

Asthma Insights: The View from the Airways

How Asthma Research Informs Clinical Care

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Science
Immunology

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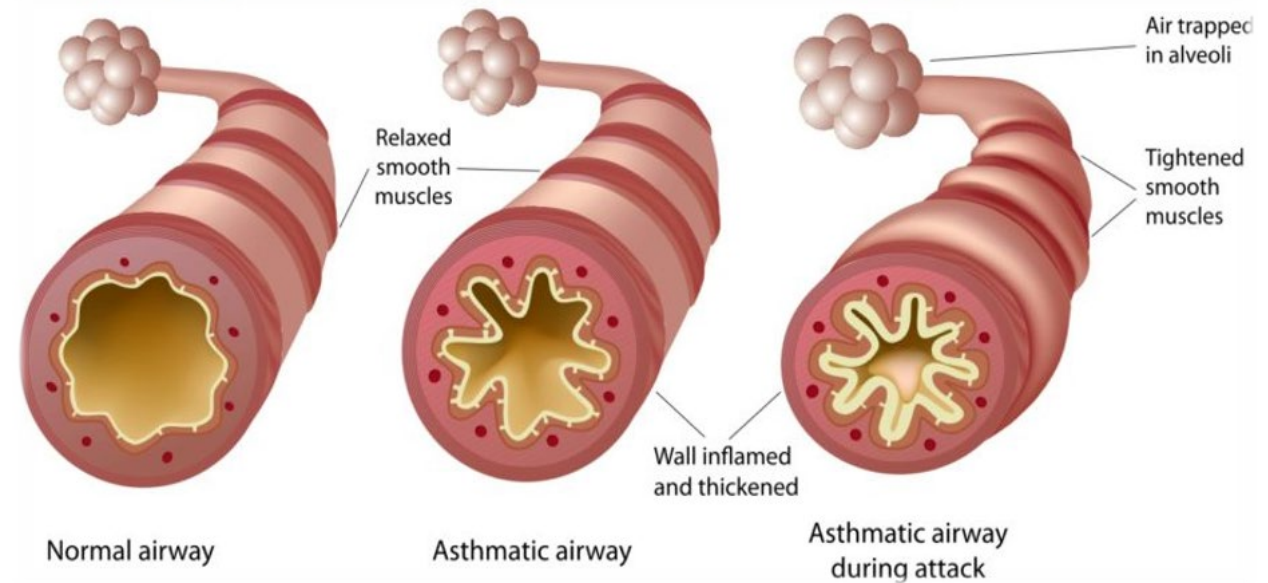


Disclosures:

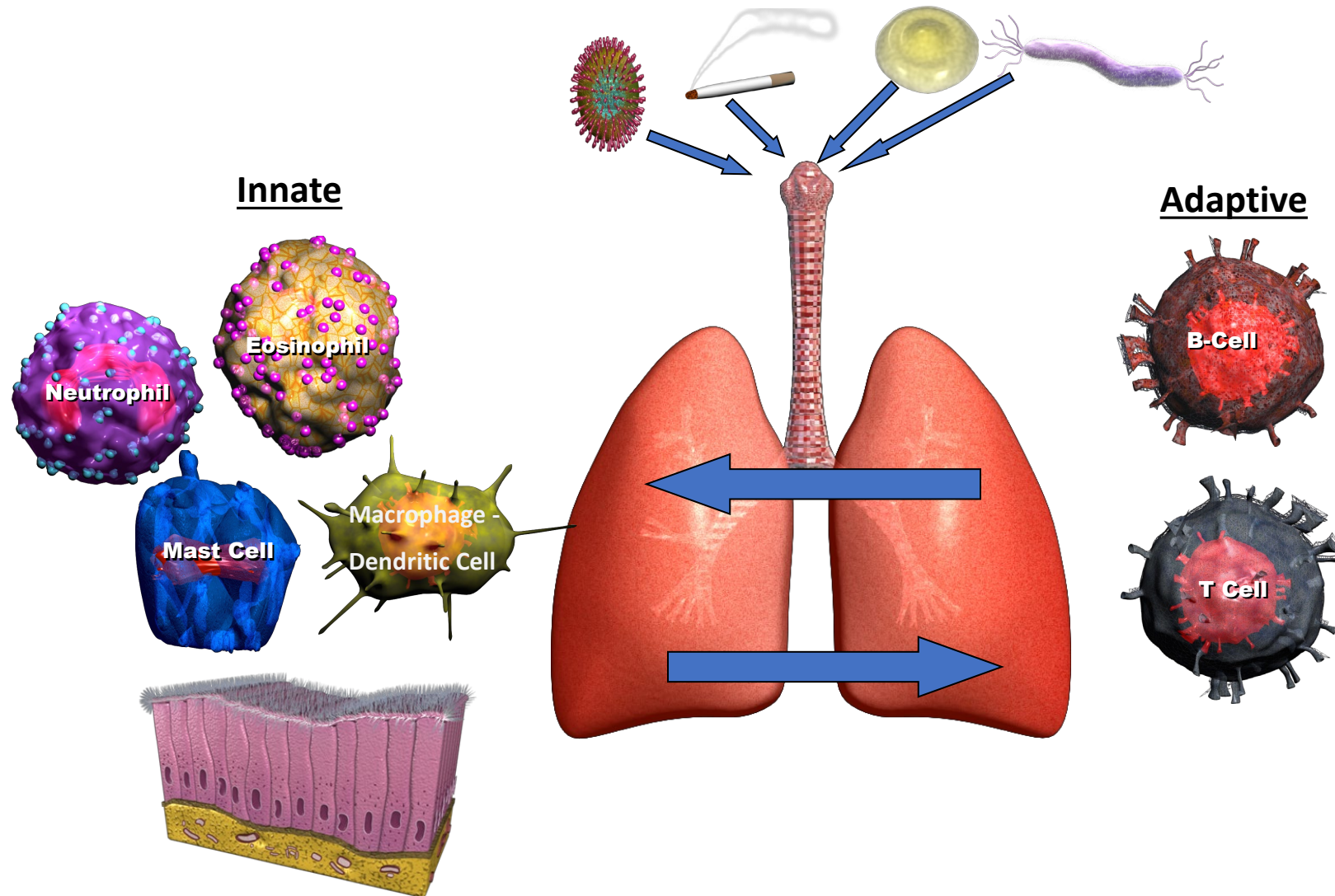
- Advisory board for Sanofi and Regeneron
- Sponsored research agreements with:
 - Sanofi
 - Regeneron

Asthma is a Common Disorder

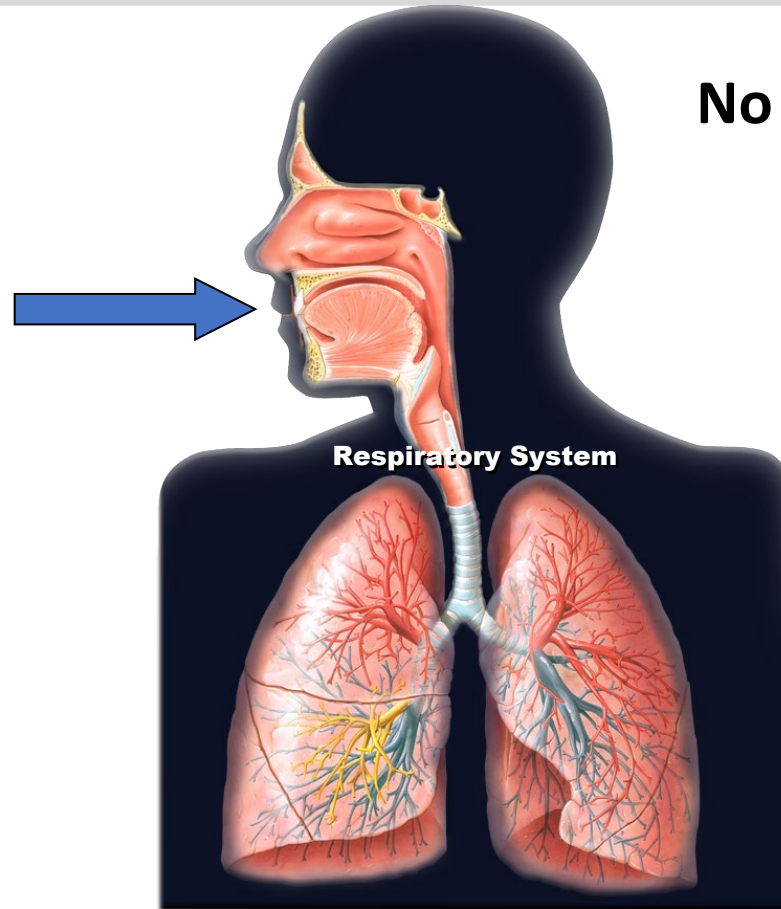
- Asthma is a chronic, heterogeneous syndrome characterized by:
 - Airway inflammation,
 - Mucus production, and
 - Airway hyper-responsiveness (AHR).
- Allergic Asthma most common form
- Even for patients with well-controlled asthma, there are significant impacts on quality of life.



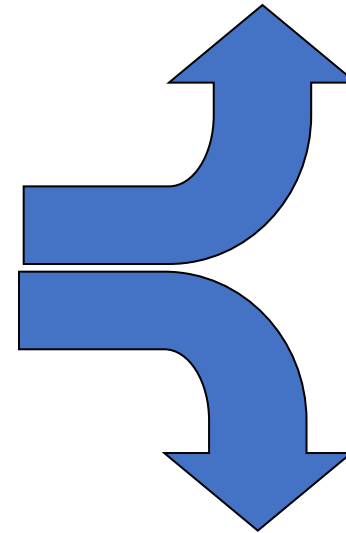
Lung Immunity



Allergic Asthma - Aberrant Immune Activation

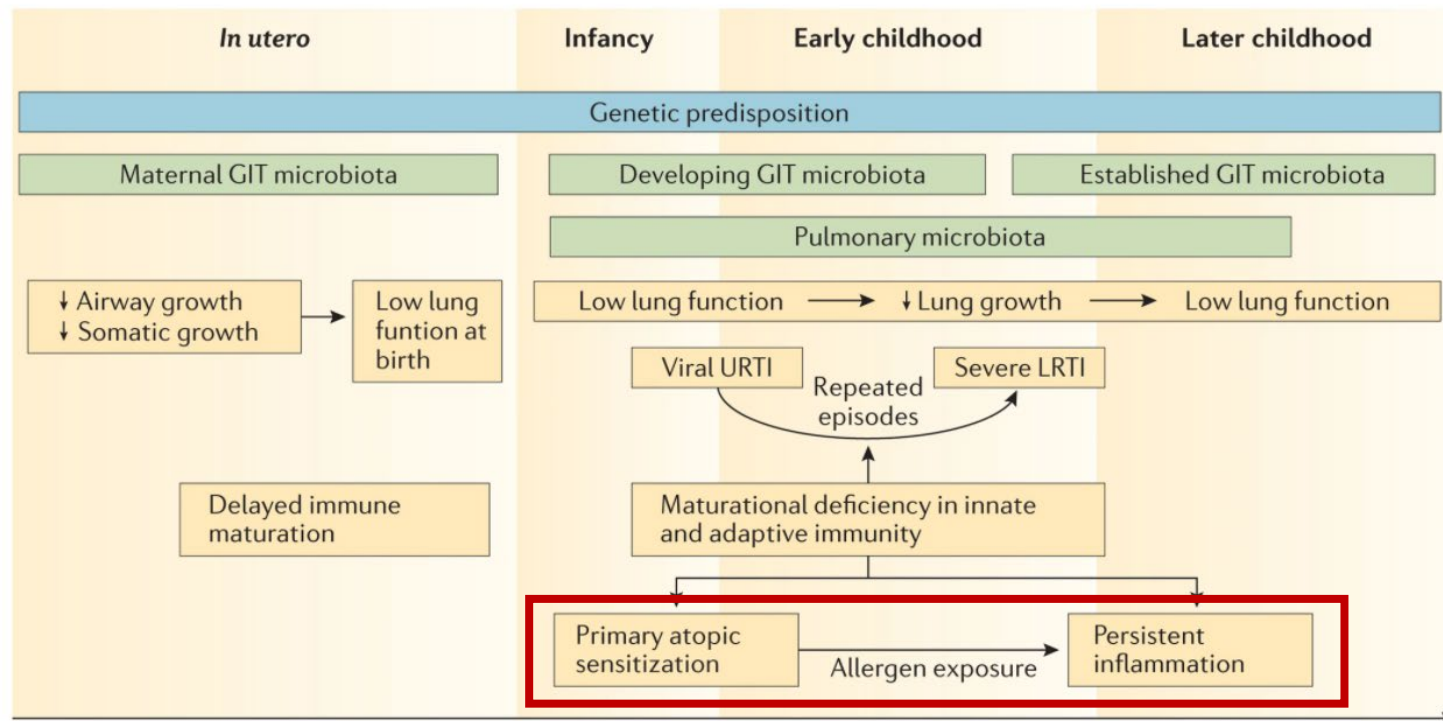
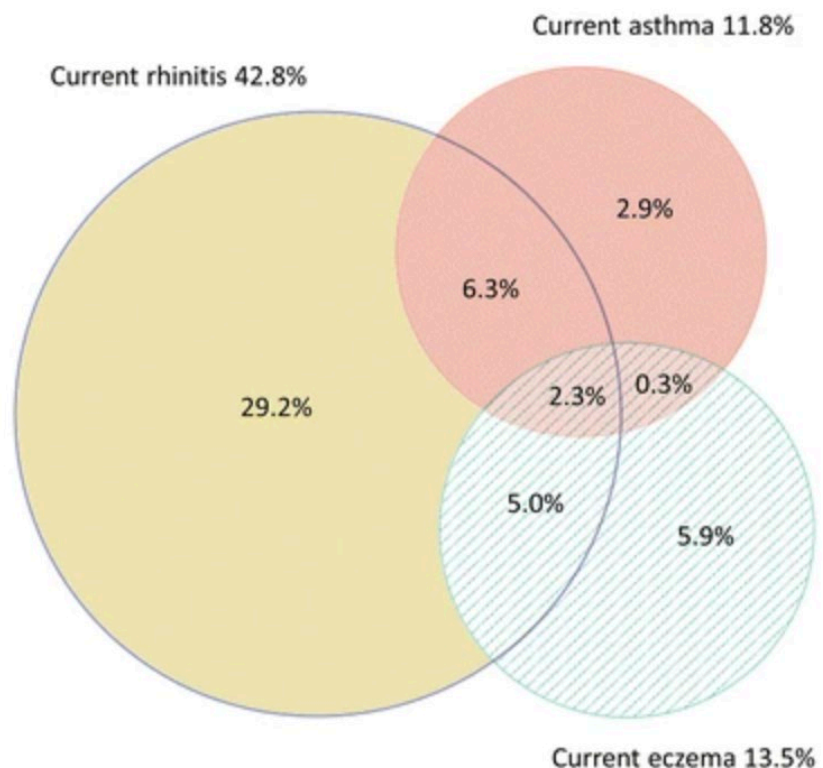


No Recognition/Tolerance



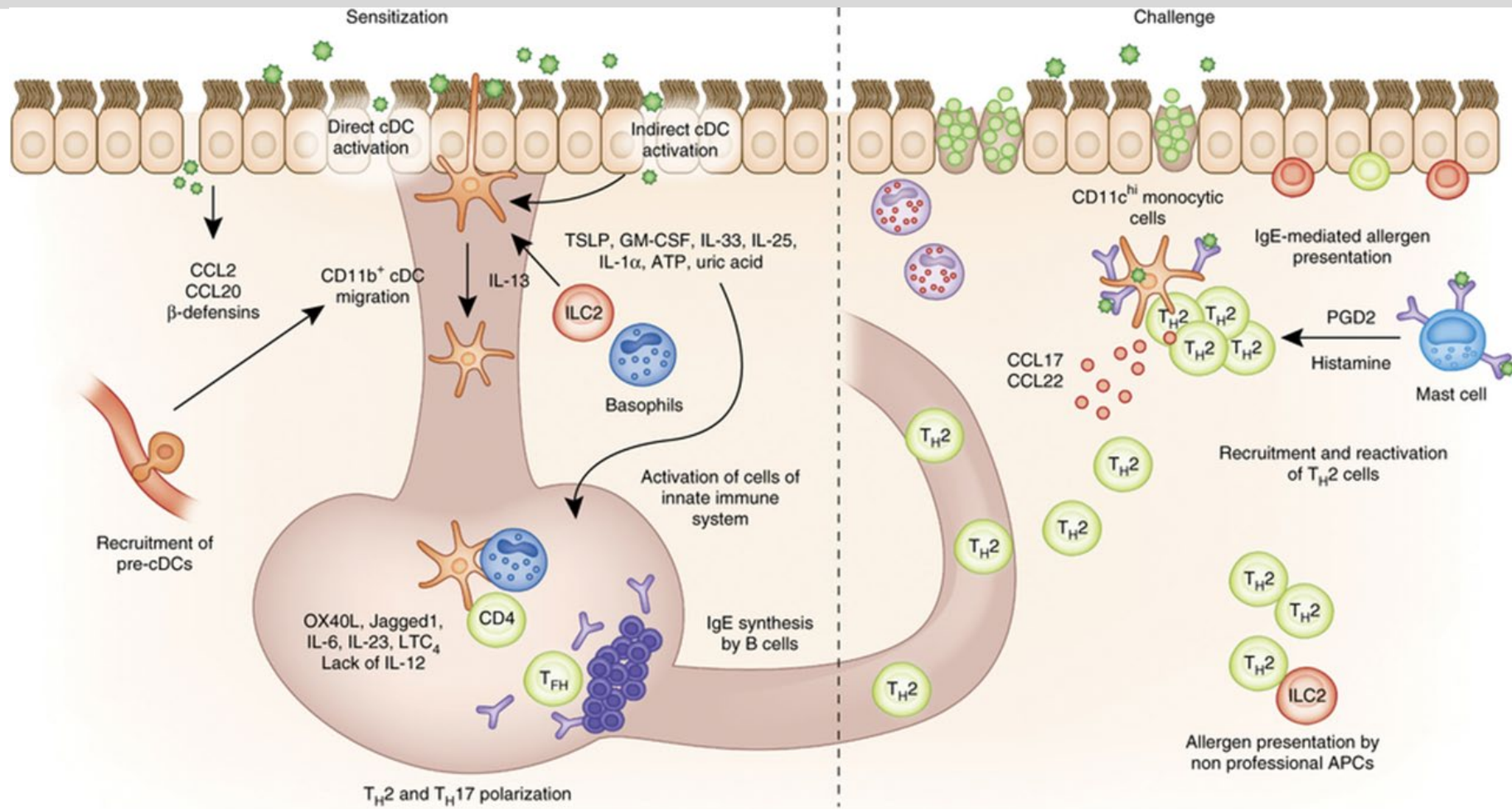
Immune Reaction
and Inflammation

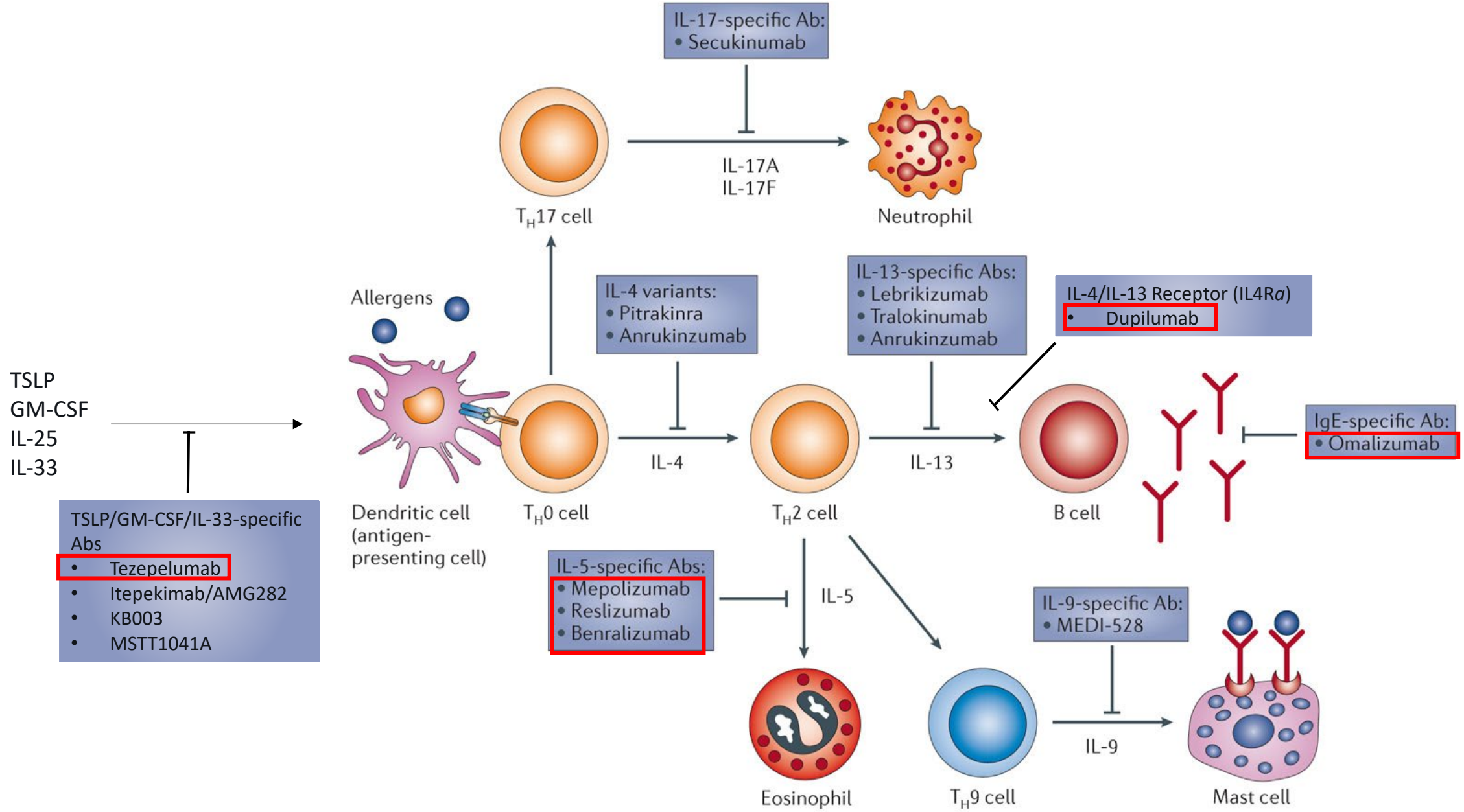
Allergy is the Strongest Risk Factor for Asthma



The development of allergic asthma may be incremental and therefore potentially reversible.

Type 2 Inflammation in Allergic Asthma





Central Hypothesis

Identification of differences in the airway response to allergen between allergic asthmatics (AA) and allergic non-asthmatic controls (AC) could provide fundamental insights into asthma pathogenesis.

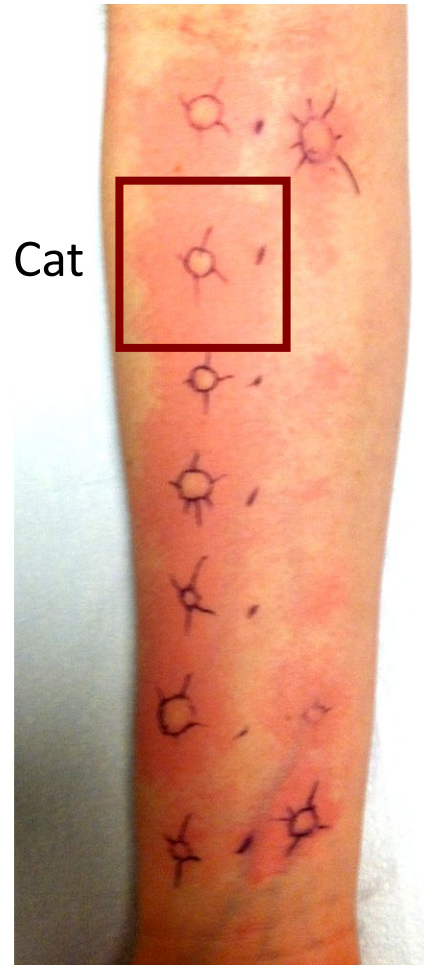
Study Design

Allergic Asthmatics (AA; n=36)	Allergic Controls (AC; n=48)	Healthy Controls (HC; n=5)
*Mild to moderate asthma	No history of asthma	No history of asthma
Allergic symptoms	**Allergic symptoms	No allergic symptoms
†Positive skin prick test	†Positive skin prick test	Negative skin prick test
Baseline FEV ₁ ≥ 75% predicted	Baseline FEV ₁ ≥ 90% predicted	Baseline FEV ₁ ≥ 90% predicted
Positive methacholine challenge (PC ₂₀ < 16 mg/ml)	Negative methacholine challenge (PC ₂₀ > 16 mg/ml)	Negative methacholine challenge (PC ₂₀ > 16 mg/ml)

*National Asthma Expert Panel's Report 3. **Allergic rhinitis, allergic conjunctivitis or contact allergy. †At least 3 mm wheal to house dust mite or cat hair extract > 0.056 BAU/AU. *Exclusion criteria:* Pregnant or breastfeeding, systemic steroids or anti-histamines, infection within 6 weeks, prior immunotherapy or omalizumab, intolerance of study medications.

Skin Prick Testing

Allergen Panel



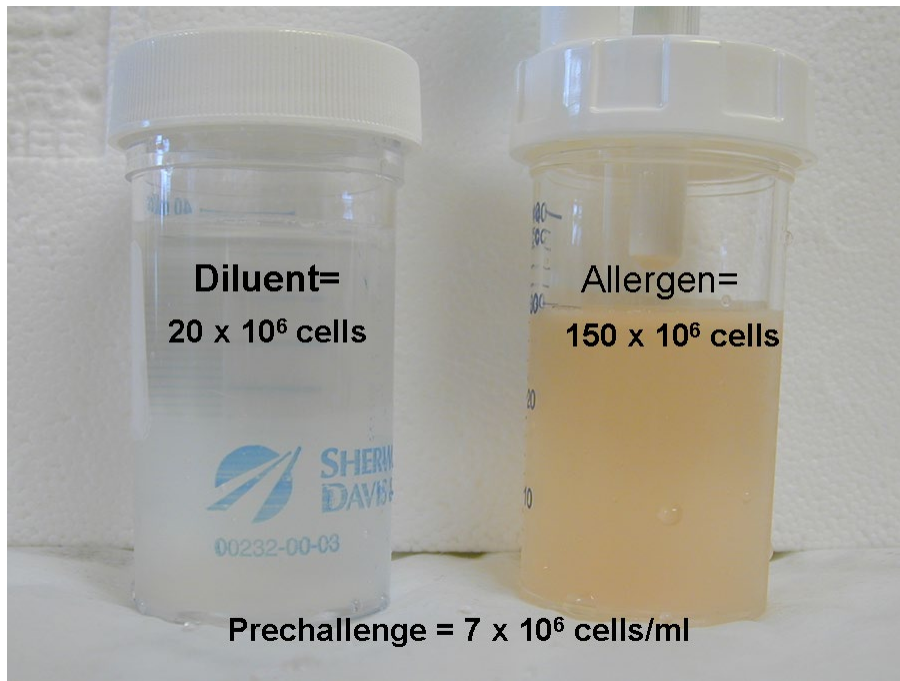
Cat

Allergen Titration

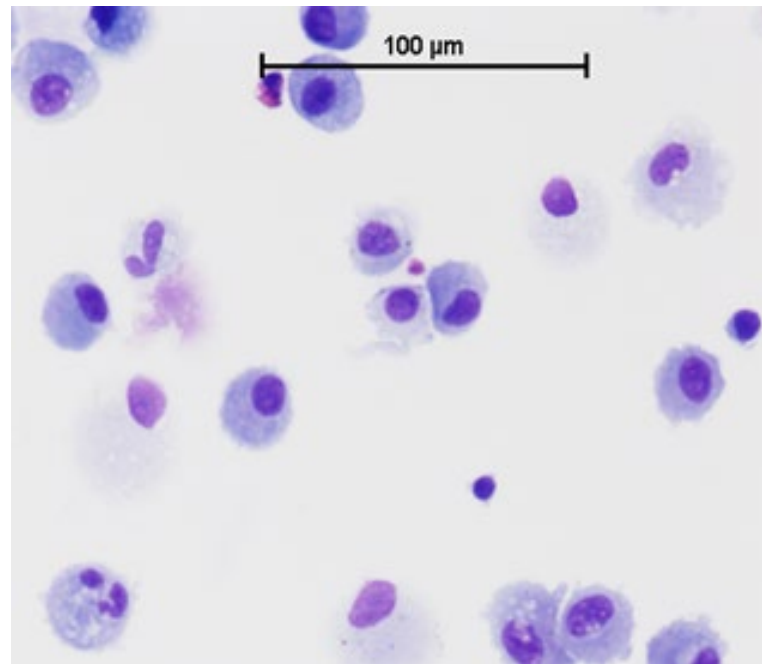


Threshold dose

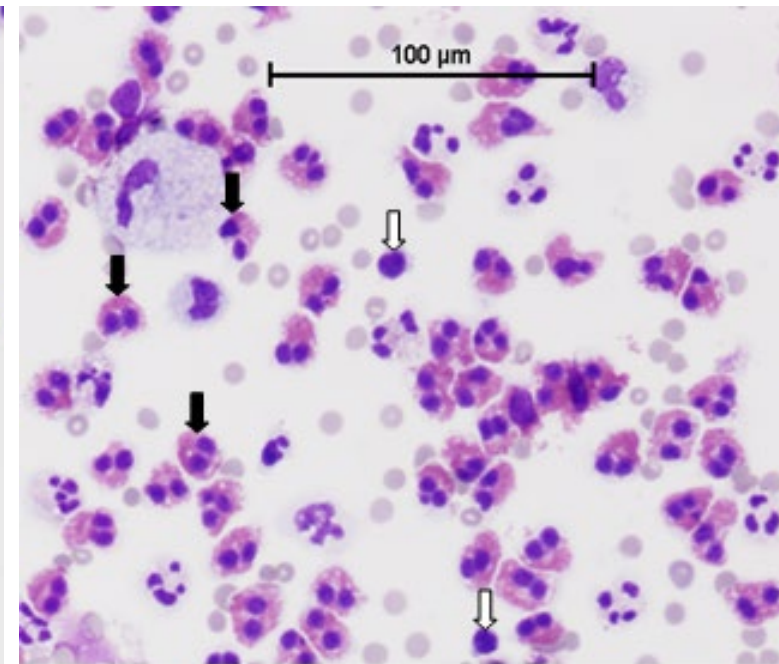
Segmental Allergen Challenge (SAC)



Baseline



Post-SAC



Allergic Control



Allergic Asthmatic

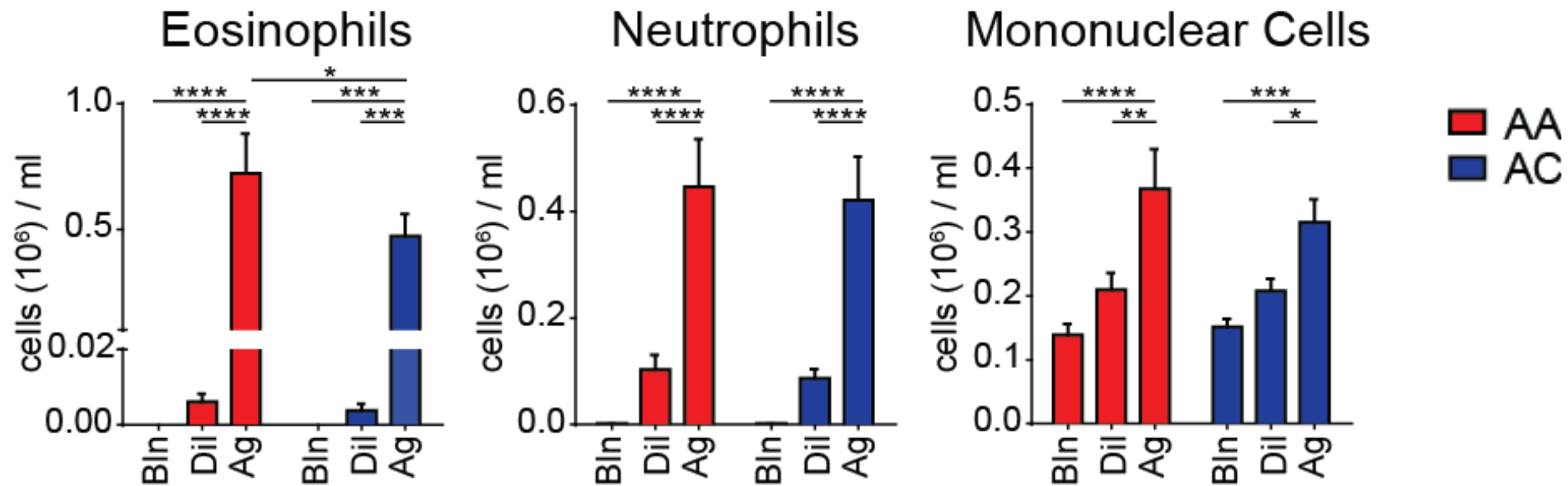


Allergic Control

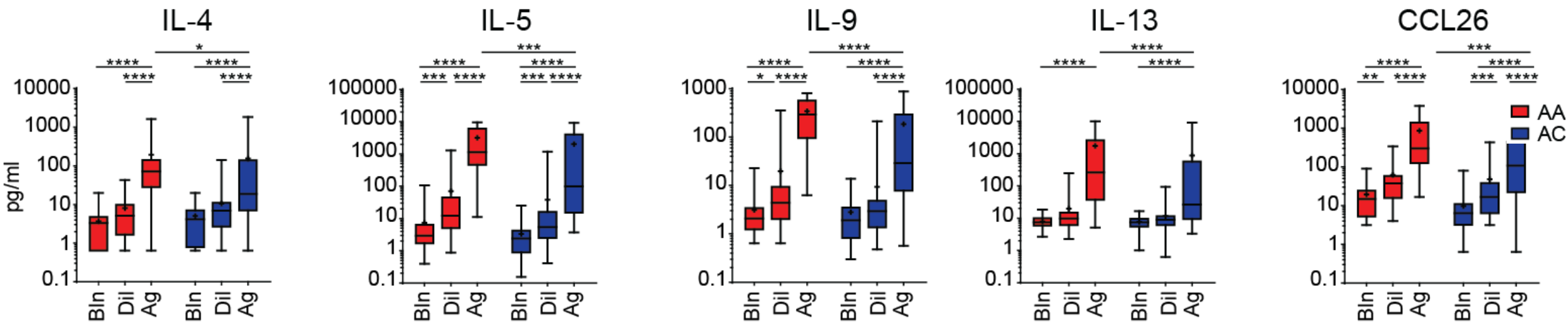


Allergic Asthmatic

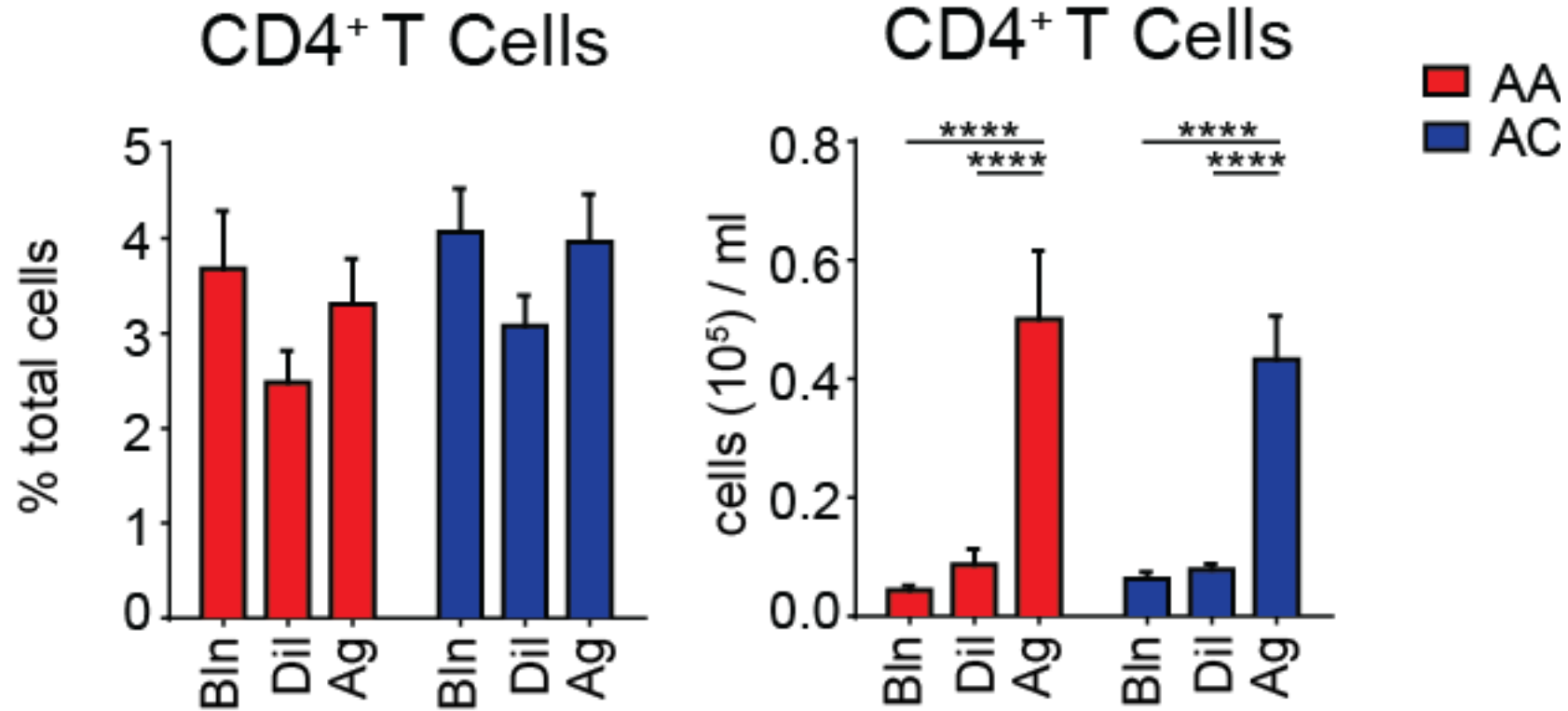
Eosinophil accumulation is higher in AA



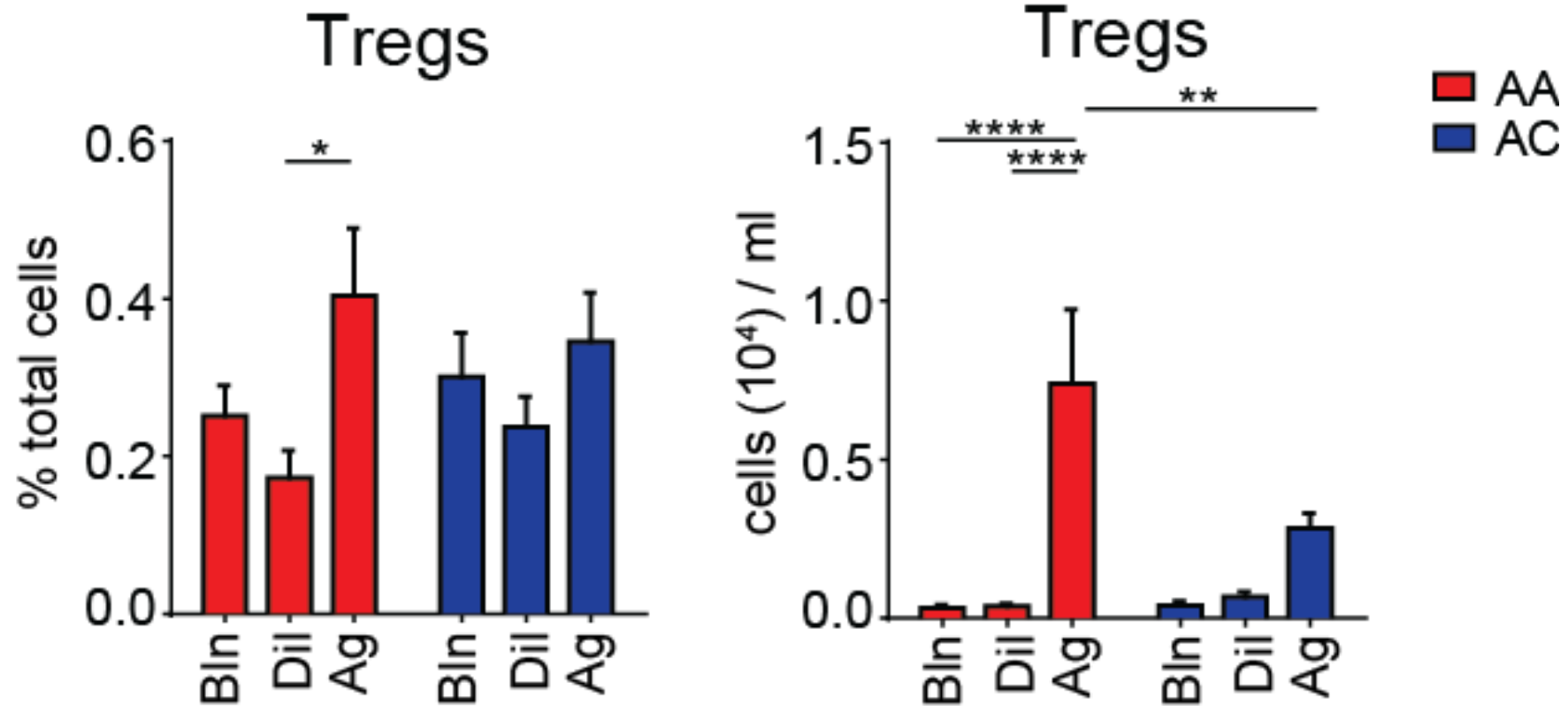
AA have higher levels of type 2 cytokines



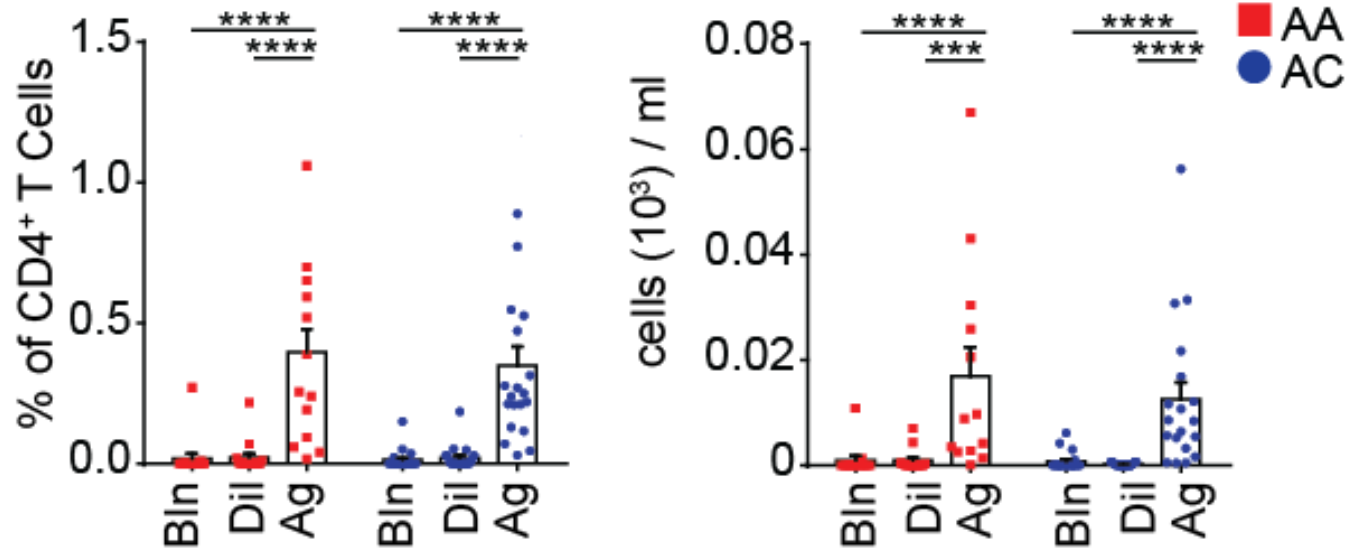
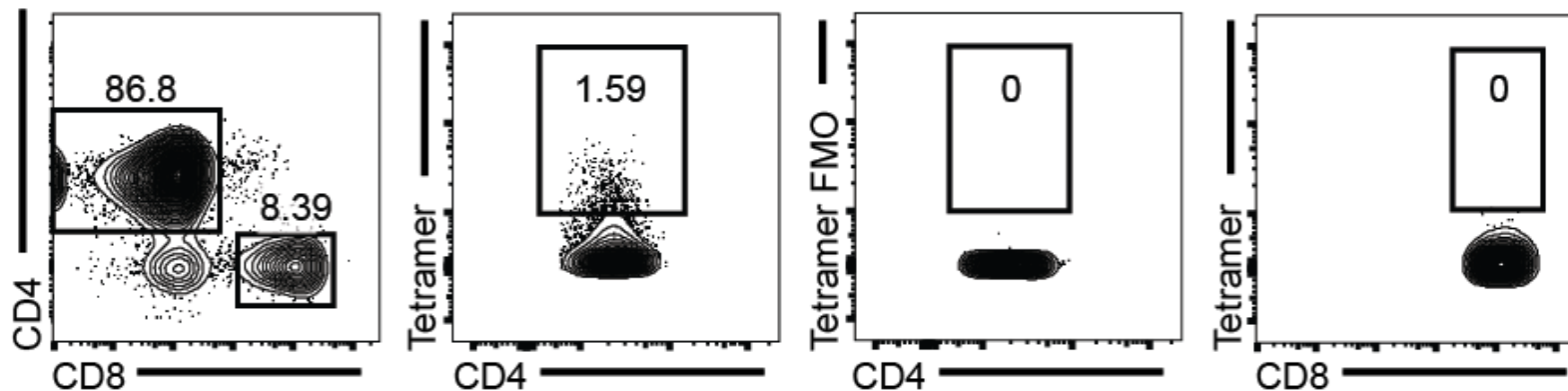
Airway CD4⁺ T cells increase after SAC



Airway Tregs increase in AA after SAC



Airway tetramer⁺ CD4⁺ T cells increase after SAC



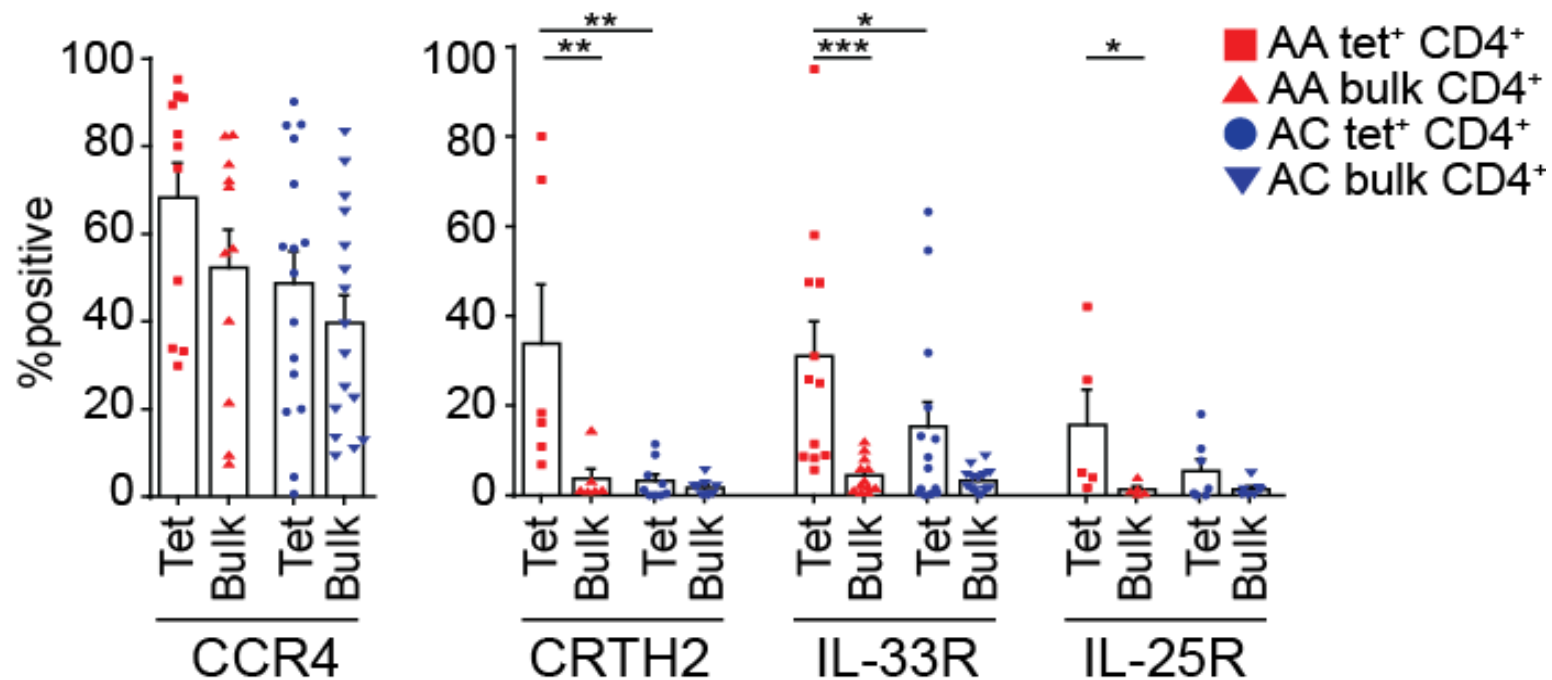
Phenotype of airway tetramer⁺ CD4⁺ T cells

Th2 chemokine receptors

- CCR4, CRTH2

Innate Type 2 cytokine receptors

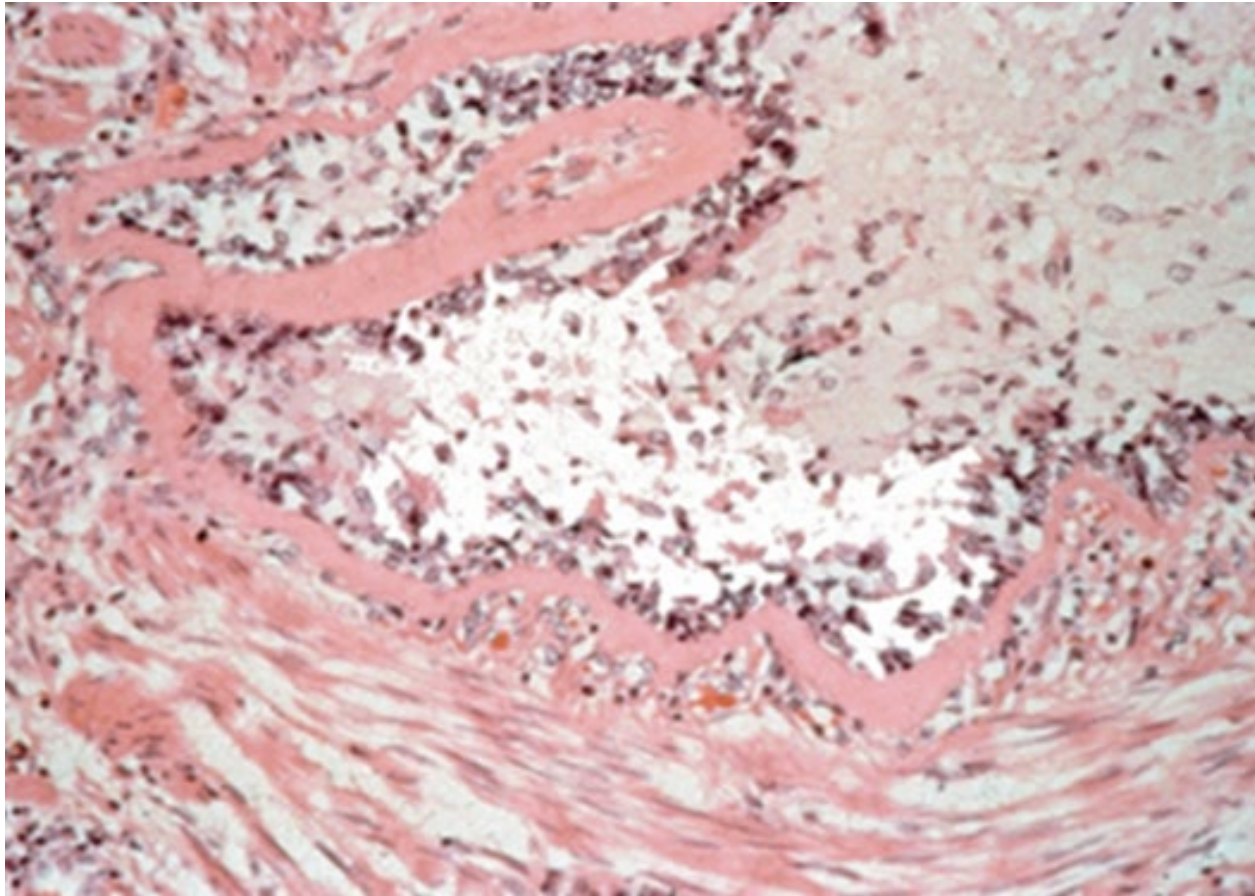
- IL-33R, IL-25R



Conclusions – Part 1

- AA have more airway eosinophils and higher levels of type 2 cytokines after SAC, but there is significant overlap between groups.
- AA and AC have similar numbers of airway allergen-specific CD4⁺ T cells after SAC.
- Allergen-specific CD4⁺ T cells in AA express higher levels of innate type 2 receptors (described as “pathogenic effectors”).
- Although type 2 inflammation is necessary for the development of allergic asthma, our data suggests it is not sufficient.
- *? Increased sensitivity of airway structural cells to allergen or type 2 inflammation is also required to manifest asthma.*

Structural Cells in Asthma



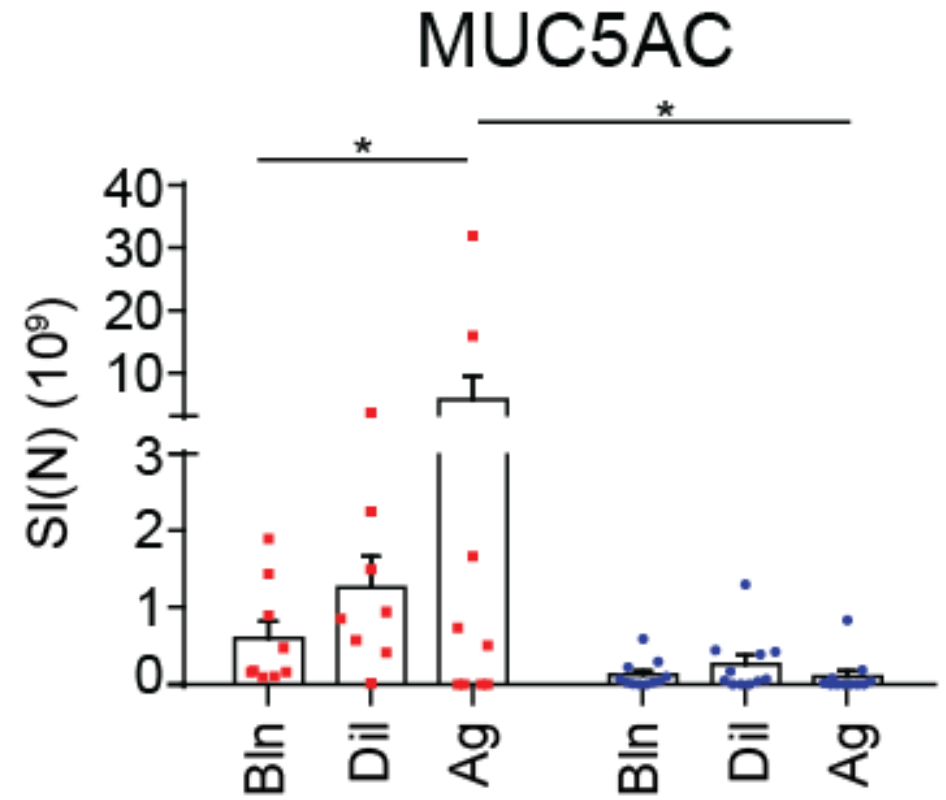
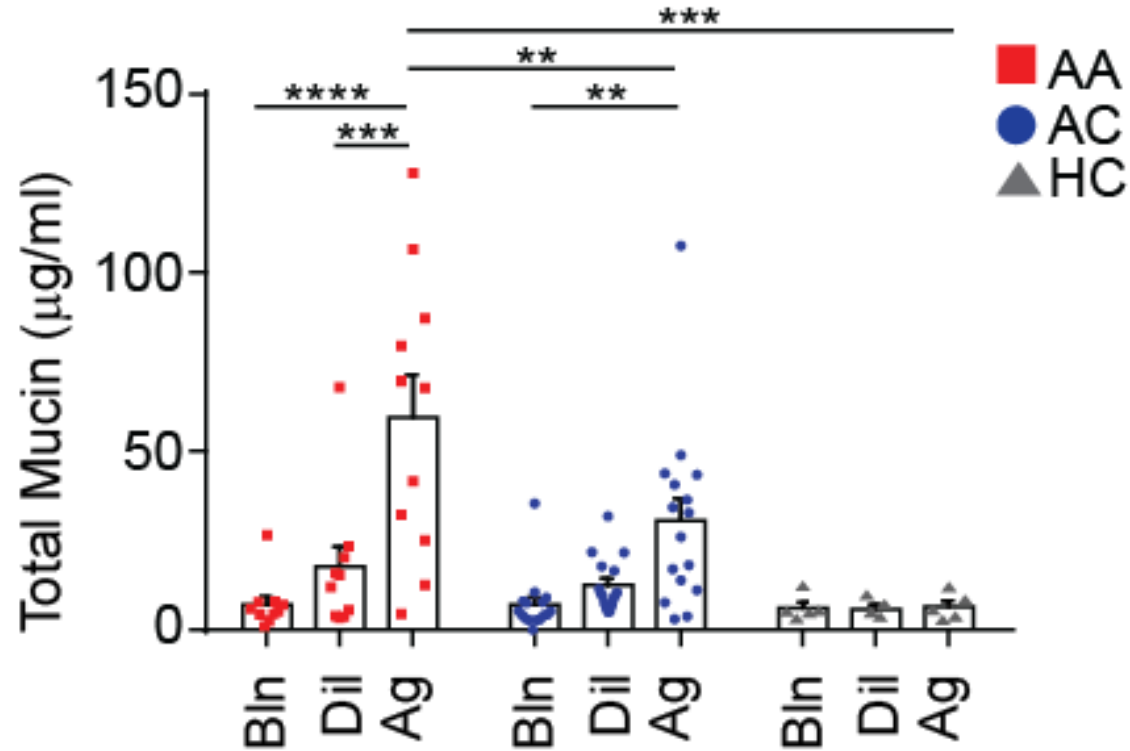
Epithelium

- Impaired barrier function
- Altered immune function
- Altered mucus response
- Altered repair response

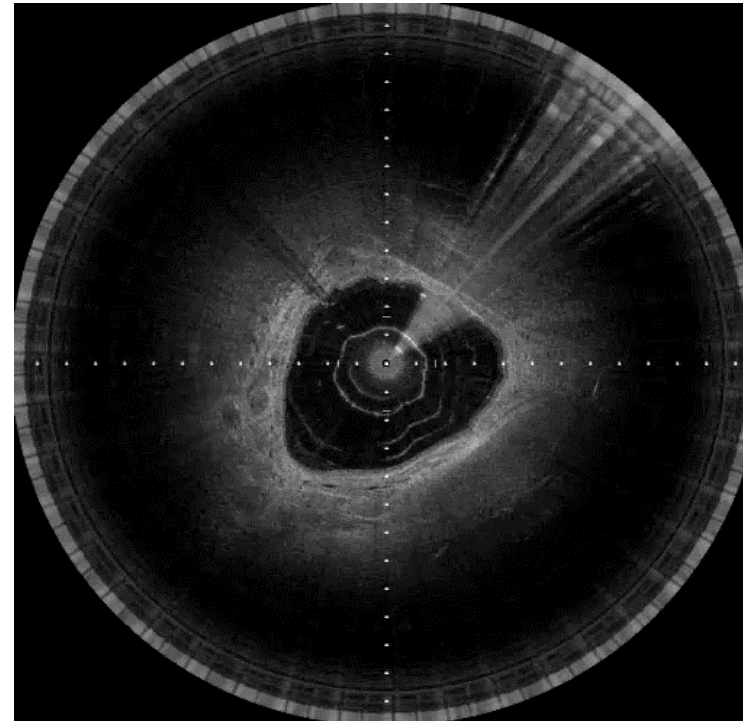
Smooth Muscle

- Increased mass
- Hyper-contractile
- Immunomodulatory

AA Produce More Mucin and MUC5AC



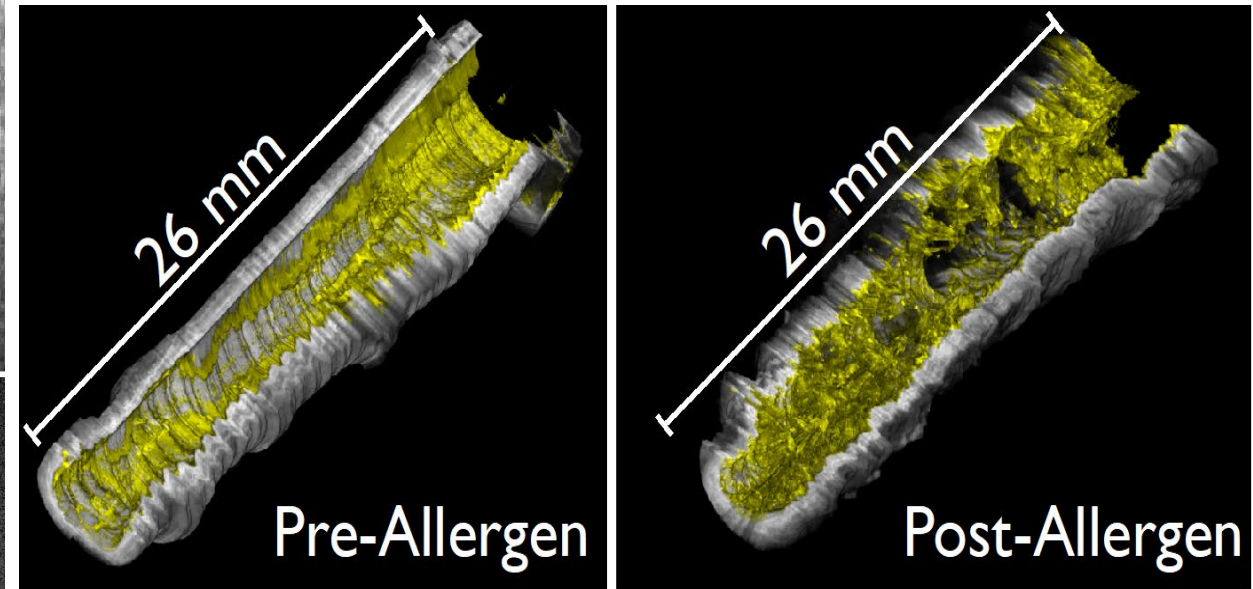
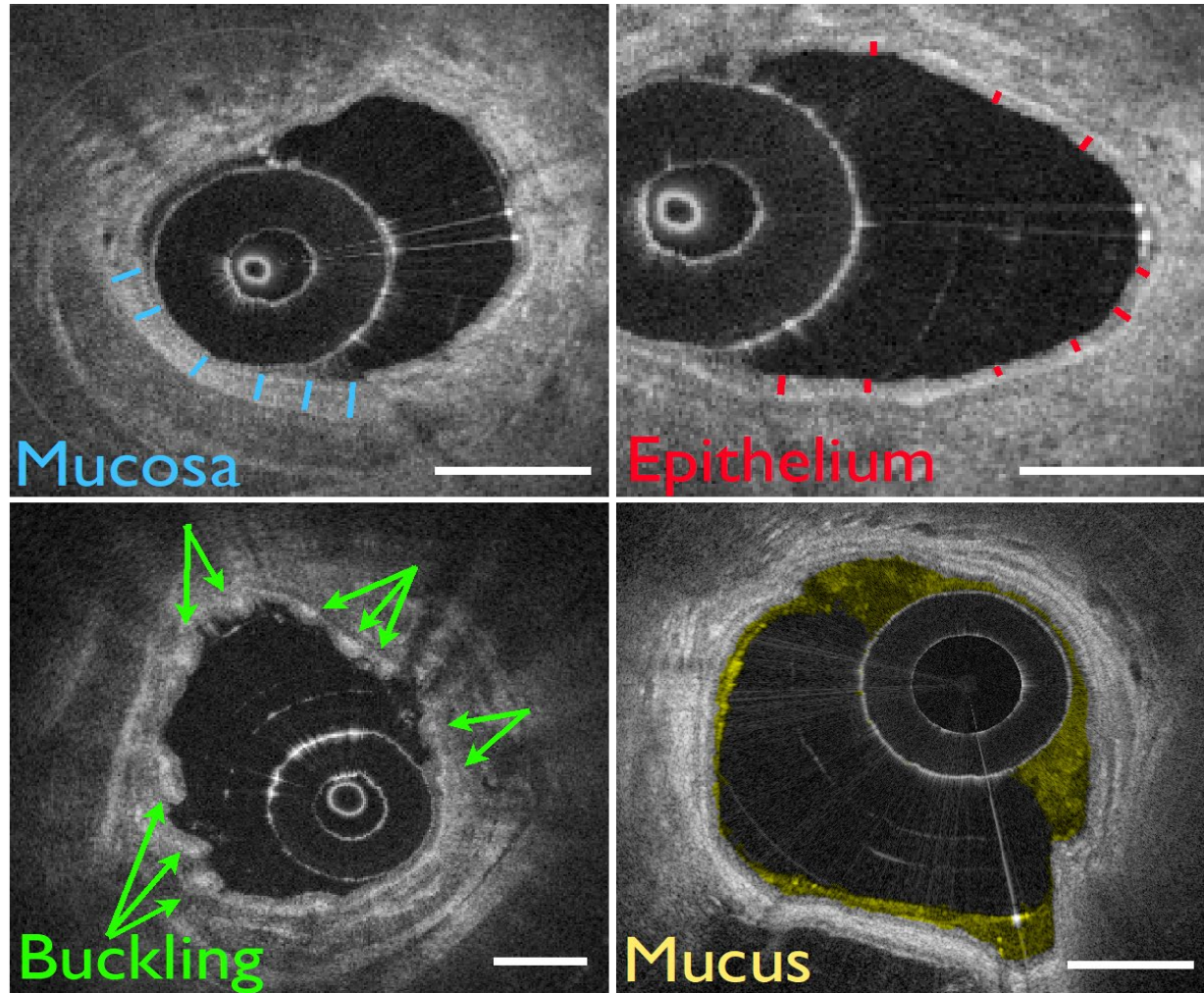
Optical Coherence Tomography (OCT)



Courtesy Melissa Suter, PhD

OCT utilizes light to capture images with μm resolution based on optical scattering.

OCT Changes in the Airway



AA have more changes in the airway after SAC

Right Middle Lobe

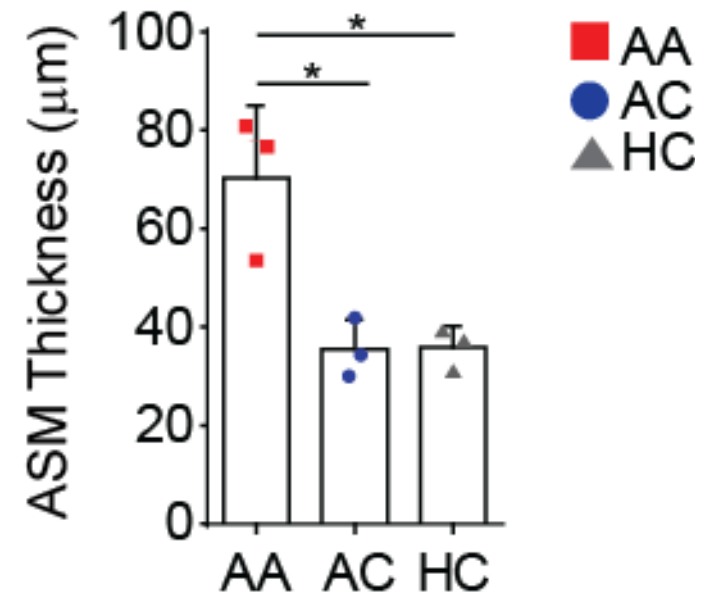
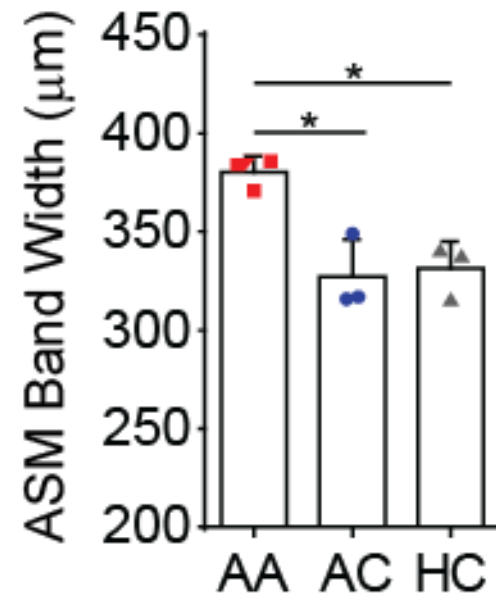
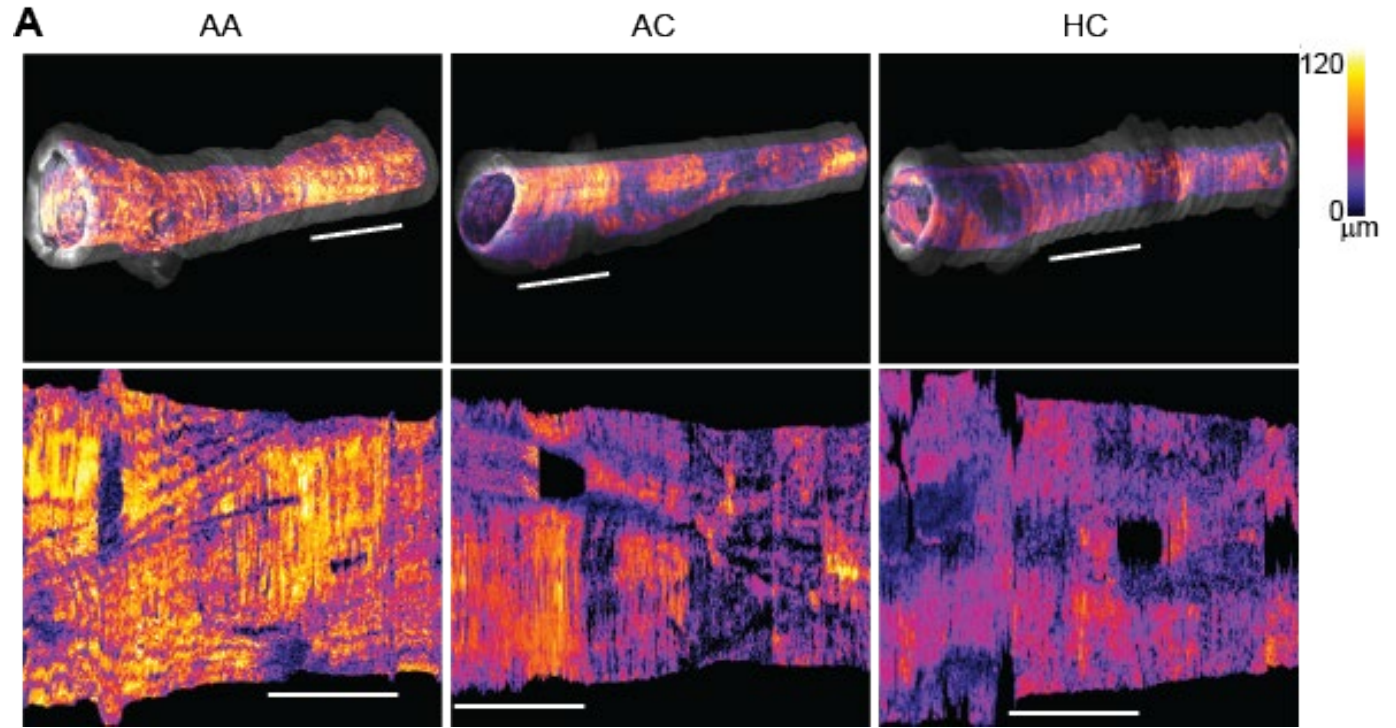
Right Upper Lobe

**

C HC

AC HC

AA have Increased ASM Mass at Baseline



Adams DC, *SciTransl Med* 2016

Conclusions – Part 2

- AA have more mucin/Muc5AC production after SAC
- AA have greater airway thickening and buckling after SAC
- AA have greater airway smooth muscle cell mass at baseline and greater constriction after SAC (not shown)
- These data suggest that tissue-level regulation of the immune and structural cell response to allergen is key to the development of asthma.

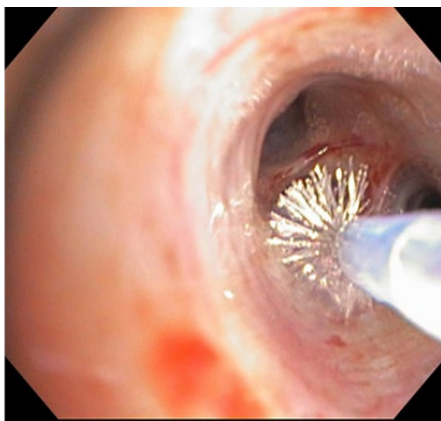
Hypothesis: Distinct local effector and regulatory programs in the lower airway are key determinants of allergic asthma pathogenesis

Tissue-Level Regulation of Allergic Inflammation

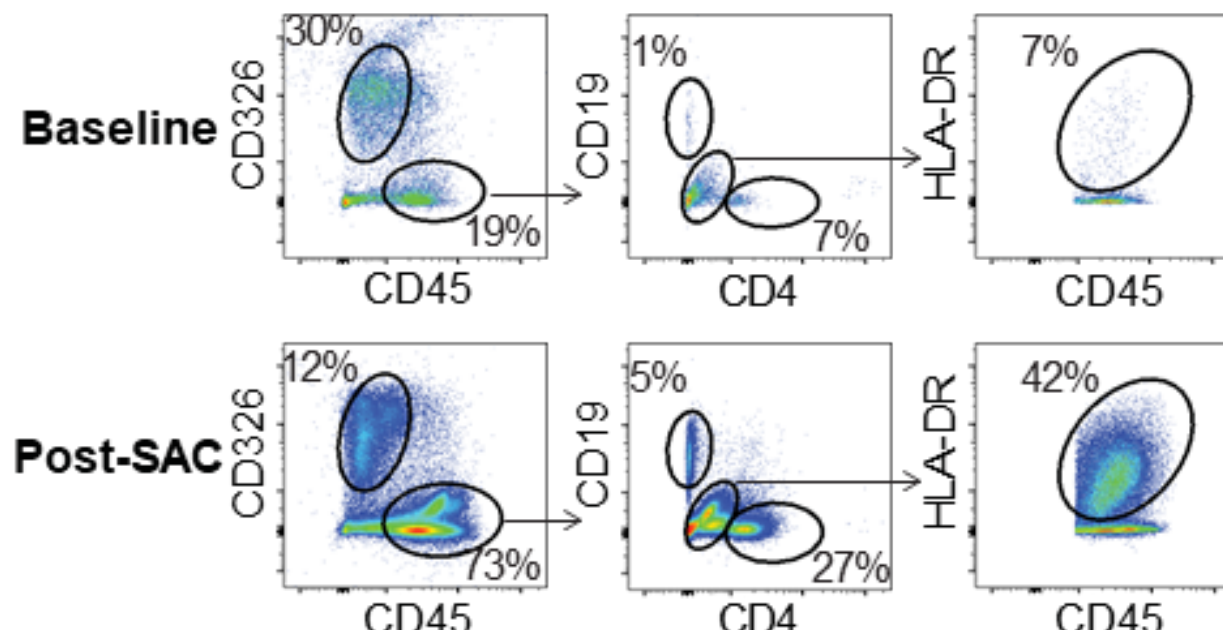
Tissue-level regulation of allergic inflammation is likely to involve cells that:

- Reside in the lungs
- Directly or indirectly interact with the environment
- Influence the adaptive immune response via soluble mediators or cell-cell interactions

**Endobronchial
Brushing**



Endobronchial brush flow cytometry



Cellular and Molecular Pathways in the Airway Unique to Allergic Asthma

Participant Recruitment

Allergic Controls (AC), n=4 **Allergic Asthmatics (AA), n=4**

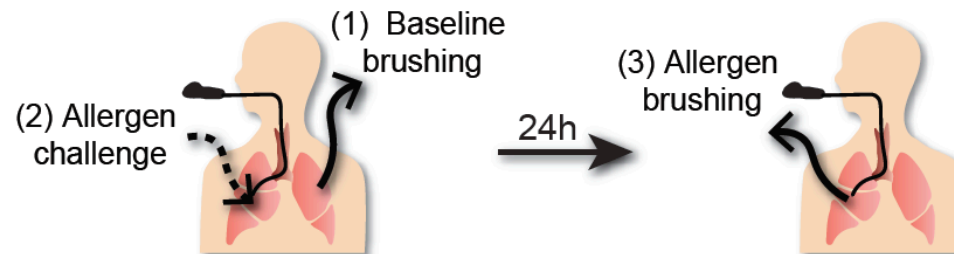


- ✓ Allergy to HDM or cat
- ✓ No history of asthma
- ✓ Negative methacholine challenge



- ✓ Allergy to HDM or cat
- ✓ Mild asthma
- ✓ Positive methacholine challenge

Segmental Allergen Challenge



Single-Cell Analysis of Endobronchial Brushings

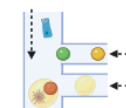
Endobronchial brushing (n=21)



Single cell suspension

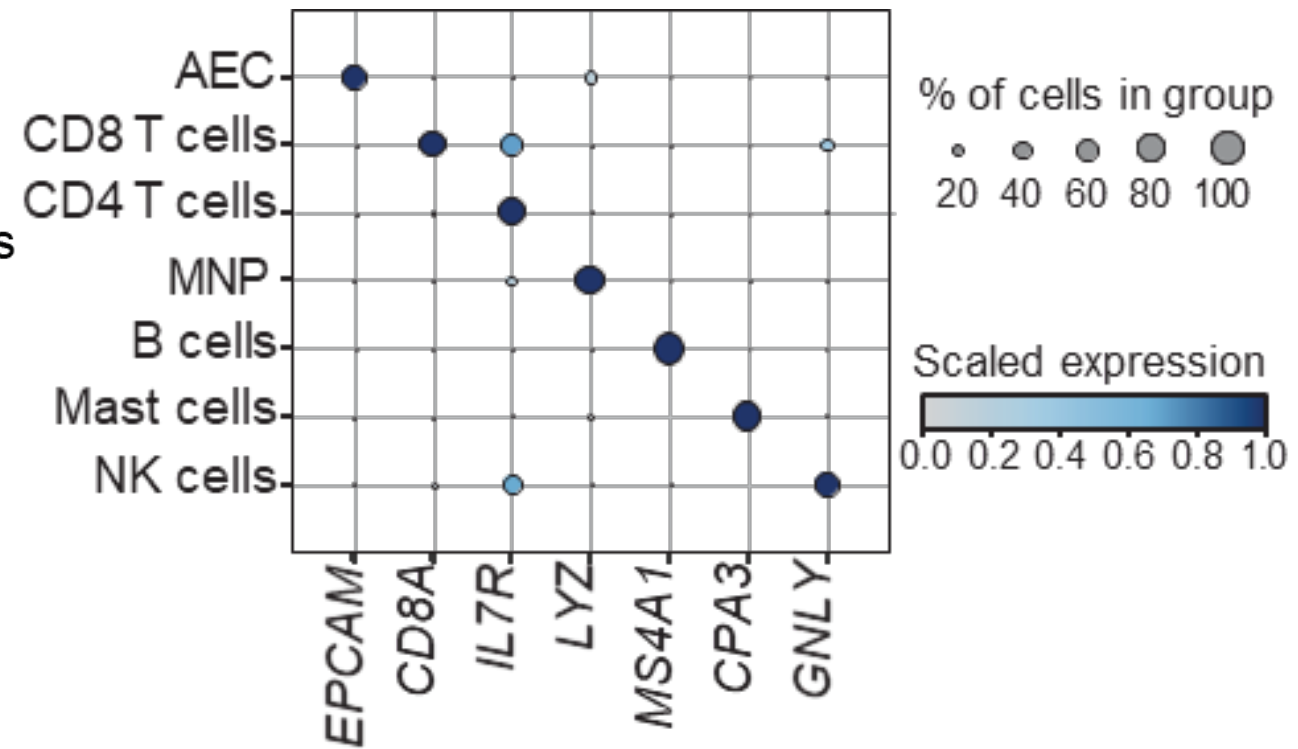
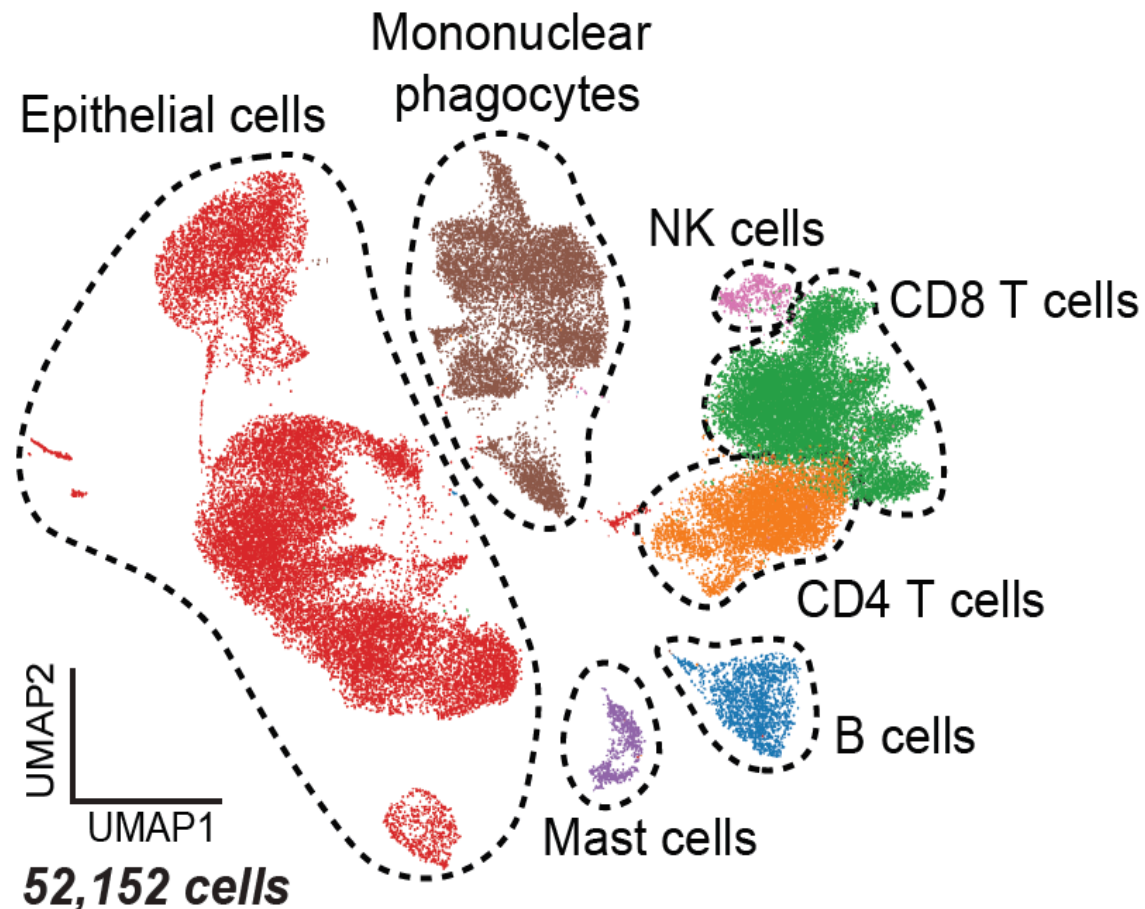


Single-cell RNA sequencing



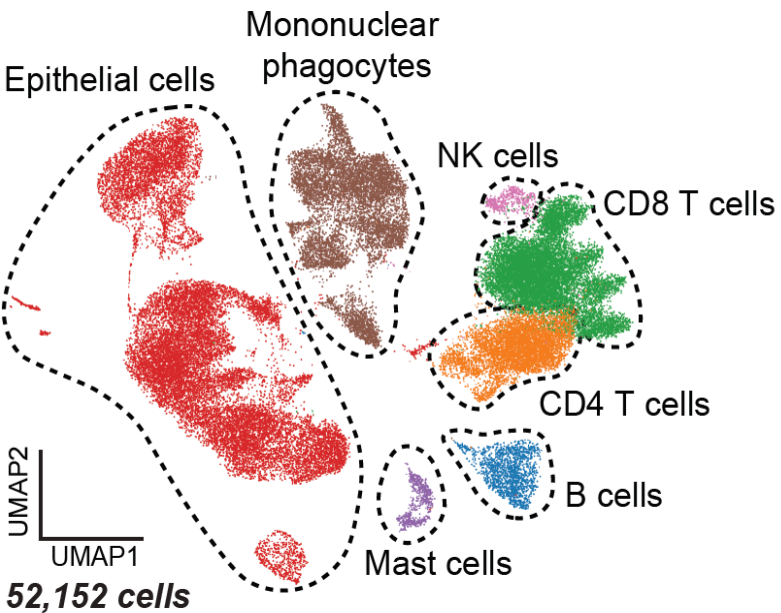
Removal of dead cells, RBCs

Lower Airway Mucosa Cellular Landscape in AA and AC

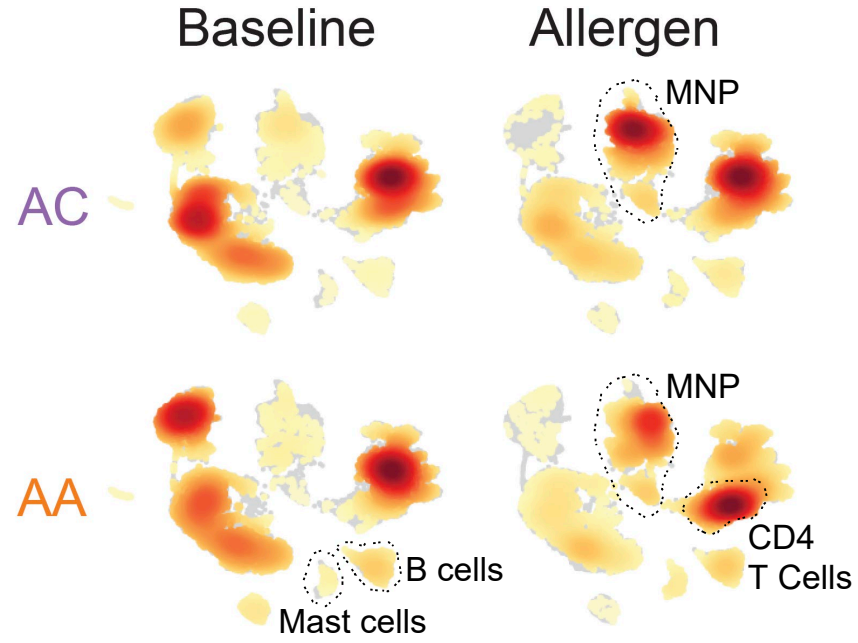


SAC transforms the airway mucosal landscape

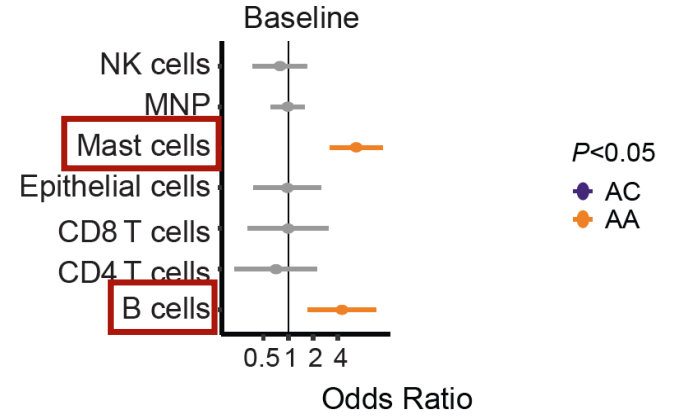
Overall clusters



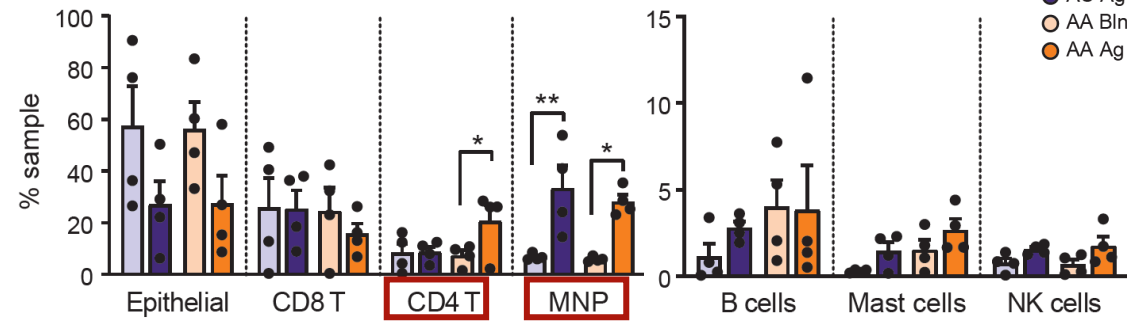
UMAP embedding of cell density



Disease association

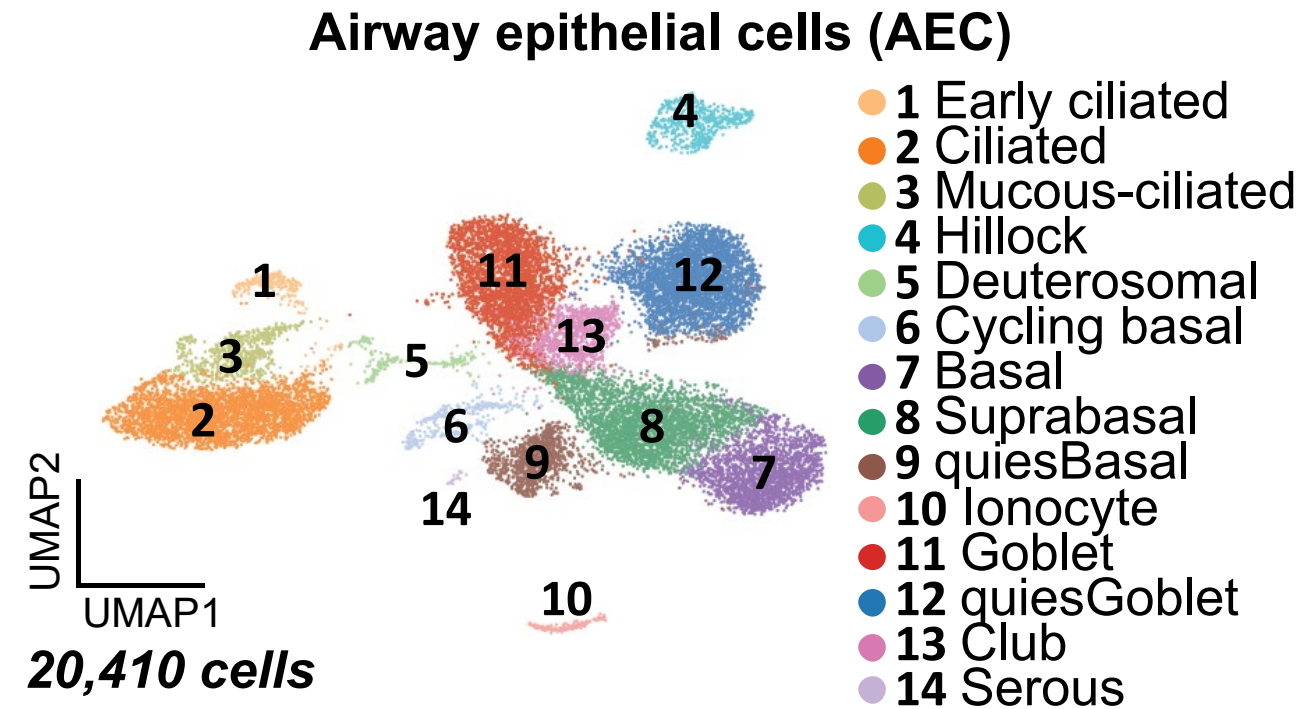


Cellular quantification

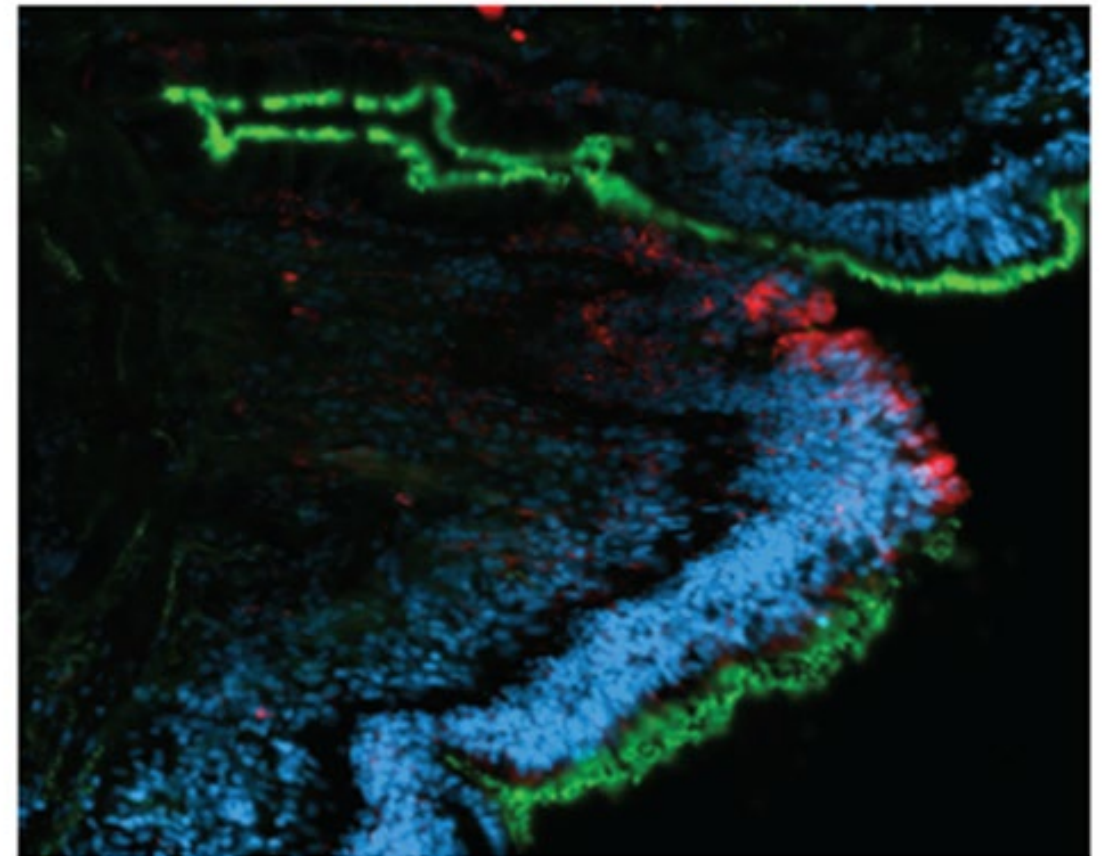


SAC induces an epithelial to immune cellular shift in allergic individuals

Airway Epithelial Cell Subsets



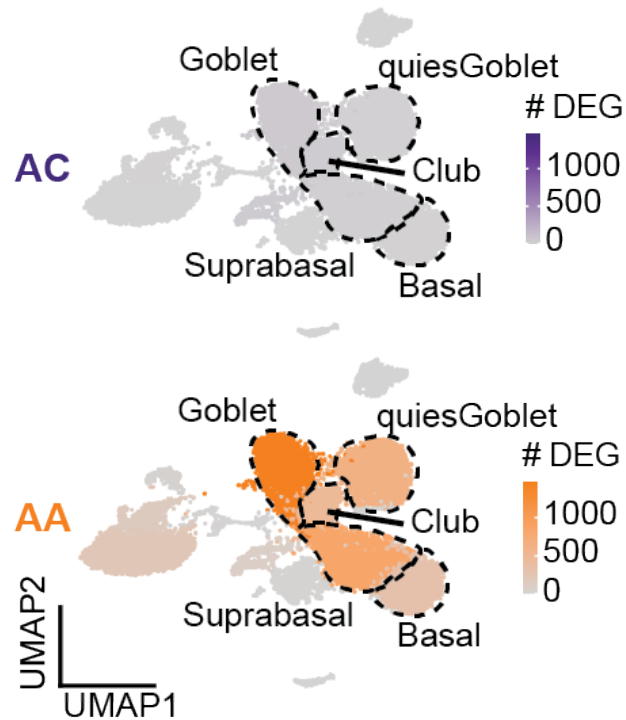
AcTub / Krt13 / DAPI



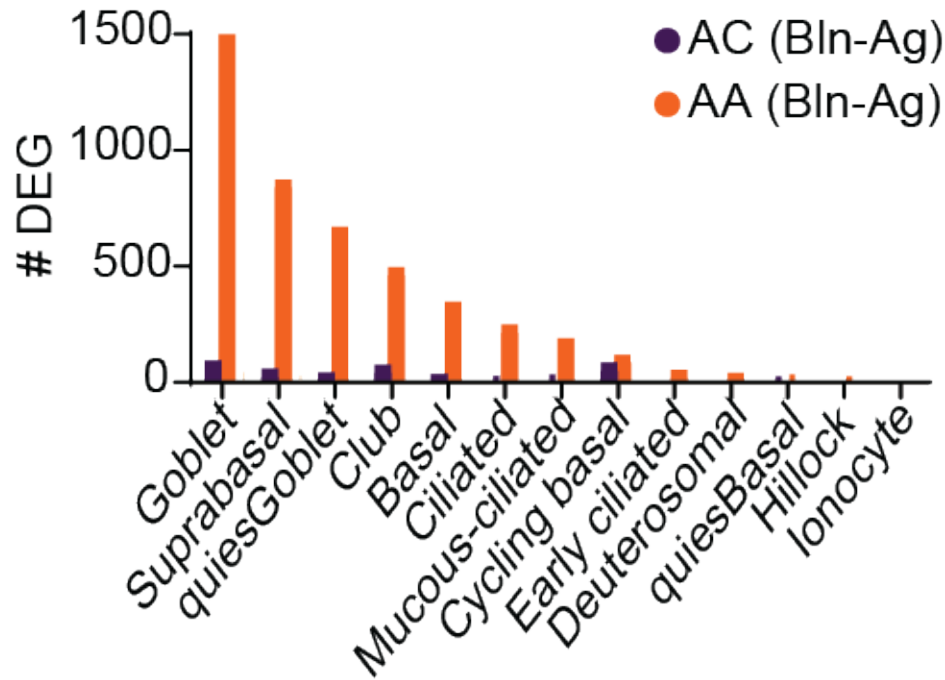
KRT13⁺ “hillocks”

SAC Induces a Robust Transcriptional Response in AEC

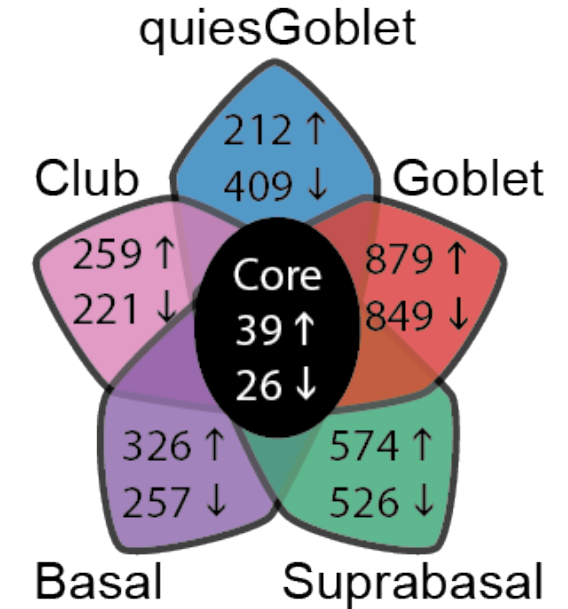
**Number of DEG
(allergen vs baseline)**



There were no differences in AEC composition between groups, but basal and secretory cells dramatically altered their transcriptional profiles after SAC

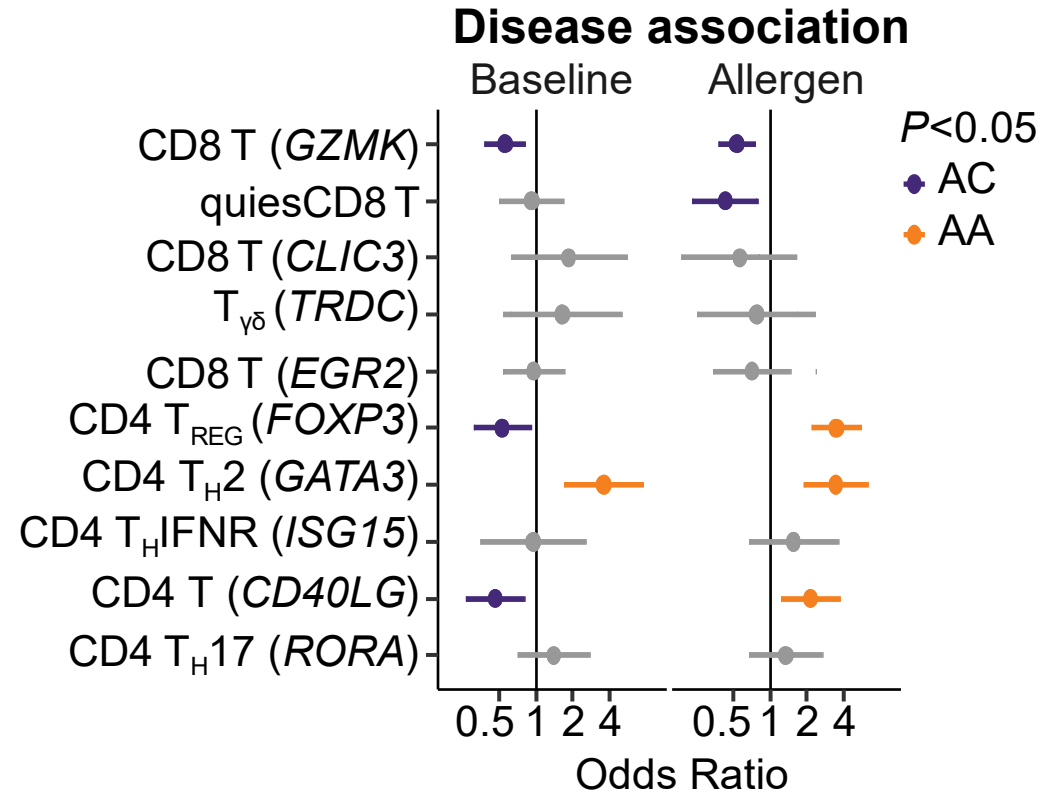
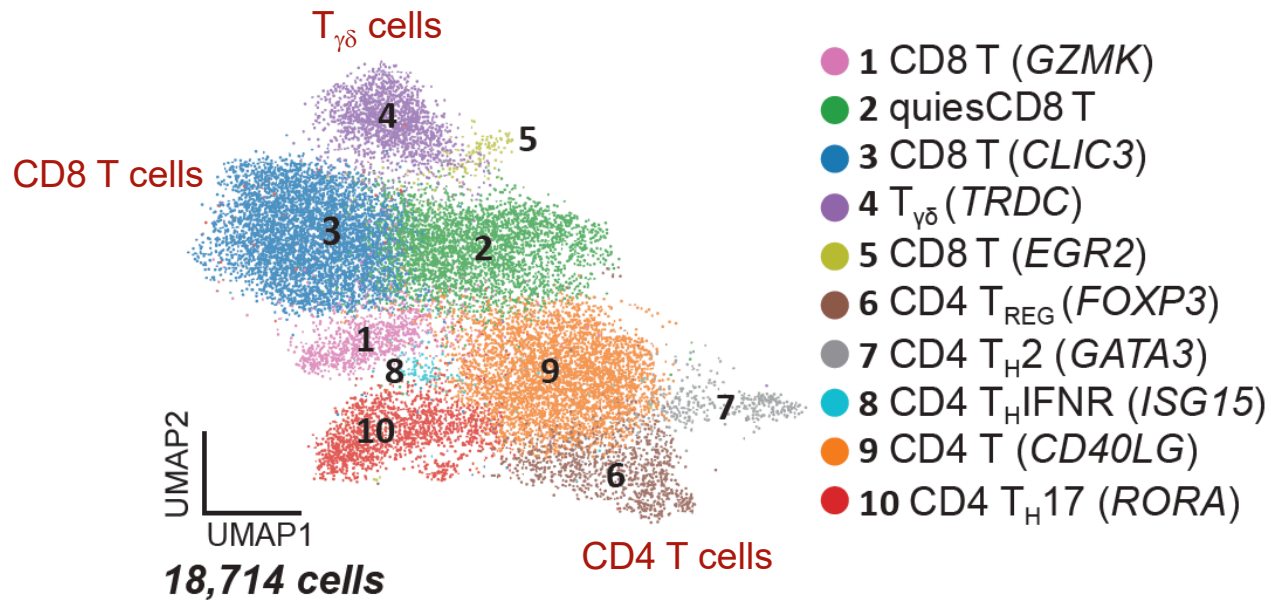


AA upregulated genes involved in inflammatory signaling, tissue remodeling, and glycolysis and downregulated antioxidant genes, compared to AC



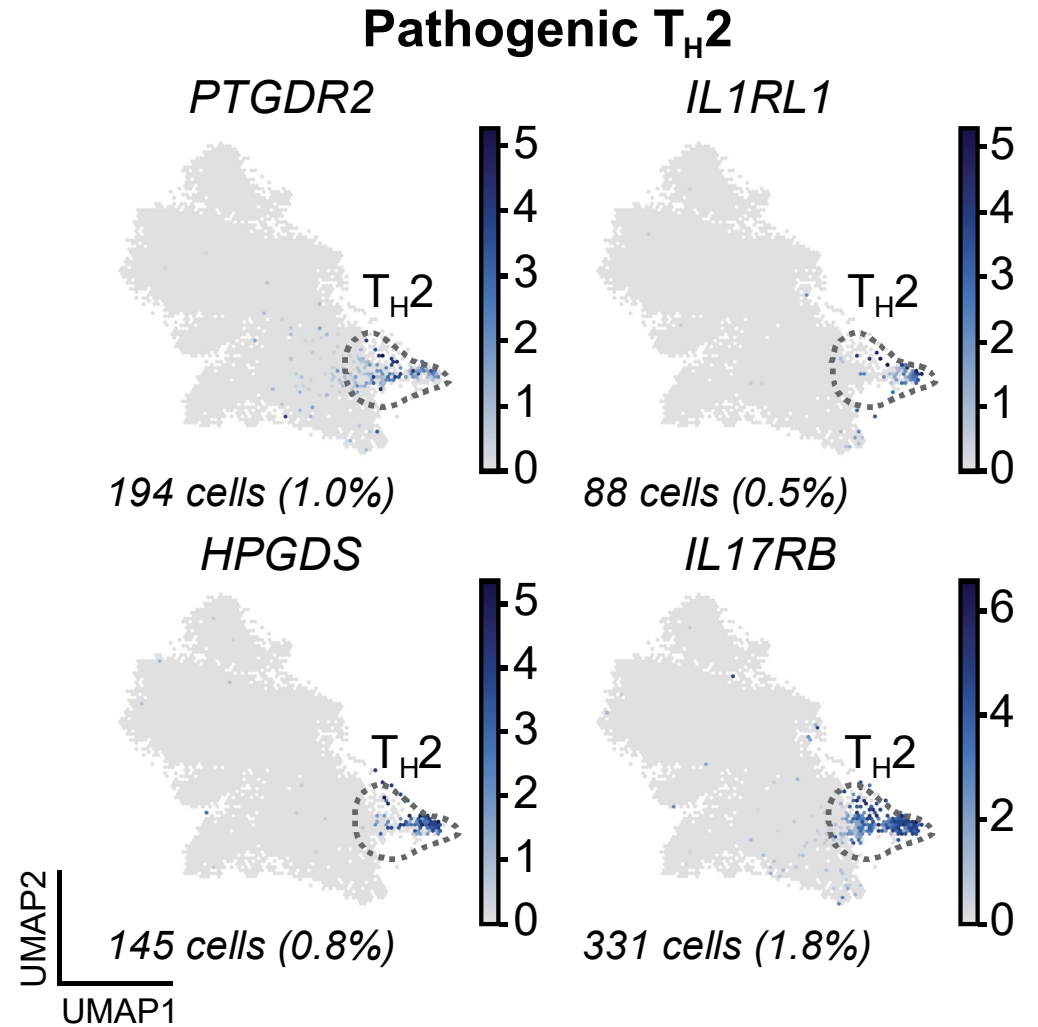
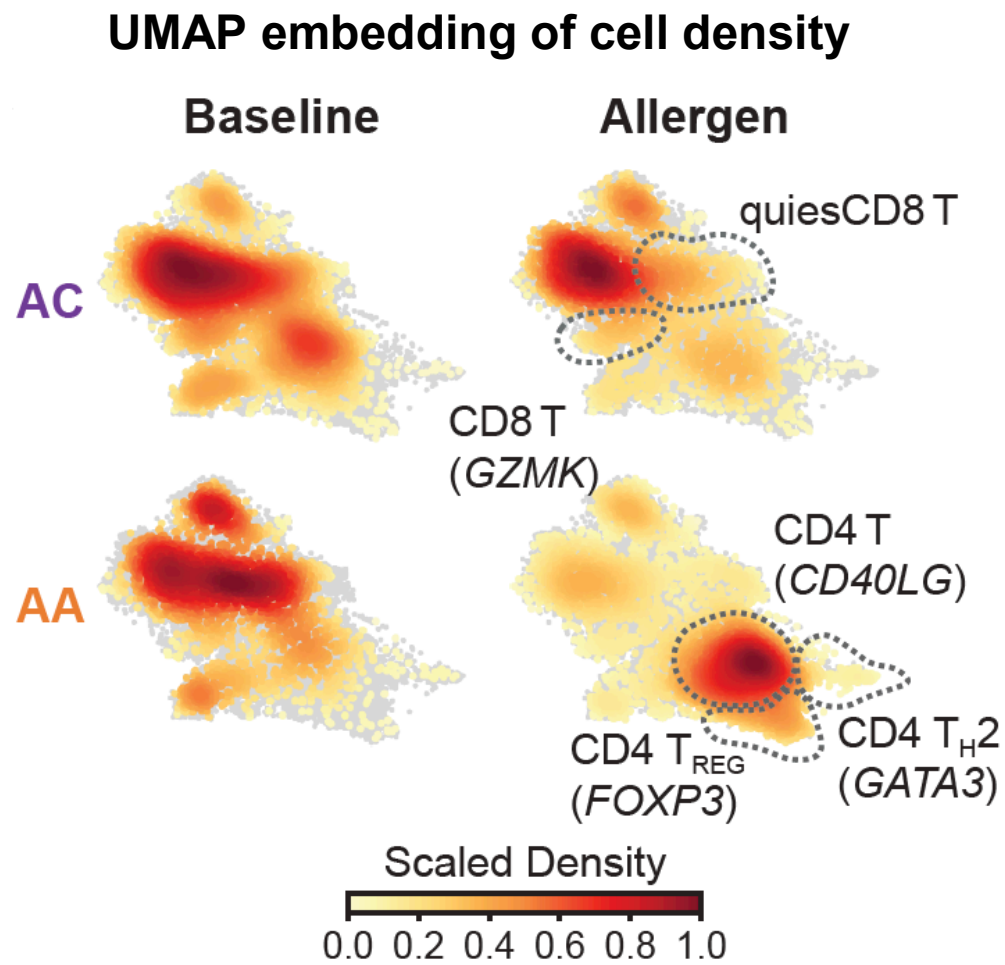
Upregulated core response	
Inflammation	<i>ALOX15, CISH, MAP2K</i>
Protease Inhibitors	<i>SERPINB4, SERPINB2, SERPINB1, CSTB</i>
Glycolysis	<i>PFKP, LDHA, HK1, TPI1, ENO1</i>
Cytoskeletal	<i>TUBA1C, TMSB4X, TUBA1B, ANXA2, CFL1, ACTG1, RAC1, MYL6, TPM4</i>
Downregulated core response	
Anti-oxidant	<i>SELENOP, NTHL1, STEAP2, CYP2F1, STOX1, STEAP4, NME3</i>

T cell subsets in the airway mucosa



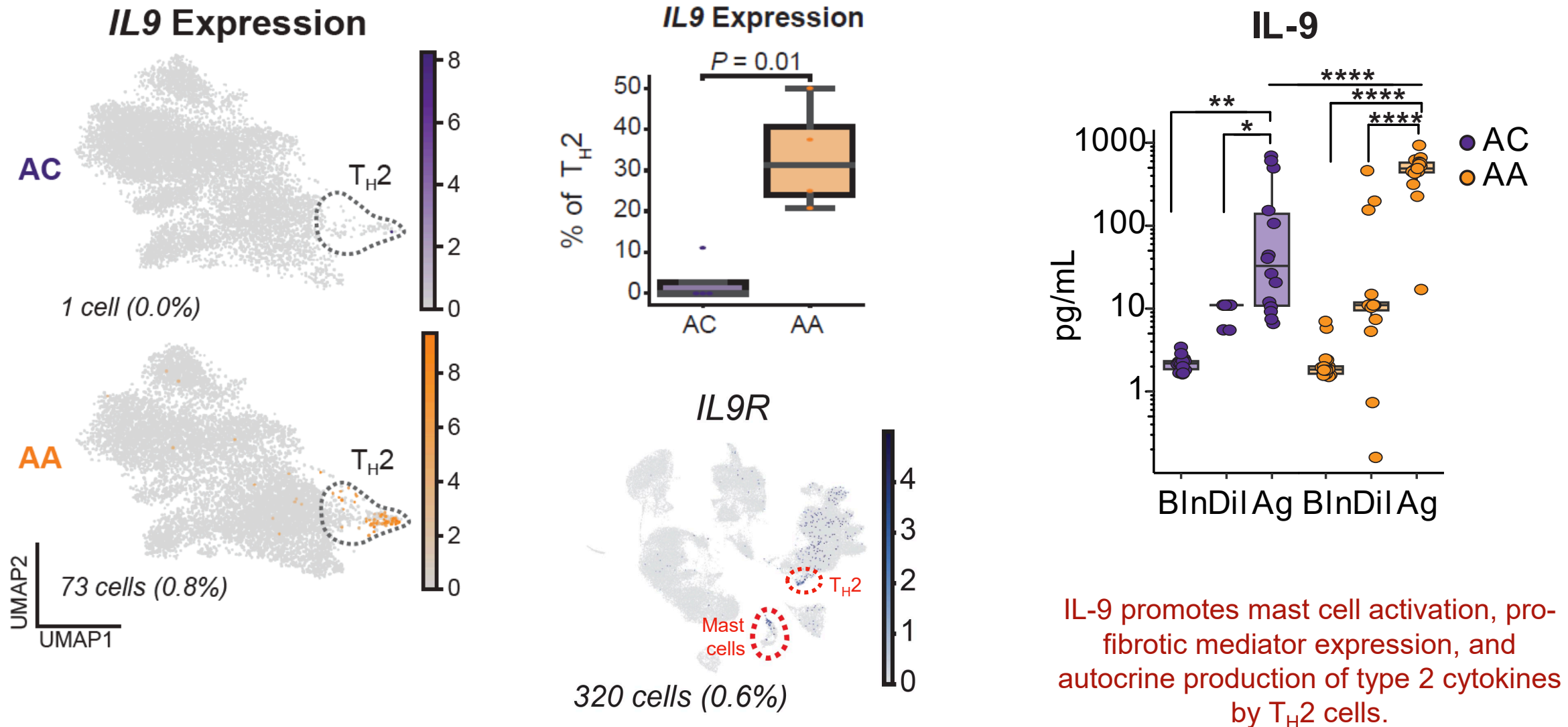
CD4 T_H2 cells are associated with AA at baseline and after SAC

CD4 T cell subsets are associated with AA after SAC



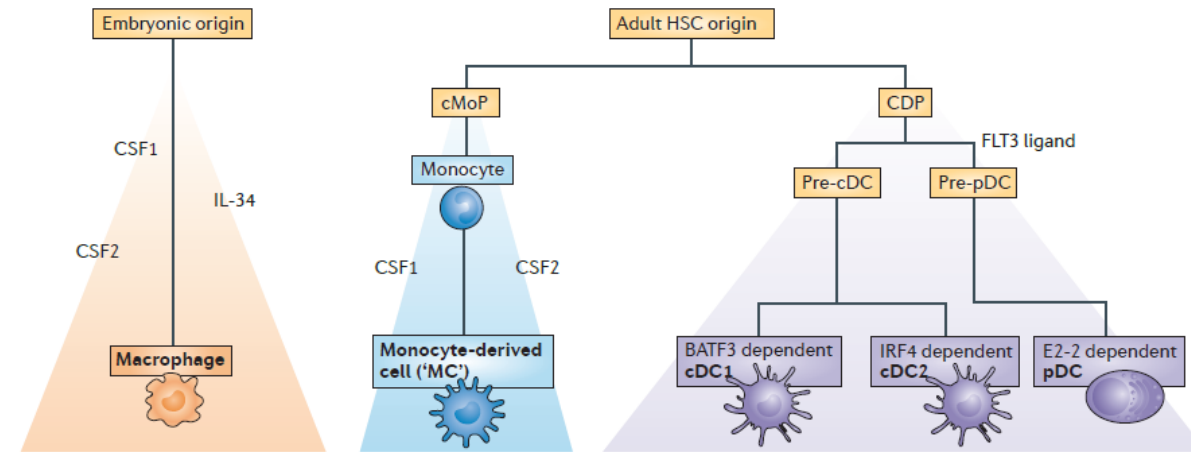
The proportion of CD4 T cells is increased in AA after SAC

IL9-expressing pathogenic TH2 cells were specific to asthmatic airways



Mononuclear phagocytes (MNP)

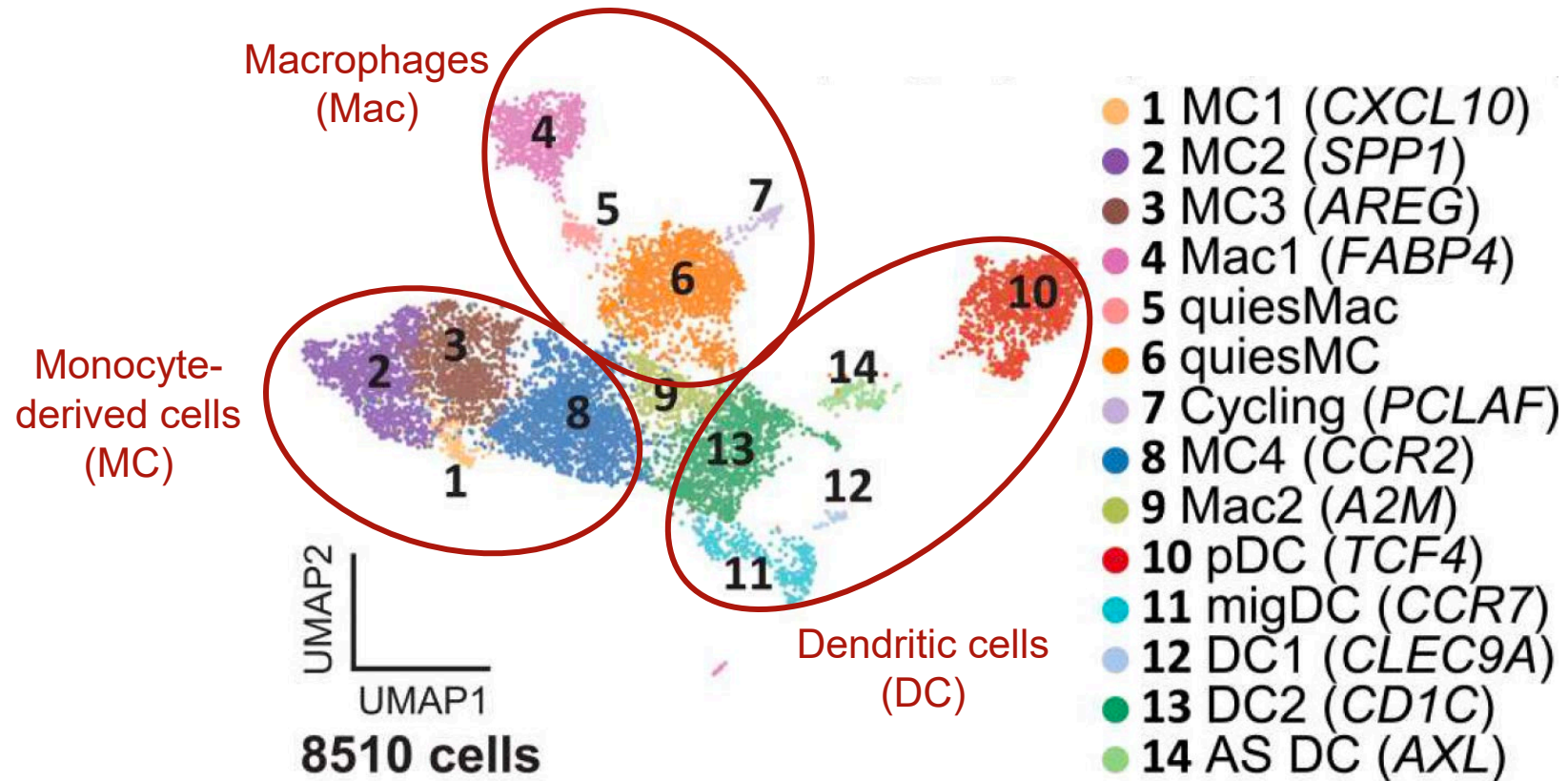
- MNP are immune sentinels and include dendritic cells (DC), macrophages, and monocyte-derived cells (MC) (Guilliams et al. *Nat Rev Immunol*, 2014)
- Macrophages play an important role in tissue homeostasis (Mosser et al. *Cell Mol Immunol*, 2021)
- DC can activate and polarize T cells and are necessary to sustain type 2 inflammation in murine models of asthma (van Rijt et al. *J Exp Med*, 2005)
- MC are recruited to the lung during allergic inflammation and programmed by the tissue microenvironment to acquire functional specialization (Geissman, F. *Science*, 2010; Jakubzick et al. *Nat Rev Immunol*, 2017)
- The precise role of human MNP subsets and their functional specialization during allergic inflammation have not been characterized



Guilliams et al. *Nat Rev Immunol*, 2014.

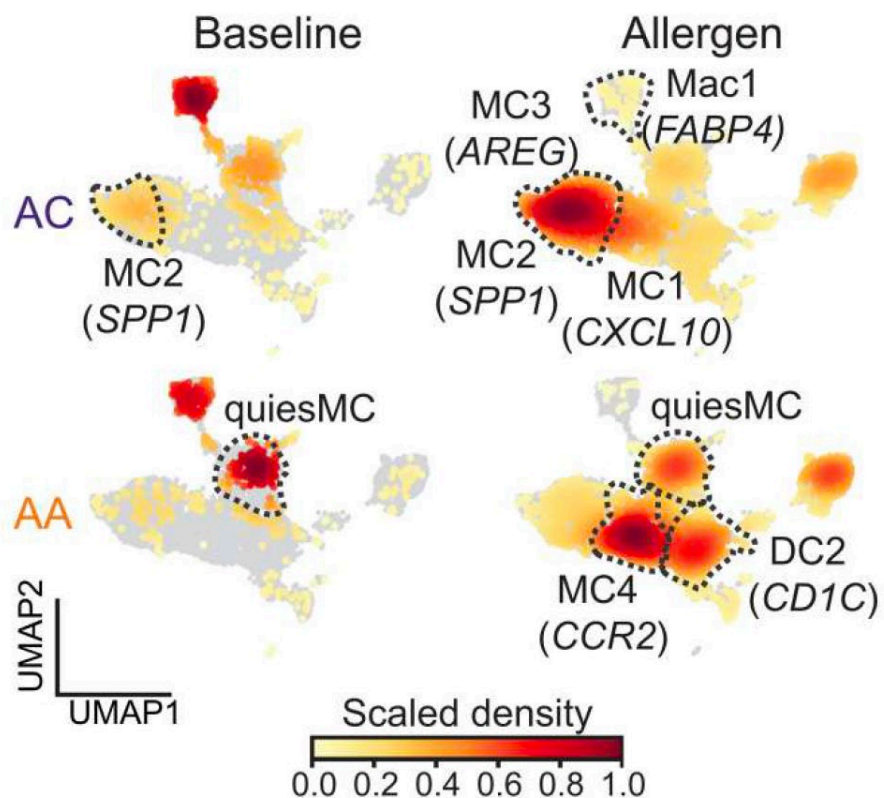
SAC identified 14 MNP clusters in the lower airway mucosa

Mononuclear phagocyte (MNP) clusters

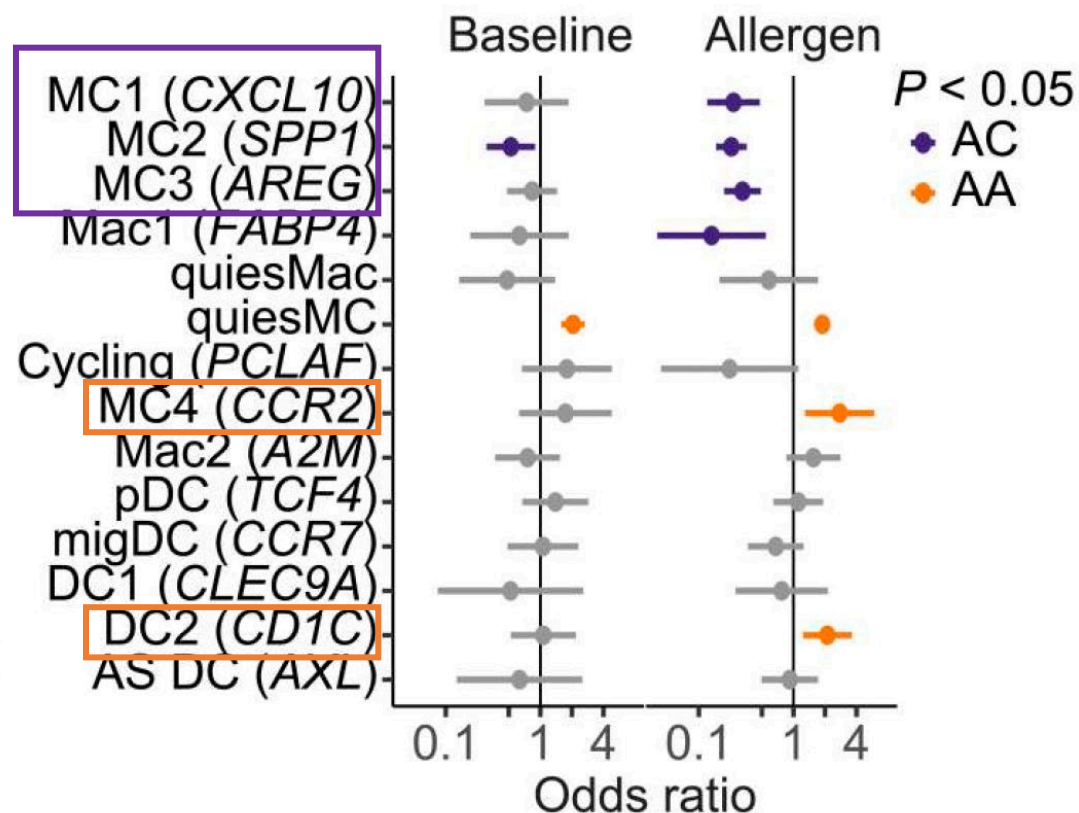


SAC reveals distinct mononuclear phagocyte profiles in AA and AC

UMAP embedding of cell density

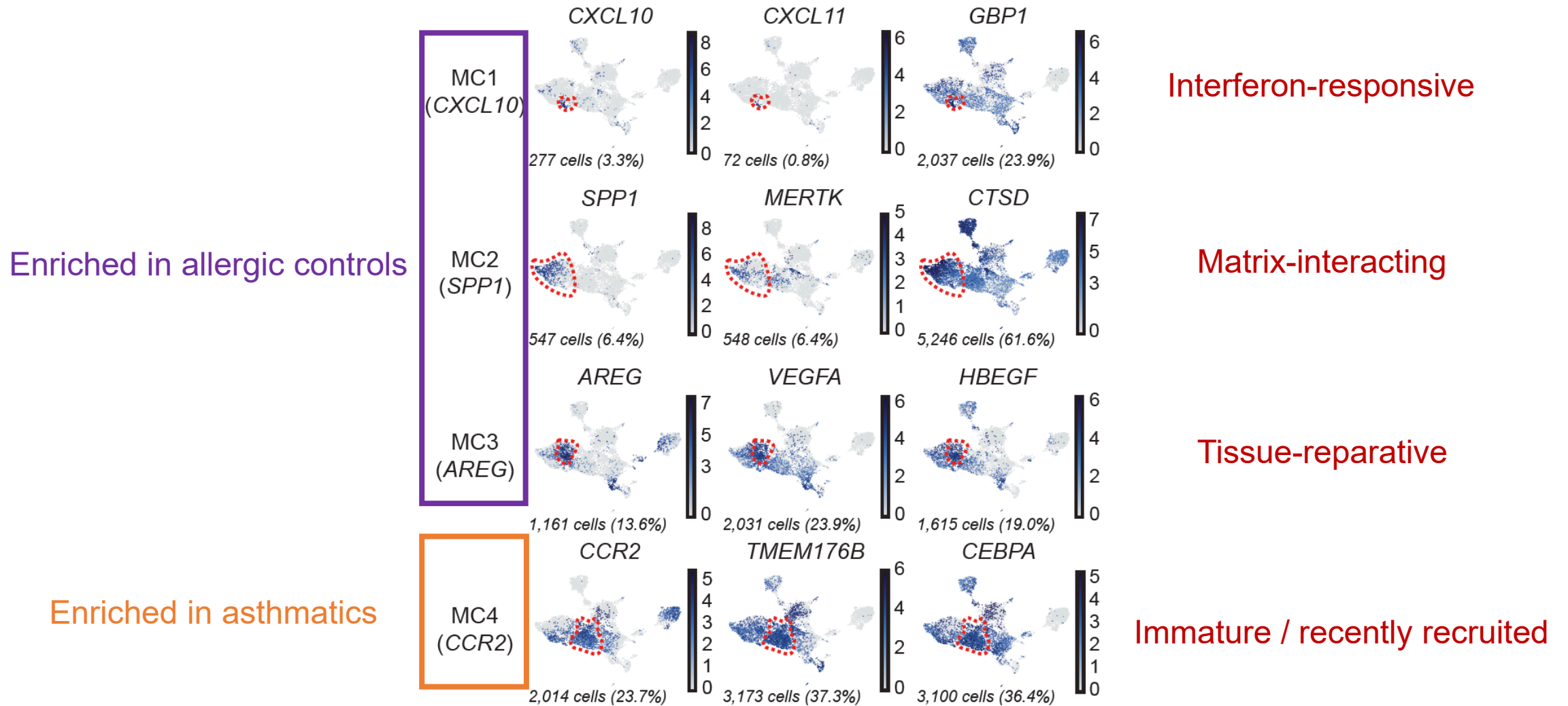


Disease Association




Macrophages and macrophage-like MC are enriched in allergic controls, while $CCR2^{hi}$ immature MC and DC2 are enriched in asthmatics after SAC

Monocyte-derived cell (MC) functional specialization



Suggests that monocyte-derived cells have specialized programs in human lung tissue

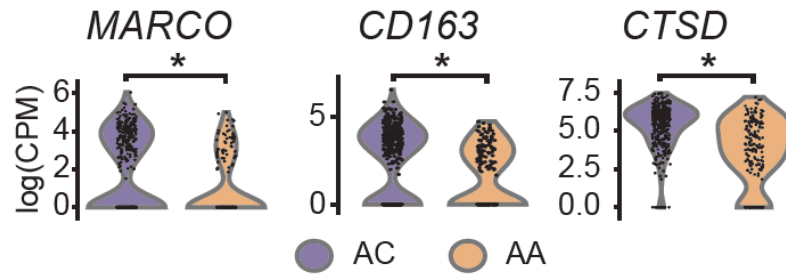
Monocyte-derived cells upregulate distinct transcriptional programs in AA vs AC after SAC


 **MC2 (*SPP1*)**

Enriched in AC after SAC

Phagolysosome Genes

↑ AC

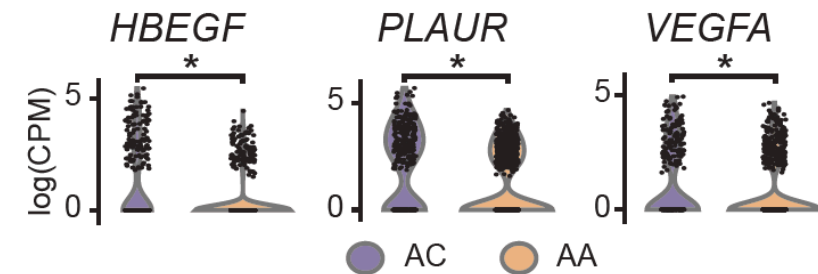


 **MC4 (*CCR2*)**

Enriched in AA after SAC

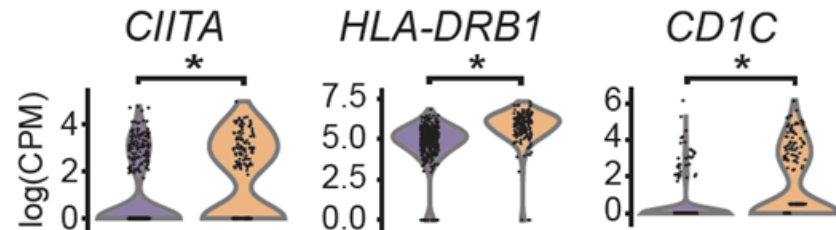
Tissue Repair Genes

↑ AC



MHC Genes

↑ AA



Tissue Remodeling Genes

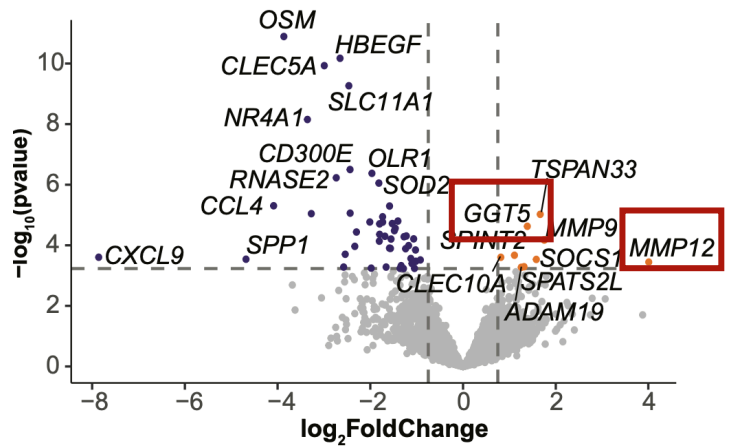
↑ AA



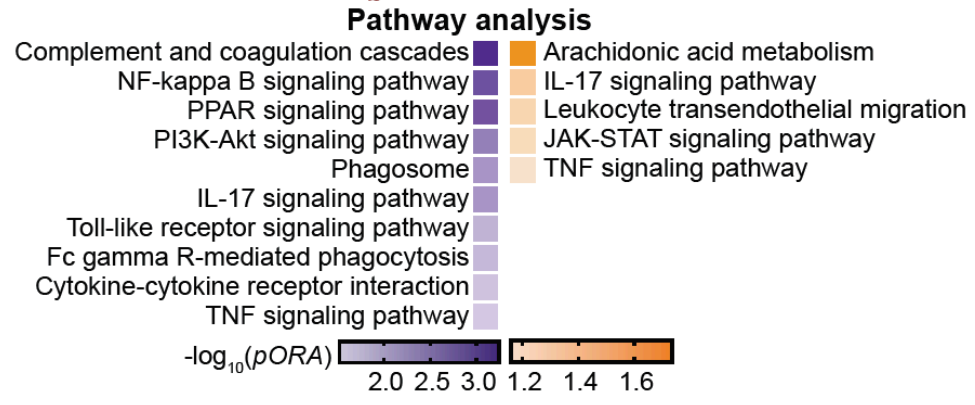
Suggests that the same cell types may be differentially programmed by local signals

MC programming in asthmatic airways

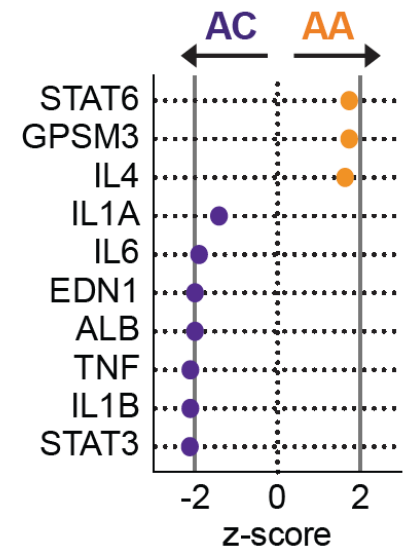
MC4 (CCR2)



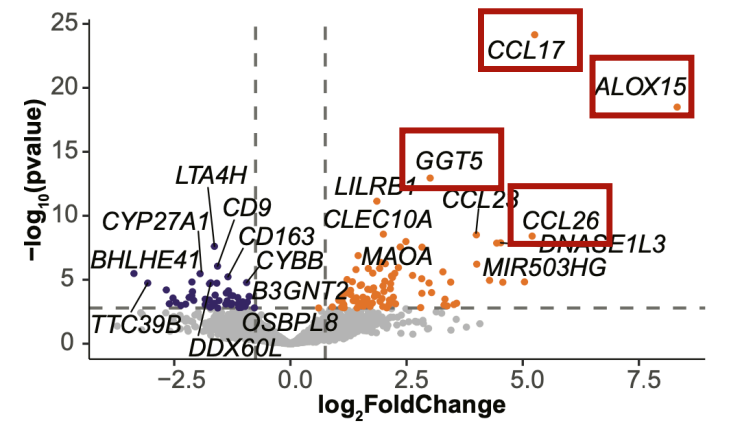
MC4 (CCR2)



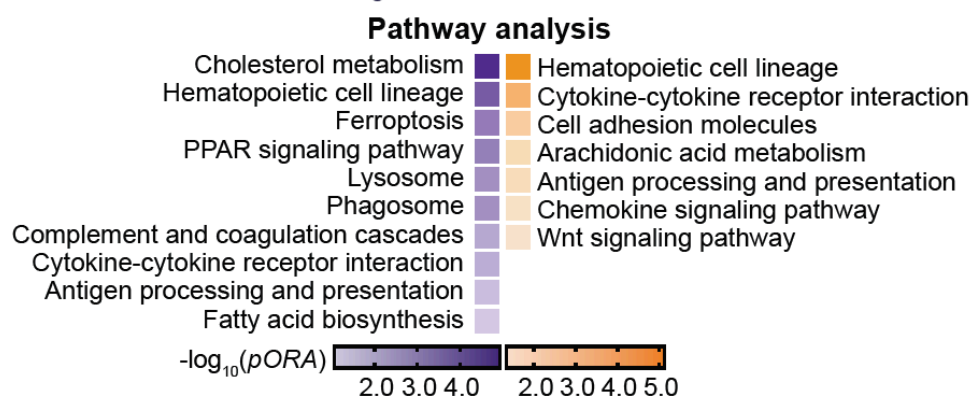
MC4
Upstream regulators



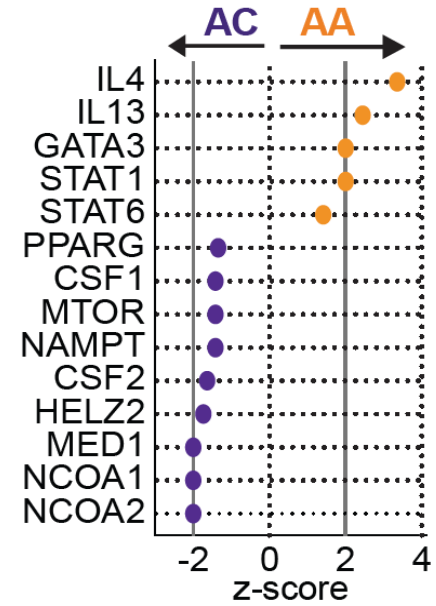
MC2 (SPP1)



MC2 (SPP1)

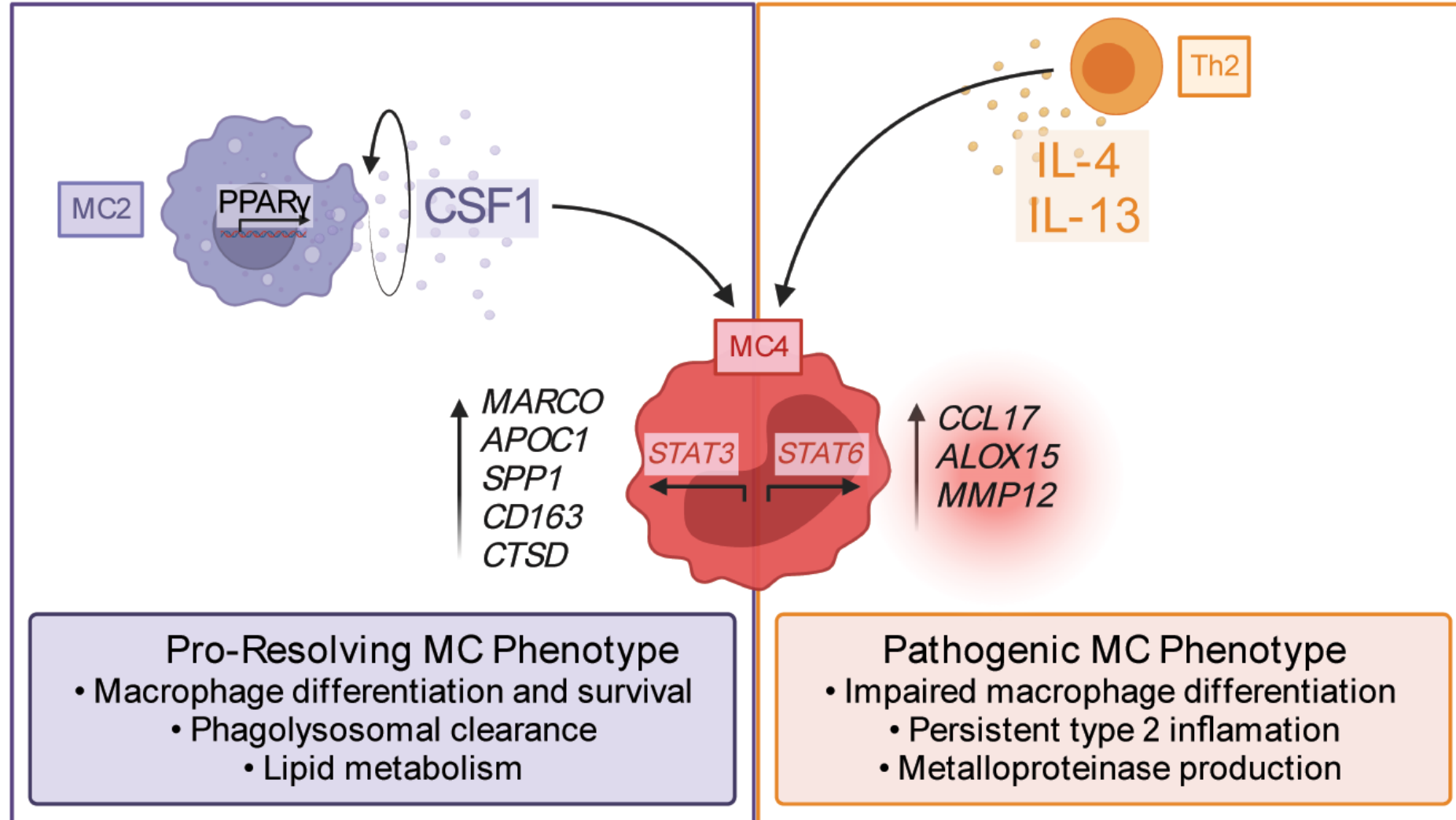


MC2
Upstream regulators

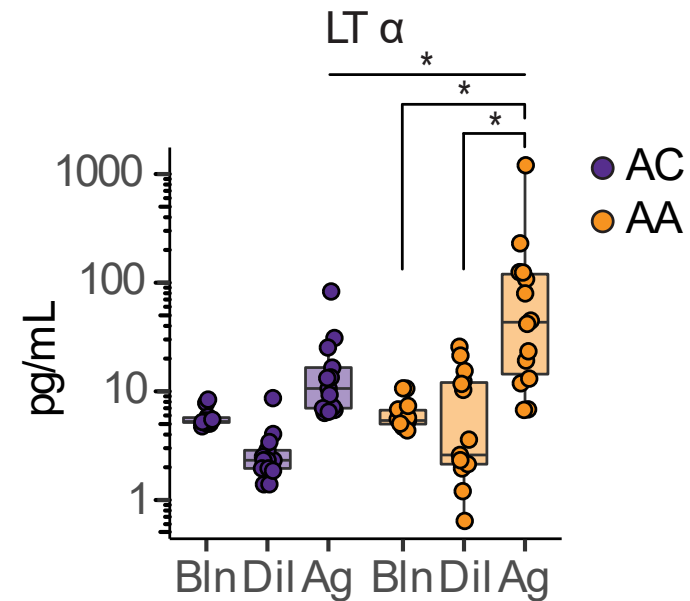
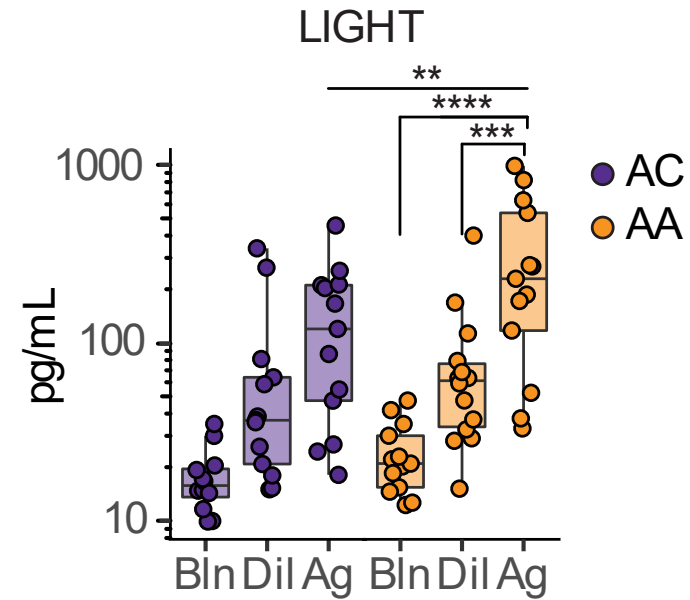
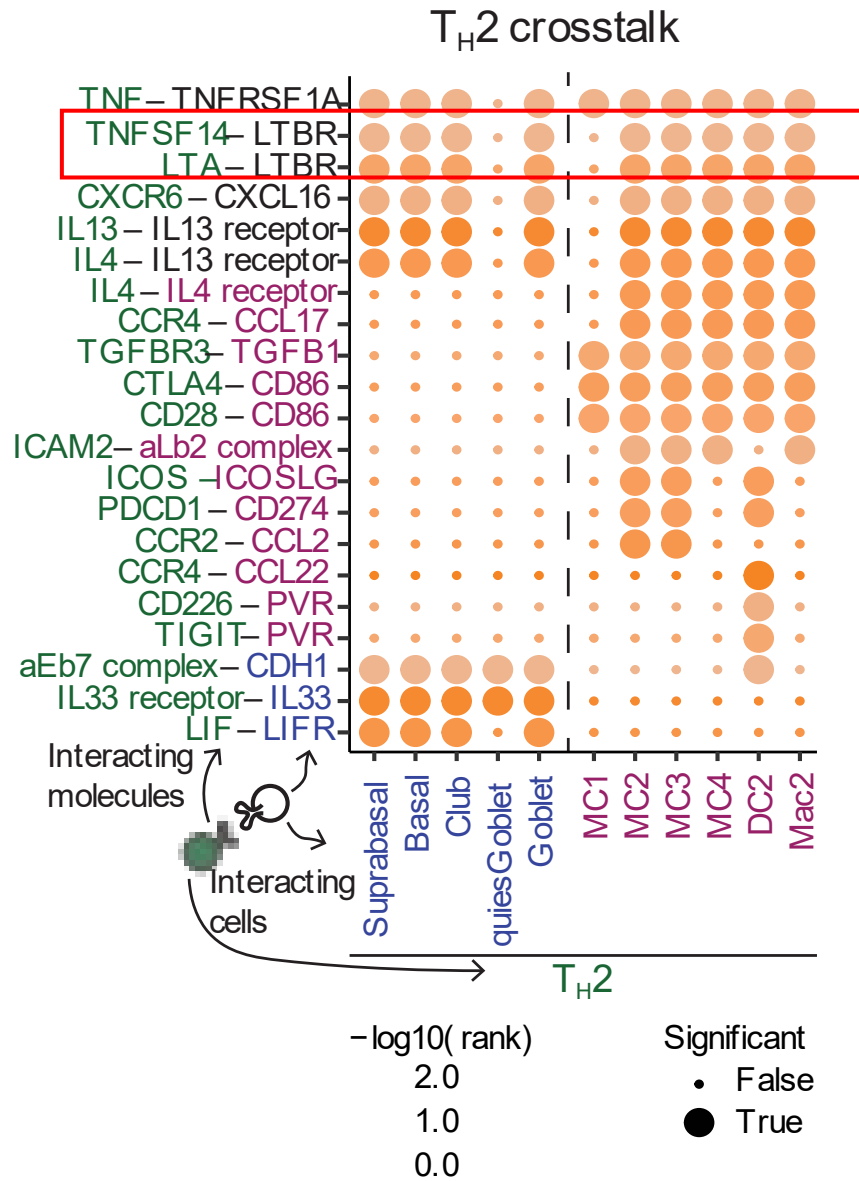


MC in asthmatics are enriched for genes involved in T_H2 chemotaxis, eicosanoid metabolism, and ECM degradation

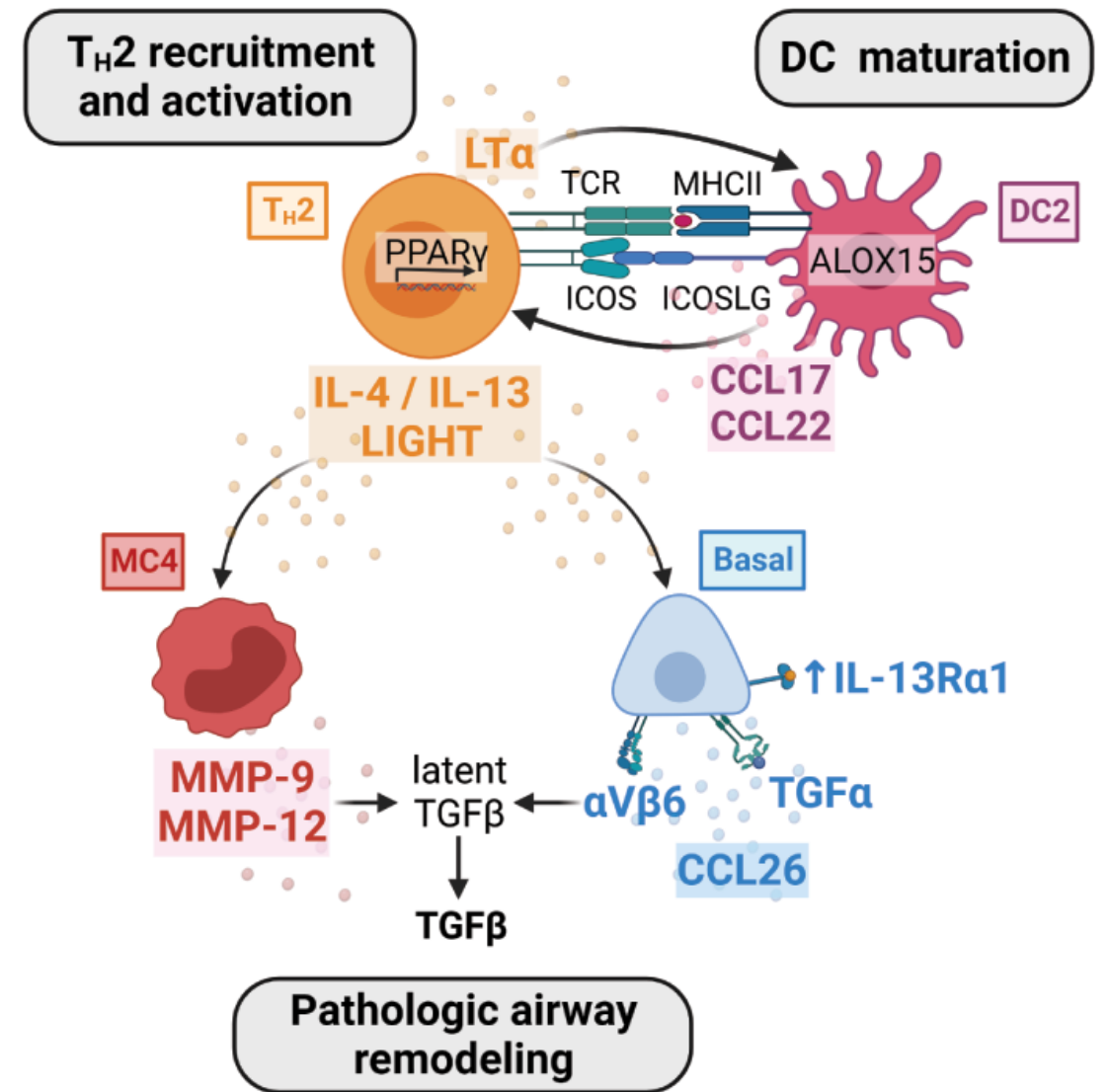
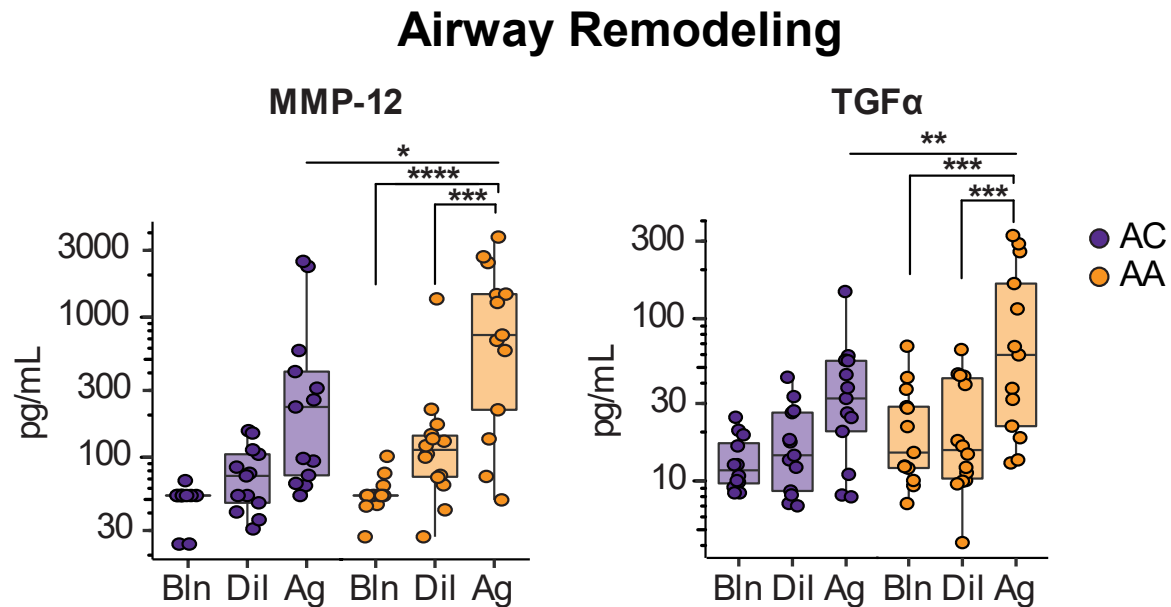
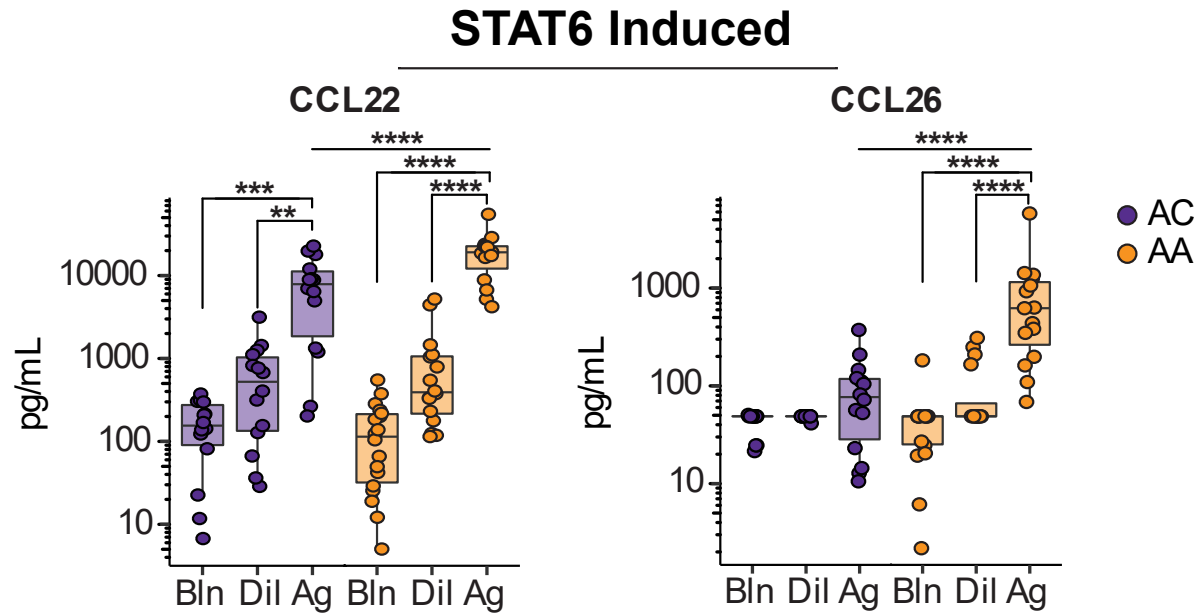
MC4 Phenotype is Different in AA compared to AC



T_H2 cellular cross-talk in asthmatic airways



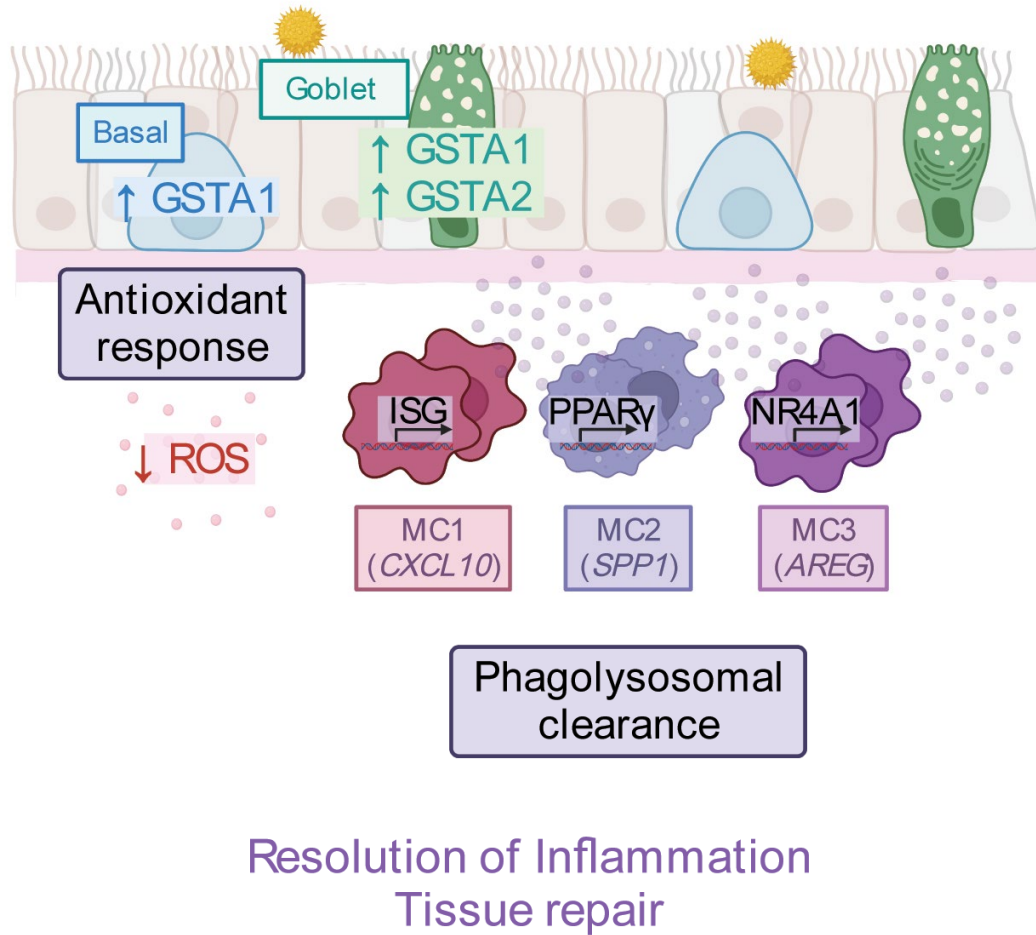
T_H2 cellular cross-talk in asthmatic airways



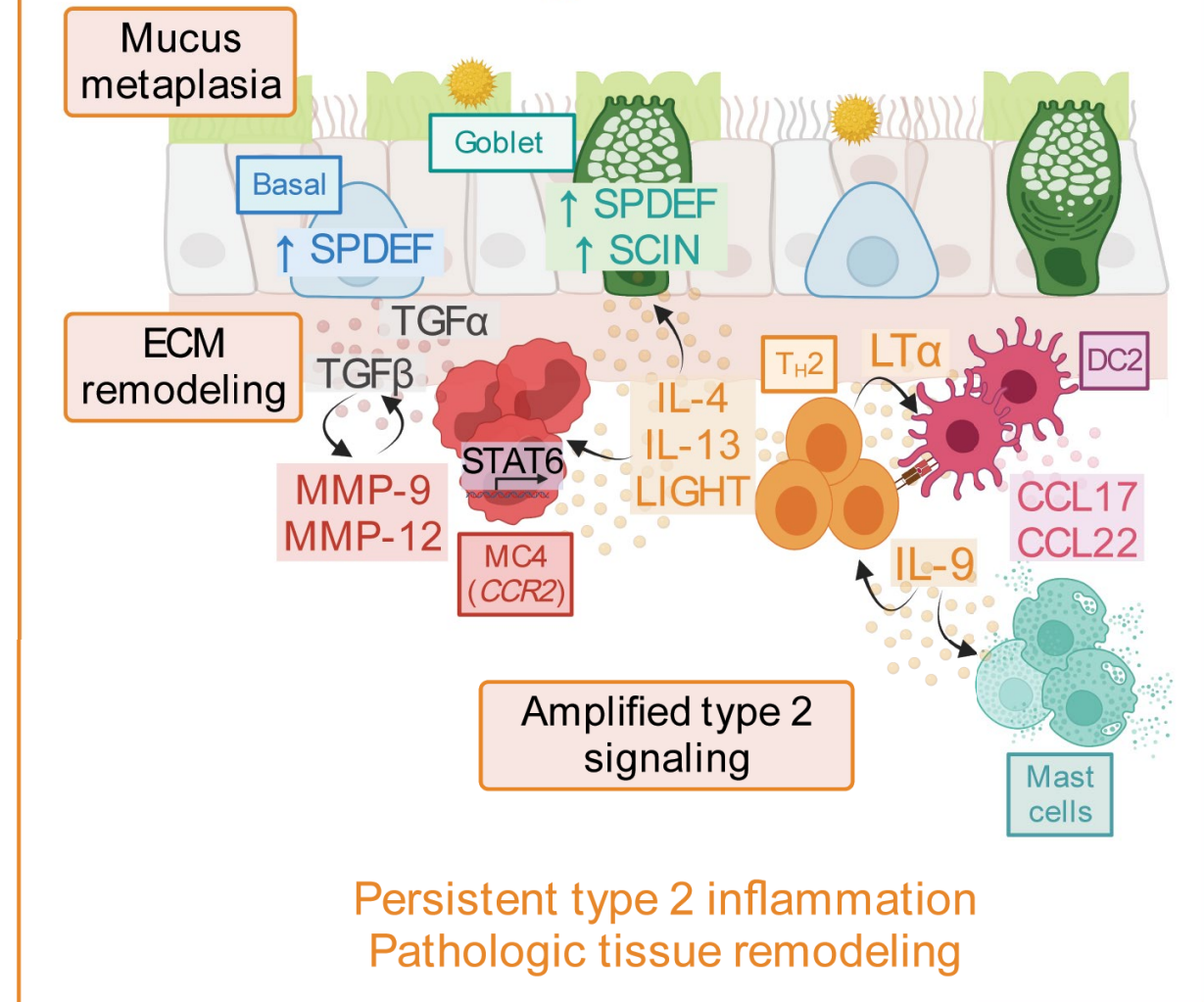
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Allergic Control



Allergic Asthmatic



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