

## Supplementary Material

### Human organotypic airway and lung organoid cells of bronchiolar and alveolar differentiation are permissive to infection by influenza and SARS-CoV-2 respiratory virus

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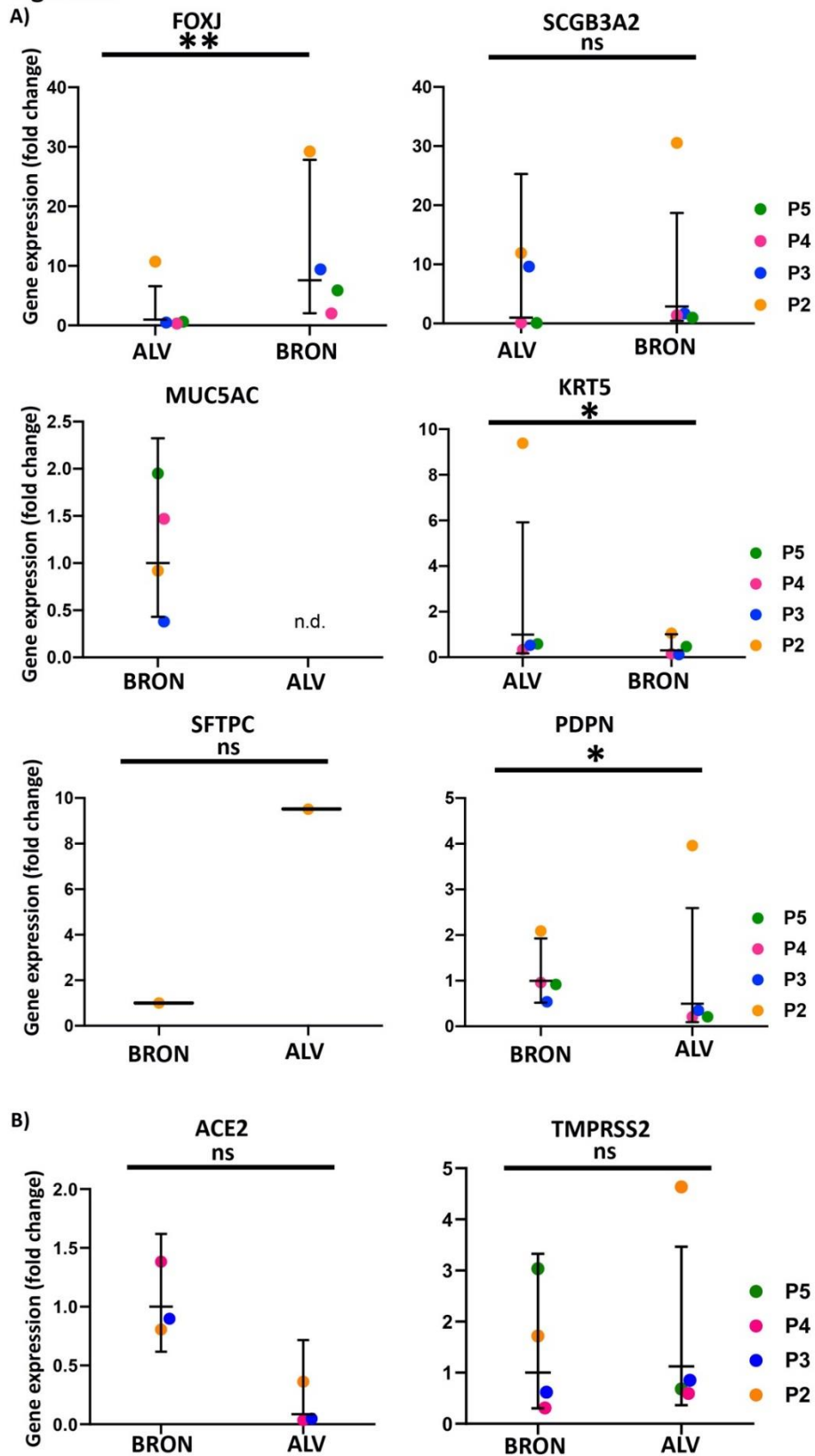
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RESULTS

Figure S1



## FIGURE S1

**A)** Relative expression of the cell-type markers, FOXJ1 (ciliated cell), SCGB3A2 (club cell), MUC5AC (goblet cell), and KRT5 (basal cell), SFTPC (AT2 cell), and PDPN (AT1 cell). Relative quantification was performed using the Livac ( $2^{-\Delta\Delta C_t}$ ) method. Bronchiolar (BRON) and alveolar (ALV) are normalized against the average delta Ct of BRON organoid for the markers, PDPN, SFTPC and MUC5AC. BRON and ALV organoids are normalized against the average delta Ct of alveolar organoids for the markers, FOXJ, SCGB3A2 and KRT5. BRON and ALV organoids have been passaged 4 times (P2-P5) in the respective differentiation media. The mean value of three technical replicates for each passage are shown here as individual points. Mean represents the  $2^{(\text{mean}(-\Delta\Delta C_t))}$  of the biological replicates harvested at different passages. GraphPadPrism v9 was used to calculate and display the geometric mean and 90% confidence interval of the biological replicates (passages). Statistics were performed on the delta Ct values using the Mann-Whitney test. ns = not significant. A significant difference in gene expression were detected for FOXJ (\*\* $P = 0,0034$ ), KRT5 ( $*P = 0,0387$ ) and PDPN ( $*P = 0,0387$ ) between ALV and BRON organoids. The gene expression profile shown here are from patient L1.

**B)** ACE2 (left) and TMPRSS2 (right) expression is detected in patient L1 organoids of bronchiolar (BRON) and alveolar (ALV) differentiation from passage 2-5. Relative quantification was performed using the Livac method and alveolar organoids are normalized to the average delta Ct of bronchiolar organoids. The mean value of three technical replicates for each passage are shown as individual data points. GraphPadPrism v9 was used to calculate and display the geometric mean and 90% confidence interval of the biological replicates (passages). Statistics were performed on the delta Ct values using the Mann-Whitney test. No statistically significant differences were observed in the median delta Ct values of ACE2 and TMPRSS2 between BRON and ALV cultures (ns = not significant).

### VIDEO S1:

IncuCyte timelapse video showing the organoid formation in alveolar (ALV) differentiation media. Weblink: <http://vedlegg.uib.no/?id=bc53ca0f221f160089306c24c67cf6fd>

### VIDEO S2:

IncuCyte timelapse video showing the organoid formation in bronchiolar (BRON) differentiation media. Weblink: <http://vedlegg.uib.no/?id=16670b783bb9633fd600b4c1ec23fba8> (BRON)

### VIDEO S3:

Video show beating cilia in organoids of bronchiolar (BRON) differentiation medium. Weblink: <http://vedlegg.uib.no/?id=dee2d0b60466c9a50761d96ee067e72b>