



A regulatory T cell Notch4-GDF15 axis licenses tissue inflammation in asthma

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Elucidating the mechanisms that sustain asthmatic inflammation is critical for precision therapies. We found that interleukin-6 and STAT3 transcription factor-dependent upregulation of Notch4 receptor on lung tissue regulatory T (T_{reg}) cells is necessary for allergens and particulate matter pollutants to promote airway inflammation. Notch4 subverted T_{reg} cells into the type 2 and type 17 helper (T_H2 and T_H17) effector T cells by Wnt and Hippo pathway-dependent mechanisms. Wnt activation induced growth and differentiation factor 15 expression in T_{reg} cells, which activated group 2 innate lymphoid cells to provide a feed-forward mechanism for aggravated inflammation. Notch4, Wnt and Hippo were upregulated in circulating T_{reg} cells of individuals with asthma as a function of disease severity, in association with reduced T_{reg} cell-mediated suppression. Our studies thus identify Notch4-mediated immune tolerance subversion as a fundamental mechanism that licenses tissue inflammation in asthma.

A hallmark of asthma is a chronic inflammatory process that is associated with airway hyperresponsiveness (AHR) and tissue remodeling^{1,2}. The persistence of asthmatic inflammation in the face of countervailing immunoregulatory mechanisms that normally limit tissue damage suggests that the latter may become compromised³. In agreement with this premise, subversion of allergen-specific Foxp3⁺ T_{reg} cells, leading to the loss of their immune regulatory activity and their conversion into type 2 helper T cell (T_H2) and type 17 helper T cell (T_H17), T effector (T_{eff})-like cells, has emerged as a key pathogenic mechanism^{3–6}. Elucidating the molecular mechanisms of T_{reg} cell subversion in asthma and the means of restoring their function would offer new approaches to therapy.

Relevant to immune tolerance breakdown in allergic airway inflammation are recent studies on mechanisms by which air-polluting, ambient particulate matter, and especially ultrafine particles (UFPs), upregulate allergic airway inflammation^{7,8}. These particles are taken up by alveolar macrophages (AMs), where they activate the aryl hydrocarbon receptor to induce the expression of the Notch ligand Jagged1 (Jag1). In turn, Jag1 engages Notch receptors on CD4⁺ T cells to promote mixed T_H2 and T_H17 cell-dependent inflammation. Antibody-mediated inhibition of the Notch receptors pointed to Notch4 as the critical Notch receptor involved in this pathway⁸. The identity of the CD4⁺ T cell subpopulation(s) expressing Notch4, the signals that regulate its induction and its downstream effector pathways remained unknown. In the present study, we identify Notch4 as a master molecular switch that subverts lung tissue T_{reg} cell function to promote allergic airway inflammation. Notch4 is mainly induced on allergen-specific-induced (i)T_{reg} cells in an allergen- and interleukin-6 (IL-6)-dependent manner, and

acts to disrupt their function by Wnt and Hippo pathway-dependent mechanisms. Importantly, Notch4 acts via the Wnt pathway to induce the expression in lung tissue T_{reg} cells of the cytokine growth and differentiation factor 15 (GDF15). The latter is a cytokine previously implicated in metabolic adaptation to inflammation^{9,10}, which we show here to upregulate allergic tissue inflammation by directly activating group 2 innate lymphoid (ILC2) cells. These findings place Notch4 at the intersection of allergen- and pollutant-driven airway inflammation, and suggest new intervention strategies targeting Notch4 to restore long-term immune tolerance in asthma and related disorders.

Results

Notch4 is inducibly expressed on T_{reg} cells in allergic airway inflammation. We determined, using real time (RT)-PCR, the identity of the Notch receptor species expressed in lung tissue T_{reg} and T_{eff} cells isolated from sham (phosphate-buffered saline (PBS)) and ovalbumin (OVA)-sensitized mice after their challenge with OVA, and from OVA-sensitized mice co-treated with intranasal UFPs during the OVA challenge phase (OVA + UFP). *Notch4* transcript expression was enriched in lung T_{reg} cells at baseline compared with T_{eff} cells, and was sharply upregulated in OVA and especially OVA + UFP-treated mice relative to *Notch1–3* species (Fig. 1a). These results were corroborated by flow cytometric analysis of the expression of respective Notch receptors in T_{reg} and T_{eff} cells, which confirmed the differential upregulation of Notch4 on lung T_{reg} cells in allergic airway inflammation (Fig. 1b,c and see also Extended Data Fig. 1).

To examine the signals driving the induction of Notch4 on iT_{reg} cells, we used an in vitro iT_{reg} cell differentiation system in which

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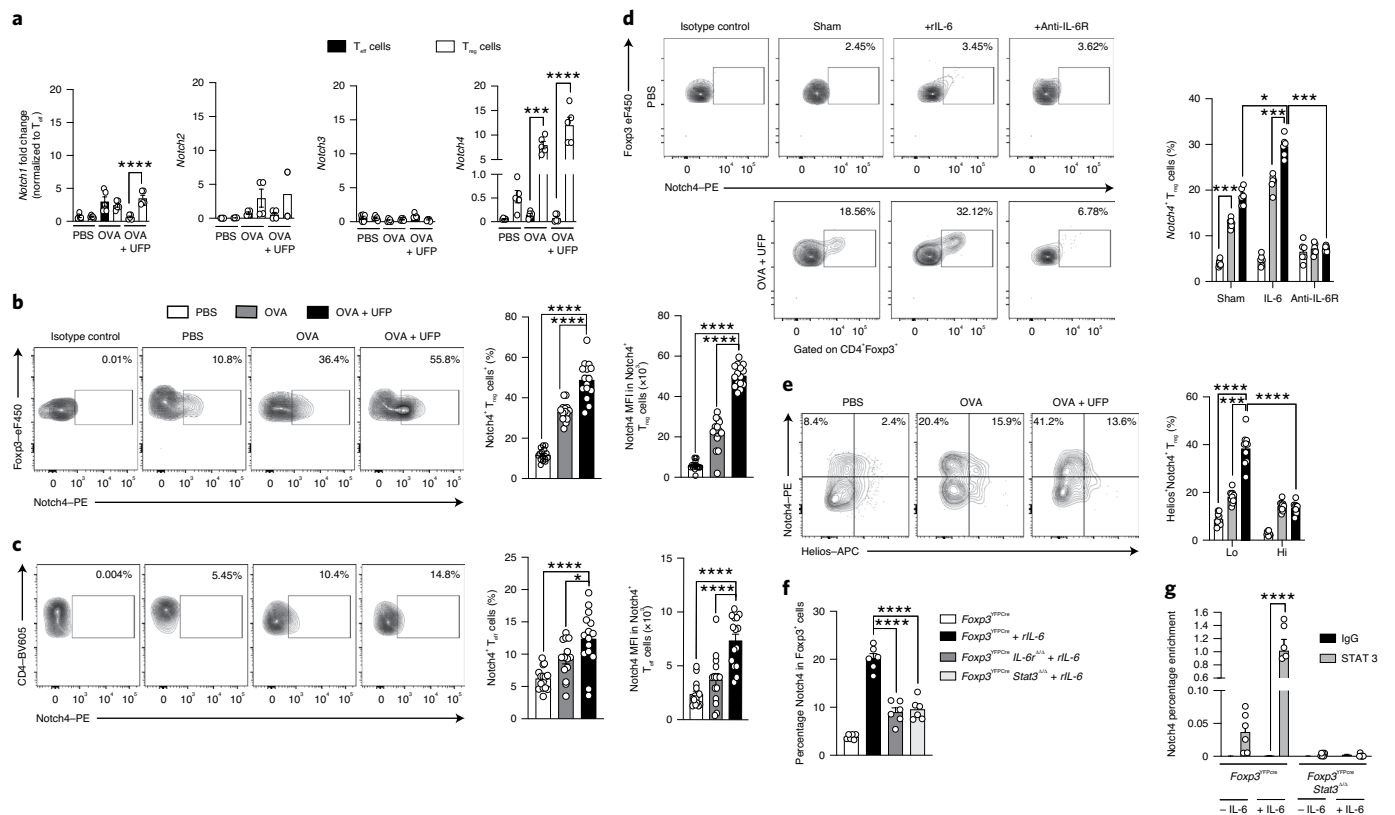


Fig. 1 | Notch4 expression on lung T_{reg} cells in allergic airway inflammation. **a**, RT-PCR of *Notch1-4* transcripts in lung T_{reg} and T_{eff} cells isolated from PBS, OVA and OVA + UFP mouse groups ($n=5$). **b,c**, Flow cytometric analysis, cell frequencies and mean fluorescence intensity (MFI) of Notch4 expression on lung T_{reg} and T_{eff} cells in the respective treatment groups ($n=15$). **d**, Flow cytometric analysis and cell frequencies of Notch4 expression on OT-II $^{+}$ CD4 $^{+}$ Foxp3 $^{+}$ T cells generated in co-cultures with sham- or OVA₃₂₃₋₃₃₉+UFP-pulsed alveolar macrophages without or with IL-6 or anti-IL-6R monoclonal antibody ($n=5$). rIL-6, recombinant IL-6. **e**, Flow cytometric analysis and cell frequencies of Notch4 $^{+}$ Helios $^{+}$ and Helios $^{+}$ lung T_{reg} cells isolated from the respective treatment groups ($n=5$). **f**, Flow cytometric analysis and cell frequencies of Notch4 expression on in vitro differentiated T_{reg} cells derived from naive CD4 $^{+}$ T cells isolated from *Foxp3*^{YFP-Cre}, *Foxp3*^{YFP-Cre}*Il6ra* Δ/Δ and *Foxp3*^{YFP-Cre}*Stat3* Δ/Δ mice and either untreated or treated with IL-6 ($n=6$). **g**, ChIP assays for the binding of STAT3 and control (IgG) antibodies to the *Notch4* promoter in lung T_{reg} cells of OVA + UFP-treated *Foxp3*^{YFP-Cre} and *Foxp3*^{YFP-Cre}*Stat3* Δ/Δ mice ($n=6$). Each symbol represents one mouse. Numbers in flow plots indicate percentages. Error bars indicate the s.e.m. Statistical tests: one-way ANOVA with Dunnett's post-hoc analysis (**b, c** and **f**); two-way ANOVA with Šidák's post-hoc analysis (**a, d, e** and **g**). * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. Data represent two or three independent experiments.

naive CD4 $^{+}$ T cells expressing the OT-II T cell receptor (TCR), specific for the OVA₃₂₃₋₃₃₉ peptide, were incubated with OVA₃₂₃₋₃₃₉-pulsed primary AMs⁸. The latter cell type potently drives iT_{reg} cell differentiation under noninflammatory conditions, and at the same time critically promotes allergic airway inflammation by allergens and UFPs by virtue of their inducible expression of Notch ligands, most notably Jag1 (ref. ⁸). Results revealed a step-wise increase in Notch4 expression in differentiating iT_{reg} cells in co-cultures with OVA₃₂₃₋₃₃₉ and OVA₃₂₃₋₃₃₉ + UFP-pulsed AMs (Fig. 1d). Addition of IL-6 to the cell cultures, but not IL-1 β , tumor necrosis factor (TNF), IL-25 or thymic stromal lymphopoietin (TSLP), resulted in super-induction of Notch4 expression on differentiating iT_{reg} cells in an antigen-dependent manner. IL-33 treatment did not induce Notch4 on its own, but augmented the expression of Notch4 induced by IL-6 (Fig. 1d and see also Extended Data Fig. 1). In contrast, treatment with an anti-IL-6 receptor (IL-6R) monoclonal antibody suppressed Notch4 expression induced by OVA₃₂₃₋₃₃₉ and OVA₃₂₃₋₃₃₉ + UFP-pulsed AMs. Notch4 differentially localized in the de novo lung tissue iT_{reg} cells as evidenced by their lack of Helios expression (Helios $^{+}$ T_{reg} cells) (Fig. 1e). Furthermore, in an antigen-presenting cell-free system of in vitro iT_{reg} cell differentiation by treatment of naive T cells with anti-CD3+anti-CD28 monoclonal antibodies,

in the presence of transforming growth factor beta-1 (TGF- β)¹¹, addition of IL-6 to the cell culture induced Notch4 on differentiating T_{reg} cells, which was attenuated by T_{reg} cell-specific deletion of the IL-6R alpha chain or the downstream transcription factor STAT3, using a *Foxp3*-driven Cre recombinase and floxed target alleles (*Foxp3*^{YFP-Cre}*Il6ra* Δ/Δ and *Foxp3*^{YFP-Cre}*Stat3* Δ/Δ , respectively) (Fig. 1f). Chromatin immunoprecipitation (ChIP) confirmed IL-6-dependent STAT3 binding to the *Notch4* promoter in T_{reg} cells, but not to those of *Notch1*, *Notch2* or *Notch3* (Fig. 1g and see also Extended Data Fig. 1). These results identified that IL-6 is a key inducer of Notch4 expression on differentiating allergen-specific lung tissue iT_{reg} cells.

Notch4 subverts T_{reg} cell-mediated immune tolerance in allergic airway inflammation. To elucidate the pathogenic role of inducible Notch4 expression on lung T cells in allergic airway inflammation, we employed *CD4*^{Cre}*Notch4* Δ/Δ mice, in which a floxed *Notch4* allele is specifically deleted in all T cells (see Extended Data Fig. 2a). Results showed that deletion of *Notch4* in CD4 $^{+}$ T cells greatly attenuated airway inflammation induced in OVA-sensitized and -challenged mice, without or with UFP treatment (Fig. 2a,b). *Notch4* deletion largely suppressed the increase in AHR induced

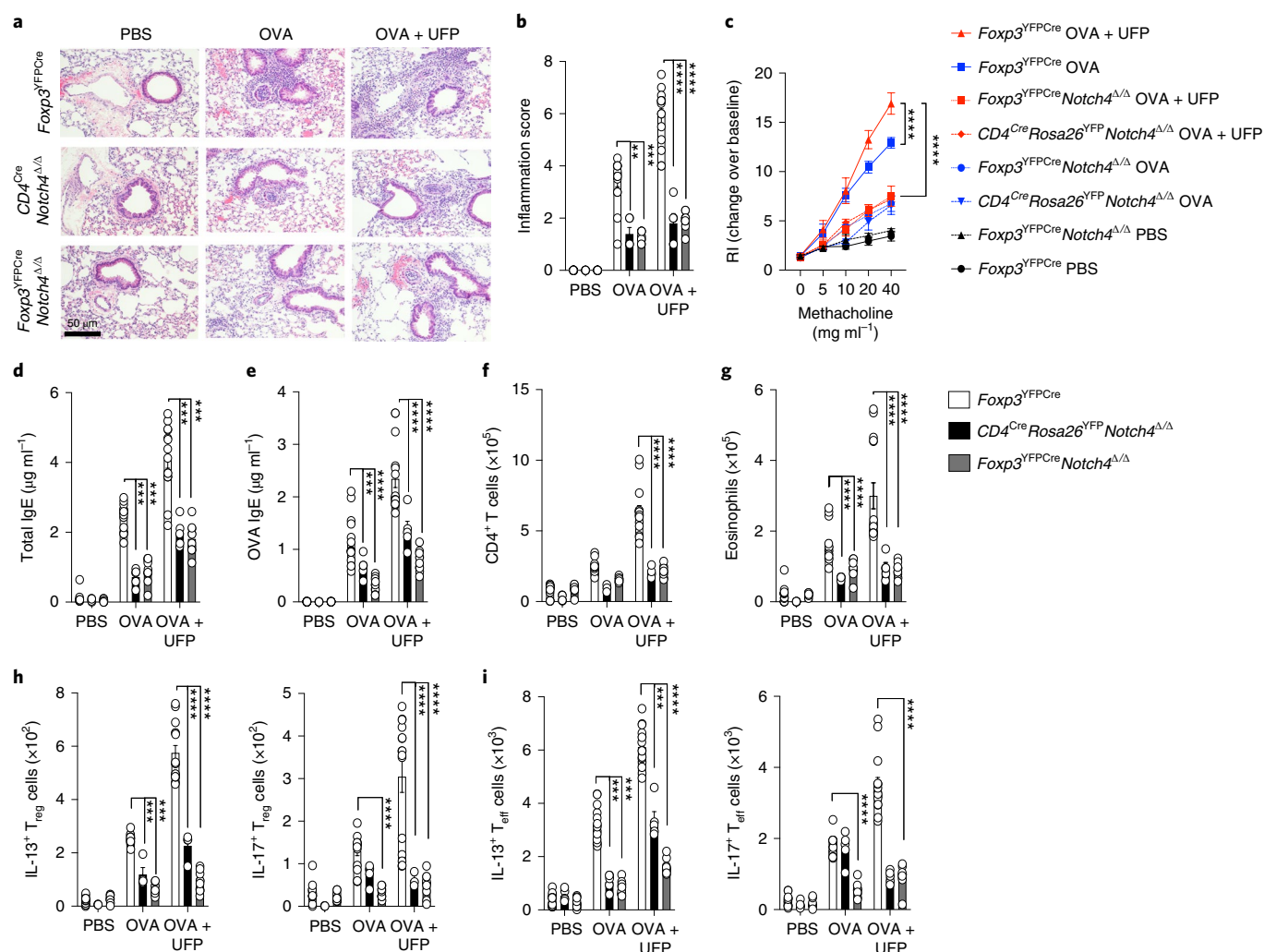


Fig. 2 | Notch4 expression on lung T_{reg} cells licenses allergic airway inflammation. **a**, Representative PAS-stained sections of lung tissues isolated from *Foxp3^{YFP}Cre*, *CD4^{Cre}Notch4^{Δ/Δ}* or *Foxp3^{YFP}CreNotch4^{Δ/Δ}* mice segregated into PBS-, OVA- or OVA + UFP-treated groups ($\times 200$ magnification). **b**, Inflammation scores in the respective lung tissues. **c**, AHR in the respective mouse groups in response to methacholine. RI, responsiveness index (a measure of airway resistance). **d**, **e**, Serum total (**d**) and OVA-specific (**e**) IgE concentrations. **f**, **g**, Absolute numbers of lung $CD4^{+}$ T cells (**f**) and eosinophils (**g**). **h**, **i**, IL-13 and IL-17 expression in lung *Foxp3^{YFP}Cre* $CD4^{+}$ T_{reg} (**h**) and *Foxp3^{YFP}Cre* $CD4^{+}$ T_{eff} cells (**i**). Each symbol represents an independent sample. Numbers in flow plots indicate percentages. Error bars indicate the s.e.m. Statistical tests: two-way ANOVA with Šidák's post-hoc analysis (**b**–**i**). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data represent two or three independent experiments (white bars $n = 15$, black bars $n = 5$ and gray bars $n = 15$).

by OVA, and its superinduction by UFP co-treatment (Fig. 2c). As Notch4 is preferentially induced in T_{reg} cells in allergic airway inflammation, we employed *Foxp3^{YFP}CreNotch4^{Δ/Δ}* mice, in which the floxed *Notch4* allele is specifically deleted in T_{reg} but not in T_{eff} cells, leading to the complete selective loss of Notch4 expression in the latter cells (see Extended Data Fig. 2a,b). The attenuated allergic airway inflammation noted in *CD4^{Cre}Notch4^{Δ/Δ}* mice was completely reproduced in *Foxp3^{YFP}CreNotch4^{Δ/Δ}* mice, indicating that the effect of Notch4 deletion is localized to T_{reg} but not T_{eff} cells (Fig. 2a–c). Notch4 deletion in $CD4^{+}$ T cells, or specifically in T_{reg} cells, suppressed total and OVA-specific immunoglobulin (Ig)E responses, and T cell and eosinophil infiltration of lung tissues (Fig. 2d–g). Notch4 deletion also suppressed lung tissue T_H2 and T_H17 cell responses and reversed the destabilization of lung tissue T_{reg} cells toward T_H2 and T_H17 cell-like phenotypes, while keeping the T_{eff} and T_{reg} cell interferon- γ response unaltered (Fig. 2h,i and see also Extended Data Fig. 2c).

To assess the disease-suppressing capacities of allergen-specific iT_{reg} cells that lack Notch4 expression, we used an adoptive transfer

model in which iT_{reg} cells were derived in vitro from naive splenic $CD4^{+}$ T cells of *Foxp3^{YFP}Cre* and *Foxp3^{YFP}CreNotch4^{Δ/Δ}* mice that concurrently expressed the OTII transgene. The cells were adoptively transferred into OVA-sensitized *Foxp3^{YFP}Cre* mice, which were then challenged with aerosolized OVA and analyzed (see Extended Data Fig. 2e,f). OTII⁺ Notch4-deficient iT_{reg} cells were superior to their Notch4-sufficient counterparts in suppressing the different attributes of OVA-induced airway inflammation, including AHR, tissue eosinophilia and lymphocytosis in the lungs of the recipient mice. They were also superior in suppressing IL-4, IL-13 and IL-17 expression in the recipient lung T_{eff} and T_{reg} cells.

The salutary effects of *Notch4* deletion on allergic airway inflammation was fully reproduced by T_{reg} cell-specific deletion of *Pofut1*, encoding an enzyme that mediates O-fucosylation of Notch receptors, a requisite event in their glycosylation modification that is essential to their function (see Extended Data Fig. 3)^{12,13}. To determine the role of the canonical versus noncanonical pathways in mediating the effects of Notch signaling on T_{reg} cells in airway inflammation, we examined the impact of T_{reg} cell-specific deletion of *Rbpj*, encoding

the canonical Notch adapter RBPJ, on airway responses^{13,14}. Results revealed that *Foxp3*^{YFP^{Cre}}*Rbpj*^{Δ/Δ} mice exhibited decreased AHR and tissue eosinophilia in between those of *Foxp3*^{YFP^{Cre}}*Pofut1*^{Δ/Δ} and *Foxp3*^{YFP^{Cre}} mice (see Extended Data Fig. 3). However, whereas the T_H2 cell responses were suppressed, the T_H17 cell responses were unaffected, indicating that the latter proceeds by a Notch noncanonical pathway. In contrast, T_{reg} cell-specific deletion of floxed *Notch1* or *Notch2* alleles, or global deletion of *Notch3*, had no impact on allergic airway inflammation (see Extended Data Fig. 4).

The relationship between Notch4 expression in T_{reg} cells and airway inflammation was also investigated by interrupting upstream pathways regulating its expression. T_{reg} cell-specific deletion of *Il6ra* or *Stat3* recapitulated the protective effect of T_{reg} cell Notch4 deficiency. Both deletions attenuated OVA-induced allergic airway inflammation, with decreased AHR, airway eosinophilia, total and OVA-specific IgE, and T_H2 and T_H17 cell responses (see Extended Data Fig. 5).

T_{reg} cell-specific *Notch4* deletion was also found to be similarly protective across different aeroallergens. Thus, in a house-dust-mite-induced model of allergic airway inflammation, it also suppressed airway inflammation and AHR, tissue eosinophilia and neutrophilia and T_H2/T_H17 cell responses (see Extended Data Fig. 6). It was also protective in a chronic model of allergic airway inflammation, in which, in addition to suppressing the inflammatory and allergic responses noted above, it also suppressed subepithelial collagen deposition, a hallmark of airway remodeling due to chronic inflammation (see Extended Data Fig. 7)¹⁵. Altogether, these results highlighted the critical role of IL-6-STAT3-dependent Notch4 signaling pathways in the destabilization and dysregulation of lung T_{reg} cells in allergic airway inflammation.

Notch4 activates the Hippo and Wnt pathways to disrupt T_{reg} cell functions. To further investigate the mechanisms by which Notch4 disrupted T_{reg} cell function, we analyzed the transcriptional profiles of T_{reg} cells isolated from the lungs of sham- and OVA + UFP-treated *Foxp3*^{YFP^{Cre}} and *Foxp3*^{YFP^{Cre}}*Notch4*^{Δ/Δ} mice. Results revealed a Notch4-dependent dysregulation of several pathways in OVA + UFP-treated mice previously shown to impact T_{reg} cell stability and/or function, with particularly prominent changes in the Hippo (*Wwtr1*, *Yap1*, *Tead1*, *Tead2*, *Tead3*, *Tead4*, *Foxo6*)^{16,17} and Wnt pathways (*Ctnnb1*, *Serpine1*, *Fzd5*, 8, 10 and *Wnt4*, 5a, 8a, 9a, 9b, 11)^{18,19} (Fig. 3a,b and Supplementary Dataset 1). Notch4-dependent upregulation of the Hippo and Wnt pathways was confirmed by flow cytometry, which revealed increased expression of the Hippo pathway effector Yap, encoded by *Yap1*, and the Wnt pathway β-catenin, encoded by *Ctnnb1*, in lung T_{reg} cells in OVA- and OVA + UFP-driven allergic airway inflammation. This increase was sharply downregulated on T_{reg} cell-specific *Notch4* deletion (Fig. 3c,d). The regulation of the Hippo pathway by Notch4 was further investigated by analyzing the phosphorylation of the Hippo pathway intermediates Mob1 and Lats1 in lung T_{reg} cells of sham- and OVA + UFP-treated *Foxp3*^{YFP^{Cre}} and *Foxp3*^{YFP^{Cre}}*Notch4*^{Δ/Δ} mice. *Notch4* deletion upregulated the phosphorylation of Mob1 and Lats1, which is consistent with dampening Hippo pathway activation by promoting Lats1/2 kinase-dependent proteolysis of the effector proteins Yap and Taz. These results indicate that Notch4 activates Hippo-dependent transcription by silencing its kinase cascade (Fig. 3e,f).

To determine the role of the Hippo and Wnt pathways in mediating T_{reg} cell subversion by Notch4, we examined the consequences of T_{reg} cell-specific deletion of genes encoding key components of the respective pathways on allergic airway inflammation induced by OVA + UFP. Combined T_{reg} cell-specific deletion of *Yap1* and *Wwtr1*, encoding the Hippo pathway transcriptional regulators Yap and Taz, respectively²⁰, partially attenuated inflammation and AHR, whereas T_{reg} cell-specific deletion of *Ctnnb1*, encoding

β-catenin²¹, largely recapitulated the effect of T_{reg} cell-specific *Notch4* deletion in suppressing those parameters, with neither deletion affecting Notch4 expression (Fig. 4a–h). *Yap1* and *Wwtr1* deletion suppressed T_H17 and, to a lesser extent, T_H2 cell responses in the airways while upregulating T_H1 cell responses (Fig. 4a–d). In contrast, *Ctnnb1* deletion profoundly suppressed the T_H2 cell-like reprogramming of Notch4^{hi} T_{reg} cells and the airway conventional T_H2 cell response, but left the T_H17 cell responses unaffected (Fig. 4e–h). Combined deletion of *Yap1*, *Wwtr1* and *Ctnnb1* in T_{reg} cells reproduced the full effect of *Notch4* deletion in suppressing allergic airway inflammation and T_H2 and T_H17 cell responses (Fig. 4i,j). These results indicated that the Hippo and Wnt pathways mediated distinct, complementary aspects of Notch4 signaling in disrupting immune tolerance in allergen and pollutant-induced allergic airway inflammation.

Notch4 promotes T_{reg} cell destabilization toward T_H2 and T_H17 cell fates. To determine whether Notch4 acted to destabilize lung T_{reg} cells to give rise to Foxp3[−] T_H2 and T_H17 ex-T_{reg} cells, we employed a lineage tracing approach using a *Rosa26* Stop-flox enhanced green fluorescent protein (EGFP) reporter (*R26*^{EGFP}) crossed to *Foxp3*^{YFP^{Cre}}. *Foxp3*^{YFP^{Cre}}*Notch4*^{Δ/Δ}*R26*^{EGFP} and control *Foxp3*^{YFP^{Cre}}*R26*^{EGFP} mice were either sham or OVA sensitized, and then challenged with aerosolized OVA without or with intranasal UFP treatment. Cytokine expression was examined in T_{reg} (YFP⁺EGFP⁺), ex-T_{reg} (YFP-EGFP⁺) and CD4⁺ T_{eff} cells (YFP-EGFP[−]). The frequencies of YFP-EGFP⁺ ex-T_{reg} cells were markedly increased in the lungs of the *Foxp3*^{YFP^{Cre}}*R26*^{EGFP} OVA-sensitized and -challenged group, and were further increased in the OVA + UFP-treated group, with the ex-T_{reg} cells reaching up to a third of the total T_{reg} lineage-derived (EGFP⁺) cells in the lung (Fig. 5a). In contrast, the ex-T_{reg} cells were markedly decreased in the equivalent *Foxp3*^{YFP^{Cre}}*Notch4*^{Δ/Δ}*R26*^{EGFP} groups, indicative of heightened T_{reg} cell instability mediated by Notch4 (Fig. 5a). Approximately half of the ex-T_{reg} cells were T_H2- and T_H17-skewed cells at a ratio of 3:1, with both being suppressed in *Foxp3*^{YFP^{Cre}}*Notch4*^{Δ/Δ}*R26*^{EGFP} mice (Fig. 5b,c).

To investigate the source of Notch4-mediated T_{reg} cell instability, we further examined the epigenetic methylation signature of the *Foxp3* CNS2 promoter region, which inversely affects T_{reg} cell lineage stability^{22,23}. There was increased methylation of the CpG elements in the *Foxp3* CNS2 of T_{reg} cells isolated from the lungs of OVA + UFP-treated mice compared with those of sham-treated mice, which segregated with high but not low Notch4 expression (Notch4^{hi} versus Notch4^{lo}) (Fig. 5d,e). Increased CNS2 CpG methylation was also reversed on T_{reg} cell-specific deletion of *Notch4* compared with that of total lung T_{reg} cells of *Foxp3*^{YFP^{Cre}} control mice. Combined *Yap1* and *Wwtr1*, but not *Ctnnb1*, deletion fully reversed the increased methylation of the *Foxp3* CNS2 in lung T_{reg} cells of OVA-/UFP-treated mice, indicating that the destabilization of T_{reg} cells by Notch4 signaling was mediated by the Hippo but not by the Wnt pathway (Fig. 5d,e).

We also investigated the role of Notch4 expression in impairing T_{reg} cell function by sorting out Notch4^{hi} versus Notch4^{lo} T_{reg} cells from the lungs of the *Foxp3*^{YFP^{Cre}} OVA + UFP-treated group as well as T_{reg} cells from untreated control *Foxp3*^{YFP^{Cre}} mice, and examining these three groups of T_{reg} cells for their suppressive capacity. Whereas the Notch4^{lo} lung T_{reg} cells from OVA + UFP-treated mice were equivalent to control lung T_{reg} cells in their capacity to inhibit in vitro T cell proliferation, the suppressive function of Notch4^{hi} lung T_{reg} cells was profoundly impaired (Fig. 5f). Combined *Yap1* and *Wwtr1*, but not *Ctnnb1*, deletion fully restored the in vitro suppressive function of Notch4^{hi} T_{reg} cells, consistent with the impact of the respective pathways on CNS2 demethylation (Fig. 5g,h). Overall, these results indicated Notch4-induced T_{reg} cell instability and T_H2 and T_H17 cell-like reprogramming in the context of allergic airway inflammation, and that this destabilization was associated

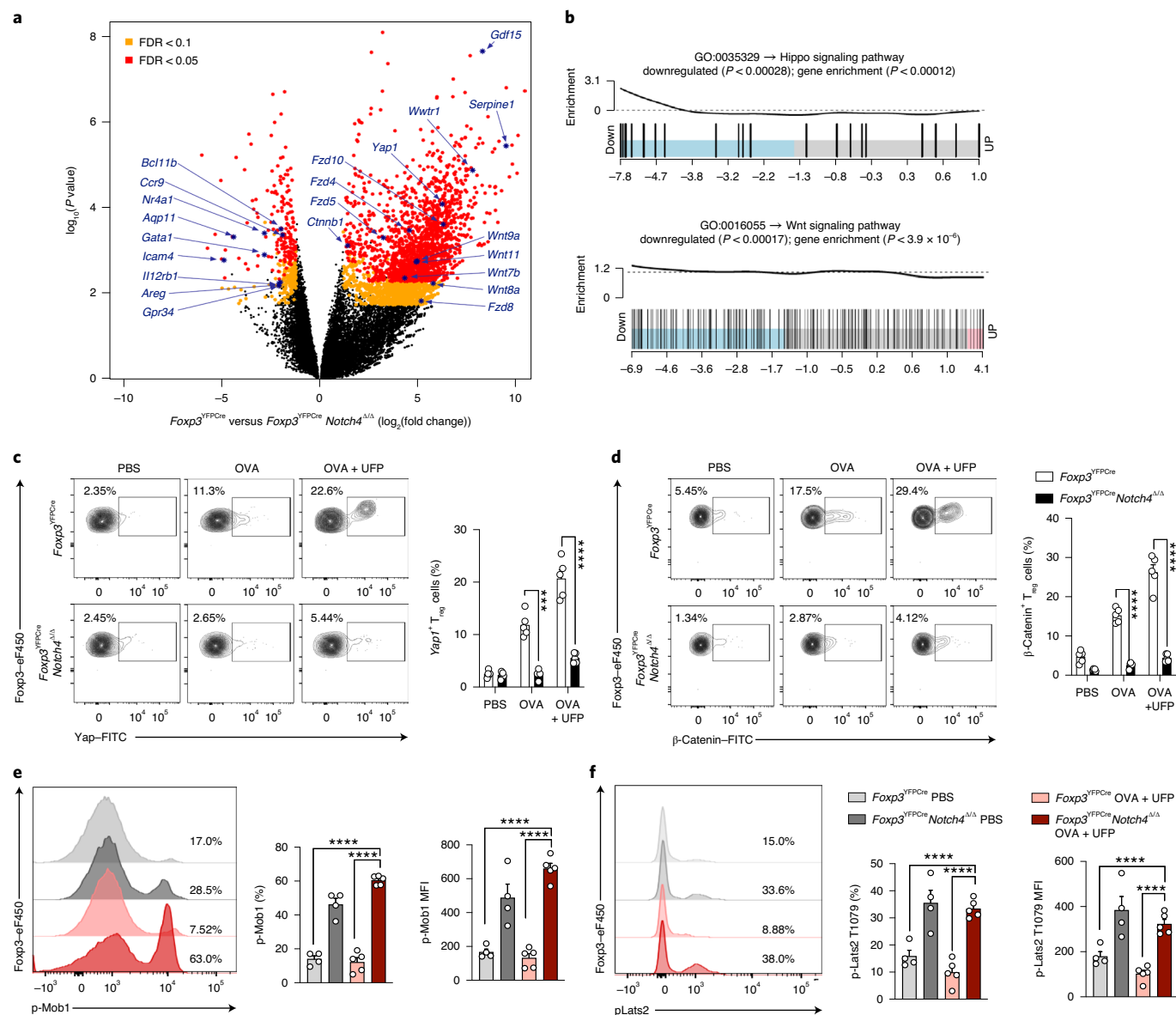


Fig. 3 | Notch4-dependent transcriptional programs in lung T_{reg} cells. **a**, Volcano plot of differential gene expression in lung T_{reg} cells isolated from $Foxp3^{YFP/Cre}$ versus $Foxp3^{YFP/Cre} Notch4^{\Delta/\Delta}$ mice that were treated with OVA + UFP. **b**, Enrichment pathway analysis of Hippo and Wnt pathways. GO, gene ontology. **c**, Flow cytometric analysis and cell frequencies of Yap1 expression on lung T_{reg} cells in the respective treatment groups ($n = 5$). **d**, Flow cytometric analysis and cell frequencies of β -catenin expression on lung T_{reg} cells in the respective treatment groups ($n = 5$). **e**, Representative histogram, cell frequencies and MFI of phospho-Mob1 expression on lung T_{reg} cells in the respective treatment groups (light gray bar $n = 4$, dark gray bar $n = 4$, light red bar $n = 5$ and dark red bar $n = 4$). **f**, Representative histogram, cell frequencies and MFI of phospho-Lats2 T1079 expression on lung T_{reg} cells in the respective treatment groups (light gray bar $n = 4$, dark gray bar $n = 4$, light red bar $n = 5$ and dark red bar $n = 4$). Each symbol represents an independent sample. Numbers in flow plots indicate percentages. Error bars indicate the s.e.m. Statistical tests: two-way ANOVA with Šidák's post-hoc analysis (**c** and **d**); one-way ANOVA with Dunnett's post-hoc analysis (**e** and **f**). *** $P < 0.001$, **** $P < 0.0001$.

with epigenetic methylation at the *Foxp3* CNS2 locus mediated by the Hippo pathway.

A T_{reg} cell Notch4–Wnt–GDF15 pathway promotes ILC2 expansion and activation. ILC2s play a key role in allergic airway inflammation by virtue of copious secretion of type 2 cytokines, most prominently IL-13 (ref. 24). Total ILC2 as well as IL-13-expressing ILC2s were sharply increased in OVA- and especially OVA + UFP-treated mice, but were dramatically reduced on deletion of *Notch4* in T_{reg} cells (Fig. 6a). The effect of T_{reg} cell-specific *Notch4* deletion on ILC2 expansion and activation was reproduced by *Ctnnb1* but not *Yap1* and *Wwtr1* T_{reg} cell-specific deletion

(Fig. 6a). In vitro studies revealed that *Notch4*^{hi} T_{reg} cells derived from OVA + UFP-treated $Foxp3^{YFP/Cre}$ mice failed to suppress the upregulation of IL-13 expression in ILC2s derived from the inflamed lungs of the same mice (Fig. 6b). In contrast, *Notch4*^{lo} lung T_{reg} cells derived from the OVA + UFP-treated $Foxp3^{YFP/Cre}$ mice or *Notch4*-deficient T_{reg} cells derived from OVA + UFP-treated $Foxp3^{YFP/Cre} Notch4^{\Delta/\Delta}$ mice potently suppressed IL-13 expression, as did treatment of *Notch4*^{hi} T_{reg} cells with an anti-*Notch4* monoclonal antibody (Fig. 6b). Further analysis revealed that T_{reg} cell-specific *Ctnnb1*, but not *Yap* and *Taz*, deletion restored the ILC2-suppressive function of *Notch4*^{hi} T_{reg} cells, thus implicating the Wnt pathway in the failure of ILC2 regulation (Fig. 6c).

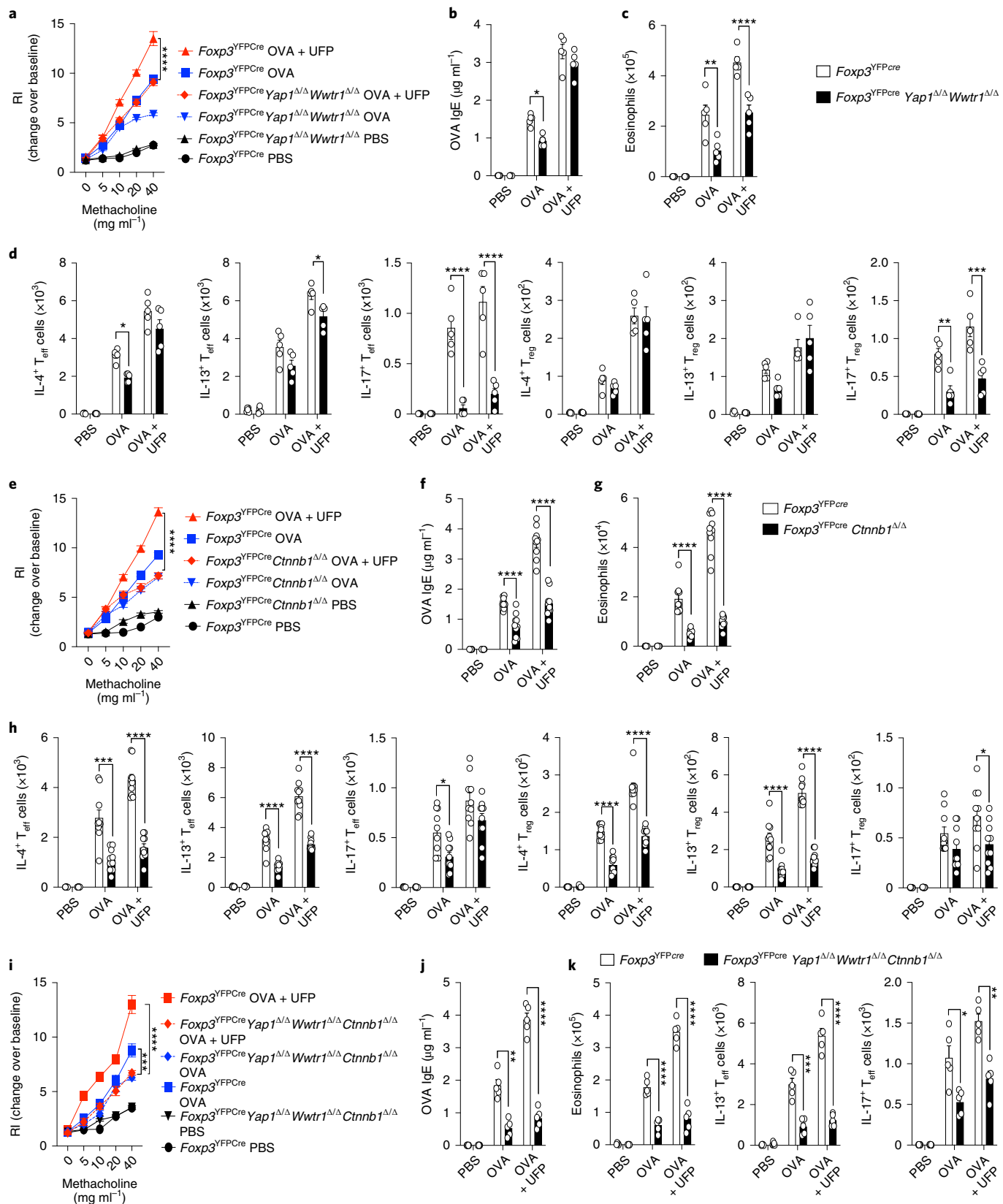


Fig. 4 | Regulation of allergic airway inflammation by Notch4-dependent Hippo and Wnt pathway. a,e,i, AHR in the respectively treated *Foxp3*^{YFPcre} *Wwtr1*^{Δ/Δ} *Yap1*^{Δ/Δ} (**a**), *Foxp3*^{YFPcre} *Ctnnb1*^{Δ/Δ} (**e**) and *Foxp3*^{YFPcre} *Wwtr1*^{Δ/Δ} *Yap1*^{Δ/Δ} *Ctnnb1*^{Δ/Δ} (**i**) mice as compared with control *Foxp3*^{YFPcre} mice in response to methacholine (**a**, *n* = 15; **e** and **i**, *n* = 5). **b,f,j**, OVA-specific IgE titers (**b**, *n* = 15; **f** and **j**, *n* = 5). **c,d,g,h,k**, Absolute lung tissue eosinophils and IL-4⁺, IL-13⁺ and IL-17⁺ T_{eff} and T_{reg} cells in *Foxp3*^{YFPcre} *Wwtr1*^{Δ/Δ} *Yap1*^{Δ/Δ}, *Foxp3*^{YFPcre} *Ctnnb1*^{Δ/Δ} and *Foxp3*^{YFPcre} *Wwtr1*^{Δ/Δ} *Yap1*^{Δ/Δ} *Ctnnb1*^{Δ/Δ} mice as compared with control *Foxp3*^{YFPcre} mice (**c** and **d**, *n* = 15; **g**, **h** and **k**, *n* = 5). Each symbol represents an independent sample. Error bars indicate the s.e.m. Statistical tests: two-way ANOVA with Šidák's post-hoc analysis (**a–k**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Data represent two or three independent experiments.

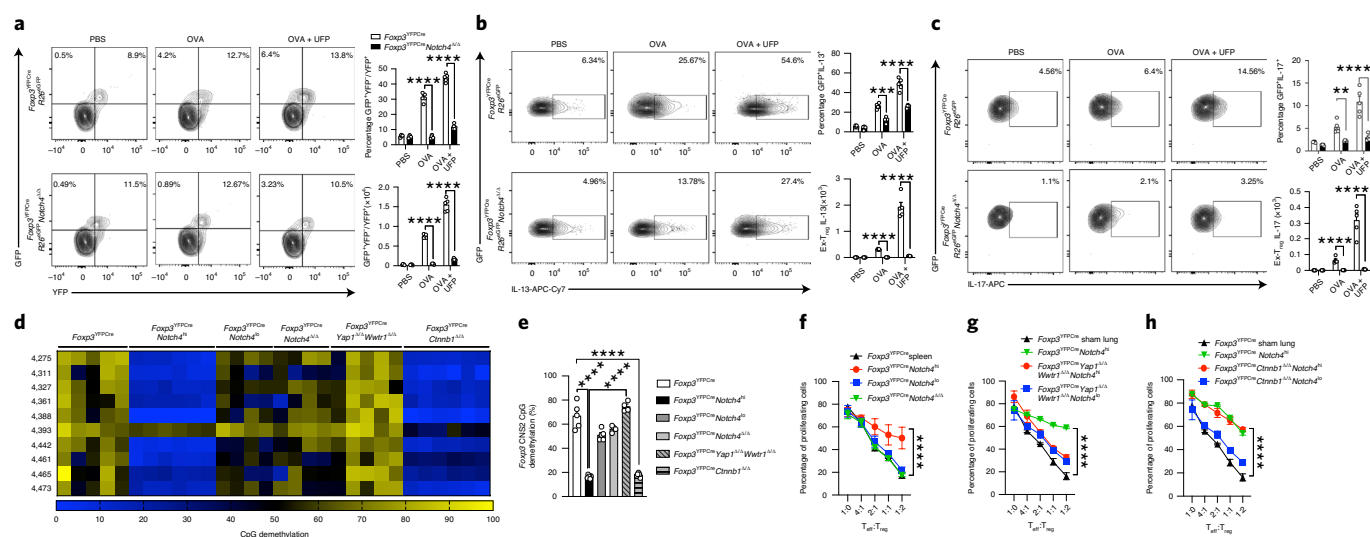


Fig. 5 | Notch4 destabilizes T_{reg} cells in a Hippo pathway-dependent manner. **a**, Flow cytometric analysis and frequencies of ex- T_{reg} (GFP⁺YFP⁺) cells, plotted as a fraction of ex- T_{reg} /total T_{reg} (YFP⁺) cells in lung tissue ($n=5$). **b,c**, Flow cytometric analysis and frequencies of IL-13- ($n=5$) (**b**) and IL-17- ($n=5$) (**c**) producing ex- T_{reg} cells in lung tissues. **d**, Methylation status of CpG motifs of the *Foxp3* CNS2 region in T_{reg} cells isolated from lung tissue of sham- versus OVA + UFP-sensitized and -challenged mice of the respectively indicated genotypes. Numbers on the left side indicate the position of the respective motifs. **e**, Global methylation status of *Foxp3* CNS2 in the respective T_{reg} cell populations (white bar $n=5$, black bar $n=6$, dark gray bar $n=5$, light gray bar $n=4$, gray line bar $n=4$ and gray line bar $n=6$). **f-h**, In vitro suppression of the proliferation of WT responder CD4⁺ T cells (T_{eff}) by the respective T_{reg} cell populations (**f**, black line $n=3$, red line $n=6$, blue line $n=6$, green line $n=3$; **g**, black line $n=3$, green line $n=3$, red line $n=3$, blue line $n=3$; **h**, black line $n=3$, green line $n=3$, red line $n=3$, blue line $n=3$). Each symbol represents an independent sample. Flow plots are representative and numbers in flow plots indicate percentages. Error bars indicate the s.e.m. Statistical tests: two-way ANOVA with Sidák's post-hoc analysis (**a-c**, **f-h**); one-way ANOVA with Dunnett's post-hoc analysis (**e**). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data represent two or three independent experiments.

RNA-sequencing (RNA-seq) analysis identified *Gdf15* transcripts to be highly enriched in lung T_{reg} cells of OVA + UFP-treated *Foxp3*^{YFP^{Cre} mice (see Fig. 3a). Consistent with this finding, transcripts encoding the cytokine GDF15 were highly induced in Notch4^{hi} lung T_{reg} cell in a β -catenin-dependent manner (Fig. 6d). Flow cytometric analysis confirmed that T_{reg} cells were the primary source of GDF15 in the inflamed lungs of OVA- and OVA + UFP-treated mice, whereas GDF15 was sharply downregulated in Notch4- and β -catenin-deficient, but not Yap/Taz-deficient, T_{reg} cells (Fig. 6e). Addition of recombinant GDF15 to in vitro cultures of ILC2s derived from naive mice upregulated IL-13 expression alone and especially in synergy with IL-33 (Fig. 6f). Furthermore, the in vitro co-culture of GDF15-expressing Notch4^{hi} T_{reg} cells, isolated from lungs of OVA + UFP-treated mice, with naive ILC2s upregulated the expression of IL-13 in the latter, an effect that was reversed by the addition of a GDF15-blocking peptide (Fig. 6g). Addition of GDF15-blocking peptide restored the ILC2-suppressive function of lung T_{reg} cells of OVA + UFP-treated mice, consistent with the critical role of the Wnt–GDF15 axis in the failure of ILC2 regulation (Fig. 6h).}

The contribution of GDF15 to the allergic airway inflammatory response was explored by intratracheal instillation of recombinant GDF15 in OVA + UFP-treated *Foxp3*^{YFP^{Cre}Notch4 ^{Δ/Δ} mice, which resulted in the upregulation of AHR and tissue inflammation, as well as the increased expression of IL-4 and IL-13 in T_{eff} cells (Fig. 7a–c). Reciprocally, instillation of the GDF15-blocking peptide suppressed the aforementioned changes in OVA + UFP-treated *Foxp3*^{YFP^{Cre} mice (Fig. 7d–f). The essential role of ILC2-derived IL-13 in mediating the effects of GDF15 on airway inflammation was established by specifically deleting an *Il4–Il13* gene cassette in ILC2s using a *Rora*-driven Cre recombinase (*Rora*^{Cre}*Il4 Δ/Δ* *Il13 Δ/Δ*). ILC2-specific deletion of *Il4–Il13* reduced OVA + UFP-driven airway inflammation, and abrogated the capacity of GDF15 to further upregulate it. GDF15 treatment did not affect the expression of}}

IL-13 in T_{eff} cells neither in control *Rora*^{Cre} mice nor in *Rora*^{Cre}*Il4 Δ/Δ* *Il13 Δ/Δ* mice (Fig. 7g,h). Overall, these findings confirmed that Notch4 expression abrogated the capacity of lung T_{reg} cells to suppress ILC2s and activated a T_{reg} cell-intrinsic Wnt–GDF15 axis that promoted ILC2 activation.

T_{reg} cell Notch4 expression segregates with disease severity in subjects with asthma. To determine the relevance of the Notch4 signaling pathways in human subjects with asthma, we analyzed the expression of Notch4 on peripheral blood mononuclear cells (PBMCs) of subjects with asthma and controls (the demographics and disease severity classification of subjects with asthma are described in Supplementary Table 1). Results revealed that subjects with asthma had elevated frequencies of circulating Notch4^{hi} T_{reg} cells, with both the cell frequencies and expression intensity progressively increasing as a function of asthma severity, reaching up to 50% of circulating T_{reg} cells in subjects with severe asthma (Fig. 8a). In contrast, Notch4 expression on circulating CD4⁺ T_{eff} cells was low and remained relatively low as a function of asthma severity (Fig. 8b). Increased expression of Notch4 in circulating T_{reg} cells of subjects with asthma was found to be independent of the atopic status of patients (see Extended Data Fig. 8). Also, expression of Notch1–3 on T_{reg} and T_{eff} cells was not increased in subjects with asthma compared with control subjects (see Extended Data Fig. 8). Subjects with other allergic diseases expressed Notch4 on their circulating T_{reg} cells at levels that were either similar to those of controls (for example, food allergy) or equivalent to those of subjects with mild asthma (for example, eczema without or with food allergy).

Further analysis revealed that Notch4 expression was restricted to the circulating Helios⁺ T_{reg} cell subpopulation (Fig. 8c). T_{reg} cell expression of the Hippo and Wnt pathway effector proteins Yap/Taz and β -catenin, respectively, localized to Notch4⁺ T_{reg} cells and similarly increased as a function of asthma severity (Fig. 8d,e). Also,

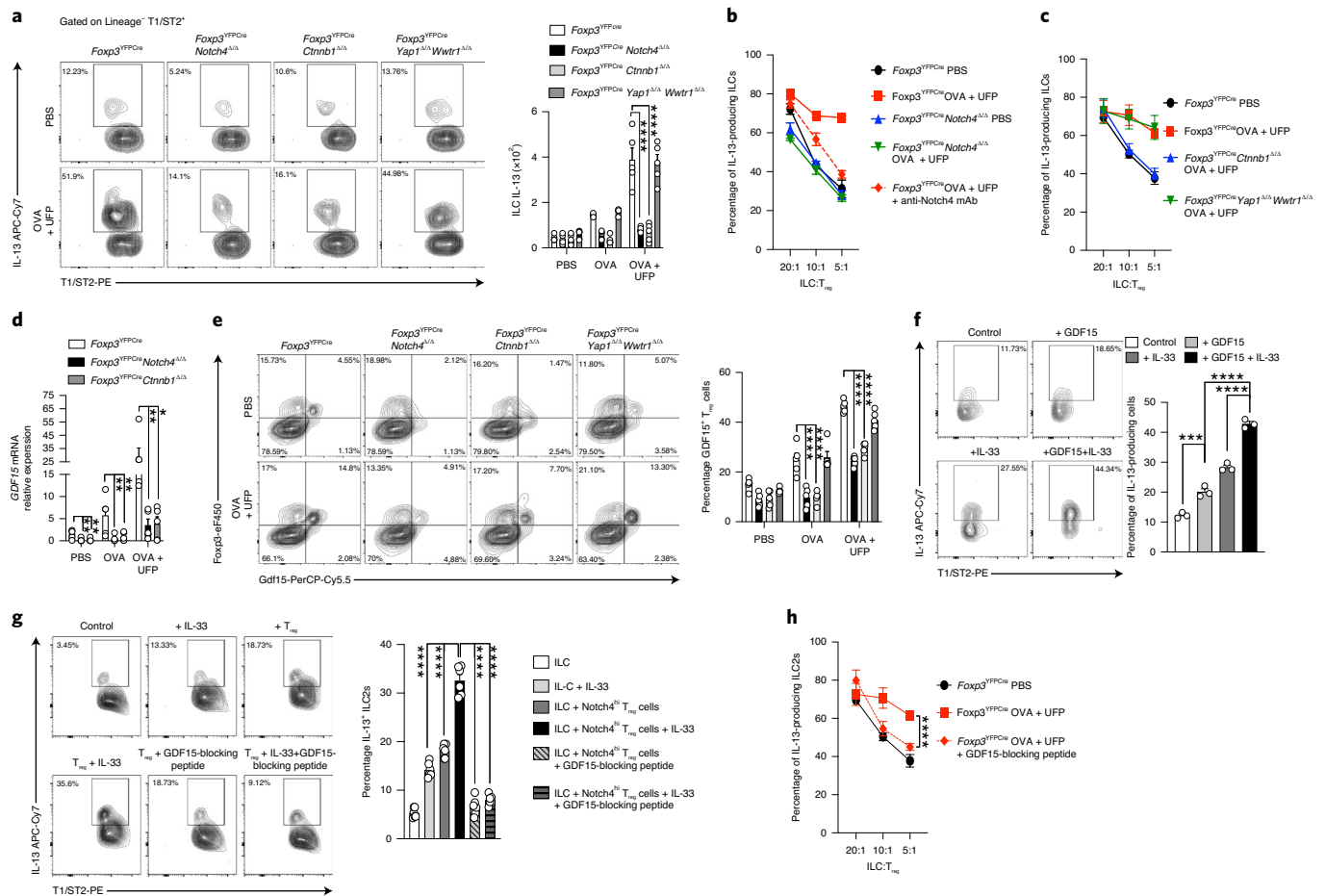


Fig. 6 | Notch4 promotes ILC2 activation via a GDF15-dependent mechanism. **a**, Flow cytometric analysis and frequencies of IL-13⁺ ILC2 (Lineage-T1/ST2⁺) cells in mice of respective genotypes treated as indicated ($n=5$). **b,c**, In vitro suppression assays using ILC2s from OVA + UFP-treated *Foxp3*^{YFP/Cre} mice and lung T_{reg} cells of the respective genotypes, treated as indicated ($n=4$). mAb, monoclonal antibody. **d**, GDF15 transcripts in T_{reg} cells of *Foxp3*^{YFP/Cre}, *Foxp3*^{YFP/Cre}*Notch4*^{Δ/Δ} and *Foxp3*^{YFP/Cre}*Ctnnb1*^{Δ/Δ} ($n=5$). **e**, Flow cytometric analysis and frequencies of GDF15⁺ lung T_{reg} cells in the respective mouse genotypes treated as indicated ($n=5$). **f**, Flow cytometric analysis and frequencies of IL-13 induced in naive ILC2s stimulated with IL-33, GDF15 or both ($n=3$). **g**, IL-13 expression in naive ILC2s incubated with Notch4^{hi} T_{reg} cells from OVA + UFP-treated mice without or with GDF15-blocking peptide ($n=6$). **h**, In vitro suppression assays using lung T_{reg} cells and ILC2s isolated from OVA + UFP-treated *Foxp3*^{YFP/Cre} mice and incubated without or with GDF15-blocking peptide ($n=4$). Each symbol represents an independent sample. Numbers in flow plots indicate percentages. Error bars indicate the s.e.m. Statistical tests: two-way ANOVA with Šidák's post-hoc analysis (**a–e** and **h**); one-way ANOVA with Dunnett's post-hoc analysis (**f** and **g**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data represent two or three independent experiments.

there were increased concentrations of GDF15 in the sera of subjects with moderate and severe asthma that positively correlated with circulating T_{reg} cell Notch4 expression, whereas those of subjects with mild asthma were similar to those of controls (Fig. 8f and see also Extended Data Fig. 8). The contribution of Notch4 signaling to T_{reg} cell dysfunction was ascertained by the demonstration that Notch4^{hi} peripheral blood T_{reg} cells poorly suppressed in vitro T cell proliferation compared with Notch4^{lo} T_{reg} cells isolated from the same asthmatic subjects or with T_{reg} cells isolated from healthy control subjects, which were overwhelmingly Notch4^{lo} (Fig. 8g). Moreover, the in vitro co-culture of Notch4^{hi} T_{reg} cells, isolated from peripheral blood of subjects with severe asthma, failed to suppress the activation of ILC2s compared with T_{reg} cells of healthy control subjects, a defect that was reversed by the addition of a GDF15-blocking peptide (Fig. 8h).

Analysis of PBMCs of a subject with severe asthma treated with the anti-IL-6R monoclonal antibody tocilizumab revealed decreased Notch4 expression on the patient T_{reg} cells after initiation of therapy, consistent with the requirement for IL-6R signaling to upregulate

Notch4 expression (Fig. 8i)²⁵. These results, which mirror those obtained in the mouse system, indicate that Notch4 expression may similarly serve as an immune regulatory switch that licenses allergic inflammation in humans with asthma.

Discussion

In the present study, we have identified a new pathway central to the pathogenesis of asthmatic airway inflammation involving the inducible expression of Notch4 on allergen-specific T_{reg} cells. This induction, synergistically mediated by allergens and ambient pollutant particles, activates downstream Hippo and Wnt pathways to subvert T_{reg} cell stability and functions. Inhibition of Notch4 expression in T_{reg} cells, but not that of other Notch receptors, suppressed airway inflammation and restored immune tolerance. Critically, Notch4 signaling upregulated the expression in T_{reg} cells of GDF15, a cytokine that we demonstrate to reinforce airway inflammation by a new mechanism involving ILC2 activation. Notch4 and its downstream effector pathways were upregulated on T_{reg} cells of subjects with asthma as a function of disease severity, thus identifying

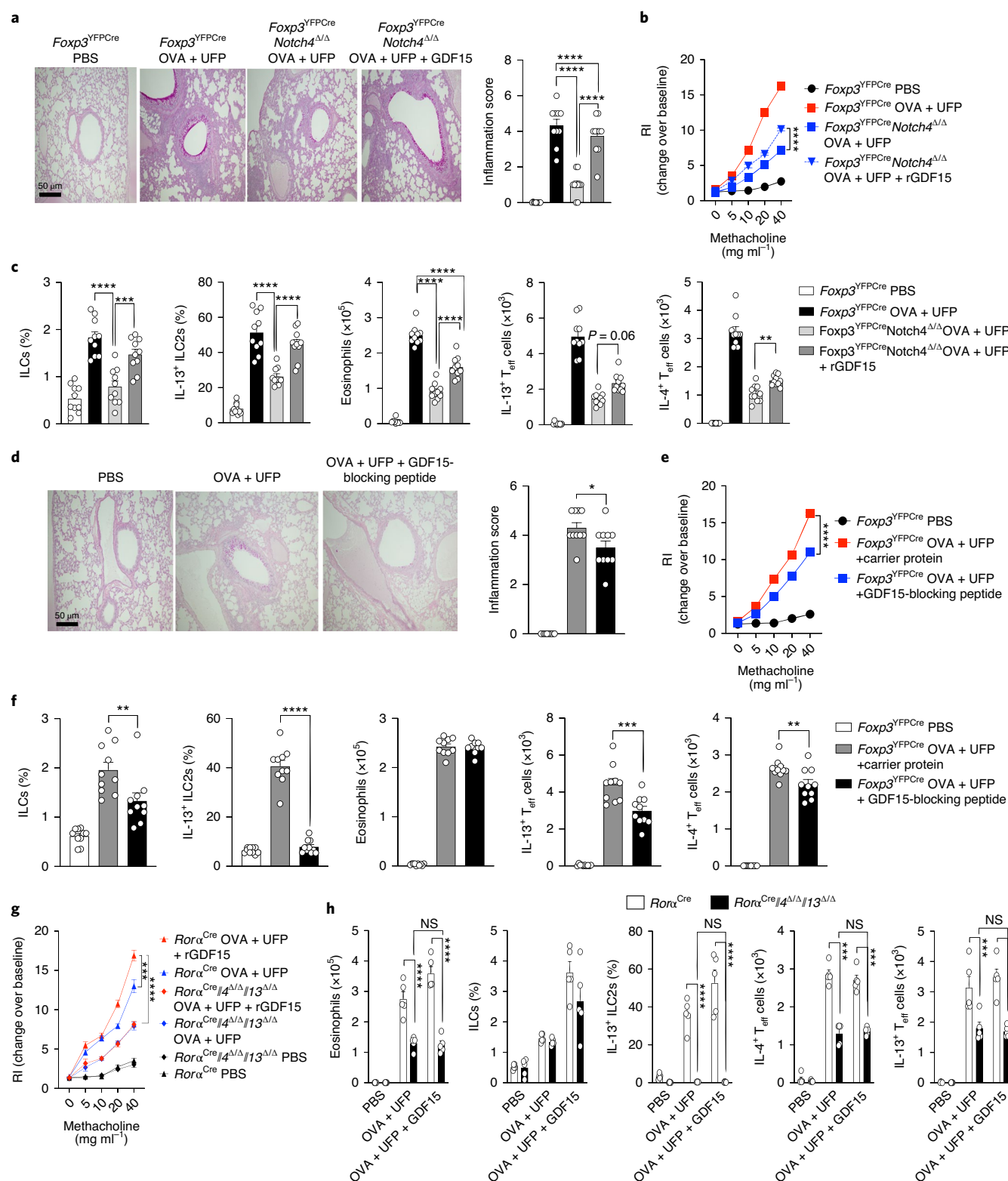


Fig. 7 | GDF15 regulates ILC2 response in airway inflammation. **a,d**, Representative PAS-stained sections of lung tissues isolated from *Foxp3^{YFP/Cre}* and *Foxp3^{YFP/Cre}Notch4^{Δ/Δ}* with either PBS or OVA + UFP, the latter either alone or supplemented with GDF15 or GDF15-blocking peptide, as indicated ($\times 200$ magnification). Inflammation score for the respective mouse groups ($n = 10$). **b,e**, AHR in *Foxp3^{YFP/Cre}* and *Foxp3^{YFP/Cre}Notch4^{Δ/Δ}* treated as indicated ($n = 10$). **c,f**, Frequencies and absolute numbers of ILC2s, eosinophils, IL-4 and IL-13; expression in lung *Foxp3^{YFP/Cre}* CD4⁺ T_{eff} cells in the respective groups ($n = 10$). **g**, AHR in *Rora^{Cre}* and *Rora^{Cre}/4^{Δ/Δ}/13^{Δ/Δ}* treated as indicated ($n = 5$). **h**, Frequencies and absolute numbers of eosinophils, ILC2s, IL-4 and IL-13; expression in lung *Foxp3^{YFP/Cre}* CD4⁺ T_{eff} cells in the respective groups ($n = 5$). NS, not significant; error bars indicate the s.e.m. Statistical tests: one-way ANOVA with Dunnett's post-hoc analysis (**a**, **c**, **d** and **f**); two-way ANOVA with Sidák's post-hoc analysis (**b**, **e**, **g** and **h**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data represent two or three independent experiments.

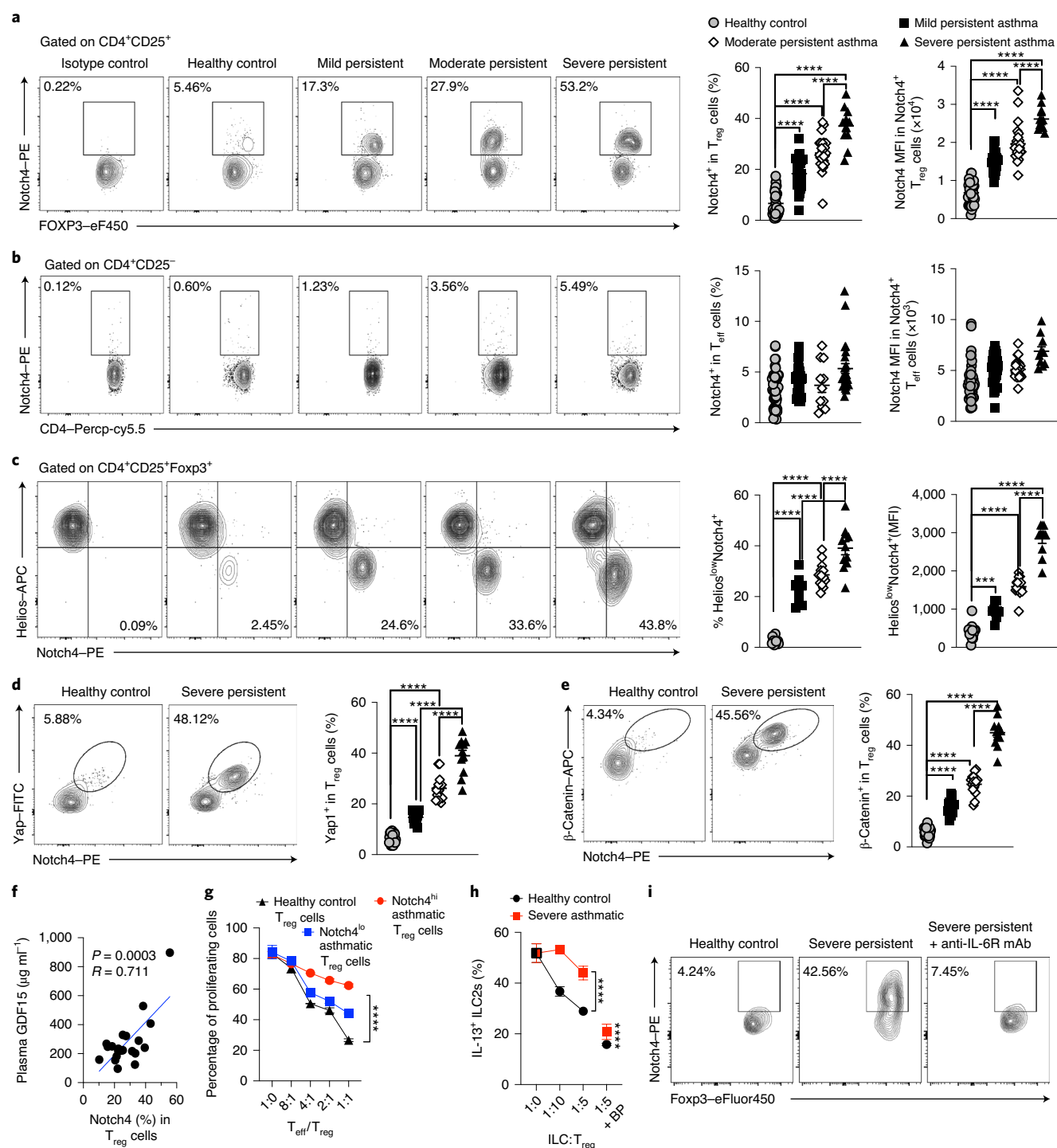


Fig. 8 | Notch4 expression on circulating T_{reg} cells segregates with asthma severity. **a, b**, Flow cytometric analysis, cell frequencies and MFI of Notch4 expression on circulating T_{reg} cells (**a**) and T_{eff} cells (**b**) of control subjects and subjects with asthma, the latter segregated for asthma severity (control: $n = 39$; mild $n = 31$; moderate: $n = 27$; severe: $n = 11$). **c**, Flow cytometric analysis, cell frequencies and MFI of Notch4 expression on Helios⁺ versus Helios⁻ circulating T_{reg} cells of control subjects and subjects with asthma (control: $n = 13$; mild $n = 9$; moderate $n = 14$; severe $n = 11$). **d, e**, Flow cytometric analysis, cell frequencies and MFI of Yap (**d**) and β-catenin (**e**) expression on circulating T_{reg} cells of control subjects and subjects with severe asthma (control $n = 24$; mild $n = 15$; moderate $n = 15$; severe $n = 11$). **f**, Serum GDF15 concentrations in subjects with moderate and severe asthma plotted as a function of Notch4 expression on circulating T_{reg} cells ($n = 21$). **g**, In vitro suppression of third-party CD4⁺ T cells (T_{eff}) by the Notch4^{hi} versus Notch4^{lo} T_{reg} cells from subjects with severe asthma as compared with T_{reg} cells of control subjects ($n = 2$ subjects, 3 replicates per dilution per subject). **h**, In vitro suppression assays of ILC2 activation using circulating Notch4^{hi} T_{reg} cells of subjects with asthma and control T_{reg} cells of healthy controls, incubated at the indicated T_{reg} cell:ILC2 ratios without or with GDF15-blocking peptide (BP) ($n = 5$). **i**, Flow cytometric analysis of Notch4 expression in T_{reg} cells of a healthy control and a subject with severe asthma before and after treatment with anti-IL-6R monoclonal antibody ($n = 1$). Error bars indicate the s.e.m. Statistical tests: one-way ANOVA with Dunnett's post-hoc analysis (**a–e**); simple linear regression analysis (**f**); two-way ANOVA with Šidák's post-hoc analysis (**g** and **h**). *** $P < 0.001$, **** $P < 0.0001$. Data represent two or three independent experiments.

Notch4 as an immune regulatory switch mechanism that licenses asthmatic inflammation and highlighting the therapeutic potential for tolerance restoration in asthma.

Induction of Notch4 on T_{reg} cells in the airway involved coordinate allergen peptide-specific TCR activation and IL-6–STAT3 signaling, a process further upregulated by IL-33. Remarkably, this pathway thus integrates several genetic loci, including *NOTCH4*, *IL6* and *IL33*, identified to impart susceptibility to asthma incidence and/or disease severity^{26–30}. T_{reg} cell-specific deletion of *Il6ra* or *Stat3* substantially attenuated Notch4 expression in T_{reg} cells in allergic airway inflammation, as did treatment of a subject with severe asthma with the anti-IL-6R α chain monoclonal antibody tocilizumab²⁵. STAT3 was demonstrated to bind to the *Notch4* promoter, consistent with direct upregulation of Notch4 expression by IL-6–STAT3 signaling. The specificity of Notch4 induction on lung T_{reg} cells may relate to a ‘niche effect’, in which the interaction with AMs normally drives differentiation of naive allergen-specific T cells into T_{reg} cells^{8,31}. The uptake of allergens and ambient particulate matter is associated with the production of IL-6 and upregulation of Notch ligands, including Jag1 (ref. ⁸), driving T_{reg} skewing toward T_{eff} cell phenotypes in a Notch4-dependent manner.

Notch4 expression on lung T_{reg} cells mobilized several downstream pathways, notably Hippo and Wnt, to derail T_{reg} cell stability and function. Expression of effectors of both pathways, including Yap and Taz (Hippo) and β -catenin (Wnt), also segregated with Notch4 expression in peripheral blood T_{reg} cells of humans with asthma and correlated with asthma severity. The two pathways acted to disrupt different aspects of T_{reg} cell functions. Whereas the Hippo pathway impaired lung T_{reg} cell in vitro suppressor function and promoted their skewing toward the T_H17 cell fate, the Wnt pathway promoted their T_H2 cell-like reprogramming and was essential to the T_H2 effector T cell response in the airways. T_{reg} cell-specific deletion of *Rbpj* segregated the T_H2 and T_H17 cell responses along the canonical and noncanonical pathways, respectively. Thus, Notch4 mobilizes distinct signaling pathways within lung T_{reg} cells that act in a modular fashion to disrupt immune tolerance in the airways.

An important pathogenic mechanism mobilized by Notch4 is the potentiation of ILC2 activation, which proceeded by a T_{reg} cell-intrinsic, β -catenin-dependent pathway. Notch4– β -catenin signaling impaired the suppression by T_{reg} cells of activated ILC2s. Furthermore, it positively promoted the activation of resting ILC2 by inducing the expression in T_{reg} cells of GDF15, which selectively activated ILC2 but not T cells in synergy with IL-33. Antagonism of GDF15 augmented the in vitro suppression of ILC2s by Notch4^{hi} T_{reg} cells and downregulated airway inflammation in vivo. These results indicated a critical role for GDF15 in mediating an ILC2-dependent forward amplification loop by which Notch4^{hi} T_{reg} cells actively promote asthmatic inflammation.

Analysis of Notch4 expression on circulating T_{reg} cells of a pediatric cohort of subjects with asthma demonstrated a step-wise increase in Notch4 expression as a function of asthma severity, with the T_{reg} cells of those with severe asthma especially marked by high expression of Notch4 and its downstream effectors Yap, Taz and β -catenin. Similar to the mouse studies, Notch4-expressing human T_{reg} cells also showed impaired in vitro suppressive function. Remarkably, there was minimal heterogeneity in Notch4 expression within each disease severity subgroup, highlighting Notch4 as a common pathogenic mechanism operative in these patients, the amplitude of which is highly informative of disease activity. These results emphasize the potential usefulness of Notch4 and its downstream Hippo and Wnt effectors as new biomarkers to monitor disease activity and response to therapy.

In conclusion, our studies identify a new mechanism central to the pathogenesis of asthmatic inflammation. These studies, together with earlier ones on the role of Notch1 expression on T_{reg} cells in promoting T_H1 cell inflammatory and autoimmune responses^{13,32}, hint

at a T_{reg} cell-specific Notch receptor code that directs different T_H cell responses in allergic and autoimmune diseases. Elucidating the respective roles of different Notch receptors in controlling disease outcome by modulating T_{reg} cell responses may offer opportunities for precision medical interventions to restore immune tolerance in a tissue- and disease-specific manner.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41590-020-0777-3>.

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References

- Lambrecht, B. N. & Hammad, H. The immunology of asthma. *Nat. Immunol.* **16**, 45–56 (2015).
- Martinez, F. D. & Vercelli, D. Asthma. *Lancet* **382**, 1360–1372 (2013).
- Noval Rivas, M. & Chatila, T. A. Regulatory T cells in allergic diseases. *J. Allergy Clin. Immunol.* **138**, 639–652 (2016).
- Krishnamoorthy, N. et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. *Nat. Med.* **18**, 1525–1530 (2012).
- Noval Rivas, M. et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* **42**, 512–523 (2015).
- Massoud, A. H. et al. An asthma-associated IL4R variant exacerbates airway inflammation by promoting conversion of regulatory T cells to T_H17-like cells. *Nat. Med.* **22**, 1013–1022 (2016).
- Xia, M. et al. Vehicular exhaust particles promote allergic airway inflammation through an aryl hydrocarbon receptor–notch signaling cascade. *J. Allergy Clin. Immunol.* **136**, 441–453 (2015).
- Xia, M., Harb, H., Saffari, A., Sioutas, C. & Chatila, T. A. A Jagged 1–Notch 4 molecular switch mediates airway inflammation induced by ultrafine particles. *J. Allergy Clin. Immunol.* **142**, 1243–1256.e17 (2018).
- Tsai, V. W. W., Husaini, Y., Sainsbury, A., Brown, D. A. & Breit, S. N. The MIC-1/GDF15–GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. *Cell Metab.* **28**, 353–368 (2018).
- Luan, H. H. et al. GDF15 is an inflammation-induced central mediator of tissue tolerance. *Cell* **178**, 1231–1244.e11 (2019).
- Chen, W. et al. Conversion of peripheral CD4⁺CD25⁺ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J. Exp. Med.* **198**, 1875–1886 (2003).
- Shi, S. & Stanley, P. Protein O-fucosyltransferase 1 is an essential component of Notch signaling pathways. *Proc. Natl Acad. Sci. USA* **100**, 5234–5239 (2003).
- Charbonnier, L. M., Wang, S., Georgiev, P., Sefik, E. & Chatila, T. A. Control of peripheral tolerance by regulatory T cell–intrinsic Notch signaling. *Nat. Immunol.* **16**, 1162–1173 (2015).
- Han, H. et al. Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. *Int. Immunol.* **14**, 637–645 (2002).
- Tachdjian, R. et al. Pathogenicity of a disease-associated human IL-4 receptor allele in experimental asthma. *J. Exp. Med.* **206**, 2191–2204 (2009).
- Shi, H. et al. Hippo kinases Mst1 and Mst2 sense and amplify IL-2R-STAT5 signaling in regulatory T cells to establish stable regulatory activity. *Immunity* **49**, 899–914.e6 (2018).
- Geng, J. et al. The transcriptional coactivator TAZ regulates reciprocal differentiation of T_H17 cells and T_{reg} cells. *Nat. Immunol.* **18**, 800–812 (2017).
- van Loosdregt, J. et al. Canonical Wnt signaling negatively modulates regulatory T cell function. *Immunity* **39**, 298–310 (2013).
- van Loosdregt, J. & Coffey, P. J. The role of WNT signaling in mature T cells: T cell factor is coming home. *J. Immunol.* **201**, 2193–2200 (2018).
- Misra, J. R. & Irvine, K. D. The Hippo signaling network and its biological functions. *Annu. Rev. Genet.* **52**, 65–87 (2018).
- Clevers, H. & Nusse, R. Wnt/ β -catenin signaling and disease. *Cell* **149**, 1192–1205 (2012).
- Feng, Y. et al. Control of the inheritance of regulatory T cell identity by a cis element in the Foxp3 locus. *Cell* **158**, 749–763 (2014).
- Li, X., Liang, Y., LeBlanc, M., Benner, C. & Zheng, Y. Function of a Foxp3 cis-element in protecting regulatory T cell identity. *Cell* **158**, 734–748 (2014).
- Vivier, E. et al. Innate lymphoid cells: 10 years on. *Cell* **174**, 1054–1066 (2018).

25. Esty, B. et al. Treatment of severe persistent asthma with IL-6 receptor blockade. *J. Allergy Clin. Immunol. Pract.* **7**, 1639–1642.e4 (2019).
26. Hirota, T. et al. Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. *Nat. Genet.* **43**, 893–896 (2011).
27. Ferreira, M. A. et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet* **378**, 1006–1014 (2011).
28. Gruzieva, O. et al. Prenatal particulate air pollution and DNA methylation in newborns: an epigenome-wide meta-analysis. *Environ. Health Perspect.* **127**, 57012 (2019).
29. Li, X. et al. Genome-wide association study identifies T_H1 pathway genes associated with lung function in asthmatic patients. *J. Allergy Clin. Immunol.* **132**, 313–320.e15 (2013).
30. Savenije, O. E. et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J. Allergy Clin. Immunol.* **134**, 170–177 (2014).
31. Soroosh, P. et al. Lung-resident tissue macrophages generate Foxp3⁺ regulatory T cells and promote airway tolerance. *J. Exp. Med.* **210**, 775–788 (2013).
32. Magee, C. N. et al. Notch-1 inhibition promotes immune regulation in transplantation via regulatory T cell-dependent mechanisms. *Circulation* **140**, 846–863 (2019).

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Methods

Mice. The following mouse strains were obtained from the JAX Laboratories: *CD4^{Cre}* (B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ)³³, floxed *Cttnb1* (*Cttnb1^{fl/fl}*) (B6(Cg)-*Cttnb1^{tm1Kw}*/J)³⁴, *Foxp3^{YFP/Cre}* (B6.129(Cg)-*Foxp3^{tm4(YFP/cre)Ayr}*/J)³⁵, *Il6^{fl/fl}* (B6;SJL-*Il6^{tm1.1Drew}*/J)³⁶, *Notch1^{fl/fl}* (B6.129S1-*Notch1^{tm2Rko/Grd}*/J)³⁷, *Notch2^{fl/fl}* (B6.129S-*Notch2^{tm3Grid}*/J)³⁸, *Notch3^{-/-}* (B6.129S1-*Notch3^{tm1Grid}*/J)³⁹, OT-II (B6.Cg-Tg(T_{cr}^α-C_β)425Cbn/J)⁴⁰, *Stat3^{fl/fl}* (B6.129S1-*Stat3^{tm1Yxfl}*/J)⁴¹, *Wwtr1^{fl/fl}* (B6.129(Cg)-*Wwtr1^{tm1Hmc}*/J) and *Yap1^{fl/fl}* (B6.129P2(Cg)-*Yap1^{tm1.1Dupa}*/J)^{42,43}, and CD45.1 (B6.SJL-*Ptprc^b Pepc^b/Boy*) mice. *Notch4^{fl/fl}* (*Notch4^{tm1c(NCOM)Mfg}*) were obtained from the Canadian mutant mouse repository. *Pofut1^{fl/fl}* (B6.Cg-Pofut1^{tm2.1Pst}/J) and *Rbpj1^{fl/fl}* (B6.129P2-*Rbpj1^{tm1Hon}*/HonRbrC) mice were kind gifts of P. Stanley and T. Honjo, respectively^{12,14}.

Particles. UFPs (≤0.18 μm) were collected in an urban area of downtown Los Angeles, as previously reported⁷. The respective particles were suspended in an aqueous solution, with the hydrophilic components becoming part of the solution, whereas the solid non-soluble UFP cores are left in suspension. The entire mixture was administered intranasally, as detailed under the “Allergic sensitization and challenge section”.

T cell co-cultures with lung alveolar macrophages. Naive CD4⁺OTII⁺ T cells were isolated from spleens of *OTII⁺Foxp3^{YFP/Cre}* mice by FACS. AMs were isolated by flow cytometry as shown in Xia et al.⁸, and were aliquoted at 2 × 10⁴ cells in 48-well plates, then either sham treated, or treated overnight with OVA_{323–339} or with OVA_{323–339} and UFPs at 10 μg ml⁻¹. The treated AMs were washed twice with PBS to remove residual UFPs, and the OTII⁺ naive CD4⁺ T cells were then added at 4 × 10⁵ cells per well in a final volume of 0.5 ml 10% fetal calf serum (FCS)/RPMI culture medium. Recombinant IL-1β, IL-25, TSLP and TNF-α (Peprotech) were added at a concentration of 10 μg ml⁻¹. Recombinant IL-6 and IL-33 (Peprotech) were titrated from 10 μg ml⁻¹ to 1 μg ml⁻¹. Rat anti-mouse IL-6 monoclonal antibody (Bioxcell, clone: MP5-20F3) was added at a concentration of 10 μg ml⁻¹.

In vitro iT_{reg} cell differentiation. Sorted naive CD4⁺CD62L⁺*Foxp3^{YFP/Cre}* T cells (1 × 10⁶ ml⁻¹) were cultured with plate-bound anti-CD28 (5 μg ml⁻¹, BioLegend), anti-CD3 (clone: 145-2C11, 5 μg ml⁻¹, BioLegend), recombinant TGF-β₁ (5 ng ml⁻¹, R&D Systems), with or without IL-4 (10 ng ml⁻¹; Peprotech), anti-IL-4 (BioLegend, clone: 11B11, 10 μg ml⁻¹) or anti-IL-6 monoclonal antibody (BioLegend, MP5-20F3, 10 μg ml⁻¹), mitogen-activated protein kinase (MEK) inhibitor PD98059 (50 μM, Sigma-Aldrich) or P38 inhibitor IV (10 μM, Sigma-Aldrich). After 4 d, the induced T_{reg} cells were analyzed by flow cytometry for Foxp3 expression and intracellular cytokines production and re-sorted on the basis of yellow fluorescent protein (YFP) expression.

In vitro suppression assays. Total CD4⁺ T cells were isolated using a CD4⁺ isolation kit (Miltenyi Biotec) followed by cell sorting on FACSARIA. Isolated T_{eff} cells were labeled with CellTrace Violet Cell Proliferation dye according to the manufacturer's instructions (Life Technologies) and were used as responder cells. T_{reg} cells were isolated on a FACSARIA on the basis of CD4, YFP and/or Notch4 expression, and were used as suppressor cells. Responder cells were co-cultured with T_{reg} cells, at the indicated ratios, and stimulated for 3 d with 2 μg ml⁻¹ of coated anti-CD3 and 5 μg ml⁻¹ of soluble anti-CD28 in 96-well, round-bottomed plates in triplicate. The responder cells (YFP⁺) were then analyzed for CellTrace dye dilution by flow cytometry. For our human suppression assay studies, T_{reg} cells were isolated on the basis of CD4⁺CD25⁺CD127⁻ (T_{reg}) whereas T_{eff} cells were isolated as CD4⁺CD25⁺CD127⁺ (T_{eff}) from either healthy controls or subjects with severe asthma. T_{eff} cells were then stained with CellTrace Violet Cell Proliferation dye (Invitrogen) and plated at a concentration of 10⁴ cells in a U-bottomed, 96-well plate with 0.1 μl of prewashed T cell activation and expansion beads (Thermo Fisher Scientific) in 100 μl of complete medium. Different numbers of T_{reg} cells are added to the culture in 100 μl of complete medium to have 1:1, 1:2, 1:4 and 1:8 ratios (respectively 10⁴, 5 × 10³, 2.5 × 10³ and 1.25 × 10³ cells) or 100 μl of complete medium for wells without T_{reg} cells. The cultures will be incubated at 37 °C in 5% CO₂ for 4 d. On the fourth day, the cells will be stained again for CD4, CD127 and CD25, and the dilution of the proliferation dye evaluated by gating on CD4⁺CD127⁺Violet Tracer⁺ cells.

In vitro ILC2 suppression assay. ILC cells were isolated from lungs of OVA + UFP-sensitized, -challenged and -treated mice. ILC2s were isolated as Lineage (Lin)⁻ (CD3, CD4, CD11c, CD11b, CD19, Siglec-F, F4/80)⁻ Thy1.1⁺ Sca-1⁺ T1/ST2⁺. IL-13 percentages were checked at the start of the experiment. ILC2s were then co-cultured with 10³ cells per well. T_{reg} cells at these different concentrations, 1:5, 1:10 or 1:20, from *Foxp3^{YFP/Cre}* mice either sham treated or treated with OVA + UFPs with high Notch4 expression, with or without the addition of anti-Notch4 monoclonal antibody (Bioxcell, clone: HMN4-14) at a concentration of 10 ng ml⁻¹, and T_{reg} cells from *Foxp3^{YFP/Cre}Notch4^{Δ/Δ}*, *Foxp3^{YFP/Cre}Cttnb1^{Δ/Δ}* or *Foxp3^{YFP/Cre}Yap1^{Δ/Δ}Wwtr1^{Δ/Δ}* mice treated with OVA + UFPs. After 48 h, IL-13 expression of ILC2s was measured by flow cytometric analysis.

In vitro ILC2–T_{reg} cell co-cultures. ILC2s were isolated from the lungs of naïve *Foxp3^{YFP/Cre}* mice. These cells were incubated either with IL-33 (10 μg ml⁻¹),

Notch4^{high} T_{reg} cells isolated from the lungs of OVA + UFP-treated mice or with both along with or without GDF15-blocking peptide (Mybiosource, cat. no. MBS9229417, 10 μg ml⁻¹)⁴⁴. The peptide was separately validated to inhibit the in vitro activation of ILC2s by *E. coli*-derived recombinant GDF15 (R&D Systems, cat. no. 8944-GD) as measured by the inhibition of GDF15-induced IL-13 expression (data not shown). Seventy-two hours later, the expression of IL-13 in ILC2s was measured by flow cytometric analysis.

In vitro ILC2–GDF15 co-cultures. ILC2s were isolated from the lungs of naïve *Foxp3^{YFP/Cre}* mice. These cells were incubated with either IL-33 (10 ng ml⁻¹), *E. coli*-derived recombinant GDF15 (R&D Systems, cat. no. 8944-GD) (10 μg ml⁻¹) or both. Seventy-two hours later, the expression of IL-13 in ILC2s was measured by flow cytometry.

Isolation of human PBMCs. Human PBMCs were isolated from full blood of healthy controls, or probands with mild, moderate or severe asthma, via density gradient using Ficoll (GE Healthcare). PBMCs were then stored frozen in FCS (Sigma-Aldrich) and 15% dimethylsulfoxide (Sigma-Aldrich). The cells were later thawed for analysis of their Notch, Yap1 and β-catenin expression by flow cytometry.

Isolation, culture and suppression assay of human peripheral ILC2s. Human ILC2s were isolated using the human ILC2 isolation kit (Miltenyi Biotec). The cells were then stained for CD294 and Lineage and sorted out as CD294⁺Lineage⁻. The ILC2s were then cultured with IL-33 (10 μg ml⁻¹, Peprotech) and IL-25 (2 μg ml⁻¹, Peprotech) for 5 d. Moreover, T_{reg} cells were isolated from either subjects with asthma or healthy controls by sorting them out as CD4⁺CD25⁺CD127⁻ cells. The T_{reg} cells were cultured with the ILC2s in a 1:5 or 1:10 ratio with and without the addition of GDF15-blocking peptide. After 48 h, the production of IL-13 by the ILCs was assessed by flow cytometric analysis.

GDF15 ELISA. Ethylenediaminetetraacetic acid plasma from 88 probands (control subjects, and patients with mild, moderate and severe persistent asthma) were used to measure GDF15 with ELISA (R&D Systems) according to the manufacturer's protocol.

Allergic sensitization and challenge. Mice were sensitized to OVA by intraperitoneal (i.p.) injection of 100 μg of OVA in 100 μl of PBS, then boosted 2 weeks later with a second i.p. injection of OVA in PBS. Control mice were sham sensitized and boosted with PBS alone. Starting on day 29, both OVA- and sham-sensitized mice were challenged with aerosolized OVA at 1%, for 30 min daily for 3 d. Then 2 h before each OVA aerosol exposure, subgroups of mice were given either intranasal (i.n.) PBS or i.n. UFP at 10 μg per 100 μl of PBS per instillation. Mice were euthanized on day 32 post-sensitization and analyzed. For dust-mite-induced allergic airway inflammation, mice received 5 μg of lyophilized *Dermatophagoides pteronyssinus* extract (Greer) in 100 μl of i.n. PBS for 3 d at the start of the protocol, and were then challenged with the same dose of *D. pteronyssinus* extract on days 15–17 with or without UFPs at the same concentration as before. Mice were euthanized on day 18 and analyzed for measures of airway inflammation. Bronchoalveolar lavage fluid and lung tissues were obtained and analyzed for cellular components and T cell cytokine expression as described⁶. For the chronic model, mice were sensitized to OVA by three i.p. injections of 10 mg OVA (Sigma) adsorbed to 1.5 mg of Al(OH)₃ (Pierce, Rockford) diluted in 200 ml of PBS on days 1, 14 and 21. The mice were challenged with OVA aerosol (1% w/v in PBS) via the airways twice a week on 2 consecutive days over a period of 12 weeks as previously described¹⁵. Sham sensitization and challenges were carried out with sterile Al(OH)₃ in PBS. Animals were analyzed after 12 weeks of OVA aerosol challenge.

Measurement of airway functional responses. AHR was measured, as previously described⁸. Anesthetized mice were exposed to doubling concentrations of aerosolized acetyl-β-methacholine (Sigma-Aldrich) by using a Buxco small-animal ventilator (Data Sciences International). The relative peak airway resistance for each methacholine dose, normalized to the saline baseline, was calculated.

Lung histopathology staining. Paraffin-embedded lung sections were stained with hematoxylin and eosin (H&E) or periodic acid–Schiff (PAS). The lung pathology was scored by blinded operators. Inflammation was scored separately for cellular infiltration around blood vessels and airways: 0, no infiltrates; 1, a few inflammatory cells; 2, a ring of inflammatory cells one-cell-layer deep; 3, a ring of inflammatory cells two- to four-cells deep; and 4, a ring of inflammatory cells more than four-cells deep¹⁵. A composite score was determined by the adding the inflammatory scores for both vessels and airways.

PCR. B cells, T_{reg} and/or T_{eff} cells were sorted using ARIA II sorter (BD Biosciences). Messenger RNA isolation was conducted according to manufacturer's protocol (QIAGEN). Complementary DNA and quantitative PCR (qPCR) were performed using an RT–PCR cDNA conversion kit (QIAGEN) and probes for Notch1, Notch2, Notch3, Notch4 and GDF15 from applied biosystems (Thermo Fisher Scientific). The mRNA expression was normalized to Notch1 expression in T_{eff} cells.

Flow cytometric analysis of mouse and human cells. Antibodies against the following murine antigens were used for flow cytometric analyses: IL-4 (BioLegend, clone 11B11, catalog no. 504104 1:300 dilution), Siglec-F (BD Pharmingen, E50-2440, catalog no. 552126, 1:300), Foxp3 (eBioscience, FJK-16S, catalog no. 48-5773-82 1:300), interferon- γ (BioLegend, XMG1.2, catalog no. 505806, 1:300), IL-13 (eBioscience, eBio13a, catalog no. 47-7133-82, 1:300), Helios (eBioscience, 22F6, catalog no. 47-9883-42, 1:200), CD11c (BioLegend, N418, catalog no. 117318 1:500), CD11b (eBioscience, M1/70, catalog no. 101222 1:500), CD4 (BioLegend, GK1.5, catalog no. 100451, 1:500), CD3 (BioLegend, 17A2, catalog no. 100203, 1:500), IL-17 (BioLegend, TC11-18H10.1, catalog no. 506922, 1:200), GR-1 (BioLegend, RB6-8C5, catalog no. 108406, 1:500), CD45 (BioLegend, 30-F11, catalog no. 103140, 1:300), Notch1 (BioLegend, HMN1-12, catalog no. 130615, 1:500), Notch2 (BioLegend, HMN2-35, catalog no. 130714, 1:500), Notch3 (BioLegend, HMN3-133, catalog no. 130512, 1:300) and Notch4 (BioLegend, HMN4-14, catalog no. 128407, 1:200). Polyclonal rabbit anti-GDF15 (Assaypro, catalog no. 32572-05171, 1:200), anti-CD16/CD32 (BioLegend, clone: 93, catalog no. 101319, 1:1,000), Alexa-Fluor-647 goat anti-rabbit IgG antibody (Thermo Fisher Scientific, clone PA5-39741, catalog no. A32733, 1:1,000), p-Mob1 antibody (CST, T35; clone D2F10, catalog no. 86995, 1:300), rabbit anti-mouse p-Lats1 antibody (CST, T35; clone D57D3, 1:300), rabbit anti-mouse p-Lats1/2 antibody (Thermo Fisher Scientific, S909/872; clone PA5-39741, catalog no. PA5-105895, 1:300), Rat anti-mouse IL-6 monoclonal antibody (Bioxcell, clone: MP5-20F3, catalog no. BE0046, 1:500), anti-CD28 (BioLegend, clone: 37.51, catalog no. 122004, 1:1,000) and anti-IL-4 (Peprotech, catalog no. 500-P54, 1:1,000). Antibodies against the following human antigens were used: CD3 (BioLegend, HIT3a, catalog no. 300318, 1:300), CD4 (BioLegend, RPA-T4, catalog no. 300530, 1:300), Foxp3 (eBioscience, PCH-101, catalog no. 48-4776-42, 1:200), Helios (eBioscience, 22F6, catalog no. 47-9883-42, 1:200), Notch1 (BD Pharmingen, HMN1-519, catalog no. 352108, 1:500), Notch2 (BD Pharmingen, HMN2-25, catalog no. 742291, 1:300), Notch3 (BD Pharmingen, HMN3-21, catalog no. 744828, 1:300), Notch4 (BD Pharmingen, HMN4-2, catalog no. 563269, 1:300), Yap1 (CST, 147295, catalog no. 14729S, 1:200) and β -catenin (R&D Systems, 196624, catalog no. IC13292V, 1:500). The specificity and optimal dilution of each antibody were validated by testing on appropriate negative and positive controls or otherwise provided on the manufacturer's website. Intracellular cytokine staining was performed as previously described¹³. Furthermore, for YFP/GFP/cytokine staining, a different protocol was used. Cells were resuspended in approximately 100 μ l of 4% formaldehyde per 1 million cells for 15 min at room temperature (20–25 °C). After centrifugation, the pellet was resuspended with 100 μ l of Triton-X for each 1 million cells. The mix was incubated at room temperature for 10 min. Cytokines were stained overnight as previously prescribed¹³. Dead cells were routinely excluded from the analysis based on the staining of eFluor-780 Fixable Viability Dye (1:1,000 dilution; eBioscience). Stained cells were analyzed on a BD LSR Fortessa cell analyzer (BD Biosciences) and data were processed using FlowJo (Tree Star Inc.).

ChIP. For ChIP analysis, two systems were used. For STAT3 ChIP, iT_{reg} cells were used after a naive T cell differentiation protocol. These cells were stimulated with or without IL-6 at a concentration of 10 μ g ml⁻¹. Cells were then directly crosslinked with 10% paraformaldehyde for 8 min at room temperature. Chromatin was then treated with lysis buffers I and II. ChIP protocol was conducted as described⁴⁶. Then qPCR was conducted to measure enrichment percentage to input controls at the *Notch1* promoter region: forward primer 5'-CACAAAGGGGTAAGGGTTC-3', reverse primer 5'-GAGGCACTAGTGAGGCTCTGA-3'; *Notch2* forward primer 5'-TTTCAAGCTCCTGCTGTCTCT-3', reverse primer 5'-CTCTGGGCATTTC GTTCATT-3'; *Notch3* forward primer 5'-AGGCTTGGCGGGTAGAAG-3', reverse primer 5'-TCCCTCCTCCCTCTTTC-3'; and *Notch4* forward primer 5'-GCTC ACAACCATCCGTAACA-3', reverse primer 5'-ACTGAAACCGGTCACTTT GG-3' promoter regions.

DNA methylation. The methylation status of the Foxp3 T_{reg} cell-specific demethylation region conserved noncoding sequence 2 (CNS2) of Foxp3^{YFPcre}, Foxp3^{YFPcre}Notch4^{Δ/Δ}, Foxp3^{YFPcre}Cttnb1^{Δ/Δ} and Foxp3^{YFPcre}Yap1^{Δ/Δ}Wwtr1^{Δ/Δ} lung T_{reg} cells of OVA + UFP mice were assessed. DNA extraction, bisulfite conversion, pyrosequencing and data analysis were done by EpigenDx as published previously^{46,47}. Then the 12 cytosine guanine dinucleotides (CpGs) of the mouse T_{reg} cell-specific demethylation region (CNS2 of FOXP3) were analyzed.

Transcriptome profiling. T_{reg} cells were isolated from either Foxp3^{YFPcre} or Foxp3^{YFPcre}Notch4^{Δ/Δ} mice that were sensitized with OVA and challenged with OVA + UFPs. The mRNA was isolated using the QIAGEN RNeasy mini kit. RNA was then converted into double-stranded DNA (dsDNA), using SMART-Seq v.4 Ultra Low Input RNA kit (Clontech). DsDNA was then fragmented to size 200–300 bp, using M220 Focused-ultrasonicator (Covaris), and utilized for construction of libraries for Illumina sequencing using KAPA Hyper Prep Kit (Kapa Biosystems). Libraries were then quantified using Qubit dsDNA HS (High Sensitivity) Assay Kit on Agilent High Sensitivity DNA Bioanalyzer.

Gene-level read counts were quantified using feature Counts and the latest UCSC mouse annotation (GRCm38/mm10). To identify differentially expressed genes, we used edgeR (v.3.28) and DESeq2 (v.1.26.0) bioconductor packages with default parameters. Count tables were normalized to transcripts per million for visualizations and quality control. Sample clustering and path analyses were performed using a customized pipeline available on request. Transcripts were called as differentially expressed when the adjusted *P* values were <0.05, fold-changes >±1.5 and false discovery rate (FDR) <0.1. Raw sequencing files will be accessible in the National Center for Biotechnology Information repository in the near future.

Statistical analysis. The two-tailed Student's *t*-test, one-way analysis of variance (ANOVA) and two-way ANOVA, and repeat measures two-way ANOVA with Sidak's post-test analysis of groups were used to compare test groups, as indicated. A *P* value <0.05 was considered statistically significant.

Study approval. Recruitment of human subjects was approved by the Institutional Review Board at Boston Children's Hospital and Marmara University. All animal studies were reviewed and approved by the Boston Children's Hospital office of Animal Care Resources.

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

Data availability

The data presented in the manuscript, including de-identified patient results, will be made available to investigators after a request to the corresponding author. Any data and materials to be shared will be released via a material transfer agreement. RNA-seq datasets have been deposited in the Gene Expression Omnibus with the accession no. [GSE151763](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE151763). Source data are provided with this paper.

References

- Lee, P. P. et al. A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. *Immunity* **15**, 763–774 (2001).
- Messerschmidt, D. et al. β -Catenin-mediated adhesion is required for successful preimplantation mouse embryo development. *Development* **143**, 1993–1999 (2016).
- Rubtsov, Y. P. et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* **28**, 546–558 (2008).
- McFarland-Mancini, M. M. et al. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. *J. Immunol.* **184**, 7219–7228 (2010).
- Yang, X. et al. Notch activation induces apoptosis in neural progenitor cells through a p53-dependent pathway. *Dev. Biol.* **269**, 81–94 (2004).
- McCright, B., Lozier, J. & Gridley, T. Generation of new Notch2 mutant alleles. *Genesis* **44**, 29–33 (2006).
- Krebs, L. T. et al. Characterization of Notch3-deficient mice: normal embryonic development and absence of genetic interactions with a Notch1 mutation. *Genesis* **37**, 139–143 (2003).
- Barnden, M. J., Allison, J., Heath, W. R. & Carbone, F. R. Defective TCR expression in transgenic mice constructed using cDNA-based α - and β -chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* **76**, 34–40 (1998).
- Moh, A. et al. Role of STAT3 in liver regeneration: survival, DNA synthesis, inflammatory reaction and liver mass recovery. *Lab. Invest.* **87**, 1018–1028 (2007).
- Zhang, N. et al. The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. *Dev. Cell* **19**, 27–38 (2010).
- Reginensi, A. et al. Yap- and Cdc42-dependent nephrogenesis and morphogenesis during mouse kidney development. *PLoS Genet.* **9**, e1003380 (2013).
- Binder, A. K. et al. Expression of human NSAID activated gene 1 in mice leads to altered mammary gland differentiation and impaired lactation. *PLoS ONE* **11**, e0146518 (2016).
- Charbonnier, L. M. et al. Functional reprogramming of regulatory T cells in the absence of Foxp3. *Nat. Immunol.* **20**, 1208–1219 (2019).
- Zheng, Y. et al. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature* **463**, 808–812 (2010).
- Povolieri, G. A. M. et al. Human retinoic acid-regulated CD161⁺ regulatory T cells support wound repair in intestinal mucosa. *Nat. Immunol.* **19**, 1403–1414 (2018).

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Author contributions

H.H. and T.A.C. designed experiments. H.H., E.S.-V, M.B., A.M., Y.C, L.-M.C. and S.A. performed experiments and developed experimental models. E.C., S.B., A.C. and W.P. recruited patients and analyzed their demographics. K.S.A. and B.G. analyzed the RNA-seq data. J.M.L.C. and R.S.G. provided *Rora*^{Cre} and *Rora*^{Cre}*Il4^{Δ/Δ}Il13^{Δ/Δ}* mice. C.S. and A.J.M provided UFPs. H.H. and T.A.C. wrote the manuscript.

Competing interests

T.A.C., H.H. and A.M. are inventors on published US patent application no. WO2019178488A1 submitted by the Children's Medical Center Corporation, titled 'Method for treating asthma or allergic disease'. B.G. is a director of Pacific Analytics and SMRTR, Australia; a founding member of the International Cerebral Palsy Genetics

Consortium; and a member of the Australian Genomics Health Alliance. W.P. is a Consultant for Genentech, Novartis, Regeneron, Sanofi Genzyme and Glaxo Smith Kline, and receives clinical trial support from Genentech, Novartis, Regeneron, Circassia, Thermo Fisher, Monaghan, Lincoln Diagnostics, Alk Abello and Glaxo Smith Kline.

Additional information

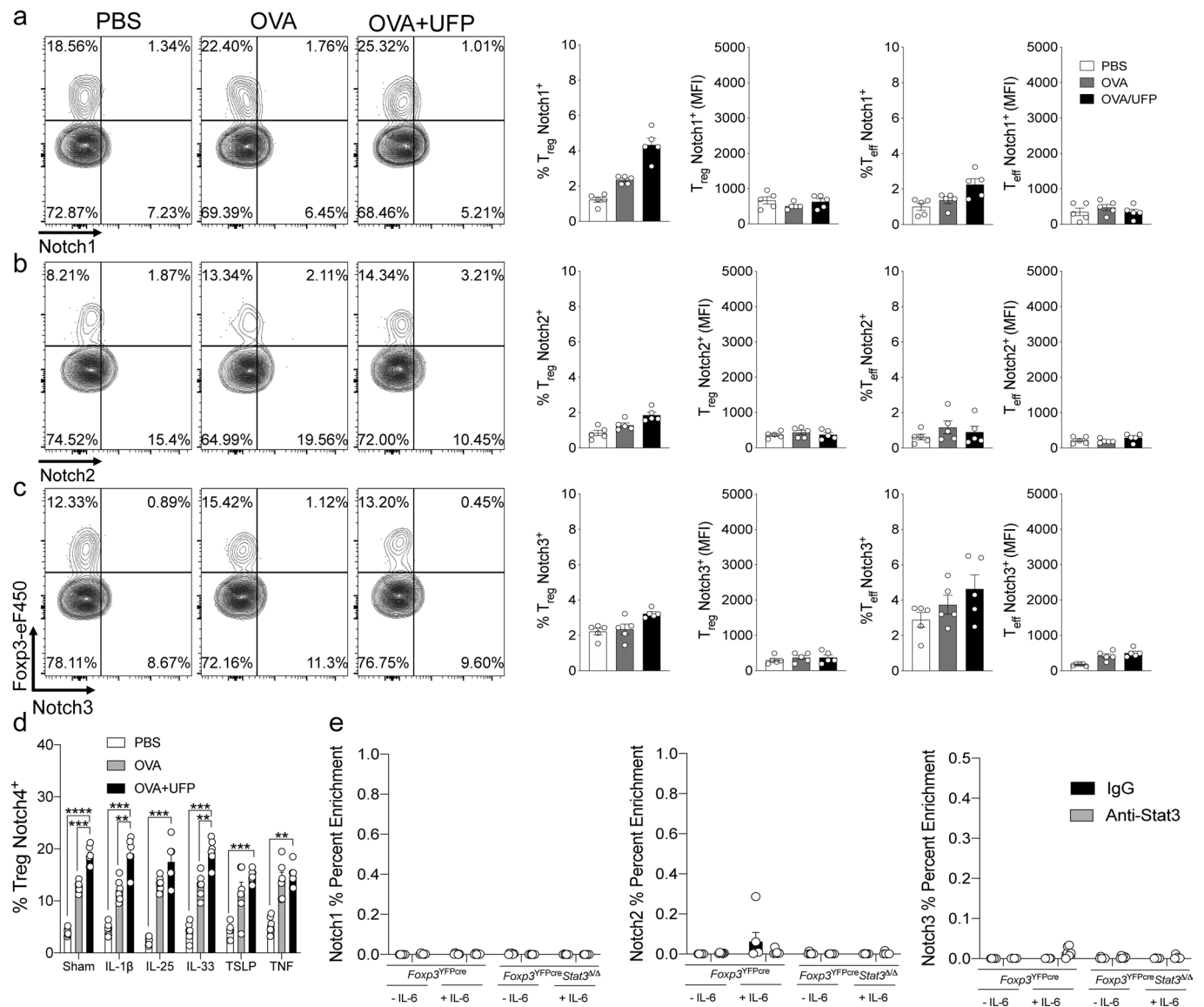
Extended data is available for this paper at <https://doi.org/10.1038/s41590-020-0777-3>.

Supplementary information is available for this paper at <https://doi.org/10.1038/s41590-020-0777-3>.

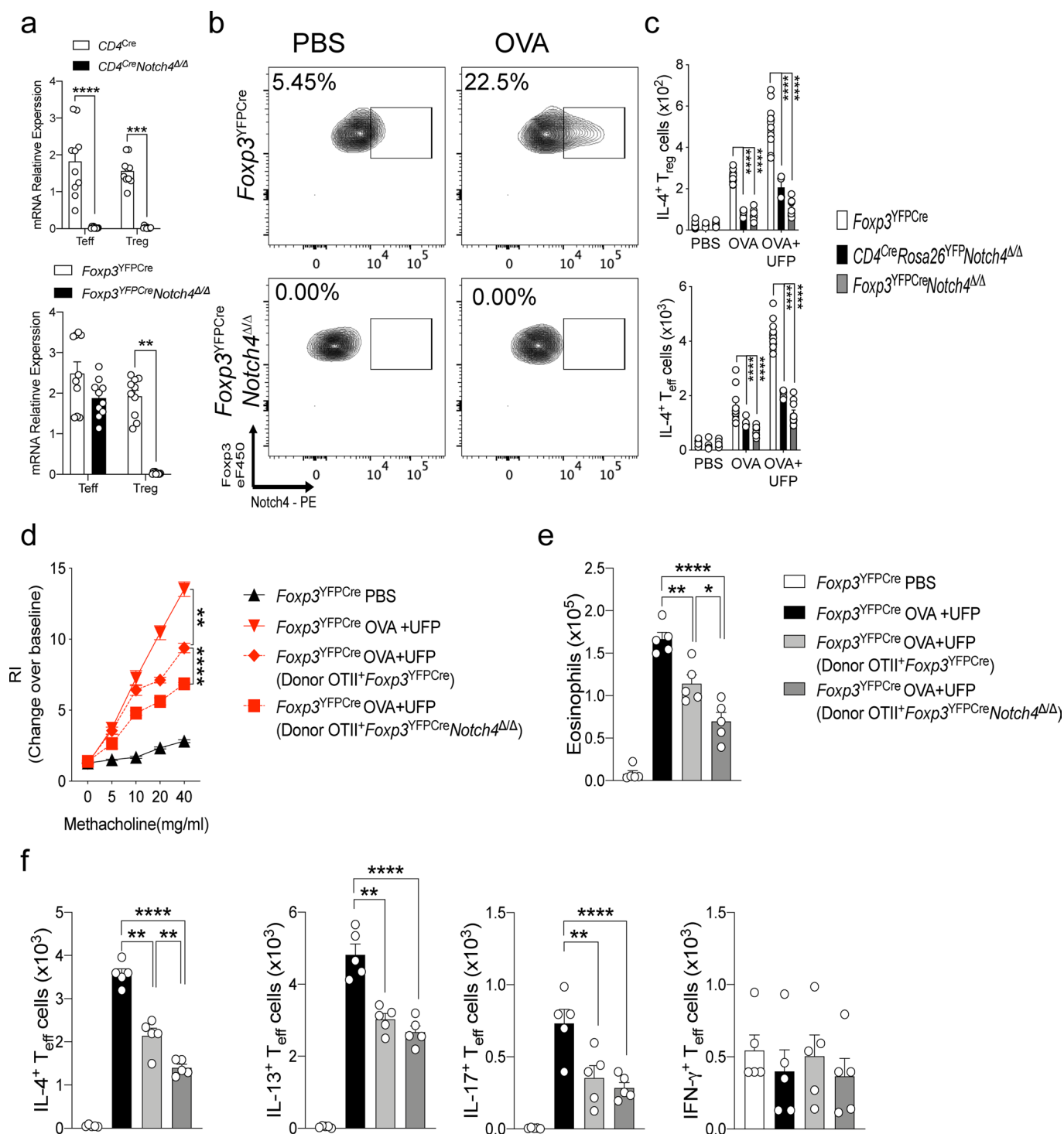
Correspondence and requests for materials should be addressed to T.A.C.

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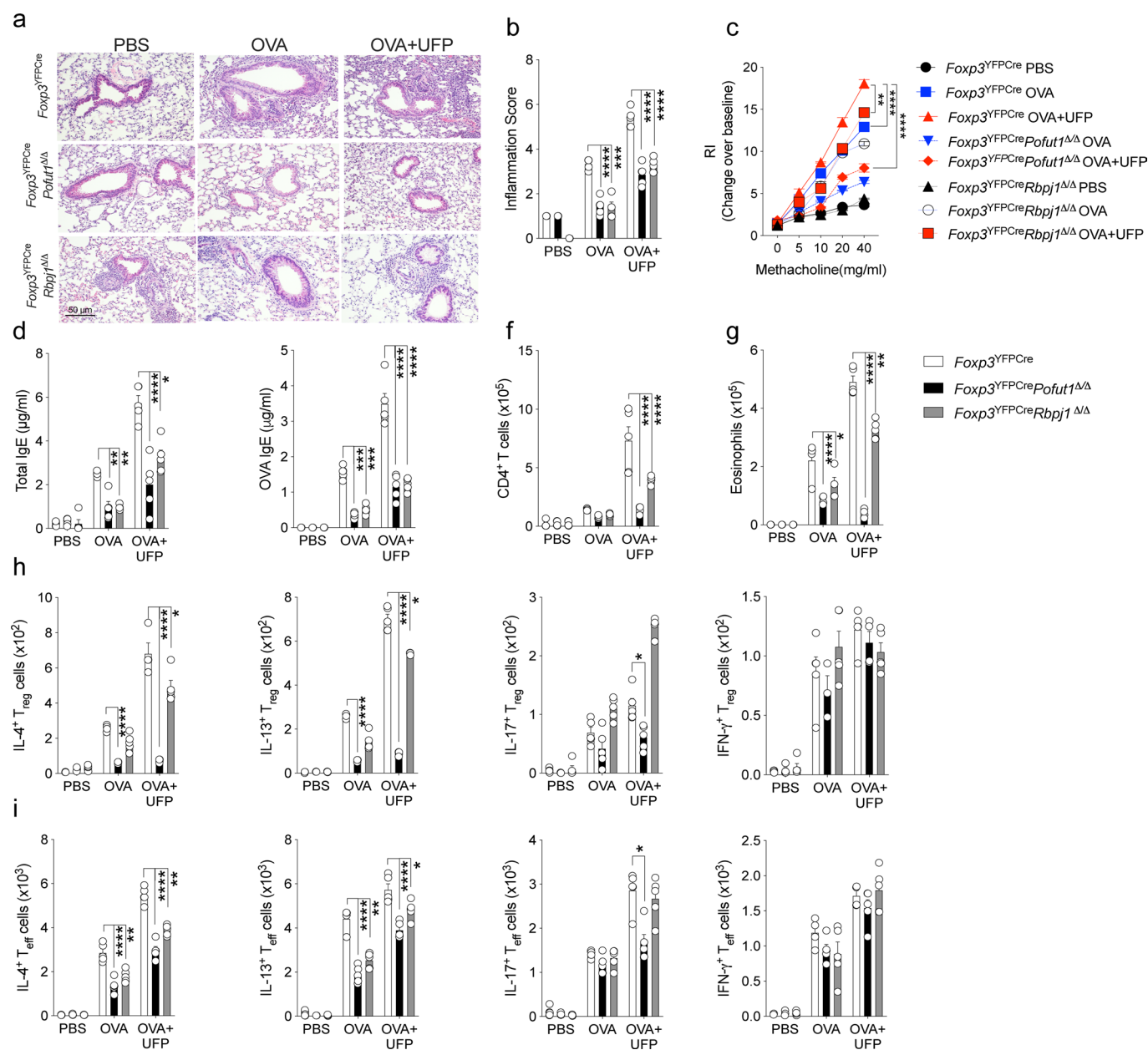
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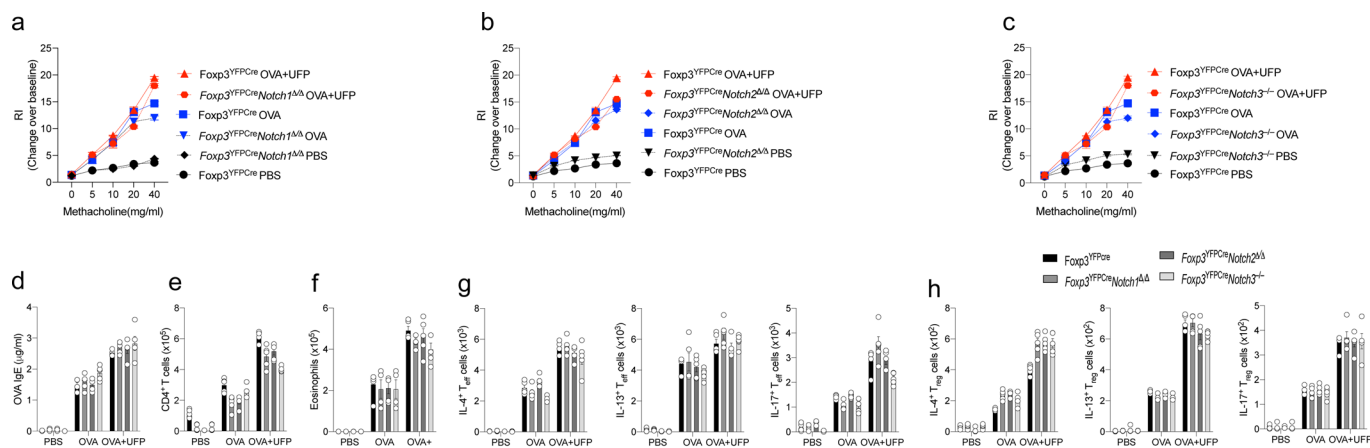
Extended Data Fig. 1 | Notch4 expression on lung T_{reg} cells in allergic airway inflammation. **a–c**, Flow cytometric analysis, cell frequencies and (MFI) of Notch1, 2 and 3 expression on lung T_{reg} and T_{eff} cells in $Foxp3^{YFP/Cre}$ ($n=5$). **d**, Cell frequencies of Notch4 expression on OT-II⁺CD4⁺Foxp3⁺ T cells generated in co-cultures with sham or OVA_{323–339} + UFP-pulsed alveolar macrophages without or with IL-1 β , IL-25, IL-33, TSLP or TNF ($n=5$). **e**, ChIP assays for the binding of STAT3 and control (IgG) antibodies to the *Notch1*, 2 and 3 promoters in lung T_{reg} cells of OVA + UFP-treated $Foxp3^{YFP/Cre}$, and $Foxp3^{YFP/Cre}Stat3^{\Delta/\Delta}$ mice ($n=5$). Each symbol represents one mouse. Numbers in flow plots indicate percentages. Error bars indicate SEM. Statistical tests: One-way ANOVA with Dunnett's post hoc analysis (**a–c**); two-way ANOVA with Sidak's post hoc analysis (**d,e**). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data representative of two or three independent experiments.



Extended Data Fig. 2 | Notch4 expression on lung T_{reg} cells licenses allergic airway inflammation. **a**, RT-PCR analysis of Notch4 expression in $CD4^{Cre}$ mice in B-cells and T-cells ($n=5$). **b**, RT-PCR analysis of Notch4 expression in $Foxp3^{YFP/Cre}$ mice in both T_{reg} and T_{eff} cells ($n=5$). **c,d**, IL-4 and IFN- γ expression in lung $Foxp3^{YFP/Cre}CD4^{+}T_{reg}$ cells (**c**) and $Foxp3^{YFP/Cre}CD4^{+}T_{eff}$ cells (**d**) derived from the respectively treated $Foxp3^{YFP/Cre}$, $CD4^{Cre}Notch4^{\Delta/\Delta}$ and $Foxp3^{YFP/Cre}Notch4^{\Delta/\Delta}$ mice ($n=5$). **e**, Airway hyperresponsiveness in $Foxp3^{YFP/Cre}$ sensitized either with PBS or OVA, then challenged with OVA + UFP following transfer of OTII $^{+}Foxp3^{YFP/Cre}$ or OTII $^{+}Foxp3^{YFP/Cre}Notch4^{\Delta/\Delta}$ T_{reg} cells ($n=5$). **f**, Eosinophil numbers for the respective mouse groups ($n=5$). **g**, IL-4, IL-13, IL-17 and IFN γ expression in lung $Foxp3^{YFP/Cre}CD4^{+}T_{eff}$ cells. Each symbol represents one mouse ($n=5$). Error bars indicate SEM. Statistical tests: two-way ANOVA with Sidak's post hoc analysis (**a,c,d**); One-way ANOVA with Dunnett's post hoc analysis (**e,f**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. Data representative of two or three independent experiments.

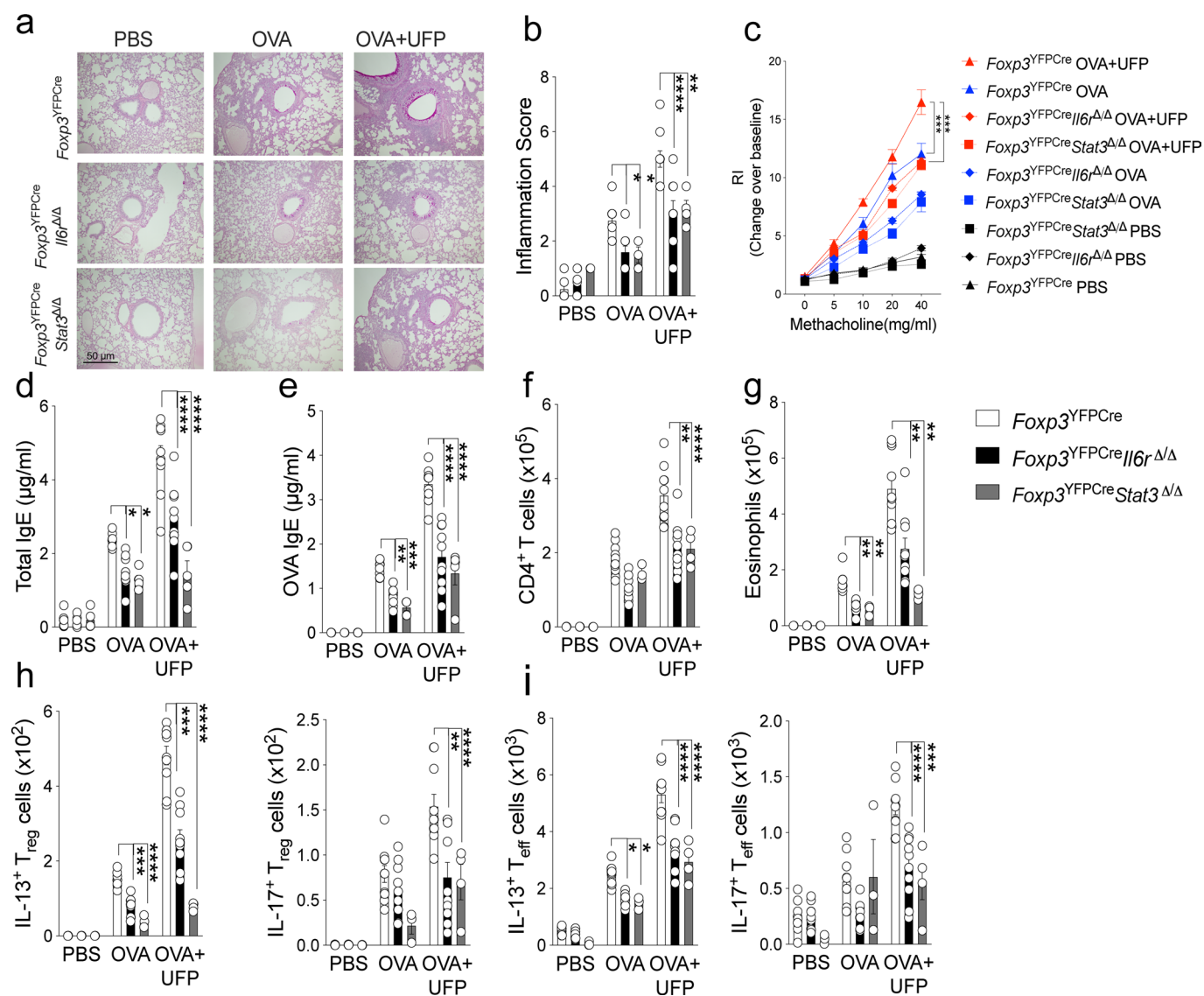


Extended Data Fig. 3 | Allergic airway inflammatory responses in mice with T_{reg} cell-specific *Pofut1* or *Rbpj1* deletion. **a**, Representative PAS-stained sections of lung tissues isolated from *Foxp3^{YFPCre}*, *Foxp3^{YFPCre}Pofut1^{Δ/Δ}* or *Foxp3^{YFPCre}Rbpj1^{Δ/Δ}* mice segregated into PBS, OVA or OVA + UFP-treated groups (200X magnification). **b**, Inflammation scores in the respective lung tissues. **c**, AHR in the respective mouse groups in response to methacholine. **d,e**, serum total and OVA-specific IgE concentrations. **f,g**, absolute numbers of lung CD4⁺ T cells and eosinophils. **h,i**, IL-4, IL-13, IL-17 and IFNγ expression in lung Foxp3⁺CD4⁺ T_{reg} (**h**) and Foxp3⁺CD4⁺ T_{eff} cells (**i**). Each symbol represents an independent sample. Error bars indicate SEM. Statistical tests: two-way ANOVA with Sidak's post hoc analysis (**b-i**). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data representative of two or three independent experiments. n = 5 mice per group.

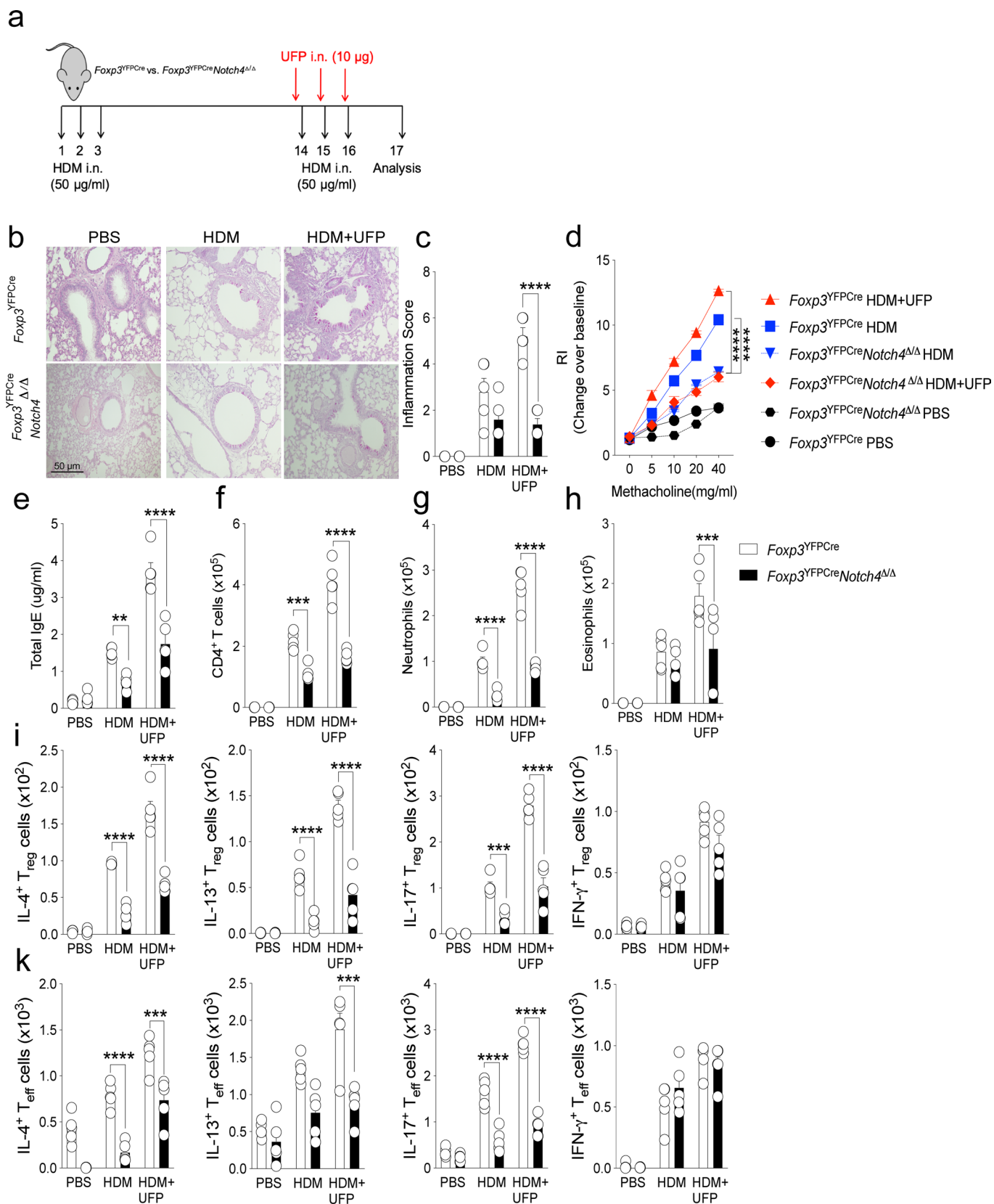


Extended Data Fig. 4 | Allergic airway inflammatory responses in mice with T_{reg} cell-specific *Notch1* or *Notch2* deletion or global *Notch3* deletion.

a–c, AHR in *Foxp3^{YFPCre}*, *Foxp3^{YFPCre}Notch1^{Δ/Δ}*, *Foxp3^{YFPCre}Notch2^{Δ/Δ}*, or *Foxp3^{YFPCre}Notch3^{−/−}* mice segregated into PBS, OVA or OVA + UFP-treated groups (200X magnification). **d**, serum OVA-specific IgE concentrations. **e,f**, absolute numbers of lung CD4⁺ T cells and eosinophils. **g,h**, IL-4, IL-13, and IL-17 expression in lung Foxp3⁺CD4⁺T_{eff} (**g**) and Foxp3⁺CD4⁺T_{reg} cells (**h**). Each symbol represents an independent sample. Error bars indicate SEM. Statistical tests: two-way ANOVA with Sidak's post hoc analysis **a–h**. Data representative of two or three independent experiments. n = 5 mice per group.

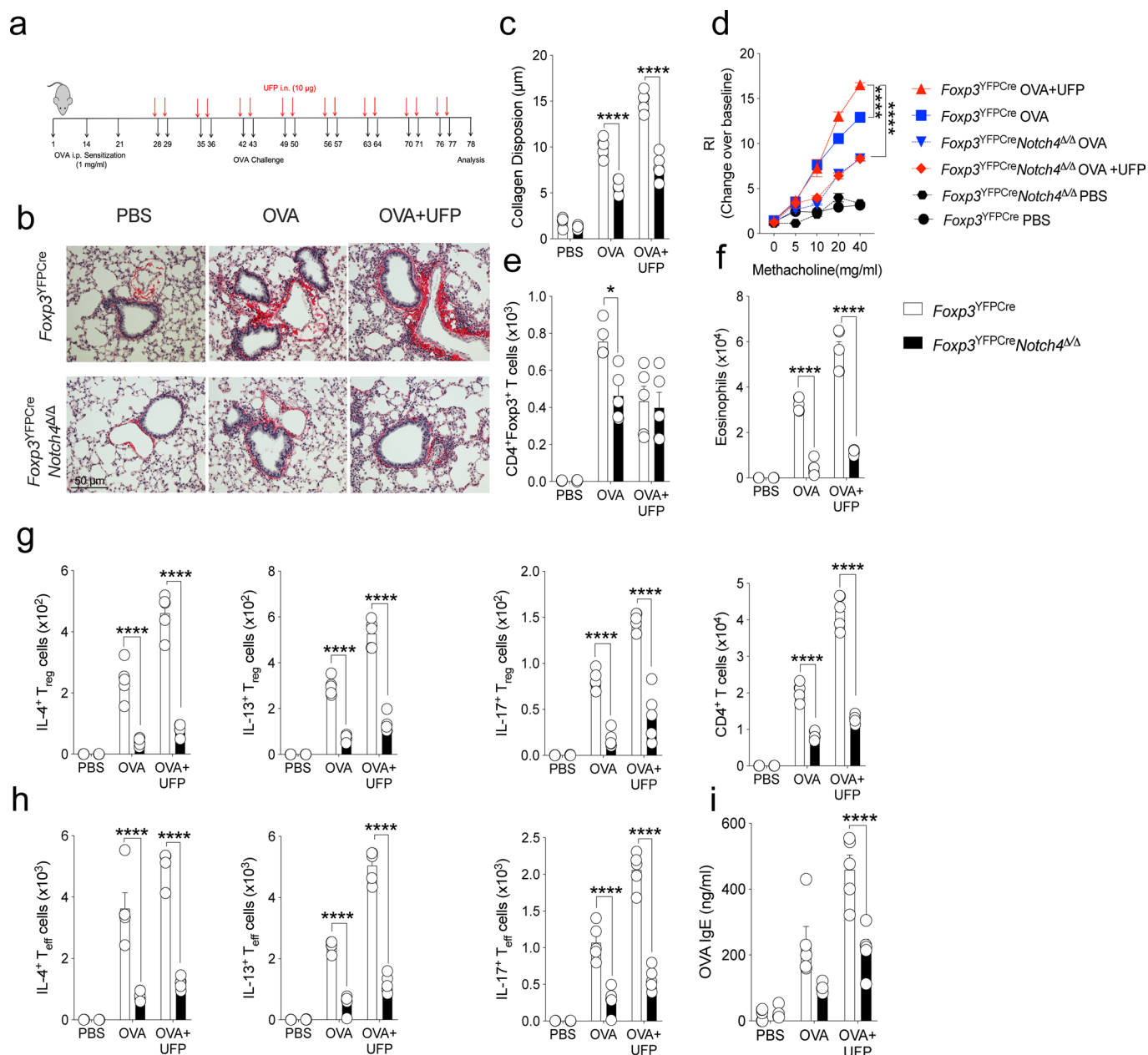


Extended Data Fig. 5 | T_{reg} cell-specific *Il6r* and *stat3* deletions attenuate allergic airway inflammation. **a**, Representative PAS-stained sections of lung tissues isolated from *Foxp3^{YFP/Cre}*, *Foxp3^{YFP/Cre}Il6^{Δ/Δ}* or *Foxp3^{YFP/Cre}Stat3^{Δ/Δ}* mice segregated into PBS, OVA or OVA + UFP-treated groups (200X magnification). **b**, Inflammation scores in the respective lung tissues. **c**, AHR in the respective mouse groups in response to methacholine. **d, e**, serum total and OVA-specific IgE concentrations. **f, g**, absolute numbers of lung CD4⁺ T cells and eosinophils. **h, i**, IL-13 and IL-17 expression in lung *Foxp3*⁺CD4⁺ T_{reg} (**h**) and *Foxp3*⁺CD4⁺ T_{eff} cells (**i**). Each symbol represents an independent sample. Error bars indicate SEM. Statistical tests: two-way ANOVA with Sidak's post hoc analysis (**b-i**). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data representative of two or three independent experiments. n = 5 mice per group.

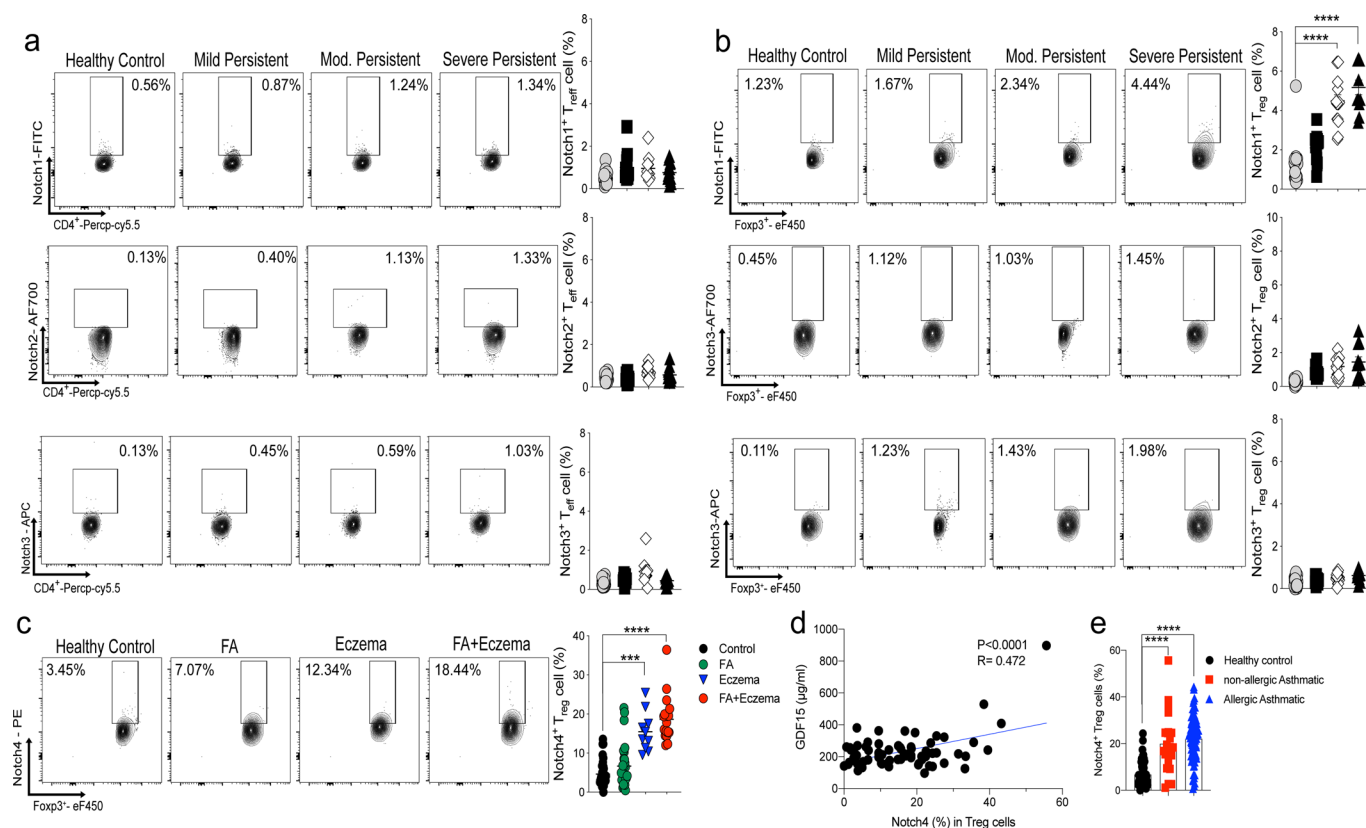


Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | T_{reg} cell-specific *Notch4* deletion rescues HDM induced allergic airway inflammation. **a**, scheme of the house dust mite (HDM) airway inflammation protocol. **b**, Representative PAS-stained sections of lung tissues isolated from *Foxp3^{YFP-Cre}* or *Foxp3^{YFP-Cre}Notch4^{Δ/Δ}* mice segregated into PBS, OVA or OVA + UFP-treated groups (200X magnification). **c**, Inflammation scores in the respective lung tissues. **d**, AHR in the respective mouse groups in response to methacholine. **e**, serum total IgE concentrations. **(f-h)**, absolute numbers of lung CD4⁺ T cells, neutrophils and eosinophils. **i,k**, IL-4, IL-13, IL-17 and IFN γ expression in lung Foxp3⁺CD4⁺ T_{reg} (**i**) and Foxp3⁻CD4⁺ T_{eff} cells (**k**). Each symbol represents an independent sample. Numbers in flow plots indicate percentages. Error bars indicate SEM. Statistical tests: two-way ANOVA with Sidak's post hoc analysis (**c-k**). **P < 0.01, ***P < 0.001, ****P < 0.0001. Data representative of two or three independent experiments. n = 5 mice per group.



Extended Data Fig. 7 | T_{reg} cell-specific *Notch4* deletion rescues chronic allergic airway inflammation. **a**, Scheme for the chronic airway inflammation mouse protocol. **b**, Representative Sirius-Red-stained sections of lung tissues isolated from *Foxp3^{YFPcre}* or *Foxp3^{YFPcre}Notch4^{Δ/Δ}* mice segregated into PBS, OVA or OVA + UFP-treated groups (200X magnification). **c**, Collagen disposition measurement in the respective lung tissues. **d**, AHR in the respective mouse groups in response to methacholine. **e**, **f**, absolute numbers of lung CD4⁺ T cells and eosinophils. **g**, **h**, IL-4, IL-13, and IL-17 expression in lung *Foxp3*⁺CD4⁺ T_{reg} (**g**) and *Foxp3*⁺CD4⁺T_{eff} cells (**h**). **i**, Serum OVA-specific IgE titers in the respective groups. Each symbol represents an independent sample. Error bars indicate SEM. Statistical tests: two-way ANOVA with Sidak's post hoc analysis (**c**–**h**). *P < 0.05, ****P < 0.0001. Data representative of two or three independent experiments. n = 5 mice per group.



Extended Data Fig. 8 | Notch receptor expression in human T_{reg} and T_{eff} cells. **a,b**, Flow cytometric analysis, cell frequencies and mean fluorescence intensity (MFI) of Notch1, 2 and 3 expression in peripheral blood T_{reg} cells (**a**) and T_{eff} cells (**b**) of control and asthmatic subjects, the latter segregated for asthma severity (control n = 22, M.P n = 15, Mod n = 16, S.P n = 11). **c**, Flow cytometric analysis and cell frequencies of Notch4 peripheral blood T_{reg} cells of healthy control, food allergy (FA), eczema and FA + eczema (Control n = 37, FA n = 28, Eczema n = 10 and FA + Eczema n = 20). **d**, Serum GDF15 concentrations in asthmatic subjects plotted as a function of Notch4 expression on circulating T_{reg} cells (n = 73). **e**, Cell frequencies of Notch4 expression in peripheral blood T_{reg} cells in healthy subjects, allergic and non-allergic asthmatics (control = 56, non-allergic n = 21, allergic n = 85). Error bars indicate SEM. Statistical tests: One-way ANOVA with Dunnett's post hoc analysis. (**a-c,e**); simple regression analysis (**d**). ***P < 0.001, ****P < 0.0001. Data representative of two or three independent experiments.

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-----|-----------|
| n/a | Confirmed |
|-----|-----------|
- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
 - ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
 - ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
 - ☐ ☒ A description of all covariates tested
 - ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
 - ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
 - ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
 - ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
 - ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
 - ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No software was used
Data analysis	Graph pad Prism version 8 was used for statistical analysis. FlowJo (Tree star) Version 10 was used for analysis of flow cytometry. RNA sequencing data was demultiplexed by using perfect matches to indices and was quality-inspected using FastQC. The sequencing data was aligned to the mm10 build (Gencode annotation) of the mouse genome using STAR62, and counts were quantified using HTSeq63. Raw counts were filtered for non-mitochondrial protein-coding genes with at least three counts in one sample, and were normalized using the DESeq2 package in R64. Pairwise comparisons of differential gene expression were computed using DESeq2. EdgeR

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data available on request from the authors

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size	Our previous studies (see Lin et al, Nat. Immunol 2007, Massoud et al. Nat Med 2016) and our findings in this report indicate that the effect size is very large (for many measures, the effect size d is anywhere between 2-4 or larger). Accordingly, assuming an alpha of 0.05 and power (1-beta) of 0.8, a sample size of 4-6 mice is required when employing a two tailed t test to detect differences between WT and mutant groups. A similarly large effect became evident from the human studies reported herein. Most studies in our manuscript are collated from 2-3 experiments, with n=7- 10 subjects per group. In a small number of studies a lesser sample size is employed, but the effect size is such that an statistical difference between groups is readily detectable.
Data exclusions	No data was excluded from the analyses
Replication	Experiments usually were repeated three times to ensure reproducibility of results. The only exceptions were the RNA-seq studies, each of which had several replicates done as part of one experiment. There were 4/5 independent samples used for the RNA-seq studies. All attempts of replication were successful
Randomization	Whenever possible, litter-mates were used as controls. In comparisons between different mouse strains, mice were segregated based on their genotype. Mice from the same genotype were randomly ascribed to different comparison groups.
Blinding	Histological analysis was blinded. Human Studies and analysis were blinded to the investigators. Other studies were not blinded

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	IL-4 (clone 11B11, cat: 504104 1:300 dilution, Biolegend), Siglec-F (E50-2440, cat: 5521261:300, BD Pharmingen), Foxp3 (FJK-16S, cat: 48-5773-82 1:300, eBioscience), IFN-γ (XMG1.2, cat: 505806 1:300, Biolegend), IL-13 (eBio13a, cat: 47-7133-82 1:300, eBioscience), Helios (22F6, cat: 47-9883-42 1:200, eBioscience), CD11c (N418, cat: 117318 1:500, Biolegend), CD11b (M1/70, cat: 101222 1:500, eBioscience), CD4 (GK1.5, cat: 100451, 1:500, Biolegend), CD3 (17A2, cat: 100203, 1:500, Biolegend), IL-17 (TC11-18H10.1, cat: 506922, 1:200, Biolegend), GR-1 (RB6-8C5, cat: 108406, 1:500, Biolegend), CD45 (30-F11, cat: 103140, 1:300, Biolegend), Notch1 (HMN1-12, cat: 130615, 1:500, Biolegend), Notch2 (HMN2-35, cat: 130714, 1:500, Biolegend), Notch3 (HMN3-133, cat: 130512, 1:300, Biolegend), Notch4 (HMN4-14, cat: 128407 1:200, Biolegend). Polyclonal rabbit anti-GDF15(cat: 32572-05171, 1:200, Assaypro), anti-CD16/CD32 (clone: 93, Cat: 101319, 1:1000, Biolegend), Alexa Fluor 647 goat anti-rabbit IgG Ab (clone PA5-39741, cat: A32733, 1:1000, Thermofischer), p-Mob1 Ab (T35; clone D2F10, Cat: 8699S, 1:300, CST), rabbit anti-mouse p-Lats1 Ab (T35; clone D57D3, 1:300, CST), rabbit anti-mouse p-Lats1/2 Ab (S909/872; clone PA5-39741, cat: PA5-105895, 1:300, Thermofischer), Rat anti-mouse IL-6 mAb (Clone: MP5-20F3, Cat: BE0046, 1:500, Biorcell), anti-CD28 (Clone: 37.51, Cat: 122004, 1:1000, Biolegend), anti-IL-4 (Cat: 500-P54, 1:1000, Peprotech). Antibodies against the following human antigens were used: CD3 (HIT3a, cat: 300318, 1:300, Biolegend), CD4 (RPA-T4, cat: 300530, 1:300, Biolegend), Foxp3 (PCH-101, cat: 48-4776-42 1:200, eBioscience), Helios (22F6, cat: 47-9883-42 1:200, eBioscience), Notch1 (HMN1-519, cat: 352108, 1:500, BD Pharmingen), Notch2 (HMN2-25, cat: 742291, 1:300, BD Pharmingen), Notch3 (HMN3-21, cat: 744828, 1:300, BD Pharmingen), Notch4 (HMN4-2, Cat: 563269, 1:300, BD Pharmingen), Yap1 (147295, Cat: 147295, 1:200, CST) and beta-Catenin (196624, Cat: IC13292V, 1:500, R&D system).
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Validation

The specificity and optimal dilution of each antibody was validated by testing on appropriate negative and positive controls or otherwise provided on the manufacturer's website.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

CD4Cre (B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ)
 floxed Ctnnb1 (Ctnnb1fl/fl) (B6(Cg)-Ctnnb1tm1Knw/J)
 Foxp3YFPcre (B6.129(Cg)-Foxp3tm4(YFP/cre)Ayr/J)
 Il6rf1/fl (B6;SJL-Il6ratm1.1Drew/J)
 Notch1fl/fl (B6.129X1-Notch1tm2Rko/Grid/J)
 Notch2fl/fl (B6.129S-Notch2tm3Grid/J)
 Notch3-/- (B6;129S1-Notch3tm1Grid/J)
 OT-II (B6.Cg-Tg(TcraTcrb)425Cbn/J)
 Stat3fl/fl (B6.129S1-Stat3tm1Xyfu/J)
 Wwtr1fl/fl (B6.129(Cg)-Wwtr1tm1Hmc/J)
 Yap1fl/fl (B6.129P2(Cg)-Yap1tm1.1Dupa/J)
 Il4/Il13fl/fl (C.129P2(Cg)-Il4/Il13tm1.1Lky/J)44.(Notch4tm1c(NCOM)Mfgc)
 RoraCre mice
 CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ)
 Notch4fl/fl (Notch4tm1c(NCOM)Mfgc) were obtained from the Canadian mutant mouse repository
 Pofut1fl/fl (B6.Cg-Pofut1tm2.1Pst/J)
 Rbpj1fl/fl (B6.129P2-Rbpjtm1Hon/HonRbrc) were kind gifts of Pamela Stanley and Tasuku Honjo, respectively
 Il4/Il13fl/fl (C.129P2(Cg)-Il4/Il13tm1.1Lky/J.
 RoraCre

Both males and females were used for the experiment (50:50). The mice were 6-8 weeks old for the experiments.

Wild animals

no wild animals were used in the study.

Field-collected samples

no field collected samples were used in the study.

Ethics oversight

Boston Children's Hospital IUCAC

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

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Both male and female subjects ages 21 years and below were included in the study. Patients were categorized based on the presence or absence of the following characteristics: asthma diagnosis (according to ATS guidelines), food allergy (positive skin testing and/or specific IgE and history of immediate reaction following food ingestion), allergic rhinitis (positive skin testing and/or specific IgE), and eczema (clinical diagnosis). Control subjects are healthy individuals with no personal history of atopic conditions. Patients and controls were recruited at Boston Children Hospital and Marmara University.

Recruitment

All subjects were recruited under clinical research protocols approved by the local Institutional Review Boards at the respective referring centers.

Ethics oversight

Boston Children's Hospital and Marmara University Institutional Review Boards (IRB)

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cells were isolated from either the lungs of experimental mice or peripheral blood of human subjects following methods detailed in the Method Section

Instrument	LSR Fortessa was used Model number: 647784L5
Software	FlowJo (Tree star) Version 10 was used for analysis of flow cytometry.
Cell population abundance	Mouse Treg cells had an abundance of 5-9% in source population, and were sorted where indicated to a purity of >98% Mouse ILC cells had an abundance of 0.5-2.3% in source population, and were sorted with a purity of >99% Human Treg cells had an abundance of 2.4-3% in source PBMC, and were sorted with a purity of >98% Human ILC2 cells had an abundance of 0.6-1% in source PBMC, and were sorted with a purity of >98%
Gating strategy	Lymphocytes were gated using their characteristic FSC and SSC profile. Doublets were then excluded and the gate were placed on live cells by viability staining). The live cells were then gated as follows: - CD4+ vs. Foxp3+ staining was employed to set a gate for Treg vs. Teff cells. The Teff cells are then gated on IL-4, IL-13, IL-17 and IFN-gamma positive populations using staining with the respective antibodies. The populations were set as per Fluorescence minus 1 (FMO) technique and employment of the appropriate isotype antibody controls. 3). For Treg cells, the same gating strategy for cytokines was used. - For Eosinophils: The FCS and SSC profiles of all collected cells were noted. The doublets and dead cells were excluded as above. for Eosinophils, we gated on CD45+ cells, CD4- cells, F4/80- cells, GR-1- cells and SiglecF+ cells. - For Neutrophils: we gated on CD45+ cells, CD4- cells, F4/80- cells, GR-1+ cells and SiglecF- cells. - For ILC cells: After FCS/SSC and doublet and dead cell exclusion, the cells were gated on Lineage- (CD3, CD4, CD11c, CD19, CD11b) and T1/ST2+ cells. The ILC compartment were then checked for IL-13, IL-17 and IFN-gamma expression by staining for the respective cytokine using the appropriate FMO and isotype antibody controls. Positive populations are showed in the figures.

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

Supplementary information

A regulatory T cell Notch4–GDF15 axis licenses tissue inflammation in asthma

In the format provided by the
authors and unedited

Supplementary Table 1: Demographics and allergic disease attributes of human study subject.

Patient ID	Gender	Age	Food allergies	Asthma	Environmental Allergies	Eczema
ID-1	M	15	no	Intermittent	yes	yes
ID-2	F	6	no	Intermittent	no	yes
ID-3	M	9	no	Intermittent	yes	no
ID-4	M	13	no	Intermittent	yes	yes
ID-5	F	9	peanut, tree nuts, lentil, kiwi	Intermittent	yes	yes
ID-6	M	9	milk, egg, peanut, tree nuts	Intermittent	yes	yes
ID-7	M	7	no	Intermittent	no	no
ID-8	F	19	tree nuts, shellfish	Intermittent	yes	no
ID-9	M	11	no	Intermittent	yes	yes
ID-10	F	10	no	Intermittent	no	yes
ID-11	F	8	milk, egg, peanut, tree nuts, fish	Intermittent	no	yes
ID-12	F	10	no	Intermittent	yes	yes
ID-13	F	3	cashew, pistachio, milk	Intermittent	yes	yes
ID-14	M	11	no	Intermittent	no	no
ID-15	M	7	peanut, tree nuts	Intermittent	yes	no
ID-16	M	6	no	Intermittent	cat	no
ID-17	M	9	no	Intermittent	no	yes
ID-18	M	13	no	Intermittent	yes	yes
ID-19	M	2	no	Intermittent	no	yes
ID-20	F	12	peanut, tree nuts	Intermittent	yes	no
ID-21	F	10	no	Intermittent	yes	no
ID-22	F	9	scallop	Intermittent	yes	yes
ID-23	F	9	no	Intermittent	yes	no
ID-24	F	13	no	Intermittent	no	no
ID-25	M	7	no	Intermittent	yes	yes

ID-26	F	4	milk, egg, peanut, tree nuts, sesame	Intermittent	yes	yes
ID-27	M	11	no	Intermittent	no	no
ID-28	F	9	peach, carrot (OAS)	Intermittent	yes	no
ID-29	M	8	no	Intermittent	yes	yes
ID-30	M	8	no	Intermittent	yes	yes
ID-31	M	10	no	Intermittent	no	no
ID-32	M	3	peanut, tree nuts, sesame	Intermittent	yes	yes
ID-33	M	9	no	Intermittent	yes	no
ID-34	M	16	egg, peanut, tree nuts, sesame	Mild Persistent	yes	yes
ID-35	M	11	shellfish	Mild Persistent	yes	no
ID-36	F	10	no	Mild Persistent	no	no
ID-37	M	10	no	Mild Persistent	no	no
ID-38	M	12	no	Mild Persistent	no	no
ID-39	M	9	no	Mild Persistent	no	yes
ID-40	F	17	no	Mild Persistent	yes	no
ID-41	F	10	peanut	Mild Persistent	yes	no
ID-42	F	11	no	Mild Persistent	no	no
ID-43	M	15	no	Mild Persistent	dust	no
ID-44	F	8	no	Mild Persistent	yes	no
ID-45	M	12	no	Mild Persistent	yes	no
ID-46	M	9	no	Mild Persistent	no	no
ID-47	M	12	no (OAS)	Mild Persistent	yes	yes
ID-48	M	8	no	Mild Persistent	yes	yes
ID-49	M	6	no	Mild Persistent	yes	no
ID-50	M	7	no	Mild Persistent	yes	no
ID-51	F	8	no	Mild Persistent	no	no
ID-52	F	9	no	Mild Persistent	yes	no

ID-53	F	10	milk, egg, peanut, sesame, fish, shellfish	Mild Persistent	yes	yes
ID-54	M	8	no	Mild Persistent	yes	no
ID-55	F	12	no	Mild Persistent	yes	yes
ID-56	F	3	egg	Mild Persistent	no	yes
ID-57	F	1	peanut, tree nuts	Mild Persistent	no	yes
ID-58	M	7	no	Mild Persistent	yes	no
ID-59	M	5	egg, peanut, tree nuts	Mild Persistent	yes	no
ID-60	F	10	no	Mild Persistent	no	no
ID-61	F	6	no	Mild Persistent	yes	no
ID-62	F	10	no	Mild Persistent	yes	yes
ID-63	F	10	no	Mild Persistent	yes	yes
ID-64	M	8	no	Mild Persistent	no	no
ID-65	F	8	no	Mild Persistent	yes	no
ID-66	M	6	no	Mild Persistent	yes	no
ID-67	F	6	no	Mild Persistent	no	no
ID-68	M	9	no	Mild Persistent	yes	no
ID-69	M	7	yes chilli peppers	Mild Persistent	no	no
ID-70	M	9	no	Mild Persistent	no	no
ID-71	M	12	no	Mild Persistent	no	no
ID-72	M	9	no	Mild Persistent	yes	no
ID-73	M	8	no	Moderate Persistent	yes	no
ID-74	F	15	Peanut, tree nuts	Moderate Persistent	yes	yes
ID-75	M	11	no	Moderate Persistent	yes	no
ID-76	M	9	no	Moderate Persistent	yes	yes
ID-77	M	6	no	Moderate Persistent	yes	yes
ID-78	F	8	no	Moderate Persistent	yes	yes
ID-79	M	13	no	Moderate Persistent	yes	no

ID-80	F	10	shellfish	Moderate Persistent	yes	no
ID-81	M	14	no	Moderate Persistent	yes	yes
ID-82	F	9	egg, peanut, tree nuts, sesame, shellfish, wheat, banana	Moderate Persistent	yes	yes
ID-83	F	12	no	Moderate Persistent	yes	yes
ID-84	M	7	no	Moderate Persistent	yes	no
ID-85	F	10	milk, peanut, tree nuts	Moderate Persistent	yes	yes
ID-86	F	11	no	Moderate Persistent	yes	yes
ID-87	M	5	no	Moderate Persistent	yes	no
ID-88	M	11	no	Moderate Persistent	yes	no
ID-89	M	4	peanut, tree nuts	Moderate Persistent	yes	yes
ID-90	M	8	no	Moderate Persistent	no	no
ID-91	F	21	multiple	Moderate Persistent	yes	yes
ID-92	F	17	no	Moderate Persistent	yes	no
ID-93	M	10	no	Moderate Persistent	yes	no
ID-94	M	4	peanut	Moderate Persistent	yes	yes
ID-95	M	4	peanut, tree nuts	Moderate Persistent	yes	yes
ID-96	M	5	peanut, tree nuts	Severe Persistent	yes	yes
ID-97	M	9	no	Severe Persistent	yes	yes
ID-98	F	3	peanut, tree nuts, orange	Severe Persistent	yes	yes
ID-99	M	2	no	Severe Persistent	yes	no
ID-100	M	12	no	Severe Persistent	yes	yes
ID-101	M	11	cow milk, peanut, tree nuts	Severe Persistent	yes	yes
ID-102	F		no	Severe Persistent	no	no
ID-103	M	12	shellfish, soy, beef	Severe Persistent	yes	yes
ID-104	F	11	no	Severe Persistent	no	no
ID-105	F	11	no	Severe Persistent	yes	no
ID-106	M	6	no	Severe Persistent	yes	yes

ID-107	F	10	banana	no	yes	no
ID-108	F	1	egg	no	no	no
ID-109	M	2	egg, peanut	no	no	no
ID-110	M	2	egg, peanut	no	no	no
ID-111	F	2	egg, peanut, tree nuts	no	no	no
ID-112	M	11	peanut, tree nuts	no	no	no
ID-113	M	14	peanut, tree nuts	no	yes	no
ID-114	M	2	peanut, tree nuts, sesame	no	yes	no
ID-115	F	20	peanut, tree nuts, sesame, shellfis	no	yes	no
ID-116	M	5	peanut, tree nuts. Sesme	no	yes	no
ID-117	F	12	pineapple	no	no	no
ID-118	M	10	sesame	no	yes	no
ID-119	F	10	shellfish	no	no	no
ID-120	F	6	tree nuts	no	yes	no
ID-121	M	10	tree nuts	no	yes	no
ID-122	M	9	tree nuts	no	yes	no
ID-123	M	10	tree nuts?	no	yes	no
ID-124	F	9	unknown	no	yes	no
ID-125	M	1	egg	no	yes	yes
ID-126	M	3	egg, peanut	no	yes	yes
ID-127	F	5	egg, peanut, tree nuts	no	yes	yes
ID-128	F	10	peanut, tree nuts	no	yes	yes
ID-129	F	2	peanut, tree nuts	no	yes	yes
ID-130	M	8	peanut, tree nuts, sesame	no	yes	yes
ID-131	F	10	sesame	no	yes	yes
ID-132	F	8	no	no	yes	yes
ID-133	M	11	no	no	yes	yes

ID-134	M	6	no	no	yes	yes
ID-135	F	11	no	no	yes	yes
ID-136	M	10	no	no	no	yes
ID-137	M	6	no	no	no	yes
ID-138	F	8	no	no	yes	yes
ID-139	F	8	no	no	no	no
ID-140	M	10	no	no	no	no
ID-141	F	8	no	no	no	no
ID-142	M	2	no	no	no	no
ID-143	M	2	no	no	no	no
ID-144	M	11	no	no	no	no
ID-145	M	1	no	no	no	no
ID-146	M	8	no	no	no	no
ID-147	M	10	no	no	no	no
ID-148	M	9	no	no	no	no
ID-149	F	9	no	no	no	no
ID-150	F	9	no	no	no	no
ID-151	M	11	no	no	no	no
ID-152	F	10	no	no	no	no
ID-153	M	8	no	no	no	no
ID-154	F	8	no	no	no	no
ID-155	F	8	no	no	no	no
ID-156	M	9	no	no	no	no
ID-157	F	7	no	no	no	no
ID-158	M	9	no	no	no	no
ID-159	M	1	no	no	no	no
ID-160	M	12	no	no	no	no
ID-161	F	7	no	no	no	no

ID-162	F	9	no	no	no	no
ID-163	M	1	no	no	no	no
ID-164	M	9	no	no	no	no
ID-165	F	12	no	no	no	no
ID-166	F	16	no	no	no	no
ID-167	M	8	no	no	no	no
ID-168	F	7	no	no	no	no
ID-169	F	8	no	no	no	no
ID-170	M	10	no	no	no	no
ID-171	M	10	no	no	no	no
ID-172	F	9	no	no	no	no
ID-173	F	8	no	no	no	no
ID-174	F	8	no	no	no	no
ID-175	M	1	no	no	no	no
ID-176	F	10	no	no	no	no
ID-177	F	12	no	no	no	no
ID-178	M	7	no	no	no	no
ID-179	F	7	no	no	no	no
ID-180	M	7	no	no	no	no
ID-181	F	10	no	no	no	no
ID-182	M	8	no	no	no	no
ID-183	F	7	no	no	no	no

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-----|-----------|
| n/a | Confirmed |
|-----|-----------|
- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
 - ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
 - ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
 - ☐ ☒ A description of all covariates tested
 - ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
 - ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
 - ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
 - ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
 - ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
 - ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No software was used
Data analysis	Graph pad Prism version 8 was used for statistical analysis. FlowJo (Tree star) Version 10 was used for analysis of flow cytometry. RNA sequencing data was demultiplexed by using perfect matches to indices and was quality-inspected using FastQC. The sequencing data was aligned to the mm10 build (Gencode annotation) of the mouse genome using STAR62, and counts were quantified using HTSeq63. Raw counts were filtered for non-mitochondrial protein-coding genes with at least three counts in one sample, and were normalized using the DESeq2 package in R64. Pairwise comparisons of differential gene expression were computed using DESeq2. EdgeR

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Data

Policy information about [availability of data](#)

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

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data available on request from the authors

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size	Our previous studies (see Lin et al, Nat. Immunol 2007, Massoud et al. Nat Med 2016) and our findings in this report indicate that the effect size is very large (for many measures, the effect size d is anywhere between 2-4 or larger). Accordingly, assuming an alpha of 0.05 and power (1-beta) of 0.8, a sample size of 4-6 mice is required when employing a two tailed t test to detect differences between WT and mutant groups. A similarly large effect became evident from the human studies reported herein. Most studies in our manuscript are collated from 2-3 experiments, with n=7- 10 subjects per group. In a small number of studies a lesser sample size is employed, but the effect size is such that an statistical difference between groups is readily detectable.
Data exclusions	No data was excluded from the analyses
Replication	Experiments usually were repeated three times to ensure reproducibility of results. The only exceptions were the RNA-seq studies, each of which had several replicates done as part of one experiment. There were 4/5 independent samples used for the RNA-seq studies. All attempts of replication were successful
Randomization	Whenever possible, litter-mates were used as controls. In comparisons between different mouse strains, mice were segregated based on their genotype. Mice from the same genotype were randomly ascribed to different comparison groups.
Blinding	Histological analysis was blinded. Human Studies and analysis were blinded to the investigators. Other studies were not blinded

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	IL-4 (clone 11B11, cat: 504104 1:300 dilution, Biolegend), Siglec-F (E50-2440, cat: 5521261:300, BD Pharmingen), Foxp3 (FJK-16S, cat: 48-5773-82 1:300, eBioscience), IFN- γ (XMG1.2, cat: 505806 1:300, Biolegend), IL-13 (eBio13a, cat: 47-7133-82 1:300, eBioscience), Helios (22F6, cat: 47-9883-42 1:200, eBioscience), CD11c (N418, cat: 117318 1:500, Biolegend), CD11b (M1/70, cat: 101222 1:500, eBioscience), CD4 (GK1.5, cat: 100451, 1:500, Biolegend), CD3 (17A2, cat: 100203, 1:500, Biolegend), IL-17 (TC11-18H10.1, cat: 506922, 1:200, Biolegend), GR-1 (RB6-8C5, cat: 108406, 1:500, Biolegend), CD45 (30-F11, cat: 103140, 1:300, Biolegend), Notch1 (HMN1-12, cat: 130615, 1:500, Biolegend), Notch2 (HMN2-35, cat: 130714, 1:500, Biolegend), Notch3 (HMN3-133, cat: 130512, 1:300, Biolegend), Notch4 (HMN4-14, cat: 128407 1:200, Biolegend). Polyclonal rabbit anti-GDF15(cat: 32572-05171, 1:200, Assaypro), anti-CD16/CD32 (clone: 93, Cat: 101319, 1:1000, Biolegend), Alexa Fluor 647 goat anti-rabbit IgG Ab (clone PA5-39741, cat: A32733, 1:1000, Thermofischer), p-Mob1 Ab (T35; clone D2F10, Cat: 8699S, 1:300, CST), rabbit anti-mouse p-Lats1 Ab (T35; clone D57D3, 1:300, CST), rabbit anti-mouse p-Lats1/2 Ab (S909/872; clone PA5-39741, cat: PA5-105895, 1:300, Thermofischer), Rat anti-mouse IL-6 mAb (Clone: MP5-20F3, Cat: BE0046, 1:500, Biorcell), anti-CD28 (Clone: 37.51, Cat: 122004, 1:1000, Biolegend), anti-IL-4 (Cat: 500-P54, 1:1000, Peprotech). Antibodies against the following human antigens were used: CD3 (HIT3a, cat: 300318, 1:300, Biolegend), CD4 (RPA-T4, cat: 300530, 1:300, Biolegend), Foxp3 (PCH-101, cat: 48-4776-42 1:200, eBioscience), Helios (22F6, cat: 47-9883-42 1:200, eBioscience), Notch1 (HMN1-519, cat: 352108, 1:500, BD Pharmingen), Notch2 (HMN2-25, cat: 742291, 1:300, BD Pharmingen), Notch3 (HMN3-21, cat: 744828, 1:300, BD Pharmingen), Notch4 (HMN4-2, Cat: 563269, 1:300, BD Pharmingen), Yap1 (147295, Cat: 147295, 1:200, CST) and beta-Catenin (196624, Cat: IC13292V, 1:500, R&D system).
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Validation

The specificity and optimal dilution of each antibody was validated by testing on appropriate negative and positive controls or otherwise provided on the manufacturer's website.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

CD4Cre (B6.Cg-Tg(Cd4-cre)1Cwi/BfluJ)
 floxed Ctnnb1 (Ctnnb1fl/fl) (B6(Cg)-Ctnnb1tm1Knw/J)
 Foxp3YFPcre (B6.129(Cg)-Foxp3tm4(YFP/cre)Ayr/J)
 Il6rf1/fl (B6;SJL-Il6ratm1.1Drew/J)
 Notch1fl/fl (B6.129X1-Notch1tm2Rko/Grid/J)
 Notch2fl/fl (B6.129S-Notch2tm3Grid/J)
 Notch3-/- (B6;129S1-Notch3tm1Grid/J)
 OT-II (B6.Cg-Tg(TcraTcrb)425Cbn/J)
 Stat3fl/fl (B6.129S1-Stat3tm1Xyfu/J)
 Wwtr1fl/fl (B6.129(Cg)-Wwtr1tm1Hmc/J)
 Yap1fl/fl (B6.129P2(Cg)-Yap1tm1.1Dupa/J)
 Il4/Il13fl/fl (C.129P2(Cg)-Il4/Il13tm1.1Lky/J)44 .(Notch4tm1c(NCOM)Mfgc)
 RoraCre mice
 CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ)
 Notch4fl/fl (Notch4tm1c(NCOM)Mfgc) were obtained from the Canadian mutant mouse repository
 Pofut1fl/fl (B6.Cg-Pofut1tm2.1Pst/J)
 Rbpj1fl/fl (B6.129P2-Rbpjtm1Hon/HonRbrc) were kind gifts of Pamela Stanley and Tasuku Honjo, respectively
 Il4/Il13fl/fl (C.129P2(Cg)-Il4/Il13tm1.1Lky/J.
 RoraCre

Both males and females were used for the experiment (50:50). The mice were 6-8 weeks old for the experiments.

Wild animals

no wild animals were used in the study.

Field-collected samples

no field collected samples were used in the study.

Ethics oversight

Boston Children's Hospital IUCAC

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

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Both male and female subjects ages 21 years and below were included in the study. Patients were categorized based on the presence or absence of the following characteristics: asthma diagnosis (according to ATS guidelines), food allergy (positive skin testing and/or specific IgE and history of immediate reaction following food ingestion), allergic rhinitis (positive skin testing and/or specific IgE), and eczema (clinical diagnosis). Control subjects are healthy individuals with no personal history of atopic conditions. Patients and controls were recruited at Boston Children Hospital and Marmara University.

Recruitment

All subjects were recruited under clinical research protocols approved by the local Institutional Review Boards at the respective referring centers.

Ethics oversight

Boston Children's Hospital and Marmara University Institutional Review Boards (IRB)

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cells were isolated from either the lungs of experimental mice or peripheral blood of human subjects following methods detailed in the Method Section

Instrument	LSR Fortessa was used Model number: 647784L5
Software	FlowJo (Tree star) Version 10 was used for analysis of flow cytometry.
Cell population abundance	Mouse Treg cells had an abundance of 5-9% in source population, and were sorted where indicated to a purity of >98% Mouse ILC cells had an abundance of 0.5-2.3% in source population, and were sorted with a purity of >99% Human Treg cells had an abundance of 2.4-3% in source PBMC, and were sorted with a purity of >98% Human ILC2 cells had an abundance of 0.6-1% in source PBMC, and were sorted with a purity of >98%
Gating strategy	Lymphocytes were gated using their characteristic FSC and SSC profile. Doublets were then excluded and the gate were placed on live cells by viability staining). The live cells were then gated as follows: - CD4+ vs. Foxp3+ staining was employed to set a gate for Treg vs. Teff cells. The Teff cells are then gated on IL-4, IL-13, IL-17 and IFN-gamma positive populations using staining with the respective antibodies. The populations were set as per Fluorescence minus 1 (FMO) technique and employment of the appropriate isotype antibody controls. 3). For Treg cells, the same gating strategy for cytokines was used. - For Eosinophils: The FCS and SSC profiles of all collected cells were noted. The doublets and dead cells were excluded as above. for Eosinophils, we gated on CD45+ cells, CD4- cells, F4/80- cells, GR-1- cells and SiglecF+ cells. - For Neutrophils: we gated on CD45+ cells, CD4- cells, F4/80- cells, GR-1+ cells and SiglecF- cells. - For ILC cells: After FCS/SSC and doublet and dead cell exclusion, the cells were gated on Lineage- (CD3, CD4, CD11c, CD19, CD11b) and T1/ST2+ cells. The ILC compartment were then checked for IL-13, IL-17 and IFN-gamma expression by staining for the respective cytokine using the appropriate FMO and isotype antibody controls. Positive populations are showed in the figures.

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