Pacific Northwest
NATIONAL LABORATORY

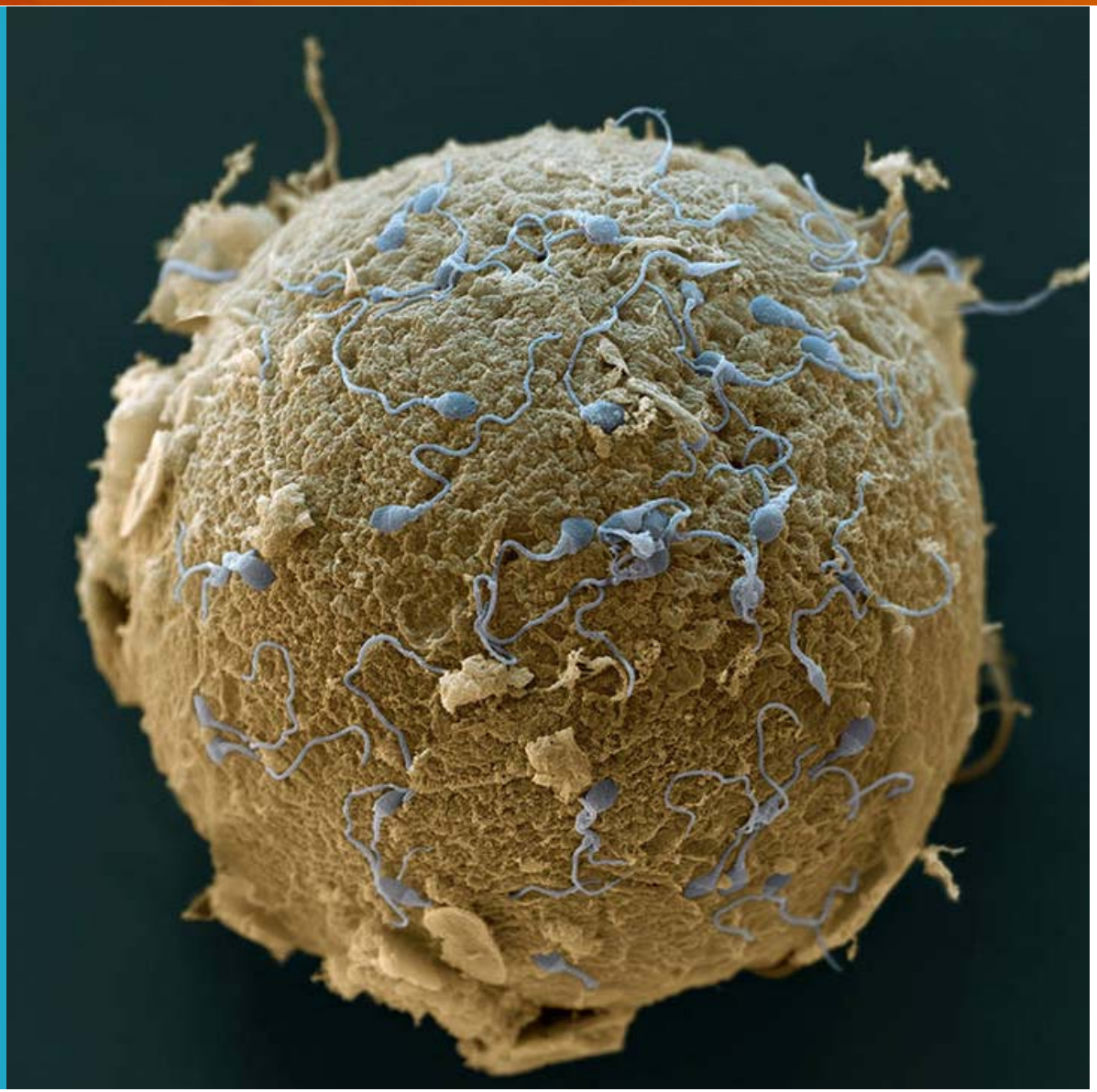
Proudly Operated by Battelle *Since 1965*

Multi-Omics analysis of the developing Lung

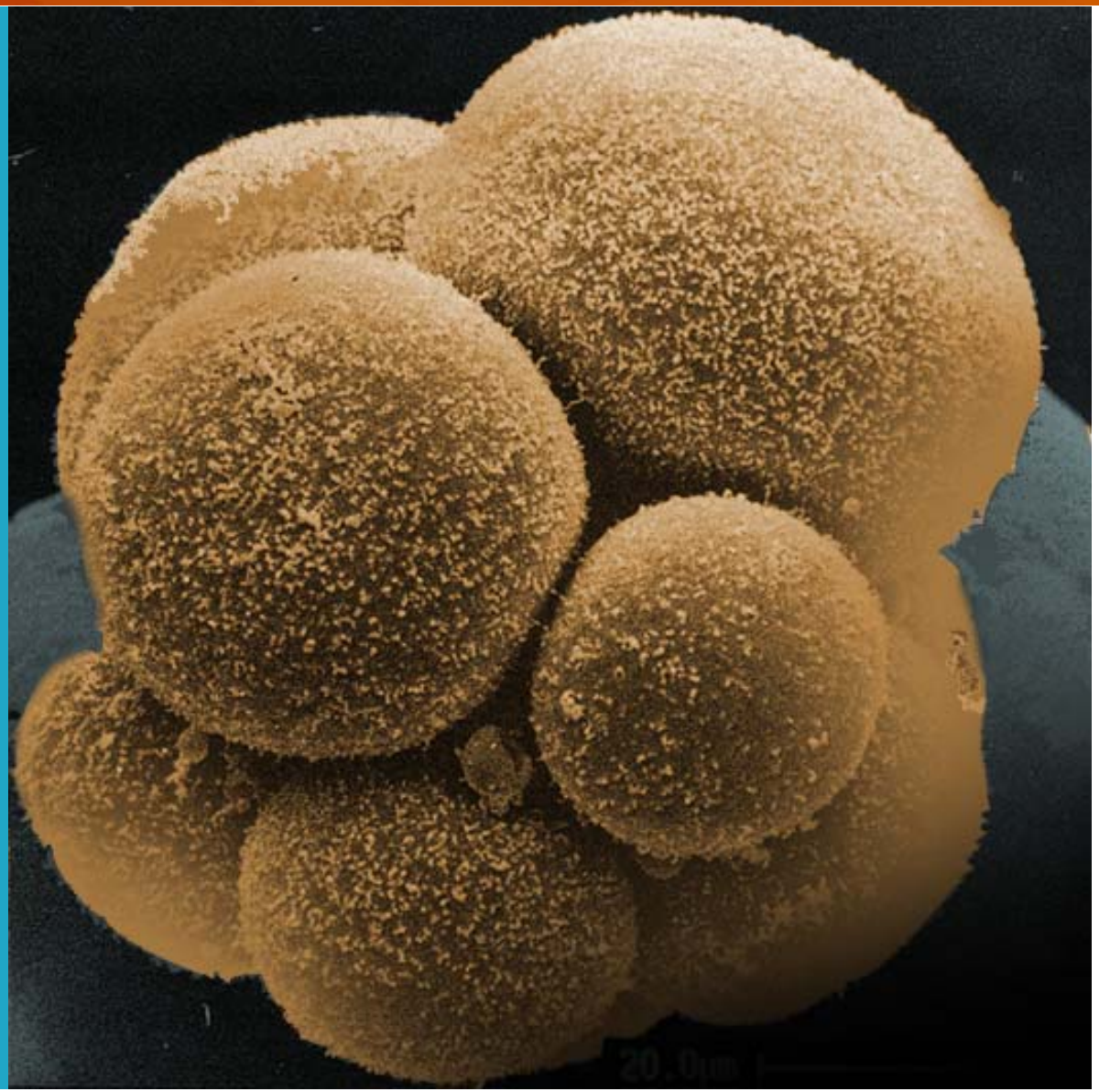
Geremy Clair

U.S. DEPARTMENT OF
ENERGY

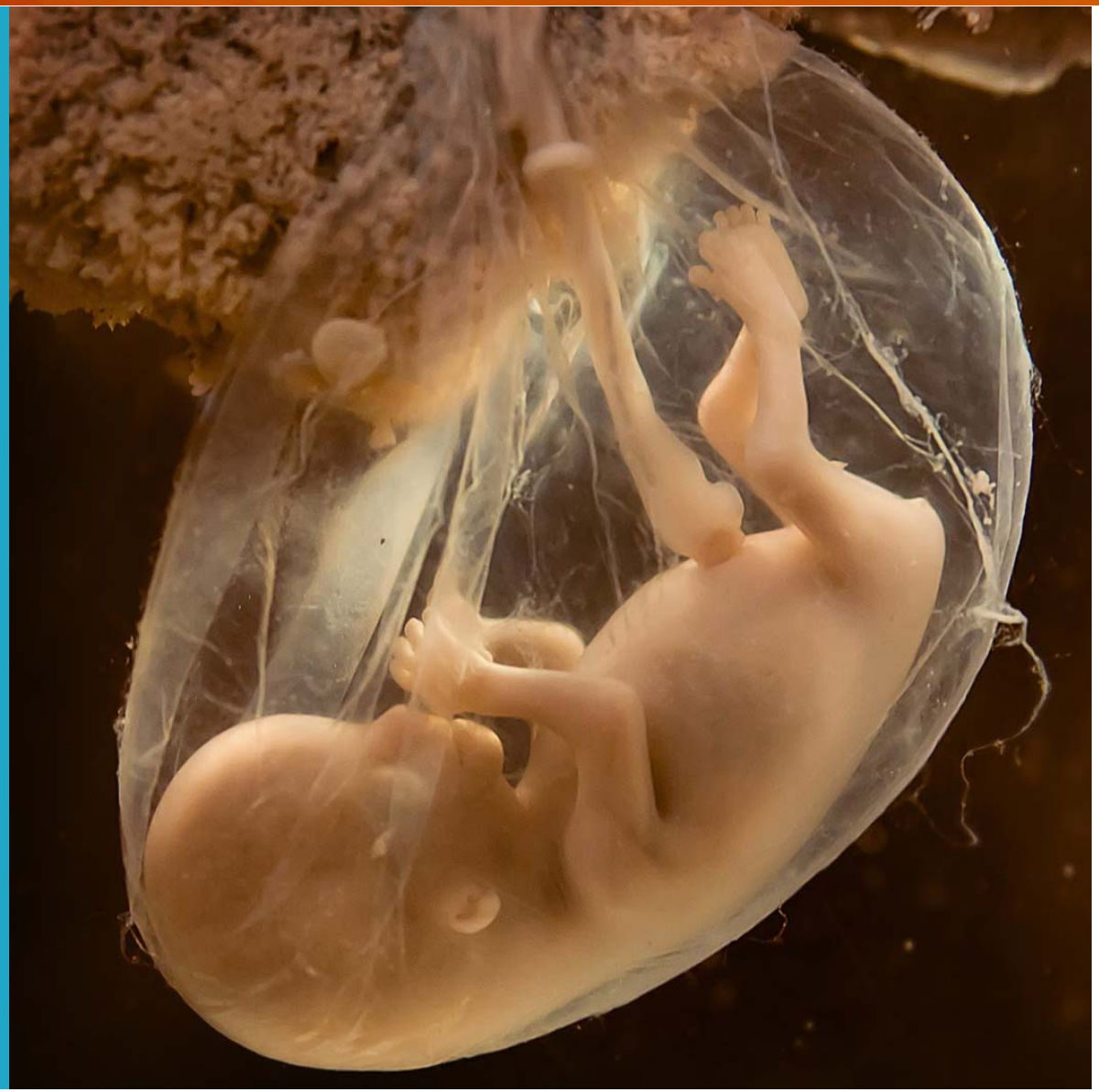
Fertilization



… initiates the developmental process…



…of an incredibly complex system



The Lung is incredibly complex

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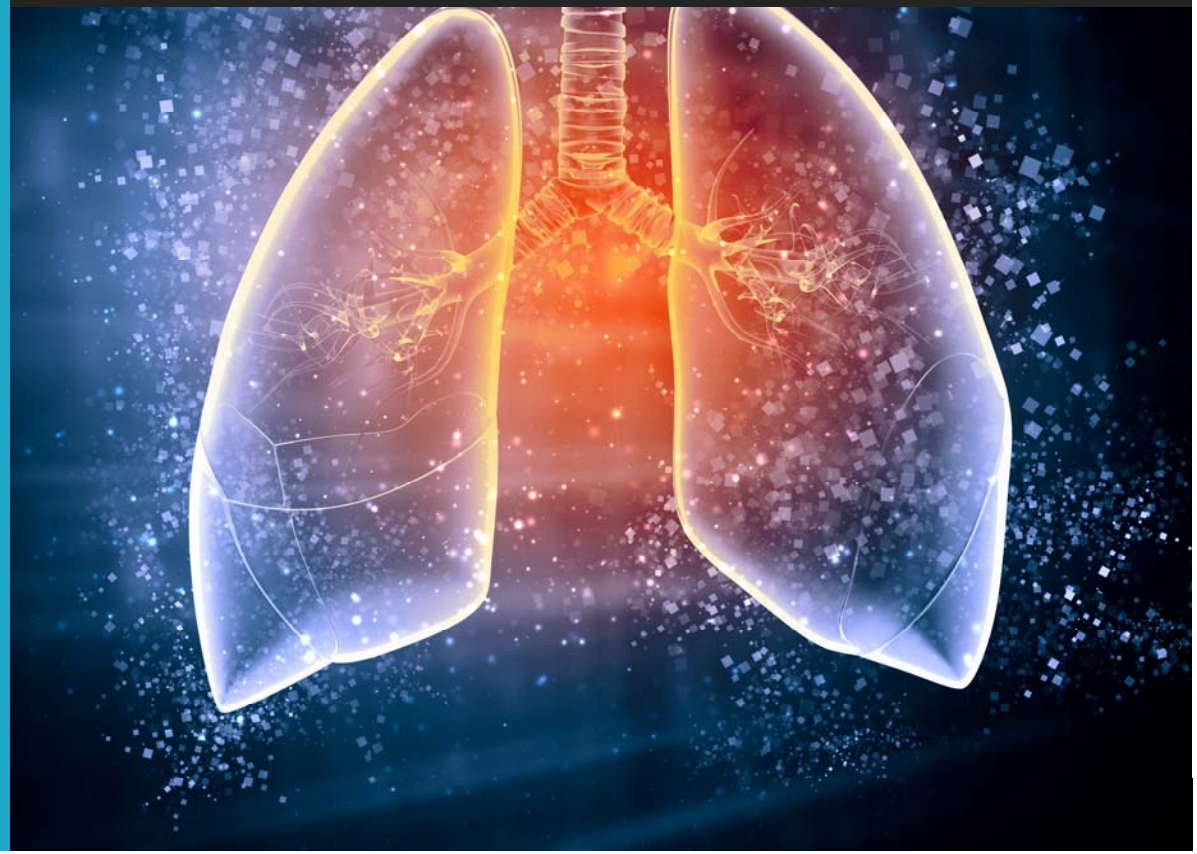
DNA **RNA** **Proteins** **Lipids** **Metabolites**



The LungMAP

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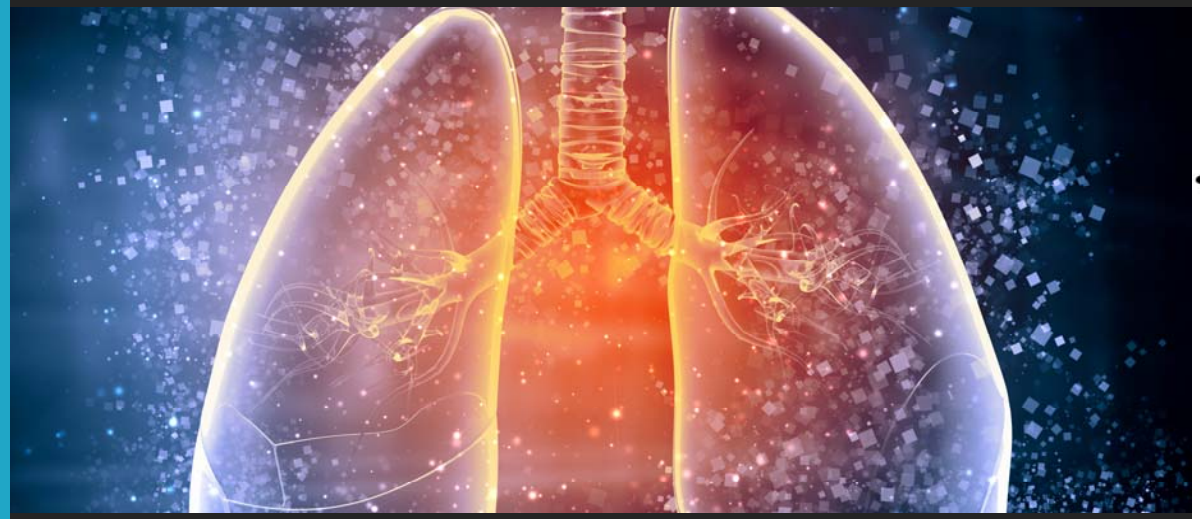
RNA **Proteins** **Lipids** **Metabolites**

Multiple spatial and temporal resolutions



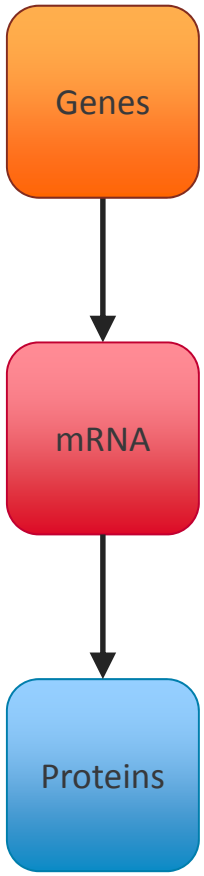
Fluorescence and Mass spectrometry imaging

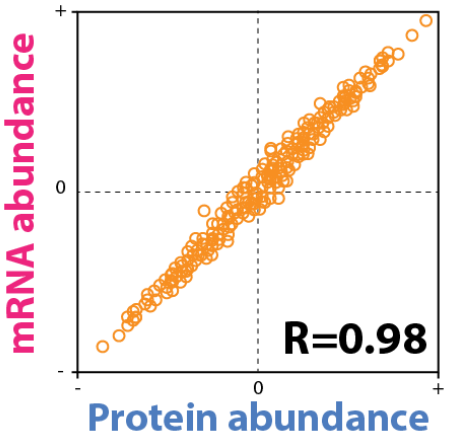
LungMAP www.lungMAP.net

NIH National Heart, Lung, and Blood Institute

E9.5	Wk4	Pseudoglandular
E16.5	Wk17	Canalicular
E17.5	Wk26	Saccular
PND5	Wk36	Alveolar
PND28	8 Years	

Transcription and translation from the textbooks





If there was only transcription and translation controlling protein abundance :
the transcripts/proteins correlation would be very high.

Transcription and translation the real world

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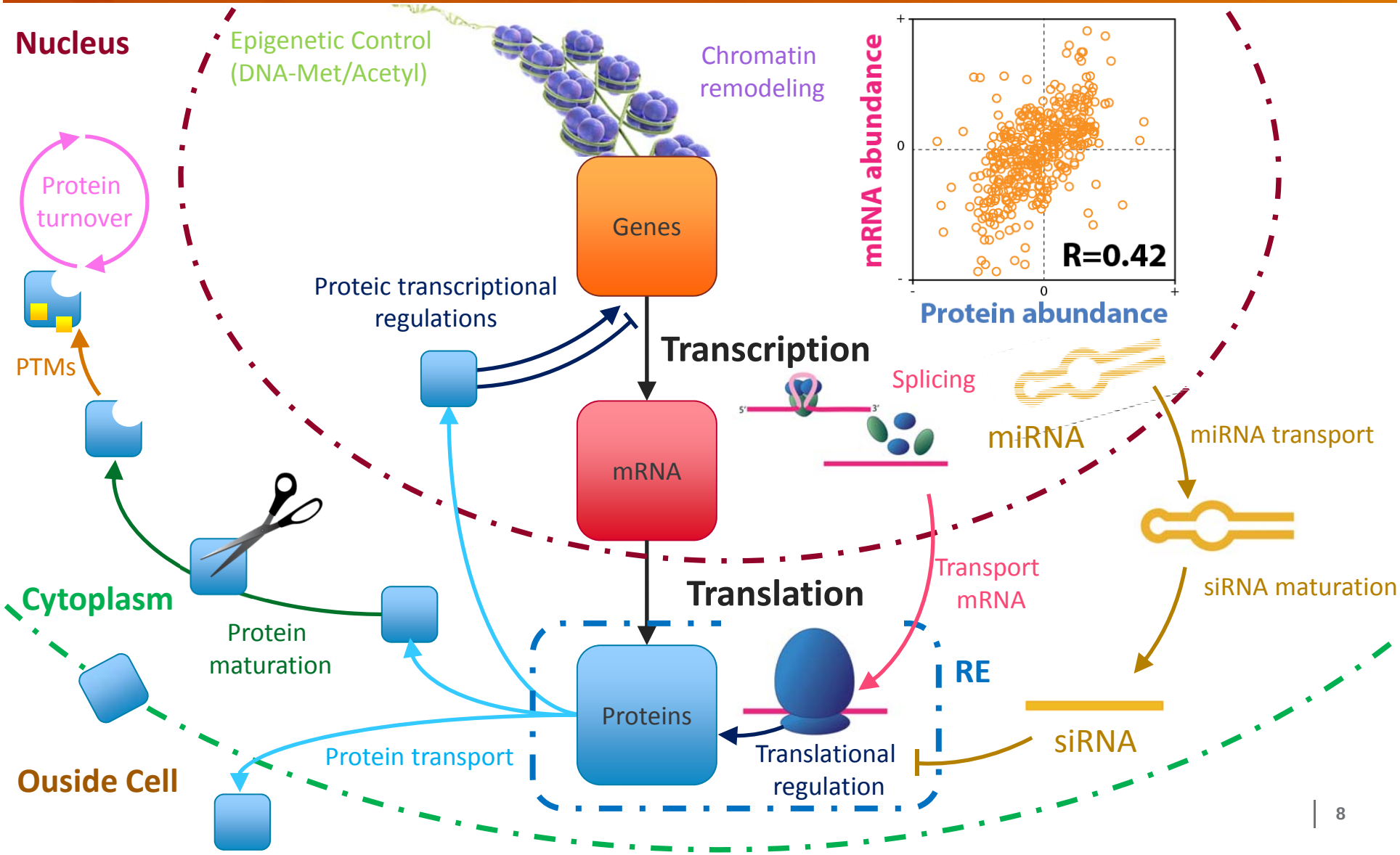

Anti-correlated mRNA and proteins during lung development

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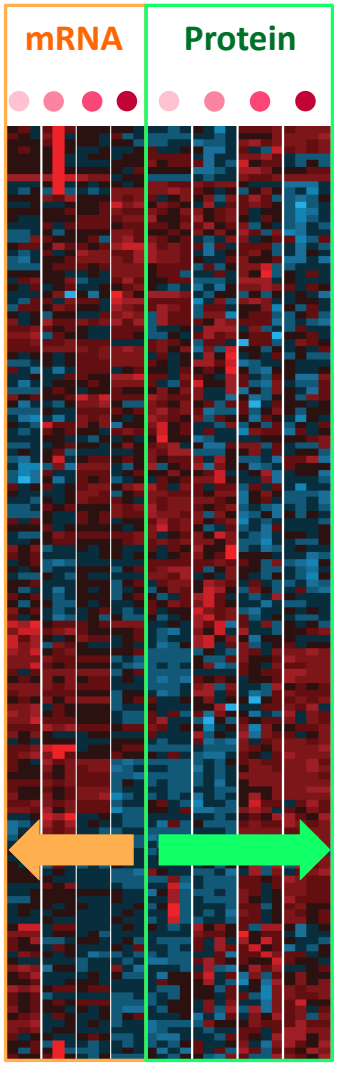
E16.5

E18.5

PND5

PND28



Protein and mRNA abundance

Low

High

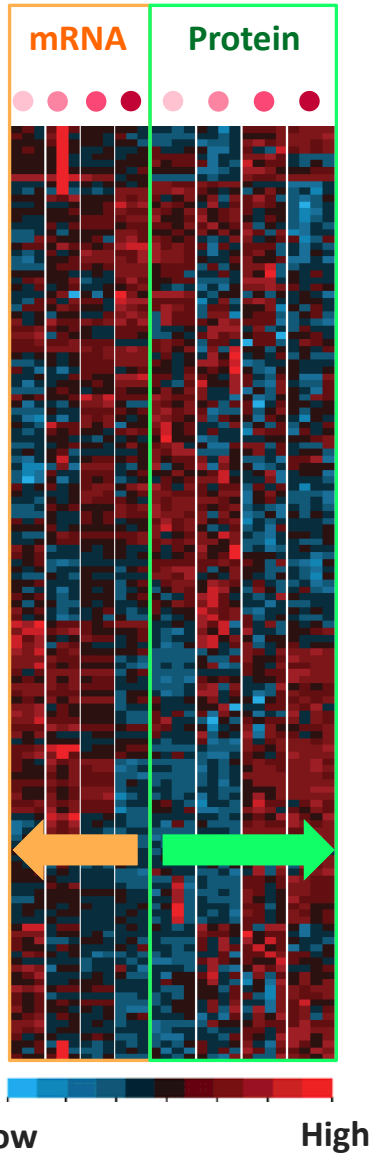
Post-transcriptional regulated functions

E16.5

E18.5

PND5

PND28



Functional enrichment :
Post-transcriptionally regulated processes

Immune system process
Lipid metabolic process
Oxidation-reduction process
Metabolic process
Translation
mRNA processing
Covalent chromatin modification
…

The lung proteome is extensively remodeled during normal development

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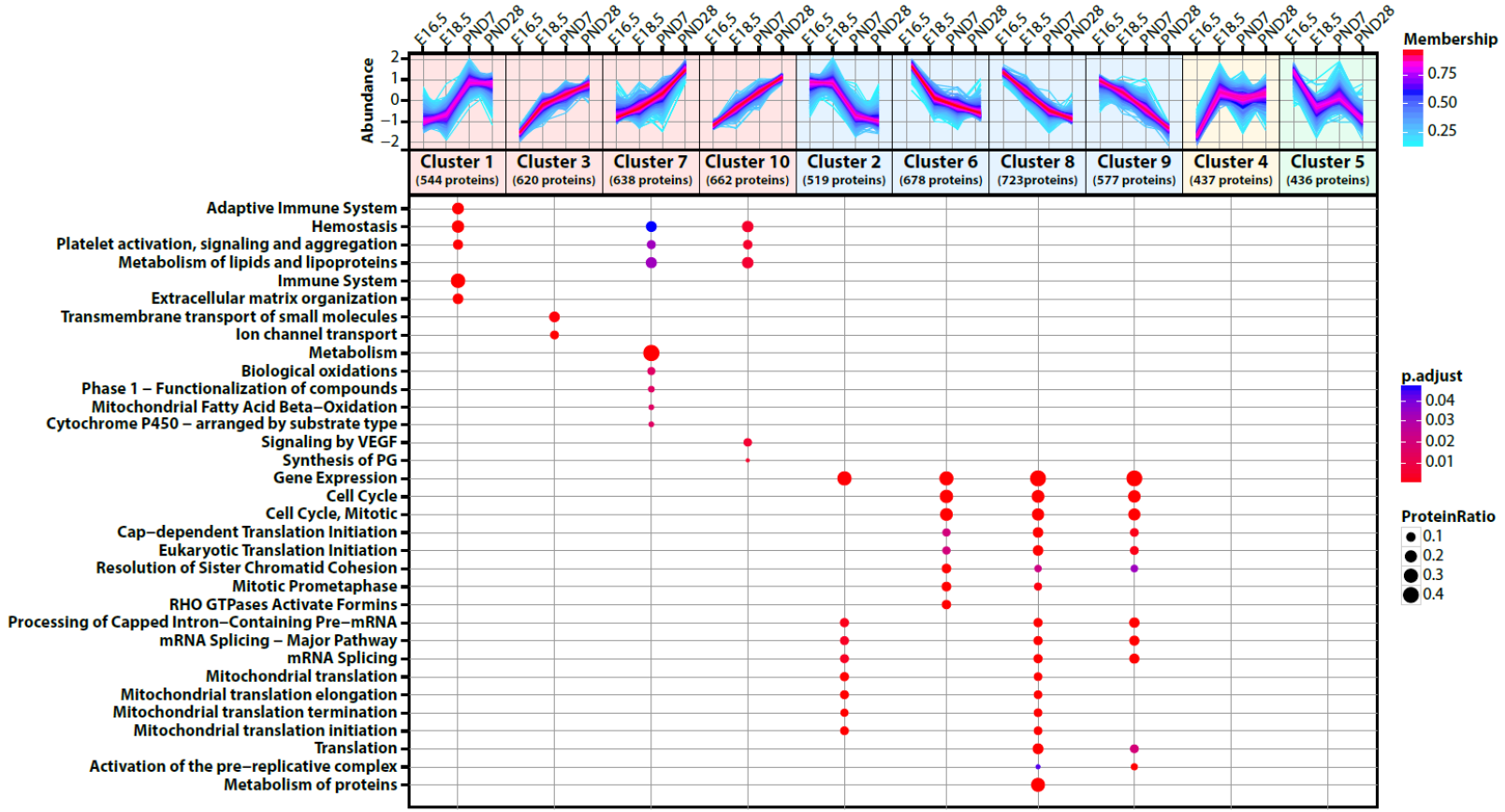
E16.5
E18.5
PND5
PND28

8,932 proteins → 8,171 quantifiable
→ 5,515 significantly changing in abundance (68%)



Transcription regulators and signaling molecules orchestrate the development

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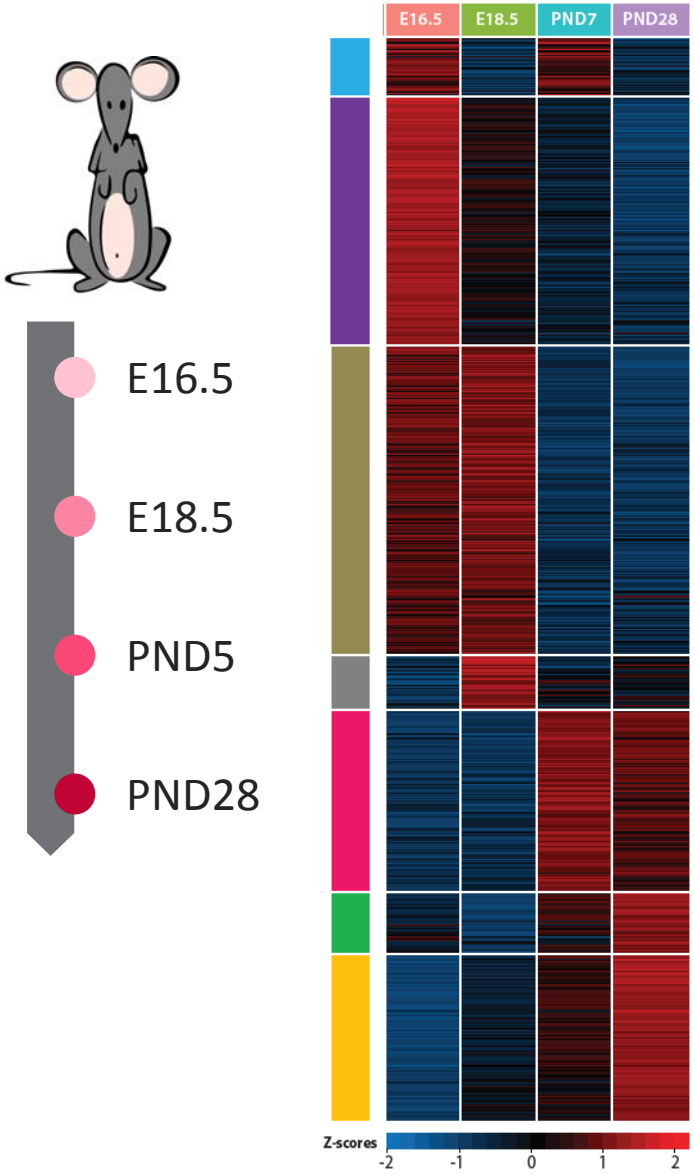

1,569 transcription/translation regulators and proteins involved in signaling

Some TR and SM are potentially highly involved in the proteome remodeling

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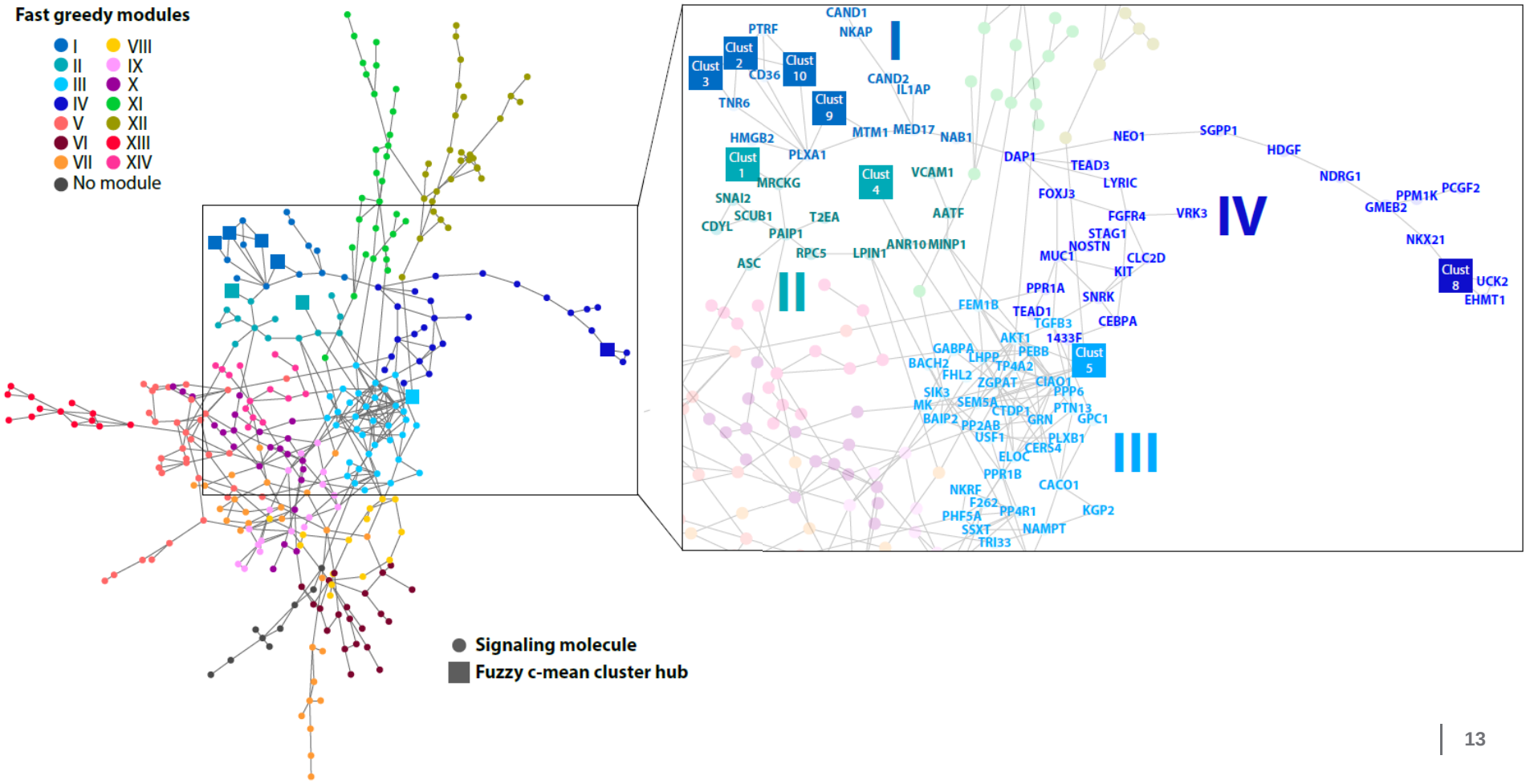
CLR unsupervised clustering method allowed us to identify 4 modules of transcriptions factors and signaling molecule that appear to be implicated in the global proteome remodeling



Lung Heterogeneity

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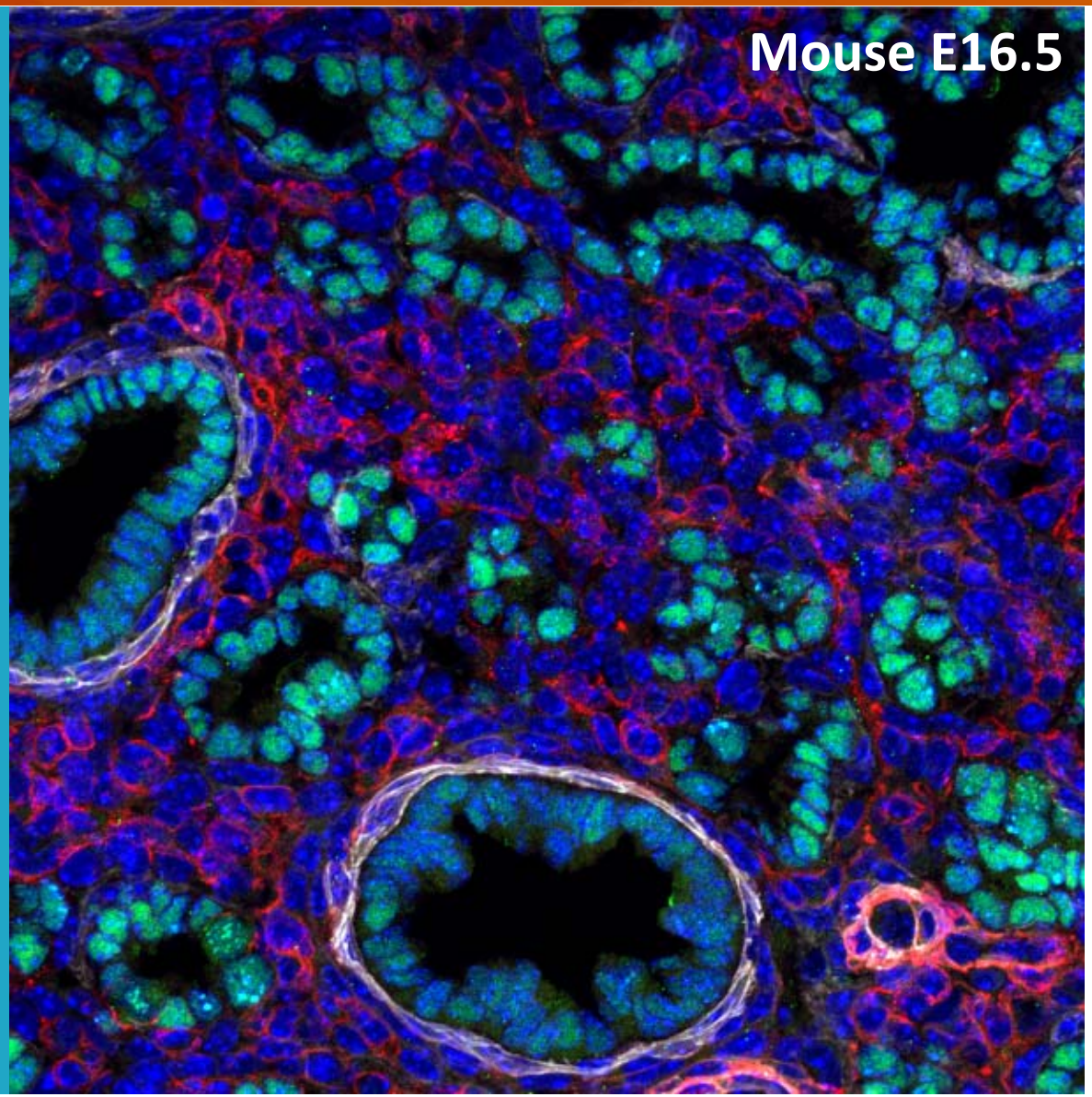

- NKX2-1
- Acta2
- Cspg4
- DAPI

LungMAP
www.lungMAP.net

> 40 cell types

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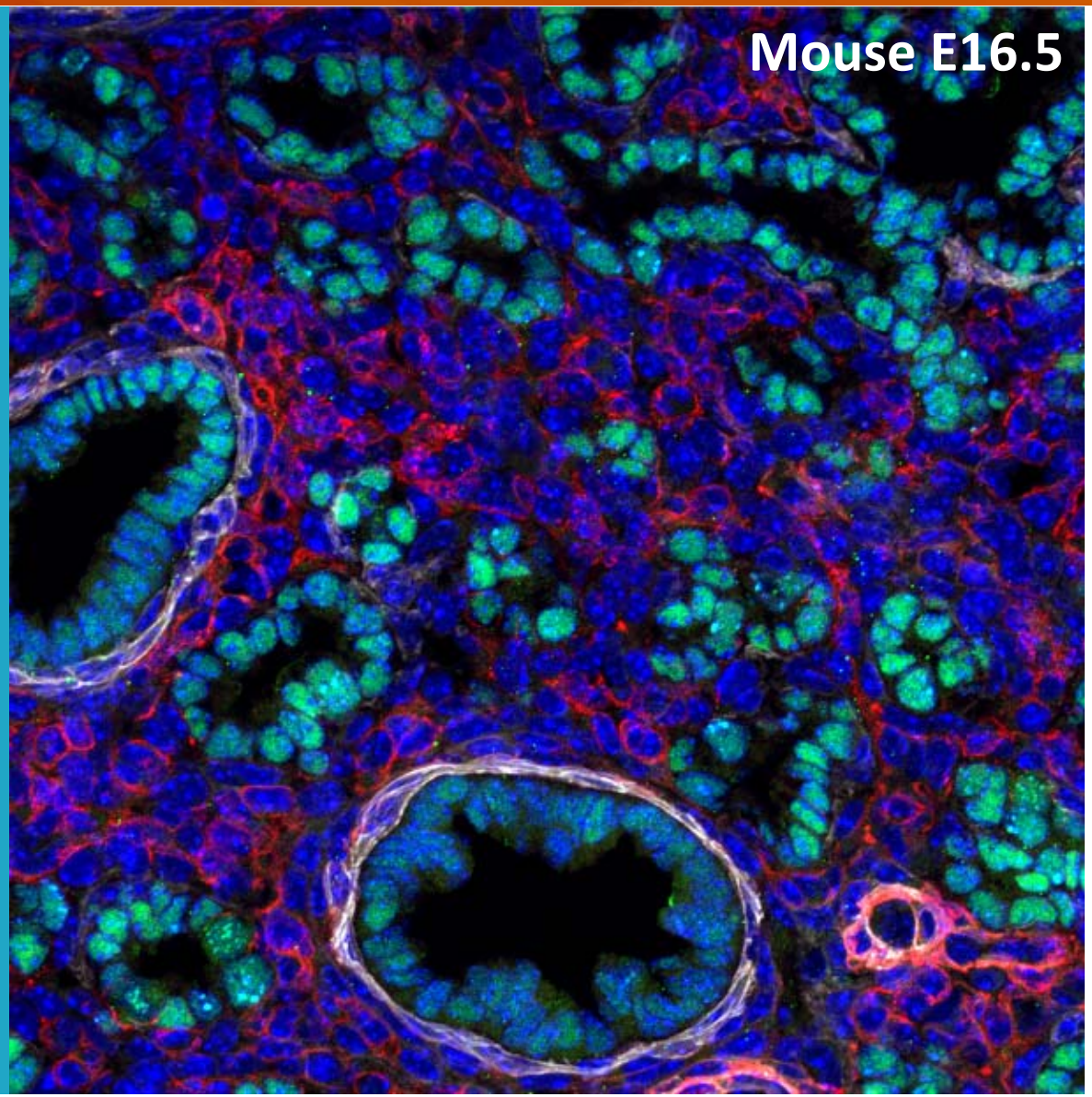
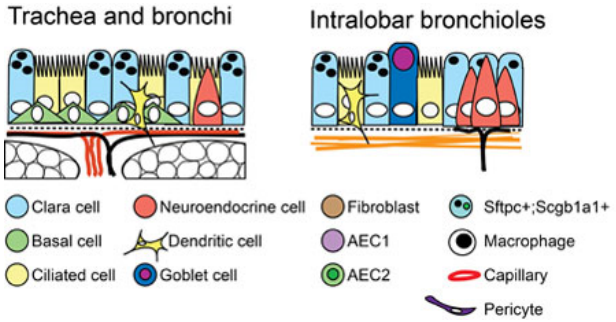
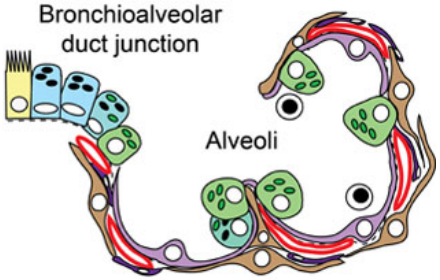


NKX2-1
Acta2
Cspg4
DAPI

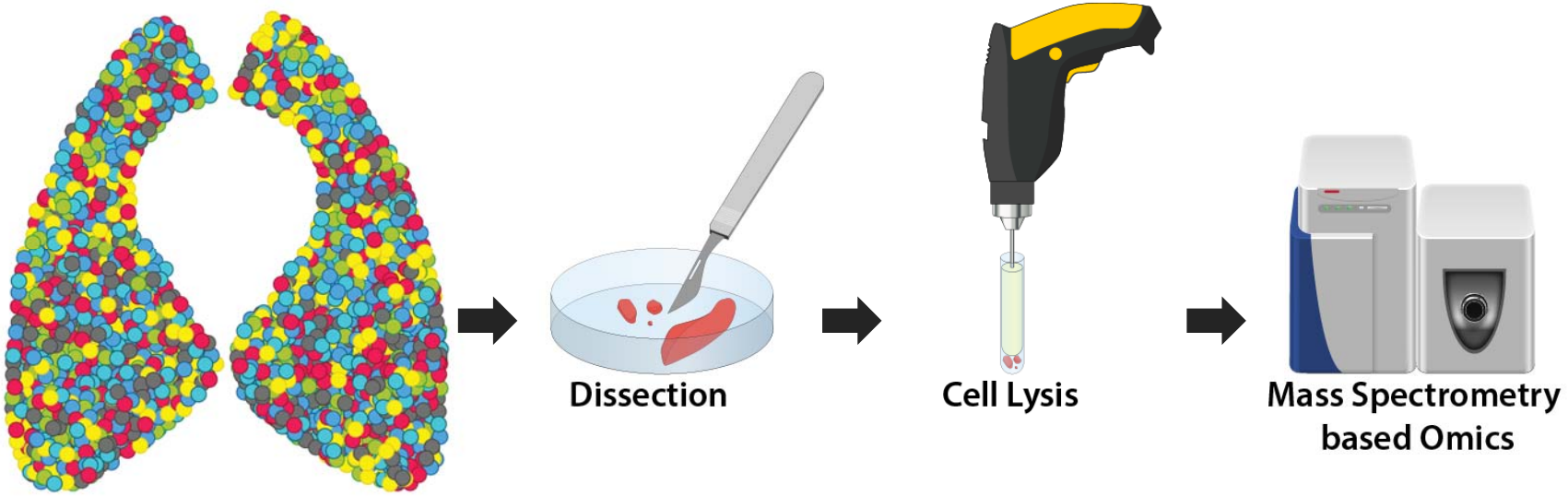
LungMAP
www.lungMAP.net



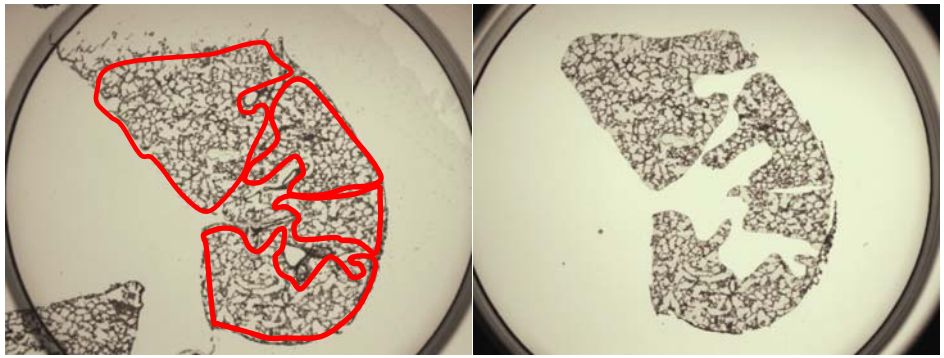


rocklab.ucsf.edu

Blending and averaging effects in omics



LCM



Isolated alveolar parenchyma

- E16.5
- PND7
- PND28

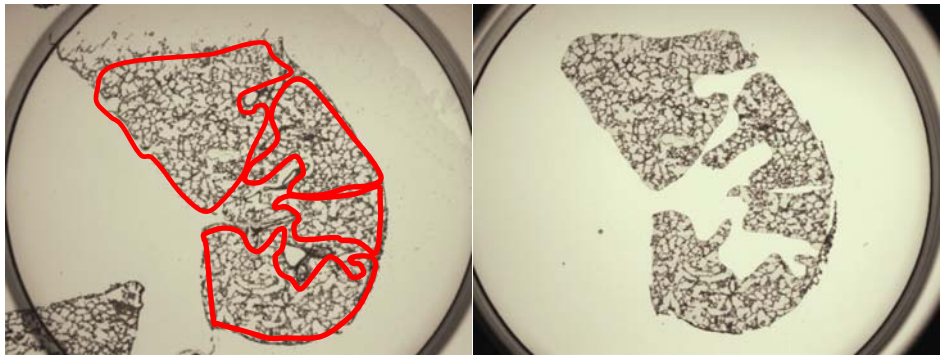
Clair *et al. Scientific report* 2016.

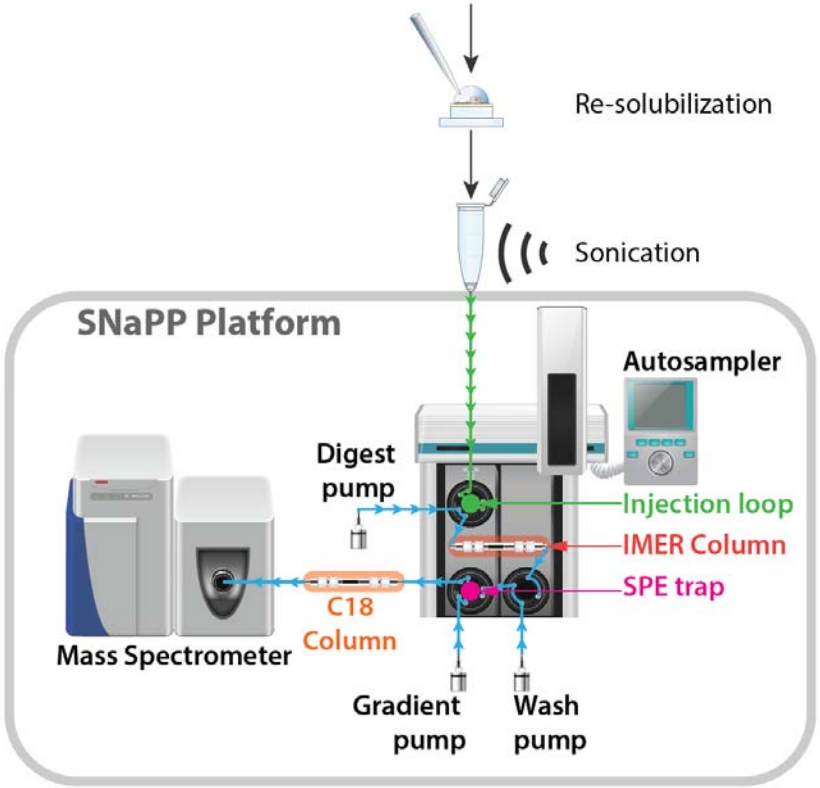
LCM and nano-proteomics

E16.5

PND7

PND28





Isolated alveolar parenchyma

Clair *et al. Scientific report* 2016.

LCM and nano-proteomics (Optimal from 2,000 to 8,000 cells)

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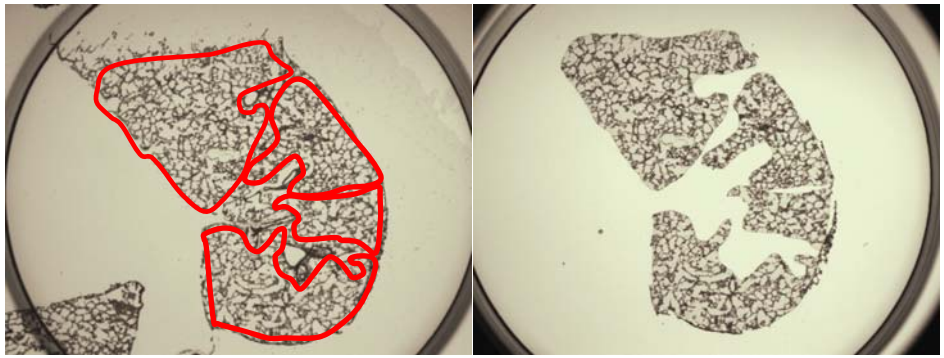
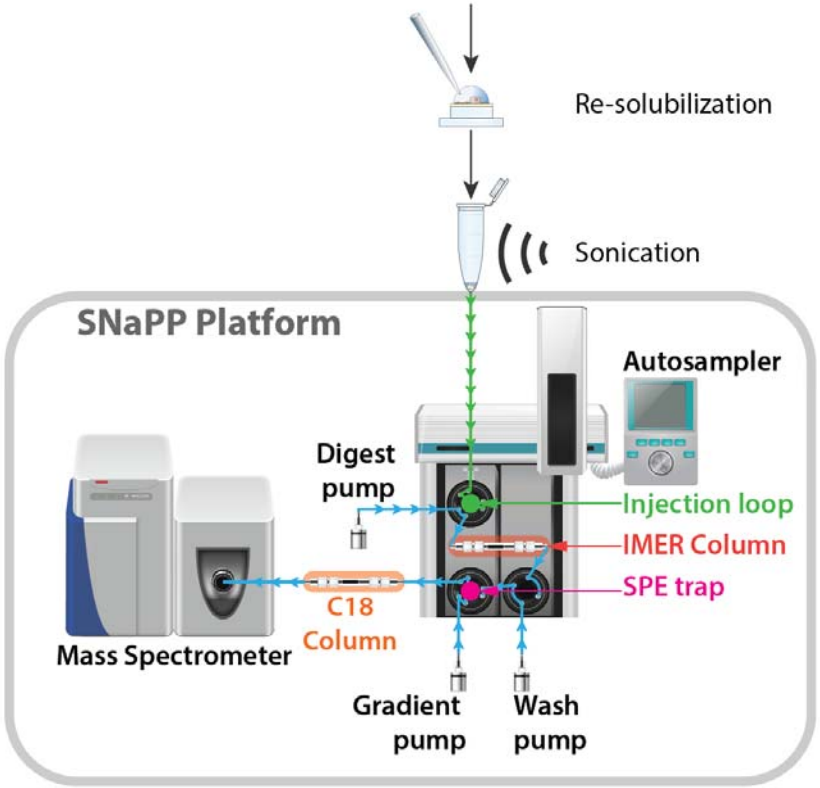
E16.5

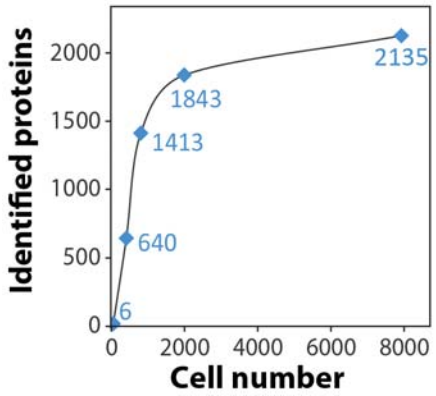
PND7

PND28





Isolated alveolar parenchyma



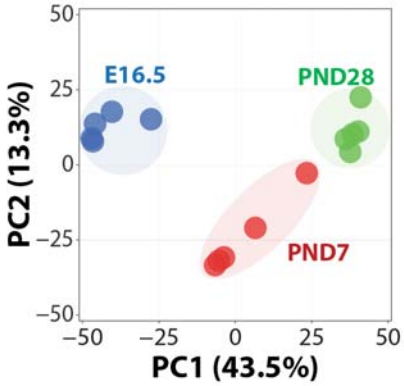
Optimal after 2,000 cells

Clair *et al. Scientific report* 2016.

Alveolar tissue proteomics

- E16.5
- PND7
- PND28

PCA



Segregation of the biological replicates by time-points

Clair *et al. Scientific report* 2016.

Alveolar tissue proteomics during development

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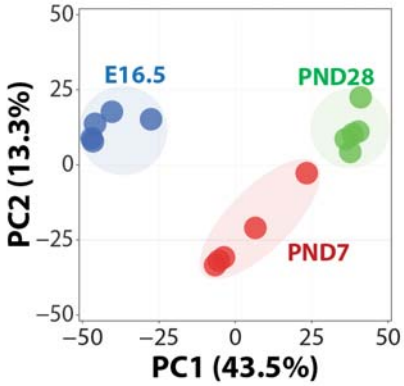
E16.5

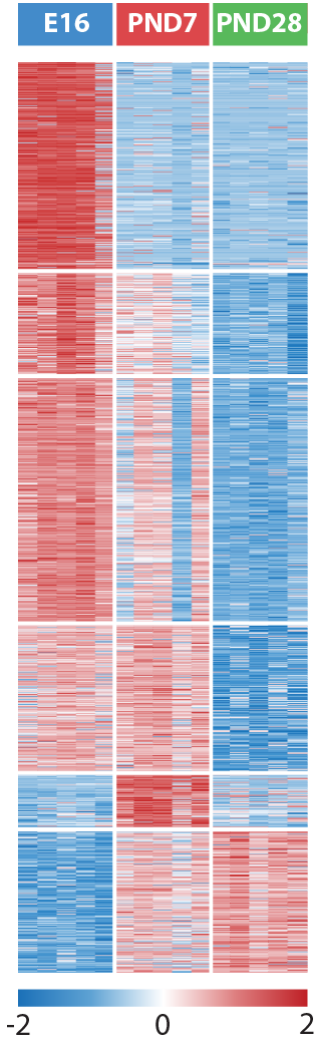
PND7

PND28

PCA



Segregation of the biological replicates by time-points



>3,400 identified proteins

1
GO:0006412 Translation (37)
GO:0022900 Electron transport chain (14)
GO:0022403 Cell cycle phase (12)

2
GO:0007049 Cell cycle (13)
GO:0006412 Translation (22)
GO:0006350 Transcription (17)

3
GO:0006412 Translation (37)
GO:0006350 Transcription (34)
GO:0006396 RNA processing (31)

4
GO:0009611 Response to wounding (23)
GO:0031960 Response to hormones (15)
GO:0010941 Regulation of cell death (8)

5
GO:0030832 Regulation of actin (7)
GO:0030029 Actin filament-based process (7)

6
GO:0007155 Cell adhesion (25)
GO:0055114 Oxidation reduction (22)
GO:0006955 Immune response (11)

Z-score

Clair *et al. Scientific report* 2016.

Alveolar tissue proteomics during development

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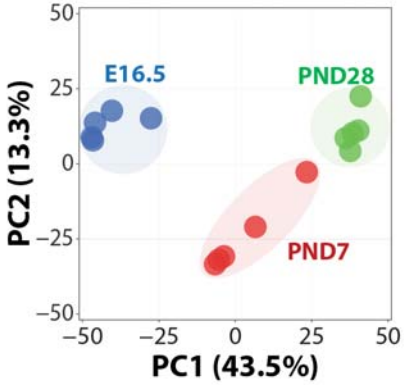
E16.5

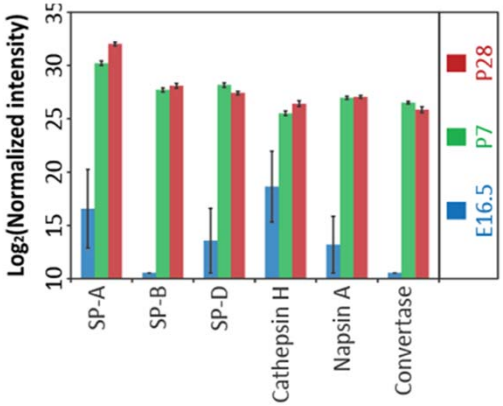
PND7

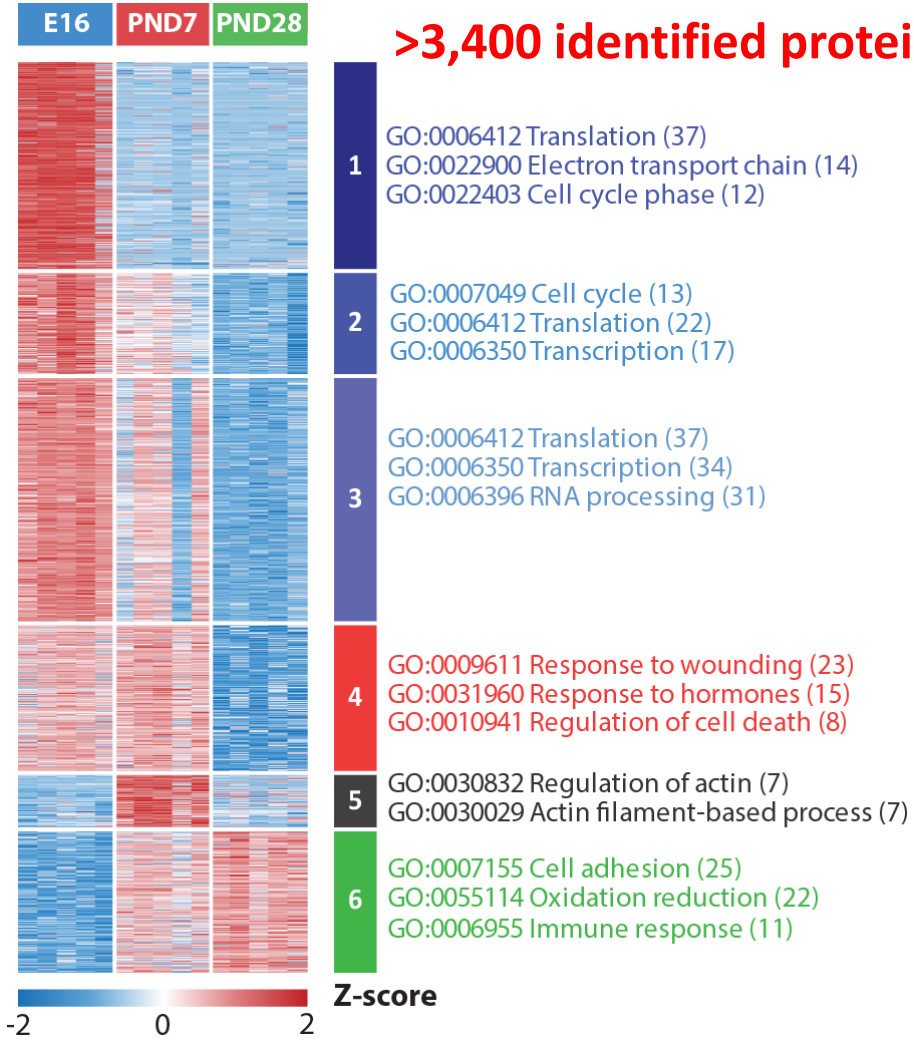
PND28

PCA



Segregation of the biological replicates by time-points





>3,400 identified proteins

Clair *et al. Scientific report* 2016.

> 300 low abundance regulatory molecules modulated during the alveolarization process

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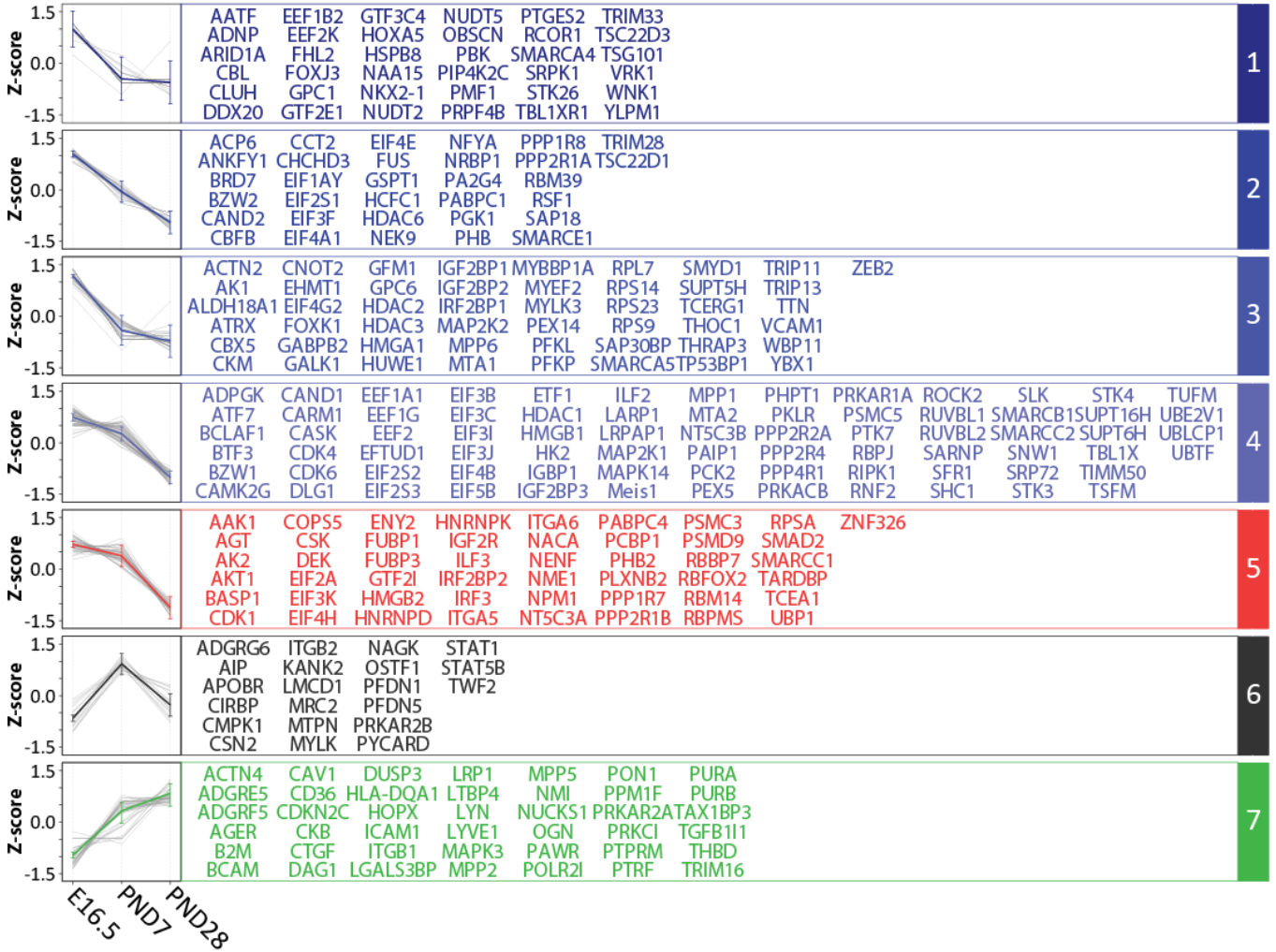
E16.5

PND7

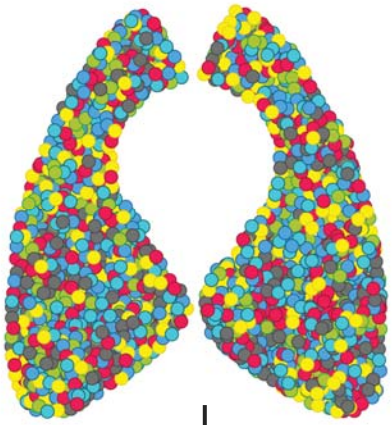
PND28



Clair *et al. Scientific report* 2016.

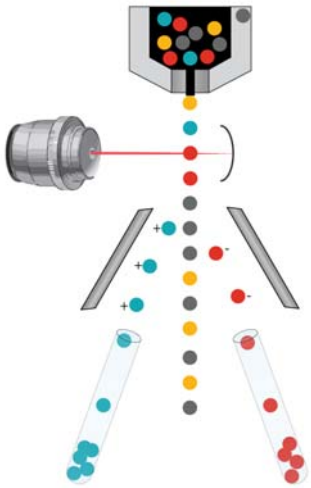
Sorting lung cell population

3 donors
20 month-old
females

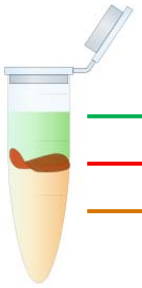


Homogenization

Unsorted mixed population	PMX
Endothelial cells	END
Epithelial cells	EPI
Mixed immune cells	MIC
Mesenchymal cells	MES



FACS cell sorting



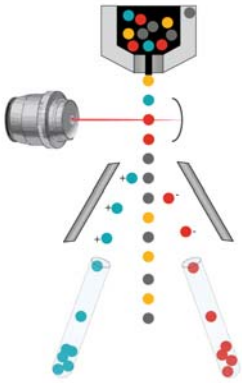
MPLeX

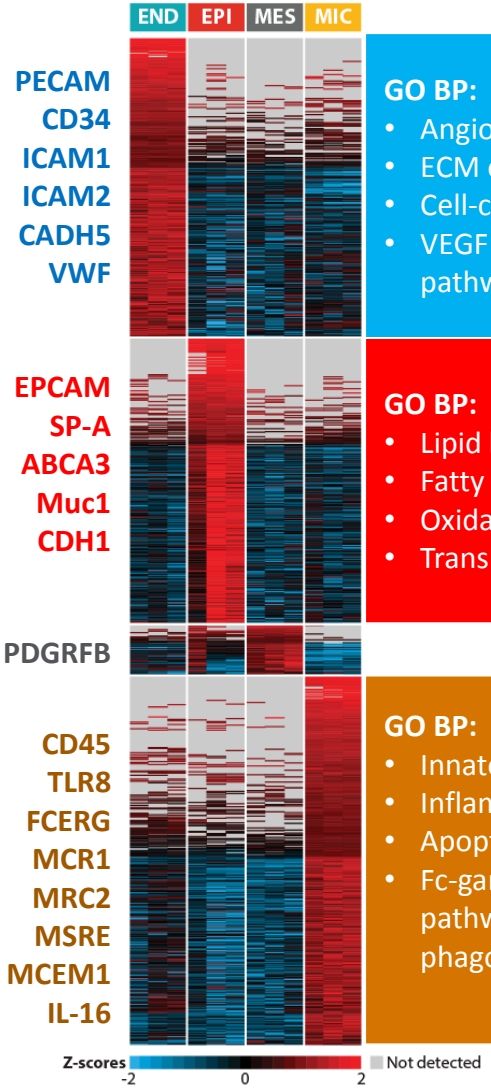
Polar metabolites
Proteins
Lipids

Mass Spectrometry
based Omics

Sorted-cells proteomics

3 donors
20 month-old
females





GO BP:
- Angiogenesis
- ECM organization
- Cell-cell adhesion
- VEGF receptor signaling pathway

In line with :
physiological role in angiogenesis and vascular permeability

GO BP:
- Lipid biosynthetic process
- Fatty acid metabolic process
- Oxidation-reduction
- Translation

In line with :
physiological role in surfactant production

GO BP:
- Innate immune response
- Inflammatory response
- Apoptotic process
- Fc-gamma receptor signaling pathway involved in phagocytosis

In line with :
physiological role in immune responses

Surface antigens in our proteomics data

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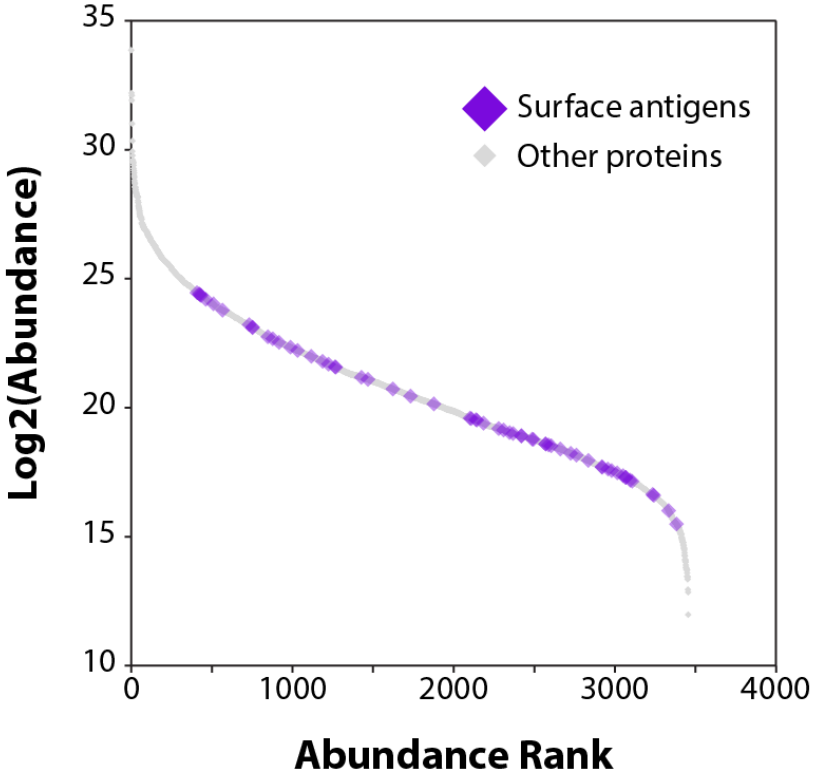
Our data contains a lot of surface antigens that could be use to further sort sub-cell types



Surface antigens enriched in epithelial cells

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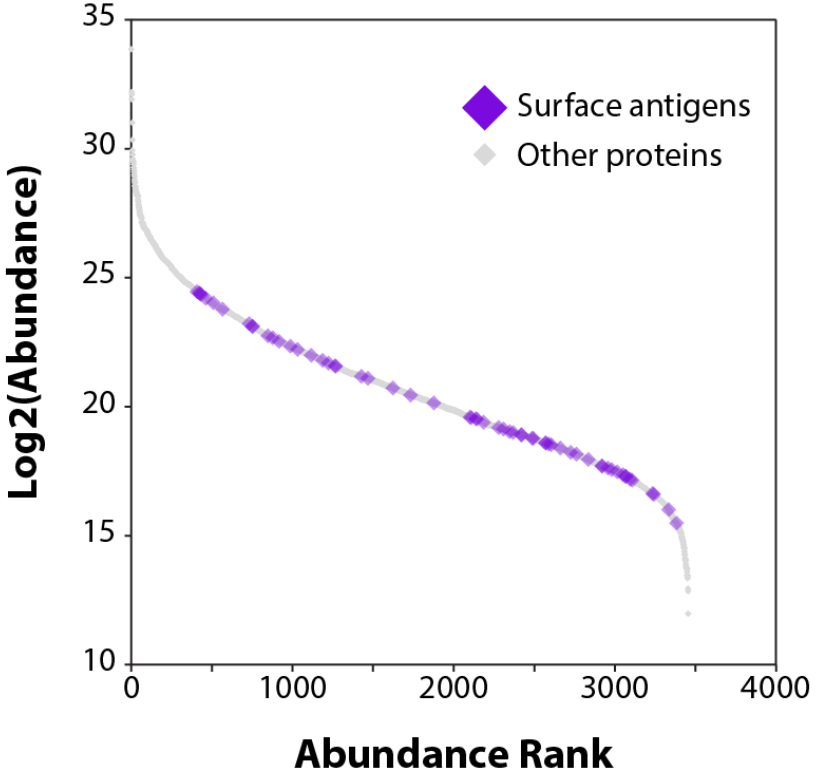
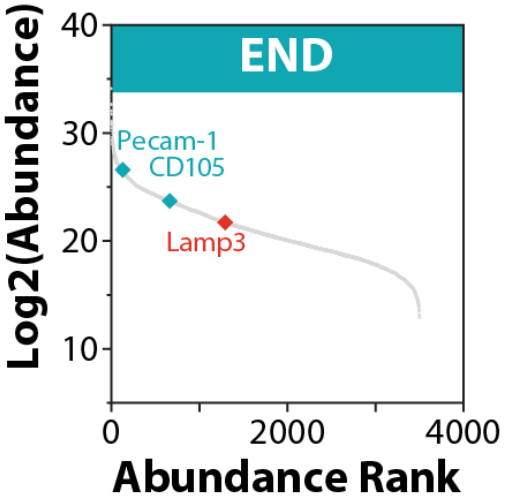
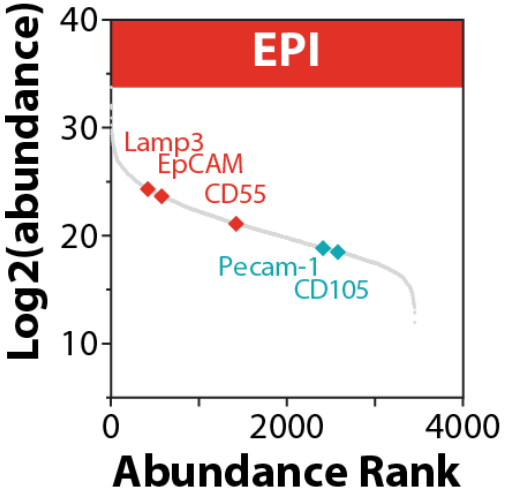
Our data contains a lot of surface antigens that could be use to further sort sub-cell types







Surface antigens enriched in epithelial cells could help to further sort sub-populations

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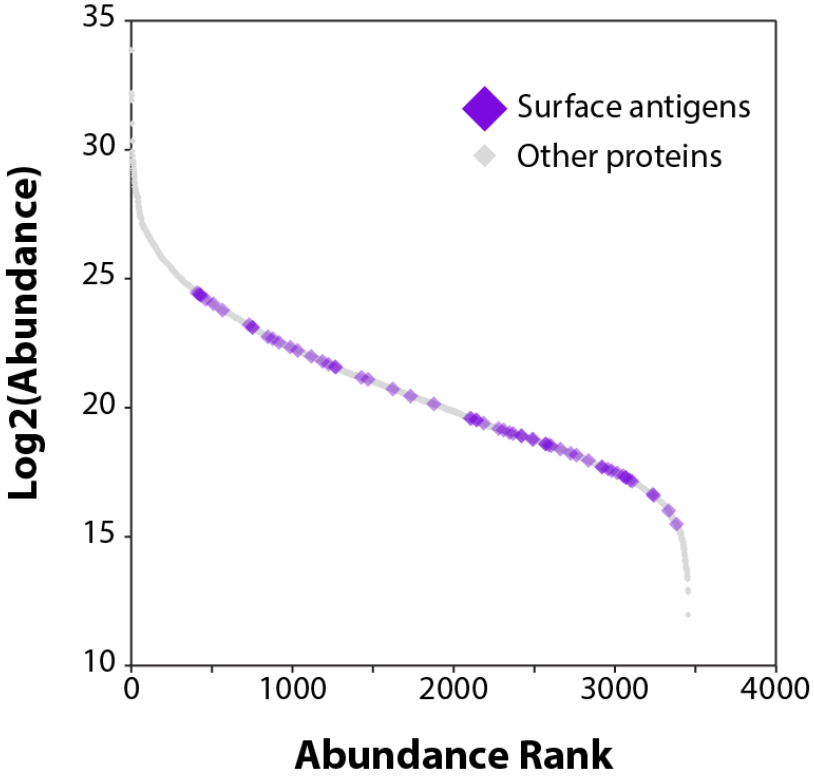
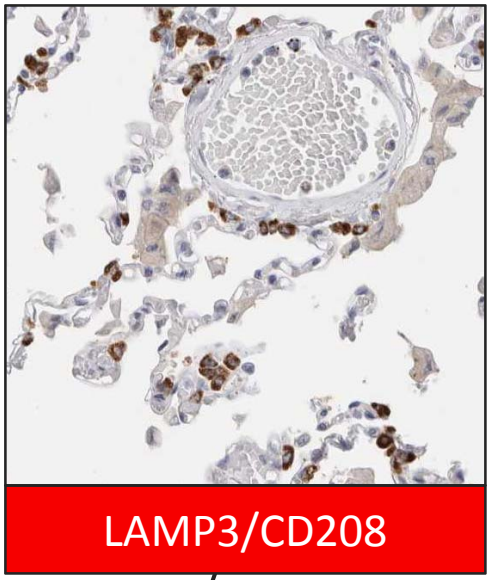
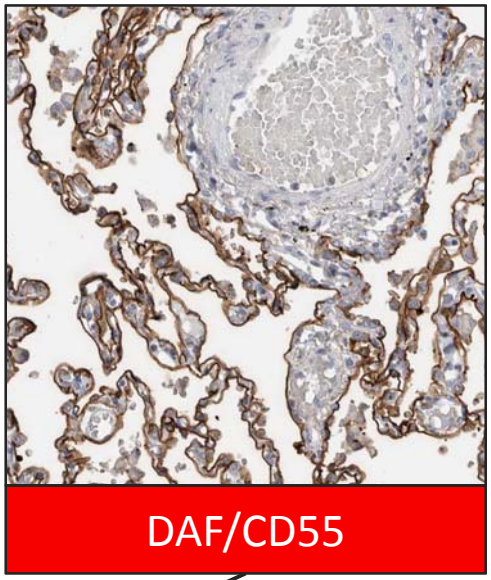
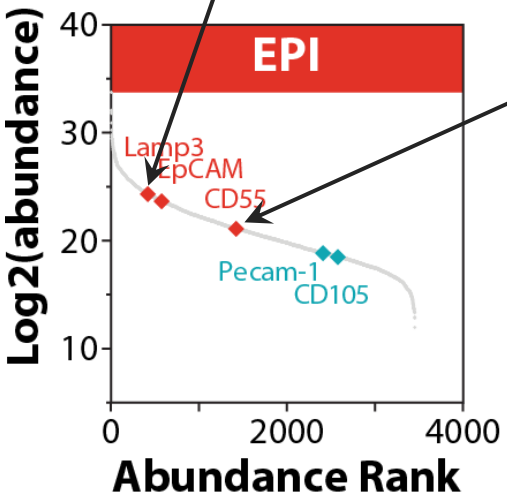
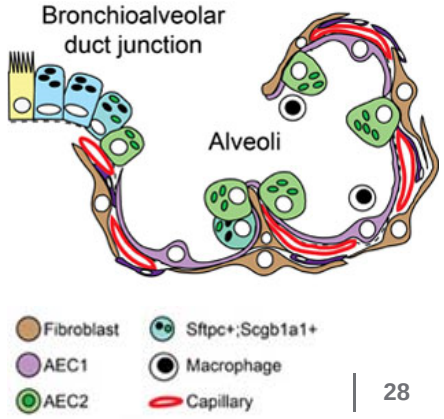
Our data contains a lot of surface antigens that could be use to further sort sub-cell types







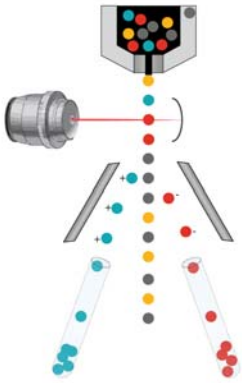


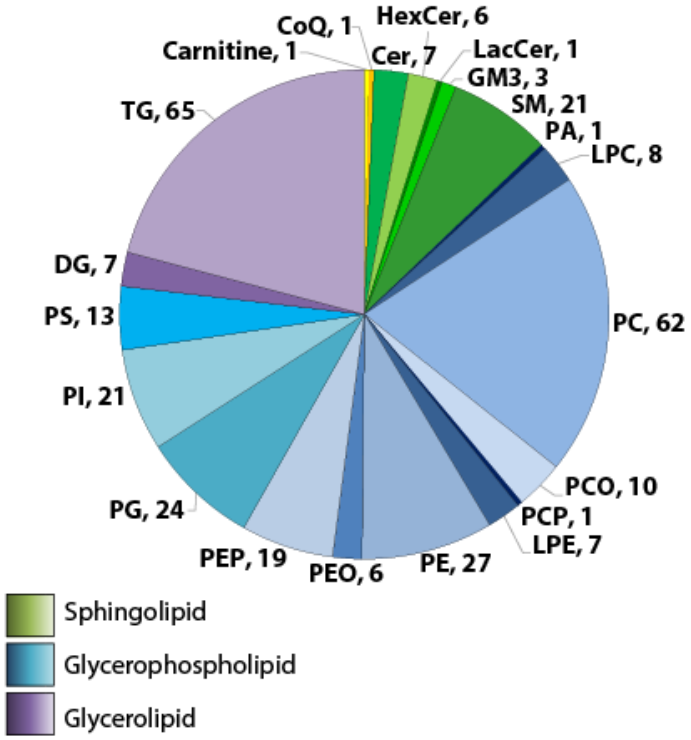


Human tissue imaging from : www.proteinatlas.org

Lipidomics of sorted cells

3 donors
20 month-old
females

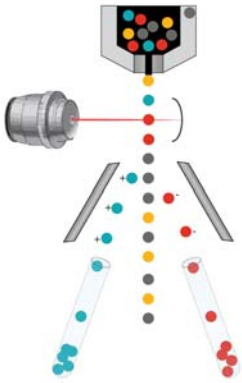


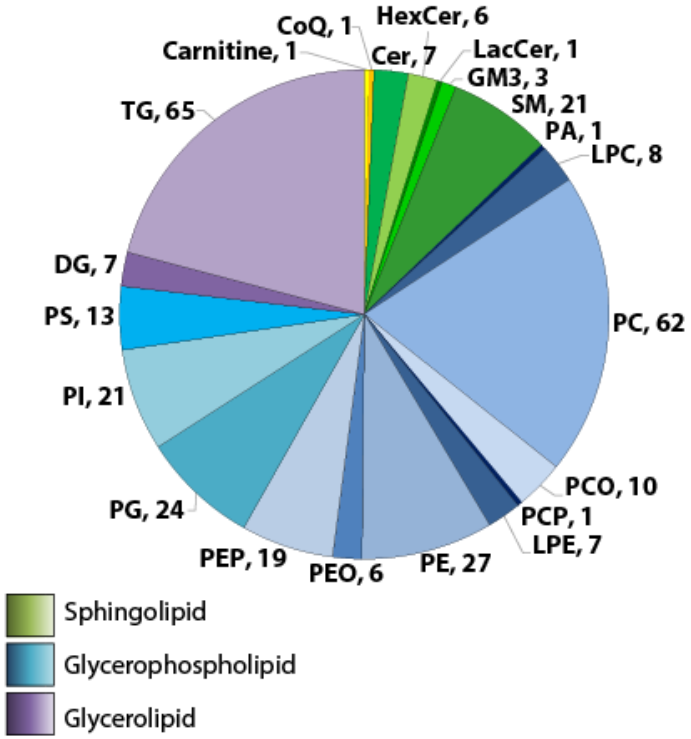


299 lipids identified
across 21 subclasses

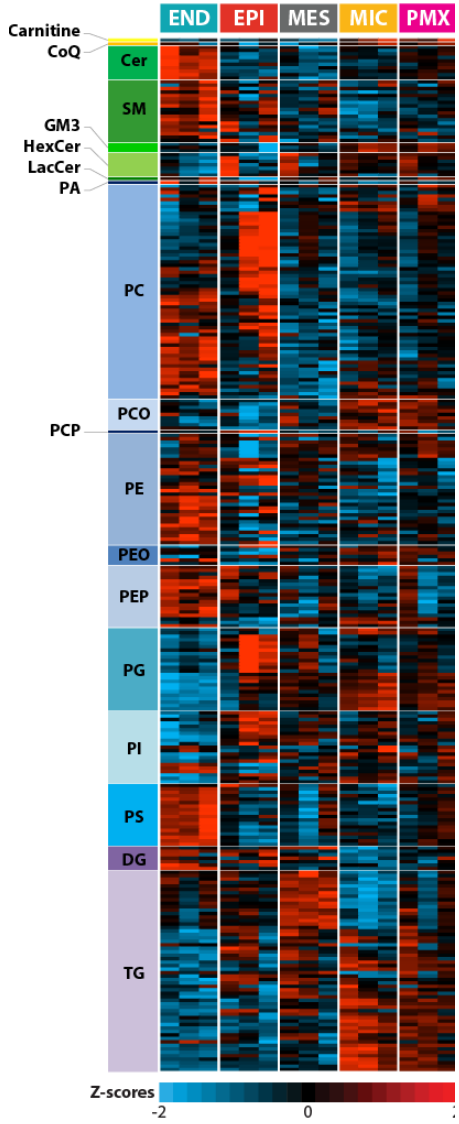
Lipidomics of sorted cells

3 donors
20 month-old
females



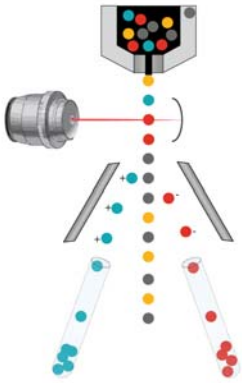


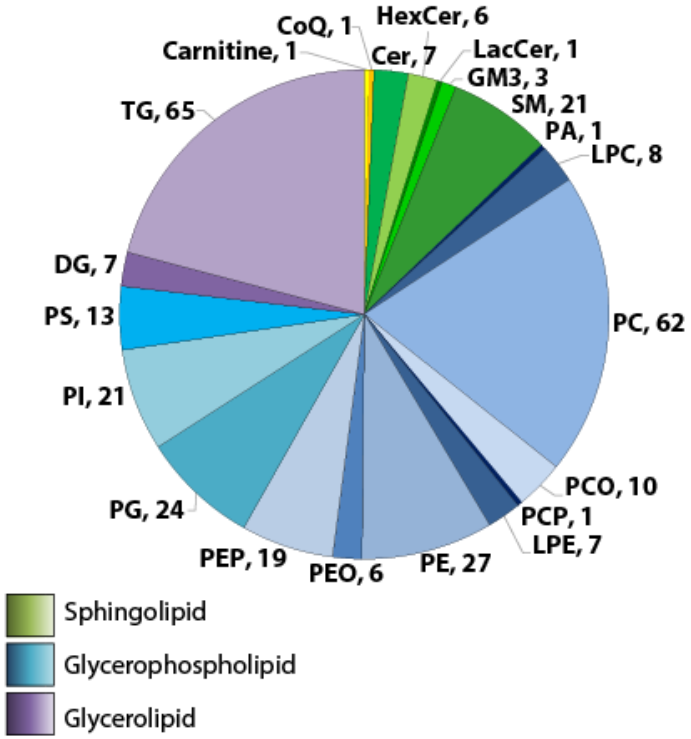
299 lipids identified
across 21 subclasses



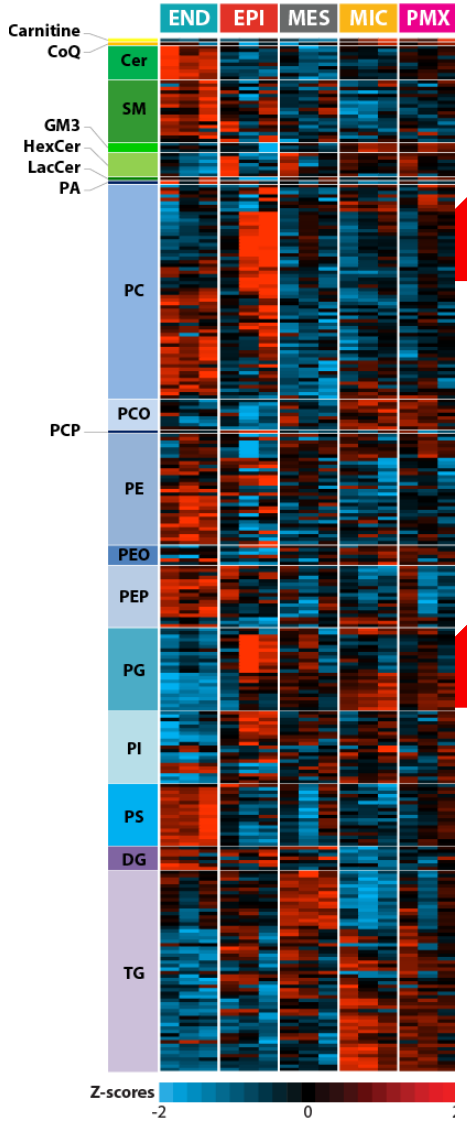
Surfactant lipids are enriched in EPI population

3 donors
20 month-old
females





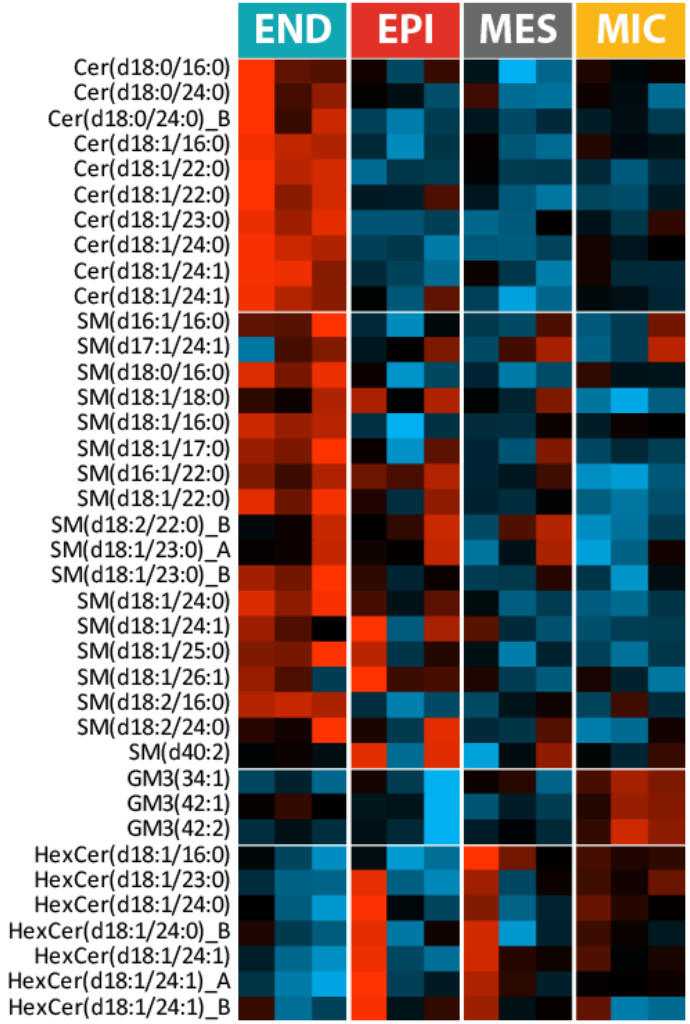
299 lipids identified across 21 subclasses



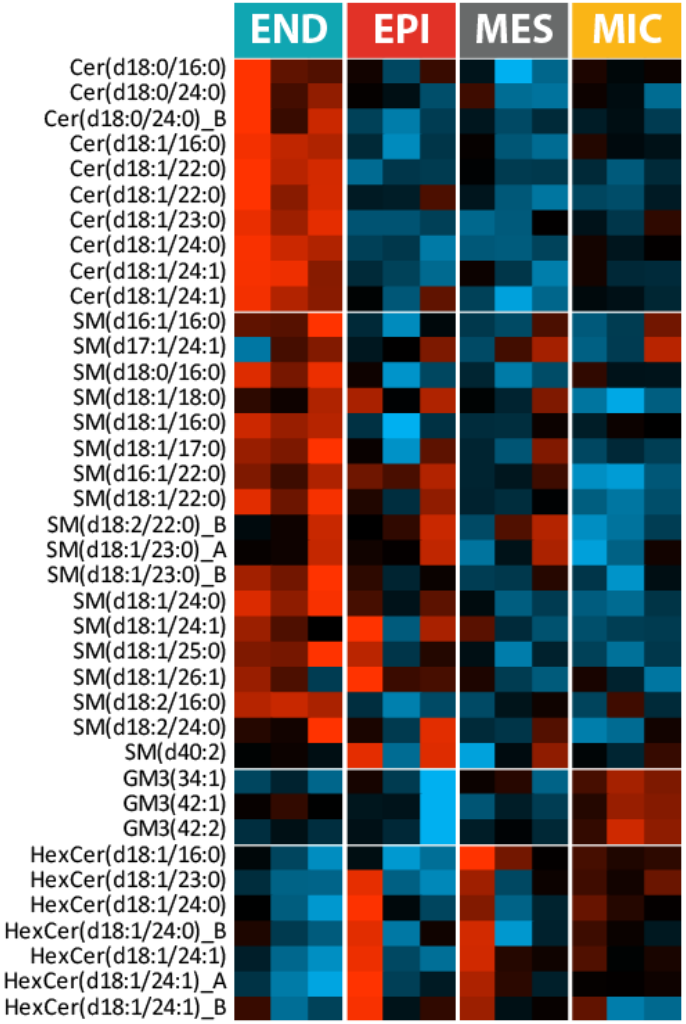
PC(16:0/16:0)
PC(16:0/14:0)

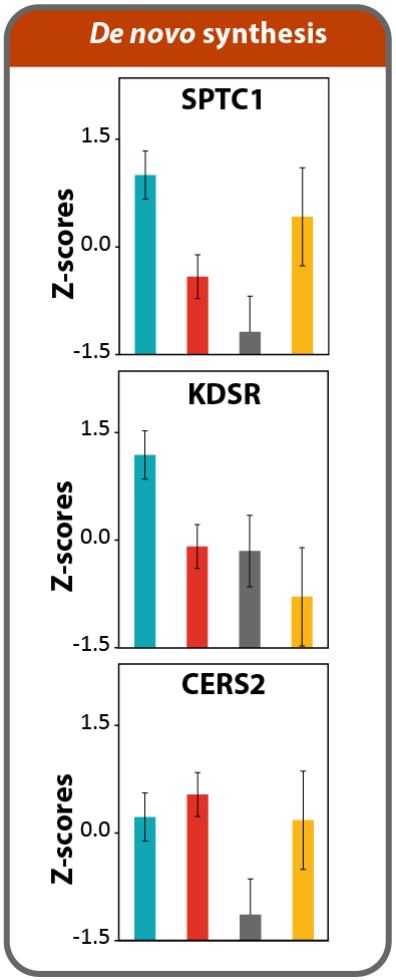
PG(16:0/16:0)
PG(16:0/14:0)

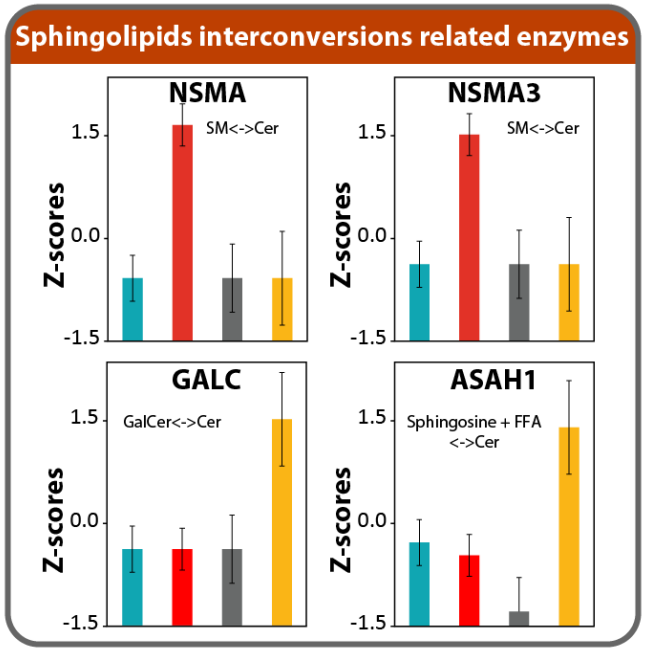
Sphingolipids origin in lungs



Sphingolipids origin in lungs

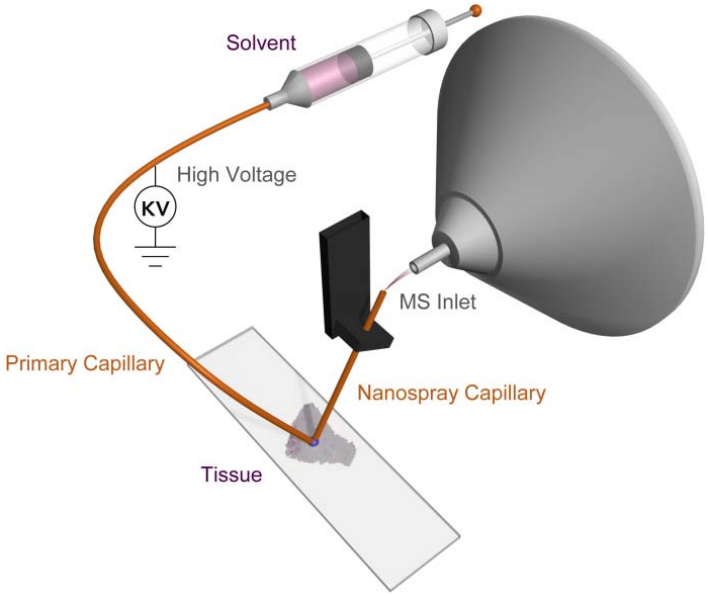






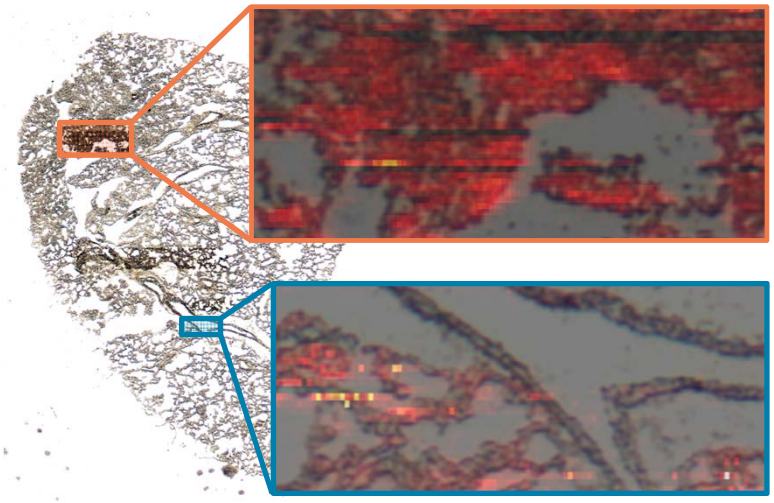
Endothelial cells
Epithelial cells
Mesenchymal cells
Mixed immune cells

Mass spectrometry imaging (Nano-DESI)

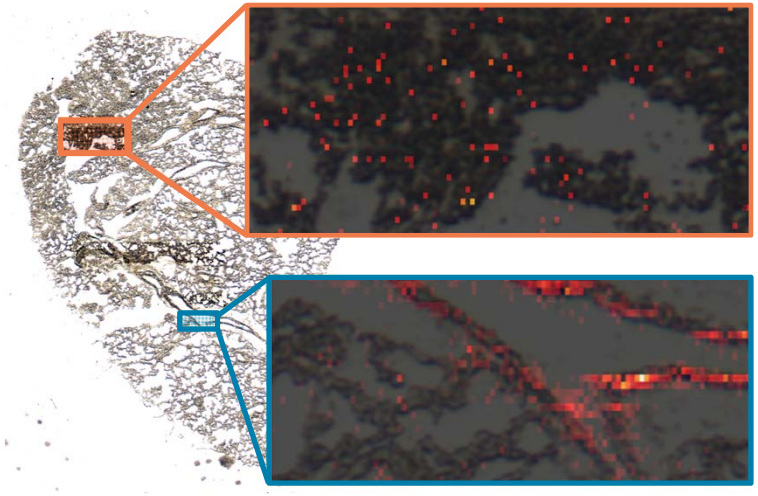


- Images hundreds of lipids and metabolites simultaneously
- No sample pre-treatment
- High spatial resolution (~10μm)
- Accurate quantification

PC(16:0/16:0)
Surfactant lipid
m/z 756.5520



PC(16:0/22:6)
Structural?
m/z 828.5520



PNNL in LungMAP consortium

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>10,000 proteins

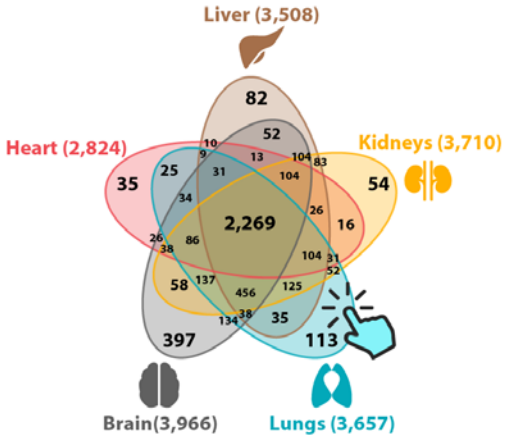
>800 lipids

>150 polar metabolites

Developmental expression

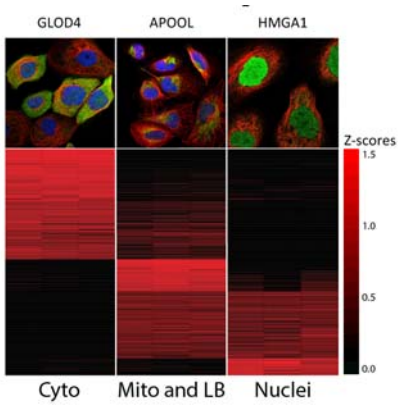
Alveolar tissue expression

Organ specificity



Cell specificity

Sub-cellular localization



ABCA3 responsible of pediatric lung disease

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American Thoracic Society
Publisher's Version of Article
Issue Featuring Article
Submissions
American Journal of Respiratory and Critical Care Medicine®

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Published online 2005 Jun 23. doi: 10.1164/rccm.200503-504OC
PMCID: PMC1403838
NIHMSID: NIHMS8259

ABCA3 Mutations Associated with Pediatric Interstitial Lung Disease

Janine E. Bullard, Susan E. Wert, Jeffrey A. Whitsett, Michael Dean, and Lawrence M. Nogee

Author information ► Article notes ► Copyright and License information ►

Abstract

Go to:

Rationale: ABCA3 is a member of the ATP-binding cassette family of proteins that mediate the translocation of a wide variety of substrates, including lipids, across cellular membranes. Mutations in the gene encoding ABCA3 were recently identified in full-term neonates with fatal surfactant deficiency.

Objective: To test the hypothesis that *ABCA3* mutations are not always associated with fatal neonatal lung disease but are a cause of pediatric interstitial lung disease.

Methods: DNA samples were obtained from 195 children with chronic lung disease of unknown etiology. The 30 coding exons of the *ABCA3* gene were sequenced in four unrelated children with a referring diagnosis of desquamative interstitial pneumonitis and who were older than 10 years at the time of enrollment.

Results: Three of four patients (ages 16, 23, and 11 years) with desquamative interstitial pneumonitis had *ABCA3* mutations identified on both alleles. All three had the same missense mutation (E292V) and a second unique mutation. The E292V mutation was not found on 200 control alleles from adults without lung disease, but seven additional patients of the remaining study patients had the E292V mutation on one allele. Immunohistochemical analysis of surfactant protein expression in three patients revealed a specific staining pattern for surfactant protein-B, which was the same pattern observed in several infants with fatal lung disease due to *ABCA3* mutations.

LungMAP ABCA3 protein ID Card

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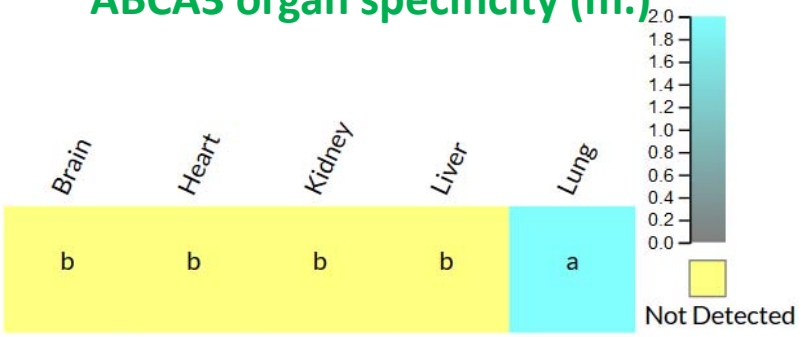
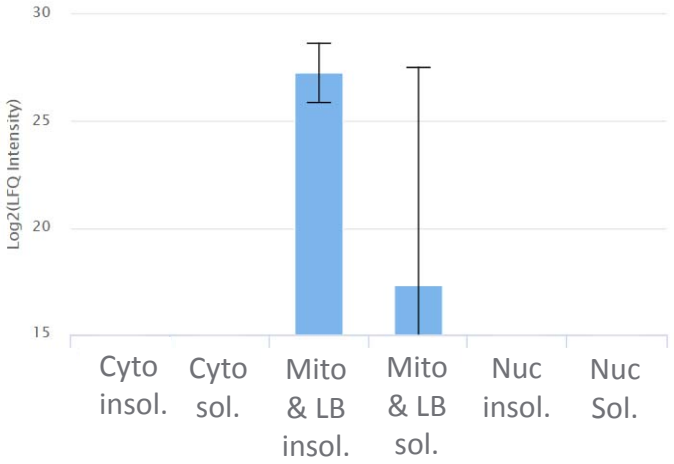
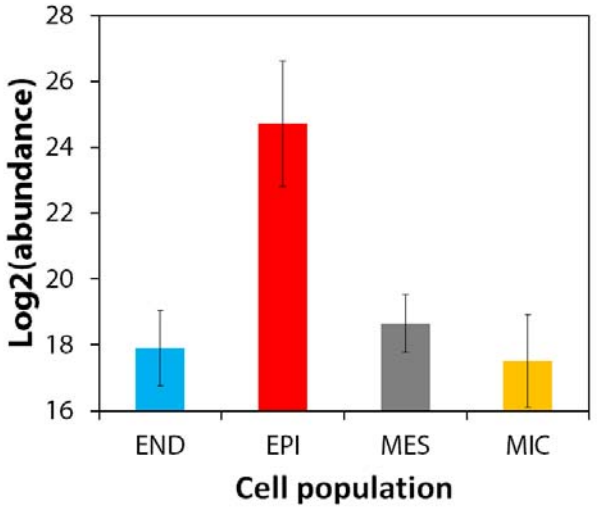
ABCA3 organ specificity (m.)



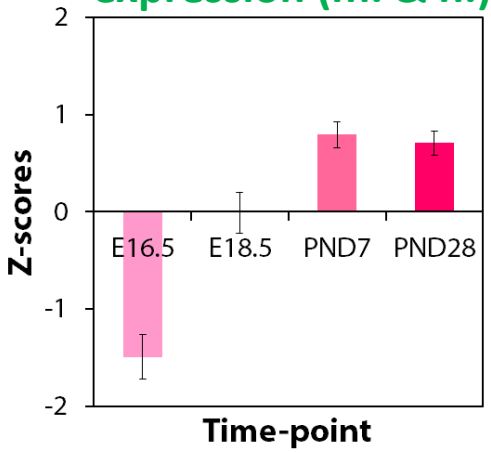
ABCA3 Sub-cellular localization (m.)



ABCA3 Cell-type specificity (h.)



ABCA3 whole lung expression (m. & h.)



IF imaging

mRNA expression

Etc.

MS-LungMAP website

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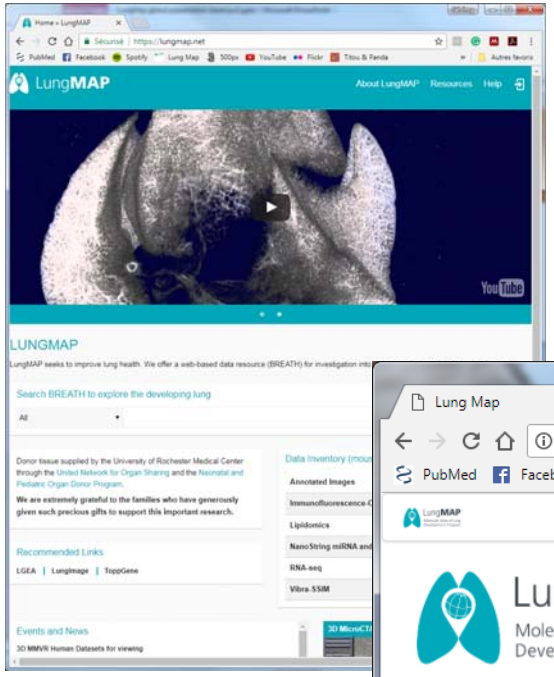
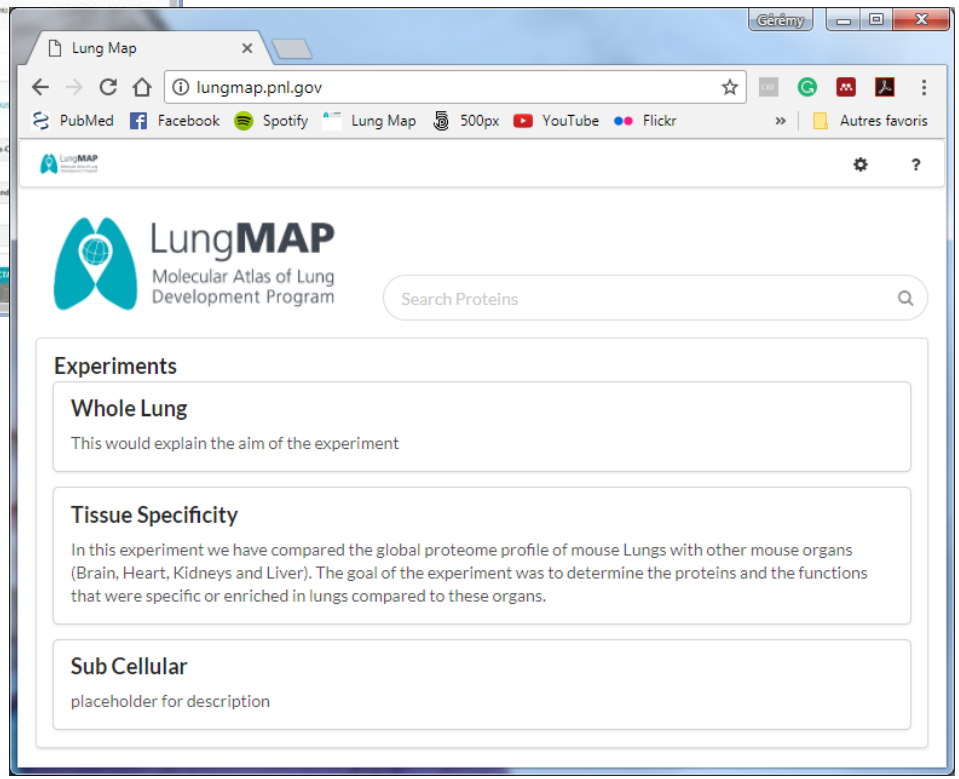
LungMAP
Molecular Atlas of Lung Development Program

lungmap.net





Acknowledgments

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LungMAP

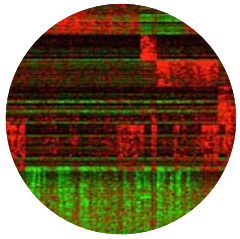
Coordinating Center

Duke & RTI

Sponsor

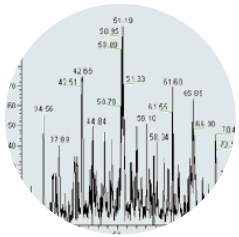
NIH National Heart, Lung, and Blood Institute

Single Cell Transcriptome Confocal Imaging



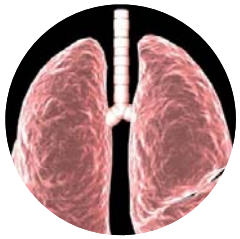
CCHMC:
Cincinnati

Proteomics, Metabolomics, Lipidomics, HT-ISH, MS Imaging



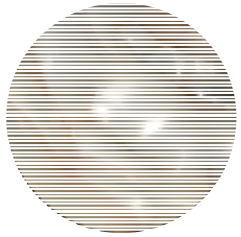
PNNL & TACC
Richland & Austin

Human Lung Tissue Procurement



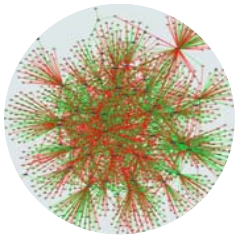
URMC:
Rochester

3-D Imaging



Saban Inst.
CHLA & USC

Systems Biology



UAB & Yale
& Pitt & UCSD

PNNL LungMAP Awesome team!

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Ahmed Mohieb
Son Nguyen
Sydney Dautel
Erika Zink
Ryan Sontag
Julia Laskin
Jason McDermott
Richard Corley