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Supplemental information

Fluvastatin mitigates SARS-CoV-2 infection in human lung cells

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Supplemental figures

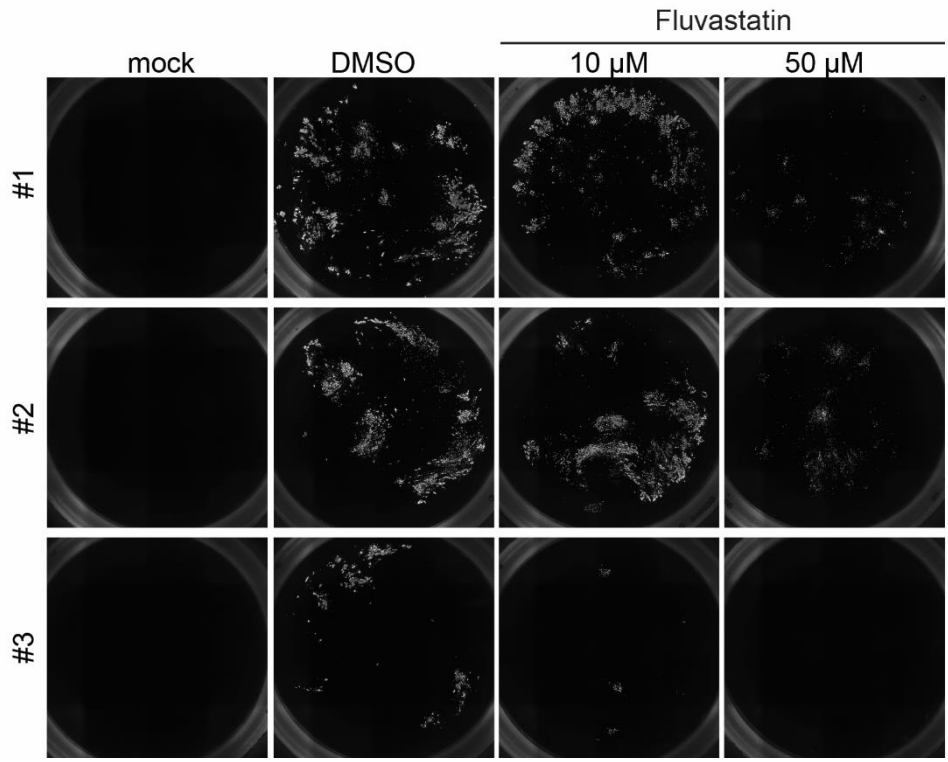

Figure S1. Infection of HBEC ALI cultures, related to figure 2. HBEC ALI cultures of three individuals were infected with SARS-CoV-2, immunostained for infection (SARS-CoV-2 N) and nuclei (Hoechst33342) and imaged by acquisition of 4x4-images per insert with a 4x object on a Cytation5 screening microscope. Depicted are representative overview images of the infection signal stitched based on the nuclear signal from each of the three donors. Stitching was performed in the BioTek Gen5+ software by alignment of images acquired with a 10% overlap using linear blend as a fusion method based on the nuclear channel and alignment was automatically transferred to the infection channel.

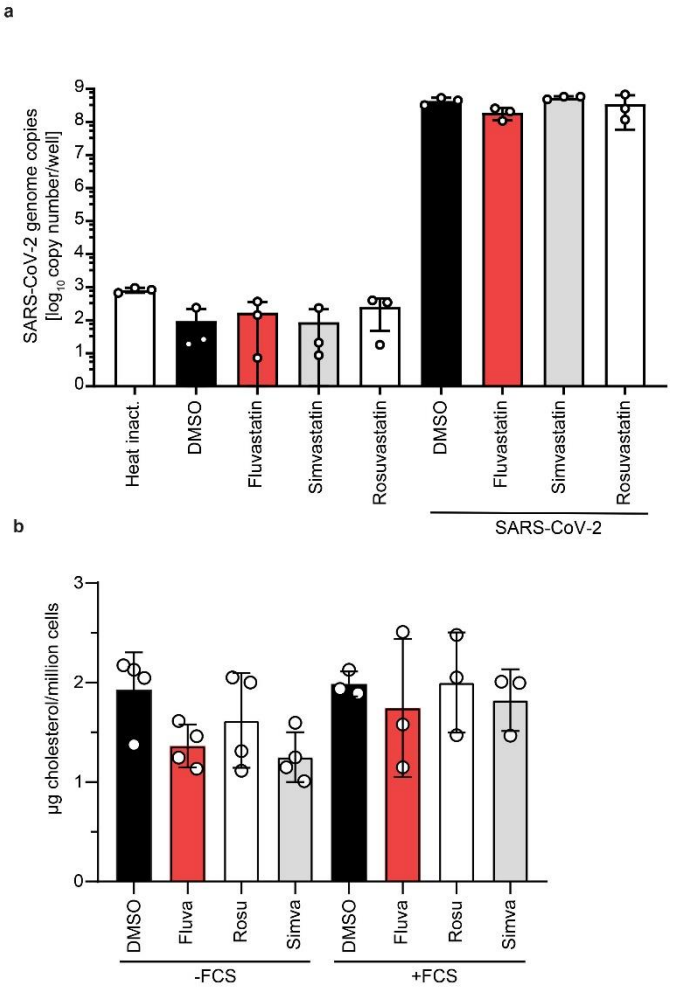

Figure S2. Confirmation of SARS-CoV-2 infection and effectiveness of statin treatment, related to figure 3. a, Viral copy numbers in the supernatants of statin treated and SARS-CoV-2 infected Calu-3 cells that were used for the mass spectrometry analysis. Calu-3 cells were pretreated with statins or DMSO solvent control for 24 h followed by infection with SARS-CoV-2 in the presence of the drug for 48 h at an MOI of 2.x10-5. Viral copies were determined by RT-qPCR. Mean ± SD of three independent biological replicates shown. **b,** Total cellular cholesterol quantification from Calu-3 cells cultured in media with or without FCS and treated with fluvastatin, rosuvastatin, simvastatin or DMSO solvent control for 3 days. Results are normalized to the corresponding DMSO solvent control. Mean ± SD of four (-FCS) or three (+FCS) independent biological replicates shown

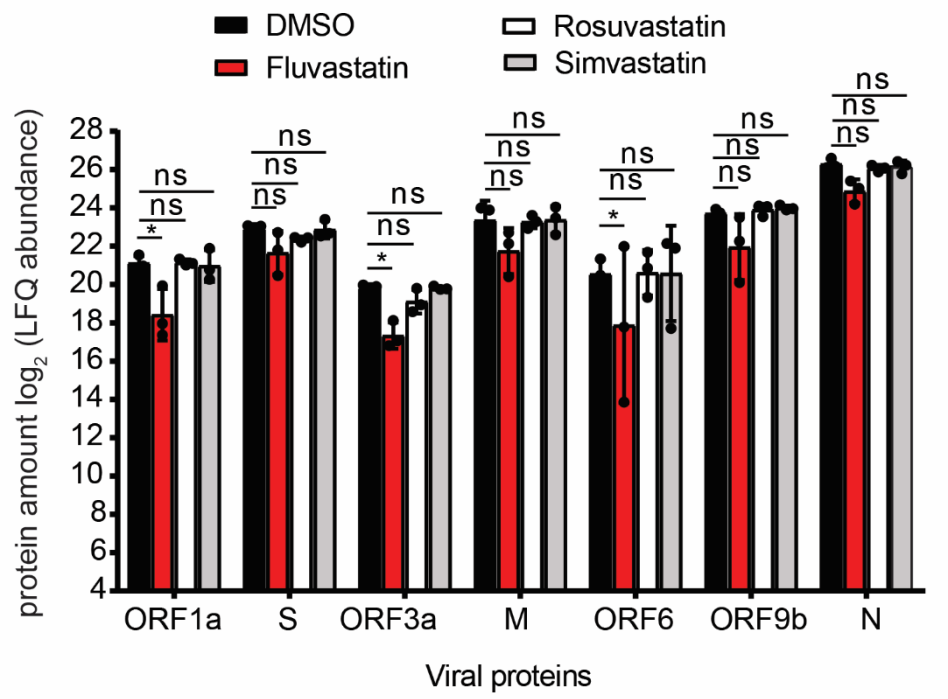

Figure S3: Viral protein abundance in SARS-CoV-2 infected Calu-3 cells, related to figure 5. Abundance (as log2 values obtained by label free quantification) of seven viral proteins in SARS-CoV-2 infected Calu-3 cells treated with statins. Viral proteins were not detected in uninfected cells. For statistical analysis missing values were replaced from normal distribution with a constant downshift. Mean ± SD of three independent biological replicates shown. One-way ANOVA, followed by Dunnett`s multiple comparison test * $p<0.05$

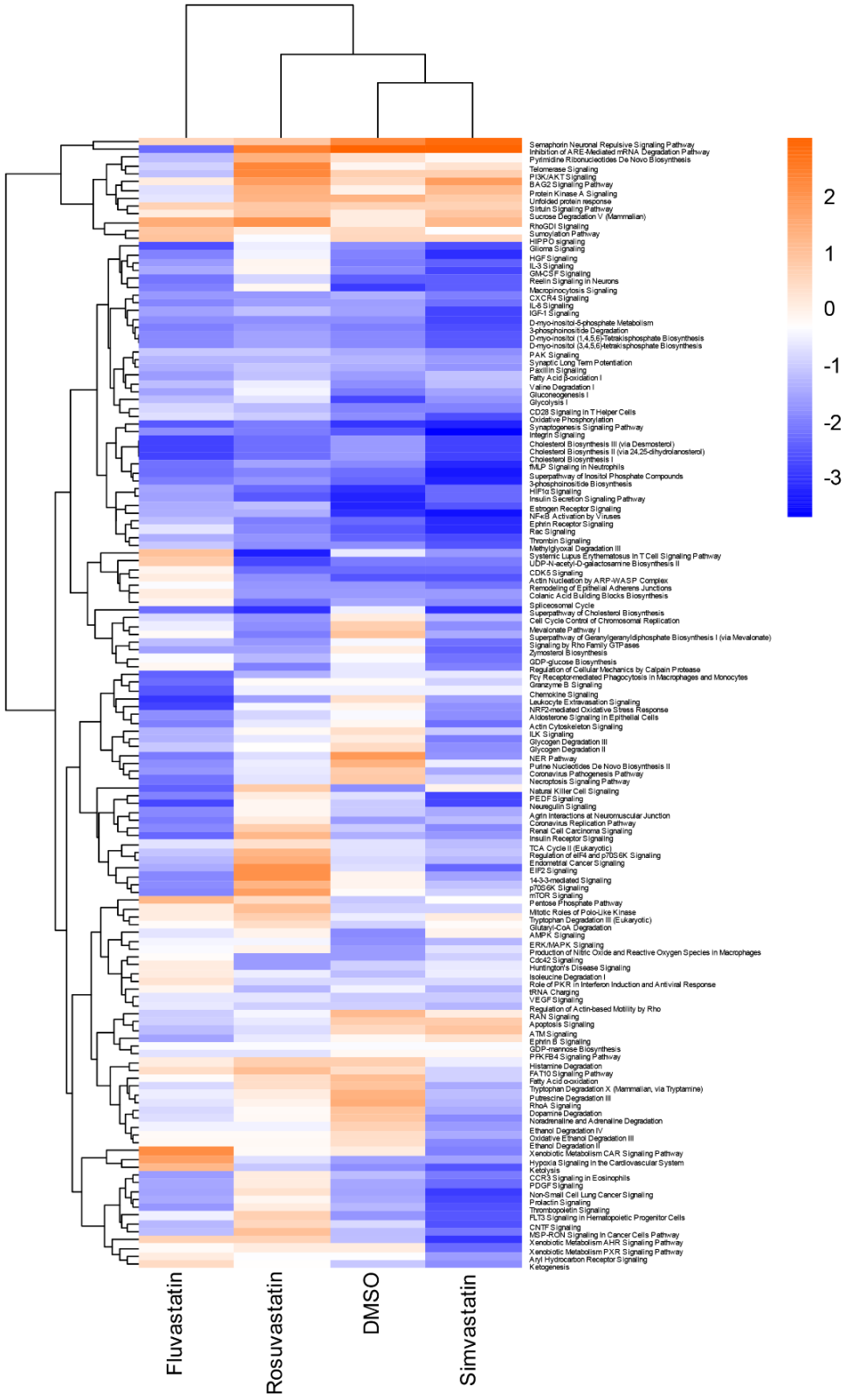

Figure S4. Canonical pathway analysis of SARS-CoV-2 infected Calu-3 cells, related to figure 5. Canonical pathway analysis for the whole-cell proteome of SARS-CoV-2 infected Calu-3 cells treated with the indicated statins or DMSO solvent control. Enriched pathways with a -log10 p-value of at least ±2 in one of the three statin treatments are depicted in the heatmap. Hierarchical clustering based on enrichment score profiles. Magnification of Fig 5b

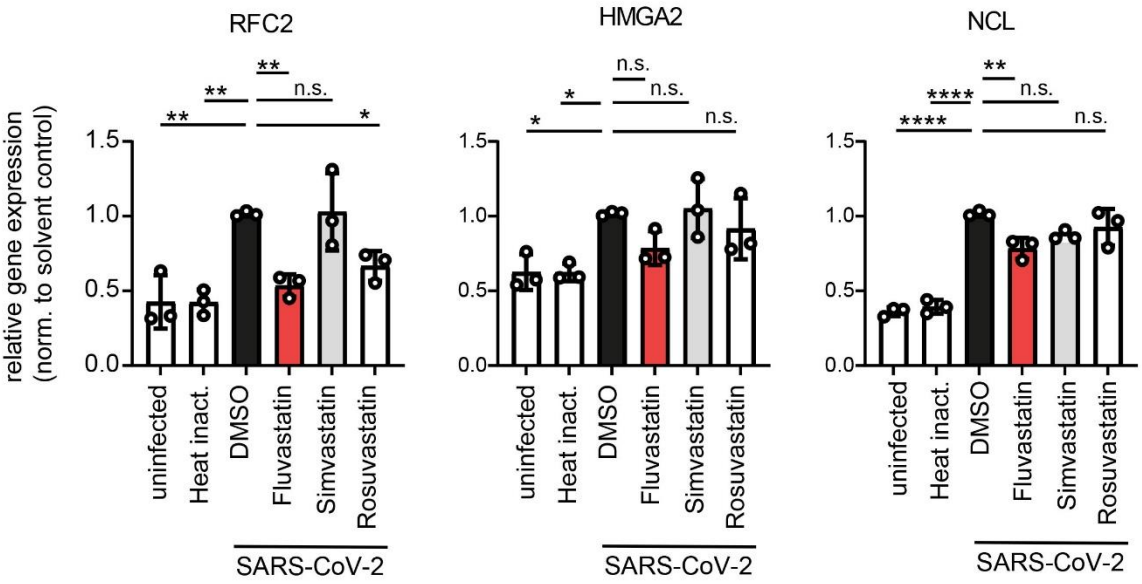

Figure S5: qPCR validation of candidate host factors downregulated during SARS-CoV-2 infection in a fluvastatin-specific manner, related to figure 5. Cells were treated with statins and infected with SARS-CoV-2 as described in figure 1e. Transcript expression levels of host factors depicted in the STRING network in figure 5d were determined by qPCR. RNA expression levels were normalized to the housekeeping gene ß-actin and then normalized to the solvent control. Mean ± SD of three independent biological replicates shown. One-way ANOVA, followed by Dunnett`s multiple comparison test * $p<0.05$, ** $p<0.005$, **** $p<0.0001$.