

Rhinovirus and the T-Cell Landscape in Childhood Asthma

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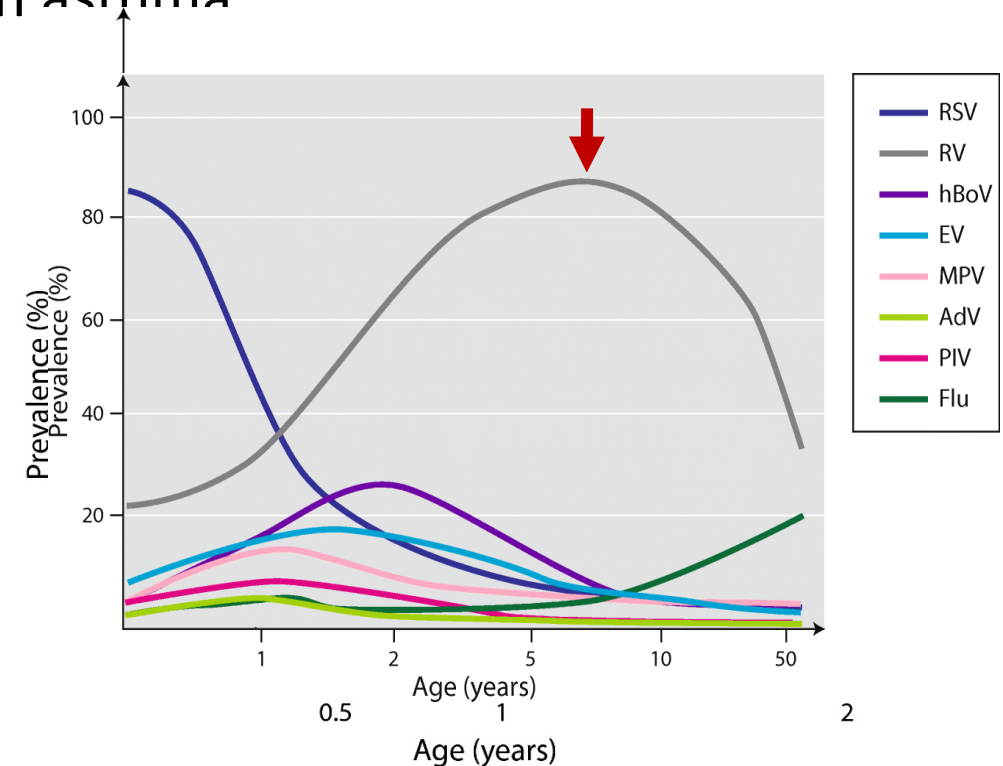
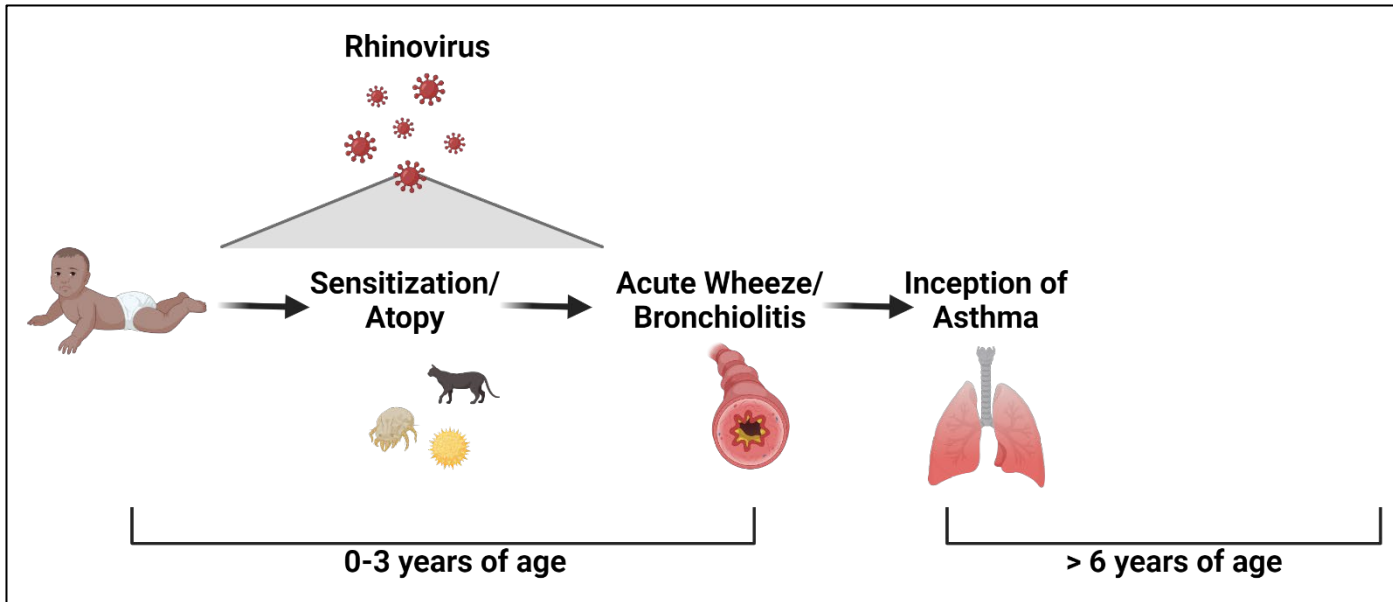
62nd Swineford Allergy Conference

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RV as a Cause and Exacerbator of Asthma

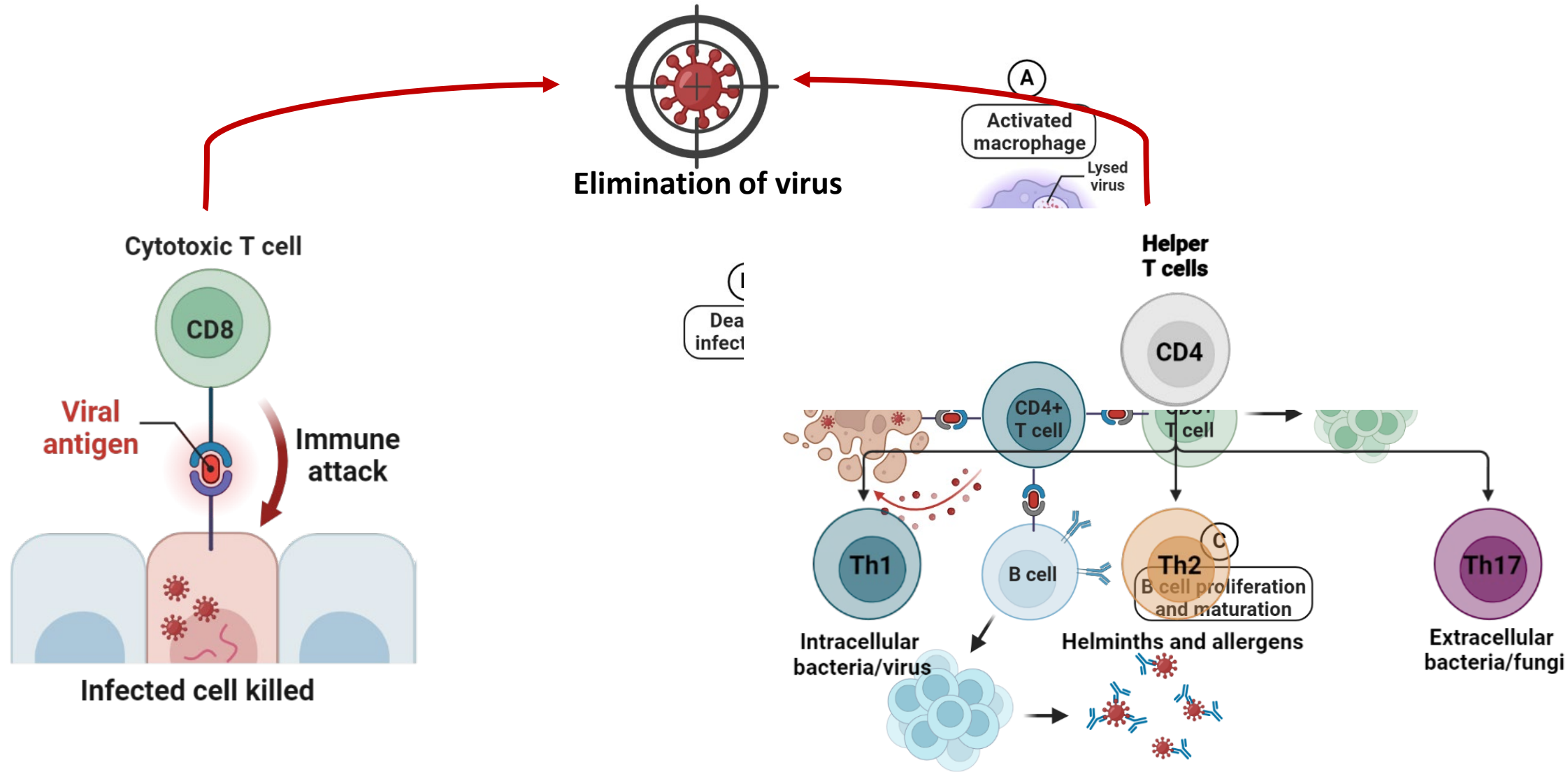
- Rhinovirus (RV) is the primary cause of the common cold
 - ~2 infections per year for children and ~1 for adults¹
- Implicated in the development of asthma²
 - Most common viral agent implicated in bronchiolitis after 1 year
- Potent trigger for acute wheezing episode in those with asthma²
 - Contributes to ~50-80% of exacerbations



[1] CDC, 2023; [2] Jackson and Gern, JACI In Practice, 2022

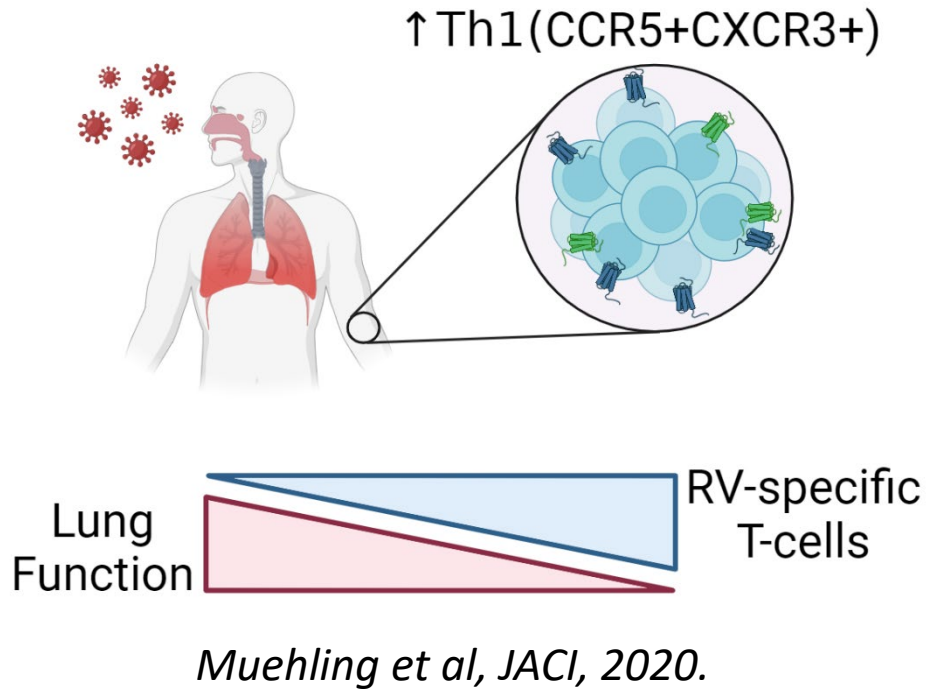
Jartti et al, *Scandinavian Journal of Allergy, 2028.*

Role of T Cells in Viral Infection

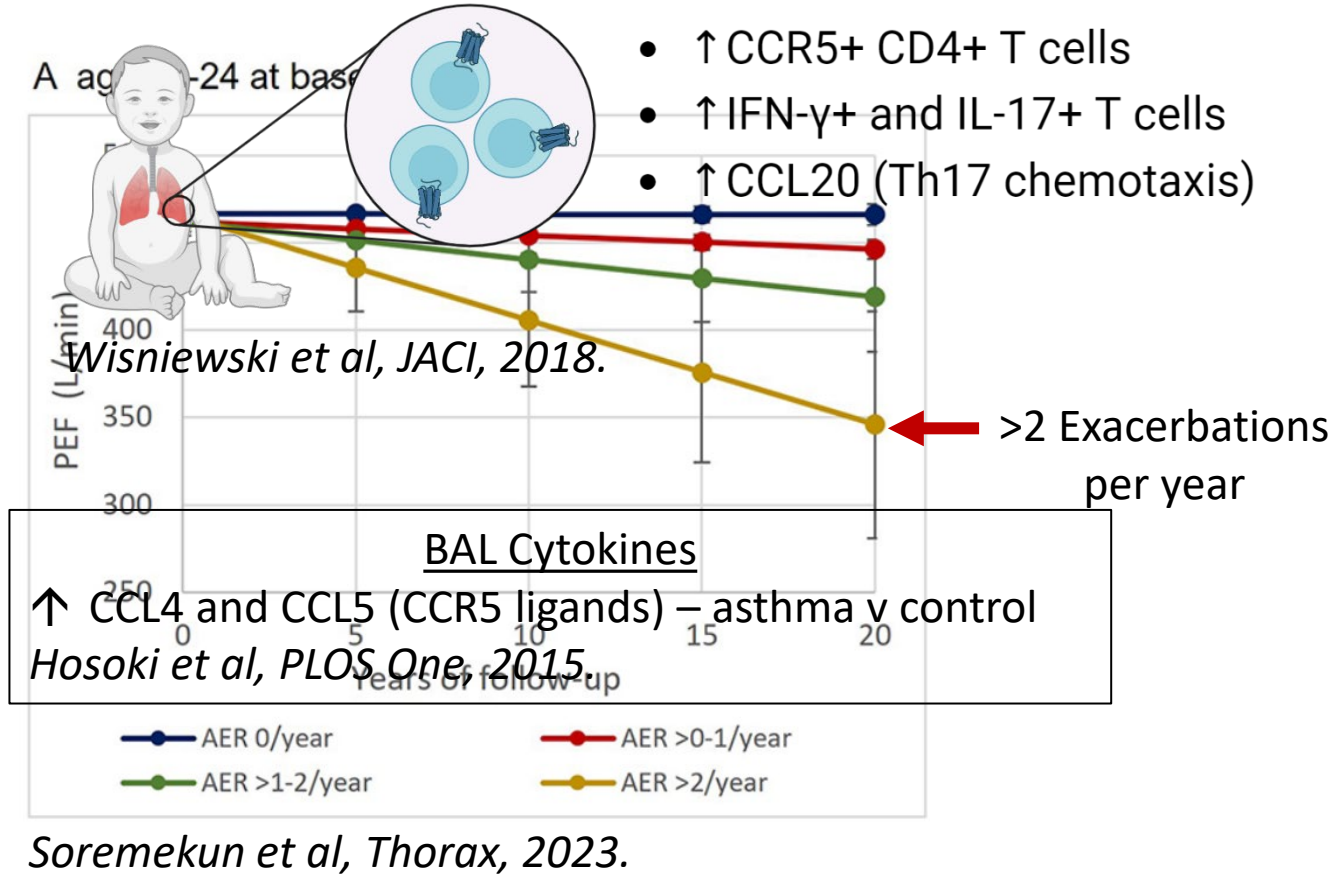


How Do Virus-specific T Cells Factor into Asthma Pathogenesis?

Adult Allergic Asthma

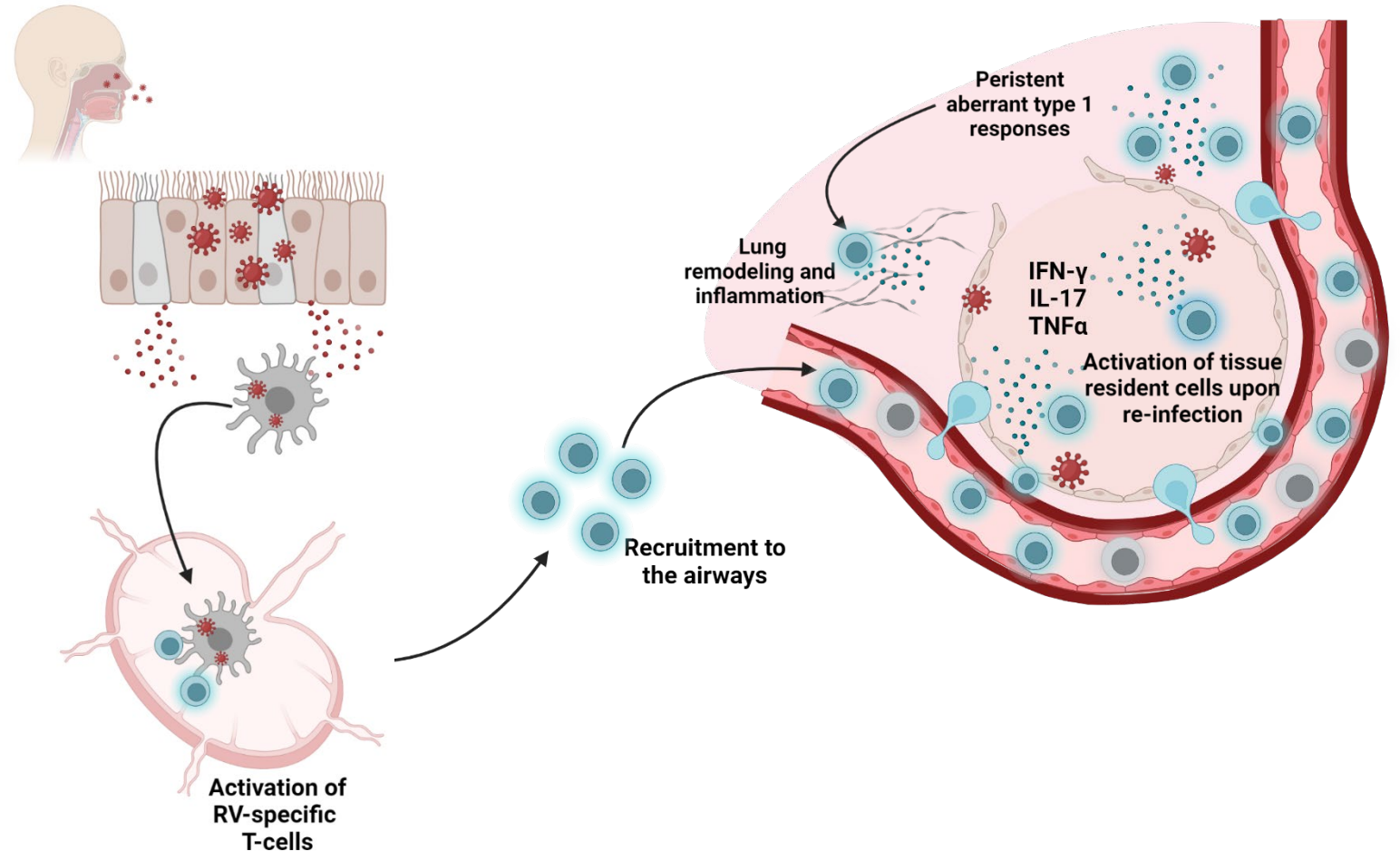


Pediatric Severe Asthma



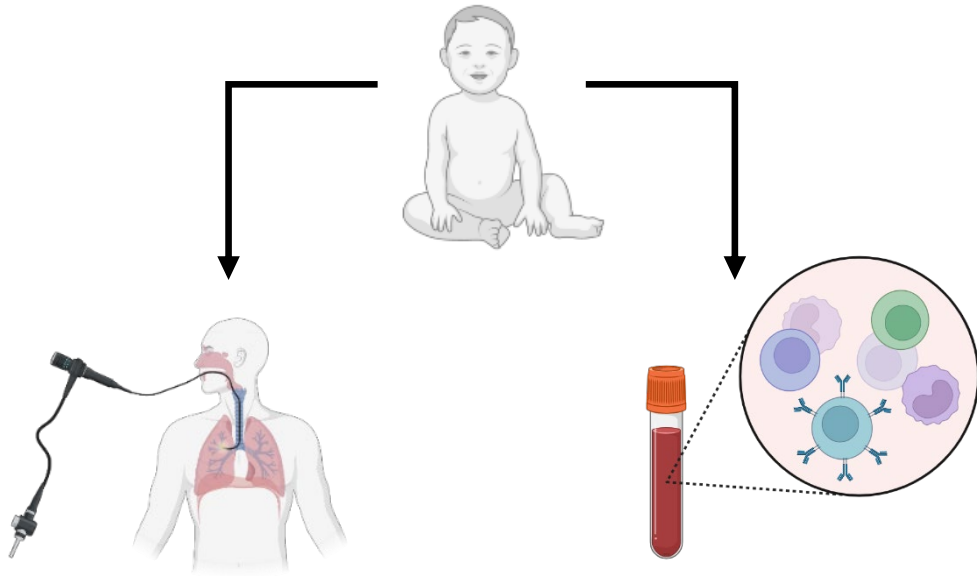
What Role Do RV-specific T Cells Play in Childhood Asthma?

- Hypothesis: RV-specific CD4+ T cells reside and persist in the lower airways of children with asthma, where they are poised to promote chronic inflammation.
- Limitations:
 - Accessibility of the lower airways
 - Difficulty in obtaining specimens
 - Limited cell numbers



Study Design

Children with treatment-refractory wheeze, ages 1-16 yrs. ($n=32$)



Treatment-refractory wheeze

Regional Referral of Children with Troublesome Cough/Wheeze/Croup

Specific Diagnosis and Guidelines-based Treatment

Clinically Indicated Bronchoscopy

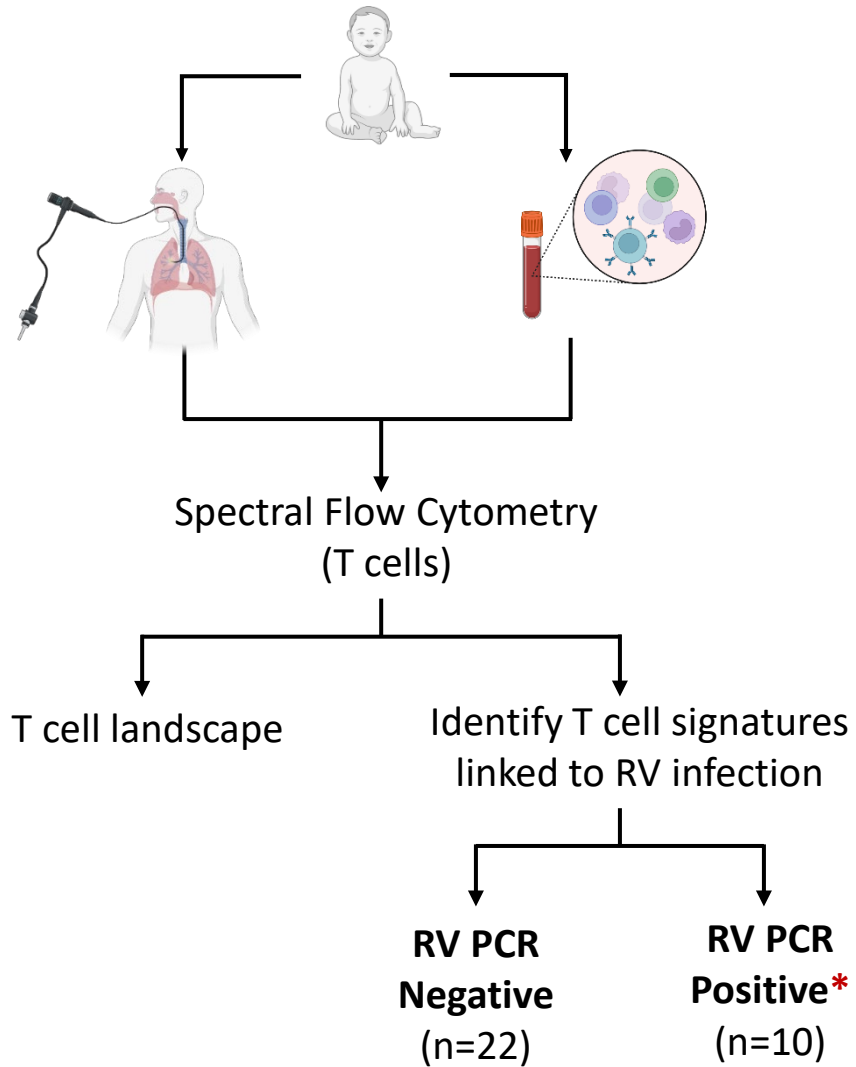
- 1) Could not attain symptom control
- 2) Recurrent healthcare utilization for wheeze despite appropriate treatment



W. Gerald Teague

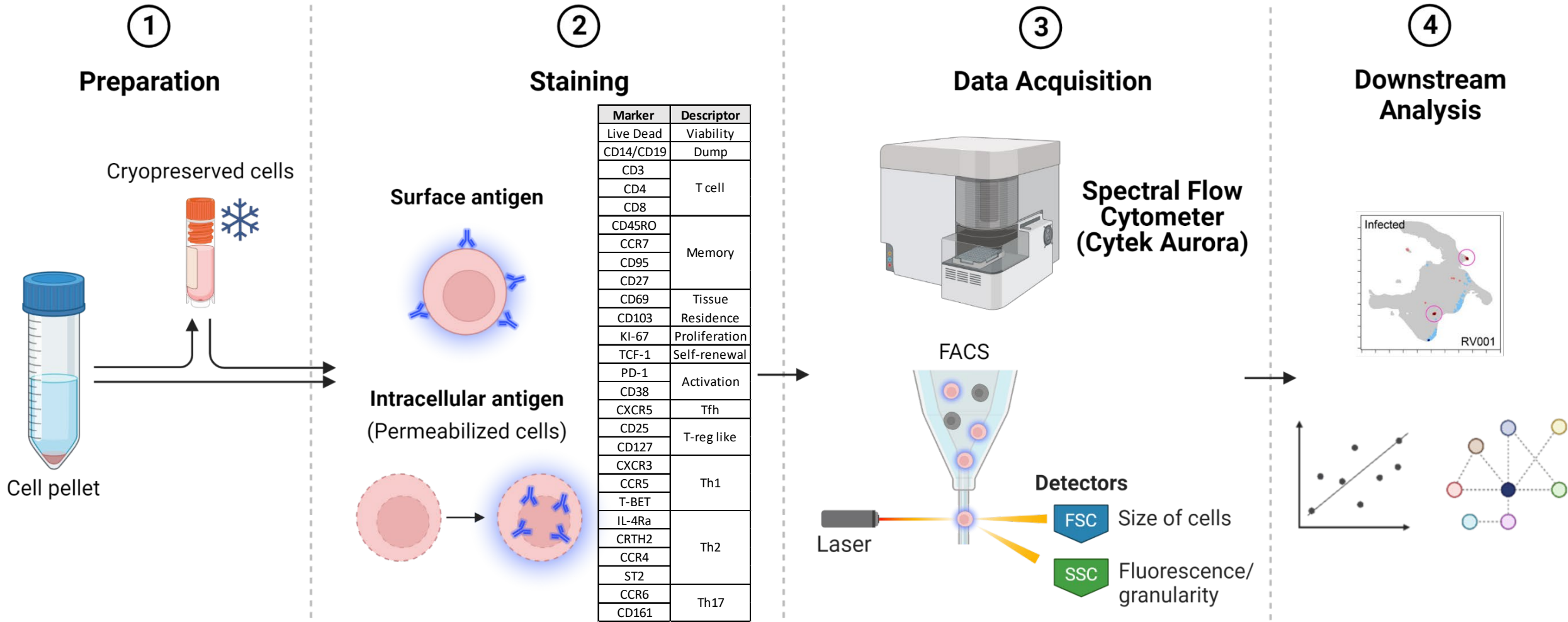
Study Design

Children with treatment-refractory wheeze, ages 1-16 yrs. (n=32)



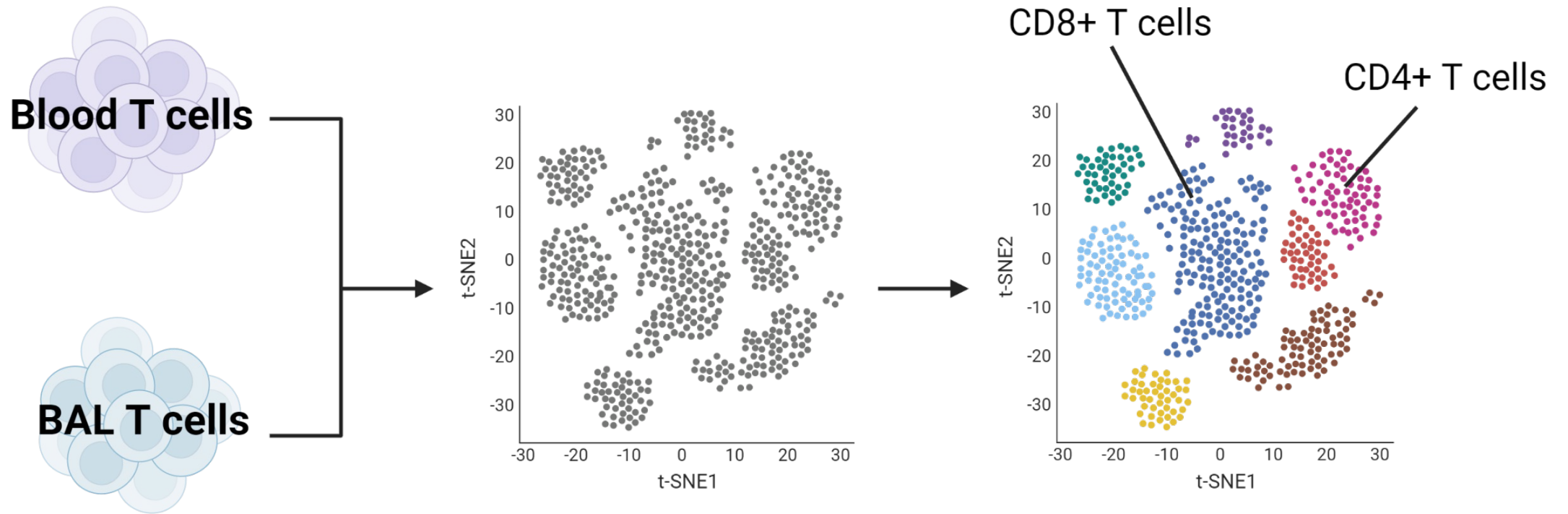
*Asymptomatic at time of bronchoscopy

High Throughput Workflow for Deep T Cell Profiling



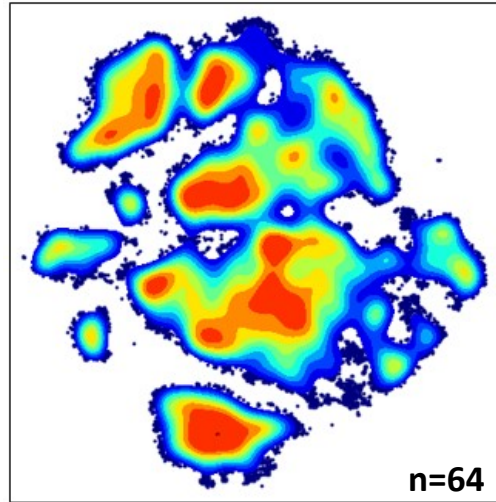
- High throughput with rigorous quality control
- Requires low cell numbers
- Identifies disease-relevant populations

Examining the T Cell Landscape in Treatment-refractory Wheeze

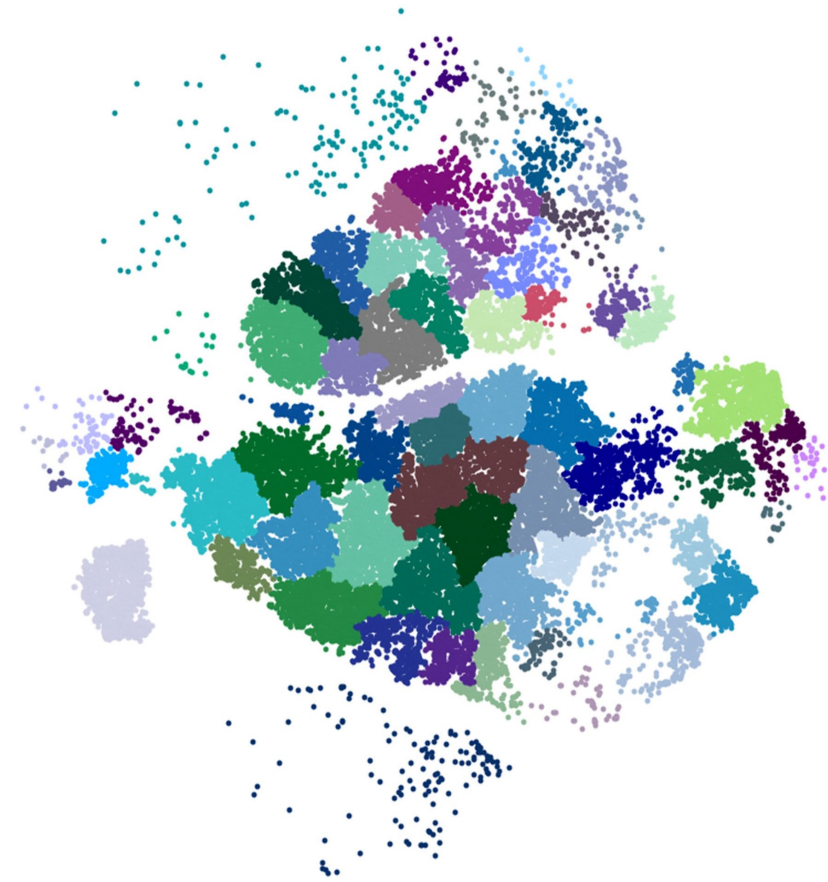


T Cell Landscape in the Lower Airways is Diverse

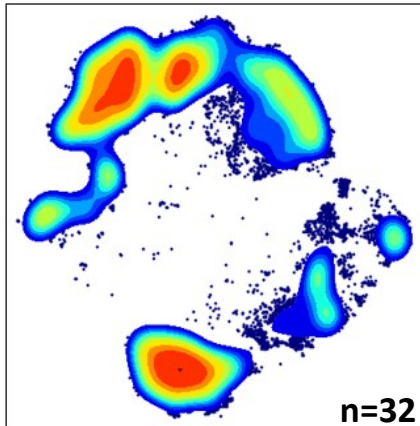
Blood & BAL



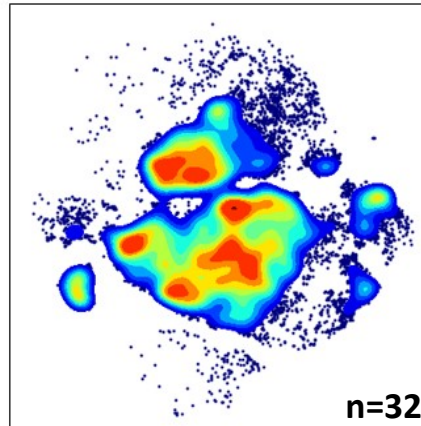
BAL



Blood



BAL

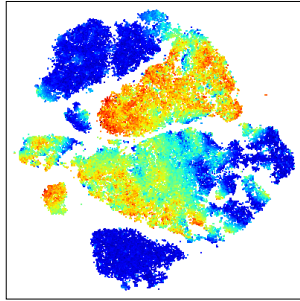
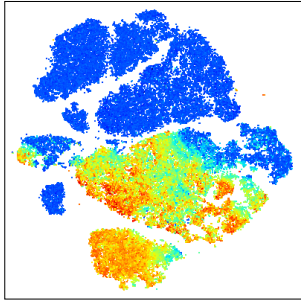
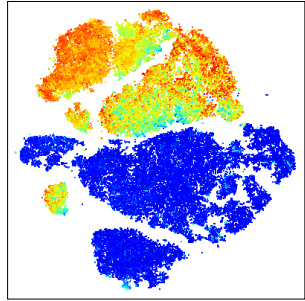


T Cell Landscape in the Lower Airways is Diverse

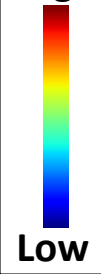
CD4

CD8

CD45RO



High

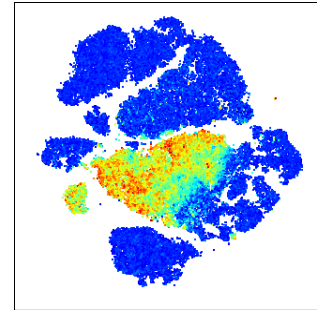
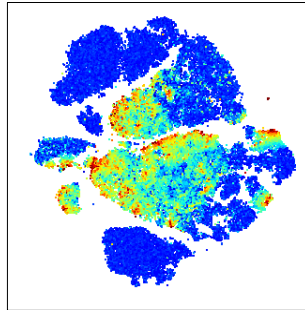


Low

Tissue Resident Memory (Trm)

CD69

CD103



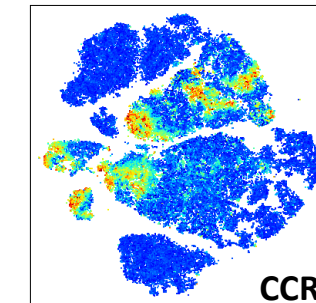
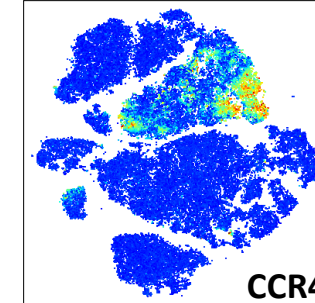
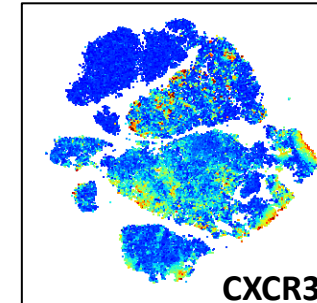
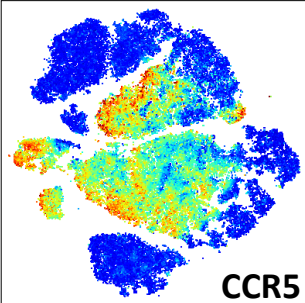
T Subsets

T1

T1

T2

T17



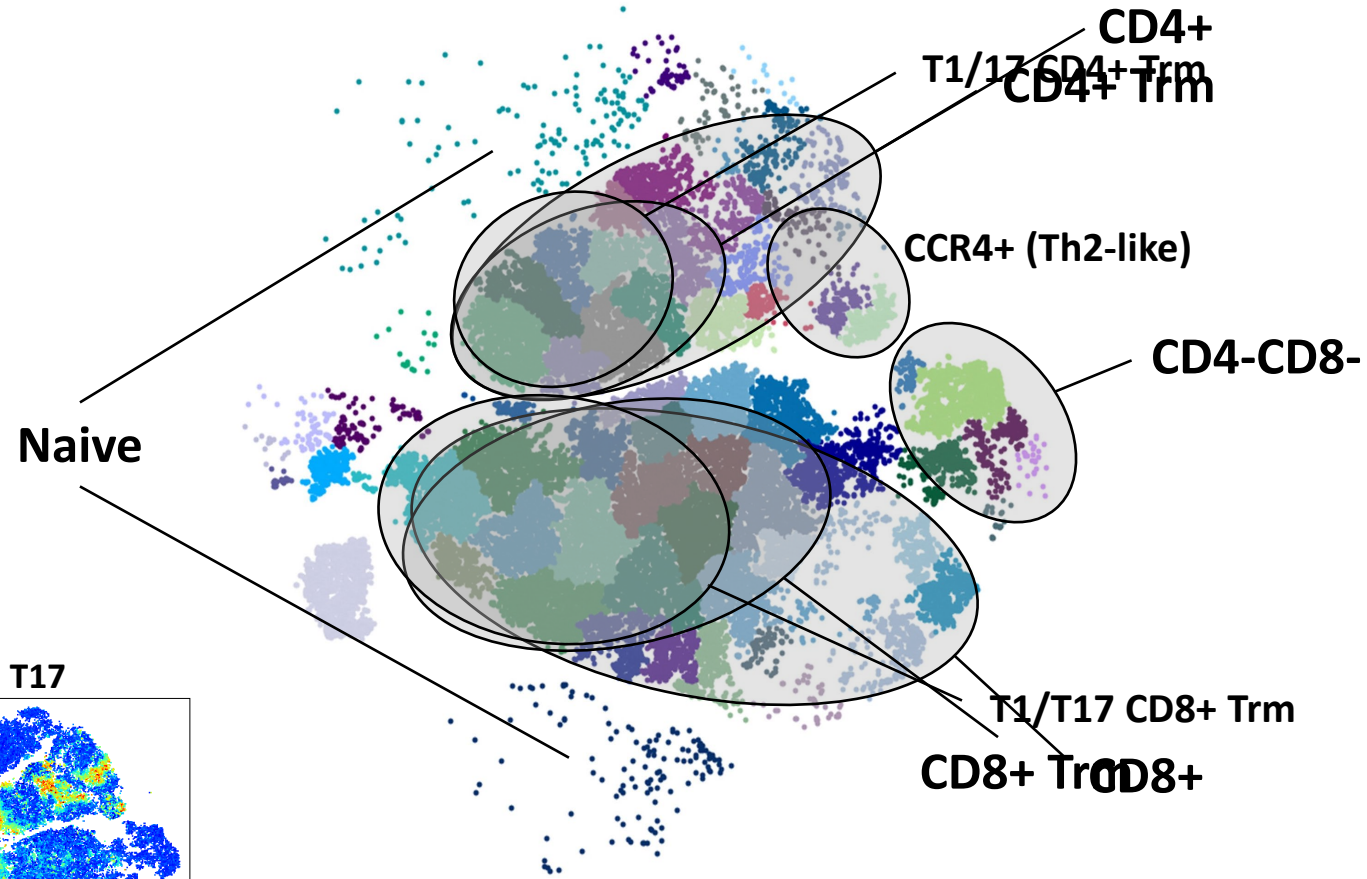
CCR5

CXCR3

CCR4

CCR6

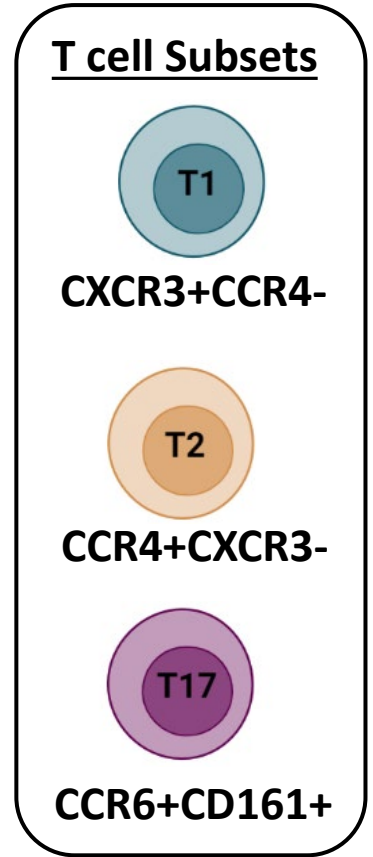
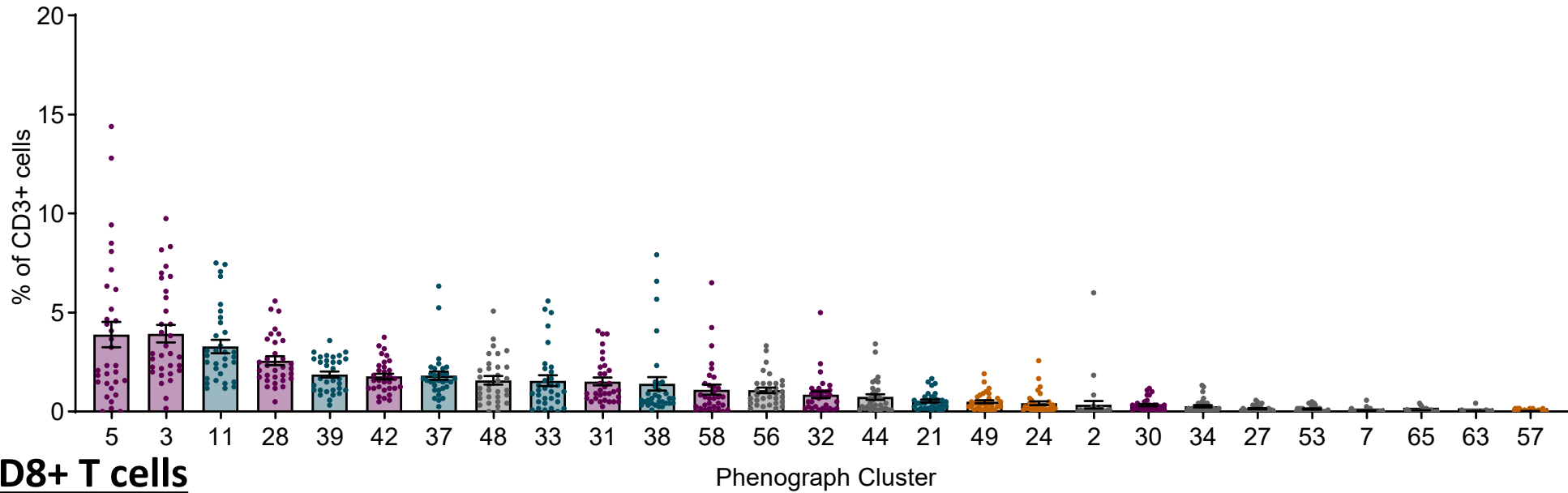
BAL



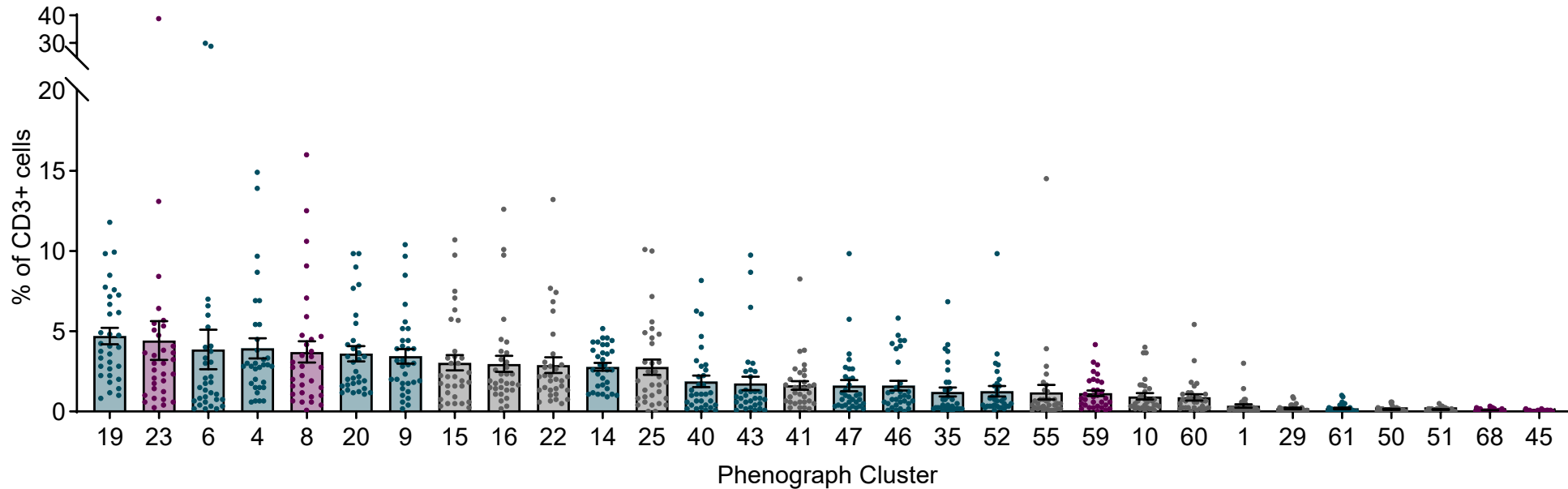
CD3+ Cells

Type 1 and 17 Cells are Enriched in the Lower Airways of Children with Wheeze

CD4+ T cells

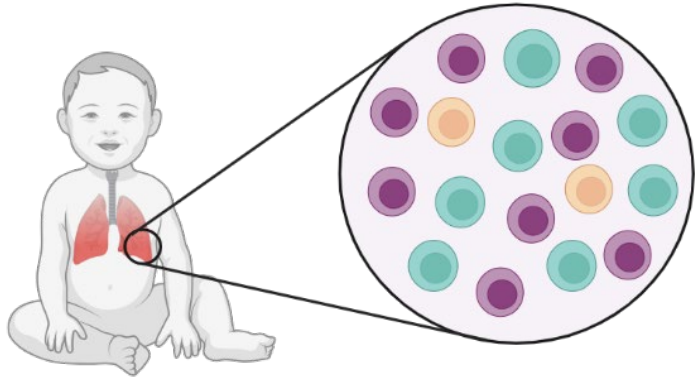


CD8+ T cells



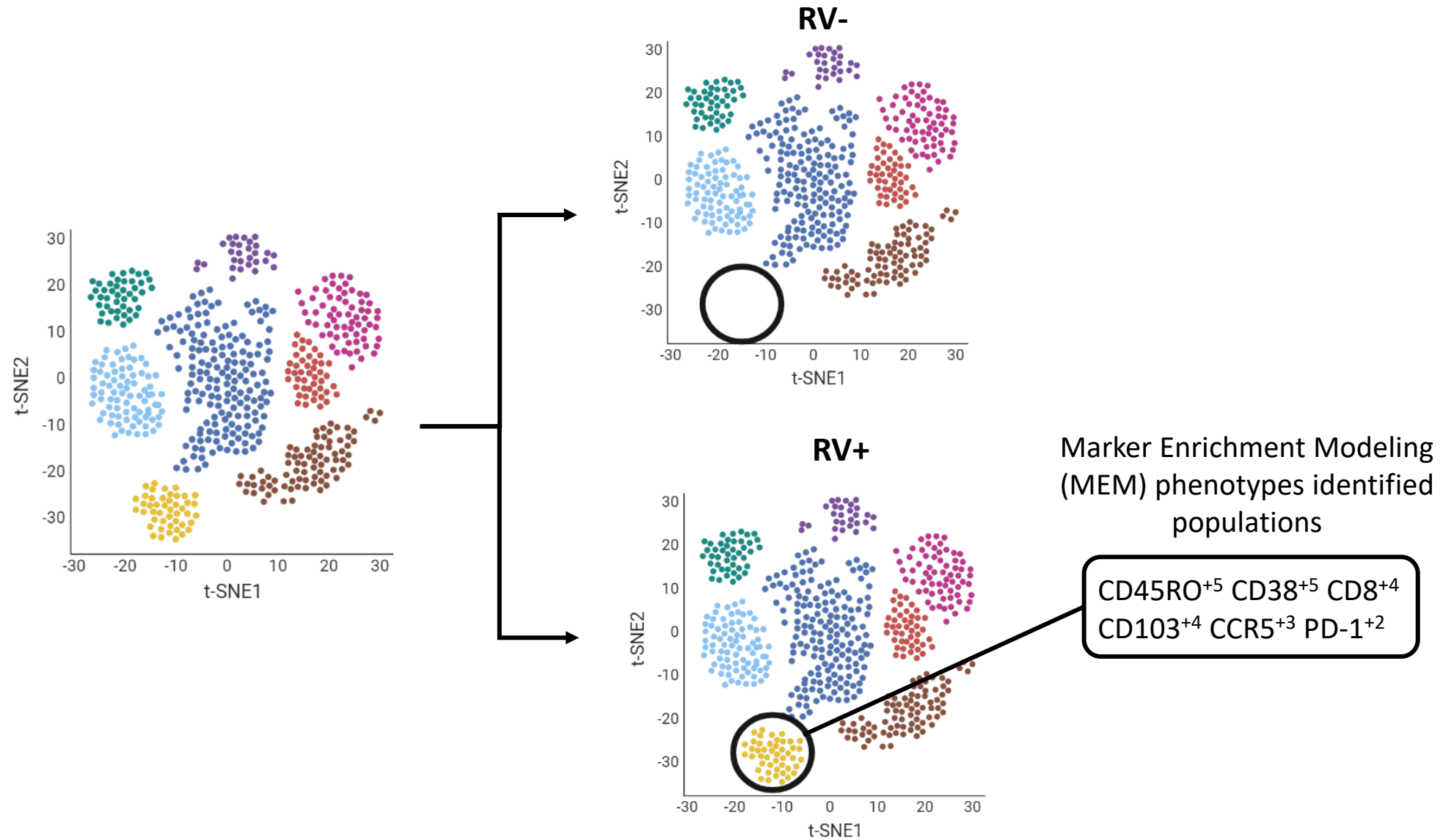
Summary

- The T cell landscape of the lower airways of children with treatment-refractory wheeze is highly complex and heterogeneous
 - \uparrow CCR5+ T cells, \uparrow CD8+ T cells, \uparrow Tissue-resident cells
 - T1/T17 > T2

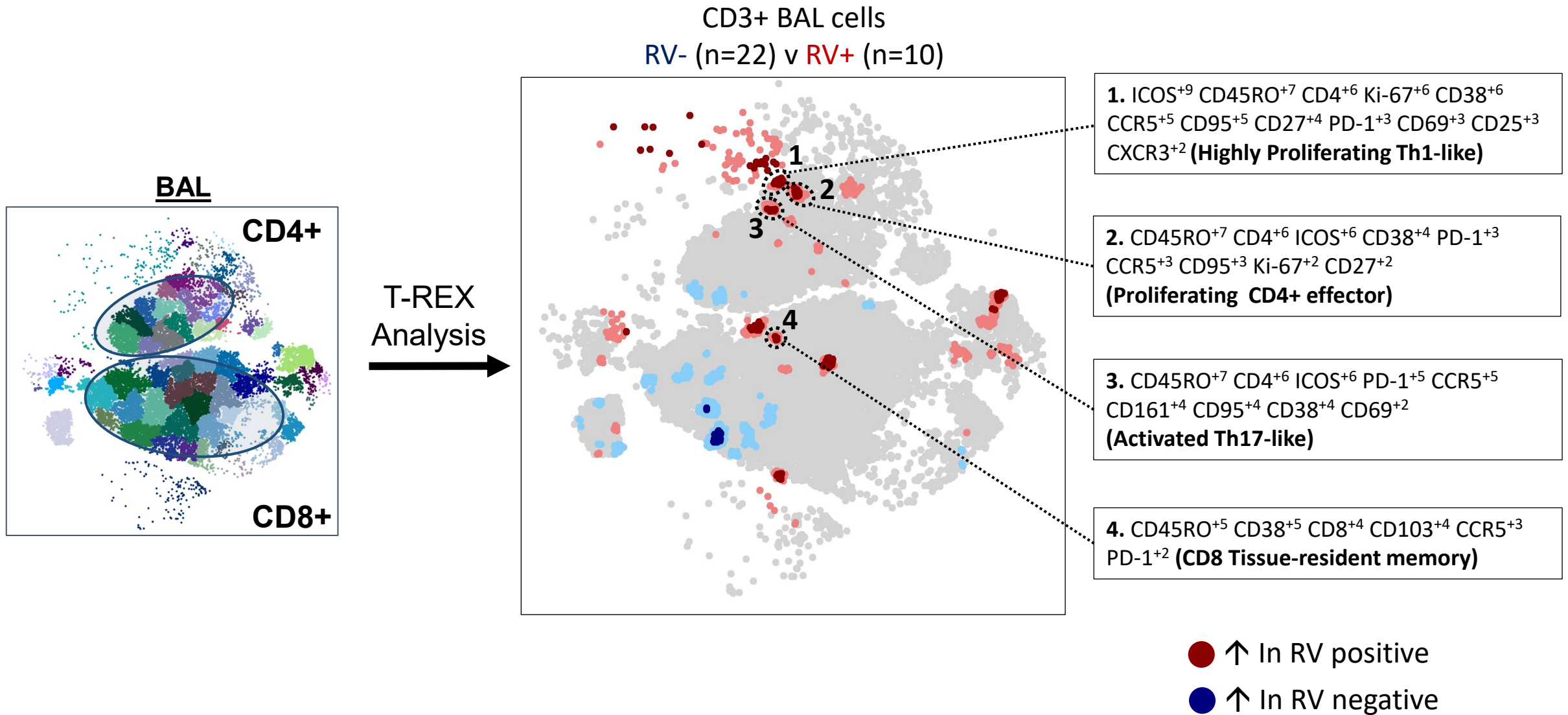


How Does RV Infection Impact the T Cell Landscape?

T-REX (Tracking Responders Expanding) Identifies Rare Virus-Responsive T Cells

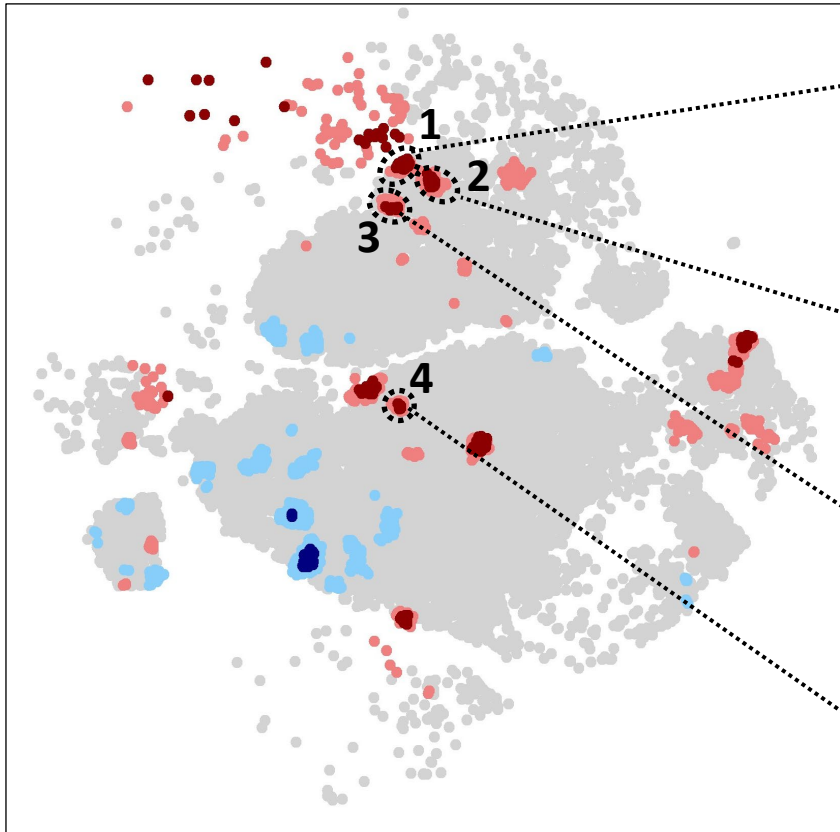


T-REX Identifies T Cell Signatures Unique to the Airways of RV+ Children



Activated Tissue Homing T Cells are Enriched in RV+ Children

CD3+ BAL cells
RV- (n=22) v RV+ (n=10)



1. ICOS⁺ CD45RO⁺ CD4⁺ Ki-67⁺
CD38⁺ CCR5⁺ CD95⁺ CD27⁺ PD-1⁺
CD69⁺ CD25⁺ CXCR3⁺ (Highly
Proliferating Th1-like)

2. CD45RO⁺ CD4⁺ ICOS⁺ CD38⁺ PD-1⁺
CCR5⁺ CD95⁺ Ki-67⁺ CD27⁺
(Proliferating CD4+ effector)

3. CD45RO⁺ CD4⁺ ICOS⁺ PD-1⁺ CCR5⁺
CD161⁺ CD95⁺ CD38⁺ CD69⁺
(Activated Th17-like)

4. CD45RO⁺ CD38⁺ CD8⁺ CD103⁺
CCR5⁺ PD-1⁺ (CD8 Tissue resident
memory)

Tissue homing

CCR5
CXCR3

Activation

PD-1
CD38
CD25

Tissue Residence

CD69
CD103

Proliferation

Ki-67

IL-17 Production

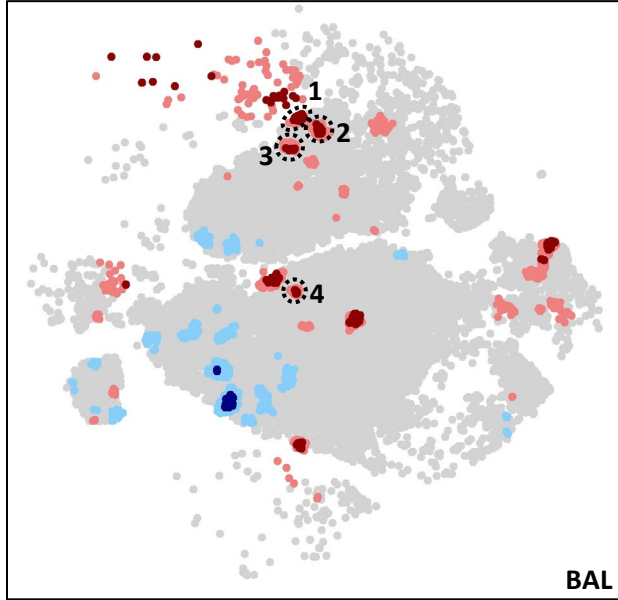
CD161

● ↑ In RV positive

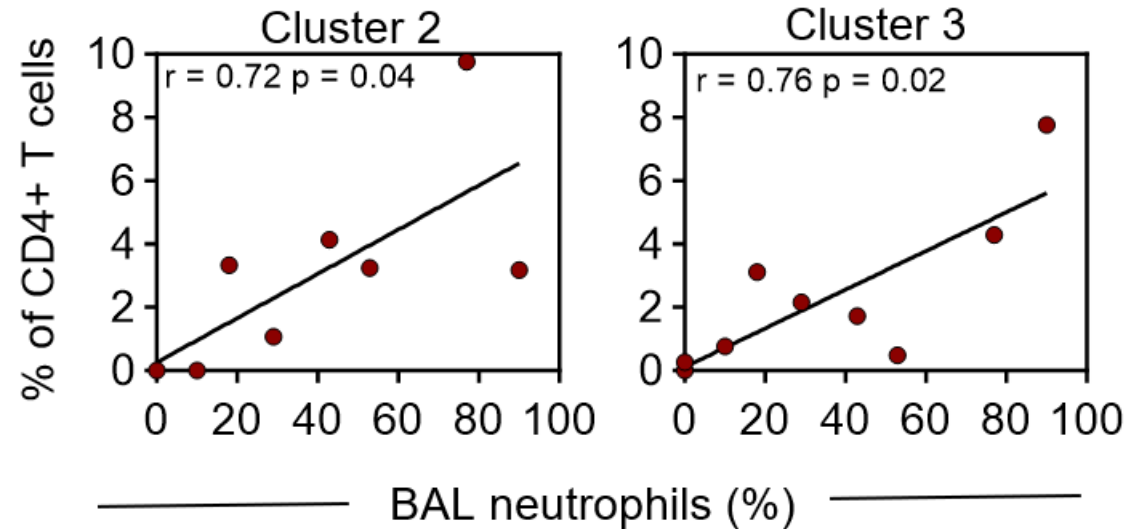
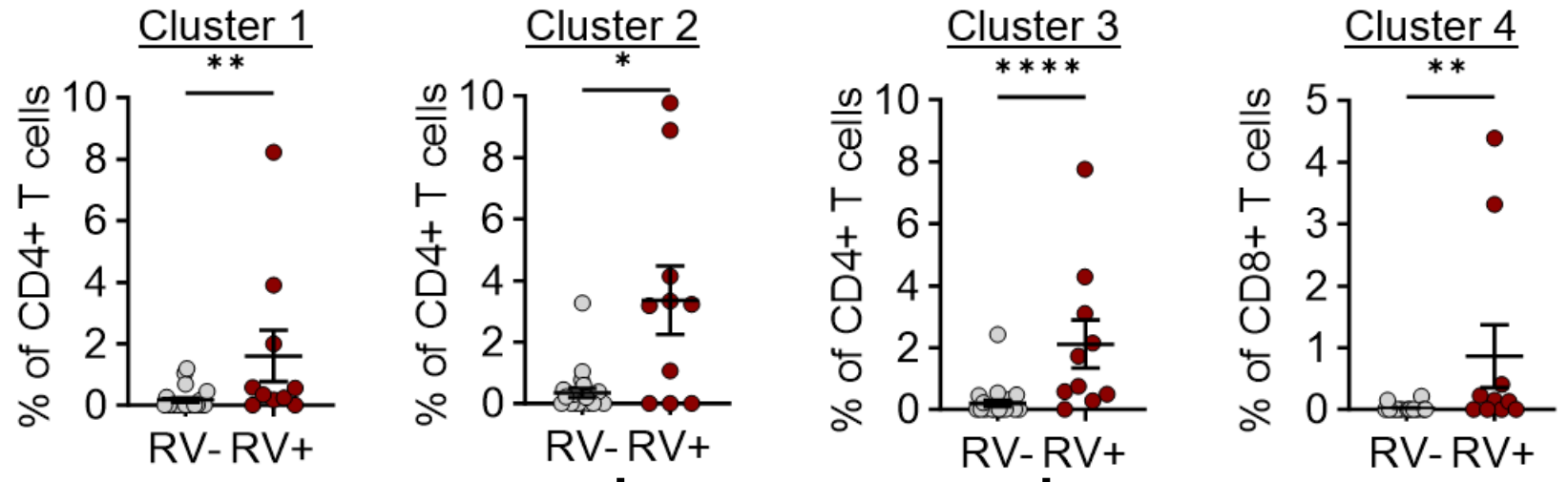
● ↑ In RV negative

T Cell Frequencies Correlate with Airway Neutrophils in RV+ Children

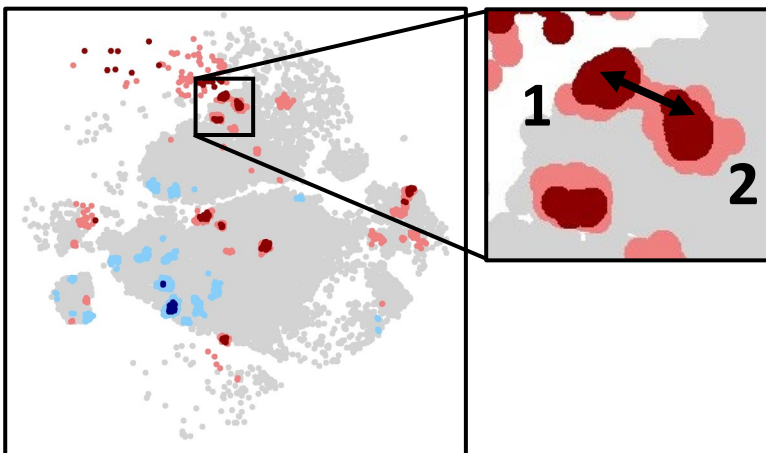
RV- (n=22) v RV+ (n=10)



- 1 Highly Proliferating Th1-like
- 2 Proliferating CD4+ effector
- 3 Activated Th17-like
- 4 CD8+ Tissue-resident memory

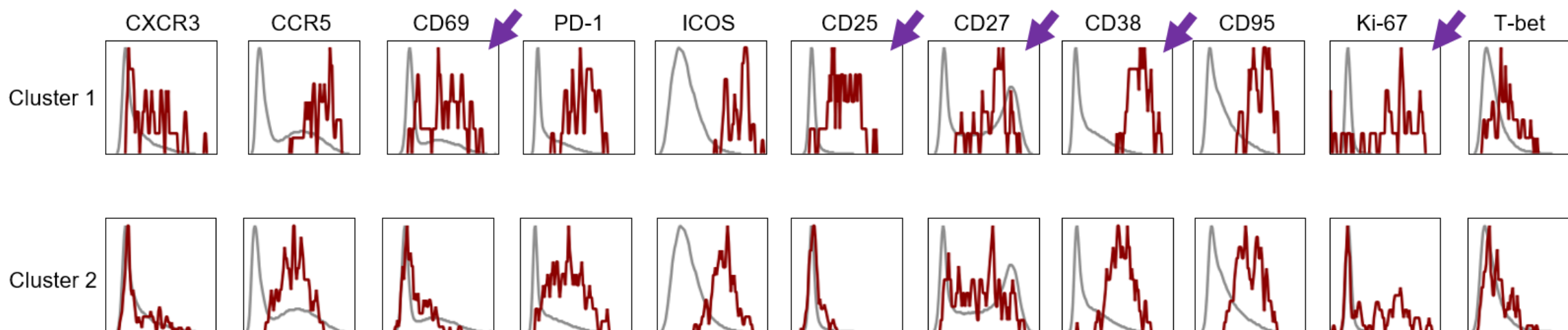


Evidence of a Dynamic T Cell Environment



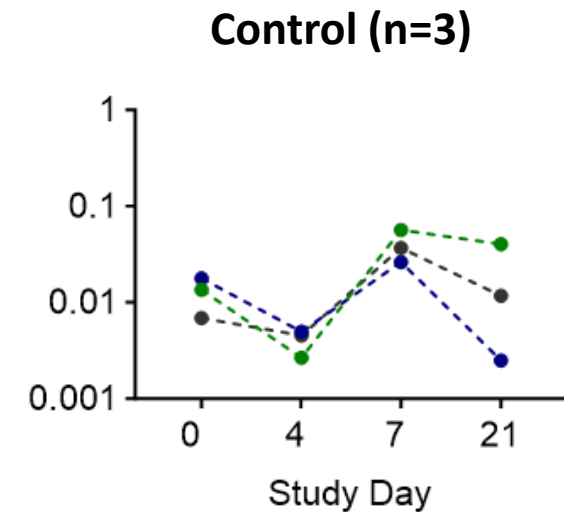
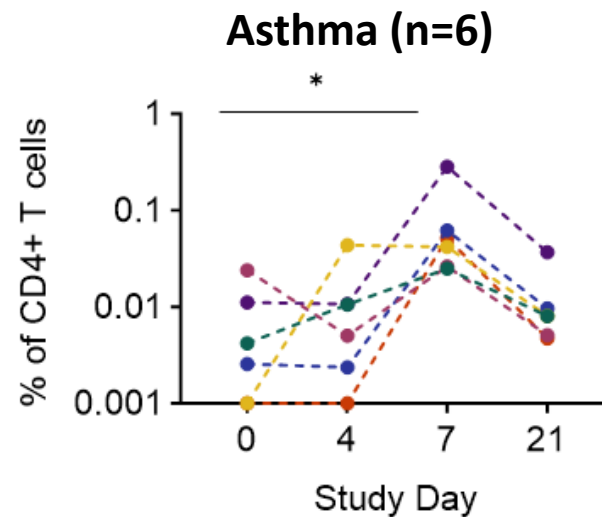
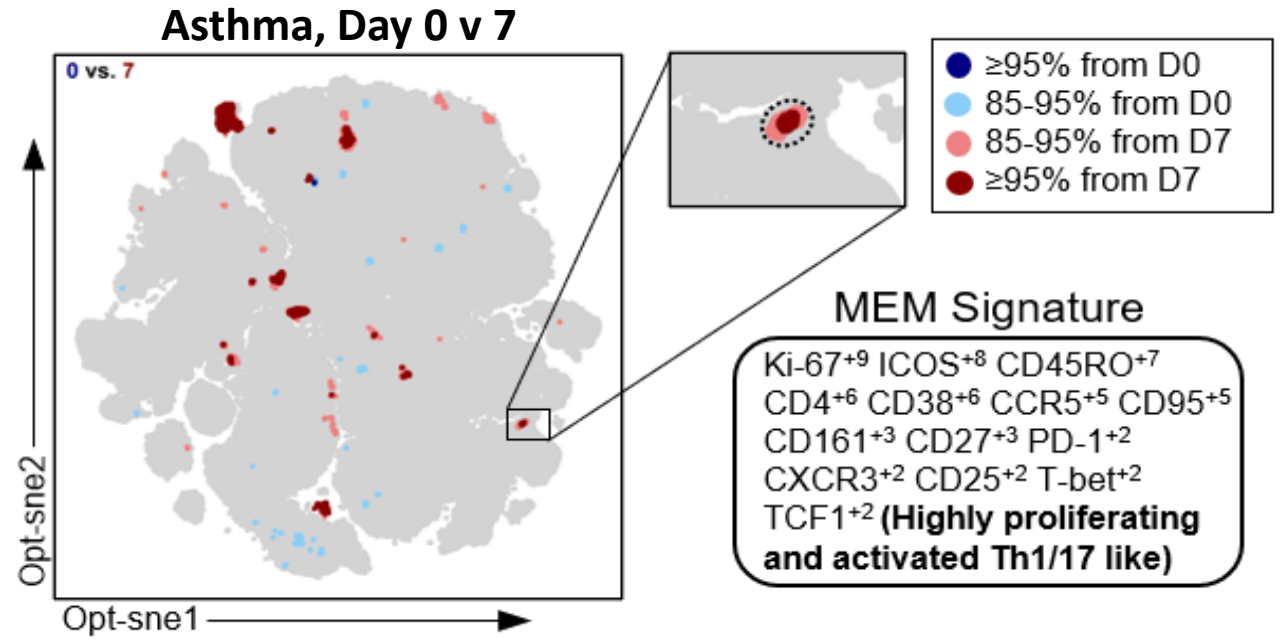
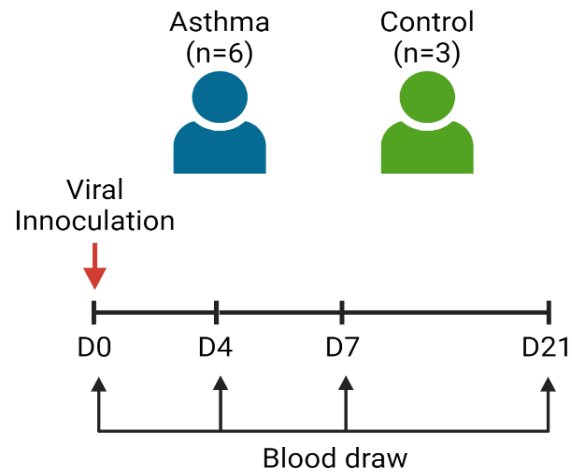
1 ICOS⁺⁹ CD45RO⁺⁷ CD4⁺⁶ Ki-67⁺⁶ CD38⁺⁶ CCR5⁺⁵ CD95⁺⁵ CD27⁺⁴ PD-1⁺³ CD69⁺³
CD25⁺³ CXCR3⁺² (**Highly - proliferating Th1-like**)

2 CD45RO⁺⁷ CD4⁺⁶ ICOS⁺⁶ CD38⁺⁴ PD-1⁺³ CCR5⁺³ CD95⁺³ Ki-67⁺² CD27⁺²
(**Proliferating CD4+ effector**)



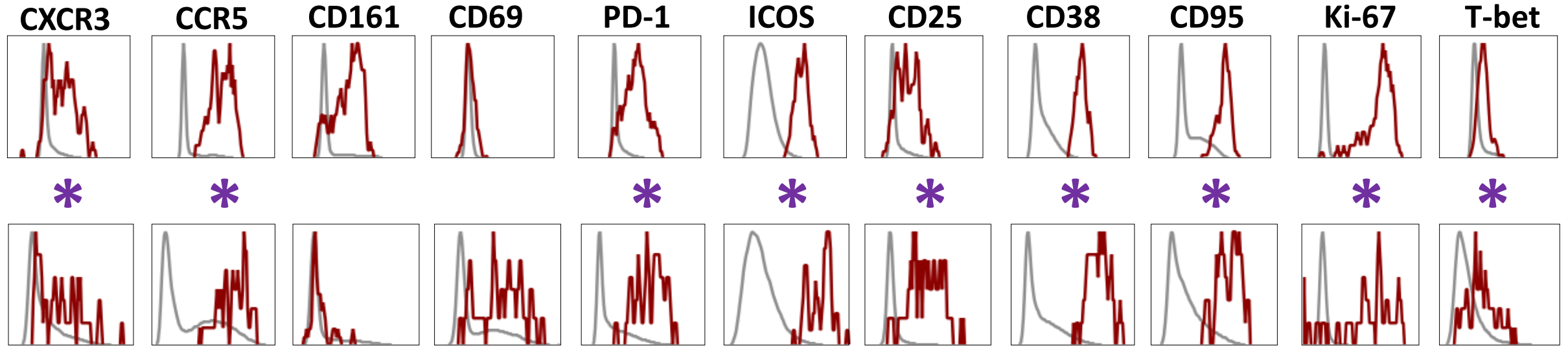
□ Total cells
■ Cluster

RV Challenge Identifies Hallmarks of Infection



RV-Responsive T Cells Mirror Those Found in RV+ Children

Adult RV Challenge: Ki-67⁺⁹ ICOS⁺⁸ CD45RO⁺⁷ CD4⁺⁶ CD38⁺⁶ CCR5⁺⁵ CD95⁺⁵ CD161⁺³ CD27⁺³ PD-1⁺² CXCR3⁺²
CD25⁺² T-bet⁺² TCF1⁺² (Highly Proliferating and activated Th1/17-like)



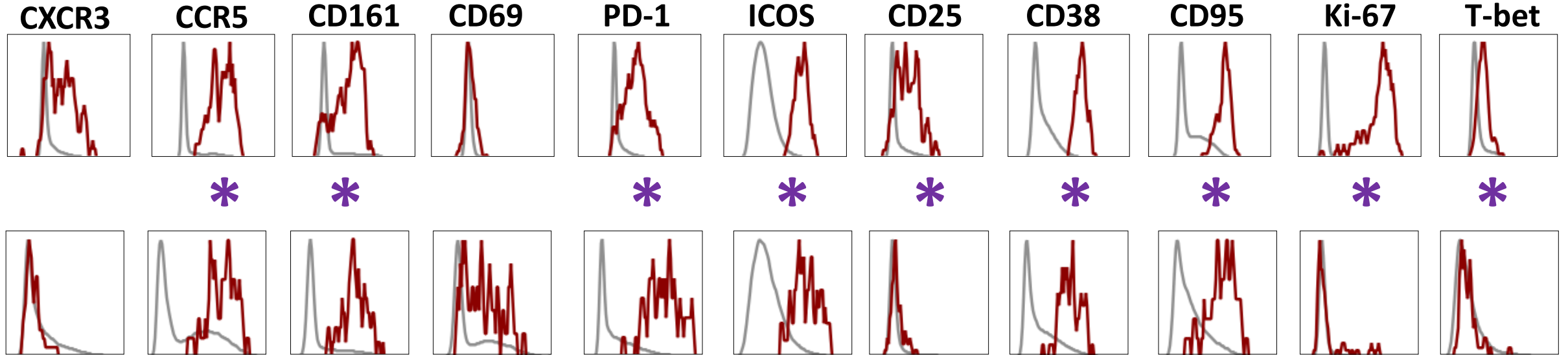
Pediatric BAL #1: ICOS⁺⁹ CD45RO⁺⁷ CD4⁺⁶ Ki-67⁺⁶ CD38⁺⁶ CCR5⁺⁵ CD95⁺⁵ CD27⁺⁴ PD-1⁺³ CD69⁺³
CD25⁺³ CXCR3⁺² (Highly Proliferating Th1-like)

Percent Similarity = 89.6

Legend:
Total cells (grey line)
Cluster (red line)

RV-Responsive T Cells Mirror Those Found in RV+ Children

Adult RV Challenge: Ki-67⁺⁹ ICOS⁺⁸ CD45RO⁺⁷ CD4⁺⁶ CD38⁺⁶ CCR5⁺⁵ CD95⁺⁵ CD161⁺³ CD27⁺³ PD-1⁺² CXCR3⁺²
CD25⁺² T-bet⁺² TCF1⁺² (Highly Proliferating and activated Th1/17-like)



Pediatric BAL #3: CD45RO⁺⁷ CD4⁺⁶ ICOS⁺⁶ PD-1⁺⁵ CCR5⁺⁵ CD161⁺⁴ CD95⁺⁴ CD38⁺⁴ CD69⁺²
(Activated Th17-like) RMSD=86.9

Percent Similarity = 86.9

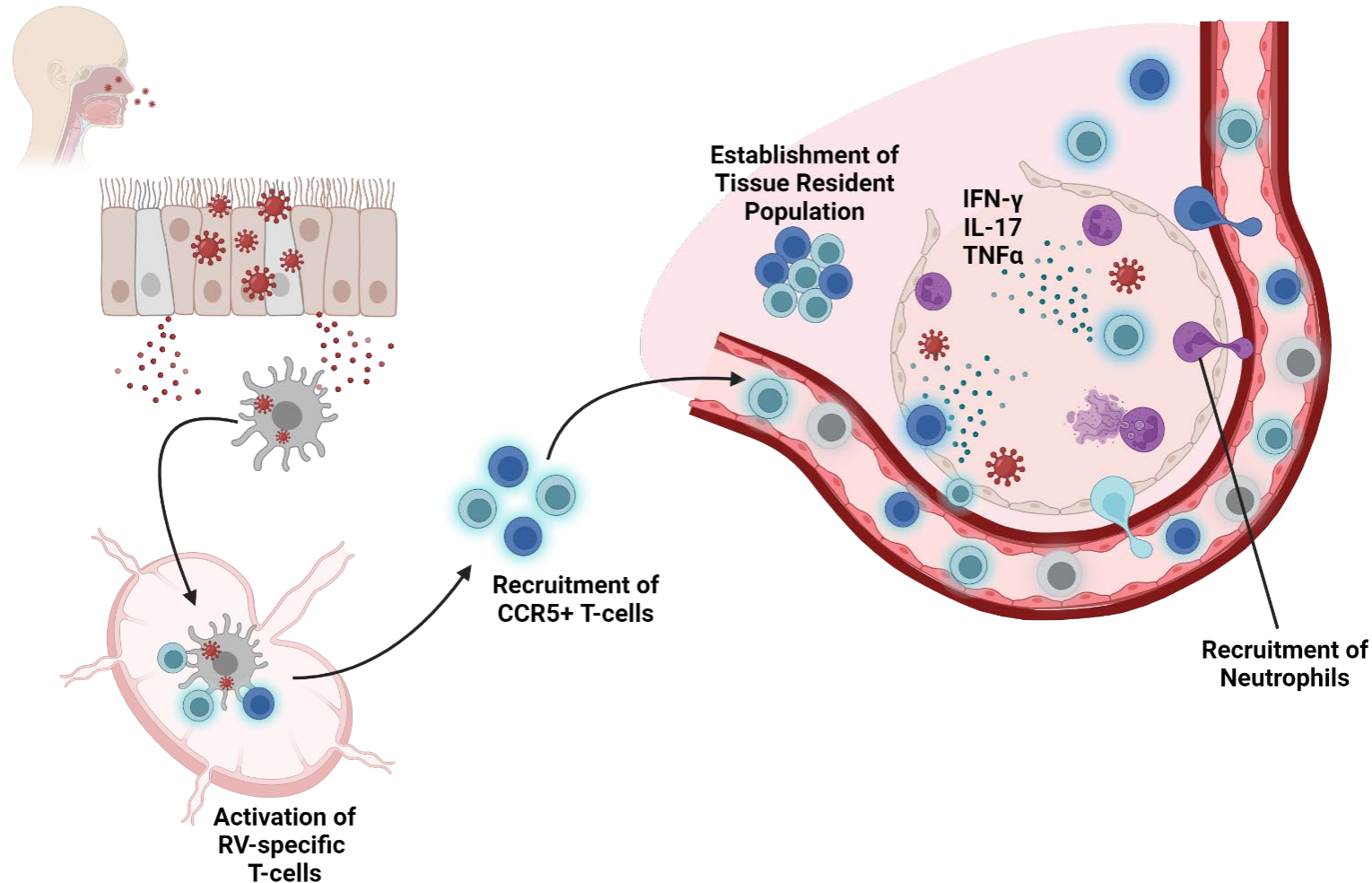
Legend:
Total cells (grey line)
Cluster (red line)

Summary

- Unsupervised machine learning tools provide a precise tool for identifying putative RV-specific T cells
- T cells enriched in the airways of RV+ children display RV-responsive hallmarks

Conclusion

- In children with treatment-refractory wheeze, RV-responsive CD4+ T cells reside within the lower airways, where they are poised to exert their pathogenic function in a dynamic T cell environment



Acknowledgments



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