A fluorescence microscopy image showing a cluster of lung organoids. The organoids are stained with various markers: blue (nuclei), red (cytokeratin 5/6, basal layer), green (surfactant protein A, type II cells), and yellow (surfactant protein C, type II cells).

# Patient iPSC-based disease modeling of chILD caused by ABCA3 mutations

**Yuliang Leon Sun**

**Darrell Kotton Lab**

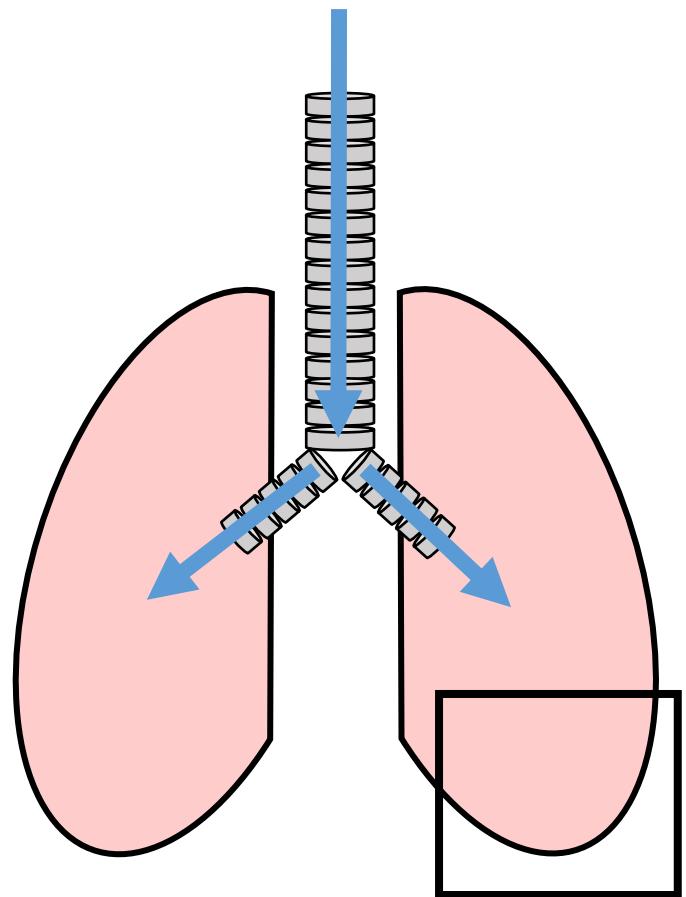
Center for Regenerative Medicine

Boston University School of Medicine

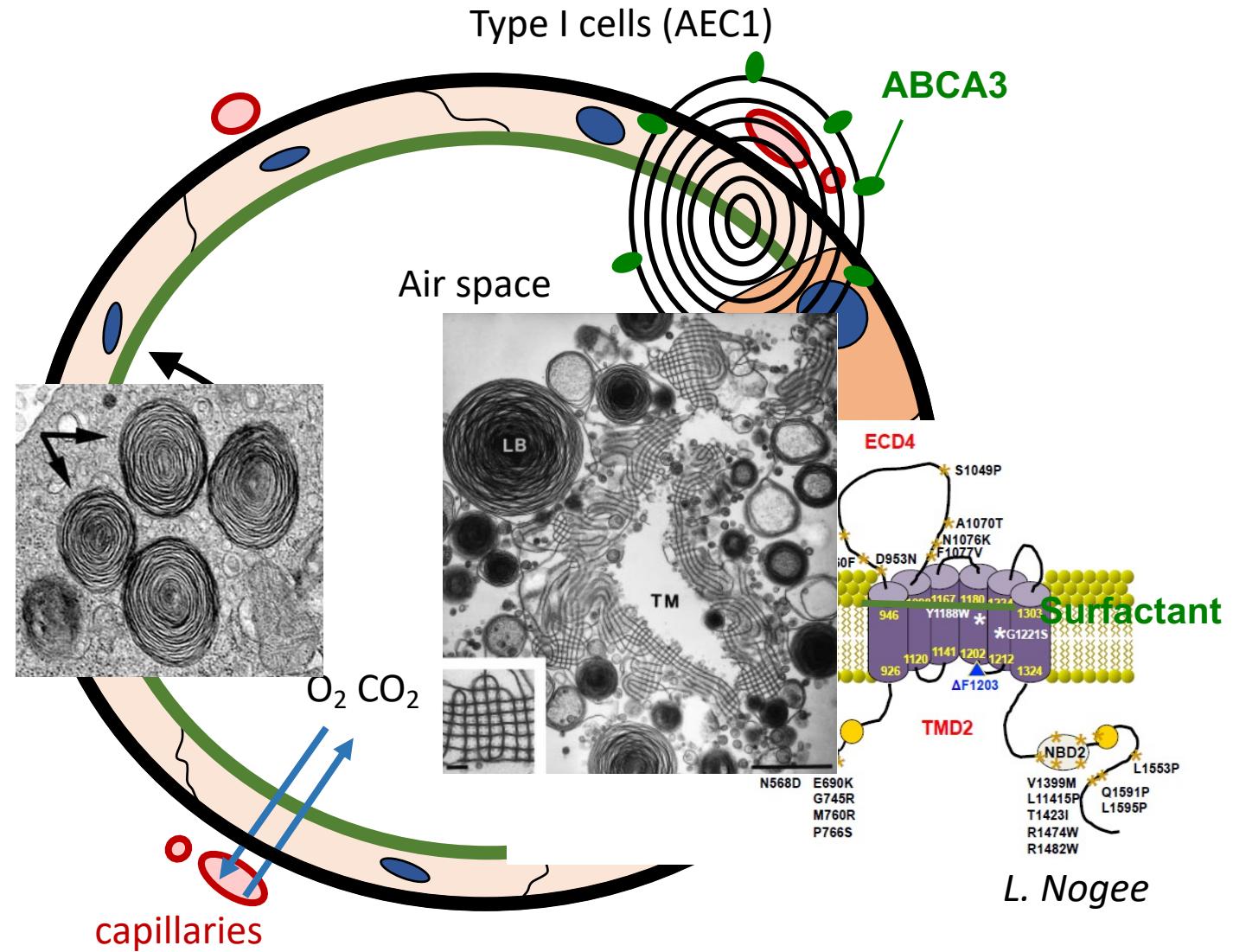
Boston Medical Center

7/7/17

# Type II alveolar epithelial cells (AEC2s) and the role of ABCA3 in surfactant metabolism

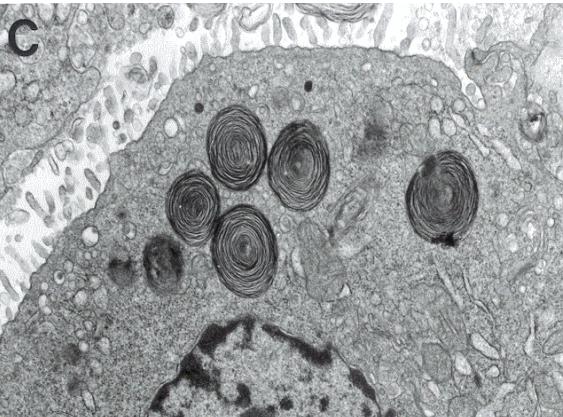
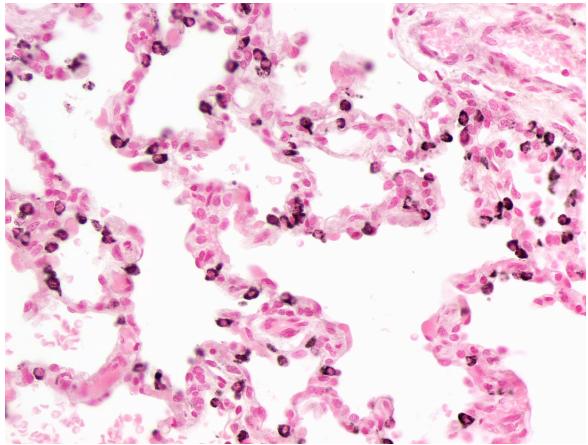


Type II cells  
(AEC2)

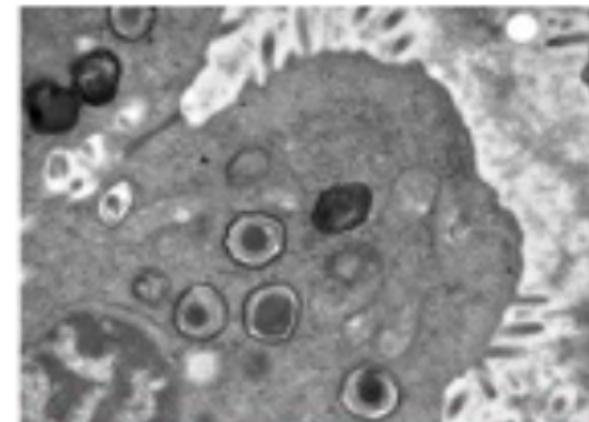
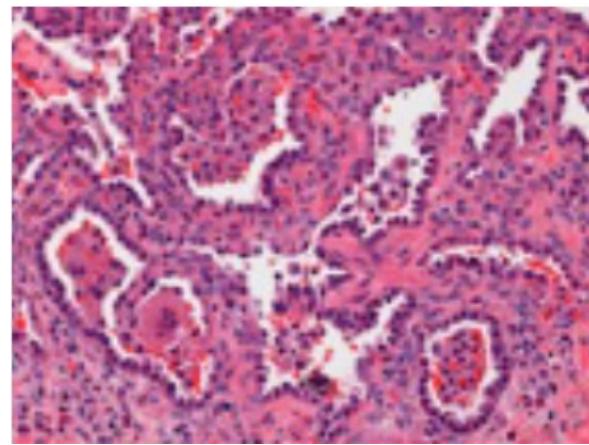


# Childhood interstitial lung disease (chILD) can be caused by disruption in pulmonary surfactant homeostasis in AEC2s

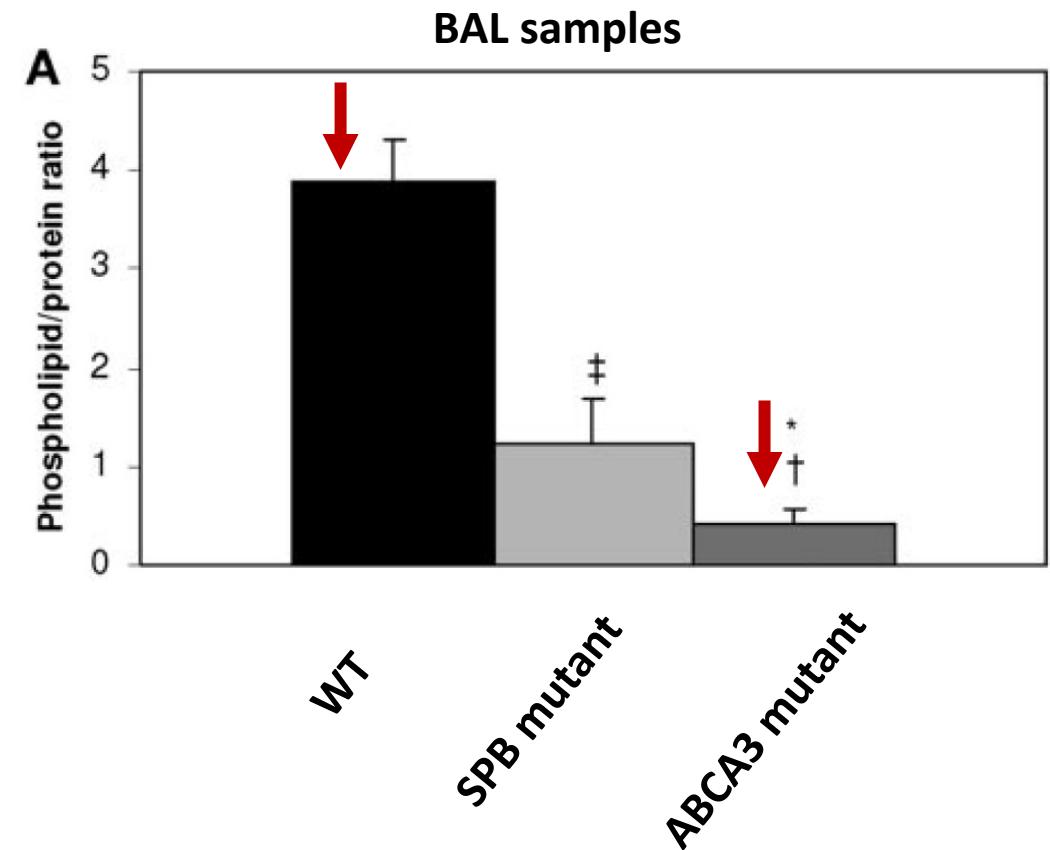
Normal human Lung



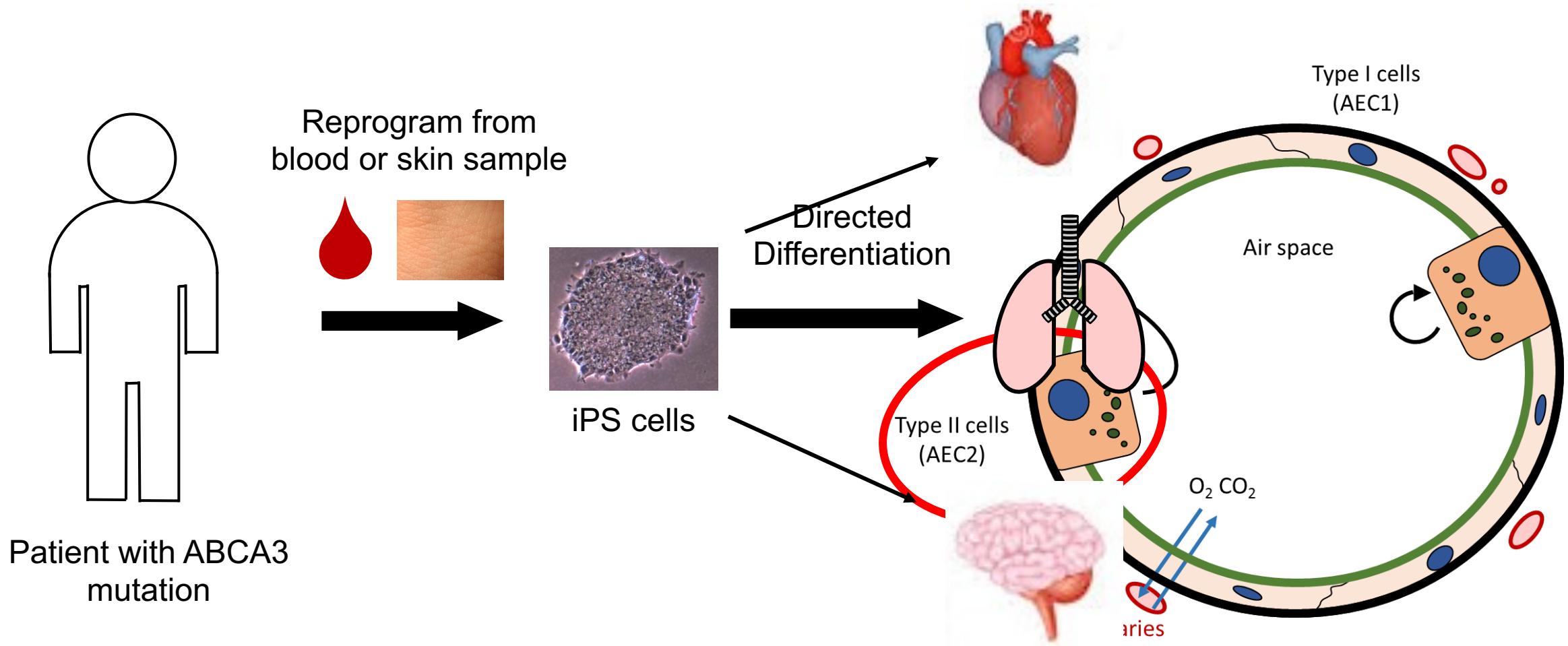
ABCA3 Mutant Diseased lung



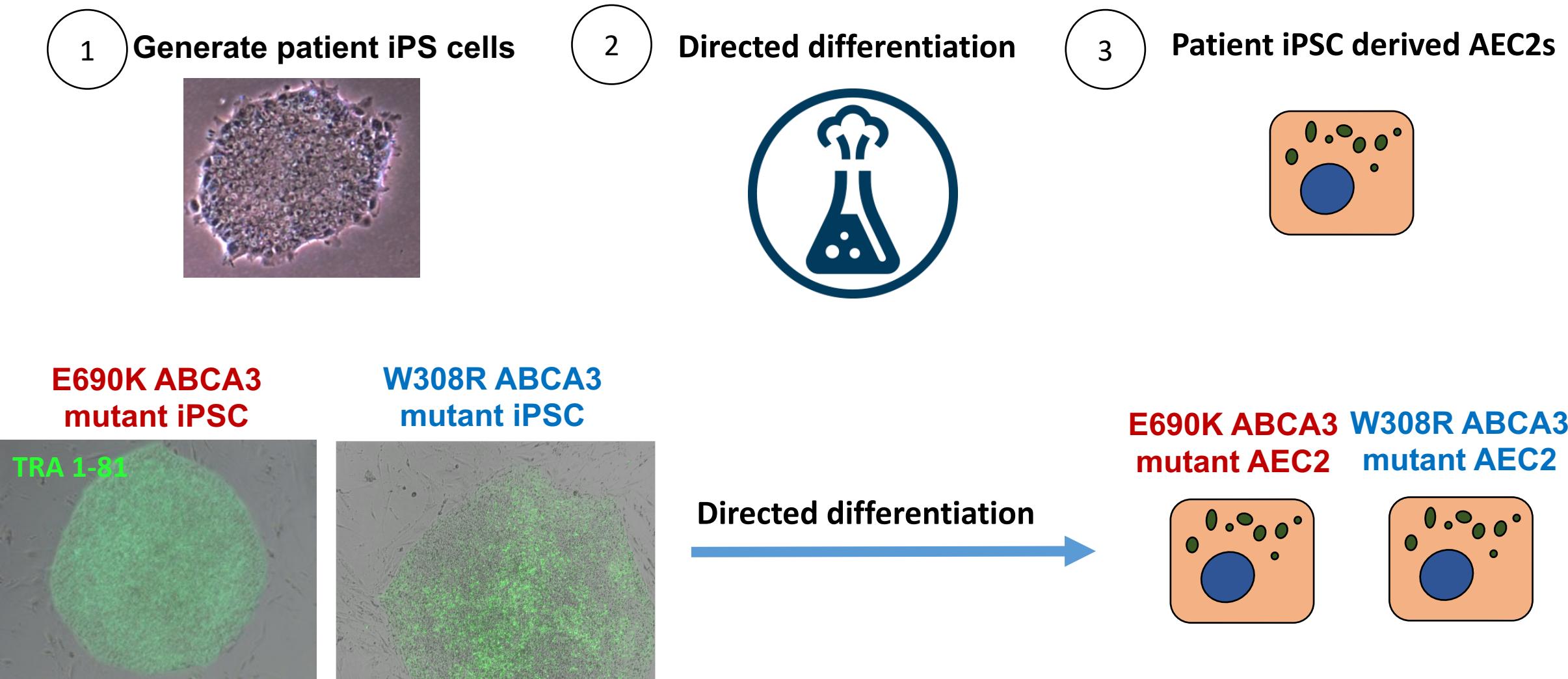
Mutations in SFTPC, SFTPB, NKX2-1, **ABCA3**



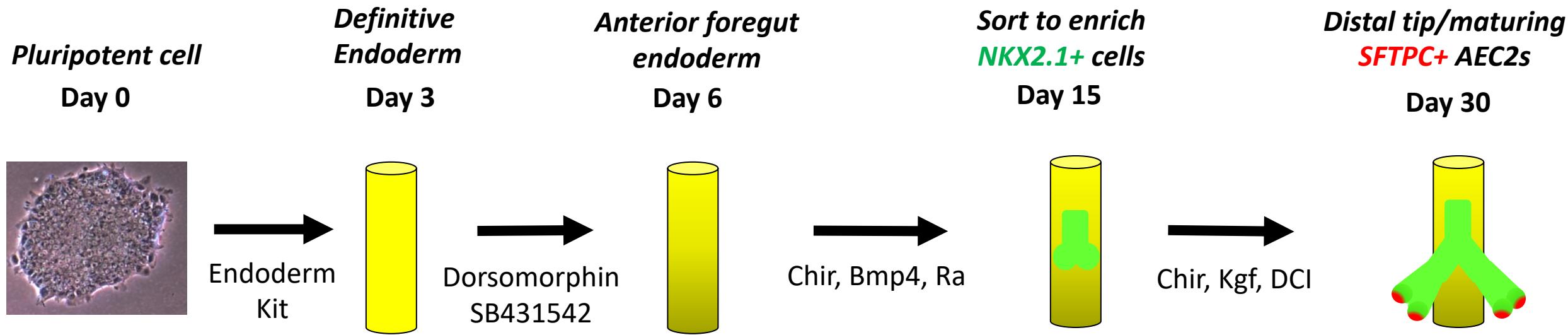
# Using AEC2s derived from patient induced pluripotent stem cells (iPSCs) as a model to study ABCA3 mutation in AEC2s



# Road to derive ABCA3 mutant patient-specific AEC2s from iPSCs

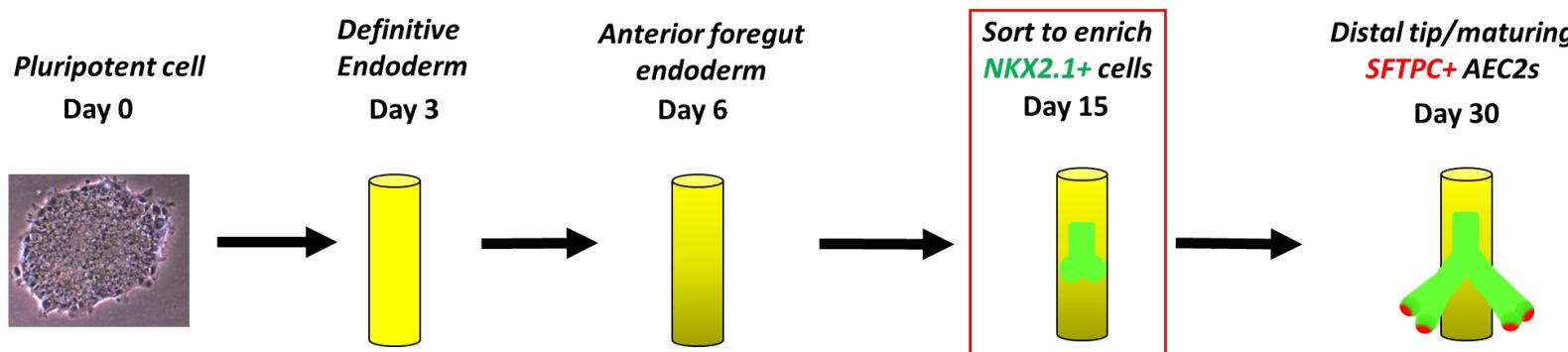
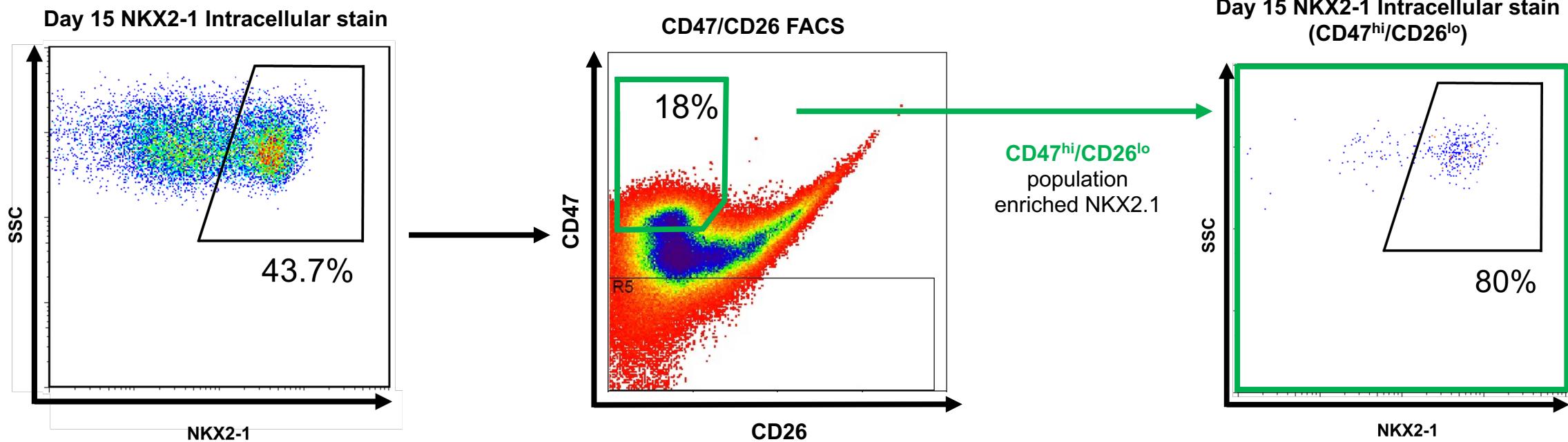


# Lung directed differentiation mirrors milestones achieved over the course of *in vivo* human lung developmental biology



Day 15 NKX2-1 expressing cells were enriched using CD47/26 cell surface markers

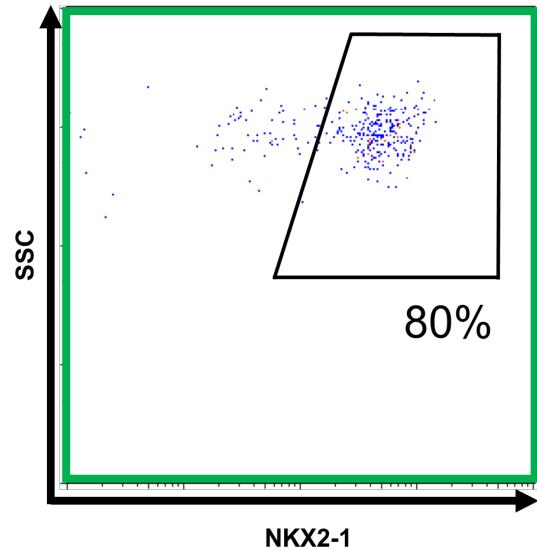
E690K ABCA3 iPSC line



# Outgrowth of day 15 NKX2.1 enriched cells formed alvelospheres expressing the SFTPC/tdTomato reporter

E690K ABCA3 iPSC line

Day 15 NKX2-1 enriched population



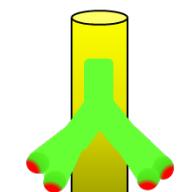
Sort to enrich  
NKX2.1+ cells

Day 15

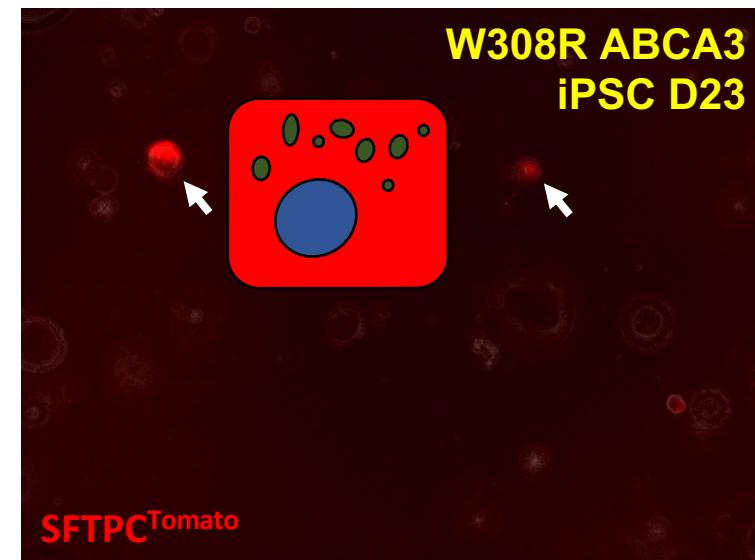
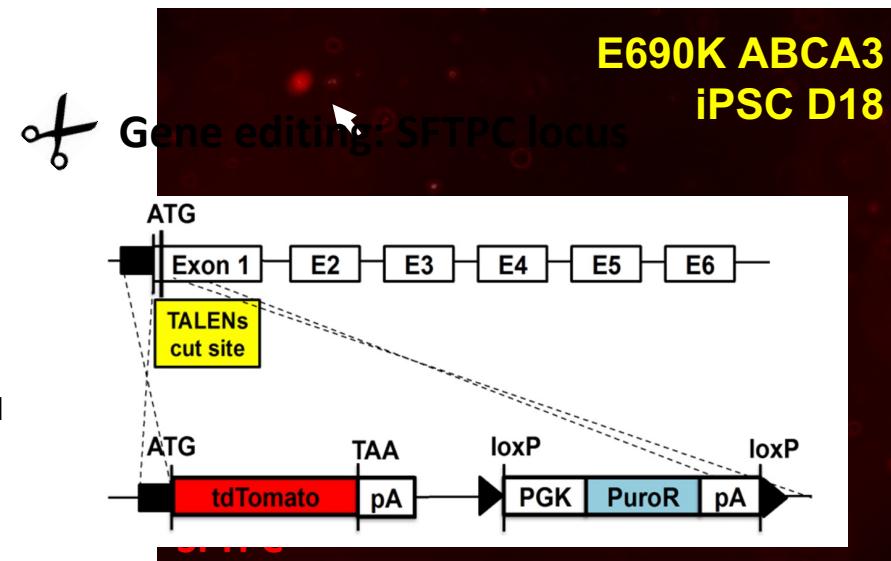


Distal tip/maturing  
SFTPC+ AEC2s

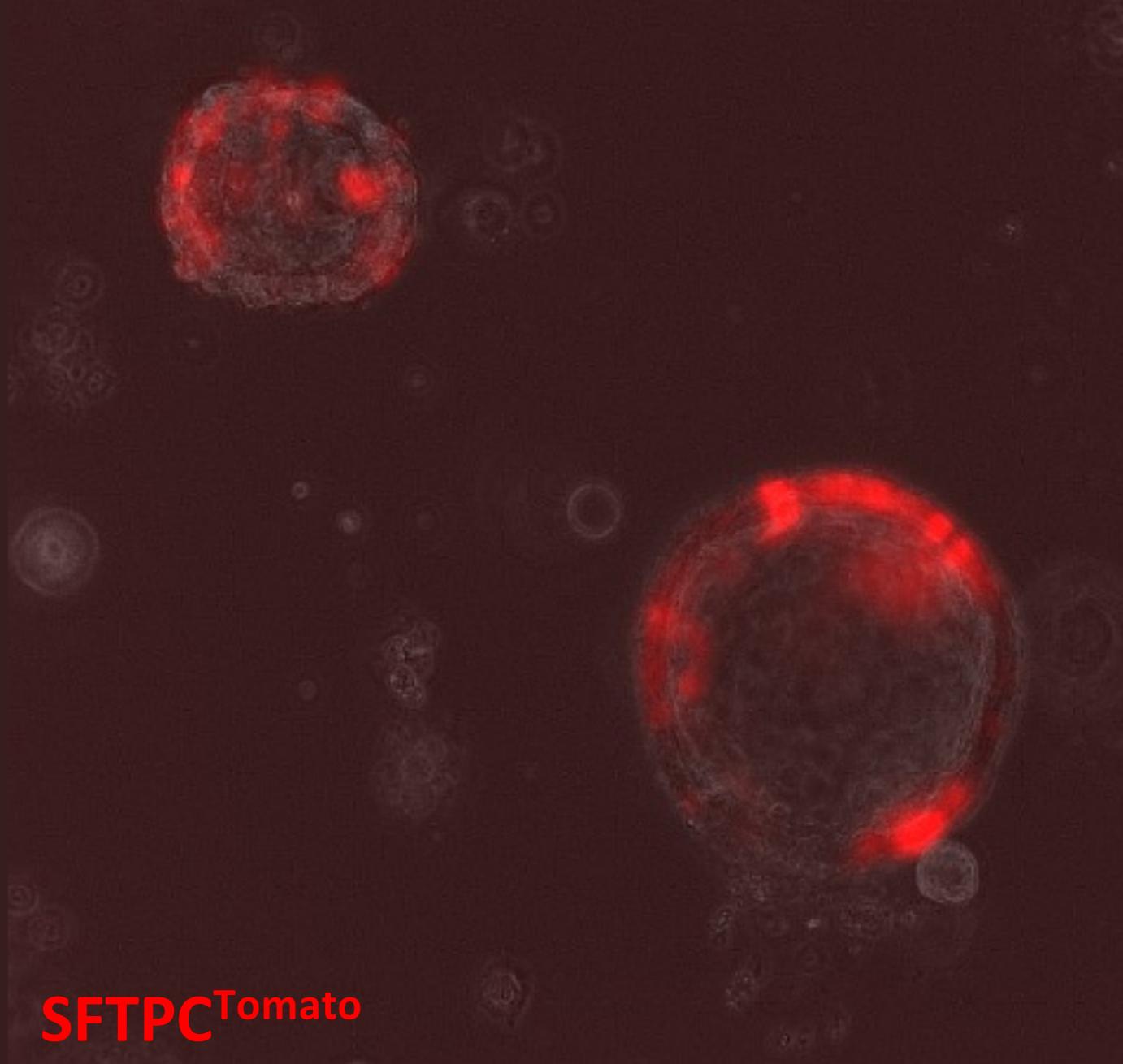
Day 30



Chir, Kgf, DCI  
Plate in 3D matrigel

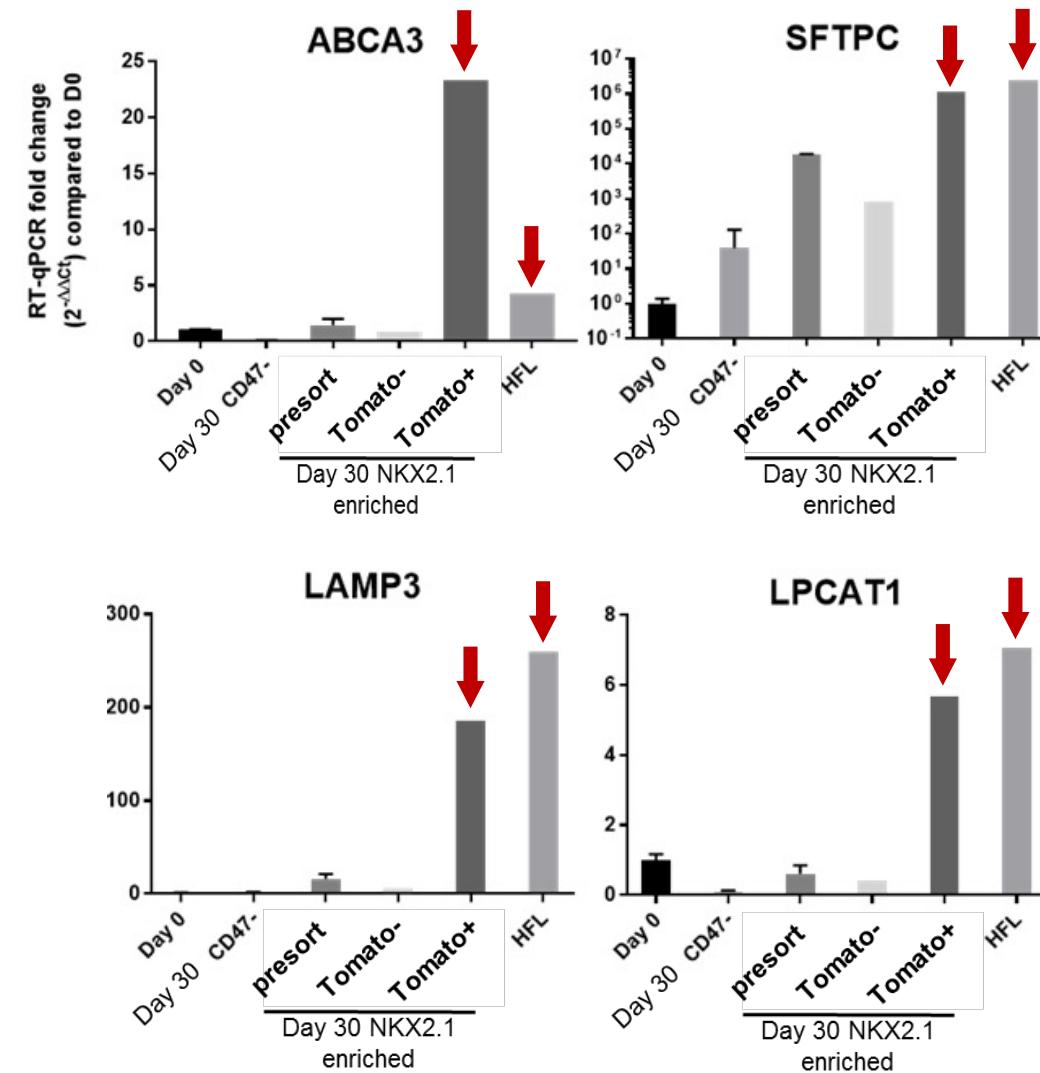
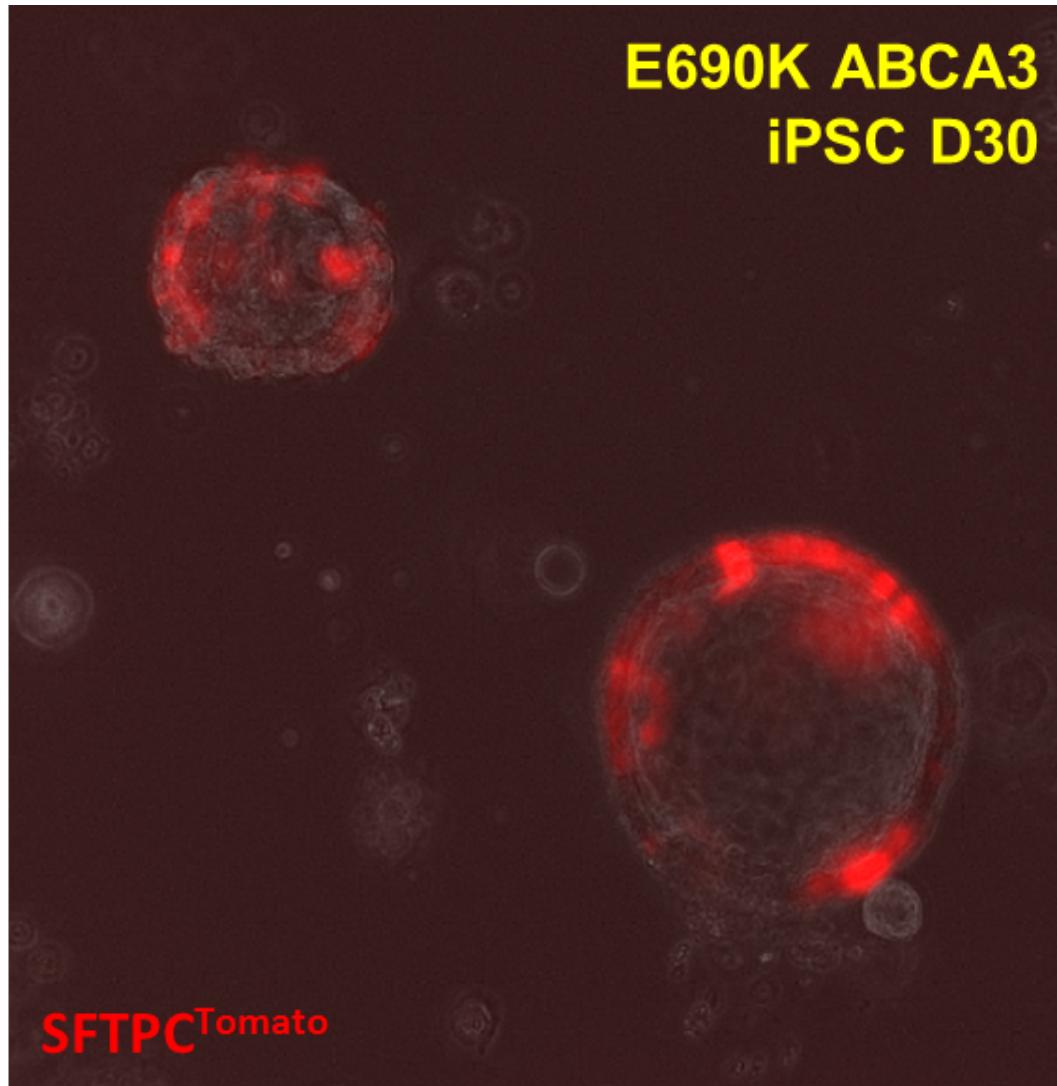


# E690K ABCA3 iPSC D30



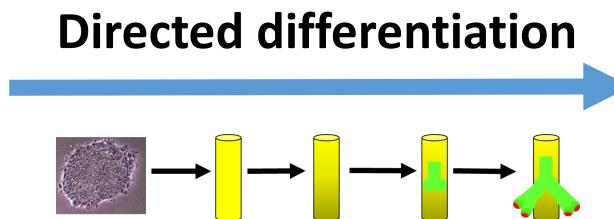
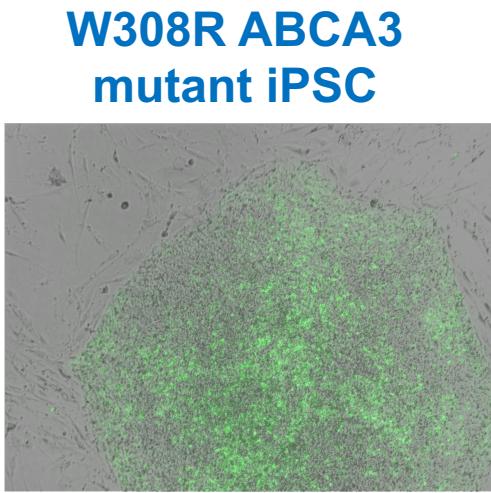
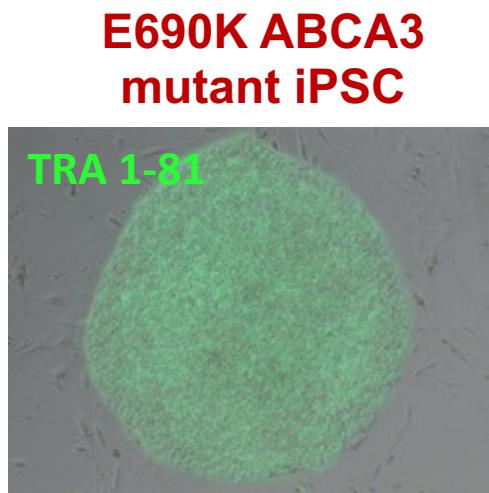
SFTPC<sup>Tomato</sup>

# Characterization of Tomato+ cells by RT-qPCR against human fetal lung positive control showed similar expression of key surfactant-related genes

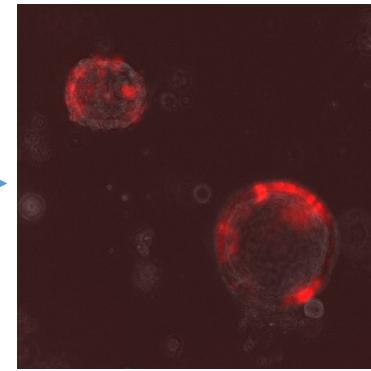


Positive CTRL= Week 21 human fetal lung enriched in AEC2s

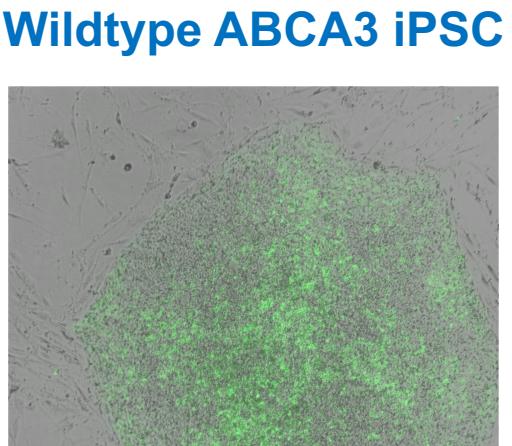
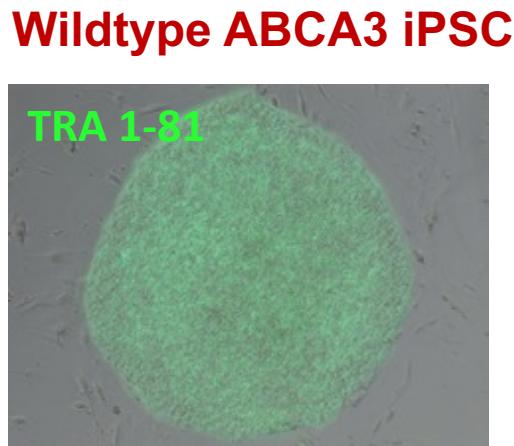
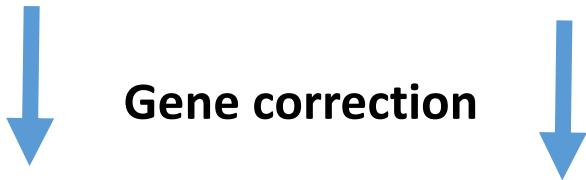
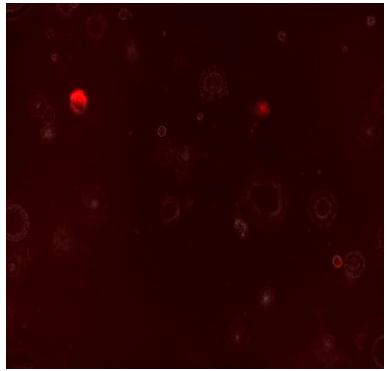
# Comparing patient iPSC-derived AEC2s with gene corrected syngeneic wildtype controls to identify ABCA3 mutant specific disease mechanisms



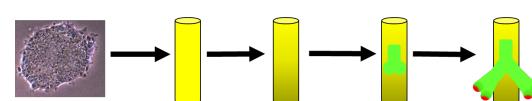
E690K ABCA3 mutant AEC2



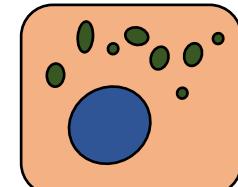
W308R ABCA3 mutant AEC2



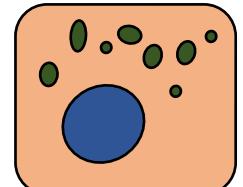
Directed differentiation



Wildtype AEC2

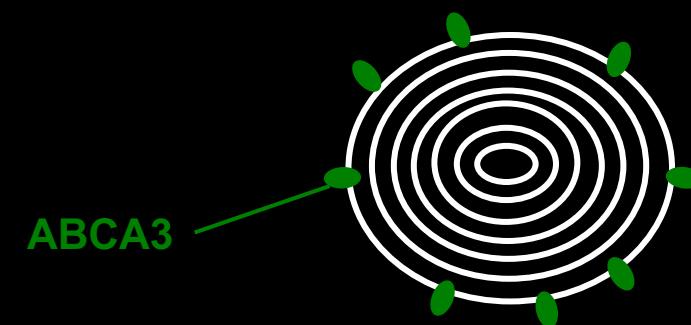
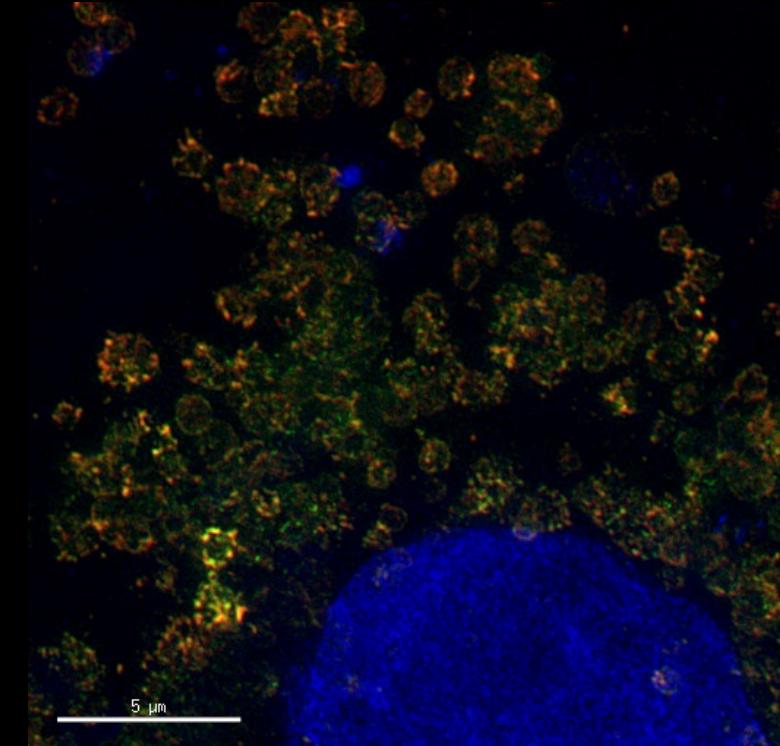
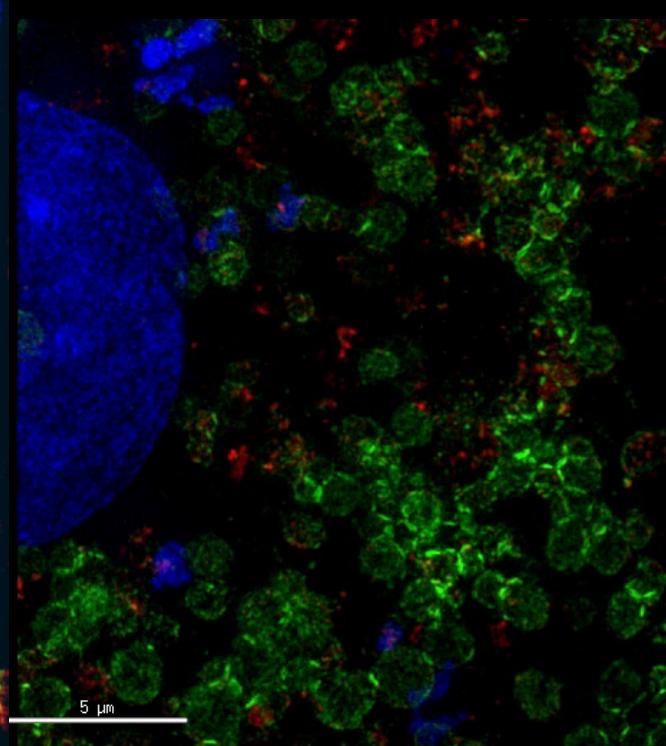
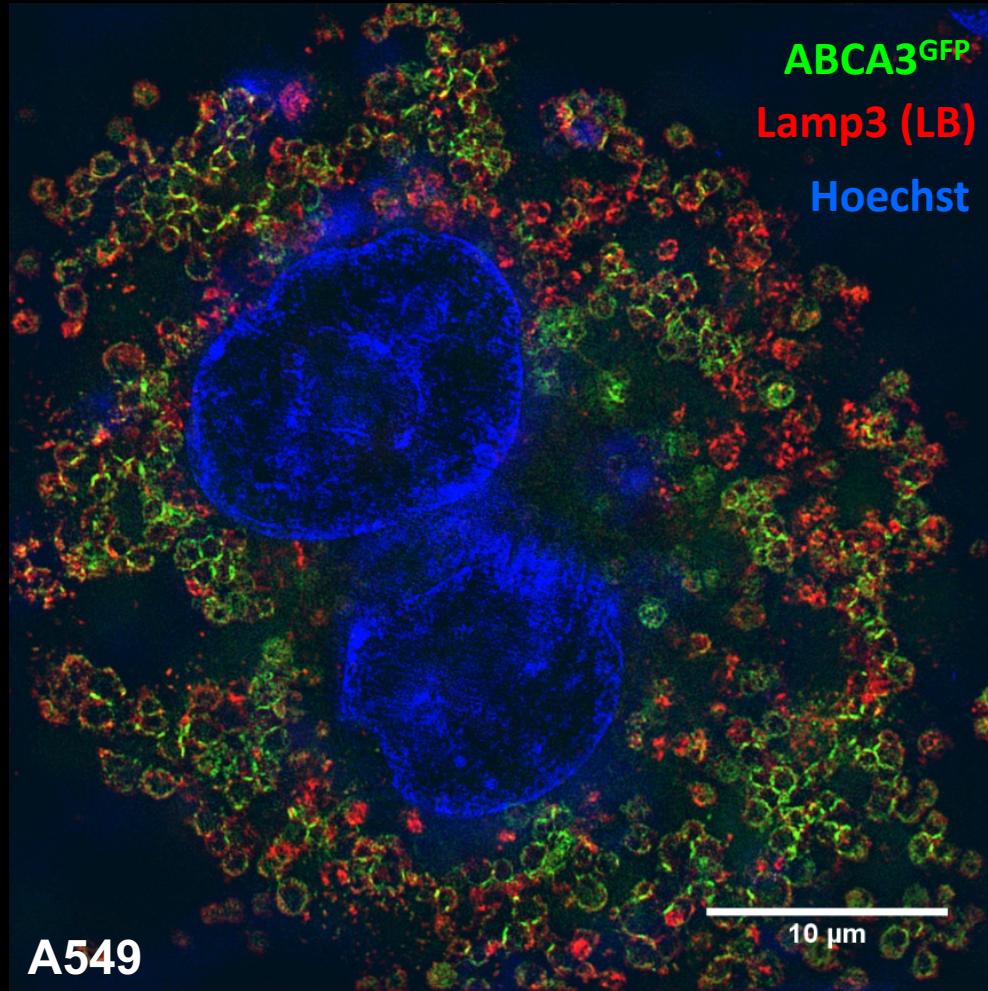


Wildtype AEC2



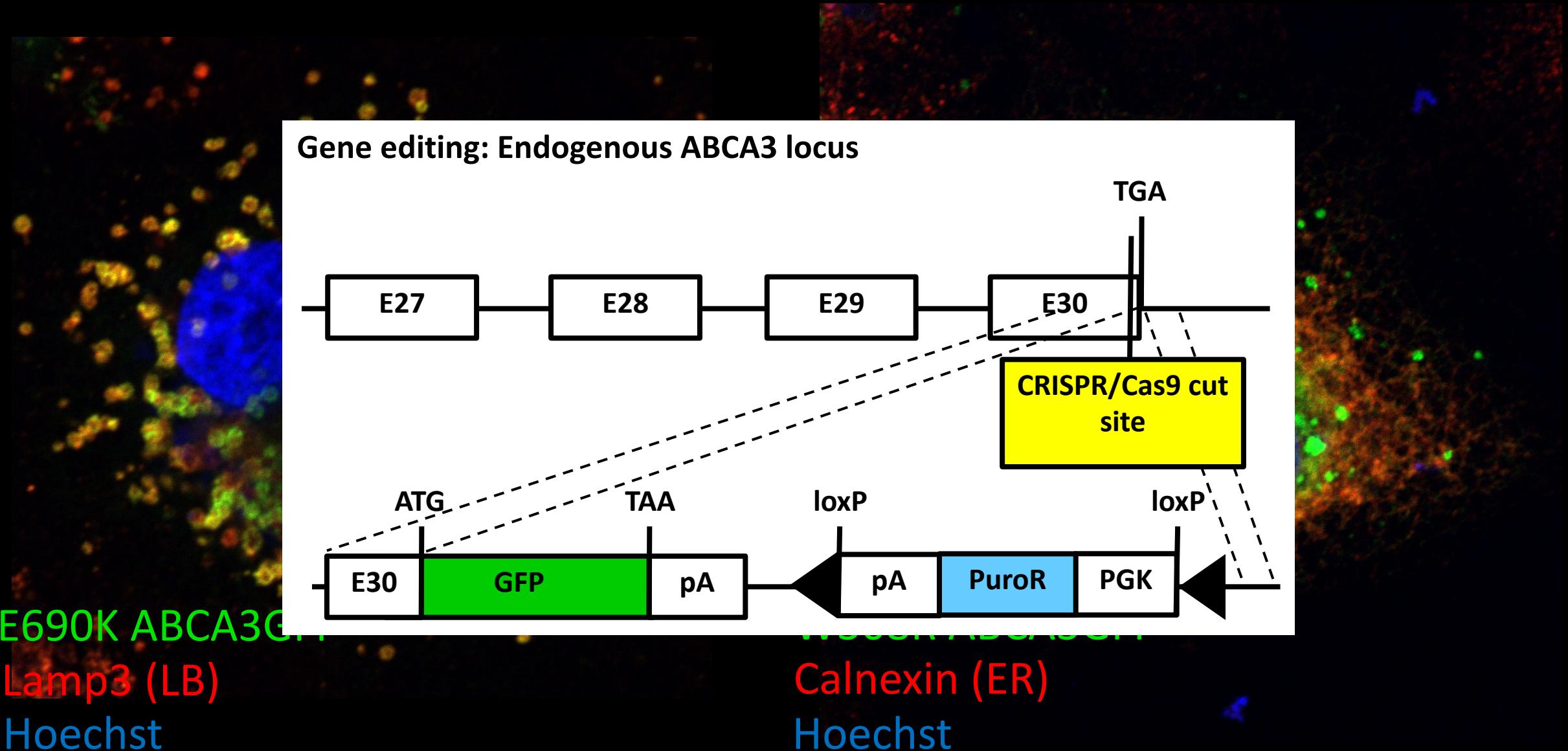
Identify ABCA3 mutant specific disease mechanisms

# ABCA3 GFP fusion protein can be used as a tool to visualize ABCA3 protein trafficking

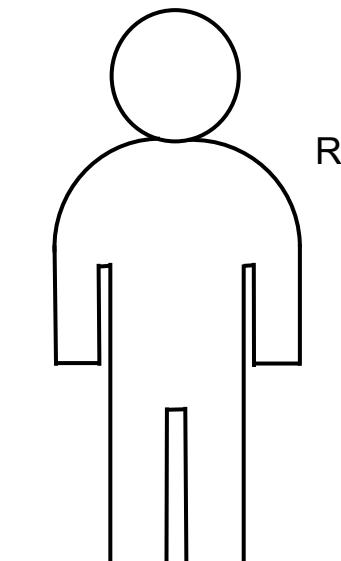


Plasmids received from Jennifer Wambach

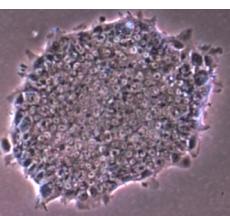
A549 cells expressing W308R mutant ABCA3GFP showed abnormal co-localized with ER marker Calnexin suggestive of protein mistrafficking



# In summary



Reprogram from blood  
or skin

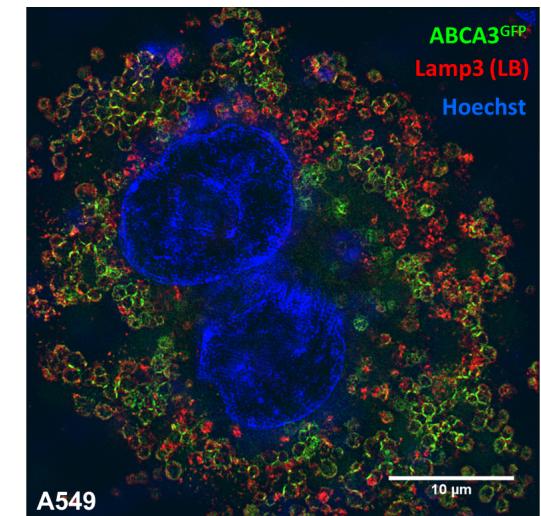
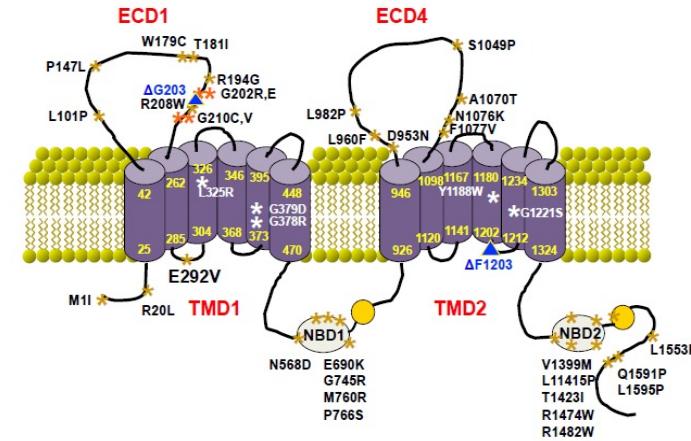
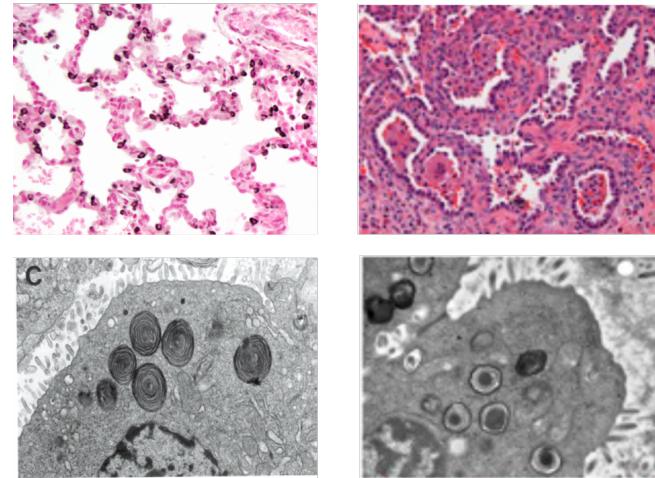


iPS cells

Differentiate into  
iAEC2s



SFTPC/Tomato+  
iAEC2s



Patient with ABCA3  
mutation

**E690K**

**W308R**

# Acknowledgements:

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Hector Marquez  
Jyn-Chang Jean  
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Michelle Higgins

## Wash U:

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Ping Yang

## U Penn:

Ed Morrissey

## Univ of Cincinnati:

Jeffrey Whitsett

## Johns Hopkins Univ:

Lawrence Nogee