



# Tissue-specific contributions of *Tmem79* to atopic dermatitis and mast cell-mediated histaminergic itch

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Atopic dermatitis (AD) is the most common skin disease in children. It is characterized by relapsing inflammation, skin-barrier defects, and intractable itch. However, the pathophysiology of itch in AD remains enigmatic. Here, we examine the contribution of *Tmem79*, an orphan transmembrane protein linked to AD in both mice and humans. We show that *Tmem79* is expressed by both keratinocytes and sensory neurons, but that loss of keratinocytic *Tmem79* is sufficient to elicit robust scratching. *Tmem79*<sup>-/-</sup> mice demonstrate an accumulation of dermal mast cells, which are diminished following chronic treatment with cyclooxygenase inhibitors and an EP3 receptor antagonist. In *Tmem79*<sup>-/-</sup> mice, mast cell degranulation produces histaminergic itch in a histamine receptor 1/histamine receptor 4 (H4R/H1R)-dependent manner that may involve activation of TRPV1<sup>-</sup> afferents. TMEM79 has limited sequence homology to a family of microsomal glutathione transferases and confers protection from cellular accumulation of damaging reactive species, and may thus play a role in regulating oxidative stress. In any case, mechanistic insights from this model suggest that therapeutics targeting PGE<sub>2</sub> and/or H1R/H4R histaminergic signaling pathways may represent useful avenues to treat *Tmem79*-associated AD itch. Our findings suggest that individuals with mutations in *Tmem79* develop AD due to the loss of protection from oxidative stress.

itch | atopic dermatitis | oxidative stress | *Tmem79* | prostaglandin E2

The development of atopic dermatitis (AD) is associated with a genetic predisposition toward immune dysregulation and skin-barrier defects as well as hypersensitivity to environmental factors and psychological triggers (1, 2). The canonical pathology of AD is often accompanied by an infiltrate of inflammatory cells (e.g., mast cells, eosinophils, and T lymphocytes) into the dermal layer of skin. In recent years, oxidative stress has also gained attention for its role in the pathogenesis of AD (3).

Several mouse models of AD have been characterized (4) including *Tmem79*<sup>-/-</sup> (or *Matt*<sup>-/-</sup>) mice. Mutations in *Tmem79*<sup>-/-</sup> mice underlie the matted hair and dermatitis phenotypes in flaky tail mice (*Flg*<sup>fl</sup>) and lead to spontaneous AD characterized by skin-barrier defects, eczematous skin lesions, and severe scratching (5, 6). Importantly, a missense SNP in *Tmem79* is associated with AD in human subjects (6). However, mechanisms contributing to itch in *Tmem79*-associated AD remain elusive. Furthermore, it is not known if *Tmem79* plays a dominant role in keratinocytes, neurons, or other skin cells that results in the development of atopy. Here we leveraged *Tmem79*-null and -conditional knockout reporter mouse lines to explore the cellular and molecular mechanisms underlying itch in this model of AD.

## Results and Discussion

Gross observation revealed that our de novo *Tmem79*<sup>-/-</sup> mouse line exhibits pelage (SI Appendix, Fig. S1A) consistent with a previously reported nonsense mutation (6). Additionally, we observed thickened dermis with immune cell infiltrate (SI Appendix, Fig. S1B and C) and exaggerated scratching behavior (SI

Appendix, Fig. S1E–G), again consistent with previous reports (5, 6). *Tmem79*<sup>-/-</sup> mice are normal in weight (SI Appendix, Fig. S1D) and do not exhibit anxiety behavior as measured by the elevated plus maze (SI Appendix, Fig. S1H).

Scratching is typically preceded by pruritoception (i.e., activation of a subset of somatosensory neurons that trigger itch). We therefore asked if *Tmem79* is expressed in neuronal tissues, in addition to skin as previously reported (5, 6). Indeed, *Tmem79* initially came to our attention as a transcript that is preferentially expressed in somatosensory ganglia rather than in other neural tissues (SI Appendix, Fig. S2C). Consistent with this, we found that *Tmem79* is transcribed in hairy and glabrous skin as well as trigeminal (TG) and dorsal root (DRG) sensory ganglia but not in brain or spinal cord (Fig. 1A). Expression by a subset of sensory neurons was confirmed by in situ hybridization histochemistry (SI Appendix, Fig. S2B) as well as by a reporter mouse line (*Tmem79*-KI) in which eGFP is produced from a *Tmem79*-2A-eGFP precursor protein (SI Appendix, Fig. S2A). In these mice, robust eGFP expression was observed in epidermal cells of hairy skin (Fig. 1B), cervix, prostate, and tongue but not in cells of the lung, adrenal medulla, brain, or spinal cord (SI Appendix, Fig. S2G). In hairy skin epithelium, eGFP was highly colocalized with keratin 14 (K14), a marker for keratinocytes (SI Appendix, Fig. S2H). Consistent with our transcriptomics, we

## Significance

Atopic dermatitis (AD) is a common skin disease affecting children, but the mechanisms underlying the accompanying itch remain poorly understood. Indeed, treatments to alleviate itch in AD and the substantial morbidity it represents for patients are often unsuccessful. Here, we shed light on a form of AD associated with the loss of *Tmem79* and provide insights into cellular and molecular players in this genetically driven form of the disease that has been described in mice and humans. We propose a link between this inherited form of AD and the recognized contribution of oxidative stress to this disease. Our analysis may suggest pharmacological strategies for treating patients who suffer from AD and bear mutations in *Tmem79*.

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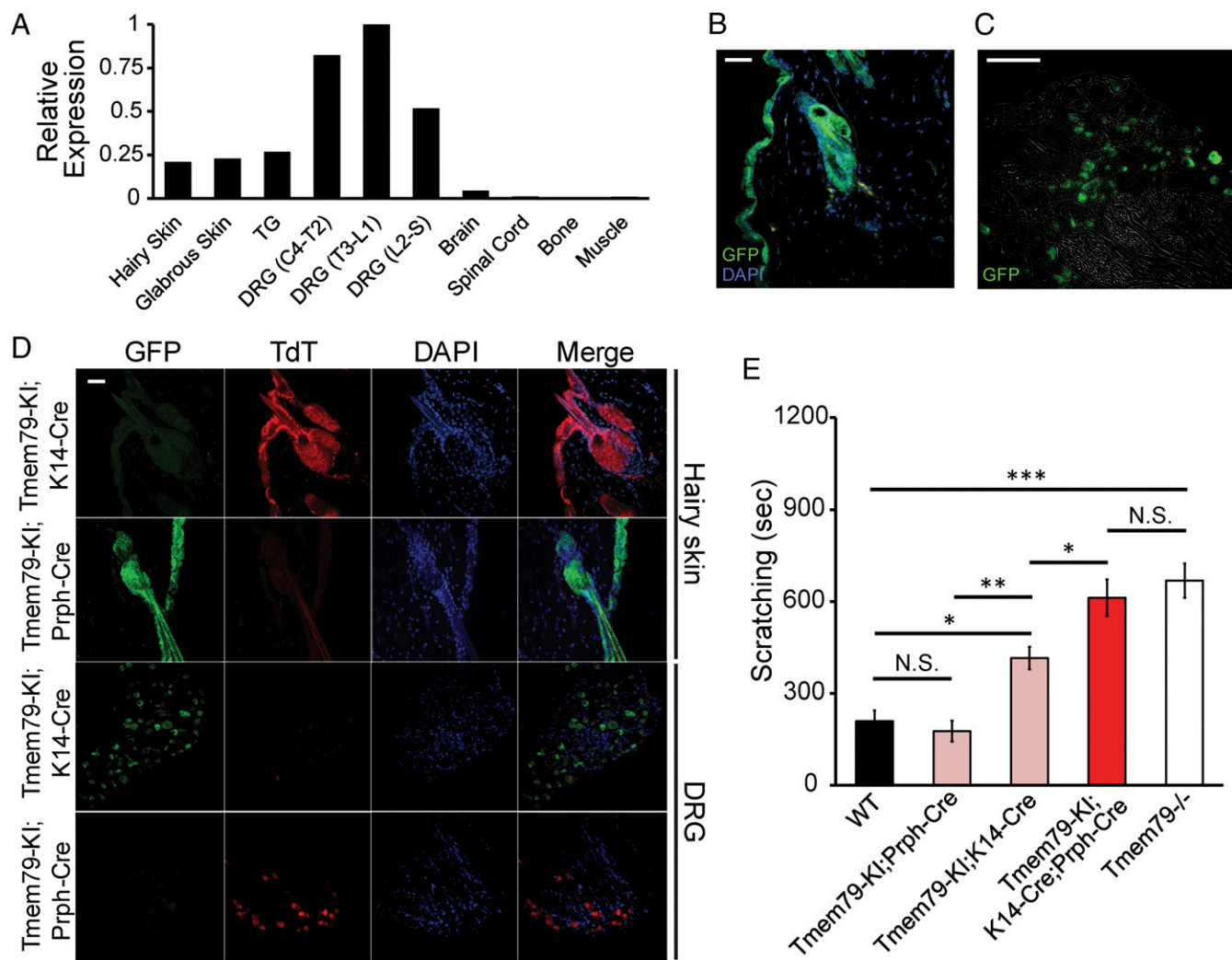
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**Fig. 1.** Loss of *Tmem79* in sensory neurons and keratinocytes produces itch. (A) Real-time qPCR analysis of *Tmem79* expression relative to *Rpl19* in tissues dissected from wild-type mice. The graph presents  $\Delta\Delta Ct$  values normalized to the tissue with greatest relative expression of *Tmem79*.  $\Delta\Delta Ct$  values were calculated from real-time PCR reactions run in triplicate from combined tissues from three wild-type mice. Tissues examined were hairy skin, glabrous (hairless) skin, TGs, DRG fourth cervical to second thoracic (DRG C4–T2), DRG third thoracic to first lumbar (DRG T3–L1), DRG second lumbar to sacral (DRG L2–S), brain, spinal cord, bone, and muscle. (B and C) Representative micrographs of tissue sections from *Tmem79-KI* reporter mice stained for immunoreactivity to GFP (green). (B) Hairy skin section (20  $\mu\text{m}$ ) with the superficial epidermis oriented to the left. Nuclei were counterstained using DAPI (blue). (Scale bar: 15  $\mu\text{m}$ .) (C) DRG section (12  $\mu\text{m}$ ). The image is superimposed with brightfield micrograph. (Scale bar: 100  $\mu\text{m}$ .) (D) Representative micrographs of hairy skin or DRG sections from *Tmem79-KI;K14-Cre* or *Tmem79-KI;Prph-Cre* mice stained for immunoreactivity to GFP (green) or TdT (red). Superficial epidermis is oriented to the left (1st Row, *Tmem79-KI;K14-Cre*) or the right (2nd Row, *Tmem79-KI;Prph-Cre*). Nuclei were counterstained with DAPI (blue). Merged images are shown in the far right column. (Scale bar: 15  $\mu\text{m}$ .) (E) Quantified scratching behavior of adult wild-type, *Tmem79-KI;Prph-Cre*, *Tmem79-KI;K14-Cre*, *Tmem79-KI;K14-Cre;Prph-Cre*, and *Tmem79<sup>-/-</sup>* mice. Data shown are the mean number of seconds of scratching in multiple mice of each genotype  $\pm$  SEM.  $n = 6$  wild-type mice, 9 *Tmem79-KI;Prph-Cre* mice, 11 *Tmem79-KI;K14-Cre* mice, 5 *Tmem79-KI;K14-Cre;Prph-Cre* mice, and 12 *Tmem79<sup>-/-</sup>* mice; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , one-way ANOVA with Holm–Sidak’s multiple comparisons correction; N.S., not significant.

found eGFP to be highly expressed in a subset of sensory neurons (Fig. 1C) of small-to-medium diameter (*SI Appendix, Fig. S2F*) and showed varying degrees of coexpression with the following markers: isolectin-B4 (IB4, 45%), NF200 (32%), peripherin (74%), TRPV1 (20%), calcitonin gene-related peptide (CGRP, 16%), substance P (SP, 32%), TRPA1 (24%), histamine receptor 1 (H1R, 61%), and histamine receptor 4 (H4R, 80%) (*SI Appendix, Fig. S2D and E*). In conclusion, we find *Tmem79* to be expressed in keratinocytes of the epidermis, epithelial tissues, and a subpopulation of somatosensory neurons.

Our *Tmem79-KI* reporter mouse line also features an inverted floxed cassette (*SI Appendix, Fig. S2A*) that allows cell-specific targeting of *Tmem79* deletion as indicated by the expression of TdTomato (TdT). As predicted, K14-TdT (*Tmem79-KI;K14-*

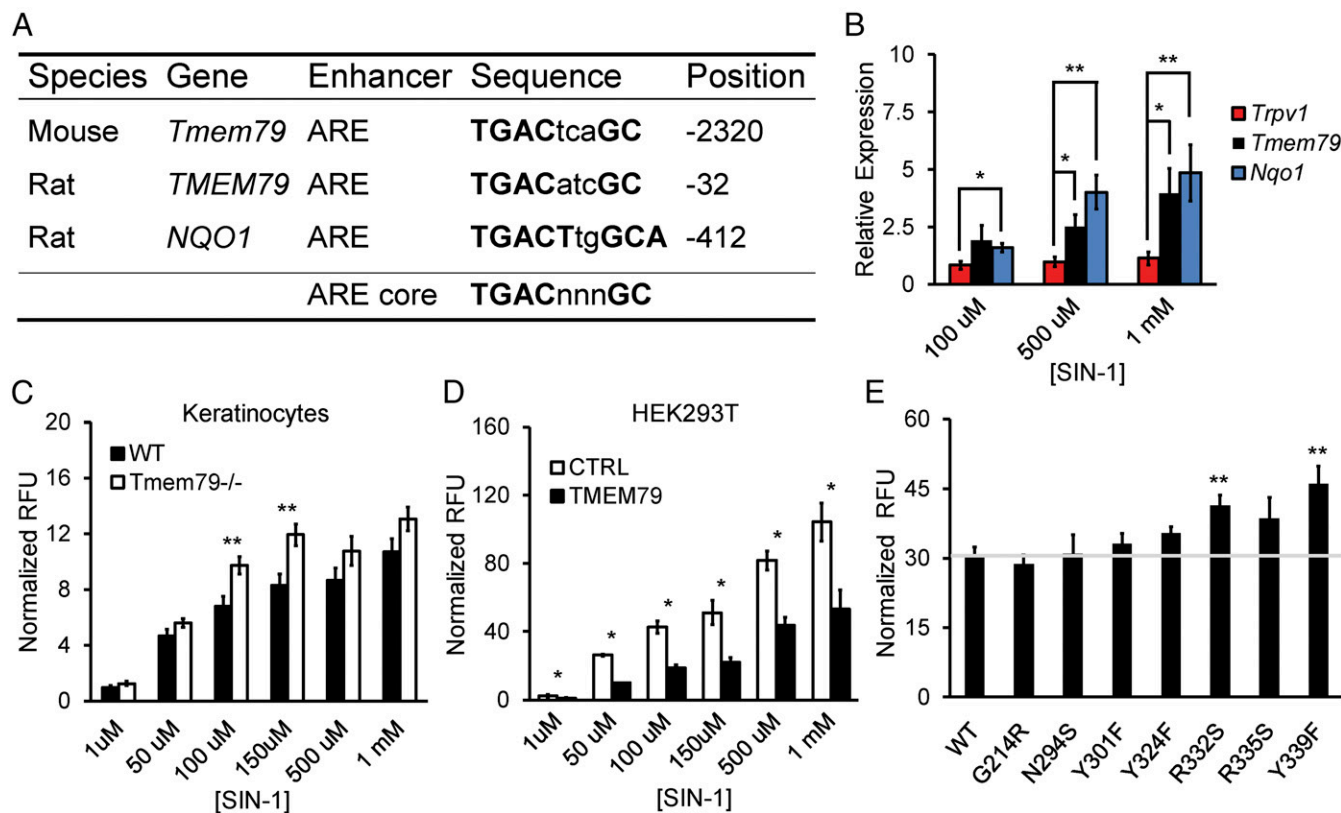
*Cre*) and Prph-TdT (*Tmem79-KI;Prph-Cre*) mice showed robust TdT expression in the epidermis and sensory neurons, respectively, with no observable expression of eGFP (Fig. 1D). As further validation of faithful, cell-specific recombination, the population of sensory neurons expressing TdT in Prph-TdT mice matched the pattern of eGFP expression in *Tmem79-KI* animals (*SI Appendix, Fig. S2F*). We next leveraged these crosses to determine the contribution of *Tmem79* in keratinocytes versus sensory neurons in regard to AD phenotypes. We found that while K14-TdT mice exhibit partially elevated scratching behavior and dermal thickening (*SI Appendix, Fig. S5F and G*), only double-deletion, K14-TdT;Prph-TdT mice completely phenocopied *Tmem79<sup>-/-</sup>* scratching (Fig. 1E). These data suggest that while the loss of keratinocytic *Tmem79* accounts for a substantial

portion of scratching and AD in *Tmem79*-null mice, the loss of neuronal *Tmem79* also contributes to the phenotype.

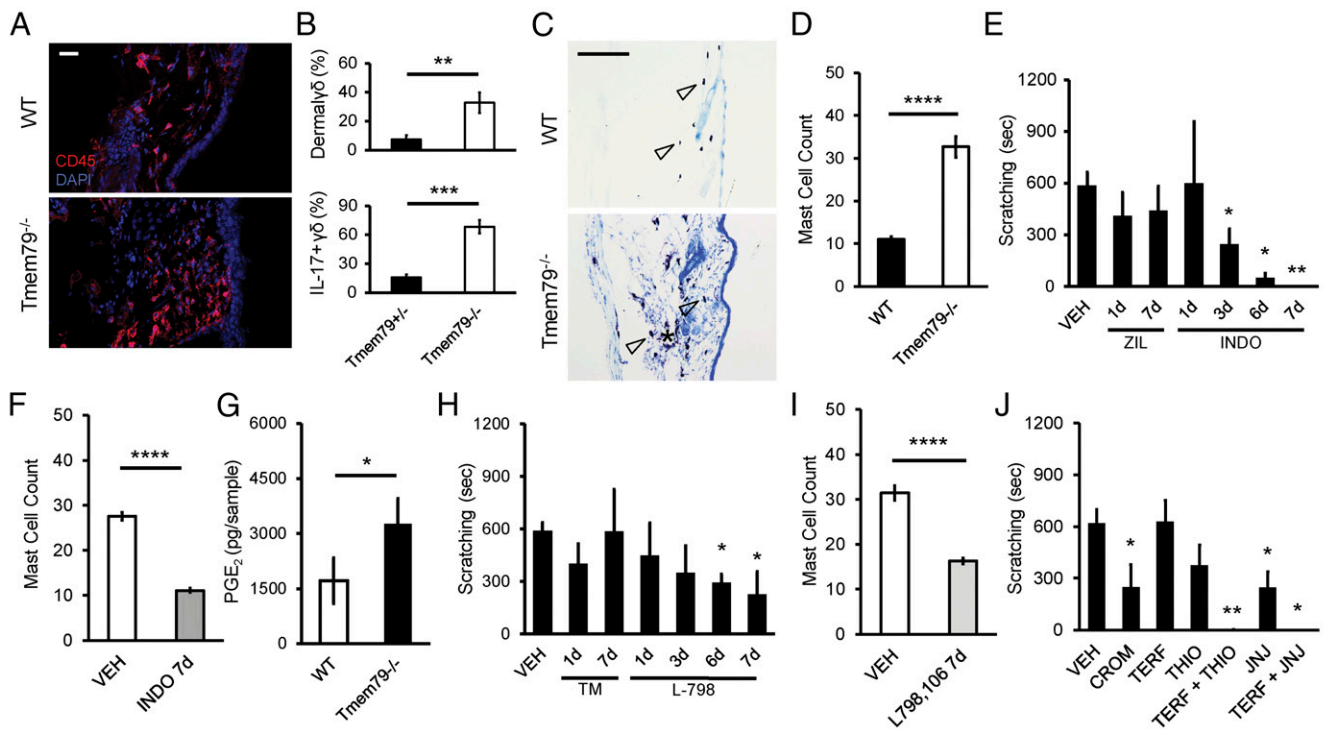
Previous work suggests that TMEM79 plays a role in secretory transport and that consequent defects in *Tmem79* mutants produce AD due to deficiencies in skin-barrier function (5). However, TMEM79 shows limited, but intriguing, primary amino acid sequence similarity (~30%) with membrane glutathione transferases (MAPEGs) such as MGST3 (7) or MGST1 (8–10) that detoxify reactive electrophiles (SI Appendix, Fig. S3 F–H) (6). In light of this, and our findings that *Tmem79* is also expressed in sensory neurons, we asked if *Tmem79* modulates oxidative stress in cellular assays. First, we found that acutely dissociated keratinocytes from *Tmem79*<sup>-/-</sup> mice accumulate higher levels of reactive species than do wild-type controls when challenged with moderate concentrations of the oxidant 3-Morpholinosydnonimine (SIN-1) (Fig. 2C). Similarly, heterologous expression of TMEM79 in HEK293T cells diminished the accumulation of reactive species in response to SIN-1 or sodium nitroprusside (but not tert-Butyl hydroperoxide or H<sub>2</sub>O<sub>2</sub>) (Fig. 2D and SI Appendix, Fig. S3 A–E). Furthermore, mutation of two putative active-site residues (R332S and Y339F) within a MAPEG-homologous region of TMEM79 eliminated the protective effect in this assay (Fig. 2E). Interestingly, the rat and mouse *Tmem79* genes contain a canonical antioxidant

response element sequence (Fig. 2A) (11–13). Consistent with this, we found that exposure of cultured rat sensory neurons to SIN-1 results in higher relative expression of *Tmem79* mRNA than *Trpv1* mRNA, which lacks the response element (Fig. 2B). Taken together, these data suggest that TMEM79 may function to neutralize reactive electrophiles, thus preventing cellular oxidative stress, a known contributor to the development of AD (3).

As noted, *Tmem79*<sup>-/-</sup> mice showed an increased presence of immune cells in the dermis as assessed by cytologic staining (SI Appendix, Fig. S1B) and CD45 immunoreactivity (Fig. 3A). Furthermore, flow cytometry (SI Appendix, Fig. S4A) revealed an expansion of IL-17-expressing  $\gamma\delta$ -T cells and mast cells (Fig. 3B and SI Appendix, Fig. S4 B–D) but no change in the number of neutrophils, eosinophils, or Ly6C<sup>hi</sup> or Ly6C<sup>low</sup> monocytes in the skin of *Tmem79*<sup>-/-</sup> mice (SI Appendix, Fig. S4D). Histological inspection revealed an increase in mast cell count in *Tmem79*<sup>-/-</sup> dermis (Fig. 3 C and D). In some instances, degranulated mast cells were apparent at higher magnifications (SI Appendix, Fig. S5A), which may explain an underestimation of mast cell count by flow cytometry (SI Appendix, Fig. S4D). We similarly observed additional CD45<sup>+</sup> immune cells (SI Appendix, Fig. S5C) and increased mast cell counts (SI Appendix, Fig. S5E) in the dermis of *Tmem79*-KI;K14-Cre mice. Of note, we failed to observe



**Fig. 2.** TMEM79 diminishes oxidative stress. (A) Table of antioxidant response elements (AREs) in rodent genes (*Tmem79*, *TMEM79*, and *NQO1*). Sequence identity and the position relative to the start codon for each gene are provided. The ARE core is shown for reference. (B) Real-time qPCR data for gene expression relative to *Rpl19* in cultured P0 rat sensory neurons following 16-h incubation with concentrations of SIN-1. Data are shown as the mean relative expression  $\pm$  SEM;  $n = 3$  independent experiments with samples run in triplicate.  $^{**}P < 0.01$ ,  $^{*}P < 0.05$ , two-tailed Holm  $t$  test. (C) Quantification of DCF fluorescence in acute cultures of keratinocytes from age-matched wild-type or *Tmem79*<sup>-/-</sup> mice following 30-min incubation with SIN-1 concentrations as noted. Data represent mean relative fluorescent units (RFU) normalized to baseline  $\pm$  SEM;  $n = 7$  mice with samples run in triplicate.  $^{**}P < 0.01$ , two-tailed Student's  $t$  test. (D) Quantification of DCF fluorescence in cultures of HEK293T cells expressing either control (CTRL) or mouse *Tmem79* (TMEM79) vector following 30-min incubation with various SIN-1 concentrations. Data represent mean RFU normalized to baseline  $\pm$  SEM;  $n = 3$  or 4 experiments with samples run in triplicate.  $^{*}P < 0.05$ , one-tailed Student's  $t$  test. (E) Quantification of DCF fluorescence in cultures of HEK293T cells expressing vectors containing mouse *Tmem79* (WT) or amino acid substitution mutants (G214R, N294S, Y301F, Y324F, R332S, R335S, and Y339F) following 30-min incubation in 150  $\mu$ M of SIN-1. Data represent mean RFU normalized to baseline  $\pm$  SEM. The horizontal gray line represents normalized RFU from mouse *Tmem79* (WT) for reference.  $n = 5$  experiments with samples run in triplicate.  $^{**}P < 0.01$ , two-tailed Holm  $t$  test.



**Fig. 3.** PGE<sub>2</sub>–EP3 interaction recruits dermal mast cells that drive histaminergic itch via H4R in *Tmem79*<sup>−/−</sup> mice. (A) Representative micrographs of stained hairy skin sections (20 μm) from age-matched wild-type (Upper) or *Tmem79*<sup>−/−</sup> (Lower) mice. Superficial epidermis is oriented to the right. Staining for immunoreactivity to CD45 (red) with nuclei counterstained using DAPI (blue). (Scale bar: 10 μm.) (B) Flow cytometry quantification of immune cells and cytokine expression in the hairy skin of littermate *Tmem79*<sup>+/+</sup> and *Tmem79*<sup>−/−</sup> mice. Data shown are the percentage of dermal immune cells that are γδ<sup>+</sup> (Upper) and the percentage of dermal γδ<sup>+</sup> cells that are also IL-17<sup>+</sup> (Lower). Data shown are the mean percentages ± SEM from four *Tmem79*<sup>+/+</sup> and four *Tmem79*<sup>−/−</sup> mice. \*\**P* < 0.01, \*\*\**P* < 0.001, one-tailed Student’s *t* test. (C) Representative micrographs of Toluidine blue O-stained hairy skin sections (20 μm) from age-matched wild-type (Upper) or *Tmem79*<sup>−/−</sup> (Lower) mice. Superficial epidermis is oriented to the right. Hollow arrowheads indicate examples of stained mast cells. (Scale bar: 100 μm.) (D) Quantification of mast cells from micrographs represented in C. Data shown are the mean mast cell count ± SEM from fields captured from wild-type or *Tmem79*<sup>−/−</sup> mice; *n* = 40 micrographs in wild-type mice and 47 micrographs in *Tmem79*<sup>−/−</sup> mice. \*\*\*\**P* < 0.0001, two-tailed Student’s *t* test. (E) Quantified scratching behavior of adult *Tmem79*<sup>−/−</sup> mice following i.p. administration of vehicle (VEH), 20 mg/kg zileuton (ZIL), or 10 mg/kg indomethacin (INDO) for 1, 3, 6, or 7 consecutive days. Data shown are the mean number of seconds of scratching ± SEM from multiple mice (*n* = 4) in each treatment group. \**P* < 0.05, \*\*\**P* < 0.01, one-tailed Holm *t* test. (F) Quantification of mast cells from micrographs of stained hairy skin sections from mice receiving vehicle (VEH) or 10 mg/kg indomethacin (INDO) for seven consecutive days. Data shown are the mean mast cell count ± SEM; *n* = 43 micrographs from vehicle-treated mice and 85 micrographs from indomethacin-treated mice. \*\*\*\**P* < 0.0001, two-tailed Student’s *t* test. (G) LC/MS/MS-based lipidomics was used to identify and quantify prostanoids, PGE<sub>2</sub>, and PGE<sub>2</sub> metabolite content from lysed acutely dissociated keratinocytes from age-matched wild-type and *Tmem79*<sup>−/−</sup> mice. Deuterated internal standards were used to correct for extraction and processing recoveries. An AB Sciex QTRAP 4500 mass spectrometer was used for analyses; prostanoids were identified and quantitated using scheduled MRM with four to six specific transition ions for each analyte. Data represent the average ± SEM PGE<sub>2</sub> content (quantified in picograms) from keratinocytes from weight-matched skin dissected from separate animals; *n* = 4 animals per group. \**P* < 0.05, two-tailed Student’s *t* test. (H) Quantified scratching behavior of adult *Tmem79*<sup>−/−</sup> mice following i.p. administration of vehicle (VEH), 5 mg/kg TM30089 (TM), or 100 mg/kg L-798,106 (L-798) for seven consecutive days. Data shown are the mean number of seconds of scratching ± SEM by multiple mice (*n* = 4) in each treatment group. \**P* < 0.05, one-tailed Holm *t* test. (I) Quantification of mast cells from micrographs of stained hairy skin sections from mice treated with vehicle (VEH) or 100 mg/kg L-796,106 (L-796) for seven consecutive days. Data shown are the mean mast cell count ± SEM. *n* = 54 micrographs from vehicle-treated mice and 62 micrographs from L-796,106-treated mice. \*\*\*\**P* < 0.0001, two-tailed Student’s *t* test. (J) Quantified scratching behavior of adult *Tmem79*<sup>−/−</sup> mice following i.p. administration of vehicle (VEH), 20 mg/kg cromolyn sulfate (CROM), 20 mg/kg terfenadine (TERF), 20 mg/kg thioperamide (THIO), 20 mg/kg terfenadine + 20 mg/kg thioperamide (TERF + THIO), 20 mg/kg JNJ-777120 (JNJ), or 20 mg/kg terfenadine + 20 mg/kg JNJ-777120 (TERF + JNJ). Data shown are the mean number of seconds of scratching ± SEM from multiple mice (*n* = 4) in each treatment group. \**P* < 0.05, \*\**P* < 0.01, one-tailed Holm *t* test.

*Tmem79* expression in the lung epithelium of *Tmem79*-KI reporter mice (SI Appendix, Fig. S2G) or any increased immune- or mast cell presence in sections of *Tmem79*<sup>−/−</sup> lung epithelium compared with wild-type mice (SI Appendix, Fig. S5B). Taken together, these observations suggest that the *Tmem79*<sup>−/−</sup> phenotype does not include airway inflammation characteristic of the “atopic march” seen in some cases of AD (14). These results suggest that TMEM79 dysregulation promotes the accumulation of IL-17-expressing γδ-T cells and mast cells which have been implicated in AD (15, 16) and pruritus (17–19).

Eicosanoids (e.g., prostaglandins and leukotrienes) are produced by keratinocytes in response to cellular oxidative stress (20, 21) and could serve as mast cell chemoattractants (22). We therefore asked if these lipid mediators contribute to phenotypes

associated with loss of *Tmem79*. Whereas daily administration of the 5-lipoxygenase inhibitor zileuton (20 mg/kg, i.p.) had no effect on scratching behavior, the cyclooxygenase inhibitor indomethacin (10 mg/kg, i.p.) abolished elevated scratching in *Tmem79*<sup>−/−</sup> after 7 d (Fig. 3E) while also reducing normal scratching in wild-type mice after 3 d (SI Appendix, Fig. S5J). Indomethacin also reduced dermal mast cell (Fig. 3F and SI Appendix, Fig. S5D) and CD45<sup>+</sup> immune cell counts (SI Appendix, Fig. S5C), but not dermal thickness in *Tmem79*<sup>−/−</sup> mice (SI Appendix, Fig. S5F and G), suggesting a prostanoid-independent mechanism. Taken together, these results show that scratching in *Tmem79* mutants is dependent on the generation of an excess of endogenous prostanoids and that *Tmem79*-dependent itch is an exacerbation of normal baseline scratching behavior.

We next sought to determine which prostaglandin(s) may be mediating mast cell chemotaxis. Indomethacin prevents the formation of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) and the downstream prostaglandins PGE<sub>2</sub> and PGD<sub>2</sub>. Blockade of PGE<sub>2</sub> signaling by the administration of an EP3 receptor antagonist (L-798,106, 100 mg/kg, i.p.) reduced scratching in *Tmem79*<sup>-/-</sup> mice (Fig. 3H). Furthermore, consistent with a known role for EP3R in PGE<sub>2</sub>-mediated chemoattraction (22), administration of this antagonist resulted in reduced dermal mast cell counts (Fig. 3I and *SI Appendix, Fig. S5D*) and in a slight reduction in CD45<sup>+</sup> immune cells (*SI Appendix, Fig. S5C*), although dermal thickness was again unaffected (*SI Appendix, Fig. S5 F and G*). By contrast, a CRTH2/DP2 receptor antagonist (TM30089, 5 mg/kg, i.p.) had no measurable effect on these parameters, suggesting that PGD<sub>2</sub> is not involved.

Do keratinocytes in the epidermis constitute a source of PGE<sub>2</sub> in *Tmem79*<sup>-/-</sup> mice (20, 21, 23)? Indeed, we observed a specific increase in PGE<sub>2</sub>, but not in the prostanoid precursor PGH<sub>2</sub> (*SI Appendix, Fig. S5 H and I*). LC-MS/MS-based lipidomic analyses confirmed a selective increase in PGE<sub>2</sub> from *Tmem79*<sup>-/-</sup> keratinocytes with no change in PGE<sub>2</sub> metabolites (13,14-dihydro-15-keto-PGE<sub>2</sub>, 13,14-dihydro-15 keto-PGA<sub>2</sub>, or bicyclo-PGE<sub>2</sub>) (Fig. 3G). Our analysis suggests that increased PGE<sub>2</sub> levels are due to enhanced PGE<sub>2</sub> synthesis rather than a general increase in COX enzymatic activity or decreased PGE<sub>2</sub> turnover. These data are consistent with observations of increased PGE<sub>2</sub> content in skin plaques from humans with AD (24) and suggest that EP3R antagonism may have a newfound application in treating AD-associated itch.

In light of these results, we asked if mast cell-mediated histamine release underlies scratching in *Tmem79*<sup>-/-</sup> mice. First, the administration of cromolyn sulfate, which prevents mast cell degranulation, diminished scratching in *Tmem79*<sup>-/-</sup> mice (Fig. 3J). Second, scratching in *Tmem79*<sup>-/-</sup> and *Tmem79*-KI;K14-Cre mice was diminished with the H4R antagonists thioperamide or JNJ-777120, but not by the H1R antagonist terfenadine (Fig. 3J). Remarkably, dual administration of H1R and H4R antagonists had a synergistic effect in which scratching was completely abolished (Fig. 3J and *SI Appendix, Fig. S5K*). The s.c. administration of pruritogens (histamine or chloroquine) to *Tmem79*<sup>-/-</sup> mice did not further enhance scratching in these animals (*SI Appendix, Fig. S8 A and B*). Our data suggest that histamine from mast cells contributes to scratching in these mice primarily via activation of H4R, with some contribution from H1R. Because dual administration of histamine receptor antagonists completely abolished scratching, similar to COX inhibition in wild-type and *Tmem79*<sup>-/-</sup> mice, we suggest that *Tmem79*-dependent itch is an exacerbation of normal baseline scratching behavior. These findings provide further support for the use of a combination of H1R/H4R antagonists in treating dermatitis as gleaned from models of both atopic and allergic dermatitis (25–27).

H1R and H4R are expressed on somatosensory neurons (27) and mast cells (Fig. 4 A and B and *SI Appendix, Fig. S6 A–C*), raising the question of their location of action when producing AD phenotypes in *Tmem79*<sup>-/-</sup> mice. In light of our observations that (i) administration of H4R/H1R antagonists has no effect on mast cell degranulation (*SI Appendix, Fig. S6D*) and (ii) administration of H4R or H4R/H1R antagonists relieves scratching within 30 min, before H4R-mediated chemotaxis of mast cells would be blocked (28), we agree that the pruritic actions of histamine are likely to be working on histamine receptors located on nerve terminals (25, 27). Indeed, we find that a subset of DRG sensory neurons responds to 4-methyl histamine (4-MH), a selective H4R agonist (Fig. 4C and *SI Appendix, Fig. S7A*), and that these responses are blocked by JNJ-777120 (*SI Appendix, Fig. S7B*). Taken together, these data suggest that H4R antagonists may reduce scratching in *Tmem79*<sup>-/-</sup> mice via their action

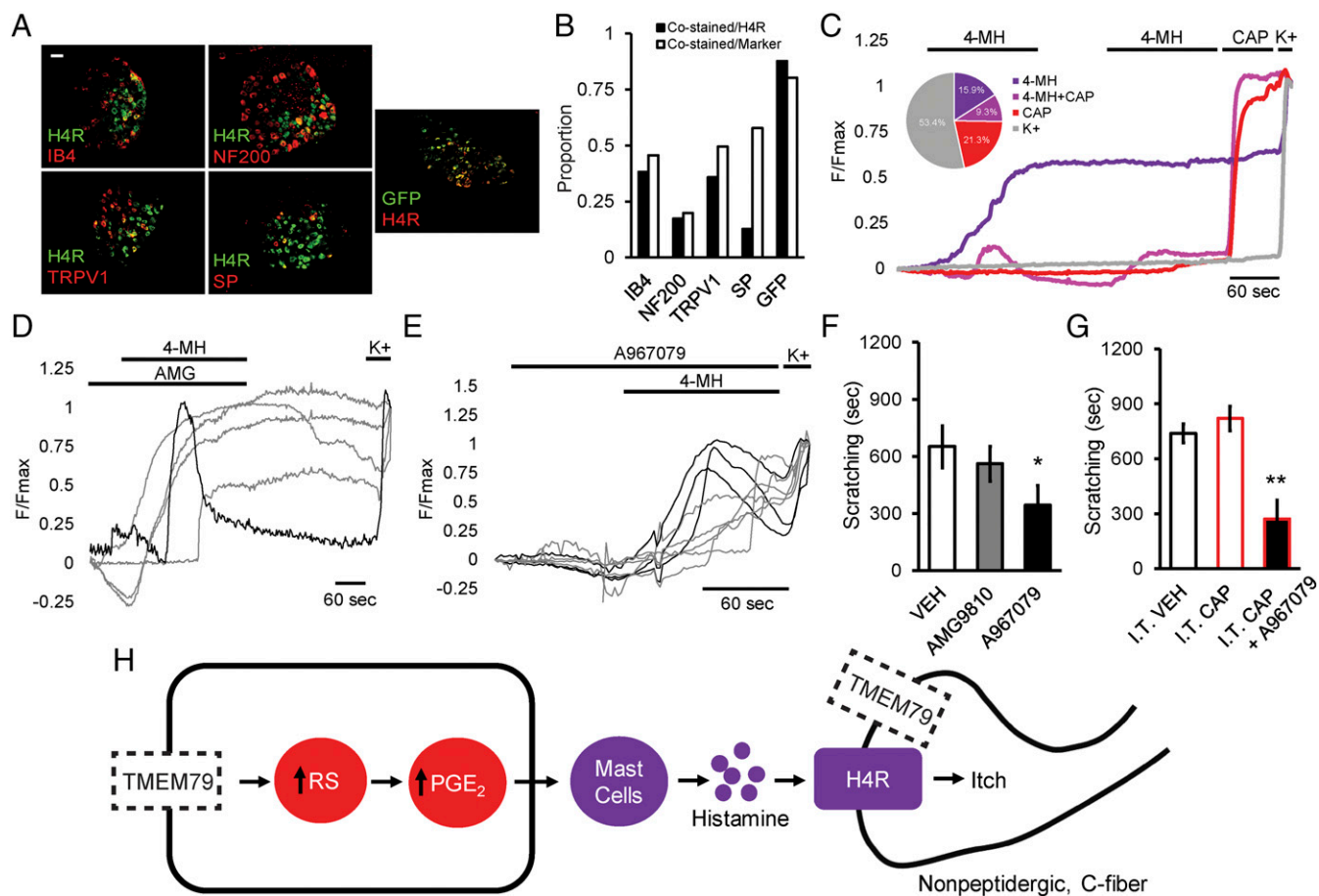
on nerve terminals, as previously shown in models of induced pruritus (29).

Last, we sought to determine the major sensory neural pathway that transduces these histaminergic signals. Since TRPV1 (25, 27, 30–32) and TRPA1 (33) have been implicated in the transmission of itch signals, we asked if either of these channels is required in this model of AD. To this end, we ablated central projections of TRPV1<sup>+</sup> neurons [which encompasses most TRPA1<sup>+</sup> fibers (34–36)] using intrathecal administration of capsaicin (I.T. CAP). Although it severely attenuated TRPV1- and TRPA1-mediated nocifensive behaviors (*SI Appendix, Fig. S8 F–H*), I.T. CAP administration had no effect on *Tmem79*<sup>-/-</sup> scratching (Fig. 4G). Consistent with a TRPV1-independent mechanism, we found that H4R<sup>+</sup> neurons are mostly nonpeptidergic with only partial (<40%) coexpression of TRPV1 and that a majority (>85%) were also positive for *Tmem79* (Fig. 4 A and B). Furthermore, we found that sensory neurons that respond to 4-MH are capsaicin insensitive (Fig. 4C). Additionally, responses to 4-MH persist in the presence of the selective TRPV1 or TRPA1 antagonists AMG9810 (AMG) or A967079, respectively (Fig. 4 D and E and *SI Appendix, Fig. S7 H–J*), despite their dependence on extracellular calcium (*SI Appendix, Fig. S7 C–G and J*).

Consistent with these observations, we found that administration of a TRPV1 antagonist (AMG9810, 30 mg/kg, i.p.) to *Tmem79*<sup>-/-</sup> mice did not diminish scratching (Fig. 4F) despite blocking the nocifensive behaviors elicited by intraplantar injection of capsaicin or elevated surface temperatures (*SI Appendix, Fig. S8 C and D*). Administration of a TRPA1 antagonist (A967079, 50 mg/kg, i.p.) produced a partial reduction in scratching (Fig. 4F), which also persisted following I.T. CAP treatment (Fig. 4G). This reduction following A967079 administration was not observed in *Tmem79*<sup>-/-</sup>; *Trpa1*<sup>-/-</sup> double-knockout animals, which were indistinguishable from *Tmem79*<sup>-/-</sup> mice (*SI Appendix, Fig. S8E*), ruling out an off-target effect. These results suggest that TRPA1 contributes in some manner to the itch phenotype, which is revealed by acute, on-target drug action, but is masked by compensation in the unconditional gene knockout. Future experiments will clarify if TRPA1 contribution arises from peripheral terminals of TRPV1<sup>+</sup>/TRPA1<sup>+</sup> neurons, TRPV1<sup>-</sup>/TRPA1<sup>+</sup> neurons, or nonneuronal TRPA1.

These data support a model of itch transmission in *Tmem79*<sup>-/-</sup> mice that does not depend on canonical TRPV1<sup>+</sup>/TRPA1<sup>+</sup> central projections (30), but instead involves nonpeptidergic, TRPV1<sup>-</sup>/TRPA1<sup>-</sup>, H4R<sup>+</sup> C fibers. The high coexpression of *Tmem79* on H4R<sup>+</sup> neurons may help explain the contribution of neuronal *Tmem79* to scratching behavior (Fig. 1E), although the precise mechanism remains unclear. The observed partial contribution of TRPA1 could derive from its activation by reactive electrophiles on peripheral nociceptor terminals with consequent localized release of proinflammatory mediators and vasoactive substances (37). Expression and activation of TRPA1 on keratinocytes has been suggested to mediate the release of interleukins, mast cell degranulation, and the development of allergic dermatitis (38), representing another potential mechanism whereby this channel contributes to *Tmem79*<sup>-/-</sup>-associated itch.

*Tmem79*-deficient mice provide a robust and compelling model of AD. Our results demonstrate that the main somatosensory component of AD, namely itch, is mediated through a keratinocyte-, PGE<sub>2</sub>/EP3-, mast cell-, and histamine-dependent signaling pathway (Fig. 4H). While the biochemical function of TMEM79 remains uncertain, our findings lead us to propose that it plays a role in scavenging reactive electrophiles or other deleterious products of oxidative stress that, when left unchecked, foster a proinflammatory state resulting in the observed phenotypes. Our genetic models also suggest that *Tmem79* expressed in keratinocytes affords the bulk of this proposed protective function, although expression in sensory nerve fibers contributes as well. Indeed, this is consistent with a model in which TMEM79 neutralizes freely diffusible endogenous irritants in a paracrine manner,



**Fig. 4.** H4R receptor-mediated calcium influx in DRG sensory neurons and *Tmem79*<sup>-/-</sup> histaminergic itch is TRPV1 independent. (A) Representative micrographs of fluorescent colabeling of 12- $\mu$ m DRG sections from adult wild-type (Left and Middle) or *Tmem79*-KI (Right) mice. Sections were stained for immunoreactivity to H4R or GFP (green) and binding to IB4 or for immunoreactivity to NF200, TRPV1, SP, or H4R as indicated (red). (Scale bar: 50  $\mu$ m.) (B) Quantification of colabeling from micrographs depicted in A. Colabeling is shown as a proportion of total H4R<sup>+</sup> and marker<sup>+</sup> neurons. Proportions were calculated from counts taken from at least eight micrographs. (C–E) Normalized  $F_{340}/F_{380}$  calcium imaging traces from adult DRG neurons. (C) Averaged responses to the application of 500  $\mu$ m 4-MH or 1  $\mu$ m capsaicin (CAP). Neurons were identified at the conclusion of each experiment by a positive response to elevated extracellular potassium (150 mM KCl, K<sup>+</sup>). Each trace represents a distinct subpopulation of responders: 4-MH (purple), 4-MH + CAP (magenta), CAP (red), or K<sup>+</sup> only (gray). (Inset) The pie chart shows the percentage of each subpopulation from seven independent experiments (4-MH, 77/483, 15.9%; 4-MH+CAP, 45/483, 9.3%; CAP, 103/483, 21.3%; K<sup>+</sup>, 258/483, 53.4%). (Scale bar: 60 s.) (D and E) Responses to the application of 500  $\mu$ m 4-MH and 10  $\mu$ m AMG (D) or 1 mM A967079 (E). Neurons were identified at the conclusion of each experiment by a positive response to elevated extracellular potassium (150 mM KCl, K<sup>+</sup>). Each trace represents a distinct responder from a single experiment. (Scale bar: 60 s.) (F) Quantified scratching behavior of adult *Tmem79*<sup>-/-</sup> mice following i.p. administration of vehicle (VEH), 30 mg/kg AMG9810, or 50 mg/kg A967079. Data shown are the mean number of seconds  $\pm$  SEM of scratching from multiple mice in each treatment group ( $n = 9$  vehicle-treated mice, 4 AMG9810-treated mice, 21 A967079-treated mice). \* $P < 0.05$ , one-tailed Holm  $t$  test. (G) Quantified scratching behavior of adult *Tmem79*<sup>-/-</sup> mice 1–14 d after intrathecal administration of vehicle (i.t. VEH) or 10  $\mu$ g capsaicin (i.t. CAP) and i.p. administration of 50 mg/kg A967079 (i.t. CAP + A967079). Data shown are the mean seconds of scratching  $\pm$  SEM from multiple mice in each treatment group ( $n = 7$  intrathecal vehicle-treated mice, 7 intrathecal capsaicin-treated mice, 4 intrathecal capsaicin + A967079-treated mice). \*\* $P < 0.01$ , one-tailed Holm  $t$  test. (H) Proposed model: Loss of keratinocytic *Tmem79* results in the accumulation of reactive species (RS) stimulating the production of PGE<sub>2</sub>. PGE<sub>2</sub> from keratinocytes serves to attract mast cells to the dermis of *Tmem79*<sup>-/-</sup> mice through activation of EP3. Mast cells in the dermis degranulate, releasing histamine and activating H4R on nonpeptidergic C-fibers that are TRPV1<sup>-</sup> and presumably have lost *Tmem79*.

irrespective of their cellular origin. In any case, *Tmem79*<sup>-/-</sup> mice represent a tractable and robust model for studying mechanisms of AD and itch and for evaluating possible treatments. In this regard, we show that interfering with PGE<sub>2</sub> synthesis/signaling or histaminergic signaling through H4 receptors, in addition to TRPA1 antagonism, are efficacious in treating the debilitating pruritic component of AD. More broadly, our work suggests that enhancing the activity of glutathione transferases or other mechanisms to reduce oxidative stress may be useful strategies for protecting against the development of AD and other inflammatory pain signals.

## Materials and Methods

**Animal Use, Husbandry, and Genotyping.** Mice were bred and housed in accordance with University of California, San Francisco (UCSF) Institutional Animal Care guidelines. Two to five mice were housed together on a 12-h

light/dark schedule with constant access to food and water. *Tmem79*<sup>-/-</sup> mice were generated by ES electroporation and homologous recombination of a pBS targeting vector containing the *Tmem79* gene lacking exon 2 with the start codon. The loss of exon 2 in *Tmem79*<sup>-/-</sup> mice was confirmed using the following primers: *Tmem79*<sup>-/-</sup> forward: 5'-AGC CTC CCA TTC CAA AGC-3' and reverse: 5'-AGT CGT GCT GCT TCA TGT G-3'. *Tmem79*-KI mice were generated by Ingenious Targeting Laboratory using ES electroporation and homologous recombination of a targeting vector containing the *Tmem79* coding sequence fused to eGFP via a 2A peptide. The vector also features and an inverted floxed (Lox71 and Lox66) tandem dimer tomato (TdT) that is knocked in to replace the *Tmem79* coding sequence at the start codon following Cre recombination. Mismatched lox sequences prohibit further recombination of the locus. The genotype of *Tmem79*-KI mice was confirmed using the following primers: *Tmem79*-KI forward: 5'-CTG ATA TAC TGG TTG ACC TTT GCT-3' and reverse: 5'-CCA GGC CTA CAA CTG TTC CA-3'. *Tmem79*-KI mice were bred to Tg(KRT14-cre)1Amc/J (K14-Cre; strain 004782; Jackson

Laboratory) or Peripherin-Cre (Prph-Cre) mice (39) to produce *Tmem79*<sup>K14-Cre</sup>, *Tmem79*<sup>K14-Cre</sup>;Prph-Cre, or *Tmem79*<sup>K14-Cre</sup>;Prph-Cre conditional-knockin mice. Cre alleles were detected and differentiated using the following primers: K14-Cre forward: 5'-TTC CTC AGG AGT GTC TTC GC-3' and reverse: 5'-GTC CAT GTC CTT CCT GAA GC-3'; Prph-Cre forward: 5'-TGA AGT CAG GCC AGT GGG AG-3' and reverse: 5'-CCT GAT CCT GGC AAT TTC GGC T-3'.

**RT-PCR from Whole Tissues.** Adult mice were euthanized by CO<sub>2</sub> asphyxiation, and tissues were dissected into TRIzol reagent (Invitrogen) and were kept on ice until processed. Tissues were homogenized using a T25 Ultra-Turrax homogenizer (IKA), and RNA was extracted and purified using TRIzol and chloroform extraction according to the recommendations of the TRIzol reagent manufacturer. Following extraction of RNA, the aqueous phase was cleaned using RNA Clean and Concentrator (Zymo), and RNA was eluted into RNase-free water. The quality and concentration of RNA was determined using the Agilent 2100 Bioanalyzer. Samples were used for downstream applications only if the RNA integrity number was greater than 7. Isolated RNA was stored at -80 °C until used for RT-PCR. For each tissue, equivalent RNA content was used to carry out first-strand cDNA synthesis using SuperScript III (Thermo Fisher) according to the manufacturer's recommendations. First-strand cDNA synthesis reactions for each tissue were carried out in triplicate and were combined. Following cDNA synthesis, RT-PCR reactions were run in triplicate using SYBR Green dye (Applied Biosciences) and Stratagene Mx3005P. Average cycle threshold (Ct) values were calculated, and relative expression of *Tmem79* was determined using *Rpl19* as a control. Primers were determined to have similar efficiencies across a series of seven twofold dilutions. Melt curves were assessed to ensure amplification of single product. Primers used were *Tmem79* forward: 5'-CTT GGT TTA CAC CCT TCG CT-3' and reverse 5'-GTA TAT CAG CCG AGA TAT GGC A-3'; *Rpl19* forward: 5'-GAT TGA CCG CCA TAT GTA TCA CAG-3' and reverse: 5'-ACA GAG TCT TGA TGA TCT CTT CCT-3'.

**RT-PCR from Cultured Rat Sensory Neurons.** P0 rats were euthanized by cooling and decapitation. TG were dissected from rats and incubated in 1 mg/mL collagenase (Worthington Biochemical) and then in 0.05% trypsin-EDTA with phenol red (25300054; Thermo Fisher) for 10 min each. TG were transferred to complete DMEM [DMEM (Thermo Fisher) with 10% FBS and 1× penicillin/streptomycin antibiotic] and then were carefully passed through the tips of P1000 and P200 Pipetman pipettes (Gilman) to mechanically triturate and dissociate the ganglia. Dissociated neurons were plated on coverslips overnight (O/N) at 37 °C and 5% CO<sub>2</sub>. After O/N incubation, neurons were incubated in SIN-1 (BML-CN245-0020; Enzo Life Science) in complete DMEM for 16 h. Following incubation, neurons were collected in an excess of TRIzol, and RT-PCR was carried out as described above. Primers used were *Tmem79* forward: 5'-CTT GGT TTA CAC CCT TCG CT-3' and reverse: 5'-GTA TAT CAG CCG AGA TAT GGC A-3'; *Trpv1* forward: 5'-GGA TGA GGT GAA CTG GACTAC-3' and reverse: 5'-GTG CTA TGC CTA TCT CGA GTG-3'; *Nqo1* forward: 5'-GAG CCC GGA TAT TGT AGC TG-3' and reverse: 5'-CTT CTT ATT CTG GAA AGG ACC C-3'; *Rpl19* forward: 5'-AGC CTG TGA CTG TCC ATT CC-3' and reverse: 5'-GGC AGT ACC CTT CCT CTT CC-3'.

**RNA Sequencing.** Sequencing libraries were prepared as described (40). Briefly, cDNA libraries were prepared from poly(A)<sup>+</sup> RNA using the Illumina mRNA-Seq Sample Prep Kit (RS-100-0801) according to the manufacturer's instructions. Libraries were then sequenced on the Illumina Genome Analyzer II using two 36-cycle sequencing kits (FC-104-3002) to read 80 nt of sequence from a single end of each insert, by standard protocols. Between 2.4 million and 12.5 million inserts were sequenced for each sample. Sequences were aligned to the mouse reference genome mm9 using TopHat v 0.7.0 (Trapnell Laboratory, Center for Bioinformatics and Computational Biology, University of Maryland) and Bowtie v0.9.7 (Trapnell Laboratory, Center for Bioinformatics and Computational Biology, University of Maryland), and the number of sequences aligned to *Tmem79* was determined for each neuronal tissue type using Cufflink (Trapnell Laboratory, Center for Bioinformatics and Computational Biology, University of Maryland).

**Tissue Dissection and Cryosectioning.** Adult mice were deeply anesthetized with pentobarbital and then were perfused transcardially with 10 mL of PBS followed by 10 mL of 10% neutral buffered formalin (NBF). Tissues were dissected, postfixed in 10% NBF at 4 °C O/N, and then cryoprotected in PBS with 30% (wt/vol) sucrose O/N at 4 °C. Just before sectioning, tissue samples were embedded in OCT compound (VWR) at -20 °C. DRGs and all other tissues were sectioned with a Leica CM3050 S cryostat at 12 μm and 20 μm, respectively, and then were thaw-captured on Diamond White Glass slides

(Globe Scientific). Slides were stored at -20 °C until use. Slides were used within 2 wk of processing to produce optimal signals.

**In Situ Hybridization of Tissue Sections.** Tissue sections were processed using the ViewRNA ISH Tissue 1-plex or 2-plex Assay Kits (Affymetrix) following the manufacturer's recommendations including frozen tissue modifications. Affymetrix was commissioned to design a type 1 probe set to mouse *Tmem79* (NM\_024246.5) and a type 6 probe set to mouse *Trpa1* (NM\_177781.4). For two-plex processing, endogenous alkaline phosphatase was inactivated by incubation in H<sub>2</sub>O with 0.1 M HCl and 300 mM NaCl. For one- and two-plex protocols, the H&E counterstaining procedure was omitted. Images were acquired with a Leica DMRB microscope and a DFC500 digital camera using Leica Application Suite v3.5.0 and then were further analyzed using ImageJ software (NIH).

**Immunohistochemical Staining of Tissue Sections.** Slides containing sections of DRG or dorsal skin were blocked for 1 h at RT in PBS with 0.1% (vol/vol) Triton X-100 (Sigma) and 10% normal goat serum (NGS). Slides were briefly rinsed once with PBS with 0.1% Triton X-100 and 2.5% NGS and were incubated O/N with primary antibody in PBS with 0.1% Triton X-100 and 2.5% NGS at 4 °C. Following O/N incubation, slides were washed three times with vigorous agitation (2 min each washing) in fresh PBS, rinsed once with PBS with 0.1% Triton X-100, and were incubated in secondary antibody for 2 h in PBS with 0.1% Triton X-100 at RT in the dark. Following incubation, slides were washed three times with vigorous agitation (2 min each washing) in fresh PBS and were mounted in ProLong Gold antifade reagent with DAPI (Life Technologies) under a coverslip. Slides were stored covered at RT until used for imaging. Epifluorescent images were acquired with a Leica DMRB microscope mounted with a DFC500 digital camera using Leica Application Suite v3.5.0 or with an Infinity 3-3UR Monochrome CCD digital camera using Infinity Analyze software. Confocal images were acquired with a Nikon Ti inverted microscope equipped with an Andor Borealis CSU-W1 spinning-disk confocal system, an Andor four-line laser launch (100 mW at 405, 561, and 640 nm; 150 mW at 488 nm), a Lumencor Spectra X six-channel LED illuminator, a Sutter excitation and emission filter wheel, and an Andor DU-888 EMCCD camera using Micro-Manager software (Open Imaging). Following capture, all images were further analyzed using ImageJ software. The following primary antibodies were used: chicken anti-GFP (1:500; A10262; Thermo Fisher), rabbit anti-RFP (1:500; 600-401-379 which recognizes tdT; Rockland), rat anti-CD45 (1:500; 103101; BioLegend), rabbit anti-H1R (1:100; AHR-001; Alomone Labs), rabbit anti-H4R (1:1,000; Biorbyt), guinea pig anti-TRPV1 (1:1,000; D.J. laboratory, UCSF), guinea pig anti-substance P (1:5,000; gift of John Maggio, University of Cincinnati, Cincinnati), mouse anti-NF200 (1:10,000; N5389; Sigma), rabbit anti-CGRP (1:10,000; T-4031.400; Peninsula Labs), rabbit anti-peripherin (1:1,000; SAB4502419; Sigma), and rabbit anti-TH (1:5,000; EP1532Y; Abcam). Fluorophore-conjugated secondary antibodies raised in goat against mouse, rat, rabbit, chicken, or guinea pig were used, as appropriate (1:1,000; Alexa-Fluor 488, 568, or 596; Life Technologies). To identify IB4-binding cells, biotinylated IB4 (1:1,000; B-1205; Vector Labs) and fluorophore-conjugated streptavidin (1:1,000; Alexa-Fluor 488; Life Technologies) were used in place of primary and secondary antibodies. Mouse anti-K14 primary antibody (ab7800; Abcam) was pre-conjugated to Alexa-Fluor 594 using the APEX Alexa-Fluor 594 Antibody Labeling Kit according to the manufacturer's recommendations and was used at 1:100.

**Immunohistochemical Staining of Transiently Transfected HEK293T Cells.** HEK293T cells were grown to confluence in a 10-cm plate and were split 1:15 into six wells of a six-well plate containing poly-L-lysine (Sigma)-coated 8-mm round coverslips. Cells were allowed to adhere for 2 h at 37 °C and 5% CO<sub>2</sub> and then were transfected as directed with Lipofectamine 3000 (Thermo Fisher) and pcDNA3.1<sup>+</sup> vectors containing the coding sequence for *Tmem79* linked to HA via Gly-Ala on either the N or C terminus (HA coding sequence: 5'-TACCATACGATGTTCAGATTACGCT-3'). Following transfection, cells were allowed to incubate O/N at 37 °C and 5% CO<sub>2</sub>; then coverslips were carefully transferred to slides. To prepare the FIX<sup>-</sup> condition, slides were washed once with PBS (note: all washes were conducted using gentle pipetting) and then were blocked with PBS for 1 h with 0.1% Triton-X 100 and 10% NGS at RT. Following blocking, slides were rinsed once with PBS, incubated in primary antibody (rabbit anti-HA; 1:500; H6908; Sigma) for 1 h in PBS with 0.1% Triton-X 100 and 2.5% NGS at RT and then were rinsed three times with PBS. After rinsing, slides were incubated with a secondary antibody (1:1,000; Alexa-Fluor 594; Life Technologies) for 30 min in PBS with 0.1% Triton-X 100 at RT, were rinsed three times with PBS, and were mounted in ProLong Gold antifade reagent with DAPI (Life Technologies) under a larger coverslip. To prepare the FIX<sup>+</sup> condition, slides were fixed in PBS with 10% NBF for 10 min and were

rinsed twice with PBS before undergoing the FIX<sup>+</sup> steps outlined above, concluding with the placement of a larger coverslip. Slides were stored in the dark at RT until used for imaging. Images were acquired using a Nikon Ti inverted microscope equipped with an Andor Borealis CSU-W1 spinning-disk confocal system, an Andor four-line laser launch (100 mW at 405, 561, and 640 nm; 150 mW at 488 nm), a Lumencor Spectra-X six-channel LED illuminator, a Sutter excitation and emission filter wheel, and an Andor DU-888 EM CCD camera using Micromanager software (Open Imaging) and then were further processed using ImageJ software.

**Histological and Cytological Staining.** Dorsal hairy skin or lung tissues were dissected and prepared as noted above and then were stained with Toluidine blue O or H&E. For Toluidine blue O staining, a stock solution was prepared by dissolving 1 g of Toluidine blue O (Sigma) into 70% ethanol. Fresh Toluidine blue working solution was prepared by diluting the stock solution 1:10 into 1% sodium chloride in distilled water. The working solution was adjusted to pH 2.4. Slides were rehydrated in tap water (40 dips) and then were stained for 2 min in working solution. Slides were rinsed in tap water (30 dips) and were cleared in 100% ethanol (30 dips) followed by xylene (6 min). For histology, slides were mounted in DPX Mountant (Sigma) under a coverslip and were allowed to set O/N at RT in the dark until used for imaging. For H&E staining, slides were rehydrated in tap water (40 dips) and were incubated twice (2 min each incubation) in hematoxylin solution (Gill no. 2; Sigma). Slides were blued in tap water (40 dips), dipped twice in eosin (Eosin Y Solution, alcoholic, with phloxine; Sigma). Following eosin staining, slides were rinsed in tap water (30 dips) and were cleared in 100% ethanol (30 dips) followed by xylene (30 dips). For histology, slides were mounted in DPX Mountant (Sigma) under a coverslip and were allowed to set O/N at RT in the dark. Slides were stored covered at RT until used for imaging. Images were acquired with a Nikon Eclipse E600 and an Infinity 3-6URC Color CCD digital camera using Infinity Analyze software. Mast cells in the dermis were counted in each unique field captured from sections of dorsal hairy skin. Degranulated mast cells were counted as a percentage of total (degranulated + nondegranulated) mast cells in each unique field captured from sections of dorsal hairy skin following treatment with vehicle or drug. Dermal thickness was calculated in each unique field as the average of 15 measurements made from deepest portion of the stratum basale to the most superficial portion of the hypodermis as identified by adipocytes.

**Cell Preparation from Tissues and Stimulation for Intracellular Cytokine Staining.** Single-cell suspensions of skin draining lymph nodes were mechanically dissociated through a 100- $\mu$ m filter, and dorsal skin (2.5 cm<sup>2</sup>) was processed as previously described (41). Single cells were washed in tissue-culture medium and filtered. Cells were counted using an automated cell counter (NucleoCounter NC-200; ChemoMetec) to determine the absolute number of specific cell populations per unit area. Single cells (2–3  $\times$  10<sup>6</sup>) were stained for flow cytometry or cultured for intracellular cytokine staining using a phorbol12-myristate13-acetate and ionomycin cell-stimulation mixture (Tonbo Biosciences).

**Flow Cytometry.** Single-cell suspensions were pelleted, and cells were washed and stained with Ghost Viability dye (Tonbo Biosciences) in PBS. Following a wash in PBS, cells were stained for surface markers in PBS containing 2% FCS. For intracellular staining, cells were fixed and permeabilized with a FoxP3 buffer set (eBioscience). Samples were run on a Fortessa analyzer (BD Biosciences) in the UCSF Flow Cytometry Core and were collected using FACS Diva software (BD Biosciences). Flow cytometry data were analyzed using FlowJo software (TreeStar). Fluorophore-conjugated antibodies specific for mouse surface and intracellular antigens were purchased from eBioscience, BD Biosciences, and BioLegend. The following antibodies and clones were used: anti-Ly6G (1A8), anti-CD11b (M1/70), anti-CD11c (HL3), anti-MHC class II (M5/114.15.2), anti-Ly6C (HK1.4), anti-IL-17 (SHLR17), anti-IFN- $\gamma$ XMG1.2), anti-CD3 (145-2C11), anti-CD4 (RM4-5), anti-CD8 (SK1), anti-TCR $\gamma\delta$ ), anti-CD45 (30-F11), Siglec-F (E50-2440), anti-CD117 (2B8), anti-Fc $\epsilon$ R1 (MAR-1), and anti-FoxP3 (FJK-16s).

**Protein Sequence Alignment and Topology Prediction.** The TMEM79 sequence was obtained from the National Center for Biotechnology Information. Blastp was used to query the TMEM79 sequence against reference proteins in the *Mus musculus* protein database. Blastp query of TMEM79 revealed a conserved domain found in Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG, pfam01124). Protein sequences encoded by mouse MAPEG family members (*Mgst1*, *Pges1*, *Mgst3*, *Flap*, *Mgst2*, and *Ltc4s*) were aligned to TMEM79 using ClustalW2 multiple sequence-alignment software. TMEM79 protein topology was predicted using TMpred software.

**Heterologous Expression of Tmem79 and Mutant Generation.** For reactive species assays, *Tmem79* was transiently expressed in HEK293T cells using

Lipofectamine 3000 (Thermo Fisher). A QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent) was used to generate amino acid substitutions in the coding sequence of *Tmem79* in pcDNA3.1<sup>+</sup>.

**DCF Assay in HEK293T Cells.** HEK293T cells were grown to confluence in a 10-cm plate and then were split 1:15 into six wells of a six-well plate. Cells were allowed to adhere for 2 h and then were transfected with 5  $\mu$ g of plasmid (2.5  $\mu$ g of mCherry and 2.5  $\mu$ g of either empty pcDNA3.1<sup>+</sup> or pcDNA3.1<sup>+</sup> containing the coding sequence for *Tmem79* or *Tmem79* amino acid substitution mutants). Cells were incubated for >24 h in transfection medium at 37 °C and 5% CO<sub>2</sub>. Following incubation, cells were detached using 0.05% trypsin-EDTA with phenol red and gentle pipetting, resuspended in complete DMEM, and plated in a poly-L-lysine-coated 96-well plate (354640; Corning). Cells were allowed to adhere O/N at 37 °C and 5% CO<sub>2</sub> and then were washed twice in Ringer's solution (280 mM NaCl, 10 mM KCl, 4 mM CaCl<sub>2</sub>·0.2H<sub>2</sub>O, 4 mM MgCl<sub>2</sub>·0.6H<sub>2</sub>O, 20 mM D-glucose, 20 mM Hepes, pH 7.4) and incubated in 5  $\mu$ M DCF (cell-permeant 2,7-dichlorodihydrofluorescein diacetate or H2DCFDA; D399; Thermo Fisher) for 10 min at 37 °C and 5% CO<sub>2</sub>. Cells were washed twice in Ringer's solution and then were introduced to oxidant in Ringer's solution. Immediately following incubation of cells in oxidant, plates were read (excitation at 495 nm/emission at 529 nm) at 37 °C using a BioTek H4 plate reader (Thermo Fisher). Fluorescence readings were collected from wells starting at time 0 and continuing every 10 min for 1 h. Oxidants were hydrogen peroxide (516813; Sigma), tert-butyl hydroperoxide (416665; Sigma), sodium nitroprusside (71780; Sigma), and SIN-1 (BML-CN245-0020; Enzo Life Science). *N*-acetyl cysteine (A7250; Sigma) was added during 10 min DCF loading.

**Acute Keratinocyte Dissociation and ELISA for Prostaglandins.** Adult mice were euthanized by CO<sub>2</sub> asphyxiation and decapitation. Hair was cut from mice dorsal and ventral surfaces using hair trimmers (Oster). A 3  $\times$  2 cm area of trimmed skin was dissected, and submucosa was removed using blunt dissection. Skins were incubated floating on the surface of 0.25% trypsin-EDTA with phenol red (Thermo Fisher) for 1 h at 37 °C and 5% CO<sub>2</sub>. Following incubation, skins were transferred to complete DMEM, and keratinocytes were dissociated into solution by scraping the surface of the segment with the blunted edge of a pair of forceps. Dermis from each skin was discarded, and the remaining solution and particulates were collected. The suspension was shaken vigorously by hand for 30 s to homogenize and dissociate keratinocytes and then was filtered using a 100- $\mu$ m cell strainer (BD). For prostaglandin quantification using ELISA, keratinocytes dissociated from age- and sex-matched wild-type or *Tmem79*<sup>-/-</sup> mice were incubated for an additional 30 min at 37 °C in complete DMEM, sonicated on ice for 30 s (10 s on/10 s off), and spun at 8,000  $\times$  g at 4 °C to pellet debris. Supernatant was collected and kept on ice until processing using ELISA kits for quantification of PGE<sub>2</sub> (514010; Cayman Chemical) or PGH<sub>2</sub> (CEA612Ge; Cloud Clone) according to the manufacturer's guidelines.

**Lipidomics.** Keratinocyte lysates were collected as described above; then cold methanol was added immediately to stabilize lipid mediators. Samples were stored at -80 °C until processing. Lipid mediators and metabolites were extracted through C-18 solid-phase columns and quantified via LC-MS/MS as described previously (42–44). Deuterated internal standards (PGE<sub>2</sub>-d<sub>4</sub>, LTBA-d<sub>4</sub>, 15-HETE-d<sub>8</sub>, LXA<sub>4</sub>-d<sub>5</sub>, DHA-d<sub>5</sub>, and AA-d<sub>8</sub>) were added to samples to calculate extraction recovery. The LC-MS/MS system consisted of an Agilent 1200 Series HPLC, Luna C18 column (Phenomenex), and AB Sciex QTRAP 4500 mass spectrometer. Analysis was carried out in negative ion mode, and lipid mediators were quantitated using the scheduled multiple reaction monitoring (MRM) mode using multiple specific transition ions.

**Dichlorofluorescein Assay in Dissociated Keratinocytes.** Following dissociation and filtration using a cell strainer as described above, keratinocytes were pelleted at 425  $\times$  g and then were washed once in complete DMEM and once in Ringer's solution. Following the washes, keratinocytes were resuspended in Ringer's solution and were incubated in 5  $\mu$ M dichlorofluorescein (DCF) for 10 min at 37 °C and 5% CO<sub>2</sub>. Keratinocytes were pelleted at 425  $\times$  g, resuspended in 1.5 mL Ringer's solution, and incubated in SIN-1. Immediately following the incubation of cells in SIN-1, plates were read (excitation at 495 nm/emission at 529 nm) at 37 °C using a BioTek H4 plate reader (Thermo Fisher). Fluorescence readings were collected from wells starting at time 0 and continuing every 1 min for 1 h.

**Primary Cultures of Adult DRG Neurons and Calcium Imaging.** Glass coverslips (8 mm; Electron Microscopy Sciences) were washed briefly with 70% ethanol, dried with a Kimwipe (Kimberly-Clark), and were coated with 40  $\mu$ L of 10  $\mu$ g/mL



laminin (23017-015; Thermo Fisher) in 0.01% poly-L-lysine (P4707; Sigma) and incubated for >1 h at 37 °C and 5% CO<sub>2</sub>. Coverslips were then rinsed once with complete DMEM. Adult mice were euthanized by CO<sub>2</sub> asphyxiation and decapitation, and vertebral columns were dissected. DRGs from a single mouse were dissected and combined in 1 mL of collagenase I (1 mg/mL in Ringer's solution) with DNase I (150 K/mL; DN25; Sigma) on ice. DRGs in 1 mL collagenase were incubated for 20 min in a 37 °C water bath; then supernatant was removed without disturbing the loose DRG pellet. DRGs were resuspended without trituration in 1 mL of 0.25% trypsin-EDTA with phenol red (Thermo Fisher) with DNase I (150 K/mL) and were incubated for 20 min in a 37 °C water bath. Supernatant was removed without disturbing the loose DRG pellet. DRGs were resuspended in 1 mL of complete DMEM (preheated to 37 °C) with DNase I (150 K/mL) and were triturated 30× each with a P1000 and P200 Pipetman to dissociate neurons into the solution. The homogenate was gently loaded onto the surface of 3 mL of 15% Percoll (vol/vol) Ringer's solution in a 15-mL Falcon tube (Corning) and was spun at 1,300 × g at 4 °C for 10 min. The supernatant was removed without disturbing the pellet. The pellet was completely resuspended in 300 μL complete DMEM, and 25 μL of suspended neurons were plated onto prepared glass coverslips (one mouse produced 12 coverslips) and were allowed to plate O/N at 37 °C and 5% CO<sub>2</sub>. Following O/N plating, Fura-2 AM working solution [10 μL of 1 mg/mL Fura-2 AM dye (F1201; Life Technologies) and 1 μL of 20% pluronic acid in 1 mL Ringer's solution] was prepared. One to two hours before imaging, 25 μL of Fura-2 AM working solution was added to coverslips; then neurons were allowed to load in the dark at RT. Coverslips containing Fura-2 AM-loaded neurons were placed in a laminar flow system with bath solutions controlled by a ValveBank8 Perfusion System (Automate Scientific) and driven by two synchronized Dynamax peristaltic pumps (Rainin). Sequential excitation of Fura at 340 and 380 nm in neurons was conducted using a Lambda LS Illuminator with a 10-2 Controller (Sutter). Emission signals were captured using an ORCA-ER camera and controller (Hamamatsu) mounted on a Nikon Eclipse TE200 microscope. Data were digitized and analyzed using MetaFluor software (Molecular Devices).

**Statistics and Experimental Design.** Sample sizes for experiments were chosen as the minimum number of independent observations required for statistically significant results, based on previous experience with these assays. No statistical methods were used to predetermine sample size. Numbers of micrographs, replicates, or animals used for each experiment are reported in the figure legends. For behavioral experiments, animals were randomly chosen for experimental cohorts, conditions were compared within the same experimental time course, and experimenters were double-blinded to experimental conditions. Animal genotype was tracked by ear tags, and genotype unblinding occurred after analysis was complete.

Data were analyzed using Prism 6 (GraphPad) or Microsoft Excel software and significance testing with a Student's *t* test, Holm *t* test, or one-way ANOVA with Holm–Sidak's multiple comparisons correction, as noted in the figure legends. Tests were either one- or two-tailed as indicated in the figure legends. The number of experiments and significance are reported in the figure legends. All significance tests were justified as appropriate given

the experimental design and the nature of the comparisons. With our statistical tests, we assume equal variance and normal distributions of data, which are common assumptions made for significance testing within these experimental paradigms as previously published by our group and others.

**Animal Behavior and Pharmacology.** Behavioral experiments were approved by the UCSF Institutional Animal Care and Use Committee and were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (45) and the recommendations of the International Association for the Study of Pain. For scratching assays, mice were allowed to acclimate in clear, ventilated cylindrical chambers for 30 min. Chambers were covered by a clear lid, and opaque barriers were placed between chambers to prohibit any visual interference among mice. Following acclimation, behavior was captured for 30 min using a HDR-CX160 High-Definition Handycam Camcorder (Sony); then scratching behavior was quantified from recordings. Scratching behavior was defined as oral contact, including licking or biting, with the dorsal or ventral hairy skin including upper portions of limbs. To assess the effects of pharmacologic compounds on scratching behavior, mice were i.p. administered drug or vehicle [10% DMSO (vol/vol) and 10% Tween 20 (vol/vol) in sterile normal saline] (doses are indicated in figure legends) by a blinded operator following the 30-min acclimation period. Behavior was captured for 30–60 min following drug or vehicle administration, and scratching behavior was quantified by a blinded observer from recordings. For experiments involving s.c. injection of histamine or chloroquine, mice were administered drug or vehicle by a blinded operator following the 30-min acclimation period, and behavior was captured immediately for 20 min. Scratching behavior in these two assays was defined as forepaw or hindpaw contact at or near the s.c. injection site and was quantified by a blinded observer from recordings. For experiments involving intraplantar injection of capsaicin or mustard oil, mice were administered drug by an operator following the 30-min acclimation period, and behavior was captured immediately for 10 min (capsaicin) or 20 min (mustard oil). Paw licking was quantified by a blinded observer from recordings. For studies of intrathecal capsaicin administration, mice (20–40 g) were anesthetized with 1.5% isoflurane (vol/vol) and injected intrathecally with 5 μL capsaicin (10 μg) or vehicle [10% ethanol (vol/vol) and 10% Tween 80 (vol/vol) in sterile normal saline] using a Hamilton syringe at the level of the pelvic girdle (46). Behavioral assays were performed 1–14 d after capsaicin injection. Anxiety-related behavior was assessed using the elevated plus maze as previously described (47). For the hot plate assay, latency to nocifensive responses (flinching, jumping, or licking) for each mouse was calculated as an average of three trials (60-s maximum and at 10-min intervals) in response to indicated surface temperatures (ThermoBrite; Abbott Molecular).

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Supplementary Information for

Tissue-specific contributions of Tmem79 to atopic dermatitis and mast cell-mediated histaminergic itch

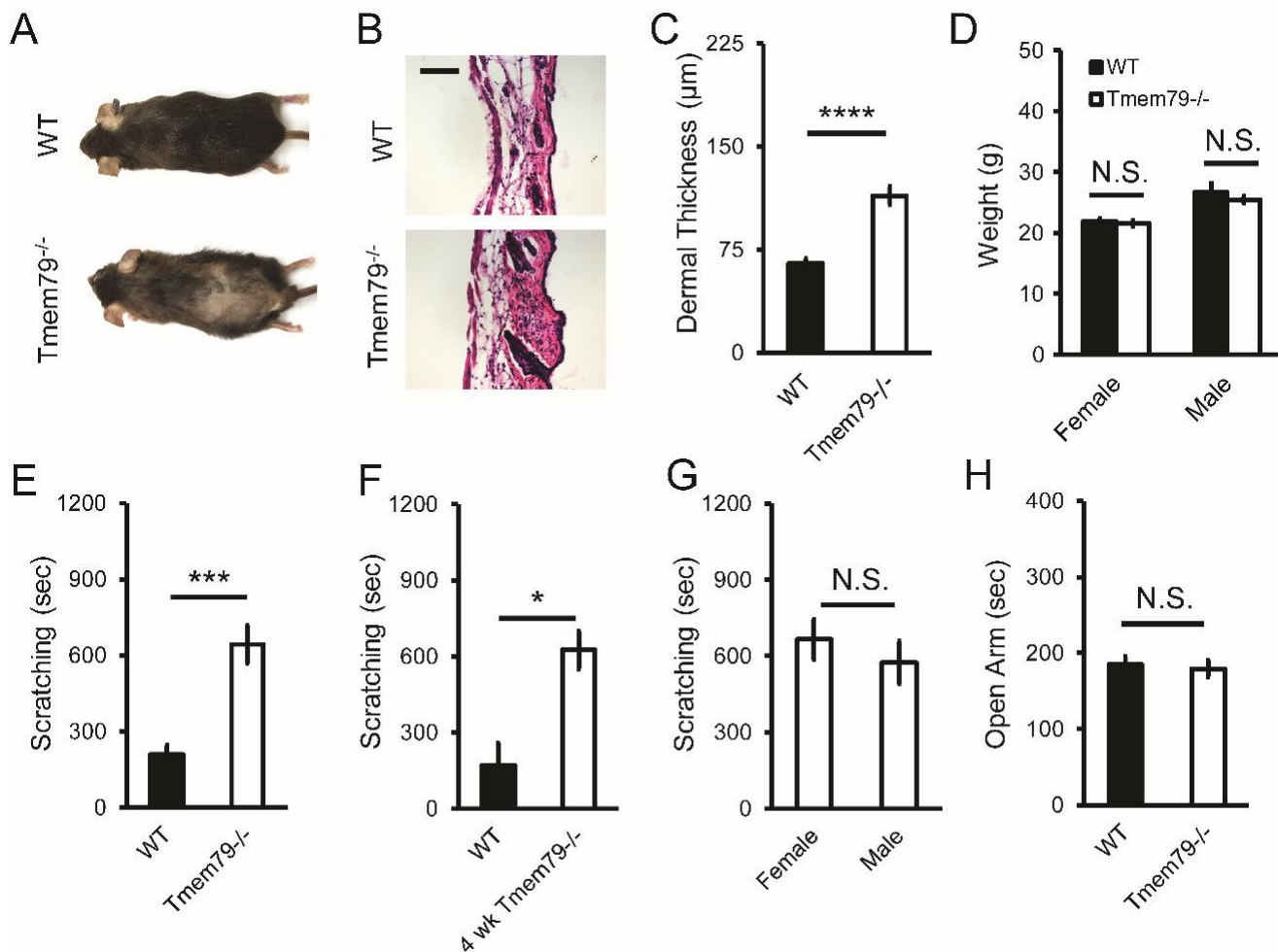
Joshua J. Emrick, Anubhav Mathur, Jessica Wei, Elena O. Gracheva, Karsten Gronert, Michael D. Rosenblum, and David Julius

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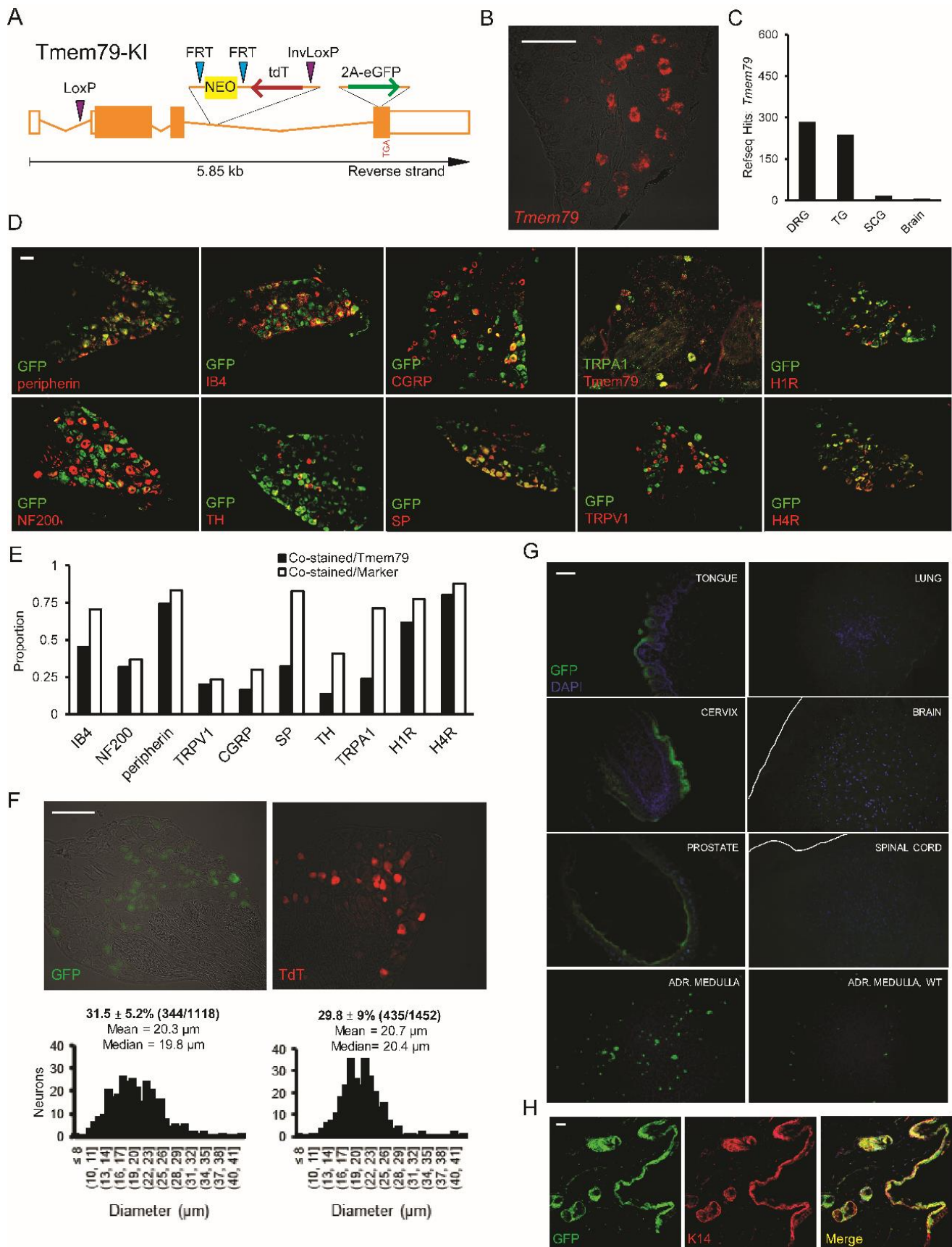
Figs. S1 to S8



**Fig. S1. *Tmem79*<sup>-/-</sup> mice develop atopic dermatitis (AD) and itch.**

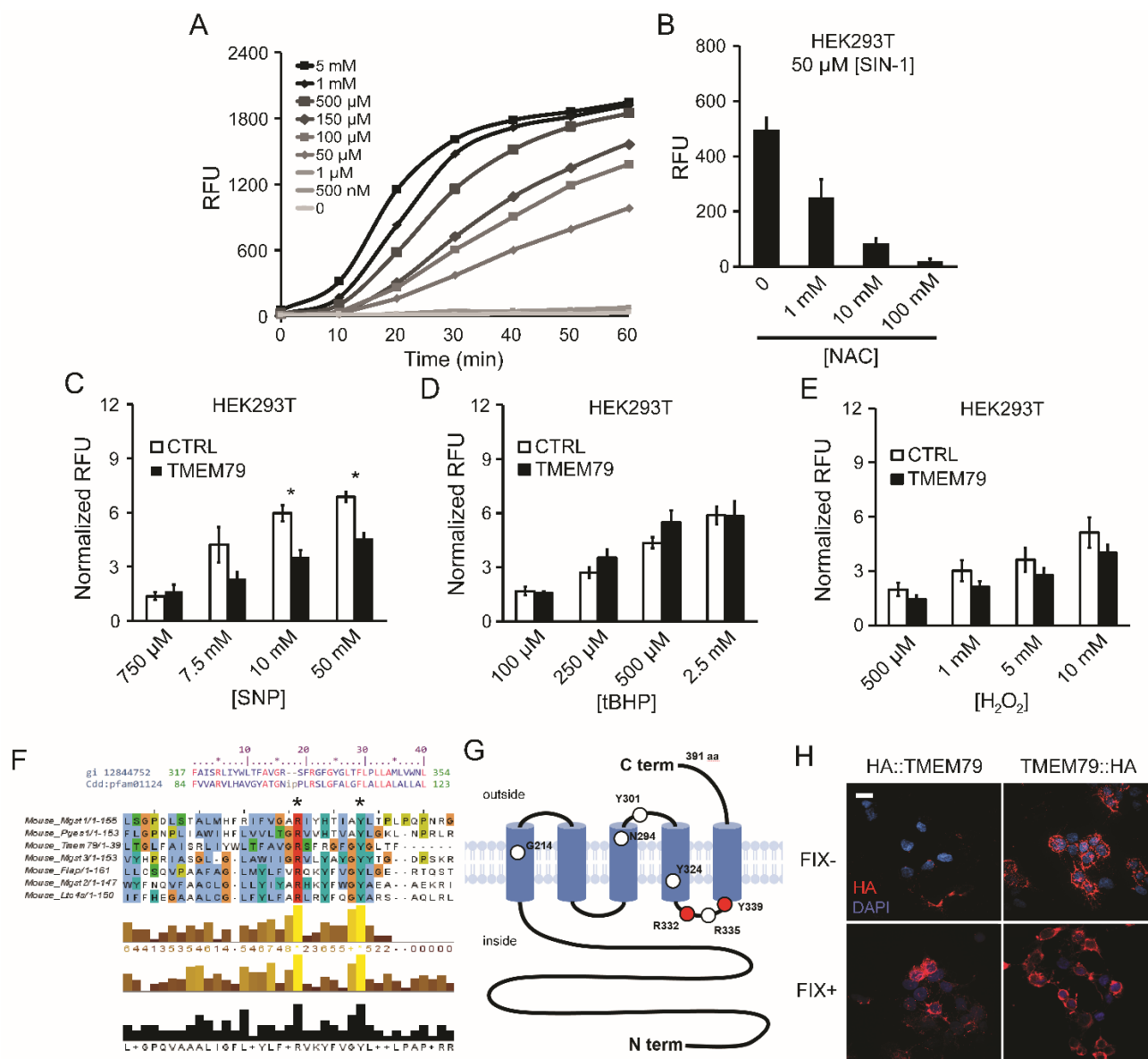
(A) Representative photographs of pelage of age-matched wild-type (WT, top panel) and *Tmem79*<sup>-/-</sup> (bottom panel) mice from an overhead vantage. (B) Representative micrographs of hematoxylin and eosin stained sections of hairy skin (20 μm) dissected from age-matched wild-type (WT, top panel) and *Tmem79*<sup>-/-</sup> (bottom panel) mice. Scale bar represents 100 μm. (C) Quantification of dermal thickness from dermal sections as represented in (B). Data shown are the mean thickness of the dermis of hairy skin sections from age-matched wild-type (WT) and *Tmem79*<sup>-/-</sup> mice ± s.e.m. (n = 13 sections; \*\*\*\*P < 0.0001, Student's t test, two-tailed). (D) Quantification of weight of male and female adult wild-type (WT) and *Tmem79*<sup>-/-</sup> mice. Data shown are the mean weight of mice ± s.e.m. (n = 13 female *Tmem79*<sup>-/-</sup>, 15 female WT, 13 male *Tmem79*<sup>-/-</sup>, 6 male WT; N.S. P > 0.05, Student's t test, two-tailed). (E) Quantified scratching behavior of adult wild-type (WT) and *Tmem79*<sup>-/-</sup> mice. Data shown are the mean seconds of scratching from multiple mice of each genotype ± s.e.m. (n = 6 WT, 10 *Tmem79*<sup>-/-</sup>; \*\*\*P < 0.001, two-tailed). (F) Quantified scratching behavior of 4 week old wild-type (WT) and *Tmem79*<sup>-/-</sup> mice. Data shown are the mean seconds of scratching from multiple mice of each genotype ± s.e.m. (n = 3 WT, 3 *Tmem79*<sup>-/-</sup>; \*P < 0.05, two-tailed). (G) Quantified scratching behavior of adult female and male *Tmem79*<sup>-/-</sup> mice. Data

shown are the mean seconds of scratching from multiple mice of each genotype  $\pm$  s.e.m. ( $n = 13$  female, 10 male;  $P > 0.05$ , Student's  $t$  test, two-tailed). (H) Elevated plus maze assay of adult wild-type (WT) and  $Tmem79^{-/-}$  mice. Data shown are the mean seconds multiple mice of each genotype  $\pm$  s.e.m. occupied the open arm of the plus maze ( $n = 6$  WT, 7  $Tmem79^{-/-}$ ; N.S.  $P > 0.05$ , Student's  $t$  test, two-tailed).



**Fig. S2. *Tmem79* is expressed in epithelial and nervous tissues.**

(A) Schematic depicting targeting construct for the generation of the *Tmem79* reporter/conditional knock-in reporter mouse line (*Tmem79*-KI). The construct results in the expression of *Tmem79*-2A-eGFP under the control of the *Tmem79* promoter. Self-cleavage of *Tmem79*-2A-eGFP produces separate products TMEM79 and eGFP. Following action of Cre recombinase, TdTomato is knocked in to replace *Tmem79*-2A-eGFP. (B) Representative micrograph of *Tmem79* labeled by fluorescent *in situ* hybridization in dorsal root ganglion (12  $\mu$ m section). Scale bar represents 100  $\mu$ m. (C) RNAseq hits for *Tmem79* in dorsal root ganglion (DRG), trigeminal ganglion (TG), superior cervical ganglion (SCG), and brain tissue of wild-type mice. (D) Representative micrographs of fluorescent co-labeling of dorsal root ganglion sections (12  $\mu$ m) from adult *Tmem79*-KI mice. Sections stained for immunoreactivity to GFP (green) and binding to IB4, or immunoreactivity to peripherin, CGRP, H1R, NF200, TH, SP, TRPV1, or H4R as indicated (red). *In situ* hybridization co-labeling for *Trpa1* (TRPA1, green) or *Tmem79* (*Tmem79*, red) also shown. Scale bar represents 50  $\mu$ m. (E) Quantification of co-labeling from micrographs as depicted in (D). Co-labeling shown as a proportion of total *Tmem79*- or marker-positive neurons. Proportions were calculated from counts taken from at least 7 micrographs. (F) Representative micrographs of staining for immunoreactivity to GFP (upper left panel, green) or TdT (upper right panel, red) in 12  $\mu$ m sections of dorsal root ganglion dissected from mice. Images superimposed with brightfield micrographs. Scale bar represents 100  $\mu$ m. Lower panels provide additional descriptive statistics of GFP- (left) or TdT-positive (right) neurons. Data include proportion of positive neurons/micrograph  $\pm$  s.e.m. with raw count and population mean and median diameters.  $n = 10$  sections (GFP) or 13 sections (TdT) from 3 separate animals. (G) Representative micrographs of staining for immunoreactivity to GFP (green) in 20  $\mu$ m sections of tongue, cervix, prostate, lung, brain, spinal cord, and adrenal medulla (as indicated) dissected from *Tmem79*-KI mice. Nuclei were counterstained with DAPI (blue). Scale bars represent 200  $\mu$ m. (H) Representative micrograph of staining in hairy skin section (20  $\mu$ m) from *Tmem79*-KI mice for immunoreactivity to GFP (left) or keratin 14 (K14, center). Merged image shown at right. Scale bar represents 10  $\mu$ m.



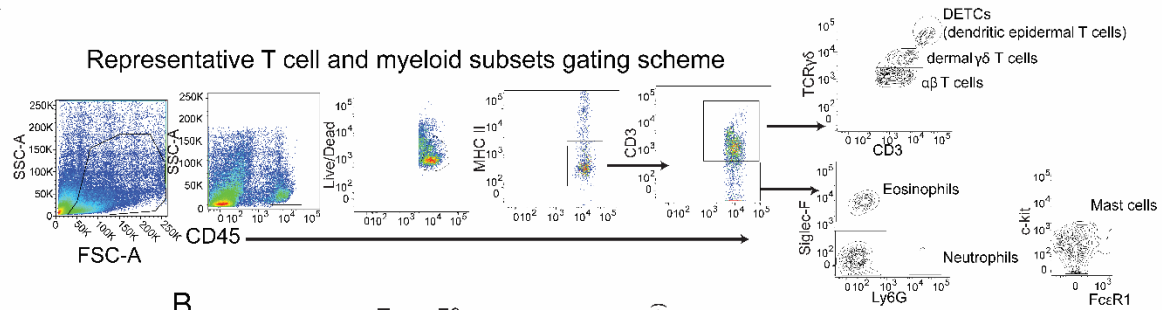
**Fig. S3. TMEM79 diminishes reactive oxidative species *in vitro* and has similarity to members of the membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG) superfamily.**

(a-b) HEK293T cells were transfected with control (CTRL) vector, loaded with DCF dye, then stimulated with SIN-1. (A) Quantification of DCF fluorescence in cultures of CTRL HEK293T cells with time in response to various concentrations. DCF fluorescence in HEK293T cells increases in a dose-dependent manner to SIN-1 concentrations with saturation at 5 mM. Data represent mean relative fluorescent units (RFU).  $n = 4$  independent experiments with samples run in triplicate. (B) Quantification of DCF fluorescence in cultures of HEK293T cells after 30 min incubation with 50  $\mu$ M SIN-1 and various concentrations of an antioxidant, N-acetylcysteine (NAC). NAC diminishes DCF fluorescence in HEK293T cells in a

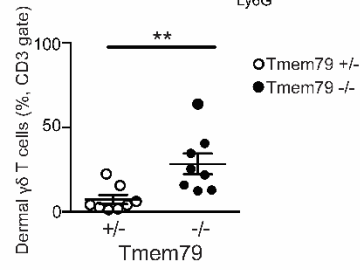
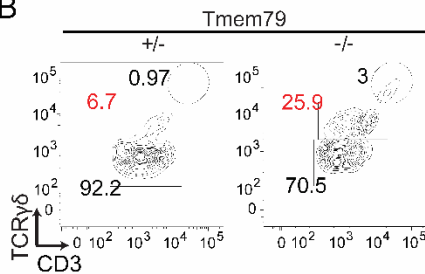


dose-dependent manner. Data represent mean RFU  $\pm$  s.e.m.  $n = 4$  independent experiments with samples run in triplicate. (C-E) Quantification of DCF fluorescence in cultures of HEK293T cells expressing either control (CTRL) vector or mouse *Tmem79* (TMEM79) following 30 minute incubation with concentrations of oxidants: sodium nitroprusside (SNP), tert-butyl hydroperoxide (tBHP), or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Data represent mean RFU normalized to baseline  $\pm$  s.e.m.  $n = 3-4$  experiments with samples run in triplicate. \* $P < 0.05$ , Student's *t* test, one-tailed. (F) BLAST query and alignment of the primary amino acid sequence of mouse *Tmem79* (top) reveals identity with the MAPEG superfamily. ClustalW2 alignment (bottom) of mouse *Tmem79* with mouse MAPEG members *Mgst1*, *Pges1*, *Mgst3*, *Flap*, *Mgst2*, and *Ltc4s*. Asterisk indicates two conserved residues within a region of sequence similarity corresponding to mouse *Tmem79* R332 and Y339. ClustalW2 alignment shown at bottom. (G) Mouse *Tmem79* membrane topology as predicted by TMpred with intracellular C terminus (inside). Residues mutated in Fig. 2e are indicated (white and red circles). Conserved residues from ClustalW2 alignment, R332 and Y339, are represented by red circles. (H) Representative micrographs of HEK293T stained for immunoreactivity to hemagglutinin (HA, red) without (FIX-, top row) or with (FIX+, bottom row) fixation for permeabilization. HEK293T cells were transfected with vectors for expression of N terminal HA-Tmem79 fusion (HA::TMEM79, left column) or C terminal HA-Tmem79 fusion protein (TMEM79::HA, right column). Scale bar represents 15  $\mu$ m. Immunostaining in micrographs suggests topology shown in (G).

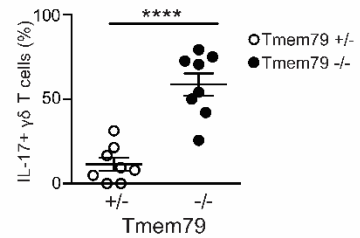
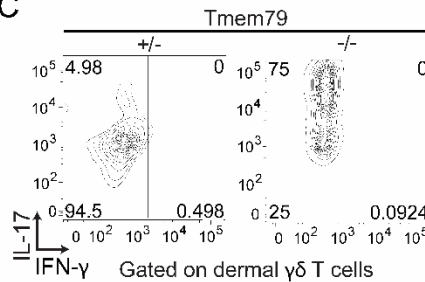
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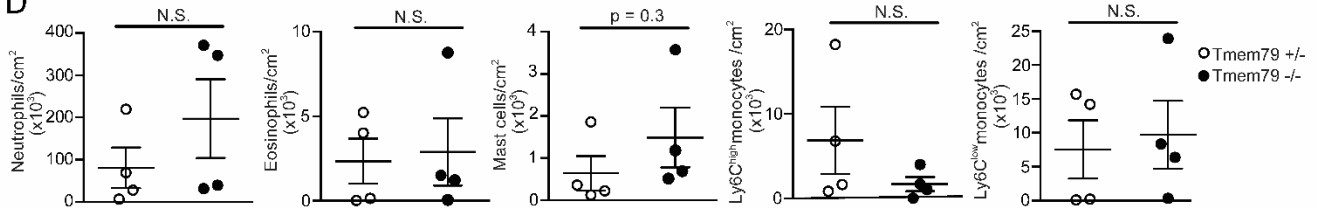
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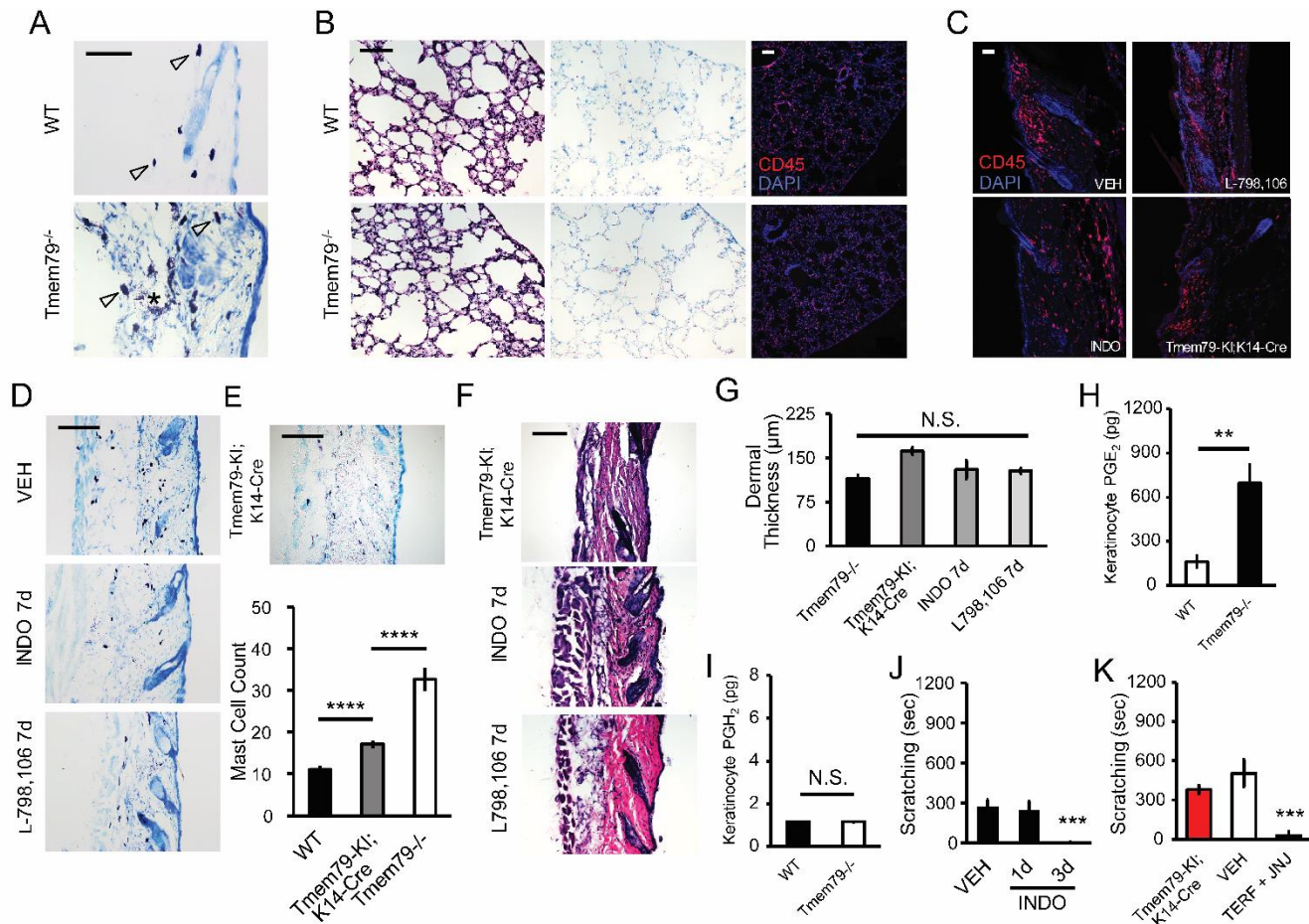
D



**Fig. S4. Preferential accumulation of IL-17-producing dermal  $\gamma\delta$  T cells in the skin of *Tmem79*<sup>-/-</sup> mice.**

(A) Representative gating scheme of immune cell phenotypes in skin by flow cytometry. The gating strategy is shown for neutrophils, eosinophils, mast cells, dendritic epidermal  $\gamma\delta$  T cells (DETCs), dermal  $\gamma\delta$  T cells, and  $\alpha\beta$  T cells. (B) Representative flow cytometry plots and percent of dermal  $\gamma\delta$  T cells in the dorsal skin of *Tmem79*<sup>-/-</sup> compared to *Tmem79*<sup>+/-</sup> littermate controls. Plots are pre-gated on live, CD45<sup>+</sup> CD3<sup>+</sup> MHC II<sup>-</sup> cells. Data are mean percentages  $\pm$  s.e.m.  $n =$  mice, 8 *Tmem79*<sup>+/-</sup>, 8 *Tmem79*<sup>-/-</sup> representing two independent pooled sets of 4 mice for each group. \*\* $P < 0.01$ , Student's  $t$  test, one-tailed. (C) Representative flow cytometry plots and percent of IL-17<sup>+</sup> dermal  $\gamma\delta$  T cells in the skin of *Tmem79*<sup>-/-</sup> compared to *Tmem79*<sup>+/-</sup> littermate controls. Plots are pre-gated on dermal  $\gamma\delta$  T cells (described in A). Data are mean percentages  $\pm$  s.e.m.  $n =$  mice, 8 *Tmem79*<sup>+/-</sup>, 8 *Tmem79*<sup>-/-</sup> representing two independent pooled sets of 4 mice for each group.

\*\*\*\* $P < 0.0001$ , Student's  $t$  test, one-tailed. (D) Absolute number of the indicated immune cells per  $\text{cm}^2$  of dorsal hairy skin of  $Tmem79^{-/-}$  mice compared to  $Tmem79^{+/+}$  littermate controls. Data are mean number  $\pm$  s.e.m.  $n =$  mice, 4  $Tmem79^{+/+}$ , 4  $Tmem79^{-/-}$  representing two independent pooled sets of 2 mice for each group. N.S.  $P > 0.05$ , Student's  $t$  test, one-tailed.

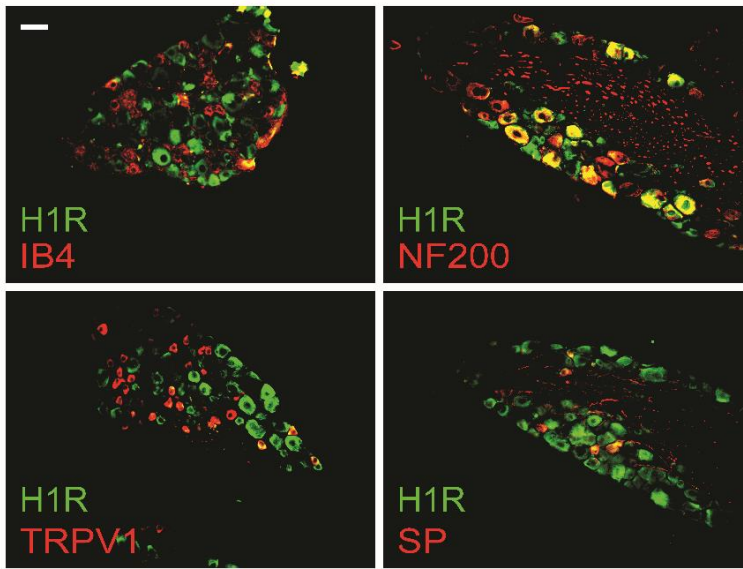


**Fig. S5. Interfering with PGE<sub>2</sub>-EP3 signaling reverses mast cell accumulation, but not dermal thickness.**

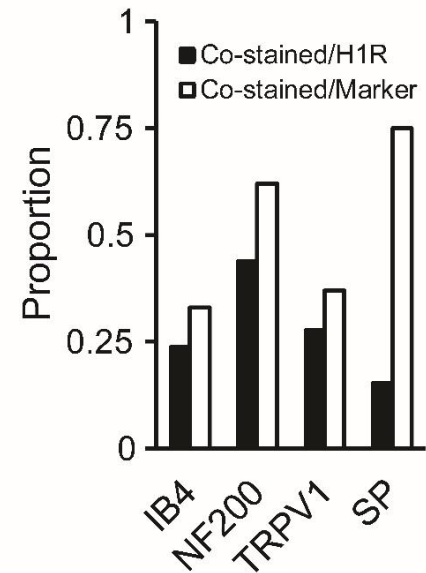
(A) Representative higher magnification micrographs of toluidine blue O stained hairy skin sections (20 µm) from age-matched wild-type (WT, top panel) or *Tmem79<sup>-/-</sup>* (bottom) mice. Superficial epidermis is oriented to the right. Hollow arrowheads bring attention to examples of stained mast cells. Asterisk indicates degranulated mast cell(s). Scale bar represents 50 µm. (B) Representative micrographs of stained lung sections (20 µm) from age-matched wild-type (WT, top panel) or *Tmem79<sup>-/-</sup>* (bottom) mice. Staining by hematoxylin and eosin (left column), by toluidine blue O (center column) or for immunoreactivity to CD45 with nuclei counterstained by DAPI (right column). Scale bar represents 100 µm except in right column where it represents 10 µm. (C) Representative micrographs of stained hairy skin sections (20 µm) from untreated *Tmem79-KI;K14-Cre* mice or *Tmem79<sup>-/-</sup>* mice receiving 7 consecutive days of intraperitoneal administration of vehicle (VEH), 10 mg/kg indomethacin (INDO), or 100 mg/kg L-798,106. Superficial epidermis is oriented to the left. Staining for immunoreactivity to CD45 (red) with nuclei counterstained using DAPI (blue). Scale bar represents 10 µm. (D) Representative micrographs of toluidine blue O stained hairy skin sections (20 µm) from *Tmem79<sup>-/-</sup>* mice receiving 7 consecutive days of intraperitoneal administration of vehicle (VEH), 10 mg/kg indomethacin (INDO), or 100 mg/kg L-798,106. Superficial epidermis is oriented to the right. Scale bar represents 100

$\mu\text{m}$ . (E) Top panel: Representative micrographs of toluidine blue O stained hairy skin sections ( $20 \mu\text{m}$ ) from *Tmem79*-KI;K14-Cre. Superficial epidermis is oriented to the right. Scale bar represents  $100 \mu\text{m}$ . Bottom panel: Quantification of mast cells from micrographs represented by top panel and from Fig. 3d. Data shown are the mean mast cell count from fields captured from wild-type (WT), *Tmem79*-KI;K14-Cre, or *Tmem79*<sup>-/-</sup> mice  $\pm$  s.e.m.  $n =$  micrographs, 40 WT, 69 *Tmem79*-KI;K14-Cre, 47 *Tmem79*<sup>-/-</sup>. \*\*\*\* $P < 0.0001$ , Holm  $t$  test, two-tailed. (F) Representative micrographs of hematoxylin and eosin stained sections of hairy skin ( $20 \mu\text{m}$ ) dissected from untreated *Tmem79*-KI;K14-Cre mice or *Tmem79*<sup>-/-</sup> mice receiving 7 consecutive days of intraperitoneal administration of 10 mg/kg indomethacin (INDO), or 100 mg/kg L-798,106. Scale bar represents  $100 \mu\text{m}$ . (G) Quantification of dermal thickness from skin sections as represented in (F) and Fig. S1b-c. Data shown are the mean thickness of the dermis in hairy skin sections from mice  $\pm$  s.e.m. ( $n = 13$  *Tmem79*<sup>-/-</sup>, 22 *Tmem79*-KI;K14-Cre, 12 INDO, 24 L-798,106; N.S.  $P > 0.05$ , Holm  $t$  test, two-tailed). (H-I) ELISA was used to quantify PGE<sub>2</sub> or PGH<sub>2</sub> content from lysed, acutely-dissociated keratinocytes from age-matched wild-type (WT) and *Tmem79*<sup>-/-</sup>. Data represent the average content of PGE<sub>2</sub> or PGH<sub>2</sub> in picograms (pg) from keratinocytes from weight matched skin dissected from separate animals  $\pm$  s.e.m. ( $n = 3$  animals per group; \*\* $P < 0.01$ , N.S.  $P > 0.05$ , Student's  $t$  test, one-tailed). (J) Quantified scratching behavior of adult wild-type mice following 1 or 3 consecutive days of intraperitoneal administration of vehicle (VEH) or 10 mg/kg indomethacin (INDO). Data shown are the mean seconds of scratching from multiple mice in each treatment group  $\pm$  s.e.m. ( $n = 4$ ; \*\*\* $P < 0.001$ , Holm  $t$  test, one-tailed). (K) Quantified scratching behavior of adult *Tmem79*-KI;K14-Cre mice at baseline and following intraperitoneal administration of vehicle (VEH) or 20 mg/kg terfenadine+20 mg/kg JNJ-7777120 (TERF+JNJ). Data shown are the mean seconds of scratching from multiple mice in each treatment group  $\pm$  s.e.m. ( $n = 12$  *Tmem79*-KI;K14-Cre, 10 VEH, 7 TERF+JNJ; \*\*\* $P < 0.001$ , Holm  $t$  test, one-tailed).

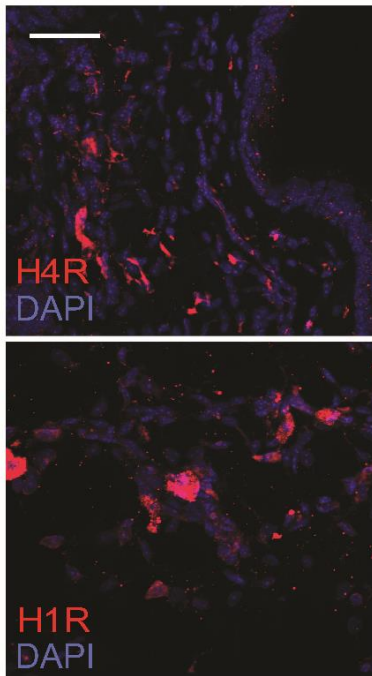
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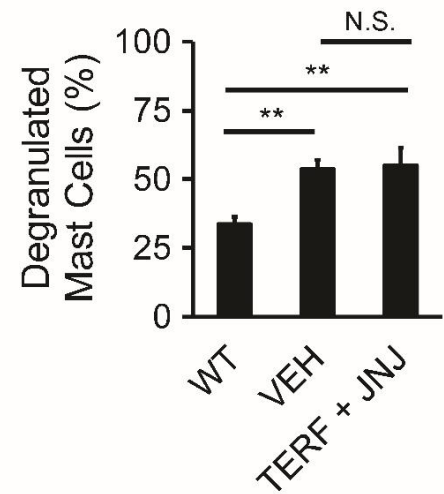
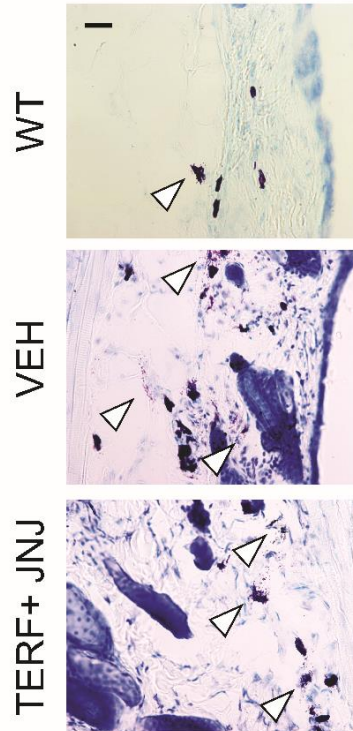
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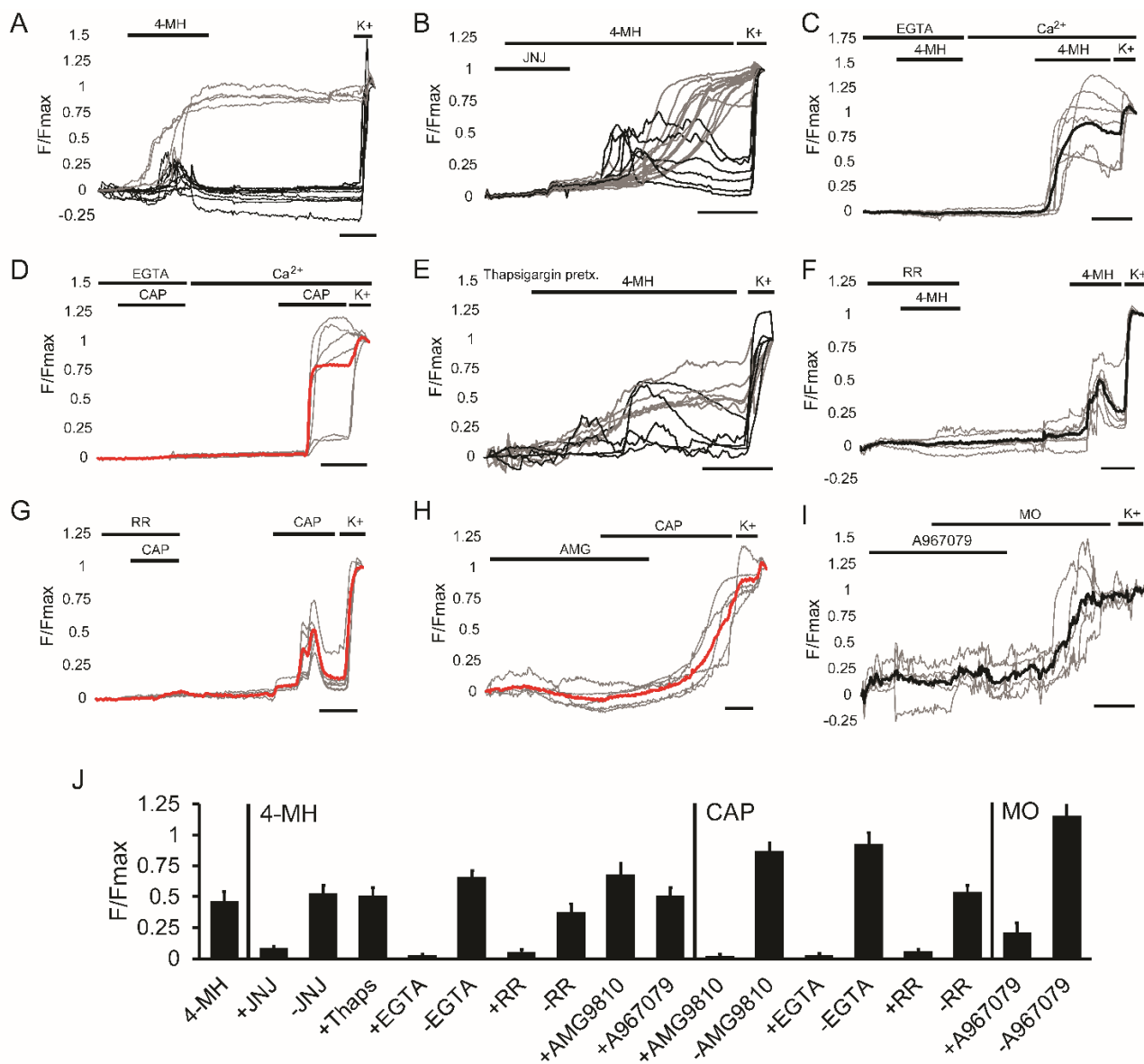
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**Fig. S6. Blockade of histaminergic signaling does not diminish mast cell degranulation suggesting that neuronal histamine receptors underlie *Tmem79*<sup>-/-</sup> itch.**

(A) Representative micrographs of fluorescent co-labeling of dorsal root ganglion sections (12  $\mu$ m) from adult wild-type mice. Sections stained for immunoreactivity to H1R (green) and binding to IB4, or immunoreactivity to NF200, TRPV1, or SP

as indicated (red). Scale bar represents 50  $\mu\text{m}$ . (B) Quantification of co-labeling from micrographs as depicted in (A). Co-labeling shown as a proportion of total H1R-positive and marker-positive neurons. Proportions were calculated from counts taken from at least 8 micrographs. (C) Representative micrographs of fluorescent co-labeling of hairy skin sections (20  $\mu\text{m}$ ) from adult wild-type mice. Sections stained for immunoreactivity to H4R or H1R (red). Nuclei were counterstained with DAPI (blue). Scale bar represents 15  $\mu\text{m}$ . (D) Representative micrographs of toluidine blue O stained hairy skin sections (20  $\mu\text{m}$ ) from age-matched wild-type (WT, top panel) or *Tmem79*<sup>-/-</sup> mice 1 hr post intraperitoneal administration of vehicle (middle) or 20 mg/kg terfenadine+20 mg/kg JNJ-7777120 (TERF+JNJ, bottom panel). Superficial epidermis is oriented to the right. Hollow arrowheads bring attention to examples of stained degranulated mast cells. Scale bar represents 100  $\mu\text{m}$ . (E) Quantification of mast cells from micrographs represented by (D). Data shown are the mean percentage of degranulated mast cells  $\pm$  s.e.m from fields captured from wild-type (WT) or *Tmem79*<sup>-/-</sup> mice that received vehicle (VEH) or 20 mg/kg terfenadine+20 mg/kg JNJ-7777120 (TERF+JNJ) treatment ( $n$  = micrographs, 15 WT, 9 VEH, 9 TERF+JNJ; \*\* $P$ <0.01, \*\*\*\* $P$ <0.0001, One-way ANOVA with Holm-Sidak's multiple comparisons correction).

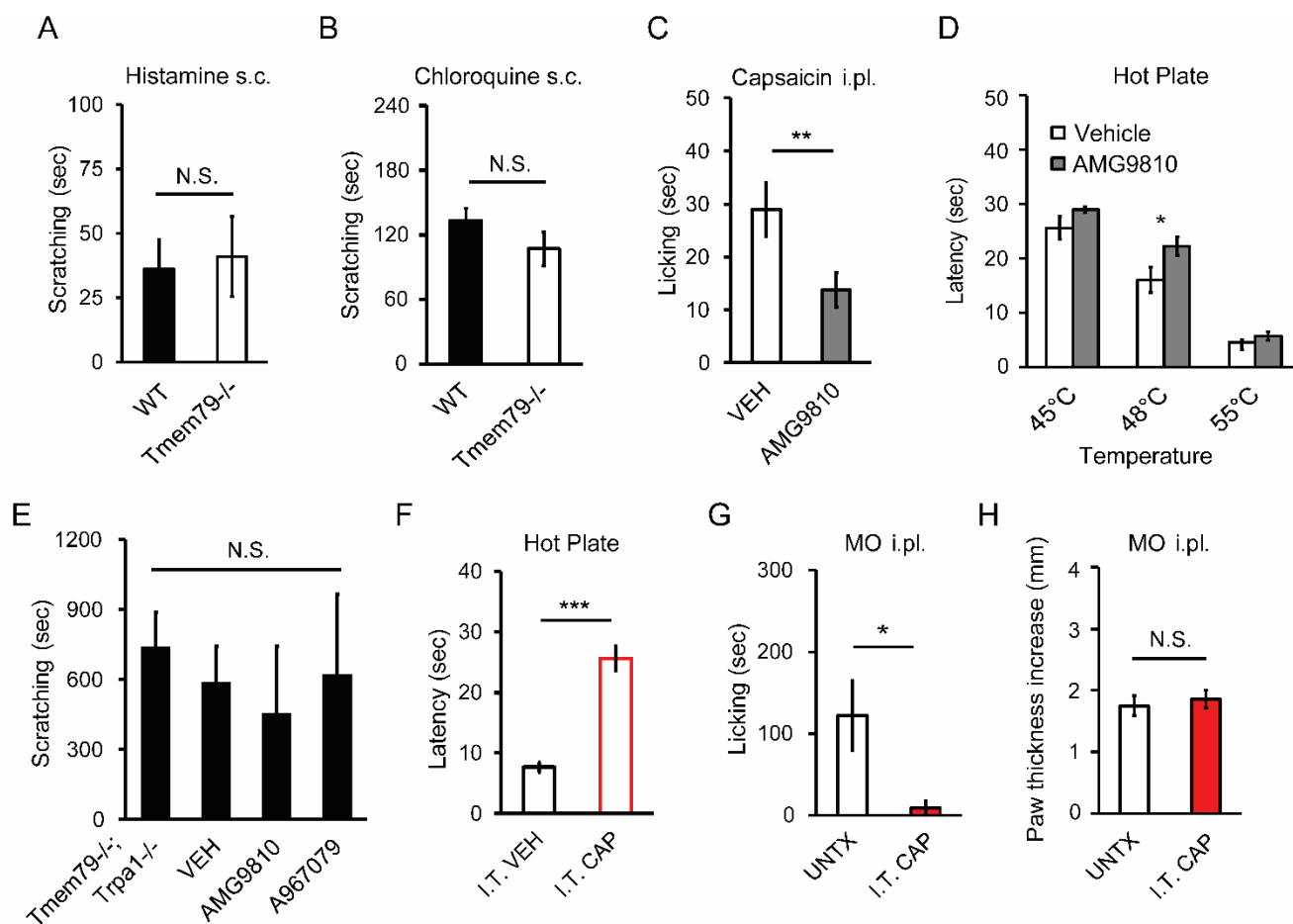


**Fig. S7. H4R-agonist triggers TRPV1- and TRPA1-independent calcium entry into sensory neurons.**

(A-I) Normalized  $F_{340}/F_{380}$  calcium imaging traces from adult dorsal root ganglion neurons. Neurons were identified at the conclusion of each experiment by a positive response to elevated extracellular potassium (150 mM KCl, K+). Each trace represents a distinct responder. Scale bars represent 60 sec. (A-B) Application of 500  $\mu$ M 4-methyl histamine (4-MH) produces transient and sustained responses that are blocked by 1 mM JNJ-777120. (C-D) Responses to 500  $\mu$ M 4-MH and 1  $\mu$ M capsaicin (CAP) are absent when extracellular calcium is removed (EGTA = Ca<sup>2+</sup>-free Ringer's solution with 2 mM EGTA). (E) Responses to 500  $\mu$ M 4-MH persist after 90 min pretreatment of neurons with 2  $\mu$ M thapsigargin. (F-G) Responses to 500  $\mu$ M 4-MH and 1  $\mu$ M CAP are blocked by 10  $\mu$ M ruthenium red (RR). (H-I) Responses to 1  $\mu$ M CAP and 1 mM AITC (MO) are blocked by 10  $\mu$ M AMG9810 (AMG) or 1 mM A967079, respectively. (J) Quantification of peak responses from (A-I) above. Data shown are mean  $\pm$  s.e.m.



of peak normalized  $F_{340}/F_{380}$  responses ( $n = 13+$  responders from 3-4 independent experiments).



**Fig. S8. *Tmem79*<sup>-/-</sup> histaminergic itch is TRPV1- and TRPA1-independent.** (A) Quantified scratching behavior of age-matched wild-type (WT) and *Tmem79*<sup>-/-</sup> mice following subcutaneous administration of histamine (500  $\mu$ g/100  $\mu$ L). Data shown are the mean seconds of scratching from multiple mice ( $n = 5$  WT, 6 *Tmem79*<sup>-/-</sup>) in each group  $\pm$  s.e.m. N.S.  $P > 0.05$ , Student's  $t$  test. (B) Quantified scratching behavior of age-matched wild-type (WT) and *Tmem79*<sup>-/-</sup> mice following subcutaneous administration of chloroquine (200  $\mu$ g/50  $\mu$ L). Data shown are the mean seconds of scratching from multiple mice ( $n = 3$  WT, 3 *Tmem79*<sup>-/-</sup>) in each group  $\pm$  s.e.m. N.S.  $P > 0.05$ , Student's  $t$  test. (C) Quantified paw licking of adult *Tmem79*<sup>-/-</sup> mice in response to 1  $\mu$ g intraplantar capsaicin following intraperitoneal administration of vehicle (VEH) or 50 mg/kg AMG9810. Data shown are the mean seconds of ipsilateral paw licking from multiple mice ( $n = 8$ ) in each treatment group  $\pm$  s.e.m. \* $P < 0.05$ , Student's  $t$  test, one-tailed. (D) Quantified latency to flinching, licking, or jumping of adult *Tmem79*<sup>-/-</sup> mice in response to various elevated surface temperatures following intraperitoneal administration of vehicle (VEH) or 50 mg/kg AMG9810. Data shown are the mean seconds to latency of event from 3-5 trials from multiple mice ( $n = 8$ ) in each treatment group  $\pm$  s.e.m. \* $P < 0.05$ , Student's  $t$  test, one-tailed. (E) Quantified scratching behavior of adult *Tmem79*<sup>-/-</sup>; *Trpa1*<sup>-/-</sup> mice at baseline or following intraperitoneal administration of vehicle (VEH), 30 mg/kg AMG9810, or 50 mg/kg A967079. Data shown are the mean seconds of scratching from

multiple mice ( $n = 4$  *Tmem79*<sup>-/-</sup>; *Trpa1*<sup>-/-</sup> baseline, 7 VEH, 4 AMG9810, 4 A967079) in each treatment group  $\pm$  s.e.m. N.S.  $P > 0.05$ , Holm *t* test, one-tailed. (F) Quantified latency to flinching, licking, or jumping of adult *Tmem79*<sup>-/-</sup> mice in response to various elevated surface temperatures following intrathecal administration of vehicle (I.T. VEH) or capsaicin (10  $\mu$ g) (I.T. CAP). Data shown are the mean seconds to latency of event from 3 trials from multiple mice ( $n = 3$ ) in each treatment group  $\pm$  s.e.m. \*\*\* $P < 0.001$ , Student's *t* test, one-tailed. (G) Quantified paw licking of adult *Tmem79*<sup>-/-</sup> mice after intrathecal administration of 10  $\mu$ g capsaicin (I.T. CAP) or wild-type mice (UNTX) in response to 20  $\mu$ L intraplantar 0.75% mustard oil. Data shown are the mean seconds of ipsilateral paw licking from multiple mice ( $n = 9$  UNTX, 7 I.T. CAP) in each treatment group  $\pm$  s.e.m. \* $P < 0.05$ , Student's *t* test, one-tailed. (H) Quantified paw thickness increase in adult *Tmem79*<sup>-/-</sup> mice after intrathecal administration of 10  $\mu$ g capsaicin (I.T. CAP) or wild-type mice (UNTX) in response to 20  $\mu$ L intraplantar 0.75% mustard oil. Data shown are the mean increase in ipsilateral paw thickness (in mm) from multiple mice in each treatment group  $\pm$  s.e.m. ( $n = 8$  UNTX, 7 I.T. CAP; N.S.  $P > 0.05$ , Student's *t* test, one-tailed).