). Single channel amplitude was determined as the amplitude difference in Gaussian fits as shown in b. The slope conductance value (γ) is the average of slope conductance values determined for each cell (mean \pm s.e.m.).

Gel band containing control sample purified from either untransfected- (Exp1) or mock-transfected (Exp2) cells

| Protein hits | Gene information | Number of peptides identified in Exp 1 | Number of peptides identified in Exp 2 |
|---|---|--|--|
| gi 1346343 Keratin, type II cytoskeletal 1 (Cytokeratin-1) (CK-1) (Keratin-1) (K1) (67 kDa cytokeratin) (Hair alpha protein) | KRT1 gene | 6 | 5 |
| gi 28317 unnamed protein product [Homo sapiens] | Human gene for acidic (type I) cytokeratin 10 | 2 | 5 |
| gi 119572363 hCG22067 [Homo sapiens] | From Homo sapiens 211000035830252 genomic scaffold, whole genome shotgun sequence | 2 | 1 |
| gi 494066 Chain A, Three-Dimensional Structure Of Class Pi Glutathione S- Transferase From Human Placenta In Complex With S- Hexylglutathione At 2.8 Angstroms Resolution | GSTP1 gene | 2 | 0 |
| gi 37460 unnamed protein product [Homo sapiens] | pancreatic trypsinogen III | 1 | 1 |
| gi 64653281 CHRNA4 protein [Homo sapiens] | CHRNA4 | 1 | 0 |
| gi 5032087 splicing factor 3a, subunit 1, 120kDa isoform 1 [Homo sapiens] | splicing factor 3a, subunit 1, 120kDa | 0 | 2 |
| gi 55956899 keratin 9 [Homo sapiens] | KRT9 gene | 0 | 1 |
| gi 16751921 dermcidin preproprotein [Homo sapiens] | dermcidin | 0 | 1 |
| gi 547754 Keratin, type II cytoskeletal 2 epidermal (Cytokeratin-2e) (CK 2e) (K2e) (keratin-2) | KRT2 gene | 0 | 1 |

Gel band containing samples purified from mpiezo1-GST transfected cells

| Protein hits | Gene information | Number of peptides identified in Exp 1 | Number of peptides identified in Exp 2 |
|---|---|--|--|
| mpiezo1-GST | mPiezo1 | 32 (14% sequence coverage) | 81 (31% sequence coverage) |
| gi 1510143 KIAA0233 [Homo sapiens] (human piezo1) | hPiezo1, likely detected from sequence homology with mPiezo1 | 7 | 12 |
| gi 307086 keratin-10 | KRT10 gene | 2 | 0 |
| gi 386850 keratin K5 | KRT5 gene | 0 | 3 |
| gi 119581150 keratin 14 (epidermolysis bullosa simplex, Dowling-Meara, Koebner), isoform CRA_b [Homo sapiens] | KRT14 gene | 0 | 2 |
| gi 6650826 PRO2044 [Homo sapiens] | Serum albumin | 1 | 0 |
| gi 6735452 B-ind1 protein [Homo sapiens] | protein tyrosine phosphatase-like A domain containing 1 | 0 | 1 |
| gi 2224907 15S-lipoxygenase [Homo sapiens] | arachidonate 15-lipoxygenase, type B | 0 | 1 |
| gi 119595160 fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2) oncogene homolog) | fibroblast growth factor 3 | 0 | 1 |
| gi 1457948 APEG-1 protein | SPEG complex locus | 0 | 1 |
| gi 1346343 Keratin, type II cytoskeletal 1 (Cytokeratin-1) (CK-1) (Keratin-1) (K1) (67 kDa cytokeratin) (Hair alpha protein) | KRT1 | 6 | 14 |
| gi 453155 keratin 9 [Homo sapiens] | KRT9 gene | 4 | 11 |
| gi 547754 Keratin, type II cytoskeletal 2 epidermal (Cytokeratin-2e) (CK 2e) (K2e) (keratin-2) | KRT2 gene | 3 | 7 |
| gi 494066 Chain A, Three-Dimensional Structure Of Class Pi Glutathione S- Transferase From Human Placenta In Complex With S- Hexylglutathione At 2.8 Angstroms Resolution | GSTP1 | 2 | 0 |
| gi 119572363 hCG22067 [Homo sapiens] | From Homo sapiens 211000035830252 genomic scaffold, whole genome shotgun sequence | 1 | 1 |
| gi 28317 unnamed protein product [Homo sapiens] | Human gene for acidic (type I) cytokeratin 10 | 2 | 11 |
| gi 37460 unnamed protein product [Homo sapiens] | pancreatic trypsinogen III | 0 | 1 |

Supplementary Table 1. Mass spectrometry results of gel band containing samples purified from either control HEK293T cells (untransfected- or mock transfected-cells) or mpiezo1-GST transfected-cells.

Keratin proteins detected in both samples were likely derived from skin contamination during sample preparation. Among the proteins only detected in the mpiezo1-GST sample (highlighted in bold in the lower table), only piezo1 proteins were consistently identified with numerous peptides detected from two independent experiments.

| Control sample purified from untransfected HEK293T cells | | | |
|---|--|--|--|
| Protein Hits | Gene Information | Number of Peptides Identified in Exp 1 (purified from whole cell lysate) | Number of Peptides Identified in Exp 2 (purified from membrane fraction) |
| gi 2554831 Chain A, Crystal Structure Of Human Glutathione S-Transferase P1- 1[v104] Complexed (9r,10r)-9-(S-Glutathionyl)-10- Hydroxy-9,10-Dihydrophenanthrene | glutathione S-transferase pi 1 (GSTP1) | 12 | 7 |
| gi 14250650 Glutathione S-transferase M3 (brain) [Homo sapiens] | glutathione S-transferase mu 3 (brain) (GSTM3) | 6 | |
| gi 157929330 glutathione S-transferase M2 (muscle) [Homo sapiens] | glutathione S-transferase mu 2 (muscle) | 3 | |
| gi 4502599 carbonyl reductase 1 [Homo sapiens] | carbonyl reductase 1 (CBR1) | 1 | 1 |
| gi 10835838 Chain L, Crystal Structure Of The Fab Fragment Of The Monoclonal Antibody Mak33 | | 1 | |
| gi 2345030 Ig kappa light chain variable region [Homo sapiens] | | 1 | |
| gi 446244 anti-GlcNAc antibody variable region:SUBUNIT=heavy chain | | 1 | |
| gi 49354807 immunoglobulin E variable region [Homo sapiens] | | 1 | |
| gi 1346343 Keratin, type II cytoskeletal 1 (Cytokeratin-1) (CK-1) (Keratin-1) | Keratin 1 | | 3 |
| gi 453155 Keratin 9 | Keratin 9 | | 1 |
| gi 5031931 nascent polypeptide-associated complex alpha subunit isoform b [Homo sapiens] | nascent polypeptide-associated complex alpha subunitprovided | | 1 |

| mpiezo1-GST samples purified from mpiezo1-GST transfected HEK293T Cells | | | |
|---|--|--|--|
| Protein Hits | Gene Information | Number of Peptides Identified in Exp 1 (purified from whole cell lysate) | Number of Peptides Identified in Exp 2 (purified from membrane fraction) |
| mpiezo1-GST | mpiezo1 | 4 (2% sequence coverage) | 8 (4% sequence coverage) |
| hCG1980844, isoform CRA_i [Homo sapiens] | hpiezo1 | | 1 |
| gi 2554831 Chain A, Crystal Structure Of Human Glutathione S-Transferase P1- 1[v104] Complexed (9r,10r)-9-(S-Glutathionyl)-10- Hydroxy-9,10-Dihydrophenanthrene | Glutathione S-transferase pi (GSTP1) | 12 | 3 |
| gi 14250650 Glutathione S-transferase M3 (brain) [Homo sapiens] | glutathione S-transferase mu 3 (brain) (GSTM3) | 2 | |
| gi 4502599 Carbonyl reductase 1 [Homo sapiens] | carbonyl reductase 1 (CBR1) | 2 | |
| gi 494186 Chain A, Crystal Structure Of Human Class Mu Glutathione Transferase Gstm2-2: Effects Of Lattice Packing On Conformational Heterogeneity | glutathione S-transferase mu 2 (muscle) (GSTM2) | 2 | |

Supplementary Table 2. Mass spectrometry results of solution samples purified from either control untransfected HEK293T cells or mpiezo1-GST transfected cells.

Control and mpiezo1-GST samples purified from either whole cell lysates (Experiment 1) or membrane fractions (Experiment 2). GSTP1, GSTM3, CBR1 proteins were consistently identified in both control and mpiezo1-GST samples, corresponding to the three lower protein bands present in both control and mpiezo1-GST sample (Supplementary Fig. 4a). mpiezo1-GST proteins were consistently identified only in the mpiezo1-GST sample. Reconstitution of the same set of samples used for experiment 2 into lipid bilayers resulted in recordings of channel activity with mpiezo1-GST but not control sample, suggesting that the observed channel activity is specifically mediated by mpiezo1-GST proteins.

| | |
|---------------------------------|---------------|
| Conductance (pS) | 118 ± 15 |
| Open Occupancy | 0.43 ± 0.14 |
| Closed occupancy | 0.57 ± 0.15 |
| τ_{open} (ms) | 13 ± 4 |
| $\tau_{1\text{closed}}$ (ms) | 5 ± 1 (0.74) |
| $\tau_{2\text{closed}}$ (ms) | 47 ± 9 (0.26) |
| Total number of openings | 19,583 |

Supplementary Table 3. Single channel properties of mpiezo1 in lipid bilayers.

Single channel current recordings, in which only one channel was open at any given time, were analyzed to determine the channel dwell times in the open and closed states (references 1 and 2). Probability density analysis provides the number of open and closed states and the channel open (τ_o) and closed (τ_c) lifetimes (refs 1 and 2). Histograms were normalized to have a total area equal to one. The data summarized in the Table provide evidence for the occurrence of a single open state and two closed states under the experimental conditions (0.5 M KCl and V=-100 mV). For τ_{c1} and τ_{c2} , the relative area under the fitted curve is shown in brackets and represents the frequency of occurrence of each state. The results are cumulative from three different experiments; error indicates s.e.m.

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