Online Data Supplement

METHODS

$CD3^+$ T cell isolation and transfer

Mouse mediastinal lymph nodes from air- or CS- sensitised C57BL/6 mice were harvested after euthanasia, and single-cell suspensions were prepared using sterile syringe plunger. Cells were finally strained through a 40-mm filter. $CD3^+$ T cells were purified with Mojosort™ mouse CD3 T cell isolation kit (Biolegend, 480024) according to the manufacturer's protocol and were delivered ($1x10^6$ cells/mouse) intratracheally in 50 μl PBS to naïve recipient under anaesthesia. Recipient mice were challenged one day after T cell transfer with 50 μg elastin per day for 3 days.

BALF collection

Forty-eight hours after the last exposure to elastin, mice were sacrificed using an overdose of 2% pentobarbital sodium and tracheotomy was performed. Each mouse was lavaged with 0.4 ml PBS by injecting into the lungs and drawing to collect cells for 3 times. The total number of BALF cells was counted, then the remaining BALF was centrifuged (400 g for 10min at 4°C). The supernatant was retained for further analysis, while the cell pellet was resuspended in PBS moderately and centrifuged on glass slides. Then cells on glass slides were stained with Wright–Giemsa stain (Baso, BA-4017), and differential counts were assessed by counting 200 total cells.

Quantitative real-time PCR

RNA from BALF cells and lung homogenates was isolated with Trizol (Invitrogen, 15596026). Reverse transcription was performed with Reverse Transcription Reagents (Takara Biotechnology, DRR037A). Real-time RT-PCR was performed using the StepOnePlus PCR system and gene expression assays (Applied Biosystems, Foster City, CA, USA). A ΔΔCt method was used to quantify mRNA levels for *Muc5ac* and *Mmp12*. Gene expression was quantified using ABI and MS Excel software. Each sample was assayed in

triplicate. PCR primers used are shown as below: (Mouse *Gapdh*: Forword: 5'-AGGTCGGTGTGAACGGATTTG-3', Reverse: 5'-GGGGTCGTTGATGGCAACA-3'; Mouse *Muc5ac*: Forword: 5'-CTGTGACATTATCCCATAAGCCC-3', Reverse: 5'-AAGGGGTATAGCTGGCCTGA-3'; Mouse *Mmp12*: Forword: 5'-CTGCCCCATGAATGACAGTG-3', Reverse: 5`-AGTTGCTTCTAAAC-3')

ELISA

The concentration of CXCL1 (MKC008B, R&D), CXCL2 (MM200, R&D), IL6 (M60000B, R&D), IFNG (MZF00, R&D), and IL17A (M1700, R&D) in BALF supernatants or Lung homogenates, mouse cotinine (EA100902, OriGene) in serum, human anti-elastin (E01E0050, Bluegene) in plasma, and IL17A (BMS2017HS, Invitrogen) in induced sputum were determined with ELISA kits following the manufacturer's protocol.

Lung histology and determination of collagen deposition

The left lobes of lungs were fixed in 4% paraformaldehyde at 4°C for 24 h. After paraffin embedding, the tissue sections were prepared (3 μm). Lung sections were stained with hematoxylin/eosin (H&E), PAS or Masson trichrome. The H&E staining sections were semi-quantitated (score: 0–4) with Olympus microscope (10 × 20 magnification) for the inflammatory situation. PAS stained goblet cells in airway epithelium were scored as described previously (3). Collagen area on the basal membrane of airway was analyzed by Leica-Qwin image-processing system (Leica Imaging Systems, Bensheim, Germany). The result was expressed as collagen staining area of per micrometer length of basement membrane of bronchioles. At least 10 bronchioles with 150 to 250 μm of internal diameter were counted on each slide. All slides were examined in a random blinded fashion by 2 independent investigators.

Immunohistochemistry and Immunofluorescence

Sections of lung tissues were immunostained with anti-MUC5AC, according to the manufacturer's instructions. Images were scanned with an Olympus BX53 inverted microscope (Olympus, Melville, NY), and image quantitative analysis was performed as

previously described [1]. MUC5AC positive bronchial epithelial cells were presented as a percentage of total epithelial cells.

Collection and processing of induced sputum

Sputum was induced as previously described [2]. A sample was considered adequate when the patient was able to expectorate at least 2 ml of sputum and the slides contained <10% squamous cells on differential cell counting. All sputum samples were kept at -80°C until analysis.

Genotyping of HLA-A alleles

EDTA-anticoagulated peripheral blood from COPD patients or healthy controls were collected, and genomic DNA was extracted using a universal genomic DNA kit (CWbiotech, China) following the manufacturer's procedure. High resolution genotyping of HLA-A alleles was performed by Immunogenetics Laboratory, Blood center for Zhejiang Province using commercial polymerase chain reaction (PCR) plus sequence-based typing (SBT) kit (One Lambda, Inc.).

PBMC isolation

Heparin-anticoagulated peripheral blood from COPD patients or healthy controls were collected and centrifuged at 400 × g for 5 min and the top layer containing plasma was removed. The remaining blood was diluted with an equal volume of normal saline, 5 ml of diluted blood was layered over 5 ml of the Ficoll-Paque PLUS (GE Healthcare). Gradients were centrifuged at 400 × g for 30 min at room temperature in a swinging-bucket rotor without the brake applied. The PBMC interface was carefully removed by pipetting and washed with normal saline by centrifugation at 400 × g for 5 min. PBMC were prepared for further use.

GVAPGVGVAPGV/HLA-A*02:01 tetramer prediction, synthesis and staining

Epitope prediction was performed on 2018/4/20 with the IEDB Analysis Resource web interface [3, 4]. Briefly, the repeating peptide VGVAPG was input as specific sequence, all

possible sub-peptides (with 8-14 residues) hosted within the full-length were predicted to bind with all human HLA-A, -B, -C alleles in IEDB recommended 2.19 database. The predicted output is given in units of IC50 (nmol/l). Peptides with IC50 values <50 nmol/l are considered with high affinity. GVAPGVGVAPGV and HLA-A*02:01 were paired as they were among those with high or intermediate affinity in prediction, and HLA-A*02:01 is a common allele in human.

PE-conjugated GVAPGVGVAPGV/HLA-A*02:01 tetramer was synthesized according to the protocol published previously [5].

PBMC pellets were suspended and incubated in human T cell culture medium containing 5% autoplasma for 7 days in the presence of IL2 (R&D systems). Cells were then harvested and stained for GVAPGVGVAPGV/HLA-A*02:01 tetramer, anti-CD3, and anti-CD8 antibodies (eBioscience) for analysis of tetramer positive cells by flow cytometry.

Flow cytometry

Mouse lung white blood cells or human peripheral blood mononuclear cells were stained with indicated antibodies and were analysed by BD Fortessa LSR. The samples were analysed using the Flowjo software (Treestar LLC. Ashland, Oregon, USA). Anti-Mouse CD45 perCP-Cyanine 5.5, Anti-Mouse CD3e APC-Cyanine7, Anti-Mouse CD8a PE-Cyanine 7, Anti-Mouse CD4 PE-Cyanine7, Anti-Mouse IFNG FITC, Anti-Mouse IL17A APC, Anti-human CD45 APC, Anti-human CD8a eFluor®450, Anti-human CD3 PE-Cyanine 7, Anti-human IFNG Alexa Fluor®488, and Anti-human IL-17A PE were all purchased from eBioscience, San Diego, CA, USA.

Measurement of lung function

Mice were sedated with 0.1 g/kg intraperitoneal and 1% pentobarbital sodium, and the dose could be adjusted as necessary. The mice were intubated with a 2-in-long, 14-gauge angiocatheter which then connected to a rodent-specific forced maneuver system (Pulmonary Function Testing, Buxco Research Systems, USA). A series of preprogrammed forced ventilation maneuvers were used to measure FVC (forced vital capacity) and FEV_{20} (forced expiratory volume in 20 seconds) within 10 minutes. Each measurement was performed a

minimum number of three times to ensure reasonable repeatability.

Morphological analysis of lung sections

Serial longitudinal sections (3-µm thick) stained with hematoxylin/eosin (H&E) were used for morphological analysis. Ten randomly selected (100x) fields per slide were photographed using Olympus BX53 inverted microscope (Olympus, Melville, NY). The images were analyzed using the modified Image J software (open source), as described previously[6]. From each field, 10-12 areas of interest, free of airways and blood vessels, were picked for measurement of the number of intersections of virtual lines of known length, with alveolar septa. An increase in the average distance between intercepts (mean linear intercept) indicates enlarged airspaces.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the statistical differences among the groups, with P values indicated in the related graphs. Differences between 2 groups were identified using the Student's t-test. All data are expressed as mean ± s.e.m.. The analyses and graphs were performed using GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA). A value of P less than 0.05 was considered statistically significant.

Reference

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Supplementary Figure S1. Dose-dependent effects of elastin challenge in inducing airway inflammation. Mice were sensitized with air or CS, and then challenged with various doses of elastin (25, 50, or 100 μg) intratracheally (i.t.) for 3 times. Mice were sacrificed 48 h after last elastin instillation, and the inflammatory cell counts (**a**) and cytokines (**b**) in BALF were detected. Mac: macrophages; Neu: neutrophils; Lym: lymphocytes; Eos: eosinophils. Throughout, data are representative of 5 mice and were replicated in at least 3 independent experiments. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA.

Supplementary Figure S2. FACS Gating strategy for identification of T cell responses in mouse lung single cell suspension. Mouse lung single-cell-suspensions were harvested and stained with fixable viability stain to exclude dead cells, mouse anti-CD45, anti-CD3, anti-CD8 were labeled to identify T cells and mouse anti-IL17A, anti-IFNG antibodies were label after fixation and permeabilization to detect specific T cell responses. Plot (**a**) Total cells. Plot (**b**) Single cells. Plot (**c**) Live $CD45^+$ white blood cells. Plot (**d**) $CD3^+CD8^+$ represents Tc subsets while $CD3^+CD8^-$ represents Th subsets. Plot (**e.f**) $IL17A^+$ represents Tc17/Th17 subsets while $IFNG^+$ represents Tc1/Th1 subsets.

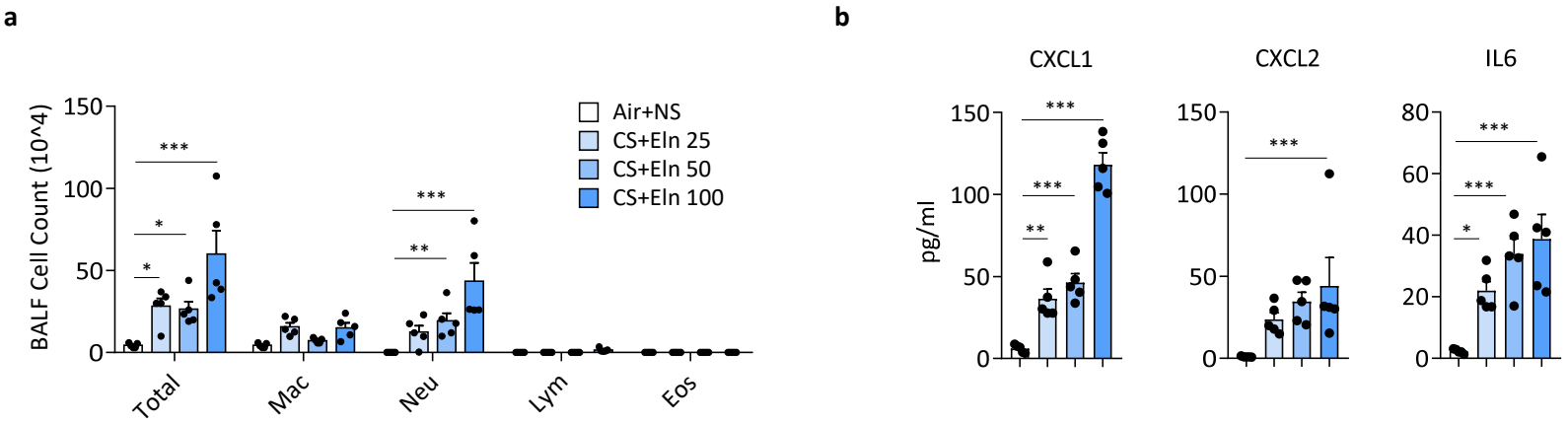
Supplementary Figure S3. Sensitization and short-term challenge with mouse elastin induce Th1 immune responses and a bronchitis-like airway inflammation in mice. (**a**) Experimental outline. Mice were sensitized with elastin (Eln, 100 μg) and CFA or normal saline (NS) intraperitoneally (i.p.) at day 1 and 14, challenged with elastin (100 μg) intratracheally (i.t.) for 3 times at day 29, 30, and 31, and sacrificed 48 h after the last elastin challenge. (**b**) Inflammatory cell counts in the BALF. (**c**) Representative images of H&E staining and the semi-quantified scoring of mouse lung sections. (**d**) Concentrations of CXCL1, CXCL2 and IL6 in BALF. (**e**) Levels of Th1, Tc1, Th17, and Tc17 in mouse lungs. (**f**) Levels of IFNG and IL17A in mouse lung homogenates. (**g**) Expression of *Muc5ac* mRNA transcripts in mouse lungs. (**h,i**) Representative images and the semi-quantified scorings of PAS (**h**) or MUC5AC (**i**) staining in mouse lung sections. Mac: macrophages; Neu: neutrophils; Lym: lymphocytes; Eos: eosinophils. Throughout, data are representative of 5-6 mice and were replicated in at least 3 independent experiments. Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 by one-way analysis of variance (ANOVA).

Supplementary Figure S4. *Rag1*$^{-/-}$ mice exhibit little mature CD3^{+} cells in lung. Representative flow cytometry images showing the depletion of CD3^{+} cells in WT and *Rag1*$^{-/-}$ mice lung tissue single-cell suspension.

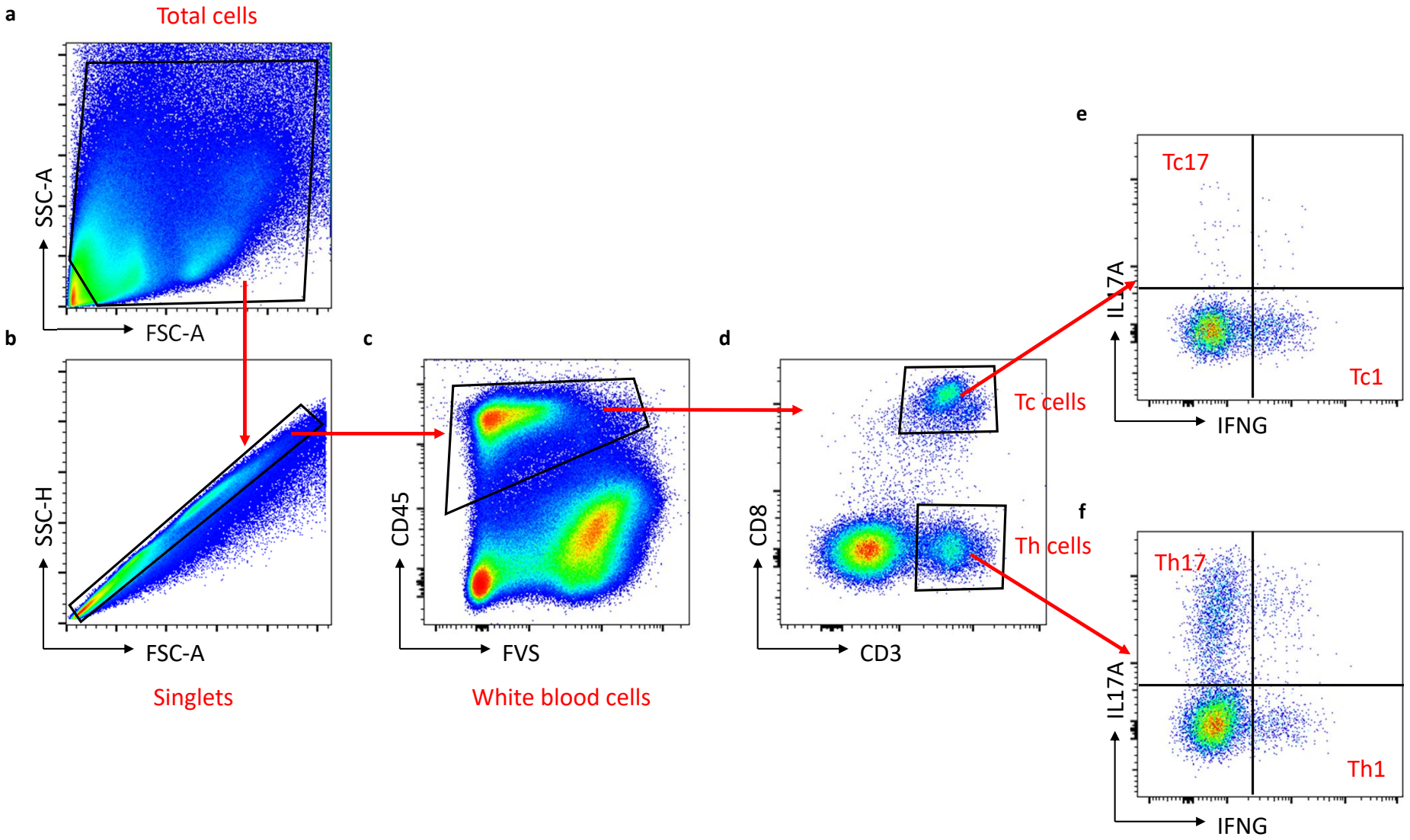
Supplementary Figure S5. Repeated CS exposure/rest cycle enhance the T cell response to elastin. Mice were subjected to CS exposure/rest for two cycles, lung red-blood-cell-free single-cell-suspensions were cultured in the presence or absence of elastin for 72 hrs, (**a. b**) cell culture supernatants were collected for ELISA analysis. Data are representative of 5 mice. Data are mean ± s.e.m. *P < 0.05, **P < 0.01 by one-way ANOVA.

Supplementary Figure S6. Exposure to CS for 6 months induces emphysema-like phenotype in mice. Exposure to CS for 6 months induces emphysema-like phenotype in mice. **(a)** Inflammatory cell counts in the BALF. **(b)** Representative images of Masson's trichrome staining of mouse lung sections and the semi-quantified scoring. **(c)** Representative images of PAS staining of mouse lung sections and the semi-quantified scoring. **(d)** Representative images of airspace and measurement of the MLI of mouse lungs (n=16). Mac: macrophages; Neu: neutrophils; Lym: lymphocytes; Eos: eosinophils. Throughout, data are representative of 7 mice unless otherwise indicated, and were replicated in at least 3 independent experiments. Data are mean ± s.e.m. ***P < 0.001 by student's *t*-test.

Supplementary Figure 1



Supplementary Figure 2



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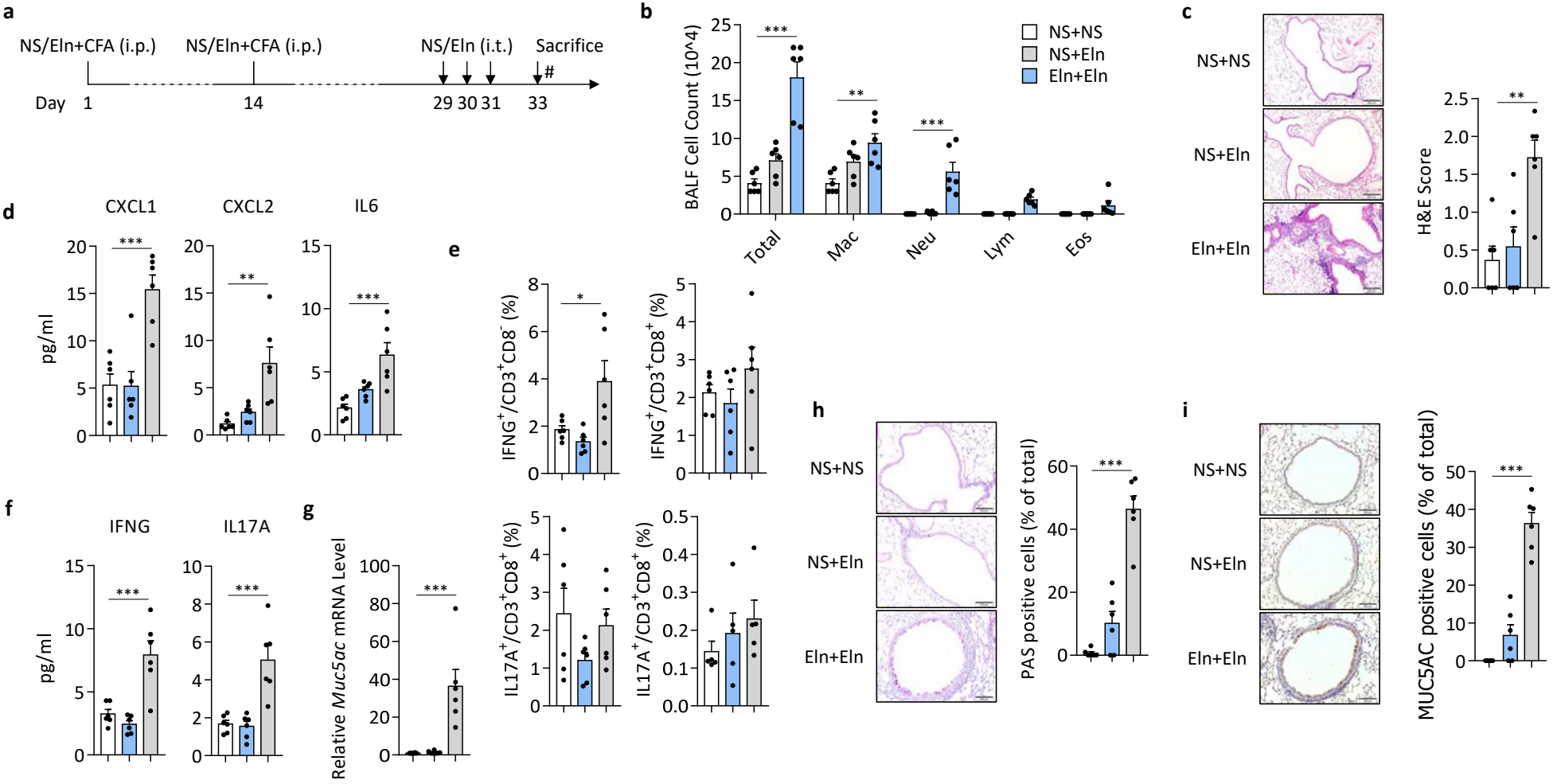

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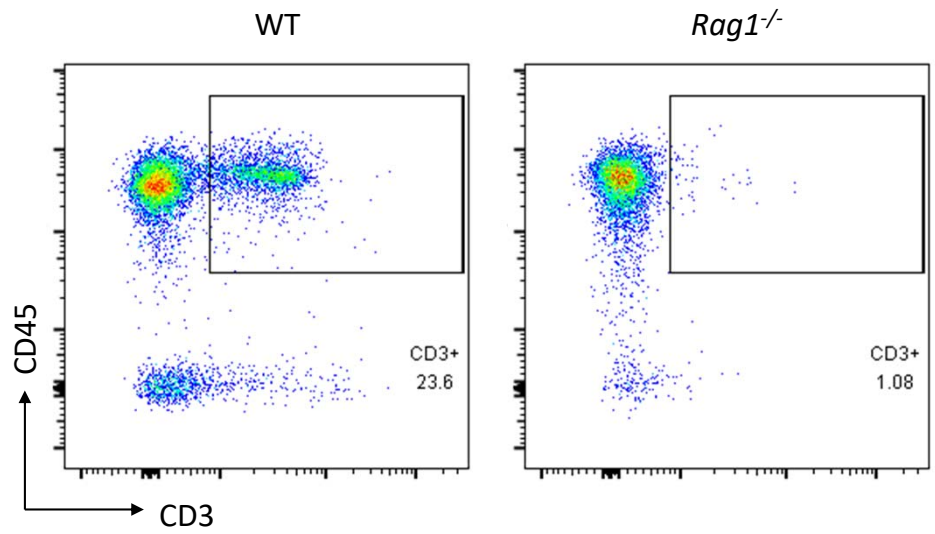
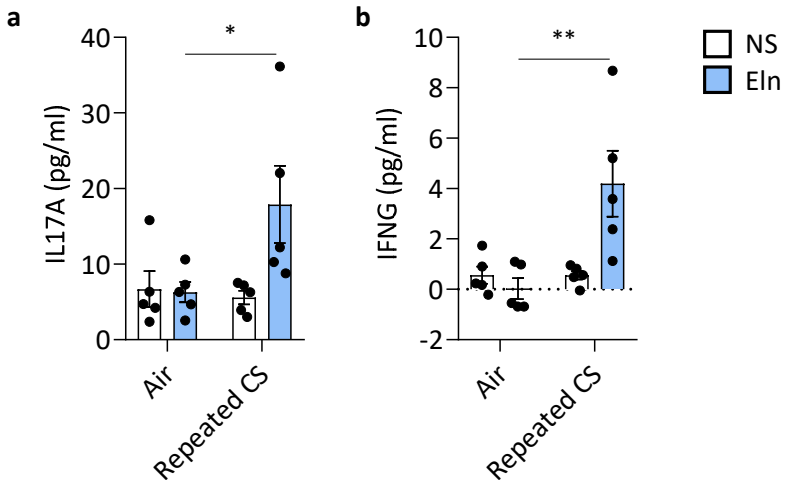


Supplementary Figure 5



Supplementary Figure 6