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Original Article

Airway epithelial STAT3 inhibits allergic inflammation via upregulation of stearoyl-CoA desaturase 1

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ARTICLE INFO

Article history:

Received 21 February 2022

Received in revised form

19 March 2022

Accepted 2 April 2022

Available online xxx

Keywords:

Airway inflammation

Asthma

Lipid metabolism

Signal transducer and activator of

transcription 3

Stearoyl-CoA desaturase

Abbreviations:

AEC, airway epithelial cell; ATII, alveolar epithelial type II; BALF, bronchoalveolar lavage fluid; DEG, differentially expressed gene; DMSO, dimethyl sulfoxide; DOX, doxycycline; HDM, house dust mite; HE, hematoxylin–eosin; i.t., intratracheally; LXA4, Lipoxin A4; PAS, periodic acid–Schiff; qPCR, quantitative real-time PCR; RNA-seq, RNA-sequencing; SCD1, stearoyl-CoA desaturase 1; STAT3, signal transducer and activator of transcription 3; WT, wild-type

ABSTRACT

Background: Airway epithelial cells (AECs) play a crucial role in the induction and development of allergic inflammation through the development and activation of immune cells, including Th2 cells and ILC2s. Recent studies have revealed that STAT3 expressed in epithelial cells protects against pathogens and maintains homeostasis in the intestine. However, the roles of STAT3 in airway epithelium are poorly understood. Therefore, we sought to elucidate the roles of airway epithelial STAT3 in allergic airway inflammation.

Methods: Allergic airway inflammation was induced by intratracheal administration of house dust mite (HDM) extract in doxycycline-induced AEC-specific STAT3-deficient (STAT3-cKO) mice and their genetic control (STAT3-WT) mice. Airway inflammation was evaluated by flow cytometric analysis of bronchoalveolar lavage fluid cells and histological analysis of the lung. Purified airway epithelial cells were analyzed by quantitative PCR and RNA-sequencing (RNA-seq).

Results: HDM-induced airway inflammation was exacerbated in STAT3-cKO mice compared with STAT3-WT mice. RNA-seq analyses revealed that *Scd1*, coding stearoyl-CoA desaturase 1, was most significantly upregulated in HDM-treated STAT3-WT mice compared to HDM-treated STAT3-cKO mice. Notably, the administration of an SCD1 inhibitor exacerbated HDM-induced airway inflammation. AECs of HDM-treated STAT3-cKO mice and those of HDM-treated SCD1 inhibitor-injected mice shared 45 differentially expressed genes (DEGs). Gene enrichment analysis of the DEGs revealed that the enriched ontology clusters included fatty acid biosynthetic process and regulation of lipid biosynthetic process, suggesting the involvement of the STAT3-SCD1-lipid metabolism axis in suppressing allergic inflammation.

Conclusions: STAT3 is crucial for suppressing HDM-induced allergic airway inflammation, possibly inducing SCD1 expression in AECs.

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Introduction

Asthma is one of the most common chronic lung diseases and is characterized by eosinophilic airway inflammation, mucus hyperproduction, and airway hyperresponsiveness, which are caused by Th2 cells and ILC2s.^{1,2}

Airway epithelial cells (AECs) not only function as barriers to the outside world but also produce cytokines and chemokines in response to pathogens, allergens, and air pollutants.^{3,4} House dust mite (HDM) extract, a major allergen for asthma, is also known to induce the secretion of IL-33, IL-25, and TSLP from AECs through activation of Toll-like receptors and protease-activated receptors.^{3,4}

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Peer review under responsibility of Japanese Society of Allergy.

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<https://doi.org/10.1016/j.alit.2022.05.002>

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Consequently, AECs play a crucial role in the induction and development of allergic inflammation through the activation of Th2 cells and ILC2s.⁴

STAT3 (signal transducer and activator of transcription 3) is a transcription factor activated by various cytokines such as IFNs, IL-2, IL-6, IL-10, IL-22, and epidermal growth factor and modulates both innate and adaptive immune responses.⁵ STAT3 also promotes

the transcription of target genes involved in apoptosis, proliferation, and differentiation in various cell types.⁶

Recent studies have revealed that the IL-22-STAT3 axis is vital for protecting against pathogens and maintaining homeostasis in the epithelium. For instance, IL-22 is required for lung epithelial repair after influenza infection.⁷ Regarding allergic airway inflammation, our recent study has shown that IL-22 induces Reg3 γ

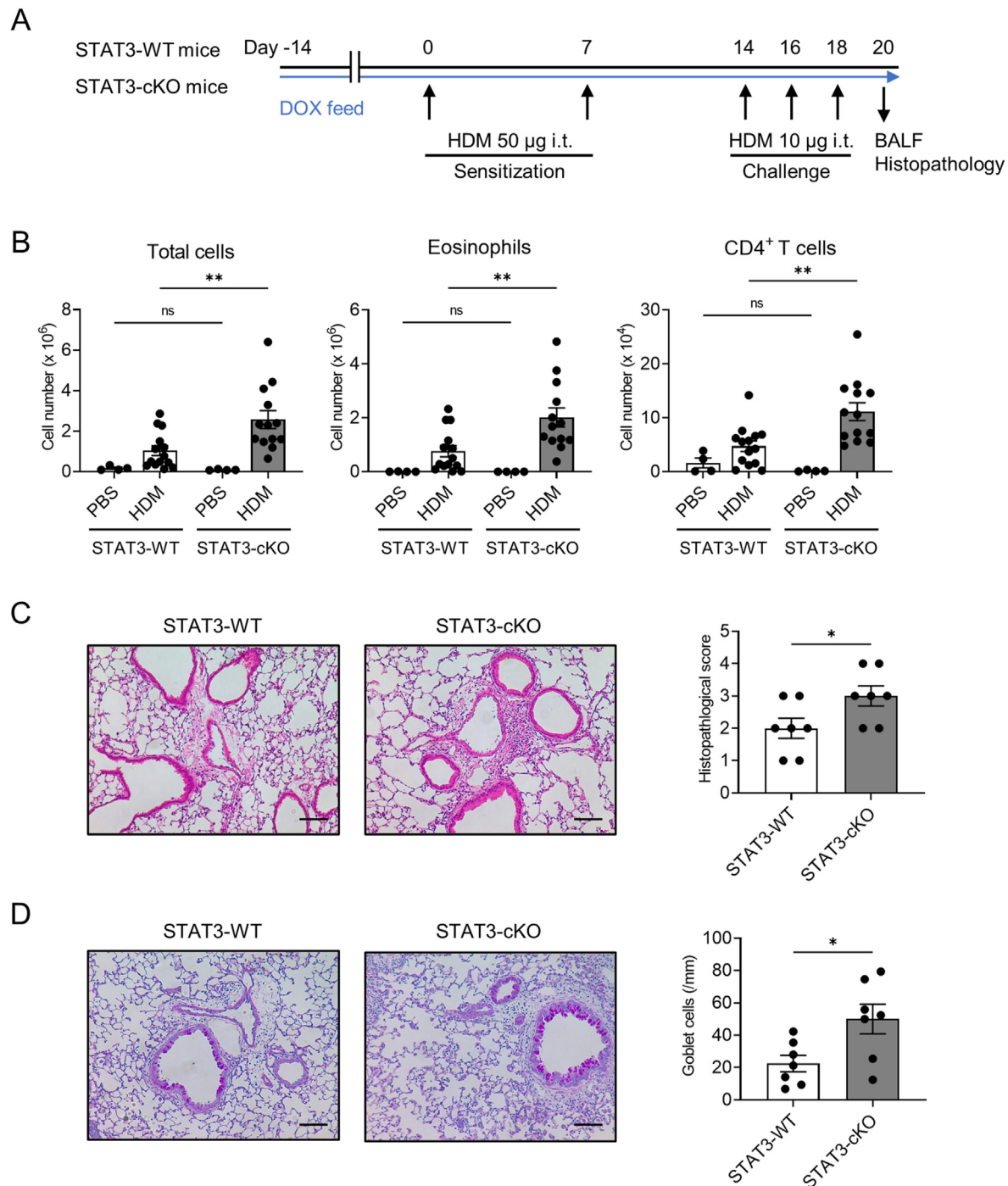


Fig. 1. HDM-induced airway inflammation is exacerbated in AEC-specific STAT3-deficient mice. DOX-inducible AEC-specific STAT3-deficient (STAT3-cKO) mice and their genetic control (STAT3-WT) mice were given DOX-containing feed and sensitized and challenged with HDM as described in the Methods. **(A)** Schematic diagram of HDM-induced asthma model. **(B)** The numbers of total cells, eosinophils (SiglecF⁺ CD11c⁻), and CD4⁺ T cells in BALF were evaluated by FACS analysis at 48 h after the last challenge. Data are means $\pm$ SEM, n = 4–14 mice per group. **p < 0.01, ns; not significant (two-way ANOVA followed by Tukey's multiple comparisons test). **(C, D)** Histopathological scores (HE stain) **(C)** and the quantification of goblet cells (PAS stain) **(D)** with representative photomicrographs of lung sections at 48 h after the last challenge. Bars indicate 100 μ m. Data are means $\pm$ SEM, n = 7 mice per group. *p < 0.05 (unpaired t-test).

production from lung epithelial cells in a STAT3-dependent manner and inhibits HDM-induced allergic airway inflammation.⁸ Meanwhile, Simeone-Penney *et al.* have shown that airway epithelial STAT3 aggravates HDM-induced allergic inflammation using mice lacking STAT3 in airway epithelial cells constitutively.⁹ However, it has been reported that STAT3 is expressed in lung epithelial cells at the fetal stage, and STAT3 signaling is involved in murine bronchiolar epithelial differentiation.^{10,11} Accordingly, mice lacking STAT3 constitutively in epithelial cells may have defects in epithelial cells before the induction of asthma. Thus, the roles of epithelial STAT3 in allergic inflammation should be investigated by a more sophisticated approach.

In this study, to address this point, we examined the role of epithelial STAT3 in HDM-induced allergic airway inflammation by using AEC-specific doxycycline (DOX)-inducible STAT3-deficient mice.

Methods

Mice

C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan). Mice carrying floxed alleles for STAT3 (STAT3^{fl/fl} mice)¹² were bred with CCSPrtTA/tetO-Cre mice¹¹ to establish mice with AEC-specific and DOX-dependent deletion of STAT3 [CCSPrtTA/tetO-Cre STAT3^{fl/fl} mice (STAT3-CKO)]. CCSPrtTA STAT3^{fl/fl} mice (STAT3-WT) were used as genetic control mice. STAT3-CKO mice and STAT3-WT mice were provided with DOX (625 mg/kg)-containing feed (CLEA Japan) from 2 weeks before the initiation of experiments to the end of experiments.^{13,14} All mice were housed in microisolator cages under specific pathogen-free conditions. All animal experiments were approved by the Chiba University Animal Care and Use Committee and conducted following the guidelines of Chiba University, which conform to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011).

HDM-induced allergic inflammation model

Mice were anesthetized as described previously¹⁵ and then sensitized intratracheally (i.t.) twice with HDM extracts (50 µg; *D. Pteronyssinus*, Greer Laboratories, Lenoir, NC) in PBS at a 7-day interval. Seven days after the last sensitization, the mice were challenged i.t. with 10 µg of HDM extracts in PBS as indicated in Figure 1A. Forty-eight hours after the last challenge, the numbers of eosinophils and CD4⁺ T cells recovered in bronchoalveolar lavage fluid (BALF) were evaluated by FACS analysis as previously described.¹⁶ Data were collected with FACSCanto II (BD Biosciences, San Diego, CA) and analyzed by FlowJo software (Tree Star, San Carlos, CA). For histological analysis, the lung was perfused with PBS to remove blood cells and was fixed with 4% paraformaldehyde (PFA) phosphate buffer solution overnight.

Isolation of lung epithelial cells

Lung epithelial cells were isolated by two-step purification as previously described.¹⁷ In brief, lung cell suspensions were prepared by using Dispase (1000 PU/ml, GODO SHUSEI, Tokyo, Japan), and then CD45⁺ cells were depleted by magnetic sorting (Mojo-Sort; BioLegend, San Diego, CA). Enriched CD45⁺ cells were subjected to further purification of club cell-rich fraction and alveolar epithelial type II (ATII) cell-rich fraction by a cell sorter SH800Z (Sony Biotechnology, Tokyo, Japan) as described elsewhere.¹⁸ The resultant cells were >95% pure by flow cytometry.

Antibodies

For FACS analysis of BALF cells, anti-CD3ε (145-2C11), anti-CD4 (RM4-5), anti-CD8α (53-6.7), and anti-CD11c (N418) were purchased from BioLegend, and anti-CD19 (1D3) and anti-Siglec-F (E50-2440) antibodies were purchased from BD Biosciences. For isolation of lung epithelial cells by FACS sorting, the following antibodies were purchased from BioLegend: anti-CD45 (30-F11), anti-CD31 (MEC13.3), anti-CD326 (EpCAM) (G8.8), and anti-I-A/I-E (M5/114.15.2).

Quantitative real-time PCR (qPCR) analysis

Total cellular RNA was extracted with an RNeasy Micro kit (QIAGEN), and reverse transcription was performed using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Quantitative PCR was performed with an ABI PRISM7300 sequence detection system (Applied Biosystems, Foster City, CA) using a SYBR green reagent (Applied Biosystems). The levels of each gene were normalized to the levels of β-actin. The sequences of PCR primers are shown in Supplementary Table 1.

Histological analysis

PFA-fixed lung sections (3 µm-thick, 4 sections per lung) were stained with hematoxylin–eosin (HE) and periodic acid-Schiff (PAS) according to standard protocols. Histopathological scoring and quantification of goblet cells were performed on HE- and PAS-stained lung sections, respectively, in a blinded manner as previously described.^{8,19,20}

RNA-sequencing (RNA-seq) analysis

Total RNA was extracted from isolated cells by using a TRIzol reagent (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. RNA-seq libraries were prepared using a NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs, Ipswich, MA). RNA-seq was performed on NextSeq 500 (Illumina, San Diego, CA) using a NextSeq 500/550 High Output Kit v2.5 (Illumina) with a single 75 base-pair read. Differentially expressed genes (DEGs) were identified as adjusted p-value <0.05 using DESeq2 package²¹ in R. Gene enrichment analyses were performed using Metascape (<https://metascape.org/gp/index.html>).²²

Effect of an SCD1 inhibitor on HDM-induced allergic inflammation

To inhibit SCD1 activity during HDM-induced allergic inflammation, 25 µg of A939572 (Cayman Chemical) in 250 µl of 10% DMSO in PBS or vehicle was administered intraperitoneally daily from day 0 to day 19.

Statistical analysis

Data are summarized as means ± SEM. The statistical analyses of the results were performed using Prism 9 (GraphPad Software, San Diego, CA). The respective statistical analyses are specified in each figure legend. p values < 0.05 were considered significant.

Results

HDM-induced airway inflammation is exacerbated in mice lacking STAT3 in airway epithelial cells

To investigate the roles of STAT3 expressed in AECs in the induction and development of HDM-induced asthma models, we

compared HDM-induced airway inflammation in AEC-specific DOX-inducible STAT3-deficient (STAT3-cKO) mice and their genetic control mice (STAT3-WT) (Fig. 1). Importantly, contrary to the previous study,⁹ HDM-induced eosinophilic airway inflammation was significantly exacerbated in STAT3-cKO mice compared to STAT3-WT mice (Fig. 1B, C). Goblet cell hyperplasia was also enhanced in STAT3-cKO mice (Fig. 1D). Prior to the experiments, we confirmed that STAT3 expression was significantly decreased in alveolar epithelial type II (ATII) cells and club cells,

formerly known as Clara cells, in STAT3-cKO mice by qPCR analyses (Supplementary Fig. 1A). No significant differences in the cellular composition of airway epithelium (Supplementary Fig. 1B) and gene expression level of tight junction protein, ZO-1 (Supplementary Fig. 1C), were found between STAT3-cKO mice and STAT3-WT mice, suggesting that the development of epithelium is intact in STAT3-cKO mice. These results suggest that STAT3 expressed in AECs suppresses HDM-induced allergic airway inflammation.

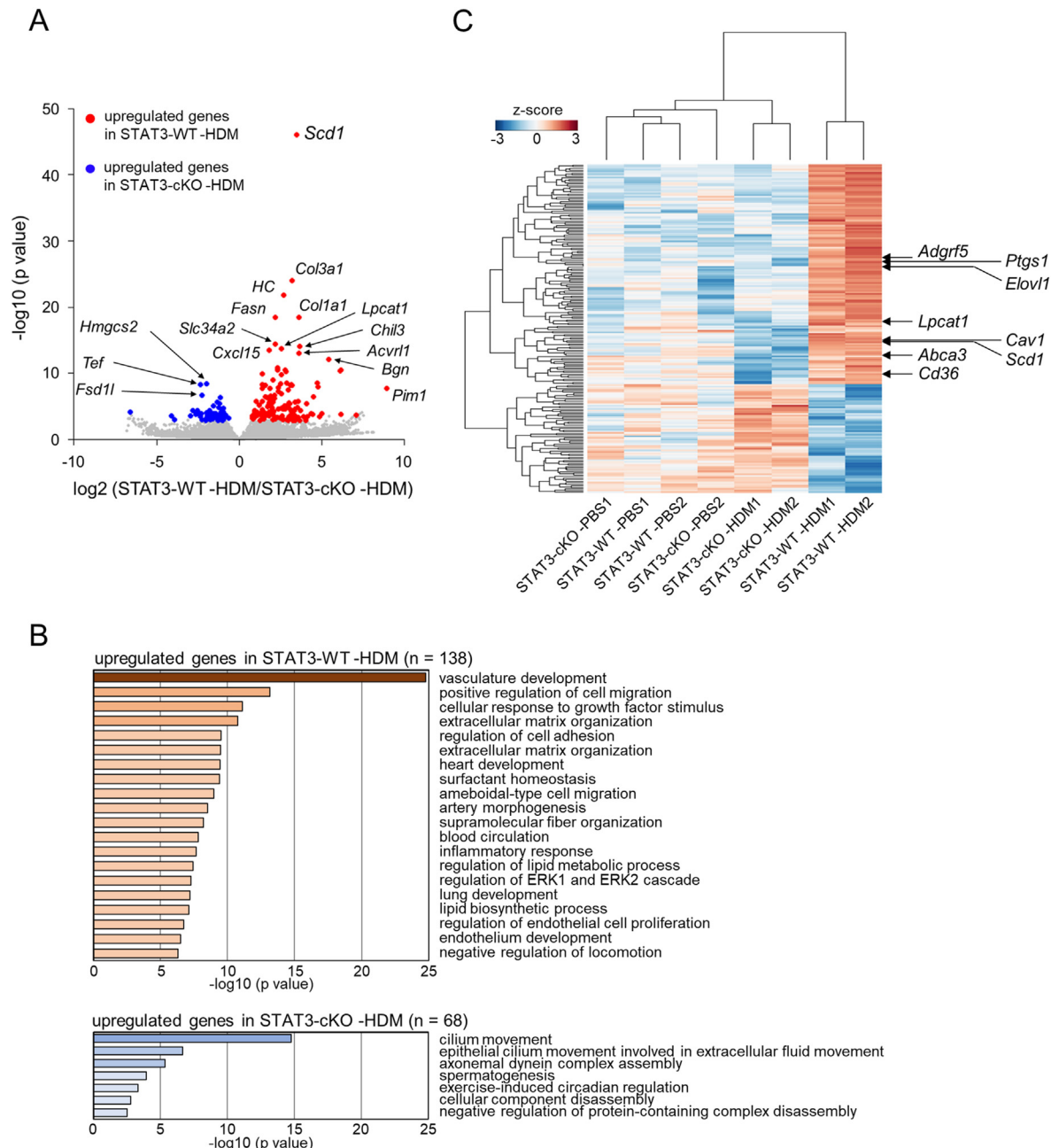


Fig. 2. The gene induction of SCD1 was impaired in club cells in HDM-treated STAT3-cKO mice. STAT3-WT and STAT3-cKO mice were sensitized with HDM or PBS as described in the Methods (n = 2 per group). On day 9 (48 h after the last sensitization), club cells were isolated from the lung by cell sorting, and total RNAs were prepared and subjected to RNA-seq analyses. DEGs were defined as adjusted p-value <0.05. One hundred thirty-eight upregulated genes and 68 downregulated genes in HDM-treated STAT3-WT mice (STAT3-WT-HDM) were identified. (A) Volcano plot of DEGs between STAT3-WT-HDM and HDM-treated STAT3-cKO mice (STAT3-cKO-HDM). (B) Enriched ontology clusters in the upregulated DEGs in STAT3-WT-HDM (upper) and those in the upregulated DEGs in STAT3-cKO-HDM (lower). (C) Heat map of hierarchical clustering of DEGs between STAT3-WT-HDM and STAT3-cKO-HDM. The log₂-transformed expressions were normalized by z-score and clustered by hierarchical clustering. The genes annotated with both surfactant homeostasis and lipid biosynthetic processes are displayed.

SCD1 upregulation is impaired in club cells isolated from STAT3-cKO mice in the HDM-induced allergic inflammation

To address the mechanisms underlying epithelial STAT3 signaling-mediated suppression of HDM-induced allergic inflammation, we sought to identify the molecules that STAT3 regulates in epithelial cells. In a preliminary experiment, we examined the expression levels of known STAT3-regulated genes in isolated AECs from wild-type (WT) mice at several time points of the HDM-induced asthma model (Supplementary Fig. 2A) to identify the timing of STAT3 activation in AECs. ATII cell-rich fraction (ATII cells) and club cell-rich fraction (club cells) were separately isolated by FACS cell sorting (Supplementary Fig. 2B) and subjected to qPCR analyses. The isolated club cells showed much higher expression of club cell marker gene *Scgb1a1* than ATII cells (Supplementary Fig. 2C), whereas the isolated ATII cells showed much higher expression of ATII marker gene *Sftpc* than club cells (Supplementary Fig. 2C), confirming the certainty of isolation. We then analyzed gene expression levels of several STAT3-regulated genes in club cells and found that many of STAT3-regulated genes were upregulated during HDM-induced allergic inflammation, with many of them being the highest at day 9 (Supplementary Fig. 2D).

We therefore isolated club cells from HDM- or PBS-treated STAT3-WT or STAT3-cKO mice on day 9 and examined their transcriptome by RNA-seq analyses. DEG analysis between HDM-treated STAT3-WT mice (STAT3-WT – HDM) and HDM-treated STAT3-cKO mice (STAT3-cKO – HDM) identified 206 DEGs (138 and 68 upregulated genes in STAT3-WT – HDM and STAT3-cKO – HDM, respectively) (Fig. 2A). The volcano plot revealed that several genes relevant to surfactant and lipid production, such as *Scd1*, *Fasn*, and *Lpcat1*, were expressed in an epithelial STAT3-dependent manner (Fig. 2A). Especially, *Scd1*, coding fatty acid desaturating enzyme, stearoyl-CoA desaturase 1 was most differentially upregulated in STAT3-WT – HDM than STAT3-cKO – HDM (Fig. 2A). We next performed gene enrichment analysis for the DEGs. Consistent with the volcano plot, the top-20 enriched ontology clusters in the upregulated DEGs in STAT3-WT – HDM included surfactant homeostasis and lipid biosynthetic process as well as vasculature development and positive regulation of cell migration (Fig. 2B). Heat map of hierarchical clustering of DEGs showed that club cells from STAT3-WT – HDM were distant from the other groups: STAT3-cKO – HDM, PBS-treated STAT3-WT mice (STAT3-WT – PBS), and PBS-treated STAT3-cKO mice (STAT3-cKO – PBS) (Fig. 2C), suggesting that AECs of STAT3-cKO mice exhibit impaired responses to HDM administration. Consistently, the genes annotated with both surfactant homeostasis and lipid biosynthetic processes, such as *Scd1* and *Lpcat1*, were down-regulated in STAT3-cKO – HDM (Fig. 2C). These results suggest that *Scd1* is one of the STAT3-regulated genes during HDM-induced allergic airway inflammation.

The administration of an SCD1 inhibitor exacerbates HDM-induced airway inflammation

Recently, it has been reported that SCD1 expression and activity in the whole lung is decreased in murine asthma models and the inhibition of SCD1 decreases methacholine-induced airway contraction.²³ However, the roles of SCD1 in AECs in allergic airway inflammation are still largely unknown. To determine whether SCD1 upregulation in AECs in HDM-treated WT mice is crucial for inhibiting allergic inflammation, we next examined the effect of an SCD inhibitor, A939572, on HDM-induced airway inflammation. As shown in Figure 3A, the numbers of total cells and eosinophils in the BALF were significantly increased in mice treated with A939572 compared to those with vehicle (DMSO). Histological analyses

showed that inflammation score and goblet cell hyperplasia were also increased in mice treated with A939572 (Fig. 3B, C). These findings suggest that SCD1 plays an inhibitory role in HDM-induced allergic airway inflammation.

STAT3-SCD1 axis might contribute to lung homeostasis via lipid metabolism

To further investigate the role of STAT3-mediated SCD1 induction in HDM-induced allergic inflammation, we performed transcriptome analyses of club cells isolated from WT mice treated with A939572 or vehicle on day 9 of HDM-induced allergic inflammation. RNA-seq analyses identified 484 DEGs between A939572-treated mice (SCD1 inhibitor – HDM) and vehicle-treated mice (DMSO – HDM) (Fig. 4A). Among 484 DEGs, 45 DEGs were shared with 206 DEGs between STAT3-WT – HDM and STAT3-cKO – HDM found in Figure 2 (Fig. 4B). Importantly, most of these genes showed the same direction in the expression between ‘STAT3-WT – HDM to STAT3-cKO – HDM’ and ‘DMSO – HDM to SCD1 inhibitor – HDM’ (Fig. 4C), supporting our notion that SCD1 is one of the functional downstream molecules of STAT3 in HDM-induced allergic inflammation. We finally performed gene enrichment analysis of the shared 45 DEGs and found surfactant metabolism, fatty acid biosynthetic process, and regulation of lipid biosynthetic process as the enriched ontology clusters in the upregulated DEGs (Fig. 4D). Given that the SCD1 inhibitor exacerbated HDM-induced allergic airway inflammation (Fig. 3), these results suggest that the STAT3-SCD1 axis might contribute to lung homeostasis via lipid metabolism during allergic airway inflammation.

Discussion

Using the inducible AEC-specific STAT3-deficient mice, we show here that epithelial STAT3 plays a crucial role in suppressing HDM-induced allergic airway inflammation (Fig. 1). RNA-seq analyses revealed that SCD1 is one of the STAT3-regulated genes in AECs during HDM-induced airway inflammation (Fig. 2). Importantly, the administration of SCD1 inhibitor exacerbates HDM-induced airway inflammation (Fig. 3). These results suggest that epithelial STAT3 is crucial for suppressing HDM-induced airway inflammation, possibly by inducing SCD1 expression.

Regarding the roles of epithelial STAT3, it has been shown that STAT3 in intestinal epithelium contributes to immune homeostasis through IL-22-dependent mucosal wound healing²⁴ and the induction of anti-microbial peptides including Reg family members.^{25,26} Our recent study also demonstrated that IL-22 inhibits HDM-induced allergic inflammation possibly via the induction of Reg3γ.⁸ Consistent with these findings, we showed that epithelial STAT3 plays an inhibitory role in HDM-induced allergic airway inflammation (Fig. 1). By contrast, Simeone-Penney *et al.* reported that epithelial STAT3 plays a proinflammatory role in allergic airway inflammation by using AEC-specific STAT3-deficient mice (CC10-Cre/STAT3^{fl/fl} mice) and STAT3 inhibitors.⁹

Although the reasons for the discrepancy between these studies are unclear, there are two speculations. First, because both CC10 (also known as CCSP)¹⁰ and STAT3²⁷ are expressed in lung epithelium during the development and STAT3 is required for normal lung development,^{10,27} it is possible that CC10-Cre/STAT3^{fl/fl} mice might have structural or functional defects in the lung epithelium, leading to the attenuated allergic inflammation. Second, since the STAT3 inhibitor was administered intranasally, airway inflammation might be attenuated by the STAT3 inhibitor via the suppression of STAT3 in immune cells such as dendritic cells in the lung, which are known to play a critical role in Th2 immune responses to HDM.¹⁶

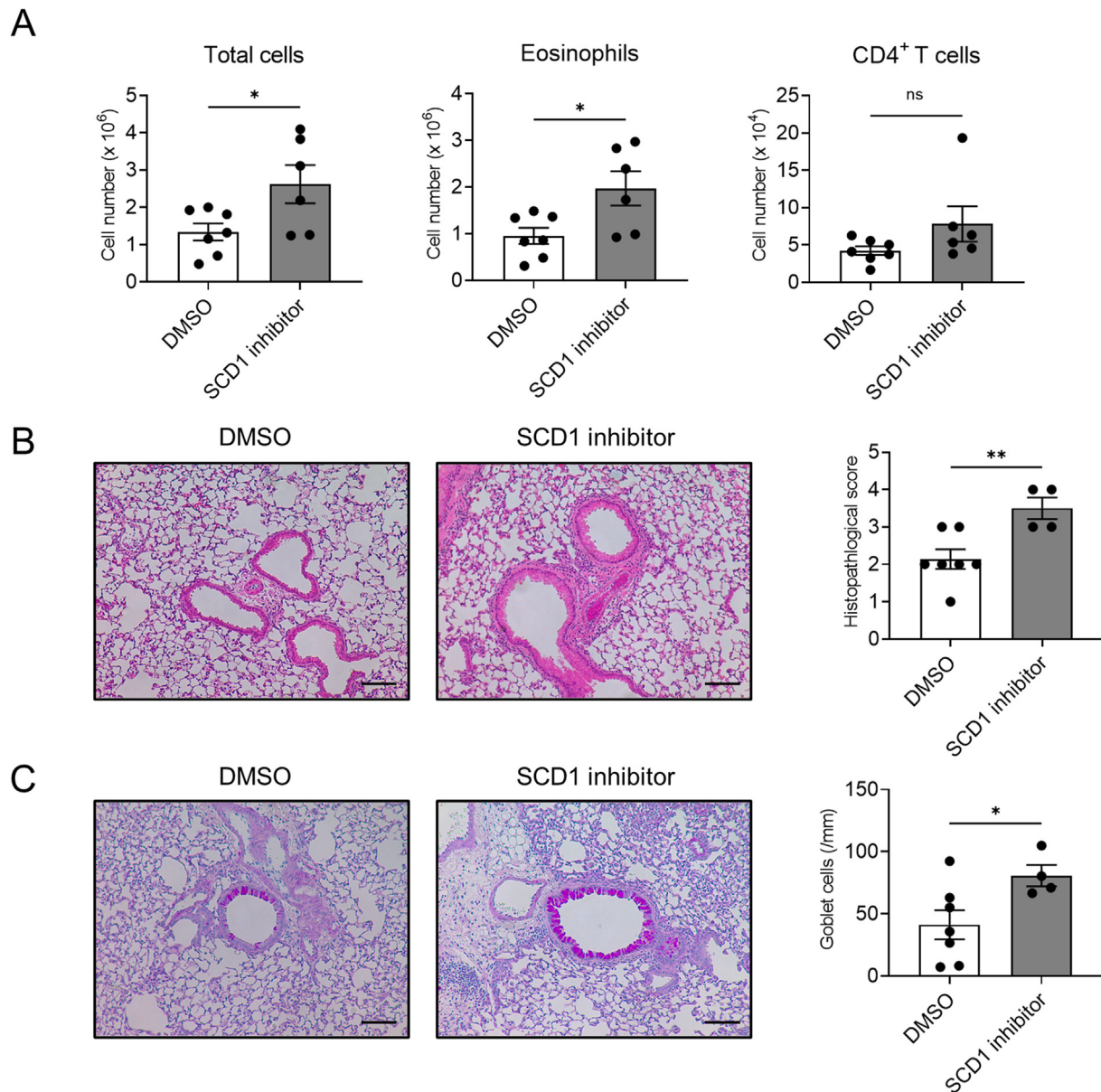


Fig. 3. SCD1 inhibitor exacerbates HDM-induced airway inflammation. WT mice were sensitized and challenged with HDM as described in the Methods. Mice were treated intraperitoneally with 25 μ g of A939572 (an SCD1 inhibitor) in 250 μ l of 10% DMSO in PBS or vehicle daily from day 0 to day 19. **(A)** The numbers of total cells, eosinophils, and CD4⁺ T cells in BALF were evaluated by FACS analysis 48 h after the last HDM challenge. Data are means $\pm$ SEM, $n = 6-7$ mice per group. * $p < 0.05$, ns; not significant (unpaired t-test). **(B)** Histopathological scores and the quantification of goblet cells (PAS stain) **(C)** with representative photomicrographs of lung sections at 48 h after the last challenge. Bars indicate 100 μ m. Data are means $\pm$ SEM, $n = 4-7$ mice per group. * $p < 0.05$, ** $p < 0.01$ (unpaired t-test).

Our findings indicate that epithelial STAT3 is involved in the upregulation of SCD1 in HDM-induced airway inflammation (Fig. 2). SCD1 catalyzes the synthesis of monounsaturated fatty acids from saturated fatty acids.²⁸ It has been shown that SCD1 deficiency protects against diet-induced and genetic obesity and liver steatosis.²⁹ In addition, the suppression of SCD1 has been shown to alter cellular function by modulating inflammation and stress in various cell types and tissues, such as adipocytes, macrophages, β -cells, endothelial cells, and skin.²⁹

Regarding the role of SCD1 in asthma, Rodriguez-Perez *et al.* reported that primary bronchial cells from patients with asthma showed decreased expression of SCD1 and that SCD1 expression was decreased in the lung in murine asthma models.²³ We found that the SCD1 inhibition exacerbated HDM-induced allergic inflammation (Fig. 3A–C) in line with the data of STAT3-cKO mice.

However, in this experiment, the SCD1 inhibitor was administered intraperitoneally because the solvent of the SCD1 inhibitor, DMSO, is potentially toxic in the case of intratracheal administration. Therefore, we cannot exclude the possibility that SCD1 expressed in other cell types than AECs affects overall responses in the airway inflammation in this experimental setting. However, because many DEGs are detected in RNA-seq analyses (Fig. 4A), it is suggested that SCD1 inhibition has significant effects on AECs. The precise roles of SCD1 in airway epithelium in HDM-induced airway inflammation should be addressed by more sophisticated experiments with AEC-specific SCD1-deficient mice in the future.

Metabolic states are closely linked to cellular function.^{30,31} Our combined analysis of two RNA-seq data sets suggests that the genes relevant to lipid metabolism might contribute to allergic inflammation downstream of the STAT3-SCD1 axis (Fig. 4D). Accumulating

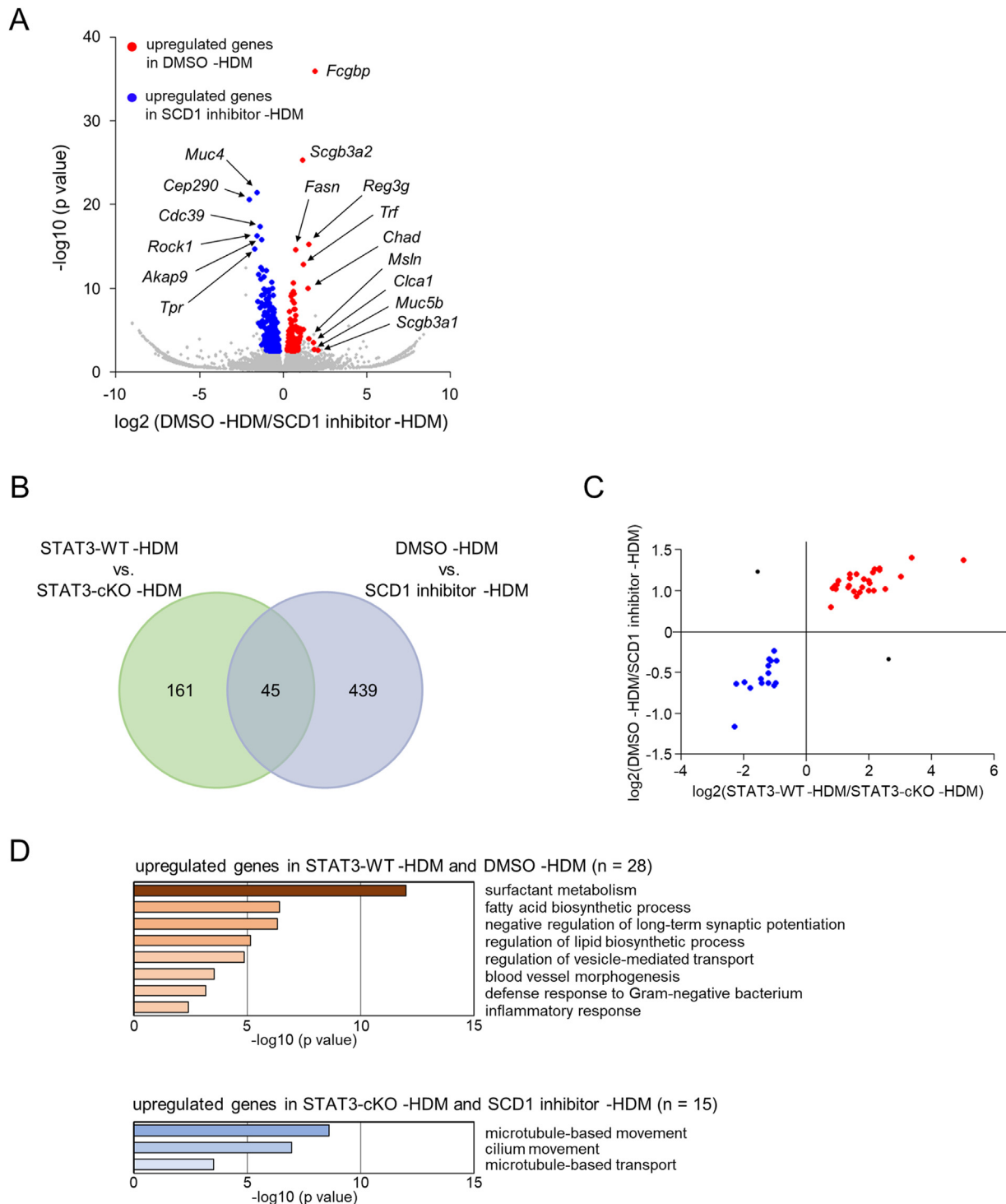


Fig. 4. STAT3-SCD1 axis might contribute to lung homeostasis via lipid metabolism. WT mice were sensitized with HDM as described in the Methods. Mice were treated intraperitoneally with A939572 or vehicle daily from day 0 to day 8 (n = 2 per group). On day 9 (48 h after the second sensitization), club cells were isolated from the lung by cell sorting, and total RNAs were prepared and subjected to RNA-seq analyses. DEGs were defined as adjusted p-value <0.05. One hundred forty-five upregulated genes and 339 downregulated genes in vehicle-treated mice (DMSO – HDM) were identified. **(A)** Volcano plot of DEGs between DMSO – HDM and A939572-treated mice (SCD1 inhibitor – HDM). **(B)** DEGs of RNA-seq (DMSO – HDM vs. SCD1 inhibitor – HDM) (484 genes) were compared to DEGs of RNA-seq (STAT3-WT – HDM vs. STAT3-cKO – HDM) (206 genes). **(C)** Scatter plot of log₂ (fold change) value of the 45 common genes. **(D)** Enriched ontology clusters in the upregulated DEGs in both STAT3-WT – HDM and DMSO – HDM (upper) and those in the upregulated DEGs in both STAT3-cKO – HDM and SCD1 inhibitor – HDM (lower).

evidence has revealed the roles of lipid mediators in the resolution of inflammation.^{32,33} Lipoxin A₄ (LXA₄) is a potent anti-inflammatory mediator and is generated by neutrophils from 15-hydroxyicosatetraenoic acid provided by epithelial cells in the

mucosa.³⁴ LXA₄ has pleiotropic effects on asthma pathophysiology.³⁵ For instance, LXA₄ increases NK-cell-induced eosinophil apoptosis and decreases IL-13 production from ILC2s.³⁶ Thus, impaired production of anti-inflammatory lipid mediators in the lung might

contribute to the exacerbated airway inflammation in STAT3-cKO mice and SCD1 inhibitor-treated mice in the present study.

Our previous study showed that IL-22-STAT3-mediated Reg3 γ induction in airway epithelial cells inhibits HDM-induced allergic inflammation.⁸ Intriguingly, we found that the expression of the Reg3 γ gene (*Reg3g*) in airway epithelial cells was decreased by the administration of SCD1 inhibitor in HDM-sensitized WT mice (Fig. 4A). Therefore, Reg3 γ could also be involved in the SCD1-mediated suppression of HDM-induced allergic inflammation.

We performed RNA-seq analyses of airway epithelial cells at day 9 in HDM-induced allergic inflammation based on the expression of STAT3-dependent genes (Supplementary Fig. 2D). However, STAT3 is suppressed throughout the experimental period in this experimental setting. Therefore, it is possible that STAT3 targets different gene sets at different time points and affects HDM-induced allergic inflammation by different mechanisms. Further analyses are needed to address this possibility.

In conclusion, we have shown that epithelial STAT3 plays a critical role in the suppression of allergic inflammation, possibly inducing SCD1 expression in AECs. Our results also suggest that SCD1 activity might contribute to lung homeostasis through the regulation of lipid metabolism. Thus, SCD1-mediated cellular processes in AECs could be a therapeutic target for the treatment of asthma.

Acknowledgments

We thank Dr. Kiyoshi Takeda for STAT3-floxed mice and Dr. Jeffrey A Whitsett for CCSPrtTA/tetO-Cre mice. We also thank Ms. Kazumi Nemoto and Ms. Maho Yoshino for their technical assistance. This work was supported in part by grants from JST [Moonshot R&D] [Grant Number JPMJMS2025], Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government, LGS (Leading Graduate School) Program, MEXT, GSK Research Grant, and Takeda Science Foundation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2022.05.002>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

NN, MY, SK, AI, TK, KH, and HN designed research; NN, MY, SK, AI, TK, TI, AS, and YM performed research; NN and MY analyzed data; and NN, MY, and HN wrote the manuscript.

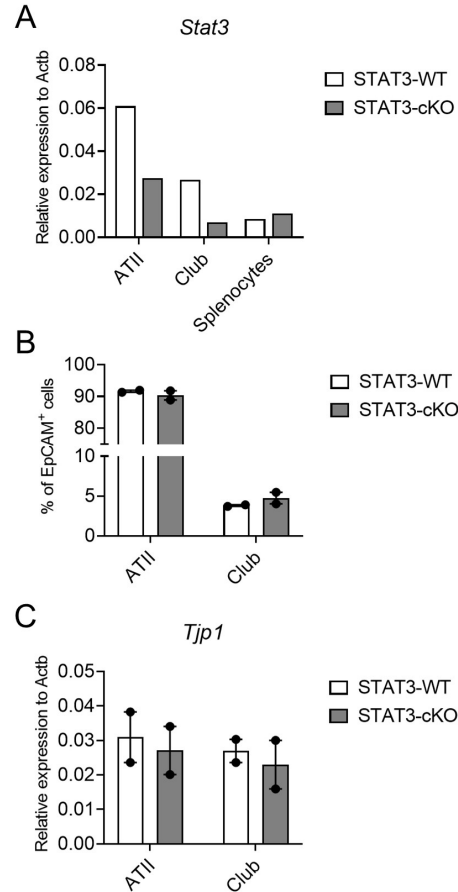
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Supplementary data

Supplementary Table 1. The sequences of primers for qPCR analysis

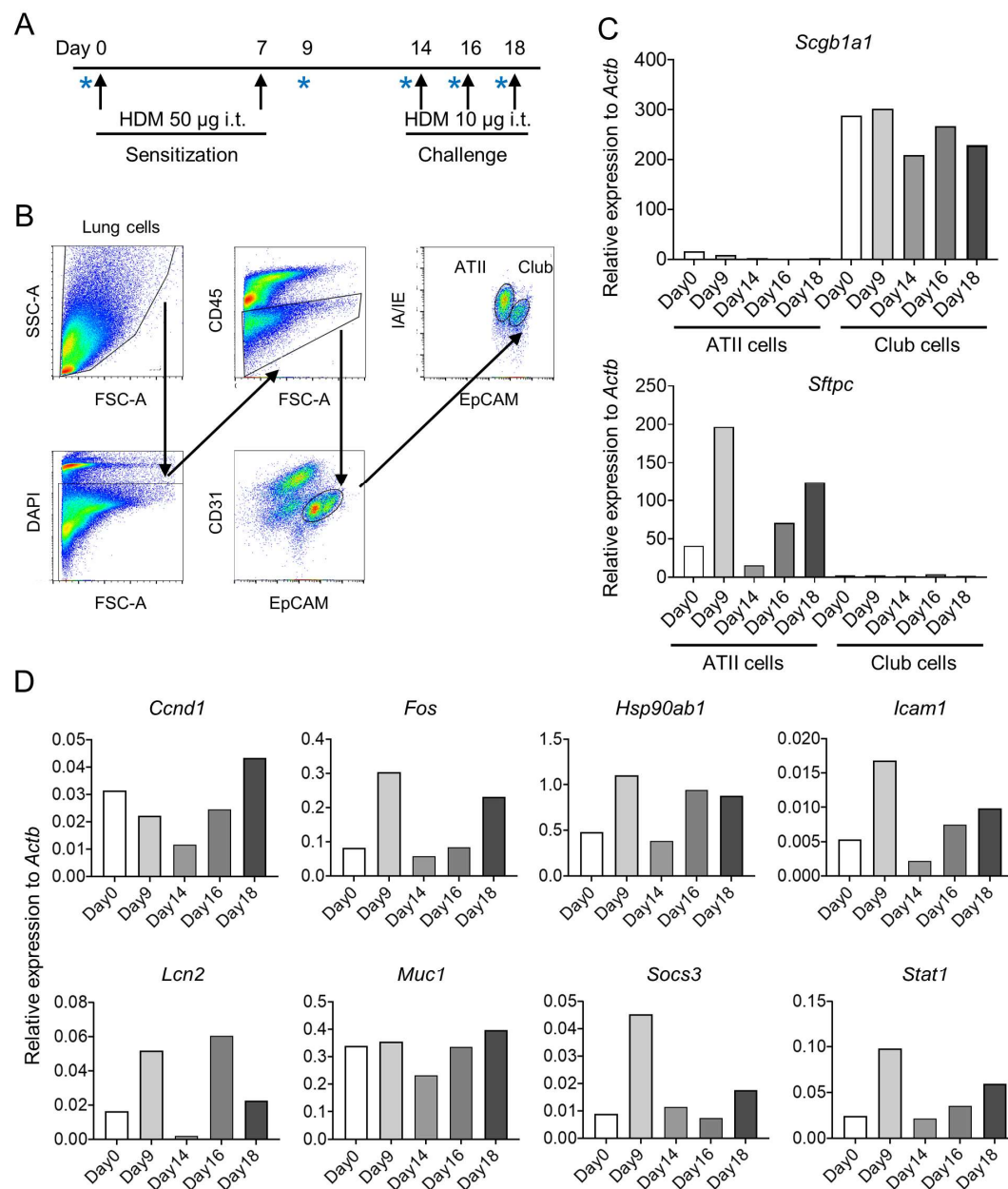
Gene symbol	Primer sequence
Actb	forward primer 5'-GCTCTGGCTCCTAGCACCAT-3' reverse primer 5'-GCCACCGATCCACACAGAGT-3'
Ccnd1	forward primer 5'-GCGTACCCTGACACCAATCTC-3' reverse primer 5'-CTCCTCTTCGCACTTCTGCTC-3'
Fos	forward primer 5'-CGGGTTTCAACGCCGACTA-3' reverse primer 5'-TTGGCACTAGAGACGGACAGA-3'
Hsp90ab1	forward primer 5'-GTCCGCCGTGTGTTTCATCAT-3' reverse primer 5'-GCACTTCTTGACGATGTTCTTGTC-3'
Icam1	forward primer 5'-GTGATGCTCAGGTATCCATCCA-3' reverse primer 5'-CACAGTTCTCAAAGCACAGCG-3'
Lcn2	forward primer 5'-TGGCCCTGAGTGTCATGTG-3' reverse primer 5'-CTCTTGTAGCTCATAGATGGTGC-3'
Muc1	forward primer 5'-GGCATTCGGGCTCCTTTCTT-3' reverse primer 5'-TGGAGTGGTAGTCGATGCTAAG-3'
Scd1	forward primer 5'-TTCTTGCGATACTCTGGTGC-3' reverse primer 5'-CGGGATTGAATGTTCTTGTCGT-3'
Scgb1a1	forward primer 5'-ATGAAGATCGCCATCACAATCAC-3' reverse primer 5'-GGATGCCACATAACCAGACTCT-3'
Socs3	forward primer 5'-ATGGTCACCCACAGCAAGTTT-3' reverse primer 5'TCCAGTAGAATCCGCTCTCCT--3'
Stat1	forward primer 5'-TCACAGTGGTTCGAGCTTCAG-3' reverse primer 5'-GCAAACGAGACATCATAGGCA-3'



Supplementary Figure 1. Normal cellular composition of airway epithelium and gene expression level of ZO-1 in STAT3-cKO mice.

STAT3-WT and STAT3-cKO mice were provided with DOX-containing feed for 14 days, and then ATII cells and club cells were isolated by cell sorting as described in the Methods. **(A)** *Stat3* expression in ATII cells, club cells and, splenocytes (as control) in STAT3-WT and STAT3-cKO mice by qPCR analysis. Representative data from 2 independent experiments. **(B)** The proportion of ATII cells and club cells in DOX-treated STAT3-WT and STAT3-cKO mice by FACS analysis. n = 2 per group. **(C)** The gene expression level of tight junction protein, ZO-1 (*Tjp1*) in ATII cells and club cells in DOX-treated STAT3-WT and STAT3-cKO mice by qPCR analysis. n = 2 per

group.



Supplementary Figure 2. STAT3 is activated in AECs in HDM-induced allergic inflammation.

WT mice were sensitized and challenged with HDM intratracheally (i.t.) as described in the Methods. ATII cell-rich fraction and club cell-rich fraction were isolated by cell sorting. **(A)** Schematic diagram of HDM-induced asthma model. Asterisks show the time points for qPCR analysis of isolated cells from the lung. **(B)** Representative flow

cytometry plots showing sorting strategy for ATII cell-rich fraction (ATII) and club cell-rich fraction (Club). **(C)** Gene expression of *Scgb1a1* and *Sftpc* in ATII cells and club cells was assessed by qPCR analysis at each time point. Representative data from 2 independent experiments. **(D)** The expression of STAT3-related genes in club cells was assessed at each time point by qPCR. Representative data from 2 independent experiments.