



# *Bifidobacteria adolescentis* regulated immune responses and gut microbial composition to alleviate DNFB-induced atopic dermatitis in mice

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## Abstract

**Purpose** Emerging studies have reported gut microbial composition plays a key role in alleviating AD clinical symptoms during the probiotic intervention, but the correlation among clinical symptoms, immune responses and gut microbial alteration needs to be explored. Therefore, the objective was to investigate the correlation during *Bifidobacterium adolescentis* intervention in DNFB-induced AD mice.

**Methods** The mice were randomly divided into nine groups and fed *B. adolescentis* for 3 weeks. At the end of the experiment, clinical and immune indicators were assessed. Flow cytometry was performed to explore the effect of *B. adolescentis* on regulatory T cells in the spleen. V3–V4 region of the 16S ribosomal RNA (rRNA) gene was sequenced to evaluate changes in the gut microbiota.

**Results** *Bifidobacteria adolescentis* treatments reduced ear and skin thickness and suppressed eosinophils and mast cells infiltration. Th1- and Th2-type responses were regulated and the Tregs population was promoted in the spleen by *B. adolescentis* treatments. *Bifidobacteria adolescentis* increased the relative abundance of *Lactobacillus* but decrease *Dorea* and *Pediococcus*. Propionic and butyric acids were increased but isovaleric acid was decreased by *B. adolescentis* treatment. Besides, the functional modules, such as fatty acid biosynthesis, antigen processing and presentation were upregulated by *B. adolescentis* Ad1 treatment compared to the DNFB group.

**Conclusion** Collectively, these results imply that *B. adolescentis* with the role of immunomodulation promotes Treg differentiation and suppresses Th2 responses, and increases the proportion of *Lactobacillus* that is positively correlated to increase in propionic acid production, and thus has the potential for AD amelioration.

**Keywords** *Bifidobacterium adolescentis* · Immune responses · Gut microbiota · Short-chain fatty acids · Atopic dermatitis

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## Introduction

Atopic dermatitis (AD) is an allergic skin disease [1]. High serum immunoglobulin (Ig) E levels are a notable feature in some AD patients [2]. The interaction of genetic and environmental factors influences the normal immune responses and thus is associated with AD [3]. Although the pathogenesis of AD remains to be elucidated, accumulating studies have evidenced that T helper (Th) 2-type immune responses are prevalent in the acute stage of AD while Th1- and Th17-type immune responses are predominant the development of AD at the chronic stage [4, 5]. Additionally, immune responses, inflammatory cells recruitment, and inflammatory infiltration play key roles in AD pathogenesis [6].

The gut is the important immune organ for animals and humans, and the gut microbiota includes up to  $10^{14}$  bacterial cells [7, 8]. Immune homeostasis is composed of the interaction with epithelial cells, antigen processing cells, lymphocytes in the gut, and intestinal microorganisms provoke the differentiation of regulatory T cells (Tregs) to increase immune tolerance through this interaction [9]. Therefore, the balance in the micro-ecology system of gut microbiota is important for immune responses [10]. Recent studies have investigated that an imbalance of gut microbiota relates to the development of allergic diseases including AD [11–13]. However, causes of the differences are difficult to determine because the gut microbiota is affected by many complex factors.

To alleviate AD symptoms, many approaches including probiotics treatment, have been used in both animal and clinical studies. Probiotics regulate intestinal flora and have good benefits for gut immunity, thereby alleviating AD symptoms. For instance, *Lactobacillus sakei* WIKIM30, a Gram-positive bacterium, ameliorates AD symptoms by inducing Tregs differentiation and altering the gut microbiota in a mouse model [14]. Additionally, supplement with a probiotic mixture including *B. longum* CECT 7347, *B. lactis* CECT 8145, and *L. casei* CECT 9104 significantly decreases Scoring Atopic Dermatitis (SCORAD) index and the use of topical steroids in patients with AD. However, the mechanism is not fully understood [15].

In this study, 2,4-dinitrofluorobenzene (DNFB) was used to induce AD symptoms in mice. This model was used to evaluate the effect of six strains of *B. adolescentis* on AD symptoms and immune responses. A study has demonstrated that in chronic asthma patients, although the proportion of *Bifidobacterium* decreased, the *B. adolescentis* species prevailed [16]. While previously study has demonstrated *B. adolescentis* CCFM 669 and CCFM667 alleviated loperamide-induced constipation through the

increase in propionic and butyric acids in BALB/c mice [17]. Additionally, some studies have reported that commensal intestinal bacteria were able to stimulate the generation of Foxp3<sup>+</sup> Tregs, frequently in a SCFAs-dependent mechanism [18]. Based on these debates, we proposed a hypothesis that *B. adolescentis* might attenuate disease symptoms through regulating gut microbiota and affecting gut microbiota-derived SCFAs production. In addition, the correlation between clinical manifestation, immune responses, and gut microbial alteration was evaluated to explore possible alleviation mechanisms.

## Materials and methods

### *Bifidobacterium adolescentis* strains

Six *B. adolescentis* strains FJSYC5M10 (Ad1), FJSYC6M8 (Ad2), FJSYC7M3 (Ad3), FAHBZ6M2 (Ad4), FHNXY72M2 (Ad5), and FAHBZ2M5 (Ad6) derived from Culture Collection of Food Microorganisms of Jiangnan University (Wuxi, China). *Bifidobacteria adolescentis* were isolated from the healthy volunteer feces or animal feces. All strains were consecutively reactivated in an anaerobic incubator (Electrotek AW500SG, England) at least three times using 2% (v/v) inoculum in MRS broth with 0.5% L-cysteine (w/v) at 37 °C (Supplementary material).

### Animals and treatment

Six-week-old, female, and specific-pathogen-free grade C57bl/6 mice were kept in a facility under light cycle (12 h light/12 h dark), temperature (25 °C), and humidity level (50%). The study protocol was approved by the Ethics Committee of Jiangnan University, China (JN. No20180115c2600531 [5]).

All mice were fed standard chow and water ad libitum. Mice were divided into the following groups: control group (fed with the vehicle), DNFB group (fed with the vehicle), Keto group (fed with ketotifen, 1 mg/kg·b.w. (bodyweight)) and *B. adolescentis* group (fed with *B. adolescentis*) and the detailed randomization process was in Supplementary material. AD symptoms were induced by DNFB as Sasso et al. described and modified [19]. 0.5% DNFB solution (substrate solution: acetone/olive oil (4:1, v/v)) was painted on the shaved dorsal skin and left ear of mice on day 1. After painting, mice were painted on days 5, 8, 11, 14 with 0.2% DNFB solution. Mice were only treated with a substrate solution in the control group. The *Bifidobacterium* spp. suspension of 0.2 ml ( $1 \times 10^9$  CFU) was orally administrated once a day for 3 weeks, and Fig. 1a showed the experimental flow chart.

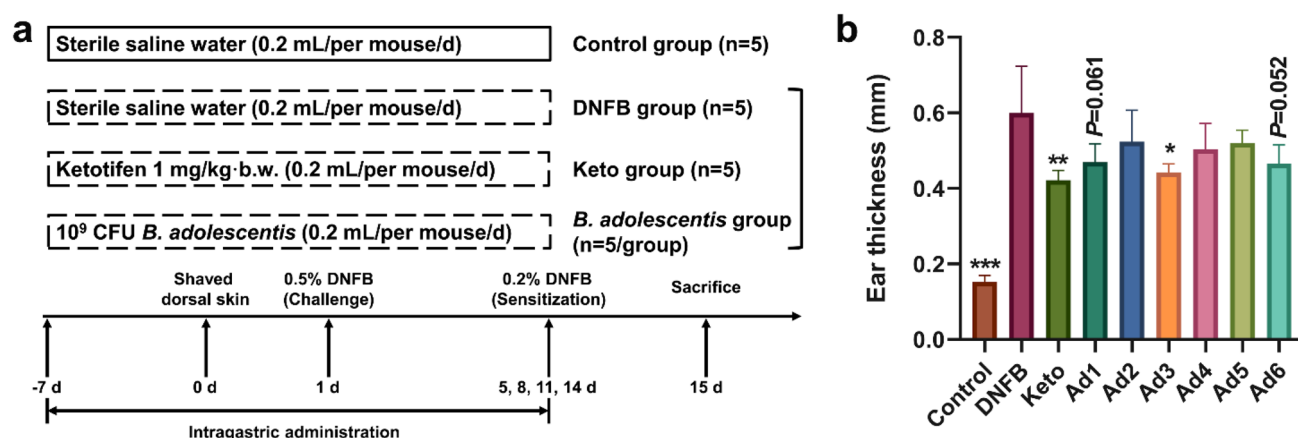


**Fig. 1** Effect of *Bifidobacterium adolescentis* on ear thickness. **a** Animal experimental schedule. **b** Effects of *B. adolescentis* on ear thickness in AD mice ( $n=5$ /group)

### Ear thickness determination

A digital vernier caliper was used to determine the thickness at the middle sections of the ear.

### Measurement of skin thickness

The dorsal skin was stained with haematoxylin and eosin. Photomicrographs and skin thickness were taken at a fixed magnification of 400 $\times$  by the digital scanner.

### Mast cells stain

Mast cells were stained by toluidine blue, and then the number of mast cells was calculated.

### Measurement of immune indicators

After sacrifice, skin tissues and serum samples were collected and determined by enzyme-linked immunosorbent assay kits (Senbeijia, Nanjing, China).

### Flow cytometry analysis

After sacrifice, referring to the protocol, single-cell suspension of spleen was prepared for CD4, CD25 and Foxp3 intracellular staining by mouse regulatory T cell staining kit #1 (Invitrogen Corporation, Carlsbad, CA, USA).

### 16S rRNA amplicon sequencing and SCFAs metabolism

To access changes in gut flora, the total DNA of feces was collected by FastDNA Spin Kits (MP Biomedicals, Santa Ana, USA). The V3–V4 region was amplified and sequenced at

the Illumina MiSeq platform (Illumina, Santiago, CA, USA) as described previously [20] (Supplementary material). The microbial biomarkers were identified by linear discriminant analysis (LDA) effect size (LEfSe) method. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUST) was performed to analyze the functional genes profiles of gut microbiota based on 16S information [21]. The functional genes predictive analysis was performed by the Galaxy website (Supplementary material).

SCFAs were measured by GCMS, referring to Mao et al. [22]. SCFAs concentrations were determined according to the external standard method (Supplementary material).

### Statistical analysis

The statistical analysis was performed with SPSS 24.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8 (GraphPad Inc., La Jolla, CA, USA). Data represent the mean  $\pm$  SD ( $n=5$ /group). The Kruskal–Wallis tests (non-parametric analysis) was used to compare the statistical difference among all groups, followed by Dunn's multiple comparisons tests (\* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  vs DNFB group). To examine the relationship between parameters associated with AD and gut microbial alteration, a principal component analysis (PCA) was carried out by XLSTAT (Addinsoft, Paris, France). The network correlation between variations was performed by R (version 3.5.1) and Gephi (version 0.9.2) [23].

## Results

### *Bifidobacteria adolescentis* alleviated AD-like clinical symptom

The inhibition effect of *B. adolescentis* on clinical symptoms in mice with AD was shown in Fig. 1b. *Bifidobacteria adolescentis* Ad1, Ad3, and Ad6 decreased the ear

thickness versus the DNFB group, but *B. adolescentis* Ad2, Ad4, and Ad5 could not alleviate ear swelling. We next performed the histological analysis. Skin thickness was improved by oral administration of *B. adolescentis* Ad1 and Ad3 (Fig. 2a, b), besides, the recruitment of mast cells was inhibited by Ad1 treatment compared to the DNFB group (Fig. 2c, d). In summary, these results demonstrated that *B. adolescentis* Ad1 and Ad3 treatments effectively alleviated AD-like clinical symptoms.

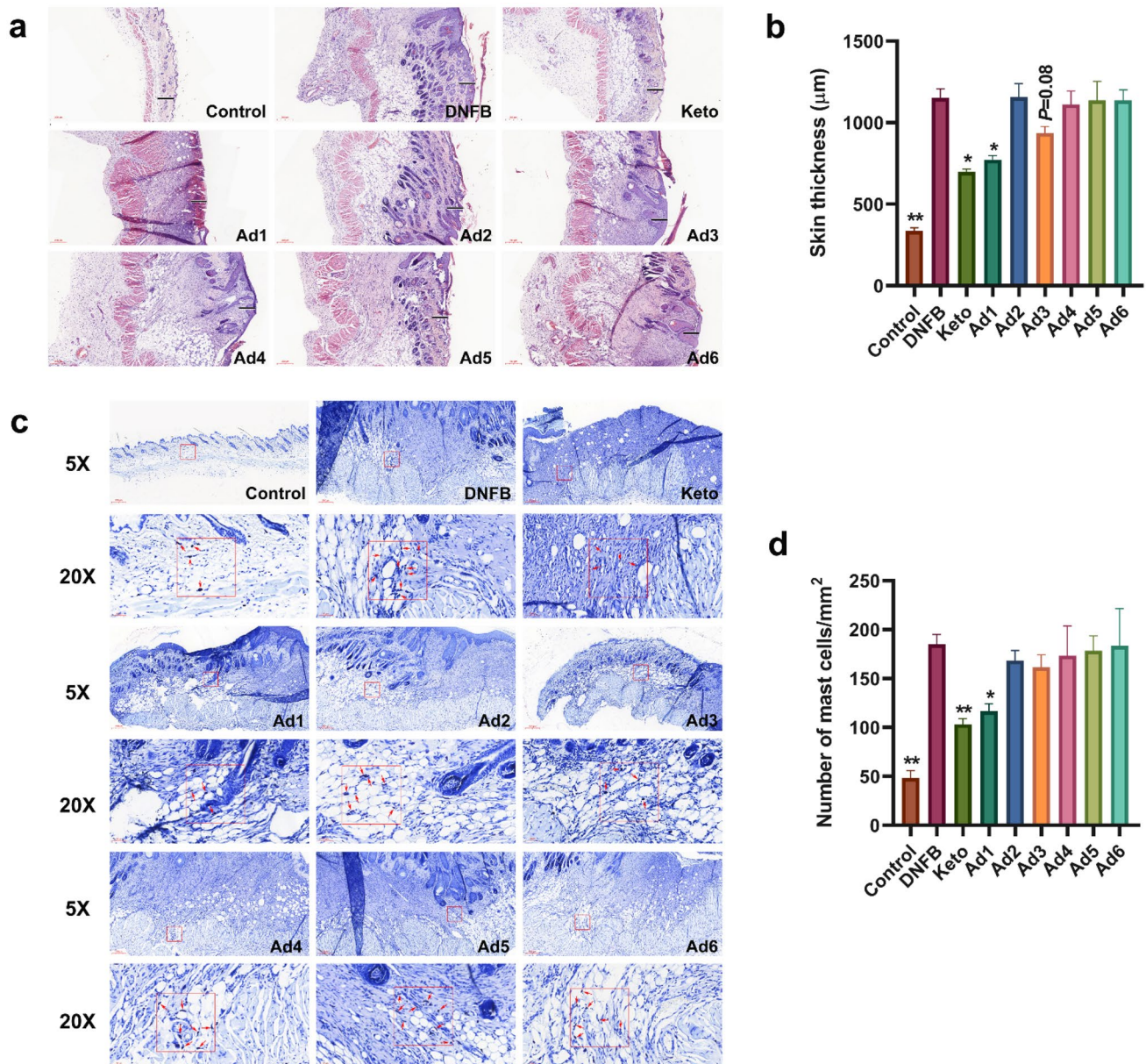


**Fig. 2** Effects of *Bifidobacterium adolescentis* treatments on symptoms of atopic dermatitis. **a** Dorsal skin sections were stained, Scale bar=200  $\mu$ m, original magnification= $\times$ 400. **b** Changes in skin thick-

ness ( $n=5$ /group). **c** Mast cells were stained (red arrowhead), Scale bar=200  $\mu$ m ( $\times$ 5), 50  $\mu$ m ( $\times$ 20), original magnification= $\times$ 400. **d** Mast cell number was evaluated ( $n=5$ /group)

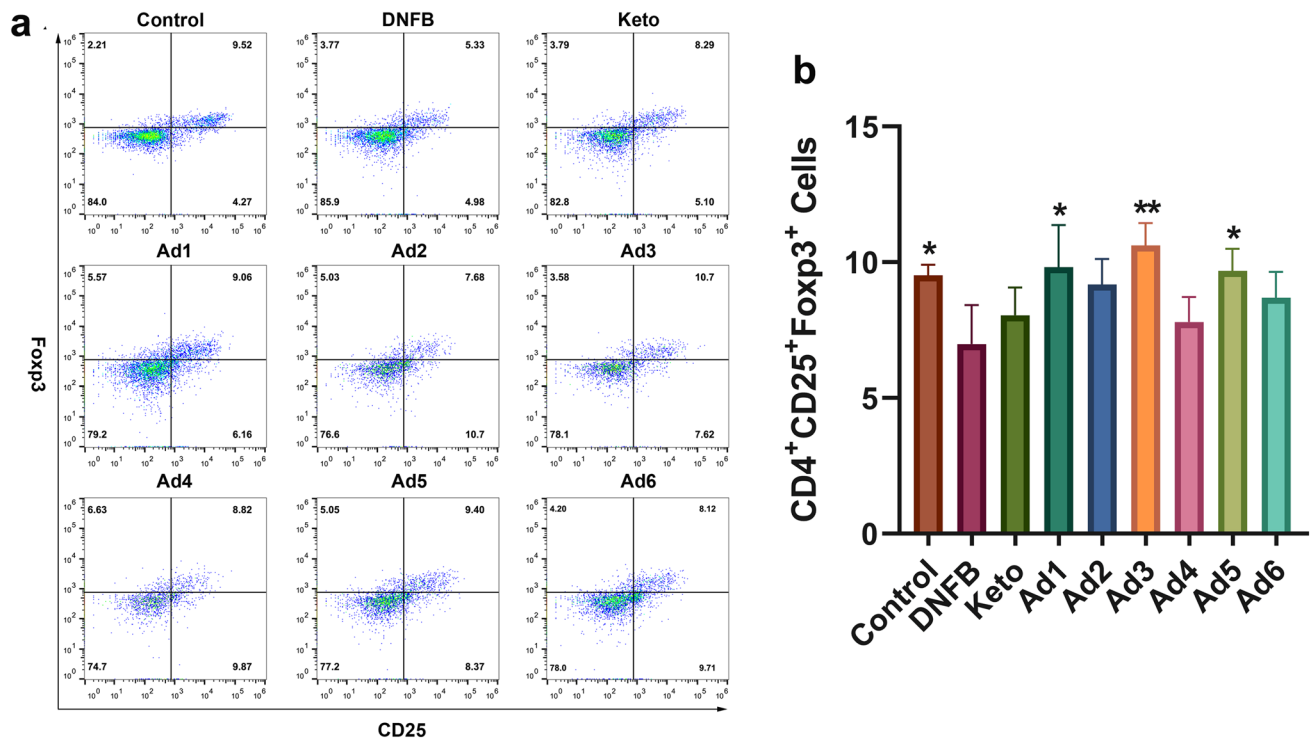


**Fig. 3** Tregs ratio in the spleen. **a** The percentages of Tregs were analyzed by flow cytometry. **b** Tregs ratio was measured ( $n=5/\text{group}$ )

### ***Bifidobacteria adolescentis* promoted Tregs differentiation in the spleen**

Tregs differentiation plays an important role in the acquisition of immunosuppression. To investigate whether *B. adolescentis* administration affected immunomodulation in the spleen, regulatory T cells (Tregs) population were measured by flow cytometry (Fig. 3a). Ad1, Ad3, and Ad5 treatments significantly increased Tregs ratio in spleen versus the DNFB group (Fig. 3b). The other three strains also promoted the Tregs differentiation although there was no statistical difference. The results suggested that oral administration of *B. adolescentis* Ad1, Ad3, and Ad5 induced Tregs differentiation, thereby resulting in suppressing the Th2-dominant immune response in AD.

### ***Bifidobacteria adolescentis* suppressed serum IgE and regulated cytokines production**

The levels of serum IgE and cytokines related to AD were determined. *Bifidobacteria adolescentis* Ad1, Ad4, Ad5, and Ad6 significantly reduced serum IgE levels compared to the DNFB group (Fig. 4a). All strains decreased IL-4 levels in dorsal skin except for Ad3, although there was no statistical significance in these groups (Fig. 4b). Compared to the DNFB group, *B. adolescentis* Ad5 and Ad6

treatments reduced the expression levels of IL-13, but there was no statistical significance (Fig. 4c). *Bifidobacteria adolescentis* Ad1 and Ad4 treatments decreased IL-5 levels versus the DNFB group (Fig. 4d). In addition, oral administration of Ad1 and Ad2 decreased the expression levels of macrophage-derived chemokine (MDC/CCL22) versus the DNFB group (Fig. 4e). *Bifidobacteria adolescentis* Ad1–Ad3 significantly increased IFN- $\gamma$  and IL-10 levels (Fig. 4f, g). These results showed that *B. adolescentis* had variable effects on immune responses in DNFB-induced AD mice.

### ***Bifidobacteria adolescentis* affected the gut microbiota diversity in AD mice**

To determine whether *B. adolescentis* can influence the gut microbiota composition, the fecal flora obtained from mice were analyzed by high-throughput sequencing. The mice from the DNFB group showed dramatic shifts in gut microbial composition and structure versus the control group (Fig. 5a). *Firmicutes* was reduced in the DNFB group, and the ratio of *Firmicutes/Bacteroidetes* (F/B), a signature of gut dysbiosis, was decreased in the DNFB group (Fig. 5b). Treatment with *B. adolescentis* strains prevented the DNFB-induced increases in *Bacteroidetes* while increased the proportion of *Firmicutes*, and *B. adolescentis* Ad2, Ad4, Ad5,

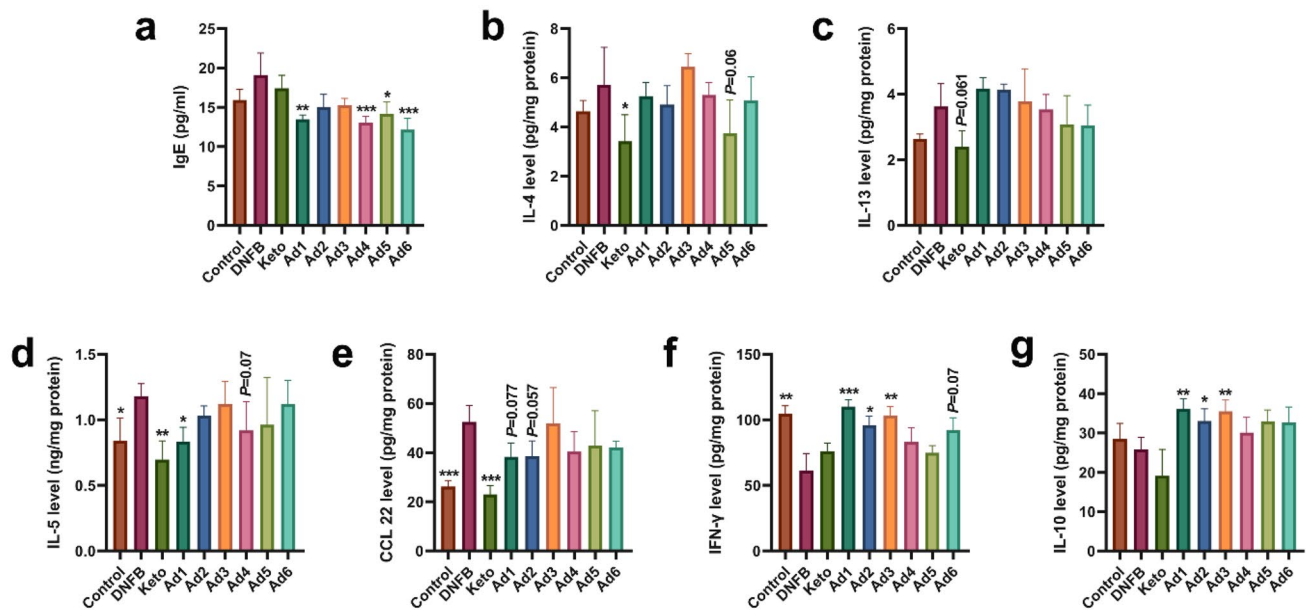


**Fig. 4** Effects of *B. adolescentis* on cytokine levels and serum IgE ( $n=5$ /group)

and Ad6 treatments significantly increased the F/B ratio versus the DNFB group (Fig. 5b).

Additionally, the Shannon and Simpson diversity index were evaluated to exhibit gut microbial diversity (Fig. 5c). Treatment with *B. adolescentis* strains increased the Chao1 index, particularly, *B. adolescentis* Ad1 significantly increased the Chao1 index versus the DNFB group. However, the Shannon index was no differences between all groups. The beta diversity showed the differences in gut microbial composition among all groups (Fig. 5d).

### ***Bifidobacteria adolescentis* regulated the gut microbiota composition and SCFAs metabolism**

To further explore changes in the gut microbiota, LEfSe analysis was performed. The taxonomic cladogram and tag exhibited different taxa in the DNFB group compared to all other groups (Fig. 6a, b, Fig. S2). *Pseudomonas*, *Sutterella*, *Enterococcus*, *Dorea*, and *Pediococcus* were the core microbiome in the DNFB group compared to all other groups. Some selected different genera were shown in Fig. 6c, the relative abundance of *Enterococcus* was significantly increased in DNFB group, while this was restored by *B. adolescentis* Ad1, A3, A4, and Ad5 treatments. *Bifidobacteria adolescentis* Ad1 treatment decreased the proportion of *Dorea*. Additionally, the reduction in *Lactobacillus* was restored by *B. adolescentis* Ad1 treatment.

To reveal changes in the metabolism of gut microbiota, the colonic SCFAs were determined. Acetic acid, propionic acid, and butyric acid were decreased while isovaleric acid was increased in DNFB group (Fig. 7a). Treatment with *B.*

*adolescentis* Ad1 increased propionic and butyric acids but decreased isovaleric acid, and could not increase acetic acid versus the DNFB group. *Bifidobacteria adolescentis* Ad4 and Ad5 treatments also decreased isovaleric acid versus the DNFB group.

### ***Bifidobacteria adolescentis* modulated functional modules of the gut microbiota**

To explore the effects of *B. adolescentis* on the physiological functions of gut microbiota, PICRUSt analysis was performed to analyze and predict the composition of the functional genes in metabolic pathways. First, based on clinical and immune indicators, and changes in gut microbiota, a PCA analysis was performed to select the possible effective strain for further research (Fig. 7b). The distance of points represented the similarity among groups. *Bifidobacteria adolescentis* Ad1, Ad2, and Ad3 located closer to the control group than other *B. adolescentis* strains. Therefore, we selected *B. adolescentis* Ad1 to conduct PICRUSt analysis. At the  $P < 0.05$  from the  $t$  test (two-sided), 11 different functional categories were identified between the control and DNFB groups (Fig. 7c). Pathways involved in C5-branched dibasic acid metabolism, phenylpropanoid biosynthesis, and valine, leucine and isoleucine biosynthesis were upregulated in DNFB group, but other eight pathways, such as polycyclic aromatic hydrocarbon degradation, fructose and mannose metabolism, and phosphotransferase system were upregulated in the control group. The differential pathways between the DNFB and Ad1 groups were exhibited in Fig. 7c. *Bifidobacteria adolescentis* Ad1 treatment also upregulated

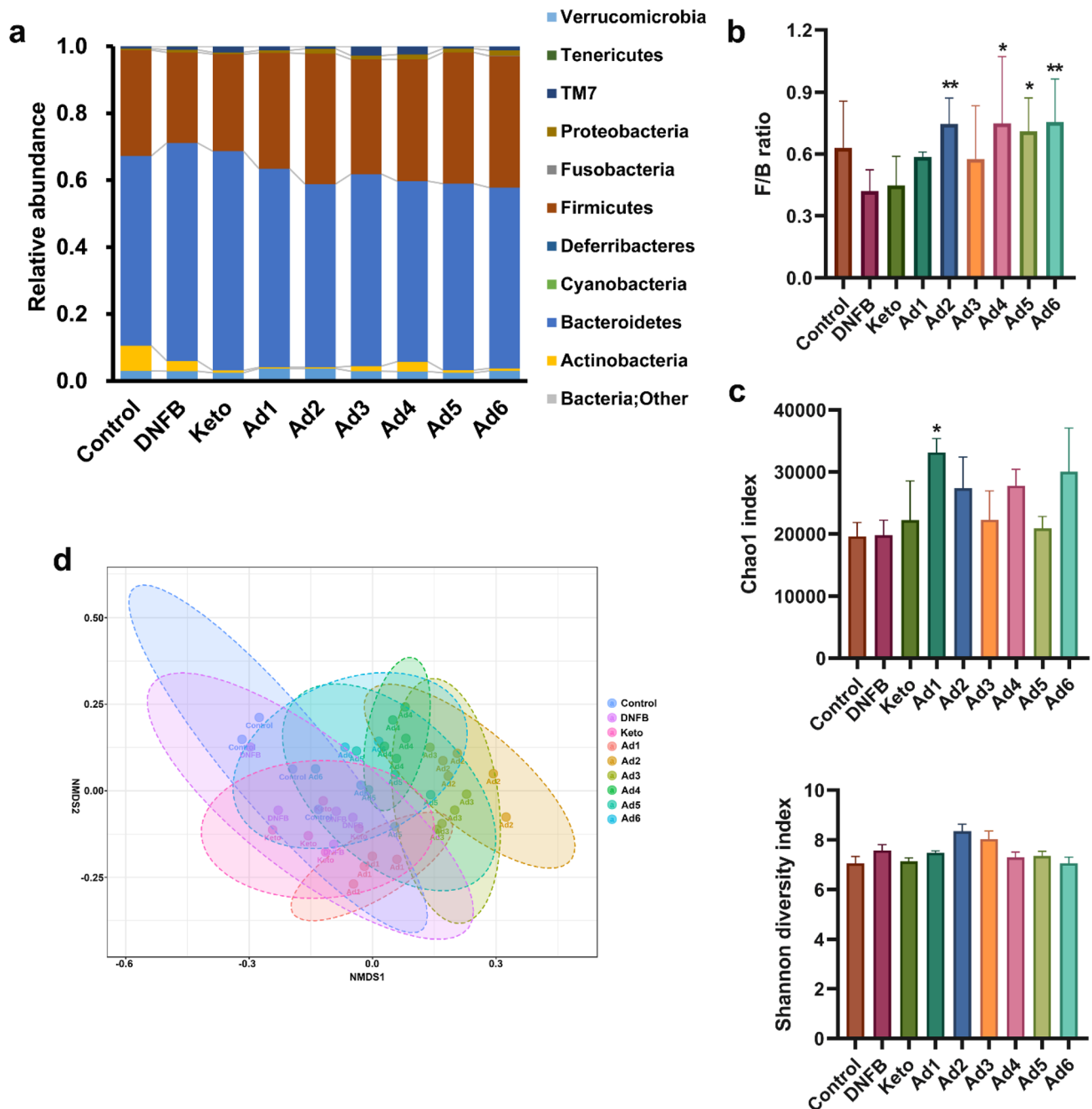


**Fig. 5** Analysis of gut microbiota composition. **a** The gut microbial composition at the phylum level. **b** Changes in the F/B ratio ( $n=4-5/\text{group}$ ). **c**  $\alpha$  diversity indicated by Chao1 and Shannon indices ( $n=4-5/\text{group}$ ). **d**  $\beta$  diversity based on Bray-Curtis similarity

fructose and mannose metabolism versus the DNFB group, and pathway involved in fatty acid biosynthesis of gut microbiota was upregulated. Additionally, antigen processing and presentation and nucleotide-binding oligomerization domain-like receptor signaling pathways were significantly upregulated by *B. adolescentis* Ad1 treatment, although it was no statistical significance in the control and DNFB groups (Fig. S3).

### Correlation between clinical symptoms, immune responses, and gut microbial alteration

To reveal the relationship among variations related to pathological and immune indicators, SCFAs production, and gut microbiota, the interaction network analysis was performed. The differential taxa were selected based on LefSe analysis in the Control, DNFB, Ad1, Ad2, and Ad3 groups (Fig. 6a

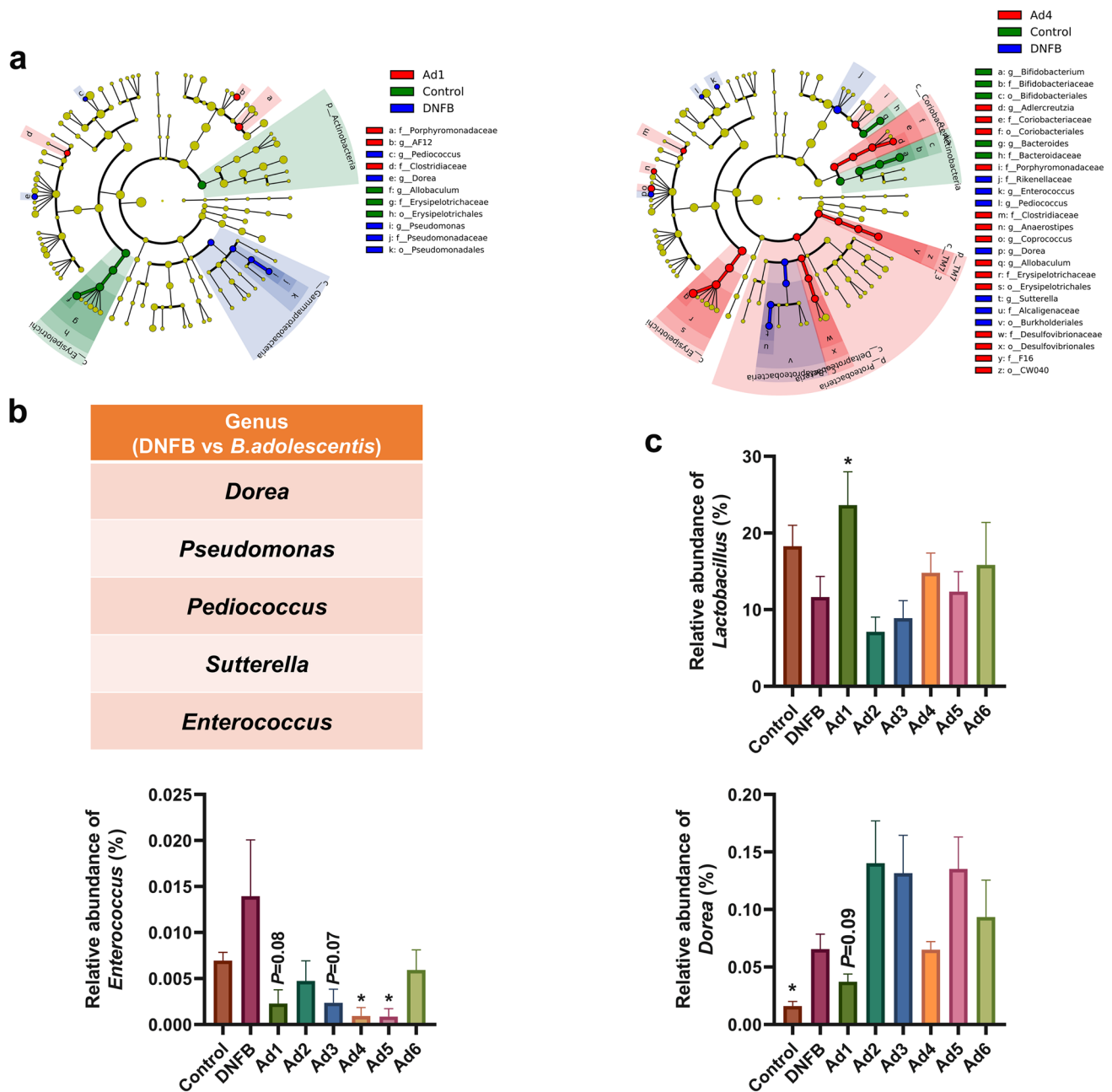


**Fig. 6** Differential gut microbiota among all groups. **a** LefSe comparison of microbiota in all groups. **b** The differential genus in the DNFB group compared to Ad1–Ad6 groups. **c** The relative abundance of *Enterococcus*, *Dorea*, and *Lactobacillus*

and Fig. S2). Figure 8a showed that skin thickness and mast cells were positively related to IL-5, CCL22, *Rikenella*, and *Dorea* but negatively related to Tregs, IFN- $\gamma$ , propionic acid, butyric acid, and *Lactobacillus* ( $P < 0.05$ ,  $r > 0.3$  or  $r < -0.3$ ). Obviously, clinical symptoms were closely associated with regulation of immune responses and gut microbiota. *Bifidobacteria adolescentis* interventions regulated the composition of gut microbiota, and thus indirectly altered SCFAs production. According to network analysis, immune responses were directly affected by the gut microbiome and

SCFAs. Tregs, IFN- $\gamma$ , and IL-10, related to inhibition of Th2 type immune responses, were positively associated with AF12, *Bifidobacterium*, and *Anaerotruncus*. Interestingly, there was a positive correlation between Tregs and butyric acid. Th2 type cytokines, such as IL-4 and IL-13 were negatively related to *Bifidobacterium* and *Allobaculum*. Additionally, the interaction between gut microbiota was important for AD amelioration. The increase in *Lactobacillus* and *Bifidobacterium* suppressed the relative abundance of *Dorea*, and AF12 and *Anaerotruncus* were contributed to

a decrease in *Pediococcus* and *Pseudomonas* (negative correlation). Collectively, *Bifidobacterium* interventions were contributed to AD amelioration through inhibition of Th2 type immune responses, SCFAs production, and competition between gut microbiota.

## Discussion

This study evaluated the effects of six *B. adolescentis* strains on skin lesions, gut microbial profiles, and their immunomodulatory properties. The correlations between immune and pathological indices and specific gut microbiota were further analyzed, and changes in the functional gene in the metabolic pathways and SCFAs were explored.

AD is a common disorder with severe pruritus on the skin and significantly decreases the life quality index of patients. To explore the effect of *B. adolescentis* on AD, we established a DNFB-induced AD mouse model with a polarized Th2 immune response. Initial and final body weight of mice had no statistical difference within the group or between groups during the experiment period (Fig. S1). DNFB markedly destroyed the physical barrier of the ear and induced ear swelling (Fig. 1b). Additionally, in the skin lesions mast cells infiltration and collagen accumulation were increased and thus leading to dermal thickening and tissue damage (Fig. 2). Treatment with *B. adolescentis* Ad1, Ad3, and Ad6 reduced ear thickness, and the ear swelling was partially suppressed by these strains. It indicated that these strains had the potential to alleviate AD-like clinical symptoms. Therefore, we next evaluated the pathological changes in the dorsal skin of AD mice. *Bifidobacteria adolescentis* Ad1 and Ad3 also suppressed the increase in dermal thickening, and *B. adolescentis* Ad1 reduced mast cells infiltration and accumulation in skin lesions. The immune role of effective probiotics on alleviating AD-like symptoms would be explored because of the development of AD involved immune disorders.

Tregs have the ability to suppress Th2 immune responses by moving to peripheral tissues and inflammation draining lymph nodes during the allergic immune responses [24]. To investigate whether *B. adolescentis* increased differentiation of Tregs in the spleen, the flow cytometry analysis was performed. DNFB significantly reduced the CD4 + CD25 + Foxp3 + Treg population while the oral administration of *B. adolescentis* Ad1, Ad3, and Ad5 significantly promoted the ratio of Tregs in the spleen (Fig. 3b). Th1- and Th2-type immune responses are pivotal for allergic diseases. Therefore, the cytokines involving the balance and serum IgE levels were evaluated in AD-like skin lesions. Treatment with *B. adolescentis* decreased serum IgE levels except for A2 and Ad3, but *B. adolescentis* A1 and A2 suppressed IL-4, IL-5, and CCL22 levels versus DNFB group

(Fig. 4a–e). However, *B. adolescentis* could not reduce the expression IL-13 levels except Ad5 and Ad6. Additionally, *B. adolescentis* A1, A2, and A3 significantly increased IFN- $\gamma$  and IL-10 levels (Fig. 4f, g). These results indicated that *B. adolescentis* promoted Th1-type immune responses and suppressed Th2-type immune responses. In other words, treatment with *B. adolescentis* restored the balance related to Th1- and Th2-type immune responses. Besides, IL-10 also contributed to Th2 immune inhabitation and the increased IL-10 production might be associated with the migration of Tregs in the spleen [25, 26]. But studies are to be needed to explore the pathways involving the migration of Tregs among gastrointestinal, general, and skin immune system.

Studies have demonstrated that changes in gut microbial composition were associated with AD [27–29]. Gut dysbiosis is one of the causes to lead to AD. Our results showed that in the DNFB group *Firmicutes* decreased but *Bacteroidetes* prevailed and thus leading to a low F/B ratio (Fig. 5a, b). Treatment with *B. adolescentis* decreased the proportion of *Bacteroidetes* and increased *Firmicutes* and resulted in an increased F/B ratio and improved gut dysbiosis. To further explore the different taxa, we performed LEfSe analysis. We found that *Pseudomonas*, *Sutterella*, *Enterococcus*, *Dorea*, and *Pediococcus* were the core microbiome in the DNFB group (Fig. 6a, b). *Sutterella* has been reported to relate to the development of autism and is prevalent in children with autism and gastrointestinal dysfunction [30]. *Dorea* spp., major gas-producing bacteria [31], have been reported to increase in IBS patients [32]. *Pseudomonas* is widely distributed on the skin of humans and animals and leads to skin infection, urinary tract infection, and central nervous system infections [33]. The development of AD might be associated with the presence of these harmful bacteria in the DNFB group. However, the proportion of *Lactobacillus* was increased by *B. adolescentis* treatments versus the DNFB group (Fig. 6c). The increase in *Lactobacillus* was contributed to restoring the balance of gut microbiota under pathological conditions. These results demonstrated that *B. adolescentis* treatment altered gut microbial composition in AD mice, and affected the gut microenvironment.

Gut microbiota-produced SCFAs serve as energy substrates for epithelial cells and affect various physiological processes in the gut [34]. A study has reported that SCFAs regulate the function of the colonic Tregs and prevent colitis from developing in mice [35]. Treatment with *B. adolescentis* increased propionic acid and butyric acid concentrations but decreased isovaleric acid versus the DNFB group (Fig. 7a). The increased propionic acid was negatively related to clinical symptoms, CCL22, and *Dorea* (Fig. 8a), and indicated that propionic acid was contributed to the inhibition of these indicators, but it was associated with the increase in *Lactobacillus*. Butyric acid was also negatively correlated with clinical symptoms but

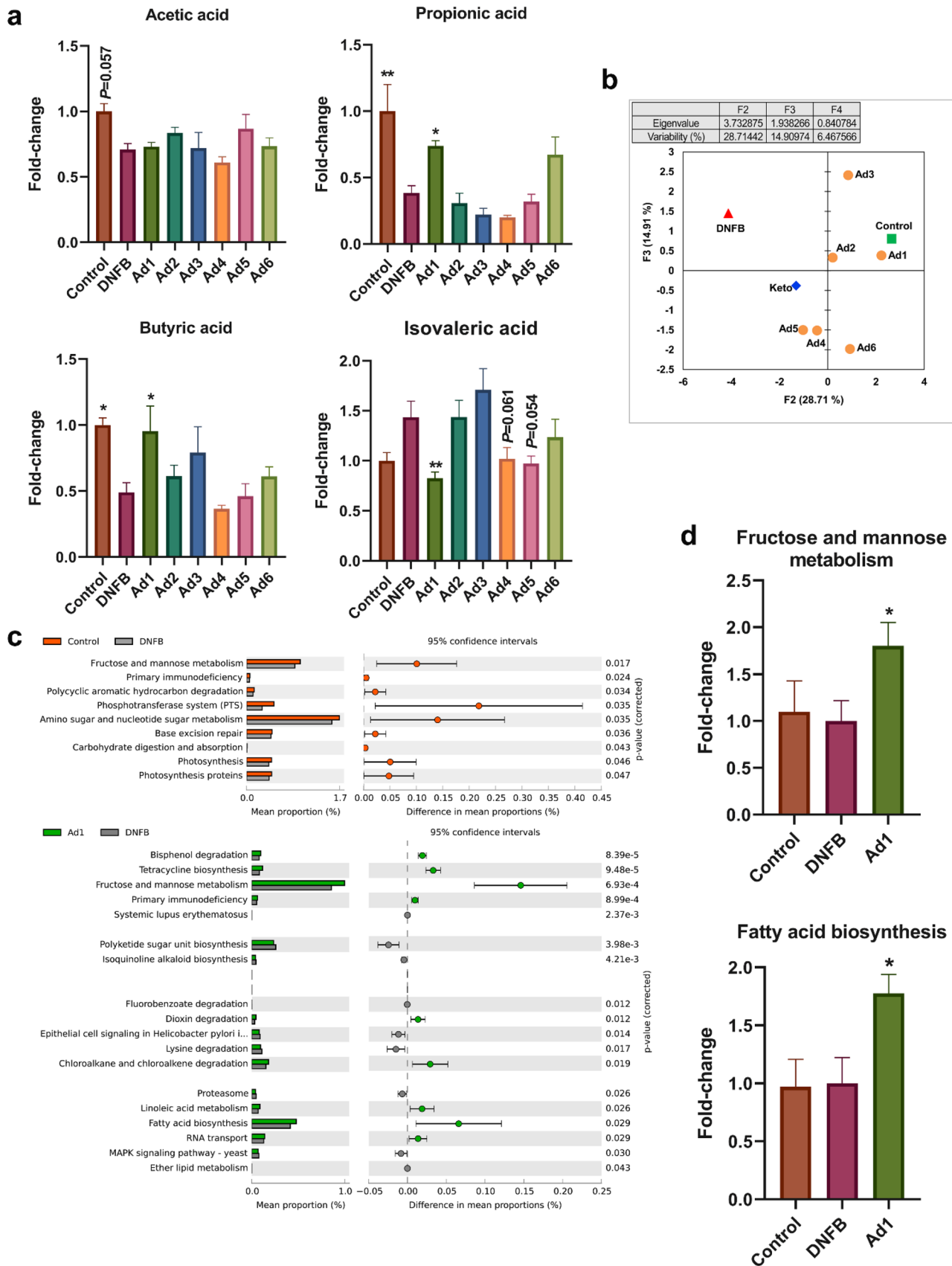


**Fig. 7** Changes in SCFAs and KEGG pathways in the control, DNFB, and Ad1 groups. **a** Changes in SCFAs ( $n=5$ /group). **b** PCA score scatter plot of clinical, immune, and intestinal measures of the six strains. **c** Differential KEGG pathways between the control and DNFB groups (Welch's  $t$  test, two-sided,  $P<0.05$ ); Differential KEGG pathways between the DNFB and Ad1 groups (Welch's  $t$  test, two-sided,  $P<0.05$ , effect size  $<1.10$ ). **d** Changes in differential KEGG pathways

positively correlated with IFN- $\gamma$ . It indicated that butyric acid suppressed the clinical pathology and increased Th1 type immune responses. However, isovaleric acid was related to the increase of IgE, CCL22, and *Dorea*, and related to the aggravation of AD.

Gut microbial alteration affected not only SCFAs metabolism but the composition of the functional genes. Taking *B. adolescentis* Ad1 as an example based on a PCA analysis (Fig. 7b). 11 involved in KEGG pathways in the control and DNFB groups were identified, and treatments with *B. adolescentis* significantly influenced the functional gene composition (Fig. 7c). Pathways involved in fructose and mannose metabolism and fatty acid biosynthesis were upregulated by *B. adolescentis* Ad1 treatment (Fig. 7d), and this might be associated with the increase in propionic and butyric acids (Fig. 7a). Additionally, a study has reported that the reduction in genes involved in antigen processing and presentation and nucleotide-binding oligomerization domain-like

receptor signaling was associated with impaired immune development in the six-month-old infants with AD versus healthy control subjects [36]. Our results showed that *B. adolescentis* Ad1 treatment also significantly upregulated both two pathways versus DNFB group (Fig. S3).

Besides the effect of SCFAs on clinical and immune indicators, gut microbiota was important in the network analysis (Fig. 8a). AF12 and *Allobaculum* were inversely related to IgE, CCL22, and IL-4, respectively, and *Bifidobacterium* and *Lactobacillus* were inversely related to IL-13 and IL-5, respectively. AF12 was positively related to Tregs, IL-10, and IFN- $\gamma$ . Obviously, the increase in these genera restored the balance related to Th1 and Th2 type immune responses, and this was contributed to the inhibition of immune responses related to AD development. Additionally, the core microbiome in the DNFB group, such as *Dorea* was negatively related to *Anaerotruncus*, *Ruminococcus*, *Lactobacillus*, and *Bifidobacterium* and this revealed the competition between these genera in the gut. The competition in the gut helped the restoration of balance between gut microbiota in AD mice. Changes in gut microbiota caused by *B. adolescentis* treatment were associated with improved clinical symptoms, suppressed Th2 type responses, and increased SCFAs production according to network analysis. Therefore, the strain-specific ameliorating effect of *B. adolescentis* on AD manifestation closely related to the alteration of gut microbiota.

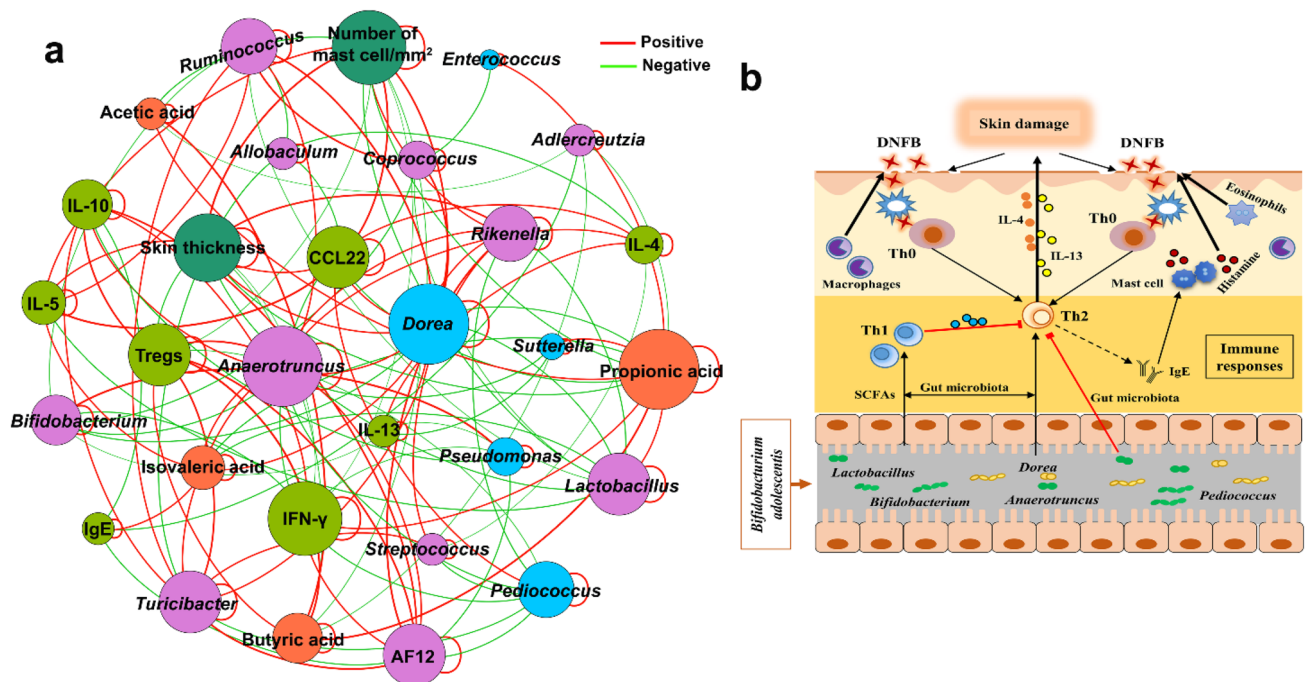


**Fig. 8** Correlation networks and schematic diagram. **a** The interaction networks among clinical symptoms, immune indicators, SCFAs production, and differential taxa. The size of the circles stands for the contribution; red represents a positive correlation, green represents

a negative correlation ( $P<0.05$ ,  $r>0.3$  or  $r<-0.3$ ). **b** Schematic diagram for the alleviating effects of *Bifidobacterium adolescentis* on atopic dermatitis

There are some limitations in this study, for instance, the communication between *B. adolescentis* and the gut immune system needs to be elucidated, and the metabolites except SCFAs contributing to immune regulation need to be identified as well as the related gut bacteria.

In summary, the results showed that the immune responses induced by DNFB were suppressed by *B. adolescentis* through promoting Tregs population in the spleen, restoring the balance between Th1- and Th2-type immune responses, and regulating the gut microbiota composition (Fig. 8b). Additionally, changes in SCFAs and functional gene profiles demonstrated the possible metabolism pathways in AD disease.

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**Author contributions** ZFF conducted the experiments, performed the statistical analysis, and wrote the manuscript; ZFF and WWL analyzed and interpreted the data. LL facilitated the experiments. WWL, HZ, and WC conceived and designed the work. WWL, YKL, JXZ, and WC reviewed and edited the manuscript.

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## Compliance with ethical standards

**Conflict of interest** There are no conflicts to declare.

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# ***Bifidobacteria adolescentis* regulated immune responses and gut microbial composition to alleviate DNFB-induced atopic dermatitis in mice**

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## **Materials and Methods**

### **Isolation and identification of *Bifidobacterium adolescentis* strains**

About one gram of fecal sample was diluted and painted to the MRS agar medium (containing 1% (w/v) mupirocin and 0.05% (w/v) cysteine), then incubated in an anaerobic environment at 37 °C. After 48-72 hours, picking up the single colony and cultured in MRS liquid medium (containing 0.5% L-cysteine (w/v)) at 37 °C for 48 hours. Total DNA was extracted and sequenced for the whole genome. Strains were identified by BLAST based on Gene Bank and stored at -80 °C with 30% (v/v) glycerin for future use.

### **Randomization process for animal experiment**

Mice were randomly divided by body weight into various experimental groups (initial body weight was ranged 16-19 g). We used Microsoft Excel (version 2016) to perform randomization process. Firstly, mice were ordered

according to body weight, and then we randomly selected 45 numbers according to the random number table. The selected number divided by 9 (number of groups), and mice were assigned to different groups according to reminder.

### **16S rRNA amplicon sequencing**

The total DNA of feces was collected by FastDNA Spin Kits (MP Biomedicals, Santa Ana, USA). The polymerase chain reaction (PCR) was used to amplify the V3-V4 region (forward primer, 5'-CCTAYGGGRBGCASCAG-3'; reverse primer, 5'-GGACTACNNGGTATCTAAT-3'). PCR products were excised from a 2.0% agarose gel, purified by TIANGel Mini Purification Kit (TIANGEN, Beijing, China). DNA concentration was measured using the Qubit BR dsDNA assay. Libraries were prepared using TruSeq DNA LT Sample Preparation Kits (Illumina, Santiago, California) and sequenced for 500 + 7 cycles on an Illumina Miseq using the MiSeq Reagent Kit.

16S rRNA sequence data were analyzed using the QIIME pipeline. The raw sequences were screened (low-quality (score <30) and short length (<200 bp) sequences were abandoned) and the qualifying sequences were spliced. Sequences with similarity > 97% were clustered into operational taxonomic units (OTUs) by QIIME software and representative sequences of each cluster were used to classify bacterial taxa. Linear Discriminant Analysis Effect Size (LEfSe) and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) were performed online (Galaxy: <http://huttenhower.sph.harvard.edu/galaxy>).

### **SCFAs measurement**

50-100 mg freeze-dried fecal sample was dispersed 500 µl of saturated NaCl solution for 30 min. The soaked sample was homogenized and acidified with 40 µl of 10% sulfuric acid. One milliliter of ether was added to the acidified homogenates and vortexed before centrifugation at 18,000g for 15 min at 4°C. Five hundred microliters of the supernatants was carefully filtered through 0.22 µm pore and transferred to gas phase vial for gas chromatography–mass spectrometry analysis.

The concentrations of SCFAs were determined by gas chromatography mass spectrometry (GCMS-QP2010 Ultra system, Shimadzu Corporation, Kyoto, Japan) with a Rtx-Wax column (30 m × 0.25 × µm ×

0.25  $\mu\text{m}$ ). The carrier gas was helium, the flow rate was 2 mL/min, the split ratio was 10:1 and the volume of sampling was 1  $\mu\text{L}$ . The injection temperature was 240  $^{\circ}\text{C}$ , and the GC temperature program was as follows: the initial temperature of 100  $^{\circ}\text{C}$  was increased to 140  $^{\circ}\text{C}$  at the rate of 7.5  $^{\circ}\text{C}/\text{min}$ , and then was increased by 60  $^{\circ}\text{C}/\text{min}$  to 200  $^{\circ}\text{C}$ , which was maintained for 3 min. The ionization temperature was 220  $^{\circ}\text{C}$ . Mass scanning was performed in full scan mode. A standard curve was generated by the external standard method, and the concentrations of SCFAs were calculated according to the standard curve.

### Results

**Fig. S1:** Changes in initial and final body weight of mice. ns: no significance (two-way mixed-effects analysis following Sidak's multiple comparisons tests (within group), or two-way ANOVA multiple comparisons following Tukey's multiple comparisons tests (between groups) ).

**Fig. S2:** LEfSe comparison of different taxa in the DNFB group versus the control, Ad2, A3, A5, and A6 groups.

**Fig. S3:** Antigen processing and presentation and nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathways between the DNFB and Ad1 groups. The Kruskal-Wallis tests was used to compare the statistical difference among all groups, followed by Dunn's multiple comparisons tests (\*  $P < 0.05$  vs DNFB group).

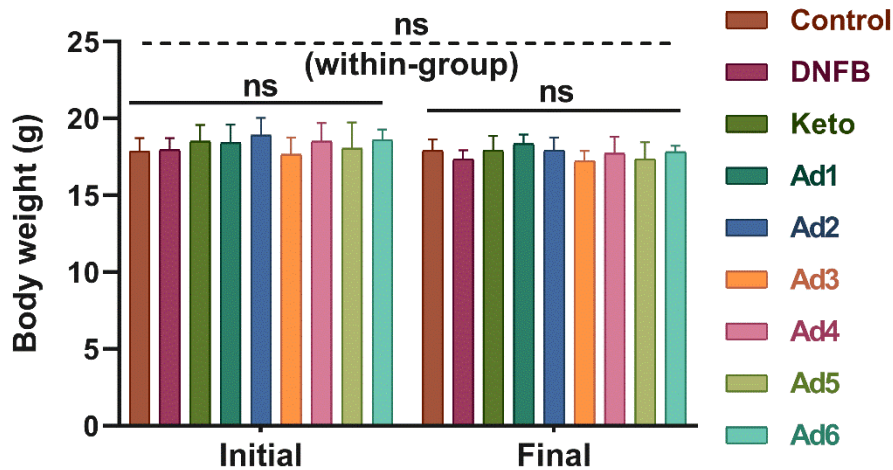


Fig. S1

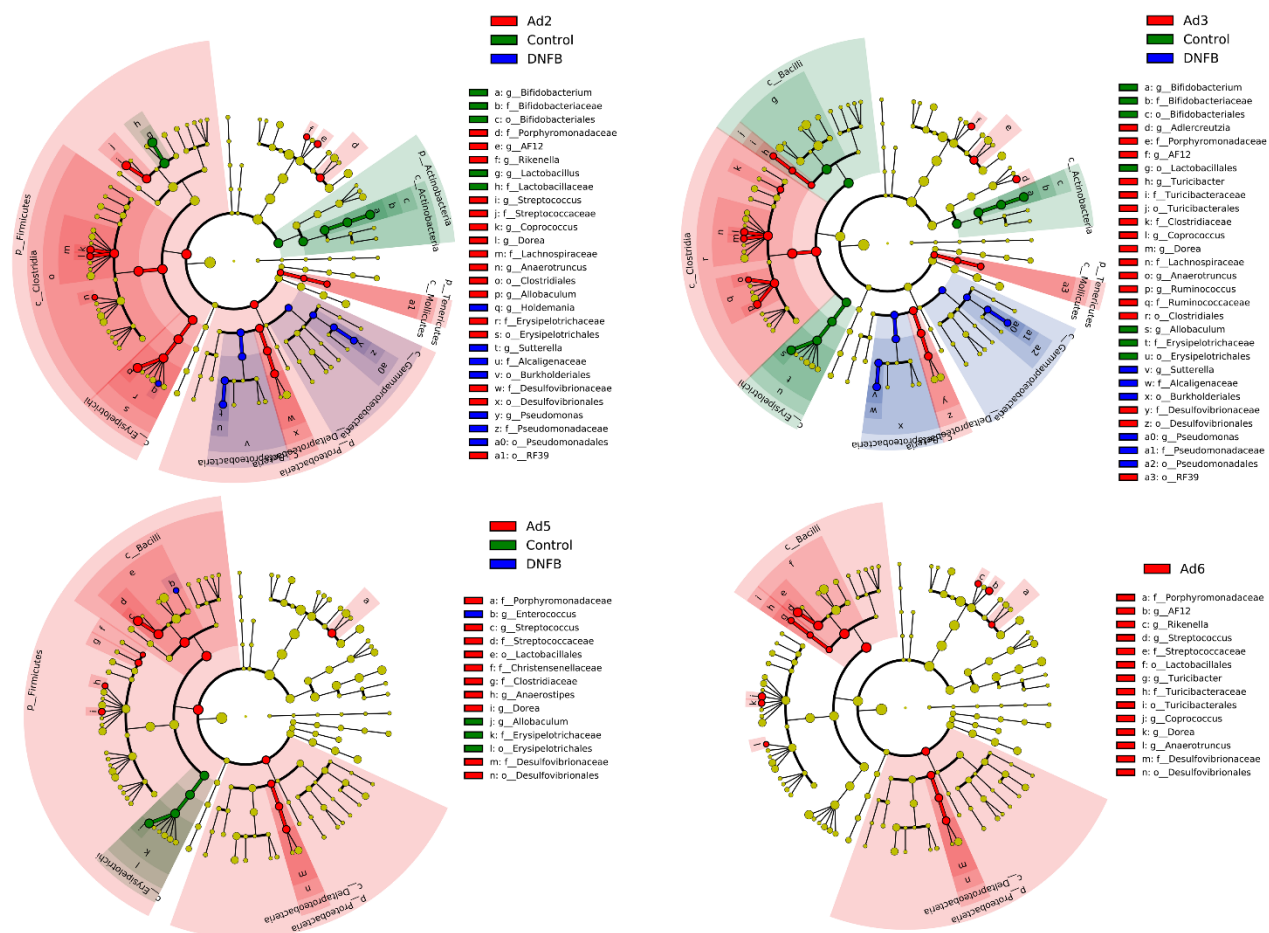


Fig. S2

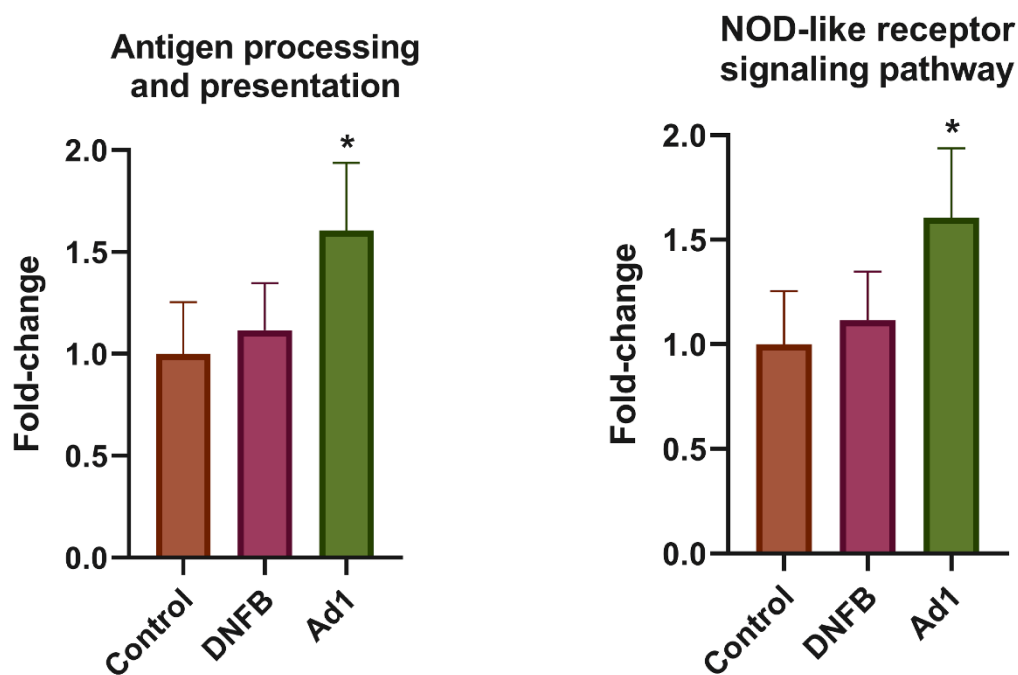


Fig. S3