**LETTER**

**Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors**

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Touch submodalities, such as flutter and pressure, are mediated by somatosensory afferents whose terminal specializations extract tactile features and encode them as action potential trains with unique activity patterns6. Whether non-neuronal cells tune touch receptors through active or passive mechanisms is debated. Terminal specializations are thought to function as passive mechanical filters analogous to the cochlea’s basilar membrane, which deconstructs complex sounds into tones that are transduced by mechanosensory hair cells. The model that cutaneous specializations are merely passive has been recently challenged because epidermal cells express sensory ion channels and neurotransmitters2,3; however, direct evidence that epidermal cells excite tactile afferents is lacking. Epidermal Merkel cells display features of sensory receptor cells2,3 and make ‘synapse-like’ contacts4 with slowly adapting type I (SAI) afferents5. These complexes, which encode spatial features such as edges and texture1, localize to skin regions with high tactile acuity, including whisker follicles, fingertips and touch domes. Here we show that Merkel cells actively participate in touch reception in mice. Merkel cells display fast, touch-evoked mechanotransduction currents. Optogenetic approaches in intact skin show that Merkel cells are both necessary and sufficient for sustained action-potential firing in tactile afferents. Recordings from touch-dome afferents lacking Merkel cells demonstrate that Merkel cells confer high-frequency responses to dynamic stimuli and enable sustained firing. These data are the first, to our knowledge, to directly demonstrate a functional, excitatory connection between epidermal cells and sensory neurons. Together, these findings indicate that Merkel cells actively tune mechanosensory responses to facilitate high spatio-temporal acuity. Moreover, our results indicate a division of labour in the Merkel cell–neurite connection between epidermal cells and sensory neurons. Together, these findings demonstrate that inward currents of touch-dome afferents lead to slowly adapting responses in vivo? Like hair cells and *Drosophila melanogaster* bristles14, Merkel-cell mechanotransduction channels display steady-state currents that are ~10% of peak responses (Extended Data Fig. 1). These currents are likely to be amplified by voltage-activated calcium channels4,10. Indeed, an accompanying manuscript demonstrates that inward currents of ~20 pA are sufficient to depolarize Merkel cells to voltage-activated ion-channel thresholds15. Moreover, computational modelling predicts that a rapidly adapting transduction current with a small steady-state component can account for SAI firing patterns16.

We next tested whether activating Merkel cells in the intact skin is sufficient to excite tactile afferents. We used optogenetics to selectively depolarize Merkel cells without directly stimulating their associated sensory afferents (Fig. 2a). A previous microarray screen identified cholecystokinin (Cck) as a Merkel-cell–specific transcript in the epidermis4. To express Channelrhodopsin-2 (ChR2) in Merkel cells *in vivo*, we crossed Cck-ires-Cre mice18 with mice harbouring a ChR2-ttdTomato fusion at the *Gt(Rosa)26Sor* locus19. Heterozygote CckCreERT2;ChR2loxP/+ mice showed strong expression of ChR2-ttdTomato in touch-dome Merkel cells, whose fluorescence was easily identifiable in intact skin (Fig. 2b).

Whole-cell recordings *in vitro* confirmed that ChR217. Merkel cells exhibited light-activated inward currents (*n* = 5, Extended Data Fig. 2). We confirmed the absence of ChR2 expression in SAI afferents via immunohistochemistry of skin cryosections (Fig. 2c) and whole-mounts (Extended Data Fig. 3a–e). ChR2 expression was not observed in any merkel cells. GFP-expressing Merkel cells were dissociated from mouse epidermis for distinct elements of discriminative touch.

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afferents that displayed light-evoked responses were touch-sensitive (Fig. 2d). When touch domes were presented with 5-s light pulses, action fibre optics with a collimator lens to restrict illumination to touch domes in some dermal cell types (Extended Data Fig. 3).

We next tested whether optogenetic silencing of Merkel cells inhibits touch-evoked firing in SAI afferents. We selectively expressed Archaerhodopsin-3 (ArchT), a green-light-sensitive, hyperpolarizing proton pump22, in Merkel cells (GckCre/ArchTloxP/lox; Extended Data Fig. 6). During 3-min displacements, touch domes were presented with a series of 10-s light pulses. We observed a tenfold reduction in median touch-evoked firing rates during light stimuli (n = 3; Fig. 2i, j). Inhibition grew progressively stronger during sustained displacement, becoming almost complete with successive light presentations (Fig. 2i). Thus, Merkel-cell depolarization is necessary for robust static phase firing in SAI afferents.

To determine whether Merkel cells also contribute to the dynamic phase of touch-evoked SAI responses, we analysed epidermal-specific Atoh1 conditional knockout (Atoh1CKO) mice (K14Cre;Atoh1lacZ/flox), which completely lack Merkel cells but have otherwise normal epidermis23. Touch domes were innervated by myelinated afferents that contain nodes of Ranvier, suggesting that they are capable of firing action potentials (Extended Data Fig. 7). A previous unbiased survey of touch-sensitive afferents reported a selective loss of SAI responses in Hoxb1lCre; Atoh1lacZ/flox mice, which lack Merkel cells from development but retain innervation of touch domes and footpads24. Here, we used a targeted approach to analyse firing properties of afferents that selectively innervate touch domes, which were identified during recording based on FM1-43 fluorophore uptake25. We found that touch domes in Atoh1CKO mice were innervated by mechanosensitive Aβ afferents (conduction velocity: 10.2–18.5 m/s, n = 6); however, their firing patterns differed markedly from SAI responses8,9 in control genotypes (Fig. 3a, b; n = 5). First, the static phase firing was truncated in Atoh1CKO mice compared with control afferents, which maintained firing throughout 5-s stimuli (Fig. 3a, b). Thus, responses of Atoh1CKO touch-dome afferents could be classified as intermediate adapting26 (IA; Fig. 3c).

Second, Atoh1CKO responses displayed markedly lower spike counts (Fig. 3d and Extended Data Table 2) and firing rates than control genotypes (Atoh1CKO mean ± s.d., 25 ± 8 Hz; control: 59 ± 25 Hz; P = 0.004). Notably, Atoh1CKO lacked high-frequency firing and short ISIs in both dynamic and static phases (Atoh1CKO peak firing rates, 79 ± 40 Hz; control, 238 ± 69 Hz; P = 0.003; Extended Data Fig. 8 and Extended Data Table 2). Together, these data indicate that touch-dome
Merkel cells are necessary and sufficient to elicit sustained action-potential trains in touch-dome afferents. a, Schematic of mouse ex vivo skin–nerve recordings. b, Confocal image of a ChR2-expressing touch dome in a living skin–nerve preparation. Scale bar, 20 μm. c, Immunostaining of skin cryosections shows expression of ChR2-tdTomato in Merkel cells (keratin 8, Krt8) but not in touch-dome afferents (Neurofilament heavy, NFH (also known as Nefh). Scale bar, 20 μm. d, During electrophysiological recording, ChR2-expressing Merkel cells and blue-light stimuli were imaged separately using different filter sets (bottom insets). Merged image illustrates the illuminated area (top panel). Confocal reconstruction of this touch dome is shown in Extended Data Fig. 2a–e. Scale bar, 200 μm. e, Light pulses of increasing intensities elicited phase-locked action potentials from the touch dome in d (left trace). Comparison of spike shapes evoked by light (blue) and touch (black) confirmed single-unit recording (right trace). f, Mean instantaneous firing frequency (IFF) versus light intensity for a single touch-dome afferent (top graph). Blue trace shows mean IFFs from e. Red trace shows mean IFFs evoked by light intensities presented in decreasing order. The averages of these stimuli (black trace) were analysed further in the lower part of the panel. Mean IFFs on a log-intensity scale (n = 12 single units; bottom graph). Data were fit with a four-parameter Weibull sigmoidal function (R² = 0.95). g, h, A sustained light-evoked response from the touch-dome afferent in d and e with corresponding ISI histogram. i–j, Optogenetic silencing of ArchT-expressing Merkel cells. i, Representative 3-min recording. j, Box-plot of firing rates during light-off (n = 3 units, n = 20 10-s periods) and light-on (same units, n = 23 10-s periods). Two outliers are firing rates from initial light-on periods, ***P = 0.001.

Our study sheds new light on the role of Merkel cells in touch reception. Our findings demonstrate that Merkel cells are touch-sensitive cells that actively tune mechanosensory afferents by conferring two features of the SAI response: sustained responses and high-frequency firing. First, by maintaining firing throughout mechanical stimulation, slowly adapting afferents inform the brain about pressure1,27. Our optogenetic approach demonstrates that Merkel-cell activation elicits, and silencing reversibly suppresses, sustained SAI firing. This provides the first direct evidence that Merkel cells are not simply passive mechanical filters in the skin. Moreover, recordings from Atoh1CKO and Piezo2CKO mice show that SAI afferents cannot properly convey static phase information without intact Merkel cells. Second, during active tactile exploration, high-frequency firing is important for encoding object features (such as edges and curvature) with high information content27,28. Although afferents have mechanosensory terminals capable of responding to touch, but do so with firing properties distinct from the canonical SAI response.

We next compared the responses of touch-dome afferents in Atoh1CKO with epidermal-specific Piezo2 knockout mice15 (Piezo2CKO). In the latter, Merkel cells and touch-dome afferents develop normally and are retained through adulthood15. Although these mutations disrupt distinct molecular pathways and cause different anatomical phenotypes, we found a remarkable degree of concordance between static-phase firing patterns. Mutant touch-dome afferents displayed a similar proportion of intermediately adapting responses (Fig. 3c)15 and showed similar increases in mean ISIs during static displacement (Fig. 3e and Extended Data Fig. 8). Together, these data indicate that Piezo2-dependent Merkel-cell signalling is essential for proper SAI responses to sustained pressure.
Our findings support two models for how Merkel cells contribute to the SAI afferent’s unique firing patterns. First, our data directly demonstrate that the Merkel cell–neurite complex is a compound sensory system with two receptor cell types that mediate different aspects of touch transduction.52 A similar division is found in the mammalian system, which also provides information on object shape and movement. In the retina, rods are optimized for low-light conditions and cones for high-acuity, colour vision. Second, the contribution of Merkel cells to dynamic firing indicates a previously unsuspected role in signal amplification, analogous to outer hair cells in the mammalian cochlea. These mechanosensory cells actively expend energy to tune the cochlea’s frequency selectivity and mechanical sensitivity. A key question that remains is the nature of the excitatory mechanisms that convey signals between Merkel cells and SAI afferents.

METHODS SUMMARY

Experimental procedures were performed in compliance with the Institutional Animal Care and Use Committees of Columbia University and Baylor College of Medicine. See the full Methods for details of immunohistochemistry, electrophysiology, live-cell imaging and statistical analyses. Unless noted, statistical comparisons were performed with Student’s t-test (unpaired, two-tailed), data are expressed as mean ± standard error of the mean (s.e.m.), and error bars denote s.e.m. Touch-sensitive units were classified as SAI afferents based on the following physiological criteria: low mechanical threshold (<1 mN), receptive field restricted to touch domes, sustained responses with irregular ISIs during ramp-and-hold stimuli, and Aβ conduction velocity.

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.

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Author Contributions S.M. screened transgenic mouse lines, and performed and analysed all ex vivo optogenetic experiments (Fig. 2 and Extended Data Figs 3–6). M.N. performed and analysed all whole-cell recordings (Fig. 1, Extended Data Fig. 1a,b and Extended Data Table 1). Y.B. performed and analysed recordings from Atoh1 and Piezo2 strains (Fig. 3, Extended Data Fig. 8 and Extended Data Table 2). A.M.N. performed qRT-PCR (Fig. 1i) and calcium imaging (Extended Data Fig. 1c–i). K.L.M. performed immunohistochemistry in Atoh1 strains (Extended Data Fig. 7) and assisted in preparation of all figures. S.A.W. and E.A.L. conceived optogenetic strategies. P.F. generated initial ChR2 transgenic mouse lines. E.A.L. conceived and supervised the project. During this manuscript’s peer-review process, we entered into a collaboration with S.H.W., S.R. and A.P., to analyse unpublished Piezo2\(^{2/0}\) mice. S.R. generated Piezo2\(^{2/0}\) mice and E.A.L. generated and validated Krt14Cre;Piezo2\(^{2/0}\) mice in the laboratory of A.P. The manuscript was written by S.M., M.N. Y.B. and E.A.L. and edited by A.M.N., K.L.M., S.A.W., P.F., S.H.W. and A.P.

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 E.A.L. (eal1166@columbia.edu).
METHODS

Experimental animals. All experimental procedures followed National Institute of Health guidelines and were approved by Columbia University Institutional Animal Care and Use Committee (IACUC). Preliminary studies performed at Baylor College of Medicine (BCM) were approved by the BCM IACUC.

Optogenetic experiments were performed on 6–10-week-old male and female mice. For optogenetic activation of Merkel cells, CkCre; ChR2EY-loxP/− and K14Cre; ChR2EY-loxP/− mice were generated by crossing Ai27D mice (B6.Cg-Gt(Rosa)26Sortm1(2CAG-GFP)15d/Tomato, which conditionally express a ChR2-tomato fusion protein from the Gt(Rosa)26Sor locus12, with one of two Cre-expressing strains. For CkCre; ChR2EY-loxP/−, we used Ck-IRES-Cre mice (CkI/E(1.5/1.5Cre)) and CkCre; Trangenic K14-Cre (K14Cre;ChR2EY-loxP/+) transgenic K14-Cre (YF;RT14;1A/lAcFmci/mci) mice were used. For optogenetic inhibition of Merkel cells, CkCre; ArchT-EYRptx mice were generated by crossing Ck-IRES-Cre mice13 with B6.Cg-Gt(Rosa)26Sor tm1(2CAG-AFP-EGFP)Hsu12, which conditionally express an ArchET–EGFP fusion protein from the GT(Rosa)26Sor locus (MGI ref: 1:191265).

Atoh1 conditional knockout (Atoh1fl/fl) mice were generated as described previously12. Mice of the K14Cre; Atoh1fl/fl genotype lacked expression of Atoh1 in K14-expressing cells and were designated as Atoh1loxp mice. Genotypes that lacked either Cre and/or LacZ were designated as controls. Female 8–15-week-old mice were used for experiments.

For whole-cell patch clamp experiments, Merkel cells were dissociated from male and female Atoh1-nGFP transgenic pups (postnatal day 2 (P2–P5)), which express nuclear-localized GFP driven by Atoh1 regulatory sequences20, and GkG (/; ChR2EY-loxP/−; P5).

Immunohistochemistry. For cryosections, mouse skin was shaved, depilated (Surgi-cream) and dissected either from the back or from the hind limb of CkCre;ChR2EY-loxP/− mice or from female Atoh1loxP mice and littermate controls (8–10-weeks of age). Tissue was fixed in 4% paraformaldehyde (PFA), cryoprotected in 30% sucrose, frozen and sectioned at a thickness of 16–20 μm. Cryosectioned skin was labelled at 4 °C overnight with primary antibodies against Keratin-8 (TROMA1, Sigma-Aldrich, 1:1000), Nestin (Aves Labs, NES, 1:200), S100 (Dako, Z0311, 1:500) and βIV spectrin (Gift from M. Rasband, 1:200 (ref. 32)). Secondary goat AlexaFluor-conjugated antibodies (Invitrogen) directed against rat (Alexafluor 488; A11034) IgG were used for 1 h at room temperature (1:1,000).

For whole-mount immunostaining, skin pieces (2–5 mm in diameter) containing two to five touch domes (recorded and neighbouring touch domes) were dissected from the skin-saphenous nerve preparation after ex vivo recording. The following steps were all performed at 4 °C: tissue was fixed in 4% paraformaldehyde (PFA) for 2–4 h and blocked overnight with 5% normal goat serum containing 0.3% TritonX-100 (5% NGST) plus 10% DMSO plus M.O.M. blocking reagent (Vector Labs, M-KB-2213, 2 drops per ml). The next day, tissue was washed with PBS containing 0.3% TritonX-100 (PBST) for several hours and incubated with primary antibodies in 5% NGST for 3–6 days. Primary antibody concentrations were the same as indicated for cryosections. After four washes (30 min to 1 h each) with PBST, tissue was incubated with secondary antibodies in 5% NGST for 2 days (1:500). After four washes with PBST (30 min to 1 h each), skin was dehydrated in serial tetrahydrofuran solutions and cleared in dibenzyl ether, according to published methods29. Skin was imaged in dibenzyl ether by confocal microscopy (Zeiss exciter equipped with x20, 0.8NA and x40,1.3 NA objective lenses). For whole mounts, we used the same antibodies as for cryosections, plus an additional two: primary antibody against Neurofilament 200 (Sigma-Aldrich, N0142, 1:300); secondary antibody against mouse IgG (Invitrogen, Pacific Blue, P31582).

In vitro electrophysiology. The Merkel-cell isolation and patch clamp recording procedures were described previously32. Currents were recorded from Merkel cells after 1–2 days in culture with an Axopatch 200B amplifier, a Digidata 1440A interface and a personal computer running pClamp 10 software (Axon Instruments). Pipette resistance ranged from 0.9–3.5 MΩ. The perforated-patch technique was used for all whole-cell recordings. Pipette tips were filled with internal solution and backfilled with internal solution supplemented with 100–180 μM NaCl, 1 μM CaCl2, 5 EGTA, 2 MgATP, and 10 HEPES (pH 7.2, adjusted with t-glucosate).

The somata of dissociated Merkel cells were stimulated for 50 ms by families of displacements (0.3-μm steps) every 5 s, with a glass probe (tip diameter: 2–3 μm) driven by a piezo-electric actuator (model PAB/12, Piezosystem Jena; power supply ENV40 C, Piezosystem Jena). The glass probe was positioned at an angle of 48° to the cover slip. Displacements were triggered by a pClamp-controlled command voltage passed to the actuator through a low-pass filter (cut-off = 500 Hz, model LPI-100a, Warner Instruments). The rise time of the driving signal was 0.6 ± 0.1 ms, calculated as the latency for the probe to travel 10–90% of a half-maximal displacement. The speed of the mechanical stimulator was 2 μm s−1. In combination with the rise time of the driving signal, the latency of the stimulator was estimated at 1 ms. Mechanically evoked currents were measured at a holding potential of −70 mV. We chose dendritic Merkel cells for recordings, which typically show fast activation and inactivation kinetics (Fig. 1c). In addition, mechanosensitive currents with fast activation kinetics were occasionally recorded from oval-shaped Merkel cells (data not shown). These tended to show both fast (<10 ms) and slow inactivation kinetics (10–200 ms).

Displacement magnitudes were visually calibrated daily. To ensure that mechanosensitive channels were not activated at rest, displacements began from an offset position located 1–2 μm away from the soma44. Thus, each stimulus family included −6 displacements that did not contact the soma. To correct for this variable offset, displacements are reported relative to each cell’s mechanical threshold (labelled as ‘0’ in Fig. 1d). Mechanical threshold was defined as the displacement that elicited a peak current ≥ 4 times the s.d. of the pre-stimulus noise level (12.5 ms before stimulus onset). For clarity, this offset has been subtracted from the stimulus families shown in Fig. 1.

Given that stimulus-response relations are normalized to each cell’s mechanical threshold, their lateral shifts likely reflect a compliance in series with mechanotransduction machinery. One possibility is the coupling between the cell and the overslip, which was not systematically controlled in these in vitro recordings. Current–displacement relationships were fitted for the normalized current with a Boltzmann equation of the form13:

\[ I_{\text{norm}}(x) = \frac{1}{1 + \exp \left( \frac{x - x_0}{s} \right) \times 100} \]

where \( x \) is the displacement (in μm), \( x_0 \) is the displacement that produces half-maximal amplitude of the peak current, and \( s \) is the current sensitivity to displacement. For estimating the activation kinetics, the period from 10 to 90% of a maximal evoked current was calculated and denoted as activation rise time \( t_{\text{activation}} \). For estimating the inactivation kinetics, single-exponential fitting was conducted with Clampfit for 47 and 50 ms from peak current time point for touch- and light-evoked current, respectively. Both activation and inactivation kinetics where the displacement was \( x_0 \) were estimated by linear interpolation of adjacent four points. For calculating the operating range, we adopted the method used to study hair cells: the operating range of the \( I_{\text{norm}}(x) \) relation was the net deflection required to evoke from 10 to 90% of the maximal response25. To estimate reversal potential, mechanical stimuli were delivered at multiple holding potentials (−75 to +50 mV in 25 mV increments). Holding potentials were stepped 100 ms before mechanical stimulation (50 ms).

For the Ruthenium red blockade experiment, 50 mM stock solution was prepared in DMSO and dissolved into extracellular solution at a final concentration of 100 μM. Mechanical stimulation was given at least five times after reaching the mechanical threshold of each Merkel cell. To quantify the blocking effects of Ruthenium red, we compared maximal inward current (peak current (\( I_{\text{peak}} \)) averaged from a 250–μs time window), with current at the end of touch stimulation (steady state current (\( I_s \)), averaged from a 5-ms time window).

FM1-43 injections. FM1-43 (Biotium; #70020) was used to visualize SAI receptive fields (1:400, 200, and 400 μM). FM1-43 was diluted in 1.5 mM in sterile PBS and injected subcutaneously (70 μl per mouse). Hindlimb skin for ex vivo skin–nerve electrophysiology was collected 12–14 h after injection.

Ex vivo skin–nerve electrophysiology. Light- and touch-evoked responses in the skin were recorded after dissecting the hindlimb skin and saphenous nerve according to published methods6. Briefly, the skin was placed epidermis-side-up in a custom chamber and perfused with carbogen-buffered synthetic interstitial fluid (SIF) kept at 32 °C with a temperature controller (model TC-34AB, Warner Instruments). The nerve was kept in mineral oil in a recording chamber, teased apart and placed onto a silver recording electrode connected with a reference electrode to a differential amplifier (model 1800, A-M Systems). The extracellular signal was digitized using a DT304 A/D board (DataWave Technologies) and recorded using Sci-Works Experimentor software (DataWave Technologies). SAI receptive fields (touch domes) were visualized using a fluorescence microscope equipped with 1 MgCl2, 0.5 CaCl2, 5 EGTA, 2 MgATP, and 10 HEPES (pH 7.2, adjusted with t-glucosate).

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For these studies, we focused on touch-dome afferents, which innervate Merkel cells in wild-type animals, as described in ref 7. The afferents generally have no spontaneous firing, respond selectively to pressure applied directly to a touch dome, are particularly sensitive to moving stimuli but are insensitive to hair tufting and skin stretch. To identify responses from these afferents in mutant and control genotypes, we used a mechanical search paradigm with a fine glass probe. Afferents were classified as ‘touch-dome afferents’ according to the following criteria: (1) AF conduction velocity (≥ 9 m s⁻¹), (2) punctate receptive fields restricted to one or more fluorescently labelled touch domes, (3) insensitive to pressure applied to skin areas adjacent to touch domes, (4) insensitive to hair tufting but responsive when the hair is bent to compress the touch dome. Touch-sensitive afferents that did not meet these criteria were not analysed further. Responses were classified as intermediately adapting (IA) if firing ceased during the first 4 s of the static phase, and slowly adapting (SA) if spikes were observed throughout the duration of the 5 s hold phase. Recordings and analysis of Piezo2Knockout and their controls were performed blind to genotype. For other strains, directed recordings could not be performed blind to genotype because transgenic and control Merkel cells differed in their appearance under the fluorescence microscope used to confirm the presence of touch domes in the field. In the Atoh1 knockout strain, FM1-43 labelling differs from control mice because the former lack FM1-43-labelled Merkel cells24 (Extended Data Fig. 7). In transgenic mice expressing ChR2 or ArchT, the presence of fluorescent Merkel cells was confirmed for touch dome analysis.

Mechanical responses were elicited with von Frey monofilaments and a custom-built mechanical stimulator. The automated mechanical stimulator applied stimuli with an indenter (tip diameter: 1.6 mm), and stimuli were commanded using a model XPS motion controller and driver system (Newport) connected to a PC computer. Movement of the indenter was controlled with custom-made software and measured with a laser distance-measuring device (OptipNCDT 1402, Micro-Epsilon). Touch stimuli consisted of ramp and hold indentations. First-order approximation of approach speed was 3.2 mm s⁻¹. Mechanical displacements ranged from 0.4–1.6 mm in depth. The period between successive displacements was 10 s light pulses was monitored with a photodiode. The period between successive stimulations (30 s) allowed for ChR2 recovery after desensitization36. To test recordings were performed in low-light conditions and a period between success-}

Statistics and sample sizes. Unless noted, P values report the results of Student’s t-test (unpaired, two-tailed), data are expressed as mean ± standard error of the mean (s.e.m.) and error bars denote s.e.m.

Fisher’s exact test was used to evaluate differences in afferent populations (Fig. 3c). Differences between population means were assessed with unpaired Student’s t-test (two tailed) for normally distributed data. Because fewer than 10 afferents were included in Fig. 3d, e and Extended Data Fig. 8, we also used a two-tailed Mann–Whitney test, which confirmed that medians of populations indicated with asterisks were statistically different (P < 0.01 for all comparisons). Variances between control and mutant genotypes were not statistically different, with the exception of static-phase ISIs from Piezo2Knockout mice (P < 0.01; Mann–Whitney U-value = 1).

Sample sizes were chosen based on published and pilot studies. For in vitro electrophysiology, we conducted pilot studies to measure touch-activated currents in four Merkel cells. Power analysis showed that at least two recordings are sufficient to discriminate touch activated currents from voltage- and calcium-activated currents, which are the majority of transmembrane currents measured in a previous study37. For optogenetic Merkel-cell silencing, the sample size was limited by the number of adult animals available for ex vivo skin-nerve preparation recording. For ex vivo recordings from Atoh1 knockout and Piezo2 knockout mice, we based samples sizes on our previous survey of touch-sensitive afferents in Atoh1 knockout mice. Among Aβ afferents, we observed eight SAI afferents in control mice (n = 8/39) but none in Atoh1 knockout genotypes (n = 0/27)37. Thus, we reasoned that at least five afferents per group would be sufficient to observe differences in response properties of touch-dome afferents in directed recordings.

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Extended Data Figure 1 | Mechanically activated responses in Merkel cells.

a, Representative trace of mechanically evoked current induced by 1-μm mechanical displacement. Application of Ruthenium red (RR, 100 μM) attenuated mechanically activated current. b, Peak currents (I_{peak}) were estimated from 250 ms around peak and steady-state currents (I_{ss}) were estimated from the last 5 ms (black bar in 1a) of mechanical displacements. Data were normalized by I_{peak} for each cell. With Ruthenium red, I_{peak} was reduced to 38 ± 7% of control condition. Steady state currents were also reduced by Ruthenium red (n = 4; control: 9 ± 1% of I_{peak}; RR: 2 ± 1% of I_{peak}). **P < 0.01; paired Student's t-test (two-tailed). c–i, Merkel cells display reversible Ca^{2+} responses to focal displacements applied to somata.

c, Representative pseudocolour images of fura-2 ratios (340:380) of a Merkel cell at rest. d, A Merkel cell activated by depolarizing (high-K^{+}) solution. e, A brightfield image showing the position of the stimulus probe. f–h, Peak responses corresponding to each displacement. 'Fold Δ' is the fold change in fluorescence ratio from baseline. Scale bar, 10 μm. i, Representative time course of mean fura-2 ratios during the touch stimuli shown above. Stimulus onset in f–h is indicated by arrows. Calcium responses were stimulus-dependent. Similar responses were observed from 11 Merkel cells.
Extended Data Figure 2 | ChR2⁺ Merkel cells display light-activated inward currents. 

**a.** Light-activated currents were recorded with whole-cell, tight-seal voltage clamp methods. **b.** Fluorescent image of a ChR2-tdTomato expressing Merkel cell. Scale bar, 10 µm. **c.** Representative trace for light-activated inward currents at a holding potential of −70 mV. Inactivation kinetics were measured by fitting a single exponential curve (red).
Extended Data Figure 3 | Immunostaining of ChR2-expressing touch domes. a–e, Whole-mount staining and confocal axial projection of the touch dome shown in Fig. 2d. a, Merged image. b–d, Expression of ChR2-tdTomato was present in Merkel cells (Krt8), but absent from sensory terminals (neurofilament heavy, NFH). e, Some terminal Schwann cells (Nestin) also expressed ChR2 (arrowheads in b and e). f–i, Immunostaining of skin cryosections. f, Merged image. g–i, ChR2-tdTomato was present in some S100⁺ Schwann cells that also expressed Nestin, a marker for type II terminal Schwann cells (arrowheads in f–i). Scale bars, 20 μm.
Extended Data Figure 4 | Light-evoked activity is specific to touch-dome illumination. a, f, Responses to light stimuli centred on a touch dome. b–e, When the light stimulus was positioned around the touch dome, no light-evoked activity was observed. Illuminating a cluster of ChR2<sup>+</sup> dermal cells did not evoke any responses (c). f, To confirm that the absence of light-evoked activity was not due to the loss of Merkel cells and/or neuronal fibres, the experiment ended by re-positioning the light stimulus over the touch dome to re-elicit light-evoked activity. Images have been thresholded for clarity. Scale bars, 200 µm.
Extended Data Figure 5 | K14Cre;ChR2loxP/ mice exhibit light-evoked SAI activity. **a**, Confocal image of a touch dome illustrating ChR2-tdTomato expression driven by K14Cre. ChR2-tdTomato expressed much stronger in Merkel cells than in neighbouring keratinocytes. **b**, Light-evoked responses from the touch dome shown in a to seven light intensities as indicated. Spike sorting and clustering analysis were used to identify the unit that fired in phase with light (lower trace with spike positions and their amplitudes). **c**, Mean IFFs for light with varying illumination intensities on a log-intensity scale (n = 3 recordings). Scale bar, 20 μm.
Extended Data Figure 6 | Confocal axial projection of a touch dome shows selective ArchT–EGFP expression in Merkel cells driven by Cck<sup>Cre</sup>. ArchT–EGFP expression was not observed in touch-dome afferents. Scale bar, 20 µm.
Extended Data Figure 7 | Structure of touch-dome afferents in Atoh1\textsuperscript{cko} mice. Immunostaining of skin cryosections from Atoh1\textsuperscript{cko} and control genotypes are shown. Antibodies labelling myelinated afferents (NFH; cyan), Merkel cells (Krt8; yellow), nodes of Ranvier (βIV spectrin; magenta) show that the general structure of touch-dome afferents, including myelinated branches and Nodes of Ranvier (arrowheads), appears normal even in the absence of Merkel cells. Scale bar, 20 μm.
Extended Data Figure 8 | Comparison of ISI distributions in Atoh1<sup>CKO</sup>, Piezo2<sup>CKO</sup> and control genotypes. a, Histogram of ISI distribution during saturating responses in Atoh1<sup>CKO</sup> (mean ± s.d., 43.4 ± 59.2 ms, median: 29.8 ms; n = 466 intervals from n = 6 units) and control genotypes (mean ± s.d., 16.5 ± 12.9 ms, median: 13.8 ms; n = 1,412 intervals from n = 5 units). Inset on the left illustrates all ISIs, including those > 150 ms, which were excluded from the main histograms (14/466 intervals in Atoh1<sup>CKO</sup> and 1/1,412 in control genotypes). At right, bar graphs show the minimum ISIs during dynamic and static phases. Minimum ISIs were longer in Atoh1<sup>CKO</sup> than control mice for both phases, indicating a loss of high-frequency firing during dynamic stimuli and static displacement (**P < 0.02, ***P < 0.01; Student’s t-test). Mann–Whitney tests indicated that median values were also significantly different (P < 0.001). b, Histogram of ISI distribution for Piezo2<sup>CKO</sup> (Mean ± s.d., 41.9 ± 32.3 ms, median: 23.4 ms; n = 792 intervals from N = 6 units) and control genotypes (mean ± s.d., 13.9 ± 1.4 ms, median: 11.8 ms; n = 1,845 intervals from n = 5 units). Main histograms excluded long intervals (>150 ms; 4/792 intervals in Piezo2<sup>CKO</sup> and 2/1,845 in control mice.) Minimum ISIs were not significantly different in the dynamic phase (P = 0.76; Student’s t-test and Mann–Whitney test); indicating that high-frequency firing is preserved in touch-dome afferents in these mice. For static phase firing, the means were not significantly different (P = 0.095; Student’s t-test); however, non-parametric analysis indicated that medians differed between genotypes (P = 0.0043; Mann–Whitney test).
Extended Data Table 1 | Properties of mechanically and light-evoked currents in Merkel cells

|                      | (mean ± s.e.m.) |
|----------------------|-----------------|
| **Mechanically**     |                 |
| **activated**        |                 |
| \(N = 6\)           |                 |
| 6 trials             |                 |
| Mean peak current (pA) | 370 ± 80       |
| Mean steady state current (pA) | 20 ± 6        |
| Activation rise time, \(t_{\text{activation}}\) (ms) | 1.0 ± 0.1     |
| \(\tau_{\text{inactivation}}\) (ms) | 8 ± 2          |
| **Light**            |                 |
| **activated**        |                 |
| \(N = 5\)           |                 |
| 56 trials            |                 |
| Mean peak current (pA) | 38 ± 2         |
| Mean steady state current (pA) | 18 ± 1        |
| Activation rise time, \(t_{\text{activation}}\) (ms) | 6.4 ± 0.4     |
| \(\tau_{\text{inactivation}}\) (ms) | 12 ± 1         |

Activation kinetics (activation rise time, \(t_{\text{activation}}\)) was estimated as the period from 10–90\% of maximally evoked currents. Inactivation kinetics (\(\tau_{\text{inactivation}}\)) were estimated by fitting single exponential functions. All values were estimated by linear interpolation at displacements nearest to the half-peak response. Measurements were conducted at \(V_H = -70\) mV.
Extended Data Table 2 | Summary of touch-dome responses from $Atoh1^{CKO}$ and control mice

| Data ID | Dynamic phase | Static phase | Ratio of IA | CoV |
|---------|---------------|--------------|-------------|-----|
|         | # of spikes   | # of spikes  |              |     |
|         | Min (ms) | Max (ms) | Ave ±SD (ms) | Median (ms) | Min (ms) | Max (ms) | Ave ±SD (ms) | Median (ms) |
| KO 1    | 41          | 15.4       | 24.5        | 19.3±2.3 | 19.5 | 132 | 20.9 | 53.3 | 30.1±5.0 | 29.6 | 33 | 0.16 |
| KO 2    | 37          | 15.4       | 61.8        | 27.3±10.6 | 25.1 | 56  | 27.1 | 300.1 | 69.2±51.9 | 49.0 | 50 | 0.75 |
| KO 3    | 49          | 6.2        | 594.3       | 35.2±88.9 | 13.6 | 39  | 10.6 | 667.3 | 101.0±137.1 | 50.9 | 100 | 1.36 |
| KO 4    | 31          | 15.0       | 42.8        | 22.9±5.9 | 22.6 | 26  | 28.4 | 71.4 | 42.6±9.7 | 41.8 | 100 | 0.23 |
| KO 5    | 18          | 15.5       | 33.6        | 22.4±5.2 | 23.6 | 24  | 26.1 | 233.8 | 58.0±46.6 | 41.5 | 100 | 0.80 |
| KO 6    | 35          | 19.3       | 26.4        | 23.2±3.5 | 23.5 | 12  | 32.1 | 286.8 | 98.6±74.9 | 61.7 | 100 | 0.76 |
|         | Wild 1      | 45          | 7.2         | 35.0       | 12.9±6.2 | 10.0 | 201 | 9.4 | 175.7 | 19.9±16.3 | 15.4 | 17 | 0.62 |
|         | Wild 2      | 77          | 4.2         | 81.7       | 18.7±11.4 | 16.4 | 95  | 22.0 | 95.4 | 42.0±13.2 | 39.1 | 0 | 0.32 |
|         | Wild 3      | 74          | 4.8         | 792.8      | 21.5±16.6 | 10.3 | 169 | 4.3 | 80.1 | 23.2±15.9 | 19.0 | 0 | 0.69 |
|         | Wild 4      | 89          | 3.3         | 11.5       | 4.9±1.8  | 4.0  | 274 | 3.9 | 48.2 | 14.5±7.2 | 13.1 | 0 | 0.51 |
|         | Wild 5      | 91          | 3.3         | 39.4       | 8.4±6.8  | 5.3  | 312 | 2.9 | 32.8 | 12.8±5.2 | 12.3 | 0 | 0.41 |

Maximum response among experimental set was chosen as representative data. Dynamic phase: period from stimulus onset (when the indenter began moving) to end of stimulation onset (when 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, averaged; s.d., standard deviation; IA, intermediate adapting response; CoV, coefficient of variation in static phase.