

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

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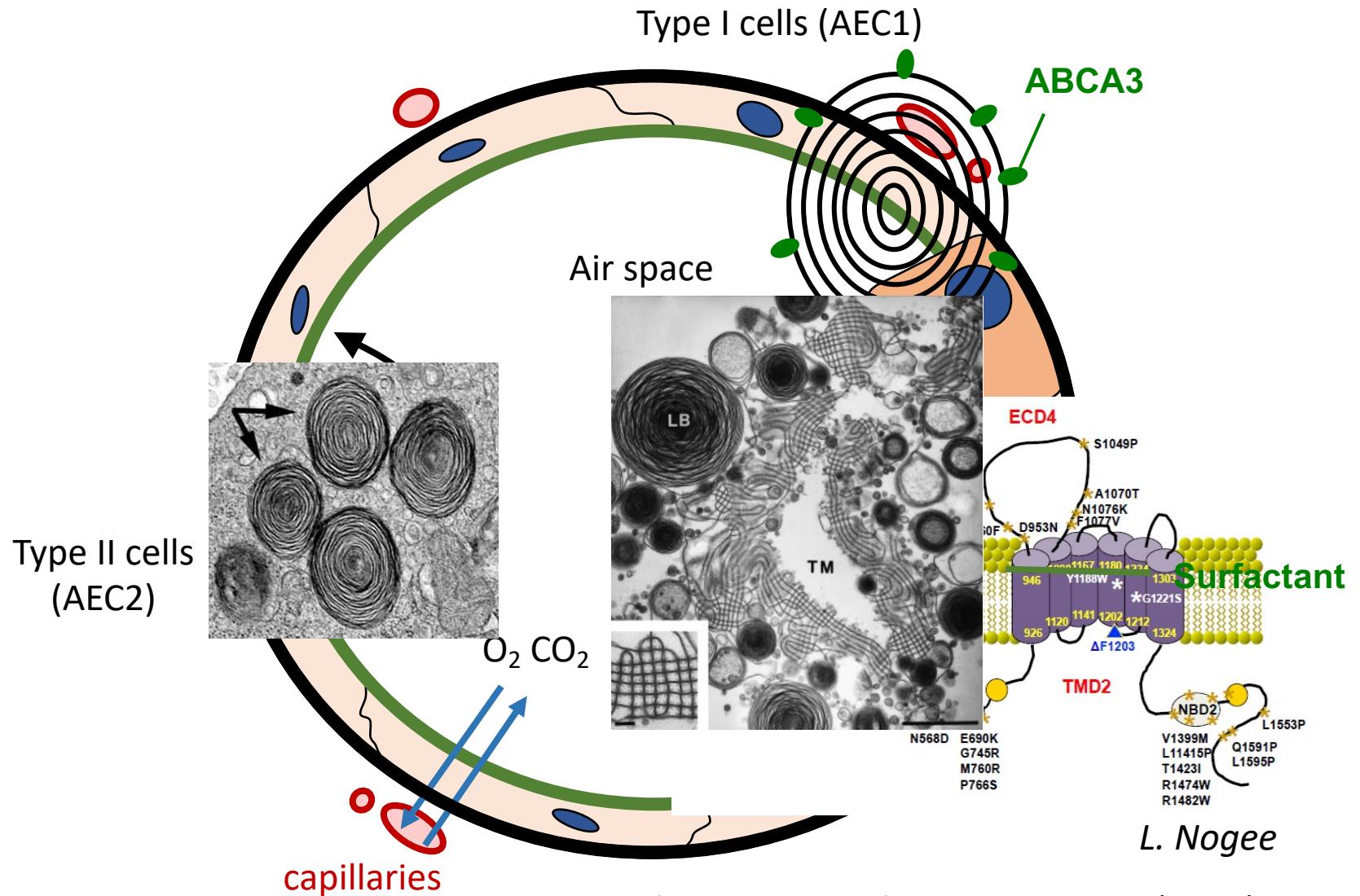
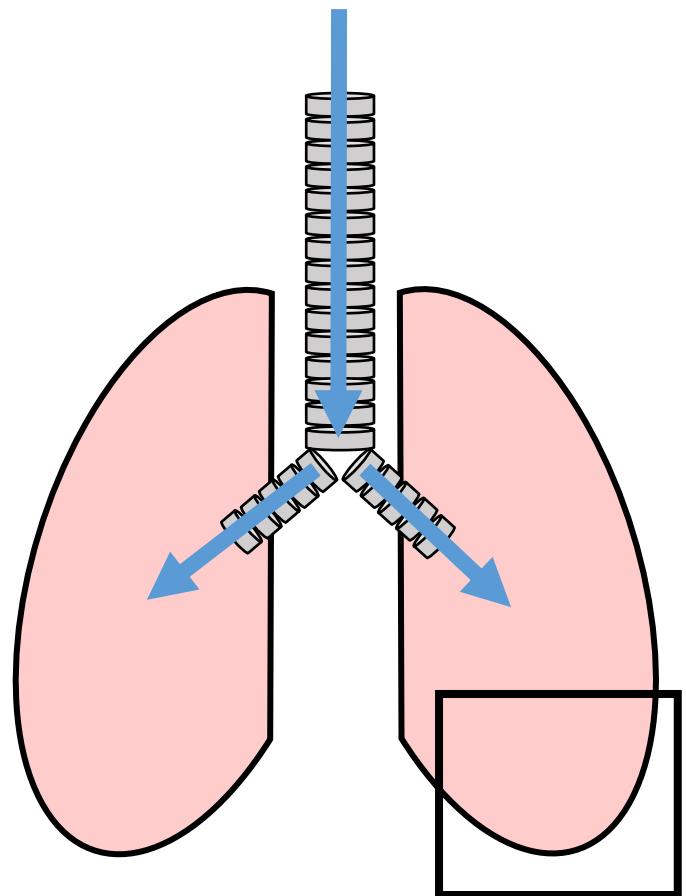
4/24/18



Outline:

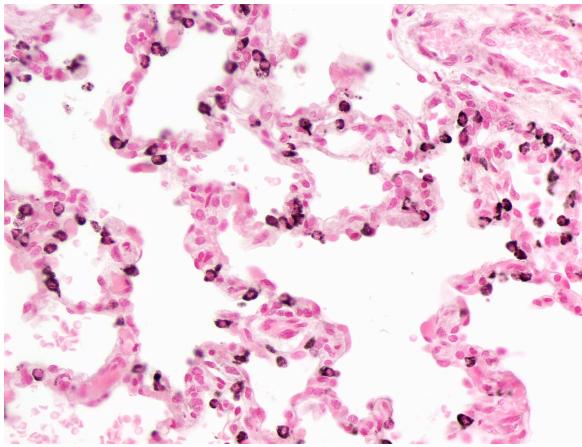
- AEC2 and the role of ABCA3
- Background on childhood interstitial lung disease (chILD) caused by ABCA3 mutations
- Patient iPSC-derived AEC2 (iAEC2) disease model
- Tool to visualize ABCA3 protein expression and trafficking in iAEC2s

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

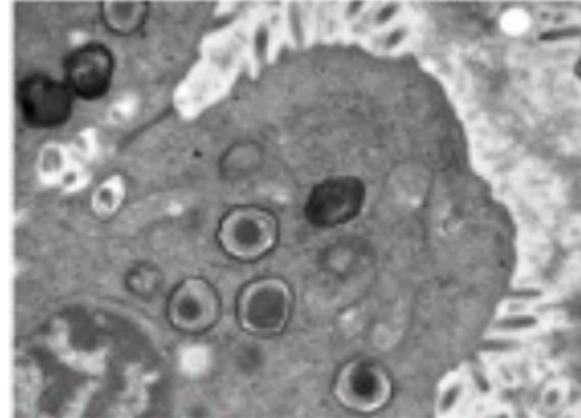
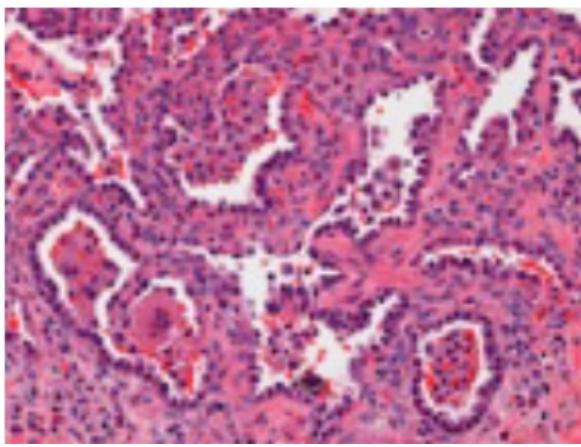


Childhood interstitial lung disease (chILD) can be caused by mutations in genes associated with pulmonary surfactant homeostasis in AEC2s

Normal human Lung



ABCA3 Mutant Diseased lung

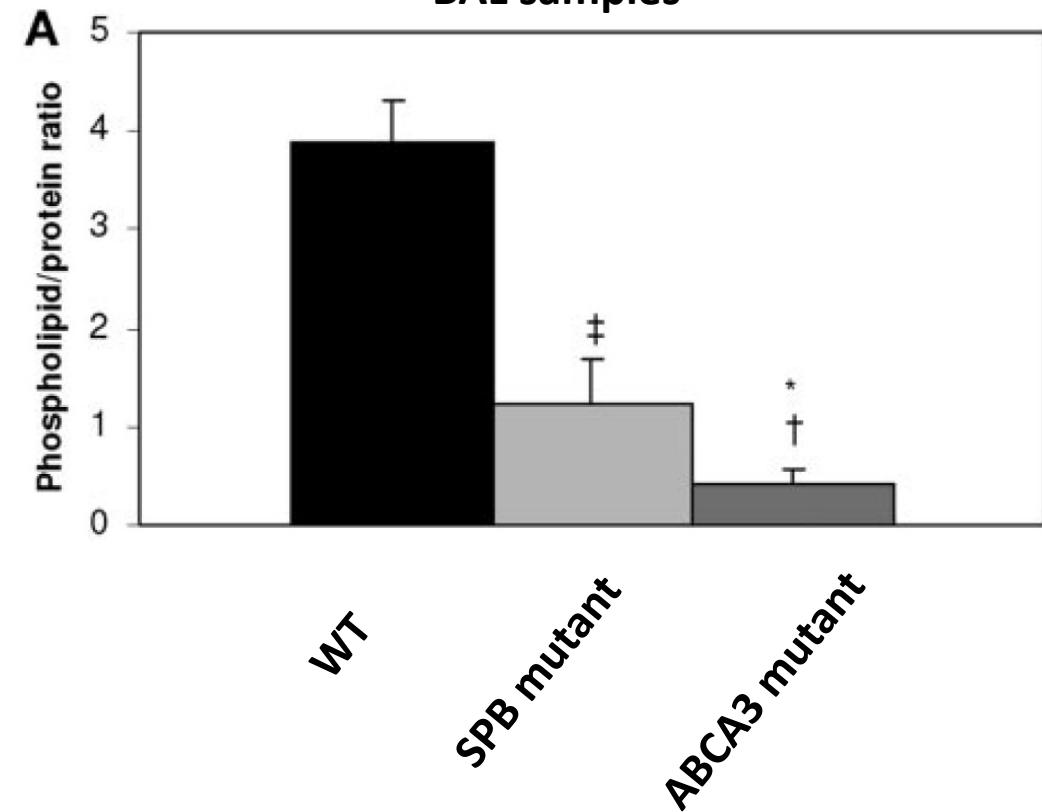


Fried egg lamellar bodies

J. Whitsett, Gower et al. 2008 NeoReviews

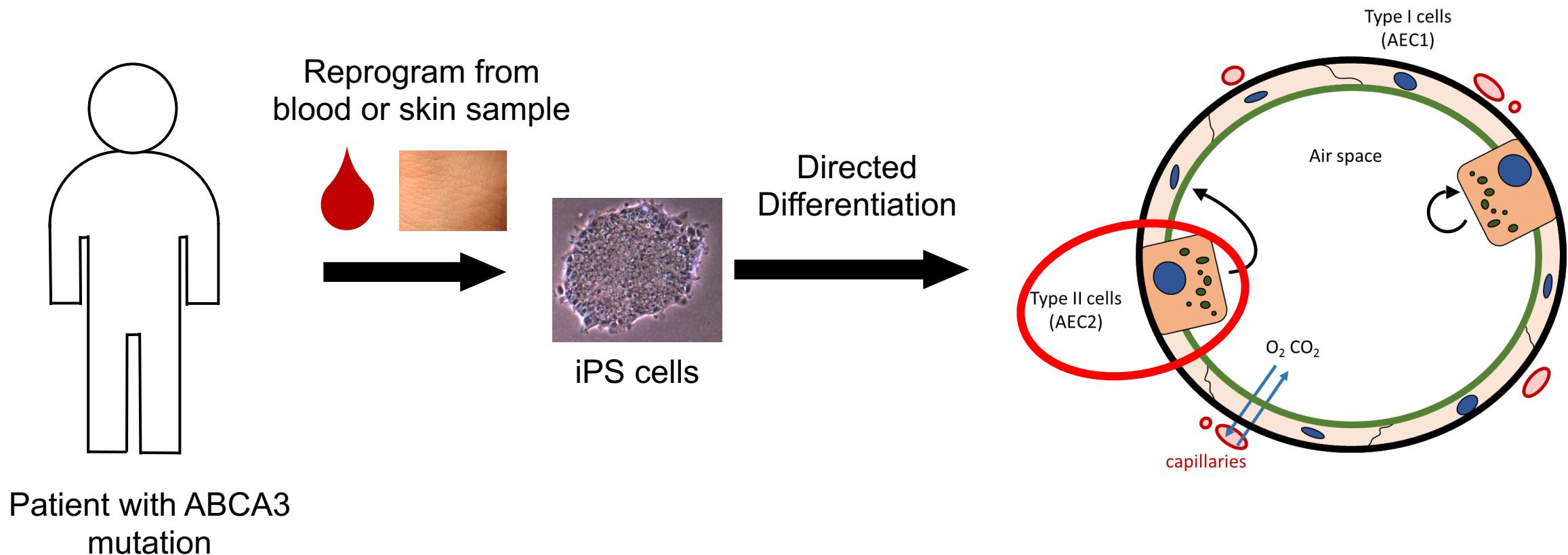
Caused by bi-allelic mutations in **ABCA3** and other genes

BAL samples

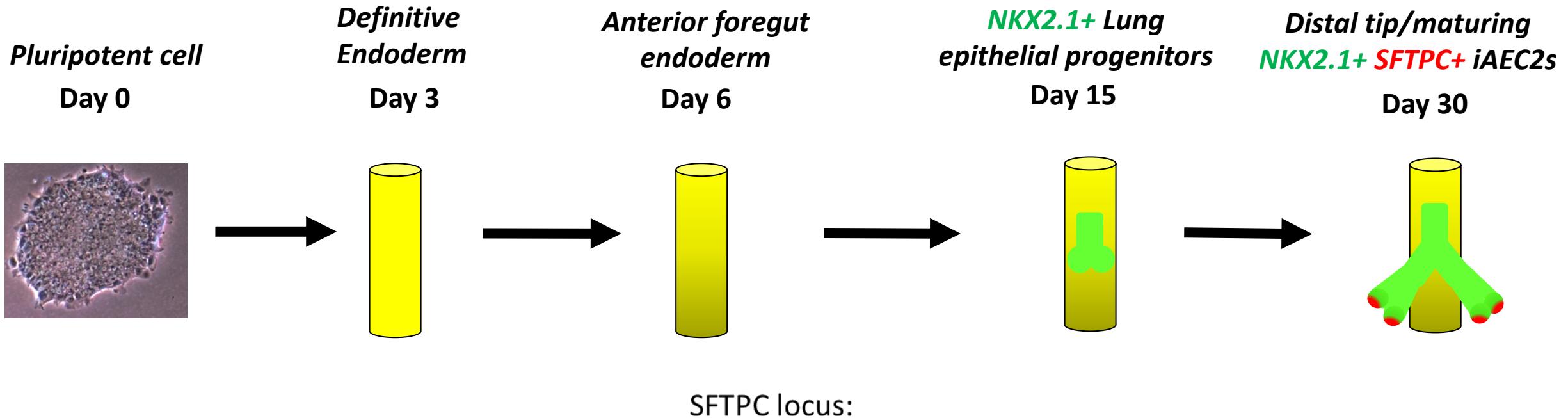


Garmann et al. 2006 Ped. Res.

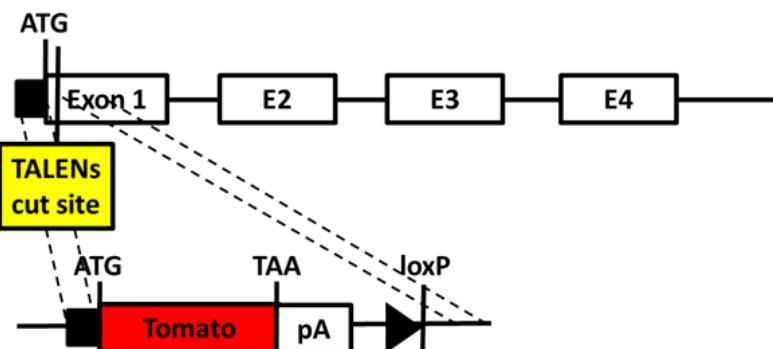
Using AEC2s derived from patient induced pluripotent stem cells (iPSCs) as a model to study ABCA3 disease signatures in AEC2s



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



SFTPC locus:

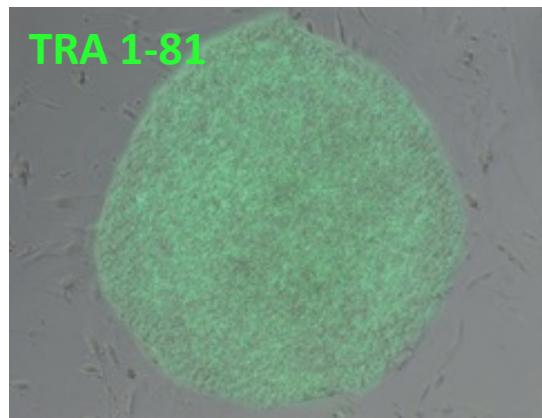


Hawk et al. 2017 *JCI*

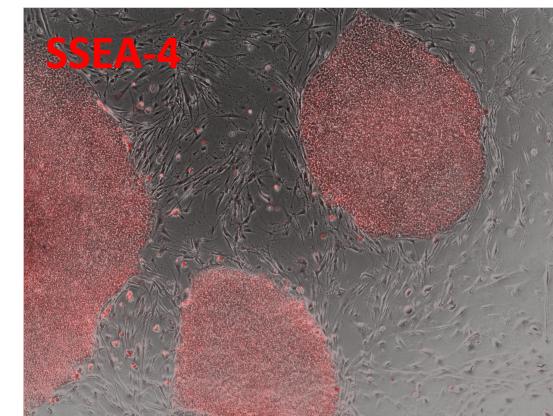
Jacob et al. 2017 *Cell Stem Cell*

iPSCs were generated from patients with homozygous E690K and W308R ABCA3 mutations

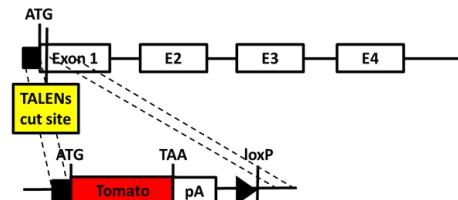
Homozygous E690K
ABCA3 mutant iPSC



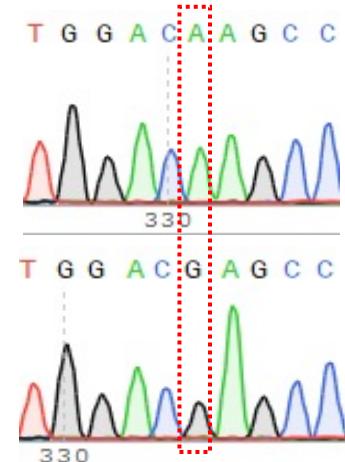
Homozygous W308R
ABCA3 mutant iPSC



SFTPC locus:

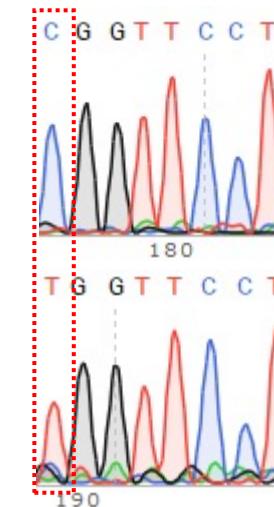


E690K mutant
(G->A)

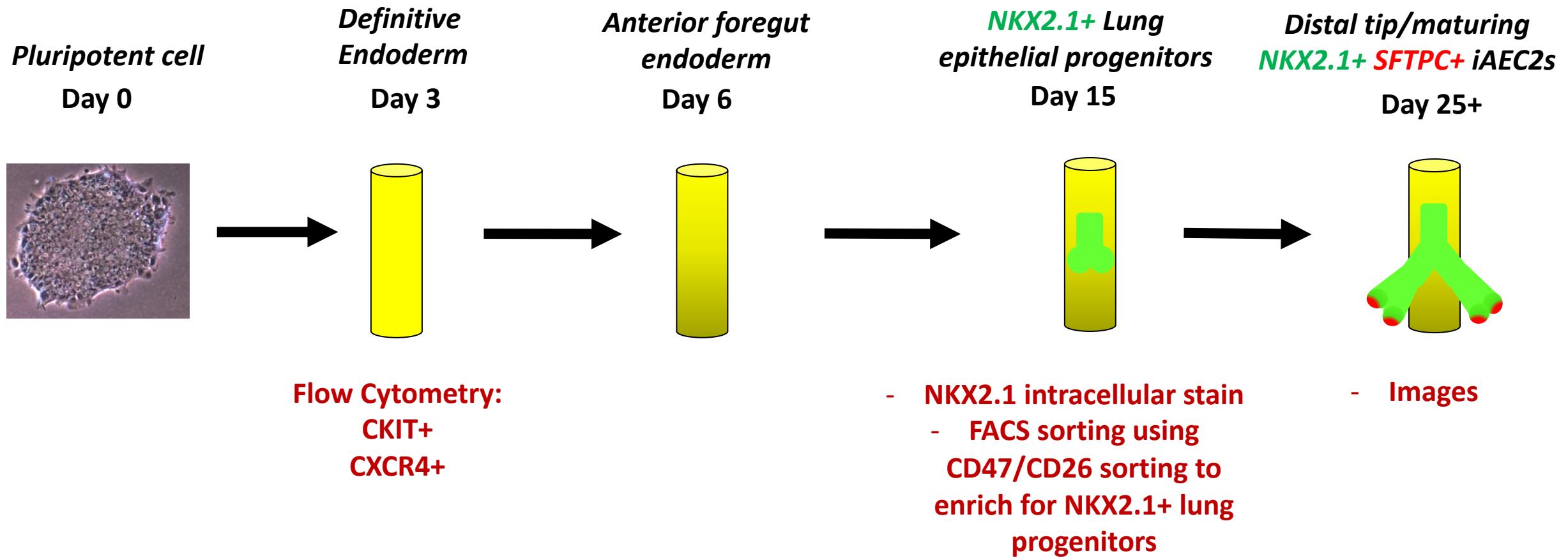


E690 corrected

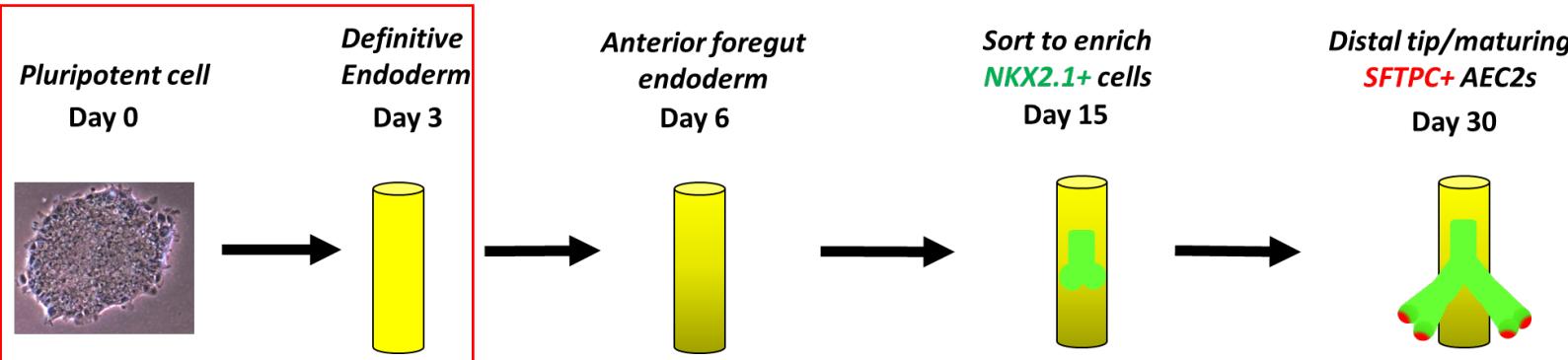
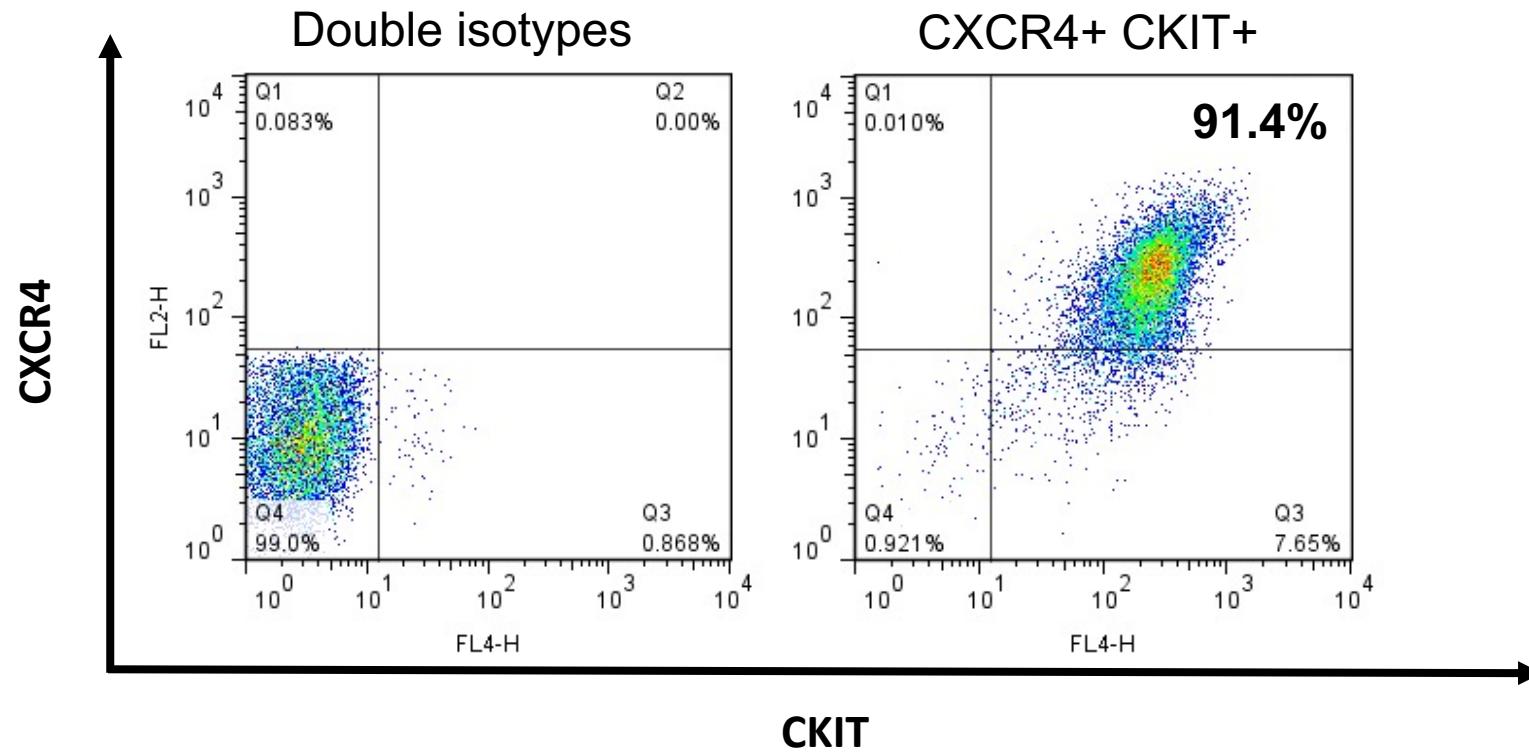
W308R mutant
(T->C)



W308 corrected

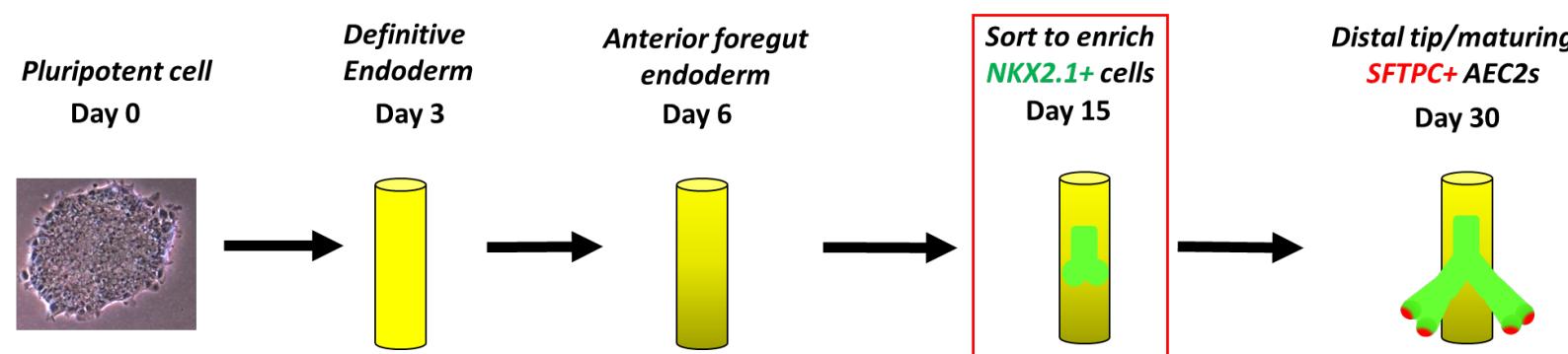
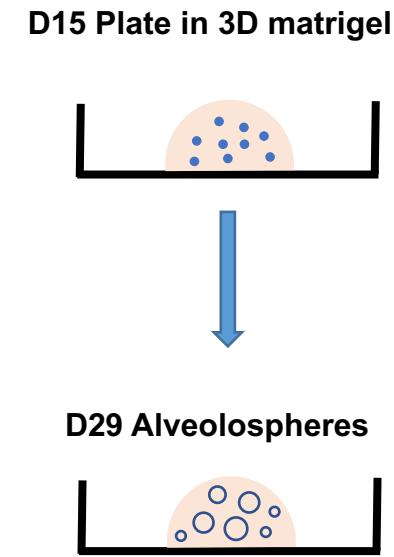
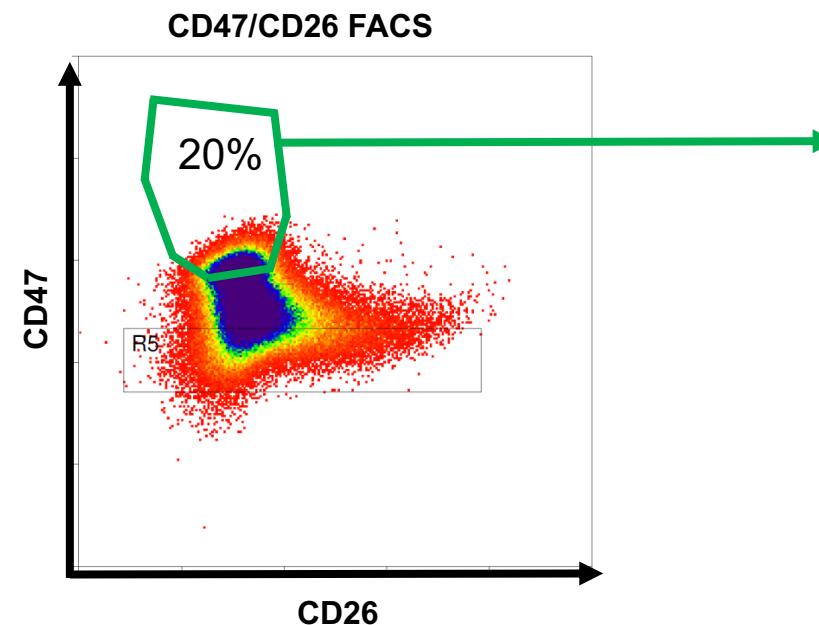
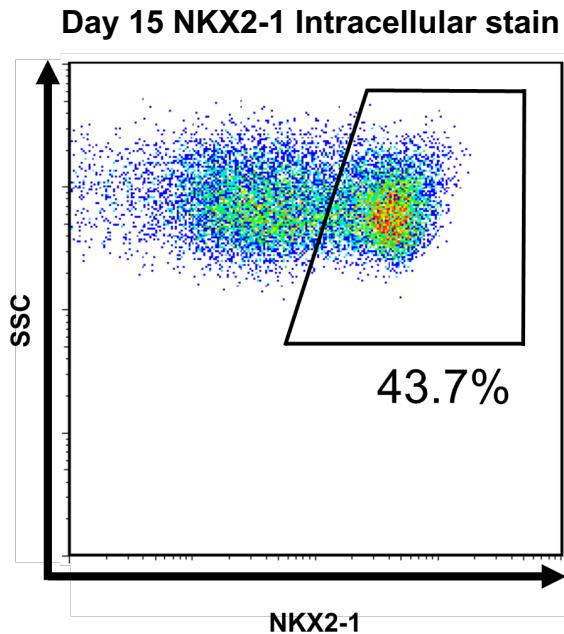


Day 3 E690K cells expressed definitive endoderm markers CKIT and CXCR4

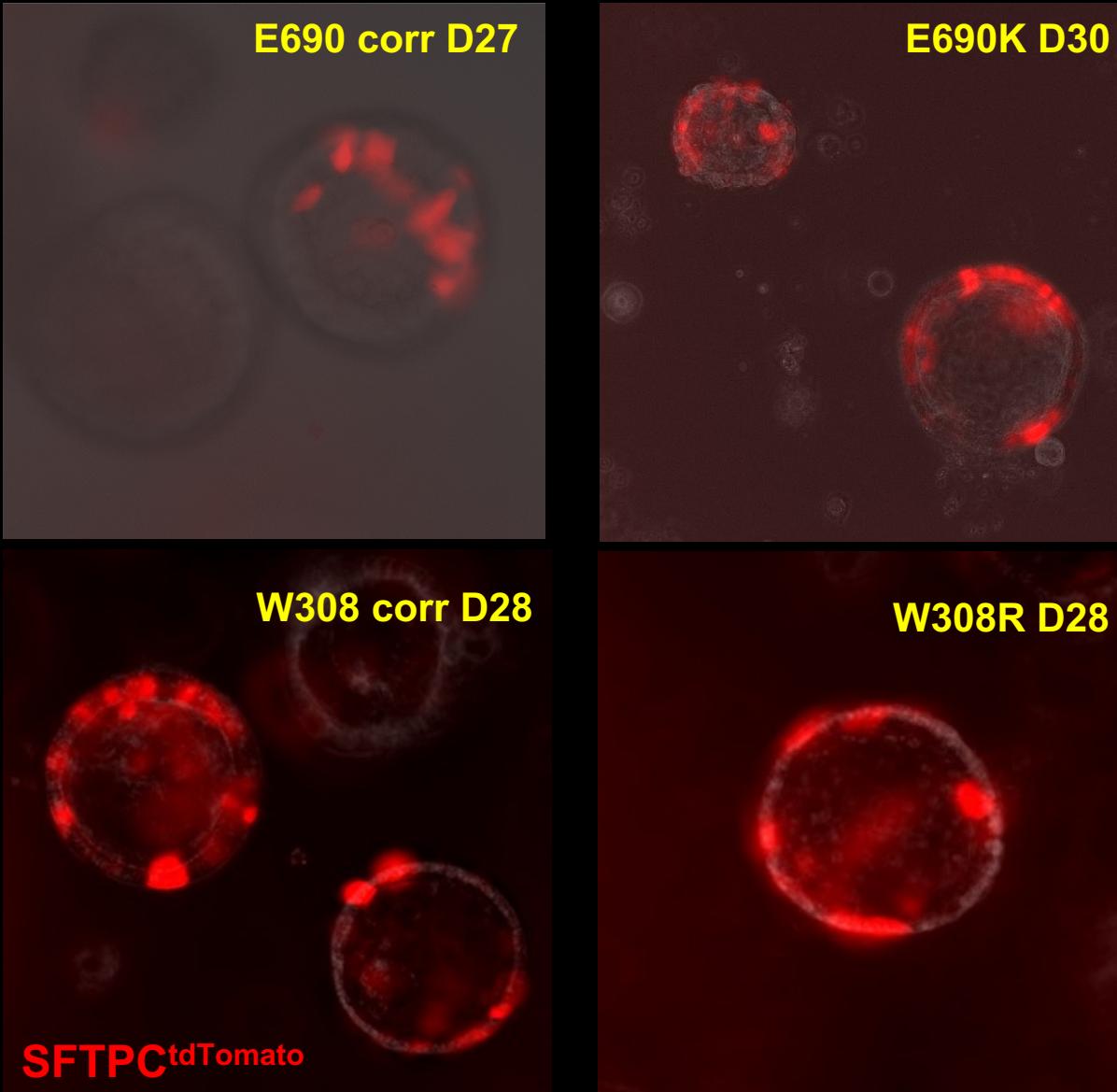


Day 15 NKX2-1 expressing cells were enriched using CD47/26 cell surface markers and plated in 3D matrigel

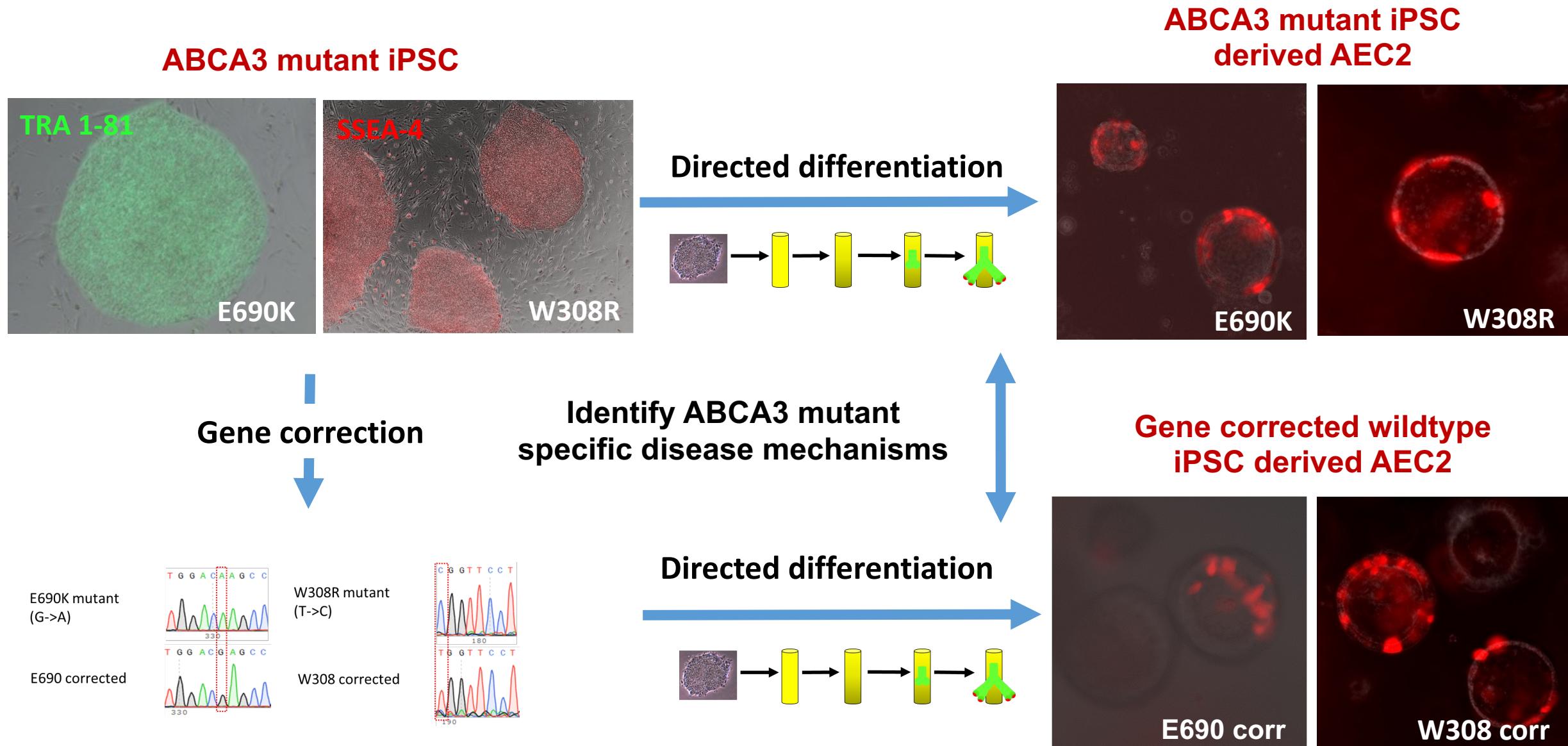
E690K ABCA3 iPSC line



Mutant and gene-corrected syngeneic iPSC lines generated $SFTPC^{tdTomato}$ + iAEC2s following lung directed differentiation



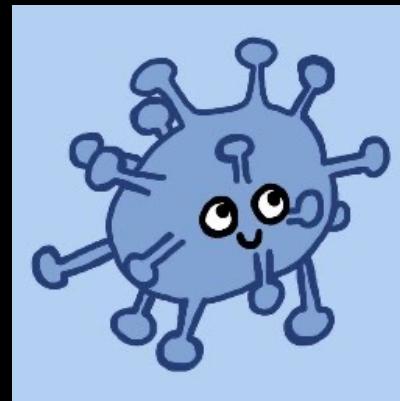
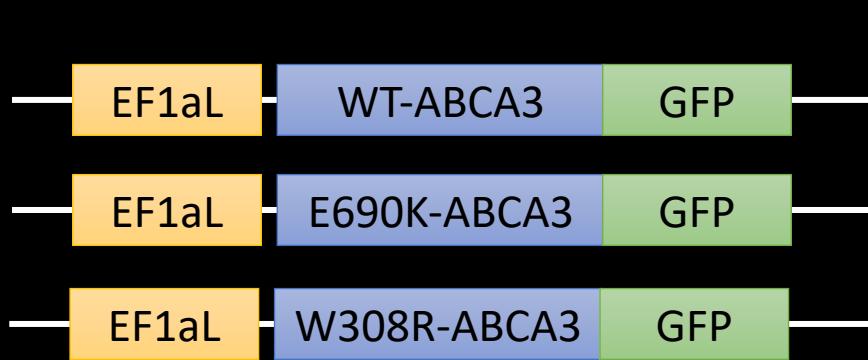
Comparing patient iPSC-derived AEC2s with gene corrected syngeneic lines will enable identification of mutant specific disease signatures



Outline:

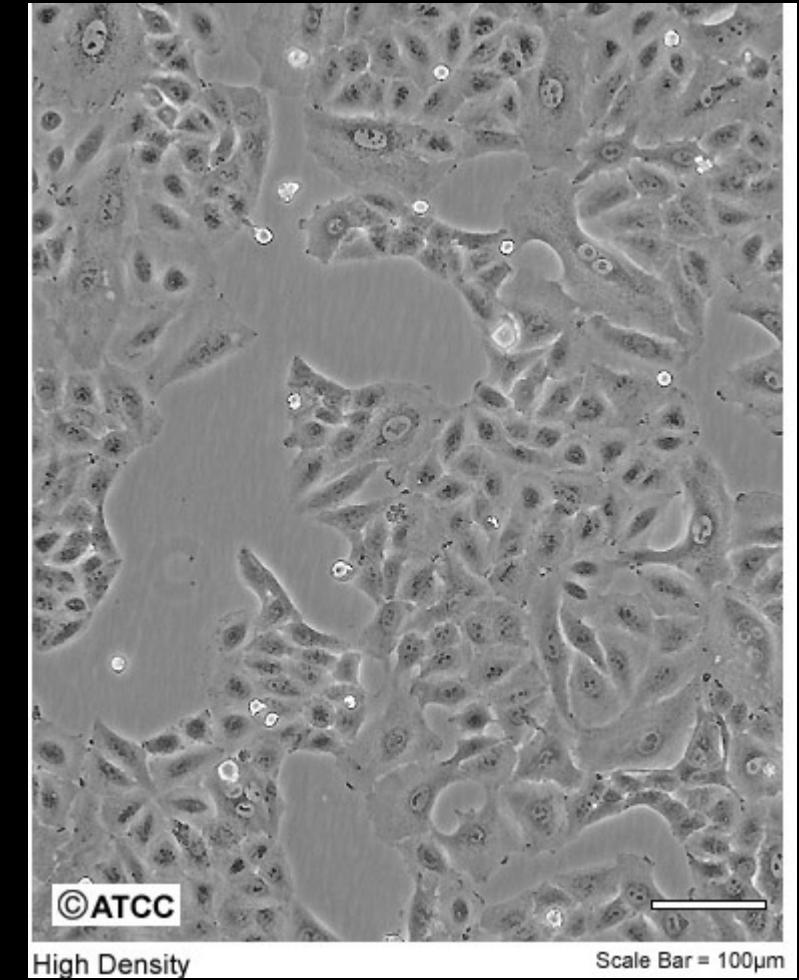
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WT and mutant ABCA3:GFP fusion protein were expressed in A549 lung adenocarcinoma line using lentiviral infection

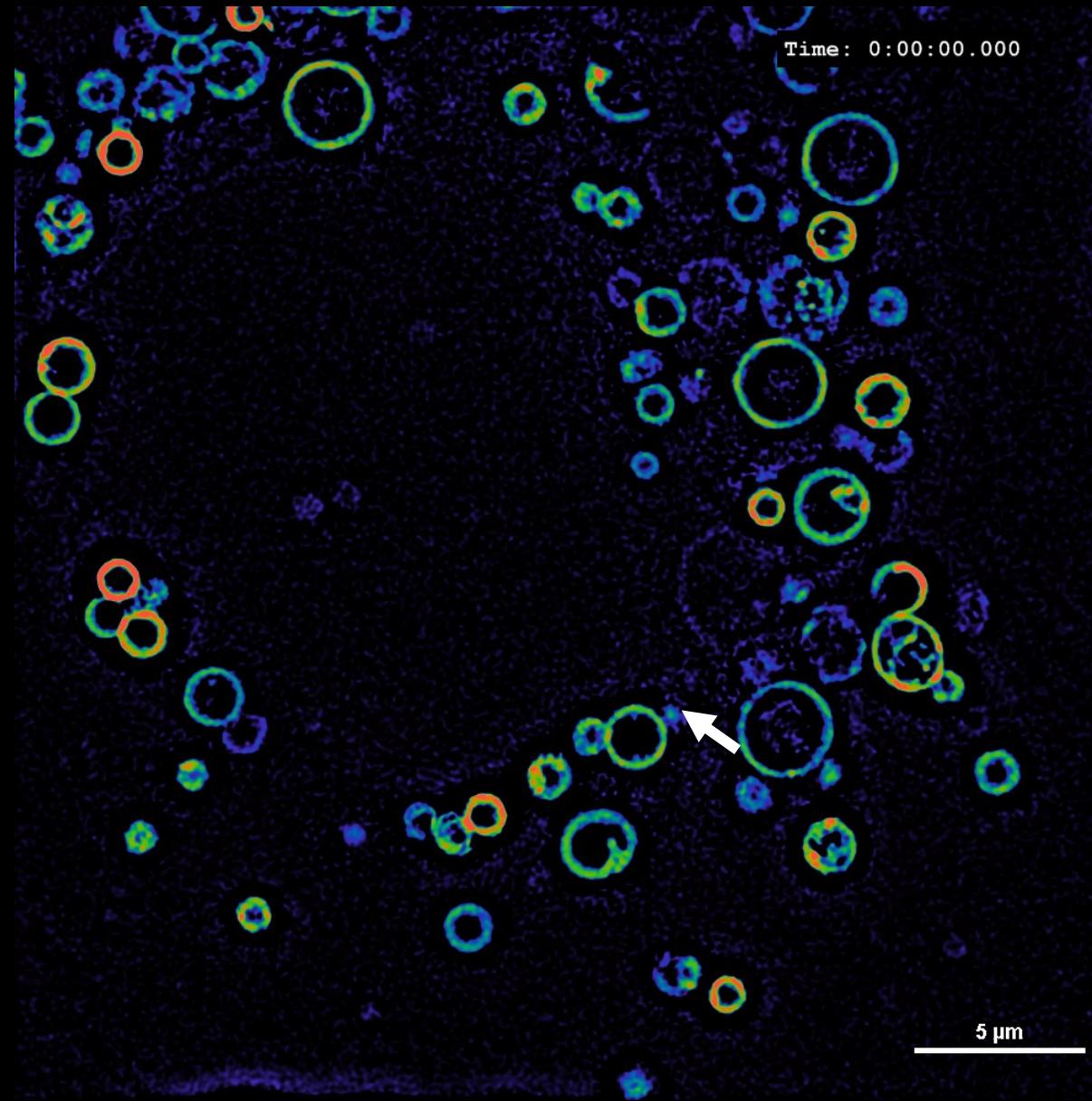


Lentivirus

A549

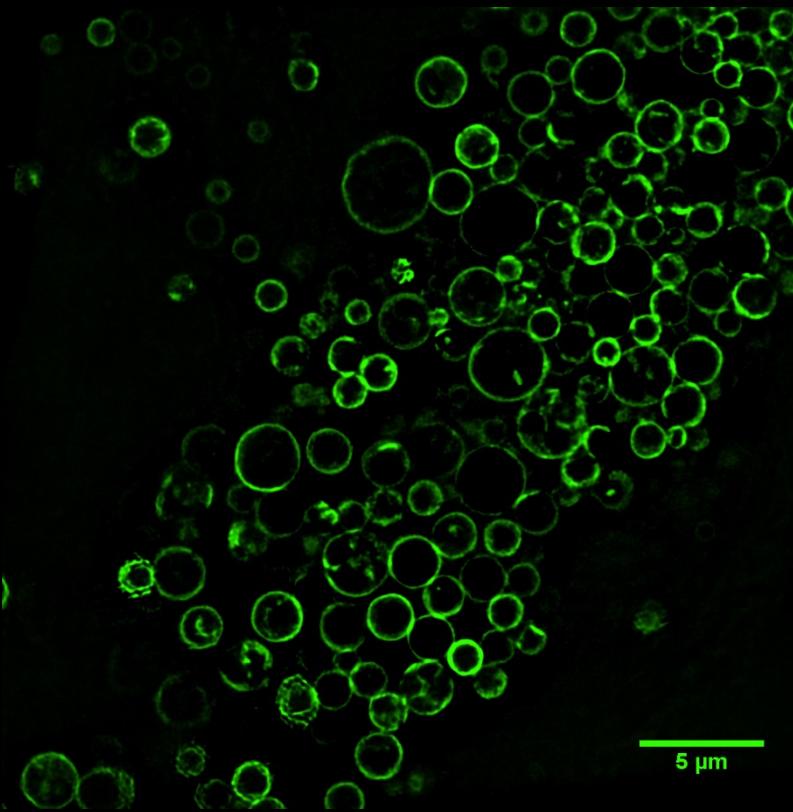


ABCA3:GFP+ lamellar body-like inclusions were expressed in A549 cells

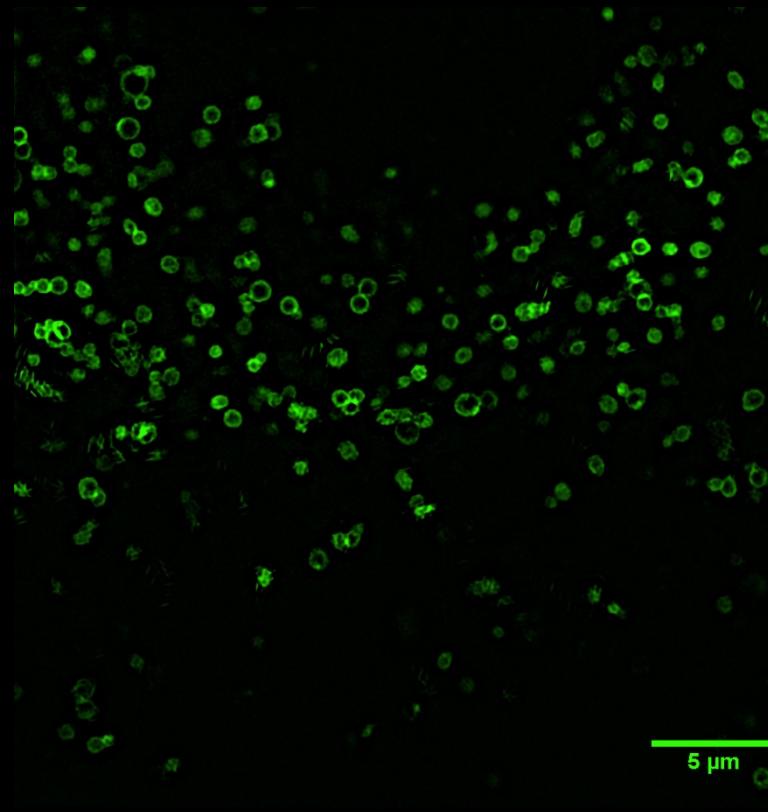


WT

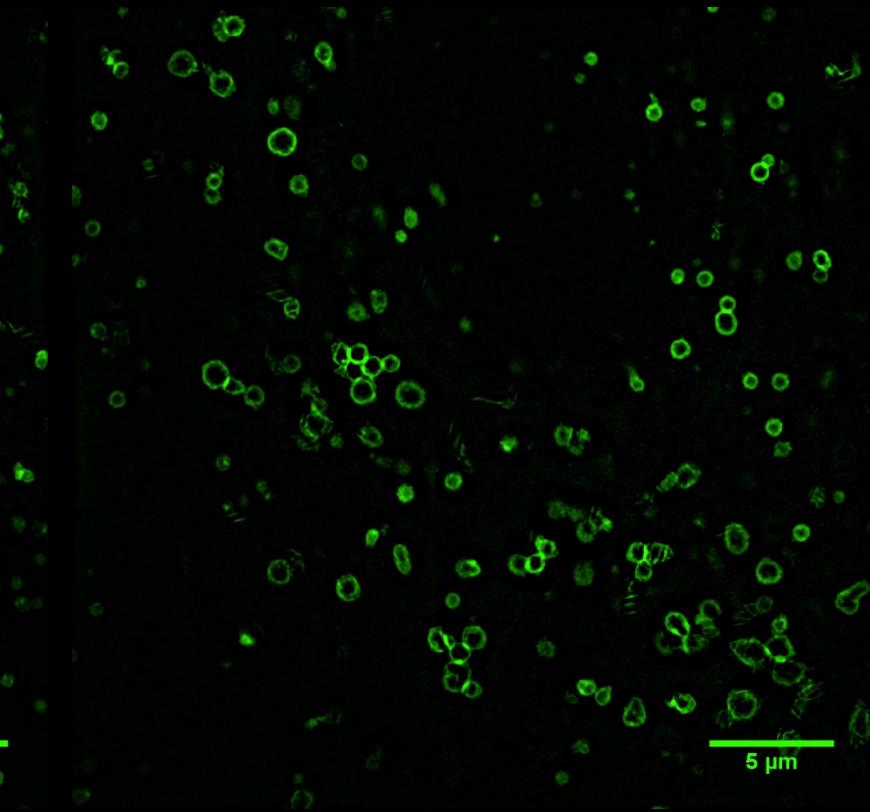
E690K and W308R mutant ABCA3:GFP fusion protein formed smaller GFP+ vesicles compared to wildtype



WT

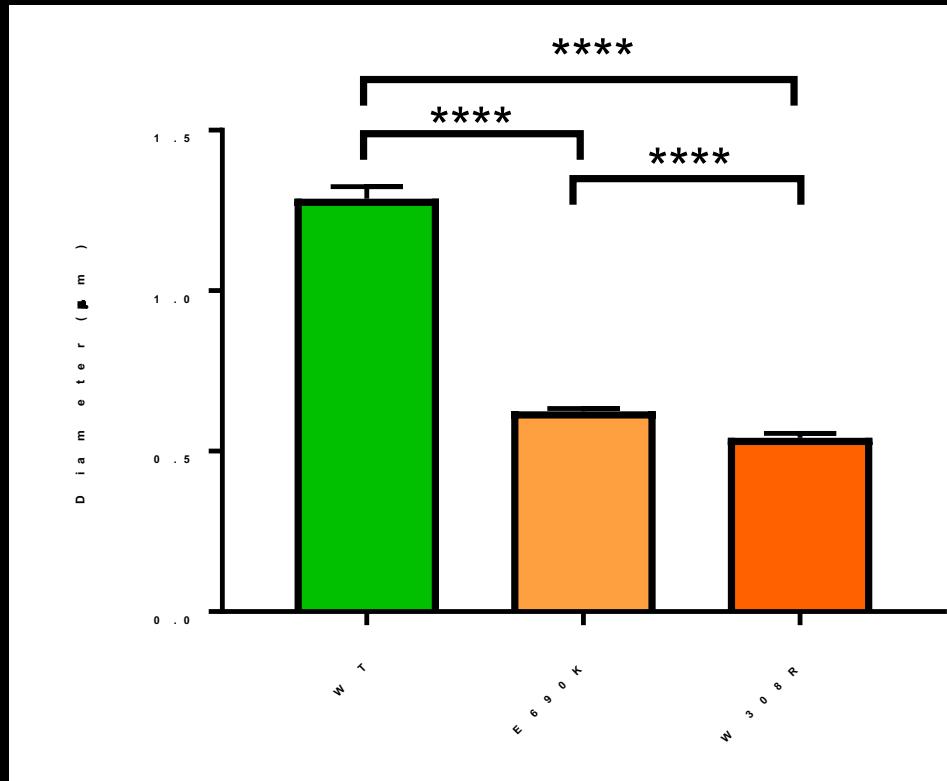


E690K

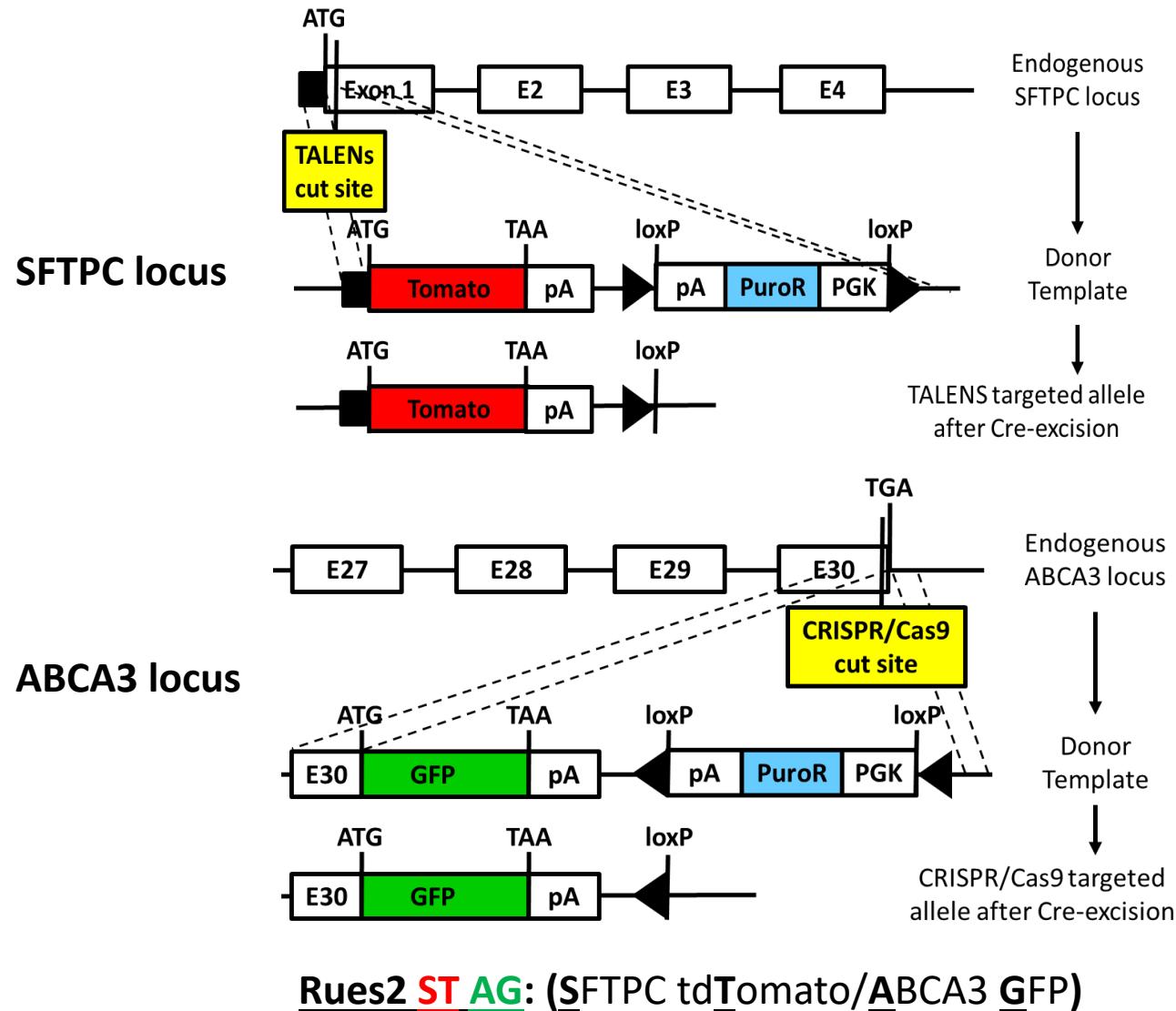


W308R

E690K and W308R mutant ABCA3:GFP fusion protein formed smaller GFP+ vesicles compared to wildtype



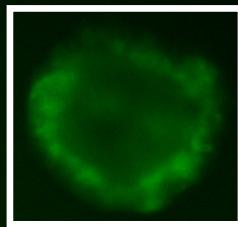
Dual fluorochrome markers will enable the study of ontogeny and localization of ABCA3 expression as lung epithelial progenitors differentiate into functional iAEC2s



Jacob et al. 2017 *Cell Stem Cell*

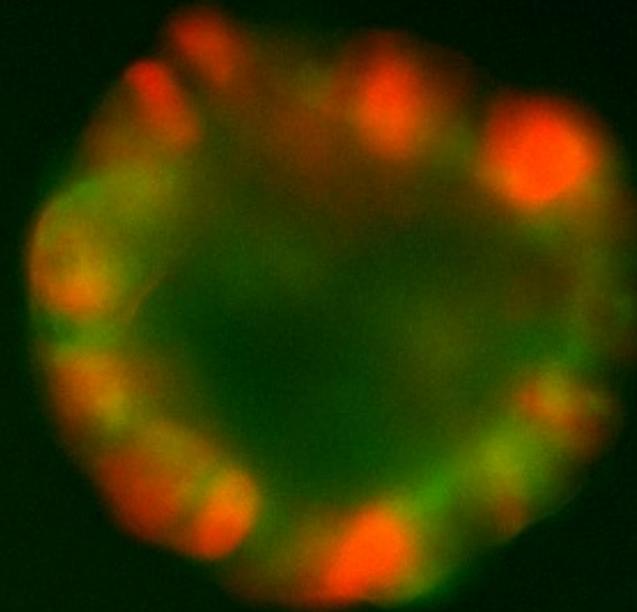
On day 29, RUES2 STAG expressed both the ABCA3:GFP and SFTPC^{tdTomato} reporters

ABCA3:GFP



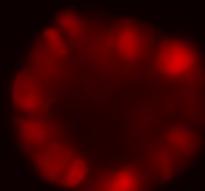
ABCA3:GFP

SPC TdTomato



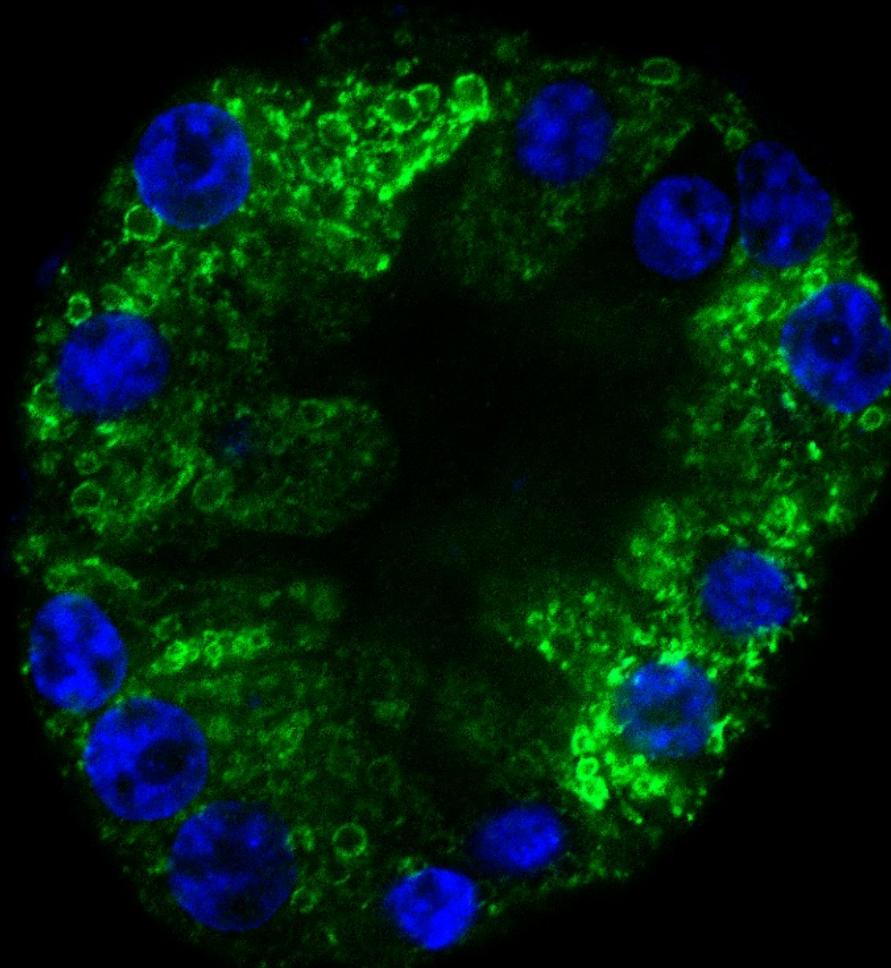
Merge

SPC TdTomato



Day 29 RUES2 STAG

Day 88 RUES2STAG

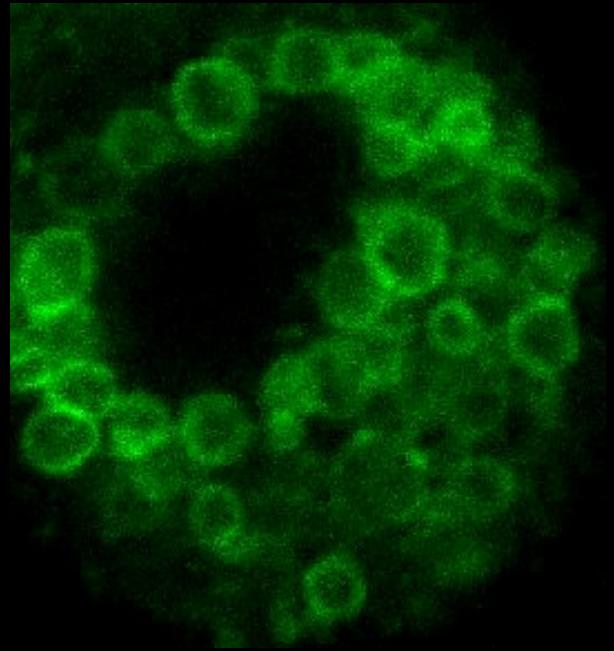


ABCA3:GFP
Hoechst (nuclei)

10 μm

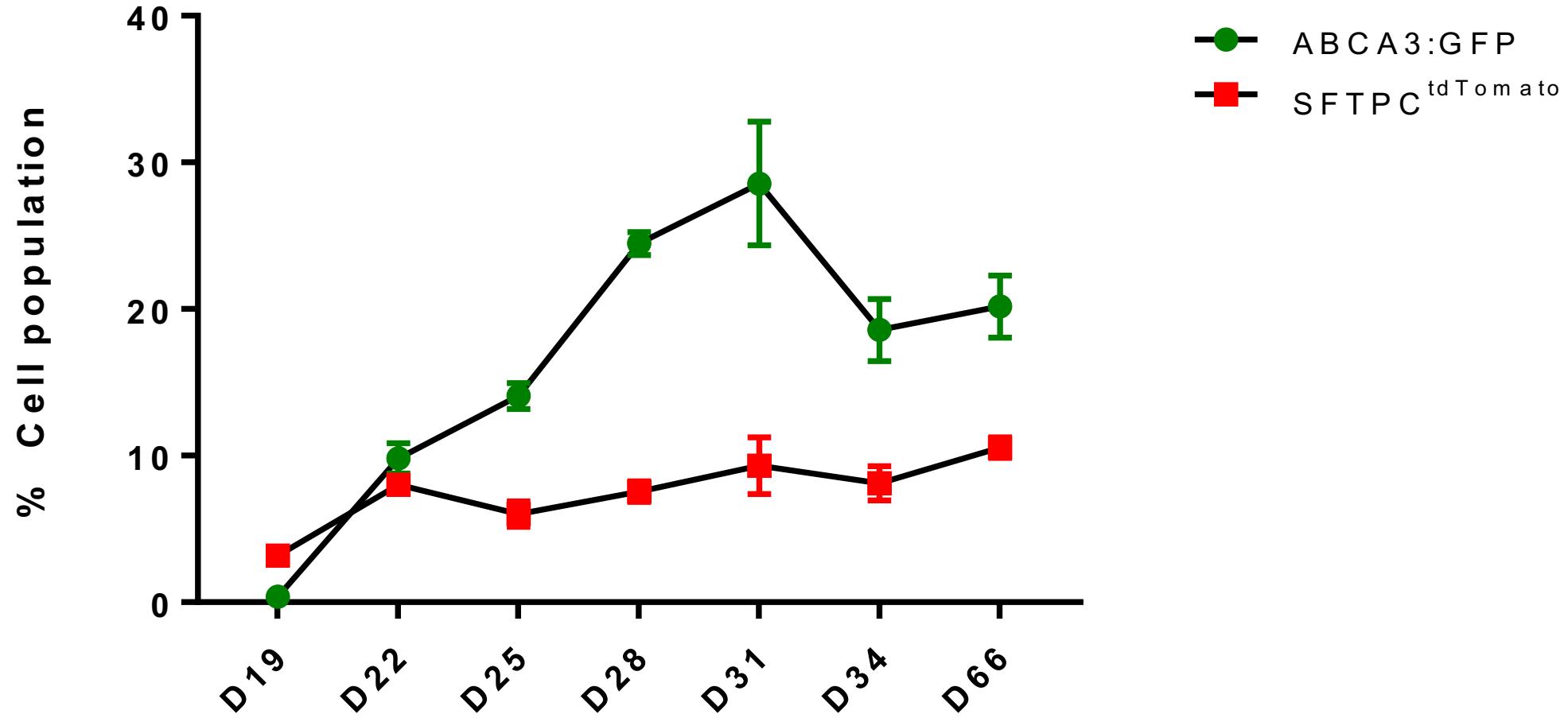
Zeiss LSM 700
Confocal microscopy

ABCA3:GFP+ intracellular vesicles can be visualized using live cell confocal microscopy

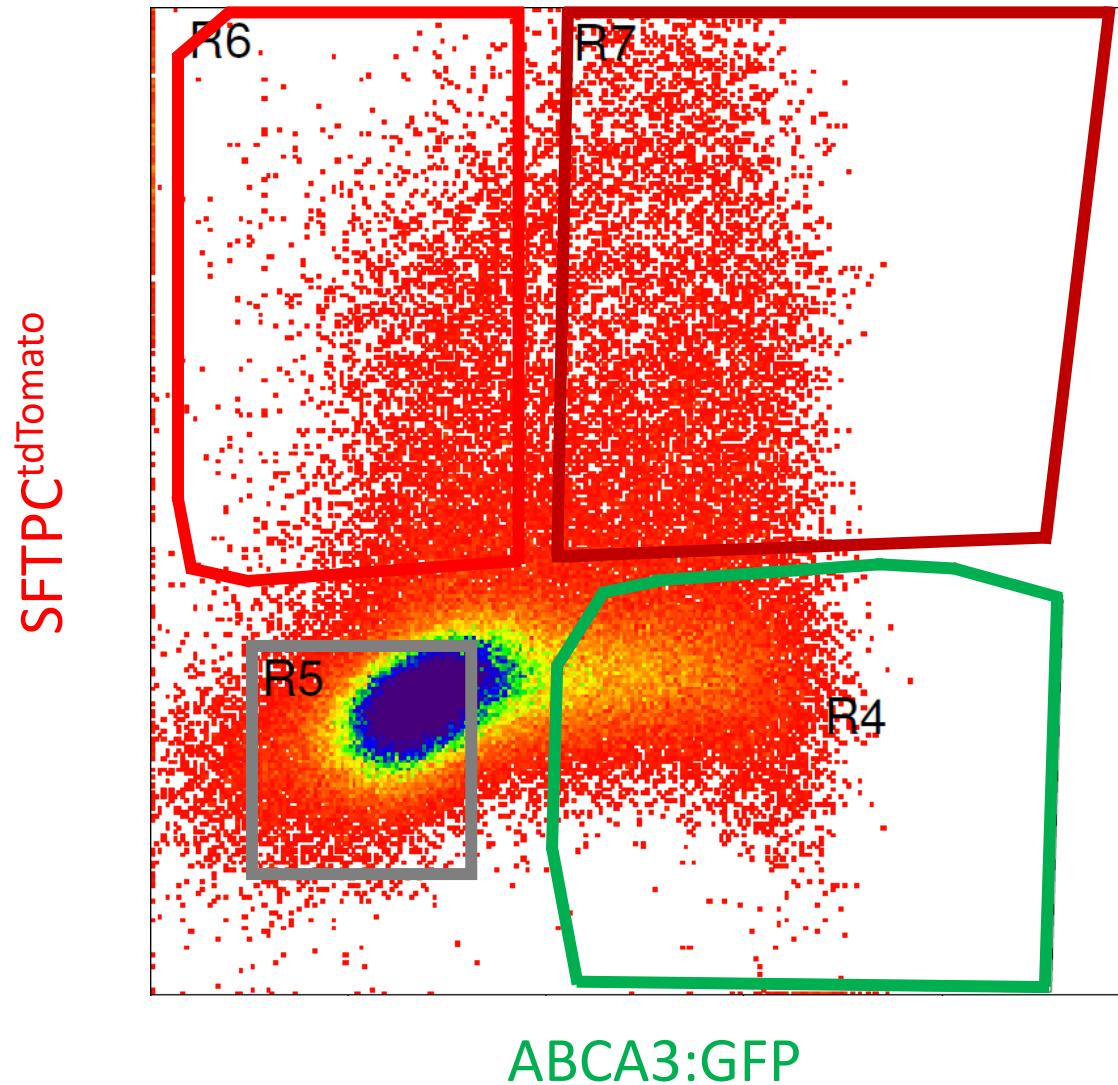


Live cell confocal

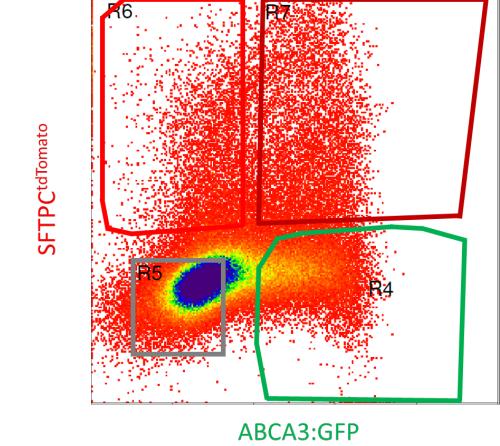
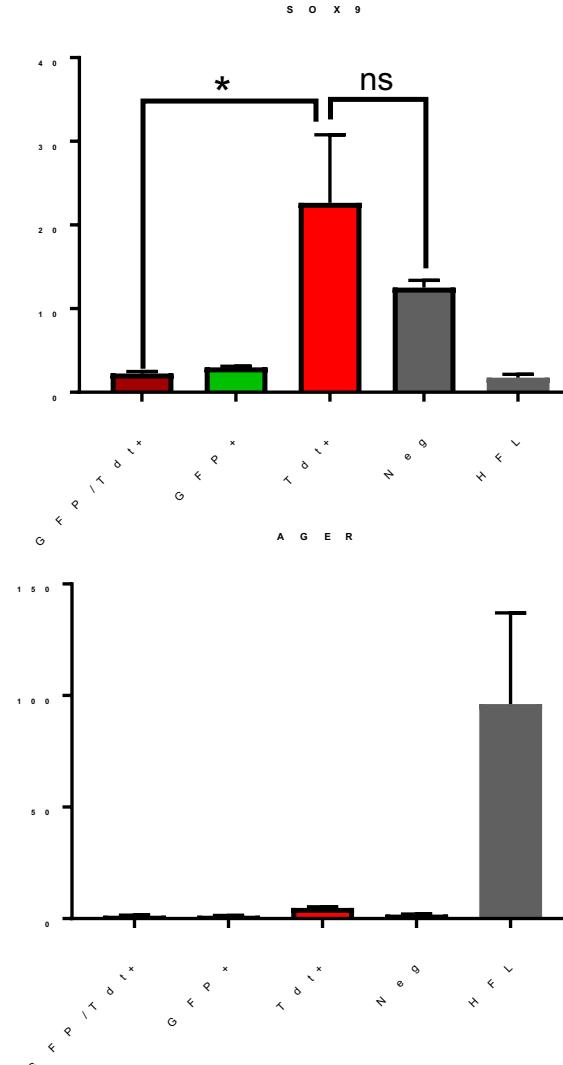
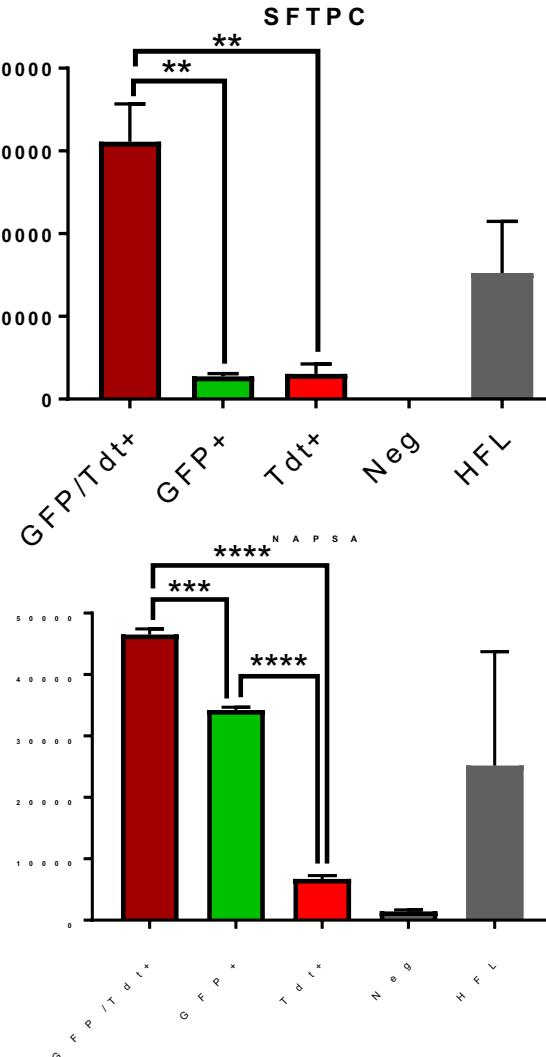
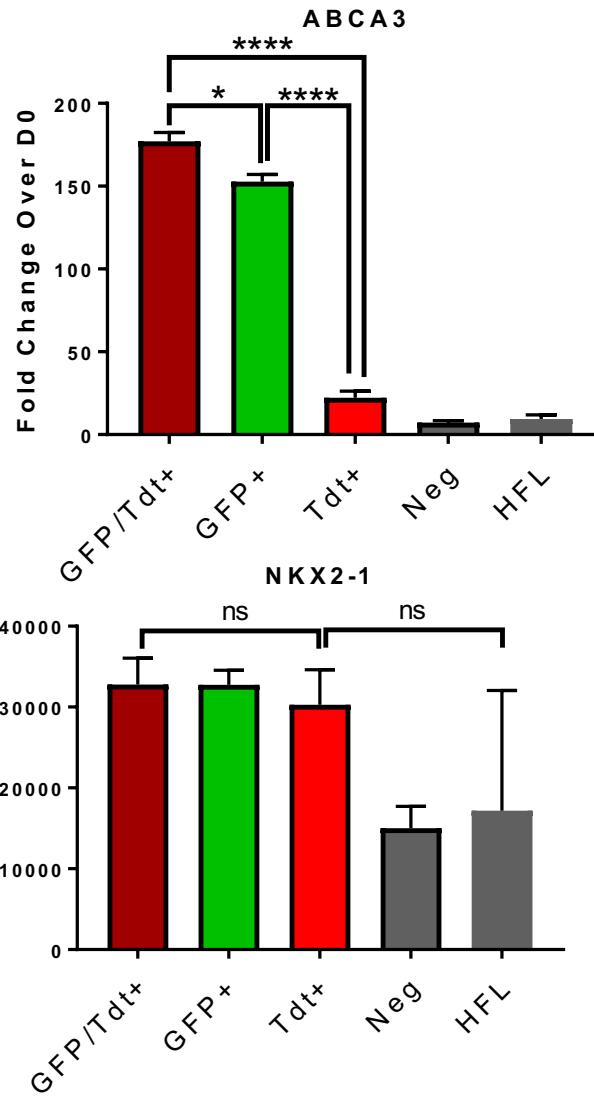
ABCA3:GFP has a delayed expression compared to SFTPC^{tdTomato} and is more broadly expressed in STAG alveolospheres



Day 66 STAG alveolospheres were sorted into **GFP+** only, **TdTomato+** only, **double positive**, and **double negative** populations for RT-qPCR

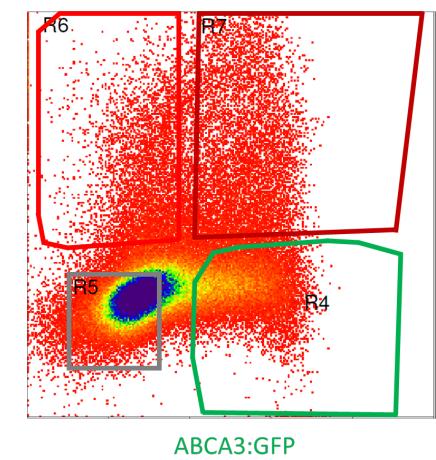


ABCA3:GFP, SFTPC^{tdTomato} double positive cells had the highest levels of ABCA3, SFTPC and surfactant processing gene expression by RT-qPCR



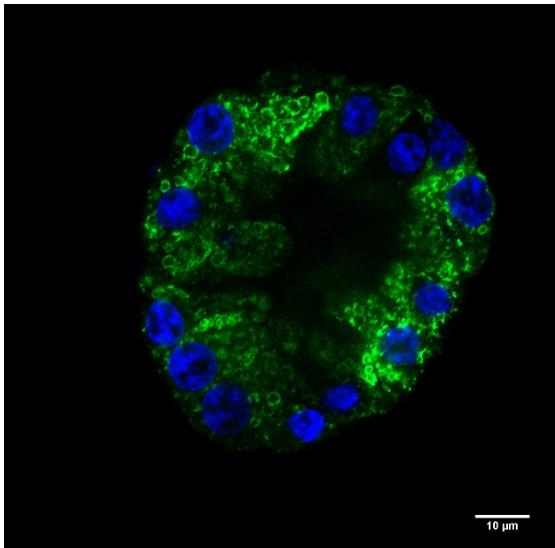
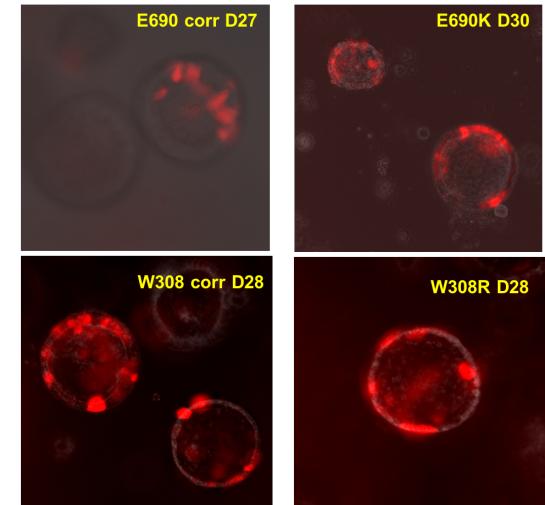
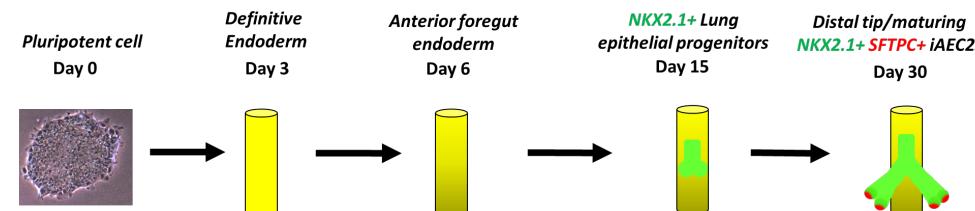
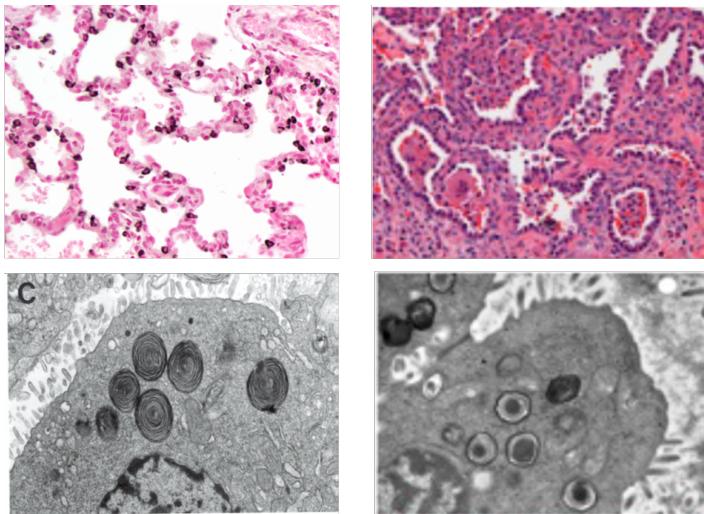
HFL= wk 21 human fetal lung enriched in AEC2

SFTPC^{tdTomato}



ABCA3:GFP

Summary and future directions:



- Identification of disease signatures for each ABCA3 mutant
- Introduce E690K, W308R mutations using CRISPR/Cas9 into the dual-reporter STAG line
- Small molecule screening for reversal of disease phenotype in ABCA3 mutant iAEC2s

Acknowledgements:

Kotton Lab:

Darrell Kotton
Anjali Jacob
Kathrine McCauley
Marally Vedaie
George Kwong
Killian Hurley
Hector Marquez
Jyn-Chang Jean
Kostas Alysandratos
Michael Herriges
David Roberts
Jinyoung Choi
Julian Undeutch

Hawk Lab:

Finn Hawkins
Dylan Thomas
Andrew Berical
Mary Lou Beermann

Wilson Lab:

Andrew Wilson
Joseph Kaserman

Mostoslavsky lab:

Dar Heinz



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