

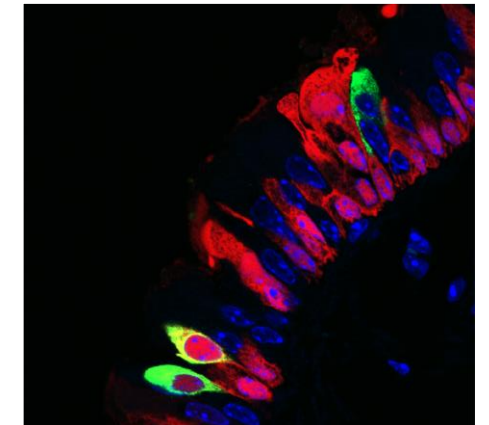
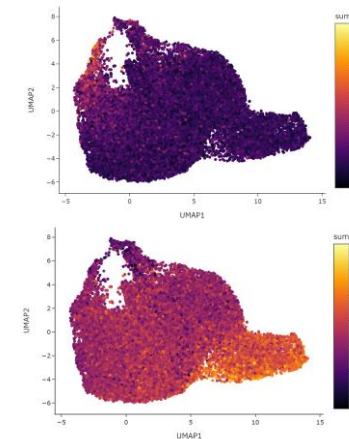
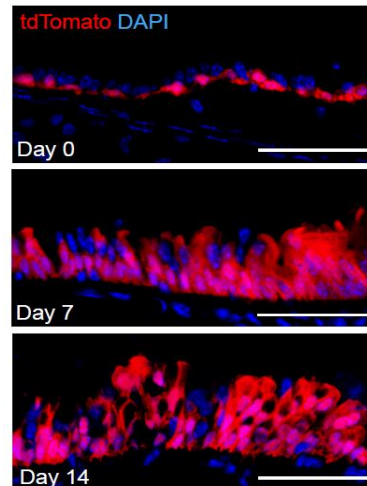
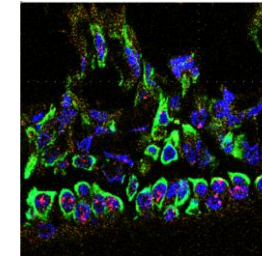
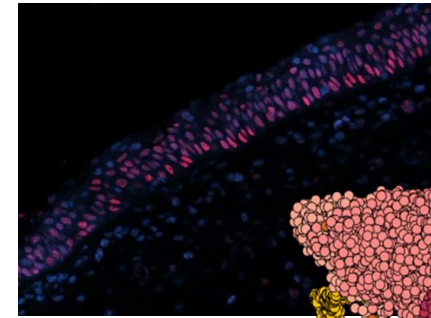
Emerging Insights in Asthma Pathobiology From Airway Omics

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Medical School



Disclosures

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Consulting: Biohaven, Regeneron, Uptodate

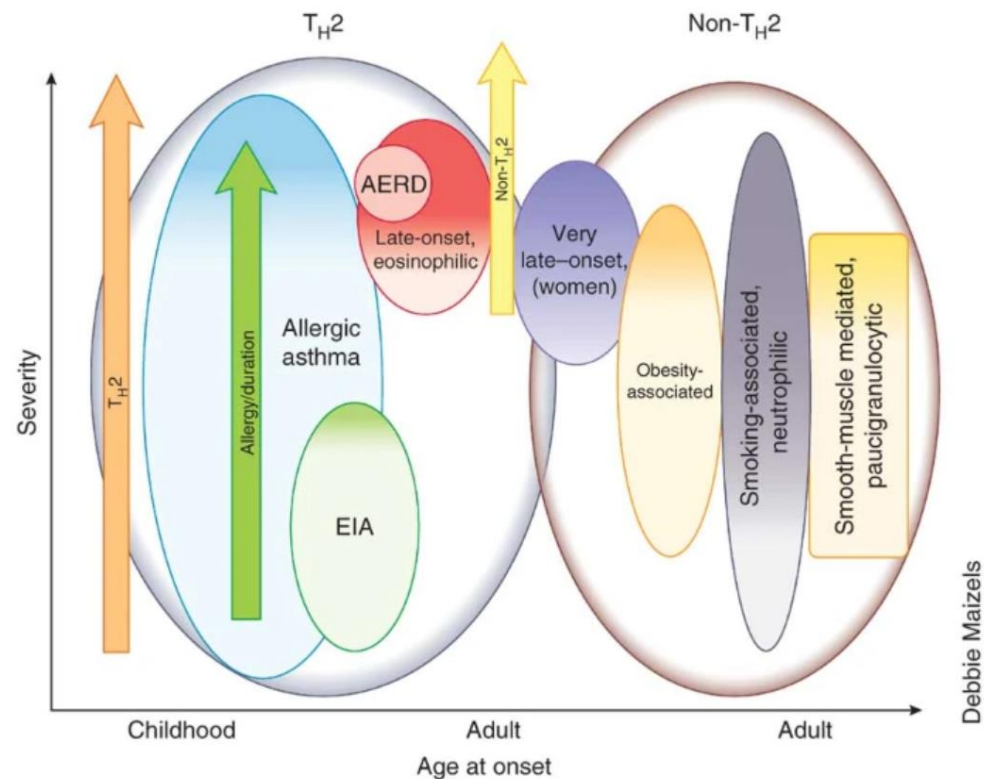
Grant review: American Lung Association and NIH

Not Relevant to Presentation Here

Outline

- Defining Asthma Endotypes in Transcriptomics
 - Sputum
 - Bronchial brushings
- Loss of a Regulatory Axis Identified in scRNA-seq
- Novel Methods to Define Pathobiologic Axes

Airway transcriptomics in severe asthma.... Are we there yet?

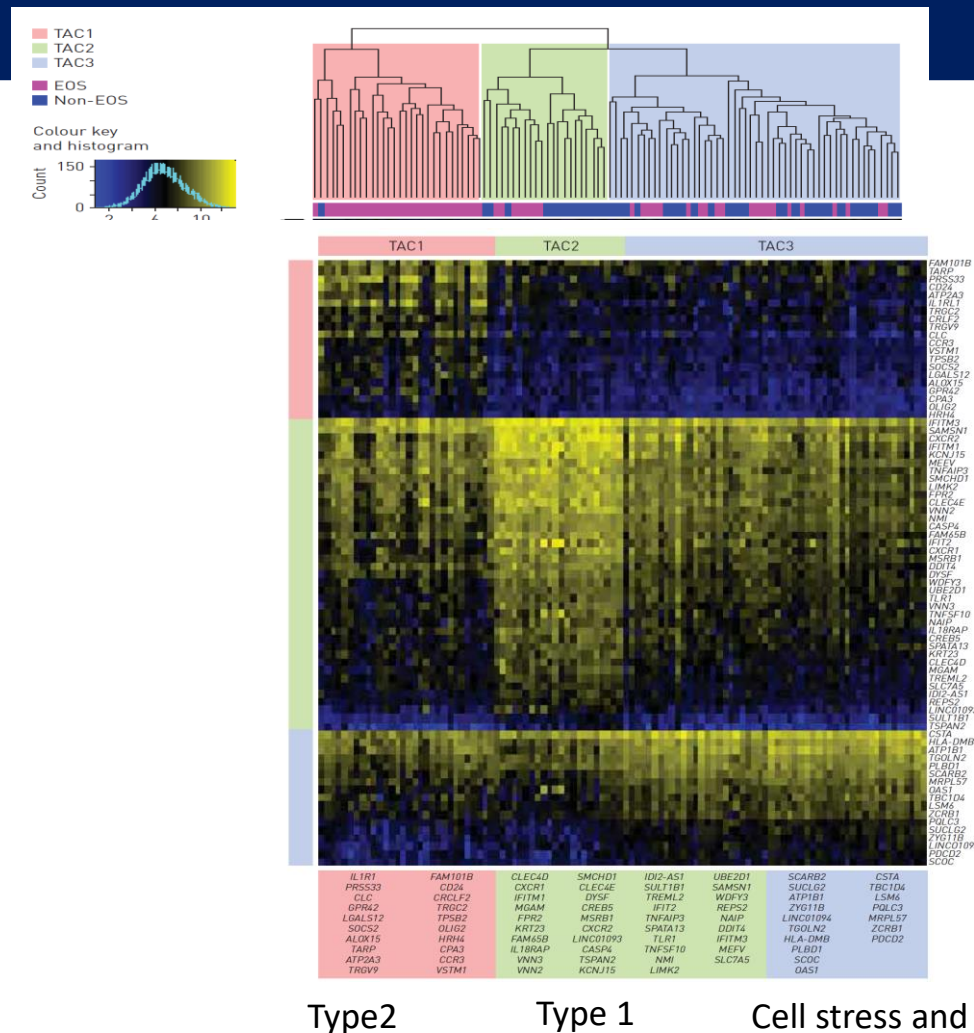


Wenzel Nat Med 2012

- Severe Asthma Research Program (SARP)
- European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA)
- Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome (U-BIOPRED)
- Airways Disease Endotyping for Personalized Therapeutics (ADEPT)

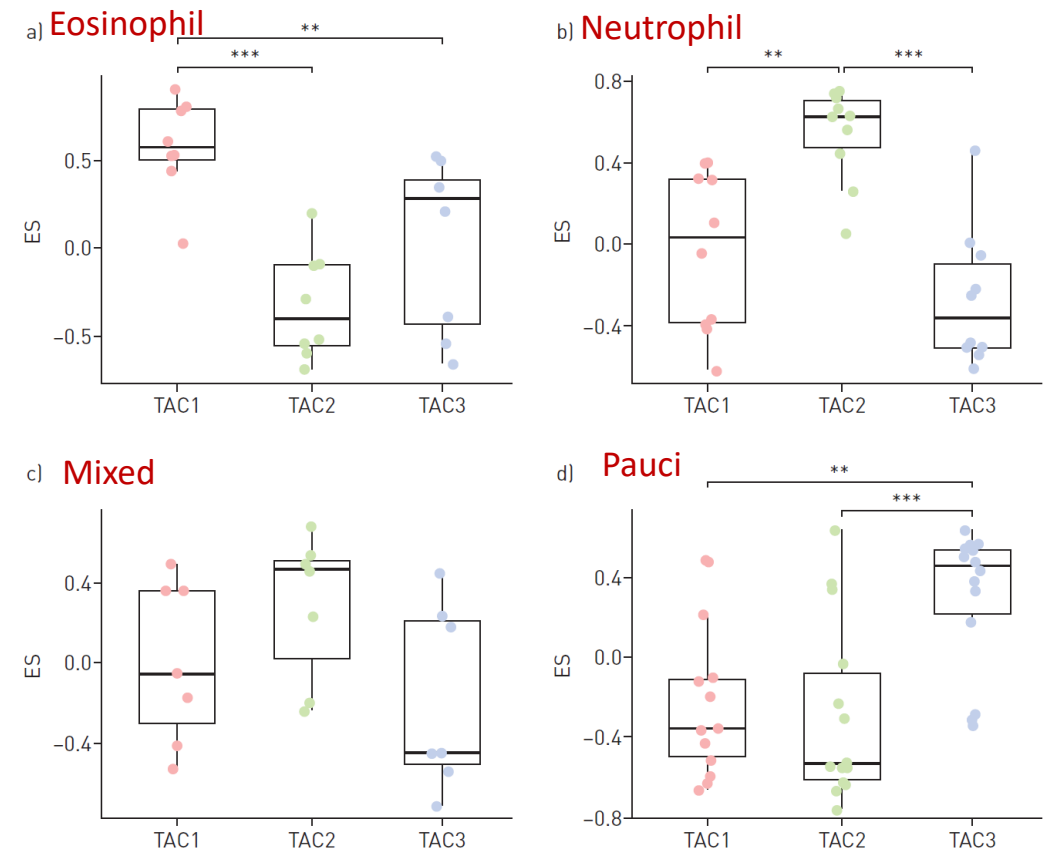
Sputum transcriptomics identifies endotypes

- U-BIOPRED 104 mod/sev asthma and 16 healthy controls
- Induced sputum transcriptomics (microarray)
- Identified 508 DEGS between asthma with sputum eos, no eos, healthy control
- Identified 3 clusters (TACs)



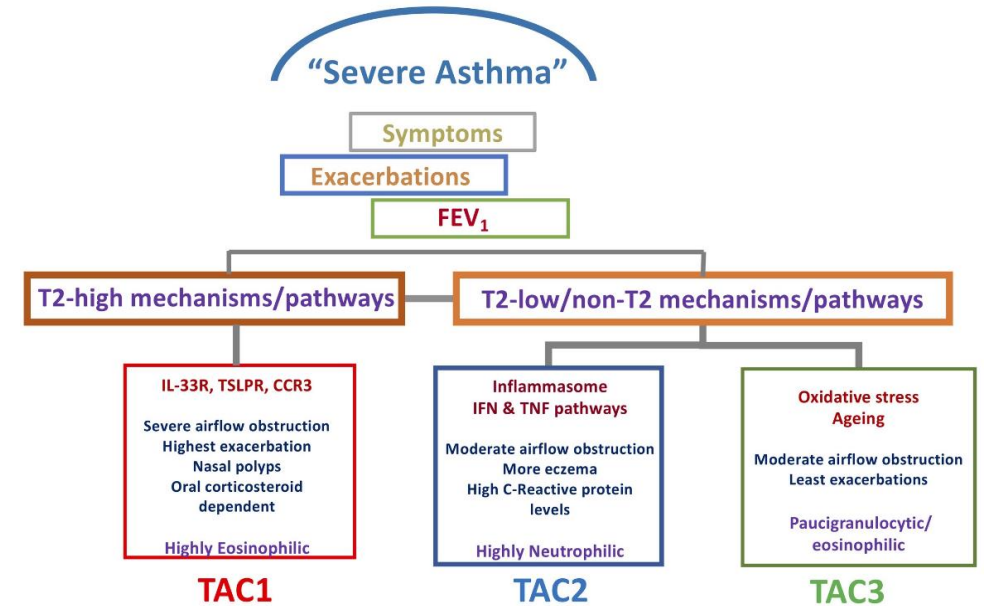
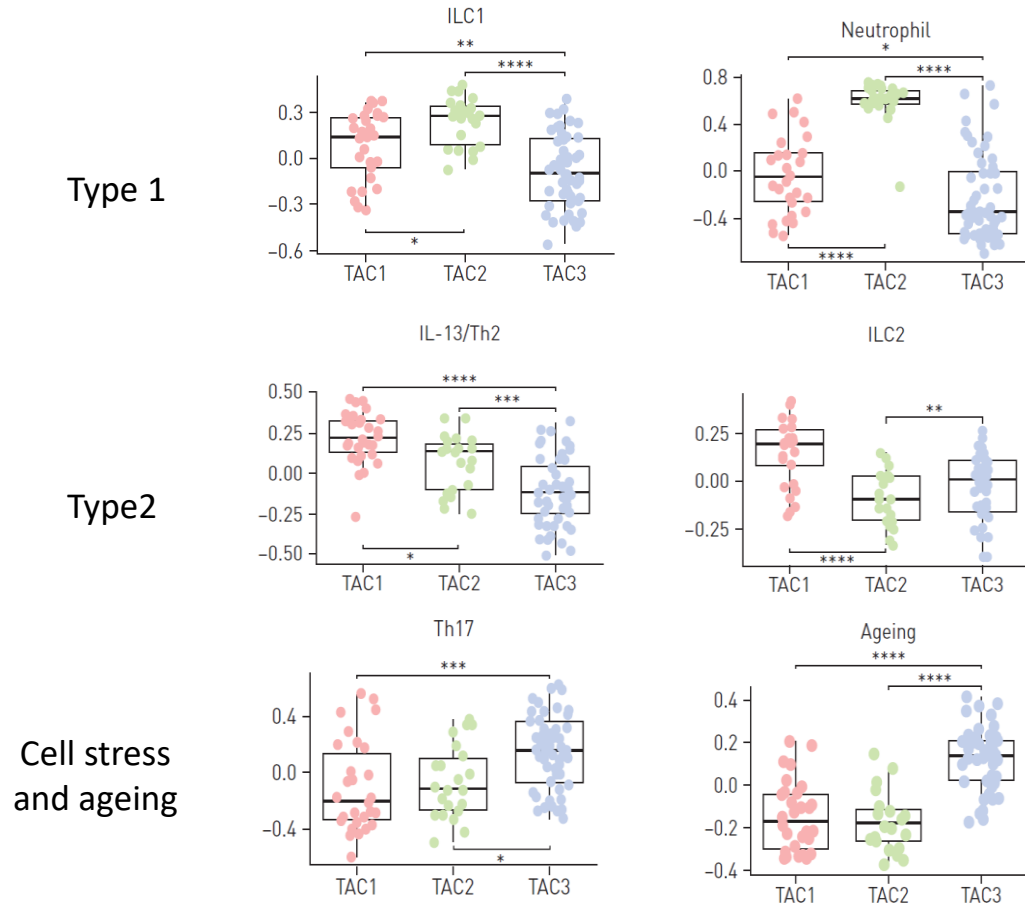
Sputum endotypes correlate with granulocyte phenotype

- U-BIOPRED 104 mod/sev asthma and 16 healthy controls
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- Identified 508 DEGS between asthma with sputum eos, no eos, healthy control
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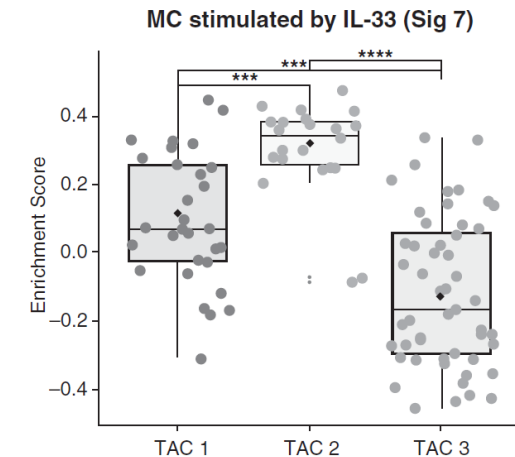
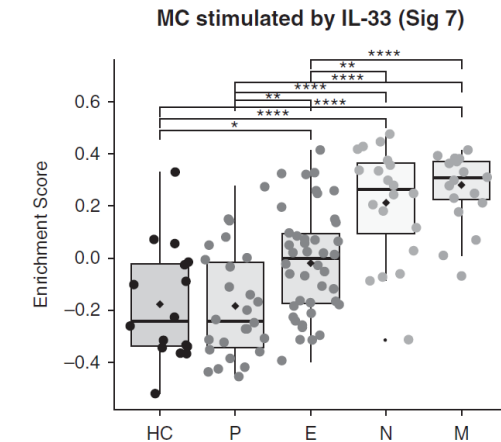
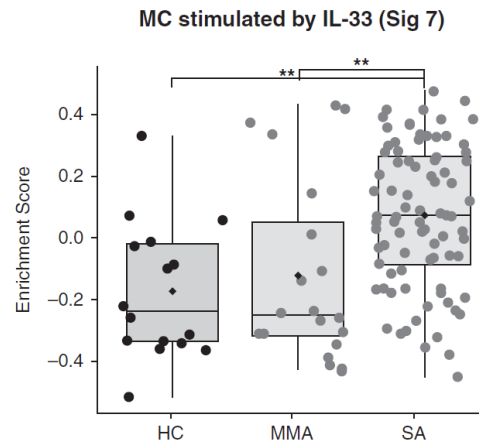
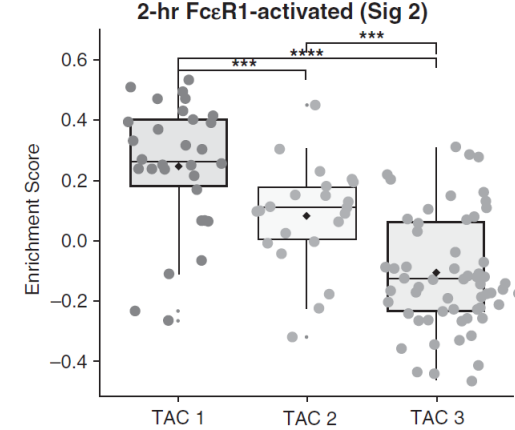
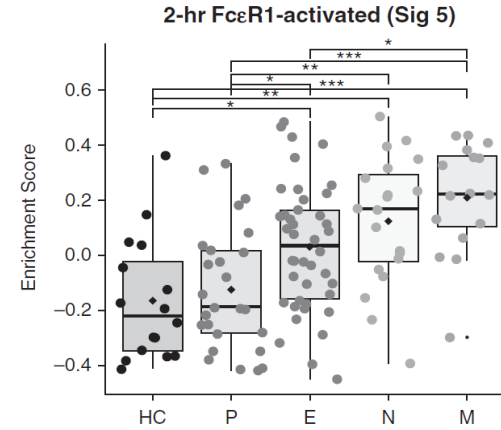
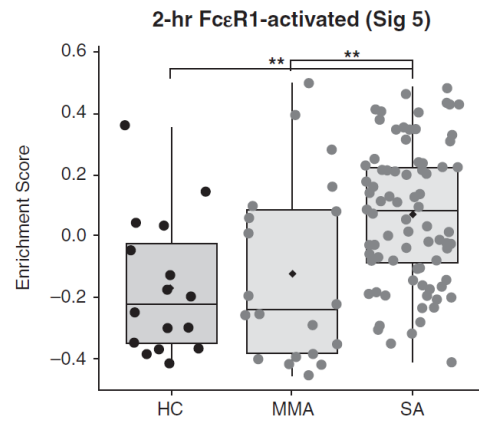


Sputum endotypes expresses distinct immunologic genes (GSVA)

Sputum transcriptomes nicely capture immunologic diversity beyond eosinophilic and neutrophilic inflammation

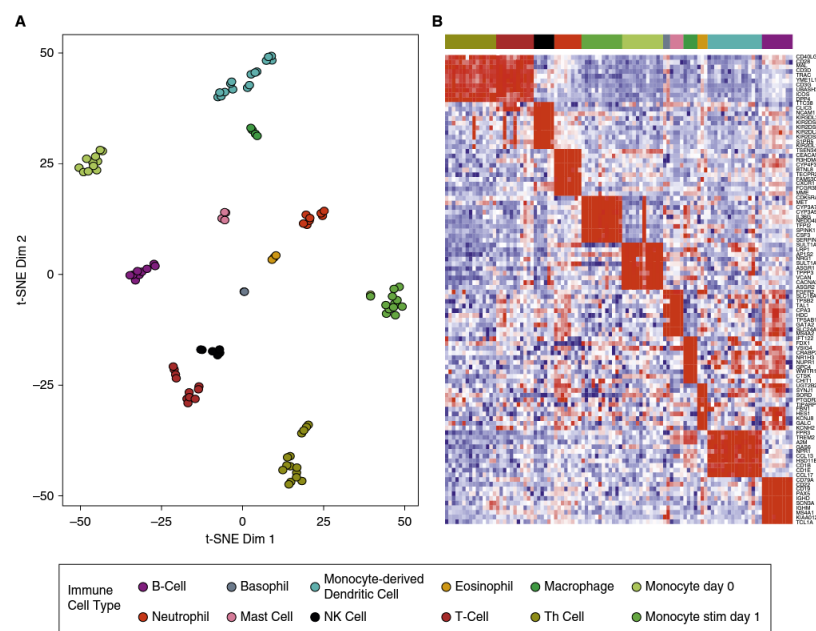


Sputum endotypes express distinct signatures of mast cell activation

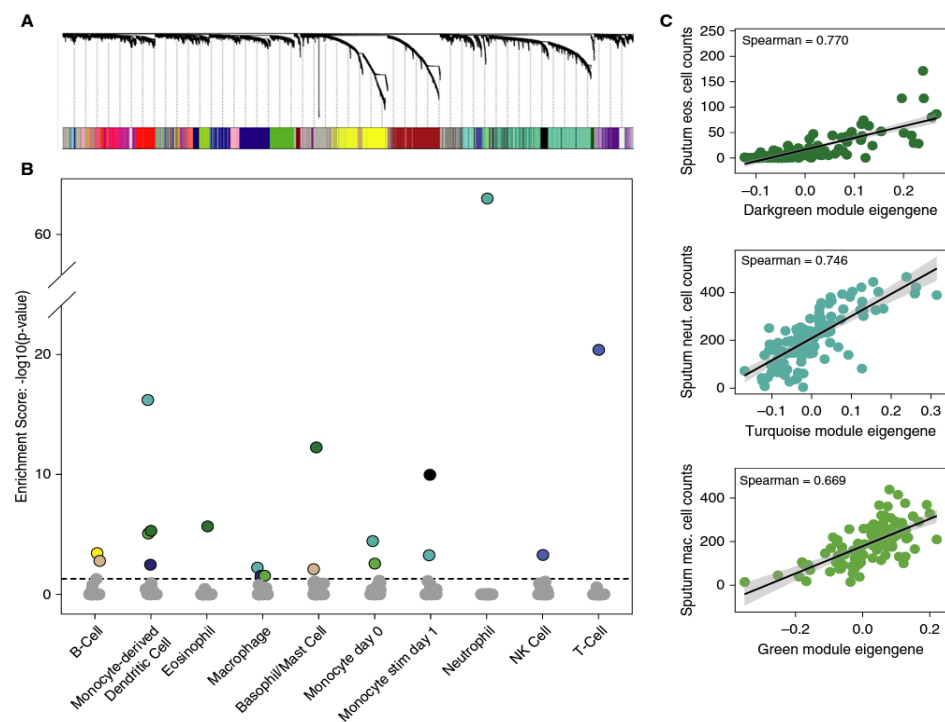


Further assessment of immunocytes in sputum of mild/mod asthma

Defining the transcriptome of immunocytes

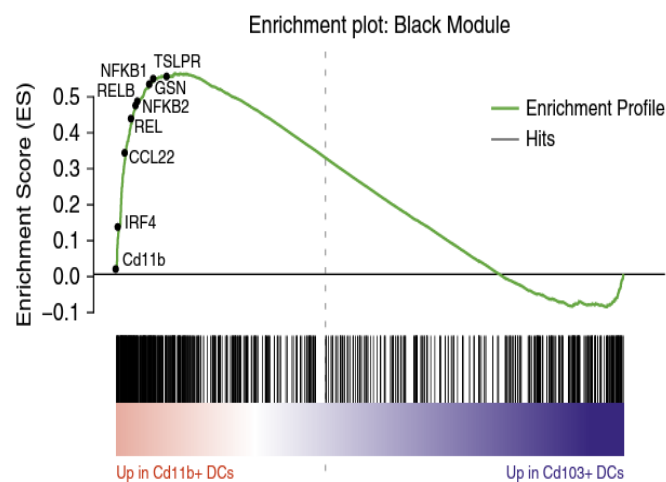


Assessing for enrichment in the sputum

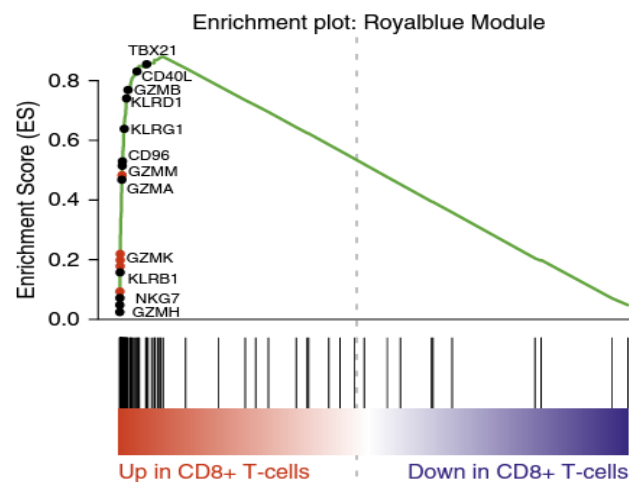


Further assessment of immunocytes in sputum of mild/mod asthma

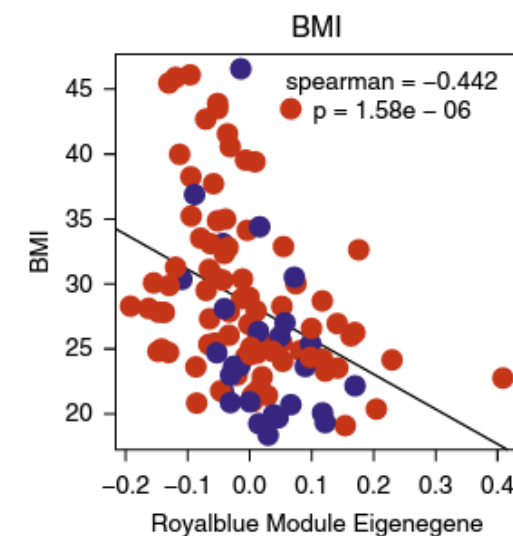
Increased CD11b+IRF4+ myeloid cells in Th2 asthma



Loss of cytotoxic cells in T2 low asthma

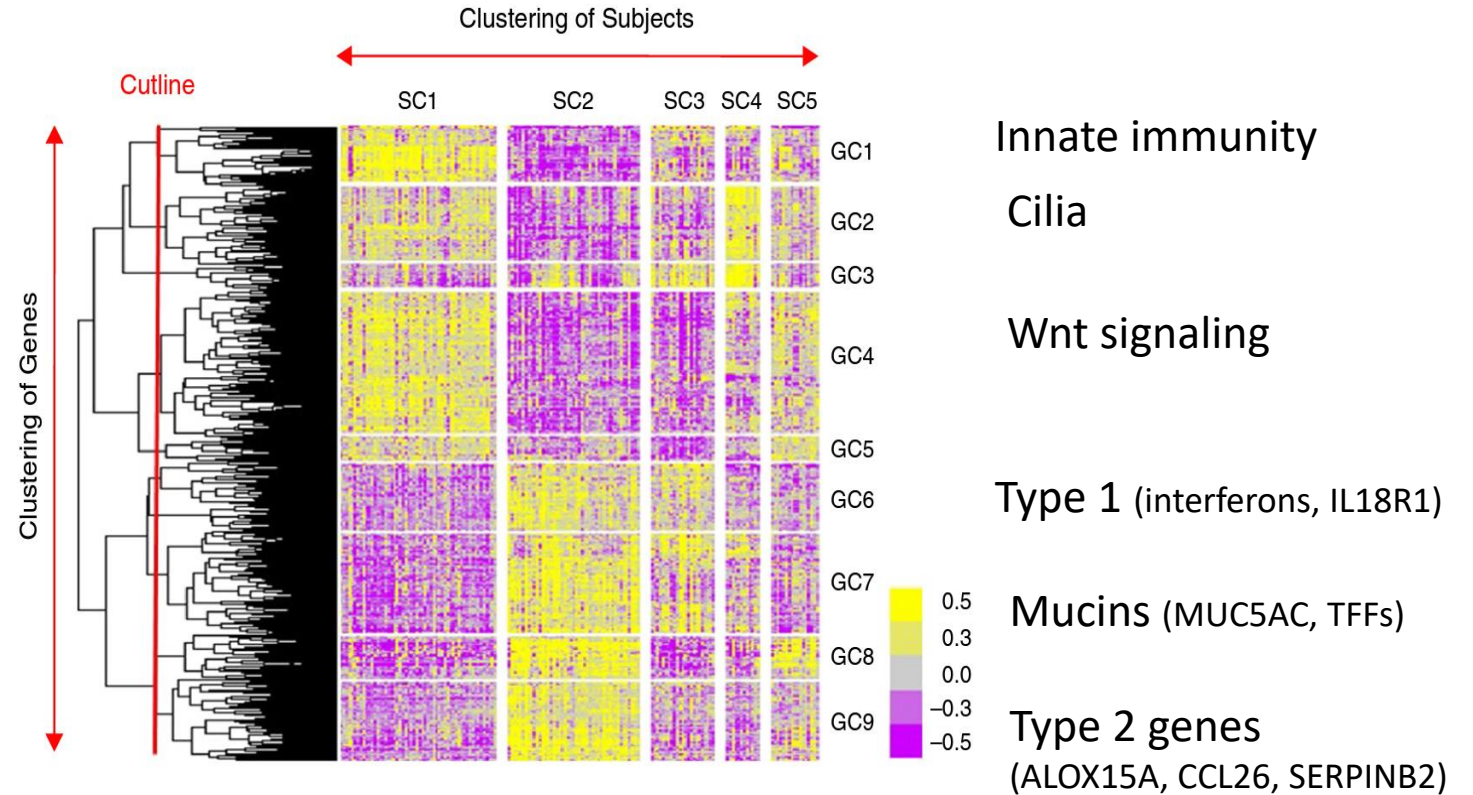


Cytotoxic module is lost in obesity



Bronchial brushing transcriptomics – supervised analysis

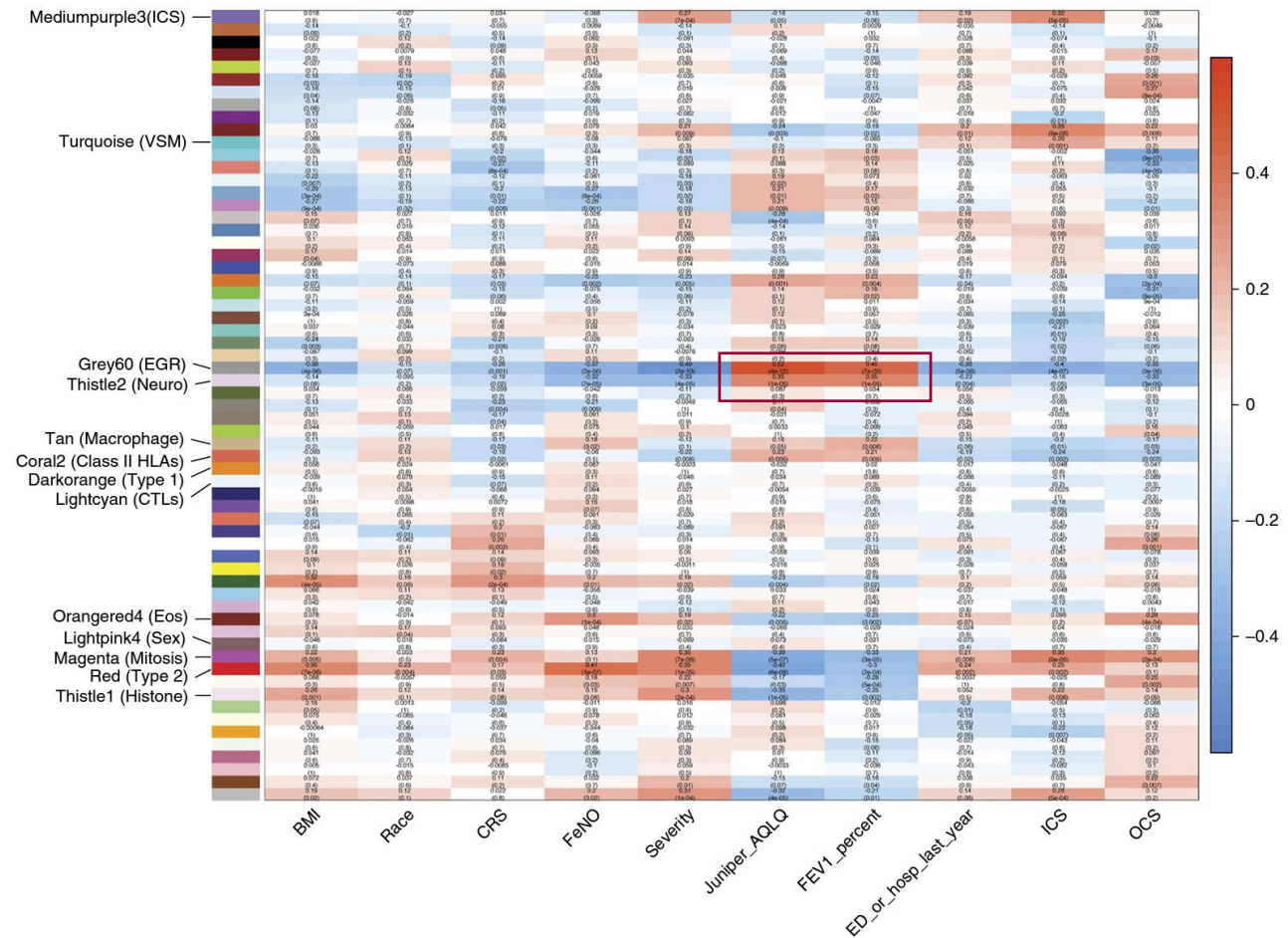
- SARP 154 patients
 - HC, MMA, SA
- Bronchial brushings, microarray
- Supervised clustering
- Atopy, FENO, eosinophilia, age of onset, and lung function



Bronchial brushing transcriptomics – unsupervised analysis

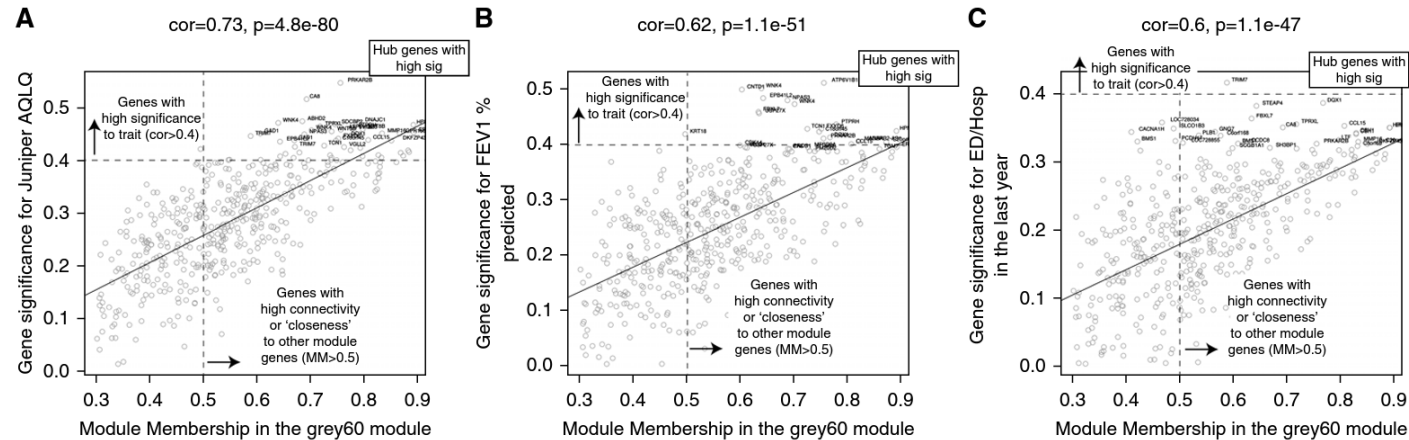
Weighted Gene Correlation Network Analysis (WGCNA)

- SARP 154 patients
 - HC, MMA, SA
- Bronchial brushings, microarray
- WGCNA

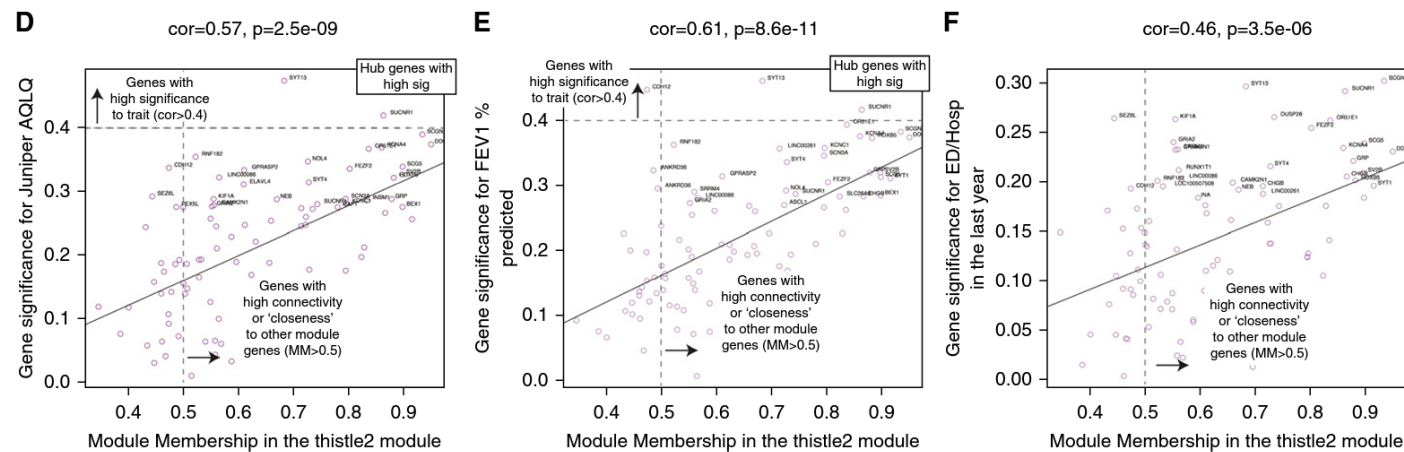


WGCNA reveals an epithelial repair module and neural module associated with AQLQ, FEV1%, and hospitalization in the last year

Epithelial growth and repair

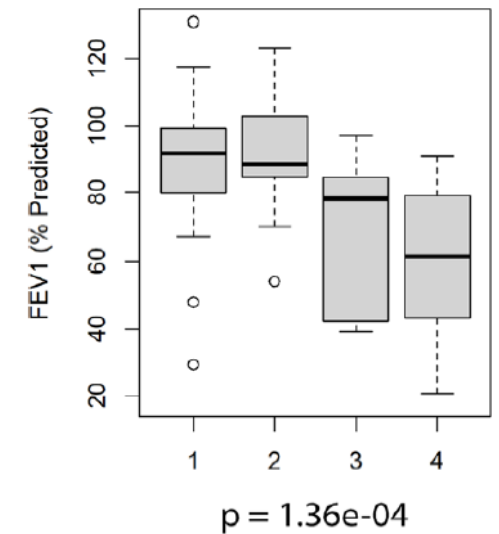
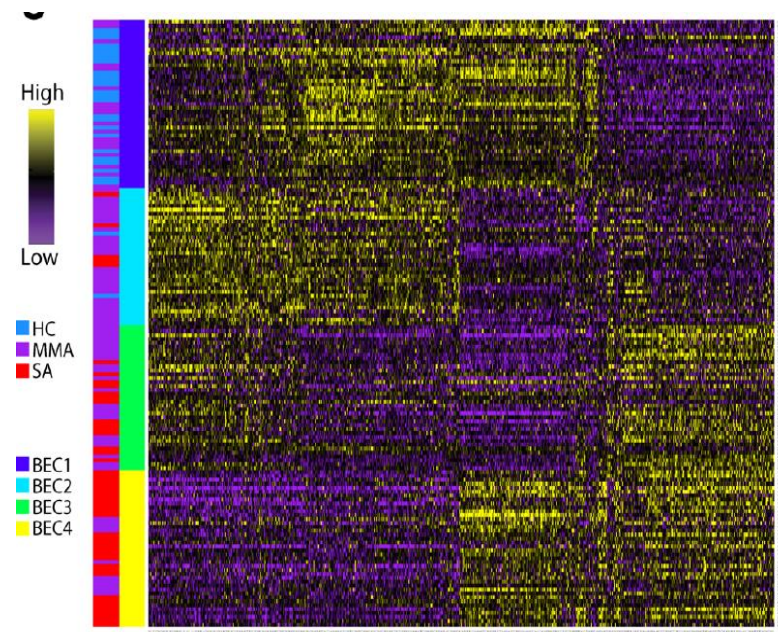


Neuronal function



Bronchial brushings transcriptomics – partially supervised analysis identify a cluster with low lung function

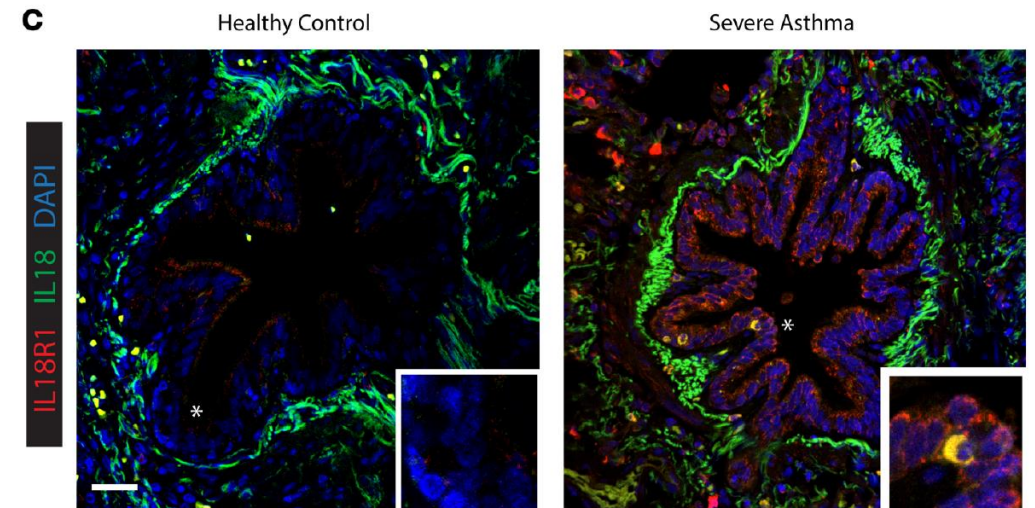
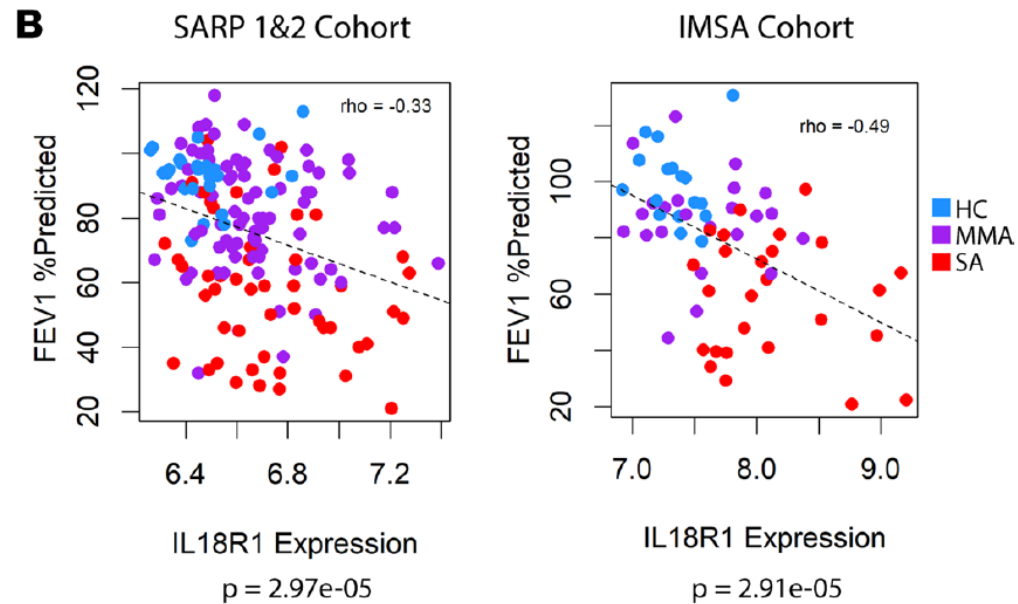
- SARP 154 patients
 - HC, MMA, SA
- Bronchial brushings, microarray
- 758 DEGs, unsupervised clustering
- 4 clusters
 - BEC1 – HCs, low T2
 - BEC2 – downregulated inflammation and ECM remodeling
 - BEC3 – high T2 status
 - BEC4 – high stress response, lowest lung function



IL18R1 expression in severe asthma with low lung function

- IL18R1, expressed in cluster, inversely correlates with lung function

IL18R1 Upregulated in SA



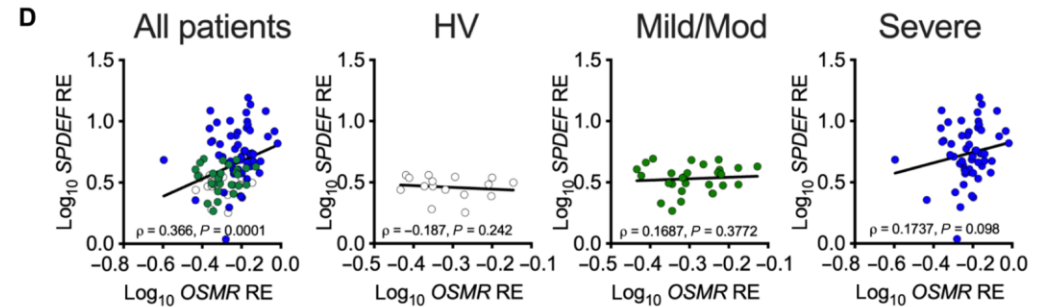
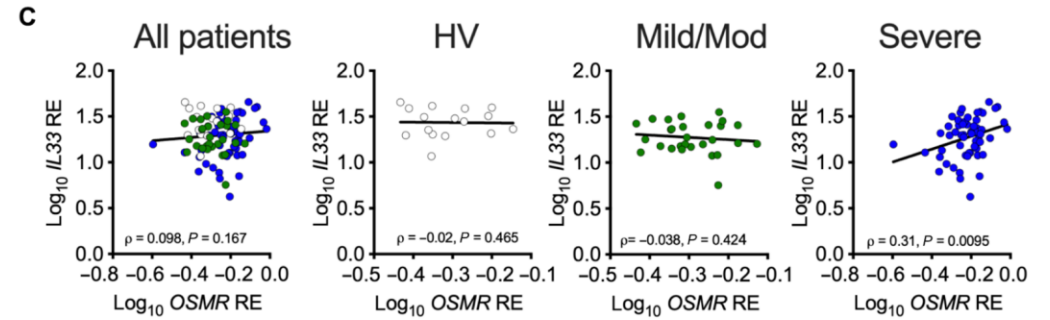
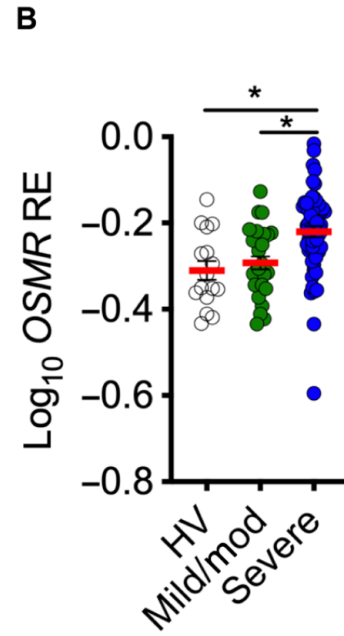
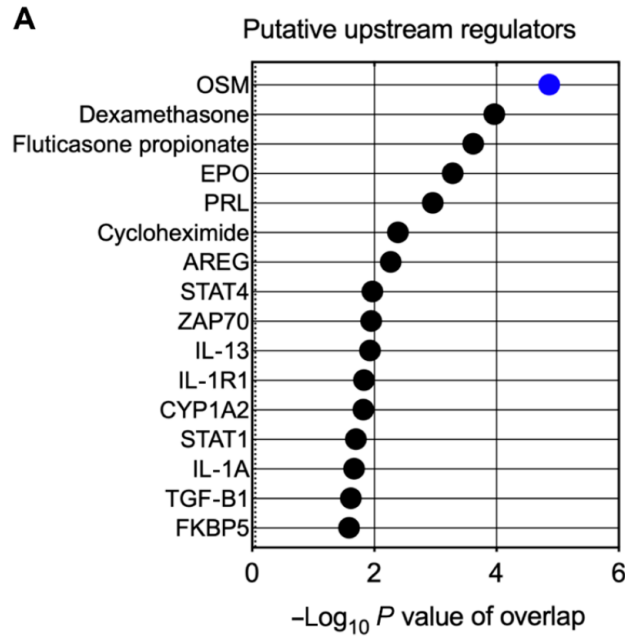
IL-18 function

- Member of the IL-1 family
 - Made as an inactive pro-IL-18 and requires cleavage for mature active form
 - Caspase-1, caspase-8, proteinase-3, chymase, granzyme B
- Promote T1 or T2 inflammation depending on the presence of IL-12
 - In basophils and NKT cells, IL-18 drives IL-4 production
 - In T cells, IL-18 + IL-12 drives IFN γ generation
- BEC4 was mixed eosinophilic and neutrophilic BAL, mixed T2 and IFN transcripts
- Subsequent paper identified patients with IFN γ + T cells

Oncostatin M (OSM) – a mediator of bacterial-elicited inflammation and mucus

- Member of the gp130 family of cytokines (IL-6 family)
- Expressed by human DCs and macrophages
- Increased in allergic diseases
- Receptor OSM receptor B (OSMRB) on epithelial cells, fibroblasts, and airway smooth muscle cells

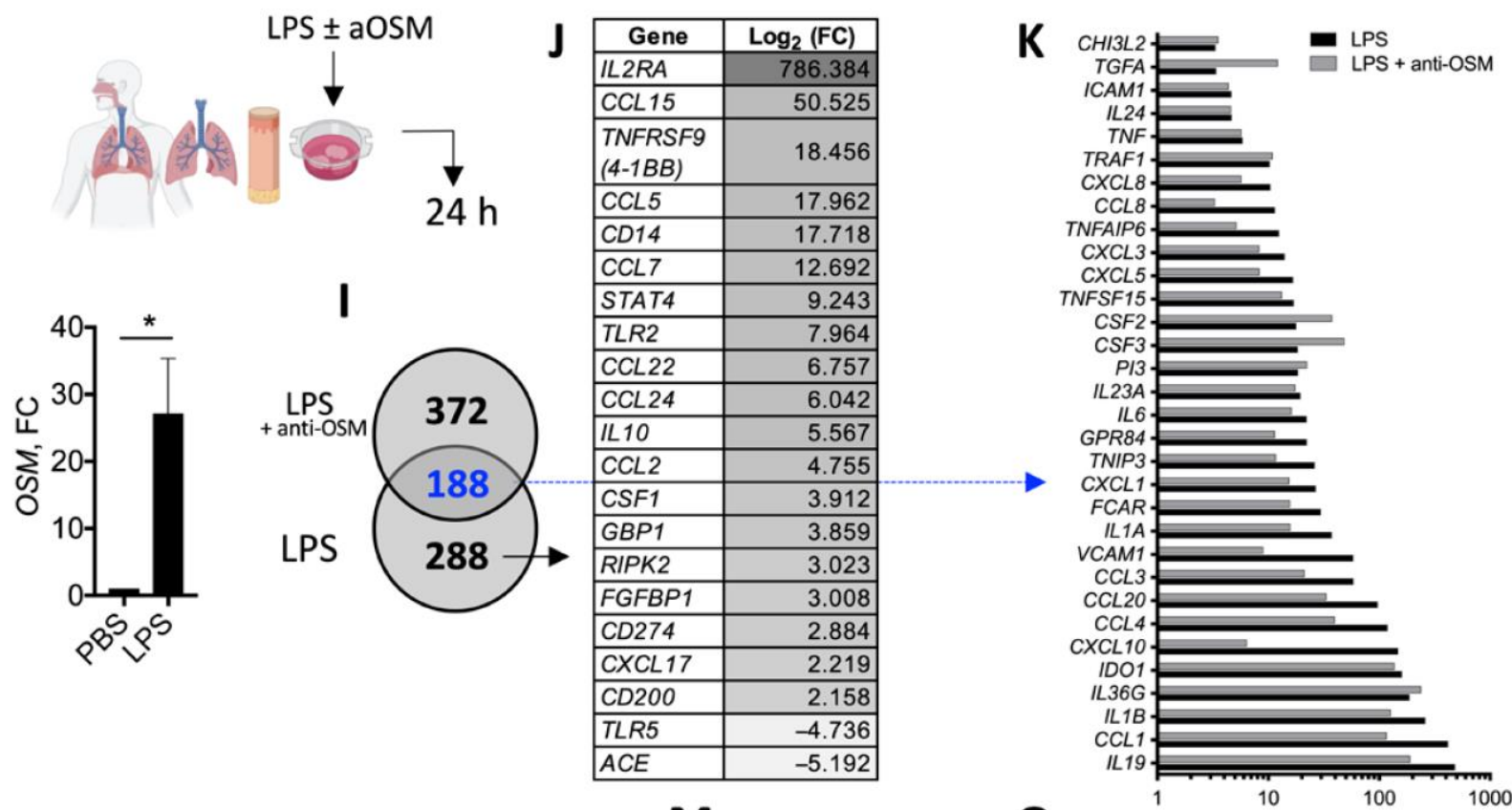
Oncostatin M: ASMR in Severe Asthma



Oncostatin M: ASMR in Severe Asthma

- OSM drives inflammatory and mucus pathways in human epcs
- OSM drives eosinophilic and neutrophilic inflammation in a murine HDM asthma model
- Deletion of OSM attenuates eosinophilic and neutrophilic inflammation in HDM asthma model
- Blocking Ab to OSM also attenuates inflammation

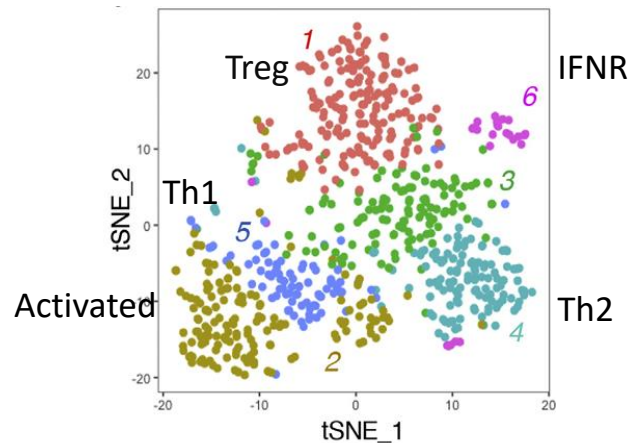
OSM neutralization prevents LPS-elicited chemokines in PCLS



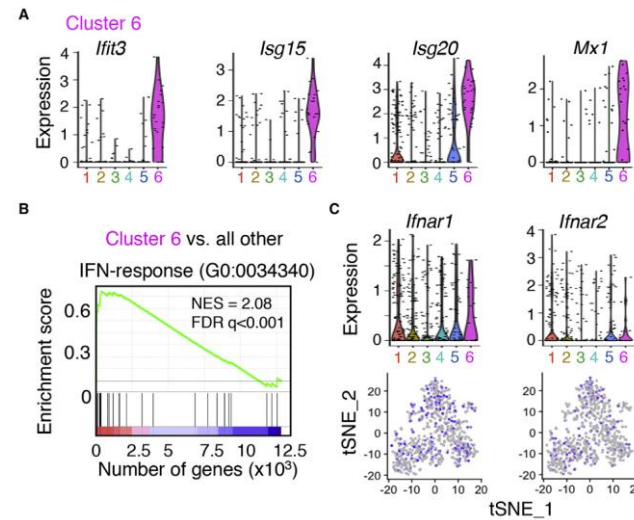
IFNR+ CD4+ T cells in HDM-elicited asthma



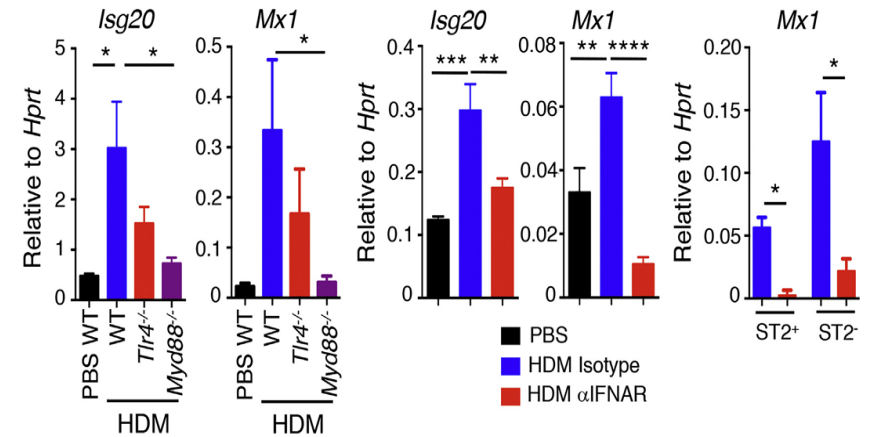
HDM-elicited CD4+ T cells



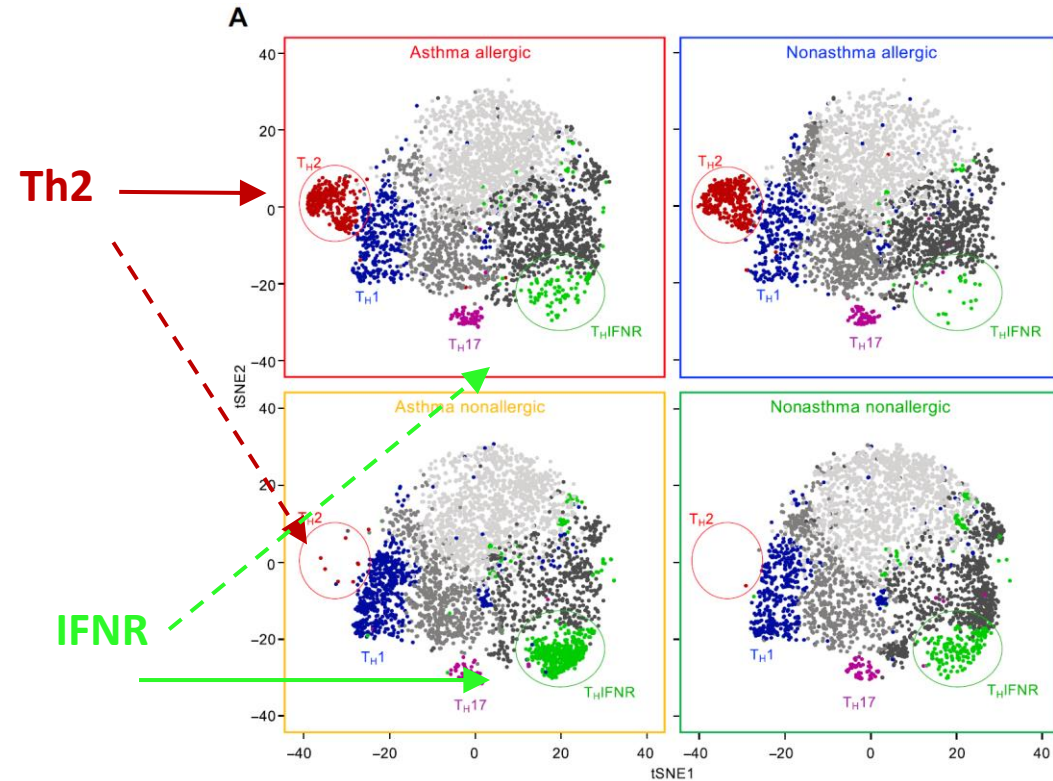
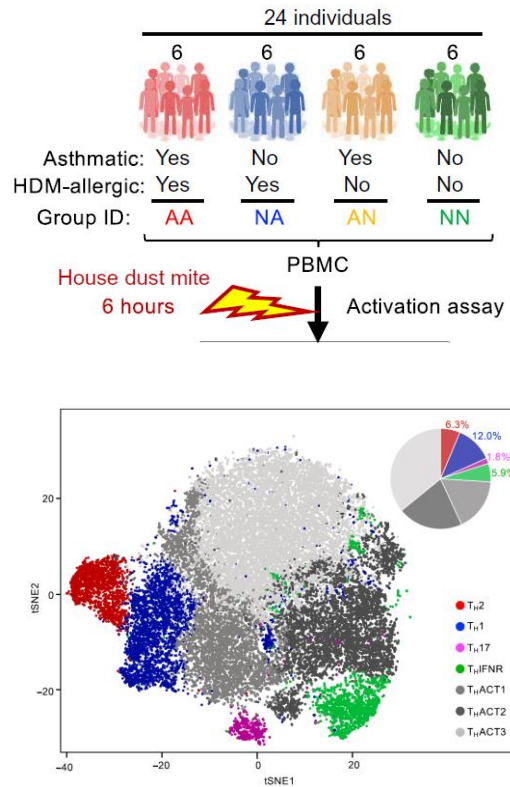
CD4+ T cell cluster expressing IFNR genes



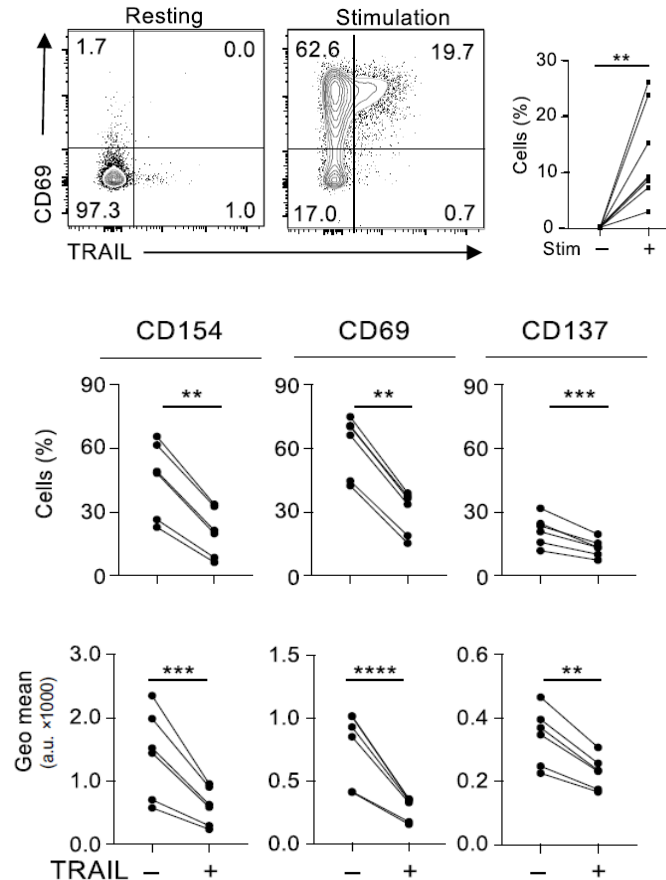
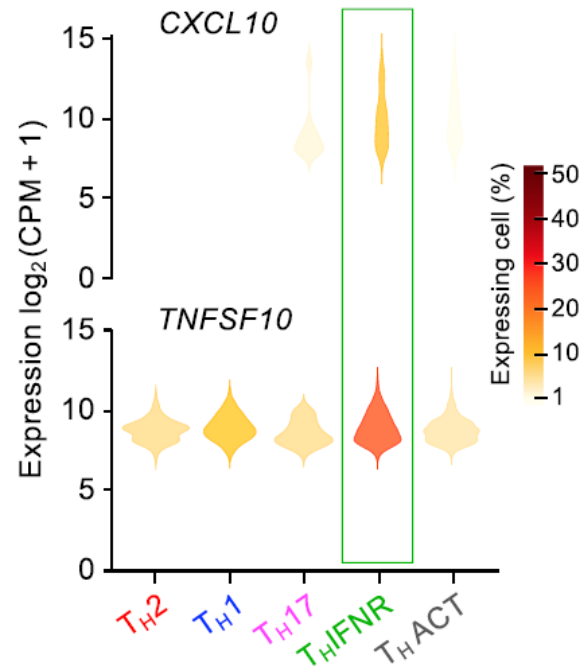
CD4+ T cell cluster is generated in response to IFNAR (binding type 1 interferons)



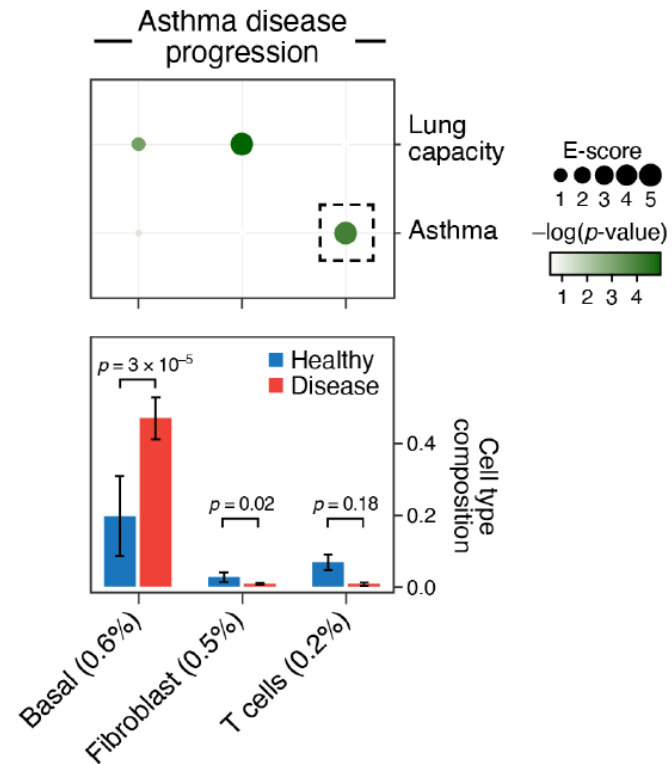
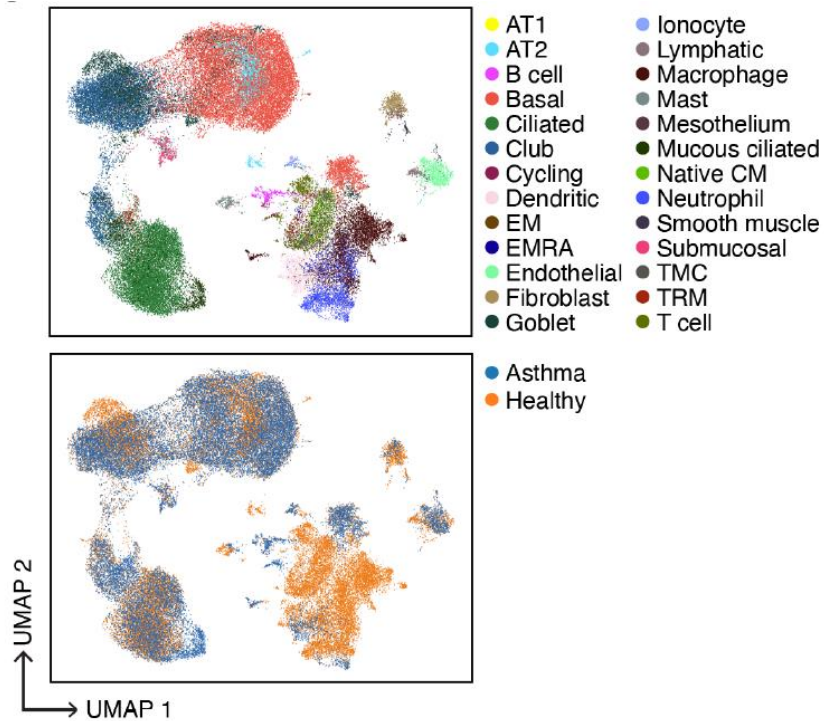
IFNR+ CD4+ T cells are detected in human PBMCs and reduced in patients with allergic asthma



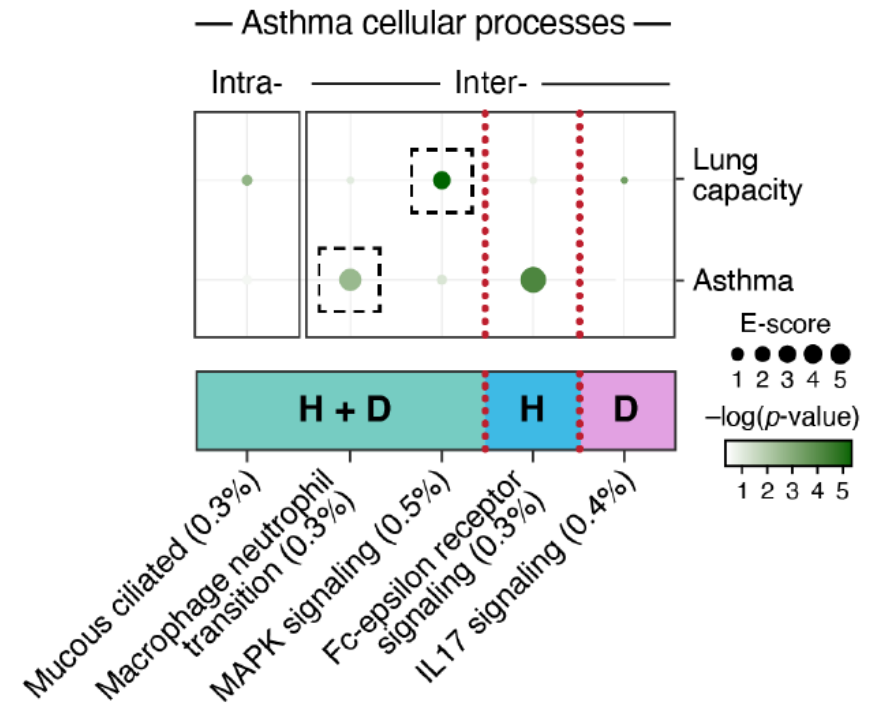
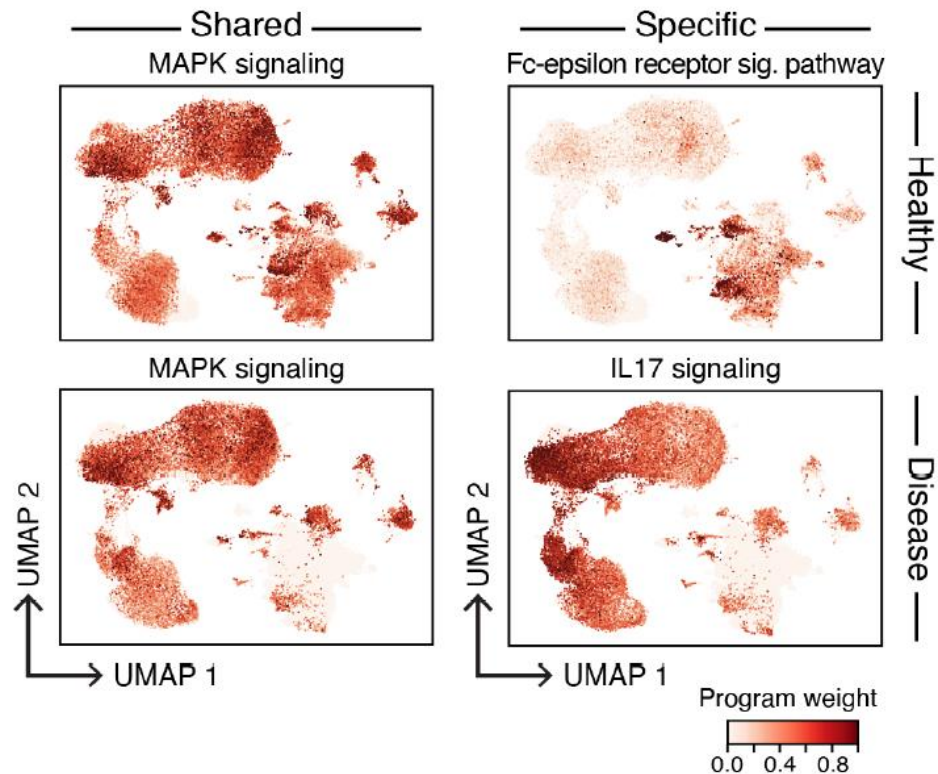
IFNR+ CD4+ T cells secrete immunoregulatory molecules such as Trail



Novel Methods to Identify Cell States Mediating Asthma and its Phenotypic Correlates



Novel Methods to Identify Cell States Mediating Asthma and its Phenotypic Correlates



Airway Transcriptomics in Asthma: Are We There Yet?

- Identify activated immunocytes from induced sputum and peripheral blood
- Identify structural alterations in bronchial brushings (epithelial and neural components). ASM is a big miss!
- IL18/IL18R and OSM/OSMR
- scRNA-seq can detect altered abundance, gene expression, and gene sets
- Novel methods integrating GWAS and scRNA-seq will allow scRNA-seq data to be used to characterize larger cohorts 'in silico'