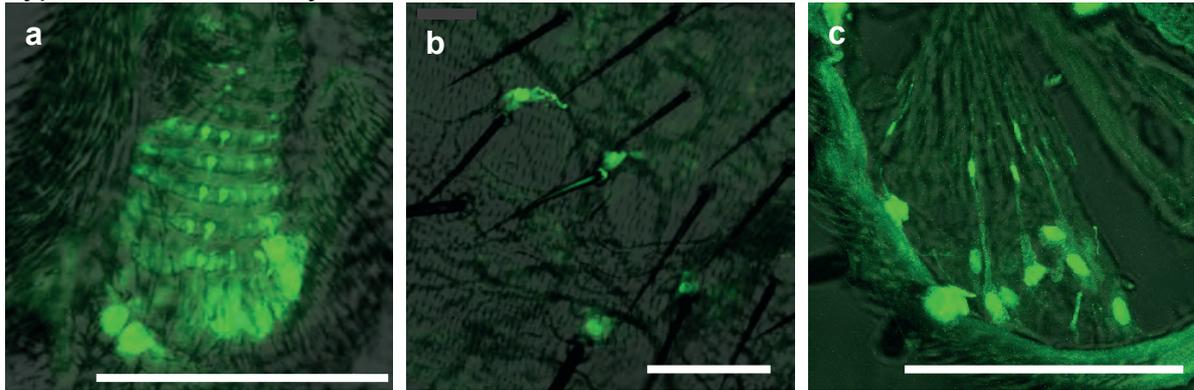


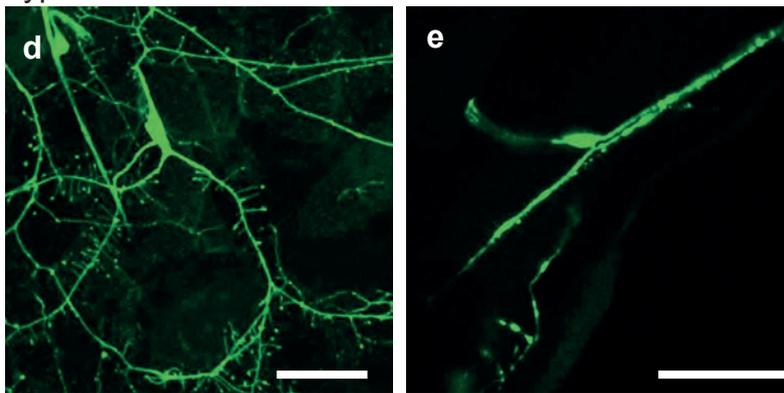
Supplementary Figure 1: Hydropathy plot of cloned *dpiezo*.

The Kyte-Doolittle hydropathy plot and TMHMM 2.0 transmembrane prediction were calculated with the cloned dPiezo protein sequence¹⁰.

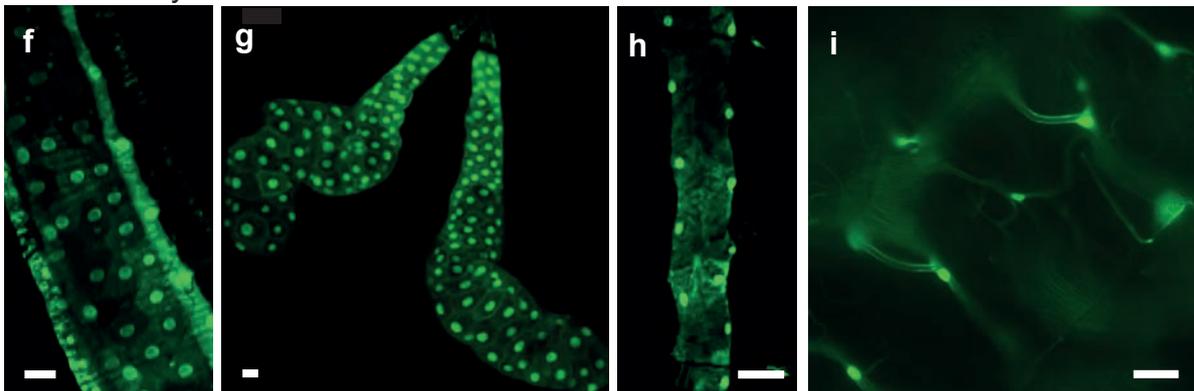
Type I ciliated sensory neurons



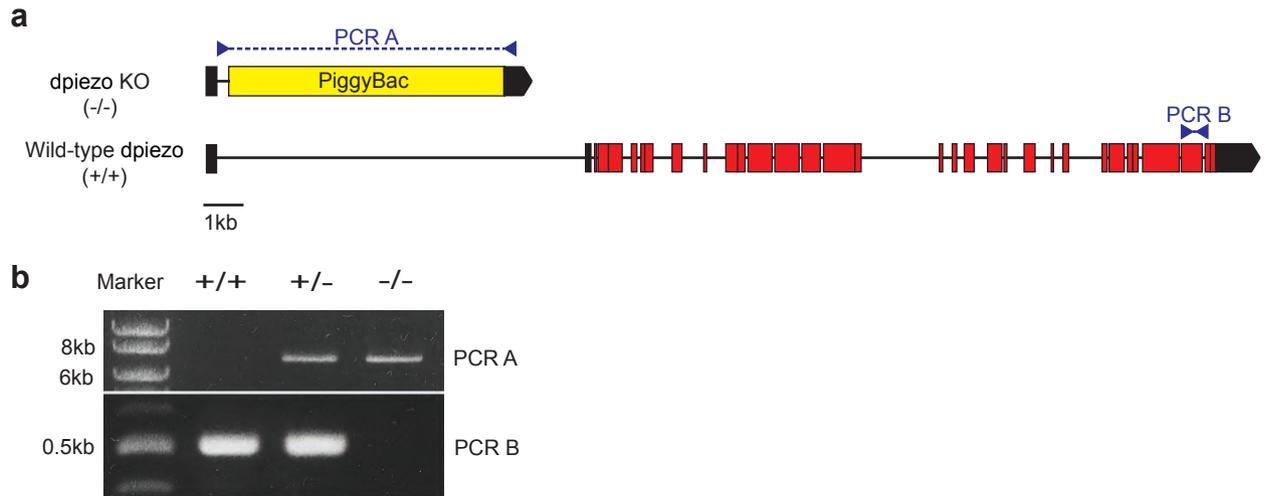
Type II multidendritic neurons



Non-sensory tissues

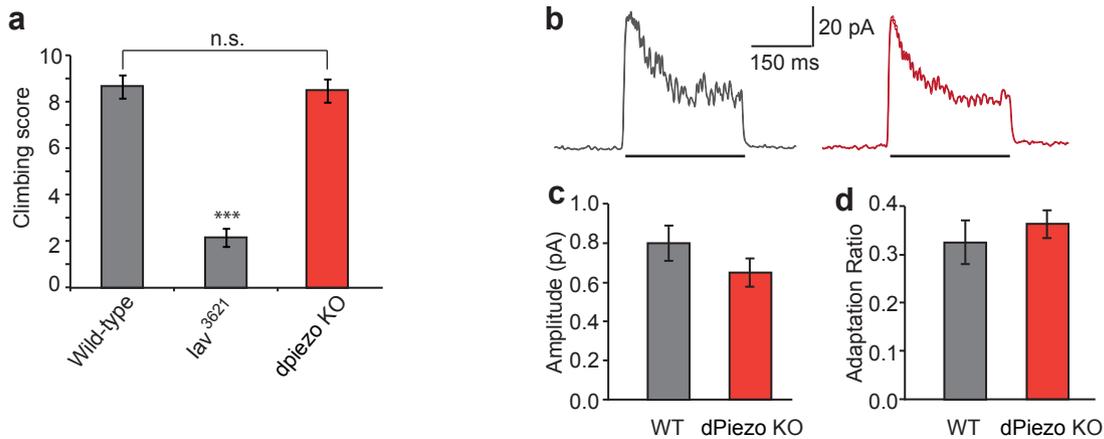
**Supplementary Figure 2: Expression pattern of dpiezo.**

GFP fluorescence, driven by *dpiezo* promoter, was detected in adult or larva. (a) Campaniform sensillar in the adult haltere. (b) External sensory neurons in the adult thoracic bristles. (c) Chordotonal neurons in the adult second antenna segment (Johnston's organ). (d) Class III dendritic arborization multidendritic (md) neurons in larva. (e) Larval bipolar md neurons. (f) Larval hindgut. (g) Larval salivary gland. (h) Larval aorta. (i) Larval trachea. Images of adult sensory structures are presented as overlays of GFP fluorescence and brightfield (a-c) and images from third instar larvae show only the fluorescence (d-i). UAS-GFP is the reporter in all panels except for (d,e) where UAS-CD8::GFP was used. Scale bars represent 50 μm .



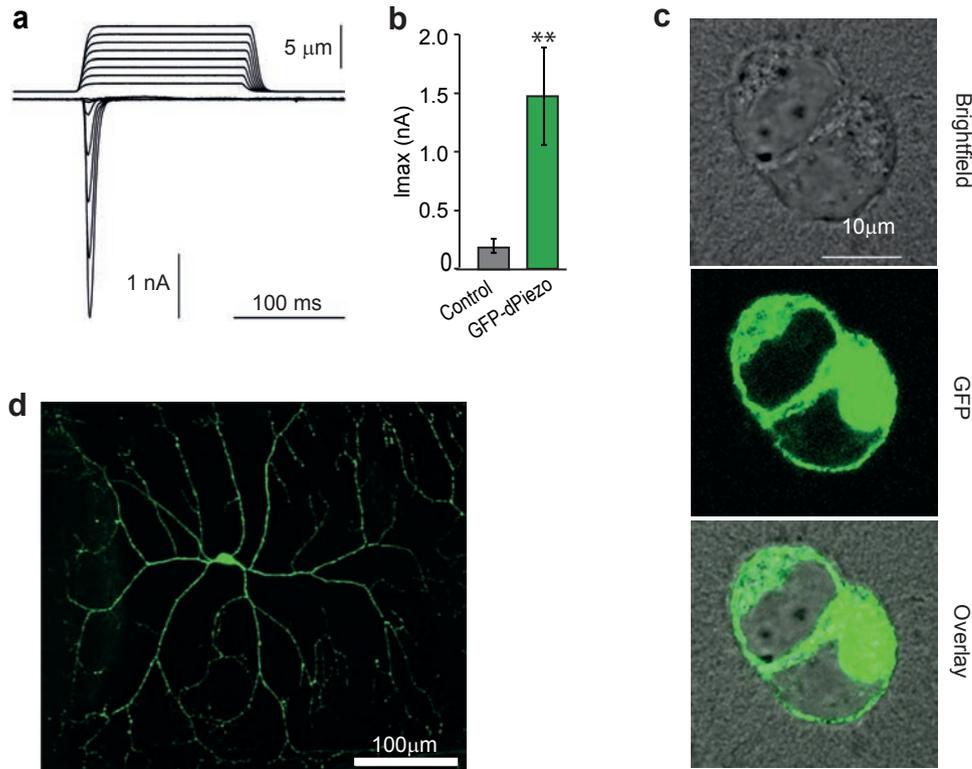
Supplementary Figure 3: PCR confirmation of *dpiezo* knockout.

The FLP-FRT recombined *dpiezo* KO was confirmed by PCRs using extracted genomic DNA from adult flies. (a) PCR A was designed to amplify 7.7 kbp only in the KO chromosome with primers, 5'-TGTGGTAAGACACCGTGGAAGTTGG-3' and 5'-GAAATTTCCATTCGAAGCCA-3'. PCR B was designed to amplify 501bp only in the wild-type chromosome with primers, 5'-CGAGCAGCACTCATTACAT-3' and 5'-GAAGACTTCATAAAGCGCG-3'. (b) PCR results of wild-type (w^{1118} , +/+), heterozygous *dpiezo* KO (+/-) and homozygous KO (-/-).



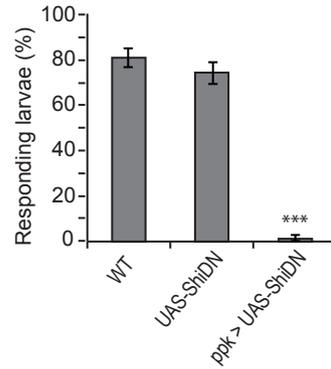
Supplementary Figure 4: Coordination and mechanoreceptor potential in adult *dpiezo* knockout flies.

(a) Climbing assay was conducted as described²³. Ten male flies of day 3 to 7 were placed in an assay tube and the number of flies climbing above 15 cm within 15 seconds was defined as the climbing score. All assays were conducted between 9 and 11 AM. Total number of trials is six. *** $p < 0.001$. Canton-S was used for wild-type. *Iav*³⁶²¹, which is an allele of *inactive* was used as a positive control and reproduced the phenotype described previously²³. Error bars represent mean \pm SEM. (b-d) Mechanoreceptor potential recordings from the anterior notopleural bristle. A trans-epithelial voltage of +40 mV was applied across the mechanosensory cell and the currents evoked by deflection of bristle toward the thorax were measured as described in Walker et al.⁵. (b) Representative trace for wild-type (black) and *dpiezo* KO (red). (c) Average current amplitudes. (d) Average adaptation, defined as the ratio between the steady-state and peak values. Wild-type is w^{1118} . $n > 5$. Error bars represent mean \pm SEM.



Supplementary Figure 5: Functionality of GFP-dPiezo.

(a, b) Mechanically activated currents of GFP-dpiezo cDNA expressed in HEK293T cells recorded in the whole-cell configuration at $V_h = -80$ mV. (a) Representative traces of mechanically activated currents in cells subjected to a series of mechanical $1 \mu\text{m}$ increment steps in applied using a glass probe. (b) Average maximal current amplitude of MA currents in GFP (control) or GFP-dPiezo transfected cells. $n = 12$. $** P < 0.01$, Mann-Whitney test. (c) A single confocal image of HEK293T cells expressing GFP fused to dPiezo. Fluorescence is observed throughout the cytoplasm and the plasma membrane. (d) GFP-dPiezo localization within the larval ppk-positive neuron. UAS-GFP-dPiezo was driven by ppk-GAL4.



Supplementary Figure 6: Silencing ppk-positive neurons.

Mechanical nociception assay with larvae when ppk-positive neurons were silenced by expressing a dominant-negative mutant of Shibere (ShiDN). $n > 65$ from three independent experiments. *** $P < 0.001$.

Supplementary Reference

- 23 Sun, Y. *et al.* TRPA channels distinguish gravity sensing from hearing in Johnston's organ. *Proc. Natl. Acad. Sci. USA* **106**, 13606-13611 (2009).