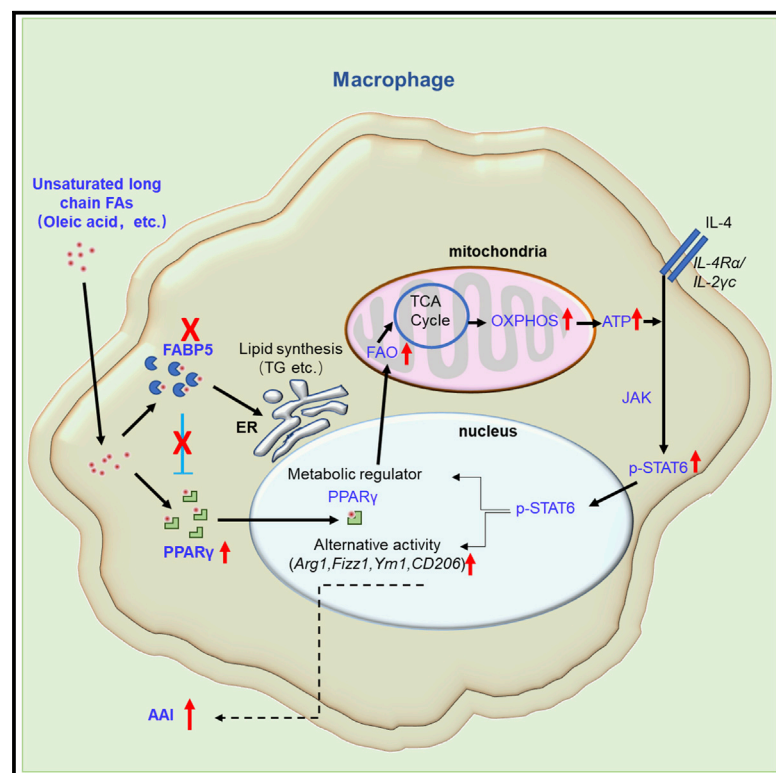


FABP5 controls macrophage alternative activation and allergic asthma by selectively programming long-chain unsaturated fatty acid metabolism

Graphical abstract



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In brief

Hou et al. report that FABP5 mainly regulates M2 polarization of macrophages by regulating long-chain unsaturated fatty acids (LCUFAs)—PPAR γ -ATP-STAT6 pathway and then regulates allergic asthma. This study provides a new strategy and idea for the prevention and treatment of allergic asthma.

Highlights

- Myeloid-specific deletion of *Fabp5* aggravates allergic airway inflammation
- FABP5 selectively regulates the accumulation of long-chain unsaturated fatty acids
- Excessive uptake of oleic acid exacerbates allergic airway inflammation
- FABP5 programs lipid metabolism to regulate M2 polarization



Article

FABP5 controls macrophage alternative activation and allergic asthma by selectively programming long-chain unsaturated fatty acid metabolism

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SUMMARY

Fatty acids (FAs) are widely involved in diverse biological functions. In mice with myeloid-specific deletion of fatty acid-binding protein 5 (FABP5), OVA-induced allergic airway inflammation (AAI) is significantly exacerbated by increasing alternatively activated macrophages (M2). *Fabp5* deficiency increases IL-4-induced M2 *in vitro*. In macrophages, *Fabp5* deletion causes significant accumulation of free long-chain unsaturated FAs, such as oleic acid, but does not cause detectable changes to other groups of FAs. Interestingly, excessive uptake of oleic acid aggravates AAI pathogenesis, with increased M2 polarization in bronchoalveolar lavage fluid. Informatics and mechanistic studies indicate that *Fabp5* deficiency may reprogram metabolic pathways by enhancing FA β oxidation, tricarboxylic acid cycle, and oxidative phosphorylation, in addition to producing more ATP through activation of the PPAR γ signaling pathway, reshaping macrophages in favor of M2 polarization. These results emphasize the importance of FABP5 and oleic acid in AAI, suggesting preventive and therapeutic strategies for allergic asthma.

INTRODUCTION

Fatty acids (FAs) are highly involved in cell proliferation, differentiation, survival, and many other processes, where they act as energy sources and signals for metabolic and gene transcriptional regulatory networks. FAs can modulate the biological functions of immune cells by regulating cellular structure and metabolism, as well as by acting through membrane transporters, cell surface proteins, and intranuclear receptors in healthy and diseased individuals.^{1–3} Increased consumption of saturated FAs is associated with increased risks of atherosclerosis, coronary heart disease, obesity, and metabolic syndrome.^{4,5} Meanwhile, consumption of unsaturated FAs can either decrease or increase susceptibility to many immune-related and metabolic diseases.^{6,7} The n-6 to n-3 ratio is important for balancing host immunological activity. Higher proportions of linoleic acid and total n-6 polyunsaturated FAs are related to an increased risk of atopic asthma, whereas higher proportions of eicosapentaenoic acid (EPA; 20:5n-3) are related to a decreased risk of nonatopic asthma.⁸ However, several studies have reported inconsistent results regarding the effect

of FAs on diseases,⁷ potentially arising from differences in formats, doses, genetics, microbiome, and other factors. FA uptake in the cell is predominately controlled by transport proteins like CD36, FAs-binding proteins and the FA transport protein family in the plasma membrane. As lipid chaperones, FABPs actively facilitate the transportation of lipids to specific cell compartments, including the lipid droplet, the endoplasmic reticulum, the mitochondria or peroxisome, and the nucleus, as well as to the outside of the cell to enable autocrine or paracrine signaling.⁹ FABP5, is a small, 15-kDa cytoplasmic protein that participates primarily in FA uptake, transport, and metabolism within the cell cytoplasm.¹⁰ Studies using *Fabp4*- and/or *Fabp5*-deficient mice found that both FABP4 and FABP5 play significant roles in the development of metabolic syndrome, including insulin resistance, diabetes mellitus, and atherosclerosis.^{11–13} It was recently reported that T cell-specific deficiency of both *Fabp4* and *Fabp5* impairs uptake of exogenous free FAs by tissue-resident memory CD8⁺ T cells and greatly reduces long-term survival *in vivo*; however, no effect was observed on the survival of central memory CD8⁺ T cells in lymph nodes.¹⁴ The roles and relevant mechanisms of the lipid chaperone protein



FABP5 in anti-inflammatory alternative macrophage activation related to allergic pathological situations have yet to be addressed.

Allergic asthma has become more prevalent in recent years.⁶ Allergic asthma is a chronic inflammatory disease of the airways characterized by infiltration of eosinophils, basophils, mast cells, and CD4⁺ T cells in the airway submucosa.¹⁵ *Fabp5* expression is upregulated in the sputum of asthmatic individuals.¹⁶ FABP5 supports the synthesis of retinoic acid from type II alveolar epithelial cells to suppress activation of innate lymphoid cell 2 (ILC2) during allergic lung inflammation.¹⁷ Macrophages play an important role in allergic airway inflammation (AAI).^{18,19} *Fabp5* downregulation in alveolar macrophages contributes to increased inflammation in chronic obstructive pulmonary disease.²⁰ In the present study, we investigated the effects of FABP5 on alternative macrophage activation (M2) and AAI pathogenesis using an OVA-induced AAI mouse model and mice with a myeloid-specific genetic deletion of *Fabp5* (*Lyzs-Fabp5KO* mice). We found that deletion of *Fabp5* in macrophages and excessive intake of oleic acid may worsen the severity of AAI, highlighting potential strategies to prevent and treat allergic asthma.

RESULTS

Fabp5 deficiency in macrophages increases M2 polarization and aggravates AAI pathogenesis in mice

We first analyzed *Fabp5* gene expression in different immune cells using available RNA sequencing (RNA-seq) data sources, which were collected from the NCBI GEO database as summarized in Table S1. The highest expression of *Fabp5* among all analyzed immune cell subsets was observed in the abdominal cavity and in bone marrow-induced macrophages (Figure 1A), and the expression of *Fabp5* in macrophages was the highest among members of the *Fabp* family, including *Fabp1–7*, 9, and 12 (Figure 1B). In addition, we downloaded single-cell RNA-seq data from human lung samples²¹ to analyze FABP5 expression in different human lung cell types. The results showed that FABP5 is also highly expressed in human lung macrophages and type 2 alveolar cells (Figure S1A). Interestingly, *Fabp5* expression in wild-type (WT) mouse macrophages increased about 2-fold under IL-4 induction (Figure 1C). Thus, it is important to study the effects of FABP5 on M2 polarization and M2-related diseases. To address this issue, we created genetically modified mice lacking *Fabp5* in all cells or in myeloid cells specifically. Details regarding genetically modified mouse construction and identification are shown in Figures 1D, 1E, and S1B–S1D. We used interleukin (IL)-4 or IL-13 to stimulate WT and *Fabp5KO* peritoneal macrophages (PEMs) and induce M2 polarization, as described previously.²² Expression of M2-related genes was significantly upregulated in IL-4-induced *Fabp5KO* macrophages, as indicated by RNA-seq analysis (Figure 1F) and confirmed by real-time PCR analysis, showing that *Fabp5* deletion significantly increases expression of M2-related marker genes, including *Arg1*, *Fizz1*, *CD206*, and *Ym1*, in PEMs or BMDMs (Figures 1G, 1H, and S1E). The percentages of CD206⁺ cells in *Fabp5KO* F4/80⁺CD11b⁺ PEMs and BMDMs were significantly higher than those in WT cells in the presence

of IL-4 or IL-13, as detected by flow cytometry (Figures 1I, S1F, and S1G). Arginase activity and protein expression were remarkably higher in *Fabp5KO* PEMs and BMDMs than in WT PEMs and BMDMs in the presence of IL-4, as detected by western blotting (Figures 1J, 1K, S1H, and S1I). Furthermore, *Fabp5KO* M2 PEMs significantly promoted Th0 cell to Th2 cell polarization *in vitro* (Figure S1J). Chitin administration recruited and induced M2 macrophages, which are important for eosinophil recruitment.²³ Chitin induced significantly more Siglec-F⁺CD11b⁺ eosinophils in the abdominal cavities of *Lyzs-Fabp5KO* mice (Figure 1M). Meanwhile, expressions of M2-related genes, including *Arg1*, *Fizz1*, *CD206*, and *Ym1*, were significantly higher in the PEMs of *Lyzs-Fabp5KO* mice after chitin treatment, as determined by real-time PCR (Figure 1M). These results support the idea that *Fabp5* deficiency in macrophages enhances their ability to become M2 polarized macrophages *in vitro* and *in vivo*.

Macrophage M2 polarization is closely relevant to AAI.²² Therefore, we used WT and *Lyzs-Fabp5KO* mice to construct OVA-induced AAI models, as reported previously.^{24,25} The results showed that *Lyzs-Fabp5KO* leads to a significant increase in the percentage and number of Siglec-F⁺CD11b⁺ eosinophils in bronchoalveolar fluid (BALF) compared with WT mice in the AAI mouse model (Figures 2A–2C, S2A, and S2B). However, *Fabp5* deletion had no detectable effect on the numbers of other infiltrated immune cells, including macrophages, neutrophils, DCs, CD4⁺ T cells, and CD8⁺ T cells, in BALF (Figures S2A and S2B). Levels of anti-OVA immunoglobulin (Ig)G1 and anti-OVA IgE were significantly higher in the serum of *Lyzs-Fabp5KO* AAI mice (Figures 2D and 2E). Hematoxylin and eosin (H&E) staining showed that the lungs of *Lyzs-Fabp5KO* AAI mice displayed more serious airway inflammation and greater immune cell infiltration (Figure 2F). Furthermore, Masson staining showed that lung sections of *Lyzs-Fabp5KO* AAI mice exhibited more blue areas than those of WT AAI mice (Figure 2G). Collagen I staining showed that lung sections from *Lyzs-Fabp5KO* AAI mice also exhibited more positive areas (Figure 2H). These results imply that myeloid-specific deletion of *Fabp5* enhances pulmonary fibrosis in the AAI model. In addition, we also measured airway hyper-responsiveness to inhaled methacholine in AAI mice and found that the sRAW of *Lyzs-Fabp5KO* AAI mice was significantly higher than that of WT AAI mice (Figure 2I). As expected, real-time PCR analysis revealed that macrophages in the BALF of *Lyzs-Fabp5KO* AAI mice displayed remarkably higher expression of M2-related genes, such as *Arg1*, *Fizz1*, *CD206*, and *Ym1* (Figure 2J). Meanwhile, *Lyzs-Fabp5KO* mice increased expression of type II-related genes (IL-5, IL-10, and IL-13) and decreased expression of type I inflammatory factors (tumor necrosis factor α and IL-12), in sorted macrophages from BALF, as detected by real-time PCR (Figure S2C). As a whole, these *in vitro* and *in vivo* studies collectively indicate that myeloid-specific deletion of *Fabp5* primarily increases macrophage M2 polarization and exacerbates allergic asthma in mice.

Fabp5 deficiency selectively causes accumulation of free long-chain unsaturated FAs in macrophages

After observing that FABP5 markedly affected M2 polarization, we next sought to understand the relevant molecular

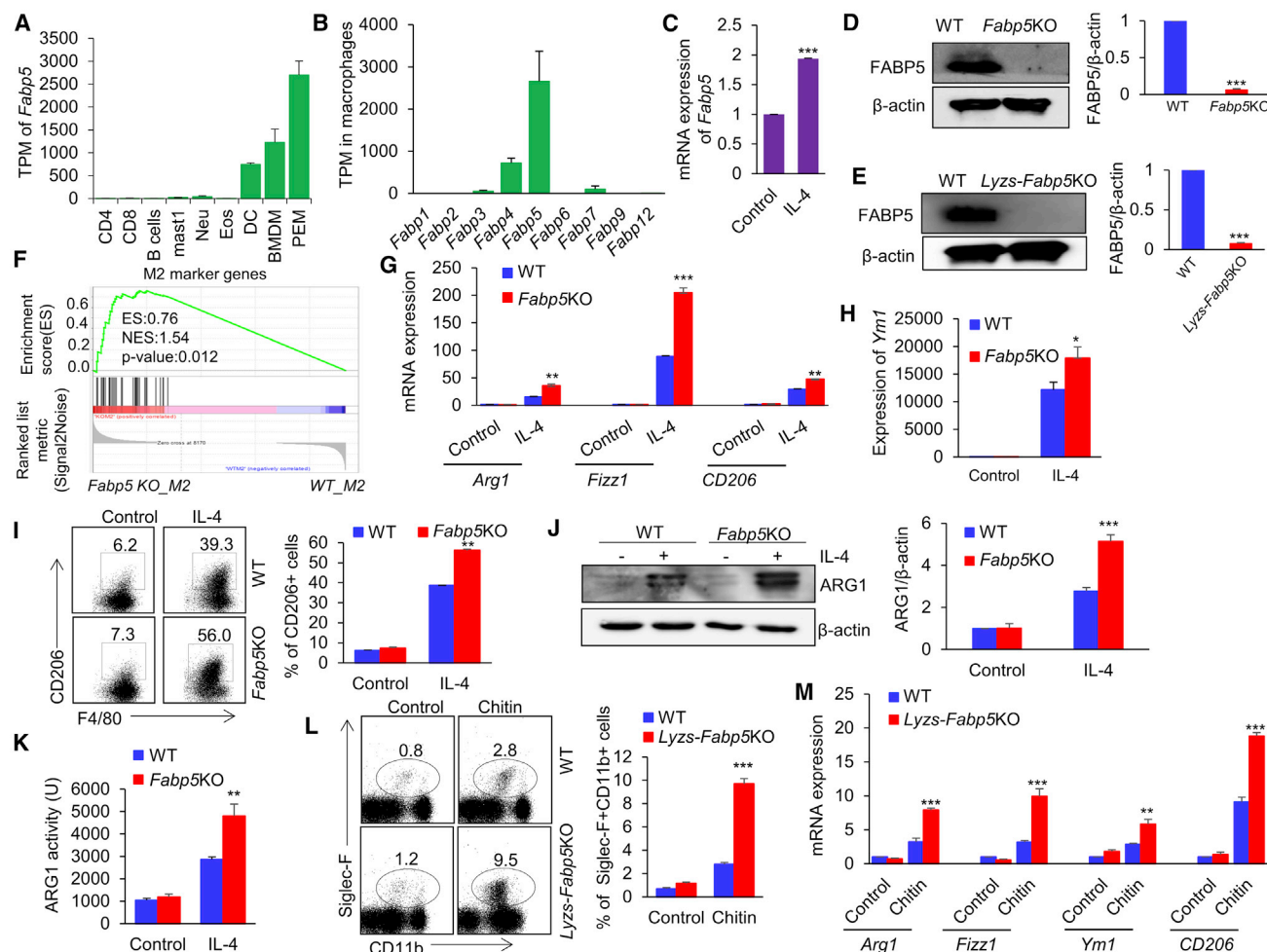


Figure 1. *Fabp5* is highly expressed in macrophages and inhibits M2 macrophage differentiation in vitro and in vivo

(A) Transcripts per million (TPM) of the *Fabp5* gene in different immune cells.
 (B) TPM values of *Fabp* family members in macrophages.
 (C) Mean relative mRNA expression of *Fabp5* in WT PEMs with or without IL-4 stimulation.
 (D) Immunoblot analysis of FABP5 protein in WT and *Fabp5*KO mouse macrophages.
 (E) Immunoblot analysis of FABP5 protein in WT and *Lyzs-Fabp5*KO mouse macrophages.
 (F) GSEA enrichment of M2 marker genes in *Fabp5*KO versus WT M2 macrophages as assayed by RNA-seq.
 (G and H) Gene expression of *Arg1*, *Fizz1*, *CD206*, and *Ym1* in WT and *Fabp5*KO mouse PEMs with or without IL-4 stimulation for 48 h as measured via quantitative PCR.
 (I) Flow cytometry analysis of CD206 expression in F4/80⁺CD11b⁺ cells in WT and *Fabp5*KO PEMs with or without IL-4 stimulation.
 (J) Immunoblot analysis of ARG1 protein in WT and *Fabp5*KO PEMs with or without IL-4 stimulation.
 (K) ARG1 activity in WT and *Fabp5*KO PEMs with or without IL-4 stimulation was determined via fluorescence.
 (L) The percentage of Siglec-F⁺CD11b⁺ eosinophils in WT and *Lyzs-Fabp5*KO mice 2 days after chitin treatment.
 (M) Quantitative PCR showing expression of *Arg1*, *Ym1*, *Fizz1*, and *CD206* mRNA in freshly isolated PEMs from WT and *Lyzs-Fabp5*KO mice at 2 days after chitin treatment. Data are representative of three independent experiments and are presented as mean $\pm$ SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, Student's *t* test. See also Figure S1.

mechanisms. *Fabp5* deficiency did not significantly affect IL-4R (CD124) protein expression in F4/80⁺CD11b⁺ PEMs, as detected by flow cytometry (Figure 3A). However, phosphorylation of the key IL-4R downstream molecule STAT6, an important transcription factor for M2 polarization,^{22,26} was significantly enhanced in *Fabp5*KO PEMs following IL-4 treatment (Figure 3B). M2 macrophages primarily use FA oxidation (FAO; β -oxidation) to fuel mitochondrial oxidative phosphorylation²⁷ and the FABP family

plays important roles in lipid transportation and metabolism. We thus performed a lipidomic analysis to detect free lipids in WT and *Fabp5*KO macrophages before and after IL-4 stimulation. Table S2 shows the abbreviations of detected FAs and their corresponding compound names. The results indicated that levels of long-chain unsaturated FAs, such as oleic acid, arachidonic acid, and linoleic acid, but not of long-chain saturated FAs or medium-chain FAs, were significantly higher in

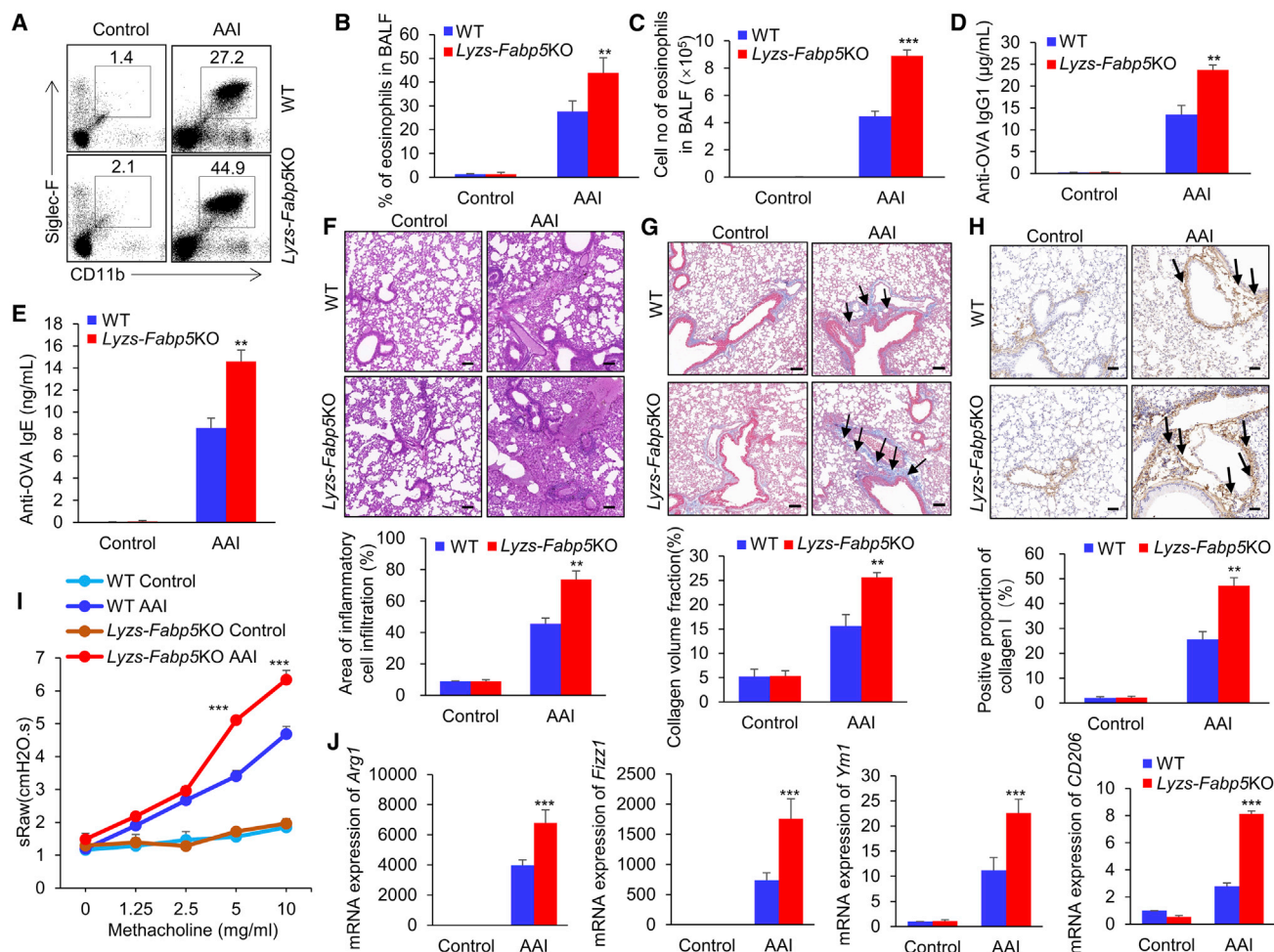


Figure 2. Deletion of *Fabp5* increases M2 polarization of alveolar macrophages and exacerbates AAI in mice

(A–C) Flow cytometry analysis of Siglec-F⁺CD11b⁺ eosinophils (A), statistical diagram of eosinophil proportions (B), statistical graph of eosinophil numbers (C) in BALF of WT and *Lyzs-Fabp5KO* mice (n = 5).

(D and E) Levels of OVA-specific IgG1 (D), and IgE (E) in the serum of WT and *Lyzs-Fabp5KO* mice.

(F) H&E staining of lung tissues from WT and *Lyzs-Fabp5KO* mice (scale bar represents 100 μ m).

(G) Masson staining of lung tissues from WT and *Lyzs-Fabp5KO* mice (scale bar represents 100 μ m). Arrows indicate sites of pulmonary fibrosis.

(H) Collagen I staining of lung tissues from WT and *Lyzs-Fabp5KO* mice (scale bar represents 50 μ m). Arrows represent positive staining.

(I) Baseline sRaw upon methacholine inhalation was measured in WT and *Lyzs-Fabp5KO* mice with or without AAI (n = 4).

(J) mRNA expression of *Arg1*, *Fizz1*, *Ym1*, and *CD206* in sorted CD45⁺CD11c⁺Siglec-F⁺ macrophages isolated from BALF of WT and *Lyzs-Fabp5KO* mice as detected by real-time PCR. Data are representative of three independent experiments and are presented as mean $\pm$ SD (n = 3). **p < 0.01 and ***p < 0.001, Student's t test. See also Figure S2.

Fabp5-deficient macrophages, no matter whether the cells were in M0 or M2 (Figures 3C–3G, and S3). These findings imply that FABP5 selectively programs the transportation and metabolism of long-chain unsaturated FAs in macrophages. To identify changes to the gene expression profiles of macrophages induced by *Fabp5* deficiency and altered levels of intracellular long-chain unsaturated FAs, we performed RNA-seq on WT and *Fabp5KO* macrophages. Heatmap analysis indicated that the transcriptional expression levels of glycerolipid metabolism-related genes (*Aldh2*, *Akr1b8*, *Aldh9a1*, *Lpin2*, *Dgkz*, *Ppap2b*, and *Dgkd*),²⁸ glycerophospholipid metabolism-related genes (*Dgkz*, *Ppap2b*, *Dgkd*, *Pld4*, *Pld2*, *Pld3*, *Pnpla7*, and *Gpcpd1*),²⁹ and sphingolipid metabolism-related genes

(*Ppap2b*, *Degs1*, and *B4galt6*)³⁰ were obviously lower in *Fabp5*-deleted macrophages (Figure 3H). These results indicate that *Fabp5* deletion may reduce lipid anabolism, potentially underlying the intracellular accumulation of free long-chain unsaturated FAs in *Fabp5*-deleted macrophages, although the potential involvement of *Fabp5* deficiency-impaired intracellular lipid transportation in this process can be excluded at present.

Increased oleic acid promotes M2 polarization and AAI pathogenesis in mice

To investigate whether long-chain unsaturated FAs regulate M2 polarization, we pretreated WT and *Fabp5KO* PEMs with oleic acid before inducing M2 polarization. Real-time PCR analysis

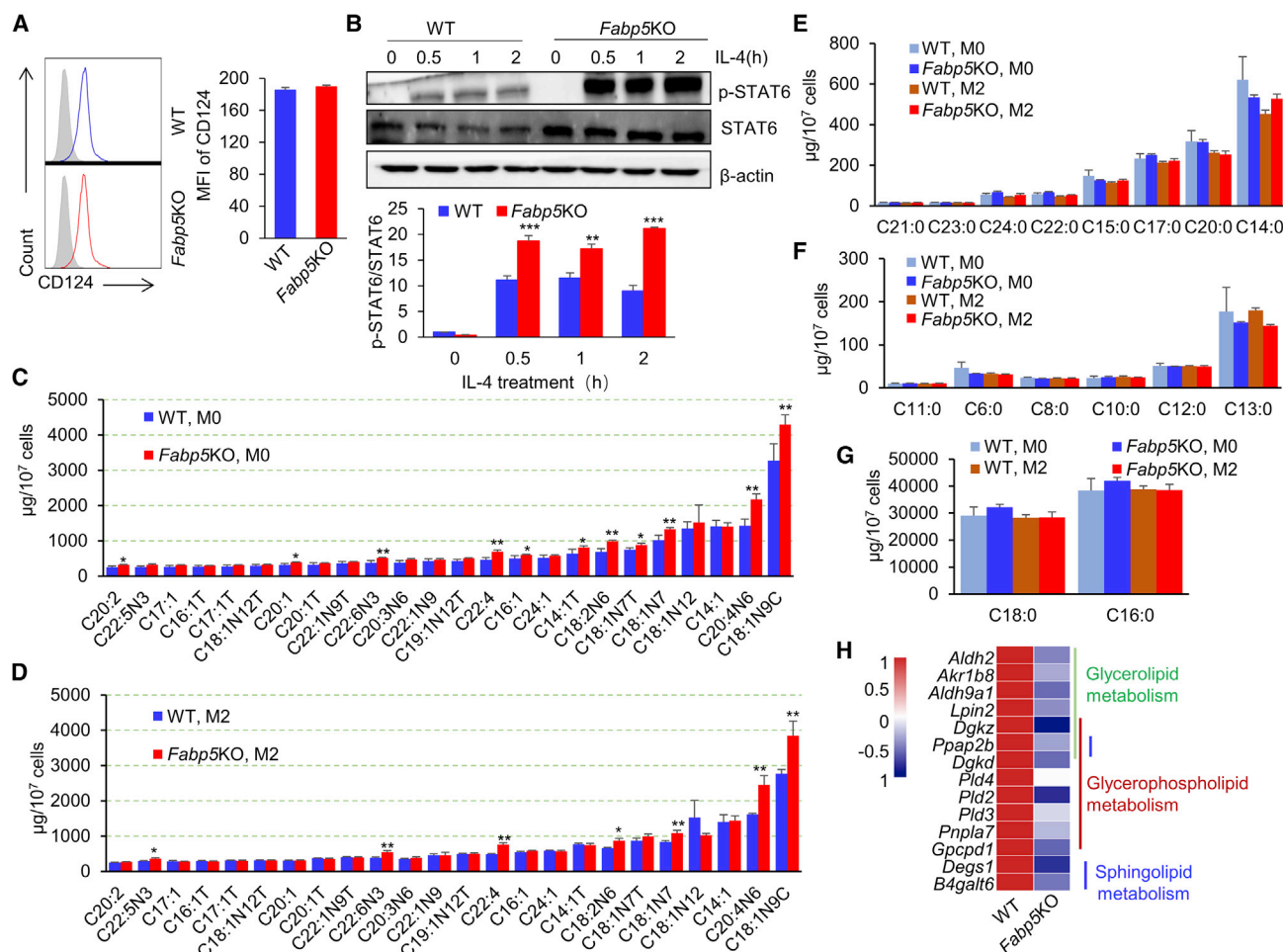


Figure 3. FABP5 regulates the accumulation of long-chain specific free unsaturated FAs and affects lipid anabolism in macrophages

(A) Median fluorescence intensity (MFI) of CD124 in F4/80⁺CD11b⁺ macrophages from WT and *Fabp5*KO mice assayed by a flow cytometry, with the associated statistical bar chart shown in the right panel.

(B) Immunoblot analysis of phosphorylated STAT6 and total STAT6 protein in WT and *Fabp5*KO macrophages with or without IL-4 stimulation for 0.5, 1, or 2 h. (C and D) Metabolomic profiling of long-chain unsaturated FAs in WT and *Fabp5*KO BMDMs, with or without IL-4 stimulation for 48 h. Samples were measured by gas chromatography-mass spectrometry (GC-MS) (n = 4).

(E–G) Metabolomic profiling of long-chain saturated FAs (E), medium-chain FAs (F), and major long-chain saturated FAs (palmitic acid and stearic acid) (G) in WT and *Fabp5*KO BMDMs, with or without IL-4 stimulation for 48 h. Samples were measured by GC-MS (n = 4).

(H) Heatmap representing the expression levels of related genes involved in glycerolipid metabolism, glycerophospholipid metabolism, and sphingolipid metabolism in WT and *Fabp5*KO macrophages. Data are representative of three independent experiments and are presented as mean $\pm$ SD (n = 3). *p < 0.05, **p < 0.01, and ***p < 0.001, Student's t test. See also Figure S3.

revealed that the expression of M2-related genes (*Arg1*, *Ym1*, and *CD206*) increased significantly in WT PEMs in a manner dependent on the dose of oleic acid. However, oleic acid did not have any detectable effects on M2 polarization in *Fabp5*KO PEMs, even at high doses (Figure 4A). Oleic acid increased protein expression of CD206 and arginase in F4/80⁺CD11b⁺ cells among IL-4-stimulated WT macrophages but not among IL-4-stimulated *Fabp5*KO macrophages (Figures 4B–4D). Consistently, western blot analysis revealed that oleic acid increased phosphorylation of STAT6 proteins in WT macrophages but not in *Fabp5*KO macrophages (Figure 4E). As peroxidase proliferator-activated receptor γ (PPAR γ) is a key regulator of lipid metabolism in macrophages, and the interaction between STAT6 and

PPAR γ is important for M2 polarization,^{31,32} we assessed levels of the PPAR γ protein via western blot analysis. Oleic acid increased protein expression of PPAR γ in IL-4-treated WT macrophages but not in *Fabp5*KO macrophages (Figure 4D). To determine whether oleic acid has similar effects on human macrophage M2 polarization, we pretreated human monocyte-derived macrophages with oleic acid, followed by IL-4 stimulation. Oleic acid increased protein levels of CD209 and CD163, which have been identified as markers of human M2 macrophages,³³ on IL-4-stimulated human CD14⁺ macrophages (Figures 4F, 4G, S4C, and S4D), as well as transcriptional expression of human M2-related genes *CD209*, *CD206*, and *CD163*,³⁴ as determined by real-time PCR analysis (Figure 4H).

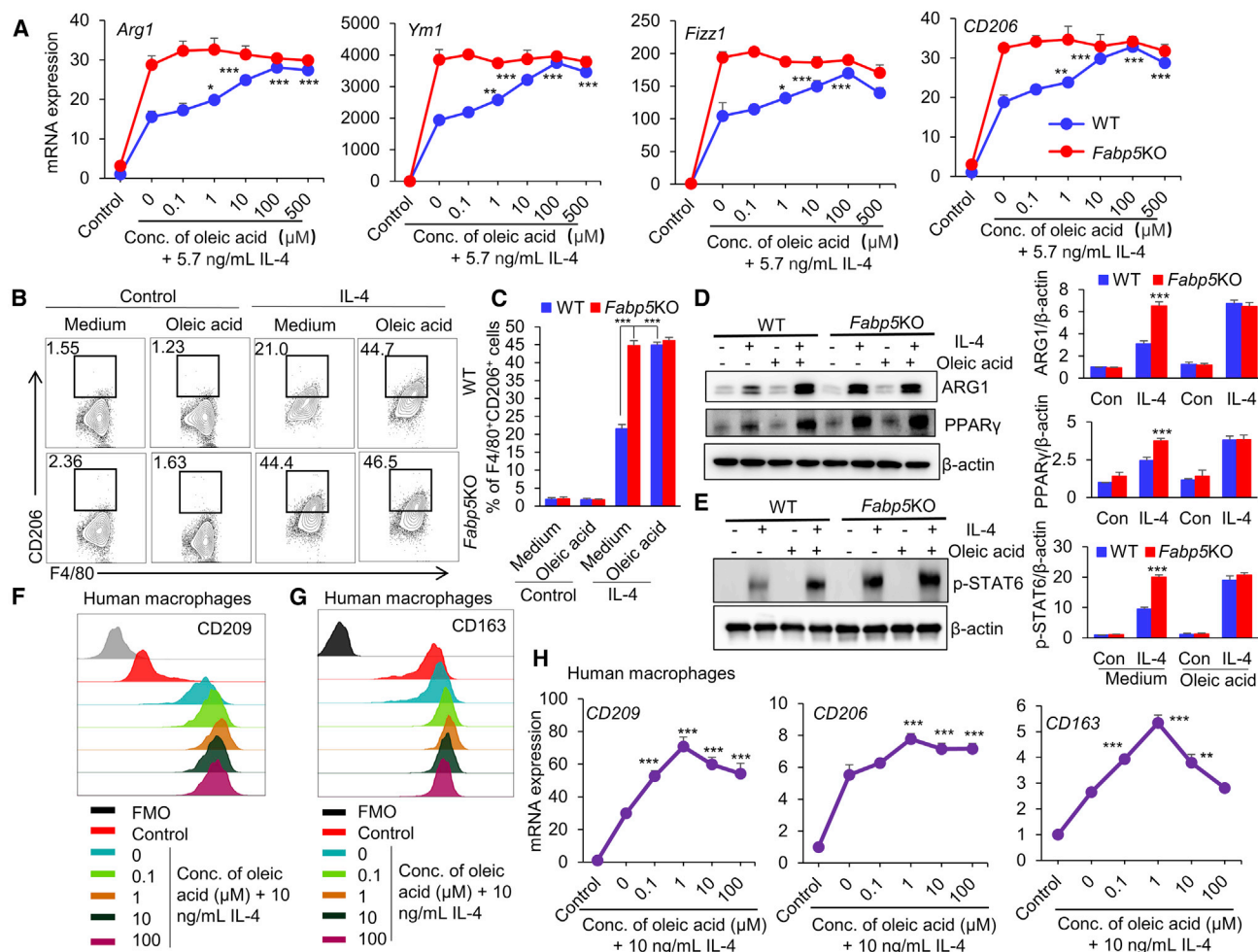


Figure 4. Oleic acid increases M2 polarization of macrophages *in vitro*

(A) mRNA expression levels of *Arg1*, *Ym1*, *Fizz1*, and *CD206* were determined by real-time PCR. Macrophages of WT and *Fabp5KO* mice were pretreated with 0, 0.1, 1, 10, 100, and 500 μ M oleic acid for 24 h, followed by stimulation with IL-4 for 48 h.

(B and C) Flow cytometry analysis of CD206 expression in F4/80⁺CD11b⁺ macrophages from WT and *Fabp5KO* mice.

(D) Immunoblot analysis of ARG1 and PPAR γ protein in WT and *Fabp5KO* macrophages. Macrophages were incubated in DMEM containing 100 μ M oleic acid for 24 h, followed by stimulation with or without IL-4 for 48 h.

(E) Immunoblot analysis of phosphorylated STAT6 protein in WT and *Fabp5KO* macrophages. Macrophages were incubated in DMEM containing 100 μ M oleic acid for 24 h, followed by stimulation with or without IL-4 for 1 h.

(F and G) Human macrophages were pretreated with 0, 0.1, 1, 10, and 100 μ M oleic acid for 24 h, followed by stimulation with human IL-4 for 48 h. Protein expression of CD209 (F) and CD163 (G) on human CD14⁺ macrophages as assessed by flow cytometry.

(H) Gene expression of *CD209*, *CD206*, and *CD163* in human macrophages as detected by real-time PCR. Data are representative of three independent experiments and are presented as mean $\pm$ SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, Student's t test. See also Figure S4.

Furthermore, we selected another long-chain unsaturated FA, linoleic acid, and performed similar studies *in vitro*. We found that adding linoleic acid also increased IL-4-induced M2 polarization in WT PEMs but not in *Fabp5KO* PEMs (Figures S4A and S4B). These results collectively imply that the exogenous addition of oleic acid or linoleic acid can promote M2 polarization in WT but not *Fabp5KO* macrophages *in vitro*.

To determine whether additional application of oleic acid could promote M2 polarization and M2-related pathogenesis *in vivo*, we used an OVA-induced WT AAI mouse model with or without excessive oleic acid provided by gavage. Interestingly, excessive addition of oleic acid increased the percentage and

number of Siglec-F⁺CD11b⁺ eosinophils in BALF significantly (Figures 5A–5C). Meanwhile, oleic acid significantly increased serum levels of OVA-specific IgE and IgG1 in AAI mice, as detected by ELISA (Figures 5D and 5E). Importantly, supplementation with oleic acid led to severe inflammatory cell infiltration and fibrosis in the lungs of AAI mice, as evidenced by H&E staining and Masson and collagen I staining (Figures 5F–5H). The sRaw of oleic acid-treated AAI mice was significantly higher than that of saline-treated AAI mice (Figure 5I). Furthermore, significantly higher expression of M2-related genes such as *Arg1*, *Fizz1*, *CD206*, and *Ym1* was detected in the alveolar macrophages of oleic acid-treated AAI mice (Figure 5J). Thus, excessive dietary

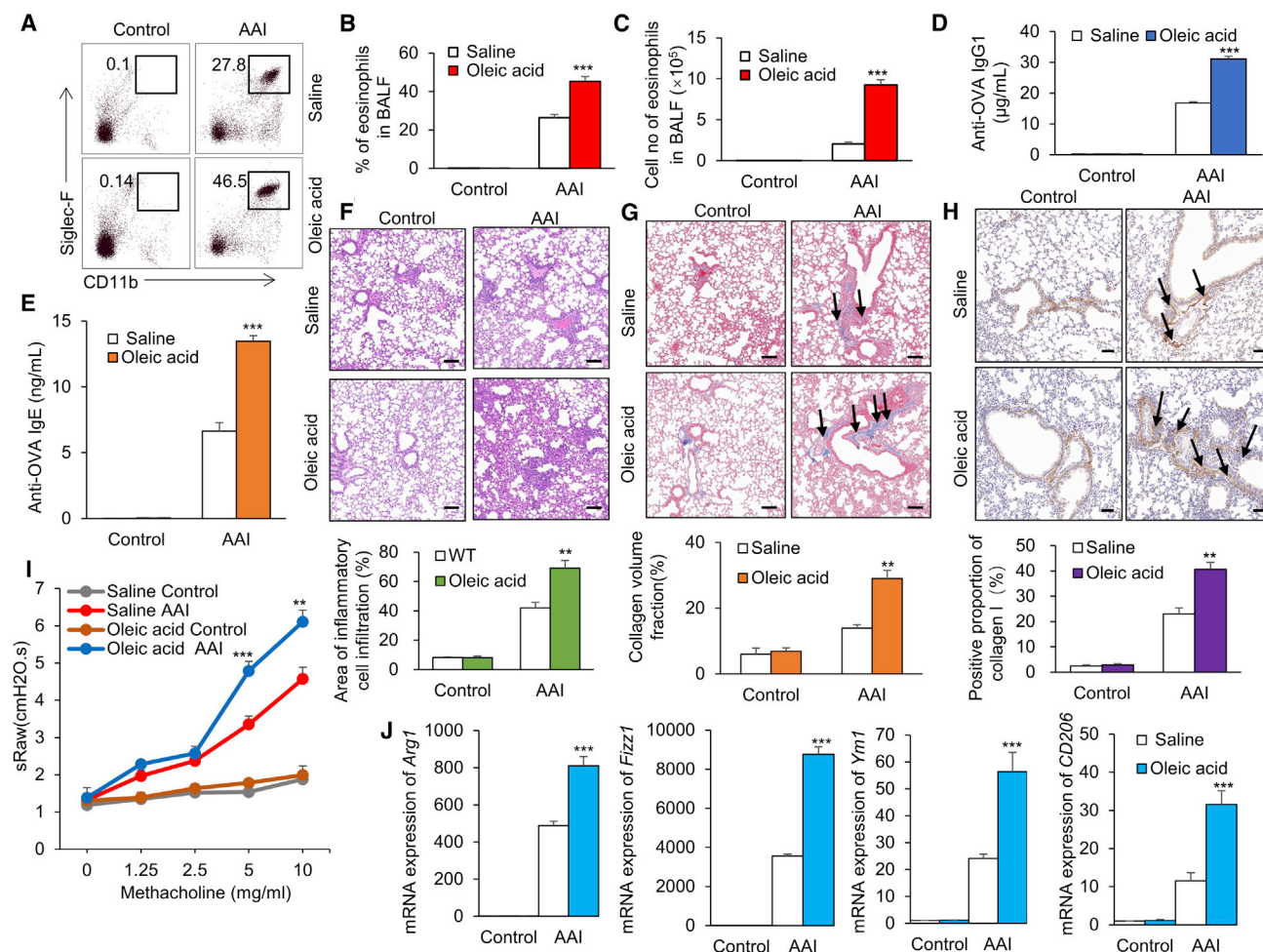


Figure 5. Excessive intake of oleic acid in WT mice enhances M2 polarization of alveolar macrophages and aggravates AAI in mice

(A–C) The percentage of Siglec-F⁺CD11b⁺ eosinophils as determined by flow cytometry (A), bar chart of eosinophil ratio statistics (B), bar chart showing eosinophil numbers (C) (n = 5).

(D and E) Levels of OVA-specific IgG1 (D) and IgE (E) in serum from the saline group and oleic acid group.

(F) H&E staining of lung tissue from saline- and oleic acid-treated mice (scale bar represents 100 μm).

(G) Masson staining of lung tissue from saline- and oleic acid-treated mice (scale bar represents 100 μm). Arrows indicate sites of pulmonary fibrosis.

(H) Collagen I staining of lung tissue from saline- and oleic acid-treated mice (scale bar represents 50 μm). Arrows represent positive staining.

(I) Baseline sRaw upon methacholine inhalation was measured in saline- and oleic acid-treated mice with or without AAI (n = 4).

(J) Quantitative PCR showing expression levels of *Arg1*, *Fizz1*, *Ym1*, and *CD206* in sorted CD45⁺CD11c⁺Siglec-F⁺ macrophages isolated from BALF of saline- and oleic acid-treated mice. Data are representative of three independent experiments and are presented as mean $\pm$ SD (n = 3). **p < 0.01 and ***p < 0.001, Student's t test.

intake of oleic acid could increase asthma risk, potentially with the involvement of M2 polarization, although the effects of excessive oleic acid intake on other immune cells and on macrophages in other tissues cannot be excluded at present.

Fabp5 deficiency enhances PPAR γ signaling during M2 polarization

After observing that *Fabp5* deficiency activates the PPAR γ pathway and promotes M2 polarization by regulating levels of oleic acid in macrophages, we performed RNA-seq on WT and *Fabp5*KO mouse macrophages. We identified the top 10 signaling pathways upregulated by M2 in WT cells through KEGG analysis and displayed them in a bubble chart; based

on gene ratio, we selected the three most obviously upregulated pathways: PPAR signaling pathway, chemokine signaling pathway and Jak-STAT signaling pathway. We then counted the gene ratios of pathways that significantly increased between *Fabp5*KO and WT M2 macrophages and found that the PPAR signaling pathway increased significantly in *Fabp5*KO M2 macrophages compared with WT M2 macrophages (Figure 6A). To investigate changes in the PPAR signaling pathway during WT and *Fabp5*KO M2 polarization, we performed differential gene enrichment for all genes in the PPAR γ signaling pathway. Network analysis of the PPAR signaling pathway showed that lipid metabolism-related genes, including *Scd1*, *Scd2*, *Fads2*, *Olr1*, *Scp2*, *Acadl*, and *Cpt1b*, exhibited significant upregulation

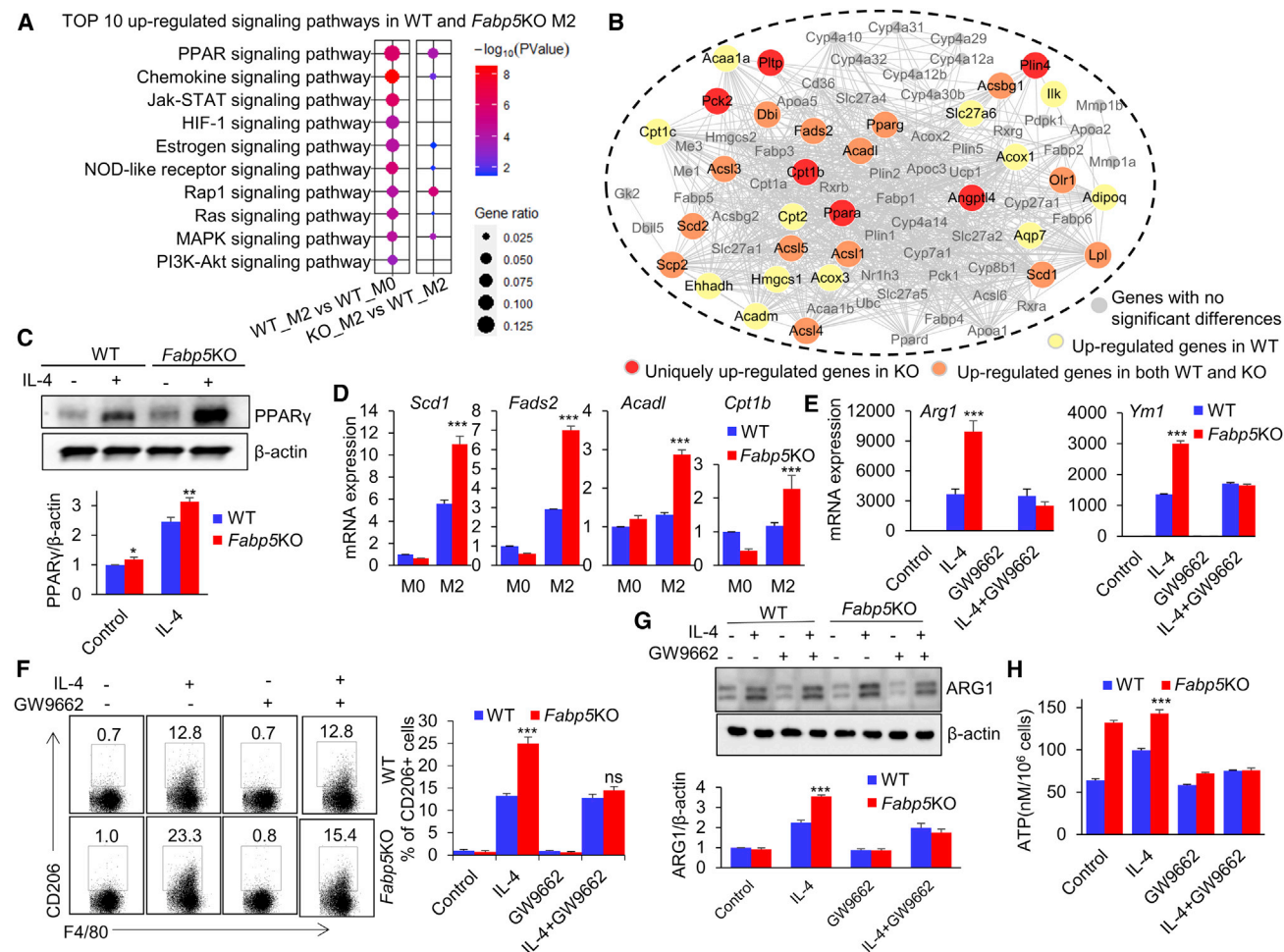


Figure 6. Deletion of *Fabp5* enhances M2 polarization by activating the PPAR γ signaling pathway

(A) Upregulated KEGG signaling pathways enriched for genes differentially expressed between WT and *Fabp5*KO BMDMs with (M2) or without (M0) IL-4 stimulation for 48 h. The top 10 pathways are shown.

(B) Network analysis of differential gene enrichment for all PPAR signaling pathway genes between WT and *Fabp5*KO BMDMs with (M2) or without (M0) IL-4 stimulation for 48 h. As shown in the figure, colored dots correspond to different meanings.

(C) Immunoblot analysis of PPAR γ protein in WT and *Fabp5*KO macrophages with or without IL-4 stimulation for 48 h.

(D) Quantitative PCR showing expression of PPAR γ pathway-related metabolic genes, including *Scd1*, *Fads2*, *Acadl*, and *Cpt1b*, between WT and *Fabp5*KO BMDMs with (M2) or without (M0) IL-4 stimulation for 48 h.

(E) Gene expression of *Arg1* and *Ym1* in WT and *Fabp5*KO macrophages pretreated with GW9662 (5 μ M) for 1 h, then stimulated with IL-4 for 48 h, as measured by quantitative real-time PCR.

(F) Flow cytometry analysis of CD206 expression in WT and *Fabp5*KO F4/80⁺CD11b⁺ macrophages pretreated with GW9662 (5 μ M) for 1 h and stimulated with IL-4 for 48 h.

(G) Immunoblot analysis of ARG1 protein in WT and *Fabp5*KO PEMs pretreated with GW9662 (5 μ M) for 1 h and stimulated with IL-4 for 48 h.

(H) ATP production levels in WT and *Fabp5*KO PEMs pretreated with GW9662 (5 μ M) for 1 h and stimulated with IL-4 for 48 h. Data are representative of three independent experiments and are presented as mean $\pm$ SD (n = 3). NS, no significant difference, *p < 0.05, **p < 0.01, and ***p < 0.001, Student's t test. See also Figures S5 and S6.

in *Fabp5*KO M2 cells (Figure 6B), suggesting that *Fabp5* deletion upregulates the PPAR γ signaling pathway. It is true that *Fabp5*KO macrophages displayed higher PPAR γ protein expression after IL-4 stimulation (Figure 6C). Expression of key genes downstream of the PPAR γ signaling pathway and of lipid metabolism-related genes, such as *Scd1*, *Fads2*, *Acadl*, and *Cpt1b*, were significantly higher in *Fabp5*KO macrophages after IL-4 treatment, as determined by real-time PCR (Figure 6D).

To determine whether the enhanced M2 polarization of *Fabp5*KO macrophages was mediated by the PPAR γ signaling pathway, we treated WT and *Fabp5*KO PEMs with GW9662, a specific PPAR γ antagonist,³⁵ and initiated IL-4-induced M2 polarization after selecting an appropriate dose of GW9662 (Figures S5A and S5B). GW9662 pretreatment fully rescued the enhanced expression of *Arg1*, *Ym1*, and CD206 expression caused by *Fabp5* deletion in IL-4-treated PEMs, such that there

was no detectable difference in the expression of these M2 marker genes between WT and *Fabp5*KO macrophages when cells were treated with a combination of IL-4 and GW9662, as determined by real-time PCR analysis (Figures 6E and S5C). Meanwhile, the increased number of CD206⁺ cells and elevated ARG1 protein expression associated with *Fabp5* deletion in M2 macrophages were not observed after the addition of GW9662 (Figures 6F–6G). In addition, no significant differences between WT and *Fabp5*KO macrophages were observed for key genes downstream of the PPAR γ signaling pathway and for lipid metabolism-related genes after PPAR γ inhibitor treatment (Figure S5D). Consistent with enhanced PPAR γ signaling and the role of lipid metabolism in ATP production,³⁶ more ATP was produced by *Fabp5*KO macrophages, regardless of whether cells were treated with IL-4 (Figure 6H). The presence of GW9662 remarkably reversed the enhanced ATP production in *Fabp5*KO macrophages, restoring them to normal level as seen in WT macrophages (Figure 6H). Furthermore, we also used small interfering RNA (siRNA) 1 and siRNA 2 to silence PPAR γ , significantly reducing PPAR γ expression in both IL-4-induced WT and *Fabp5*KO macrophages as determined by western blot (Figures S6A and S6B). Treatment with siRNA 1 or siRNA 2 reduced CD206⁺ expression in IL-4-induced *Fabp5*KO macrophages to the same levels as in IL-4-induced WT macrophages, as assayed by flow cytometry (Figures S6C and S6D). Thus, these results collectively indicate that *Fabp5* deficiency enhances the PPAR γ signaling pathway, increases ATP production, and subsequently promotes M2 polarization in macrophages.

Absence of *Fabp5* reprograms the metabolic profile to enhance M2 polarization

PPAR γ acts as a key regulator of lipid metabolism and regulates a series of metabolic processes, such as FA β oxidation, the tricarboxylic acid cycle (TCA), and oxidative phosphorylation, in macrophages.²⁷ Given the enhanced PPAR γ signaling pathway in *Fabp5*KO macrophages, we ascertained whether FABP5 reprograms macrophage metabolism using a deep informative analysis of these metabolism pathways, and we found that genes related to β oxidation, such as *Acadl*, *Echs1*, *Hadha*, and *Hadhb*,³⁷ were significantly upregulated in *Fabp5*KO M2 macrophages compared with WT M2 macrophages (Figure 7A). Meanwhile, genes related to the TCA cycle or to oxidative phosphorylation were also significantly upregulated in *Fabp5*KO M2 macrophages (Figures 7B and 7C). However, glycolysis-related genes did not show any significant difference (Figure S7). Consistent with RNA-seq results, real-time PCR analysis revealed that TCA- and oxidative phosphorylation-related genes, such as *Echs1*, *Hadha*, *Mdh2*, *Aco1*, and *Sdhc*, were significantly enhanced in *Fabp5*KO M2 macrophages (Figure 7D). Functionally, the oxygen consumption rate (OCR) of *Fabp5*KO M2 macrophages was significantly higher (Figures 7E and 7F). These data suggest that during macrophage M2 polarization, FABP5 mainly affects lipid and oxidative metabolism, not glucose metabolism.

Oligomycin is an inhibitor of oxidative phosphorylation energy transfer that is often used in experimental studies to inhibit ATP production.¹⁴ Oligomycin treatment significantly decreased ATP production in both WT and *Fabp5*KO macrophages. Meanwhile,

oligomycin greatly rescued the enhanced ATP production and the enhanced STAT6 phosphorylation observed in IL-4-treated *Fabp5*KO macrophages to more closely resemble WT macrophages (Figures 7G and 7H). Importantly, oligomycin fully rescued the enhanced M2 polarization of *Fabp5*KO macrophages, indicated by similar expression levels of *CD206*, *Arg1*, *Fizz1*, and *Ym1* in WT and *Fabp5*KO macrophages treated with both oligomycin and IL-4, as assayed by flow cytometry and real-time PCR (Figures 7I and 7J). These data imply that FABP5 predominately regulates FA β oxidation, TCA, and oxidative phosphorylation to control ATP production and M2 polarization.

DISCUSSION

Fabp5 is highly and selectively expressed in macrophages among immune cells and is the *Fabp* family member most highly expressed in macrophages. *Fabp5* deficiency significantly increases M2 polarization. *Lyzs-Fabp5*KO AAL mice exhibited increased infiltration of eosinophils in the lungs, more anti-OVA IgE in the sera, increased lung fibrosis, increased expressions of M2 marker molecules, and airway hyper-responsiveness to inhaled methacholine. Therefore, myeloid-specific deletion of *Fabp5* aggravates allergic asthma, likely by regulating macrophage M2 polarization, although our present results do not exclude the potential involvement of neutrophils and DCs in this process.

As lipid chaperones, FABPs actively facilitate the intracellular transport of lipids to specific cellular compartments and regulate cell metabolism.⁹ Lipidomic analysis showed that concentrations of long-chain unsaturated FAs, especially oleic acids, were significantly increased in *Fabp5*-deficient macrophages; however, levels of other FAs, including long-chain saturated FAs and medium-chain FAs, were identical in *Fabp5*-deficient and WT macrophages. These results indicate that FABP5 may selectively affect the transportation and metabolism of long-chain unsaturated FAs. It is true that the transcriptional expression of glycerolipid metabolism-related genes, glycerophospholipid metabolism-related genes, and sphingolipid metabolism-related genes were obviously lower in *Fabp5*KO macrophages compared with WT macrophages, as detected by RNA-seq and real-time PCR assays. The reduced lipid anabolism might be one reason for the specific accumulation of free long-chain unsaturated FAs observed in *Fabp5*KO macrophages. However, the detailed mechanisms underlying the selectivity of FABP5 toward free long-chain unsaturated FAs in macrophages remain to be clarified in future experiments.

After observing excessive accumulation of oleic acid in *Fabp5*-deficient macrophages, we studied the potential roles of oleic acid and linoleic acid in anti-inflammatory macrophage M2 polarization. The addition of oleic acid or linoleic acid significantly increased expression of M2 marker on both mRNA and protein levels in IL-4-treated WT macrophages in a dose-dependent manner, simultaneously accompanied by enhanced PPAR γ expression and p-STAT6 activity. However, the addition of oleic acid or linoleic acid failed to significantly increase expression of M2 marker in IL-4-treated *Fabp5*-deficient macrophages, even in the presence of high doses of oleic acid. These data indicate

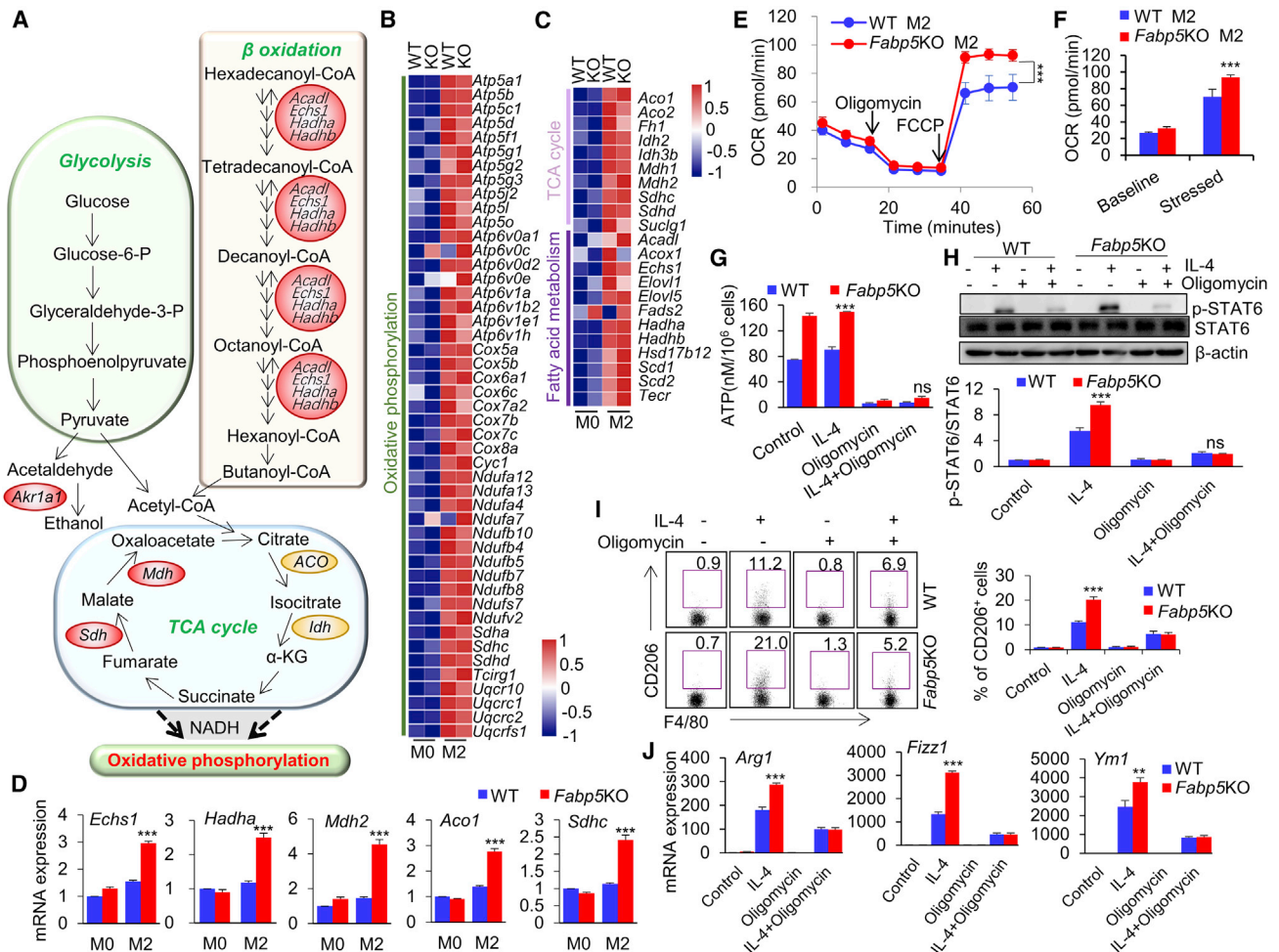


Figure 7. *Fabp5* deficiency reprograms macrophage metabolism to increase M2 polarization

(A) Diagram of changes to lipid and glucose metabolism between WT and *Fabp5* KO BMDMs with IL-4 (M2) stimulation for 48 h. Genes in the red circle represent genes upregulated in *Fabp5* KO cells ($p < 0.05$).

(B) Heatmap representing expression levels of related genes involved in oxidative phosphorylation between WT and *Fabp5* KO BMDMs with (M2) or without (M0) IL-4 stimulation for 48 h.

(C) Heatmap representing the expression levels of related genes involved in the TCA cycle and FA metabolism in WT and *Fabp5* KO BMDMs with (M2) or without (M0) IL-4 stimulation for 48 h.

(D) Quantitative PCR showing mRNA expression levels of factors involved in the TCA cycle and FA β oxidation (*Echs1*, *Hadha*, *Mdh2*, *Aco1*, and *Sdhc*) in WT and *Fabp5* KO BMDMs with (M2) or without (M0) IL-4 stimulation for 48 h.

(E and F) PEMs of WT and *Fabp5* KO mice were induced with IL-4 (M2), and OCR profiles were measured at baseline and after indicated injections (E). Basal oxygen consumption and maximal oxygen consumption bar charts are shown (F).

(G) ATP production levels in WT and *Fabp5* KO PEMs pretreated with oligomycin (1 μ M) for 1 h and stimulated with IL-4 for 48 h.

(H) Immunoblot analysis of phosphorylated and total STAT6 protein in WT and *Fabp5* KO PEMs pretreated with oligomycin (1 μ M) for 1 h and stimulated with IL-4 for 1 h.

(I) Flow cytometry analysis of CD206 expression in WT and *Fabp5* KO F4/80⁺CD11b⁺ PEMs pretreated with oligomycin (1 μ M) for 1 h and stimulated with IL-4 for 48 h.

(J) Quantitative PCR showing the expression of *Arg1*, *Fizz1*, and *Ym1* in WT and *Fabp5* KO PEMs pretreated with oligomycin (1 μ M) for 1 h and stimulated with IL-4 for 48 h. Data are representative of three independent experiments and are presented as mean $\pm$ SD ($n = 3$). NS, no significant difference, ** $p < 0.01$ and *** $p < 0.001$, Student's t test. See also Figure S7.

that the promoting effects of oleic acid and linoleic acid on M2 polarization are either dependent on the presence of FABP5 or require the high intracellular levels of oleic acid or linoleic acid associated with *Fabp5*-deficient macrophages to promote M2 polarization, which needs to be clarified in the future. Importantly, excessive intake of oleic acid by gavage generated signif-

icantly higher numbers of eosinophils in BALF, higher expression of M2-related genes in alveolar macrophages, increased lung fibrosis, higher OVA-specific IgE levels in sera, and enhanced airway hyper-responsiveness to inhaled methacholine in AAI mice. These results show that excessive dietary oleic acid intake can aggravate allergic asthma in mice. Whether excessive intake

of oleic acid solely or primarily acts on lung macrophages is unclear and needs to be clarified. Oleic acid is a monounsaturated, omega-9 long-chain FA found in most animal and vegetable sources and is an essential nutrient for humans.^{38–40} Therefore, it will be interesting to investigate the relationship between excess oleic acid and asthma in humans, as well as to determine whether FABP5 is involved in this relationship.

Our results show that *Fabp5* deficiency increases long-chain unsaturated FA accumulation while simultaneously activates the PPAR γ signaling pathway in IL-4-stimulated macrophages. This observation is consistent with a previous report showing that *Fabp5* deficiency increases PPAR γ expression to promote CD36 expression in macrophages.⁴¹ *Fabp5* deficiency in macrophages decreased inflammatory factors, such as lipopolysaccharide-induced COX2 and IL-6 expression, and suppressed early atherosclerosis in mice.⁴¹ It seems that FABP5 controls the M1/M2 balance by enhancing M1 polarization and inhibiting M2 polarization, although this will require future confirmation. On the other hand, FABP5 may interact with PPAR γ via a coordinated mechanism to facilitate malignant progression in prostatic cancer.⁴² PPAR γ is activated by ligands, which mainly include natural ligands and synthetic ligands. Long-chain unsaturated FAs, such as oleic acid, linoleic acid, arachidonic acid, and some arachidonic acid derivatives, are natural ligands of PPAR γ .^{32,43} Greater levels of long-chain unsaturated FAs, including oleic acid, caused by *Fabp5* deficiency may interact directly with PPAR γ to activate the PPAR γ signaling pathway on a high level. Thus, *Fabp5* deficiency is likely to activate the PPAR γ pathway directly by regulating PPAR γ expression and indirectly by increasing PPAR γ expression through increased levels of intracellular long-chain unsaturated FAs. PPAR γ expression is important for the full expression of genes characteristic of M2 macrophages.^{44,45} It has been reported that *Fabp5* deficiency in human macrophages increases proinflammatory response and decreases IL-10 expression after LPS and IFN- γ stimulation, in addition to decreasing IL-10 and IL-33 expression after IL-4 stimulation, FABP5 and PPAR γ interact with one another and promote each other's transcriptional expression in unstimulated macrophages and in cell lines.⁴⁶ However, we observed that *Fabp5* deficiency increases PPAR γ protein expression in IL-4-stimulated mouse macrophages. The reasons for this discrepancy are unclear, but may be related to differences in macrophage polarization (M0, M1, or M2), cell lines, animal models, and genetic modification (knockout versus short hairpin RNA) used by different laboratories; the effects of these differences need to be studied in the future. In addition, PPAR γ agonists have shown beneficial effects on airway inflammation and hyper-responsiveness to asthma.⁴⁷ Inhaled PPAR γ agonist analogues are under development for the treatment of chronic inflammatory airway diseases and were suggested as possibly beneficial in cases of non-eosinophilic asthma in 2009.⁴⁸ The different roles of the PPAR γ pathways in allergic asthma may be due to the different types of inflammatory airway diseases studied and the different approaches used; we studied myeloid-specific *Fabp5* deleted mice in which PPAR γ expression was selectively upregulated in macrophages or myeloid cells, while others used PPAR γ agonists to activate PPAR γ in all cells.

In summary, *Fabp5* deficiency in myeloid cells exacerbates OVA-induced AAI in mice and increases macrophage M2 polarization. Interestingly, FABP5 selectively regulates the accumulation of long-chain unsaturated FAs, such as oleic acid, in macrophages. Unexpectedly, excessive uptake of oleic acid leads to increased M2 polarization in BALF and significantly aggravates AAI pathogenesis, suggesting that high dietary levels of oleic acid may exacerbate allergic asthma. Furthermore, *Fabp5* deficiency activates PPAR γ , causing a series of metabolic reactions that include FA β oxidation, the TCA cycle, and oxidative phosphorylation, producing more ATP, and enhancing STAT6 activation to promote M2 polarization and M2-related allergic asthma.

Limitations of the study

These studies were conducted mainly in mice. While it remains to be determined whether similar effects will be observed in humans, *FABP5* is highly expressed in human macrophages. It is of great clinical significance to explore the effects of FABP5 on human asthma samples. In addition, our results show that *Fabp5*-deficient macrophages accumulate more long-chain unsaturated FAs and exhibit reduced lipid anabolism. It is worthwhile to study the effects of FABP5 on FA intake and intracellular transportation in macrophages via FA labeling. Whether the FA binding region of the FABP5 protein contains a unique domain compared to other FABP family proteins also needs to be clarified to help understand the ability of FABP5 to selectively bind long-chain unsaturated FAs.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.celrep.2022.111668>.

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AUTHOR CONTRIBUTIONS

Y.H. and Y.Z. conceived the project and designed the experiments. Y.H., D.W., Z.Z., S.L., J.Z., and H.G. carried out the experiments. Y.H. and D.W. carried out most experiments, analyzed data, and wrote the manuscript. Z.Z. analyzed RNA-seq data. H.G. performed studies with human peripheral blood mononuclear cells. S.L. and J.Z. assisted with flow cytometry and the allergic airway inflammation mouse model. P.Z. bred the mice and genotyped genetically modified mice. L.Z. produced the *Fabp5* KO mice and *Fabp5*-flox mice and revised the manuscript. Y.Z. oversaw the project, designed experiments, and carefully revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti- β -actin	Sigma	Cat#A5441; RRID:AB_476744
Anti-Arginase	Santa Cruz	Cat#sc-20150; RRID:AB_2058955
Anti-FABP5	Cell Signaling Technology	Cat#39926S; RRID:AB_2799165
Anti-p-STAT6	Cell Signaling Technology	Cat# 9361; RRID:AB_331595
Anti-STAT6	Cell Signaling Technology	Cat# 5397S; RRID:AB_11220421
Anti-PPAR γ	Cell Signaling Technology	Cat# 2435; RRID:AB_2166051
FITC anti-mouse CD206 Antibody	BioLegend	Cat# 141703; RRID:AB_10900988
PE anti-mouse F4/80 Antibody	BioLegend	Cat# 123110; RRID:AB_893486
PE/Cy5 anti-mouse CD11b Antibody	Thermo Fisher	Cat# 15-0112-83; RRID:AB_468715
PE anti-mouse CD124 Antibody	BD Biosciences PharMingen	Cat# 552509; RRID:AB_394407
BV510 anti-mouse CD11b Antibody	BioLegend	Cat#101245; RRID:AB_2561390
FITC anti-mouse Ly6G Antibody	BioLegend	Cat#127606; RRID:AB_1236494
APC anti-mouse F4/80 Antibody	Thermo Fisher	Cat# 17-4801-82; RRID:AB_2784648
PE/Cy5 anti-mouse CD11c Antibody	BioLegend	Cat#117316; RRID:AB_493566
BV395 anti-mouse CD45 Antibody	BD Biosciences PharMingen	Cat# 564279; RRID:AB_2651134
PE anti-mouse CD8a Antibody	Thermo Fisher	Cat# 12-0081-82; RRID:AB_465530
APC anti-mouse CD4 Antibody	BioLegend	Cat# 100412; RRID:AB_312697
PE anti-mouse IL-4 Antibody	BioLegend	Cat# 504104; RRID:AB_315318
PE anti-mouse Siglec-F Antibody	BD Biosciences PharMingen	Cat# 552126; RRID:AB_394341
FITC anti-human CD14 Antibody	BioLegend	Cat# 367116; RRID:AB_2571929
BV510 anti-mouse/human CD11b Antibody	BioLegend	Cat# 101263; RRID:AB_2629529
PE/Cy7 anti-human CD163 Antibody	BioLegend	Cat# 333614; RRID:AB_2562641
APC anti-human CD209 (DC-SIGN) Antibody	BioLegend	Cat# 330108; RRID:AB_1134045
Chemicals, peptides, and recombinant proteins		
Recombinant murine IL4	R&D Systems	Cat# 404-ML
Recombinant murine IL13	Pepro Tech	Cat# 210-13
Ultra-LEAF TM Purified anti-mouse CD28 Antibody	BioLegend	Cat# 102116
Ultra-LEAF TM Purified anti-mouse CD3 ϵ Antibody	BioLegend	Cat# 100340
Recombinant Murine M-CSF	Pepro Tech	Cat# 315-02
Recombinant Human M-CSF	R&D Systems	Cat# 216-MC-025
Ovalbumin	Sigma	Cat# A5253
Aluminum hydroxide	Sigma	Cat# 239186
Thioglycollate	BD BBL	Cat# 211716
GW9662	MedChem Express	Cat# HY-16578
Methacholine Chloride	9 Ding Chemistry	Cat# VZ179
Oleic acid	Sigma	Cat# O1383
Linoleic acid	Sigma	Cat# L1012
Oligomycin	Sigma	Cat# 495455
Chitin	Sigma	Cat# C7170
Trizol	Thermo Fisher	Cat# 15596018
Oligo dT18	Thermo Fisher	Cat# 18418020

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Recombinant RNase Inhibitor	TaKaRa	Cat# 2313A
dNTP Mixture	TaKaRa	Cat# 4030
SYBR Green Mixture	TaKaRa	Cat# RR420A
AMV Reverse transcriptase	TaKaRa	Cat# 2621
FICOLL PAQUE PREMIUM	Cytiva	Cat# 17544202
Critical commercial assays		
Mouse-IgE ELISA Kit	Biolegend	Cat# 439807
Mouse-IgG1 ELISA Kit	Cayman	Cat# 500830
Lipofectamine 3000 Transfection Kit	Thermo Fisher	Cat# L3000008
BCA Protein Assay Kit	Thermo Fisher	Cat# 23225
EasySep™ Human Monocyte Isolation Kit	STEMCELL	Cat# 19359
Deposited data		
Bulk RNA-seq data	This paper	NCBI: PRJNA786866
Experimental models: Organisms/strains		
Mouse: WT C57BL/6J	This paper	N/A
Mouse: FABP5 flox/flox	This paper	N/A
Mouse: FABP5 KO	This paper	N/A
Mouse: Lyzs-cre	This paper	N/A
Oligonucleotides		
For genotyping primer sequence, please see Table S3	This paper	N/A
Software and algorithms		
GraphPad Prism 7	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
FlowJo	FlowJo	https://www.flowjo.com/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yong Zhao (zhaoy@ioz.ac.cn).

Materials availability

No reagents were generated in the study.

Data and code availability

The data supporting the findings of this study are available within the article and its supplemental information. The RNA sequencing datasets generated in this study can be found in the NCBI SRA database (<http://www.ncbi.nlm.nih.gov/sra/>) using the access number PRJNA786866 (SAMN23720062, SAMN23720060, SAMN23720063, SAMN23720065).

This study did not generate original code.

Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

C57BL/6 (B6) mice were purchased from Beijing University Experimental Animal Center (Beijing, China) or Sipeifu Biotechnology Co. (Beijing, China). *Fabp5*KO mice and *Fabp5* flox/flox and *LysMCre* mice were obtained from Dr. Lianfeng Zhang (Key Laboratory of Human Diseases Comparative Medicine, Ministry of Health, Institute of Laboratory Animal Science, CAMS & PUMC) and allowed to adapt to housing in the animal facility of the Institute of Zoology for one week prior to random breeding cage assignment. To obtain myeloid-specific *Fabp5* knockout mice, *Fabp5* flox/flox mice were bred with transgenic mice harboring Cre-recombinase driven by a

myeloid-specific lysozyme M promoter to generate the following genotypes: *Fabp5* flox/flox (control) and *LysM^{Cre}-Fabp5* (*Lyzs-Fabp5KO*). They were genotyped at 2–3 weeks of age using the supplier's suggested primer set (see Table S3). Sex-matched WT and *Fabp5KO* mice 6–8 weeks of age were used primarily for *in vitro* experiments. In *in vivo* experiments using *Lyzs-Fabp5KO* mice, *Fabp5* flox/flox littermates were used as controls. Mice were fed and bred under a specific pathogen-free status and given free access to water and food in the animal facility at the Institute of Zoology, Chinese Academy of Science. All experiments were operated with the consent of the Animal Ethics Committee of the Institute of Zoology.

Mouse macrophage preparation

Bone marrow-derived macrophages (BMDMs) were harvested and cultured as previously described.⁴⁹ After intraperitoneal injection of 3% thioglycolate (TG) for 5 days, primary peritoneal macrophages were obtained from the peritoneal exudates of mice. Peritoneal exudate cells were washed twice with PBS solution, adjusted to a concentration of 1×10^6 cells/mL in DMEM, and cultured for 2 h at 37°C and 5% CO₂ in a cell incubator.²² Cells in the supernatant were washed away, and F4/80⁺CD11b⁺ cells accounted for more than 90% of adherent cells as assessed by flow cytometry staining, indicating that the purity of macrophages among adherent cells was above 90%.

Human macrophage preparation

Buffy coats were collected from healthy donors at the Chinese People's Liberation Army General Hospital as approved by the hospital's ethics committee. Monocytes were isolated using an immunomagnetic negative selection kit (Stemcell Technologies, Vancouver, Canada), followed by Ficoll density gradient centrifugation with Ficoll Paque Premium (GE Healthcare, Chicago, USA). Monocyte purity (CD14⁺ cells) was above 90%, as assessed by flow cytometry. Human monocyte-derived macrophages were obtained by culturing human CD14⁺ monocytes for six days in RPMI-Glutamax medium (GIBCO) supplemented with antibiotics (penicillin and streptomycin) and 5% fetal calf serum in the presence of 50 ng/mL M-CSF (R&D Biotechne).

Polarization of CD4⁺ T cells by macrophages *in vitro*

Naive CD4⁺ T cells (CD4⁺CD44[−]CD62L⁺) were sorted from the spleens of WT mice using the MoFlo XDP (Beckman Coulter, Fullerton, Calif). Naive CD4⁺ T cells were cultured in RPMI 1640 supplemented with 10% FCS (Gibco, Carlsbad, Calif), 100 U/mL penicillin and 100 mg/mL streptomycin. Naive CD4⁺ T cells were activated with plate-bound anti-CD3 (2 mg/mL; 145-2C11; BD Biosciences, San Jose, Calif) and soluble anti-CD28 (2 mg/mL; 37.51; BD Biosciences). For T cell polarization, cells were cultured with M0 or M2 macrophages from WT and *Fabp5KO* mice. After three days of differentiation, IL-4 expression was assessed using FACS intracellular staining.

METHOD DETAILS

Flow cytometry

BMDMs or peritoneal macrophages (PEMs) were treated with IL-4 (5.7 ng/mL) and IL-13 (10 ng/mL) to induce M2 polarization. M2 macrophages were harvested after 48 h. GW9662 (5 μM) or oligomycin (1 μM) were used to pretreat BMDMs or PEMs for 1 h before IL-4 administration. Different doses of oleic acid (0.1, 1, 10, 100, and 500 μM) were used to pretreat PEMs for 24 h before the administration of IL-4. Bronchoalveolar lavage fluid (BALF) was collected according to Haque et al.'s method.⁵⁰ After treatment, BMDMs, PEMs, or BALF cells were collected using cold PBS.⁵¹ For surface staining, BMDMs and PEMs were stained with anti-mouse CD11b, anti-mouse F4/80, anti-mouse CD206, and anti-mouse CD124 for 30 min at 4°C. Chitin-recruited PECs were stained with anti-mouse CD11b, anti-mouse F4/80, and anti-mouse Siglec-F for 30 min at 4°C. After staining and two washes, cells were immediately analyzed using flow cytometry. BALF cells were stained with anti-mouse CD11c, anti-mouse CD45, and anti-mouse Siglec-F for 30 min at 4°C, and alveolar macrophages (CD45⁺CD11c⁺SiglecF⁺) were sorted by FACSaria II.

Human monocyte-derived macrophages were treated with recombinant human IL-4 (10 ng/mL) to induce M2 polarization. Different doses of oleic acid (0.1, 1, 10, and 100 μM) were pre-added to the macrophage culture system for 24 h before IL-4 treatment. Next, cells were collected and stained with anti-human CD11b, anti-human CD14, anti-human CD209, or anti-human CD163 for 30 min at 4°C. After two washes, cells were analyzed using flow cytometry.

Western blotting

PEMs or BMDMs were cultured in DMEM medium containing 10% fetal bovine serum. After stimulation, cells were lysed in RIPA buffer in the presence of protease and phosphatase inhibitor cocktails (Roche). Protein concentrations were detected using a BCA Protein Assay Kit. Lysates were mixed with 5× SDS-PAGE loading buffer and boiled at 100°C for 5 min. Protein samples were subjected to 10% SDS-PAGE and transferred to PVDF membranes (Millipore, CA). Membranes were blocked for 1 h in TBS containing 5% nonfat dry milk, then probed overnight at 4°C with primary antibodies specific for FABP5, β-actin, ARG1, STAT6, p-STAT6 (Tyr641), or PPARγ. Membranes were washed and incubated for 1 h at room temperature with peroxidase-conjugated secondary antibodies, then detected via chemiluminescence (Millipore). Image J software was used to detect protein signal levels.

Real-time PCR

RNA was extracted using a total RNA Kit or Trizol, and reverse transcription was performed with AMV reverse transcriptase according to the manufacturer's instructions. Quantitative real-time PCR was performed in 96-well plates using Power SYBR® PCR Master Mix (Applied Biosystems) and a two-step PCR program (95°C for 5 s, 60°C for 60 s, 40 cycles). The cDNA melt curves were single-peaked. Plates were read using a C (Bio-Rad). Real-time PCR data were analyzed using the comparative CT method and normalized to the housekeeping gene HPRT. Primer sequences are shown in [Table S3](#).

SiRNA transfection

Using the Lipofectamine 3000 Transfection Kit (Catalog #L3000008, Thermo Fisher Scientific, USA) kit and associated transfection protocol, BMDMs were transfected with 20 nM control siRNA or siRNA against PPAR γ . siRNA 1 corresponds to siPPAR γ 1, siRNA 2 corresponds to siPPAR γ 2, and siRNA negative corresponds to a meaningless RNA sequence. All siRNAs were purchased from Tsingke Biotechnology. Primer sequences are shown in [Table S3](#). After 6 h of incubation at 37°C with 5% CO $_2$, the culture medium was replaced with fresh DMEM. Cells were stimulated with IL-4 (5.7 ng/mL) for 48 h, then harvested for RT-PCR, western blotting, and flow cytometry described above.

ATP measurements

Cellular ATP levels were measured using an ATP Assay Kit according to the manufacturer's instructions. First, PEMs were obtained from the peritoneal exudates of mice after five days of treatment with 3% TG. PEMs were pretreated with oligomycin or GW9662, then induced with IL-4. We ensured that the number of cells in each tested sample was 1×10^6 . After treatment, a luminometer was used for analysis.

Seahorse respirometry

Real-time changes in the OCR of BMDMs were analyzed with a Seahorse XFp Analyzer (Agilent Technologies) as described by Zhang.⁴⁹ Briefly, TG-induced PEMs from WT or *Fabp5*KO mice were seeded in XF24 cell culture microplates (Agilent Technologies; 40K per well) for 48 hours with IL-4 stimulation before the experiments. Subsequently, the cells were switched to Seahorse media. The OCRs were measured three times at baseline, and kinetics were recorded for the indicated periods after injection with inhibitors (Oligomycin, FCCP). Oligomycin (1 μ M) was used to inhibit ATP synthase, and carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP; 2 μ M) was used to fully uncouple respiration.

FA metabolite analysis

BMDMs were cultured and harvested from M-CSF-induced bone marrow cells from WT and *Fabp5*KO mice for seven days. BMDMs were induced with or without IL-4 stimulation for 48 h. After stimulation, macrophage samples were collected and immediately frozen in liquid nitrogen. Each sample contained 1×10^7 macrophages. FAs were detected by Novogene technology company limited using GC-MS (Agilent 6890N/5975B; Santa Clara, CA, USA). Chromatographic conditions were as follows: Thermo TG-FAME capillary column (50 m $\times$ 0.25 mm ID $\times$ 0.20 μ m), injection volume of 1 μ L, split ratio of 8:1, inlet temperature of 250°C, ion source temperature of 230°C, transmission line temperature of 250°C, and quadrupole temperature of 150°C. The initial temperature of the program was 80°C, which was maintained for 1 min. The temperature was increased to 160°C at 20°C/min and maintained for 1.5 min, then raised to 196°C at 3°C/min and maintained for 8.5 min. Finally, the temperature was increased to 250°C at 20°C/min and maintained for 3 min. The carrier gas was helium with a flow rate of 0.63 mL/min. MS conditions were as follows: electron bombardment ionization (EI) source, SIM scanning mode, electron energy 70 eV. A total of 51 fatty acids were detected according to the total ion flow chromatogram (TIC) chart. The internal standard (methyl salicylate) was clearly separated from the other fatty acid standards, indicating that the method is optimal. The relative standard deviations (RSDs) of all QCS were calculated by the peak area ratio of the standard to the internal standard, and the stability of the method was evaluated. The results showed that the RSDs of all substances were below 15%, indicating that the method is stable, reliable, and can be applied to the detection of samples. All samples were quantitatively analyzed according to established sample pretreatment and instrumental analysis methods. Fatty acids were quantified in μ g/sample.

Arginase assay

Cells were lysed in 0.1% Triton X-100. Lysates were supplemented with Tris-HCl (12.5 mM) and MnCl $_2$ (1 mM) and heated for 10 min at 56°C to activate the arginase. L-arginine substrate (250 mM) was added to the system, incubated at 37°C for 30 min, and stopped by the addition of H $_2$ SO $_4$ /H $_3$ PO $_4$. After addition of α -isonitrosopropiophenone and heating for 30 min at 95°C, urea production was measured using absorbance at 540 nm.²³ Data were normalized to total protein content.

ELISA

Mouse OVA-specific IgE and IgG1 were detected from sera using quantitative ELISA kits for mouse anti-OVA IgE (Chondrex) and mouse anti-OVA IgG1 (Chondrex).²⁵ All kits were used according to the manufacturers' instructions. The specific detection range of the mouse anti-OVA IgE kit was 20.7 pg/mL to 50 ng/mL, whereas the specific detection range of the mouse anti-OVA IgG1 kit was 1.56 to 200 ng/mL.

Chitin administration

Chitin (Sigma) was sonicated for 30 min with a UR-20P device (Tomy). After filtration and dilution in PBS, 800 ng of chitin was intraperitoneally injected into each mouse.²³ Peritoneal cells were collected 2 days after administration. Cells were analyzed by flow cytometry and real-time PCR as described above.

OVA-induced AAI mouse model

OVA-induced acute allergic inflammation was elicited, as previously described, via i.p. sensitization with 10 μ g OVA mixed with 1 mg alum on Days 1 and 14.^{22,23} From Day 21, mice were treated by nebulizer-delivered airway challenges with 1% OVA for three consecutive days. Mice were then assessed 24 h after the last OVA challenge. Thereafter, the mice's lungs were lavaged three times *in* with 1.5 mL cold PBS to collect cells from the bronchoalveolar region. Lung tissues were fixed with formaldehyde and embedded in paraffin. Thin sections of the embedded tissues were stained with hematoxylin and eosin (H&E) stain, Masson trichrome stain and collagen I stain. Image J software was used to quantitate histological images.

Intragastric administration

Mice were given oleic acid (0.6 g/kg) by intragastric administration twice weekly for three weeks before the OVA challenge. The control group was given the same amount of saline by intragastric administration. Similarly, all mice were put into an OVA-induced AAI mouse model using the induction method described above. The OVA-induced AAI model was applied to saline and oleic acid-treated mice.

Evaluation of airway hyper-responsiveness to methacholine

Mice airway hyper-responsiveness was evaluated based on airway response to methacholine. Methacholine aerosols were generated and delivered into the chambers using the Buxco Research Systems (DSI, USA), according to the Buxco NAM guide. Methacholine solutions were prepared in concentrations of 0, 1, 2.5, 5, and 10 mg/mL. After the mice had inhaled saline solution and each methacholine aerosol for 30 s, sRaw was measured after 2 min of reaction. Airway responsiveness to methacholine was detected as increased sRaw levels at the same concentration methacholine gradient.

RNA-seq assays

Downloaded data were collected from the NCBI GEO database as summarized in Table S1. In addition, human lung single-cell RNA data were analyzed from the Human Protein Atlas website, as available through the following link: <https://www.proteinatlas.org/ENSG00000164687-FABP5/single+cell+type/lung>.

BMDMs were cultured and obtained as previously described. Furthermore, BMDMs in WT and *Fabp5*KO mice were induced with (M2) or without (M0) IL-4 stimulation for 48 h. Each sample contained 1×10^6 macrophages. Total RNA was extracted using Trizol (Thermo Fisher Scientific, 15596018), according to the manufacturer's instructions. First, magnetic Oligo (dT) beads were used to enrich for eukaryotic mRNA. Then, fragmentation buffer was added to break mRNAs into short fragments. The mRNA fragments were used as a template to synthesize single-stranded cDNA using random hexamer primers. Buffer, dNTPs, DNA polymerase I and RNase H were added to synthesize double-stranded cDNA, which was then purified using AMPure XP. AMPure XP beads were used for fragment size selection. Finally, we performed PCR amplification and used AMPure XP beads to purify the PCR products and obtain the final library. After library quantification, libraries were pooled to match to the effective concentration and target data volume requirements of the flow cell. After cBOT clustering, the Illumina NovaSeq 6,000 high-throughput sequencing platform was used for sequencing. From the raw data, low-quality reads ($Q < 20$) were identified using FastQC, and adaptor sequences were filtered by Trimgalore. Then, the mapping software HISAT2⁵² was used to map reads to the mm10 mouse reference genome, and StringTie⁵² was used to construct transcript models independently for each cell. We utilized DESeq to identify differentially expressed genes between WT M0, *Fabp5*KO M0, WT M2, and *Fabp5*KO M2 cells. A p -value < 0.05 and $|\log_2(\text{foldchange})| > 0$ were selected as significant difference thresholds.

GO and KEGG pathway enrichment analysis

Gene ontology (GO) functional annotation and KEGG pathway analysis were performed on differential genes using the DAVID Bioinformatics Resources 6.8 online search tool (<https://david.ncifcrf.gov/>)⁵³ and the KOBAS online search tool (<http://kobas.cbi.pku.edu.cn/>).^{54,55}

Network analysis

The protein interaction network was obtained using STRING (<https://string-db.org/>), Network Analyst (<https://www.networkanalyst.ca/NetworkAnalyst/home.xhtml>)^{56,57} was used for enrichment analysis, and Cytoscape was used for visualization.⁵⁸

QUANTIFICATION AND STATISTICAL ANALYSIS

The results are presented as means $\pm$ SD. An unpaired student's t -test was used to test differences between the two groups, as based on the assessment of data variance. All data were analyzed using GraphPad Prism software (version 7). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Statistical values can be found in the figure legends.