

A $\gamma\delta$ T cell–IL-3 axis controls allergic responses through sensory neurons

<https://doi.org/10.1038/s41586-024-07869-0>

Received: 19 January 2023

Accepted: 23 July 2024

Published online: 4 September 2024



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In naive individuals, sensory neurons directly detect and respond to allergens, leading to both the sensation of itch and the activation of local innate immune cells, which initiate the allergic immune response^{1,2}. In the setting of chronic allergic inflammation, immune factors prime sensory neurons, causing pathologic itch^{3–7}. **Although these bidirectional neuroimmune circuits drive responses to allergens, whether immune cells regulate the set-point for neuronal activation by allergens in the naive state is unknown.** Here we describe a $\gamma\delta$ T cell–IL-3 signalling axis that controls the allergen responsiveness of cutaneous sensory neurons. We define a poorly characterized epidermal $\gamma\delta$ T cell subset⁸, termed GD3 cells, that produces its hallmark cytokine IL-3 to promote allergic itch and the initiation of the allergic immune response. Mechanistically, IL-3 acts on *Il3ra*-expressing sensory neurons in a JAK2-dependent manner to lower their threshold for allergen activation without independently eliciting itch. This $\gamma\delta$ T cell–IL-3 signalling axis further acts by means of STAT5 to promote neuropeptide production and the initiation of allergic immunity. **These results reveal an endogenous immune rheostat that sits upstream of and governs sensory neuronal responses to allergens on first exposure.** This pathway may explain individual differences in allergic susceptibility and opens new therapeutic avenues for treating allergic diseases.

Sensory neurons densely innervate the skin and respond to inflammatory, pathogenic and allergic triggers that direct downstream immune responses^{1,2,9–11}. **Allergens activate sensory neurons directly through their enzymatic activity, leading to the sensation of itch and the release of neuropeptides at the site of allergen exposure^{1,2}.** Sensory neuronal activation leads to the migration of mature, allergen-bearing dendritic cells (DCs) from the periphery to draining lymph nodes (dLNs), where they initiate T helper 2 (T_H2) cell differentiation and allergic sensitization^{1,12}. In the setting of chronic allergen exposure, these T_H2 cells, as well as other inflammatory cells, can traffic back to the skin, releasing immune factors that activate or alter the responsiveness of sensory neurons. The allergic factors TSLP³, LTC₄ (refs. 4,5) and IL-31 (ref. 6) directly elicit itch and scratching, whereas IL-4 and IL-13 prime sensory neurons⁷, lowering their threshold for activation by other pruritogens without independently causing itch. **However, in the absence of allergic inflammation or in naive skin, whether the immune system plays a role in establishing the primary ‘set-point’ for neuronal activation by allergens is unknown.** We proposed that a cutaneous innate immune cell and its production of a priming factor controls the allergen activation of sensory neurons on their first exposure to an allergen, governing itch

and the initiation of allergic immunity. We identify a poorly characterized epidermal $\gamma\delta$ T cell population, which we term **GD3 cells, that produces IL-3 under homeostatic conditions and tunes the responsiveness of *Il3ra*-expressing sensory neurons to allergens through distinct JAK2 and STAT5-dependent effects.** This $\gamma\delta$ T cell–IL-3 axis sits upstream of and regulates the initial sensory neuronal response to allergens, constituting a new pathway for the innate immune control of allergic itch and allergen sensitization.

$\gamma\delta$ T cells promote allergen-induced itch

Using the itch and scratch response to the model allergen papain in naive mice as a readout of allergen activation of sensory neurons^{1,13,14}, we performed a behavioural screen of mice genetically deficient in specific immune cell populations, none of which showed spontaneous non-specific itch behaviour (Extended Data Fig. 1a,b). Despite well-characterized roles in IgE and MRGPR-mediated itch^{5,15}, mast cell or basophil deficiency had no effect on papain-induced scratching (Extended Data Fig. 1c). Instead, papain-induced itch was defective in lymphocyte (*Rag2*^{−/−}) and $\gamma\delta$ T cell-deficient (*Tcrd*^{−/−}) mice, but intact in

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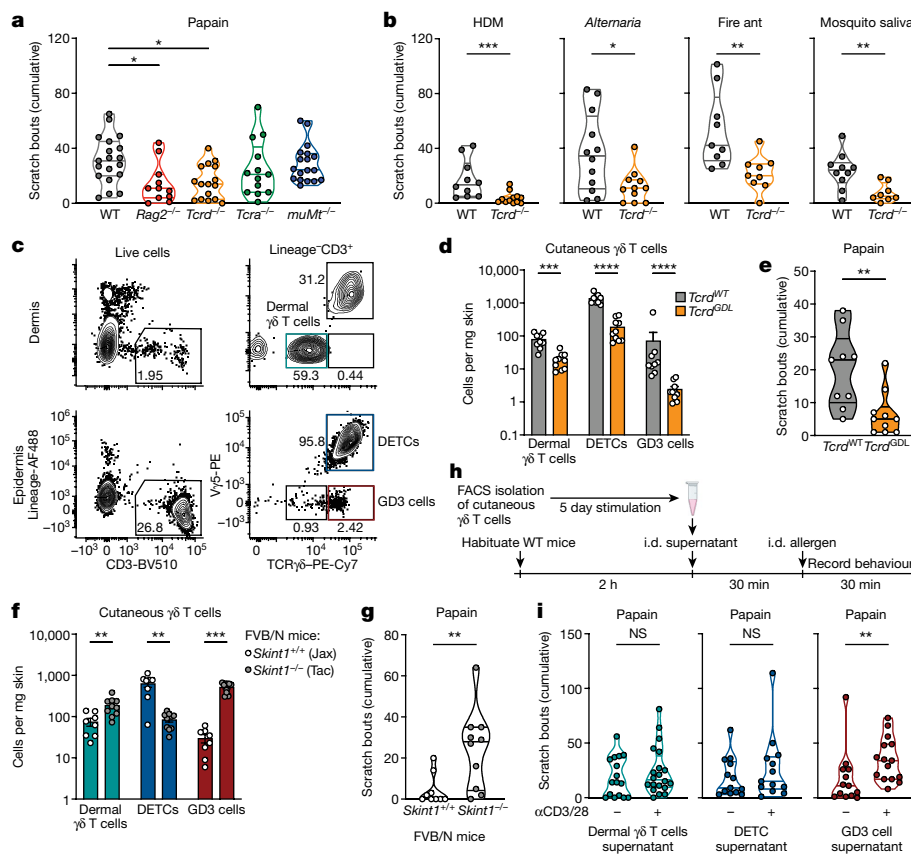


Fig. 1 | $\gamma\delta$ T cells promote allergic itch through a secreted factor. **a**, Cumulative cheek scratch bouts following i.d. injection with papain ($n = 19$ WT (C57BL/6); $n = 11$ $Rag2^{-/-}$; $n = 16$ $Tcrd^{-/-}$; $n = 13$ $Tcr\alpha^{-/-}$; $n = 18$ $muMT^{-/-}$). **b**, Cumulative cheek scratch bouts following i.d. injection with HDM ($n = 10$ WT; $n = 12$ $Tcrd^{-/-}$), *Alternaria* ($n = 12$ WT; $n = 11$ $Tcrd^{-/-}$), fire ant ($n = 9$ per group) or mosquito saliva ($n = 10$ WT; $n = 8$ $Tcrd^{-/-}$). **c**, Flow cytometry of dermis or epidermis, from naive WT pinnae. **d, f**, Flow cytometric quantification of skin (dermis + epidermis) $\gamma\delta$ T cells from pinnae (**d**, $n = 9$ $Tcrd^{WT}$; $n = 10$ $Tcrd^{GDL}$; **f**, $n = 8$ $Skint1^{+/+}$; $n = 10$ $Skint1^{-/-}$) of $Tcrd^{GDL}$ mice (**d**) or $Skint1^{-/-}$ mice (**f**). **e, g**, Cumulative cheek scratch bouts following i.d. injection with papain (**e**, $n = 9$ $Tcrd^{WT}$; $n = 10$ $Tcrd^{GDL}$; **g**, $n = 9$ $Skint1^{+/+}$; $n = 10$ $Skint1^{-/-}$) of $Tcrd^{GDL}$ mice (**e**) or $Skint1^{-/-}$ mice (**g**). **h**, Schematic of $\gamma\delta$ T cell

supernatant transfer and behaviour protocol. **i**, Cumulative cheek scratch bouts following i.d. injection of $\gamma\delta$ T supernatant, then papain ($n = 16$ dermal $\gamma\delta$ T cell unstimulated; $n = 20$ dermal $\gamma\delta$ T cell α CD3/28; $n = 13$ DETC unstimulated, DETC α CD3/28 or GD3 unstimulated; $n = 15$ GD3 α CD3/28). Symbols represent individual mice. Violin plots show the median and quartiles. Bar plots are mean $\pm$ s.e.m. Data represent at least two independent experiments and were combined. Statistical tests: ordinary one-way ANOVA with Tukey's multiple comparisons test (**a**), two-sided unpaired *t*-test (**b**, *Alternaria*, fire ant, mosquito saliva) or two-sided Mann–Whitney *U*-test (**b**, HDM; **d–g**, **i**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; NS, not significant. Schematics in **h** created using BioRender (<https://biorender.com>).

mice deficient in B or $\alpha\beta$ T cells (Fig. 1a). The effect of $\gamma\delta$ T cell deficiency on itch was shared among diverse allergens, including extracts of house dust mite (HDM), *Alternaria*, fire ant and mosquito saliva (Fig. 1b). Itch to histamine or chloroquine and pain to capsaicin were intact in $Tcrd^{-/-}$ mice (Extended Data Fig. 1d,e), and there was no difference in cutaneous innervation between wild-type (WT) and $Tcrd^{-/-}$ mice (Extended Data Fig. 1f). These data indicate that although $\gamma\delta$ T cells are required for allergen-induced itch, their absence does not cause broad defects in sensory neuronal function or innervation.

The axons of peripheral sensory neurons extend from their cell bodies in the dorsal root ganglia (DRG) to the skin. $\gamma\delta$ T cells (live CD45⁺ Lineage⁺ CD3⁺ TCR $\gamma\delta$ ⁺) were detected in the skin, but not the DRG (Extended Data Fig. 2a,b). Two populations of $\gamma\delta$ T cells with distinct TCR rearrangement and effector function seed the dermis and epidermis⁸: dermal $\gamma\delta$ T cells are TCR $\gamma\delta$ ^{intermediate}, whereas dendritic epidermal T cells (DETCs) are Vy5⁺ and TCR $\gamma\delta$ ^{high}. Flow cytometry of naive WT mouse skin confirmed the presence of these stereotypical populations and identified an extra Vy5⁺ TCR $\gamma\delta$ ^{high} epidermal subset (Fig. 1c and Extended Data Fig. 2c,d), which we termed GD3 cells. The frequency of these $\gamma\delta$ T cell populations, including GD3 cells, was unaffected by chronic allergic inflammation (Extended Data Fig. 2e–g), so we focused our efforts on understanding exactly how these innate immune cells control the sensory neuronal response to initial allergen exposure in naive skin.

$\gamma\delta$ T cells are important in the development and homeostasis of immune cells and tissues¹⁶. To rule out off-target effects from the constitutive loss of $\gamma\delta$ T cells, we inducibly depleted cutaneous $\gamma\delta$ T cells in $Tcrd^{GDL}$ and $iTcrd^{DTA}$ mice, which reduced papain-induced itch (Fig. 1d,e and Extended Data Fig. 3a–d). Epidermal DETCs and GD3 cells were preferentially depleted in $iTcrd^{DTA}$ mice, suggesting that epidermal, but not dermal $\gamma\delta$ T cells, are required for allergic itch. To assess the role of DETCs in allergic itch, we examined FVB/N (sensitivity to Friend leukaemia virus B strain) mice deficient in DETCs due to a spontaneous point mutation in the *Skint1* gene¹⁷. Compared to *Skint1*^{+/+} (FVB/N-Jax) controls, *Skint1*^{-/-} (FVB/N-Tac) mice lacked DETCs, which were largely replaced by GD3 cells (Fig. 1f and Extended Data Fig. 3e). Despite a deficiency in DETCs, *Skint1*^{-/-} mice had no defect in papain, HDM, histamine or chloroquine-induced itch (Fig. 1g and Extended Data Fig. 3f).

With our data suggesting a limited role for dermal $\gamma\delta$ T cells and DETCs in establishing the allergen responsiveness of sensory neurons, we asked whether GD3 cells control this pathway through a secreted factor. Stimulated GD3 cell supernatant, but not dermal $\gamma\delta$ T cell, DETC or cell-free supernatant, enhanced papain-induced itch (Fig. 1h,i and Extended Data Fig. 3g). These data suggest that GD3 cells produce a secreted factor that tunes the sensory neuronal response to a primary allergen exposure.

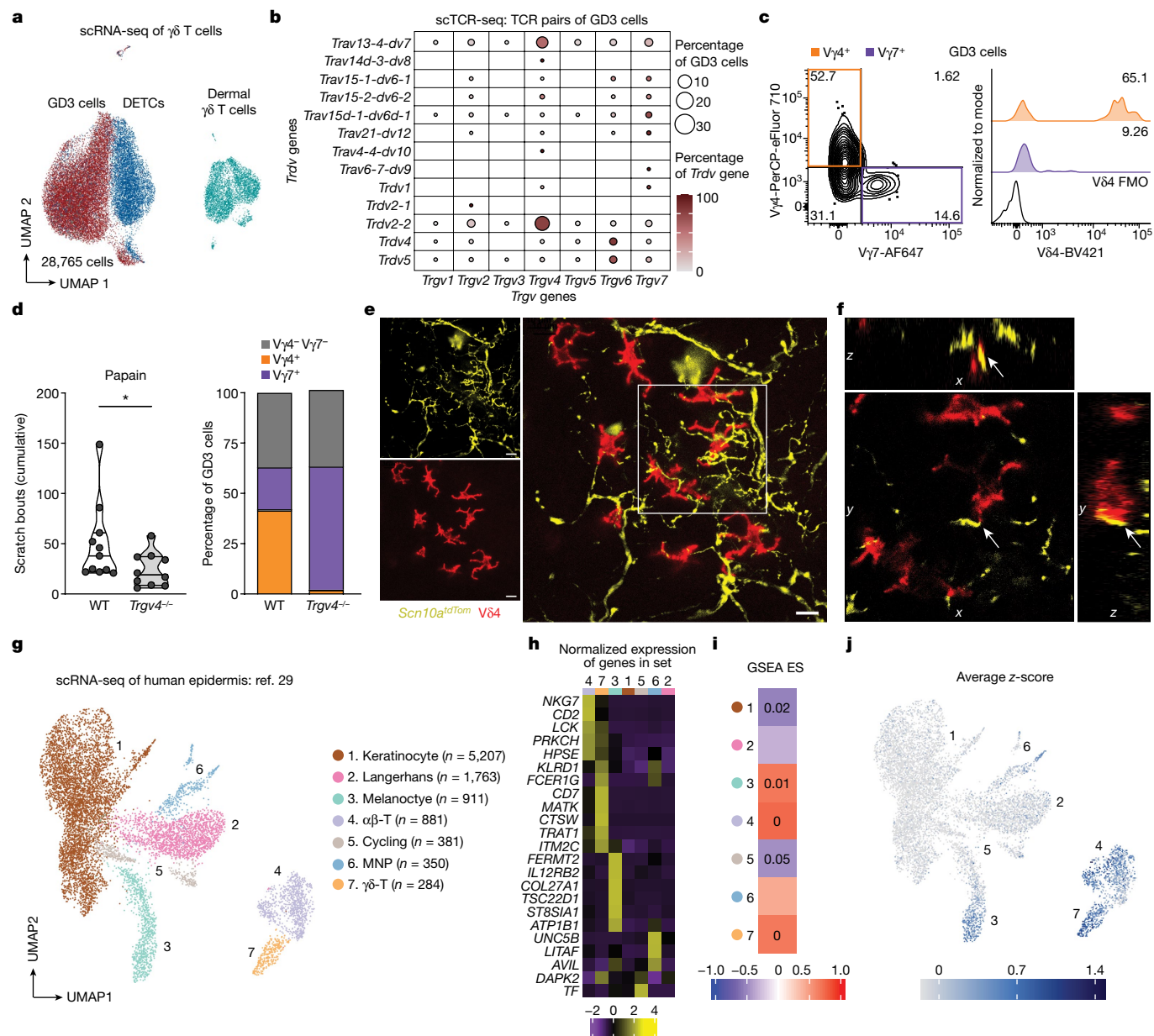


Fig. 2 | GD3 cells are required for allergic itch, associated with sensory neurons and transcriptionally related to human epidermal T cells. **a**, UMAP plot of naive WT pinnae $\gamma\delta$ T cells analysed by scRNA-seq. **b**, Bubble plot of *Trdv* and *Trgv* genes in clones of GD3 cells analysed by scTCR-seq (size denoting the percentage of GD3 cells expressing a *Trdv* gene pair; colour denoting the percentage of cells with a given *Trdv* gene that has the given *Trgv* gene). **c**, Flow cytometry of naive WT pinnae. **d**, Cumulative cheek scratch bouts following i.d. injection with papain (left panel; $n = 16$ WT; $n = 10$ *Trgv4*^{-/-}). Flow cytometry of naive pinnae (right panel; $n = 11$ per group). **e**, Confocal microscopy z-stack of naive *Scn10a*^{tdTomato} pinnae. **f**, Orthogonal depiction of the x, y and z planes of the z-stack in e. Arrows show the same location in x, y and z space. **g**, UMAP plot

of healthy human epidermal cells reclustered from the Gellatly et al.²⁹ publicly available dataset. **h**, Heatmap (z score) of the human homologues of the top 25 differentially expressed mouse GD3 cell genes compared to dermal $\gamma\delta$ T cells. **i**, Heatmap of the enrichment scores (ES) from GSEA comparing the genes in h to the human clusters from g. $P < 0.05$ is shown. **j**, UMAP plot of the z-score of the genes from h. Symbols represent individual mice. Violin plots show the median and quartiles. Bar plots are the mean. Mouse data represent at least two independent experiments (c–f), combined in d. Statistical tests: two-sided Mann–Whitney *U*-test (d). * $P < 0.05$. Scale bar, 10 μ m. FMO, fluorescence minus one.

GD3 cells are a unique $\gamma\delta$ T cell subset

Despite previous visualization of V γ 5⁻ epidermal $\gamma\delta$ T cells^{18–20}, they have never been characterized. To determine the transcriptional signature of GD3 cells, we performed single-cell RNA (scRNA-seq) and TCR $\gamma\delta$ (scTCR-seq) sequencing on fluorescence-activated cell sorting (FACS)-isolated cutaneous $\gamma\delta$ T cells. Pooled transcriptomic analysis revealed that **GD3 cells were transcriptionally distinct from dermal $\gamma\delta$ T cells and DETCs** (Fig. 2a). As expected, we identified V γ 4⁺ (*Trgv4*) and V γ 6⁺ (*Trgv6*) dermal $\gamma\delta$ T cells and V γ 5⁺ (*Trgv5*) DETCs^{18,21,22}, whereas

V γ 4 (*Trgv4*) primarily characterized GD3 cells (Supplementary Fig. 1a). Compared to highly clonal DETCs ($n = 285$ clones), **oligoclonal GD3 cells ($n = 948$ clones) showed more diversity, with the *Trgv4*-*Trdv2-2* pairing being the dominant clonotype** (Fig. 2b and Supplementary Fig. 1b). Flow cytometry validated that most GD3 cells were marked by V γ 4, often paired with V δ 4 (*Trdv2-2*), with a smaller population of V γ 7⁺ cells absent of V δ 4 (Fig. 2c and Supplementary Fig. 1c,d). Nonetheless, TCR usage did not define transcriptionally independent subsets of GD3 cells (Supplementary Fig. 2a); rather, RNA velocity identified a gene expression programme gradient (Supplementary Fig. 2b,c).

Transcriptional analysis suggested that GD3 cells have higher expression of *Ly6a* (Sca-1) and lower expression of *Il18r1* (IL-18R α ; compared to DETCs and dermal $\gamma\delta$ T cells) and *Cd9* (CD9; compared to DETCs), which we validated with flow cytometry (Supplementary Fig. 2d–f). *Trgu4* deficiency led to an absence of $V\gamma4^+$ GD3 cells and phenocopied the papain-induced itch defect in *Tcrd*^{-/-} mice (Fig. 2d and Extended Data Fig. 4a). These data indicate that **GD3 cells are a bona fide cutaneous $\gamma\delta$ T cell subset and that the dominant $V\gamma4^+$ clones promote allergen activation of sensory neurons.**

Ageing alters the epidermal compartment²³ and was associated with reduced GD3 cell numbers (Extended Data Fig. 4b). Commensal microbes regulate cutaneous immune cells, including dermal $\gamma\delta$ T cells²⁴. Dermal $\gamma\delta$ T cells, GD3 cells and allergic itch were deficient in germ-free (GF) mice (Extended Data Fig. 4c,d). Reintroduction of commensal microbiota into adult mice by cohousing specific-pathogen-free (SPF) with GF mice (exSPF and exGF) restored GD3 cell numbers (Extended Data Fig. 4e). GD3 cells were detected in genetically diverse and microbially rich pet shop mice (Extended Data Fig. 4f). Given the positive association between dry skin and allergic sensitization^{25,26}, we proposed that GD3 cells accumulate in dry skin. **Indeed, GD3 cells increased in the skin of WT mice treated with acetone:diethylether and water (AEW) compared to sham controls, even in the absence of CD45⁺ immune cell infiltration (Extended Data Fig. 4g,h).** Together, these data suggest that physiologic and environmental factors control GD3 cell numbers.

The epidermis is a thin epithelium that must balance its role in sensing the external environment through innervation by free nerve endings with that as a physical barrier. Confocal immunofluorescence microscopy of epidermal sheets showed that $V\delta4^+$ GD3 cells (of the dominant $V\gamma4^+V\delta4^+$ GD3 clonotype) were found in clusters adjacent to DETCs and mirrored their hallmark dendritic morphology^{20,27} (Supplementary Fig. 3a). The presence of GD3 cell clusters is consistent with local proliferative units and clonal self-renewal, as previously observed in $V\gamma5^+$ DETCs²⁷. **$V\delta4^+$ GD3 cells and DETCs formed direct contacts with *Scn10a*^{tdTomato} (Nav1.8⁺) nociceptive and pruriceptive free nerve endings (Fig. 2e,f and Supplementary Fig. 3b), demonstrating the intimate association and neuroimmune network formation between epidermal $\gamma\delta$ T cells and sensory neurons.**

Through several in-frame premature termination codons, humans are functionally devoid of *Skint1* (ref. 28), suggesting there is no direct human equivalent to mouse $V\gamma5^+$ DETCs¹⁶. Given the comparable lack of *Skint1* between humans and *Skint1*^{-/-} (FVB/N-Tac) mice that contain abundant GD3 cells, we compared mouse GD3 cells to human epidermal cells using the Gellatly et al.²⁹ scRNA-seq dataset. Mouse GD3 cells showed significant transcriptional similarity to human $\alpha\beta$ and $\gamma\delta$ T cells, although we also detected statistically significant similarities between mouse GD3 cells and human melanocytes (Fig. 2g–j and Supplementary Fig. 4a). An analogous comparison of mouse GD3 cells to normal human cutaneous T and innate lymphoid cells (ILCs) from Reynolds et al.³⁰ revealed that the populations with the highest normalized enrichment scores were natural killer cells and $\gamma\delta$ T cells (Supplementary Fig. 4b–e). Despite differences in the sequencing methods and isolation protocols used among the datasets, in both cases, **mouse GD3 cells shared a common transcriptional signature with human $\gamma\delta$ T cells in normal skin.**

Allergic itch requires GD3-derived IL-3

Our data indicate that a GD3 cell-secreted factor heightens the allergen responsiveness of sensory neurons (Fig. 1i). **A multiplex assay with validation by enzyme-linked immunosorbent assay (ELISA) revealed that the only unique factor secreted by α CD3/CD28-stimulated GD3 cells was IL-3, whereas dermal $\gamma\delta$ T cells produced IL-17A (Fig. 3a and Extended Data Fig. 5a,b).** $V\gamma4^+$ GD3 cells produced significantly more IL-3 than $V\gamma7^+$ or $V\gamma4^+V\gamma7^+$ GD3 cells (Extended Data Fig. 5c). There were no differences between DETC and GD3 cell viability and proliferation

following their stimulation in vitro (Extended Data Fig. 5d). GD3 cells produced more IL-3 than cutaneous $\alpha\beta$ T cells, ILCs or other remaining CD45⁺ cells, indicating that they are the major source of IL-3 in naive, steady-state skin (Extended Data Fig. 5e). *Il3* expression in naive skin was specific to GD3 cells (Extended Data Fig. 6a,b). This was independent of papain, as GD3 cells only expressed *Il3* or produced IL-3 when stimulated with PMA/Ionomycin or α CD3/CD28 (Extended Data Fig. 6c,d).

Given the unique production of IL-3 by GD3 cells, we proposed that this was the factor that drives allergen-induced itch. **Although IL-3 was unable to elicit itch on its own (Fig. 3b), IL-3 enhanced allergen-induced itch in WT mice when intradermally (i.d.) injected before diverse allergens (Fig. 3c and Extended Data Fig. 7a) and rescued papain-induced itch in *Tcrd*^{-/-} mice (Fig. 3d).** IL-3, however, had no effect on IL-31-induced itch (Extended Data Fig. 7b), demonstrating the specificity of IL-3 priming to allergen-induced itch. The i.d. injection of IL-17A, produced only by dermal $\gamma\delta$ T cells, or CCL3, produced non-specifically by dermal $\gamma\delta$ T and GD3 cells, before papain did not affect itch (Extended Data Fig. 7c). Although the involvement of IL-4 and IL-13 in chronic itch was previously reported in an atopic dermatitis mouse model⁷, papain-induced itch was unaffected in *Il4ra*^{-/-} mice (Extended Data Fig. 7d). Consistent with a necessary role for IL-3 in sensory neuronal responses to allergens, papain-induced itch depended on global *Il3* and specific *Il3* expression in $\gamma\delta$ T cells (Fig. 3e,f). Because GD3 cells are the only apparent $\gamma\delta$ T cell source of IL-3, these data indicate that **GD3-derived IL-3 is required for allergen activation of sensory neurons.**

IL-3 primes PEP1 neurons to allergens

We postulated that sensory neurons respond directly to IL-3 by means of the IL-3-specific receptor component IL-3R α (*Il3ra*) and the IL-3, IL-5 and GM-CSF common component CD131 (*Csf2rb*) heterodimer³¹. *Il3ra* and *Csf2rb* were detected in acutely dissected DRG from mice or human participants³² (Supplementary Fig. 5). To determine whether *Il3ra* was enriched within specific sensory neuronal subsets, we performed RNA fluorescence in situ hybridization (FISH) of DRG sections and paired *Il3ra* detection with that for marker genes of sensory neuronal subsets previously identified by scRNA-seq^{33–36}. ***Il3ra* was significantly enriched in PEP1 and virtually absent in NP3 sensory neurons (Fig. 3g), explaining the lack of effect of IL-3 on IL-31-induced itch (Extended Data Fig. 7b).** In mice lacking *Il3ra*, papain-induced itch was impaired (Fig. 3h). To determine whether sensory neurons directly respond to IL-3 to promote allergic itch, we deleted *Il3ra* in sensory neurons through *Scn10a*. **IL-3 enhanced papain-induced itch in control *Scn10a*^{cre/+} mice expressing only one functional copy of the sodium channel *Scn10a* (Fig. 3i), but not when *Il3ra* was eliminated from these cells (Fig. 3j).**

In vitro calcium imaging revealed that 8% of DRG neurons responded to IL-3, 20% to papain or AITC (a TRPA1 ligand) and 50% to capsaicin (a TRPV1 ligand) (Extended Data Fig. 7e–g). IL-3-responsiveness overlapped with papain- and capsaicin-responsive neurons but not with AITC-responsive neurons (Extended Data Fig. 7h). **In the absence of TRPV1⁺ neurons, GD3 cells decreased, suggesting that there is bidirectional communication between these cells (Extended Data Fig. 7i).** **IL-3 pretreatment of DRG neurons in vitro increased the percentage of papain-responsive neurons (Fig. 3k).** We replicated these findings in vivo, assessing the effect of IL-3 on papain-induced calcium responses in trigeminal ganglia sensory neurons in live mice. Consistent with the in vitro results, we observed a significant increase in papain-induced sensory neuronal calcium activity among mice that underwent IL-3 pretreatment, in contrast to mice pretreated with sham Dulbecco's phosphate buffered saline (DPBS) (Fig. 3l,m). Given the enrichment of *Il3ra* in PEP1 sensory neurons, we leveraged their expression of *Tac1* (refs. 33–36) to assess their role in allergic itch. Chemogenetic activation of *Tac1*-expressing cells caused mixed itch and pain responses (Extended Data Fig. 7j), whereas chemogenetic

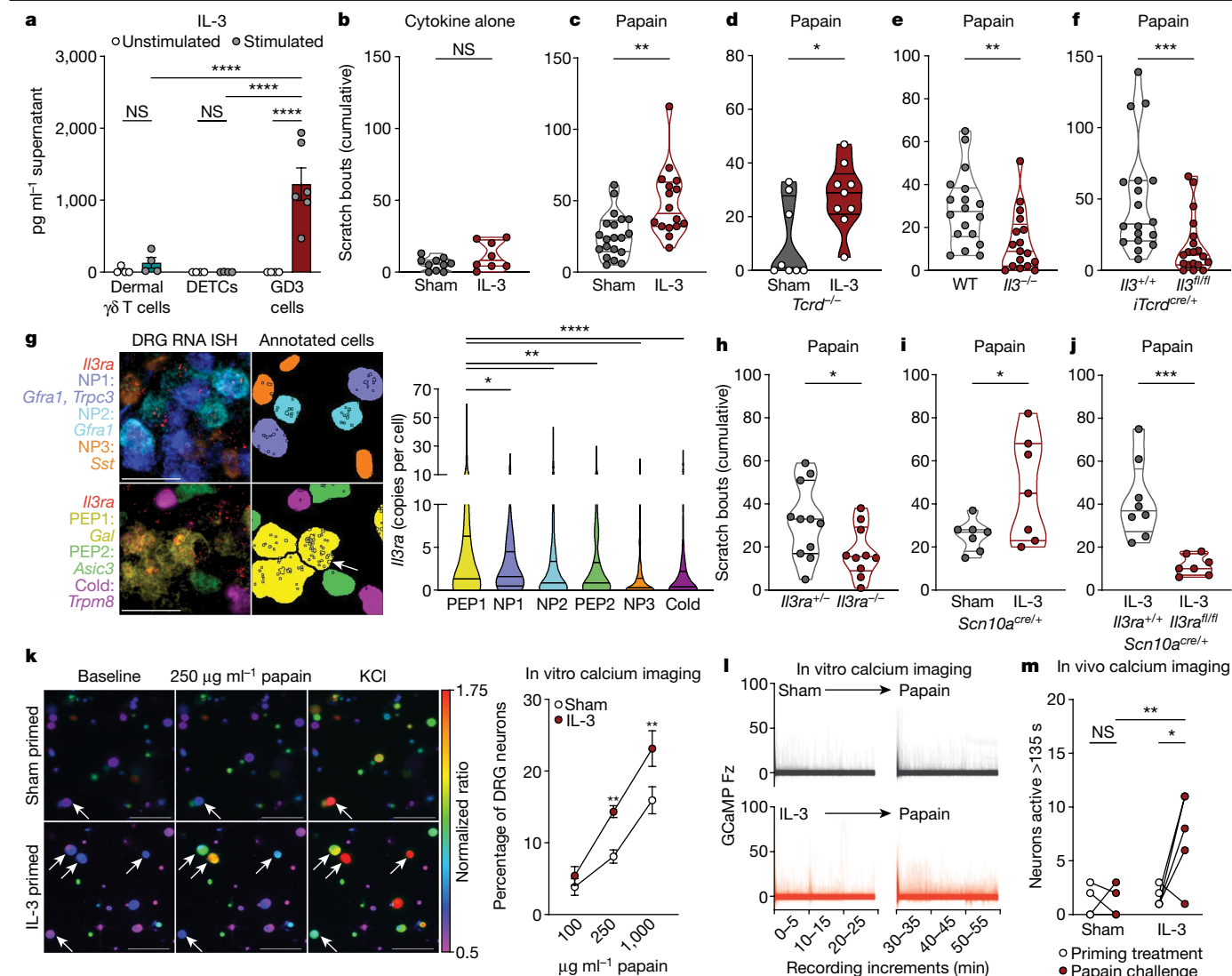


Fig. 3 | GD3 cell IL-3 primes the allergen responsiveness of sensory neurons through *IL3ra*. **a**, IL-3 ELISA of cell-free supernatant from FACS-isolated $\gamma\delta$ T cells stimulated as indicated ($n = 4$ per group, except for stimulated GD3 cells ($n = 6$)). **b–d, i–j**, Cumulative cheek scratch bouts following i.d. injection of IL-3 (**b**, $n = 10$ sham; $n = 8$ IL-3), then papain (**c**, $n = 20$ sham; $n = 16$ IL-3; **d**, $n = 8$ sham; $n = 9$ IL-3; **i**, $n = 7$ per group; **j**, $n = 8$ *Scn10a^{cre/+}IL3ra^{+/+}*; $n = 7$ *Scn10a^{cre/+}IL3ra^{fl/fl}*) in WT (**b, c**), *Tcrd^{-/-}* (**d**) or *Scn10a^{cre/+}* (**i, j**) mice. **e, f, h**, Cumulative cheek scratch bouts following i.d. injection of papain (**e**, $n = 18$ WT; $n = 17$ *IL3^{-/-}*; **f**, $n = 18$ *iTcrd^{cre/+}IL3^{+/+}*; $n = 19$ *iTcrd^{cre/+}IL3^{fl/fl}*; **h**, $n = 11$ *IL3ra^{+/+}*; $n = 10$ *IL3ra^{-/-}*). **g**, RNA FISH of mouse DRG tissue sections. The arrow indicates a cell with *IL3ra* expression (open circles) ($n = 134$ PEP1; $n = 114$ NP1; $n = 139$ NP2; $n = 256$ NP2; $n = 192$ NP3; $n = 152$ cold). **k**, Ratiometric calcium imaging of DRG neurons stimulated with papain. Arrows show neurons excited by $250 \mu\text{g ml}^{-1}$ papain ($n = 11$ sham; $n = 10$ IL-3).

I, m, GCaMP imaging of trigeminal ganglia neurons from mice i.d. immunized with sham DPBS or IL-3, then papain ($n = 4$ sham; $n = 5$ IL-3). **I**, Calcium traces from individual neurons; **m**, number of neurons with sustained calcium activity per mouse. Symbols represent individual wells (**a**), mice (**b–f, h–j, m**) or mean $\pm$ s.e.m. of experiments (**k**). Bar and dose–response plots are mean $\pm$ s.e.m. Violin plots show the median and quartiles. Data represent at least two independent experiments and were combined, except in **a**. Statistical tests: two-way ANOVA with Tukey's multiple comparisons test (**a**), two-way repeated measures ANOVA with Tukey's multiple comparisons test (**k**), two-way repeated measures ANOVA (**m**), one-way ANOVA with Tukey's multiple comparisons test (**g**), two-sided unpaired *t*-test (**b, h–j**) or two-sided Mann–Whitney *U*-test (**c–f**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Scale bars, $50 \mu\text{m}$ (**g**), $20 \mu\text{m}$ (**k**).

inhibition prevented papain, but not IL-31, induced itch (Extended Data Fig. 7k). These data indicate that **IL-3 primes the allergen responsiveness of *IL3ra*-expressing PEP1 neurons**.

IL-3 drives allergic immune initiation

The initiation of the allergic immune response, characterized by the migration of T_H2 -skewing CD301b⁺ DCs from the skin to the dLN followed by T_H2 differentiation, depends on the release of substance P from allergen-activated sensory neurons^{1,12}. Given the requirement for $\gamma\delta$ T cells in allergen activation of sensory neurons, we proposed that the downstream allergic immune response would be defective in

mice deficient in the GD3–IL-3 axis (Extended Data Fig. 8a). We tracked the migration of Kaede^{red} T_H2 -skewing CD301b⁺ DCs from the skin to the dLN (Extended Data Fig. 8b) and found that **papain immunization induced the migration of T_H2 -skewing CD301b⁺ DCs in WT Kaede, but not *Tcrd^{-/-} Kaede mice* (Fig. 4a and Extended Data Fig. 8c, d). This finding was recapitulated in mice where *IL3* or *IL3ra* were deleted from *Tcrd*- or *Scn10a*-expressing cells, respectively; **in these models, migratory antigen-bearing and activated T_H2 -skewing CD301b⁺ DCs were reduced in the absence of *IL3* in $\gamma\delta$ T cells or *IL3ra* in sensory neurons** (Fig. 4b and Extended Data Fig. 8e, f). Although papain elicits itch, acute scratching was not required for T_H2 -skewing CD301b⁺ DC migration to the dLN (Extended Data Fig. 8g).**

migration was dependent on both STAT5 (Fig. 4k) and the presence of DRG neurons in the culture, as supernatant from cell-free wells treated with papain did not induce appreciable CD301b⁺ DC migration (Extended Data Fig. 10e). These data suggest that JAK2 and STAT5 exert distinct effects on the neuroimmune response to allergens: sensory neuronal JAK2 promotes allergic itch, whereas STAT5 governs the initiation of the allergic immune response.

Here we describe a $\gamma\delta$ T cell–IL-3 pathway central to the allergen responsiveness of sensory neurons (Extended Data Fig. 10f). Previous studies found that sensory neurons direct the initial immune response to allergens¹ and that chronic allergic inflammation of the skin elicits sensory neuronal activation^{3–7}. Sitting upstream of this bidirectional neuroimmune circuit, we now propose that during naive homeostatic conditions, GD3 cells and IL-3 determine the allergen activation ‘set-point’ in sensory neurons through JAK2–STAT5 signalling to control sensory responses and neuropeptide transcription. Once an initial allergen exposure overcomes this threshold and chronic allergic inflammation is established, $\alpha\beta$ T cells enter the skin and may further sensory neuronal responses through IL-3, IL-4 and/or IL-13 (refs. 7,15). **However, the expression patterns of the relevant receptors (*Il3ra* in PEP1 neurons and *Il4ra* more broadly expressed^{7,33,35}) explain why IL-3 promotes the activity of protease allergens that preferentially target PEP1 neurons^{1,2,33,35}, whereas IL-4 and IL-13 promote the responsiveness of chemical pruritogens, including histamine and chloroquine^{7,33,35}.** Given that a subset of atopic dermatitis patients fail dupilumab (anti-IL-4R α monoclonal) therapy but respond to JAK inhibitors, this suggests that alternative pathways to IL-4R α and JAK1 (ref. 37), such as IL-3R α and JAK2, are new targets in atopic dermatitis and other itch disorders³⁸.

In addition to these findings, we characterized the physiological role of a rare epidermal $\gamma\delta$ T cell lineage in mice. Similar to *Skint1*^{−/−} FVB/N-Tac mice, hominoids, including humans, harbour a *SKINT1*-like gene that is inactivated by several mutations, rendering these primates devoid of a direct analogue to mouse V γ 5⁺ DETCs²⁸. Nonetheless, humans contain a population of epidermal $\gamma\delta$ T cells with a poorly understood aetiology and function^{29,39}. Comparing mouse GD3 cells to healthy human skin cells revealed that GD3 cells are transcriptionally similar to human $\gamma\delta$ T cells. **Thus, our data suggest that GD3 cells may be the relevant mouse counterpart to human epidermal $\gamma\delta$ T cells.** Whether GD3 cells have other physiological roles or if their production of IL-3 affects other cells expressing IL-3R α has not yet been established in homeostatic or pathophysiological situations. The allergic immune response may have evolved to protect the host from environmental toxins, offering a potential beneficial role of GD3 cells for the organism⁴⁰. **Given that the number of GD3 cells is highly receptive to environmental cues, we propose that this pathway could explain individual differences in allergic susceptibility.** Those questions notwithstanding, our study suggests that correcting an overabundance of GD3 cells, their production of IL-3, sensory neuronal IL-3R α or sensory neuronal JAK2–STAT5, would improve the outcome of patients with allergic disease and prevent allergen sensitization.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-024-07869-0>.

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Methods

Mice

Experiments involving animals were approved by the Massachusetts General Hospital or Harvard Medical School Institutional Animal Care and Use Committee. Mice were maintained and bred in a SPF facility in ventilated cages, a maximum of five mice per cage, on a 12 hour day–night cycle, at 20–25 °C and 35–65% humidity and provided food and water ad libitum. Experimental mice were 5–16 weeks of age unless otherwise noted. Male and female mice were used in all experiments unless specifically noted, and comparisons were made between age- and sex-matched controls. Power calculations were conducted to determine sample sizes. Mice were placed into groups depending on their genotype; when possible, mice were randomized. Behavioural experiments were blinded and immunologic experiments were not.

WT C57BL/6 (556) mice were purchased from Charles River Laboratories. C57BL/6 WT (000664), *Tcrd*^{tm1.1Cgn} (B6.129P2-*Tcrd*^{tm1.1Cgn}/J; 002120), *Rag2*^{tm1.1Cgn} (B6.Cg-*Rag2*^{tm1.1Cgn}/J; 008449), *Tcra*^{tm1.1Cgn} (B6.129S2-*Tcra*^{tm1.1Cgn}/J; 002116), *muMT*^{tm1.1Cgn} (B6.129S2-*Ighm*^{tm1.1Cgn}/J; 002288), *Skint1*^{+/+} FVB/NJ (001800), *Tcrd*^{CreER} (B6.129S-*Tcrd*^{tm1.1(cre/ERT2)Zhu}/J; 031679), *ROSA-DTA* (B6.129P2-*Gt(ROSA)26Sor*^{tm1(DTA)Jky}/J; 009669), *Scn10a*^{Cre} (B6.129(Cg)-*Scn10a*^{tm2(cre)Woj}/Tjpj; 036564), *Ai9* (B6.Cg-*Gt(ROSA)26Sor*^{tm9(CAG-tdTomato)Hze}/J; 007909), *Ai9SD* (B6J.Cg-*Gt(ROSA)26Sor*^{tm9S.1(CAG-GCaMP6f)Hze}/MwarJ; 028865), *Vglut2*^{Cre} (B6J.129S6(FVB)-*Slc17a6*^{tm2(cre)Lowl}/MwarJ; 028863), *Tac1*^{Cre} (B6.129S-*Tac1*^{tm1.1(cre)Hze}/J; 021877), *GqDREADD* (B6N;129-Tg(CAG-CHRM3*,*-mCitrine*)1Ute/J; 026220) and *GiDREADD* (B6.129-*Gt(ROSA)26Sor*^{tm1(CAG-CHRM4*,*-mCitrine*)Ute}/J; 026219) mice were purchased from The Jackson Laboratory. *Skint1*^{+/+} FVB/NTac mice were purchased from Taconic Biosciences. *Cpa3*^{Cre} mice were originally from Hans-Reimer Rodewald (German Cancer Research Center). *Tcrd*^{GDL} mice were originally from Immo Prinz (Medizinische Hochschule Hannover). GF mice were acquired from the Massachusetts Host-Microbiome Center. Skin samples from pet shop mice were kindly provided by D. Masopust (University of Minnesota). *IL3*^{+/+}, *IL3ra*^{+/+}, *IL3*^{GFPfl/fl} and *IL3ra*^{fl/fl} mice were generated and provided by C. S. McAlpine, B. Kleinstiver and F. Swirski at Massachusetts General Hospital^{41,42}. *Trpv1*^{DTR} mice were originally from D. Mucida (Rockefeller). *IL4ra*^{+/+} mice were originally from F. Finkelman (University of Cincinnati). *Trpv1*^{DTR} mice were originally from M. Hoon (NIH). Kaede mice were originally from O. Kanagawa (Riken). *Tcrd*^{CreER} (referred to as *iTcrd*^{Cre}) were crossed to *ROSA-DTA* or *IL3*^{GFPfl/fl} to generate tamoxifen-inducible *iTcrd*^{DTA} or *iTcrd*^{Cre/+} *IL3*^{GFPfl/fl} mice, respectively. *Scn10a*^{Cre} were crossed to *Ai9* or *IL3ra*^{fl/fl} to generate *Scn10a*^{Cre/+} *tdTomato* or *Scn10a*^{Cre/+} *IL3ra*^{fl/fl} mice, respectively. *Vglut2*^{Cre} was crossed to *Ai9SD* to generate mice for in vivo calcium imaging. *Tac1*^{Cre} were crossed to *GqDREADD* or *GiDREADD* to generate *Tac1*^{Cre/+} *GqDREADD* or *Tac1*^{Cre/+} *GiDREADD* mice, respectively. Kaede was crossed to *Tcrd*^{+/+} to generate Kaede × *Tcrd*^{+/+} mice.

To control for differences in commensal microbes among colonies, all experiments using *Cpa3*^{Cre/+}, *Tcrd*^{GDL}, *iTcrd*^{Cre/+}, *Scn10a*^{Cre/+} and *Tac1*^{Cre/+} *GqDREADD* mice were performed with littermate controls bred in our facility. All other experiments comparing WT to experimental mouse lines were performed with colony control WT mice originally from The Jackson Laboratory, but bred in our facility, except in Fig. 1b, Extended Data Fig. 1d (chloroquine) and Extended Data Figs. 1e and 8i, which used controls purchased from Charles River Laboratories. In experiments that only used WT mice, the mice were purchased from Charles River Laboratories and used immediately on arrival in our laboratory. FVB/N mice came from different colonies to enable *Skint1* studies (*Skint1*^{+/+}, Jax; *Skint1*^{+/+}, Taconic). In supernatant transfer models, donor cells were the same strain and colony of origin as the recipient mice.

Experimental mouse models

Immunizations. Under brief isoflurane anaesthesia, mice were i.d. immunized with 25 µl in the cheek (behaviour), top of the foot

(DC migration and T cell differentiation) or pinnae (immune cell collection and delayed-type hypersensitivity). Immunizations included papain (50 µg previously frozen, 1 µg freshly prepared (Millipore Sigma) or 5 µg of freshly prepared (Creative Enzymes), to normalize for differences in enzymatic activity); 100 or 20 µg (low-dose) of HDM and 100 or 10 µg (low-dose) of *Alternaria alternata* (Greer Laboratories); 2,500 or 500 NU (low-dose) fire ant (ALK); five mosquitoes-worth of mosquito saliva; 50 µg histamine, chloroquine and ovalbumin (ova; Millipore Sigma); 20 µg of capsaicin (Cayman Chemical); 2.5 µg of IL-31 (Peprotech); 1.25 µg clozapine-*N*-oxide (CNO) (Millipore Sigma); culture supernatant as indicated. Mosquito saliva was generated as previously described⁴³ from adult *Aedes aegypti* females (Liverpool strain). Soluble substances were dissolved in DPBS (Corning) and insoluble substances were dissolved in corn oil or dimethylsulfoxide (DMSO) (Millipore Sigma). For chronic papain injections, mice were i.d. injected with 5 µg of papain in 25 µl of DPBS in the pinnae on days 0, 2, 4, 7, 9 and 11. Mice were euthanized on day 14, and pinnae were harvested and processed following the section on the 'Isolation of immune cells from mouse dermis and epidermis' protocol.

Behaviour. Mice were moved from the animal facility to a private room in our laboratory and separated into individual cages where they habituated for 2 h. Following i.d. cheek injection with 25 µl of indicated stimuli, a video was recorded from above and scratching at the injection site (with the hind paw) was tabulated. A 20 min video was recorded for chloroquine, HDM, capsaicin and *Alternaria* in WT versus *Tcrd*^{+/+} mice (Fig. 1b and Extended Data Fig. 1d,e) and papain in WT versus *IL4ra*^{+/+} mice (Extended Data Fig. 8i). A 60 min video was recorded for mosquito saliva experiments. In all other behaviour experiments, a 30 min video was recorded. In spontaneous non-specific itch experiments (Supplementary Fig. 1b), no injection was performed and scratch at any skin site (cheek, pinnae, flank, side, back and so on) was quantified over 30 min.

Depletion of γδ T cells in *Tcrd*^{GDL} mice. *Tcrd*^{GDL} mice express the human diphtheria toxin receptor (DTR) in *Tcrd*-expressing cells⁴⁴. *Tcrd*^{WT} and *Tcrd*^{GDL} mice were injected intraperitoneally (i.p.) with 0.5 µg of diphtheria toxin (Millipore Sigma) every other day for two total injections, then rested for 3 days. Behaviour and flow cytometry were performed after the rest period.

Tamoxifen-induced cre in *iTcrd*^{Cre} mice. We crossed *iTcrd*^{Cre} with *ROSA-DTA* mice to generate tamoxifen-inducible diphtheria toxin A in *Tcrd*-expressing cells^{45,46}. *iTcrd*^{control} and *iTcrd*^{DTA} or *iTcrd*^{Cre/+} *IL3*^{+/+} and *iTcrd*^{Cre/+} *IL3*^{fl/fl} mice were injected i.d. with 0.5 mg of tamoxifen (Cayman Chemical) on two consecutive days, then rested for 4 days. Behaviour, flow cytometry of the pinnae, DC migration or T cell differentiation assays were performed after the rest period. Epidermal DETCs and GD3 cells were preferentially depleted *iTcrd*^{DTA} mice, probably due to their high expression of *Tcrd*, as previously found⁴⁵.

Mouse model of dry skin. To induce dry skin, we modified the acetone:diethyl ether + water (AEW) model as previously described^{47,48}. Pinnae were treated twice daily with AEW or sham for 4 days. A 1:1 ratio of acetone:diethyl ether was applied to the skin using a soaked gauze for 15 s, then H₂O was applied to the same site for 30 s. Following a rest period, experiments were performed on day 8.

Depletion of Trpv1⁺ sensory neurons in *Trpv1*^{DTR} mice. Over a 14 day period, WT and *Trpv1*^{DTR} mice were injected i.p. with 0.2 µg of DT (Millipore Sigma) every day for 5 days, then rested for 2 days. After the final DT injection, mice rested for 7 days. Deletion of *Trpv1*-expressing sensory neurons was confirmed using the tail-flick assay¹. Flow cytometry was performed after the rest period.

Article

Supernatant transfer and cytokine instillation. Tissue-culture-treated U-bottom 96-well plates (Corning) were incubated with 50 μ l of purified α CD3 (2 μ g ml⁻¹, BioLegend) for 2 h at 37 °C, then washed four times with DPBS. Dermal γ δ T cells, DETCs and GD3 cells were FACS isolated and resuspended in fully supplemented Roswell Park Memorial Institute medium (RPMI) (Corning, supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, 1% glutamax, 1% HEPES, 1% NEAA (Gibco), 1% sodium pyruvate (Gibco) and 0.1% 2-mercaptoethanol (Gibco). Next, 1,000–4,000 cells per well were incubated at 37 °C in 5% CO₂. In wells coated with α CD3, purified α CD28 (2 μ g ml⁻¹) was added to the fully supplemented RPMI. In one control experiment, wells were coated with α CD3 and purified α CD28 (2 μ g ml⁻¹) was added to the fully supplemented RPMI, but no cells were plated. After 5 days, cell-free supernatant was harvested, snap-frozen and stored at -80 °C.

Following 2 h of habituation, as described in the section on the 'Behaviour' protocol, mice were i.d. immunized with 25 μ l of cell-free supernatant derived from unstimulated or stimulated dermal γ δ T cells, DETCs or GD3 cells. After 30 min, mice were i.d. immunized with 1 or 5 μ g of papain. Videos were recorded following papain injection, and scratch bouts were tabulated. In the indicated experiments, DPBS or 5 μ g recombinant-mouse IL-3, IL-17A or CCL3 (Peprotech) in DPBS was injected 30 min before papain, HDM, *Alternaria alternata*, fire ant or IL-31. Videos were recorded in the 30 min following cytokine immunization (when indicated) or following the subsequent papain injection.

Cohousing GF mice. To re-introduce a commensal microbiota to GF mice, female C57BL/6 SPF mice from Charles River were cohoused along with female GF mice for 5 weeks in our colony. These mice became exSPF and exGF. Following cohousing, flow cytometry was performed.

Trigeminal ganglion in vivo calcium imaging. *Vglut2^{cre}* were crossed to *Ai95D* to allow for calcium imaging of trigeminal ganglia as described previously⁴⁹. Briefly, mice were induced and maintained at surgical plane anaesthesia by i.p. injection of ketamine (100 mg kg⁻¹) and xylazine (10 mg kg⁻¹), and a unilateral craniotomy and hemispherectomy performed to expose the surface of the trigeminal ganglion. Illumination was provided with a collimated 470 nm light emitting diode through a $\times$ 10, 0.3 numerical aperture air objective. Images were acquired at 10 Hz with 35 ms exposure time using a CMOS camera. For each animal, we designated a single field of view guided by the criterion that it contained neurons showing robust calcium responses on the indentation of the cheek skin. In each experiment, before assessing the effect of IL-3, GCaMP activity was imaged in response to cheek indentation and 5 min of baseline activity. Subsequently, mice were i.d. injected with 5 μ g (25 μ l) IL-3 or sham DPBS (25 μ l) at the location where mechanical indentation was performed. This was followed by three 5 min imaging sessions, covering 0–5, 10–15 and 20–25 min postinjection. Then 30 min after IL-3 or DPBS priming, the mice received an i.d. challenge injection of 5 μ g (25 μ l) papain followed again by three 5 min imaging sessions capturing 0–5, 10–15 and 20–25 min postpapain treatment.

Images of GCaMP fluorescence were normalized to a low-pass spatial filtered image of each frame to correct for fluorescence bleaching and non-focal plane-derived fluorescence transients, and motion corrected using a Python implementation of TurboReg⁵⁰. Regions of interest containing active cells were identified by adaptive thresholding of heat maps constructed by measuring correlation of each pixel to neighbouring pixels, with manual curation of region of interests defined automatically. Region of interest masks were refined by constrained non-negative matrix factorization⁵¹, after which modified z-scored activity traces were extracted as mean fluorescence intensity normalized to median absolute deviation of each trace. Proportion of time above threshold (z-score above 3.5) was used as a metric of continuing activity in each neuron during baseline measurement and after papain injection, with cells above threshold in at least 15% of time points over a 15 min imaging time period classified as spontaneously active.

Chemogenetic manipulation of Tac1-expressing cells. CNO is a pharmacologically inert ligand that binds to DREADDs (designer receptors activated only by designer drugs), leading to the chemogenetic activation of *Tac1*-expressing cells in the *GqDREADD* mouse and inhibition in the *GiDREADD* mouse⁵². To elicit the chemogenetic activation of *Tac1*-expressing cells, *Tac1^{cre/+} GqDREADD* mice were i.d. immunized with 1.25 μ g of CNO in the cheek. A 90 min video was recorded from above. Cheek wipes (with the fore paw) and cheek, flank or pinnae scratch bouts (with the hind paw) were tabulated. To elicit the chemogenetic inhibition of *Tac1*-expressing cells, *Tac1^{cre/+} GiDREADD* mice were i.d. immunized with 1.25 μ g of CNO in the cheek 30 min before 5 μ g of papain or 2.5 μ g of IL-31 was injected at the same site. A 30 min video was recorded from above, and scratching at the injection site was counted.

DC migration. Kaede mice express a photoconvertible protein that switches from green to red on exposure to violet light⁵³. Following the exposure of Kaede skin to violet light, cell migration throughout the organism can be tracked by assessing cells that were photoconverted from Kaede^{green} to Kaede^{red}. The skin of Kaede mice was photoconverted by exposing the dorsal aspect of the feet to light generated by a Bluewave LED visible light curing unit (Dymax) with a 420 nm bandpass filter (Andover Corp). The exposure lasted 5 min, and the light was positioned 7.5 cm away from the dorsal feet. Immediately after photoconversion, the dorsal foot was immunized with 25 μ l as indicated. Internal controls were used in these experiments, as one dorsal foot received ova alone (control antigen), while the other received ova + papain. In some experiments, cell-free supernatant from DRG neuron cultures was injected into the dorsal foot as indicated (culture supernatant was prepared as described in the section on the 'Substance P release for DRG neurons' protocol). In all experiments, paired analyses were used to evaluate the migration of DCs to the dLN. Then 24 h after immunization, the popliteal LN was harvested and digested at 37 °C in digestion buffer: RPMI supplemented with DNase I (100 μ g ml⁻¹; Roche), Dispase II (800 μ g ml⁻¹; Millipore Sigma), Collagenase P (200 μ g ml⁻¹; Millipore Sigma) and 1% FBS. Every 7 min, the digestion buffer was removed and placed into RPMI supplemented with 2 mM EDTA and 1% FBS on ice. Fresh digestion buffer was added to the LNs. Tubes were vigorously shaken before and after each addition of fresh digestion buffer. This process was repeated until no fragment of LN tissue remained. Single-cell suspensions were filtered through a 70 μ m cell strainer before flow cytometry was performed.

To track the migration of DCs in other mouse strains that lack photoconvertible skin cells, 5 μ g of fluorescently labelled ova-AF647 (Fisher Scientific) was i.d. injected with 45 μ g ova and 50 μ g papain in the dorsal aspect of the feet. One foot received ova, while the other received ova + papain. Then 24 h after immunization, the popliteal LNs were gathered and processed as above. In migratory (CD11c⁺IAIE^{high}) CD301b⁺ DCs, ova-AF647⁺PDL2⁺ cells marked activated and antigen-bearing T_H2-skewing DCs.

In some experiments, DC migration was assessed in mice anaesthetized with isoflurane (less than 30 s of sedation) or ketamine and xylazine (more than 30 min of sedation). With ketamine and xylazine, mice remain sedated throughout the itch response to primary allergen immunization, largely completed within 20 min after injection¹. In *Scn10a^{cre/+}* experiments, only male mice were used.

T cell differentiation. Mouse footpads were immunized with 25 μ l as indicated. Internal controls were used in these experiments, as one dorsal foot received ova alone (control antigen), while the other received ova + papain. Then 5 days following immunization, the dLN was collected and dissociated in DPBS using the coarse end of a Super-Frost Plus Microscope Slide and the base of a curved forcep. Single-cell suspensions were filtered through a 70 μ m cell strainer. 2 $\times$ 10⁶ LN cells were added to a 1.5 ml Eppendorf tube (Fisher Scientific) and resuspended in 1 ml of fully supplemented RPMI with PMA (50 ng ml⁻¹;

Millipore Sigma), Ionomycin ($1 \mu\text{g ml}^{-1}$; Millipore Sigma) and Protein Transport Inhibitor (1:1,000, BD Biosciences). Samples were incubated for 3 h at 37°C in 5% CO_2 . Samples were washed twice with FACS Buffer, then cells were incubated in FACS Buffer containing fluorescent-conjugated antibodies targeting surface antigens for 15 min at 4°C . Following fixation with 4% paraformaldehyde (PFA) for 8 min at room temperature, fluorescent-conjugated antibodies targeting intracellular cytokines were added to each sample in $1\times$ eBioscience Permeabilization Buffer for 1 h at 4°C . Cells were washed twice with DPBS, then assessed by flow cytometry.

Delayed-type hypersensitivity. WT and *Tcrd*^{-/-} mice were i.d. injected in bilateral cheeks with 25 μl of 50 μg ova and 50 μg papain on days 0 and 2. On day 12, one pinna was injected with 25 μl of 50 μg ova. Pinnae thickness was measured on day 12 before the injection, and 24 (day 13) and 48 (day 14) h later.

Inhibitor treatment. Mice were i.p. injected with 100 μl of 10 mg kg^{-1} fedratinib (targeting JAK2; Selleck Chemicals), 10 mg kg^{-1} upadacitinib (targeting JAK1; Selleck Chemicals) or 10 mg kg^{-1} STAT5-IN-1 (targeting STAT5; Millipore Sigma) dissolved in corn oil containing 10% DMSO (Millipore Sigma). Behaviour to papain or histamine was evaluated 2 h later in WT mice treated with or without the inhibitors. Then 2 h fedratinib injection, the dorsal feet of Kaede mice were photoconverted and immunized with ova or ova + papain. The dLN was collected 24 h later to assess the migration of DCs. Next, 2 h after fedratinib injection, the dorsal feet of WT mice were immunized with ova or ova + papain. Then 5 days later, T cell differentiation was assessed in the popliteal dLN.

Isolation of immune cells from mouse dermis and epidermis

To isolate cutaneous cells from the skin, pinnae were removed from euthanized mice and weighed. Forceps were used to pull apart the dorsal and ventral sides of the pinnae. Once separated, the epidermis was attached to a piece of Flexigrid tape. In some experiments, to isolate cutaneous cells from the cheek and dorsal foot, mice were anaesthetized with ketamine and xylazine, and hair was removed from the cheek or dorsal foot skin using Veet. Then 24 h later, the cheek and dorsal foot skin were harvested from euthanized mice and weighed. The epidermis was then attached to a piece of Flexigrid tape. With the dermis-side down, the skin was floated on Dispase II (3 mg ml^{-1} , Millipore Sigma) in DMEM (Corning) for 90–120 min at 37°C in 5% CO_2 . The dermis was removed from the epidermis and vigorously minced before digestion in 5 ml Eppendorf tubes (Fisher Scientific) containing Liberase ($25 \mu\text{g ml}^{-1}$, Millipore Sigma), DNase I ($75 \mu\text{g ml}^{-1}$, Millipore Sigma) and 10 μM HEPES (Corning) in DMEM. The epidermis was digested in 5 ml Eppendorf tubes containing Collagenase IV (2 mg ml^{-1} , Millipore Sigma) in DPBS. Next, 5 ml samples were placed at 37°C in 5% CO_2 for 45 min and vigorously shaken every 9 min. Tubes were immediately placed on ice, and 500 μl of heat-inactivated FBS (Millipore Sigma) and 15 μl of 0.5 M EDTA (Invitrogen) were added. Dermal fragments were repeatedly aspirated and flushed through an 18-gauge needle. Finally, dermal and epidermal suspensions were twice filtered through a 70 μm cell strainer (Corning or Biologix) before downstream use.

Isolation of cells from mouse DRG

DRG were carefully dissected from mice and placed into a 15 ml Falcon tube (Corning) containing DMEM supplemented with 10% FBS and 1% penicillin-streptomycin (Millipore Sigma). DRGs were centrifuged at 1,000 rpm for 5 min, then digested for 70 min in 3 ml of Collagenase A (1.25 mg ml^{-1} , Millipore Sigma) and Dispase II (2.5 mg ml^{-1} , Millipore Sigma) on a 37°C shaker set to 150 rpm. Then 10 ml of DMEM supplemented with 10% FBS and 1% penicillin-streptomycin was added, then the dissociated DRGs were washed twice in DPBS supplemented with 1% penicillin-streptomycin and 1% GlutaMax (Gibco) before being resuspended in 1 ml of Neurobasal media A (NBM; Thermo

Fisher Scientific) supplemented with $1\times$ B-27 (Thermo Fisher Scientific) and 1% penicillin-streptomycin. DRGs were gently pipetted for 5–10 min using a P1000 pipette tip. This process was repeated using a P200 pipette tip. DRGs were layered on top of a 28 and 12.5% gradient of Percoll (VWR) diluted in supplemented NBM. The DRG cell suspension was centrifuged at 1,300g for 10 min at room temperature with no acceleration or brake. The cell pellet was then resuspended in supplemented NBM and filtered through a 70 μm cell strainer before downstream use. To isolate immune cells from DRGs, the Percoll gradient was not performed.

Flow cytometry and cell sorting

Single-cell suspensions were incubated at 4°C for 15 min in FACS Buffer (0.5% FBS in DPBS) containing fluorescent-conjugated antibodies. Dead cells were excluded using Fixable Viability Dye eFluor 780 (eBioscience) added to antibody (Ab) preparations at a dilution of 1:2,000. TruStain fcX (antimouse CD16/32; BioLegend) was added to Ab preparations at a dilution of 1:200. Ab-tagged cells were washed twice with DPBS. If necessary, single-cell suspensions were fixed in 4% PFA (Electron Microscopy Sciences) for 8 min at room temperature and washed twice with DPBS. Owing to the autofluorescence of dermal macrophages, the lineage markers CD68, CD11b and CD11c were used in the indicated skin analyses to exclude them. Purified antimouse TCR V γ 7 (BioLegend) was conjugated to AF647 using a Lightning-Link Alexa Fluor 647 Conjugation kit (Abcam). Ab-tagged cells were analysed on a CytoFLEX S flow cytometer (Beckman Coulter) or a Cytek Aurora (Cytek Biosciences) or sorted on a MA900 multi-application cell sorter (SONY, Cell Sorter Software v.3.3.2). Flow cytometric data were acquired using CytExpert v.2.3 (Beckman Coulter) or SpectroFlo v.3.2.1 (Cytek Biosciences) software, and .fcs files were analysed using FlowJo v.10 (TreeStar). The Abs used in this study are listed in Supplementary Table 3.

The following gating strategies were used: dermal $\gamma\delta$ T cells were live Lineage⁻CD3⁺TCR $\gamma\delta$ ^{intermediate}. DETCs were live Lineage⁻CD3⁺TCR γ δ^{high} V γ 5⁺. Epidermal GD3 cells were live Lineage⁻CD3⁺TCR $\gamma\delta^{\text{high}}$ V γ 5⁺. In Extended Data Fig. 2b, $\gamma\delta$ T cells were live CD45⁺Lineage⁻CD3⁺TCR $\gamma\delta$ ⁺. In Extended Data Fig. 2f, $\alpha\beta$ T cells were live Lineage⁻CD3⁺TCR β ⁺. Lineage markers were CD68, CD11b and CD11c. In Extended Data Fig. 9e, GD3 cells were live CD45⁺Lineage1⁻CD3⁺TCR $\gamma\delta^{\text{high}}$ V γ 5⁺, $\alpha\beta$ T cells were live CD45⁺Lineage1⁻CD3⁺TCR β ⁺, helper-like ILCs were live CD45⁺Lineage1⁻Lineage2⁻CD3⁺Thy1.2⁺ and other were all remaining CD45⁺ cells. Lineage1 was CD68, CD11b and CD11c. Lineage2 was NK1.1, B220 and CD19. T_H2-skewing DCs were live CD11c⁺IAIE^{high}CD301b⁺. The migration of T_H2-skewing DCs to the dLN was assessed by Kaede^{red} positivity, whereas activation and antigen uptake were monitored by PDL2 and ova-AF647 positivity, respectively. IL-4⁺ T_H2 cells were live CD4⁺CD44^{high}IL-4⁺. IL-4⁺IL-13⁺ T_H2 cells were live CD4⁺CD44^{high}IL-4⁺IL-13⁺.

Mouse scRNA-seq and scTCR-seq

A single-cell suspension of skin cells was generated from 30 pooled WT mice. Dermal $\gamma\delta$ T cells, DETCs and GD3 cells were FACS sorted into 50% fully supplemented RPMI and 50% FBS. After washing the sorted cells with FACS Buffer, each population was loaded as a separate channel on the Chromium 10X Genomic v.1.1 platform (10X Genomics). Libraries were prepared using the Chromium Single Cell 5' Library Construction Kit. Gene expression and TCR $\gamma\delta$ (TCR) libraries were generated from the complementary DNA (cDNA) produced through the Chromium chemistry. For the TCR libraries, custom primers were used to amplify TCR $\gamma\delta$ chains following a protocol that was previously described in ref. 54. Nucleic acid products were separated using SPRIselect beads (Beckman Coulter). Libraries were quantified using the Bioanalyzer High-Sensitivity DNA analysis kit (Agilent). Sequencing of gene expression libraries was performed using the NextSeq 500/550 High Output kit v.2.5 (75 cycles; Illumina), whereas TCR libraries were sequenced with the NextSeq 500/550 High Output kit v.2.5 (150 cycles; Illumina). Sequencing was performed on the NextSeq 550 (Illumina).

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Read alignment and quantification. Raw sequencing data was pre-processed with Cell Ranger (v.6.1.2, 10X Genomics) to demultiplex FASTQ reads, align reads to the mouse reference genome (mm10, v.2020-A from 10X Genomics) and count unique molecular identifiers (UMI) to produce a cell $\times$ gene count matrix⁵⁵. All count matrices were then aggregated with Pegasus (v.1.6.0, Python) using the aggregate_matrices function⁵⁶. Droplets with more than 20% mitochondrial UMI or fewer than 500 unique genes detected were deemed low-quality cells or empty droplets and were filtered out of the matrix before proceeding with downstream analyses. The counts for each remaining cell in the matrix were then log-normalized by computing the $\log_{10}$ (counts per 100,000). Following the application of quality control filters on the data, 28,765 high-quality cutaneous $\gamma\delta$ T cells were recovered (Supplementary Table 1).

scRNA-seq analysis. For clustering, 2,000 highly variable genes were selected using the highly_variable_features function in Pegasus and used as input for principal component analysis. The resulting principal components were used as input for uniform manifold approximation and projection (UMAP) algorithm (spread=1, min-dist=0.5). The number of principal components used for each clustering (global 35; GD3 26; DETC 20) was decided by means of molecular cross-validation (MCV)⁵⁷. In this MCV approach, the raw counts for each cell were randomly split to obtain two count matrices, A and B, whose sum was equal to the original count matrix. The top K principal components and loadings of the centred and scaled matrix A' were then used to reconstruct matrix B' and estimate an MCV loss with the mean squared error. The MCV losses for the top K principal components between 2 and 100 were estimated and the K that minimized MCV loss was used in downstream analyses. Comparisons between the different cell types were performed using the de_analysis function from Pegasus. $\log_2$ FC values were visualized with ggplot2 (R v.3.3.3).

Single-cell TCR-seq analysis. Raw sequencing data was preprocessed with Cell Ranger (v.7.0.0, 10X Genomics) to demultiplex FASTQ reads and align reads to the mouse immune repertoire genome (GRCm38, v.7.0.0 from 10X Genomics). Resulting TCRs were filtered to those that were productive (that is, no stop codons), had at least two UMI, and aligned with the single-cell data. For cells with several captured rearranged TCR chains, the one with the greatest number of UMIs was used for downstream analysis.

Estimation of RNA velocity. Count matrices of spliced and unspliced transcript abundances were calculated using velocity (v.0.17.15, Python)⁵⁸. These matrices underwent dimensionality reduction by means of principal component analysis (as described above and were used to compute a k -nearest neighbour graph ($k = 30$) that was used as input to estimate cellular velocity with scVelo (v.0.2.4, Python)⁵⁹. CellRank (v.1.5.1, Python) was used to estimate initial and terminal states and these estimations were used to recover latent time with the recover_latent_time function with scVelo⁶⁰. Velocity streamlines were plotted on UMAP embeddings with the velocity_embedding_stream function and the lineage driver genes associated with the inferred trajectory were calculated with the lineage_drivers function from CellRank. The top 100 genes driving the RNA velocity of the GD3 lineage are presented in Supplementary Table 2.

Human scRNA-seq

Two independent public datasets of human skin were re-analysed^{29,30} to compare the relationship between mouse GD3 cells and human skin cells.

Datasets used and reviews of their cohorts. The raw gene count matrix from ref. 29 was obtained from <https://vitiligo.dolphinnext.com/index.html>. Cells derived from this study were collected through

suction blistering of the skin and sequenced using a modified inDrop protocol. This method enriches epidermal cells, which was of particular interest given that GD3 cells are found in the mouse epidermis. The public dataset was filtered to include only healthy control participants, that is, those with no auto-immune or inflammatory skin diseases. This dataset included seven individuals, consisting of four women and three men aged 11 to 57.

Raw gene count matrices from ref. 30 were obtained from ref. 61. Skin cells from this study were obtained from mammoplasty surgery surplus and sequenced following 10X protocol. As genes in the healthy dataset (healthy.h5ad) were already filtered, raw counts from the full dataset (submission_210120.h5ad) were obtained by matching droplets by barcode. The final dataset included five healthy female individuals aged 25 to 60.

Preprocessing and clustering. Gene count matrices from both human datasets were reprocessed similarly to the processing of the mouse data. The Reynolds dataset was first filtered to include only cells previously annotated as T and natural killer cells; all lineages in the Gellatly dataset were kept due to the smaller dataset size. Higher-quality droplets were then selected on the basis of the number of unique genes and percentage of mitochondrial UMIs (more than 250 genes, fewer than 20% mitochondrial UMIs in the Reynolds dataset; more than 250 genes in the Gellatly dataset (no mitochondrial UMIs provided)). Similar to the mouse dataset, counts were log-normalized by computing the $\log_{10}$ (counts per 100,000).

To cluster cells in each dataset, 2,000 highly variable genes were selected using the highly variable features function of Pegasus. These genes were then used in MCV to obtain the number of principal components for downstream analyses (20 principal components Gellatly, 40 principal components Reynolds). To account for variability between donors and sequencing time, the resulting principal component scores were batch-corrected by sample using harmony-pytorch v.0.1.4. The harmonized principal components were used as input for Leiden clustering and creating a MAP.

Gene set scoring. Differential expression analysis was run between the GD3 and dermal $\gamma\delta$ T cell types of the mouse dataset using Pegasus's de_analysis function, and genes were ranked by t -statistic to obtain a list of the top 25 distinguishing genes between cell types. The V γ 5⁺ DETC cell type was excluded as it is not observed in humans due to the inactivation of the *SKINT1*-like gene, which its equivalent is required for V γ 5⁺ DETC development in mice²⁸. Mouse genes were then converted to their human homologues to be used as reference gene sets in Gene Set Enrichment Analysis (GSEA) using Ensembl's biomaRt release 108. Cells in the human datasets were pseudobulked by sample, and differential expression analysis was run using limma v.3.54.2 to compare one cluster against all other clusters to obtain genes distinguishing the individual clusters. The R package fgsea v.1.24.0 was then used to run GSEA on each human cluster, in which the distinguishing genes ranked by t -statistic were provided as input to investigate trends relating to the GD3 gene set used as a reference.

One limitation of the previous approach is that clustering resolution may affect what signal is picked up in the lists of distinguishing genes. To identify potential signals on a smaller scale, average z -scores of gene expression were computed for each cell using the GD3 gene set. These averaged z -scores were then plotted in UMAP space to find areas of high expression.

Confocal immunofluorescence of epidermal sheets and intact skin

Pinnae were removed from euthanized mice, and forceps were used to pull apart the dorsal and ventral sides of the pinnae. The epidermis was attached to a piece of Flexigrid tape. Pinnae were floated on prewarmed 0.47 M ammonium thiocyanate (Millipore Sigma) in DPBS for 15 min

at 37 °C in 5% CO₂, with the dermal side facing down. The dermis was carefully peeled away from the epidermis. Pieces of tape with epidermis attached to them were rinsed twice in DPBS. Epidermal sheets were fixed by floating them on 4% PFA for 8 min at room temperature. Epidermal sheets were fixed with gentle shaking on a plate shaker. Samples were washed three times in 2% heat-inactivated goat serum (Jackson ImmunoResearch) and 0.2% Triton X-100 (Millipore Sigma) in DPBS for 10 min during each wash cycle, then incubated for 2 h at room temperature in 500 µl of 10% heat-inactivated goat serum and 0.2% Triton X-100 in DPBS containing fluorescent-conjugated antibodies of interest. Confocal immunofluorescence of epidermal sheets used the following antibodies: 1:50 anti-TCRγδ (BV421; clone GL3; 118120; BioLegend), 1:50 Vδ4 (eFluor 600; clone GL2; 50-5702-80; eBioscience) and 1:200 Vγ5 (PE; clone 536; 137504; BioLegend). Samples were washed three times in 2% heat-inactivated goat serum and 0.2% Triton X-100 in DPBS for 10 min during each wash cycle. Epidermal sheets were mounted onto SuperFrost Plus Microscope Slides (Fisher Scientific) with one drop of ProLong Diamond Antifade Mountant (Invitrogen). In experiments using intact pinnae, wherein the dermis was not separated from the epidermis, pinnae from *Scn10a^{tdTomato}* mice were removed, forceps were used to pull apart the dorsal and ventral sides, then immediately fixed in 4% PFA for 1 h. All steps following fixation were performed as described above. Confocal immunofluorescence of intact pinnae used the following antibodies: 1:50 Vδ4 (eFluor 600; clone GL2; 50-5702-80; eBioscience) and 1:200 Vγ5 (fluorescein isothiocyanate; clone 536; 553229; BioLegend). To visualize free nerve endings in the whole skin, fixed skin was incubated with 1:100 anti-Tuj1-biotin (clone Tuj1; MAB1195; R&D Systems) overnight at 4 °C. The next day, samples were incubated with 1:100 streptavidin-PE (405204; BioLegend). Fixation and washing of these samples were performed as described above. Imaging was performed on a Zeiss laser scanning confocal microscope using Zen Black v.2.3, and processing was performed using Zen Blue v.3.7 (Zeiss). The 3D tool generated an orthogonal projection of GD3 cells in contact with *Scn10a^{tdTomato}* free nerve endings.

To quantify the contact between epidermal γδ T cells and free nerve endings, images from *Scn10a^{tdTomato}* skin were analysed. Using the orthogonal tool to account for the *x*, *y* and *z* axes, the number of times GD3 cells or DETCs came into direct contact were counted. Quantitative analysis of Tuj1 sensory nerve fibre density was performed using the HALO imaging analysis platform (Indica Laboratories). A strict positivity threshold was manually selected after reviewing each image. HALO's Area Quantification FL module was used to enumerate the percentage of the total area covered by pixels that reached this threshold, effectively characterizing the overall density of Tuj1 for each image.

Multiplex and ELISA

Cell-free culture supernatant from unstimulated and stimulated γδ T cells, collected as described in the section on the 'Supernatant transfer and cytokine instillation' protocol, was assessed for secreted factors using the Mouse Cytokine/Chemokine 44-Plex Discovery Assay Array (MD44; Eve Technologies). γδ T cell-free supernatant was tested for the presence of IL-3 or IL-17A using the BD OptEIA Mouse IL-3 ELISA Set (BD Biosciences) or IL-17A using the IL-17A ELISA MAX Deluxe ELISA Kit (BioLegend). In one experiment, cell-free supernatant from stimulated skin immune cells, including sorted GD3 cells, αβ T cells, helper-like ILcs and all remaining CD45⁺ cells (other), was assayed for IL-3 as above. These immune populations were stimulated overnight with PMA (50 ng ml⁻¹) and Ionomycin (1 µg ml⁻¹). For all assays, the manufacturer's instructions were followed. ELISA samples were assessed on a SpectraMax iD5 microplate reader (Molecular Devices). Concentrations were determined from a standard curve.

Proliferation of DETCs and GD3 cells

To assess the viability of DETCs and GD3 cells following 5 days in culture, we isolated the cells following the section on the 'Supernatant transfer

and cytokine instillation' protocol. Before plating, single-cell suspensions were incubated with 1:1,000 CellTrace Violet (Thermo Fisher Scientific) at 37 °C in 5% CO₂ for 20 min. Some 1:1 fully supplemented RPMI was added for 5 min before pelleting and resuspension in the appropriate culture medium.

Overnight stimulation of MACS-enriched or FACS-sorted γδ T cells

Single-cell suspensions of mouse epidermis were prepared from *Skint1^{-/-}* Taconic FVB/N mice following the section on the 'Isolation of immune cells from mouse dermis and epidermis' protocol. These mice were selected for this protocol because the epidermal γδ T cell compartment lacks DETCs and contains high numbers of GD3 cells. Single-cell suspensions were enriched for γδ T cells following the TCRγδ⁺ T Cell Isolation Kit, mouse (Miltenyi Biotec). Single-cell suspensions of mouse dermis and epidermis were prepared from WT or *Il3^{GFP/WT}* mice following the section on the 'Isolation of immune cells from mouse dermis and epidermis' protocol. Dermal γδ T cells, DETCs and GD3 cells were then FACS sorted.

Here, 15,000 MACS-enriched GD3 cells or 4,000 FACS-sorted γδ T cells were plated in a tissue-culture-treated flat-bottom 96-well plate and stimulated with papain (5 µg ml⁻¹), PMA (50 ng ml⁻¹) and Ionomycin (1 µg ml⁻¹) or Dynabeads Mouse T-Activator CD3/CD28 beads (Gibco) at a bead-to-cell ratio of 5:1. Following overnight incubation at 37 °C in 5% CO₂, cells were incubated with fluorescent-conjugated antibodies in FACS Buffer for 15 min at 4 °C, then fixed with 4% PFA for 8 min at room temperature. Fluorescent-conjugated antibodies targeting intracellular cytokines were added to each well in 1× eBioscience Permeabilization Buffer (Invitrogen) for 1 h at 4 °C. Cells were washed twice with DPBS and then assessed by flow cytometry.

qPCR

Dermal γδ T cells, DETCs and GD3 cells were FACS isolated, and RNA was extracted using the QIAGEN RNeasy Micro Kit in accordance with the manufacturer's protocol. Here, 50 µl of cDNA per reaction was generated using random hexamers, Oligo (dT), magnesium chloride, dNTPs (10 mM), Reverse Transcriptase and RNase inhibitor (all from Thermo Fisher Scientific). Gene expression was measured using a LightCycler 96 Real-Time PCR System (Roche) and SYBR Green Master Mix (Roche) reagents. The reaction cycles were: 95 °C for 600 s, then 95, 60 and 72 °C for 10 s each for 45 total cycles, followed by 95 °C for 10 s, 65 °C for 60 s and finally 97 °C for 1 s. Fluorescence was quantified during each amplification. LightCycler 96 Software v.1.1 (Roche) was used to determine quantification cycle (Cq) values for each cell type. Cq values of *Il3* were divided from *Gapdh* to determine the ratio of copies per *Gapdh*. The *Gapdh* primers were: forward 5' TTG ATG GCA ACA ATC TCC AC 3' and reverse 5' CGT CCC GTA GAC AAA ATG GT 3'. The *Il3* primers were: forward 5' GCC TGC CTA CAT CTG CGA AT 3' and reverse 5' GGT TAG GAG AGA CGG AGC CA 3'.

RNA FISH of mouse DRGs

Tissue preparation. All materials and reagents were RNase-free. WT DRGs were carefully removed and placed in optimal cutting temperature compound, then snap-frozen on dry ice within 5 min of collection. Tissue blocks were stored at -80 °C and used within 3 months. Frozen tissue blocks were equilibrated to -20 °C inside a Leica Jung Frigocut 2800E cryostat (Leica Biosystems) for 1 h. Then 10 µm tissue sections were cut onto SuperFrost Plus Microscope Slides (Fisher Scientific) and kept at -20 °C for 1 h to enhance tissue adherence, then stored at -80 °C and used within 3 months.

RNA Scope reagents (ACDBio) were used to prepare DRG tissue sections for RNA FISH. In brief, sections were removed from storage and placed in prechilled 4% PFA (Electron Microscopy Sciences) at 4 °C for 1 h. Sections were rinsed twice in 1× DPBS and then serially dehydrated in 50, 70, 100 and 100% EtOH (Decon Laboratories) for 5 min each.

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Slides were dried for 10 min, and an ImmEdge pen (Vector Laboratories) was used to create a hydrophobic barrier around the tissue section. Tissue sections were incubated in H_2O_2 for 10 min and washed twice with H_2O . Tissue sections were incubated in Protease IV for 10 min and washed twice with H_2O . Following these steps to prepare tissue sections for probe hybridization, the manufacturer's instructions were followed. To determine whether *Il3ra* transcript was enriched within specific neuronal subsets, we paired *Il3ra* detection with that for marker genes of the NP1 (*Gfra1*, *Trpc3*), NP2 (*Gfra1*), NP3 (*Sst*), PEP1 (*Gal*), PEP2 (*Asic3*) and cold (*Trpm8*) sensory neuronal subsets previously identified by scRNA-seq^{33–36}. To determine whether *Tac1* transcript levels were decreased in the absence of sensory neuronal *Il3ra*, we paired *Tac1* with *Trpv1*. Probes were visualized by pairing with the following fluorescent probes: TSA vivid 520 was 1:750, TSA vivid 570 was 1:1,500, TSA vivid 650 was 1:1,500 and TSA-DIG (1:3,000) was paired with Opal Polaris 780 (1:1,000; Akoya Biosciences). Fluorophore concentrations were optimized to balance the signal intensity of each channel. Glass coverslips were mounted on microscope slides with Prolong Diamond Antifade Mountant (Thermo Fisher Scientific).

Image acquisition. Images of tissue slides were acquired on an Akoya Phenolmager HT multi-spectral slide scanner (Akoya Biosciences) at $\times 20$ (0.5 μm per pixel) resolution. Exposures were set for each image individually to avoid saturation or underexposure. Akoya Biosciences inForm v.2.7.1 software was used to spectrally separate signals from each fluorophore and to mitigate the effect of native tissue autofluorescence. The spectral library was created using the synthetic Opal spectra and autofluorescence spectra from unstained mouse DRG tissue. Unmixed tiles produced by inForm were fused to reproduce a whole-slide pyramidal TIF using Indica Laboratories HALO software v.3.6. In *Tac1* experiments, images of tissue slides were acquired with Zen Blue v.3.7 on a Zeiss Axio Imager M2 Upright Microscope at $\times 20$ resolution. Exposures were set for each image individually to avoid saturation or underexposure.

Image analysis. Quantitative image analysis was performed using the HALO image analysis platform from Indica Laboratories. To minimize potential bias in data analysis, we used an artificial intelligence and machine learning approach to annotate 987 individual DRG neurons on the basis of the expression of sensory neuronal subset marker genes, and then quantitated *Il3ra* copies per neuron. In *Tac1* experiments, we annotated 925 individual DRG neurons. Areas of interest containing in-focus DRG tissue sections were manually annotated. Cell detection and segmentation were performed using two HALO AI v.3.6 artificial neural network classifiers, one per staining panel. Each segmentation network was trained on datasets of 150 hand-annotated cells per panel until segmentation results were stable and sufficiently accurate. The segmentation networks were trained at the whole-cell level using a weighted average of the stains that were diffuse and spread across the cell body, which were all stains except *Il3ra*, to properly detect cell boundaries. Two HALO AI Object Phenotyper artificial neural networks were then trained to classify the cells into neuronal phenotypes of interest per staining panel. The training sets for the phenotyper networks consisted of the same cells used to train the segmentation networks. After cells were detected and phenotyped using both neural network classifiers, each staining channel was quantified per cell using the HALO FISH-IF v.2.2.5 module. The number of *Il3ra* ISH copies per cell was determined on the basis of the size and intensity of detected dots. The fluorescence intensity of *Tac1* in *Tac1⁺Trpv1⁺* and *Tac1⁺Trpv1⁻* cells was determined.

Calcium imaging of DRG neurons

Here, 20 μl of poly-D-lysine and laminin solution (Millipore Sigma) was pipetted as a small dot on a fluorodish cell culture petri dish (World Precision Instruments). Fluorodishes were then incubated at 37 °C in

5% CO_2 for 2 h to coat the dot's location with poly-D-lysine and laminin. DRGs were collected and dissociated as described in the section on the 'Isolation of cells from mouse DRG' protocol. Then 10 μl of cells (at 5 μl per DRG collected) was plated at the exact spot of the poly-D-lysine and laminin dot on the fluorodishes. Dishes were incubated at 37 °C in 5% CO_2 for 2 h. After DRG neurons attached to the poly-D-lysine and laminin-coated fluorodish, plates were flooded with 1 ml of supplemented NBM. Fluorodishes were incubated overnight at 37 °C in 5% CO_2 . The following day, the medium was aspirated and replaced with supplemented NBM containing 5 mM of the calcium indicator dye Fura-2 (Invitrogen). In some experiments, 5 $\mu\text{g ml}^{-1}$ IL-3 was added during this step. Fluorodishes were returned to 37 °C in 5% CO_2 for 30 min. Fluorodishes were washed three times with Krebs–Ringer Solution (Alfa Aesar) before the plate was completely flooded with Krebs–Ringer Solution. Krebs–Ringer solution contained 20 mM sodium chloride, 5 mM potassium chloride, 2 mM calcium chloride, 1 mM magnesium chloride, 25 mM sodium bicarbonate and 5.5 mM D-glucose, pH 7.3.

The ValveBank 8 Pinch Valve Perfusion System (AutoMate Scientific, Inc.) was used in calcium imaging experiments to perfuse cells. The perfusion pencil was held in a PatchStar Micromanipulator (Scientifica). Fluorodishes were placed on an Inverted Motorised Movable Top Plate with a petri dish and slide adaptor ring (Scientifica) that was placed over a Nikon Ti2-A Intelligent Inverted Microscope (Nikon Instruments). Fura-2-loaded DRG neurons were illuminated every second with alternating 340 and 380 nm wavelengths produced by a RETRA FURA III light engine (Lumencor). Images were captured with a pco.edge 4.2 LT sCMOS Camera (PCO). Ratiometric assessment of 340/380 signals was performed with NIS-Elements v.5.30.02 (Nikon Instruments). DRG neurons were identified by drawing a region of interest around each cell. Neurons were considered live and excitable if they responded to capsaicin, AITC or KCl with a 15% ratio increase from baseline. IL-3 and papain excited neurons if their perfusion led to a 15% ratio increase from baseline.

In sequential stimuli experiments, Fura-2-loaded DRG neurons were perfused with Krebs–Ringer solution, followed by 5 $\mu\text{g ml}^{-1}$ recombinant-mouse IL-3, 1 $\mu\text{g ml}^{-1}$ papain, 0.5 μM capsaicin, 200 μM AITC (Millipore Sigma) and 40 mM KCl (Millipore Sigma). Between each stimulus, Krebs–Ringer solution was perfused through the system. The percentages of neurons that responded to IL-3, papain, AITC or capsaicin were plotted as a proportion of total live and excitable neurons. In priming experiments, control DPBS or 5 $\mu\text{g ml}^{-1}$ IL-3 was added to the Neurobasal-A-Fura-2 mixture. Fura-2-loaded, DPBS or IL-3 primed DRG neurons were perfused sequentially with Krebs–Ringer solution, followed by increasing doses of papain (100, 250 and 1,000 $\mu\text{g ml}^{-1}$), capsaicin, AITC and KCl. The percentages of neurons that responded to the increasing doses of papain were plotted as a proportion of total live and excitable DRG neurons.

DRG neuron ex vivo culture

Here, 40 μl of poly-D-lysine and laminin solution (Millipore Sigma) was pipetted in a tissue-culture-treated flat-bottom 96-well plate (Corning) and incubated for 2 h before washing four times with DPBS. DRGs were collected and dissociated as described in the section on the 'Isolation of immune cells from mouse DRG' protocol. Then 10,000 DRG neurons per well were cultured for 7 days in supplemented NBM containing 25 ng ml^{-1} NGF (Alomone Laboratories), 2 ng ml^{-1} GDNF (Peprotech) and cytosine β -D-arabinofuranoside hydrochloride (Millipore Sigma). In the indicated experiments, 500 nM fedratinib or 100 μM STAT5-IN-1 was added to wells on day 2, and 2.5 $\mu\text{g ml}^{-1}$ IL-3 was added on day 6. Experiments were performed on day 7.

STAT5 phosphorylation in DRG neurons

Glass coverslips were treated with 20 μl of poly-D-lysine and laminin solution (Millipore Sigma) and incubated for 2 h before washing four times with DPBS. Sensory neurons were prepared following the section

on the 'DRG neuron ex vivo culture' protocol, but cultured overnight on a glass coverslip in 400 μ l of supplemented NBM. Fedratinib was added to the overnight culture medium when indicated. The following day, cells were treated with 2.5 μ g ml⁻¹ IL-3 or DPBS for 5 min, then fixed for 5 min with ice-cold 4% PFA. The fixed neurons were washed three times with DPBS for 10 min each time, then incubated overnight at 4 °C in 1.715 μ g ml⁻¹ anti-pSTAT5 (Cell Signaling Technology) and 1:300 Neurotrace 435/455 (Thermo Fisher Scientific). The following day, cells were washed three times with DPBS for 10 min each time, then incubated in 0.483 mg ml⁻¹ of fluorescently conjugated goat anti-rabbit IgG (Abcam) for 2 h at room temperature. After washing the cells four times with DPBS for 10 min each time, glass coverslips were mounted on microscope slides with Prolong Diamond Antifade Mountant (Thermo Fisher Scientific). Images of slides were acquired on a Zeiss Axio Imager M2 Upright Microscope at $\times$ 20 resolution. Exposures were set to avoid saturation or underexposure. Images were assessed in FIJI v.2.14.0 to identify neurotrace⁺ neurons. Images were converted to 32-bit grayscale, and then regions of interest of neurotrace⁺ cells were determined using the intensity of neurotrace and cell morphology. Regions of interest were applied to the pSTAT5 channel images, and then the intensity of pSTAT5 fluorescence was measured. Cells were considered pSTAT5⁺ using a threshold that corresponded to the negative control.

ChIP assay

We evaluated the *Tac1* promoter region and identified a putative STAT5 binding site using the Eukaryotic Promoter Database ($P < 0.001$). We prepared sensory neurons following the section on the 'DRG neuron ex vivo culture' protocol and plated 200,000 cells per well in a 12-well plate. The Abcam High-Sensitivity ChIP Kit (ab185913) (Abcam) was used, and the manufacturer's instructions were followed. Next, 100 μ l of chromatin lysate was sonicated at 190 W for 30, 5 min cycles, with 15 s on and 30 s off (Qsonica). DNA associated with *Tac1* was precipitated using anti-STAT5 (R&D Systems), with anti-IgG as a control (included in the chromatin immunoprecipitation (ChIP) Kit). Following precipitation of DNA associated with STAT5, quantitative PCR (qPCR) primers were designed to target the specific region of the *Tac1* promoter with a predicted STAT5 binding site: forward 5' ACC GTG GTT TGC TAA AGA CT 3' and reverse 5' TTA AGC CTT GTT GGA GGG CT 3'.

Substance P release from DRG neurons

We prepared sensory neurons following the section on the 'DRG neuron ex vivo culture' protocol. On day 7, the media was replaced with supplemented NBM for 10 min. This supernatant was considered 'unstimulated'. Then, 5 μ g ml⁻¹ (low-dose) papain in supplemented NBM was added to the wells for 10 min. This supernatant was considered 'stimulated'. The dose of 5 μ g ml⁻¹ (low-dose) papain was selected because it caused limited (non-significant) release of substance P in the absence of IL-3 priming. Cell-free supernatant was assayed for substance P using the Substance P ELISA Kit (Cayman Chemical), and the data were analysed following the manufacturer's instructions. To control for the non-specific effects of papain on the ELISA, we included 'no cells control' wells that were unstimulated or stimulated with papain as described. No cell control concentrations were subtracted (corrected) from wells containing cells.

Statistical methods

Results are shown as follows: violin plots show the median and quartiles, and bar plots, dose-response curves and time courses are mean $\pm$ s.e.m. Symbols represent individual wells, experimental replicates or mice, or as described in figure legends. Unless otherwise noted, data were processed in Excel v.16.86 and statistical analyses were performed using Prism v.10 (GraphPad). Data were assessed for normality by the following tests: D'Agostino and Pearson test, Anderson-Darling test, Shapiro-Wilk test and Kolmogorov-Smirnov test. When two or more

normality tests indicated that the distribution of data was not normal, the appropriate nonparametric test was performed. When comparing one variable among three or more groups or two variables, analysis of variance (ANOVA) was always used as it is robust to violations of normality. As indicated in the figure legends, two-sided *t*-tests, two-sided Mann-Whitney *U*-tests, one-way ANOVA with Tukey's multiple comparisons tests, two-way ANOVA with Tukey's multiple comparisons (comparing all groups) or two-way repeated measures ANOVA (with paired data) were used throughout the study. Paired analyses were used when samples were derived from the same subject. Data were significant if they met the following *P* value criteria: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ and NS, not significant.

Inclusion and ethics statement

Roles and responsibilities were agreed among all authors during the inception of this study and before any work was conducted. Experiments involving animals were approved by the Massachusetts General Hospital or Harvard Medical School Institutional Animal Care and Use Committee.

Reporting summary

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

Data availability

scRNA-seq data generated in this project are deposited in the Gene Expression Omnibus database (under accession number GSE223220). scRNA-seq from human skin^{29,30} (<https://vitiligo.dolphinnext.com/index.html> and <https://zenodo.org/record/4569496> (ref. 61)) and bulk RNA-seq from mouse and human DRG³² (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7305999/>) were obtained from publically available sources. In the mouse scRNA-seq experiment, raw sequencing data was aligned to the mouse reference genome (mm10, v.2020-A from 10X Genomics). In the mouse scTCR-seq experiment, raw sequencing data was aligned to the mouse immune repertoire genome (GRCm38, v.7.0.0 from 10X Genomics). Source data are provided with this paper.

Code availability

Source code for data analysis is available on GitHub (https://github.com/villani-lab/gdt_allergic_response). There are no access restrictions.

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Acknowledgements This work was supported by grant no. T32HL116275 and a National Eczema Association Catalyst Research grant (to C.H.F.), grant nos. National Institutes of Health (NIH) K99/R00 HL151750, R01 HL158534, R01 AG082185 and the Cure Alzheimer's Fund (to C. S. McAlpine), grant nos. R35 HL135752 (to F.K.S.), NIH R35 NS105076-01 and R01 AT011447 (to C.J.W.), grant nos. DP2CA247831 (to A.-C.V.), R01AI15116, AAAAI Foundation and D.Y.M. Leung/JACI Editors Faculty Development Award, Food Allergy Science Initiative, Massachusetts General Hospital Howard Goodman Scholarship, and the Broad Institute Next Generation Scholar (to C.L.S.) and Massachusetts General Hospital Transformative Scholar Award (to A.-C.V. and C.L.S.). C.L.S. receives extra sponsored research support from GlaxoSmithKline (GSK). We thank J. Kagan for his advice and mentorship. We thank J. Boyce for providing *Cpa3^{cre}* mice. We thank D. Mathis (Harvard Medical School) for providing *Tcrd^{Cre}* mice. We thank S. Bromley (Massachusetts General Hospital) for providing *Il4ra^{-/-}* mice. We thank D. Masopust for providing pet shop mice. We thank D. Mucida and B. Reis for providing *Trgv4^{-/-}* mice. E. Pondeville, MRC-University of Glasgow Centre for Virus Research, UK, kindly supplied mosquito eggs. Through the Harvard Catalyst programme, we consulted with a biostatistician to review our statistical approaches and methods. We thank the Massachusetts General Hospital Cancer Center Translational Cartography Core for supporting the imaging

and analysis of confocal microscopic and RNA FISH images. Schematics in Fig. 1h and Extended Data Figs. 1a, 8a and 10f were created using BioRender.com.

Author contributions Experiments were designed, performed and analysed by C.H.F. (all), I.J.K. (Fig. 2a,b,g-j and Supplementary Figs. 1a,b, 2a-d and 4), P.R.M. (Extended Data Figs. 2d-g and 6b,c and Supplementary Fig. 2f), X. Zeng (Fig. 3l,m), D.A.Y. (Fig. 3l,m), C.H. (Fig. 4e), P.R.N. (Figs. 1b and 4h and Extended Data Figs. 1b, 3f and 10b), D.R.B. (Extended Data Figs. 1b and 4g,h), P.A.A. (Fig. 1a and Extended Data Fig. 7d), R.B.C. (Fig. 1a and Extended Data Fig. 7d), X. Zhu (Extended Data Fig. 4f), A.J.T. (Fig. 2a,b,g-j and Supplementary Figs. 1a,b, 2a-d and 4), J.T.M. (Fig. 2a,b,g-j and Supplementary Figs. 1a,b, 2a-d and 4), N.P.S. (Fig. 2a,b,g-j and Supplementary Figs. 1a,b, 2a-d and 4) and C.L.S. (all). C. S. McKimmie generated the mosquito saliva and offered advice. C. S. McAlpine and F.K.S. generated and provided the *Il3* and *Il3ra* mouse models and offered advice. C.J.W. designed and guided in vivo calcium imaging experiments. A.-C.V. designed and guided the single-cell transcriptomics. C.H.F. and C.L.S. wrote the paper. C.L.S. provided resources, reagents and funding. C.L.S. supervised the study.

Competing interests C.L.S. is a paid consultant for Bayer and Merck and receives sponsored research support from GSK. P.A.A. is a current employee of Werewolf Therapeutics. C. S. McAlpine is a paid consultant of Granite Bio. C.J.W. is a founder of Nocion Therapeutics, QurAlis and BlackBox Bio, and is on the scientific advisory board of Lundbeck Pharma, Axonis and Tafalgie Therapeutics. A.-C.V. has a financial interest in 10X Genomics. The company designs and manufactures gene sequencing technology for use in research, and such technology is being used in this research. A.-C.V.'s interests were reviewed by The Massachusetts General Hospital and Mass General Brigham in accordance with their institutional policies.

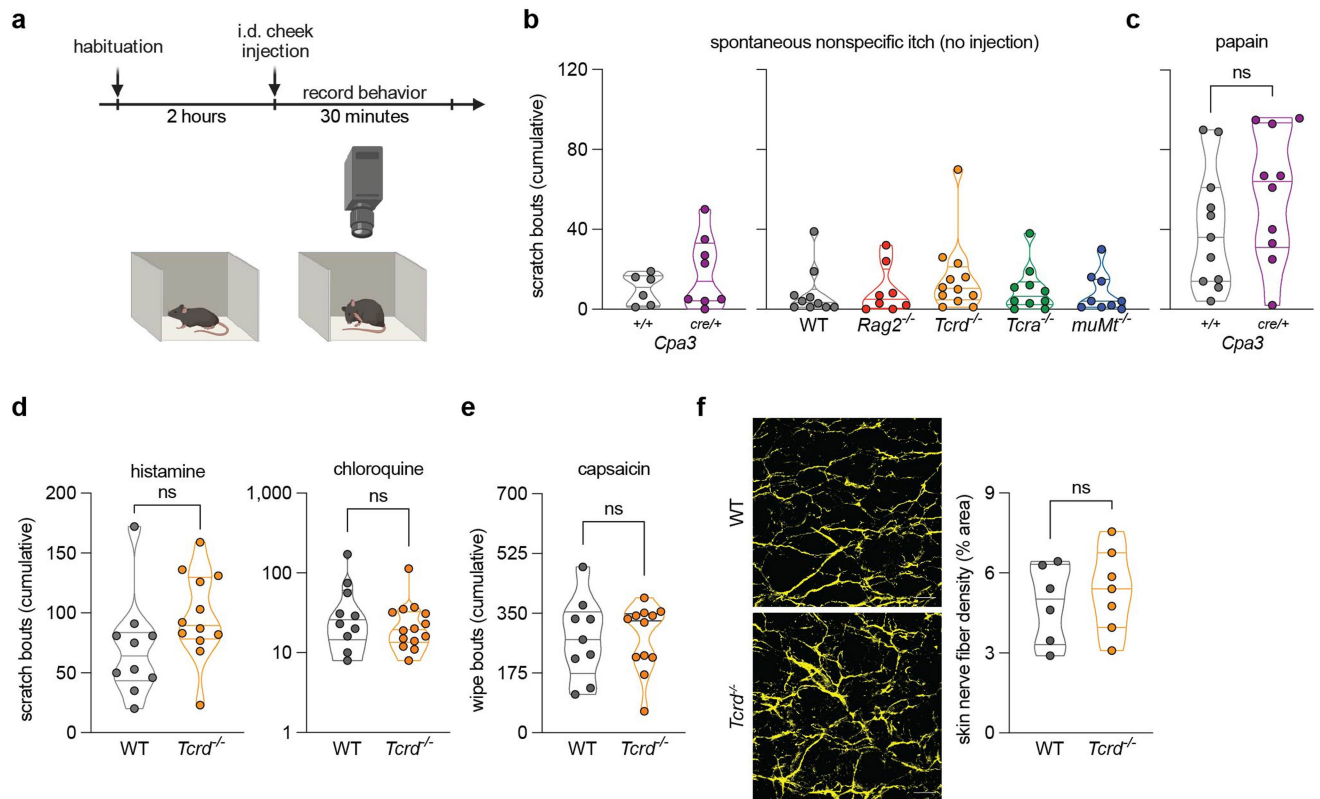
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-024-07869-0>.

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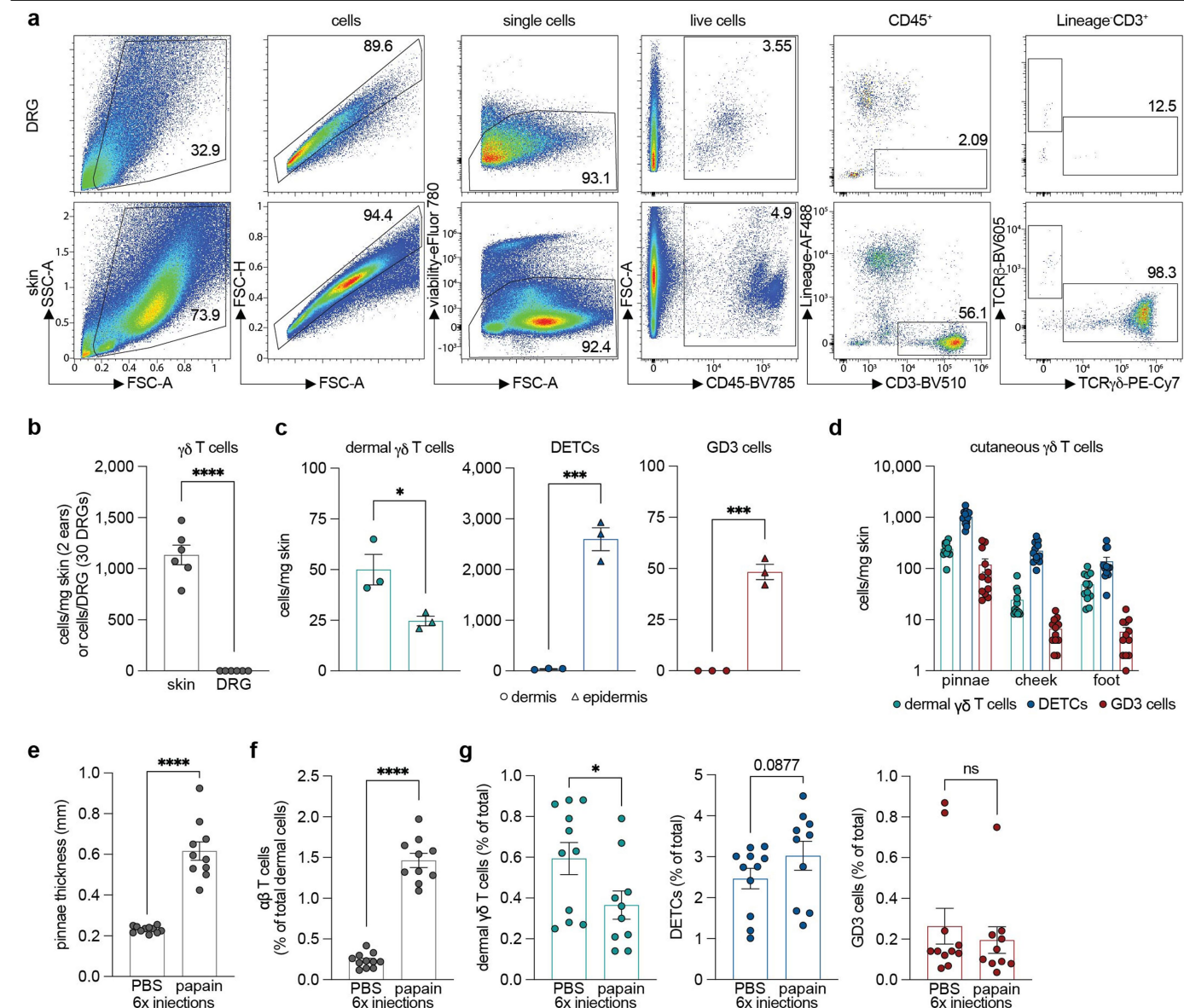
Peer review information *Nature* thanks Brian Kim and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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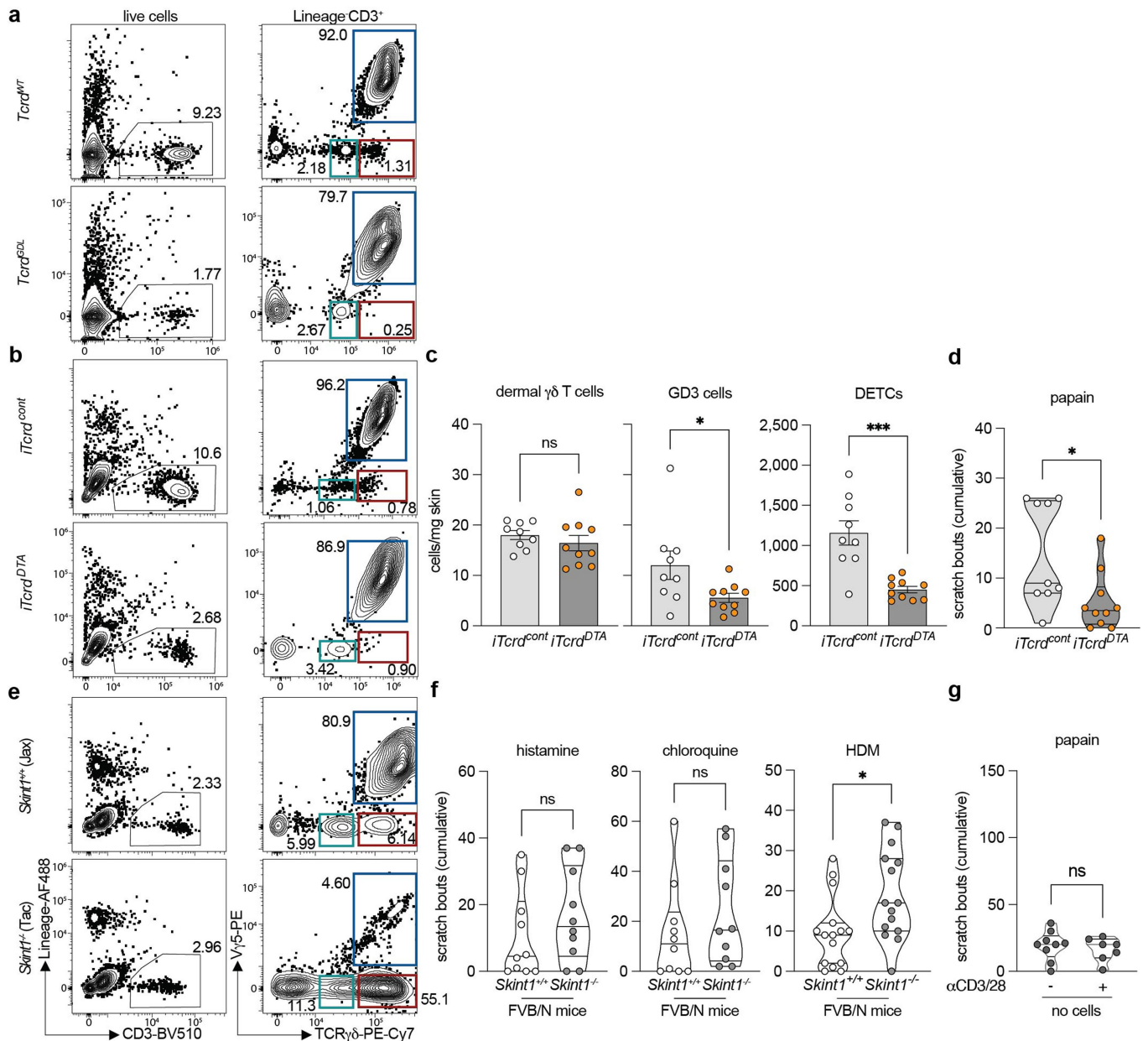
Extended Data Fig. 1 | Mice lacking $\gamma\delta$ T cells have no broad defect in itch, pain, or neuronal innervation in the skin. **a, Schematic of standard behaviour protocol. **b**, Cumulative spontaneous nonspecific scratch at any skin site (n = 6 *Cpa3^{+/+}*; n = 8 *Cpa3^{cre/+}*; n = 10 WT; n = 8 *Rag2^{-/-}*; n = 12 *Tcrd^{-/-}*; n = 10 *Tcra^{-/-}*; n = 9 *muMT^{-/-}*). **c-e**, Cumulative cheek scratch bouts following intradermal (i.d.) injection as indicated (**c**, n = 11 *Cpa3^{+/+}*; n = 10 *Cpa3^{cre/+}*; **d**, histamine: n = 10 WT; n = 12 *Tcrd^{-/-}*; **e**, chloroquine: n = 10 WT; n = 14 *Tcrd^{-/-}*; **f**, capsaicin: n = 9 WT; n = 12 *Tcrd^{-/-}*). **f**, Confocal microscopy z-stack of Tuj1 in naive WT and *Tcrd^{-/-}* mice. The scale**

bar is 50 μ m (n = 6 WT; n = 7 *Tcrd^{-/-}*). Symbols represent individual mice (**b-e**) or images (**f**). Violin plots show the median and quartiles. Data represent at least two independent experiments and were combined. Statistical tests: one-way ANOVA with Tukey's multiple comparisons test (**b**, WT versus indicated mouse strains), two-sided unpaired *t*-test (**b**, *Cpa3*; **c**, **d**, histamine; **e**, **f**), or two-sided Mann Whitney *U*-test (**d**, chloroquine). ns=not significant. Schematic in **a** created using BioRender (<https://biorender.com>).



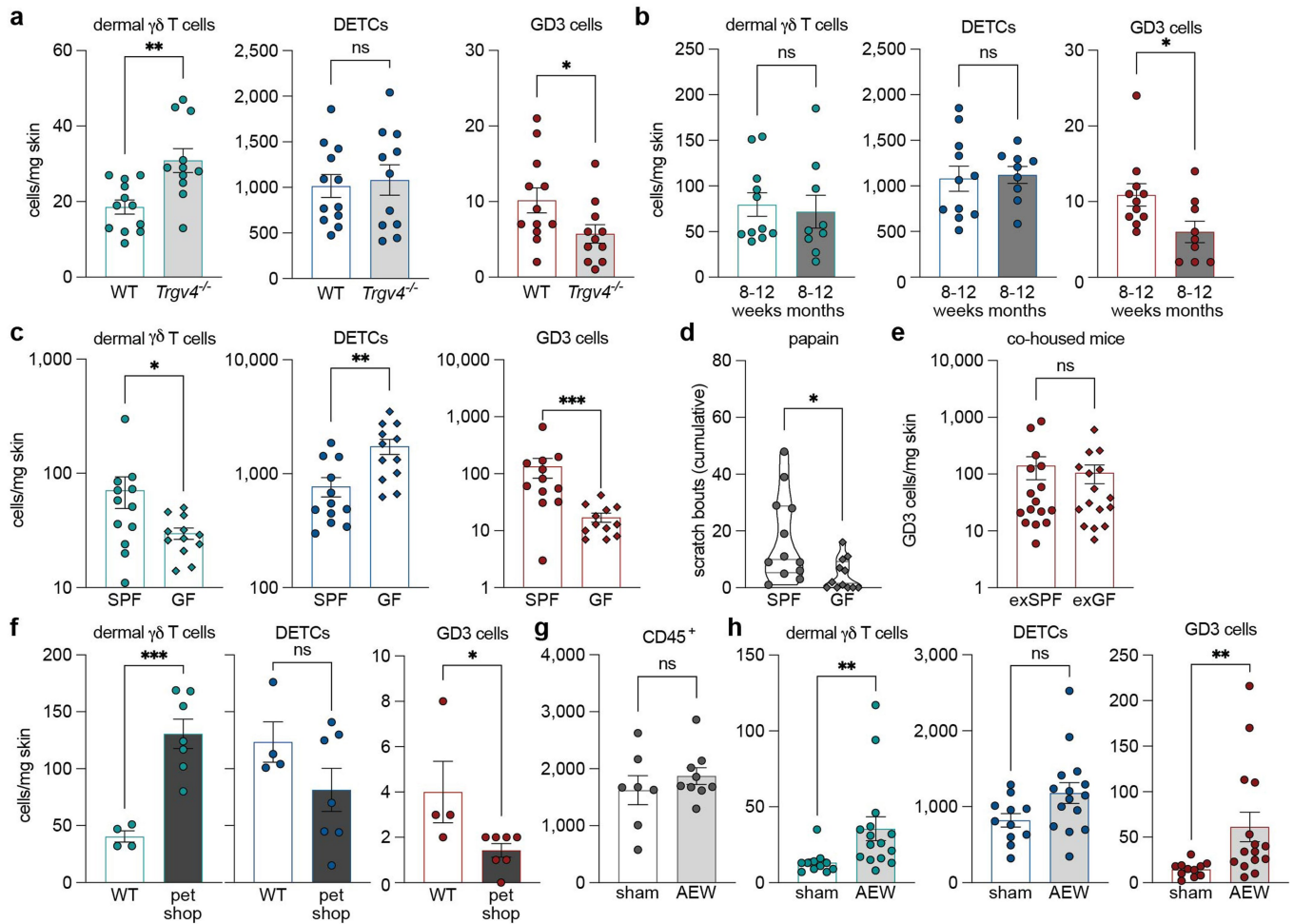
Extended Data Fig. 2 | GD3 cells are Vγ5⁺ epidermal γδ T cells. **a**, Flow cytometry of naive WT pinnae or dorsal root ganglia (DRG). **b-d, f, g**, Flow cytometric quantification of naive WT pinnae or DRG (**b**), naive WT dermis or epidermis (**c**), naive WT pinnae, cheek, or foot (**d**), or WT pinnae (**b**, **n** = 6 per group; **c**, **n** = 3 per group; **d**, **n** = 12 per group; **f, g**, **n** = 11 PBS; **n** = 10 papain). **e**, Pinnae thickness was measured following chronic papain exposure

(**n** = 11 PBS; **n** = 10 papain). Symbols represent individual mice. Bar plots are mean ± SEM. Data represent at least two independent experiments and were combined, except in **c**. Statistical tests: two-sided unpaired *t*-test (**b-f**; dermal γδ T cells) or two-sided Mann Whitney *U*-test (**g**, DETCs and GD3 cells). * *p* < 0.05, *** *p* < 0.001, **** *p* < 0.0001, ns = not significant.



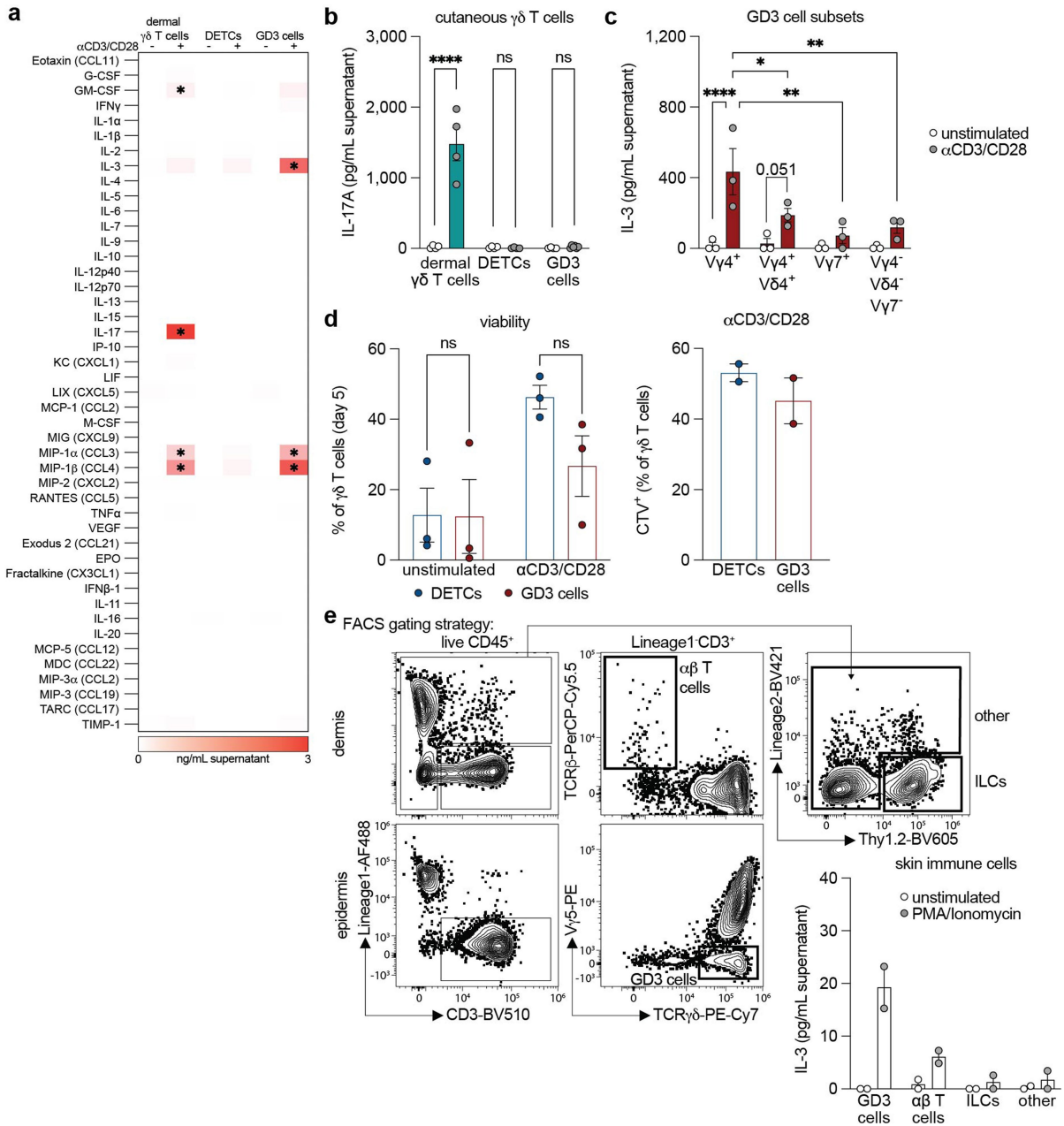
Extended Data Fig. 3 | Dermal γδ T cells and DETCs are not required for allergic itch. **a, b, e.** Flow cytometry of naive pinnae (**a**, gating strategy corresponding to Fig. 1d; **e**, gating strategy corresponding to Fig. 1f). **c.** Flow cytometric quantification of naive WT pinnae ($n = 9$ *iTcrd^{cont}*; $n = 10$ *iTcrd^{DTA}*). **d, f, g.** Cumulative cheek scratch bouts following i.d. injection as indicated

(**d**, $n = 9$ *iTcrd^{cont}*; $n = 10$ *iTcrd^{DTA}*; **f**, $n = 10$ histamine and chloroquine; $n = 15$ HDM; **g**, $n = 9$ αCD3/28⁻; $n = 7$ αCD3/28⁺). Symbols represent individual mice. Bar plots are mean ± SEM. Data represent at least two independent experiments and were combined. Statistical tests: two-sided unpaired *t*-test (**c, g**) or two-sided Mann Whitney *U*-test (**d, f**). * $p < 0.05$, *** $p < 0.001$, ns = not significant.



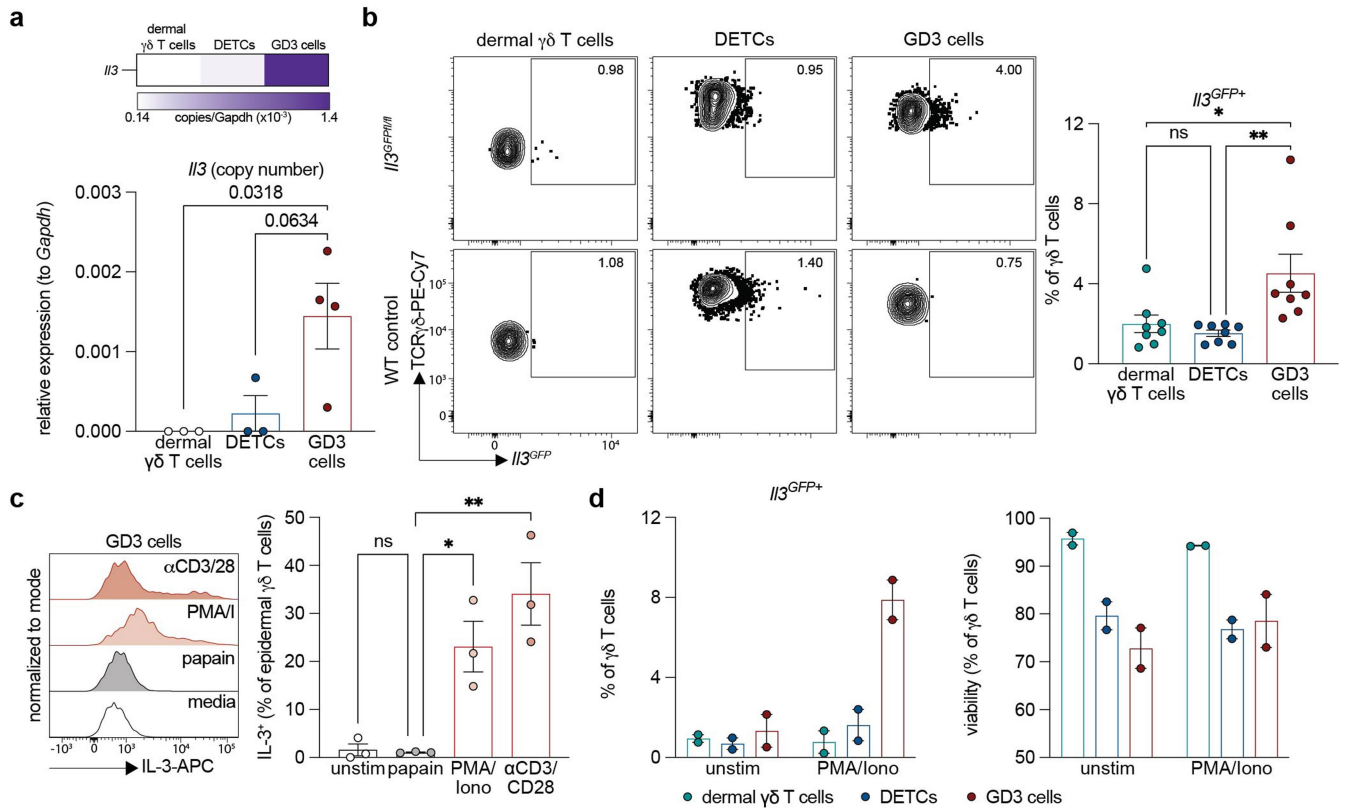
Extended Data Fig. 4 | GD3 cells are regulated by *Trgv4*, age, microbial factors, and dry skin. a-c, e-h. Flow cytometric quantification from pinnae (a, n = 12 WT; n = 11 *Trgv4^{-/-}*; b, n = 11 8–12 weeks; n = 9 8–12 months; c, n = 12 per group; e, n = 16 per group; f, n = 4 WT; n = 7 per shop; g, n = 7 sham; n = 9 AEW; h, n = 11 sham; n = 15 AEW). d, Cumulative cheek scratch bouts following i.d. injection of papain (n = 12 per group). Symbols represent individual mice.

Bar plots are the mean $\pm$ SEM. Violin plots show the median and quartiles. Data represent at least two independent experiments and were combined, except in f. Statistical tests: two-sided unpaired *t*-test (a; b, DETCs; f, dermal $\gamma\delta$ T cells and DETCs; g; h, DETCs) or two-sided Mann-Whitney *U*-test (b, dermal $\gamma\delta$ T cells and GD3 cells; c-e; f, GD3 cells; h, dermal $\gamma\delta$ T cells and GD3 cells). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant.



Extended Data Fig. 5 | V γ 4⁺ GD3 cells are the major source of IL-3 in naive skin. **a**, Heatmap of multiplex assay of cell-free supernatant from FACS sorted $\gamma\delta$ T cells stimulated as indicated (n = 3 per group). * symbols represent a cytokine or chemokine that significantly increased following stimulation. **b,c,e**, IL-17A or IL-3 ELISA of cell-free supernatant from FACS sorted $\gamma\delta$ T cells or immune cells stimulated as indicated (**b**, n = 4 per group, except stimulated GD3 cells (n = 6); **c**, n = 3 per group; **e**, n = 2 per group). **d**, Flow cytometry of

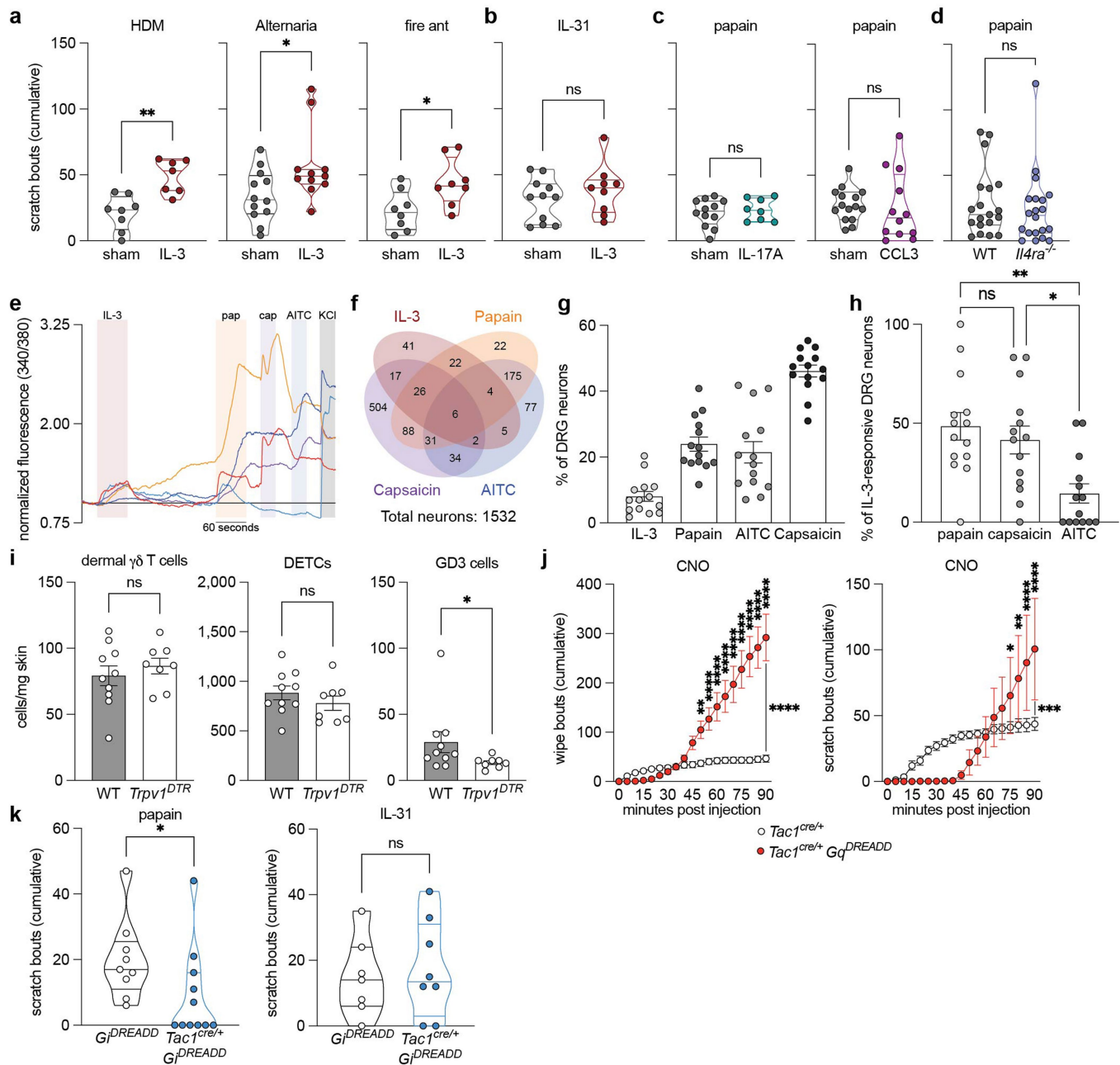
FACS isolated $\gamma\delta$ T cells. CTV = cell trace violet (viability: n = 3 per group; CTV: n = 2 per group). Symbols represent individual wells (**b**) or experimental replicates (**c-e**). Bar plots are mean $\pm$ SEM. Data represent at least two independent experiments combined, except in **a**, a single pilot experiment, and **b**, a representative experiment with n = 4–6 wells. Statistical tests: two-way ANOVA with Tukey's multiple comparisons test (**a-c**) or two-way ANOVA (**d**, viability). * p < 0.05, ** p < 0.01, **** p < 0.0001, ns = not significant.



Extended Data Fig. 6 | Expression and production of IL-3 by GD3 cells.

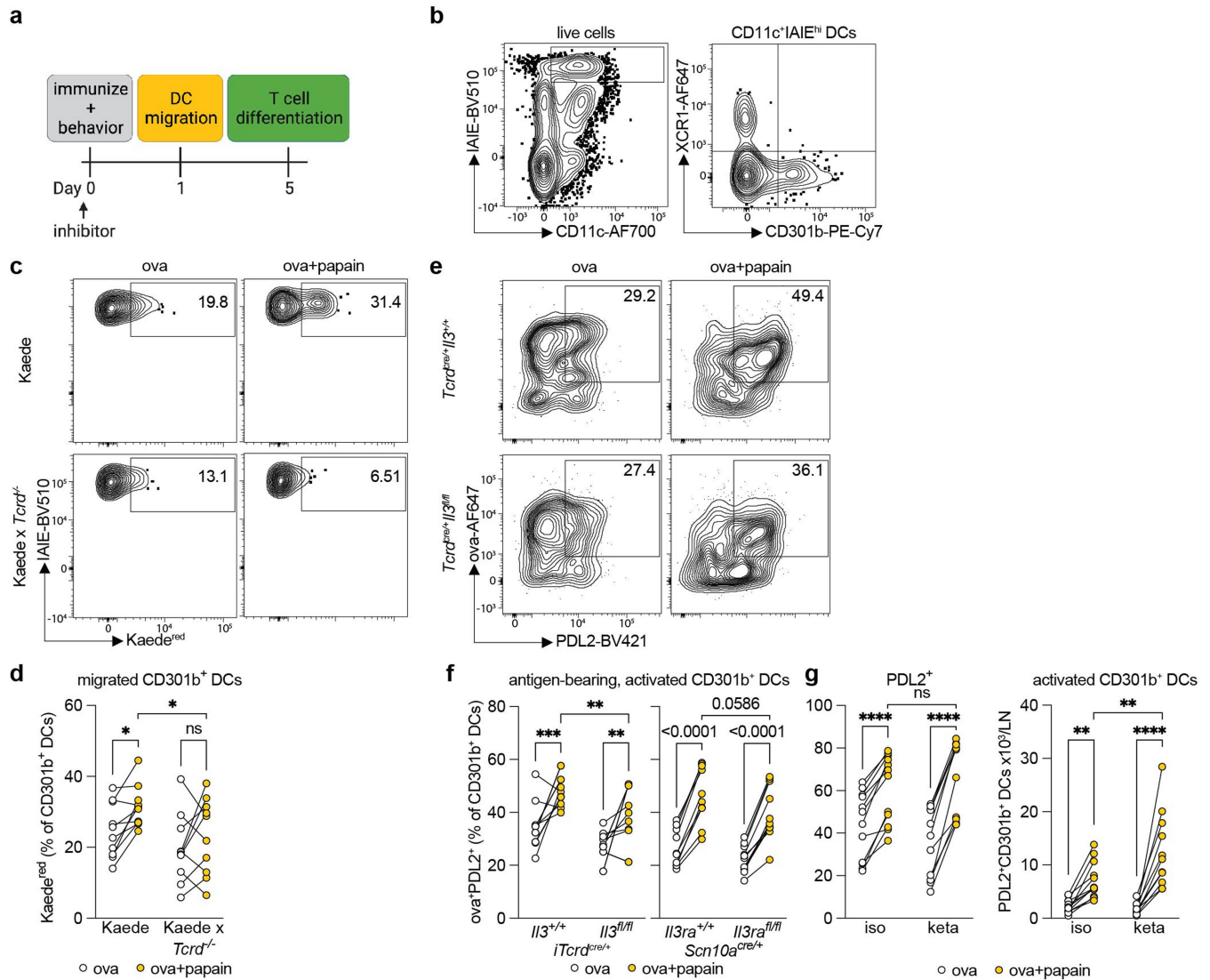
a, QPCR of *Il3* (normalized to *Gapdh*) from FACS sorted $\gamma\delta$ T cells from naïve WT pinnae (n = 3 per group, except GD3 cells (n = 4)). **b**, Flow cytometric quantification of naïve *Il3^{GFP+/J}* pinnae (n = 8 per group). **c, d**, Flow cytometric quantification of MACS enriched GD3 cells from *Skint1^{-/-}* FVB/N pinnae (**c**) or FACS sorted $\gamma\delta$ T cells from *Il3^{GFP+/J}* pinnae (**d**) left unstimulated (unstim) or stimulated as indicated (**c**, n = 3 per group; **d**, n = 2 per group). Symbols

represent individual experimental replicates of pooled mice (**a, c, d**) or mice (**b**). Bar plots are mean $\pm$ SEM. Data represent at least two independent experiments and were combined. n = 3-4 experimental replicates of pooled mice (**a**), n = 8 mice (**b**), or n = 2-3 experimental replicates (**c, d**). Statistical tests: one-way ANOVA with Tukey's multiple comparisons test (**a-c**). *p < 0.05, **p < 0.01, ns=not significant.



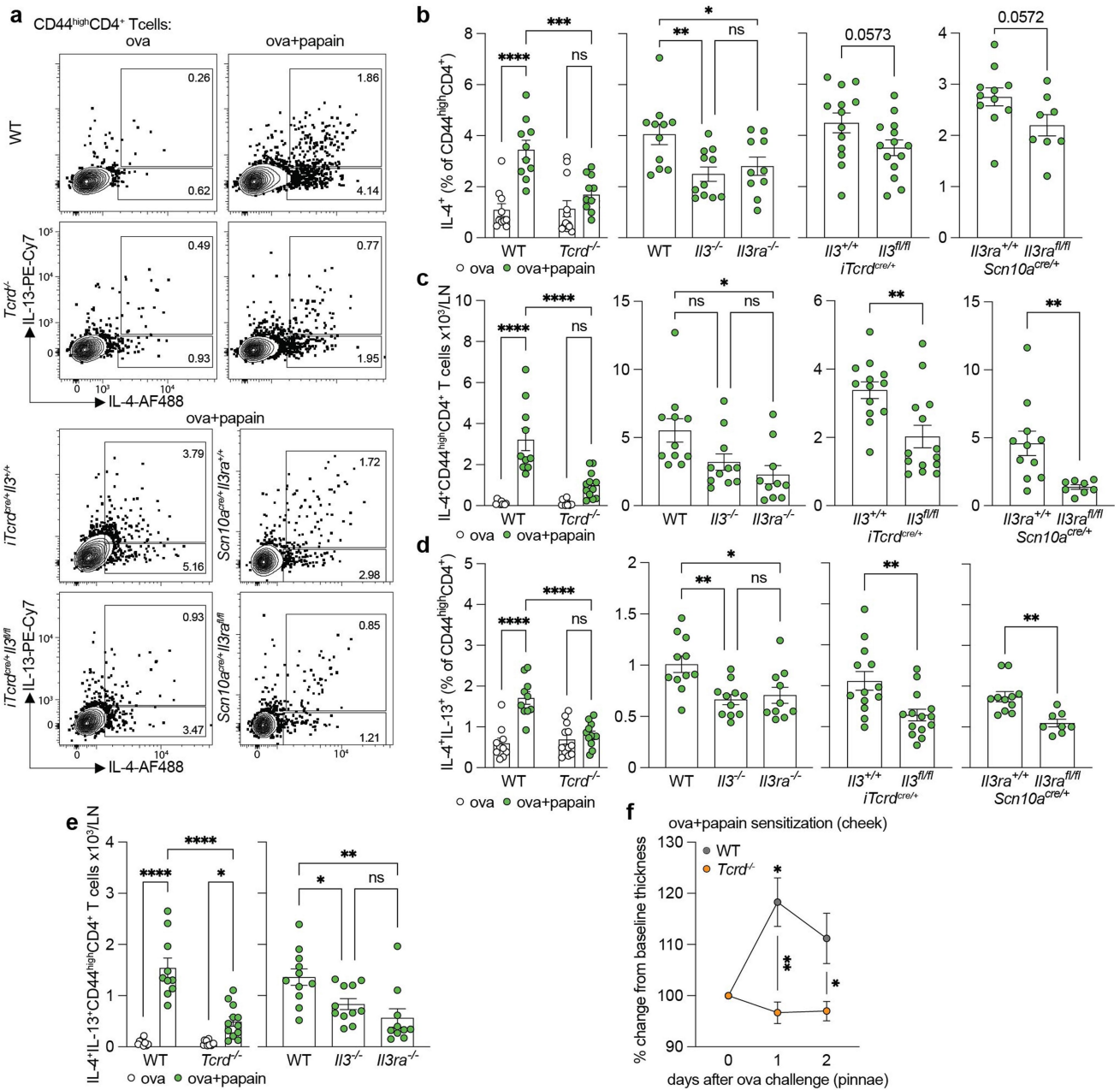
Extended Data Fig. 7 | IL-3 targets PEP1 sensory neurons, which encode mixed itch and pain and are required for allergic itch. a-c, Cumulative cheek scratch bouts following i.d. injection of recombinant cytokine, then allergen or IL-31 (**a**, $n = 8$ HDM sham; $n = 7$ HDM IL-3; $n = 12$ Alternaria sham; $n = 11$ Alternaria IL-3; $n = 8$ fire ant; **b**, $n = 11$ sham; $n = 9$ IL-3; **c**, $n = 12$ papain sham; $n = 8$ papain IL-17A; $n = 15$ papain sham; $n = 12$ papain CCL3). **d**, Cumulative cheek scratch bouts following i.d. injection of papain ($n = 19$ WT; $n = 20$ *Il4ra*^{-/-}). **e**, Representative traces of ratiometric calcium imaging of DRG neurons sequentially stimulated as indicated. **f**, Venn diagram indicating the overlapping responsiveness of individual DRG neurons. **g**, Percent of DRG neurons that responded to the indicated stimuli ($n = 14$ per group). **h**, Percent of IL-3 responsive DRG neurons that also responded to papain, capsaicin, or AITC ($n = 14$ per group). **i**, Flow cytometric quantification of naive pinnae ($n = 10$ WT; $n = 8$ *Trpv1*^{DTR}). **j**, Cumulative

cheek wipe or scratch bouts following i.d. injection of CNO ($n = 10$ *Tac1*^{cre/+}; $n = 8$ *Tac1*^{cre/+} *Gq*^{DREADD}). **k**, Cumulative cheek wipe or scratch bouts following i.d. injection of CNO, then papain or IL-31 ($n = 9$ *Gf*^{DREADD} papain; $n = 11$ *Tac1*^{cre/+} *Gf*^{DREADD} papain; $n = 7$ *Gf*^{DREADD} IL-31; $n = 8$ *Tac1*^{cre/+} *Gf*^{DREADD} IL-31). Symbols represent mice (**a-d, i, k**) or individual wells (**g-h**). Violin plots show the median and quartiles. Bar plots and time courses are mean $\pm$ SEM. Data represent at least two independent experiments and were combined. Statistical tests: two-sided unpaired *t*-test (**a**, HDM and fire ant; **b, c, i**, dermal $\gamma\delta$ T cells and DETCs; **k**, IL-31), two-sided Mann Whitney *U*-test (**a**, Alternaria; **d, i**, GD3 cells; **k**, papain), one-way ANOVA with Tukey's multiple comparisons test (**h**), or two-way ANOVA with Tukey's multiple comparisons test (**j**). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns = not significant.



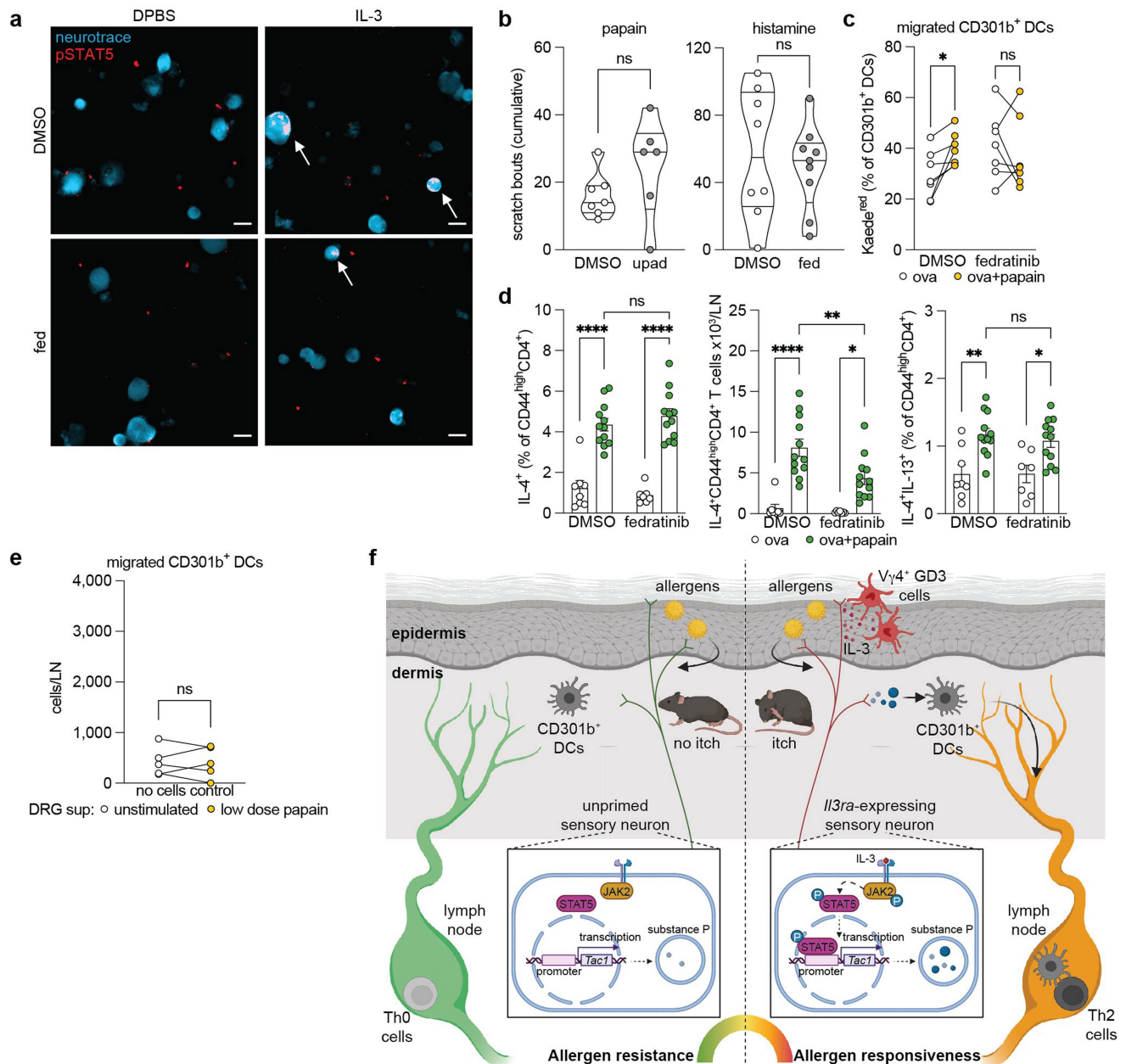
Extended Data Fig. 8 | The absence of the GD3-IL-3-sensory neuron axis inhibits papain-induced Th2-skewing dendritic cell migration. **a**, Schematic of the allergic immune response protocol. **b,c,e**, Flow cytometry of the draining lymph node (dLN) 24 h after immunization with ova $\pm$ papain (gating strategy corresponding to Fig. 4a, b, i, k). **d,f,g**, Flow cytometric quantification of the draining lymph node (dLN) 24 h after immunization with ova $\pm$ papain (**d**, $n = 10$ per group; **f**, $n = 12$ $iTcrd^{cre/+} Il3^{+/+}$; $n = 11$ $iTcrd^{cre/+} Il3^{fl/fl}$; $n = 10$ $Scn10a^{cre/+} Il3ra^{+/+}$;

$n = 11$ $Scn10a^{cre/+} Il3ra^{fl/fl}$; **g**, $n = 12$ iso; $n = 11$ keta). Symbols represent individual mice. Symbols connected by lines indicate paired samples derived from the same mouse. Data represent at least two independent experiments and were combined. Statistical tests: two-way repeated measures ANOVA (**d,f,g**). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, ns=not significant. Schematic in **a** created using BioRender (<https://biorender.com>).



Extended Data Fig. 9 | The absence of the GD3-IL-3-sensory neuron axis impairs papain-induced Th2 differentiation. **a**, Flow cytometry of the draining lymph node (dLN) 5 days after immunization with ova ± papain (gating strategy corresponding to Fig. 4c, j). **b-e**, Flow cytometric quantification of the draining lymph node (dLN) 5 days after immunization with ova ± papain (n = 10 WT; n = 12 *Tcrd*^{-/-}; n = 11 WT; n = 11 *Il3*^{-/-}; n = 10 *Il3ra*^{-/-}; n = 13 *iTcrd*^{cre/+} *Il3*^{fl/fl}; n = 14 *iTcrd*^{cre/+} *Il3*^{fl/fl} *Scn10a*^{cre/+}; n = 11 *Scn10a*^{cre/+} *Il3ra*^{fl/fl}; n = 8 *Scn10a*^{cre/+} *Il3ra*^{fl/fl}). **f**, Pinnae thickness after papain challenge following the induction of delayed-type hypersensitivity (n = 10 WT;

n = 6 *Tcrd*^{-/-}). Symbols represent individual mice. Symbols connected by lines indicate paired samples. Bar plots and line graphs are mean ± SEM. Data represent at least two independent experiments and were combined. Statistical tests: two-way ANOVA with Tukey's multiple comparisons test (**b-d**; **e**, WT v *Tcrd*^{-/-}; **g**), two-way repeated measures ANOVA (**i**), one-way ANOVA with Tukey's multiple comparisons test (**b-d**; **e**, WT v *Il3*^{-/-} v *Il3ra*^{-/-}), two-sided unpaired *t*-test (**b,c**; **d**, *iTcrd*^{cre/+} and *Scn10a*^{cre/+}), or two-sided Mann-Whitney *U*-test (**e**). * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001, **** *p* < 0.0001, ns = not significant.



Extended Data Fig. 10 | Inhibition of the JAK2-STAT5 pathway reduces the responsiveness of sensory neurons to allergens. **a**, Representative fluorescence microscopic images of pSTAT5 in DRG neurons. The scale bar is 20 μ m. **b**, Cumulative cheek scratch bouts following i.d. injection of papain or histamine (papain: $n = 7$ DMSO; $n = 6$ upad; histamine: $n = 8$ DMSO; $n = 9$ fed). **c**, **e**, Flow cytometric quantification of the draining lymph node (dLN) 24 h after immunization as indicated (**c**, $n = 7$ per group; **e**, $n = 5$ per group). **d**, Flow cytometric quantification of the draining lymph node (dLN) 5 days after immunization with ova $\pm$ papain ($n = 8$ DMSO ova; $n = 12$ DMSO ova+papain; $n = 7$ fedratinib ova; $n = 12$ fedratinib ova+papain). **f**, Graphical abstract: a $\gamma\delta$ T cell-IL-3 axis controls allergic responses through sensory neurons. $V\gamma 4^+$ GD3 cells are the major source of IL-3 in naïve skin and control the allergen responsiveness of *Il3ra*-expressing sensory neurons. Upon allergen exposure, IL-3-primed sensory neurons respond with robust itch, Th2-skewing DC

migration to the dLN, and Th2 differentiation in the dLN. Mechanistically, IL-3 activates JAK2 and STAT5, leading to the transcription of the substance P gene *Tac1*. While JAK2 is necessary for allergic itch and the initiation of the allergic immune response, STAT5 is only required for the initiation of the allergic immune response. Together, the GD3-IL-3 axis controls the initial sensory neuronal response to allergens, dictating whether an organism is resistant or sensitive to allergens. Symbols represent individual mice (**b**, **d**), while symbols connected by lines indicate paired samples (**c**, **e**). Violin plots show the median and quartiles. Bar plots are mean $\pm$ SEM. Data are representative of at least two experiments and combined. Statistical tests: two-way repeated measures ANOVA (**c**, **e**), two-way ANOVA with Tukey's multiple comparisons test (**d**), or two-sided unpaired *t*-test (**b**). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, ns = not significant. Schematic in **f** created using BioRender (<https://biorender.com>).

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	CytExpert v2.3, SpectroFlo v3.2.1, SONY MA900 Cell Sorter Software v3.3.2, inForm v2.7.1, HALO v3.6, NIS-Elements v5.30.02, ZEN Black v2.3, ZEN Blue v3.7, LightCycler 96 v1.1
Data analysis	Excel v16.86, Graphpad Prism v10, FlowJo v10, CellRanger v6.1.2 & v7.0.0, Pegasus v1.6.0, velocity v0.17.15, harmony-pytorch v0.1.4, CellRank v1.5.1, Ensembl release 108, R 3.3.3, limma v3.54.2, fgsea v1.24.0, HALO v3.6, HALO-AI v3.6, HALO FISH-IF v2.2.5, FIJI v2.14.0, NIS-Elements v5.30.02, ZEN Blue v3.7, LightCycler 96 v1.1

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

scRNAseq data generated in this project are deposited in the GEO database (under accession number GSE223220). Source code for data analysis shown in all figures

are available on GitHub (https://github.com/villani-lab/gdt_allergic_response). scRNAseq from human skin (<https://vitiligo.dolphinnext.com/index.html>, <https://zenodo.org/record/4569496>) and bulk RNAseq from mouse and human DRG (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7305999/>) were obtained from publicly available sources. In the mouse scRNAseq experiment, raw sequencing data was aligned to the mouse reference genome (mm10, v2020-A from 10x Genomics). In the mouse scTCRseq experiment, raw sequencing data was aligned to the mouse immune repertoire genome (GRCm38, v7.0.0 from 10x Genomics). Source data are provided.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\)](#), [and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Power calculations were conducted to determine sample sizes.
Data exclusions	No data were excluded from the analysis.
Replication	All experiments were replicated at least twice using independent cohorts.
Randomization	Mice were placed into groups depending on their genotype; when possible, mice were randomized.
Blinding	Behavioral experiments were blinded, while immunologic experiments were not.

Reporting for specific materials, systems and methods

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	The antibodies used in this study and their dilutions are listed in Extended Data Table 3.
Validation	Antibody validation information can be found at the manufacturer's website with the following links: CD45-BV785 (30-F11): https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-cd45-antibody-10636 CD68-AF488 (FA-11): https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-cd68-antibody-6619 CD11b-AF488 (M170): https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-human-cd11b-antibody-2700 CD11c-AF488 (N418): https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-cd11c-antibody-2702 CD3-BV510 (17A2): https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-cd3-antibody-7990 TCRgd-PE/Cy7 (GL3): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-tcr-gamma-delta-antibody-7822 TCRb-BV605 (H57-597): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-tcr-beta-chain-antibody-13533 Vg5-PE (536): https://www.biolegend.com/en-us/products/pe-anti-mouse-tcr-vgamma3-antibody-6525 Vg5-FITC (536): https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fits-hamster-anti-mouse-v-3-tcr.553229 Vg4-PerCP-eFluor710 (UC3-10A6): https://www.thermofisher.com/antibody/product/TCR-V-gamma-2-Antibody-clone-UC3-10A6-Monoclonal/46-5828-82 Vg7-unconjugated (F2.67): https://www.biolegend.com/en-us/products/purified-anti-mouse-tcr-vgamma7-antibody-20318 Vd4-BV421 (GL2): https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv421-hamster-anti-mouse-v-4-tcr.744799 Vd4-eFluor600 (GL2): https://www.thermofisher.com/antibody/product/TCR-V-delta-4-Antibody-clone-GL2-Monoclonal/50-5702-80 Vd4-BV786 (GL2): https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv786-hamster-anti-mouse-v-4-tcr.744686 Ly-6A/E-BV605 (D7): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-ly-6a-e-sca-1-antibody-8664 CD9-PE/Dazzle594 (MZ3): https://www.biolegend.com/en-us/products/pedazzle-594-anti-mouse-cd9-antibody-17299 IL-18Ra-eFluor450 (P3TUNYA): https://www.thermofisher.com/antibody/product/CD218a-IL-18Ra-Antibody-clone-P3TUNYA-Monoclonal/48-5183-80 IL-3-APC (MP2-8F8): https://www.biolegend.com/en-us/products/apc-anti-mouse-il-3-antibody-17696 TCRb-PerCP-cy5.5 (H57-597): https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-tcr-beta-chain-antibody-5603 CD45-AF700 (30-F11): https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd45-antibody-3407 CD19-BV421 (6D5): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd19-antibody-7160 CD45R/B220-BV421 (RA3-6B2): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-human-cd45r-b220-antibody-7158 NK1.1-BV421 (PK136): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-nk-1-1-antibody-7150 Thy1.2-BV605 (30-H12): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd90-2-thy1-2-antibody-13864 I-A/I-E-PerCP-Cy5.5 (M5/114.15.2): https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-i-a-i-e-antibody-4282 I-A/I-E-BV510 (M5/114.15.2): https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-i-a-i-e-antibody-7997 CD11c-BV510 (N418): https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-cd11c-antibody-8491 CD11c-AF700 (N418): https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-cd11c-antibody-3429 CD11c-BV605 (N418): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd11c-antibody-7865 CD11c-AF488 (N418): https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-cd11c-antibody-2702 XCR1-AF647 (ZET): https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-rat-xcr1-antibody-10402 XCR1-PE (ZET): https://www.biolegend.com/en-us/products/pe-anti-mouse-rat-xcr1-antibody-10217 CD301b-PE/Cy7 (URA-1): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd301b-mgl2-antibody-9659 CD273-BV421 (TY25): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd273-b7-dc-pd-l2-antibody-15573 CD4-BV605 (RM4-5): https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd4-antibody-7627 CD44-BV510 (IM7): https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-human-cd44-antibody-7994 IL-4-AF488 (11B11; BioLegend): https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-il-4-antibody-2717 IL-4-AF488 (11B11; eBioscience): https://www.thermofisher.com/antibody/product/IL-4-Antibody-clone-11B11-Monoclonal/53-7041-82 IL-13-PE/Cy7 (eBio13A): https://www.thermofisher.com/antibody/product/IL-13-Antibody-clone-eBio13A-Monoclonal/25-7133-82 Beta-III tubulin-biotin (Tuj1): https://www.rndsystems.com/products/neuron-specific-beta-iii-tubulin-biotinylated-antibody-tuj-1_bam1195 pSTAT5 (Rabbit mAb, C11C5): https://www.cellsignal.com/products/primary-antibodies/phospho-stat5-tyr694-c11c5-rabbit-mab/9359 STAT5 (Rabbit IgG, polyclonal): https://www.rndsystems.com/products/human-mouse-stat5a-b-pan-specific-antibody_af2168 Rabbit IgG-AF488 (polyclonal): https://www.abcam.com/products/secondary-antibodies/goat-rabbit-igg-hl-alexa-fluor-488-ab150077.html

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Wild-type C57BL/6 (WT; 556) mice were purchased from Charles River Laboratories (Wilmington, MA). C57BL/6 WT (000664), Tcrd-/- (B6.129P2-Tcrdtm1Mom/J; 002120), Rag2-/- (B6.Cg-Rag2tm1.1Cgn/J; 008449), Tcrα-/- (B6.129S2-Tcratm1Mom/J; 002116), muMT-/- (B6.129S2-Ighmtm1Cgn/J; 002288), Skint1+/+ FVB/NJ (001800), TcrdCreER (B6.129S-Tcrdtm1.1(cre/ERT2)Zhu/J; 031679), ROSA-DTA (B6.129P2-Gt(ROSA)26Sortm1(DTA)Lky/J; 009669), Scn10acre (B6.129(Cg)-Scn10atm2(cre)Jwo/TjPj; 036564), Ai9 (B6.Cg-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J; 007909), Ai95D (B6.J-Cg-Gt(ROSA)26Sortm95.1(CAG-GCaMP6f)Hze/Mwar/J; 028865),
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Vglut2cre (B6J.129S6(FVB)-Slc17a6tm2(cre)Low/MwarJ; 028863), Tac1cre (B6;129S-Tac1tm1.1(cre)Hze/J; 021877), GqDREADD (B6N;129-Tg(CAG-CHRM3*, -mCitrine)1Ute/J; 026220), and GiDREADD (B6.129-Gt(ROSA)26Sortm1(CAG-CHRM4*, -mCitrine)Ute/J; 026219) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Skint1-/- FVB/NTac mice were purchased from Taconic Biosciences (Germantown, NY). Cpa3cre mice were originally from Hans-Reimer Rodewald (German Cancer Research Center). TcrdGDL mice were originally from Immo Prinz (Medizinische Hochschule Hannover). Il3-/-, Il3ra-/-, Il3GFPfl/fl, and Il3rafl/fl mice were generated and provided by Cameron McAlpine, Ben Kleinstiver, and Filip Swirski at Massachusetts General Hospital. Trgv4-/- mice were originally from Daniel Mucida (Rockefeller). Il4ra-/- mice were originally from Fred Finkelman (University of Cincinnati). Trpv1DTR mice were originally from Mark Hoon (NIH). Kaede mice were originally from Osami Kanagawa (Riken). TcrdCreER (referred to as iTcrdcre) were crossed to ROSA-DTA or Il3GFPfl/fl to generate tamoxifen-inducible iTcrdDTA or iTcrdcre/+Il3GFPfl/fl mice, respectively. Scn10acre were crossed to Ai9 or Il3rafl/fl to generate Scn10atdTomato or Scn10acre/+Il3rafl/fl mice, respectively. Vglut2cre was crossed to Ai95D to generate mice for in vivo calcium imaging. Tac1cre were crossed to GqDREADD or GiDREADD to generate Tac1cre/+GqDREADD or Tac1cre/+GiDREADD mice, respectively. Kaede was crossed to Tcrd-/- to generate Kaede x Tcrd-/- mice.

Mice were maintained and bred in a specific-pathogen-free facility in ventilated cages, a maximum of 5 mice per cage, on a 12-hour day-night cycle, at 20-25 degrees C and 35-65% humidity, and provided food and water ad libitum. Experimental mice were 5-16 weeks of age unless otherwise noted. Male and female mice were used in all experiments unless specifically noted, and comparisons were made between age and sex-matched controls.

Wild animals	No wild animals were used in the study.
Reporting on sex	Unless specifically indicated, all experiments included male and female mice.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	Experiments involving animals were approved by the Massachusetts General Hospital or Harvard Medical School Institutional Animal Care and Use Committee (IACUC).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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

To assay surface markers, single-cell suspensions were incubated at 4°C for 15 minutes in FACS Buffer (0.5% FBS in DPBS) containing fluorescent-conjugated antibodies. Dead cells were excluded using Fixable Viability Dye eFluor 780 (eBioscience) added to antibody (Ab) preparations at a dilution of 1:2000. TruStain fcX™ (anti-mouse CD16/32; BioLegend) was added to Ab preparations at a dilution of 1:200. Ab-tagged cells were washed twice with DPBS. If necessary, single-cell suspensions were fixed in 4% Paraformaldehyde (PFA; Electron Microscopy Sciences) for 8 minutes at room temperature and washed twice with DPBS, then assayed by flow cytometry.

To assay surface markers and intracellular cytokines in FACS or MACS enriched gd T cells, cells were plated in a tissue-culture-treated flat-bottom 96-well plate and stimulated with papain (5 µg/mL), PMA (50 ng/mL)/Ionomycin (1 µg/mL), or Dynabeads Mouse T-Activator CD3/CD28 beads (Gibco) at a bead-to-cell ratio of 5:1. Following overnight incubation at 37°C in 5% CO₂, cells were incubated with fluorescent-conjugated antibodies in FACS Buffer for 15 minutes at 4°C (following the above protocol), then fixed with 4% PFA for 8 minutes at room temperature. Fluorescent-conjugated antibodies targeting intracellular cytokines were added to each well in 1x eBioscience™ Permeabilization Buffer (Invitrogen) for 1 hour at 4°C.

	Cells were washed twice with DPBS and then assessed by flow cytometry.
	To assay surface markers and intracellular cytokines in dLN T cells, single-cell suspensions were filtered through a 70 µm cell strainer. 2x10 ⁶ LN cells were added to a 1.5 mL Eppendorf tube (Fisher Scientific) and resuspended in 1 mL fully-supplemented RPMI with PMA (50 ng/mL; Millipore Sigma), Ionomycin (1 µg/mL; Millipore Sigma), and Protein Transport Inhibitor (1:1000, BD Biosciences). Samples were incubated for 3 hours at 37°C in 5% CO ₂ . Samples were washed twice with FACS Buffer; then, cells were incubated in FACS Buffer containing fluorescent-conjugated antibodies targeting surface antigens for 15 minutes at 4°C. Following fixation with 4% PFA for 8 minutes at room temperature, fluorescent-conjugated antibodies targeting intracellular cytokines were added to each sample in 1x eBioscience™ Permeabilization Buffer for 1 hour at 4°C. Cells were washed twice with DPBS, then assessed by flow cytometry.
Instrument	CytoFLEX S, Aurora, MA900 multi-application cell sorter
Software	CytExpert v2.3, SpectroFlo v3.2.1, SONY MA900 Cell Sorter Software v3.3.2
Cell population abundance	Cell abundance was sufficient for downstream applications.
Gating strategy	The following gating strategies were used: Dermal gd T cells were live Lineage-CD3+TCRgdintermediate. Dendritic epidermal T cells (DETCs) were live Lineage-CD3+TCRgdhighVg5+. Epidermal GD3 cells were live Lineage-CD3+TCRgdhighVg5-. In Extended Data Figure 2b, gd T cells were live CD45+Lineage-CD3+TCRgd+. In Extended Data Figure 2f, ab T cells were live Lineage-CD3+TCRb+. Lineage markers: CD68, CD11b, CD11c. In Extended Data Figure 9e, GD3 cells were live CD45+Lineage1-CD3+TCRgdhighVg5-, ab T cells were live CD45+Lineage1-CD3+TCRb+, helper-like innate lymphoid cells (ILCs) were live CD45+Lineage1-Lineage2-CD3-Thy1.2+, and other were all remaining CD45+ cells. Lineage1 was CD68, CD11b, and CD11c. Lineage2 was NK1.1, B220, and CD19. Th2-skewing dendritic cells (DCs) were live CD11c+IAIEhighCD301b+. The migration of Th2-skewing dendritic cells to the draining lymph node was assessed by Kaedered positivity, while activation and antigen uptake were monitored by PDL2 and ova-AF647 positivity, respectively. IL-4+ Th2 cells were live CD4+CD44highIL-4+. IL-4+IL-13+ Th2 cells were live CD4+CD44highIL-4+IL-13+.

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