Using chILD patient-derived induced pluripotent stem cells to model ABCA3 dysfunction in vitro

Yuliang L. Sun[1], Anjali Jacob[1], Jyn-Chang Jean[1], Anusha Sridharan[2], Jeffery Whitsett[2], and Darrell Kotton[1]
[1]Boson University School of Medicine, Boston, MA, USA; [2]University of Cincinnati College of Medicine

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Introduction

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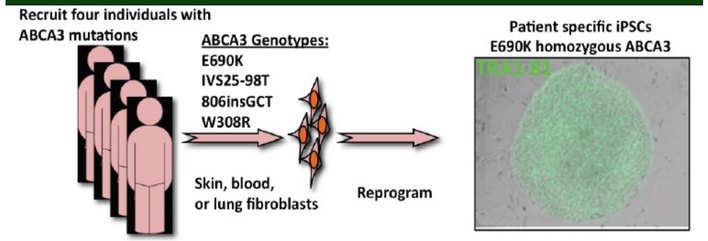

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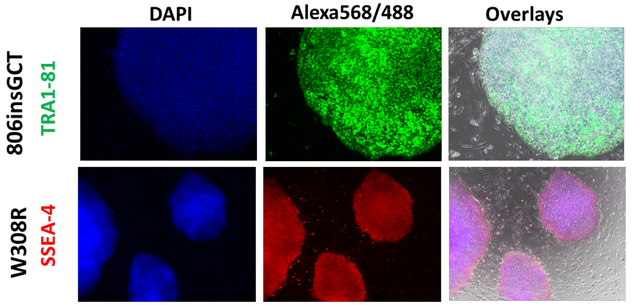

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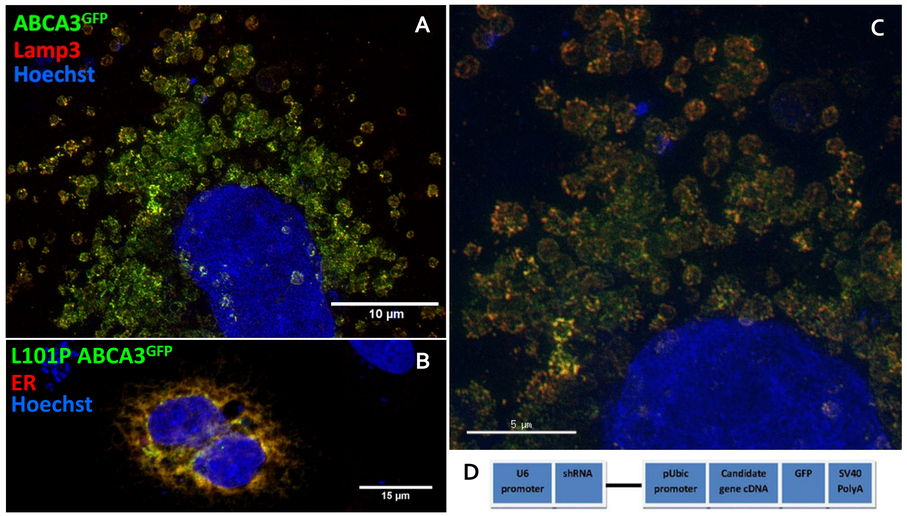

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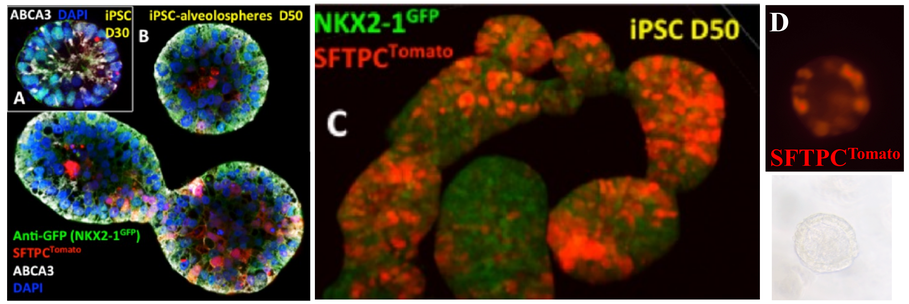

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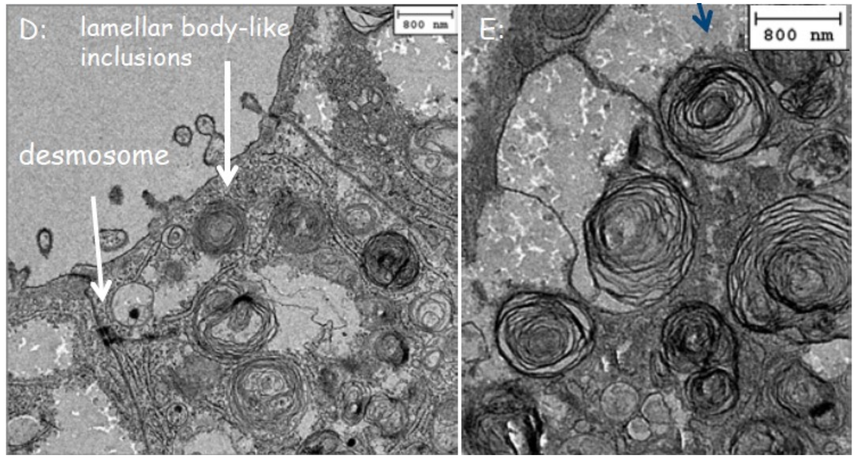

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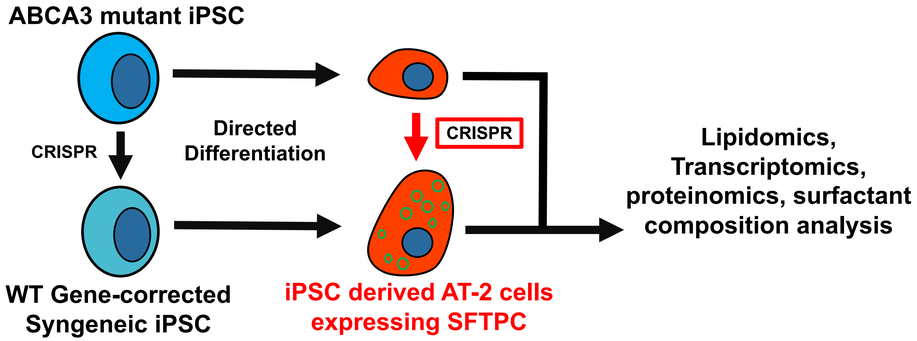
Path to Disease Modeling and Clinical Translation



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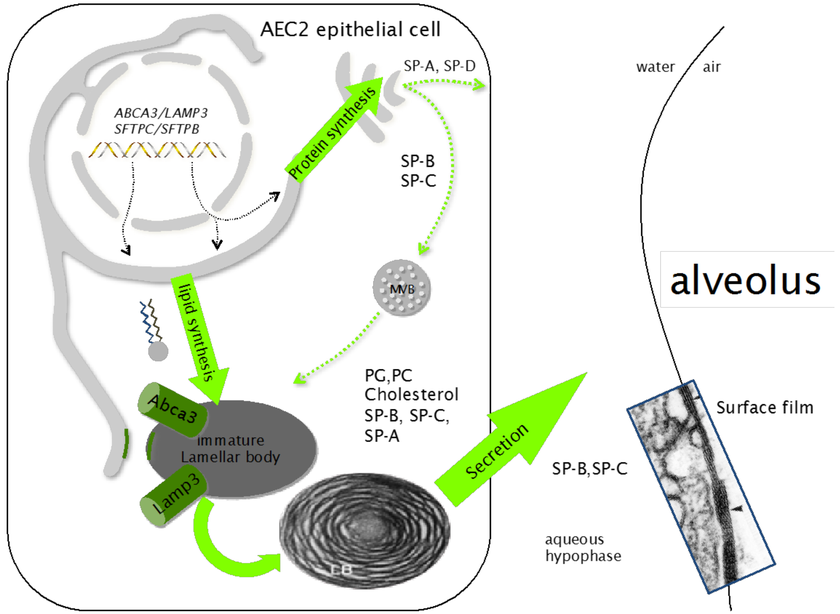
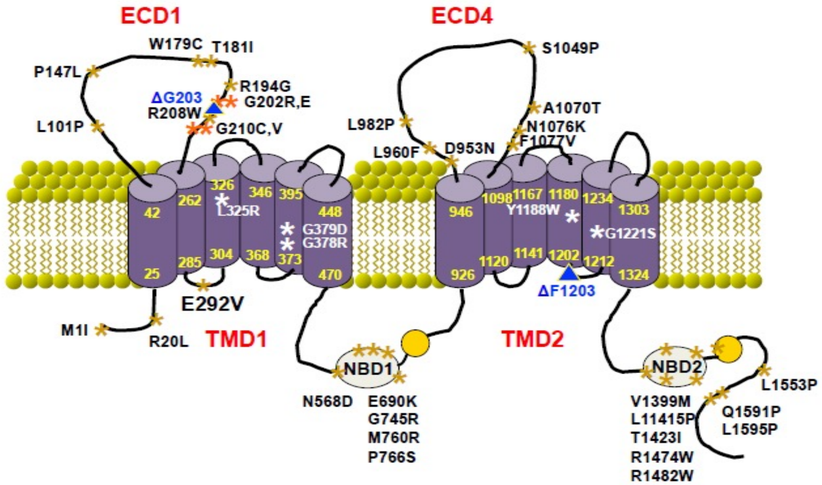
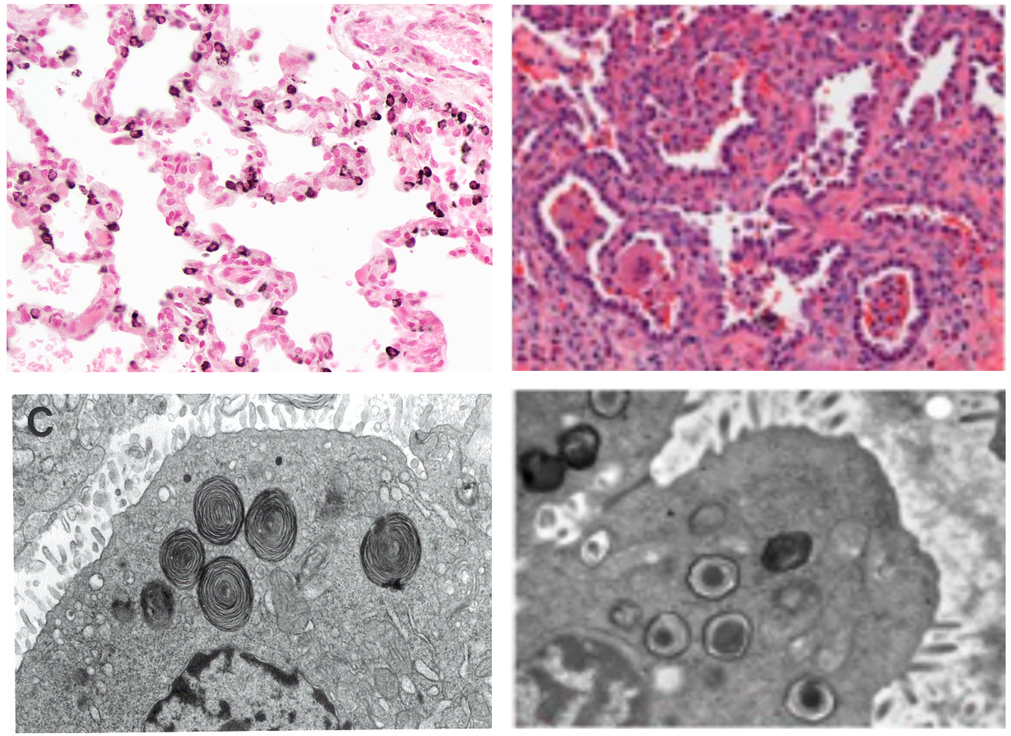
Autosomal recessive mutations in the ABCA3 gene can result in Childhood Interstitial Lung Disease (chILD)





L. Nogee

Normal human Lung | **ABCA3 mut (S1049P)**



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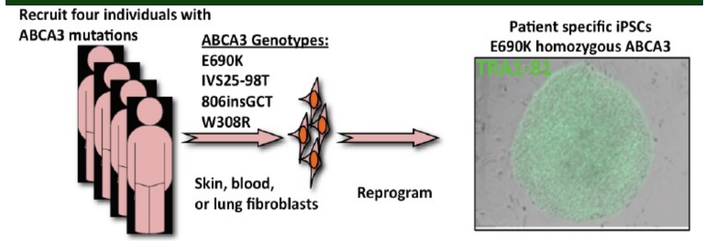

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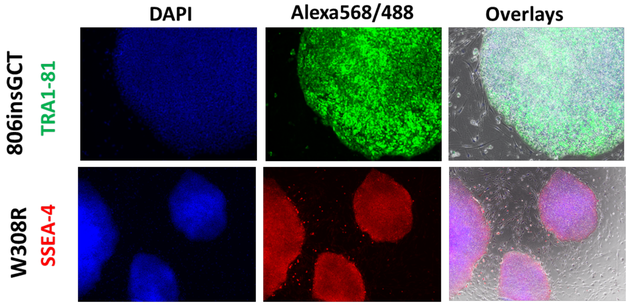

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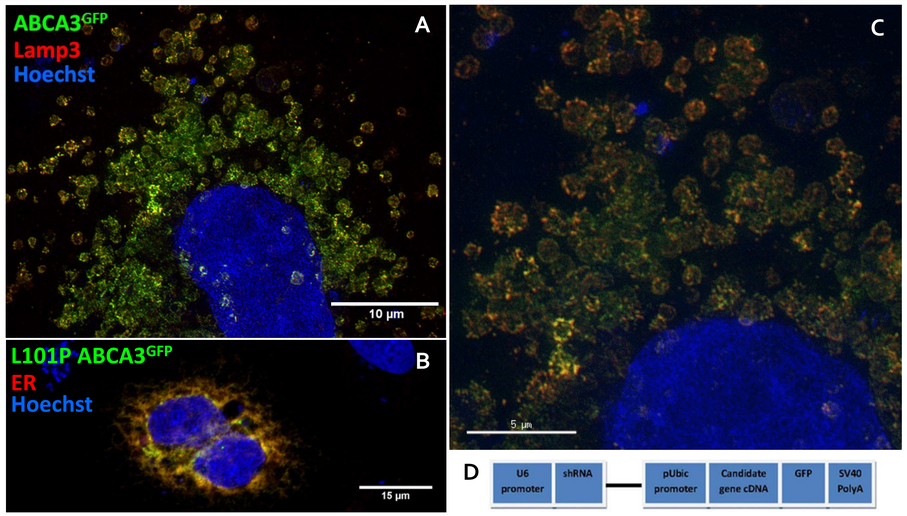

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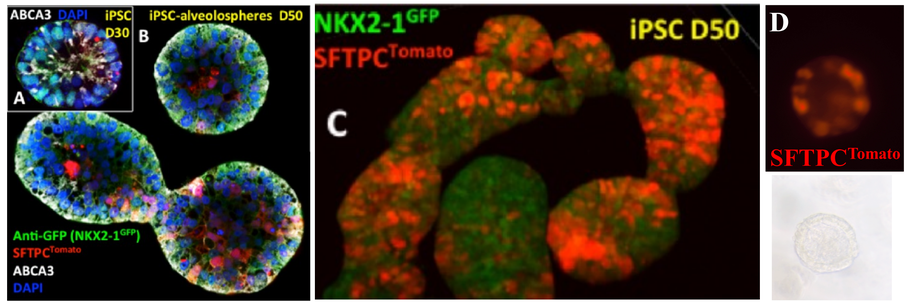

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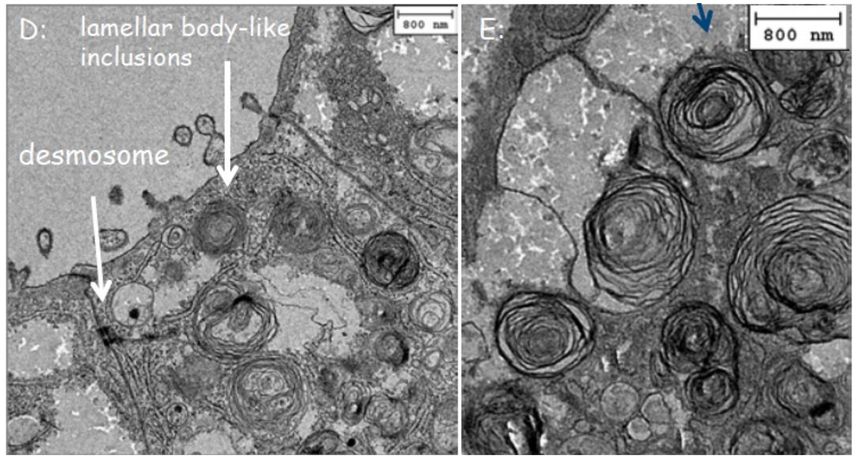

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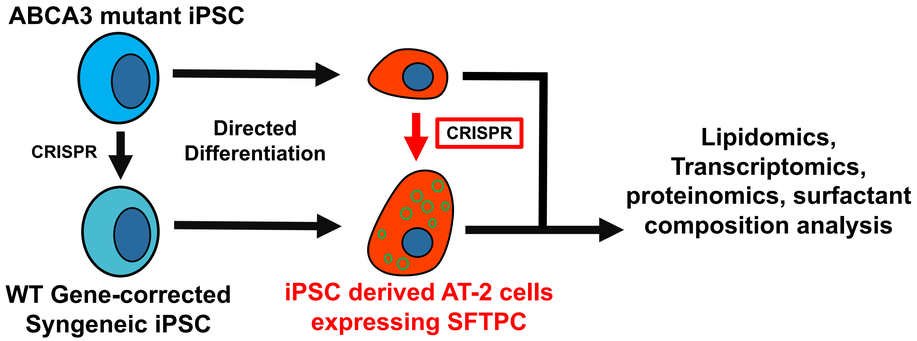
Path to Disease Modeling and Clinical Translation



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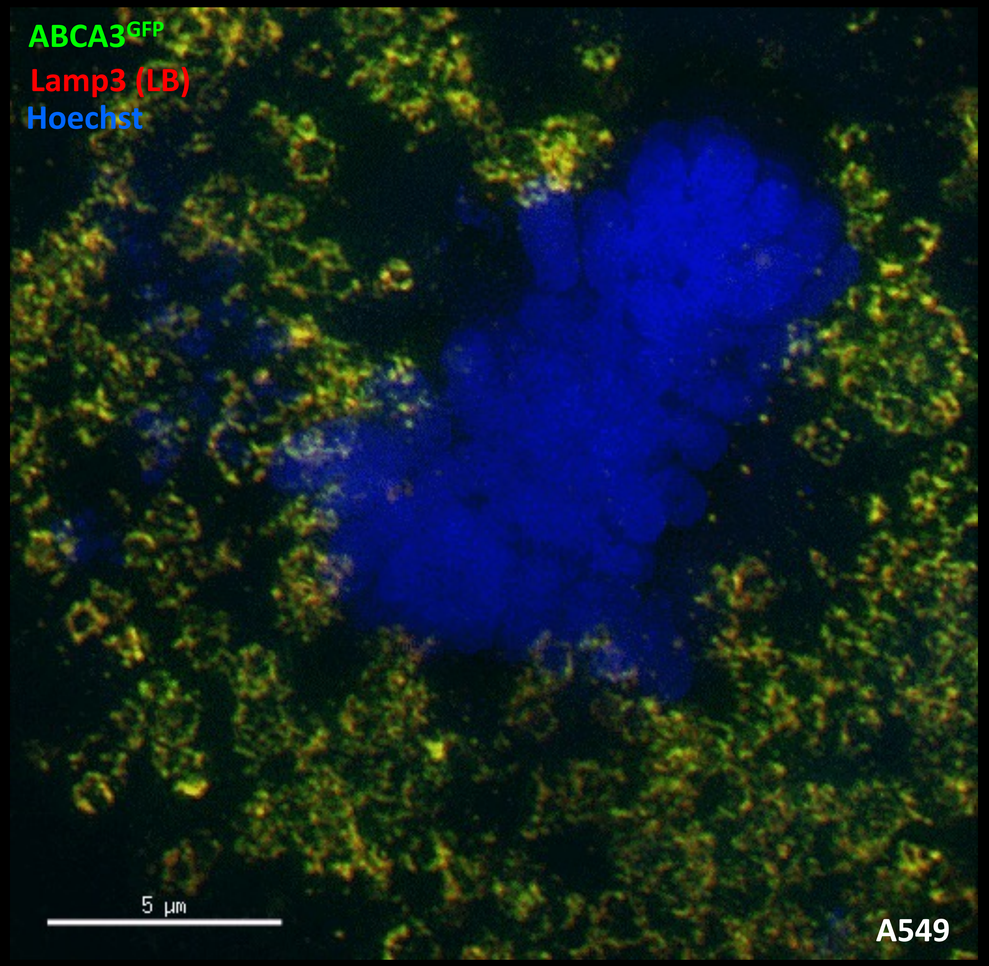

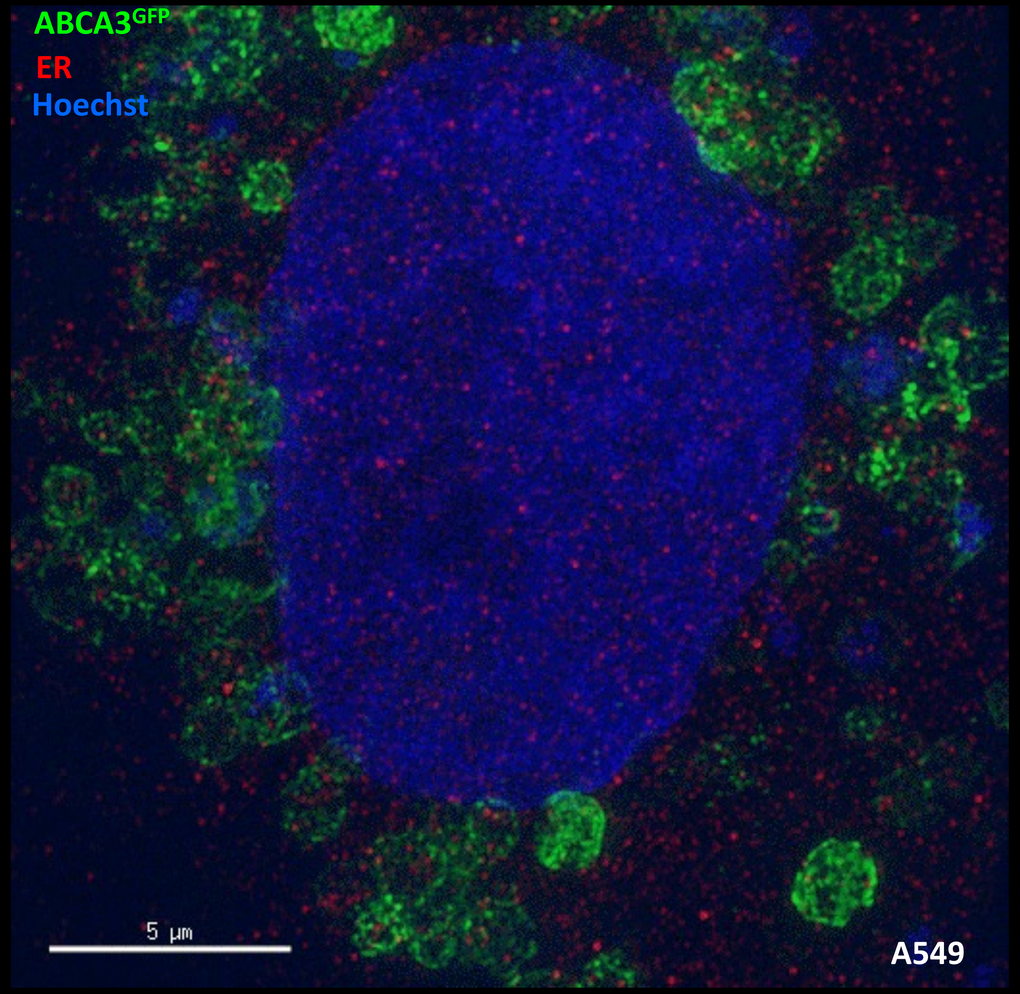

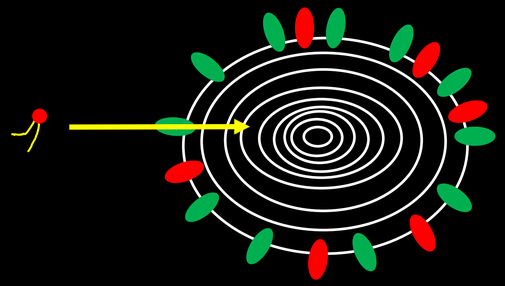

Phospholipid

ABCA3GFP

LAMP3

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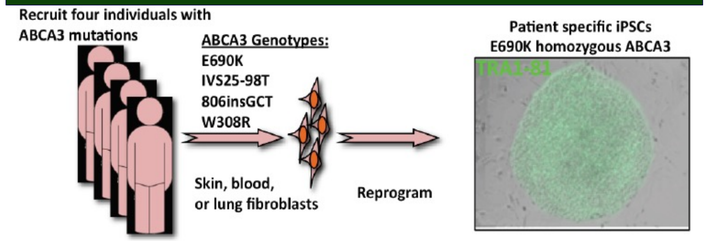

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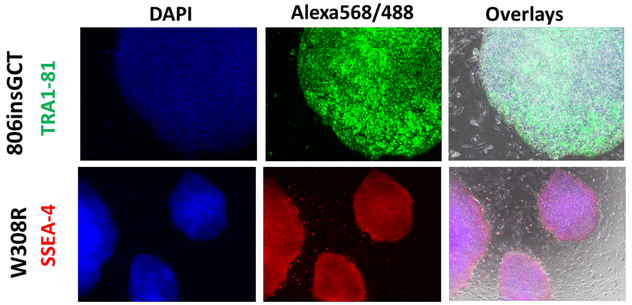

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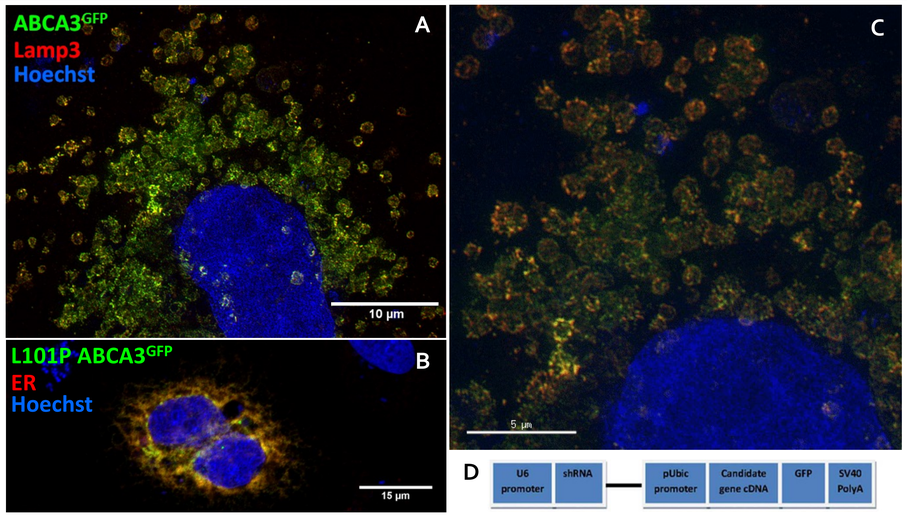

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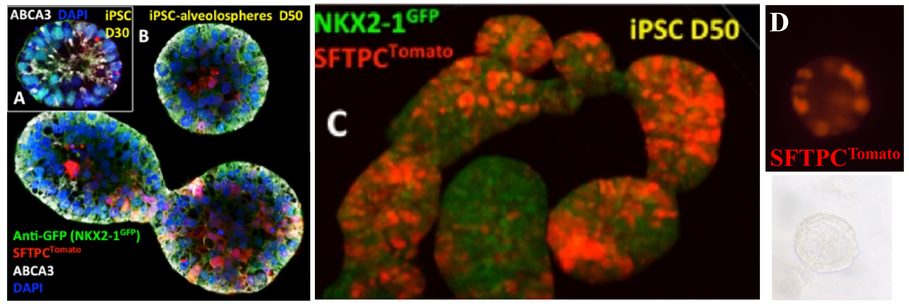

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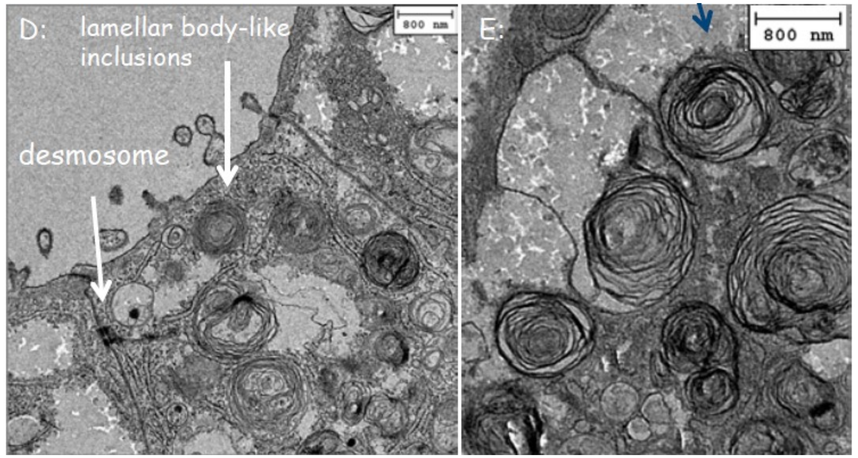

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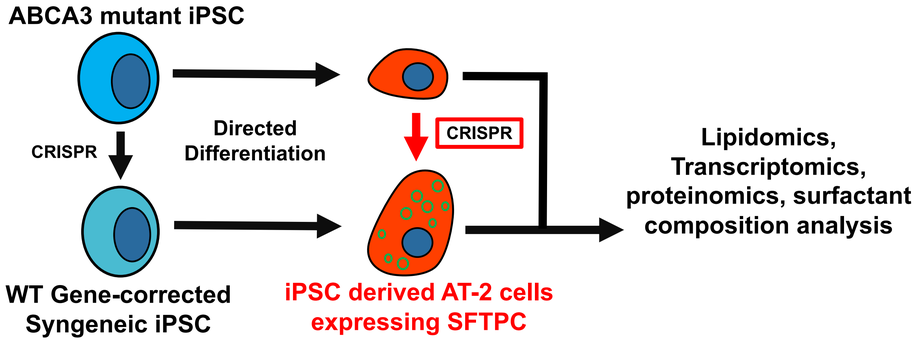

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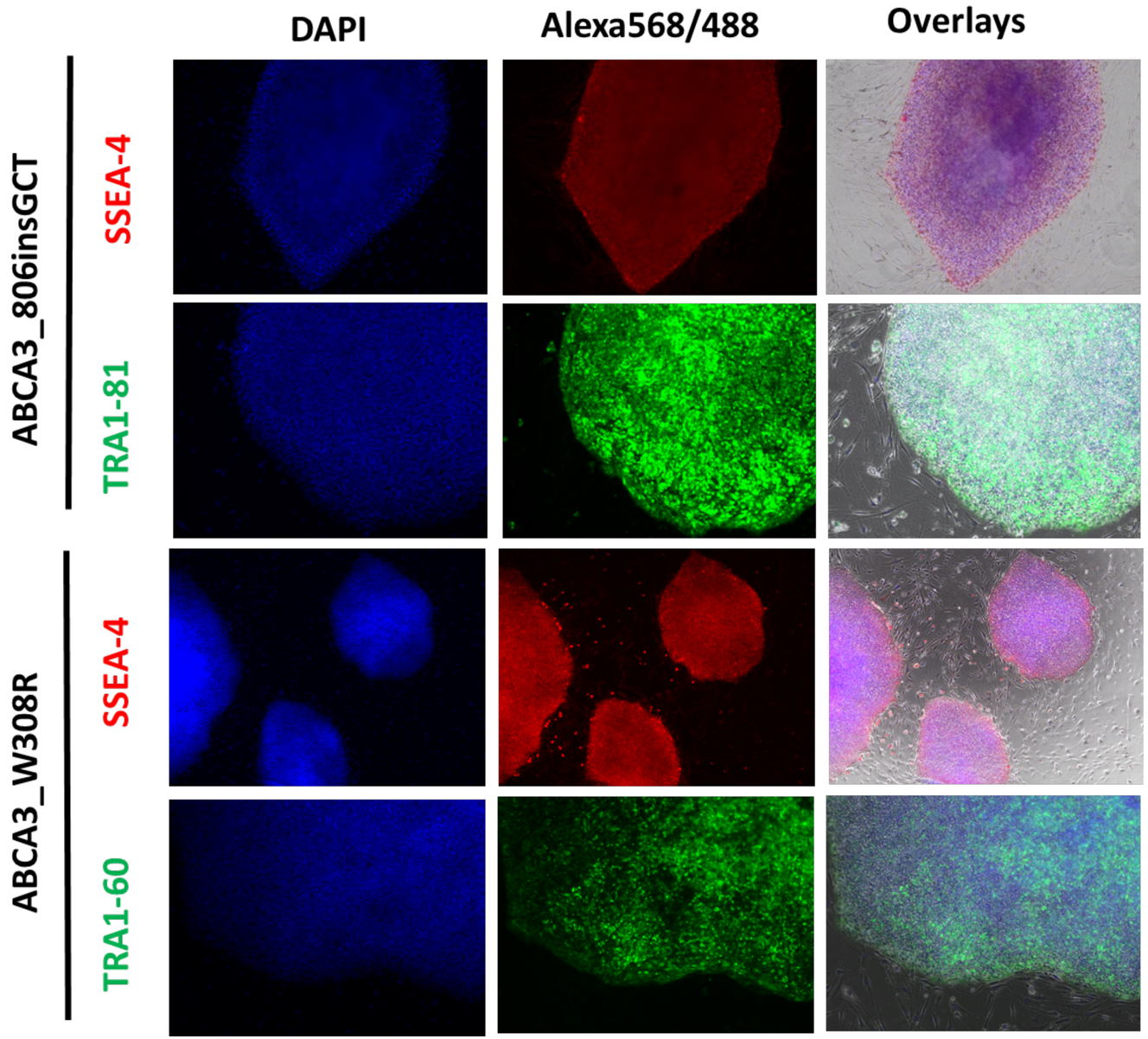
Goal: Generate iPSC-derived AECII model of ABCA3 diseased lines for pharmacological screening and CRISPR-based gene therapy



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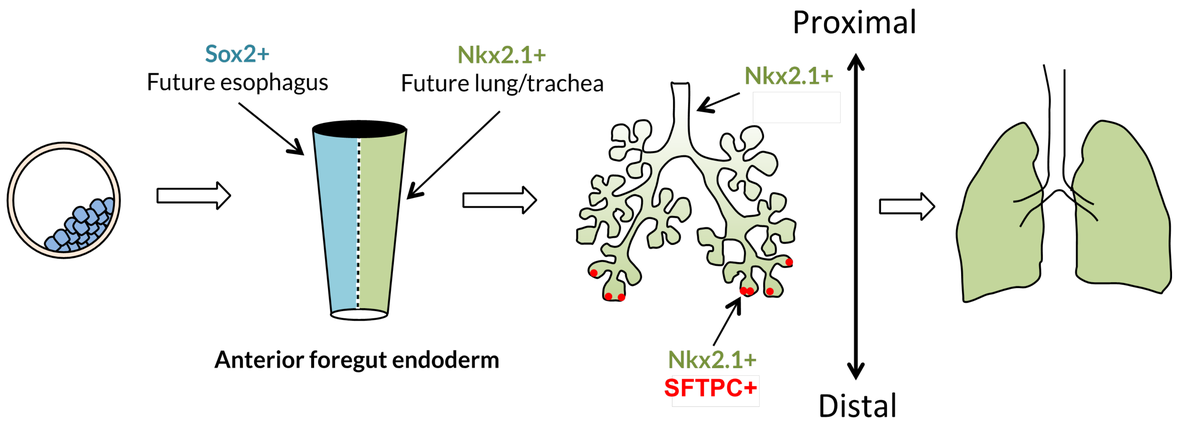

Fig.1 Schematic showing the stages of lung development from inner cell mass to ventral anterior endoderm with Nkx2.1 expression, a marker found in all developing lung epithelia. SFTPC is the first locus specifically activated during differentiation of distal lung epithelial progenitors.

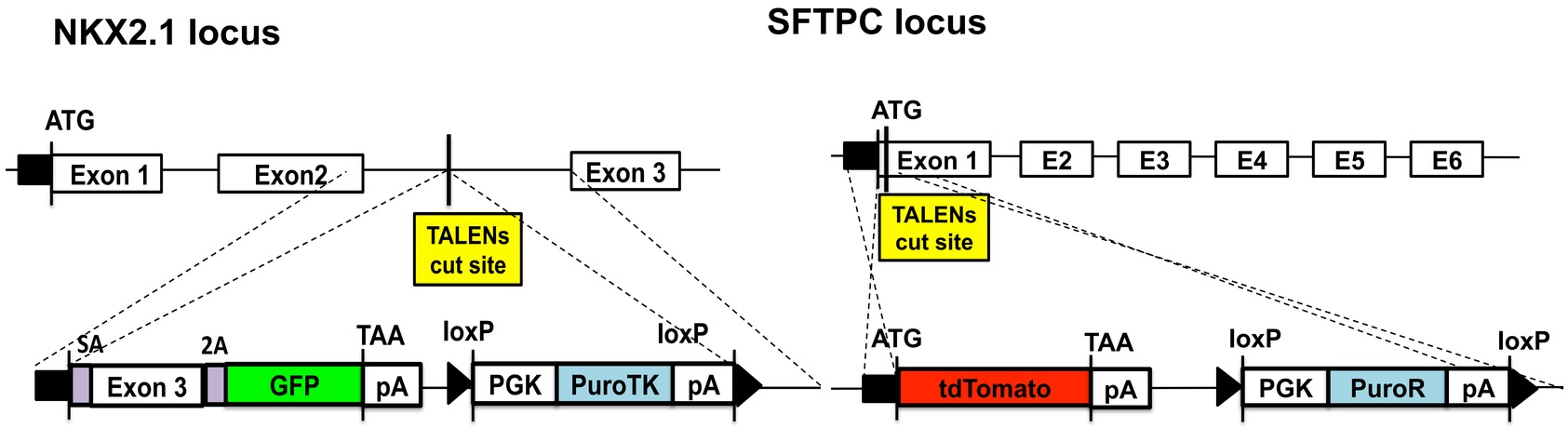

Fig.2 Using gene editing technology, we targeted a GFP fluorochrome reporter to the NKX2.1 locus and a Tomato fluorochrome reporter to the Surfactant protein C (SFTPC) gene, the first locus specifically activated during the differentiation of distal lung epithelial progenitors.

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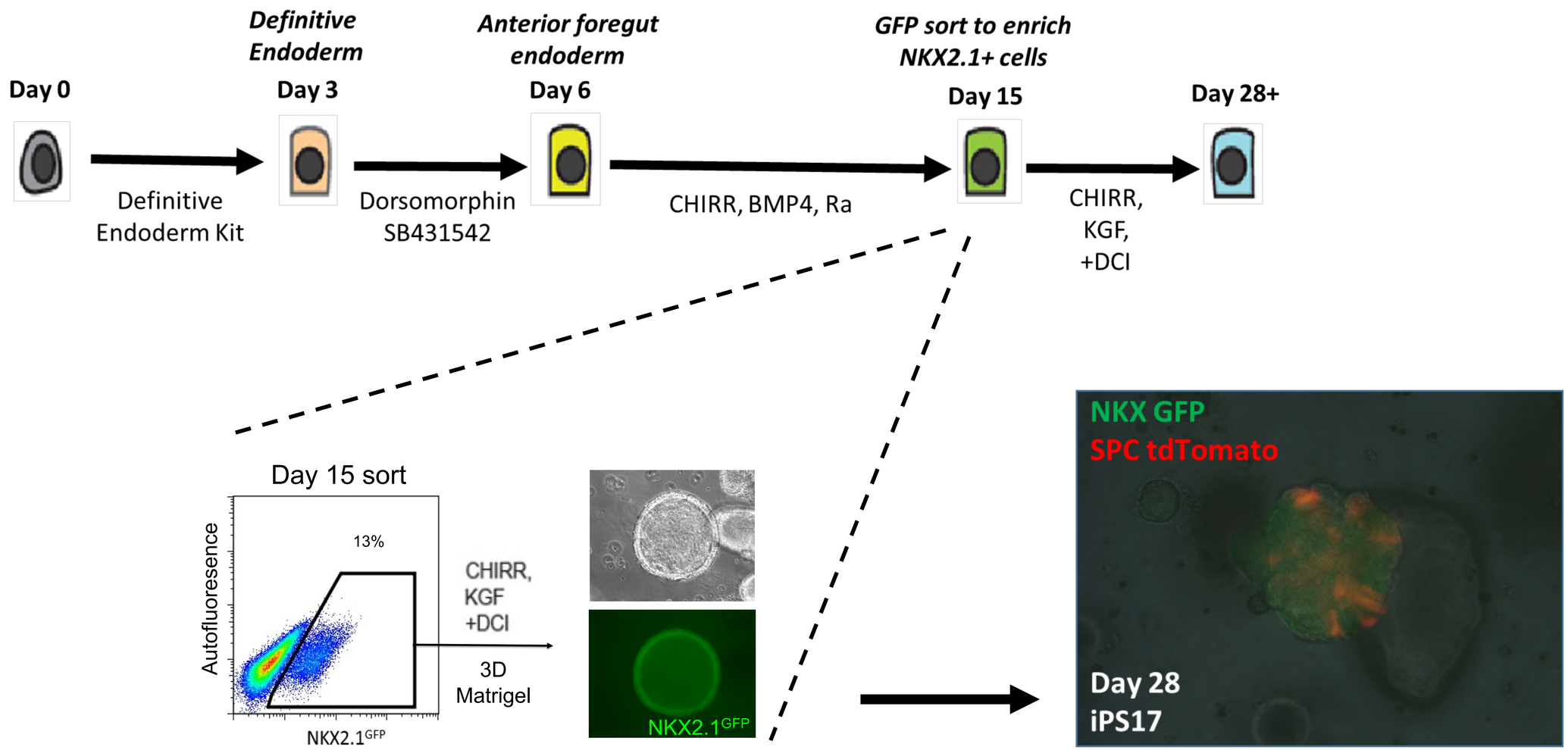

Fig.3 Using the reporters to identify, track, and purify iPSC-derived cells undergoing lung differentiation, we established a protocol which mimics the natural *in-vivo* stages of embryonic lung development.

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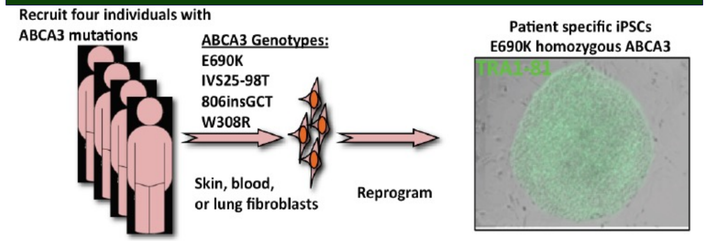

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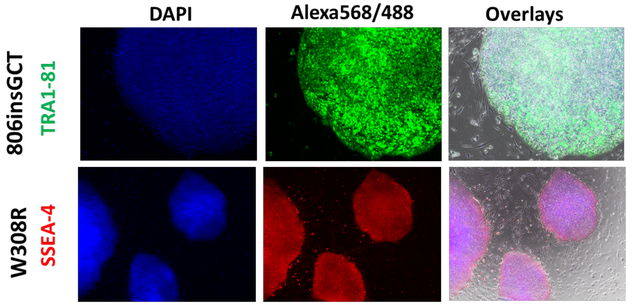

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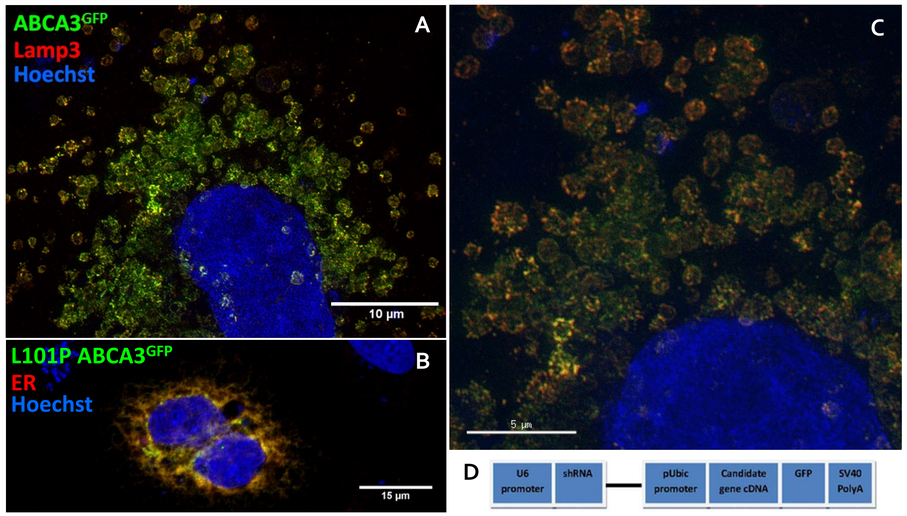

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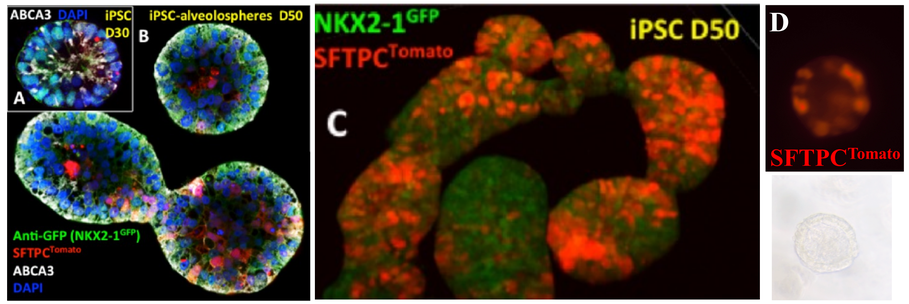


Fig.4 iPSC-derived "alveolospheres" with AT-2 like cells: A) iPS17 cell line sorted on day 16 for NKX2-1^{GFP} immunostained for ABCA3. B) Day 50 culture of the same cells showed co-expression of ABCA3 and NKX2-1^{GFP}. C) 3D confocal reconstruction of cells from B showed both NKX2-1 and SFTPC positive AT-2 like cells. D) AT2 proliferative potential demonstrated by sorting SFTPC$^{tomato+}$ /NKX2-1^{GFP+} cells from alveolospheres on day 36 shows clonal proliferation on day 45.

Characterization of iPSC-derived Lung Epithelial Progenitors

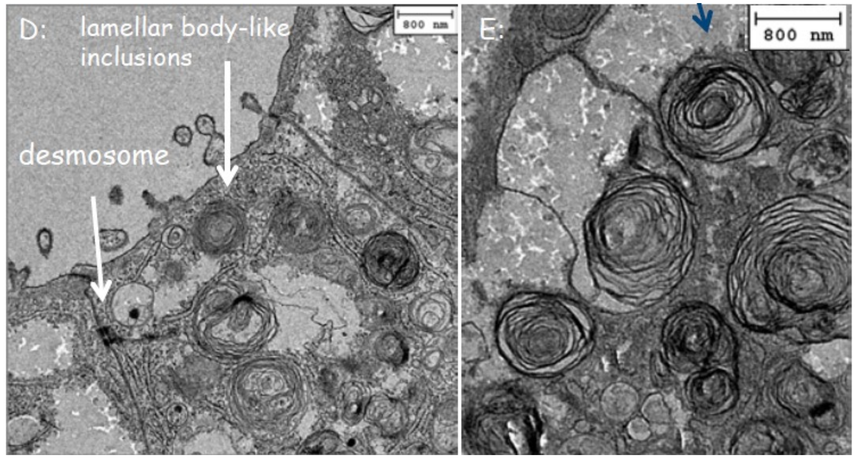
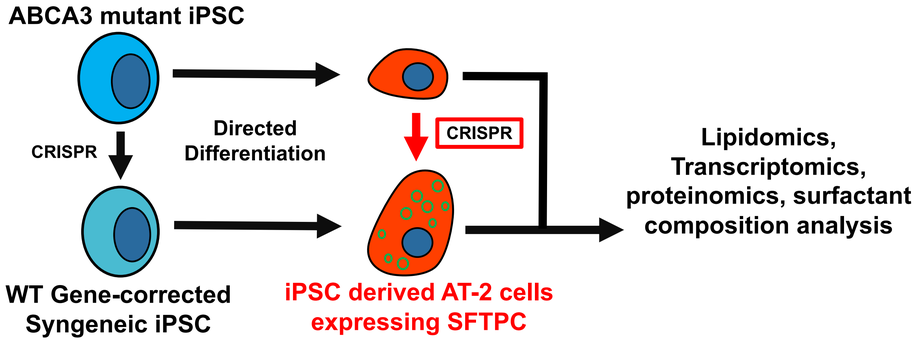


Fig.5 Ultrastructural analysis using TEM revealed lamellar body-like inclusions in iPSC-derived alveolar type 2 cells.

Path to Disease Modeling and Clinical Translation



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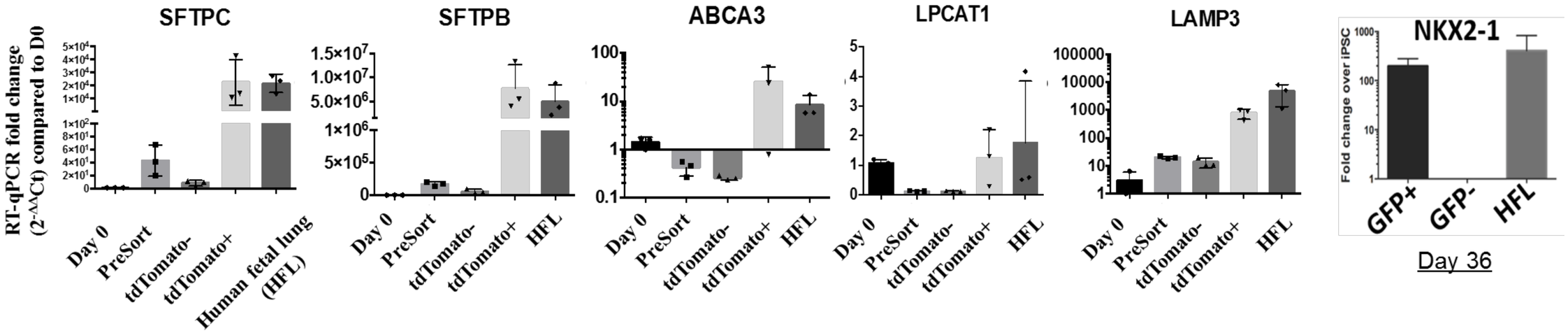

Gene expression to human fetal lung (HFL) control using RT-qPCR showed similar levels of SFTPC and transcripts encoding lamellar body associated proteins, including-- ABCA3, LAMP3, LPCAT, and SFTPB on Day 30. Similar level of NKX2-1 levels was also observed on Day 36 analysis.

Using chILD patient-derived induced pluripotent stem cells to model ABCA3 dysfunction in vitro

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BOSTON UNIVERSITY

CReM CENTER FOR REGENERATIVE MEDICINE

Introduction

Childhood interstitial lung disease (chILD) can be caused by autosomal recessive mutations in ATP binding cassette member A3 (ABCA3), a lamellar body associated lipid transporter expressed in alveolar epithelial type II cells (AEC2s). Dysfunction of ABCA3 is thought to cause AEC2 injury by disrupting surfactant biogenesis, resulting in lung remodeling. AEC2s are difficult to study in cell culture due to their propensity to transdifferentiate, and inability to adequately proliferate. Using patient-specific induced pluripotent stem cells (iPSCs) as an inexhaustible source of AEC2s, we sought to engineer an *in vitro* model of ABCA3 deficiency.

ABCA3 Disease Modeling Using Human Pluripotent Stem Cells

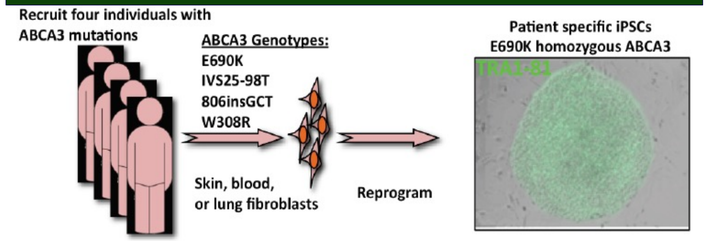

Fig.1 Schematic showing the work flow of generating 4 different homozygous ABCA3 mutant lines. Patient cell samples were taken either from blood PBMC or dermal fibroblasts. We have already reprogrammed a blood sample from a patient with lung disease from a homozygous E690K ABCA3 mutation.

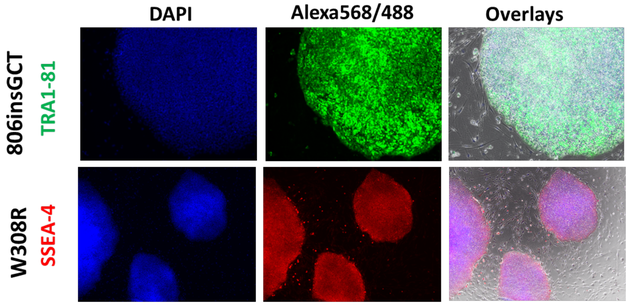

Fig.2 Immunofluorescent staining of nucleus (blue) pluripotent markers TRA1-81(green) and SSEA-4 (red) on two homozygous ABCA3 mutant lines reprogrammed from human dermal fibroblasts: 806insGCT and W308R.

Modeling ABCA3 disease in A549 Lung Carcinoma Cell Line

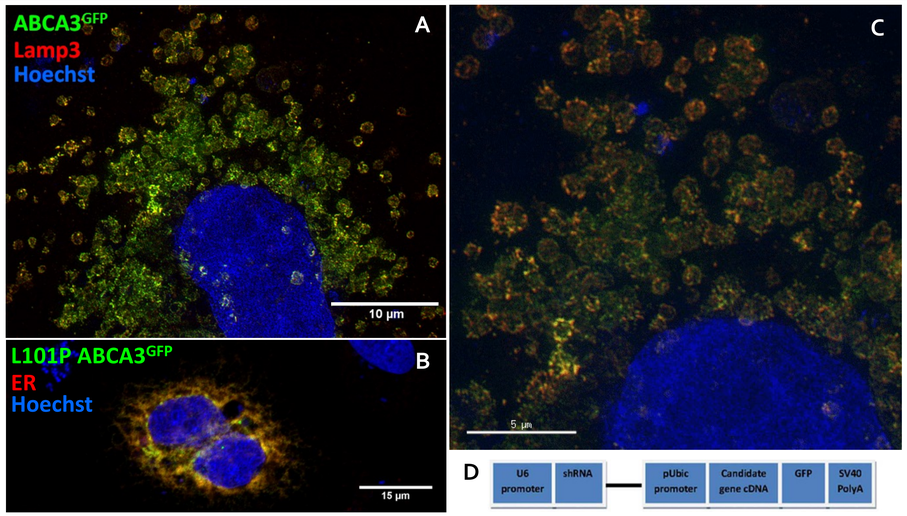

Fig.3 Immunofluorescence of A549 lung adenocarcinoma cell transiently expressing wildtype or L101P mutant ABCA3^{GFP} (green) was stained with Hoechst (nucleus-blue) and (A) lamellar body associated protein 3 (Lamp3-red) or (B) Calnexin (ER-red). (C) 3D reconstruction of (A) shows spherical patterning of lamellar bodies. (D) Construct used to allow transient expression of ABCA3^{GFP}.

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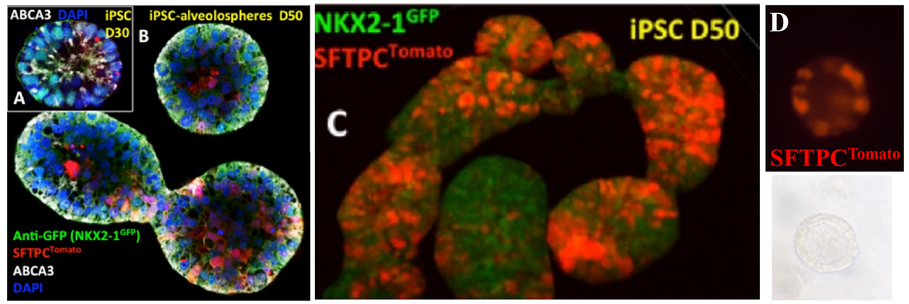

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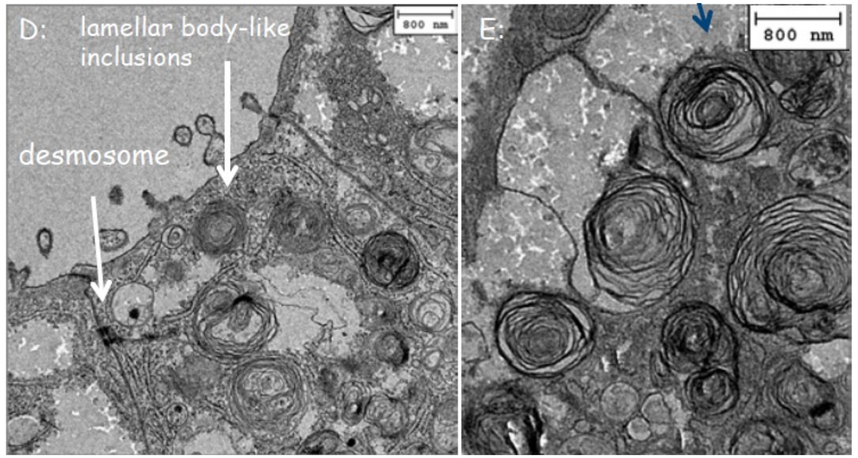

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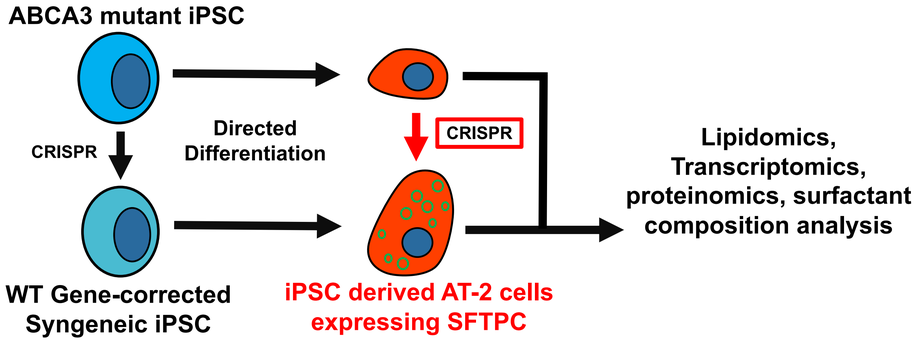

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Steve Borkan