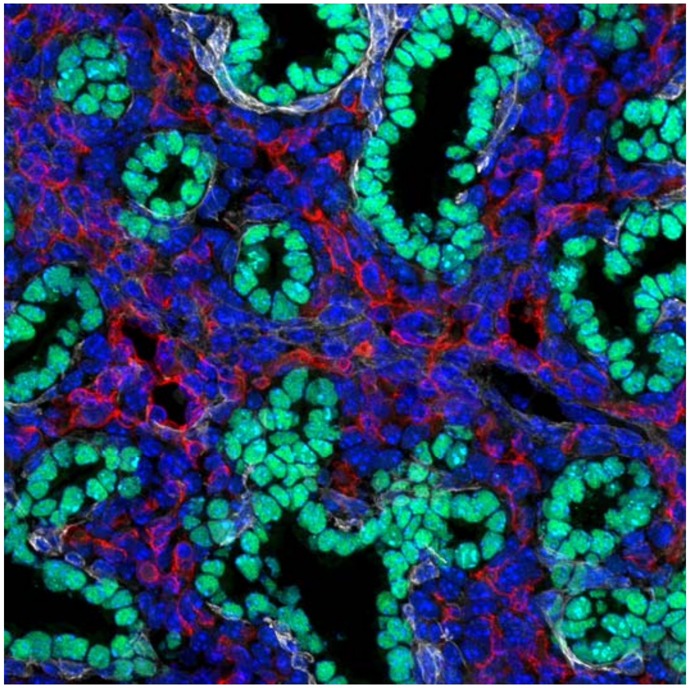
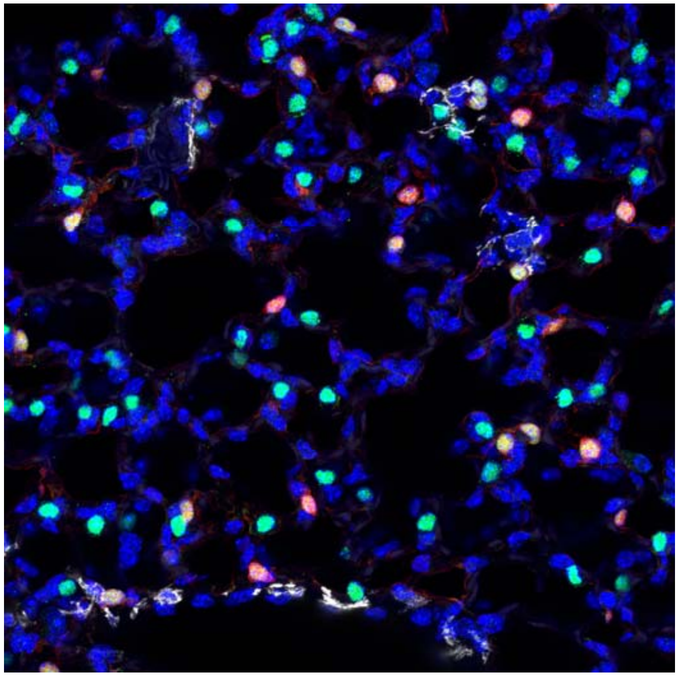
SanDiego 2017:Rare Pediatric Lung Disease

Single Cell Expression Analysis to Understand Interstitial Pulmonary Diseases



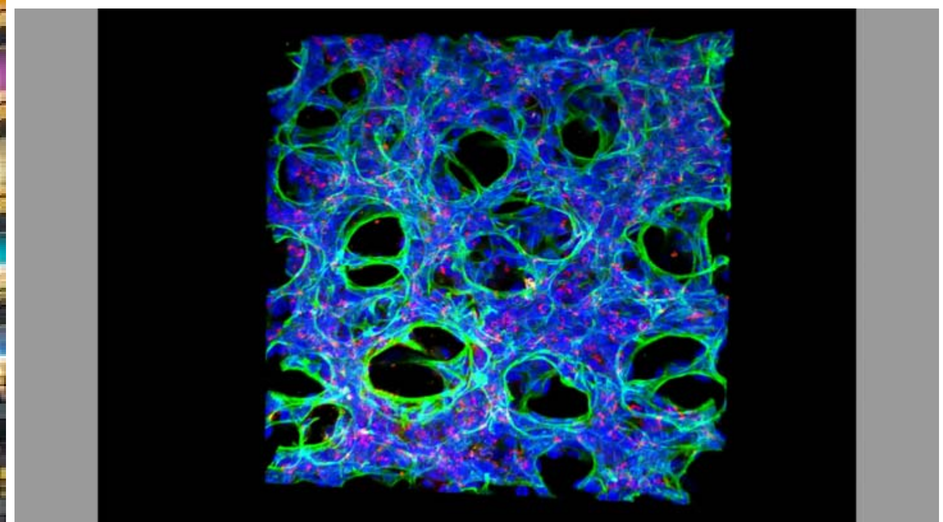


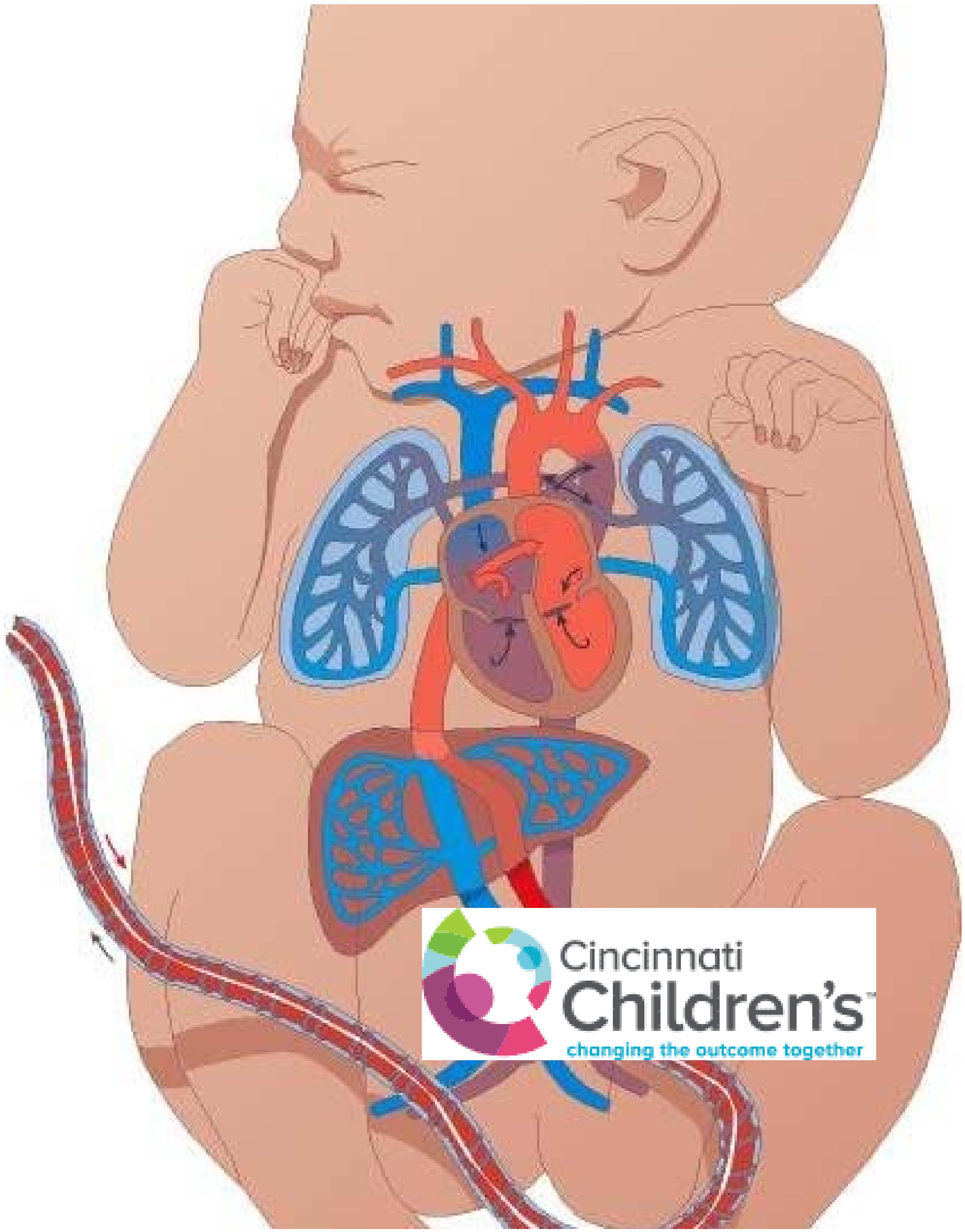
Jeff Whitsett: Cincinnati Children’s Hospital Medical Center

jeff.whitsett@cchmc.org

Cincinnati Children's™

changing the outcome together





Cincinnati Children's
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Cincinnati Children's
Hospital Medical Center

Molecular Tools to Explore Lung Biology

SP-A, SP-B, SP-C, SP-D

↓

cDNAs, genes

↓

Cell specific promoters

↓

Lung specific gene expression

↓

transcriptional networks
Conditional gene expression/deletion

↓

Disease models

↓

Diagnosis/Therapy
Genetic Basis of "Idiopathic Pulmonary Disease"

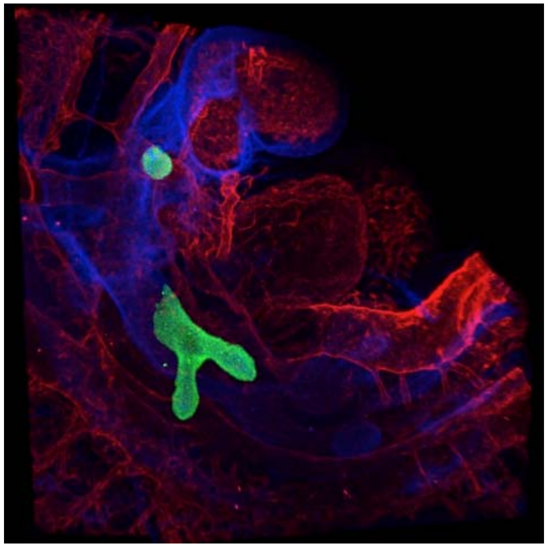
↓

"Surfactopathies"
ILD/RDS/ARDS/PAP/IPF

Cincinnati Children's
change the outcome

'Maturation' of the Alveolar Epithelium

Lung Buds



FOXA2, SOX17

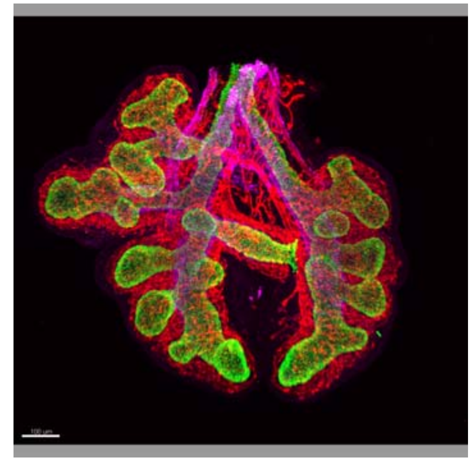
Endoderm

E10

Foregut

E12

Branching Morphogenesis



NKX2-1

Respiratory epithelium

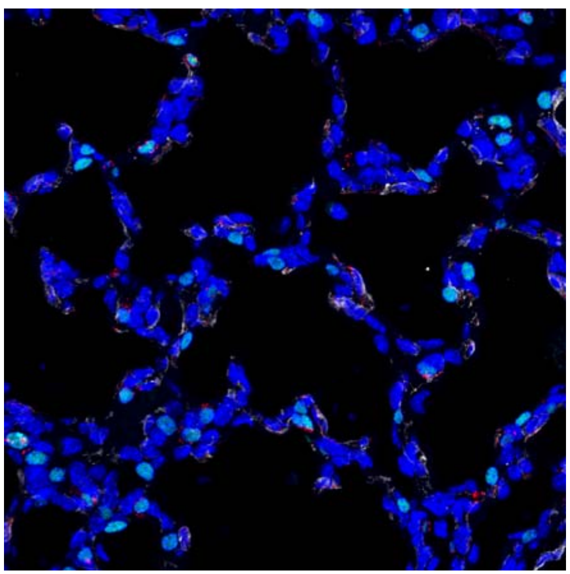
Proximal Progenitors

SOX2

Peripheral Progenitors

SOX9, NKX2-1

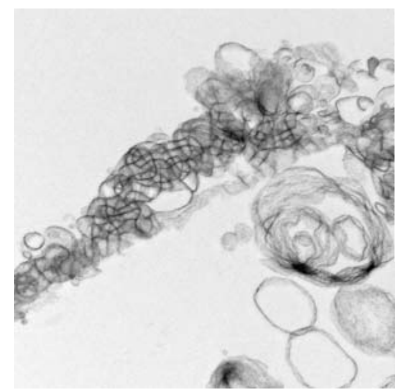
PND7



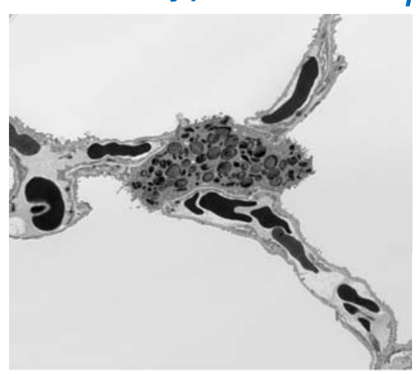
HOPX/AGER/AQP5

Alveolar Type I

Alveolar Type II



Alveoli

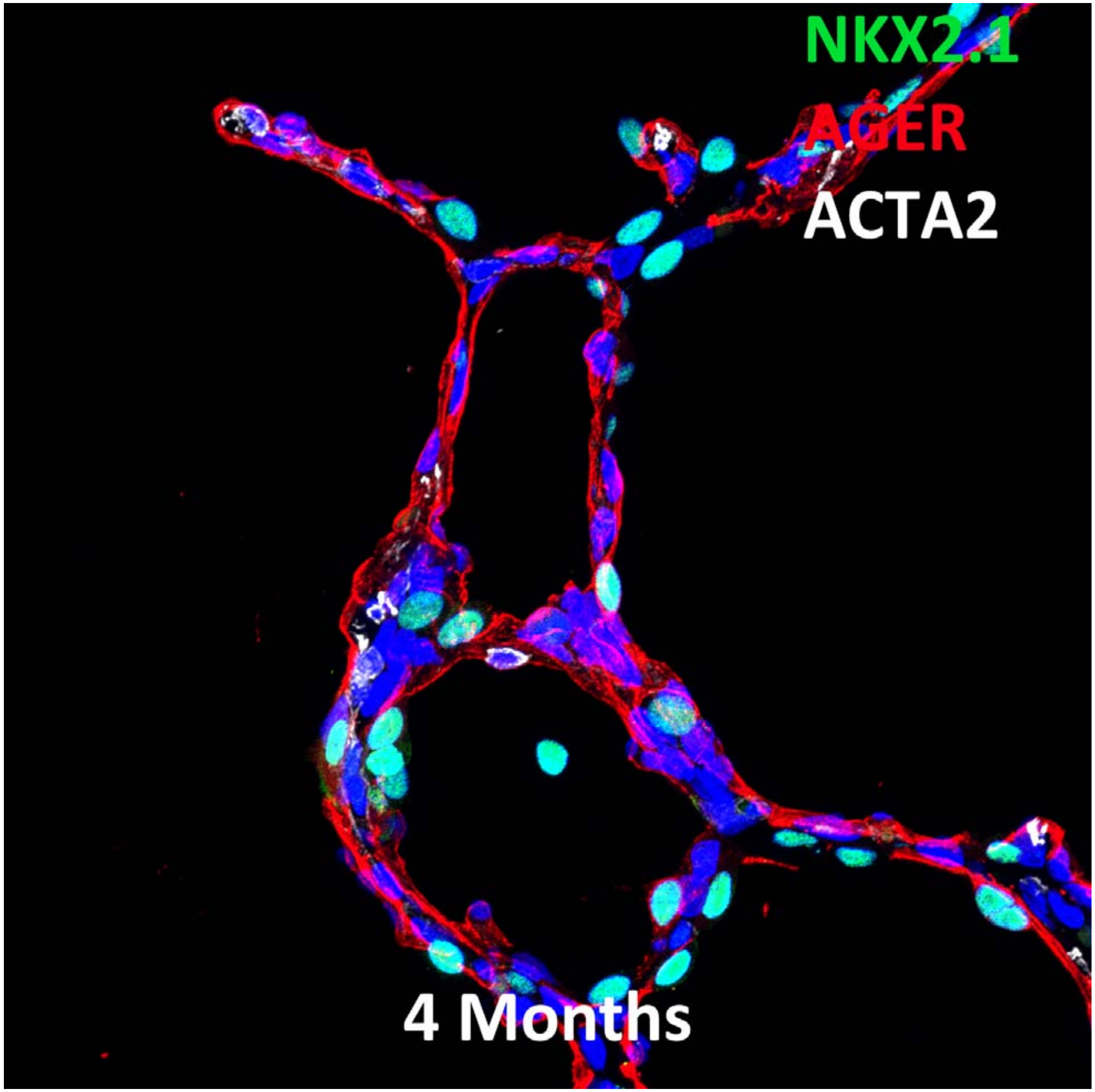


Alveolar Surfactant

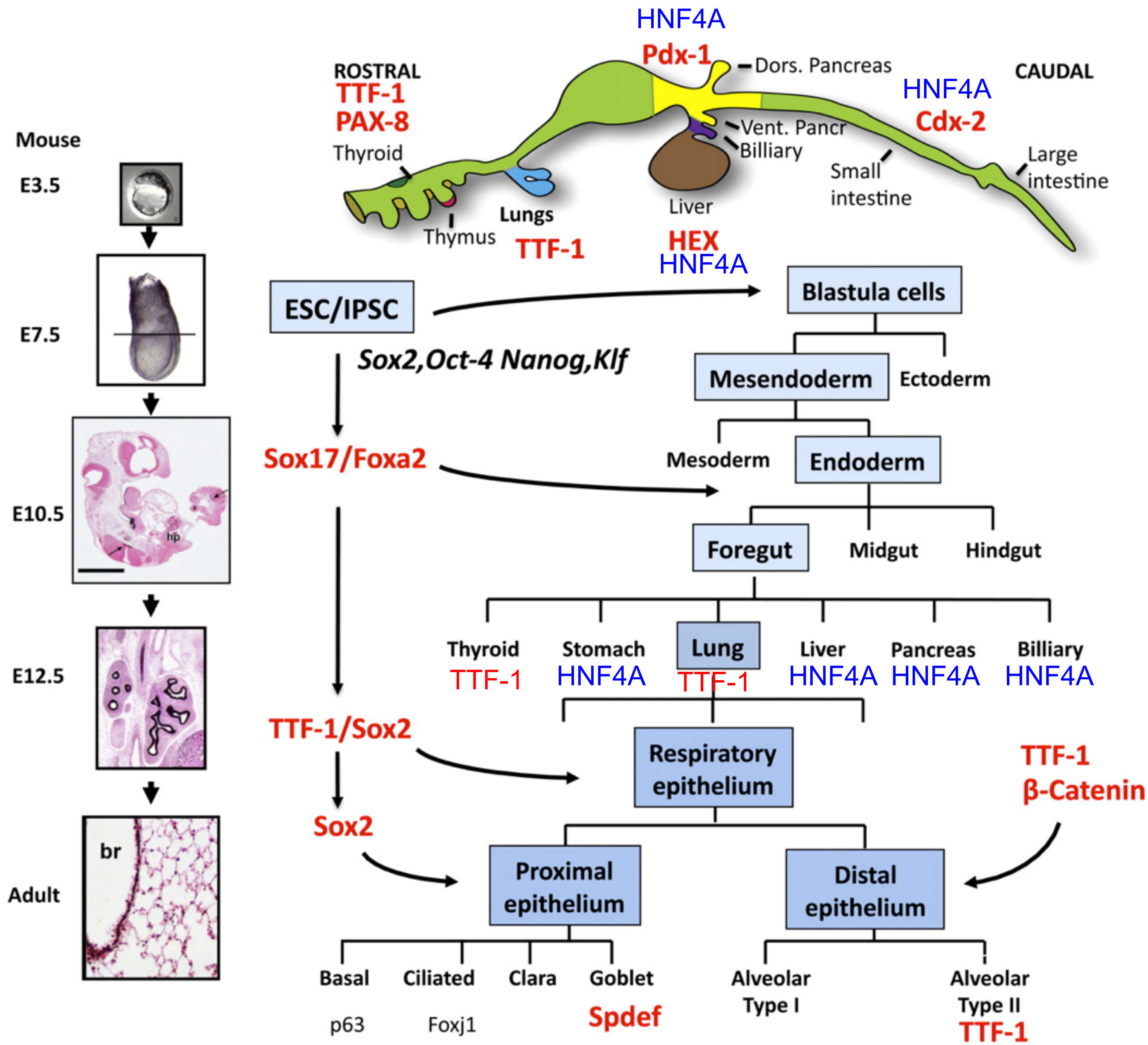
SFTPA/B/C/D

ABCA3,GPR116,NAPSA

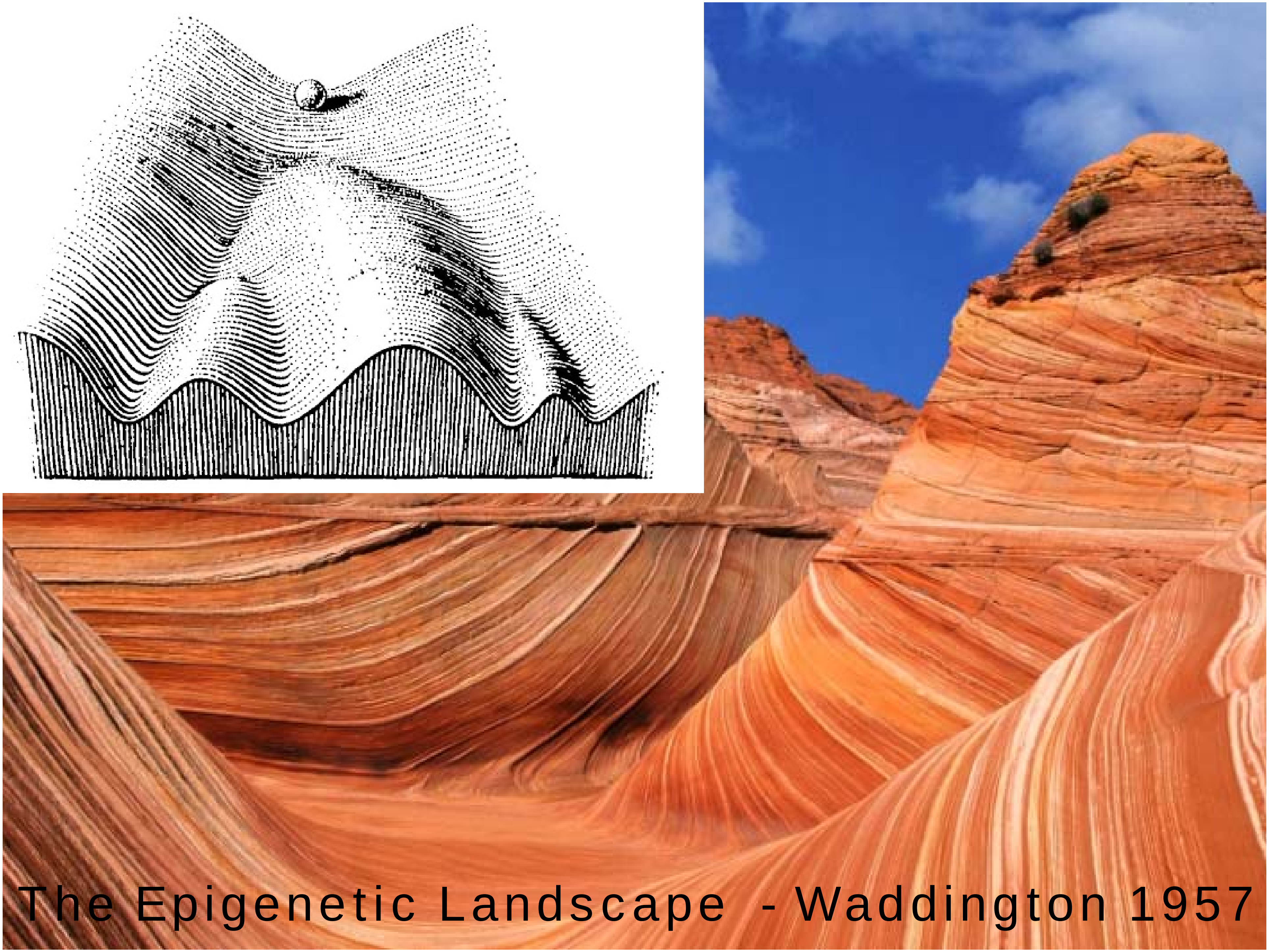
Alveolar AT1 [AGER],AT2[NKX2-1] and Myofibroblasts [ACTA2] in Human Lung



TTF-1/NKX2-1 keeps lung integrity by activating lung specific genes and suppressing liver specific genes

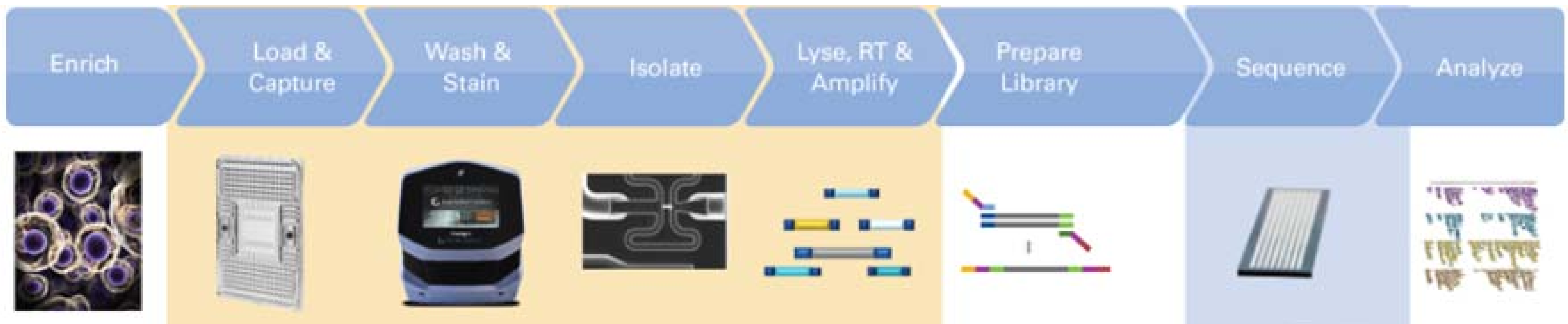


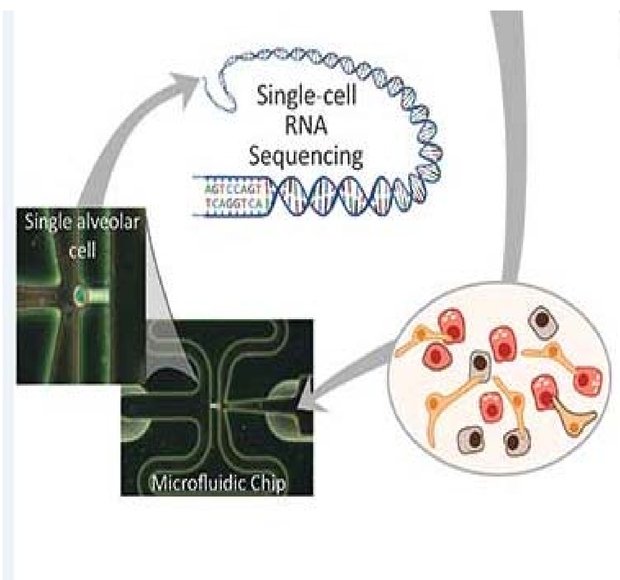
Modified from Whitsett et al., Am J Respir Crit Care Med 2011



The Epigenetic Landscape - Waddington 1957

Single Cell RNA-Seq





isolate single cells from protease-dispersed lungs

⬇

separate them using the Fluidigm C1™ Single-Cell Auto Prep System

⬇

convert RNA into a sequencing library using Fluidigm PN100-5989

⬇

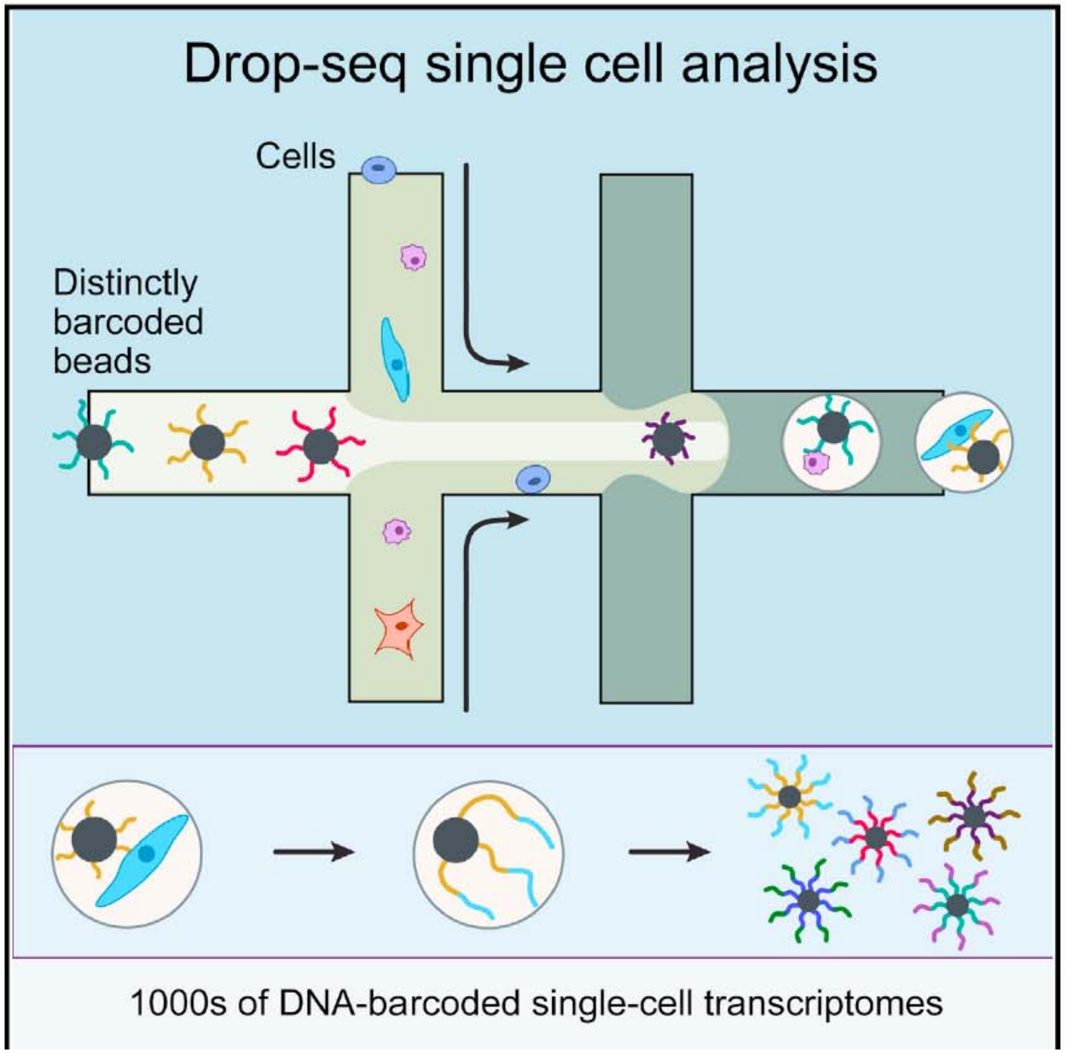
RNA-Seq on 96 individual lung cells per run on Illumina 2500 Hi-Seq

⬇

RNA-seq data QC, alignment and analysis

Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Graphical Abstract



Authors

Evan Z. Macosko, Anindita Basu, ...,
Aviv Regev, Steven A. McCarroll

Correspondence

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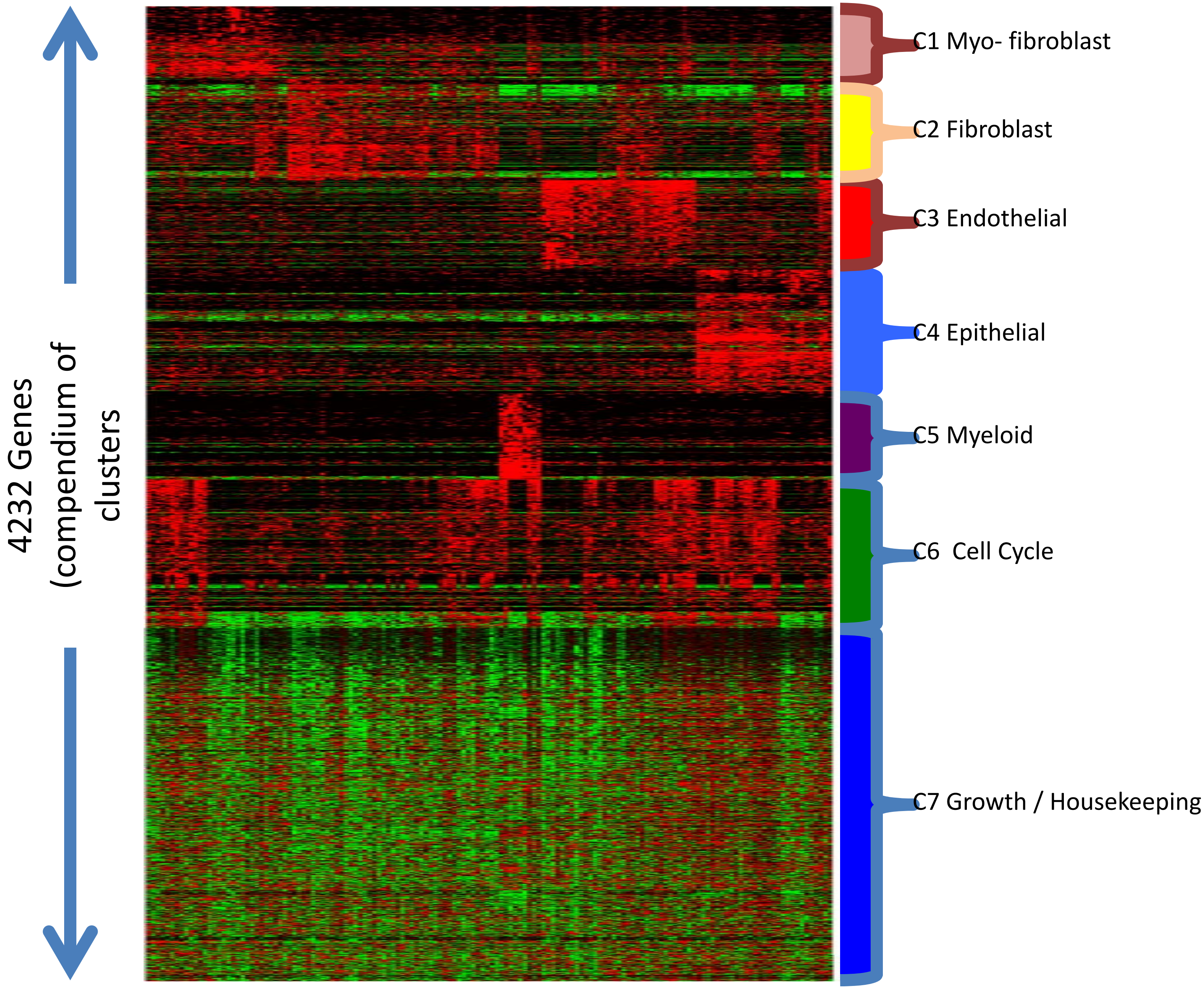
(E.Z.M.),
mccarroll@genetics.med.harvard.edu
(S.A.M.)

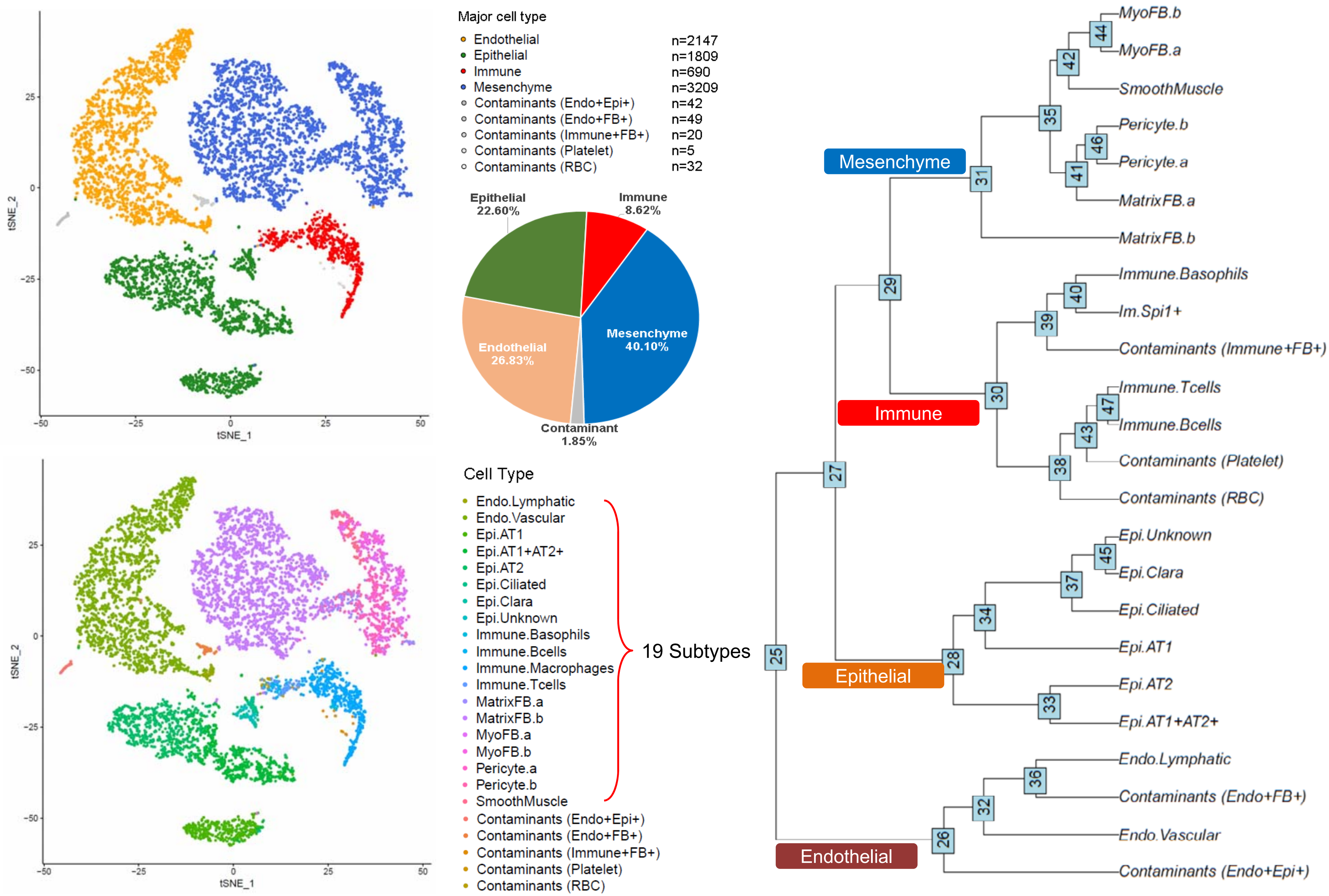
In Brief

Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers of each type.

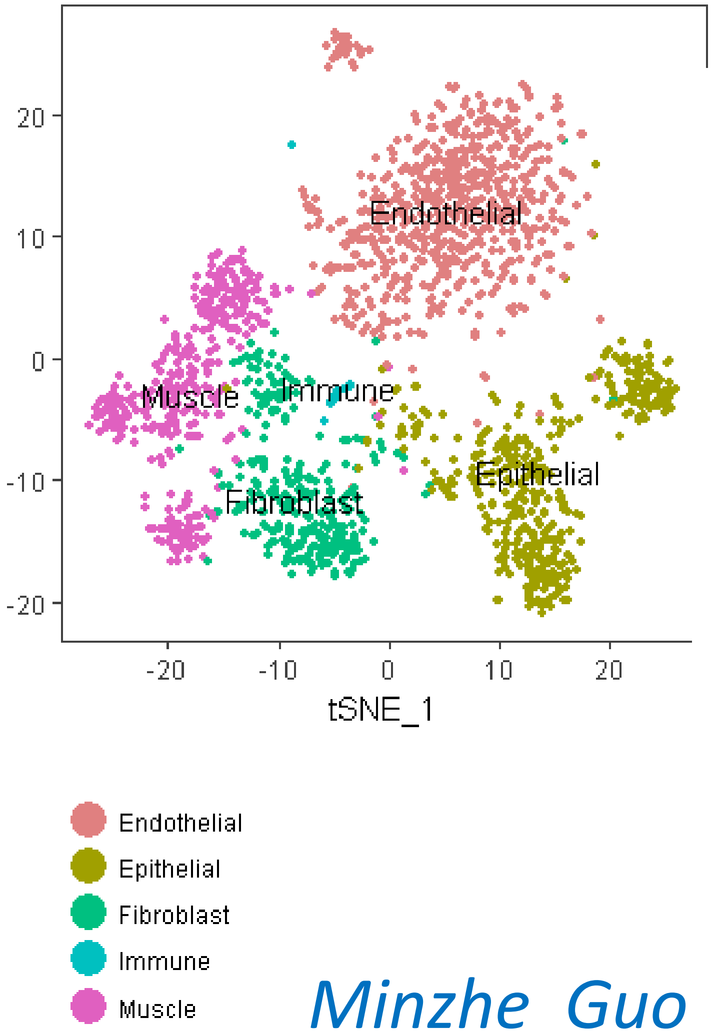


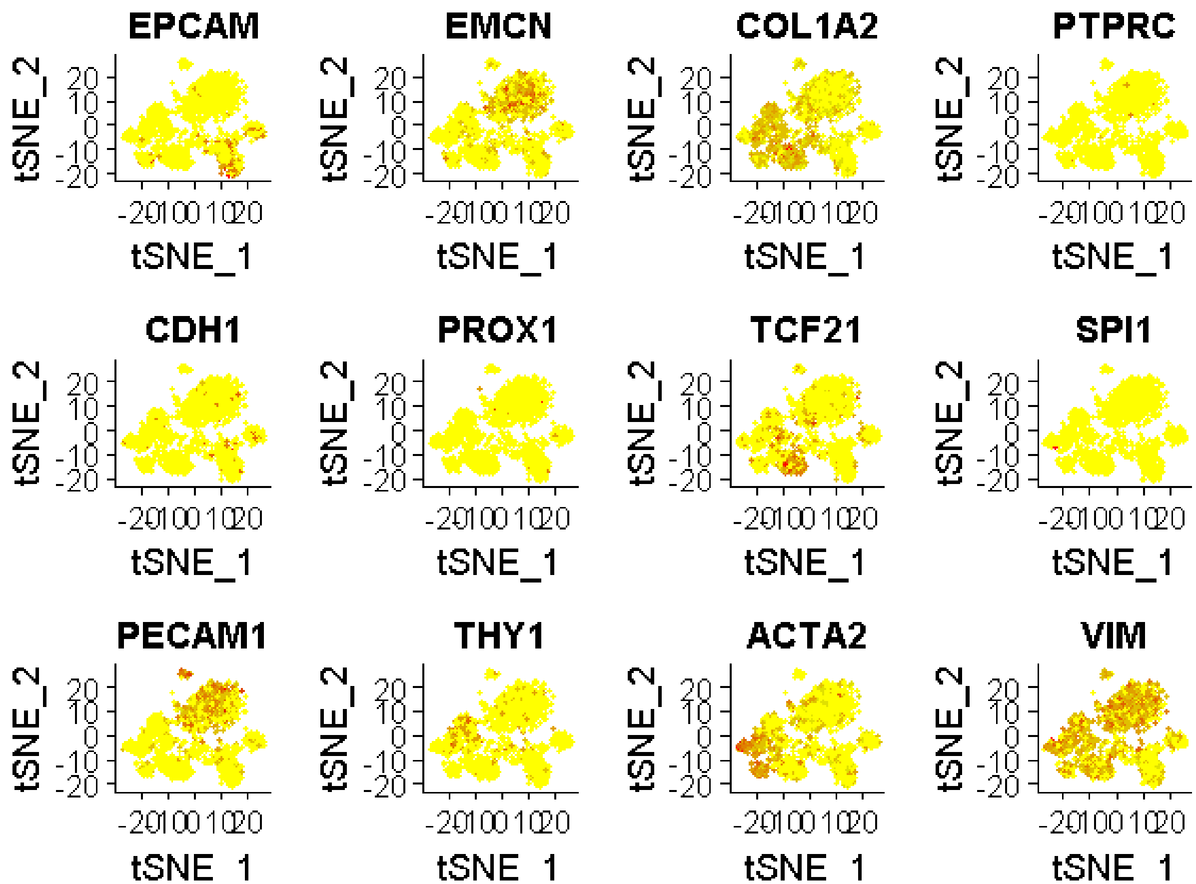
4232 Genes
(compendium of
clusters
C1 Myo- fibroblast
C2 Fibroblast
C3 Endothelial
C4 Epithelial
C5 Myeloid
C6 Cell Cycle
C7 Growth / Housekeeping

Mouse P1 Cell Types Identification



Human Infant-DAY1 Drop-seq: Major Cell Types and Marker Expression (HTC)



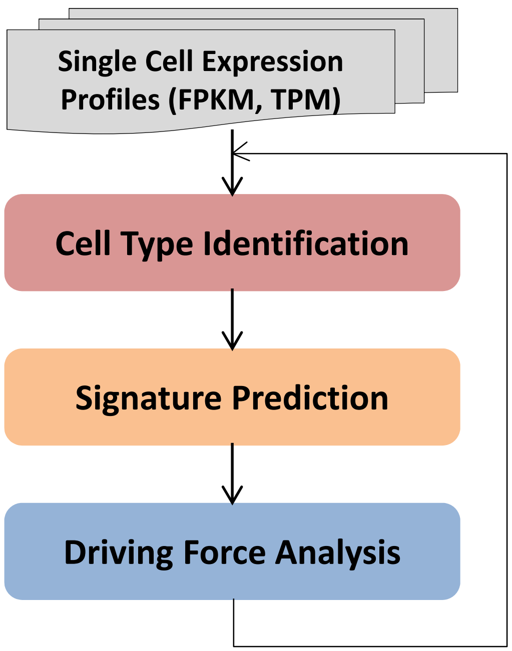


Minzhe Guo

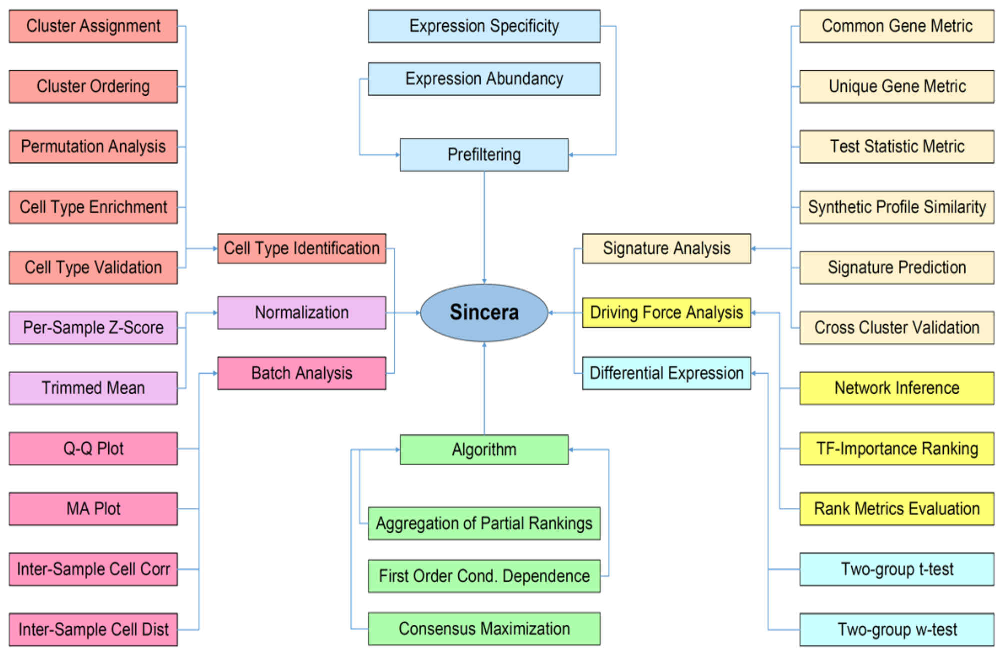
CCHMC/U of Rochester

Sincera: a computational pipeline for SINgle CEll Rna-seq profiling Analysis

Main Analysis Components



Program Structure of Sincera



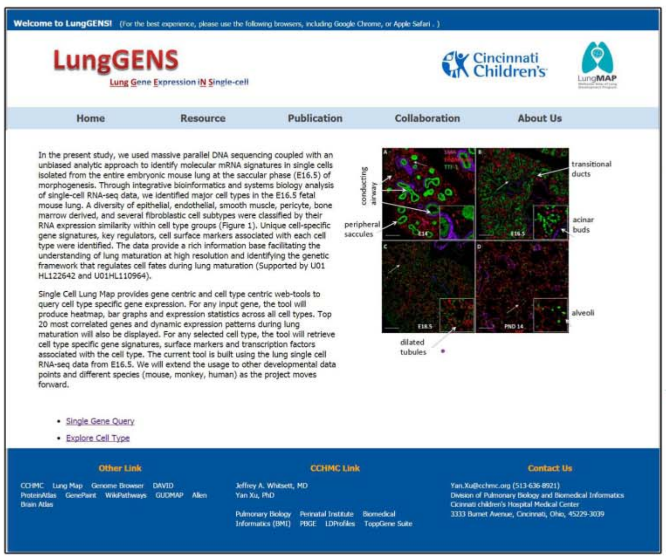
https://research.cchmc.org/pbge/sincera.html

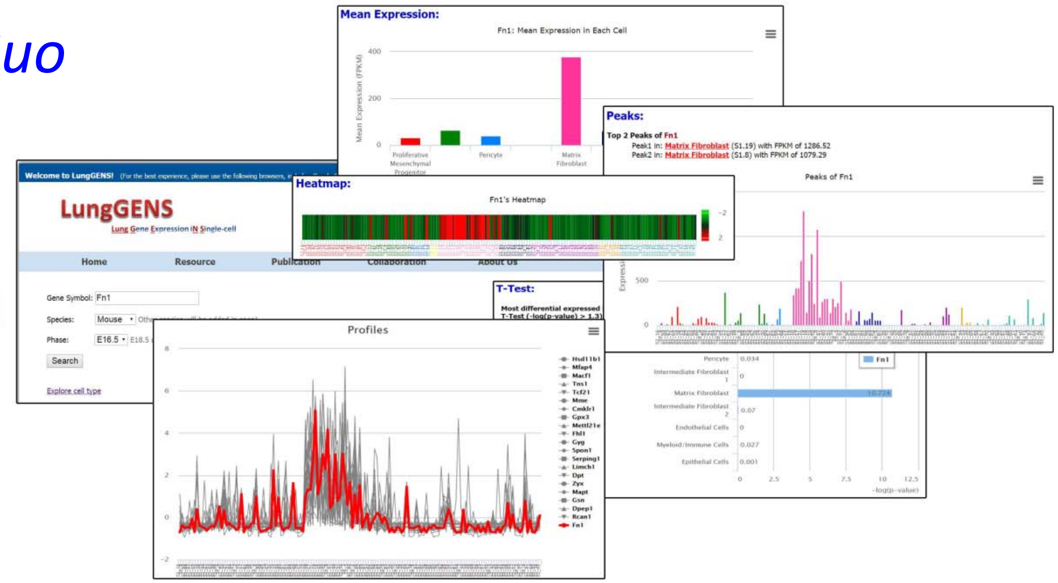
Screenshot of LungGENS

(https://research.cchmc.org/pbge/lunggens/default.html)

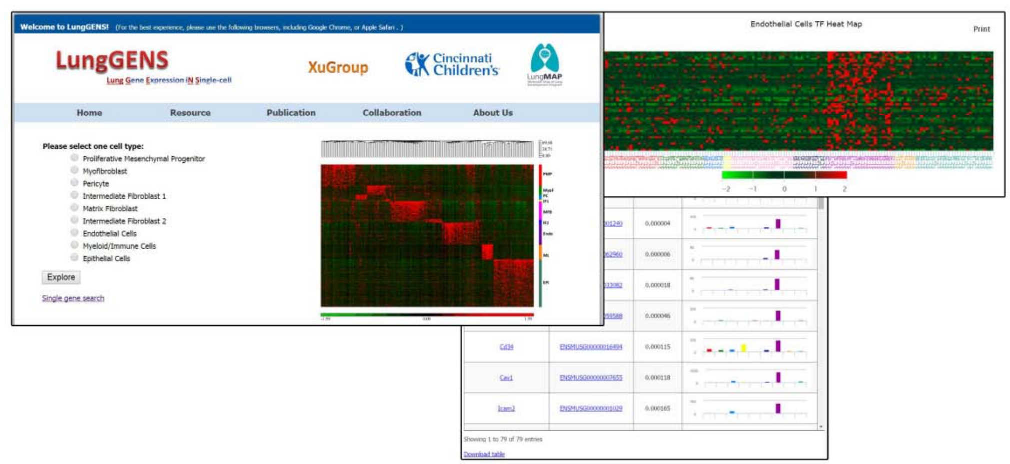
Yan Xu,Yina Du,Mizhe Guo

Query by Gene

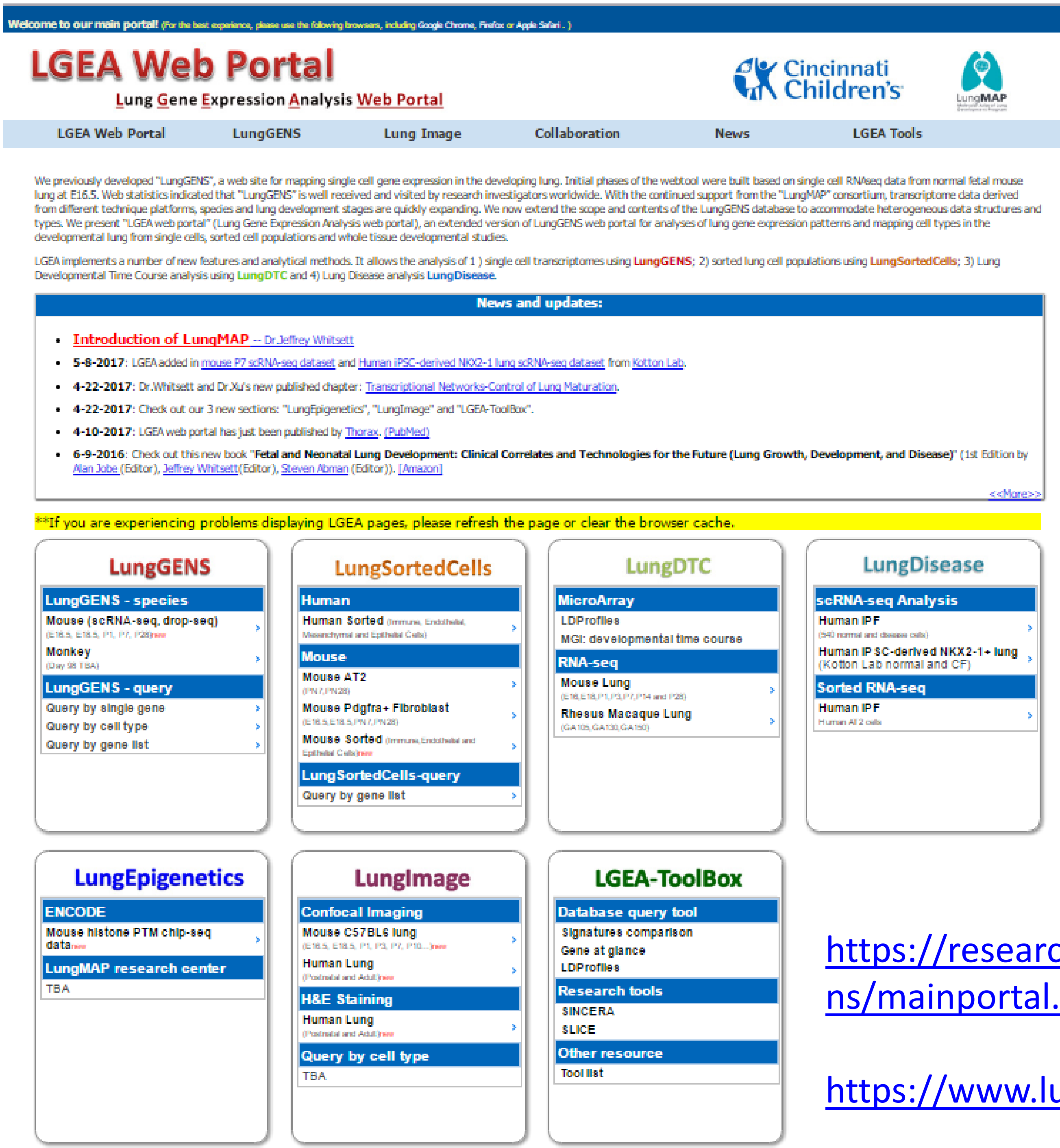




Query by Cell Type



CCHMC



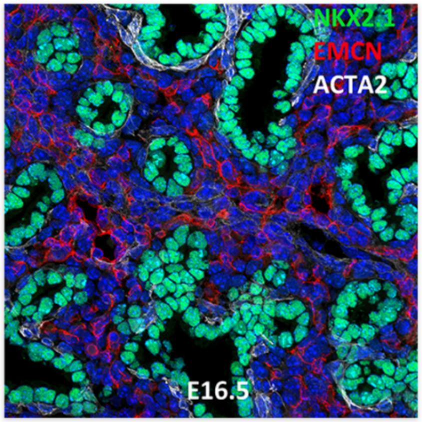
Current version and under development

https://research.cchmc.org/pbge/lunggens/mainportal.html

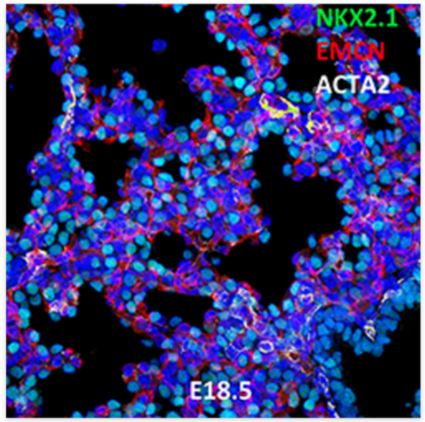
https://www.lungmap.net/

“LGEA “and “Breath”:Atlas of Mouse Lung Cell Types During Development

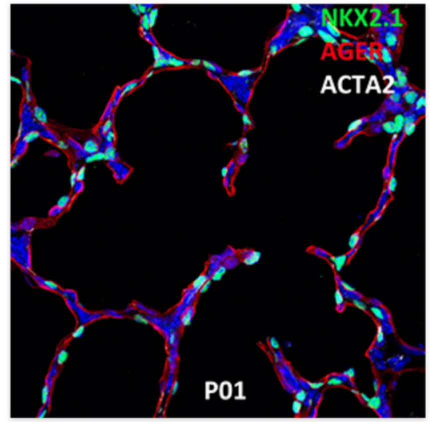
C57BL/6



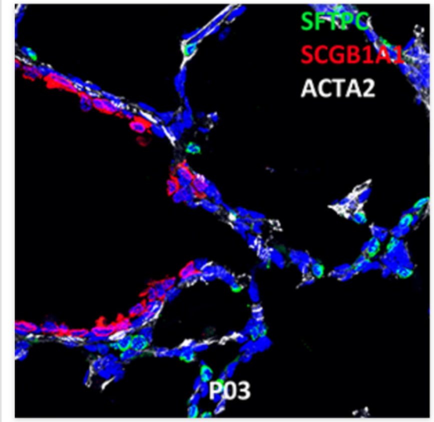
E16.5 C57BL6 Confocal Imaging



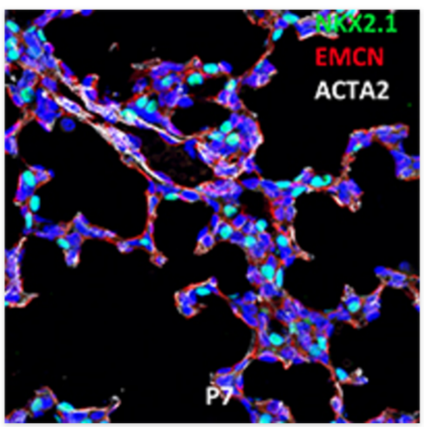
E18.5 C57BL6 Confocal Imaging



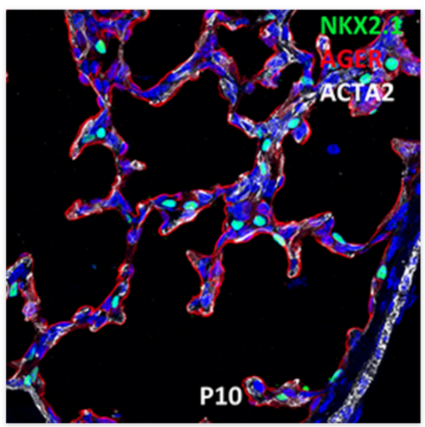
Postnatal Day 1 C57BL6 Confocal Imaging



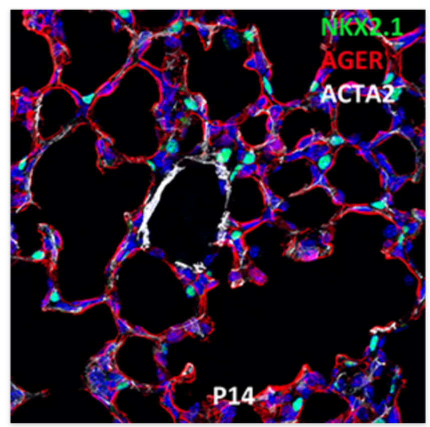
Post Natal Day 3 C57BL6 Confocal Imaging



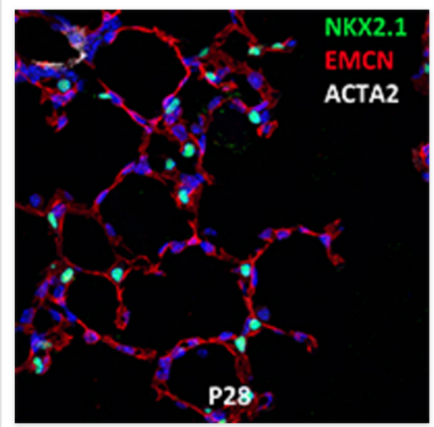
Postnatal Day 7 C57BL6 Confocal Imaging



Postnatal Day 10 Confocal Imaging

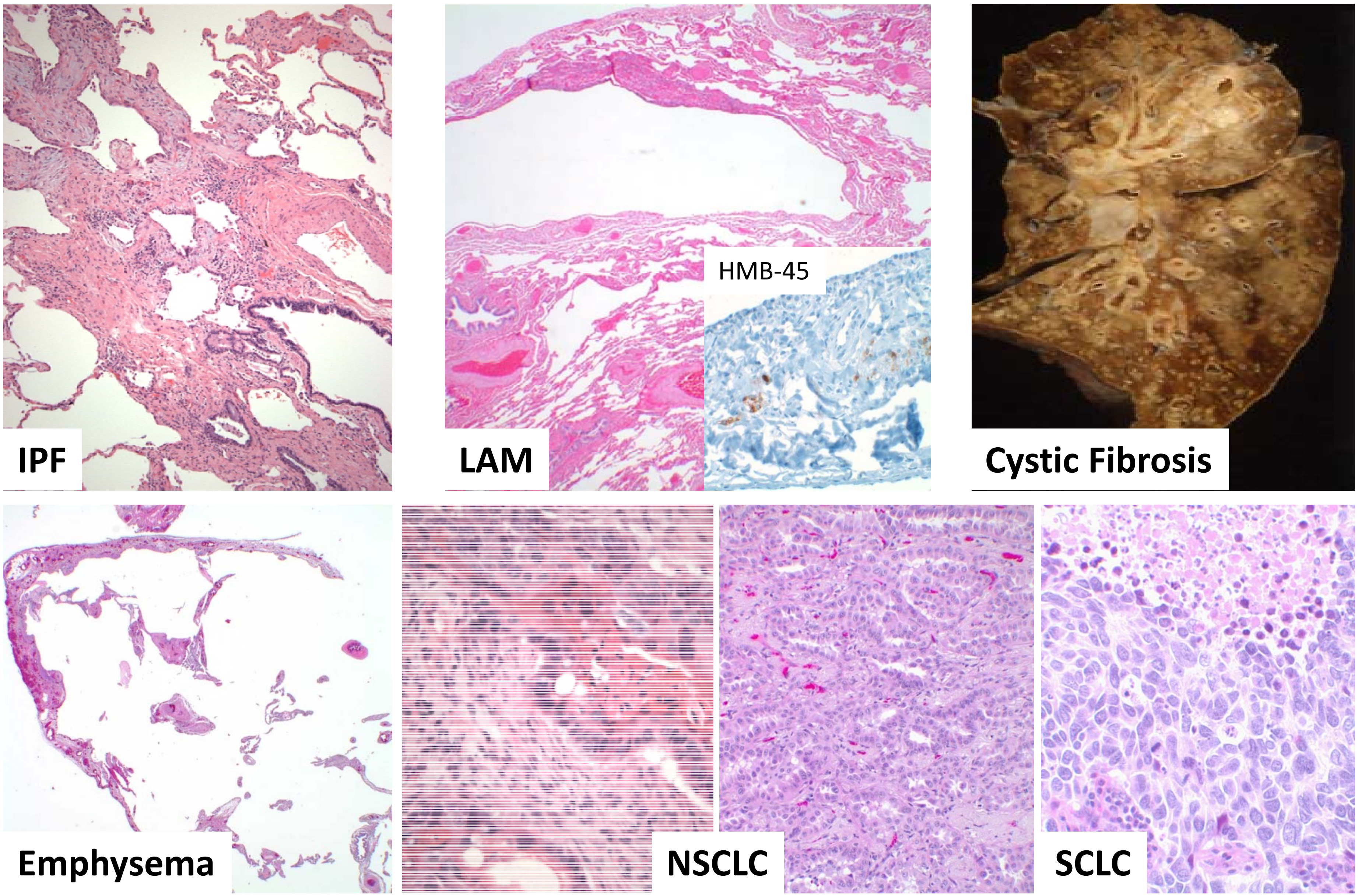


Postnatal Day 14 C57BL6 Confocal Imaging



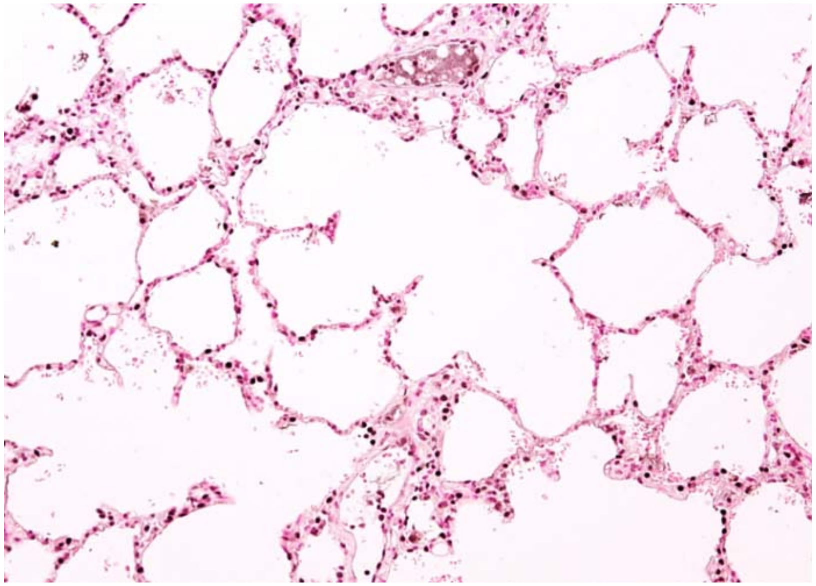
Postnatal Day 28 C57BL6 Confocal Imaging

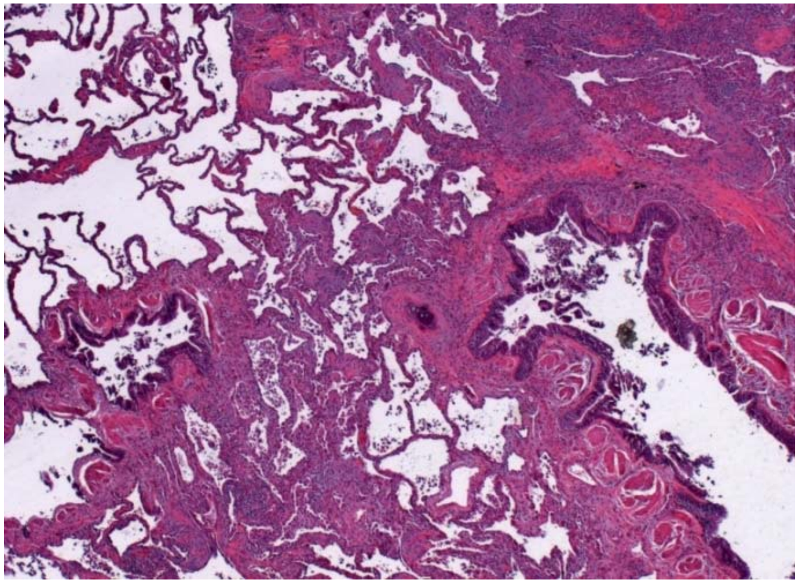
Challenge of Chronic Pulmonary Diseases



Idiopathic Pulmonary Fibrosis

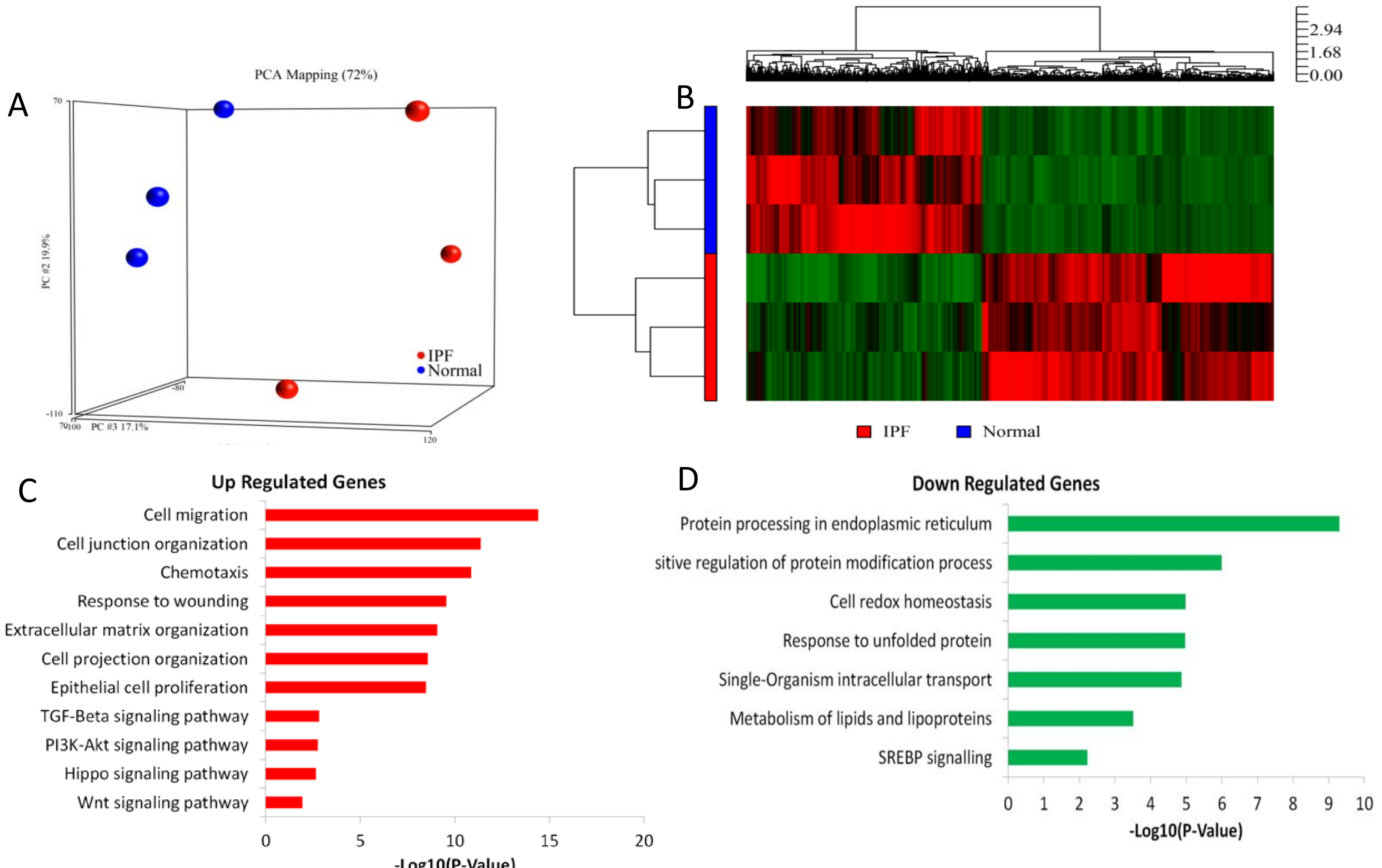
- Normal IPF





Barry Stripp-Cedar Sinai

Gene Expression in Human IPF :Sorted Epithelial Cells



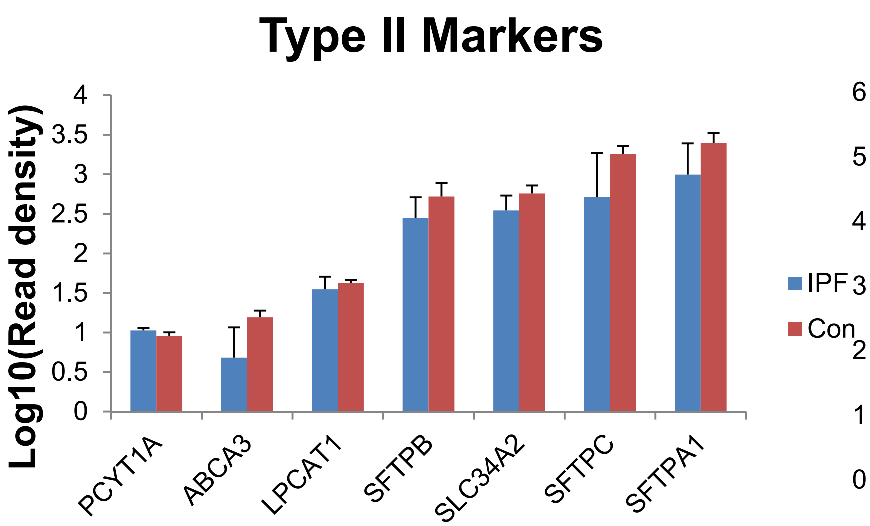
JCI Insight 2016:Yan Xu et al

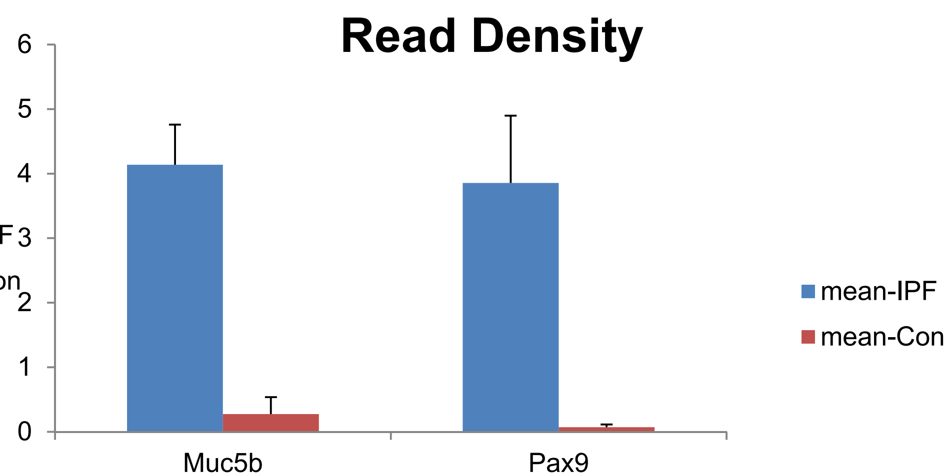
Expression of Proximal Epithelial Markers in IPF Type 2 Cells

Loss of Normal Epithelial Cell Identity

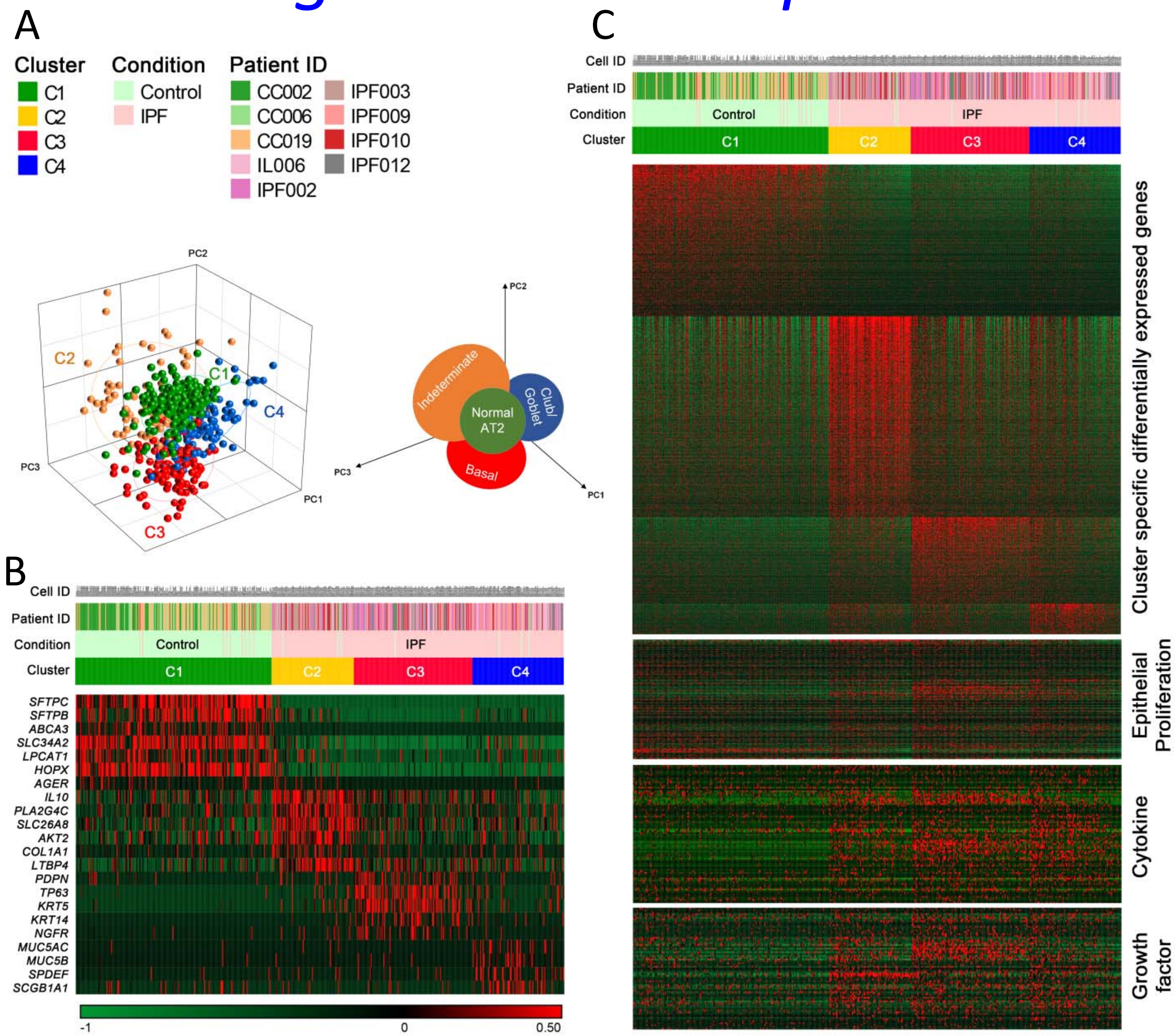
SFTPA1,SFTPB,SFTPC, PCYT1A,
ABCA3, SLC34A1,LPCAT1

Muc5b Pax9





Single Cell RNA Expression Patterns in IPF



D

C1	Functional terms	P-value
GO:0009636	response to toxic substance/detoxification	1.79E-10
GO:0055114	oxidation-reduction process	2.23E-08
GO:0006629	lipid metabolic process	1.50E-05
GO:0006869	lipid transport	2.16E-05
GO:0043129	surfactant homeostasis	6.67E-05
GO:0042599	lamellar body	1.62E-07
Transcription Factor Binding Site	V$CEBP_Q2_01	3.15E-04
Transcription Factor Binding Site	V$GATA6_01	2.64E-03

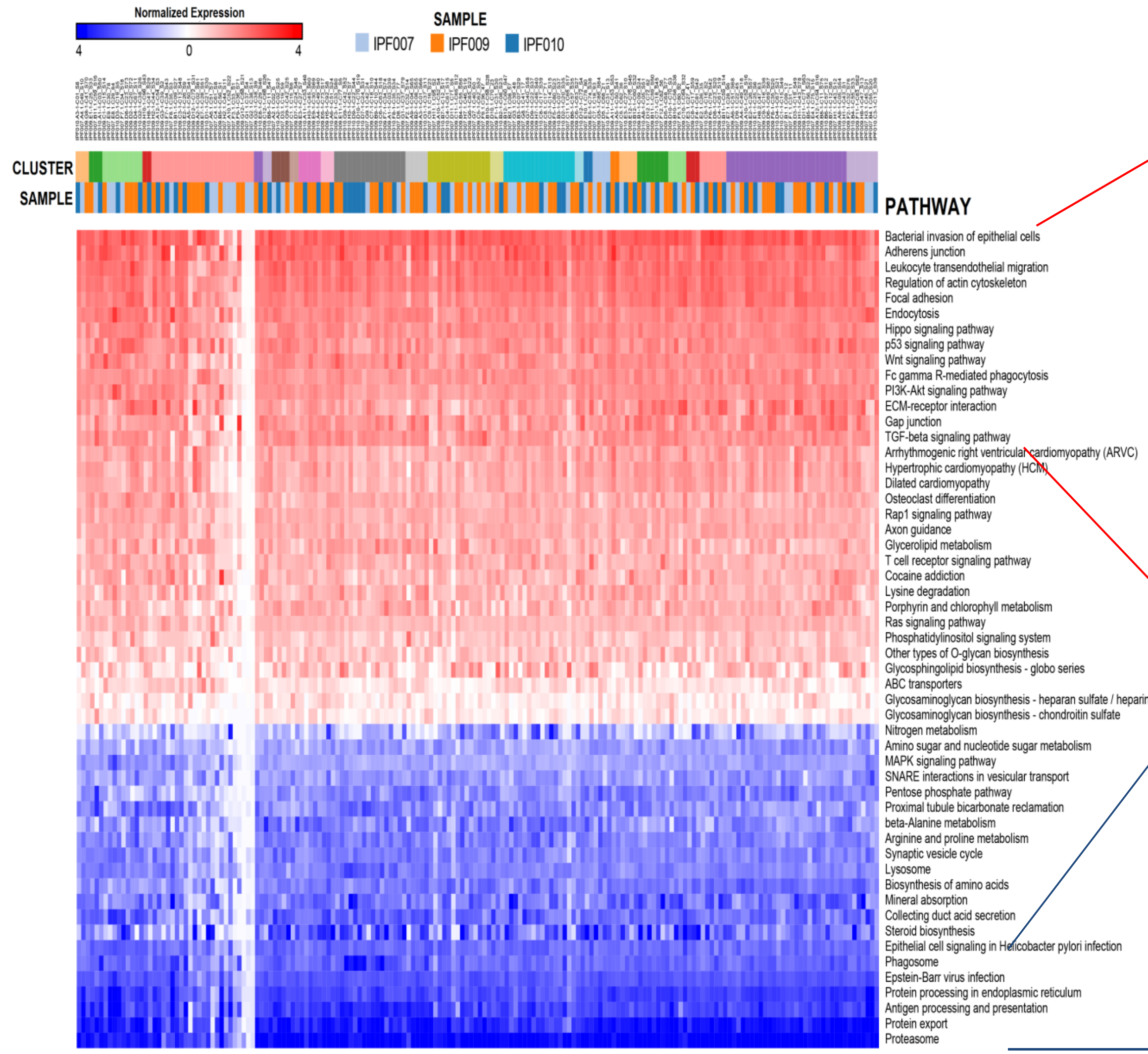
C2	Functional terms	P-value
GO:0006820	anion transport	3.73E-03
GO:0043409	negative regulation of MAPK cascade	3.77E-03
GO:0043547	positive regulation of GTPase activity	6.85E-03
GO:0050709	negative regulation of protein secretion	9.47E-03
GO:0042098	T cell proliferation	9.52E-03
GO:0045936	negative regulation of phosphate metabolic process	2.13E-02
GO:0006954	inflammatory response	2.87E-02
GO:0006869	lipid transport	3.40E-02
GO:0019752	carboxylic acid metabolic process	4.48E-02

C3	Functional terms	P-value
GO:0031581	hemidesmosome assembly	2.39E-13
GO:0034330	cell junction organization	7.07E-12
GO:0016477	cell migration	2.05E-10
GO:0042060	wound healing	4.66E-10
GO:0005604	basement membrane	1.80E-10
Pathway	Laminin interactions	5.48E-08
Pathway	PI3K-Akt signaling pathway	2.37E-05
Transcription Factor Binding Site	CATTGTYY_V$SOX9_B1	3.94E-03

C4	Functional terms	P-value
GO:0009617	response to bacterium	1.17E-06
GO:0009100	glycoprotein metabolic process	2.18E-05
GO:0030855	epithelial cell differentiation	1.14E-04
GO:0070085	glycosylation	5.24E-04
GO:0005902	microvillus	2.15E-06
GO:0005794	Golgi apparatus	1.50E-03
MP:0000511	abnormal intestinal mucosa morphology	1.02E-04
MSigDB M5885	Ensemble of genes encoding ECM-associated proteins including ECM-affiliated proteins, ECM regulators and secreted factors	2.36E-05

JCI Insight 2016/Yan Xu et al

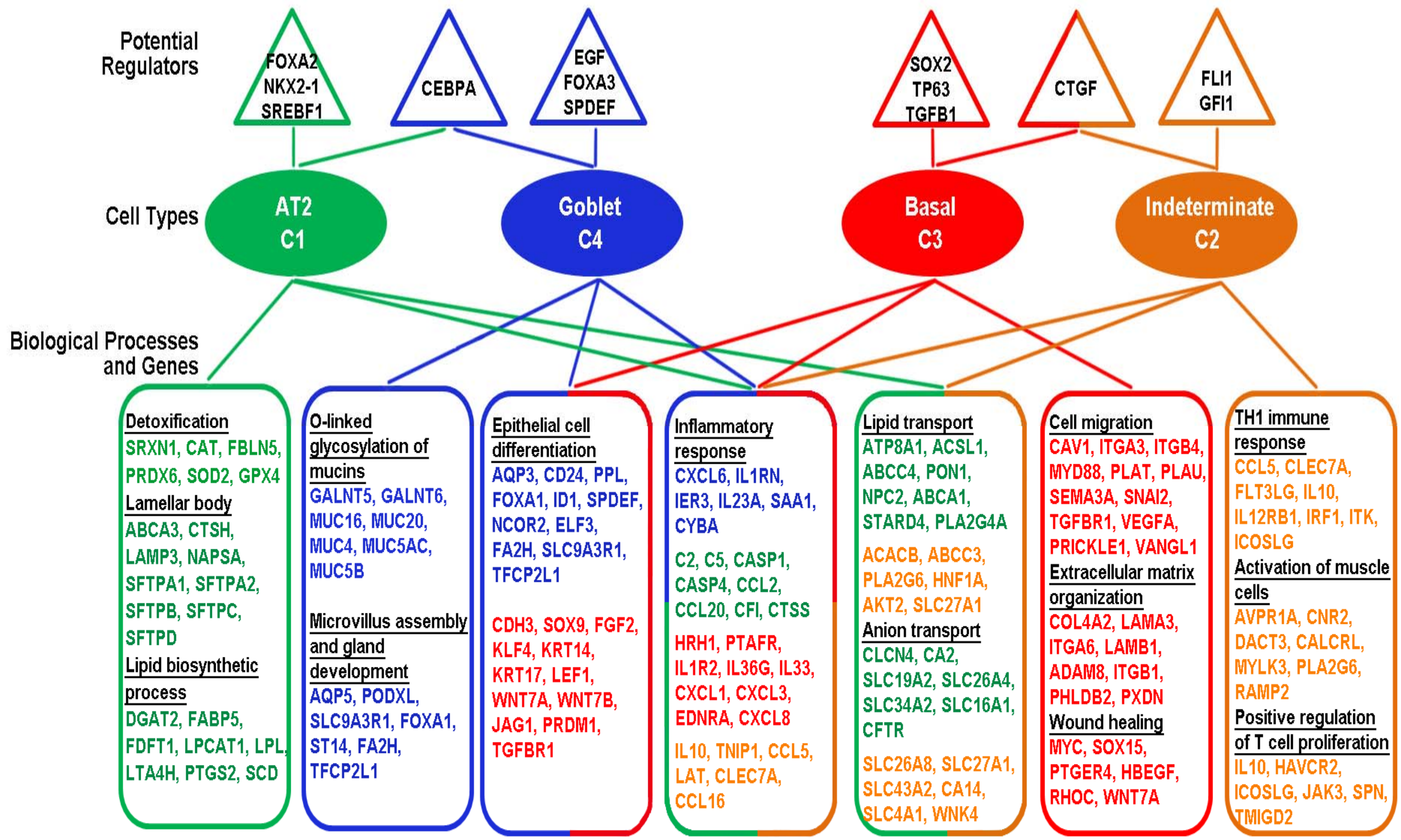
Enrichment of KEGG Pathway in IPF SC



- Bacterial invasion of epithelial cells
- Adherens junction
- Leukocyte transendothelial migration
- Regulation of actin cytoskeleton
- Focal adhesion
- Endocytosis
- Hippo signaling pathway
- p53 signaling pathway
- Wnt signaling pathway
- Fc gamma R-mediated phagocytosis
- PI3K-Akt signaling pathway
- ECM-receptor interaction
- Gap junction
- TGF-beta signaling pathway

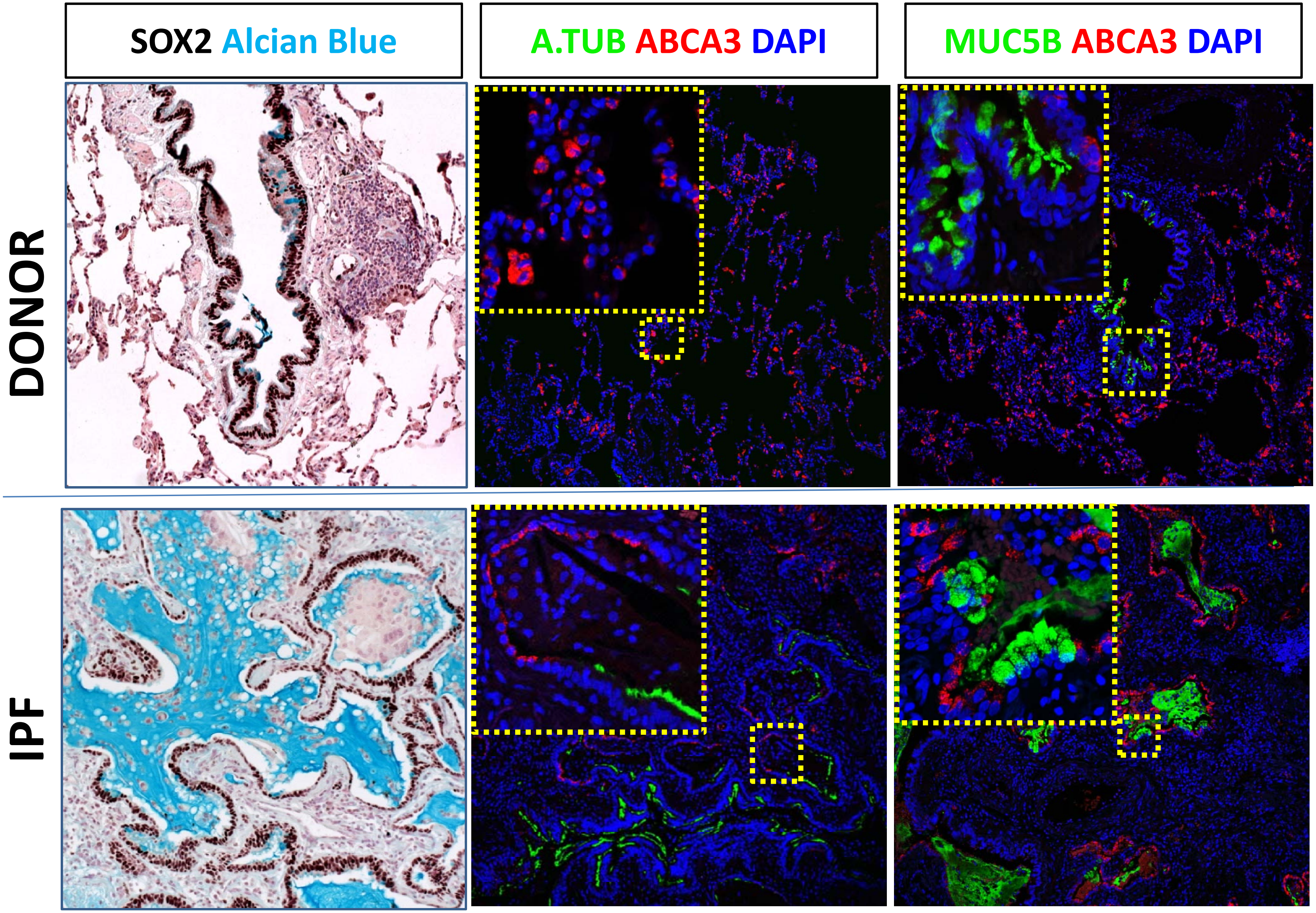
- Proteasome
- Protein export
- Antigen processing and presentation
- Protein processing in endoplasmic reticulum
- Epstein-Barr virus infection
- Phagosome

Normal AT2 Cells *IPF Epithelial Cells*

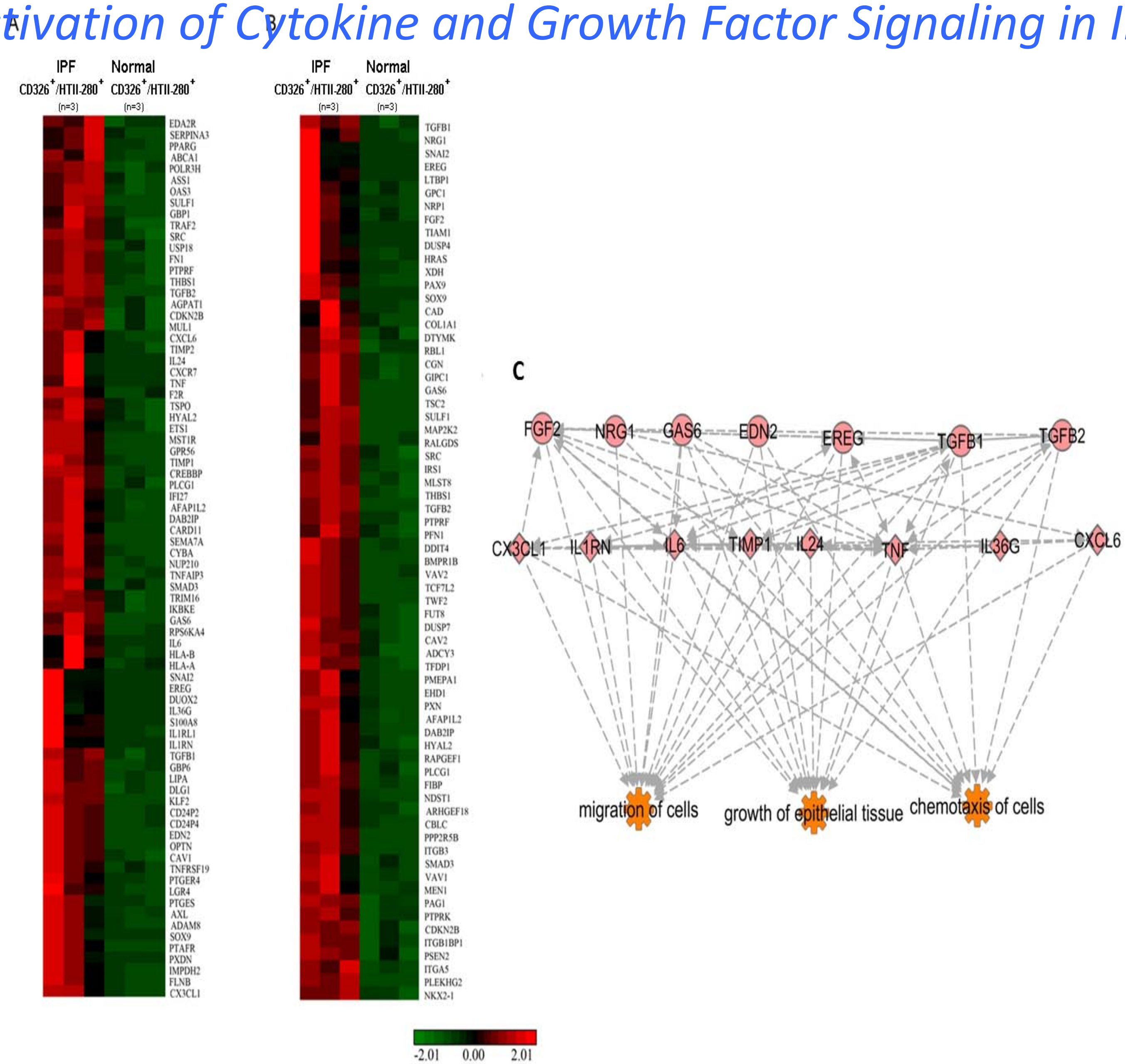


Loss of Alveolar Cell Identity in IPF

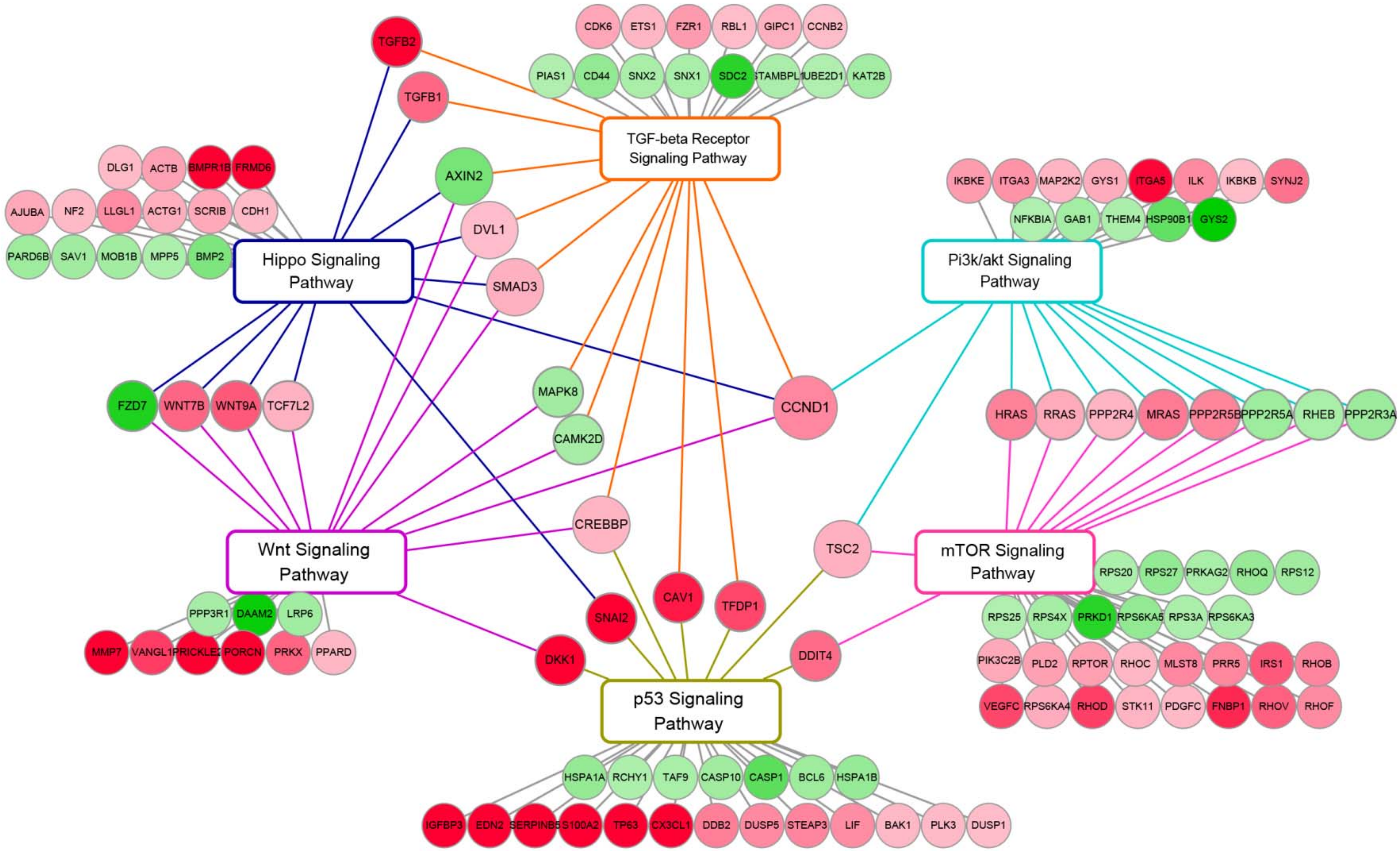
"Proximalization" of the peripheral lung in IPF

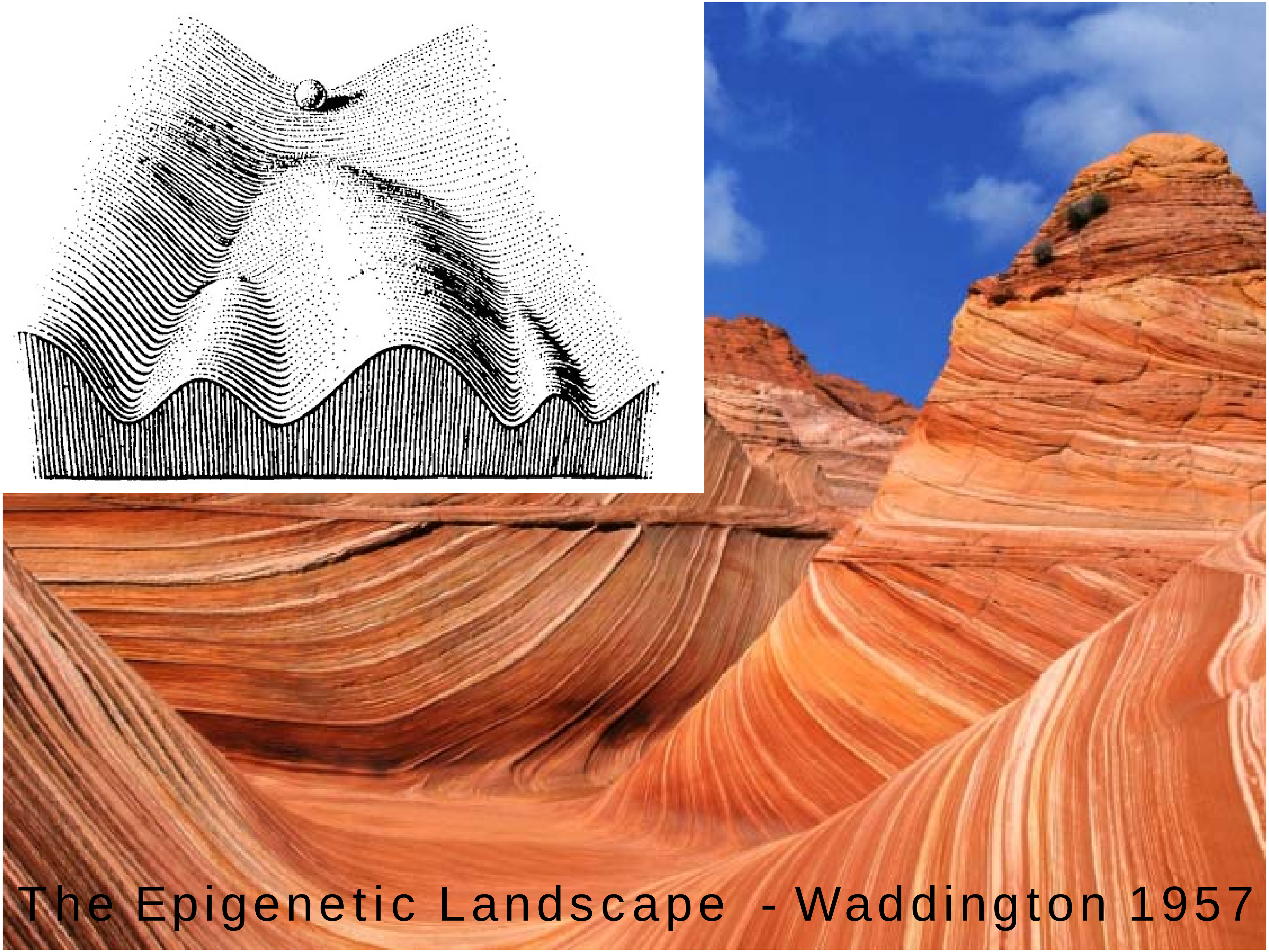


Activation of Cytokine and Growth Factor Signaling in IPF



Prediction of Abnormal Cell Signaling Driving IPF





The Epigenetic Landscape - Waddington 1957

Monogenic Diseases of Surfactant Homeostasis

ABCA3: Surfactant lipid transport in AT2 cells

SFTPB: Intra cellular packaging and function of surfactant

SFTPC: Surfactant function / host defense

SFTPA: Host defense, surfactant structure

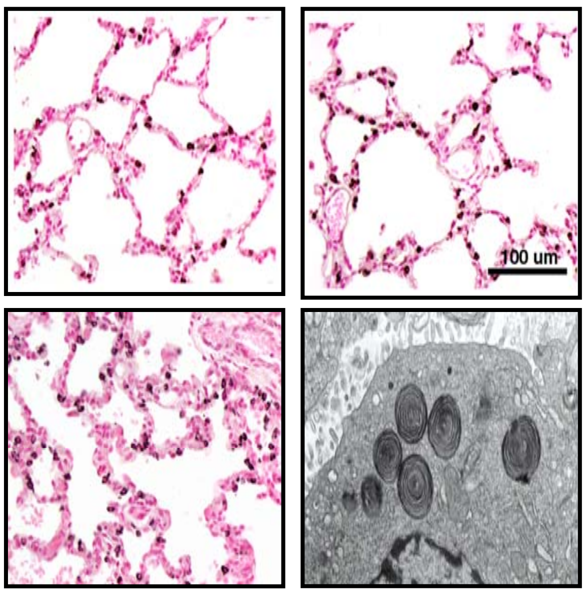
NKX2-1: AT2 cell differentiation and function

GMCSFa/b Receptors:Hereditary PAP/Macrophage

Cincinnati Children's
changing the outcome together

Histopathology of ABCA3 Deficiency

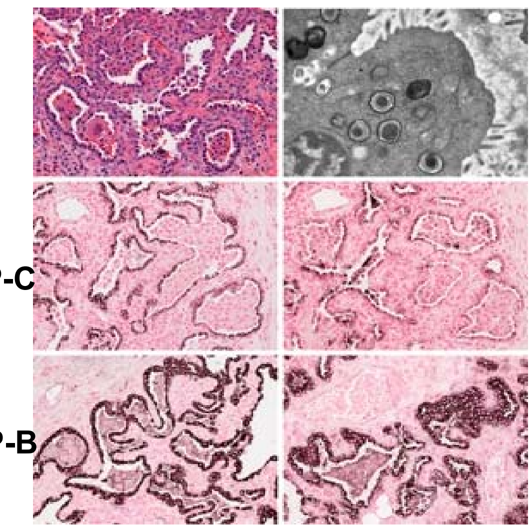
A



Normal Human Lung

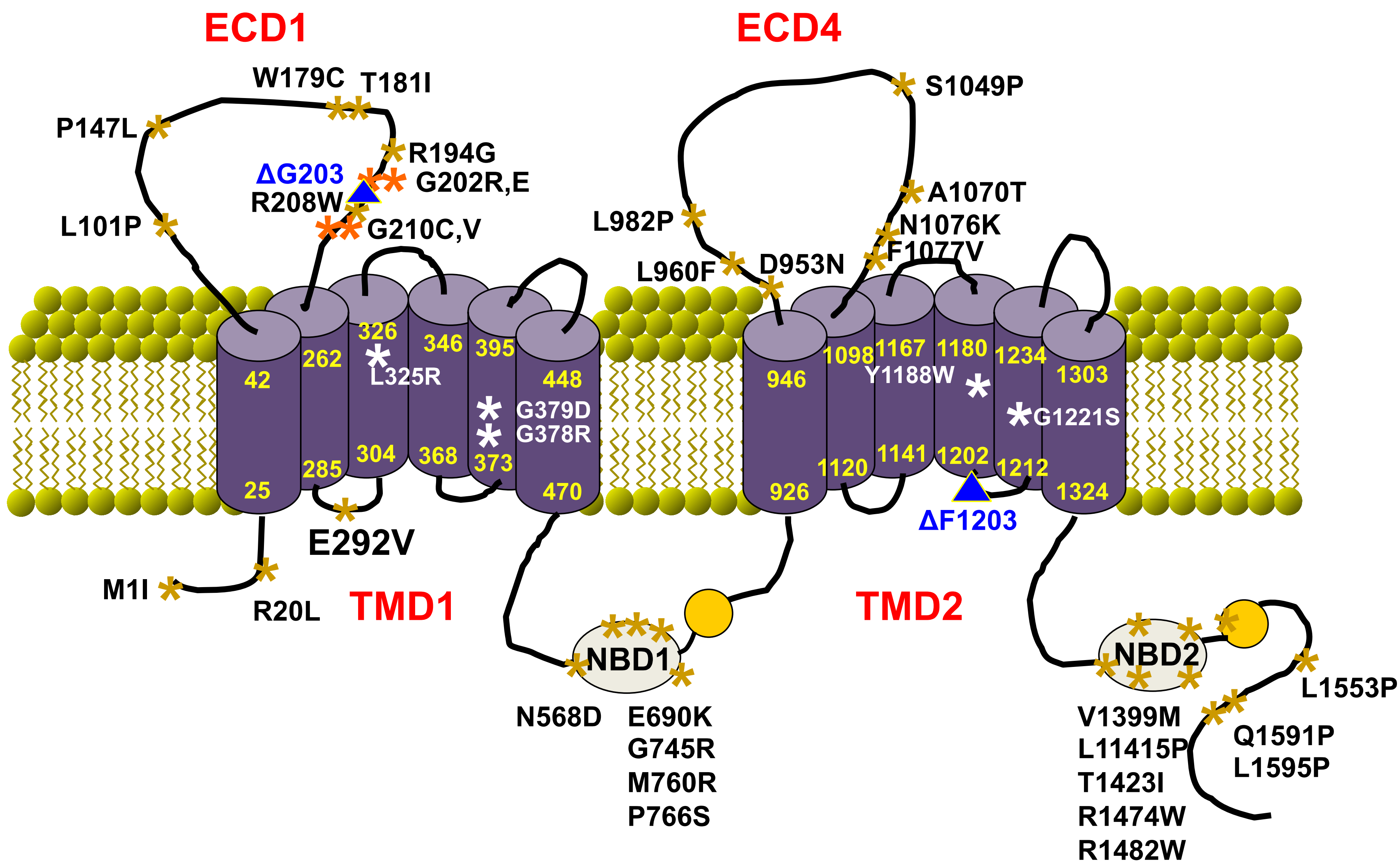
B

ABCA3 Mutation S1049P



Partial Map of ABCA3 Mutations

Larry Nogee –Johns Hopkins

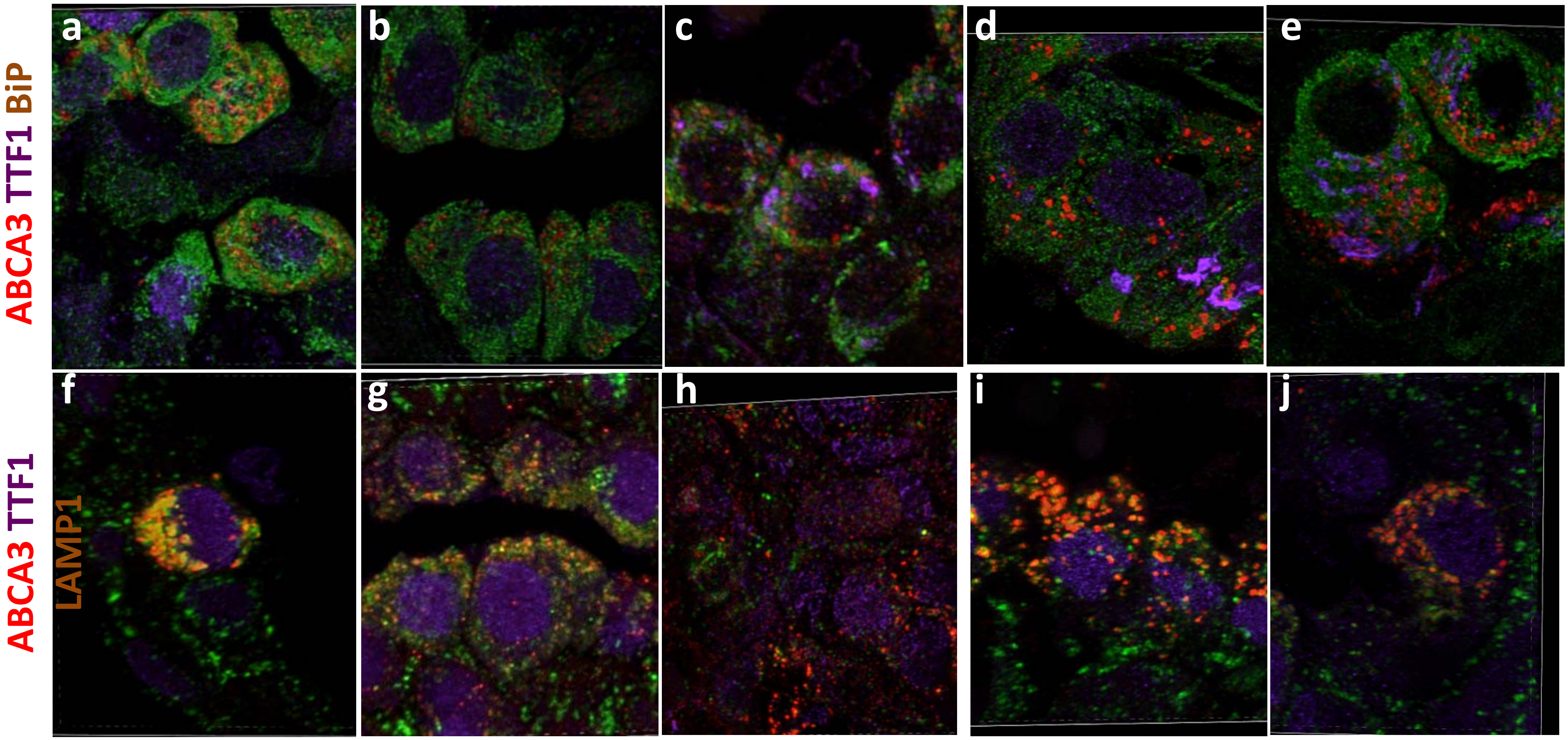


Current Classification of ABCA3 Mutations

Clinical Phenotype	Type I Mutations Impaired Processing / Traffickng Localized to ER	Type II Mutations Impaired ATP Hydrolysis / Lipid Transport Localized to LB-like vesicles
NRF (11)	L101P (1,2,6) L982P (2) W1142X (2) L1553P (2) Gly1518Val*1 (2) Q215K (9,10)	R43L (6,8) R295C (severely impaired)(5) N568D (1,2,4) E690K (severely impaired) (4) T1114M (severely impaired) (4) G1221S (1, 2) L1580P (2)
chILD (11)	R280C (partial) (6) Q1591P (2)	E292V (moderately impaired) (4,9,10) D253H (7) T1173R (7)

NRF, Neonatal Respiratory Failure; chILD, Children's Interstitial Lung Disease

Subcellular Localization of ABCA3 Mutant Proteins



<u>Expression of ABCA3 in human lungs.</u> Confocal images were acquired by oversampling in x, y and z, deconvolved by landweber 3D. Control **(a and f)**and ABCA3 mutants S1049P/S1049 (**b and g)** , Q1591P/Q1131R (**c and h)** , W1142X/W1142X **(d and i)**, and L982P/G1221S (**e and j)** stained with ABCA3 (red), TTF1 (purple) and the ER marker GRP78 (BiP) (green **top row panels a-e**) or lysosome marker LAMP1 (green **bottom row panels f-j**).

Progenitor Cell Translational Consortium -

Towards Correction of Monogenic Pulmonary Disorders

Collaborative Multi-PI Program

Jeff Whitsett – CCHMC

Ed Morrisey – University of Pennsylvania

Darrell Kotton – Boston University

F. Session Cole & Jennifer Wambach – Washington University

Larry Nogee – The Johns Hopkins University

Charles Ansong – Pacific Northwest National Laboratory

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Towards Pathogenesis and Treatment of Monogenic Disorders of Surfactant Homeostasis

Model Human Surfactant Disorders: Mice, IPSC's, Organoids

Identify AT2 cell progenitors

Phenotype human/mouse AT2 s, IPSC's, Progenitors

Target and Deliver: RNA's, c DNA's, Gene editing tools to AT2 cell progenitors.

Pathogenesis of ABCA3 related disease:

- *Molecular pathways: Modeling ABCA3 mutations*
- *Proteomics, Lipidomics, Transcriptomics*
- *Mechanisms of tissue remodeling and lung dysfunction*
- *Correction of ABCA3 disease: Replacement Editing*

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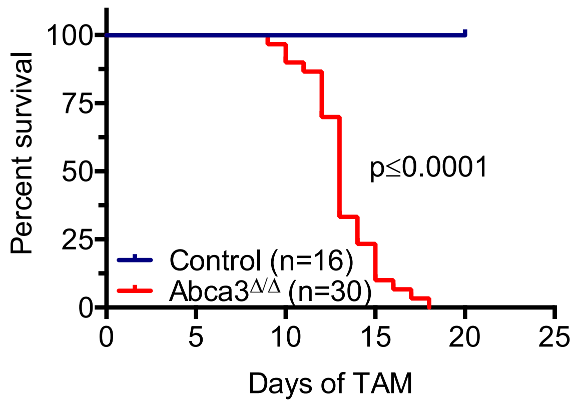
Disease Modeling in Transgenic Mice:

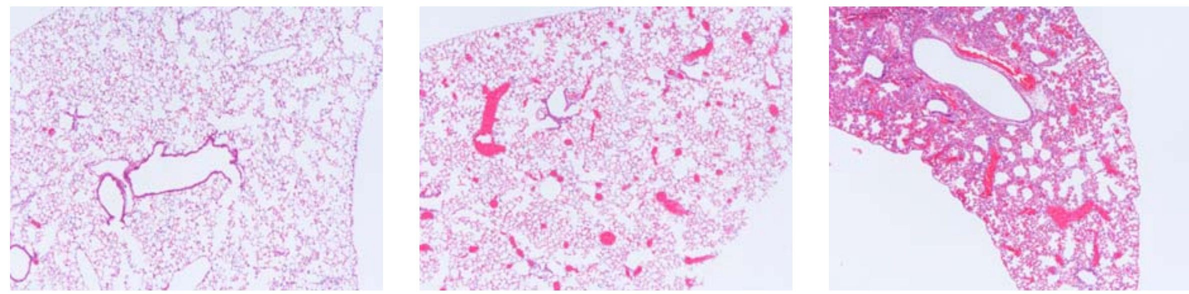
Conditional ABCA3 Deletion in Type II Cells Recapitulates Disease Phenotype in Mice

SftpcCreT/Lox/Stop ABCA3

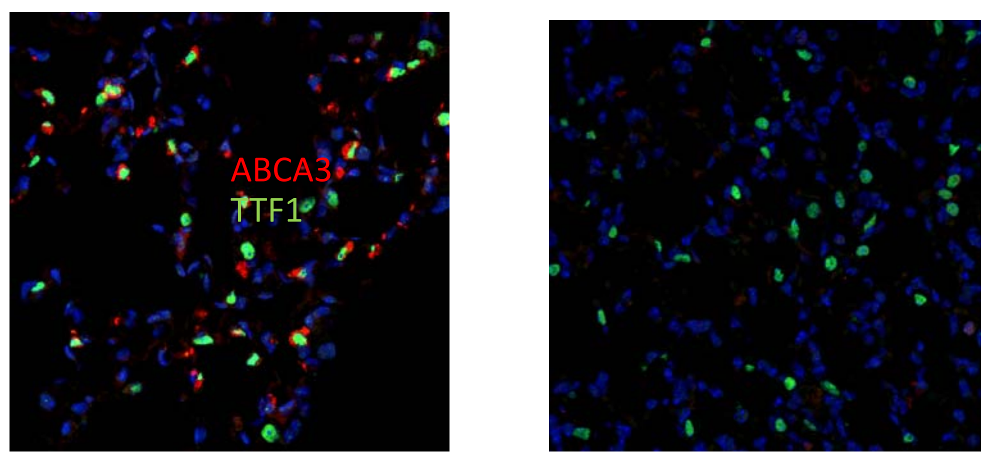


ABCA3$^{\Delta/\Delta}$

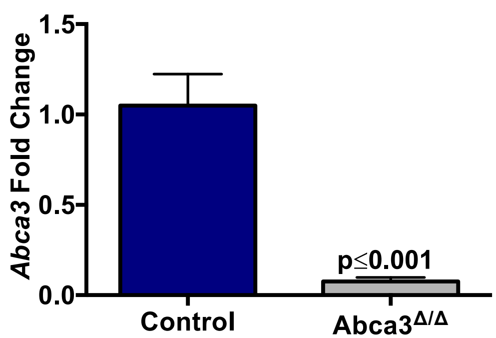
Control | Day 11 - mild | Day 11 - severe



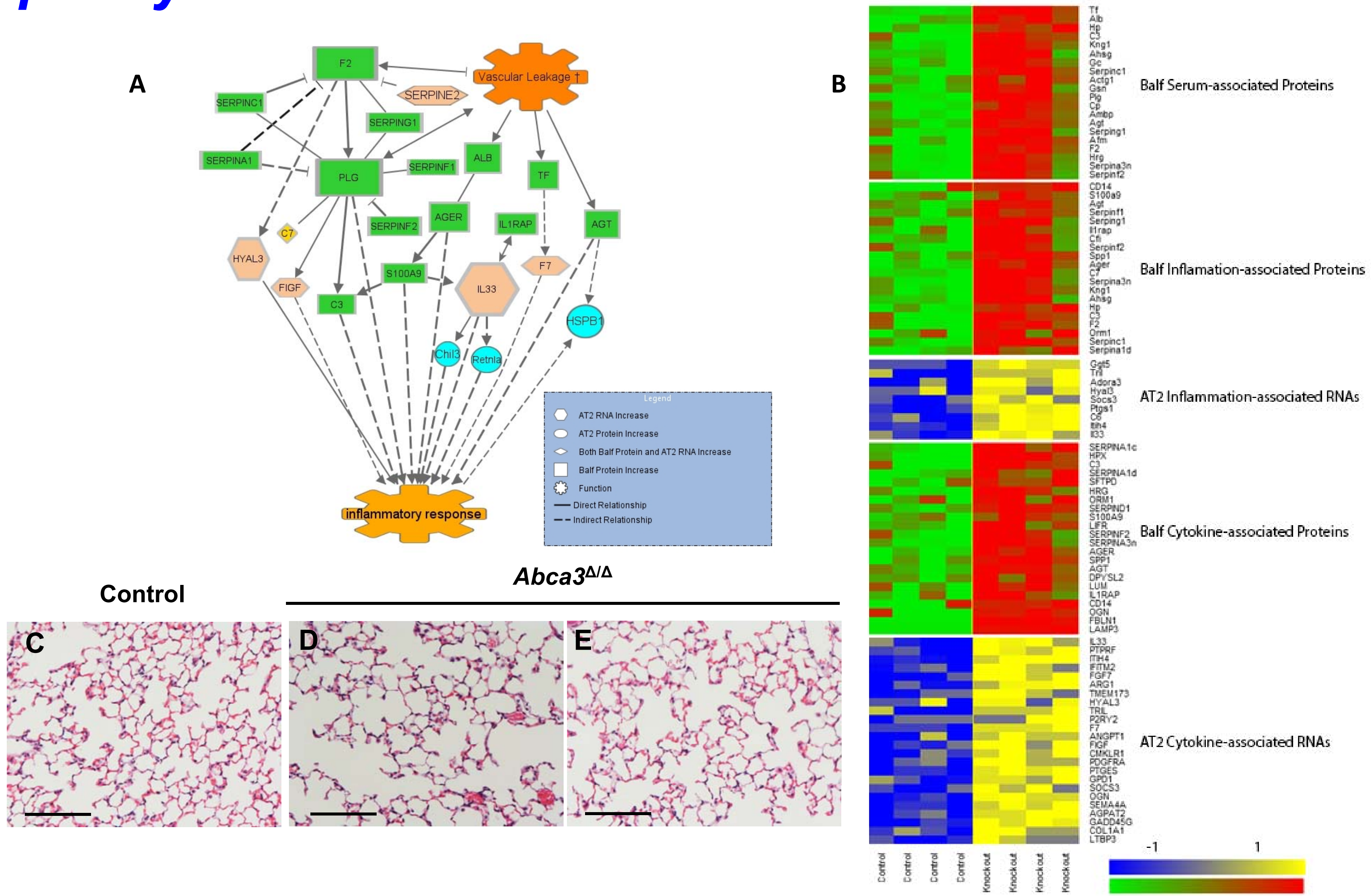
Control | ABCA3$^{\Delta/\Delta}$



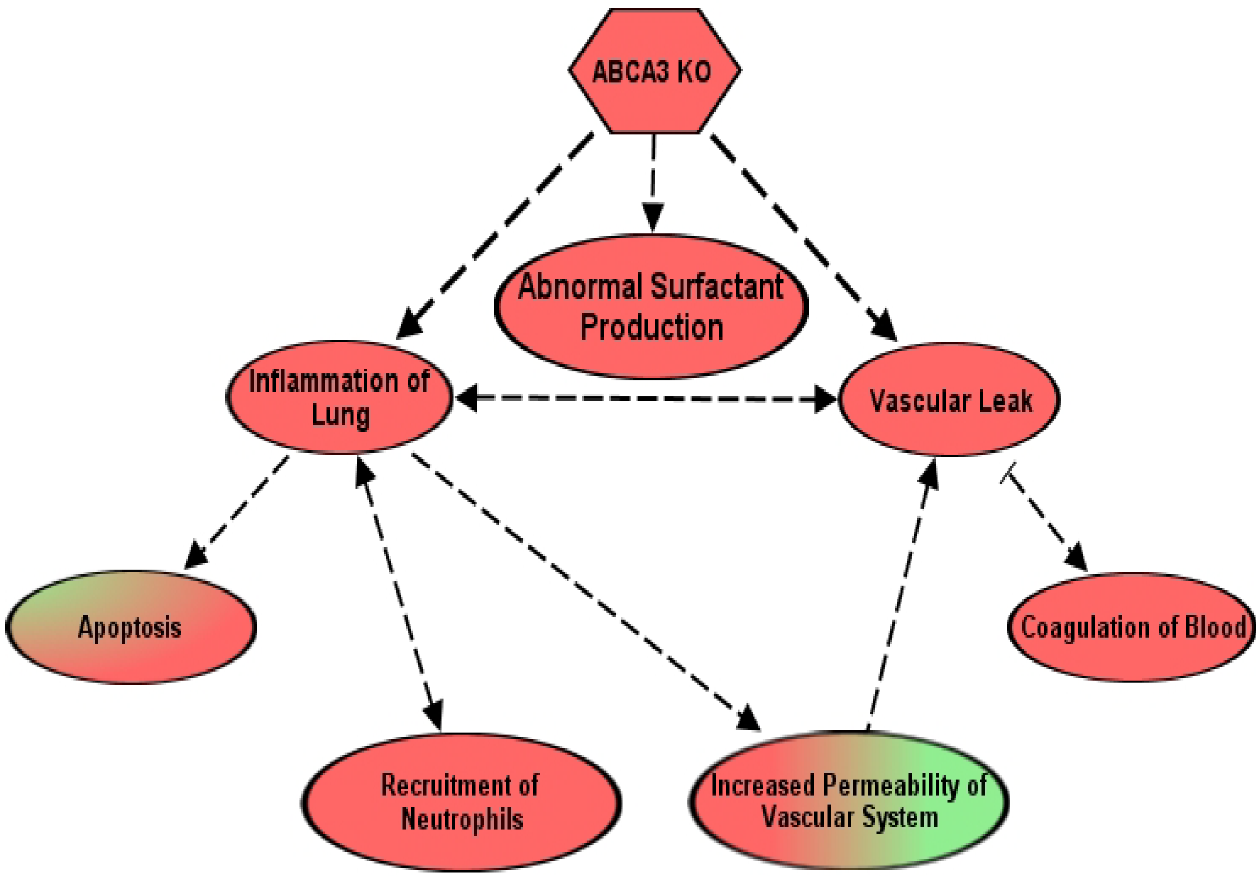
Abca3 mRNA



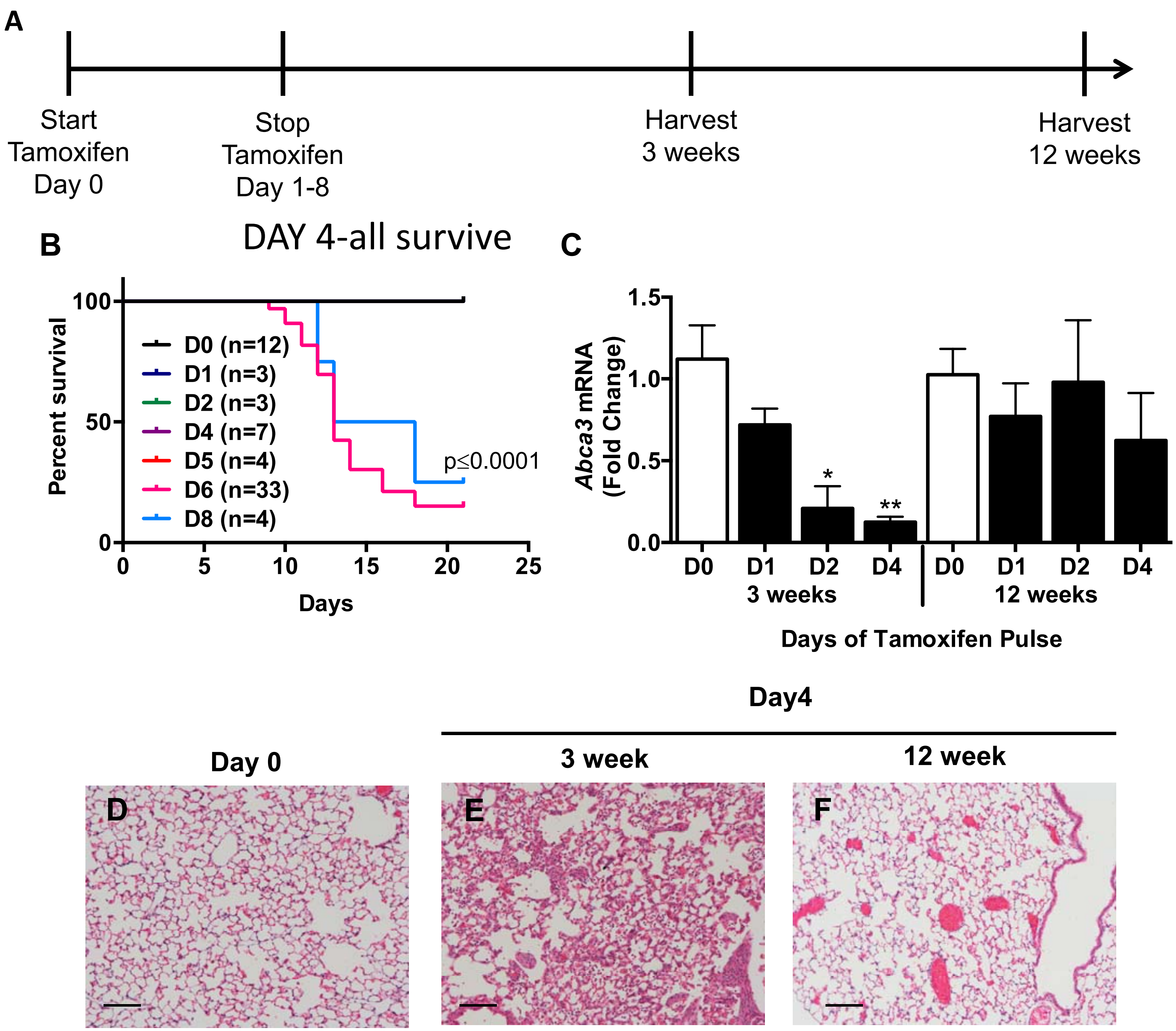
Deletion of ABCA3 initiates inflammation and capillary leak



Pathogenesis of Respiratory Failure in ABCA3 Deficiency

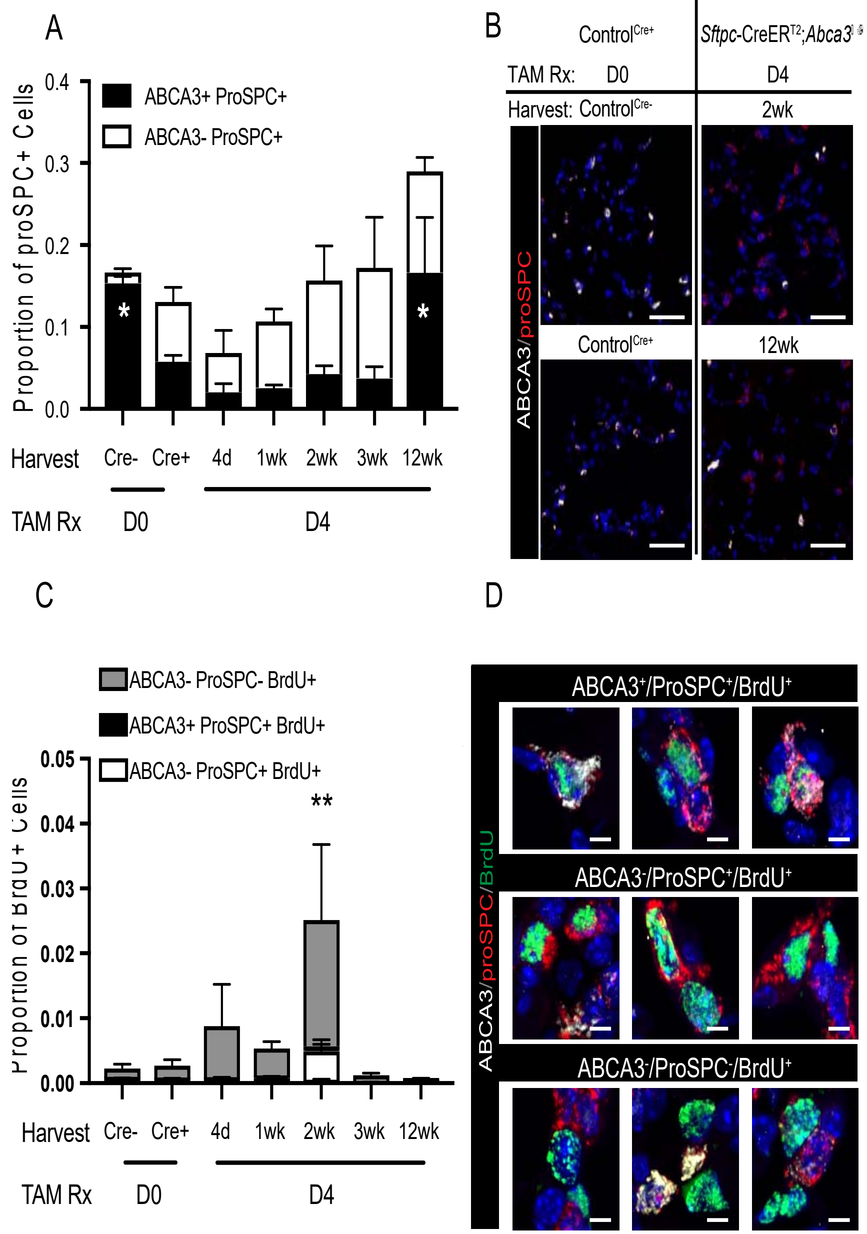


Remarkable Regeneration of the Peripheral Lung from ABCA3 Sufficient Cells

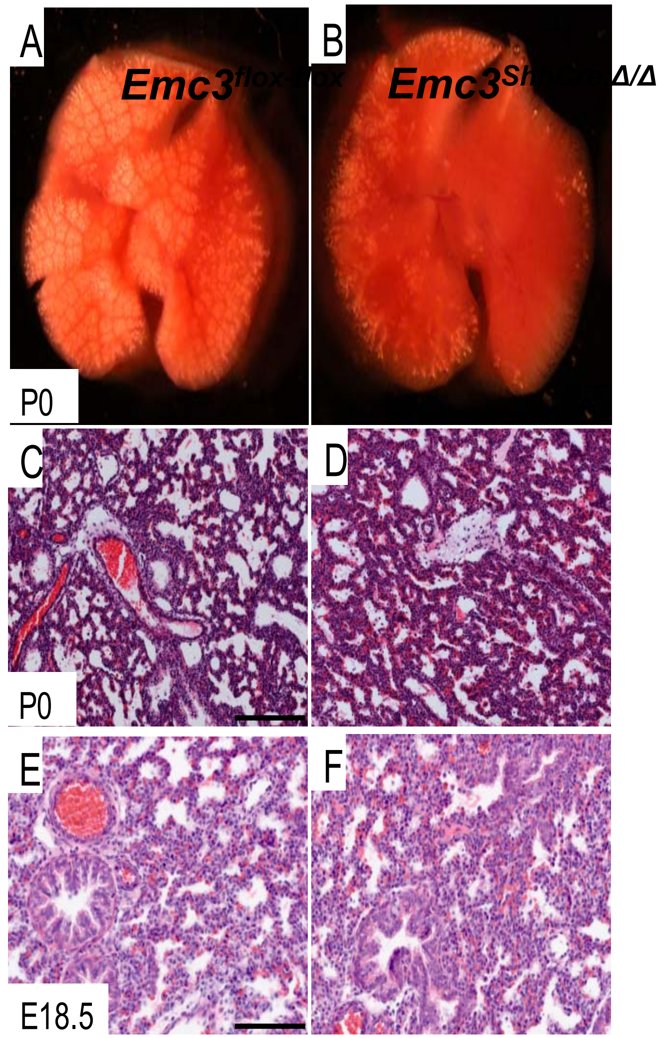


Complete

Alveolar Repair after Deletion of ABCA3

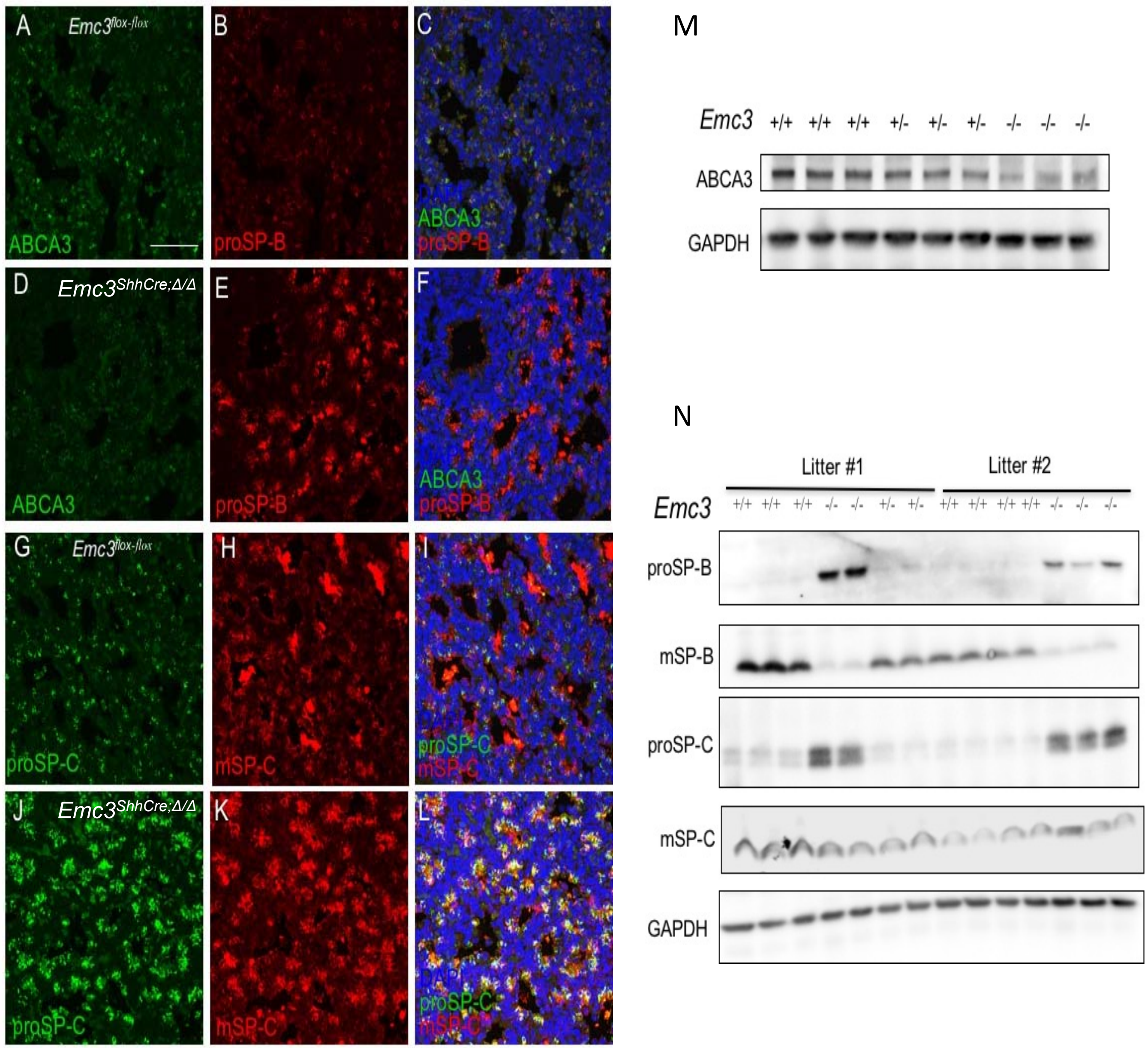


Cellular Mechanisms Controlling ABCA3/SP-B/SP-C Trafficking and Processing

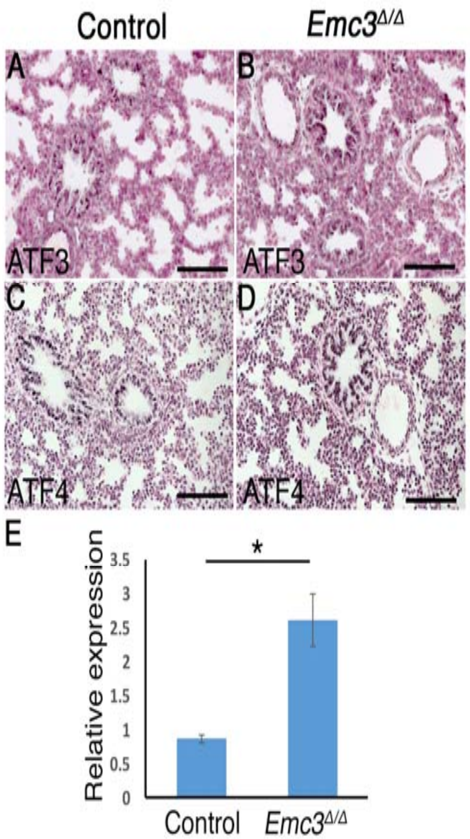


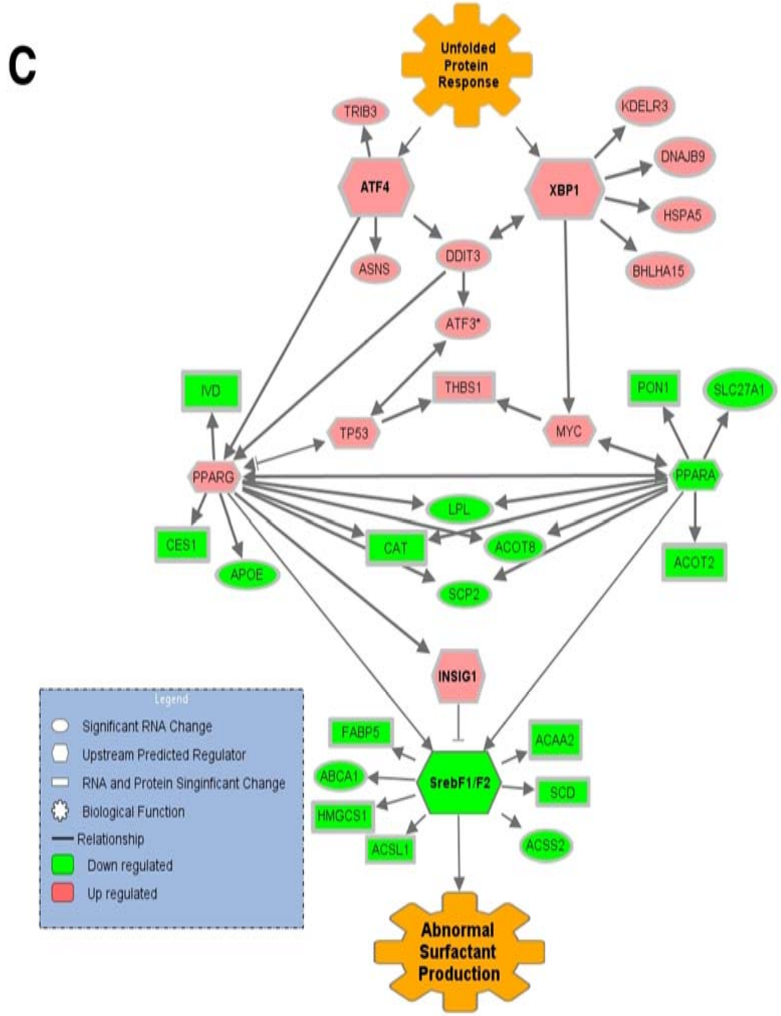
Respiratory Failure in EMC3 Deficient Mice

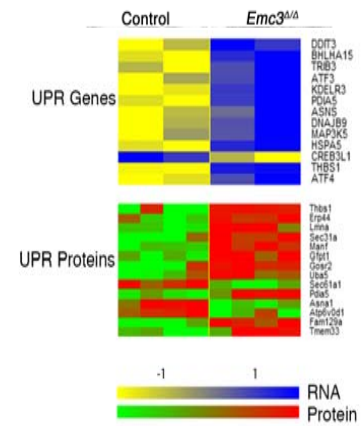
Decreased ABCA3 and Abnormal Surfactant Protein Processing in Emc3$^{ShhCre;\Delta/\Delta Mice}$

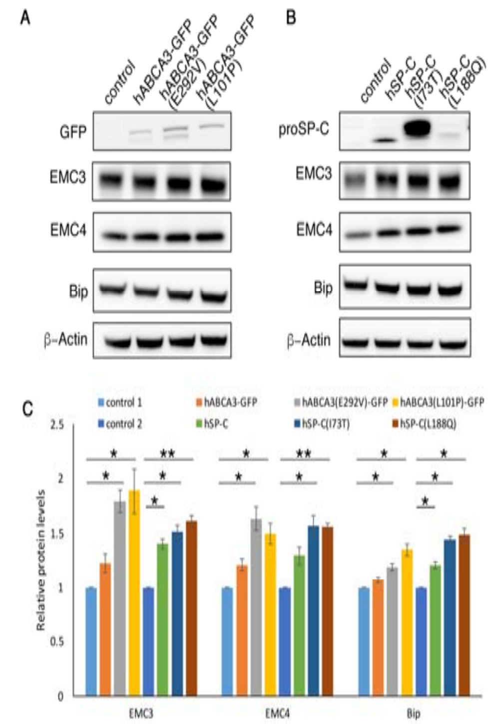


Role of EMC3 in UPR Modeled by Integration of RNA Sequencing and Proteomics









Genetic Basis of Disease

Model Systems

Patient Tissue
Organoids
IPSCs

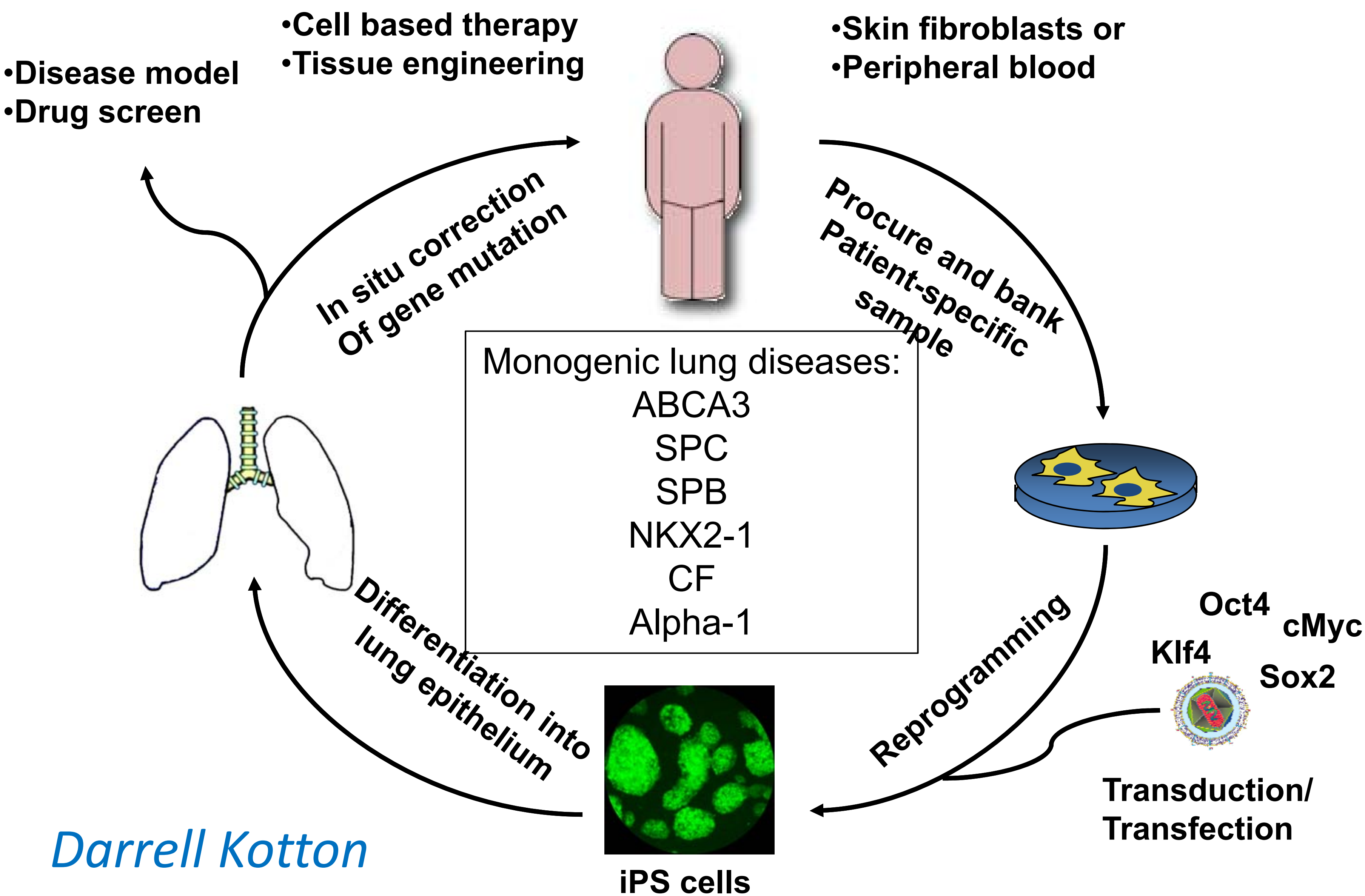
Advancing Technologies

"Systems Biology"

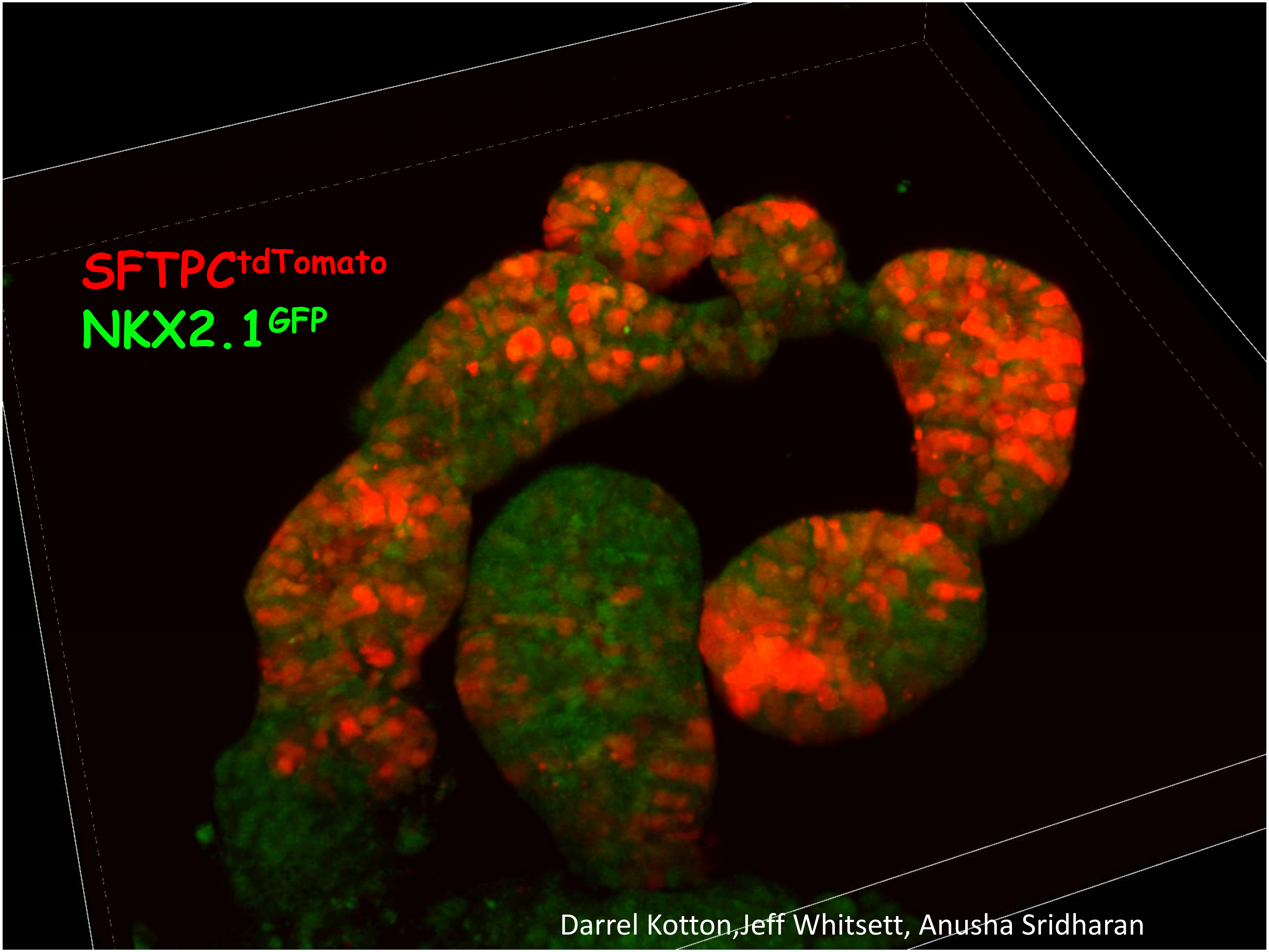
Mechanisms of Disease Pathogenesis

DX and RX

IPSCs to Model Disorders of Surfactant Homeostsis



Darrell Kotton


$SFTPC^{tdTomato}$
$NKX2.1^{GFP}$
Darrel Kotton, Jeff Whitsett, Anusha Sridharan

Vectors for Gene Transfer to the Respiratory Epithelium

AAV9/2 and AAV6/2: Single RNA's, Cas9, SP-C/SP-B

BOCA virus: ABCA3 ~ 5Kb

Lenti: ABCA3 and mutants

SNIMs: Stabilized, Modified RNA's: Cas9 sg RNA's

Can We Correct Monogenic Disorders of Surfactant Homeostasis?

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Lung MAP and "Single Cell-NexGen Sequencing to Identify Mechanisms Mediating Lung Formation Prior to Birth"

Pulmonary Biology

Cincinnati Children's
change the outcome

Yan Xu ,Yina Du
Anusha Sridharan
Joseph Kitzmiller
Minzhe Guo /XiaoFang Tang/Xinhua Lin
Anna-K Perl

Jim Bridges/Tara Rindler/John Snowball

Developmental Biology

Steven Potter
Eric Brunskill
Matthew Kofron

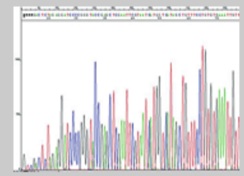
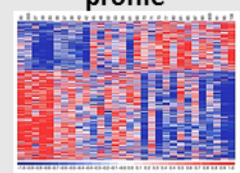
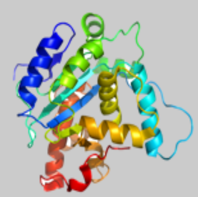
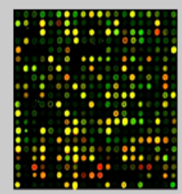
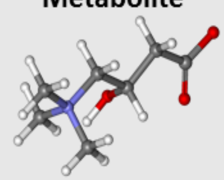
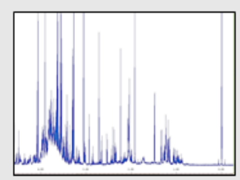
DNA Core CCHMC
Imaging Core CCHMC

Division of Bioinformatics

Bruce Aronow
Phillip Dexheimer

LungMap"omics": Mouse and Human Lung

Data Integration Access and Display

-Omics Technology	Molecule	Large-Scale Data
Genomics	DNA 	DNA Sequence 
Transcriptomics	RNA 	mRNA transcript profile 
Proteomics	Protein 	Protein Profiling 
Metabolomics	Metabolite 	Metabolite Profiling 

Lung Development to Disease

Thanks!

LungMAP

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Research Centers

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