

Supplementary Materials for

# **A High-Throughput, High-Containment Human Primary Epithelial Airway Organ-on-Chip Platform for SARS-CoV-2 Therapeutic Screening**

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## **This file includes:**

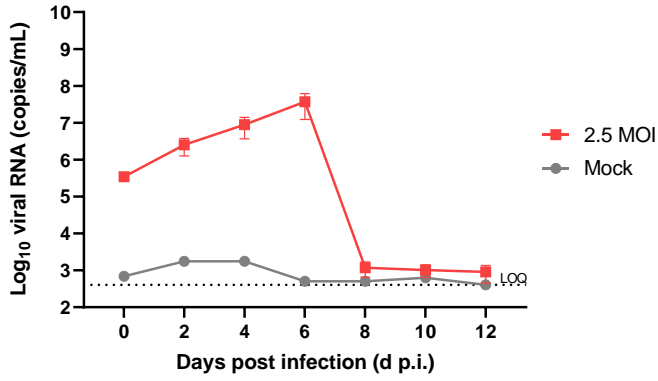
Table S1

Figures S1 to S4

Primers, Probes and TaqMan® Assays	Vendor, Catalogue/Assay Number
nCOV_N1 Forward Primer	IDT, 10006830
nCOV_N1 Reverse Primer	IDT, 10006831
nCOV_N1 Probe	IDT, 10006832
nCOV_N2 Forward Primer	IDT, 10006833
nCOV_N2 Reverse Primer	IDT, 10006834
nCOV_N2 Probe	IDT, 10006835
RNase P Forward Primer	IDT, 10006836
RNase P Reverse Primer	IDT, 10006837
RNase P Probe	IDT, 10006838
ACE2 TaqMan Gene Expression Assay	Thermo Scientific, Hs01085333_m1
TMPRSS2 TaqMan Gene Expression Assay	Thermo Scientific, Hs01122322_m1
SCGB1A1 TaqMan Gene Expression Assay	Thermo Scientific, Hs00171092_m1
Muc5AC TaqMan Gene Expression Assay	Thermo Scientific, Hs01365616_m1
FoxJ1 TaqMan Gene Expression Assay	Thermo Scientific, Hs00230964_m1
TP63 TaqMan Gene Expression Assay	Thermo Scientific, Hs00978340_m1

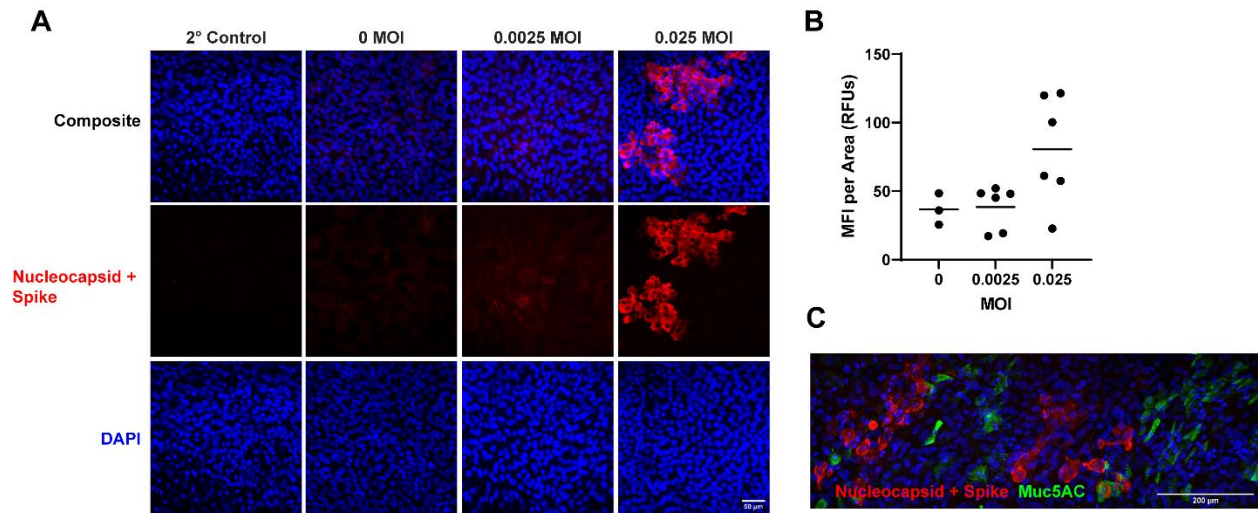
**Table S1.**

Primers, probes and TaqMan® Assays used in RT-qPCR assays.



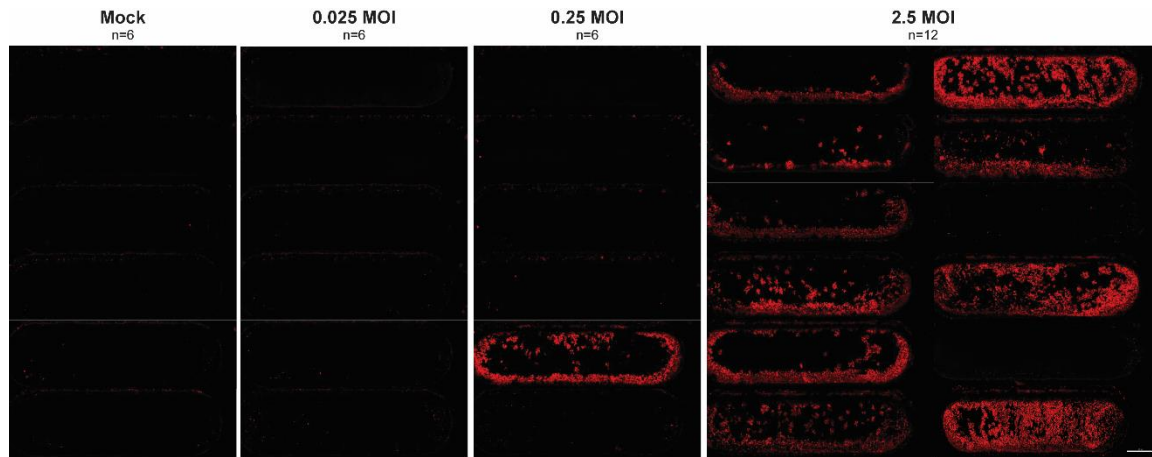
**Figure S1.**

Type RT-qPCR analyses for SARS-CoV-2 nucleoprotein RNA copies in the apical wash of PREDICT96-ALI airway tissue from donor 04401 (N = 6 tissue replicates for mock, N = 6 for 2.5 MOI) at 0, 2, 4, 6, 8, 10, and 12 days post infection (d p.i.). Infection peaked at 6 d p.i. Limit of quantification (LOQ, dotted line): Log<sub>10</sub> viral RNA (copies/ml) corresponding to cycle threshold values  $\geq 37$ .



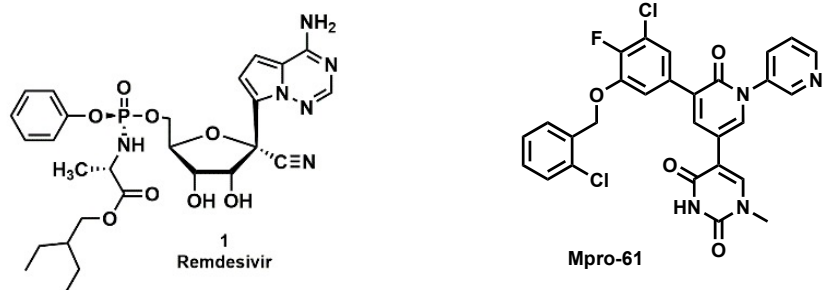
**Figure S2.**

Immunofluorescence staining of SARS-CoV-2-inoculated PREDICT96-ALI airway tissues from donor DH01. (A) Max projection immunofluorescence images depicting positive staining of the SARS-CoV-2 spike and nucleocapsid proteins (S and N, respectively, red) and dsDNA nucleic acids (Hoechst 33342, blue) within PREDICT96-ALI airway tissue from donor DH01. Tissues were fixed with 4% paraformaldehyde at 6 d p.i. following inoculation with SARS-CoV-2 USA-WA1/2020 (MOI 0, 0.0025 or 0.025). Secondary antibody control showed low background noise. Images were taken at 40x magnification and are shown with a 50 µm scale bar. One representative region of interest (ROI) from N = 1 tissue device shown per MOI. (B) Mean Fluorescent Intensity (MFI) image quantification of SARS-CoV-2 infection in PREDICT96-ALI airway tissues associated with panel A. MFI from the red channel (S and N proteins) indicate a trend of increased signal in the 0.025 MOI condition, but is not significant compared to the 0 or 0.0025 MOI conditions. The average MFI of the 0 (N = 3 tissue replicates), 0.0025 (N = 6 tissue replicates), and 0.025 MOI (N = 6 tissue replicates) are  $36.67 \pm 11.45$ ,  $38.38 \pm 15.75$ ,  $80.52 \pm 39.69$  RFUs (mean  $\pm$  standard deviation), respectively. Images were taken at 10x magnification and analyzed for MFI using ImageJ Fiji. (C) Representative max projection immunofluorescence image of SARS-CoV-2 positivity (S and N proteins, red), goblet cells (Muc5AC, green) and dsDNA nucleic acids (Hoechst 33342, blue) within PREDICT96-ALI airway tissue from donor DH01 at 6 d.p.i. following inoculation with SARS-CoV-2 at 0.025 MOI. Image was taken at 40x magnification and is shown with a 200 µm scale bar.



**Figure S3.**

Tile scan images depicting SARS-CoV-2 positivity (Nucleocapsid, red) in 30 devices of PREDICT96-ALI airway tissues from donor 04401 at 6 d p.i. following inoculation with SARS-CoV-2 at 0, 0.025, 0.25, and 2.5 MOI. Images were used for MFI quantification (see figure 3B). Images were taken at 10x magnification and are shown with a 500  $\mu\text{m}$  scale bar.



**Figure S4.**

Antivirals evaluated in SARS-CoV-2-inoculated PREDICT96-ALI tissues. Remdesivir, a SARS-CoV-2 RNA-dependent RNA polymerase inhibitor (left) and Mpro-61, a SARS-CoV-2 protease inhibitor (right).