A fluorescence microscopy image showing a cluster of cells. The nuclei are stained blue. Within the cells, there are punctate areas of red and green fluorescence, indicating the presence and localization of the ABCA3 protein.

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

Yuliang Leon Sun

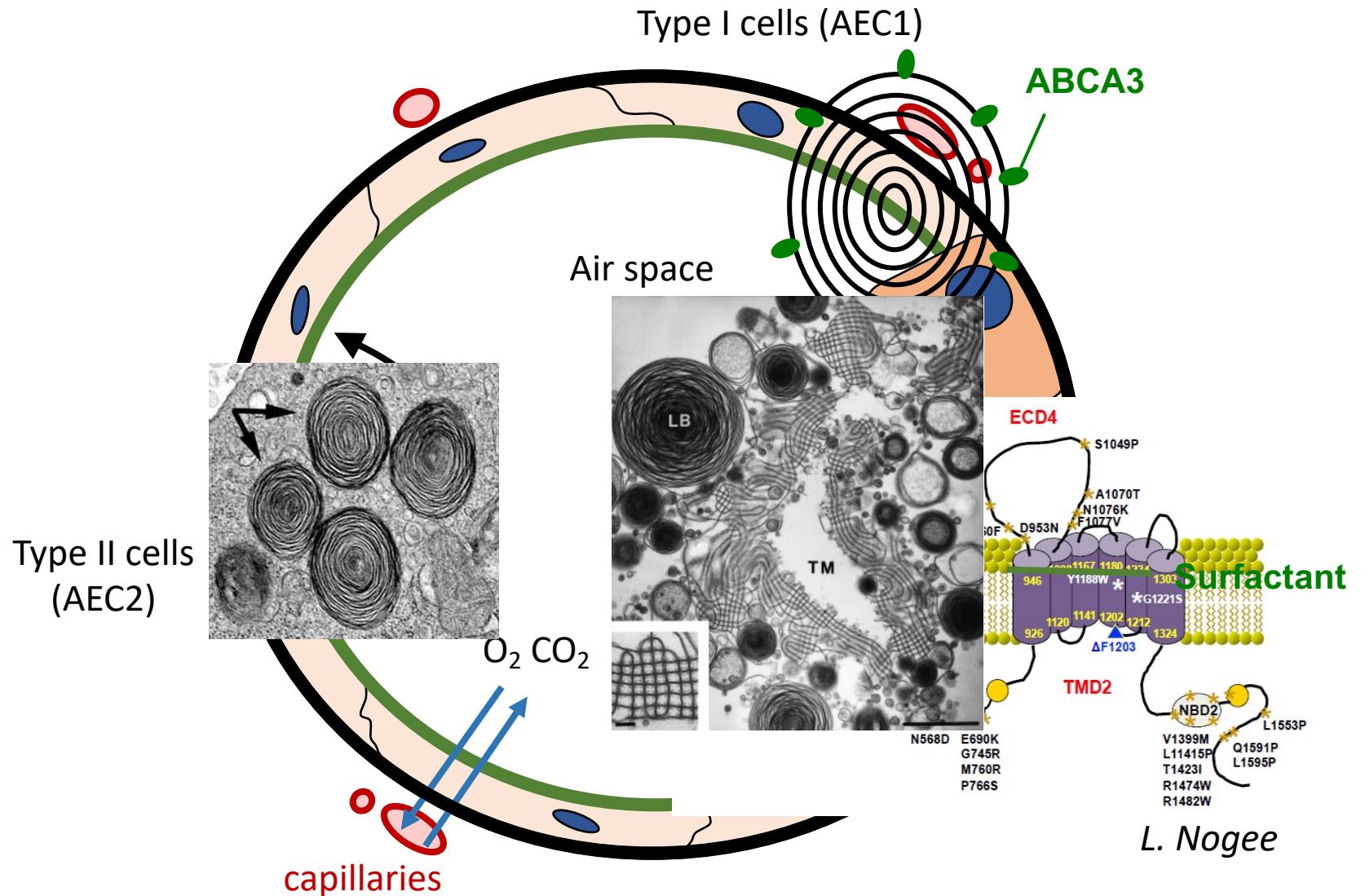
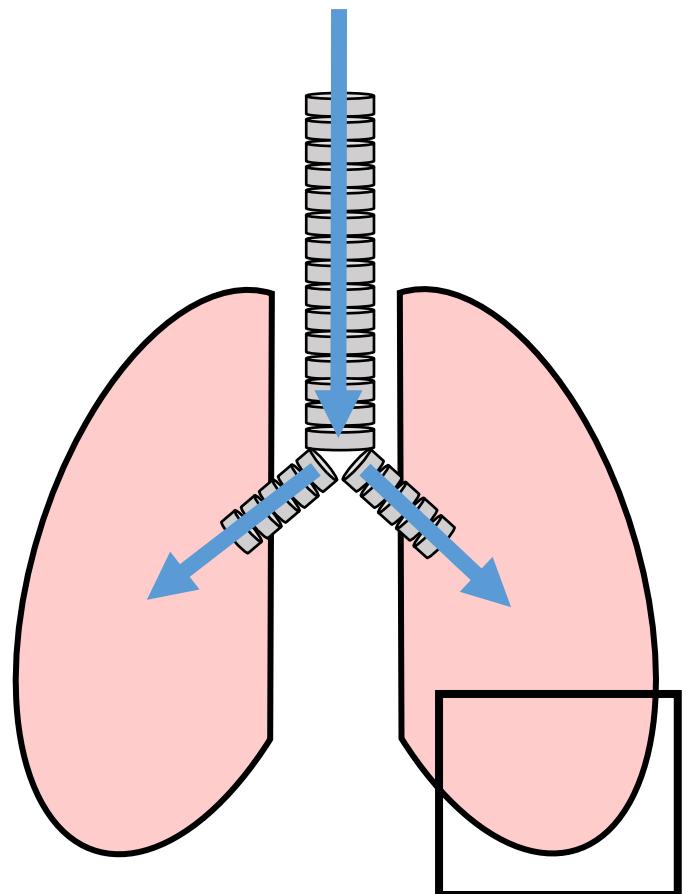
Darrell Kotton Lab, Whitsett Research Hub

Center for Regenerative Medicine

Boston University and Boston Medical Center

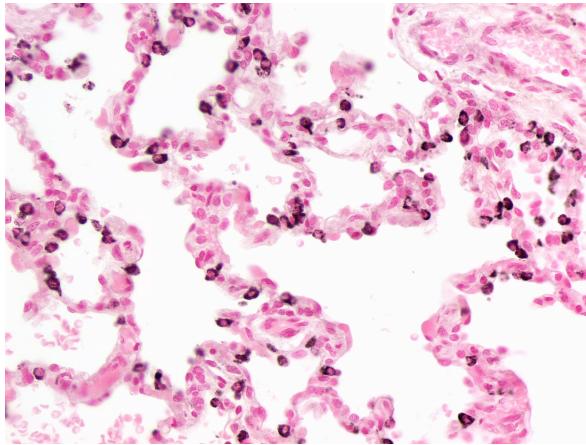
9/25/17

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

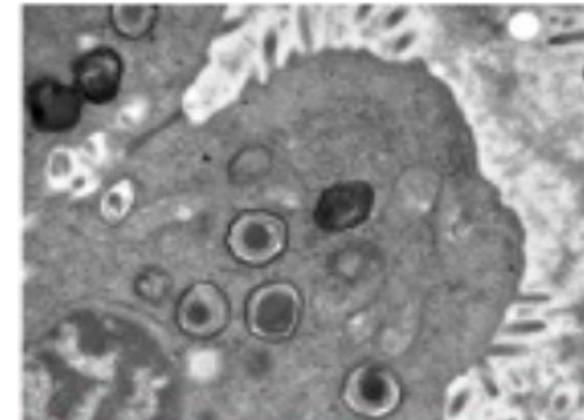
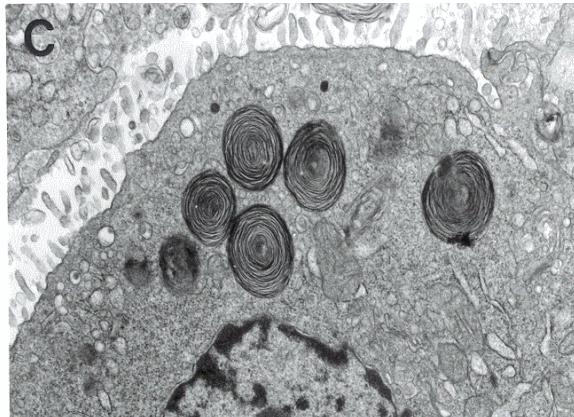
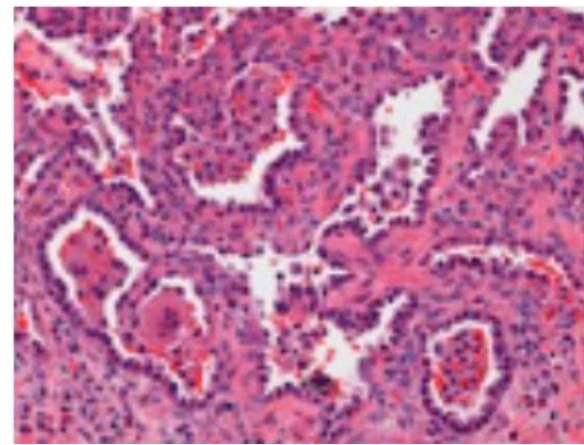


Childhood interstitial lung disease (chILD) can be caused by disruption in pulmonary surfactant homeostasis in AEC2s

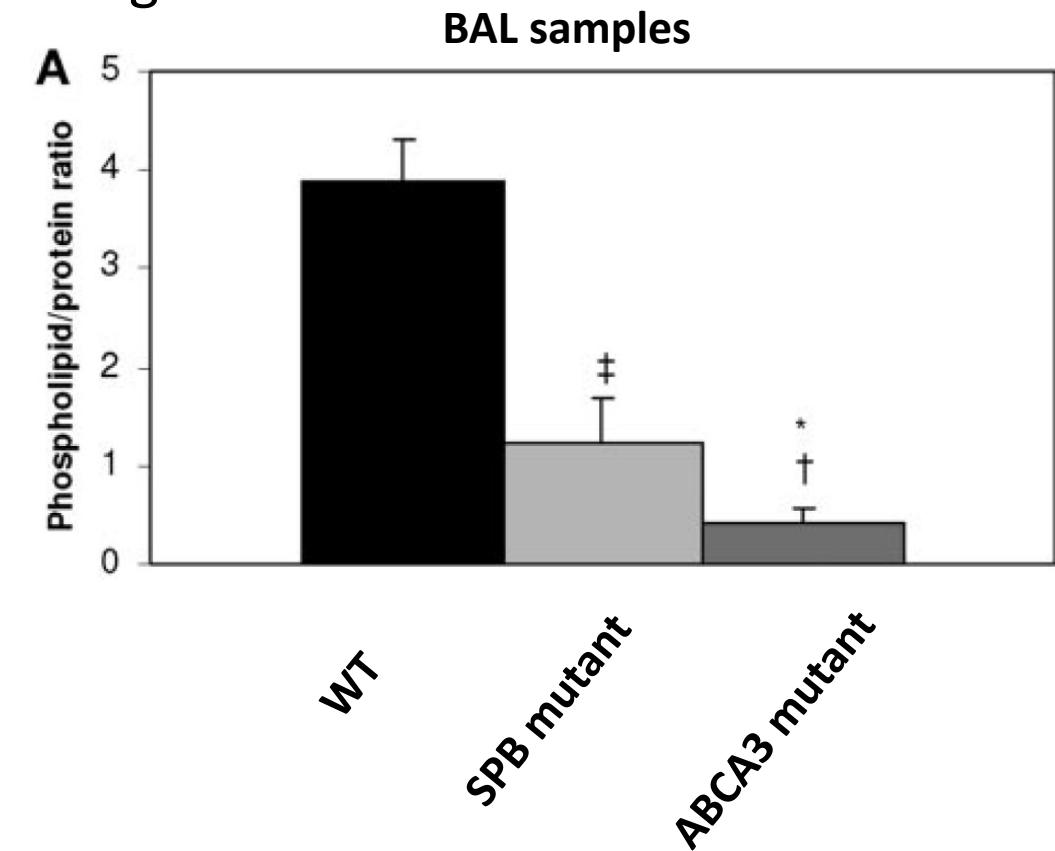
Normal human Lung



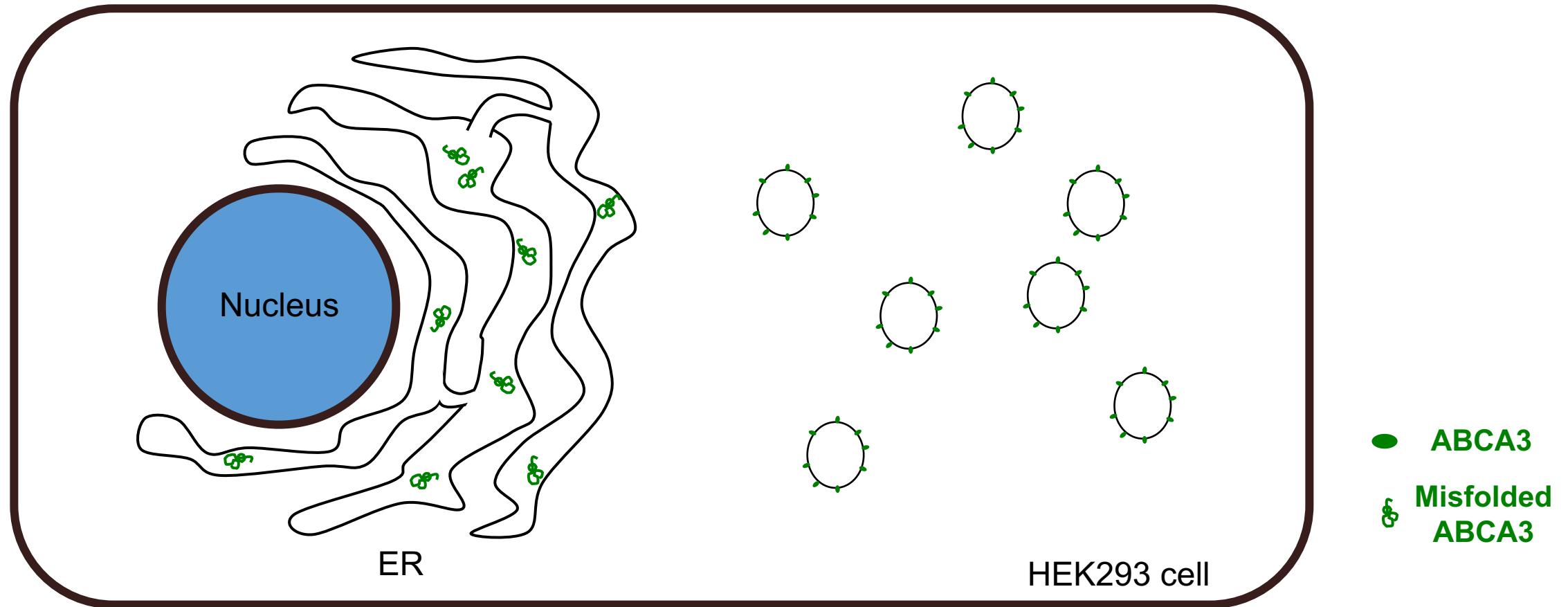
ABCA3 Mutant Diseased lung



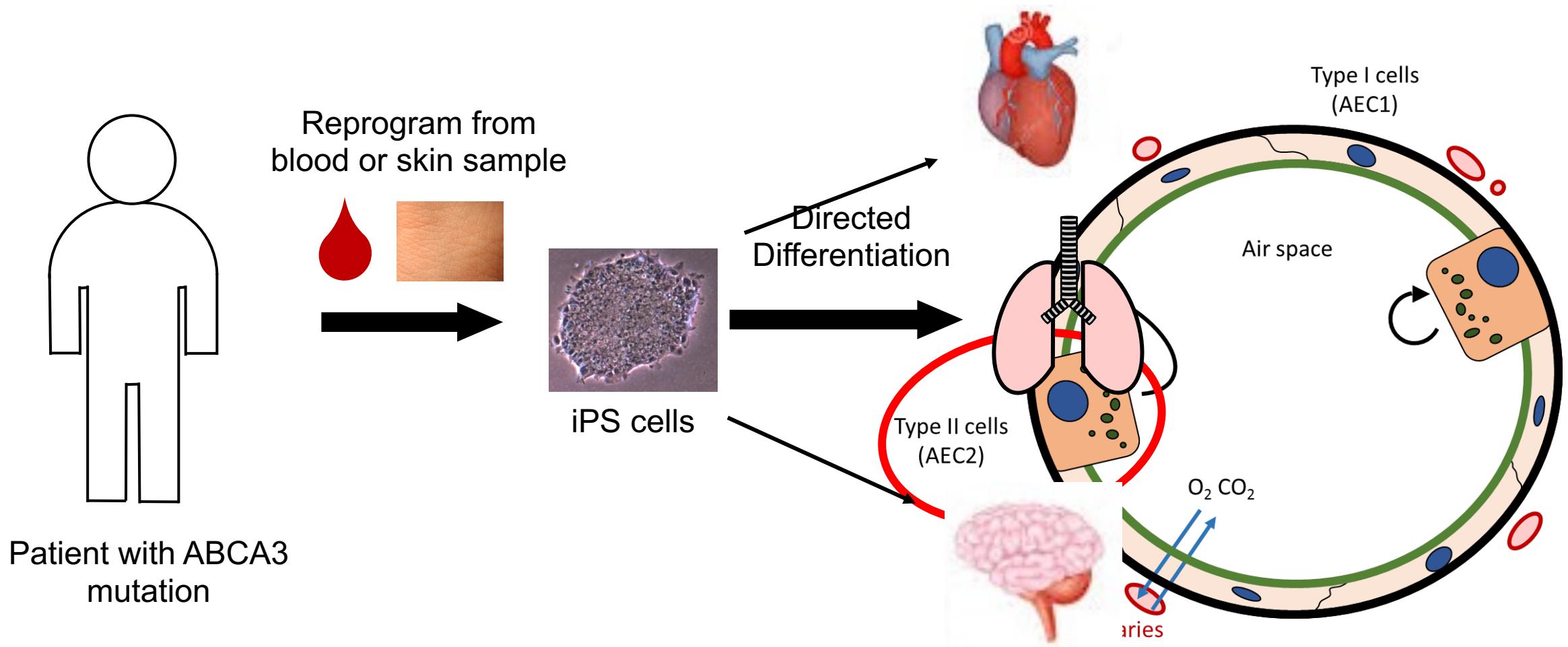
Caused by bi-allelic mutations in **ABCA3** and other genes



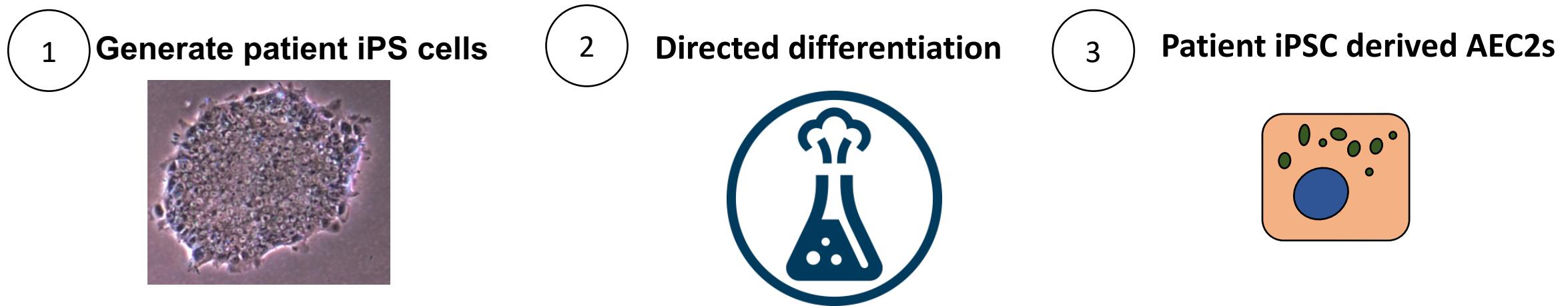
ABCA3 mutations may be classified into two categories:
functional mutants and protein mis-trafficking mutants



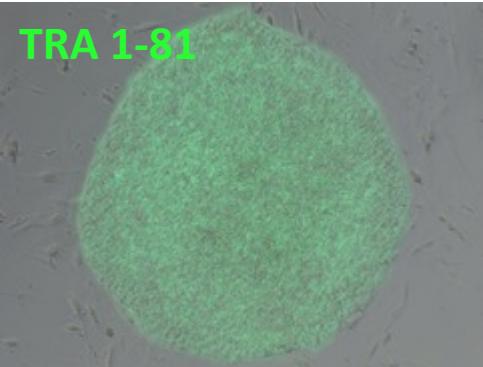
Using AEC2s derived from patient induced pluripotent stem cells (iPSCs) as a model to study ABCA3 mutation in AEC2s



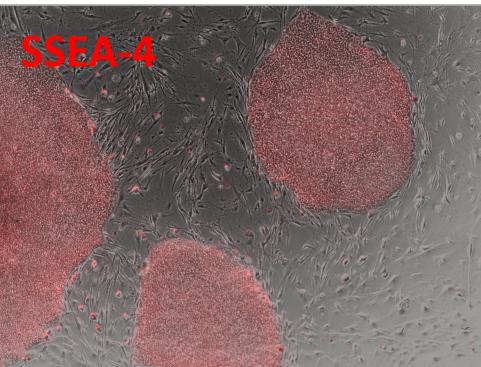
Road to derive ABCA3 mutant patient-specific AEC2s from iPSCs



Homozygous E690K
ABCA3 mutant iPSC



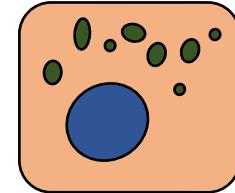
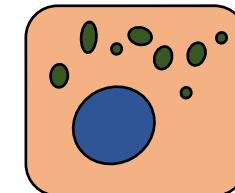
Homozygous W308R
ABCA3 mutant iPSC



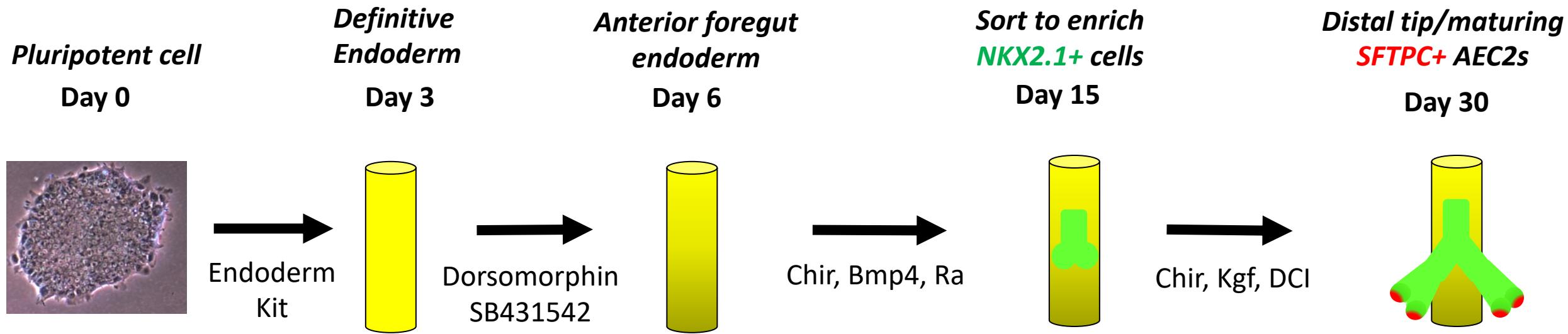
Directed differentiation



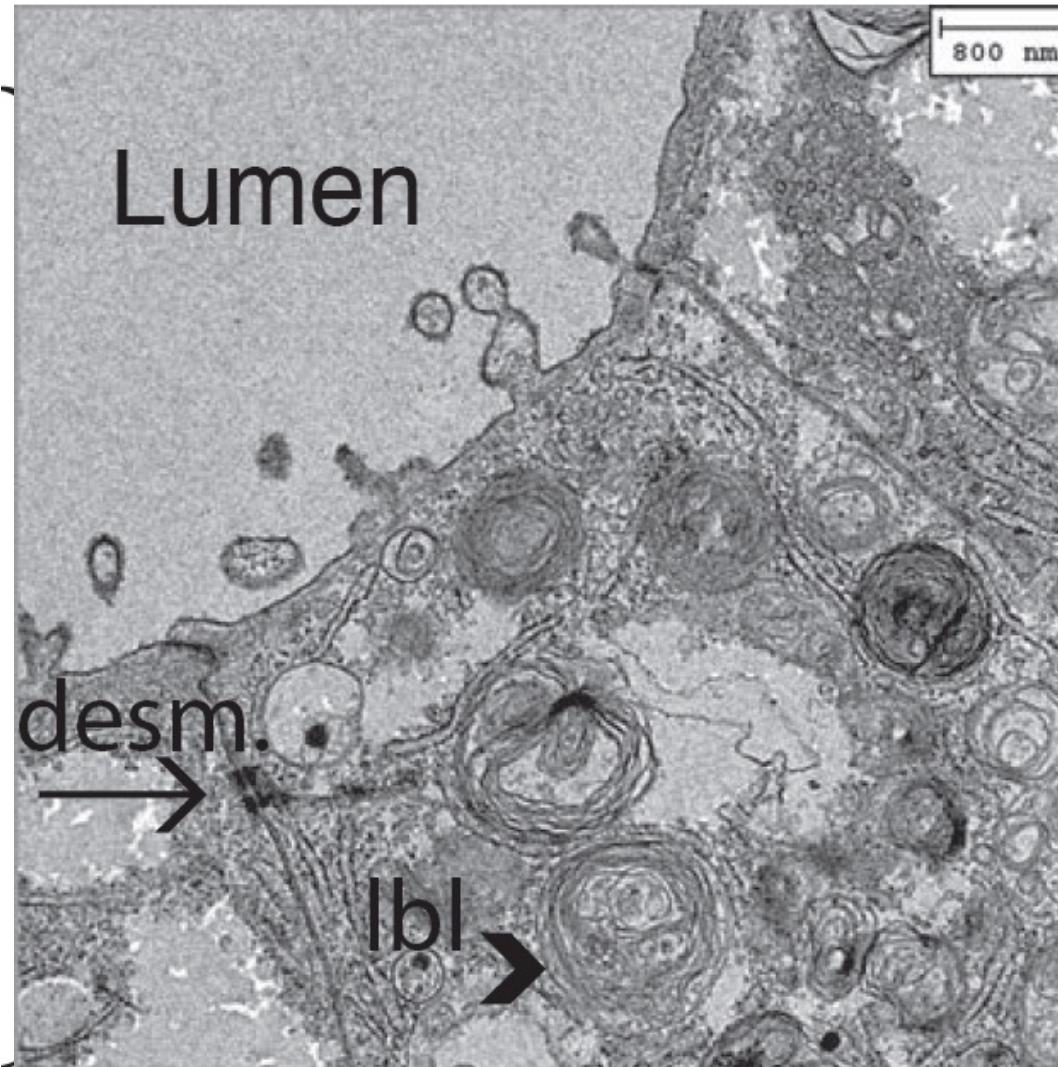
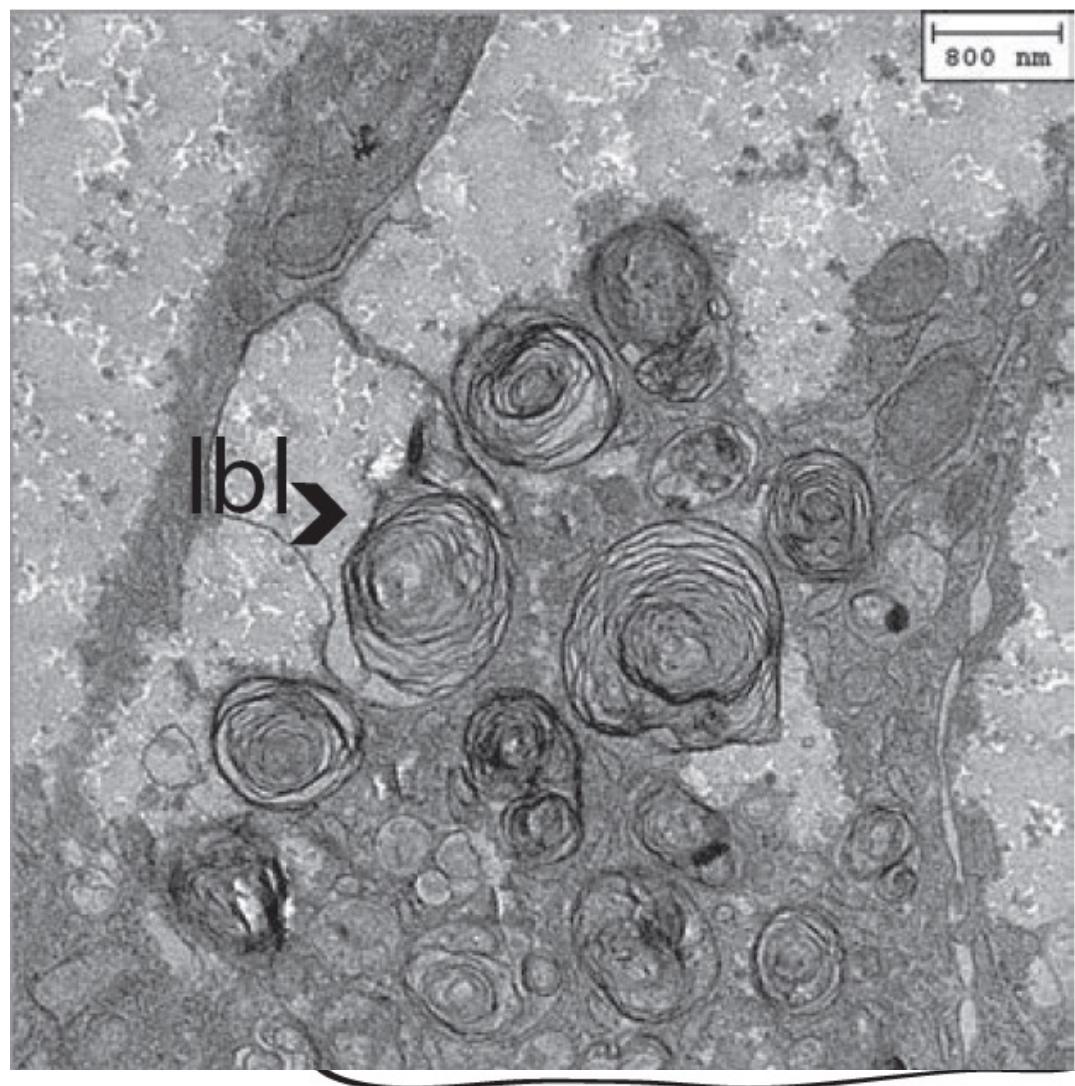
E690K ABCA3 W308R ABCA3
mutant AEC2 mutant AEC2



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

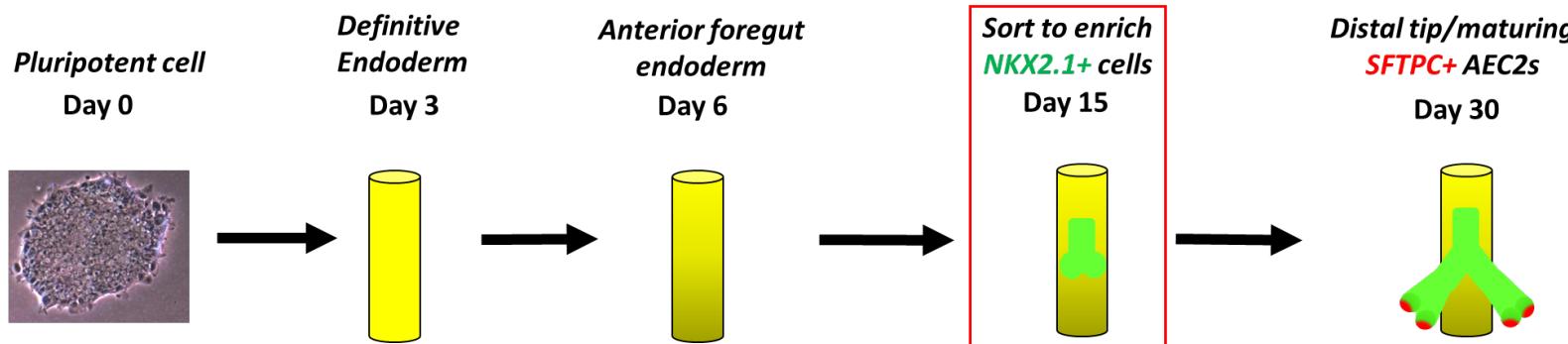
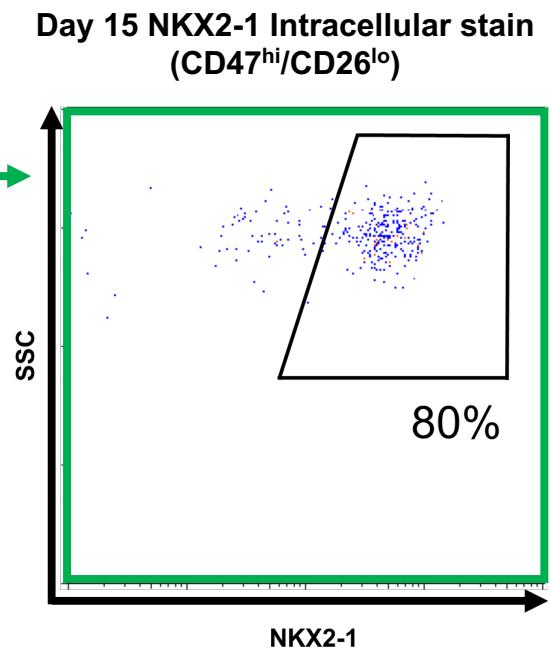
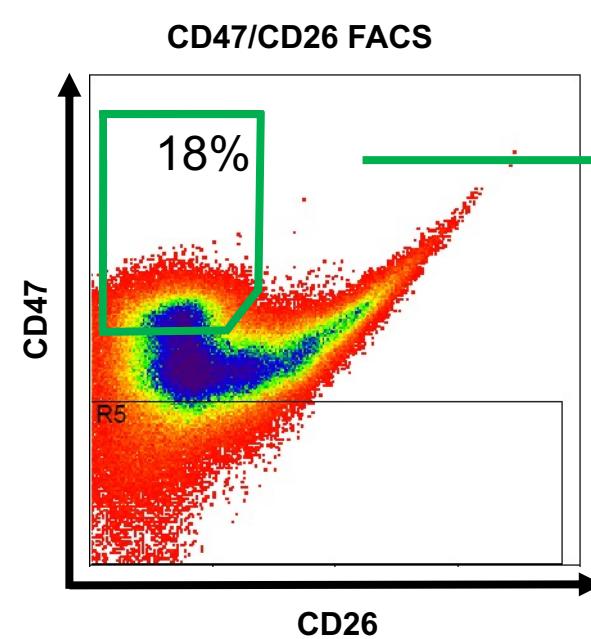
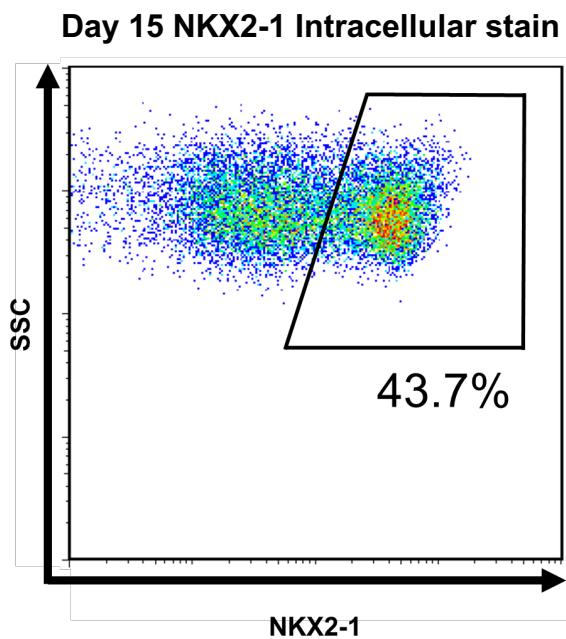


D30 cells following lung directed differentiation contained functional lamellar bodies characterized by SFTPB protein processing



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

E690K ABCA3 iPSC line

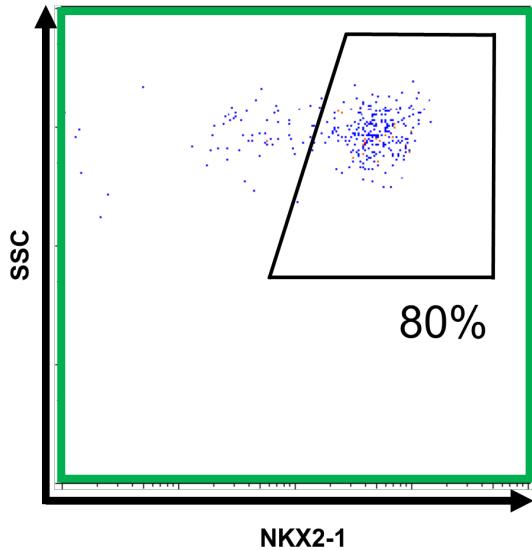


Hawkins et al. 2017 JCI

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



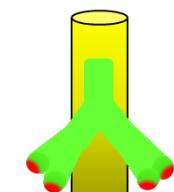
Sort to enrich
NKX2.1+ cells

Day 15

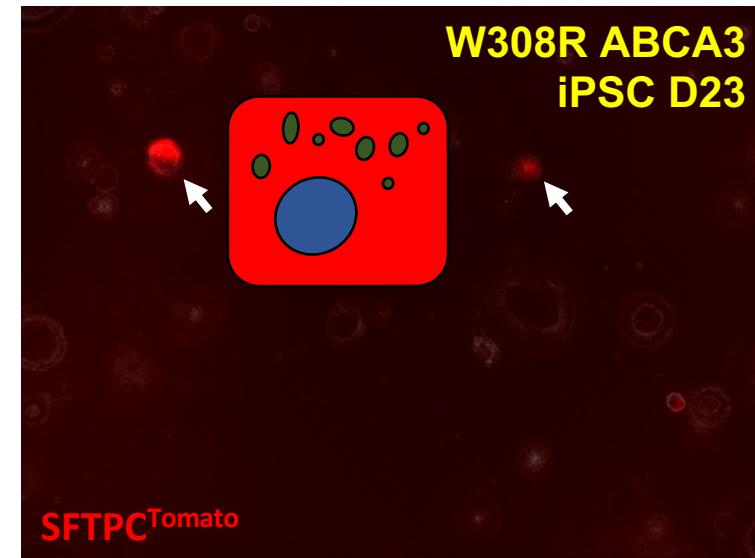
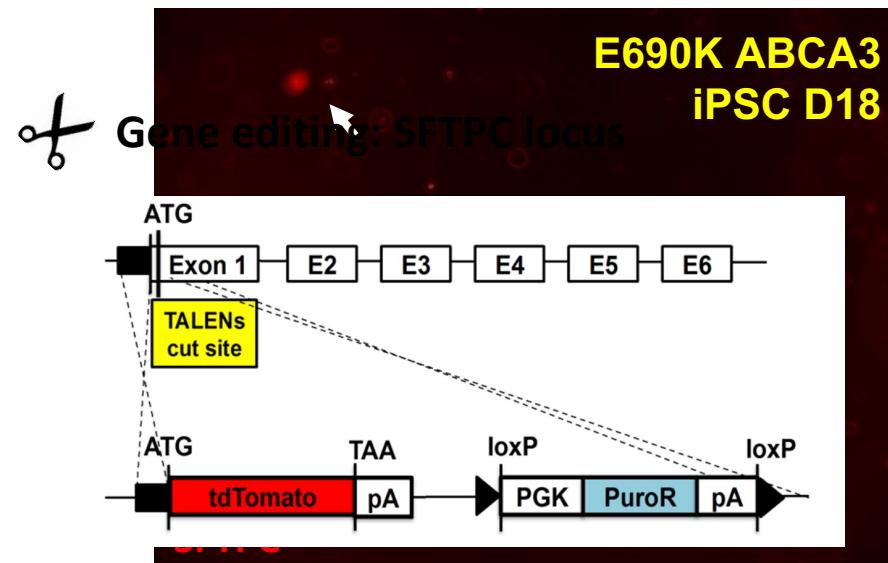


Distal tip/maturing
SFTPC+ AEC2s

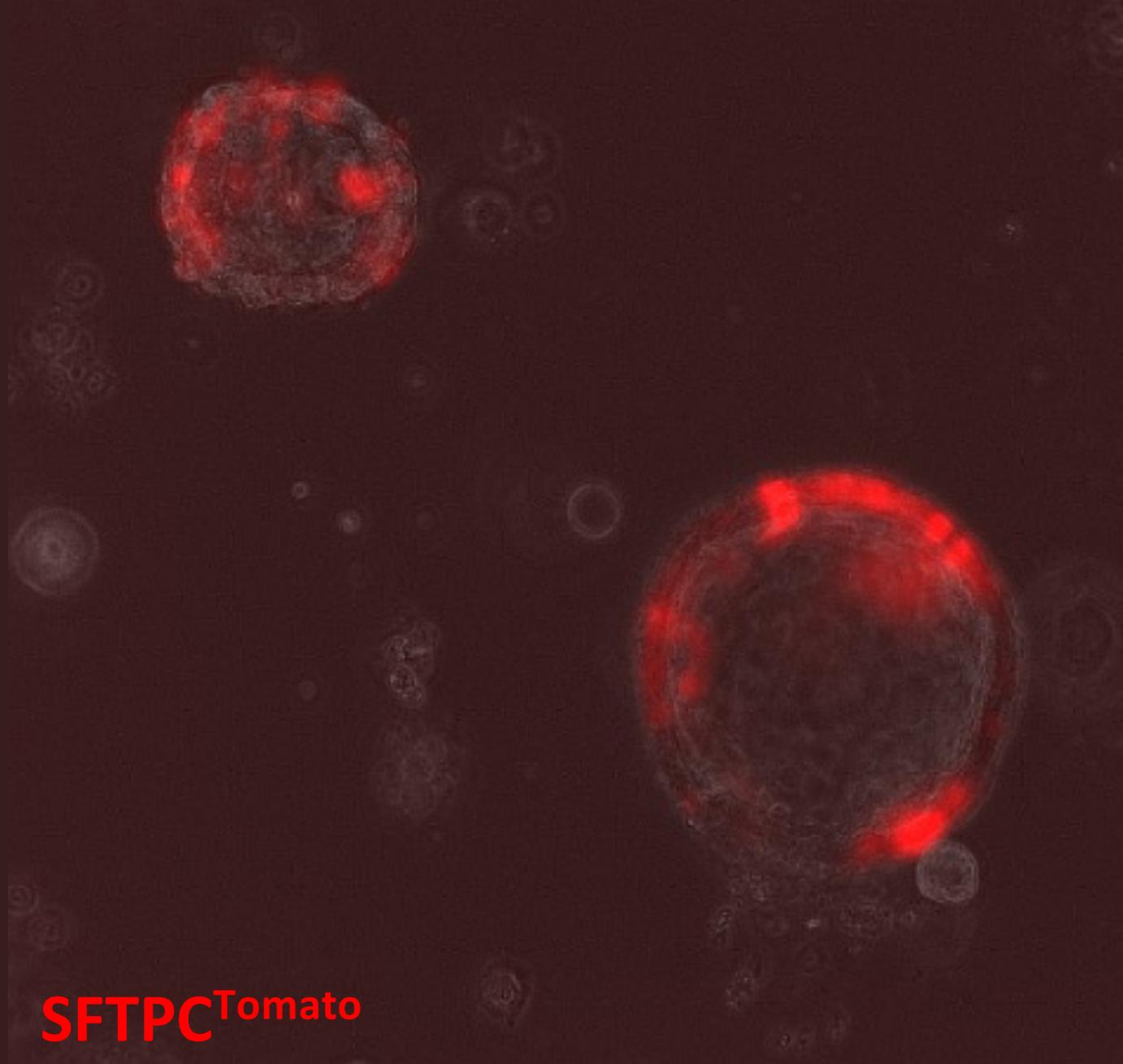
Day 30



Chir, Kgf, DCI
Plate in 3D matrigel

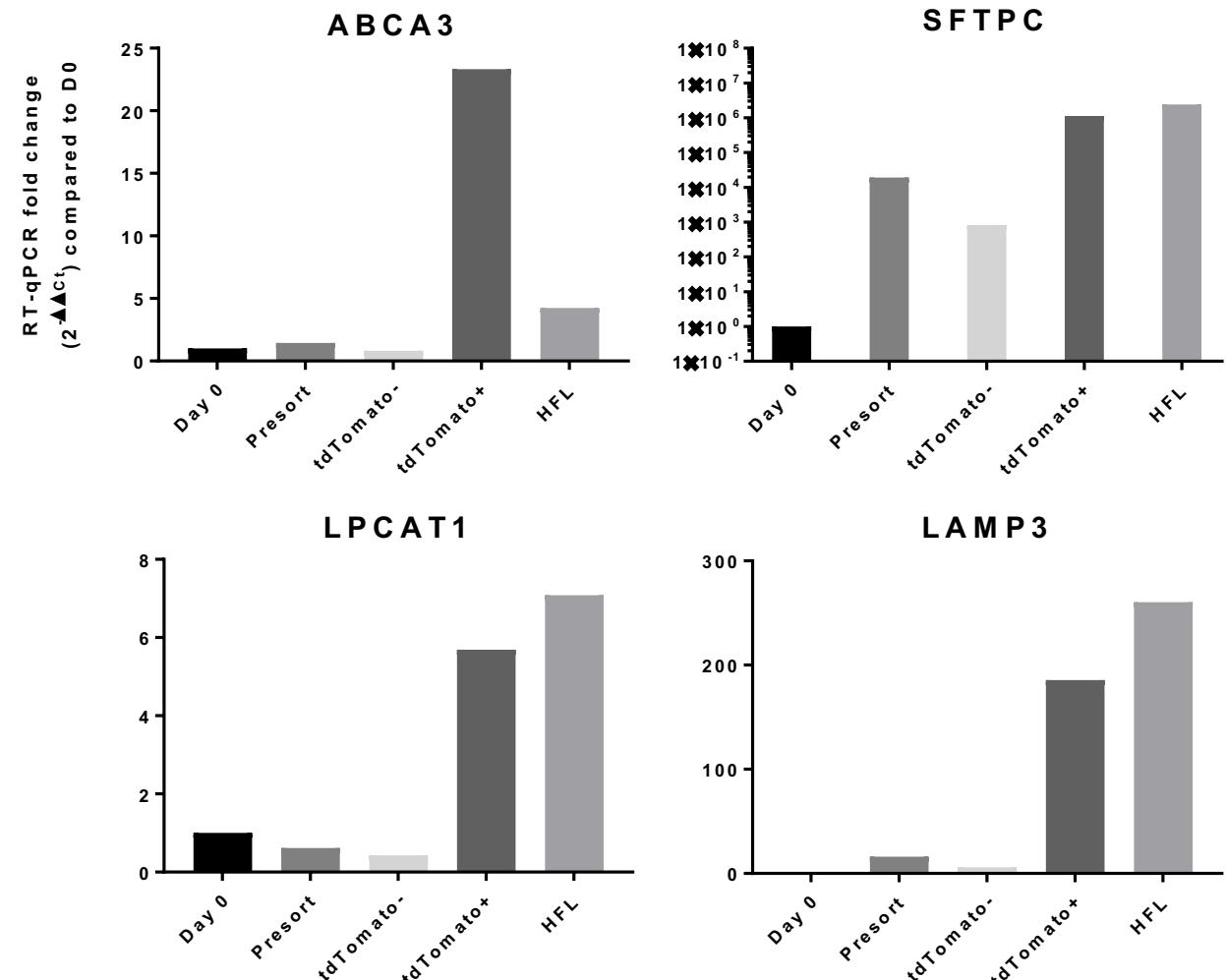
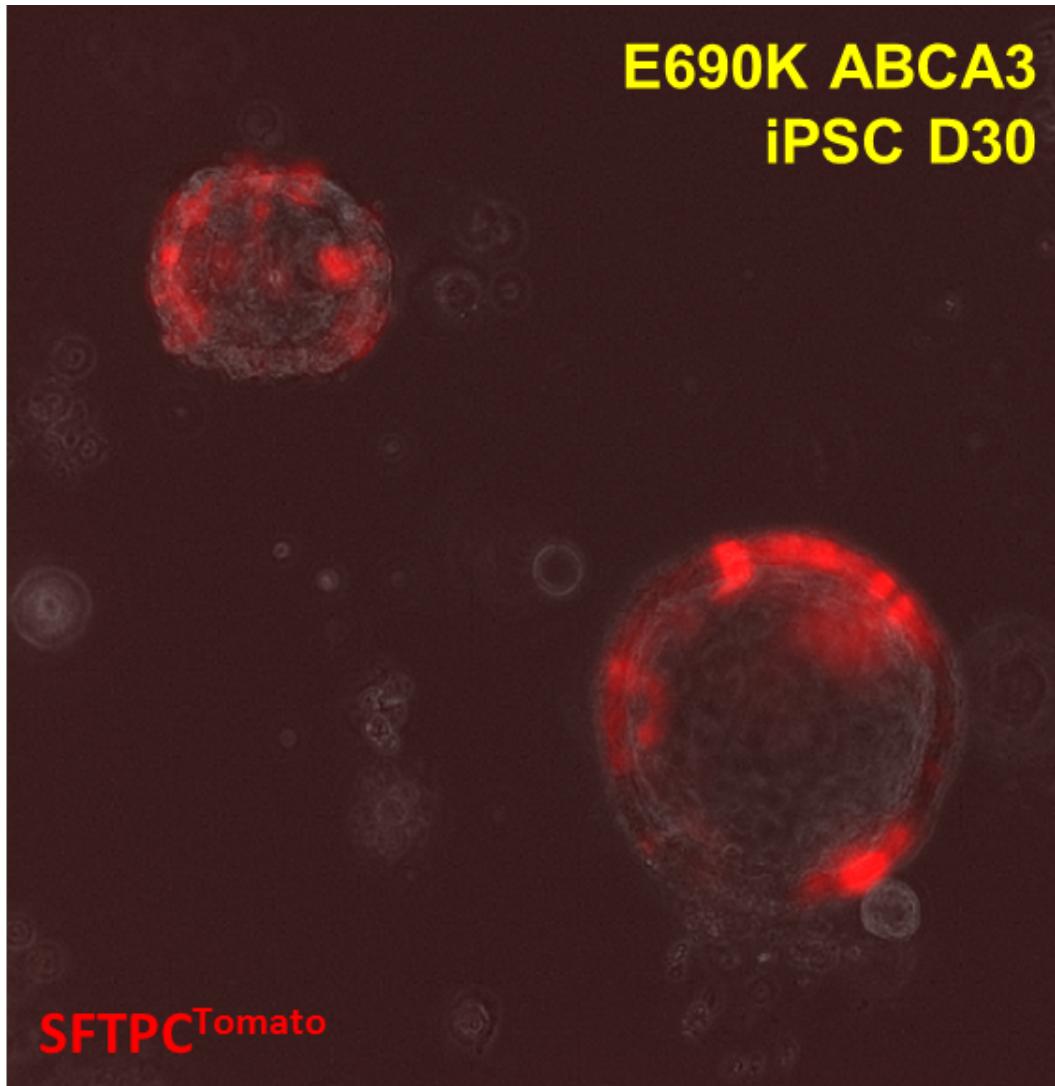


E690K ABCA3 iPSC D30



SFTPC^{Tomato}

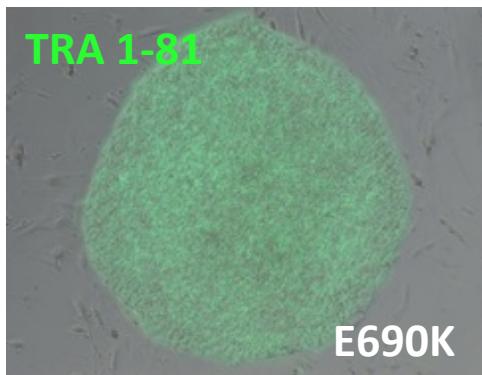
Day 30 Tomato+ cells by RT-qPCR against human fetal lung positive control express of key surfactant-related genes



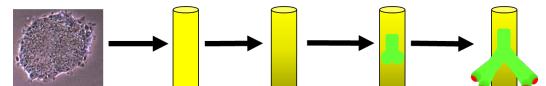
Positive CTRL= Week 21 human fetal lung enriched in AEC2s

Comparing patient iPSC-derived AEC2s with gene corrected syngeneic lines will enable identification of mutant specific disease mechanisms

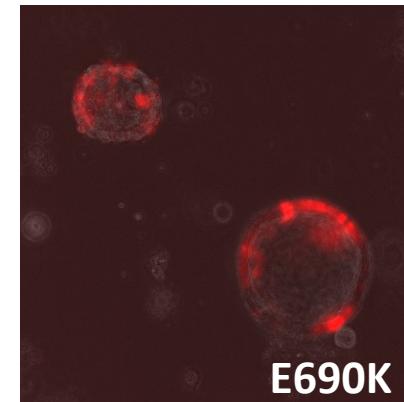
ABCA3 mutant iPSC



Directed differentiation



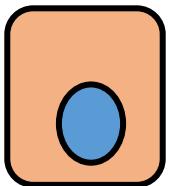
ABCA3 mutant iPSC derived AEC2



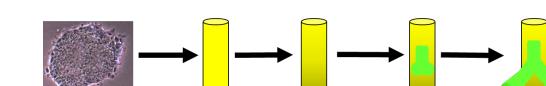
Gene correction



Wildtype ABCA3 iPSC



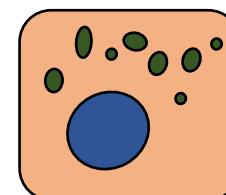
Directed differentiation



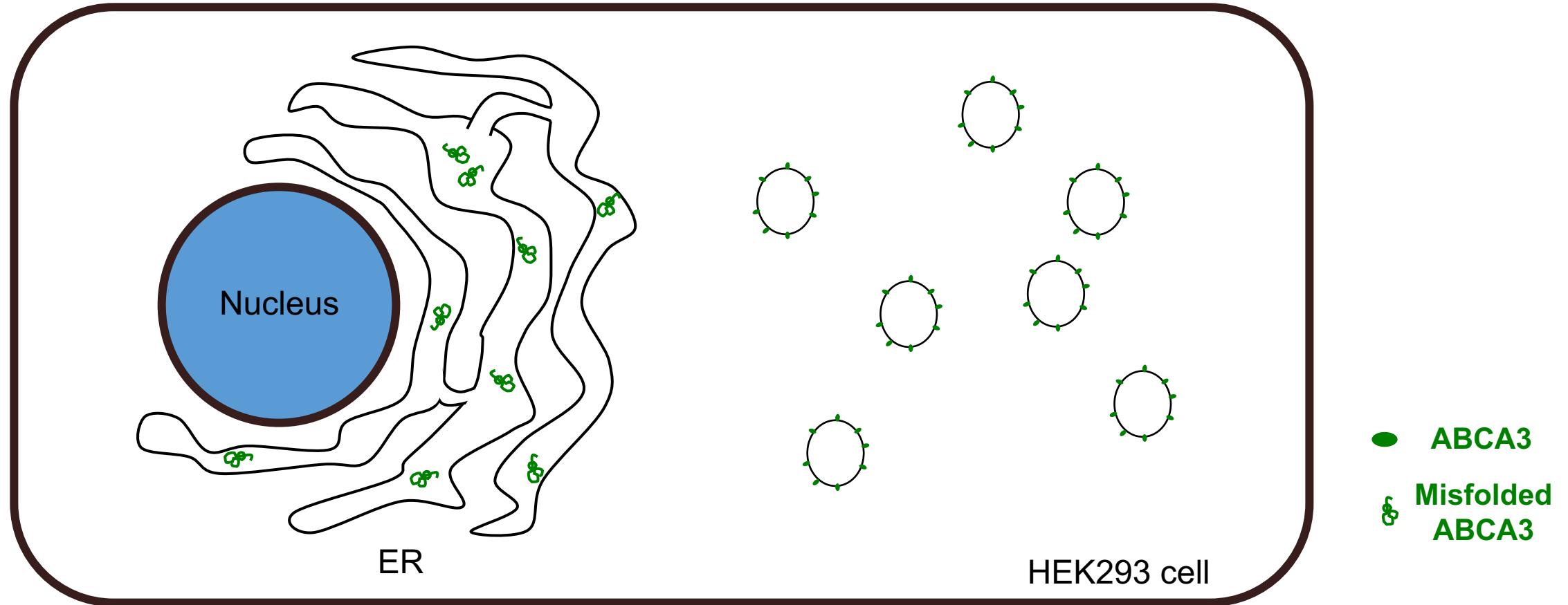
Identify ABCA3 mutant specific disease mechanisms



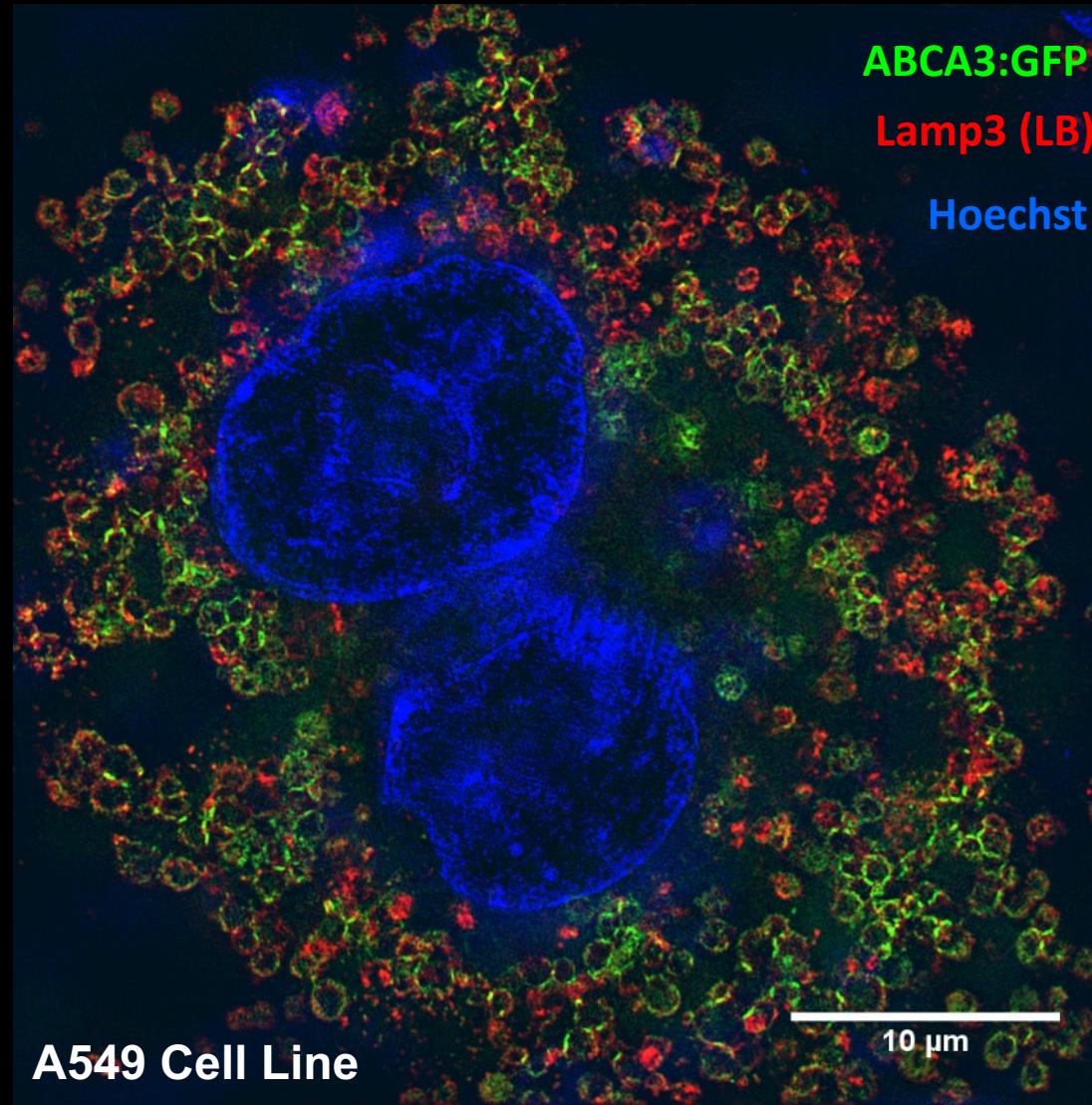
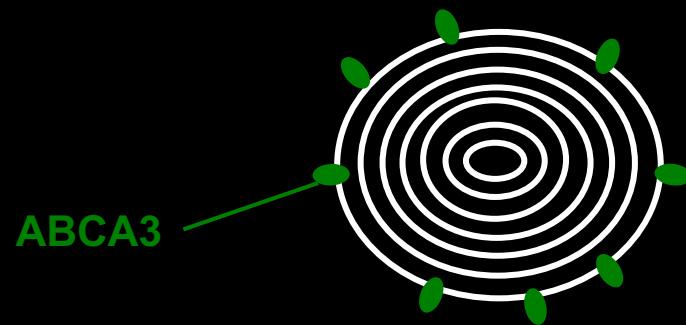
Gene corrected wildtype iPSC derived AEC2



ABCA3 mutations may be classified into two categories:
functional mutants and protein mis-trafficking mutants



ABCA3:GFP fusion protein can be used to visualize ABCA3 protein trafficking



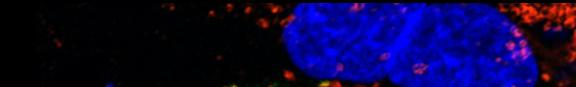
Plasmids received from Jennifer Wambach

A549 cells expressing E690K and W308R mutant ABCA3:GFP fusion proteins displayed distinct protein trafficking patterns

E690K ABCA3:GFP

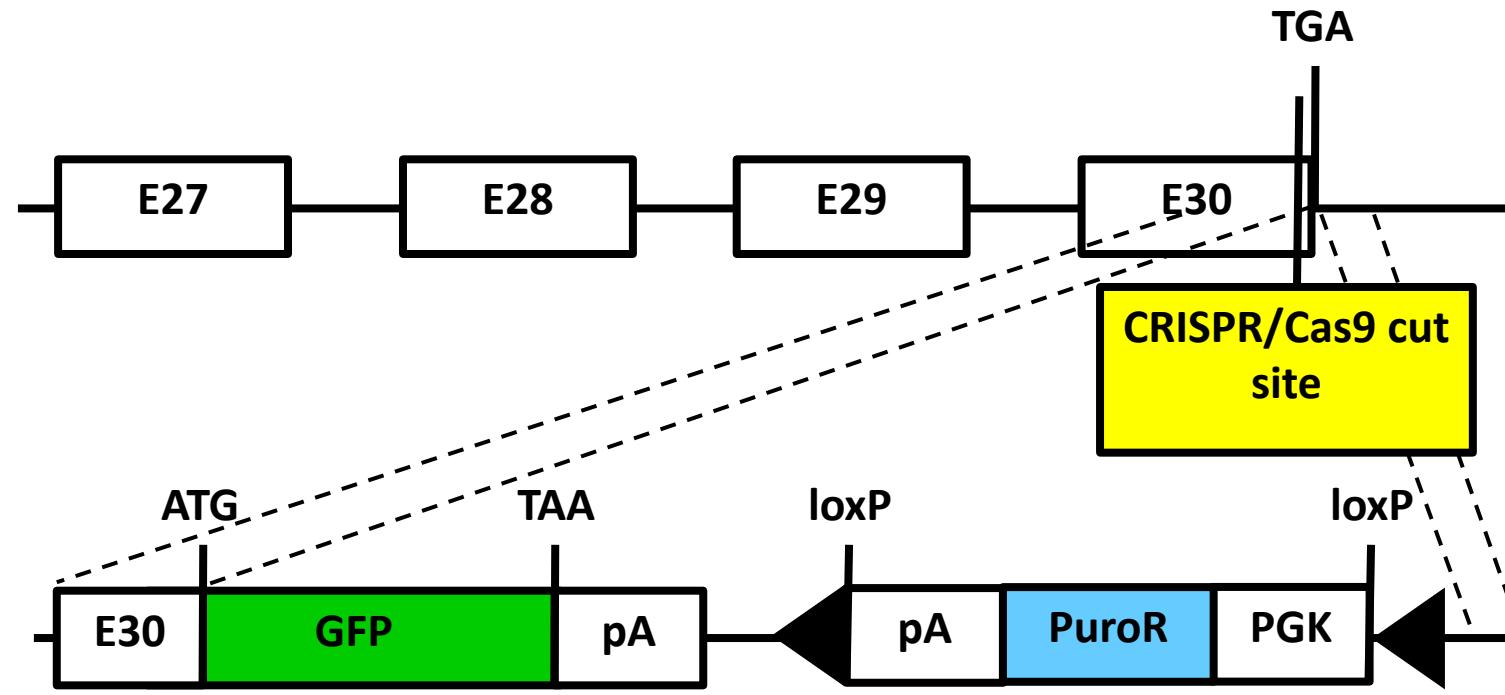


W308R ABCA3:GFP

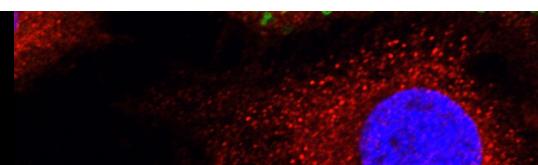


Gene editing: Endogenous ABCA3 locus

Lamp3 (red)

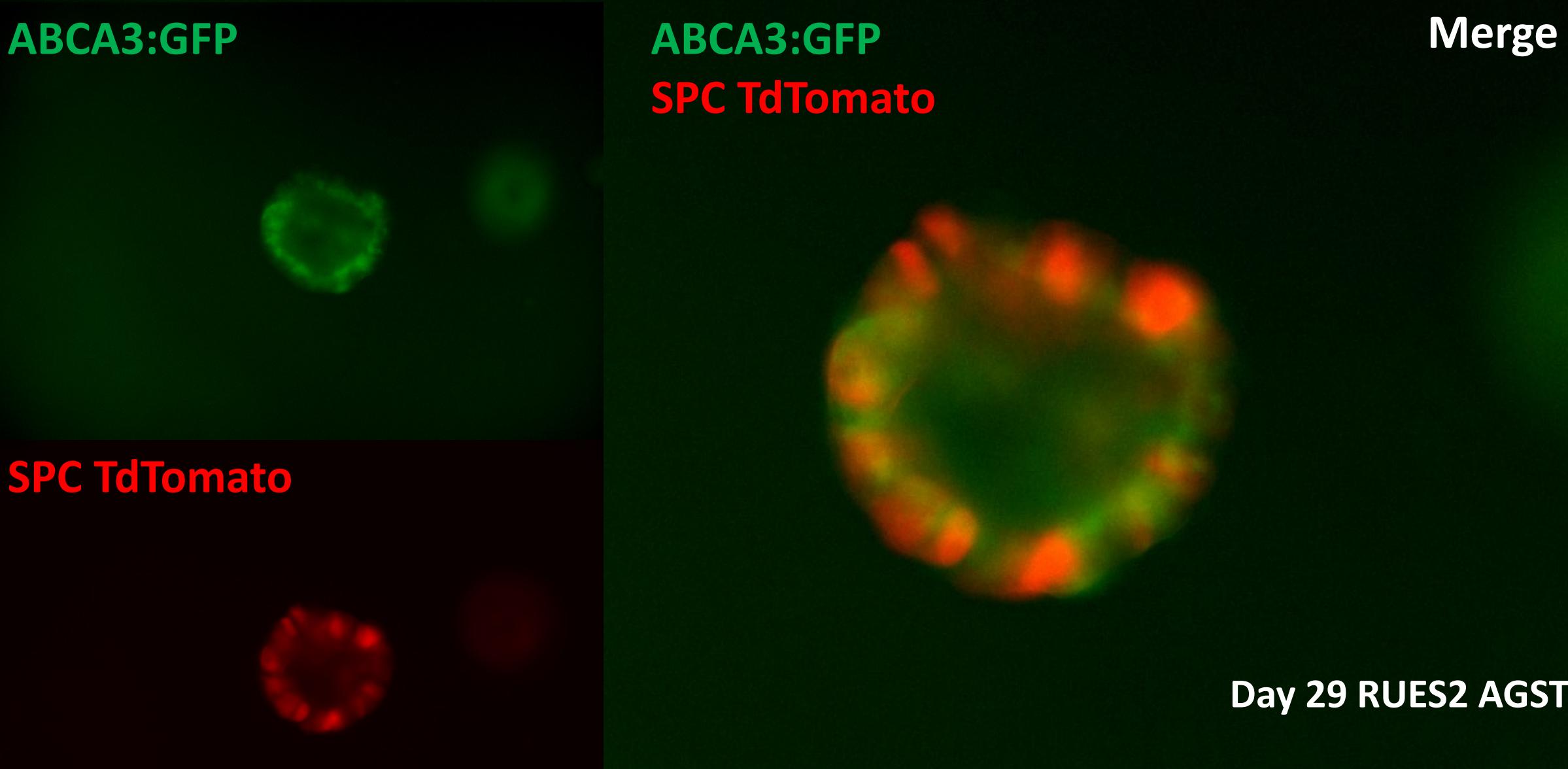


Calnexin (red)



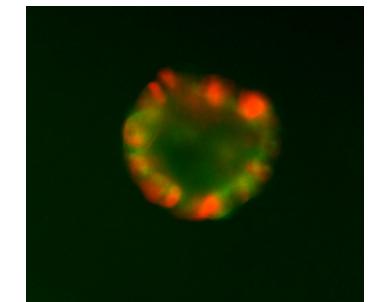
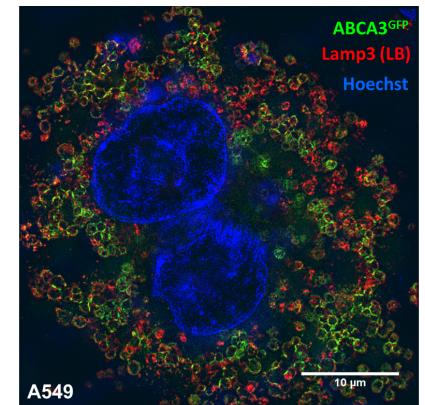
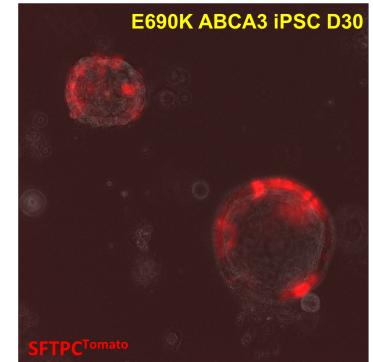
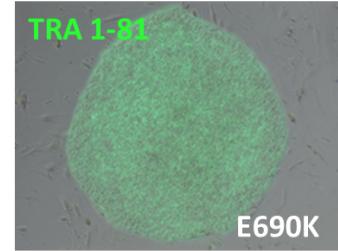
Hoechst (Nuclei)

Wildtype human ES line targeted with SPC^{TdTomato} and ABCA3:GFP fusion expressed both reporters on day 29 of directed differentiation



In summary:

- Reprogrammed two novel patient derived ABCA3 mutant iPSC lines
- ABCA3 mutant iPSC lines can be differentiated into cells that resemble type II alveolar epithelial cells using directed differentiation protocol
- Development of gene corrected syngeneic lines will enable discovery of ABCA3 mutant specific disease mechanisms
- Generation of an ABCA3:GFP fusion reporter to further investigate ABCA3 protein trafficking in the context of disease pathogenesis and in vivo lung development



Acknowledgements:

Kotton Lab:

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