

## **Piezo1 plays a role in erythrocyte volume homeostasis**

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Manuscript received on February 10, 2013. Manuscript accepted on July 15, 2013.

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## Methods

### Zebrafish strains and husbandry.

Zebrafish were maintained under standardized conditions and experiments were conducted in accordance with the European Communities council directive of November 1986. Embryos were staged according to Kimmel *et al* (11). Transgenic lines used in this manuscript: the CD41:GFP line has been described previously (12), the MPEG:mCherry line was generated by the Lutfalla lab based on a previous report (13), the Lmo2:dsRed line has been described previously (14).

### Morpholinos and injections

Morpholino oligonucleotides (MOs) were obtained from Gene Tools (Philomath, OR, USA) and injected into one-cell stage embryos. *Pz1 MO1* targets the start codon of *piezo1*, *Pz1 MO2* targets the splice site at the boundary between intron 1 and exon2 of *piezo1*. The sequences and amounts of injected MO are the following :

*Pz1 MO1*: 5'-ATCCGCATACCACCTGAAGCTCCAT-3' ; 8 ng

*Pz1 MO2* : 5'-AATATGCAGGCTGTGGAAAACACAT -3' ; 10 ng

### o-dianisidine staining

48 hpf dechorionated embryos were incubated for 15 min in the dark with staining solution (0.6 mg/mL o-dianisidine (Sigma), 10mM sodium acetate pH5.2, 0.65% hydrogen peroxide and 40% ethanol) then fixed in 4% PFA for 1 hour at room temperature.

### Sudan Black staining

Embryos were stained with Sudan Black as described previously (15).

### Blood smear

Embryonic blood was collected by cutting the tail of 2 dpf embryos (10 embryos for wild-type and 30 embryos for *Pz1* morphants) in a 1x PBS, 1% BSA, 10U/ml heparin solution. Blood was collected into a microtube and centrifuged for 3 min at 750 rpm. The pellet was resuspended in 5  $\mu$ L of the same solution, deposited on a slide and air-dried overnight. The blood preparation was then stained with Wright-Giemsa solution following manufacturer's

instructions (Sigma). For immunostaining, blood smears were treated as described (16). Images were taken using a Zeiss AxioImager Z1.

### **RT-PCR analysis**

30 hpf embryos non-injected or injected with 10 or 20 ng of *piezol* MO2 were collected and RNA was isolated with TRIzol® Reagent. RT-PCR was performed on 1 µg of RNA using *piezol* specific primers and amplification PCR was performed using the same primers.

For : 5'-ATGGAGCTTCAGGTGGTATG-3'

Rev : 5'-AGTTGTGTCCCAGTGCGTCA-3'

### **Acridine Orange**

Live embryos were incubated for 10 min in a 1 µg/mL acridine orange solution in the dark.

### **Erythrocyte counting**

To count the cells, 10 movies of wild-type and 10 movies of *piezol* morphants were taken using a Zeiss stereomicroscope and a 20X M PLAN APO 0.42 objective. For each movie, 5 time frames were picked randomly and cells were counted using the Cell-count ImageJ plugin.

### **Erythrocyte volume calculation**

Wildtype erythrocytes were assumed to be ellipsoid with equal major and minor axis diameters. The following formula was used to calculate the volume of wildtype erythrocytes.

$V = \pi/6(abc)$ , a=major axis, b=minor axis and c=vertical axis

*Piezol* morphant erythrocytes were assumed to be spherical with equal radii. The following formula was used to calculate the volume of *piezol* morphant erythrocytes.

$V = (4/3) \times \pi \times r^3$

### **In situ hybridisation**

*In situ* hybridisation was performed as described previously (17). The *piezol* sense and antisense probes were generated by cloning an approximately 500bp fragment from embryonic cDNA. The fragments was ligated into the pGEM-T® EASY vector and

subsequently linearized. For the *gatal*, *hbbe1* and *tall/scl* probes the full length genes were cloned from embryonic cDNA. These were ligated into the pGEM-T® EASY vector and subsequently linearized. The *band3* probe was generated by cloning the 250bp exon 16 from genomic zebrafish DNA. This was ligated into the pGEM-T® EASY vector and subsequently linearized. Anti-sense and sense probes were synthesized as described previously (18).

### **Immunohistochemistry**

Embryos were fixed with 4% paraformaldehyde (Sigma) in PBS for 2 hours at room temperature, rinsed in PBS-Tween 0.1% and permeabilized by PBS-Tween 0.1%-Triton 1%. Embryos were then blocked for 2 hours in 10% sheep serum/PBSDT (PBS, 1% DMSO, 0.1% Triton) at room temperature, incubated overnight with anti- $\alpha$ -tubulin (Sigma, mouse, 1:1000) over-night at 4°C, and washed with PBSDT for 6 hours at room temperature. Secondary antibody (Invitrogen, anti-mouse, 1:500) was applied overnight then rinsed in PBS-Tween 0.1%, 4 hours and observed by confocal fluorescence microscopy.

### **Confocal fluorescence imaging of live zebrafish embryos or stained embryos.**

Anesthetized or fixed embryos were immobilized in 1% LMP agarose on 35 mm glass bottom dishes (Iwaki, Chiba, Japan), covered respectively with tricaine containing embryo medium or PBS and imaged on Leica SPE confocal microscope (Leica).

### **Image processing.**

Images stacks and time-lapses obtained from confocal Leica microscopes were processed using either the Leica Las software (Leica) or Fiji ImageJ software.

CLUSTAL 2.1 multiple sequence alignment

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zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  MELQVVCGLLYCCLLP IFLLAACIFRYNALSLVYLLYLLLLLPWFQWPNKHTLRGHTGCIYI 60

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  KALFSTSLIFILGHVTFQICLYTIPSLDDALGHNCSSWETLSRHVGVSRPLPLEDPQGVLW 120

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  LLTPDLGVFIMSLITLILCSRLLKKRDEGSVPHMSALLHEADETEDDEDVEGEDEGMLS 180

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  CSETEDEESPSSSSAAQLAARLRATAQRFLRNMGRILAVTLLALAGITLPSAFSAFYFLL 240

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  FIGVCTWWACHFPI SHLGFNALCVMVAFFTGGHVLVCLYLYQSSFAQAMFSPAGLWARLFG 300

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  LKDLVTPGNCTTFFEVTLNTOYDWPVYVNP GILLLLYISVTIVLKINSNSLGDADKAREGE 360

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  AVKTVQGEAGEEVELRLWEARRQSS EDDTKQTLTVDSTLSESHITKEPVI NGSSQHDI 420

zf_PIEZO_2a -----
zf_PIEZO_2b -----
zf_PIEZO_1  GPSGEATVDNPLRLVGRMVLQ QSYICALIAMMVWSITYHSWLT FVLLLWSCVIWMLRTRQ 480
                :*****.***** *****:*:***.:**:* *:

zf_PIEZO_2a  KYAMLTSPFMVAYGNFLLVLQYIWC FEKLKPVSGFFVKM-----EVPFTELSTKV 91
zf_PIEZO_2b  KYSMVTSPFMVYFNLLIILQYIWSFE CLQVPVGLFLKK-----EVPFRELGSKT 141
zf_PIEZO_1  RFAAYCSPFILLYGLALCCLQYVWAM DLETLPQHIGSMSLHQLGLDRAQYPC LRLGALL 540
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zf_PIEZO_2a  LCQLSFWLLL RQTLMERQERKEE EAALSDIKVDEQKKVEEKSEEEVDENDLMQV LGKLV 151
zf_PIEZO_2b  LCLLSFWLLL RQFLTERREKHTDDP QLSDVTVDGHKLG--LQDEAAGHREMMEV LGSSLM 199
zf_PIEZO_1  LFTLTFWLLL RQSVKDTFSRKK-----SLTVPLQEV T--TGESTGRNESILKVFGGLVM 592
*  *:*:***** : :  :::  :.*  ::  :.  .....:** * :*

zf_PIEZO_2a  ALLVKYWIYVCGGMFFVVSFE GDMATYKIIYMMMLLSCVALYQVHYEYWR RILKYFWMSV 211
zf_PIEZO_2b  AMLNKYWIYICGGMFFVVSFE GRIVMYKIIYMMMF LFCVALYQLHYERWRWMLKYFWMSV 259
zf_PIEZO_1  SLYAKYWIYVCGGMFIMV SFAGKLVA YKIVYMLLFLLCMCLYQVYYS LWRLLKAFWMMV 652
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zf_PIEZO_2a  VVYTMLVLILIIYTSQFEDGIPTWTRMTGLDKDVLKDIGLEKFSLSNLFTRIFIPITSFLLV 271
zf_PIEZO_2b  VVYTMLVLILVYTFQFESSINVWSNMTGMSREKLEDLGLEKFSVPALFTRIFIPWFLLV 319
zf_PIEZO_1   VAYTMVVLIAIYTFQFEDFPGYWGNTGFTEQQLADMGLETFKLSSELFTSIVIPGFFLLA 712
*.:***:*** :** ***. * .:***: .: * *:*:***.*.:. *** *.* ***.

zf_PIEZO_2a  CILHLHYFHDFHLELTDLKAVVSKQESTIYRLAHPDGSLADLTIVSSSPEPLLKDEKEKE 331
zf_PIEZO_2b  CILHLHYFHERFLELTDIKTVADKQKSTISR LCS---GCALVSPEYDQDN NYHNEDEDED 376
zf_PIEZO_1   CILQLHYFHKPFMKITDLENVSPPIHR-----KRGID 743
***:*****. *::***: * ..

zf_PIEZO_2a  SLNEVLVVDCVGEEKKSDLSIYSAQMSQEDVHQSSAATDPELPSEQSSEMKNKWHLVV 391
zf_PIEZO_2b  ERVELEEDVKQTEEQKMEDSLFTCPMTADPVLEQTSGLN-----SKWHLVV 424
zf_PIEZO_1   NPDLVQSTEEVRPEEEELIVDQDDYPSEADEVIP-----SKWGLVM 784
. : : *::: . :: : .** **:.

zf_PIEZO_2a  DRLTVLFLKFLFLEYFHKLKLFVWWLLEMHIIKIVATYIILLVSVKEVSLLNLFVFLISWAFAL 451
zf_PIEZO_2b  ERLTVLFLKFLDCIQKQLFIWILELHIIKIVS-----SWAFAL 464
zf_PIEZO_1   DRLMVLRSRKFSDTLTHVQSFIVRVLELHIVKIVAFFVWVVALLEPSAMNLVVLVLSFAM 844
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zf_PIEZO_2a  PYQFRVLSSSVCTVWTCVIIIVCKLFYQLKTINPANYSSDCIMPN--YTETEKKDMKTSL 509
zf_PIEZO_2b  PFCQFRPLASSVCTVWTCIIIVCKMLYQLTSPAAAYARNCSMPDN-YTEAQRMDMAKSL 523
zf_PIEZO_1   PYGRFRAMASCLSTIWVCVIIIVCKMLYQLSVVNP AEYSNNCTAPMVNETSLEPDEVLNST 904
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zf_PIEZO_2a  LYRDPVDPANWVGLRKFHDHGIILPNLRNNLLMLLALLAFEVTIYRHQEFFRLRNKLSPPPS 569
zf_PIEZO_2b  LYIGVDPANWIGLRKFS--LLEYLRNNLLMLLALLAFEVTIYRHQDFYRMKNNITPPVT 581
zf_PIEZO_1   LYRASVDPASWFGVRKDDT--VLGYVKDHLVLMVLFVFEATVYRHOIHHRQQRSPPI 962
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zf_PIEZO_2a  RTIFHDITRQHLDNNGIIECAKYFINYFFYKFGLEVCFLLAINVGMQRMDFYSMVHGLALA 629
zf_PIEZO_2b  RTIFHNITRQHLDDGIVNCAKYFINYFFYKFGLETFCFLAVNVIGQRMDFFAMLHAFGLI 641
zf_PIEZO_1   PVIFPQATRDTLDQGLLHCIKYMLNYSFYKFGLEICFLMTVNVIGQRMNFLVIIHGCVLV 1022
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zf_PIEZO_2a  VVMFRRRRKAI AEIWPKYCCFLACMITFOYFICIGIPPAACADYPWRF-PNSSMTSNVIK 688
zf_PIEZO_2b  AVMYQRRRKAI AHVWSKYCCFLACLLSFOYLMCIGIPPAACTDYPWRR-SSSSMDSNVIK 700
zf_PIEZO_1   AIMVRRRRANIATIWSKYCLFLVVFMIYQYVLCVGGIPPALCIDYPWRWRSTVTIHSALVK 1082
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zf_PIEZO_2a  WLYLPDFHTPPSSMFLGYDFMLLL CASLQRQVFDEENKAAVRLMAGDNVEICRDLDAASF 748
zf_PIEZO_2b  WLYFPDFHTKPNLSFLLYDFMLLL CASLQGRVFEEECVSPVQLLAGDNVEICRDLDAATF 760
zf_PIEZO_1   WMYLPDFYTVPN SKNLISDFLLMCASQOWTVFDNEKKEEWMVMGGENRDNP---DPMEN 1139
*.:***:* *.* * **:*:*** * **:*: * :.:*** : *

zf_PIEZO_2a  SVHNPVPDFIHCRSYLDMLKVMFSYLFWFVLTIIIFITGTTRISIFCMGYLVACFYFLLF 808
zf_PIEZO_2b  SQHNPVPDFIHCRSYLDMLKVMVFSYLFWFVLTIIIFITGTTRISIFCMGYLVACFYFLIF 820
zf_PIEZO_1   MLFNPA PNFINCRSYLDM AKILVFRYMFVFLSVVFTGATRISVFGGLGYLMACFFFLLF 1199
.*.*:*:***:*** **:*: * *:*:***:***:***:***:***:***:***:***:***:***:***

zf_PIEZO_2a  GGQLLLKPIKEILRYWDFLIAYNVFVITMKNVFAILACGYINSLVKNSCWLIQLLSLACT 868
zf_PIEZO_2b  GGELLLKPIKSILHYWDFLIAYNVFVITMKN----- 851
zf_PIEZO_1   GTKLLTKPSRTRLVMWDCLIIYNVAVIISKNILSILACVFMEMQKNFCWVIQLFSLVCT 1259
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zf_PIEZO_2a  IKDYQPKTDLPGVDCEL PKDEAGIIWDSICFTFLLLQRRVFMSSYYFLHVIADIRAGQILA 928
zf_PIEZO_2b  -----ILSRRVFMSSYYFLHV VADIRSSQLLA 877
zf_PIEZO_1   VKGYYDPESVCNKDCSLPVEEAGIIWDSICFFLLLQRRVFLSFYFLHVSADLQASARQA 1319

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zf\_PIEZO\_2a SRGAELFQASIVKAVRARLEEEKKSMEQLKQMDRIKTRQOKFKRGKEMLSIAQESGDG 988  
zf\_PIEZO\_2b SRGAELFQATIVKAVKARIEEEKKSMDLLKQMDRIKSRQOKFKRGKERMLSLAQESGER 937  
zf\_PIEZO\_1 SRGFELFRASIIKNMHFHQQAERKSIQQLKESMKRIRGKQOKYKDGGRITSDIEEQSPDPK 1379  
\*\*\* \*\*\*:\*:\*:\*: \* : : : \*:\*:\*: \*\*..\*.\*\*: :\*:\*:\* \* : \* \* .

zf\_PIEZO\_2a EKLIPPVDNEEEEEDEEKKNKVQKKQWWRPWVDHASMVRSYDYLFFETDSEEEEEEEEEK 1048  
zf\_PIEZO\_2b HSVLK----EEEEEEE-----VVRSGNYLFFETDSEEEEEDEDK 973  
zf\_PIEZO\_1 EQKKETKLSKSKSWQYP-----WVDHATVLHSGEYMFESDSGEEDE-TFQE 1425  
.. :. . . : : : \*:\*:\*:\*:\*:\* \* : \* : :

zf\_PIEZO\_2a DEEPPKRSFAQRAIRKFSALVALPRSVIKLPKTILOYLIRAAKFVYHTWIAESKAALKE 1108  
zf\_PIEZO\_2b DEEP-KRSFQ-----FVYHAWITDSKTAMKE 999  
zf\_PIEZO\_1 EQKPRRQTAQ-----LAYQAWVTSKDALKE 1452  
: : \* : : \* \* \* : : \* : : \* \* \* : : \* : : \* \* \* : : \* \* \* : : \*

zf\_PIEZO\_2a RGKGKR-FWKRYGRR--LKKEKKEEHVAIEIGEDERQSSEEEKTDGPD-----NIIK 1157  
zf\_PIEZO\_2b RRKEKRRFWQKYTESNTNKREQKEANVTVEEG-DQDGVTEDELSDSPD-----NMMK 1050  
zf\_PIEZO\_1 RQQRQKQRAEAQQAQATDDAAEEGETVFDGENSQHAEMQEEGEDAASQATGSDMVQ 1512  
\* : : \* . . : . . . : : \* : . \* : : \* :

zf\_PIEZO\_2a RVFNIIKFTWALFLTTVESITKWLNSVCREYIDISTVLRIERCMLTREVKKGNVPSRESI 1217  
zf\_PIEZO\_2b RVFNMVKFSWVLFLLALDSFTAWLNSICQEHIDISSVLRIERCMLTHEAKGNVPSRDSI 1110  
zf\_PIEZO\_1 RILDILKFLWVLFAMVDGFTMWLNLTKQYVDTSMVLSEERYLFIHNVSQR----- 1564  
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zf\_PIEZO\_2a HVYYQKQMKLNGSRESGLDRISEEDSASNRERRRRRAQHSLDSFASRDIS SAYTEATMLF 1277  
zf\_PIEZO\_2b RMYQRQMCRPASTQSSLEPTEEAYS-----F 1138  
zf\_PIEZO\_1 -----ASRENMDQISHDSED-----L 1581  
. \* : . : . . : :

zf\_PIEZO\_2a SRQSTLDDLDDMPQNIPKTSERARPKLRKMYGLDMSNSSADSGSSVISSEATQCVTLFSR 1337  
zf\_PIEZO\_2b SPLRTPDLPPQEHTELQTSTEN-----LASEHTQGTTELYSR 1175  
zf\_PIEZO\_1 TLETCLDETDTDN-----ICDTNVVESETLGGF 1609  
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zf\_PIEZO\_2a QGTNDTIDEAEDEREQLEHKQKTEEAELRDKPEEEQVEEISDQKQEVLPVSHLVTQEVK 1397  
zf\_PIEZO\_2b QGTSDTVEECG----- 1186  
zf\_PIEZO\_1 AKSRD----- 1614  
: \*

zf\_PIEZO\_2a EDVLPVSDQLTGEVTEEEVEVEVKEMEEVEKEIGAKVEKEVEAEKEEEEAEKEVGEEV 1457  
zf\_PIEZO\_2b -----  
zf\_PIEZO\_1 -----

zf\_PIEZO\_2a QWDLVDPEEAEECVSDGDGGEQMQQDEGEWGVENVVSGPLFTPDTDAPNTSDADVPPSY 1517  
zf\_PIEZO\_2b -----ADTLWSSASDLLQTPHRDVPPSY 1209  
zf\_PIEZO\_1 -----FSSTLSELTLLELDCEPFY 1634  
: : . . \* . \* \* \*

zf\_PIEZO\_2a SKAVSFDRLSVSSDSDSKRLMLMTPDSKSDLDDPLLPSMTTDMTASELLLNKMFYDEM 1577  
zf\_PIEZO\_2b SRATGLDCICD-----ERKDKIQTPEDESS---SGTPHTSSHRLTASELLQTRMFYDAD 1259  
zf\_PIEZO\_1 SQQT-----HRPHRSRTASELLSERPFRVDE 1660  
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zf\_PIEZO\_2a LDSSDRFYKSQPLGLQLCYALYNLLVAHSEMVCYLVIIILNHMISASMATLVLPIILFLWA 1637

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zf_PIEZO_2b    LEQSERFYRSQPQLLQLCYSLYNVLVSHSQMVCYLVIILNHMISASVATLVLVPLVFLWA 1319
zf_PIEZO_1    LIQSREFYSSQNRLKLLFALYNVLAHSELVCYFIIIVLNNLVTASVISLVLVFLWA 1720
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zf_PIEZO_2a    MLSVPRPSKRFWMTAIVYTEVTIVIKYFFQFSFFPFN--QONLEVNGKGPYHPPNILGTEK 1695
zf_PIEZO_2b    MLSVPRPSKRFWMTAIVYTEVTIVIKYFFQFGFFPFN--QSIEVDKSKPFHPPNIIIGVEK 1377
zf_PIEZO_1    MLSVPRPSKRFWMTAIIYTEVMVVVKYLFQFGFFPWNTTEYELKLNEDKPFPPRILGLEK 1780
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zf_PIEZO_2a    KGYVHYDLVQLLALFFHRSILKCHGLWDEDDPKVPRKDECPRQES-VDDGKLSVVMSPD 1754
zf_PIEZO_2b    KEGYVHYDLVQLLALFFHRSILKCYGLWDEEPEPS--VSPHTHSGA-EEKAECFSEKSPV 1434
zf_PIEZO_1    TDNYIRYDLLQLLVLFHRSLLQRYGLWDOEGPLEEKSDLSPAENSKNENDEKKFQHSDE 1840
. . * * * * * : * * * * * : * * * * * : * * * * * : * * * * *

zf_PIEZO_2a    EDRKSSPAYSLKSMNLGMSVDSSQVHVQMRYPEQRPYLRRQSTSGSH---FShRSSARS 1810
zf_PIEZO_2b    EDT-YTHALSHTLRLARKQSS-----AGVRRQSSS-----ISQRS-ARS 1473
zf_PIEZO_1    EDSQAQPEPNTTEVLSLTPSAETPTDAEIQTDEPNSHTNNTKTSDTVKNKDKKSHFLRF 1900
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zf_PIEZO_2a    KRGSTSTRHSTHGENSTTESEVPQKSRKEMIMEKIREQLIKAKVYLLKRLVEFYQPIRQF 1870
zf_PIEZO_2b    KTGSSVSRSSVRRSLS-----AQQKSRKELLMEKLRQQLIRAKRFTVKTVDVYIPIRQF 1528
zf_PIEZO_1    RKKKKAEPDAVQKEPKKKKKCKTKHSETSKKLLNAIEGKFKSLFLSVVKNVYRPTWDF 1960
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zf_PIEZO_2a    FYNLVHPEYSAVTDVYVLMFLADTVDFIIIVFGFWAFGKHQGGADITSSLSQDQVPGPFL 1930
zf_PIEZO_2b    FYSLIHPEYSAVTDVYVLMFLADTVDFIIIVFGFWAFGKHSAAADITSSLSQDQVPEAF 1588
zf_PIEZO_1    FQNILHAEYRASTDVYALMFLTDVVDVFIIFFGFWAFGKHSAAADIASSLSQDQVPEAF 2020
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zf_PIEZO_2a    VMVLIQFGTMVVDRALYLRKTVVGKVFQVILVFGIHFWMFFILPGITERRFSQNTIAQL 1990
zf_PIEZO_2b    VMVLIQFGTMVVDRALYLRKTVMGKVFQVILVFGIHFWMFFILPGVTERRFSQNTVAQL 1648
zf_PIEZO_1    VMLLIQFSTMIIDRALYLRKLSILGKLIFQVILVFGIHLWMFFILPAVTERMFNHNSVAQL 2080
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zf_PIEZO_2a    WYFVKCIYFGLSAYQIRCGYPTRILGNFLTksynyanLFLFQGFRLIPFLTELRAVMDWV 2050
zf_PIEZO_2b    WYFVKCIYFGLSAYQIRCGYPTRVLGNFLTksynYLNLFQGFRLVPFLTELRAVMDWV 1708
zf_PIEZO_1    WYFFKCIYFTLSAYQIRCGYPTRILGNFLTkkfnHLNLFQGFRLVPFLVELRAVMDWV 2140
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zf_PIEZO_2a    WDTTTLSSLSSWICVEDIYAHIFILKCWRESEKRYQPQKGGKVVKYGMGMIVMLLIC 2110
zf_PIEZO_2b    WDTTTLSSLSSWICVEDIYAHIFVLKCWRESEKRYQPQKGGKVVKYGMGMIVMLLIC 1768
zf_PIEZO_1    WDTTTLSSLNWMCVEDIYANIFIICSRETEKKYPQPKGQKKKRIVKYGMGLIILFLIC 2200
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zf_PIEZO_2a    IVWFPLLFMSLVKSVAGVVNKPLeVSVSLTLGGLQPIFTMSAQONHIKSVTGAEFKKFTD 2170
zf_PIEZO_2b    IVWFPLLFMSLVKSVAGVVNKLPLDVSVTITLGGFQPIFTMSAQONQLKELTESDFKNFIN 1828
zf_PIEZO_1    IIWFPLLFISLVKSVGVVNHVPDVTVTVKLGGYEPLFTMSVQQQSIQPFTESRYNQLNN 2260
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zf_PIEZO_2a    HYKSNANAIMFLESYFPEDLTIAHLEGSSNSLWTISPPSKENLNMLNDTREPFLTFTW 2230
zf_PIEZO_2b    SYNIVPSAMQFLEAYRAEDVTVAELEGSSNSLWTISPPSRKNLMDVLSKE-DQFPVTMTW 1887
zf_PIEZO_1    QFSKNAVAMQFITMYSYEDIVTANIEGSSGSVWRISPPSRQELIKELLSSTGDMTLRLDW 2320
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zf_PIEZO_2a    SIQRNLSLGAKAETAMGYFIELDDET--RKGLTDQLGNS-SQRVTIKKIFPRYIRAPSD 2287
zf_PIEZO_2b    SIQRNLSLGAKEFAMDKHVTYLDIKT--RQELIALLNGTRNTPVLIEQVFCFIRAPSD 1945
zf_PIEZO_1    NFQRDLGKGGTVENTFDKHSISLAPKNPVRADLASLLGTRKDPVHVPHMFPNYIRAPSG 2380
. : * * * * * : * * * * * : * * * * * : * * * * * : * * * * *

```



```

zf_PIEZO_2a      SNAKPIDQLYNDGE--YMDITLFLNK-KNSSEG-VQEWIVNQTDSGPLPGLNGSGLQIY 2343
zf_PIEZO_2b      SNAKPIEQLYEDGD--YKSIQLDLERSANSSED-LQEWIVDELRSAQKQ-QREAGLQLY 2001
zf_PIEZO_1       AEAKPVMQLFDGDEEGFQDVTVSLMREASVNTTGAQEWWDISIADCKESC----NVLPMI 2436
:****: **:...: : .: : * : . . **** :. . * :

zf_PIEZO_2a      IISDQVSPPSLGFLAGYGIMGLYMSVVLVIGKQVREFFSGISHTIMFEELPNVDRIKLC 2403
zf_PIEZO_2b      VFSDKVSPPSLGFLAGYGIMGLYASVVLVIGKQVREFFSGISHSIMFEELPCVDRIKLC 2061
zf_PIEZO_1       IFNDKVSPPSLGFLAGYGIMGLYVSVVLVIGKQVRFVGFSEISHSIMFEELPCADRILKLC 2496
:..*:***** ***** *** **:***** .*****

zf_PIEZO_2a      TDIFLVRETGELDLEEDLYAKLIFLYRSPETMIKWTREKSE- 2444
zf_PIEZO_2b      TDIFLVRETGELELEEDLYAKLIFLYRSPETMIKWTREKNK- 2102
zf_PIEZO_1       MDIFLVRETGELELEEEELYSKLIFLYRSPETMIKWTREKNHN 2538
*****:***:***:*****..

```

An \* (asterisk) indicates positions which have a single, fully conserved residue.  
A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix.  
A . (period) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

Seq1	Length	Seq2	Length	%Identity
zf_PIEZO 1	2538	zf_PIEZO 2a	2444	40.63
zf_PIEZO 1	2538	zf_PIEZO 2b	2102	46.62
zf_PIEZO 2a	2444	zf_PIEZO 2b	2102	65.46

**Supplemental.figure.1. Protein homology analysis of zebrafish PIEZO1, PIEZO2a and PIEZO2b.**

CLUSTAL 2.1 multiple sequence alignment

```

human_PIEZO_1  MEPHVLGAVLYWLLLPCALLAACLLRFSGLSLVYLLFLLLLPWFPGPTRCGLQGHTGRLL 60
zf_PIEZO_1     MELQVVCGLLYCCLLPIFLLAACIFRYNALSLVYLLYLLLLPWFQWPNKHTLRGHTGCYI 60
** :*: .:** *** *****:*.:.*****:***** *.: *:*:** :

human_PIEZO_1  RALLGLSLLFLVAHLALQICLHIVPRLDQLLGPSCSRWETLSRHIGVTRLDLKDIPNAIR 120
zf_PIEZO_1     KALFSTSLIFILGHVTFQICLYTIPSLDDALGHNCSSWETLSRHVGVSRPLLEDPOGVW 120
:***. **:*:..*::*****: :* **:* ** .* ** *****:***:* *:* ..:

human_PIEZO_1  LVAPDLGILVSSVCLGICGRLARNTROSPHP-----RELDDDERVDVAS-----P 166
zf_PIEZO_1     LLTPDLGVFIMSLITLILCSRLLKKRDEGSVPHMSALLHEADETEDDDEDVEGEDEGMLS 180
*:*:*:*:*:*:* : * :*.** :: :.. * * :*:*:*:*:*.. .

human_PIEZO_1  TAGLQEAATLAPTRRSRLAARFRVTAHWLLVAAGRVLAVTLLALAGIAHPSALSSVYLLL 226
zf_PIEZO_1     CSETEDEESPSSSSAAQLAARLRATAQRFLRNMGRILAVTLLALAGITLPSAFSAFYFL 240
: :: : :. : :*****:*.**:* * **:******: *:*:*:*:*:**

human_PIEZO_1  FLALCTWWACHFPPISTRGFSRLCVAVGCFGAGHLICLYCYQMPLAQALLPPAGIWARVLG 286
zf_PIEZO_1     FIGVCTWWACHFPISHLGFNALCVMVAFFTGGLVCLYLYQSSFAQAMFSPAGLWARLFG 300
*:.:***** **.* ** * .***:** ** .:***:..***:**:.*

human_PIEZO_1  LKDFVGPNTNCSSPHALVLNTGLDWPVYASPGVLLLLCYATASLRKLRAYRP----SGQRK 342
zf_PIEZO_1     LKDLVTPGNCTT-FEVTLNTQYDWPVYVNP GILLLLYISVTIVLKINSNLSGDADKAREG 359
***:* * **:*: . :.*** *****.***:** ** :. : *:.: ..:..

human_PIEZO_1  EAAKGYEARELELAELDQWPQERESDQHVVPAPTDEADNCIVHELTGQSSVLRRPVPRPK 402
zf_PIEZO_1     EAVKTVQGEAGEEVELRLWEARRQSEDDTKQTLLTTVDSTLSESHITKEPVINGSSQHD 419
**.* :.. * .** * .*:*.:. . : * *. : .. :.*:. . : .

human_PIEZO_1  R---AEPREASPLHSLGHLIMDQSYVCALIAMMVWSITYHSWLTFVLLWACLIIWTVRSR 459
zf_PIEZO_1     IGPSGEATVDNPLRLVGRMVLOQSYICALIAMMVWSITYHSWLTFVLLWSCVIWMLRTR 479
.*. .**:* :*:~::~*****:*****:*****:***: *:*

human_PIEZO_1  HQLAMLCSPCILLYGMTLCCRLRYVWAMDRLPELPTTLGPVSLRQLGLEHTRYPCLDLGM 519
zf_PIEZO_1     QRFAYCSPFILLYGLALCCLOYVWAMDLETLPQHIGMSMLHQLGLDRAQYPCRLRGAL 539
:~:* ** * **:*:~::~*****.*** :*:.**:*:*:*:~::~***** **:*

human_PIEZO_1  LLYTLTFWLLLRQFVKEKLLKWAESPAALTEVTVADTEPTRTQTLQSLGELVKGVYAKY 579
zf_PIEZO_1     LLFTLTFWLLLRQSVKDTFSRKSLTVPLQEVTTGESTG-RNESILKVFGLVMSLYAKY 598
**:****** **:.: : . ...* **..:~::~ *:.:~::~ :* ** .:****

human_PIEZO_1  WIYVCAGMFIVVSFAGRLVYKIVYMFLFLLCLTLFQVYYSLWRKLLKAFWWLVVAYTML 639
zf_PIEZO_1     WIYVCGGMFIMVSFAGKLVAYKIVYMLLFLLCMCLYQVYYSLWRLLKAFWWMVVAYTMV 658
*****.***:*:*:*:*:* **.* **:*:~::~*****:*****:*****:*****:*****:

human_PIEZO_1  VLIAYVTFQFQDFPAYWRNLTGFTDEQLGDLGLEQFSVSELSILVPGFFLLACILQLH 699
zf_PIEZO_1     VLIAIYTFQFEDFPYWGNTGFTTEQQLADMGLETFKLSLFTSIVIPGFFLLACILQLH 718
***:*:*:*:*:*.* ** *:*:*:*:~::~*.*:* ** .:***:*:*:~::~*****

human_PIEZO_1  YFHRPFMQLTDMHVSLPGTRLPRWAHRQDAVSGTPLLREEQOEHQOQOQEEEEEEEDSR 759
zf_PIEZO_1     YFHKPFMKITDLENVSP-----IHRKRGIDNPDVQS----TEEVRPEEEELIVDQD 766
***:*:*:*:*:* **:* :.~::~ *:.: :~::~ * ** * .

human_PIEZO_1  DEGLGVATPHQATQVPEGAAKWGLVAERLLELAAGFSDVLSRVQVFLRRLLELHVFKLVA 819
zf_PIEZO_1     D-----YPSEADEVIP--SKWGLVMDRLMVLRSKFSDTLTHVQSFIVRVLELHIVKIVA 818
* * :* :* :***** :*:* * : **.*:*:*:* * :*:*:*:*:*

```

human\_PIEZO\_1 LYTVVVALKEVSMNLLLVLVWAFALPYPRFRPMASCLSTVWTCVIIIVCKMLYQLKVVNP 879  
zf\_PIEZO\_1 FVVVVALLEPSAMNLVVLVWFSAMPYGRFRAMASCLSTIIVCVIIIVCKMLYQLSVVNP 878  
: : . \*\*\*\*\* \* \* . \*\*\* : \*\*\*\*\* : \*\*\* : \*\* \*\*\* . \*\*\*\*\* : \* . \*\*\*\*\* . \*\*\*\*\*

human\_PIEZO\_1 QEYSSNCTEFPFNSTNLLPTEISQSLLYRGPVDPANWFGVRKGFNLYGIONHLOVLLLL 939  
zf\_PIEZO\_1 AEYSNNCTAPMVNETSLEPDEVLNSTLYRASVDPASWFGVRKDDTVLGYVKDHLVLMML 938  
\*\*\* . \*\*\* \* : \* . \* . \* \* \* : : \* \*\*\* . \*\*\*\*\* . \*\*\*\*\* . \*\*\*\*\* : : \* \* \* : \*\*

human\_PIEZO\_1 VFEAIVYRROEHYRROHQLAPLPAQAVFASGTROQLDQDLLGCLKYFINFFFYKFGLEIC 999  
zf\_PIEZO\_1 VFEATVYRHQIHHYRQQORSPPPIPVIFPQATRDTLDQGLLHCIKYMLNYSFYKFGLEIC 998  
\*\*\*\* \* \* : \* \* : \* \* \* . : \* . . \* \* : \* \* . \* \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 FLMAVNVIGQRMNFLVTLHGCVLVAAILTRRRHRQAIARLWPNYCLFLALFLLYQYLLCCLGM 1059  
zf\_PIEZO\_1 FLMTVNVIGQRMNFLVLIHGCWLVAIMVRRRRANIATIWSKYCLFLVVFMIYQYVLCVGI 1058  
\*\*\* : \*\*\*\*\* : \*\*\*\*\* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 PPALCIDYPWRWSRAVPMNSALIKWLYLPDFFRAPNSTNLISDFLLLLCASQOQVFSAE 1119  
zf\_PIEZO\_1 PPALCIDYPWRWRSTVTIHSALVKWMLPDPFYTPVNSKNLISDFLLLMCASQOQWTVFDNE 1118  
\*\*\*\*\* : \* . : : \* \* : \* \* : \* \* : \* \* : \* \* . \*\*\*\*\* : \*\*\*\*\* \* \* . \*

human\_PIEZO\_1 RTEEWQRMAGVNTDRLEPLR-GEPNPVPNFIHCRSYLDMLKVAVFRYLFWLVLVVVFTG 1178  
zf\_PIEZO\_1 KKEEWMVMGGENRDNPDPMENMLFNAPNFINCERSYLDMAKILVFRYMFVFLSVVFTG 1178  
: . \*\*\* \* . \* \* \* . : \* . \* \* . \*\*\*\*\* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 ATRISIFGLGYLLACFYLLLFGTALLQRDTRARLVLDCLILYNVTVIIISKNMLSLLACV 1238  
zf\_PIEZO\_1 ATRISVFGGLGYLMACFFLLFGTKLLTKPSRTRLVMWDCLIIYNVAVIIISKNILSILACV 1238  
\*\*\*\*\* : \*\*\*\*\* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 FVEQMOTGFCWVIQFLSLVCTVKGYDPEKEMDRDQDCLLPVEEAGIIWDSVCFLLLO 1298  
zf\_PIEZO\_1 FVMEMQKNFCWVIQFLSLVCTVKGYDPEVCN--KDCSLPVEEAGIIWDSICFFLLLO 1296  
\* \* : \* \* . \*\*\*\*\* : : : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 RRVFLSHYYLHVRADLQATALLASRGFALYNAANLKSIDFHRRIEEKSLAQKQRMERIR 1358  
zf\_PIEZO\_1 RRVFLSFYFLHVSADLQASARQASRGFELFRASIIKNMHFHQAERKSIQQLKESMKRIR 1356  
\*\*\*\*\* . : \* \* \* \* \* : \* \* \* \* \* \* : \* . : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 AKQEKHRQGRVDRSRPQDTLGPKDPGLEPGDPSGGSSPPRRQWWRPWLHDATVIHSGDY 1418  
zf\_PIEZO\_1 GKQQKYKDGRITSDIEEQPSDPKEQKQKTKLSKSKS-----KSWQYPWVDHATVLSHGEY 1409  
. \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 FLFESDSEEEEEAVPEDPRPSAQSAFOLAYQAVWTNAQAVLRRRQEQEQARQEQ----- 1473  
zf\_PIEZO\_1 YMFESDSGEEDETFQEEQKPRROTAFOLAYQAVVTSAKDALKERQORQRKQRAEAEKQON 1469  
: : \*\*\*\*\* \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 -----AGQLPTGGGPSQEVEPAEGPEEAAAAGR---SHVVQVRLSTAQFLWMLGQALV 1522  
zf\_PIEZO\_1 AQATDDAEEGETVFDGENSQHAEEMQEEGEDAASQATGSDMVQRILDILKFLWVFLAMV 1529  
\* : \* \* \* \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 DELTRWLQEFTRHHGTMSDVLRAERYLLTQELLOGGEVHRGVLDQLYTSQAEATLPGPTE 1582  
zf\_PIEZO\_1 DGFTMWLNLTKQYVDTSMVLSEERYLFIHNVSQRASREN-MDDQISHDSEDLTLETCLD 1588  
\* : \* \* \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 APNAPSTVSSGLGAEPLSSMTDDMGSPSTGYHTRSGSEEAVTDPGEREAGASLYQGLM 1642  
zf\_PIEZO\_1 ETDTDNICDTNVVESETLGGFAKSR--DFSSTLSELTELEDCPEFYSQOQTHRPHR--S 1644  
. : . . : . \* . \* . : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \*

human\_PIEZO\_1 RTASELLLDRRLRIPELEEAELFAEGQGRALRLLRAVYQCVAHSELLCYFIIILNHMVT 1702

zf\_PIEZO\_1 RTASELLSERPFRVDELIQSREFYSSQNRLKLLFALYNVLAHSELVCYFIIVLNNLVT 1704  
\*\*\*\*\* :\* :\*: \*\* :. \* ..\*.\* \*:\*: \*:\*: :\*\*\*\*\*:\*\*\*\*\*:\*\*\*:\*\*\*

human\_PIEZO\_1 ASAGSLVLPVLVFLWAMLSIPRPSKRFWMTAIVFTEIAVVVKYLFQFGFFPWNSHVLR 1762  
zf\_PIEZO\_1 ASVISLVLVFLVFLWAMLSVPRPSKRFWMTAIYTEVMVVVKYLFQFGFFPWNTTEYELKL 1764  
\*\* . \*\*\*\*\*:\*\*\*\*\*:\*\*\*: \*\*\*\*\*: . \* :

human\_PIEZO\_1 YENKPYFPPRILGLEKTDGYIKYDLVQLMALFFHRSQLLCYGLWDHE-----EDSPSK 1815  
zf\_PIEZO\_1 NEDKPFPPRILGLEKTDNYIRYDLLQLLVFFHRSLLQRYGLWDQEGPLEEKSDLSPAE 1824  
\*:\*:\*\*\*\*\*.\*\*\*:\*\*\*:\*\*\*.\*\*\*\*\* \* \*\*\*\*\*:\* : \*\*\*:

human\_PIEZO\_1 EHDKSGEEEGAEEGPGVPAATTEDHIOVEARVGPTDGTPEPQVELRPRDTRRISLRFRR 1875  
zf\_PIEZO\_1 NSKNENDEKFKFQHSDEEDSQAQPEPNTTEVLSLTPSAETPTDAEIQTDEPNSTNTTKTS 1884  
: .....\*:: ... . \* .\* : :\* : . ::: .....: \* . . : .

human\_PIEZO\_1 RKKEGPARKGAAAEADREEEEGEEKEAPTGREK-----RPSRSGGRVRAAGRRLQG 1929  
zf\_PIEZO\_1 DTVKNDGMKKSFLRFRKKAEPDAVQKEPKKKKCKTKHSETSKLLNAIEGKFKS 1944  
. :. . \* : :. ....: \* : :\*: \*. :\*: . \* :.\* :::.

human\_PIEZO\_1 FCLSLAQGTYPRLRRFFHDILHTKYRAATDVYALMFLADVDFIIIIIFGFWAFGKHSAA 1989  
zf\_PIEZO\_1 LFLSVVKNVYRPTWDFQNILHAEYRASTDVYALMFLTDVDFIIIIIFGFWAFGKHSAAA 2004  
: \*\*::...\*\*\* \*\*::\*\*\*:\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:

human\_PIEZO\_1 DITSSLSDDQVPEAFLVMLLIQFSTMVVDRALYLRKTVLGKLAFOVALVLAHLMWFFIL 2049  
zf\_PIEZO\_1 DIASSLSEDQVPEAFLVMLLIQFSTMIIDRALYLRKSILGKLIFQVILVFGIHLWFFIL 2064  
\*\*:\*:\*:\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*:\*\*\*\*\*

human\_PIEZO\_1 PAVTERMFNQNVVAQLWYFVKCIYFALSAYQIRCGYPTRILGNFLTCKYNHLNLFQGF 2109  
zf\_PIEZO\_1 PAVTERMFNHNSVAQLWYFFKCIYFTLSAYQIRCGYPTRILGNFLTCKFNHLNLFQGF 2124  
\*\*\*\*\*:\* \*\*\*\*\*.\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:

human\_PIEZO\_1 RLVPFVLELRAVMDVWWTDTTSLSSWMCVEDIYANIFIICKSRETEKKYPQPKGQK 2169  
zf\_PIEZO\_1 RLVPFVLELRAVMDVWWTDTTSLSNWMCVEDIYANIFIICKSRETEKKYPQPKGQK 2184  
\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:

human\_PIEZO\_1 IVKYGMGGLIILFLIAIIWFPLLFMSLVRVGVVNVQPIDVTVTLKGGYEPLFTMSAQ 2229  
zf\_PIEZO\_1 IVKYGMGGLIILFLICIIWFPLLFISLVKSVGVVNVHPVDVTVTLKGGYEPLFTMSV 2244  
\*\*\*\*\*.\*\*\*\*\*:\*\*\*:\*\*\*\*\*:\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*.\*\*\*

human\_PIEZO\_1 PSIIPTAQAYEELSRQFDPQLAMQFISQYSPEDIVTAQIEGSSGALWRISPPSRAQMK 2289  
zf\_PIEZO\_1 QSIQPFTESTRYNQLNNQFSKNAVAMQFITMYSYEDIVTANIEGSSGSVWRISPPSRQELI 2304  
\*\* \*\*\* . \*::\*..\*\* . :\*:\*\*\*\*\*: \*\* \*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:

human\_PIEZO\_1 RELYNGTADITLRFWTNFORDLAKGGTVEYANEKHMALAPNSTARRQLASLLEGTSDQS 2349  
zf\_PIEZO\_1 KELLSSSTGDMTLRLDWNFORDLGKGGTVENTFDKHSISLAPKNPVRADLASLLLGRKDP 2364  
:\* ..\*.\*:\*\*\*: \*\*\*\*\*.\*\*\*\*\* : :\*\* :\*:\*\*\*:..\* :\*\*\*\*\* \*\* ..

human\_PIEZO\_1 VVIPNLFPKYIRAPNGPEANPVKQLQPNEEADYLGVRIQLRREQGAGATGFLEWVIELQ 2409  
zf\_PIEZO\_1 VHVPHMFPNYIRAPSGAEAKPVMQLFDGDEEGFQDVTVSLMREASVNTTGAQEWWDISIA 2424  
\* :\*:\*\*\*:\*\*\*\*\*.\*.\*:\* \*\* .:\* .: .\* :.\* \*\* ...:\* \*\* \*.:

human\_PIEZO\_1 ECRTDCNLLPMVIFSDKVSPSLGFLAGYGIMGLYVSVLVIGKFVRGFFSEISHSIMFE 2469  
zf\_PIEZO\_1 DCKESCNVLPMIIFNDKVSPSLGFLAGYGIMGLYVSVLVIGKFVRGFFSEISHSIMFE 2484  
:\*: .\*\*:\*:\*:\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:

human\_PIEZO\_1 ELPCVDRILKLCQDIFLVRETRELEEEELYAKLIFLYRSPETMIKWTREKE-- 2521  
zf\_PIEZO\_1 ELPCADRILKLCMDIFLVRETGELEEEELYSKLIFLYRSPETMIKWTREKNHN 2538  
\*\*\*\*.\*\*\*\*\* \*\*\*\*\* \*\*\*\*\*:\*\*\*\*\*:\*\*\*\*\*:

An \* (asterisk) indicates positions which have a single, fully conserved residue.

A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix.

A . (period) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

<b>Seq1</b>	<b>Length</b>	<b>Seq2</b>	<b>Length</b>	<b>%Identity</b>
human_PIEZO 1	2521	zf_PIEZO 1	2538	56.0

**Supplemental.figure.2. Protein homology analysis of human PIEZO1 and zebrafish PIEZO1.**

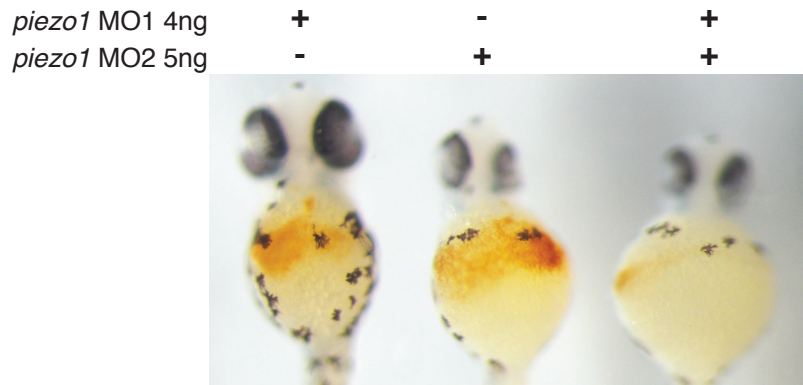
**Bold underlined:** *piezo1* MO1 target sequence.

zf\_Pz1 **ATGGAGCTTCAGGTGGTATGCGGAT**TGCTCTACTGCTGTCTGCTGCCCATCTTTTTACTG  
zf\_Pz2a ATGAAGCAGAGCTACATCTGCGCTCTCATCGCTATGATGGCGTGGAGTATAACGTATGTC  
zf\_Pz2b ATGATTACAGAATCCAGCAACAGATCTCCTCTTTTTTCAGAGAGCCTGGACTGCGGTGTT

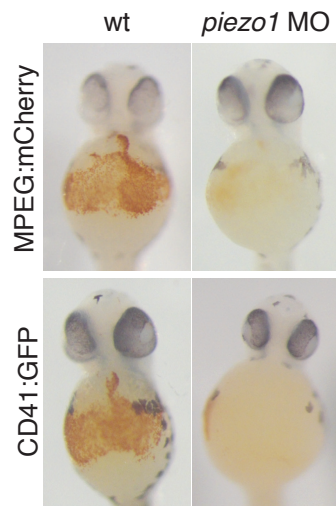
**Bold underlined:** *piezo1* MO2 target sequence. Lower case denotes intron1, uppercase denotes exon2.

zf\_Pz1 ttttttct**catgttttccacagCCTGCATATTT**CGCTACAATGCCTT  
zf\_Pz2a actggtctgtttgtctttctcaag**CCGGATAACTACACCGAGGCCAGC**  
zf\_Pz2b catgtttcttattctctttaag**GCGTGGAGTATAACGTATGTCAGCT**

**Supplemental.figure.3. Sequence analysis indicating the target sequences of the *piezo1* MO1 and *piezo1* MO2 morpholinos.**



**Supplemental.figure.4. Co-injection of *piezo1* morpholinos at suboptimal concentrations affects erythrocyte homeostasis.** 2dpf *piezo1* morphant embryos injected with suboptimal concentrations of either *piezo1* MO1(4ng)(left), *piezo1* MO2 (5ng)(centre) or co-injected with both *piezo1* MO1(4ng) and *piezo1* MO2 (5ng)(right). Embryos were subsequently stained with o-dianisidine to label erythrocytes.



**Supplemental.figure.5. Transgenic *piezo1* morphants.**

O-dianisidine staining of 2dpf MPEG:mCherry embryos, either non-injected (wt column) or injected with *piezo1* MO1 (*piezo1* MO1column). O-dianisidine staining of 2dpf CD41:GFP embryos, either non-injected (wt column) or injected with *piezo1* MO1 (*piezo1* MO1column).



**Supplemental.movie.1.** Blood circulation in a wildtype uninjected embryo.

A movie taken through a light microscope of a region in the trunk of a 3dpf wildtype embryo. The dorsal aorta is above the cardinal vein. Blood cells can be seen circulating through these vessels.

**Supplemental.movie.2.** Blood circulation in a *piezol* morphant embryo.

A movie taken through a light microscope of a region in the trunk of a 3dpf *piezol* morphant embryo. The dorsal aorta is above the cardinal vein. Note that fewer blood cells are circulating than in the wildtype movie.