

Dynamic keratinocyte Piezo2 expression drives itch and nerve remodeling in atopic dermatitis

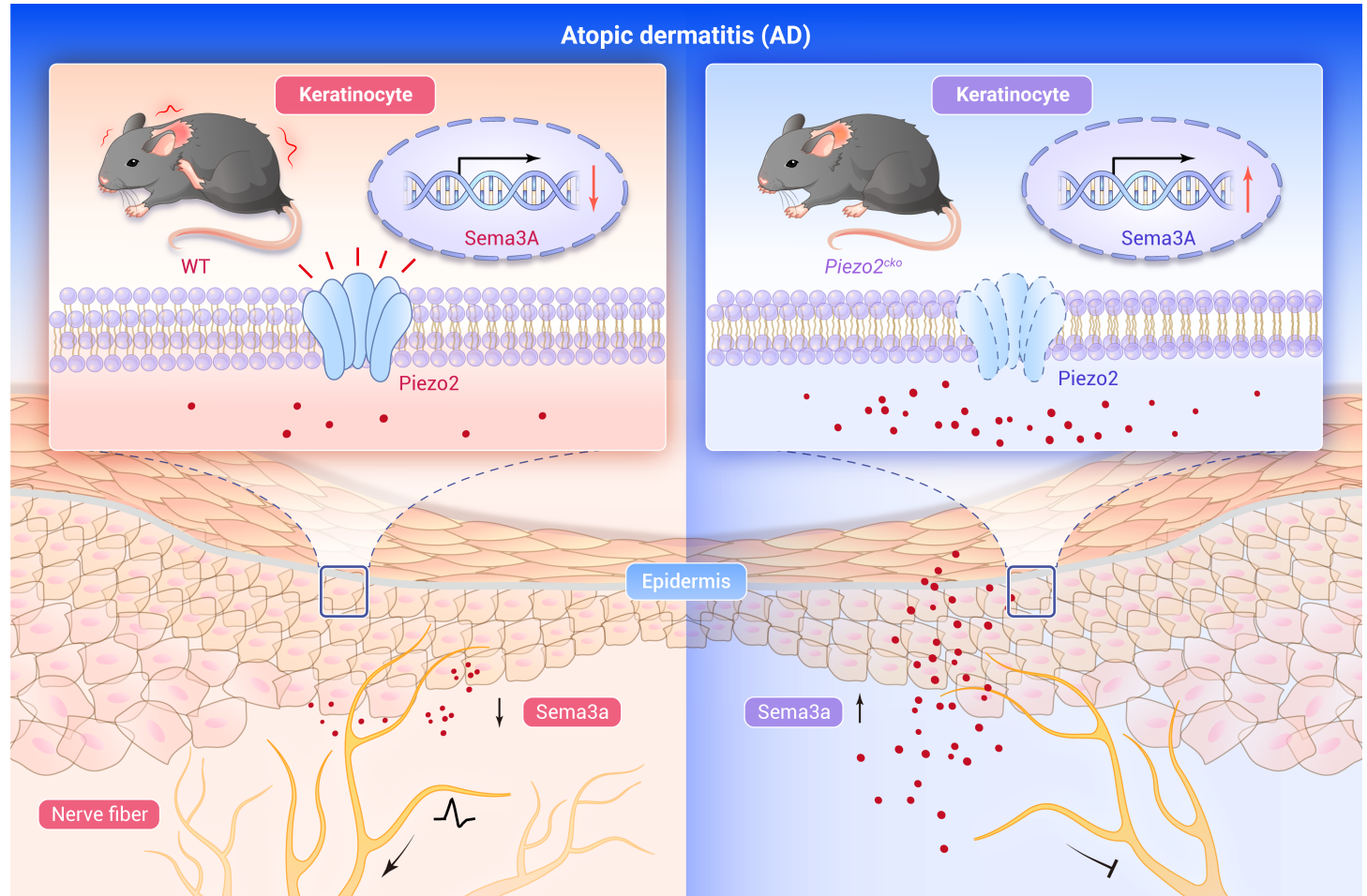
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Keratinocyte Piezo2 is selectively upregulated in AD.
- Piezo2-Sema3a axis drives itch and nerve remodeling.
- Piezo2 is involved in AD itch.

Dynamic keratinocyte Piezo2 expression drives itch and nerve remodeling in atopic dermatitis

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Keratinocytes actively contribute to somatosensory signaling in chronic skin diseases, yet the mechanotransduction mechanisms involved in atopic dermatitis (AD)-associated itch remain unclear. Here we identify keratinocyte-expressed mechanosensitive ion channel Piezo2 as a critical driver of chronic itch in AD. While Piezo1 was constitutively expressed and dispensable for chronic itch in the MC903-induced AD model, Piezo2 expression was markedly upregulated in keratinocytes of lesional AD skin. Keratinocyte-specific Piezo2 deletion attenuated spontaneous scratching and spontaneous C fiber activity, while having minimal impact on skin inflammation. Mechanistically, Piezo2 suppressed the neurorepulsive factor Semaphorin 3A (Sema3a), a key regulator of sensory nerve architecture. Loss of Piezo2 restored Sema3a expression in the epidermis, reduced nerve fiber branching, and dampened C fiber hyperexcitability. Furthermore, keratinocyte-specific ablation of Sema3a exacerbated AD-related itch and nerve fiber outgrowth. These findings establish a non-neuronal epithelial mechanism wherein keratinocyte Piezo2 modulates sensory nerve remodeling and pruritus in chronic skin inflammation, offering potential targets for therapeutic intervention.

INTRODUCTION

Atopic dermatitis is a common chronic inflammatory skin disease characterized by persistent itch, skin barrier dysfunction, and immune dysregulation.¹⁻³ It affects up to 20% of children and 10% of adults, imposing substantial physical and psychological burden.⁴ Studies have identified several key mediators of AD pathogenesis, including thymic stromal lymphopoietin (TSLP) and interleukin-4 (IL-4), which promote type 2 immune responses.^{5,6} JAK inhibitors that block downstream signaling of IL-4/IL-13 show clinical efficacy, yet pruritus remains difficult to manage in many patients.^{7,8} These gaps highlight the need for mechanism-based therapeutic strategies, particularly those targeting itch.

Recent studies have redefined keratinocytes as active contributors to sensory transduction.^{9,10} Beyond serving as structural components of the skin barrier, keratinocytes engage in dynamic crosstalk with sensory neurons through release of neuroactive mediators such as ATP, glutamate, cytokines, and growth factors.^{10,11} Previous studies demonstrated that optogenetic activation of keratinocytes expressing channelrhodopsin can trigger action potentials in cutaneous sensory afferents and elicit nociceptive behaviors, while inhibiting keratinocyte activity dampens primary afferent firing.^{12,13} Other findings also suggest that injured keratinocytes promote afferent hyperexcitability and chronic pain by releasing inflammatory mediators and altering neuronal ion channel expression.¹⁴ Together, these results support the idea that keratinocytes function as upstream initiators and modulators of nociceptive signals, potentially contributing to both acute and chronic sensory pathologies.

Mechanosensitive Piezo channels, comprising Piezo1 and Piezo2, are key transducers of mechanical force in the skin.¹⁵⁻¹⁸ Piezo1 is highly expressed in keratinocytes and has been implicated in regulating nociceptive signaling and wound healing.¹⁹⁻²¹ Previous work showed that Piezo1 activation in

keratinocytes can modulate cellular migration by enhancing retraction during wound healing.²² In the sensory domain, Piezo1 in keratinocytes has also been implicated in both innocuous and noxious mechanical sensation.^{20,23} Piezo2, in contrast, is predominantly expressed in specialized skin structures including Merkel cells and Meissner's corpuscles, where it plays a crucial role in light touch detection.^{24,25} Merkel cell-Piezo2 signaling has also been implicated in the modulation of mechanical itch.^{26,27} Despite these advances, it remains unclear whether Piezo1 or Piezo2 channels contribute to chronic itch, particularly in the setting of inflammatory skin disorders such as AD.

In this study, we reveal that Piezo2 is upregulated in keratinocytes during AD-like inflammation. Keratinocyte-specific deletion of Piezo2 attenuates spontaneous itch and C fiber hyperactivity, without affecting skin inflammation. Mechanistically, Piezo2 suppresses Semaphorin 3A (Sema3a), a neurorepulsive factor that regulates nerve density. Ablation of Piezo2 restores Sema3a expression, limits aberrant nerve sprouting, and alleviates chronic itch. These findings uncover a previously unrecognized epithelial mechanotransduction pathway that drives sensory nerve remodeling and pruritus in AD.

MATERIALS AND METHODS

Animals

C57BL/6J (#000664), *K14^{cre/+}* (#018964), *Piezo1^{flax/flax}* (#029213), and *Piezo2^{flax/flax}* (#027720) mice obtained from the Jackson Laboratories (Bar Harbor, ME, USA). *Sema3a^{flax/flax}* (#20346) and *Atoh1^{flax/flax}* (#11921) mice were purchased from Cyagen Biosciences (Suzhou, China). Body weight-matched male or female mice were used for all experiments. Mice used within each experimental comparison were bred to generate littermates, cohoused under identical conditions, and randomly assigned to experimental groups by lab managers blinded to the study design. Scratching behavior and dermatitis severity did not differ significantly between males and females. All animal procedures were performed in strict accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Mouse model of MC903-induced atopic dermatitis

To induce AD-like inflammation and itch, mice received daily topical applications of 2 nmol MC903 (calcipotriol, Sigma-Aldrich) dissolved in 20 μ L ethanol on the nape of the neck for 7 consecutive days.^{28,29} Control mice were treated with ethanol alone under the same conditions. After the treatment period, mice were euthanized and skin samples were collected for analysis.

Pharmacological interventions in the MC903-induced AD model

Pharmacological treatments were administered as follows: The Sema3a agonist TCS1105 (1 mM, 100 μ L; MCE, #HY-103511) or recombinant Sema3a protein (1 μ g in 100 μ L; MCE, #HY-P74566) was applied to the mice according to the experimental design. Both the Sema3a agonist TCS1105 and recombinant Sema3a protein were administered once daily through repeated intraperitoneal injections for 7 consecutive days.

Open field test

Behavioral assessments were conducted using an animal behavior analysis system (Xinruan Information Technology Co., Ltd., Shanghai, China). Each mouse was placed in a square open-field chamber enclosed within sound-attenuating walls. The mice were allowed to freely explore the chamber for 10 min to evaluate locomotor activity and habituation. Each session began with the animal placed at the center of the chamber.

Scratching behavior assessment

Spontaneous itch behavior was evaluated after mice were acclimated to the recording environment for at least three days. Mice were further acclimated to the recording room and chambers for 30 minutes before video monitoring. Itch behavior was recorded for one hour and analyzed by a blinded researcher. A scratching bout was defined as one or more rapid hind paw movements directed toward the MC903-treated area, ending when the paw touched the floor or the animal licked/bit the toes.

Mouse RNA isolation and qRT-PCR

For RNA isolation from primary keratinocytes, the harvested epidermis from mouse skin samples was homogenized in Trizol Reagent (Invitrogen) following the manufacturer's instructions. mRNA was reverse-transcribed into cDNA using the Maxima H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), according to the manufacturer's protocol. Quantitative PCR (qPCR) was performed using Power SYBR™ Green PCR Master Mix (Bio-Rad). Gene expression was analyzed using the $\Delta\Delta C_t$ method relative to β -actin as a housekeeping gene, and fold changes were normalized to the control group. Primers were synthesized by Integrated DNA Technologies. The sequences used were as follows: β -actin (forward: GTGACGTTGACATCCTGTAAGA, reverse: GCCGGACTCATCTACTCC), *Sema3a* (forward: TGGGCTGGTTCACCTGGGATTGC, reverse: CTGGAGCTGTTGGCCAAGCCAT), *Piezo1* (forward: CTGCATGGCTAGTGATAGG, reverse: CTATGAGCCACTGTTTCCAT), and *Piezo2* (forward: ATGTGCGTTCGGGTACAATGG, reverse: TGTGTCCTTGATCATGCTTGTCT).

RNAscope in situ hybridization

RNAscope in situ hybridization was performed using the Multiplex Fluorescent Reagent Kit v2 (Advanced Cell Diagnostics, 322300-USM) following the manufacturer's instructions. Briefly, 12- μ m skin sections were fixed in 4% paraformaldehyde at 4°C for 15 minutes, then dehydrated in increasing concentrations of ethanol (50%, 70%, and 100%). After air drying, sections were treated with hydrogen peroxide for 10 minutes, followed by antigen retrieval and Protease III treatment to enhance tissue permeability. Probes targeting mouse *Piezo1* (#400181-C2), *Piezo2* (#400191-C2), and *Sema3a* (#412961-C1) were hybridized at 40°C for 2 hours using a HybEZ Oven. Amplification and fluorophore labeling steps were performed according to the RNAscope protocol. Co-localization analysis was conducted via immunofluorescence staining as described below.

Immunofluorescence assay

Skin sections were first blocked in 10% normal donkey serum (NDS) containing 0.1% Triton X-100 for 2 hours at room temperature. They were then incubated overnight at 4°C with primary antibodies against K14 (rabbit anti-K14, 1:500, BioLegend, #906004) and PGP9.5 (rabbit anti-PGP9.5, 1:500, Abcam, #ab108986). The following day, Alexa Fluor 488- or 594-conjugated secondary antibodies (Thermo Fisher Scientific) were applied for 2 hours at room temperature. Nuclei were counterstained with DAPI before mounting and imaging. 3-5 skin sections from 3 mice each group were analyzed to quantify the intraepidermal density of PGP9.5⁺ skin fibers. Adobe Photoshop 2022 and ImageJ were used to quantify neurite length and branching.

Ex vivo skin-nerve preparations

Ex vivo skin-nerve recordings were performed on MC903-treated skin samples as previously described.^{25,30,31} A segment of MC903-treated dorsal skin (2 × 3 cm), which was innervated by thoracic DRG, was dissected and cleared of underlying tissue. The corresponding nerve bundle was isolated into an adjacent oil-filled chamber and mounted on a gold recording electrode. The myelin sheath was carefully removed without disturbing the axons.

Spontaneous C-fiber activity was recorded for 30 minutes, followed by mechanical stimulation of the receptive field using a dual-mode lever system (Aurora Scientific Inc.) delivering ascending forces (1-150 mN) at 1-minute intervals. Conduction velocities were used to classify fiber types: A β (> 10 m/s), A δ (1.2-10 m/s), and C fibers (< 1.2 m/s). Neuronal responses were captured and analyzed using Spike2 software (Cambridge Electronic Design).

Hematoxylin and Eosin (H&E) staining

Mouse dorsal skin samples were embedded in OCT compound and stored at -80°C. Sections of 12 μ m thickness were cut using a cryostat, washed with PBS, and stained with hematoxylin for 2 minutes. After differentiation in 1% hydrochloric acid-ethanol for 5 seconds and washing, eosin staining was performed for 2 minutes. The sections were sequentially dehydrated with 70%, 85%, 95%, and 100% ethanol, followed by mounting with neutral gum and visualization under a light microscope.

Western blot assay

To separate the epidermis and dermis, skin tissues were incubated dermis side down in 4 mg/mL Dispase II (Sigma) at 37°C for 2 hours with gentle shaking. The isolated epidermis was lysed in ice-cold RIPA buffer containing 1% phosphatase and protease inhibitors, then sonicated and centrifuged at 12,000 rpm for 15 minutes at 4°C. Protein concentration was determined by BCA assay. Equal amounts of protein were boiled with loading buffer, separated by 8% SDS-PAGE, and transferred onto a PVDF membrane. After blocking with 5% milk in TBST for 2 hours, membranes were incubated overnight at 4°C with anti-Sema3a antibody (1:500, Santa Cruz, sc-74554). Following three TBST washes, membranes were incubated with HRP-conjugated goat anti-mouse secondary antibody for 2 hours. Protein bands were detected by ECL and quantified using ImageJ software.

Quantification and statistical analyses

All data are expressed as mean $\pm$ standard error of the mean (SEM). Comparisons between two groups were analyzed using unpaired two-tailed Student's *t*-test. For comparisons involving three or more groups, one-way or two-way ANOVA with appropriate post hoc tests was used. A *p*-value < 0.05 was considered statistically significant. Significance is indicated as: *P* < 0.05 (*), *P* < 0.01 (**), *P* < 0.001 (***), and NS (not significant).

RESULTS AND DISCUSSION

To examine the involvement of PIEZO channels in AD-related chronic itch, we induced AD-like symptoms using topical calcipotriol (MC903) and assessed PIEZO1 and PIEZO2 expression. *PIEZO1* was broadly and stably expressed in keratinocytes (Figures 1A-B). In contrast, *PIEZO2* was sparsely expressed under baseline conditions but markedly upregulated after MC903 treatment (Figures 1C-D), suggesting its dynamic regulation in response to AD-associated stimuli.

We next generated keratinocyte-specific *Piezo1* and *Piezo2* knockout mice (Figures 1E & G, S1 & S2). Under baseline conditions, deletion of *Piezo1* or *Piezo2* did not affect scratching behavior or skin morphology (Figures S3 & S4). While *Piezo1* deletion had no effect on MC903-induced spontaneous scratching (Figure 1F), *Piezo2* ablation in keratinocytes significantly reduced scratching bouts (Figure 1H), indicating that *Piezo2* plays a critical role in mediating AD-related itch. Although *Piezo2* is also expressed in Merkel cells, Merkel cell-deficient mice showed no change in itch behavior or skin thickness, reinforcing the keratinocyte-specific function of *Piezo2* (Figure S5).

While keratinocyte-derived mediators are known to influence immune cell activation and inflammation in AD,³² deletion of *Piezo1* or *Piezo2* in keratinocytes did not affect skin inflammation (Figures 2A-F). Interestingly, we observed a notable reduction in intraepidermal PGP9.5-positive nerve fibers in *Piezo2*-deficient mice, but not in *Piezo1*-deficient mice (Figures 2G-L). This decrease in nerve density raised the possibility that *PIEZO2* may influence the structural remodeling of sensory fibers, potentially disrupting neural signaling involved in AD-associated itch.

To assess the functional relevance of these anatomical changes, we conducted ex vivo skin-nerve recordings and found that *Piezo2*-deficient mice exhibited a reduction in spontaneous firing rates and a diminished

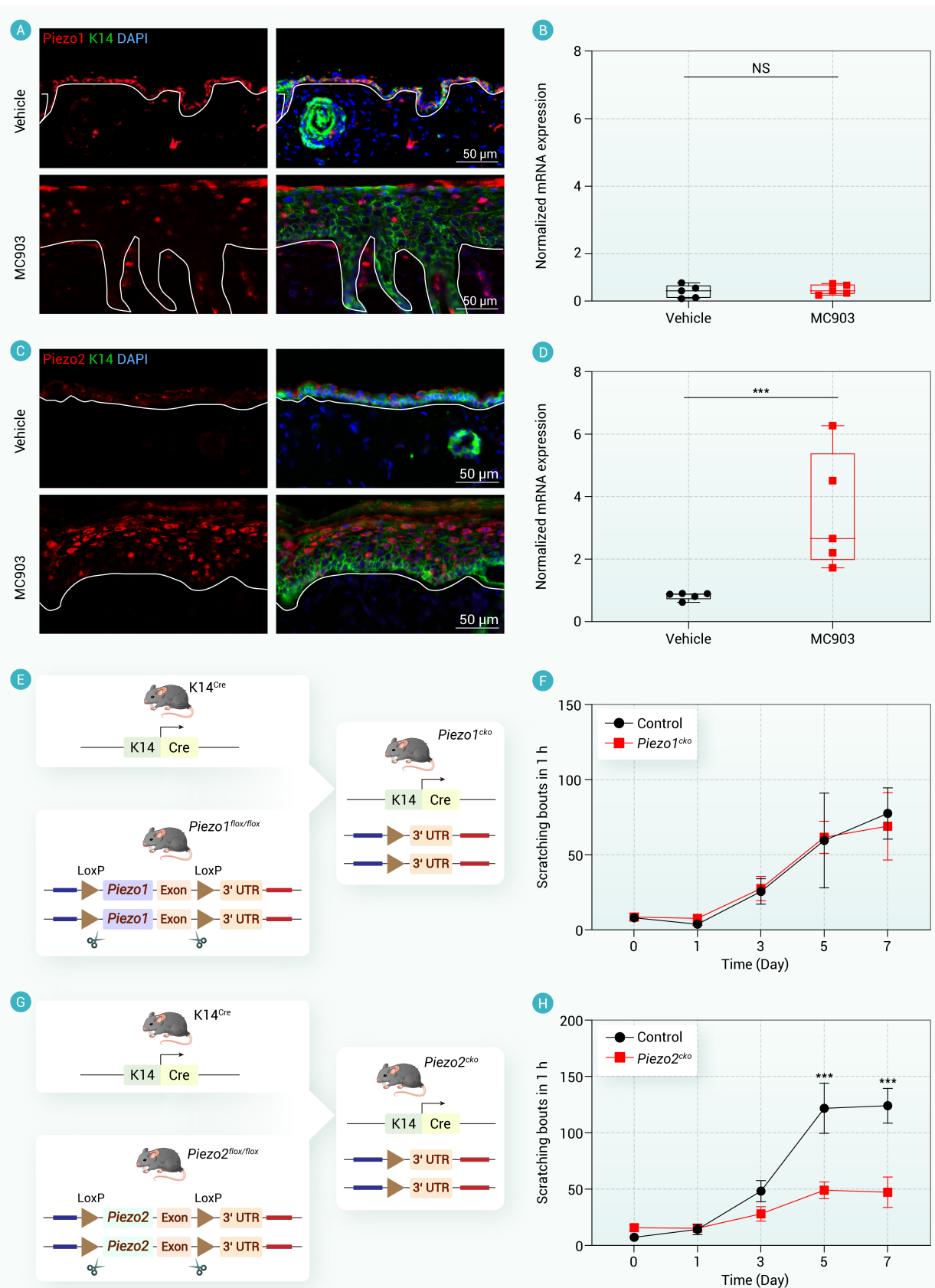


Figure 1. Keratinocyte *Piezo2* expression is upregulated in AD-like skin and drives chronic itch (A) Representative RNA-scope fluorescent images showing *Piezo1* expression in keratinocytes of vehicle- and MC903-treated mouse skin. n = 3-5 sections from 3 mice. Scale bar, 50 μ m. (B) Quantification of *Piezo1* expression levels in keratinocytes. n = 5 mice per group. (C) Representative RNA-scope fluorescent images showing *Piezo2* expression in keratinocytes of vehicle- and MC903-treated mouse skin. n = 3-5 sections from 3 mice. Scale bar, 50 μ m. (D) Quantification of *Piezo2* expression levels in keratinocytes. n = 5 mice per group. (E) The schematic diagram of the generation of *Piezo1*^{cko} mice. (F) Quantification of spontaneous scratching bouts after MC903 treatment in *Piezo1*^{cko} mice. n = 4-5 mice per group. (G) The schematic diagram of the generation of *Piezo2*^{cko} mice. (H) Quantification of spontaneous scratching bouts after MC903 treatment in *Piezo2*^{cko} mice. n = 4-5 mice per group. Data are presented as mean $\pm$ SEM. Two-tailed unpaired Student's *t*-test in B & D. Two-way ANOVA with Bonferroni post hoc test in F & H. NS, not significant, *** P < 0.001.

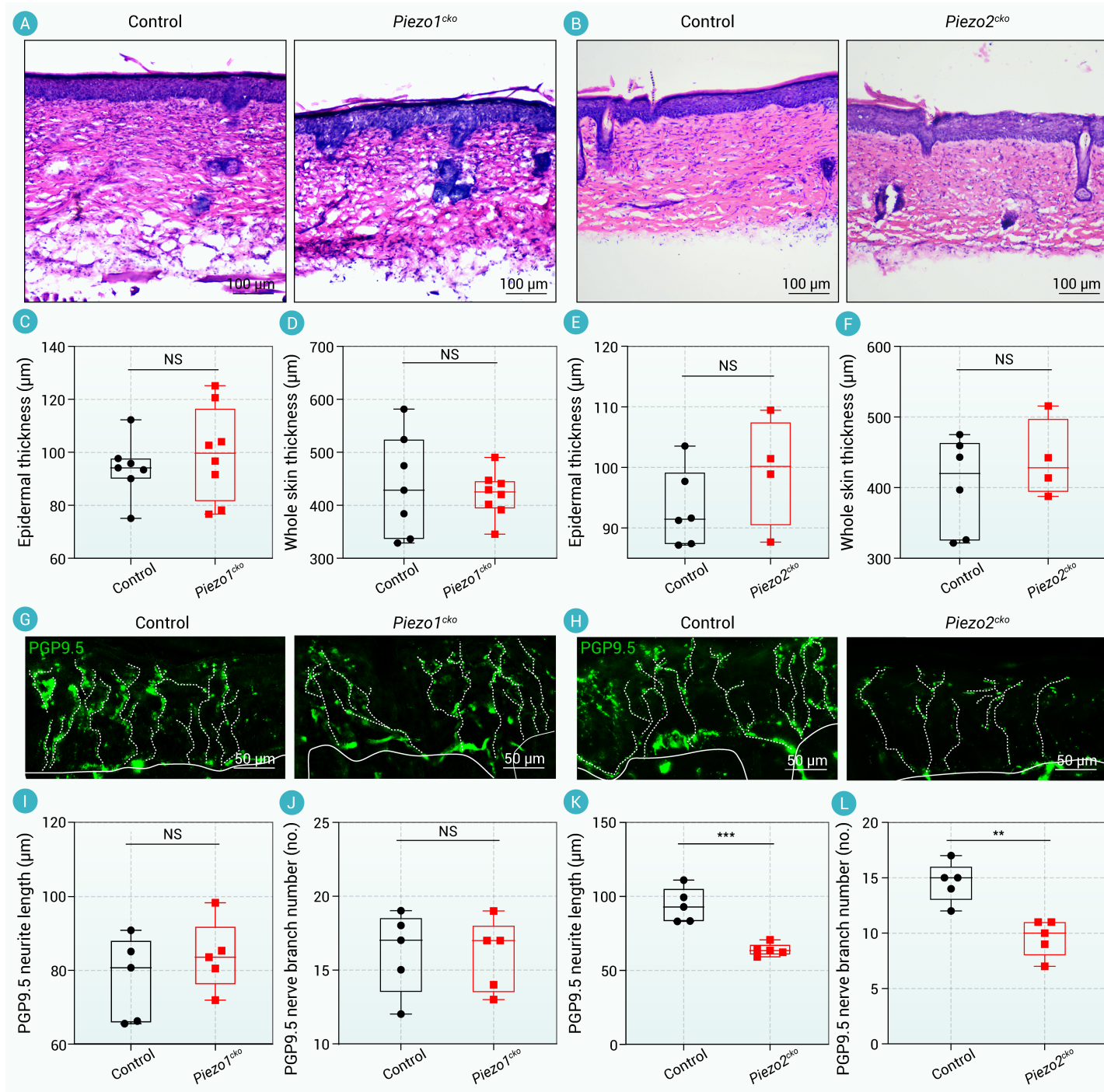


Figure 2. Keratinocyte-expressed Piezo2 promotes epidermal sensory fiber remodeling, but does not affect skin inflammation in MC903-induced AD (A-B) H&E-stained sections of MC903-treated skin from *Piezo1*^{cko} (left) and *Piezo2*^{cko} (right) mice. *n* = 3-5 sections from 3 mice per group. Scale bar, 100 μm. (C-E) Epidermal (C & E) and whole skin thickness (D & F) quantification in *Piezo1/2*^{cko} mice and control littermates. *n* = 7-8 mice per group. (G-H) Representative images of PGP9.5⁺ nerve fibers in the epidermis of *Piezo1*^{cko} (left) and *Piezo2*^{cko} (right) mice after MC903 treatment. Scale bar, 50 μm. (I-L) Quantification of PGP9.5⁺ neurite length (I & K) and branch number (J & L) in the epidermis of *Piezo1/2*^{cko} mice and the control littermates after MC903 treatment. *n* = 5 mice per group. Data are presented as mean ± SEM. Two-tailed unpaired Student's *t*-test. NS, not significant, ***P* < 0.01, ****P* < 0.001.

response to mechanical stimuli (Figure 3). These findings demonstrate that Piezo2 in keratinocytes is crucial for maintaining afferent C-fiber excitability under inflammatory conditions and plays a key role in mediating the neural transmission of itch.

To explore how Piezo2 influences epidermal innervation, we examined downstream guidance molecules regulating sensory nerve growth. We prioritized Semaphorin 3A (Sema3a), a well-established neuronal repulsive cue, based on its known role in restricting axonal outgrowth and prior reports of its downregulation in AD skin coinciding with nerve hyperinnervation. Although neurotrophic factors such as NGF are also elevated in AD, they are more associated with pain sensitization than itch. In contrast, the function of

repulsive cues like Sema3a in chronic itch is less understood.³³ In control mice, Sema3a was robustly expressed in keratinocytes but was downregulated following MC903 treatment (Figures 4A-D & S6). Strikingly, this downregulation did not occur in Piezo2-deficient mice (Figures 4E-H & S6), suggesting that Piezo2 is required for suppressing Sema3a expression in the context of AD.

Sema3a transcription in keratinocytes is regulated by the calcium-sensitive MAPK/ERK-AP-1 signaling pathway.³⁴ The known role of Piezo2 in mediating calcium influx suggests a plausible mechanism through which Piezo2 might modulate Sema3a expression, linking mechanotransduction to neurorepulsive signaling in keratinocytes under inflammatory conditions.³⁵ To

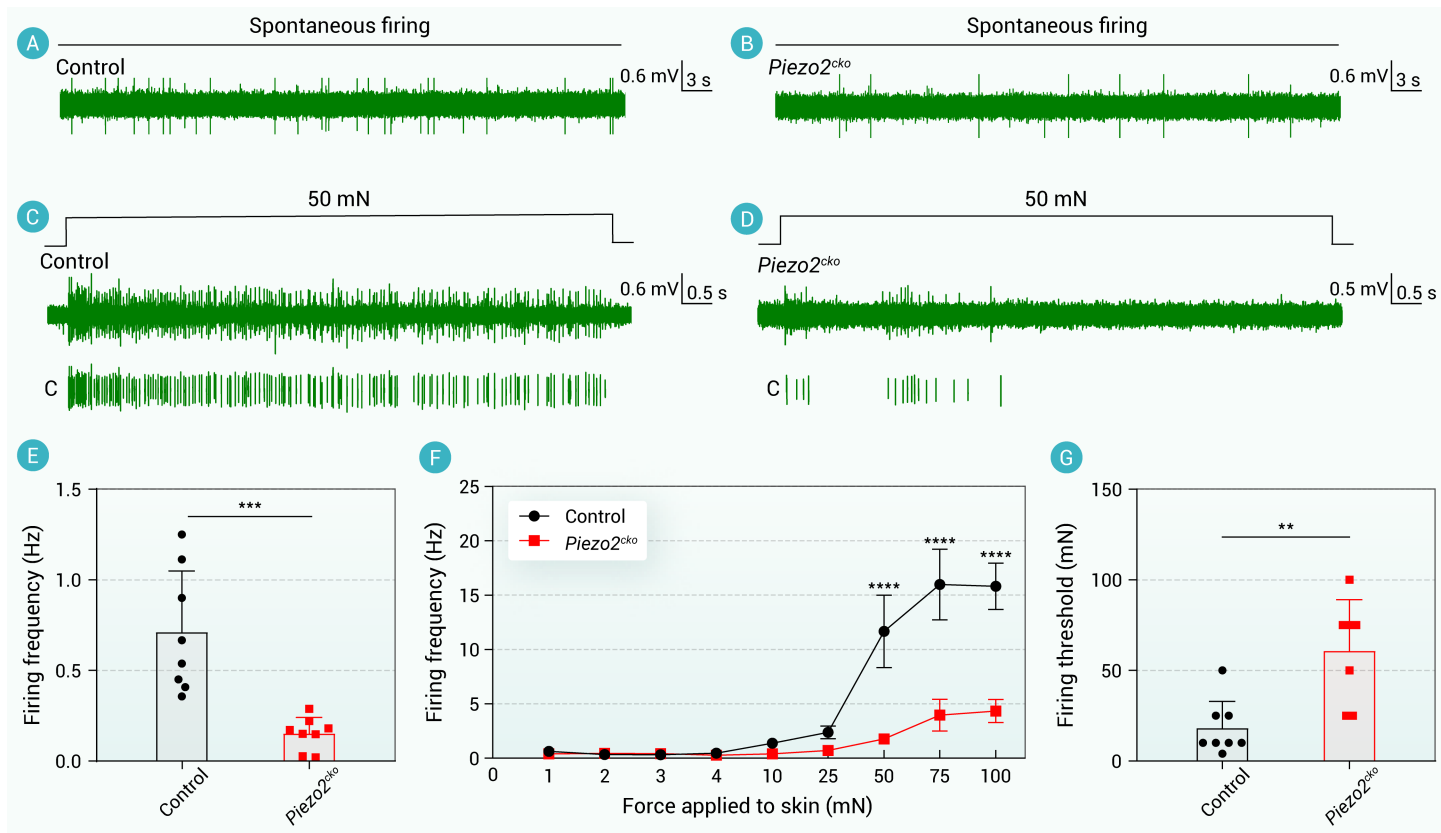


Figure 3. Keratinocyte-expressed Piezo2 enhances C fiber excitability during AD-associated itch (A-B) Representative traces of spontaneous C fiber firings recorded *ex vivo* from MC903-treated skin of a control littermate (left) and a *Piezo2^{cko}* (right) mice. (C-D) Representative traces of mechanically evoked C fiber firings in response to 50 mN force in MC903-treated skin from a control littermate (left) and a *Piezo2^{cko}* (right) mice. (E-G) Summarized data showing spontaneous C fiber firing frequency (E), mechanically evoked C fiber firing frequency (F), and mechanical firing threshold (G). *n* = 8 units from 4 mice. Data are presented as mean $\pm$ SEM. Two-tailed unpaired Student's *t*-test in E & G. Two-way ANOVA with Bonferroni post hoc test in F. ***P* < 0.01, ****P* < 0.001.

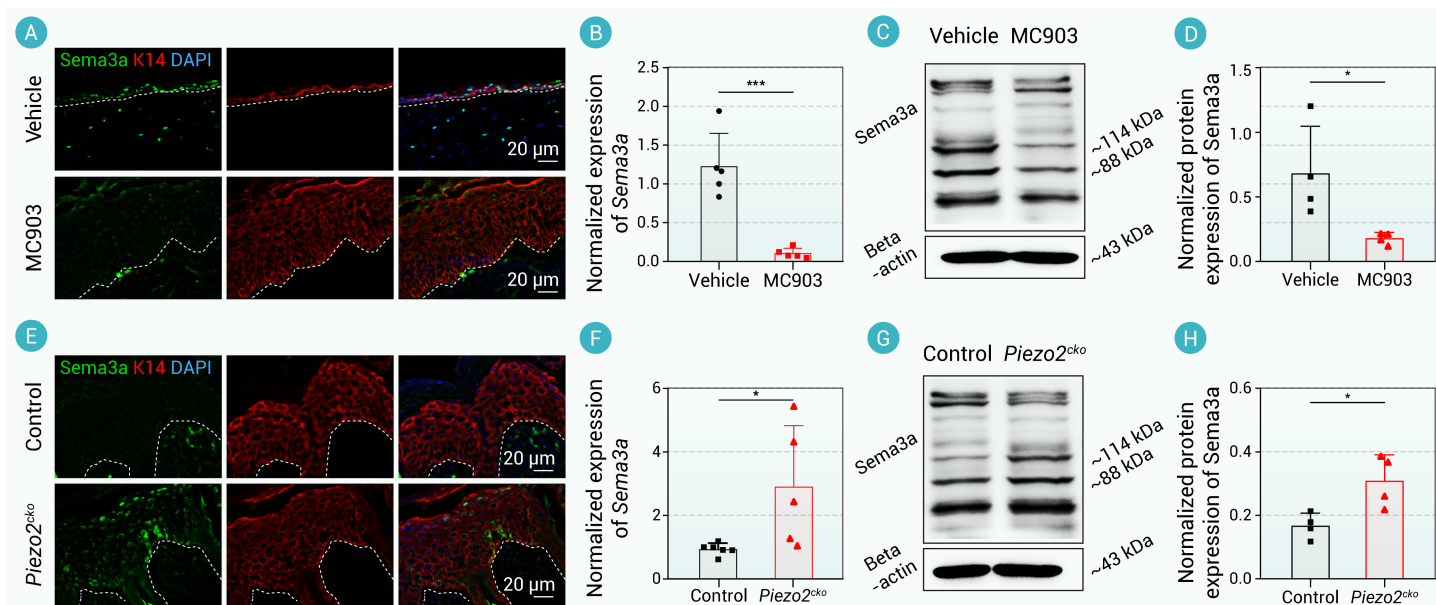


Figure 4. PIEZO2 is required for suppressing Sema3a expression in the context of AD (A) Representative images of Sema3a RNA-scope fluorescent staining in the skin of vehicle-treated and MC903-treated mice. *n* = 3–5 sections from 3 mice per group. Scale bar, 20 μ m. (B) Quantification of Sema3a mRNA levels by qPCR in keratinocytes isolated from vehicle-treated and MC903-treated mice. *n* = 5 mice per group. (C-D) Western blot analysis (C) and quantification (D) of SEMA3A protein levels in keratinocytes isolated from vehicle- and MC903-treated mice. *n* = 4 mice per group. (E) Representative images of Sema3a expression in the epidermis of MC903-treated control littermates and *Piezo2^{cko}* mice. *n* = 3–5 sections from 3 mice per group. Scale bar, 20 μ m. (F) qPCR analysis of Sema3a mRNA levels in keratinocytes isolated from MC903-treated control littermates and *Piezo2^{cko}* mice. *n* = 5 mice per group. (G-H) Western blot analysis (G) and quantification (H) of SEMA3A protein levels in keratinocytes from MC903-treated control littermates and *Piezo2^{cko}* mice. *n* = 4 mice per group. Data are presented as mean $\pm$ SEM. Two-tailed unpaired Student's *t*-test. **P* < 0.05, ****P* < 0.001.

assess the functional relevance of Sema3a in this context, we administered exogenous Sema3a, which significantly reduced scratching behavior in MC903-treated wild-type mice (Figures 5A-B). To further delineate the

keratinocyte-specific contribution of Sema3a, we generated keratinocyte-specific Sema3a knockout mice (Figures 5C & S7). At baseline, these mice exhibited no significant differences in scratching behavior or skin morphol-

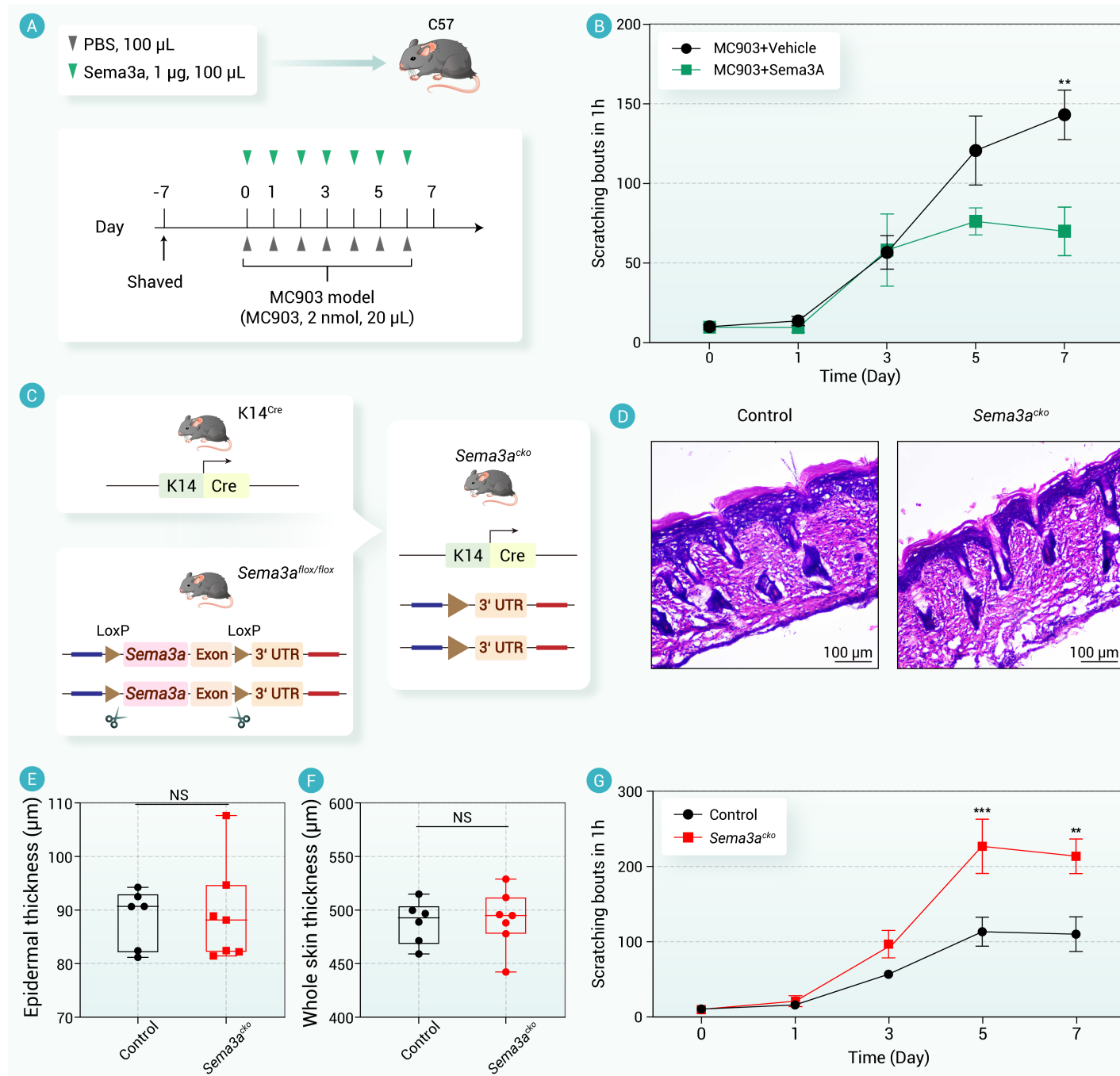


Figure 5. Keratinocyte Sema3a is essential for AD-associated chronic itch (A) Schematic of Sema3a protein administration in MC903-treated WT mice. (B) Time course of spontaneous scratching in the MC903-treated WT mice treated with vehicle or Sema3a. n = 5 mice per group. (C) The schematic diagram of the generation of *Sema3a^{cko}* mice. (D) Representative H&E-stained histological images of skin from MC903-treated control littermates (left) and *Sema3a^{cko}* mice (right), collected from the nape of the neck. n = 3-5 sections from 3 mice per group. Scale bar, 100 μ m. (E-F) Quantification of epidermal thickness (E) and total skin thickness (F) in MC903-treated control littermates and *Sema3a^{cko}* mice. n = 6-7 mice per group. (G) Quantification of spontaneous scratching bouts after MC903 treatment in *Sema3a^{cko}* mice. n = 4-5 mice per group. Data are presented as mean $\pm$ SEM. Two-tailed unpaired Student's *t*-test in E-F. Two-way ANOVA with Bonferroni post hoc test in B & G. NS, not significant, ** $P < 0.01$, *** $P < 0.001$.

ogy (Figure S8). Upon MC903 treatment, Sema3a deletion did not alter skin inflammation (Figures 5D-F), but markedly increased spontaneous scratching (Figure 5G), C-fiber excitability (Figures 6A-G), and intraepidermal nerve fiber density (Figures 6H-J). Notably, pharmacological inhibition of Sema3a using TCS1105 in *Piezo2^{cko}* mice significantly suppressed these phenotypes (Figures 6K-L), phenocopying the effects of Piezo2 activation.

CONCLUSION

Taken together, our results identify a keratinocyte-intrinsic Piezo2-Sema3a signaling axis that modulates both the excitability and structural plasticity of cutaneous sensory neurons in AD. This mechanistic insight links keratinocyte mechanotransduction to the regulation of peripheral nerve remodeling and

chronic itch. By dynamically responding to inflammatory cues and suppressing neurorepulsive signals, Piezo2 facilitates the expansion and sensitization of sensory fibers in the skin. These findings position Piezo2 as a central mediator in the neuroepidermal interface and highlight its potential as a therapeutic target for chronic itch in atopic dermatitis.

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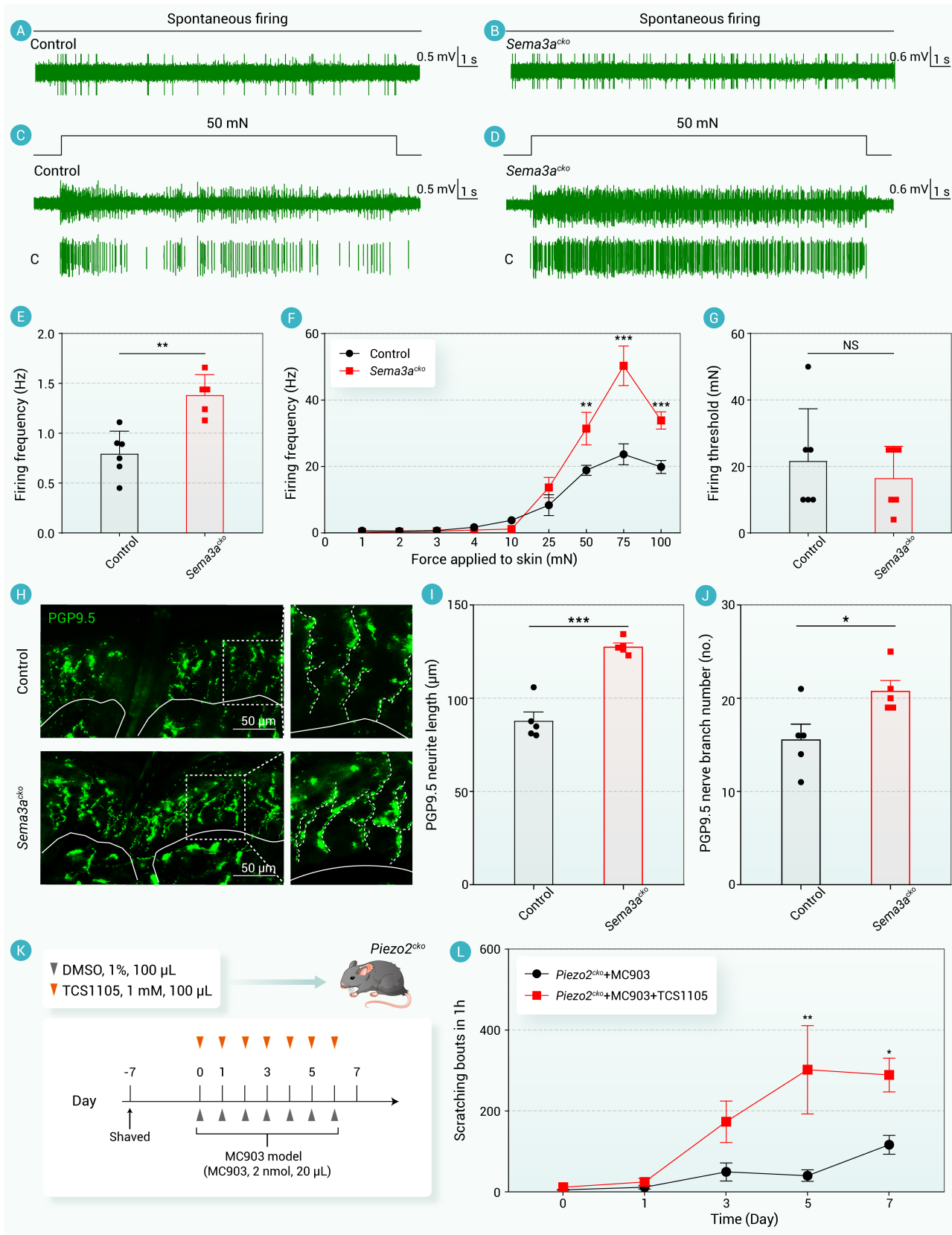


Figure 6. Piezo2-Sema3a signaling is essential for AD-associated chronic itch (A-B) Representative traces of spontaneous C fiber firing recorded *ex vivo* from MC903-treated skin of control littermates (A) and *Sema3a^{cko}* mice (B). (C-D) Representative traces of C fiber firing evoked by 50 mN mechanical stimulation in MC903-treated skin from control littermates (C) and *Sema3a^{cko}* mice (D). (E-G) Summarized data showing spontaneous C fiber firing frequency (E), mechanically activated C fiber firing threshold (F), and firing frequency (G). $n = 6$ units from 3 mice. (H) Representative images of PGP9.5⁺ nerve fibers in the epidermis of the control littermates (up) and *Sema3a^{cko}* mice (down) after MC903 treatment. $n = 3-5$ sections from 3 mice. Scale bar, 50 μm . (I-J) Quantification of PGP9.5⁺ neurite length (I) and branch number (J) in the epidermis of *Sema3a^{cko}* mice and the control littermates after MC903 treatment. $n = 5$ mice per group. (K) Schematic of TCS1105 treatment in MC903-treated *Piezo2^{cko}* mice. (L) Time course of spontaneous scratching in *Piezo2^{cko}* mice treated with vehicle or Sema3a inhibitor TCS1105. $n = 5$ mice per group. Data are presented as mean $\pm$ SEM. Two-tailed unpaired Student's *t*-test in E, G, I & J. Two-way ANOVA with Bonferroni post hoc test in F and L. NS, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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AUTHOR CONTRIBUTIONS

Conceptualization: J.F., J.L., R.Y.; Funding acquisition: J.F., T.W.; Investigation: X.H., L.W., G.Z., T.S., Y.L., Y.H., L.Z., L.L.; Analysis: Y.Z., X.H., L.W.; Supervision: J.F.; Writing – Original Draft Preparation: J.F.; Writing – Review and Editing: J.F., J.L., R.Y. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

All animal procedures were performed in strict accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (IACUC-2023-04-FJ-008; IACUC-2024-02-FJ-015; IACUC-2025-03-FJ-025).

DATA AND CODE AVAILABILITY

Data are available from the corresponding author upon reasonable request.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-life.2026.100187>