

Supplementary information

Figure S1. (A) Compliance of the respiratory system (Crs) was determined after i.n., PBS- (blue line), OVA-challenged (red line), and FDE treatment (purple line). Crs was measured as airway resistance in response to methacholine exposure. Data are presented as mean \pm SEM. n = 3 mice per group. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test; *p < 0.05, **p < 0.01, ***p < 0.001. **(B)** Elastance of the respiratory system (ERS), determined after i.n. PBS- (blue line), OVA-challenged (red line), and FDE treatment (purple line). Data are presented as mean \pm SEM. n = 3 mice per group. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test. **(C)** Gating strategy to identify pulmonary, CD103+ cDCs, CD11b+ cDCs and moDCs in BALB/c mice. CD11b+ cDCs were identified as SiglecF⁻ lin⁻ CD11C⁺ MHC-IIhi CD103⁻ CD11b⁺ CD64⁻ cells. moDCs were identified as SiglecF⁻ lin⁻ CD11C⁺ MHC-IIhi CD103⁻ CD11b⁺ CD64⁺ cells and CD103+ cDCs were identified as SiglecF⁻ lin⁻ CD11C⁺ MHC-IIhi CD103⁺ cells. **(D)** MFI of CD103 on DC, PBS- (solid Turquoise), OVA-challenged (solid red), and FDE treated (solid purple), or i.p. dexamethasone treatment (solid blue). **(E)** Fluorescence-minus-one (FMO) controls for Foxp3 and CD25 in Treg cells. The gating strategy included pre-gating on CD4⁺CD3⁺ cells to accurately identify the regulatory T cell population. **(F)** Gating strategy to identify cells in BALF, Contour plots of lung cells from PBS (upper left panel), OVA (lower right panel), FDE (upper right panel), and Dexamethasone (lower left panel) treated mice. Eosinophils (Eos) were identified as SiglecF⁺ CD11C⁻, alveolar macrophages (AMs) were identified as SiglecF⁺ CD11C⁺, neutrophils were identified as SiglecF⁻ Ly6g⁺, T cells were identified as SiglecF⁻ CD4⁺ cells.

Figure S2. BALF level of IL-17A, IL2, and TNF- α and serum level of IL-22, IL-17a, TNF- α , and IFN- γ . PBS (upper left panel), OVA (lower right panel), FDE (upper right panel), and Dexamethasone (lower left panel) treated mice. Data shown are the mean \pm SEM; n = 3-6. Data were analyzed by ANOVA followed by a Tukey test.

Figure S3. (A) Number of cells in the epithelial compartment analyzed by single-cell RNA sequencing, comparing FDE- and PBS-treated lungs. **(B)** Percentage of cells within the non-epithelial compartment after FDE treatment, based on single-cell analysis. **(C)** Number of cells in the immune cell compartment analyzed by single-cell RNA sequencing, comparing FDE- and PBS-treated lungs. **(D)** Percentage of cells within the immune compartment after FDE treatment, based on single-cell analysis. **(E)** Predicted cell-cell communication analysis among all immune cells, illustrating the interaction networks identified by single-cell analysis. **(F)** Treg cell-specific, cell-cell communication patterns. Abbreviations: Monocyte-derived macrophages (MDM), Cytotoxic T cells (CTL), Cycling macrophages (CyM), Immature neutrophils (ImNs), Lung-resident macrophages, (LRM) Mast cells/Basophils (MCB)

Figure S4. (A) Heat map analysis in Neutrophil cells based on key surface marker expression profiles. (B) Heat map analysis in AT2 cells based on gene expression profiles of key MHC-II genes. (C) Heat map analysis in goblet cells based on key gene expression profiles associated with mucus formation. (D) Heat map analysis in club cells based on key gene expression profiles associated with mucus formation. (E) Heat map analysis in T cells based on gene expression profiles of key MHC-II genes (F) Monocyte-derived macrophages (MDM)s based on gene expression profiles of key MHC-II genes. (G) Quantification of AMs numbers upon PBS- (white bar), OVA-challenged (red bar), and FDE (purple bar) or Dexamethasone-treated mice (blue bar). Data were analyzed by ANOVA followed by a Tukey test; Data show $\Delta\text{MFI} \pm \text{SEM}$; $n = 3-6$. (H) Quantification of IMs numbers upon PBS- (white bar), OVA-challenged (red bar), and FDE (purple bar) or Dexamethasone-treated mice (blue bar). Data were analyzed by ANOVA followed by a Tukey test; Data show $\Delta\text{MFI} \pm \text{SEM}$; $n = 3-6$.

Figure S5. (A) Frequency of PAS-positive cells (PBS- white bar), OVA-challenged (red bar), and FDE (purple bar), or i.p. dexamethasone treatment (blue bar). Data were analyzed by ANOVA followed by Tukey's post hoc test. (B) Airway inflammation by H&E scoring (PBS- white bar), OVA-challenged (red bar), FDE (purple bar), or i.p. dexamethasone treatment (blue bar). Data were analyzed by ANOVA followed by Tukey's post hoc test.

Figure S6. Assessment of fibrosis- and COPD-related gene expression following FDE treatment using single-cell RNA sequencing. (A) Gene expression profiles of canonical COPD markers across immune (A) and epithelial (B) compartments. (C–D) Expression of key fibrosis-associated genes in immune (C) and epithelial (D) cell populations.

Figure S7. Heat map analysis in macrophages based on genes associated with the canonical TGF- β signaling pathway. Group 1, which includes genes that previously have been strongly associated with severe wheezing and chronic cough in human populations, and Group 2, comprises genes less associated with these symptoms.

Figure S1

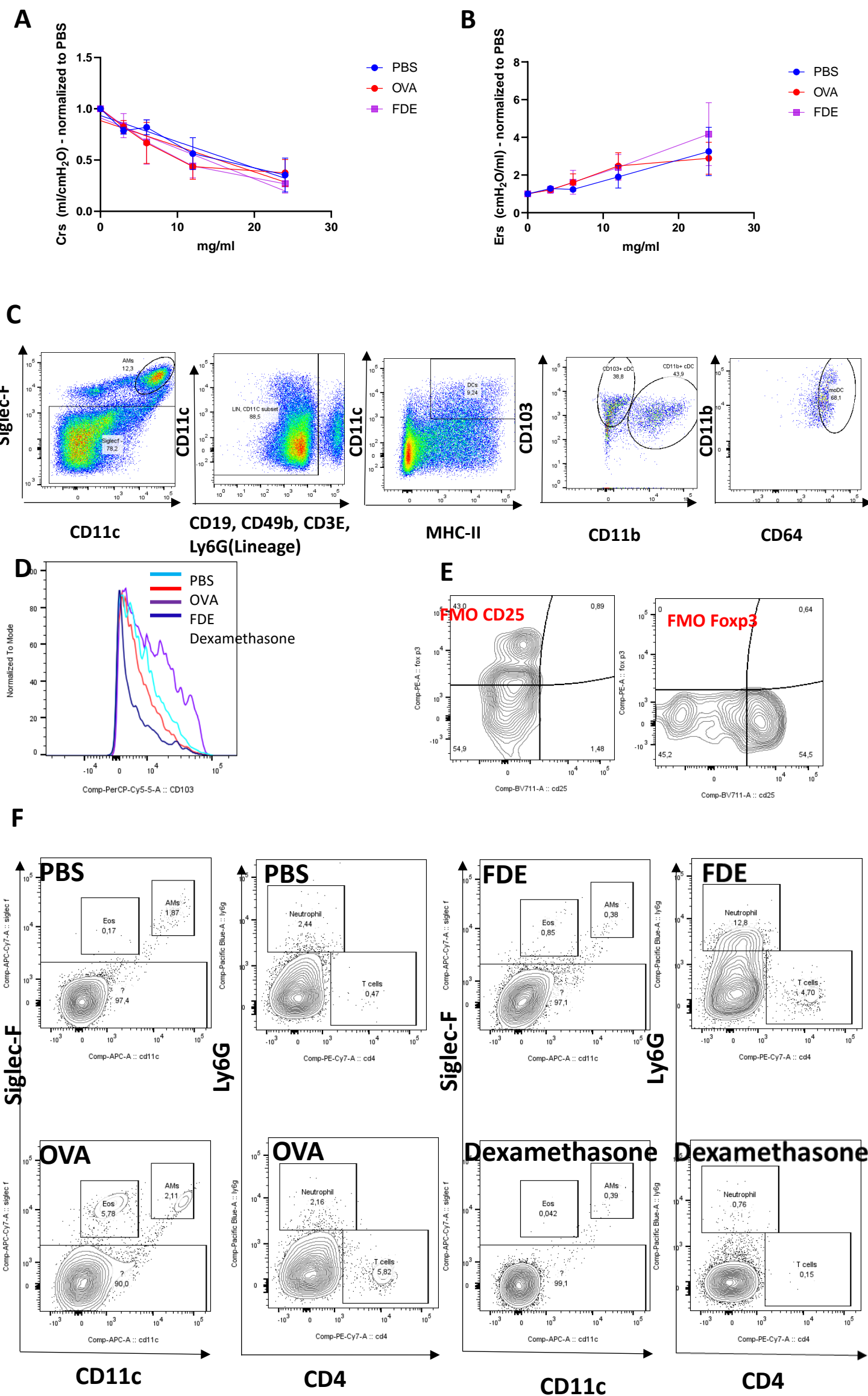


Figure S2

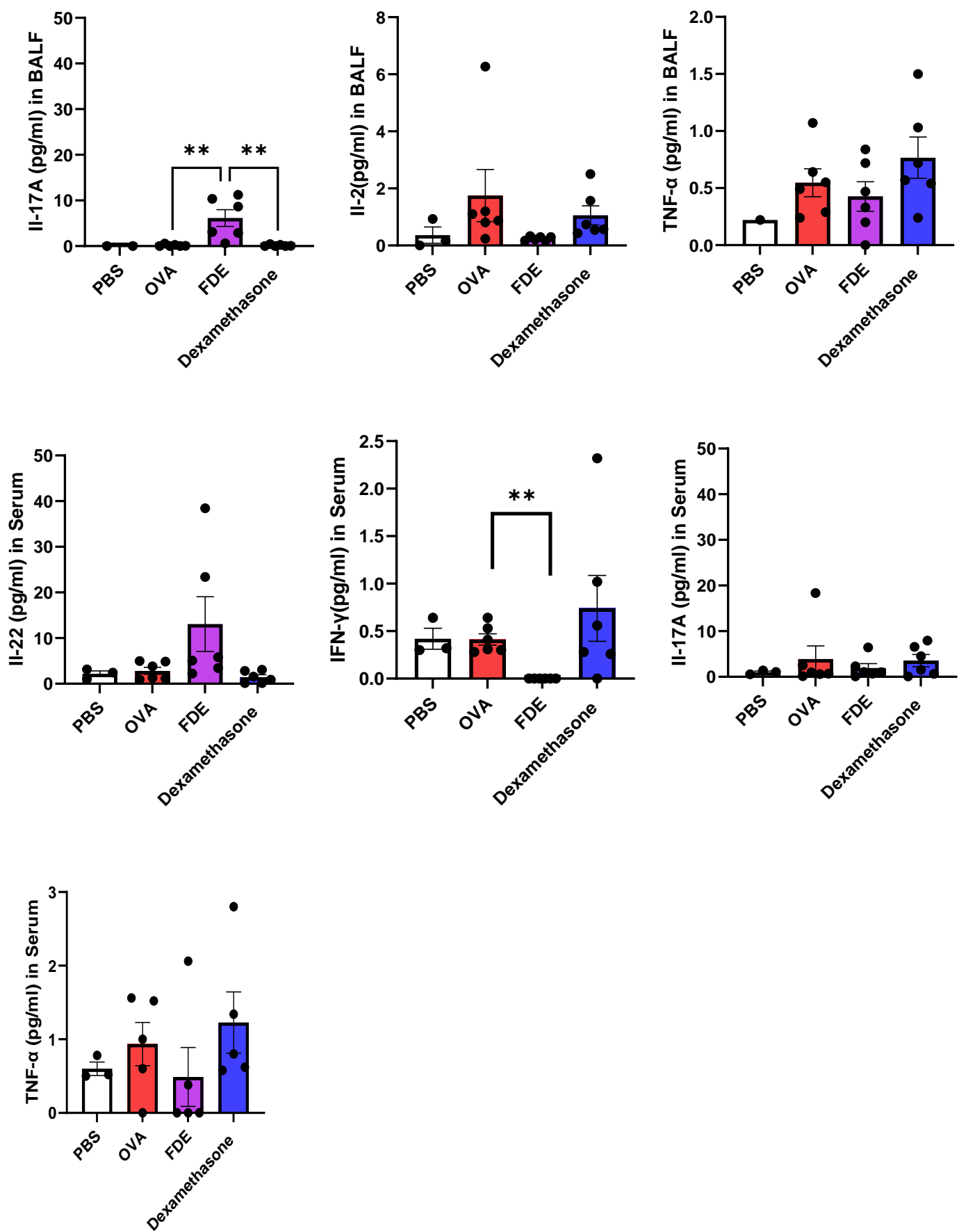


Figure S3

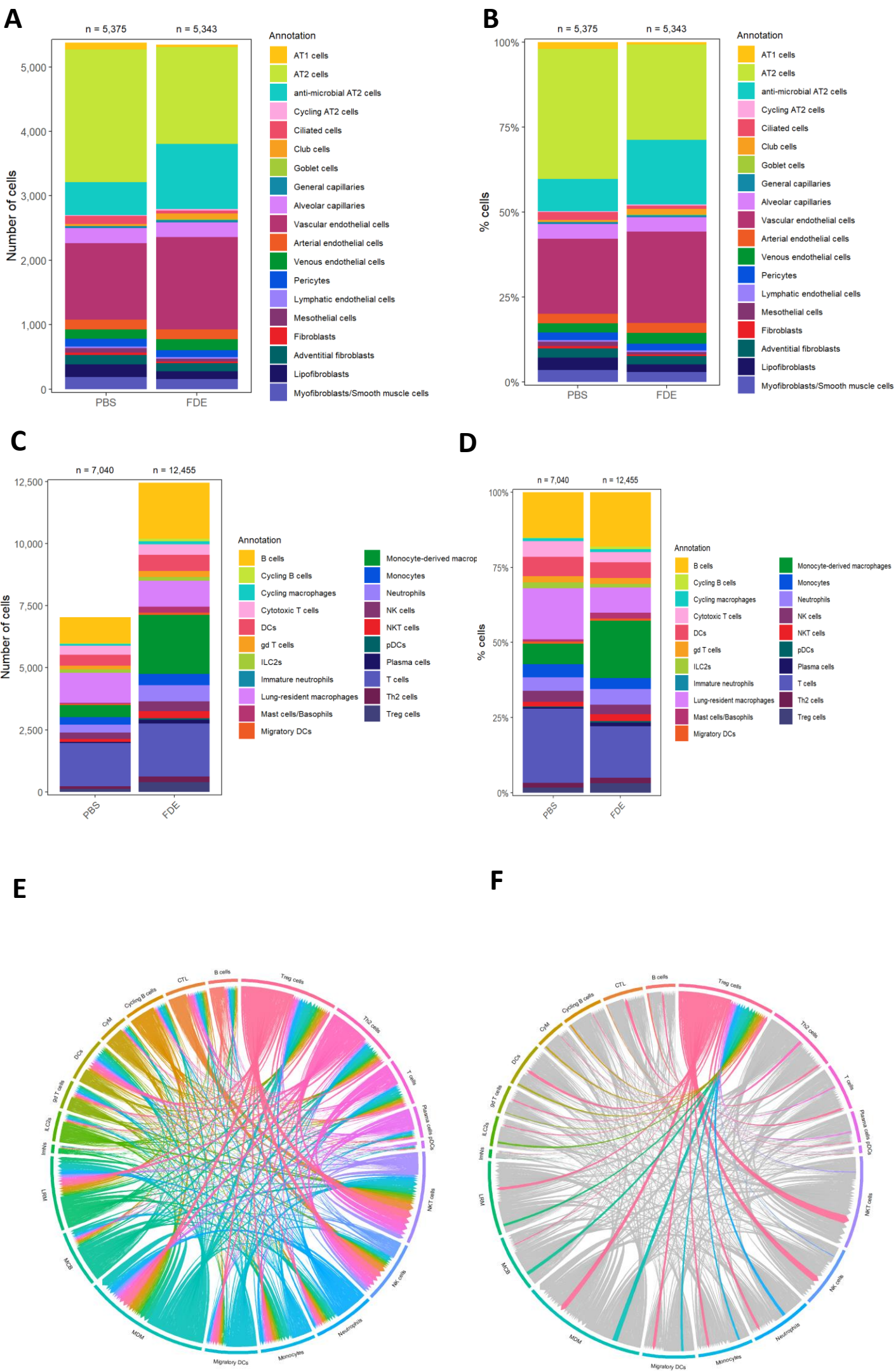


Figure S4

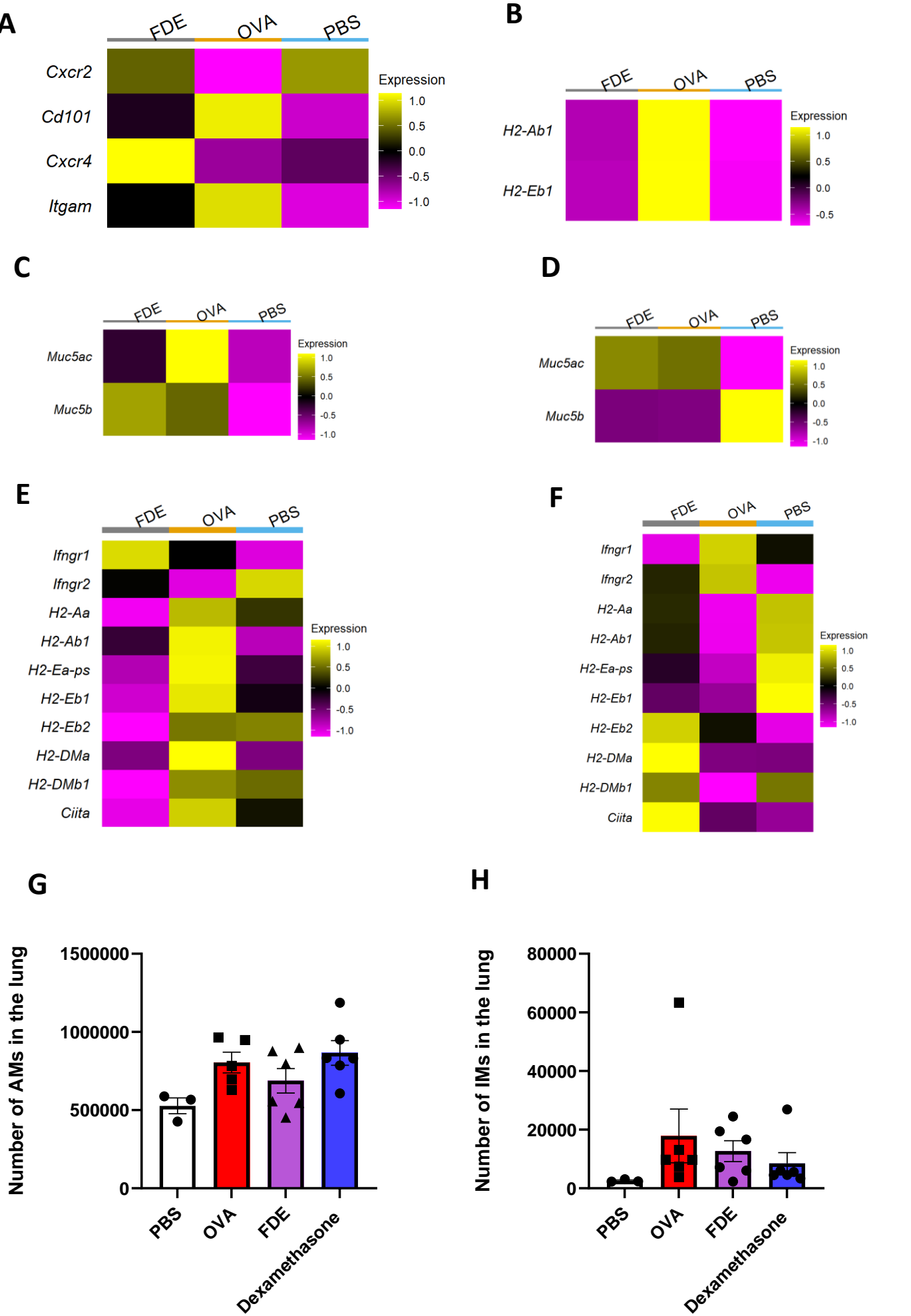
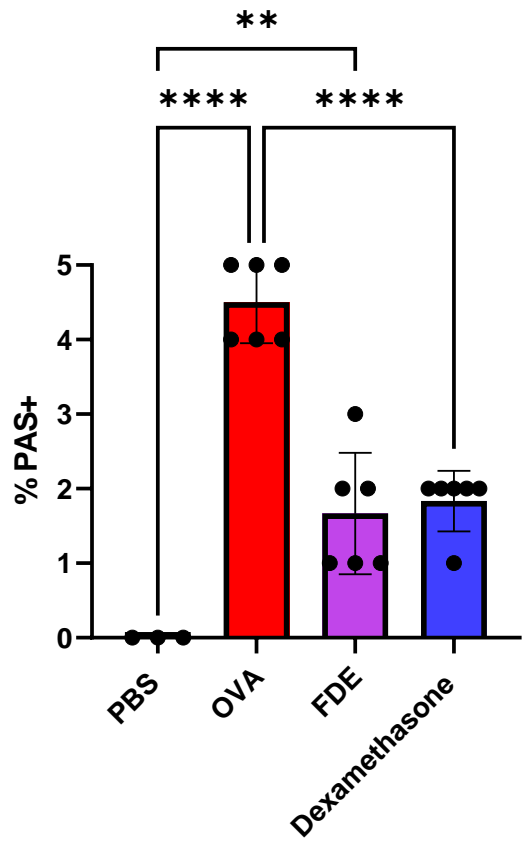
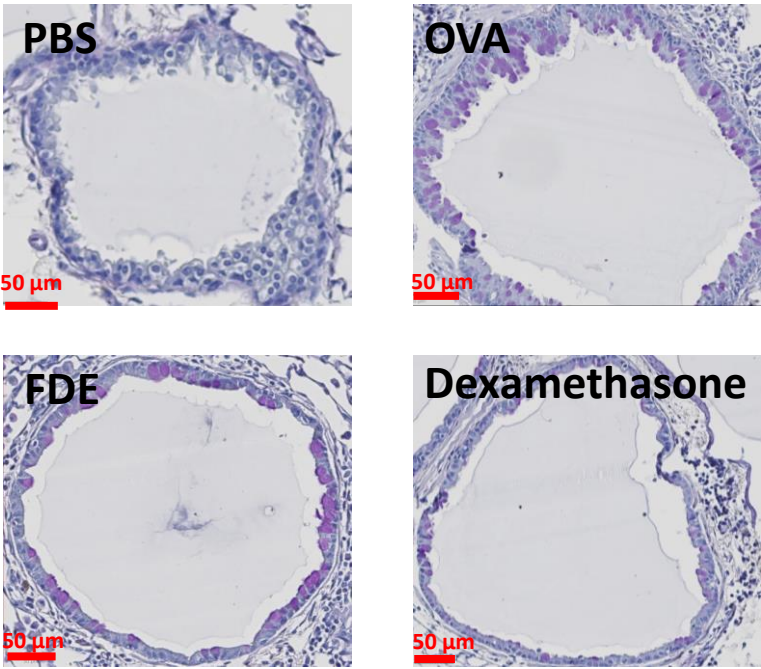


Figure S5

A



B

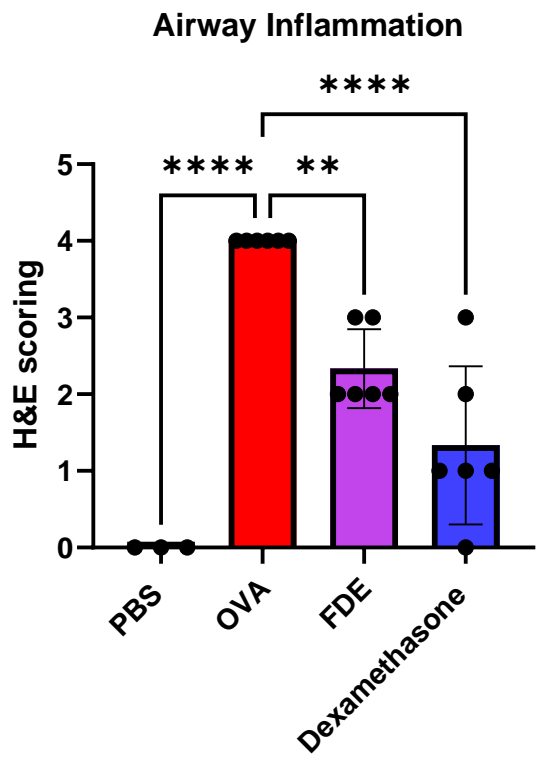
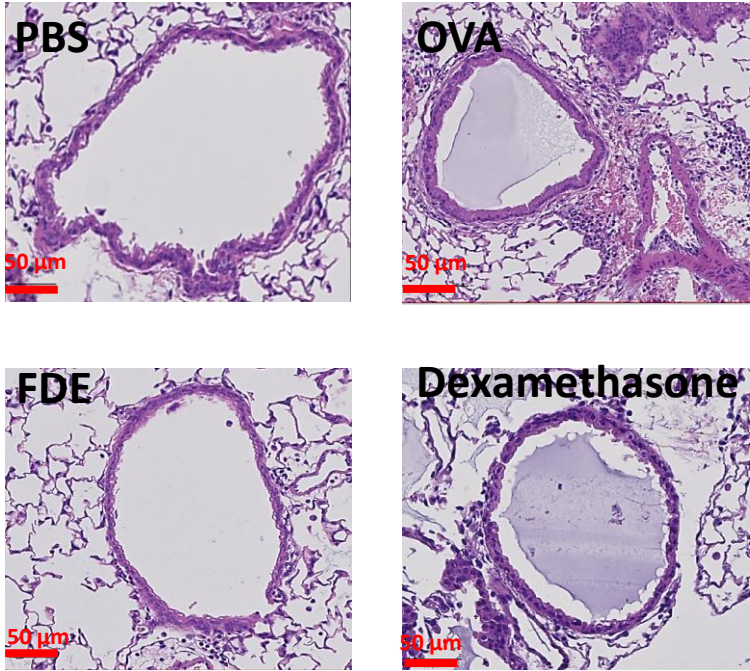


Figure S6

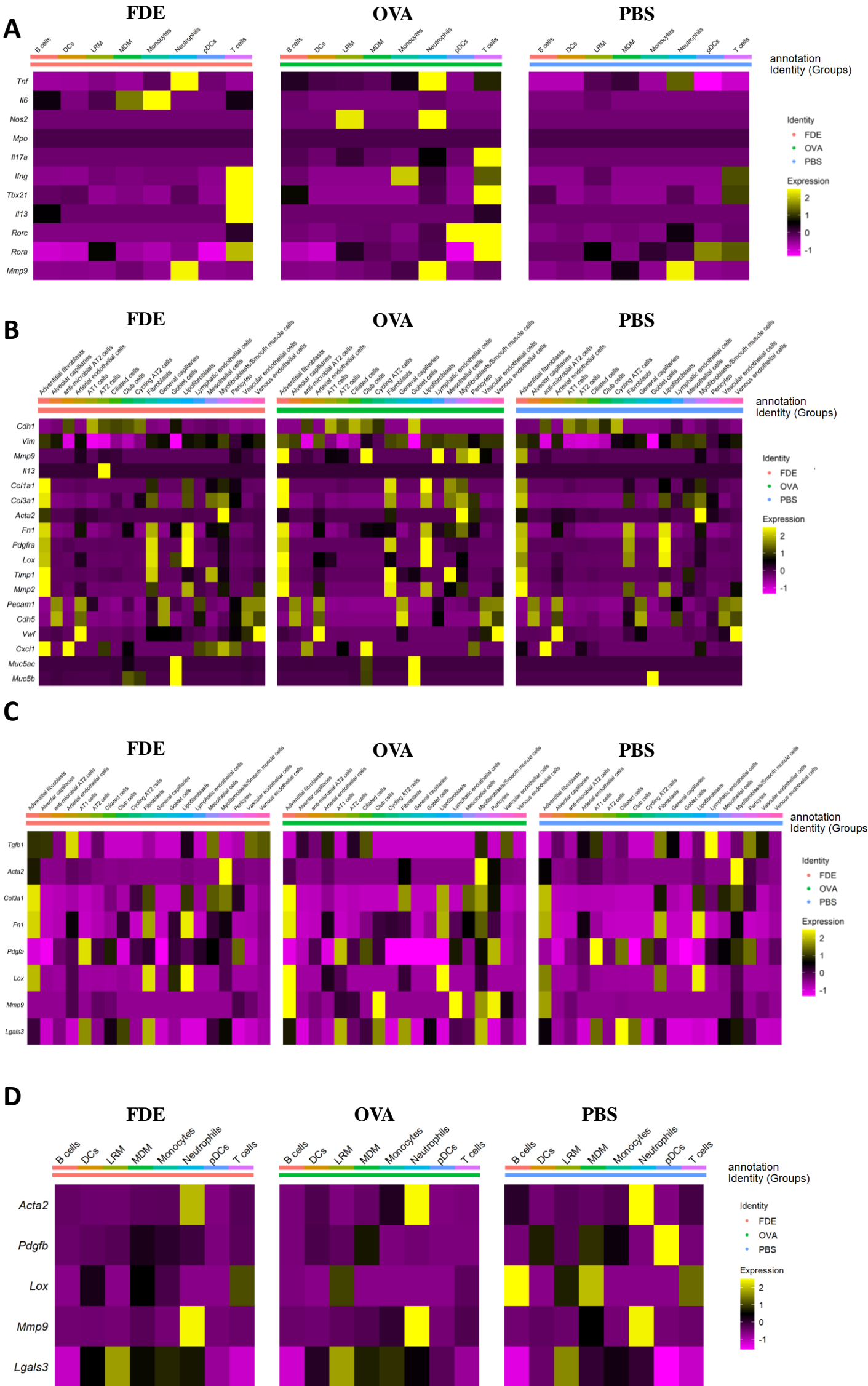


Figure S7

