

Supplementary Table S1: Optimization strategies

Media types	
PneumaCult ALI	StemCell 05001
B/D complete w/o hydrocortisone	ScienCell custom order
PneumaCult ALI w/o hydrocortisone	StemCell 05001
Vertex ALI medium	Neuberger et al, 2011 ¹
Airway organoid medium	Sachs et al, 2019 ²
Cell culture compounds	
Retinoic acid	15; 150; 1500 ng/ml; Sigma R2625
Dexamethasone	0.1; 1; 10 μ M; Sigma D4902
Inhibitors	
<i>AG-1478</i> Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor	30; 150; 300 nM; Sigma T4182
<i>PD098059</i> Mitogen-Activated Protein Kinase inhibitor	10 μ M; Sigma P215
<i>Y-27632</i> Rho-associated, coiled-coil containing protein kinase (ROCK) inhibitor	5 μ M; Cayman Chemical 10005583
<i>GM6001</i> Matrix metalloprotease (MMP) inhibitor	0.2; 0.5; 25 nM; Sigma CC1000
<i>SB202190 + A83-01</i> P38 MAPK inhibitor + TGF β kinase/activin receptor-like kinase (ALK 5) inhibitor	500 nM; 500 nM; Sigma S7067; Tocris 2939
<i>SB202190 + A83-01 + noggin</i> P38 MAPK inhibitor + TGF β ALK inhibitor + Bone Morphogenetic Protein (BMP) Inhibitor	500 nM; 500 nM; 100 ng/ml Sigma S7067; Tocris 2939; Peprotech 120-10c

References:

1. Neuberger, T., Burton, B., Clark, H. & Van Goor, F. Use of primary cultures of human bronchial epithelial cells isolated from cystic fibrosis patients for the pre-clinical testing of CFTR modulators. *Methods Mol Biol* **741**, 39–54 (2011).
2. Sachs, N. *et al.* Long-term expanding human airway organoids for disease modeling. *EMBO J* **38**, (2019).