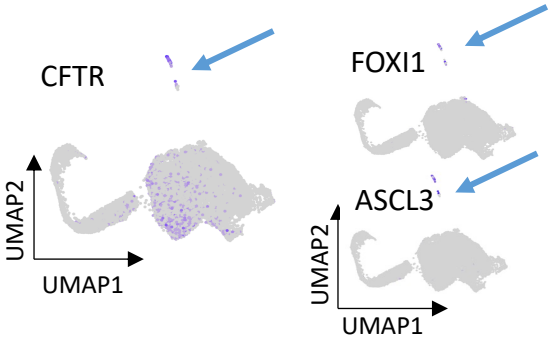
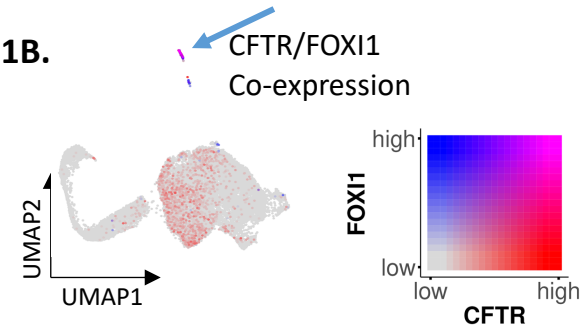
Supplemental Figure E1: scRNA-seq profiling of iPSC-ionocytes and CFTR

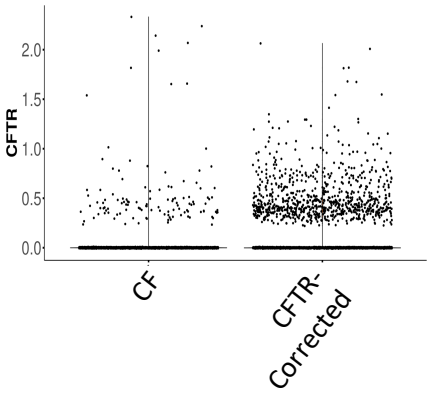
E1A.



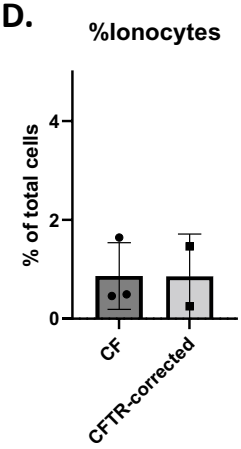
E1B.



E1C.



E1D.



E1E. Enrichr-based comparison of iPSC-ionocytes to available lineage datasets

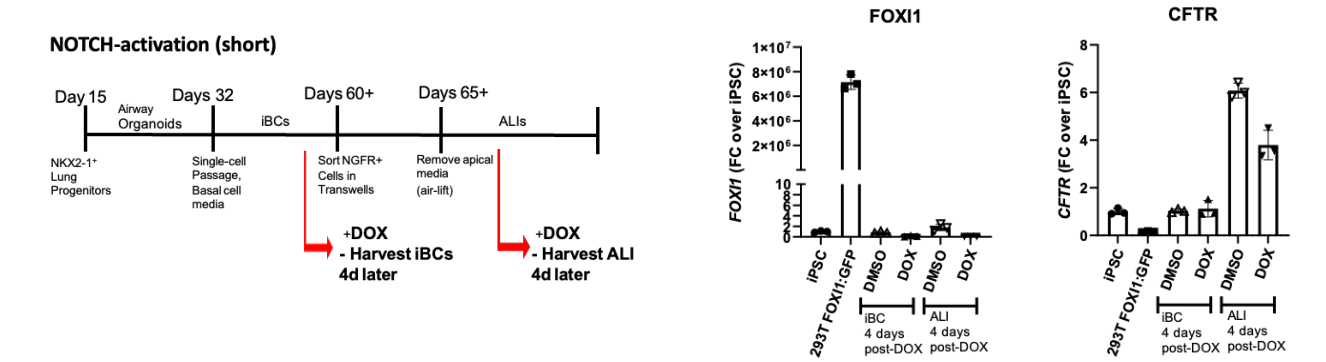
Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	Ionocyte cell:Lung	2.462e-92	1.236e-89	147.57	31126.92
2	Mesenchymal Stem cell:Synovium	0.0008709	0.02397	58.00	408.65
3	Germ cell:Ovary	0.001862	0.02397	36.90	231.95
4	Stem cell:Undefined	0.001862	0.02397	36.90	231.95
5	Lymphatic Endothelial cell:Lymph Node	0.02475	0.09414	50.24	185.84
6	Plasma cell:Peripheral Blood	0.02475	0.09414	50.24	185.84
7	Muscle-derived cell:Skeletal Muscle	0.02475	0.09414	50.24	185.84
8	Infiltrated Mononuclear cell:Kidney	0.02475	0.09414	50.24	185.84
9	Embryonic Stem cell:Embryonic Stem Cell	0.02475	0.09414	50.24	185.84
10	Limbal Epithelial Stem cell:Limbal Epithelium	0.02475	0.09414	50.24	185.84

LEGEND SUPPLEMENTAL FIGURE E1:

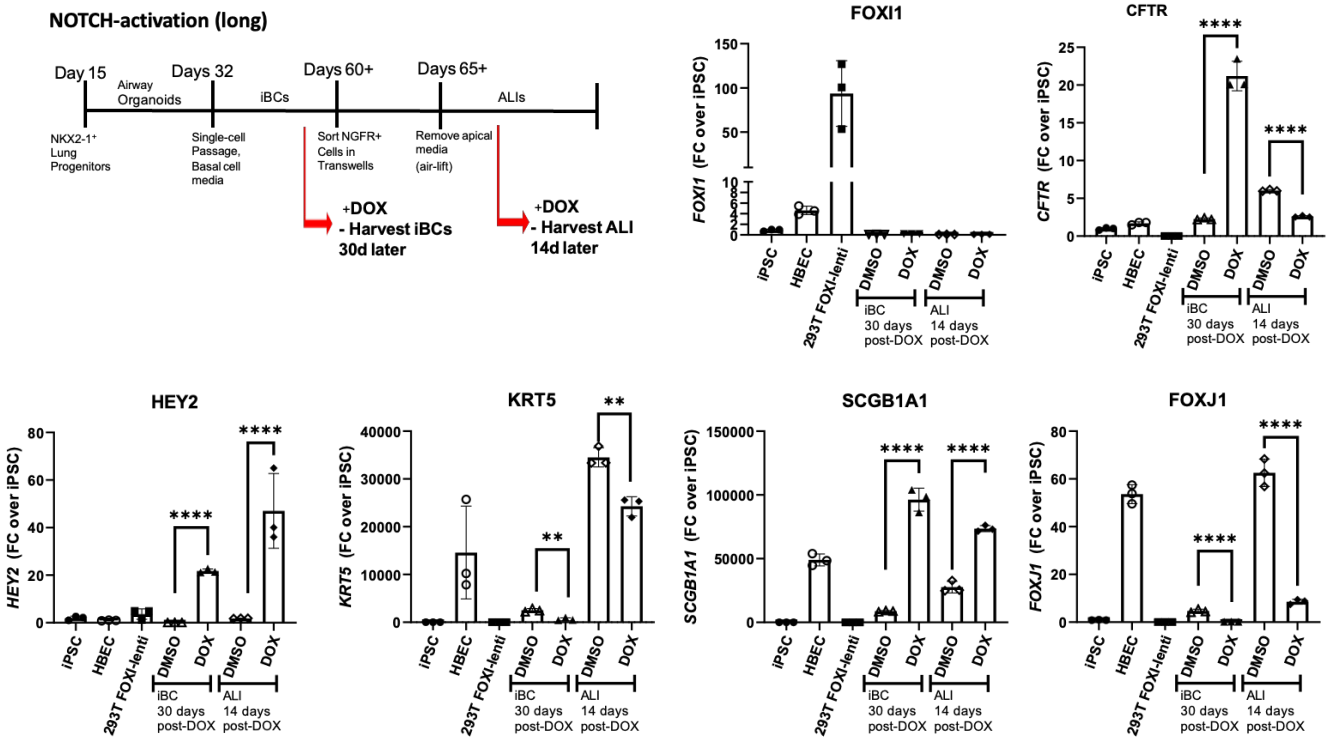
A) UMAP of iPSC-airway showing expression of CFTR, FOXI1, and ASCl3.
B) UMAP of iPSC-airway showing CFTR/FOXI1 co-expression.
C) scRNA-seq analysis of F508del homozygous CF iPSC-airway (line 1565) and syngeneic CFTR-corrected iPSC-airway (line 1564) showing CFTR expression.
D) Percentage of ionocytes generated from total iPSC-airway cells from scRNA-seq from CF iPSC-airway (lines 1565, 1567), and syngeneic iPSC-airway (lines 1564, 1566).
E) Gene set enrichment analysis of top 100 DEG of iPSC-airways (Enrichr), using gene-set library comparisons for Cell-type (CellMarker Augmented 2021).

Supplemental Figure E2: Assessment of the role of NOTCH in regulating ionocyte specification from human basal cell precursors

E2A.



E2B.



SUPPLEMENTAL FIGURE E2:

A) Schematic and RT-qPCR of FOXI1, and CFTR after short (4 days) of NOTCH-activation at iPSC-basal cell organoid stage and iPSC-airway cultured at ALI (iPSC-line Dox-NICD[1])

B) Schematic and RT-qPCR of FOXI1, CFTR, HEY2, KRT5, SCGB1A1 and FOXJ1 after long (13-30 days) of NOTCH-activation at iPSC-basal cell organoid stage and iPSC-airway cultured at ALI (iPSC-line Dox-NICD[1])

1.Heinze, D.; Park, S.; McCracken, A.; Haratianfar, M.; Lindstrom, J.; Villacorta-Martin, C.; Mithal, A.; Wang, F.; Yang, M. W.; Murphy, G.; Mostoslavsky, G., Notch activation during early mesoderm induction modulates emergence of the T/NK cell lineage from human iPSCs. *Stem Cell Reports* **2022**.