Expanded View Figures

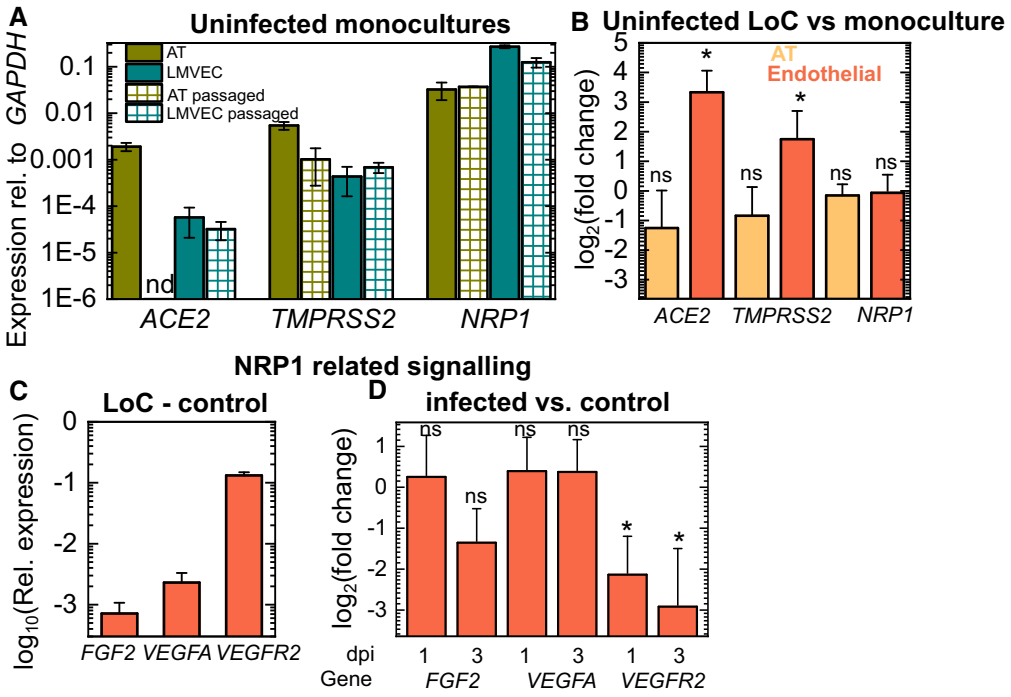

Figure EV1. Characterization of ACE2 and NRP1 expression on-chip versus monocultures and alterations in NRP1-related signalling upon infection.

A Plots of expression of the cell receptors *ACE2* and *NRP1*, and the protease *TMPRSS2* relative to *GAPDH* expression for alveolar epithelial cells (ATs) obtained from a commercial supplier at passage 3, alveolar epithelial cells postpassage in the lab ("AT passaged"), freshly isolated lung microvascular endothelial cells from a commercial supplier ("LMVEC") and lung microvascular endothelial cells postpassage ("LMVEC passaged"). "nd" refers to not detected. Data obtained from $n = 2$ technical replicates from $n = 2$ biological replicates.

B Plot of the fold change in expression of viral entry factors in cells from the epithelial and endothelial layers of uninfected control LoC ($n = 3$ biological replicates) versus monocultures ($n = 2$ biological replicates for ATs and $n = 3$ biological replicates for endothelial cells).

C Plot of the expression of basic fibroblast growth factor (*FGF2*), vascular endothelial growth factor A (*VEGFA*) and vascular endothelial growth factor receptor 2 (*VEGFR2*) relative to *GAPDH* from the endothelial layer of uninfected control LoCs ($n = 3$ biological replicates).

D Plot of the fold change in expression of *FGF2*, *VEGFA* and *VEGFR2* in the endothelial layer of infected LoCs at 1 dpi ($n = 3$ biological replicates) and infected LoCs ($n = 3$ biological replicates) at 3 dpi versus uninfected controls ($n = 3$ biological replicates).

Data information: In all plots, the bars represent the mean values, and the error bars represent the standard deviation. *P*-values are calculated using a one-way Kruskal–Wallis ANOVA test, * represents $P \leq 0.05$, ** represents $P \leq 0.01$, and ** represents $P \leq 0.001$.

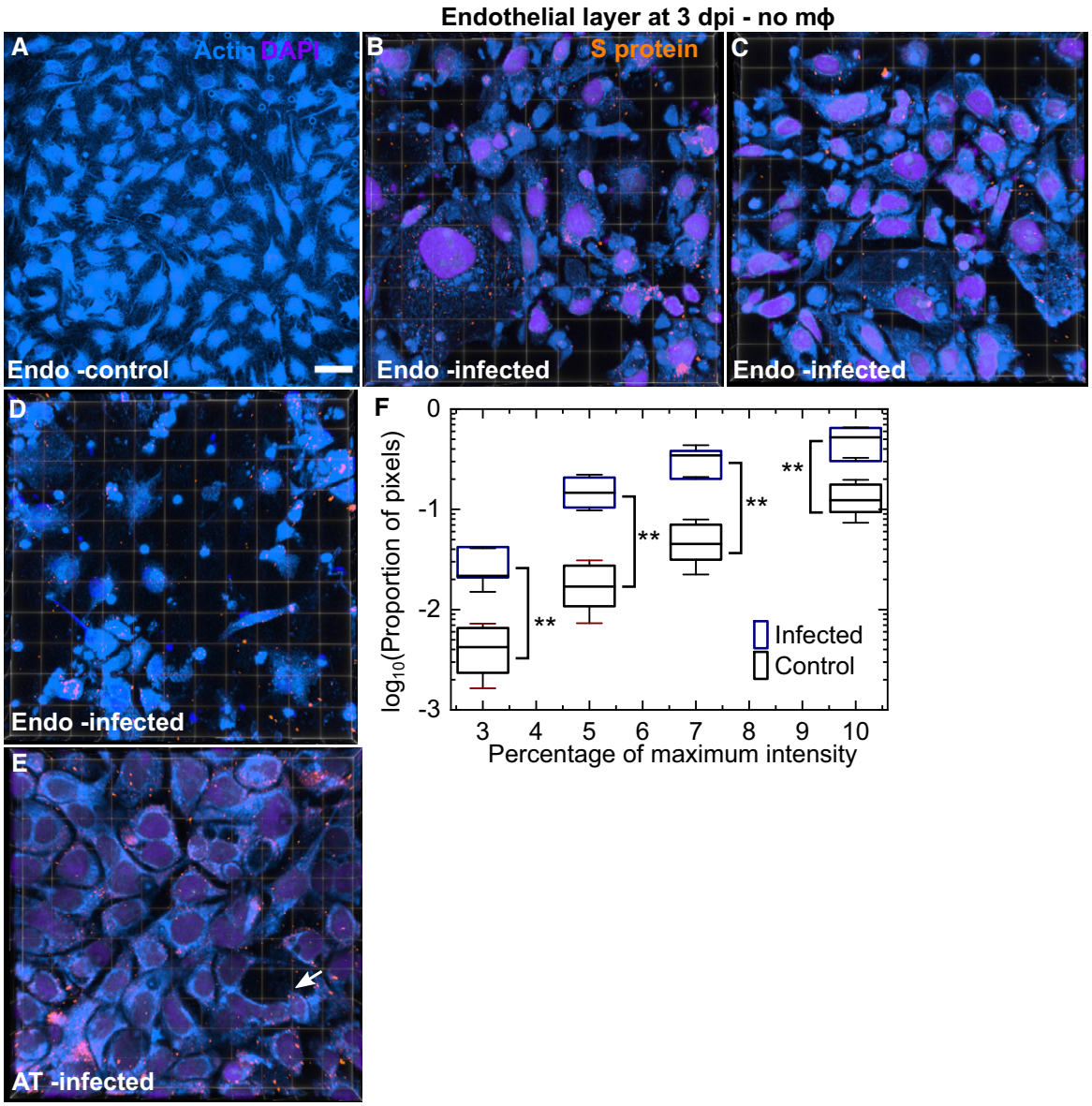

Figure EV2. Further characterization of the disruption of endothelial cell layer integrity.

A A maximum-intensity projection of a 465 × 465 μm^2 field of view of the endothelial layer in an uninfected chip at 3 days post-air–liquid interface shows intact vasculature. Scale bar = 50 μm.

B–D Additional examples of endothelial damage—3D views of 232 × 232 μm^2 fields of view from LoCs infected without macrophages at 3 dpi. S protein identified via antibody labelling, actin and nuclear labelling is shown in amber, azure and electric indigo LUTs, respectively. Many areas with little or no actin staining are observed.

E An additional example of a 3D view of a 155 × 155 μm^2 field of view of the epithelial layer from the same LoC shown in (B–D). The integrity of the layer is better maintained, and cell loss is indicated by a white arrow.

F A plot of the proportion of pixels (shown on a logarithmic scale) with regions of low F-actin intensity identified via cut-off thresholds defined as a percentage of the maximum intensity in 4–5 fields of view (technical replicates) each for one control and infected LoC. The solid bar represents the median, the boxes represent the 25–75 percentile range, and the whiskers represent the standard deviation. *P*-values are calculated using a one-way Kruskal–Wallis ANOVA test, * represents $P \leq 0.05$, ** represents $P \leq 0.01$, and ** represents $P \leq 0.001$.

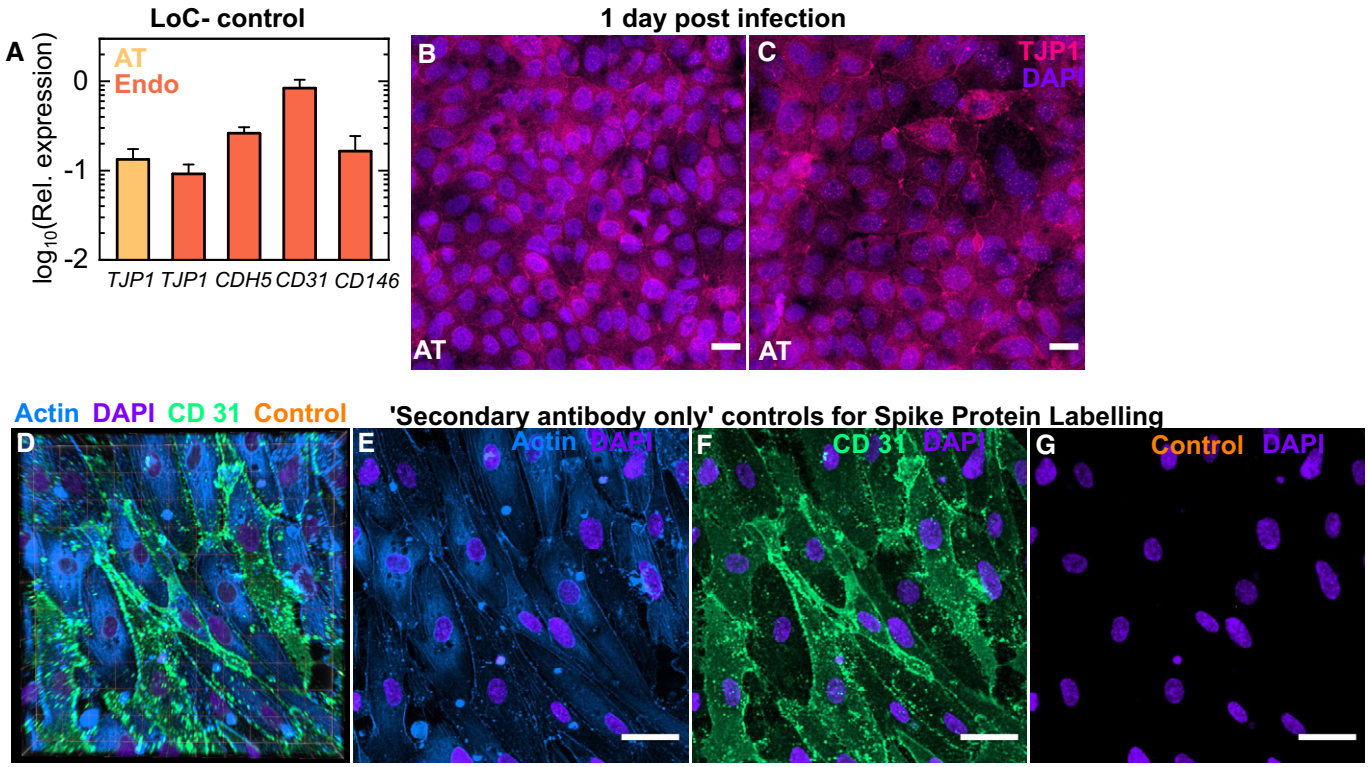

Figure EV3. Characterization of the endothelial cell monolayer on-chip.

A Plot of the expression of the tight junction markers PECAM-1 (*CD31*), VE-Cadherin (*CDH5*) and melanoma cell adhesion molecule MCAM (*CD 146*) in cells from the endothelial layer and of ZO-1 (*TJP1*) in cells from both the endothelial and epithelial layers of uninfected controls (n = 3 biological replicates) relative to *GAPDH* expression. The bars represent the mean, and the error bars represent the standard deviation.

B, C Maximum-intensity projection of two additional fields of view from the epithelial layer of an infected LoC reconstituted without macrophages at 1 dpi. TJP1 identified via antibody labelling and nuclear labelling is shown in bright pink and electric indigo LUTs, respectively.

D–G Secondary antibody only controls for spike protein labelling. (D) 3D view of a 116.36 × 116.36 μm^2 field of view of the endothelial layer of an uninfected control LoC. Actin, CD31, secondary antibody only ("control") and nuclear labelling are indicated in azure, spring green, amber and electric indigo LUTs, respectively. (E–G) Maximum-intensity projections of actin (E), CD31 (F) and control staining (G) for the same field of view. Nuclear labelling is shown in all panels.

Data information: Scale bar = 20 μm.

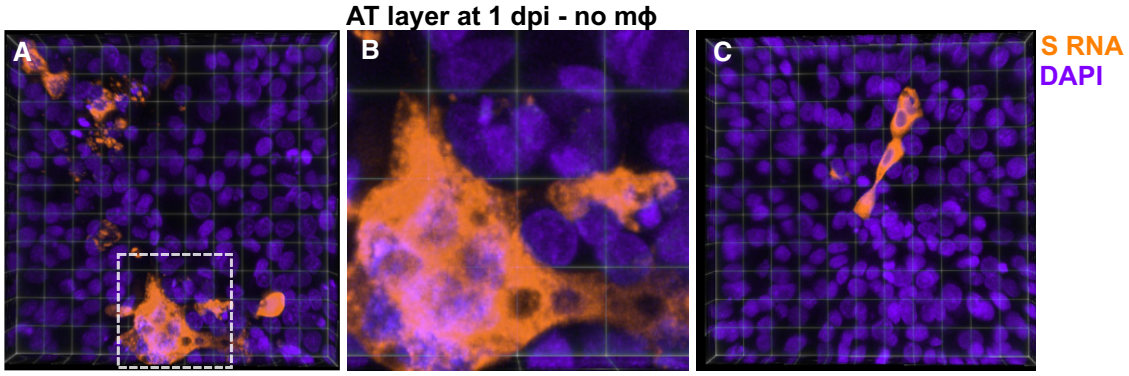

Figure EV4. RNAscope images of highly infected AT cells at 1 dpi.

A–C 3D views of two representative 232 × 232 μm^2 fields of view of the epithelial layer of an LoC reconstituted without macrophages at 1 dpi. *S* RNA is identified by RNAscope assay and false-coloured in amber, and nuclear labelling is false-coloured in indigo. Each field of view shows examples of heavily infected cells. (B) Zoom corresponding to the area marked by the white box in (A), a collection of heavily infected cells is visible together with syncytia formation.

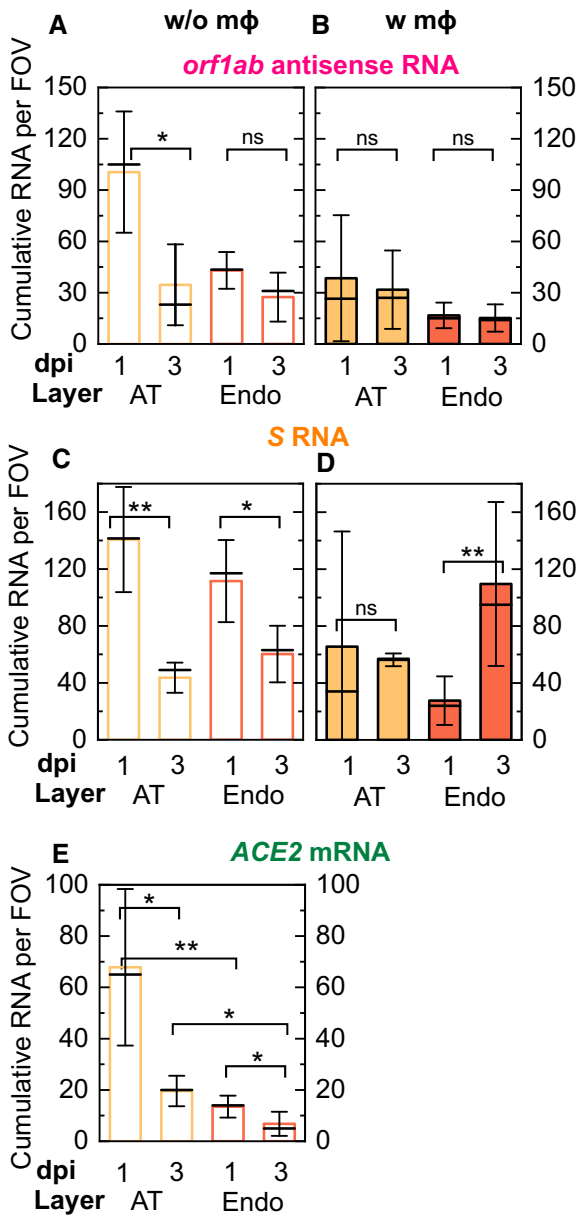

Figure EV5. Quantification of levels of viral antisense RNA, genomic RNA and ACE2 mRNA via RNAscope per field of view.

A–D Quantification of viral antisense RNA (A, B), viral genomic RNA (C, D) and *ACE2* mRNA from pairs of otherwise identical LoCs reconstituted without (A, C, E) and with macrophages (B, D) and analysed at 1 and 3 dpi. Plots show the cumulative number of spots imaged per field of view from 4 to 6 fields of view (technical replicates) detected using RNAscope and confocal imaging using identical imaging conditions for all chips.

E SARS-CoV-2 infection reduces *ACE2* expression per field of view in both epithelial cells and endothelial cells by 3 dpi in LoCs reconstituted without macrophages. Data obtained from 4 to 6 fields of view (technical replicates) from one LoC at each timepoint.

Data information: Bars represent the mean value, the solid line represents the median, and error bars represent the standard deviation. *P*-values are calculated using a Kruskal–Wallis one-way ANOVA test, * represents $P \leq 0.05$, ** represents $P \leq 0.01$, and ** represents $P \leq 0.001$.