

# Transcriptomic characterization of metabolic competency in reconstructed human respiratory epithelium (RHRE)

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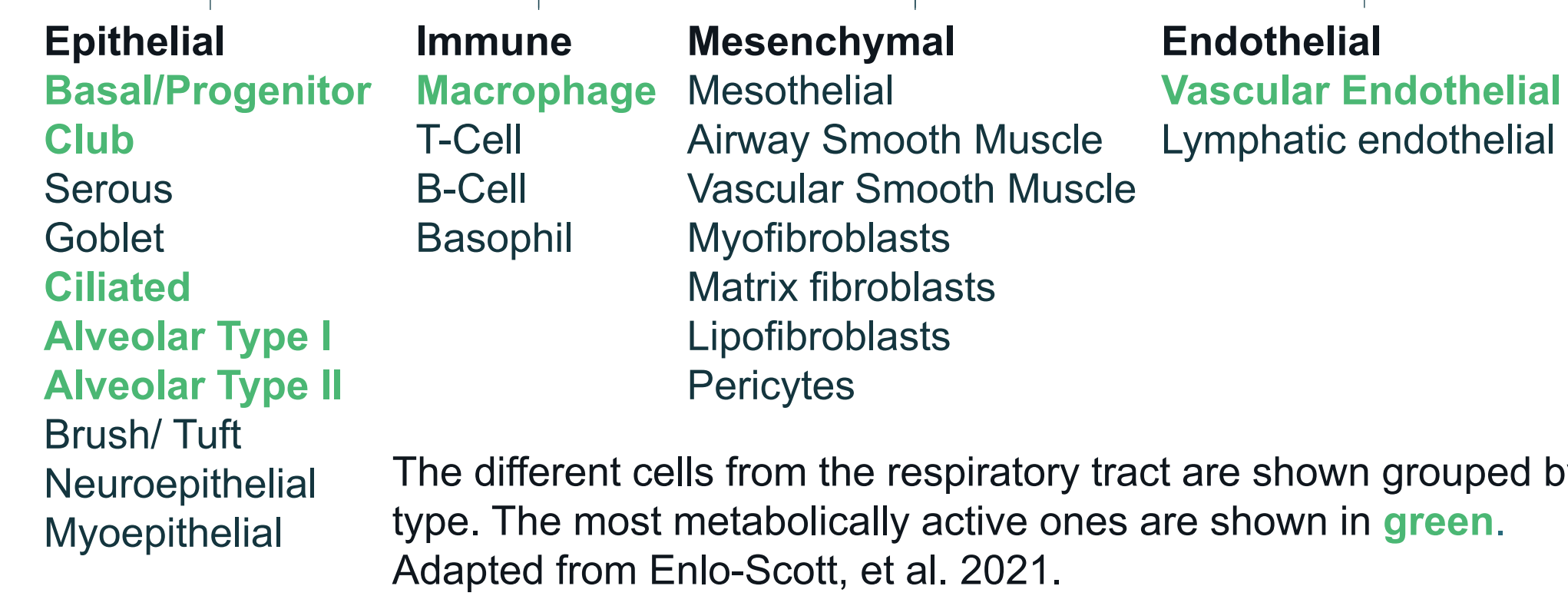
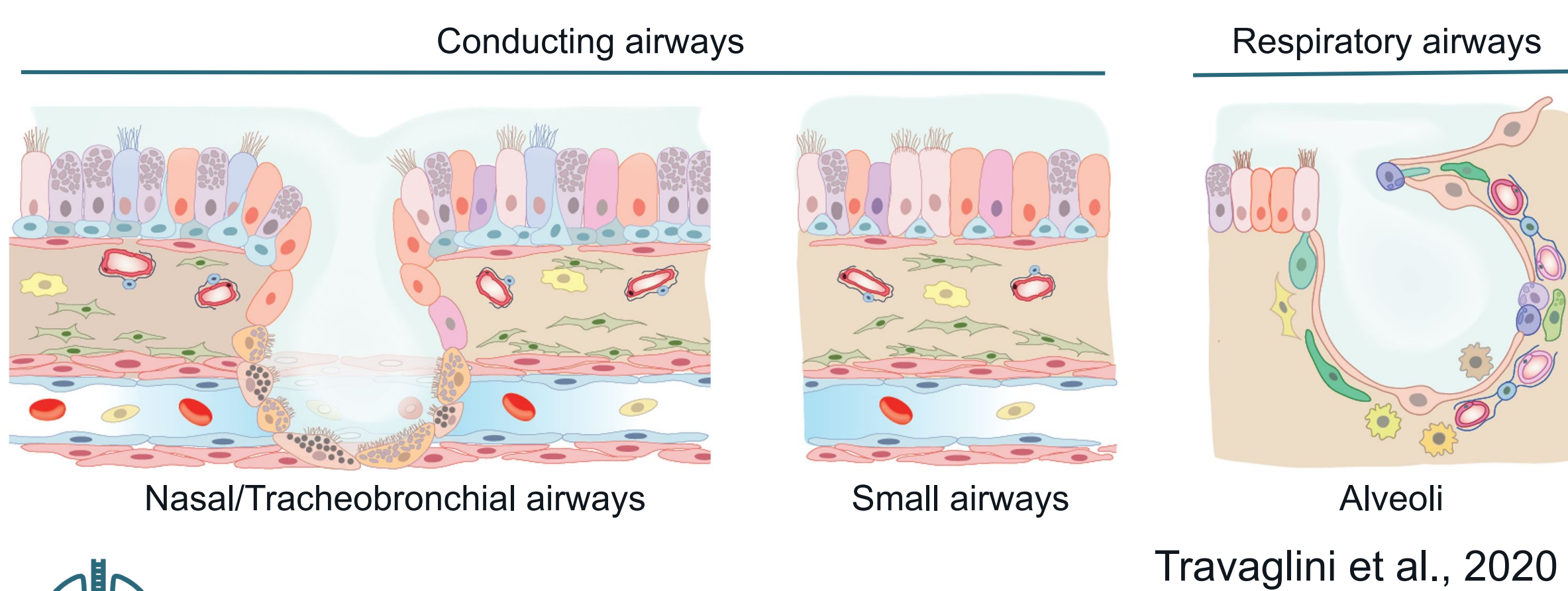
Abstract/Poster  
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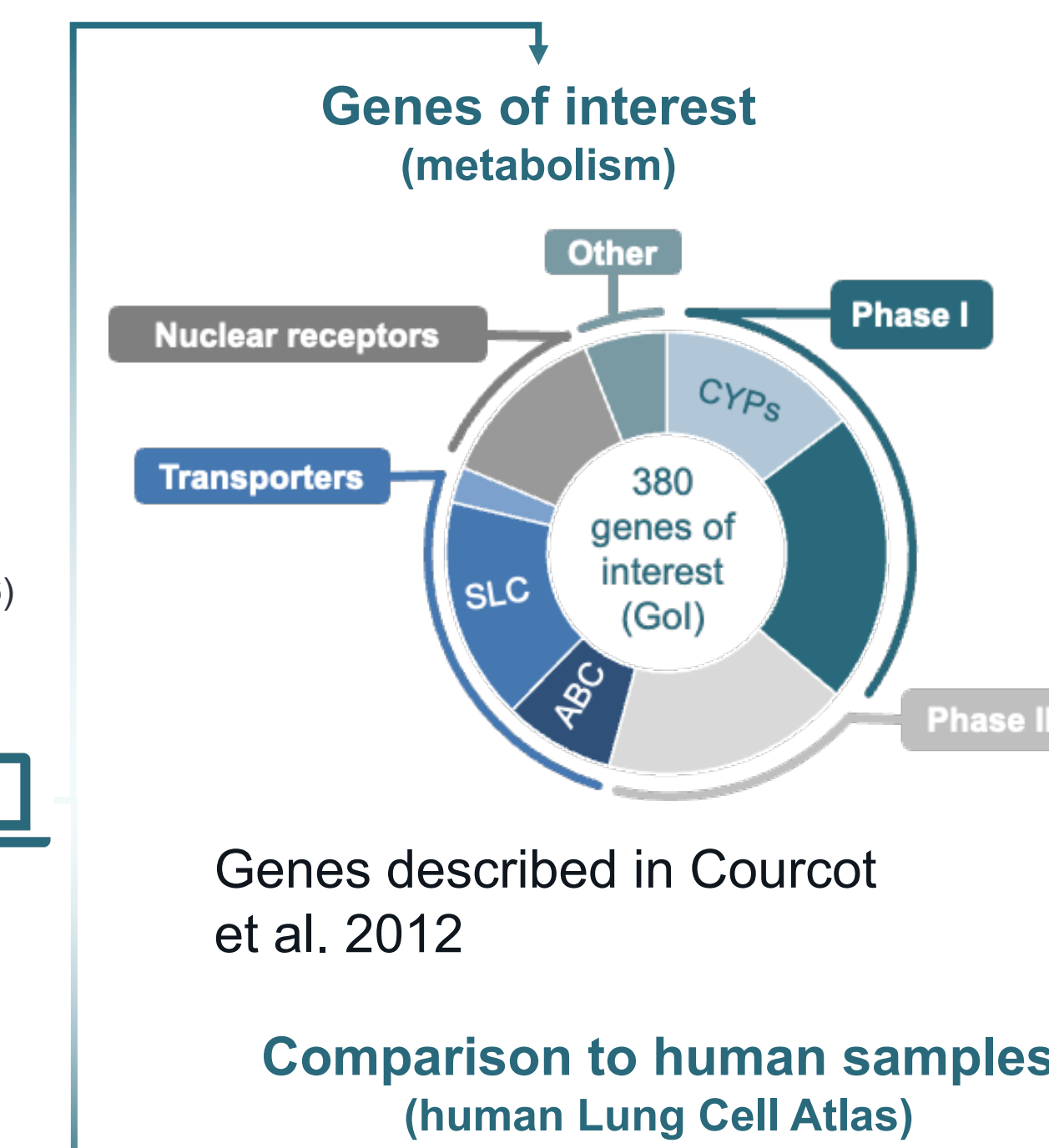
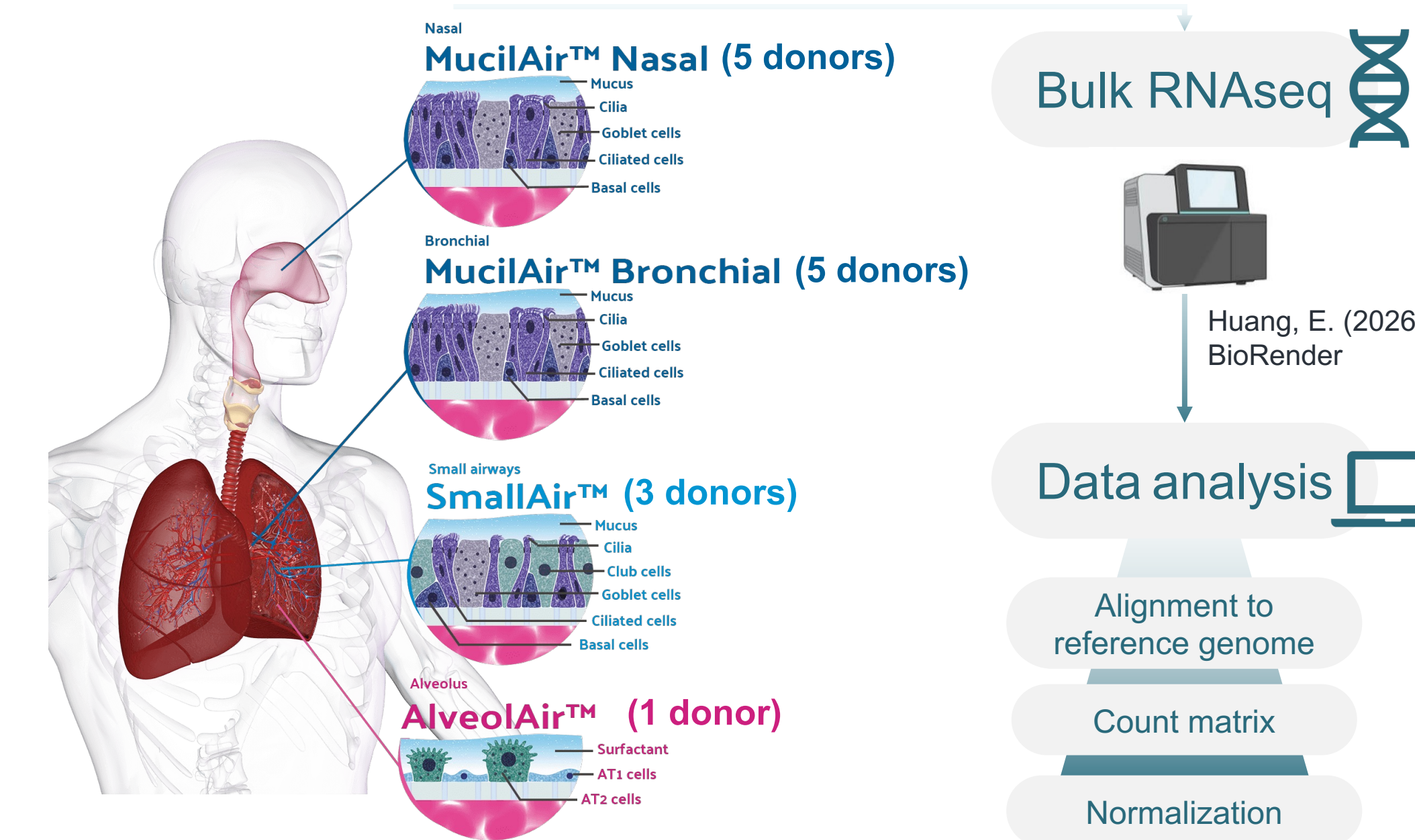
## Background and purpose

Following inhalation and deposition of a xenobiotic within the respiratory tract, local enzymes may metabolically activate or deactivate such xenobiotic. Because cell types vary along the respiratory tract, each region exhibits distinct metabolic competency. Consequently, xenobiotics may have different effects in different regions.



Reconstructed human respiratory epithelium (RHRE) are differentiated *in vitro* cell cultures that replicate the *in situ* characteristics of the respiratory tract epithelium. Characterizing their metabolic competency is key to using these models to generate data for toxicological assessments of inhaled xenobiotics. In this study, the gene expression of four RHRE models, representing different regions of the respiratory tract were compared: MucilAir nasal, MucilAir bronchial, SmallAir, and AlveolAir.

## Testing strategy



After filtering for adult and healthy datasets, two human reference samples were generated from the scRNAseq dataset in the human Lung Cell Atlas (HLCA) comprising the following annotated cells (level 3):

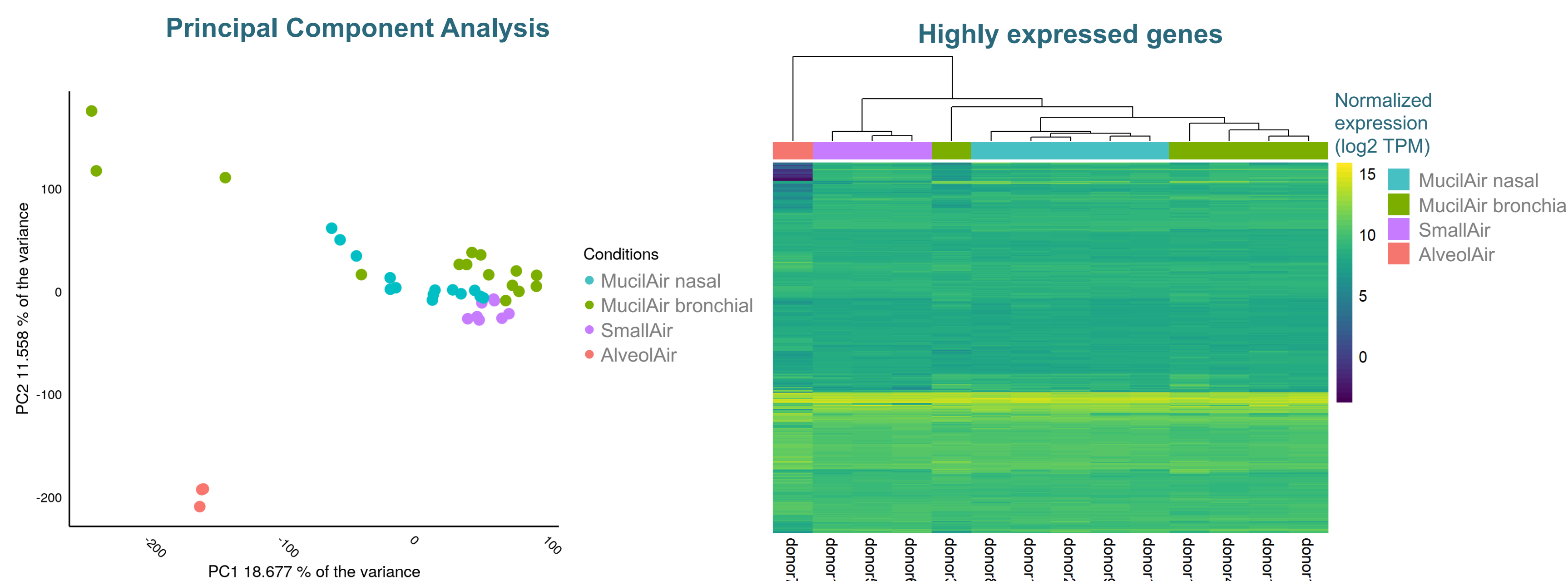
- **Conducting airways** – basal, secretory, multiciliated lineage, squamous, rare (epithelial), submucosal secretory, and none (epithelial)
- **Respiratory airways (alveolar)** – alveolar type 1, alveolar type 2, and endothelial capillary cells. When filtering for adult/healthy, no endothelial capillary datasets were available.

To compare relative gene expression, percentile (P) thresholds (25th, 50th, and 75th) were calculated separately for RHRE and HLCA datasets. Genes were classified as "Not detectable" (no signal, -), "Very low" (<P25, ...), "Low" (P25-50, +), "Moderate" (P50-P75, ++), or "High" (>P75, +++).

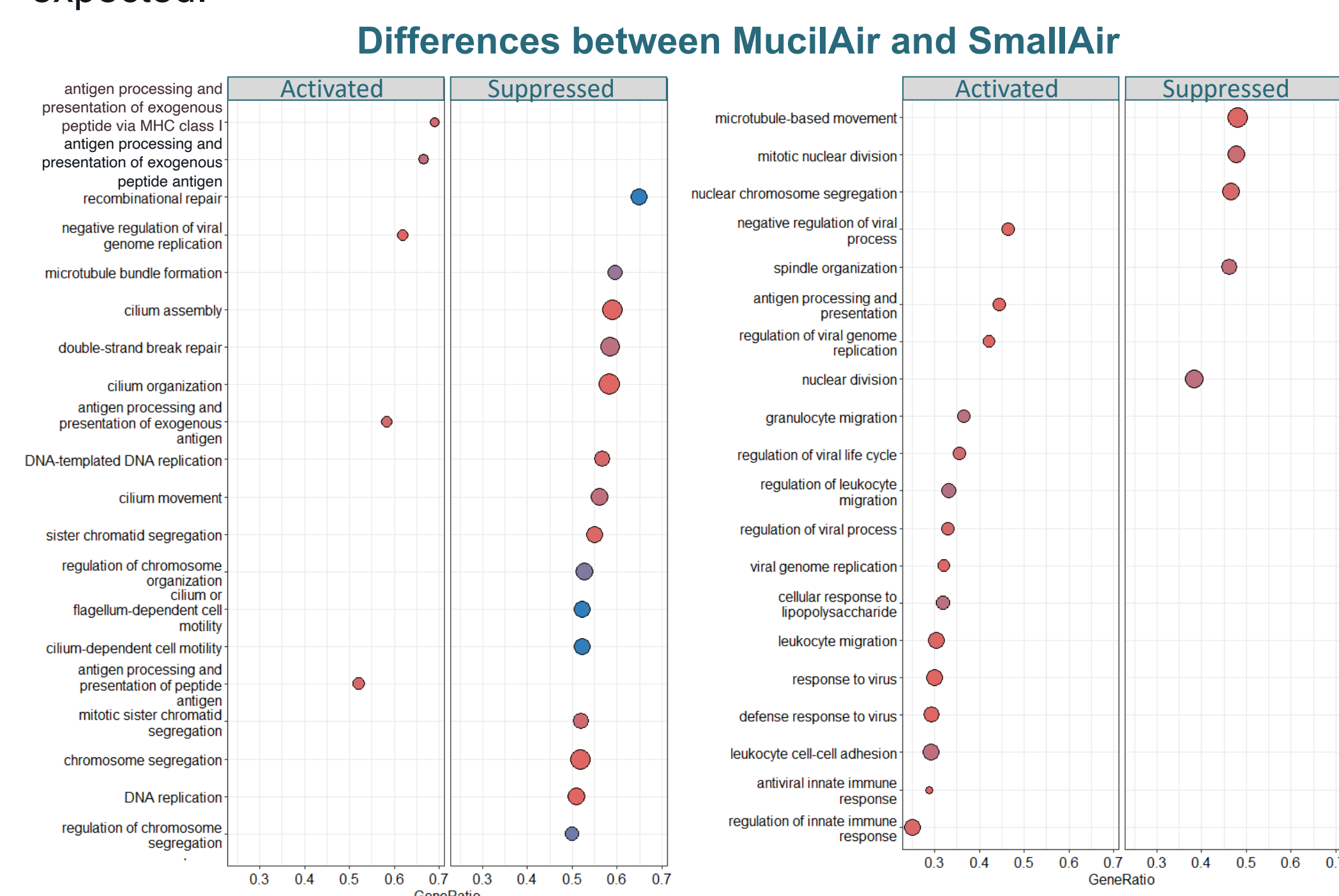
Threshold of RNA-seq data				Threshold of HLCA data			
Category	Symbol	Lower_bound	Upper_bound	Category	Symbol	Lower_bound	Upper_bound
Not detectable	-	0.000	0.000	Not detectable	-	0.000	0.000
Very low	...	0.000	0.470	Very low	...	0.000	0.305
Low	+	0.470	6.157	Low	+	0.305	4.197
Moderate	++	6.157	27.043	Moderate	++	4.197	24.108
High	+++	27.043	Inf	High	+++	24.108	Inf

## Results

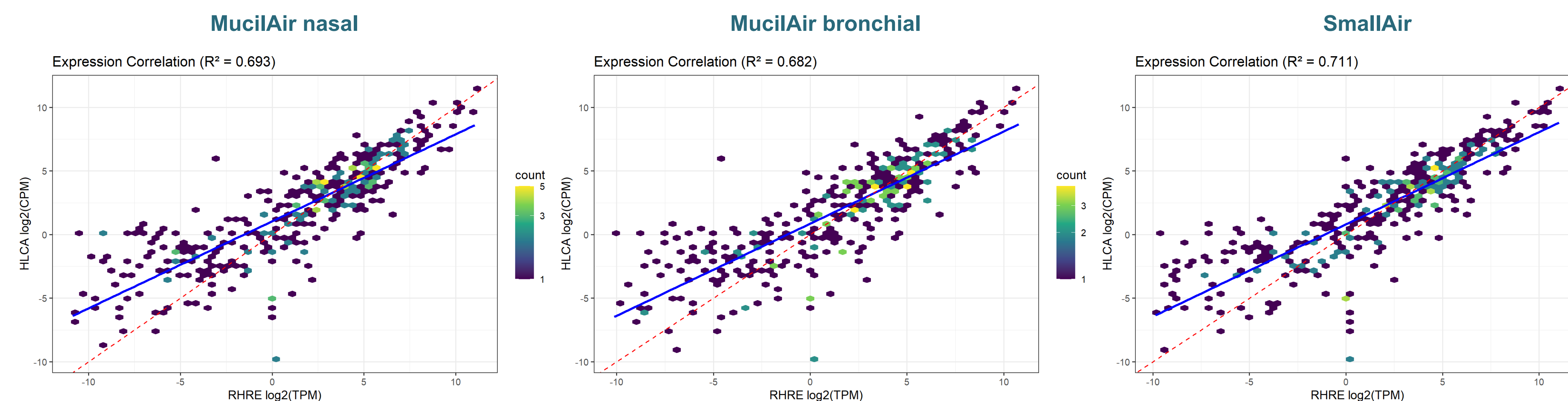
### Gene expression of RHRE from conducting airways is similar



Most samples from the conducting airways (nasal, bronchial, and small airways) are distributed within a narrow variance range along Principal Component 1 (PC1) and PC2 axis, indicating similar gene expression. Similar gene expression patterns for the conducting airways RHRE are also observed using hierarchical clustering. Shorter branches indicate greater similarity between the clustered samples. Hence, MucilAir nasal is most similar to MucilAir bronchial, followed by SmallAir. AlveolAir, which contains markedly different cell types, is clustered separately, as expected.

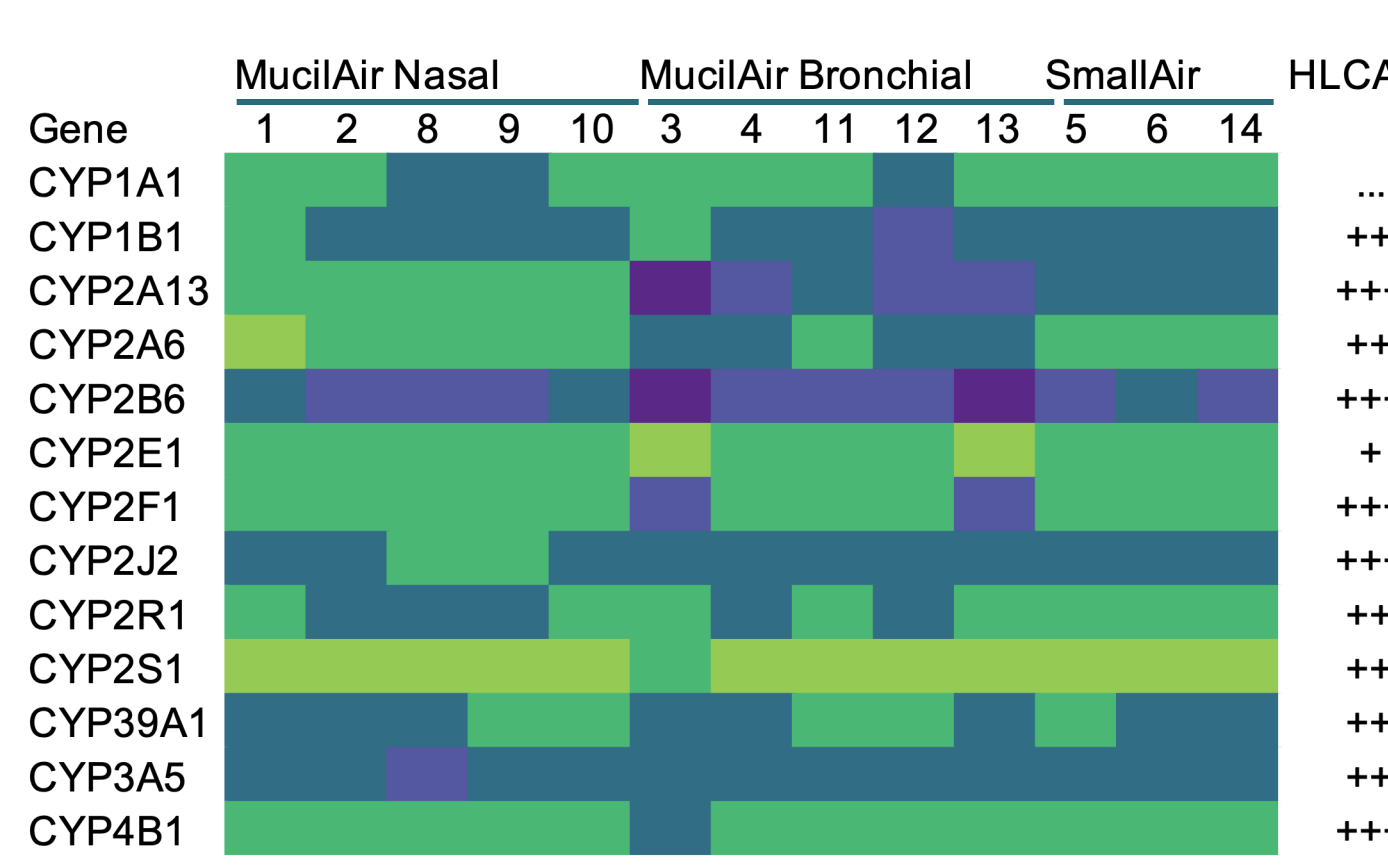


### MucilAir (nasal and bronchial) and SmallAir show a similar expression of metabolic genes compared to reference human samples (HLCA)

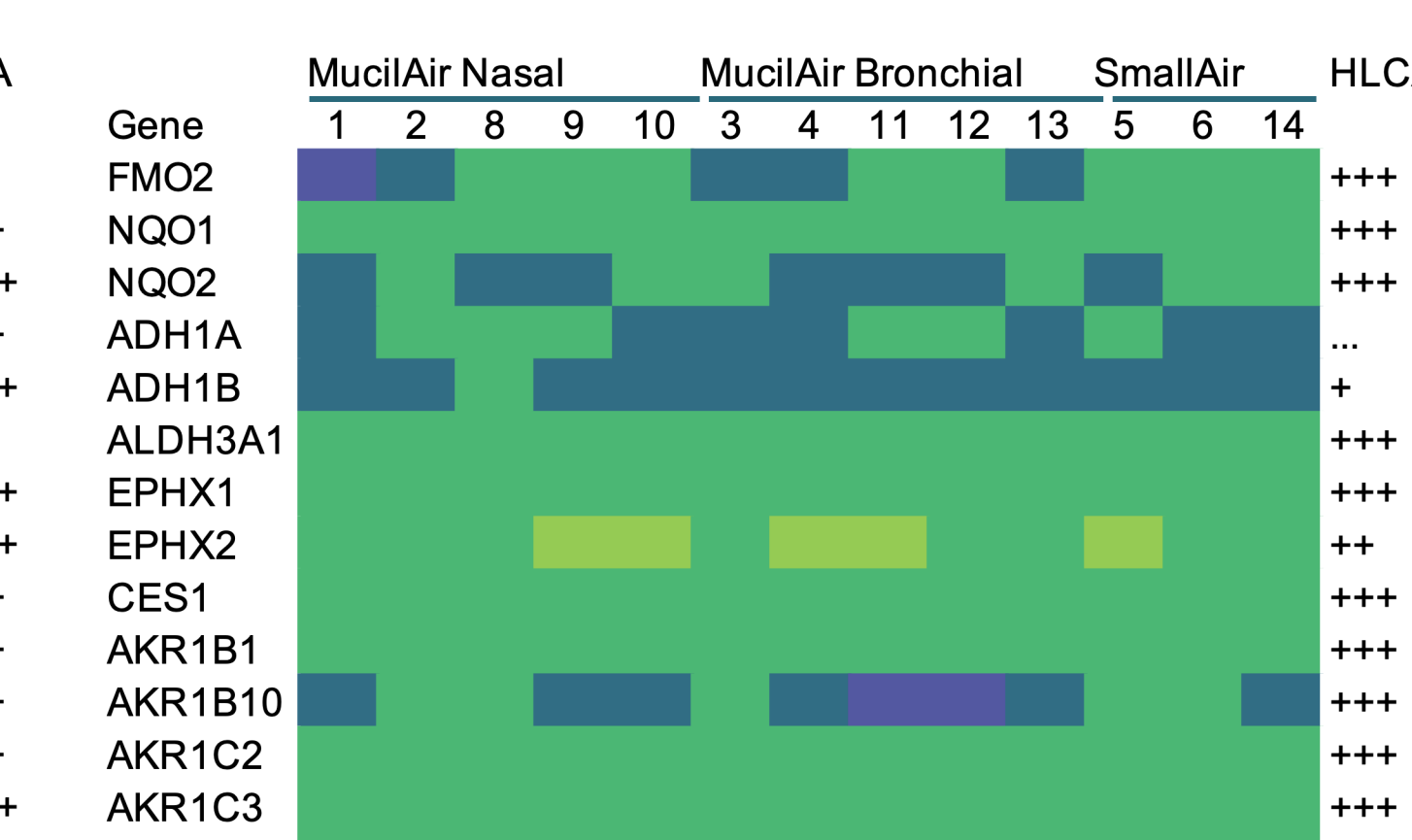


The expression of the genes of interest for RHRE of the conducting respiratory regions follows a linear correlation with the reference human sample.

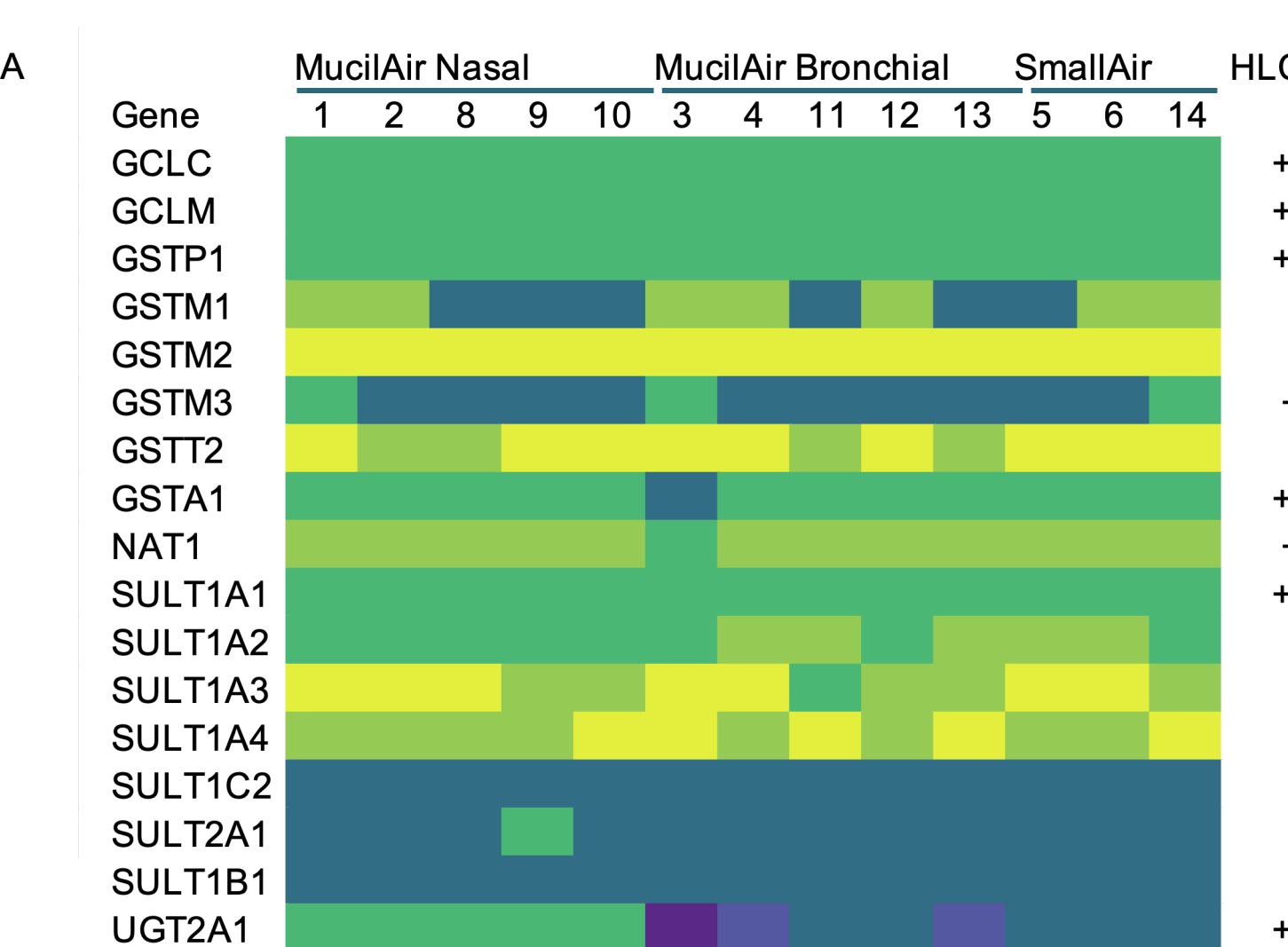
#### Phase I metabolism (CYPs)



#### Phase I metabolism (non-CYP)



#### Phase II metabolism



Regarding the expression of CYP enzymes, the RHRE that best recapitulates the gene expression of the conducting airways reference sample (HLCA) is the nasal RHRE. MucilAir bronchial shows a lower expression, and SmallAir shows a mixed pattern. The non-CYP phase I enzymes display a similar gene expression in the proximal RHRE when compared to the human reference dataset. Most RHRE (nasal, bronchial, and small airways) seem to have a similar gene expression. Most phase II metabolism associated genes shown here are expressed to a similar or higher level than the reference sample, with a few enzymes expressed lower in the RHRE.

## Main learnings

- RHRE of the conducting airways (MucilAir nasal, MucilAir bronchial, and SmallAir) display similar gene expression patterns.
- RHRE of the respiratory airways (AlveolAir) shows marked differences in gene expression from the other RHRE studied in this work. This can be explained by its different cell composition, including alveolar type I and II epithelial cells, as well as endothelial cells.
- Due to the lack of an endothelial component in the reference for the distal airways, the alveolar RHRE (AlveolAir) could not be properly compared and requires further study.
- Generally, the gene expression of metabolism-associated genes for RHRE of the conducting airways (MucilAir nasal, MucilAir bronchial, and SmallAir) is similar to that of the human reference sample which supports the human relevance of these RHRE to the gene expression level. Studying the functionality of these genes was beyond the scope of this study.

## How to use the data

You are a...



RHRE user

1. **Background** on your substance (metabolism, physchem, etc.)
2. **Where** does it deposit in the respiratory tract?
3. Select a proper RHRE and check our **database** for tissue/genes of interest to help interpret generated data
4. **Optional: Functional assays** (to demonstrate activity)



RHRE developer

1. Conduct **RNA sequencing**
2. Run pipeline in R (our analysis pipeline will be open-source)
3. **Compare results** to a human database/ our data
4. **Share data** to expand on a public database

### Acknowledgements

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