

Supplementary information

PIEZO ion channel is required for root mechanotransduction in *Arabidopsis thaliana*

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S.A.R.M., A.E.D. and A.P. designed research; S.A.R.M., A.E.D., W.Z.Z., A.M.C., K.D., D.A.G., W.T.K., C.G., and Y.Z., performed research; S.A.R.M., A.E.D. and A.P. analyzed data; and S.A.R.M., A.E.D. and A.P. wrote the paper.

Competing interests

The authors declare no competing financial interests.

Keywords: PZO1, PIEZO, mechanosensation, ion channel, *Arabidopsis*, Ca²⁺ imaging.

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Movies S1 to S4

Table S1. Electrophysiological characterization of mPiezo1 and mPiezo1/ PZO1 chimera.

SAC Parameter	mPiezo1	mPiezo1/PZO1
I_{\max} (pA)	-28 ± 8 (n=16)	-25 ± 14 (n=8)
Threshold (mmHg)	-31 ± 6 (n=16)	-31 ± 7 (n=8)
P_{50} (mmHg)	-47 ± 7 (n=15)	-61 ± 6 (n=8)
V_{rev} of current in cell-attached patch (mV) ^a	0.4 ± 2.3 (n=6)	0.7 ± 1.3 (n=2)
Inactivation rate, tau (ms)	67 ± 13 (n=15)	>250 ms (n=8) ***
Percent of peak current at 250 ms (%)	23 ± 6 (n=16)	92 ± 4 (n=8) ***
^a V_{pipette} at which SAC reversed under cell-attached patch recording conditions used here to increase intracellular K^+ ; the pipette solution (extracellular) contained high Na^+ (see Methods).		

Table S2. List of the sequence of the primers used to generate myc tags.

primer name	direction	Sequence
Myc tag1	forward	gaacaaaaacttatttctgaagaagatctgctgctgaccagcctggctgctc
Myc tag1	reverse	cagatcttctcagaaataagttttgttcctgcagagccagaaagtacatg
Myc tag2	forward	gaacaaaaacttatttctgaagaagatctgctgtacttctatctgggatataac
Myc tag2	reverse	cagatcttctcagaaataagttttgttcgctgataaatccgctcagcc
Myc tag3	forward	cagatcttctcagaaataagttttgtcccttcatacttgacggctc
Myc tag3	reverse	gaacaaaaacttatttctgaagaagatctgagcgtcgacccctggatctg
Myc tag4	forward	taccgaacaaaaacttatttctgaagaagatctgcagcaacacgctgctggcttgg
Myc tag4	reverse	ctgcagatcttctcagaaataagttttgttcggtaggatatatgctccaagc
Myc tag5	forward	aatgaaagaacaaaaacttatttctgaagaagatctgcaaggcgaggcaacgagtaactc
Myc tag5	reverse	gccttgcatcttctcagaaataagttttgttccttcattgatatagaagcatgatc

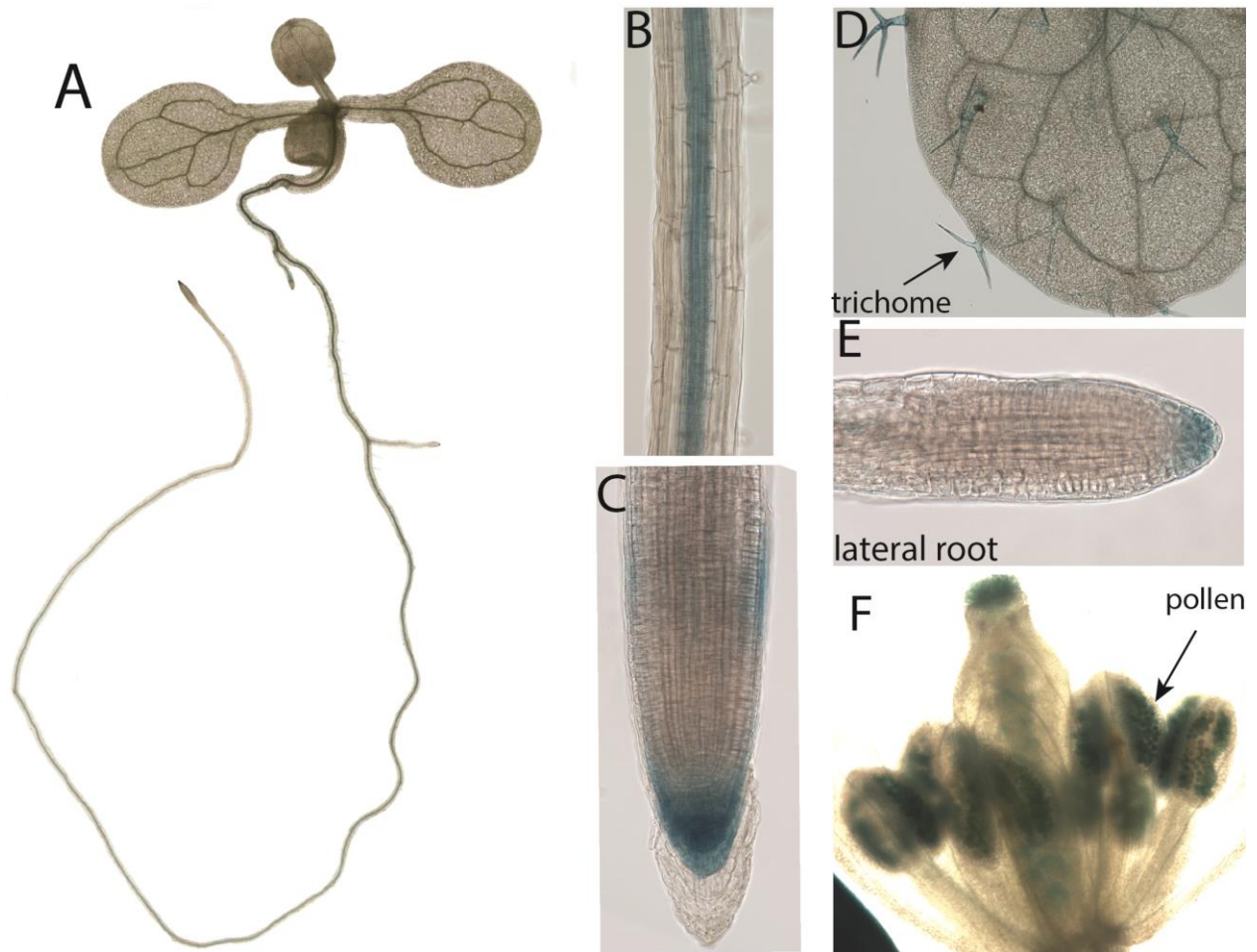


Figure S1. Representative images of the expression pattern of *PZO1* promoter activities in a *PZO1::GUSPlus* reporter line. **A**, The expression pattern of *PZO1* promoter activity in the transgenic reporter line with the 823 bp *PZO1* promoter (*PZO1* (*short*)::*GUSPlus*). **B and C**, The expression pattern in the upper root and root tip in the line with the 823 bp *PZO1* promoter. Note that the expression pattern of the *PZO1* in the lines short and long promoter (Fig.1) are similar. **D-F**, The expression pattern of *PZO1* in the line with 2000 bp *PZO1* promoter (*PZO1* (*long*)::*GUSPlus*) in the trichome of the leaf, lateral root cap and pollen.

PZO1	-----MASFLVGFLLPSL LLAALINWSVISFLDLIAFL LVHYIAPEIGYRFQRRHWLLW	55
mPiezo1	MEPHVLGAGLYWLLLPCT LLAASLRFNALSLVYLLFLL LLPWLPGP-----	47
	: : * : * * . * * * : : * : * * : :	
	TM2 Myc1	
PZO1	PIFIFSAVFLAQVVYLV WAALGQDWDTPDTGWMRVIGFMILKSWRN TVMYFLAQLL	115
mPiezo1	-----S-----	48
	.	
	TM3	
PZO1	TSLVALADI SSRFGFARWRDTWWSHFGIFEHLGSHLRVASCLL PAVQLAVGICNP	173
mPiezo1	-----RHSIPGHTGRLLRALLCLSLFLVAHLAFQICLH	82
	. * * * * . * * * . : * * . * *	
PZO1	SWVSLPFFITGSCAGLVDSLT SNVSGLFRW WRVLYIYAGFNIVLLYLYQLP INFSDMIRW	233
mPiezo1	TVPHLDQFLGQNGSL WVKVSHIGVTRL DLKDIFN -----TTRL	121
	: * * * . . * * . * : * * : * * * *	
	TM4	
PZO1	IASFIGLFRISLET EGPDICSGFLVLYIMLSYV RSDLEDMDF -----IMSTSENN	285
mPiezo1	VAPDLGVLL -----ASSLCLGLCGRRLTRKARQSRRTQELDDDDDDDDDEDI DAAPAVG	175
	: * : * : . . * * * * . . * * * * * * : : .	
	TM5	
PZO1	L-AERLLPPKYSFFI RESRAGVRHTNVLLRGAVFKTFSI -----NFFTYGF-PVSLF	335
mPiezo1	LKGAPALATKRRLWLASRFVTAHWLLMTSGRTLVIIVLLALAGIAHPSAFSSVYLVVFLA	235
	* . * * * : : . * * : * : . : . * : * *	
	TM6 TM7	
PZO1	ALSFWSFHFA -----SICAFGLLAYVGYI --IYAFPSLFQLHRLNGLLVFILLWAVS	386
mPiezo1	ICTWWSCHFLPLSGFNLCVMVSCFGAGHLICLYCYQTPFIQDMLPP-GNIWARLFLGK	294
	: * * * * * : * : . * : : * : : * * * : * : : *	
	TM8	
PZO1	TYIFNVAFSFLN TKVKGKMQI WEMVGLWHYTIPGFFLLAQFGLGMLVALGNLVNNSVFLY	446
mPiezo1	NFVDLPNYS SPNALV LNTKHAWP -----TYVSPGILLLLY TATSLLKLLHKSCPSE -----	345
	: : : * * : * : * * * * : * * : * : * : *	
	TM9	
PZO1	LSEESSRSSNERSY ---VEADE ---ETKVLVATI AWGLRKCRAIMLA -----	489
mPiezo1	LRKETPREDEE HELELDHLEPEP QARDATQ GEMPMTTEP DLNCTVHVLT SQSPVRQRFV	405
	* : * : * . : * : . * : : * * * : * : : *	
	TM10	
PZO1	---LIFLIAM -----KPGFFHA -----VYVIFFLMYLLSHNINRKIR	523
mPiezo1	RPRLAELKEMS PLHGLGHLIM DQSYVCALIAM MVWSIMYHSW LTFVLLWACL IWTVRSR	465
	* * * . . : * * : : * * : * : * *	
PZO1	KSLILLCEVHFAL -----LYILEIDL VSNSLKQEGSASRE VLFQLGLLRSESSWDFI	575
mPiezo1	HQLAMLCSP CILLYGLTL CLRLYVAMELPELP -----TTLGPVSLH QLGLEHTR --YPC	519
	: * : * * : * * : * : * * : * * * * : : *	
	Chimera6 TM11 Chimera7	
PZO1	EIAL ACFC AIHNHGFEV LFSFAIVRHT PSPPIGFSILKAGLNK SVLLSVYSSPSSSYS	635
mPiezo1	DLGAM -----LLYLLTFWLLR -----QFVK EKLLKKQKVPAALE	555
	: : : * : * : * : * * : * * : * : * * : * : *	
	TM12 TM13	
PZO1	QD-NTTYERHIAS FLSAIGQKFLS MYRSCGTYIAFITILISVYLVKPNYVSFGYIFLLLL	694
mPiezo1	VTVADTEPTQT QTLRLSLGELVTGIYV KYWYVYV CAGMFI VVSEAGRLVYKIVMFLFL	615
	* : * : * * : * : * * . * : : : * : * * * * *	
	TM14 Myc2	
PZO1	WI---TGRQLFEETKRRLWFPLKAYAVLVFMFIYCLSSFVSLQLW--LSGFI DLYFYLG	748
mPiezo1	CLTLFQVY YTLWRKLLRVFWL VVAYATMLVLI AVYTFQFQDFP TYWRNL TGF DEQL--G	673
	: . * : : * * * : * * * * : * * * * * * * * * * * * * * * * *	
	TM15	
PZO1	YNSKAPLLDNVWESLAVLIVMQLYSYERRQSGHYIPGQSSLLHPGVFGF-----FE-	799
mPiezo1	-----DLGLE -----QFSVSEL -----FSSILIPGF LLACILQLHYFHR	708
	* * * : * . * * * * * * : *	
	TM16	
PZO1	-----RFLAWHGQKIL-FAALFYASLSPISVFGF--VYLLGLVIC TTFP	840
mPiezo1	PFMQLTDLEHVPPPGTRHRPRAHQRDAVSEAPLEHQE EEEVFREDGQSM DGPQATQVP	768
	* . * : : * : . * * . * * : * * . * * *	
	TM17	
PZO1	KSSSIPSKSFLIYTGFLVSAEYLFQLWGMQAQMFPGQK-----YAELSF	884
mPiezo1	-----EGTASKWGLVADRLLDLAASFSAVLTRIQV FVRRLLELHV	808
	* * * * * : : . * * *	
	TM18 TM19	
PZO1	YLGLRVYEPGFWGIESGL--RG-KVLVVA ACTLQYNVFRWLER TSGLTVIKGKYEPCPL	941
mPiezo1	EKLVALY--TVVVALKEV SVMNLLLV WAFALPYPRFRPMAS--CLSTV WTCIIIVCKM	864
	: : * * . * : . : : * * * * * * : * : : * : *	

		Myc5	
PZO1	FVSAEDTTASVSSSNGEN--PSSTDHASIC-----MKQGEATSNSWPFPSPRGNQGAGFL		994
mPiezo1	LYQLKIVNPHEYSSNCTEPPFNNTNLQPLEIN ^R SLLYRGFPVDPANWFG-VRKGYPNLGYI		923
	: * * * : * . * : . . : : * . . * : * . * : :		
	TM20		
PZO1	HPKTGGSESGSSRRKFSFGHFWSGI-KESHRWNRRR---I-----LALKKERFETQKNLLK		1045
mPiezo1	QNHLLQI-----L LLLVFEAVVYRRQEHYRRQHQQAPLPAQAVCADGTRQRLD-QDLL		974
	: : : : * . . : : : : : : : : * : : . . * . . : : *		
	TM21 TM22		
PZO1	IYLFKWIENMFNLYGLEINMIALLLASFALLNAISMVYIALLAACV-----LLRRRVIQ		1099
mPiezo1	SCLKYFINFFYKFGLEICFLMAVNV-----IGQRMNFMVILHGCWLVAILTRRRREAIA		1029
	* * : * : * : * * * : : . . : . * : : * : * * * * * * * * *		
	TM23		
PZO1	KLWPVVVFLFASIL-----AIEYVATWNSFLPSDQAPSETSVHCHDCWSIA		1145
mPiezo1	RLWPNYCLFLLTFLLYQYLLCLGMPALCIDYPWRWSKAI PMNSALI-----KWLYL		1081
	: * * * : : : : * : * * * * * * * * * * * * * * * * *		
	TM24		
PZO1	ALYFKFCRECLVGRVDDPRTLISYFVVFMLACFKLRADHISSFSSESTYHQMKSQRKNS		1205
mPiezo1	PDFD-----RAPNSTNLISDFLLLL--CASQQQVVF-SAERTEEWQRMAGINTDH		1128
	: * : * : * * * * * : : * : : : * : : : * : : : * : : : * : : *		
	Tm25 Chimera1 (mPiezo1/PZO1)		
PZO1	FVWRDLSFETK-----SMWTVLDYLRLCYVHLLDVVLIILILITGTLEYDILHLGYL ^R AF		1259
mPiezo1	L--EPLRGENPFI PNFIHCRSYLDMLKVAVFRYLFWLVLVVVFVAGATRISIFGLGYL ^R LA		1186
	: . * * * : : * * * : : * * : : * * : * * : : * * : * * : * * * * *		
	TM26 TM27		
PZO1	ALVFARMRLIEILKKNK---IFRFLRVYNFVLIIFSL---AYQSPFVGNFNDGKCETVD		1312
mPiezo1	CFYLLLFGTLLQKDRALQVLWDCILLYNVTVIISKNLSLLSCVFVEQMOSNFCWVIQ		1246
	: : : : * * : * : : *		
	TM28		
PZO1	---YIYEVIGFYKYDYG-----FRITARSALVEIIIFMLVSLQSYMFSSEQEFDYVSR		1361
mPiezo1	LFSLVCTVKGYDPEKEMMTRDRDCLLPVEEAGI I WDSICFFLLQRRIFLSHYFLHVA		1306
	: * * * . . : : : : * * : : * * : * * : * * : * * : * * * * *		
	TM29		
PZO1	YLEAEQIGAI-----VREQEKAARKTEQLQQIREAEKKRQRNLQVE		1404
mPiezo1	DLKATALQASRGFALYNAANLKSINFHRQIEEKSLAQLKRMKRI RAKQEKYRQSQASRG		1366
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *		
	TM30 Chimera2		
PZO1	KMKSEMNLRVQLHRMNSDSNFGVASPRTEGLRRRKSPYLI PDSGAASPEIDGVVHRKEE		1464
mPiezo1	QLQSKDP--QDPSQEPGPDSPGGSSPPRRQWV---RPWLDHA-----TVIHSGDY		1411
	: : * : : : . . * * * : * * : * * : * * : * * : * * : * * : *		
	TM31		
PZO1	QPIDEDSQYPFEAHEFPVSTTPEALDSPEYSFGASPCFI-TEVQQDLVMSMERERKQKS		1523
mPiezo1	FLFESDSE-----EEEEALPEDPRPAAQSAFQMAVQAWVTNAQTVLRQRERARQERAEQLAS		1469
	: : * * : * . : * . * * . : : : * : * : * : * : * * : * * : *		
	TM32		
PZO1	EGKENPLISAVQL-----		1536
mPiezo1	GGDLNPDVEFVDVPEDEMAGRSHMQRVLSTMQFLWVLGQATVDGLTRWLRAFTKHHRTM		1529
	* . * * . . * : :		
	TM33		
PZO1	-----IGDVSQVQFIGNQAVNN-----		1554
mPiezo1	SDVLC AERYLLTQELLRVGEVRRGVLDQLYVGEDEATLSGPMETRDGPSTASSGLGAEEP		1589
	: * * : : * : : . .		
	TM34		
PZO1	LVNFL--NISPENSDTNEQSSVDDEVYDEME-----SQKRKHT--PF		1592
mPiezo1	LSSMTDDTSSPLSTGYNTRSGSEEIVTDAGDLQAGTSLHGSQELLANARTMRMTASELLL		1649
	* . . : * * . . * * . : * * : : * * : : * . : :		
	TM35		
PZO1	ERSTSLQSDRSSDGTSTFQIGR---IFRHIWSRMQSNNDIVCYCCFIIAFLWNFSLLSMVY		1649
mPiezo1	DRRLHIPELEEAERFEAQGRTRLRLRAGYQCVAAHSELLCYFIIILNHMVTASAASLVL		1709
	: : . . : : . * * * : * * : : * * : * * : * * : * * : * * : * * *		
	TM36 Chimera2		
PZO1	LAALFLYALCVHTGPTHIFW ^R IVIMLYTEIYIILLQYLYQIIIQHCGLSIDAPLL-HELGF		1708
mPiezo1	PVLVFLWAMLTIPRPSKRFW ^R MTAIVFTEVMVVTKYLFQFGFFPWNSVYVLRRYENKPYFP		1769
	. : * * * : . * * * : * * : : * * : * * : * * : * * : * * : * * *		
	TM37		
PZO1	TQRIK---SSFVSSLPFLFIYIFTLIQSSITVKDGDWVPSADFTSRRNARGSQKDLTR		1764
mPiezo1	PRILGLEKTD SYIKYDLVQLMAL---FFHRSQLLCYGLWDHEEDRYPKDHCSSVKDREA		1826
	: : : . * : : * : : * : * * . * : : * * * * *		
	TM38		
PZO1	IRLSQRILDVFKKLRDSAKLVIRSIYRYWISLTRGAESPYPYFVQVTMDVHMMWPEDGIQPE		1824
mPiezo1	KEEPEA-----KLESQS-----ETGTGHPKEPVLAGT-----PRDHIQKG		1861
	. : : * * : * . * * * . . * * * * :		

PZO1	RVECRMNQLLRVHNERCEKGNPDLCPYSSRVHVQSIERSTETPNEA-LVVLEVEYASPT	1883
mPiezo1	GS-IR-----SKDVIQDPPEDLKRHRHISIRFRRRKETPGPKGTAVMTEHEE-G * : : * * * : * : * . * * . * : * * : *	1911
PZO1	NGCSSAEWYKSL--TPASDVAKIEI----RKAQHSGLGEGTGFPYPILSV-----IGGGKR	1932
mPiezo1	EGKETTERKRPRHTQEKSKFRERMKAGRRLLQSFVSLAQSFYQPLQRFHFDILHTKYRA : * . : * * : * : : * : * : * : * : * : * : * : * : * : * : *	1971
	Chimera3 TM33 TM34	
PZO1	D TDLYAYIFGADLIVFFLVAI-FYQSVIKNKSEFIDVYQLEDQFPDFVVIILMVIFFLIV	1991
mPiezo1	A TDVYALMFLADIVDIIIIIFGFWAFGKHSAAATDIASSLSDQVPAFLFMLLVQFGTMV *	2031
	M35 Myc4 M36	
PZO1	VDRVIYLCSEFATGKVYVYLFSLILFTYAVTEYAWSIYPTQ---QHAAGLALRIIFLAKAM	2048
mPiezo1	IDRALYLRKTVLGLKLAQVVLVVA---IHIWMFFILPAVTE MFSONAVAQLWYFVKCI : *	2087
PZO1	SLALQAIQIRYGLPHKSTLYRQFLTSEVSRINYYGYRLYRALPFLYELRCVLDW SCTATS	2108
mPiezo1	YFALSAYQIRCGYPTR--ILGNFLTCKYHNLNLFQGFRLV PFLVELRAVMDWVWTDTT : *	2145
	TM37	
PZO1	LTMYDWLKLLEDVNASLYLVKCDTVLNR-THKHGEKQTKMTCCKNGICLFFIILCVIWA	2167
mPiezo1	LSLSNWMCVEDIYANIFIICKSRETEKYPQPKGQKQKIVKYGMGLIILFLIAI I WFP * : : *	2205
PZO1	MLMYSSGNP-TNIANPIKDASVQIDLKTVGGKLTLYQTT-----LCKERISGDN	2214
mPiezo1	LLFMSLIRSVVGVVNPIDVTVTLK---LGGYELPFTMSAQQPSIVPFTPAAYEELSQQ- : *	2261
PZO1	IDLGLDLGSQSFLPTYNKNDIQLICCCQADASVVLVLPDVTVVTRFIQS-LDWDTDMDITFT	2273
mPiezo1	--FDPYPLAMQFISQSPEDIVTAQIEGSSGALWRISPPSRAQMKQELYNGTADITLRFT : . : *	2319
	Myc3	
PZO1	WVLNRDRPKGKETVKYER SVDPLDLPKRSD---IQMVLNG-SMDGFRVHNLYPKFFRVT	2328
mPiezo1	WNFQRDLAKGGT-VEY NEKHTLELAPNSTARRQLAQLLEGRPDQSVVIPHLPKYIRAP * : *	2378
PZO1	GSGDVRSFEDQTDDEV-----SADILINHANFKWWSFHNLKASE	2367
mPiezo1	NGPEANPVKQLQPDEEEDYLGVRILRREQVGTGASGEQAGTKASDFLEWWVIELQDCK- .. : : : *	2437
	TM38	
PZO1	NISACEGMDGPVAIIMSEET-PPQGLGDTLSKFSIWGLYITFVLAVGRFIRLQCSDLRM	2426
mPiezo1	--ADCNL--LPMVIFSDKVSPPSLGF---LAGYGIVGLYVSIVLVVVGKFKVIRGFFSEISH : *	2489
PZO1	RIPYENLPSCDRLIAICEDLYAARAEGELGVVEEVLYWTLVKIYRSPHMLLEYTKLDYDA	2485
mPiezo1	SIMFEELPCVDRIILKLCQDIFLVRETRELEEEELYAKLIFLYRSPETMIKWTRERE-- * : *	2546

Figure S2. Alignment between PZO1 and mouse Piezo1 highlights the residues of interest. A multiple sequence alignment between mPiezo1 and PZO1 was generated using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The transmembrane topology prediction for TM1-TM14 was obtained using on TOPCONS software (<http://topcons.cbr.su.se/>) and the topology from TM15 to TM38 was derived from the structure of mPiezo1(1). Residues highlighted in grey indicate the transmembrane domains. Residues in pink are transmembrane domains predicted to be only in PZO1, but not mPiezo1. Note that there is higher homology between mPiezo1 and PZO1 in the regions where the structure of mPiezo1 is resolved. Residues highlighted in green indicate the junction between mPiezo1/ and PZO1 in the chimeras. Chimera 1 was used for electrophysiological recording. Residues highlighted in red indicate the position of the Myc tag on mPiezo1(2). Residues highlighted in yellow, indicate the position of the Myc tag on PZO1. The PFEW motif highlighted in blue is conserved among plants, mammals and protozoa (3).

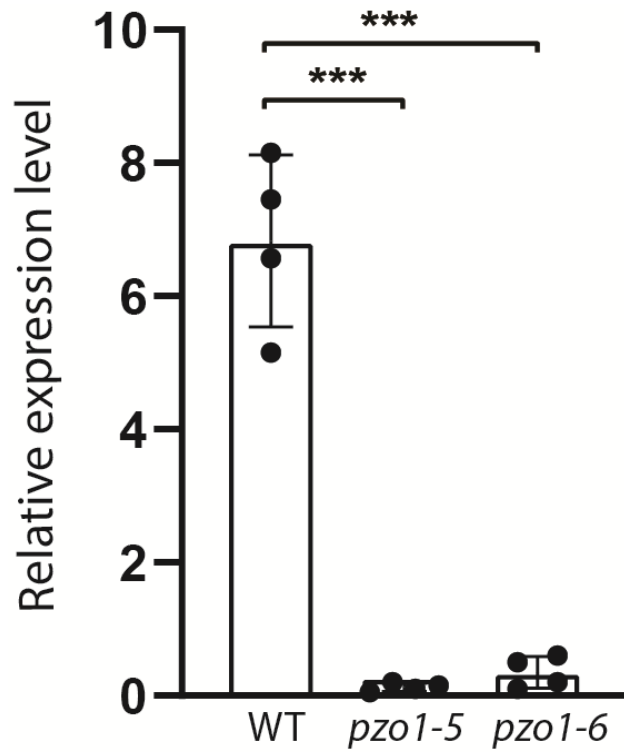


Figure S3. *PZO1* transcript level in roots of WT and mutant plants. RT-qPCR was performed on samples harvested from whole seedlings (root and leaf) from 4 different plants. *** $P < 0.001$, $N=4$ (mean \pm SD).

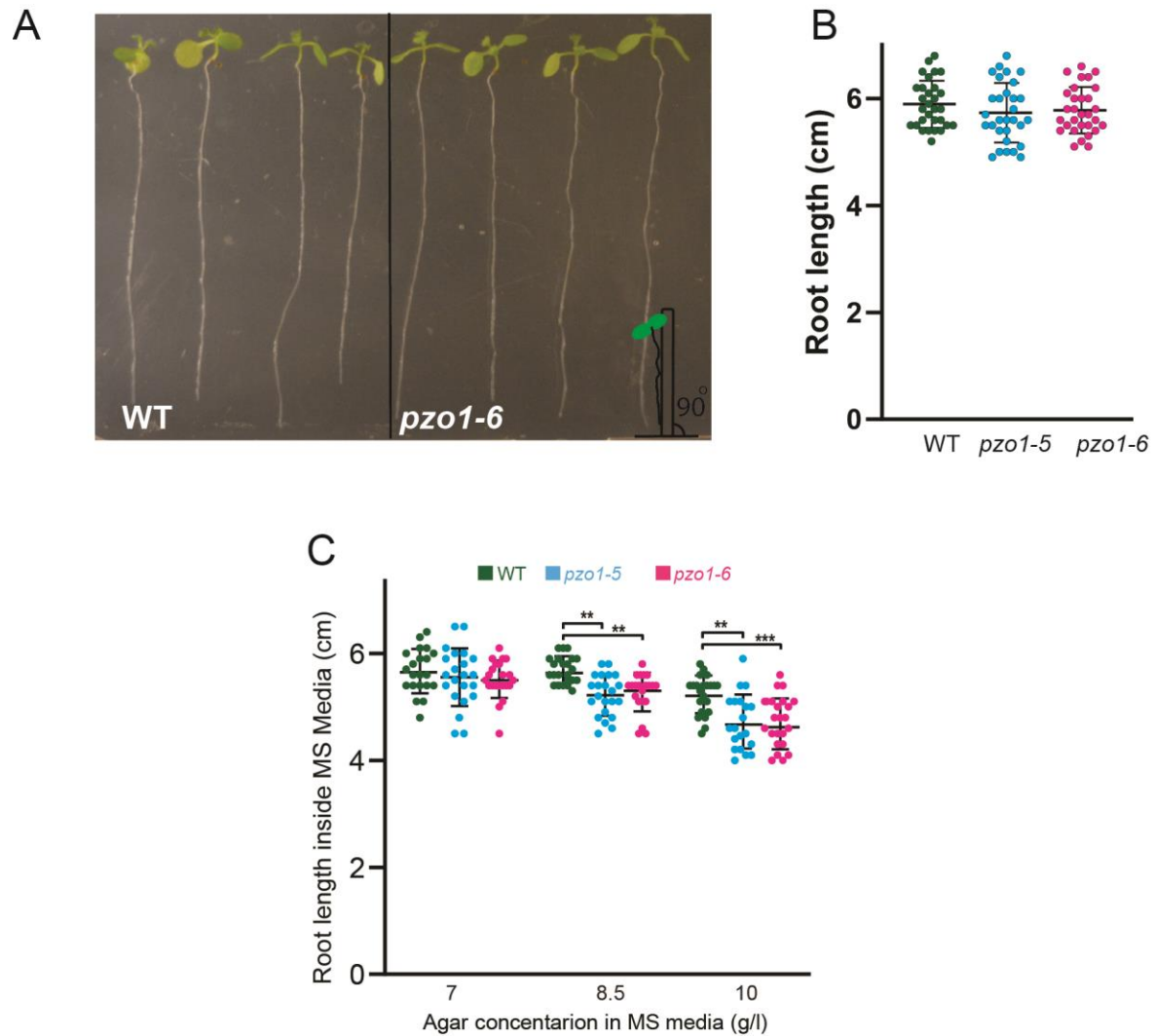


Figure S4. Root length of *pzo1* mutants. **A**, Representative image indicating the root lengths of *pzo1* mutants when grown on top of MS media in plates tilted at a 90° angle. **B**, Root length of WT and both *pzo1* mutants *pzo1-5* and *pzo1-6* (n=30). **C**, Root length of *pzo1* mutants when grown inside the MS media containing the indicated agar concentrations in plates positioned at a 60° angle. Data shown are for roots growing inside the MS media. *** $P < 0.001$ (N=23, mean±SD).

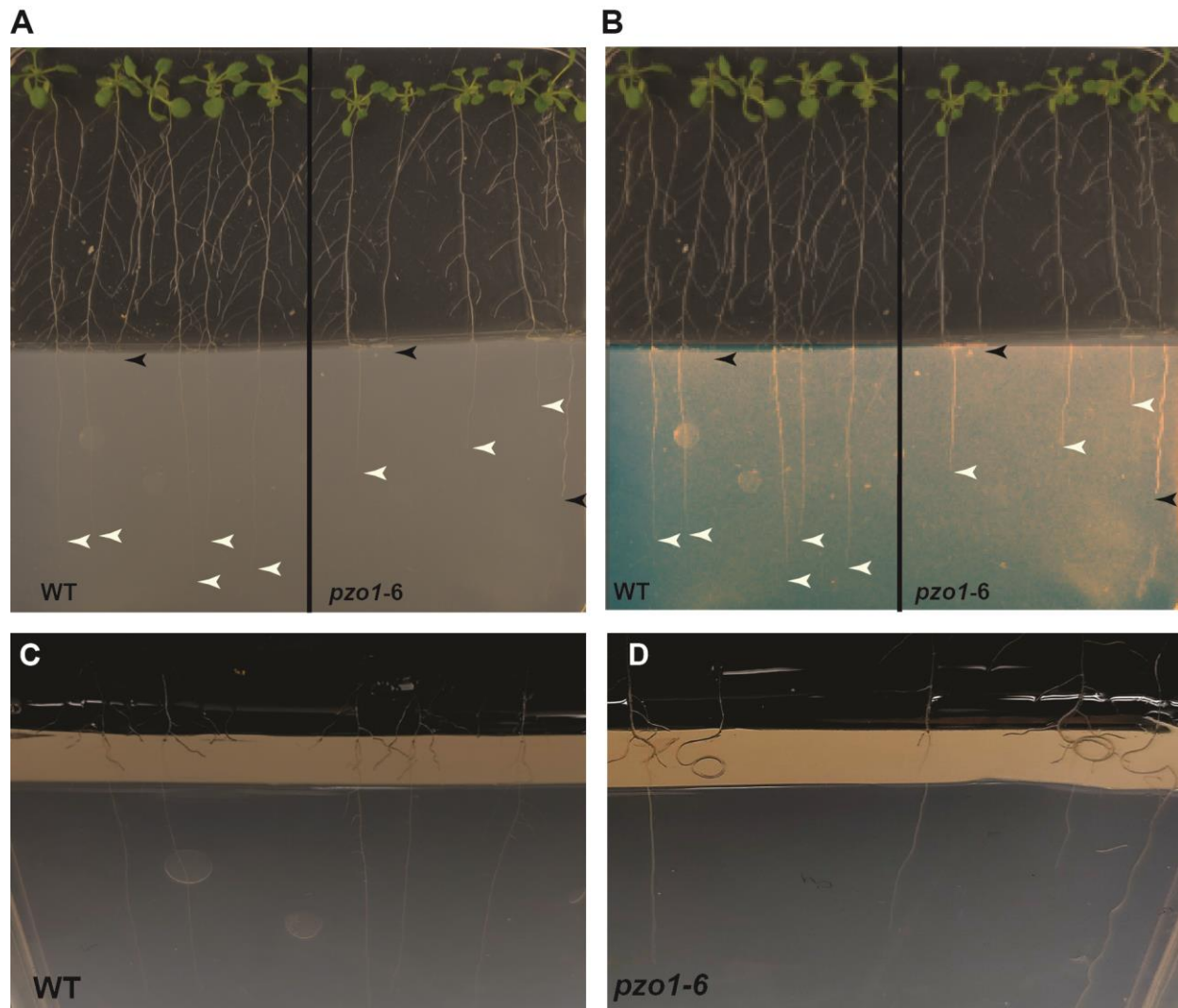


Figure S5. *Pzo1* mutants are defective at penetrating a hard barrier. **A**, Original image of the plant root that was challenged by barriers from Fig. 2e. **B**, Same image with adjusted light exposure for better visibility of roots grown inside the MS media. Black arrowheads indicate roots within the MS media or at the barrier interface, white arrowheads indicate root growth on the surface of MS media. **C** and **D**, The barrier is imaged at an angle that enables visualization of the root tips at the 5 mm wide barrier (brown; same data shown in panel A). **C**, Usually WT roots are observed to grow fairly straight through the harder barrier. **D**, Some of *pzo1-5* roots are observed to also form swirls at the barrier.

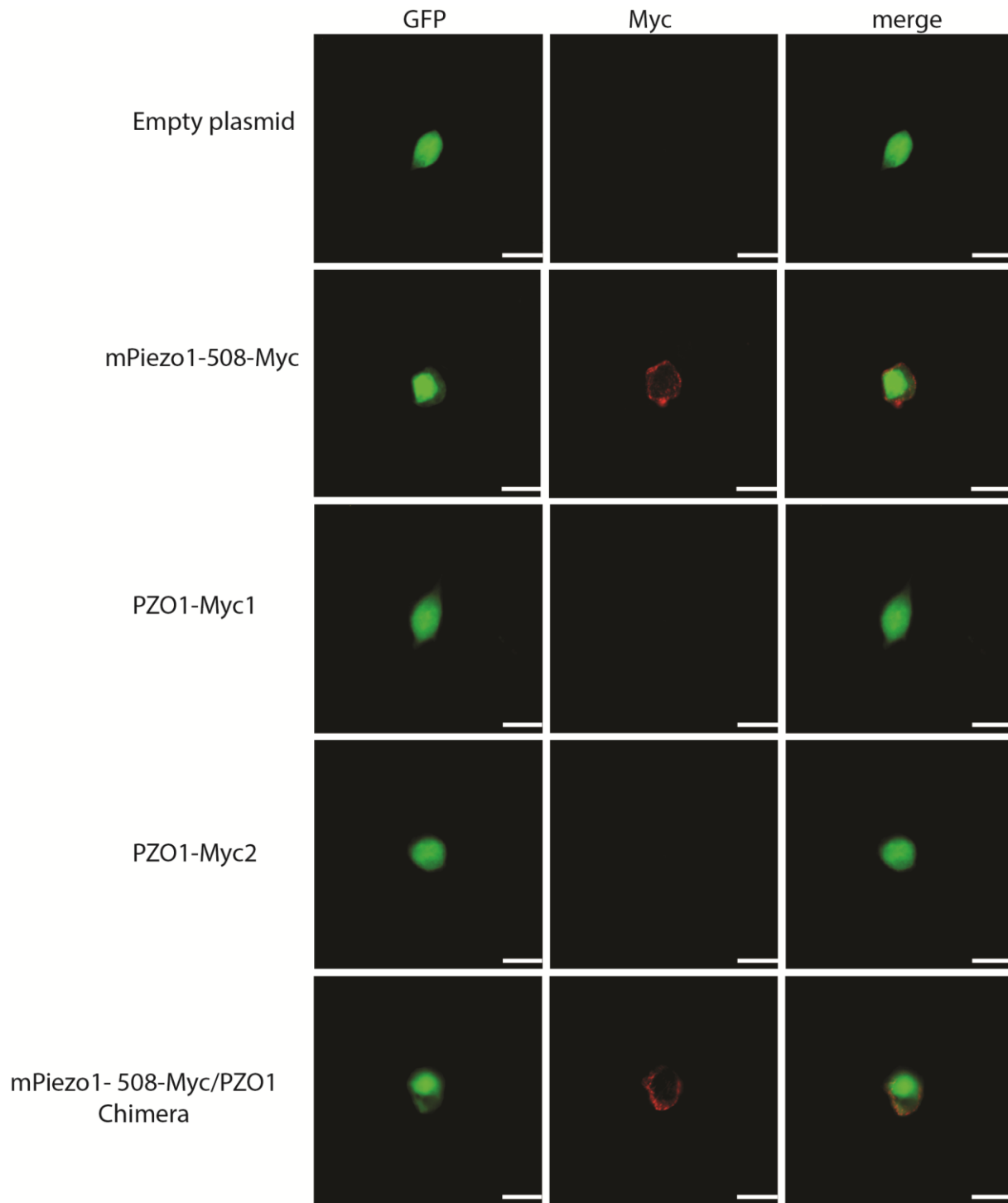


Figure S6. Myc tag staining of PZO1 and chimera. Representative images of non-permeabilized staining using an anti-Myc antibody (red) in *PZO1*-myc –IRES2- eGFP transfected cells, mPiezo1-508-Myc tag (myc tag located after amino acid 508) and mPiezo1-508-Myc/PZO1 chimera. mPiezo1-508-myc used as positive control which indicates mPiezo1 trafficked to the plasma membrane (red staining on the surface of non-permeabilized cells) (2). GFP expression indicates cells were transfected. Scale bar is 20 μ m.

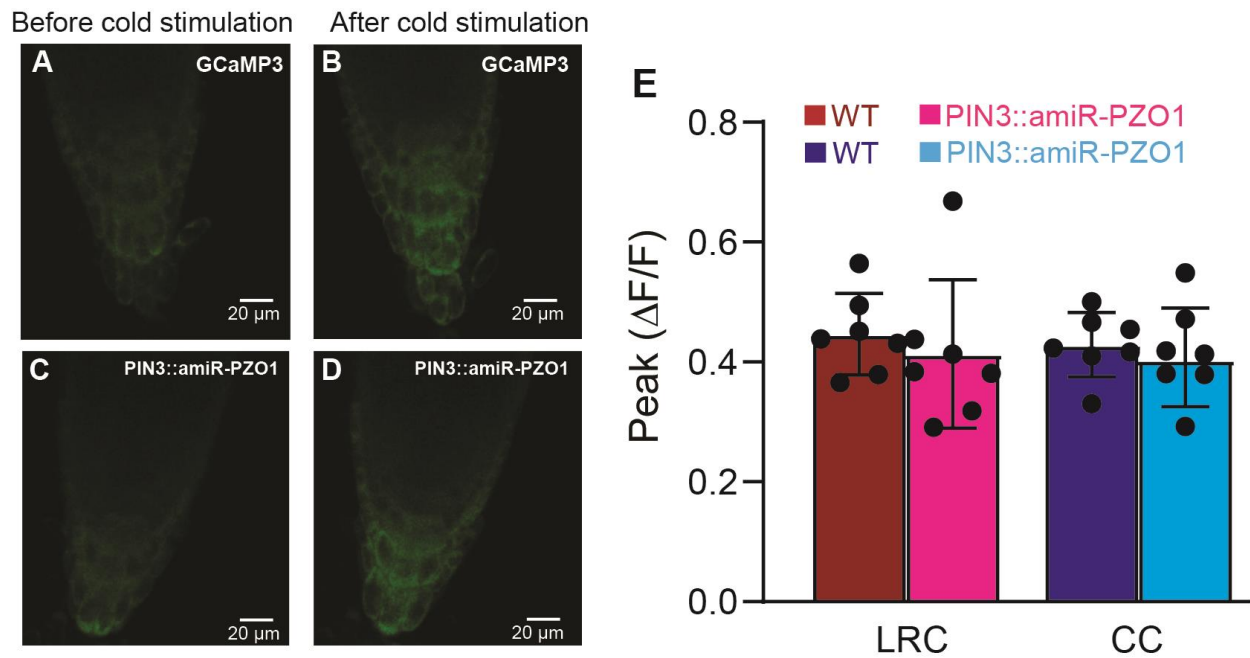


Figure S7. Ca^{2+} signal in roots in response to cold stress. A and B, representative images of the Ca^{2+} signal in root tip before and after applying 20 μl of cold water (4°C) to the root of GCaMP3 seedling (1 cm from the root tip) (images are from Movie S2). C and D. Representative images of the Ca^{2+} signal in the root tip before and after applying 20 μl of cold water (4°C) to the root of PIN3::amiR-PZO1 seedling (1 cm from the root tip). (images are from Movie S4). The Ca^{2+} recording images are representative from two different transgenic lines. E. Maximum peak fluorescence in WT and PIN3::amiR-PZO1 (piezo knockdown) (N=6, mean ±SD.) **P < 0.001.

Supplementary Video 1. *pzo1* mutant poorly penetrates hard MS media. Seeds of WT and *pzo1-CT* mutant plated on the surface of agar 2 cm above the barrier (12 g/l agar in MS media).

Supplementary Video 2. Mechanical indentation causes Ca^{2+} responses in the lateral root cap cells and columella cells in the WT expressing GCaMP3. Four mechanical stimuli were applied to the root cap beginning at 30s and followed in increasing increments of 20 μm at 15s intervals. 20-30 sec after last mechanical indentation, 20 μl of cold water (4 $^{\circ}\text{C}$) was applied to the seedling (1 cm from the root tip).

Supplementary Video 3. Extensive mechanical stimulation (100 μm) leads to wound/systemic Ca^{2+} fluxes that travel in both directions from the stimulation site. Five mechanical stimuli were applied to the upper root beginning of 25s followed in increasing increments of 20 μm at 15s intervals. At the 100 μm of mechanical stimulation, Ca^{2+} responses travel bidirectionally.

Supplementary Video 4. Mechanical indentation causes Ca^{2+} responses only in the lateral root cap cells in *pzo1* knockdown (PIN3::amiRNA-PZO1) mutant expressing GCaMP3. Four mechanical stimuli were applied to the root cap beginning at 30s and followed in increasing increments at 15s intervals. 20-30 sec after last mechanical indentation, 20 μl of cold water (4 $^{\circ}\text{C}$) was applied to the seedling (1 cm from the root tip).

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