SUPPLEMENTAL INFORMATION

Merkel cells transduce and encode tactile stimuli to drive Aβ-afferent impulses

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Figure S1. Voltage-activated currents in Merkel cells *in situ*. Related to Figure 2.

(A-C) Voltage-activated outward currents. **(A)** Sample traces of voltage-activated outward currents in a Merkel cell. Vh = -75 mV. Voltage steps were applied from -135 to 65 mV in increments of 20 mV. The outward currents have two components, the inactivating (initial, ▲) and non-inactivating (late, Δ) components. (**B)** I-V relationship of initial and late components of voltage-activated outward currents of Merkel cells (n = 34). The inactivating component had a lower voltage threshold than that of non-inactivating component. (**C)** Voltage-activated outward currents recorded from a Merkel cell with holding potential at -55 mV (top panel) or -15 mV (bottom panel). Holding at -15 mV removed inactivating outward currents but non-inactivating currents remained. Similar results were obtained from two other Merkel cells. All recordings from **A** to **C** were made with K^+-based internal solution. (**D-G**) Voltage-gated Ca^{2+} currents in Merkel cells. **(D)** A set of sample traces shows membrane currents in response to voltage steps in a Merkel cell. The recording was made in normal Krebs solution under the voltage-clamp mode with the Cs^+-based internal solution. (**E)** Recordings were made from the same cell in **D** following the bath application of 300 μM Cd^{2+} (+Cd^{2+}). In both **D** and **E**, voltage steps are indicated below the two sets of current traces. Some voltage steps and corresponding current traces are omitted for clarity. Complete voltage steps were from -90 mV to 60 mV in increments of 10 mV. Vh = -70 mV. (**F)** I-V relationship of the voltage-activated currents illustrated in **D** (orange) and **E** (blue). The curve in red is the difference between **D** and **E**, showing the Cd^{2+}-sensitive current component. The dashed

black line was extrapolated from the linear part (from -90 to -50 mV) of the orange curve to estimate the expected passive membrane currents at different voltages. (**G)** Summary data showing the I-V relationship of the Cd^{2+}-sensitive current component (n = 8). (**H-K**) L-type voltage-gated Ca^{2+} currents in Merkel cells revealed by felodipine. **(H and I)** Sample traces of voltage-activated currents in a Merkel cell *in situ* before (**H**) and following the application of 0.1 μM felodipine (**I**). The recording was made under the voltage-clamp mode with the Cs^{+}-based internal solution. In both **H** and **I**, voltage steps are indicated below the two sets of current traces. Some voltage steps and corresponding current traces are omitted for clarity. Complete voltage steps were from -90 mV to 60 mV in increments of 10 mV. Vh = -70 mV. (**J**) Traces show L-type voltage-gated Ca^{2+} current component that is blocked by 0.1 μM felodipine (Felodipine-sensitive Ca^{2+}-current component). The felodipine-sensitive Ca^{2+}-current component was obtained by subtracting the voltage-activated currents in the presence of 0.1 μM felodipine (**I**) from those before felodipine application (**H**). (**K**) I-V relationship (n = 4) of the experiments represented in **J.** The I-V curve shows the L-type voltage-gated Ca^{2+} current component that is sensitive to the block by 0.1 μM felodipine. Data represent the mean ± SEM.

Figure S2: Steady-state component of MA currents in Merkel cells and lack of MA currents in non-Merkel cells. Related to Figure 3. (A) A sample trace (left panel) shows the MA current elicited by an indirect displacement of 4 μm in a Merkel cell. The right panel shows the steady-state component of MA current at an expanded current scale. (**B**) Summary results of the relationship between steady-state current amplitude and

the distances of indirect displacement (n = 25). (**C)** Sample traces of whole-cell voltage-clamp recordings made from a non-Merkel cell following indirect displacement. (**D)** Similar to **C** except direct displacement was applied to a non-Merkel cell. (**E)** No significant MA currents were detected in non-Merkel cells with indirect (n = 16) or direct displacement stimulation (n = 24, not illustrated) at displacement distances up to 7.5 μm. Data represent the mean ± SEM. ***$P < 0.001$, compared with the value at 0 μm displacement distance (baseline), NS, not significant, one-way ANOVA with Bonferroni post-hoc tests.

Figure S3. MA currents in Merkel cells and cultured DRG neurons are similarly inhibited by a Piezo2 antibody, but the Piezo2 antibody has no effect on MA currents in N2A cells and does not affect electrophysiological properties of Merkel cells. Related to Figure 4. (A) Sample traces of MA currents recorded from 3 Merkel cells with normal electrode internal solution without Piezo2 antibody (no Piezo2Ab, left panel), internal solution that contained a Piezo2 antibody (Piezo2Ab, middle panel), or the Piezo2Ab plus its blocking peptide (Piezo2Ab+BP, right panel). In each panel, the solid trace represents the current recorded immediately after establishing the whole-cell mode, and the dashed trace is the current recorded 10 min after establishing the whole-cell mode. (**B)** Comparison between initial MA currents and the MA currents recorded 10 min later under the conditions illustrated in **A.** The currents were normalized to the initial currents recorded immediately after establishing the whole-cell mode. The values were 1.1 ± 0.2 for no Piezo2Ab group (n = 5); 0.4 ± 0.1 with Piezo2Ab (n = 10); 1.0 ± 0.2 with

Piezo2Ab+BP (n = 6), and 1.2 ± 0.3 with inactivated Piezo2Ab (n = 5). The indirect displacement stimulation was applied at 3.5 µm. (**C&D)** Similar to **A**&**B** except the recordings were made from cultured dorsal root ganglion (DRG) neurons that had rapidly adapting MA currents. The currents were normalized to the initial currents immediately after achieving the whole-cell model. The values were 1.1 ± 0.1 for no Piezo2Ab group (n = 11), 0.7 ± 0.1 with Piezo2Ab (n = 12), and 1.1 ± 0.1 with Piezo2Ab+BP (n = 8). The direct displacement stimulation was applied at 6 µm. (**E**) Piezo1-mediated MA currents were recorded from N2A cells with the electrode internal solution that contained the Piezo2 antibody (Piezo2AB). Three sets of traces represent the MA currents recorded immediately (left, Ctr), 5 min (middle) and 10 min (right) after establishing the whole-cell mode. Each panel is averaged traces of 6 recordings. The direct membrane displacement was applied at 7 µm. (**F**) Summary data (n = 6) of the experiment represented in **E**. (**G**) Sample traces of voltage-activated currents in a Merkel cell immediately (Ctr, left) and 10 min (right) after establishing the whole-cell mode. The recording was performed with the electrode internal solution that contained Piezo2AB. The circles and triangles above the two sets of traces indicate initial and late current components, respectively. (**H**) I-V relationship (n = 7) of the experiment represented in **G**. Open circles and solid circles are initial current components evoked by voltage steps immediately (control) and 10 min after establishing the whole-cell mode, respectively; there is no significant difference between them (n = 7, two-way ANOVA). Open triangles and solid triangles are late current components evoked by voltage steps immediately (control) and 10 min after establishing the whole-cell mode, respectively; there is no

significant difference between them (n = 7, two-way ANOVA). (**I**) Sample traces of AP firing in a Merkel cell immediately (left, Ctr) and 10 min (right) after establishing the whole-cell mode. The recording was performed with the electrode internal solution that contained Piezo2AB. (**J**) Summary data (n = 8) of AP firing numbers (right panel), rheobase for AP firing (middle panel), and AP amplitude (right panel) for the experiment represented in **I**. Data represent the mean ± SEM. NS, no significant difference, *** $P < 0.001$, paired Student's t-test, comparing to the currents immediately after establishing the whole-cell mode.

Figure S4. Lentiviral particle-mediated expression occurs preferentially in Merkel cell regions of whisker hair follicles and is not detectable in whisker afferents. Related to Figure 5. (A) Whisker hair follicles were injected with GFP control lentiviral particles. The expression of GFP was examined 15 days later. Left: bright field image of the front half of a whisker hair follicle. Right: fluorescent image of the same field, showing GFP expression in the initial and enlargement segments (indicated by arrows) of the hair follicle. Similar results were obtained in 9 other whisker hair follicles. (**B**) The rear half of the whisker hair follicle of **A** viewed under bright field (left) and florescent microscope (right). Expression of GFP was negligible. Similar results were observed in 9 other whisker hair follicles. An arrow indicates the attached whisker afferent bundle. Images in both **A** and **B** were taken under a 10X objective with a 1:3 image coupler. (**C**) The whisker afferent bundle in **B** is viewed under a 40X objective. Left: bright field image. Right: fluorescent image shows GFP-negative whisker afferent fibers. Similar

results were observed in 9 other whisker afferent bundles. (**D**) Bright field image (left) and fluorescent image (right) taken under a 40X objective show an example of the lack of GFP-expression in the ipsilateral trigeminal ganglion (TG) neurons. N = 40 fields of 2 ipsilateral trigeminal ganglions. (**E**) A positive control shows retrograde transport of DiI by whisker afferents to the ipsilateral TG. Five right-side whisker hair follicles were injected with DiI (each 1.5 µl DiI, 25 mg/ml) and retrograde DiI-labeling of whisker afferent neurons were examined in the ipsilateral TG 14 days later. Left, fluorescent image under a 10X objective shows some retrograde DiI-labeled whisker afferent neurons. Right, illustration of the approximate location of the retrograde DiI-labeled neurons in the ipsilateral TG. (**F**) An example of a DiI-labeled large-sized whisker afferent neuron (indicated by *) viewed under a 40x objective. Left: bright field. Right, fluorescent image. (**G**) Bright field image (left) and fluorescent image (right) taken under a 40X objective show retrograde DiI-labeling of whisker afferent bundle. (**H**) Summary of size-distribution of retrograde DiI-labeled whisker afferent neurons pooled from two ipsilateral TGs. About 80% of retrograde DiI-labeled whisker afferent neurons are medium (30-45 µm) to large (> 45 µm) size. All images were taken from freshly dissected whisker hair follicles or ipsilateral TGs without fixation and sectioning. **(I-K)** Piezo2 shRNA lentiviral particles were injected into whisker hair follicles. Ten days after the injection, the injected whisker hair follicles and their ipsilateral trigeminal ganglions were dissected out for genomic DNA analysis of the lentiviral construct WPRE, an indication of genomic integration. Genomic Gapdh (housekeeping gene) was used as a reference gene. (**I**) Amplification curves of qPCR show WPRE in whisker hair follicles

(▲) but not in ipsilateral TG neurons (Δ on baseline). On the other hand, the genomic Gapdh was present in both whisker hair follicles (●) and TG neurons (○). (**J**) Dissociation curves of amplified qPCR products for WPRE in whisker hair follicles (▲) and Gapdh in both whisker hair follicles (●) and ipsilateral TG neurons (○). Open triangles (Δ) on baseline indicate that WPRE is undetectable in ipsilateral TG neurons. (**K**) Gel electrophoresis of qPCR products shows WPRE in whisker hair follicle samples (2^{nd} lane) but not in TG samples (4^{th} lane). Gapdh is present in both whisker hair samples (1^{st} lane) and TG samples (3^{rd} lane). NTC, no template control. Two sets of hair follicles (6 hair follicles each set) and 2 sets of TGs (1 ipsilateral TG each set) were used for genomic DNA analysis by qPCR.

Figure S5. Detection of Piezo2 knockdown in whisker hair follicles by qPCR, Piezo2 knockdown has no effect on electrophysiological properties of Merkel cells, and Piezo1 shRNA lentiviral particles have no effect on MA currents in Merkel cells. Related to Figure 5. (**A-C**) Piezo2 shRNA lentiviral particles (treated) or scrambled shRNA lentiviral particles (control) were injected into whisker hair follicles. Ten days after the injection, the whisker hair follicles were dissected out for qPCR measurement of Piezo2 mRNA in treated group (●) and control group (○). Gapdh was used as a reference gene and measured in treated group (▲) and control group (Δ). (**A**) Amplification curves of qPCR show higher C_t numbers for Piezo2 in the treated whisker hair follicles (●) in comparison with the control group (○). Amplification curves for the reference gene Gapdh of treated (▲) and the control group (Δ) are also shown in the figure. The dashed

horizontal line indicates the threshold of Ct for the qPCR. (**B**) Dissociation curves of the final products of qPCR show both treated group (●) and control group (○) peak at the same melting temperature. (**C**) Gel electrophoresis of final qPCR products at 45 cycles in treated (1st and 2nd lane) and control group (3rd and 4th lane). NTC, no template control. Similar results were obtained from three other treated experiments and three other control experiments. The summary results based on C_t numbers are shown in Figure 5D. (**D**) Sample traces show voltage-activated currents in a Merkel cell of a whisker hair follicle injected with scrambled shRNA lentiviral particles (left) and a Merkel cell of a different whisker hair follicle injected with Piezo2 shRNA lentiviral particles (right). The circles and triangles above the traces indicate initial and late current components, respectively. **(E)** I-V relationship of the experiment represented in **D.** Open circles and solid circles are initial current components evoked by voltage steps in Merkel cells of scrambled (n = 28) and Piezo2 shRNA groups (n = 38), respectively; there is no significant difference between the two groups, two-way ANOVA. Open triangles and solid triangles are late current components evoked by voltage steps in Merkel cells of scrambled (n = 28) and Piezo2 shRNA groups (n = 38), respectively; there is no significant difference between the two groups, two-way ANOVA. **(F)** Sample traces show AP firing in a Merkel cell of a whisker hair follicle injected with scrambled shRNA lentiviral particles (left, Scrambled) and a Merkel cell of a different whisker hair follicle injected with Piezo2 shRNA lentiviral particles (right, Piezo2 shRNA). **(G)** Summary data (n = 28 for scrambled group, n = 43 for Piezo2 shRNA group) of AP firing numbers (left panel), rheobase for AP firing (middle panel), and AP amplitude (right panel) for the experiment

represented in **F.** Data represent the mean ± SEM. NS, no significant difference, Student's t test. (**H-I**) Piezo1 shRNA lentiviral particles or scrambled shRNA lentiviral particles were injected into whisker hair follicles. Eight days after injection, the whisker hair follicles were dissected out and MA currents were recorded from Merkel cells *in situ*. **(H)** Sample traces of MA currents recorded from a Merkel cell of a normal whisker hair follicle (left, Ctr), a Merkel cell of a whisker hair follicle micro-injected with scrambled shRNA lentiviral particles (middle), and a Merkel cell of a whisker hair follicle injected with Piezo1 shRNA lentiviral particles (right). The indirect membrane displacement was applied at 5 µm. (**I**) Summary data of the experiment represented in **H**. N = 28 for normal control (Ctr, ▲), n = 28 for scrambled group (○), and n = 8 for Piezo1 shRNA group (●). Data represent the mean ± SEM. There is no significant difference among the three groups, two-way ANOVA.

Figure S6. Cd^{2+} neither blocks whisker afferent conduction nor inhibits MA currents. Related to Figure 7. (A) Sample traces show membrane responses and APs in response to depolarizing current steps in a cultured trigeminal ganglion (TG) neuron in normal Krebs bath solution (control, Ctr). (**B)** Same neuron and same protocol as **A** except that 300 µM Cd^{2+} was applied in the bath solution. In both **A** and **B**, right panels are at an expanded time scale. Depolarizing steps were from -20 to 420 pA in increments of 40 pA for both **A** and **B**. (**C)** Summary data (n = 5) show resting membrane potential (left), rheobase that elicited APs (middle), and AP amplitude (right). Open bars, in the

absence of Cd^{2+} (Ctr); closed bars, in the presence of Cd^{2+}. The cell size averaged 44 ± 3.3 µm (35-55 µm) in diameter. **(D)** Schematic diagram shows the focal application of Cd^{2+} onto whisker afferent fibers while recording SA1 responses. Note that the whisker hair follicle is continuously perfused with normal Krebs bath solution. The Krebs bath flow is in the direction that prevents Cd^{2+} from diffusing to Merkel cells in whisker hair follicle. (**E**) Sample traces of SA1 responses before (left, Ctr) and following focal application of 300 µM Cd^{2+} for 10 min (Right, Cd^{2+}). (**F**) Summary data (n = 5) of the experiment represented in **E**. SAI spike frequency in dynamic phase and static phase are shown in Left panel and right panel, respectively. **(G and H)** A positive control for blocking whisker afferent conduction of SAI impulses by TTX. Similar to **E** and **F** except 0.5 µM TTX was focally applied onto whisker afferent fibers to block the conduction of SAI by whisker afferent fibers (n = 5). (**I)** Sample traces show MA currents recorded from a TG neuron (left panel) and a Merkel cell (right panel). Solid line, before Cd^{2+} application; dashed line, following the application of 300 µM Cd^{2+}. (J) Bars are summary data of TG neuron (TGN) MA currents (open bar, n = 6) and Merkel cell (MC) MA currents (n = 16) recorded in the presence of Cd^{2+}. The currents were normalized by the MA currents recorded before Cd^{2+} application. Direct displacement was applied at 9 - 12 µm for TG neurons and indirect displacement was applied at 2.5 µm for Merkel cells. Data represent the mean ± SEM. NS, no significant difference, $^{**}P < 0.01$, $^{***}P < 0.001$, paired Student's t-test.

Figure S7. Verapamil inhibits whisker afferent conduction, and felodipine neither inhibits the conduction of SAI impulses by whisker afferent fibers nor affects MA currents in Merkel cells. Related to Figure 7. (A) Sample traces show SAI responses recorded from a whisker afferent bundle following whisker hair displacement. Left, control in normal Krebs bath solution. Right, recording following the bath application of 200 µM verapamil for 10 min. Note that verapamil bath application covers both the whisker hair follicle and its attached whisker afferent bundle. (**B**) Summary data (n = 7) show significant inhibition of both dynamic phase (left panel) and static phase (right panel) of SAI responses by verapamil. (**C and D**) Similar to **A** and **B** except verapamil (200 µM) was focally applied onto whisker afferent fibers for 10 min (n = 5). Note that the whisker hair follicle was continuously perfused in normal Krebs bath solution while verapamil was focally applied to the whisker afferent bundle. (**E**) Sample traces show membrane responses and AP firing of a cultured TG neuron before (left), during the application of 10 µM verapamil (middle), and after wash off verapamil (right). (**F**) Summary data of the experiment represented in (**E**) to show the effects of verapamil on resting membrane potentials (left panel) and action potentials (right panel) in TG neurons. On the right panel the membrane responses to the current step of 220 pA are used; the open bar is action potential amplitude before verapamil and the closed bar is abortive potential amplitude after verapamil. Seven TG cells were tested with 10 µM verapamil (n = 7) and 2 other TG neurons were tested with 200 µM verapamil (n = 2), and the results of these 9 TG neurons are pooled together. The size of the TG neurons was 35 – 60 µm. **(G)** Sample traces of SAI responses recorded from the whisker afferent

bundle of a whisker hair follicle before (top, Ctr) and follow the focal application of 0.1 µM felodipine onto the whisker afferent bundle for 10 min (bottom, Felodipine). The whisker hair follicle was perfused with normal Krebs bath solution while felodipine was focally applied onto the whisker afferent bundle. (**H**) Summary data (n = 6) of the experiment represented in **G**. Left panel, SAI responses in dynamic phase. Right panel, SAI responses in static phase. (**I**) Sample traces of MA currents recorded from a Merkel cell before (left, Ctr) and following the bath application of 0.1 µM felodipine for 10 min onto the recorded Merkel cell (right, Felodipine). Indirect displacement was applied at 3 µm. (**J**) Summary data (n = 7) of the experiment represented in **I**. Data represent the mean ± SEM. NS, no significant difference, *$P < 0.05$, **$P < 0.01$, paired Student t-test, comparing to control.

Table S1. Membrane properties of Merkel cells and non-Merkel cells. Related to Figure 1.

	RMP (mV)	Rm (GΩ)	Cm (pF)	Rheobase (pA)	AP Threshold (mV)	AP Peak (mV)	AP Amplitude (mV)	AP Width (ms)
MCs (K^+-in)	-60±2 n=122	2.1±0.1 n=122	4.3±0.1 n=122	36±2 n=48	-14±1 n=48	5.5±0.9 n=48	28±1 n=48	16±1 n=48
MCs (Cs^+-in)	-44±3‡ n=32	2.9±0.6* n=32	4.7±0.2 n=32	20±0‡ n=15	-16±1 n=15	26.7±2.4‡ n=15	NA	NA
Non-MCs (K^+-in)	-44±2‡ n=19	0.3±0.1‡ n=19	10.9±4.0‡ n=19	NA	NA	NA	NA	NA

MCs, Merkel cells; Non-MCs, non-Merkel cells; K^+-in, K^+-based internal solution; Cs^+-in, Cs^+-based internal solution; RMP, resting membrane potentials; Rm, membrane input resistance; Cm, membrane capacitance; AP, action potential; NA, not applicable. Of 122 Merkel cells in the recordings with K^+-based internal solution, 48 cells were tested with current steps to evoke APs. For Merkel cells recorded using Cs^+-based internal solution, AP threshold and AP peak refer the starting point of rapid membrane depolarization phase and maximal depolarization potentials, respectively; repolarization did not occur due to the block of voltage-gated K^+ channels by Cs^+. Data represent the mean ± SEM. *$P < 0.05$, ‡$P < 0.001$, Student's t test, comparing to Merkel cell group recorded with K^+-based internal solution.

EXTENDED EXPERIMENTAL PROCEDURES

Merkel cell *in situ* preparations. Sprague Dawley rats aged 10-22 days were used, unless otherwise indicated. Animal care and use conformed to NIH guidelines for care

and use of experimental animals. Experimental protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Whisker hair follicles were dissected out from whisker pads of rats and placed in a dish filled with ice cold L-15 medium. Under a dissection microscope, the capsule of each follicle was removed. The follicles with their hair shafts were then fixed in a recording chamber with a tissue anchor and submerged in a Krebs solution that contained (in mM): 117 NaCl, 3.5 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$ and 11 glucose; the solution was saturated with 95 % O_2 and 5% CO_2, had pH of 7.3 and osmolarity of 325 mOsm, and was maintained at 23 ^oC. The recording chamber was mounted on the stage of an Olympus IX50 microscope that was equipped with IR-DIC and fluorescent imaging systems. The follicles were exposed to 0.05% dispase II plus 0.01% collagenase in the Krebs solution for 8-15 min, and the enzymes were then washed off with the Krebs solution. Under a 40X objective, ring sinus cells and the glassy membranes were peeled off using a glass electrode; the movement of the electrode was controlled by a micromanipulator. The follicles were then incubated with 0.3 μM quinacrine in the Krebs solution for 15 min to vital-stain Merkel cells *in situ*. After the staining, the follicles were continually perfused with the Krebs solution at a flow rate of 1.5 ml/min. The quinacrine-stained cells (Merkel cells) were identified using a fluorescent imaging system that was controlled by the MetaFluor Imaging System software (Molecular Devices, Downingtown, PA).

Patch-clamp recordings. Recordings were made at room temperature from quinacrine-stained cells. For most experiments recording electrodes were filled with a K^+-based internal solution containing (in mM): 135 K-gluconate, 5 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 Na_2ATP and 0.5 GTP-TRIS salt; the pH of the solution was adjusted to 7.3 with KOH. In some experiments, Cs^+-based internal solution was used and the solution contained (in mM): 70 Cs_2SO_4, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 Na_2ATP and 0.5 GTP-TRIS salt; the pH of the solution was adjusted to 7.3 with CsOH. Unless otherwise stated, signals were amplified and filtered at 2 kHz using the Multiclamp 700A amplifier and sampled at 4 kHz using pCLAMP 10 software (Molecular Devices). To determine membrane and action potential properties of Merkel cells, the K^+-based internal solution was used and recordings were under the whole-cell current clamp mode. Step current pulses were injected into cells through patch-clamp electrodes from -60 pA to 220 pA in increments of 20 pA per step and the duration of each pulse was 200 ms. Under the voltage-clamp mode, voltage-activated outward currents were recorded with the K^+-based internal solution and voltage-activated inward currents recorded with Cs^+-based internal solution. Unless otherwise indicated, Merkel cells were held at -75 mV in voltage-clamp experiments, and voltage steps were applied from -135 mV to 65 mV with increments of 20 mV each step and a step duration of 100 ms. To determine whole-cell currents evoked by mechanical stimulation (MA currents), K^+-based internal solution was used and Merkel cells were voltage-clamped at -75 mV, unless otherwise indicated. To determine action potential firing in Merkel cells following mechanical stimulation, action potential spike currents were recorded under cell-attached

recording mode. The spike currents were recorded with electrode command voltages being set to have ~ 0 pA baseline holding current (Perkins, 2006). After recording under cell attached mode, whole-cell mode was obtained in some cells to record MA currents and action potential firing evoked by mechanical stimulation. Recordings of non-Merkel cells were performed on quinacrine-negative cells in a similar fashion as those of Merkel cells except non-Merkel cells were held at -55 mV in voltage clamp experiments due to their less negative resting membrane potentials. In all electrophysiology experiments, unless otherwise indicated, membrane voltages mentioned in the texts have been corrected for calculated junction potentials.

Calcium imaging. Fluo-3 (Invitrogen) was loaded into Merkel cells through patch clamp recording electrodes. This was achieved by including Fluo-3 (100 μM) in the K^+-based recording internal solution with EGTA omitted. The recording internal solution also contained 85 μM Alexa 555 to help view the processes of Merkel cells. Fluo-3 was excited at 450 nm and fluorescence emission was collected at 550 nm; Alexa 555 was excited at 546 nm and fluorescence emission was collected at 567 nm. The excitation UV light was produced by a mercury lamp, and the wave-lengths of excitation and emission were achieved by two fluorescence filter sets for Fluo-3 and Alexa 555. Fluo-3 fluorescence intensity changes in Merkel cells were detected with a peltier-cooled charge-coupled device (CCD) camera (Photometrics Cool SNAP^{TM} HQ^2) under a 40× objective. Image exposure time was 200 ms per frame and acquisition interval 190 ms

using the MetaFluor Imaging System software. Images were acquired before and after current steps that induced action potential firing.

Whisker afferent fiber recordings. Whisker hair follicles with attached whisker afferent fiber bundles were prepared from whisker pads of Sprague Dawley rats aged 42-56 days. Older animals were used in order to obtain whisker afferent fiber bundles with suitable length for suction recordings. The hair follicle was fixed in a recording chamber that had a pair of anchors and perfused with Krebs solution. The anchors were made by two small blocks of silicone elastomer (Sylgard) attached parallel to each other on the bottom of the recording chamber. The distance between the two blocks was slightly narrower than the width of the hair follicle so that the hair follicle could be held steady when it was inserted in the gap. A small cut was made at the end of the capsule to open up a hole to facilitate drug diffusion into the Merkel cell region. The whisker hair was attached to a piezo device for hair movement. The whisker afferent nerve bundle was sucked into a tightly fitted recording electrode that was filled with Krebs solution. Compound action potentials in whisker afferent fibers were recorded using a Multiclamp 700A amplifier and signals were sampled at 10 KHz with low pass filter set at 1 KHz.

Mechanical Stimulation. Mechanical stimulation was applied either by displacing whisker hair follicle tissues or by whisker hair movement. For displacing whisker hair follicle tissues, a fire-polished blunted glass probe was used. It was connected to a computer-programmable piezo device (E-625 LVPZT; Physik Instrumente). The tip of

the glass probe was ~3 μm in diameter. It was positioned at an angle of 30 degrees to the surface (the outer root sheath layer) of the hair follicle section. The distance from the probe tip to the surface of the hair follicle tissue was set in such a way that the tip would contact the surface if the probe had one step (0.5 μm) forward movement. The stepwise forward movement of the probe was delivered by the piezo device. In most experiments, Merkel cells were displaced indirectly by the probe (indirect displacement stimulation). This was achieved by displacing non-recorded cells so that mechanical force was transmitted across two adjacent cells (~15 μm) to the recorded Merkel cells. In some cases, the recorded Merkel cells were directly displaced by the probe (direct displacement stimulation). For each displacement step, the forward movement was exponentially increased to reach the targeted displacement distance with an equation $f(t) = D(1-e^{t/\tau'})$, where D was the targeted displacement distance, τ' was the time constant of distance increase and was at the same value of 2 ms for different displacement distances.

To use hair movement as a tactile stimulus, a 26-gauge injection needle was bent into an "L" shape and the end of the needle was connected to the piezo device. A whisker hair shaft was then inserted into the tip of the needle. The dead space between hair shaft and the wall of the needle was eliminated by positioning the hair shaft tightly against the side wall of the needle. The piezo device drove step movements of the needle, which yielded deflections of the hair shaft. The piezo-driven step movement was delivered in a similar manner as that for displacing hair follicle tissues described above. For whisker afferent fiber recordings to determine dynamic and static SAI (slowly adapting type I) responses, hair movement was induced by a 38-μm forward step for the duration of 2.7 s;

the step had a 100-ms ramp at the speed of 0.38 μm/ms (dynamic phase) before reaching the 38-μm step (static phase). In all the above described stimulation paradigms, the piezo step movement and cell electrophysiological responses were synchronously recorded using pCLAMP 10 software.

In every experiment with mechanical stimulation, membrane movement of the recorded Merkel cells was imaged under a 40x objective, captured by a high resolution camera, and visualized in real-time on a 12-inch analog monitor. For indirect displacement stimulation, the membrane displacement of recorded Merkel cells sometimes did not occur until a forward step of 1 to 1.5 μm was applied. Accordingly, the displacement step that just produced visible membrane movement on the recorded Merkel cells was taken as the starting point of the displacement in pharmacological and Piezo2 knockdown experiments.

Pharmacology. To test effects of tetrodotoxin (TTX, a voltage-gated Na^+ channel blocker), Cd^{2+} (a voltage-gated Ca^{2+} channel blocker), felodipine (a selective L-type voltage-gated Ca^{2+} channel blocker) or low Ca^{2+} on Merkel cell action potentials, depolarizing currents were injected through patch clamp electrodes to the recorded cells before and following the bath application of 0.5 μM TTX, 300 μM Cd^{2+}, 0.1 μM felodipine or low Ca^{2+} bath solution. The drugs were usually applied for 10 min. The low Ca^{2+} bath solution was similar to Krebs solution except Ca^{2+} concentration was reduced to 20 μM.

To test pharmacological properties of Merkel cell MA currents, MA currents evoked by indirect displacement stimulation were tested in Krebs solution as controls (Ctr), then Krebs solutions that contained gadolinium (Gd^{3+}, 30 μM), ruthenium red (RR, 30 μM) or 300 μM Cd^{2+} were applied for 10 min. MA currents were determined again in the same manner as that of the controls. A Piezo2 antibody against an intracellular segment of Piezo2 (FAM38B G-20, Santa Cruz Biotechnology, Inc) was used to test its inhibitory effects on MA currents. The epitope for the antibody is 20 amino acids long and lies between amino acids 270-320 of Piezo2. The antibody was included in the recording electrode internal solution at 1 μg/ml and applied intracellularly through patch-clamp electrodes. In another set of experiments, a blocking peptide for the Piezo2 antibody (2 μg/ml) as well as the Piezo2 antibody (1 μg/ml) were pre-incubated for ~2 h, and then applied intracellularly through electrode internal solution to test MA currents. The inactivated Piezo2 antibody (1 μg/ml) after thermal denaturing was also tested in a different set of experiments. For all of the above experiments involving the tests of Piezo2 antibody, MA currents were recorded initially after establishing the whole-cell mode (controls) and then recorded again 10 min later. In addition to the tests on Merkel cells, the Piezo2 antibody was tested for its effects on the rapidly adapting MA currents recorded from cultured dorsal root ganglion (DRG) neurons, and its effects on the Piezo1 MA currents recorded from cultured N2A cells. The methods of testing MA currents in cultured DRG neurons were described in our previous study (Jia et al., 2012), and the effects of the Piezo2 antibody on MA currents in DRG neurons and N2A cells were

tested in a similar manner as those tested in Merkel cells. Similarly, the Piezo2 antibody was also tested for its effect on voltage-activated currents and AP firing in Merkel cells.

To test the effects of TTX, Cd^{2+}, verapamil or felodipine on SAI responses in whisker afferent fibers following hair movement, TTX (0.5 μM), Cd^{2+} (300 μM), verapamil (200 μM) or felodipine (0.1 μM) was bath applied continuously to whisker hair follicles. Hair movement-induced afferent SAI impulses were examined every 10 min for up to 60 min. To test the effect of ω-conotoxin MVIIV on SAI responses, whisker hair follicles were incubated with the drug for up to 95 min. To test the effects of Cd^{2+} on afferent neuron excitability and MA currents, trigeminal ganglion neurons (TGN) in culture (24 to 72 hours) were used. Cultured TGN neurons were prepared in the same manner as cultured DRG neurons. Action potentials were elicited by injecting depolarizing currents before and 10 min after the application of 300 μM Cd^{2+}. To test effects of Cd^{2+} on MA currents in TGNs, MA currents were evoked in the same manner as those in DRG neurons and effects of Cd^{2+} were tested 10 min after the application of 300 μM Cd^{2+}. To test the effects of Cd^{2+} on the conductance of SAI impulses by whisker afferent fibers, 300 μM Cd^{2+} was focally applied to whisker afferent fibers while SAI impulses were evoked by hair movement at a displacement of 38 μm. TTX (0.5 μM) was also applied focally onto whisker afferent fibers as a positive control. Using the same methods as those for Cd^{2+}, verapamil (10 and 200 μM) was tested for its effects on the excitability and AP firing of TG neurons and on the conductance of SAI impulses by whisker afferent fibers; felodipine (0.1 μM) was tested for its potential effects on MA

currents in Merkel cells and also its potential effects on the conductance of SAI impulses by whisker afferent fibers.

Ion permeability ratios of Merkel cell MA channels. To determine ion permeability ratios of Merkel cell MA channels, MA currents were recorded from Merkel cells *in situ* using electrode internal solution containing (in mM): 70 Cs_2SO_4, 2 $MgCl_2$, 5 EGTA and 10 HEPES. The relative permeability of Na^+ or Ca^{2+} in reference to Cs^+ was measured with bath solutions containing Na^+ (140 mM) or Ca^{2+} (100 mM) as the only extracellular cation, respectively. To determine Na^+ permeability relative to K^+, K^+-based electrode with K^+ (140 mM) as the only intracellular cation was used and the bath solution contained 140 mM Na^+ as the only extracellular cation. Merkel cell MA currents were determined at different holding voltages from -60 to +60 mV (before liquid junction potential correction). The junction potentials were corrected during data analysis. I-V relationships were constructed for each cell, and reversal potentials for each cell were determined by interpolation of the respective current-voltage data. The ratio of permeability, PX/PY was determined for cations X and Y for each cell from the reversal potential of the whole-cell MA current when X was the major external cation and Y the major internal cation. The Goldman-Hodgkin-Katz (GHK) equation, simplified for a single permeant cation on each side of the membrane, was employed:

$$E_{rev} = \frac{RT}{zF} \ln \frac{P_X[X]_o}{P_Y[Y]_i}$$

where RT/zF has the value of 25.5 at 23ºC. For the divalent cation Ca^{2+} permeability in reference to Cs^{+} ion, the appropriately modified equation was used.

$$E_{rev} = \frac{RT}{zF} \ln\{\sqrt{\frac{4P_{Ca}[Ca]_o}{P_{Cs}[Cs]_i} + \frac{1}{4}} - \frac{1}{2}\}$$

RT-PCR for detecting Piezo2 expression in Merkel cells. To isolate individual Merkel cells for RT-PCR, whisker hair follicles that were prepared for electrophysiological recordings were further incubated with 0.125% trypsin and 0.01% collagenase for 10 min. Quinacrine-stained cells (~30 cells) were then individually aspirated into micropipettes. After washing the outside of the aspiration micropipettes with distilled water, the tips of the aspiration micropipettes were broken in test tubes that contained a lysis buffer (with 1% 2-mercaptoethanol) from a PureLink RNA Mini Kit (Ambion/Life Technologies, Carlsbad CA) and Merkel cells were released in the lysis buffer at 4 ^{o}C. RNA was isolated following the manufacturer's instructions. Briefly, tissue lysate was passed through a nucleic acid binding column, treated with DNAse (Fisher Scientific; Pittsburgh, PA), washed, and eluted in 50 µl of supplied RNA elution solution. Due to the limited amount of RNA obtained, each sample was precipitated in the presence of 1/10th volume of 3M sodium acetate and 1 µg of glycogen (as a carrier) in 3 volumes of 100% ethanol overnight at -80 °C, then reconstituted in DEPC-treated H_2O. The total RNA of each sample was reverse transcribed with the iScript cDNA kit (Bio-Rad; Hercules CA) containing a mixture of random hexamer and oligo(dT) primers according to manufacturer's instructions, under the following thermal cycler conditions: 5 min at 25

°C, 40 min at 45 °C, 5 min at 85 °C, and held at 4 °C. Primers were designed with NCBI/Primer-BLAST tool and BLASTed against the rat Refseq_mRNA database to test for specificity and contained at least one sequence that crossed an exon-exon boundary to minimize amplification of genomic DNA. The specificity of all primers was confirmed using gel electrophoresis. PCR primers were: Piezo1 forward ACAGGTCGCCTGCTTCGTGC, reverse TGCCACCAGCACTCCCAGGT; Product: 227 bp. Piezo2 forward TTCGGAAGTGGTGTGCGGGC, and reverse GTAAGCGGGTGCGATGCGGT; Product: 277 bp. RT-PCR was performed as a 50 µl reaction on an iCycler thermal cycler (Bio-Rad) with an initial 95 °C Taq activation step for 3 min, followed by 40 cycles of 95 °C for 15 sec, 60 °C for 30 sec, and 72 °C step for 30 sec followed by 1 cycle at 72 °C for 5 min. Following cycling, samples were evaluated by gel electrophoresis on a 2% agarose gel and imaged using a Kodac Gel Logic imager and 1D 3.6 imaging software (Kodac Rochester, NY).

Piezo2 knockdown by Piezo2 shRNA lentiviral particles. Piezo2- or scrambled shRNA lentiviral particles (Santa Cruz Biotechnology, Inc) were injected into rat whisker hair follicles 1 week before patch-clamp recordings or 11 days before whisker afferent fiber recordings. The Piezo2 lentiviral particles were a pool of 3 different shRNA plasmids:
Hairpin sequence-A, GATCCCGAACTGTTCACACGAATATTCAAGAGATATTCGT GTGAACAGTTCGTTTTT. Corresponding siRNA sequences, sense: CGAACUGUUCACACGAAUAtt, antisense: UAUUCGUGUGAACAGUUCGtt

Hairpin sequence-B, GATCCCCAGACCTAGCCATTATGATTCAAGAGATCATAA TGGCTAGGTCTGGTTTTT. Corresponding siRNA sequences, sense: CCAGACCUA GCCAUUAUGAtt, antisense: UCAUAAUGGCUAGGUCUGGtt

Hairpin sequence-C, GATCCCACAAGGTTACATCTAGATTTCAAGAGAATC TAGATGTAACCTTGTGTTTTT. Corresponding siRNA sequences, sense: CACAAGGUUACAUCUAGAUtt, antisense: AUCUAGAUGUAACCUUGUGtt.

For control experiments using scrambled shRNA lentiviral particles, the sequences are scrambled and do not target any known genes. For intra-follicle injection, animals were anesthetized by isoflurane, a long whisker hair (D2 whisker or adjacent whisker) was lifted vertically by a forceps, and a sharp glass electrode containing lentiviral particle solutions was inserted along the whisker hair into the hair follicle. The glass electrode was affixed on an injection holder of a stereotaxic apparatus to assist a precise insertion of the electrode tip into Merkel cell region in a follicle (distance from follicle opening: 1 mm). A small volume of lentiviral particle solution (1 µl) was injected into the follicle over 2.5 min using a micro-injection system. Animals were injected two times in 2 consecutive days, and the injected hair follicles were harvested 6-9 days after the first injection. Patch-clamp recordings of MA currents from Merkel cells were then performed.

In a separate group of rats, GFP control lentiviral particles were injected into whisker hair follicles for examining lentiviral particle-mediated expression in whisker hair follicles. The injected whisker hair follicles were harvested between 6 to 15 days after the injection, and GFP-expression was examined under the fluorescent microscope.

Merkel cells including those expressing GFP were then identified by quinacrine-staining. In addition to the cells within the injected whisker hair follicles, whisker afferents that innervate the injected whisker hair follicles were also examined to determine if GFP was expressed in the whisker afferent fibers and their somas in the trigeminal ganglions. As a positive control of retrograde transport, DiI (1.5 µl DiI, 25 mg/ml) was injected into whisker hair follicles and retrograde DiI-labeling in whisker afferents was examined 14 days after the intra-follicle DiI injection.

Quantitative PCR (qPCR) for detecting genomic integration of Piezo2 shRNA lentiviral construct. Sprague Dawley rats aged 35-42 days were used. Ten days after intra-follicle injection of Piezo2 shRNA lentiviral particles (2 µl), the injected follicles as well as the ipsilateral trigeminal ganglia were harvested from rats and placed in separate tubes. Genomic DNA was isolated using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Briefly, genomic DNA was isolated using Proteinase K and residual RNA was digested using RNase digestion. The genomic DNA was purified by silica-based membrane binding. To detect if Piezo2 shRNA lentiviral genomic integration in host genome occurred in whisker hair follicles or trigeminal ganglions, WPRE (Woodchuck Posttranscriptional Regulatory Element) of Piezo2 shRNA lentiviral construct was measured by qPCR using the primer set as follows:

Forward: GGCACTGACAATTCCGTGGT; Reverse: AGGGACGTAGCAGAAGGACG; Product: 108 bp. The genomic Gapdh gene was used

as a reference gene with the primer set that was designed to cross an exon/intron border as below: Forward: CCGCATTGCAGAGGATGGTA; Reverse: ACAGCACAGCCCCGAATTTA; Product: 213 bp. Quantitative PCR was performed as for expression analysis (see the next section) using 50 ng of purified genomic DNA per reaction. Following the qPCR, the product samples were further evaluated by gel electrophoresis on a 2% agarose gel and imaged using a Kodac Gel Logic imager and 1D 3.6 imaging software (Kodac Rochester, NY).

Quantitative PCR (qPCR) for detecting Piezo2 knockdown. Sprague Dawley rats aged 12 days were used. Ten days after intra-follicle injection of Piezo2 shRNA lentiviral particles or scrambled shRNA lentiviral particles (1 µl), the injected follicles were harvested and their enlargement segments were dissected out and used for RNA isolation. Total RNA was isolated and first strand DNA synthesized by the method described above for RT-PCR. Piezo2 primers were also the same as those mentioned above for RT-PCR. The specificity of all primers was confirmed using qPCR melting point analysis (MxPro, Stratagene; Santa Clara, CA, USA) and gel electrophoresis. Primers for several candidate reference genes were designed and tested for expression stability using BestKeeper software under the experimental conditions employed in this study, resulting in the choice of Gapdh as a reference gene. The Gapdh primers are: Fwd: ACTTTGGCATCGTGGAAGGG; Rev: ACTTGGCAGGTTTCTCCAGG. For qPCR, each sample was run in sets of three technical replicates, using 0.5 µl of the reverse transcriptase reaction of cDNA, 300 nM of the target-specific primer working solution,

10 µl of prepared SYBR Select Master Mix (Invitrogen) to a final volume of 20 µl per reaction. Quantitative PCR was performed on a Stratagene MxPro 3500 thermal cycler with an initial 95 °C Taq activation step for 2 min, followed by 45 cycles of the following: 95 °C denaturing step for 15 s, 60 °C annealing step for 30 s, and final 72 °C extension step for 30 s, with a fluorescence value recording step after every cycle. Following cycling, samples were evaluated for correct product by melting point analysis and considered contamination-free if no-template controls were negative (MxPro, Stratagene). Following the qPCR, the product samples were further evaluated by gel electrophoresis on a 2% agarose gel and imaged using a Kodac Gel Logic imager and 1D 3.6 imaging software (Kodac Rochester, NY).

Behavioral whisker tactile tests. The whisker tactile test (Defensor et al., 2012) was performed to examine behavioral responses to whisker tactile stimulation by a thin plastic filament (0.5 mm in diameter). Rats aged 7-10 weeks were placed in a cage and habituated for 10 min. During habituation and subsequent experiments, the testing room only had a red light on so that animals could not see the examiners and the tactile stimulation filament. After the habituation, a single whisker hair (right side D2 whisker) was displaced up to 2 mm in caudal-rostral direction by the tactile stimulation filament, and the whisker tactile test was performed 10 times with an interval of ~30 s between trials. Although the whisker tactile stimulation could result in tactile responses such as eye blink and head movement, quantitatively measuring the behavioral tactile responses was difficult due to quick adaptation and large variations. To produce a reliable

measurement of whisker tactile responses, we subcutaneously injected capsaicin (305 µg in 100 µl) to right facial areas 60 min before the whisker tactile test. Under this condition, all testing rats responded, without adaptation, to the whisker tactile stimulation by exhibiting nocifensive behaviors such as attempting to grab, bite or avoid the contact filament. The nocifensive behavioral responses could last for > 2 hours following capsaicin injection. Effects of drugs on the behavioral tactile responses were tested by microinjecting TTX (48 ng in 3 µl), Cd^{2+} (33 µg in 3 µl), felodipine (0.058 µg in 3 µl) or ω-conotoxin MVIIC (0.79 µg in 3 µl) into the D2 whisker hair follicles. The drugs were injected into D2 whisker hair follicle with the animals under anesthesia by isoflurane. After the injection, isoflurane was immediately discontinued to allow the animals to recover from anesthesia for 60 min. The whisker tactile tests were then performed to examine the effects of injected drugs. To test the effects of Piezo2 knockdown on whisker tactile-induced nocifensive behavioral responses, Piezo2 shRNA lentiviral particles (3 µl) or scrambled shRNA lentiviral particles (3 µl) were injected into right side of D2 whisker hair follicles 2 times in 2 consecutive days. Whisker tactile tests were performed on the day 0, 3, 5, 7, 9 and 14 after the first injection. The intra-follicle injections were made in the same manner as that for intra-follicle injection of lentiviral particles described in Piezo2 Knockdown section.

Data Analysis. Electrophysiological data were analyzed using Clampfit software. For MA current kinetics, latency was defined as the time lapse from the trigging of the piezo device to the current deflection point of the MA rising phase; rising kinetics were

described by MA rising slope measured between 10 to 90% of peak, decay time constants of MA were obtained by curve fitting of the decay phase of MA between 10 to 90% of peak with a single exponential equation. For Ca^{2+}-imaging experiments on quinacrine labeled Merkel cells, quinacrine fluorescence was digitally subtracted from the total fluorescence and the ratio of Fluo-3 fluorescence (F/F_0) was then obtained using the MetaFluor Analyst software. For recording Merkel cell action potential spikes induced by mechanical stimulation, single AP spike cells were arbitrarily defined as cells that fired only a single spike following an additional 3 forward displacement steps (0.5 μm increment each) after the threshold distance. Data are presented as mean ± SEM. Statistical significance was evaluated using Student's t-test for two groups, as appropriate; one-way or two-way ANOVA with Bonferroni post-hoc tests for multiple groups, * $p<0.05$, ** $p<0.01$, and *** $p<0.001$.

For qPCR data analysis, raw fluorescence data were normalized to an internal reference dye (ROX) using MxPro software (Stratagene) in order to control for well-to-well pipetting error. These data were imported into LinReg software (Heart Failure Research Center, Amsterdam, The Netherlands) to obtain threshold cycles (C_t), N_0 values, and average reaction efficiencies generated from sample amplification kinetics (Ruijter et al., 2009). Replicate outliers were defined as having either a difference of three C_t values from the sample mean or efficiency outside of 5% of the plate mean, and were excluded from further analysis. Data were analyzed according to the "gene expression's C_t difference" formula described previously (Schefe et al., 2006) which incorporates a correction for efficiency in its calculation of fold change in expression.

Briefly, the C_t for the target gene (Piezo2) was normalized to a reference gene (Gapdh) expression from the same sample. Differences in expression were qualitatively assessed by plotting LinReg-derived N_0 values against the mean N_0 value for Gapdh gene for all samples. Significance was determined using the Student's t-test. A value of $p < 0.05$ was considered significant. N refers to the number of animals in a given group. Data are expressed as means ± SEM.

Supplemental References:

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