UC Supporting information

Methods

Peptide treatment in HBE cells and confocal microscope

A human bronchial epithelial (HBE) cell line was obtained from Dr. Reen Wu, University of California, Davis. HBE cells were cultivated in BEGM media (BEBM media supplemented with 0.5 ng/mL hEGF, 5 μg/mL insulin, 0.5 μg/mL hydrocortisone, 0.5 μg/mL epinephrine, 50 μg/mL gentamycin, 50 μg/mL amphotericin B, 6.5 ng/mL triiodothyronine, 10 μg/mL transferrin, 0.13 mg/mL bovine pituitary extract and 100 μg/mL penicillin, streptomycin, and fungizone (all supplied by Lonza). HBE cells (2×10^5 cells/mL) were seeded on a 4-well chamber slide (Thermo Fisher Scientific™ Nunc™) for 24 h. Cell were then washed with HBSS and incubated with 100 nmol MPS-FITC, A-MPS-FITC, and D-MPS-FITC at 37°C for 5 min. After peptide incubation, the cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) and subjected to confocal microscopy. NHBE cells were visualized and images were captured using the Leica TCS SP2/SP8X confocal microscope (Heidelberg, Germany).

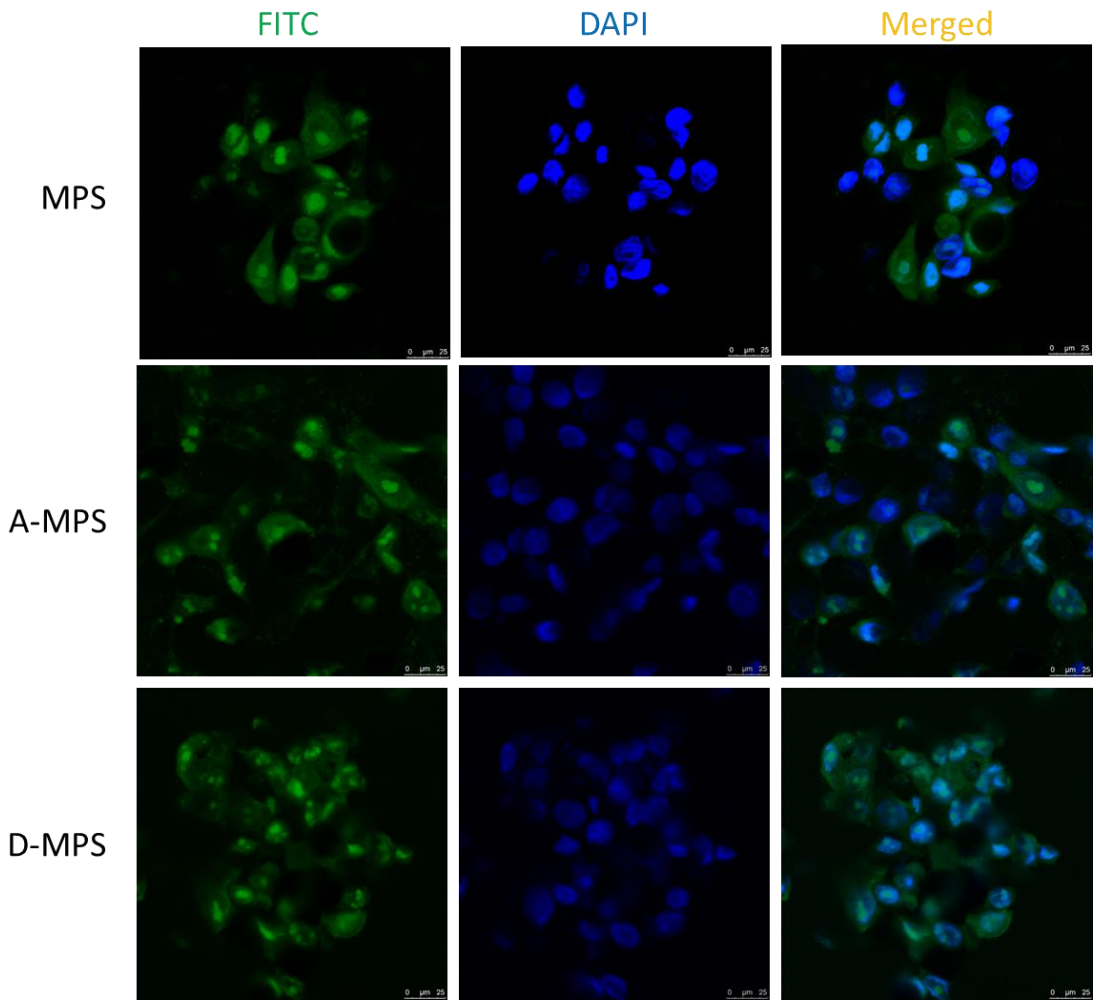


Figure S1. Intracellular localization of MPS, A-MPS, and D-MPS peptides in NHBE cells. NHBE cells were seeded in a chamber slide for 24 h and then incubated with 100 nmol MPS-FITC, A-MPS-FITC, and D-MPS-FITC at 37°C for 5 min. The cells were washed thrice with 1 ml HBSS and then subjected to confocal microscopy. Nuclei were stained with DAPI which showed blue fluorescence. Green fluorescence indicates different MPS peptides. Scale bar: 25 µm, N = 5.

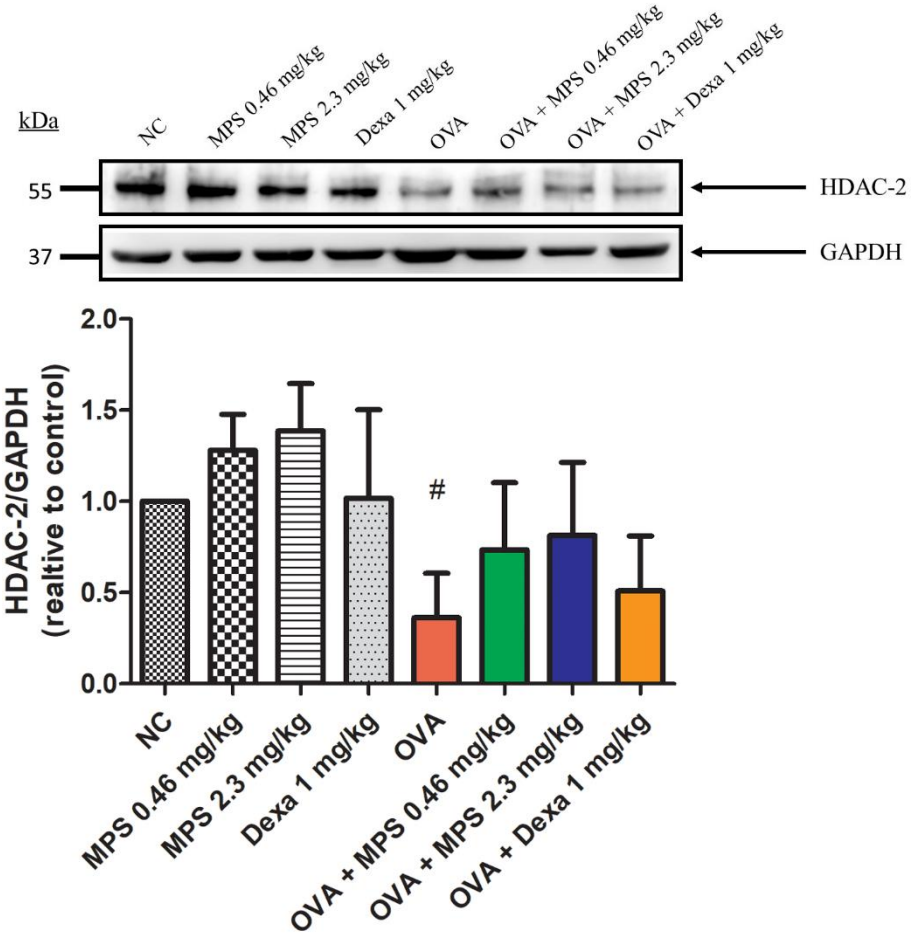
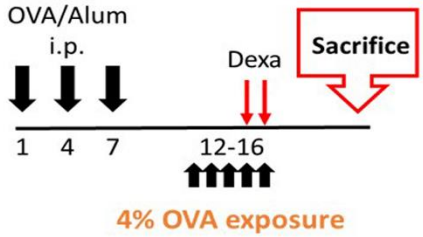
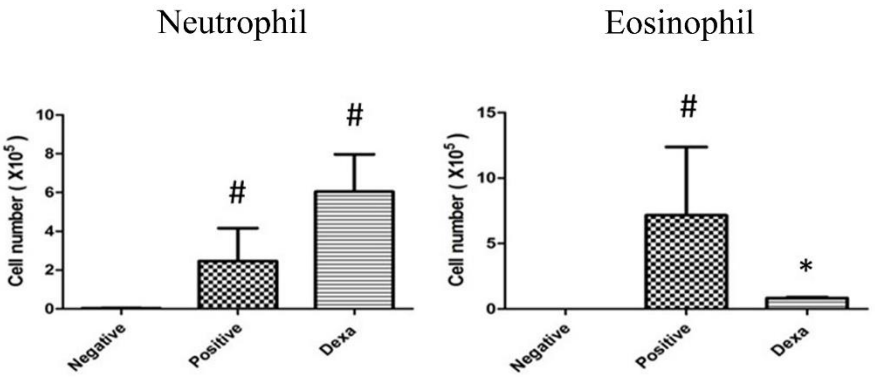


Figure S2. The effect of HDAC-2 protein expression in a murine model of steroid-resistant like asthma. The lung proteins were subjected to western blot analysis to assess the expression of HDAC-2 and GAPDH. The proteins were quantified using Image J. Data are expressed as the mean ± S.D. (n = 5). $^{\#}P < 0.05$, as compared with the NC group.

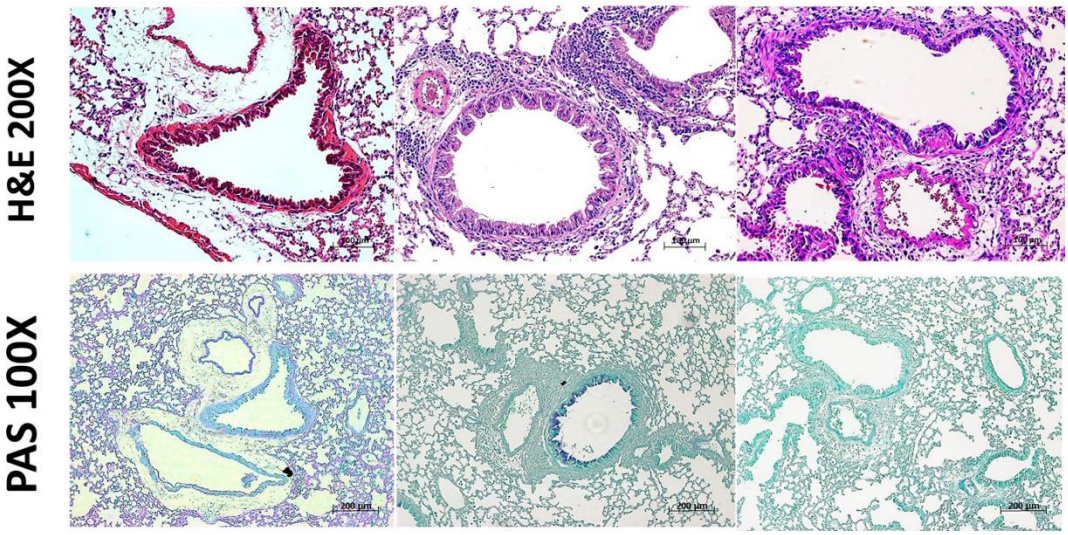
A

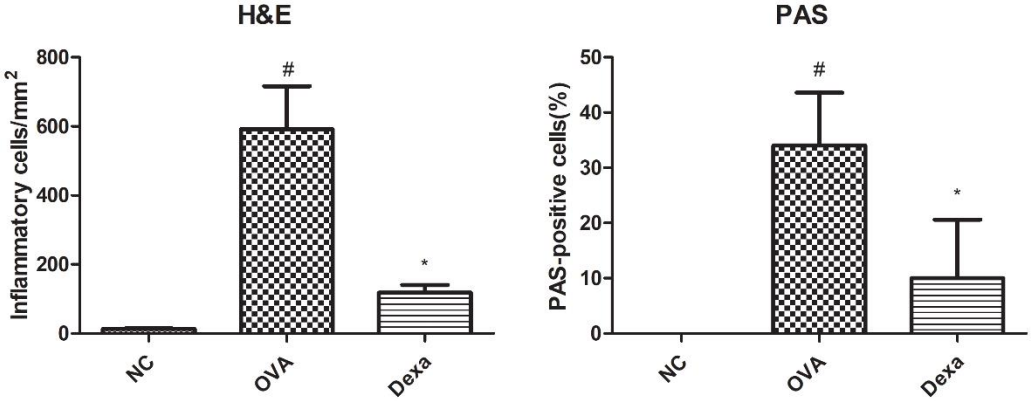

OVA/Alum
i.p.
Dexa
Sacrifice
1 4 7
12-16
4% OVA exposure

B

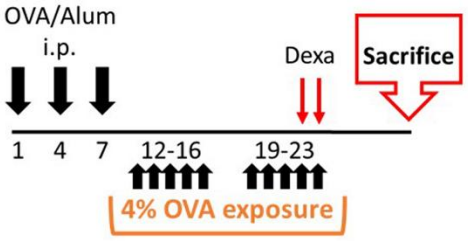

Neutrophil
Eosinophil
Cell number (X10^5)
Negative
Positive
Dexa
#
*

C

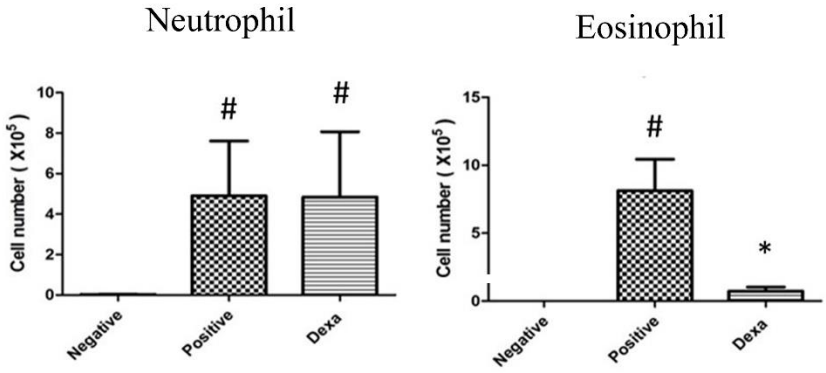

H&E 200X
PAS 100X


H&E
PAS
Inflammatory cells/mm2
PAS-positive cells(%)
NC
OVA
Dexa
#
*

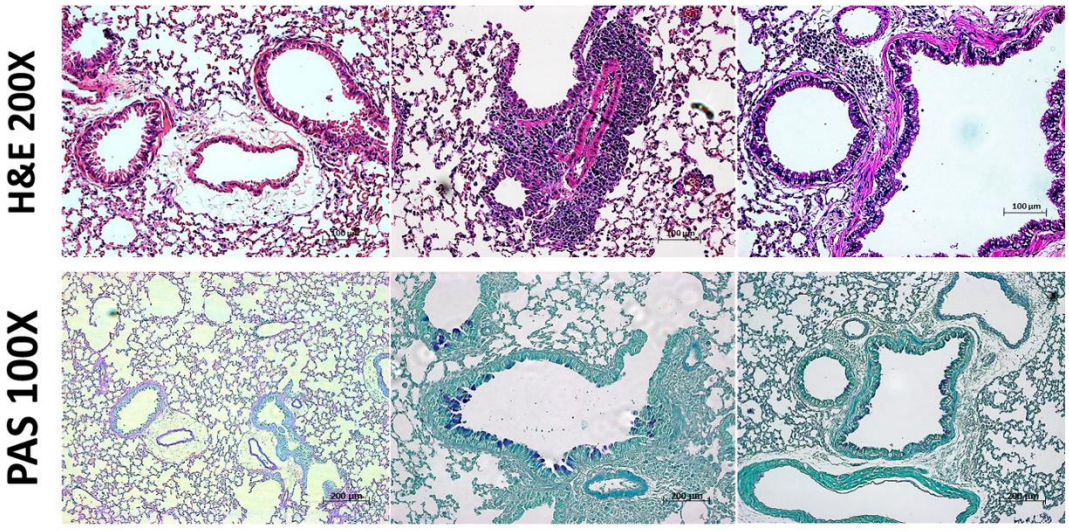
D

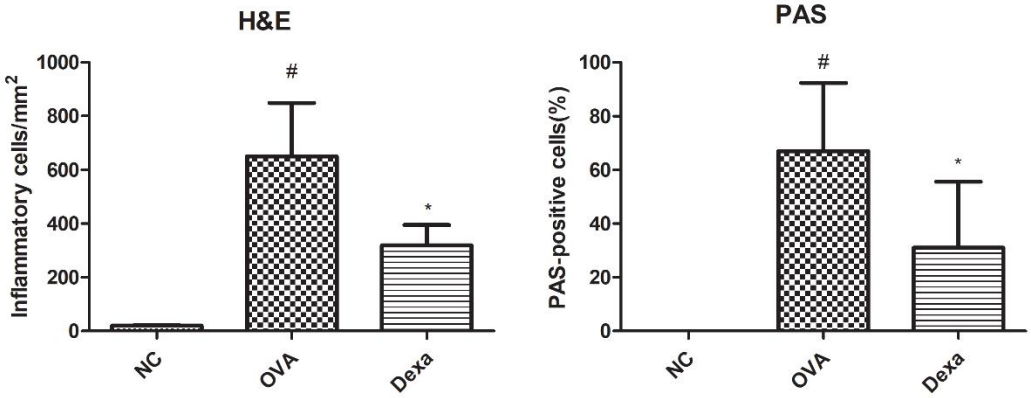

OVA/Alum
i.p.
Dexa
Sacrifice
1 4 7
12-16
19-23
4% OVA exposure

E


Neutrophil
Eosinophil
Cell number (X10^5)
#
*
Negative
Positive
Dexa

F


H&E 200X
PAS 100X


H&E
PAS
Inflammatory cells/mm^2
PAS-positive cells(%)
#
*
NC
OVA
Dexa

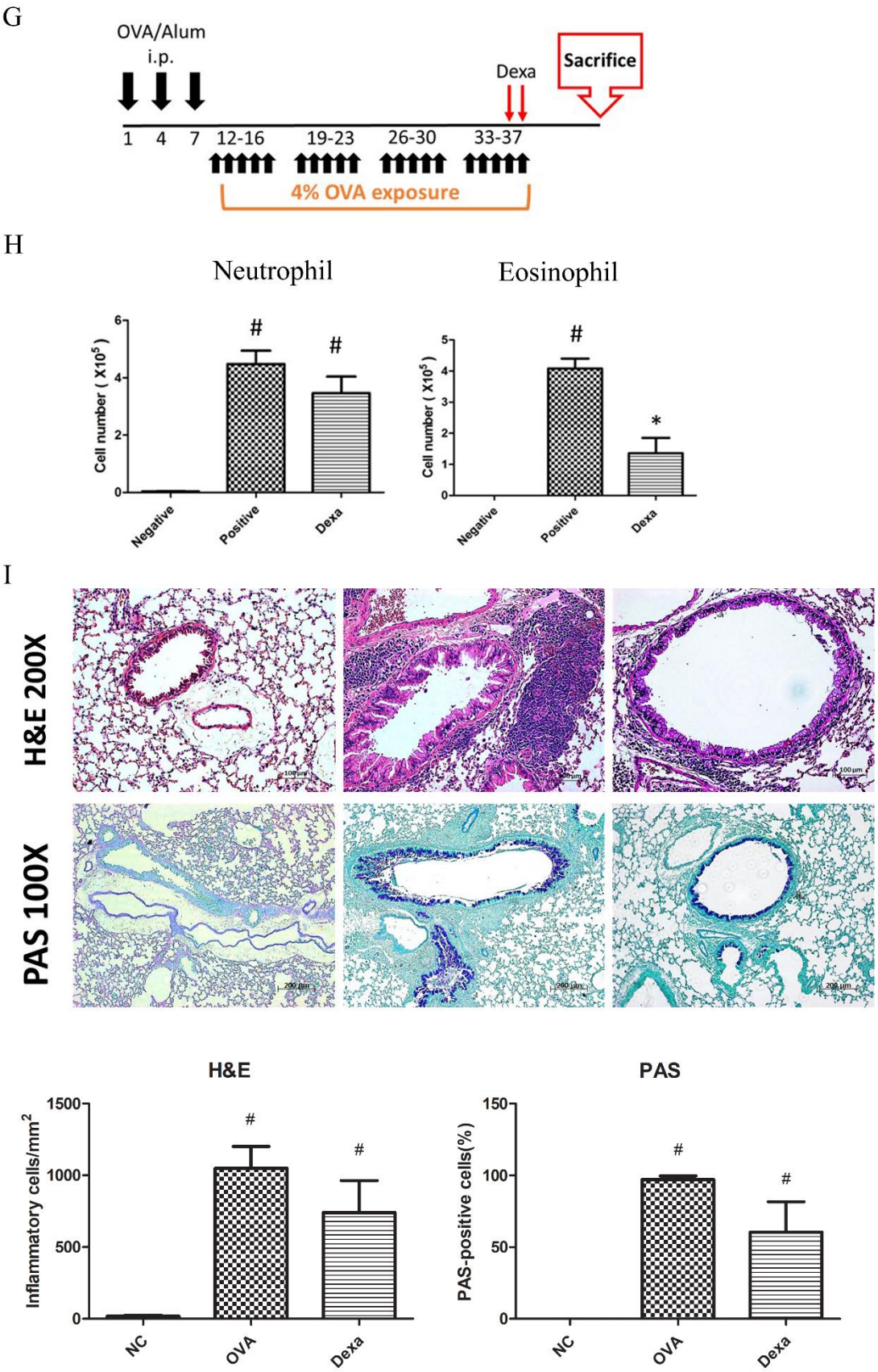


Figure S3. Pulmonary inflammation and pathology at different times of OVA exposure.

(A, D, G) Mice at 6 weeks of age were sensitized with OVA-alum. On day 12, the mice were exposed to PBS or 5 mL 4 % OVA for five days per week for 1, 2, and 4 weeks, respectively. (B, E, H) Mice were intratracheally instilled with 1 mg/kg of dexamethasone twice, at 24 h and 48 h before dissection. On day 17, 24, and 38, the mice were sacrificed and differential cell counts in the BAL were assessed by May-Giemsa staining. (C, F, I) Lungs were subjected to hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and Masson's trichrome (MT) staining. High magnification (bar represents 100 μm for H&E and MT, 200 μm for PAS). Data are expressed as mean ± S.D. ($n \geqq 5$). $^{\#}P < 0.05$, compared to the NC group.; $^{*}P < 0.05$ compared to OVA group.

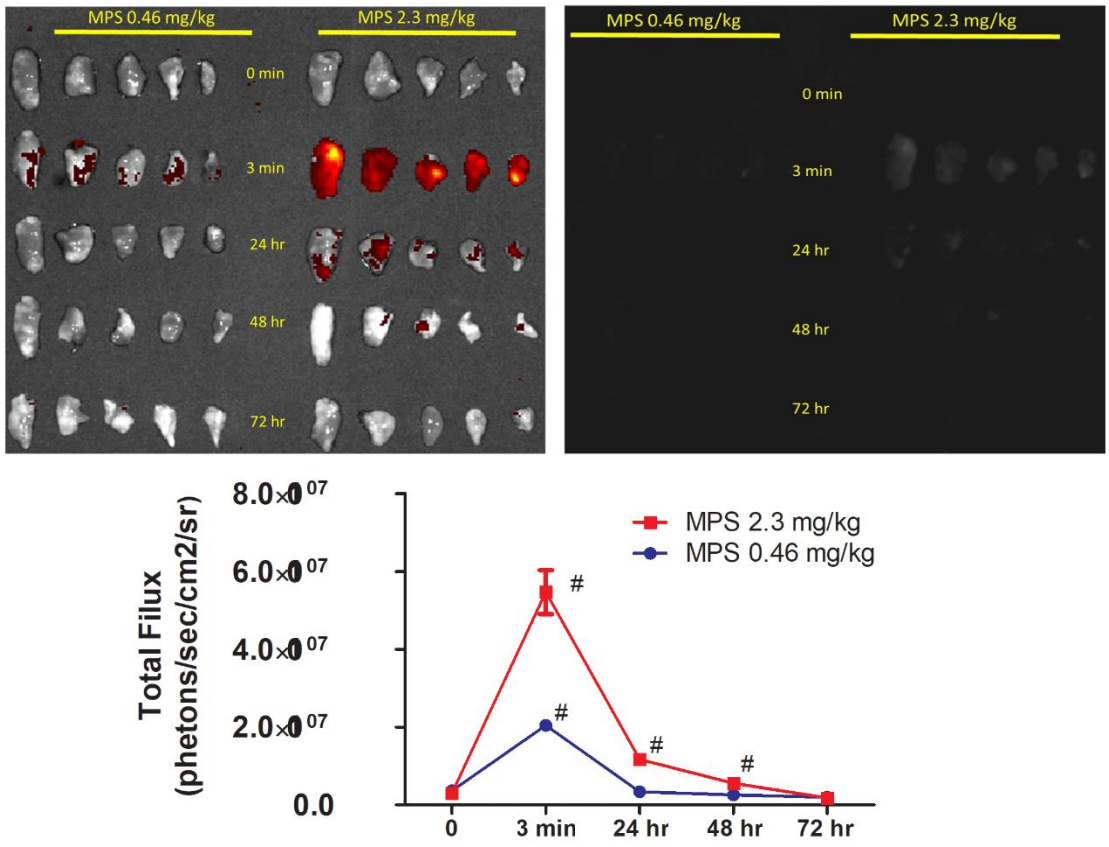


Figure S4. Retention time of MPS peptides in the airway. MPS-FITC peptides (0.46 mg/kg and 2.3 mg/kg) were intratracheally instilled in mice at different time points (0 min, 3 min, 24 h, 48 h, and 72 h), and the lungs were then dissected and observed under an IVIS System to measure and quantify the fluorescence intensity. Data are expressed as mean ± S.D. (n $\geqq$ 5). $^{\#}P < 0.05$, as compared with the NC.

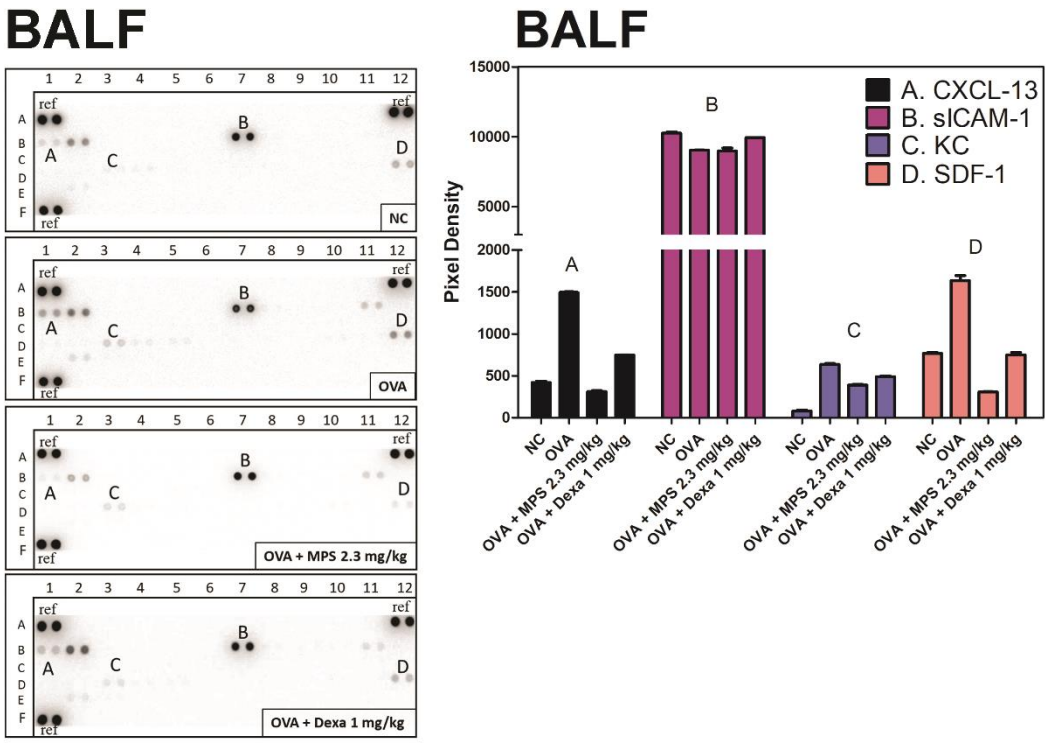


Figure S5. Cytokine expression after MPS treatment using a cytokine array in a murine model of steroid-resistant asthma. BAL fluid (BALF) was analyzed with a mouse cytokine array to screen for 40 cytokines, chemokines, and complement and acute-phase proteins in the BAL. Pixel density plots of chemokine (C-X-C motif) ligand (CXCL)-13 (A), soluble intercellular adhesion molecule-1 (sICAM-1) (B), KC (C) and stromal cell-derived factor (SDF)-1 (D) in BALF, and their quantification using Image J.

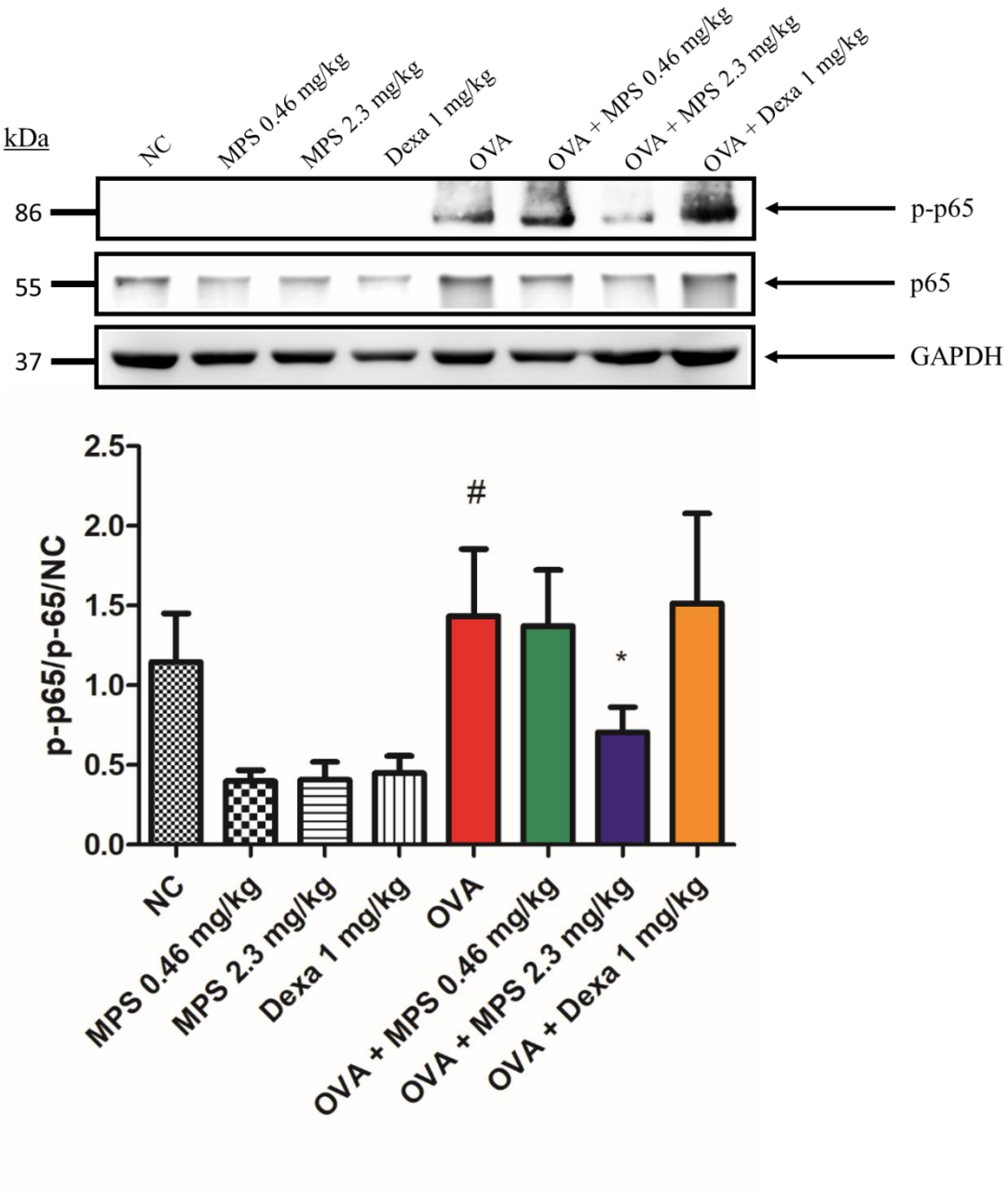


Figure S6. The effect of phospho-p65 and p-65 in mice with steroid-resistant like asthma. Lung proteins were subjected to western blot analysis to assess the expression of p-p65, p-65, and GAPDH. The proteins were quantified using Image J. Data are expressed as the mean ± S.D. (n = 5). $^{\#}P < 0.05$, as compared with the NC group. $^{*}P < 0.05$, as compared with the OVA group.

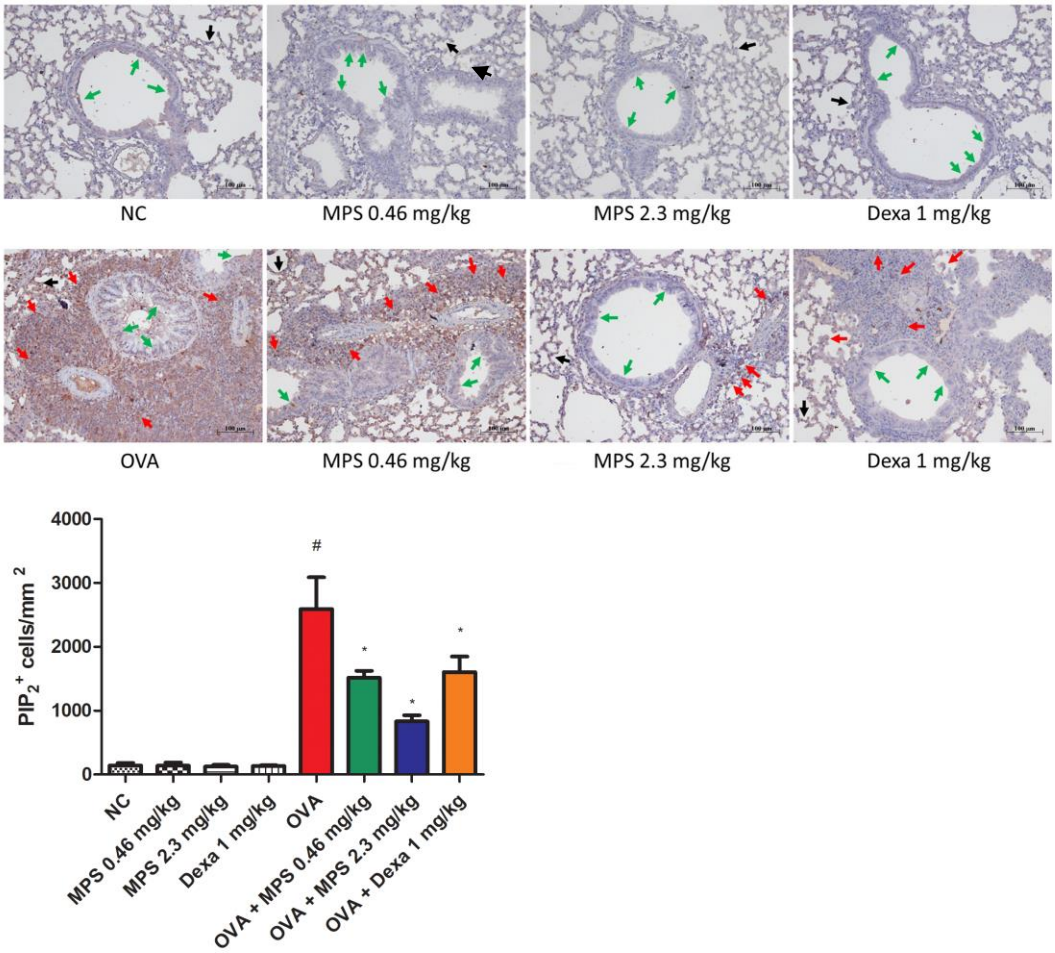


Figure S7. The effect of phosphatidylinositol 4,5-biphosphate (PIP2) levels on the airways of OVA-immunized mice. Mice at 6 weeks of age were sensitized with OVA/alum, as described in **Figure 1B**. Paraffin sections of mouse airway tissues were deparaffinized and IHC stained with PIP_2 antibody (green arrows represent bronchial epithelial cells, black arrows represent alveolar epithelial cells, and red arrows represent for inflammatory cells). High magnification (bar represents 100 μm).