

Elevated faecal 12,13-diHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes immune tolerance

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Neonates at risk of childhood atopy and asthma exhibit perturbation of the gut microbiome, metabolic dysfunction and increased concentrations of 12,13-diHOME in their faeces. However, the mechanism, source and contribution of this lipid to allergic inflammation remain unknown. Here, we show that intra-abdominal treatment of mice with 12,13-diHOME increased pulmonary inflammation and decreased the number of regulatory T (T_{reg}) cells in the lungs. Treatment of human dendritic cells with 12,13-diHOME altered expression of PPAR γ -regulated genes and reduced anti-inflammatory cytokine secretion and the number of T_{reg} cells in vitro. Shotgun metagenomic sequencing of neonatal faeces indicated that bacterial epoxide hydrolase (EH) genes are more abundant in the gut microbiome of neonates who develop atopy and/or asthma during childhood. Three of these bacterial EH genes (3EH) specifically produce 12,13-diHOME, and treatment of mice with bacterial strains expressing 3EH caused a decrease in the number of lung T_{reg} cells in an allergen challenge model. In two small birth cohorts, an increase in the copy number of 3EH or the concentration of 12,13-diHOME in the faeces of neonates was found to be associated with an increased probability of developing atopy, eczema or asthma during childhood. Our data indicate that elevated 12,13-diHOME concentrations impede immune tolerance and may be produced by bacterial EHs in the neonatal gut, offering a mechanistic link between perturbation of the gut microbiome during early life and atopy and asthma during childhood.

The development of severe asthma during childhood is often preceded by eczema and/or atopy—a heightened immune response to multiple food allergens and aeroallergens¹. Although genetic risk factors have been identified, the increased prevalence of atopy, eczema and asthma, predominantly in developed nations, suggests that changes in lifestyle and environmental exposures play a key role in the development of these diseases. Indeed, a number of early-life exposures have been identified that increase childhood atopy and asthma risk, including formula feeding, birth by caesarian section, and prenatal and postnatal antibiotic use, whereas early-life exposure to livestock and furred pets confer protection^{2–6}. Many of these exposures shape the composition and function of the human microbiome, which has now been established as a critical driver of immune function^{7–9}.

Independent US and Canadian birth cohorts revealed a link between the early-life gut microbiome and the subsequent development of childhood atopy and asthma^{10–12}. The gut microbiota of infants and neonates at increased risk of childhood atopy and/or asthma are distinct, characteristically depleted of bacterial genera and exhibit fungal expansion and metabolic reprogramming^{10–12}. Faecal metabolic alterations in high-risk infants include depletion of anti-inflammatory short-chain or polyunsaturated fatty acids and enrichment of monohydroxy fatty acids, including 12,13-diHOME^{10–12}. Importantly, in mice, gut-microbiome-derived

short-chain and polyunsaturated fatty acids protect against allergic inflammation of the airways^{13,14}. By contrast, in vitro co-culture studies of human dendritic cells (DCs) and T cells revealed that pretreatment of DCs with 12,13-diHOME caused a dose-dependent decrease in the frequency of T_{reg} cells¹¹, a characteristic immunological feature of untreated children with asthma¹⁵.

12,13-diHOME is a relatively uncharacterized linoleic acid (LA) metabolite previously shown to be induced in the airways of adults with asthma following bronchial provocation with an allergen¹⁶. More recent in vivo studies have linked 12,13-diHOME with increased thermal hyperalgesia and fatty-acid uptake in adipose and skeletal muscle^{17–19}, implicating it as an important lipid mediator in a range of physiological processes. However, the mechanism and source of this lipid in the faeces of neonates at risk of asthma remain unknown. Given our previous observations¹¹, we hypothesized that elevated faecal concentrations of 12,13-diHOME promote allergic inflammation by inducing DC dysfunction, resulting in a subsequent reduction in the number of anti-inflammatory T_{reg} cells. Furthermore, we hypothesized that this lipid is produced by gut microorganisms in high-risk neonates. Here we show that—at elevated concentrations—12,13-diHOME caused an increase in pulmonary inflammation, a reduction in the number of lung T_{reg} cells in vivo and reprogrammed human DC activity in vitro. We identified three strain-specific bacterial genes within the neonatal

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gut microbiome that catalyse production of 12,13-diHOME and are sufficient, when introduced to the gut microbiome of mice, to recapitulate the effects of 12,13-diHOME on pulmonary T_{reg} cells *in vivo*. Finally, we show that an increase in the faecal copy number of these three bacterial epoxide hydrolase genes (3EH) or the concentration of their lipid product in one-month-old neonates significantly increases the probability of developing childhood allergies, eczema and asthma.

Results

12,13-diHOME exacerbates lung inflammation in mice challenged with allergens. Initially, we examined whether 12,13-diHOME exacerbates allergic sensitization *in vivo* by treating mice with 12,13-diHOME (30 mg kg⁻¹) or vehicle control (10% DMSO) by peritoneal injection before subjecting them to airway sensitization and challenge with cockroach antigen (CRA). Treated animals showed significantly increased peribronchial and perivascular inflammatory infiltrates and total serum IgE compared with those treated with vehicle alone (Fig. 1a–d, Supplementary Fig. 1a,b). Furthermore, 12,13-diHOME-treated animals also exhibited increases in lung-resident T cells, neutrophils and monocytes, and pulmonary expression of pro-inflammatory innate cytokines IL-1 β , IL-1 α and TNF, as well as a significant decrease in the number of lung T_{reg} cells and a decrease in the number of alveolar macrophages in the lungs (Fig. 1e–g, Supplementary Fig. 1j–m).

To evaluate whether oxylipins injected into the peritoneum reach the circulation, we quantified the concentration of 12,13-diHOME in plasma using targeted liquid chromatography with mass spectrometry (LC–MS)²⁰ 3 h after peritoneal injection of 12,13-diHOME and observed a significant increase in plasma concentration compared with vehicle-treated animals (Fig. 1h). Furthermore, the 12,13-diHOME-treated animals exhibited a significantly increased concentration of 12,13-diHOME in their lungs (Supplementary Fig. 1n). Together, these data suggest that—following peritoneal injection—12,13-diHOME can enter the circulatory system and may interact directly with the lung mucosa to exacerbate allergic inflammation of the airways. This does not preclude local synthesis of 12,13-diHOME by pulmonary microbiota or by pulmonary tissue, but rather indicates that lower-airway allergic inflammation may be exacerbated by circulating metabolites originating in the gut.

12,13-diHOME reprograms lipid handling and IL-10 expression of DCs. To determine a mechanism by which 12,13-diHOME exacerbates inflammatory responses, we considered its structural similarity to known ligands of peroxisome proliferator-activated receptor- γ (PPAR γ)—a lipid-activated nuclear receptor that regulates adipogenesis, fatty-acid uptake, inflammation, glucose metabolism and intestinal microbiota^{21–23}. In mice, loss of PPAR γ in DCs leads to a loss of immune tolerance and exacerbation of pulmonary inflammation²². We therefore hypothesized that 12,13-diHOME induces pro-allergic immune dysfunction through PPAR γ signalling in DCs. Human DCs treated with 12,13-diHOME exhibit decreased secretion of IL-10, an anti-inflammatory cytokine that protects against allergic inflammation²⁴ (Fig. 2a). Co-culture of 12,13-diHOME-treated DCs and autologous T cells altered the distribution of CD4⁺ T cells, specifically decreasing the frequency of T_{reg} cells without decreasing cell viability (Fig. 2b, Supplementary Fig. 2c–e), confirming that the observed reduction in IL-10 secretion by DCs has a functional consequence for T_{reg} cell populations.

To evaluate whether 12,13-diHOME exerts this effect through PPAR γ , we isolated RNA from human DCs treated with 12,13-diHOME *in vitro* and studied PPAR γ -regulated gene expression. Treatment with 12,13-diHOME mimicked previously characterized effects of PPAR γ activation, including decreased expression of CD1a—an immune marker involved in lipid-antigen presentation²⁵—and increased expression of fatty acid binding protein

4 (FABP4) and hydroxyacyl-CoA-dehydrogenase (HADH)—genes involved in fatty-acid uptake and oxidation, respectively²⁵ (Fig. 2c). Using flow cytometry, we confirmed that 12,13-diHOME activated PPAR γ in human DCs *in vitro*, decreasing expression of CD1a, CD80 and CCR7—surface proteins involved in lipid presentation, antigen presentation and cell trafficking in human DCs²⁵ (Fig. 2d–f). To test whether 12,13-diHOME acts on PPAR γ directly, we used a modified PPAR γ reporter assay²⁶ and found that treatment with 12,13-diHOME in excess of 50 μ M leads to PPAR γ activation (Fig. 2g). These observations are consistent with recently reported increases in lipid uptake by adipose tissue treated with 12,13-diHOME¹⁷.

Although PPAR γ activation in DCs is traditionally considered to be anti-inflammatory²⁷, more-recent studies suggest that it may promote allergic inflammation²⁸. Alternatively, 12,13-diHOME may act as a weak agonist of PPAR γ and compete with endogenous PPAR γ ligands²⁵. Emerging data indicate that 12,13-diHOME influences a range of mammalian tissues^{17–19}. Our data provide evidence that the effects of 12,13-diHOME extend to antigen-presenting cells and that elevated concentrations of this lipid cause a change in lipid handling and a reduction in IL-10 secretion by DCs. Given the emerging importance of 12,13-diHOME in a number of different tissues, it is plausible that it may also signal through other lipid receptors. Indeed, 12,13-diHOME was recently identified as an activator of transient receptor potential vanilloid 1 (TRPV1)²⁹, the loss of which is associated with decreased asthma prevalence in humans and protection against airway inflammation in animal models^{30,31}. Thus, 12,13-diHOME may have multiple cellular targets and its effects are probably not mediated exclusively by PPAR γ .

12,13-diHOME is enriched in the faeces of neonates who are at risk of atopy and asthma. Given the evidence for 12,13-diHOME as a driver of allergic inflammation in mice and humans, we focused on determining the concentration and potential sources of 12,13-diHOME in neonatal faeces. Initially, using LC–MS, we quantified the concentration of 12,13-diHOME in a subset of one-month-old neonates from the Wayne County Health, Environment, Allergy and Asthma Longitudinal Study (WHEALS; Supplementary Tables 6 and 11; total $n=41$; atopic $n=7$; asthmatic $n=8$; atopy and/or asthma $n=19$)¹¹. These included 26 stool samples from one-month-old neonates—microbiologically representative of the variance in the composition of the gut microbiome observed across a larger cohort of 130 one-month-old neonates¹¹—and a further 15 randomly selected samples (with >50 mg of stool and >10 ng of extracted faecal DNA) from the same cohort of one-month-old neonates for whom childhood atopy and asthma outcomes were available. Targeted LC–MS demonstrated that 12,13-diHOME was present in all neonatal stool; however, significantly higher concentrations were detected in the stool of neonates who subsequently developed atopy and/or asthma, even after adjusting for potential confounding factors (Fig. 3a, Supplementary Table 8). By contrast, the concentration of 9,10-diHOME—an enantiomer of 12,13-diHOME—did not differ between the two groups (Supplementary Fig. 3a, Supplementary Table 8).

Specific bacterial EH genes produce 12,13-diHOME and decrease lung T_{reg} cells in CRA-challenged mice. 12,13-diHOME is a terminal product of linoleic acid (LA) metabolism, which is initially converted to 12,13-EpOME either enzymatically via a cytochrome P450 epoxygenase³² or spontaneously through oxidation. Then, 12,13-EpOME is converted to 12,13-diHOME by an epoxide hydrolase (EH)—enzymes encoded by humans, bacteria and fungi^{33–35}. To screen for potential sources of 12,13-diHOME in neonatal faeces, the aforementioned 26 neonatal stool samples were sequenced using shotgun metagenomic sequencing. A database of known bacterial (~73,000), fungal (~5,000) and human (~50) EH

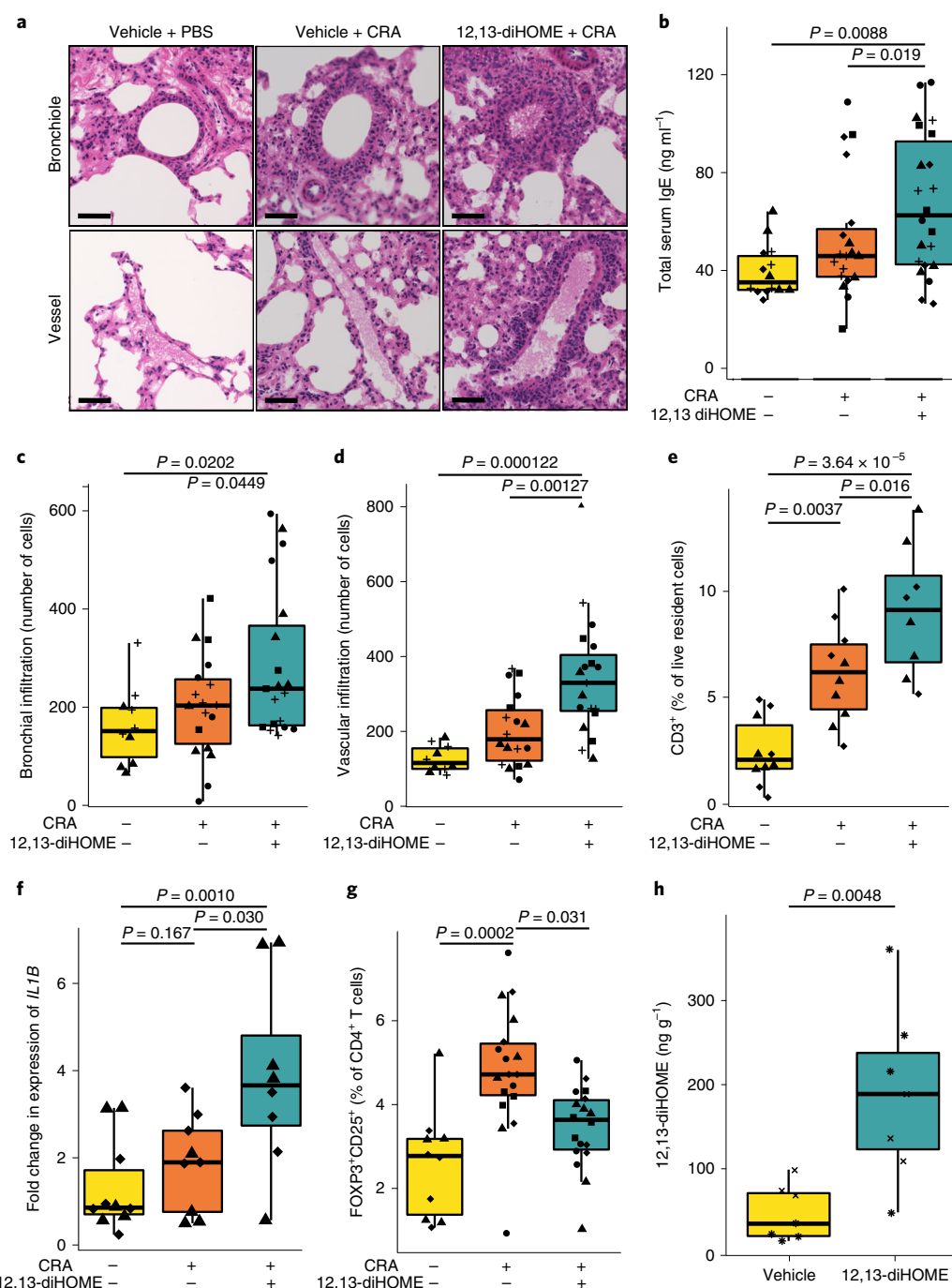


Fig. 1 | Peritoneal treatment with 12,13-diHOME exacerbates lung inflammation in mice challenged with CRA. **a**, Haematoxylin and eosin-stained bronchioles and blood vessels from the lungs of mice treated with vehicle (10% DMSO) and challenged with PBS or CRA, or treated with 30 mg kg⁻¹ 12,13-diHOME (solubilized in 10% DMSO) and challenged with CRA. Scale bars, 50 μ m. Representative of four experimental repeats. **b**, Peritoneal 12,13-diHOME treatment ($n = 22$ mice) increases serum IgE compared with vehicle-treated CRA-challenged mice ($n = 19$ mice; linear mixed effects (LME), $P = 0.019$) or vehicle-treated, PBS-challenged mice ($n = 14$ mice; LME, $P = 0.0088$). **c, d**, 12,13-diHOME treatment ($n = 19$ mice) increases the number of infiltrating cells surrounding the bronchioles (**c**) and veins (**d**) of CRA-challenged mice compared with mice treated with vehicle and challenged with PBS ($n = 10$ mice; LME, $P = 0.0202$ (bronchial), $P = 0.000122$ (venous)) or mice treated with vehicle and challenged with CRA ($n = 18$ mice; LME, $P = 0.0449$ (bronchial), $P = 0.00127$ (venous)). **e**, Peritoneal 12,13-diHOME treatment increases the number of lung-resident T cells (CD3⁺) in CRA-challenged mice ($n = 8$ mice) compared with mice treated with vehicle and challenged with PBS ($n = 10$ mice; LME, $P = 4.69 \times 10^{-6}$) or mice treated with vehicle and challenged with CRA ($n = 10$ mice; LME, $P = 0.016$). **f**, 12,13-diHOME treatment of mice challenged with CRA ($n = 8$ mice) increases the expression of *IL1B* compared with expression in mice treated with vehicle and challenged with PBS ($n = 9$ mice; LME, $P = 0.0010$) or mice treated with vehicle and challenged with CRA ($n = 9$ mice; LME, $P = 0.030$). **g**, 12,13-diHOME treatment of mice challenged with CRA ($n = 18$ mice) decreases the number of T_{reg} cells compared with the number in mice treated with vehicle and challenged with CRA ($n = 18$ mice; LME, $P = 0.031$). **h**, Peritoneal treatment of mice with 12,13-diHOME significantly increases the concentration of 12,13-diHOME in the lungs ($n = 7$ mice; LME, $P = 0.0048$) at 3 h after delivery. Unique symbols (triangles, squares, diamonds, circles, pluses, asterisks and crosses) represent mice from independent repeats. All data were plotted using boxplots from ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5 $\times$ the interquartile range (whiskers). See also Supplementary Fig. 1.

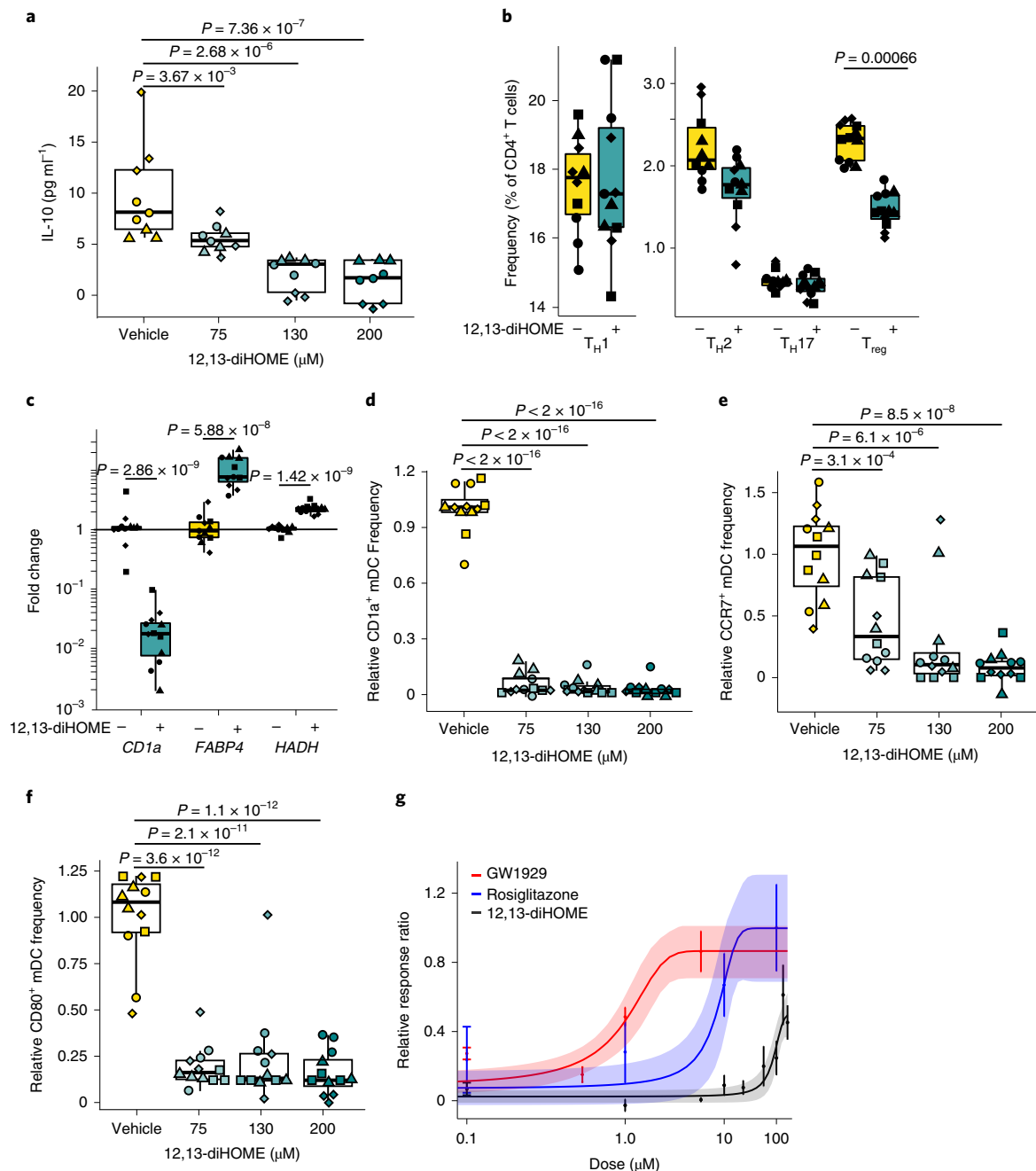


Fig. 2 | 12,13-diHOME acts through PPAR γ on DCs to decrease the number of Tregs. **a**, Treatment with 12,13-diHOME causes a dose-dependent decrease in IL-10 secretion from human DCs ($n = 3$ human donors; LME, $P = 3.67 \times 10^{-3}$, $P = 2.68 \times 10^{-6}$ and $P = 7.36 \times 10^{-7}$ for concentrations of 75 μ M, 130 μ M and 200 μ M, respectively). **b**, Treatment of human DCs with 130 μ M 12,13-diHOME causes a specific decrease in the frequency of T_{reg} cells ($n = 4$ human donors; CD3⁺CD4⁺CD25⁺FOXP3⁺; LME, $P = 0.00066$). **c**, Treatment of human DCs with 130 μ M 12,13-diHOME causes changes in gene expression consistent with activation of PPAR γ ($n = 4$ human donors; LME, $P = 2.86 \times 10^{-9}$, $P = 5.88 \times 10^{-8}$ and $P = 1.42 \times 10^{-9}$ for CD1a, FABP4 and HADH, respectively). Fold changes ($2^{-\Delta\Delta C_t}$) relative to vehicle control are presented on a log scale. **d–f**, 12,13-diHOME decreased the expression of CD1a (**d**), CCR7 (**e**) and CD80 (**f**) on human DCs (CD3⁺CD19⁺CD11c⁺; $n = 4$ human donors; LME, comparing vehicle with 75 μ M 12,13-diHOME the P values were $P < 2.0 \times 10^{-16}$, $P < 3.1 \times 10^{-4}$ and $P < 3.6 \times 10^{-12}$ for CD1a, CCR7 and CD80, respectively). **g**, Raw264.7 cells transfected with a PPAR γ -activated luciferase reporter and treated with 12,13-diHOME or known PPAR γ agonists GW1929 ($K_d = 1.4$ nM)⁵⁶ and rosiglitazone ($K_d = 40$ nM)⁵⁶ ($n = 3$ independent experiments; graph depicts median and s.e.m. for all datapoints). For **a–f**, data are presented as boxplots generated using ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5 $\times$ the interquartile range (whiskers). For **a–g**, experiments were performed with $n = 3–4$ treatment replicates using cells isolated from three or four independent donors (biological replicates are represented by triangles, diamonds, circles and squares). See also Supplementary Fig. 2.

genes was assembled and analysed using ShortBred³⁶ to probe the neonatal metagenomic data for sequence reads with EH homology. No fungal or human EH genes were detected in the neonatal

shotgun sequence dataset; however, approximately 1,400 putative bacterial EH genes were identified. Bacterial EH genes were significantly more abundant in the stool of neonates who developed

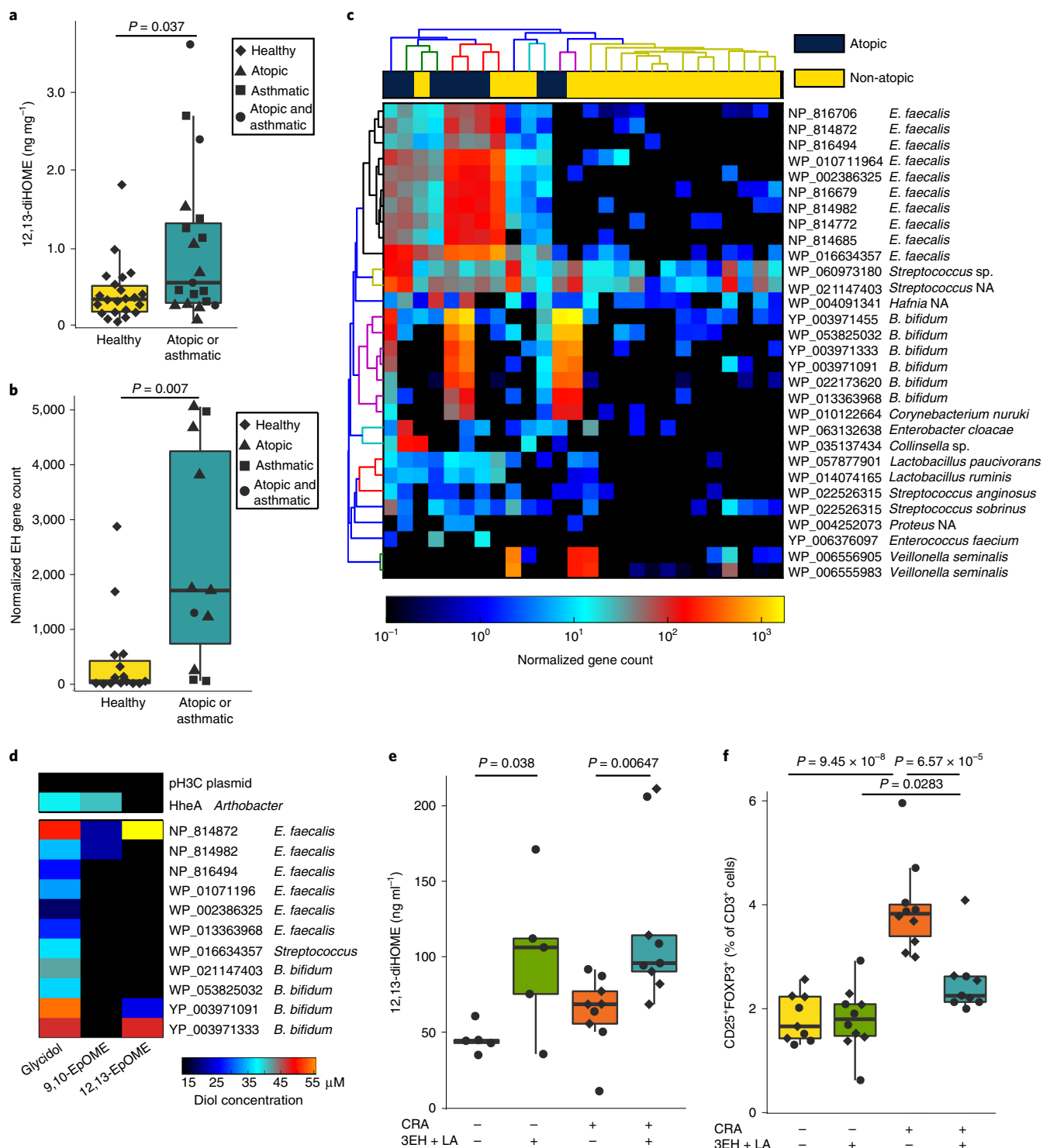


Fig. 3 | Neonatal gut-microbiome-derived EH genes from *B. bifidum* and *E. faecalis* produce 12,13-diHOME and decrease the number of lung T_{reg} cells in mice challenged with CRA. **a**, Neonates who develop childhood atopy and/or asthma exhibit increased faecal concentrations of 12,13-diHOME ($n = 41$ neonates; LR adjusting for race, $P = 0.037$; see Supplementary Table 6 for a full summary of the statistical analysis). **b**, Neonates who develop atopy and/or asthma during childhood have more bacterial EH genes in their stool ($n = 26$ neonates; LR adjusting for formula feeding; $P = 0.007$; Supplementary Table 7). **c**, The 30 most abundant EH genes identified in stool are enriched in neonates who develop atopy. Dendrograms represent hierarchical clustering of bacteria and genes generated using HClust2. NA, not applicable. **d**, Three of the candidate EH genes, *E. faecalis* NP_814872, *B. bifidum* YP_003971091 and *B. bifidum* YP_003971333, convert 12,13-EpOME to its conjugate diol, 12,13-diHOME. **e**, Mice orally gavaged with LA and *E. coli* overexpressing NP_814872, YP_003971091 and YP_003971333 (3EH and LA) have significantly more 12,13-diHOME in their plasma than those supplemented with vehicle (LME; no CRA $n = 5$ mice, $P = 0.038$; CRA-challenged $n = 9$ mice, $P = 0.0065$). **f**, Mice treated with 3EH and LA and challenged intratracheally with CRA had significantly fewer lung T_{reg} cells than mice treated with vehicle and challenged with CRA (LME; CRA-challenged $n = 9$ mice, $P = 6.57 \times 10^{-5}$). For **a**, **b**, **e** and **f**, data are presented as boxplots that were generated using ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5x the interquartile range (whiskers). See also Supplementary Fig. 3 and Supplementary Tables 6 and 7.

atopy and/or asthma during childhood (Fig. 3b, Supplementary Table 9). The 30 most abundant of which were primarily encoded by the strains *Enterococcus faecalis*, *Streptococcus*, *Bifidobacterium bifidum* and *Lactobacillus* (Fig. 3c), common early-life gut microbiome members.

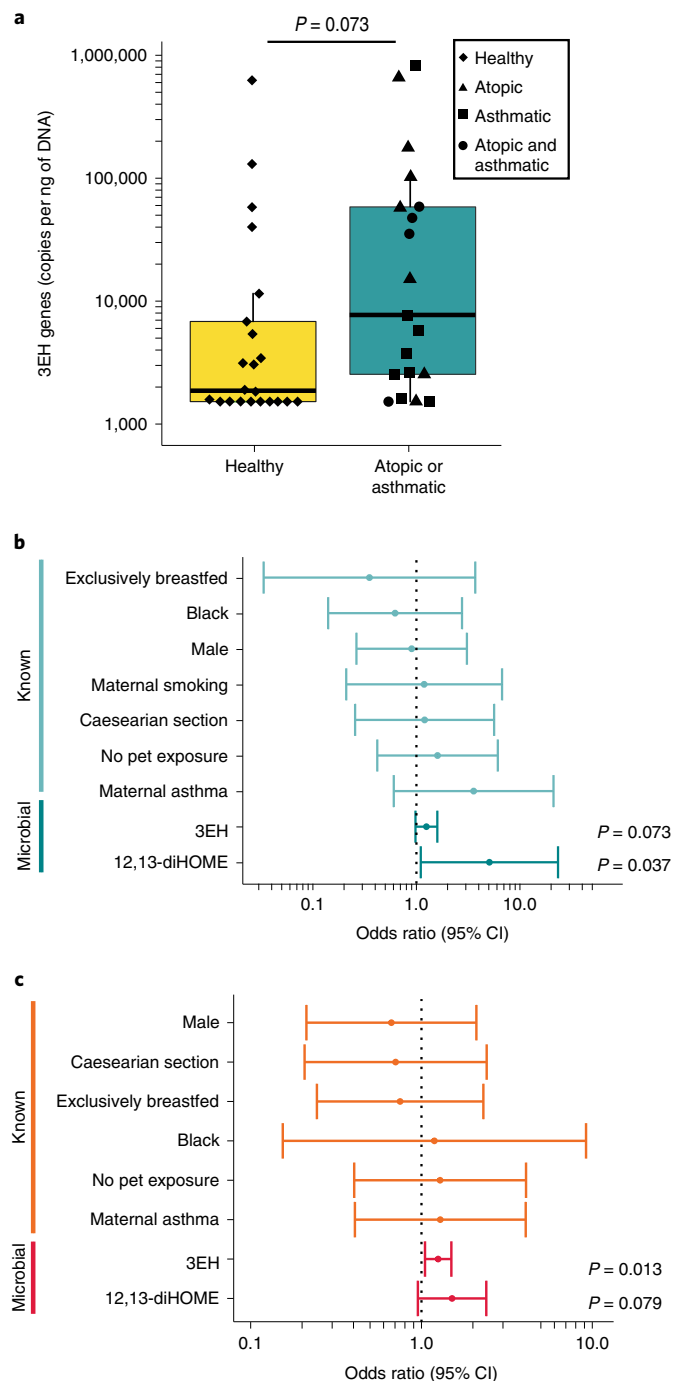
To evaluate whether these putative EH genes are capable of hydrolysing epoxides and producing 12,13-diHOME, we developed a cell-based functional assay. A subset ($n=11$) of the most frequently detected putative bacterial EH genes with $\geq 75\%$ of homologous EH marker regions identified in the metagenomic data were selected for functional screening (Supplementary Table 1). These genes were synthesized, cloned into *Escherichia coli* for expression and EH activity was measured using modifications of a previously described colorimetric assay³⁷. All 11 genes screened were capable of hydrolysing glycidol, a generic epoxide, to its conjugate diol, glycerol, and three of them hydrolysed 9,10-EpOME to 9,10-diHOME. However, only NP_814872 (*E. faecalis*), YP_003971091 (*B. bifidum*) and YP_003971333 (*B. bifidum*) converted 12,13-EpOME to 12,13-diHOME (Fig. 3d), indicating that—although bacterial EH activity is common in the neonatal gut microbiome—bacterial capacity to produce 9,10-diHOME or 12,13-diHOME appears less common and strain specific.

Next, we examined whether the introduction of the 3EHs capable of synthesizing 12,13-diHOME could recapitulate the reduction in T_{reg} cells observed in 12,13-diHOME-treated mice (Fig. 1). To test this, mice were orally supplemented with *E. coli* strains that overexpressed NP_814872 (*E. faecalis*), YP_003971091 (*B. bifidum*) and YP_003971333 (*B. bifidum*), and LA, a precursor to 12,13-diHOME. Mice were then subjected to the CRA model of allergic airway inflammation used in Fig. 1 (Supplementary Fig. 3e). Mice supplemented with the 3EH strains and LA showed significantly higher concentrations of 9,10-diHOME and 12,13-diHOME in their plasma compared with vehicle-treated controls (Fig. 3e, Supplementary Fig. 3f). Although the plasma concentrations of 12,13-diHOME in this experiment were almost an order of magnitude lower than those observed after peritoneal injection (compare Fig. 3e with Supplementary Fig. 1n), we still observed a significant decrease in the number of T_{reg} cells in the lungs of CRA-challenged

mice supplemented with the 3EH-expressing bacteria and LA (Fig. 3f). Together, these results indicate that oral supplementation with 12,13-diHOME-producing bacteria and LA is sufficient to recapitulate the decrease in mouse lung T_{reg} cells, a cardinal immune feature of airway allergic inflammation.

An increase in neonatal faecal 3EH gene copy number and 12,13-diHOME concentration are associated with atopy, eczema and asthma during childhood. Our data indicated that elevated concentrations of 12,13-diHOME promote allergic inflammation in vitro and in vivo, and that specific bacterial epoxide hydrolases identified in the gut microbiome of neonates at high risk of asthma both produce 12,13-diHOME and promote airway allergic inflammation in mouse models. We therefore investigated whether neonatal gut microbiome 3EH copy number or the concentration of

Fig. 4 | Increased concentrations of 12,13-diHOME and the 3EH genes in neonatal stool are associated with the development of childhood atopy, eczema and/or asthma in two US cohorts. a, Increased bacterial 3EH copy number is observed in neonates who developed atopy and/or asthma, and corresponds with the significant increase in overall faecal EH genes identified by ShortBred analysis ($n=41$ neonates; LR, $P=0.0387$; Fig. 3b, Supplementary Table 10). Data are presented as a boxplot that was generated using ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5x the interquartile range (whiskers). **b**, In a subset of the WHEALS cohort, an increase in 12,13-diHOME concentration in neonatal stool significantly increased the relative probability of developing atopy at two years of age and/or asthma at four years of age ($n=41$ neonates; LR, 3EH⁺: OR=1.25, 95% CI=0.98–1.59, $P=0.073$; 12,13-diHOME adjusted for race: OR=5.07, 95% CI=1.10–23.3, $P=0.037$; see Supplementary Table 12 for a full summary of the statistical analysis). For 3EH, analysis was performed using a log₂ transformation of the 3EH copy number. **c**, In a subset of the TIPS birth cohort, an increase in faecal 3EH copy number was associated with a significantly increased relative probability of developing eczema in two-year-old children and/or asthma in four-year-old children ($n=50$ neonates; LR, 3EH⁺: OR=1.25, 95% CI=1.05–1.50, $P=0.013$; 12,13-diHOME: OR=1.51, 95% CI=0.95–2.40, $P=0.079$; see Supplementary Table 14 for a full summary of the statistical analysis). For 3EH, analysis was performed using a log₂ transformation of the 3EH copy number. The OR and CI for each risk factor are shown in **b** and **c**. See also Supplementary Fig. 3 and Supplementary Tables 6–14.



12,13-diHOME could be used to predict risk of atopy or asthma in childhood. Indeed, faecal 3EH copy number was significantly increased in one-month-old neonates who subsequently developed atopy and/or asthma during childhood (Fig. 4a, Supplementary Table 10; $n=41$). Using our subset of the WHEALS cohort ($n=41$; atopic/asthmatic, $n=19$; Supplementary Tables 8 and 11) and logistic regression (LR), we examined the probability of children developing atopy at two years of age and/or asthma at four years of age on the basis of neonatal faecal 12,13-diHOME concentration, faecal 3EH copy number or known early-life risk factors selected a priori on the basis of previously published literature, including gender, race, maternal asthma, lack of pets, maternal smoking, delivery by caesarean section and formula feeding at one month of age^{38–41}.

In our subset of the WHEALS cohort, we found that increased 12,13-diHOME concentration in neonatal stool was associated with a significantly increased probability of developing atopy and/or asthma during childhood (Fig. 4b, Supplementary Table 12). Logistic regression (LR) was used to model this relationship and test for potential confounding by known risk factors. Multivariate LR identified race as a potential confounding factor of the relationship between faecal 12,13-diHOME concentration and disease development (see Supplementary Table 8 for the full analysis). Adjusting for race, we found that each additional ng of 12,13-diHOME per mg of neonatal faeces increased the probability of developing childhood atopy and/or asthma by a factor of 5 (adjusted odds ratio (OR)=5.07, 95% confidence interval (CI)=1.10–23.3, $P=0.016$; Supplementary Table 12).

Given our small sample size, we sought to validate these findings in an independent neonatal cohort. Accordingly, we quantified 12,13-diHOME concentration and 3EH copy number in one-month stool samples from the placebo arm of the Trial of Infant Probiotic Supplementation (TIPS) study⁴². This trial—performed in a predominantly white birth cohort in San Francisco, California—examined the effect of probiotic supplementation on the development of eczema and asthma. Those in the placebo arm with >300 mg of stool collected at one month of age, eczema outcomes collected at two years of age and asthma outcomes collected at 4 years of age were included in our analysis (Supplementary Tables 7 and 13; total $n=50$, eczema $n=22$, asthma $n=6$, eczema and/or asthma $n=23$).

In our subset of the TIPS cohort, increased faecal 3EH copy number was associated with the development of eczema at two years of age and/or asthma at four years of age (OR=1.25, 95% CI=1.05–1.50, $P=0.013$; Supplementary Table 14), whereas increased faecal 12,13-diHOME concentration did not reach our threshold for significance (12,13-diHOME, OR=1.51, 95% CI=0.95–2.40, $P=0.079$; Fig. 4c, Supplementary Table 14). In this predominantly white cohort, the associations between one-month-old faecal 3EH copy number or 12,13-diHOME and the development of eczema and/or asthma during childhood did not show strong evidence of confounding by known risk factors (all change in OR <10%; Supplementary Table 14). Despite the differences in geography and study design between the WHEALS and TIPS cohorts (see Methods), neonates who went on to develop eczema and/or asthma during childhood exhibited a significant increase in the faecal abundance of 3EH genes. Although these microbial risk genes require validation in additional larger cohorts, they represent exciting targets for future study and potential intervention.

Discussion

Our previous study determined that one-month-old neonates who subsequently developed childhood atopy and/or asthma exhibited a perturbed gut microbiome and increased concentrations of 12,13-diHOME¹¹. In this study, we aimed to determine whether this oxylipin promotes proallergic immune dysfunction in vitro and in vivo, in an attempt to establish a mechanistic link between metabolic dysfunction of the gut microbiome during early life and the

development of childhood disease. Consistent with this hypothesis, intra-abdominal delivery of 12,13-diHOME exacerbated airway inflammation, reduced the frequency of lung T_{reg} cells and increased the concentration of serum IgE in mice (Fig. 1). In vitro, we found that 12,13-diHOME interacts directly and agonistically with PPAR γ on DCs, altering expression of PPAR γ -regulated genes involved in fatty acid uptake, metabolism and presentation (Fig. 2c). We observed that the profile of DC gene expression is consistent with that shown by Lynes et al., who demonstrated that 12,13-diHOME leads to an increase in fatty-acid uptake in skeletal muscle and brown adipose tissue^{17,18}. Here we showed that 12,13-diHOME upregulated FABP4 and HADH—PPAR γ -regulated genes involved in fatty acid uptake and metabolism—indicating that common cellular mechanisms may underlie the effect of this molecule on distinct mammalian cell populations and tissues.

Given that 12,13-diHOME promoted allergic inflammation in vitro and in vivo, we aimed to identify potential sources of 12,13-diHOME in the neonatal gut microbiome. Metagenomic screening identified a large number of putative bacterial EHs and indicated that the faeces of high-risk neonates are enriched with these bacterial genes (Fig. 3c). Of the most frequently detected putative EH genes, several distinct genes were encoded by *B. bifidum* and *E. faecalis*, indicating that strain-specific variance in EH gene content and function exists in the neonatal gut microbiome. Indeed of the 11 EHs screened, only 3 were capable of producing 12,13-diHOME (Fig. 3d). The copy number of these three bacterial genes was both significantly enriched in the stool of neonates at high risk for childhood atopy and/or asthma and associated with an increased probability of allergic disease in two small, but racially, geographically and socioeconomically distinct birth cohorts. Notably, the TIPS and WHEALS cohorts were collected in the 2000s, in different cities, several years apart. Previous studies have demonstrated that collection and storage conditions alter the microbial and metabolic profiling of stool^{43,44}. Although this represents a limitation, the associations between 12,13-diHOME, the 3EH genes and development of allergic disease remained consistent between these distinct cohorts. Although further testing in larger cohorts is required, this suggests that the observed associations may be generalizable to a broader population.

Our data indicate that an increased copy number of specific bacterial EH genes and elevated concentration of their product—12,13-diHOME—in the gut microbiome during early life are related to clinical outcomes of atopy, eczema and asthma during childhood. Humans have the capacity to produce 12,13-diHOME³⁵, and all of the infants in our study showed baseline levels of faecal 12,13-diHOME. However, neonates whose gut microbiomes encode bacterial EH genes with the confirmed ability to produce this lipid exhibited increased concentrations of 12,13-diHOME, highlighting the importance of bacterial production of 12,13-diHOME in high-risk neonates. Our data support a role for 12,13-diHOME in promoting allergic inflammation both in vitro and in vivo. The observation that 12,13-diHOME induces altered lipid uptake, metabolism and presentation, and reduced IL-10 secretion by human DCs suggests that it may mediate its proallergic effect in part by altering immune cell metabolism. We acknowledge that other microbial-derived products likely contribute to early-life immune dysfunction associated with childhood atopy and asthma. This study exemplifies just one component of a complex microbiome–immune interaction in high-risk neonates that promotes allergic inflammation and offers a mechanism by which metabolic products of neonatal gut bacteria may increase susceptibility to allergies and asthma during childhood.

Methods

Animal models. Six-week-old female C57BL/6 mice were obtained from Jackson Laboratories and randomized to treatment groups. All experiments were performed with 3–5 mice per cage; mice were cohoused for 2–3 weeks before

treatment to minimize the effect of cages. Each treatment group was housed independently to mitigate potential effects of cohousing and coprophagy. Housing from the same assay were stored in the same rack and handled identically to minimize microbial variation⁴⁵. To determine sample size, a pilot study was performed to estimate the effect size ($d=5.47$) of 12,13-diHOME on T_{reg} cell frequency in mouse lungs. This estimated effect size was used to determine the minimal sample size ($n=3$) given an $\alpha=0.05$ and a $\beta=0.80$.

For the studies presented in Fig. 1a–g and Supplementary Fig. 1a–k, mice were treated on days 1, 2, 3, 14 and 21 with 30 mg kg⁻¹ 12,13-diHOME solubilized in 10% dimethylsulfoxide (DMSO) or vehicle (10% DMSO) by peritoneal injection. Three hours after injection, mice were challenged intratracheally with either PBS or CRA (20,000 PNU ml⁻¹; Greer). Twenty-four hours after the final challenge, mice were anaesthetized, injected retro-orbitally with 100 µl CD45-APC (1:10), allowed to recover from anaesthesia and subsequently euthanized. Serum and lung tissue were collected from all mice subjected to allergen challenge. Serum concentrations of IgE were determined for all animals from which more than 20 µl of serum was obtained by cardiac puncture ($n=57$). After euthanasia, the right and left lungs were separated. In the majority of repeats, the inferior lobes of the left lungs were processed for histological analysis ($n=47$; Fig. 1c,d, Supplementary Fig. 1a,b). The remaining lobes of the left lung were processed for flow cytometry and stained for T_{reg} cells ($n=47$; Fig. 1g, Supplementary Fig. 1d), whereas the right lung was either processed for qPCR ($n=27$) or flow cytometry ($n=28$). There were two exceptions to this: in repeat P1A6 (represented by plus symbols in Fig. 1) the left lung was not processed or stained for T_{reg} cells; in repeat P1A5 (represented by diamond symbols in Fig. 1) histological analysis was not performed.

Serum was isolated, and serum IgE levels were measured using a mouse IgE ELISA Max kit (Biolegend). Lungs were sent to the Mouse Pathology Core at University of California, San Francisco (UCSF) for haematoxylin and eosin staining of paraffin-embedded tissue sections. Two bronchioles and two vessels from each stained tissue section were scored on a 0–4 scale in which 0 represents structures with no inflammatory infiltrates; 1 represents few inflammatory cells; 2 represents a ring of inflammatory cells that is 1 cell layer wide; 3 represents a ring of inflammatory cells that is 2–4 cells wide; and 4 represents a ring of inflammatory cells more than 4 cells wide. Each structure was scored by two blinded individuals, and scores were averaged for each animal. Cell counts were determined for each structure by a blinded individual. In brief, the ImageJ freehand selection tool was used to trace the perimeter of each bronchiole and vessel. The area extending beyond the perimeter of the vessel was cleared and the colour threshold of the image was adjusted using the default method with the following parameters: hue = 0–255, saturation = 0–255, brightness = 130–255, threshold colour = white, background = dark, colour space = HSB. The image was converted to an 8-bit greyscale and the threshold was adjusted using the B&W defaults and a range of 0–150. Counts were outlined and summarized using the analyse particles window (size = 0–infinity, circularity = 0.0–1.00).

Lung cell subsets were assessed using flow cytometry. Lung tissue was manually dissected, digested with 5 mg per sample collagenase (Sigma-Aldrich) and passed through a 40 µm filter to generate single-cell suspensions. CD45⁺ lung cells were considered to be resident cells and further classified as T cells (CD3⁺), neutrophils (Ly6G⁺CD11b⁺), monocytes (F4/80⁺Ly6C⁺Ly6G⁺CD11b⁺) and alveolar macrophages (Siglec-F⁺F4/80⁺CD11c⁺CD11b⁺). The gating strategy can be found in Supplementary Fig. 1c. T_{reg} cells were identified as CD3⁺CD4⁺CD25⁺FOXP3⁺ cells using the human T cell gating strategy. A representative flow plot can be found in Supplementary Fig. 1d. Investigators were blinded to treatment groups while collecting and gating flow cytometry data.

Lung tissue was preserved in RNAlater (Ambion). Preserved lung tissue was homogenized in Lysing Matrix E Tubes (MP Biomedicals) using a FastPrep 24 homogenizer (MP Biomedicals) and extracted using an RNeasy Mini Kit (Qiagen). An RT2 First Strand Kit (Qiagen) was used to synthesize cDNA, and expression of *IL1A*, *IL1B* and *TNF* relative to *GAPDH* was measured using a Power SYBR Green PCR Master Mix (ThermoFisher Scientific) and a QuantStudio 6 Real-Time PCR system (ThermoFisher Scientific). Primers are summarized in Supplementary Table 3.

For the quantification of 12,13-diHOME in mouse lung and plasma samples (Fig. 1h, Supplementary Fig. 1n), six-week-old female C57BL/6 mice were purchased from Jackson Laboratories and treated with 30 mg kg⁻¹ 12,13-diHOME solubilized in 10% DMSO or vehicle (10% DMSO) by peritoneal injection. Three hours after a single injection, mice were euthanized and lung tissue and plasma were collected and frozen immediately in liquid nitrogen. Then, 12,13-diHOME was extracted from the tissue and plasma using an established solid-phase extraction protocol⁴⁶. In brief, flash-frozen tissue was massed and added to a Lysing Matrix E tube containing 1 ml of methanol, 10 µl 0.2 mg ml⁻¹ BHT/EDTA and 1.25 ng 12,13-diHOME-D4 (internal standard, Cayman Chemical) then homogenized as described above. Tissue samples were centrifuged for 10 min at 2,125g, and the supernatant was transferred to a Falcon tube containing 19 ml deionized water to generate a 5% methanol solution. Plasma samples were thawed on ice and 10 µl 0.2 mg ml⁻¹ BHT/EDTA and 1.25 ng 12,13-diHOME-D4 were added to 250 µl of thawed plasma. All samples were extracted using Waters Oasis HLB Cartridges (60 mg of sorbent, 30 µm particle size) as previously described²⁰.

Extracted samples were resuspended in methanol, and LC–MS was performed using a QTRAP 6500 mass spectrometer (AB Sciex) and a Nexera X2 HPLC (Shimadzu Scientific Instruments) as previously described²⁰. Linear standard curves were generated using six injections of 12,13-diHOME, 9,10-diHOME and 12,13-diHOME-D4 (internal standard). Peaks were manually integrated, and recovery of the internal standard was used to correct for extraction efficiency.

For the mouse studies shown in Fig. 3e,f and Supplementary Fig. 3e–h, six-week-old female C57BL/6 mice were obtained from Jackson Laboratories and orally gavaged with the three *E. coli* strains that overexpress NP_814872, YP_003971091 and YP_003971333, and LA before intratracheal challenge with CRA. In brief, BL21 cells containing recombinant plasmids (described below in the ‘Colorimetric detection of EH Activity’ section) were streaked onto Lauria–Bertani (LB) agar plates supplemented with 50 µg ml⁻¹ kanamycin. Isolated colonies were selected and used to inoculate 10 ml LB supplemented with 50 µg ml⁻¹ kanamycin. Starter cultures were grown for 18 h at 37 °C and 200 r.p.m. and used to inoculate 120 ml LB supplemented with kanamycin. These cultures were grown until the optical density at 660 nm (OD₆₆₀) reached 0.6, at which point expression was induced with 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG; Sigma-Aldrich). The temperature was reduced to 25 °C and incubation continued for 18 h at 200 r.p.m. Cultures were then centrifuged for 15 min at 3,000g, and the OD₆₆₀ was adjusted to 6. The three strains were combined to make a 25% glycerol stock solution with approximately 1 × 10⁹ colony forming units per strain and frozen in 500 µl aliquots.

Mice were treated by oral gavage and subjected to intratracheal challenge on days 1, 2, 3, 14, and 21 (Supplementary Fig. 3e). Each challenge was preceded by 4 d of oral gavage with either 75 µl 3EH and LA or 25% glycerol. Immediately before treatment, 250 µl filter-sterilized LA (Sigma-Aldrich) was added to each 500 µl 3EH glycerol stock. Mice were challenged intratracheally with either 50 µl CRA or PBS. On day 22, mice were euthanized and their lungs and plasma were collected. The concentration of 12,13-diHOME and 9,10-diHOME in the plasma and the frequency of lung T_{reg} cells were determined as described above. All mouse studies were reviewed and approved by the University of California San Francisco’s Institutional Care and Use Program. Investigators were blinded to treatment groups while collecting and scoring all flow cytometry and mass spectrometry data.

Human immune assays. 12,13-diHOME purchased from Cayman Chemical was stored at –20 °C in methyl acetate until use. Although 12,13-diHOME may be stably stored in organic solvents for several years, it is relatively unstable in aqueous solutions; this was reflected in the need for high concentrations of this lipid in our *in vitro* assays. We attempted to mitigate these limitations by solubilizing 12,13-diHOME in DMSO and preparing aqueous solutions immediately before use; however, the concentrations used in our *in vitro* assays are unlikely to directly reflect those found in tissue.

To prevent degradation, fresh solutions of 12,13-diHOME solubilized in DMSO were prepared immediately before use (<2 h). For all experiments, methyl acetate was removed under a nitrogen stream, and the compound was resuspended in DMSO (maximum solubility 20 mg ml⁻¹) before addition of PBS or media. Fresh media containing 12,13-diHOME was added every 48 h to *in vitro* assays. This strategy of solvent evaporation and subsequent resuspension in DMSO was used for several related oxylipins sold by Cayman Chemical, including 9,10-diHOME, 12,13-EpOME, 9-HODE and 13-HODE, none of which had a significant effect on the frequency of T_{reg} cells (Supplementary Fig. 2b).

IL-10 secretion was measured using a cytometric bead array (BD Biosciences). Human DCs were isolated from the peripheral blood mononuclear cells (PBMCs) of two healthy human donors as previously described⁴¹ and treated with increasing concentrations of 12,13-diHOME (Cayman Chemical) solubilized in 0.2% DMSO. Human PBMCs were isolated from human plasma that was purchased from the Blood Centers of the Pacific and were not subject to UCSF institutional review board (IRB) approval. Supernatant was collected after 24 h. IL-10 concentrations were determined according to the manufacturer’s instructions.

Co-culture of human DCs and T cells in the presence of 12,13-diHOME was performed as previously described⁴¹. In brief, DCs were isolated from the PBMCs of two healthy human donors and treated for 5 d with 130 µM 12,13-diHOME solubilized in 0.2% DMSO or vehicle (0.2% DMSO) in the presence of 20 ng ml⁻¹ IL-4 (R&D Systems) and 10 ng ml⁻¹ GM-CSF (R&D Systems). Fresh treatment media was added every 48 h throughout the course of the study. Then, 18 h before co-culture, DCs were stimulated with 10 ng ml⁻¹ TNF, IL-1β and IL-6 (Peprotech) and 1 mM prostaglandin E2 (Stemcell Technologies). DCs were subsequently washed and co-cultured with autologous T cells in the presence of 10 ng ml⁻¹ anti-CD28 and anti-CD49d antibodies (BD Bioscience). Flow cytometry was used to authenticate human DCs and T cells before co-culture, and primary cells were not tested for mycoplasma.

After 5 d of co-culture, T cell subsets were analysed by flow cytometry. To assess cytokine secretion, cells were stimulated for 16 h with phorbol myristate acetate and ionomycin (ACROS) and GolgiPlug (Gplug; BD Biosciences). Antibodies used for staining are summarized in Supplementary Table 2. Flow cytometry data were collected on a BD LSR II flow cytometer. Helper T cell subsets were defined as follows: T_H1 , CD3⁺CD4⁺IFNγ⁺; T_H2 , CD3⁺CD4⁺IL-4⁺; T_H17 ,

CD3⁺CD4⁺IL-17⁺; T_{reg} cells, CD3⁺CD4⁺CD25⁺FOXP3⁺. The human T cell gating strategy can be found in Supplementary Fig. 1e.

Human DC maturation was assessed by flow cytometry. PBMCs were isolated from two healthy human donors using Ficoll–Hypaque gradient centrifugation as previously described⁴¹ and cultured for 5 d in the presence of 20 ng ml⁻¹ IL-4 and 10 ng ml⁻¹ GM-CSF. Monocytic DCs were defined as CD3⁻CD19⁻CD11c⁺. The DC gating strategy can be found in Supplementary Fig. 1h.

Gene expression was examined in human DCs. DCs were isolated as described above and treated for 2 d with 130 µM 12,13-diHOME. Cells were washed twice in PBS and RNA was extracted using the RNeasy Micro Kit (Qiagen). An RT2 First Strand Kit (Qiagen) was used to synthesize cDNA, and expression of *CD36*, *CD1A*, *FABP4* and *HADH* relative to *ACTB* was measured using Power SYBR Green PCR Master Mix (ThermoFisher Scientific) and a QuantStudio 6 Real-Time PCR system (ThermoFisher Scientific). Fold changes (2^{-ΔΔC_t}) relative to vehicle treatment were plotted on a log scale in Fig. 1d,e. Primers are summarized in Supplementary Table 3.

Luciferase assay. A modified PPARγ luciferase assay was performed as described in Ye et al.⁴⁵. A PPRE-luciferase reporter plasmid, PPRE X3-TK-luc from B. Spiegelman (1015; Addgene), and a PPARγ overexpression plasmid, pGST-PPARγ from B. Vogelstein (16549; Addgene), were purified using a Plasmid Plus Maxi Kit (Qiagen). Raw264.7 cells were obtained from and authenticated by the UCSF Cell Culture Facility and were not tested for mycoplasma contamination. Cells were grown in R10 medium (Roswell Park Memorial Institute (RPMI) medium 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 100 U ml⁻¹ penicillin–streptomycin) were transfected with PPRE reporter:PPARγ:Renilla luciferase plasmid DNA at a ratio of 1:20:40 using 50 ng per well of reporter plasmid DNA. Eugene HD transfection reagent (Promega) was combined with plasmid DNA at a ratio of 4:1 and the total volume was brought to 5 µl per well with RPMI. The transfection mixture was gently combined with diluted Raw264.7 cells and plated in black clear-bottom 96-well plates at a density of 50,000 cells per well. Twenty-four hours after transfection, cells were treated with 12,13-diHOME, rosiglitazone or GW1929 (Cayman Chemical) solubilized in 0.1% DMSO. Twenty-four hours after treatment, Firefly luciferase and Renilla luminescence were measured using the Dual-Glo Luciferase Assay kit (Promega) on a Cytation 3 plate reader (BioTek Instruments). Negative controls included the following: Raw264.7 cells transfected with the reporter plasmids (but no PPARγ plasmid) and treated 12,13-diHOME; untransfected untreated cells; and transfected untreated cells (Supplementary Fig. 1i).

Study population and definitions for the WHEALS cohort. WHEALS recruited pregnant women (*n* = 1,258) between the ages of 21 and 49 between August 2003 and November 2007 in southeast Michigan⁴⁷. Women were considered to be eligible if they lived in a predefined cluster of contiguous zip codes near Detroit, Michigan, had no intention of moving out of the area and provided informed written consent. Follow-up interviews were conducted at 1, 6, 12, 24 and 48 months after birth. The appointment at 24 months occurred at a standardized study clinic, in which the child underwent evaluation by a board-certified allergist. Stool samples from children were collected during home visits at one month and used in this study.

Samples were shipped to the UCSF on dry ice, where they were stored at -80 °C until processing. Faecal DNA extracted using the modified cetyl trimethylammonium bromide (CTAB) method⁹ and previously used for fungal and bacterial profiling was stored at -20 °C until analysed in this study. Atopy was defined as per Havstad et al.¹. Children with asthma were identified by parent-reported doctor diagnosis of asthma during interview at 48 months. All research on human participants was reviewed and approved by the Henry Ford Hospital Institutional Review Board.

Characteristics of the individuals in our subset of the WHEALS birth cohort.

A subset of 41 neonates were selected from the WHEALS cohort (median age = 35 d when stool samples were taken) who had previously undergone faecal fungal and bacterial microbiota profiling¹¹ and had >50 mg of stool and >10 ng of extracted faecal DNA remaining from the samples collected during home visits at one month of age (Supplementary Table 6; total *n* = 41, atopic *n* = 7, asthmatic *n* = 8, atopic asthmatic *n* = 4). These included 26 stool samples, microbiologically representative of the variance in gut microbiota observed in this larger (*n* = 130) cohort, that had previously undergone untargeted metabolomic profiling¹¹ and 15 randomly selected faecal samples with >50 mg of stool and >10 ng of DNA, all of which had available childhood atopy and/or asthma outcomes. Power calculations were performed in G*Power v.3.1. Effect size was estimated on the basis of the relative concentration of 12,13-diHOME in high- and low-risk neonates, shown in supplementary figure 9 of Fujimura et al. 2016¹¹. The estimated effect size (*d* = 1.228), *α* = 0.05 and power = 0.95 were used to estimate the required sample size (*n* = 38, assuming allocation of N2/N1 = 1). Of the 41 samples in our subset, 19 (46%) had atopy and/or asthma, 20 (48.8%) were male and 32 (78%) were black, based on maternal self-reports. Furthermore, 8 (19.5%) were born by caesarean section, 4 (9.75%) were exclusively breastfed until one month of age, 7 (17%) reported diagnosis by a doctor of maternal asthma, 6 (14.6%) reported maternal

smoking during pregnancy and 13 (31.7%) lived with an indoor or outdoor dog or cat before delivery. The results presented in this study require replication in a larger neonatal population.

Study population and definitions for the TIPS birth cohort. The TIPS study recruited infants in the San Francisco Bay area who had at least one parent with a history of asthma and parents who were willing to supplement breast milk or formula with a probiotic supplement or placebo for at least one feed each day⁴⁸. Eligible infants were randomized to either the intervention arm, in which they received probiotic supplementation, or the placebo arm, in which they were supplemented daily for six months with 325 mg daily inulin⁴²—a fermentable prebiotic shown to increase the frequency of *Bifidobacterium* species in paediatric and adult populations⁴². Stool was collected throughout the study. Demographics data were collected after the birth of an infant and obstetric medical records were used to confirm the method of delivery. Physical examinations were conducted by study clinicians at 1, 3, 6, 12 and 24 months of age, and interviews with the family were conducted monthly for the first year of life and semi-annually in all subsequent years⁴². The TIPS study was reviewed and approved by the UCSF IRB (10-02968).

Stool samples collected from one-month-old participants in the placebo arm of randomized control trial were used in this study. Stool was collected from diapers, mailed overnight to the study team and immediately stored at -80 °C until processing. Faecal DNA was extracted using the previously described modified CTAB method⁴⁹ and stored at -20 °C until analysis.

Characteristics of the individuals in our subset of the TIPS cohort. A random subset of 50 neonates from the placebo arm of the TIPS cohort (median infant age = 32 d when stool samples were collected) that had >300 mg stool remaining from collection at one month of age were selected (total *n* = 50, eczema *n* = 22, asthma *n* = 6, eczema and asthma *n* = 23; Supplementary Table 7). Of the 50 neonates, 20 (40%) were male and 4 (8%) were black, based on a family self-report. Furthermore, 15 (30%) were born by caesarean section, 28 (56%) were exclusively breastfed until one month of age, 31 (62%) reported doctor-diagnosis of maternal asthma, 1 (2%) reported maternal smoking during pregnancy and 18 (36%) did not live with a cat or dog.

Mass spectrometry in human samples. Faecal oxylipin (9,10-diHOME and 12,13-diHOME) concentrations were assessed in our subset of the WHEALS cohort (*n* = 41). Oxylipins were extracted from approximately 50 mg of neonatal stool using the protocol described above. LC–MS was performed on a Thermo LTQ-Orbitrap-XL mass spectrometer equipped with an electrospray ionization source (ThermoFisher Scientific). The concentrations of 12,13-diHOME used in our ex vivo cell assays (~40 µg g⁻¹) and in vivo animal models (~0.2 µg g⁻¹) were within an order of magnitude of the concentrations measured in stool from high-risk neonates (0.2–4 µg g⁻¹). The stability of oxylipins in faeces is unknown. Although 12,13-diHOME is relatively stable in organic solvent, we noticed that the median concentration of 12,13-diHOME was higher in the TIPS samples compared with the older WHEALS samples (*P* = 0.0143; Supplementary Fig. 3i), indicating the potential for lipid-signal degradation in long-term-stored samples.

Metagenomic data analysis. DNA from the 26 stool samples from the WHEALS cohort that had previously undergone untargeted LC–MS¹¹ and targeted oxylipin quantification (described above) was extracted using the modified CTAB method and sent to the Vincent J. Coates Genomic Sequencing Laboratory at the California Institute for Quantitative Biosciences for 150-bp paired-end sequencing on an Illumina HiSeq 4000 (www.qb3.berkeley.edu/gsl). Recognizing that the duration of sample storage affects concentration and integrity of DNA^{45,50,51} and that the WHEALS stool samples were collected in the early 2000s, metagenomic sequencing was only performed on samples that had sufficient concentrations of DNA with sufficient integrity to produce high-quality sequencing data for our metagenomic screen (*n* = 26). Sequencing efforts resulted in an average of 4.41 Gb (range of 3.05–7.68 Gb) of data per sample with an average coverage of 66.5 reads per contig (range of 21.8–148.8) as determined by Megahit⁵². Sequencing reads were quality-trimmed to Q17 with BBduk (<https://sourceforge.net/projects/bbmap/>). A database of approximately 78,000 known bacterial (~73,000), fungal (~5,000) and human (~50) EH genes was generated using the NCBI protein database. All genes that had been tagged as ‘epoxide hydrolases’ were included in the database. The EH database and the UniRef50 database⁵³ were input into the ShortBred identify pipeline³⁶ and used to generate EH-specific markers. These markers were inputted into ShortBred quantify and were used to probe the quality-trimmed metagenomes for EH markers. The normalized marker abundance for each gene was summed and used to generate the normalized EH gene counts in Fig. 3b. The 30 most abundant EH genes were visualized using a heatmap generated by HClust2 (<https://bitbucket.org/nsegata/hclust2>) and Canberra distance matrices (Fig. 3c).

Colorimetric detection of EH activity. A subset of 13 of the most-abundant EH genes were selected for functional investigation on the basis of the number of EH markers per gene identified in the metagenomic analysis (Supplementary Table 1). Genes with fewer than 75% of markers were excluded from further analysis. The

13 genes were structurally aligned with EH genes with known crystal structures from *Mycobacterium tuberculosis* (PDB ID: 2BNG), *Pseudomonas aeruginosa* (PDB ID: 4DLN) and *Rhodococcus erythropolis* (PDB ID: 1NWW) using the Promals3D server (<http://prodata.swmed.edu/promals3d/>; Supplementary Fig. 4). Amino acids extending beyond the EH domain were trimmed. Amino acid sequences were converted into DNA sequences using the default codon usage table for *E. coli* (http://www.bioinformatics.org/sms2/rev_trans.html), and restriction enzyme sites for EcoRI and SalI were added to the 5' and 3' ends of the DNA fragment, respectively. Genes were synthesized as gBlock gene fragments by IDT (<https://www.idtdna.com/>). Sequences of the synthetic constructs are summarized in Supplementary Table 4.

Synthetic genes were sub-cloned into the EcoRI and SalI sites of a pH3C plasmid—provided by O. Rosenberg—to generate proteins with an N-terminal 8xHis fusion. Constructs were verified by forward and reverse sequencing of the T7 promoter (5'-TAATACGACTCACTATAGG-3') and terminator (5'-GCTAGTTATTGCTCAGCGG-3') performed by Quintara Bio. Subsequently, 11 of the 13 plasmids (NP_814872, NP_814982, NP_816494, WP_01071196, WP_002386325, WP_013363968, WP_01663157, WP_021147403, WP_053825032, YP_003971091 and YP_003971333) were validated and transformed into BL21(DE3) competent *E. coli* (New England Biolabs) for expression. BL21 cells containing recombinant plasmids were streaked onto LB agar plates supplemented with 50 µg ml⁻¹ kanamycin and grown overnight. Isolated colonies were selected and used to inoculate 200 µl LB supplemented with 50 µg ml⁻¹ kanamycin and 1 mM IPTG (Sigma-Aldrich).

Inoculates in 96-well V-bottom plates were incubated for 18 h at 37 °C at 200 r.p.m. After incubation, plates were centrifuged for 10 min at 3,000 r.p.m. and washed with 20 mM HEPES pH 7.5. Cells were resuspended and 40 µl of resuspended cells per well were added to 96-well flat-bottom plates. The OD₆₀₀ was measured to estimate the protein concentration, and 40 µl of 13 mM epoxide was added to each well. Known concentrations of the conjugate diol plated in triplicate were included on each plate and used to generate standard curves (Supplementary Fig. 3b–d). To each well, 20 µl of sodium nitrite was added (final concentration 20 mM). Plates were incubated for 1 h at 37 °C at 200 r.p.m. The reaction was stopped by centrifugation for 10 min at 4 °C and 3,000g. Then, 85 µl of supernatant was added to a new 96-well flat bottom plate containing 50 µl 8 mM sodium periodate (final concentration of 2 mM). Plates were incubated for 30 min at room temperature with shaking. Background absorbance at 490 nm was recorded before addition of 50 µl 12 mM adrenaline (final concentration of 3 mM; Sigma-Aldrich). Plates were incubated for 5 min at room temperature with shaking, and the absorbance at 490 nm was recorded. BL21 cells containing the pH3C plasmid or expressing HheA—a previously characterized EH⁵⁴—were included on each plate as controls. Enzymatic activity was assessed in triplicate for three epoxides, glycidol (Sigma-Aldrich), 9,10-EpOME and 12,13-EpOME (Cayman Chemical), and their conjugate diols, glycerol, 9,10-diHOME and 12,13-diHOME.

Quantification of 3EH copy number by qPCR. The abundance of the three active EH genes—NP_814872, YP_003971091 and YP_003971333—was quantified in faecal DNA that was extracted using the modified CTAB method⁴⁹. Gene fragments containing the target sequences (Supplementary Table 5) were ordered from IDT (<https://www.idtdna.com/>) and used to generate a standard curve. In brief, gene fragments were amplified by PCR, normalized to 2 × 10⁶ copies µl⁻¹ and eight 1:10 serial dilutions were made for use as standards. qPCR was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems) and a QuantStudio 6 Real-Time PCR system (ThermoFisher Scientific). The 3EH copy number represents the total number of copies of all three genes per ng of faecal DNA. Sequences of gene fragments, primers and probes are summarized in Supplementary Tables 3 and 5.

Statistical analysis. All analyses were conducted using the R statistical programming environment (v3.2.3). All comparisons made in mouse models (Fig. 1, Supplementary Fig. 1) were made using a LME (R package lmerTest) with the random effect attributed to the experimental repeat and visualized using Visualization of Regression Models (visreg; R package visreg) and boxplots generated using ggplot2 (R package ggplot2). All human cytokine, T cell, DC and gene expression differences (Fig. 2, Supplementary Fig. 2) were tested using a LME with the random effect attributed to donor and visualized using visreg. Boxplots were generated using ggplot2. Univariate LR was used to determine the statistical significance of faecal concentrations of 12,13-diHOME and 9,10-diHOME—normalized EH gene count—and 3EH copy number differences (LR, R; Figs. 3a,b, 4a, Supplementary Fig. 3a, Supplementary Tables 8–10). Multivariate LR was used to identify potential confounding factors of associations between faecal factors and the development of atopy and/or asthma during childhood. Potential confounding factors were defined⁵⁵ as risk factors that changed the adjusted OR by >10%. Risk factors were selected a priori and included race, gender, maternal asthma, maternal smoking before delivery, lack of pets before delivery, caesarean delivery and formula feeding at one month^{38–41}.

The characteristics of cohorts were compared using Fisher exact tests, with the exception of the age comparisons, which used a two-sided Student's *t*-test (Supplementary Tables 1, 2, 11 and 13). Univariate LR was used to model the

relationship between known risk factors (selected a priori), log₂-transformed 3EH copy number or 12,13-diHOME concentration (ng ml⁻¹) and the development of atopy and/or asthma during childhood (Supplementary Table 12) or eczema and/or asthma during childhood (Supplementary Table 14). Multivariate LR was used to identify potential confounders of significant associations, as described above and summarized in Supplementary Tables 12 and 14. Forest plots were created using OR- and CI-determined regression models with adjustment for potential confounding factors (Fig. 4b,c). All comparisons presented in this manuscript were analysed for effects of potential outliers. We identified potential outliers as points outside of the whiskers. Removal of these datapoints from the analysis did not change the significance of any comparison. The comparisons presented in the manuscript include all data; however, this additional outlier analysis is available in our GitHub repository (<https://github.com/srlevan>).

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

Data availability

Metagenomic data generated in this study are available in the EMBL repository as PRJEB24006 (<https://www.ebi.ac.uk/ena/>). Further datasets and materials are available from the corresponding author on reasonable request.

Code availability

Datasets and R scripts used for statistical analysis and figures are available on GitHub (<https://github.com/srlevan/>).

Received: 26 February 2019; Accepted: 29 May 2019;

Published online: 22 July 2019

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Acknowledgements

We thank the WHEALS and TIPS study participants; A. Iavarone and the mass spectrometry facility and genomic sequencing laboratory at QB3 Berkeley (<http://qb3.berkeley.edu/>), O. Rosenberg, B. Vogelstein and B. Spiegelman for plasmid donations and N. Lukacs for assessment of the manuscript. This research was funded by NIH/NIAID award AI089473.

Author contributions

S.R.L. designed the study, performed immune assays, animal models, metagenomic analysis, biochemical assays, mass spectrometry and statistical analyses, and developed the manuscript. K.A.S. assisted with animal models, performed all microscopy analysis and contributed to the manuscript. D.L.L. assisted with animal models and human immune assays. A.R.P. assisted with animal models and manuscript editing. K.E.F. and K.M. assisted with metagenomic and statistical analysis. E.F. assisted with microscopy. D.R.O., E.M.Z. and C.C.J. provided WHEALS cohort samples and data. M.M. and M.D.C. provided TIPS cohort samples and data. H.A.B. contributed to manuscript development. S.V.L. designed and supervised the study and developed the manuscript.

Competing interests

S.V.L. is co-founder of Siolta Therapeutics Inc., and serves as both a consultant and a member of its Board of Directors. Furthermore, the Regents of the University of California, UCSF have filed a provisional patent application (Application number 62/637,175) on behalf of S.V.L. and S.R.L. relating to the methods and compositions of EH genes.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41564-019-0498-2>.

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| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
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qPCR: QuantStudio version 1.0
Plate Reader: Gen5 Version 2.3.0
Mass Spectrometry: Xcalibur version 2.3.0 and Analyst 1.7

Data analysis

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Metagenomics: BBmap version 37.25, ShortBred version 0.9.3, bioBakery version 1.7, MegaHit version 1.1.4
Heatmap Generation: HClust2 version 1.0.0
Statistical Analysis: R version 3.2.3, G*Power 3.1, visreg version 2.3-0, lmerTest version 2.0-33, ggplot2 version 2.2.1

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Life sciences study design

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Sample size	Mouse Studies: A pilot study was performed to estimate the effect size ($d=5.47$) of 12,13 DiHOME on Treg frequency in mouse lungs. This estimated effect size was used to determine the minimum sample size ($n=3$) given an $\alpha=0.05$ and $\beta=0.80$. Human Primary Cell Studies: Ex vivo studies were performed with 3-4 treatment replicates per sample using cells isolated from 3-4 independent human donors. Previously published studies by our group (Fujimura et. al. 2016) were used to estimate the effect size of 12,13 DiHOME treatment of human dendritic cells on Treg frequency ($d=1.96$). This estimated effect size was used to calculate the required sample size ($n=6$) given an $\alpha=0.05$ and a $\beta=0.80$. Human Neonatal Studies: Effect size was estimated based on the relative concentration of 12,13 DiHOME in high- and low-risk neonatal stool shown in Figure S9 of Fujimura et. al. 2016. The estimated effect size ($d=1.228$), $\alpha=0.05$, and $\text{power}=0.95$ were used to estimate the required sample size ($N=38$, assuming allocation of $N_2/N_1=1$).
Data exclusions	No data was excluded from the analysis. Per the request of reviewer 2 statistical calculations were repeated excluding outliers (defined as points outside the whiskers on boxplots generated in GGPlot2 and plotted in the Figures); however, this analysis did not change the significance of any comparison is not included in the main text, but is available on our GitHub if there is interest.
Replication	Mouse studies: All experiments were performed with 3-5 mice per cage; mice were co-housed for 2-3 weeks prior to treatment to minimize cage effects; each treatment group was housed independently to mitigate potential effects of co-housing and coprophagy; cages from the same assay were stored in the same rack with identical handling to minimize microbial variation. All reported data was replicated across cages. Human Primary Cell Studies: Primary immune cells were isolated from 3-4 healthy human donors in all experiments. All reported data was replicated across all experiments; however, we were not able to replicate an earlier observation of the effect of 12,13 DiHOME on CD36 expression by dendritic cells. Two out of three human donors had increased CD36 expression with increased 12,13 DiHOME concentration; however, the third showed no changes in expression. Dendritic cells used in this experiment were isolated from human donors, who may have variable expression of CD36 at baseline. Human Neonatal Studies: We attempted to replicate our observations in our subset of the WHEALS cohort in a subset of the TIPS cohort, a geographically distinct neonatal cohort collected several years after the WHEALS cohort. While there were differences in the absolute concentrations of 12,13 DiHOME, the overall trends remained consistent across both cohorts.
Randomization	Mouse Studies: Mice were randomly grouped into cages by the UCSF animal facility and treatments were assigned by cage. Human Primary Cell Studies: Known early-life risk factors were including race, sex, maternal asthma, birth by Caesarian section, breastfeeding, maternal smoking, and pet exposure as well as the age at the time of stool collection were identified for both cohorts. Comparisons of these demographics in healthy neonates vs. neonates who developed allergic disease in both cohorts are available in Tables S6 and S7. These risk factors were selected a priori and potential confounders were defined as risk factors that modified the adjusted odds ratio by $>10\%$ (Maldonado and Greenland 1993) and displayed in tables S8-10.
Blinding	Mouse Studies: Scoring of peribronchial and perivascular inflammation was performed by two blinded individuals. Scores were averaged for each image and the average values were used for analysis. Investigators were blinded to the treatment groups while collecting and gating mass spectrometry and flow cytometry data after tissue samples had been removed from the mice. Human Primary Cell Studies: Investigators were not blinded to cell treatment conditions during data collection; however, they were blinded to the treatment conditions during gating of flow cytometry data.

Human Neonatal Studies: Investigators were blinded to health outcomes during metagenomic, mass spectrometry, and qPCR data collection and analysis.

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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

All antibodies are displayed in the following format: Antibody Clone Vendor Dilution Catalog # Lot #

Human T Cell Panel

anti-CD4 L200 BD 1:100 563913 5138713

anti-CD25 M-A251 BD 1:25 557741 6014823

LIVE-DEAD Aqua Life Technologies 1:300

anti-CD3 SP34-2 BD 1:100 557917 5121996

anti-FoxP3 PCH101 eBioscience 1:20 L34957 1729808

anti-IFNg B27 BD 1:200 554700 5230522

anti-IL4 7A3-3 Miltenyi Biotec 1:20 130-091-647 5160118145

anti-IL-17 64DEC17 eBioscience 1:20 50-7179-42 E15769-102

Human DC Panel

anti-CD11c B-ly6 BD 1:50 559877 4160524

anti-CD19 SJ25C1 BD 1:100 341093 6175589

LIVE-DEAD Aqua Life Technologies 1:300 L34957 1729808

anti-CD3 SP34-2 BD 1:100 557917 6200871

anti-CCR7 3D12 BD 1:35 560548 4153791

anti-CD80 2D10 Biolegend 1:50 305225 B219135

anti-CD86 2331 BD 1:50 561129 5338949

anti-HLA-DR G46-6 BD 1:50 552764 3241958

anti-CD36 CB38 BD 1:50 561535 6078701

Anti-CD1a HI149 BD 1:40 555807 4141654

Mouse T Cell Panel

anti-CD4 RM4-5 BD 1:100 563726 6217936

anti-CD25 PC61 BD 1:50 557658 5263790

LIVE-DEAD Aqua Life Technologies 1:300 L34957 1729808

anti-CD3e 500A2 BD 1:150 557984 4218820

anti-FoxP3 FJK-16s eBiosciences 1:70 45-5773-82 E08398-1634

Mouse Innate Panel

anti-CD11b M1/70 Biolegend 1:100 101257 B205713

anti-CD11c N418 Thermofisher Scientific 1:100 MCD11C28 1555781A

LIVE-DEAD Aqua Life Technologies 1:300 L34957 1729808

anti-CD45 30-F11 Thermofisher Scientific 1:10 MCD4505 1660868

anti-SiglecF E50-2440 BD 1:100 565528 7051805

anti-F4/80 BM8 Biolegend 1:100 123147 B210629

anti-Ly6c AL-21 BD 1:100 560593 7037863

anti-Ly6g 1A8 Biolegend 1:100 127624 B218644

DC Isolation Antibodies

anti-CD28 CD28.2 BD Biosciences NA 555725

anti-CD49d 9F10 BD Biosciences NA 555501

Validation

All antibodies were selected based on previously published literature (Fujimura et. al. 2016, Van Dyken et. al. 2017). Validations statements from the manufacturer's can be found at the links below. Additionally, all four panels were validated and titrated in our lab. The human T cell panel was validated and titrated with primary human T cells that had been cultured with autologous dendritic cells, treated with golgi plug, and treated with PMA/ionomycin. The human dendritic cell panel was validated and titrated with peripheral blood mononuclear cells. The mouse T cell panel was validated and titrated using a single cell suspension isolated from a mouse spleen. The mouse innate panel was validated and titrated using a single cell suspension isolated from a mouse lung.

Antibody and Manufacturer Validation

Human T Cell Panel
 anti-CD4 <http://www.bdbiosciences.com/ds/pm/tds/563913.pdf>
 anti-CD25 <http://www.bdbiosciences.com/ds/pm/tds/557741.pdf>
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD3 <http://www.bdbiosciences.com/ds/pm/tds/557917.pdf>
 anti-FoxP3 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=14-4776-82
 anti-IFN γ <http://www.bdbiosciences.com/ds/pm/tds/554700.pdf>
 anti-IL4 <https://admin-shop-live.miltenyibiotec.com/DE-en/shop/comMiltenyiDatasheet/product?productId=15167>
 anti-IL-17 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=50-7179-42
 Human DC Panel
 anti-CD11c <http://www.bdbiosciences.com/ds/pm/tds/559877.pdf>
 anti-CD19 <http://www.bdbiosciences.com/ds/pm/tds/341093.pdf>
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD3 <http://www.bdbiosciences.com/ds/pm/tds/557917.pdf>
 anti-CCR7 <http://www.bdbiosciences.com/ds/pm/tds/560548.pdf>
 anti-CD80 <https://www.biolegend.com/en-us/global-elements/pdf-popup/brilliant-violet-605-anti-human-cd80-antibody-7663?filename=Brilliant%20Violet%20605%20anti-human%20CD80%20Antibody.pdf&pdfgen=true>
 anti-CD86 <http://www.bdbiosciences.com/ds/pm/tds/5338949.pdf>
 anti-HLA-DR <http://www.bdbiosciences.com/ds/pm/tds/552764.pdf>
 Anti-CD1a <http://www.bdbiosciences.com/ds/pm/tds/555807.pdf>
 Mouse T Cell Panel
 anti-CD4 <http://www.bdbiosciences.com/ds/pm/tds/563726.pdf>
 anti-CD25 <http://www.bdbiosciences.com/ds/pm/tds/557658.pdf>
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD3e <http://www.bdbiosciences.com/ds/pm/tds/557984.pdf>
 anti-FoxP3 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=13-5773-80
 Mouse Innate Panel
 anti-CD11b <https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-anti-mouse-human-cd11b-antibody-349?filename=PE%20anti-mousehuman%20CD11b%20Antibody.pdf&pdfgen=true>
 anti-CD11c https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=17-0114-82
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD45 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=12-0451-82
 anti-SiglecF <http://www.bdbiosciences.com/ds/pm/tds/552125.pdf>
 anti-F4/80 <https://www.biolegend.com/en-us/global-elements/pdf-popup/fitc-anti-mouse-f4-80-antibody-4067?filename=FITC%20anti-mouse%20F480%20Antibody.pdf&pdfgen=true>
 anti-Ly6c <http://www.bdbiosciences.com/ds/pm/tds/560593.pdf>
 anti-Ly6g <https://www.biolegend.com/fr-fr/global-elements/pdf-popup/purified-anti-mouse-ly-6g-antibody-4767?filename=Purified%20anti-mouse%20Ly-6G%20Antibody.pdf&pdfgen=true>
 DC Isolation Antibodies
 anti-CD28 <http://www.bdbiosciences.com/ds/pm/tds/555725.pdf>
 anti-CD49d <http://www.bdbiosciences.com/ds/pm/tds/555501.pdf>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Raw 264.7 cells were purchased from the UCSF cell culture facility.
Authentication	Authenticated Raw264.7 cells were purchased from the UCSF cell culture facility and their identity was confirmed by morphology. No genetic authentication was performed by our lab.
Mycoplasma contamination	The Raw264.7 cells used in this study were not tested for mycoplasma contamination by our lab.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines

Animals and other organisms

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

Laboratory animals	Four week old C57B6 female mice were purchased from Jackson Laboratories (Sacramento, CA) and used in all experiments at ages 5-6 weeks.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field sample collection.

Ethics oversight

UCSF IACUC provided ethical oversight and approval of this animal study. Protocol AN171803

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

The WHEALS cohort was collected in southeastern Michigan from 2003-2007. Our subset included 41 neonates (stool median age=35 days). Of the 41, 19 (46%) had atopy and/or asthma, 20 (48.8%) were male and 32 (78%) were black, based on a maternal report of race. Additionally, 8 (19.5%) were born by Cesarean section, 4 (9.75%) were exclusively breastfed until one month of age, 7 (17%) reported doctor-diagnosis of maternal asthma, 6 (14.6%) reported maternal smoking during pregnancy, and 13 (31.7%) lived with an indoor or outdoor dog or cat prior to delivery

The TIPS cohort was collected in the San Francisco Bay area from 2006-2006. Our subset of 50 neonates from the placebo arm of the TIPS cohort (stool median age = 32 days) that had more than 300 mg of one-month stool remaining were selected (Table S7; total n=50; eczema=22; asthma=6; eczema and asthma=23). Of the 50 neonates, 20 (40%) were male and 4 (8%) were black, based on a family report of demographics. Additionally, 15 (30%) were born by Cesarean section, 28 (56%) were exclusively breastfed until one month of age, 31 (62%) reported doctor-diagnosis of maternal asthma, 1 (2%) reported maternal smoking during pregnancy, and 18 (36%) did not live with a cat or dog.

Additionally, plasma donated by healthy human donors was used for human immune cell assays. Plasma was purchased from Blood Centers of the Pacific and all samples were de-identified prior to purchase.

Recruitment

The WHEALS cohort recruited pregnant women age 21-49 who lived in a predefined cluster of contiguous zip codes near Detroit, Michigan and had no intention of leaving the area. The TIPS cohort recruited newborns from the San Francisco Bay area, who had at least one parent with a history of asthma and were willing to supplement breast milk or formula with probiotics or placebo for at least one feed each day. Populations used in this study likely reflect neighborhoods from which study participants were recruited and in particular those who sought care obstetric care in the area. Participants opted to enroll their children in this study and to participate in follow up over the first five years of life, which is likely reflected in the self-selection bias of the participants. Additionally, participants in the TIPS study were randomized to the placebo arm; however, they had agreed to provide daily pro-biotic supplementation to their infant for the first six months of life, which may have limited the potential participants.

Ethics oversight

Wayne State University IRB provided ethical oversight for the WHEALS cohort, while the UCSF IRB provided ethical oversight for the TIPS cohort.

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

Human T Cell Assays: DCs were isolated from the PBMCs of two healthy human donors and treated for five days with 130 μ M 12,13 DiHOME solubilized in 0.2% DMSO or vehicle (0.2% DMSO) in the presence of 20 ng mL⁻¹ IL-4 (R&D Systems, Minneapolis, MN) and 10 ng mL⁻¹ GM-CSF (R&D Systems, Minneapolis, MN). Fresh treatment media was added every 48 hrs throughout the course of the study. Eighteen hours prior to co-culture, DCs were stimulated with 10 ng mL⁻¹ TNF α , IL1 β , and IL-6 (Peprotech, Rocky Hill, NJ) and 1 mM prostaglandin E2 (Stemcell Technologies, Cambridge, MA). DCs were subsequently washed and co-cultured with autologous T cells in the presence of 10 ng mL⁻¹ anti-CD28 and anti-CD49d (BD Bioscience, San Jose, CA). After five days of co-culture, T cell subsets were analyzed by flow cytometry. To assess cytokine secretion, cells were stimulated for 16 hrs with Phorbol Myristate Acetate-Ionomycin (ACROS, Morris Plains, NJ) and GolgiPlug (Gplug; BD Biosciences, San Jose, CA)

Human DC Assays: PBMCs were isolated from two healthy human donors using Ficoll-Hypaque gradient centrifugation as previously described¹ and cultured for five days in the presence of 20 ng mL⁻¹ IL-4 and 10 ng mL⁻¹ GM-CSF. Monocytic DCs (DCs) were defined as CD3-CD19-CD11c+. The DC gating strategy can be found in Supplemental Fig. 1e.

Mouse Lung Tissue: Mouse lung tissue was manually dissected, digested with 5 mg per sample collagenase (Sigma-Aldrich, St. Louis, MO), and passed through a 40 μ m filter to generate single cell suspensions. CD45- lung cells were considered resident cells.

Instrument

BD LSRII

Software	Data was collected using FACS Diva version 8.0 and analyzed using FlowJo version 10.1 (flow cytometry) or FCAP Array version 3.0.1 (CBA assay). Graphs and statistical analysis were performed using R version 3.2.3. All R scripts can be found at https://github.com/srlevan/ .
Cell population abundance	Cells were not sorted.
Gating strategy	Human T Cells: All boundaries were initially identified in human T cells that were stained with the full panel +/- the antibody of interest. Lymphocytes were selected by FSC-A (25K-75K) and SSC(25K-75K). Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: Live : Live-Dead-AmCyan<500 CD3+ : CD3-A700>2000 CD4+ : CD4-BV711>700 CD8+ : CD8-BD605>700 IFNg+ : IFNg-FITC >6000 IL4+ : IL-4-PE>3000 IL17+ : IL-17-APC>500 FoxP3+ : FoxP3-PB>500 CD25+ : CD25-PE-Cy7>1000 Human DCs: All boundaries were initially identified in human peripheral blood mononuclear cells that were stained with the full panel +/- the antibody of interest. Forward and side scatter were used to select monocytes [FSC (0-250K) and SSC (50K-250K)], excluding lymphocytes [FSC(25K-75K) and SSC(25K-75K)]. Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: Live : Live-Dead-AmCyan<3000 CD3- : CD3-A700<1000 CD19- : CD19-PE-Cy7<800 CD11c+ : CD11c-APC>1050 CD36+ : CD36-PB>10,000 CD1a+ : CD1a-PE>2000 CCR7+ : CCR7-FITC>2000 CD80+ : CD80-BV605>1000 Mouse Tregs: All boundaries were initially identified in mouse splenic and lung cells that were stained with the full panel +/- the antibody of interest. Forward and side scatter were used to select lymphocytes [FSC(25K-75K) and SSC(25K-75K)] were excluded. Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: Live : Live-Dead-AmCyan<3000 CD3+ : CD3-A700>2000 CD4+ : CD4-BV711>500 CD8+ : CD8-BD605>1050 FoxP3+ : FoxP3-PB>1000 CD25+ : CD25-PE-Cy7>800 Mouse Innate Cells: All boundaries were initially identified in mouse lungs that were stained with the full panel +/- the antibody of interest. Forward and side scatter were used to select granulocytes [FSC (0-250K) and SSC (50K-250K)]. Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: CD45- : CD45-APC<1000 Live : Live-Dead-AmCyan<1000 CD11b+ : CD11b-QDot 605 >500 Eosinophils : CD11b-QDot605 4000-20,000 SiglecF-PerCP 1000-10,000 Siglec-F+ : Siglec-F-PerCP>2000 CD11b - : CD11b-QDot605 <500 Ly6G - : Ly6G-APC<1000 Ly6G + : Ly6G-APC>1000 F4/80+ : F4/80-QDot705 > 2000 CD11c + : CD11c-PB>1000 Ly6C + : Ly6C-PE-Cy7>500 Ly6C - : Ly6C-PE-Cy7 <500

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

In the format provided by the authors and unedited.

Elevated faecal 12,13-diHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes immune tolerance

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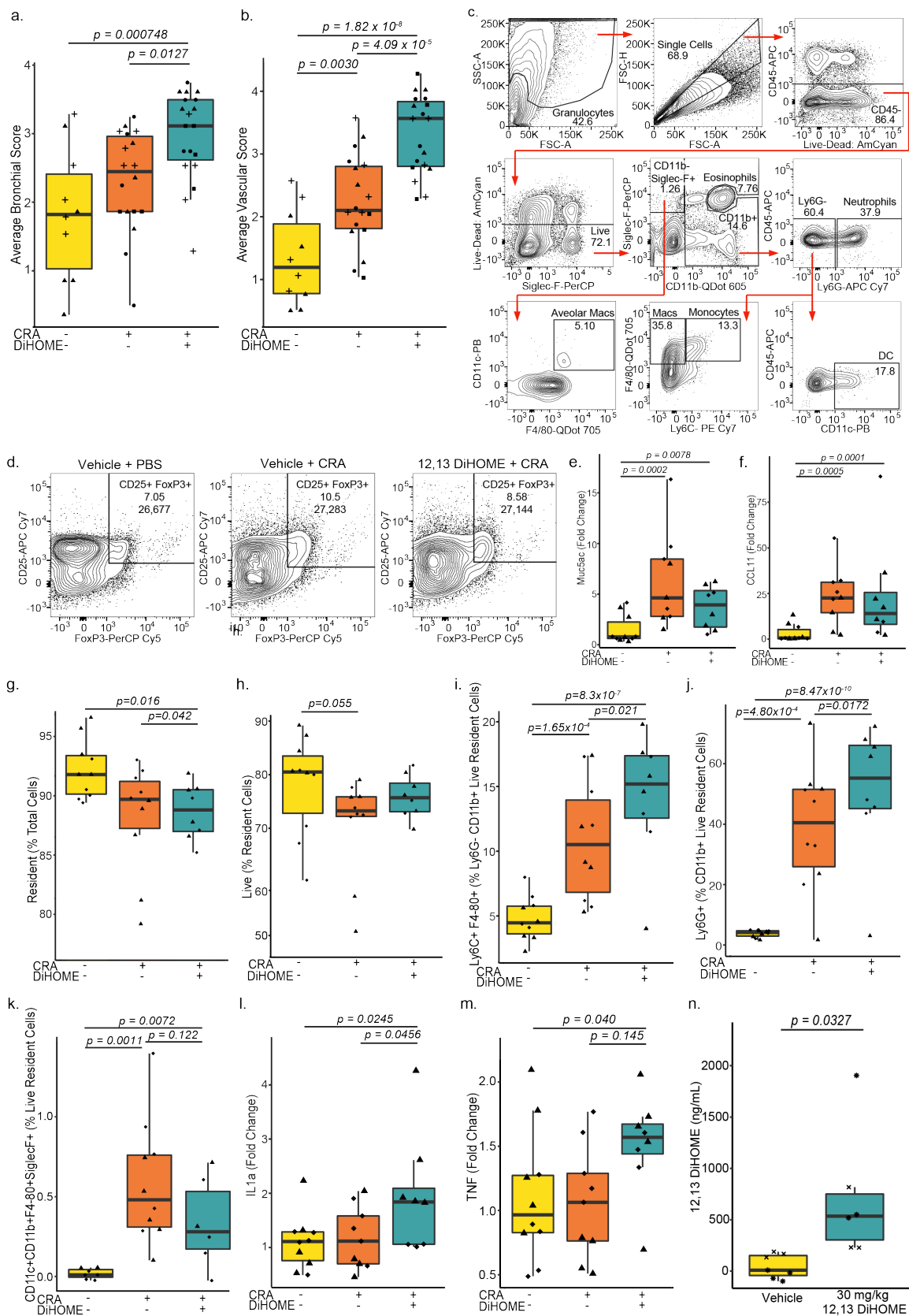
Elevated fecal 12,13 DiHOME in neonates at high-risk for asthma is produced by gut bacteria and impedes immune tolerance.

Authors: Sophia R. Levan¹, Kelsey A. Stamnes¹, Din L. Lin¹, Ariane R. Panzer¹, Elle Fukui¹, Katherine McCauley¹, Kei E. Fujimura¹, Michelle McKean², Dennis R. Ownby³, Edward M. Zoratti⁴, Homer A. Boushey⁵, Michael D. Cabana⁶, Christine C. Johnson⁷, Susan V. Lynch^{1*}

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SUPPLEMENTARY INFORMATION

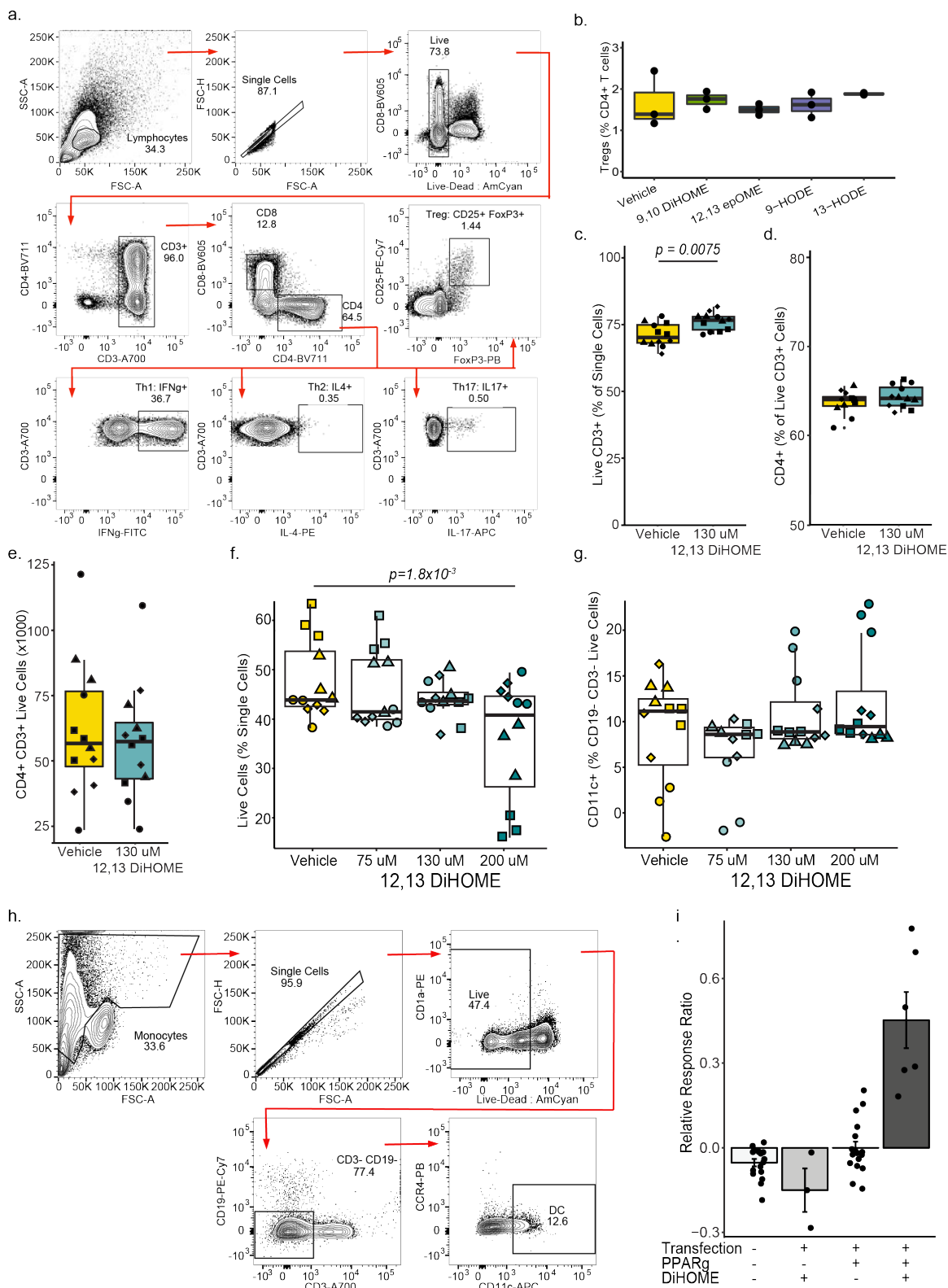
Supplementary Figures 1 - 4
Supplementary Tables 1 - 14



Supplementary Figure1. Related to Figure 1

Peritoneal treatment with 12,13 DiHOME exacerbates innate immune infiltration in the lungs of CRA-challenged mice. (A,B) Peribronchial and perivascular infiltration were scored in H&E stained lung sections (0

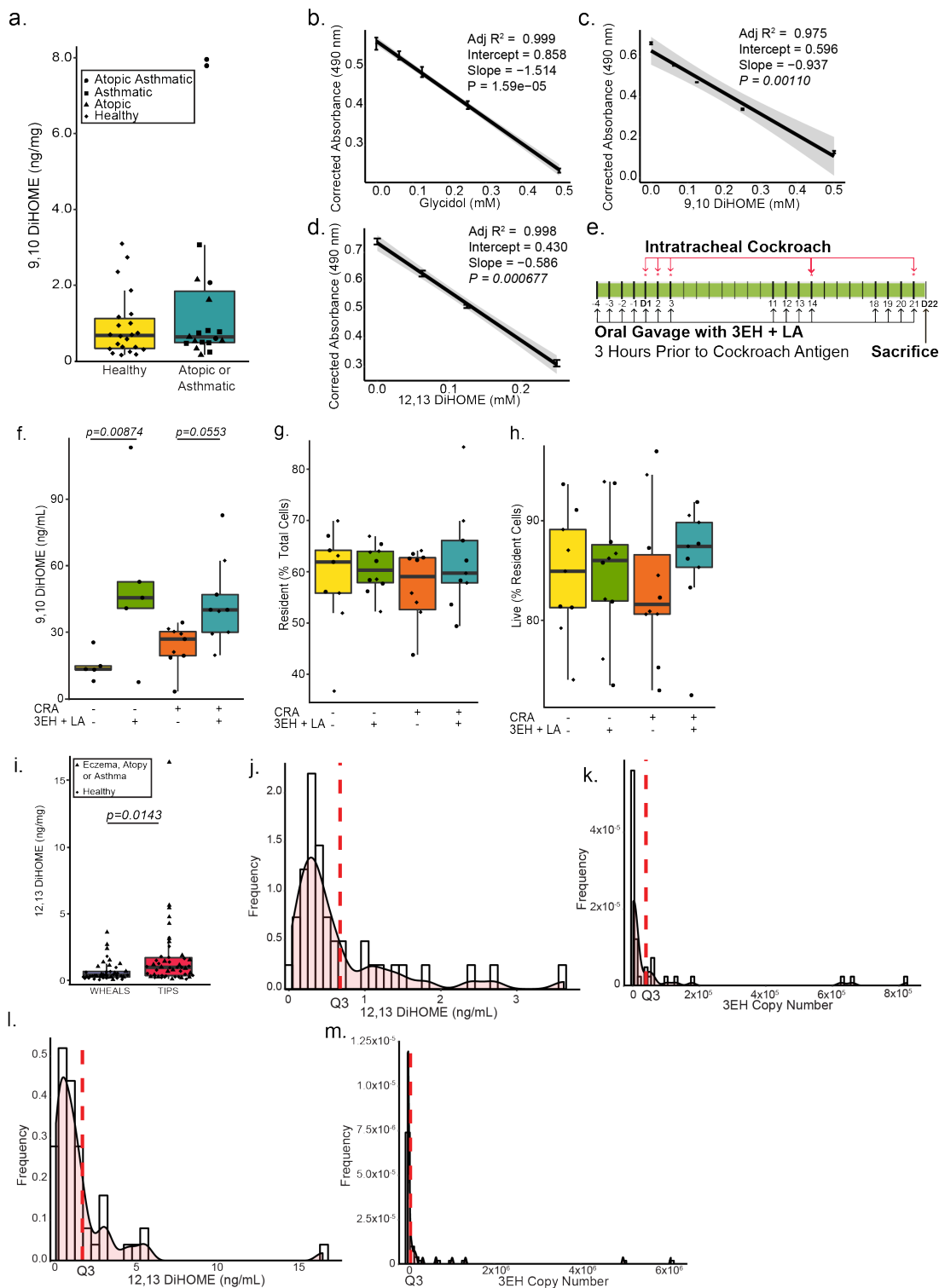
- no inflammatory infiltrates; 1 - few inflammatory cells; 2 - ring 1 cell-layer wide; 3 - ring 2-4 cells wide; 4 - ring > 4 cells wide). 12,13 DiHOME treatment (n=19 mice) increases the peribronchial and perivascular inflammation score compared to vehicle treated, PBS challenged [n=10 mice; LME; $p=0.0000748$ (bronchial), $p=1.82 \times 10^{-8}$ (venous)] or vehicle treated, CRA-challenged [n=18 mice; LME; $p=0.0127$ (bronchial), $p=4.09 \times 10^{-5}$ (venous)] animals. (C) Gating strategy used to assess resident immune cells in mouse lungs. (D) Representative flow plots displaying lung Tregs in mice (FoxP3+CD25+CD4+CD3+). This experiment was repeated in four independent experiments with similar results as summarized in Figure 1g. (E,F) Intra-peritoneal treatment with 12,13 DiHOME (n=8 mice) did not increase the expression of Muc5ac or CCL11 in CRA-challenged mice. (G) 12,13 DiHOME treatment of CRA-challenged mice (n=7 mice) decreases the frequency of resident lung cells compared to vehicle treated, PBS challenged (n=9 mice; LME; $p=0.016$) or vehicle treated, CRA challenged animals (n=9 mice; LME; $p=0.042$). (H) 12,13 DiHOME treatment of CRA-challenged mice (n=7 mice) does not change the frequency of live resident cells in the lungs (vehicle treated, PBS challenged n=9 mice; vehicle treated, CRA challenged n=9 mice). (I) 12,13 DiHOME treatment of CRA-challenged mice (n=7 mice) significantly increases the frequency of lung resident monocytes (Ly6C+F4-80+Ly6G-CD11b+) compared to vehicle treated, PBS challenged (n=9 mice; LME; $p=8.3 \times 10^{-7}$) or vehicle treated, CRA challenged animals (n=9 mice; LME; $p=0.021$) (J) 12,13 DiHOME treatment of CRA-challenged mice (n=7 mice) significantly increases the frequency of lung resident neutrophils (Ly6G+CD11b+) compared to vehicle treated, PBS challenged (n=9 mice; LME; $p=8.47 \times 10^{-10}$) or vehicle treated, CRA challenged animals (n=9 mice; LME; $p=0.0172$). (K) 12,13 DiHOME treatment of CRA-challenged mice (n=6 mice) decreases the frequency of lung resident alveolar macrophages (CD11c+CD11b+F4/80+Siglec-F+) compared to vehicle treated, CRA challenged animals (n=6 mice; LME; $p=0.122$) but increases the frequency compared to vehicle treated, PBS challenged animals (n=6 mice; LME; $p=0.0072$). (L,M) 12,13 DiHOME treatment increases the expression of IL1 α and TNF in 12,13 DiHOME treated, CRA challenged mice (n=8 mice) compared to vehicle treated, PBS challenged [n=9 mice; LME; $p=0.0245$ (IL1 α), $p=0.040$ (TNF)] or vehicle treated, CRA challenged animals [n=9 mice; LME; $p=0.0456$ (IL1 α), $p=0.145$ (TNF)]. (N) Peritoneal treatment with 12,13 DiHOME significantly increases the concentration of 12,13 DiHOME in plasma (n=6 mice, two-sided student t-test: $p=0.0327$). Unique symbols ▲, ■, ◆, ●, +, *, × represent mice from independent repeats. All boxplots were generated in Ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5 times the interquartile range (whiskers).



Supplementary Figure2. Related to Figure 2

12,13 DiHOME treatment of DCs decreases IL-10 secretion and Treg frequency without affecting cell viability. (A) Gating strategy used to assess T cell subsets following co-culture of 12,13 DiHOME treated DCs

with autologous T cells. (B) Treatment of human DCs with oxylipins 9,10 DiHOME, 12,13 epOME, 9-HODE, and 13-HODE did not impact the frequency of Tregs in co-culture (n=3 treatment replicates). (C) Frequency of live CD3+ cells following co-culture with vehicle or 130 μ M 12,13 DiHOME-treated DCs [n=4 biologic replicates ($\blacktriangle$, $\blacksquare$, $\blacklozenge$, $\bullet$) LME; p=0.0075]. (D) Frequency of CD4+ T cells following co-culture with vehicle or 130 μ M 12,13 DiHOME-treated DCs [n=4 biological replicates ($\blacktriangle$, $\blacksquare$, $\blacklozenge$, $\bullet$); LME]. (E) CD4+ CD3+ Live cell counts following co-culture with treated DCs [n=4 biological replicates ($\blacktriangle$, $\blacksquare$, $\blacklozenge$, $\bullet$); LME]. (F) Frequency of live CD3- cells following PBMC treatment with vehicle or 12,13 DiHOME [n=4 biological replicates ($\blacktriangle$, $\blacksquare$, $\blacklozenge$, $\bullet$); LME; 200 μ M p=0.0018]. (G) Frequency of CD3- CD19- CD11c+ live cells following PBMC treatment with vehicle or 12,13 DiHOME [n=4 biological replicates ($\blacktriangle$, $\blacksquare$, $\blacklozenge$, $\bullet$); LME]. (H) Gating strategy used to assess CD3- CD19- CD11c+ cells. (I) Raw264.7 cells transfected with PPAR γ and a PPAR γ -activated luciferase reporter and treated with 200 μ M 12,13 DiHOME were compared with un-transfected cells; cells treated with 200 μ M 12,13 DiHOME lacking the PPAR γ gene; and untreated transfected cells. Treatment with 12,13 DiHOME and transfection with PPAR γ were required to generate a response. All boxplots were generated using Ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5 times the interquartile range (whiskers).



Supplementary Figure3. Related to Figure 3 and Methods

Bacterial EH genes produce 9,10 and 12,13 DiHOME (A) The fecal concentration of 9,10 DiHOME did not significantly differ between healthy neonates and those who develop atopy and/or asthma in childhood in the WHEALS cohort (n=41 neonates). The following symbols, $\blacklozenge$, $\blacktriangle$, $\blacksquare$, $\bullet$, represent neonates who develop into healthy, atopic, asthmatic, and atopic asthmatic children, respectively. (B,C,D) Calibration curves generated using a modified colorimetric assay for glycidol, 9,10 DiHOME, and 12,13 DiHOME, respectively (n=3 independent experiments). Error bars represent the standard deviation, and the center represents the mean. Best-fit curves were generated using the lm (linear modeling) function in R. Grey shading represents confidence

interval of 95%. (E) Schematic of supplementation 3EH+LA and challenge used in Figure 3e,f and S3f-h (F) Supplementation with linoleic acid and *E. coli* overexpressing the 3EH genes increased the concentration of 9,10 DiHOME in mouse plasma (No CRA; n=5 mice; LME; p=0.0087; CRA-challenged; n=9 mice; LME; p=0.55). (G,H) The frequency of resident and live lung cells did not differ between the four groups of orally supplemented and intra-tracheally challenged mice used in Figure 3e,f and S3f (n=9 mice; LME). (I) The concentration of 12,13 DiHOME was significantly higher in stool from the TIPS cohort when compared to the WHEALS cohort (WHEALS n=41 neonates, TIPS n=50 neonates; LME; p=0.143). The following symbols (◆, ▲) represent healthy v. allergic children in this figure. (J,K,L,M) In our subset of the WHEALS (n=41 neonates) and TIPS (n=50 neonates) cohorts, the distributions of 12,13 DiHOME concentration and 3EH copy number were skewed. The red vertical lines indicate the third quadrant (Q3). All boxplots were constructed in Ggplot2, which displays the median, the 25th and 75th percentiles, and the smallest and largest values within 1.5 times the interquartile range (whiskers).

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NP_814772.1 4 ---LIAIDL-----DGTLLNAQ-----SLISPKTEQ----- 26

NP_814685.1 4 ---LIASDM-----DGTLLDAK-----MSITNDNAS----- 26

WP_010711964.1 5 ---AIVMDI-----DGTLLTSE-----KKISPSTRQ----- 27

NP_816494.1 29 ---AVLTDL-----DNTLIAWN-----NPDGTEELKT----- 52

NP_814872.1 5 ---LVAIDI-----DGTLLNSQ-----HKITPRVKE----- 27

WP_002386325.1 12 ---TILPDL-----DGTITDSG-----SGIMRSILYA-----TEQLGWPAPEETLRSFIPGPLYESFLHMAPSABAAQA-----VGHYRAYYQRKGMF 88

WP_016634357.1 121 MEDIVISAGEVQPS-DMEVIDGK-----VEANEALITG-----ESDLIEKEIGDITLLS----- 168

WP_013363968.1 4 ---MLPLDI-----DGTLVGKD-----QRIPQSAIR----- 26

YP_003971333.1 24 ---AASFPI-----DGTILTSFV-----THVIPQSSID----- 47

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WP_053825032.1 8 ---LIVTDL-----DGTLLHDAPTFEERFITORSD----- 35

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4dln.pdb_chainA_s002 1 -----AEEFPVPNGFESAYRE-----VDGVKLHYVKGQGPLVVMLVHGFGQTWYEHQ----- 49

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Consensus_ss: eeeee hhhh

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NP_814772.1 27 -----TLKKAIDNGHYVSIATGRP-----YRMS----- 49

NP_814685.1 27 -----AIREAERLGIIFMVATGRA-----YTEA----- 49

WP_010711964.1 28 -----ALVAAQKQCLSLILASGRP-----TNMG----- 50

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NP_814872.1 28 -----ALQKANEQGVRIVLCTGRP-----LPGV----- 50

WP_002386325.1 89 ENHVPYGIPEVLTRLKEAGAKLYIATSKP-----EEFA----- 121

WP_016634357.1 169 -----GSPIVSGQAYARVHVGAENYAVKTIQEAQVHKPIQSELVNSIRKVKFTSWVIIPLGILFVEAFWLRDAGIKTSVVASAAALLGMLPKGLVLLISIALTTGVIKLAKK 278

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YP_003971091.1 105 YNSVYAGIPEQLAALRADGLHLAIACTCKP-----QYQA----- 137

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YP_003971091.1 138 ---EPVCEHFILD--- 147

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WP_053825032.1 74 ---MMSIDAVETFFH---IGSSNGLLVYTE--- 96

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NP_816494.1 90 -----ALKPTARGFKLAEKKLGLK--PSEMLM-VGDQI--MTDIR 124

NP_814872.1 168 AKLPLHFT-EKYTVKSTP-----FYEIMMKNKASGNALAKLADHLGLN--KEVMA-IGDNE--NDLS 226

WP_002386325.1 132 ---RYFTGIYGASM---DGHSRKKADVQYALTEAQLDP-TKEAITM-VGDRN--HDIL 180

WP_016634357.1 449 LAYLKEEG-IDLKVISGDNPVTVSNIAARRAGLPGYESYIDLSTKTTAEVREAVQYTVFGRVSP--QQRRTIVRELKDT-----EHVVM-TGDGV--NDVL 540

NP_013363968.1 75 -----GRAARRHG-----GDRR--QRQ 89

WP_003971333.1 181 EAKLSGMC-RNVRGVWRHP-----DFTDLIPADGGKPEGMKRFMRHYGWT--REQTIA-FGDGG--NDAD 239

YP_003971091.1 148 ---TMVDGIYGAST-----DNSRIDKQVIRYCFDSIGFDADAGDRALM-VGDRW--TDVD 197

WP_053825032.1 176 AHLKTLVP-PDFDVAISEGS-----MMWMLSPLANKRQALKTLCEMRMDVR--LDGTVS-FGDDL--IDIG 234

2bng.pdb_chainA_s001 79 ---RTDAL-IIG-----PLRVQVWCVGVFVD--DGRITL-WRDYF--DVID 116

4dln.pdb_chainA_s002 187 ---HASN-----NTEEVFSERLLDLYARSYAKP--HSLNASFEYRYALNESVRQNAELAKTRQMI 241

1nww.pdb_chainA_s003 97 ---RVDVLLRALP-----TGKSYNLSILGVFQLT--EGKITG-WRDYF--DLRE 137

Consensus_aa: phps.h.....p.p.h.h.h.phshp.....hh..hgd.....s.....

Consensus_ss: hhhhhh eeeee eee hhh

WP_021147403.1 542 ---ALREAD---CSIVMAEG---DPATRQ-IANLVLM-DSEFKDIPILFEGRRVNNIAHIAPIFLIKTVSFLGLICTIASIVFGKAERYLLVFPPIQVQMT 633

NP_814982.1 216 ---LFEACD---YGIAMGNA---REELKE-KATFIST-KNTENGIVNGLKFF--DLL 259

NP_814772.1 223 ---MISYAG---WGVAMNNA---TDKIKS-VANDVTEKTNDQDGLADYLENY--LDL 267

NP_814685.1 244 ---MIQWAG---VSFAMGNA---ELEKE-YAKYETA-TNLENGVGEAILRA--IREDL 289

WP_010711964.1 236 ---MLEWAG---TGIA MENA---VDELKN-IATVEVTL-SNNDNGIAVALAKI--VG 278

NP_816494.1 125 ---GNAAG---IRNVLVQP--IVDT--DGNWTR--INRFFERKIMKY--LSKKHPMTWRGGLE 175

NP_814872.1 227 ---MIDYAG---IGVAMGNA---TENVKT-IADVHTT-SNDEEDGVAQIIEKM--VLI 270

WP_002386325.1 181 ---GAQONG---LDSIGVLYGFGEETELQEGAGATFLVQ--SPKDLGAILQN--S 225

WP_016634357.1 541 ---ALREAD---CSIAMAG---DGATRQ-ISNLVLL-DSDFTTLPDVLVFEGRRRVNNVTRVSSVFFIKTIYSFILSICALTAIAFFPIQVTLIDLAIEG 632

WP_013363968.1 240 ---MLAFAG---IGVAMGNA---TEPAKA-AADYITD-DVDHDGIMNALKHFF--NVL 283

YP_003971333.1 198 ---GAIACG---LDCIGRCWGYAEAGLEEHEGAYRIID--TVDELAANVNDY--FAK 244

WP_053825032.1 235 ---HMTTSE---TGAVANA--NPEVIK-IADEICP-PNNDCCVAGQWIERH--LLA 278

2bng.pdb_chainA_s001 117 ---MFKG---LLRLGLAL--VVPSS 132

4dln.pdb_chainA_s002 242 TTMTLACGGHGMGTFLQEQMKAYAEADVEGHVLPGC--GHWLPE--ECAAPMKNRLVIDF--LSRGRHH 304

1nww.pdb_chainA_s003 138 ---FEEAVD---LPLRG----- 148

Consensus_aa: END SEQUENCES HERE

Consensus_ss: hhp.ts.....ht.hhhs.t.....h.....ps.l.p.l.ph.....

hhhh eeee hhhh h eee hhhhhhhhhh h

Supplementary Figure4. Related to Methods: Colorimetric Detection of EH Activity

Alignment of thirteen EH candidate genes with *Mycobacterium tuberculosis* (pdbid: 2bng), *Pseudomonas aeruginosa* (pbdid: 4dln), and *Rhodococcus erythopolis* (pdbid: 1nww), the crystal structures of which have been solved. Alignment was performed using Promals3D. The keys for consensus amino acid symbols (consensus_aa) and consensus secondary structure symbols (consensus_ss) can be found at <http://prodata.swmed.edu/promals3d/info/consensus.html> and http://prodata.swmed.edu/promals3d/info/consensus_ss.html, respectively.

Supplementary Table 1. Related to Methods: Colorimetric Detection of EH Activity

Conserved marker regions for the top differential EH genes found in the metagenomic sequencing data. Fold changes were calculated comparing neonates who developed atopy to those who did not (n=26 neonates). P-values were generated using a two-sided Wilcox test and adjusted using the Benjamini & Hochberg (BH) correction. The frequency of conserved markers found in the metagenomic data were used to select candidate genes for further analysis.

Gene	Taxa	Fold Change	Adjusted p-value	Markers	% Markers
NP 814685	<i>Enterococcus faecalis</i>	4.79	0.0237	3 of 4	75%
NP 814772	<i>Enterococcus faecalis</i>	3.85	0.0237	5 of 6	83%
NP 814872	<i>Enterococcus faecalis</i>	4.03	0.0237	4 of 5	80%
NP 814982	<i>Enterococcus faecalis</i>	3.92	0.0237	5 of 6	83%
NP 816494	<i>Enterococcus faecalis</i>	4.12	0.0237	2 of 2	100%
NP 816679	<i>Enterococcus faecalis</i>	3.73	0.0237	4 of 6	67%
NP 816706	<i>Enterococcus faecalis</i>	4.45	0.0237	3 of 5	60%
WP 002386325	<i>Enterococcus faecalis</i>	3.29	0.0237	6 of 7	86%
WP 010711964	<i>Enterococcus faecalis</i>	3.9	0.0237	6 of 7	86%
WP 016634357	<i>Enterococcus faecalis</i>	4.21	0.0237	14 of 15	93%
WP 021147403	<i>Streptococcus</i>	2.16	0.042	7 of 9	78%
WP 060973180	<i>Streptococcus</i> sp	2.17	0.038	4 of 6	67%
WP 010122664	<i>Corynebacterium nuruki</i>	5.33	0.0131	1 of 7	14%
WP 013363968	<i>Bifidobacterium bifidum</i>	5.05	0.004	1 of 1	100%
WP 022173620	<i>Bifidobacterium bifidum</i>	5.39	0.0253	1 of 3	33%
WP 053825032	<i>Bifidobacterium bifidum</i>	5.2	0.0262	6 of 6	100%
YP 003971091	<i>Bifidobacterium bifidum</i>	6.82	0.0185	3 of 3	100%
YP 003971333	<i>Bifidobacterium bifidum</i>	4.87	0.0393	5 of 5	100%
YP 003971455	<i>Bifidobacterium bifidum</i>	4.92	0.0028	9 of 13	69%
WP 035137434	<i>Collinsella</i> sp	2.52	0.0422	4 of 6	67%
WP 063132638	<i>Enterobacter cloacae</i>	7.33	0.0095	4 of 6	67%
WP 014074165	<i>Lactobacillus ruminis</i>	2.62	0.0093	1 of 9	11%
WP 057877901	<i>Lactobacillus paucivorans</i>	2.57	0.009	1 of 7	14%

Supplementary Table 2. Related to Methods: Human Immune Assays and Animal Models
Antibodies used for human and mouse immune cell staining.

Human T Cell Panel			
Antibody	Clone	Vendor	Dilution
anti-CD4	L200	BD	1:100
anti-CD25	M-A251	BD	1:25
LIVE-DEAD Aqua		Life Technologies	1:300
anti-CD3	SP34-2	BD	1:100
anti-FoxP3	PCH101	eBioscience	1:20
anti-IFN γ	B27	BD	1:200
anti-IL4	7A3-3	Miltenyi Biotec	1:20
anti-IL-17	64DEC17	eBioscience	1:20
Human DC Panel			
Antibody	Clone	Vendor	Dilution
anti-CD11c	B-ly6	BD	1:50
anti-CD19	SJ25C1	BD	1:100
LIVE-DEAD Aqua		Life Technologies	1:300
anti-CD3	SP34-2	BD	1:100
anti-CCR7	3D12	BD	1:35
anti-CD80	2D10	Biolegend	1:50
anti-CD86	2331	BD	1:50
anti-HLA-DR	G46-6	BD	1:50
anti-CD36	CB38	BD	1:50
Anti-CD1a	HI149	BD	1:40
Mouse T Cell Panel			
Antibody	Clone	Vendor	Dilution
anti-CD4	RM4-5	BD	1:100
anti-CD25	PC61	BD	1:50
LIVE-DEAD Aqua		Life Technologies	1:300
anti-CD3e	500A2	BD	1:150
anti-FoxP3	FJK-16s	eBiosciences	1:70
Mouse Innate Panel			
Antibody	Clone	Vendor	Dilution
anti-CD11b	M1/70	Biolegend	1:100
anti-CD11c	N418	Thermofisher Scientific	1:100
LIVE-DEAD Aqua		Life Technologies	1:300
anti-CD45	30-F11	Thermofisher Scientific	1:10
anti-SiglecF	E50-2440	BD	1:100
anti-F4/80	BM8	Biolegend	1:100
anti-Ly6c	AL-21	BD	1:100
anti-Ly6g	1A8	Biolegend	1:100

Supplementary Table 3. Related to Methods: Human Immune Assays, Animal Models, and Quantification of 3EH Copy Number by qPCR

Primers used for human, mouse, and bacterial qPCR.

Human qPCR Primers		
Gene	Primer	Sequence
HADH	Forward	ATCAACACTGCCAGTGTGGCT
HADH	Reverse	AGTGTCATGCCCCACTATTCCC
FABP4	Forward	GGATGGAAAATCAACCACCA
FABP4	Reverse	GGAAGTGACGCCTTTCATGA
CD1a	Forward	ACCTGTCCTGTCGGGTGAA
CD1a	Reverse	CCCACGGAACTGTGATGCT
CD36	Forward	TCTTTCCTGCAGCCCAATG
CD36	Reverse	AGCCTCTGTTCCAACTGATAGTGA
Beta-actin	Forward	AAGATGACCCAGATCATGTTTGAGACC
Beta-actin	Reverse	AGCCAGTCCAGACGCAGGAT
Mouse qPCR Primers		
Gene	Primer	Sequence
IL1a	Forward	CGAAGACTACAGTTCTGCCATT
IL1a	Reverse	GACGTTTCAGAGGTTCTCAGAG
IL1b	Forward	GAAATGCCACCTTTTGACAGTG
IL1b	Reverse	TGGATGCTCTCATCAGGACAG
TNF	Forward	CCCTCACACTCAGATCATCTTCT
TNF	Reverse	GCTACGACGTGGGCTACAG
GAPDH	Forward	CCTCGTCCCGTAGACAAAATG
GAPDH	Reverse	TCTCCACTTTGCCACTGCAA
Bacterial EH qPCR Primers and Probes		
Gene	Primer	Sequence
NP_814872	Forward	CGA CGG AAC ATT GCT AAA TTC AC
NP_814872	Reverse	CAA AGG ACG GCC TGT ACA TAA
NP_814872	Probe	/56-FAM/ACGGGTCAA/ZEN/AGAAGCGCTCCAAA/3IABkFQ/
YP_003971091	Forward	GTC GTT CAG ACG CAA CCA TA
YP_003971091	Reverse	TTT GTG GCC GGG ATT GTT
YP_003971091	Probe	/56-FAM/TACTATGCG/ZEN/GACAAGGCGGTGTTC/3IABkFQ/
YP_003971333	Forward	GCA CCG ACC GTG AAC AT
YP_003971333	Reverse	GGA CAG CTT CGC TTC ATC TT
YP_003971333	Probe	/56-FAM/CGCTGAAGC/ZEN/ATGAGACGTTCCAGA/3IABkFQ/

Supplementary Table 4. Related to Colorimetric Detection of EH Activity

Sequences of the synthetic EH gene constructs used in the *in vitro* screen of EH activity.

Synthetic Epoxide Hydrolase Genes	
Gene	Sequence
NP_814685	aattgcagaattcatgattaaactgattgagcgatgatggatggcaccctgctggat gcgaaaatgagcattaccaacgataacgcgagcgcgattcggaagcggaacgc ctgggcattgaatttatggtggcgaccggcgcgctataccgaagcgaaaccggc gctggaagaagcgggcattgattgagcgatgattaccctgaacggcgcgagggtgt ttgataaagatggccatagcctgtttaccgcgggcattgaaaaagaaaccgtgacc gaagtgtgaccattctgagccagcataacgtgtattatgaaattagcaccaacaaa ggcatttttagcgaacatcaggaaaaacgcattgaaaactttgcggcgcatattgcg gaaagcatgccgcatctgacctataaagtggcgattgcatggcgagcgcgcatct gagcctgctgcatattacctatgttgatcgctggatgatattctgaaagatgatagca ttgaagtgtgaaaattattggcttttagcatggatggcccgaagtgtggggccggc gggcatggaagtgaagaactggatgatctggtgtgaccagcagcgcgctgaac aacattgaaattaaccatcgctggcgagaaaggcattgcggtggcgcgcgctggc gaaagaacgcggcattccggcggaacagggtgatgaccattggcgataacctgaac gatgtgagcatgattcagtgggcgggcgtagctttgcatgggcaacgcggaact ggaactgaaagaatatgcgaatatgaaaccgcgaccaacctggaaaacggcgt ggggaagcgattctgcgcgagattcggaagatctggtgtcgacttatccggcc
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	cgccgcaaccatatgccggatgaactgctggatcgcgcgctggaaatttatcgga atattatgaggataaagcgggtgttgatgatccgaacaaccgggccataaaattcc gggcccctgtataacagcgtgtatgcgggcattccggaacagctggcgcgctgc gcgcggatggcctgcatctggcgattgcgacctgcaaaccgcagtatcaggcgga accgggtgtgcaacattttcatctggataccatggtggatggcatttatggcgcgagc accgataacagccgcattgataaagatcagggtattcgctattgctttgatagcattgg ctttgatgcggatgcgggcgatcgcgcgctgatggtggcgatcgctggaccgatgt ggatggcgcgattgcgtgcggcctggattgcctgggctgcgctggggctatgcgga agcggggcaactggaagaacatggcgcgatcgcatattgataccgtggatgaac tggcggcggcggtgaacgattatttgcgaaagtgtcgacttatccggcc
YP_003971333	aattgcagaattcatgattaaagcggcggtttttgatattgatggaccctgaccagctt gtgacccatgtgattccgcagagcagcattgatgcgctgcatgaactgcaggatcgc ggcgtgaaagtgtttatttgcagcgggccgcgcgccagccatatgaccgtggtgtg gatatgatccggtgcattttgatggcattattgcgctgaacggccagattgctttgatg atcatggcctgctggaaaaagaaagcctgctgccggaagatattgtgaccattacc gctggtggatgaacatccgatgtggtggcgaactattgcgaaaaagattatgtgt tttaaccagattaccgatgcgatgcgcgcgacctggcgccagctgggcaaaaccg cgccgaccgtgaacattgatgatccgcataacgcgcgctgaaacatgaaacctt cagattagcccgtatattagctttgaagatgaagcgaactgagcggcattgtccgc aacgtgcgcggcgctgcgctggcatccggattttaccgatctgattccggcggatggc ggcaaacgggaaggcatgaaacgctttatgcgccattatggctggaccgcgaac agaccattgcgtttggcgatggcggaacgatgcggatatgctggcggttgcgggca ttggcgtggcgatgggcaacgcgaccgaaccggcgaaagcggcggcggattatat taccgatgatgtgatcatgatggcattatgaacgcgctgaaacattttaacgtgctgg ttgtcgacttatccggcc

Supplementary Table 5. Related to Quantification of 3EH Copy Number by qPCR**Gene fragments used to generate the standard curves for quantification of the 3EH genes by qPCR.**

Bacterial EH Gene Fragments	
Gene	Sequence
NP_814872	ATGTCAATTAAGTTAGTTGCTATTGATATCGACGGAACAT TGCTAAATTCACAACACAAGATTACCCACGGGTCAAAGA AGCGCTCCAAAAAGCAAATGAGCAAGGTGTTTCGTATTGTT TTATGTACAGGCCGTCCTTT
YP_003971091	ACCGGCCATCATCGAGTCGTTTCAGACGCAACCATATGCC GGACGAGCTGCTGGACCGCGGCGTGGAGATATACCGCG AATACTATGCGGACAAGGCGGTGTTTCGACGACCCGAACA ATCCCGGCCACAAAATTCCCGGACGAC
YP_003971333	CCAACTCGGCAAGACCGCACCGACCGTGAACATCGACG ATCCGCACGAGAGGGCGCTGAAGCATGAGACGTTCCAG ATCAGCCCGTACATCAGCTTTGAAGATGAAGCGAAGCTG TCC GGCATGTGTTCGC

Supplementary Table 6. Comparison of sample characteristics for development of atopy at age two and/or asthma at age four for a subset of the WHEALS cohort. P values were calculated using a Fisher Exact test for all comparisons except age at the time of stool collection, which was calculated using a two-sided student T-test.

Comparison of children who developed atopy at 2 years and/or asthma at 4 years from a subset of the WHEALS cohort			
	Atopic and/or Asthmatic (n=19)	Not atopic or asthmatic (n=22)	P Value
Race: Black, no. (%)	14 (73.7)	18 (81.8)	0.709
Male, no. (%)	9 (47.4)	11 (50.0)	1.000
Age of Stool, median (SD)	35 (11.4)	35 (23.4)	0.522
Maternal Asthma, no. (%)	5 (26.3)	2 (9.1)	0.219
Cesarean section, no. (%)	4 (21.1)	4 (18.2)	1.000
Exclusively Breastfed, no. (%)	1 (5.3)	3 (13.6)	0.610
Maternal Smoking, no. (%)	3 (15.8)	3 (13.6)	1.000
No Pet Exposure, no. (%)	14 (73.7)	14 (63.6)	0.524

Supplementary Table 7. Comparison of sample characteristics for development of eczema at age two and/or asthma at age four for a subset of the TIPS cohort. P values were calculated using a Fisher Exact test for all comparisons except age at the time of stool collection, which was calculated using a two-sided student T-test.

Comparison of children who developed eczema at 2 years and/or asthma at 4 years from a subset of the TIPS cohort			
	Eczema and/or Asthma (n=23)	No eczema or asthma (n=27)	P Value
Race: Black, no. (%)	2 (8.7)	2 (7.4)	1.000
Male, no. (%)	8 (34.8)	12 (44.4)	0.569
Age of Stool, median (SD)	33 (5.55)	31 (4.06)	0.245
Maternal Asthma, no. (%)	15 (65.2)	16 (59.3)	0.773
Cesarean section, no. (%)	6 (26.1)	9 (33.3)	0.758
Exclusively Breastfed, no. (%)	12 (52.2)	16 (59.3)	0.776
Maternal Smoking, no. (%)	0 (0.0)	1 (3.7)	1.000
No Pet Exposure, no. (%)	9 (39.1)	9 (33.3)	0.771

Supplementary Table 8. Related to Figure 3a, Supplementary Figure 3a

Logistic regression analysis of the association between fecal oxylipins and the development of atopy and/or asthma. Univariate logistic regression was used to model the relationship between the concentration of 12,13 DiHOME or 9,10 DiHOME in neonatal stool and the development of atopy at age two and/or asthma at age four. Multivariate Logistic regression indicates that the association between 12,13 DiHOME and the development of atopy and/or asthma is confounded by race (shown in bold; change in OR = 14.6%). The adjusted p-value is presented in Figure 3a.

Logistic regression modeling of DiHOME concentration and atopy at age 2 and/or asthma at age 4

Predictor	Estimate	Error	Odds Ratio (95% CI)	P value
12,13 DiHOME	1.487	0.726	4.42 (1.07-18.35)	0.041
9,10 DiHOME	0.29	0.24	1.34 (0.84-2.13)	0.22

Multivariate regression modeling of DiHOME concentration and atopy at age 2 and/or asthma at age 4 and potential confounders

Predictor	Adjusting for	Estimate	Error	Adjusted OR (95% CI)	% Change OR
12,13 DiHOME	Maternal Asthma (n=7)	1.526	0.787	4.60 (0.98-21.52)	4.02
12,13 DiHOME	No Pets (n=28)	1.440	0.736	4.22 (1.00-17.86)	-4.55
12,13 DiHOME	Maternal Smoking (n= 6)	1.574	0.727	4.83 (1.16-20.07)	9.13
12,13 DiHOME	Male (n=20)	1.492	0.727	4.44 (1.07-18.47)	0.47
12,13 DiHOME	Exclusively Breastfed (n=4)	1.440	0.742	4.22 (0.99-18.07)	-4.58
12,13 DiHOME	Race : Black (n=32)	1.623	0.779	5.07 (1.10-23.33)	14.60
12,13 DiHOME	C-section (n=8)	1.495	0.728	4.46 (1.07-18.59)	0.85
12,13 DiHOME	Age Stool (median = 35 d)	1.472	0.719	4.36 (1.07-17.81)	-1.54
9,10 DiHOME	Maternal Asthma (n=7)	0.31	0.26	1.36 (0.82-2.28)	1.60
9,10 DiHOME	No Pets (n=28)	0.28	0.23	1.33 (0.85-2.08)	-1.08
9,10 DiHOME	Maternal Smoking (n= 6)	0.29	0.24	1.34 (0.84-2.14)	-0.04
9,10 DiHOME	Male (n=20)	0.30	0.24	1.36 (0.84-2.18)	1.10
9,10 DiHOME	Exclusively Breastfed (n=4)	0.26	0.23	1.30 (0.82-2.06)	-2.92

9,10 DiHOME	Race : Black (n=32)	0.35	0.26	1.42 (0.86-2.35)	5.93
9,10 DiHOME	C-section (n=8)	0.31	0.24	1.36 (0.85-2.19)	1.56
9,10 DiHOME	Age Stool (median = 35 d)	0.28	0.24	1.32 (0.83-2.09)	-1.69

Supplementary Table 9. Related to Figure 3b

Logistic regression analysis of the association between the normalized fecal EH count and the development of atopy and/or asthma. Univariate Logistic regression was used to model the relationship between base 2 log transformed normalized EH count in neonatal stool and the development of atopy at age two and/or asthma at age four. Multivariate logistic regression indicates that the association between total EH count and the development of atopy and/or asthma is confounded by exclusive breastfeeding (shown in bold; change in OR = 22.9%). The adjusted p-value is presented in Figure 3b. ⁺Analysis was performed using a base 2 log transformation of total EH count.

Logistic regression modeling of total EH count and atopy at age 2 and/or asthma at age 4

Predictor	Estimate	Error	Odds Ratio (95% CI)	P value
Total EH Count ⁺	0.510	0.197	1.66 (1.13-2.45)	0.009

Multivariate regression modeling of total EH count and atopy at age 2 and/or asthma at age 4 and potential confounders

Predictor	Adjusting for	Estimate	Error	Adjusted OR (95% CI)	% Change OR
Total EH Count ⁺	Maternal Asthma (n=3)	0.525	0.212	1.69 (1.12-2.56)	1.49
Total EH Count ⁺	No Pets (n=18)	0.580	0.220	1.79 (1.16-2.75)	7.22
Total EH Count ⁺	Maternal Smoking (n= 0)	0.510	0.197	1.67 (1.13-2.45)	0.00
Total EH Count ⁺	Male (n=19)	0.579	0.225	1.78 (1.15-2.77)	7.10
Total EH Count⁺	Exclusively Breastfed (n=3)	0.716	0.265	2.05 (1.22-3.44)	22.8
Total EH Count ⁺	Race : Black (n=20)	0.574	0.225	1.78 (1.14-2.76)	6.61
Total EH Count ⁺	C-section (n=4)	0.505	0.202	1.66 (1.12-2.46)	-0.50
Total EH Count ⁺	Age Stool (median = 32.5 d)	0.522	0.199	1.69 (1.14-2.49)	1.19

Supplementary Table 10. Related to Supplementary Figure 4a

Logistic regression analysis of the association between the 3EH genes and the development of atopy and/or asthma. Univariate logistic regression was used to model the relationship between base 2 log transformed 3EH copy number in neonatal stool and the development of atopy at age two and/or asthma at age four. Multivariate logistic regression indicates that there were no strong confounders of the association between 3EH gene copy number and development of atopy and/or asthma (all changes in OR <10%). The univariate logistic regression p value is presented in Figure 4a. ⁺Analysis was performed using a base 2 log transformation of 3EH copy number.

Logistic regression modeling of total EH count and atopy at age 2 and/or asthma at age 4					
Predictor		Estimate	Error	Odds Ratio (95% CI)	P value
3EH Genes ⁺		0.510	0.197	1.25 (0.98-1.59)	0.073
Multivariate regression modeling of total EH count and atopy at age 2 and/or asthma at age 4 and potential confounders					
Predictor	Adjusting for	Estimate	Error	Adjusted OR (95% CI)	% Change OR
3EH Genes ⁺	Maternal Asthma (n=7)	0.221	0.127	1.25 (0.98-1.60)	-0.10
3EH Genes ⁺	No Pets (n=28)	0.236	0.128	1.27 (0.99-1.63)	1.45
3EH Genes ⁺	Maternal Smoking (n= 6)	0.228	0.125	1.26 (0.98-1.60)	0.62
3EH Genes ⁺	Male (n=20)	0.225	0.126	1.25 (0.98-1.60)	0.24
3EH Genes ⁺	Exclusively Breastfed (n=4)	0.263	0.134	1.30 (1.00-1.69)	4.17
3EH Genes ⁺	Race : Black (n=32)	0.232	0.127	1.26 (0.98-1.62)	0.97
3EH Genes ⁺	C-section (n=8)	0.267	0.135	1.31 (1.00-1.70)	4.60
3EH Genes ⁺	Age Stool (median = 35 d)	0.228	0.125	1.26 (0.98-1.61)	0.56

Supplementary Table 11. Comparison of sample characteristics for atopy at age two and asthma at age four for a subset of the WHEALS cohort. P values were calculated using a two-sided Fisher Exact test for all comparisons except age at the time of stool collection, which was calculated using a two-sided student T-test.

	Comparisons for atopy at age 2			Comparisons for asthma at age 4		
	Atopic (n=11)	Non- atopic (n=30)	P value	Asthmatic (n=12)	Non- asthmatic (n=29)	P value
Race: Black, no. (%)	9 (81.8)	23 (76.7)	1.000	9 (75)	23 (79.3)	1.000
Male, no. (%)	4 (36.4)	16 (53.3)	0.484	6 (50)	14 (48.3)	1.000
Age of Stool, median (SD)	35 (4.24)	34.5 (21.5)	0.284	33 (14.3)	35 (20.5)	0.829
Maternal Asthma, no. (%)	3 (27.3)	4 (13.3)	0.361	3 (25)	4 (13.8)	0.398
Cesarean section, no. (%)	1 (9.1)	7 (23.3)	0.412	3 (25)	5 (17.2)	0.672
Exclusively Breastfed, no. (%)	1 (9.1)	3 (10.0)	1.000	0 (0)	4 (13.8)	0.302
Maternal Smoking, no. (%)	1 (9.1)	5 (16.7)	1.000	3 (25)	3 (10.3)	0.334
No Pet no. (%)	8 (72.7)	20 (66.7)	1.000	9 (75)	19 (65.5)	0.719

Supplementary Table 12. Related to Supplementary Figure4b

Logistic regression models of associations between risk factors and development of atopy at age two and/or asthma at age four in a subset of the WHEALS cohort. Univariate logistic regression was used to model associations between known early-life risk factors, fecal concentration of 12,13 DiHOME (ng/mg), and base 2 log-transformed 3EH copy number, and the odds of developing atopy at age two and/or asthma at age four. Increased fecal 12,13 DiHOME was associated with significantly increased odds of developing atopy and/or asthma in childhood. Multivariate logistic regression was used to test for confounding. As seen in Supplementary Table 6, the relationship between 12,13 DiHOME and the development of atopy and/or asthma is confounded by race. This adjusted odds ratio is presented in Supplementary Figure4b. In contrast, the association between 3EH copy number and the development of atopy and/or asthma showed no strong evidence of confounding (Supplementary Table 10). ⁺Analysis was performed using a base 2 log transformation of 3EH copy number.

Univariate Logistic regression modeling associations between known and microbial risk factors and atopy at age 2 and/or asthma at age 4 in the WHEALS cohort.				
Predictor	Estimate	Error	Odds Ratio (95% CI)	P value
Race: Black	-0.474	0.760	0.62 (0.14-2.76)	0.532
Male	-0.105	0.627	0.90 (0.26-3.07)	0.867
Age of Stool	-0.012	0.018	0.99 (0.95-1.02)	0.517
Maternal Asthma	1.273	0.906	3.57 (0.60-21.1)	0.160
Cesarean Section Exclusively Breastfed	0.182	0.789	1.20 (0.26-5.63)	0.817
	-1.045	1.201	0.35 (0.03-3.70)	0.384
Maternal Smoking	0.172	0.884	1.19 (0.21-6.72)	0.846
No Pet Exposure	0.470	0.684	1.60 (0.42-6.11)	0.492
3EH Genes⁺	0.222	0.124	1.25 (0.98-1.59)	0.073
12,13 DiHOME	1.487	0.726	4.42 (1.07-18.4)	0.041

Multivariate regression modeling of the association between 12,13 DiHOME and atopy at age 2 and/or asthma at age 4.

Predictor	Adjusting for	Estimate	Error	Adjusted OR (95% CI)	% Change OR	P value
12,13 DiHOME	Race: Black	1.62	0.78	5.07 (1.10-23.33)	14.60	0.037

Supplementary Table 13. Comparison of sample characteristics for eczema at age two and asthma at age four for a subset of the TIPS cohort. P values were calculated using a Fisher Exact test for all comparisons except age at the time of stool collection, which was calculated using a two-sided student T-test. Only 41 of the 50 patients had asthma outcomes at 4 years. Those without asthma outcomes were excluded from the comparisons below.

	Comparisons for eczema at age 2			Comparisons for asthma at age 4		
	Eczema (n=22)	No Eczema (n=28)	P value	Asthmatic (n=6)	Non- asthmatic (n=35)	P value
Race: Black, no. (%)	2 (9.1)	2 (7.1)	1.000	1 (16.7)	3 (8.6)	0.483
Male, no. (%)	8 (36.4)	12 (42.9)	0.773	2 (33.3)	14 (40.0)	1.000
Age of Stool, median (SD)	32.5 (5.15)	31.5 (4.61)	0.627	31.5 (5.56)	32 (4.16)	0.483
Maternal Asthma, no. (%)	14 (63.6)	17 (60.7)	1.000	4 (66.7)	23 (65.7)	1.000
Cesarean section, no. (%)	6 (27.3)	9 (32.1)	0.765	2 (33.3)	11 (31.4)	1.000
Exclusively Breastfed, no. (%)	11 (50.0)	17 (60.7)	0.568	3 (50.0)	22 (62.9)	0.662
Maternal Smoking, no. (%)	0 (0.0)	1 (3.6)	1.000	0 (0.0)	1 (2.9)	1.000
No Pet Exposure, no. (%)	8 (36.4)	10 (35.7)	1.000	4 (66.7)	12 (34.3)	0.187

Supplementary Table 14. Related to Supplementary Figure4c

Logistic regression modeling of associations between risk factors and the development of eczema at age two and/or asthma at age four in a subset of the TIPS cohort. Univariate logistic regression was used to model the associations between known early-life risk factors, fecal concentration of 12,13 DiHOME (ng/mg), and base 2 log-transformed 3EH copy number and the odds of developing eczema at age two and/or asthma at age four. Increased fecal 3EH copy number was associated with significantly increased odds of developing atopy and/or asthma in childhood. Multivariate logistic regression was used to test for confounding. The associations between fecal 3EH copy number or 12,13 DiHOME and the development of eczema and/or asthma showed no strong evidence of confounding. ⁺Analysis was performed using a base 2 log transformation of 3EH copy number.

Univariate Logistic regression modeling associations between known and microbial risk factors and eczema at age 2 and/or asthma at age 4 in the TIPS cohort.

Predictor	Estimate	Error	Odds Ratio (95% CI)	P value
Race: Black	0.174	1.043	1.190 (0.154-9.193)	0.867
Male	-0.405	0.585	0.667 (0.212-2.096)	0.488
Age of Stool	0.072	0.062	1.075 (0.952-1.213)	0.244
Maternal Asthma	0.254	0.587	1.289 (0.408-4.077)	0.666
Cesarean Section	-0.288	0.572	0.750 (0.244-2.303)	0.615
Exclusively Breastfed	-0.348	0.626	0.706 (0.207-2.409)	0.578
Maternal Smoking	-15.443	1455	0.000 (0.000-Inf)	0.992
No Pet Exposure	0.251	0.591	1.286 (0.404-4.094)	0.671
3EH Genes⁺	0.225	0.091	1.253 (1.049-1.496)	0.013
12,13 DiHOME	0.413	0.235	1.511 (0.953-2.396)	0.079

Multivariate regression modeling of the association between 12,13 DiHOME and eczema at age 2 and/or asthma at age 4 in the TIPS cohort.

Predictor	Adjusting for	Estimate	Error	Adjusted OR (95% CI)	% Change OR
12,13 DiHOME	Race: Black	0.489	0.264	1.631 (0.972-2.739)	7.99
12,13 DiHOME	Male	0.455	0.249	1.577 (0.969-2.567)	4.38
12,13 DiHOME	Age of Stool	0.442	0.253	1.556 (0.947-2.555)	2.97
12,13 DiHOME	Maternal Asthma	0.401	0.234	1.493 (0.943-2.364)	-1.15
12,13 DiHOME	Cesarean Section	0.409	0.236	1.506 (0.949-2.389)	-0.34
12,13 DiHOME	Exclusively Breastfed	0.406	0.235	1.500 (0.946-2.380)	-0.68
12,13 DiHOME	Maternal Smoking	0.393	0.234	1.481 (0.937-2.341)	-1.98
12,13 DiHOME	No Pet Exposure	0.415	0.237	1.514 (0.952-2.408)	0.21
3EH Genes ⁺	Race: Black	0.23	0.09	1.255 (1.050-1.499)	0.19
3EH Genes ⁺	Male	0.23	0.09	1.257 (1.052-1.503)	0.38
3EH Genes ⁺	Age of Stool	0.22	0.10	1.245 (1.031-1.504)	-0.61
3EH Genes ⁺	Maternal Asthma	0.23	0.09	1.257 (1.051-1.503)	0.36
3EH Genes ⁺	Cesarean Section	0.23	0.09	1.265 (1.054-1.518)	0.98
3EH Genes ⁺	Exclusively Breastfed	0.23	0.09	1.253 (1.047-1.498)	0.00
3EH Genes ⁺	Maternal Smoking	0.23	0.09	1.259 (1.052-1.507)	0.53
3EH Genes ⁺	No Pet Exposure	0.22	0.09	1.252 (1.047-1.497)	-0.07

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| ☐ | ☒ 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's 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

Flow Cytometry: FACS Diva version 8.0
qPCR: QuantStudio version 1.0
Plate Reader: Gen5 Version 2.3.0
Mass Spectrometry: Xcalibur version 2.3.0 and Analyst 1.7

Data analysis

Cytometric Bead Array: FCAP Array version 3.0.1
Flow Cytometry: FloJo version 10.1
Histology: ImageJ version 1.50i
Mass Spectrometry: Xcalibur version 2.3.0 and Analyst 1.7
Alignment: PROMALS3D version 1, Sequence Manipulation Suite version 1,
Metagenomics: BBmap version 37.25, ShortBred version 0.9.3, bioBakery version 1.7, MegaHit version 1.1.4
Heatmap Generation: HClust2 version 1.0.0
Statistical Analysis: R version 3.2.3, G*Power 3.1, visreg version 2.3-0, lmerTest version 2.0-33, ggplot2 version 2.2.1

All computer code used in this study is now publicly available in our github repository at www.github.com/srlevan

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

Metagenomic data generated in this study is available in the EMBL repository Accession #PRJEB24006 (<https://www.ebi.ac.uk/ena/>). Additional data sets and materials are available from the corresponding author upon request. All data sets and R scripts used for statistical analysis and figures are available on GitHub (<https://github.com/srlevan/>).

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

Life sciences study design

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

Sample size	Mouse Studies: A pilot study was performed to estimate the effect size ($d=5.47$) of 12,13 DiHOME on Treg frequency in mouse lungs. This estimated effect size was used to determine the minimum sample size ($n=3$) given an $\alpha=0.05$ and $\beta=0.80$. Human Primary Cell Studies: Ex vivo studies were performed with 3-4 treatment replicates per sample using cells isolated from 3-4 independent human donors. Previously published studies by our group (Fujimura et. al. 2016) were used to estimate the effect size of 12,13 DiHOME treatment of human dendritic cells on Treg frequency ($d=1.96$). This estimated effect size was used to calculate the required sample size ($n=6$) given an $\alpha=0.05$ and a $\beta=0.80$. Human Neonatal Studies: Effect size was estimated based on the relative concentration of 12,13 DiHOME in high- and low-risk neonatal stool shown in Figure S9 of Fujimura et. al. 2016. The estimated effect size ($d=1.228$), $\alpha=0.05$, and $\text{power}=0.95$ were used to estimate the required sample size ($N=38$, assuming allocation of $N2/N1=1$).
Data exclusions	No data was excluded from the analysis. Per the request of reviewer 2 statistical calculations were repeated excluding outliers (defined as points outside the whiskers on boxplots generated in GGPlot2 and plotted in the Figures); however, this analysis did not change the significance of any comparison is not included in the main text, but is available on our GitHub if there is interest.
Replication	Mouse studies: All experiments were performed with 3-5 mice per cage; mice were co-housed for 2-3 weeks prior to treatment to minimize cage effects; each treatment group was housed independently to mitigate potential effects of co-housing and coprophagy; cages from the same assay were stored in the same rack with identical handling to minimize microbial variation. All reported data was replicated across cages. Human Primary Cell Studies: Primary immune cells were isolated from 3-4 healthy human donors in all experiments. All reported data was replicated across all experiments; however, we were not able to replicate an earlier observation of the effect of 12,13 DiHOME on CD36 expression by dendritic cells. Two out of three human donors had increased CD36 expression with increased 12,13 DiHOME concentration; however, the third showed no changes in expression. Dendritic cells used in this experiment were isolated from human donors, who may have variable expression of CD36 at baseline. Human Neonatal Studies: We attempted to replicate our observations in our subset of the WHEALS cohort in a subset of the TIPS cohort, a geographically distinct neonatal cohort collected several years after the WHEALS cohort. While there were differences in the absolute concentrations of 12,13 DiHOME, the overall trends remained consistent across both cohorts.
Randomization	Mouse Studies: Mice were randomly grouped into cages by the UCSF animal facility and treatments were assigned by cage. Human Primary Cell Studies: Known early-life risk factors were including race, sex, maternal asthma, birth by Caesarian section, breastfeeding, maternal smoking, and pet exposure as well as the age at the time of stool collection were identified for both cohorts. Comparisons of these demographics in healthy neonates vs. neonates who developed allergic disease in both cohorts are available in Tables S6 and S7. These risk factors were selected a priori and potential confounders were defined as risk factors that modified the adjusted odds ratio by $>10\%$ (Maldonado and Greenland 1993) and displayed in tables S8-10.
Blinding	Mouse Studies: Scoring of peribronchial and perivascular inflammation was performed by two blinded individuals. Scores were averaged for each image and the average values were used for analysis. Investigators were blinded to the treatment groups while collecting and gating mass spectrometry and flow cytometry data after tissue samples had been removed from the mice. Human Primary Cell Studies: Investigators were not blinded to cell treatment conditions during data collection; however, they were blinded to the treatment conditions during gating of flow cytometry data.

Human Neonatal Studies: Investigators were blinded to health outcomes during metagenomic, mass spectrometry, and qPCR data collection and analysis.

Reporting for specific materials, systems and methods

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

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ 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

All antibodies are displayed in the following format: Antibody Clone Vendor Dilution Catalog # Lot #

Human T Cell Panel

anti-CD4 L200 BD 1:100 563913 5138713

anti-CD25 M-A251 BD 1:25 557741 6014823

LIVE-DEAD Aqua Life Technologies 1:300

anti-CD3 SP34-2 BD 1:100 557917 5121996

anti-FoxP3 PCH101 eBioscience 1:20 L34957 1729808

anti-IFNg B27 BD 1:200 554700 5230522

anti-IL4 7A3-3 Miltenyi Biotec 1:20 130-091-647 5160118145

anti-IL-17 64DEC17 eBioscience 1:20 50-7179-42 E15769-102

Human DC Panel

anti-CD11c B-ly6 BD 1:50 559877 4160524

anti-CD19 SJ25C1 BD 1:100 341093 6175589

LIVE-DEAD Aqua Life Technologies 1:300 L34957 1729808

anti-CD3 SP34-2 BD 1:100 557917 6200871

anti-CCR7 3D12 BD 1:35 560548 4153791

anti-CD80 2D10 Biolegend 1:50 305225 B219135

anti-CD86 2331 BD 1:50 561129 5338949

anti-HLA-DR G46-6 BD 1:50 552764 3241958

anti-CD36 CB38 BD 1:50 561535 6078701

Anti-CD1a HI149 BD 1:40 555807 4141654

Mouse T Cell Panel

anti-CD4 RM4-5 BD 1:100 563726 6217936

anti-CD25 PC61 BD 1:50 557658 5263790

LIVE-DEAD Aqua Life Technologies 1:300 L34957 1729808

anti-CD3e 500A2 BD 1:150 557984 4218820

anti-FoxP3 FJK-16s eBiosciences 1:70 45-5773-82 E08398-1634

Mouse Innate Panel

anti-CD11b M1/70 Biolegend 1:100 101257 B205713

anti-CD11c N418 Thermofisher Scientific 1:100 MCD11C28 1555781A

LIVE-DEAD Aqua Life Technologies 1:300 L34957 1729808

anti-CD45 30-F11 Thermofisher Scientific 1:10 MCD4505 1660868

anti-SiglecF E50-2440 BD 1:100 565528 7051805

anti-F4/80 BM8 Biolegend 1:100 123147 B210629

anti-Ly6c AL-21 BD 1:100 560593 7037863

anti-Ly6g 1A8 Biolegend 1:100 127624 B218644

DC Isolation Antibodies

anti-CD28 CD28.2 BD Biosciences NA 555725

anti-CD49d 9F10 BD Biosciences NA 555501

Validation

All antibodies were selected based on previously published literature (Fujimura et. al. 2016, Van Dyken et. al. 2017). Validations statements from the manufacturer's can be found at the links below. Additionally, all four panels were validated and titrated in our lab. The human T cell panel was validated and titrated with primary human T cells that had been cultured with autologous dendritic cells, treated with golgi plug, and treated with PMA/ionomycin. The human dendritic cell panel was validated and titrated with peripheral blood mononuclear cells. The mouse T cell panel was validated and titrated using a single cell suspension isolated from a mouse spleen. The mouse innate panel was validated and titrated using a single cell suspension isolated from a mouse lung.

Antibody and Manufacturer Validation

Human T Cell Panel
 anti-CD4 <http://www.bdbiosciences.com/ds/pm/tds/563913.pdf>
 anti-CD25 <http://www.bdbiosciences.com/ds/pm/tds/557741.pdf>
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD3 <http://www.bdbiosciences.com/ds/pm/tds/557917.pdf>
 anti-FoxP3 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=14-4776-82
 anti-IFN γ <http://www.bdbiosciences.com/ds/pm/tds/554700.pdf>
 anti-IL4 <https://admin-shop-live.miltenyibiotec.com/DE-en/shop/comMiltenyiDatasheet/product?productId=15167>
 anti-IL-17 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=50-7179-42
 Human DC Panel
 anti-CD11c <http://www.bdbiosciences.com/ds/pm/tds/559877.pdf>
 anti-CD19 <http://www.bdbiosciences.com/ds/pm/tds/341093.pdf>
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD3 <http://www.bdbiosciences.com/ds/pm/tds/557917.pdf>
 anti-CCR7 <http://www.bdbiosciences.com/ds/pm/tds/560548.pdf>
 anti-CD80 <https://www.biolegend.com/en-us/global-elements/pdf-popup/brilliant-violet-605-anti-human-cd80-antibody-7663?filename=Brilliant%20Violet%20605%20anti-human%20CD80%20Antibody.pdf&pdfgen=true>
 anti-CD86 <http://www.bdbiosciences.com/ds/pm/tds/5338949.pdf>
 anti-HLA-DR <http://www.bdbiosciences.com/ds/pm/tds/552764.pdf>
 Anti-CD1a <http://www.bdbiosciences.com/ds/pm/tds/555807.pdf>
 Mouse T Cell Panel
 anti-CD4 <http://www.bdbiosciences.com/ds/pm/tds/563726.pdf>
 anti-CD25 <http://www.bdbiosciences.com/ds/pm/tds/557658.pdf>
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD3e <http://www.bdbiosciences.com/ds/pm/tds/557984.pdf>
 anti-FoxP3 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=13-5773-80
 Mouse Innate Panel
 anti-CD11b <https://www.biolegend.com/en-us/global-elements/pdf-popup/pe-anti-mouse-human-cd11b-antibody-349?filename=PE%20anti-mousehuman%20CD11b%20Antibody.pdf&pdfgen=true>
 anti-CD11c https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=17-0114-82
 LIVE-DEAD Aqua http://tools.thermofisher.com/content/sfs/manuals/live_dead_fixable_dead_cell_stains_man.pdf
 anti-CD45 https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=12-0451-82
 anti-SiglecF <http://www.bdbiosciences.com/ds/pm/tds/552125.pdf>
 anti-F4/80 <https://www.biolegend.com/en-us/global-elements/pdf-popup/fitc-anti-mouse-f4-80-antibody-4067?filename=FITC%20anti-mouse%20F480%20Antibody.pdf&pdfgen=true>
 anti-Ly6c <http://www.bdbiosciences.com/ds/pm/tds/560593.pdf>
 anti-Ly6g <https://www.biolegend.com/fr-fr/global-elements/pdf-popup/purified-anti-mouse-ly-6g-antibody-4767?filename=Purified%20anti-mouse%20Ly-6G%20Antibody.pdf&pdfgen=true>
 DC Isolation Antibodies
 anti-CD28 <http://www.bdbiosciences.com/ds/pm/tds/555725.pdf>
 anti-CD49d <http://www.bdbiosciences.com/ds/pm/tds/555501.pdf>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Raw 264.7 cells were purchased from the UCSF cell culture facility.
Authentication	Authenticated Raw264.7 cells were purchased from the UCSF cell culture facility and their identity was confirmed by morphology. No genetic authentication was performed by our lab.
Mycoplasma contamination	The Raw264.7 cells used in this study were not tested for mycoplasma contamination by our lab.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines

Animals and other organisms

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

Laboratory animals	Four week old C57B6 female mice were purchased from Jackson Laboratories (Sacramento, CA) and used in all experiments at ages 5-6 weeks.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field sample collection.

Ethics oversight

UCSF IACUC provided ethical oversight and approval of this animal study. Protocol AN171803

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

The WHEALS cohort was collected in southeastern Michigan from 2003-2007. Our subset included 41 neonates (stool median age=35 days). Of the 41, 19 (46%) had atopy and/or asthma, 20 (48.8%) were male and 32 (78%) were black, based on a maternal report of race. Additionally, 8 (19.5%) were born by Cesarean section, 4 (9.75%) were exclusively breastfed until one month of age, 7 (17%) reported doctor-diagnosis of maternal asthma, 6 (14.6%) reported maternal smoking during pregnancy, and 13 (31.7%) lived with an indoor or outdoor dog or cat prior to delivery

The TIPS cohort was collected in the San Francisco Bay area from 2006-2006. Our subset of 50 neonates from the placebo arm of the TIPS cohort (stool median age = 32 days) that had more than 300 mg of one-month stool remaining were selected (Table S7; total n=50; eczema=22; asthma=6; eczema and asthma=23). Of the 50 neonates, 20 (40%) were male and 4 (8%) were black, based on a family report of demographics. Additionally, 15 (30%) were born by Cesarean section, 28 (56%) were exclusively breastfed until one month of age, 31 (62%) reported doctor-diagnosis of maternal asthma, 1 (2%) reported maternal smoking during pregnancy, and 18 (36%) did not live with a cat or dog.

Additionally, plasma donated by healthy human donors was used for human immune cell assays. Plasma was purchased from Blood Centers of the Pacific and all samples were de-identified prior to purchase.

Recruitment

The WHEALS cohort recruited pregnant women age 21-49 who lived in a predefined cluster of contiguous zip codes near Detroit, Michigan and had no intention of leaving the area. The TIPS cohort recruited newborns from the San Francisco Bay area, who had at least one parent with a history of asthma and were willing to supplement breast milk or formula with probiotics or placebo for at least one feed each day. Populations used in this study likely reflect neighborhoods from which study participants were recruited and in particular those who sought care obstetric care in the area. Participants opted to enroll their children in this study and to participate in follow up over the first five years of life, which is likely reflected in the self-selection bias of the participants. Additionally, participants in the TIPS study were randomized to the placebo arm; however, they had agreed to provide daily pro-biotic supplementation to their infant for the first six months of life, which may have limited the potential participants.

Ethics oversight

Wayne State University IRB provided ethical oversight for the WHEALS cohort, while the UCSF IRB provided ethical oversight for the TIPS cohort.

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

Human T Cell Assays: DCs were isolated from the PBMCs of two healthy human donors and treated for five days with 130 μ M 12,13 DiHOME solubilized in 0.2% DMSO or vehicle (0.2% DMSO) in the presence of 20 ng mL⁻¹ IL-4 (R&D Systems, Minneapolis, MN) and 10 ng mL⁻¹ GM-CSF (R&D Systems, Minneapolis, MN). Fresh treatment media was added every 48 hrs throughout the course of the study. Eighteen hours prior to co-culture, DCs were stimulated with 10 ng mL⁻¹ TNF α , IL1 β , and IL-6 (Peprotech, Rocky Hill, NJ) and 1 mM prostaglandin E2 (Stemcell Technologies, Cambridge, MA). DCs were subsequently washed and co-cultured with autologous T cells in the presence of 10 ng mL⁻¹ anti-CD28 and anti-CD49d (BD Bioscience, San Jose, CA). After five days of co-culture, T cell subsets were analyzed by flow cytometry. To assess cytokine secretion, cells were stimulated for 16 hrs with Phorbol Myristate Acetate-Ionomycin (ACROS, Morris Plains, NJ) and GolgiPlug (Gplug; BD Biosciences, San Jose, CA)

Human DC Assays: PBMCs were isolated from two healthy human donors using Ficoll-Hypaque gradient centrifugation as previously described¹ and cultured for five days in the presence of 20 ng mL⁻¹ IL-4 and 10 ng mL⁻¹ GM-CSF. Monocytic DCs (DCs) were defined as CD3-CD19-CD11c+. The DC gating strategy can be found in Supplemental Fig. 1e.

Mouse Lung Tissue: Mouse lung tissue was manually dissected, digested with 5 mg per sample collagenase (Sigma-Aldrich, St. Louis, MO), and passed through a 40 μ m filter to generate single cell suspensions. CD45- lung cells were considered resident cells.

Instrument

BD LSRII

Software	Data was collected using FACS Diva version 8.0 and analyzed using FlowJo version 10.1 (flow cytometry) or FCAP Array version 3.0.1 (CBA assay). Graphs and statistical analysis were performed using R version 3.2.3. All R scripts can be found at https://github.com/srlevan/ .
Cell population abundance	Cells were not sorted.
Gating strategy	Human T Cells: All boundaries were initially identified in human T cells that were stained with the full panel +/- the antibody of interest. Lymphocytes were selected by FSC-A (25K-75K) and SSC(25K-75K). Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: Live : Live-Dead-AmCyan<500 CD3+ : CD3-A700>2000 CD4+ : CD4-BV711>700 CD8+ : CD8-BD605>700 IFNg+ : IFNg-FITC >6000 IL4+ : IL-4-PE>3000 IL17+ : IL-17-APC>500 FoxP3+ : FoxP3-PB>500 CD25+ : CD25-PE-Cy7>1000 Human DCs: All boundaries were initially identified in human peripheral blood mononuclear cells that were stained with the full panel +/- the antibody of interest. Forward and side scatter were used to select monocytes [FSC (0-250K) and SSC (50K-250K)], excluding lymphocytes [FSC(25K-75K) and SSC(25K-75K)]. Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: Live : Live-Dead-AmCyan<3000 CD3- : CD3-A700<1000 CD19- : CD19-PE-Cy7<800 CD11c+ : CD11c-APC>1050 CD36+ : CD36-PB>10,000 CD1a+ : CD1a-PE>2000 CCR7+ : CCR7-FITC>2000 CD80+ : CD80-BV605>1000 Mouse Tregs: All boundaries were initially identified in mouse splenic and lung cells that were stained with the full panel +/- the antibody of interest. Forward and side scatter were used to select lymphocytes [FSC(25K-75K) and SSC(25K-75K)] were excluded. Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: Live : Live-Dead-AmCyan<3000 CD3+ : CD3-A700>2000 CD4+ : CD4-BV711>500 CD8+ : CD8-BD605>1050 FoxP3+ : FoxP3-PB>1000 CD25+ : CD25-PE-Cy7>800 Mouse Innate Cells: All boundaries were initially identified in mouse lungs that were stained with the full panel +/- the antibody of interest. Forward and side scatter were used to select granulocytes [FSC (0-250K) and SSC (50K-250K)]. Single cells were gated using FSC-A and FSC-H. The following boundaries were used in gating: CD45- : CD45-APC<1000 Live : Live-Dead-AmCyan<1000 CD11b+ : CD11b-QDot 605 >500 Eosinophils : CD11b-QDot605 4000-20,000 SiglecF-PerCP 1000-10,000 Siglec-F+ : Siglec-F-PerCP>2000 CD11b - : CD11b-QDot605 <500 Ly6G - : Ly6G-APC<1000 Ly6G + : Ly6G-APC>1000 F4/80+ : F4/80-QDot705 > 2000 CD11c + : CD11c-PB>1000 Ly6C + : Ly6C-PE-Cy7>500 Ly6C - : Ly6C-PE-Cy7 <500

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