

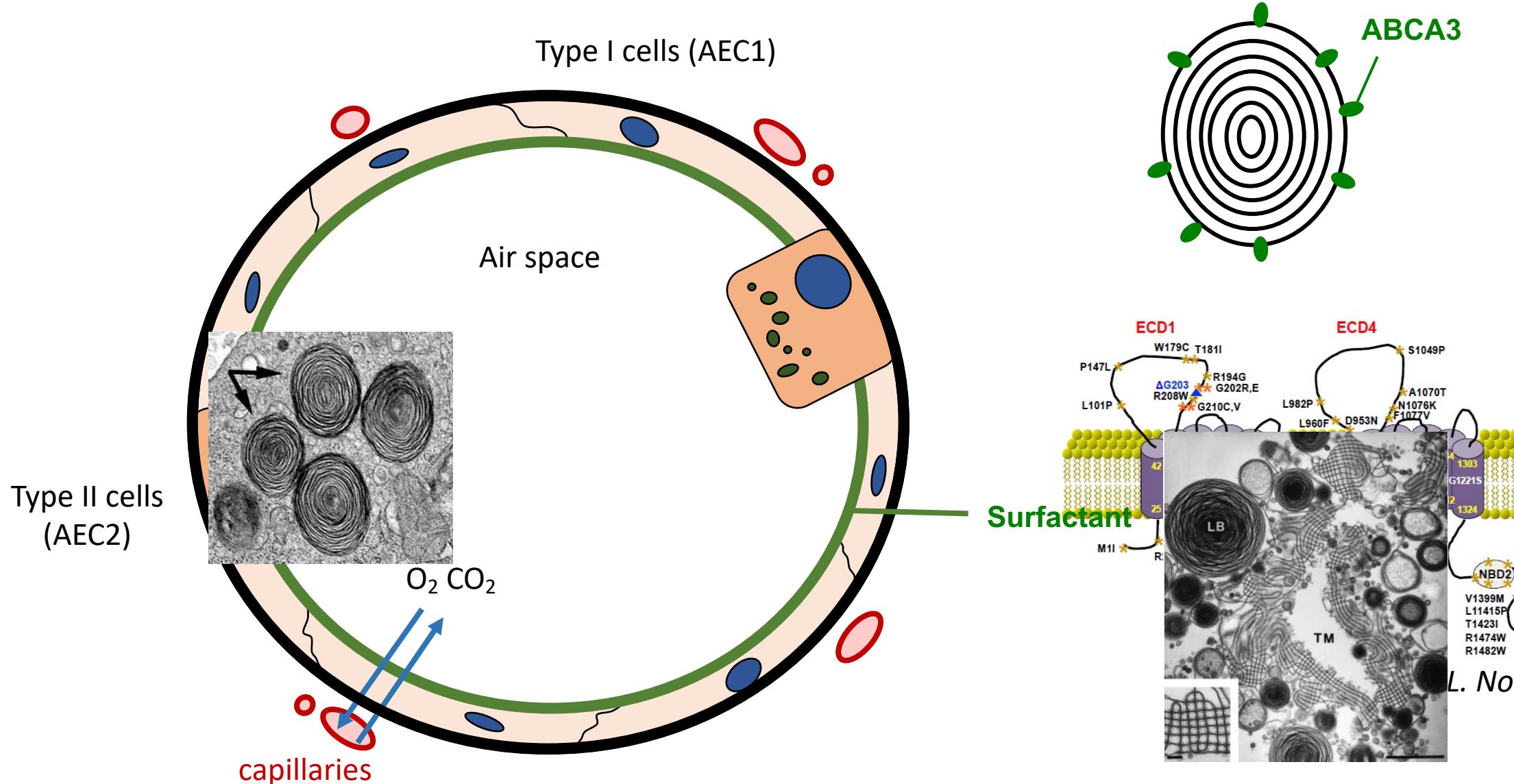
A novel tool to study ABCA3 biology in PSC-derived AEC2s

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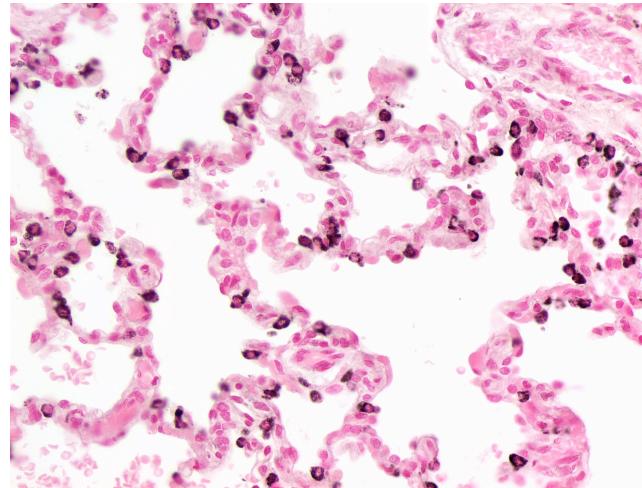
8/2/18

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

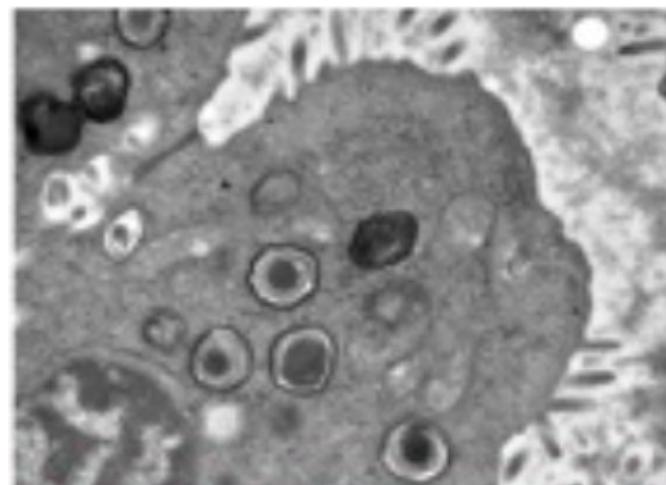
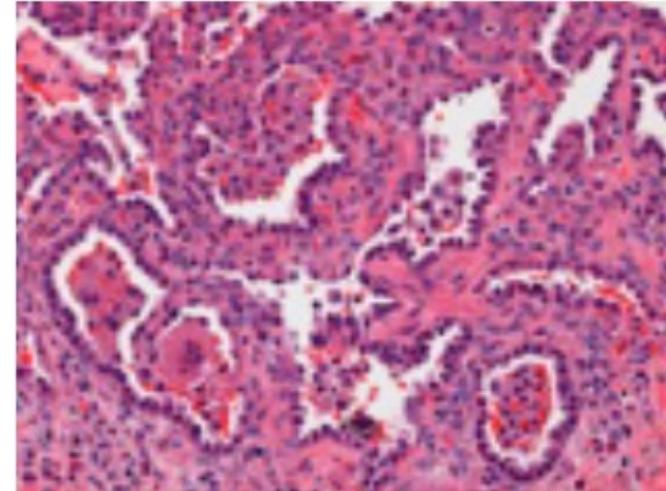


Autosomal recessive mutations in the ABCA3 gene represent the most common genetic cause of childhood interstitial lung disease (chILD)

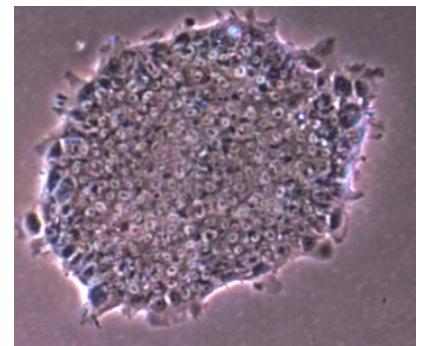
Normal human lung



ABCA3 mutant diseased lung

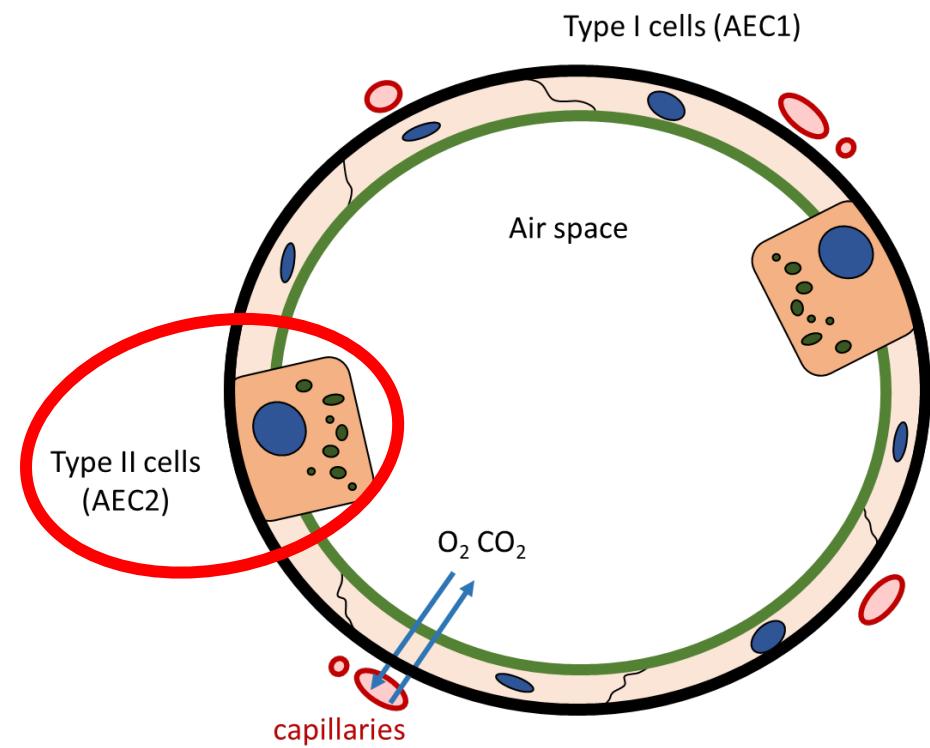


Using AEC2s derived from pluripotent stem cells (PSCs) as a model to study ABCA3 mutations in AEC2s

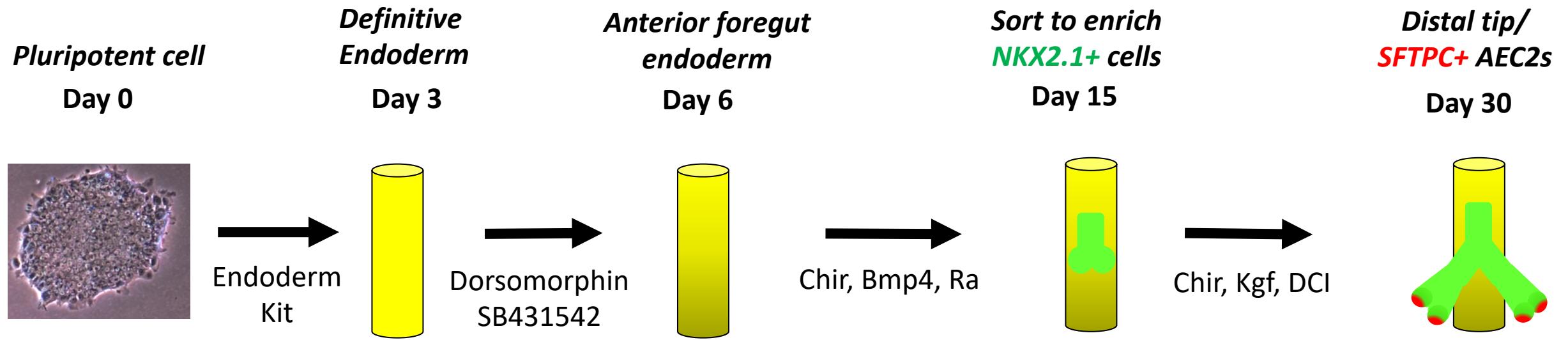


Pluripotent stem cells

Directed
Differentiation



Engineering primary-like AEC2s from PSCs to study ABCA3 biology

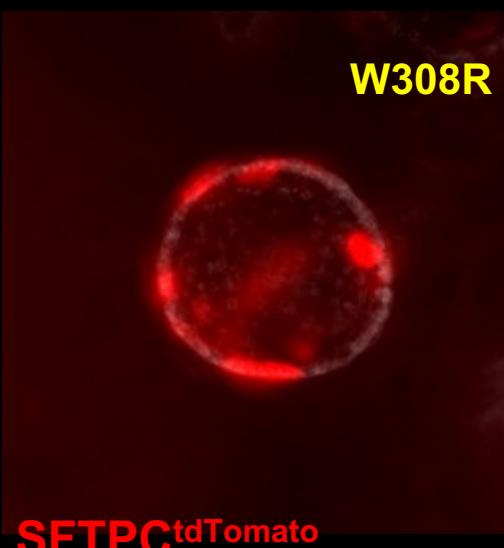
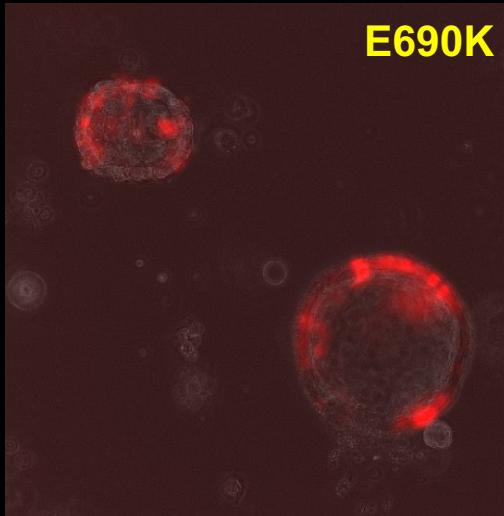


Patient iPSCs containing homozygous ABCA3 mutations generated SFTPC^{tdTomato}+ AEC2s following lung directed differentiation

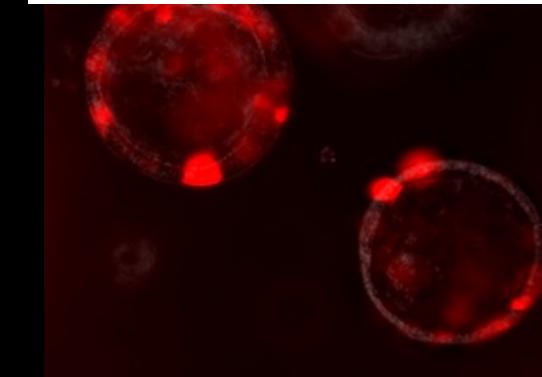
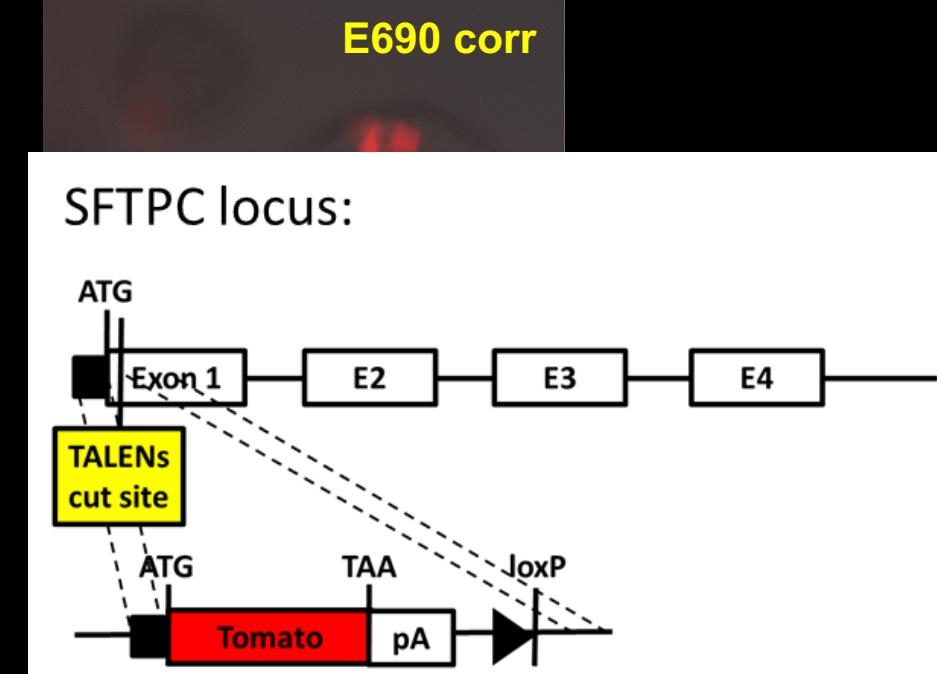
Patient iPSCs



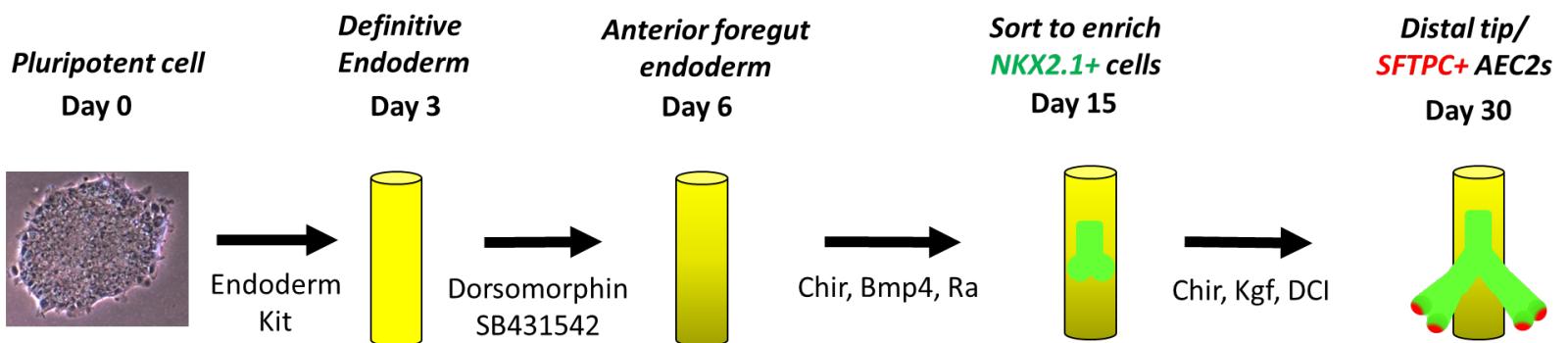
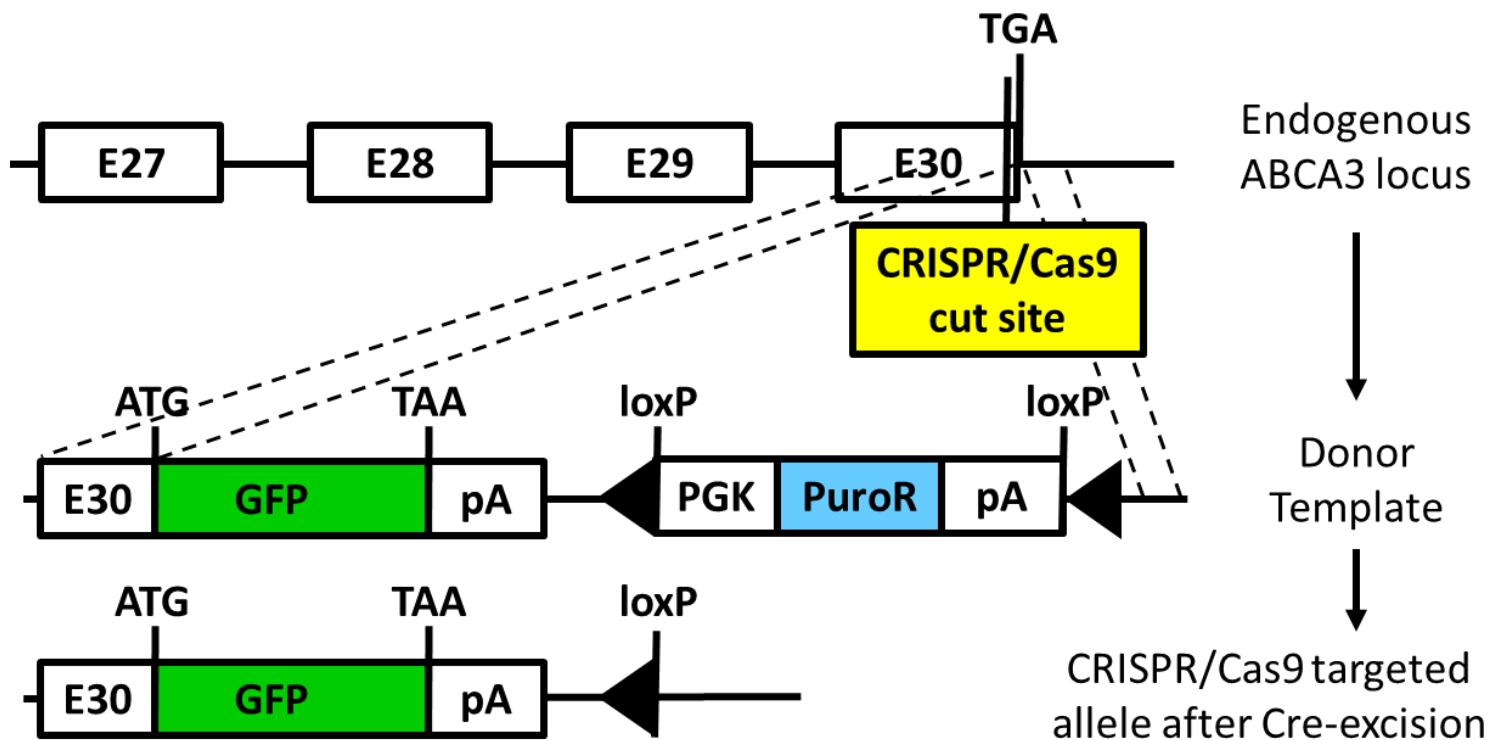
ABCA3 mutant patient AEC2s



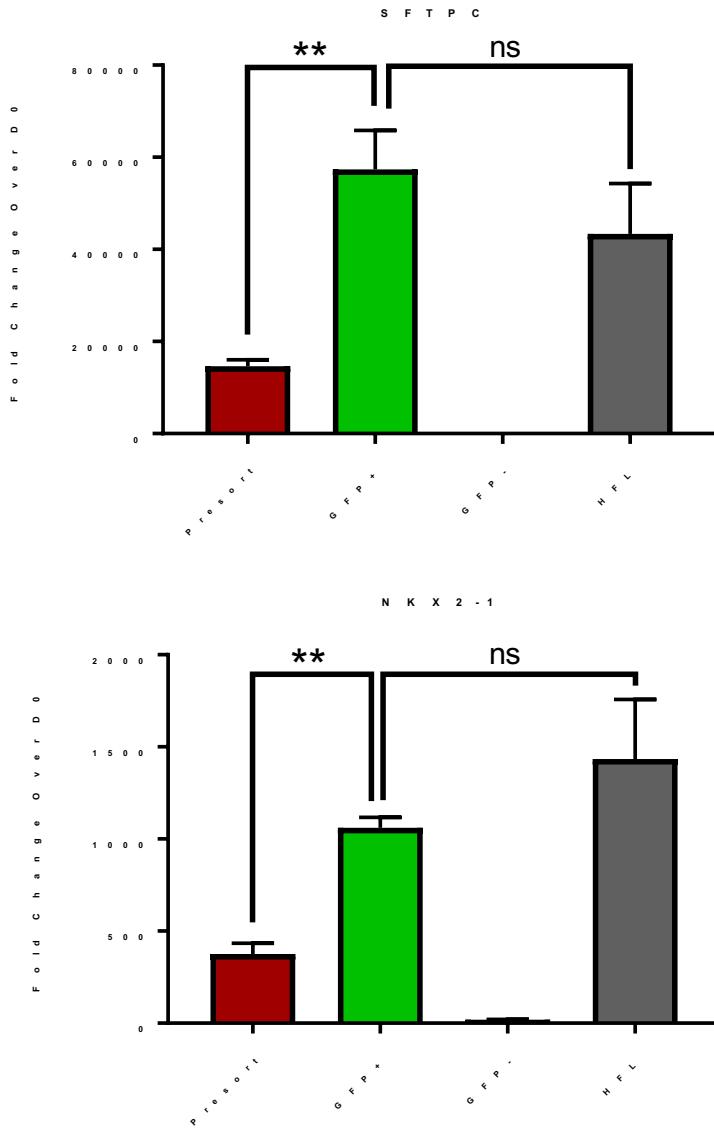
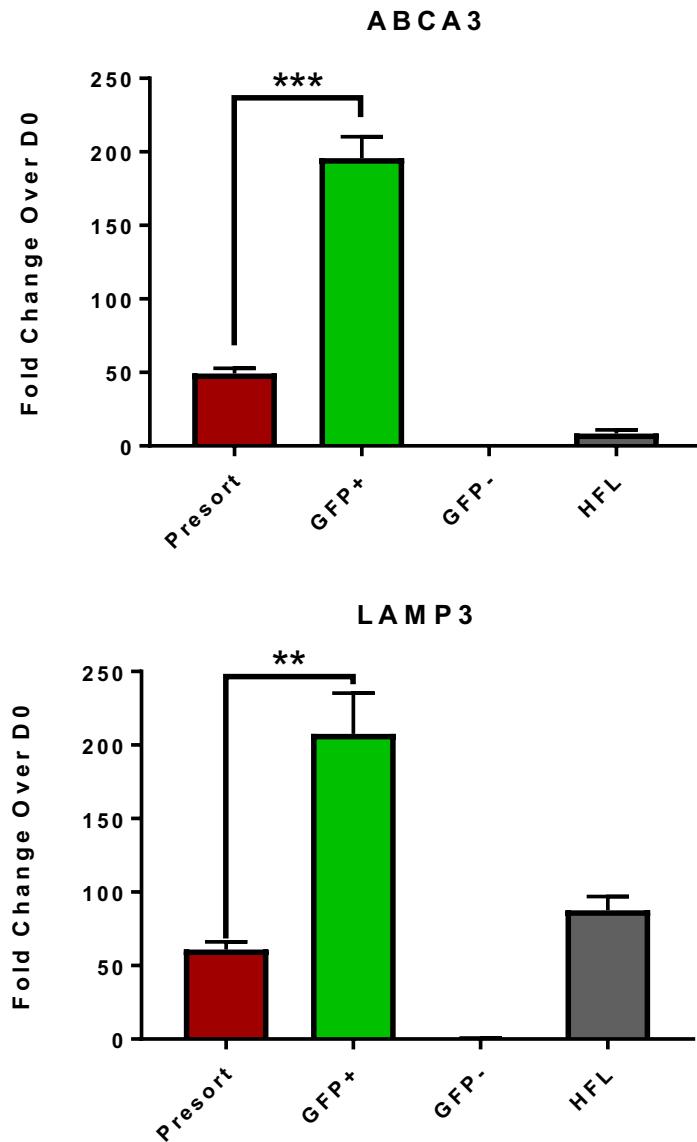
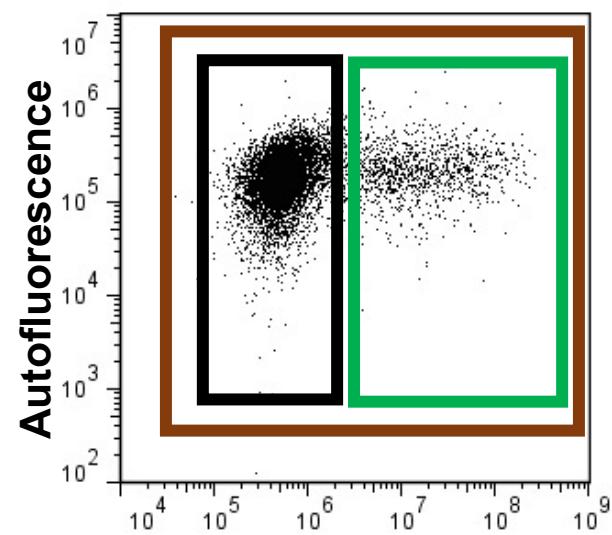
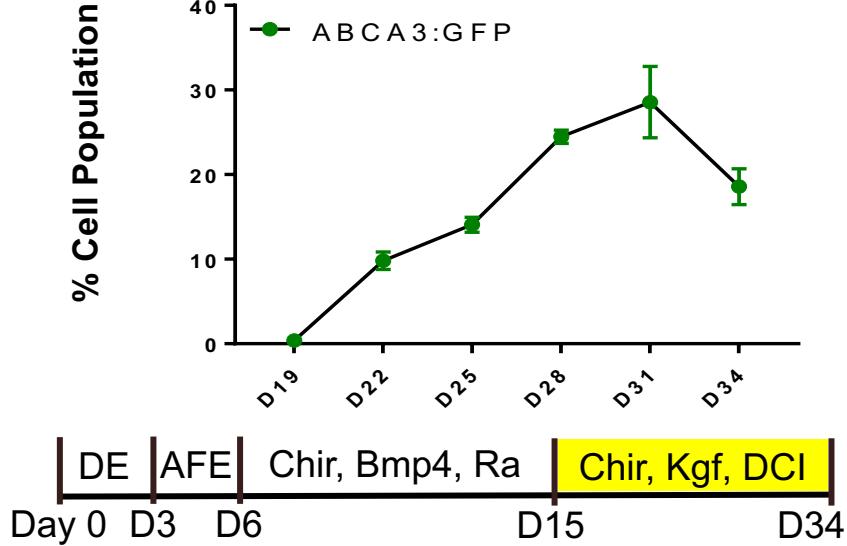
Gene corrected wildtype AEC2s



Using a fluorochrome reporter to study ABCA3 protein in PSC-derived AEC2s

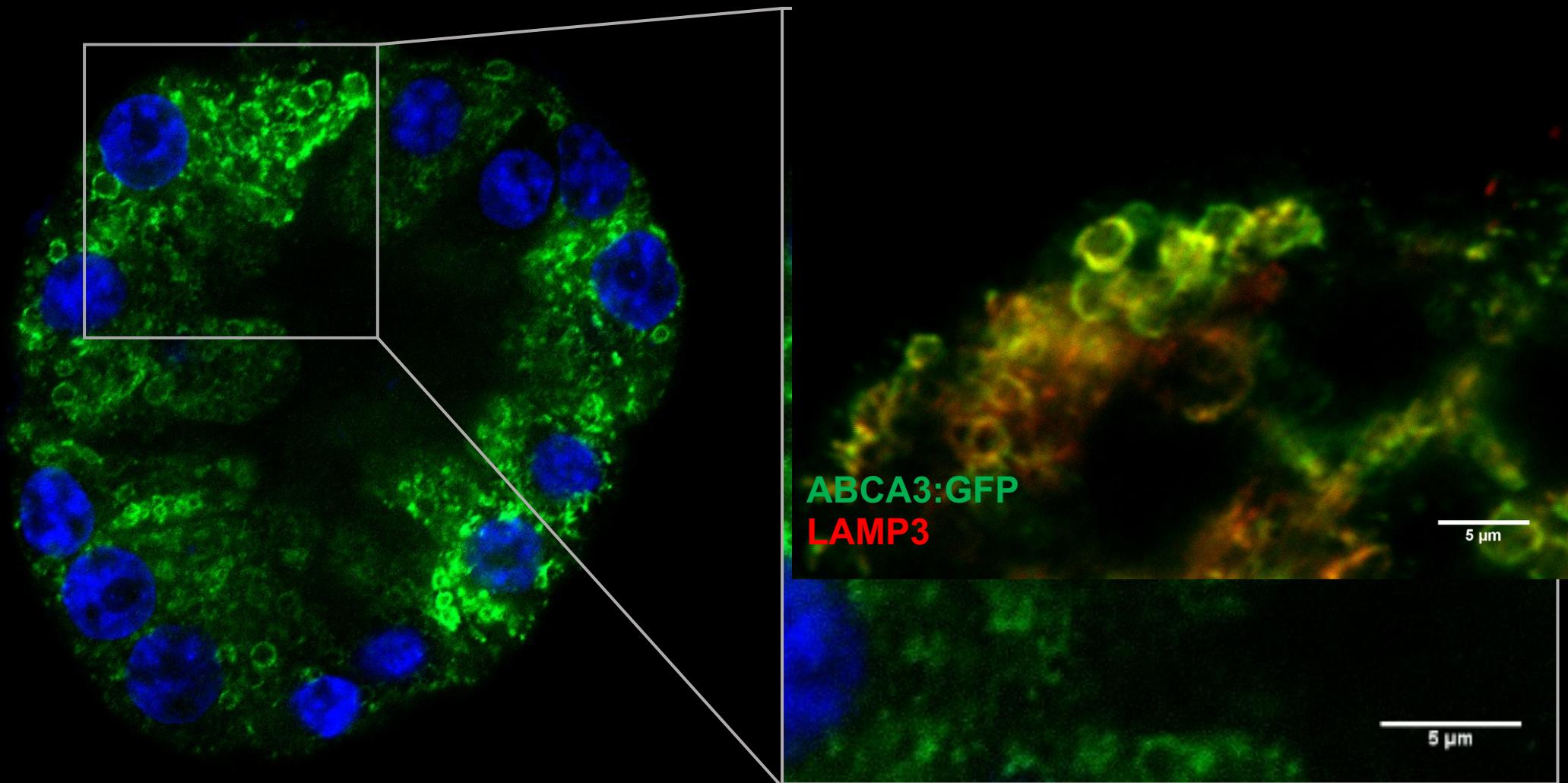


Day 34 GFP+ cells enriched for ABCA3 as well as other AEC2 markers including surfactant processing genes



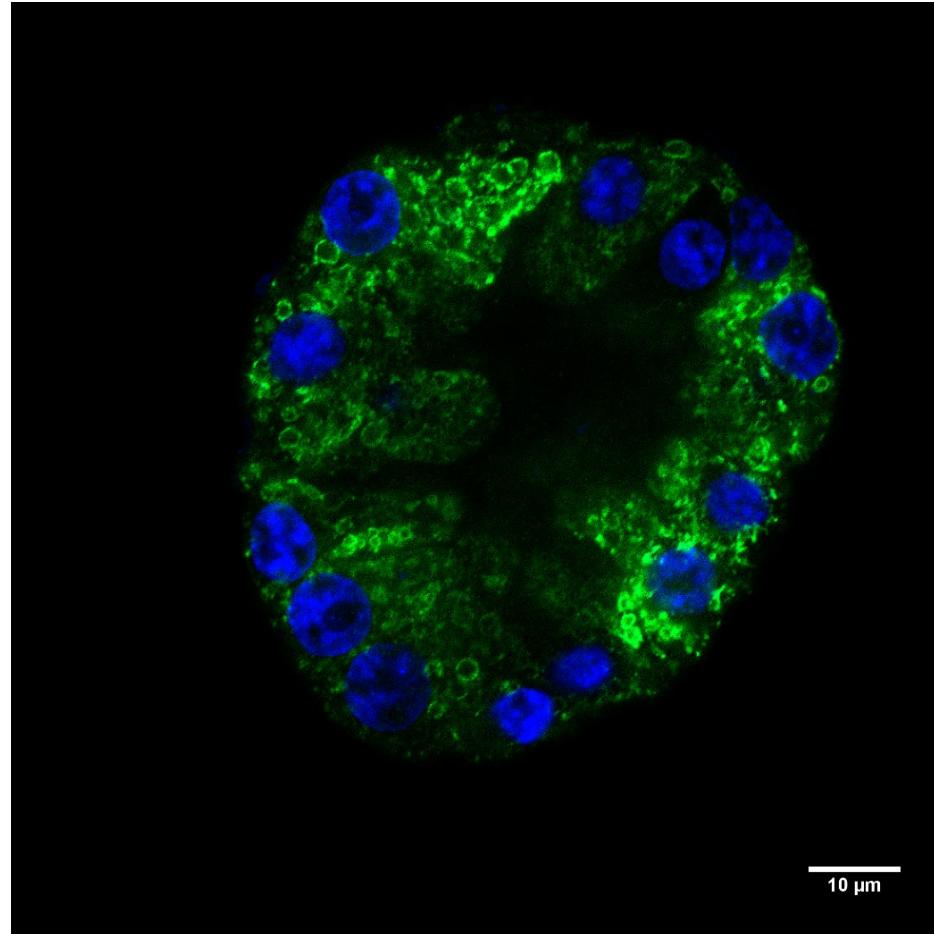
HFL = wk 21 human fetal lung enriched in AEC2; ** p<0.01 , *** p<0.001, student's t test

Day 88 PSC-derived alveolosphere

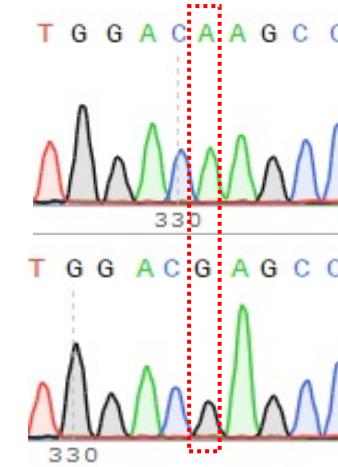


ABCA3:GFP
Hoechst (nuclei)

CRISPR/Cas9 mutagenesis of ABCA3:GFP fusion PSC line to study mutant ABCA3 localization and lamellar body morphology

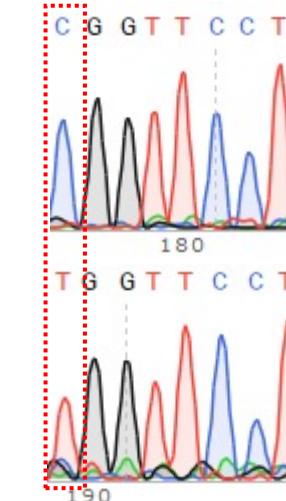


E690 mutant
(G->A)



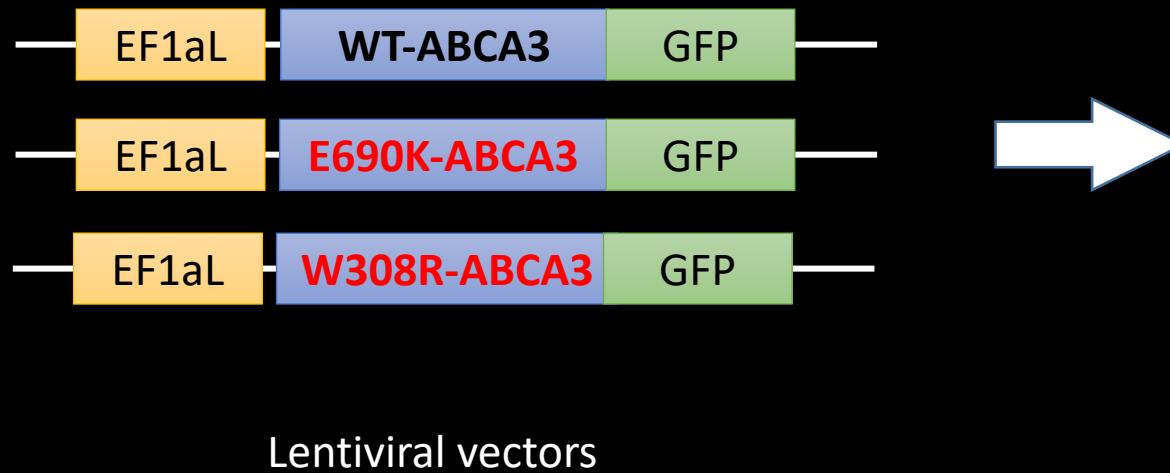
E690 wildtype

W308 mutant
(T->C)

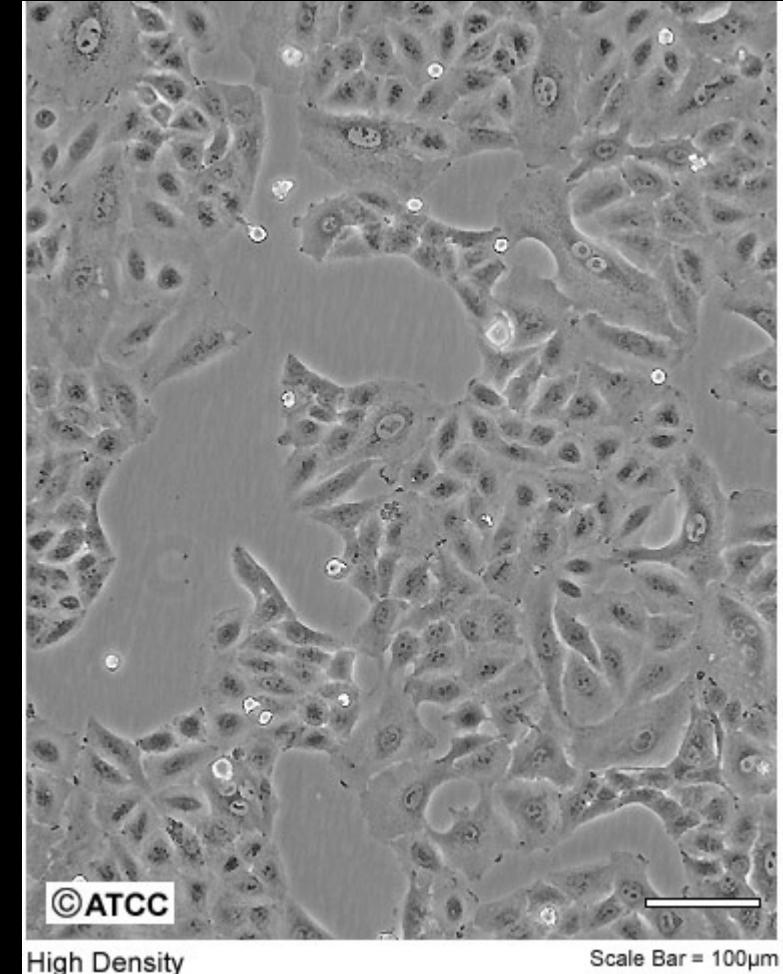


W308 wildtype

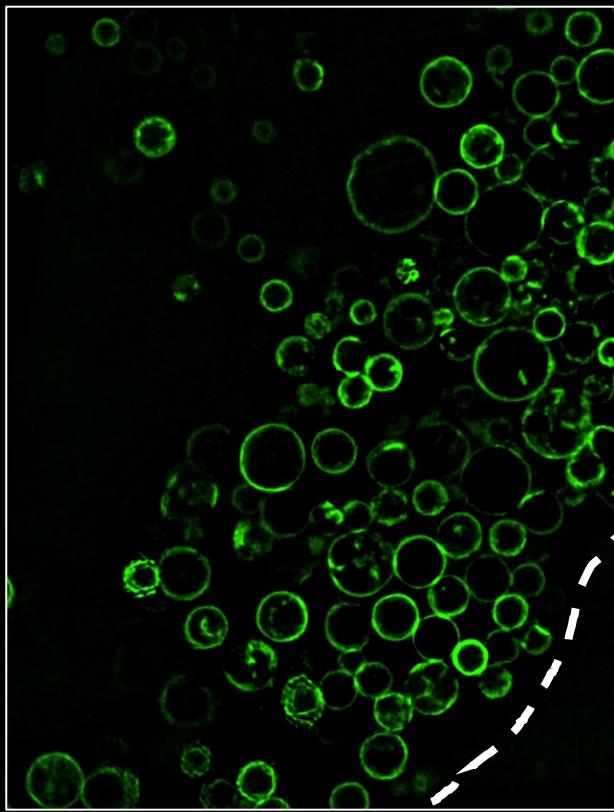
WT and mutant ABCA3:GFP fusion protein were expressed in A549 lung adenocarcinoma line using lentiviral infection



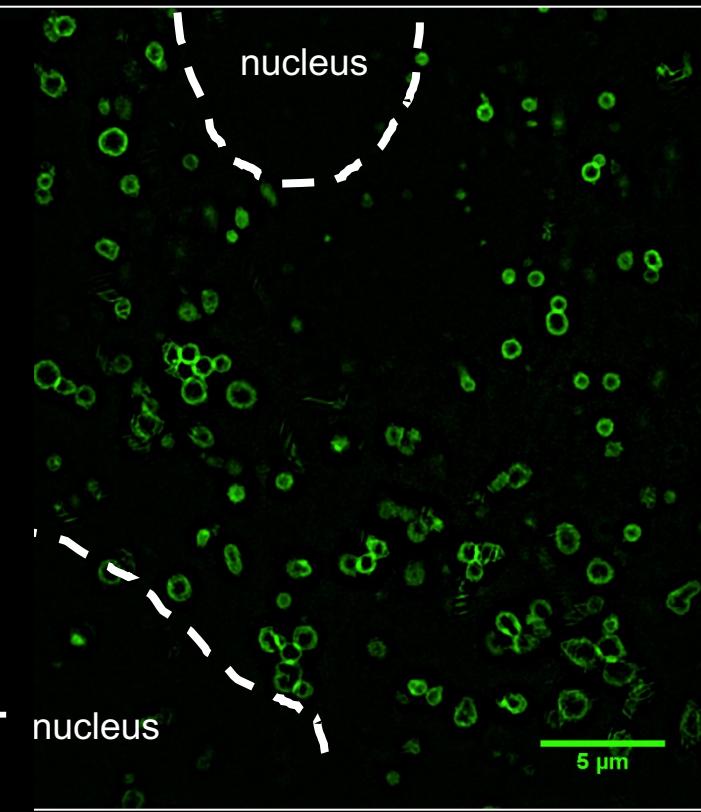
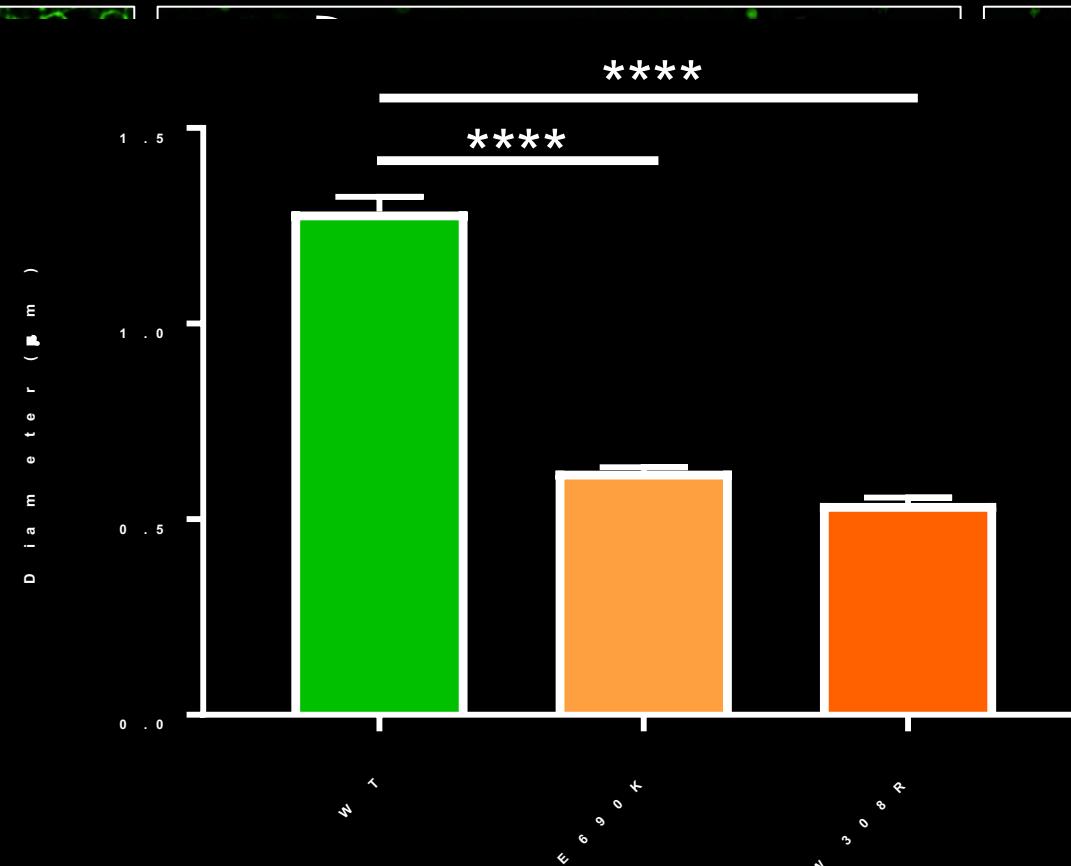
A549



In A549 cells, E690K and W308R mutant ABCA3:GFP fusion proteins formed smaller GFP+ vesicles compared to wildtype



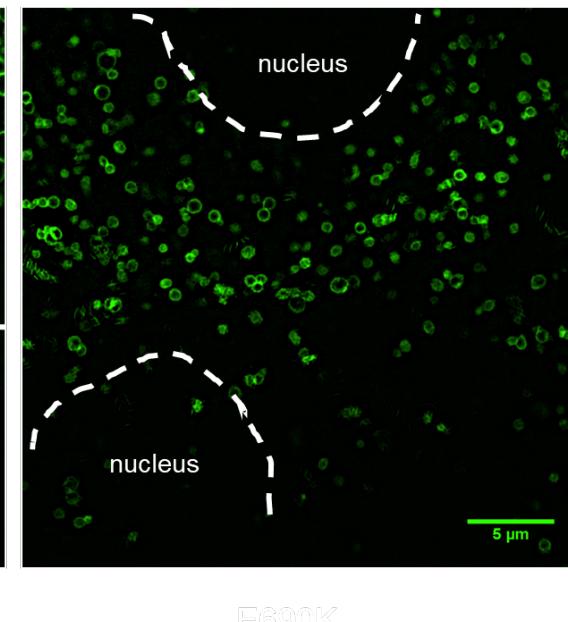
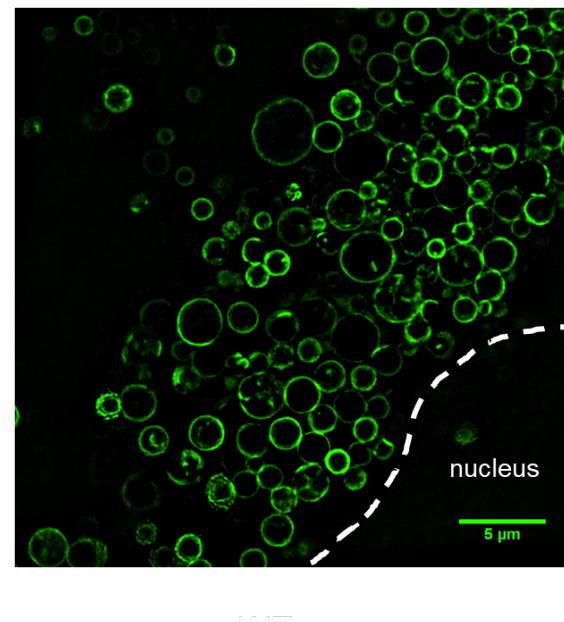
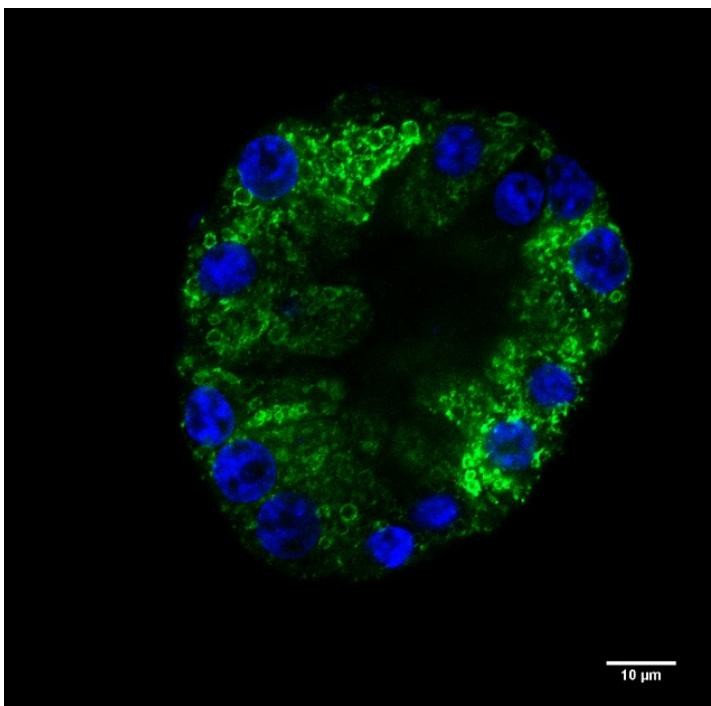
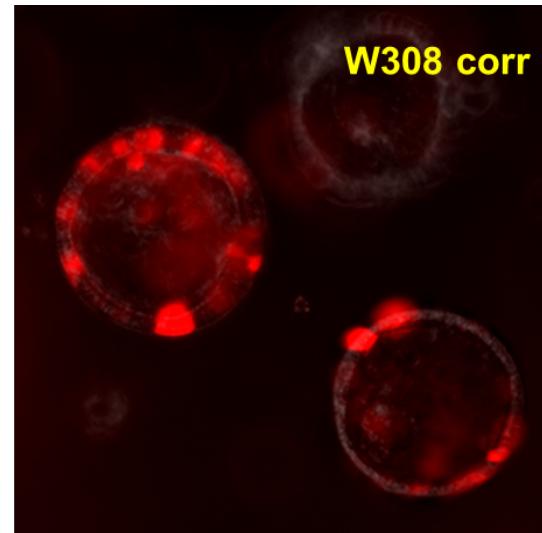
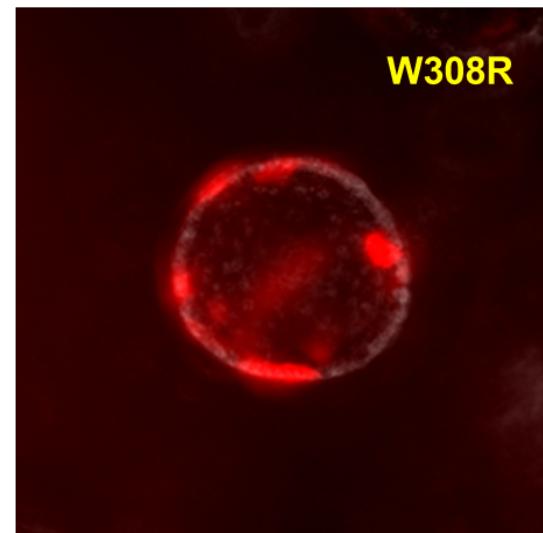
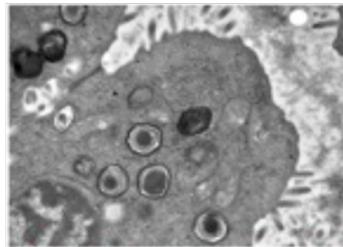
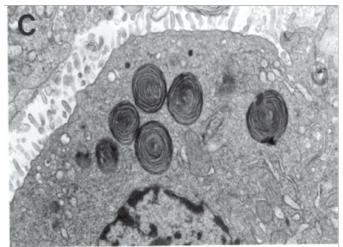
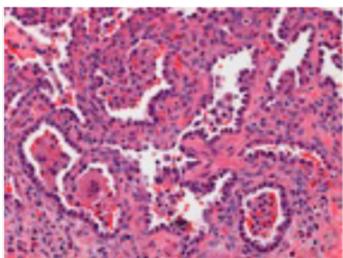
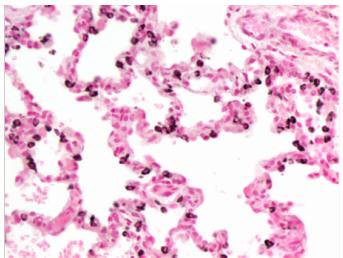
WT



W308R

A549 cells

Summary and future directions:



WT

E690K

Acknowledgements:

Kotton Lab:

Darrell Kotton

Anjali Jacob

Kathrine McCauley

Marally Vedaie

George Kwong

Killian Hurley

Hector Marquez

Jyn-Chang Jean

Kostas Aly sandratos

Michael Herriges

David Roberts

Claire Burgess



Wash U:

F. Sessions Cole

Jennifer Wambach

Ping Yang

U Penn:

Ed Morrissey

Univ of Cincinnati:

Jeffrey Whitsett

Johns Hopkins Univ:

Lawrence Nogee



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