

Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis

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Metabolites from intestinal microbiota are key determinants of host-microbe mutualism and, consequently, the health or disease of the intestinal tract. However, whether such host-microbe crosstalk influences inflammation in peripheral tissues, such as the lung, is poorly understood. We found that dietary fermentable fiber content changed the composition of the gut and lung microbiota, in particular by altering the ratio of Firmicutes to Bacteroidetes. The gut microbiota metabolized the fiber, consequently increasing the concentration of circulating short-chain fatty acids (SCFAs). Mice fed a high-fiber diet had increased circulating levels of SCFAs and were protected against allergic inflammation in the lung, whereas a low-fiber diet decreased levels of SCFAs and increased allergic airway disease. Treatment of mice with the SCFA propionate led to alterations in bone marrow hematopoiesis that were characterized by enhanced generation of macrophage and dendritic cell (DC) precursors and subsequent seeding of the lungs by DCs with high phagocytic capacity but an impaired ability to promote T helper type 2 (T_H2) cell effector function. The effects of propionate on allergic inflammation were dependent on G protein-coupled receptor 41 (GPR41, also called free fatty acid receptor 3 or FFAR3), but not GPR43 (also called free fatty acid receptor 2 or FFAR2). Our results show that dietary fermentable fiber and SCFAs can shape the immunological environment in the lung and influence the severity of allergic inflammation.

In recent decades, there has been a well-documented increase in the incidence of allergic asthma in developed countries¹, and coincident with this increase have been changes in diet, including reduced consumption of fiber². Dietary fibers have been linked with beneficial effects in gastrointestinal inflammatory disorders and protection from colon cancer; however, little is known about the consequences of dietary fiber intake on inflammation outside of the intestine. Dietary fibers are complex carbohydrates consisting of both soluble and insoluble components. The insoluble fibers have important bulking properties, whereas the soluble forms can be fermented by certain species of gut bacteria, leading to physiologically active byproducts. SCFAs are among the most abundant of these dietary metabolites, and they have a crucial role as a fuel source for intestinal epithelial cells³ and exert effects on gut morphology and function⁴. Moreover, SCFAs are an energy source for certain bacterial species^{5–7}. SCFAs are thought to elicit their effects through binding to endogenous receptors such as GPR41 and GPR43 (refs. 8–10) and through their capacity to inhibit histone deacetylase activity^{11–13}. Acetate, propionate and butyrate are the most extensively described SCFAs and are found in the intestinal tract at a molar ratio of 60:20:20, respectively¹⁴. Notably, SCFAs are not restricted to the intestinal tract but can disseminate systemically and be detected in the blood¹⁵.

Very little is known about whether dietary fiber, the microbiota and the resulting metabolites affect inflammation in the lung.

We unraveled a mechanism by which dietary fiber content shapes the gut microbiota, increasing the circulating levels of SCFAs, which act to drive bone marrow hematopoiesis and impair the capacity of DCs to instigate T_H2 cell-mediated allergic airway inflammation (AAI).

RESULTS

Dietary fiber content influences susceptibility to AAI

To address whether dietary fiber influences pulmonary inflammatory responses, mice were raised on a diet with either standard 4% fiber content (control diet) or low fiber content (<0.3%). We exposed adult mice on either diet to house dust mite extract (HDM) through intranasal administration and monitored the ensuing allergic airway inflammation. Inflammatory cell infiltration was increased in the airways of mice on a low-fiber diet (Fig. 1a). This inflammation was dominated by enhanced eosinophilic and lymphocytic infiltrates (Fig. 1b) that were also evident in histological sections (Fig. 1c). In line with the increased lymphocytic infiltration, the concentration of interleukin-4 (IL-4), IL-5, IL-13 and IL-17A were enhanced in lung tissue homogenates from mice on the low-fiber diet (Fig. 1d). Serum levels of total IgE and HDM-specific IgG1 antibodies were also increased in the low-fiber diet conditions as compared to the control diet (Fig. 1e), supporting the conclusion that dietary fiber content influences the overall magnitude of the inflammatory response

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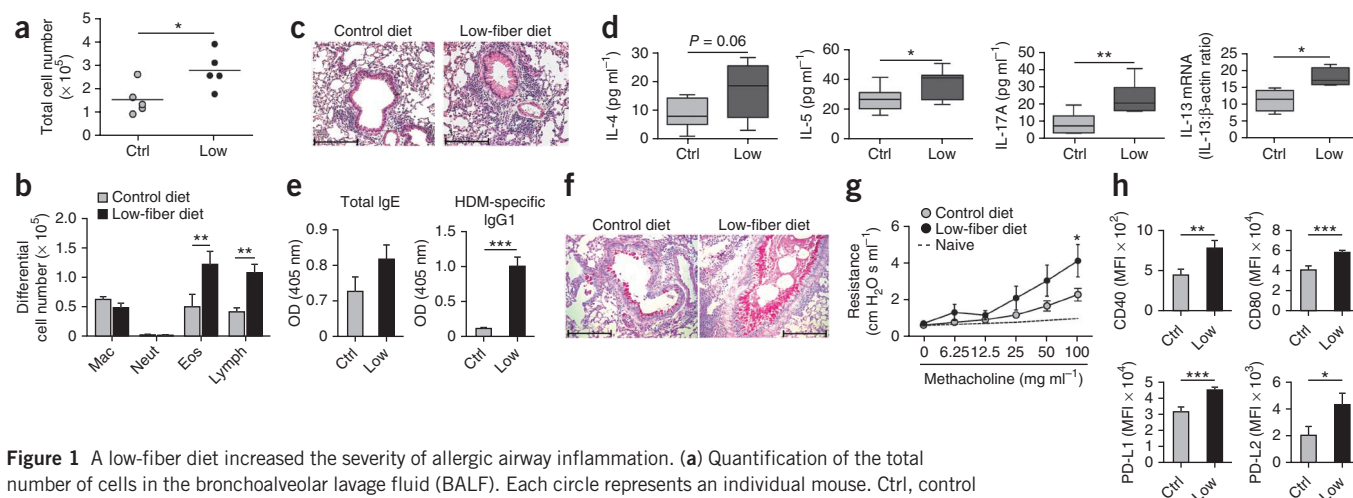


Figure 1 A low-fiber diet increased the severity of allergic airway inflammation. **(a)** Quantification of the total number of cells in the bronchoalveolar lavage fluid (BALF). Each circle represents an individual mouse. Ctrl, control diet; low, low-fiber diet. **(b)** Differential cell counts in the BALF. Mac, macrophages; neut, neutrophils; eos, eosinophils; lymph, lymphocytes. **(c)** Representative H&E-stained lung tissue from mice on either a control or low-fiber diet. Scale bars, 200 μm. **(d)** Concentration of IL-4, IL-5 and IL-17A and analysis of IL-13 mRNA expression levels in the lungs. The box and whisker plots represent the maximum and minimum values (whiskers), the upper and lower quartiles (boxes) and the median (middle horizontal line). **(e)** Measurement of the total amount of IgE or HDM-specific IgG1 in the serum. OD, optical density. **(f)** Representative Periodic acid–Schiff (PAS)-stained lung tissue from mice on either control or low-fiber diet. Scale bars, 200 μm. **(g)** Measurement of AHR, as assessed by airway resistance to increasing doses of methacholine. **(h)** Surface expression of the co-stimulatory molecules CD40, CD80, PD-L1 and PD-L2 on CD11b^{hi} DCs in the lungs. MFI, mean fluorescence intensity. All results are representative of data generated in three different experiments and are expressed as the mean ± s.e.m. ($n = 5$ control mice and $n = 5$ mice on the low-fiber diet). Statistical significance was determined with Student's *t* test, except in **b** and **g**, in which two-way analysis of variance (ANOVA) test was used. * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$.

against HDM. Further histological analysis of lung tissue sections from HDM-exposed mice revealed increased goblet cell hyperplasia and mucus production in mice fed a low-fiber diet (Fig. 1f). The increased inflammation and mucus secretion in animals on the low-fiber diet translated into increased airway hyper-reactivity (AHR), as measured by increased airway resistance after methacholine challenge (Fig. 1g). DCs isolated from the lung exhibited a more activated phenotype that was characterized by increased surface expression of the co-stimulatory molecules CD40, CD80, programmed death ligand 1 (PD-L1) and PD-L2 (Fig. 1h and Supplementary Fig. 1a).

We next performed the complementary experiment by increasing dietary fiber content. Accordingly, we fed mice a diet that we supplemented with either the poorly fermentable fiber cellulose as a control or the readily fermentable fiber pectin. We used the cellulose diet as a comparison in this model to control for possible alterations in mineral and vitamin uptake caused by the supplementation^{16–18}. Mice receiving a pectin-rich diet exhibited reduced cellular infiltration into the airways after HDM challenge (Fig. 2a), which was due primarily to the reduced recruitment of eosinophils (Fig. 2b). Similarly, we found reduced perivascular and peribronchial inflammatory cell infiltration in tissue sections from the lung (Fig. 2c). The amount of IL-4, IL-5, IL-13 and IL-17A were reduced in the lungs of mice fed the pectin-rich diet as compared to mice receiving the control diet (Fig. 2d), as were the serum levels of total IgE and HDM-specific IgG1 (Fig. 2e). Goblet cell hyperplasia and mucus production were also reduced in the mice fed the pectin-rich diet (Fig. 2f). As a result, animals that received the high-fiber diet showed improved AHR (Fig. 2g), and DCs that were present in the lungs of mice fed a pectin-rich diet were less activated, as demonstrated by reduced surface expression of CD40, CD80, PD-L1 and PD-L2 molecules (Fig. 2h and Supplementary Fig. 1b).

Dietary fiber changes the gut and lung microbiota

As recent evidence has indicated that microbiota can influence immune cell homeostasis¹⁹ and susceptibility to allergic inflammation^{20–25}, we next analyzed the effect of dietary changes on the

intestinal microbiota. To do this, we generated 16S ribosomal DNA (rDNA) amplicons and sequenced them from fecal samples of mice fed the cellulose-enriched control diet, the pectin-enriched high-fiber diet, the regular unsupplemented diet or the low-fiber diet. We performed the analysis of the variance between microbial communities from mice fed diets of varying fiber content using weighted UniFrac distances that we analyzed using principal component analysis. Dietary fiber content resulted in markedly diverse gut microbial communities (Fig. 3a). Measurements of ecological metrics revealed that a low-fiber diet markedly diminished the richness and diversity of the gut microbiota, whereas supplementing a normal chow with more fermentable fiber (pectin) did not increase the richness or diversity of the microbiota further as compared to a nonfermentable-fiber (cellulose) diet (Fig. 3b). Analysis of the microbiota at various taxonomic levels indicated alterations to the composition of the gut microbiota depending on the fiber content of the diet. At the family level, we found that a diet rich in fiber increased the proportion of Bacteroidaceae and Bifidobacteriaceae. Notably, SCFAs, particularly propionate, have been reported to be potent growth stimulators for *Bifidobacterium*⁵. Comparatively, a low-fiber diet led to microbiota that were dominated by Firmicutes, particularly those of the Erysipelotrichaceae family (Fig. 3c,d), which has also been noted in mice exposed to western high-fat diets²⁶. To validate these data at a phylum level, we used quantitative PCR to determine the relative proportions of Firmicutes to Bacteroidetes and confirmed that fiber content in the diet altered the ratio of these two bacterial phyla (Fig. 3e). We performed analysis on the lung tissue of mice from the respective diets and found a similar trend in the ratios of Bacteroidetes and Firmicutes, albeit one that was not statistically significant (Supplementary Fig. 2a,b).

Bacteria from the Bacteroidetes phylum are known to be potent at fermenting fiber into SCFAs such as acetate and propionate^{27,28}. We thus performed HPLC analysis on cecal and serum samples from mice on the respective diets and found that the levels of SCFAs in both cecal (Fig. 3f) and serum (Fig. 3g) samples were proportionally increased with higher amounts of soluble dietary fiber

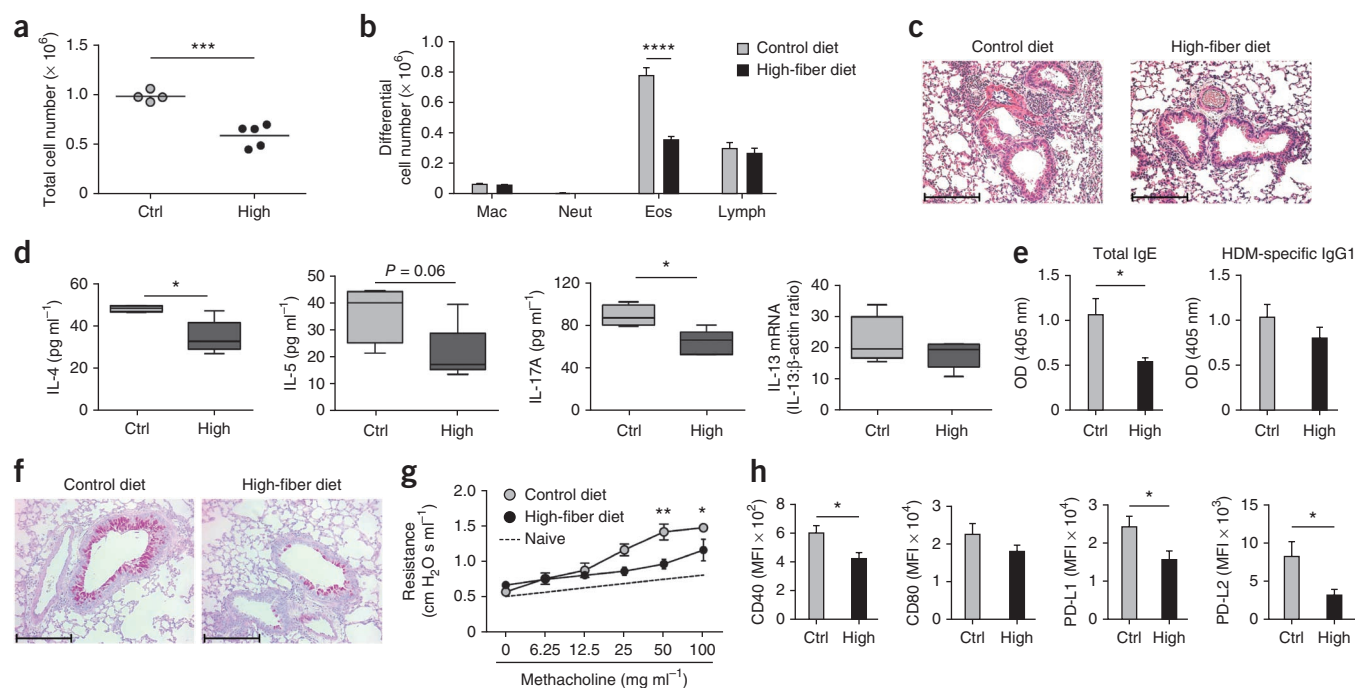


Figure 2 A high-fiber diet decreased susceptibility to allergic airway inflammation. (a) Total number of cells infiltrating the airways in the BALF. High, high-fiber diet. (b) Differential cell counts in the BALF. (c) Representative H&E-stained lung tissue from mice on either a control or high-fiber diet on day 16. Scale bars, 200 μ m. (d) Concentration of IL-4, IL-5 and IL-17A and IL-13 mRNA expression levels in the lungs. The box and whisker plots represent the maximum and minimum values (whiskers), the upper and lower quartiles (boxes) and the median (middle horizontal line). (e) Total amount of IgE or HDM-specific IgG1 in the serum. (f) Representative PAS-stained lung tissue from mice on either control or high-fiber diet on day 16. Scale bars, 200 μ m. (g) AHR in mice fed a control or high-fiber diet or in naive mice. (h) Cell-surface expression of the co-stimulatory molecules CD40, CD80, PD-L1 and PD-L2 on CD11b^{hi} DCs in the lungs. All results are representative of data generated in three different experiments and are expressed as the mean $\pm$ s.e.m. ($n = 5$ control mice and $n = 6$ mice on the high-fiber diet; $n = 5$ control mice and $n = 4$ mice on the high-fiber diet for the lung function analysis). Statistical significance was determined with Student's *t* test, except in **b** and **g**, in which two-way ANOVA test was used. * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$, **** $P = 0.0001$.

(acetate, 8.35-fold increase; propionate, 1.45-fold increase; butyrate, 0.64-fold decrease in mice fed a pectin-rich diet compared to those fed the control diet) and decreased with lower amounts of fiber (acetate, 0.2-fold decrease; propionate, 0.8-fold decrease; butyrate, 0.3-fold decrease in mice fed a lower-fiber compared to those fed the control diet). The systemic ratio of the three major SCFAs, acetate, butyrate and propionate, was 55:35:10, respectively, which is similar to that described in previous reports¹⁴. Notably, we were not able to detect these SCFAs in lung tissue (data not shown).

Propionate protects against AAI in a GPR41-dependent manner

We hypothesized that the increase in circulating SCFAs might mediate the observed protective effect of dietary fiber against allergic airway inflammation. Notably, in mice fed a pectin-rich diet, acetate and propionate were the two SCFAs that increased the most at the systemic level. Thus, we next investigated whether administering acetate or propionate to mice would mediate the same protective effect that was elicited by a high-fiber diet. Indeed, supplementing drinking water with either acetate or propionate reduced cellular infiltration into the airways after HDM exposure (Supplementary Fig. 3a), which was due primarily to a dampened recruitment of eosinophils (Supplementary Fig. 3b). We then proceeded with mechanistic studies with systemic propionate treatment. The immediate inflammatory response (in terms of immune cell infiltration into the airways) 1 d after HDM challenge was comparable between saline- and propionate-treated mice (Fig. 4a); however, the inflammation waned rapidly in the propionate-treated mice, whereas it was maintained in the saline-treated

mice up to 6 d after challenge (Fig. 4a). The reduced inflammation in the propionate-treated mice from day 2 after challenge onwards was due primarily to reduced eosinophilia, which is suggestive of an impaired T_H2 cell response (Fig. 4b). Indeed, analysis of cytokine levels in the lung tissue showed that although the immediate peak of cytokine production 1 d after HDM challenge was comparable between saline- and propionate-treated mice, at later stages of the response (days 3–6), the concentration of IL-4, IL-5 and IL-17A in the lung were reduced in the propionate-treated group (Fig. 4c). Thus, although the immediate induction of an inflammatory response against HDM was not affected, the response could not be maintained in mice that had been treated with propionate. The dampened inflammatory response in the presence of propionate was reflected in the histological analysis of the lungs, which showed reduced cellular infiltration (Fig. 4d), goblet cell hyperplasia, mucus production and levels of IL-13 in propionate-treated animals (Fig. 4e). Furthermore, serum levels of total IgE and HDM-specific IgG1 were significantly reduced (Fig. 4f).

We next investigated whether the SCFA receptors GPR41 and GPR43 are involved in this process, as propionate has been shown to bind to both of these receptors²⁹. We treated wild-type, GPR41-deficient and GPR43-deficient mice with propionate and exposed them to HDM, as described above. In line with our expectations, propionate-treated wild-type mice exhibited reduced airway eosinophilia on day 5 after HDM challenge as compared to their saline-treated counterparts (Fig. 4g). Similarly, pretreatment of GPR43-deficient mice with propionate also reduced eosinophilic infiltration in the

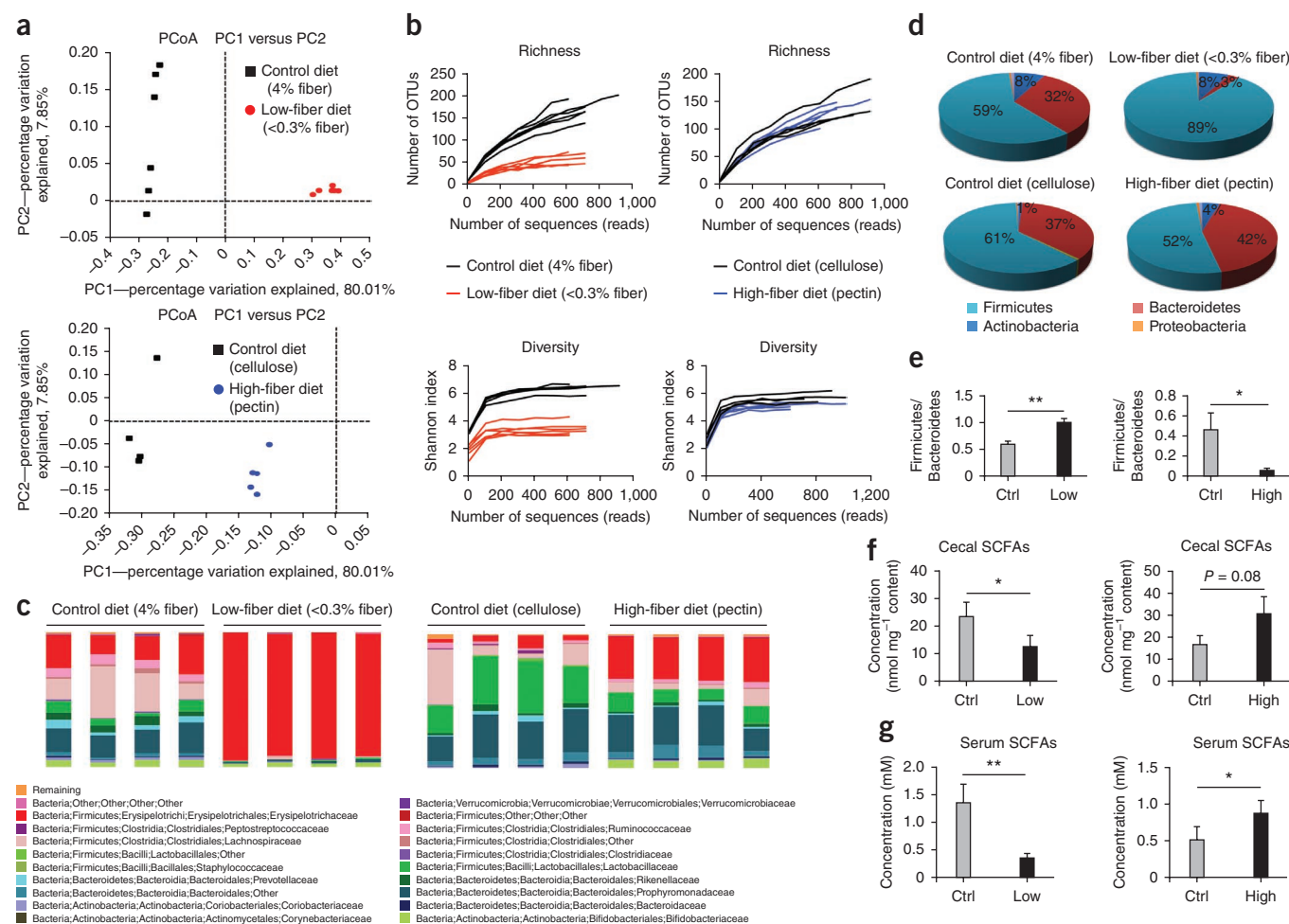


Figure 3 Dietary fiber content alters the intestinal microbiota and both local and systemic levels of SCFAs. **(a)** Analysis of the variance between microbial communities from the feces of mice fed diets of varying fiber content assessed by the average relative abundance using principal component analysis. PC, principal component. **(b)** Dietary fiber-derived changes in the microbiota richness and diversity analyzed using the QIIME pipeline. Differences are expressed by weighted UniFrac distances, and the significance of differences between the groups was tested by the Adonis method (analysis of variance). OTUs, operational taxonomic units. **(c,d)** 454 pyrosequencing analysis of the fecal microbiota composition from mice fed the different fiber diets at the family level **(c)** and the proportions of the Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria phyla **(d)**. **(e)** The ratio of Firmicutes to Bacteroidetes in the feces of mice fed high-fiber, low-fiber or control diets by quantitative PCR. **(f,g)** HPLC quantification of SCFA levels in the cecal contents **(f)** or serum **(g)**. All results are representative of data generated in two to three different experiments and are expressed as the mean $\pm$ s.e.m. ($n = 6$ control mice and $n = 6$ mice on the low-fiber diet for the control versus low fiber experiments; $n = 4$ control mice and $n = 5$ mice on the high-fiber diet for the control versus high fiber experiments). Statistical significance was determined with Student's *t* test, except in **f** and **g**, in which Mann-Whitney test was used. * $P = 0.05$, ** $P = 0.01$.

lung, indicating that this receptor is not required for the protective effect of propionate (**Fig. 4h**). However, the propionate-mediated effect required the GPR41 receptor, as GPR41-deficient mice treated with saline or propionate developed comparable airway eosinophilia (**Fig. 4i**). Thus, these data show that propionate treatment elicits a protective effect against allergic airway inflammation by signaling through the receptor GPR41.

Impaired T_H2 differentiation in SCFA-treated mice

Considering the central role DCs have in the induction and maintenance of allergic airway inflammation^{30–32}, the fact that they express GPR41 (**Supplementary Fig. 4a,b**) and that the observed protective effect of propionate treatment or high-fiber diet was not associated with increased FoxP3⁺CD25⁺ regulatory CD4⁺ T (T_{reg}) cells (**Supplementary Fig. 5**), we next sought to establish whether propionate treatment influenced DC recruitment and activation

in the lung-draining lymph nodes. We characterized different DC populations by their expression of CD11c, F4/80, major histocompatibility complex II (MHCII) and CD11b by flow cytometry (**Fig. 5a**). We assessed DC phenotypes and numbers on both days 1 and 4 after the final HDM challenge (**Fig. 5b**), at which points we found either no difference or a significant reduction in airway inflammation, respectively (**Fig. 4a**). No difference in the proportion of DC subpopulations in the lymph node was evident in the presence or absence of exogenous propionate (**Fig. 5b** and **Supplementary Fig. 6a**). However, after closer examination, it was evident that on day 4 after HDM challenge, the CD11b^{hi} DCs in the lymph nodes of propionate-treated mice exhibited an altered activation state, characterized by reduced expression of CD40, PD-L2 and CD86 (**Fig. 5c** and **Supplementary Fig. 7a,b**). The CD11b^{int} and CD11b⁺ DC subsets showed similar activation states between the control and propionate-treated animals (**Supplementary Fig. 6b**).

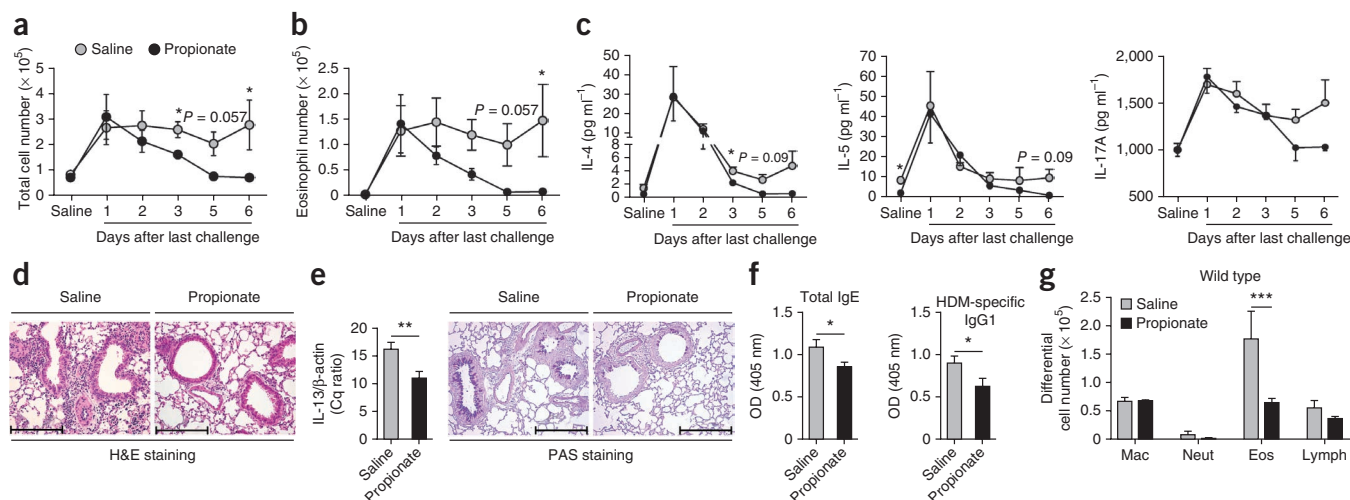


Figure 4 Mice treated with propionate are protected against the development of allergic airway inflammation. **(a,b)** The total number of cells **(a)** and eosinophils **(b)** in the BALF. **(c)** Concentration of IL-4, IL-5 and IL-17A in the lungs. **(d)** Representative H&E-stained lung tissue from mice treated or not with sodium propionate on day 5 after the last HDM challenge. Scale bars, 200 μ m. **(e)** Analysis of IL-13 mRNA expression levels in the lungs and representative PAS-stained lung tissue from mice treated with saline or sodium propionate 5 d after the last challenge. Scale bars, 200 μ m. **(f)** Total amount of IgE or HDM-specific IgG1 in the serum at day 5 after the last challenge. **(g-i)** BALF differential cell counts in wild-type **(g)**, GPR43-deficient (*Ffar2*^{-/-}) **(h)** and GPR41-deficient (*Ffar3*^{-/-}) **(i)** mice. All results are representative of data generated in three different experiments and are expressed as the mean $\pm$ s.e.m. ($n = 5$ control mice and $n = 5$ sodium propionate-treated mice). Statistical significance was determined with Student's *t* test, except in **a** and **b**, in which Mann-Whitney test was used, and **g**, in which two-way ANOVA was used. * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$.

We next assessed the phenotype and function of CD4⁺ T cells in the lung-draining lymph nodes after HDM challenge. Similar to the DC data, on both days 1 and 4 after the final HDM challenge, there was no difference in the proportion of CD4⁺ T cells in the lymph

nodes (**Fig. 5d**); however, on day 4 after HDM challenge, the CD4⁺ T cells from propionate-treated mice exhibited a reduced activation state, characterized by decreased expression of CD44 and PD-1 and increased expression of CD45RB (**Fig. 5e** and data not shown).

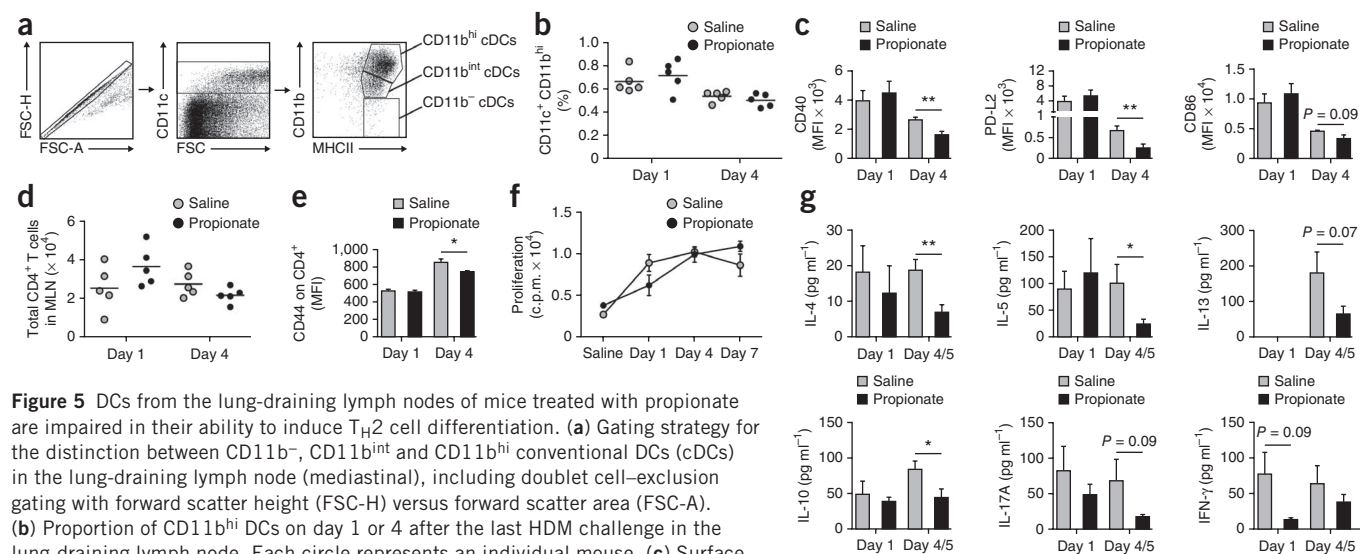


Figure 5 DCs from the lung-draining lymph nodes of mice treated with propionate are impaired in their ability to induce TH2 cell differentiation. **(a)** Gating strategy for the distinction between CD11b^{hi}, CD11b^{int} and CD11b^{lo} conventional DCs (cDCs) in the lung-draining lymph node (mediastinal), including doublet cell-exclusion gating with forward scatter height (FSC-H) versus forward scatter area (FSC-A). **(b)** Proportion of CD11b^{hi} DCs on day 1 or 4 after the last HDM challenge in the lung-draining lymph node. Each circle represents an individual mouse. **(c)** Surface expression of CD40, PD-L2 and CD86 on CD11b^{hi} DCs in the lung-draining lymph node. **(d)** Total number of CD4⁺ T cells in the lung-draining lymph nodes of mice on day 1 or 4 after the last HDM challenge. Each circle represents an individual mouse. **(e)** Cell surface expression of CD44 on CD4⁺ T cells in the lung-draining lymph node. **(f)** HDM-specific cell proliferation as determined by thymidine incorporation in cell suspensions from the lung-draining lymph nodes from mice euthanized on the indicated days after the last HDM challenge. **(g)** Concentration of cytokines in the supernatant of HDM-stimulated cells isolated from lung-draining lymph nodes on the indicated days after the last HDM challenge. Day 4/5 indicates day 4 or day 5. All results are representative of data generated in two different experiments and are expressed as the mean $\pm$ s.e.m. ($n = 5$ mice per group). The data in **g** are pooled from two experiments in which lymph nodes were isolated on day 1 and on either day 4 or 5 ($n = 10$ mice per group). Statistical significance was determined with Student's *t* test. * $P = 0.05$, ** $P = 0.01$.

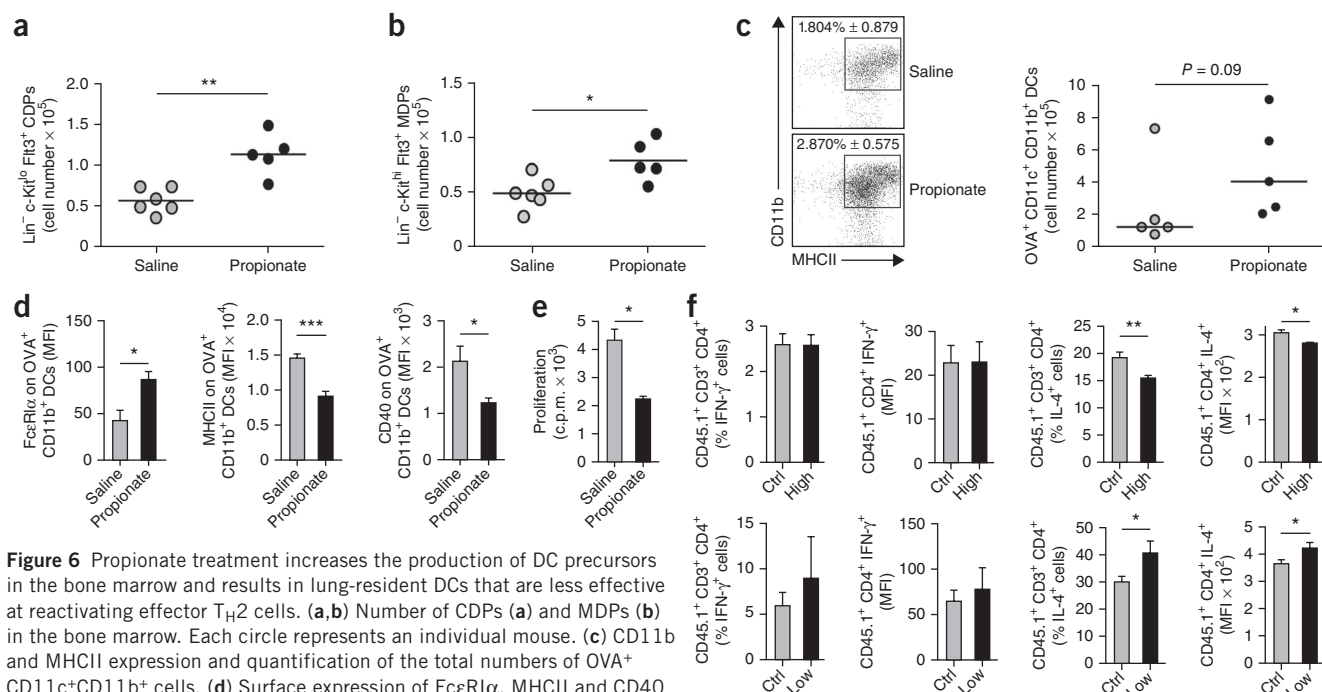


Figure 6 Propionate treatment increases the production of DC precursors in the bone marrow and results in lung-resident DCs that are less effective at reactivating effector T_H2 cells. (a,b) Number of CDPs (a) and MDPs (b) in the bone marrow. Each circle represents an individual mouse. (c) CD11b and MHCII expression and quantification of the total numbers of OVA⁺ CD11c⁺CD11b⁺ cells. (d) Surface expression of FcεRIα, MHCII and CD40 on OVA⁺CD11c⁺CD11b⁺ lung cells determined by flow cytometry. (e) Proliferation at 72 h of FACS-purified OVA⁺CD11c⁺CD11b⁺ lung DCs from mice treated with saline or propionate cultured together with *in vitro*-differentiated T_H2 cells from OT-II TCR transgenic mice. (f) Analysis of the IL-4- and IFN-γ-producing T_H2 -polarized CD45.1⁺CD4⁺ T cell recall response in the lungs of HDM-sensitized Ly5.2 wild-type recipient animals that were fed a control diet or a low- or high-fiber diet. All results are representative of data generated in two different experiments. All data are expressed as the mean ± s.e.m. ($n = 6$ saline-treated mice and $n = 5$ sodium propionate-treated mice in a and b; $n = 5$ mice per group in c–f). Statistical significance was determined with Student's *t* test. * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$.

Although these cells exhibited an altered activation state, they retained the ability to proliferate *ex vivo* after re-exposure to HDM *ex vivo* at all of the time points examined (Fig. 5f). However, further analysis of the effector function of the CD4⁺ T cells showed that although the cells exhibited normal effector function on day 1 after HDM challenge, cells isolated from propionate-treated mice on either day 4 or 5 after challenge produced less IL-4, IL-5, IL-13, IL-10 and IL-17A after antigen-specific re-stimulation, which is in line with the impaired DC activation state at these time points (Fig. 5g).

Propionate enhances hematopoiesis of DC precursors

It was notable that the protective effect of propionate treatment started to manifest 2 d after the final HDM challenge, suggesting that newly recruited DCs might be responsible. Also, although we were able to detect altered SCFA levels in the circulation of mice fed a high-fiber diet (Fig. 3g), we could not detect SCFAs in the lung (data not shown). We thus sought to assess whether SCFA treatment influenced newly recruited lung DCs. We first enumerated DC precursors in the bone marrow and found that propionate treatment increased the numbers of both the common DC precursors (CDPs) (Fig. 6a) and the macrophage and DC precursors (MDPs)³³ (Fig. 6b). To determine whether this increased hematopoiesis influenced cells seeding the lung, we treated mice with saline or propionate and exposed them to HDM as described above; however, we supplemented the last HDM challenge with a fluorochrome-conjugated ovalbumin (OVA) protein that enabled the specific identification of local antigen-presenting cells. Similar to what we observed in the bone marrow, we found that there was a trend toward increased numbers of DCs that had captured OVA in the lungs of propionate-treated mice (Fig. 6c), and these DCs expressed higher levels of FcεRIα (Fig. 6d), which is a

marker that characterizes newly recruited inflammatory DCs^{32,34}. However, recruited DCs also expressed lower levels of MHCII and CD40 (Fig. 6d).

To examine the ability of lung DCs to reactivate effector T_H2 cells, we differentiated OT-II T cell receptor (TCR) transgenic T cells *in vitro* under T_H2 -polarizing conditions and then cocultured them with FACS-purified OVA⁺ lung DCs. Lung DCs purified from propionate-treated mice exhibited a reduced capacity to elicit effector T_H2 cell proliferation as compared to DCs purified from saline-treated mice (Fig. 6e). To address whether this impaired reactivation of effector T_H2 cells occurred *in vivo* in the lung under different dietary conditions, we differentiated OT-II CD4⁺ cells into T_H2 effector cells *in vitro* and transferred them intravenously to HDM-challenged mice that were on a control diet, a high-fiber diet or a low-fiber diet. In line with the altered DC phenotype described above, the proportion of IL-4-producing T_H2 effector CD4⁺ T cells and the amount of IL-4 on a per-cell basis were significantly reduced when the recipient mice had been fed a high-fiber diet (Fig. 6f). Interferon-γ (IFN-γ) production was not influenced by the dietary conditions (Fig. 6f). Recipients fed the low-fiber diet showed increased IL-4 production by the adoptively transferred cells, suggesting that the low-fiber diet led to a lung environment that was more prone to T_H2 cell responses (Fig. 6f).

DISCUSSION

Host-microbial mutualism is an integral part of maintaining health and immune homeostasis. This mutualism is best recognized within the context of the intestinal tract, but it is increasingly clear that host-microbial interactions occur in, or have an impact on, all tissues that are exposed to the environment^{35–37}. Beyond the direct interaction between host cells and microbes is an additional layer of complexity

whereby dietary components and microbial communities both influence metabolite availability. Such metabolites are not always restricted to the intestinal tract but may enter the circulation and influence cells that are located within peripheral tissues^{38,39}. Our core finding is that fermentable fibers in the diet promote the outgrowth of bacteria from the Bacteroidetes phylum, leading to increased local and systemic levels of SCFAs, which in turn influence DC hematopoiesis and functionality. This process requires the receptor GPR41 but not its related receptor, GPR43. GPR43 has been associated previously with the beneficial effects of acetate in protection against colitis and arthritis in mouse models⁹, and thus it was surprising that propionate-mediated regulation of airway inflammation did not involve GPR43. Within this context, it is also interesting to note the recent studies that showed a GPR43-mediated expansion of colonic T_{reg} cells⁴⁰ and that reported that certain SCFAs could influence T_{reg} cell expansion in either the spleen or colon⁴¹. Comparatively, SCFAs do not lead to an accumulation of T_{reg} cells in the lung indicating that these cells are probably not associated with the failure of lung DCs to sustain allergic inflammation. There are a number of possible explanations for these apparently disparate findings. The two receptors could be expressed on distinct cell subpopulations or tissues and thus influence different aspects of immune responses. It is also plausible that the influences of GPR41 and GPR43 receptor engagement could be tightly linked with co-receptors or specific inflammatory cell differentiation states. Future studies are warranted to further dissect the likely pleiotropic roles of GPR41 and GPR43.

It was previously reported that GPR43-deficient mice are more susceptible to inflammation after systemic priming and airway challenge with OVA⁹. Comparatively, we found that GPR43- and GPR41-deficient mice in fact have reduced AAI as compared to wild-type controls. In addition to the different allergen used in our study (HDM) and in the previous study (OVA), these models also employ different sensitization routes: only the intranasal route is used for HDM, whereas the OVA model involves systemic immunizations in combination with alum adjuvant. Thus, one difference between the two studies could be that the enhanced inflammation seen in the knockout mice after OVA sensitization and challenge is due to systemic influences after the initial OVA and alum immunization, which are absent in the lung. It is also important to note that these receptors could themselves influence a number of metabolic and physiological pathways, and consequently, interpreting data from direct comparisons between knockout and wild-type mice needs to be considered carefully.

Our data show that DCs in the lung and draining lymph node are affected by increased levels of propionate in the circulation, but notably, the major differences in allergic airway response became evident only at 2 d after the final HDM exposure. Although we were able to detect SCFAs in the cecum and serum, we were unable to detect SCFAs in the lung itself. We thus hypothesize that the lung is typically sequestered from substantial SCFA exposure, and in the absence of inflammation and accelerated cell turnover, DCs that are resident in the lung develop normally. However, after inflammation, the lung DC compartment is replenished with inflammatory monocyte-derived DCs that have been exposed to SCFAs in the bone marrow and circulation, leading to a maturation profile that is ineffective at driving T_H2 cell responses. Indeed, DCs appear to be less mature in propionate-treated mice, given that their phagocytic activity was high and their MHCII expression was low. It is particularly interesting that SCFA exposure appears to influence hematopoiesis and the generation of CDP and MDP cell subsets directly. This link between diet, microbes,

metabolites and hematopoiesis might be a fundamental mechanism through which diet influences disease in general and warrants further investigation.

A further observation from our study was that changes in diet led to a change in both the intestinal microbiota and, to a lesser extent, the microbiota that are present in the lung. Given that no SCFAs were detectable in the lung itself, we hypothesize that these lung-resident bacteria did not contribute to SCFA levels, possibly because of the absence of suitable substrates. The functional role and implications of the airway microbiota are unknown, but this is a promising avenue for future investigations, particularly given the recent literature that has associated different airway microbiota with healthy or diseased lungs^{35,36,42}. Within this context, the possible gut-lung axis for formation of the airway microbiota⁴³ could have important implications for our understanding of the crosstalk between mucosal tissues.

In summary, these data demonstrate that dietary fiber content influences the intestinal microbiota and thus the circulating levels of SCFAs. SCFAs, such as propionate, enhance the hematopoiesis of DC precursors from bone marrow, and these DCs exhibit an impaired ability to activate T_H2 effector cells in the lung. As a consequence, allergic airway inflammation cannot be sustained and rapidly resolves. Our work highlights the importance of dietary fermentable fibers and provides a cellular mechanism for an intestinal–bone marrow–lung axis in controlling allergic airway inflammation. Moreover, these data can be interpreted within the context of epidemiological studies showing that as fiber content in the diet has decreased, the incidence of allergies has increased^{1,2}. Taken together, our findings support the concept that intervention strategies targeting diet are a valuable approach for not only intestinal diseases but also respiratory inflammatory diseases.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

B.J.M. conceived the study. B.J.M. and A.T. designed the study. A.T., E.S.G., K.Y. and A.K.S. performed experiments. A.T. and E.S.G. analyzed data. N.S. performed SCFA analysis. C.N.-B., A.T. and B.J.M. performed microbiota analysis. T.J. provided *Ffar3*^{−/−} and *Ffar2*^{−/−} mice. T.J., C.B., N.L.H., L.P.N., E.S.G., A.T. and B.J.M. provided critical analysis and discussions. B.J.M. and A.T. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the [online version of the paper](#).

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ONLINE METHODS

Mice. 8- to 12-week-old C57BL/6 female mice were purchased from Charles River Laboratories (L'Arbresle, France) and housed under specific pathogen-free conditions. GPR41 and GPR43 knockout mice were obtained from Novartis Institutes for Biomedical Research, Basel, Switzerland. Animal experiments were performed in accordance with institutional guidelines and Swiss federal and cantonal laws on animal protection.

Rodent diets. Mice were fed either normal chow (Provimi Kliba diet 3202) or a low-fiber diet (Provimi Kliba diet 2122). When studying the effect of a high-fiber diet, mice were given normal chow supplemented with 30% cellulose (J. Rettenmaier & Söhne, Rosenberg, Germany) or 30% pectin (Herbstreith & Fox, Neuenbürg, Germany). All diets were purchased from Kliba Nafag AG, Kaiseraugst, Switzerland.

SCFA treatment. Mice were given saline as a control or sodium propionate (Sigma-Aldrich, St. Louis, MO) for 2 weeks before being exposed to HDM and were treated throughout the duration of the experiment by intraperitoneal injection every other day at 1 g per kg body weight. Alternatively, mice were also given either sodium acetate or sodium propionate in the drinking water at a final concentration of 200 mM for 3 weeks before HDM exposure and throughout the study.

Animal model of allergic airway inflammation. Mice were anaesthetized with a mixture of ketamine and xylazine (Dr. E. Graeb AG, Bern, Switzerland) and challenged intranasally with 15 μ g of HDM (Greer Laboratories Inc., Lenoir, NC) in 30 μ l sterile saline every other day for a total of six exposures. Airway inflammation was examined from day 1 to day 6 after the last HDM challenge.

Measurement of lung function. Lung resistance was quantified using the whole body-restrained plethysmograph system flexiVent (SCIREQ). Mice were anesthetized by intramuscular injection of 100 mg per kg body weight ketamine and intraperitoneal injection of 50 mg per kg body weight pentobarbital (Esconarkon, Streuli Pharma). Eight minutes later, mice were tracheotomized and mechanically ventilated at a rate of 200 breaths per min and a tidal volume of 10 ml per kg body weight. Airway hyper-responsiveness was measured by administering increasing doses of acetyl methylcholine (Sigma).

Adoptive transfer experiments. OT-II TCR transgenic CD4⁺ T cells were isolated from the spleens of naive Ly5.1 OT-II mice by positive selection using magnetic beads (magnetic-activated cell sorting (MACS); Milteny Biotec, Germany). Isolated OT-II CD4⁺ T cells were subsequently T_H2 polarized *in vitro* using anti-CD3-coated plates (BioXcell, 145-2C11) and a cocktail of 1 μ g ml⁻¹ CD28-specific antibody (BioXcell, 37.51), recombinant IL-4 (Peprotech, 214-14) at a 10 ng ml⁻¹ final concentration, antibody to IFN- γ (XMG1.2) at a 10 μ g ml⁻¹ final concentration and recombinant IL-2 (Biolegend, 575402) at a 10 ng ml⁻¹ final concentration for 5 d before intravenous transfer into Ly5.2 wild-type recipient animals that were fed a control diet or a low- or high-fiber diet and that had been previously challenged six times with 15 μ g of HDM extract. The day after the transfer, mice received 100 μ g of OVA together with the HDM allergen. The recall responses of T_H2 cells in the lungs of recipient mice were assessed 2 d later.

In vivo tracking of DCs. Mice were administered 100 μ g of FITC-labeled OVA (Invitrogen, Grand Island, NY) together with 15 μ g HDM intranasally. OVA-FITC-loaded airway DCs migrating to the lung-draining lymph nodes were monitored by flow cytometry.

Cellular infiltration of the airways. BALF was collected to assess the cellular compartment of the airway lumen. Total cell numbers in the BALF were determined using a Coulter Counter (IG Instrumenten-Gesellschaft AG, Basel, Switzerland). Differential cell counts were performed on cytopins stained with Diff-Quik solution (Dade Behring, Siemens Healthcare Diagnostics, Deerfield, IL). Percentages of eosinophils, neutrophils, macrophages and lymphocytes were determined by counting 200 cells per sample.

Cytokine levels. IL-4, IL-5, IL-13, IL-17A, IL-10 and IFN- γ levels were quantified in lung homogenates and cell culture supernatants using a Milliplex MAP assay kit (Millipore, Billerica, MA). Assays were performed according to manufacturer's instructions and read on the Luminex 200TM Multiplexing Instrument (Merck Millipore).

ELISAs. Systemic levels of total IgE and HDM-specific IgG1 were determined by ELISA. Briefly, NUNC MaxiSorp 96-well plates were coated with either 2 μ g ml⁻¹ goat anti-mouse IgE (1110-01) or 5 μ g ml⁻¹ HDM overnight at 4 °C. Samples were added the next day and incubated overnight at 4 °C before the addition of a 1 μ g ml⁻¹ final concentration of alkaline phosphatase-conjugated goat anti-mouse IgE (1110-04) or IgG1 (1070-04) (all antibodies were from SouthernBiotech, Birmingham, AL). 4-nitrophenyl phosphate sodium salt hexahydrate (pNPP) (Sigma) was used as a substrate, and the colorimetric reaction was read at 405 nm on the Synergy H1 microplate reader (Biotek, Luzern, Switzerland).

Histology. The left lobes of the lungs were fixed in 10 ml of 10% buffered formalin at 4 °C and embedded into paraffin. Prepared sections (4 μ m) were stained with either H&E or PAS reagents using standardized protocols and analyzed with an Axioskop 2 plus microscope equipped with an Axio-Cam HRC (Carl Zeiss Microimaging GmbH, Jena, Germany).

Flow cytometry. Characterization and phenotyping of the various DC subsets and CD4⁺ T cells were performed by flow cytometry. Lung tissue and mediastinal lymph nodes were digested using collagenase IV (BioConcept, Worthington, Lakewood, NY) in Iscove's modified Dulbecco's medium for 45 min at 37 °C. Samples were then filtered through a 70- μ m cell strainer, washed and resuspended in PBS supplemented with 0.2% BSA. Total cell counts were determined using a Coulter Counter. Cells were then stained for flow cytometry with antibodies to CD11c-allophycocyanin (APC)/Cy7 (117324, 1:800), CD11b-PerCP/Cy5.5 (101228, 1:800), F4/80-AF647 (123122, 1:500), I-A/I-E-Alexa700 (107622, 1:1,000), Ly-6c-Pacific blue (128014, 1:600) and Fc ϵ RI α -phycoerythrin (PE) (134307, 1:400), and activation state was assessed by staining with antibodies to CD40-PE (102806, 1:400), PD-L2-PE (107205, 1:400) and CD86-PE (105008, 1:400). CD4⁺ T cells were characterized by staining with antibodies to CD3-Pacific blue (100214, 1:400), CD4-APC (100412, 1:800) or CD4-PerCP/Cy5.5 (100434, 1:400), CD8-PE/Cy7 (100722, 1:1,000), CD44-biotin (103003, 1:400), PD-1-PE (135205, 1:400), CD45RB-PE (103308, 1:1,000) and streptavidin-APC/Cy7 (405208, 1:1,000). T_{reg} CD4⁺ cells were stained using antibodies to CD3-Pacific blue, CD4-PerCP/Cy5.5, CD25-PE/Cy7 (102016, 1:400) and FoxP3-AF647 (126408, 1:200). Cytokine production by CD4⁺ T cells was assessed by intracellular staining with antibodies to IFN- γ -APC (505810, 1:100) and IL-4-PE (504104, 1:100). All antibodies were purchased from Biolegend (San Diego, CA). Fluorescence minus one (FMO) internal staining controls were set up by pooling cells from all the different experimental groups. Cells were acquired on BD FACS Canto (BD Biosciences, San Jose, CA). Samples were analyzed using FlowJo 9.6.3 software (Tree Star Inc., Ashland, OR).

Ex vivo re-stimulation assay. Cells from lung-draining lymph nodes were plated at a final concentration of 5×10^5 ml⁻¹ and stimulated for 72 h with 0, 8, 40 or 200 ng or 1 or 5 μ g of HDM. Proliferation was determined by the addition of ³H-thymidine at 2.5 a final concentration of 2.5 μ Ci ml⁻¹ for the last 18 h. Supernatants were partly harvested before the addition of ³H-thymidine to quantify cytokines that had been secreted by primed CD4⁺ T cells.

Flow cytometric sorting of CD11b⁺ DCs and coculture assay. Mice were rendered allergic as described above, and in the last challenge, 100 μ g of OVA-FITC was instilled together with HDM. Lungs from five mice were digested as described above, pooled and stained for sorting by flow cytometry. CD11b⁺ DCs were gated as described in Figure 6 and sorted into 100% FCS. FACS-purified DCs (10⁴) were cocultured in a round-bottom 96-well plate with MACS-sorted splenic OT-II TCR transgenic CD4⁺ T cells (5×10^4) that had been preactivated *in vitro* and polarized toward a T_H2 cell phenotype.

Bacterial DNA isolation from mouse lungs and feces. Lungs and feces from mice housed under specific pathogen-free conditions were harvested under sterile conditions in a 2-ml Biopure tube (Eppendorf, Hamburg, Germany) and immediately snap frozen in liquid nitrogen. Samples were stored at -80°C until processing for DNA isolation. To isolate the total bacterial as well as the cellular DNA, lung tissue was weighed, and sterile PBS was added to a final concentration of up to $300\text{ }\mu\text{g }\mu\text{L}^{-1}$. Lungs were homogenized for 5 min at 25 Hz using sterile 3-mm tungsten carbide beads (Qiagen, Valencia, CA) and the TissueLyser system (Qiagen). $80\text{ }\mu\text{L}$ of the lung homogenate was transferred into a fresh 2-ml Biopure tube and digested with proteinase K (provided with the QiaAMP DNA Mini Kit, Qiagen) at 56°C for 2.5 h with shaking set at 400 r.p.m. Feces were weighed, and 25 mg was transferred to a sterile 2-ml Biopure tube before being digested as described above, but for at least 4 h to allow for complete digestion of the fecal pellets.

DNA was then isolated using the QiaAMP DNA Mini Kit according to the manufacturer's instructions. DNA was eluted twice with $100\text{ }\mu\text{L}$ buffer AE (provided with the kit) in PCR-grade 1.5-ml HydroLogix tubes (Molecular BioProducts, San Diego, CA), and DNA concentration was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Asheville, NC). DNA was either used directly for quantitative PCR or stored at -20°C until further use.

454 pyrosequencing. To ascertain the composition of the bacterial phyla present in feces of the mice that were fed the different diets, isolated bacterial DNA was tagged and sequenced using 454 pyrosequencing technology. PCR amplification was performed using a set of primers targeting the hypervariable regions V3 to V1 (V123) of the 16S rRNA gene. The primers used were as follows: V123 forward primer 1, 5'-CTATGCGCCTTGCCAGCCGCTCAGTCTCAGAGTTTGATYMTGGCTCAG-3'; V123 forward primer 2, 5'-CTATGCGCCTTGCCAGCCGCTCAGTCTCAGGGTTTCGATTCTGGCTCAG-3'; V123 forward primer 3, 5'-CTATGCGCCTTGCCAGCCGCTCAGTCTCAGAGTTTGATCCTGGCTTAG-3'; V123 forward primer 4, 5'-CTATGCGCCTTGCCAGCCGCTCAGTCTCAGAATTTGATCTTGTTTCAG-3'; V123 reverse primer, 5'-CGTATCGCCTCCCTCGCGCCATCAGNNNNNNNNGGTTACCGCGGCTGCTGGCAC-3' (where the adaptor sequences for Roche 454 FLX Titanium sequencing are italicized, the linkers are underlined, NNNNNNNN sequences designate the sample-specific eight-base barcodes used to tag each PCR product, and bold sequences correspond to broadly conserved 16S rRNA gene regions). V123 forward primers 1, 2, 3 and 4 were combined in 4:1:1:1 ratios. Each PCR amplification reaction contained $2\text{ }\mu\text{L}$ of DNA extract, $50\text{ }\mu\text{M}$ dNTPs, 200 nM forward primer (a mix of forward primers for the V123 region), 200 nM reverse primers, $1\times$ Expand High Fidelity Reaction Buffer and 2.5 U Expand High Fidelity enzyme blend (Roche Applied Science, Switzerland). The PCR conditions were 94°C for 2 min followed by 25 cycles of 94°C for 30 s, 49°C for 30 s and 72°C for 1 min and ending with a last step of 72°C for 7 min. After purification and pooling in equimolar amounts, the PCR products were sequenced using the 454 FLX Titanium technology (Microsynth AG, Balgach, Switzerland). Raw data were analyzed using the QIIME software package with default parameters, except that no barcode correction was allowed, and reverse primers were removed when they were present. Samples described by less than 200 sequencing reads were excluded from the analysis. Quality-filtered sequencing reads were analyzed using the Uclust method at a similarity threshold of 95% identity for OTUs clustering. Assignment of OTUs into the Bergey's bacterial taxonomy was done using RDP Classifier with a confidence value threshold of 60%.

Quantitative PCR. To validate the pyrosequencing data, bacterial DNA was submitted to quantitative PCR and amplified using previously described primers (pan-bacteria: forward: 5'-GCAGGCCTAACACATGCAAGTC-3' and reverse: 5'-CTGCTGCCTCCCGTAGGAGT-3'; Bacteroidetes: forward: 5'-CR AACAGGATTAGATACCCT-3' and reverse: 5'-GGTAAGGTTCCCTCGCGT AT-3'; Firmicutes: forward: 5'-TGAACTYAAAGGAATTGACG-3' and reverse: 5'-ACCATGCACCACCTGTC-3'; Actinobacteria: forward: 5'-TACGGCCGCAAGGCTA-3' and reverse: 5'-TCRTCCCCACCTTCCTC CG-3'; Gammaproteobacteria: forward: 5'-TCGTCAGCTCGTGTGTGA-3' and reverse: 5'-CGTAAGGGCCATGATG-3'). GPR41 and GPR43 expression levels in the lungs and in FACS-purified lung CD11b⁺ DCs were determined using the following primers: GPR41: forward: 5'-TCCTGCCGTTTCGCATGGT GG-3' and reverse: 5'-ACCGCCGTCAGGAAGAGGGAG-3'; GPR43: forward: 5'-CCCTGTGCACATCCTCCTGC-3' and reverse: 5'-GCGTT CCATGCTGATGCCCG-3'. Lung IL-13 mRNA expression was assessed by quantitative RT-PCR using the following primer set: forward: 5'-TCCAGC CTCCCCGATACC-3' and reverse: 5'-AGCAAAGTCTGATGTGAGAAA GG-3'. Ten-microliter PCR reactions were set up containing $4\text{ }\mu\text{L}$ of template DNA at a concentration of $25\text{ ng }\mu\text{L}^{-1}$, $5\text{ }\mu\text{L}$ SsoAdvanced SYBR Green reaction mix (BioRad, Hercules, CA), $0.25\text{ }\mu\text{L}$ of each primer at a concentration of $20\text{ }\mu\text{M}$ and $0.5\text{ }\mu\text{L}$ of nuclease-free water. Quantitative PCR was performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using the following conditions: one cycle at 50°C for 2 min, one cycle at 95°C for 10 min and then 45 cycles at 95°C for 15 s and 61.5°C for 1 min, followed by a dissociation stage at 65°C for 31 s and cycles of 5 s starting at 65°C , raising 0.5°C per cycle, to obtain melting curves for specificity analysis. After amplification, Cq values were obtained using the CFX ManagerTM software 2.1 (Bio-Rad).

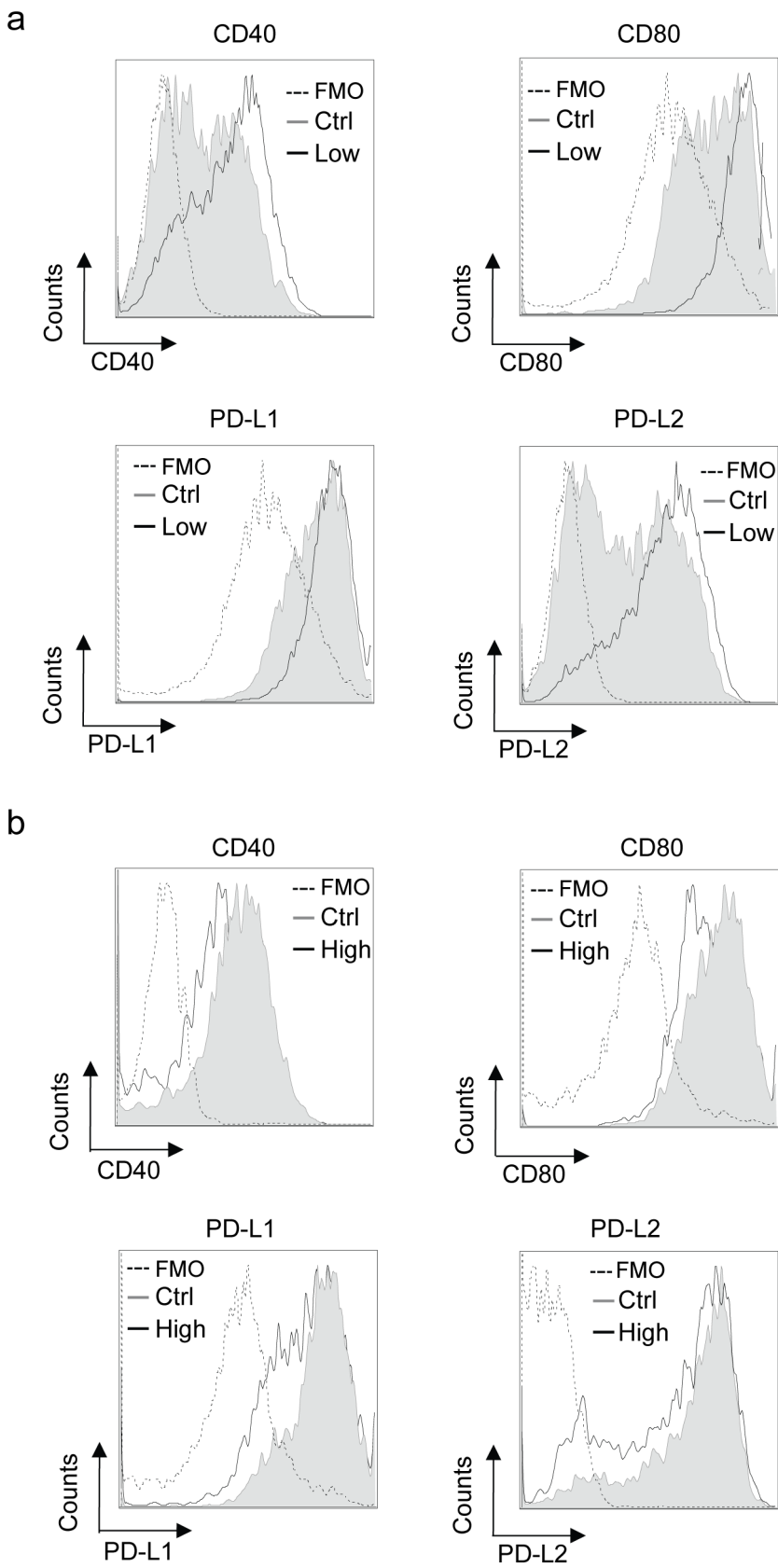
Cecal and serum SCFA quantification. Organic acids—lactate, acetate, propionate, butyrate, isovalerate and valerate—present in the cecal contents and serum of the mice fed our different diets were analyzed by the HPLC Ultimate 3000 (Dionex, Sunnyvale, CA) equipped with an U3000 RS diode array detector (Dionex). Briefly, cecal contents (100 mg) were first homogenized in $400\text{ }\mu\text{L}$ of deionized water. Thereafter, sera and cecal samples were acidified with 25% metaphosphoric acid at a 1:5 ratio (1 volume of acid for 5 volumes of sample) for 30 min on ice. Samples were then centrifuged at $12,000g$ for 15 min at 4°C , and supernatants were filtered over Ultrafree MC columns (Millipore). The eluate was stored at -80°C until it was analyzed by HPLC. SCFAs were separated on a Hi-Plex H column (8 mm ; $300\times 7.7\text{ mm}$; Polymer Laboratories (Varian), Shropshire, UK) run isocratic with 5 mM sulfuric acid at a flow rate of 0.6 mL min^{-1} and a temperature of 55°C . Organic acids were detected at a wavelength of 210 nm and quantified using external standard curves from 0.5 to 100 mM of the respective authentic organic acids (Fluka, Buchs, Switzerland). Dionex Chromeleon software version 6.8 (Thermo Fisher Scientific) was employed to pilot the HPLC and determine the concentrations of each individual organic acid.

Statistical analyses. Depending on the data distribution, Student's *t* test (unpaired, two tailed) or a Mann Whitney test was used to calculate significance levels between treatment groups. Differences in microbiota richness and diversity between the control diet and either a low-fiber or high-fiber diet were tested by analysis of variance. $P < 0.05$ was considered significant. Graph generation and statistical analyses were performed using Prism version 5.0d software (GraphPad, La Jolla, CA).

Dietary fiber influences the severity of allergic airway inflammation through GPR41 mediated host-microbial-metabolite cross-talk.

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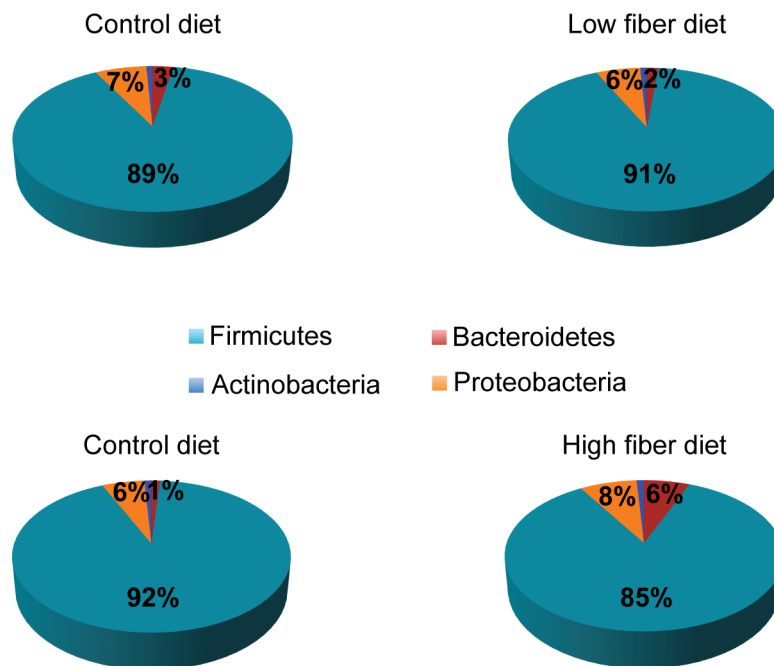
Supplementary Figure 1



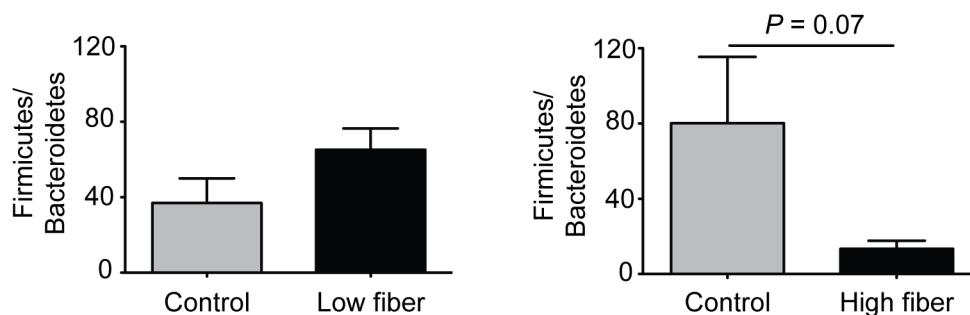
Supplementary Figure 1. Activation profile of CD11b^{high} Dendritic cells from lung draining lymph node of mice fed a low or high fiber diet differs from mice on control diet. (a) Histograms depicting the surface expression of CD40, CD80, PD-L1 and PD-L2 on the CD11b^{high} DC population of low fiber diet versus regular diet fed mice. (b) Histograms depicting the surface expression of CD40, CD80, PD-L1 and PD-L2 on the CD11b^{high} DC population of high fiber diet versus regular diet fed mice. Results are representative of data generated in three different experiments. Data are expressed as mean $\pm$ SEM (n = 5 control mice and 5 low fiber diet mice/ group or n = 5 control mice and 6 high fiber diet mice/ group).

Supplementary Figure 2

a



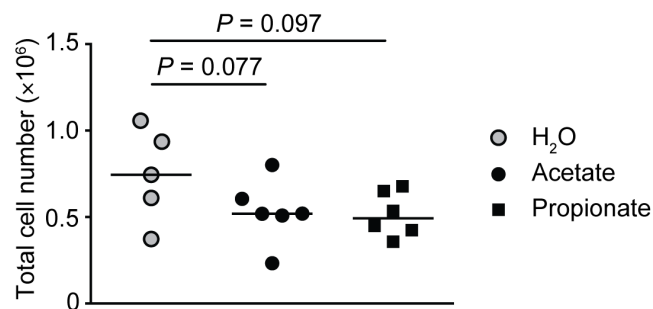
b



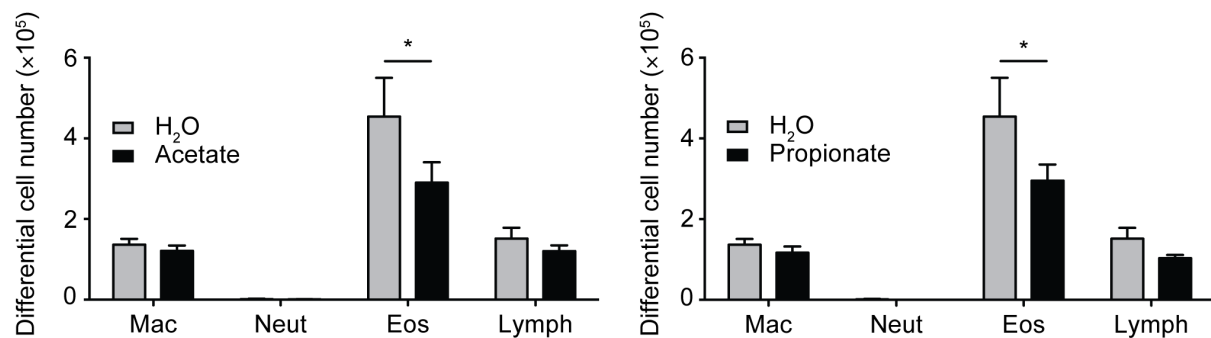
Supplementary Figure 2. Dietary fiber content influences the constituents of the lung microbiota. (a) Analysis of the different microbial communities (Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria) from the lungs of mice fed either low, high fiber or control diets by quantitative PCR. (b) Representation of the Firmicutes/Bacteroidetes ratios. Data are expressed as mean $\pm$ SEM ($n = 5$ control and 5 low fiber diet fed mice/ group; 4 control and 5 high fiber diet fed mice/ group).

Supplementary Figure 3

a



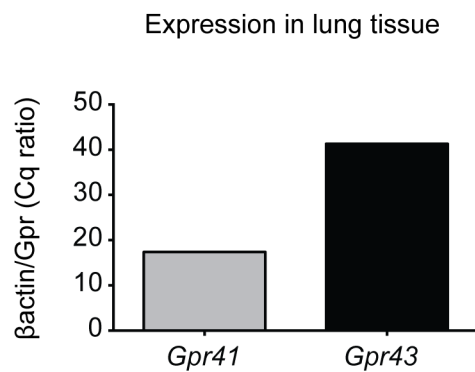
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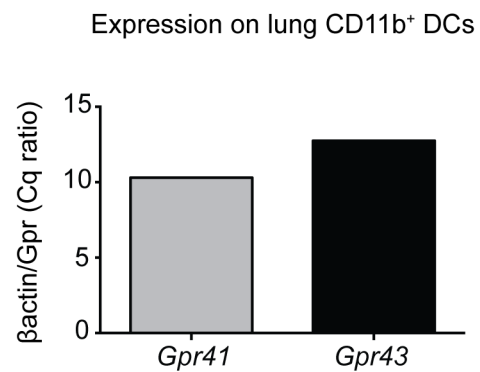
Supplementary Figure 3. Acetate and propionate supplementation in the drinking water dampens allergic airway inflammation to HDM. (a) Quantification of the total number of cells infiltrating the airways in the BALF. (b) Analysis and quantification of the differential cell counts in the BALF. Results are representative of data generated in two different experiments. Data are expressed as mean $\pm$ SEM ($n = 5$ control mice and 6 treated mice/ group). * $p = 0.05$.

Supplementary Figure 4

a

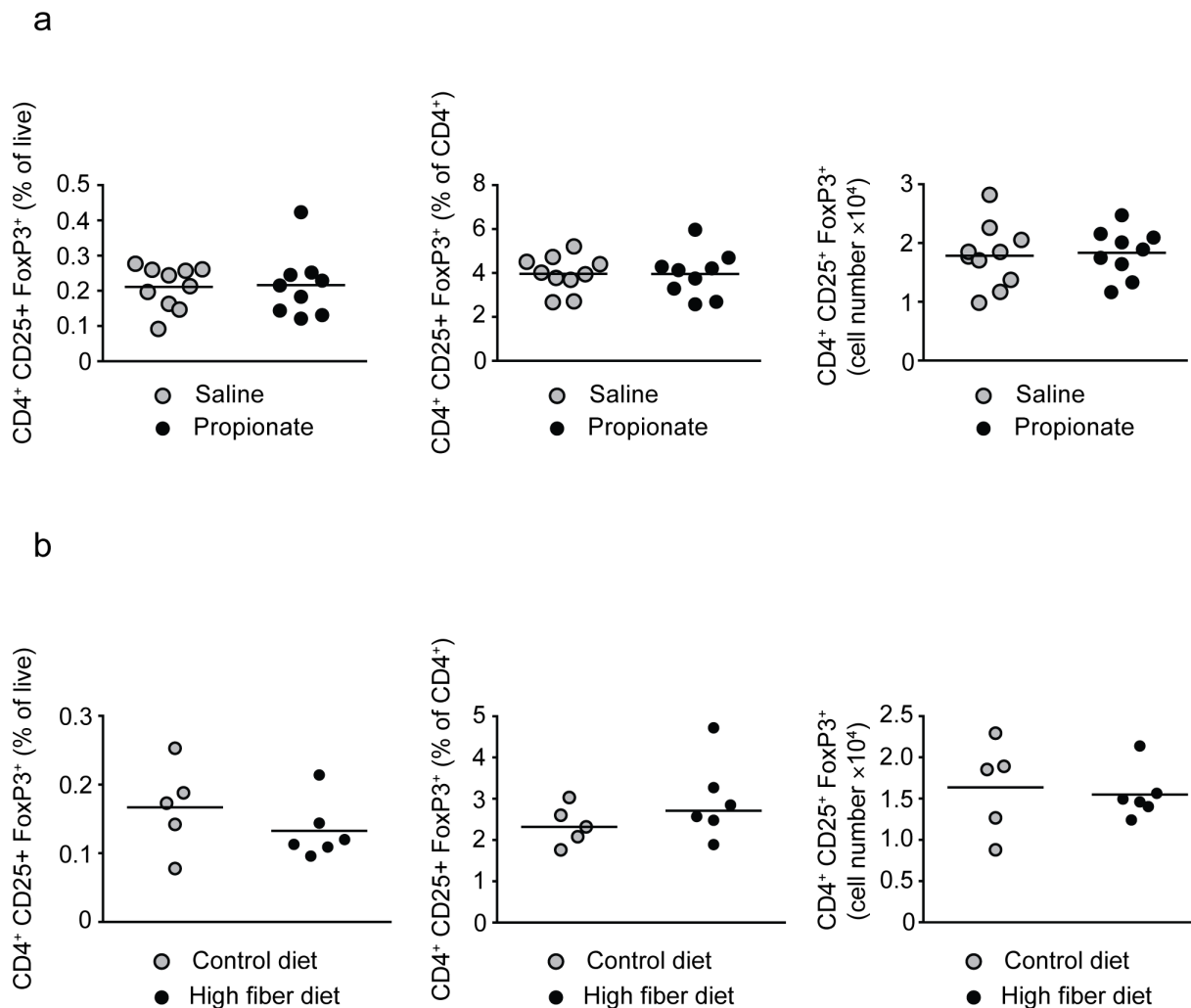


b



Supplementary Figure 4. Lung CD11b⁺ Dendritic cells express both SCFA receptors GPR41 and GPR43. (a) Analysis of GPR41 and GPR43 mRNA expression levels in the whole lungs, or on (b) FACS-purified CD11c⁺ CD11b⁺ lung DCs.

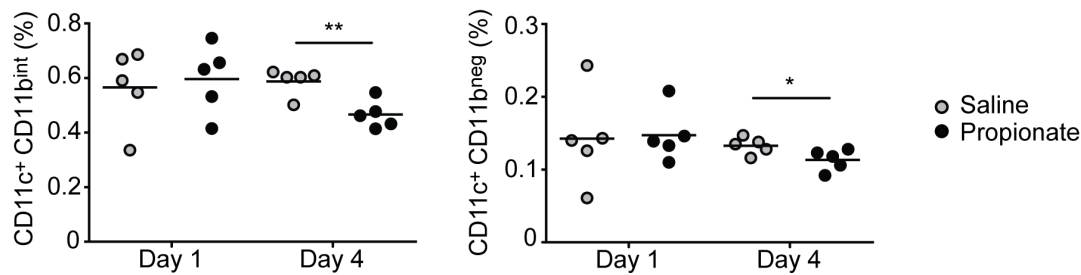
Supplementary Figure 5



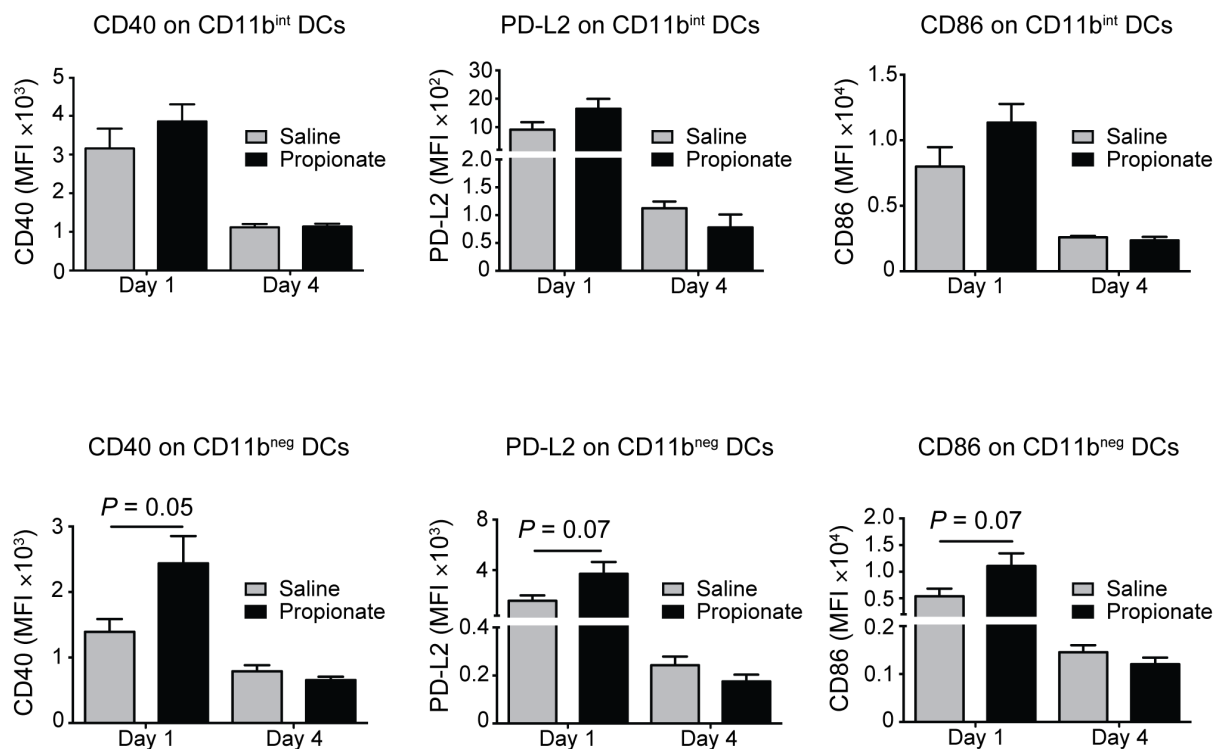
Supplementary Figure 5. The protective effect of propionate or high fiber diet on allergic airway inflammation is not associated with increased regulatory CD4⁺ T cell numbers. (a) Proportion and total numbers of FoxP3⁺ CD25⁺ CD4⁺ regulatory T cells in mice treated with saline or sodium propionate, or (b) on a control diet supplemented with cellulose or enriched with pectin. Results are representative of data generated in two different experiments. Data are expressed as mean ± SEM (n = 10 control mice and 9 propionate treated mice; 5 control and 6 high fiber diet fed mice/ group).

Supplementary Figure 6

a



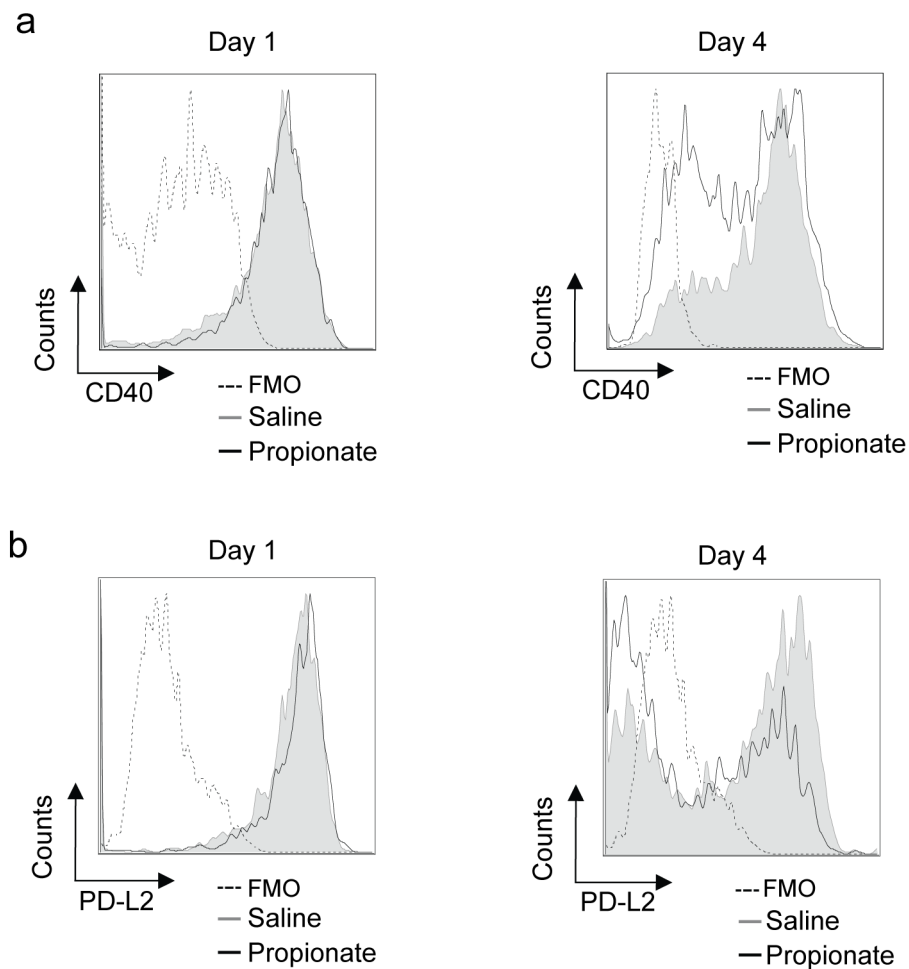
b



Supplementary Figure 6. CD11b^{neg} and CD11b^{int} Dendritic cells from the lung draining lymph node of mice treated with propionate behave similarly to their CD11b^{hi} counterparts. (a) The percentage and total number of the indicated DC subpopulations either 1 day or 4 days post the final HDM challenge. (b) Surface expression analysis of CD40, PD-L2 and CD86 on the CD11b^{int} DC population. (c)

Surface expression analysis of CD40, PD-L2 and CD86 on the CD11b^{neg} DC population. Results are representative of data generated in two different experiments. Data are expressed as mean $\pm$ SEM (n = 5 mice/ group). *p = 0.05, **p = 0.01.

Supplementary Figure 7



Supplementary Figure 7. Activation profile of CD11b^{high} Dendritic cells from lung draining lymph node of propionate treated mice differs from control mice.

(a) Histograms depicting the surface expression of CD40 on the CD11b^{high} DC population of propionate treated versus control mice on day 1 and day 4 after the last HDM challenge. (b) Histograms depicting the surface expression of PD-L2 on the CD11b^{high} DC population of propionate treated versus control mice on day 1 and day 4 after the last HDM challenge. Results are representative of data generated in two different experiments. Data are expressed as mean $\pm$ SEM (n = 5 control mice/group).