

# Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites

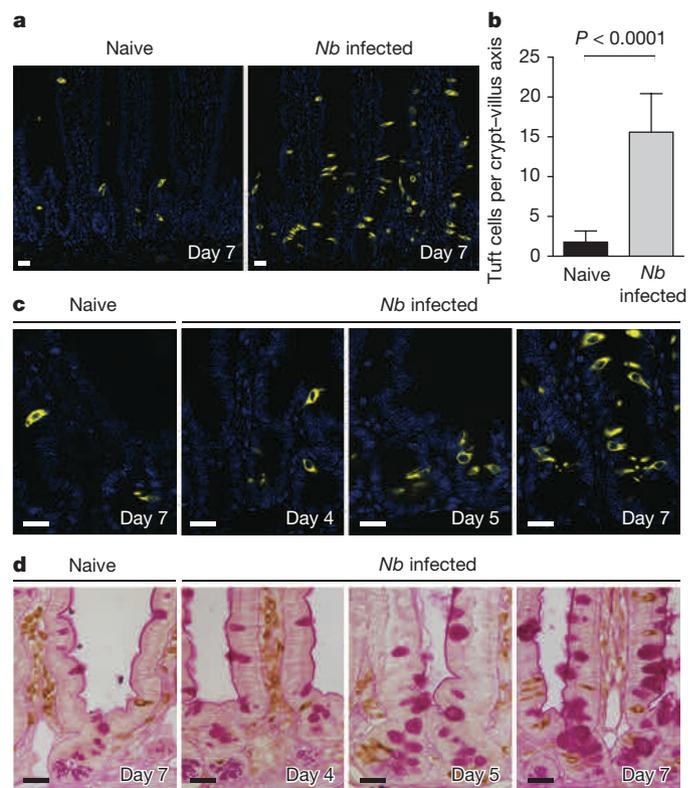
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Helminth parasitic infections are a major global health and social burden<sup>1</sup>. The host defence against helminths such as *Nippostrongylus brasiliensis* is orchestrated by type 2 cell-mediated immunity<sup>2</sup>. Induction of type 2 cytokines, including interleukins (IL) IL-4 and IL-13, induce goblet cell hyperplasia with mucus production, ultimately resulting in worm expulsion<sup>3,4</sup>. However, the mechanisms underlying the initiation of type 2 responses remain incompletely understood. Here we show that tuft cells, a rare epithelial cell type in the steady-state intestinal epithelium<sup>5</sup>, are responsible for initiating type 2 responses to parasites by a cytokine-mediated cellular relay. Tuft cells have a Th2-related gene expression signature<sup>6</sup> and we demonstrate that they undergo a rapid and extensive IL-4R $\alpha$ -dependent amplification following infection with helminth parasites, owing to direct differentiation of epithelial crypt progenitor cells. We find that the *Pou2f3* gene is essential for tuft cell specification. *Pou2f3*<sup>-/-</sup> mice lack intestinal tuft cells and have defective mucosal type 2 responses to helminth infection; goblet cell hyperplasia is abrogated and worm expulsion is compromised. Notably, IL-4R $\alpha$  signalling is sufficient to induce expansion of the tuft cell lineage, and ectopic stimulation of this signalling cascade obviates the need for tuft cells in the epithelial cell remodelling of the intestine. Moreover, tuft cells secrete IL-25, thereby regulating type 2 immune responses. Our data reveal a novel function of intestinal epithelial tuft cells and demonstrate a cellular relay required for initiating mucosal type 2 immunity to helminth infection.

Experimental subcutaneous infection of mice with *N. brasiliensis* (*Nb*) stage 3 larvae induces a typical type-2 response that involves a remodelling of epithelial cell populations, with goblet cell hyperplasia visible as soon as 5 days post-infection<sup>3,4</sup>. *Nb* L3 larvae first migrate from their injection site to the lungs, where they moult to the L4 stage, are coughed up, and swallowed to reach the intestines (day 2 post infection) where they mature and lay eggs (starting 5 days post-infection). *Nb* induces a rapid and robust type 2 response, resulting in worm expulsion by 6–8 days post infection.

While the doublecortin-like kinase 1 (Dclk1)-expressing tuft cells represent only 0.4% of intestinal epithelial cells in naive mice<sup>5</sup>, we found that *Nb* infection resulted in a 8.5-fold expansion in tuft cells (Fig. 1a, b), first detected by 5 days post-infection in intestinal crypts, where proliferative epithelial progenitor cells reside, and also in the villi by 7 days post infection (Fig. 1c, Extended Data Fig. 1a). The kinetics of tuft cell expansion was equivalent to that of goblet cells (Fig. 1d, Extended Data Fig. 1b). Neo-differentiated tuft cells were indistinguishable from tuft cells present in naive mice, as evaluated by expression of established tuft cell markers, including Dclk1, Sry-related transcription factor 9 (Sox9), and phospholipase C gamma 2 (Plc $\gamma$ 2) (Extended Data Fig. 1c)<sup>6–8</sup>. All tuft cells, characterized by Dclk1 and growth factor

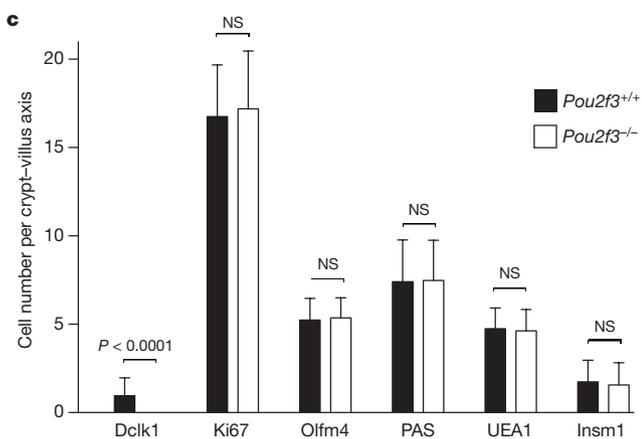
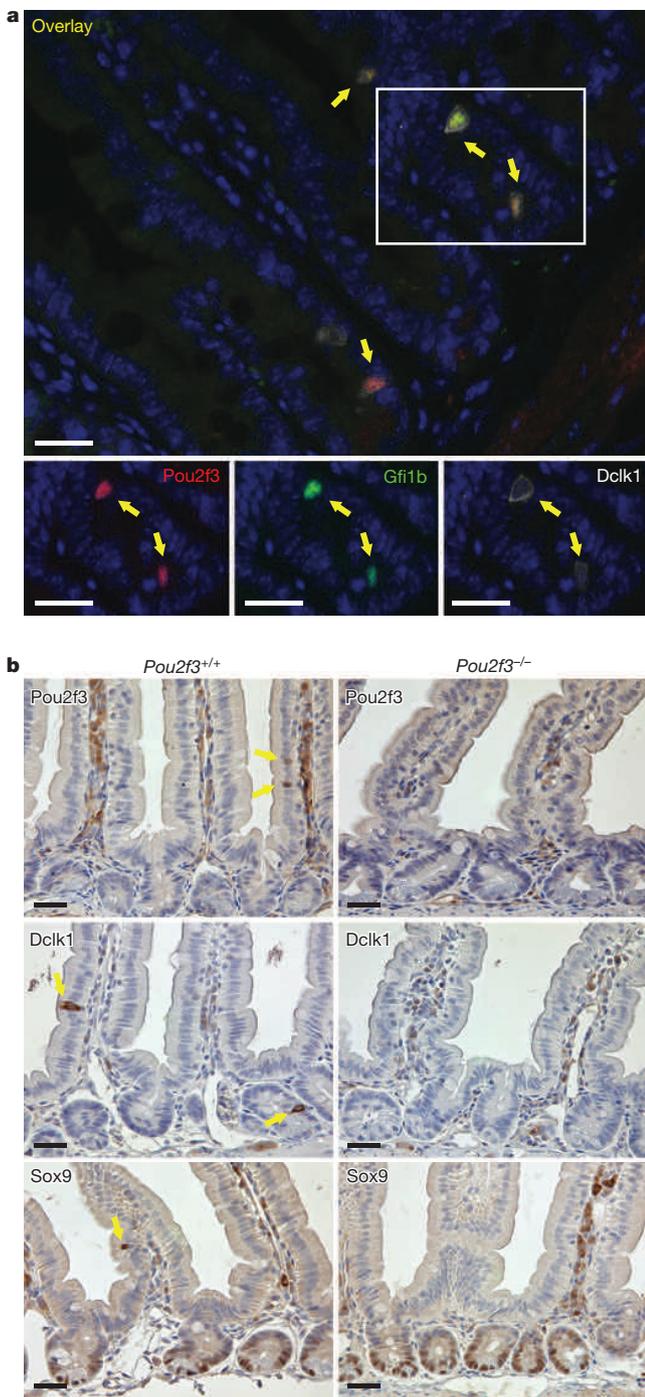
independent 1b (Gfi1b)<sup>8</sup> expression also co-expressed the Pou domain, class 2, transcription factor 3 (Pou2f3) (Fig. 2a). In addition, rare (<3%,  $n = 400$  cells counted) Pou2f3<sup>+</sup>;Dclk1<sup>low</sup> or Pou2f3<sup>+</sup>;Dclk1<sup>-</sup> cells



**Figure 1 | Rapid amplification of the tuft cell lineage following infection with *Nb*.**

**a**, Presence of tuft cells in the intestinal epithelia of naive and *Nb*-infected mice 7 days post infection, visualized by expression of the Dclk1 marker. **b**, 8.7-fold increase of tuft cell numbers ( $1.8 \pm 1.4$  to  $15.6 \pm 4.8$  per crypt-villus axis) in *Nb*-infected mice compared to naive mice, 7 days post infection. ( $n = 50$  crypt-villus units per mouse; 3 mice per condition). Data are shown as means  $\pm$  s.d. ( $P < 0.0001$ , two-tailed Student's *t*-test with Welch's correction). **c**, Changes in the Dclk1-expressing tuft cell population in intestinal crypts are presented at the indicated time points post infection. Quantification is shown in Extended Data Fig. 1a. **d**, Corresponding goblet cell hyperplasia associated with numerous and larger mucus vacuoles, detected by periodic acid-Schiff (PAS) staining. Dclk1 cells are also visualized in brown. Quantification is shown in Extended Data Fig. 1b. Scale bars, 20  $\mu$ m. All panels show representative pictures of experiments replicated 3 times in 3 mice per condition.

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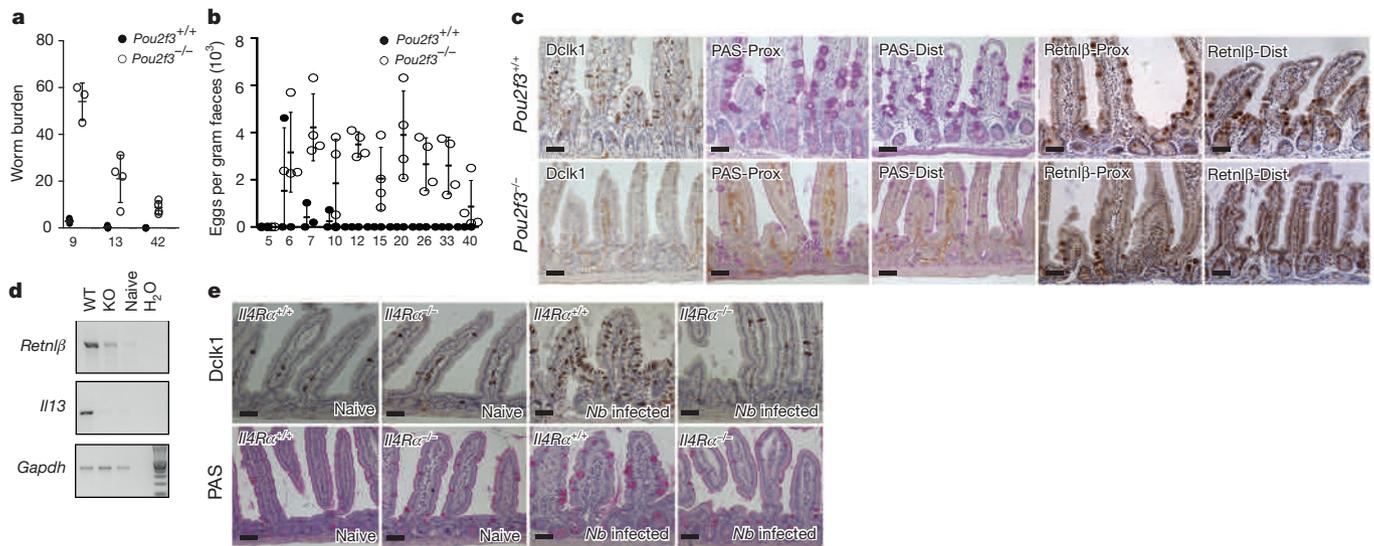


**Figure 2 | Absence of tuft cells in the intestinal epithelium of *Pou2f3*<sup>-/-</sup> mice.** **a**, *Pou2f3* is expressed specifically in tuft cells of the intestinal epithelium as determined by co-staining for *Pou2f3* and established markers of tuft cells such as *Dclk1* and *Gfi1b*. **b**, *Pou2f3* deletion results in the absence of tuft cells as monitored by staining intestinal epithelium from *Pou2f3*<sup>+/-</sup> and *Pou2f3*<sup>-/-</sup> mice with *Pou2f3*, *Dclk1*- and *Sox9*-specific antibodies. **a**, **b**, Three mice per genotype were used for staining experiments. Scale bars, 20  $\mu$ m. **c**, *Pou2f3* deficiency does not affect the proliferation zone ( $P = 0.22$ ), stem cell compartment ( $P = 0.66$ ), enterocyte (not counted), goblet ( $P = 0.83$ ), Paneth ( $P = 0.60$ ) or enteroendocrine ( $P = 0.23$ ) cell lineages as monitored by *Ki67*, *Olfm4*, alkaline phosphatase, PAS staining, UEA1 lectin, and *Insm1*, respectively. ( $n = 50$  crypt-villus units per mouse; 3 *Pou2f3*<sup>-/-</sup> and 3 wild-type mice). Data are shown as means  $\pm$  s.d. A two-tailed Student's *t*-test was used. Pictures show representative experiments replicated 3 times.

were found at the base of crypts, probably representing early differentiating tuft cells since villus *Pou2f3*<sup>+</sup> cells always co-express *Gfi1b* and *Dclk1*. Following infection, the percentage of proliferating tuft cells in crypts increased from  $13 \pm 5.6\%$  to  $24 \pm 14.9\%$  ( $P = 0.035$ ), indicating that cell proliferation contributes to the amplification of the tuft lineage during type 2 responses (Extended Data Fig. 1d, e). Examination of the location of tuft cells present in *Nb*-infected mice revealed that some tuft cells differentiate close to the stem cell zone (Extended Data Fig. 1d), suggesting that biased differentiation from the recently described *Lgr5*<sup>+</sup> slowly cycling early secretory progenitors<sup>9</sup> and *Dll1*<sup>+</sup> secretory progenitors<sup>10</sup> also contributes to tuft cell lineage amplification. The increase in tuft cells was not due to a non-specific amplification of all secretory cell lineages as the number of enteroendocrine cells expressing the insulinoma-associated 1 (*Insm1*) marker<sup>11</sup>, another secretory lineage of the intestinal epithelium, was significantly ( $P = 0.008$ ) reduced (Extended Data Fig. 1f, g).

To determine whether the increase in the tuft cell population following infection with *Nb* was specific to C57BL/6 mice, we infected BALB/c mice and also observed a significant increase in tuft cell numbers (14-fold,  $P < 0.0001$ ; Extended Data Fig. 2a, b). Moreover, this response seems to be a common adaptation to helminth infection in general, as infection of C57BL/6 and BALB/c mice strains with *Heligmosomoides polygyrus*<sup>12</sup> also resulted in a significant increase in tuft cell numbers (6.1- and 8.3-fold, respectively,  $P < 0.0001$ ; Extended Data Fig. 2c, d). Tuft cell hyperplasia following *Nb* infection also occurred in *Rag*<sup>-/-</sup> mice (10-fold;  $P < 0.0001$ ) and therefore does not require functional adaptive immunity (Extended Data Fig. 2e, f).

Epithelial remodelling following helminth infection includes goblet cell hyperplasia and changes in mucus composition, associated with protective type 2 immunity<sup>13,14</sup>. To investigate the role of tuft cells in this process, we identified and characterized a tuft-cell-deficient mouse line. Mice deficient for the *Pou2f3* transcription factor lack all *Pou2f3*-expressing taste receptor cells including sweet, umami and bitter taste cells<sup>15</sup>, as well as *Trpm5*-expressing chemosensory cells in the nasal cavity<sup>16</sup> and olfactory epithelium<sup>17</sup>. Analysis of *Pou2f3*-deficient mice revealed a unique phenotype in the intestinal epithelium, with a complete absence of tuft cells as assessed by the absence of *Pou2f3*, *Dclk1* and *Sox9* expression outside the crypt compartment (Fig. 2b). The stem cell compartment, proliferation zone, and differentiation of enterocytes, goblet, enteroendocrine and Paneth cells were not affected (Fig. 2c and Extended Data Fig. 3). Furthermore, the distribution of immune cells in lymph nodes, mesenteric lymph nodes, spleen and lamina propria of *Pou2f3*<sup>+/-</sup> and *Pou2f3*<sup>-/-</sup> mice was equivalent (Extended Data Fig. 4) and lymphocytes were capable of responding to immune stimulation (Extended Data Fig. 5). Notably, type 2 innate lymphoid cells (ILC2s), a lineage that plays a critical role in secreting type 2 cytokines in response to helminth infection<sup>18,19</sup>, were present in both the mesenteric lymph nodes and lamina propria of *Pou2f3*<sup>-/-</sup> mice, at levels that were not significantly different from wild-type mice. (Extended Data Fig. 6a–c). Therefore, the absence of



**Figure 3 | Impaired type 2 responses in tuft cell-deficient mice.**

**a**, Live adult worm counts in the small intestines of wild-type and *Pou2f3*<sup>-/-</sup> mice at days 9, 13 and 42 post infection with *Nb* ( $n = 3$  wild-type mice and 4 *Pou2f3*<sup>-/-</sup> mice for each time point except for day 9 where  $n = 3$  *Pou2f3*<sup>-/-</sup> mice). **b**, Kinetic of *Nb* infection in 3 wild-type and 4 *Pou2f3*<sup>-/-</sup> mice, assessed by faecal eggs count. **a**, **b**, Each circle represents an individual mouse. The  $x$  axis indicates time (days) post-infection. Average values  $\pm$  s.d. are shown. **c**, Immunohistochemistry illustrating the proximal and distal small intestinal epithelium of infected wild-type and *Pou2f3*<sup>-/-</sup> mice 7 days after infection ( $n = 3$  mice per genotype).

*Pou2f3* does not affect global immunity or intestinal epithelium formation. Rather, *Pou2f3* represents the first identified transcription factor that is specifically required to specify the tuft cell lineage in the intestinal epithelium, analogously to *Sox9* for Paneth<sup>20,21</sup> and *Ngn3* for enteroendocrine<sup>22</sup> cell lineages. Thus, *Pou2f3*<sup>-/-</sup> mice represent a powerful model to study the function of tuft cells.

*Pou2f3*<sup>+/+</sup> and *Pou2f3*<sup>-/-</sup> mice were infected with *Nb* and analysed at several time points. In *Pou2f3*<sup>+/+</sup> mice, only few worms were found after 9 days and expulsion was nearly complete after 13 days. In sharp contrast, numerous worms were found in *Pou2f3*<sup>-/-</sup> mice up to 42 days post infection (Fig. 3a, b), not only in the proximal part of the small intestine, their normal site of attachment<sup>23</sup>, but also in more distal locations. Together, these data strongly suggest that a compromised type-2 response is responsible for prolonged worm survival in *Pou2f3*<sup>-/-</sup> tuft-cell-deficient mice.

To understand the mechanisms underlying the delayed worm expulsion in *Pou2f3*-deficient mice, we analysed the type-2 response-dependent remodelling of the intestinal epithelium 7 days after infection, a time point at which adult worms were detected in all infected animals. In *Pou2f3*<sup>+/+</sup> mice, the intestinal epithelium displayed extensive and generalized goblet cell hyperplasia, with large mucus vacuoles, and tuft cell hyperplasia (Fig. 3c). Expectedly, *Pou2f3*<sup>-/-</sup> mice completely lacked tuft cells and, in contrast to *Pou2f3*<sup>+/+</sup> mice, were devoid of overt goblet cell hyperplasia, with focal and moderate hyperplasia limited to the most proximal small intestine, and lower goblet cell numbers than wild-type mice (Fig. 3c, Extended Data Fig. 7a, and Supplementary Information 1 and 2). Therefore, tuft-cell-deficient mice have a delayed type 2 response, with deficient mucosal goblet cell hyperplasia and delayed control of *Nb* infection.

The goblet cell-produced Resistin-like beta (Retnl $\beta$ ) molecule, strongly induced by type 2 cytokines, has direct anti-helminth activity that facilitates expulsion<sup>3,24</sup>. We compared expression of Retnl $\beta$  in wild-type and *Pou2f3*<sup>-/-</sup> mice 7 days after *Nb* infection, when worm expulsion had started in wild-type mice. Retnl $\beta$  was strongly expressed in hyperplastic goblet cells in *Pou2f3*<sup>+/+</sup> mice, but was only weakly expressed in *Pou2f3*<sup>-/-</sup> mice (Fig. 3c, d and Extended Data Fig. 7a).

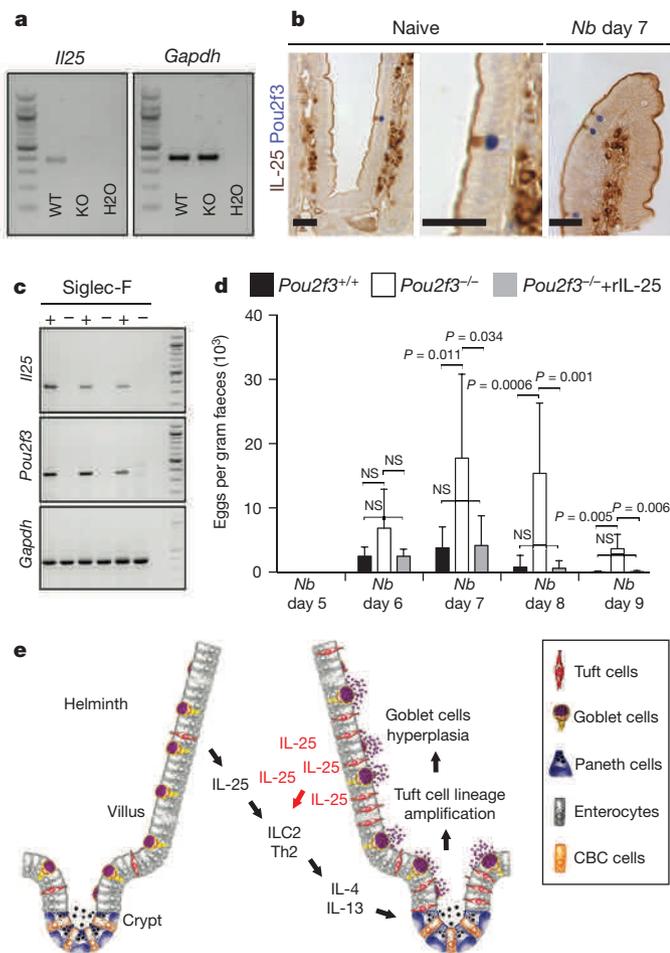
Dclk1 and PAS stainings, respectively, reveal tuft and goblet cells, as well as Retnl $\beta$  production. **d**, Quantification of IL-13 and Retnl $\beta$  in the intestinal mucosa of naive, and *Nb*-infected *Pou2f3*<sup>+/+</sup> and *Pou2f3*<sup>-/-</sup> mice by RT-PCR, 7 days after infection. Representative gels are shown with relative *Gapdh* expression presented as an internal control. **e**, Histological analysis showing tuft (Dclk1 staining) and goblet (PAS staining) cells in naive and *Nb*-infected *Il4R $\alpha$* <sup>+/+</sup> and *Il4R $\alpha$* <sup>-/-</sup> mice 7 days post infection ( $n = 3$  mice per genotype). Scale bars, 20  $\mu$ m. All panels show representative pictures of experiments replicated 3 times.

Moreover, while IL-4 levels were equivalent in mucosal tissue of *Nb*-infected *Pou2f3*<sup>+/+</sup> and *Pou2f3*<sup>-/-</sup> mice, IL-13 levels were markedly decreased in the latter (Fig. 3d). As both IL-4 and IL-13 type 2 cytokines are known to regulate Retnl $\beta$  expression<sup>3</sup>, and IL-4 is dispensable during type 2 responses to *Nb*<sup>25</sup>, our data strongly suggest that defective IL-13 production is responsible for the decreased Retnl $\beta$  expression in *Nb*-infected *Pou2f3*<sup>-/-</sup> mice. Thus, we identify a defective IL-13/Retnl $\beta$  axis in tuft-cell-deficient mice with impaired worm expulsion.

We next studied the link between tuft cells and type-2-mediated mucosal adaptation following *Nb* infection. IL-4R $\alpha$  signalling is essential for both goblet cell hyperplasia and type 2 immune responses occurring upon helminth infection, and deletion of the *Il4r $\alpha$*  gene abrogates *Nb* expulsion<sup>23,26</sup>. Importantly, the *Nb*-induced tuft cell hyperplasia occurring in wild-type mice 7 days post infection was absent in *Il4r $\alpha$* <sup>-/-</sup> mice, as was goblet cell hyperplasia (Fig. 3e, Extended Data Fig. 7b). This demonstrates the critical role of IL-4R $\alpha$  signalling in the expansion of the tuft cell population following helminth infection.

We then examined whether IL-4R $\alpha$  signalling is sufficient to trigger tuft cell lineage hyperplasia by injecting naive C57BL/6 mice with recombinant murine IL-4 and/or IL-13 (rIL-4/rIL-13) for 5 days and assessing the histology of the intestinal epithelium. rIL-4/rIL-13 injection induced goblet cell hyperplasia together with tuft cell expansion (Extended Data Fig. 7c). Importantly, treatment of *Pou2f3*<sup>-/-</sup> mice with rIL-4/rIL-13 also resulted in goblet as well as Paneth cell hyperplasia, indicating a function of tuft cells upstream of IL-4/IL-13 (Extended Data Fig. 7c, d). Moreover, ectopic IL-4/IL-13 induced Retnl $\beta$  expression in goblet cells, independently of the *Pou2f3* genotype. Retnl $\beta$  expression was found predominantly in crypts and was therefore delayed compared to the onset of goblet cell hyperplasia (Extended Data Fig. 7c), and quantitatively lower than in an infectious context (Fig. 3c). Thus, IL-4R $\alpha$  signalling is sufficient to induce an expansion of the tuft cell lineage. Furthermore, ectopic stimulation of this signalling cascade obviates the need for tuft cells in the epithelial cell remodelling of the intestine, including induction of Retnl $\beta$  expression by hyperplastic goblet cells.

To determine whether the IL-4/IL-13-induced goblet cell hyperplasia was epithelial-cell-autonomous, we used an *ex vivo* organoid culture



**Figure 4 | Tuft cells express IL-25, and rIL-25 is sufficient to initiate type 2 mucosal responses in the absence of tuft cells.** **a**, Analysis of *Il25* mRNA expression in *Pou2f3*<sup>+/+</sup> and *Pou2f3*<sup>-/-</sup> mice infected with *Nb*, 9 days post-infection, by RT-PCR. *Gapdh* expression is presented as an internal control. **b**, Immunohistochemistry showing IL-25 expression in naive and *Nb*-infected wild type mice. Blue staining, nuclear *Pou2f3* expression revealed with NBT/BCIP. Brown staining, IL-25 expression revealed with DAB ( $n = 3$  naive and 3 infected mice). Scale bars, 20  $\mu\text{m}$ . **c**, PCR with reverse transcription (RT-PCR) showing predominant *Il25* and *Pou2f3* mRNA expression in the FACS-enriched tuft cells fractions (+) and the other epithelial cells (-), obtained from 3 independent mice. *Gapdh* is shown as an internal control. **d**, Rescue of the *Pou2f3* deficiency by treatment with exogenous rIL-25, as assessed by egg counts during a time course of infection with *Nb* ( $n = 7$  mice for the wild-type and *Pou2f3*<sup>-/-</sup> mice, and  $n = 6$  for the rIL-25-treated *Pou2f3*<sup>-/-</sup> mice). Average  $\pm$  s.d. are presented, as well as exact *P* values when  $< 0.05$  (two-tailed Mann-Whitney *U*-test). **e**, Scheme illustrating the function of tuft cells in initiating type 2 responses following infection with intestinal helminths. Left, normal epithelium undergoing infection with a helminth. Right, tuft cell-dependent epithelial remodelling during type 2 responses. All panels show representative pictures of experiments replicated 3 times.

system<sup>27</sup> that allows physiological responses of an isolated intestinal epithelium to be analysed in the absence of stromal cues. As expected, tuft cells were absent in *Pou2f3*<sup>-/-</sup> organoid cultures (Extended Data Fig. 8a). Moreover, in wild-type organoids, the tuft cell population increased as early as 48 h following addition of rIL-4/rIL-13 (Extended Data Fig. 8a, b). Treatment with rIL-4 or rIL-13 alone yielded identical results to the rIL-4/rIL-13 mixture (Extended Data Fig. 8c). Treatment of *Pou2f3*<sup>-/-</sup> organoids with rIL-4/rIL-13 also triggered goblet cell hyperplasia equivalent to that detected in *Pou2f3*<sup>+/+</sup> organoids, as indicated by *Retnl3* expression (Extended Data Fig. 8d), revealing the critical role of type 2 cytokine signalling downstream of the tuft

cell lineage. Furthermore, these data demonstrate that the intestinal epithelial response to IL-4/IL-13 is epithelium-autonomous and does not require additional stromal signals. Together, our data identify a novel function of tuft cells in initiating the mucosal type 2 responses with a positive feedback loop through IL-13-producing immune cells that, in turn, amplify the tuft cell lineage.

Finally, we investigated the physiological function of the tuft cell hyperplasia, fully established by 7 days post-infection when worm expulsion starts. IL-25 is an alarmin molecule produced by an as yet unidentified intestinal epithelial cell type, capable of initiating type 2 responses by stimulating ILC2s to produce IL-4 and IL-13<sup>18,19</sup>. We thus analysed *Il25* messenger RNA expression in *Pou2f3*<sup>+/+</sup> and *Pou2f3*<sup>-/-</sup> mice infected with *Nb*. Nine days after infection, *Il25* expression was higher in the intestinal mucosa of *Pou2f3*<sup>+/+</sup> mice than in tuft-cell-deficient *Pou2f3*<sup>-/-</sup> mice (Fig. 4a). Moreover, IL-25 protein expression was restricted to tuft cells in naive mice (Fig. 4b and Extended Data Fig. 9a) and consistent with these data, *Il25* mRNA was only detected in the FACS-enriched tuft cell fraction of the intestinal epithelium (Fig. 4c and Extended Data Fig. 9b). Following *Nb* infection, IL-25 expression remained restricted to tuft cells (Fig. 4b). Concomitant with tuft cell hyperplasia, epithelial IL-25 expression peaks 9 days after infection with *Nb*, at the time of worm expulsion, for which it is required<sup>28</sup>. In accord with a critical role for IL-25-secreting tuft cells in the expansion of ILC2s, we found that the percentage of Lin<sup>-</sup>CD127<sup>+</sup>Gata3<sup>+</sup>KLRG1<sup>+</sup> ILC2s was not significantly augmented by *Nb* infection of *Pou2f3*<sup>-/-</sup> mice, but was significantly augmented in wild-type mice. Indeed, tuft cells were required for the global induction of an adaptive immune response as helminth infection induced an approximately 2.5-fold expansion of both ILC2 and Th2 subsets in mesenteric lymph nodes, whereas these subsets remained unchanged in the infected *Pou2f3*<sup>-/-</sup> mice ( $P = 0.02$ ,  $P = 0.0005$ , respectively; Extended Data Fig. 6d–f). It is likely that these immune defects are directly due to the paucity of IL-25 as treatment of *Nb*-infected *Pou2f3*<sup>-/-</sup> mice with rIL-25 almost completely compensated for the absence of tuft cells, promoting an efficient worm expulsion (Fig. 4d). IL-25 thus provides a mechanistic link between tuft cells, promotion of type 2 responses and worm expulsion.

Taken together, our data reveal a critical function of tuft cells in initiating mucosal type 2 responses following infection with helminths through IL-25 secretion. In the absence of tuft cells, IL-25 and IL-13 expression remain low, and type 2 mucosal responses and worm expulsion are delayed. Our study demonstrates a requirement for tuft cells upstream of IL-4/IL-13, with these cytokines driving tuft cell hyperplasia, thereby amplifying a feed-forward loop to orchestrate a rapid and effective anti-helminth immunity (Fig. 4e).

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Author Contributions** F.G. performed the majority of the experiments. E.S., D.J.S., B.B. and P.C. contributed to mouse studies, M.O. and I.M. to characterization of the *Pou2f3*-deficient mouse line, E.S., L.G. and M.B. to organoid experiments, V.D., M.P. and V.S.Z. to immune studies and Y.H. to parasite life cycle experiments. P.J. and F.G. conceived the study. P.J., F.G. and R.M.M. designed experiments with contributions from V.D., V.S.Z. and N.T.; P.J. wrote the manuscript with inputs from F.G. and N.T.

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## METHODS

**Animal strains.** The *Pou2f3*-deficient mice (*Pou2f3tm1Abek*) have been previously described<sup>15</sup>. *Il4ra*-deficient mice<sup>29</sup> were provided by M. Kopf (Basel Institute for Immunology, Switzerland). C57BL/6 and BALB/c mice were obtained from Charles River Laboratories. All the mice were maintained in an SPF animal facility and were naive before the experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee of Monell Chemical Senses Center or by the French Agriculture and Forestry Ministry. Unless specified, all mice were on a C57BL/6 genetic background. Mice were analysed at 10 weeks of age, regardless of the sex. For comparisons of wild-type and KO mice, littermates were analysed. Three mice per condition were analysed in all experiments. No statistical methods were used to predetermine sample size, no criteria of exclusion were defined and the experiments were not randomized nor blinded to the investigator.

**Immunophenotyping and flow cytometry analyses.** Cells, isolated from peripheral lymph nodes, mesenteric lymph nodes, spleen and lamina propria were stained with Sytox blue or Live/dead fixable viability dye (Life Technologies) and eBioscience respectively together with the appropriate conjugated anti-CD3, CD45.2, CD62L, CD4, CD8, CD44, TCR- $\gamma\delta$ , CD19, NK1.1, Gr1, CD11b, CD11c, and Foxp3 antibodies (eBioscience or Becton Dickinson, San Diego, CA). For ILC2 staining, cells were stained with a lineage cocktail and CD45.2<sup>+</sup> (clone 104) and lineage-negative CD45<sup>+</sup> cells were assessed for expression of CD127 (clone SB14), KLRG1 (clone 2F1), Sca-1 (clone D7), CD25 (clone 7D4), and intracellular expression of Gata-3 (clone L50-823). Th2 cells were identified on the basis of Gata-3-expressing cells within the CD3<sup>+</sup>CD4<sup>+</sup> subset.

IL-6, IL-12, TNF $\beta$ , IL-10, MCP-1 and IFN- $\gamma$  production was assessed in the culture supernatant of LPS/IL-4-activated splenocytes using a Cytometric Bead Array (CBA) Kit (BD Biosciences). To assess intracellular cytokine production, freshly isolated and anti-CD3/CD28 stimulated LN cells were activated with PMA (Sigma-Aldrich; 100 ng ml<sup>-1</sup>)/ionomycin (Sigma-Aldrich; 1  $\mu$ g ml<sup>-1</sup>) in the presence of brefeldin A (Sigma-Aldrich; 10  $\mu$ g ml<sup>-1</sup>) for 4 h at 37°C, fixed, permeabilized and then stained with specific antibodies against IL-2 and IFN- $\gamma$ .

Foxp3 staining was performed following fixation/permeabilization (eBioscience). Stained cells were assessed by flow cytometry (LSR Fortessa, Becton Dickinson, San Jose, CA) and data were analysed by FACSDiva (v.8.0, BD Biosciences) and FCAP Array Software (CBA analysis).

**Ex vivo stimulations.** LN cell activation was performed using plate-bound anti-CD3 (clone 2C11; 1  $\mu$ g ml<sup>-1</sup>) and anti-CD28 (clone PV-1 1  $\mu$ g ml<sup>-1</sup>) mAbs in RPMI 1640 media (Life Technologies) supplemented with 10% FCS, 2 mM glutamine and 1% penicillin/streptomycin. Exogenous IL-2 (100 U ml<sup>-1</sup>) was added every other day starting at day 2 post-activation. Cell proliferation was monitored by labelling with CFSE (Life Technologies; 2.5  $\mu$ M) for 3 min at room temperature. Splenocytes were activated with LPS (20  $\mu$ g ml<sup>-1</sup>) and IL4 (25 ng ml<sup>-1</sup>). Supernatants were collected 40 h following activation.

**Immunoglobulin detection.** IgG detection in supernatants of LPS/IL-4-stimulated splenocytes was assayed by ELISA. Microtiter plates (Maxisorb, Nunc) were saturated overnight at 4°C with 100  $\mu$ l of anti-IgG2a, anti-IgG2b, anti-IgA antibodies or anti-IgG (Fab'2) resuspended in PBS (5  $\mu$ g ml<sup>-1</sup>). Plates were washed 3 times with 0.1% Tween-containing PBS (PBST). Samples (1/2 dilution) were diluted in a final volume of 100  $\mu$ l per well of PBST-1%BSA and incubated for 2 h at RT. Following washes, peroxidase-conjugated anti-mouse anti-IgG2a, anti-IgG2b, anti-IgA (Serotech) or anti-IgG gamma-chain (SIGMA) antibodies were added in PBST-1%BSA (1:1,000 dilution; 100  $\mu$ l per well) and incubated for 1 h at 37°C. Immunoglobulin levels were then revealed with *o*-phenylenediamine (Sigma; 4  $\mu$ g ml<sup>-1</sup>) in 0.1 M Na citrate and 0.03% hydrogen peroxide. Absorbance was measured at 450 nm using an automated plate reader (InfiniteM200Pro, TECAN) after 5 min at room temperature.

**Tuft cell sorting.** Freshly isolated small intestines of BC57BL/6 mice were flushed with PBS and incised along their length. The tissue was then incubated in 30 mM EDTA (Sigma) in HBSS pH 7.4 (Life Technologies) on ice, and transferred in DMEM (Life Technologies) supplemented with 10% FBS (Sigma). Vigorous shaking yielded the epithelial fraction that was then incubated with 100  $\mu$ l of Dispase (BD Biosciences) in 10 ml of HBSS, supplemented with 100  $\mu$ l of DNase I at 2,000 Kunitz (Sigma). Single cell preparation obtained by filtration on a 30  $\mu$ m mesh was incubated with a phycoerythrin rat anti-mouse Siglec-F antibody for 30 min at 4°C (BD pharmigen 552126), washed with HBSS and resuspended in appropriate volume of HBSS pH 7.4 supplemented with 5% FBS before staining with 7-aminocytinomylin D (Life Technologies) to exclude dead cells. Siglec-F<sup>+</sup> live cells were sorted using a FACSAria (Becton Dickinson), directly in RLT lysis buffer (Qiagen) for subsequent RNA extraction.

**Parasite infections.** *Pou2f3*<sup>+/+</sup>, *Pou2f3*<sup>-/-</sup>, *Il4ra*<sup>+/+</sup> and *Il4ra*<sup>-/-</sup>, C57BL/6 and BALB/c wild-type mice were used for *Nb* infection experiments. Mice were infected with 250 L3 infective *Nb* larvae by sub-cutaneous injection<sup>23</sup> or with 200

*H. polygyrus* L3 larvae by gavage. Infection parameters were monitored by enumeration of live adult worms in the small intestinal tissue by two different investigators blinded to the study groups.

**Reagents.** Recombinant murine IL-4 (214-14), recombinant murine IL-13 (210-13) were purchased from PeproTech, and recombinant murine IL-25 (1399) was from R&D Systems. For animal treatment, mice were injected intraperitoneally daily with a mixture of both interleukins or with rIL-25 (40  $\mu$ g per kg of body weight). For rescue experiments in *Pou2f3*<sup>-/-</sup> mice, rIL-25 was injected from day 3 post infection.

**Organoid culture.** Organoid cultures were performed as previously described<sup>27</sup> using intestinal crypts from *Pou2f3*<sup>+/+</sup> and *Pou2f3*<sup>-/-</sup> mice. Organoid lines were passaged up to 10 times before experiments to ensure pure epithelial cultures. When indicated, cultures were stimulated with recombinant murine IL-4 (400 ng ml<sup>-1</sup>), recombinant murine IL-13 (400 ng ml<sup>-1</sup>) or an equimolar mixture of the two cytokines. For histological studies, organoids were washed twice in cold PBS to dissolve the Matrigel, fixed overnight in neutral-buffered formalin at 4°C and included in Histogel (Thermo Scientific) before paraffin embedding ( $n = 3$  experiments from independent mice).

**RNA extraction and PCR.** Total RNA from intestinal organoids or snap-frozen intestinal tissues was isolated using TRIzol (Life Technologies) followed by precipitation with isopropanol. Further RNA purification was carried out on RNeasy columns (Qiagen, 74104) and DNase treatment. In the case of Siglec<sup>+</sup> sorted tuft cells, extraction and DNase treatment were performed using RNeasy Micro KIT following the manufacturer's instructions. Reverse transcription was performed with 500 ng–2  $\mu$ g of purified RNA using Transcriptor First Strand cDNA synthesis KIT (Roche) according to the manufacturer's instructions. For qRT-PCR experiments, gene expression was quantified on the LightCycler 480 using LightCycler 480 SYBR Green I Master (Roche). The results from three independent organoid cultures were normalized to the expression level of *Gapdh* and *Hprt* and relative expression was obtained using the  $\Delta\Delta C_t$  method. Primer sets for each gene are listed in Extended Data Table 1. PCR analyses were performed on an Eppendorf Mastercycler, using the primer sets listed in the Extended Data Table 1.

**In situ hybridization.** Single colorimetric and double fluorescent *in situ* hybridization analyses were carried out as described previously<sup>30</sup>. Briefly, digoxigenin- and fluorescein-labelled antisense RNAs were synthesized and used as probes after fragmentation to about 150 bases under alkaline conditions. Small intestines were dissected from mice shortly after euthanasia and embedded in frozen O.C.T. compound (Sakura Finetech). Fresh-frozen sections were prepared using a cryostat (CM1900, Leica Microsystems), fixed with 4% paraformaldehyde, hybridized with probe(s), and then washed under stringent conditions. Hybridized probes were immunohistochemically detected using alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche Diagnostics) and biotin-conjugated anti-fluorescein antibody (Vector Laboratories). Signals were developed using 4-nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate as chromogenic substrates for single colorimetric analyses or the Tyramid Signal Amplification method and HNPP Fluorescent Detection Set (Roche Diagnostics) for double-fluorescent analyses. Stained and fluorescent images were obtained on a Nikon eclipse 80i microscope (Nikon Instruments Inc.) equipped with a DXM1200C digital camera (Nikon) and a Leica SP2 confocal scanning microscope (Leica), respectively. RNA probes generated were as follows: nucleotides 72–2363 of *Pou2f3* (GenBank accession number NM\_011139), nucleotides 1–2228 of *Slc15a1* (GenBank accession number BC116248), nucleotides 1–3255 of *Muc2* (GenBank accession number BC034197), nucleotides 1–1102 of *Gcg* (GenBank accession number BC012975), nucleotides 1–584 of *Gip* (GenBank accession number BC104314), nucleotides 27–400 of *Defcr6* (GenBank accession number M33225), nucleotides 1–1628 of *Olfm4* (GenBank accession number BC141127), nucleotides 1–2750 of *Dcl1* (GenBank accession number BC050903), and nucleotides 1–2797 of *Ptgs1* (GenBank accession number BC005573).

**Fluorescent and bright-field immunohistochemistry on paraffin-embedded tissue.** Tissue dissection, fixation, and immunohistochemistry on thin sections of paraffin-embedded tissue were performed essentially as described previously<sup>5</sup>. Primary antibodies used in this study were as follows: anti-Sox9 (AB5535; Millipore), anti-Cox1 (sc-1754; Santa Cruz), anti-PCNA (sc-56; Santa Cruz), anti-Plc $\gamma$ 2 (sc-5283, Santa Cruz), anti-Gfi1b (Sc-8559; Santa Cruz), anti-*Pou2f3* (sc-330, Santa Cruz and HPA019652, Prestige Antibodies), anti-*Dcl1* (ab31704; AbCam), anti-Ki67 (ab16667; AbCam), anti-Retn $\beta$  (ABIN465494, Antibodies online), anti-IL-25 (mAb 1258; R&D Systems). Anti-*Insm1* was a gift from C. Birchmeier (Max-Delbrück-Center for Molecular Medicine; Berlin; Germany). Slides were washed twice times with 0.1% PBS-Tween (Sigma-Aldrich) before incubation with fluorescent secondary antibodies conjugated with either Alexa 488, cyanin-3, or cyanin-5 (Jackson ImmunoResearch Laboratories, Inc.) and Hoechst at 2  $\mu$ g ml<sup>-1</sup> (Sigma-Aldrich) in PBS-Triton X-100 0.1%

(Sigma-Aldrich). Stained slides were washed again in PBS before mounting with FluoroMount (Sigma-Aldrich). Methods used for bright-field immunohistochemistry were identical, except that Envision+ (Dako) was used as a secondary reagent. Signals were developed with DAB (Sigma-Aldrich) and a haematoxylin counterstain (DiaPath) was used. After dehydration, sections were mounted in Pertex (Histolab). All experiments were performed on formalin-fixed tissues and 10 mM sodium citrate (pH 6.4) treated slides, except for IL-25 staining where Carnoy's fixation and 10 mM Tris-EDTA (pH 9.0) treatment were used. Enterocytes-alkaline phosphatase activity was revealed with Fast-red substrate (Sigma-Aldrich). All stainings were repeated in 3 mice per group in 3 independent experiments and scored by three different investigators blinded to the study groups.

**Microscopy and imaging.** Fluorescent pictures were acquired at room temperature on an AxioImager Z1 microscope (Carl Zeiss, Inc.) equipped with a camera (AxioCam MRm; Carl Zeiss, Inc.), EC Plan Neofluar (5×, NA 0.16; 10×, NA 0.3; 20×, 0.5 NA; 100×, NA 1.3) and Plan Apochromat (40×, NA 0.95; 63×, NA 1.4) objectives, the Apotome Slider system equipped with an H1 transmission grid (Carl Zeiss, Inc.), and AxioVision software (Carl Zeiss, Inc.). Bright-field immunohistochemistry pictures were taken at room temperature on an Eclipse 80i microscope (Nikon) with Plan Fluor (10×, NA 0.3; 20×, NA 0.5; 40×, NA 0.75; and 60×, NA 0.5–1.25) lenses (Nikon) and a digital camera (Q-Imaging Retiga 2000R with a Q-Imaging RGB Slider). Pictures were captured with Q-Capture Pro software (Nikon). Post-treatment of pictures (level correction), annotations, and panel composition were performed using Photoshop software (Adobe).

**Statistical analyses.** The Prism software was used for descriptive statistical analyses. For infection monitoring, sample ( $n$ ) was defined as the number of eggs per gram of faeces per mouse. As normal distribution assumption was not met, a two-tailed Mann–Whitney  $U$ -test was used to calculate the  $P$  value. For histological data quantification, sample ( $n$ ) was defined as the number of cells per crypt–villus unit. Unless otherwise stated, 50 crypt–villus axes were counted per histological sections from 3 mice of each genotype or condition. According to the central limit theorem ( $n > 30$ ), data comparison was achieved with a two-tailed Student's  $t$ -test. Welch's correction was applied to  $P$ -value calculation when homoscedasticity assumption was not met (determined with  $F$ -test).

Results are shown as histograms representing means as centre values and standard deviation as error bars for each genotype or conditions, except when  $n < 5$ , where individual data point are plotted.

No statistical methods were used to predetermine sample size and the experiments were not randomized. Unless otherwise stated, the investigators were not blinded to allocation during experiments and outcome assessment.

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