

Maternal stress triggers early-life eczema through fetal mast cell programming

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Nadine Serhan¹, Nasser S. Abdullah^{1,15}, Nadine Gheziel^{1,2,15}, Alexia Lose¹, Rüçhan Ekren¹, Elodie Labit¹, Anne-Alicia Gonzalez¹, Giulia Oliva³, Pauline Tarot³, Camille Petitfils², Gaëlle Payros², Paolo D'Avino⁴, Allison Voisin⁵, Holly Freya Grace Tinsley⁶, Rebecca Gentek⁶, Carole Brosseau⁷, Marie Bodinier⁷, Laurent Reber¹, Pierre Val⁵, Cezmi A. Akdis⁴, Yasutaka Mitamura⁴, Anand Kumar Andiappan^{8,9}, Jerry Kok Yen Chan^{10,11}, Florent Ginhoux^{8,12,13}, Amaury François³, Nicolas Cénac², Lilian Basso¹ & Nicolas Gaudenzio^{1,14✉}

Prenatal stress (PS) is a repeated exposure to aversive situations during pregnancy, including high emotional strain, which is suspected to affect homeostatic systems in infants. Paediatric eczema develops quickly after birth at flexural sites subjected to continuous mechanical constraints^{1,2}. Although epidemiological studies have suggested an association between PS and a higher risk of eczema in children^{3–6}, no causative biological link has yet been identified. Here we show that eczema at birth originates from molecular dysregulations of neuroimmune circuits in utero, triggered by fluctuations in the maternal hypothalamic–pituitary–adrenal axis. We found that offspring of stressed pregnant dams have dysregulated mast cells and skin-projecting neurons and quickly develop eczema in response to harmless mechanical friction. We demonstrated that PS transiently modulates amniotic fluid corticosterone concentrations, which directly alters the activation program of skin mast cells expressing the glucocorticoid receptor *Nr3c1* and the adjacent sensory neurons conveying mechanosensation. Therapeutic normalization of maternal corticosterone concentrations or genetic depletion of *Mcpt5*⁺ mast cells during stressed gestation prevents fetal immune dysregulation and protects against eczema development after birth. Our findings support a new model in which early-onset paediatric eczema originates from dysregulations in the fetal immune system, caused by fluctuations in maternal glucocorticoids induced by stress.

To study the effect of prenatal stress (PS) on atopic dermatitis at birth, we used a non-infectious mouse model of PS⁷, in which pregnant dams were restrained and exposed to bright light for 30 min, 3 times a day, from embryonic day (E)13 to E18 (Fig. 1a). The PS protocol did not impact the weight of pregnant dams, the litter size or the weight of offspring after birth (Extended Data Fig. 1a–c). The skin of 8-week-old (W8) PS offspring was visually and histologically comparable to that of control (CT) offspring, with no detectable changes in key macroscopic and microscopic features, including in the distribution pattern and abundance of key barrier proteins (Fig. 1b,c and Extended Data Fig. 1d,e). However, both W3 and W8 offspring displayed a relatively high transepidermal water loss (TEWL) (Fig. 1d and Extended Data Fig. 1f) at steady state, a well-known functional indicator of loose barrier in atopic dermatitis, especially in children⁸. The serum of W8 offspring showed, at steady state, increased concentrations of interleukin (IL)-7,

CXCL9 and of the type 2 cytokines IL-5 and IL-9 (Fig. 1e). These findings indicate that, although appearing to have normal skin, PS offspring exhibit subtle alterations in skin barrier and underlying indications of a predisposition to type 2 immune responses at steady state.

Compared with adult atopic dermatitis, paediatric atopic dermatitis often develops at flexural sites exposed to continuous mechanical constraints. We established a model of soft mechanical skin damage induced by tape stripping (Fig. 1f) using a pattern that did not induce any sign of inflammation in CT offspring. Conversely, both W3 and W8 PS offspring rapidly developed eruptions of eczematous lesions, revealing pronounced inflammation, with a significant increase in epidermal thickness (Fig. 1g,h and Extended Data Fig. 1g,h). We next patched the back skin of the mice with sterile PBS-filled gauze pads occluded with a transparent dressing to induce a light but continuous wet friction (Fig. 1i) to mimic the wet flexural locations of babies.

¹Toulouse Institute for Infectious and Inflammatory Diseases (Infinity) INSERM UMR1291 – CNRS UMR5051 – University of Toulouse, Toulouse, France. ²Institut de Recherche en Santé Digestive, University of Toulouse, INSERM, INRAe, ENVT, UPS, Toulouse, France. ³Institut de Génétique Fonctionnelle, Université de Montpellier, CNRS, INSERM, Montpellier, France. ⁴Swiss Institute of Allergy and Asthma Research, University of Zurich, Davos, Switzerland. ⁵IGRED, Clermont-Ferrand, France. ⁶Centre for Reproductive Health, Institute for Regeneration and Repair, University of Edinburgh, Edinburgh, UK. ⁷Unité de Recherche 1268 Biopolymères Interactions Assemblages, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement, Nantes, France. ⁸Singapore Immunology Network, Agency for Science, Technology and Research, Singapore, Singapore. ⁹A*STAR Skin Research Labs, Agency for Science, Technology and Research, Singapore, Singapore. ¹⁰Department of Reproductive Medicine, KK Women's and Children's Hospital, Singapore, Singapore. ¹¹Obstetrics and Gynaecology Academic Clinical Program, Duke-NUS Medical School, Singapore, Singapore. ¹²INSERM U1015, Gustave Roussy Cancer Campus, Villejuif, France. ¹³Translational Immunology Institute, SingHealth Duke-NUS Academic Medical Centre, Singapore, Singapore. ¹⁴Genoskin SAS, Toulouse, France. ¹⁵These authors contributed equally: Nasser S. Abdullah, Nadine Gheziel. ✉e-mail: nicolas.gaudenzio@inserm.fr

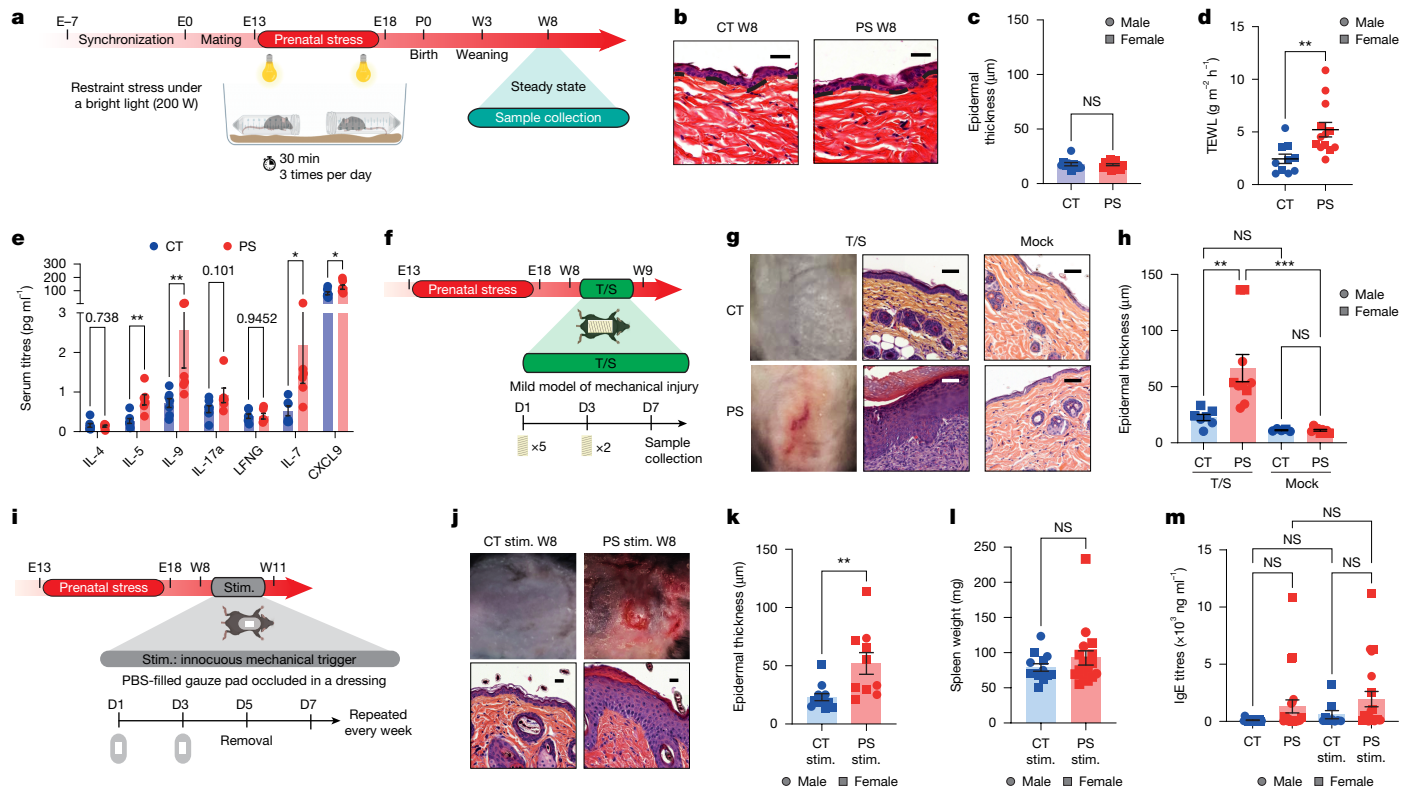


Fig. 1 | Mild mechanical trigger drives the development of skin inflammation in PS offspring. **a**, Timed-pregnant dams were restrained under a bright light for 30 min, 3 times a day and from E13 to E18. P0, parturition. **b, c**, Representative photographs and haematoxylin and eosin (H&E) staining of back skin sections from CT and PS W8 offspring (**b**) and epidermal thickness (in μm ; $n = 10$; **c**). **d**, Baseline TEWL measurements (in $\text{g m}^{-2} \text{h}^{-1}$; $n = 11$). **e**, Serum cytokine concentrations (in pg ml^{-1}) in CT ($n = 7$) and PS ($n = 6$) W8 offspring at steady state. **f**, Mild mechanical skin injury protocol (tape stripping (T/S)). Skin injury was induced by low repeats of tape stripping. **g, h**, Representative photographs and H&E staining of CT and PS back skin sections from T/S ($n = 7$ (CT) and 10 (PS)) and mock W8 ($n = 5$ (CT) and 9 (PS)) offspring (**g**) and epidermal thickness (in μm ; **h**). **i**, Back skin of CT ($n = 15$) and PS ($n = 17$) W8 offspring was patched with PBS-filled gauze pads occluded in a dressing to favour innocuous

mechanical trigger. **j, k**, Representative photographs and H&E staining of CT and PS back skin sections from Stim. W8 offspring (**j**) and epidermal thickness (in μm ; $n = 8$; **k**). **l**, Spleen weight (in mg; $n = 14$ (CT) and 17 (PS)). **m**, IgE serum titres (in ng ml^{-1} ; $n = 18$ (CT and CT PBS) and 22 (PS and PS PBS)). Data were obtained from at least three independent experiments. Bars represent mean values, and one dot or square corresponds to a single male or female mouse. Mean $\pm$ s.e.m.; * $P < 0.05$; ** $P < 0.01$; Mann–Whitney U -test (**c–e, h, k, l**); ordinary one-way analysis of variance (ANOVA) for multiple comparisons using Šidák correction (**h** ($F = 12.30$; $P < 0.0001$), **m** ($F = 2.621$; $P = 0.0568$)); two-sided. IgE, immunoglobulin E; NS, nonsignificant; stim., mechanical stimulation. Scale bars, 50 μm (**b, g**), 20 μm (**j**). Illustrations in **f** and **i** were created using BioRender (<https://biorender.com>).

Although the W8 CT offspring did not show any signs of inflammation, the PS offspring rapidly developed severe lesions characterized by outbreaks of eczema with significant increase in epidermal thickness (Fig. 1j, k). However, compared with conventional allergen-induced models of adult atopic dermatitis⁹, there was no evidence of systemic type 2 inflammation (Fig. 1l, m and Extended Data Fig. 1i, j). However, we found a strong expression of keratin 6, a marker of inflammation in keratinocytes (Extended Data Fig. 1i, j). Finally, a relatively mixed inflammatory signature of genes encoding type 1 (such as *Ifng* and *Stat4*), type 2 (such as *Tslp*, *Il13* and *Stat6*), regulatory (such as *Tgfb1*) and type 17 (such as *Il17a*, *Il17f*, *Ccl20* and *Stat3*) immune responses (Extended Data Fig. 1k) was observed in the skin of the PS offspring, a common clinical feature of atopic dermatitis often found in paediatric patients^{10,11}. Both male and female mice were included, but no significant sex-specific differences were observed. This phenotype also seemed to be restricted to skin tissue because no differences were observed in models of mast cell-dependent MrgprB2-mediated passive cutaneous anaphylaxis (Extended Data Fig. 1l), peanut-induced food allergy (Extended Data Fig. 1m) and house dust mite (HDM)/lipopolysaccharide-induced acute asthma (Extended Data Fig. 1n–q).

Longitudinal clinical studies have consistently documented instances of spontaneous remission in paediatric atopic dermatitis, indicating a favourable prognosis for many children as they transition

into adolescence². Thus, we investigated whether the W24 PS offspring would also be susceptible to our protocol of light continuous wet friction (Extended Data Fig. 1r). Compared with their W8 counterpart, the W24 PS offspring displayed only a quickly resolving dryness, without any signs of eczematous lesions or epidermal thickening (Extended Data Fig. 1s). Overall, these data demonstrate that this model of PS is associated with the development of eczema-like skin lesions in progeny in response to otherwise harmless mechanical friction. The lesions exhibit different pathological features than adult atopic dermatitis, and this phenotype can naturally resolve with age.

PS disrupts neurons conveying mechanical sensations

Beyond tissue inflammation, increased mechanical sensory sensitivity, which can worsen itch, disrupt sleep and impact the quality of life, is an important factor that participates in the full spectrum of discomfort experienced by children with atopic dermatitis^{12–14}. Mechanoception is notably encoded by specialized populations of dorsal root ganglia (DRG) neurons that respond to different types of mechanical stimuli: A β and A δ low-threshold mechanoreceptors (LTMRs), c-LTMR responding to light touch and non-peptidergic nociceptors, notably responding to noxious mechanical stimuli^{15–18}. We assessed the W8 PS offspring mechanical sensitivity using three complementary

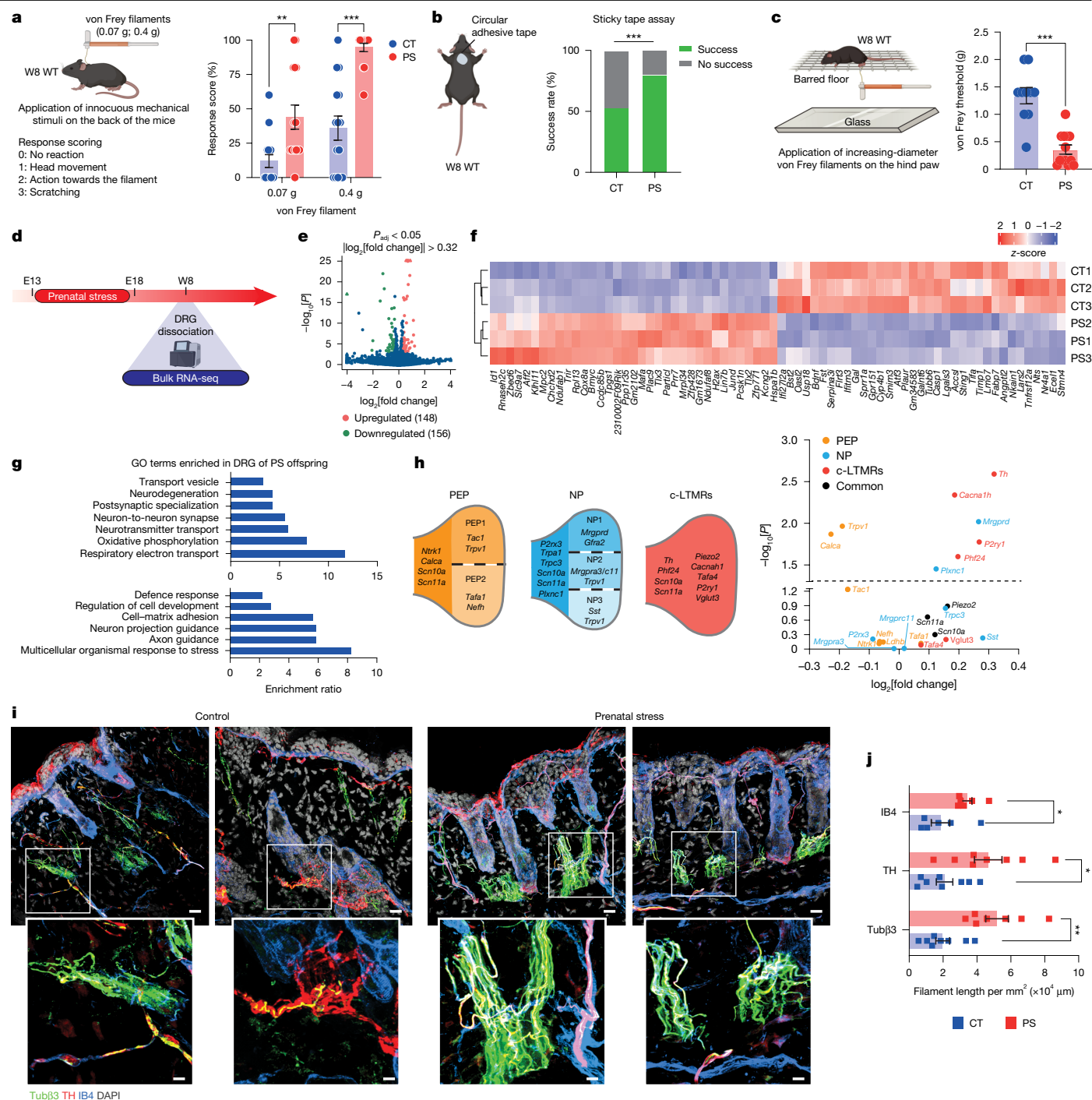


Fig. 2 | Early-life stress enhances mechanical sensitivity in adult offspring through perturbation of sensory neurons involved in skin mechanical sensation. **a**, Mechanical allodynia test and allodynia scores in W8 CT and PS ($n = 15$) offspring. **b**, Sticky-tape assay and success rate in CT versus PS groups ($n = 15$). **c**, von Frey filament test and thresholds (in g) of CT ($n = 10$) and PS ($n = 13$) W8 offspring. **d**, DRG from CT and PS offspring ($n = 3$) were collected at W8 and bulk RNA sequenced. **e**, Volcano plot of the significantly upregulated (red) and downregulated (green) DEGs in PS W8 offspring. Minimum fold change greater than 1.25 and $P_{adj} < 0.05$. **f**, Heat map of the top 50 DEGs between the DRG neurons of PS and CT W8 offspring. **g**, Gene ontology terms enriched in DRG of PS W8 offspring (top and bottom panels for upregulated and downregulated genes, respectively). **h**, Suggested markers for the identification of PEP,

non-peptidergic (NP) and c-LTMRs (left panel). Volcano plot representing specific genes of PEP (orange), NP (blue) neurons and c-LTMRs (red) DEGs in PS W8 offspring (right panel). **i**, **j**, Representative confocal microscopy images of Tubβ3 (green; $n = 8$ (CT) and 7 (PS)), TH (red; $n = 8$) and IB4 (blue; $n = 6$) (**i**) and filament length (in μm; **j**) in hair follicles in back skin sections of W8 CT and PS offspring. Data were obtained from two or three independent experiments. Bars represent mean values, and each dot or square corresponds to a single mouse (**a**, **c**, **j**). Mean + s.e.m.; * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; two-way ANOVA with multiple comparisons using Sidák corrections (**a** ($P < 0.0001$; $F = 44.34$)), Fisher's exact test (**b**) and Mann–Whitney U -test (**c**), two-sided. Scale bars, 20 μm (**i** (top)), 7 μm (**i** (bottom)). GO, gene ontology. Illustrations in **a**–**c** were created using BioRender (<https://biorender.com>).

behavioural assays (Fig. 2a–c): an allodynia experiment by applying a series of calibrated monofilaments with increasing forces (von Frey filaments) to the back skin and recording of the elicited responses,

a sticky-tape response assay measuring the success rate of adhesive tape removal and a mechanoreception assay using the von Frey filament test applied to the glabrous skin and recording the threshold at which

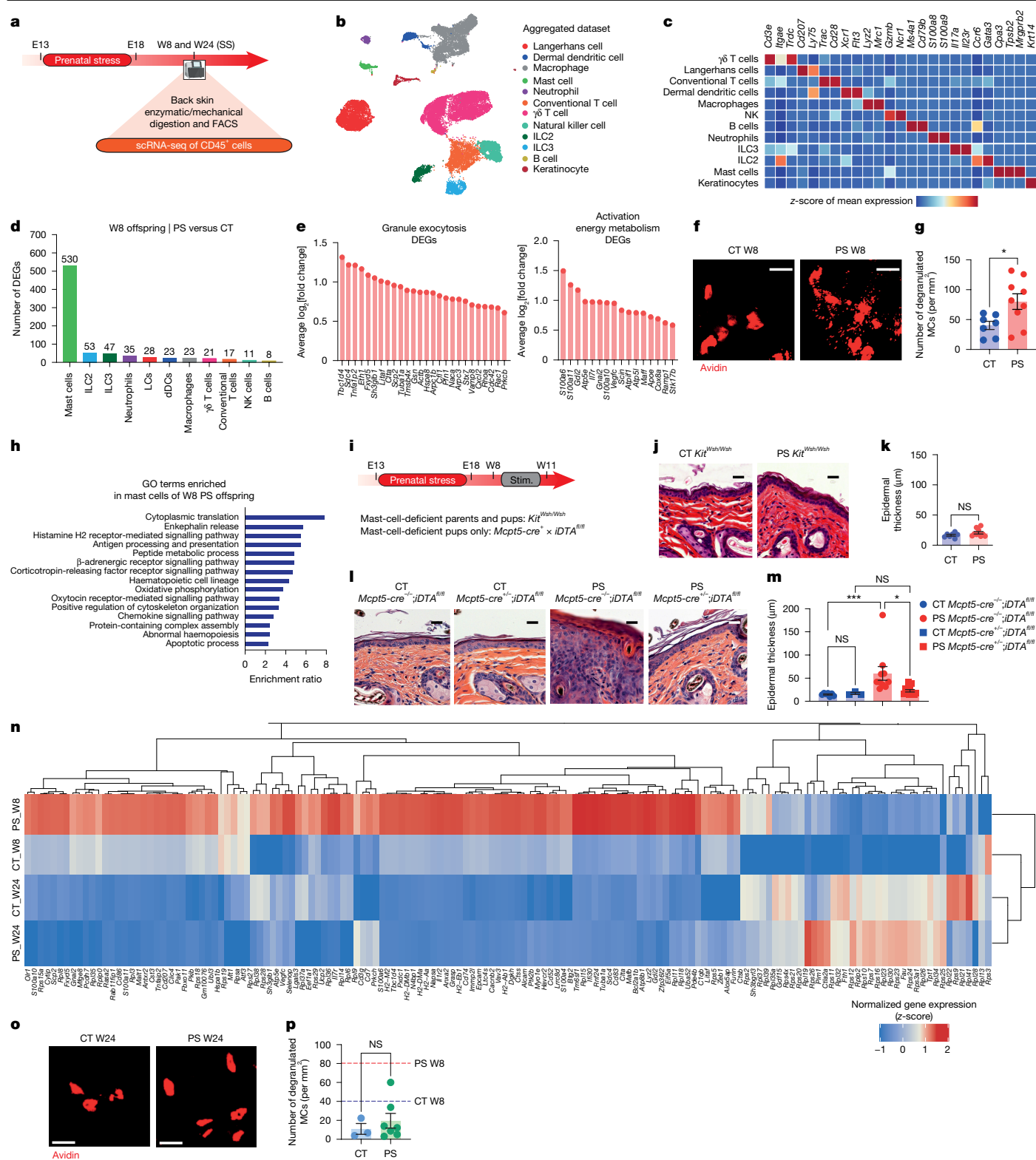


Fig.3 | See next page for caption.

a sensory response is elicited. In line with our previous findings as shown in Fig. 1f–k, we found that PS offspring displayed significantly higher somatic mechanical sensitivity than CT offspring in all tested behavioural experiments (Fig. 2a–c and Extended Data Fig. 2a–c), suggesting a development of mechanical hypersensitivity upon exposure to PS. We next isolated DRG of W8 CT and PS offspring and performed a comparative transcriptomic analysis using bulk RNA sequencing

(RNA-seq) (Fig. 2d). We found more than 300 differentially expressed genes (DEGs) between CT and PS offspring with a minimum fold change more than 1.25 and an adjusted P value (P_{adj}) < 0.05 (Fig. 2e,f). Among gene ontology terms enriched in PS DRG neurons at W8, we observed various pathways associated with metabolism and energy regulation, neuron development/differentiation and axon guidance (Fig. 2g). We next examined the expression of genes reported to be expressed by

Fig. 3 | Mast cells in PS offspring are dysregulated and required for the development of skin inflammation. **a**, Single-cell RNA sequencing of W8 and W24 ($n = 3$) CD45⁺ immune cells from back skin. **b**, Uniform Manifold Approximation and Projection (UMAP) plot and cell annotation of CT and PS, W8 and W24 aggregates. **c**, Heat map of main canonical marker expression. **d**, Number of DEGs in PS compared with CT W8 offspring. **e**, Top-enriched genes in mast cells. **f**, Representative confocal microscopy images of avidin SRho⁺ mast cells (red) in the back skin of W8 CT and PS offspring. **g**, Mast cell (MC) counts per square millimetre ($n = 7$ (CT) and 9 (PS)). **h**, Gene ontology terms enriched in PS W8 mast cells. **i**, Mating pairs were designed as follows: *Kit*^{Wsh/Wsh} females with *Kit*^{Wsh/Wsh} males and *iDTA*^{f/f} females with *Mcpt5-cre*⁺ males. **j–m**, Representative photographs and H&E staining of CT and PS back skin sections from W8 stim. *Kit*^{Wsh/Wsh} and *Mcpt5-cre*^{+/−}; *iDTA*^{f/f} mice and littermate CTs (**j,l**) and epidermal thickness (in μm ; $n = 7$ (CT *Kit*^{Wsh/Wsh}), 10 (PS *Kit*^{Wsh/Wsh}),

7 (CT *Mcpt5-cre*^{+/−}; *iDTA*^{f/f}), 3 (CT *Mcpt5-cre*^{+/−}; *iDTA*^{f/f}), 11 (PS *Mcpt5-cre*^{+/−}; *iDTA*^{f/f}) and 13 (PS *Mcpt5-cre*^{+/−}; *iDTA*^{f/f}) (**k,m**). **n**, Heat map of the top 100 W8 DEGs and normalized gene expression. **o**, Representative confocal microscopy images of avidin SRho⁺ mast cells (red) in the back skin of W24 CT and PS offspring. **p**, MC counts per square millimetre ($n = 3$ (CT) and 7 (PS)). Dotted blue and red lines indicate mean counts in CT and PS W8 offspring, respectively. Data were obtained from three independent experiments. Each dot or square corresponds to a single mouse (**g,k,m,p**). Mean $\pm$ s.e.m.; * $P < 0.05$ and *** $P < 0.001$; Mann–Whitney *U*-test (**g,k,p**), Kruskal–Wallis test for multiple comparison using Dunn's correction (**m**), two-sided. dDC, dermal dendritic cells; ILC, innate lymphoid cells; LC, Langerhans cells; MC, mast cells; NK, natural killer; SS, steady state. Scale bars, 30 μm (**f,o**), 20 μm (**j,l**). Illustrations in **a** were created using BioRender (<https://biorender.com>).

either peptidergic (PEP), non-peptidergic or c-LTMR (Fig. 2h). Genes associated with PEP nociceptors were globally downregulated in PS offspring, including preprotachykinin 1, *Trpv1* and *Calca* (Fig. 2h). We observed a significant upregulation of genes associated with non-peptidergic neurons, including *MrgprD* and plexin-C1, as well as with c-LTMRs, such as *Th*, *Cacna1h* and P2Y purinoceptor 1 (Fig. 2h), with both neuronal populations being specialized in conveying cutaneous mechanical sensation. To investigate potential modified functional responses in the different populations, we next dissociated and cultured DRG neurons in vitro (Extended Data Fig. 2d). We monitored intracellular calcium concentrations in DRG neurons after the addition of 10 nM capsaicin (an agonist of TRPV1 mainly expressed on PEP neurons), 100 μM β -alanine (an agonist of *MrgprD* in non-peptidergic neurons) or 2 nM MRS2365 (a synthetic agonist of P2Y1 expressed on LTMRs). We observed that a significantly higher proportion of neurons responded to MRS2365 in DRG from PS offspring, whereas no significant differences were observed in response to β -alanine and capsaicin (Extended Data Fig. 2d). However, the amplitude of the response of responding neurons remained unchanged between the two groups (Extended Data Fig. 2e–g).

Finally, we used three-dimensional (3D) confocal microscopy to count the number of sensory neurons in DRG and map the axonal density of back and glabrous skin-projecting sensory neurons in W8 CT versus PS offspring at steady state. Compared with CT offspring, PS offspring displayed a significantly higher density of tubulin $\beta 3$ (Tub $\beta 3$)⁺ fibres (pan neuronal marker), Tub $\beta 3$ ⁺TH⁺ fibres (identifies mainly c-LTMR and dopaminergic neurons in the skin) and Tub $\beta 3$ ⁺ isolectin B4⁺ fibres (IB4; identifies non-peptidergic neurons) in the back skin (Fig. 2i,j). In accordance, PS offspring also showed a higher density of Tub $\beta 3$ ⁺ neurofilament H (NFH)⁺ fibres (stains all myelinated LTMR neurons) and glial cell line-derived neurotrophic factor family receptor alpha 2 (Gfra2)⁺ (identifies non-peptidergic neurons) in the glabrous skin (Extended Data Fig. 2h–j). In DRG, although IB4⁺ non-peptidergic neurons remained unchanged, PS offspring displayed a higher percentage of TH⁺ neurons and a reduced percentage of substance P⁺ PEP neurons (Extended Data Fig. 2k,l). In line with these findings, the chemical ablation of TRPV1⁺ PEP neurons using resiniferatoxin (RTX)⁹ did not impact the development of inflammation in response to soft mechanical skin damage (Extended Data Fig. 3a–c). We also used *Nav1.8-cre*; *Dta*^{f/f} mice (*Nav1.8* is also known as *Scn10a*), in which sensory neurons, including c-LTMRs and, possibly, a subset of sympathetic neurons, are genetically depleted to assess whether PS-induced neuronal alterations also contribute to the inflammatory phenotype or are instead more restricted to heightened skin sensitivity. Compared with CT offspring, both PS *Nav1.8-cre*; *Dta*^{f/f} and PS littermate CTs developed comparable eczema-like skin lesions in response to otherwise harmless mechanical friction (Extended Data Fig. 3d), suggesting that sensory neurons are not required for inflammation onset. Finally, peripheral chemical sympathectomy using neurotoxin 6-hydroxydopamine (6-OHDA) also did not abrogate

the hypersensitivity phenotype because PS offspring still exhibited reduced sensitivity thresholds and increased c-LTMR filament length (Extended Data Fig. 3e–h).

These results show that PS was associated with transcriptomic, functional and anatomical modifications in non-peptidergic and c-LTMR neuronal compartments, along with abnormal responsiveness to mechanical stimuli. Although not involved in the development of the observed inflammatory reaction, such heightened sensitivity may help explain, at least in part, the sensory discomfort frequently reported in paediatric eczema, underscoring the need to address both immune and sensory dimensions of the disease.

PS alters mast cell gene program in utero and after birth

We, along with others, have previously reported that tissue-resident immune cells, including mast cells, play an important role in maintaining skin homeostasis^{9,19–21}. We used flow cytometry to investigate the abundance of skin-resident immune cells (Extended Data Fig. 4a–c) in W8 CT versus PS offspring and found no significant differences in lymphoid and myeloid compartments (Extended Data Fig. 4d,e). To understand the potential molecular impact of stress during gestation on skin-resident immune cells, we used fluorescence-activated cell sorting (FACS) to isolate CD45⁺ immune cells from the skin of W8 and W24 mice (previously shown to be responsive and unresponsive to light continuous wet friction, respectively; Extended Data Fig. 1r,s) and performed a single-cell RNA sequencing (scRNA-seq) to generate transcriptional profiles for each individual cell (Fig. 3a and Extended Data Fig. 4f). We profiled and annotated a total of 28,015 cells sorted from the skin of CT and PS offspring (7,211 cells from CT W8, 4,474 cells from PS W8, 6,812 cells from CT W24 and 9,004 cells from PS W24), as previously described²². By exploring canonical markers of immune populations in the different Seurat clusters, 11 immune populations were identified (Fig. 3b,c and Extended Data Fig. 4g,h). We next analysed the DEGs between the two conditions at W8 and found that skin mast cells (expressing *Cpa3*, *Tpsb2* and *Mrgprb2*) presented the most significant number of transcriptomic modifications with 530 DEGs (Fig. 3d). Among the identified DEGs enriched in PS mast cells, many of them were found to be involved in granule formation/trafficking, such as *Vamp8*, syndecin 4 and *Rac1*, as well as in cell activation/metabolism, such as apolipoprotein E, *S100a6* and *S100a11*, *Atp5e* and neuropeptide receptor *Ramp1* (Fig. 3e). Using fluorescent avidin, which specifically binds to mast cell granules in the skin²³, we found that skin mast cells from PS offspring were already highly activated/degranulated (presence of exteriorized cytoplasmic granules) at steady state (Fig. 3f,g). Among other gene ontology terms enriched in PS mast cells, we found stress-associated neuroendocrine pathways, oxidative phosphorylation and abnormal immune system physiology (Fig. 3h). Skin-resident mast cells of stressed pregnant dams were not more degranulated (Extended Data Fig. 5a), indicating that the observed phenotype is restricted to fetal tissue.

We then investigated the role played by mast cells in PS-associated eczematous lesions in response to our model of light continuous wet friction using two complementary transgenic models (Fig. 3i): *Kit^{W^{Sh}/W^{Sh}}* mice (deficient in mast cells but have other abnormalities) and *Mcpt5-cre;Dta* mice (selectively lack most of the skin mast cells; Extended Data Fig. 5b). Compared with their respective littermate CTs, we found that both mast cell-deficient strains were almost completely protected from the development of PS-associated eczematous lesions in this model (Fig. 3j–m). However, *Mcpt5-cre^{+/+};Dta* mice did not show signs of improvement in von Frey mechanical sensitivity and neuron density in the skin (Extended Data Fig. 5c–e), suggesting that the mast cell and sensory dimensions of the disease might not be directly interconnected in this model. Compared with W8 PS mast cells, both the transcriptomic program and activation/degranulation status of W24 PS mast cells were back to normal, that is, similar to that found in W8 and W24 CT mast cells (Fig. 3n–p). We next investigated the presence of epigenetic modifications in W8 and W24 PS versus CT mast cells using a single-nucleus assay for transposase-accessible chromatin sequencing (snATAC-seq) on CD45⁺ cells isolated from skin tissue (Extended Data Fig. 5f–h). We found that W8 PS mast cells showed a relatively high number of differentially accessible regions (DARs) compared with W8 CT mast cells, indicating epigenetic modifications induced by PS. However, such epigenetic imprinting was strongly reduced at W24 (Extended Data Fig. 5i). These data demonstrate that PS-associated eczematous lesions in response to light wet friction depend on *Mcpt5*⁺ skin mast cells for full development through profound dysregulation of their transcriptomic/epigenetic program. However, such dysregulations are no longer present in skin mast cells isolated at W24, a stage at which the pathogenic phenotype has naturally reversed.

We, along with others, previously reported that skin mast cells are of fetal origin, long-lived and independent of bone marrow for renewal^{22,24,25}. We next investigated whether PS could impact fetal skin mast cell programming in utero (Fig. 4a). We first assessed the number and degranulation status of skin mast cells in whole-mount fetuses by 3D confocal microscopy at E18.5, after the last session of PS, a time point at which the vast majority of them are suspected to be yolk-sac-derived²⁴. Although the number and distribution of mast cells were unchanged (Fig. 4b,c), these cells were already highly activated/degranulated at steady state in PS fetal skin compared with CT (Fig. 4b,d). We next investigated which population of fetal mast cells would be the most affected by PS. We administered a single dose of 4-hydroxytamoxifen (4OHT) to pregnant *Cdh5-creERT2^{+/+};m_Tm_C* (cell membrane-localized tdTomato (*m_T*) and cell membrane-localized EGFP (*m_C*)) dams at E7.5 or E10.5 to fate-map yolk-sac-derived or haematopoietic stem cell (HSC)-derived mast cells, respectively (Extended Data Fig. 5j). Using confocal imaging of thick fetal skin sections of E18.5 PS fetuses, we observed that most of degranulated mast cells were of yolk-sac origin at E18.5, whereas only a minority of them were HSC-derived (Extended Data Fig. 5k–m). We next used *Cdh5-creERT2^{+/+};Tdt* mice to fate-map yolk-sac-derived mast cells during postnatal development (Extended Data Fig. 5j). We found that the majority of mast cells were yolk-sac-derived at W8, but at W24, these cells were not detectable anymore (Extended Data Fig. 5n,o). These data are in line with reports suggesting that the natural turnover of long-lived skin mast cells is at least 24 weeks^{22,24,25}. Altogether, these results could explain, at least in part, why the PS-hypersensitive skin phenotype naturally reversed at W24.

The neuronal branching was found unchanged at E18.5 (Fig. 4e,f), which was consistent with previous studies describing that full branching of sensory neurons occurs over several weeks after birth^{26,27}. To analyse putative molecular changes responsible for such a phenotype, we sorted fetal skin mast cells (as CD45⁺CD64⁺F480⁺CD117⁺ST2⁺ skin cells) from three pools of four E18.5 CT and PS fetuses (Fig. 4g) and conducted a bulk RNA-seq analysis. Using a principal component

analysis (PCA), we found that skin mast cells from CT and PS fetuses clustered separately (Fig. 4h), indicating distinct transcriptomic signatures in PS and CT offspring. Fetal mast cells from PS fetuses exhibited 1,284 DEGs (minimum fold change greater than 1.25; $P_{\text{adj}} < 0.05$), with 874 upregulated genes and 478 downregulated genes (Fig. 4i,j). In line with our scRNA-seq dataset at W8 (Fig. 3e,h), most of the identified DEGs were also associated with processes of granule formation/remodelling, maturation and activation of fetal mast cells (Fig. 4k). We also observed a strong enrichment in genes associated with glucocorticoid signalling and neuronal synaptic plasticity (Fig. 4k). In conclusion, such a large transcriptomic modification upon exposure to early-life stress highlights a significant perturbation in the development/biology of fetal mast cells in utero, including genes inherent to granule formation, cell activation/maturation and neuronal modulation.

Maternal corticosterone drives eczema after birth

During pregnancy, environmental stress can activate neuroendocrine stress response systems, such as the hypothalamic–pituitary–adrenal (HPA) axis, leading to the release of corticosterone or the sympathetic–adrenal–medullary axis, leading to the release of adrenalin and noradrenalin, which can affect both the mother and the developing fetus²⁸. We investigated in silico whether embryonic sensory neurons and/or mast cells could express the glucocorticoid receptor *Nr3c1* or the β 2-adrenergic receptor *Adrb2* during development, potentially integrating signals from maternal HPA or sympathetic–adrenal–medullary axes, respectively. We used publicly available scRNA-seq datasets of mouse skin²⁹ and DRG development¹⁵ (Fig. 5a and Extended Data Fig. 6a). We found that sensory neurons expressed *Nr3c1* but not *Adrb2* during development (Extended Data Fig. 6b). We isolated mast cells and macrophages (as another yolk-sac-derived myeloid cell control) from fetal skin identified by their respective expression of *Cpa3* and *Csf1r* (Fig. 5b). At E13.5, we found that macrophages, but not mast cells, expressed the gene *Adrb2*. Compared with macrophages, E13.5 skin mast cells expressed high levels of *Nr3c1*, which stayed relatively stable over time (Fig. 5c,d). In line with these findings, when exploring our previously described sequencing datasets (Figs. 3 and 4), we found that PS (but not CT) E18.5 skin mast cells expressed a strong signature of genes well known to be activated in response to glucocorticoid signalling (Fig. 5e). As fetal skin is directly in contact with the amniotic fluid, we sought to determine corticosterone concentrations at the maternal–fetal interface. Corticosterone concentrations were found significantly upregulated in the amniotic fluid and maternal blood of stressed dams (Fig. 5f and Extended Data Fig. 6c) after stress exposure. However, this increase was not observed in E18.5 embryos (Fig. 5f) or after birth (Extended Data Fig. 6d). We found a significant enrichment of pro-inflammatory cytokines in the amniotic fluid from PS yolk sacs compared with CT (Fig. 5g). In line with these observations, we sought to investigate whether corticosterone and/or corticosterone-enriched amniotic fluid could directly modulate fetal mast cell activation. We sorted CD117⁺ mast cells from E18.5 fetal skin and monitored mast cell degranulation dynamics upon the addition of different stimuli in vitro²³ (Fig. 5h). We first validated our experimental framework and observed efficient activation of fetal mast cells upon the addition of substance P as a positive CT (Fig. 5i). Corticosterone addition resulted in a significant increase in mean fluorescence intensity (MFI) compared with vehicle CT, showing that it can directly act on fetal mast cell activation program (Fig. 5j). In line with this finding, the amniotic fluid from PS yolk sacs, and not from CT, also induced fetal skin mast cells to degranulate (Fig. 5j). We also monitored the intracellular calcium concentrations of W8 cultured DRG neurons after the addition of corticosterone. Although not being statistically significant, there was a strong tendency to observe calcium flux in DRG neurons from both CT and PS offspring (Extended Data Fig. 6e).

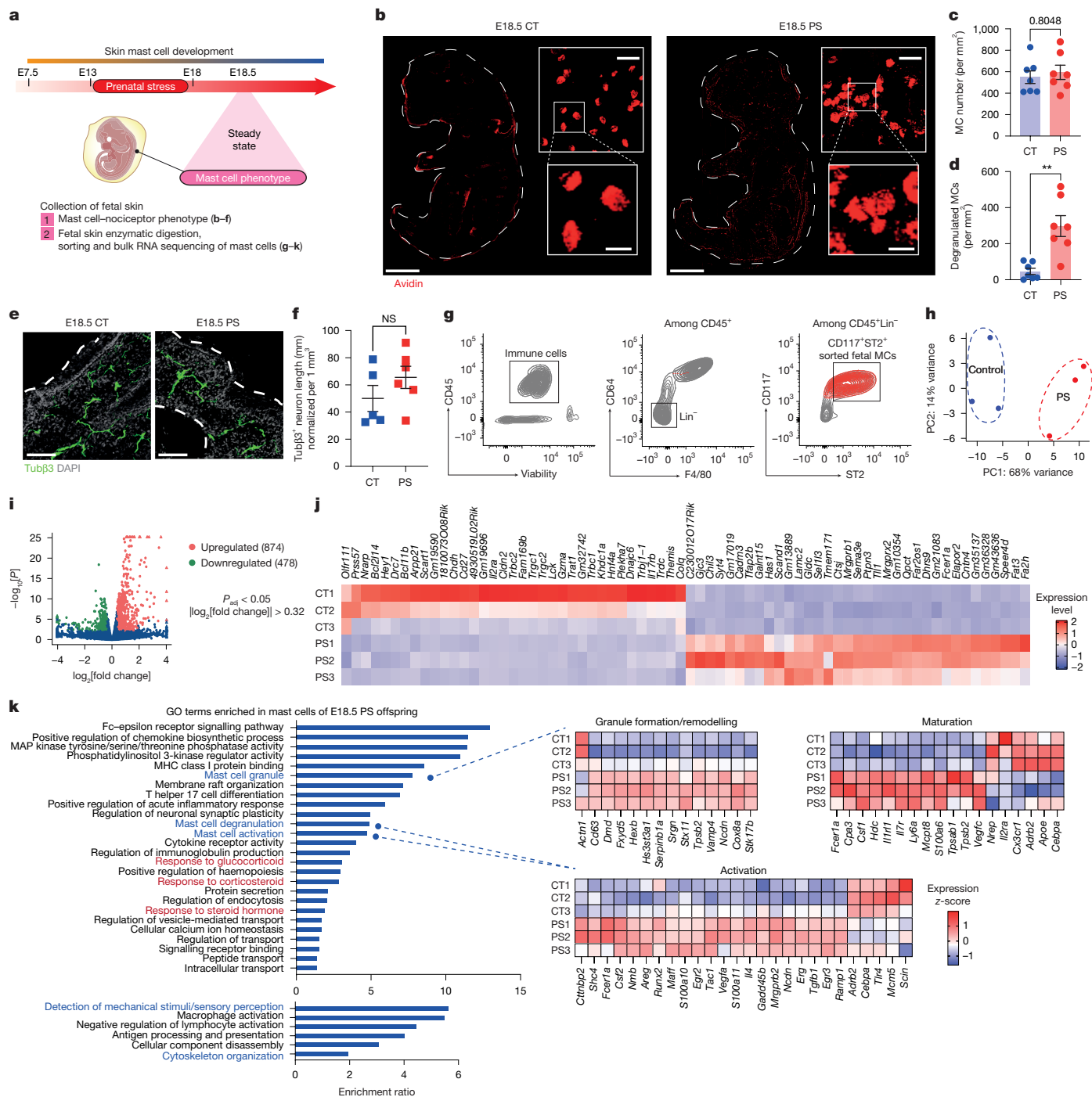


Fig. 4 | Fetal mast cells are already dysregulated in utero after stress exposure and exhibit a hyper-activated phenotype. **a–f**, Fetal skin from E18.5 embryos was collected (**a**) and analysed using confocal microscopy (**b–f**). **g–k**, Fetal mast cells were then sorted and analysed using bulk RNA-seq. **b**, Representative microscopy images of avidin SRho⁺ mast cells in whole embryo sagittal section (left) and 3D back skin (right) in CT and PS E18.5 offspring. **c**, **d**, Total (**c**) and degranulated (**d**) mast cell counts per square millimetre of back skin ($n = 7$). **e**, **f**, Representative 3D confocal microscopy images of Tubβ3 staining (green) in fetal skin of CT and PS E18.5 offspring (**e**) and associated filament length (in μm; $n = 5$ (CT) and 6 (PS); **f**). Data were obtained from three independent experiments. Bars represent mean values, and each dot corresponds to a single mouse (**c**, **d**, **f**). Mean + s.e.m.; $^{**}P < 0.01$; Mann–Whitney *U*-test (**c**, **d**, **f**), two-sided.

Investigating the role of corticosterone in PS-associated eczematous lesions is complex because glucocorticoids are essential during gestation for ensuring the structural and functional maturation of

the fetal organs^{30,31}. This led us to develop a protocol on the basis of repeated intraperitoneal injections of 50 mg kg⁻¹ of metyrapone (a selective inhibitor of CYP11B1 responsible for corticosterone

of AF cytokine concentrations. Upper rows show average values from **g**, **n**, Representative photographs and H&E after stim. and epidermal thickness (in μm ; $n = 8$ (CT-Met) and 10 (PS-Met)). **o**, von Frey thresholds ($n = 9$ (CT-Met) and 15 (PS-Met)). **p**, Representative confocal microscopy images of avidin SRho in back skin. Counts per square millimetre at E18.5 ($n = 4$ (CT-Met) and 12 (PS-Met)) and W8 ($n = 5$ (CT-Met) and 11 (PS-Met)). **q**, Representative confocal microscopy pictures of avidin SRho and MFI ($n = 771$ (AF CT-Met) and 752 (AF PS-Met)). Blue and red lines indicate mean CT and PS values (**o**, **p**) or AF CT and AF PS values (**q**), respectively. Data were obtained from at least three independent experiments. Bars represent mean values. Each dot represents one mouse (**f**, **i**, **n-p**) or one mast cell (**i**, **j**, **q**). Mean + s.e.m., * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; Mann-Whitney U -test (**f**, **i**, **j**, **n-q**) and Kruskal-Wallis test for multiple comparison (Dunn's correction; **i**), two-sided. Scale bars, 5 μm (**i**, **j**, **q**), 25 μm (**n**), 10 μm (**p**).

synthesis) before each stress session to restore optimal corticosterone concentrations in stressed pregnant dams (Fig. 5k). This protocol significantly blocked the increase in corticosterone triggered by PS (Fig. 5l). Such a ‘normalization’ of corticosterone using metyrapone did not impact the weight of pregnant dams, the litter size or the weight of male and female offspring after birth (Extended Data Fig. 6f). It did not dampen the concentrations of cytokines found in the amniotic fluid after PS (Fig. 5m), suggesting a corticosterone-independent induction of such a pro-inflammatory environment. However, W8 PS offspring born from metyrapone-treated dams were almost completely protected from the development of eczematous lesions upon mild continuous wet friction, with restored normal epidermal thickness (Fig. 5n) and mechanosensation, according to von Frey threshold analyses (Fig. 5o). Although this reversal was not associated with the reestablishment of a normal density of sensory neurons in the skin, neurons from CT-Met and PS-Met offspring exhibited similar responses to in vitro stimulation with MRS2365 and β -alanine (Extended Data Fig. 6g–i). Transient normalization of corticosterone concentrations prevented abnormal activation programs in E18.5 fetal and W8 adult skin mast cells (Fig. 5p), a phenomenon required for PS-associated phenotype (Fig. 3i–m). The amniotic fluid of metyrapone-treated PS dams failed to activate fetal mast cells in vitro, thereby reversing the previously observed phenotype and confirming that the presence of high concentrations of corticosterone, but not of inflammatory cytokines, is responsible for fetal mast cell activation (Fig. 5q). Finally, to confirm that the observed effect of corticosterone on mast cell biology was not only inherent to our mouse model of PS, we used transgenic AdKOv2 mice, in which the adrenal-specific ablation of *Prkar1a* (also known as *Cnc1*) reproduces the phenotype of adrenocorticotrophic hormone-independent Cushing’s syndrome³² (hypercorticonemia; Extended Data Fig. 6j). Compared with littermate wild-type CTs, we found that the skin of AdKOv2 mice exhibited abnormally activated/degranulated mast cells, a phenotype that resembles that of PS offspring (Extended Data Fig. 6k). Taken together, our findings in mice demonstrate that enhanced maternal corticosterone production induces transient alterations of fetal immune programming and predisposition to develop eczematous lesions at birth.

In humans, atopic individuals frequently exhibit heightened anxiety and are prone to stress-induced exacerbations of their condition, as well as an increased risk of eczema in their offspring^{3–6}. Although inherited genetic factors are well documented, growing evidence suggests that psychological stress and emotional states contribute to atopic disease development from birth. For example, newborns with a family history of atopy display an enhanced HPA axis response to a feet heel prick stressor^{33,34}. We used publicly available scRNA-seq datasets from human embryos at different stages of development to investigate the presence of *NR3C1* and *ADRB2* in sensory neurons, mast cells and macrophages^{35,36} (Extended Data Fig. 7a,d). Mirroring our findings in mice, we found that developing sensory neurons and skin embryonic mast cells expressed high levels of *NR3C1* but very low levels of *ADRB2*, whereas embryonic skin macrophages expressed high levels of *ADRB2* but not *NR3C1* (Extended Data Fig. 7b,c,e,f). We next had access to maternal blood collected from atopic (here clinically defined as having an HDM-positive basophil activation test (BAT)) and non-atopic (HDM-negative BAT) 58 pregnant women from the Healthy Early Life Moments in Singapore (HELMs) cohort³⁷ at two distinct time points: 6–10 gestational weeks and during delivery (Extended Data Fig. 7g). We found that atopic pregnant women had significantly higher circulating concentrations of cortisol than non-atopic pregnant women during the gestational period (Extended Data Fig. 7h). However, by the time of delivery, cortisol concentrations in both groups converged, consistent with previous findings of a physiological glucocorticoid surge during labour^{38,39} (Extended Data Fig. 7i,j).

These results strongly suggest a potential translational relevance of our findings obtained in mouse models, linking elevated blood glucocorticoid concentrations with enhanced atopic predisposition.

Discussion

Our findings support a new model in which the inflammatory and sensory features of early-onset paediatric eczema originate from molecular dysregulations of neuronal and immune compartments in utero triggered by fluctuations in the maternal HPA axis. These results provide a unique view on potent tissue-specific imprinting by maternal glucocorticoids and identify fetal skin mast cells as critical for full development of eczematous lesions at birth in response to otherwise innocuous mechanical friction (Extended Data Fig. 8). Previous studies have examined the effects of postnatal stress on organ physiology^{40–43}, whereas others have explored how maternal IgE can sensitize fetal mast cells⁴⁴. However, little is known about the impact of psychological PS on eczema outcome. Mast cell–sensory neuron units in the skin are key regulators of models of allergic skin inflammation in adult mice^{9,45–50}. Other findings have also implicated neuroimmune interactions in neurodevelopment and behavioural response^{48,51–53}. In this study, we did not find evidence of bidirectional neuroimmune communication. Our results show a parallel impact of maternal stress on fetal mast cells and somatosensory systems, potentially influencing the inflammatory and sensory aspects of the disease through distinct mechanisms. We found that *Mcpt5-cre;Dta* mice showed a persistent alteration of neuronal branching. However, those mice are still mast cell-sufficient in embryos; thus, it is difficult to strictly rule out a possible role of dysregulated fetal mast cells in the alteration of neuronal branching.

Early life is a critical period for the establishment of the microbiome and skin colonization⁵⁴. Other models of stress have been linked to PS-associated microbiota dysbiosis, particularly at the gut level^{7,55–57}. In addition, the introduction of a new solid diet several weeks after birth is often accompanied by a weaning reaction, which has been found to enhance the maturation of the immune system^{54,58–60}. Although most of the observed perturbations in our PS model arise in utero, before colonization at birth or weaning, we cannot totally exclude a potential role of the skin or gut microbiota. It is also attractive to place our study in perspective with the ‘epithelial barrier hypothesis’, which proposes that the increase in epithelial cell-damaging agents could loosen barriers^{61,62}. In this context, it would be interesting to investigate whether PS offspring could be hypersensitive to even very low doses of polluting agents at birth.

In summary, our results bring a new concept, in which early-onset eczema with remission could have a prenatal origin. It is interesting to speculate that such PS-associated ‘fragilized’ skin barrier at birth could then favour skin colonization by pathogenic microbiota⁵⁴, sensitization by domestic allergens and thus pave the way towards the so-called atopic march^{63–65}. A deeper understanding of the delicate equilibrium at the maternal–fetal interface could lead to new interventions to alleviate the impact of environmental factors during pregnancy, ultimately reducing the extremely high incidence of paediatric eczema and eventually other allergic disorders.

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/s41586-025-09419-8>.

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Methods

Mice

Mice were bred and housed in the local animal facilities of the Centre Régional d'Exploration Fonctionnelle et de Ressources Expérimentales (CREFRE) in pathogen-free sanitary conditions, with free access to water and food, and were submitted to alternating cycles of 12 h of light and darkness at an ambient temperature of 22 °C. C57BL/6j mice were purchased from Janvier Labs or Charles River laboratories. *Mcpt5-cre*⁶⁶, *iDTA*, *AdKOv2* and *Cdh5-creERT2;mT/mG* mice were kindly provided by A. Roers (Heidelberg University Hospital), J.-C. Guéry, P. Val and F. Lenfant, respectively. On the basis of the results obtained in our laboratory in models of atopic dermatitis⁹, we estimate that, on the basis of the difference in the mean values of the clinical scores and with a predicted 3.5 standard deviation for individual measurements, we need six mice per group to give us 90% power to detect a difference in clinical score at statistical significance of $*P \leq 0.05$ using a two-tailed unpaired *t*-test. Sample sizes for the behavioural tests were chosen to ensure equal numbers of male and female animals and equal group sizes, consistent with previous publications⁶⁷. Sample sizes for the in vitro culture experiments (DRG neuron cultures and mast cell activation assays) were also selected to ensure equal numbers of replicates per experimental condition. The number of biological replicates was on the basis of previous publications using similar methods^{9,23}, providing sufficient power to detect meaningful effects while accounting for biological variability. To minimize bias, after allocating CT and PS groups (to ensure equal representation of groups), the investigators were blinded to the group and/or genotype of the mice whenever possible during animal model and data analysis. Although we did not expect to find differences in responses of male versus female mice, we have examined the data by sex. Both age-matched male and female mice were used in experiments. Littermate CT mice were used in all experiments in which transgenic mice were used.

Animal study approval

All animal care and experimentation were conducted in France in compliance with the guidelines of the European Union (86/609/EEC) and the French Committee of Ethics (87/848) policies and with the specific approval from the local ministry-approved committee on ethics in animal experimentation (Ethics Committee UMS006 CEEA-122; project no. 21938-2019090417341270v5). Upon arrival in the animal facility, the animals were housed in accordance with European Directive 2010/63/EU. The mice were kept in cages at 20–24 °C with 45–65% humidity, in groups of three for females and two for males, and litters were housed in groups of five.

Timed pregnancies

W8 to W10 male and female mice were subjected to timed pregnancies and introduced to a breeding diet (SAFE A03; 69.2% cereals, 6% animal proteins, 20.2% plant proteins and 4.6% vitamins and minerals) at E0. Successful mating was judged by the presence of vaginal plugs, which defined E0.5 post-conception. Female weight was monitored all along the gestation period, and gestation was confirmed by a significant increase in body weight, where a weight gain of greater than 3.5 g from E0 to E13 was indicative of gestation. In addition to wild-type pairing, mating pairs were designed as follows: *Kit*^{Wsh/Wsh} females with *Kit*^{Wsh/Wsh} males, *Kit*^{+/Wsh} females with *Kit*^{+/Wsh} males and *iDTA*^{fl/fl} females with *Mcpt5-cre*⁺ males.

Chronic PS model

PS was induced, as previously described⁷. Briefly, pregnant dams were randomly attributed to CT and PS groups. The PS group was then subjected to chronic restraint stress in transparent drilled 50-ml Falcon Tubes (up to 6 mice per cage), put under a bright light (200 W), 3 times a day for 30 min each, from E13 until E18. The CT pregnant females

were left undisturbed. Female dams were monitored every day during pregnancy and kept housed in groups of two or three animals until E18.5 after the last stress session when they were put in individual cages to prepare their nests. After birth, all pups were kept in the same cage with their biological mother. The pups were then weaned at the age of 21–28 days in distinct sex groups in new cages and introduced to a maintenance diet (SAFE A04; 84.1% cereals, 4% animal proteins, 8% plant proteins and 3.9% vitamins and minerals). Experiments were conducted at the desired age of offspring.

Metrapone treatment

CT and PS dams were injected intraperitoneally with 50 µg g⁻¹ of metrapone (Bio-Techne), once on E11 and E12 and 30 min before each stress session from E13 to E18. The injection pattern and dose were determined to obtain an effective corticosterone synthesis inhibition while keeping the required levels for gestation. Serum for corticosterone dosage was collected immediately after the last session of stress on E18.5 and assessed for corticosterone concentrations.

In utero tamoxifen administration and fate mapping

For *Cdh5-creERT2* fate mapping, *ROSA*^{mT/mG} or *ROSA*^{Tdt/Tdt} females were subjected to timed matings with *Cdh5-creERT2*^{+/+} *ROSA*^{mT/mG} or *ROSA*^{Tdt/Tdt}, respectively. For induction of reporter recombination in the offspring, a single dose of 4OHT was delivered by intraperitoneal injections to pregnant females at E7.5 or E10.5. When the Cre is expressed in the *Cdh5-creERT2*; *ROSA*^{Tdt} and *Cdh5-creERT2*; *ROSA*^{mT/mG} mice, it excises the loxP-flanked stop cassette, allowing the expression of Tdt or the switching of the fluorescence from red (mT) to green (mG), respectively, permanently marking the Cre-expressing cells and their descendants. To counteract the adverse effects of 4OHT on pregnancies, 4OHT solutions were supplemented with progesterone. Females received a dose of 1.2-mg 4OHT and 0.6-mg progesterone. Pregnant dams were then subjected to PS, and offspring were euthanized at E18.5, W8 and W24. In cases when females could not give birth naturally, pups were delivered by C-section and cross fostered with lactating CD1 females.

Model of mild wet skin friction

Mouse back skin was shaved and treated with a PBS-filled gauze pad occluded in Tegaderm Transparent Dressing (3M Health Care) every 2 days. On day 5, the gauze pads were removed, and the mice were left without any treatment for 2 days. This cycle was repeated for extra 2 weeks. Two days after the last cycle of treatment, the mice were euthanized, and back skin specimens corresponding to the treated areas were obtained for analysis. Skin inflammation was graded by unbiased measurement of the epidermal thickness. Only skin lesions found in the patching area were taken into consideration.

Mild model of tape stripping and TEWL measurements

The back skins of W3 and W8–W12 mice were shaved using an electrical razor. To disrupt the skin barrier, tape strips (autoclave tapes) were pressed firmly against the shaved area and removed immediately after application. This procedure was repeated on day 1 (five tape strips for W8 and three tape strips for W3) and day 6 (two tape strips for W8 and three tape strips for W3). Tweezers were used to remove the tape in a fluent stroke. The tape strip application was always conducted by the same person to avoid any bias owing to strength and applied pressure differences. Barrier permeability was monitored using TEWL measurements (in g m⁻² h⁻¹) that were recorded in optimal room conditions (temperature 20 °C; 40–60% relative humidity). The Tewameter probe (wireless open chamber probe; Courage + Khazaka electronic) measures the density gradient of the water evaporation from the skin indirectly by two pairs of sensors (temperature and relative humidity) according to the diffusion law. The mice were euthanized on day 8, and CT versus tape-stripped skin were collected.

Multiplex proximity extension assay (Olink)

Skin sections were disrupted in RIPA Lysis Buffer (1:10; EMD Millipore Sigma-Aldrich) supplemented with protease inhibitor cocktail tablets (cOmplete Mini EDTA-free tablets; EASYpack; Roche) and homogenized using CKMix beads (Bertin) and Precellys 24 homogenizer. Skin sections were then processed with two cycles of 6,500 rpm for 20 s. The protein concentrations of clarified tissue were measured by Pierce BCA Protein Assay Kits (Thermo Fisher Scientific). Samples were subsequently normalized to a concentration of 0.5 mg ml⁻¹ with the 1× RIPA Lysis Buffer.

The obtained skin lysates, amniotic fluid and serum samples were analysed using a proximity extension assay using the Olink Target 48 Mouse Cytokine panel in the presence of internal (incubation, extension and detection CTs) and external CTs. Data were analysed using a preprocessing normalization procedure. For each sample and data point, the corresponding quantification cycle (Cq) value for the extension CT was subtracted, thus normalizing for technical variation in one run. Normalization between runs was then performed for each assay by subtracting the corresponding Δ Cq value for the median of the three calibrator replicates from the Δ Cq values generated. The normalized protein expression unit generated was on the basis of a log₂ scale. The protein concentration in standard concentration units (in pg ml⁻¹) was obtained by fitting the normalized protein expression value to a standard curve. The results were reported in standard concentration units (in pg ml⁻¹).

Mechanical alloknesis test

W8–W12 mice were shaved at the nape of the neck and acclimated for 30 min in the experimental room and then in Plexiglas boxes of the same size for 30 min, one day before the alloknesis test. The same habituation was carried out on the day of the test. Each mouse received five innocuous mechanical stimuli at the nape of the neck from two von Frey filaments (0.07 g and 0.4 g; Bioseb BIO-VF-M). The response of the mice after each stimulation was scored (0, no reaction; 1, head movement; 2, action towards the filament; 3, scratching), and the percentage of total response was plotted.

Sticky-tape assay

W8–W12 mice were acclimated for 30 min in the experimental room and then in Plexiglas boxes for 20 min. A white 12.7-mm circular adhesive tape was gently placed to the upper back of the mice between shoulders. The tape-directed responses of the mice were monitored as follows⁶⁷: wet-dog shake, one bout; trying to reach tape with snout, one bout; burst of directed scratching with hind leg, one bout; neck grooming with front paw, one bout; grooming of head, lower back, paws, legs and ventral areas or tail, no bout. The recording was stopped when the mouse managed to get the tape off or after a trial time of 5 min. The following parameters were analysed: tape riddance (success/timeout), total number of bouts over the total trial time and bouts per minute, no response time (sum of time throughout which no bout occurred for 15 s or more) and time course of bouts represented as area under the curve of the time course plot (cumulative bouts versus time). Fisher's exact test was used to test whether PS affects the rate of mice managing to get the tape off in time. Time courses of bouts were plotted per second and then averaged for each time point. The area under these averaged time course curves (baseline at 0 bouts) was calculated using the trapezoid rule for every $\Delta x = 1$ s using GraphPad Prism. The yielded averages $\pm$ s.e.m. and the averages of the total bouts and total bouts per minute were then statistically analysed by two-way ANOVA followed by Holm–Šidák multiple comparison tests.

von Frey filament test

W8–W12 were acclimated for 1 h in the testing arena. Increasing diameter filaments delivering increasing predetermined forces were applied perpendicularly to the hind paw surface of the mouse until they buckle.

The 0.02-g von Frey filament was used as the starting filament. Subsequently, filaments of 0.04 g, 0.07 g, 0.16 g, 0.4 g, 0.6 g, 1.4 g and 2 g were used. Any brisk behaviour, including paw withdrawal, licking or shaking during the probing or immediately after the filament was removed, was considered as a positive response. For each mouse, the paw withdrawal threshold was determined as the filament force that induced a positive response on three of five trials.

Passive cutaneous anaphylaxis

The ears of W8 offspring were injected intradermally with 8- μ g ciprofloxacin, an MrgprB2 agonist in 20- μ l PBS. Ear swelling was measured every 10 min under light anaesthesia (2% isoflurane), starting just before injection (T0) and ending 2 h after injection (T120).

Peanut-induced anaphylaxis

Mice were sensitized with 1 mg of peanut extract (clinical-grade preparations used for skin testing; Stallergenes Laboratories) along with 10 μ g of cholera toxin (Sigma-Aldrich) in 100- μ l water (HCO₃⁻) administered by means of oral gavage once a week for 4 weeks. One week after the last sensitization with peanut extract, the mice were challenged with the intraperitoneal injection of 1 mg of crude peanut extract (Greer Laboratories) in 100- μ l PBS. Rectal temperature measurements were performed every 30 min, starting immediately before challenge (T0) and ending 2 h after challenge (T120).

Short model of asthma and lung dissociation

W8 offspring were sensitized by intranasal administration of lipopolysaccharide on day 0 (D0) and either PBS or 20 μ g (30 μ l) of the HDM strain *Dermatophagoides pteronyssinus* on D1 and D8, under general anaesthesia (not too long so that the mouse can breathe through its nose and inhale the liquid). On D11, the mice were euthanized, and lungs were collected and dissociated using mechanical and enzymatic dissociation. Briefly, lung lobes were collected in RPMI medium in Miltenyi C Tubes and then digested with 0.5 mg ml⁻¹ of DNase I (Sigma-Aldrich) and 0.25 mg ml⁻¹ of Liberase (Roche) using the Miltenyi gentleMACS (protocol: mouse lungs). Cell suspensions were then filtered, and red blood cells were lysed using ammonium chloride–potassium lysis buffer for 5 min. The pellet was recovered and stained for FACS analysis.

Resiniferatoxin treatment

To ablate TRPV1⁺ nociceptors, W4 CT or PS mice were subcutaneously injected with increasing RTX doses of 30, 70 and 100 μ g kg⁻¹ in 100- μ l PBS for three consecutive days. Some mice were injected with similar volumes of dimethyl sulfoxide (DMSO) in 100- μ l PBS. Four weeks later, denervation was assessed using the classical tail flick assay.

Chemical sympathectomy

6-OHDA (Sigma) was dissolved in vehicle solution (0.01% ascorbic acid) in saline solution. CT and PS mice received two intraperitoneal injections of 6-OHDA (150 mg kg⁻¹) at 24-h interval. The mice were sacrificed 2 days after the first injection, and back skin was collected for imaging analysis.

Section preparation, histology, immunofluorescence and confocal microscopy

Mouse samples were either frozen in optimal cutting temperature compound (Tissue-Tek) or fixed in 10% formalin and embedded in paraffin. Frozen or paraffin-embedded mouse skin sections pretreated using a heat-induced epitope retrieval method (in 10 mM sodium citrate buffer (pH 6.0)) were permeabilized for 30 min in PBS 0.5% (w/v) BSA and 0.3% Triton X-100 and incubated overnight at 4 °C with fluorophore-coupled antibodies or unconjugated antibodies: anti-Filaggrin (Ozyme; BLE905801; 1:50), anti-Loricrin (Ozyme; BLE905101; 1:50), anti-mouse Keratin 6A (Ozyme; BLE905701; 1:50), Anti-Claudin 1 antibody (Abcam; ab15098; 1:50), Alexa Fluor 647 anti-mouse CD45 (BioLegend; 103124;

1:400), anti-TH (Sigma; AB1542; 1:100), Alexa Fluor 647 anti-Tubulin β 3 (BioLegend; 801210; 1:50), anti-human/mouse GFR α 2 (R&D Systems; AF429; 1:200), anti-mouse NFH (Sigma; AB1989; 1:1,000), anti-GFP (Proteintech; pabg1; 1:200), anti-RFP (Takara; 632496; 1:200), Anti-Substance P (Merck Millipore; MAB356; 1:200) and anti- α SMA (Thermo Fisher Scientific; 14-9760-82; Clone 1A4; 1:100). The sections were then washed 3 times in PBS 0.5% (w/v)% BSA and 0.3% Triton X-100 and incubated, if needed, with the following secondary antibodies in PBS 0.5% (w/v)% BSA and 0.3% Triton X-100 for 2 h at room temperature in the dark: Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Invitrogen; A31571; 1:200), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen; A11012; 1:200), Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (Invitrogen, A21099; 1:200), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Invitrogen; A11008; 1:200) and Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Thermo Fisher Scientific; A21247; 1:200). The slides were then washed, mounted in Mowiol medium and sealed with a coverslip. Z-stack images (512 $\times$ 512) were acquired using Leica STELLARIS, Leica SP8 and Zeiss LSM 710 Meta inverted confocal laser scanning microscopes and were processed using ImageJ (v.2.16.0) and Imaris (Bitplane; v.9.5.1) software. The MFIs of key epidermal proteins were analysed using the 'measurement function' of ImageJ software (v.2.16.0) on randomly chosen epidermal zones of identical areas on three non-overlapping sections (same total number of pixels). The cell bodies of neurons were counted on at least three images of three non-overlapping DRG sections (from lower thoracic to upper lumbar DRG) per mouse. Data were presented as percentage of Tdtomato⁺ neurons.

Haematoxylin and eosin

Mouse back skin sections were stained according to standard procedures at the histopathology department CREFRE U006. Slides were then scanned using a Panoramic Digital Slide Scanner (3DHISTECH). For measurement of epidermal thickness, three randomly selected measurements of the distance between the stratum corneum and the basal layer of the epidermis were recorded using the slide viewing application CaseViewer 2.3.

Computational analysis of filament length of neurons in the skin

The 3D high-resolution images were taken using a Leica TCS SP8 MP Meta inverted confocal microscope, as described above. The images were then processed using the software Imaris (Bitplane; v.9.5.1). The filament tracer algorithm was applied in the Tub β 3, TH, IB4, NFH and GFR α 2 channels for precise tracing of the trajectories and shapes of Tub β 3⁺ (pan neuronal marker), TH⁺ (back skin, mainly c-LTMRs and can also stain sympathetic neurons), Tub β 3⁺ IB4⁺ neurons (back skin and non-peptidergic), NFH⁺ (glabrous skin and LTMRs) and GFR α 2⁺ (glabrous skin and non-peptidergic). Filament traces were then computed, and the sum of the filament lengths was calculated and normalized per square millimetre for all the samples.

Mouse tissue enzymatic digestion and gradient separation

Cell suspensions from embryonic and adult mouse tissues were obtained through mechanical dissociation and enzymatic digest. Briefly, adult back skin from W8 or W24 mice was collected in pre-digestion medium, finely minced and incubated at 37 °C on a rotating plate to remove epithelial cells and other impurities. The samples were then digested for 45 min on a rotating plate with 1.25 mg of Liberase (Sigma) and 2.5 mg of DNase I (Sigma) to disaggregate the tissue. The samples were further dissociated with the Miltenyi gentleMACS Dissociator, and cells were then enriched with a Percoll gradient.

Fetal skin was collected, finely minced and digested in RPMI medium containing 0.1 mg ml⁻¹ of DNase I (Sigma), 400 U ml⁻¹ of Collagenase I (Sigma), 0.1 mg ml⁻¹ of Liberase DL (Roche) and 1 mg ml⁻¹ of Dispace II

(Roche) for 45 min on a rotating plate at 37 °C. The digested tissue samples were filtered.

Single-cell suspensions were then used for flow cytometry.

Flow cytometry

The cell suspensions were blocked with anti-mouse CD16/32 (S17011E; BioLegend) for 15 min at 4 °C. Surface staining was performed in FACS buffer for 1 h at 4 °C using the following antibodies: anti-mouse CD45 BV786 (BD Biosciences; 564225; 1:200), anti-mouse/human CD11b Brilliant Violet 605 (BioLegend; 101237; 1:200), anti-mouse CD117 SB436 (Thermo Fisher Scientific; 62-1171-82; 1:200), anti-mouse CD117 PE (BioLegend; 105807; 1:200), anti-mouse Ly6C Alexa Fluor 488 (BD Biosciences; 553104; 1:200), anti-mouse Ly6G APC Cy7 (BD Biosciences; 560600; 1:200), anti-mouse/human CD207 PE-Cy7 (BioLegend; 144209; 1:200), PerCP/Cyanine5.5 anti-mouse CD3 (BD Biosciences; 551163; 1:200), BV650 Anti-Mouse γ 8 T-Cell Receptor (BD Biosciences; 563993; 1:200), anti-mouse CD4 PE Cy7 (BD Biosciences; 552775; 1:200), Alexa Fluor 488 anti-mouse CD8 (BioLegend; 100726; 1:200), anti-mouse CD25 PE (BD Biosciences; 553075; 1:200), anti-mouse NK1.1 APC Cy7 (BD Biosciences; 560618; 1:200), anti-mouse Nkp46 BV605 (BioLegend; 137619; 1:200), anti-human/mouse KLRG1 BV421 (BioLegend; 138413; 1:200), anti-mouse CD11c PE CF594 (BD Biosciences; 562454; 1:200), anti-mouse MHCII AF700 (BioLegend; 107621; 1:200), anti-mouse CD24 PE Cy7 (BD Biosciences; 560536; 1:200), anti-mouse F4/80 BV711 (BioLegend; 123147; 1:200), anti-mouse CD170 (Siglec F) eFluor 660 (Thermo Fisher Scientific; 50-1702-82; 1:200), Alexa Fluor 647 anti-mouse CD64 (BD Biosciences; 558539; 1:200), anti-mouse ST2 APC (BioLegend; 146605; 1:200), anti-mouse FcER1 PE (Miltenyi; 130-118-896; 1:200) and anti-mouse FcER1 PE-Cy7 (BioLegend; 134317; 1:200). Data were acquired on a BD FACSymphony cytometer and were analysed using FlowJo (Tree Star; v.10.10.0) software. Cell sorting was performed on BD FACSAria.

Immune cell populations were gated as follows:

γ 8 T cells: CD45⁺CD11b⁻Ly6G⁻CD3⁺TCR γ 8⁺
CD4⁺ T cells: CD45⁺CD11b⁻Ly6G⁻CD3⁺TCR γ 8⁻CD4⁺CD8⁻
CD8⁺ T cells: CD45⁺CD11b⁻Ly6G⁻CD3⁺TCR γ 8⁺CD4⁻CD8⁺
Regulatory T cells: CD45⁺CD11b⁻Ly6G⁻CD3⁺CD25⁺
Natural killer cells: CD45⁺CD11b⁻Ly6G⁻CD3⁺NK1.1⁺
Natural killer T cells: CD45⁺CD11b⁻Ly6G⁻CD3⁺NK1.1⁺
ILC2: CD45⁺CD11b⁻Ly6G⁻Nkp46⁺KLRG1⁺
ILC3: CD45⁺CD11b⁻Ly6G⁻Nkp46⁺KLRG1⁻
Dermal dendritic cell: CD45⁺CD11b⁺CD11c⁺MHC2⁺
Langerhans cell: CD45⁺CD11b⁺CD24⁺
Neutrophil: CD45⁺CD11b⁺Ly6C⁺Ly6G⁺
Monocyte: CD45⁺CD11b⁺Ly6C⁺Ly6G⁻
Macrophage: CD45⁺CD11b⁺F4/80⁺
Alveolar macrophage (adult lung): CD45⁺CD11c⁺Siglec-F⁺MertK⁺
Basophil: CD45⁺CD117⁺FceRI⁺
Eosinophil: CD45⁺CD11b⁺Siglec-F⁺
Mast cell (adult tissues): CD45⁺CD11b⁻CD117⁺FceRI⁺
Mast cell (fetal skin): CD45⁺F4/80⁻CD64⁺CD117⁺ST2⁺
All cells were pregated as viable (using SYTOX viability dye) singlets.

Tissue RNA extraction

Skin samples or DRG from adult mice were stored immediately in RNAlater then dissociated in TRIzol with the Miltenyi gentleMACS Dissociator. Tissue RNA was extracted using the RNeasy Qiagen Mini Kit according to the manufacturer's guidelines. Fetal mast cells were sorted, as described above. RNA was then extracted using RNeasy Qiagen Micro Kit according to the manufacturer's guidelines. RNA quality and titres were analysed using NanoDrop. RNA was either used for bulk RNA-seq or real-time quantitative polymerase chain reaction (qPCR).

Preparation of libraries for bulk RNA-seq

Sample quality CT and libraries were prepared at the GeT-Santé facility^{38,39}. RNA concentration and purity were determined using a

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NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). The integrity of RNA was checked with a Fragment Analyzer (Agilent Technologies) using the RNA standard sensitivity kit. The 260/280 purity ratios were all greater than 1.8. Integrity indices revealed acceptable and homogeneous values for the RNA integrity number (8.2–8.6) and 28S/18S ratios (0.8–1.2). The RNA-seq paired-end libraries were prepared at the GeT-Santé facility according to Illumina's protocol with some adjustments, using the TruSeq Stranded mRNA library prep Kit (Illumina). Briefly, messenger RNAs were first selected from either 3,000 ng (DRG sequencing) or 500 ng (sorted fetal mast cell sequencing) of total RNA using poly-T beads. RNAs were then fragmented for 2' and retrotranscribed to generate double-stranded complementary DNA (cDNA). Compatible adaptors were ligated, allowing the barcoding of the samples with unique single indices. Eleven cycles of polymerase chain reaction (PCR) were applied to amplify libraries, and an extra double-sized purification step allowed to obtain 280–1,000 pb fragments. The quality of libraries was assessed using the HS NGS kit on the Fragment Analyzer (Agilent Technologies).

Bulk RNA-seq

The quantification and sequencing of libraries were performed at the GeT-PlaGe core facility (Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement). Libraries were quantified by means of qPCR using the KAPA Library Quantification Kit (Roche) to obtain an accurate quantification. Libraries were equimolarly pooled, and RNA-seq was then performed on one S prime lane of the Illumina NovaSeq 6000 instrument (Illumina) using the NovaSeq 6000 S prime v.1 Reagent Kit (300 cycles) and a paired-end 2 × 150-pb strategy.

FastQC was performed on sequence reads to evaluate the sequencing quality. Reads were then aligned on GRCm39 (mm10) as a reference genome using STAR (v.2.4.0)⁶⁸ aligner and counted with HTseq (v.0.9.1)⁶⁹, and a counting matrix was generated. Count matrix values were transformed using regularized log transformation implemented in DESeq2 (v.1.34.0)⁷⁰. We performed data exploration using RStudio (v.2024.12.1).

Preparation of libraries for scRNA-seq

Approximately 16,000 cells per sample were encapsulated into droplets using Chromium Next GEM Single Cell 3' Reagent Kits v.3.1 according to the manufacturer's protocol (10x Genomics). Briefly, after generation of nanolitre-scale Gel bead-in-Emulsion (GEM) using Next GEM Chip G, GEMs were reverse transcribed in a C1000 Touch Thermal Cycler (Bio-Rad) programmed at 53 °C for 45 min and 85 °C for 5 min and held at 4 °C. After reverse transcription, single-cell droplets were broken, and cDNA was isolated and cleaned with Clean-Up Mix containing Dynabeads (Thermo Fisher Scientific). Then, cDNA was amplified with a C1000 Touch Thermal Cycler programmed at 98 °C for 3 min, 12 cycles of (98 °C for 15 s, 63 °C for 20 s and 72 °C for 1 min) and 72 °C for 1 min and held at 4 °C. Subsequently, the amplified cDNA was fragmented, end-repaired, A-tailed, index adaptor ligated and cleaned with Clean-Up Mix containing SPRIselect Reagent Kit (Beckman Coulter) in between steps. Post-ligation product was amplified and indexed with a C1000 Touch Thermal Cycler programmed at 98 °C for 45 s, 14 cycles of (98 °C for 20 s, 54 °C for 30 s and 72 °C for 20 s) and 72 °C for 1 min and held at 4 °C. The sequencing-ready libraries were cleaned up with SPRIselect beads.

Libraries prepared using 10x Genomics technology were pooled and charged with 1% PhiX on one S prime lane of the NovaSeq 6000 instrument (Illumina) using the NovaSeq 6000 S prime Reagent Kit v.1.5 (200 cycles) and the following sequencing parameters: 28 bp read 1, 8 bp index 1 (i7) and 150 bp read 2. The S prime lane generated a total of 434 × 10⁶ reads.

Single-cell RNA sequencing analysis

Preprocessing. Transcript reads were demultiplexed, aligned to reference mouse genome (mm10 - GENCODE vM23/Ensembl 98) and

quantified using the Cell Ranger v.7.0.1 (ref. 71). Quantified gene counts of the samples were preprocessed to remove ambient RNA decontamination using the decontX function from the celda package v.1.6.0 (ref. 72). The raw unfiltered matrix output of the Cell Ranger count of each sample was used as the respective background for the ambient RNA inference. Seurat package v.4.3.0.1 (ref. 73) was preferred as the main suite of analysis. Cells with more than 10% of their reads were assigned to mitochondrial genes, and cells with less than 100 detected genes were excluded from the dataset. After this quality control trimming, a total of 28,015 cells (7,211 cells from CT W8, 4,474 cells from PS W8, 6,812 cells from CT W24 and 9,004 cells from PS W24) were retained for further analysis.

Processing. We then ran the Seurat standard analysis pipeline: normalization, finding highly variable genes, scaling and PCA. Variance explainability of the principal component dimensions was observed, and the first 20 principal components were chosen for downstream analysis. The dataset consisted of two batches, which were integrated using the Harmony package v.1.0.3 (ref. 74), with the inclusion of the first 20 principal component dimensions. The *k*-nearest neighbour graph, UMAP and t-distributed stochastic neighbour embedding projections were computed on the basis of the first 20 corrected principal components. To scrutinize the distinct communities within the dataset, unsupervised clustering was performed using the Louvain algorithm with a resolution of 0.3.

Cell identification. FindAllMarkers() function was used to determine the marker genes of the computed unsupervised clusters of the cells. By examining the expression of a set of canonical markers within our clusters, we successfully identified a total of 13 distinct cell types: *Cd3e*, *Itgae* and *Trdc* identifying γδ T cells; *Cd207* and *Ly75* identifying Langerhans cells; *Xcr1* and *Flt3* identifying dermal dendritic cells; *Lyz2* and *Mrc1* identifying conventional macrophages; *Gzmb* and *Ncr1* identifying natural killer cells; *Ms4a1* and *Cd79b* identifying B cells; *S100a9* and *S100a8* identifying neutrophils; *Il17a* and *Il23r* identifying ILC3s; *Ccr6* and *Gata3* identifying ILC2s; *Cpa3* and *Tpsb2* identifying mast cells; and *Krt14* identifying keratinocytes.

Differential gene expression analysis. For the annotated cell types, the Poisson test within Seurat's FindMarkers() function was run to compare one group to another. Genes with $P_{adj} < 0.05$ and an absolute average log₂(fold change) greater than 0.58 were determined to be significant in the given DEG test.

Preparation of libraries for single-nucleus Multiome ATAC and Gene Expression

The back skin of W8 and W24, CT and PS offspring (*n* = 4) was processed, and single-cell suspensions were enriched in CD45⁺ immune cells.

Nuclei isolation. Single nuclei were isolated following the 10x Genomics Nuclei Isolation for Single Nucleus Multiome ATAC + Gene Expression Sequencing protocol (CG000365-Rev C). Briefly, fresh cells were counted using a Countess 3 Automated Cell Counter (Thermo Fisher Scientific), and 150,000 cells were used. The cell pellet was homogenized in 100 μl of chilled 0.025% IGEPAL lysis buffer (10x Genomics). Lysate was incubated on ice for 2 min. At the lysis end, 1 ml of chilled wash buffer (10x Genomics) was added. After centrifugation (500 relative centrifugal force; 5 min; 4 °C), the supernatant was carefully removed, and the nuclei were resuspended in 1 ml of wash buffer. The nuclei suspension was then filtered through a 40-μm strainer and centrifuged (500 relative centrifugal force; 5 min; 4 °C) for a total of three washes to remove debris. Depending on nuclei number, the pellet was resuspended in between 3.4 and 15.5 μl of diluted nuclei buffer (10x Genomics) to have 8,060 nuclei per microlitre.

Single-nucleus partitioning and library preparation. Nuclei were immediately treated using the 10x Genomics Chromium Next GEM

Single Nuclei Multiome ATAC + Gene Expression protocol (CG000338-Rev F). After transposition, the nuclei were diluted to optimal concentration and loaded into the Chromium Next GEM Chip J, followed by partitioning into GEMs using the Chromium X (10x Genomics). After GEM rupture, cDNA and transposed DNA fragments were purified using Dynabeads MyOne Silane (Thermo Fisher Scientific). Transposed DNA fragments underwent seven PCR cycle amplification to generate chromatin accessibility libraries, whereas cDNA fragments were further processed through end-repair, adaptor ligation and ten indexing PCR cycles to generate enriched gene expression libraries.

Sequencing. The resulting gene expression and ATAC libraries were quantified using a Qubit Fluorometer (Thermo Fisher Scientific) and analysed for fragment size distribution using an Agilent TapeStation before sequencing. The libraries were pooled at the recommended ratio and sequenced on an Illumina NovaSeq 6000 platform at the Technology Cluster of the Toulouse Cancer Research Centre (CRCT) using paired-end reads (read 1, 50 bp; read 2, 49 bp; index 1, 8 bp; index 2, 24 bp) and using paired-end reads (read 1, 28 bp; read 2, 90 bp; index 1, 10 bp; index 2, 10 bp), targeting a sequencing depth of approximately 50,000 read pairs per nucleus for gene expression to achieve a sequencing depth of 3,350 median fragments per cell for 19,946 cells post-aggregation.

Multiome data processing and integration

Preprocessing and quality control. Single-nucleus multiome sequencing data, including ATAC-seq and single-nucleus RNA-seq reads, were processed using Cell Ranger ARC (v.2.0.2). Reads were demultiplexed, aligned to the reference mouse genome (mm10 - GENCODE vM23/Ensembl 98) and quantified to generate filtered feature matrices and ATAC fragment files. The resulting datasets were loaded into Seurat (v.5.1.0) and Signac (v.1.14.0) for downstream analysis. Raw read files from all samples were merged into a single Seurat object, followed by quality control filtering to remove low-quality or multiple nuclei. Nuclei were retained if they met the following criteria: (single-nucleus RNA sequencing) 300 or more RNA features, total RNA counts of less than 5,000 and less than 10% mitochondrial RNA content; (snATAC-seq) ATAC peak counts between 100 and 20,000, nucleosome signal less than 0.75 and transcription start site enrichment score above 2. After filtering, a total of 12,499 high-quality nuclei were retained for downstream analysis: 2,130 nuclei from CT W8, 4,094 nuclei from PS W8, 3,767 nuclei from CT W24 and 2,508 nuclei from PS W24.

Single-nucleus RNA sequencing data processing. Gene expression values were log-normalized and scaled. Highly variable genes were identified; PCA was performed, retaining 50 principal components. Batch correction was applied using Harmony, and the corrected embeddings were stored for downstream analysis.

snATAC-seq data processing. For chromatin accessibility data, peaks with a minimum of five reads were retained. Term frequency-inverse document frequency normalization was applied, followed by singular value decomposition, keeping 50 latent semantic indexing components. Batch correction was applied using Harmony, and corrected embeddings were stored.

Multiomic integration and clustering. A weighted nearest neighbour graph was constructed using Harmony-corrected embeddings from RNA and ATAC data. The first 50 dimensions from RNA and 2–50 dimensions from ATAC were used. A joint UMAP was generated, and clustering was performed using the Louvain algorithm with a resolution of 0.1. For cell identification, similar markers to those listed above were used.

Differential analysis of accessible regions. To identify DARs of chromatin, pairwise comparisons were performed across experimental

conditions. The analysis was conducted using the FindMarkers() function in Seurat with the model-based analysis of single-cell transcriptomics test, considering the chromatin accessibility data (ATAC assay). Comparisons were investigated for PS W8 versus CT W8, PS W24 versus CT W24 and PS W24 versus PS W8 groups. Only regions with a minimum detection rate of 5% across cells were considered. Genes associated with DARs were annotated using the ClosestFeature() function of Signac package (v.1.14.0). Significant DAR-associated genes were defined on the basis of a false discovery rate threshold of 0.05 and a $\log_2$ (fold-change) cutoff of 1.5. Summary statistics were recorded for each comparison, and the results were visualized using dot plots displaying the number of genes associated with DARs.

Real-time qPCR analysis

RNA (1 μ g) preparations from mouse skin were used for total RNA reverse transcription with SuperScript III First-Strand Synthesis System kit (Invitrogen) using random hexamers (Thermo Fisher Scientific) for priming. Transcripts encoding chemokine (C–C motif) ligand 20, thymic stromal lymphopoietin, interferon- γ , ILs (IL-10, IL-12p35, IL-12p40, IL-13, IL-17A, IL-17F, IL-22, IL-4, IL-5 and IL-6), oncostatin M, retinoic acid receptor-related orphan nuclear receptor- γ t, signal transducer and activator of transcription (Stat; Stat3, Stat4 and Stat6) and transforming growth factor- β 1 were quantified using real-time PCR using specific forward and reverse primers, the Takyon SYBR 2X MasterMix blue dTTP kit (Eurogentec) and quantified using the Light-Cycler 480 II (Roche Diagnostics).

Total IgE and corticosterone enzyme-linked immunosorbent assay

Blood samples were collected and centrifuged at 15,000g for 5 min. Sera were then collected and stored at -20°C until use. Total IgE titres and corticosterone concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen and Abcam, respectively) following the manufacturer's recommendations.

Single-cell analysis of mast cell degranulation dynamics

Mast cells from E18.5 fetal skin ($n = 24$) were sorted and plated into poly-D-lysine-coated ($5\text{ }\mu\text{g ml}^{-1}$; Sigma-Aldrich) six-well glass bottom plates with coverglass (IBL Baustoff + Labor) in Tyrode's buffer at 37°C for 30 min, supplemented with $5\text{ }\mu\text{g ml}^{-1}$ avidin-sulforhodamine 101 (avidin SRho; Sigma-Aldrich). Cells were then stimulated with vehicle (Tyrode $\pm$ chloroform (negative CT)), $100\text{ }\mu\text{M}$ substance P (positive CT), 100 ng ml^{-1} of corticosterone and amniotic fluid (from CT, PS, CT-Met and PS-Met yolk sacs). Fluorescence was recorded after 30 min in a controlled atmosphere (using a ZEISS stage incubation system with objective heater; 37°C) using Leica Sp8 confocal laser scanning microscope. MFI was quantified using the measurement function of ImageJ software for each defined single cell.

DRG dissociation, culture and calcium imaging

DRG from all spinal levels were collected in Hanks' balanced salt solution without Ca and Mg and were digested with a mixture of 5 mg ml^{-1} of dispase and 1 mg ml^{-1} of collagenase type I at 37°C for 45 min. DRG were then washed and further triturated with increasing gauge syringes in DMEM supplemented with 0.15 mg ml^{-1} of DNase I. After dissociation, cells were spun at 300g and resuspended in medium before being plated in Nunc Lab-Tek II CC2 Chamber Slides with cover (Thermo Fisher Scientific). DRG were cultured for 24 h in DMEM supplemented with 50 ng ml^{-1} of nerve growth factor and 1:50 NeuroCult (STEMCELL) at 37°C . The cells were loaded with Fluo-4 for 30 min in the dark at 37°C in HEPES/Hanks' balanced salt solution immediately before imaging. The cells were imaged for 20 s to establish a baseline. DRG were then stimulated with $100\text{ }\mu\text{M}$ β -alanine, 10 nM capsaicin, 2 nM MRS2365 or 400 ng ml^{-1} of corticosterone. At the end of every imaging trial, 50 mM KCl was added as a positive CT. Damaged,

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detached, high-baseline and motion-activated cells were excluded from analysis.

Pregnant women cohort and cortisol ELISA

This study was registered with the identifier NCT05207059 at ClinicalTrials.gov³⁷. All subjects were recruited after obtaining necessary ethical approvals from the SingHealth Centralised Institutional Review Board in Singapore, and informed consent was obtained for every participant. Eligible participants were women aged 21–40 years with a body mass index of 25–40 kg m⁻²; intending to reside in Singapore for the next 4 years; identifying as Chinese, Malay, Indian or a combination of these ethnic groups; and planning to conceive within a year. Exclusion criteria included current pregnancy; known type 1 or type 2 diabetes; and recent use (past month) of anticonvulsants, oral steroids, certain contraceptives, fertility treatments or medications for human immunodeficiency virus or hepatitis B/C. Maternal blood was collected from pregnant women at two distinct time points: 6–10 gestational weeks and during delivery. The freshly processed blood was used to evaluate basophil reactivity using the BAT. BAT was performed, as described previously⁷⁵, using HDM allergen, and responders were defined as those exhibiting greater than 38% degranulation upon HDM stimulation. Cortisol measurements in plasma were performed using ELISA kit (Abcam) following the manufacturer's recommendations.

Statistics

Statistical tests were performed with the software Prism 8 (GraphPad software v.10.4.2). Kruskal–Wallis test for multiple comparisons using Dunn's correction, two-way ANOVA for multiple comparisons using Šidák corrections, Fisher's exact test and Mann–Whitney *U*-test were performed on samples, as noted in the respective figure legends. All statistical tests were two-sided. A *P* value of less than 0.05 was considered statistically significant.

Reporting summary

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

Data availability

The reference genome used in this study was mm10 - GENCODE vM23/Ensembl 98. The raw and processed datasets are available in the Gene Expression Omnibus under the following accession numbers: GSE271943 (bulk RNA-seq of fetal mast cells), GSE272046 (bulk RNA-seq of adult DRG), GSE272169 (single-cell RNA-seq of adult CD45⁺ cells)

and GSE290848 (Multiome ATAC + Gene Expression of adult skin). Reanalysed publicly available data were from previously published studies^{15,29,35,36}. Source data are provided with this paper.

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Author contributions N. Gaudenzio conceived the project. N.S., L.B. and N. Gaudenzio were involved in experimental design. N.S., N.S.A., N. Ghezzi, A.L., R.E., E.L., L.B. and N. Gaudenzio performed most experiments and compiled the data. A.-A.G., G.O., P.T., C.P., G.P., A.V., H.F.G.T., R.G., P.V., C.A.A., Y.M., A.F., F.G., A.K.A., J.K.Y.C. and N.C. assisted with the experiments. All authors participated in analysing the data and writing or editing the paper.

Competing interests N. Gaudenzio is (or was) collaborating, consulting or serving as a member of the scientific advisory board for Genoskin (where he is CSO and a shareholder), Escient pharmaceuticals, Aikium, CEVA, MaxiVAX, Boehringer Ingelheim, Novartis, Sanofi, Allegria and argenx. L.R. is (or was) collaborating or consulting for Neovacs, Novartis, CEVA and argenx. The other authors declare no competing interests.

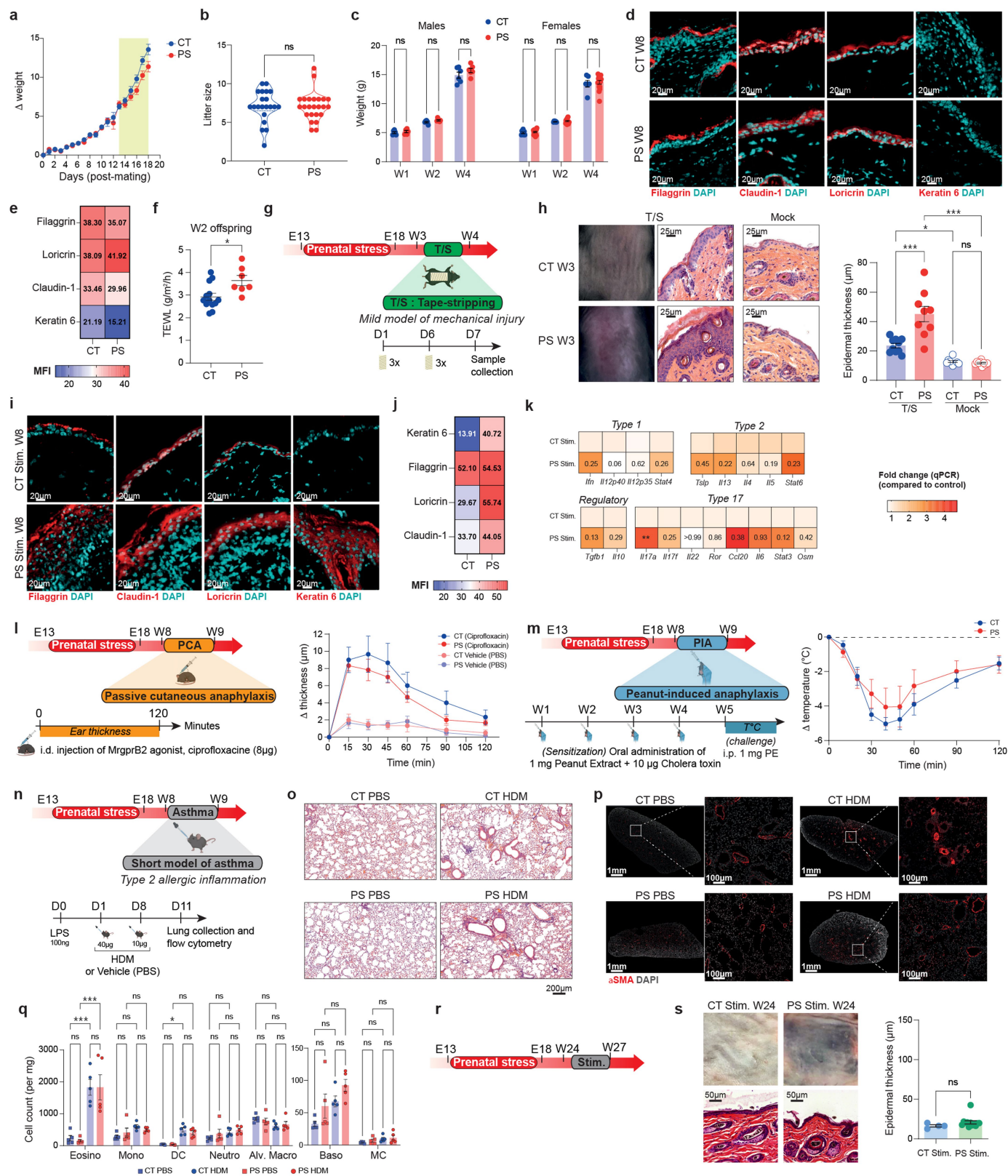
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-025-09419-8>.

Correspondence and requests for materials should be addressed to Nicolas Gaudenzio.

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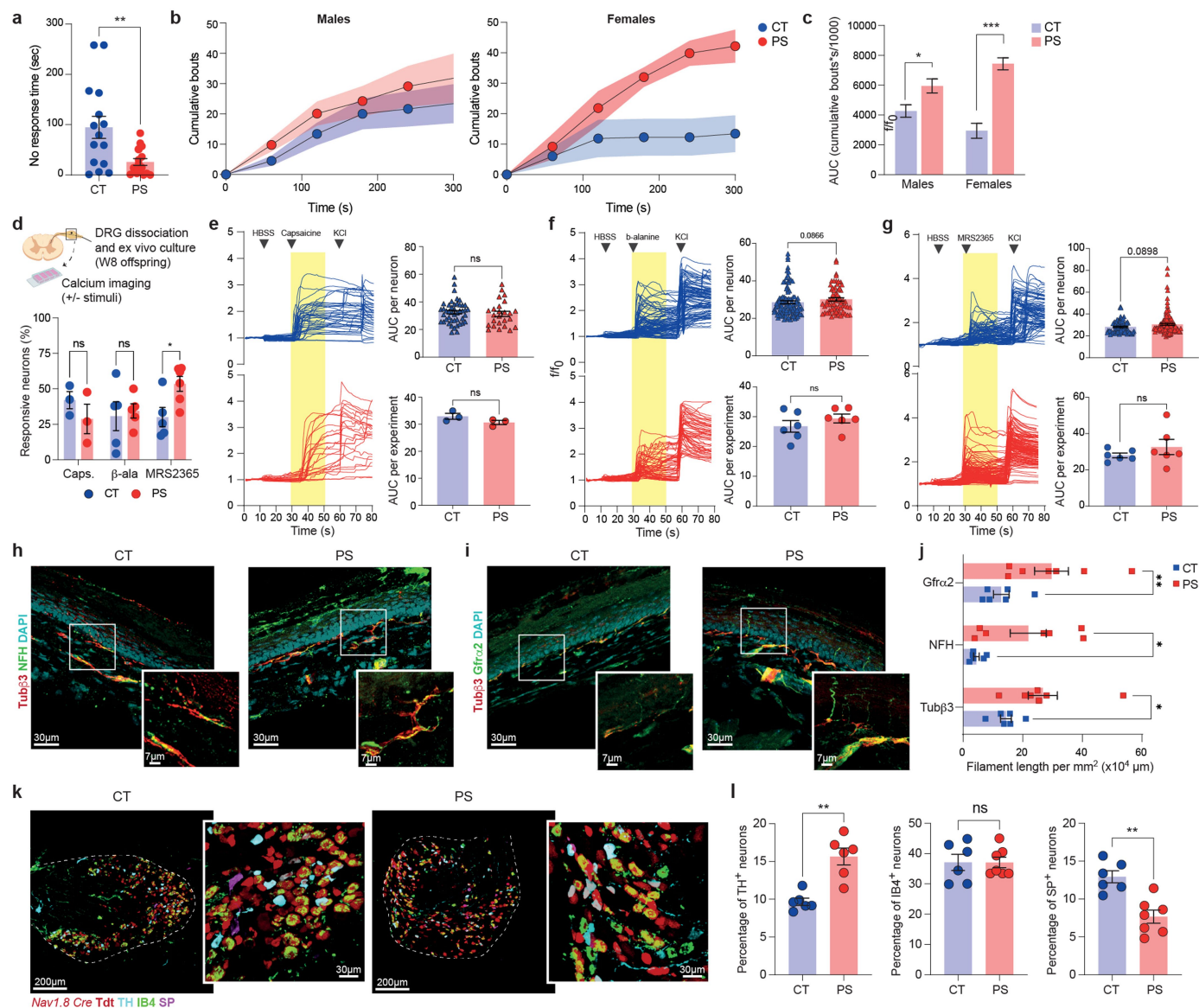


Extended Data Fig. 1 | See next page for caption.

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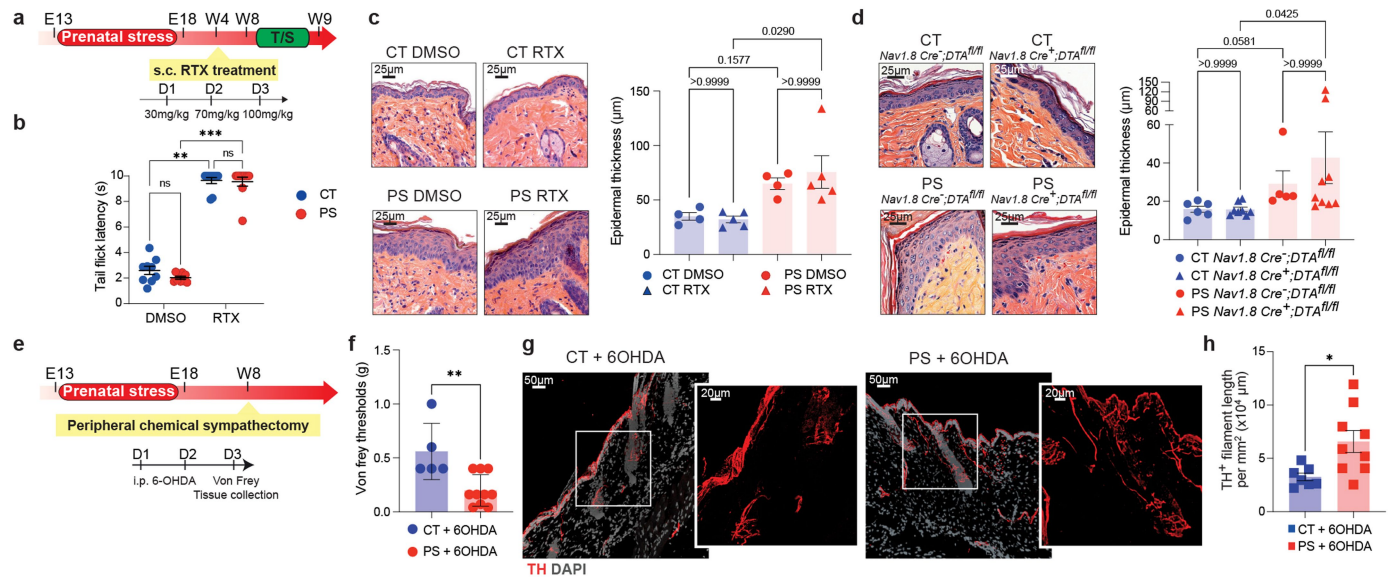
Extended Data Fig. 1 | PS-associated inflammation differs from antigen-mediated atopic dermatitis. **a**, Dams weight follow-up (n = 22 [CT], 18 [PS]). **b**, Litter size (n = 25 [CT], 29 [PS]). **c**, Weight of W1 (n = 6 M/8 F [CT], 6 M/16 F [PS]), W2 (n = 6 M/8 F [CT], 6 M/16 F [PS]), W4 (n = 6 M/6 F [CT], 6 M/16 F [PS]) offspring. **d,e**, Representative confocal microscopy images of W8 skin (d) and MFI (e). **f**, Baseline TEWL (g/m²/h; n = 13 [CT], 7 [PS]). **g**, Tape-stripping protocol (T/S). **h**, Representative photographs and H&E in W3 skin, epidermal thickness (μm, CT [n = 10], PS [n = 9]). **i,j**, Representative confocal microscopy images of Stim. W8 skin (i) and MFI (j). **k**, Relative expression in Stim. W8 skin. Numbers indicate p values. **l**, Passive cutaneous anaphylaxis. Changes in ear thickness (mm, [n = 6]). **m**, Peanut-induced anaphylaxis. Changes in temperature (°C, [n = 7 (CT), 9 (PS)]). **n**, Asthma model. **o**, Representative H&E of W8 lung.

p, Representative confocal microscopy images of α-smooth muscle actin (αSMA) in W8 lungs. **q**, Cell counts (/mg of lung, [n = 5]). **r**, Mechanical stimulation (Stim.). **s**, Representative photographs and H&E of Stim. W24 skin and epidermal thickness (μm; n = 4 [CT], 12 [PS]). Data are from at least two/three independent experiments. Bars represent mean values. Each dot represents one mouse (b, c, f, h, q-s). Scale bars: 20 μm (d, i), 25 μm (h), 200 μm (o), 1 mm (cropped to 100 μm, p), 50 μm (s). Mean + SEM; *P < 0.05, **P < 0.01, ***P < 0.001, ns: not significant; Mann-Whitney test (b, c, f, s), Kruskal Wallis test for multiple comparisons (Dunn's correction [h, q]), 2-way ANOVA with multiple comparisons (Šidák correction, l [P = 0.0003, F = 10.30], m [P = 0.5679, F = 0.342], r [P = 0.0001, F = 22.45]), two-sided. Illustrations in **l,m,n** were created using BioRender (<https://biorender.com>).



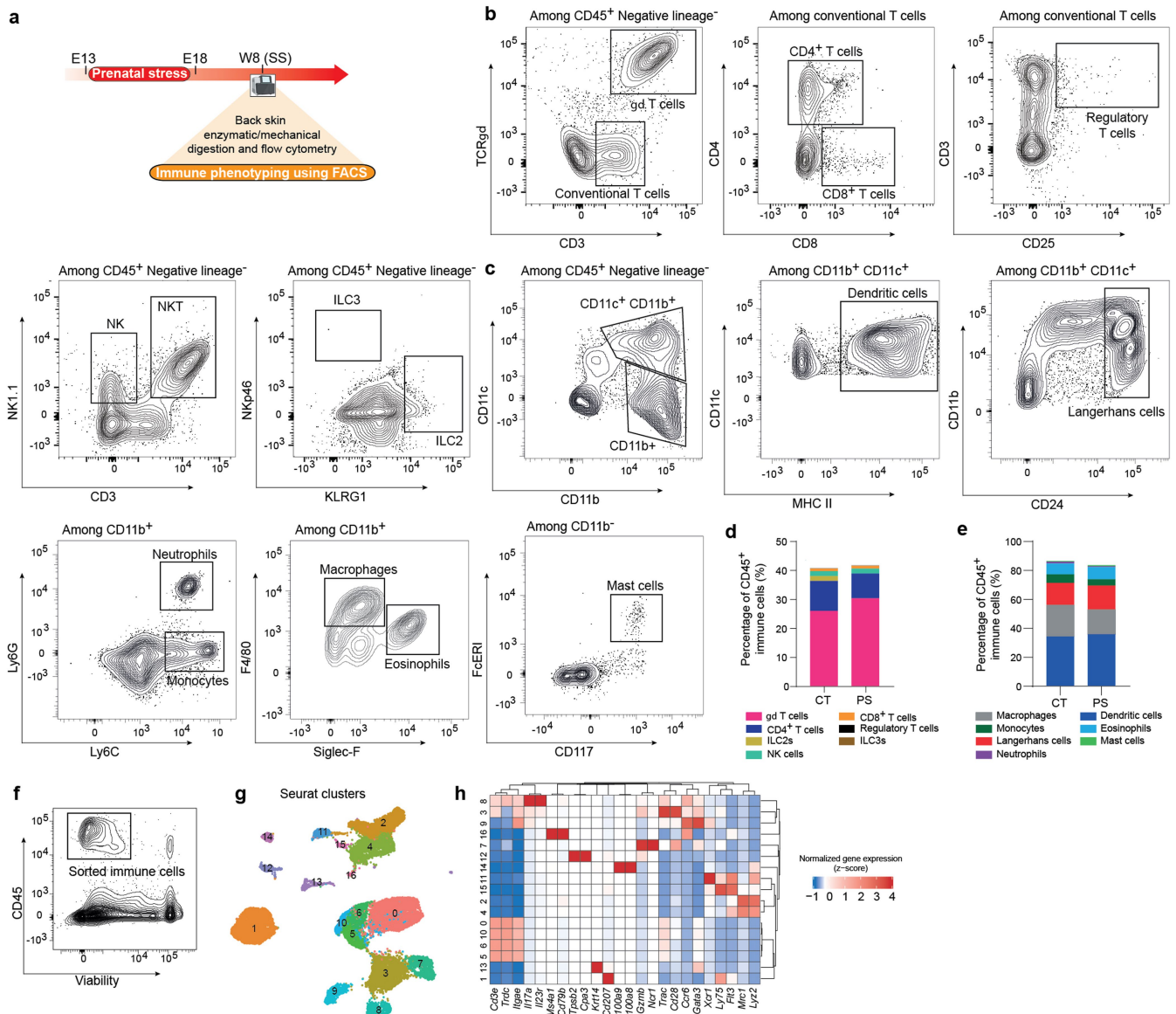
Extended Data Fig. 2 | Sensory neurons are disrupted in the dorsal root ganglia and the glabrous skin of PS offspring. **a**, Cumulative time spent not responding to the tape (no-response time, seconds). **b,c**, Averaged time courses $\pm$ SEM of tape-directed bouts (b) and AUC (c) of CT (n = 8 M/7 F) and PS (n = 9 M/6 F) offspring. **d**, Ex vivo DRG neuron stimulation and calcium imaging. Percentage (%) of responsive neurons. **e-g**, Traces of the calcium-induced changes of fluorescence exclusively in responding neurons after addition of capsaicine (e, n = 35 [CT], 21 [PS]), b-alanine (f, n = 97 [CT], 57 [PS]) and MRS2365 (g, n = 87 [CT], 131 [PS]), and amplitude of the neuronal response measured as AUC of the highlighted duration (for 20 s after the addition of the stimulus), normalized per cell (upper panel) or per experiment (lower panel). Each triangle is a responding neuron, and each dot represents an experiment including 2 mice per group (d-g). **h-j**, Representative confocal microscopy

images of neurofilament H (NFH, h) and GDNF Family Receptor Alpha 2 (Gfra2, i) in glabrous skin sections and filament length (μ m, j) per mm² in steady state W8 CT (n = 6) and PS (n = 7) offspring. Scale bars, 30 μ m (cropped to 7 μ m). **k,l**, Representative confocal microscopy images of Tub β 3, TH, IB4 and Substance P staining (k) and associated percentages (l) among all Tdt⁺ neurons, in DRG from *Nav1.8-Cre⁺;Tdt* CT (n = 6) and PS (n = 6 [TH⁺], 7 [IB4⁺, SP⁺]) at steady state. Scale bars, 200 μ m (cropped to 30 μ m). Data are from at least two or three independent experiments. Bars represent mean values, and each dot or square corresponds to a single mouse (a,j,l). Mean $\pm$ SEM; *P < 0.05, **P < 0.01, ***P < 0.001, ns: not significant; Unpaired t-test (a, c), Mann-Whitney test (d-g, j, l), two-sided. Illustrations in d were created using BioRender (<https://biorender.com>).



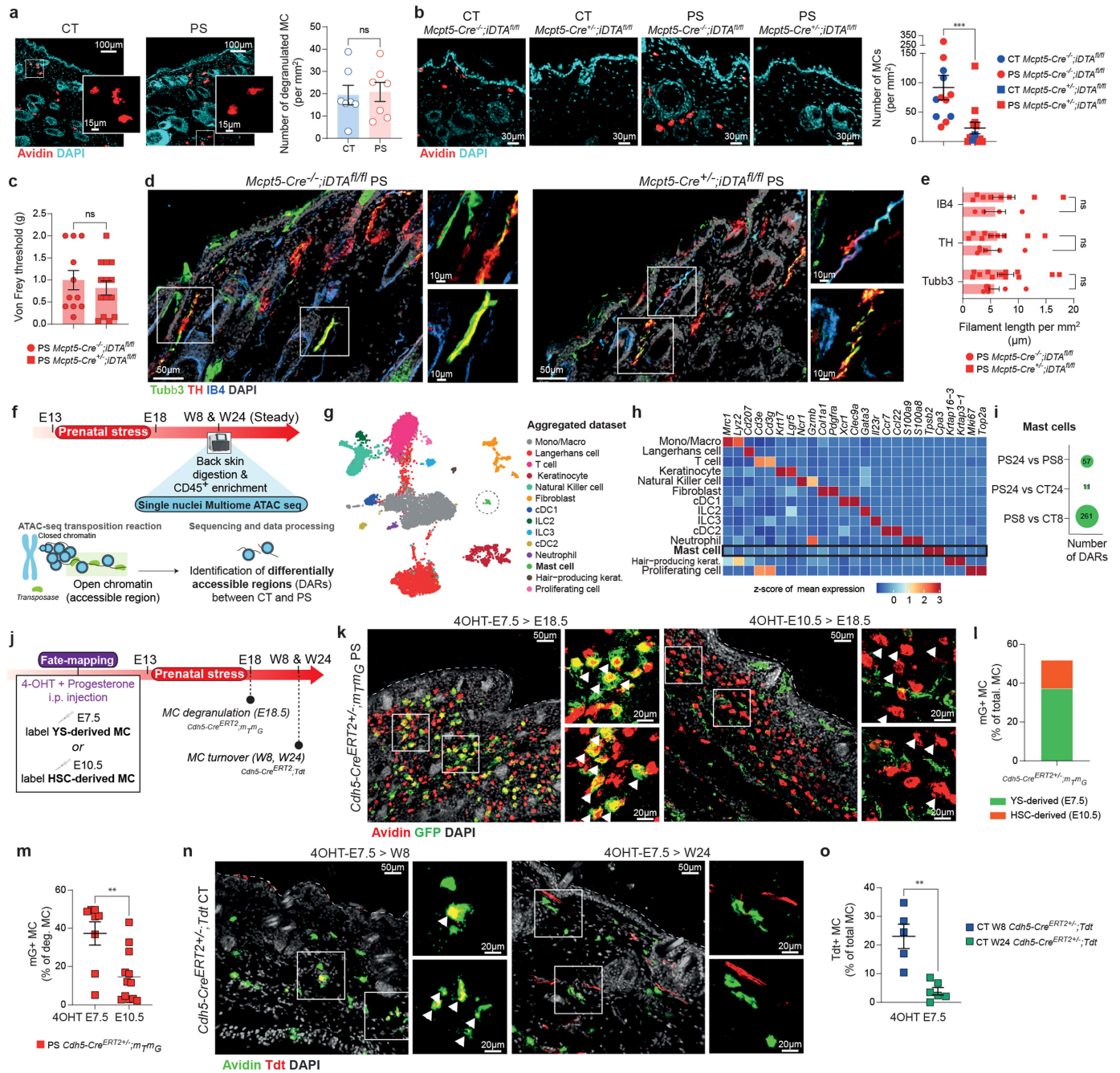
Extended Data Fig. 3 | Sensory neurons are not required for inflammation onset in PS offspring. **a**, Resiniferatoxin treatment. W4 offspring was subcutaneously injected with increasing resiniferatoxin (RTX) doses of 30 μg/kg, 70 μg/kg and 100 μg/kg for three consecutive days. 4 weeks later, denervation was assessed using the classical tail flick assay, and a mild model of skin damage (T/S) was conducted. **b**, Tail flick latency (s) for CT and PS (n = 9 [DMSO], 10 [RTX]) offspring. **c**, Representative H&E staining and corresponding epidermal thickness (μm) of CT and PS (n = 4 [DMSO], 5 [RTX]) back skin sections of W8 offspring. Scale bar, 25 μm. **d**, Representative H&E staining and corresponding epidermal thickness (μm) of CT (n = 6 [*Nav1.8-Cre⁺;DTA^{fl/fl}*], 8 [*Nav1.8-Cre⁺;DTA^{fl/fl}*]) and PS (n = 5 [*Nav1.8-Cre⁺;DTA^{fl/fl}*], 9 [*Nav1.8-Cre⁺;DTA^{fl/fl}*]) back skin sections of

W8 offspring. Scale bar, 25 μm. **e**, Peripheral chemical sympathectomy. W8 offspring were intraperitoneally injected with the neurotoxin 6-hydroxydopamine (6-OHDA, 150 mg/kg). **f**, Von Frey thresholds (g). **g**, **h**, Representative confocal microscopy images of Tyrosine Hydroxylase (TH, red) (g) and associated filament length (μm, h) in back skin sections of 6-OHDA-treated W8 CT (n = 7) and PS (n = 9) offspring. Scale bars: 50 μm (cropped to 20 μm). Data are from one or two independent experiments. Bars represent mean values, and each dot, triangle or square corresponds to a single mouse (c, d, f, h). Mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001; Kruskal Wallis test for multiple comparisons with Dunn's correction (b-d), Mann-Whitney test (f, h), two-sided.



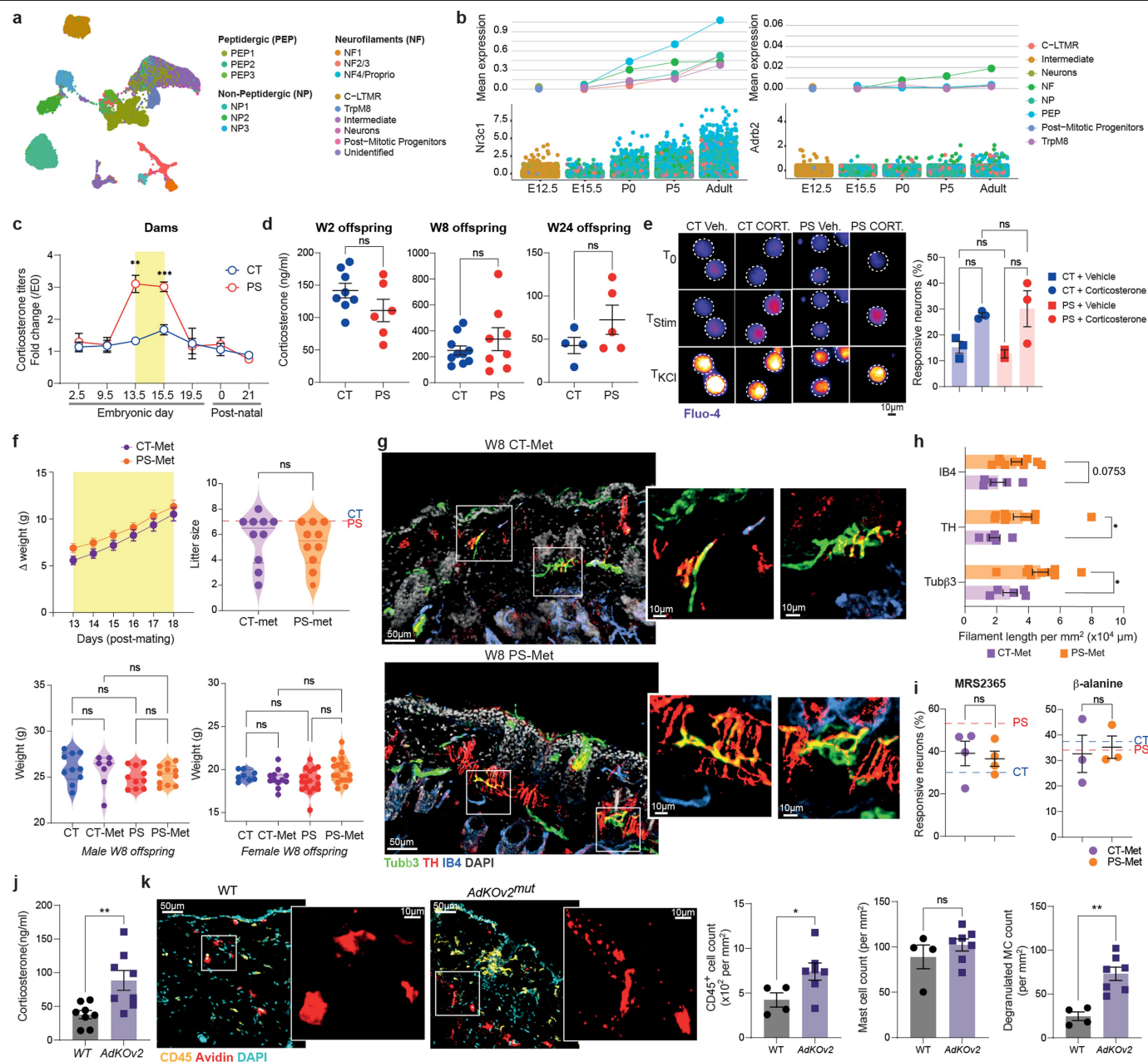
Extended Data Fig. 4 | Prenatal stress offspring show normal proportions of skin-resident immune cells at steady state. **a**, Back skin of CT and PS W8 offspring was collected and dissociated using mechanical and enzymatic digestion. Immune phenotyping was then conducted using flow cytometry. **b-e**, Gating strategies of lymphoid (b) and myeloid (c) populations and

corresponding cell proportions (d,e; n = 7). **f**, Enrichment of immune cells and sorting strategy. **g**, W8 and W24 immune cells transcriptome (n = 27,501) visualized with UMAP, colored according to unsupervised Seurat clustering. **h**, Heatmap of expression of the main canonical markers used for identification of Seurat clusters. Data are from two independent experiments.



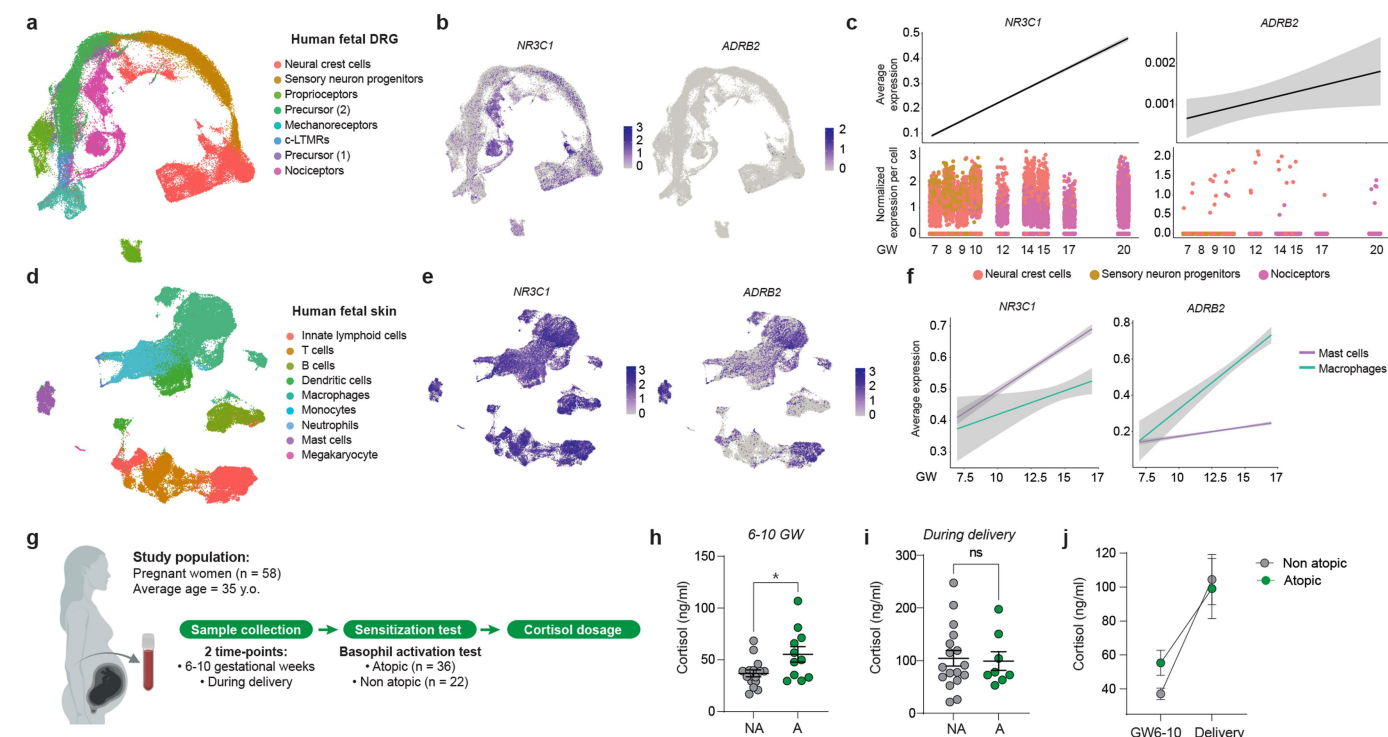
Extended Data Fig. 5 | Prenatal stress alters yolk sac-derived mast cells and induces transient epigenomic changes. a, b, Representative confocal microscopy images of Avidin SRho⁺ mast cells in back skin of pregnant dams (n = 7, a), *Mcpt5-Cre^{+/+};iDTA^{fl/fl}* (n = 12 [*Mcpt5-Cre^{+/+};iDTA^{fl/fl}*], 13 [*Mcpt5-Cre^{+/+};iDTA^{fl/fl}*]), and counts per mm². Scale bars, 100 μ m (cropped to 15 μ m, a) and 30 μ m (b). **c,** Von Frey thresholds (n = 11 [PS *Mcpt5-Cre^{+/+};iDTA^{fl/fl}*], 14 [PS *Mcpt5-Cre^{+/+};iDTA^{fl/fl}*]). **d,** Representative confocal microscopy images and filament length (μ m) of Tub3 (n = 8 [Cre⁻], 12 [Cre⁺]), TH (n = 6 [Cre⁻], 9 [Cre⁺]) and IB4 (n = 4 [Cre⁻], 8 [Cre⁺]) in PS back skin sections. Scale bars: 50 μ m (cropped to 10 μ m). **e,** Quantification of filament length per mm² of skin. **f,** Single nuclei Multiome ATAC seq. **g,** UMAP plot of W8 and W24 ATACseq aggregate and cell annotation. **h,** Heatmap of main canonical markers expression. **i,** Number of differentially accessible

chromatin regions (DARs). **j,** Fate mapping. **k-m,** Representative confocal microscopy images of Avidin SRho⁺ mast cells and GFP (k), percentages (%) of m_G (GFP)⁺ mast cells among total mast cells (l) and degranulated mast cells (m), in fetal back skin of PS offspring, fate-mapped at E7.5 (n = 8) or E10.5 (n = 12). Scale bars, 50 μ m (cropped to 20 μ m). **n, o,** Representative confocal microscopy images of Avidin⁺ mast cells and Tdt (n) and percentages (%) of Tdt⁺ mast cells among total mast cells (o), in back skin of CT *Cdh5-Cre^{ERT2}^{+/+};Tdt* W8 (n = 5) and W24 (6) offspring, fate-mapped at E7.5. Scale bars, 50 μ m (cropped to 20 μ m). Arrows indicate YS-derived mast cells (k, n). Data are from at least two or three independent experiments. Bars represent mean values, and each dot/square corresponds to a single mouse (a-d, m, o). Mean + SEM; **P < 0.01, ***P < 0.001, ns: not significant; Mann-Whitney test (a-d, m, o), two-sided.



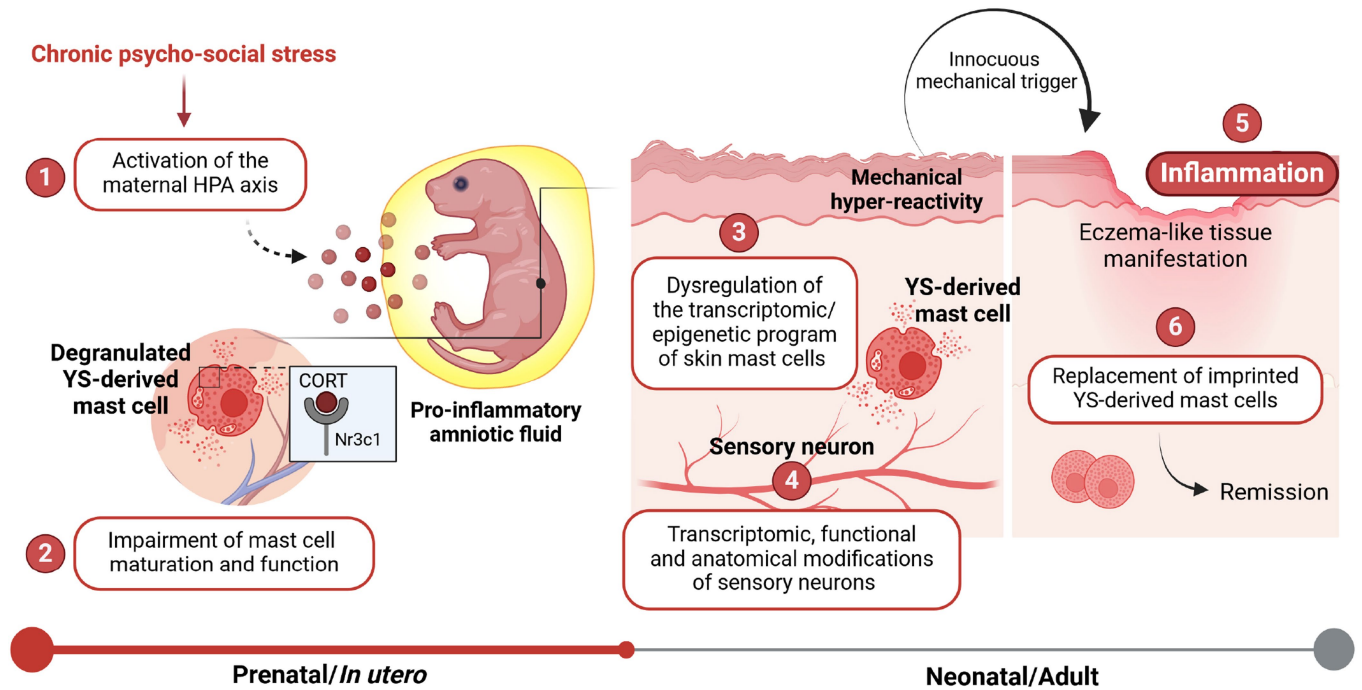
Extended Data Fig. 6 | Impact of corticosterone on offspring in different models. a, UMAP plot of E12.5, E15.5, P0, P5 and adult DRG scRNAseq. **b**, Expression levels. **c,d**, Corticosterone levels (ng/ml) in dams serum (**c**), W2 (**d**) (n = 8 [CT], 6 [PS]), W8 (n = 10 [CT], 8 [PS]) and W24 (n = 4 [CT], 5 [PS]) offspring (**d**). **e**, Representative pictures of DRG neurons. Percentage (%) of responsive neurons. **f**, Weight follow-up of dams (n = 8) and litter size (n = 10, [upper]). Weight of male (n = 10 [CT], 7 [CT-Met], 10 [PS], 11 [PS-Met]) and female (n = 8 [CT], 12 [CT-Met], 18 [PS/PS-Met]) offspring (lower). **g**, Representative confocal microscopy images in W8 back skin. Scale bars, 50 μm (cropped to 10 μm). **h**, Filament length (μm) per mm² of Tubβ3⁺ (n = 5 [CT], 9 [PS]), TH⁺ (n = 5 [CT], 10 [PS]) or Tubβ3⁺ IB4⁺ neurons (n = 5 [CT], 10 [PS]). **i**, Percentage (%) of responsive neurons. **j**, Corticosterone levels (ng/ml, n = 8). **k**, Representative confocal

microscopy images of CD45 and AvidinSRho staining in back skin. Scale bars, 50 μm (cropped to 10 μm). Counts of CD45⁺ immune cells, total and degranulated mast cells per mm² (n = 4 [WT], 7 [AdKOv2]). Data are from at least three independent experiments. Bars represent mean values. Each dot/square corresponds to a single mouse (**d**, **f**, **h**, **j**, **k**) or one experiment (2 mice/experiment, **e**, **i**). Dotted blue/red lines indicate the mean percentage from CT/PS, respectively. Mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001, ns: not significant; 2-way ANOVA for multiple comparisons using Šidák correction (**c** [P = 0.0022, F = 15.00], **f** [upper left, P = 0.2029, F = 1.785]), Mann-Whitney test (**d**, **f** [upper right], **h**, **j**, **k**), Kruskal Wallis for multiple comparison with Dunn's correction (**e**, **f** [lower]), two-sided.



Extended Data Fig. 7 | Atopic pregnant women exhibit significantly higher circulating levels of cortisol than non-atopic pregnant women during the gestational period. **a**, UMAP plot of the publicly available scRNAseq dataset for human fetal DRG performed at gestational week (GW) 7-10, 12, 14, 15, 17 and 20. **b**, Feature plot of the expression of *NR3C1* and *ADRB2*. **c**, Expression levels of *NR3C1* and *ADRB2* in neural crest cells, sensory neuron progenitors and nociceptors during development. **d**, UMAP plot of the publicly available scRNAseq dataset for human skin performed at GW 7.5, 10, 12.5, 15 and 17.

e, Feature plot of the expression of *NR3C1* and *ADRB2* in macrophages (blue) and mast cells (purple) during development. **f**, Expression levels of *NR3C1* and *ADRB2* in macrophages (blue) and mast cells (purple) during development. **g**, Description of the study cohort, including demographic and clinical characteristics, immune sensitization status (non atopic: NA, atopic: R), and cortisol levels. **h-j**, Cortisol levels (ng/ml) at GW6-10 (**h**, n = 16 [NR], 11 [R]) and during delivery (**i**, n = 17 [NR], 8 [R]), and associated averages (**j**). Mean + SEM; * $P < 0.05$, ns: not significant; Mann-Whitney test (**h**, **i**), two-sided. Illustration in **g** was created using BioRender (<https://biorender.com>).



Extended Data Fig. 8 | Summary scheme. Activation of the HPA axis in pregnant dams upon exposure to chronic stress leads to increase in corticosterone-enriched and inflammatory *in utero* environment (1), which subsequently disrupts the development of long-lived yolk sac (YS)-derived fetal skin mast cells (2). At birth, the dysregulation of the mast cell transcriptomic/epigenetic program (3) and the modifications in the transcriptomic, functional and

anatomical features of sensory neurons (4) altogether contribute to the mechanical hyper-reactivity of the skin (5). Upon exposure to innocuous mechanical trigger (i.e. mild wet friction), the offspring develops severe eczematous lesions (5) that resolve with age, after the natural replacement of imprinted YS-derived mast cells (6). Schematic was created using BioRender (<https://biorender.com>).

Extended Data Table 1 | Clinical characterization of human subjects in the HELMS cohort

Sample ID	Timepoint (/delivery)	BAT HDM Status	Final status
10001	During	Responder	Atopic
10001	Pre	Responder	Atopic
10002	During	Non-responder	Non-Atopic
10002	Pre	Non-responder	Non-Atopic
10020	During	Non-responder	Non-Atopic
10020	Pre	Non-responder	Non-Atopic
10045	During	Non-responder	Non-Atopic
10045	Pre	Non-responder	Non-Atopic
10052	During	Responder	Atopic
10052	Pre	Responder	Atopic
10053	During	Non-responder	Non-Atopic
10053	Pre	Non-responder	Non-Atopic
10064	Pre	Non-responder	Non-Atopic
10081	Pre	Non-responder	Non-Atopic
10089	During	Responder	Atopic
10089	Pre	Responder	Atopic
10104	Pre	Responder	Atopic
10105	During	Non-responder	Non-Atopic
10105	Pre	Non-responder	Non-Atopic
10115	Pre	Responder	Atopic
10117	During	Non-responder	Non-Atopic
10117	Pre	Non-responder	Non-Atopic
10135	During	Non-responder	Non-Atopic
10135	Pre	Non-responder	Non-Atopic
10136	During	Non-responder	Non-Atopic
10136	Pre	Non-responder	Non-Atopic
10140	Pre	Non-responder	Non-Atopic
10152	During	Non-responder	Non-Atopic
10152	Pre	Non-responder	Non-Atopic
10153	Pre	Responder	Atopic
10155	During	Non-responder	Non-Atopic
10155	Pre	Non-responder	Non-Atopic
10157	During	Responder	Atopic
10157	Pre	Responder	Atopic
10165	During	Responder	Atopic
10165	Pre	Responder	Atopic
10167	During	Responder	Atopic
10167	Pre	Responder	Atopic
10174	During	Responder	Atopic
10174	Pre	Responder	Atopic
10186	During	Non-responder	Non-Atopic
10186	Pre	Non-responder	Non-Atopic
10191	During	Non-responder	Non-Atopic
10191	Pre	Non-responder	Non-Atopic
10209	During	Responder	Atopic
10209	Pre	Responder	Atopic
10234	During	Non-responder	Non-Atopic
10234	Pre	Non-responder	Non-Atopic
MB24	During	Non-responder	Non-Atopic
MB25	During	Non-responder	Non-Atopic
MB36	During	Non-responder	Non-Atopic
MB41	During	Non-responder	Non-Atopic

Sample ID denotes the unique identifier for each sample. Timepoint specifies when the sample was collected relatively to delivery. BAT HDM Status indicates the results (responder vs non responder) of the Basophil Activation Test (BAT) to House Dust Mites. Final status reflects the classification assigned following analysis (atopic vs non atopic).

Reporting Summary

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n/a	Confirmed
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☐	☒ 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	Zen software, DIVA, 3D HISTECH Case Viewer, OLINK, Illumina NovaSeq 6000, Fragment Analyzer (Agilent Technologies), MPA WL FP (TEWL), Roche LightCycler
Data analysis	Softwares: Graphpad prism (Version 10.4.2), Imaris (Version 9.5.1), ImageJ (Fiji, Version 2.16.0), FlowJo (Version 10.10.0), R studio (Version 2024.12.1) Codes and algorithms (published literature): STAR(v2.4.0), HTseq(v0.9.1), DESeq2(v1.34.0), Cell Ranger v(7.0.1), celda package (v1.6.0), Seurat package(v4.3.0.1), Harmony package (v1.0.3), Signac package (v1.14.0)

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](#)

The reference genome used in this study is mm10 - GENCODE vM23 / Ensembl 98.

The raw and processed datasets are available in the Gene Expression Omnibus (GEO) under the following accession numbers: GSE271943 (bulk RNA-seq of fetal mast cells), GSE272046 (bulk RNA-seq of adult DRG), GSE272169 (single cell RNA-seq of adult CD45+ cells) and GSE290848 (Multiome ATAC + Gene Expression of adult skin).

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

In this study, only women planning to conceive within the next 6 months were recruited. Participants were identified as females based on self-reporting during recruitment. Since all participants were pregnant women, the data are not disaggregated by sex or gender.

Reporting on race, ethnicity, or other socially relevant groupings

Ethnicity in this study was self-reported by participants as Chinese, Malay, Indian, or a combination of these groups. These classifications were used to reflect sociocultural and demographic diversity, without serving as proxies for other variables.

Population characteristics

Eligible participants were women aged 21-40 years with a BMI of 25-40 kg/m², intending to reside in Singapore for the next four years, identifying as Chinese, Malay, Indian, or a combination of these ethnic groups, and planning to conceive within a year. Exclusion criteria included current pregnancy, known type 1 or type 2 diabetes, recent use (past month) of anticonvulsants, oral steroids, certain contraceptives, fertility treatments, or medications for HIV or Hepatitis B/C. Maternal blood was collected from pregnant women at two distinct time points: 6-10 gestational weeks and during delivery. The freshly processed blood was used to evaluate the basophil reactivity using the basophil activation test (BAT). BAT was performed as described previously⁶⁶ using HDM allergen, and responders were defined as those exhibiting greater than 38% degranulation upon HDM stimulation. Cortisol measurements in plasma were performed using enzyme-linked Immunosorbent Assay kit (Abcam) following the manufacturer's recommendations.

Recruitment

This study was registered at clinicaltrials.gov with the identifier NCT05207059. Participants for this single-arm implementation study (HELMS) were actively recruited at KK Women's and Children's Hospital in Singapore. All subjects in the study were recruited after obtaining necessary ethical approvals from the respective committees in Singapore, and informed consent was obtained for every participant.

Ethics oversight

The study protocol was approved by the SingHealth Centralised Institutional Review Board (CIRB).

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](https://www.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

We have adapted the size of each group of mice related to the in vivo experiments to the following:

- Models of skin inflammation: we have estimated the numbers of mice that were needed to analyze and detect differences in epidermal thickness based on previous preliminary data. We estimated that the difference in the mean value of the epidermal thickness with an estimated 1.2 standard deviation, we needed at least 5/6 mice per group (total) to give us 90% power to detect a difference at statistical significance of $p \leq 0.05$ using Man Whitney test.
- Behavioral tests: sample sizes for the behavioral tests were chosen to ensure equal numbers of male and female animals and equal group sizes, consistent with previous publications that successfully implemented the behavioral methods, ensuring adequate power to detect biologically relevant effects (e.g., Michel et al. 2020). No formal power calculation was performed specifically for this study, but sample sizes are in line with those typically reported in comparable studies and considered sufficient to ensure statistical validity while adhering to ethical principles of animal use.
- Steady state studies: for each parameter assessed in the steady state studies, we included between 5 and 16 mice per group to ensure

robust and reliable statistical analysis. For single comparisons, we used the Mann-Whitney test, and for multiple comparisons, we used 2-way ANOVA or the Kruskal-Wallis test. The sample size for each group and the specific statistical tests conducted are indicated in the legend of each figure.

- In vitro experiments: sample sizes for the in vitro culture experiments (DRG neuron cultures, mast cell activation assays) were also selected to ensure equal numbers of replicates per experimental condition. The number of biological replicates was based on prior publications using similar methods (e.g., Serhan et al., 2019; Gaudenzio et al., 2016), providing sufficient power to detect meaningful effects while accounting for biological variability.

- Sequencing: The single-cell RNA sequencing of CD45 immune cells included 4 groups (CT W8, PS W8, CT W24, PS W24), with 3 mice per group. A total of 28,015 cells was analyzed. The bulk RNA sequencing of fetal mast cells included 3 samples per group (CT, PS), with each sample being a pool of 4 fetuses. The bulk RNA sequencing of adult DRG included 3 mice per group (CT, PS). The ATAC seq multiome of CD45 immune cells included 4 groups (CT W8, PS W8, CT W24, PS W24), with 4 mice per group.

Data exclusions	No data were excluded.
Replication	All experiments were repeated at least 2-3 times and each experiments gave similar results.
Randomization	Allocation of mice in most of the experiments was random, with efforts made to ensure equal representation of males and females and to include offspring from different dams. For experiments involving transgenic mice, all litters were included regardless of genotype or sex, and data were analyzed at the end of the experimental procedures.
Blinding	To minimize bias, after allocating CT and PS groups (to ensure equal representation of groups), investigators were blinded to the group and/or genotype of the mice whenever possible during animal models and/or data analysis. Mice from different groups and genotypes were coded with unique identifiers and the codes were only revealed after the completion of data analysis.

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

Anti-Filaggrin Polyclonal Antibody (Ozyme, BLE905801, Poly19058, 1/50)
 Anti-Loricrin Polyclonal Antibody (Ozyme, BLE905101, Poly19051, 1/50)
 Anti-mouse Keratin 6A (Ozyme, BLE905701, Poly19057, 1/50)
 Anti-Claudin 1 Antibody (abcam, ab15098, 1/50)
 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Invitrogen, A31571, 1/200)
 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Invitrogen, A11012, 1/200)
 Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (Invitrogen, A21099, 1/200)
 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Invitrogen, A-11008, 1/200)
 Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (ThermoFisher, A21247, 1/200)
 Anti-mouse CD45 Alexa Fluor 647 (Biolegend, 103124, Clone 30-F11, 1/400)
 Anti-TH (Sigma Aldrich, AB1542, Polyclonal antibody, 1/100)
 Anti-Tubulin β 3-Alexa Fluor 647 (Biolegend, 801210, Clone TUJ1, 1/50)
 Anti-mouse CD45 BV786 (BD Biosciences, 564225, Clone 30-F11, 1/200)
 Anti-mouse/human CD11b Brilliant Violet 605 (Biolegend, 101237, Clone M1/70, 1/200)
 Anti-mouse CD117 SB436 (ThermoFisher, 62-1171-82, Clone 2B8, 1/200)
 Anti-mouse CD117 PE (Biolegend, 105807, Clone 2B8, 1/200)
 Anti-mouse Ly6C Alexa Fluor 488 (BD Biosciences, 553104, Clone AL-21, 1/200)
 Anti-mouse Ly6G APC Cy7 (BD Biosciences, 560600, Clone 1A8, 1/200)
 Anti-mouse/human CD207 PE-Cy7 (Biolegend, 144209, Clone 4C7, 1/200)
 Anti-mouse CD3 PercP-Cy5.5 (BD Biosciences, 551163, Clone 145-2C11, 1/200)
 Anti-mouse TCRgd BV650 (BD Biosciences, 563993, Clone GL3, 1/200)
 Anti-mouse CD4 PE Cy7 (BD Biosciences, 552775, Clone RM4-5, 1/200)
 Anti-mouse CD8 Alexa Fluor 488 (Biolegend, 100726, Clone 53-6.7, 1/200)
 Anti-mouse CD25 PE (BD Biosciences, 553075, Clone 3C7, 1/200)
 Anti-mouse NK1.1 APC Cy7 (BD Biosciences, 560618, Clone PK136, 1/200)
 Anti-mouse Nkp46 BV605 (Biolegend, 137619, Clone 29A1.4, 1/200)

Anti-human/mouse KLRG1 BV421 (Biolegend, 138413, Clone 2F1/KLRG1, 1/200)
 Anti-mouse CD11c PE CF594 (BD Biosciences, 562454, Clone HL3, 1/200)
 Anti-mouse MHCII AF700 (Biolegend, 107621, Clone M5/114.15.2, 1/200)
 Anti-mouse CD24 PE Cy7 (BD Biosciences, 560536, Clone M1/69, 1/200)
 Anti-mouse F4/80 BV711 (Biolegend, 123147, Clone BM8, 1/200)
 Anti-mouse CD170 (Siglec F) efluor 660 (Thermofisher, 50-1702-82, Clone 1RNM44N, 1/200)
 Anti-mouse CD64 Alexa Fluor 647 (BD Biosciences, 558539, Clone X54-5/7.1, 1/200)
 Anti-mouse ST2 APC (Biolegend, 146605, Clone DIH4, 1/200)
 Anti-mouse FcERI PE (Miltenyi, 130-118-896, Clone REA1079, 1/200)
 Anti-mouse FcERI PE-Cy7 (Biolegend, 134317, Clone MAR-1, 1/200)
 Anti-human/mouse GFR α 2 (R&D systems, AF429, Polyclonal, 1/200)
 Anti-mouse NFH (Sigma, AB1989, Polyclonal, 1/1000)
 Anti-GFP (ProteinTech, pabg1, Polyclonal, 1/200)
 Anti-RFP (Takara, 632496, Polyclonal, 1/200)
 Anti Substance P (Merck Millipore, MAB356, Clone NC1, 1/200)
 Anti-mouse CD16/32 (S17011E, Biolegend, 1/100)
 Anti aSMA (Thermofisher, 14-9760-82, Clone 1A4, 1/100)

Validation

All primary antibodies used in this study were validated for species and application according to the manufacturer's specifications. Validation statements are available on the manufacturers' websites, and antibody selection was supported by relevant published studies (citations provided in the manuscript where applicable). The Research Resource Identifiers (RRIDs) for each antibody have is provided below to ensure transparency and reproducibility.

Anti-Filaggrin Polyclonal Antibody, RRID:AB_2616957
 Anti-Loricrin Polyclonal Antibody, RRID:AB_2565046
 Anti-mouse Keratin 6A antibody, RRID:AB_2565052
 Anti-Claudin 1 Antibody, RRID:AB_301644
 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647, RRID:AB_162542
 Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, RRID:AB_141359
 Donkey anti-Sheep IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568, RRID:AB_10055702
 Anti-mouse CD45 Alexa Fluor 647, RRID: AB_493534
 Anti-TH (Sigma Aldrich, AB1542, RRID:AB_90755)
 Anti-Tubulin β 3-Alexa Fluor 647, RRID:AB_2686930
 Anti-mouse CD45 BV786, RRID: AB_2716861
 Anti-mouse/human CD11b Brilliant Violet 605, RRID: AB_11126744
 Anti-mouse CD117 SB436, RRID:AB_2637141
 Anti-mouse CD117 PE, RRID:AB_313216
 Anti-mouse Ly6C Alexa Fluor 488, RRID: AB_394628
 Anti-mouse Ly6G APC Cy7, RRID: AB_1727561
 Anti-mouse/human CD207 PE-Cy7, RRID: AB_2876490
 Anti-mouse CD3 PercP-Cy5.5, RRID: AB_394082
 Anti-mouse TCRgd BV650, RRID: AB_2738530
 Anti-mouse CD4 PE Cy7, RRID:AB_394461
 Anti-mouse CD8 Alexa Fluor 488, RRID:AB_493423
 Anti-mouse CD25 PE, RRID:AB_394605
 Anti-mouse NK1.1 APC Cy7, RRID:AB_1727569
 Anti-mouse Nkp46 BV605, RRID: AB_2562452
 Anti-human/mouse KLRG1 BV421, RRID: AB_10918627
 Anti-mouse CD11c PE CF594, RRID: AB_2737617
 Anti-mouse MHCII AF700, RRID:AB_493726
 Anti-mouse CD24 PE Cy7, RRID: AB_1727452
 Anti-mouse F4/80 BV711, RRID: AB_2564588
 Anti-mouse CD170 (Siglec F) efluor 660, RRID: AB_2574187
 Anti-mouse CD64 Alexa Fluor 647, RRID: AB_647120
 Anti-mouse ST2 APC, RRID:AB_2728174
 Anti-mouse FcERI PE, RRID:AB_2801716
 Anti-mouse FcERI PE-Cy7, RRID: AB_10643996
 Anti-human/mouse GFR α 2, RRID: AB_2294621
 Anti-GFP, RRID: AB_2749857
 Anti aSMA, RRID: AB_2572996
 Anti SP, RRID: AB_94639

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

This study involved different mouse strains:
 - C57BL/6J WT
 - KitW-sh/W-sh
 - Mcpt5-Cre;DTA
 - AdKOv2
 - Cdh5-CreERT2 MTMG

- Cdh5-CreERT2 Tdt
- Nav1.8 Cre Tdt
- Nav1.8 Cre DTA

Both male and female mice were used at these different ages: embryonic day 18.5, 8-weeks old, 24-weeks old.
Upon arrival in the animal facility, animals were housed in accordance with European Directive 2010/63/EU. Mice were kept in cages at 20–24°C with 45–65% humidity: in groups of three for females, groups of two for males, and litters were housed in groups of five.

Wild animals

No wild animals were used in this study.

Reporting on sex

Data analysis disaggregated by sex showed no significant impact of sex on the results. Consequently, both female and male mice were included in the experiments, and their data were pooled for analysis. However, separated data by sex is available.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All animal care and experimentation were conducted in France in compliance with the guidelines of the European Union (86/609/EEC) and the French Committee of Ethics (87/848) policies and with the specific approval from the local ministry approved committee on ethics in animal experimentation (Ethics Committee UMS006 CEEA-514 122, project n°21938-2019090417341270v5).

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

Clinical data

Policy information about [clinical studies](#)

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

Clinical trial registration

This study was registered at clinicaltrials.gov with the identifier NCT05207059.

Study protocol

The full protocol can be accessed on clinicaltrials.gov with the identifier mentioned above.

Data collection

All subjects were recruited after obtaining necessary ethical approvals from the respective committees in Singapore, and informed consent was obtained for every participant. The HELMS study was carried out at KK Women's and Children's Hospital, Singapore. Recruitment commenced on March 18, 2022, and is ongoing until December 2028. Data were collected longitudinally at preconception, during each pregnancy trimester, and across six postpartum visits spanning up to 18 months post-delivery. Eligible participants were women aged 21-40 years with a BMI of 25-40 kg/m², intending to reside in Singapore for the next four years, identifying as Chinese, Malay, Indian, or a combination of these ethnic groups, and planning to conceive within a year. Exclusion criteria included current pregnancy, known type 1 or type 2 diabetes, recent use (past month) of anticonvulsants, oral steroids, certain contraceptives, fertility treatments, or medications for HIV or Hepatitis B/C. Maternal blood was collected from pregnant women at two distinct time points: 6-10 gestational weeks and during delivery.

Outcomes

In order to study XXX, we took advantage of this cohort and only analyzed freshly processed blood, first to evaluate the basophil reactivity using the basophil activation test (BAT), and second, to measure cortisol levels in plasma. BAT responders were defined as those exhibiting greater than 38% degranulation upon HDM stimulation. Cortisol measurements in plasma were performed using enzyme-linked Immunosorbent Assay kit following the manufacturer's recommendations. Comparisons in cortisol levels at two timepoints during pregnancy, between atopic and not atopic pregnant women were conducted.

The clinical trial altogether has the following primary and secondary outcomes:

Primary outcomes include:

- Pregnancy rate (confirmed by clinical or ultrasound diagnosis),
- Maternal metabolic health (assessed by metabolic syndrome criteria),
- Maternal mental health (assessed by Edinburgh Postnatal Depression Scale).

Secondary outcomes cover:

- Reproductive outcomes (fecundability, pregnancy loss, live birth rates, gestational duration, hypertensive disorders, gestational diabetes),
 - Maternal cardiometabolic measures (lipids, glycaemic markers, blood pressure, heart rate, body composition, visceral fat),
 - Maternal lifestyle factors (dietary patterns, nutrient intake, physical activity, sedentary behaviour, sleep, emotional well-being),
 - Maternal personality traits, pain, ocular health, and complications during pregnancy, delivery, and postpartum,
 - Offspring outcomes (birth anthropometry, growth up to 18 months, feeding practices, gut microbiome),
 - Maternal gut microbiome and parental genotypes' influence on outcomes,
 - Program feasibility metrics (acceptability, adherence, and satisfaction with HELMS program).
- Outcomes are assessed at predefined time points through validated questionnaires, clinical and anthropometric measurements, biosample analyses (blood, stool, cord blood), and medical record reviews during preconception, pregnancy, and postpartum periods (up to 18 months for offspring and up to 40 months overall for mothers).

Plants

Seed stocks

NA

Novel plant genotypes

NA

Authentication

NA

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

Cell suspensions from embryonic and adult mouse tissues were obtained through mechanical dissociation and enzymatic digest. Briefly, adult back skin from W8 or W24 mice was harvested in pre-digestion medium, finely minced and incubated at 37°C on a rotating plate to remove epithelial cells and other impurities. The samples were then digested for 45 minutes on a rotating plate with 1.25 mg of Liberase TM (Sigma) and 2.5 mg of DNase I (Sigma) to disaggregate the tissue. Samples were further dissociated with the Miltenyi gentleMACS Dissociator and cells were then enriched with a Percoll gradient. Fetal skin from E18.5 embryos was harvested, finely minced and digested in RPMI medium containing 0.1 mg/ml DNase I (Sigma), 400U/ml Collagenase I (Sigma), 0.1 mg/ml Liberase DL (Roche) and 1 mg/ml Dispase II (Roche) for 45 minutes on a rotating plate at 37°C. Digested tissue samples were filtered and single cell suspensions were used for flow cytometry. Cell suspensions from mouse skin were blocked with anti-mouse CD16/32 (S17011E, Biolegend) for 15 min at 4°C. Surface staining was performed in FACS buffer for 1 hour at 4°C.

Instrument

Data were acquired on a FACSymphony (BD) cytometer.
Cell sorting was performed on FACS Aria (BD).

Software

Data were analyzed using FlowJo (Tree Star) software.

Cell population abundance

Cell population abundance was represented and calculated as a percentage of total immune cells (CD45+ cells) for the following cell types:

- gd T cells: ~26.1% (CT), ~30.45% (PS)
- CD4+ T cells: ~10.3% (CT), ~8.52% (PS)
- ILC2: ~1.7% (CT), ~0.015% (PS)
- NK cells: ~1.64% (CT), ~1.68% (PS)
- CD8+ T cells: ~0.91% (CT), ~0.95% (PS)
- T reg: ~0.11% (CT), ~0.10% (PS)
- ILC3: ~0.006% (CT), ~0.00004% (PS)
- Dermal dendritic cells: ~34.4% (CT), ~35.92% (PS)
- Macrophages: ~21.57% (CT), ~17.0625% (PS)
- Langerhans cells: ~15.357% (CT), ~16.59% (PS)
- Eosinophils: ~7.58% (CT), ~8.39% (PS)
- Monocytes: ~5.91% (CT), ~4.41% (PS)
- Neutrophils: ~1.15% (CT), ~0.7% (PS)
- Mast cells (adult): ~0.57% (CT), ~0.65% (PS)

Gating strategy

Immune cell populations were gated as follows:

- gd T cells: CD45+ CD11b- Ly6G- CD3+ TCRgd+
- CD4+ T cells: CD45+ CD11b- Ly6G- CD3+ TCRgd- CD4+ CD8-
- CD8+ T cells: CD45+ CD11b- Ly6G- CD3+ TCRgd- CD4- CD8+
- Regulatory T cells: CD45+ CD11b- Ly6G- CD3+ CD25+
- NK cells: CD45+ CD11b- Ly6G- CD3- NK1.1+
- NK T cells: CD45+ CD11b- Ly6G- CD3+ NK1.1+
- ILC2: CD45+ CD11b- Ly6G- Nkp46- KLRG1+
- ILC3: CD45+ CD11b- Ly6G- Nkp46+ KLRG1-

- Dermal dendritic cell: CD45+ CD11b+ CD11c+ MHC2+
- Langerhans cell : CD45+ CD11b+ CD24+
- Neutrophil: CD45+ CD11b+ Ly6C+ Ly6G+
- Monocyte: CD45+ CD11b+ Ly6C+ Ly6G-
- Macrophage: CD45+ CD11b+ F4/80+
- Eosinophil: CD45+ CD11b+ Siglec-F+
- Mast cell (adult tissue): CD45+ CD11b- CD117+ FcεRI+
- Mast cell (embryonic tissues): CD45+ F4/80- CD64- CD117+ ST2+
- Alveolar macrophage (adult lung): CD45+ CD11c+ Siglec-F+ MertK+
- Basophil : CD45+ CD117- FcεRI+

All cells were pre-gated as viable (using SYTOX viability dye) singlets.

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