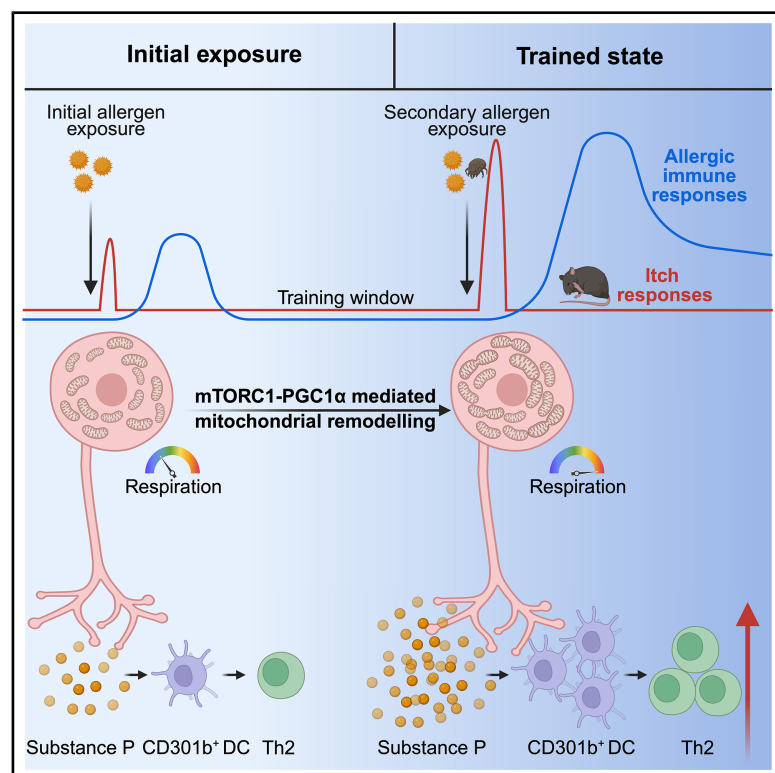


Immunity

Sensory neuronal mTORC1 kinase signaling establishes neuroimmune memory that initiates allergic immunity

Graphical abstract



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In brief

How cumulative environmental allergen exposures are encoded by peripheral sensory neurons to shape immune responsiveness is unclear. Zhu et al. show that protease allergens induce an mTORC1-dependent, metabolically trained state in sensory neurons that amplifies later neuroimmune responses to allergens and enables cross-sensitization to antigenically distinct allergens.

Highlights

- Protease allergen exposure induces a trained state in sensory neurons
- Trained neurons amplify itch, DC migration, and Th2 cell differentiation to allergens
- The trained neuronal state requires mTORC1-driven mitochondrial remodeling
- Neuronal training enables cross-sensitization to distinct protease allergens

Article

Sensory neuronal mTORC1 kinase signaling establishes neuroimmune memory that initiates allergic immunity

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SUMMARY

Environmental allergens are enriched in protease activity, which activates cutaneous sensory neurons, triggering itch and substance P release to promote migration of T helper (Th)2 cell-skewing CD301b⁺ dendritic cells and initiate allergic immunity. However, allergens are typically encountered through repeated sub-threshold exposures, and how these cumulatively induce sensitization is unknown. We identified a sensory neuron-intrinsic mechanism of neuroimmune memory. Protease allergen exposure induced sustained mechanistic target of rapamycin complex 1 (mTORC1) kinase signaling and transcriptional activator peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α)-associated mitochondrial remodeling in sensory neurons, establishing a metabolically primed state with enhanced neuronal responsiveness. Upon allergen re-exposure, this state drove enhanced itch, CD301b⁺ dendritic cell migration, and Th2 cell differentiation. Disrupting neuronal mTORC1 signaling or mitochondrial stability abrogated this amplification while sparing primary responses. This mechanism generalized across distinct protease allergens, revealing mTORC1-driven metabolic reprogramming in sensory neurons as a form of innate neuroimmune memory underlying allergen cross-sensitization and polysensitization.

INTRODUCTION

The type 2, or allergic, immune response is characterized by CD4⁺ T helper (Th)2 cell differentiation and immunoglobulin E (IgE) production and is triggered by diverse stimuli, including helminths, venoms, and environmental allergens.^{1–3} A unifying feature of many such stimuli is protease activity.^{4–6} We and others have shown that protease allergens are directly detected by transient receptor potential vanilloid subtype 1 (TRPV1)⁺ peptidergic sensory neurons in the skin, which elicit acute itch and release sub-

stance P (SP) to promote mast cell activation and migration of Th2 cell-skewing CD301b⁺ dendritic cells (DCs) to draining lymph nodes (dLNs), initiating allergic immunity.^{7–9} These findings position sensory neurons as critical sentinels of allergen detection. Yet, in both natural and experimental settings, allergens are typically encountered via recurrent low-dose exposures.^{10–13} How these individually subthreshold encounters cumulatively drive allergic sensitization remains unclear.

Neurons are among the most energetically demanding cells in the body, relying heavily on mitochondrial oxidative

phosphorylation (OXPHOS) to sustain excitability and neurotransmission.^{14,15} Sensory neurons, particularly unmyelinated C-fibers, are especially vulnerable to energetic stress given their long axons and high ATP demands.^{16,17} To meet these demands, they employ adaptive metabolic strategies, including mitochondrial remodeling, acquisition, and biogenesis.^{18–22} However, such adaptations can become maladaptive; in models of inflammatory pain, enhanced mitochondrial function drives persistent pain sensitization, termed hyperalgesic priming.²³ Whether similar metabolic priming occurs in response to allergen-induced itch remains unknown.

The mechanistic target of rapamycin complex 1 (mTORC1) integrates diverse environmental inputs to coordinate mitochondrial function, protein synthesis, and energy production.^{24,25} One key mechanism involves activation of the transcriptional co-activator peroxisome proliferator-activated receptor gamma co-activator-1 α (PGC-1 α), a master regulator of mitochondrial biogenesis. By upregulating PGC-1 α expression and activity, mTORC1 promotes mitochondrial biogenesis and enhances spare respiratory capacity (SRC), thereby enabling cells to meet increased energy demands.^{26,27} In neurons, mTORC1 supports axonal integrity and plasticity,^{25,28,29} whereas aberrant activation contributes to chronic pain.^{30–33} Given its position at the intersection of environmental sensing and neuronal plasticity, mTORC1 is well positioned to regulate how sensory neurons adapt to repeated environmental stimuli such as allergens.

Emerging evidence suggests that “trained immunity,” the long-term functional reprogramming of innate immune cells to enhance subsequent responsiveness, extends beyond traditional immune cells to include non-immune cell types.^{34–38} We reasoned that a similar principle might apply to sensory neurons, which directly detect protease allergens and exhibit activity-dependent plasticity influenced by their metabolic state.³⁹ We therefore hypothesized that recurrent low-dose protease allergen exposure would induce a neuron-intrinsic “memory” or trained state that gives rise to neuroimmune memory. We define neuroimmune memory as an emergent phenotype in which a neuron-intrinsic adaptation amplifies both sensory and immune responses upon allergen re-exposure. In this model, initial allergen exposure would lead to sustained mTORC1 signaling, driving mitochondrial adaptations and neuronal hyper-responsiveness, thereby amplifying allergic immune initiation upon re-exposure. Consistent with this hypothesis, we found that repeated exposure to the model protease allergen papain established a neuron-intrinsic state of neuroimmune memory, marked by augmented itch, enhanced migration of Th2 cell-skewing CD301b⁺ DCs to dLNs, and increased Th2 cell differentiation. This state arose independently of adaptive immune cells or systemic cues but required initial sensory neuron activation. Mechanistically, protease allergens induced sustained mTORC1 activation and PGC-1 α -associated mitochondrial remodeling in sensory neurons, conferring heightened responsiveness upon allergen re-exposure. Disruption of mTORC1 signaling or mitochondrial stability abrogated training without affecting primary responses. Notably, allergen-trained sensory neurons conferred heightened responses not only to the same allergen but also to antigenically distinct protease allergens. Together, these findings established mTORC1-driven metabolic reprogramming in sensory neurons as a mechanism

of neuroimmune memory, providing a mechanistic basis for allergen cross-sensitization and the progressive accumulation of allergen sensitivities observed in atopic disease.

RESULTS

Repeated allergen exposure amplifies sensory and immune responses upon re-exposure

To test whether repeated allergen exposure induced a memory-like neuroimmune response, we developed a repeated allergen exposure model adapted from protocols of hyperalgesic priming.^{40,41} Wild-type C57BL/6 mice were intradermally (i.d.) injected with allergens at a 7-day interval, allowing acute effects of the first injection to resolve and minimizing confounding inflammation at the injection site. Sensory neuronal activation was assessed by quantifying itch behavior in response to the model protease allergen papain, and immune responses were evaluated by DC migration and Th2 cell differentiation (Figure 1A). As expected, a single injection of papain, but not the inert protein ovalbumin (Ova), elicited acute scratching.⁸ Papain-induced scratching after an initial Ova injection was indistinguishable from that following a single papain injection, confirming that neither Ova nor the injection itself primed neuronal responses (Figure 1B). In contrast, mice exposed to papain on days 0 and 7 exhibited markedly enhanced scratching upon re-exposure (Figure 1B). This amplification required papain protease activity, as re-exposure with heat-inactivated papain (HIP) elicited no detectable scratching (Figure 1B). Thus, initial exposure to a proteolytically active allergen-primed sensory responses to subsequent exposure.

We next asked whether this enhanced sensory response was accompanied by amplified allergic immunity. In the skin, CD301b⁺ DCs drive Th2 cell responses, whereas CD103⁺ DCs include the XCR1⁺ subset that drives Th1 cell responses.^{42–45} Prior studies showed that allergen-activated sensory neurons, via local SP release, selectively promote the migration of activated PDL2⁺CD301b⁺ dermal DCs, but not CD103⁺ or XCR1⁺ DCs, from the skin to the dLNs.^{8,9} We therefore analyzed Th1 and Th2 cell-skewing DCs in the popliteal dLN after repeated allergen exposure (Figure S1A). Consistent with prior work, a single papain injection triggered migration of activated CD301b⁺ DCs, but not CD103⁺ DCs (Figures 1C and 1D).^{8,9} Repeated papain exposure further enhanced CD301b⁺ DC migration, while CD103⁺ DCs were minimally affected, indicating biased amplification of Th2 cell-skewing DC trafficking (Figures 1C and 1D). Because CD301b⁺ DC migration is essential for Th2 cell priming,^{43,46} we next examined T cell responses by flow cytometry (Figure S1B). Repeated papain exposure significantly increased interleukin (IL)-4⁺ and IL-13⁺ CD4⁺ T cells compared with Ova-injected controls, an effect largely lost upon re-exposure to HIP (Figures 1E and 1F). Both the frequency and total number of IL-4⁺ and IL-4⁺IL-13⁺ CD4⁺ T cells were reduced with HIP, whereas the frequency of IL-13⁺ single-positive CD4⁺ T cells remained comparable despite a reduction in total cell number. Thus, proteolytically active papain is required for optimal amplification of the type 2 immune response, and these findings show that repeated protease allergen exposure establishes a memory-like neuroimmune phenotype, characterized by enhanced itch, amplified CD301b⁺ DC migration, and augmented Th2 cell

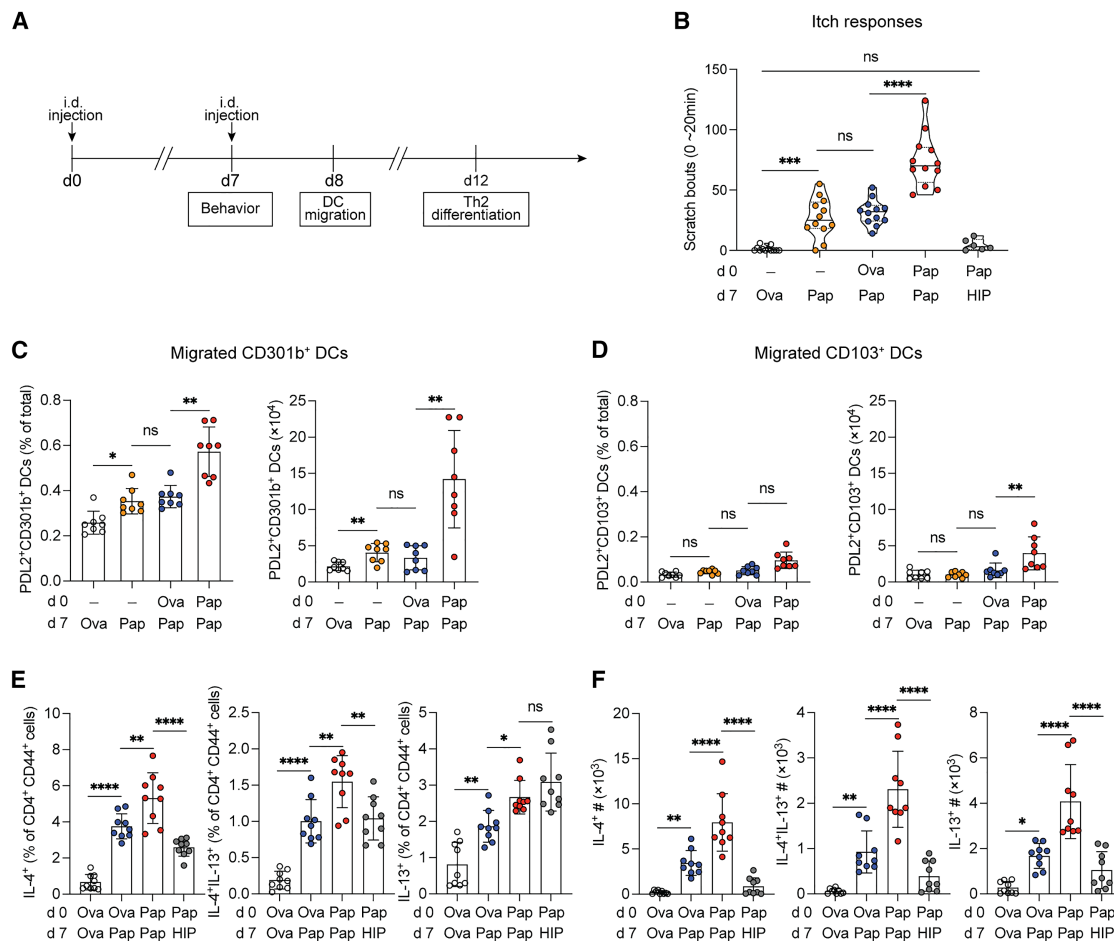


Figure 1. Repeated allergen exposure enhances sensory and immune responses

(A) Experimental design to assess scratching after i.d. cheek injection and dendritic cell (DC) migration and Th2 cell differentiation after i.d. footpad injection. (B) Scratching bouts following day 7 i.d. cheek challenge in wild-type (WT) mice treated with ovalbumin (Ova), papain (Pap), heat-inactivated papain (HIP), or no injection (–) on days 0 and 7 as indicated. (C and D) Frequency and number of PDL2⁺CD301b⁺ DCs (C) and PDL2⁺CD103⁺ DCs (D) in the popliteal draining lymph nodes (dLNs) on day 8. (E and F) Frequency (E) and number (F) of IL-4⁺, IL-4⁺IL-13⁺, and IL-13⁺ cells among CD4⁺CD44⁺ T cells in the popliteal dLNs on day 12. Statistical tests: one-way ANOVA with Tukey's multiple comparisons test (B, E, and F); Welch's ANOVA (C and D). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant. Data are pooled from three independent experiments in (B), except the Pap/HIP group, which was analyzed in two experiments, and from two independent experiments in (C)–(F). Each point represents an individual mouse. Violin plots show median and quartiles; bar graphs show mean ± SD.

See also Figure S1.

differentiation upon allergen re-exposure. Having established this allergen-induced memory-like phenotype, we next asked whether this phenomenon is intrinsic to neurons or involves systemic or adaptive immune factors.

Allergen-induced neuroimmune training is independent of adaptive lymphocytes

Sensory neurons and immune cells communicate bidirectionally during allergic inflammation. Epithelial- and immune-derived mediators, including thymic stromal lymphopoietin (TSLP), leukotriene C4 (LTC₄), and IL-31, directly trigger itch, whereas IL-3, IL-4, and IL-13 prime sensory neuron responsiveness.^{9,47–51} To assess whether systemic factors contributed to enhanced itch upon allergen re-exposure, we examined whether

neuroimmune training was site specific. Mice injected with papain into the right cheek on day 0 exhibited robust scratching upon ipsilateral re-exposure on day 7 (Figure 2A). However, contralateral re-exposure failed to augment scratching (Figure 2B), indicating that training was locally imprinted at the initially exposed site rather than mediated by circulating factors.

We next examined the role of adaptive lymphocytes. *Tcrα*^{–/–} mice, which lack αβ T cells, and *μMT*^{–/–} mice, which lack B cells, retained enhanced itch and CD301b⁺ DC migration upon allergen re-exposure (Figures 2C–2F). *Rag2*^{–/–} mice lack all T and B cells and have an impaired primary response to protease allergens because they also lack IL-3-producing γδ T cells that prime initial neuronal responses to allergens.⁹ As expected, *Rag2*^{–/–} mice exhibited a blunted primary response but retained

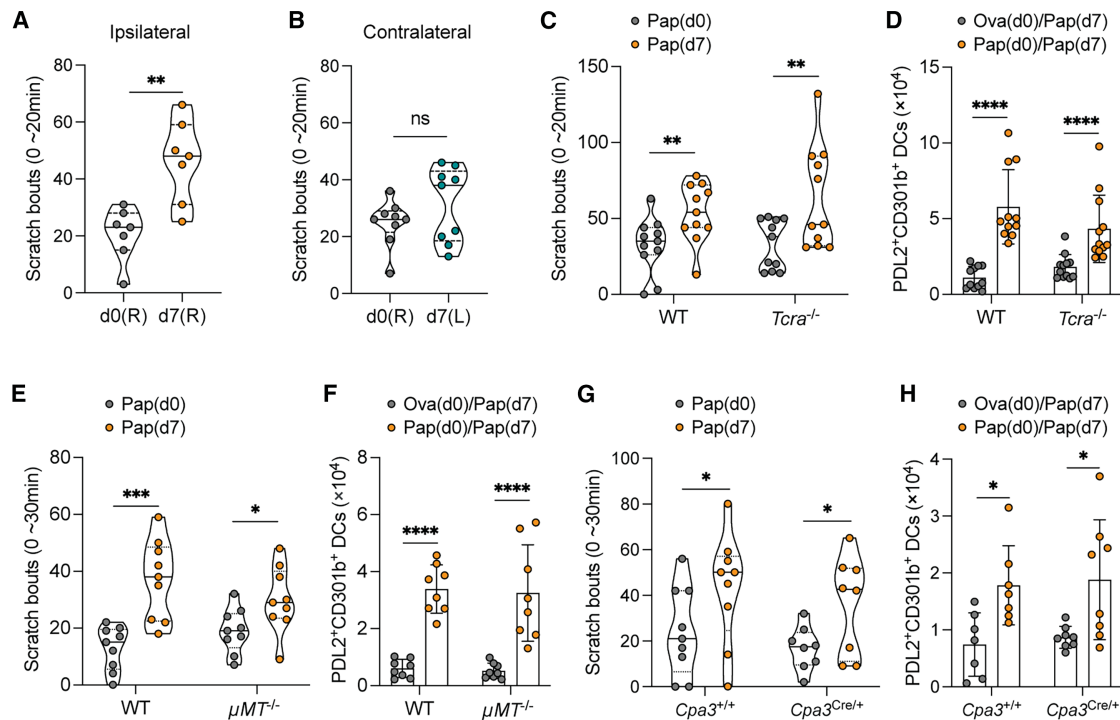


Figure 2. Allergen-induced amplification of neuroimmune responses occurs independently of lymphocytes, mast cells, and basophils

(A and B) Scratching bouts after i.d. papain (Pap) injection into right cheek on day 0 and either the right (ipsilateral; A) or left (contralateral; B) cheek on day 7. (C and D) Scratching bouts after i.d. cheek injection on days 0 and 7 (C) and numbers of PDL2⁺CD301b⁺ DCs in the popliteal dLN on day 8 after i.d. footpad injection (D) in WT and *Tcra*^{-/-} mice.

(E and F) Scratching bouts after i.d. cheek injection on days 0 and 7 (E) and numbers of PDL2⁺CD301b⁺ DCs in the popliteal dLN on day 8 after i.d. footpad injection (F) in WT and *μMT*^{-/-} mice.

(G and H) Scratching bouts after i.d. cheek injection on days 0 and 7 (G) and numbers of PDL2⁺CD301b⁺ DCs in the popliteal dLN on day 8 after i.d. footpad injection (H) in *Cpa3*^{+/+} or *Cpa3*^{Cre/+} littermates.

Statistical tests: Welch's *t* test (A and B); two-way ANOVA with Tukey's multiple comparisons test (C-H). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Data are pooled from two independent experiments in (A), (B), and (F)-(H), and three in (C)-(E). Each point represents one mouse. Violin plots show the median and quartiles; bar graphs show mean ± SD.

See also Figure S2.

enhanced scratching and DC migration upon re-exposure (Figures S2A and S2B), further indicating that training did not require adaptive immunity. Because mast cells and basophils mediate itch through both IgE- and Mas-related G protein-coupled receptor (MRGPR)-dependent pathways,^{49,52} we also assessed *Cpa3*^{Cre/+} mice, which lack both cell types.⁵³ Repeated papain exposure still enhanced scratching and CD301b⁺ DC migration in these mice (Figures 2G and 2H). Thus, allergen-induced amplification of sensory and immune responses is independent of adaptive lymphocytes, mast cells, basophils, and circulating mediators, supporting a locally encoded mechanism.

Allergen-induced neuroimmune training requires neuronal activation

Protease allergen-sensing neurons are enriched in their expression of the calcium channel TRPV1 and the sodium channel Nav1.8, encoded by *Scn10a*.^{8,9} Nav1.8 is expressed exclusively in sensory neurons, where it mediates depolarization and transmission of sensory stimuli, such as those detected by TRPV1. To determine whether sensory neuronal depolariza-

tion was required for the establishment of neuroimmune memory, we transiently blocked neuronal excitability during the initial allergen exposure using QX314, a lidocaine derivative that enters TRPV1- and transient receptor potential ankyrin 1 (TRPA1)-expressing neurons and blocks sodium channels.^{54,55} Mice initially injected with papain alone developed robust scratching upon re-exposure, whereas initial co-injection of QX314 abolished this augmentation (Figure 3A). Using Kaede transgenic mice to track migration of photoconverted (Kaede^{Red}) skin DCs,⁵⁶ we found that initial co-injection with QX314 similarly abrogated enhanced CD301b⁺ DC migration upon allergen re-exposure (Figure 3B). Initial co-injection of allergen with the selective Nav1.8 sodium channel blocker A-803467 likewise prevented enhanced scratching and CD301b⁺ DC migration upon re-exposure (Figures 3C and 3D).⁵⁷ We next asked whether neuronal activity was required during the secondary allergen challenge. Acute QX314 treatment during re-exposure markedly reduced scratching and abrogated enhanced CD301b⁺ DC migration (Figure 3A; Figure S3A). Together, these findings suggested that sensory neuronal activation was required both during initial allergen

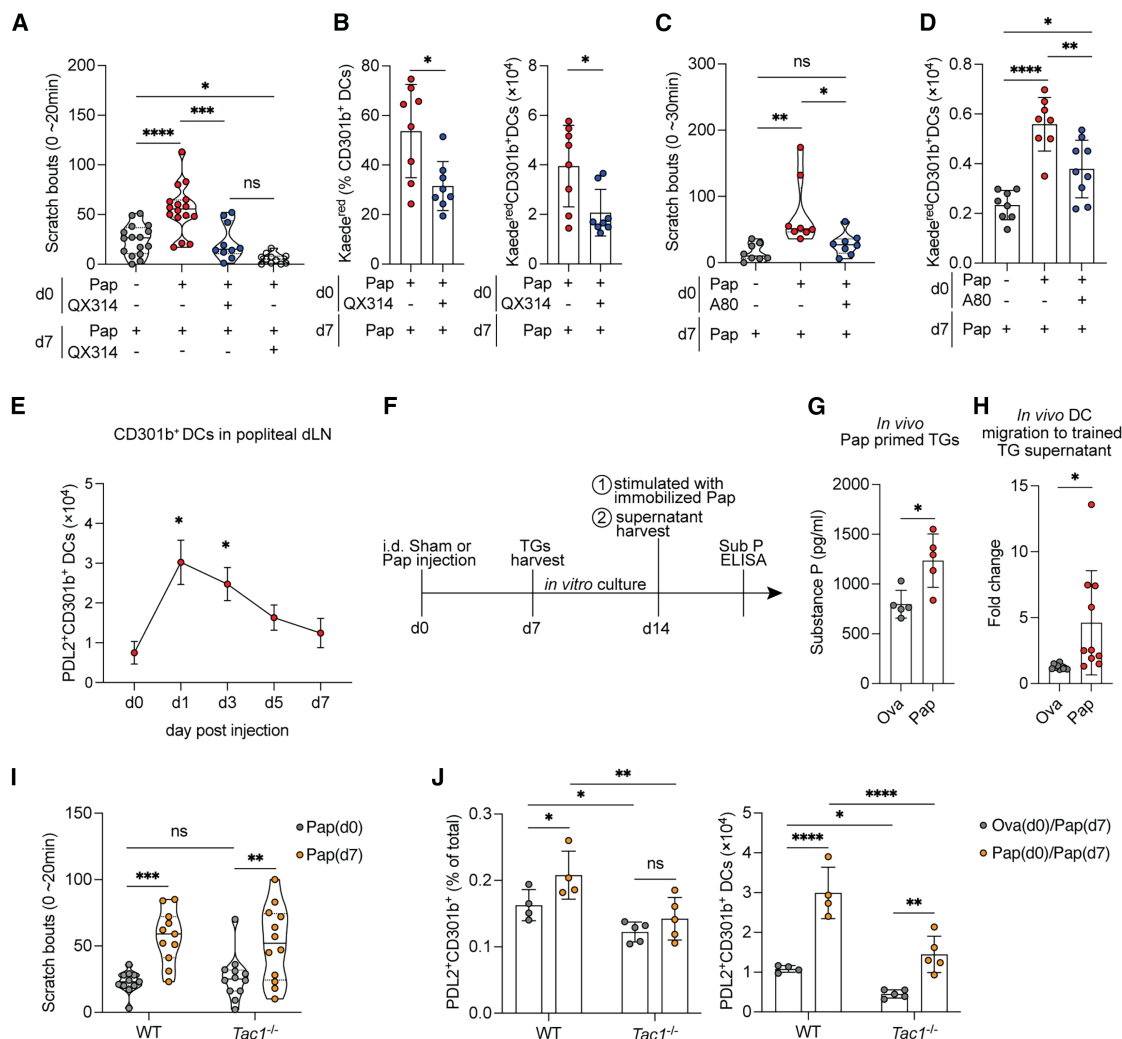


Figure 3. Allergen-induced neuroimmune training requires sensory neuronal activation and enhanced SP release

(A–D) WT mice (A and C) or Kaede mice (B and D) received Pap on days 0 and 7 as indicated. QX314 was co-administered with Pap in (A) and (B), and vehicle or A-803467 (A80) was administered intraperitoneal (i.p.) 4 h before day 0 treatment in (C) and (D). Kaede footpads were photoconverted immediately prior to day 0 injection. Day 7 scratching after cheek challenge (A and C) and frequency and number of Kaede^{Red} CD301b⁺ DCs in the popliteal dLN 22–24 h after footpad challenge (B and D) are shown.

(E) Kinetics of PDL2⁺CD301b⁺ DC accumulation in the popliteal dLNs after a single Pap footpad injection.

(F–H) Experimental design (F), substance P (SP) in supernatants (G), and fold change over media-only control in popliteal dLN Kaede^{Red}CD301b⁺ DC numbers after footpad injection of supernatants (H) from TG cultures of Ova- or Pap-primed mice restimulated *in vitro* with immobilized Pap.

(I and J) WT or *Tac1*^{-/-} mice received injections on days 0 and 7 as indicated. Scratching bouts after cheek injection (I) and frequency and number of PDL2⁺CD301b⁺ DCs in the popliteal dLNs 22–24 h after day 7 footpad challenge (J) are shown.

Statistical tests: one-way ANOVA with Tukey's multiple comparisons test (A and C–E); paired *t* test (B); unpaired Welch's *t* test (G and H); and two-way ANOVA with Tukey's multiple comparisons test (I and J). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Data are pooled from four independent experiments in (A), two in (B)–(E), and three in (H) and (I). Data in (G) and (J) are representative of four and two independent experiments, respectively. Each point represents one mouse. Violin plots show median and quartiles; bar and line graphs show mean ± SD.

See also Figure S3.

exposure to establish training and during re-exposure to express amplified sensory and immune responses.

We next investigated how repeated allergen exposure amplified CD301b⁺ DC migration. Flow cytometric analysis showed that CD301b⁺ DC numbers in the dLNs returned to baseline within days of initial allergen exposure (Figure 3E), ruling out DC retention as a cause of increased accumulation in the lymph node. Instead, trigeminal ganglia (TG) neurons from papain-in-

jected mice released significantly more SP than naive controls when restimulated *in vitro* with immobilized papain (Figures 3F and 3G), suggesting that prior allergen exposure enhanced neuropeptide release upon re-exposure. To determine whether neuron-derived factors were sufficient to augment DC migration, we applied supernatants from papain-restimulated TG cultures to the photoconverted skin of Kaede mice. Supernatants from papain-exposed neurons induced greater CD301b⁺ DC

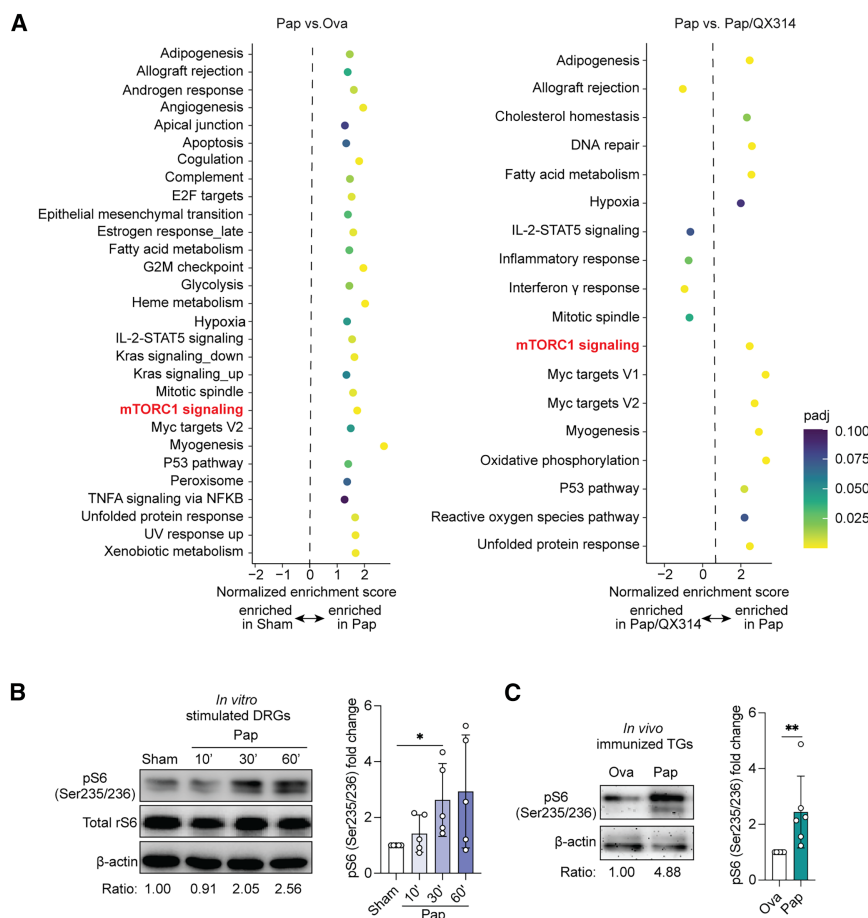


Figure 4. Protease allergen-stimulated sensory neurons exhibit mTORC1 activation

(A) Gene set enrichment analysis (GSEA) of hallmark pathways in pooled TG neurons 7 days after i.d. cheek injection of Ova, Pap, or Pap with QX314 (Pap/QX314).

(B and C) Representative immunoblots and quantification of pS6 (Ser235/236) levels in dorsal root ganglia (DRG) neurons stimulated with Pap *in vitro* for the indicated times (B) and TG neurons isolated from WT mice 7 days after Pap cheek injection (C). pS6 was normalized to β -actin.

Statistical tests: two-tailed ratio paired *t* test (B and C). **p* < 0.05; ***p* < 0.01. Bulk RNA-seq (A) was performed with three independent replicates per group. Each point in (B) and (C) represents an independent experiment. Bar plots show mean $\pm$ S.D. See also Figure S4 and Tables S1 and S2.

set enrichment analysis (GSEA) identified upregulation of metabolic pathways, including fatty acid metabolism and mTORC1 signaling, after papain exposure (Figure 4A; Figure S4C). Papain co-administration with QX314 attenuated these signatures (Figure 4A), indicating that neuronal activation was required for mTORC1 pathway induction.

mTORC1 activity is commonly assessed by phosphorylation of ribosomal protein S6 (pS6).^{24,25} Guided by our transcriptomic results, we examined pS6 in primary dorsal root ganglia (DRG) neurons stimulated with papain *in vitro*. Papain induced robust pS6 within 30 min (Figure 4B), which was abolished by the mTORC1 inhibitor sirolimus (rapamycin) (Figure S4D), confirming mTORC1-dependent S6 phosphorylation. *In vivo* cheek administration of papain produced an approximately 2-fold increase in pS6 that persisted in TG neurons 7 days after exposure (Figure 4C). Thus, protease allergen stimulation induces sustained mTORC1 signaling in sensory neurons, providing a potential link between initial neuronal activation and establishment of the trained neuronal state.

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Sensory neuronal mTORC1 activation is required for allergen-induced neuroimmune memory

Previous studies have shown that mTORC1 supports neuronal plasticity,^{25,28} which can have pathologic outcomes in chronic pain by promoting neuronal responses to non-painful stimuli.^{30–33} Based on these observations, we hypothesized that sustained mTORC1 activation in sensory neurons mediated allergen-induced neuroimmune memory. To test this, we used two structurally distinct mTORC1-specific inhibitors, sirolimus (rapamycin) and temsirolimus (CCI-779 or Torisel). In contrast to a previous report examining itch responses to chemical pruritogens,⁵⁸ neither inhibitor affected acute papain-induced scratching or CD301b⁺ DC migration on day 0 (Figures 5A and 5B; Figures S5A and S5B). However, both abrogated the

migration to dLNs than those from Ova-exposed neurons (Figure 3H). We then assessed the functional contribution of SP using *Tac1*^{−/−} mice, which lack SP, neurokinin A, neuropeptide K, and neuropeptide γ . *Tac1* deficiency did not impair enhanced scratching upon allergen re-exposure (Figure 3I), indicating that amplified itch was largely SP-independent. In contrast, enhanced CD301b⁺ DC migration was markedly reduced in *Tac1*^{−/−} mice (Figure 3J). Consistent with this, glutamine-tryptophan-phenylalanine (QWF), a broad antagonist of SP-responsive receptors including TACR1, TACR2, MRGPRB2, and MRGPA1, attenuated amplified CD301b⁺ DC migration during secondary exposure (Figure S3B). Together, these findings show that allergen-induced neuroimmune training requires sensory neuronal activation and primes neurons for enhanced SP release upon re-exposure, thereby amplifying Th2 cell-skewing DC migration, whereas enhanced itch proceeds through SP-independent pathways.

Protease allergen stimulation induces sustained mTORC1 activation in sensory neurons

To define sensory neuron-intrinsic mechanisms underlying allergen-induced neuroimmune training, we performed bulk RNA sequencing (RNA-seq) on pooled TGs from wild-type mice 7 days after i.d. exposure to Ova, papain, or papain plus QX314. Although overall transcriptional differences were minimal at this time point (Tables S1 and S2; Figures S4A and S4B), gene

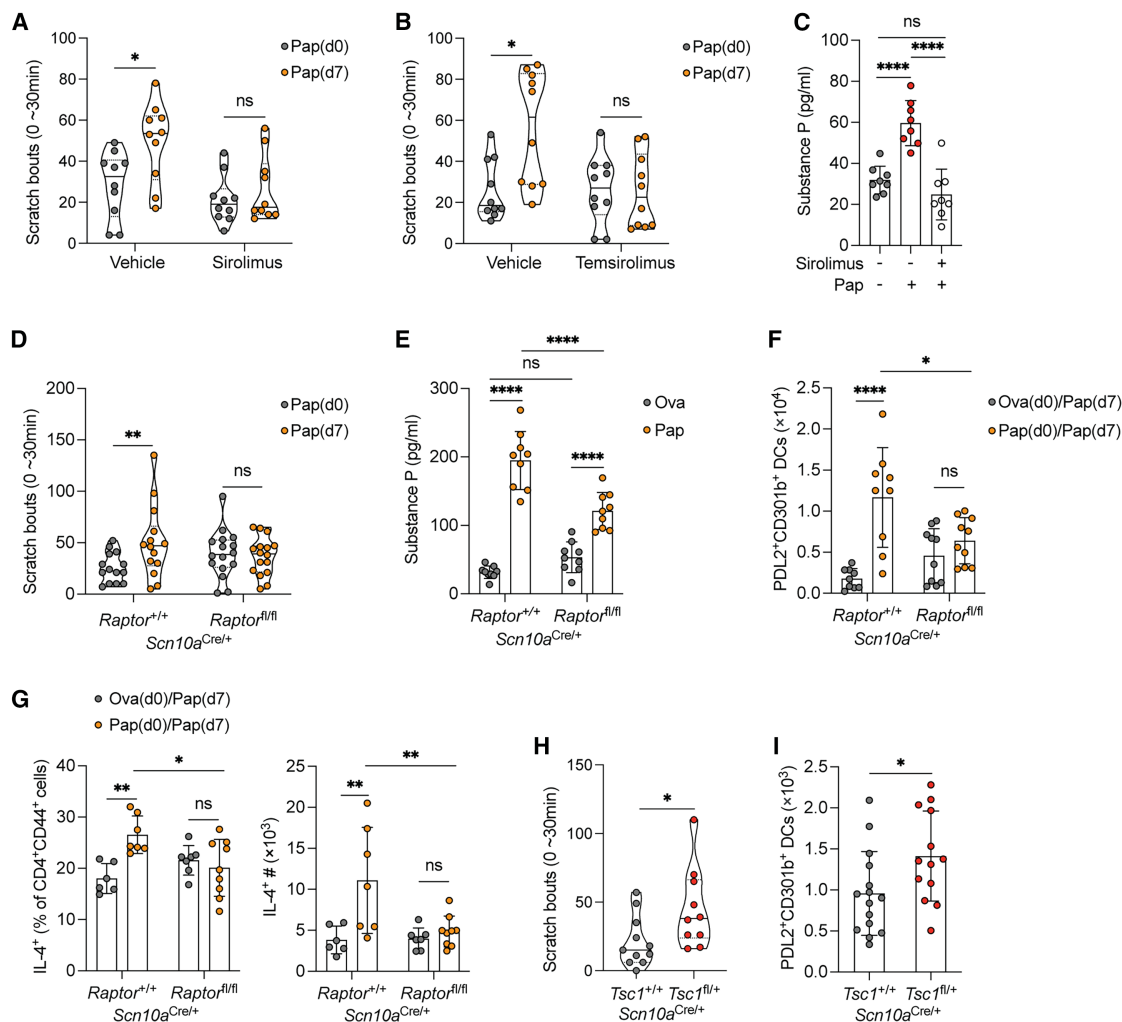


Figure 5. Sensory neuronal mTORC1 activation is required for allergen-induced neuroimmune memory

(A and B) WT mice were treated daily with vehicle, sirolimus (A), or temsirolimus (B) on days 0–7, with dosing 2 h before i.d. Pap cheek injection on days 0 and 7. Scratching bouts are shown.

(C) SP in supernatants of TG cultures stimulated with immobilized Pap with or without sirolimus.

(D–G) *Scn10a^{Cre/+}Raptor^{+/+}* and *Scn10a^{Cre/+}Raptor^{fl/fl}* mice were treated as indicated. Scratching bouts after cheek injection on days 0 and 7 (D), SP from Pap-restimulated TG cultures 7 days after Ova or Pap priming (E), PDL2⁺CD301b⁺ DC numbers in popliteal dLNs 22–24 h after day 7 footpad challenge (F), and frequency and number of IL-4⁺ cells among CD4⁺CD44⁺ T cells in the dLN on day 12 (G) are shown.

(H and I) Scratching bouts after a single Pap cheek injection (H) and popliteal dLN PDL2⁺CD301b⁺ DC numbers after a single Pap footpad injection (I) in *Scn10a^{Cre/+}Tsc1^{+/+}* and *Scn10a^{Cre/+}Tsc1^{fl/fl}* mice.

Statistical tests: two-way ANOVA with Tukey's multiple comparisons test (A, B, and D–G); one-way ANOVA with Tukey's multiple comparisons test (C); Welch's *t* test (H and I). **p* < 0.05; ***p* < 0.01; *****p* < 0.0001; ns, not significant. Data are pooled from three independent experiments in (A), (B), (D), (F), (H), and (I) and two independent experiments in (C), (E), and (G). Each point represents an individual biological replicate. Violin plots show median and quartiles; bar graphs show mean ± SD.

See also Figure S5.

enhanced scratching and CD301b⁺ DC migration upon papain re-exposure on day 7 (Figures 5A and 5B; Figures S5A and S5B). Consistent with a role for mTORC1 in regulating neuronal output, sirolimus also significantly reduced SP release from papain-primed TG neurons (Figure 5C), suggesting that impaired SP release contributed to reduced DC migration upon mTORC1 inhibition. Thus, mTORC1 was dispensable for acute responses but was required for the trained state.

To confirm these findings genetically, we deleted *Raptor*, an essential component of the mTORC1 complex,⁵⁹ in Nav1.8⁺ sen-

sory neurons (*Scn10a^{Cre/+}Raptor^{fl/fl}*). These mice exhibited normal itch, SP release, CD301b⁺ DC migration, and Th2 cell differentiation upon initial papain exposure and intact cutaneous innervation compared with littermate controls (Figures 5D–5G; Figures S5C and S5D). In contrast, enhanced scratching, CD301b⁺ DC migration, and Th2 cell differentiation upon re-exposure were absent in *Scn10a^{Cre/+}Raptor^{fl/fl}* mice (Figures 5D, 5F, and 5G). To examine the underlying neuronal mechanism, we assessed neuropeptide release. SP release from papain-primed TG neurons was significantly reduced in

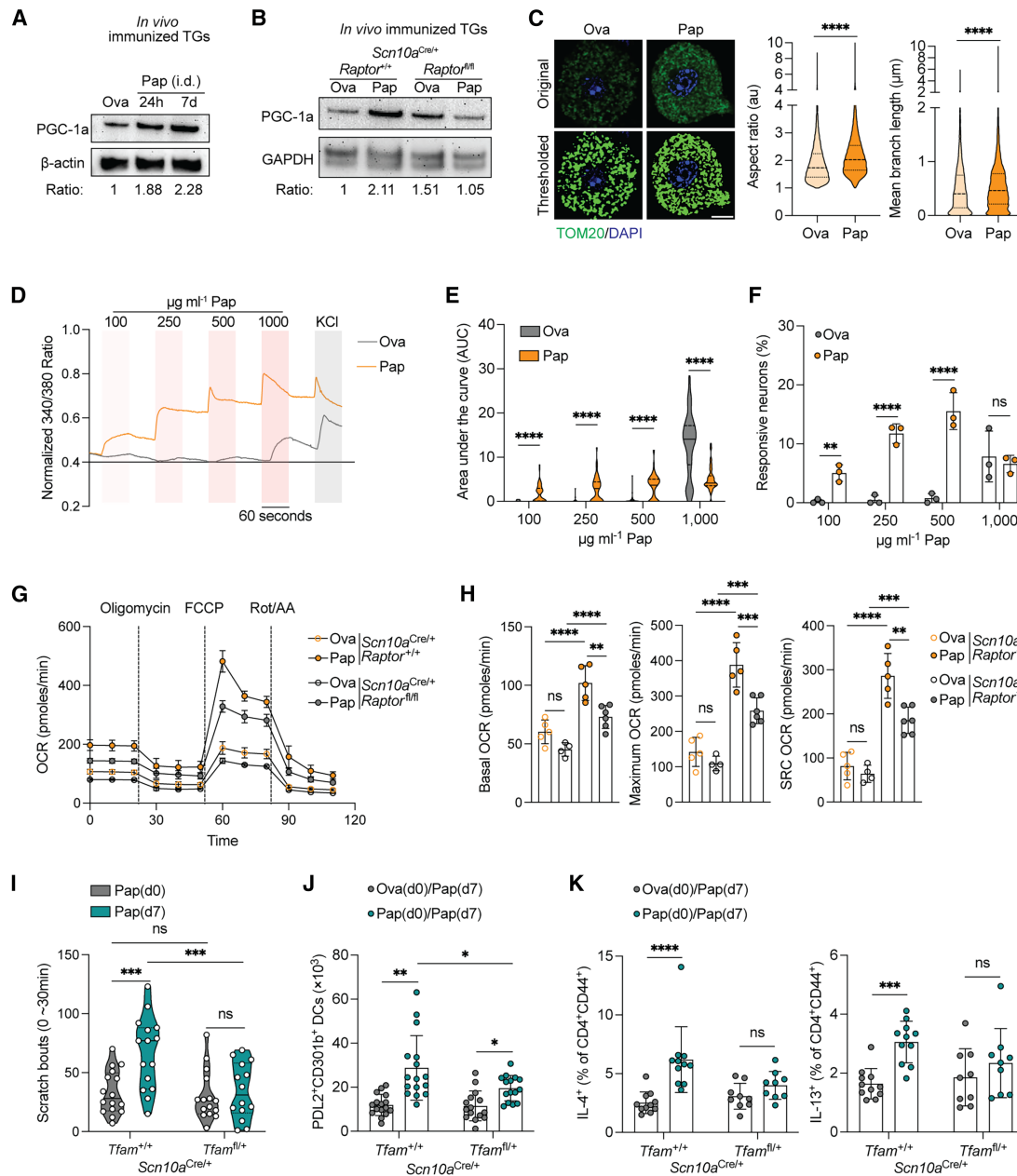


Figure 6. mTORC1 promotes mitochondrial function required for allergen-induced neuroimmune memory

(A and B) Representative immunoblots of PGC-1α in TG neurons from WT mice 24 h or 7 days after i.d. cheek injection of Ova or Pap (A) and from *Scn10a^{Cre/+}Raptor^{+/+}* and *Scn10a^{Cre/+}Raptor^{fl/fl}* mice 24 h after injection (B). PGC-1α/β-actin (A) and PGC-1α/GAPDH (B) ratios are shown.

(C) Representative TOM20 staining and thresholded images of TG neurons isolated 7 days after Ova or Pap injection. Mitochondrial aspect ratio and mean branch length were quantified using the Mitochondrial Analyzer ImageJ plugin (Ova, 170 neurons; Pap, 194 neurons). Scale bars indicate 5 μm.

(D–F) TG neurons isolated 7 days after Ova or Pap injection were stimulated with increasing Pap concentrations followed by KCl. Representative calcium traces (D), area under the curve (AUC) (E), and frequencies of responsive neurons (F) are shown.

(G and H) Oxygen consumption rate (OCR) profiles of TG neurons from *Scn10a^{Cre/+}Raptor^{+/+}* and *Scn10a^{Cre/+}Raptor^{fl/fl}* mice 7 days after Ova or Pap injection (G), with basal and maximal OCR and spare respiratory capacity (SRC) quantified in (H).

(I–K) *Scn10a^{Cre/+}Tfam^{+/+}* and *Scn10a^{Cre/+}Tfam^{fl/fl}* mice received injections on days 0 and 7 as indicated. Scratching bouts after cheek injection (I), number of PDL2⁺CD301b⁺ DCs in the popliteal dLNs after footpad injection (J), and frequencies of IL-4⁺ and IL-13⁺ cells among CD4⁺CD44⁺ T cells in popliteal dLNs (K) are shown.

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Raptor-deficient mice, while baseline SP release remained intact (Figure 5E). Consistent with the established role of SP in promoting CD301b⁺ DC migration, this reduction was accompanied by impaired CD301b⁺ DC migration and Th2 cell differentiation upon allergen re-exposure (Figures 5F and 5G), linking mTORC1-dependent neuronal output to downstream immune amplification. Conversely, persistent sensory neuronal mTORC1 activation through heterozygous deletion of the mTORC1 inhibitor *Tsc1* in Nav1.8⁺ neurons (*Scn10a^{Cre/+}Tsc1^{fl/+}* mice)⁶⁰ was sufficient to enhance scratching and CD301b⁺ DC migration after a single papain exposure (Figures 5H and 5I), phenocopying a trained wild-type response. Together, these results demonstrated that sensory neuron-intrinsic mTORC1 signaling was both necessary and sufficient to establish allergen-induced neuroimmune memory.

mTOR signaling is mediated through two functionally distinct complexes, mTORC1 and mTORC2 (mammalian target of rapamycin complex 2).⁶¹ *Rictor*, an essential component of mTORC2, has also been implicated in neuronal development and function.^{24,29,62} Because prolonged sirolimus exposure can indirectly inhibit mTORC2,⁶³ we next assessed whether mTORC2 contributed to training. Papain induced only a transient increase in phosphorylated Akt (Ser473), a canonical mTORC2 target,⁶⁴ in cultured DRG neurons (Figure S5E). Moreover, conditional deletion of *Rictor* in Nav1.8⁺ neurons (*Scn10a^{Cre/+}Rictor^{fl/fl}*) had no effect on acute or memory responses (Figures S5F and S5G). Collectively, these findings establish that sensory neuronal mTORC1, but not mTORC2, is required for the long-term adaptations that amplify allergic reactivity.

mTORC1 drives mitochondrial remodeling and metabolic reprogramming to promote neuroimmune memory

To investigate how mTORC1 supports neuroimmune memory, we focused on PGC-1 α , a transcriptional coactivator that regulates mitochondrial biogenesis and oxidative metabolism.⁶⁵ Because mTORC1 directly regulates PGC-1 α expression and activity to enable metabolic adaptation to environmental and energetic stress,⁶⁶ we asked whether allergen exposure induced this axis in sensory neurons. Initial papain exposure increased PGC-1 α protein in TG neurons within 24 h, and this increase persisted for at least 7 days (Figure 6A; Figure S6A). Sirolimus partially reduced PGC-1 α induction (Figure S6B), and induction was markedly impaired in papain-primed TG neurons from *Scn10a^{Cre/+}Raptor^{fl/fl}* mice (Figure 6B; Figure S6C), establishing that allergen-induced PGC-1 α upregulation required sensory neuronal mTORC1 signaling *in vivo*.

PGC-1 α promotes mitochondrial biogenesis and network connectivity, features associated with enhanced oxidative metabolism.^{26,27,65} Consistent with this, TG neurons from papain-injected mice exhibited increased mitochondrial DNA (mtDNA) copy number compared with Ova controls (Figure S6D). Immunostaining for the mitochondrial outer membrane marker translo-

case of outer membrane-20 (TOM20) revealed enlarged, elongated, and more complex mitochondria, reflected by increased aspect ratio (length), form factor (length and branching), and mean branch length (Figure 6C; Figures S6E and S6F).^{67–69} We next asked whether these adaptations were accompanied by altered neuronal responsiveness. Calcium imaging showed that TG neurons from papain-exposed mice exhibited greater calcium influx, increased area under the curve (AUC), and a higher proportion of responsive neurons following low-dose papain restimulation *in vitro* (Figures 6D–6F). Papain-primed neurons responded robustly to papain concentrations that elicited minimal responses from Ova-primed neurons, indicating a reduced activation threshold. Thus, allergen exposure induced mitochondrial remodeling accompanied by enhanced neuronal sensitivity and responsiveness to allergen restimulation.

We next asked whether this remodeling enhanced mitochondrial respiration. Hyperalgesic priming has been linked to enhanced mitochondrial activity, characterized by increased SRC and elevated mitochondrial oxidized/reduced nicotinamide adenine dinucleotide (NAD⁺/NADH) ratios.²³ 7 days after i.d. papain exposure, TG neurons exhibited increased basal and maximal oxygen consumption rates (OCRs), SRC, and ATP production compared with Ova controls (Figures S6G and S6H). In contrast, extracellular acidification rate (ECAR) remained largely unchanged (Figure S6I), indicating preferential enhancement of OXPHOS rather than glycolysis. TG neurons from *Scn10a^{Cre/+}Raptor^{fl/fl}* mice exhibited defective allergen-induced OXPHOS enhancement, despite maintaining baseline respiration and glycolysis (Figures 6G and 6H; Figures S6J and S6K). Thus, mTORC1 was required for allergen-induced enhancement of mitochondrial respiratory capacity.

To test whether mitochondrial integrity was required for training, we examined *Scn10a^{Cre/+}Tfam^{fl/+}* mice. *Tfam* is a nuclear-encoded mitochondrial transcription factor required for mtDNA replication, and its haploinsufficiency reduces mitochondrial content and stability.^{70–72} *Scn10a^{Cre/+}Tfam^{fl/+}* mice, in which *Tfam* haploinsufficiency is restricted to Nav1.8⁺ sensory neurons, exhibited normal acute itch, CD301b⁺ DC migration, and Th2 cell differentiation after initial papain exposure (Figures 6I–6K). Cutaneous innervation and baseline SP release were also preserved (Figures S7A–S7C), indicating intact baseline neuronal structure and function. However, *Scn10a^{Cre/+}Tfam^{fl/+}* mice failed to develop enhanced scratching upon allergen re-exposure (Figure 6I), and SP release from papain-primed TG neurons was significantly reduced (Figure S7C). Consistent with the role of SP in promoting DC migration, CD301b⁺ DC migration and Th2 cell differentiation were also impaired upon re-exposure (Figures 6J and 6K). In line with these functional defects, *Tfam*-haploinsufficient TG neurons failed to increase basal or maximal OCR or SRC after allergen exposure while maintaining baseline metabolic activity (Figures S7D–S7F). Thus, mitochondrial stability and inducible respiratory

Statistical tests: Welch's *t* test (C); two-way ANOVA with Tukey's multiple comparisons test (E, F, and I–K); one-way ANOVA with Tukey's multiple comparisons test (H). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Data are representative of at least three independent experiments (A, B, and D) or pooled from three (C, E, F, and I–K) or two (G and H) independent experiments. Each symbol represents an individual neuron (C and E), an independent experiment (F), or individual biological replicates (H–K). Violin plots show median and quartiles; bar and line graphs show mean $\pm$ SD. See also Figures S6 and S7.

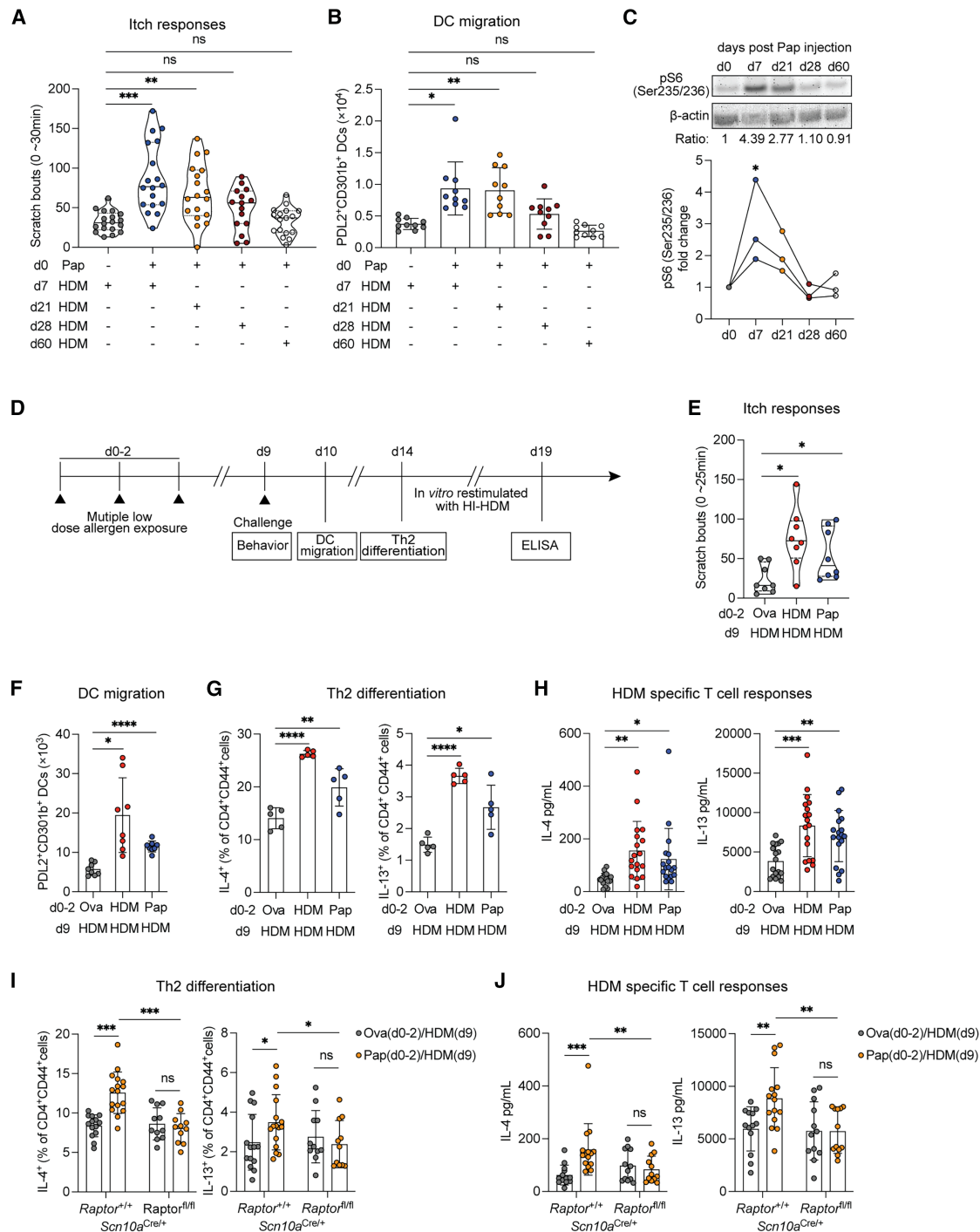


Figure 7. Allergen-induced neuroimmune memory promotes responsiveness to recurrent allergen exposure in an antigen-independent manner

(A and B) WT mice were primed with Pap on day 0 and challenged with house dust mite (HDM) on the indicated day. Scratching after cheek injection (A) and PDL2⁺CD301b⁺ DC numbers in the dLN 22–24 h after secondary footpad injections (B) are shown.

(C) Representative immunoblot and quantification of pS6 (Ser235/236) in TG neurons isolated at the indicated days after Pap cheek injection. pS6 was normalized to β -actin and expressed relative to baseline.

(D–H) WT mice received low-dose Ova, HDM, or Pap on days 0–2 and HDM challenge on day 9. Experimental design (D), scratching bouts (E), dLN PDL2⁺CD301b⁺ DC numbers (F), and frequencies of IL-4⁺ and IL-13⁺ cells among CD4⁺CD44⁺ T cells (G) are shown. (H) IL-4 and IL-13 production after heat-inactivated HDM (HI-HDM) restimulation of day 14 dLN cells.

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remodeling were required for sensory neuronal training and downstream immune activation.

Finally, we asked whether neuronal activation alone was sufficient to induce training. Capsaicin exposure before papain challenge failed to enhance scratching or DC migration upon allergen re-exposure (Figures S7G and S7H), indicating that TRPV1-mediated neuronal activation alone was insufficient to induce the trained state. Together, these findings demonstrate that allergen-induced mTORC1-PGC-1 α signaling promotes mitochondrial remodeling and oxidative metabolism in sensory neurons, lowering the activation threshold of sensory neurons and enhancing SP release. Mitochondrial integrity is therefore required to establish the neuronal memory state that amplifies downstream neuroimmune responses.

Allergen-induced neuroimmune training enables antigen-independent cross-sensitization

Our findings suggested that mTORC1-mediated neuroimmune training, characterized by augmented itch, enhanced CD301b⁺ DC migration, and increased Th2 cell differentiation, depended on the protease activity of allergens rather than antigen recognition. We therefore hypothesized that antigenically distinct protease allergens could cross-sensitize neuroimmune responses. We first assessed the durability of this trained response by challenging mice with house dust mite (HDM) at increasing intervals after initial papain exposure. Enhanced itch and DC migration upon protease allergen re-exposure persisted for at least 21 days but were no longer evident by day 28 (Figures 7A and 7B; Figure S8A). Consistent with this functional resolution, neuronal mTORC1 activation, assessed by pS6 (Ser235/236), returned to near-baseline levels by day 28 (Figure 7C). Thus, the allergen-induced neuronal state persisted within a defined temporal window rather than indefinitely and extended to other protease allergens.

We next tested whether recurrent low-dose allergen administration could promote cross-sensitization between papain, a plant-derived cysteine protease, and antigenically unrelated HDM, which contains the cysteine protease Der p 1.⁴ Mice received low-dose papain, HDM, or Ova as a sham injection on days 0–2, followed by HDM challenge on day 9 (Figure 7D). As expected, repeated HDM exposure enhanced scratching, activated CD301b⁺ DC migration, and Th2 cell differentiation relative to Ova controls (Figures 7E–7G). Despite lacking antigenic overlap with HDM, low-dose papain priming produced comparable enhancement of these responses (Figures 7E–7G). To confirm that this reflected enhanced HDM-specific adaptive immunity, we restimulated lymph node cultures with heat-inactivated HDM (HI-HDM) and found that both HDM and papain priming elicited equivalent HDM-specific IL-4 and IL-13 production (Figure 7H). We next exam-

ined whether cross-sensitization extended beyond allergens with shared cysteine protease activity using *Alternaria alternata* (*A. alternata*), a clinically relevant allergen enriched in serine proteases.⁴ Papain priming similarly enhanced scratching, CD301b⁺ DC migration, and Th2 cell differentiation after *A. alternata* challenge (Figures S8E–S8G). Thus, protease-mediated neuronal reprogramming was not restricted to a single class of protease allergens but instead represented a broader mechanism by which distinct protease-containing allergens could cross-sensitize neuroimmune responses.

We next tested whether sensory neuronal mTORC1 was required for this papain-to-HDM cross-sensitization. Sirolimus treatment during papain priming reduced the enhanced Th2 cell polarization and HDM-specific cytokine production after HDM challenge (Figures S8I and S8J). However, because mTORC1 directly regulates CD4⁺ T cell priming and function, this pharmacologic approach could not distinguish neuron-intrinsic from broader immunologic effects.⁷³ We therefore examined sensory neuron-specific Raptor-deficient mice (*Scn10a^{Cre/+}Raptor^{fl/fl}*). In this model, papain priming enhanced total Th2 cell differentiation and HDM-specific T cell responses in littermate controls, but not in *Scn10a^{Cre/+}Raptor^{fl/fl}* mice (Figures 7I and 7J), establishing that sensory neuronal mTORC1 was required to enhance adaptive immune responses to a distinct allergen. Likewise, papain-primed *Scn10a^{Cre/+}Tfam^{fl/+}* mice failed to mount enhanced total or HDM-specific Th2 cell responses after HDM challenge (Figures S8K and S8L). Together, these findings demonstrate that mTORC1-driven mitochondrial adaptation in sensory neurons underlies cross-sensitization between antigenically distinct protease allergens, providing a neuronal mechanism for the progressive accumulation of allergen sensitivities characteristic of atopic disease.

DISCUSSION

Environmental allergens are most often encountered through repeated low-dose exposures, yet how these individually sub-threshold encounters cumulatively trigger allergic immunity remains unclear. Here, we identified a sensory neuron-intrinsic mechanism of neuroimmune memory, in which protease allergen exposure induced sustained mTORC1 activation and mitochondrial remodeling. This trained state increased neuronal sensitivity to allergen restimulation, amplifying itch, CD301b⁺ DC migration, and Th2 cell priming upon re-exposure. Training persisted for weeks but was not permanent, defining a temporally restricted window of heightened responsiveness. Because it generalized across antigenically distinct protease allergens, this mechanism provides a potential neuronal basis for polysensitization in atopic disease and places sensory neurons alongside innate immune cells as loci of trained immunity.

(I and J) *Scn10a^{Cre/+}Raptor^{fl/fl}* and *Scn10a^{Cre/+}Raptor^{fl/fl}* mice were treated as in (D). Frequencies of IL-4⁺ and IL-13⁺ cells among CD4⁺CD44⁺ T cells (I) and HI-HDM restimulated IL-4 and IL-13 production (J) are shown.

Statistical tests: Welch's ANOVA (A, B, E, and H); one-way ANOVA with Dunnett's multiple comparisons test (C) or Tukey's multiple comparisons test (F and G); two-way ANOVA with Tukey's multiple comparisons test (I and J). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Data are pooled from three independent experiments (A and H–J) or two independent experiments (B and E). Data in (F) and (G) are representative of three independent experiments. In (C), the immunoblot is representative of three independent experiments, each shown in the graph. Each symbol represents an individual mouse except in (C), where each line represents an independent experiment. Violin plots show median and quartiles; bar graphs show mean $\pm$ SD.

See also Figure S8.

Analogous to hyperalgesic priming in pain pathways, allergen exposure imprinted sensory neurons with heightened excitability that persisted beyond the initial encounter.^{23,74} This neuroadaptive response required protease activity, as inert Ova did not induce training, but the relevant sensors of protease activity remain undefined. Protease-activated receptors (PARs), such as PAR1, have been implicated in neuronal detection of bacterial serine proteases and may contribute to atopic dermatitis pathogenesis.⁷⁵ The HDM cysteine protease Der p 1 cleaves PAR2 to generate a non-canonical peptide that may activate PAR2 and the MRGPR family of receptors.^{76,77} However, PAR2 is dispensable for sensory neuronal activation in response to HDM.⁷ Alternatively, protease-induced stress caused by membrane protein cleavage, ion influx through membrane damage, or endoplasmic reticulum (ER) stress may activate mTORC1. Although chronic ER stress canonically inhibits mTORC1 activity, acute ER stress can activate mTORC1 via activating transcription factor 6 (ATF6).⁷⁸ Such mechanisms could initiate mTORC1 signaling, but how activity is sustained remains unclear. Persistent metabolic remodeling within sensory neurons, epigenetic stabilization of that state, or longer-lived tissue-derived signals from surrounding cells, such as satellite glial cells, may contribute.⁷⁹ Defining the pathways that initiate and maintain neuronal training will be an important next step.

Allergen-induced neuroimmune training was neuron-intrinsic and independent of adaptive lymphocytes, mast cells, basophils, and circulating mediators. Although tissue-derived inflammatory signals may further modulate neuronal responsiveness *in vivo*, several findings supported sensory neurons as the primary cellular substrate of the trained state. Acute neuronal silencing during initial allergen exposure prevented training, and selective disruption of mTORC1 signaling in Nav1.8⁺ nociceptive neurons abolished amplified responses upon re-exposure while sparing primary responses. Conversely, persistent neuronal mTORC1 activation was sufficient to enhance neuroimmune responsiveness. These findings identify mTORC1 as a molecular switch determining whether allergen encounters are “remembered” by sensory neurons.

Mechanistically, this mTORC1-dependent trained state acted at least in part through PGC-1 α -associated mitochondrial remodeling, which increased OXPHOS capacity without altering glycolysis. Allergen-primed neurons exhibited larger and more interconnected mitochondria, increased oxygen consumption, enhanced reserve capacity, and heightened calcium responses upon restimulation. *Tfam* haploinsufficiency abolished training while sparing acute responses, demonstrating that mitochondrial integrity was required for the trained state. Enhanced SP release likewise depended on mTORC1 signaling and mitochondrial integrity, and selectively mediated amplified CD301b⁺ DC migration, but not enhanced itch. Thus, mTORC1-driven mitochondrial adaptation coupled heightened neuronal responsiveness to amplified immune activation. Other neuron-derived mediators may also contribute, and whether increased mitochondrial output facilitates vesicular release through ATP availability, calcium handling, or purinergic signaling remains to be determined.

This pathway generalized across allergens. Papain priming enhanced neuroimmune responses to antigenically distinct HDM and *A. alternata*, establishing a protease-driven mechanism

of cross-sensitization despite the absence of antigenic overlap. Restimulation with HI-HDM confirmed that the enhanced T cell response was HDM-specific, demonstrating that papain priming amplified immunity to the challenge antigen rather than merely preserving or enhancing responses to the priming antigen. By contrast, capsaicin-mediated activation of TRPV1⁺ sensory neurons did not induce training, indicating that neuronal activation alone was insufficient and that protease allergen-associated context was required. This antigen-independent cross-sensitization parallels heterologous memory in innate immune cells and may contribute to progression from monosensitization to polysensitization. Its dependence on sensory neuronal mTORC1 signaling and mitochondrial integrity further identifies this metabolic pathway as a tractable therapeutic target.

The trained neuronal state persisted for approximately 3 weeks following initial exposure, providing a window that may bridge sequential allergen encounters and allow exposure to one allergen to enhance sensitization to an unrelated allergen encountered days or weeks later. This may be particularly relevant during seasonal allergen exposure, when individuals encounter multiple allergens over a compressed interval, and could contribute to the progressive acquisition of additional sensitizations. However, training was not indefinitely maintained. Reversibility may be particularly important in long-lived sensory neurons, in which permanent reprogramming after successive allergen encounters could otherwise produce progressively cumulative changes in responsiveness. Unlike shorter-lived immune populations that can be replaced by newly generated, untrained cells, sensory neurons remain functional over the lifetime of an organism. A temporally restricted trained state may therefore amplify neuroimmune communication during periods of recurrent exposure while avoiding permanent pathological activation. Although direct evidence for sensory neuronal training in humans is currently lacking, common patterns of allergen exposure illustrate its potential clinical relevance.

The duration of neuroimmune training may reflect the persistence and turnover of the mTORC1-dependent mitochondrial program. Alternatively, or additionally, epigenetic remodeling could transiently stabilize the associated metabolic and transcriptional state. Trained immunity in myeloid cells and memory phenotypes in astrocytes involve epigenetic reprogramming,^{34–37} and epigenetic memory has also been described in tissue-resident stromal cells that regulate allergic inflammation, supporting a broader paradigm of non-immune cellular memory across tissues.³⁸ Whether chromatin remodeling similarly contributes to the duration of neuronal training remains to be determined. Future studies should also establish whether neuronal training accelerates later features of allergic disease, including IgE production, mast cell activation, or anaphylaxis.

Together, our findings established mTORC1-driven metabolic adaptation in sensory neurons as a form of neuroimmune memory that amplifies allergic sensitization. By shifting the focus from immune-centric to neuro-immune-metabolic mechanisms, this work reframes our knowledge of how allergens initiate and broaden allergic responses. Future studies should identify the upstream sensors of protease activity, define the neuronal subsets responsible, and determine the mechanisms governing its persistence and resolution. Targeting sensory neuronal mTORC1 signaling or mitochondrial

fitness may offer strategies to limit polysensitization and halt the atopic march.

Limitations of the study

This study demonstrates that protease allergens induce a sensory neuron-intrinsic trained state through mTORC1-dependent mitochondrial remodeling, but several limitations remain. First, the upstream mechanisms linking protease activity to neuronal mTORC1 activation are undefined. Although neuronal activation was required, the molecular sensors that detect protease allergens remain to be identified. Second, contributions from other tissue-resident cells, including epithelial, stromal, and immune populations, cannot be excluded. Interactions between sensory neurons and the tissue microenvironment may shape the magnitude and persistence of training. Third, although the trained state persisted for weeks, its full temporal dynamics and mechanisms of maintenance remain unsolved. Epigenetic stabilization, persistent metabolic adaptation, and ongoing tissue-derived signals may each contribute. Fourth, our model examines early events in allergic sensitization, including itch, DC migration, and Th2 cell priming, but does not directly address later manifestations such as IgE production or mast cell activation. Finally, although cross-sensitization occurred between antigenically distinct allergens containing cysteine or serine proteases, whether neuronal training extends to non-protease allergens remains to be tested. Despite these limitations, our findings establish a framework for understanding how sensory neuronal memory may promote polysensitization in atopic disease.

RESOURCE AVAILABILITY

Lead contact

Requests for further information, resources, and reagents should be directed to, and will be fulfilled by, the lead contact, Caroline L. Sokol (clsokol@mgh.harvard.edu).

Materials availability

This study did not generate any new, unique reagents.

Data and code availability

- Bulk RNA-seq data generated in this study have been deposited in the NCBI BioProject database and are publicly available from the date of publication. The accession number is listed in the [key resources table](#).
- The code used for bulk RNA-seq analysis in this study is publicly available on GitHub at <https://github.com/XZ924/Neuroimmune-memory-bulkRNAseq>.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: X.Z. and C.L.S.; data curation: X.Z. and C.L.S.; formal analysis: X.Z., H.Y., H.Z., I.J.K., N.P.S., and C.L.S.; funding acquisition: C.L.S.; investigation: X.Z., H.Y., H.Z., I.J.K., D.R.B., N.L., C.H., P.R.N., L.R.D., L.M.A., N.P.S., R.L., and Z.S.; methodology: X.Z., H.Y., H.Z., I.J.K., C.H., L.M.A., N.P.S., E.W., C.H.F., A.-C.V., R.A.R., and C.L.S.; project administration: C.L.S.; resources: C.L.S., A.E.R., and A.-C.V.; supervision: C.L.S.; visualization: X.Z., H.Y., H.Z., I.J.K., and C.L.S.; writing – original draft: X.Z. and C.L.S.; and writing – review & editing: X.Z., P.R.M., S.Z., and C.L.S. All authors provided discussion, participated in revising the manuscript, and agreed to the final version.

DECLARATION OF INTERESTS

C.L.S. is a paid consultant for Bayer, Merck, and Lilly; received sponsored research support from GSK; is on the scientific advisory board for Granite Biosciences; and is on the advisory board for *Immunity*. A.-C.V. has a financial interest in 10x Genomics. A.-C.V.'s interests were reviewed by The Massachusetts General Hospital and Mass General Brigham in accordance with their institutional policies. C.H.F. is a current employee of Gilead.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to assist with copy editing and language refinement. The authors reviewed and edited the output as needed and take full responsibility for the content of the published article.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.immuni.2026.08.012>.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Brilliant Violet 605™ anti-mouse CD11c Antibody (clone N418)	BioLegend	Cat# 117334; RRID: AB_2562415
Alexa Fluor® 488 anti-mouse CD11c Antibody (clone N418)	BioLegend	Cat# 117311; RRID: AB_3893006
FITC anti-mouse/human CD11b Antibody (clone M1/70)	BioLegend	Cat# 101206; RRID: AB_312789
Brilliant Violet 605™ anti-mouse/human CD11b Antibody (clone M1/70)	BioLegend	Cat# 101257; RRID: AB_2565431
APC anti-mouse/human CD11b Antibody (clone M1/70)	BioLegend	Cat# 101212; RRID: AB_312795
Brilliant Violet 605™ anti-mouse CD103 Antibody (clone 2E7)	BioLegend	Cat# 121433; RRID: AB_2629724
Alexa Fluor® 647 anti-mouse/rat XCR1 Antibody (clone ZET)	BioLegend	Cat# 148214; RRID: AB_2564369
PE/Cyanine7 anti-mouse CD301b (MGL2) Antibody (clone URA-1)	BioLegend	Cat# 146808; RRID: AB_2563390
PerCP/Cyanine5.5 anti-mouse I-A/I-E Antibody (clone M5/114.15.2)	BioLegend	Cat# 107626; RRID: AB_2191071
Brilliant Violet 421™ anti-mouse CD273 (B7-DC, PD-L2) Antibody (clone TY25)	BioLegend	Cat# 107219; RRID: AB_2728127
PE anti-mouse CD86 Antibody (clone GL-1)	BioLegend	Cat# 105008; RRID: AB_313151
Brilliant Violet 605™ anti-mouse CD4 Antibody (clone RM4-5)	BioLegend	Cat# 100548; RRID: AB_2563054
PerCP/Cyanine5.5 anti-mouse CD4 Antibody (clone GK1.5)	BioLegend	Cat# 100434; RRID: AB_893324
PE anti-mouse CD8a Antibody (clone 53-6.7)	BioLegend	Cat# 100708; RRID: AB_312747
PerCP/Cyanine5.5 anti-mouse CD8a Antibody (clone 53-6.7)	BioLegend	Cat# 100743; RRID: AB_2075238
Alexa Fluor® 700 anti-mouse/human CD44 Antibody (clone IM7)	BioLegend	Cat# 103026; RRID: AB_493713
eBioscience IL-4 Monoclonal Antibody Alexa Fluor™ 488 (clone 11B11)	Thermo Fisher Scientific	Cat# 53-7401-82; RRID: AB_469922
eBioscience IL-13 Monoclonal Antibody PE-Cyanine7 (clone eBio13A)	Thermo Fisher Scientific	Cat# 25-7133-82; RRID: AB_2573530
Brilliant Violet 421™ anti-mouse IL-10 Antibody (clone JES5-16E3)	BioLegend	Cat# 505022; RRID: AB_2563240
APC anti-mouse IFN-γ Antibody (clone XMG1.2)	BioLegend	Cat# 505810; RRID: AB_315404
eBioscience™ Fixable Viability Dye eFluor™ 780	Thermo Fisher Scientific	Cat# 65-0865-14
Neuron-specific beta-III Tubulin Biotinylated Antibody	R&D Systems	Cat# BAM1195; RRID: AB_356859
Phospho-S6 Ribosomal Protein (Ser235/236) Rabbit Monoclonal Antibody #4858 (clone D57.2.2E)	Cell Signaling Technology	Cat# 4858L; RRID: AB_916516
beta Actin Polyclonal Antibody, HRP	Thermo Fisher Scientific	Cat# PA1-183-HRP; RRID: AB_2608409
GAPDH Polyclonal antibody	Thermo Fisher Scientific	Cat# PA1-183-HRP; RRID: AB_2608409

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Anti-PGC1 alpha antibody (clone EPR25162-281)	Abcam	Cat# ab313559; RRID: AB_2914107
BD Pharmingen™ Purified Mouse anti-Akt (pS473)	BD Biosciences	Cat# 560397; RRID: AB_1645553
TOMM20 Polyclonal Antibody	Thermo Fisher Scientific	Cat# PA5-52843; RRID: AB_2648808
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647)	Abcam	Cat# ab150079; RRID: AB_2722623
Chemicals, peptides, and recombinant proteins		
Ovalbumin	Sigma-Aldrich	Cat# A5503
Papain	Sigma-Aldrich	Cat# 5125
Papain	Spectrum Chemical	Cat# PA125
Papain	Thermo Fisher Scientific	Cat# 416760100
Immobilized Papain (Agarose Resin)	Thermo Fisher Scientific	Cat# 20341
Mite, house dust	Greer Laboratories Inc	Cat# NC9756554
Mite, house dust	Greer Laboratories Inc	Cat# NC1887739
Alternaria alternata Fungi	Greer Laboratories Inc	Cat# NC1620293
Fetal Bovine Serum (FBS)	Sigma-Aldrich	Cat# F4135
Penicillin-Streptomycin (10,000 U/mL)	Gibco	Cat# 15140122
GlutaMAX	Gibco	Cat# 35050061
HEPES (1M)	Gibco	Cat# 15630080
MEM Non-Essential Amino Acids Solution (100X)	Gibco	Cat# 11140050
Sodium Pyruvate (100 mM)	Gibco	Cat# 11360070
2-Mercaptoethanol	Gibco	Cat# 21985023
RPMI 1640, 1X	Corning	Cat# 15-040-CV
DMEM	Corning	Cat# 10-014-CV
PMA	Sigma-Aldrich	Cat# P1585
Ionomycin	Sigma-Aldrich	Cat# I0634
BD GolgiPlug Protein Transport Inhibitor (Containing Brefeldin A)	BD Biosciences	Cat# 555029
QWF	Tocris	Cat# 6642
QX314 chloride	Tocris	Cat# 2313
A-803467	Selleck Chemicals	Cat# S2785
Sirolimus	Stemcell Technologies	Cat# 73364
Temsirolimus	Sigma-Aldrich	Cat# PZ0020
Capsaicin	Sigma-Aldrich	Cat# M2028
Leibovitz's L-15 Medium	Gibco	Cat# 11415064
Percoll	Cytiva	Cat# 17-0891-01
Neurobasal-A Medium	Gibco	Cat# 10888022
Neurobasal™ Medium, minus phenol red	Gibco	Cat# 12348017
B-27™ Supplement (50X), minus antioxidants	Gibco	Cat# 10889038
Cytosine beta-D-arabinofuranoside	Sigma-Aldrich	Cat# C6645
Nerve Growth Factor 2.5S	Invitrogen	Cat# 13257019
GDNF	Sigma-Aldrich	Cat# G1401
Laminin	Sigma-Aldrich	Cat# L2020
Poly-D-lysine	Gibco	Cat# A3890401
Normal Goat serum (NGS)	Jackson ImmunoResearch	Cat# 005-000-121
Triton X-100	Sigma-Aldrich	Cat# T9284
Potassium chloride solution	Sigma-Aldrich	Cat# 60142

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Fura-2, AM	Invitrogen	Cat# F1201
Krebs-Ringer Bicarbonate Buffer	Boston Bioproducts	Cat# BSS-250
PBS	Corning	Cat# 21-031-CV
Red blood cell lysis buffer	Sigma-Aldrich	Cat# R7757
0.5 M EDTA, pH 8.0	Gibco	Cat# 15575-038
Collagenase A	Sigma-Aldrich	Cat# 10103578001
Collagenase P	Sigma-Aldrich	Cat# 11213857001
Dispase II	Sigma-Aldrich	Cat# D4693
DNase I, grade II, from bovine pancreas	Sigma-Aldrich	Cat# 10104159001
Tris-HCl, 0.5M buffer soln., pH 8.0, low endotoxin	Thermo Fisher Scientific	Cat# J67510.AK
Sodium dodecyl sulfate	Sigma-Aldrich	Cat# 436143
Sodium chloride	Sigma-Aldrich	Cat# S5886
2-Propanol	Sigma-Aldrich	Cat# I9030
Ethyl alcohol, Pure	Sigma-Aldrich	Cat# 459844
Roche Diagnostics FS ESSENTIAL DNA GREEN MASTER	Roche	Cat# 6402712001
EMS 16% Paraformaldehyde Aqueous Solution	Electron Microscopy Sciences	Cat# 15710
Tris Buffered Saline with Tween 20 (TBST 10X)	Cell Signaling Technology	Cat# 9997S
Invitrogen novex NuPAGE MES SDS Running Buffer (20X)	Life Technologies	Cat# NP0002
Critical commercial assays		
Substance P ELISA Kit	Abcam	Cat# ab288318
BD Cytofix/Cytoperm	BD Biosciences	Cat# 554714; RRID: AB_2869008
Seahorse XFp Cell Mito Stress Test Kit	Agilent Technologies	Cat# 103010-100
iBlot™ Transfer Stack, nitrocellulose, mini	Invitrogen	Cat# IB301002
Deposited data		
Bulk RNA-seq data generated in this study	This paper	NCBI Bioproject: PRJNA1457553
Experimental models: Organisms/strains		
Mouse: C57BL/6	Charles River Laboratory	RRID: IMSR_CRL: 027
Mouse: C57BL/6	The Jackson Laboratory	RRID: IMSR_JAX: 000664
Mouse: Kaede transgenic	Tomura et al. ⁵⁶	N/A
Mouse: B6.Cg-Tac1tm1Bbm/J	The Jackson Laboratory	RRID: IMSR_JAX: 004103
Mouse: B6.Cg-Rag2tm1.1Cgn/J	The Jackson Laboratory	RRID: IMSR_JAX: 008449
Mouse: B6.129S2-Tcratm1Mom/J	The Jackson Laboratory	RRID: IMSR_JAX: 002116
Mouse: B6.129S2-Ighmtm1Cgn/J	The Jackson Laboratory	RRID: IMSR_JAX: 002288
Mouse: <i>Cpa3</i> ^{Cre/+}	Hans-Reimer Rodewald (German Cancer Research Center)	N/A
Mouse: B6.129(Cg)-Scn10atm2(cre)Jwo/TjpJ	The Jackson Laboratory	RRID: IMSR_JAX: 036564
Mouse: B6.Cg-Rptortm1.1Dmsa/J	The Jackson Laboratory	RRID: IMSR_JAX: 013188
Mouse: B6.129S4(Cg)-Tsc1tm1Djk/MhnyJ	The Jackson Laboratory	RRID: IMSR_JAX: 038428
Mouse: B6.Cg-Tfamtm1.1Ncdl/J	The Jackson Laboratory	RRID: IMSR_JAX: 026123
Mouse: B6.Ptprcb Thy1a Ricorttm1.1Klg/SjmJ	Dr. Leonid Pobezinsky (University of Massachusetts, Amherst, MA, USA)	RRID: IMSR_JAX: 020649
Oligonucleotides		
H2K FWD (5'-GCCAGCCTCTCCTGATTTAGTGT-3')	Integrated DNA Technologies, Quiros et al. ⁸⁰	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
H2K REV (5'-GGGAACACAAAAGACCTCTTCTGG-3')	Integrated DNA Technologies, Quiros et al. ⁸⁰	N/A
16s rRNA FWD (5'-CCGCAAGGGAAAGATGAAAGAC-3')	Integrated DNA Technologies, Quiros et al. ⁸⁰	N/A
16s rRNA REV (5'-TCGTTTGGTTTCGGGGTTTC-3')	Integrated DNA Technologies, Quiros et al. ⁸⁰	N/A
ND1 FWD (5'-CTAGCAGAAA CAAACCGGGC-3')	Integrated DNA Technologies, Quiros et al. ⁸⁰	N/A
ND1rRNA REV (5'-CCGGCTGCGTATTCTACGTT-3')	Integrated DNA Technologies, Quiros et al. ⁸⁰	N/A
Software and algorithms		
Prism Version 11.0.0 (93)	GraphPad Software	https://www.graphpad.com/demos
Zen Blue	Carl Zeiss	https://www.zeiss.com/microscopy/en/products/software/zeiss-zen.html
CytExpert	Beckman Coulter	https://www.beckman.com/flow-cytometry/research-flow-cytometers/cytoflex/software
FlowJo v10	BD Bioscience	https://www.bdbiosciences.com/en-us/products/software/flowjo-software?tab=flowJo-v11-software
LAS X	Leica Microsystems	https://www.leica-microsystems.com/products/microscope-software/p/leica-las-x-ls/
Fiji (Image J)	NIH	https://fiji.sc/?utm_source.com
Mitochondrial Analyzer plugin	Chaudhry et al. ⁶⁹	https://github.com/AhsenChaudhry/Mitochondria-Analyzer

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Mice

Experiments involving animals were approved by the Massachusetts General Hospital or Harvard Medical School Institutional Animal Care and Use Committee. Mice were maintained and bred in an SPF facility in ventilated cages, with a maximum of five adult mice per cage, on a 12-hour day-night cycle, at 20–25 °C and 35–65% humidity and provided food and water *ad libitum*. All experimental mice were on the C57BL/6 background and were 5–16 weeks of age. Male and female mice were used in all experiments, and comparisons were made between age- and sex-matched controls. Power calculations were conducted to determine sample sizes. Mice were placed into groups depending on their genotype; when possible, mice were randomized. Behavioral experiments were blinded, and immunologic experiments were not. WT C57BL/6 (556) and *Tac1*^{−/−} mice were purchased from Charles River Laboratories (Wilmington, MA). WT C57BL/6 (000664), *Rag2*^{−/−} (B6.Cg-*Rag2*^{tm1.1Cgn}/J; 008449), *Tcra*^{−/−} (B6.129S2-*Tcra*^{tm1.Mom}/J; 002116), *μMT*^{−/−} (B6.129S2-*Ighmt*^{tm1.Cgn}/J; 002288), *Scn10a*^{Cre} (B6.129(Cg)-*Scn10a*^{tm2(Cre)Jwo}/TjPj; 036564), *Raptor*^{fl/fl} (B6.Cg-*Rptor*^{tm1.1Dmsa}/J; 013188), *Tsc1*^{fl/+} (B6.129S4(Cg)-*Tsc1*^{tm1Djk}/MhnyJ; 038428) and *Tfam*^{fl/fl} (B6.Cg-*Tfam*^{tm1.1Ncdl}/J; 026123) mice were purchased from The Jackson Laboratory. *Cpa3*^{Cre/+} mice were originally from Hans-Reimer Rodewald (German Cancer Research Center). *Rictor*^{fl/fl} mice, originally described by Magee et al.,⁸¹ were generously provided by the laboratory of Dr. Leonid Pobeizinsky (University of Massachusetts, Amherst, MA, USA). *Scn10a*^{Cre} were crossed to *Raptor*^{fl/fl}, *Rictor*^{fl/fl}, *Tsc1*^{fl/+}, *Tfam*^{fl/+} to generate *Scn10a*^{Cre/+} *Raptor*^{fl/fl}, *Scn10a*^{Cre/+} *Rictor*^{fl/fl}, *Scn10a*^{Cre/+} *Tsc1*^{fl/+} and *Scn10a*^{Cre/+} *Tfam*^{fl/+} respectively. Kaede mice were originally from Osami Kanagawa as described previously.⁸ To control for differences in commensal microbes among colonies, all experiments using *Cpa3*^{Cre/+}, *Scn10a*^{Cre/+}, *Scn10a*^{Cre/+} *Raptor*^{fl/fl}, *Scn10a*^{Cre/+} *Rictor*^{fl/fl}, *Scn10a*^{Cre/+} *Tsc1*^{fl/+} and *Scn10a*^{Cre/+} *Tfam*^{fl/+} mice were performed with littermate controls bred in our facility. All experiments comparing WT to experimental mouse lines were performed with colony control WT mice originally from The Jackson Laboratory but bred in our facility. In experiments that only used WT mice, the mice were purchased from Charles River Laboratories and housed in the MGH animal facility for one to two weeks prior to undergoing behavioral testing.

METHOD DETAILS

Intradermal Immunizations

Mice were briefly anesthetized and injected intradermally (i.d.) with 25 μ L of the indicated stimulus. For behavioral testing, injections were administered to the right cheek unless otherwise indicated. For dendritic cell (DC) migration experiments, injections were delivered into the plantar surface of the footpad or, for Kaede photoconversion experiments, into the dorsal surface. Injections included: Papain, 50 μ g previously frozen, 10 μ g freshly prepared (Millipore Sigma), 10 μ g freshly prepared (Creative Enzymes), 1 μ g, 2.5 μ g or 5 μ g freshly prepared (Fisher Scientific) to normalize for differences in enzymatic activity; heat-inactivated papain (2 μ g or 50 μ g, matching the doses used for papain injections; Millipore Sigma); Ovalbumin (Ova, Millipore Sigma); and capsaicin (20 μ g for behavioral analysis and 10 μ g for DC migration; Cayman Chemical). For DC migration and T cell differentiation in the multiple low dose exposure model, mice received daily administrations (days 0-2) of 1 μ g of freshly prepared papain (Fisher Scientific); 1 μ g of house dust mite (HDM) (Greer Laboratories, XPB91D3A2.5) or 1 μ g of *Alternaria alternata* (*A. alt*) (Greer Laboratories, XPM1D3A2.5), followed by injections with 5 μ g HDM or 3 μ g *A. alt* on day 9. For behavioral testing in the multiple low-dose exposure model, mice were administered 2 μ g of freshly prepared papain (Fisher Scientific) or 5 μ g of HDM (Greer Laboratories, XPB91D3A2.5) daily on days 0-2, followed by a challenge with 10 μ g papain or 100 μ g HDM on day 9. All reagents were diluted in sterile PBS (Corning) except for capsaicin, which was diluted in PBS containing 6.4% DMSO. For experiments involving trigeminal ganglia (TG) neuron collection, mice that underwent cheek immunization were anesthetized with an intraperitoneal injection (i.p.) of ketamine (100 mg/kg) and xylazine (10 mg/kg) prior to allergen administration to minimize potential confounding effects of itch and acute inflammation.

Behavioral Analysis

As described previously,^{8,9} mice were transferred from the animal facility to a private behavioral testing room where they were separated into individual cages and underwent a two-hour habituation period. A white noise machine (Marpac) was used in the testing room to reduce distractions. After habituation, mice received an i.d. injection of 25 μ L of the indicated stimulus into the cheek. Behavioral responses were recorded via overhead video, and the number of scratching bouts directed at the injection site using the hind paw was quantified. A 20-, 30-, or 40-minute video was recorded for papain, and a 25-minute video was recorded for HDM, as indicated in each of the behavior-related figures.

Inhibitor Treatment

QX314 (Tocris) was dissolved in PBS (Corning) at 10% (w/v) and stored at -20°C for up to 3 months. 1% QX314 was co-injected with papain i.d. into the cheek or footpad to assess behavioral responses or DC migration. A-803467 (Selleck) was dissolved in corn oil containing 5% DMSO (Sigma-Aldrich) to a final concentration of 5 mg/mL and stored at 4°C for up to 10 hours. Mice received i.p. injections of A-803467 (50 mg/kg) 4 hours prior to papain exposure for behavioral testing and DC migration experiments. Sirolimus (StemCell Technologies) and temsirolimus (Sigma-Aldrich) were dissolved in DMSO to prepare 10 mM stock solutions and stored at -20°C for up to 3 months. For *in vitro* experiments, dissociated dorsal root ganglia (DRG) cultures were treated with sirolimus at a final concentration of 200 nM. For *in vivo* experiments, sirolimus (15 nmol in 25 μ L PBS) or temsirolimus (12.5 nmol in 25 μ L PBS) was administered via i.d. injection in the cheek (behavioral analysis) or footpad (DC migration) 2 hours before papain or Ova injections as indicated. QWF was prepared as a 10 mM stock solution in DMSO and diluted in PBS containing 5% DMSO to a final concentration of 500 μ M for i.d. administration.⁸²

DC migration

Mice were briefly anesthetized with isoflurane and injected i.d. in the dorsal footpad with 25 μ L of the indicated allergen or 30 μ L of TG neuron culture supernatant. For internal controls, one foot received Ova while the other received Ova plus indicated allergens in initial and secondary injections. In experiments using Kaede transgenic mice, which express a photoconvertible protein that changes from green (Kaede^{Green}) to red (Kaede^{Red}) upon exposure to violet light,⁵⁶ DC migration was tracked post-photoconversion. Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and the dorsal surface of the feet was exposed to 420 nm light (Bluewave LED curing unit with bandpass filter; Dymax/Andover Corp) for 5 minutes at a distance of 7.5 cm. Allergen was injected i.d. into the photoconverted area immediately afterward. Then, 22-24 hours after allergen injection, draining lymph node (dLNs) were collected and digested at 37°C in RPMI containing DNase I (100 μ g/mL; Roche), Dispase II (800 μ g/mL; Millipore Sigma), Collagenase P (200 μ g/mL; Millipore Sigma), and 1% FBS. Every 7 min, the digestion buffer was removed and transferred into RPMI supplemented with 2 mM EDTA and 1% FBS on ice. Fresh digestion buffer was added to the LNs. Tubes were vigorously shaken before and after each addition of fresh digestion buffer. This process was repeated until no fragment of LN tissue remained. Single-cell suspensions were filtered through a 70 μ m cell strainer (Corning or Biologix) before flow cytometry was performed.

T cell differentiation

Mice received 25 μ L i.d. injections into both dorsal footpads with Ova alone or Ova co-administered with the indicated allergens. 5 days after the last injections, the popliteal dLNs were harvested and mechanically dissociated in PBS using the frosted end of a Superfrost Plus microscope slide and curved forceps. Single-cell suspensions were filtered through a 70 μ m cell strainer (Corning or Biologix). A total of 2×10^6 dLN cells were resuspended in 1 mL of fully supplemented RPMI medium supplemented with PMA (50 ng/mL; Millipore Sigma), ionomycin (500 ng/mL; Millipore Sigma), and protein transport inhibitor (1:1,000 dilution; BD

Biosciences). Cells were incubated for 2.5 to 3 hours at 37 °C with 5% CO₂. Following stimulation, cells were washed twice with FACS buffer and stained per our flow cytometry protocol described below. Additionally, cells were fixed with 4% paraformaldehyde (PFA) for 8 minutes at room temperature (RT) and permeabilized using 1 × eBioscience Permeabilization Buffer. Intracellular cytokine staining was performed using fluorescently labeled antibodies for 1 hour at RT or overnight at 4 °C. After two final washes with FACS buffer, samples were analyzed by flow cytometry.

Flow cytometry

Single-cell suspensions were prepared by enzymatic digestion and stained in FACS Buffer (1% FBS in DPBS) with fluorescently conjugated antibodies for 30 minutes at 4°C. To ensure viability assessment, Fixable Viability Dye eFluor™ 780 (eBioscience) was added into antibody preparations at a 1:2,000 dilution. Fc receptor blocking was performed using TruStain fcX™ (anti-mouse CD16/32; BioLegend) at a 1:250 dilution. Following staining, cells were washed twice with FACS Buffer to remove unbound antibodies. Flow cytometry analysis was performed using either a CytoFLEX S flow cytometer (Beckman Coulter) or a Cytex Aurora spectral cytometer (Cytex Biosciences). Data acquisition was conducted using manufacturer-specific software (CytExpert v2.3 for CytoFLEX or SpectroFlo v3.2.1 for Aurora). All flow cytometry data (.fcs files) were subsequently analyzed using FlowJo software (v10, TreeStar) with appropriate gating strategies to identify specific cell populations.

Isolation of DRG/TG neurons

DRG or TG neurons were isolated from mice as previously described.^{9,83} Briefly, tissues were placed in 15 mL Falcon tubes (Corning) containing DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Millipore Sigma). Samples were centrifuged at 1,000 rpm for 5 minutes and digested in 3 mL of enzyme solution containing Collagenase A (1.25 mg/mL) and Dispase II (2.5 mg/mL) at 37 °C for 70-90 minutes with shaking at 150 rpm. Following digestion, 10 mL of DMEM with 10% FBS and 1% penicillin-streptomycin was added. Cells were washed twice with DPBS containing 1% penicillin-streptomycin and 1% GlutaMax (Gibco), then resuspended in 1 mL of Neurobasal Medium A (NBM; Thermo Fisher Scientific) supplemented with 1 × B-27 and 1% penicillin-streptomycin. Tissues were gently dissociated by pipetting with a P1000 for 10 minutes, then filtered through a 70 µm cell strainer (Corning or Biologix). The resulting suspension was layered onto a 28%/12.5% Percoll gradient (VWR) prepared in Leibovitz's L-15 Medium (Thermo Scientific) and centrifuged at 1,300 × g for 10 minutes at RT without acceleration or braking. The cell pellet was resuspended in fresh supplemented NBM and filtered through a 70 µm cell strainer (Corning or Biologix) for downstream use.

Culture and stimulation of DRG/TG neurons

To prepare the culture substrate, 50 µL of a poly-D-lysine and laminin solution (Millipore Sigma) was added to each well of a tissue-culture-treated, flat-bottom 96-well plate (Corning) and incubated for at least 2 hours at 37°C. The wells were then washed three times with DPBS. DRG or TG neurons (5,000–10,000 cells/well) were cultured for 7 days in supplemented NBM containing 25 ng/mL nerve growth factor (NGF; Alomone Laboratories), 2 ng/mL glial cell line-derived neurotrophic factor (GDNF; PeproTech), and 0.01 mM cytosine β-D-arabinofuranoside hydrochloride (Ara-C; Millipore Sigma). For experiments detecting pS6 and pAKT, the culture medium was replaced with 200 µL/well of supplemented Neurobasal-A medium lacking exogenous growth factors. DRG neuron cultures were either left unstimulated, stimulated with papain (10 µg/mL; Sigma-Aldrich) for 10 min, 30 min, or 1 h, or co-treated with papain (10 µg/mL) and sirolimus (200 nM; StemCell Technologies) for 1 h. After stimulation, supernatants were removed, and cells were harvested for Western blot analysis as described in the 'Protein extraction and immunoblotting' protocol. To assess Substance P release from TG neuron cultures in response to papain, the culture medium was replaced with 200 µL/well of supplemented Neurobasal-A medium lacking exogenous growth factors. TG neurons were either left unstimulated or stimulated with immobilized papain (Thermo Fisher, 10mg/mL) for 10 minutes. Cell-free supernatants were collected, snap-frozen and stored at –80 °C for subsequent substance P quantification (ELISA) and DC migration assays.

Calcium imaging of TG neurons

TG neurons were isolated and cultured as mentioned in "isolation of DRG/TG neurons" protocol. Calcium imaging was performed using Fura-2 AM (Thermo Fisher Scientific) based ratiometric imaging as previously described.⁹ Briefly, TG neurons were plated on poly-D-lysine/laminin-coated glass-bottom dishes and cultured overnight in supplemented Neurobasal-A medium without phenol red. TG Neurons were loaded with Fura-2 AM for 30 minutes at 37°C, followed by washing and equilibration in Krebs-Ringer buffer (Alfa Aesar). Calcium imaging was performed using alternating excitation at 340 and 380 nm, and images were acquired at ~1 seconds intervals. Cells were continuously perfused with Krebs-Ringer buffer, and stimuli were applied sequentially as indicated. TG neurons were considered live and excitable if they responded to KCl with a 15% ratio increase from baseline. TG neurons were considered responsive if stimulation induced an increase in the 340/380 fluorescence ratio exceeding 15% above baseline. TG neurons were sequentially stimulated with increasing concentrations of papain as indicated, and responsive neurons were defined using the same threshold criteria. Calcium responses were quantified as changes in the 340/380 fluorescence ratio, and response magnitude was assessed by area under the curve (AUC) following baseline subtraction. Data were analyzed using GraphPad Prism.

Protein extraction and immunoblotting

Cells were lysed in RIPA lysis buffer (G Biosciences) with $1 \times$ protease inhibitor (Thermo Scientific). After a 30-minute incubation on ice, cell lysate was centrifuged at $16000 \times g$ for 15 minutes at 4°C . The cell lysates were boiled in SDS loading buffer at 95°C for 5 to 10 minutes and proteins were either run on 10–12% SDS-polyacrylamide gels, followed by transferring to PVDF membranes, or the up-layer supernatants were incubated in SDS loading buffer at 75°C for 10 minutes and proteins run on 4–12% precast gels transferred to nitrocellulose membranes. For blocking non-specific binding and antibody incubation, 5% BSA in $1 \times$ TBST (Cell Signaling Technology) was used. After primary antibody addition (Rabbit anti-pS6(Ser235/236), CST (4858S), 1:1000; Rabbit anti-pAKT (Ser473), CST (9271T), 1:1000; Rabbit anti-PGC1 α , Abcam (ab313559), 1:500), samples were incubated at 4°C overnight with shaking. Secondary antibody (Peroxidase (HRP) Anti-Rabbit IgG Goat Secondary Antibody, CST (7074P2), 1:8000) incubation occurred at RT for 1 hour. Following secondary incubation, the membranes were washed in $1 \times$ TBST three times. Signal was scanned using BIO-RAD (Hercules, CA, USA) ChemiDocTM MP imaging system or Protein Simple (Biotechne) FluorChem M system.

Confocal immunofluorescence of intact ear skin

Confocal imaging of sensory nerve fibers was performed as previously described.⁹ Briefly, ears were depilated (Veet), and separated into dorsal and ventral halves. Whole-mount ear skin was then fixed in 4% PFA for 1 hour at RT or 4°C without shaking. To visualize free nerve endings, fixed ear skin was incubated overnight at 4°C with anti-Tuj1-biotin antibody (1:100; clone TuJ1, MAB1195; R&D Systems). The following day, samples were incubated with streptavidin-PE (1:100; BioLegend, 405204) to visualize staining. Samples were washed three times with DPBS containing 2% heat-inactivated goat serum and 0.2% Triton X-100, with 10-minute incubations per wash. Intact ear skin was then mounted on SuperFrost Plus microscope slides (Fisher Scientific) using ProLong Diamond Antifade Mountant (Invitrogen). Quantification of Tuj1⁺ sensory nerve fiber density was performed using ImageJ software.

Immunofluorescence staining of TOM20 and confocal imaging analyses

Coverslips were circled with an ImmEdge® Hydrophobic Barrier PAP Pen (Vector Laboratories) and coated with 200 μL of poly-D-lysine/laminin solution (Millipore Sigma). Coated coverslips were incubated for ≥ 2 h at 37°C and subsequently washed three times with DPBS. TG neurons were isolated from mice 7 days after i.d. cheek injections as indicated, following the “TG/DRG neuron isolation” protocol. Approximately 20,000–30,000 neurons were seeded per coated circle and maintained overnight in supplemented NBM containing 25 ng/mL nerve growth factor (NGF; Alomone Laboratories), 2 ng/mL glial cell line-derived neurotrophic factor (GDNF; PeproTech), and 0.01 mM cytosine β -D-arabinofuranoside hydrochloride (Ara-C; Millipore Sigma). For TOM20 staining, cells were fixed with 4% PFA for 8–10 minutes at RT and washed three times with DPBS. Cells were incubated with anti-TOM20 antibody (#PA5-52843, Thermo Fisher Scientific; 1:200 in 1% BSA) overnight at 4°C in a humidified chamber, protected from light. Following three DPBS washes, cells were incubated with the Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150079, Abcam; 1:400 in 1% BSA) at RT for 1 hour in the dark. Coverslips were mounted with ProLong Glass Antifade Mountant containing DAPI (Invitrogen, Thermo Fisher Scientific) and stored protected from light. Confocal images were acquired on a Leica Stellaris system equipped with a Power HyD detector, using a $63\times$ oil immersion objective under identical imaging settings for all groups. Mitochondrial morphology quantification was conducted by the Mitochondrial Analyzer Plugin through Image J software.⁶⁹ In brief, 3–4 different regions of the image stack were selected, and an average intensity projection of two subsequent stacks per region were calculated. Brightness and contrast were then adjusted to 30–255. Mitochondrial thresholding was then performed in 2D by the Mitochondrial Analyzer, with block size 1.05 and a C-Value of 9 with default settings. Particles lower than 30-pixel units were then removed and “fill holes” was applied. Analysis was then performed with Mitochondrial Analyzer on a per mitochondrion basis.

Isolation of Genomic DNA from TG neurons

TG neurons were isolated following the “isolation of DRG/TG neurons” protocol. Cells were lysed in 500 μL of lysis buffer (20 mM Tris-HCl, pH 7.4; 100 mM NaCl; 10 mM EDTA; 0.5% SDS) supplemented with 100 $\mu\text{g}/\text{mL}$ proteinase K and incubated at 37°C for 30 min. Subsequently, 300 μL of supersaturated NaCl solution was added, mixed thoroughly, and the samples were incubated on ice for 30 min. The lysates were centrifuged at $12,000 \times g$ for 15 minutes at 4°C , and the supernatant (aqueous phase) was carefully transferred to a new 1.5 mL microcentrifuge tube. DNA was precipitated by adding 600 μL of isopropanol, mixed gently, and incubated on ice for 30 minutes, followed by centrifugation at $12,000 \times g$ for 10 minutes at 4°C . The supernatant was carefully removed to avoid disturbing the pellet, which was then washed with 500 μL of 70% ethanol and centrifuged at $12,000 \times g$ for 5 minutes at 4°C . The pellet was air-dried and resuspended in 100 μL of nuclease-free water. DNA concentration and purity were determined using a NanoDrop spectrophotometer.

Quantitative PCR (qPCR) and quantification of mitochondrial DNA (mtDNA) copy number

Genomic DNA extracted from TG cells was first diluted with double-distilled water to a final concentration of 10 ng/ μL for qPCR analysis. qPCR was performed for the mitochondrial genes 16S ribosomal RNA gene (16S rRNA), NADH dehydrogenase 1 (ND1), and the nuclear-encoded housekeeping gene Hexokinase 2 (HK2) using SYBR Green detection (FastStart Essential DNA Green Master Mix, Roche) on a LightCycler 96 Instrument (Roche), as previously described.⁸⁰

Relative mtDNA copy number was determined by comparing the expression of ND1 and 16S rRNA to that of the nuclear DNA (nDNA) reference gene HK2. The mtDNA copy number was calculated using the following formulas:

$$\Delta Ct = Ct (\text{nDNA gene}) - Ct (\text{mtDNA gene});$$

$$\text{Copies of mtDNA} = 2 \times 2^{\Delta Ct}.$$

Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) measurements of TG neurons

OCR and ECAR were measured using Seahorse XFp or XFe96 extracellular flux analyzers (Agilent Technologies). TG neurons were isolated as described in the ‘Isolation of DRG/TG Cells’ protocol. Cells (4,000–5,000 cells/well) were seeded in Agilent 8-well Seahorse XFp Cell Culture Miniplates or Agilent Seahorse XFe96 Pro cell culture microplate and cultured for 24 hours in supplemented Neurobasal Medium (NBM) under conditions outlined in the ‘Culture and Stimulation of DRG/TG neurons’ protocol. Prior to the assay, cells were gently washed three times and equilibrated in serum-free, unbuffered Seahorse XF RPMI medium (Agilent Technologies) supplemented with 10 mM glucose, 2 mM glutamine, and 1 mM sodium pyruvate. The plate was then incubated at 37°C in a non-CO₂ environment for 60 minutes to allow temperature and pH stabilization. Mitochondrial function was assessed using the Seahorse XFp Cell Mito Stress Test Kit (Agilent Technologies). Sequential injections of metabolic modulators were performed: Oligomycin (5 μM), ATP synthase inhibitor (to assess ATP-linked respiration); FCCP (2 μM), Mitochondrial uncoupler (to induce maximal respiration); Rotenone + Antimycin A (2 μM), then Complex I/III inhibitors (to determine non-mitochondrial respiration). Metabolic Parameter Calculations were derived as follows:

- (1) Basal respiration = [OCR (basal)] – [OCR (Rotenone/Antimycin A)].
- (2) ATP-linked respiration = [OCR (basal)] – [OCR (Oligomycin)].
- (3) Maximal respiratory capacity = [OCR (FCCP-induced peak)] – [OCR (Rotenone/Antimycin A)].
- (4) Spare respiratory capacity = [OCR (FCCP-induced peak)] – [OCR (basal)].
- (5) Coupling efficiency = ATP-linked respiration / Basal respiration.

In vitro stimulation of draining lymphocytes

Draining lymphocytes enzymatically digested from the dLN after completing the ‘DC migration’ protocol were added to a 96 well flat bottom plate (Corning) at 5×10^5 cells/per well. 30 μg or 100 μg of heat inactivated HDM (HI-HDM) in 200 μL of fully supplemented RPMI was added to the cells. Plates were incubated at 37 °C with 5% CO₂ for 4–5 days until the medium turned yellow, then cell-free supernatant was harvested, snap-frozen and stored at –80 °C for downstream analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Substance P (SP) levels were quantified using a competitive mouse SP ELISA kit (Abcam) following the manufacturer’s protocol. For cytokine analysis, cell-free culture supernatant from stimulated whole dLN cells was collected. High-binding 96 well plates (Corning) were coated with capture antibodies overnight at 4 °C. IL-4 and IL-13 were captured with anti-mouse IL-4 (0.25 μg/ml, BioLegend) and anti-mouse IL-13 (1.0 μg/ml, BioLegend), respectively. Plates were then washed three times with 0.1% Tween 20 in PBS and blocked with 1% (wt/vol) BSA for 2 hours at 37 °C before cell-free culture supernatant was plated overnight at 4 °C. Next, plates were washed three times with 0.1% Tween 20 in PBS. IL-4 and IL-13 were detected by adding biotinylated anti-mouse IL-4 (0.25 mg/ml, BioLegend) or biotinylated anti-mouse IL-13 (0.25 mg/ml, eBioscience) for 2 hours at RT. Following this, plates were washed four times, incubated with Streptavidin-horseradish peroxidase (BioLegend) for 30 minutes at RT, washed 7 times, and finally incubated with OPD solution (Sigma-Aldrich) for development. Development was stopped with 2M H₂SO₄. ELISA samples were assessed on a SpectraMax iD5 microplate reader (Molecular Devices). Concentrations were determined from a standard curve.

Bulk RNA-seq data generation

To prepare sequencing libraries, total RNA was extracted from ground TG tissue using TRIzol® reagent (Invitrogen). Then, 20 ng of RNA lysate was processed using a modified SMART-Seq2 protocol⁸⁴ entailing RNA secondary structure denaturation (72°C for 3 min), reverse transcription with Maxima Reverse transcriptase (Life Technologies), and whole-transcriptome amplification (WTA) with KAPA HiFi HotStart ReadyMix 2X (Kapa Biosystems) for 12 cycles. WTA products were purified with AMPure XP beads (Beckman Coulter), quantified with a Qubit double-stranded DNA HS Assay Kit (Thermo Fisher Scientific), and quality assessed with a high-sensitivity DNA chip (Agilent). The purified WTA product (0.2 ng) was used as input for the Nextera XT DNA Library Preparation Kit (Illumina). Uniquely barcoded libraries were pooled and sequenced with a NextSeq 500/550 high-output V2 75-cycle kit (Illumina) using 2 × 38-bp paired-end reads.

Bulk RNA-seq read alignment and quantification

Raw sequencing data were processed using the bulk_rna_seq (xavier-genomics/bulk_rna_seq, v24) workflow from the Broad Institute’s online platform Terra. Reads from each sample were aligned to the mouse reference genome GRCm38 through STAR alignment by using “GRCm38_ens93filt” as the reference and “star” as the aligner. Output expected count matrices were combined to produce one sample x gene matrix.

Differential expression analysis

The package DESeq2 (v1.38.2, R) was used to identify changes in gene expression between treatments using $\text{gene} \sim \text{treatment}$ as the model design. The raw count matrix was provided as input.

Gene set enrichment analysis

GSEA was performed using the `fgsea` function from the `fgsea` package (v1.18.0, R) with 10,000 permutations to test for independence. Input gene sets tested were derived from the HALLMARK pathways database and limited to those with a minimum of 10 genes per set. For all comparisons of treatment, input gene rankings were based on decreasing Wald statistic values from DE analysis. The package `biomaRt` (v2.65.0, R) was used to map mouse genes to their human orthologs by referencing the Ensembl database.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analysis of data was performed using GraphPad Prism version 10.3.1 (GraphPad Software). Comparison of data between two groups was performed using Welch's *t*-tests. Comparison of data between more than two groups with one independent variable was performed using one-way ANOVA with Tukey's multiple comparisons tests. Comparison of two independent variables was performed using two-way ANOVA with Tukey's multiple comparisons tests. *P*-values of less than 0.05 were considered statistically significant. Statistical details are listed in the respective figure legends.

INCLUSION AND ETHICS STATEMENT

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

Supplemental information

**Sensory neuronal mTORC1 kinase signaling
establishes neuroimmune memory
that initiates allergic immunity**

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Figure S1

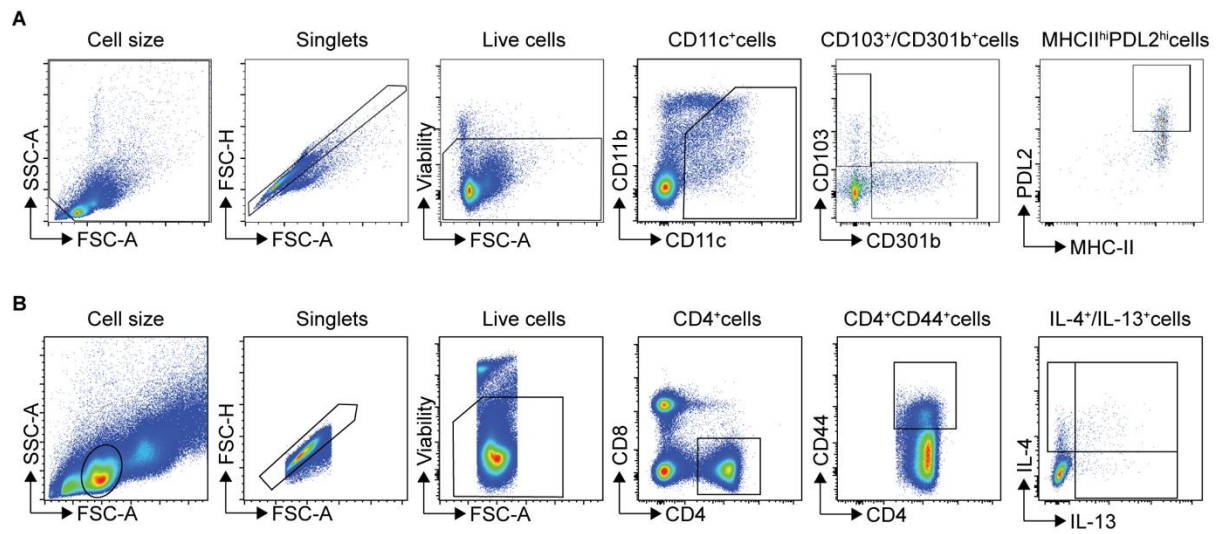


Figure S1. Gating strategies for DC and Th2 cell cytokine analyses, related to Figure 1.
(A and B) Representative flow cytometry gating strategies for DCs **(A)** and CD4⁺ T cell cytokine analyses **(B)** in popliteal dLNs.

Figure S2

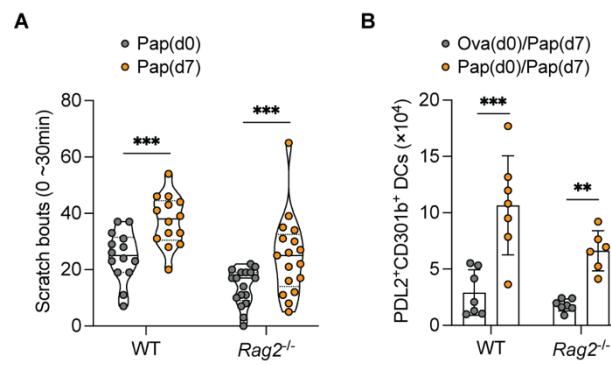


Figure S2. Allergen-induced neuroimmune memory occurs independently of lymphocytes, related to Figure 2.

(**A** and **B**) WT and *Rag2*^{-/-} mice received Ova or Pap on days 0 and 7 as indicated. Scratching after cheek injection (**A**) and PDL2⁺CD301b⁺ DC numbers in popliteal dLNs on day 8 after footpad injection (**B**) are shown.

Statistical tests: two-way ANOVA with Tukey's multiple comparisons test (**A**) and one-way ANOVA with Welch's correction (**B**). ***p* < 0.01; ****p* < 0.001. Data are pooled from three independent experiments in (**A**) and two independent experiments in (**B**). Each symbol represents an individual mouse. Violin plots show median and quartiles; bar graphs show mean ± SD.

Figure S3

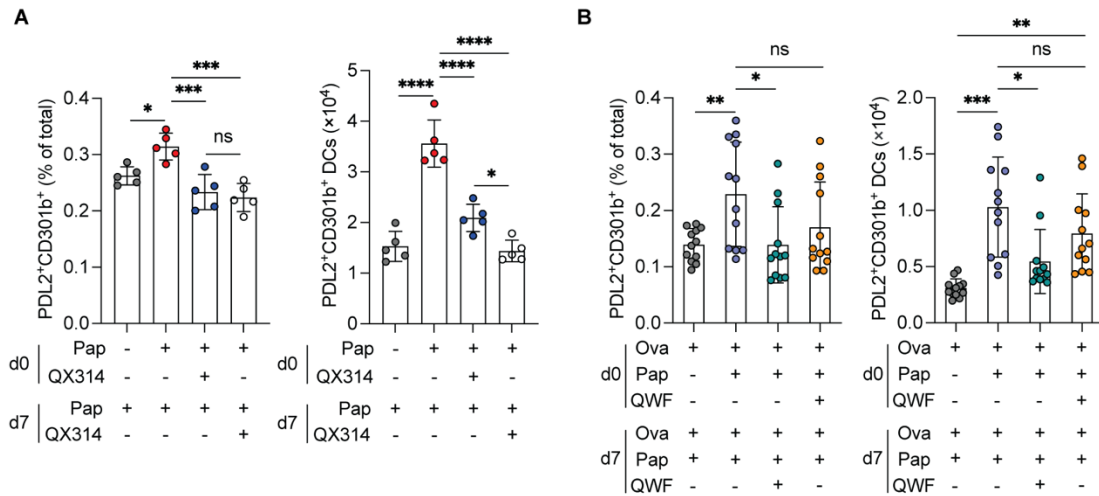


Figure S3. Allergen-induced enhancement of DC migration requires initial neuronal activation, related to Figure 3.

(**A** and **B**) WT mice received i.d. footpad injections on days 0 and 7 as indicated. Frequency and number of PDL2⁺CD301b⁺ DCs in the popliteal dLNs are shown 22-24 h after the day 7 injections of Pap with or without QX314 (**A**) or Ova and Pap with or without QWF (**B**).

Statistical analysis: one-way ANOVA with Tukey's multiple comparisons test (**A**); and one-way ANOVA with Welch's correction (**B**). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant. Data in (**A**) are representative of three independent experiments; data in (**B**) are pooled from three independent experiments. Each symbol represents an individual mouse. Bar graphs show mean $\pm$ SD.

Figure S4

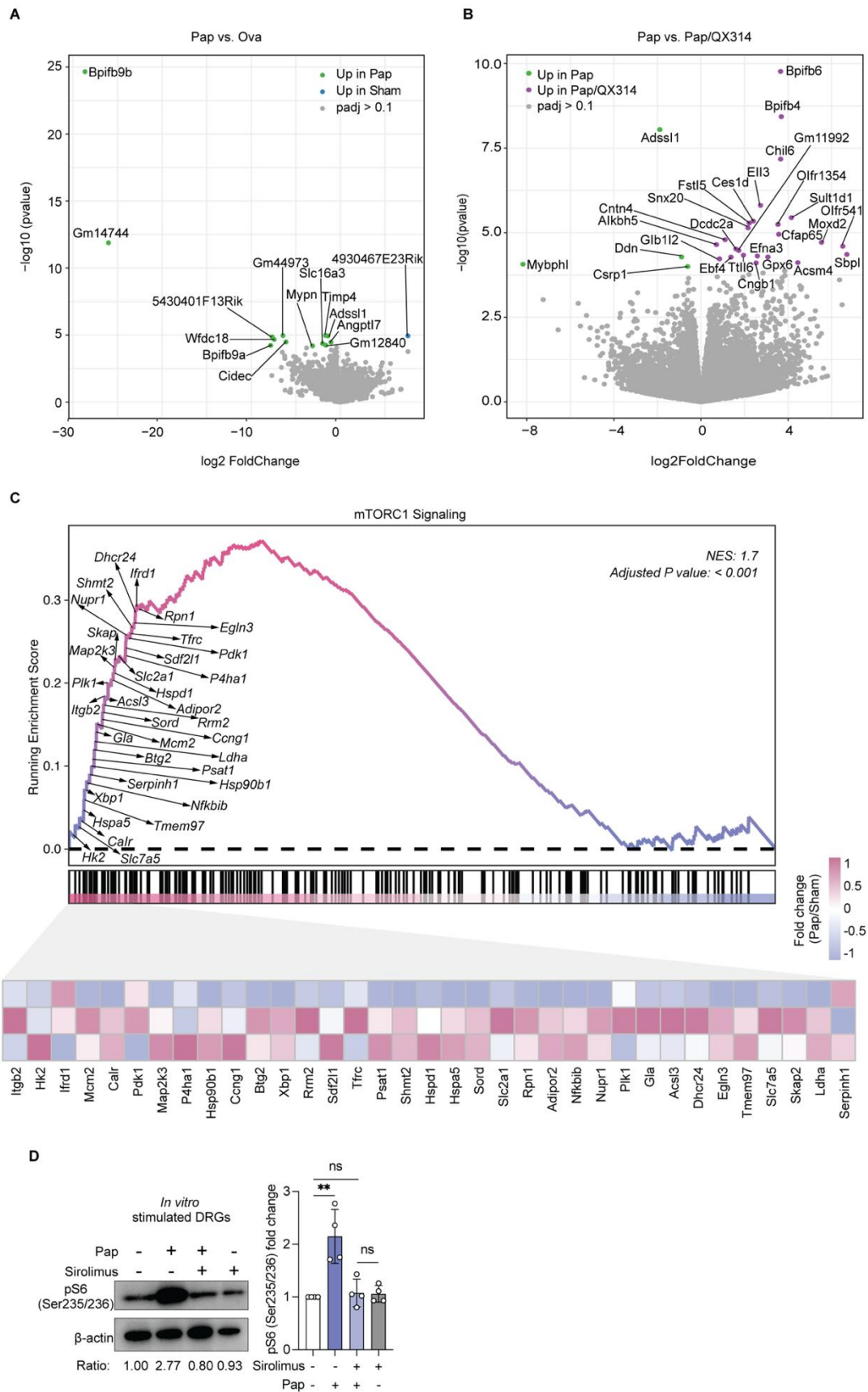


Figure S4. Protease allergen-stimulated sensory neurons exhibit mTORC1 activation, related to Figure 4.

(A and B) Volcano plots showing differentially expressed genes in pooled trigeminal ganglia (TG) 7 days after a single i.d. cheek injection with ovalbumin (Ova), papain (Pap), or Pap co-administered with QX314 (Pap/QX314). Pap vs. Ova (A) and Pap vs. Pap/QX314 (B) are shown. Volcano plots show $-\log_{10}(p \text{ value})$ versus $\log_2$ fold change. Genes with adjusted p values ≤ 0.1 are colored, and selected differentially expressed genes are labeled.

(C) Gene set enrichment analysis (GSEA) identified enrichment of the mTORC1 signaling pathway in Pap versus Ova TG neurons. The enrichment plot shows the distribution of the top 35 signature genes involved in the mTORC1 signaling pathway, and the heatmap shows their relative expression (fold change was calculated as the per-gene counts in each papain-exposed TG neuron sample divided by the mean gene counts of OVA-exposed TG neurons). Fold change values in the heatmap were normalized by row. NES, normalized enrichment score.

(D) Representative immunoblot (left) and quantification (right) of pS6 (Ser235/236) levels in dorsal root ganglia (DRG) neurons stimulated *in vitro* with or without papain and sirolimus. pS6 was normalized to β -actin.

Statistical tests: two-tailed ratio paired t -test (D), $**p < 0.01$; ns, not significant. Bulk RNA-seq in (A-C) was performed with three independent replicates per group. Each symbol in (D) represents an independent experiment. Bar graphs show mean $\pm$ SD.

Figure S5

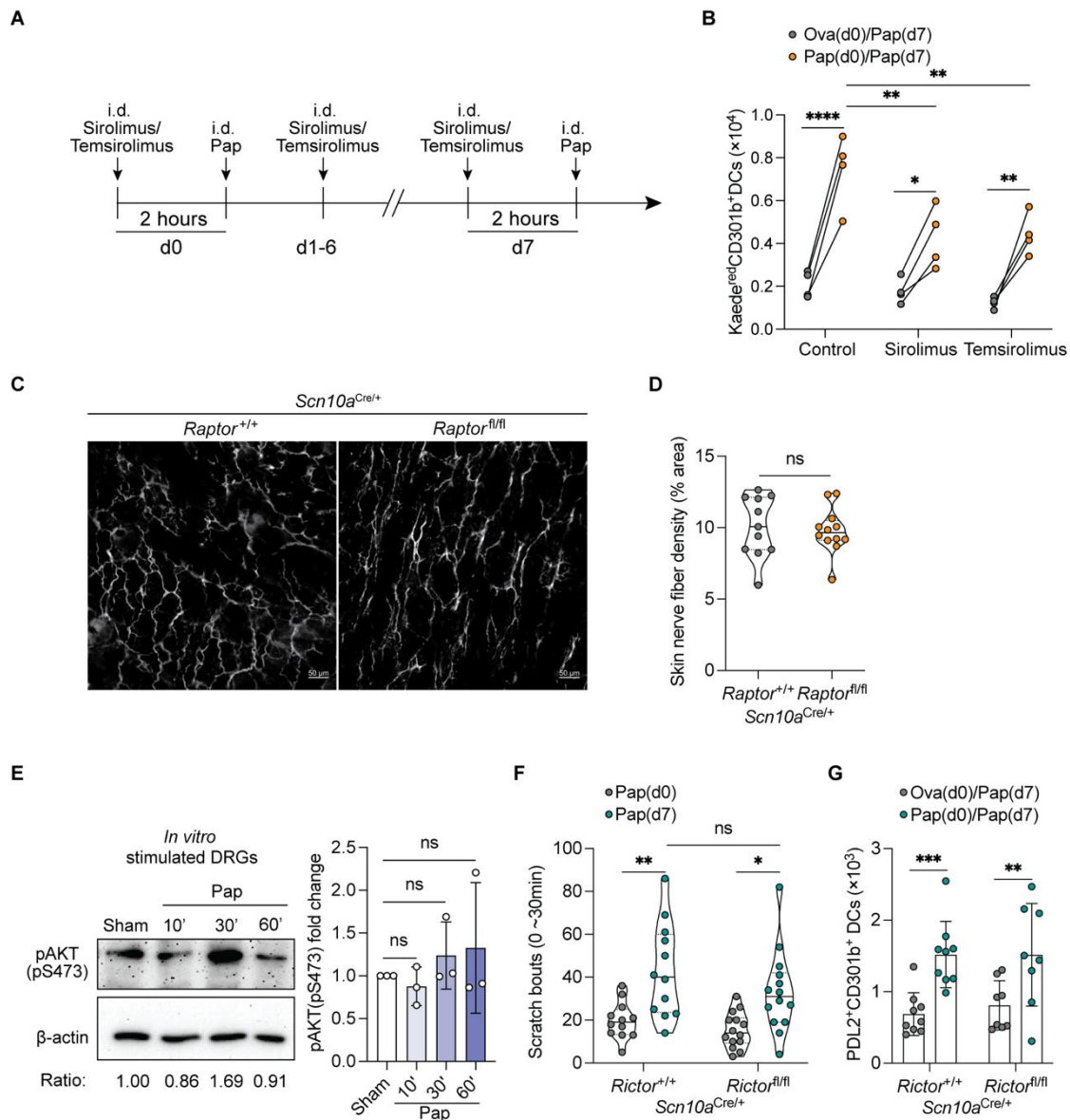


Figure S5. Sensory neuronal mTORC1 activation is required for allergen-induced neuroimmune memory, related to Figure 5.

(A) Schematic of sirolimus or temsirolimus treatment protocol for assessing behavioral responses and DC migration. WT mice received i.d. cheek (for behavioral assays) or footpad (for DC migration) injections of sirolimus, temsirolimus, or vehicle 2 h before papain injection on day 0, followed by daily dosing on days 1-6 and a final dose 2 h before papain injection on day 7. Scratching bouts were assessed after cheek injection, and DC migration into the popliteal dLNs was analyzed 22-24 h after day 7 injections.

(B) Kaede mice were treated with vehicle, sirolimus, or temsirolimus as in **(A)** and received i.d. footpad injections of Ova or Pap on day 0. The initial injection site was photoconverted

immediately before Pap injection on day 7. The total number of Kaede^{Red}CD301b⁺ DCs in the popliteal dLN was quantified by flow cytometry 22-24 h after injections on day 7.

(C) Representative confocal microscopy z-stacks showing Tuj1 staining in the skin of *Scn10a*^{Cre/+}*Raptor*^{+/+} and *Scn10a*^{Cre/+}*Raptor*^{fl/fl} mice. The scale bar is 50 μ m.

(D) Quantification of skin nerve fiber density. Each symbol represents an individual image; images were collected from 4 mice per group.

(E) Representative immunoblot (left) and quantification (right) of pAKT (Ser473) levels in DRG neurons stimulated *in vitro* with Pap. pAKT was normalized to β -actin.

(F and G) *Scn10a*^{Cre/+}*Rictor*^{+/+} and *Scn10a*^{Cre/+}*Rictor*^{fl/fl} mice received injections on days 0 and 7 as indicated. Scratching after cheek injection (F) and PDL2⁺CD301b⁺ DC numbers in popliteal dLNs 22-24 h after i.d. footpad injections (G) are shown.

Statistical tests: two-way ANOVA with Tukey's multiple comparisons test (B, F, G); two-tailed unpaired *t*-test (D); two-tailed ratio paired *t*-test (E). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Data in (D), (F), and (G), are pooled from three independent experiments. Data in (B) and (E) are representative of three independent experiments. Each symbol represents an individual mouse, unless otherwise indicated; each symbol in (E) represents an independent experiment. Violin plots show the median and quartiles; bar graphs show mean $\pm$ SD.

Figure S6

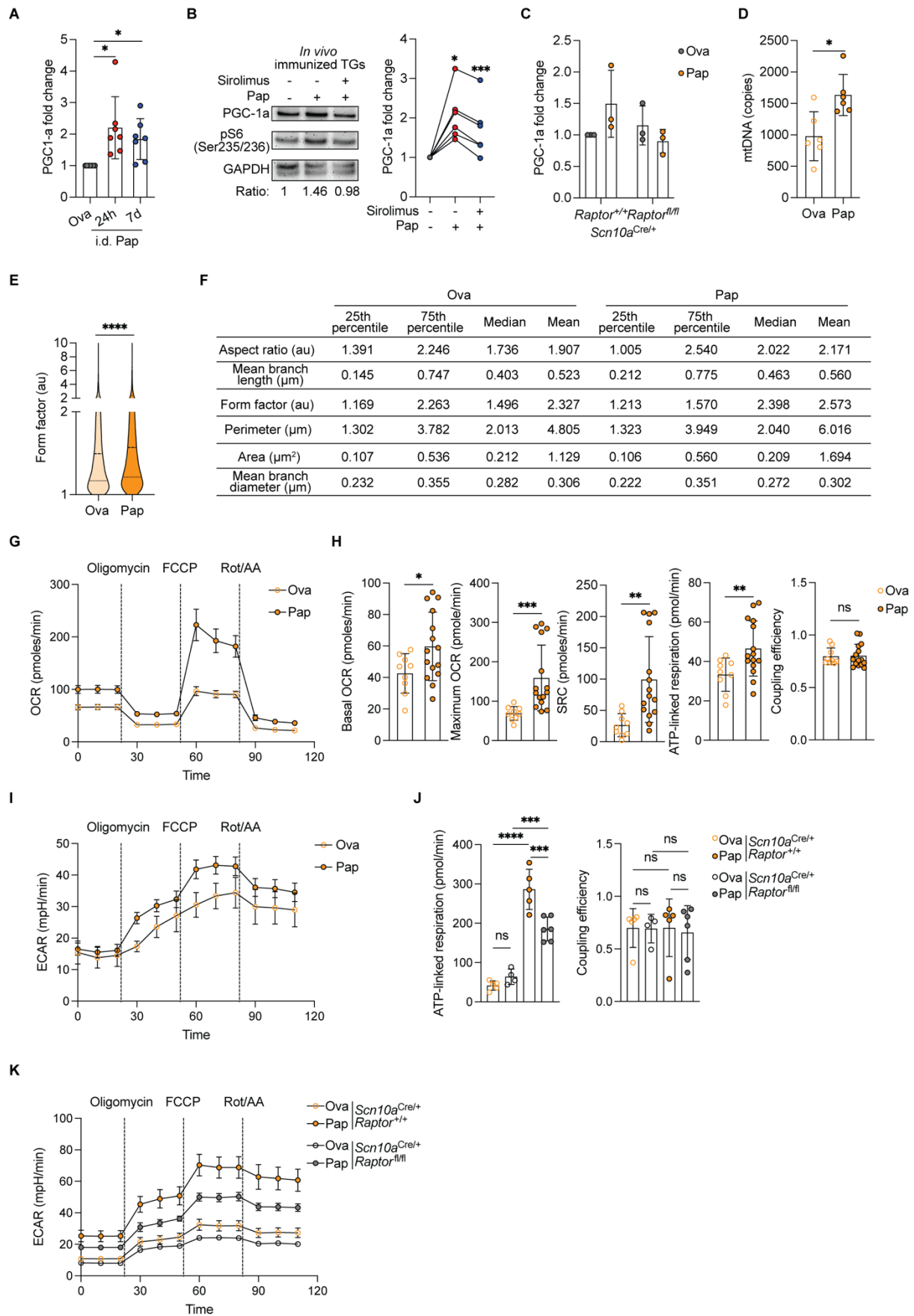


Figure S6. mTORC1 promotes mitochondrial function required for allergen-induced neuroimmune memory, related to Figure 6.

(A) PGC-1 α protein levels in TG neurons from WT mice 24 h or 7 days after i.d. cheek injection of Ova or Pap.

(B) Representative immunoblots of PGC-1 α and pS6 (Ser235/236) and quantification of PGC-1 α in TG neurons from WT mice 24 h after Pap injection with or without sirolimus treatment. PGC-1 α was normalized to GAPDH.

(C) PGC-1 α protein levels in TG neurons from *Scn10a*^{Cre/+}*Raptor*^{+/+} and *Scn10a*^{Cre/+}*Raptor*^{fl/fl} mice 24 h after Ova or Pap injection. Fold change values were normalized to Ova-treated control.

(D) Mitochondrial DNA (mtDNA) copy number in TG neurons isolated from WT mice 7 days after Ova or Pap cheek injections.

(E) Mitochondrial form factor in Ova- or Pap-primed TG neurons analyzed in Figure 6C using the Mitochondrial Analyzer ImageJ plugin. au, arbitrary units.

(F) Morphometric summary of the mitochondria analyzed in Figure 6C. Values show 25th and 75th percentiles, median, and mean for each parameter.

(G and H) Oxygen consumption rate (OCR) profiles of TG neurons from WT mice 7 days after Ova or Pap injections and assessed by Seahorse metabolic flux analysis. Responses to oligomycin, FCCP, and Rot/AA are shown (G), with corresponding statistical analysis in (H).

(I) Extracellular acidification rate (ECAR) profiles of TG neurons treated as in (G).

(J and K) ATP-linked respiration and coupling efficiency (J) and ECAR profiles (K) of TG neurons from *Scn10a*^{Cre/+}*Raptor*^{+/+} and *Scn10a*^{Cre/+}*Raptor*^{fl/fl} mice 7 days after Ova or Pap injection.

Statistical tests: one-way repeated measures ANOVA with Greenhouse-Geisser correction (A, C); one-way ANOVA with Tukey's multiple comparisons test (B, J); two-tailed unpaired *t*-test with Welch's correction (D-E, H); two-way repeated measures ANOVA with Tukey's multiple comparisons test (G, I, K). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Each symbol represents an independent experiment (A-C), each point in (D, H, J) represents an individual mouse. Data are pooled from independent experiments as follows: (E, F, G-I) 3 independent experiments; (J and K) 2 independent experiments. Panel D shows representative data from one experiment; similar results were obtained in 2 independent experiments. Violin plots show the median and quartiles; bar graphs show mean $\pm$ SD; line graphs in (B) show paired values from individual experiments; line graphs in (G, I, K) show mean $\pm$ SD.

Figure S7

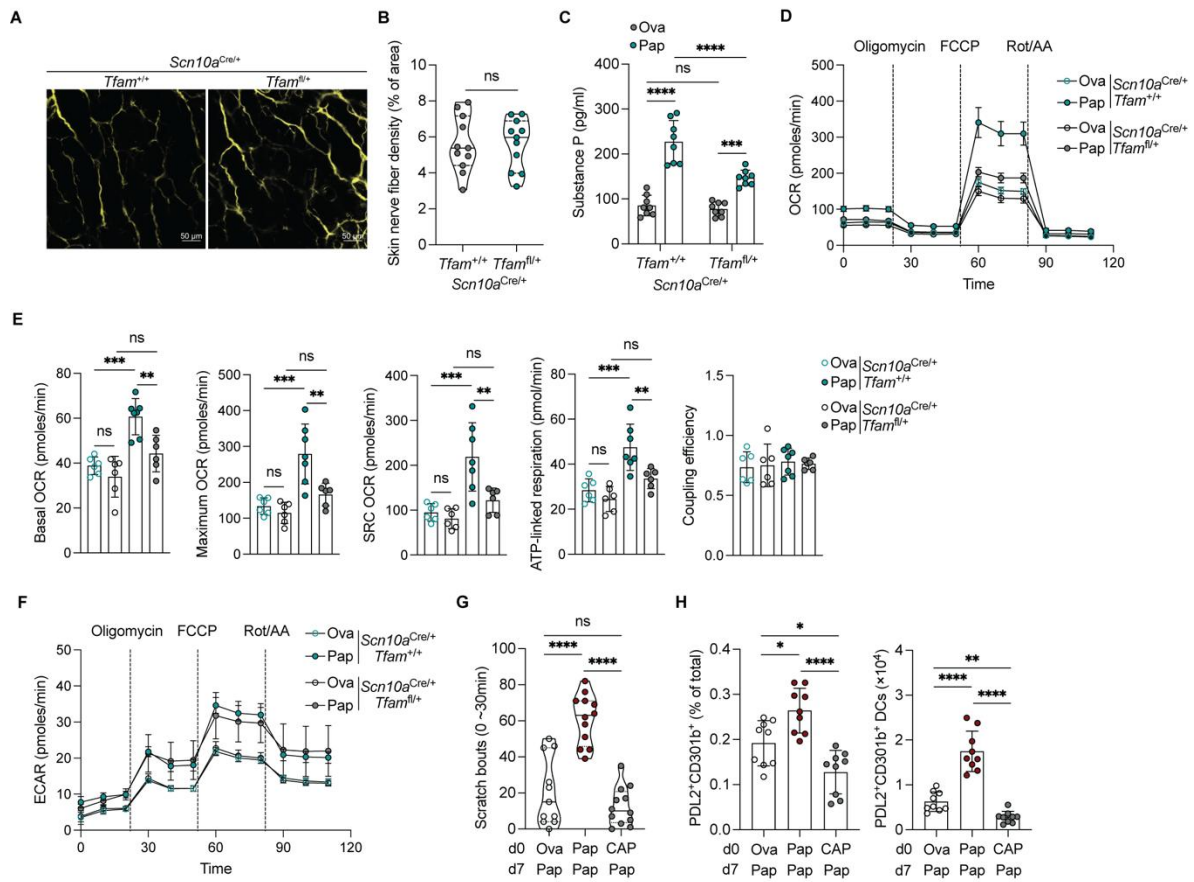


Figure S7. Mitochondrial stability is required for allergen-induced neuroimmune memory, related to Figure 6.

(A) Representative confocal z-stack images of Tuj1 staining in the skin of *Scn10a^{Cre/+} Tfam^{+/+}* and *Scn10a^{Cre/+} Tfam^{fl/+}* mice. Scale bars, 50 μ m.

(B) Quantification of skin nerve-fiber density. Each symbol represents one image; images were collected from four mice per group.

(C) Substance P (SP) in supernatants of Pap-restimulated TG neuron cultures from *Scn10a^{Cre/+} Tfam^{+/+}* and *Scn10a^{Cre/+} Tfam^{fl/+}* mice, harvested 7 days after intradermal (i.d.) injection of Ova or Pap.

(D and E) OCR profiles of TG neurons from mice treated as in (C) and assessed by Seahorse metabolic flux analysis following oligomycin, FCCP, and Rot/AA treatment (D), with corresponding quantification and statistical analysis (E).

(F) ECAR profiles of TG neurons treated as in (D).

(G and H) WT mice received Ova, Pap, or capsaicin (CAP) on day 0 and Pap on day 7 as indicated. Scratching bouts after i.d. cheek injection (G) and frequencies and numbers of PDL2⁺CD301b⁺ DCs in the popliteal dLNs 22-24 h after i.d. footpad injections (H) are shown.

Statistical tests: two-tailed unpaired *t*-test with Welch's correction (B); two-way ANOVA with

Tukey's multiple comparisons test (**C**); one-way ANOVA with Tukey's multiple comparisons test (**E**); one-way ANOVA with Welch's correction (**G**, **H**). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant. Data are pooled from independent experiments as follows: (**A-F**, **H**) 2 independent experiments; (**G**) 3 independent experiments. Each symbol represents an individual mouse unless otherwise indicated. Violin plots show median and quartiles; bar graphs show mean $\pm$ SD; line graphs show mean $\pm$ SD.

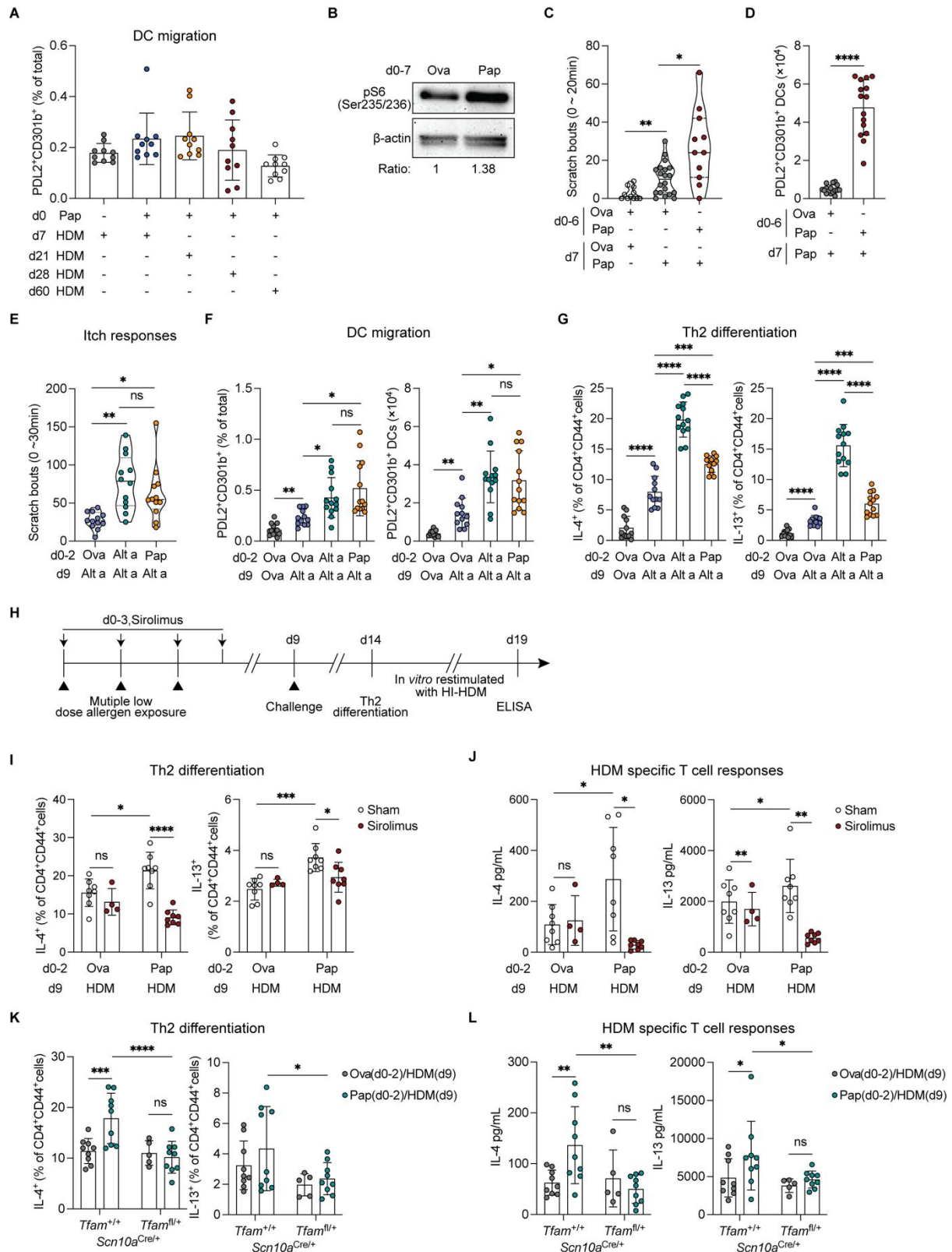


Figure S8: Allergen-induced neuroimmune memory promotes responsiveness to recurrent low-dose allergen exposure in an antigen-independent manner, related to Figure 7.

(A) WT mice received Pap on day 0 and house dust mite (HDM) challenge at the indicated

time. Frequencies of PDL2⁺CD301b⁺ DCs in the popliteal dLNs were quantified 22-24 h after secondary injections.

(B) Representative immunoblot of pS6 (Ser235/236) in TG neurons from WT mice following daily Ova or Pap injections from days 0-7. pS6 was normalized to β -actin.

(C) Scratching bouts after day 7 cheek injection in WT mice exposed to Ova or Pap on days 0-6 and Ova or Pap on day 7 as indicated.

(D) PDL2⁺CD301b⁺ DC numbers in popliteal dLNs 22-24 h after day 7 Pap footpad challenge in WT mice exposed to Ova or Pap on days 0-6.

(E-G) WT mice received Ova, *Alternaria alternata* extract (Alt a), or Pap on days 0-2 and Ova or Alt a on day 9 as indicated. Scratching bouts after cheek challenge **(E)**, frequencies and numbers of PDL2⁺CD301b⁺ DCs in popliteal dLNs 22-24 h after footpad challenge **(F)**, and frequencies of IL-4⁺ and IL-13⁺ cells among CD4⁺CD44⁺ T cells on day 14 **(G)** are shown.

(H) Sirolimus-treatment protocol for the recurrent low-dose allergen exposure model. WT mice received vehicle or sirolimus 2 h before allergen exposure on days 0-2 and an additional dose on day 3, followed by challenge on day 9. Th2 cell differentiation was assessed on day 14, and HDM-specific cytokine production was assessed after heat-inactivated HDM (HI-HDM) restimulation.

(I and J) WT mice received Ova or Pap on days 0-2 and HDM on day 9 and were treated with vehicle or sirolimus as in **(H)**. Frequencies of IL-4⁺ and IL-13⁺ CD4⁺CD44⁺ T cells on day 14 **(I)** and IL-4 and IL-13 production after HI-HDM restimulation of dLN cells **(J)** are shown.

(K and L) *Scn10a*^{Cre/+}*Tfam*^{+/+} and *Scn10a*^{Cre/+}*Tfam*^{fl/+} mice received Ova or Pap on days 0-2 and HDM on day 9. Frequencies of IL-4⁺ and IL-13⁺ CD4⁺CD44⁺ T cells in the popliteal dLNs on day 14 **(K)** and IL-4 and IL-13 production after HI-HDM restimulation of dLN cells **(L)** are shown.

Statistical analysis: one-way ANOVA with Welch's correction **(C, E-G)**; two-tailed unpaired *t*-test with Welch's correction **(D)**; two-way ANOVA with Tukey's multiple comparisons test **(I-L)**. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant. Data are pooled from independent experiments as follows: **(A, I-L)** 2 independent experiments; **(C-G)** 3 independent experiments. **(B)** is representative of 2 independent experiments. Each symbol represents an individual mouse. Violin plots show median and quartiles; bar graphs show mean $\pm$ SD.

Table S1. Genes with significant changes in the Pap group compared to the Ova group, related to Figure 4 and Figure S4.

Ensembl id	Gene name	Log2 Fold Change	Padj
ENSMUSG00000067996	Bpifb9b	-28.64685615	4.8404E-21
ENSMUSG00000079522	Gm14744	-26.01174197	1.4214E-08
ENSMUSG00000067998	Bpifb9a	-7.832501272	0.098545577
ENSMUSG00000094113	5430401F13Rik	-7.669237713	0.042343459
ENSMUSG00000000983	Wfdc18	-7.425704115	0.058533276
ENSMUSG00000109129	Gm44973	-6.448664583	0.041893189
ENSMUSG00000030278	Cidec	-6.110942803	0.074629886
ENSMUSG00000020067	Mypn	-3.129987684	0.099502886
ENSMUSG00000025161	Slc16a3	-2.015184048	0.084969459
ENSMUSG00000030317	Timp4	-1.669565406	0.041893189
ENSMUSG00000086320	Gm12840	-1.648854478	0.098545577
ENSMUSG00000011148	Adssl1	-1.410895602	0.041893189
ENSMUSG00000028989	Angptl7	-1.06219617	0.074629886
ENSMUSG00000096265	4930467E23Rik	7.563724755	0.041893189

Table S2. Genes with significant changes in the Pap group compared to the Pap/QX314 group, related to Figure 4 and Figure S4.

Ensembl id	Gene name	Log2 Fold Change	Padj
ENSMUSG00000068745	Mybphl	-8.174298805	0.086869462
ENSMUSG00000011148	Adssl1	-1.892529047	8.18221E-05
ENSMUSG00000059213	Ddn	-0.893404183	0.062934233
ENSMUSG00000026421	Csrp1	-0.612726418	0.098558689
ENSMUSG00000042650	Alkbh5	0.708914294	0.044068459
ENSMUSG00000036395	Glb1l2	0.851266202	0.067922579
ENSMUSG00000064293	Cntn4	1.104214982	0.036791624
ENSMUSG00000053552	Ebf4	1.377028494	0.062934233
ENSMUSG00000035910	Dcdc2a	1.605668888	0.052045988
ENSMUSG00000040978	Gm11992	1.730921761	0.054310088
ENSMUSG00000038756	Tll6	1.949044722	0.062934233
ENSMUSG00000031662	Snx20	2.160331246	0.019772283
ENSMUSG00000034098	Fstl5	2.213739447	0.017336648
ENSMUSG00000056973	Ces1d	2.401269865	0.017336648
ENSMUSG00000031789	Cngb1	2.527419195	0.082744956
ENSMUSG00000028039	Efna3	2.590732543	0.062934233
ENSMUSG00000027246	Eil3	2.731096286	0.008559875
ENSMUSG00000004341	Gpx6	3.072736528	0.062934233
ENSMUSG00000094673	Olfr1354	3.536006739	0.017336648
ENSMUSG00000047021	Cfap65	3.572849738	0.027778915
ENSMUSG00000068009	Bpifb6	3.654785331	4.67847E-06
ENSMUSG00000027902	Chil6	3.656895403	0.000457565
ENSMUSG00000074665	Bpifb4	3.688802149	5.09902E-05
ENSMUSG00000029273	Sult1d1	4.151079096	0.016434241
ENSMUSG00000047026	Acsm4	4.445290602	0.082744956
ENSMUSG00000029885	Moxd2	5.534619866	0.040729649
ENSMUSG00000057997	Olfr541	6.512282733	0.046196584
ENSMUSG00000024125	Sbpl	6.698008125	0.062934233