

Supplementary Information

MSL10 is a high-sensitivity mechanosensor in the tactile sense of the Venus flytrap

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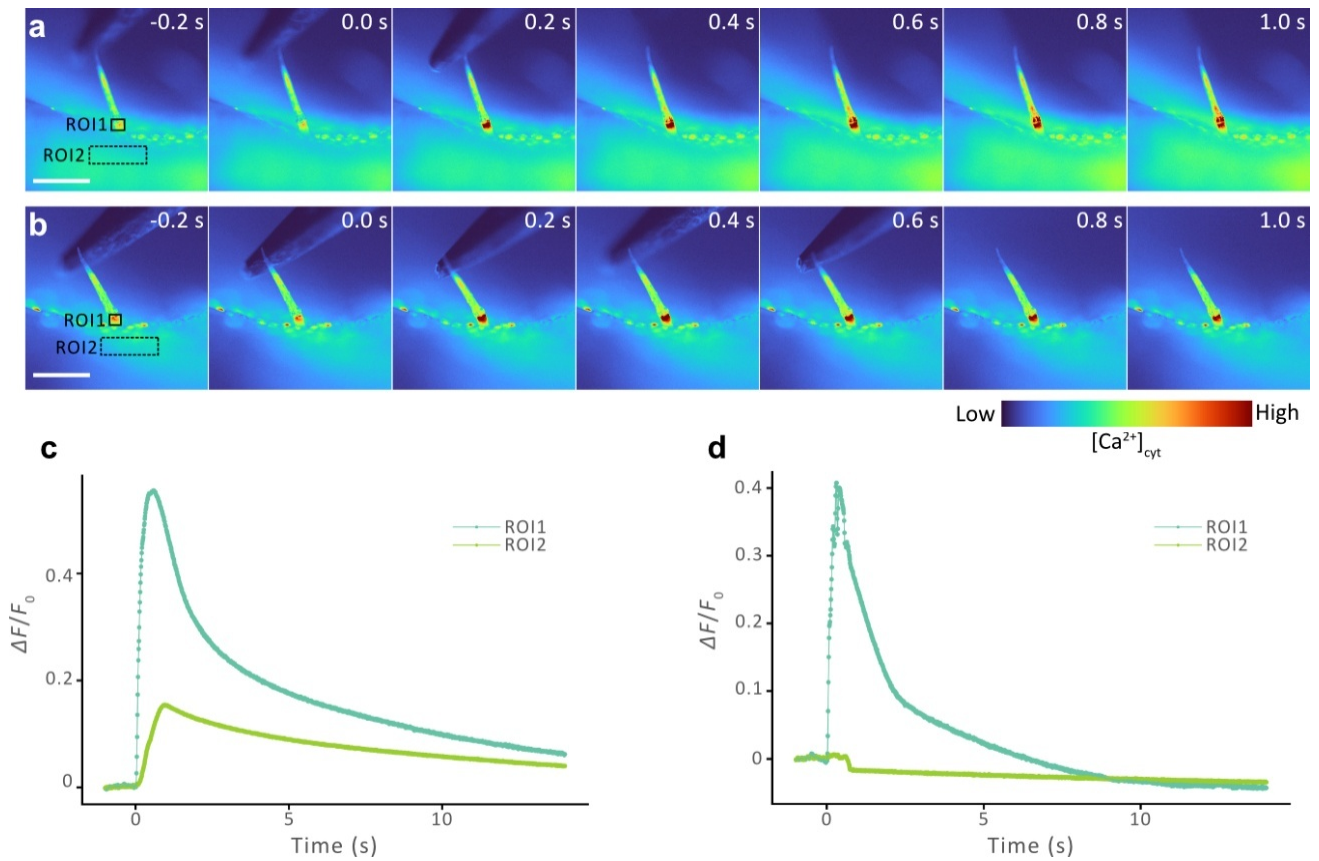
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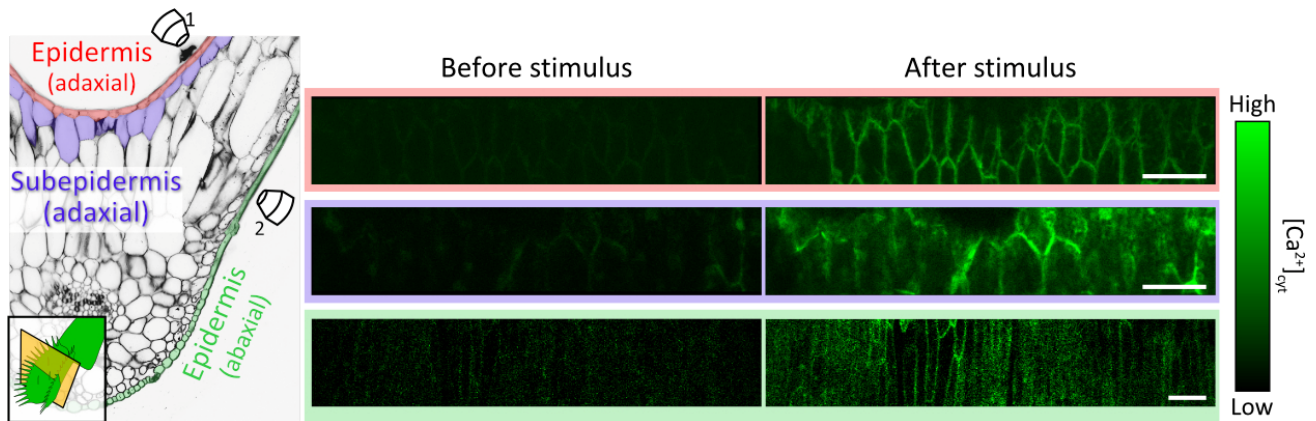
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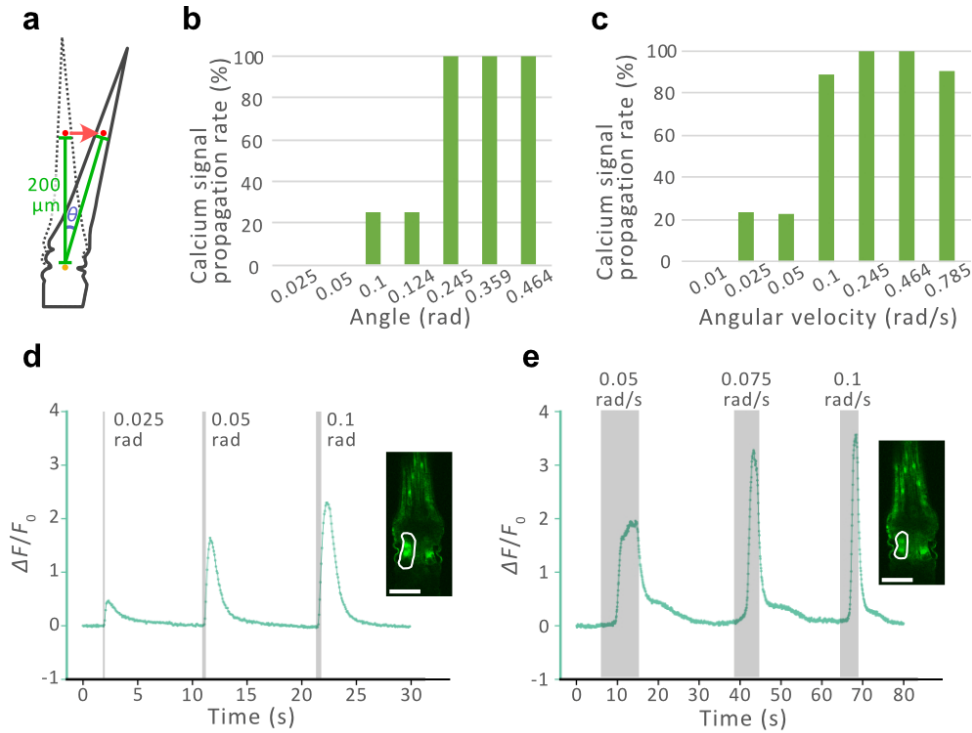
Supplementary Fig. 1 Strong mechanical stimulus triggers calcium signal propagation in the Venus flytrap.

a, b, Fluorescence images of sensory hairs of the Venus flytrap harbouring GCaMP6f with a strong touch stimulus (a deflection of approximately 0.157 rad at 3.91 rad/s to the hair) (**a**) and a weak touch stimulus (a deflection of approximately 0.0715 rad at 0.595 rad/s to the hair) (**b**). The sensory tissues and leaf blade cells were denoted as ROI1 and ROI2, respectively. The time at which the mechanical stimulus was applied was set at 0 s. **c, d**, Average standardised fluorescence intensity changes in the ROIs in **a** (**c**) and ROIs in **b** (**d**) are plotted. Data show representative results from three biologically independent hair samples. Scale bars, 500 μ m.



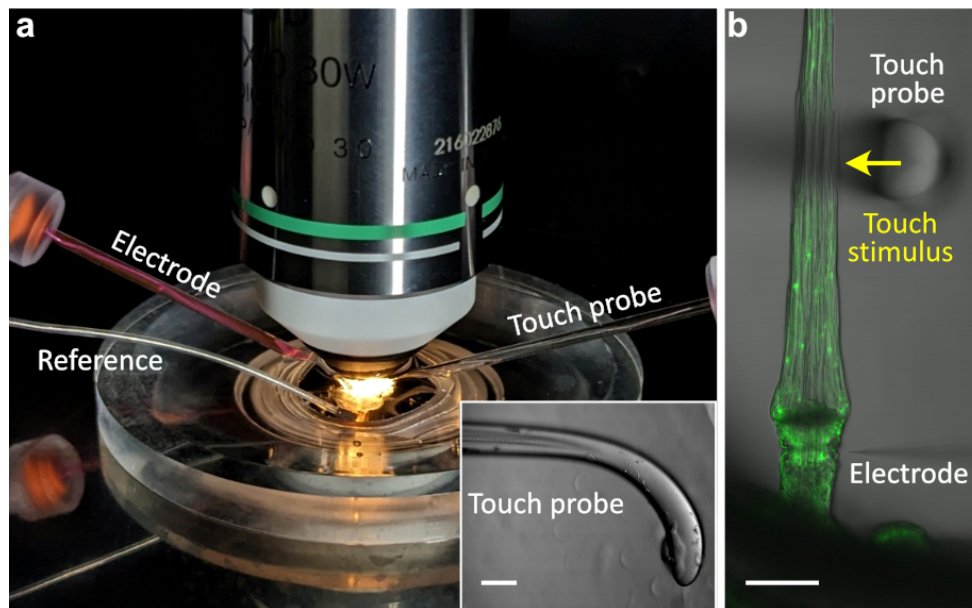
Supplementary Fig. 2 Long-range calcium signals propagated across all layers of the leaf blade.

Confocal microscope images of the leaf blade cells at the adaxial side epidermal cell layer (red), subepidermal cell layer (violet), and abaxial side epidermal cell layer (green) in response to mechanical stimuli applied to the sensory hair. The left panel shows a leaf's cross-section, which was sectioned perpendicular to the midrib, with an inset indicating where the cross-section was taken from. The orientations of the objective lenses for observation are shown in the schematic diagrams of the lenses, with 1 for the adaxial side and 2 for the abaxial side. Each layer represents the representative result from three biologically independent leaves. Scale bars, 50 μm .



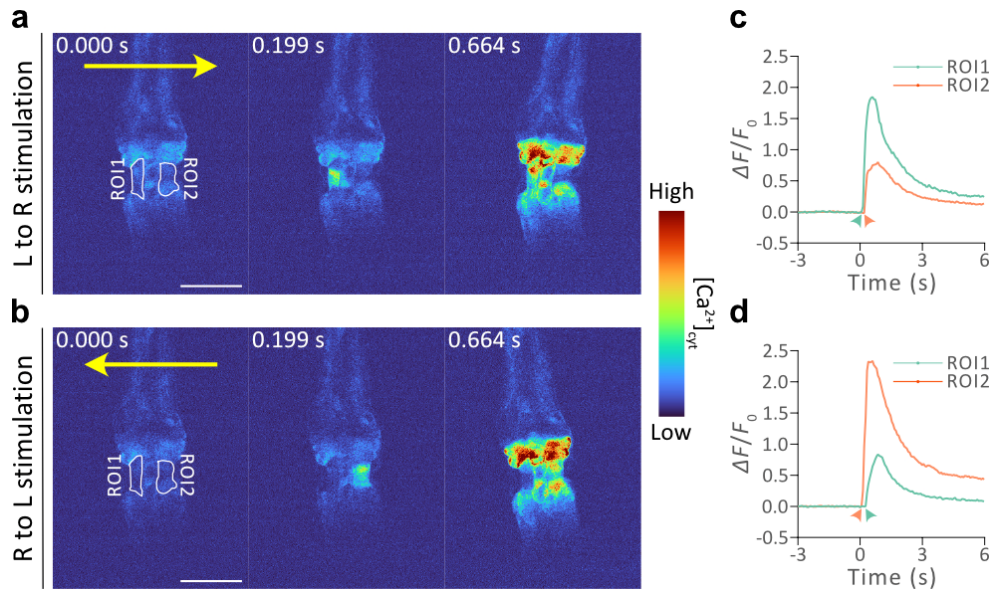
Supplementary Fig. 3 Calcium signals in response to deflection at various distances and speeds.

a, Schematic diagram of the mechanical stimulation system. The sensory hair position is shown before (grey dotted line) and after (grey solid line) stimulation. The mechanical stimuli (red arrow) with varying travel speeds and distances were applied to the apical side of the sensory hair (red dots), positioned 200 μm apically from the centre of the central cell of the sensory tissue (orange dot). Angles and angular velocities were calculated from deflection angles indicated by θ . **b**, **c**, Propagation rate of the calcium signal in response to the deflection at various distances (0.025–0.464 rad) with constant speed (0.245 rad/s) (**b**) or at various speeds (0.01–0.785 rad/s) with constant distance (0.464 rad) (**c**). In **b**, sample sizes were as follows: $n = 3$ for 0.359 rad, $n = 5$ for 0.025 and 0.05 rad, $n = 8$ for 0.1, 0.124 and 0.245 rad, and $n = 17$ for 0.464 rad. In **c**, sample sizes were as follows: $n = 4$ for 0.01 rad/s, $n = 9$ for 0.05, 0.1, and 0.464 rad/s, $n = 10$ for 0.785 rad/s, $n = 13$ for 0.025 rad/s, and $n = 17$ for 0.245 rad/s. Data were collected from three biologically independent hair samples. **d**, **e**, Average of standardised signal intensities of GCaMP6f fluorescence at stretch side indented cells in ROIs (inset) in response to the deflection at various distances (0.025–0.1 rad) with constant speed (0.245 rad/s) (**d**) or various speeds (0.05–0.1 rad/s) with constant distance (0.464 rad) (**e**). The insets show sensory hair with indented cells (white outlines) used as ROIs. The times during which the deflection was applied are indicated by grey boxes. Each plot shows representative result from three biologically independent hairs. Scale bars, 50 μm .



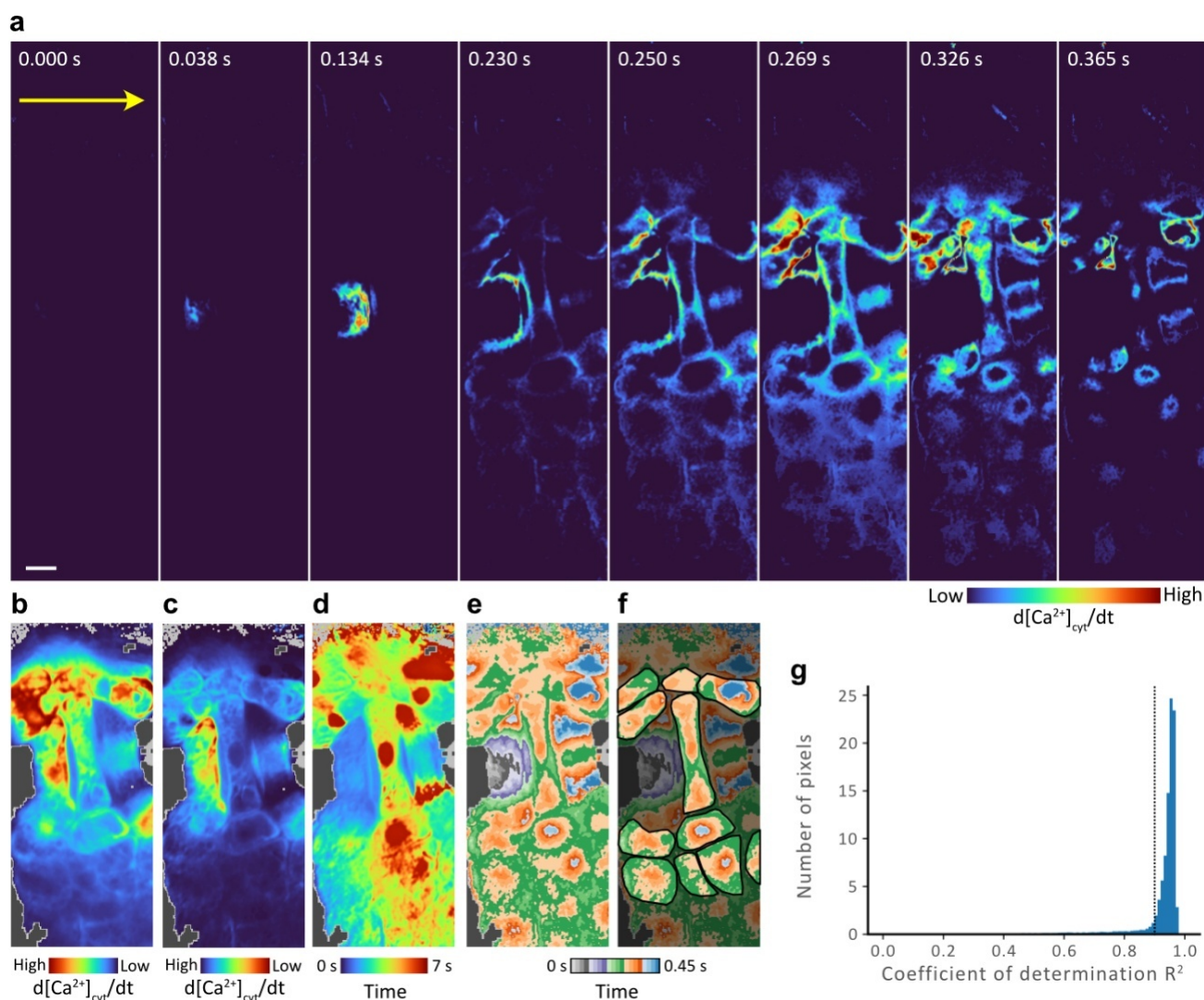
Supplementary Fig. 4 Recording setting under the two-photon microscope.

a, Recording setup under a two-photon microscope. A touch probe for the mechanical stimulus and its magnified image are shown. The recording and reference electrodes for the electrical potential measurements are indicated as “Electrode” and “Reference”, respectively. **b**, The entire image of the sensory hair is the merged bright-field and GCaMP6f-fluorescence images. Mechanical stimuli were applied by pulling the apical part of the sensory hair in the direction indicated by the yellow arrow, using a touch probe. Scale bars, 100 μm .



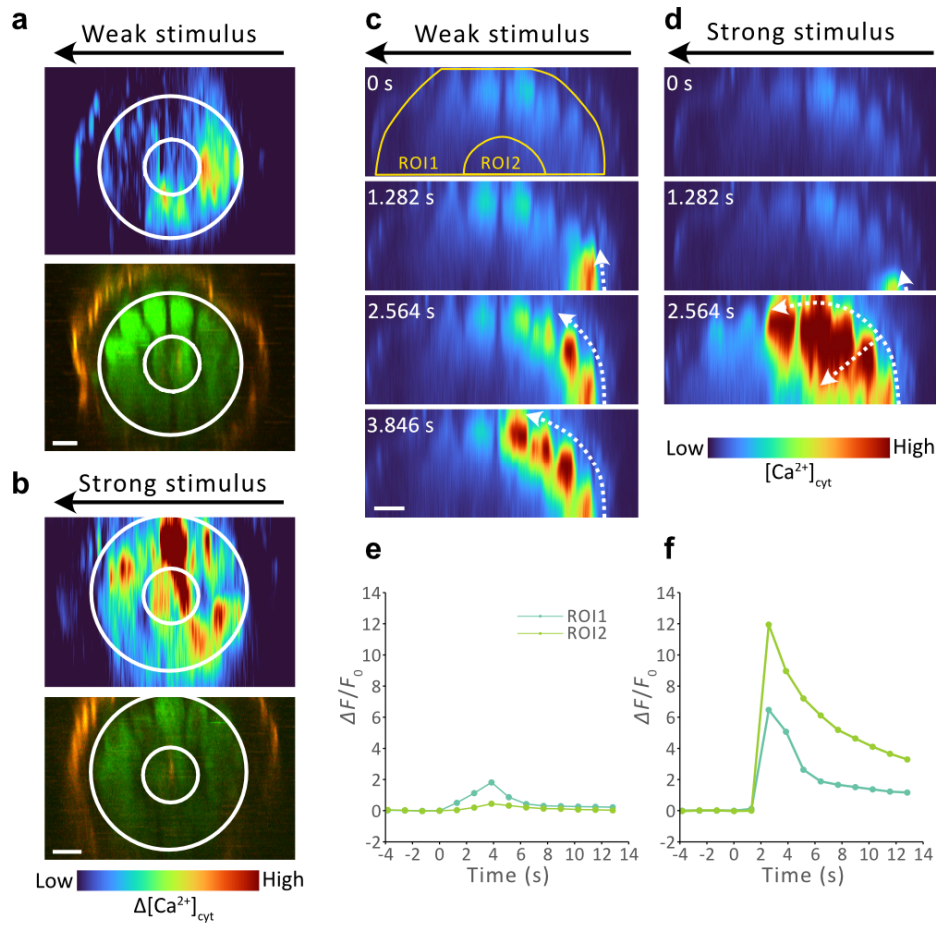
Supplementary Fig. 5 Calcium signal initiation at the indented cell on the stretched side.

a, b, The sensory hair was mechanically bent from left (L) to right (R) (**a**) and then from R to L (**b**). Mechanical stimuli were applied at a speed of 0.245 rad/s and distance of 0.245 rad. Yellow arrows indicate the directions of the mechanical stimuli. ROIs for the fluorescent intensity analyses are indicated by white enclosures. Scale bars, 50 μm . **c, d,** The standardised GCaMP6f fluorescent intensity changes in the indented cells (ROI1 and ROI2) during mechanical stimulation from L to R (**c**) and R to L (**d**). The arrowheads on the graphs indicate the onset time of the increased fluorescent signal. The time at which the mechanical stimulus was applied was set at 0 s. Data shown are the representative results from four biologically independent hairs.



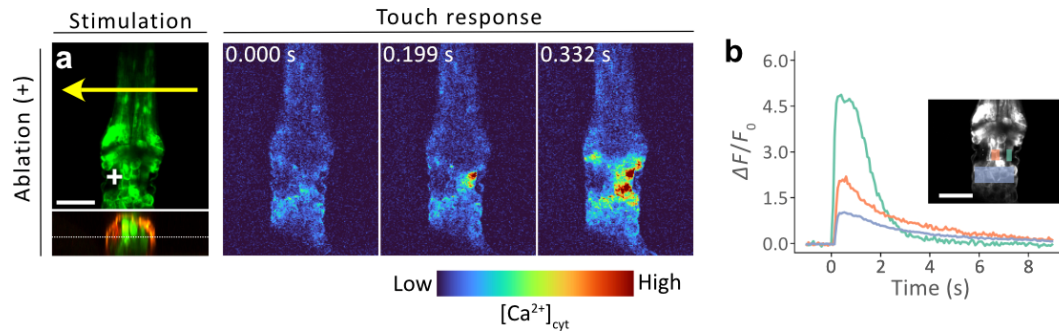
Supplementary Fig. 6 Temporal derivative images of fluorescence changes reveal the front line of calcium signal propagation.

a, Temporal derivative images of fluorescence changes calculated from the calcium signal shown in Fig. 1c. A yellow arrow indicates the direction of the mechanical stimulus. The time at which the mechanical stimulus was applied was set at 0 s. The image's upper part indicates the sensory hair's apical side. Scale bar, 10 μ m. **b**, **c**, Rise (**b**) and decay (**c**) slopes are calculated at half-maximum of the signal intensity for each pixel. **d**, Duration between rising and decaying half-maxima of the signal intensity at each pixel. **e**, **f**, Signal arrival time, calculated from when the half-maximum intensity was reached at each pixel (**e**), and the same plot with cell contours of adjacent cells and surrounding cells indicated by black lines (**f**). **g**, A histogram of the coefficients of determination calculated from all pixels in **a**. The coefficients are derived from time-course intensity changes before fitting and from fitted functions. The dotted line indicates the position of the coefficient of determination, which was 0.9. The images and plots show representative results from three biologically independent hairs.



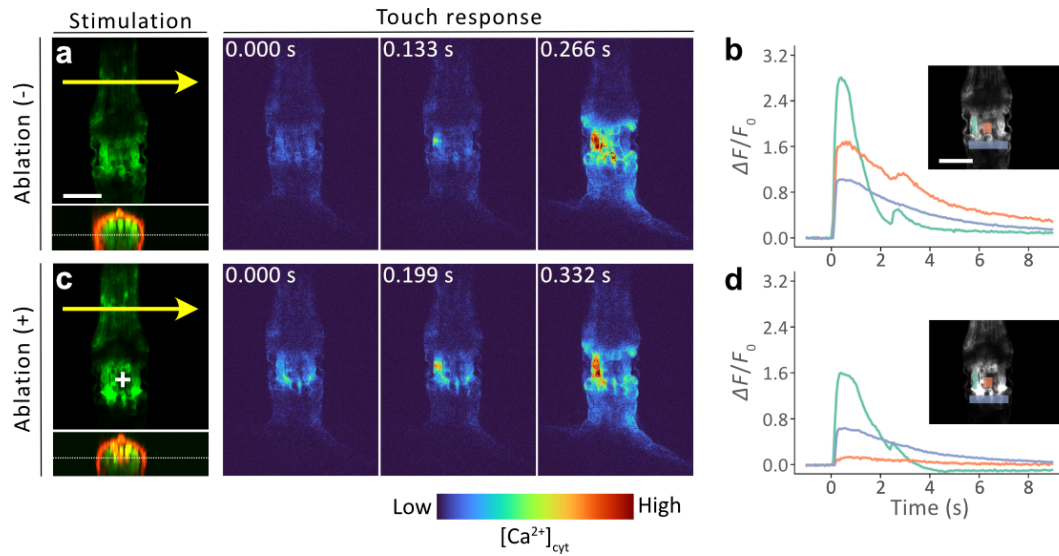
Supplementary Fig. 7 Optical sections perpendicular to the longitudinal axis of the sensory hairs reveal calcium signal propagation through the cell layers.

a, b, Maximum fluorescence intensity changes in sensory hairs following either a weak (**a**; 0.245 rad, 0.025 rad/s) or strong (**b**; 0.245 rad, 0.245 rad/s) mechanical stimulus (top). Autofluorescence is shown in red and GCaMP6f fluorescence in green (bottom). Optical sections were obtained perpendicular to the longitudinal axis of the sensory hairs. Large and small circles indicate the outer boundaries of indented cell layer and the central cell layer, respectively. **c–f**, Time-course imaging in the optical section perpendicular to the longitudinal axis of the sensory hair, in response to a weak (**c** and **e**; 0.245 rad, 0.05 rad/s) or strong (**d** and **f**; 0.245 rad, 0.245 rad/s) mechanical stimulus. White dotted arrows indicate the directions of the calcium signal propagations. The time point immediately before stimulation was set to 0 s. Average standardised fluorescence intensity changes in ROIs (**c**) are plotted in (**e**) and (**f**), with ROI1 corresponding to the indented cell layer and ROI2 to the central cell layer. All optical sections perpendicular to the longitudinal axis of the hairs were obtained via piezo-driven scanning from the top to the bottom of the panels. Arrows indicate the directions of the mechanical stimuli. The image's upper part faces the objective lens. Data show representative results from three biologically independent hairs. Scale bars, 10 μ m.



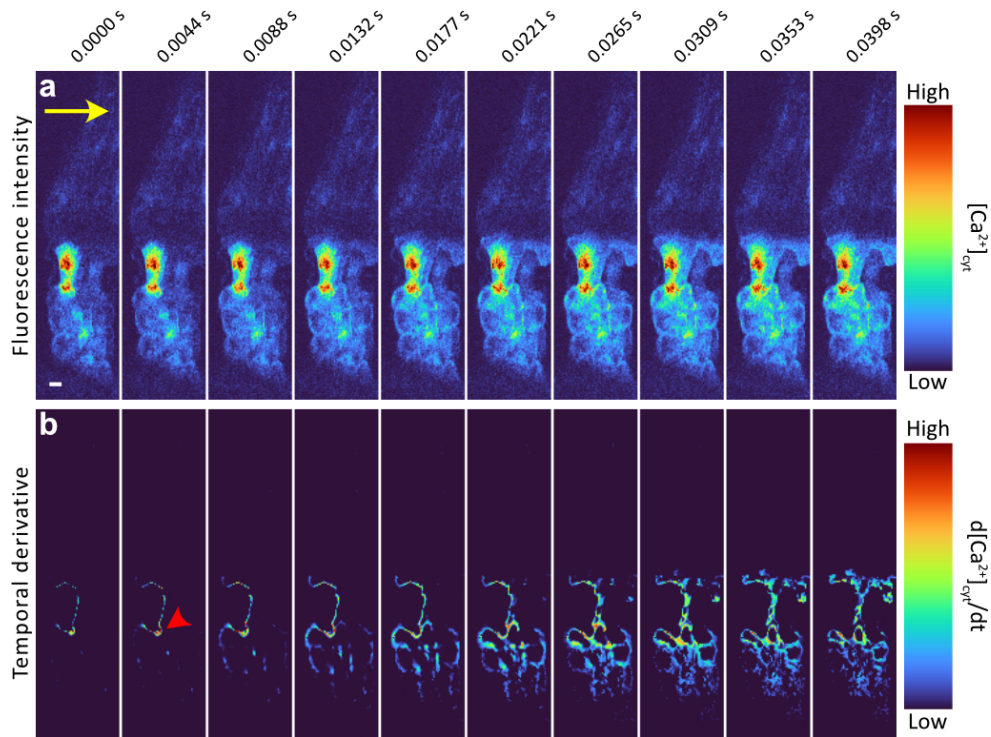
Supplementary Fig. 8 Long-range calcium signal propagation is triggered by a mechanical stimulus from the side opposite the laser-ablated cell.

a, b, Calcium signal propagation in response to a mechanical stimulus from the opposite side of the laser-ablated cell. The stimulated hair was the same as shown in Fig. 2a–d. Captured images (**a**) and average standardised fluorescence intensity changes in ROIs from the captured images (**b**). An optical section of the sensory tissue, perpendicular to the longitudinal axis of the hair, is shown at the bottom of the stimulation column in panel (**a**), with a dotted line indicating the plane of the section shown at the top of each panel. Autofluorescence is shown in red and GCaMP6f fluorescence in green. Mechanical stimuli were applied at a speed of 0.245 rad/s and a distance of 0.05 rad. The laser-ablated cell is indicated by a cross symbol. A yellow arrow indicates the direction of the mechanical stimulus. The time at which the mechanical stimulus was applied was set at 0 s. The ROIs are indicated in the inset of the panel, corresponding to the colours of the plotted lines (**b**). The image's upper part indicates the sensory hair's apical side, except in the lower optical section of (**a**), where it faces the objective lens. Data show representative results from three biologically independent hair samples. Scale bars, 50 μm .



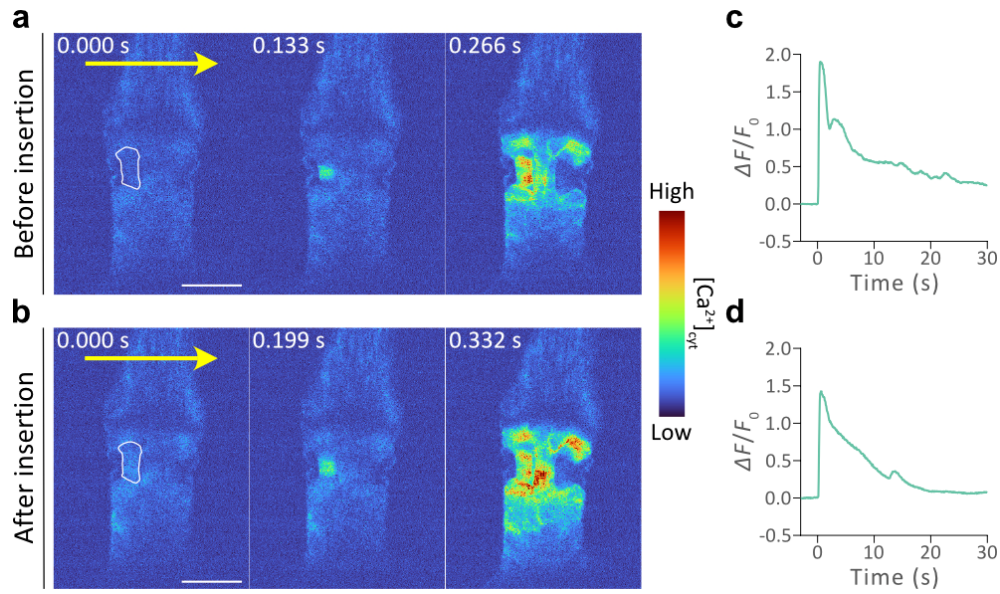
Supplementary Fig. 9 Long-range calcium signal propagation is triggered by a mechanical stimulus after laser ablation of a central cell of the sensory tissue.

a–d, Calcium signal propagation in response to mechanical stimuli before (**a** and **b**) and after (**c** and **d**) cell destruction by laser ablation of the central cell of the sensory tissue in the same sensory hair of the GCaMP6f line. Captured images (**a** and **c**) and average standardised fluorescence intensity changes in ROIs from the captured images (**b** and **d**). Optical sections of the sensory tissue, perpendicular to the longitudinal axis of the hair, are shown at the bottom of the stimulation column in panels (**a**) and (**c**), with dotted lines indicating the plane of the section shown at the top of each panel. Autofluorescence is shown in red and GCaMP6f fluorescence in green. Mechanical stimuli were applied at a speed of 0.245 rad/s and a distance of 0.025 rad. The laser-ablated cell is indicated by a cross symbol. Yellow arrows indicate the directions of the mechanical stimuli. The time at which the mechanical stimulus was applied was set at 0 s. The ROIs are indicated in the insets of the panels, corresponding to the colours of the plotted lines (**b** and **d**). The image's upper part indicates the sensory hair's apical side, except in the lower optical sections of (**a**) and (**c**), where it faces the objective lens. Data show representative results from three biologically independent hair samples. Scale bars, 50 μm .



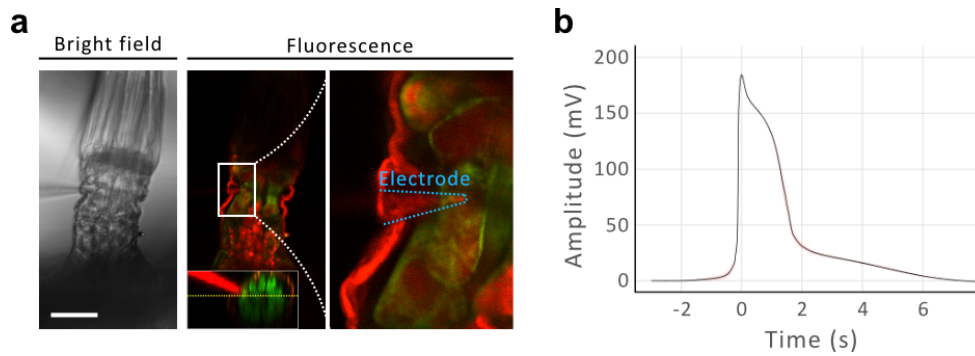
Supplementary Fig. 10 High-speed imaging revealed the initiation of long-range calcium signal propagation on the basal side of the indented cells.

a, b, High-speed imaging of the fluorescence intensity (**a**) and its temporal derivative (**b**) in response to a mechanical stimulus in the GCaMP6f line. A yellow arrow indicates the direction of the mechanical stimulus. The time at which the long-range calcium signal propagation started was set to 0 s. A red arrowhead indicates the initiation of long-range calcium signal propagation. The image's upper part indicates the sensory hair's apical side. Data show representative results from four biologically independent hair samples. Scale bar, 10 μm .



Supplementary Fig. 11 Calcium signal at the indented cell before and after the electrode insertion.

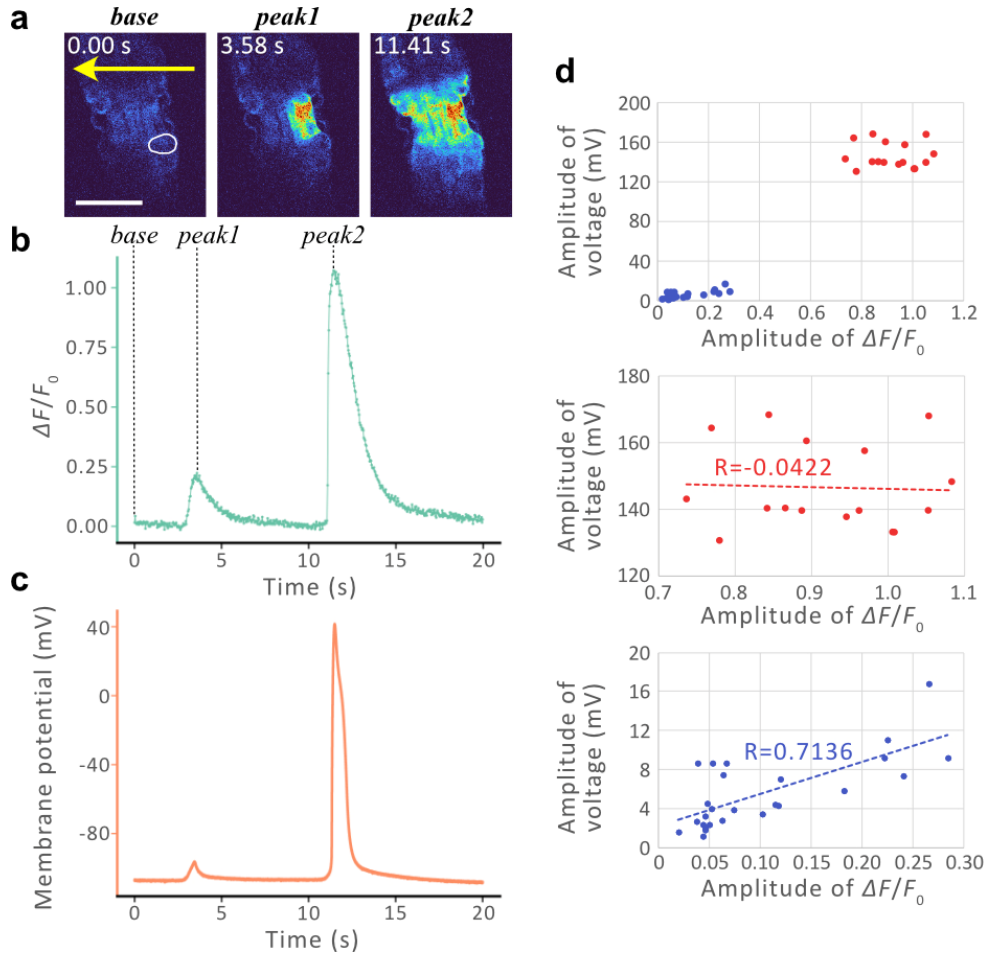
a, b, Calcium signal propagating in the same wild-type sensory hair before (**a**) and after (**b**) electrode insertion into the indented cell. The electrode was inserted into an indented cell indicated by white enclosures (ROIs for the fluorescent intensity analyses). Mechanical stimuli were applied at a speed of 0.245 rad/s and distance of 0.245 rad. Yellow arrows indicate the directions of the mechanical stimuli. Scale bars, 50 μm . **c, d**, The standardised GCaMP6f fluorescent intensity changes at the ROIs before (**c**) and after (**d**) electrode insertion into the indented cell of the same wild-type sensory hair. The time at which the mechanical stimulus was applied was set at 0 s. Data shown are the representative results from three biologically independent hairs.



Supplementary Fig. 12 Representative intracellular APs and electrode position during measurements.

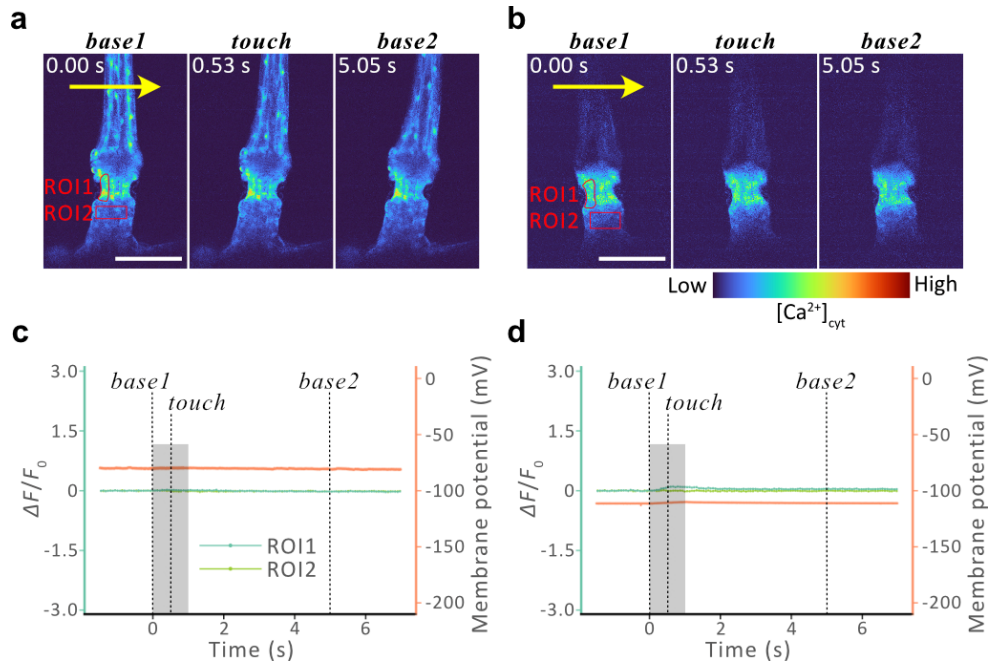
a, Insertion of the electrode for the intracellular recording system of electrical potentials, as shown in Supplementary Fig. 4. Autofluorescence of sensory hair (red) and cytosolic fluorescence of GCaMP6f (green) are shown in the middle and right panels. An optical section of the sensory tissue, perpendicular to the longitudinal axis of the hair, is shown in the inset of the middle panel. The upper inset image faces the objective lens. The yellow dotted line indicates the plane of the section shown in the middle panel. The dotted blue lines indicate the electrode tip inserted into the indented cell. Scale bar, 50 μm .

b, A typical electrical potential change recorded by intracellular measurements. The time of peak amplitude was recorded and set to 0 s. The mean $\pm$ SEM values calculated from 7 measurements using a single hair are shown. The plot is a representative result from three biologically independent hairs.



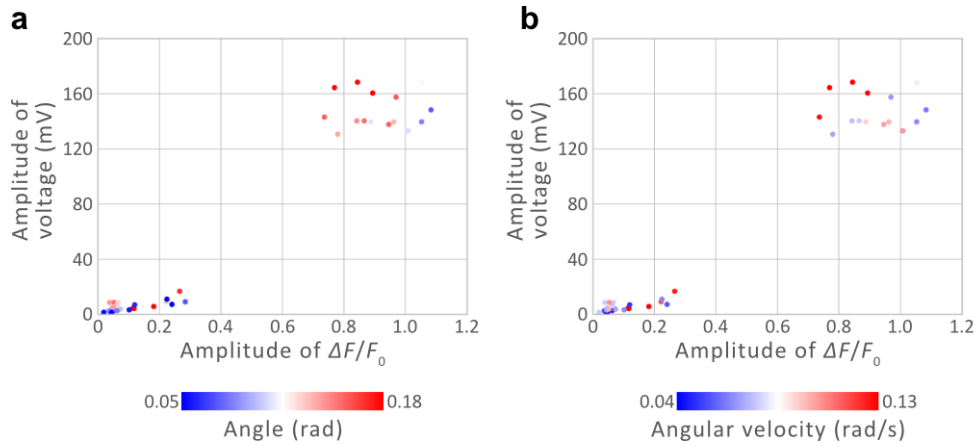
Supplementary Fig. 13 Small potential changes and APs are qualitatively distinct electrical signals.

a–c, Simultaneous recordings of fluorescence intensity and electrical potential changes in the cells adjacent to indented cells in response to two successive mechanical stimuli: a first weak stimulus (approximately 0.0375 rad, 0.0323 rad/s) and a second strong stimulus (approximately 0.0723 rad, 0.079 rad/s). The average standardised signal intensity of GCaMP6f fluorescence in the ROI (**a**, inset) is plotted as a green line (**b**). The electrical potential of the cell adjacent to the indented cell is indicated by an orange line (**c**). The italicised words *base*, *peak1*, and *peak2* indicate the key time points, corresponding to images (**a**) and a plot (**b**), respectively. The arbitrary time before the stimulus was set as 0 s. Data show representative results from five biologically independent hairs. Scale bar, 50 μm . **d**, Amplitudes of electrical potential changes and fluorescence intensity changes (upper panel). Data points were classified into a high-amplitude group (red dots) and a low amplitude group (blue dots). Magnified plots of the high- (red dots in middle panel) and low-amplitude groups (blue dots in bottom panel) are shown. The correlation coefficients calculated from the linear approximation using the least-squares method are indicated as R in the plots. $n = 42$ from two biologically independent hairs.



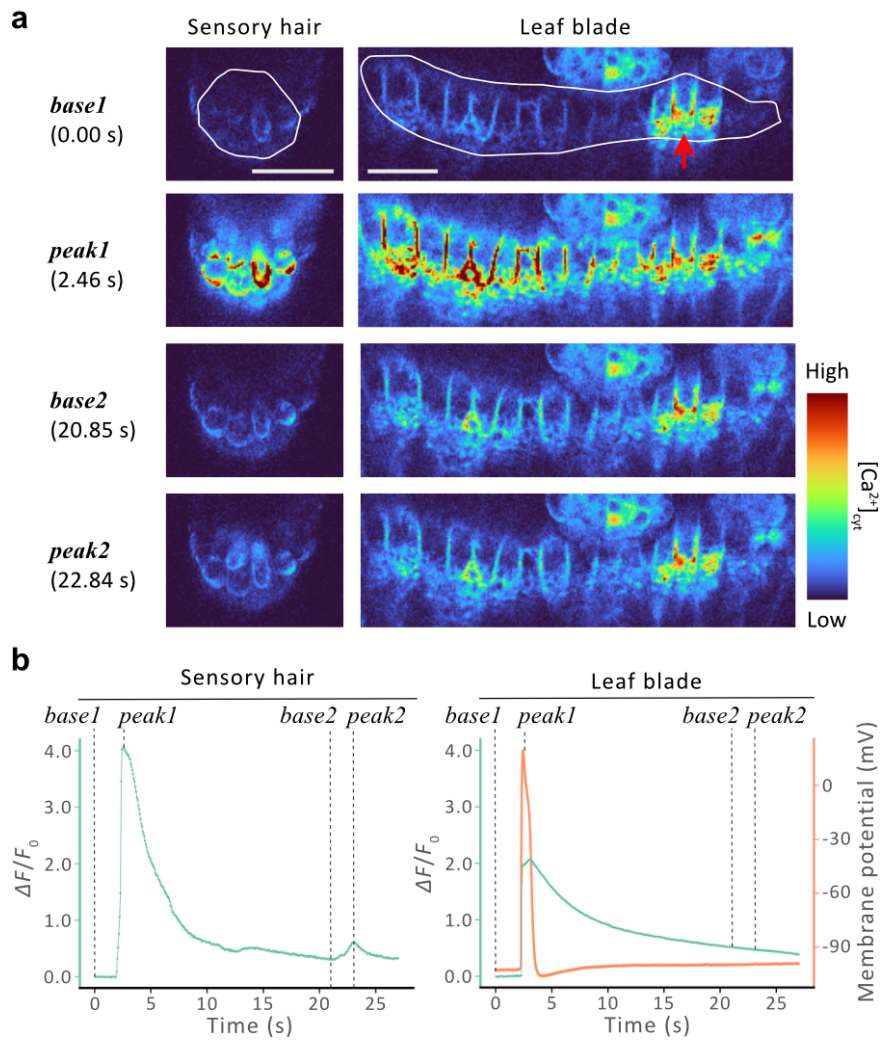
Supplementary Fig. 14 Electrical potential changes and calcium signal propagation were attenuated by calcium channel inhibitors.

Simultaneous recordings of fluorescence intensity and electrical potential changes in response to mechanical stimuli at a speed of 0.245 rad/s and distance of 0.245 rad after a 240 min treatment with 2 mM LaCl_3 (**a** and **c**) or 90 min treatment with 2 mM GdCl_3 (**b** and **d**) in the GCaMP6f line. **a, b**, Captured images used for measurements in **c** and **d**. The time elapsed since applying the mechanical stimulus is indicated in the inset image. Key time points are indicated by italicised words *base1*, *touch*, and *base2*, corresponding to images (**a** and **b**) and plots (**c** and **d**). Yellow arrows indicate the directions of the mechanical stimuli. The upper part of the image indicates the apical side of the sensory hair. **c, d**, Average standardised fluorescence intensity changes in ROI1 (indented cell) and ROI2 (basal side cells), which are indicated in the inset in (**a** and **b**), are plotted using green lines. The electrical potentials of the indented cell are plotted using orange lines. The time at which the mechanical stimulus was applied was set at 0 s. The times during which the deflection was applied are indicated by grey boxes. The images and plots represent the results of three biologically independent hairs, respectively. Scale bars, 100 μm .



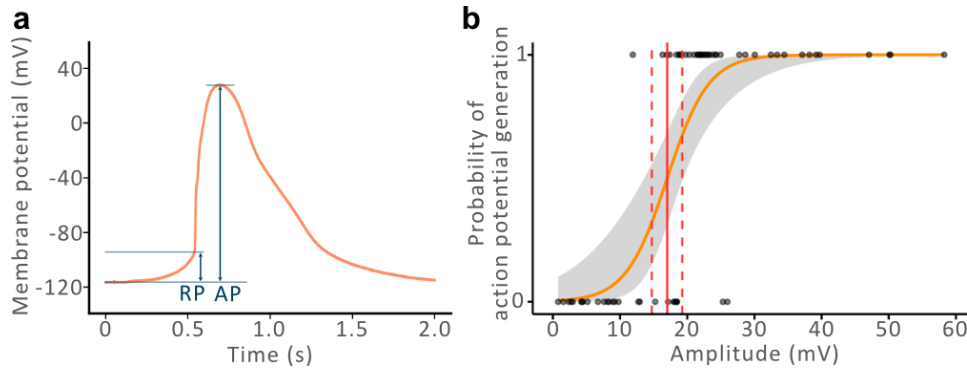
Supplementary Fig. 15 Fluorescent intensity, electrical potential change, and deflection.

Amplitudes of electrical potential changes, fluorescence intensity changes, and deflections. **a**, Angles of the applied stimuli in Supplementary Fig. 13d are plotted in pseudocolour. **b**, Angular velocities of the applied stimuli in Supplementary Fig. 13d are plotted in pseudocolour. $n = 42$ from two biologically independent sensory hair samples.



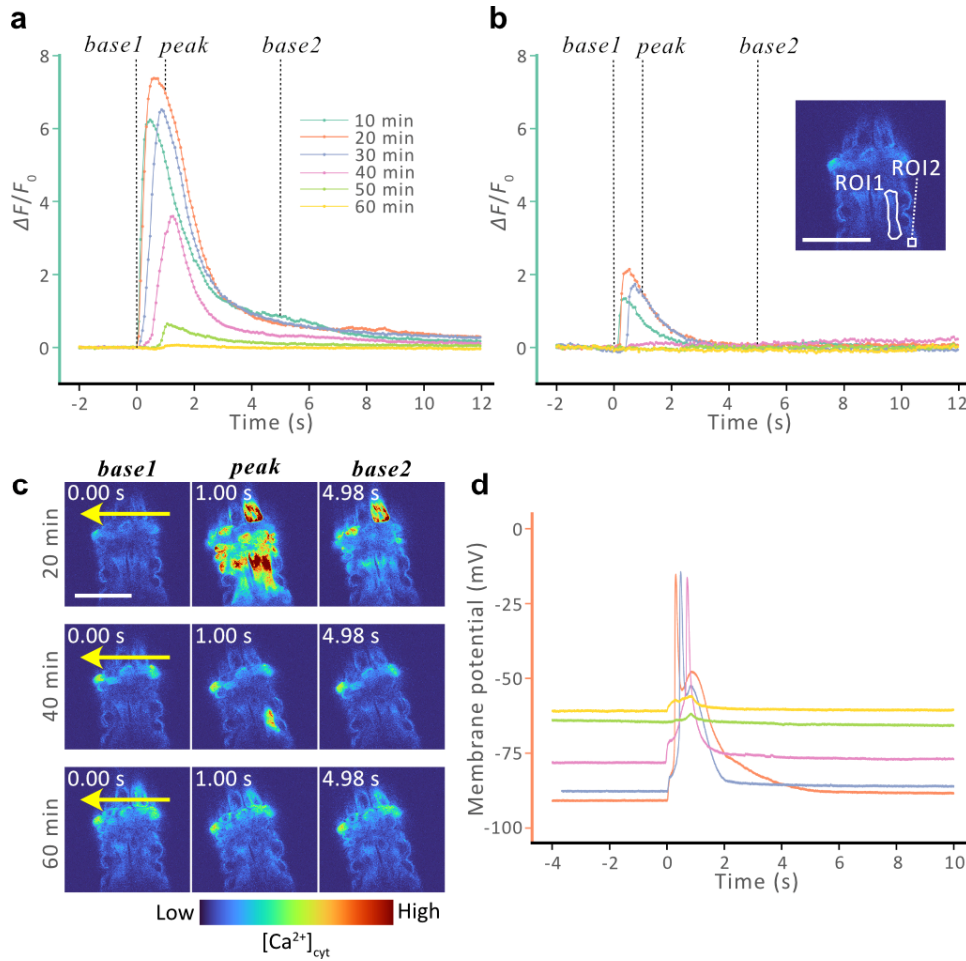
Supplementary Fig. 16 Small potential changes do not propagate to the leaf blades.

a, b, Simultaneous recordings of fluorescence intensity changes and electrical potential changes in sensory hair and leaf blades in response to two successive mechanical stimuli in the GCaMP6f line. Keyframes (**a**) and signal intensity changes (**b**) are shown. The average standardised signal intensities of GCaMP6f fluorescence in the ROI outlined with white lines (**a**, inset) are plotted with green lines (**b**). An orange line indicates electrical potential at the leaf blade cell (**b**). A red arrow indicates the electrode-inserted cell. The key time points are indicated by the italicised words *base1*, *peak1*, *base2*, and *peak2*, corresponding to images (**a**) and plots (**b**). The arbitrary time before the stimulus was set as 0 s. Data show representative results from four biologically independent hair samples. Scale bars, 50 μ m.



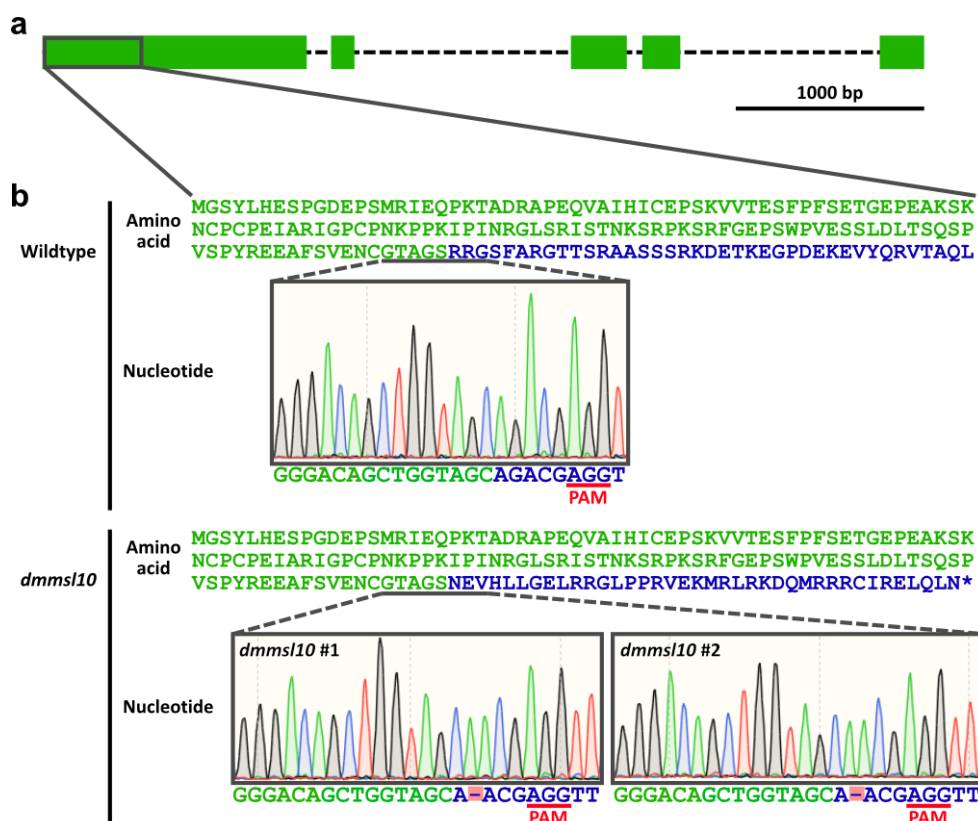
Supplementary Fig. 17 Logistic regression analysis reveals a threshold for the RP amplitude that triggers APs.

a, A representative AP recorded in the indented cell is indicated by the orange line. The RP amplitude (RP) and peak amplitude of the AP (AP) are indicated. The mechanical stimulus was applied at 0 s with a speed of 0.245 rad/s and distance of 0.245 rad. **b**, Logistic regression analysis results for wild-type plants using the amplitude of RPs as an explanatory variable. The threshold value of the RP amplitude to trigger calcium signal propagation in 50% of the sensory hairs was predicted at 17.02 mV (95% confidence interval: 14.72–19.26 mV) with statistical significance ($P < 0.0000001$ by likelihood ratio test), and odds ratio was predicted as 1.384409 (95% confidence interval: 1.21–1.71) with statistical significance ($P = 0.00018$ by likelihood ratio test). Action potential generation states (black dots: 1, propagated; 0, not propagated), regression curve (orange line), 95% confidence interval of the regression curve (grey regions), predicted threshold (solid red line), and 95% CI of the predicted threshold (dashed red lines) are shown. $n = 67$ from 11 biologically independent sensory hair samples.



Supplementary Fig. 18 Calcium and electrical signals are diminished by chloride channel inhibitor treatment.

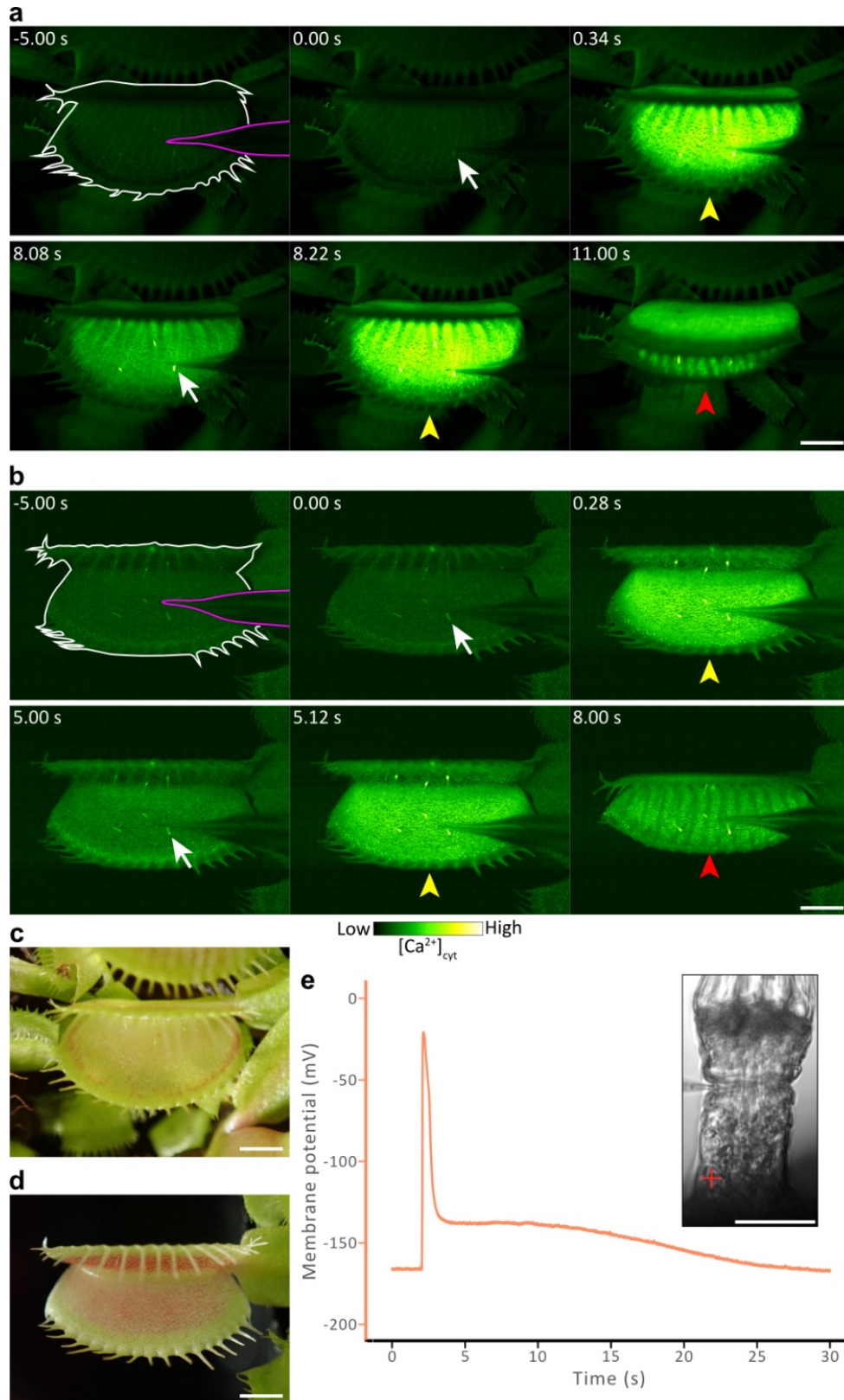
Fluorescence intensity changes (**a–c**) and electrical potential changes (**d**) in response to the mechanical stimuli at a speed of 0.245 rad/s and distance of 0.245 rad after the treatment with 500 μ M A-9-C in GCaMP6f line. **a, b**, Average standardised fluorescence intensity changes in ROI1 (**a**; indented cell) and ROI2 (**b**; part of the basal side cell), which are indicated in the inset in (**b**). **c**, Captured images used for measurements in **a** and **b**. Key time points are indicated by italicised words *base1*, *peak*, and *base2*, corresponding to plots (**a** and **b**). The treatment time for A-9-C is indicated on the left side of the panel. The time elapsed since applying the mechanical stimulus is indicated in the inset of the images. Yellow arrows indicate the directions of the mechanical stimuli. The upper part of the image indicates the apical side of the sensory hair. **d**, Electrical potential changes measured at the indented cell. The time at which the mechanical stimulus was applied was set at 0 s. The line colours in **a, b**, and **d** correspond to the treatment times with A-9-C indicated in **a**. The images and plots in **a–c** and **d** represent the results of five and four biologically independent hairs, respectively. Scale bars, 50 μ m.



Supplementary Fig. 19 Gene structure diagram and knockout target sequences of DmMSL10.

a, Gene structure diagram of DmMSL10. Green boxes indicate exons, and dotted lines indicate introns.

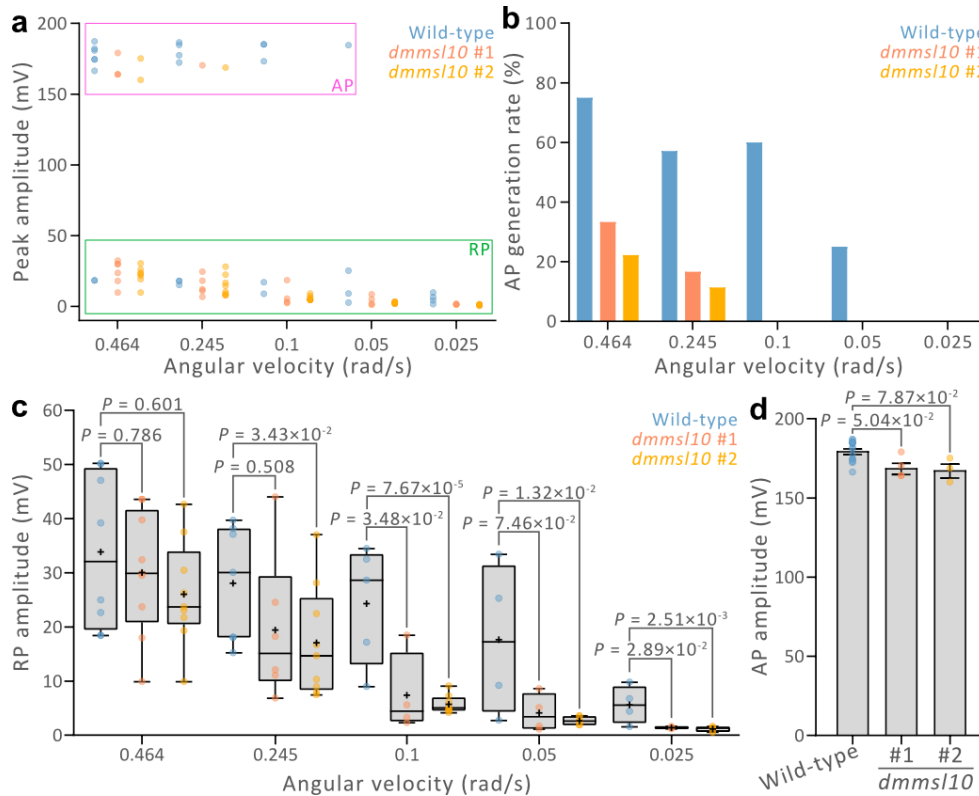
b, Target sequences of gene knockouts and sequencing chromatograms of wild-type plants and two *dmmsl10* knockout lines. Both *dmmsl10* lines showed one base deletion in the first exon with a frameshift, causing the insertion of a stop codon in the first exon. Protospacer adjacent motifs (PAM) are indicated by red underlines. Amino acid and nucleotide sequences that are downstream of the targeted site are indicated by blue letters.



Supplementary Fig. 20 Calcium and electrical signals in *dmmsl10* plants when stimulated by manual touch or laser ablation.

a, b, In both wild-type (**a**) and *dmmsl10* (**b**), first touches to sensory hairs (0.00 s in **a** and **b**, white

arrows) generated calcium signals that propagated through the leaf blades (0.34 s in **a** and 0.28 s in **b**, yellow arrowheads). Second touches to sensory hairs (8.08 s in **a** and 5.00 s in **b**, white arrows) generated the calcium signals again (8.22 s in **a** and 5.12 s in **b**, yellow arrowheads) and then, the leaves were closed (11.00 s in **a** and 8.00 s in **b**, red arrowheads). The leaves and touch probes are outlined by white and magenta lines, respectively. The images show representative results from five (wild-type) or six (*dmmsl10* #1) biologically independent leaves. **c**, **d**, Bright-field images of wild-type (**c**) and *dmmsl10* #1 leaves (**d**). **e**, Electrical potential recorded at the indented cell in response to the laser ablation stimulus in *dmmsl10*. The red cross symbol in the inset indicates the laser-ablated cell. The arbitrary time before the stimulus was set as 0 s. The plot shows a representative result from three biologically independent hair samples. Scale bars, 2 mm (**a–d**) and 50 μ m (**e**).



Supplementary Fig. 21 RP amplitudes and AP generation rates are reduced in *dmmsl10* mutants compared with wild-type plants.

a, Peak amplitudes of touch-induced electrical signals in sensory hair-indented cells of the wild-type (blue) and two independent *dmmsl10* mutants (#1 [orange] and #2 [yellow]). Electrical signals with APs (pink rectangle) and RPs (green rectangle) were clearly distinguished by their peak amplitudes. Dots indicate individual data. **b**, AP generation rates in response to touch stimuli in sensory hairs from the wild-type and two independent *dmmsl10* mutants. **c**, RP amplitudes in touch-induced electrical signals from sensory hairs of the wild-type and two independent *dmmsl10* mutants. Boxes indicate the interquartile range. The whiskers below and above the boxes indicate the minimum and maximum values, respectively. The horizontal lines in the boxes are the medians, and the plus signs represent the means. Dots indicate individual data. In **a–c**, sensory hairs were bent with a touch probe at the various speeds shown in each graph and at the constant distances (0.245 rad). Sample sizes are as follows. $n = 3$ for *dmmsl10* #1 (0.025 rad/s). $n = 4$ for wild-type (0.025 and 0.05 rad/s) and *dmmsl10* #1 (0.05 and 0.1 rad/s). $n = 5$ for wild-type (0.1 rad/s) and *dmmsl10* #2 (0.025 rad/s). $n = 6$ for *dmmsl10* #1 (0.245 rad/s) and #2 (0.05 rad/s). $n = 7$ for wild-type (0.245 rad/s). $n = 8$ for wild-type (0.464 rad/s) and *dmmsl10* #2 (0.1 rad/s). $n = 9$ for *dmmsl10* #1 (0.464 rad/s) and #2 (0.245 and 0.464 rad/s). Wild-type data were obtained from three independent sensory hairs. *dmmsl10* #1 data were obtained from three (0.025 rad/s), four (0.05 and 0.1 rad/s), five (0.245 rad/s), or six (0.464 rad/s) independent sensory

hairs. *dmmsl10* #2 data were obtained from three (0.025 and 0.05 rad/s) or five (0.1, 0.245, and 0.464 rad/s) independent sensory hairs. **d**, Amplitudes of touch-induced APs from the sensory hairs of the wild-type and two independent *dmmsl10* mutants. Mean $\pm$ SEM values are displayed. Dots indicate individual data ($n = 14$ from three independent sensory hairs for wild-type, $n = 4$ from two independent sensory hairs for *dmmsl10* #1, and $n = 3$ from one sensory hair for *dmmsl10* #2). In **c** and **d**, data were statistically analysed using two-tailed Steel's multiple comparison test.