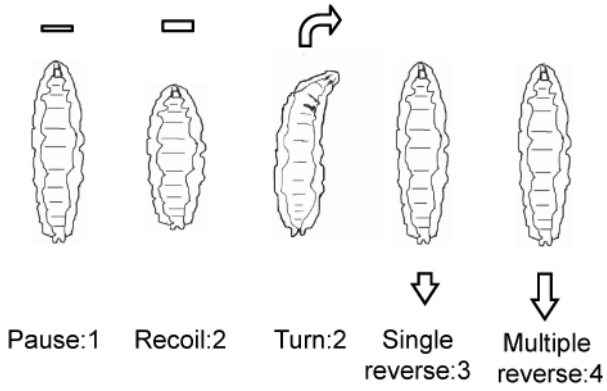
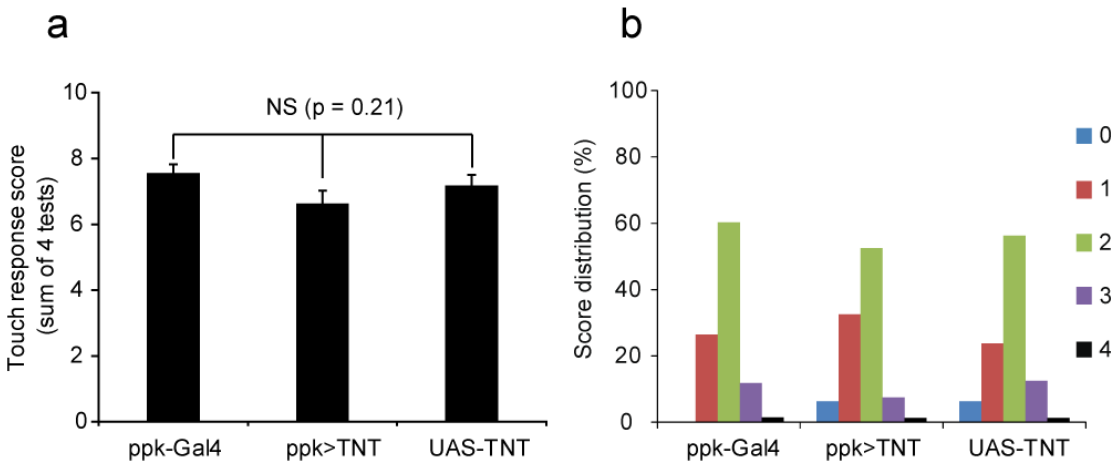
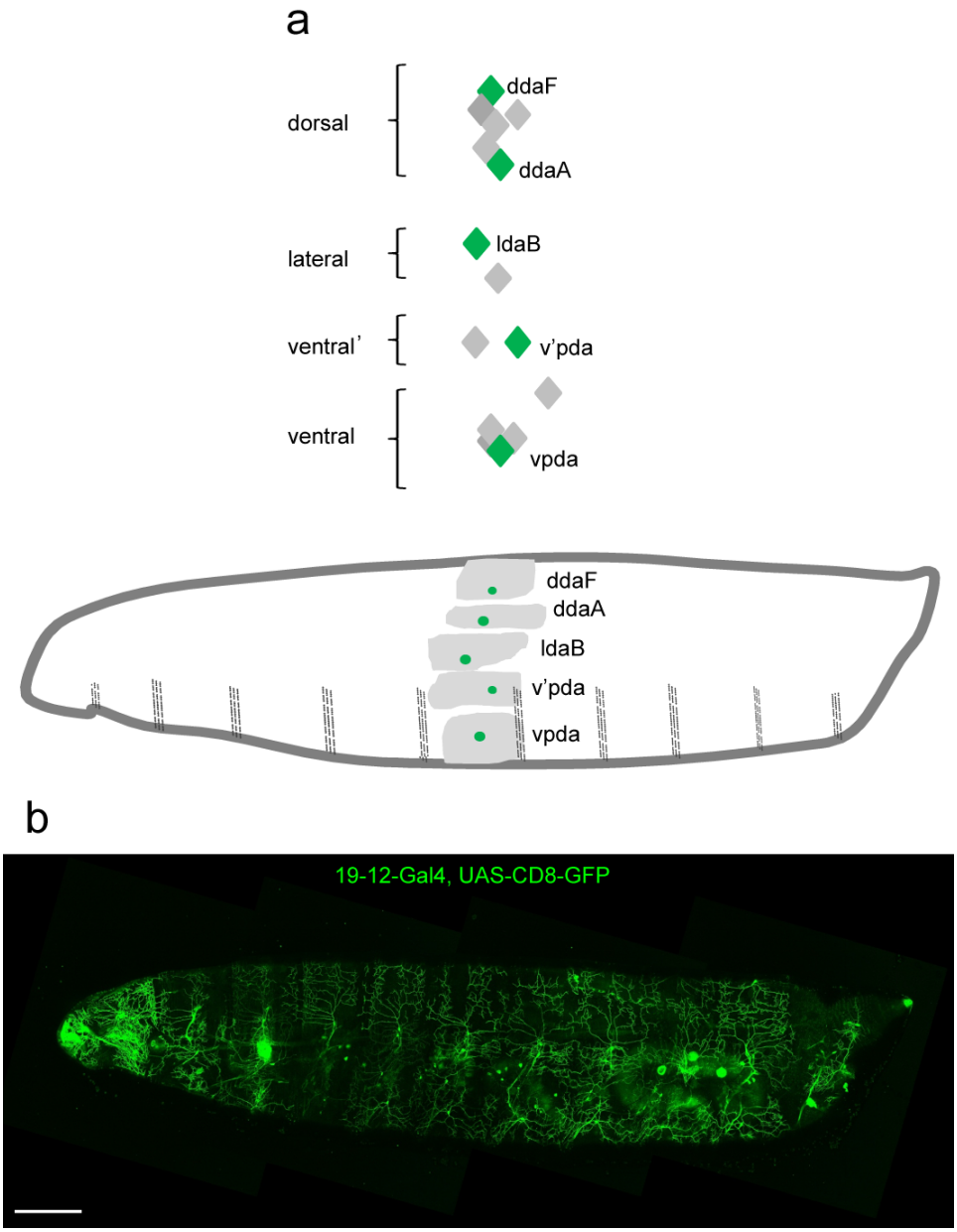


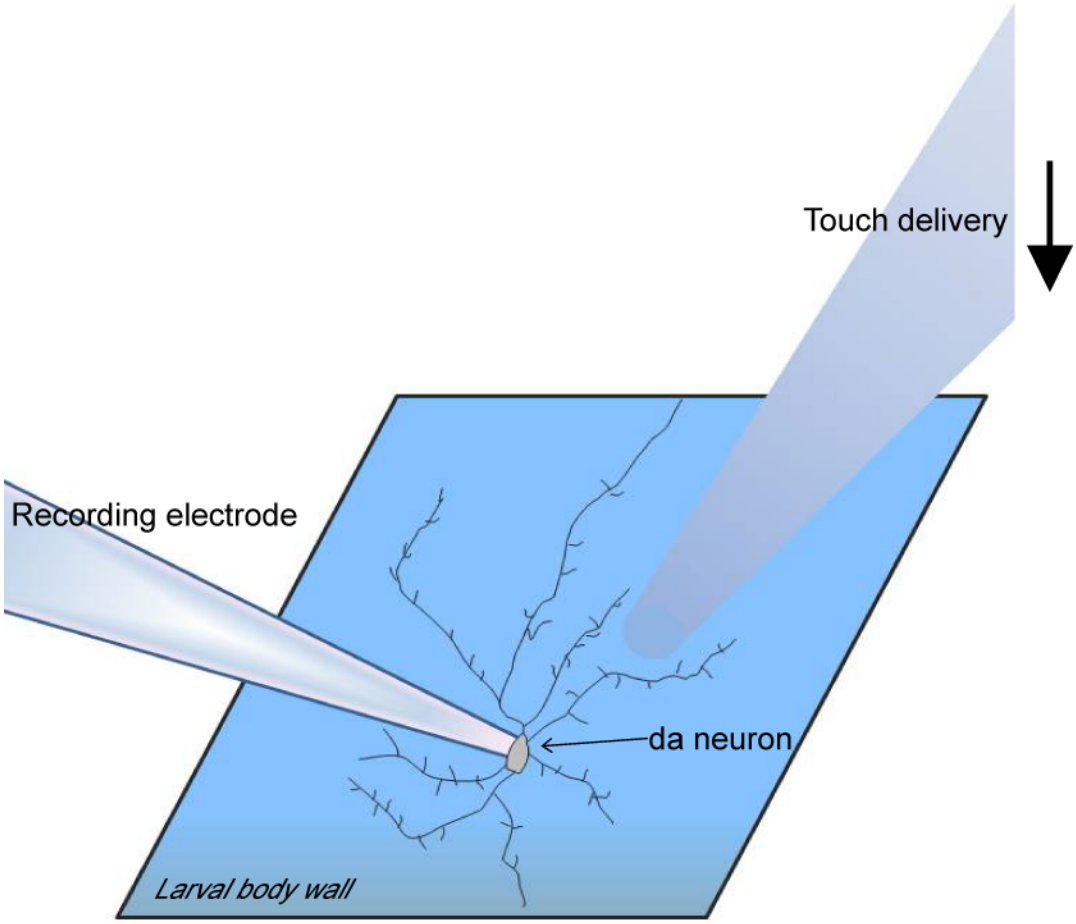
Supplementary Figure 1. Scoring method for the behavioral assay as in an earlier study[1]. 0: no response; 1: pause (hesitate); 2: recoil (anterior withdraw) with or without a turn; 3: single reverse contractile wave; 4: multiple waves of reverse contraction.



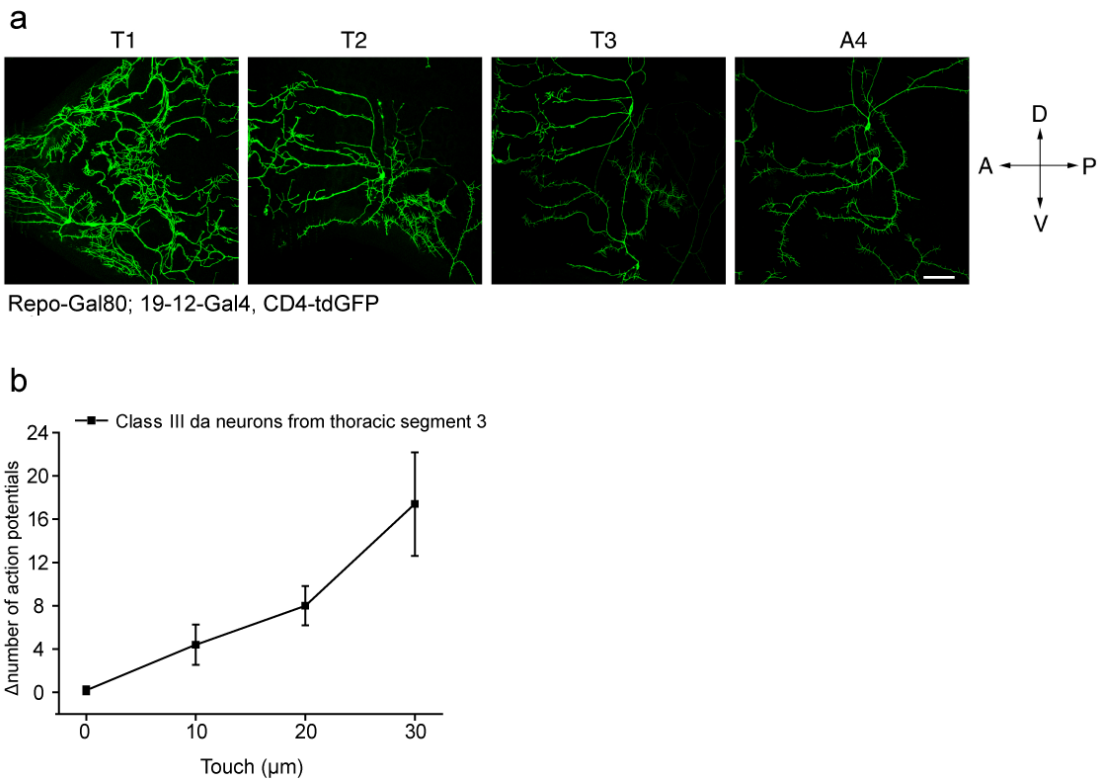
Supplementary Figure 2. a, Larval behavioral responses to gentle touch were normal with class IV da neurons silenced via TNT expression. TNT was specifically expressed in class IV da neurons by ppk-Gal4. UAS-TNT and ppk-Gal4 are present in single copies in all experiments. One-way ANOVA test was followed by Tukey's comparison, p = 0.21, n =17-20. **b,** Touch response score distribution of different genotypes. 0: no response; 1: pause (hesitate); 2: recoil (anterior withdraw) with or without a turn; 3: single reverse contractile wave; 4: multiple waves of reverse contraction.



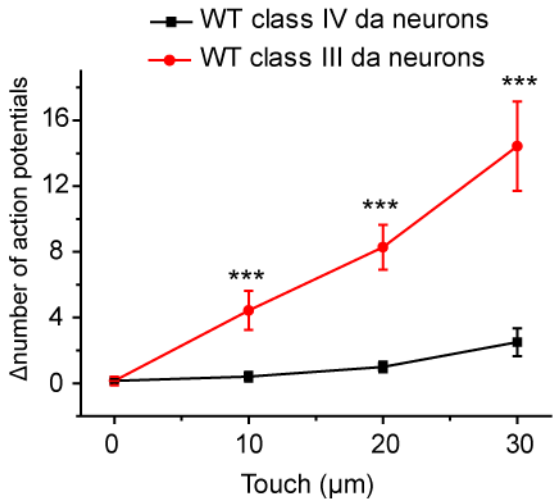
Supplementary Figure 3. a, Schematic of class III da neurons in an abdominal hemisegment of the larval body wall. Upper panel, green diamonds: class III da neurons, grey diamonds: other da neurons, d, l, v', v represent dorsal, lateral, ventral prime and ventral cluster. Lower panel, territories of different class III da neurons are marked in grey in the body wall[2], cell bodies of class III da neurons are marked with green dots. **b,** Class III da neurons labeled with 19-12-Gal4 driven CD8-GFP cover the larval body wall, scale bar: 200 μm.



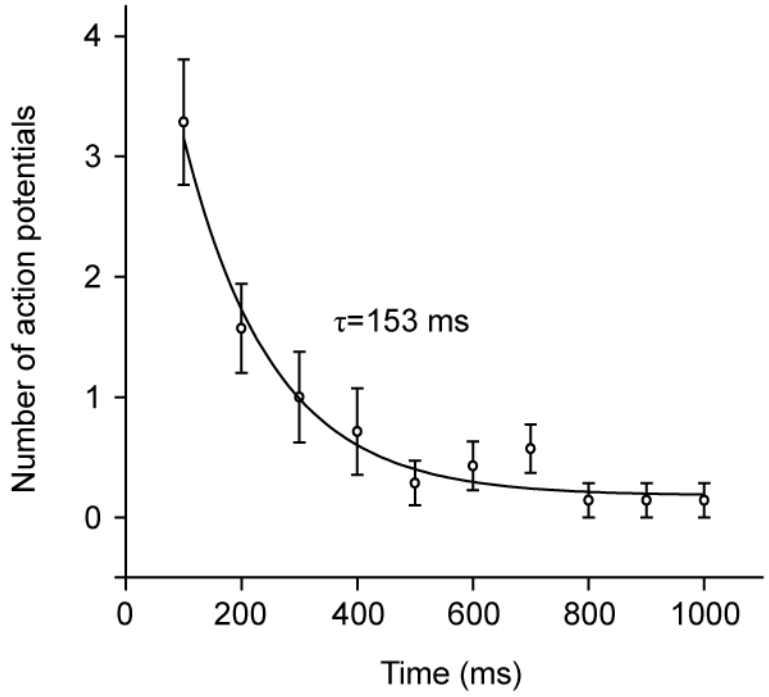
Supplementary Figure 4. Diagram for extracellular recording of a class III da neuron in response to touch, in the fillet preparation of a 3rd instar larva after removal of the muscle covering the da neurons. A touch stimulus was delivered using a sealed and smooth glass electrode to the larval body wall to produce a certain displacement in the dendrite field of a da neuron and the response was recorded.



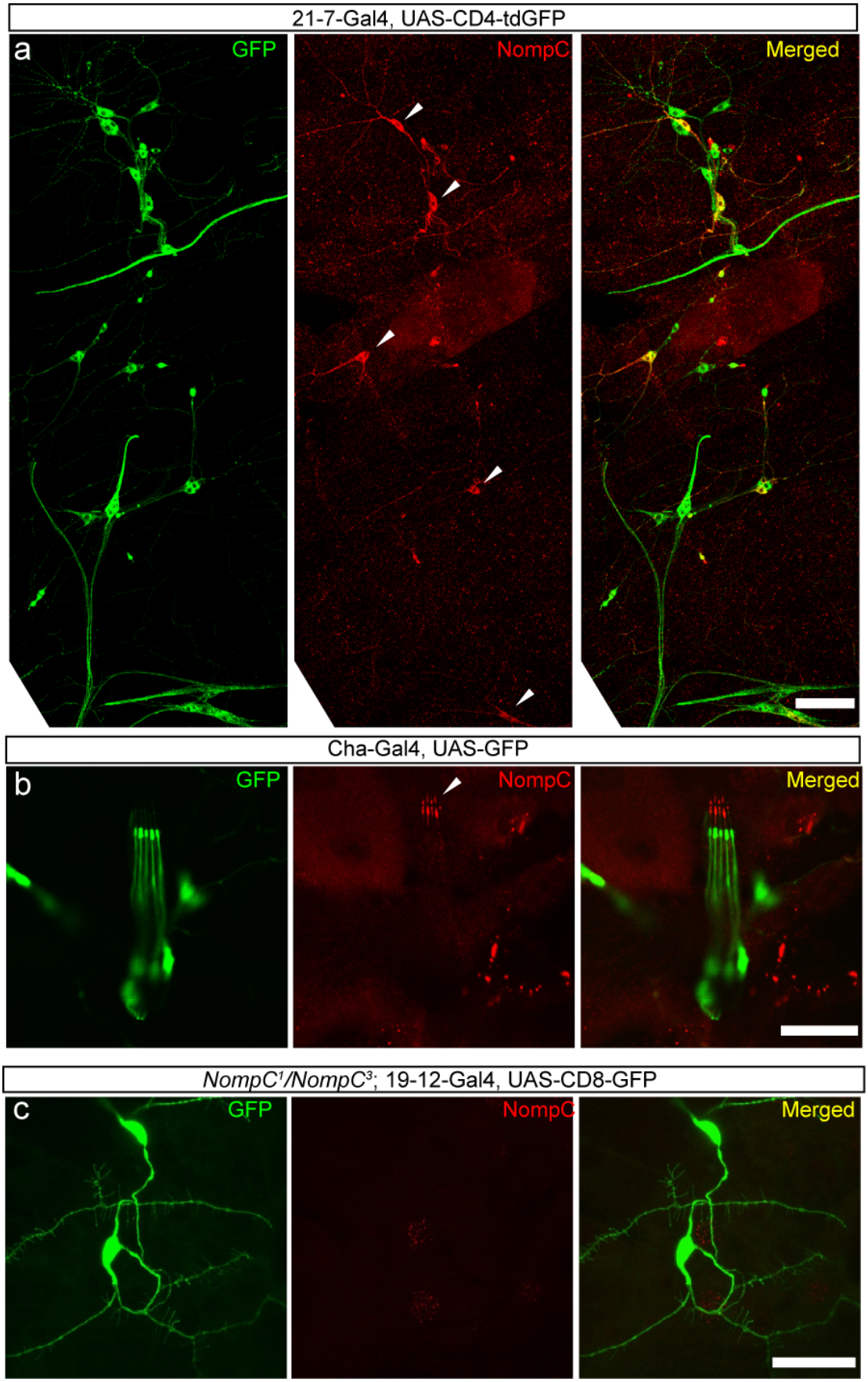
Supplementary Figure 5. a, Morphology of class III da neurons from thoracic segments (T1, T2 and T3). Shown on the right are class III da neurons in the fourth abdominal segment (A4) for comparison. A: anterior, P: posterior, D: dorsal, V: ventral. scale bar: 50 μm. **b,** Summary of action potential firing of class III da neurons in the third thoracic segment (T3) in response to progressively greater mechanical stimuli. Δnumber of APs: increase of the number of action potentials in 1 second after stimulus onset compared to 1 second before stimulus onset.



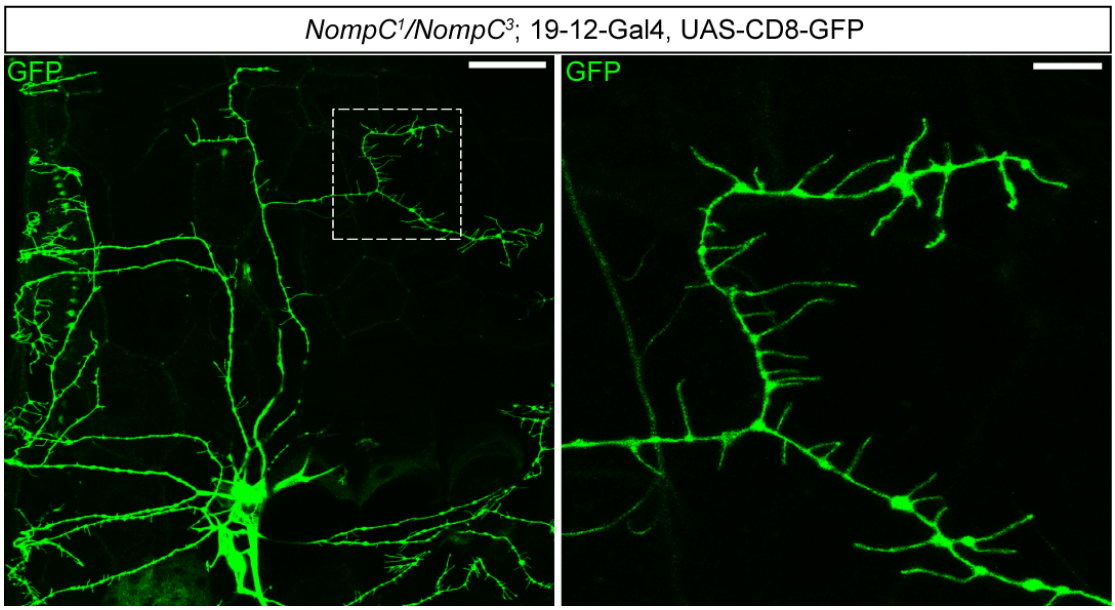
Supplementary Figure 6. Summary of action potential firing of class IV da neurons in abdominal segments in response to progressively greater mechanical stimuli. Wild type class IV da neurons were labeled by ppk-Gal4 and wild type class III da neurons were labeled by 19-12-Gal4. Class IV da neurons are insensitive to the touch stimuli that elicited response in class III da neurons. n = 8 and 6, ***p < 0.001, unpaired *t*-test.



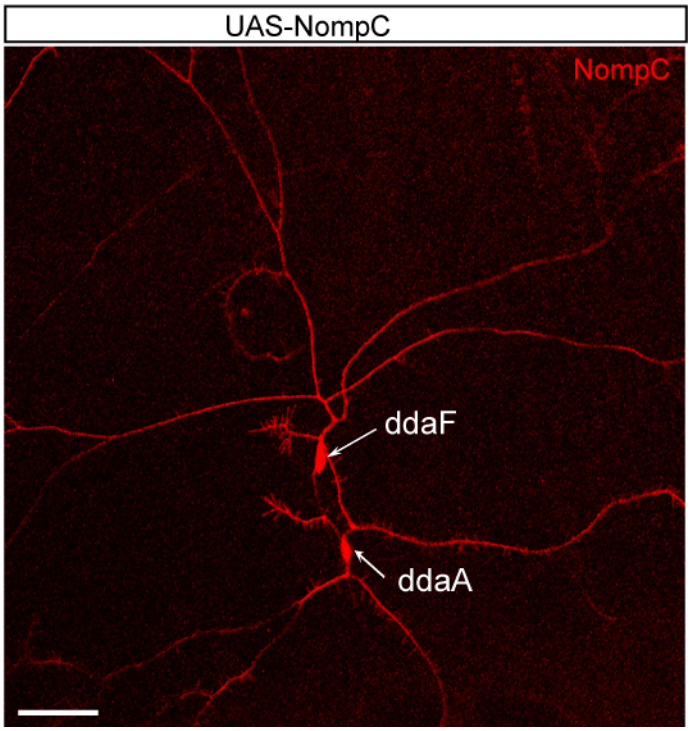
Supplementary Figure 7. Number of action potentials (bin width 100 ms) plotted as a function of time for the first second after onset of the stimulus, 20 μm touch displacements was used. Averages from 8 neurons displayed exponential decay (solid line). The time constant (τ = 153 ms) indicates the adaptation rate. Error bars represent s.e.m..


21-7-Gal4, UAS-CD4-tdGFP
a
GFP
NompC
Merged
Cha-Gal4, UAS-GFP
b
GFP
NompC
Merged
NompC1/NompC3; 19-12-Gal4, UAS-CD8-GFP
c
GFP
NompC
Merged

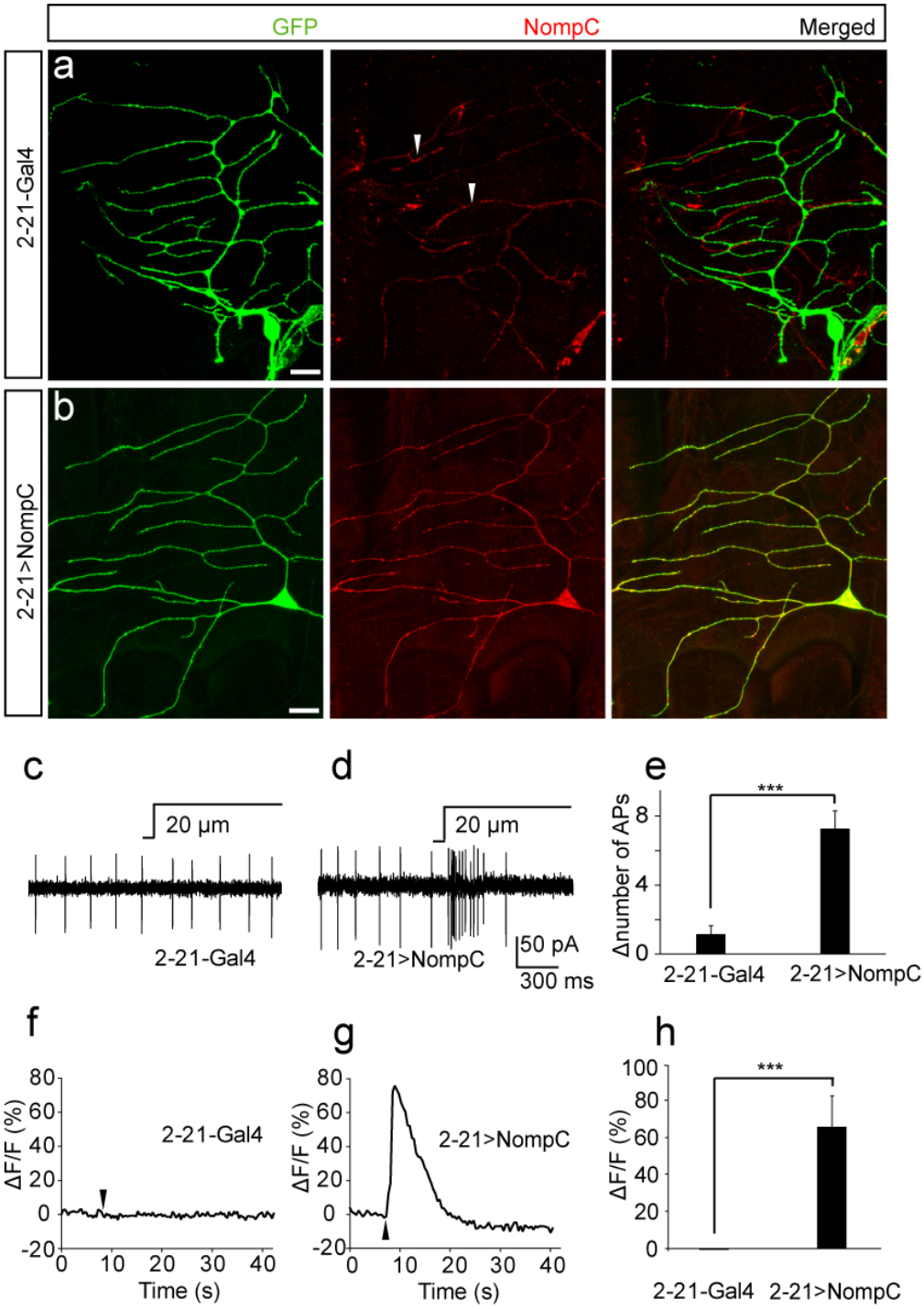
Supplementary Figure 8. a, All five class III da neurons of one hemisegment in the larval body wall showed strong NompC staining. GFP was expressed in all MD neurons driven by 21-7-Gal4. From top to bottom, arrowheads indicate the five class III da neurons (ddaF, ddaA, ldaB, v'pda, vpda) that tile the larval body wall. Scale bar: 50 μm. **b,** Chordotonal neurons showed strong NompC staining in wildtype larvae. The arrowhead indicates that the NompC staining is enriched in the dendritic tip. Scale bar: 25 μm. **c,** NompC staining was absent in class III da neurons in the *NompC* mutant. Scale bar: 25 μm.



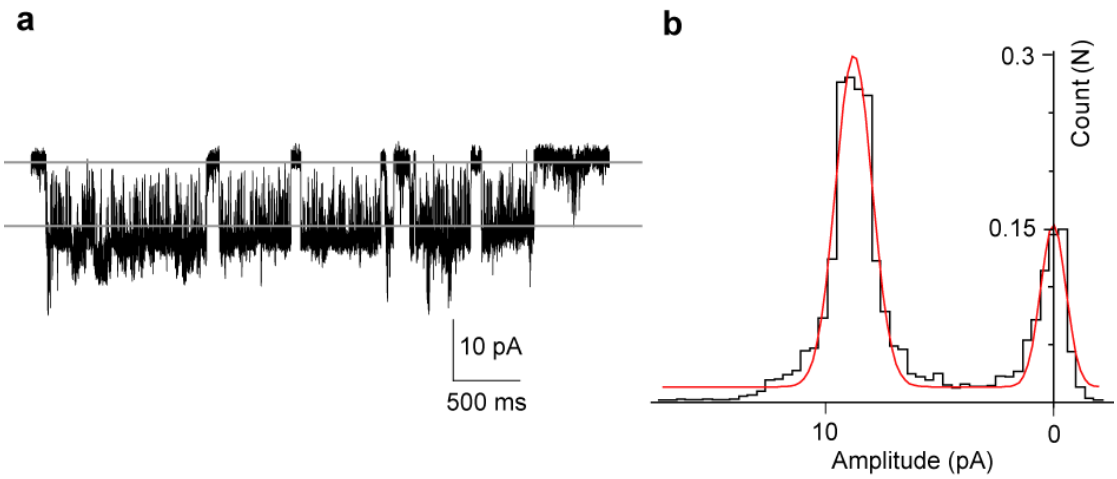
Supplementary Figure 9. Class III da neurons in the *NompC* mutant showed no obvious morphological abnormality. Left, class III da neurons in *NompC* mutant. Right, high magnification image from boxed region in the left panel showing the spike-like protrusions. Scale bar, left: 50 μm, right: 10 μm.



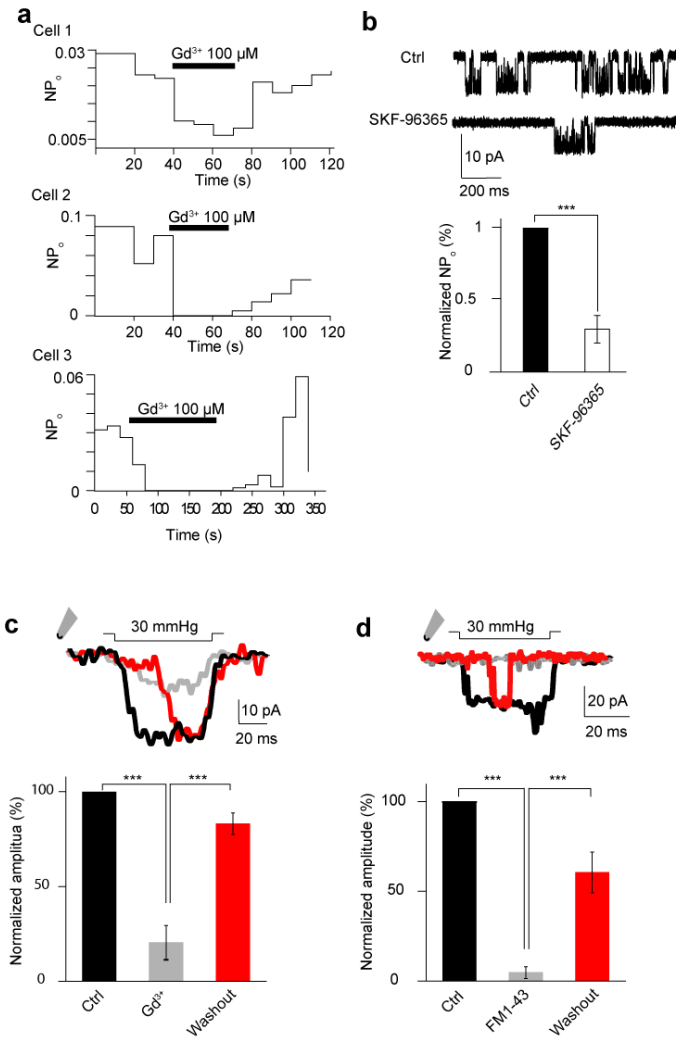
Supplementary Figure 10. NompC staining in UAS-NompC control in wild type background. NompC clearly labeled the class III da neurons ddaA and ddaF in the dorsal cluster but not class IV or class I da neurons in the dorsal cluster (reference schematic, Supplementary Figure 3), scale bar: 50 μm.


GFP
NompC
Merged
a
2-21-Gal4
b
2-21>NompC
c
20 μm
2-21-Gal4
d
20 μm
2-21>NompC
50 pA
300 ms
e
***
Δnumber of APs
8
4
0
2-21-Gal4
2-21>NompC
f
2-21-Gal4
ΔF/F (%)
80
60
40
20
0
-20
0
10
20
30
40
Time (s)
g
2-21>NompC
ΔF/F (%)
80
60
40
20
0
-20
0
10
20
30
40
Time (s)
h
***
ΔF/F (%)
100
80
60
40
20
0
2-21-Gal4
2-21>NompC

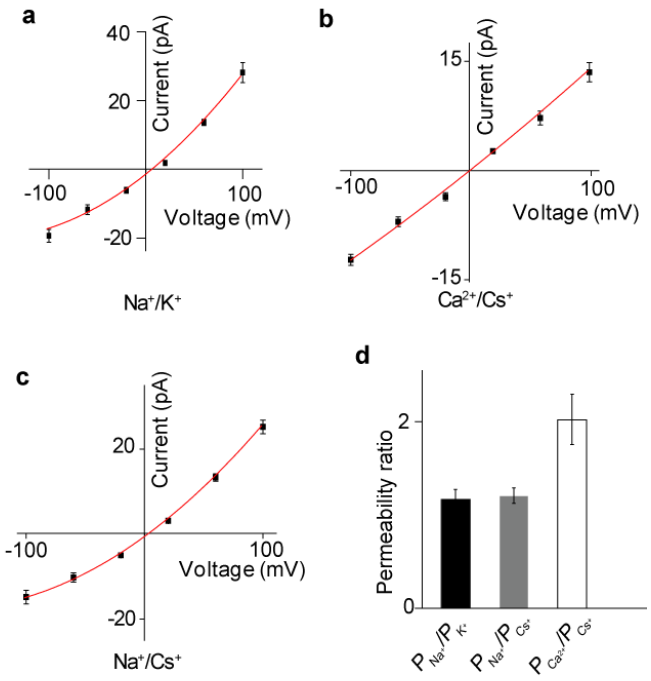
Supplementary Figure 11. Overexpression of NompC confers mechanosensitivity to normally touch insensitive class I da neurons. a, Class I da neurons showed no detectable NompC expression, while dendrites of class III da neurons nearby displayed NompC staining (indicated by arrowheads). 2-21-Gal4 was used to label class I da neurons with GFP. **b,** Class I da neurons showed strong NompC staining when NompC was overexpressed via 2-21-Gal4. Scale bars in (**a**, **b)**: 20 μm. (**c**, **f**) Class I da neurons did not show detectable electrophysiological (**c**) or calcium (**f**) response to mechanical stimuli, note class I da neurons often fire low frequency spontaneous action potentials. (**d**, **f)** Class I da neurons with NompC overexpression displayed action potential firing upon 20 μm touch displacements (**d**) and calcium rise with 40 μm touch displacements lasting 300 ms (**g)**. The same mechanical stimuli were used in (**f, g**); arrowheads indicate onset of the stimulus. (**e**, **h**) Group data of electrophysiological (**e**) and calcium (**h**) response to mechanical stimulation of class I da neurons from control larvae bearing the 2-21-Gal4 driver, and larvae with NompC expression in class I da neurons. Δnumber of APs: increase of the number of action potentials in 1 second after stimulus onset compared to 1 second before stimulus onset. Error bars indicate s.e.m., n = 7-10, ***$p < 0.001$, unpaired *t*-test.



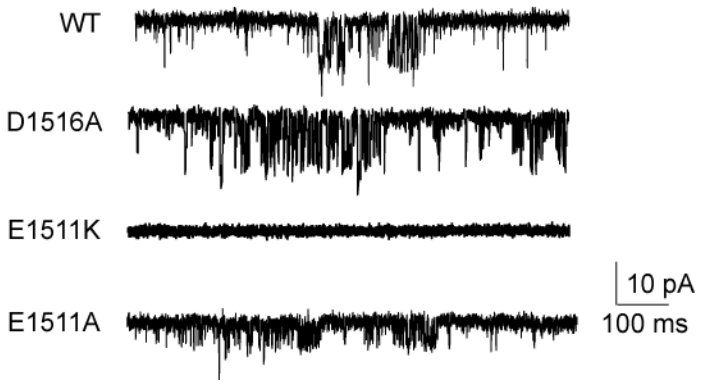
Supplementary Fig. 12. a, A 5 s current trace of spontaneously active channels from whole-cell recording of an S2 cell transfected with NompC-GFP at -60 mV. **b**, An all-event amplitude histogram plotted from NompC-induced spontaneous currents. Red line indicates the Gaussian fit for the histogram.



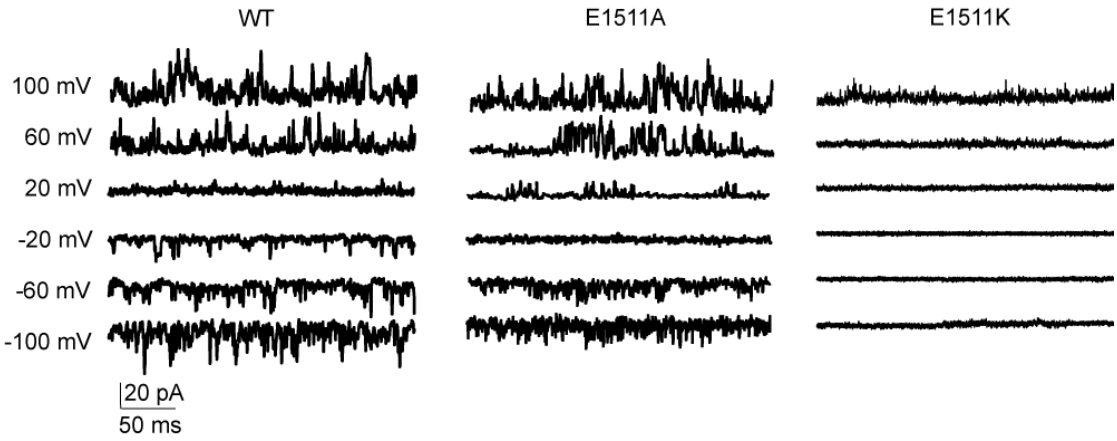
Supplementary Fig. 13. a, Time course plots of NP_o throughout the entire experiment to show channel blockage by 100 μM Gd^{3+} and recovery after washout (bin width = 10 s for cell 1 and 2, bin width = 20 s for cell 3). **b,** Sample traces and group data of spontaneous currents in S2 cells expressing NompC-GFP before and after application of the TRP channel blocker SKF-96365. **c,** Gd^{3+} blockage of negative pressure-induced mechanical current on excised patches (black trace: Ctrl; grey: Gd^{3+}; red: washout. n = 5, ***p < 0.001, paired *t*-test). **d,** FM1-43 blockage of pressure-induced mechanical current on excised patches (black trace: Ctrl; grey: FM1-43; red: washout. n = 6, ***p < 0.001, paired *t*-test).



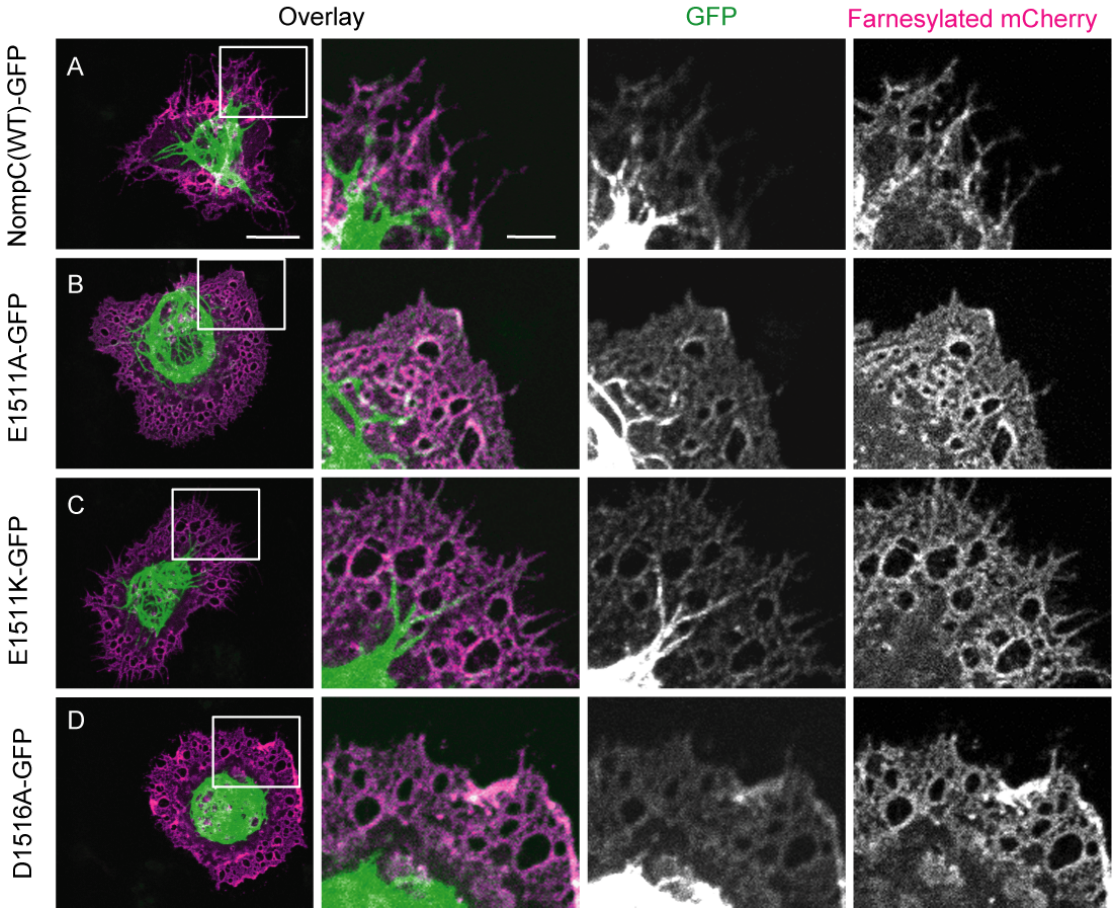
Supplementary Fig. 14. Single-channel I-V curves of spontaneously active NompC channel in different bi-ionic conditions. **a**, Extracellular Na^+/intracellular K^+. **b**, Extracellular Ca^{2+}/intracellular Cs^+. **c**, Extracellular Na^+/intracellular Cs^+. **d**, Single channel conductance at -60 mV.



Supplementary Fig. 15. Spontaneous current traces from cells transfected with WT or mutant NompC channels. Cells were held at -60 mV (extracellular Na^+/intracellular Cs^+ solution).



Supplementary Fig. 16. Spontaneous current traces of WT and mutant NompC at different membrane potentials to show reversal of the current in the bi-ionic extracellular Na^+/intracellular Cs^+ condition.



Supplementary Fig. 17. WT and mutant NompC showed similar distribution in membrane of S2 cells. Farnesylated mCherry is a cell membrane marker.. Scale bar: 30 μm; 10 μm for zoom-in.

MOTHODS

Fly Stocks

The *NompC* mutant lines were provided by C. Zuker. Class III (19-12-Gal4) and class I (2-21-Gal4) drivers were isolated from a Gal4 insertion library provided by U. Heberlein. The UAS-GCaMP5 line was a gift from G. Rubin and L. L. Looger. The UAS-TNT line was from U. Heberlein and the Repo-Gal80 line was from T. Lee. The UAS-Cut line was from R. Bodmer. The *iav*[1] mutant was from Bloomington stock center.

Behavior assay

Animals were raised at 25 °C in an incubator with 12 h light/dark cycles and humidity control (Darwin Chamber Company). Ninety-six hours after egg laying, third instar larvae were gently picked up from the vial, washed twice with PBS and transferred to a 30-mm agar plate. The larvae were allowed to crawl freely at room temperature. The behavioral assay for larvae's response to gentle touch was carried out as described before[1]. The larvae were touched with an eyelash at one side of the thoracic segments gently and the behavioral responses were scored. When the wildtype larvae were touched with an eyelash, typically they would contract the head, then turn to one side or begin reverse locomotion. Larvae that did not respond to touch were scored 0. A score of 1 was given to those larvae which stopped or hesitated, those that retracted their heads or turned were scored as 2, those that retracted with a single wave were scored as 3, and those that retracted and moved in the opposite direction were scored 4. Each larva was touched and scored four times and the values were summed as the

final score. A possible score range is from 0 to 16, and wildtype larvae show a score around 8.

Electrophysiological recordings

Fillet preparations were made by dissecting 3rd instar larvae in haemolymph-like saline containing (in mM): 103 NaCl, 3 KCl, 5 TES, 10 trehalose, 10 glucose, 7 sucrose, 26 $NaHCO_3$, 1 NaH_2PO_4 and 4 $MgCl_2$, adjusted to pH 7.25 and 310 mOsm. 2 mM Ca^{2+} was added to the saline before use. Muscles covering the neurons were gently removed with a fine forceps and the da neurons were exposed. Specific types of da neurons were visualized and identified by fluorescent markers driven by appropriate drivers. Glass electrodes for electrophysiological recording were pulled with a P-97 puller (Sutter instruments) from thick wall borosilicate glass, and filled with external saline solution. The glass electrode was mounted to the probe and the action potentials were recorded extracellularly with a sample rate of 10 kHz and low-pass filtered at 2 kHz. Multiclamp 200B amplifier, DIGIDITA 1440A and Clampex 10.3 software (Molecular Devices) were used to acquire and process the data.

Whole-cell recordings of S2 cells were carried out under Zeiss LSM5 510 microscopy equipped with a 40X water immersion lens. Transfected cells were identified by fluorescence. Sample rate was 10 kHz and filtered at 2 kHz (low-pass). Patch electrodes with 5-7 MΩ resistance were used. The pipette solution contains: 140 mM potassium Gluconic acid, 10 mM HEPES. The bath solution contains: 140 mM NaMES (sodium methanesulfonate), 10 mM HEPES. For ion selectivity experiments,

the Cs^+ pipette solution contains: 140 mM CsMES in bi-ionic conditions, 10 mM HEPES. The Ca^{2+} bath solution consisted of 70 mM CaMES, 10 mM HEPES, 100 mM sucrose. All solutions were adjusted to 320 mOsm and pH 7.2. Voltages were corrected for the liquid junction potential for calculating the ion selectivity.

Drug application

$GdCl_3$ was dissolved in the bath solution to the final concentration. SKF-96365 was prepared in DMSO at the concentration of 100 mM and diluted in saline to the working concentration before experiments. FM1-43 (Invitrogen) was dissolved in water at a stock concentration of 3 mM (1000X) and diluted in saline to the working concentration before experiments.

The drug-containing solution was perfused to the recording chamber and the drug was washed out with the normal bath solution. The single channel activity was detected by multiple step search run by Clampfit at the fixed amplitude. The NP_o indicates the population activity of channels. A time window of 10 s was used to calculate the NP_o. For each cell, the NP_o was normalized to the basal activity before drug application.

Calcium imaging of da neurons

Calcium imaging of larval da neurons was carried out using the same fillet preparation and solution as in electrophysiological recordings. The stimuli were also delivered by the same device and protocol. The imaging data were acquired in a Zeiss LSM510 confocal microscope. A newly available genetically coded calcium indicator GCaMP5 was used to measure the calcium signal. GCaMP5 and red fluorescent proteins were excited by 488 nm and 543 nm laser respectively and the fluorescent

signals were collected. The GCaMP5 showed dramatic increase of fluoresce upon touch whereas the red fluorescent proteins showed no fluorescence change when the same stimulus was delivered to the neuron. Average GCaMP5 signal from the first 5 s before stimulus was taken as F_0 and $\Delta F/F_0$ was calculated for each data point. GCaMP5 signals from the soma were analyzed.

Mutagenesis of NompC channel

The full length cDNA has been previously described[3]. All point mutations were introduced by site-directed mutagenesis (quick change) and verified by sequencing the entire NompC coding region. Mutants were made in the pBluescript vector and then cloned into the pUAST-GFP vector.

S2 cell culture

Drosophila S2 cells were cultured in Schneider' medium supplied with 5% FBS at 25°C. Cells were plated into 35 mm petri dishes before transfection. Effectene was used to transfect cells according to the product protocol. pUAST-NompC-GFP (wildtype or mutants) constructs were co-transfected with pGal4. Recordings were carried out 1-2 days after transfection.

Immunohistochemistry

Immunostaining of *Drosophila* larvae was performed as described before[2]. Briefly, 3rd instar larvae were dissected in PBS, fixed in 4% paraformaldehyde solution for 20 min at room temperature, and treated with the primary antibody overnight at 4°C and secondary antibody for 2 hours at room temperature. The primary antibody is a mouse monoclonal antibody against NompC (1:100, from J. Howard) and the secondary antibodies were the appropriate fluorescence conjugated anti-mouse IgG (1:200,

Jackson ImmunoResearch). Images were acquired with Leica SP5 confocal microscope.

Mechanical stimulation

A glass probe was driven by a piezo actuator mounted on a micromanipulator to give mechanical stimulation. The movements were triggered and controlled by the piezo amplifier which is synchronized with the programmed signals from pClamp software. For larval body wall stimulation to induce electrophysiological responses, the stimulation pipette was sealed and polished to a diameter around 20 μm. The stimulation pipette was driven downward vertically to press the larval body wall to specified displacements for 6 or 10 seconds by a piezo actuator. In the experiments for calcium imaging, a brief touch (40 μm) lasting for 300 ms was given by a glass pipette (sealed and polished to a diameter around 20 μm) driven by Sutter MP285 micromanipulator. The time and distance of the stimulus was specified by a program of the MP285 micromanipulator controller. For cultured cells, the pipette was sealed and polished by microforge to a diameter around 1 μm. The probe moved along an angle around 45 degrees to the cell surface. To apply different forces, a series of mechanical steps in 0.4 μm increment were applied every 8 s. Cells were held at -60 mV.

High speed pressure clamp

After forming whole cell recording, an excised outside-out patch was obtained from pulling the electrode away from the cell body. Negative pressure was applied to the excised membrane via a High Speed Pressure Clamp (HSPC, ALA-scientific). Signals

generated from pClamp software were sent to HSPC to control the timing and intensity of the pressure.

To record the dose-response curve of the mechanosensitive current, pressure steps of 500 ms with 10 mmHg increment were applied to the membrane patch through the recording pipette.

Data analysis

The extracellularly recorded action potentials of da neurons were detected by threshold-based search on the single-unit recordings. A fixed-length time window (1 second) was taken before and after stimulation onset time. The response firing number was calculated by the difference of the two time windows. To estimate the decay time, the post stimulus time histogram (PSTH) for a single cell was plotted for the number of spikes in a 100 ms bin. Then the PSTHs from different cells were averaged and fitted with an exponential decay curve. The time constant τ indicates the adaptation rate. To calculate the reversal potentials for Spontaneous NompC channels, each cell in each solution was held with voltage step ranging from -100 mV to 100 mV with 40 mV increments. The channel conductance for each voltage step was calculated from the amplitude-histograms of the current amplitude. Then the reversal potentials were determined from fitting of the I-V curves. The I-V curves for mechanical current were plotted and fitted from the response amplitude measured from -100 to 100 mV with 40 mV increments. The piezo displacement was 2.8 μm and the negative pressure was 50 mmHg, respectively.

To estimate the relative permeability ratio of P_X/P_Y for monovalent cations, we used

the following equation derived from the Goldman-Hodgkin-Katz model as previously described:

$$E_{rev} = \frac{RT}{zF} \ln \frac{P_X [X]_{out}}{P_Y [Y]_{in}}$$

where E_{rev} is the reversal potential of the current.

To calculate the permeability ratio for Ca^{2+}/Cs^{+}, the following equation was used:

$$E_{rev} = \frac{RT}{zF} \ln \left(\sqrt{\frac{1}{4} + \frac{4P_{Ca2+}[Ca^{2+}]_{out}}{P_{Cs^{+}}[Cs^{+}]_{in}}} - \frac{1}{2} \right)$$

The single channel conductance was then calculated as:

$$\gamma = i/(V_m - E_{rev})$$

where V_m is the holding potential, i is the single channel current amplitude and E_{rev} is the reversal potential of the current.

1. Kernan, M., Cowan, D. & Zuker, C. Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of *Drosophila*. *Neuron* **12**, 1195-1206 (1994).
2. Grueber, W. B., Jan, L. Y. & Jan, Y. N. Tiling of the *Drosophila* epidermis by multidendritic sensory neurons. *Development* **129**, 2867-2878 (2002).
3. Cheng, L. E., Song, W., Looger, L. L., Jan, L. Y. & Jan, Y. N. The role of the TRP channel NompC in *Drosophila* larval and adult locomotion. *Neuron* **67**, 373-380 (2010).