



SUPPLEMENTARY FIG. S3. Matured lung epithelium can be cultured in 3D and retains a lung epithelial phenotype. **(A)** NKX2.1/FOXP2 cells form branching clusters of cells when cultured in 3D. **(B)** Immunocytochemistry of branching cells lung endoderm expressing E-cadherin, FOXP2, GATA6, and NKX2.1. **(C)** Distal airway cells cultured in 3D form cystic structure. **(D)** Immunocytochemistry showing 3D cysts continue to express distal airway markers Pro-SFTPC and MUC1. **(E)** QPCR analysis showing that 3D cysts continue to express distal airway markers (NKX2.1, GATA6) as well as genes of AECTI (AQP5, P2X7) and AECTII (ABCA3, SFTPC). White bars = 100 μ M. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$. 3D, three-dimensional; FL, human fetal lung control; AL, adult lung control; C, undifferentiated hESC control; 3D, airway epithelium cultured for 25 days in 3D Matrigel.