

House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation

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Allergic skin diseases, such as atopic dermatitis, are clinically characterized by severe itching and type 2 immunity-associated hypersensitivity to widely distributed allergens, including those derived from house dust mites (HDMs). Here we found that HDMs with cysteine protease activity directly activated peptidergic nociceptors, which are neuropeptide-producing nociceptive sensory neurons that express the ion channel TRPV1 and *Tac1*, the gene encoding the precursor for the neuropeptide substance P. Intravital imaging and genetic approaches indicated that HDM-activated nociceptors drive the development of allergic skin inflammation by inducing the degranulation of mast cells contiguous to such nociceptors, through the release of substance P and the activation of the cationic molecule receptor MRGPRB2 on mast cells. These data indicate that, after exposure to HDM allergens, activation of TRPV1⁺ *Tac1*⁺ nociceptor-MRGPRB2⁺ mast cell sensory clusters represents a key early event in the development of allergic skin reactions.

Altered tissue sensitivity to environmental triggers is thought to contribute to the development of allergic inflammation, frequently starting with type 2 (allergic) skin diseases such as atopic dermatitis (AD)¹ during childhood. AD is a chronic, relapsing inflammatory skin disease characterized by severe itching of the skin, eczema and hypersensitivity to otherwise innocuous environmental substances². The etiology of AD is incompletely understood, but many factors, such as genetic or environmental elements and immune or structural cells, are thought to contribute to the development of skin lesions².

Aberrantly activated type 2 immunity against common sources of indoor allergens such as HDMs, and increased numbers of activated mast cells and eosinophils in the lesional skin^{2–4}, may have important roles in the pathology of AD. Moreover, 80–100% of patients with AD are colonized with *Staphylococcus aureus* compared to only 5–30% of healthy individuals⁵, and patients whose skin is colonized with exotoxin-producing *S. aureus*, such as staphylococcal enterotoxins B (SEB)^{4,6,7}, have a higher severity of AD than those who are colonized with non-toxicogenic strains⁶. Finally, although abnormalities of the epidermis leading to loss of skin barrier have been reported to contribute to the development of skin lesions^{8–10}, many patients with AD do not have loss-of-function mutations of epidermal structural proteins such as filaggrin^{11–13}. Therefore, the full spectrum of factors that contribute to skin barrier disorganization and the development of type 2 immunity and skin lesions remains to be determined.

The skin is innervated by an intricate network of nociceptive sensory neurons, known as nociceptors, with cell bodies located in the dorsal root or trigeminal ganglia¹⁴. Their primary function is the transmission of sensations of temperature, pain and itch to elicit appropriate behavioral responses such as withdrawal (to avoid tissue injury) and scratching (to remove irritants). Nociceptors may be involved in manifestations of AD by promoting itch and scratching behavior^{13,15}, an idea supported by clinical studies showing that the serum of patients with AD exhibits high amounts of neuropeptides, such as the cationic neuropeptide substance P (SP), and that the amounts detected correlate with the severity of the disease^{15,16}. While much remains to be learned about itching sensations, peripheral neurons also have the potential to influence immune cells and inflammatory responses^{17–22}.

Mast cells are innate immune cells thought to be involved in allergic diseases, including AD, in which they recognize specific antigens through their high-affinity receptors for immunoglobulin E (IgE) (FcεRI)²³. Under homeostatic conditions, skin and peritoneal mast cells in mice specifically express MRGPRB2, a receptor for cationic molecules from the Mas-related G protein-coupled receptor family²⁴. MRGPRX2, the human ortholog of MRGPRB2, and not the neurokinin 1 receptor, is the main receptor for cationic molecules, including SP, in human skin mast cells^{25,26}. MRGPRX2- or MRGPRB2-mediated activation of mast cells can result in a remarkably fast degranulation dynamics, which is associated with the development of rapid and localized mast cell-dependent inflammation²⁵.

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Whether specialized neuro-immune cross-talk has a role in the development of allergic skin inflammation remains unclear. Combining genetically modified mouse models with intravital imaging approaches, we show here that a large proportion of TRPV1⁺ nociceptor projections form physical contacts with MRGPRB2⁺ mast cells. These neuro-immune clusters can sense the presence of allergic cues and can trigger pathogenic type 2 immune responses and associated lesions in the skin.

Results

***Tac1* expression is required for allergic skin inflammation.** We used a mouse model of allergic skin inflammation, driven by six epicutaneous exposures over one month, to two antigens found together in the lesional skin of 80–100% of AD patients^{2–7,26}, 10 µg of the HDM strain *Dermatophagoides farinae* and 500 ng of the bacterial exotoxin SEB from *S. aureus* (Supplementary Fig. 1a,b). Compared to healthy subjects, AD patients often suffer from *S. aureus* skin infections which are thought to play an important role in the pathogenesis and/or worsening of the condition⁶. This model recapitulates moderate to severe AD-like disease, associated with both the histopathological features of an exacerbated type 2 immune response and a global gene expression pattern statistically similar to that seen in human AD^{8,27}. Wild-type (WT) mice sensitized with a combination of SEB and *D. farinae* developed more severe skin inflammation than those treated with SEB or *D. farinae* alone, while *D. farinae* alone induced more skin lesions than SEB alone (Supplementary Fig. 1c,d). Compared to vehicle, treatment with *D. farinae*+SEB induced a systemic *D. farinae*-specific T_H2 response (Supplementary Fig. 1e,f). In accord with reports that patients with moderate to severe AD were treated successfully with dupilumab²⁸, a human monoclonal antibody against the interleukin-4 (IL-4) receptor α (IL-4Rα) that inhibits both IL-4 and IL-13 signaling, systemic treatment with blocking antibodies for mouse IL-4Rα significantly decreased skin lesions and histopathological features in the AD-like mouse model (Supplementary Fig. 1g,h). These data indicate that treatment with *D. farinae*+SEB led to the development of AD-like allergic skin inflammation dependent on type 2 cytokines.

The neuropeptide SP is thought to be released primarily from a unique subpopulation of TRPV1⁺ TRPA1[−] peptidergic nociceptors that highly express *Tac1* (ref. ²⁹), the gene encoding the SP precursor. Using publicly available gene expression data³⁰, we mapped the expression of *Trpv1*, *Tac1* and *Trpa1* among different mouse tissues and various subpopulations of immune cells. *Trpv1*, *Tac1* and *Trpa1* were highly (or exclusively, in the case of *Trpv1* and *Trpa1*) expressed in dorsal root ganglia (DRG), where the somas of sensory neurons are located, with weak additional expression of *Tac1* in the central and the enteric nervous systems (Fig. 1a and Supplementary Fig. 1i). In whole-mounted skin biopsies from C57BL/6J WT mice, SP expression was restricted to PGP9.5⁺ cutaneous neuronal fibers (Fig. 1b).

To analyze the role of SP in the development of allergic skin inflammation, we treated WT and *Tac1*^{−/−} mice with *D. farinae*+SEB and assessed the development of key pathological features associated with AD⁸. Compared to vehicle-treated WT mice, *D. farinae*+SEB-treated WT mice developed macroscopic skin lesions (Fig. 1d), increased epidermal thickness, strong infiltration of eosinophils and neutrophils (Fig. 1e) and elevated serum *D. farinae*-specific IgG₁ and IgE (Fig. 1f), along with a profound alteration of filaggrin protein expression (Fig. 1g,h), a key component of skin barrier function linked to human AD¹². In addition, *D. farinae*+SEB-treated WT mice showed increased expression of keratin6 (K6), a marker of inflammatory stress in keratinocytes (Supplementary Fig. 2a,b) and alterations in claudin-1, K14 and K10 expression (Supplementary Fig. 2c–h). Expression of two other structural proteins, loricrin and E-cadherin, was not

significantly affected (Supplementary Fig. 2i–l). By contrast, *D. farinae*+SEB-treated *Tac1*^{−/−} mice were mostly protected from disease, with substantial reduction in skin lesion development, histological abnormalities, infiltration of immune cells, serum levels of *D. farinae*-specific IgG₁ and IgE and changes in skin barrier architecture (Fig. 1c–h and Supplementary Fig. 2). Taken together, these data suggest that expression of *Tac1* and SP was restricted to the neuronal compartment of the skin and that expression of *Tac1* was required for the full development of the pathological features associated with allergic skin inflammation in this model.

HDMs directly activate SP-producing TRPV1⁺ neurons. To analyze the role of TRPV1⁺ nociceptors in the development of allergic skin inflammation, we treated WT mice systemically with resiniferatoxin (RTX), which is a potent TRPV1 agonist selectively ablating TRPV1⁺ nociceptors^{17,18} (Supplementary Fig. 3a,b). RTX-treated mice and control dimethylsulfoxide (DMSO)-treated mice were subsequently treated with *D. farinae*+SEB to induce allergic skin inflammation. RTX-treated mice showed a marked reduction in skin lesions (Fig. 2a,b) and lesion-associated histopathological features compared to control DMSO-treated mice (Fig. 2c), along with restored filaggrin organization and decreased expression of the stress marker K6 (Supplementary Fig. 3), suggesting that TRPV1⁺ nociceptors are required for the full development of allergic skin inflammation in this model.

The primary function of nociceptors is to detect potentially damaging stimuli and initiate appropriate behavioral responses such as removal or scratching. We investigated whether *D. farinae* and SEB were directly detected by TRPV1⁺ DRG neurons cultured ex vivo. We found that 5 ng ml^{−1} SEB alone induced only a weak (barely detectable in some experiments) influx of calcium in DRG neurons (Fig. 2d,e), while concentrations ranging from 5 ng ml^{−1} of *D. farinae*, either alone (Fig. 2d,f) or in combination with 5 ng ml^{−1} SEB (Fig. 2d,g), triggered an increase in intracellular calcium in a subpopulation of DRG neurons that also responded to the TRPV1 agonist capsaicin (Fig. 2h,i).

D. farinae alone or in combination with SEB also triggered the secretion of SP from DRG neurons with a tendency, in some experiments, for *D. farinae*+SEB to trigger less SP secretion compared to *D. farinae* alone (Fig. 2j). Moreover, cultured DRG neurons from RTX-treated mice did not secrete SP in response to *D. farinae* alone or *D. farinae*+SEB, while those from DMSO-treated mice secreted SP (Fig. 2k). Importantly, DRG neurons from RTX-treated mice were also unresponsive to capsaicin (Fig. 2k), indicating that in vivo RTX treatment efficiently depleted TRPV1⁺ neurons. These data indicate that *D. farinae* directly triggered the secretion of SP from TRPV1⁺ DRG neurons cultured ex vivo.

Next, we investigated the mechanism by which *D. farinae* activates TRPV1⁺ DRG neurons. Treatment of DRG neurons with NBP2-29328, a MYD88 inhibitory peptide⁸, did not prevent *D. farinae*-induced activation of DRG neurons compared to those treated with a non-relevant control peptide (Supplementary Fig. 4a,b), indicating that, at least under the culture conditions used here, DRG neuron activation is not dependent on MYD88-dependent signaling through Toll-like receptors. Next, we investigated whether the proteolytic activity of *D. farinae* is required for triggering of DRG neuron activation ex vivo. Like other common environmental allergens, HDM strains including *D. farinae*³¹ have intrinsic proteolytic activity that can degrade epithelial junctions^{32–35} or full-length IL-33 (ref. ³⁶) in vivo. To characterize the type of proteolytic activity present in the *D. farinae* extracts used, we measured the proteolytic activities of *D. farinae* with or without specific and irreversible inhibitors of cysteine or serine protease activities. Pretreatment with the cysteine protease inhibitor E64, but not with the serine protease inhibitor AEBSE, completely inhibited the proteolytic activity of *D. farinae* at levels comparable

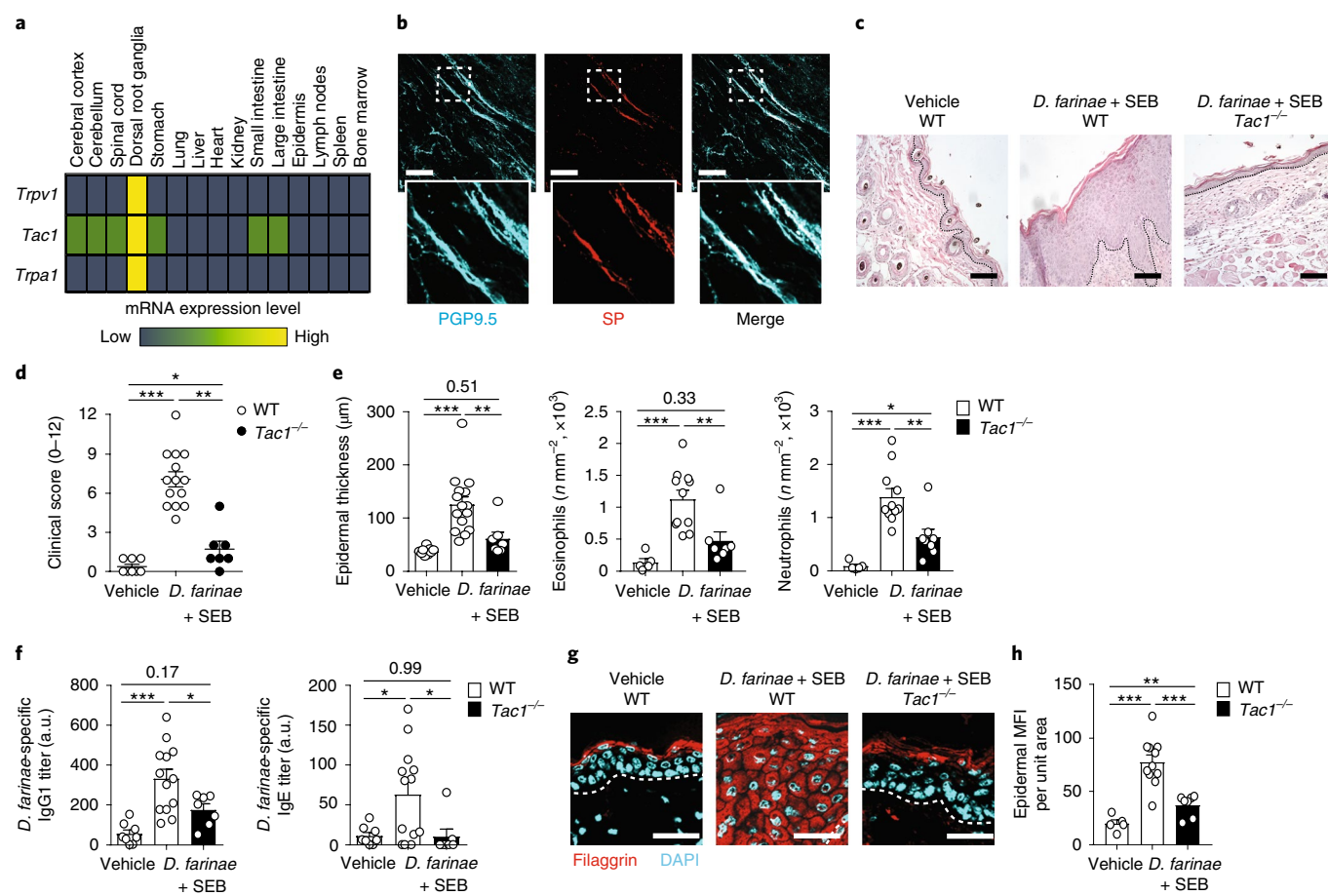


Fig. 1 | *Tac1* gene expression is required for the full development of pathological features in a model of allergic skin inflammation. **a**, Publicly available microarray gene expression data of *Trpv1*, *Tac1* and *Trpa1* in different mouse tissues (GSE 10246); data are shown using a heat map of messenger RNA expression levels. **b**, Representative 3D confocal microscopy picture of whole-mounted normal dorsal skin stained for PGP9.5 (a pan-neuronal marker, cyan) and substance SP (red). **c**, Representative hematoxylin and eosin (H&E) staining of vehicle- or *D. fariniae* + SEB-treated areas in WT or *Tac1*^{-/-} mice. **d**, Clinical scores (0–12) of vehicle- or *D. fariniae* + SEB-treated areas in WT or *Tac1*^{-/-} mice treated as in **c**. **e**, Epidermal thickness (μ m) (left), number of eosinophils (middle) and neutrophils (right) in skin sections in WT or *Tac1*^{-/-} mice treated as in **c**. **f**, Serum levels (a.u., arbitrary units) of *D. fariniae*-specific IgG1 (left) and IgE (right) antibodies in vehicle- or *D. fariniae* + SEB-treated WT or *Tac1*^{-/-} mice as in **c**. **g,h**, Representative confocal microscopy pictures of dorsal skin sections (**g**) and fluorescence analysis (**h**) of filaggrin staining in the epidermis of vehicle- or *D. fariniae* + SEB-treated areas in WT or *Tac1*^{-/-} mice treated as in **c**. Scale bars, 100 μ m; dotted black (**c**) or white (**g**) lines indicate the epidermis/dermis junction. Each circle represents one mouse. Number of mice: (**b**) $n = 3$; (**c–h**) $n = 6$ (WT vehicle), $n = 14$ (WT *D. fariniae* + SEB), $n = 7$ (*Tac1*^{-/-}). (**e,f,h**) Open bars, WT mice; black bars, *Tac1*^{-/-} mice. **b–h**, Data from three independent experiments, mean $\pm$ s.e.m. (**d**), mean $\pm$ s.e.m. (**e,f,h**); one-way ANOVA with Tukey's test for multiple comparisons, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

to heat inactivation of *D. fariniae* (Fig. 3a). No proteolytic activity was measured in the absence of dithiothreitol (DTT), a reducing agent required for cysteine protease activity under cell-free conditions (Fig. 3a), indicating the presence of cysteine protease activities in the *D. fariniae* extract. SEB alone neither exhibited protease-like activity nor enhanced *D. fariniae* proteolytic activity (Supplementary Fig. 4c).

Heat-induced inactivation or treatment with E64 prevented the influx of calcium in TRPV1⁺ DRG neurons by *D. fariniae* ex vivo (Fig. 3b–e). However, TRPV1⁺ DRG neuron activation was independent of expression of the protease-activated receptor PAR-2 by neurons (Supplementary Fig. 4a,b). We next investigated whether other clinically relevant environmental allergens with either predominant cysteine protease activity (such as the HDM strain *Dermatophagoides pteronyssinus*³¹) or serine-like protease activities³⁶ from pollen (ragweed), fungus (*Alternaria alternata*) or German cockroach could also directly activate DRG neurons. Only *D. pteronyssinus* triggered significant influx of calcium in DRG neurons, comparable to that obtained after stimulation with *D. fariniae* (Fig. 3f). These data indicate that two common HDM strains, with

predominant cysteine protease activity involved in allergic inflammation and suspected to have major roles in various allergic disorders, could trigger the activation of a subset of nociceptors directly, predominantly via a cysteine protease activity-dependent mechanism.

Functional MRGPRB2 is required for allergic skin inflammation. Mouse MRGPRB2 and its human ortholog, MRGPRX2, are mast cell-restricted receptors for several cationic substances, including the neuropeptide SP. We used *Kit*^{W-sh/W-sh} mice, which have an inversion mutation affecting expression of KIT, the receptor for the mast cell growth and survival factor (SCF), and *Kit*-independent *Cpa3-Cre⁺Mcl-1^{fl/fl}* mast cell-deficient mice, in which the anti-apoptotic factor Mcl-1 is deleted in the mast cell lineage (Supplementary Figs. 5 and 6a–d), to study the role of mast cells in the development of allergic skin inflammation. After treatment with *D. fariniae* + SEB, both *Kit*^{W-sh/W-sh} and *Cpa3-Cre⁺Mcl-1^{fl/fl}* mice showed a marked reduction in skin lesions compared to *Kit*^{+/+} and *Cpa3-Cre⁺Mcl-1^{fl/fl}* littermate controls, respectively (Supplementary Fig. 5). Importantly, *Tac1*^{-/-} mice or RTX-treated WT mice had

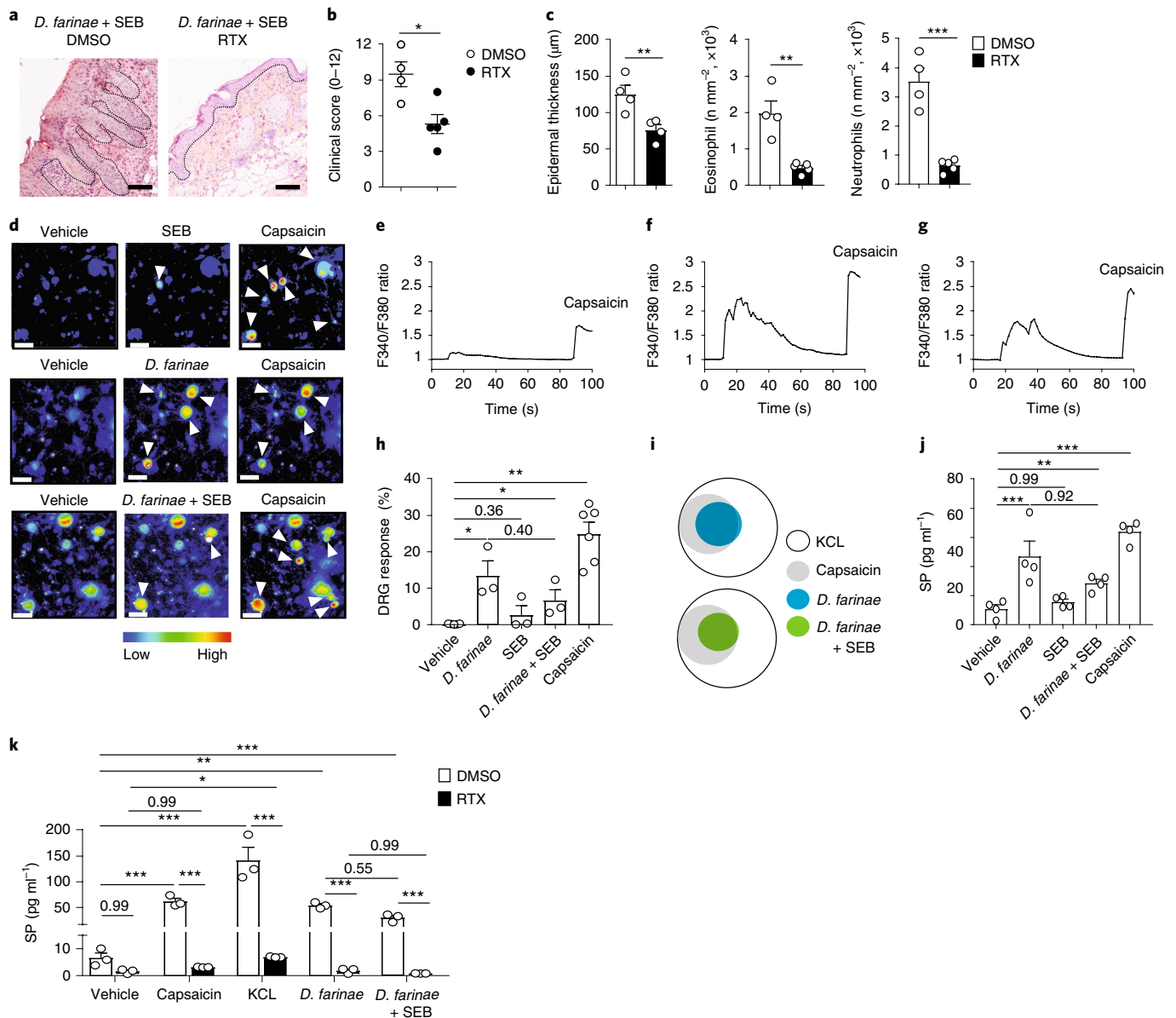


Fig. 2 | TRPV1⁺ nociceptors are required for the full development of allergic skin inflammation, and *D. farinae* extracts directly induce neuronal activation. **a**, Representative H&E staining of *D. farinae* + SEB-treated areas in mock (DMSO)-treated or RTX-treated (TRPV1⁺ nociceptor-ablated) mice. **b**, Clinical scores (0–12) of *D. farinae* + SEB-treated areas in DMSO- or RTX-treated mice as in **a**. **c**, Epidermal thickness (μm) (left), number of eosinophils (middle) and neutrophils (right) of *D. farinae* + SEB-treated areas in DMSO- or RTX-treated mice as in **a**. **a–c**, $n = 4$ (DMSO) and $n = 5$ (RTX); data are from two independent experiments; mean $\pm$ s.e.m., two-tailed, unpaired *t*-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **d–h**, Representative Fura-2 ratiometric fields (**d**), associated calcium traces for SEB (**e**), *D. farinae* (**f**) and *D. farinae* + SEB (**g**) and proportion (%) of responding DRG neurons (**h**) of ex vivo-cultured DRG neurons (expressed as percentage of DRG neurons responding to 50 mM KCL) stimulated with 5 ng ml⁻¹ SEB (**d** (top), **e**, **h**) or 5 ng ml⁻¹ *D. farinae* (**d** (middle), **f**, **h**) or both (**d** (bottom), **g**, **h**) and 1 μM capsaicin (**d–h**). **d**, Scale bars, 50 μm ; white arrowheads indicate neurons with increased Fura-2 fluorescence. **d–h**, Data are from three independent experiments performed with DRG from $n = 3$ (for the condition of vehicle-, *D. farinae*-, SEB- or *D. farinae* + SEB-treated DRG neurons) and $n = 6$ (for the condition of capsaicin-treated DRG neurons) different mice; mean $\pm$ s.e.m., two-tailed, unpaired *t*-test, $^*P < 0.05$, $^{**}P < 0.01$. **i**, Venn diagrams of responding DRG neurons. **j**, SP secretion in DRG neurons cultured ex vivo from (**j**) WT mice and (**k**) DMSO (open bars) versus RTX (black bars)-treated mice and stimulated as indicated. Data are from four (**j**) or three (**k**) independent experiments, mean $\pm$ s.e.m., two-tailed, unpaired *t*-test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Each circle represents one mouse.

normal numbers of dermal mast cells (Supplementary Fig. 6e,f), indicating that the reduced pathological features in these mice after treatment with *D. farinae* + SEB could not be attributed simply to a lack of, or reduction in, the number of skin mast cells.

To assess whether MRGPRB2 contributed to the development of allergic skin inflammation, we used *Mrgprb2*^{mut/mut} mice (in which MRGPRB2 is genetically inactivated by mutation) that have normal numbers of mast cells²⁴ (Supplementary Fig. 6g,h). In contrast to

Mrgprb2^{+/+} littermates, *Mrgprb2*^{mut/mut} mice did not develop allergic skin inflammation after treatment with *D. farinae* + SEB, and had substantial reductions in skin lesions (Fig. 4a,b), histological abnormalities, infiltration of eosinophils and neutrophils (Fig. 4c), *D. farinae*-specific IgE (Fig. 4d) and disruption of skin barrier architecture (Fig. 4e,f and Supplementary Fig. 7), suggesting that MRGPRB2 expressed on mast cells contributed to the development of this model of allergic skin inflammation induced by *D. farinae* + SEB.

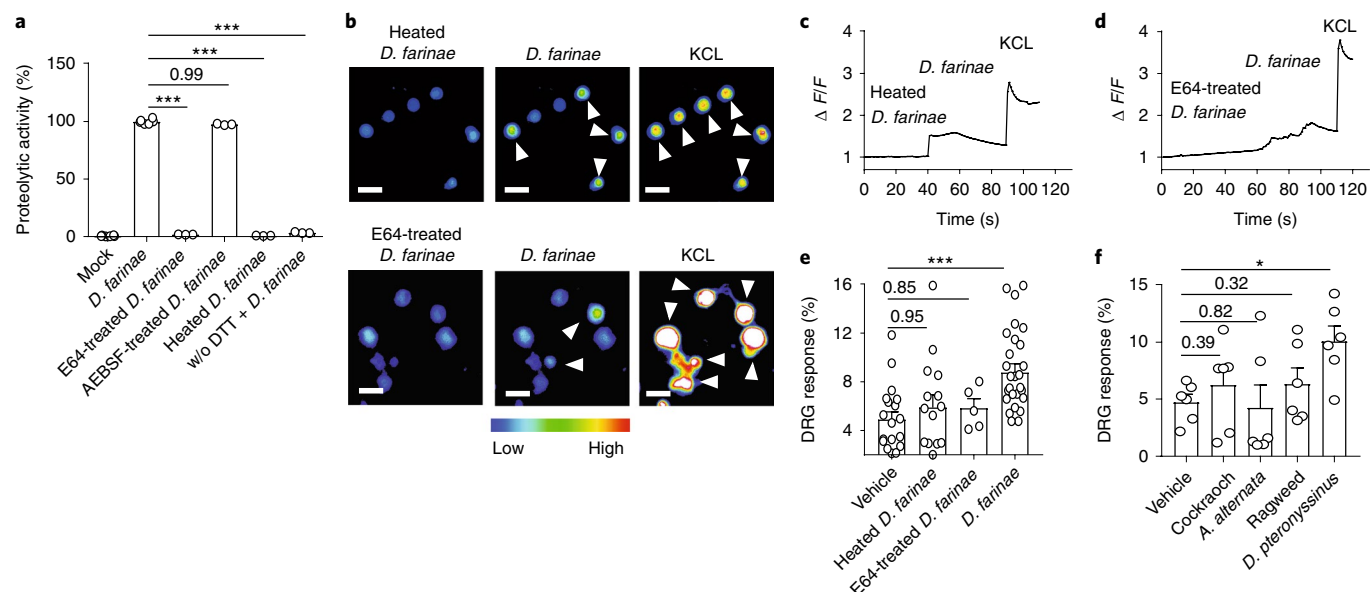


Fig. 3 | Activation of DRG neurons by *D. farinae* extract depends on cysteine protease activity. **a**, Protease activity in *D. farinae* extracts heat inactivated at 95 °C for 60 min (heated), or treated with cysteine protease inhibitor (E64, 100 nM) or serine protease inhibitor (AEBSF, 1 mM), with or without DTT. Data are from three independent experiments performed with three independent biological samples, mean + s.e.m., one-way ANOVA and Holm-Sidak's post hoc test, *** $P < 0.001$. **b–e**, Representative Fura-2 ratiometric fields (**b**), associated calcium traces showing F_{340}/F_{380} ratio ($\Delta F/F$) (**c,d**), and proportion (%) of responding DRG neurons (**e**) in ex vivo-cultured DRG neurons (expressed as percentage of DRG neurons responding to 50 mM KCL) stimulated sequentially with 5 ng ml⁻¹ heated *D. farinae* (**b** (top), **c**) or 5 ng ml⁻¹ E64 (2.5 pM)-treated *D. farinae* (**b** (bottom), **d**), 5 ng ml⁻¹ *D. farinae* and 50 mM KCL. Data are from five independent experiments with $n = 22$ (for the conditions vehicle and *D. farinae*), $n = 14$ (for the condition heated *D. farinae*) and $n = 5$ (for the condition E64-treated *D. farinae*) independent experiments performed with independent biological samples, mean + s.e.m., two-tailed, unpaired t -test, *** $P < 0.001$. **f**, Proportion of responding DRG neurons stimulated with either vehicle, 5 ng ml⁻¹ *D. pteronyssinus*, 5 ng ml⁻¹ German cockroach, 5 ng ml⁻¹ *A. alternata* or 5 ng ml⁻¹ ragweed, expressed as percentage of DRG neurons responding to 50 mM KCL. Data are from six independent experiments performed with six independent biological samples, mean + s.e.m., two-tailed, unpaired t -test, * $P < 0.05$. Each circle represents one mouse.

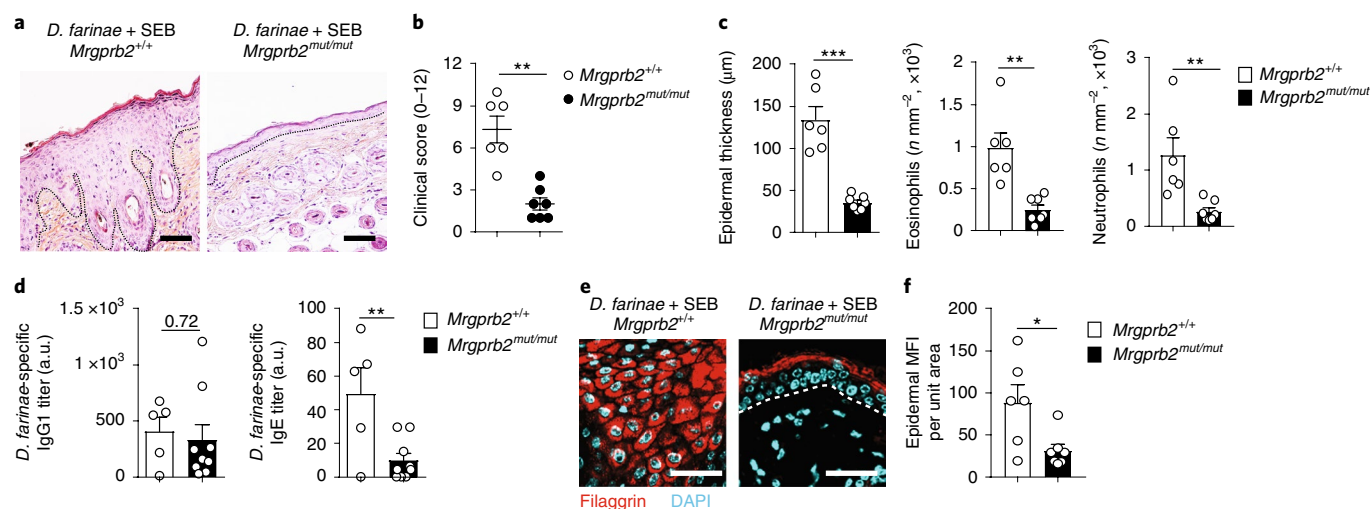


Fig. 4 | Genetic inactivation of MRGPRB2 largely prevents development of pathology in a model of allergic skin inflammation. **a**, Representative H&E staining of *D. farinae* + SEB-treated areas in *Mrgprb2*^{mut/mut} and *Mrgprb2*^{+/+} mice; dotted black lines indicate the epidermis/dermis junction. **b**, Clinical scores (0–12) of *D. farinae* + SEB-treated areas in *Mrgprb2*^{mut/mut} and *Mrgprb2*^{+/+} mice as in **a**. **c**, Epidermal thickness (μ m) (left), number of eosinophils (middle) and neutrophils (right) in *D. farinae* + SEB-treated areas in *Mrgprb2*^{mut/mut} and *Mrgprb2*^{+/+} mice as in **a**. **d**, Serum levels of *D. farinae*-specific IgG1 (left) and IgE (right) antibodies of *D. farinae* + SEB-treated *Mrgprb2*^{mut/mut} and *Mrgprb2*^{+/+} mice as in **a**. **e,f**, Representative confocal microscopy of dorsal skin section (**e**) and fluorescence analysis (**f**) of filaggrin staining of *D. farinae* + SEB-treated areas in *Mrgprb2*^{mut/mut} and *Mrgprb2*^{+/+} mice as in **a**. White broken line indicates the epidermis/dermis junction. Scale bars, 100 μ m. Each open circle represents one mouse. Number of mice: **a–c,e,f**, $n = 6$ (*Mrgprb2*^{+/+}), $n = 7$ (*Mrgprb2*^{mut/mut}); **d**, $n = 5$ (*Mrgprb2*^{+/+}), $n = 9$ (*Mrgprb2*^{mut/mut}). Mean $\pm$ s.e.m. (**b–d,f**), two-tailed, unpaired t -test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TRPV1⁺ nociceptors activate skin mast cells through MRGPRB2. We next explored whether *D. farinae* and SEB would trigger the degranulation of dermal mast cells in vivo through the activation

of TRPV1⁺*Tac1*⁺ nociceptors and MRGPRB2. A single intradermal (i.d.) injection of sulforhodamine 101-labeled avidin (Av-SRho) can be used to monitor skin mast cell granule structures specifically

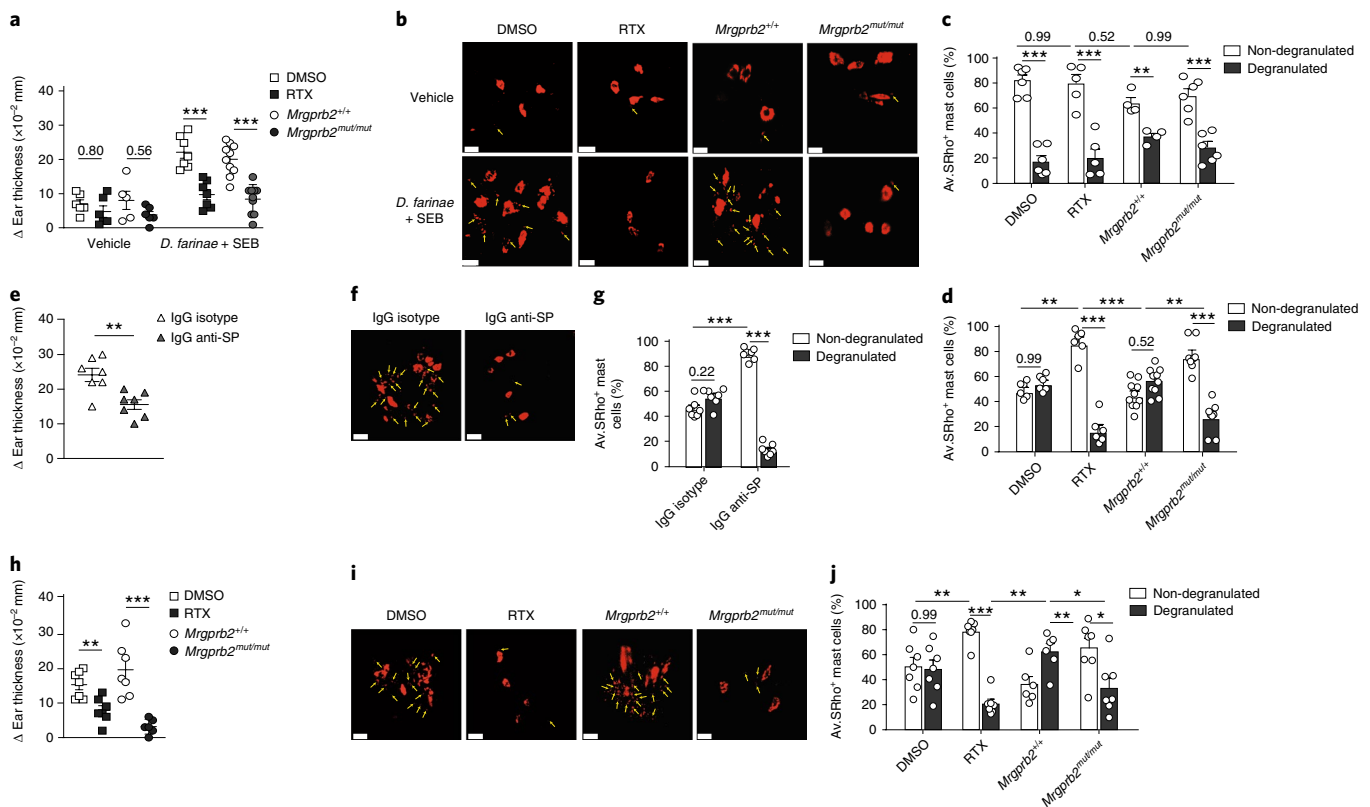


Fig. 5 | Sensory neurons, MRGPRB2 and SP are required to trigger in vivo mast cell degranulation and associated skin swelling in response to *D. farinae* and SEB antigens. **a**, Changes (Δ) in ear thickness 45 min after i.d. injection of vehicle or *D. farinae* + SEB in DMSO-treated (open squares), RTX-treated (gray squares), *Mrgprb2*^{+/+} (open circles) or *Mrgprb2*^{mut/mut} (gray circles) mice. **b**, 3D representative confocal microscopy images of Av.SRho fluorescent signal (red) in whole-mounted ears in mice treated as in **a**. **c**, Quantification of non-degranulated (that is, lacking exteriorized granules, white bars) or degranulated mast cells (that is, presence of exteriorized Av.SRho⁺ granule structures, indicated by yellow arrows in **b** and gray bars) in DMSO-treated, RTX-treated, *Mrgprb2*^{+/+} or *Mrgprb2*^{mut/mut} mice 45 min after i.d. injection of vehicle. **d**, Same experiment as in **c** but 45 min after i.d. injection of *D. farinae* + SEB. **e**, Changes in ear thickness 45 min after i.d. injection of *D. farinae* + SEB with either IgG control isotype (open triangles) or IgG anti-SP (gray triangles) in WT mice. **f**, 3D representative confocal microscopy images of Av.SRho fluorescent signal (red) in whole-mounted ears in mice treated as in **e**. **g**, Quantification of non-degranulated or degranulated mast cells in mice treated as in **e**. **h**, Same experiment as in **a** but 45 min after i.d. injection of capsaicin. **i**, Same experiment as in **b** but 45 min after i.d. injection of capsaicin. **j**, Same experiment as in **d** but 45 min after i.d. injection of capsaicin. Scale bars, 20 μ m. All data are from two independent experiments with the following number of mice: **a–d**, $n = 6$ (DMSO vehicle), $n = 5$ (RTX vehicle), $n = 5$ (*Mrgprb2*^{+/+} vehicle), $n = 6$ (*Mrgprb2*^{mut/mut} vehicle), $n = 7$ (DMSO *D. farinae* + SEB), $n = 7$ (RTX *D. farinae* + SEB), $n = 9$ (*Mrgprb2*^{+/+} *D. farinae* + SEB), $n = 9$ (*Mrgprb2*^{mut/mut} *D. farinae* + SEB); **e–g**, $n = 7$ (IgG control isotype), $n = 7$ (IgG anti-SP); **h–j**, $n = 7$ (DMSO), $n = 7$ (RTX), $n = 7$ (*Mrgprb2*^{+/+}), $n = 7$ (*Mrgprb2*^{mut/mut}). Mean $\pm$ s.e.m. (**a,e,h**), mean $\pm$ s.e.m. (**c,d,g,j**), two-tailed, unpaired *t*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

in living mice by two-photon microscopy, or ex vivo by confocal microscopy³⁷. We injected either vehicle or 1 μ g *D. farinae* + 50 ng SEB i.d. into the pinna of Av.SRho-injected mice and measured tissue swelling, as an early readout of inflammation and Av.SRho⁺ mast cell degranulation, in whole-mounted ear skin using ex vivo volumetric confocal microscopy 45 min following *D. farinae* + SEB injection (Supplementary Fig. 8a). Intradermal injection of vehicle induced very mild swelling of the ear tissue (Fig. 5a) and did not trigger substantial skin mast cell degranulation (Fig. 5b,c) in DMSO-treated WT or *Mrgprb2*^{+/+} mice, while injection of *D. farinae* + SEB induced strong ear swelling (Fig. 5a) and the abundant degranulation of Av.SRho⁺ mast cells (Fig. 5b,d). After injection of *D. farinae* + SEB, RTX-treated WT or *Mrgprb2*^{mut/mut} mice showed a substantial reduction in both ear swelling (Fig. 5a) and degranulation of Av.SRho⁺ mast cells (Fig. 5b,d) compared to DMSO-treated WT or *Mrgprb2*^{+/+} mice. Wild-type mice injected i.d. with blocking antibodies against SP showed a marked reduction in both ear swelling (Fig. 5e) and mast cell degranulation (Fig. 5f,g) compared to IgG isotype-treated control mice. In addition, 30-min pretreatment of *D. farinae* + SEB with the cysteine protease inhibitor E64 decreased ear swelling significantly (Supplementary Fig. 8b,c).

D. farinae and/or SEB did not trigger the degranulation of in vitro cultured mouse or human mast cells at the tested concentrations (Supplementary Fig. 8d,e).

We next tested whether activation of TRPV1⁺ nociceptors in the skin using the TRPV1 agonist capsaicin would result in MRGPRB2-dependent mast cell degranulation in vivo. Intradermal injection of 1 μ M capsaicin induced neither significant ear tissue swelling (Fig. 5h) nor Av.SRho⁺ mast cell degranulation (Fig. 5i,j) in RTX-treated WT or *Mrgprb2*^{mut/mut} mice, while marked ear swelling (Fig. 5h) and linked degranulation of Av.SRho⁺ mast cells (Fig. 5i,j) were observed in DMSO-treated WT and *Mrgprb2*^{+/+} mice, indicating that the effect is largely dependent on TRPV1⁺ nociceptors and MRGPRB2. These results indicate that, following i.d. injection of *D. farinae* + SEB or capsaicin, the observed mast cell degranulation and associated early signs of inflammation require functional TRPV1⁺ nociceptors, release of SP and signaling through MRGPRB2.

Next, we adapted the fluorescent avidin-based mast cell imaging approach to visualize the activation of TRPV1⁺ nociceptors and the activation of mast cells simultaneously using intravital two-photon microscopy³⁷. For this purpose we used Pirt-GCaMP3 mice, in which expression of the calcium tracer GCaMP3 is driven by the

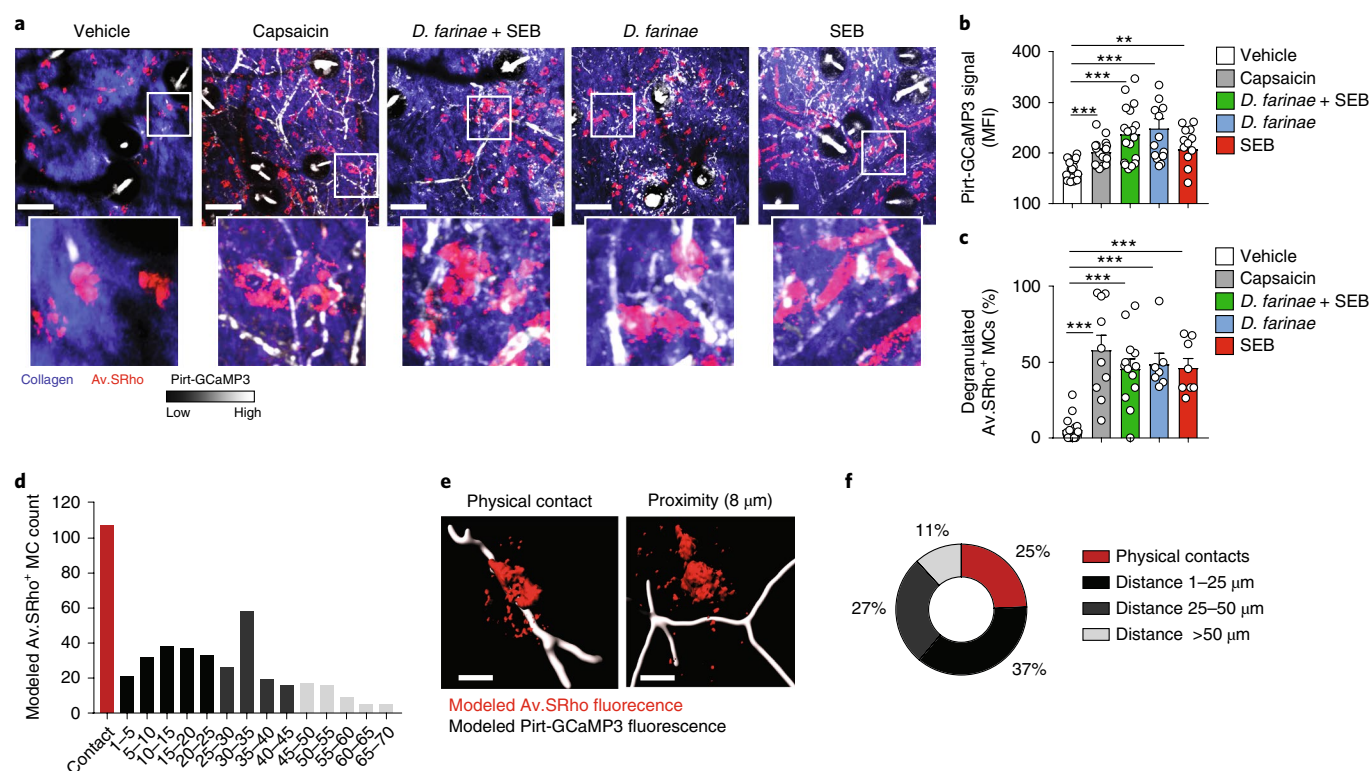


Fig. 6 | Dermal mast cells and TRPV1⁺ nociceptors form sensory clusters in the skin that respond to *D. farinae* and SEB. **a**, Representative high-resolution 3D images of the dermis; merged fluorescence of Av.SRho (red), GCaMP3 calcium tracer (pseudocolor intensity scale) and collagen structures (blue) in Av.SRho-labeled pinna of Pirt-GCaMP3 mice 30–60 min after i.d. injection of vehicle, 1 μ M capsaicin or 1 μ g *D. farinae* and 50 ng SEB (alone or in combination). White lines denote magnified areas shown in lower images. Scale bars, 100 μ m. **b**, Mean fluorescence intensity (MFI) of GCaMP3 fluorescent signal per field of view in mice treated as in **a**. **c**, Proportion (%) of Av.SRho⁺ mast cells with exteriorized granule structures (that is, activated to degranulate) per field of view in mice treated as in **a**. One symbol represents one field analyzed. MC, mast cell. **a–c**, All data are from three independent experiments with the following number of mice: $n = 4$ (vehicle), $n = 5$ (capsaicin), $n = 5$ (*D. farinae*), $n = 3$ (SEB), $n = 5$ (*D. farinae* + SEB). Mean + s.e.m., two-tailed, unpaired *t*-test, ** $P < 0.01$, *** $P < 0.001$. **d**, Automated computational analysis of the minimum distance (μ m) between modeled Av.SRho⁺ mast cell bodies detected and modeled capsaicin-activated TRPV1⁺ Pirt-GCaMP3⁺ sensory neurons. **e**, Examples of modeled Av.SRho⁺ mast cell bodies forming physical contact with (left) or in proximity to (right) modeled capsaicin-activated TRPV1⁺ Pirt-GCaMP3⁺ sensory neurons. **f**, Proportion (%) of 453 modeled Av.SRho⁺ mast cell bodies from the pinnae of three different mice in physical contact with (red), at 1–25 μ m (black), at 25–50 μ m (dark gray) or at >50 μ m (light gray) from modeled capsaicin-activated TRPV1⁺ Pirt-GCaMP3⁺ sensory neurons.

Pirt promoter in sensory neurons³⁸ to track the spatiotemporal dynamics of skin mast cell granule structures and TRPV1⁺ nociceptor activation in living mice (Supplementary Fig. 8f). Low basal expression of GCaMP3 fluorescence (Fig. 6a,b and Supplementary Fig. 8g) in sensory neurons, but no degranulation of Av.SRho⁺ mast cells (Fig. 6a,c and Supplementary Fig. 8h), were detected after i.d. injection of vehicle. In contrast, i.d. injection of 1 μ M capsaicin induced marked increases in GCaMP3 fluorescence (Fig. 6a,b and Supplementary Fig. 8g), revealing the presence of an abundant network of activated TRPV1⁺ nociceptors in the mouse dermis as well as degranulation of ~50–60% of Av.SRho⁺ mast cells (Fig. 6a,c and Supplementary Fig. 8h). After i.d. infusion of 1 μ g *D. farinae* + 50 ng SEB, either in combination or separately, in Av.SRho-labeled Pirt-GCaMP3 mice we observed a significant increase in GCaMP3 fluorescence in skin neurons compared to vehicle-injected mice (Fig. 6a,b), and degranulation of ~50% Av.SRho⁺ mast cells (Fig. 6a,c), suggesting that the presence of *D. farinae* and/or SEB antigens in the dermis could lead to the activation of TRPV1⁺ nociceptors and mast cells in vivo. Finally, we used an automated computer-assisted calculation method to perform an unbiased analysis of the spatial organization of activated TRPV1⁺ nociceptors and Av.SRho⁺ mast cells in the dermis of Pirt-GCaMP3 mice as assessed by live imaging. About 62% of Av.SRho⁺ mast cells either formed contacts with activated TRPV1⁺ nociceptors in the skin

(25%) or resided in close proximity to activated nociceptors (37% of Av.SRho⁺ mast cells were within <25 μ m of activated TRPV1⁺ nociceptors) (Fig. 6d–f). These data suggest that mast cells and TRPV1⁺ nociceptors form cellular clusters in the skin that can be activated in the presence of *D. farinae* and SEB. Together, these results indicate that the ear swelling observed following i.d. injection of *D. farinae* + SEB resulted from the activation of skin TRPV1⁺ nociceptors that induced the degranulation of mast cells through the release of SP and activation of MRGPRB2.

Discussion

Here we found that TRPV1⁺*Tac1*⁺ nociceptors and MRGPRB2⁺ mast cells formed clusters in the mouse skin that could be activated by allergens such as HDMs. This, in turn, drove the development of type 2 immunity-associated skin inflammation in mice that mimicked many features of human AD. In addition, our results identified MRGPRB2 as a key receptor that facilitated communication between skin mast cells and TRPV1⁺*Tac1*⁺ nociceptors.

Based on the high-resolution, three-dimensional (3D) images generated, it is interesting to speculate that the close anatomical co-localization of mast cells and TRPV1⁺ nociceptors in the mouse dermis might enable the accumulation of sufficiently high amounts of secreted neuropeptide such as SP to reach the previously reported high activation threshold of MRGPRB2 (ref. ³⁹). Fixed biopsies

have indicated proximity or contact between nerve fibers and mast cells in mice, pigs and humans^{40–43}, while mouse and human mast cells can be directly activated by neuropeptides (including SP) in vitro^{25,42,44}. A bi-directional activation process between mast cells and submucosal neurons in cultured gut biopsies has been reported⁴⁵. Our study offers direct evidence of functional interactions between nociceptors and mast cells in vivo, and identified these interactions as a critical element in the development of type 2 skin inflammation. Targeting SP-enabled interactions between TRPV1⁺ nociceptors and MRGPRX2⁺ mast cells might therefore represent a promising therapeutic approach for the prevention and/or treatment of human AD pathology.

Recent studies have reported that MRGPRB2 on mast cells has an important role in the regulation of inflammatory pain⁴⁶ and itch sensation⁴⁷. SP and MRGPRB2 were reported to be directly involved in the regulation of inflammatory mechanical and thermal hyperalgesia, and in the recruitment of innate immune cells at the site of injury⁴⁶. In addition, compared to activation through canonical FcεRI, mast cell activation through MRGPRB2 induces itch distinct from classical histaminergic itch⁴⁷. MRGPRB2-mediated activation of mast cells results in the differential release of granule-associated mediators (including some with known pruritogen function such as histamine, serotonin or tryptase), and MRGPRB2 deficiency significantly decreases itch in different models of allergic contact dermatitis, which is thought to be associated with pathogenic CD8⁺ T cell responses⁴⁸. These observations indicate that MRGPRB2 activation on mast cells modulates the transmission of sensations of pain and itch in different inflammatory contexts.

Mast cells can drive certain types of IgE-independent pseudo-allergy reactions through the binding of a wide range of cationic drugs (such as the U.S. Food and Drug Administration-approved mivacurium or icatibant) to MRGPRX2 or MRGPRB2 (refs. 24,49,50). It is tempting to speculate that MRGPRX2- or MRGPRB2-mediated mast cell activation by cationic drugs could lead to the activation of nociceptors at the site of injection and the subsequent development of pain and/or itch. However, further in vivo work is needed to define whether the clusters of TRPV1⁺Tac1⁺ nociceptors and MRGPRB2⁺ mast cells described here are involved in drug-induced adverse events.

We found that *D. farinae*, but not SEB, directly activated TRPV1⁺ DRG neurons ex vivo through a cysteine protease-dependent mechanism. We cannot rule out the possibility that direct effects of *D. farinae* and/or SEB on skin mast cells, apart from effects on degranulation, may also have contributed to our findings. However, the data presented suggest that, under the conditions studied, *D. farinae* + SEB-induced cutaneous inflammation required TRPV1⁺ nociceptors and the release of SP, which then activated skin mast cells.

Most patients with severe AD are colonized with exotoxin-producing *S. aureus*^{4,6,7}. In agreement with that, we found that *D. farinae* in combination with SEB triggered the development of more severe skin inflammation than when the two antigens were used separately. While SEB alone did not activate DRG neurons cultured ex vivo, following i.d. injection in vivo it triggered GCaMP3 fluorescence in skin neurons comparable to that observed after injection of *D. farinae*. This might indicate that SEB activates nociceptors through an indirect mechanism; however, the precise mechanism(s) remain to be elucidated. It is possible that SEB, which is known to be a potent superantigen, could also favor the proliferation of T cells in our model. However, SEB treatment alone in WT mice triggered less severe skin inflammation and skin lesions than treatment with *D. farinae* alone. It is therefore possible that a direct mechanism of nociceptor activation by *D. farinae* cysteine protease activity might be required to efficiently trigger the development of this model of *D. farinae* + SEB-induced allergic skin inflammation.

The sensing of allergen-associated proteolytic activity has been proposed to be an important mechanism of environmental allergen detection that could contribute to the initiation of allergic

diseases^{31,36}. Our data suggest that the clusters of TRPV1⁺ nociceptors and MRGPRB2⁺ mast cells might represent tissue-resident 'sensory systems' that would detect allergens with cysteine-like protease activity in the skin and initiate type 2 immunity-associated allergic skin disease. Sensory nerve fibers and mast cells are also present in the upper airways, lungs and gastrointestinal tract, which are organs continuously exposed to environmental allergens and within which allergic diseases are also known to develop. However, the extent to which neuron–mast cell clusters are involved in the development of other allergic disorders in different organs remains to be investigated.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at <https://doi.org/10.1038/s41590-019-0493-z>.

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Author contributions

S.J.G. and N.G. conceived the project. All authors were involved in experimental design. N.S., L.B. and N.G. performed most experiments and compiled the data. R.S., C.B., J.M., C.P., T.M., P.S., L.L.R., N.C., X.D. and M.T. helped with experiments. All authors participated in analyzing the data and writing or editing the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Mice. Four- to 8-week-old C57BL/6J and *Tac1*^{-/-} mice were purchased from Charles River or the Jackson Laboratory; both age-matched male and female mice were used in experiments. *Mrgprb2*^{mut/mut} mice (which are homozygous for a mutation that induces a genetic inactivation of *Mrgprb2* (ref. 24)) and *Pirt*-GCaMP3 mice (in which a genetically encoded calcium tracer (GCaMP3) is driven into sensory neurons by the *Pirt* promoter³⁸) were provided by X.D., and both male and female mice were used in experiments. The mast cell-deficient *Kit*^{W-sh/W-sh} and *Cpa3-cre*⁺; *Mcl-1*^{fl/fl} mice have been described previously^{25,37}; both male and female mice were used in experiments. PAR-2-deficient mice were a generous gift from N. Vergnolle. Mice were bred and housed in the local animal facilities of CREFRE (Toulouse, France) or Stanford University (CA, USA), and littermate control mice were used in all experiments. All animal care and experimentation were conducted in France and the USA. Experiments conducted in the USA (Galli Lab, Stanford University, CA) were in compliance with the guidelines of the National Institutes of Health (NIH) and the Institutional Animal Care and Use Committee of Stanford University. Experiments conducted in France (Gaudenzio Lab, INSERM, University of Toulouse) were in compliance with the guidelines of the European Union (No. 86/609/EEC) and the French Committee of Ethics (No. 87/848) policies, and with the specific approval of the local ministry-approved committee on ethics in animal experimentation (Ethics Committee UMS006 CEEA-122, project No. 13283 2018031416055447V3). Eight- to 12-week-old mice were used in experiments, except for RTX treatment in which 4-week-old mice were used.

Reagents and antibodies. Sodium citrate, bovine serum albumin (BSA), DMSO, saponin, capsaicin, RTX and SEB were obtained from Sigma-Aldrich. HDM extracts of the strain *D. farinae* were purchased from Greer Laboratories. The following antibodies were obtained from Covance: anti-Keratin (K) 14, anti-K6, anti-K10, anti-loricrin and anti-filaggrin. Anti-Claudin 1 was acquired from Abcam. Alexa594-conjugated goat anti-rabbit IgG, Alexa488-conjugated avidin and DAPI were obtained from Life Technologies Invitrogen. The following reagent and antibodies were acquired from eBioscience Thermo Fisher Scientific: CellTrace CFSE Cell Proliferation Kit, anti-CD4-APC, anti-IL-4-PE, anti-IL-5-PE, anti-IL-13-PE and anti-IFN γ -PE. Purified NA/LE Rat Anti-Mouse CD124 (mIL4R-M1) or control Istoppe (R35-95) were acquired from BD Bioscience. MYD88 inhibitory peptide (that is, a cell-permeant peptide sequence that selectively blocks MYD88 homodimerization) and a control peptide (that is, an inactive and cell-permeant truncated form of the MYD88 inhibitory peptide) were obtained from Novus. *Alternaria alternata*, *D. pteronyssinus*, ragweed and German cockroach were all acquired from Stallergenes Greer.

Model of allergic skin inflammation. Allergic skin inflammation was induced as previously described^{8,27} (see Supplementary Fig. 1). Briefly, dorsal skin was shaved and a solution of 500 ng SEB (Sigma-Aldrich) and 10 μ g *D. farinae* extract (HDM, Stallergenes Greer) in PBS was applied on a gauze pad placed on the shaved skin and occluded with a Tegaderm Transparent Dressing (3 M HealthCare). Three days later, the gauze pads were replaced. Mice were monitored on a daily basis and, if a mouse removed the bandage, a new dressing was immediately applied and also to all of the other mice within the same experiment (so that they received the same treatment and equal amounts of antigens). Four days later, dressings were removed and mice were kept without treatment for the next week. This '3 + 4-d' pattern of treatment was repeated twice more, so that the mice were subjected to three cycles of such treatment. Two days after the last cycle of treatment, the mice were euthanized and dorsal skin specimens corresponding to the treated areas were obtained for analyses. In a set of experiments, mice were also treated with either IgG isotype control or anti-CD124 antibody (100 μ l intraperitoneally) at 2.8 mg kg⁻¹ twice per week for the entire period of treatment. The last treatment with either of these antibodies was done 1 d before sacrifice. This model has been efficiently used in both Inserm Toulouse and Stanford University, and key experiments have been repeated in both animal facilities.

Ablation of Trpv1⁺ nociceptors in mice. To ablate Trpv1⁺ nociceptors, 4-week-old C57BL/6J mice were subcutaneously injected with increasing RTX doses of 30, 70 and 100 μ g kg⁻¹ in 100 μ l of PBS for three consecutive days. Control mice were injected with similar volumes of DMSO in 100 μ l of PBS. Four weeks later, denervation was assessed using the classical tail flick assay.

Peripheral blood mononuclear cell-derived human mast cell culture. Peripheral blood mononuclear cells were obtained from the buffy coat of healthy blood donors at the Etablissement Français du Sang and were cultured as described in ref. 25. Briefly, isolated CD34⁺ cells (EasySep Human CD34 Positive Selection Kit, STEMCELL Technologies) were cultured for 1–2 weeks in StemSpan medium (STEMCELL Technologies) supplemented with recombinant human IL-6 (50 ng ml⁻¹; Peprotech), human IL-3 (10 ng ml⁻¹; Peprotech), ciprofloxacin (10 μ g ml⁻¹; Sigma-Aldrich) and 3% supernatant of Chinese hamster ovary transfectants secreting mouse SCF. After 2 weeks, cells were transferred to IMDM Glutamax supplemented with sodium pyruvate, 2- β -mercaptoethanol (50 μ M), 0.5% BSA, 1% insulin transferrin selenium (all from Invitrogen), ciprofloxacin (10 μ g ml⁻¹; Sigma-Aldrich), IL-6 (50 ng ml⁻¹) and 3% supernatant of Chinese hamster ovary

transfectants secreting mouse SCF. Mast cells were usually ready for experiments after ~10 weeks in culture.

Bone marrow-derived cultured mouse mast cells (BMCMCs). Hematopoietic cells were collected from C57BL/6 mouse femurs and cultured in DMEM supplemented with Glutamax, 2- β -mercaptoethanol (50 μ M; Invitrogen), murine IL-3 (10 ng ml⁻¹; Peprotech), 10% fetal bovine serum (FBS, Invitrogen) and pen/strep (100 $\times$). Twenty-four hours later, adherent cells were removed and fresh culture medium was added to non-adherent cells. Three days later, cells were resuspended in fresh culture medium. The same procedure was repeated twice per week. Cells were used for experiments between weeks 4 and 10.

β -Hexosaminidase release assay. Human mast cells were incubated in culture medium with or without human IgE (1 μ g ml⁻¹; Sigma-Aldrich) overnight at 37 °C. BMCMCs were incubated with or without anti-dinitrophenol (DNP) IgE (0.5 μ g ml⁻¹; Sigma-Aldrich) overnight at 37 °C. The cells were then washed and distributed in 96-well, flat-bottom plates at a density of 10⁵ cells in 50 μ l of Tyrode's buffer at 37 °C. Forty minutes later, cells were treated with 50 μ l of prewarmed stimuli diluted in Tyrode's buffer for 45 min at 37 °C. As a positive control of functional activation, human mast cells were stimulated with anti-IgE (10 ng ml⁻¹; Euromedex) and BMCMCs with DNP-BSA (10 ng ml⁻¹; Sigma-Aldrich). To assess whether AD antigens could activate mast cells, human mast cells and BMCMCs were stimulated with increasing concentrations of *D. farinae* + SEB (5, 50 and 500 ng ml⁻¹), alone or together. β -Hexosaminidase release in the supernatants was measured as previously described²⁵.

Intracellular flow cytometry of *D. farinae*-specific CD4⁺ T cells. Following induction of allergic skin inflammation, spleens from vehicle-treated or *D. farinae* + SEB-treated mice were harvested and dissociated to obtain a suspension of cells. A total of 200,000 spleen cells was stained with CFSE for 7 min at 37 °C and incubated for 5 d with 10 μ g ml⁻¹ *D. farinae* in RPMI 1640 supplemented with 10% FCS GlutaMAX-I, sodium pyruvate, 2-mercaptoethanol and 10 μ g ml⁻¹ ciprofloxacin. Intracellular cytokines were analyzed by gating on proliferating (CFSE^{low}) CD4⁺ T cells after a 5-h restimulation with phorbol 12-myristate 13-acetate (50 ng ml⁻¹; Sigma) and ionomycin (1 mg ml⁻¹; Sigma) in the presence of GolgiStop (BD Pharmingen). Cells were fixed, permeabilized (0.1% saponin in PBS 0.5% BSA) and stained with antibodies directed against mouse IL-4, IL-5, IL-13 or IFN γ . Flow cytometric data were acquired on a BD FACSCanto cytometer and were analyzed using FlowJo software (Tree Star, Inc.).

Skin section preparation, histology, immunofluorescence and confocal microscopy. Mouse dorsal skin (1–2 cm²) samples were fixed in 10% formalin and embedded in paraffin. Four-micrometer-thick sections were stained with H&E, and photographs were taken using a Nikon H600L microscope and analyzed with NIS-Elements imaging Software. All sections were coded so that the evaluator was not aware of their identity, as previously described⁸. Eosinophils and neutrophils were counted on H&E-stained skin sections from all mouse strains. For immunostaining of mouse specimens, 4- μ m-thick sections were pretreated using a heat-induced epitope retrieval method⁹ in 10 mM sodium citrate buffer (pH 6.0), then permeabilized for 30 min in PBS supplemented with 0.5% BSA and 0.1% saponin. Permeabilized skin sections were incubated overnight at 4 °C with primary antibodies, extensively washed and incubated with appropriate secondary antibodies for 2 h at room temperature in the dark. Images of 1,024 $\times$ 1,024 pixels were acquired using Zeiss LSM780 and LSM710 Meta inverted confocal laser-scanning microscopes. Images were processed using Zen software (Zeiss). Epidermal K6, K17, Claudin 1, filaggrin, loricrin and e-cadherin mean fluorescence intensities were analyzed using the 'measurement function' of ImageJ software on randomly chosen epidermal areas of identical size (that is, same total number of pixels).

Detection of levels of serum *D. farinae*-specific IgG1 and IgE antibodies. Each incubation step described below was followed by three to five washing steps using PBS containing 0.05% Tween-20. MaxiSorp enzyme-linked immunosorbent assay plates (Nunc) were coated with 5 μ g ml⁻¹ *D. farinae* at 4 °C overnight. Plates were then blocked with 1% BSA in PBS for at least 2 h at room temperature. Sera diluted in PBS containing 1% BSA were added and incubated in the blocked wells for 2 h at 37 °C. Afterwards, biotinylated detection antibodies (rat anti-mouse IgG1 and rat anti-mouse IgE, 1/1,000; BD Biosciences) were then added for 1 h at room temperature followed by incubation with horseradish peroxidase-conjugated streptavidin (1/2,000, BD Biosciences) for 30 min at room temperature. Reaction was then revealed using supersensitive 3,3',5,5'-tetramethylbenzidine (Sigma-Aldrich) substrate, and OD was measured at 450 nm.

***D. farinae* proteolytic activity assay.** *Dermatophagoides farinae* samples were diluted to 200 μ g ml⁻¹ in a reaction buffer composed of sodium phosphate 100 mM pH 6.0, EDTA 10 mM and DTT 1 mM. The extract was incubated or not with irreversible protease inhibitors (cysteine protease inhibitor E64 100 nM or serine protease inhibitor AEBSF 1 mM) in a reaction volume of 50 μ l. Activity was measured using Boc-Gln-Ala-Arg-AMC fluorogenic substrate (200 μ M, diluted in the same reaction buffer). *D. farinae* extracts and substrate solutions were

incubated separately for 20 min at 37 °C. Immediately after the addition of 50 μ l of substrate solution to *D. farinae* extracts, the release of AMC fluorescence was followed over time using a Varioskan Flash apparatus (Thermo Fisher Scientific). The initial velocity reflecting proteolytic activity was calculated from the kinetics curves. A control was performed by heat inactivation of *D. farinae* extract for 60 min at 95 °C before the addition of substrate. An additional control was performed in a reaction buffer devoid of DTT.

DRG dissociation, culture and Ca^{2+} imaging. Dorsal root ganglion neurons from all spinal levels were collected in ice-cold DMEM/F-12 supplemented with 10% FBS, penicillin (100 U ml^{-1}) and streptomycin (100 $\mu\text{g ml}^{-1}$). DRG were digested with a mixture of dispase (5 mg ml^{-1}) and collagenase type I (1 mg ml^{-1}) enzyme at 37 °C for 45 min. After dissociation, cells were spun at 300g and resuspended in medium before being plated on glass coverslips coated with poly-D-lysine (0.5 mg ml^{-1}) and laminin (10 $\mu\text{g ml}^{-1}$; Invitrogen) or Lab-tek II chambers slide with cover (Thermo Fisher Scientific). DRG were cultured in medium supplemented with 50 ng ml^{-1} nerve growth factor at 37 °C overnight (12–24 h) before experimentation. In some experiments either control peptide or MyD88 inhibitory peptide (100 μM) was added to the DRG cultures for 16 h. Cells were imaged in calcium imaging buffer (CIB; 10 mM HEPES, 1.2 mM NaHCO_3 , 130 mM NaCl, 3 mM KCl, 2.5 mM CaCl_2 , 0.6 mM MgCl_2 , 20 mM glucose and 20 mM sucrose at pH 7.4 and 290–300 mOsm) or Hanks' buffered salt solution (HBSS). To monitor changes in intracellular $[\text{Ca}^{2+}]$ ($[\text{Ca}^{2+}]_i$), cells were loaded with Fura-2-AM or Fluo-4 for 30 min in the dark at 37 °C in CIB or HEPES/HBSS immediately before imaging. Emission at 520 nm was monitored after excitation at 340 nm (Ca^{2+} bound) or 380 nm (unbound). Cells were imaged for 20 s to establish a baseline before compounds were added. DRG were stimulated with 5 ng ml^{-1} *D. farinae*, 5 ng ml^{-1} heat-treated *D. farinae* (95 °C for 60 min), 5 ng ml^{-1} *D. farinae* treated with 2.5 pM E64 (37 °C for 30 min), 5 ng ml^{-1} SEB or 1 μM capsaicin. At the end of every imaging trial, 50 mM KCl was added as a positive control. Cells were identified as responding if the intracellular $[\text{Ca}^{2+}]$ rose by either 50% compared to baseline or 50% compared to the $[\text{Ca}^{2+}]_i$ change assayed during the addition of 50 mM KCl (neurons only). Damaged, detached, high-baseline and motion-activated cells were excluded from analyses. Those experiments were performed in the Dong Lab at Johns Hopkins University (and confirmed in the Gaudenzio Lab at INSERM, Toulouse France) in compliance with Johns Hopkins University ethical guidelines and Inserm Toulouse guidelines.

Quantification of SP secretion. Dorsal root ganglia of mice were cultured as described above, rinsed in HBSS (Sigma) and incubated in L-cysteine containing 1% papain (Sigma) for 10 min at 37 °C. After one wash with filtered Leibovitz's L-15 medium (Gibco) containing 10% FBS (Sigma), they were incubated in HBSS containing collagenase (1 mg ml^{-1} ; Sigma) and dispase (4 mg ml^{-1} ; Sigma) at 37 °C for 10 min. After titration, cells were plated in a poly-D-lysine–laminin (Sigma)-coated 12-well-plate (Corning) and recovered with the complete culture medium (DMEM–Glutamax, 2.5% FBS, 1% pen/strep and ARAC, FUDR, uridine (10 μM each; all from Sigma)). Cells were incubated for 5 min with vehicle or 5 ng ml^{-1} *D. farinae* or 5 ng ml^{-1} SEB and/or 125 nM capsaicin. Cell supernatants were tested with a Substance P Elisa kit (Enzo) following the manufacturer's recommendations.

Pinna injection, mast cell staining and ear thickness measurement. Av.SRho (8 μg) in 20 μl of PBS was injected i.d. into both pinnae of WT, *Mrgprb2^{mut/mut}* and RTX-treated mice, and the respective *Mrgprb2^{+/+}* or DMSO-treated control mice, to selectively label mast cell secretory granules in vivo, as previously described³⁷. Seven days later, ear thickness was measured and the right pinna injected i.d. with 1 μg *D. farinae* + 50 ng SEB (pre-incubated or not for 30 min at 37 °C with 100 nM E64 cysteine protease inhibitor) or 1 μM capsaicin in 20 μl of PBS. In some experiments, pinnae were injected i.d. with 1 μg *D. farinae* + 50 ng SEB with 15 μg of IgG anti-SP (Sigma-Aldrich) in 20 μl of PBS. Left pinnae were injected with 20 μl of the respective control solutions: vehicle alone or in combination with IgG isotype control. Forty-five minutes later, ear thickness was measured

again and mice were euthanized before ear excision. Whole ears were fixed in 4% paraformaldehyde overnight, then mounted on microscopy slides and placed under a LSM710 Meta inverted confocal laser-scanning microscope. High-resolution z-stack images (1,024 $\times$ 1,024 pixels) of Av.SRho fluorescent signal were acquired using a $\times 20$ objective. Images were then processed using ImageJ and Imaris (Bitplane) software.

In vivo two-photon microscopy of living mice. Experiments were conducted as previously described^{25,37}. Briefly, 8 μg Av.SRho in 20 μl of PBS was injected i.d. into the pinna of Pirt-GCaMP3 mice. One week later, mice were injected i.d. with vehicle, 1 μM capsaicin or 1 μg *D. farinae* and 50 ng SEB (used alone or in combination) in a final volume of 20 μl , then placed under the two-photon microscope on a custom-built 3D printed mouse platform; anesthesia was maintained by a mixture of isoflurane and O_2 , and the animal's pinna was kept at 36 °C using a heating pad system. Fluorescence corresponding to Av.SRho⁺ mast cell granule structures or GCaMP3⁺ skin neurons was measured using a Prairie Ultima IV two-photon microscope (Spectra Physics Mai Tai HP Ti:sapphire laser, tunable from 690 to 1,040 nm). Images were acquired in 3D at up to 100–150 μm depth, with a $\times 20/0.95$ numerical aperture Olympus XLUM Plan Fl water-immersion objective and a software zoom setting of 1 or 3 (eight bits per pixel 1,024 $\times$ 1,024, scaling $x = 0.228 \mu\text{m}$, $y = 0.228 \mu\text{m}$, $z = 0.5 \mu\text{m}$). Modeling and analysis of fluorescent signals were performed on untreated image sequences, as previously described²⁵, using Imaris software (Bitplane) and ImageJ software version Fiji, respectively.

Automated computational analysis of the minimum distance between mast cells and neurons in the dermis of living mice. Three-dimensional, high-resolution images were taken using a Prairie Ultima IV two-photon microscope as described above. The following steps have been automated in the software Imaris Bitplane v.9.2. (1) Hair follicle autofluorescent signals were modeled into matched 3D objects using the isosurface algorithm. (2) Autofluorescent signals corresponding to the generated isosurfaces were depleted from the GCaMP3 fluorescence detection channel so that hair follicles were no longer detectable in that particular channel. (3) The filament tracer algorithm was applied in the GCaMP3 fluorescence detection channel for precise tracing of the trajectories and exact shapes of GCaMP3 fluorescent signals. Filament traces were then converted into fluorescent signals in a new fluorescent channel. (4) Those newly generated fluorescent signals were modeled into matched 3D objects using the isosurface algorithm. The distance transformation algorithm was applied to those new isosurfaces, resulting in the generation of a new distance transformation channel. (5) Av.SRho fluorescent signals were modeled into matched 3D objects using the isosurface algorithm. The intensity minimum (that is, distance minimum in micrometers) to the distance transformation channel (that is, modeled sensory neurons) was calculated for all Av.SRho⁺ isosurfaces of at least 5 μm diameter (corresponding to Av.SRho⁺ mast cell cellular bodies and excluding small exteriorized Av.SRho⁺ granule structures). (6) Results per field of view were generated into separated Excel sheets. The same procedure was automatically applied to all 3D images analyzed.

Statistics. Statistical tests were performed with the software Prism 6 (GraphPad Software). Two-tailed unpaired/paired Student's *t*-tests, one-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons or Holm–Sidak's post hoc test were performed on samples as noted in the respective figure legends. $P < 0.05$ was considered statistically significant.

Reporting Summary. Further information on research design is available in the Life Sciences Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

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Data collection

Zen software, Prairie/Brucker, Diva

Data analysis

Image J, GraphPad Prism 6, Imaris, Flow Jo

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Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	<i>We have reduced the number of mice as far as possible. We have estimated the numbers of mice that were needed to analyze and detect differences in clinical scores based on previous preliminary data. We estimated that the difference in the mean value of the clinical score with an estimated 1.2 standard deviation, we needed 5/6 mice per group (total) to give us 90% power to detect a difference in clinical score at statistical significance of $p \leq 0.05$ using a two tailed, unpaired t test based on preliminary results using vehicle vs. treated WT C57Bl/6 mice. Based on this estimation, we have adapted the size of each group of mice related to in vivo experiments employing our model of allergic skin inflammation.</i>
Data exclusions	<i>No data were excluded.</i>
Replication	<i>All experiments were repeated at least 2-3 times and each experiments gave similar results.</i>
Randomization	<i>Allocation of mice in each experimental group was random as we used all littermate controls in each experiments. Therefore, we performed experiment independently of the genotype or sex and analyze the results for each mouse at the end of the experimental procedure.</i>
Blinding	<i>Regarding experiments with mice treated to develop an allergic skin inflammation, investigators were blinded during data collection until the genotype was revealed. Regarding intravital imaging, we needed transgenic mice expressing a genetically-encoded calcium tracer to visualize neurons and mast cells in vivo, therefore mice were genotyped before performing imaging experiments described in Figure 4. However, we developed an automated computational analysis of the minimum distance between mast cells and neurons in the dermis of living mice. This enabled us to perform an unbiased analysis of neuron-mast cell clusters in the skin of those transgenic mice.</i>

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).</i>
Research sample	<i>State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.</i>
Sampling strategy	<i>Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.</i>
Data collection	<i>Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.</i>
Timing	<i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Non-participation	<i>State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.</i>
Randomization	<i>If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.</i>

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.</i>
Research sample	<i>Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i>, all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i>

Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
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Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field work?	☐ Yes ☐ No

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access and import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-Filaggrin Polyclonal Antibody (Ozyme, BLE905801, Poly19058, 1/50) Anti-Loricrin Polyclonal Antibody (Ozyme, BLE905101, Poly19051, 1/50) Anti-Keratin 14 Polyclonal Antibody (Ozyme, BLE905301, Poly19053, 1/50) Anti-Keratin 10 Polyclonal Antibody (Ozyme, BLE905401, Poly19054, 1/50) Anti-mouse Keratin 6A (Ozyme, BLE905701, Poly19057, 1/50) Mouse Anti-E-Cadherin (BD Biosciences, 610181, Clone 36/E-Cadherin, 1/50) Anti-Claudin 1 Antibody (abcam, ab15098, 1/50) Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Invitrogen, A31571, 1/200) Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, A11012, 1/200) F(ab') ₂ -Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, A11072, 1/200) Anti-CD4-APC (eBioscience, 17-0041-82, Clone GK1.5, 1/200) Anti-IL4-PE (eBioscience, 12-7041-82, Clone 11B11, 1/200) Anti-IL5-PE (eBioscience, 12-7052-82, Clone TRFK5, 1/200) Anti-IL13-PE (eBioscience, 12-7133-82, Clone eBIO13A, 1/200)
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Anti-IFN-g-PE (eBioscience, 12-7311-82, Clone XMG1.2, 1/200)
 Purified NA/LE Rat Anti-Mouse CD124 (BDPharmingen, 552288, mIL4R-M1, in vivo 2.8mg/kg)
 Purified NA/LE Rat IgG2a k Isotype control (BDPharmingen, 554687, R35-95, in vivo 2.8mg/kg)
 Anti-IgE (BD bioscience, 553419, R35-118)
 Anti-IgG1 (BD bioscience, 550331, A85-1)
 Polyclonal Rabbit Anti-Substance-P (Millipore, AB1566, Lot 3184744, 15µg/ear)

Validation

Anti-Filaggrin Polyclonal Antibody, RRID:AB_2616957
 Anti-Loricrin Polyclonal Antibody, RRID:AB_2565046
 Anti-Keratin 14 Polyclonal Antibody, RRID:AB_2565048
 Anti-Keratin 10 Polyclonal Antibody, RRID:AB_2565049
 Anti-mouse Keratin 6A antibody, RRID:AB_2565052
 Mouse Anti-E-Cadherin, RRID:AB_397580
 Anti-Claudin 1 Antibody, RRID:AB_301644
 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647, RRID:AB_162542
 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, RRID:AB_141359
 F(ab')₂-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, RRID:AB_2534116
 Anti-CD4-APC, RRID:AB_469320
 Anti-IL4-PE, RRID:AB_466156
 Anti-IL5-PE, RRID:AB_763587
 Anti-IL13-PE, RRID:AB_763559
 Anti-IFN-g-PE, RRID:AB_466193
 Purified NA/LE Rat Anti-Mouse CD124, RRID:AB_394356, in vivo use has been published in Li, G. et al. IL-4 receptor blockade abrogates satellite cell: rhabdomyosarcoma fusion and prevents tumor establishment. *Stem cells* 31, 2304-2312, doi:10.1002/stem.1491 (2013).
 Purified NA/LE Rat IgG2a k Isotype control, RRID:AB_479678
 Anti-IgE, RRID:AB_394850
 Anti-gG1, RRID:AB_2296342
 Polyclonal Rabbit Anti-Substance-P, RRID:AB_2271494, in vivo use has been published in Green, D. P., Limjunyawong, N., Gour, N., Pundir, P. & Dong, X. A Mast-Cell-Specific Receptor Mediates Neurogenic Inflammation and Pain. *Neuron* 101, 412-420 e413, doi:10.1016/j.neuron.2019.01.012 (2019).

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	State the source of each cell line used.
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.
Mycoplasma contamination	Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology

Specimen provenance	Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	This study involve different mouse strains: C57Bl/6J WT, Tac1ko, Mrgprb2mutant, KitW-sh/W-sh, Cpa3-cre;Mcl1fl/fl, PAR2ko and Pirt-GCaMP3 mice. Mice were male and female, 8-12-week old of age (excepted noted otherwise)
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal care and experimentation were conducted in France and in the USA. Experimentations conducted in USA (Galli Lab, Stanford University, CA) were in compliance with the guidelines of the National Institutes of Health (NIH) and the Institutional

Animal Care and Use Committee of Stanford University. Experimentations conducted in France (Gaudenzio Lab, INSERM, University of Toulouse) were in compliance with the guidelines of the European Union (86/609/EEC) and the French Committee of Ethics (87/848) policies and with the specific approval from the local ministry-approved committee on ethics in animal experimentation (Ethics Committee UMS006 CEEA-122, project n° 13283 2018031416055447V3).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."
Recruitment	Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.
Ethics oversight	Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. UCSC)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Following induction of allergic skin inflammation, spleens from vehicle-treated or D. farinae + SEB-treated mice were harvested and dissociated to obtain a suspension of cells. 200,000 spleen cells were stained with CFSE for 7 minutes at 37°C and incubated for 5 days with 10 ug/ml of D. farinae in RPMI 1640 supplemented with 10% FCS, GlutaMAX-I, sodium pyruvate, 2-mercaptoethanol, and ciprofloxacin. Intracellular cytokines were analyzed by gating on proliferating (CFSElow) CD4-APC+ T cells after 5-hour restimulation with phorbol 12-myristate 13-acetate (50 ng/mL, Sigma) and ionomycin (1 mg/mL, Sigma) in the presence of GolgiStop (BD Pharmingen). Cells were fixed, permeabilized (0.1% saponin in PBS 0.5% BSA), and stained with antibodies directed against mouse IL-4-PE, IL-5-PE, IL-13-PE or IFN-g-PE. Flow cytometric data were acquired on a BD FACSCanto cytometer and were analyzed using FlowJo software (Tree Star, Inc, Ashland, Ore).

Instrument

BD FACS CANTO

Software

FlowJo software (Tree Star, Inc, Ashland, Ore)

Cell population abundance

We analyzed CD4+ T cells that represented ~ 20-25% of recovered splenic cells.

Gating strategy

We gated on lymphocytes based on size and then on CD4+ cells to study CD4+ T cells cytokine profile (Supplementary Figure 1f)

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ BothStatistic type for inference
(See [Eklund et al. 2016](#))

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a | Involved in the study

☐ ☐ Functional and/or effective connectivity☐ ☐ Graph analysis☐ ☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

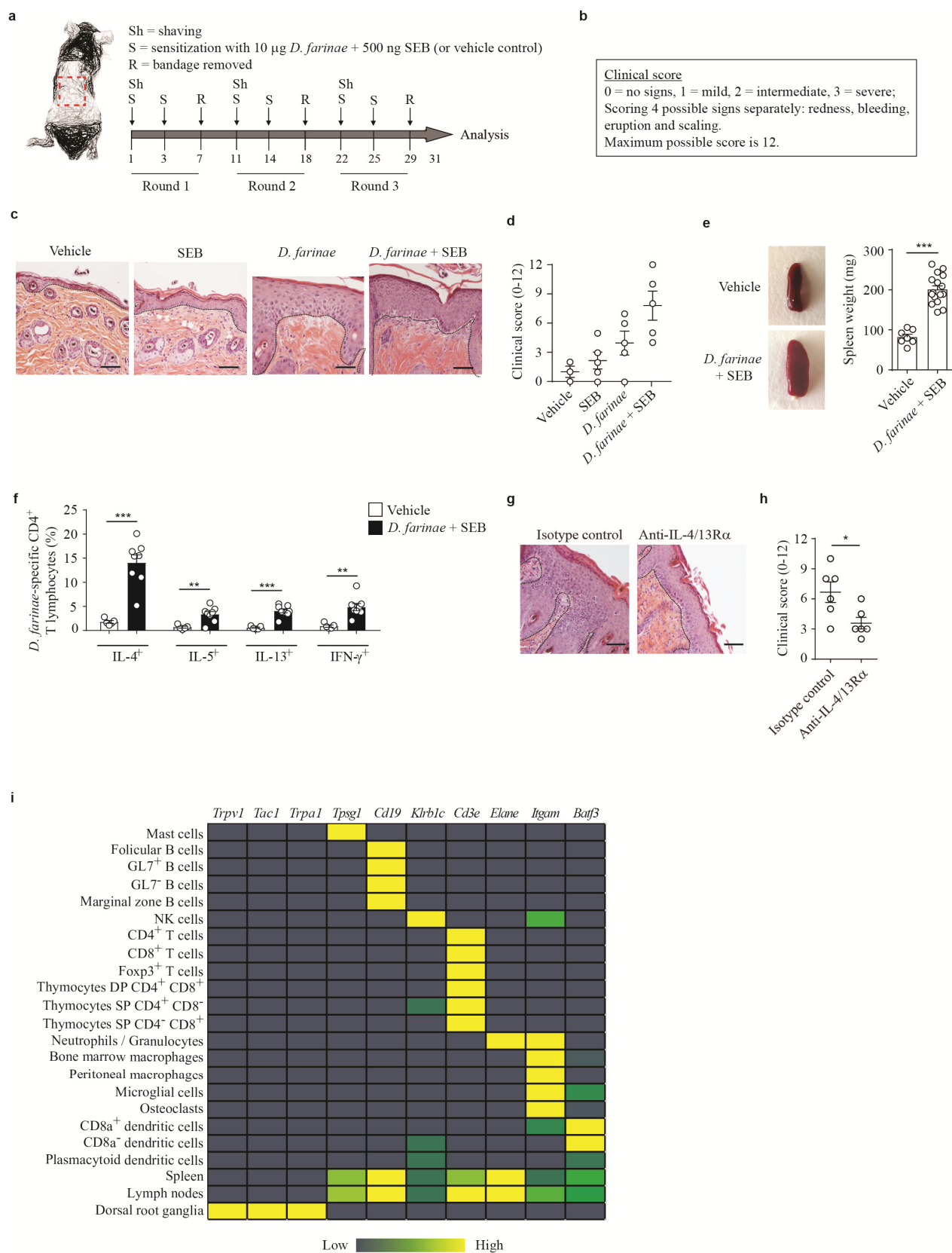
Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

In the format provided by the authors and unedited.

House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation

Nadine Serhan^{1,14}, Lilian Basso^{1,14}, Riccardo Sibilano^{2,3}, Camille Petitfils⁴, James Meixiong⁵,
Chrystelle Bonnart⁴, Laurent L. Reber^{6,7}, Thomas Marichal^{8,9}, Philipp Starkl^{10,11}, Nicolas Cenac⁴,
Xinzhong Dong^{5,12}, Mindy Tsai^{2,3}, Stephen J. Galli^{2,3,13,15*} and Nicolas Gaudenzio^{1,15*}

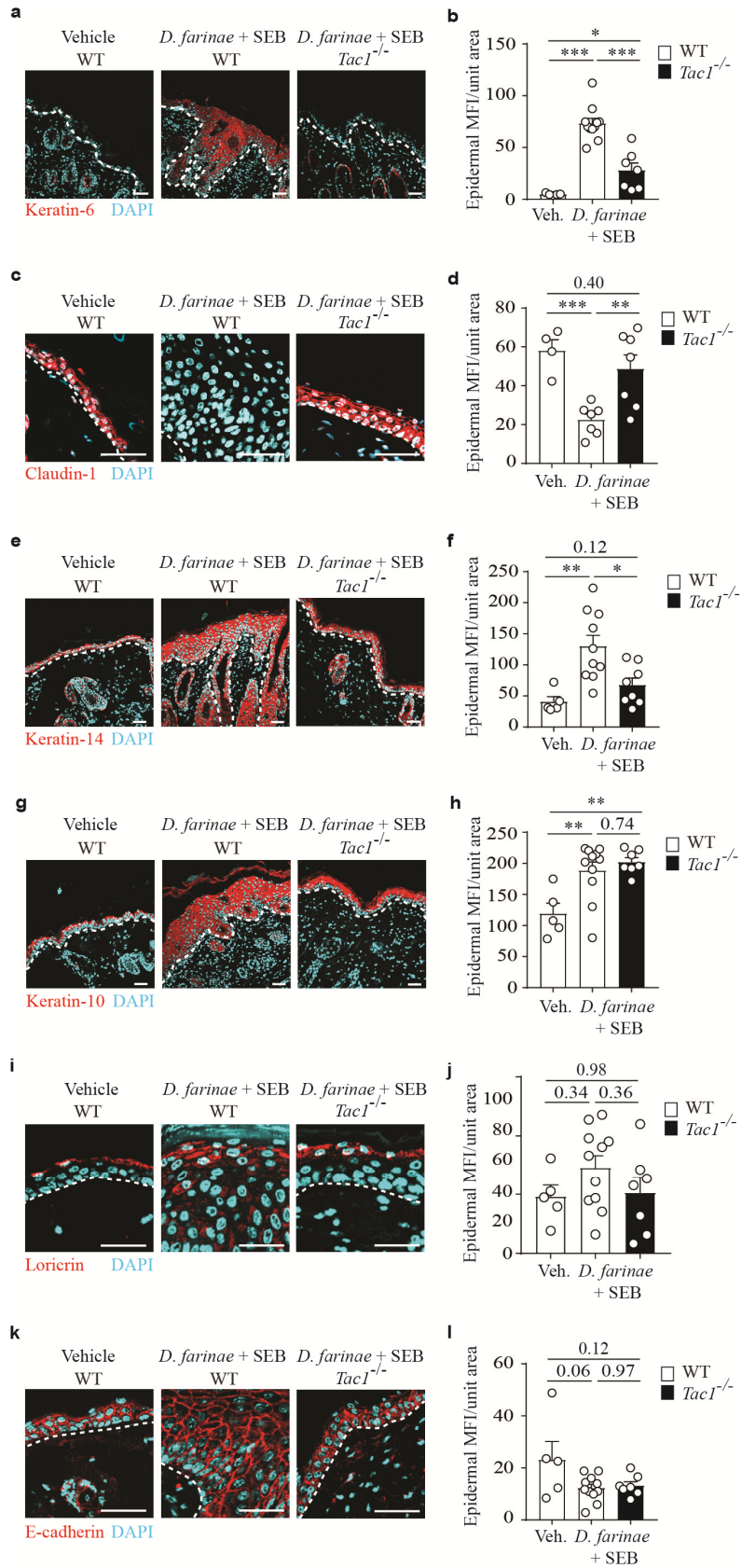
¹Unité de Différenciation Epithéliale et Autoimmunité Rhumatoïde, UMR 1056, INSERM, Université de Toulouse, Toulouse, France. ²Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA. ³Sean N. Parker Center for Allergy and Asthma Research, Stanford University School of Medicine, Stanford, CA, USA. ⁴IRSD, INSERM, INRA, INP-ENVIT, Université de Toulouse 3 Paul Sabatier, Toulouse, France. ⁵The Solomon H. Snyder Department of Neuroscience, Center for Sensory Biology, Johns Hopkins University School of Medicine, Baltimore, MD, USA. ⁶Unit for Antibodies in Therapy and Pathology, Institut Pasteur, UMR1222 INSERM, Paris, France. ⁷Center for Pathophysiology Toulouse Purpan, INSERM U1043, CNRS UMR 5282, Toulouse III University, Toulouse, France. ⁸GIGA Institute and Faculty of Veterinary Medicine, Liege University, Liege, Belgium. ⁹WELBIO, Walloon Excellence in Life Sciences and Biotechnology, Wallonia, Belgium. ¹⁰CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria. ¹¹Laboratory of Infection Biology, Department of Medicine I, Medical University of Vienna, Vienna, Austria. ¹²Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA. ¹³Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA, USA. ¹⁴These authors contributed equally: Nadine Serhan, Lilian Basso. ¹⁵These authors jointly directed this work: Stephen J. Galli, Nicolas Gaudenzio. *e-mail: sgalli@stanford.edu; nicolas.gaudenzio@inserm.fr



Supplementary Figure 1

***D. farinae* and SEB treatment induces the development of systemic *D. farinae*-specific type 2 immunity and AD-like pathology.**

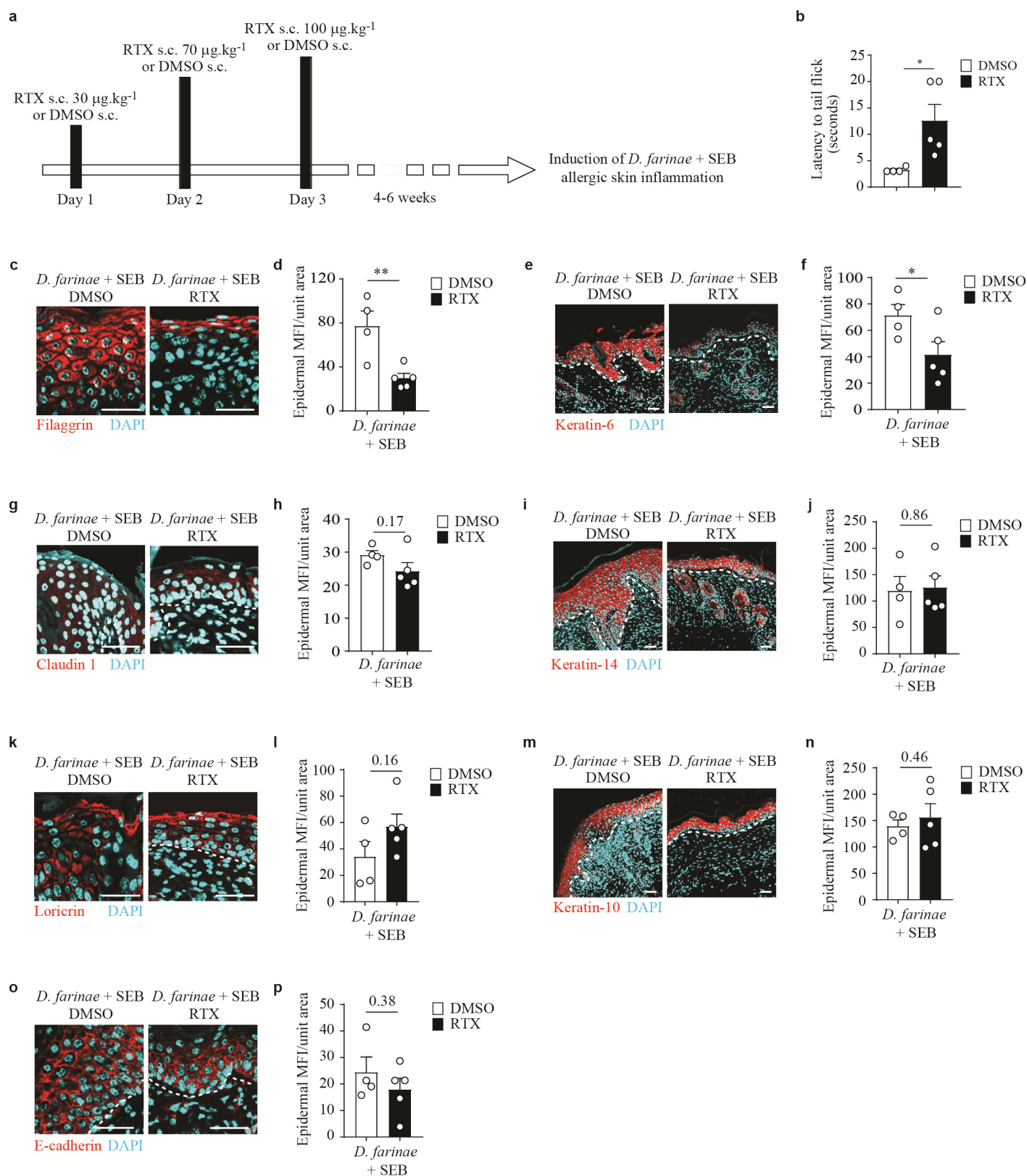
a, Protocol to induce *D. farinae* + SEB-mediated allergic skin inflammation. **b**, Clinical score calculation. **c-e**, WT mice were treated with SEB, *D. farinae* or *D. farinae* + SEB (or vehicle control) to induce the development allergic skin inflammation. Representative hematoxylin & eosin (H&E) staining of vehicle-, SEB-, *D. farinae*-, or *D. farinae* + SEB-treated areas. **d**, Clinical score (0-12) of vehicle- (n = 3 mice), SEB-(n = 5 mice), *D. farinae*-(n = 5 mice), or *D. farinae* + SEB-treated (n = 5 mice) areas from two independent experiments, mean $\pm$ SEM. **e**, Spleen weight measurements of vehicle- (n = 6 mice) or *D. farinae* + SEB-treated (n = 15 mice) mice from five independent experiments. **f**, Flow cytometry analysis of the proportion (%) of splenic CD4⁺ T lymphocytes from vehicle- (open bars) or *D. farinae* + SEB-treated (black bars) mice expressing intracellular IL-4, IL-5, IL-13 or IFN- γ after *ex vivo* restimulation with *D. farinae*, from vehicle- (n = 5 mice) or *D. farinae* + SEB-treated (n = 7 mice) mice from three independent experiments, mean $\pm$ SEM; two-tailed, unpaired *t*-test, **P<.01 ***P<.001. **g,h**, WT mice were treated with *D. farinae* + SEB and injected intraperitoneally twice a week with an anti-mouse IL-4/13R α blocking antibody (n=6 mice) or an isotype control (n = 6 mice), data are from two independent experiments. **g**, Representative H&E staining of *D. farinae* + SEB-treated areas. **h**, Clinical score (0-12), isotype control-treated vs. anti-IL-4/13R α -treated *D. farinae* + SEB-treated, mean $\pm$ SEM; two-tailed, unpaired *t*-test, *P<.05. **c,g**, dotted black lines indicate the junction epidermis/dermis, bars = 100 μ m. Each open circle = one mouse. **i**, Publicly available microarray gene expression data of *Trpv1*, *Tac1* and *Trpa1* in mouse DRG and different immune cell subpopulations (GSE 10246); data are shown using a heat map of mRNA expression levels.



Supplementary Figure 2

Extended analysis of epidermal proteins in WT and *Tac1*^{-/-} mice.

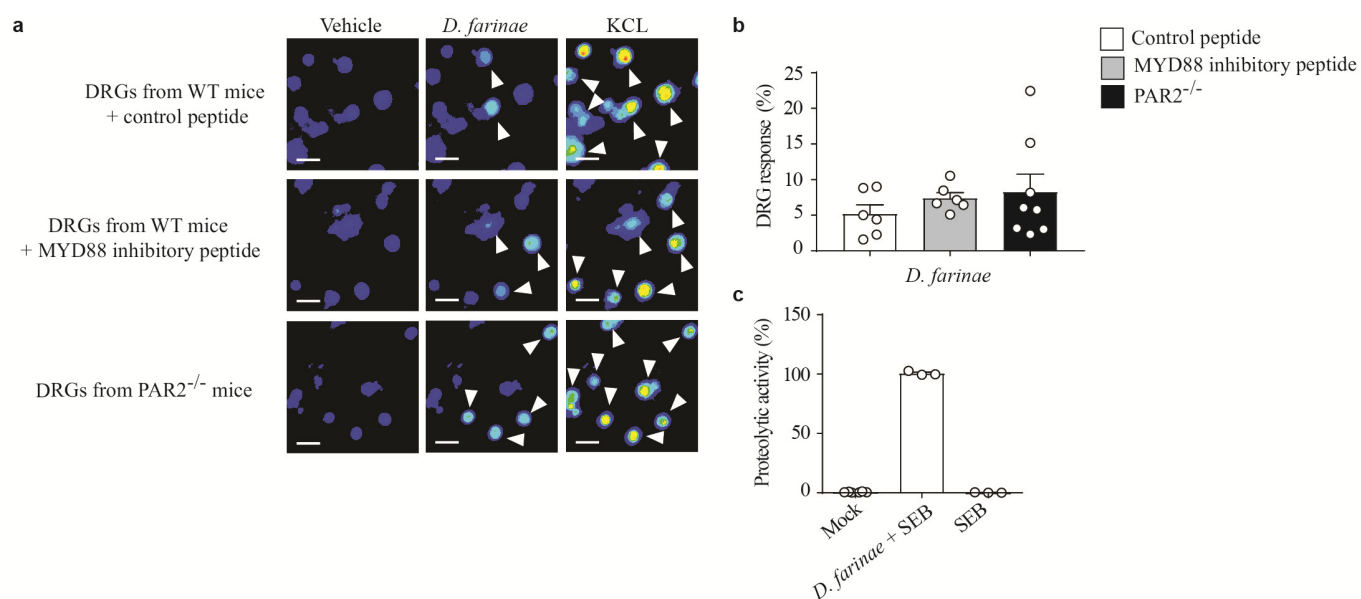
Representative confocal microscopy of back skin sections (**a,c,e,g,i,k**) and corresponding fluorescence analysis (**b,d,f,h,j,l**) of the indicated staining. Bars = 100 μ m; dotted white lines indicate the epidermal/dermal junction. Each open circle = one mouse. Open bars: WT mice treated either with vehicle (n = 5 mice) or *D. farinae* + SEB (n = 14 mice); black bars: *Tac1*^{-/-} mice treated with *D. farinae* + SEB (n = 8 mice); from three independent experiments, mean + SEM; 1-way ANOVA with Tukey's test for multiple comparisons; *P<.05 **P<.01 ***P<.001.



Supplementary Figure 3

Extended analysis of epidermal protein organization in DMSO-treated and RTX-treated mice.

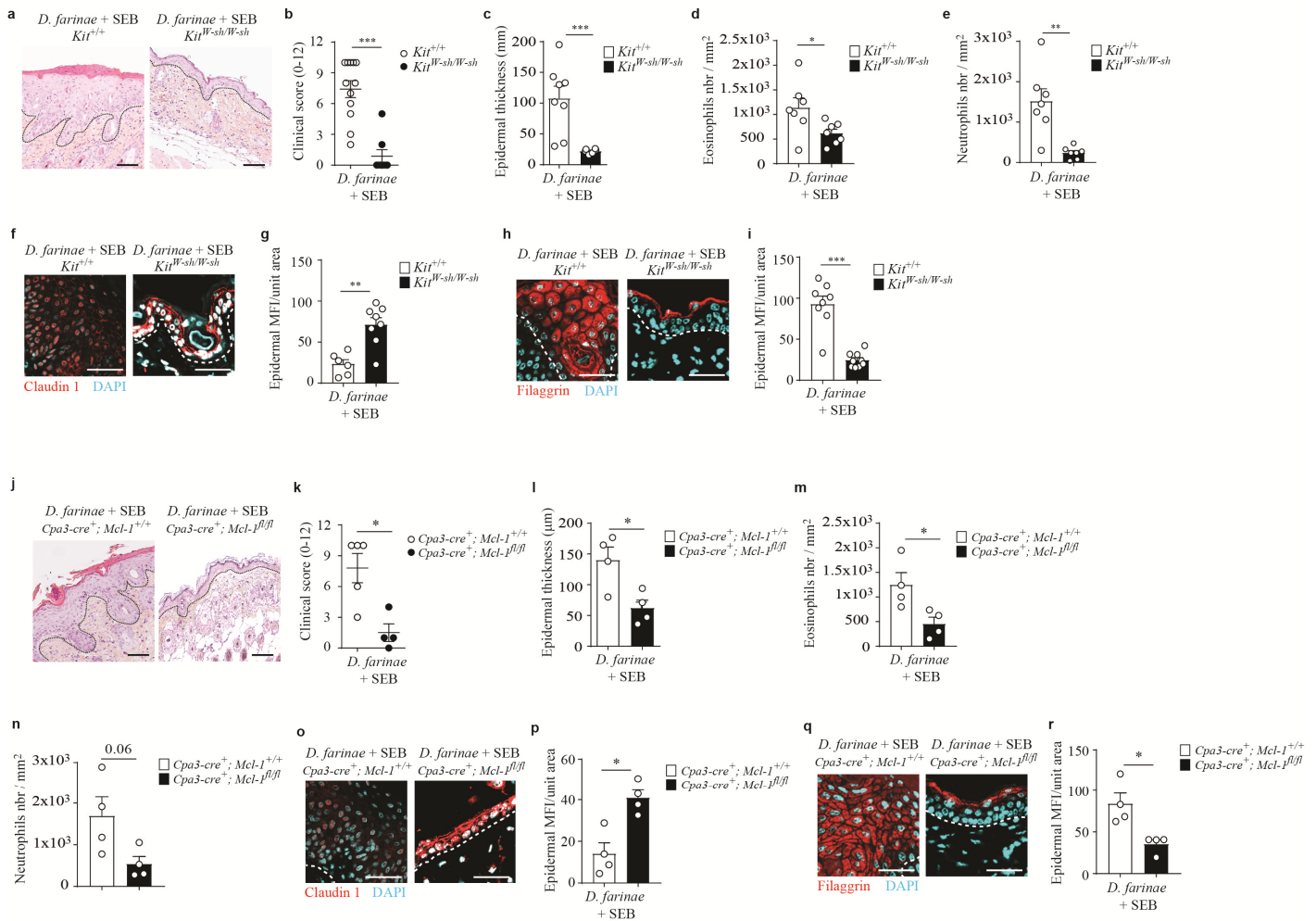
a, 4-week-old *C57BL/6J* WT mice were treated subcutaneously (s.c.) into the back with DMSO (Mock treated) or RTX in three escalating doses (30, 70 and 100 $\mu\text{g.kg}^{-1}$) on consecutive days and mice were rested for 4-6 weeks before *D. farinae* + SEB treatment. **b**, Confirmation of TRPV1⁺ nociceptors ablation by immersion of the tail into a water bath maintained at 52°C¹⁷ and comparison of the latency to the first tail movement elicited by hot water in DMSO-treated (open bars, n = 4 mice) vs RTX-treated (black bars, n = 5 mice) group, from two independent experiments, mean + SEM; two-tailed, unpaired *t*-test, *P<.05. **c-p**, Representative confocal microscopy from two independent experiments of back skin section (**c,e,g,i,k,m,o**) and corresponding fluorescence analysis (**d,f,h,j,l,n,p**) of the indicated staining. Bars = 100 μm ; dotted white lines indicate the epidermal/dermal junction. Each open circle = one mouse. All the following groups are *D. farinae* + SEB-treated, open bars: Mock DMSO-treated mice (n = 4 mice), black bars: RTX-treated mice (n = 5 mice); from two independent experiments mean + SEM; two-tailed, unpaired *t*-test, *P<.05 **P<.01.



Supplementary Figure 4

***D. farinae*-mediated activation of DRG neurons is not dependent on MyD88 or PAR-2 signaling and analysis of the proteolytic activity of *D. farinae* and/or SEB.**

DRG neurons were isolated from WT mice then treated with control or MyD88 inhibitory peptide or from PAR-2-deficient mice (**a,b**). **a**, Representative Fluo-4 fields of *ex vivo* cultured DRG neurons from WT mice, incubated with control peptide (**a, up**) or MyD88 inhibitory peptide (**a, mid**) or from PAR-2-deficient mice (**a, low**) and treated with vehicle, 5 ng/ml *D. farinae*, or 50 mM KCL. Bars = 50 μ m. **b**, Proportion of responding DRG neurons incubated with control peptide (white bars, n = 6 mice) or MyD88 inhibitory peptide (grey bars, n = 6 mice) or from PAR-2-deficient mice (black bars, n=8 mice) stimulated with 5 ng/ml *D. farinae* and expressed as % of DRG neurons responding to 50 mM KCL, each open circle = one mouse. (**a,b**) Data are from three independent experiments, (**b**) mean + SEM. **c**, Proteolytic activity of *D. farinae* extracts in presence of SEB or SEB alone and normalized to the 100% proteolytic activity obtained in the control condition *D. farinae*, from three independent experiments, mean + SEM.

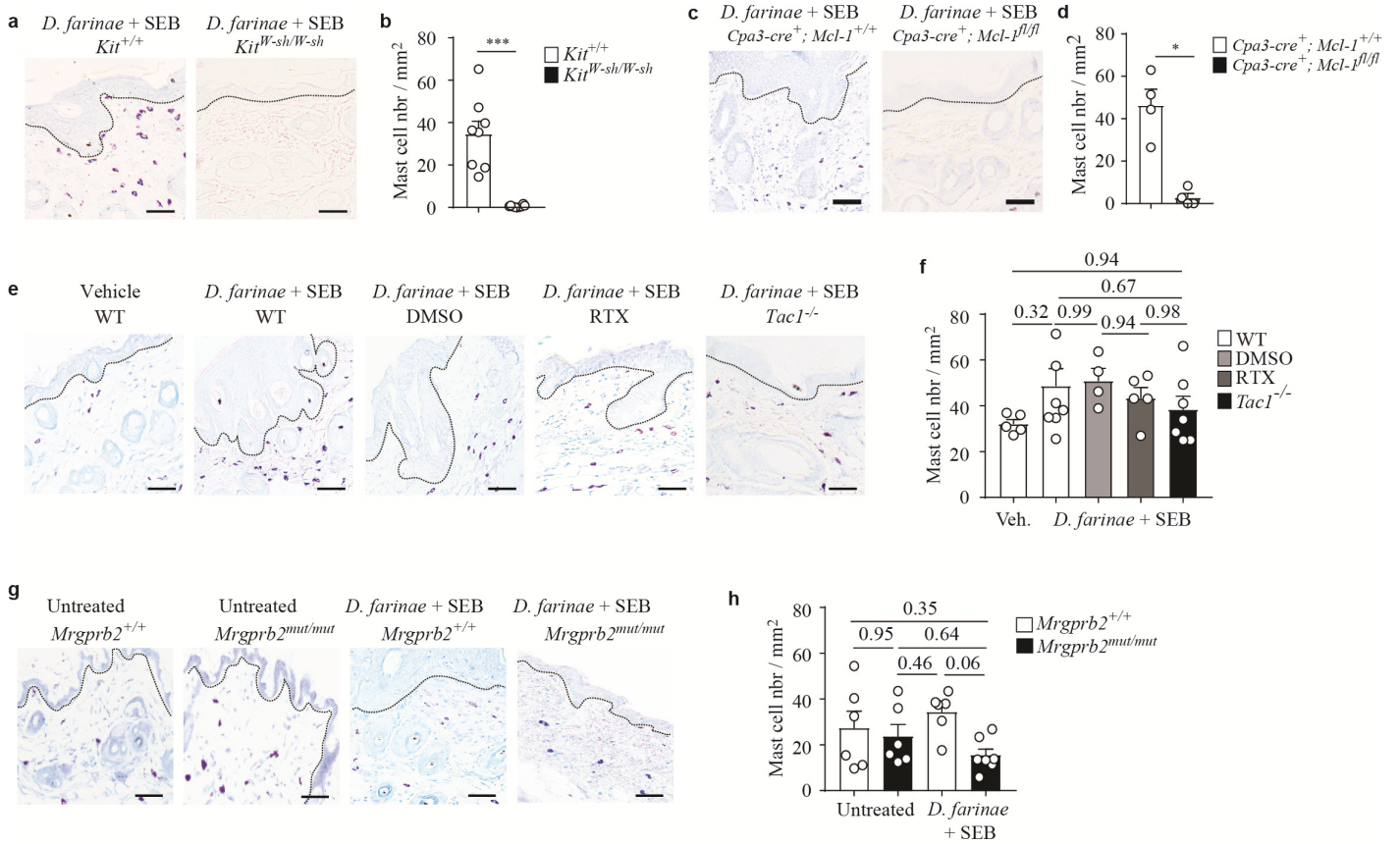


Supplementary Figure 5

***Kit*-dependent and *Kit*-independent mast cell-deficient mice are protected from the development of a model of allergic skin inflammation.**

Kit-dependent *Kit*^{W-sh/W-sh} mast cell-deficient mice versus *Kit*^{+/+} mast cell-sufficient littermate control mice (**a-i**) and *Kit*-independent *Cpa3-cre*⁺; *Mcl-I*^{fl/fl} mast cell-deficient mice and *Cpa3-cre*⁺; *Mcl-I*^{+/+} mast cell-sufficient littermate control mice (**j-r**) were treated with *D. farinae* + SEB to induce the development allergic skin inflammation. **a**, Representative H&E staining of *D. farinae* + SEB-treated areas. **b**, Clinical scores (0-12), open circles: *Kit*^{+/+} mice (n = 8 mice), closed circles: *Kit*^{W-sh/W-sh} mice (n = 8 mice), from three independent experiments, mean ± SEM, two-tailed, unpaired *t*-test, ***P<.001. **c**, Epidermal thickness (μm). **d,e**, Number of eosinophils (**d**) and neutrophils (**e**) in skin sections. **f-i**, Representative confocal microscopy of back skin section (**f,h**) and fluorescence analysis (**g,i**) of claudin-1 (**f,g**) and filaggrin (**h,i**) stainings. Bars = 100 μm, dotted black (**a**) or white (**f,h**) lines indicate the junction epidermis/dermis. **c-e,g,i**, Each open circle = one mouse; white bars: *Kit*^{+/+} mice (number of mice [**c-e**] n = 7, [**g**] n = 6, [**i**] n = 8), black bars: *Kit*^{W-sh/W-sh} mice (number of mice [**c-e**] n = 7, [**g-i**] n = 8) from three independent experiments, mean ± SEM; two-tailed, unpaired *t*-test, *P<.05 **P<.01 ***P<.001. (**j-r**) same experiment as in (**a-i**) but in *Kit*-independent *Cpa3-cre*⁺; *Mcl-I*^{fl/fl} mast cell-deficient mice and *Cpa3-cre*⁺; *Mcl-I*^{+/+} mast cell-sufficient littermate control mice. Each open circle = one

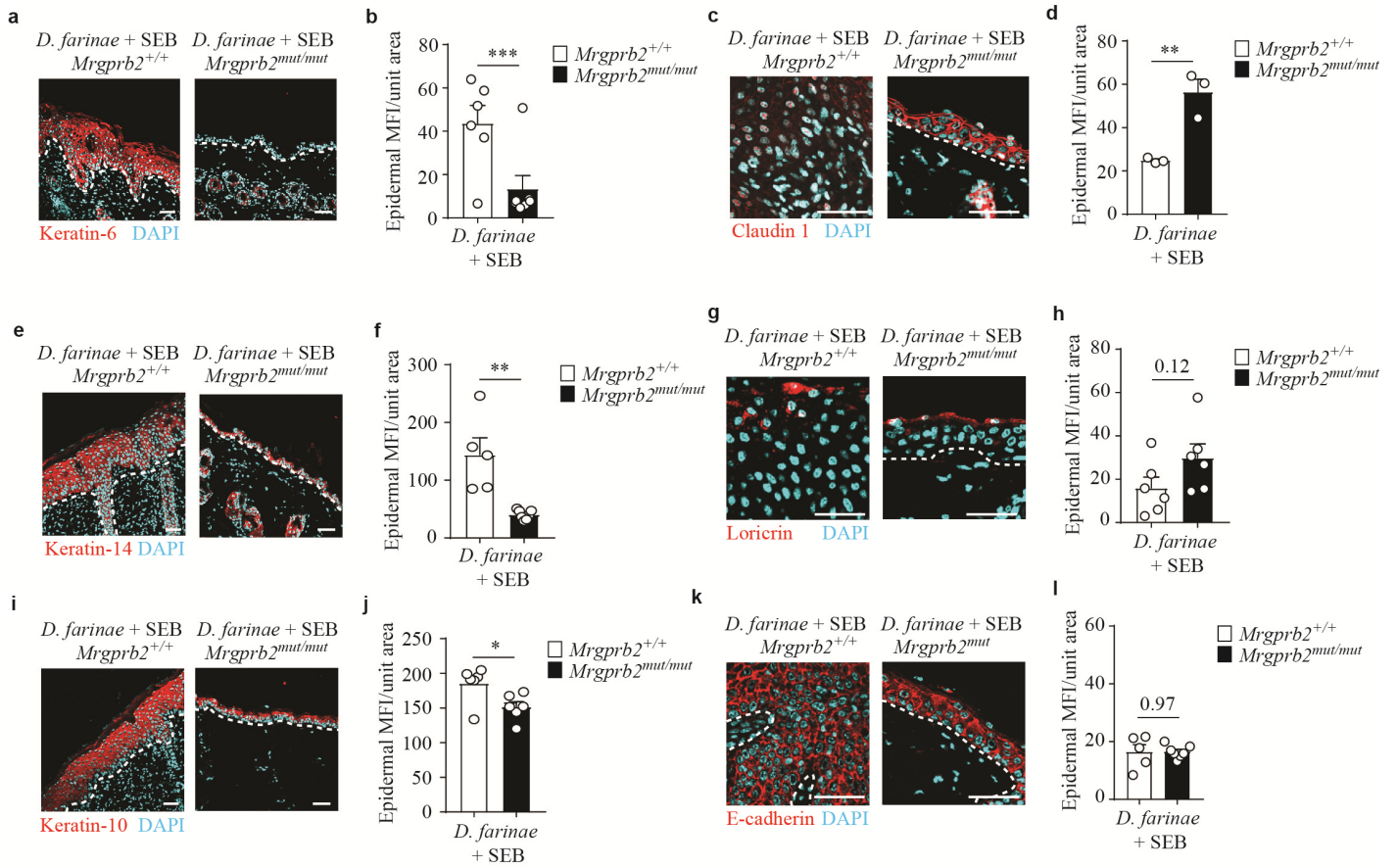
mouse, open symbols or bars: *Cpa3-cre*⁺;*Mcl-1*^{+/+} mice (n = 4 mice), black symbols or bars: *Cpa3-cre*⁺;*Mcl-1*^{fl/fl} mice (n = 4 mice); from two independent experiments mean + SEM; two-tailed, unpaired *t*-test, *P<.05.



Supplementary Figure 6

RTX-treated, *Tac1*^{-/-} or *Mrgprb2*^{mut/mut} mice are not deficient in skin mast cell.

a,b, Representative Toluidine blue (TB) staining (**a**) and associated mast cell counts (**b**) of *D. farinae* + SEB-treated area in *Kit*^{+/+} (n = 8 mice) and *Kit*^{W-sh/W-sh} mice (n = 7 mice), from three independent experiments. **c,d**, Same experiment in *Cpa3-cre*⁺; *Mcl-I*^{+/+} mice (n = 5 mice) and *Cpa3-cre*⁺; *Mcl-I*^{fl/fl} mice (n = 5 mice), from two independent experiments. **b,d**, mean + SEM; two-tailed, unpaired *t*-test, **P* < .05 ****P* < .001. **e,f**, Same experiment but in vehicle-treated WT mice (open bar, n = 5 mice) or *D. farinae* + SEB-treated WT (open bars, n = 7 mice, from three independent experiments), DMSO-treated (light grey bars n = 4 mice, from two independent experiments), RTX-treated (dark grey bars n = 5 mice, two independent experiments) and *Tac1*^{-/-} mice (black bars, n = 7 mice, from three independent experiments). **g,h**, Same experiment but in untreated or *D. farinae* + SEB-treated in *Mrgprb2*^{+/+} mice (n = 6 mice) and *Mrgprb2*^{mut/mut} mice (n = 7 mice) (**f,h**). Mean + SEM; two-tailed, 1-way ANOVA with Tukey's test for multiple comparisons. Each open circle = one mouse.



Supplementary Figure 7

Extended analysis of epidermal protein organization in *Mrgprb2*^{+/+} and *Mrgprb2*^{mut/mut} mice.

Representative confocal microscopy, of back skin section (**a,c,e,g,i,k**) and corresponding protein fluorescence analysis (**b,d,f,h,j,l**) of the indicated staining. Bars = 100 μ m; dotted white lines indicate the junction epidermis/dermis. Each open circle = one mouse. All the following groups are *D. farinae* + SEB-treated, white bars: *Mrgprb2*^{+/+} mice ([**b,h,j**] *n* = 6, [**d**] *n* = 3, [**f,l**] *n* = 6 mice), black bars: *Mrgprb2*^{mut/mut} mice ([**b,f,h,j,l**] *n* = 6, [**d**] *n* = 3 mice); all from three independent experiments, mean + SEM; two-tailed, unpaired *t*-test, **P* < .05 ***P* < .01 ****P* < .001.

Single i.d. injection of **Av.SRho** in both ears

Single i.d. injection of **vehicle** in the left and **stimuli** in the right ear pinnae

1 μ g *D. farinae* + 50 ng SEB (+/- IgG isotype or IgG anti-SP) or 1 μ M Capsaicin

Ear thickness measurement

Ex vivo 3D confocal microscopy

Timeline: 0 1 week 0 45 minutes

DMSO vs RTX mice
Mrgprb2^{+/+} vs *Mrgprb2*^{mut} mice
WT mice

Left ear pinnae
1 μ g *D. fariniae* + 50 ng SEB

1 μ g *D. fariniae* + 50 ng SEB
pretreated with E64 for 30 minutes at 37°C

Ear thickness measurement

45 minutes



Figure 3 is a scatter plot showing the effect of E64-pretreatment on the thickness of the ear. The y-axis is labeled Δ ear thickness ($\times 10^{-2}$ mm) and ranges from 0 to 25. The x-axis is labeled *D. farinae* + SEB. There are two data series: Untreated (open circles) and E64-pretreated (filled circles). The Untreated group has a mean thickness of approximately 14, while the E64-pretreated group has a mean thickness of approximately 8.5. An asterisk (*) indicates a significant difference between the two groups.

Treatment	β -hexosaminidase release (%)
Vehicle	~5
Anti-IgE 10 ng/ml	~85
<i>D. farinae</i> 500 ng/ml	~5
<i>D. farinae</i> 50 ng/ml	~5
<i>D. farinae</i> 5 ng/ml	~5
SEB 500 ng/ml	~5
SEB 50 ng/ml	~5
SEB 5 ng/ml	~5
<i>D. farinae</i> + SEB 500 ng/ml	~5
<i>D. farinae</i> + SEB 50 ng/ml	~5
<i>D. farinae</i> + SEB 5 ng/ml	~5

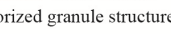
Treatment	Mean Release (%)	Standard Deviation (%)
Vehicle	~8.5	~1.5
DNP-HSA	~38.0	~5.0
10 ng/ml	~35.0	~5.0
<i>D. fariniae</i> 500 ng/ml	~8.5	~1.5
<i>D. fariniae</i> 50 ng/ml	~9.0	~1.5
<i>D. fariniae</i> 5 ng/ml	~8.5	~1.5
SEB 500 ng/ml	~8.5	~1.5
SEB 50 ng/ml	~8.5	~1.5
SEB 5 ng/ml	~8.5	~1.5
<i>D. fariniae</i> + SEB 500 ng/ml	~8.5	~1.5
<i>D. fariniae</i> + SEB 50 ng/ml	~8.5	~1.5
<i>D. fariniae</i> + SEB 5 ng/ml	~8.5	~1.5

The diagram illustrates the experimental timeline in four stages:

- A single intradermal injection of Av.SRho**: A syringe injects red liquid into a white mouse. Below the mouse, it states: "Pirt-GCaMP3 mice (the calcium tracer GCaMP3 is genetically-encoded into sensory neurons)".
- A single intradermal injection of vehicle or stimuli**: A syringe injects blue liquid into the same mouse. A horizontal arrow labeled "1 week" connects this stage to the next.
- 3D image acquisition using a 2-photon microscope**: A microscope focuses on the injection site. A horizontal arrow labeled "10-15 minutes" connects this stage to the final analysis.
- Image processing and single cell analysis of fluorescence**: A box contains the following information:
 - Collagen (blue text)
 - Av.SRho⁺ MC granule structures (red text)
 - Pirt-GCaMP3 (neuron calcium level) with a grayscale bar ranging from Low to High.

Av.SRho⁺ MCs without exteriorized granule structures

Example #1 Example #2



Av.SRho⁺ MCs with exteriorized granule structures

Example #1 Example #2



Av.SRho (to identify MC granule structures)

Supplementary Figure 8

Imaging methods used *ex vivo* and *in vivo*, pretreatment with E64 prevents ear swelling and AD antigens do not activate mast cells *in vitro*.

a, Protocol to analyze release of mast cell granules *ex vivo* in whole-mounted ear skin (related to Figure 5). 8 μ g of sulforhodamine 101-labeled avidin (Av.SRho) in 20 μ l PBS were intradermally (i.d.) injected into the ear pinna of WT, *Mrgprb2^{mut/mut}*, or RTX-treated mice and their respective control mice to selectively label mast cell secretory granules *in vivo*³⁷. 1 week later, ear thickness was measured and right ear pinnae were injected i.d. with 1 μ g *D. farinae* + 50 ng SEB or 1 μ M Capsaicin. In some experiments, right ear pinnae were injected i.d. with 1 μ g *D. farinae* + 50 ng SEB with 15 μ g of IgG anti-SP. Left ear pinnae were injected with control solutions: vehicle alone or vehicle in combination with IgG isotype. 45 minutes later, ear thickness was measured again and mice were euthanized before ear excision. 3-D images of untouched whole-mounted ears were acquired using volumetric confocal microscopy. **b**, 1 μ g *D. farinae* + 50 ng SEB alone or pretreated with cysteine protease inhibitor E64 for 30 minutes at 37°C were injected respectively into the left and right ear pinna of each WT mouse (so that each mouse is its own control) in 20 μ l PBS. 45 minutes later, ear thickness was measured. **c**, Changes (Δ) in ear thickness over time after i.d. injection of stimuli, untreated, white circles n = 10 mice and E64-pretreated, n = 10 mice, from two independent experiments, mean $\pm$ SEM, two-tailed, paired *t*-test, *P<.05. Each open circle = one mouse. **d**, β -hexosaminidase release from human mast cells (hMC) upon treatment with increasing concentrations of *D. farinae* and SEB, alone or together. For positive control, hMCs were sensitized with human IgE and then stimulated with 10 ng/mL anti-IgE. n = 6 independent experiments with three independent donors, mean + SEM, one-way ANOVA with Tukey's test for multiple comparisons, ***P<.001. **e**, β -hexosaminidase release from mouse BMCMCs upon treatment with increasing concentrations of *D. farinae* and SEB, alone or together. For positive control, BMCMCs were sensitized with murine anti-DNP IgE and then challenged with 10 ng/mL DNP-BSA, n = 8 independent experiments, with BMCMCs from four mice, mean + SEM, one-way ANOVA with Tukey's test for multiple comparisons ***P<.001. **f**, Protocol to analyze sensory neuron calcium levels and mast cell granules release simultaneously in living mice. 8 μ g of Av.SRho in 20 μ l of PBS were injected i.d. into the ear pinna of Pirt-GCaMP3 mice. 1 week later, mice were injected i.d. with vehicle, 1 μ M capsaicin, 1 μ g *D. farinae* and 50 ng SEB (used alone or in combination) in a final volume of 20 μ l then placed under the two-photon microscope. 3-D images from three independent experiments were acquired and fluorescence corresponding to Av.SRho⁺ mast cell granule structures or GCaMP3⁺ neuron calcium levels were analyzed using ImageJ and/or Imaris Bitplane software. **g**, Examples of mean fluorescence intensity (MFI) detected in vehicle (left) and capsaicin-activated (i.e., with non-degranulated mast cells, right) sensory neurons. **h**, Examples of Av.SRho⁺ mast cells without (left) or with (i.e., degranulated mast cells, right) exteriorized mast cell granule structures.

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Zen software, Prairie/Brucker, Diva

Data analysis

Image J, GraphPad Prism 6, Imaris, Flow Jo

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

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All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

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Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	<i>We have reduced the number of mice as far as possible. We have estimated the numbers of mice that were needed to analyze and detect differences in clinical scores based on previous preliminary data. We estimated that the difference in the mean value of the clinical score with an estimated 1.2 standard deviation, we needed 5/6 mice per group (total) to give us 90% power to detect a difference in clinical score at statistical significance of $p \leq 0.05$ using a two tailed, unpaired t test based on preliminary results using vehicle vs. treated WT C57Bl/6 mice. Based on this estimation, we have adapted the size of each group of mice related to in vivo experiments employing our model of allergic skin inflammation.</i>
Data exclusions	<i>No data were excluded.</i>
Replication	<i>All experiments were repeated at least 2-3 times and each experiments gave similar results.</i>
Randomization	<i>Allocation of mice in each experimental group was random as we used all littermate controls in each experiments. Therefore, we performed experiment independently of the genotype or sex and analyze the results for each mouse at the end of the experimental procedure.</i>
Blinding	<i>Regarding experiments with mice treated to develop an allergic skin inflammation, investigators were blinded during data collection until the genotype was revealed. Regarding intravital imaging, we needed transgenic mice expressing a genetically-encoded calcium tracer to visualize neurons and mast cells in vivo, therefore mice were genotyped before performing imaging experiments described in Figure 4. However, we developed an automated computational analysis of the minimum distance between mast cells and neurons in the dermis of living mice. This enabled us to perform an unbiased analysis of neuron-mast cell clusters in the skin of those transgenic mice.</i>

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).</i>
Research sample	<i>State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.</i>
Sampling strategy	<i>Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.</i>
Data collection	<i>Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.</i>
Timing	<i>Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Non-participation	<i>State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.</i>
Randomization	<i>If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.</i>

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All studies must disclose on these points even when the disclosure is negative.

Study description	<i>Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.</i>
Research sample	<i>Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i>, all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i>

Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
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Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field work?	☐ Yes ☐ No

Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access and import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-Filaggrin Polyclonal Antibody (Ozyme, BLE905801, Poly19058, 1/50) Anti-Loricrin Polyclonal Antibody (Ozyme, BLE905101, Poly19051, 1/50) Anti-Keratin 14 Polyclonal Antibody (Ozyme, BLE905301, Poly19053, 1/50) Anti-Keratin 10 Polyclonal Antibody (Ozyme, BLE905401, Poly19054, 1/50) Anti-mouse Keratin 6A (Ozyme, BLE905701, Poly19057, 1/50) Mouse Anti-E-Cadherin (BD Biosciences, 610181, Clone 36/E-Cadherin, 1/50) Anti-Claudin 1 Antibody (abcam, ab15098, 1/50) Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Invitrogen, A31571, 1/200) Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, A11012, 1/200) F(ab') ₂ -Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, A11072, 1/200) Anti-CD4-APC (eBioscience, 17-0041-82, Clone GK1.5, 1/200) Anti-IL4-PE (eBioscience, 12-7041-82, Clone 11B11, 1/200) Anti-IL5-PE (eBioscience, 12-7052-82, Clone TRFK5, 1/200) Anti-IL13-PE (eBioscience, 12-7133-82, Clone eBIO13A, 1/200)
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Anti-IFN-g-PE (eBioscience, 12-7311-82, Clone XMG1.2, 1/200)
 Purified NA/LE Rat Anti-Mouse CD124 (BDPharmingen, 552288, mIL4R-M1, in vivo 2.8mg/kg)
 Purified NA/LE Rat IgG2a k Isotype control (BDPharmingen, 554687, R35-95, in vivo 2.8mg/kg)
 Anti-IgE (BD bioscience, 553419, R35-118)
 Anti-IgG1 (BD bioscience, 550331, A85-1)
 Polyclonal Rabbit Anti-Substance-P (Millipore, AB1566, Lot 3184744, 15µg/ear)

Validation

Anti-Filaggrin Polyclonal Antibody, RRID:AB_2616957
 Anti-Loricrin Polyclonal Antibody, RRID:AB_2565046
 Anti-Keratin 14 Polyclonal Antibody, RRID:AB_2565048
 Anti-Keratin 10 Polyclonal Antibody, RRID:AB_2565049
 Anti-mouse Keratin 6A antibody, RRID:AB_2565052
 Mouse Anti-E-Cadherin, RRID:AB_397580
 Anti-Claudin 1 Antibody, RRID:AB_301644
 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647, RRID:AB_162542
 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, RRID:AB_141359
 F(ab')₂-Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, RRID:AB_2534116
 Anti-CD4-APC, RRID:AB_469320
 Anti-IL4-PE, RRID:AB_466156
 Anti-IL5-PE, RRID:AB_763587
 Anti-IL13-PE, RRID:AB_763559
 Anti-IFN-g-PE, RRID:AB_466193
 Purified NA/LE Rat Anti-Mouse CD124, RRID:AB_394356, in vivo use has been published in Li, G. et al. IL-4 receptor blockade abrogates satellite cell: rhabdomyosarcoma fusion and prevents tumor establishment. Stem cells 31, 2304-2312, doi:10.1002/stem.1491 (2013).
 Purified NA/LE Rat IgG2a k Isotype control, RRID:AB_479678
 Anti-IgE, RRID:AB_394850
 Anti-gG1, RRID:AB_2296342
 Polyclonal Rabbit Anti-Substance-P, RRID:AB_2271494, in vivo use has been published in Green, D. P., Limjunyawong, N., Gour, N., Pundir, P. & Dong, X. A Mast-Cell-Specific Receptor Mediates Neurogenic Inflammation and Pain. Neuron 101, 412-420 e413, doi:10.1016/j.neuron.2019.01.012 (2019).

Eukaryotic cell lines

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State the source of each cell line used.

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Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

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Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

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Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

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Animals and other organisms

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Laboratory animals

This study involve different mouse strains: C57Bl/6J WT, Tac1ko, Mrgprb2mutant, KitW-sh/W-sh, Cpa3-cre;Mcl1fl/fl, PAR2ko and Pirt-GCaMP3 mice. Mice were male and female, 8-12-week old of age (excepted noted otherwise)

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animal care and experimentation were conducted in France and in the USA. Experimentations conducted in USA (Galli Lab, Stanford University, CA) were in compliance with the guidelines of the National Institutes of Health (NIH) and the Institutional

Animal Care and Use Committee of Stanford University. Experimentations conducted in France (Gaudenzio Lab, INSERM, University of Toulouse) were in compliance with the guidelines of the European Union (86/609/EEC) and the French Committee of Ethics (87/848) policies and with the specific approval from the local ministry-approved committee on ethics in animal experimentation (Ethics Committee UMS006 CEEA-122, project n° 13283 2018031416055447V3).

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Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.
Files in database submission	Provide a list of all files available in the database submission.
Genome browser session (e.g. UCSC)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

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Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Following induction of allergic skin inflammation, spleens from vehicle-treated or D. farinae + SEB-treated mice were harvested and dissociated to obtain a suspension of cells. 200,000 spleen cells were stained with CFSE for 7 minutes at 37°C and incubated for 5 days with 10 ug/ml of D. farinae in RPMI 1640 supplemented with 10% FCS, GlutaMAX-I, sodium pyruvate, 2-mercaptoethanol, and ciprofloxacin. Intracellular cytokines were analyzed by gating on proliferating (CFSElow) CD4-APC+ T cells after 5-hour restimulation with phorbol 12-myristate 13-acetate (50 ng/mL, Sigma) and ionomycin (1 mg/mL, Sigma) in the presence of GolgiStop (BD Pharmingen). Cells were fixed, permeabilized (0.1% saponin in PBS 0.5% BSA), and stained with antibodies directed against mouse IL-4-PE, IL-5-PE, IL-13-PE or IFN-g-PE. Flow cytometric data were acquired on a BD FACSCanto cytometer and were analyzed using FlowJo software (Tree Star, Inc, Ashland, Ore).

Instrument

BD FACS CANTO

Software

FlowJo software (Tree Star, Inc, Ashland, Ore)

Cell population abundance

We analyzed CD4+ T cells that represented ~ 20-25% of recovered splenic cells.

Gating strategy

We gated on lymphocytes based on size and then on CD4+ cells to study CD4+ T cells cytokine profile (Supplementary Figure 1f)

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐

Used

☐

Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

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If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

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Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

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Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ BothStatistic type for inference
(See [Eklund et al. 2016](#))

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a | Involved in the study

- ☐ ☐ Functional and/or effective connectivity
- ☐ ☐ Graph analysis
- ☐ ☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.