LETTER

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The role of *Drosophila* Piezo in mechanical nociception

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Transduction of mechanical stimuli by receptor cells is essential for senses such as hearing, touch and pain[1–4]. Ion channels have a role in neuronal mechanotransduction in invertebrates[1]; however, functional conservation of these ion channels in mammalian mechanotransduction is not observed. For example, no mechanoreceptor potential C (NOMPC), a member of transient receptor potential (TRP) ion channel family, acts as a mechanotransducer in *Drosophila melanogaster*[5] and *Caenorhabditis elegans*[6,7]; however, it has no orthologues in mammals. Degenerin/epithelial sodium channel (DEG/ENaC) family members are mechanotransducers in *C. elegans*[8] and potentially in *D. melanogaster*[9]; however, a direct role of its mammalian homologues in sensing mechanical force has not been shown. Recently, Piezo1 (also known as Fam38a) and Piezo2 (also known as Fam38b) were identified as components of mechanically activated channels in mammals[10]. The Piezo family are evolutionarily conserved transmembrane proteins. It is unknown whether they function in mechanical sensing *in vivo* and, if they do, which mechanosensory modalities they mediate. Here we study the physiological role of the single Piezo member in *D. melanogaster* (Dm*piezo*; also known as CG8486). Dm*piezo* expression in human cells induces mechanically activated currents, similar to its mammalian counterparts[11]. Behavioural responses to noxious mechanical stimuli were severely reduced in Dm*piezo* knockout larvae, whereas responses to another noxious stimulus or touch were not affected. Knocking down Dm*piezo* in sensory neurons that mediate nociception and express the DEG/ENaC ion channel *pickpocket* (*ppk*) was sufficient to impair responses to noxious mechanical stimuli. Furthermore, expression of Dm*piezo* in these same neurons rescued the phenotype of the constitutive Dm*piezo* knockout larvae. Accordingly, electrophysiological recordings from *ppk*-positive neurons revealed a Dm*piezo*-dependent, mechanically activated current. Finally, we found that Dm*piezo* and *ppk* function in parallel pathways in *ppk*-positive cells, and that mechanical nociception is abolished in the absence of both channels. These data demonstrate the physiological relevance of the Piezo family in mechanotransduction *in vivo*, supporting a role of Piezo proteins in mechanosensory nociception.

D. melanogaster is widely used to study mechanotransduction, and genetic studies have identified several ion channels that are essential for mechanosensation[5,9,12–14]. However, none of the identified proteins have been shown to be activated by mechanical force when expressed in heterologous systems. Because expression of mouse Piezo proteins in a variety of mammalian cells induces mechanically activated currents[10], we investigated whether the *Drosophila* counterpart is also sufficient to induce mechanosensitivity. Similar to its mammalian counterparts, the Dm*piezo* gene is predicted to consist of a large number of transmembrane domains (39; Supplementary Fig. 1). Although fly and mammalian *piezo* genes do not exhibit extensive sequence conservation (24% identity), expression of Dm*piezo* in cultured human cells induced mechanically activated cationic currents, suggesting a role of Dm*piezo* in mechanotransduction[11].

To characterize Dm*piezo* expression in flies we used a fusion between the Dm*piezo* enhancer/promoter region and GAL4 (Dm*piezoP*–GAL4). Four independent Dm*piezoP*–GAL4 transgenic insertions were examined to avoid insertional effects on GAL4 expression. We used green fluorescent protein (GFP) regulated by upstream activating sequence elements (UAS) (UAS-GFP) for labelling cells, except for arborized neurons that were optimally visualized using the membrane-targeted UAS-CD8::GFP. We found fluorescent labelling induced by Dm*piezo* enhancer/promoter region in all types of sensory neurons and several non-neuronal tissues in both adults and larvae (Supplementary Fig. 2). This diverse pattern of Dm*piezo* expression observed in *Drosophila* is in accord with the expression of Piezo1 and Piezo2 in mice[10].

We created Dm*piezo* knockout flies in which all 31 coding exons were deleted using genomic recombination[15] (Fig. 1a, see Supplementary Fig. 3 for details). The knockout flies were viable, fertile and did not show a lack of coordination or a defect in bristle mechanoreceptor potential (Supplementary Fig. 4). We studied whether Dm*piezo* knockout larvae have mechanical nociception deficits by using a mechanically induced escape behaviour assay[9,14,16]. Stimulation with von Frey filaments that ranged from 2–60 milliNewton (mN) demonstrated that Dm*piezo* knockout larvae have a severe response deficit over a wide range (Fig. 1b). Repeated stimulations of the same larvae resulted in comparable responsiveness in both wild-type and Dm*piezo* knockout, indicating that the stimuli did not induce considerable damage to the sensory system (Fig. 1c, d). A 153 ± 11.0 mN filament elicited responses only to the first of three stimulations in wild-type larvae, arguing that this amount of force is damaging (data not shown). For further experiments, we chose to stimulate the larvae using a 45 mN filament, which has been used in a previous study[14], and elicits a substantial response in both wild-type and Dm*piezo* mutant larvae. Thirty four ± 4.4% of Dm*piezo* knockout larvae showed a response to 45 mN filament stimulation, compared to over 80% of wild-type or heterozygote larvae (Fig. 1e). As a control for the genetic background, we used larvae that carry the Dm*piezo* knockout allele on one chromosome and a deficiency in which the entire Dm*piezo* genomic region is deleted on the homologous chromosome. The defect in the trans-heterozygous larvae was similar to the knockout homozygote phenotype (51 ± 3.9%, $P = 0.091$). In contrast, Dm*piezo* knockout larvae were indistinguishable from wild type in an assay for responses to high temperature, a different noxious stimulus that elicits the same escape response[14] (Fig. 1f). Therefore, Dm*piezo* knockout larvae retain a normal ability to elicit the escape behaviour in response to noxious stimuli, whereas Dm*piezo* is specifically required for the mechanical modality of nociception. To evaluate the possible role of Dm*piezo* in other modes of larval mechanical sensing, we tested the sensitivity of Dm*piezo* knockout to gentle touch, which is mediated through ciliated neurons[17,18]. We observed no defect in the sensitivity of Dm*piezo* knockout larvae to innocuous gentle touch (Fig. 1g).

A mechanical nociception phenotype was previously observed in mutants of *ppk*, a DEG/ENaC channel[9] and *painless* (*pain*), a TRPA

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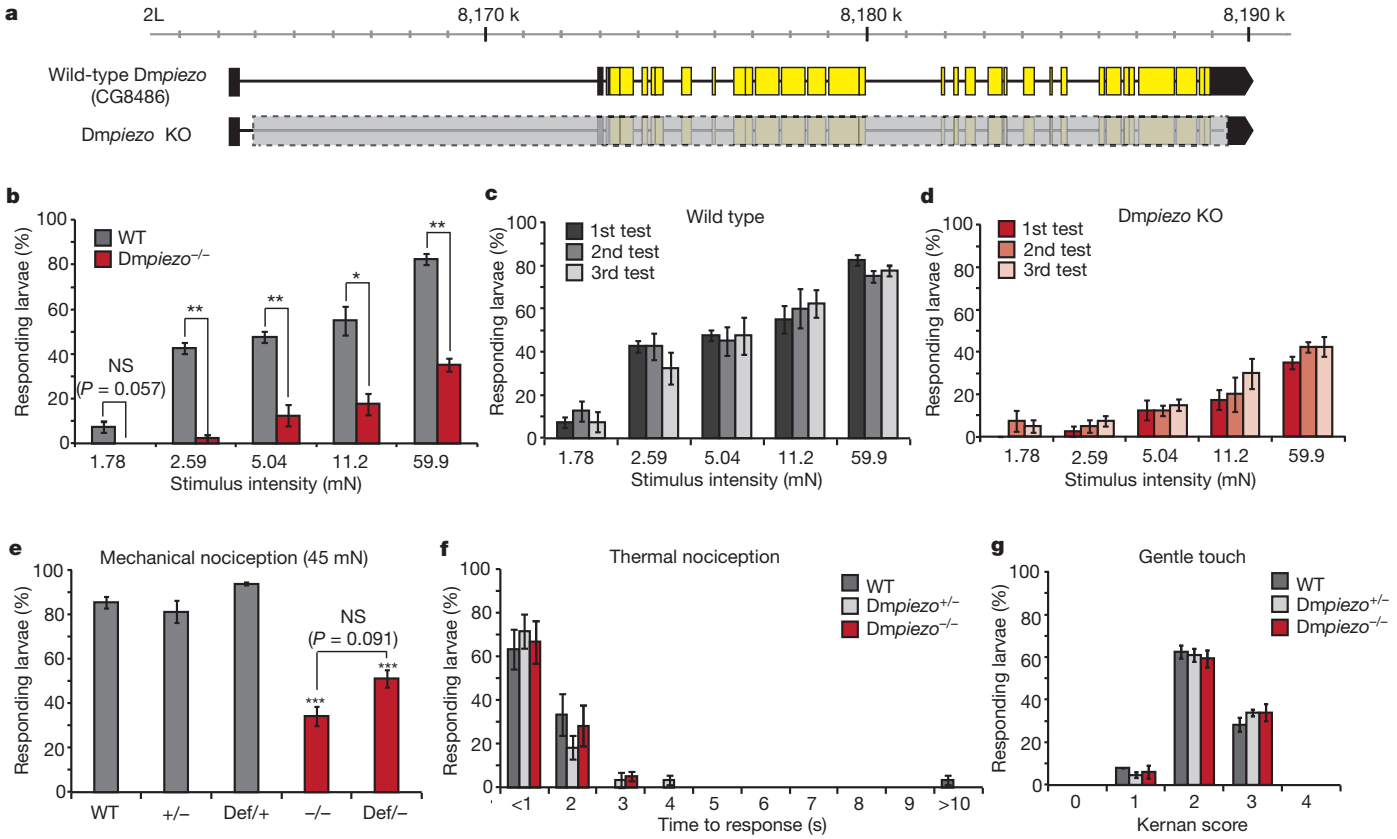

Figure 1 | Mechanical nociception defect in Dm*piezo* knockout larvae. **a**, Genomic map showing wild-type Dm*piezo* gene (top) and engineered Dm*piezo* knockout (bottom). Yellow and black boxes represent coding and non-coding exons, respectively. The segment deleted from the left arm of chromosome 2 (2L) in Dm*piezo* knockout is marked with a grey box. **b**, Mechanical nociception assay using a range of stimulus forces in wild-type (WT) and Dm*piezo* knockout larvae. $n = 40$ from four independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ from two-tailed paired Student *t*-test. **c**, **d**, Mechanical nociception assay using repeated stimuli of the same larvae. $n = 40$. KO, knockout. **e**, Mechanical nociception assay using a 45 mN von Frey filament with wild type (+/+), heterozygous knockout (+/−), heterozygous deficiency (Def/+), homozygous KO (−/−) and trans-heterozygous KO (Def/−). $n > 85$. $^{***}P < 0.001$. **f**, Thermal nociception assay using heated probe (45 °C). $n = 60$. **g**, Gentle touch assay[17]. For details about the Kernan score, see Methods. $n > 150$. Error bars indicate mean ± s.e.m. NS, not significant.

ion channel[14]. The specificity of Dm*piezo* knockout to mechanical nociception resembles the phenotype of *ppk*, as *pain* is also essential for sensing thermal nociception[14]. We therefore tested the role of Dm*piezo* in *ppk*-positive cells using *ppk*-GAL4, which labels subclasses of multidendritic neurons[19,20]. The multidendritic neurons are non-ciliated receptor cells that tile the body wall of the larvae and respond to a variety of external stimuli such as mechanical forces, temperature and light[9,14,16,21]. We used enhanced (E)GFP driven directly by the regulatory regions of the *ppk* gene (*ppk*-EGFP)[22] together with a red fluorescent protein expression in Dm*piezo*-positive cells to probe Dm*piezo* and *ppk* co-expression. Indeed, we did observe that all *ppk*-positive cells also expressed Dm*piezo* (Fig. 2a). Next we used *ppk*-GAL4 to drive the expression of Dm*piezo* RNA interference (RNAi) to test whether Dm*piezo* function is specifically required in *ppk*-expressing cells. The restricted knockdown of Dm*piezo* resulted in a mechanical nociceptive phenotype (Fig. 2b) similar to the phenotype observed in Dm*piezo* knockout larvae (Fig. 1e). In a complementary approach, we used *ppk*-GAL4-driven expression of Dm*piezo* complementary DNA in an attempt to rescue the mechanical nociception phenotype of Dm*piezo* knockout larvae. We used a fusion between DmPiezo and GFP to monitor expression levels in *ppk* cells and DmPiezo localization within the neurons. GFP–DmPiezo fusion protein induces mechanically activated currents in human cell lines, similar to untagged DmPiezo,

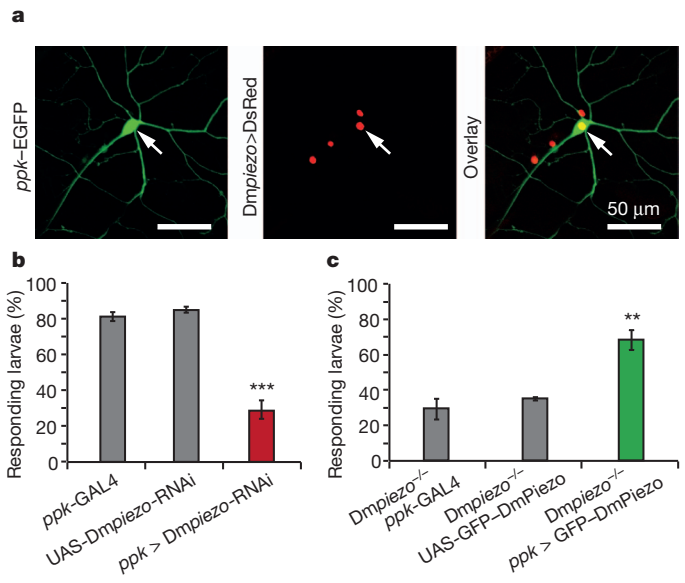

Figure 2 | Dm*piezo* functions in *ppk*-positive type II sensory neurons. **a**, Double fluorescence labelling using *ppk*-EGFP (green) and Dm*piezo*P-GAL4 that drives the expression of the nucleus targeted UAS-DsRed-NLS (red). A representative high-magnification image shows one *ppk*-positive neuron (arrow). All three *ppk*-positive cells in each hemisegment expressed Dm*piezo* in all segments. **b**, Mechanical nociception assay with Dm*piezo* knockdown larvae in *ppk*-expressing cells by *ppk*-GAL4 and UAS-Dm*piezo*-RNAi. $n > 85$, $^{***}P < 0.001$. **c**, Mechanical nociception assay in rescued Dm*piezo* knockout. GFP–DmPiezo was expressed in *ppk*-cells using *ppk*-GAL4 and UAS-GFP–DmPiezo. $n > 60$. $^{**}P < 0.01$. Error bars indicate mean ± s.e.m.

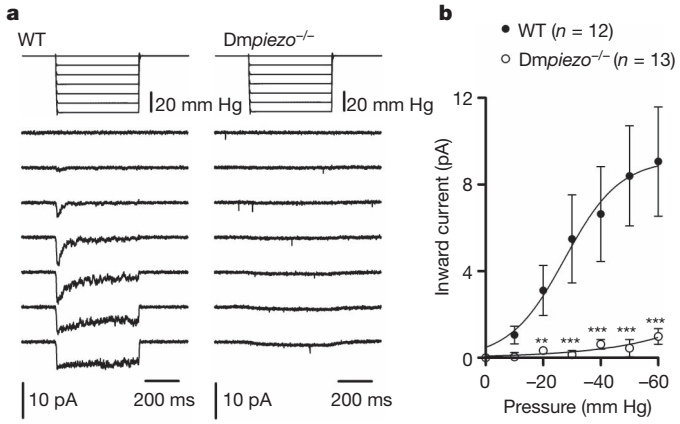

Figure 3 | Dm*piezo* mediates mechanically activated currents in *ppk*-positive neurons. **a**, Representative currents elicited by negative pipette pressure (0 to −60 mm Hg, Δ10 mm Hg) in cell-attached configuration at −80 mV in wild type (left) and Dm*piezo*$^{-/-}$ (right). **b**, Average peak current–pressure relationship of stretch-activated currents in wild type ($n = 12$ cells) and Dm*piezo*$^{-/-}$ ($n = 13$ cells). Data points are mean ± s.e.m. fitted with a Boltzmann equation. $^{**}P < 0.01$, $^{***}P < 0.001$, Mann–Whitney test.

confirming functionality (Supplementary Fig. 5a–c). When expressed in *Drosophila*, GFP–DmPiezo fluorescence was present throughout cell bodies, axons and dendritic arborizations of *ppk*-positive neurons (Supplementary Fig. 5d). Importantly, expression of GFP–DmPiezo in *ppk*-positive neurons alone was sufficient to rescue the mechanical nociception defect of Dm*piezo* knockout larvae (Fig. 2c). These data suggest that Dm*piezo* functions in *ppk*-positive neurons to mediate mechanical nociception.

To test if the *ppk*-positive neurons respond to mechanical stimuli and if Dm*piezo* mediates such responses, we performed electrophysiological recordings from isolated cells. Larvae that had GFP labelling in *ppk*-positive neurons were dissociated using enzymatic digestion and mechanical trituration. Plated fluorescent neurons were then tested using patch-clamp recordings in the cell-attached configuration, and they were stimulated using negative pressure through the recording pipette[10]. Stimulating wild-type neurons resulted in a current that was rapidly activated and had a half-maximal activation (P_{50}) of 27.6 ± 7.6 mm Hg (Fig. 3). These currents were not observed in the Dm*piezo* knockout mutant neurons (Fig. 3). Therefore, *ppk*-positive neurons, which mediate the avoidance response to noxious stimuli, display Dm*piezo*-dependent, mechanically activated currents.

Silencing of *ppk* cells resulted in complete abolition of noxious mechanosensation (Supplementary Fig. 6), in accord with the severe defect previously observed[16]. In contrast, only a moderate deficit is observed upon eliminating or knocking down *ppk* in the same cells[9], suggesting that there are multiple pathways for mechanical sensing. We tested mechanical nociception in larvae that are deficient in Dm*piezo* and either *pain* or *ppk* to gain insight into cellular pathways that involve mechanotransduction in these cells. Once again, we used a 45 mN filament, enabling us to monitor both Dm*piezo*-dependent and independent mechanisms (Fig. 1b). The Dm*piezo*::*pain* double mutant had a defect that was comparable to each one of the mutants separately, suggesting that Dm*piezo* and *pain* might function in the same pathway (Fig. 4a). Larvae that are heterozygous for both Dm*piezo* and *pain* showed a response deficit whereas each one of them separately was normal (Fig. 4b), further demonstrating their role in a common signalling mechanism. Remarkably, combining both Dm*piezo* and *ppk* knockdowns resulted in a nearly complete abolishment of responses to noxious mechanical stimuli (Fig. 4c). Importantly, responses to noxious temperatures and touch were normal in larvae with both Dm*piezo* and *ppk* knocked down (Fig. 4d, e). These data indicate that Dm*piezo* and *ppk* function in two parallel pathways in *ppk*-positive sensory neurons, and that together they constitute the response to noxious mechanical stimuli. There could be many reasons why the mechanically activated currents we observe are entirely dependent on DmPiezo (Fig. 3). This could either be because PPK responds to a different modality of mechanical stimulus or due to the specific experimental settings (for example, level of applied forces, solutions, applied voltage). Future experiments should resolve this issue.

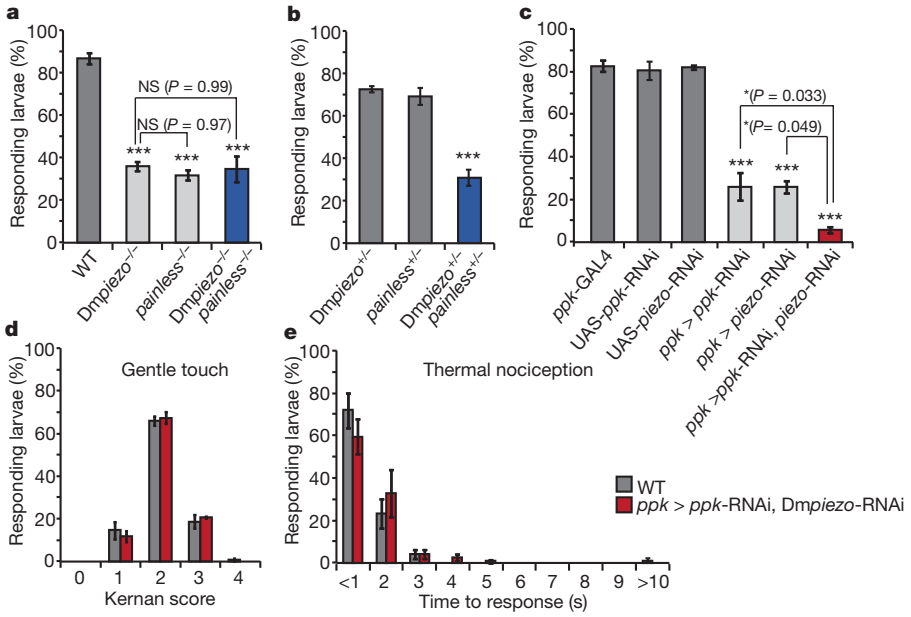

Figure 4 | Dm*piezo* and *ppk* function in parallel pathways. **a**, Mechanical nociception assay using a 45 mN von Frey filament with double-null mutant of Dm*piezo* and *painless*. Single-knockout strains were used as controls and the wild-type strain is w^{1118}. $n > 60$. **b**, Mechanical nociception assay on heterozygous larvae for Dm*piezo* and/or *pain*. *n* (heterozygote Dm*piezo* knockout) = 74 from three trials, *n* (heterozygote *painless*^{1}) = 169 from five trials, *n* (trans-heterozygote) = 166 from five trials. **c**, Mechanical nociception assay with *ppk* and Dm*piezo* knockdown. *ppk* and/or Dm*piezo* RNAi were driven by *ppk*-GAL4. $n > 90$. $^{*}P < 0.5$, $^{***}P < 0.001$. **d**, Gentle touch sensitivity assay with *ppk* and Dm*piezo* knockdown. For details about the Kernan score, see Methods. Wild type is w^{1118}. $n > 90$. **e**, Thermal nociception assay using 45 °C probe with *ppk* and Dm*piezo* knockdown. $n > 75$. Error bars indicate mean ± s.e.m.

Using the *Drosophila* model system we have demonstrated that *piezo* is essential for sensing noxious mechanical stimulus *in vivo*. This is the first demonstration that a Piezo family member is essential for mechanotransduction in the whole animal. Indeed, Dm*piezo* is, to our knowledge, the first eukaryotic excitatory channel component shown to be activated by mechanical force in a heterologous expression system and required for sensory mechanotransduction *in vivo*. Piezo2 is expressed in mouse dorsal root ganglion neurons that are involved in sensing nociception, and is required for rapidly adapting mechanically activated currents in such isolated neurons[10]. This study raises the possibility that mammalian Piezo2 is also required for mechanical pain transduction *in vivo*. Furthermore, *Drosophila* genetics can now be used to map cellular pathways involved in *piezo*-dependent mechanotransduction in sensory neurons and beyond.

METHODS SUMMARY

Fly stocks. PiggyBacs (PBac{WH}CG8486-f02291, PBac{RB}CG8486-e00109; Exelixis Collection at the Harvard Medical School), *ppk*-GAL4 (Bloomington *Drosophila* Stock Center (BDSC), 32078, 32079), Deficiency (Df(2L)Exel7034/CyO; BDSC, 7807), UAS-Dm*piezo*-RNAi (National Institute of Genetics, Japan, 8486R-3), UAS-*ppk*-RNAi (Vienna *Drosophila* RNAi Center, 108683), *ppk*-EGFP5 (ref. 22; Y. N. Jan), *painless*[1] (BDSC, 27895).

Generating Dm*piezo* knockout flies. The Dm*piezo* knockout fly was generated by FLP-FRT recombination with two PiggyBac lines as described previously[15]. The recombined knockout fly was confirmed by PCR (Supplementary Fig. 3). The genetic background was cleaned using meiotic recombination with w^{1118}.

Imaging. Fluorescence in adult fly or larva was detected by Nikon C2 Confocal Laser Point Scanning Microscope, Olympus FluoView500 Confocal Microscope or Olympus AX70 microscope.

Cloning. To clone the enhancer/promoter of the Dm*piezo* gene, the genomic region between 1.0 kb upstream of the beginning of transcription and the start codon of Dm*piezo* was amplified by PCR and cloned into the pPTGAL vector. The GFP–DmPiezo construct has three alanines as a linker between the carboxy-terminal GFP and amino-terminal DmPiezo. The construct was cloned in modified pUAST vector to generate transgenic flies and in modified pIRES2-EGFP vector for electrophysiology recordings.

Behavioural assays and statistics. The mechanical nociception was tested as described previously[9,14,16] using calibrated von Frey filaments. The thermal nociception was tested as described previously[14] using a 45 °C heated metal probe. All error bars represent mean ± s.e.m.

Isolation of *ppk*-positive neurons. Third instar larvae that had GFP labelling in *ppk*-positive neurons were dissected, digested with collagenase and mechanically triturated. The cells were collected by centrifugation and plated on a poly-D-lysine-coated glass coverslip. The fluorescent *ppk*-positive cells were recorded after incubating for 2 h at room temperature (23–25 °C).

Electrophysiology. HEK cells were studied in the whole cell configuration using a polished glass probe for stimulation[10] and *ppk*-positive neurons were stimulated using negative pressure in the cell attached configuration[10].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions S.E.K. conducted most experiments. B. Coste performed the electrophysiology experiments shown in Fig. 3 and Supplementary Fig. 5. A.C. performed the fly electrophysiology experiments shown in Supplementary Fig. 4. S.E.K., A.P. and B. Cook designed experiments and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to B.C. (bcook@scripps.edu) or A.P. (apatapou@gnf.org).

METHODS

Fly stocks. We used the following stocks: PiggyBacs (PBac{WH}CG8486-f02291, PBac{RB}CG8486-e00109, Exelixis Collection at the Harvard Medical School), *ppk*-GAL4 (Bloomington *Drosophila* Stock Center (BDSC), 32078, 32079), Deficiency (Df(2L)Exel7034/CyO, BDSC, 7807), UAS-Dm*piezo*-RNAi (National Institute of Genetics, Japan, 8486R-3), UAS-*ppk*-RNAi (Vienna *Drosophila* RNAi Center, 108683), *ppk*-EGFP5 (ref. 22; Y. N. Jan), *painless*[1] (BDSC, 27895) and UAS-DsRed-NLS (J. W. Posakony). The following stocks were from BDSC: UAS-GFP, UAS-CD8::GFP, CyO-GFP, w^{1118} and *Canton-S*.

Engineering Dm*piezo* knockout flies. The Dm*piezo* knockout fly was generated as described in previously described[15]. Two PiggyBac lines that carry the FRT sequence were selected for FLP-FRT recombination. PBac{WH}CG8486-f02291 is inserted in the first intron and PBac{RB}CG8486-e00109 in the 3′ untranslated region (UTR) of the Dm*piezo* gene. After FLP-FRT recombination, 20 kb of the Dm*piezo* gene, including all 31 coding exons, was removed and replaced with 7 kb of PiggyBac insertion that contained the FRT sequence and white gene. The recombined knockout fly was confirmed by PCR reactions (Supplementary Fig. 2). The genetic background was cleaned using meiotic recombination with w^{1118}.

Molecular biology. To clone the enhancer/promoter of the Dm*piezo* gene, the genomic region between 1.0 kb upstream of the beginning of transcription and the start of the Dm*piezo* coding region was amplified by PCR using forward primer, 5′-ATCTGGCGGCCGCTATCTATTTTTTAACTAGTGGAAGTCT-3′ and reverse primer, 5′-TTACTGGTACCATGGATGCCTCCGGCGCCGTTC TCCTCCAG-3′. The amplified sequence was cloned into pPTGAL vector (*Drosophila* Genomic Resource Center, 1225) using NotI and KpnI sites and the sequence was verified.

For rescue experiments, Dm*piezo* cDNA was amplified from the plasmid reported in ref. 11, using forward primer 5′-TATTAGCGGCCGCAGTCTTCA GCTATGCGTGCATGGTG-3′ and reverse primer 5′-TAATTCGGTCCGTTAT TGCGGTTGCTGTGGCTGCAGTTGCTCCGG-3′ and cloned into a modified pUAST vector using NotI and RsrII. NotI restriction enzyme site was used as a linker by providing three alanine residues between EGFP and DmPiezo. The order of sequences in the pUAST vector is the following: UAS-kozak-EGFP-3×(Ala)-DmPiezo. To generate transgenic flies, DNA was injected into the isogenized w^{1118} embryos along with transposase Δ2–3. For the electrophysiology experiment, EGFP–DmPiezo was cloned into mammalian expression vector with CMV promoter.

Behaviour assays. Mechanical nociception was tested as described previously[9,14,16] using calibrated von Frey filaments. Thermal nociception was tested as described previously[14] using a calibrated heated metal probe. For both nociception assays, the number of larvae that showed at least one 360° rotation was counted for each trial. The gentle touch assay was performed and each stimulated larva was scored as described previously[17]. 0 = no response, 1 = hesitates, 2 = turns or withdraws anterior segments, 3 = single reverse contractile wave, and 4 = multiple waves. For all behaviour assays each third instar larva was stimulated only once. All data were generated from at least three trials.

The von Frey filaments for larvae behaviour experiments were modified from Touch-Test sensory Evaluator (North Coast Medical) or from monofilament fishing lines. Each monofilament was cut to a length of 18 mm, glued into a pipette tip so that 9 mm of it protruded and mounted on a hand manipulator with a 90° angle. Each von Frey filament was calibrated as described previously[9]. The force of each von Frey stimulator was determined by measuring the weight upon filament bending and converting the value into the force: force (mN) = mass (g) × gravity acceleration constant (*g*; 9.8). Each stimulator was calibrated 15 times and its mean value was used in figures. The calibrated forces (mean ± s.e.m.) of each stimulator are as follows (in mN): 1.78 ± 0.15, 2.59 ± 0.15, 5.04 ± 0.19, 11.2 ± 0.66 and 59.9 ± 1.79.

Fluorescence imaging. For identifying tissues or cells expressing fluorescence by the Dm*piezo* promoter, both adult flies and third instar larvae carrying Dm*piezo*P-GAL4 and UAS-GFP, or UAS-CD8::GFP, were dissected or whole-mounted. For double fluorescent labelling in multidendritic neurons, second instar larvae carrying *ppk*-EGFP, Dm*piezo*P-GAL4 and UAS-DsRed were whole-mounted. For imaging *ppk*-cells expressing GFP–DmPiezo, third instar larvae carrying *ppk*-GAL4 and UAS-GFP–DmPiezo were whole-mounted. Fluorescence images were obtained either by Nikon C2 Confocal Laser Point Scanning Microscope, Olympus FluoView500 Confocal Microscope or Olympus AX70 microscope.

Isolation of larvae *ppk*-positive neurons. In both wild-type and Dm*piezo* knockout larvae, *ppk*-positive neurons were fluorescently labelled by *ppk*-EGFP, which is a direct fusion of *ppk* genomic regulatory regions with EGFP. Third instar larvae were dissected in M3 media containing 10% heat inactivated FBS. Each larva was cut twice and its internal organs were removed. The cleaned body wall was treated with 5 mg ml^{-1} collagenase type IV at 25 °C for 1 h in serum-free M3 media and washed with serum containing M3 media. The enzyme-treated body wall was triturated with fire-polished Pasteur pipettes in M3 media with 2 mM EGTA and 10% FBS. The cuticle and debris were removed by centrifugation at 40*g* and the small size cells including neurons were collected by centrifugation at 360*g* for 10 min. The cell pellet was resuspended with serum containing M3 media and plated into a poly-D-lysine-coated coverslip in a small droplet. After 2 h of incubation at room temperature (23–25 °C), the coverslips were transferred to the electrophysiology rig for recording.

Electrophysiology. For whole-cell recordings in HEK293T cells, patch pipettes had resistances of 2–3 MΩ when filled with an internal solution consisting of (in mM) 133 CsCl, 10 HEPES, 5 EGTA, 1 $CaCl_2$, 1 $MgCl_2$, 4 MgATP and 0.4 Na2GTP (pH adjusted to 7.3 with CsOH). The extracellular solution consisted of (in mM) 130 NaCl, 3 KCl, 1 $MgCl_2$, 10 HEPES, 2.5 $CaCl_2$, 10 glucose (pH adjusted to 7.3 with NaOH). Mechanical stimulation was achieved using a fire-polished glass pipette (tip diameter 3–4 μm). The probe had a velocity of 1 μm ms^{-1} during the ramp segment of the command for forward motion and the stimulus was applied for 150 ms.

For cell-attached recordings in *ppk*-positive dissociated neurons, patch pipettes had resistances of 3–3.5 MΩ when filled with a solution consisting of (in mM) 130 NaCl, 5 KCl, 10 HEPES, 1 $CaCl_2$, 1 $MgCl_2$, 10 TEA-Cl (pH 7.3 with NaOH). External solution used to zero the membrane potential consisted of (in mM) 140 KCl, 10 HEPES, 1 $MgCl_2$, 10 glucose (pH 7.3 with KOH). Membrane patches were stimulated with brief negative pressure pulses through the recording electrode using a Clampex controlled pressure clamp HSPC-1 device (ALA-scientific). Stretch-activated channels were recorded at a holding potential of −80 mV with pressure steps from 0 to −60 mm Hg (−10 mm Hg increments). Current–pressure relationships were fitted with a Boltzmann equation of the form: $I(P) = (1 + \exp(-(P - P_{50})/s))^{-1}$, where I is the peak of stretch-activated current at a given pressure, P is the applied patch pressure (in mm Hg), P_{50} is the pressure value that evoked a current value which is 50% of I_{max}, and s reflects the current sensitivity to pressure.

All experiments were performed at room temperature. Currents were sampled at 50 or 20 kHz and filtered at 5 or 2 kHz. Voltages were not corrected for a liquid junction potential. Leak currents before mechanical stimulations were subtracted off-line from the current traces.