

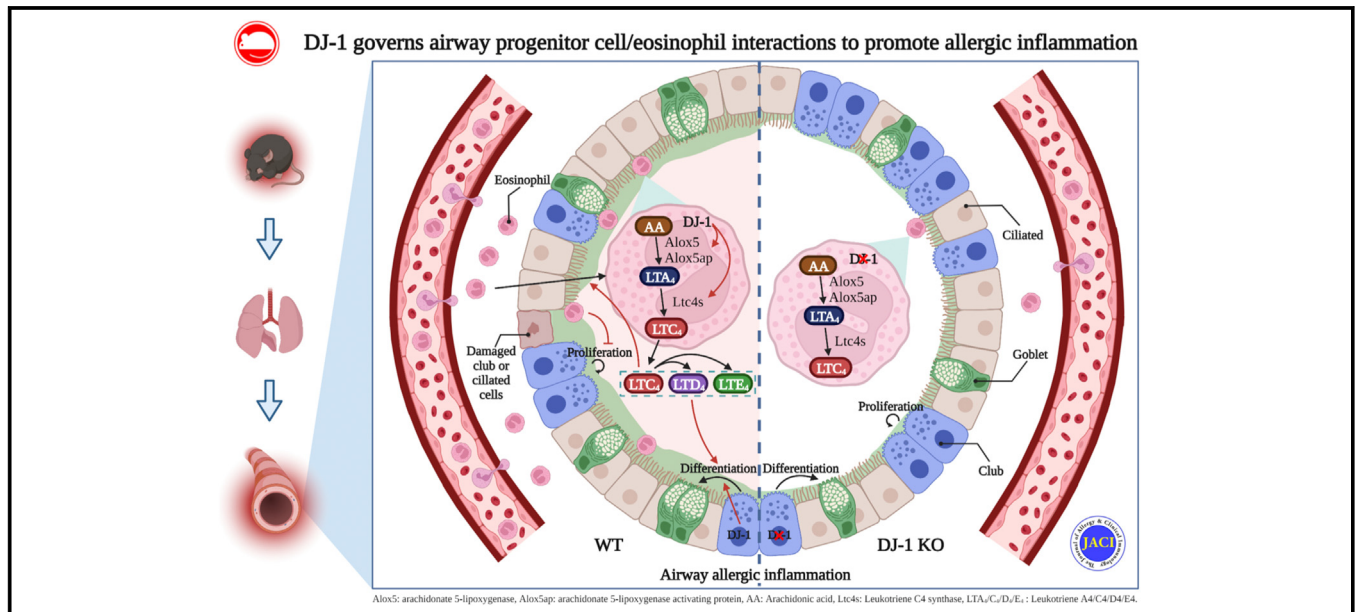
DJ-1 governs airway progenitor cell/eosinophil interactions to promote allergic inflammation



Kuan Li, MS,^{a,b,c,d,*} Qiuyang Zhang, BS,^{a,b,c,d,*} Li Li, MD,^{d,e,*} Shuangyan Li, MS,^{c,*} Qing Yue, MS,^c Xue Li, MS,^{a,b,c,d} Yu Li, MS,^{a,b,c,d} Jianhai Wang, PhD,^{a,b,c,d} Sisi Wang, MS,^c Zengqiang Yuan, PhD,^f and Huaiyong Chen, PhD^{a,b,c,d}

Tianjin and Beijing, China

GRAPHICAL ABSTRACT



Background: DJ-1 is an antioxidant protein known to regulate most cell-mediated allergic response, but its role in airway eosinophilic interactions and allergic inflammation is not known. **Objective:** The aim of this study was to investigate the role of DJ-1 in airway eosinophilic inflammation *in vitro* and *in vivo*. **Methods:** Ovalbumin-induced airway allergic inflammation was established in mice. ELISA was adopted to analyze DJ-1 and cytokine levels in mouse bronchoalveolar lavage fluid. Transcriptional profiling of mouse lung tissues was conducted by single-cell RNA-sequencing technology. The role of DJ-1 in the differentiation of airway progenitor cells into goblet cells was examined by organoid cultures, immunofluorescence

staining, quantitative PCR, and cell transplantation in normal, DJ-1 knockout (KO), or conditional DJ-1 KO mice. **Results:** This study observed that DJ-1 was increased in the lung tissues of ovalbumin-sensitized and challenged mice. *DJ-1* KO mice exhibited reduced airway eosinophil infiltration and goblet cell differentiation. Mechanistically, we discovered that eosinophil-club cell interactions are reduced in the absence of *DJ-1*. Organoid cultures indicated that eosinophils impair the proliferative potential of club cells. Intratracheal transplantation of DJ-1-deficient eosinophils suppresses airway goblet cell differentiation. Loss of DJ-1 inhibits the metabolism of arachidonic acid into cysteinyl leukotrienes in eosinophils

From ^athe Department of Basic Medicine, Haihe Hospital, Tianjin University; ^bthe Key Research Laboratory for Infectious Disease Prevention for State Administration of Traditional Chinese Medicine, Tianjin Institute of Respiratory Diseases; ^cthe Department of Basic Medicine, Haihe Clinical School, Tianjin Medical University; ^dthe Department of Respiratory Medicine, Haihe Clinical School, Tianjin Medical University; ^eTianjin Key Laboratory of Lung Regenerative Medicine; and ^fThe Brain Science Center, Beijing Institute of Basic Medical Sciences.

*These authors contributed equally to this work.

Supported by the National Natural Science Foundation of China, China (grants 82070001, 81970001, 81630026, 81930029, and 82100077), Natural Science Foundation of Tianjin, China (grants 20JCQNJC01790, 20JCYBJC01250, 21JCZDJC00430, 21JCQNJC00550, 21JCQNJC00510, 21JCQNJC00410), Science and Technology Planning Project of Tianjin Jinnan District, China (grants 20200118, 20200112), and Haihe Hospital Fund, China (grants HHYY-202012, HHYY-202008). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Some of the images were created with BioRender.com.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication September 29, 2021; revised March 9, 2022; accepted for publication March 29, 2022.

Available online June 18, 2022.

Corresponding author: Huaiyong Chen, PhD, Tianjin Key Laboratory of Lung Regenerative Medicine, Haihe Hospital, Tianjin University, Tianjin 300350, China. E-mail: huaiyong.chen@foxmail.com. Or: Zengqiang Yuan, PhD, The Brain Science Center, Beijing Institute of Basic Medical Sciences, Beijing 100850, China. E-mail: zqyuan@bmi.ac.cn.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/\$36.00

© 2022 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaci.2022.03.036>

while these secreted metabolites promote airway goblet cell fate in organoid cultures and *in vivo*.

Conclusions: DJ-1–mediated interactions between airway epithelial progenitor cells and immune cells are essential in controlling airway goblet cell metaplasia and eosinophilia. Blockade of the DJ-1 pathway is protective against airway allergic inflammation. (J Allergy Clin Immunol 2022;150:1178-93.)

Key words: Airway epithelial progenitor cells, eosinophilic inflammation, DJ-1, arachidonic acid, cell transplantation, organoid cultures, cell-cell interaction, allergic inflammation, goblet cell metaplasia

Bronchial asthma is characterized by airway oxidative stress, goblet cell hyperplasia, and chronic inflammation with significant infiltration of inflammatory cells, including neutrophils and eosinophils (EOSs).^{1,2} The current standard of care includes anti-inflammatory steroids and bronchodilators to improve airflow limitation.³ Inflamed airway epithelia release many chemoattractants that drive the recruitment of neutrophils and EOSs to the lungs.⁴ Co-culture with airway epithelial cells has been shown to increase the production of cysteinyl leukotrienes (LTs) by activated EOSs.⁵ In turn, EOSs produce a range of proinflammatory proteins and cytotoxic molecules, causing epithelial alterations and damage.^{6,7} Clinical evidence indicates that the extent of the increase in EOSs and the level of their secreted products often correlates with the severity of bronchial asthma and the frequency of acute exacerbations.⁸⁻¹¹ By adopting an ovalbumin (OVA)-induced allergic inflammation mouse model, we previously demonstrated that a diminished pool of airway epithelial progenitor cells might contribute to epithelial damage in asthma.¹² The altered immunological microenvironment in asthma may affect the fate of these epithelial progenitor cells. However, it remains largely unknown how EOSs crosstalk with airway epithelial progenitor cells during the progression of allergic airway inflammation.

Bronchial asthma exacerbation is nonpredictable and refractory and is closely associated with impaired airway mucociliary clearance. Chronic mucin hypersecretion could lead to ciliary dyskinesia and mucus plugs of smaller airways, where inhaled microbes are easily trapped and replicate to elicit infection, amplify existing inflammation, and impair gas conduction and exchange.^{13,14} Submucosal gland mucous/secreting cells and airway epithelial goblet cells are the major sources of mucin. Club cells are abundantly distributed from the proximal to distal airways, serving as a part of the airway protective barrier by secreting antimicrobial proteins.¹⁵ Club cells are also believed to be facultative progenitor cells because of their ability to generate goblet cells.¹⁶ The goblet cell differentiation process is tightly controlled in the lungs at a steady state. However, the underlying mechanisms that drive goblet cell metaplasia during allergic airway inflammation are not yet fully understood. This may explain why therapeutic drugs targeting goblet cell metaplasia are not currently available for the treatment of patients with asthma. Thus, tackling airway goblet cell metaplasia may be fundamental to cure asthma.

DJ-1, the Parkinsonism-associated deglycase gene (*PARK7*) located on chromosome 1p36, has broad biological functions, including antioxidant activity, transcription modulation, and

Abbreviations used

APC:	Allophycocyanin
BALF:	Bronchoalveolar lavage fluid
CFE:	Colony-forming efficiency
DEG:	Differentially expressed gene
DJ-1:	Parkinsonism-associated deglycase gene (<i>PARK7</i>)
DMEM:	Dulbecco modified Eagle medium
EOS:	Eosinophil
GO:	Gene Ontology
ILC:	Innate lymphoid cell
KEGG:	Kyoto Encyclopedia of Genes and Genomes
KO:	Knockout
LT:	Leukotriene
NK:	Natural killer
OCT:	Optimal cutting temperature
OVA:	Ovalbumin
qPCR:	Quantitative PCR
scRNA-seq:	Single-cell RNA-sequencing
UMI:	Unique molecular identifier
WT:	Wild-type

chaperone-like functions. It also attenuates oxidative stress-induced injury to epithelial cells in the kidney and retina.^{17,18} Loss of *DJ-1* was shown to augment oxidative stress and promote mast cell–mediated allergic responses in a mouse model of passive cutaneous anaphylaxis.¹⁹ *DJ-1*–deficient mice exhibit exacerbated lung injury and increased cellular infiltration during LPS-induced sterile lung injury.²⁰ It appears that DJ-1 is cytoprotective against alveolar epithelial cells in peripheral lung inflammation mouse models and human distal lung diseases.²¹⁻²⁴ However, it remains unknown whether DJ-1 orchestrates allergic inflammation in the conducting airways.

In this study, we used loss-of-function genetically modified mice, an OVA-induced airway allergic inflammation mouse model, single-cell RNA-sequencing (scRNA-seq), and 3-dimensional organoid cultures to investigate the role of DJ-1 in the development of airway allergic inflammation. DJ-1 expression was found to be increased in the sputum of patients with asthma and in the lungs of OVA-allergic mice. Loss of DJ-1 resulted in reduced eosinophilic infiltration and inhibition of goblet cell differentiation in the airways. These results suggest that DJ-1 is a promoter of allergic airway inflammation.

METHODS

Human subjects

The study of DJ-1 (*PARK7*) levels in the sputum of healthy individuals and patients with asthma was approved by the Ethics Committee of Tianjin Haihe Hospital (number: 2021HHKT-019-recheck-01), complying with all relevant ethical regulations. All sputum samples were obtained using noninvasive methods. Only discarded sputum samples were used in this study. Informed consent was obtained from all participants, including 11 healthy subjects and 12 patients with asthma (see Table E1 in this article's Online Repository at www.jacionline.org).

Mice

Mice were exposed to a 12-hour light/dark cycle and had free access to food and water. *DJ-1*^{−/−} mice were obtained from Dr Jie Shen of Harvard University Medical School (Cambridge, Mass). The obtained *DJ-1*^{−/−} mice were backcrossed with C57BL/6 mice 5 times. *DJ-1*^{fl/fl} mice were purchased from Gempharmatech (Nanjing, China). *Scgblal*^{CreER} mice

were obtained from the Jackson Laboratory (Bar Harbor, Maine). *Scgblal^{CreER};DJ-1^{fl/fl}* mice were established by crossing *Scgblal^{CreER}* mice with *DJ-1^{fl/fl}* mice. Tamoxifen was injected intraperitoneally at 200 mg/kg every other day, 3 times, to induce the deletion of DJ-1 in club cells. C57BL/6 mice were purchased from Sipeifu Biotechnology (Beijing, China). All mice were housed in a specific pathogen-free facility at the Tianjin Haihe Hospital. All animal procedures were performed according to the protocol approved by the Tianjin Haihe Hospital Animal Care and Use Committee (number: 2021HHKT(A)-004). All animal experiments were performed under anesthesia with 1% pentobarbital sodium (80 mg/kg) via intraperitoneal injection.

Cell cultures

The mouse lung fibroblast cell line MLg2908 (see Table E2 in this article's Online Repository at www.jacionline.org) was purchased from ATCC (Manassas, Va) and cultured in a 100-mm petri dish in a humidified 37°C incubator with 5% CO₂. MLg2908 cells were maintained in advanced Dulbecco modified Eagle medium (DMEM; Gibco, Thermo Fisher Scientific, Waltham, Mass) containing 10% FBS (Gibco, Thermo Fisher Scientific), 100 IU/mL penicillin, and 100 µg/mL streptomycin (Gibco, Thermo Fisher Scientific).

Lung tissue dissociation and flow cytometry

Lung tissue was processed as a single-cell suspension via enzyme digestion, as described previously.²⁵ In brief, whole lung irrigation was performed 3 times with 1 mL of 0.2 mmol/L ethyleneglycol-bis-(β-aminoethylether)-NN,N',N'-tetraacetic acid (Sigma-Aldrich, St Louis, Mo) diluted in PBS. Lung tissue was digested with 4 U/mL elastase (Worthington Biochemical Corporation, Lakewood, NJ) at 37°C for 25 minutes. The tissue was minced into 2 mm³ of chipping. The sample was incubated with 5 mL of 0.5% DNase I (Sigma-Aldrich) diluted in Ham's F12 (Corning, Corning, NY) at 37°C for 15 minutes. The sample was filtered using a 70-µm FALCON Cell Strainer (Corning). Lung cells were resuspended in advanced Hank's balanced saline solution (Solarbio Beijing, China) containing 2% FBS, 10 mmol/L HEPES (Sigma-Aldrich), and 1% penicillin/streptomycin. For scRNA-seq, a cell viability ratio of >85% was required. For club cell sorting, primary antibodies, including CD24-phycoerythrin, Epithelial cell adhesion molecule-phycoerythrin–Cyanine7, Sca-1-allophycocyanin (APC), CD31-biotin, CD34-biotin, and CD45-biotin, were added to the cell suspension. Biotin-binding antibodies CD31, CD34, and CD45 were labeled with streptavidin APC-eFluor 780 (eBioscience, Thermo Fisher Scientific). A FACS Aria 3III sorter (BD Biosciences, San Jose, Calif) was used for fluorescence-activated cell sorting of labeled lung cells.

Organoid cultures

Mouse EpCAM⁺CD24^{high}Sca-1⁺ club cells were isolated as previously described.²⁵ Briefly, 3 × 10³ club cells were mixed with 2 × 10⁵ MLg2908 lung fibroblasts in 100 µL of Matrigel (BD Pharmingen, San Diego, Calif) and DMEM/F12 basic medium (1:1) mixture. A 100-µL cell suspension was plated into each Transwell insert (pore size: 0.4 µm) of a 24-well plate (Greiner Bio-One, Kremsmünster, Austria). The cell mixture was incubated at 37°C, and 450 µL of advanced DMEM/F12 (Corning) containing 10% FBS, 1% penicillin/streptomycin, 1% insulin/transferrin/selenium (Sigma-Aldrich), 10 µmol/L SB41542 (Sigma-Aldrich), and Y27632 (Sigma-Aldrich) was added to the bottom of each chamber. Cells were cultured at 37°C in a constant-temperature incubator with 5% CO₂. Y27632 was added to limit cell death during the first 2 days of culture. Starting from the third day of culture, the spent culture medium was replaced with fresh medium every other day. For the co-culture of EOSs and club cells, 2 × 10⁴ EOSs were placed in each Transwell. For the indicated experiments, IL-13 (10 ng/mL, Sigma-Aldrich) was added to promote goblet cell differentiation.²⁶ Club cell-derived organoids were visualized using a DP80 inverted fluorescence microscope (Olympus, Tokyo, Japan) on day 8 after seeding.

The colony-forming efficiency (CFE) was determined by calculating the number of organoids as a percentage of the total number of club cells with a diameter ≥50 µm. For pathological examination, Matrigel disks were fixed with 4% paraformaldehyde (Solarbio) for 2 hours at 4°C. The fixed Matrigel disks were then embedded in optical cutting temperature (OCT) compound (Sakura, Torrance, Calif). Frozen sections (5-µm thickness) were then sliced for staining. For gene expression analysis, Matrigel disks were lysed in TRIzol reagent to extract RNA from the cultures.

OVA-induced allergic lung inflammation

Six- to 12-week-old female mice were used for OVA-induced allergic lung inflammation. Animals were randomly assigned and were age- and sex-matched. Mice in the OVA group were sensitized on days 0 and 7 by intraperitoneal injection of 10 µg chicken OVA (Sigma-Aldrich) emulsified in 44% Al(OH)₃ (Imject Alum Adjuvant, Thermo Fisher Scientific) to a total volume of 100 µL with PBS. The mice then underwent ultrasonic atomization inhalation of 2% OVA diluted in PBS for 0.5 hours daily from days 14 to 18. Mice in the control group were subjected to a similar process in which OVA was replaced with PBS. The mice were sacrificed on the day after the final challenge. Bronchoalveolar lavage fluid (BALF) was washed out with 1 mL PBS and centrifuged at 300g for 5 minutes at 4°C, after which the cells were resuspended in 80 µL PBS and spread on slides. The abundance of OVA-triggered leukocytes, including EOSs, neutrophils, and macrophages, was analyzed using Hema 3 staining (Fisherbrand, Pittsburgh, Pa) according to the manufacturer's protocol. Lung tissues were harvested using elastase (Worthington Biochemical Corporation) to allow sorting between airway club cells and EOSs. The lung tissues were then fixed for further pathological staining or frozen at –80°C for subsequent gene expression or Western blot analysis.

Histological staining

Paraffin sections (5-µm thickness) were routinely dewaxed and rehydrated, and then stained with 1% Alcian blue solution (Sigma-Aldrich) for 10 minutes. Slides were rinsed in double-distilled water and stained with Lillie-Mayer solution (Solarbio) for 1 minute. For hematoxylin and eosin staining, dewaxed and rehydrated sections were processed with hematoxylin staining (Solarbio) for 2 to 5 minutes. They were then washed, dehydrated, and eosin-stained (Solarbio) for 10 to 15 seconds. For periodic acid–Schiff staining, dewaxed and rehydrated sections were treated with periodic acid (Solarbio) for 10 minutes and then stained with Schiff solution (Solarbio) for 10 to 15 minutes. For Alcian blue, hematoxylin and eosin, and periodic acid–Schiff staining, the slides were dehydrated and sealed with neutral balsam for imaging.

Immunofluorescence staining

Paraffin sections (5-µm thickness) were prepared after the lung tissues were fixed with 4% paraformaldehyde for 2 hours at room temperature. For organoid cultures, the Matrigel disks were fixed with 4% paraformaldehyde for 2 hours at 4°C, after which they were embedded in OCT compound. OCT compound-embedded Matrigel disks were quickly frozen with liquid nitrogen and then sliced into 6-µm cryosections. After antigen retrieval using citric acid (10 mmol/L, pH 6), paraffin sections and cryosections were incubated with 0.2% Triton-X/PBS (Sangon Biotech, Shanghai, China) diluted in 5% BSA (BOSTER, Wuhan, Hubei, China) for 30 minutes at room temperature. Slides were incubated with primary antibodies specific for Cyp2f2 (1:100, Santa Cruz Biotechnology), and CLCA3 (1:200; Abcam, Cambridge, Mass), and KI67 (1:200, eBioscience, Thermo Fisher Scientific) overnight at 4°C. After washing, the slides were incubated with fluorochrome-conjugated secondary antibodies (1:200; Invitrogen, Carlsbad, Calif) at room temperature for 2 hours. Nuclei were counterstained with 4'-6-diamidino-2-phenylindole, dihydrochloride (1:1000; Roche, Basel, Switzerland) for 20 minutes. Slides were then mounted with VECTA-SHIELD (Vector Laboratories, Burlingame, Calif) for 20 minutes at room temperature. Images of stained tissues and organoids were obtained using a DP80 inverted fluorescence microscope (Olympus).

EOS transplantation

Wild-type (WT) and *DJ-1* knockout (KO) mice were induced with OVA to trigger allergic lung inflammation, as described above. Lung tissues were harvested and digested with elastase to sort lung EOSs, as follows: Single-cell suspensions were incubated with primary antibodies, including CD11c-APC, Siglec-F-Alexa Fluor 488, and CD45-biotin (eBioscience, Thermo Fisher Scientific), followed by incubation with streptavidin APC-eFluor 780 (eBioscience, Thermo Fisher Scientific). Mouse lung CD45⁺Siglec F⁺CD11c⁺ EOSs were isolated as previously described.²⁷ Freshly sorted EOSs or *DJ-1*^{-/-} EOSs were intratracheally delivered to WT mice (5×10^5 cells per mouse). The mice were then sacrificed on day 5, and the lung tissues were harvested and fixed for further immunological staining.

Ultrasonic atomization inhalation of LTC₄, LTD₄, and LTE₄

LTC₄, LTD₄, and LTE₄ powder (Cayman, Ann Arbor, Mich) were dissolved in ethyl alcohol to prepare a stock solution (16 μ mol/L LTC₄, 20 μ mol/L LTD₄, and 20 μ mol/L LTE₄). Mice in the LTC₄ group inhaled aerosolized 16 nmol/L LTC₄ diluted in PBS for 1 hour daily on days 0, 1, 2, 3, and 4. Control group mice were forced to inhale PBS instead of LTC₄ using the same system. Mice in the LTD₄ and LTE₄ groups were subjected to a similar process, in which LTC₄ was replaced with 20 nmol/L LTD₄ and 20 nmol/L LTE₄, respectively. The day after the final aerosol inhalation, the mice were sacrificed. Lung tissue was fixed for further pathological examination or lysed using TRIzol reagent (Invitrogen) for the gene expression analysis.

RNA extraction and gene expression analysis

Lung tissues, sorted airway epithelial cells, EOSs, or organoids were lysed in TRIzol reagent (Invitrogen) to extract total RNA. Then, 0.8 μ g total RNA was reverse-transcribed into cDNA using HiScript III RT SuperMix (Vazyme, Nanjing, Jiangsu, China) following the manufacturer's instructions. Afterward, 1.5 μ L cDNA was subjected to real-time PCR using ChamQ Universal SYBR qPCR Master Mix (Vazyme) in a Roche LightCycler 96 real-time PCR system (Roche) with the following amplification conditions: 95°C for 2 minutes, followed by 40 cycles of 95°C for 10 seconds to denature cDNA, 60°C for 20 seconds for cDNA renaturation, and 72°C for 20 seconds for primer extension. The relative expression levels of target genes were normalized to β -actin using the $2^{-\Delta\Delta Ct}$ method. The primer sequences (see Table E3 in this article's Online Repository at www.jacionline.org) used to detect target gene expression were designed using the PrimerQuest Tool (Integrated DNA Technologies, Inc, Coralville, Iowa; available at <https://sg.idtdna.com/pages>) and are shown in the supplemental data.

Western blot

Frozen whole lungs were crushed using a homogenizer, and the cells were disrupted using an ultrasonic disintegrator. Total proteins were extracted using ice-cold radioimmunoprecipitation assay lysis buffer (BOSTER) containing a protease inhibitor. Then, 200 μ g of total protein from each sample was subjected to 12% SDS-PAGE and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, Calif). After the samples were washed and blocked with 5% nonfat milk, the membrane was incubated with the primary antibody DJ-1 (1:500, Santa Cruz Biotechnology). After washing in PBS with Tween 20 (PBST), the membrane was treated with a horseradish peroxidase-conjugated secondary antibody (goat anti-mouse IgG, Invitrogen, Thermo Fisher Scientific). Proteins were visualized using chemiluminescence reagents (5200 Multi, Tanon, Shanghai, China).

ELISA

The sputum specimens from patients with asthma were diluted 1:1 with PBS. The diluent was centrifuged at 2000g for 10 minutes to obtain the supernatant. A human PARK7 ELISA Kit (Abcam) was used to detect the level of PARK7 in the sputum specimens of patients with asthma, following the manufacturer's instructions.

scRNA-seq library construction and sequencing

After dissociating the lung tissue, cell viability was detected using a TC20 Automated Cell Counter (Bio-Rad). A cell viability >85% was required for the construction of a scRNA library. A total of 8000 cells per library were obtained, for which single-cell gel beads in emulsions were generated. Then, the input cells were converted to barcoded single-cell sequencing libraries using a Chromium Single Cell B Chip Kit (version 3, 10x Genomics, Pleasanton, Calif), Chromium Single Cell 3 Reagent Kits (version 3, 10x Genomics), Chromium Single Cell 3' gel beads in emulsion, and a Library and Gel Bead Kit (version 3, 10x Genomics) following the manufacturer's instructions. Libraries were sequenced with 150-bp paired-end reads on an Illumina NovaSeq sequencer by Novogene (Tianjin, China). The raw scRNA-seq data is available at GEO: GSE203079.

Single-cell gene expression quantification and determination of cell types

The FastQC (Babraham Bioinformatics, Cambridge, United Kingdom)-qualified raw reads of Read1 (containing the sequence of the 16 bp 10x Barcode and 12 bp unique molecular identifier [UMI]) and Read2 (the sequence of the cDNA fragment) were analyzed using the default parameters with Cell Ranger 3.0 (10x Genomics; <https://support.10xgenomics.com>). Alignment of reads to the reference genome and generation of gene-cell matrices were obtained after cell anger analysis. The gene-cell matrices loaded into the Seurat package (version 3.0) were subjected to the Seurat alignment method canonical correlation analysis²⁸ for data normalization, dimensionality reduction, clustering, and differential expression. All cells that satisfied ≥ 1 of the conditions were removed to ensure high data quality. For the OVA-challenged WT (WT-OVA) group, of cells that had either >4500 or <200 expressed genes, >20% UMI related to mitochondrial genome or >5% UMI related to hemoglobin. For the DJ-KO-OVA group, of cells that had either >5000 or <200 expressed genes, >26% UMI related to mitochondrial genome or >5% UMI related to hemoglobin. After filtering, a total of 10,508 cells (6020 and 4488 cells from the WT-OVA and DJ-1 KO-OVA groups, respectively) were used for downstream analysis. After the filtered data were normalized using "LogNormalize," using "FindVariableFeatures" calculated the highly variable genes by utilizing "mean.cutoff (0.0125–5) and dispersion cutoff (1, Inf)" parameters were used for principal component analysis. The number of principal components was confirmed using an Elbowplot function. A nonlinear dimensional reduction (*t*-distributed stochastic neighbor embedding, t-SNE) of the principal components was confirmed using the default settings of the RunTSNE function.

Cluster-specific marker genes between the 2 groups were analyzed using the edgeR package.²⁹ The cell-specific markers combined with cluster-enriched genes were used to identify the cell types in each cluster. Cluster-specific markers were manually and repeatedly checked using a loupe cell browser. Gene length bias-corrected marker genes were analyzed with the clusterProfiler R package to obtain Gene Ontology (GO) enrichment items between the WT and DJ-1 KO OVA groups.³⁰ GO terms were considered significantly enriched when *P* values were <.05. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (available <http://www.genome.jp/kegg/>) were used to examine the statistical enrichment of the marker genes using the clusterProfiler R package.³¹

Bulk RNA-seq library construction and sequencing

After scRNA-seq, 1×10^7 cells were lysed with TRIzol reagent to extract total RNA. The integrity and total RNA content were examined using a model 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif). A total of 0.4 μ g of RNA per sample was used for library construction with NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, Mass) following the manufacturer's instructions on an Illumina NovaSeq (Illumina, San Diego, Calif) sequencer by Novogene. The "insert size" of the RNA libraries was detected with an Agilent Bioanalyzer to ensure that the libraries met the quality requirements. The libraries were then sequenced on an Illumina Novaseq platform. The raw and processed bulk RNA-seq data reported in this paper is GEO: GSE207415.

Bulk RNA-seq data processing

Establishment of the mouse reference genome (mm9) and alignment of paired-end clean reads to mm9 were performed. After assembling mapped reads using StringTie (version 1.3.3b),³² the number of mapped reads for each gene was counted to calculate the fragments per kilobase per million (FPKM). Differential expression analysis between the WT-OVA and DJ-1 KO OVA groups was performed using the DESeq2 R package (version 1.20.0). Differentially expressed genes (DEGs) were determined with *P* values < .05. GO and KEGG enrichment was performed with clusterProfiler R package, and *P* values < .05 were considered significantly enriched.

Cell-cell communication analysis

To determine global communication among cells, intercellular communication networks were analyzed and visualized using scRNA-seq data with CellChat (<https://github.com/sqjin/CellChat>).³³ Genes expressed in >10% of cells of any type were further evaluated. A ligand-receptor pair with a *P* value < .05, was considered an existing cell-cell communication. For the EOS club cell communication analysis, only selected genes are shown in the bubble plot. EOS and club cell interaction scores were calculated using modules exploited by Novogene.³⁴⁻³⁷ The following equation was used to calculate this score:

$$\text{Interaction score (receptor, ligand, cell type 1, cell type 2)} = \frac{1}{n_{\text{cell type 1}}} \sum_{i \in \text{cell type 1}} e_{i,\text{receptor}} \times \frac{1}{n_{\text{cell type 2}}} \sum_{j \in \text{cell type 2}} e_{j,\text{ligand}},$$

where e_{ij} = expression of gene *j* in cell *i* and n_c = number of cells of cell type *c*.

Statistical analysis

Data were collected from 3 independent experiments and expressed as the mean ± SD. A 2-tailed unpaired Student *t*-test was used to measure the differences in variables between the experimental and control groups by calculating the *P* values. Statistical significance was set at **P* < .05, ***P* < .01, ****P* < .001.

RESULTS

Lack of DJ-1 mitigates OVA-induced allergic airway inflammation

DJ-1 has been suggested to be associated with tissue inflammation and injury.^{20,38} Here, we found that DJ-1 levels were higher in sputum samples from patients with asthma than in healthy controls (see Fig E1 in this article's Online Repository at www.jacionline.org). We adopted an OVA-induced airway allergic inflammation mouse model (Fig 1, A). In this model, IL-13, as opposed to IL-5, was elevated in BALF after the OVA challenge (Fig 1, B). Similarly, remarkably elevated DJ-1 levels were observed in the BALF and lungs of mice during OVA-induced allergic airway inflammation (Fig 1, C and D). Total lung cells from 3 randomly selected PBS-treated WT mice or OVA-treated WT mice were pooled for scRNA-seq analysis (Fig 1, E). As a result, 4197 cells were collected from the lungs of the PBS-treated WT group and 6020 cells were collected from the WT-OVA group. We identified 6 different epithelial cell types: club, ciliated, goblet, basal, AT2, and AT1 cells (Fig 1, F, and see Fig E2 in this article's Online Repository at www.jacionline.org). Within the non-epithelial cell population, we identified 9 different cell types: fibroblasts, EOSs, neutrophils, innate lymphoid cells (ILCs), macrophages, natural killer (NK)

cells, T cells, B cells, and endothelial cells (Fig 1, F, Fig E2). We found that DJ-1 expression was increased in club cells and EOSs and decreased in fibroblasts, macrophages, T cells, NK cells, and ILCs in the lung during OVA-induced airway allergic inflammation (Fig 1, G and H).

To examine the functional importance of DJ-1 in airway inflammation, DJ-1 KO mice were used. As expected, the numbers of EOSs and neutrophils were significantly higher in the BALF of OVA-challenged mice than in the BALF of control mice (Fig 2, A). Alleviated inflammatory infiltration was observed in DJ-1 KO mice compared to control mice, as the numbers of EOSs and neutrophils were significantly reduced in BALF after OVA (Fig 2, A). IL-13, but not IL-5, was decreased in the BALF of DJ-1 KO mice after the OVA challenge (Fig 2, B). Quantitative PCR analysis indicated that mRNA levels of IL-4, IL-6, IL-9, IL-10, and IL-31 in the lung remained unchanged in the absence of DJ-1 during OVA-induced allergic inflammation (see Fig E3 in this article's Online Repository at www.jacionline.org). In addition, based on histological staining,

a reduced abundance of mucus-producing cells was also observed in the airways of DJ-1 KO mice (Fig 2, C). Airway epithelial progenitor club cells express markers such as Cyp2f2; Clca3 levels are an indicator of goblet cell differentiation. The percentage of Clca3⁺ cells was significantly reduced in the airways of DJ-1 KO mice compared to that in control mice after OVA-induced allergic airway inflammation (Fig 2, D). These data suggest that DJ-1 is associated with goblet cell differentiation and EOS recruitment during OVA-induced allergic airway inflammation.

Club cell-EOS crosstalk is attenuated in the absence of DJ-1

Next, we conducted scRNA-seq profiling to analyze the association between EOS infiltration and goblet metaplasia. After OVA challenge, total lung cells from 3 randomly selected WT mice or DJ-1 KO mice were pooled for scRNA-seq analysis (Fig 3, A). Six epithelial cell types and 9 non-epithelial cell populations were identified (Fig 3, B, and see Fig E4, A and B in this article's Online Repository at www.jacionline.org). Consistent with the observations above (Fig 2, A), a similar decrease was noted in the proportion of EOSs in the lungs of DJ-1 KO-OVA mice (1.6%) as compared to the WT-OVA mice (2.5%) (Fig 3, C). Similarly, a decrease was observed in the fraction of goblet cells or neutrophils in the lungs of DJ-1 KO-OVA group compared to those from the WT-OVA group (Fig 3, C). However, the fraction of T cells was increased in the lungs in the absence of DJ-1 after the OVA challenge (Fig 3, C). Macrophages can be divided into 2 distinct polarization states: classically activated phenotype (M1) and alternatively activated phenotype (M2). Alveolar macrophages (*Itgax*⁺, *Cd200r1*⁺) and monocyte-derived macrophages (*Apoe*⁺, *Cx3cr1*⁺) were observed in the lungs during OVA-induced airway

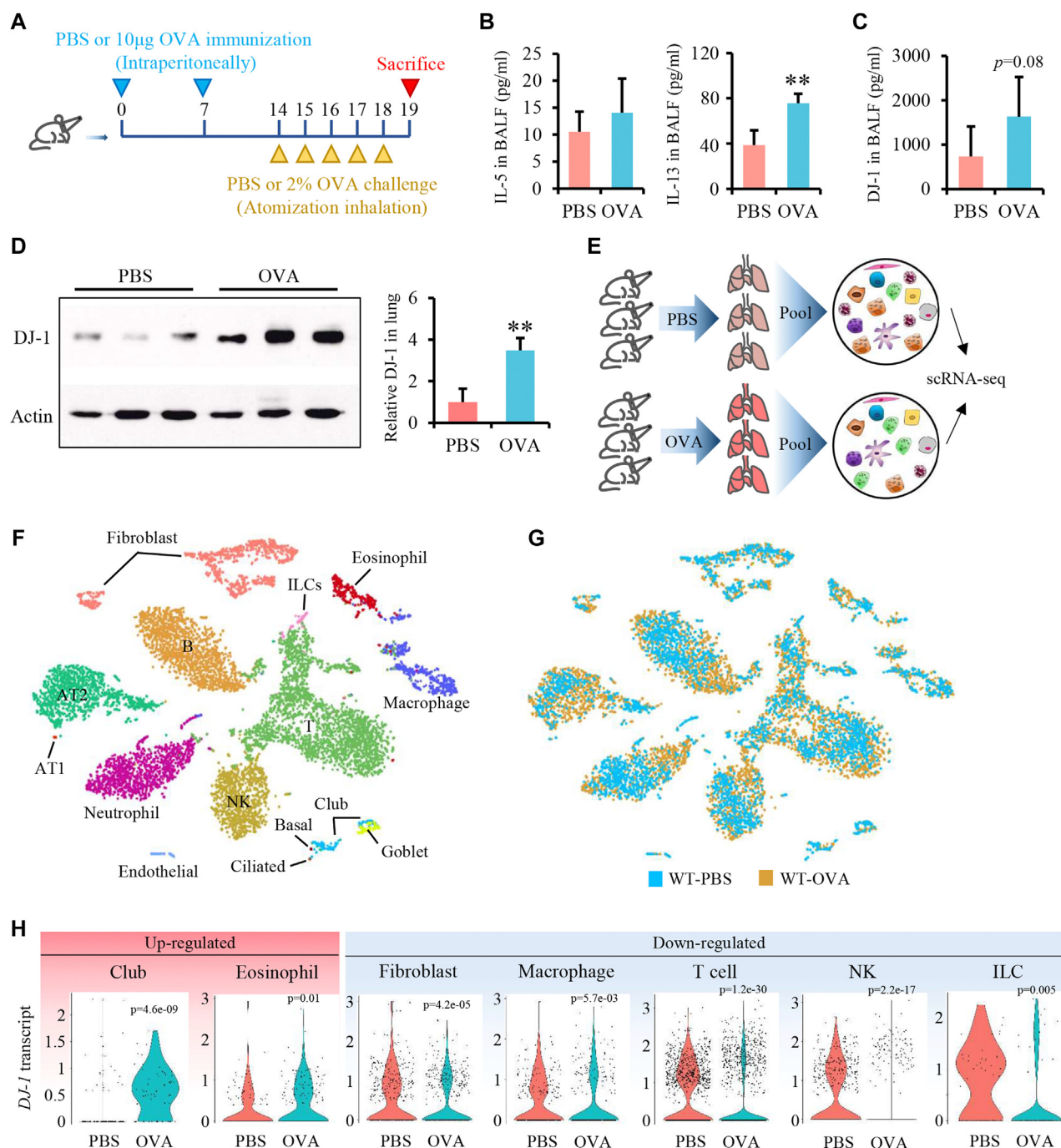


FIG 1. Altered expression of *DJ-1* during OVA-induced airway allergic inflammation. **(A)** Schematic of the OVA-induced allergic lung inflammation model. **(B)** ELISA analysis of IL-5 and IL-13 in BALF from control ($n = 3$) and OVA-challenged ($n = 3$) mice. **(C)** ELISA analysis of DJ-1 in BALF. **(D)** Western blot analysis of DJ-1 expression in lung tissues. **(E)** Schematic representation of the study flowchart of scRNA-seq. Total lung cells isolated from 3 randomly selected WT mice treated with PBS or OVA were pooled to construct a scRNA library. **(F)** *t*-Distributed stochastic neighbor embedding (*tSNE*) plots of 10,217 cells with different cell types isolated from lungs of WT mice treated with PBS or OVA. **(G)** Sample origin. Blue: PBS-treated WT (WT-PBS) group. Yellow: WT-OVA group. **(H)** scRNA-seq analysis showed violin plots depicting DJ-1 expression in different lung cells isolated from PBS-treated or OVA-challenged mice. $^{**}P < .01$.

allergic inflammation (Fig 3, D). Both alveolar and monocyte-derived macrophage populations contained the M1 phenotype (Tnf^+ , $Cxcl2^+$, $Arg1^-$, $Retnla^-$, $Chil3^-$) and M1/M2 phenotype (Tnf^+ , $Cxcl2^+$, $Arg1^+$, $Retnla^+$, $Chil3^+$). More alveolar M1/M2 macrophages were observed in the lungs of DJ-1 KO mice than

in those from WT mice after the OVA challenge (Fig 3, D). We found 3 subsets of T cells: naïve T cells ($Sell^+$, $Ccr7^+$), T_H cells ($CD4^+$), and cytotoxic T cells ($CD8a^+$) in the lungs after the OVA challenge. Compared to WT mice, DJ-1 KO mice displayed more cytotoxic T cells in the lungs during OVA-induced airway

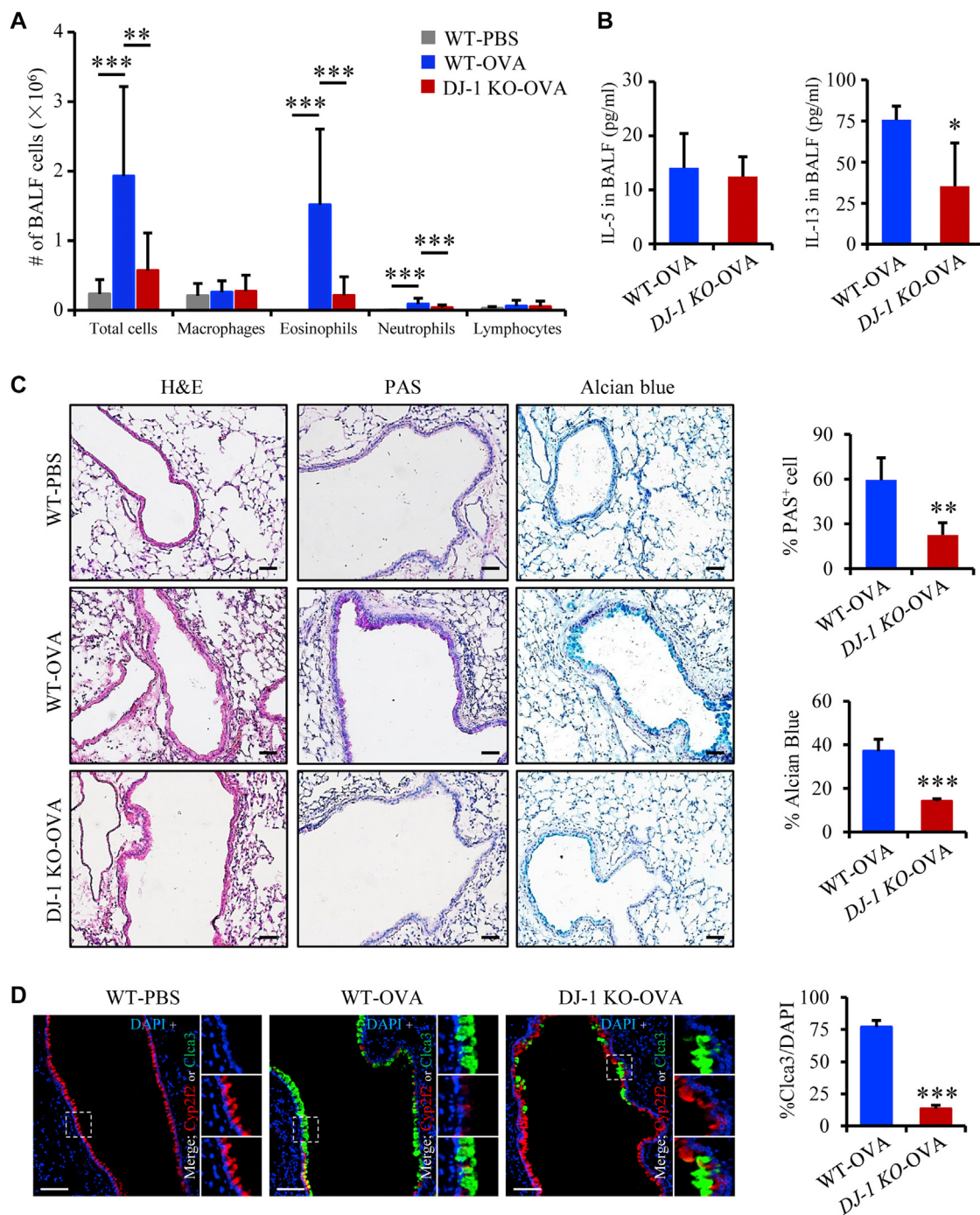


FIG 2. Loss of *DJ-1* attenuates OVA-induced allergic lung inflammation. **(A)** Infiltrated inflammatory cells in BALF harvested from the control ($n = 12$) or OVA-challenged WT ($n = 10$) or *DJ-1* KO ($n = 10$) mice were quantified using Hema 3 staining. **(B)** ELISA analysis of IL-5 and IL-13 in BALF. **(C)** Hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Alcian blue staining of lungs from control, OVA-challenged WT, or *DJ-1* KO mice. Bars = 50 μ m. **(Right)** Bar graphs represent quantification of PAS-positive cells or Alcian blue-positive cells in total lung epithelial cells of OVA-challenged WT ($n = 4$) and *DJ-1* KO ($n = 3$) mice. **(D)** Immunofluorescence staining of lungs from controls or OVA-challenged WT or *DJ-1* KO mice. Bar = 50 μ m. **(Right)** Bar graphs represent quantification of Clca3⁺ cells in the total epithelial cells from the lungs of OVA-challenged WT ($n = 3$) and *DJ-1* KO ($n = 3$) mice. Results represent the mean $\pm$ SD. * $P < .05$; ** $P < .01$; *** $P < .001$; using Student *t*-test. DAPI, 4'-6-diamidino-2-phenylindole, dihydrochloride.

allergic inflammation (Fig 3, D). Three clusters of B cells were identified in the lung: B1 cells (*Spn*⁺, *Cd5*⁺, *Cd27*⁺), mature B cells (*Tnfrsf13c*⁺, *Ms4a1*⁺), and activated B cells (*Fcgr2a*⁺). It

was observed that all 3 subsets of B cells were diminished in the lungs of *DJ-1* KO mice as compared to that of WT mice during OVA-induced airway allergic inflammation (Fig 3, D). We found

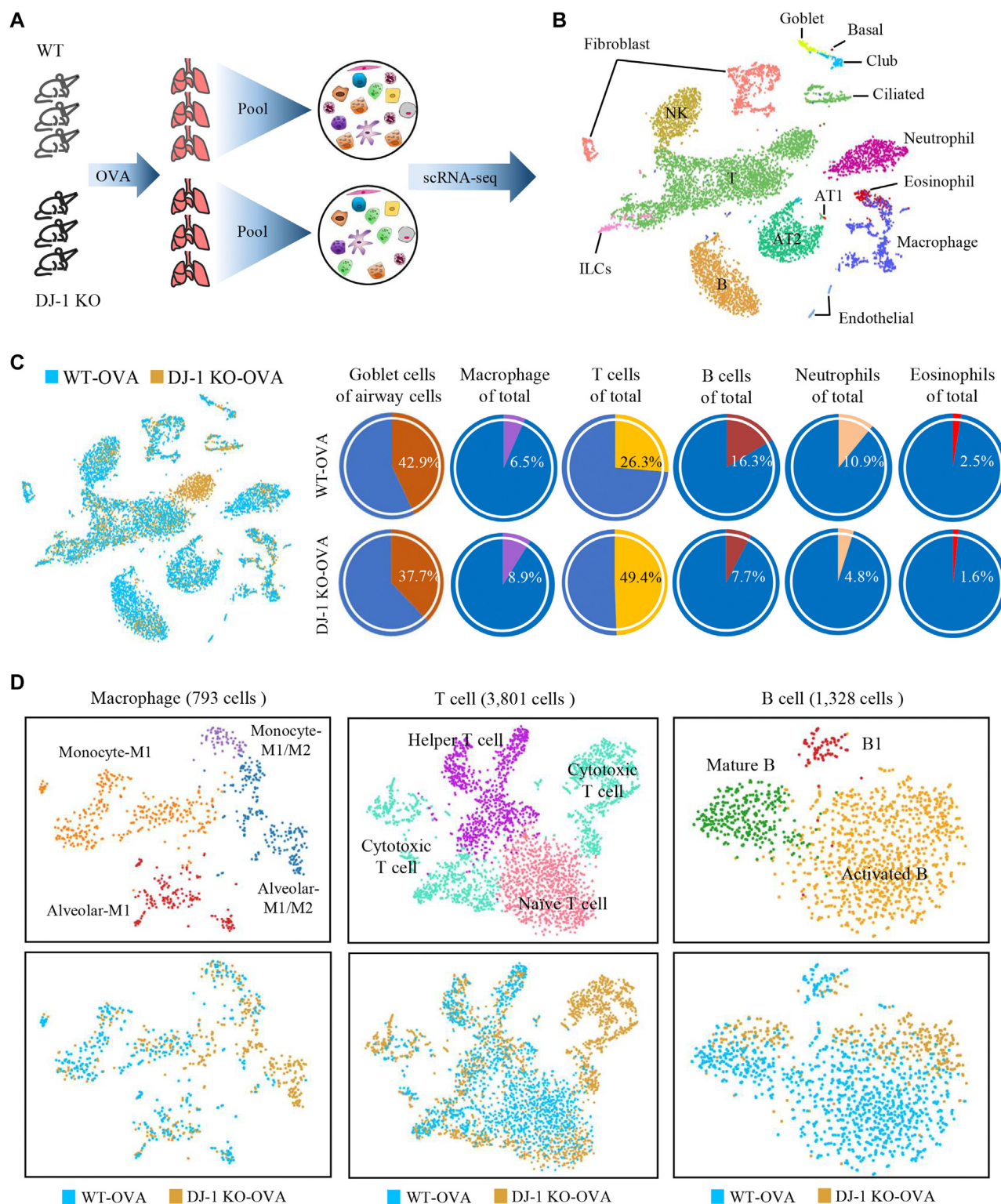


FIG 3. scRNA-seq profiling of lung cells after OVA challenge. **(A)** Schematic representation of the study flowchart. After OVA challenge, total lung cells isolated from 3 randomly selected WT mice or *DJ-1* KO mice were pooled to construct an scRNA library. **(B)** tSNE plots of 10,508 cells with different cell types. **(C)** Sample origin. Blue: WT-OVA group. Yellow: DJ-1 KO-OVA group. Pie chart shows the percentages of macrophage, T cells, B cells, neutrophils, or EOSs as a percentage of total lung cells and goblet cells as a percentage of airway epithelial cells in the WT-OVA and *DJ-1* KO-OVA mice, respectively. **(D)** tSNE plot of macrophages, T cells, or B cells, color-coded by the indicated cell types (top) or the sample type of origin (bottom).

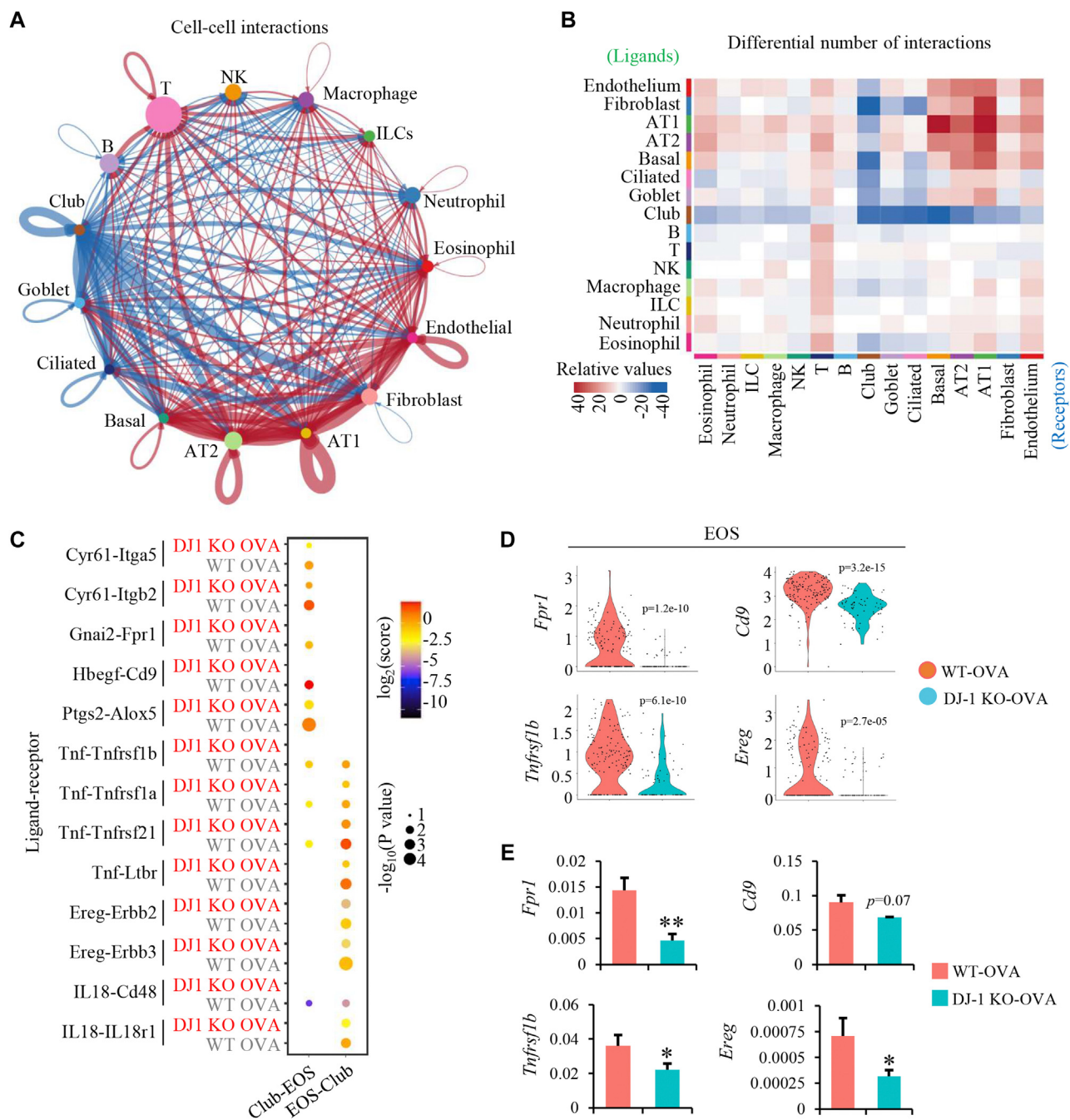


FIG 4. Attenuated EOS-club interactions in the absence of *DJ-1*. **(A)** Network of putative ligand-receptor pairs showing the cell-cell differential interactions across all cell types in *DJ-1* KO-OVA lungs compared to those in WT-OVA lungs. The colors of the dots represent individual cell types. The size of the dot is relative to the number of cells of each type. *Red line*: increased cell-cell interactions in lungs of *DJ-1* KO-OVA mice. *Blue line*: decreased cell-cell interactions in lungs of *DJ-1* KO-OVA mice. The weight of the connecting lines from thin to thick indicates the counts of cell-cell interaction pairs from low to high. All the *arrows* are pointing to the receptors. **(B)** Heatmap showing differences in the numbers of putative ligand-receptor pairs in each cell type. The color key from *blue* to *red* indicates the counts of putative ligand-receptor pairs from a decrease to an increase in *DJ-1* KO-OVA lungs compared to WT-OVA lungs. **(C)** Dot plot analysis showing EOS-club cell interactions between *DJ-1* KO-OVA lungs and WT-OVA lungs. **(D)** Violin plot depicting expressions of ligands and receptors as indicated in EOS. **(E)** Validation of the expression of genes indicated in **(D)** by qPCR analysis of EOSs isolated from OVA-challenged WT mice or OVA-challenged *DJ-1* KO mice. Results represent the mean $\pm$ SD. * $P < .05$, ** $P < .01$; using Student *t*-test.

only 1 cluster of neutrophils showing high expression of activated genes, including *Itgb2*, *Cd24a*, *Itgam*, *Cxcr2*, and *Il17ra* (see Fig E5, A in this article's Online Repository at www.jacionline.org).

The abundance of activated neutrophils was reduced in the lungs of *DJ-1* KO mice compared to WT mice after OVA challenge (Fig E5, B).

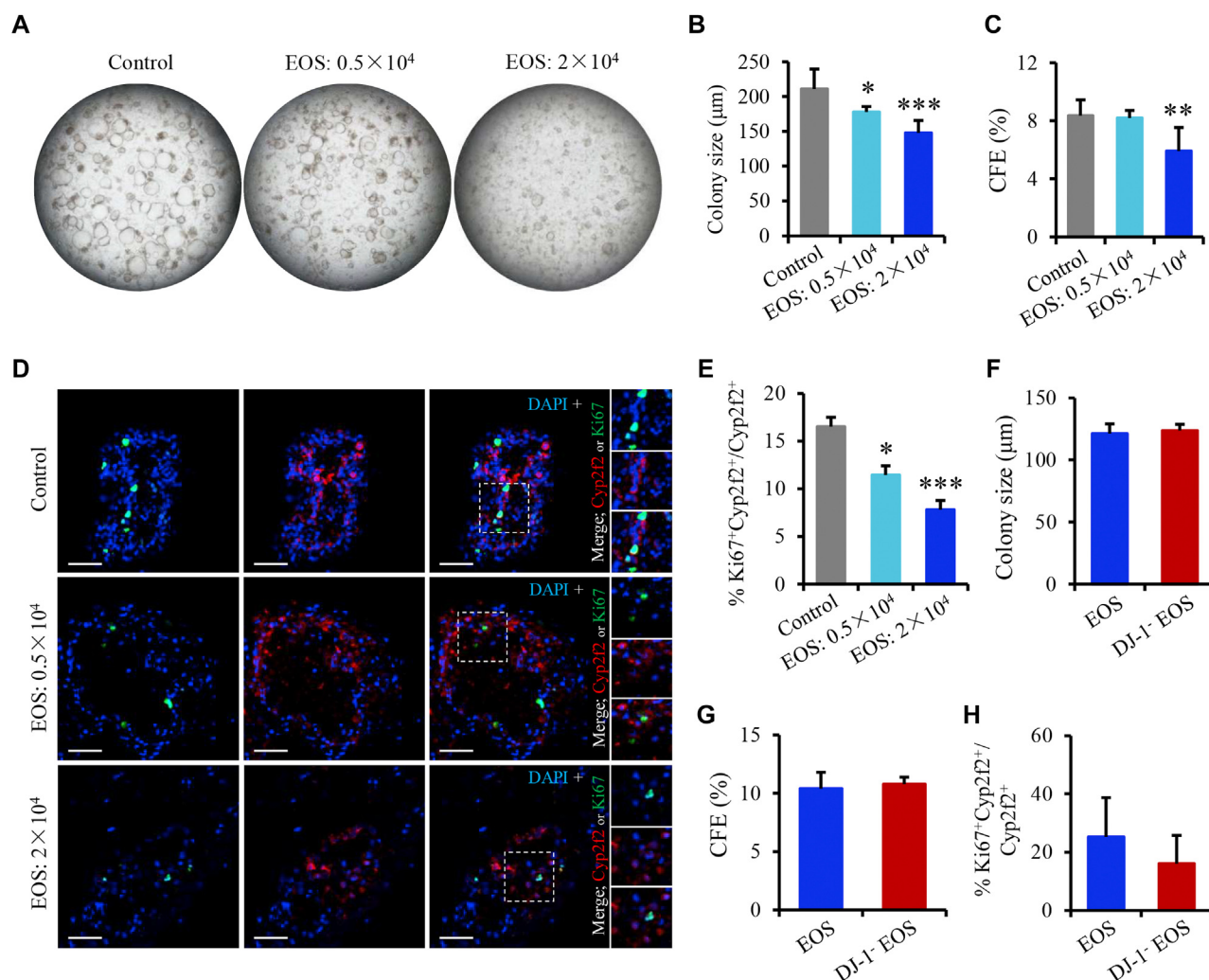


FIG 5. EOSs inhibit club cell proliferation in organoid culture. **(A)** Representative images of EOS and club cell organoid co-cultures at day 8 after plating. Bar = 500 μm . **(B)** Diameter of colonies in club cells co-cultured with 5×10^3 EOSs and 2×10^4 EOSs from WT-OVA mice at day 8 after plating. **(C)** CFEs of club cultures under conditions described in **(B)**. **(D)** Immunofluorescence staining of EOS and club cell organoid co-cultures at day 8 after plating. Bars = 50 μm . **(E)** Quantification of Ki67⁺Cyp2f2⁺ cells in the total Cyp2f2⁺ cells for EOS and club organoid co-cultures. **(F)** The diameters of colonies in club cells co-cultured with 2×10^4 EOSs from WT-OVA mice or DJ-1 KO-OVA mice. **(G)** CFEs of club cell cultures under conditions described in **(F)**. **(H)** Quantification of Ki67⁺Cyp2f2⁺ cells in the total Cyp2f2⁺ cells for EOS and club cell organoid co-cultures in which EOSs were sorted from WT-OVA mice or DJ-1 KO-OVA mice. Results represent the mean $\pm$ SD. * $P < .05$; ** $P < .01$; *** $P < .001$; using Student t-test.

To further understand the effects of DJ-1 on cellular crosstalk during OVA-induced allergic inflammation, we analyzed the cell-cell interactions between immune cells and other types of pulmonary cells (Fig 4, A). We found a decline in the number of predicted cell-cell interactions between EOSs and club cells in DJ-1 KO mice during OVA-induced airway allergic inflammation (Fig 4, B). In addition, club cell interactions with neutrophils, ILCs, macrophages, and NK cells declined in the absence of DJ-1 after the OVA challenge (see Fig E6, A in this article's Online Repository at www.jacionline.org). Furthermore, EOS interactions with goblet cells declined (Fig E6, B). Analysis of these cell-cell interactions indicated that cell chemotaxis and leukocyte migration were reduced in DJ-1 KO mice during OVA-induced airway allergic inflammation (Fig E6, A and B). The expression of *Fpr1*, *Cd9*, and *Tnfrsf1b* in EOSs, which are receptors for chemotactic ligands *Gnai2*, *Hbegf*, and *Tnf*, respectively, were

significantly downregulated in DJ-1 KO-OVA as compared to WT-OVA (Fig 4, C and D). In addition, the expression of epithelial proliferative and differentiation modulator *Ereg*³⁹ was significantly decreased in EOSs, and interactions with their receptors on club cells were reduced in the absence of DJ-1 (Fig 4, C and D). In addition, the mRNA expression of *Fpr1* was reduced in macrophages in the lungs of DJ-1 KO mice as compared to WT mice after the OVA challenge (see Fig E7 in this article's Online Repository at www.jacionline.org). The expression of *Cd9* decreased in ILCs but increased in macrophages in the lungs in the absence of DJ-1 after the OVA challenge (Fig E7). Loss of DJ-1 resulted in a reduction in the expression of *Tnfrsf1b* in neutrophils, macrophages, and NK cells in the lungs after the OVA challenge (Fig E7). Altogether, these data suggest that DJ-1 may be required for club cell-EOS crosstalk during OVA-induced allergic inflammation.

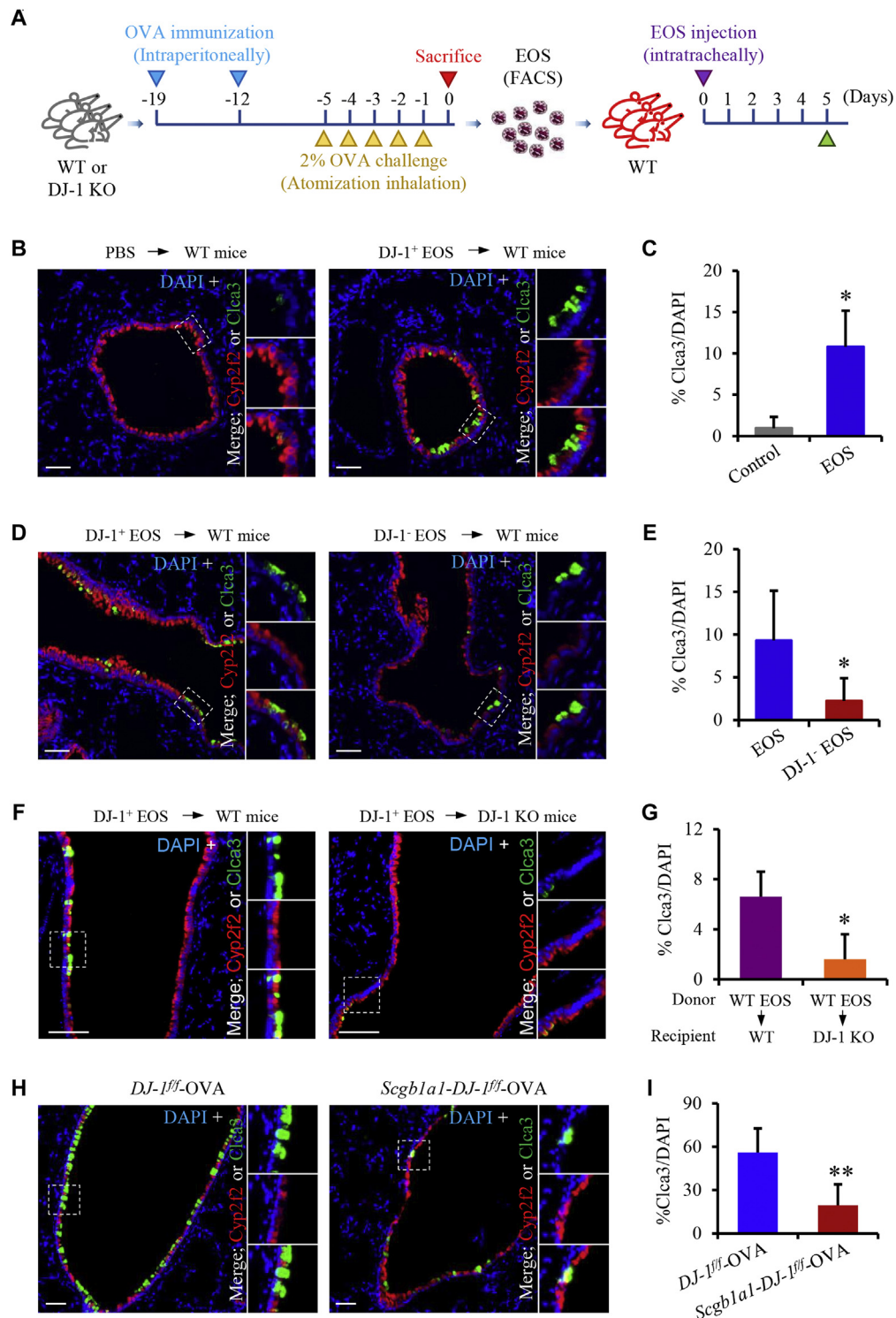


FIG 6. Loss of *DJ-1* in EOSs reduces goblet cell differentiation. **(A)** Schematic of the animal model used. Mice were challenged with OVA to induce EOS infiltration. Then, EOSs from donor mice were transplanted to the airways of receptor mice via noninvasive intratracheal instillation. **(B,C)** Immunofluorescence staining and quantification of Clca3⁺ cells in WT lungs that received intratracheal instillation of PBS or EOS collected from airways of OVA challenged WT mice. **(D,E)** Immunofluorescence staining and quantification of Clca3⁺ cells in WT lungs that received intratracheal instillation of EOS (collected from airways of OVA-challenged WT mice) or DJ-1⁻ EOS (collected from airways of OVA-challenged DJ-1 KO mice). **(F,G)** Immunofluorescence staining and quantification of Clca3⁺ cells in WT lungs that received intratracheal instillation of EOS (collected from airways of OVA-challenged WT mice) or DJ-1 KO mice that received intratracheal instillation of EOS (collected from airways of OVA-challenged WT mice). **(H,I)** Immunofluorescence staining and quantification of Clca3⁺ cells in *DJ-1^{ff}* lungs or *Scgb1a1^{CreER};DJ-1^{ff}* mice after OVA challenge. Results represent the mean ± SD. **P* < .05. ***P* < .01. using Student *t*-test. Bars = 50 μm.

EOSs inhibit club cell proliferation independent of DJ-1

We established an EOS and club cell organoid co-culture model to assess the role of EOSs in club cell proliferation (Fig 5, A). We found that co-culture with EOSs reduced the CFE and size of club cell–derived organoids in a dose-dependent manner (Fig 5, B and C). Consistent with this finding, immunofluorescence staining showed a decreased ratio of proliferative Cyp2f2⁺KI67⁺ club cells to Cyp2f2⁺ club cells in the EOS/club cell organoid co-cultures compared to club cell organoid cultures alone (Fig 5, D and E). We further investigated whether DJ-1 is involved in the regulation of club cell proliferation by EOSs. There was no significant difference in CFE and organoid size in club cell–derived organoid cultures between *DJ-1*[−] and *DJ-1*⁺ EOSs (Fig 5, F and G). The loss of DJ-1 also had no effect on the proportion of Cyp2f2⁺KI67⁺ club cells from the total number of club cells (Fig 5, H). Altogether, these data suggest that EOSs may suppress club cell proliferation independent of DJ-1.

EOS-derived DJ-1 promotes goblet cell differentiation

To determine the effect of EOSs on club cell differentiation, we sorted EOSs from the lungs after OVA-induced allergic airway inflammation and intratracheally transplanted them (Fig 6, A). Immunofluorescence staining of the lung tissues of the recipient WT mice indicated that the abundance of Clca3⁺ goblet cells increased after EOS transplantation (Fig 6, B and C). *DJ-1*–deficient EOSs isolated from the lungs of *DJ-1* KO mice after OVA challenge induced airway Clca3 expression to a lesser extent than *DJ-1*–sufficient EOSs did (Fig 6, D and E). We transferred DJ-1⁺ EOSs to the lungs of WT and DJ-1 KO mice and found that the airway of recipient DJ-1 KO mice displayed fewer Clca3⁺ cells than those of the recipient WT mice did (Fig 6, F and G). This suggests that non-EOS-derived DJ-1 is also required for airway goblet cell differentiation. We then challenged *DJ-1*^{fl/fl} or *Scgb1a1*^{CreER};*DJ-1*^{fl/fl} mice (specific knockout of DJ-1 in airway club cells) with OVA and found fewer Clca3⁺ cells in the airways of these mice (Fig 6, H and I). These data suggest that club cell–derived DJ-1 drives goblet cell differentiation. These data suggest that DJ-1 in both airway club cells and EOSs enhances airway goblet cell differentiation.

LTs regulate airway goblet cell differentiation

To reveal the underlying mechanism by which DJ-1 in EOSs contributes to club cell differentiation into goblet cells, we analyzed the biological function of EOSs using GO analysis. We performed GO analysis on all DEGs between the DJ-1 KO-OVA and WT-OVA groups (Fig 7, A, and see Fig E8, A in this article's Online Repository at www.jacionline.org). Insulin is known to aggravate the inflammatory response^{40,41} and IFN-γ is necessary for the inhibition of airway EOSs.⁴² Negative regulation of cellular responses to insulin stimuli and responses to IFN-γ was significantly enriched in the GO analysis of upregulated DEGs (Fig 7, A). In contrast, LT metabolic processes were enriched in the downregulated DEGs (Fig 7, A). GO analysis suggested that eosinophilic airway inflammation was alleviated in *DJ-1* KO mice after OVA challenge. The expression of *Ltc4s*, *Alox5*, and *Alox5ap*, which are key enzymes in the conversion

of arachidonic acid into cysteinyl LTs, was significantly downregulated in EOSs from *DJ-1* KO-OVA mice compared with EOSs from WT-OVA mice (Fig 7, B). EOSs were the prominent cell type expressing *Ltc4s* and *Alox5* in the lungs (Fig 7, B). KEGG analysis of lung bulk RNA-seq indicated that arachidonic acid metabolism was enriched in the downregulated DEGs between *DJ-1* KO-OVA and WT-OVA mice (Fig 7, C, Fig E8, B and C). Lung-specific expression of *Ltc4s* was significantly downregulated, as observed via bulk RNA-seq from *DJ-1* KO-OVA versus WT-OVA mice (Fig 7, D). Quantitative PCR (qPCR) analysis revealed that *Ltc4s* was downregulated in the lung tissue of *DJ-1* KO-OVA mice compared to that in WT-OVA mice (Fig 7, E). These findings suggest that *DJ-1* KO inhibited the expression of *Ltc4s* in EOSs after OVA-induced allergic airway inflammation.

It is widely known that eosinophilic airway inflammation can be amplified by LTC₄, LTD₄, and LTE₄, which originate from arachidonic acid via *Ltc4s* catalyzation.⁴³ We next explored the role of cysteinyl LTs in the regulation of club cell function. Organoid culture indicated that the CFE of club cells was barely affected in the presence of LTC₄, LTD₄, or LTE₄ (see Fig E9 in this article's Online Repository at www.jacionline.org). However, the expression of goblet cell markers, including *Clca1* (CLCA3-coding gene), *Muc5ac*, and *Spdef*, was promoted in organoid cultures of club cells in the presence of LTC₄ (Fig 8, A–C). Furthermore, immunofluorescence staining for Clca3 showed an uptrend in goblet cell abundance in the lung after aerosol inhalation of LTC₄ (Fig 8, D and E). Similarly, LTD₄ and LTE₄ also promoted the differentiation of club cells into goblet cells in *in vitro* organoid cultures and *in vivo* (Fig 8, F–O). These data suggest that cysteinyl LTs can accelerate goblet cell differentiation from club cells.

DISCUSSION

In this study, we showed that DJ-1 levels are increased in the sputum of patients with asthma and in the lung tissue of OVA-induced allergic mice. Using *DJ-1* KO mice, we found that *DJ-1* deficiency significantly decreased OVA-induced EOS filtration and goblet cell metaplasia in the airways. scRNA-seq suggested that DJ-1 is involved in the crosstalk between airway club cells and EOSs. In turn, the recruited EOSs abrogated the proliferation potential of club cells independent of DJ-1. However, EOS-derived DJ-1 favors the metabolism of arachidonic acid into cysteinyl LTs, which may interact with club cells and promote goblet cell differentiation *in vitro* and *in vivo*.

EOS infiltration and airway epithelial barrier dysfunction are typical pathological airway alterations associated with asthma.⁴⁴ Many investigations have reported that airway epithelial cells initiate the recruitment of EOSs by upregulating the expression of chemotactic factors and type 2 cytokines.⁴⁵ We revealed that DJ-1 mediates the crosstalk between EOSs and club progenitor cells and orchestrates airway allergy-related inflammation. EOSs seem to impair the proliferation potential of club cells. We previously demonstrated that a diminished pool of airway progenitor cells abrogates the regenerative capacity of the airway epithelium.¹² The long-term retention of EOSs in the airway may cause damage to the lung epithelium. This proposition supports the concept that an injured and disrupted airway epithelium contributes to a more chronic and persistent inflammatory phenotype in

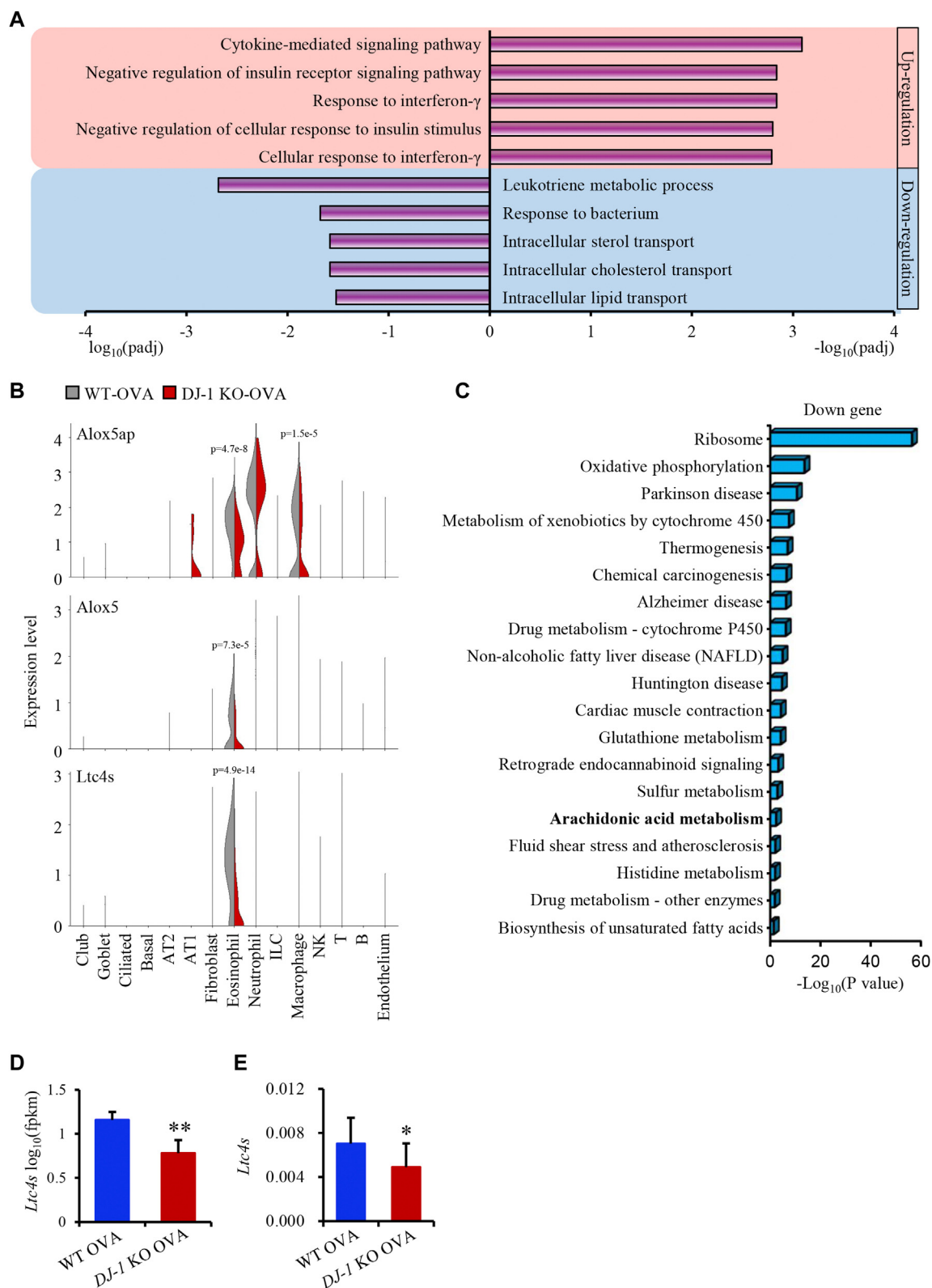


FIG 7. DJ-1 regulates arachidonic acid metabolism in EOSs after OVA-induced allergic airway inflammation. **(A)** Histogram showing top the 5 terms of the GO analysis of upregulated (*top*) or downregulated (*bottom*) DEGs between DJ-1 KO-OVA and WT-OVA EOSs. **(B)** Violin plot showing expressions of *Alox5ap*, *Alox5*, and *Ltc4s* in each cell type. **(C)** Histogram showing KEGG terms enrichment analysis ($P < .05$) of downregulated DEGs between DJ-1 KO-OVA and WT-OVA lungs as determined via bulk RNA-seq. **(D)** Bulk RNA-seq analysis of *Ltc4s* expression in lungs of WT-OVA and DJ-1 KO-OVA. **(E)** qPCR analysis of *Ltc4s* expression in the lungs of WT-OVA and DJ-1 KO-OVA mice. Results represent the mean $\pm$ SD. * $P < .05$; ** $P < .01$ using Student *t*-test.

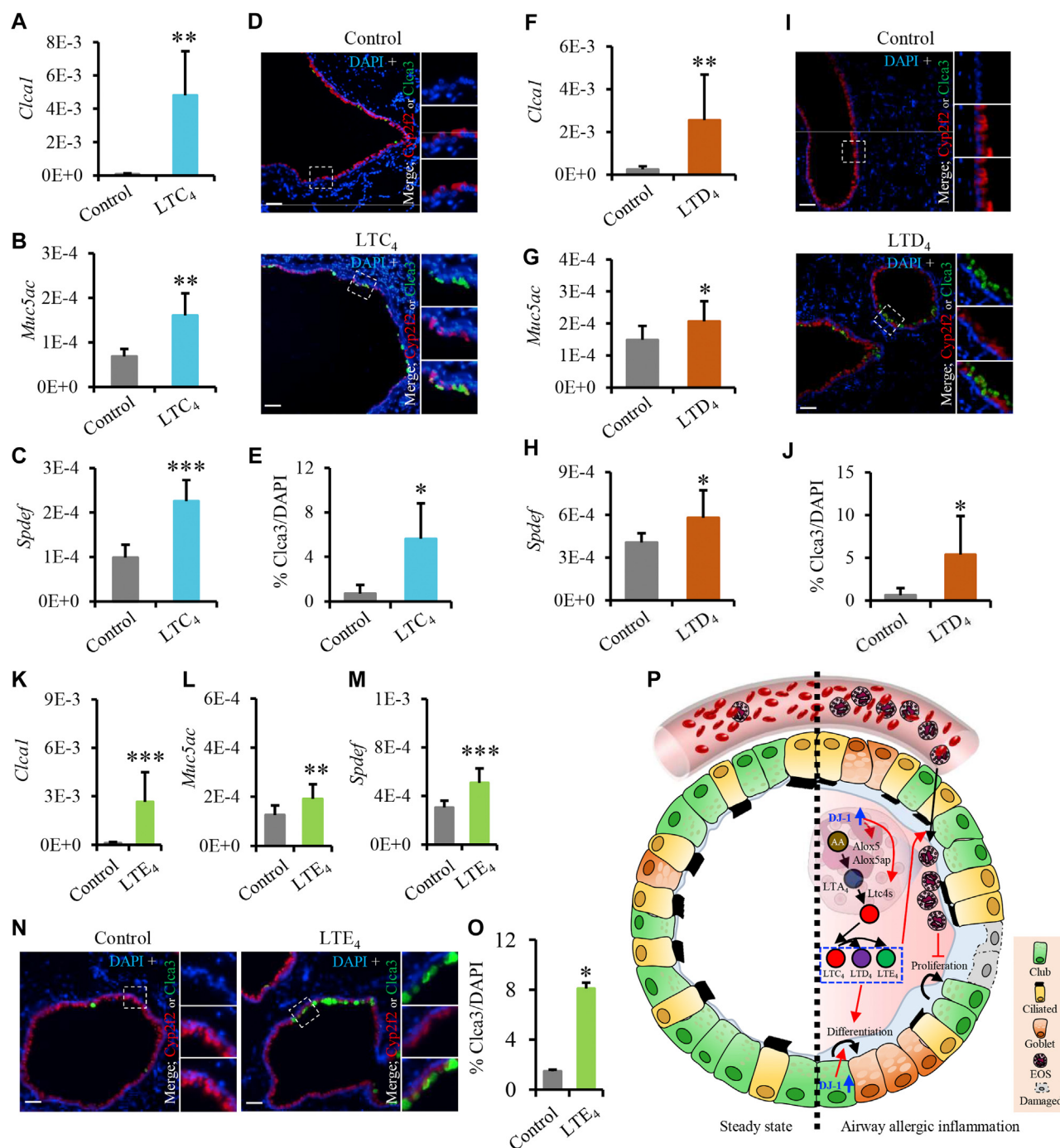


FIG 8. LTs promote airway goblet cell differentiation. (A–C) qPCR analysis of *Clca1* (A), *Muc5ac* (B), and *Spdef* (C) expressions (relative to β -actin) in organoid cultures of club cells in the presence of LTC₄ (16 nmol/L) at day 8 after seeding. (D) Immunofluorescence staining of the lungs of LTC₄ aerosol-treated mice. Bars = 100 μ m. (E) Quantification of CLCA3⁺ cells in the airways of LTC₄ aerosol-treated WT mice. (F–H) qPCR analysis of *Clca1* (F), *Muc5ac* (G), and *Spdef* (H) expressions (relative to β -actin) in organoid cultures of club cells in the presence of LTD₄ (20 nmol/L) at day 8 after seeding. (I) Immunofluorescence staining of the lungs of LTD₄ aerosol-treated WT mice. Bars = 50 μ m. (J) Quantification of CLCA3⁺ cells in the airways of LTD₄ aerosol-treated mice. (K–M) qPCR analysis of *Clca1* (K), *Muc5ac* (L), and *Spdef* (M) expressions (relative to β -actin) in organoid cultures of club cells in the presence of LTE₄ (20 nmol/L) at day 8 after seeding. (N) Immunofluorescence staining of the lungs of LTE₄ aerosol-treated WT mice. Bars = 50 μ m. (O) Quantification of CLCA3⁺ cells in the airways of LTE₄ aerosol-treated mice. (P) Schematic model dissecting the role of DJ-1-mediated EOS-club interaction during airway allergic inflammation in mice. Results represent the mean $\pm$ SD. * P < .05; ** P < .01, *** P < .001; using Student t -test.

patients with asthma.^{46,47} Resident EOSs were discovered in the lung and showed an inhibitory role in T_H2 cell responses to inhaled allergens.⁴⁸ Whether the interaction between resident EOSs and club cells differs from that of recruited EOSs and club cells during airway allergic inflammation is unknown. Club cell necroptosis exacerbates house dust mite-induced allergic inflammation in mice.⁴⁹

DJ-1 is a driver of eosinophilic inflammation in the mouse model of allergic asthma. *DJ-1* loss appears to be beneficial against airway allergic inflammation as the number of EOSs and the abundance of goblet cells was reduced in *DJ-1*-deficient mice after OVA sensitization and challenge. DJ-1 is required for EOSs to promote club cell differentiation into goblet cells, but not club cell proliferation. In club cells, the loss of DJ-1 suppresses the differentiation of club cells into goblet cells after the OVA challenge. In EOSs, DJ-1 may accelerate arachidonic acid production and secretion of LTC₄, LTD₄, and LTE₄. These LTs are known to contribute to EOS recruitment.⁵⁰⁻⁵² Our data suggest that they also promote airway goblet cell fate in *in vitro* organoid cultures and in *in vivo* animal models, supporting previous findings.⁵³⁻⁵⁵

In the lung, DJ-1 appears to be a double-edged regulator, and its regulation is cell-specific. *DJ-1* deficiency amplifies antigen-induced degranulation and the production of both types 1 and 2 cytokines in bone marrow-derived mast cells.¹⁹ Direct binding of Lyn to DJ-1 is important for full Lyn kinase activation and consequent human mast cell responses.⁵⁶ Our experiments suggest that DJ-1 is a proinflammatory factor in the OVA-induced airway allergy mouse model. EOSs contain a variety of proteins and lipids, including major basic proteins, peroxidase, cationic protein, and neurotoxin.^{57,58} Further studies are needed to determine whether these granule-derived proteins affect the fate determination of airway progenitor cells.

In addition, DJ-1 transcript levels were lowered in airway macrophages and T cells after the OVA challenge. DJ-1 loss resulted in an increase in the abundance of CD8(+) cytotoxic T cells in the airway. It has been revealed that CD8(+) T cells inhibit airway inflammation and the depletion of CD8(+) T cells enhances airway remodeling in an OVA-induced allergic rat model.⁵⁹ However, CD8(+) T cells were found to express IL-13, and mice lacking CD8(+) T cells exhibited reduced airway inflammation and airway response to spasmogen in a house dust mite-induced allergic mouse model.^{60,61} It is possible that cytotoxic T cells respond differently to different allergens. Alveolar macrophages suppress airway allergic inflammation and abrogate bronchial hyperresponsiveness.⁶²⁻⁶⁴ Traditionally, M1 macrophages are linked to proinflammatory responses, while M2 macrophages suppress inflammatory reactions.⁶⁵ However, alveolar macrophages show M2 polarization in mice after the OVA challenge, and targeting M2 macrophages alleviates OVA-induced airway inflammation.⁶⁶ In this study, abundance of alveolar macrophages expressing both M1 and M2 markers increased in the DJ-1 KO mice compared to WT mice after the OVA challenge. M1/M2 macrophages could be a transitional population that responds to the OVA allergen that is regulated by DJ-1.

In summary, DJ-1-mediated interaction between EOSs and club cells promotes eosinophilic inflammation and goblet cell metaplasia (Fig 8, P). Therefore, the suppression of DJ-1 via neutralizing antibodies or appropriate small molecular inhibitors may be a therapeutic target for airway allergic inflammation in the future.

Key messages

- DJ-1 was elevated in airway club cells and EOSs during OVA-induced allergic inflammation in mice.
- DJ-1 regulates interactions between airway club cells and EOSs.
- DJ-1 loss in either club cells or EOSs suppresses airway goblet cell differentiation.
- DJ-1 controls the metabolism of arachidonic acid into cysteinyl LTs in EOSs while these secreted metabolites promote airway goblet cell fate.

REFERENCES

- Hammad H, Lambrecht BN. The basic immunology of asthma. *Cell* 2021;184:1469-85.
- Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci O. Oxidative stress in asthma: part of the puzzle. *Pediatr Allergy Immunol* 2018;29:789-800.
- Anderson SD. Repurposing drugs as inhaled therapies in asthma. *Adv Drug Deliv Rev* 2018;133:19-33.
- Kato Y, Fujisawa T, Shibano M, Saito T, Gatto W, Kamiya H, et al. Airway epithelial cells promote transmigration of eosinophils in a new three-dimensional chemotaxis model. *Clin Exp Allergy* 2002;32:889-97.
- Jawien J, Chlopicki S, Olszanecki R, Lorkowska B, Gryglewski RJ. Eosinophil-epithelial cell interaction augments cysteinyl leukotrienes synthesis. *J Physiol Pharmacol* 2002;53:127-32.
- Pegorier S, Wagner LA, Gleich GJ, Pretolani M. Eosinophil-derived cationic proteins activate the synthesis of remodeling factors by airway epithelial cells. *J Immunol* 2006;177:4861-9.
- Shimizu S, Kouzaki H, Ogawa T, Takezawa K, Tojima I, Shimizu T. Eosinophil-epithelial cell interactions stimulate the production of MUC5AC mucin and profibrotic cytokines involved in airway tissue remodeling. *Am J Rhinol Allergy* 2014;28:103-9.
- Kumar RM, Pajanivel R, Koteeswaran G, Menon SK, Charles PM. Correlation of total serum immunoglobulin E level, sputum, and peripheral eosinophil count in assessing the clinical severity in bronchial asthma. *Lung India* 2017;34:256-61.
- Fujimoto K, Kubo K, Matsuzawa Y, Sekiguchi M. Eosinophil cationic protein levels in induced sputum correlate with the severity of bronchial asthma. *Chest* 1997;112:1241-7.
- Mallah N, Rodriguez-Segade S, Gonzalez-Barcala FJ, Takkouche B. Blood eosinophil count as predictor of asthma exacerbation: a meta-analysis. *Pediatr Allergy Immunol* 2021;32:465-78.
- Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715-21.
- Li K, Li M, Li W, Yu H, Sun X, Zhang Q, et al. Airway epithelial regeneration requires autophagy and glucose metabolism. *Cell Death Dis* 2019;10:875.
- Frey A, Lundberg LP, Ehlers JC, Weckmann M, Zissler UM, Wegmann M. More Than just a barrier: the immune functions of the airway epithelium in asthma pathogenesis. *Front Immunol* 2020;11:761.
- Bonser LR, Zlock L, Finkbeiner W, Erle DJ. Epithelial tethering of MUC5AC-rich mucus impairs mucociliary transport in asthma. *J Clin Invest* 2016;126:2367-71.
- Hamilton JR, Sachs D, Lim JK, Langlois RA, Palese P, Heaton NS. Club cells surviving influenza A virus infection induce temporary nonspecific antiviral immunity. *Proc Natl Acad Sci U S A* 2016;113:3861-6.
- Wang J, Li X, Chen H. Organoid models in lung regeneration and cancer. *Cancer Lett* 2020;475:129-35.
- Shen ZY, Sun Q, Xia ZY, Meng QT, Lei SQ, Zhao B, et al. Overexpression of DJ-1 reduces oxidative stress and attenuates hypoxia/reoxygenation injury in NRK-52E cells exposed to high glucose. *Int J Mol Med* 2016;38:729-36.
- Shadrach KG, Rayborn ME, Hollyfield JG, Bonilha VL. DJ-1-dependent regulation of oxidative stress in the retinal pigment epithelium (RPE). *PLoS One* 2013;8:e67983.
- Kim DK, Kim HS, Kim AR, Kim JH, Kim B, Noh G, et al. DJ-1 regulates mast cell activation and IgE-mediated allergic responses. *J Allergy Clin Immunol* 2013;131:1653-62.
- Amatullah H, Maron-Gutierrez T, Shan Y, Gupta S, Tsoporis JN, Varkouhi AK, et al. Protective function of DJ-1/PARK7 in lipopolysaccharide and ventilator-induced acute lung injury. *Redox Biol* 2021;38:101796.

21. Bahmed K, Boukhenouna S, Karim L, Andrews T, Lin J, Powers R, et al. The effect of cysteine oxidation on DJ-1 cytoprotective function in human alveolar type II cells. *Cell Death Dis* 2019;10:638.
22. Lin CR, Bahmed K, Tomar D, Marchetti N, Criner GJ, Bolla S, et al. The relationship between DJ-1 and S100A8 in human primary alveolar type II cells in emphysema. *Am J Physiol Lung Cell Mol Physiol* 2019;317:L791-804.
23. Bahmed K, Lin CR, Simborio H, Karim L, Aksoy M, Kelsen S, et al. The role of DJ-1 in human primary alveolar type II cell injury induced by e-cigarette aerosol. *Am J Physiol Lung Cell Mol Physiol* 2019;317:L475-85.
24. Kosmider B, Lin CR, Vlasenko L, Marchetti N, Bolla S, Criner GJ, et al. Impaired non-homologous end joining in human primary alveolar type II cells in emphysema. *Sci Rep* 2019;9:920.
25. Chen H, Matsumoto K, Brockway BL, Rackley CR, Liang J, Lee JH, et al. Airway epithelial progenitors are region specific and show differential responses to bleomycin-induced lung injury. *Stem Cells* 2012;30:1948-60.
26. Danahay H, Pessotti AD, Coote J, Montgomery BE, Xia D, Wilson A, et al. Notch2 is required for inflammatory cytokine-driven goblet cell metaplasia in the lung. *Cell Rep* 2015;10:239-52.
27. Lei AH, Xiao Q, Liu GY, Shi K, Yang Q, Li X, et al. ICAM-1 controls development and function of ILC2. *J Exp Med* 2018;215:2157-74.
28. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol* 2018;36:411-20.
29. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res* 2012;40:4288-97.
30. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS* 2012;16:284-7.
31. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* 2000;28:27-30.
32. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. String-Tie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol* 2015;33:290-5.
33. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, Kuan CH, et al. Inference and analysis of cell-cell communication using CellChat. *Nat Commun* 2021;12:1088.
34. Kumar MP, Du J, Lagoudas G, Jiao Y, Sawyer A, Drummond DC, et al. Analysis of single-cell RNA-seq identifies cell-cell communication associated with tumor characteristics. *Cell Rep* 2018;25:1458-68.e1454.
35. Skelly DA, Squiers GT, McLellan MA, Bolisetty MT, Robson P, Rosenthal NA, et al. Single-cell transcriptional profiling reveals cellular diversity and intercommunication in the mouse heart. *Cell Rep* 2018;22:600-10.
36. Schiebinger G, Shu J, Tabaka M, Cleary B, Subramanian V, Solomon A, et al. Optimal-transport analysis of single-cell gene expression identifies developmental trajectories in reprogramming. *Cell* 2019;176:1517.
37. Cohen M, Giladi A, Gorki AD, Solodkin DG, Zada M, Hladik A, et al. Lung single-cell signaling interaction map reveals basophil role in macrophage imprinting. *Cell* 2018;175:1031-44.e1018.
38. Choi DJ, Yang H, Gaire S, Lee KA, An J, Kim BG, et al. Critical roles of astrocytic-CCL2-dependent monocyte infiltration in a DJ-1 knockout mouse model of delayed brain repair. *Glia* 2020;68:2086-101.
39. Xu X, Chen W, Leng S, Padilla MT, Saxton B, Hutt J, et al. Muc1 knockout potentiates murine lung carcinogenesis involving an epiregulin-mediated EGFR activation feedback loop. *Carcinogenesis* 2017;38:604-14.
40. Belmonte KE, Fryer AD, Costello RW. Role of insulin in antigen-induced airway eosinophilia and neuronal M2 muscarinic receptor dysfunction. *J Appl Physiol* (1985) 1998;85:1708-18.
41. Dror E, Dalmás E, Meier DT, Wueest S, Thevenet J, Thienel C, et al. Postprandial macrophage-derived IL-1 β stimulates insulin, and both synergistically promote glucose disposal and inflammation. *Nat Immunol* 2017;18:283-92.
42. Isogai S, Athiviraham A, Fraser RS, Taha R, Hamid Q, Martin JG. Interferon-gamma-dependent inhibition of late allergic airway responses and eosinophilia by CD8 $^{+}$ $\gamma\delta$ T cells. *Immunology* 2007;122:230-8.
43. Liu T, Garofalo D, Feng C, Lai J, Katz H, Laidlaw TM, et al. Platelet-driven leukotriene C4-mediated airway inflammation in mice is aspirin-sensitive and depends on T prostanoid receptors. *J Immunol* 2015;194:5061-8.
44. Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Björnsson E, Roomans GM, et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* 2000;162:2295-301.
45. Lampinen M, Rak S, Venge P. The role of interleukin-5, interleukin-8 and RANTES in the chemotactic attraction of eosinophils to the allergic lung. *Clin Exp Allergy* 1999;29:314-22.
46. Holgate ST. Epithelium dysfunction in asthma. *J Allergy Clin Immunol* 2007;120:1233-1244; quiz 1245-36.
47. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445-54.
48. Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirotton D, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest* 2016;126:3279-95.
49. Oikonomou N, Schuijs MJ, Chatzigiagkos A, Androulidaki A, Aidinis V, Hammad H, et al. Airway epithelial cell necroptosis contributes to asthma exacerbation in a mouse model of house dust mite-induced allergic inflammation. *Mucosal Immunol* 2021;14:1160-71.
50. Wang HB, Akuthota P, Kanaoka Y, Weller PF. Airway eosinophil migration into lymph nodes in mice depends on leukotriene C4. *Allergy* 2017;72:927-36.
51. Chan CC, McKee K, Tagari P, Chee P, Ford-Hutchinson A. Eosinophil-eicosanoid interactions: inhibition of eosinophil chemotaxis in vivo by a LTD4-receptor antagonist. *Eur J Pharmacol* 1990;191:273-80.
52. Deykin A, Belostotsky O, Hong C, Massaro AF, Lilly CM, Israel E. Exhaled nitric oxide following leukotriene E(4) and methacholine inhalation in patients with asthma. *Am J Respir Crit Care Med* 2000;162:1685-9.
53. Kim DC, Hsu FI, Barrett NA, Friend DS, Grenningloh R, Ho IC, et al. Cysteinyl leukotrienes regulate Th2 cell-dependent pulmonary inflammation. *J Immunol* 2006;176:4440-8.
54. Tamaoka M, Hassan M, McGovern T, Ramos-Barbon D, Jo T, Yoshizawa Y, et al. The epidermal growth factor receptor mediates allergic airway remodelling in the rat. *Eur Respir J* 2008;32:1213-23.
55. Paruchuri S, Tashimo H, Feng C, Maekawa A, Xing W, Jiang Y, et al. Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. *J Exp Med* 2009;206:2543-55.
56. Kim DK, Beaven MA, Metcalfe DD, Olivera A. Interaction of DJ-1 with Lyn is essential for IgE-mediated stimulation of human mast cells. *J Allergy Clin Immunol* 2018;142:195-206.e198.
57. Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem* 2014;289:17406-15.
58. Spencer LA, Bonjour K, Melo RC, Weller PF. Eosinophil secretion of granule-derived cytokines. *Front Immunol* 2014;5:496.
59. Tsuchiya K, Isogai S, Tamaoka M, Inase N, Akashi T, Martin JG, et al. Depletion of CD8 $^{+}$ T cells enhances airway remodelling in a rodent model of asthma. *Immunology* 2009;126:45-54.
60. Raemdonck K, Baker K, Dale N, Dubuis E, Shala F, Belvisi MG, et al. CD4(+) and CD8(+) T cells play a central role in a HDM driven model of allergic asthma. *Respir Res* 2016;17:45.
61. Miyahara N, Takeda K, Kodama T, Joetham A, Taube C, Park JW, et al. Contribution of antigen-primed CD8 $^{+}$ T cells to the development of airway hyperresponsiveness and inflammation is associated with IL-13. *J Immunol* 2004;172:2549-58.
62. Zaslona Z, Przybranowski S, Wilke C, van Rooijen N, Teitz-Tennenbaum S, Osterholzer JJ, et al. Resident alveolar macrophages suppress, whereas recruited monocytes promote, allergic lung inflammation in murine models of asthma. *J Immunol* 2014;193:4245-53.
63. Draijer C, Speth JM, Penke LRK, Zaslona Z, Bazzill JD, Lugogo N, et al. Resident alveolar macrophage-derived vesicular SOCS3 dampens allergic airway inflammation. *FASEB J* 2020;34:4718-31.
64. Careau E, Bissonnette EY. Adoptive transfer of alveolar macrophages abrogates bronchial hyperresponsiveness. *Am J Respir Cell Mol Biol* 2004;31:22-7.
65. Cheng P, Li S, Chen H. Macrophages in lung injury, repair, and fibrosis. *Cells* 2021;10:436.
66. Wang Q, Hong L, Chen M, Shi J, Lin X, Huang L, et al. Targeting M2 macrophages alleviates airway inflammation and remodeling in asthmatic mice via miR-378a-3p/GRB2 pathway. *Front Mol Biosci* 2021;8:717969.

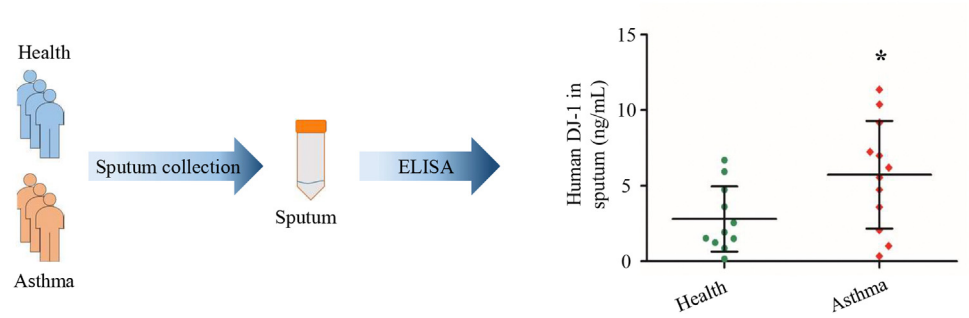


FIG E1. Altered DJ-1 levels in sputum samples from patients with asthma. (*Left*) Flowchart of the samples. Sputum samples were collected from healthy controls (n = 11) and patients with asthma (n = 12). Sputum DJ-1 levels were measured by ELISA. **P* < .05.

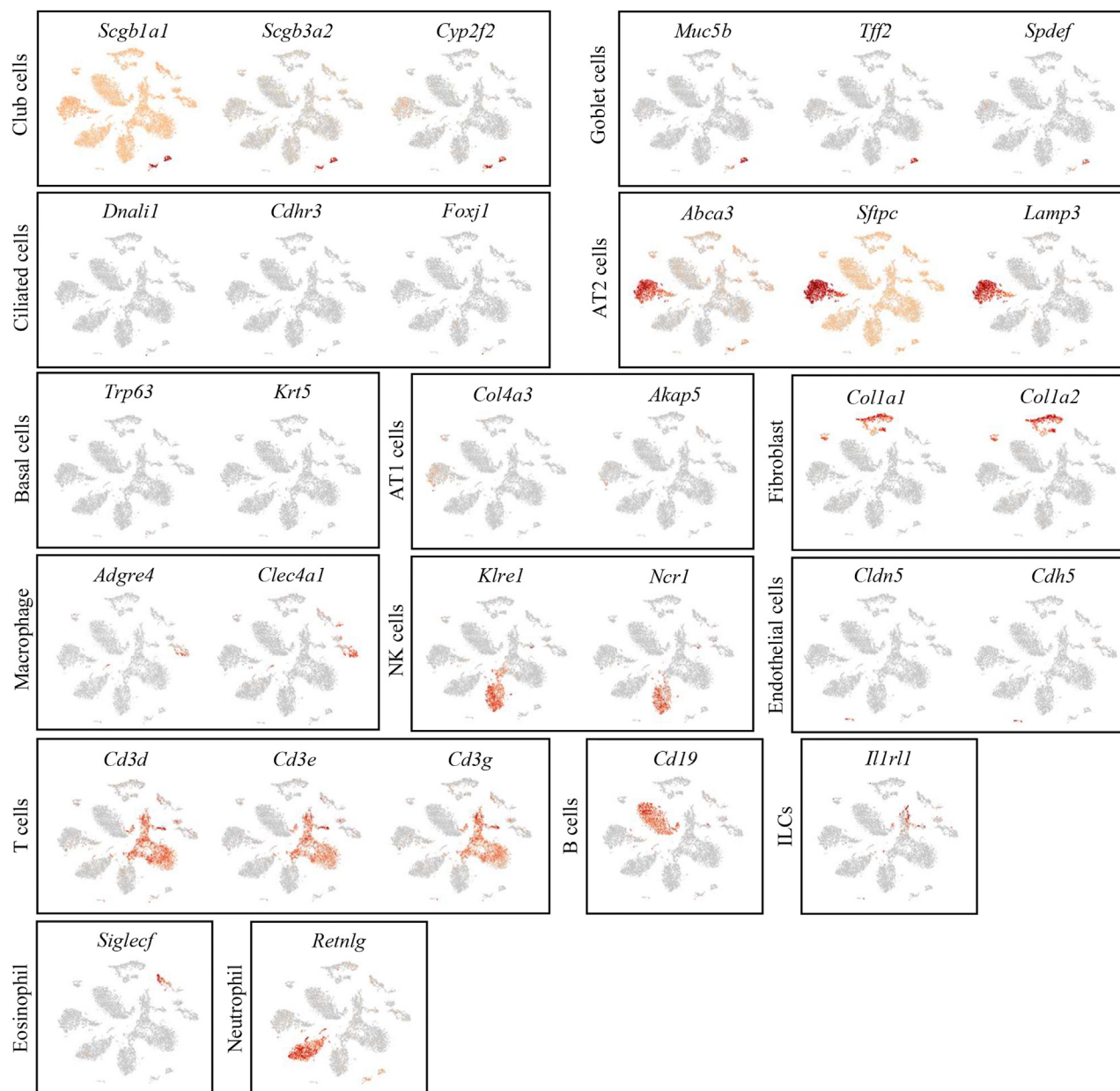


FIG E2. Identification of cells from PBS-challenged WT and OVA-challenged WT mouse lung samples. tSNE plot color-coded for expression (gray to red) of cell type-specific marker genes based on scRNA-seq transcriptome data.

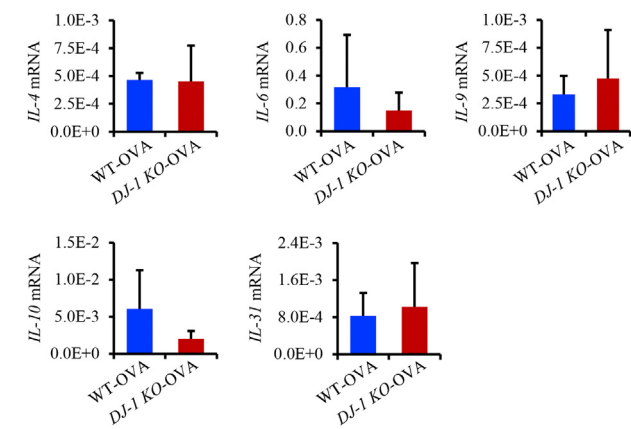


FIG E3. Expression levels of type 2 cytokines in OVA-challenged WT and DJ-1 KO mouse lung samples. qPCR analysis of *IL-4*, *IL-6*, *IL-9*, *IL-10*, and *IL-31* expression in the lungs of WT-OVA and DJ-1 KO-OVA mice. **P* < .05.

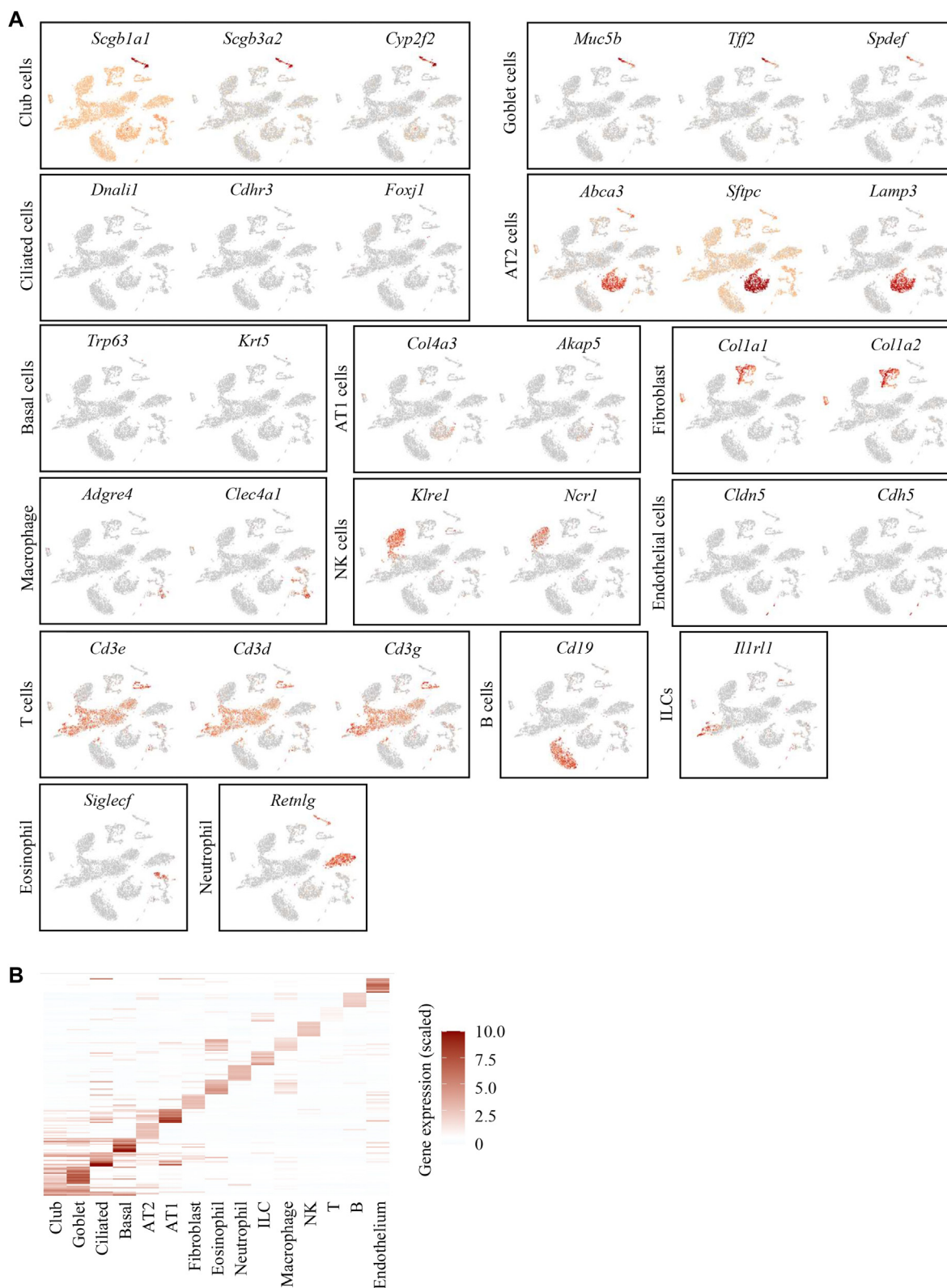


FIG E4. Identification of cells from OVA-challenged WT and DJ-1 KO mouse lung samples. Each cell population was identified based on the scRNA-seq transcriptome data. **(A)** Marker genes in different cell types. **(B)** Heatmap of expression signatures of the top 10 marker genes for each cell population. Each column represents 1 cell type and each row represents 1 gene. The color key from red to white indicates gene expression from high to low.

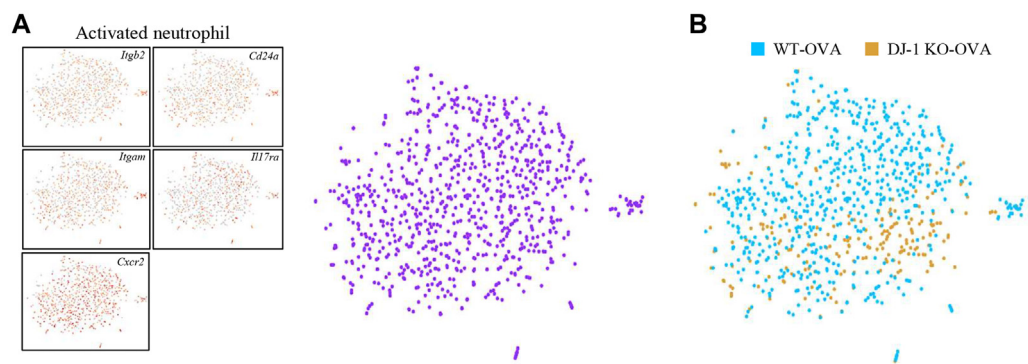


FIG E5. Neutrophil clusters characterized by scRNA-seq. **(A, Left)** tSNE plot color-coded for expression (gray to red) of cell type-specific marker genes for activated neutrophils. **(Right)** tSNE plot of 870 neutrophils. **(B)** tSNE plot of neutrophils, color-coded by the sample type of origin.

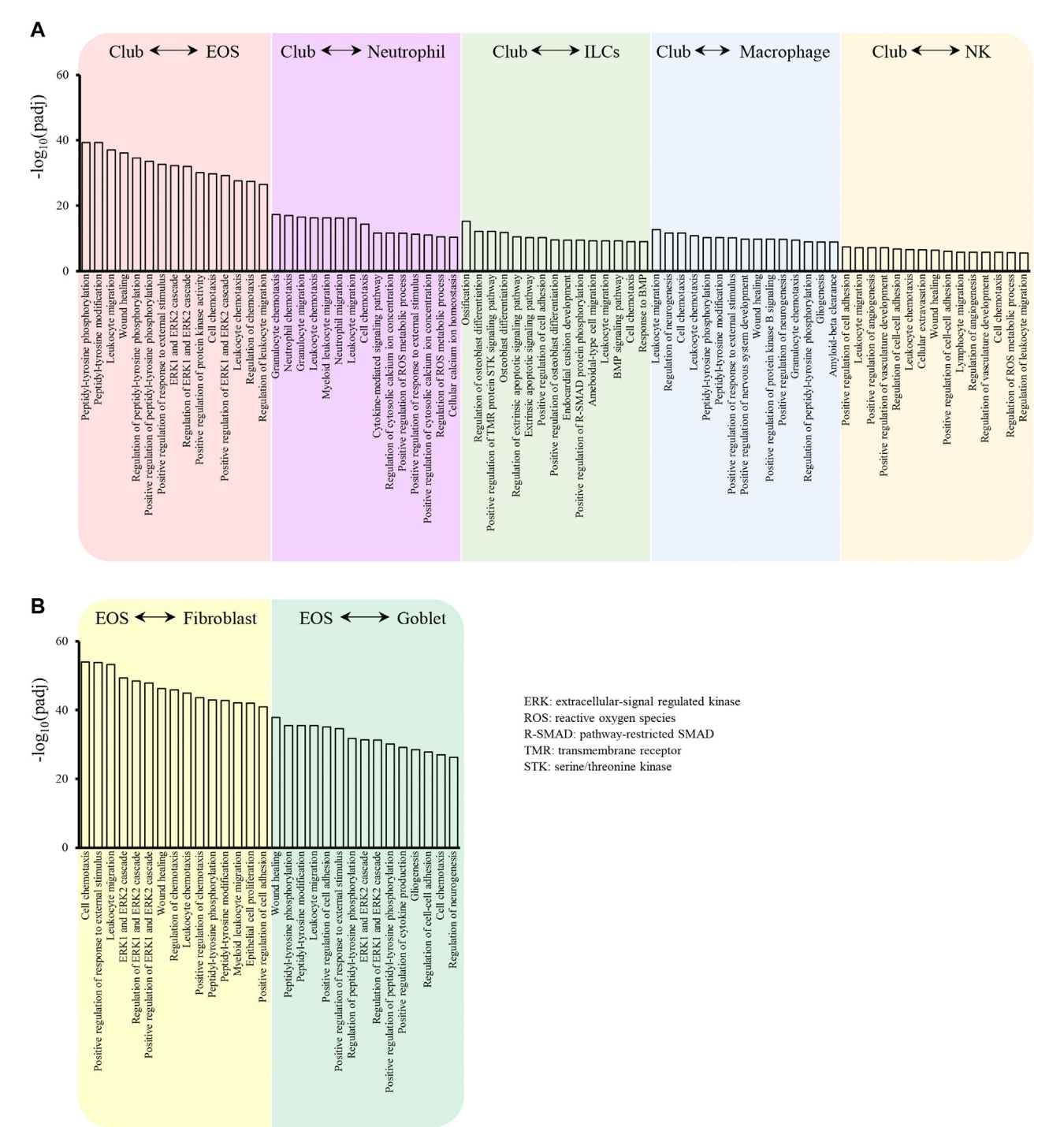


FIG E6. GO enrichment analyses based on the genes of cell-cell interaction. **(A)** GO enrichment analyses of selected club- EOS, club-neutrophil, club-ILC, club-macrophage, and club-NK interactions enriched in WT-OVA group but absent in DJ-1 KO-OVA group. **(B)** GO enrichment analyses of selected EOS-fibroblast and EOS-goblet interactions enriched in WT-OVA group but absent in DJ-1 KO-OVA group.

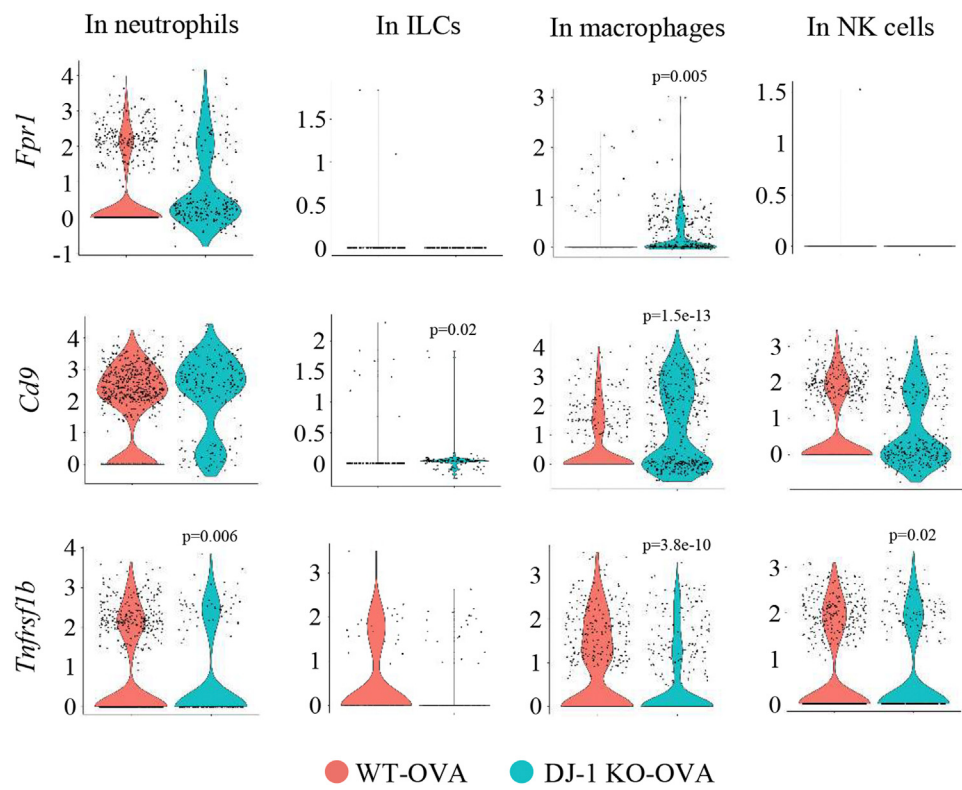


FIG E7. Violin plot depicting expressions of *Fpr1*, *Cd9*, and *Tnfrsf1b* in neutrophils, ILCs, macrophages, and NK cells.

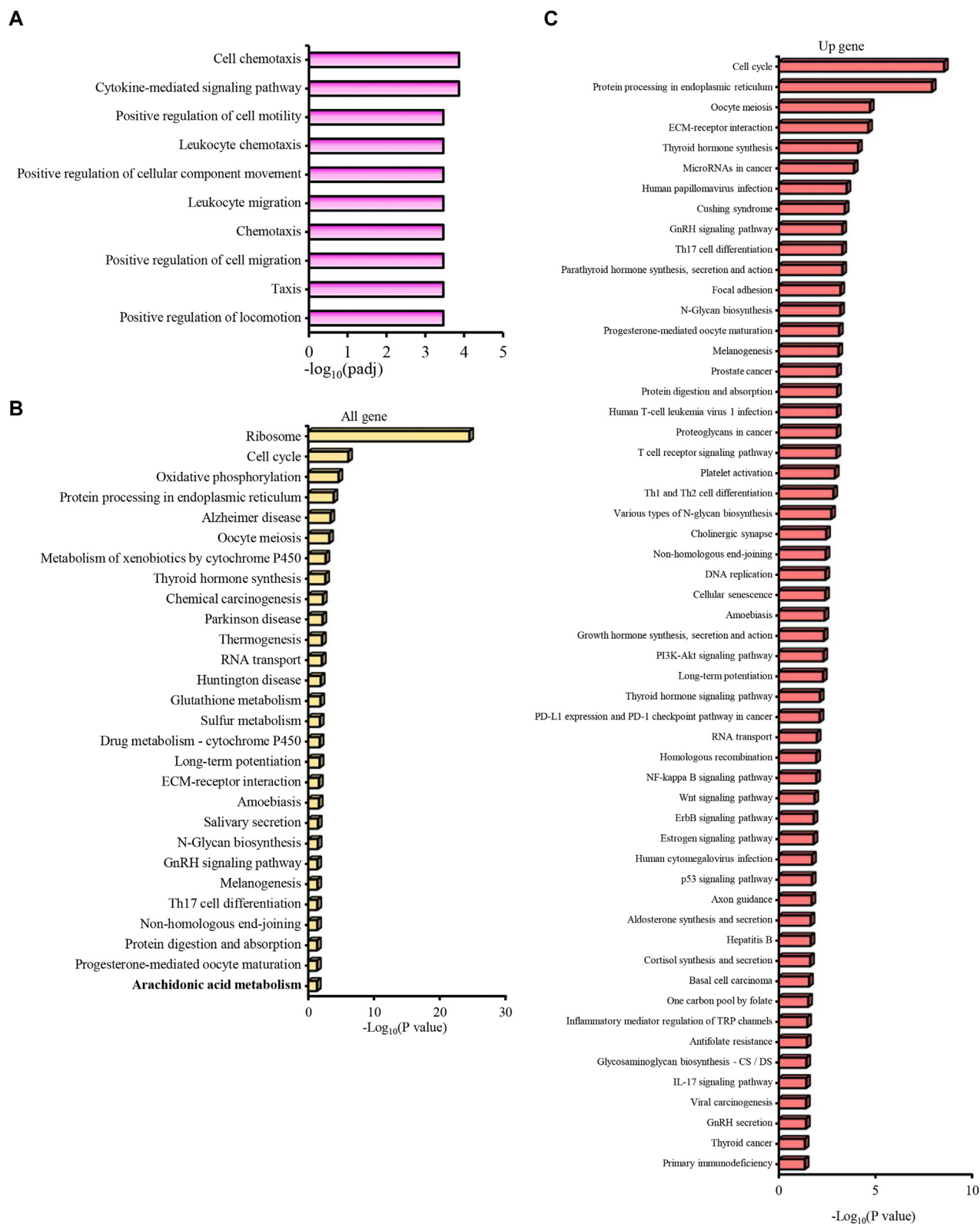


FIG E8. Altered pathways in the absence of DJ-1 during allergic inflammation. **(A)** Histogram showing the top 10 terms of GO analysis in all DEGs in the scRNA-seq of EOSs (DJ-1 KO-OVA vs WT-OVA). **(B,C)** Histogram showing KEGG terms enrichment analysis ($P < .05$) of all DEGs in the bulk RNA-seq of lungs from mice in different treatment groups (DJ-1 KO-OVA vs WT-OVA).

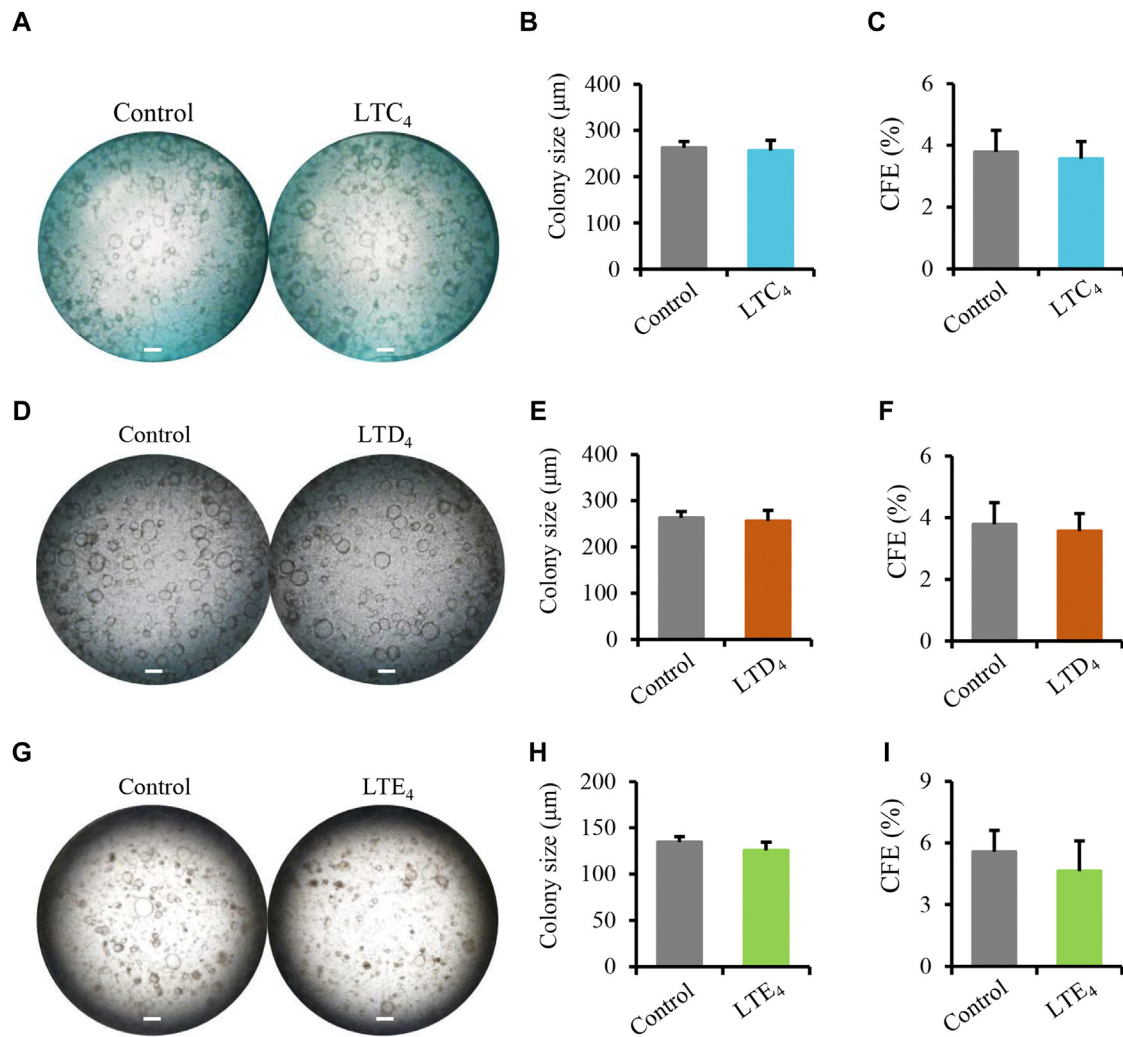


FIG E9. LTs have no effect on the colony-forming ability of club cells. **(A,D,G)** Representative images of organoid-cultured club cells in the presence of LTC₄ (16 nmol/L), LTD₄ (20 nmol/L), and LTE₄ (20 nmol/L) at day 8 after plating. **(B,E,H)** Diameters of club cell colonies from the LTC₄, LTD₄, and LTE₄ groups under the conditions described in **(A)**, **(D)**, and **(G)**. **(C,F,I)** CFEs of club cells from the LTC₄, LTD₄, and LTE₄ groups under the conditions described in **(A)**, **(D)**, and **(G)**. Bars = 500 μm.

TABLE E1. Patient demographics and clinical characteristics

Characteristic	Asthma (n = 12)	Healthy (n = 11)
Age (y) median (range)	55 (21-77)	46 (24-55)
Sex (M)	5 (41.7)	5 (45.5)
Acute exacerbation	8 (66.7)	NA
PaO ₂ (mm Hg), mean (range)	86.8 (62.5-130)	NA
PaCO ₂ (mm Hg), mean (range)	40.6 (35.9-45.9)	NA
Currently prescribed treatment, n	12	NA
LT receptor antagonist	9 (75)	NA
ICS/LABA	10 (83.3)	NA
SABA	1 (8.3)	NA
LAMA	2 (16.7)	NA
ICS	5 (41.7)	NA
PDEI	8 (66.7)	NA
Duration of hospitalization (d), median (range)	8.25 (6-13)	NA

Values are n (%) unless otherwise indicated. For the analysis of PaO₂ and PaCO₂, n=7.

ICS, Inhaled corticosteroid; LABA, long-acting beta-2 agonist; LAMA, long-acting muscarinic antagonists; M, male; NA, not available; PaCO₂, partial pressure of carbon dioxide; PaO₂, partial pressure of oxygen; PDEI, phosphodiesterase inhibitor; SABA, short-acting beta-2 agonist.

TABLE E2. Reagents

Name	Source	Catalog no.
Antibodies		
Anti-Mouse CD24 PE	eBioscience	12-0242-83
Anti-Mouse CD45 Biotin	eBioscience	13-0451-85
Anti-Mouse CD34 Biotin	eBioscience	13-0341-85
Anti-Mouse CD31 Biotin	eBioscience	13-0311-85
Anti-Mouse Ly-6A/E (Sca-1) APC	eBioscience	17-5981-81
PE/Cy7 anti-mouse CD326 (EpCAM)	eBioscience	118216
7-AAD Viability Staining Solution	eBioscience	00-6993-50
Streptavidin APC-eFluor 780	eBioscience	47-4317-82
Anti-Mouse CD11c APC	eBioscience	17-0114-82
Anti-Mouse CD170 (Siglec F) Alexa Fluor 488	eBioscience	53-1702-82
Anti-Clca3	abcam	Ab46512
Anti-ki67	eBioscience	14-5698-82
Anti-Cyp2f2	Santa Cruz	sc-374540
Anti-DJ-1	Santa Cruz	sc-55572
DAPI	Roche	10236276001
Goat Anti-Mouse IgG	Invitrogen	31430
Donkey Anti-Mouse IgG, Alexa Fluor 594	Invitrogen	A21203
Donkey Anti-Rabbit IgG, Alexa Fluor 488	Invitrogen	A21206
Chemicals, peptides, and recombinant proteins		
Matrigel Matrix	BD Pharmingen	356231
DMEM-F12	Corning	16-405-CVR
Ham's F12	Corning	10-080-CVR
DMEM (1X)	Gibco	11965092
FBS	Gibco	16000-044
Penicillin-streptomycin	Gibco	15140-122
SB431542	Sigma-Aldrich	S4317-5MG
Y27632	Sigma-Aldrich	Y0503
ITS	Sigma-Aldrich	I3146
HEPES	Sigma-Aldrich	H0087
Hematoxylin-Eosin/HE Staining Kit	Solarbio	G1120
PAS/Hematoxylin Stain Kit	Solarbio	G1281
Hema 3	Fisherbrand	400937
Deoxyribonuclease I from bovine pancreas	Sigma-Aldrich	DN25-5G
Alcian Blue solution	Sigma-Aldrich	B8438-250ML
EGTA	Sigma-Aldrich	E8145
Albumin from chicken egg white (OVA)	Sigma-Aldrich	A5503-50g
TRIzol reagent	Invitrogen	15596018
HBSS	Solarbio	H1025
4% paraformaldehyde	Solarbio	P1110
Triton X-100	Sangon Biotech	A600198-0500
5% BSA	BOSTER	AR0004
RIPA lysis buffer	BOSTER	AR0102-100
Elastase	Worthington Biochemical Corporation	LS002279
Leukotriene C ₄	Cayman	20210
Leukotriene D ₄	Cayman	20310
Leukotriene E ₄	Cayman	20410
HiScript III RT SuperMix	Vazyme	R323-01
ChamQ Universal SYBR qPCR Master Mix	Vazyme	Q711
VECTASHIELD	Vectorlabs	H1000
Imject Alum Adjuvant	Thermo Fisher Scientific	77161
OCT compound	Tissue-Tek	4583
Lillie-Mayer	Solarbio	G4070
Critical commercial assays		
Human PARK7 ELISA Kit	abcam	ab215535
Mouse IL-5 ELISA Kit	BOSTER	EK0408
Mouse IL-13 ELISA Kit	BOSTER	EK0425
Chromium Single Cell B Chip Kit	10x Genomics	PN-1000153
Chromium Single Cell 3' Reagent Kits	10x Genomics	CG000183
Chromium Single Cell 3' GEM, Library & Gel Bead Kit	10x Genomics	PN-1000075
Chromium i7 Multiplex Kit	10x Genomics	PN-120262

(Continued)

TABLE E2. (Continued)

Name	Source	Catalog no.
MLg cell line		
MLg2908 mouse lung fibroblast cell line	ATCC	CCL-206
Software and algorithms		URL
ImageJ	NIH	https://imagej.nih.gov/ij/
FastQC (version: 0. 11. 3)	Babraham Bioinformatics	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
edgeR (version: 3. 18. 1)	McCarthy et al, ²⁹ 2012	http://bioconductor.org/packages/release/bioc/html/edgeR.html
clusterProfiler (version: 3. 4. 4)	Yu et al, ³⁰ 2012	http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html
DESeq2 R (version: 1. 20. 0)	Love et al, 2014*	https://bioconductor.org/packages/release/bioc/html/DESeq2.html
Cell Ranger (version: 3. 1. 0)	10x Genomics	https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest
StringTie (version: 1. 3. 3b)	Pertea et al, ³² 2015	http://ccb.jhu.edu/software/stringtie
Seurat (version: 3. 1. 0)	Stuart et al 2019†; Butler et al, ²⁸ 2018	https://satijalab.org/seurat/

*Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550.

†Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM 3rd, Hao Y, Stoeckius M, Smibert P, Satija R. 2019. Comprehensive Integration of Single-Cell Data. *Cell* 2019;177:1888-1902.e21

EGTA, ethyleneglycol-bis-(β-aminoethylether)-NN,N',N'-tetraacetic acid; *EpCAM*, epithelial cell adhesion molecule; *GEM*, gel beads in emulsion; *HBSS*, Hank's balanced saline solution; *ITS*, insulin/transferrin/selenium; *NIH*, National Institutes of Health; *PE*, phycoerythrin; *PE/Cy7*, EpCAM-phycoerythrin-Cyanine7; *RIPA*, radio immunoprecipitation assay.

TABLE E3. Sequences of primers for qPCR

Genes	Forward primer	Reverse primer
<i>β-actin</i>	5'-GGCCAACCGTGAAAAGATGA-3'	5'-CAGCCTGGATGGCTACGTACA-3'
<i>Clca3</i>	5'-GGCATCGTCATCGCCATAG-3'	5'-CACCATGTCCTTTATGTGTTGAATG-3'
<i>Cyp2f2</i>	5'-CGACTGCTTCCTCACAAAGA-3'	5'-GTCATCAGCAGGGTATCCATATT-3'
<i>Muc5ac</i>	5'-TGA CTCAATCTGCGTGCCTT-3'	5'-AGGCCTTCTTTTGGCAGGTT-3'
<i>Spdef</i>	5'-GACTGTGGAATTCCTGGGGG-3'	5'-ATTGTGGCAGGAGCAGAGAC-3'
<i>DJ-1</i>	5'-CGGCTGCAGTCTTTAAGAAATAG-3'	5'-CAGGAATCACTGTCTCCATCTC-3'
<i>Alox5</i>	5'-ACAGCTTATCTGCGAGTATGG-3'	5'-GGGAAACACAGGGAGGAATAG-3'
<i>Alox5ap</i>	5'-CATGAAAGCAAGGCGCATAAT-3'	5'-CCCAGATAGCCGACAAAGTATT-3'
<i>Ltc4s</i>	5'-TCTGTTGCAAGCCTACTTCTC-3'	5'-AGTACTCGCTGCAGTTTACC-3'
<i>IL-4</i>	5'-TTGAGAGAGATCATCGGCATTT-3'	5'-CTCACTCTCTGTGGTGTCTTC-3'
<i>IL-6</i>	5'-CTTCCATCCAGTTGCCTTCT-3'	5'-CTCCGACTTGTGAAGTGGTATAG-3'
<i>IL-9</i>	5'-GGGCATCAGAGACACCAATTA-3'	5'-ATCAGTTGGGACGGAGAGA-3'
<i>IL-10</i>	5'-TTGAATTCCTGGGTGAGAAG-3'	5'-TCCACTGCCTTGCTCTTATT-3'
<i>IL-31</i>	5'-GGATGTCAGCAGACGAATCAA-3'	5'-TGACTTTCTCCAGATGTGCATAG-3'