Piezo2 is required for Merkel–cell mechanotransduction

Seung-Hyun Woo1, Sanjeev Ranade1, Andy D. Weyer2, Adrienne E. Dubin1, Yoshichika Baba3, Zhaozhu Qi1,4, Matt Petrus4, Takashi Miyamoto4†, Kritika Reddy4, Ellen A. Lumpkin5, Cheryl L. Stucky2 & Ardem Patapoutian1

How we sense touch remains fundamentally unknown1,2. The Merkel cell–neurite complex is a gentle touch receptor in the skin that mediates slowly adapting responses of Aβ sensory fibres to encode fine details of objects3–6. This mechanoreceptor complex was recognized to have an essential role in sensing gentle touch nearly 50 years ago3–5. However, whether Merkel cells or afferent fibres themselves sense mechanical force is still debated, and the molecular mechanism of mechanotransduction is unknown6–12. Synapse-like junctions are observed between Merkel cells and associated afferents13–15, and yet it is unclear whether Merkel cells are inherently mechanosensitive or whether they can rapidly transmit such information to the neighbouring nerve16,17. Here we show that Merkel cells produce touch-sensitive currents in vitro. Piezo2, a mechanically activated cation channel, is expressed in Merkel cells. We engineered mice deficient in Piezo2 in the skin, but not in sensory neurons, and show that Merkel-cell mechanosensitivity completely depends on Piezo2. In these mice, slowly adapting responses in vivo mediated by the Merkel cell–neurite complex showed reduced static firing rates, and moreover, the mice displayed a decrease in behavioural responses to gentle touch. Our results indicate that Piezo2 is the Merkel-cell mechanotransduction channel and provide the first line of evidence that Piezo channels have a physiological role in mechanosensation in mammals. Furthermore, our data present evidence for a two-receptor-site model, in which both Merkel cells and innervating afferents act together as mechanosensors. The two-receptor system could provide this mechanoreceptor complex with a tuning mechanism to achieve highly sophisticated responses to a given mechanical stimulus13,18,19.

We recently discovered Piezo proteins as an evolutionarily conserved mechanically activated (MA) cation channel family20–22. Drosofihla melanogaster Piezo and zebrafish Piezo2b have been shown to be involved in somatosensory mechanotransduction20–22. Of the two mammalian Piezo members, Piezo1 and Piezo2, Piezo2 is expressed in dorsal root ganglion (DRG) sensory neurons and is required for a subset of mechanically activated currents in DRGs23. Here we focused on whether Piezo2 also has a role in somatosensory mechanotransduction in mammalian skin.

We generated a Piezo2-GFP-IRES-Cre knock-in reporter mouse line to detect Piezo2 expression in vivo. The Piezo2-GFP-IRES-Cre (Piezo2GFP) allele contains enhanced green fluorescent protein (EGFP) fused to the carboxy (C)-terminal end of the Piezo2 coding region, followed by Cre recombinase expressed through an internal ribosome entry site (IRES) (Fig. 1a, 1). Mice carrying this allele express Piezo2–GFP fusion protein as well as Cre recombinase driven by the endogenous Piezo2 promoter. Expression of the Piezo2–GFP fusion protein in human embryonic kidney (HEK293T) cells gives rise to mechanically activated currents indistinguishable from wild-type Piezo2-dependent currents (not shown). Using the Piezo2–GFP portion of the construct as a Piezo2 reporter, we examined Piezo2 expression in DRGs isolated from Piezo2−/− mice as a positive control tissue23. When we co-stained using anti-GFP and anti-Piezo2 antibodies, GFP and Piezo2 expression patterns overlapped (Extended Data Fig. 1).

We examined both hairy and glabrous skin of Piezo2+/GFP mice for Piezo2 expression. Piezo2 was previously shown to be present at low levels in the skin by quantitative polymerase chain reaction with reverse transcription (qRT–PCR)20, and here we found that GFP was specifically expressed in Merkel cells (~0.05–0.1% of total epithelial cells from dorsal skin) within whisker pad, dorsal skin, and footpad (Fig. 1b–f and Extended Data Fig. 2a–c). We used antibodies against keratin 8 (Krt8, a marker for Merkel cells) and neurofilament heavy polypeptide (Nefh, a marker for myelinated sensory afferents) in conjunction with GFP antibody to visualize the precise localization of Piezo2 within Merkel–neurite complexes. GFP was expressed in Merkel cells, preferentially on the side adjacent to afferent fibre innervation (Fig. 1b–f and Extended Data Fig. 2d–h). GFP was also present in Nefh+ sensory afferents, including the fibres that innervated Merkel cells (Fig. 1c, d and Extended Data Fig. 2d–h).

Because of the close proximity between Merkel cells and innervating afferents, it was difficult to convincingly conclude that GFP and Piezo2 were indeed present in Merkel cells. Therefore, we used atonal homologue 1 fused to GFP (Atoh1GFP) reporter mice expressing GFP in Merkel cells to purify these cells and perform qRT–PCR analysis for Piezo2 (ref. 24). GFP+ and GFP– cells from Atoh1GFP dorsal skin were purified by fluorescence-activated cell sorting (FACS), and total RNA from these samples was subjected to qRT–PCR for Krt8, Piezo2 and keratin 14 (Krt14, a marker for basal keratinocytes and Merkel cells) (Fig. 1g, h). As expected, GFP+ cells showed high expression levels of Krt14 (1.1, left), indicating that they were indeed Merkel cells. Importantly, GFP+ cells also showed high Piezo2 levels comparable to DRG neurons, confirming Piezo2 expression in Merkel cells (Fig. 1h, middle). Piezo2 levels in GFP cells were minimal, consistent with our GFP immunofluorescence results in Piezo2−/− mouse skin as well as our previous qRT–PCR results from skin (Fig. 1b–f)20. As expected, Krt14 expression was observed in both GFP+ and GFP– epithelial cells, but not in DRGs. This is in agreement with GFP+ epithelial cells being mainly comprised of basal keratinocytes (Fig. 1h, right).

To probe the role of Piezo2 in Merkel cells, we engineered skin-specific Piezo2 knockout mice. We generated floxed Piezo2 mice (Piezo2flox), which contained two loxP sites flanking exons 43 through 45 (Fig. 2a). We targeted this specific region close to the C terminus as it is highly conserved across different species, and moreover, the Cre excision of exons 43–45 causes a frameshift mutation in Piezo2, introducing an early stop codon (Extended Data Fig. 3). We crossed Piezo2−/− mice to Krt14Cre mice to generate Krt14Cre;Piezo2flox conditional knockout (cKO) mice, in which Piezo2 expression is ablated in all epithelial cells including Merkel cells (Fig. 2a, bottom). Krt14Cre;Piezo2flox conditional knockout mice developed normally, and their skin, including Merkel cell–neurite complexes, appeared normal compared to Piezo2−/− wild-type littermates (Extended Data Fig. 4). To study a Piezo2 deletion specifically in Merkel cells, we further generated Krt14Cre;Piezo2flox;Atoh1GFP mice. This particular mouse line allowed for the purification of GFP+ Piezo2-deficient and wild-type Merkel cells for qRT–PCR (Fig. 2b, c). In GFP+ Piezo2-deficient Merkel

1Howard Hughes Medical Institute, Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, California 92037, USA. 2 Departments of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA. 3 Departments of Dermatology & Physiology and Cellular Biophysics, Columbia University, New York, New York 10032, USA. 4 Genomics Institute of the Novartis Research Foundation, San Diego, California 92121, USA. 5 Present address: Gladstone Institute of Neurological Disease, San Francisco, California 94158, USA.

©2014 Macmillan Publishers Limited. All rights reserved
cells, nearly 95% of Piezo2 transcript was degraded compared to wild type, whereas Piezo2 levels in DRGs from these knockout mice were normal (Fig. 2b, c). Moreover, immunofluorescence using the Piezo2 antibody in Piezo2-deficient whisker follicles revealed that Piezo2 expression was abolished in Merkel cells (Fig. 2d, e) but still intact in afferent fibres that innervated Merkel cells (Fig. 2e, f). These results indicate that our skin-specific Piezo2 conditional knockout mice efficiently ablated Piezo2 only in the epidermal lineage, including Merkel cells.

Next, we asked whether Merkel cells are mechanosensitive, and if they are, whether their mechanosensitivity is dependent on Piezo2. Using Krt14Cre;Piezo2\(^{fl/fl}\);Atoh1GFP and littermate control mice, GFP\(^+\) wild-type and Piezo2-deficient Merkel cells were FACs-purified for whole-cell patch clamp electrophysiological recordings. When Merkel cells were stimulated by gentle poking using a blunt glass probe, wild-type cells (n = 15) responded robustly in a stimulus-dependent manner, producing rapidly adapting currents (τ = 6.4 ± 1.5 ms, Fig. 3a, left). However, none of the Piezo2-deficient Merkel cells (n = 13) showed mechanically activated currents (Fig. 3a, right). The lack of mechanosensitivity in Piezo2-deficient cells could not be accounted for by compromised membrane properties (Extended Data Table 1).

Mechanosensitive currents were rapidly adapting in voltage clamp mode. In current clamp mode, sustained depolarization was induced by gentle mechanical stimuli in all wild-type but not in Piezo2-deficient Merkel cells (Fig. 3b, c, top). Subsequent voltage clamp recordings in these same cells confirmed activation of rapidly adapting currents in wild-type cells upon mechanical stimulation (Fig. 3b, c, middle). Importantly, both wild-type and Piezo2-deficient Merkel cells were depolarized similarly by small current injections (Fig. 3b, c, bottom, and Extended Data Table 1). We next asked whether mechanically activated currents through Piezo2 could be responsible for the prolonged depolarization in wild-type cells (Fig. 3b, top). To answer this, we injected short-duration currents with amplitudes similar to Piezo2-driven currents, and indeed observed sustained depolarization in wild-type Merkel cells (Extended Data Fig. 5), indicating that a predominant rapidly adapting current could account for the observed sustained receptor potentials in these cells. Other conductances may also contribute to this prolonged depolarization, including voltage-gated calcium currents, which are reported to have a threshold near −20 mV (ref. 17), and stochastic gating of Merkel-cell-membrane ion channels, which could cause significant voltage fluctuations due to the high \(R_m\) of these cells (Extended Data Table 1). Thus, activation of Merkel cells by gentle touch produces a long-lasting depolarization that may ultimately contribute to the slowly adapting firing of SAI nerve fibres that innervate them. Collectively, these data show for the first time that Merkel cells are indeed mechanosensitive, and that Piezo2 is required in Merkel cells to produce their mechanical currents in vitro.

Next, we asked whether Piezo2 ablation in Merkel cells has any effect on slowly adapting responses of A\(\beta\) touch dome afferents. To answer this, we performed ex vivo skin–saphenous nerve recordings in Krt14Cre;Piezo2\(^{fl/fl}\) (cKO) and Piezo2\(^{WT}\) (WT) littermates (Fig. 4). The overall proportions of A\(\beta\) fibre subtypes found by an electrical search stimulus were similar in both groups (Fig. 4a). Interestingly, firing
freedoms of overall slowly adapting Aβ fibres were comparable between Piezo2-deficient and wild-type mice (Extended Data Fig. 7 and Ex-

Figure 2 | Generation and characterization of skin-specific Piezo2 conditional knockout mice. a, A schematic diagram of the Piezo2\(^{\text{fl}}\) allele generation. Flp, flippase. b, Representative FACS plots (out of 9 experiments) of live epithelial cells isolated from wild-type (WT) and conditional knockout (cKO) dorsal skin. c, qRT–PCR analysis (\(n = 3\)) showing Piezo2 levels in GFP\(^{+}\) cells and DRG neurons from wild-type (WT) and conditional knockout (cKO) mice. Bars represent mean ± s.e.m. ***\(p < 0.001\); NS, not significantly different, unpaired t-test with Welch’s correction. d, Piezo2 and Krt8 co-
staining in wild-type (d) and conditional knockout (e) whiskers pads. In e, arrows mark the position of Krt8\(^{+}\) Merkel cells. f, Piezo2, Krt8 and Nefh co-
staining in Piezo2 conditional knockout whisker pad. Arrowheads mark the co-localization of Piezo2 and Nefh in sensory afferents. Scale bars d–f, 20 μm.
We next performed somatosensory behavioural assays in Piezo2 conditional knockout and control littermate mice. Because Merkel cell–neurite complexes are known to mediate gentle touch sensation, we subjected these animals to tests for gentle touch, texture and pain, which included von Frey filaments, cotton swab sensitivity of the hindpaw, texture discrimination assay and a Randall Selitto test. For most of the above-mentioned assays, Piezo2 conditional knockout animals behaved similarly to control littermates (data not shown). In the automated von Frey filament test, Piezo2-deficient mice showed a significant decrease in per cent paw withdrawal response only at lower forces (1.0 g–1.5 g), but showed comparable responses to controls at higher forces (Fig. 5a). At low forces, not only were the responses less frequent, but fewer knockout mice responded (Fig. 5b). These results indicate that Piezo2-deficient mice are affected in feeling only low force mechanical stimuli. This phenotype is in agreement with our skin–nerve recordings, which show a reduction, but not complete ablation of slowly adapting responses in Piezo2-deficient mice.

We have provided answers to critical questions regarding the functionality of the Merkel cell–neurite complex. Our study is the first, to our knowledge, to show that Merkel cells display touch-sensitive currents, and that Piezo2 is required for Merkel-cell mechanotransduction. This represents the first definitive evidence for a mammalian Piezo family member to be involved in mechanotransduction in vivo. Moreover, we show that Merkel cells have a partial role in the generation of slowly adapting responses and in gentle touch perception in mice. Interestingly, the accompanying manuscript demonstrates that Krt14Cre;Atoh1CKO animals, which completely lack epidermal Merkel cells in their skin, show similar SAI firing deficits as Piezo2 conditional knockout mice in skin–nerve recordings. This reinforces the hypothesis that Piezo2 is the principal Merkel-cell mechanotransduction channel in vivo. Our current data are most consistent with a two-receptor-site model, which proposes that both Merkel cells and Aβ sensory afferents are necessary for mediating proper mechanically activated slowly adapting responses. This model explains why slowly adapting fibres are only partially affected when Merkel cells are present but not mechanosensitive. The observed expression of Piezo2 in the afferents that innervate Merkel cells may also support this model. Analysis of the sensory afferent-specific Piezo2-deficient animal model will provide further clues to reveal the extent to which mechanotransduction is directly dependent on the nerves. Most peripheral sensory afferents in Piezo2-deficient mice produced intermediating adapting firing patterns (Fig. 4e). The number of spikes fired (Fig. 4f and Extended Data Table 3) and overall mean firing rates were significantly diminished in touch dome afferents of Piezo2-deficient mice (17 ± 3 Hz; n = 33 displacements from 6 afferents), compared to wild type (27 ± 4 Hz; n = 28 displacements from 5 afferents). This difference was more pronounced during the static stimulation phase, resulting in reduced static firing rates (Fig. 4f). Together, our data suggest that Piezo2 is required for proper mechanosensory encoding in Merkel cell–neurite complexes in the intact skin.
receptors are thought to have evolved with a single receptor site for sensory transduction. The Merkel cell–neurite complex is known to have the highest spatial resolution among other cutaneous mecanoreceptors, and this allows for deciphering fine spatial details such as shape, edge and curvature. We speculate that mechanosensors present both at nerve endings and in Merkel cells act together to convey the exquisite mechanosensitivity of this complex.

METHODS SUMMARY

Detailed information regarding mouse lines, immunofluorescence, Merkel-cell isolation, qRT–PCR, FACS, whole-cell electrophysiology, skin-nerve recordings and behavioural assays is provided in the Methods.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 20 November 2013; accepted 14 March 2014.

Published online 6 April 2014.

1. Abraira, V. E. & Ginty, D. D. The sensory neurons of touch. Neuron 79, 618–639 (2013).
2. Maksimovic, S., Baba, Y. & Lumpkin, E. A. Neurotransmitters and synaptic components in the Merkel cell-neurilemma complex, a gentle-touch receptor. Ann. NY Acad. Sci. 1279, 13–21 (2013).
3. Chambers, M. R. & Iggo, A. Slowly-adapting cutaneous mechanoreceptors. J. Physiol. (Lond.) 132, (suppl.) 26P–27P (1967).
4. Iggo, A. & Muir, A. R. The structure and function of a slowly adapting touch corpuscle in hairy skin. J. Physiol. (Lond.) 200, 763–796 (1969).
5. Johnson, K. O. The roles and functions of cutaneous mechanoreceptors. Curr. Opin. Neurobiol. 11, 455–461 (2001).
6. Halata, Z., Grimm, M. & Bauman, K. I. Friedrich Sigmund Merkel and his “Merkel cell”, morphology, development, and physiology: review and new results. Anat. Rec. A Discov. Mol. Cell. Evol. Biol. 271A, 225–239 (2003).
7. Ikeda, I., Yamashita, Y., Ono, T. & Ogawa, H. Selective phototoxic destruction of rat Merkel cells abolishes responses of slowly adapting type I mechanoreceptor units. J. Physiol. (Lond.) 479, 247–256 (1994).
8. Mills, L. R. & Diamond, J. Merkel cells are not the mechanosensory transducers in the touch dome of the rat. J. Neurocytol. 24, 117–134 (1995).
9. Senok, S. S., Baumann, K. I. & Halata, Z. Selective phototoxic destruction of quinacrine-loaded Merkel cells is neither selective nor complete. Exp. Brain Res. 110, 325–334 (1996).
10. Kinkel, L., Stucky, C. L. & Kolzenburg, M. Postnatal loss of Merkel cells, but not of slowly adapting mechanoreceptors in mice lacking the neurotrophin receptor p75. Eur. J. Neurosci. 11, 3963–3969 (1999).
11. Marichich, S. M. et al. Merkel cells are essential for light-touch responses. Science 324, 1580–1582 (2009).
12. Marichich, S. M., Morrison, K. M., Mathes, E. L. & Brewer, B. M. Rodents rely on Merkel cells for texture discrimination tasks. J. Neuroscience 32, 3296–3300 (2012).
13. Hartschuh, W. & Weihe, E. Fine structural analysis of the synaptic junction of Merkel cell-axon-complexes. J. Invest. Dermatol. 75, 159–165 (1980).
14. Gu, J., Polak, J. M., Tapia, F. J., Marangos, P. J. & Pearse, A. G. Neuron-specific enolase in the Merkel cells of mammalian skin. The use of specific antibody as a simple and reliable histologic marker. Am. J. Pathol. 104, 63–68 (1981).
15. Fagan, B. M. & Cahusac, P. M. Evidence for glutamate receptor mediated transmission at mechanoreceptors in the skin. Neurreport 12, 341–347 (2001).
16. Diamond, J., Holmes, M. & Nurse, C. A. Are Merkel cell-neurite reciprocal synapses involved in the initiation of tactile responses in salamander skin? J. Physiol. (Lond.) 376, 101–120 (1986).
17. Yamashita, Y., Akaike, N., Wakamori, M., Ikeda, I. & Ogawa, H. Voltage-dependent currents in isolated single Merkel cells of rats. J. Physiol. (Lond.) 450, 143–162 (1992).
18. Cahusac, P. M. & Mavuluri, S. C. Non-competitive metabotropic glutamate 1 receptor antagonists block activity of slowly adapting type I mechanoreceptor units in the rat sinus hair follicle. Neuroscience 163, 933–941 (2009).
19. Press, D., Multi, S. & Guclu, B. Evidence of fast serotonin transmission in frog slowly adapting type 1 responses. Somatosens. Res. 27, 174–185 (2010).
20. Coste, B. et al. Piezo 1 and Piezo 2 are essential components of distinct mechanically activated cation channels. Science 330, 55–60 (2010).
21. Coste, B. et al. Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 483, 176–181 (2012).
22. Kim, S. E., Coste, B., Chadha, A., Cook, B. & Patapoutian, A. The role of Drosophila Piezo in mechanical nociception. Nature 483, 209–212 (2012).
23. Fauver, A., Nargeot, J., Mangoni, M. E. & Jolting. C. Piezo2 regulates vertebrate light touch response. J. Neurosci. 33, 17089–17094 (2013).
24. Rose, M. F. et al. Math 1 is essential for the development of hindbrain neurons critical for peripheral breathing. Neuron 64, 341–354 (2009).
25. Dassule, H. R., Lewis, P., Bei, M., Maas, R. & McMahon, A. P. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 127, 4775–4785 (2000).
26. Lasniak, D. et al. Computation identifies structural features that govern neuronal firing properties in slowly adapting touch receptors. eLife 3, e01488 (2014).
27. Welnitz, S. A., Lasniak, D. R., Gelting, G. J. & Lumpkin, E. A. The regularity of sustained firing reveals two populations of slowly adapting touch receptors in mouse hairy skin. J. Neurophysiol. 103, 3378–3388 (2010).
28. Maksimovic, S. et al. Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. Nature http://dx.doi.org/10.1038/nature13250 (2013).
29. Yamashita, Y. & Ogawa, H. Slowly adapting mechanoreceptor afferent units associated with Merkel cells in frogs and effects of direct currents. Somatosens. Res. 8, 87–95 (1991).
30. Milenkovic, N., Wetzal, C., Moshourab, R. & Lewin, G. R. Speed and temperature dependences of mechanotransduction in afferent fibers recorded from the mouse saphenous nerve. J. Neurophysiol. 100, 2771–2783 (2008).

Acknowledgements We would like to thank S. Murthy and B. Coste for suggestions. Research was supported by the Howard Hughes Medical Institute (to A.P.) and NIH grants R01 DE022358 (to A.P.) and R01 AR051219 (to E.A.L.).

Author Contributions S.-H.W. conducted experiments for Figs 1–3 and Extended Data Figs 1–5. A.E.D. conducted and analysed in vitro electrophysiology experiments in Fig. 3 and Extended Data Fig. S. A.D.W. performed and analysed general skin-nerve recordings in Fig. 4a, b and Extended Data Figs 6 and 7. M.P. and K.R. performed behavioural experiments in Fig. 5. S.R. generated Piezo2½ mice. Z.Q. and T.M. generated Piezo2fl/fl mice. During this manuscript’s peer-review process, we entered into a collaboration with Y.B. and E.A.L. E.A.L. conceived, and Y.B. performed and analysed targeted skin-nerv recordings in Fig. 4c-f and E.A.L. and C.L.S. contributed to the editing of the manuscript. S.-H.W. and A.P. designed experiments and wrote the manuscript.

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

©2014 Macmillan Publishers Limited. All rights reserved
DNA was isolated. For electrophysiological recordings, cells were sorted into CiT-02 media with 10% FBS. Merkel cells were cultured overnight and subjected to electrophysiological recordings the following day.

**Western blotting.** HEK293T cells were transfected with pIRES2-EGFP, mPiezo1- pcDNA3.1(-)–IRES-EGFP, or mPiezo2-sport-IRESEGFP vectors using Lipofectamine 2000. Cells were collected 48 h post-transfection and cell lysates were processed for western blotting as previously described22.

**Whole-cell electrophysiology.** Whole-cell voltage clamp recordings were performed as previously described22. Current clamp recordings were performed as previously described24. Briefly, cells were continuously perfused with (in mM) 127 NaCl, 3 KCl, 1 MgCl₂, 2.5 CaCl₂, 10 dextrose, 10 HEPES (pH 7.3). Electrodes had resistances of 3.7 ± 0.2 MΩ (n = 25) when filled with standard CaCl₂ based intracellular solution (in mM): 133 CsCl, 1 MgCl₂, 5 EGTA, 10 HEPES, 4 Mg-ATP, 0.5 Na-GTP (pH 7.3 with CsOH). Access resistance was not different between genotypes (9 ± 1 MΩ; n = 19). To determine membrane potential changes in current clamp, cells were recorded using a low Cl⁻ intracellular solution (in mM): 125 K-gluc, 7.5 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, 1 tetraK-BAPTA, 4 Mg-ATP, 0.5 Na-GTP (pH 7.3 with KOH) (electrode resistances were 6 ± 1 MΩ (n = 12)). The probe displacement was advanced in increments of 0.25 μm.

In current clamp experiments, depolarizing current was injected in increments of 5 pA from membrane potential near −60 mV (achieved by applying hyperpolarizing bias current). Cell capacitance was determined as previously described22. Rm was determined using 20 mV steps at the beginning of each sweep for mechanically activated current acquisition, and membrane time constant tau was calculated as (Rm × Cm) (Extended Data Table 1). Membrane time constant was also determined by fitting membrane depolarizations elicited by small amplitude current injections with a single exponential (Extended Data Table 1). All recordings and data analyses were performed in a blinded manner.

Wild-type Merkel cells were very sensitive to mechanical stimulation: during advancement of the probe in 0.25 μm increments, initial responses were sometimes observed as the probe visibly touched the cell and on average were observed with 0.4 ± 0.1 μm displacement beyond the initial touch (n = 15, mean ± s.e.m.). On the other hand, Piezo2-deficient cells were not activated by 2.6 ± 0.3 μm (n = 13) above touching the cell (range: 1.8–4.5 μm before the recording was lost).

**Skin-nerve recordings (general method for Fig. 4a, b).** Piezo2 conditional knockout (n = 12) or wild-type littermates (n = 15) aged 7–17 weeks old were euthanized, and the hairy skin of the left and right hindpaws was shaved and dissected from the leg along with the innervating saphenous nerve. The skin-nerve preparation was then moved to a bath containing oxygenated synthetic interstitial fluid consisting of the following (in mM): 123 NaCl, 3.5 KCl, 0.7 MgSO₄, 1.7 NaH₂PO₄, 2.0 CaCl₂, 9.5 sodium glutamate, 5.5 glucose, 7.5 sucrose and 10 HEPES. The buffer was brought to a pH of 7.45 ± 0.05, and all recordings were performed with a bath temperature of 32 ± 0.5 °C. All recordings were performed with the corium side up.

The saphenous nerve was then placed on a mirror plate in a mineral oil-filled chamber and was teased apart into fine filaments and placed on an AgCl recording electrode. Most functionally independent fibres were identified using an electrical search stimulus, although a small number of fibres were found using a mechanical search stimulus. Fibres showing percentages of fibre types were made using only those fibres found with an electrical search stimulus (Fig. 4a). After fibre isolation, the most sensitive spot of the receptive field was determined electrically, and conduction velocity was calculated. Fibres were categorized as Aβ, Aδ, or C based on their conduction velocity as previously described24,25, with Aβ fibres conducting at velocities greater than 10 m per sec, Aδ fibres between 1.2–10 m per sec, and C fibres below 1.2 m per sec. Fibres were also grouped based on the adaptation to mechanical stimuli (rapidly adapting (RA) fibres; slowly adapting (SA) fibres; mechanically insensitive (MI) fibres). Note that intermediate adapting (IA) fibres were included within the SA category in Fig. 4a. Mechanical thresholds were obtained for each fibre type using von Frey filaments (0.044 mN (0.0045 g) to 147 mN (14.99 g)). Skin-nerve recordings were digitized on a oscilloscope and recorded on a computer for analysis using a PowerLab data acquisition system and Chart 5 software. Once a functionally independent fibre was found, two-minute baseline recordings were made to determine spontaneous activity. Fibres were then subject to a series of forces (5 mN (0.51 g), 10 mN (1.02 g), 20 mN (2.04 g), 40 mN (4.08 g), 100 mN (10.2 g), 150 mN (15.3 g), and 200 mN (20.4 g)) for 10 s using a feedback-controlled, computer-driven mechanical stimulator with a flat ceramic tip (diameter 1.0 mm). Action potential responses to these stimuli were recorded in LabChart, and 1-min intervals were given between stimuli to prevent sensitization/desensitization. All recordings and data analysis were performed in a blinded manner.

**Single-cell recordings from dorsal root afferents identified by FM1–43 for Fig. 4c–f.** Éx vivo skin-nerve recordings from identified touch dome afferents were performed as described24. Adult female mice (7–15-weeks-old) were injected subcutaneously with FM1–43 (70 µl of 1.5 mM in sterile PBS) to label Merkel cells and cutaneous sensory afferents in touch domes of
Piezo2 conditional knockout and control mice. Hindlimb skin was shaved, depilated and harvested 12–14 h after injection. Skin was mounted epidermis-side-up and was continuously perfused with carbogen-buffered synthetic interstitial fluid (SIF) at 32 °C. The nerve was kept in mineral oil in a recording chamber, teased and placed onto a silver recording electrode connected with a reference electrode to differential amplifier. Ramp-and-hold voltages (0.1–1.3 mm) were delivered with a custom-built indenter probe (1.6-mm ceramic tip; 5-s hold phases). Each indentation was delivered from a point 0.6 mm above the skin’s surface, as indicated in Fig. 4c. One-minute intervals were given between successive stimuli. Extracellular signals were digitized and recorded using Scientiffic Workbench software (DataWave Technologies). FM1-43-labelled Merkel cell–neurite complexes in each afferent’s receptive field were visualized using a fluorescence microscope equipped with a long-pass GFP filter set. Responses from touch dome afferents were identified with a mechanical search protocol as described4,27,28. Responses were recorded from touch dome afferents identified with a mechanical search protocol as described4,27,28. Responses were classified as immediately adapting if spikes persisted through only a portion of the first 4 s of the static phase, and slowly adapting if spikes were observed throughout the duration of the 5-s hold phase. All recordings and analysis were performed blind to genotype.

**Behavioural assay (von Frey filament stimulation).** Males and females aged 6–15 weeks old were used in behavioural experiments. An automated von Frey apparatus (Dynamic Plantar Aesthesiometer, UGO Basile) was used to conduct experiments as described previously29. Mice were allowed to acclimate on a wire mesh and then were probed with von Frey filaments of defined force (g). The hind paw was probed for one second and scored for a withdrawal response. Each force was probed four times, and per cent withdrawal responses were calculated. The same set of withdrawal response data was also used to calculate per cent responders to von Frey stimulation. Out of four stimulations, an animal that gave at least one withdrawal response was considered as a responder.

**Statistics.** Statistical analyses for qRT–PCR, whole cell current density, and behavioural assays were performed using an unpaired t-test with Welch’s correction. For skin–nerve recordings, firing frequencies between wild-type and Piezo2 conditional knockout Aβ fibres over different forces were compared using a two-way ANOVA with Bonferroni post-hoc analysis. Percentage of fibre types between genotypes was determined using a contingency table with the chi squared test. Analysis of conduction velocities between the two genotypes was performed using Student’s t-test, and von Frey thresholds were compared using a Mann–Whitney U-test. For targeted skin–nerve recordings, spike counts and mean firing rates in identified touch dome afferents (by FM1-43) were compared with Student’s t-test (two-tailed, unpaired). The proportion of immediately adapting responses between genotypes was compared using a contingency table with Fisher’s exact test.

**Sample size choice.** For Merkel-cell isolation using FACs and all immunofluorescence studies, we performed more than 10 separate experiments with different samples, and we observed consistent results from all experiments that were conducted independently. For qRT–PCR analysis, sample sizes were chosen based on our previous studies, in which we performed at least 3 separate experiments per sample to ensure statistical significance20. For whole-cell electrophysiological recordings, we observed that in vitro recorded MA currents were either present (in all wild-type Merkel cells) or not (in all conditional knockout cells). Therefore, we recorded from 15 wild-type Merkel cells and 13 Piezo2 conditional knockout Merkel cells to ensure consistent results and statistical significance. For ex vivo skin–nerve recordings, we chose sample sizes according to the previous work of touch-sensitive afferents in Atoh1−/− mice13. Among Aβ afferents, they observed 8 SA1 afferents in WT littermates (n = 8/39) but none in Atoh1−/−genotypes (n = 0/27)13. Therefore, we concluded that a minimum of five afferents per genotype would be sufficient to observe differences in response properties of touch dome afferents in our targeted recordings. For behavioural assays, sample sizes were chosen based on our previous studies in which we used a minimum of 8–10 animals per genotype to ensure statistical significance20. For all our assays, multiple cohorts were tested on multiple days so that our results were not biased. We tested 32 mice (wild-type littermates) and 33 mice (Piezo2 conditional knockouts) in order to ensure multiple cohorts were tested.
Extended Data Figure 1 | Validation of anti-Piezo2 antibody.  
a, Piezo2 detection by western blotting using anti-Piezo2 antibody (see full methods for antibody generation) in HEK293T cells overexpressing pIres2-EGFP (left lane), mPiezo1-pcDNA3.1-Ires-EGFP (middle lane), and mPiezo2-sport6-Ires-EGFP (right lane). 
b, Piezo2 immunofluorescence in mPiezo2-sport6-Ires-EGFP-transfected HEK293T cells. The left panel shows EGFP epifluorescence in transfected cells, and the middle panel shows Piezo2 immunofluorescence in these same cells.  
c, d, Immunofluorescence of GFP and Piezo2 in adult Piezo2GFP reporter (c) and wild-type littermate (d) DRG. Piezo2 expression is observed in ~45.6% of DRG neurons (587 neurons/1,287 total neurons). Of the Piezo2-expressing neurons, high Piezo2 expression was seen in 159/587 neurons. Scale bars (c, d), 100 μm.
Extended Data Figure 2 | GFP immunofluorescence in wild-type control and Piezo2GFP reporter mice. a, GFP, Krt8, and Nefh co-staining in wild-type littermate whisker follicle. b, c, GFP and Krt8 co-staining in wild-type littermate touch dome (b) and glabrous skin (c). Arrows mark the position of Krt8+ Merkel cells. d–h, GFP and Nefh co-staining in Piezo2GFP whisker follicle. e–h shows magnified views of the bracketed area in d. Arrows mark GFP expression only. Closed arrowheads mark the co-localization of GFP and Nefh. Scale bars (a–h), 20 μm. epi, epidermis; der, dermis.
Extended Data Figure 3 | Generation of Piezo2 null allele (Piezo2$^{-/-}$) and characterization of Piezo2 constitutive knockout mice. 

**a**, A schematic diagram of Piezo2$^{-/-}$ allele generation. qRT–PCR ($n = 2$) showing Piezo2 levels in Piezo2$^{+/+}$, Piezo2$^{+/}$, and Piezo2$^{-/-}$ E19.5 lungs. Error bars represent mean ± s.e.m. **P < 0.01; NS, not significantly different, unpaired t-test with Welch’s correction.**

**b**, Piezo2 immunofluorescence in wild-type littermate (c) and Piezo2$^{-/-}$ newborn DRG (d). Scale bars (c, d), 100 μm.
Extended Data Figure 4 | Characterization of Piezo2<sup>WT</sup> (wild type) and Krt14Cre;Piezo2<sup>cKO</sup> (conditional knockout) adult skin. 

**a, b**, Haematoxylin and eosin (H&E) staining of wild-type (WT) and Piezo2 conditional knockout (cKO) dorsal skin. 

**c, d**, Immunofluorescence of Krt14 and a-SMA (alpha smooth muscle actin) in wild-type and conditional knockout dorsal skin. 

**e–g, j–l**, Epidermal touch domes co-stained with Krt8, Nefh and VGLUT2 (vesicular glutamate transporter 2, a marker for Merkel cells) in wild-type and conditional knockout dorsal skin. 

**h, m, n**, Lanceolate endings and circumferential fibres co-stained with S100 (S100 calcium binding protein, a marker for Schwann cells) and Nefh in wild-type and conditional knockout dorsal skin. Closed arrowheads mark circumferential fibres, and arrows mark lanceolate endings. 

**i, o**, Meissner’s corpuscles co-stained with S100 and Nefh in wild-type and conditional knockout footpads. Closed arrows mark Meissner’s corpuscle. Scale bars, 100 μm (a, b), 20 μm (other panels). epi, epidermis; der, dermis.
Extended Data Figure 5 | Current injections simulating Piezo2-mediated currents produce prolonged depolarizations in Merkel cells in vitro.

a, Representative traces of mechanically activated inward currents evoked by a gentle poking stimulus in a wild-type Merkel cell. A ramp (1 μm s⁻¹)-and-hold displacement stimulus (0.25 μm increments) was applied to the cell in whole-cell voltage clamp configuration. The steady state current at the end of a 125 ms displacement was $-6 \pm 2 \text{pA} (V_{\text{hold}} = -80 \text{mV}, n = 15), 4\%$ of the maximal current observed ($-146 \pm 29 \text{pA}$).

b, Representative current clamp recordings from a wild-type Merkel cell, displaying a change in membrane potential in response to gentle mechanical stimuli.

c, Piezo2-dependent currents were simulated by injecting short current pulses followed by different levels of long-lasting but small current injections. In wild-type Merkel cells ($n = 4$), membrane potential changes are elicited by applying a short (2.5 ms) 150 pA current injection followed by additional current injections of 0 pA (black), +0.5 pA (blue), +1 pA (orange) and +2 pA (red) from the bias holding current ($-10 \text{pA}$). In the absence of any continuous current, the membrane potential slowly decays after cessation of the initial 150 pA injection, consistent with a contribution of passive membrane properties (black trace). Importantly, long-lasting depolarizations are observed when these short pulses are followed by very small current injections (0.5–1 pA, which are $\sim 10\%–15\%$ of the average observed Piezo2 current remaining at the end of the 125 ms mechanical stimulation (Fig. 3b, see above)).

d, In wild-type cells ($n = 4$), membrane potential changes are elicited by a short 100 pA current injection followed by +3 pA (half the average Piezo2-dependent mechanically activated sustained current) from the bias holding current ($-10 \text{pA}$). Orange line, 2.5 ms initial pulse; Blue line, 5 ms initial pulse. These data indicate that long-lasting Piezo2 channel activity at levels below that observed during mechanical stimulation (panel a and Fig. 3b) is crucial for sustained membrane depolarizations in Merkel cells. The high $R_m$ of these cells can enable small current fluctuations to produce large voltage fluctuations.
Extended Data Figure 6 | Conduction velocity and von Frey thresholds of all slowly adapting Aβ afferents in wild-type and Piezo2 conditional knockout mice. Conduction velocity (*P < 0.05, Student’s t-test) and von Frey thresholds (P = 0.0516, Mann–Whitney U-test) of all slowly adapting Aβ fibres from wild-type and Piezo2 conditional knockout mice. Error bars represent mean ± s.e.m.
Extended Data Figure 7 | Characterization of rapidly adapting Aβ afferents in wild-type and Piezo2 conditional knockout mice. a, Firing rates of rapidly adapting Aβ fibres in response to an increasing series of mechanical forces in wild-type and Piezo2 conditional knockout mice. b, c, Conduction velocity (b) and von Frey thresholds (c) of rapidly adapting Aβ fibres from wild-type and Piezo2 conditional knockout mice.
Extended Data Table 1 | Membrane properties of wild-type and Piezo2 conditional knockout Merkel cells

| Parameter                                                                 | WT            | Piezo2 cKO      |
|---------------------------------------------------------------------------|---------------|-----------------|
| Cell capacitance (pF)                                                     | 2.3 ± 0.2 (n=17) | 2.4 ± 0.1 (n=17) |
| Current at -100mV (pA)                                                   | -5 ± 1 (n=17)  | -2 ± 1 (n=17)  |
| $R_{in}$ in K-glucanate (GΩ)                                              | 15 ± 2 (n=10)  | 14, 17 (n=2)   |
| $R_{m} \times C_{m}$ (calculated membrane time constant) in K-glucanate (msec) | 35 ± 6 (n=10) | 33, 41 (n=2) |
| Membrane time constant determined from current clamp recordings (msec)   | 70 ± 17 (n=11) | 60 ± 24 (n=3)  |
| Current injection required to elicit membrane potential fluctuations (not passive change) (pA, 125 msec injection) | 17 ± 5 (4) | 14, 20 (n=2) |
| Resting potential in K-Gluconate (mV)                                    | -22 ± 3 (n=8)  | -15, -25 (n=2) |
Extended Data Table 2 | Afferent properties of Aβ, Aδ, and C fibres from wild-type and *Piezo2* conditional knockout animals

| Fiber Type | Conduction Velocity (m/s) | von Frey Threshold (mN) | n  |
|------------|---------------------------|-------------------------|----|
| Aβ fiber WT | 13.59 ± 0.36              | 2.94 ± 0.47             | 73 |
| cKO        | 13.08 ± 0.37              | 3.89 ± 0.43             | 70 |
| Aδ fiber WT | 6.41 ± 0.49               | 4.37 ± 1.37             | 22 |
| cKO        | 5.08 ± 0.75               | 6.77 ± 1.16             | 16 |
| C fiber WT  | 0.57 ± 0.04               | 3.67 ± 0.84             | 17 |
| cKO        | 0.60 ± 0.05               | 5.52 ± 0.80             | 21 |

No statistically significant differences were found in conduction velocity or von Frey threshold between the two genotypes.
Extended Data Table 3 | Summary of touch dome responses for wild-type and *Piezo2* conditional knockout animals

### a

| Data ID | Dynamic phase | Static phase |
|---------|---------------|--------------|
|         | Min | Max | Ave±SD | Median | Min | Max | Ave±SD | Median |
| KO 1    | 3.9 | 13.4 | 6.5±1.9 | 6.2    | 6.9 | 67.6 | 16.0±9.8 | 13.3 |
| KO 2    | 3.2 | 67.8 | 28.7±17.3 | 36.6 | 38.7 | 200.8 | 76.7±37.8 | 60.9 |
| KO 3    | 3.2 | 20.6 | 6.3±3.7 | 4.5 | 9.2 | 149.4 | 50.9±31.4 | 45.9 |
| KO 4    | 6.1 | 332.5 | 24.2±56.1 | 10.4 | 8.6 | 76.5 | 19.3±10.1 | 15.8 |
| KO 5    | 5.6 | 28.6 | 13.4±4.6 | 13 | 17.2 | 447.5 | 54.8±61.4 | 38.6 |
| KO 6    | 4.9 | 17.4 | 8.7±2.2 | 8.2 | 8.2 | 86.9 | 27.0±11.1 | 26.3 |

**Piezo2 KO**

| Data ID | Dynamic phase | Static phase |
|---------|---------------|--------------|
|         | Min | Max | Ave±SD | Median | Min | Max | Ave±SD | Median |
| Wild 1  | 5.1 | 23.5 | 9.6±4.0 | 8.1 | 4.1 | 50.6 | 14.7±5.0 | 14.9 |
| Wild 2  | 9.1 | 295.3 | 22.2±48.2 | 11.0 | 4.8 | 54.0 | 16.7±7.2 | 14.6 |
| Wild 3  | 2.5 | 51.1 | 5.4±5.4 | 3.4 | 3.0 | 190.3 | 12.4±16.8 | 6.1 |
| Wild 4  | 3.3 | 23.3 | 6.2±3.5 | 4.9 | 3.9 | 40.2 | 11.8±5.3 | 10.3 |
| Wild 5  | 4.1 | 245.7 | 10.2±26.9 | 6.1 | 6.4 | 42.8 | 14.2±5.7 | 13.1 |

**Control**

### b

| Data ID | Ratio of IA response | Num. of SP in Dynamic | Num. of SP in Static | CoV |
|---------|----------------------|-----------------------|----------------------|-----|
| KO 1    | 0.42                 | 79                    | 250                  | 0.61|
| KO 2    | 1                    | 25                    | 29                   | 0.49|
| KO 3    | 1                    | 95                    | 78                   | 0.62|
| KO 4    | 0.14                 | 35                    | 207                  | 0.52|
| KO 5    | 0.89                 | 58                    | 70                   | 1.12|
| KO 6    | 0.60                 | 57                    | 147                  | 0.41|

**Piezo2 KO**

| Data ID | Ratio of IA response | Num. of SP in Dynamic | Num. of SP in Static | CoV |
|---------|----------------------|-----------------------|----------------------|-----|
| Wild 1  | 0                    | 61                    | 271                  | 0.34|
| Wild 2  | 0                    | 45                    | 239                  | 0.43|
| Wild 3  | 0.13                 | 110                   | 321                  | 1.35|
| Wild 4  | 0.10                 | 102                   | 338                  | 0.45|
| Wild 5  | 0.07                 | 81                    | 282                  | 0.40|

**Control**

---

**a.** Summary of inter-spike intervals (ISIs) for touch-evoked responses. For each afferent, the maximal response was selected as representative data for further analysis. Dynamic phase: period from stimulus onset (when the indenter began moving) to end of ramp phase (when the indenter reached the hold displacement depth). Static phase: initial 4 s period after indenter reached commanded displacement. Unit of all values were milliseconds. Min, minimum; max, maximum; ave, average; SD, standard deviation. **b.** Summary of touch dome responses. IA, intermediately adapting. Num of SP: number of spikes. CoV: coefficient of variation of ISIs in the static phase.