

LXR is an epithelial transcriptional checkpoint that restrains mucosal type 2 immunity

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LXR is an epithelial transcriptional checkpoint that restrains mucosal type 2 immunity

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Abstract

Protective type 2 immunity requires robust amplification during helminth infection, yet the epithelial mechanisms that restrain this feed-forward response remain poorly understood. Here, we identify the oxysterol-sensing nuclear receptor liver X receptor (LXR) as an epithelial transcriptional checkpoint that limits mucosal type 2 immunity. Pharmacological LXR activation reduced tuft cell and group 2 innate lymphoid cell (ILC2) expansion at steady state and following succinate stimulation. ILC2 responsiveness remained intact, as recombinant IL-25 fully restored tuft cell and ILC2 expansion, placing LXR upstream of IL-25-dependent circuit amplification. Consistently, epithelial-specific deletion of LXR α and LXR β abolished the suppressive effects of LXR activation, demonstrating that epithelial LXR signaling is required to restrain the tuft cell-ILC2 circuit. Single-cell transcriptomic analysis further revealed that LXR preserves canonical tuft cell sensory programs while selectively limiting maturation toward the immune-associated tuft-2 state, thereby restricting inducible circuit amplification without disrupting epithelial chemosensing. Functionally, LXR activation impaired protective immunity against helminth infection, resulting in increased parasite burden. Together, these findings identify LXR as an epithelial transcriptional checkpoint that establishes the activation threshold of the tuft cell-ILC2 circuit, revealing a mechanism by which metabolic sensing controls mucosal type 2 immunity through regulation of epithelial cell states.

Summary:

LXR activation acts through the intestinal epithelium to limit inducible tuft cell expansion and downstream ILC2 amplification, with impaired helminth control as a functional consequence.

Keywords: Type 2 immunity; tuft cells; Liver X receptors; small intestine; helminth infection;

INTRODUCTION

The intestinal mucosa serves as the frontline defense against pathogens, undergoing rapid cellular adaptation during parasitic helminth infection. Classically, type 2 immunity is boosted, with type 2 helper cells (Th2) and group 2 innate lymphoid cells (ILC2) serving as the central effectors in pathogen clearance¹⁻³. Among the diverse cell types that orchestrate intestinal immunity, tuft cells have emerged as key sentinels of the intestinal epithelium. These cells express a repertoire of chemosensory receptors coupled to canonical taste-transduction machinery, including TRPM5, GNAT3 and PLC β 2⁴⁻⁹, to detect microbial and parasite-associated metabolites. Upon stimulation, tuft cells produce alarmins such as Interleukin-25 (IL-25), cysteinyl leukotrienes (CysLTs), and thymic stromal lymphopoietin (TSLP), initiating a cascade in which activated ILC2s release IL-4, IL-5, and IL-13¹⁰⁻¹². In turn, ILC2-derived IL-13 promotes further tuft cell expansion, forming a feed-forward circuit that amplifies intestinal type 2 immunity. This tuft-ILC2 circuit serves as a critical mechanism for the "weep and sweep" response via enhancing mucus production and smooth muscle contraction which ultimately drive helminth expulsion^{10,13-16}. While tuft cells and type 2 immunity are essential elements for protective immunity, their dysregulation contributes to pathological type 2 inflammation^{12,17,18}. Signals that activate this circuit are well characterized, however less is known about the mechanisms that restrain its amplification. Recent studies have begun to uncover intrinsic regulatory mechanisms within tuft cells, implicating pathways such as IL-17RB signaling and SpiB transcription factor-dependent transcriptional control in maintaining immune homeostasis^{12,19}. However, it remains largely unknown what upstream cues, beyond these intrinsic factors, keep tuft cells in check, thereby setting the threshold for type 2 immunity.

Nuclear receptors are uniquely positioned to translate metabolic state into transcriptional programs that calibrate immune activation thresholds. Among them, the cholesterol-sensing receptor Liver X receptors (LXRs)^{20,21} has emerged as a regulator of intestinal homeostasis. LXR signaling shapes mucosal adaptive immunity by controlling Th17 and ROR γ t⁺ regulatory T-cell responses and promotes epithelial regeneration while uncoupling tissue repair from tumorigenesis^{21,23}. More broadly, other lipid-responsive nuclear receptors, including retinoid X receptors (RXRs)^{24,25} and farnesoid X receptor (FXR)²⁶, have been shown to modulate epithelial immunity and ILC2 activation thresholds²⁶, suggesting that metabolic sensing may represent a broader mechanism for

regulating barrier immune responses. Whether LXR similarly regulates epithelial initiation of type 2 immunity has remained unknown.

Here, we hypothesize that nuclear receptors control the tuft cell-ILC2 circuit balance to adapt to the rapid environmental changes in the intestine. We report that LXR activation acts on the epithelial compartment to interfere with tuft cell state transition and disrupts the amplification of the tuft cell-ILC2 circuit upon succinate exposure, thus restraining type 2 immune response. LXR disruption of the tuft cell-ILC2 circuit was rescued by exogenous rIL-25 and depletion of LXR in the intestinal epithelial compartment failed to disrupt the circuit. Single-cell transcriptomics showed altered composition of tuft cell states, suggesting reduced preferential expansion of immune-associated tuft-2 cells. In addition, LXR-mediated disruption of the tuft cell-ILC2 circuit leads to a failure in mounting the appropriate anti-helminth response, which can be rescued by exogenous rIL-25. Together, our findings identify epithelial LXR signaling as a master regulator of inducible tuft cell–ILC2 circuit amplification, linking endogenous lipid sensing to anti-helminth defense.

RESULTS

LXR activation in the small intestine transcriptionally affects lymphoid cells differentiation.

To define how systemic LXR activation reshapes the intestinal immune landscape, we performed spatial and transcriptional profiling of the small intestine following pharmacologic LXR stimulation *in vivo*. Wild type mice were fed a diet containing the synthetic LXR agonist GW3965 or control diet for 10 days as previously described^{22,23,27}. Quantitative PCR analyzing transcription from intestinal biopsies confirmed robust induction of *Abca1*, a proxy for LXR activation²⁸, across all intestinal segments in GW3965-treated animals (**Fig. 1a**). Spatial transcriptomics (ST) analysis in Swiss rolls encompassing the ileum to jejunum^{29,30}, revealed a homogenous induction of *Abca1*, *Abcg1* and, *Srebf1* in the SI upon ectopic LXR activation (**Fig. 1b**). To assess whether LXR activation modulates immune differentiation programs at a systems level, we performed gene set enrichment analysis (GSEA)³¹ on distal small intestinal biopsies. GSEA using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database³² revealed down-regulation of genes associated with T helper cell differentiation, including Th2 in GW3965-fed mice (**Fig. 1c**). Similarly, proximal small intestine samples showed suppression of primary immunodeficiency related pathways (**Extended Data Fig.1 a-b**), collectively indicating that ectopic LXR activation broadly modulates lymphocyte-associated transcriptional programs along the small intestine.

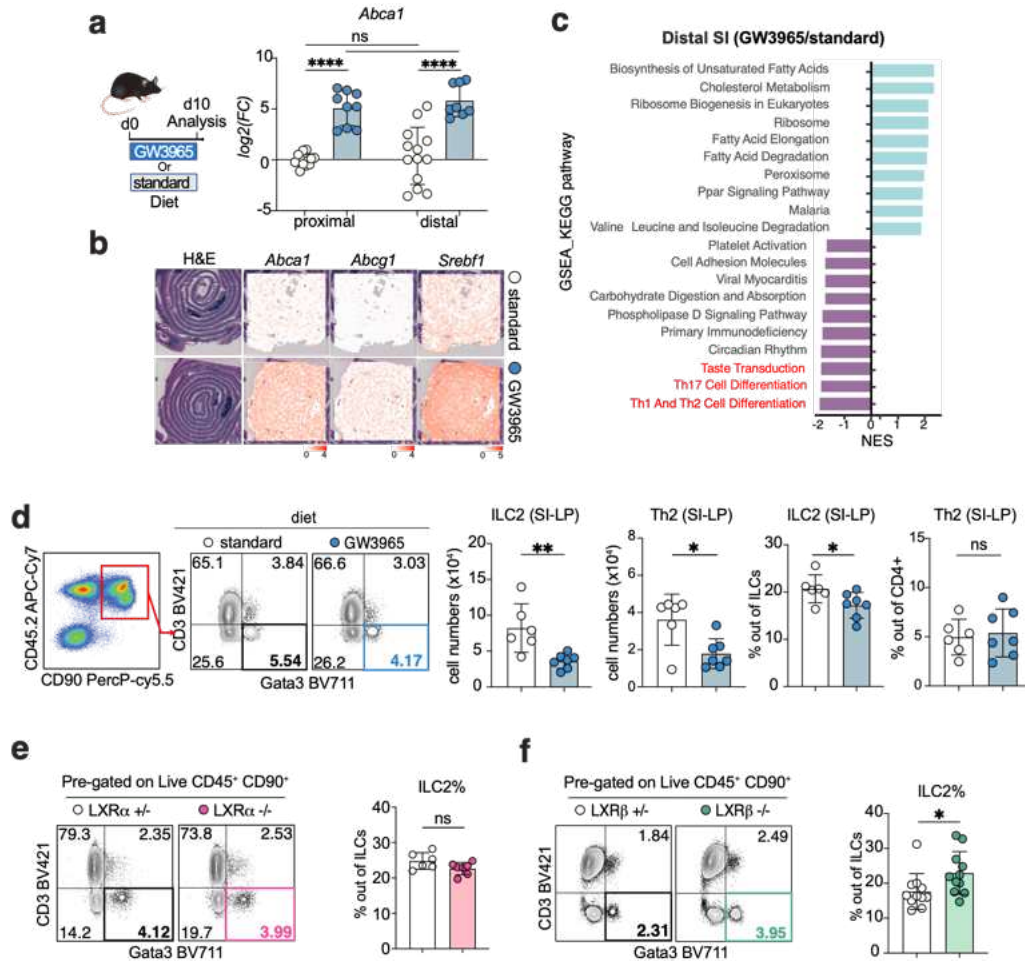
Figure 1

Fig. 1. Ectopic liver X receptor (LXR) activation diminishes type 2 lymphocytes in the small intestine. **a**, Schematic representation of the experimental design and qPCR analysis of *Abca1* expression, normalized to *Hprt*, in proximal and distal small-intestinal biopsies from mice fed with GW3965 or standard diet. **b**, Hematoxylin and eosin (H&E) staining and spatial transcriptomic analysis showing *Abca1*, *Abcg1*, and *Srebf1* expression in the proximal small intestine. **c**, Gene set enrichment analysis (GSEA) of Kyoto Encyclopedia of Genes and Genomes for (KEGG) pathway (both up and down-regulated, $P < 0.01$) between GW3965-treated and control animals during homeostasis. **d-f**, Flow cytometric analysis of small-intestinal lamina propria cell suspensions. Representative flow cytometry plots and quantification of the proportions and numbers of Th2 cells and ILC2s in the small-intestinal lamina propria (**d**) and quantification of ILC2 proportions in LXR α -deficient (**e**) or LXR β -deficient (**f**) mice compared with their respective control littermates under homeostatic conditions. Where applicable in (**a** and **d-f**), each dot represents one biological replicate (one mouse), unless otherwise specified. Data were pooled from two or more independent experiments ($n = 3-13$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using an unpaired Student's *t*-test for comparisons between two groups or one-way ANOVA followed by Tukey's multiple-comparisons test when more than two groups were compared.

LXR modulates ILC2 numbers in the small intestine.

The modulation of T helper subsets by LXR is consistent with previous findings showing reduced Th17 and ROR γ t⁺ regulatory T cells (Treg) upon ectopic LXR activation^{23,33–35}. Since the role of LXR in modulating type 2 immunity has not been previously addressed, we focused our analysis on Th2 and group 2 innate lymphoid cells (ILC2). Flow cytometry analysis of the whole small intestine lamina propria (SI-LP) revealed diminished ILC2 (CD45⁺ CD90⁺ CD3^{neg} GATA3⁺) and Th2 (CD45⁺ CD90⁺ CD3⁺ CD4⁺ GATA3⁺) numbers after 10 days of exposure to the GW3965 diet compared to standard-diet fed animals (**Fig. 1d and Extended Data Fig. 2a**). While the frequencies of Th2 out of T helper cells (CD45⁺ CD90⁺ CD3⁺ CD4⁺) remain comparable, ILC2 frequencies out of total ILCs (CD45⁺ CD90⁺ CD3^{neg}) were reduced in the SI-LP from GW3965-fed compared to standard-fed counter parts (**Fig. 1d**). Type 2 immune responses are characterized by the production of IL-5 and IL-13, which are predominantly attributed to ILC2s during intestinal homeostasis^{10,36,37}. Correspondingly, we detected IL-13 and IL-5 to be produced mostly by CD90⁺ CD3^{neg} ILCs (**Extended Data Fig. 2b and c**), which was significantly reduced upon ectopic LXR activation *in vivo* (**Extended Data Fig. 2c**). Such reduction in IL5⁺ IL13⁺ ILCs was comparable between B6 and BALB/c mice (**Extended Data Fig. 2d**), indicating that LXR-mediated repression of ILC2 numbers did not depend on the genetic background. We then sought to investigate which LXR isoform, namely LXR α (*Nr1h3*) and LXR β (*Nr1h2*), account for ILC2 regulation. Using publicly available spatial transcriptomics (Visium HD)³⁸ and single cell²² analysis of the small intestine we found that both isoforms are expressed in epithelial cells and lamina propria (**Extended Data Fig. 3a**). Specifically, our previous single cell data (22) showed that LXR β and LXR α both express in immune, stromal and epithelial compartments in the small intestine, but LXR β is the dominant isoform, displaying an enriched expression level in the immune compartment (**Extended Data Fig. 3b and c**). To ascertain whether LXR α , LXR β or both isoforms are required to modulate type 2 lymphocytes in the small intestine, we examined the Th2 and ILC2 in either LXR α - or LXR β -deficient mice. While LXR α -deficient mice showed comparable Th2 and ILC2 frequencies and numbers compared to their control littermates (**Fig. 1e and Extended Data Fig. 3d**), LXR β -deficient mice exhibited higher Th2 and ILC2 frequencies compared to their littermate controls without altering cytokine production (**Fig. 1f and Extended Data Fig. 3e**), suggesting that overall type 2 immunity is predominantly regulated by LXR β in the

LXR signaling modulates intestinal tuft cell homeostasis.

While the pathway analysis revealed a significant enrichment in lymphocyte differentiation, we next sought to examine the specific differentially expressed genes (DEGs) underpinning this transcriptional immune remodeling. First, we analyzed DEGs from the RNA-seq analysis on both proximal and distal SI biopsies from GW3965- and Standard-fed mice. The most upregulated genes in GW3965-exposed SI compared to control, included known LXR target genes *Abcg1*, *Abca1*, and *Srefb1* (**Fig. 2a and Extended Data Fig. 1b**). Among the distal SI downregulated genes was *Gnat3* (**Fig. 2a**), which is highly expressed in mouse and human tuft cells, and acts as an essential signal transduction element for the production of alarmins sensed by ILC2^{8,39}. Given that ectopic LXR activation inhibited the expression of genes associated with the “taste transduction” pathway (**Fig. 1c**) and significantly decreased *Gnat3* transcript levels compared to controls (**Fig. 2a**), we hypothesized that LXR activation compromises tuft cell homeostasis. Analysis of the RNA-seq dataset for tuft cell signature genes, such as *Dclk1*, *Pclb2*, *Gnat3*, *Trpm5*, *Pou2f3* and *Sucnr1*⁸ showed a tendency for LXR activation to down-regulate tuft cell markers in the distal SI tissue compared to control samples (**Fig. 2b**). Reflecting the distal enrichment of tuft cells in the epithelium⁴⁰, alterations in the tuft cell markers *Gnat3*, *Sucnr1*, and *Trpm5* were more pronounced in the distal versus proximal small intestine of GW3965 and standard-fed mice (**Fig. 2c**). Flow cytometry analysis further confirmed a higher CD24⁺, doublecortin-like kinase 1 (DCLK1)⁺ tuft cell frequency out of total EpCAM⁺ IECs in the distal compared to the proximal SI from standard diet-fed animals (**Fig. 2d-e**)⁸, which was inhibited by GW3965 (**Fig. 2d-e**). Total tuft cells numbers by measuring DCLK1⁺ cells⁴¹ in tissue sections by IHC were instead decreased in distal compared to proximal small intestine, and this reduction was more pronounced in the presence of GW3965 (**Fig. 2f-g**), without affecting the number of DCLK1⁺ cells in the crypts (**Fig. 2h**). Further investigation into whether LXR α , LXR β or both were required to modulate DCLK1⁺ tuft cell numbers without GW3965 presence revealed an expansion of villus DCLK1⁺ tuft cells in LXR β -deficient animals compared to littermates (**Fig. 2i**), while no differences were found in LXR α -deficient animals compared to their littermate controls (**Fig. 2j**), indicating that DCLK1⁺ tuft cell homeostasis depends mostly on signaling through LXR β .

Figure 3

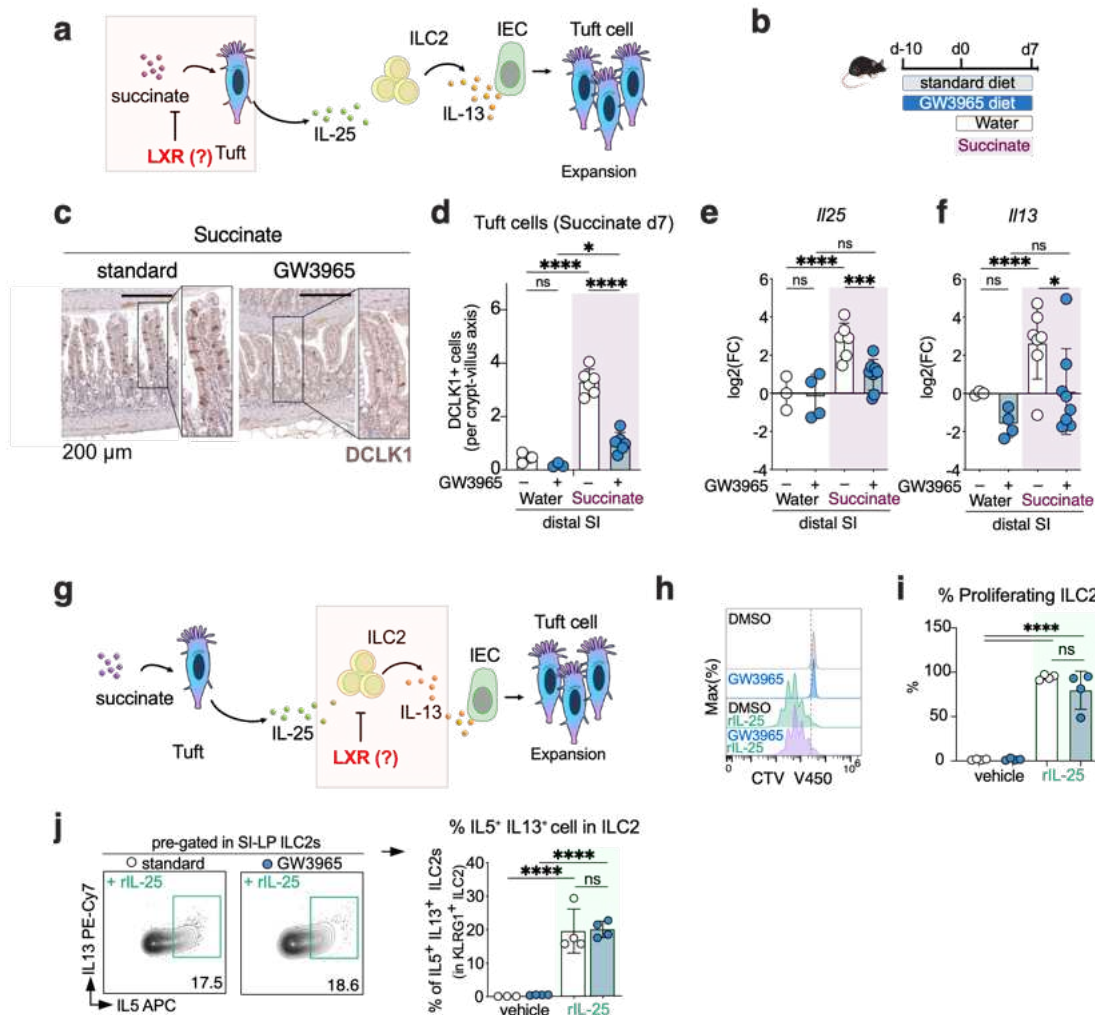


Fig. 3. LXR activation inhibits succinate-induced tuft cell hyperplasia without impairing ILC2 effector functions. **a**, Schematic representation of the ILC2–tuft cell axis and the proposed site of LXR action. **b**, Schematic representation of the experimental design. **c–d**, Representative images of DCLK1 immunohistochemical staining (**c**) and quantification of DCLK1⁺ cells per crypt–villus axis (**d**) in the distal small intestine. Scale bar, 200 μ m. **e–f**, qPCR analysis of *Il25* (**e**) and *Il13* (**f**) expression, normalized to *Hprt*, in distal small-intestinal biopsies from mice treated as depicted in (**b**). **g**, Schematic representation of the ILC2–tuft cell axis and the proposed site of LXR action. **h–i**, Representative CellTrace Violet histograms (**h**) and quantification of mean fluorescence intensity (MFI; **i**) in sorted ILC2s cultured for 3 days with DMSO or GW3965 in the presence or absence of recombinant IL-25 (rIL-25) *in vitro*. **j**, Representative flow cytometry contour plots and analysis of small-intestinal lamina propria cell suspensions from mice fed a GW3965-containing or standard diet and cultured *in vitro* overnight in the presence or absence of rIL-25. Data are from one experiment with 3–4 mice per group. In (**d–f**, **i**, and **j**), unless otherwise specified, each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 3–8$ mice), unless otherwise specified, and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Fisher’s least significant difference (LSD) test (**d–f**) or Tukey’s multiple-comparisons test (**i** and **j**).

LXR activation impairs succinate-induced tuft cell hyperplasia.

Having established that LXR restrains the tuft-ILC2 circuit at baseline, we next asked whether this checkpoint persists under conditions of strong inductive stimulation. Because succinate is a potent microbiota-derived activator of tuft cells^{7,8,42}, we tested whether exogenous succinate could bypass LXR-mediated suppression (**Fig. 3a**). Mice pretreated for 10 days with standard or GW3965-containing diets were challenged with succinate in the drinking water for 7 days, followed by quantification of tuft cells and their associated transcripts (**Fig. 3b**). In control animals, succinate robustly induced expression of tuft cell markers *Dclk1*, *Gnat3*, and *Sucnr1*, confirming effective activation of the tuft cell program (**Extended Data Fig. 4a**). In contrast, LXR-activated mice failed to upregulate these transcripts in response to succinate, indicating that LXR signaling renders tuft cells refractory to canonical agonist-driven activation (**Extended Data Fig. 4a**). Consistent with impaired downstream type 2 remodeling, succinate-induced upregulation of the goblet cell marker *Muc2* was also blunted by LXR activation (**Extended Data Fig. 4a**). Immunohistochemical analysis confirmed a robust expansion of DCLK1⁺ tuft cells in both proximal and distal small intestine following succinate treatment in control mice (**Fig. 3c-d**, **Extended Data Fig. 4b**). This expansion was markedly reduced in GW3965-treated mice (**Fig. 3c-d**, **Extended Data Fig. 4b**). Together, these findings demonstrate that LXR activation suppresses succinate-induced tuft cell hyperplasia. Notably, tuft cell expansion upon succinate challenge was comparable between LXR β -deficient and control mice (**Extended Data Fig. 4c-d**), indicating that while LXR β -deficiency increases tuft cell numbers at steady state, it does not alter their response to succinate stimulation. Of note, the reduction in tuft cell abundance upon GW3965 diet was paralleled by a significant decrease in *Il25* expression within the intestine (**Fig. 3e**). Consistent with a disruption of the upstream signal of ILC2 activation, we also observed a marked decrease in *Il13* expression (**Fig. 3f**), confirming that the tuft-ILC2 circuit was dampened following GW3965 diet upon succinate challenge.

LXR activation does not impair ILC2 response to IL-25.

The reduced tuft cell numbers and diminished *Il25* and *Il13* observed *in vivo* implicated LXR activation as a negative regulator of the tuft-ILC2 circuit. However, whether LXR acts directly on tuft cells, on ILC2s, or on both remained unclear. To investigate whether LXR activation intrinsically affects ILC2, sorted ILC2s (CD45⁺Lin⁻Klrg1⁺Sca1⁺) were stimulated *in vitro* with

recombinant IL-25 in the presence or absence of GW3965 (**Fig. 3g and Extended Data Fig. 6a**). LXR activation had no effect on ILC2 proliferation, CD25 expression, or capacity to produce IL-13 after 72 hours or 8 days rIL-25 stimulation (**Fig. 3h-i and Extended Data Fig. 5b-c**). Likewise, rIL-25 stimulation of SI-LP *ex-vivo* cultures after 10-day special diet treatments revealed a comparable level of IL-5 and IL-13 producing capacity in ILC2 (**Fig. 3j**), indicating LXR does not impair ILC2 fitness or responsiveness to *in vitro* IL-25 stimulation.

rIL-25 rescues LXR activation induced tuft cell inhibition.

IL-25 is a tuft cell-derived cytokine essential for initiation and amplification of succinate-induced type 2 immunity in the intestine^{10,11}. Given that LXR activation suppressed succinate-driven tuft cell expansion, we hypothesized that LXR functions upstream of IL-25 by limiting its production from tuft cells (**Fig. 4a**). To test this, we employed a functional rescue approach and asked whether administration of recombinant IL-25 could bypass the requirement for tuft cell-derived signaling and restore downstream immune activation in the context of LXR activation (**Fig. 4b**). To visualize IL-13-producing ILC2, we took advantage of the Smat13 reporter mouse line⁴³ combined with Ki67 intracellular staining enabling us to determine ILC2 proliferation, as a proxy of their activation. We observed that 4 days succinate stimulation already resulted in a 2.7-fold expansion of Sm13⁺ ILC2s (81.7% ± 17.7) and a 9.5-fold increase in proliferating ILC2s (76.5% ± 16.8) compared to untreated mice (30.5%±13.9 of Sm13⁺ ILC2, 8.0%±6.2 of Ki67⁺ ILC2) (**Fig. 4c-e**), whereas, mice fed with GW3965 diet showed a 1.9-fold decrease in Sm13⁺ ILC2s (43.2% ± 11.0) and a 2.2-fold decrease in proliferating ILC2s (34.1% ± 10.8) compared to succinate-exposed mice fed with a standard diet (**Fig. 4c-e**). Notably, rIL-25 administration promoted proliferating Sm13⁺ ILC2, regardless of ectopic LXR activation by the GW3965 diet, at levels comparable to succinate exposed controls animals (**Fig. 4e**). Collectively, these results indicate that LXR constrains type 2 immunity by limiting tuft cell derived IL-25, establishing IL-25 as the key downstream effector of LXR-mediated immune restraint.

Figure 4

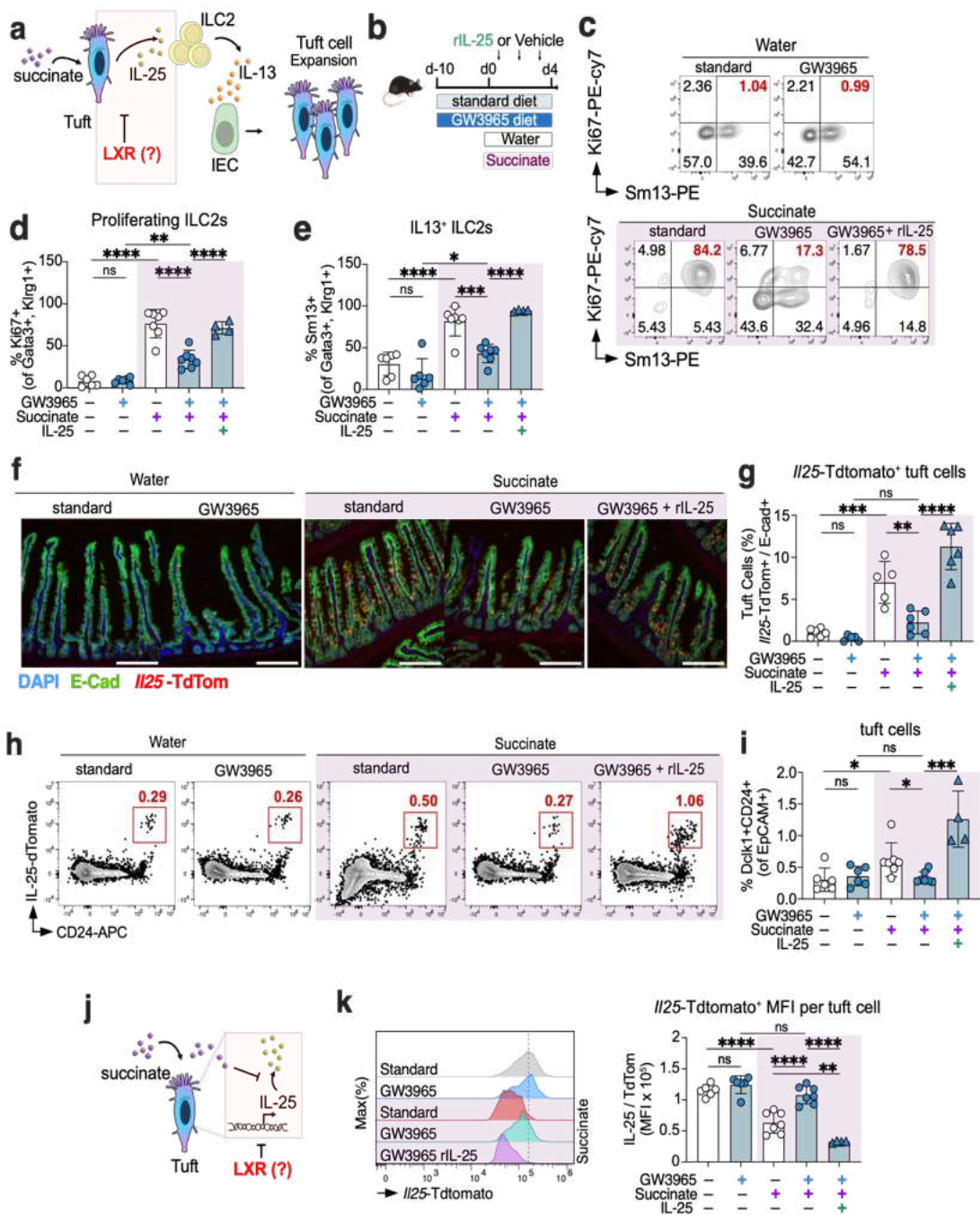


Fig. 4. IL-25 signaling rescues LXR-mediated inhibition of the tuft cell-ILC2 circuit. **a**, Schematic representation of the ILC2-tuft cell axis and the proposed site of LXR action. **b**, Schematic representation of the experimental design. **c**, Representative flow cytometry contour plots of Ki-67⁺ and Sm13⁺ ILC2s in proximal small-intestinal lamina propria cell suspensions. **d-e**, Quantification of Ki-67⁺ (**d**) and Sm13⁺ (**e**) ILC2s. **f-g**, Representative immunofluorescence images showing the *IL25*-tdTomato reporter, E-cadherin, and DAPI (**f**) and quantification of *IL25*-tdTomato⁺ tuft cells as a percentage over total E-cadherin⁺ cells (**g**).

Fig. 4. IL-25 signaling rescues LXR-mediated inhibition of the tuft cell-ILC2 circuit. (continues from last page) Each dot in (g) represents one field of view; $n = 2$ biological replicates per condition. Scale bar, 200 μm . **h-i**, Representative flow cytometry contour plots of DCLK1⁺ tuft cells in proximal small-intestinal epithelial cell suspensions (**h**) and quantification of tuft cells as a percentage of total EpCAM⁺ cells (**i**). **j**, Schematic representation of the predicted LXR target involved in regulating IL-25 expression in tuft cells. **k**, Representative histogram showing *Il25*-tdTomato mean fluorescence intensity (MFI) in tuft cells under the indicated conditions. Where applicable in (**d-k**), each dot represents one biological replicate (one mouse), except in (**g**) as specified above. Unless otherwise specified, data were pooled from two or more independent experiments ($n = 6-7$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. For comparisons involving more than two groups, statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test.

Having established that IL-25 rescues ILC2 activation despite LXR signaling, we next sought to determine whether LXR directly restricts tuft cell hyperplasia, IL-25 production, or both. Because IL-25 protein was undetectable by ELISA from either the 2D or 3D organoid cultures upon stimulation (*H.p* excretory and secretory production, ionomycin or succinate), we employed the *Il25*-TdTom reporter mice to assess *Il25* expression *in vivo*¹⁰. Immunofluorescence analysis revealed a marked increase in IL-25⁺ tuft cells (*Il25*-TdTom) following succinate stimulation, whereas GW3965 treatment significantly suppressed this succinate-induced tuft cells hyperplasia (**Fig. 4f** and **4g**). Importantly, exogenous rIL-25 administration rescued tuft cell hyperplasia despite LXR activation (**Fig. 4f** and **4g**). Flow cytometry analysis corroborates these findings as succinate exposure increased IL-25⁺CD24⁺ cell and DCLK1⁺ tuft cell frequencies, which was again rescued by exogenous rIL-25 (**Fig. 4h** and **4i**). To determine whether LXR also affects IL-25 production on a per-cell basis (**Fig. 4j**), we quantified *Il25*-TdTomato mean fluorescence intensity (MFI) in EpCAM⁺SiglecF⁺CD24⁺ tuft cells. Consistent with prior reports of feedback regulation during prolonged succinate exposure¹⁹, *Il25* reporter intensity decreased following succinate challenge in control mice (**Fig. 4k**). In contrast, LXR activation disrupted this dynamic regulation maintaining *Il25*-TdTomato MFI at steady-state levels irrespective of succinate exposure (**Fig. 4k**). Of note, treatment with exogenous IL-25 resulted in a strong succinate-mediated suppression of *Il25*-reporter expression accumulation per tuft cell (**Fig. 4k**), demonstrating that LXR limits both tuft cell expansion and IL-25 output.

Given that additional alarmins, such as IL-33 can partially compensate within type 2 immune circuits, we asked whether IL-33 might overcome LXR-mediated suppression of the tuft-ILC2 axis. To address this, we administered recombinant IL-33 to mice under steady-state conditions and quantified tuft cells and ILC2s in the distal small intestine, using rIL-25 in parallel as a positive control. We found that both rIL-33 and rIL-25 at steady state rescued the LXR-inhibited tuft cell number and ILC2 in the distal SI to a similar level as shown in the standard diet fed animals (**Extended Data Fig. 6a-d**), further confirming that LXR does not intrinsically affect ILC2 activation. Additionally, we measured the relative *Ii33* expression in the small intestine and found succinate stimulation did not induce *Ii33* (**Extended Data Fig. 6e**). No difference in *Ii33* transcriptional levels between two dietary groups regardless of the presence of succinate (**Extended Data Fig. 6e**), indicating LXR activation dampened tuft cell-ILC2 circuit independently of IL-33. Together, these data identify LXR as an epithelial checkpoint that acts upstream of alarmin-driven ILC2 activation to restrict tuft cell expansion, IL-25 output, thus the amplification of the tuft-ILC2 circuit.

Epithelial intrinsic LXR activation is required to inhibit tuft cell hyperplasia upon succinate challenge

We then sought to investigate whether this regulatory axis requires epithelial-intrinsic LXR signaling *in vivo*. Towards this, we employed genetic loss-of-function models using epithelial-specific LXR deficient mice (Villin cre-LXR α^{ff} LXR β^{ff} mice, hereafter termed LXR $\alpha\beta^{\Delta IEC}$). Under steady-state conditions, epithelial LXR deletion did not alter tuft cell numbers per crypt-villus structural unit (**Fig. 5a**). However, LXR $\alpha\beta^{\Delta IEC}$ mice exhibited a modest but significant increase in the proportion of ILC2s within the small intestinal lamina propria, (18.4% $\pm$ 3.0 vs. 22.2% $\pm$ 1.6 among SI-LP total ILCs, **Fig. 5b**), suggesting that epithelial LXR signaling influences immune composition through epithelial-immune crosstalk. Notably, absolute ILC2 numbers and their capacity to produce type 2 cytokines were unaffected (**Extended Data Fig. 6f**), indicating that epithelial LXR is dispensable for maintaining the tuft cell and ILC2 numbers at homeostasis. We next examined whether epithelial LXR is required to constrain the circuit during inductive challenge. Upon succinate administration, pharmacologic LXR activation suppressed tuft cell expansion in control mice. Strikingly, this inhibitory effect was completely abolished in LXR $\alpha\beta^{\Delta IEC}$ mice, demonstrating that epithelial LXR signaling is necessary for limiting tuft cell

289 hyperplasia in response to strong luminal stimulation (**Fig. 5c**). Together, these findings reveal an
 290 asymmetric role for epithelial LXR in regulating the tuft-ILC2 circuit: while dispensable for
 291 steady-state maintenance, epithelial LXR activation is essential to restrain circuit amplification
 292 during succinate challenge.

Figure 5

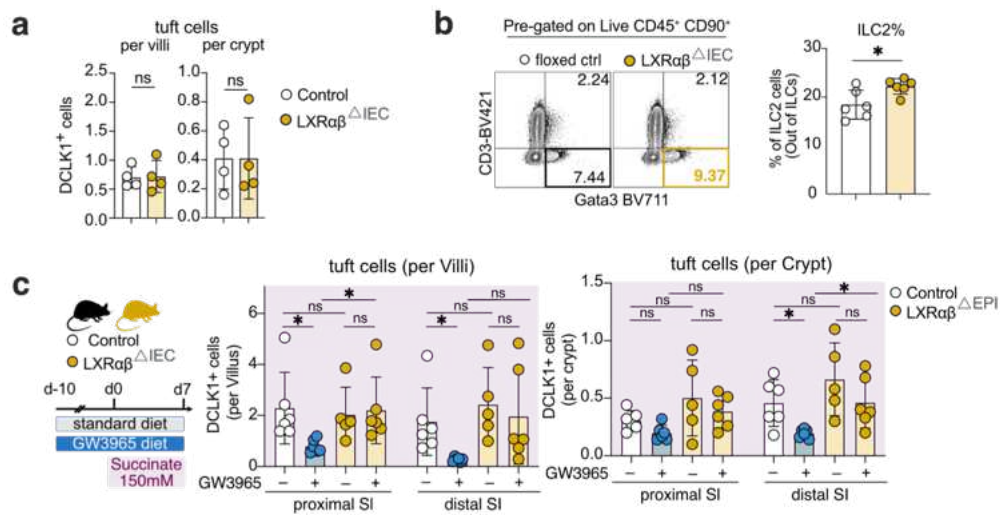


Fig. 5. Epithelial-intrinsic LXR activation is required to inhibit tuft cell hyperplasia following succinate challenge. **a**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus and crypt in the distal small intestine of LXRαβ^{ΔIEC} mice and control littermates under homeostatic conditions. **b**, Representative flow cytometry contour plots and quantification of ILC2s in whole small-intestinal lamina propria cell suspensions. **c**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus and crypt in the distal small intestine of LXRαβ^{ΔIEC} mice and control littermates following 7 days of treatment with 150 mM succinate. In (**a-c**), each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 4-6$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using an unpaired Student's t -test (**a** and **b**) or two-way ANOVA followed by Fisher's least significant difference (LSD) test (**c**).

LXR activation preserves tuft cell canonical chemosensory programs

To examine epithelial responses associated with LXR-mediated restriction of tuft cell expansion, we performed single-cell RNA sequencing of sorted EpCAM⁺ intestinal epithelial cells. To this end, we performed single-cell RNA sequencing analysis of sorted EpCAM⁺ epithelial cells isolated from the small intestine at steady state and following 4-day succinate challenge under control or GW3965 diet. The scRNAseq clustered on all epithelial cells into 6 major epithelial subtypes in 4 conditions including intestinal stem cells (ISC), enterocytes (EC), transit-amplifying cells (TA), Paneth and goblet cells (Paneth-GC), enteroendocrine cells (EEC) and tuft cells based on their canonical epithelial cell subtypes markers (**Extended Data Fig. 7a-c**). Among these, tuft cells exhibited the most pronounced proportional changes in response to succinate (**Extended Data Fig. 7d**). Tuft cells sub-clustering revealed intermingled distributions across all experimental groups without distinct treatment-specific clusters (**Fig. 6a-b**), indicating that LXR activation does not induce overt lineage re-specification. Succinate increased the number and proportion of tuft cells in control mice (3.2-fold increase in percentage), whereas this response was substantially blunted under LXR activation (**Fig. 6c**).

Next, we hypothesized that the dampened tuft cell response could arise from a blockade of sensing (e.g., downregulation of SUCNR1, impairment in alarmin production/release). To test this, we examined a shared transcriptional program induced by succinate in all 4 conditions (**Extended Data Fig. 7e**). Analysis of this shared gene set identified a core adaptive program in tuft cells commonly invoked by succinate, featuring an acute response enriched for functions related to inflammatory response regulation, GPCR signaling, and cellular response to chemical stimuli. Its key components include GPCR-associated genes (*Sucnr1*, *Vmn2r26*, *Drd3*), the NF- κ B pathway inhibitor *Nfkbia*⁴⁴, the potassium channel *Kcnj16*, and the immune-modulating cysteine-rich intestinal protein *Crip1*⁴⁵ (**Extended Data Fig. 7e**). All together, these data indicate that the core transcriptional response to succinate is independent of LXR activation. Supporting this, other tuft cell associated genes, including lineage markers (*Pou2f3*, *Trpm5*, *Spib*), GPCR signaling components (*Itpr2*, *Plcb2*, *Alox5*), and effector molecules (*Ltc4s*, *Chat*, *Tslp*) remained unchanged across conditions (**Extended Data Fig. 7f**). *Il25* was undetectable by scRNA-seq. We therefore evaluated the *Il25*-TdTomato reporter and observed no corresponding reduction in reporter intensity per tuft cell following GW3965 treatment during homeostasis (**Fig. 4k**). To functionally

assess whether LXR activation modulates GPCR-dependent tuft cell chemosensory machinery, we measured CysLT protein levels in the culture medium of monolayer cultures of small intestinal epithelium (**Extended Data Fig. 8a**). We reasoned that because CysLTs are key inflammatory mediators produced by tuft cells in an ALOX5- and TRPM5-dependent manner, changes in CysLT levels reflect alterations in tuft cell function¹¹. Induced CysLTs levels were comparable between control cells and GW3965 treated cells (1h or 24h), suggesting that LXR does not impair the critical tuft cell elements ALOX5 and TRPM5, despite the rapid elevation of its target gene *Abca1* within 1 hour (**Extended Data Fig. 8b and c**). Together, these findings indicated that LXR activation preserves the canonical chemosensory and effector transcriptional program of tuft cells, supporting a model in which LXR restrains type 2 immunity by regulating tuft cell state transitions rather than epithelial sensing.

Reciprocal regulation of tuft-1 and tuft-2 cell signature genes by LXR

Recent studies have defined two distinct tuft cell subsets, tuft-1 and tuft-2 in mouse and human, which display differential functional properties and thereby influence tuft cell-mediated chemosensory responses^{6,46–48}. This emerging heterogeneity led us to investigate whether LXR instead reprograms tuft cell identity. Direct comparison of tuft cells from standard diet and GW3965-treated mice revealed reciprocal regulation of subtype-defining genes at baseline: *Gng13* (tuft-1 marker) expression was higher upon LXR activation, whereas *Gnat3* (tuft-2 marker)^{46,47} was reduced (**Fig. 6d**). These genes localized to spatially distinct but partially overlapping regions within the tuft cell UMAP (**Fig. 6e and Extended Data Fig. 8d**). Representative tuft-1-associated genes included *Nrep*, *Gng13*, *Il13ra1* and *Sox4*^{46,47,49}, whereas tuft-2-associated genes included *Folr1*, *Gnat3*, *Chat*, *Il17rb* and *Sucnr1*^{6,46,47}. Additionally, we found a cluster of cells enriched in *Top2a*, *Mki67*, *Tgfr1* and *Ccnd1* which are the signatures of tuft cell progenitors (tuft-p) and recapitulating the regenerative tuft cell described in human⁴⁸ (**Extended Data Fig. 8d**).

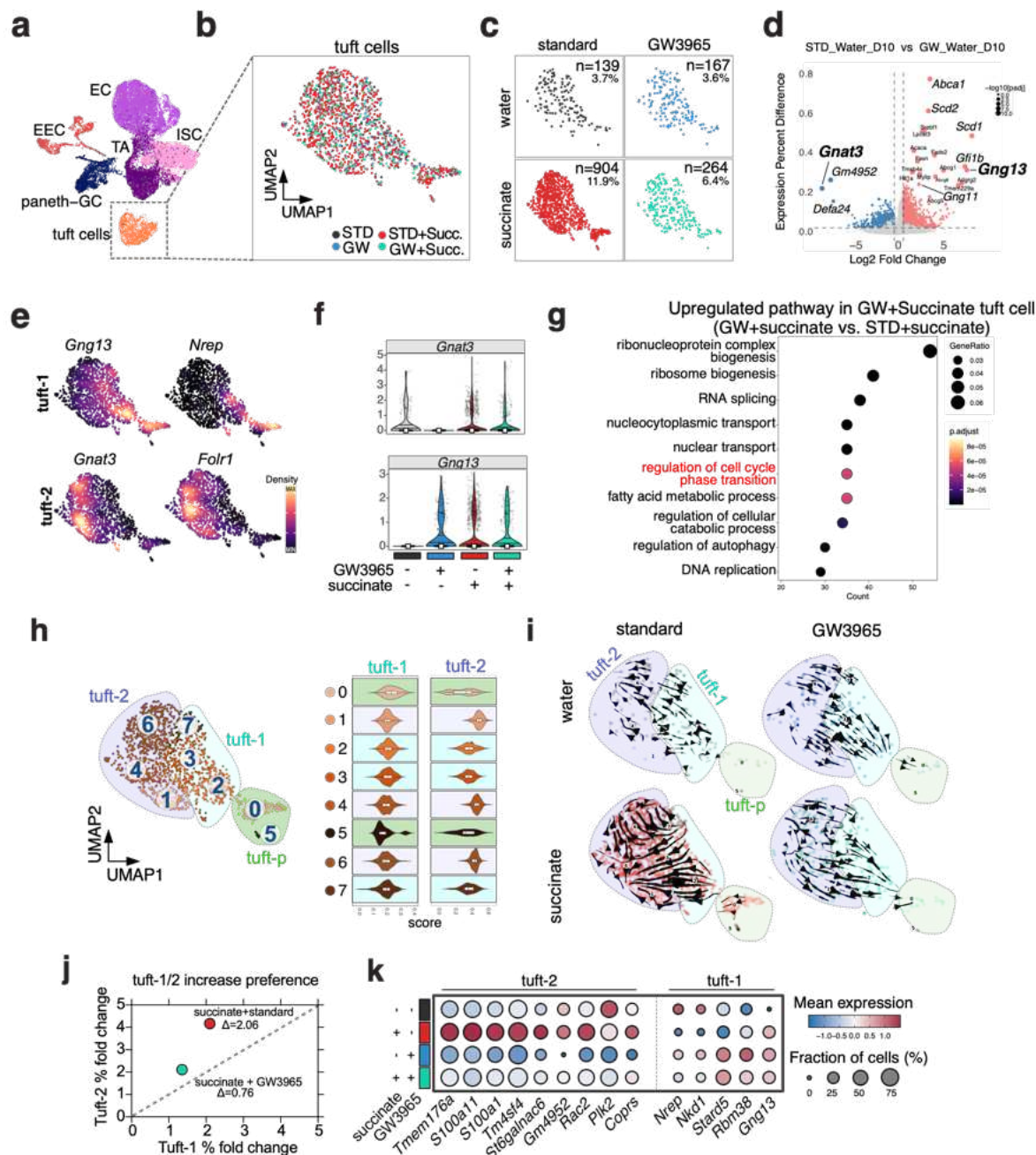


Fig. 6. LXR signaling enhances the tuft-1 cell signature. **a-c**, Uniform manifold approximation and projection (UMAP) visualization of scRNA-seq data from EpCAM⁺ epithelial cells (**a**) and tuft cells (**b-c**) isolated from the whole small intestine under the four indicated treatment conditions. Three mice were pooled per treatment condition. In (**c**), *n* indicates the number of tuft cells, and % indicates the percentage of tuft cells among all analyzed EpCAM⁺ epithelial cells in each treatment group. **d**, Volcano plot showing differentially expressed genes in tuft cells from mice fed a standard or GW3965 diet under homeostatic conditions. **e**, Expression density of tuft cell-associated genes projected onto the UMAP. **f**, Violin plots showing *Gnat3* and *Gng13* expression in the scRNA-seq dataset under the indicated conditions.

Fig. 6. LXR signaling enhances the tuft-1 cell signature. (continues from the previous page) **g**, Gene Ontology analysis showing the top 10 biological processes enriched among genes upregulated in tuft cells from mice receiving a GW3965 diet plus succinate compared with those receiving a standard diet plus succinate. **h**, UMAP visualization of eight tuft cell clusters and violin plots showing tuft-1 and tuft-2 signature scores in each subcluster. **i**, RNA velocity-based trajectory analysis overlaid on UMAP visualizations of tuft cell clusters under the four indicated conditions. **j**, Scatter plot comparing fold changes in tuft-1 and tuft-2 cell abundance following succinate challenge in mice fed a standard or GW3965-containing diet. The diagonal line ($y = x$) indicates equivalent expansion of the two cell populations, and the distance above the diagonal ($\Delta = \text{Fold Change}_{\text{tuft2}} - \text{Fold Change}_{\text{tuft1}}$) represents the magnitude of preferential tuft-2 cell expansion. **k**, Bubble plot comparing the expression of tuft-1 and tuft-2 signature genes under the four indicated conditions.

Strikingly, succinate uniformly upregulated both *Gngl3* and *Gnat3* across all tuft cells regardless of diet (**Fig. 6f**), indicating that the acute sensory response overrides baseline subtype bias. Succinate challenge triggered a pronounced enhancement of cell cycle phase transition pathway in LXR-activated tuft cells (**Fig. 6g**). Among the most prominent were checkpoint genes associated with cell cycle arrest, including *Cdc25b*⁵⁰ and *Psme3*⁵¹, both of which were uniquely upregulated in tuft cells isolated from mice on a GW3965 diet and exposed to succinate (**Extended Data Fig. 8e**). Interestingly, despite the apoptotic gene *Casp2* was seen upregulated by GW3965 diet in tuft cells (**Extended Data Fig. 8e**), the apoptotic tuft cells marked by a universal apoptosis probe Apo-15 peptide did not differ between diet treatments at steady state or after 4-day 150mM succinate challenge (**Extended Data Fig. 8f and G**). These findings indicate that impaired tuft cell expansion following LXR activation is not explained by increased apoptosis.

To systematically resolve tuft cell states, we re-clustered tuft cells into eight transcriptionally distinct subpopulations (**Fig. 6h**). Gene ontology analysis revealed distinct functional characteristics across tuft cell state clusters. Clusters 0 and 5 exhibited features of tuft cell progenitors, with overrepresented pathways associated with epithelial cell proliferation and regeneration (**Extended Data Fig. 9a**). Clusters 2, 3, and 7 displayed interconnected pathways indicative of developing and immature tuft-1 cells⁴⁷. This progression encompassed RNA synthesis and enhanced translational capacity (cluster 2), Golgi organization (cluster 3), and regulation of cytoskeleton dynamics and cell polarity (cluster 7) (**Extended Data Fig. 9a**). By contrast, clusters 1, 4 and 6 were enriched for tuft-2-related signatures and immune-response-associated pathways^{6,52}. These clusters showed evidence of metabolic reprogramming in response to stimuli (cluster 1), immune defense mechanisms (clusters 1 and 4), and cellular structural

adaptations (cluster 6). To quantitatively validate the identity of the eight subclusters, we scored each cell using tuft-1 and tuft-2 gene top differentially expressed genes generated from the previous report ⁴⁶ (**Table S1**). Tuft-2 signature scores robustly identified clusters 1,4, and 6 as terminal effector states, whereas tuft-1 states displayed greater transcriptional heterogeneity (**Fig. 6h**).

In contrast, the tuft-1 signature showed less discriminatory power, displaying similar scores across multiple subclusters, including progenitor-like populations (**Fig. 6h**). This likely reflects the more graded and heterogeneous nature of tuft-1 states compared to the specialized, terminal nature of tuft-2 cells. Collectively, these annotations provided a descriptive framework for examining how LXR activation was associated with tuft cell-state abundance during succinate-induced expansion.

LXR activation is associated with reduced preferential expansion of tuft-2-related states

Having established the transcriptional landscape of tuft cell states, we next investigated how these states are dynamically connected during differentiation. We performed RNA velocity analysis which allows a snapshot prediction of transient cell-state dynamic and maturation direction. Under control conditions, tuft cells converged from cluster 2,3 and 7 (tuft-1-like) toward a dominant tuft-2 endpoint (cluster 6), consistent with progressive maturation as earlier reported ⁵⁰ (**Fig. 6i**). Upon succinate stimulation, a new cell-state endpoint of other streams was consolidated toward the interface between clusters 4 and 1 (representing a mixed tuft-2 state). Notably, LXR-imprinted trajectory persisted even after succinate challenge, demonstrating that LXR establishes a stable cell state transition bias resistant to acute stimulation (**Fig. 6i**). By quantifying tuft cell number in each stratified cell states, we found LXR activation did not cause major cell number changes in different cell states at homeostasis (**Extended Data Fig. 9b**). Likewise, baseline proportions of tuft-1 and tuft-2 were comparable among epithelial cells (**Extended Data Fig. 9c**). In contrast, upon expansion, tuft-2 substantially outnumbered both tuft-1 and tuft-p cells (**Extended Data Fig. 9c**). The distance from the diagonal represents the preferential expansion of tuft-2 over tuft-1 in percentage out of total epithelial cells (calculated as $\% \text{Fold Change}_{tuft2} - \% \text{Fold Change}_{tuft1}$) (**Fig. 6j**). The standard diet control induced a much greater preferential expansion ($\Delta=2.06$) compared to the GW3965 diet ($\Delta=0.76$) (**Fig. 6j**), suggesting that LXR activation inhibited the tuft-2 cell expansion preference upon succinate exposure. Concordantly, LXR upregulated tuft-1-associated

genes (*Rbm38*, *Stard5*, *Nkd1*, *Nrep*) while suppressing succinate-induced tuft-2 effector genes (*Coprs*, *Rac2*, *St6galnac6*, *Tm4sf4*, *S100a1*, *Tmem176a*) (**Fig. 6k**). Collectively, these findings associate LXR activation with a shift in tuft cell-state composition that favors tuft-1–like over immune-associated tuft-2 states during type 2 immune activation.

LXR activation impairs parasitic clearance.

The tuft cell-ILC2 circuit serves as a critical axis driving type 2 immunity during parasitic infection⁵³. Succinate producing *Tritrichomonas muris* is a well-studied protozoan that requires functional SUCNR1 to be able to clear the helminth in the intestine⁸. After establishing that LXR controls succinate-induced tuft cell expansion, we aimed to extend our findings to succinate-independent type 2 immune responses and its relevant physical relevance. To confirm the generalizable nature of the LXR pathway controlling tuft cell-ILC2-mediated immune responses, we utilized *Nippostrongylus brasiliensis* (*N. b*), a model that does not require SUCNR1 to achieve worm expulsion and is comparable to human hookworm infections (**Fig. 7a**)^{8,54}. Tuft cell quantification at 10 dpi showed expansion of tuft cell numbers in the distal small intestine, which was inhibited by ectopic LXR activation (**Fig. 7b**). While control animals completely cleared the worms, as evidenced by virtually no eggs in the stool at 10 dpi, GW3965-fed animals retained a significant number of eggs in the stool after 10 days of infection (**Fig. 7c**). This persistent enhanced egg count in GW3965 treated mice, using two distinct helminth infection models, indicates impaired host-response due to LXR activation.

Figure 7

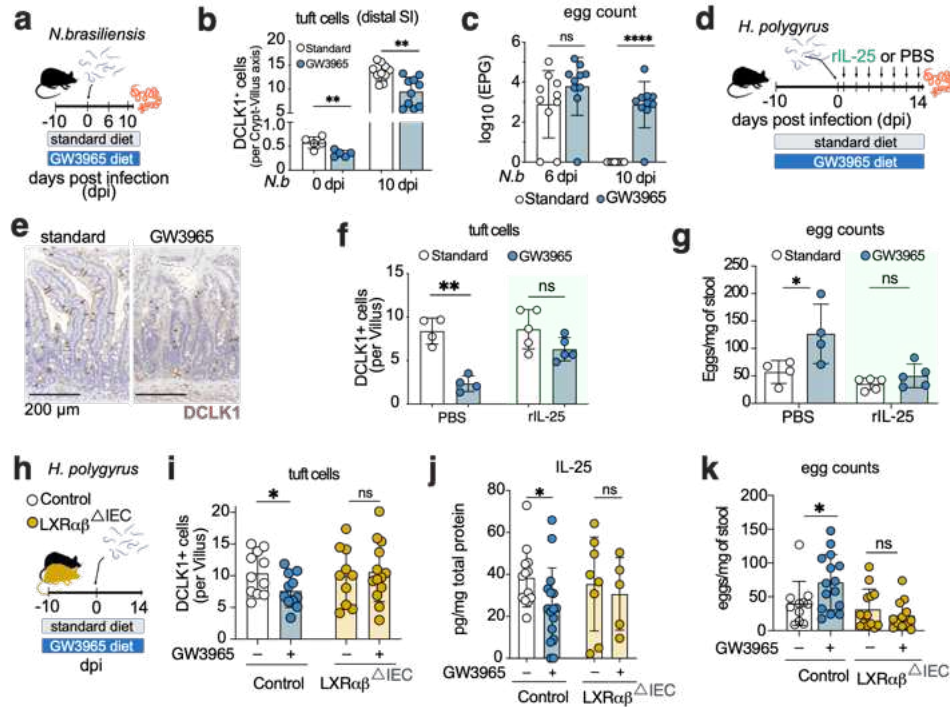


Fig. 7. LXR activation impairs the anti-helminth response. **a**, Schematic representation of the *N. brasiliensis* infection experiment. **b**, Immunohistochemistry-based quantification of DCLK1⁺ cells per crypt-villus axis at the indicated time points. dpi, days post-infection. **c**, Quantification of eggs per gram of stool from mice treated as depicted in (a). **d**, Schematic representation of the *H. polygyrus* infection experiment using wild-type mice. **e-f**, Representative images of DCLK1 immunohistochemical staining (e) and quantification of DCLK1⁺ cells per villus (f) in the proximal small intestine of mice treated as depicted in (d). Scale bar, 200 μm. **g**, Quantification of eggs per milligram of stool at 13 dpi from mice treated as depicted in (d). **h**, Schematic representation of the *H. polygyrus* infection experiment using LXRα^{ΔIEC} mice and littermate controls. **i**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus in the proximal small intestine of mice treated as depicted in (h). **j**, Quantification of IL-25 concentrations in proximal small-intestinal tissue homogenates from mice treated as depicted in (h), as measured by ELISA. **k**, Quantification of eggs per milligram of stool at 13-14 dpi from mice treated as depicted in (h). Where applicable in (b-k), each dot represents one biological replicate (one mouse), unless otherwise specified. Data were pooled from two or more independent experiments ($n = 3-16$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test (b-c and f-g) or Fisher's least significant difference (LSD) test (i-k).

IL-25 signaling rescues the LXR-mediated inhibition of the tuft cell-ILC2 circuit during *H.polygyrus* infection.

Having demonstrated that LXR activation impairs worm clearance in the *N. brasiliensis* model of intestinal helminth infection, we next sought to determine whether this effect is generalizable to other helminth species, and whether IL-25 plays a similar role in this context. To address these questions, we examined the impact of LXR activation on the host response in mice infected with *Heligmosomoides polygyrus* (*H. polygyrus*)⁵⁵, and simultaneously administered rIL-25 to a subset of infected mice to assess the role of IL-25 in regulating anti-helminth response (**Fig. 7d**). At 14 days post-infection (dpi), an 8-fold expansion of tuft cell numbers per villus occurred in the proximal small intestine compared to the tuft cell numbers at base line in standard diet-fed mice (**Fig. 7d-f**). Notably, ectopic LXR activation resulted in only 2-fold tuft cell expansion which cell numbers were significantly lower than the non LXR activated mice in villi and similar trend observed in the crypt (**Fig. 7d-f and Extended Data Fig. 10a**). rIL-25 rescued tuft cell and goblet cell repression induced by ectopic LXR activation during *H. polygyrus* infection (**Fig. 7f, Extended Data Fig. 10a and 10b**). Analysis of SI-LP cell suspensions from *H. polygyrus* infected animals (6 dpi) revealed reduced frequencies and numbers of GATA3⁺ ILC2s in GW3965-fed compared to control animals (**Extended Data Fig.10c**), whereas Th2 cells were comparable (**Extended Data Fig. 10d**). The IL-13 levels measured in the tissue homogenates from proximal SI displayed a decreased yet not significant change in mice fed with GW3965 diet compared to the controls, reflecting a modest tissue response regulated by LXR activation (**Extended Data Fig. 10e**). Importantly, exogenous rIL-25 administration rescued ILC2 frequencies and numbers in GW3965-fed animals during *H. polygyrus* infection (**Extended Data Fig. 10c**), resulting in a restored egg counts and 14dpi worm burden (**Fig. 7g and Extended Data Fig. 10f**). Interestingly, although rIL-25 is known for its pro-inflammatory properties, when administered to *H.polygyrus* infected mice, no exaggerated response was observed in host defenses such as tuft cells and goblet cells hyperplasia at 14dpi (**Fig. 7f and Extended Data Fig. 10b**), intestinal ILC2 and Th2 cell numbers at 6dpi (**Extended Data Fig. 10c and d**), nor worm fecundity (**Fig. 7g**). This non-additional effect by rIL-25 may suggests a self-limiting or saturation via IL-25 signaling in this helminth infection model and may possibly involve a host-pathogen co-evolution, preventing from excessive inflammation in the intestine⁵⁶. Collectively, LXR activation represses tuft cell hyperplasia and ILC2 expansion in response to parasitic infection.

LXR activation in epithelium is necessary to control anti-helminth responses.

To determine whether epithelial LXR was required for the inhibitory effects of GW3965 during infection, we infected Villin cre-LXR $\alpha^{f/f}$ LXR $\beta^{f/f}$ mice with *H. polygyrus* in combination with LXR agonist GW3965 diet or standard diet as the control (**Fig. 7h**). Epithelial-specific deletion of LXR did not significantly alter the host response to *H. polygyrus* infection, as evidenced by comparable tuft cell numbers, IL-25 protein concentrations in SI homogenates, and egg counts compared to controls (**Fig. 7i-k**). In LXR floxed control mice, LXR activation replicated our previous findings in C57BL/6 mice, resulting in both suppressed tuft cell numbers and reduced IL-25 protein concentrations in small intestinal homogenates compared to standard diet-fed controls (**Fig. 7i and j**). However, this LXR activation-induced suppression of tuft cells and IL-25 expression was abolished in LXR $\alpha\beta^{\Delta IEC}$ mice, indicating that LXR activation within the intestinal epithelium is necessary to influence tuft cell number and IL-25 production during *H. polygyrus* infection (**Fig. 7i and j**). Consistent with this, the enhanced worm fecundity observed with GW3965 treatment in LXR-sufficient mice did not occur in mice lacking epithelial LXR (**Fig. 7k**). Although LXR activation in the floxed mice showed comparable worm burden as the standard diet-fed controls, LXR $\alpha\beta^{\Delta IEC}$ mice showed a subtle but significant decrease from approximately 110 to 75 worm counts per mouse intestine in two diet groups compared to the LXR-sufficient mice (**Extended Data Fig. 10h**). In single LXR isoform whole body knock-out mice, however, the susceptibility to *H. polygyrus* infection was unchanged in LXR $\alpha^{-/-}$ or LXR $\beta^{-/-}$ mice compared to their own littermate controls, shown by similar egg and worm counts (**Extended Data Fig. 10i**). This result may indicate the importance of both isoforms of LXR in regulating the host response upon *H. polygyrus* infection.

In conclusion, our results establish that LXR activation within the intestinal epithelium drives a suppressive effect on tuft cell-mediated immunity, resulting in impaired host resistance to helminth infection.

DISCUSSION

Our study identifies LXR as an epithelial transcriptional checkpoint that actively restrains the initiation of mucosal type 2 immunity. Rather than suppressing canonical tuft cell sensory programs or downstream ILC2 responsiveness, LXR activation limits tuft cell expansion and is

associated with reduced preferential expansion of tuft 2-related states. These findings establish a previously unrecognized mechanism by which metabolic sensing is coupled to epithelial cell-state regulation to set the threshold for type 2 inflammation.

The tuft cell-ILC2 circuit amplifies intestinal type 2 immunity through reciprocal IL-25 and IL-13 signaling^{10,13–16}. Most previous studies have defined signals that initiate or reinforce this circuit, whereas less is known about how epithelial amplification is restrained. Our findings identify LXR as such a brake. Importantly, LXR does not directly suppress immune cell function, and our single cell data indicate that LXR does not abolish epithelial chemosensing neither. Instead, it selectively limits the expansion of the immune-responsive tuft-2 compartment, thereby uncoupling environmental sensing from immune amplification. We propose that this mechanism allows the intestinal epithelium to remain responsive to environmental cues while preventing excessive feed-forward activation. This model is supported by several independent observations. First, our single-cell analyses indicate that tuft cells can sense exogenous succinate (**Supp Fig 8E**) and yet failed to overcome LXR-mediated suppression of tuft cell expansion, indicating that LXR acts within, rather than upstream of, the tuft cell sensory circuit. Second, recombinant IL-25 fully restored tuft cell and ILC2 expansion, placing LXR upstream of IL-25-dependent circuit amplification. Third, epithelial-specific deletion of LXR abolished the inhibitory effects of pharmacological LXR activation, establishing the intestinal epithelium as the critical site of action. Together, these findings position LXR as an epithelial rheostat that controls the gain of the tuft-ILC2 amplification circuit rather than an on/off regulator of type 2 immunity.

Our single-cell analyses further provide mechanistic insight into how this regulation is achieved. Previous studies identified distinct tuft cell states, including tuft-1, tuft-2, and progenitor-like populations, as previously identified by others^{46–48}. Using scRNA-seq across homeostatic and succinate-challenged conditions, we confirmed these populations and found that LXR activation selectively remodels their relative abundance. Canonical tuft cell sensory genes, including *Pou2f3*, *Trpm5*, *Plcb2*, *Spib*, and *Sucnr1*, remained largely unchanged, arguing against impaired chemosensory competence. Instead, LXR redirected differentiation away from the immune-associated tuft-2 state toward tuft-1 and progenitor-like states. Thus, metabolic regulation of epithelial immunity appears to occur through modulation of cell-state composition rather than

519 suppression of core sensory machinery. Tuft cells have long been regarded as terminally
520 differentiated epithelial cells ⁵⁷, but this finding points to a possible route for dedifferentiation
521 during epithelium damage and regeneration as previously reviewed ⁵⁸. RNA velocity analysis
522 further suggested that LXR reshapes the differentiation landscape of the tuft cell compartment.
523 Whereas succinate normally reinforced progression toward tuft-2 effector cells, LXR activation
524 redirected predicted trajectories toward tuft-1 and progenitor-like states.

525
526 Although RNA velocity cannot establish long-term lineage relationships, these observations raise
527 the intriguing possibility that tuft cell states retain greater plasticity than previously appreciated.
528 Tuft-1-associated cells have previously been reported to localize preferentially to intestinal crypts
529 ^{47,59}. In our histological analyses, GW3965 reduced tuft cell numbers in villi but not crypts.
530 Conversely, LXR β deletion increased tuft cell numbers selectively in villi (**Fig. 2f–J**). These
531 spatial observations were compatible with a preferential effect on villus-associated tuft cells.
532 However, the histological markers used here did not directly assign cells to the transcriptomic tuft-
533 1 or tuft-2 states. Thus, the combined data supported altered tuft cell-state composition during
534 LXR activation, while leaving the direction of state transitions unresolved. Whether immune-
535 associated tuft-2 cells can revert to earlier differentiation states, and whether such plasticity
536 contributes to epithelial regeneration following tissue injury, remain important questions for future
537 investigation.

538
539 Beyond orchestrating type 2 immunity, tuft cells have recently emerged as multifunctional
540 epithelial cells involved in tissue regeneration and tumorigenesis ^{60,61} or serving as a backup for
541 Paneth cells to maintain stem cell homeostasis ⁶². DCLK1⁺ tuft cells can function as reserve stem-
542 like cells following epithelial injury and have been implicated as cells of origin in intestinal cancer
543 ^{63–65}. Our transcriptomic analyses revealed that LXR activation altered pathways associated with
544 cell-cycle regulation and p53-related genes such as *Trp53*, *Atr*, and *Ddb1* within tuft cells.
545 Together with our previous study showing that LXR uncouples epithelial regeneration from
546 intestinal tumorigenesis ²², these findings suggest that LXR may broadly coordinate epithelial
547 plasticity beyond immune regulation. Whether regulation of tuft cell state contributes directly to
548 regeneration or tumor initiation will require lineage-tracing and functional studies.

LXR is a ligand-activated nuclear receptor that integrates oxysterol availability with cellular cholesterol metabolism. Our findings extend the function of LXR beyond lipid metabolism by identifying its restriction in tuft cell-ILC2 circuit and protective type 2 responses during helminth infection. Whether physiological LXR ligands or defined nutritional states reproduce the effects of GW3965 remains unknown. Consumption of high-fat diets is widely believed to increase circulating oxysterol levels ^{66,67} and may therefore affect LXR activity. Because endogenous oxysterols activate LXR ⁶⁸, our findings raise the possibility that metabolic status influences the epithelial threshold for type 2 immunity. However, dietary lipids alter numerous metabolic and inflammatory pathways in addition to LXR signaling, and therefore our data should not be interpreted as evidence that high-fat diets suppress type 2 immunity through LXR alone. Instead, our work identifies a molecular mechanism through which metabolic cues could, in principle, influence epithelial immune function, providing a foundation for future studies investigating how nutrient sensing shapes barrier immunity in physiological and pathological settings.

Together, our study identifies LXR as a previously unrecognized epithelial checkpoint that links metabolic sensing to tuft cell state transitions. By establishing the activation threshold of the tuft–ILC2 feed-forward circuit, LXR calibrates the magnitude of mucosal type 2 immunity while preserving epithelial sensory function. These findings reveal an additional layer of regulation governing barrier immunity and establish epithelial cell-state control as a fundamental mechanism for tuning tissue immune responses.

MATERIALS AND METHODS

Mice. All mice used were between 7-20-week age and housed in specific pathogen-free conditions at AKM, KMB and KMF animal facility unless specified. Control mice were gender- and age-matched littermate controls. C57BL/6 (B6) and BALB/c mice were purchased from Taconic (B6), Janvier Lab (B6), Charles River (B6 and BALB/c) or locally bred in our animal facility (B6). LXR α and LXR β knock-out mice and *Nr1h3*^{f/f} (LXR α ^{f/f}) and *Nr1h2*^{f/f} (LXR β ^{f/f}) mice were kindly provided by Dr. Jan-Åke Gustafsson (Karolinska Institutet). Villin cre-*Nr1h3*^{f/f}*Nr1h2*^{f/f} (Villin cre-LXR α ^{f/f} LXR β ^{f/f} mice) were obtained by crossing *Nr1h3*^{f/f} (LXR α ^{f/f}) and *Nr1h2*^{f/f} (LXR β ^{f/f}) to *B6.Cg-Tg(Vill-Cre)1000Gum/J* (Jackson Laboratory). The triple reporter mice expressing Arginase-1^{YFP} (Yarg; *Arg1*^{YFP}), IL-13^{hCD4} (Smart13; *Il13*^{hCD4}) and IL-25^{fl-tdTomato} (B6(C)-

Il25^{tm1.1Lky/J})¹⁰ were bred and housed under specific pathogen-free conditions, free of *Tritrichomonas* in University of Zurich. All genetically modified mice were on a C57BL/6 background. For ectopic LXR activation, the LXR agonist GW3965 *ad libitum* (Adooq Bioscience) was administered in formulated diet (AIG93G + Inulin) manufactured by SSNIFF, Germany. An identical formulated diet without GW3965 were used as the standard diet (standard or STD). For rIL-33 and rIL-25 administration experiment at homeostasis, 0.5 µg murine rIL-25 (587306, BioLegend) or rIL-33 (3626-ML-010, R&D system) was administered i.p in 200 µl 0.1% BSA in PBS daily for 3 days after 8-day special diet. During OVA diet, GW3965 (30mg/kg bodyweight, Adooq Bioscience) in PBS was administered via i.p injection at indicated time point, DMSO was given in PBS as the vehicle control. All mice were maintained and handled under the regulation of Institutional Animal Care and Use Committee in University of Zurich (Zurich, Switzerland), Karolinska Institutet and Karolinska Hospital (Stockholm, Sweden).

Helminth infection model. *H. polygyrus* infection All mice were housed in individually ventilated cages in BSL2 at KMB animal facility in Karolinska Institutet. Mice were inoculated with 200 third stage (L3) *Heligmosomoides polygyrus* (*H.p.*) by oral gavage in 200 µl 0.05% agarose (A9539, Sigma) ddH₂O. In ectopic LXR activation experiments, mice received 8-10 days of STD or GW3965 diet prior to infection and were under special diet during infection. For rIL-25 rescue experiment, 1 µg murine rIL-25 (587306, BioLegend) was administered i.p in 200 µl 0.1% BSA in PBS every other day. Stool samples were collected on the indicated time points. For counting fecal *H.p.* eggs, fresh fecal samples were weighed and homogenized with flotation buffer (26% Sucrose (% w/w) and 21% Sodium Chloride (% w/w) water), counted by McMaster counting slide. Worm burden was quantified by manually counting the adult *H.p* in RPMI media (SH30027.FS, Hyclone) and remained in the SI after incubating the dissected 14 dpi SI in RPMI for 3-4h at 37 °C.

***N. brasiliensis* infection** 8-wk-old female C57BL/6 mice purchased from Envigo UK housed in individually ventilated cages were used throughout this study. Infections employed *N. brasiliensis* maintained as previously described⁶⁹. Animals were infected with 250 L3 *N. brasiliensis* larvae subcutaneously. All animal studies were performed under UK Home Office Licence and approved by the University of Glasgow Ethical Review Board.

Succinate treatment. 150mM succinate prepared from sodium succinate hexahydrate (6106-21-4, Alfa Aesar) or 0.22 μ m-filtered neutralized 100 mM succinic acid (S3674, Sigma) was administrated via drinking water for 7 or 4 days prior to euthanasia and organ harvesting. In ectopic LXR activation experiments, mice received 8-10 days of STD or GW3965 diet prior to infection and maintained under the same special diet during the succinate treatment. For rIL-25 rescue experiment, 4 consecutive days prior to euthanasia and organ harvest, mice received 0.8 μ g murine rIL-25 (587306, BioLegend) i.p. To detect apoptotic tuft cells, 400nM Apo-15 peptide (ApoTracker™ Green 427401, Biolegend) was included in the surface staining in epithelial cells fraction and analyzed by flow cytometry.

Food allergy model. Food allergy experiment was performed as previously described^{70,71}. Briefly, 50 μ g OVA (A5378, Sigma) and 1mg Alum (Alhydrogel® adjuvant 2% vac-alu-50, Invivogen) was injected i.p (in 200 μ L PBS) twice with a 2-week interval (on d-28 and d-14). Standard or GW3965 diets were administered 4 days (d-10) after the final OVA injection. After 10 days of diet exposure (d0), mice changed to OVA diet (OVA containing diet, 20% protein, 57.47% starch, 5.0% sugar, 5.0% cellulose, 6.0% soy oil, 5.0% mineral mixture, 1.3% vitamin mixture (AIN93M composition) and 0.23% choline chloride, manufactured in SSNIF, Germany) containing OVA (approx. 250mg OVA digested per day). Alternatively, allergy response in mice was induced by 3 consecutive days of daily oral gavage 50mg OVA dissolved in 200 μ L PBS under standard or GW3965 diet condition. For rIL-25 rescue experiment, 0.5 μ g murine rIL-25 (587306, BioLegend) was administrated i.p in 200 μ L 0.1% BSA in PBS at d0 and d2 post the OVA challenge.

Tissue harvest and processing. Tissue harvesting was performed immediately after sacrifice by CO₂. Biopsies (0.5 cm) from the proximal SI were collected 2 cm apart of the stomach-intestine junction and distal SI biopsies were taken from the intestine-cecum junction. The luminal content was removed with cold PBS and cleaned biopsies were snap-frozen in dry ice and moved later -80°C for protein extraction or kept in RNeasy lysis buffer (AM7020, Thermo Fisher) at -20°C for later RNA extraction. Swiss rolls were prepared as described elsewhere^{29,30}. Briefly, 12cm proximal SI (starting from the stomach-intestine junction) and 12cm distal SI (ending in the intestine-cecum junction) were cut opened longitudinally and rolled from proximal to distal (for 12cm proximal SI), or from distal to proximal SI (for 12 cm distal SI). For single cells suspension preparation, the first 2cm stomach-intestine junction was discard and the rest was regarded as the whole SI length and digested for intraepithelial lymphocytes fraction (IELs) and lamina propria (LP) cells fraction

as previously described ²³. Briefly, proximal, distal or the whole SI was open longitudinally and cut into 0.5~1cm small pieces after removing luminal content and cleaned in cold PBS, stored in collection buffer containing 5% FBS (A5256701, Gibco), 1X Glutamax (35050038, Thermo Scientific) , 10 mM HEPES (15630-080, Thermo Scientific), 100 U/mL penicillin-streptomycin (15140122, Gibco) in HBSS (12082739, Gibco) on ice. Cells from intra epithelial layer (IEL) were obtained by incubating small intestinal tissue pieces in epithelial strip buffer containing 5% FBS (A5256701, Gibco), 1mM DTT (D9779-5G, Sigma), 5mM EDTA (15575-020, Thermo Scientific) and 10 mM HEPES (15630-080, Thermo Scientific), in HBSS (12082739, Gibco) for 30 mins at 37 °C and filtering through 70 µm cell strainer. Span down at 500g, 6 mins at 4 °C and resuspended the IEL pallets in FACS buffer containing 2% FBS and 2mM EDTA (15575-020, Thermo Scientific) in PBS, proceeded tuft cell staining for FACS analysis. To further isolate cells from the LP, method was adapted from the previously described method ⁷². Briefly, the epithelial strip buffer treated SI tissue pieces were collected and washed once with PBS with Ca²⁺ and Mg²⁺. Incubated in 10ml pre-warm digestion mix (10% FBS (steady state) or 20% FBS (succinate and helminth infection experiments), 1mg/ml Collagenase A (10103578001, Roche), 0.05 mg/ml DNase, 1x Glutamax, 10mM HEPES, 100 U/mL penicillin-streptomycin (15140122, Gibco) in RPMI (R0883, Sigma)) for 30 mins at 37 °C. Stopped the enzymatic digestion by filling 25 ml cold stop buffer (5% FBS, 0.05mg/ml DNase (10104159001, Sigma), 10mM HEPES, 100 U/mL penicillin-streptomycin in HBSS). Obtained LP cells suspension by filtering through first 100 µm and following 40 µm cell strainers. Span down at 400g, 8 mins at 4 °C and resuspended the cells pallet in cold 1ml complete medium (10%FBS, 1X Glutamax, 10mM HEPES, 100 U/mL penicillin-streptomycin in RPMI). Proceeded for in-vitro stimulation or antibody labelling for flow cytometry assay. For ILC2 sorting, (44%/67%) percoll (GE17-0891-01, Sigma) gradience was performed for lymphocyte enrichment. For succinate experiment using the triple reporter mice carried out in the university of Zurich 4 cm of proximal SI tissue was processed as previously described ²⁶.

Flow cytometry and cell sorting. Tuft cell staining from IEL cell fraction was adapted from O’leary, 2021 ²⁶. Briefly, IEL cells were incubated with 1:500 Fc-Block (14-0161-85, eBioscience) and 1:1000 fixable viability dye eFluor780 (65-0865-18, eBioscience) for 15 mins at 4 °C. Epithelial surface staining was performed with a 1:300 antibody mix including CD45-APC (for CD45.2: 558702, BD Biosciences; for CD45.1: 110713, Biolegend), Epcam-FITC (118208,

673 Biolegend) and CD24-BV421 (101825, Biolegend) for 20 mins at 4 °C in FACS buffer. After
 674 washing in FACS buffer, IEL cells were fixed with 4% PFA for 7 mins and stained for 1:1000
 675 DCAMKL1 (DCLK1) (ab31704, abcam) in perm buffer (00-8333-56, eBioscience). Incubated in
 676 1:2000 secondary antibody (A32731, Goat anti-Rabbit Alexa Fluor Plus 488, Invitrogen) for 6
 677 mins. Tuft cells were gated as Live/Epcam⁺ CD45^{neg}/CD24⁺ DCLK1⁺. To identify ILC2
 678 (Lymphocyte/Live CD45⁺/CD90⁺ CD3^{neg}/Gata3⁺) and Th2 (Lymphocyte/Live CD45⁺/CD90⁺
 679 CD3⁺/CD4⁺/Gata3⁺ Foxp3^{neg}), LP cells after harvesting from the enzymatic digestion, were
 680 blocked with 1:1000 Fc-Block (14-0161-85, eBioscience) and fixable viability dye eFluor506 (65-
 681 0866-18, eBioscience) in PBS for 10 mins at 4 °C. Proceeded LP surface staining for transcription
 682 factor panel with 1:200 antibody mix containing CD45-APC-Cy7 (For CD45.2: 47-0454-82,
 683 eBioscience; for CD45.1: 110716, Biolegend), CD90-PerCP (105322, Biolegend), CD3-BV421
 684 (100227, Biolegend), CD4-FITC (100510, Biolegend), Klrg1-PE-Cy7 (25-5893-80, eBioscience)
 685 in FACS buffer for 15 mins at 4 °C. Then cells were fixed overnight in Foxp3/Transcription Factor
 686 Fixation/Permeabilization buffer (00-5521-00, eBioscience) at 4 °C followed by LP intracellular
 687 transcription factor staining with 1:200 antibody mix containing Gata3-BV711 (565449, BD
 688 Bioscience), Foxp3-APC (17-5773-82, eBioscience) and RORγt -PE (562607, BD Bioscience) in
 689 perm buffer for 1h at RT. To examine type 2 cytokines after PMA/Ionomycin incubation,
 690 stimulated cells were harvested and blocked with 1:1000 Fc-Block (14-0161-85, eBioscience) and
 691 fixable viability dye eFluor506 (65-0866-18, eBioscience) in PBS for 10 mins at 4 °C. Proceeded
 692 LP surface staining for cytokine panel with 1:200 antibody mix containing CD45-APC-Cy7 (For
 693 CD45.2: 47-0454-82, eBioscience; for CD45.1: 110716, Biolegend), CD90-PerCP (105322,
 694 Biolegend), CD3-BV421 (100227, Biolegend), CD4-PE-Cy7 (25-0042-82, eBioscience) in FACS
 695 buffer for 15 mins at 4 °C. Then cells were fixed overnight in Foxp3/Transcription Factor
 696 Fixation/Permeabilization at 4 °C followed by LP intracellular cytokine staining with 1:200
 697 antibody mix containing Gata3-BV711 (565449, BD Bioscience), IL-4-AF488 (504109,
 698 Biolegend), IL-5-APC (504305, Biolegend) and IL-13-PE (12-7133-81, eBioscience) in perm
 699 buffer for 1h at RT. Added counting beads (ACBP-100-10, Spherotech) to all FACS stained
 700 samples before reading in flowcytometry (BD LSRFortessa). To sort ILC2, LP cells were
 701 incubated with 1:1000 Fc-Block (14-0161-85, eBioscience) for 10 mins and surface stained with
 702 1:200 antibody mix containing CD45- CD45-APC-Cy7 (For CD45.2: 47-0454-82, eBioscience;
 703 for CD45.1: 110716, Biolegend), Lineage-AF700 (133313, Biolegend, Alexa Fluor® 700 anti-

704 mCD3/ Alexa Fluor® 700 anti-mLy-6G (Ly-6C)/ Alexa Fluor® 700 anti-mCD11b/ Alexa Fluor®
 705 700 anti-mCD45R (B220)/ Alexa Fluor® 700 anti-mTer-119), CD90-PerCP (105322, Biolegend),
 706 Klrp1-PE (25-5893-80, eBioscience) and Sca1-AF488 (108115, Biolegend) for 15 mins at 4 °C.
 707 Cells were added DAPI and sorted using SONY SH800S cell sorter. Sorting strategy of ILC2:
 708 Live/CD45⁺/Lin^{neg}/Klrp1⁺ Sca1⁺. Sorted cells were collected in complete media, centrifuged, and
 709 counted for following culture.

710 For experiments using triple reporter mice, single cell suspensions of either small intestinal
 711 epithelia or lamina propria tissue, were initially incubated (10 mins, on ice) with Fc-receptor
 712 blocking antibody diluted in FACS-buffer, prior to resuspension in a FACS-buffer based cocktail
 713 of antibodies directed towards surface antigens (specified below). Following incubation (20 mins,
 714 on ice), cells were resuspended in zombie red dye (423110, BioLegend), according to the
 715 manufacturers' instructions. After another incubation (10 mins, on ice), epithelial samples were
 716 fixed for 3 minutes using 4 % PFA (15710, Lucerna-Chem), and lamina propria suspensions fixed
 717 for 10 minutes using the Foxp3/Transcription factor staining set (00-5523-00, Thermo Fisher).
 718 Cells were then resuspended in 1x permeabilization buffer (00-8333-56, Thermo Fisher)
 719 containing all antibodies directed towards intracellular targets (specified below). Acquisition was
 720 performed using the spectral analyzer Cytex Aurora. Lamina propria surface staining: Anti-mouse
 721 CD45 in BUV395 (564279, BD Horizon), Anti-mouse CD8a in BV510 (100752, BioLegend),
 722 Biotinylated anti-mouse ST2 (145308, BioLegend), Streptavidin in BV605 (405229, BioLegend),
 723 Anti-mouse CD4 in BV650 (100555, BioLegend), Anti-mouse CD11b in BV711 (101241,
 724 BioLegend), Anti-mouse Thy1.2 in BV785 (105331, BioLegend), Anti-mouse KLRG1 in
 725 PerCPeFluor710 (46-5893-82, Thermo Fisher), Anti-human CD4 in PE (300508, BioLegend),
 726 Anti-mouse Ly6G in AF700 (127622, BioLegend), Anti-mouse SiglecF in APC-Cy7 (155532,
 727 BioLegend), and a lineage cocktail consisting of Anti-mouse CD11c (117343, BioLegend), Anti-
 728 mouse CD19 (115549, BioLegend), Anti-mouse NK1.1 (108741, BioLegend), and Anti-mouse
 729 Ter119 (116234, BioLegend), all in BV421. Epithelial surface staining: Anti-mouse CD45 in
 730 BUV395 (564279, BD Horizon), Biotinylated anti-mouse CD24 (101804, BioLegend),
 731 Streptavidin in APC (405207, BioLegend), Anti-mouse SiglecF in APC-Cy7 (155532,
 732 BioLegend). Lamina propria intracellular staining: Anti-mouse GATA3 in AF488 (TWAJ,
 733 Invitrogen), Anti-mouse Ki-67 in PE-Cy7 (25-5698-82, eBioScience), Anti-mouse RORγt (17-
 734 6981-82, Invitrogen). Epithelial intracellular staining: Rabbit anti-mouse DCAMKL1 (ab31704,

Abcam), Goat anti-rabbit IgG in AF488 (A32731, Thermo Fisher), Anti-mouse CD326 in PerCP-Cy5.5 (118220, BioLegend). In case the epithelial fraction was not fixed, for the purpose of maintaining TdTomato signal, anti-mouse CD326 antibody was instead added to the surface staining mix, and DCLK1 staining excluded.

In-vitro cell culture. For PMA-Ionomycin stimulation, 3 million live LP cells were stimulated for 4 hours at 37 °C, 5% CO₂ and with 100ng/ml PMA (P1585-1MG, Sigma) and 1 µg /ml Ionomycin (I0634-1MG, Sigma) in complete medium for 30 mins and added 1000X Golgi Plug (555029, BD Bioscience). For rIL-25 *in vitro* stimulation, 5 million live LP cells were stimulated with vehicle or 200ng/ml rIL-25 (587306, Biolegend) in complete media overnight. The day after, cells were treated 1500X Golgi Stop (554724, BD Bioscience) for 3h and proceeded the antibody staining for FACS assay. To examine ILC2 proliferation, 2500-10000 sorted ILC2s in each well in 96-well plate were treated with 200 µl 10ng/ml mrIL-2 (575404, BioLegend), 10ng/ml mrIL-7 (217-17-2UG, Peprotech) and 10ng/ml rIL-25 (587306, Biolegend) in complete media and the media was refreshed 50% every two days until day 8. Viable ILC2s were counted under microscope.

Intestinal monolayer culture. Intestinal monolayer culture used in this study was adapted from previously described ^{11,73}. Crypts from whole length of SI were harvested as described above. Precoated the 48-well plate with 100 µl Matrigel: Advanced DMEM/F12 (1:20) in 37 °C 5% CO₂ for at least 1 h. After removing the residue uncoated Matrigel mix, in each well, plated 1500-1800 suspended crypts in 100 µL basic organoid culture medium supplemented with 50ng/ml of murine recombinant epidermal growth factor (i.e. EGF, 2028-EG-200, from R&D), 250-500 ng/ml recombinant murine R-Spondin (3474-RS-250, R&D) and 100ng/ml recombinant murine Noggin (250-38, Peprotech), 10 µM CHIR99021 (SML1046-5MG, Sigma), 50nM A83-01 (909910-43-6, Sigma) and 10 µM Y-27632 (Y0503-1MG, Sigma). Allowed attachment overnight in cell incubator at 37 °C, 5% CO₂. The next day the non-adherence fragment was discarded and washed once with 400 µL pre-warmed PBS. Replaced with 100 µL pre-warmed RPMI with 1 µM DMSO or 1 µM GW3965 and incubated for 1h. After incubation, replaced the 100 µL media with or without 1 µg/ml Ionomycin, DMSO or GW3965 presented during the entire stimulation. After 30 mins stimulation at 37 °C 5% CO₂, the cell supernatant was collected after spinning down the unattached cells, stored at -80 °C until ELISA assay.

ELISA. Cysteinyl leukotrienes level in the epithelial monolayer cell culture supernatant was measured by using Cysteinyl Leukotriene Express ELISA Kit (154-10009291, Caymanchem) according to product manual. To test IL-25 and IL-13 levels in SI tissue homogenate, for each sample, total protein was extracted from 1cm frozen SI by 300µl RIPA lysis buffer (20-188, Millipore) supplemented with Halt™ Protease and Phosphatase Inhibitor Cocktail (100X) (78440, Thermo Scientific). Tissue homogenate cytokines were measured by Mouse IL-17E DuoSet ELISA kit (DY1399, R&D Systems) and Mouse IL-13 DuoSet ELISA kit (DY413, R&D Systems). Data were normalized to total protein measured by Pierce™ Detergent Compatible Bradford Assay Kit (23246, Thermo Scientific)

Immunohistochemistry (IHC) and quantification. Tissue rolls were fixed in 10% buffered formalin immediately after sacrifice. Unstained sample slides were obtained by tissue paraffin embedded and sectioned (5 µm) by FENO (Morphological Phenotype Analysis) facility (Karolinska Institutet). For staining, unstained slides were deparaffinized in xylene for 10 mins x 3 times and then rehydrated with graded alcohols. Antigen retrieval was performed in 1mM EDTA buffer (pH - 8.0) at 121°C for 20 minutes using 2100 Antigen Retriever. After cooling down and washing in RT PBS for 3 times, samples were blocked by using BLOXALL blocking solution (VESP-6000-100, Vector Labs) for 1h at RT. Then the blocked samples were incubated with primary antibodies diluted in PBS overnight at 4 °C. Primary antibody used: 1:300 Anti-DCAMKL1 (DCLK1) antibody (ab31704, Abcam) for tuft cell staining; 1:500 Anti-Mucin2 antibody (PAA705Mu01, Cloud-clone corp) for goblet cell staining. 1:300 secondary anti-rabbit antibody (BA-1000, Vector Laboratories) was incubated for 1h the next day after 3 times of PBS wash at RT. The slides were washed four times in PBS, then incubated at RT with VECTASTAIN Elite ABC HRP Kit (PK-6100, Vector Labs) for 30 minutes. Staining was developed using DAB staining (VESK-4100, DAB peroxidase staining kit, Vector Labs). After being counterstained with hematoxylin (Harris Hematoxylin, Leica) and washed in tap water, the sections were dehydrated in increasing grades of alcohols and then xylene before drying. Finally, the slides were mounted using permanent mounting medium and scanned using Hamamatsu NanoZoomer Slide Scanner. Quantification of tuft cell (DCLK1⁺) and goblet cell (Muc2⁺) were performed in blinded conditions by randomly selecting 50-100 villus and crypts per section in Qupath software, then quantified the stained and averaged as DAB⁺ cells per villus, per crypt and per crypt-villus axis.

Immunofluorescent staining and quantification. For DCLK1 Immunohistochemistry in samples from *N. brasiliensis* experiment, the small intestine was taken and prepared for processing and embedding in paraffin using the Swiss-rolling technique ⁷⁴. Transverse sections were made using a microtome at a thickness of 5 µm before mounting on glass slides. Sections were deparaffinized by immersing slides in xylene, then hydrated through 100%, 90%, and 70% ethanol successively. Heat-induced epitope retrieval was performed in citrate buffer, and then sections were blocked using 2.5% normal horse serum blocking solution (Vector Laboratories) for 1 h at room temperature. Slides were then incubated overnight at 4°C with rabbit anti-mouse DCLK1 (ab31704, Abcam) at 1:1000 in 2.5% normal horse serum blocking solution. After washing, sections were incubated with ImmPRESS® HRP Horse Anti-Rabbit IgG Polymer Detection Kit, Peroxidase (Vector Laboratories) and developed with the ImmPACT® DAB Substrate Kit, Peroxidase (HRP) (Vector Laboratories). Finally, slides were dehydrated and mounted with DPX before scanning on the NanoZoomer-SQ Digital slide scanner (Hamamatsu). The resulting image files were analysed using NDP.view 2 software (Hamamatsu). For staining tissue sections from *Il25*-tdTomato mice, sections were incubated for 1 h at room temperature in blocking buffer (1× PBS supplemented with 2% BSA, 0.1% Triton X-100 and 5% goat serum). All staining was subsequently performed in blocking buffer. Incubation with primary antibodies (DCLK1 (Abcam, ab31704, 1:1,000; E-Cadherin – AF647 (Biolegend, 147308, 1:100)) was performed at 4 °C overnight, and incubation with the secondary antibody (goat anti-rabbit IgG-AF488 (Thermo Fisher, A32731, 1:2,000)) was performed for 1 h at room temperature. DAPI was added separately for 5 min, and samples were washed with PBS. For mounting, Vectashield mounting medium was diluted 1:3 in a glycerol solution with added Tris (50 mM). 2-3 representative images per section were acquired using a Leica DMI8 Thunder Imager equipped with a 20X objective and a DFC9000GT Camera. Individual channel images were combined using the tiff file library and segmented using the “fluorescent nuclei and cells” model from Instanseg ⁷⁵. Single cell intensity measurements were extracted using the steinbock package ⁷⁶ and tuft cells were identified as E-Cadherin, DCLK1 double positive cells. Epithelial cells were identified as E-Cadherin positive cells.

RNA extraction and quantitative RT-PCR. Tissues were recovered to 4 °C from frozen in RNAlater (AM7020, Thermo Fisher) reserved at -20 °C. Tissues were transferred to RLT Plus buffer (74136, Qiagen) + 2% β-mercaptoethanol (31350010, Gibco) and lysed by TissueLyser LT

(Qiagen). RNA extraction from tissue or organoid RLT homogenate were performed by using RNAeasy Mini Kit (74136, Qiagen) according to manufacturer instructions and the concentration of RNA was measured using Nanodrop. iScript RT Supermix (1708841, Biorad) was used for reverse transcription to cDNA. Quantitative real-time PCR analysis was performed using iTaq Universal SYBR Green Supermix (1708880, Biorad), and log2 fold changes (log₂FC) were determined in relation to either *Hprt* (for tissues) or *B2m* (for organoid) using the using $2^{-(\Delta\Delta CT)}$ method. Primer pair sequences used in the study are listed in Table S2.

Bulk RNAseq analysis. cDNA libraries were constructed from the extracted SI RNA and sequenced by Novogene Co Ltd (UK) at a sequencing depth of 20 million reads per sample using paired-end 150 bp reads on a NovaSeq 6000 sequencer and v1.0 reagent kit. Raw sequences were quantified and annotated using Kallisto⁷⁷ and GRCm38 (mm10) cDNA assembly⁷⁸, and resulting gene counts were compiled into gene expression matrices. Gene expression analysis was performed in R Statistical Software v3.6.3 and v4.1.2⁷⁹. Differential expression was evaluated using edgeR v3.36.0 and v4.0.16⁸⁰, with a quasi-likelihood test between diet groups. The resulting log2 (fold-change) values were used to rank the genes prior to identification of enriched KEGG pathways⁸¹ using Gene Set Enrichment Analysis³¹ in GSEA_4.2.1 software. The threshold for data visualization is described specifically in the corresponding figure legend.

Sample preparation for scRNAseq. Wild type C57BL/6 mice were fed a standard or GW3965 diet for 10 days, followed by 4 days of 150mM succinate in drinking water or normal water as control (n=3 per group). Next, the whole SI of cell suspensions were prepared as described above from four conditions (standard diet with normal drinking water n=3; GW3965 diet with normal drinking water; standard diet with 150mM succinate n=3; GW3965 diet with 150mM succinate n=3). After sacrifice, IEL and LP fractions were obtained individually from each mouse. Cell suspensions were then blocked with Fc-blocking solution (eBioscience, 1:1000) and surface stained at 4 °C for 15 min with 1:200 antibody mix containing Epcam-FITC (118208, Biolegend), CD45-PeCy7 (109830, BioLegend), Ter119-Pacific Blue (116232, BioLegend). Prior to sorting, cells from the three biological replicates were pooled by treatment group for each fraction. EPCAM⁺/CD45⁻/Ter119⁻/DAPI⁻ epithelial cells (IECs) and EPCAM⁻/CD45⁺/Ter119⁻/DAPI⁻ immune cells were sorted from IEL and LP fractions respectively using the SONY SH800S cell sorter. Sorted IEC and immune cells were counted, adjusted to 800 cells/μl in FACS buffer, and mixed in a 1:1 volume ratio. Mixed IECs and immune cells in 1:1 volume ratio (50 μl: 50μl) from

each condition. Approximately 19,000 IEC cells and 19,000 LP cells per sequencing sample were loaded onto the 10x chip and the samples were processed using the Chromium Next GEM single cell 3' reagents kit v4 (dual index). Libraries were constructed following the manufacturer's guidelines. Libraries prepared for scRNA-seq were sequenced by Xpress Genomics at a sequencing depth of 1600 million reads per sample using paired-end 150 bp reads on the NovaSeq 6000 sequencer.

Analysis for scRNAseq reference. For analysis, we employed Cell Ranger v6.0.2⁸², CellBender v0.3.2⁸³, Seurat v4.4.0⁸⁴ and Scanpy v1.1.1⁸⁵. Pre-processing and initial quality control were performed using the Cell Ranger pipeline (10X) on the mm10 reference genome. Each cellranger output was processed with CellBender pipeline with default parameters. All cells were processed together in Seurat as follows: Cells expressing below 200 unique genes were filtered out as well as cells with mitochondrial RNA content higher than 10% and ribosomal RNA content lower than 2%. Genes with 3 counts or fewer in the entire dataset were removed. Genes annotated as protein coding and TCR/BCR complex according to ENSEMBL were retained, excluding mitochondrial genes which were removed from the dataset. After quality control, 15,266 genes and 19,956 cells were kept. Gene expression data were normalized using `normalize_total(target_sum=1e4)` and `log1p`. Highly variable genes across all samples were chosen using `highly_variable_genes(n_top_genes=2e3, flavor='seurat_v3', batch_key='sample')`. Integration was performed using `scvi-tools` (87) 1.3.0 with `n_layers=2`, `n_latent=30`, `gene_likelihood='nb'`. The `scvi` model was trained for up to 400 epochs with early stopping enabled. The learned latent representation was used to construct a nearest-neighbor graph. Then the preliminary clustering was performed by running the `neighbors(n_pcs=30, use_rep='X_scvi')` and `leiden(resolution=0.5, flavor='igraph')`. Clusters were annotated based on canonical marker gene expression obtained by `rank_genes_groups(method='wilcoxon')`. Then the Tuft cell cluster was extracted and subclustered as follow. Highly variable genes across all samples were chosen using `highly_variable_genes(n_top_genes=8e, flavor='seurat_v3', batch_key='sample')` and scaled. Dimensionality reduction was performed using PCA to capture major axes of variation in neighborhood structure. Integration was performed using `harmonypy`⁸⁷ 0.0.1 with default parameters. Then the Tuft cell sub-clustering was performed by running the `neighbors(n_pcs=30, use_rep=X_pca_harmony)` and `leiden(resolution=0.5, flavor='igraph')`. DEGs were obtained by `rank_genes_groups(method='wilcoxon')`.

Functional enrichment analysis. To interpret the biological relevance of each factor, gene GO enrichment analysis was performed on the selected gene sets ($p_val_adj < 0.05$ & $avg_log2FC > 0.5$) using clusterProfiler (89) v4.14.6 in combination with the org.Mm.eg.db v3.20.0 annotation resource. Gene symbols were first converted to ENTREZ identifiers using mapIds, and enrichment was performed using enrichGO.

RNA velocity and dynamical modeling. RNA velocity analysis on Tuft cells was performed using scvelo ⁸⁹ v0.3.3. To account for potential sample-specific dynamics, all analyses were conducted separately for each biological sample. For each sample, raw spliced and unspliced count matrices were preprocessed using gene filtering and normalization. Genes with low shared counts across spliced and unspliced layers were excluded using a minimum shared count threshold of 20, and the top 1,200 highly variable genes were retained for downstream analysis. Normalized expression values were then used to compute first- and second-order moments across neighboring cells, based on a neighborhood graph constructed using 30 principal components and 20 nearest neighbors. To infer transcriptional dynamics, the dynamical model implemented in scVelo was applied. A velocity graph was subsequently constructed to model transition probabilities between cells in the high-dimensional expression space.

Statistics. GraphPad Prism9 software was utilized for data analysis, with the statistical tests performed as detailed in the figure legends.

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Author Contributions

XL, NH, QZ, TA, MM, RAMC, MCP, CC, PF, JLM, FC, FLF, AO, SMP, BCK and SD performed experiments. NH, JF, and XL performed bioinformatics analysis of the transcriptomic data. EJV, SD, and XL conceived the idea. EJV, CS, RMM, JHB, and CH provided resources. XL and EJV wrote the paper. All authors discussed the data, read, and approved the manuscript.

Competing interests

E.J.V. has been paid consultant for Pfizer, Kancera AB, NanoString, Novome Biotechnologies, Ono Pharma, Mabydon, and Ferring. E.J.V. has received sponsor research support from Mabydon and F. Hoffmann-La Roche AG, serves as lead Scientific Advisory Board member and holds equity in Evogence Biologics OY Ltd, and is founder of PaperVids. C. R. H. Hedin served as a speaker and/or advisory board member for AstraZeneca, Abbvie, Celltrion, Dr Falk Pharma and the Falk Foundation, Galapagos, Janssen, Lilly, Pfizer, Pharmacosmos, Ferring, Takeda, Tillotts Pharma, and received grant support from Tillotts and Takeda, outside the submitted work.

Data availability

The datasets generated in this study have been uploaded to the Gene Expression Omnibus (GEO) database and can be accessed with the following accession codes: GSE324544 for single cell RNA

1142 sequencing, GSE260593 and GSE260594 for bulk RNAseq. Spatial transcriptomics dataset was
1143 re-visualized from a public dataset GSE227742 ²².

1144

1145 **Code availability**

1146 Code for bulk tissue RNAseq analysis is available at

1147 https://github.com/xinluoki/DistalSI_STDvsGW_bulkRNAseq_analysis_2024.git

1148 https://github.com/xinluoki/ProximalSI_0_14dpi-bulkRNAseq_analysis_2024.git

1149

1150 Code for single cell RNAseq analysis is available at

1151 https://github.com/ejvillablancaLab/LXR_Succinate_on_Tuft

1152

Main Figure legends

Fig. 1. Ectopic liver X receptor (LXR) activation diminishes type 2 lymphocytes in the small intestine. **a**, Schematic representation of the experimental design and qPCR analysis of *Abca1* expression, normalized to *Hprt*, in proximal and distal small-intestinal biopsies from mice fed with GW3965 or standard diet. **b**, Hematoxylin and eosin (H&E) staining and spatial transcriptomic analysis showing *Abca1*, *Abcg1*, and *Srebf1* expression in the proximal small intestine. **c**, Gene set enrichment analysis (GSEA) of Kyoto Encyclopedia of Genes and Genomes for (KEGG) pathway (both up and down-regulated, $P < 0.01$) between GW3965-treated and control animals during homeostasis. **d-f**, Flow cytometric analysis of small-intestinal lamina propria cell suspensions. Representative flow cytometry plots and quantification of the proportions and numbers of Th2 cells and ILC2s in the small-intestinal lamina propria (**d**) and quantification of ILC2 proportions in LXR α -deficient (**e**) or LXR β -deficient (**f**) mice compared with their respective control littermates under homeostatic conditions. Where applicable in (**a** and **d-f**), each dot represents one biological replicate (one mouse), unless otherwise specified. Data were pooled from two or more independent experiments ($n = 3-13$ mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using an unpaired Student's *t*-test for comparisons between two groups or one-way ANOVA followed by Tukey's multiple-comparisons test when more than two groups were compared.

Fig. 2. LXR signaling modulates intestinal tuft cell homeostasis. **a**, Heatmap of genes differentially expressed in distal small-intestinal tissue from mice fed with GW3965 or standard diet (from Bulk RNASeq analysis, $P < 0.001$ and $|\log_2FC| > 2$). **b**, Heatmap of normalized TPM values for selected tuft cell signature genes identified by bulk RNA-seq analysis of distal small-intestinal tissue from mice fed with GW3965 or standard diet. **c**, qPCR analysis of *Gnat3*, *Trpm5*, and *Sucnr1* expression, normalized to *Hprt*, in proximal and distal small-intestinal biopsies from mice fed with GW3965 or standard diet for 10-17 days. **d-e**, Representative flow cytometry plots (**d**) and quantification (**e**) of tuft cells in the proximal and distal small intestine under homeostatic conditions. **f-g**, Representative images of DCLK1 immunohistochemical staining (**f**) and quantification of DCLK1⁺ cells per villus and crypt (**g**) in the distal small intestine under homeostatic conditions. Three biological replicates were analyzed per condition. Scale bar, 200 μ m. **h-i**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus and crypt in the distal small intestine of LXR β -deficient (**h**) or LXR α -deficient (**i**) mice compared with their respective control littermates under homeostatic conditions. Where applicable in (**a-i**), each dot represents one biological replicate (one mouse), unless otherwise specified. Data were pooled from two or more independent experiments ($n = 3-7$ mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using an unpaired Student's *t*-test (**c** and **g-i**) or one-way ANOVA followed by Tukey's multiple-comparisons test (**e**).

Fig. 3. LXR activation inhibits succinate-induced tuft cell hyperplasia without impairing ILC2 effector functions. **a**, Schematic representation of the ILC2–tuft cell axis and the proposed site of LXR action. **b**, Schematic representation of the experimental design. **c–d**, Representative images of DCLK1 immunohistochemical staining (**c**) and quantification of DCLK1⁺ cells per crypt–villus axis (**d**) in the distal small intestine. Scale bar, 200 μ m. **e–f**, qPCR analysis of *Il25* (**e**) and *Il13* (**f**) expression, normalized to *Hprt*, in distal small-intestinal biopsies from mice treated as depicted in (**b**). **g**, Schematic representation of the ILC2–tuft cell axis and the proposed site of LXR action. **h–i**, Representative CellTrace Violet histograms (**h**) and quantification of mean fluorescence intensity (MFI; **i**) in sorted ILC2s cultured for 3 days with DMSO or GW3965 in the presence or absence of recombinant IL-25 (rIL-25) *in vitro*. **j**, Representative flow cytometry contour plots and analysis of small-intestinal lamina propria cell suspensions from mice fed a GW3965-containing or standard diet and cultured *in vitro* overnight in the presence or absence of rIL-25. Data are from one experiment with 3–4 mice per group. In (**d–f**, **i**, and **j**), unless otherwise specified, each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 3–8$ mice), unless otherwise specified, and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Fisher’s least significant difference (LSD) test (**d–f**) or Tukey’s multiple-comparisons test (**i** and **j**).

Fig. 4. IL-25 signaling rescues LXR-mediated inhibition of the tuft cell-ILC2 circuit. **a**, Schematic representation of the ILC2–tuft cell axis and the proposed site of LXR action. **b**, Schematic representation of the experimental design. **c**, Representative flow cytometry contour plots of Ki-67⁺ and Sm13⁺ ILC2s in proximal small-intestinal lamina propria cell suspensions. **d–e**, Quantification of Ki-67⁺ (**d**) and Sm13⁺ (**e**) ILC2s. **f–g**, Representative immunofluorescence images showing the *Il25*–tdTomato reporter, E-cadherin, and DAPI (**f**) and quantification of *Il25*–tdTomato⁺ tuft cells as a percentage over total E-cadherin⁺ cells (**g**). Each dot in (**g**) represents one field of view; $n = 2$ biological replicates per condition. Scale bar, 200 μ m. **h–i**, Representative flow cytometry contour plots of DCLK1⁺ tuft cells in proximal small-intestinal epithelial cell suspensions (**h**) and quantification of tuft cells as a percentage of total EpCAM⁺ cells (**i**). **j**, Schematic representation of the predicted LXR target involved in regulating IL-25 expression in tuft cells. **k**, Representative histogram showing *Il25*–tdTomato mean fluorescence intensity (MFI) in tuft cells under the indicated conditions. Where applicable in (**d–k**), each dot represents one biological replicate (one mouse), except in (**g**) as specified above. Unless otherwise specified, data were pooled from two or more independent experiments ($n = 6–7$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. For comparisons involving more than two groups, statistical significance was determined using one-way ANOVA followed by Tukey’s multiple-comparisons test.

Fig. 5. Epithelial-intrinsic LXR activation is required to inhibit tuft cell hyperplasia following succinate challenge. **a**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus and crypt in the distal small intestine of LXR α ^{ΔIEC} mice and control littermates under

homeostatic conditions. **b**, Representative flow cytometry contour plots and quantification of ILC2s in whole small-intestinal lamina propria cell suspensions. **c**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus and crypt in the distal small intestine of LXRαβ^{ΔIEC} mice and control littermates following 7 days of treatment with 150 mM succinate. In (**a-c**), each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 4-6$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using an unpaired Student's t -test (**a** and **b**) or two-way ANOVA followed by Fisher's least significant difference (LSD) test (**c**).

Fig. 6. LXR signaling enhances the tuft-1 cell signature. **a-c**, Uniform manifold approximation and projection (UMAP) visualization of scRNA-seq data from EpCAM⁺ epithelial cells (**a**) and tuft cells (**b-c**) isolated from the whole small intestine under the four indicated treatment conditions. Three mice were pooled per treatment condition. In (**c**), n indicates the number of tuft cells, and % indicates the percentage of tuft cells among all analyzed EpCAM⁺ epithelial cells in each treatment group. **d**, Volcano plot showing differentially expressed genes in tuft cells from mice fed a standard or GW3965 diet under homeostatic conditions. **e**, Expression density of tuft cell-associated genes projected onto the UMAP. **f**, Violin plots showing *Gnat3* and *Gng13* expression in the scRNA-seq dataset under the indicated conditions. **g**, Gene Ontology analysis showing the top 10 biological processes enriched among genes upregulated in tuft cells from mice receiving a GW3965 diet plus succinate compared with those receiving a standard diet plus succinate. **h**, UMAP visualization of eight tuft cell clusters and violin plots showing tuft-1 and tuft-2 signature scores in each subcluster. **i**, RNA velocity-based trajectory analysis overlaid on UMAP visualizations of tuft cell clusters under the four indicated conditions. **j**, Scatter plot comparing fold changes in tuft-1 and tuft-2 cell abundance following succinate challenge in mice fed a standard or GW3965-containing diet. The diagonal line ($y = x$) indicates equivalent expansion of the two cell populations, and the distance above the diagonal ($\Delta = \text{Fold Change}_{\text{tuft2}} - \text{Fold Change}_{\text{tuft1}}$) represents the magnitude of preferential tuft-2 cell expansion. **k**, Bubble plot comparing the expression of tuft-1 and tuft-2 signature genes under the four indicated conditions.

Fig. 7. LXR activation impairs the anti-helminth response. **a**, Schematic representation of the *N. brasiliensis* infection experiment. **b**, Immunohistochemistry-based quantification of DCLK1⁺ cells per crypt-villus axis at the indicated time points. dpi, days post-infection. **c**, Quantification of eggs per gram of stool from mice treated as depicted in (**a**). **d**, Schematic representation of the *H. polygyrus* infection experiment using wild-type mice. **e-f**, Representative images of DCLK1 immunohistochemical staining (**e**) and quantification of DCLK1⁺ cells per villus (**f**) in the proximal small intestine of mice treated as depicted in (**d**). Scale bar, 200 μm . **g**, Quantification of eggs per milligram of stool at 13 dpi from mice treated as depicted in (**d**). **h**, Schematic representation of the *H. polygyrus* infection experiment using LXRαβ^{ΔIEC} mice and littermate controls. **i**, Immunohistochemistry-based quantification of DCLK1⁺ cells per villus in the proximal small

intestine of mice treated as depicted in **(h)**. **j**, Quantification of IL-25 concentrations in proximal small-intestinal tissue homogenates from mice treated as depicted in **(h)**, as measured by ELISA. **k**, Quantification of eggs per milligram of stool at 13-14 dpi from mice treated as depicted in **(h)**. Where applicable in **(b-k)**, each dot represents one biological replicate (one mouse), unless otherwise specified. Data were pooled from two or more independent experiments ($n = 3-16$ mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test (**b-c** and **f-g**) or Fisher's least significant difference (LSD) test (**i-k**).

Supplementary Figure legends

Extended Data Fig.1. LXR activation exerts immunosuppressive effects in the proximal small intestine, related to Fig. 1. **a**, Gene set enrichment analysis (GSEA) showing enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in proximal small-intestinal bulk RNA-seq data from GW3965-fed and standard-fed mice (both up and down-regulated, $P < 0.01$). **b**, Heatmap of genes differentially expressed in proximal small-intestinal biopsies from GW3965-fed and standard-fed mice from by bulk RNA-seq ($P < 0.001$ and $|\log_2FC| > 2$).

Extended Data Fig.2. Small intestine lamina propria type 2 cytokine producing ILC2 numbers upon ectopic LXR activation, related to Fig. 1. **a**, Flow cytometry gating strategy for identifying ILC2s and Th2 cells in the small-intestinal lamina propria (SI-LP). **b**, Flow cytometry gating strategy for analyzing IL-5⁺ IL-13⁺ ILC2s. Cytokine production was measured after 4 h of PMA and ionomycin stimulation of total SI-LP cells *in vitro*. **c**, Representative flow cytometry contour plots and quantification of IL-13⁺ and IL-5⁺ ILCs per million SI-LP cells following PMA and ionomycin stimulation *in vitro*. Cells were isolated from mice fed with GW3965 or standard diet. **d**, Representative flow cytometry plots and quantification of IL-5⁺ IL-13⁺ ILCs per million SI-LP cells following 4 h of PMA and ionomycin stimulation *in vitro* from mice fed or not with GW3965. In (**c-d**), each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 6-9$ mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using an unpaired Student's *t*-test.

Extended Data Fig.3. LXR isoforms LXR α and LXR β expression and type 2 lymphocytes in the small intestine, related to Fig. 1. **a**, Representative hematoxylin and eosin (H&E)-stained images of mouse small intestine overlaid with Visium HD spatial transcriptomic signals for *Nr1h3* (LXR α) and *Nr1h2* (LXR β). **b**, UMAP visualization of the public scRNA-seq dataset GSE227726, showing epithelial, immune, and stromal cells from the mouse small intestine. **c**, UMAP visualization and violin plots showing *Nr1h3* and *Nr1h2* expression in immune, stromal, and epithelial cells from mouse small intestine in scRNAseq dataset GSE227726. **d-e**, Flow cytometric analysis of Th2 cells and ILC2s in SI-LP cell suspensions from LXR α -deficient (**d**) or LXR β -deficient (**e**) mice and their respective control littermates. In (**d-e**), each dot represents one biological replicate (one mouse). Unless otherwise specified, data were pooled from two independent experiments ($n = 6-11$ mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using an unpaired Student's *t*-test.

Extended Data Fig.4. LXR activation, but not LXR β deficiency affects succinate-induced tuft cell, related to Fig. 3. **a**, qPCR analysis of *Dclk1*, *Sucnr1*, *Gnat3*, and *Muc2* expression, normalized to *Hprt*, in distal small-intestinal biopsies under the indicated conditions. **b**,

Quantification of DCLK1⁺ cells per villus and crypt in the distal small intestine under the indicated conditions. **c**, Quantification of DCLK1⁺ cell frequencies among EpCAM⁺ cells along the entire small intestine of LXR β -deficient mice and littermate controls (after 4-day 100mM succinate stimulation, exp $n = 2$) and DCLK1⁺ cells number per villus and per crypt (after 7-day 150mM succinate stimulation, exp $n = 1$) in LXR β mice and their littermate controls. Each dot represents one biological replicate (one mouse). Unless otherwise specified, data were pooled from two or more independent experiments ($n = 3$ -13 mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test (**a**), Fisher's least significant difference (LSD) test (**b-c**) or unpaired Student's t -test (**d**).

Extended Data Fig.5. GW3965 activation induced comparable activation and proliferation in SI-LP sorted ILC2s after rIL-25 stimulation, related to Fig. 3. **a**, Flow cytometry gating strategy for sorting ILC2s. **b**, Representative flow cytometry contour plots and quantification of CD25⁺ and CD25⁻ ILC2 frequencies following 72 h of stimulation with rIL-25 in the presence or absence of GW3965. **c**, ILC2 numbers following 8 days of *in vitro* culture with rIL-25, rIL-2, and rIL-7 at 10 ng/ml each, starting from flow cytometry-sorted ILC2s on day 0. Each dot in (**c**) represents the mean of three biological replicates collected across two independent experiments. In (**b-c**), data were pooled from two independent experiments ($n = 4$ mice per condition) and are presented as mean $\pm$ SD in (**b**) and mean $\pm$ SEM in (**c**). ns, not significant. Statistical significance was determined using two-way ANOVA (**b**) or two-way ANOVA followed by Šidák's multiple-comparisons test (**c**).

Extended Data Fig.6. LXR activation acts upstream of ILC2s in the tuft cell-ILC2 circuit, related to Figs. 4 and 5. **a-d**, Representative flow cytometry plots of distal small-intestinal tuft cells (**a**) and Th2 cells and ILC2s (**b**) under the indicated conditions, with quantification of tuft cell frequencies (**c**) and ILC2 frequencies and numbers (**d**). **e**, qPCR analysis of *Il33* expression, normalized to *Hprt*, in distal small-intestinal biopsies under the indicated conditions. **f**, Quantification of ILC2 numbers and the frequencies and numbers of IL-5⁺ IL-13⁺ cells per million small-intestinal lamina propria (SI-LP) cells in LXR $\alpha\beta^{\Delta IEC}$ mice and littermate controls under homeostatic conditions. In (**c-f**), each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 4$ -7 mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Statistical significance was determined using an unpaired Student's t -test for comparisons between two groups or one-way ANOVA followed by Tukey's multiple-comparisons test when more than two groups were compared.

Extended Data Fig.7. scRNAseq analysis shows shared regulation in response to succinate between standard-fed and GW3965-fed animals, related to Fig. 6. **a**, Bubble plot showing marker gene expression across epithelial cell subtypes, colored by cell type. TA, transit-amplifying cells; EC, enterocytes; Paneth-GC, Paneth and goblet cells; ISC, intestinal stem cells; EEC,

enteroendocrine cells; tuft, tuft cells. **b-c**, UMAP visualization of analyzed cells pooled across all conditions or separated by treatment group. **d**, Proportions of each epithelial cell subtype under the indicated treatment conditions. **e**, Heatmap of genes differentially expressed in response to succinate in both standard-fed and GW3965-fed mice, as determined by scRNA-seq ($P < 0.01$ and $|\log_2FC| > 0.5$). **f**, Violin plots showing the expression of the indicated genes under the specified conditions.

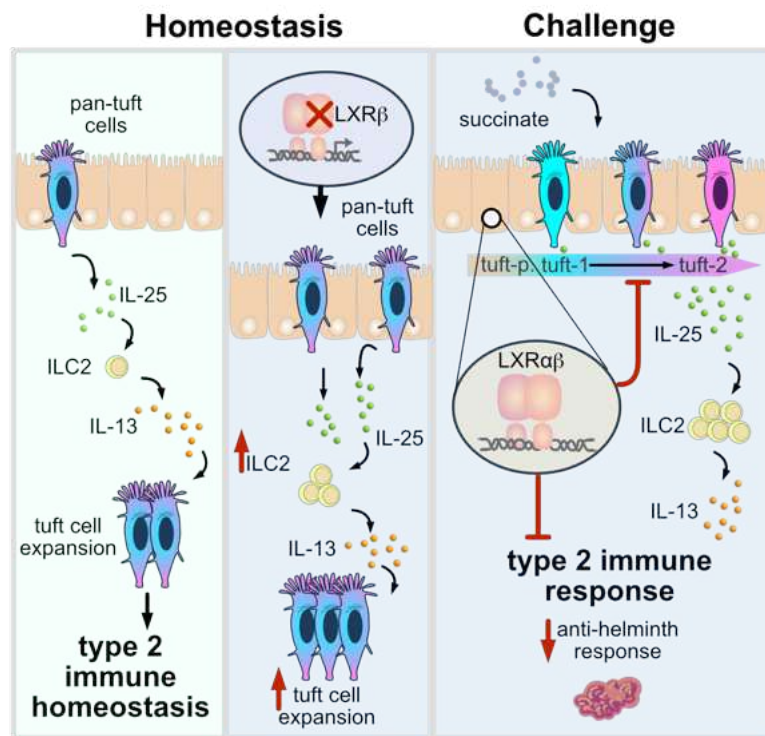
Extended Data Fig.8. scRNAseq analysis in tuft cells revealed cell-state heterogeneity between standard-fed and GW3965-fed animals, related to Fig. 6. **a**, Experimental scheme. **b-c**, Small-intestinal crypt monolayers were cultured *in vitro* with or without GW3965, beginning 1 or 24 h before stimulation with ionomycin for 30 min. qPCR analysis of *Abca1* expression in organoids (**b**) and quantification of cysteinyl leukotriene (CysLT) concentrations in culture supernatants by ELISA (**c**). **d**, Expression density of tuft cell-related genes in the UMAP. **e**, Heatmap of core genes contributing to the “regulation of cell cycle phase transition” pathway from scRNA-seq analysis. **f-g**, Representative flow cytometry plots of Apo-15 peptide⁺ apoptotic tuft cells within the EpCAM⁺ Singlet-F⁺ CD24⁺ tuft cell population (**f**) and quantification of tuft cell frequencies among total epithelial cells and apoptotic tuft cell frequencies among total tuft cells (**g**) following 4 days of treatment with 150 mM succinate. Where applicable in (**b-c** and **f-g**), each dot represents one biological replicate (one mouse). Data were obtained from two independent experiments for (**b-c**) and one experiment for (**f-g**) ($n = 3-7$ mice) and are presented as mean $\pm$ SD. ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Dunnett’s multiple-comparisons test against the DMSO control group (**b**) or Tukey’s multiple-comparisons test (**c** and **g**).

Extended Data Fig.9. Tuft cell state differences between standard-fed and GW3965-fed mice, related to Fig. 6. **a**, Barplot showing the top 10 Gene Ontology biological processes associated with the indicated tuft cell states. **b**, Cell number in the indicated tuft cell subclusters from scRNA-seq analysis. **c**, Frequencies of tuft-p cells (subclusters C0 and C5), tuft-1 cells (subclusters C2, C3, and C7), and tuft-2 cells (subclusters C1, C4, and C6) among all analyzed epithelial cells under each indicated condition.

Extended Data Fig.10. Anti-helminth response characterization upon *H.polygyrus* infection in animals with LXR activation or deficiency, related to Fig. 7. **a**, Quantification of DCLK1⁺ cells per crypt-villus axis in the proximal small intestine of mice treated as depicted in Fig. 7d. **b**, Quantification of MUC2⁺ cells per villus in the proximal small intestine of mice treated as depicted in Fig. 7d. **c-d**, Flow cytometric analysis of ILC2 (**c**) and Th2-cell (**d**) frequencies and numbers in small-intestinal lamina propria (SI-LP) cell suspensions from GW3965-fed and standard-fed mice at 6 days post-infection (dpi), with or without recombinant rIL-25 treatment. **e**, Quantification of IL-13 concentrations in proximal small-intestinal tissue homogenates from mice treated as depicted in Fig. 7d, as measured by ELISA. **f**, Adult *H. polygyrus* counts in the small intestine at 14 dpi in experiments using wild type mice. **g**, Adult *H. polygyrus* counts in the small intestine at

14 dpi in experiments using LXR $\alpha\beta^{\Delta IEC}$ mice and littermate controls. **h**, Egg counts per milligram of stool and adult *H. polygyrus* counts in the small intestine at 14 dpi in experiments using LXR α -deficient or LXR β -deficient mice and their own littermate controls by the strain. In (**a-h**), each dot represents one biological replicate (one mouse). Data were pooled from two or more independent experiments ($n = 3-12$ mice) and are presented as mean $\pm$ SD. ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple-comparisons test (**a-d**), one-way ANOVA followed by Fisher's least significant difference (LSD) test (**e-g**), or an unpaired Student's *t*-test (**h**).

Graphical Abstract



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