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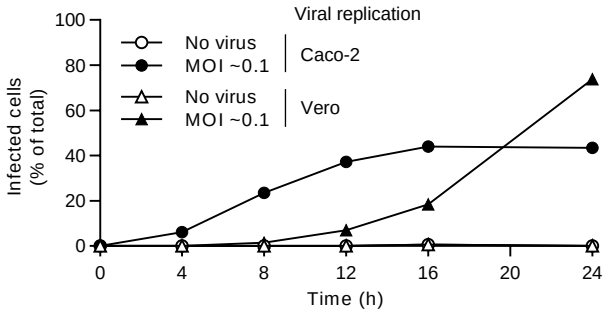
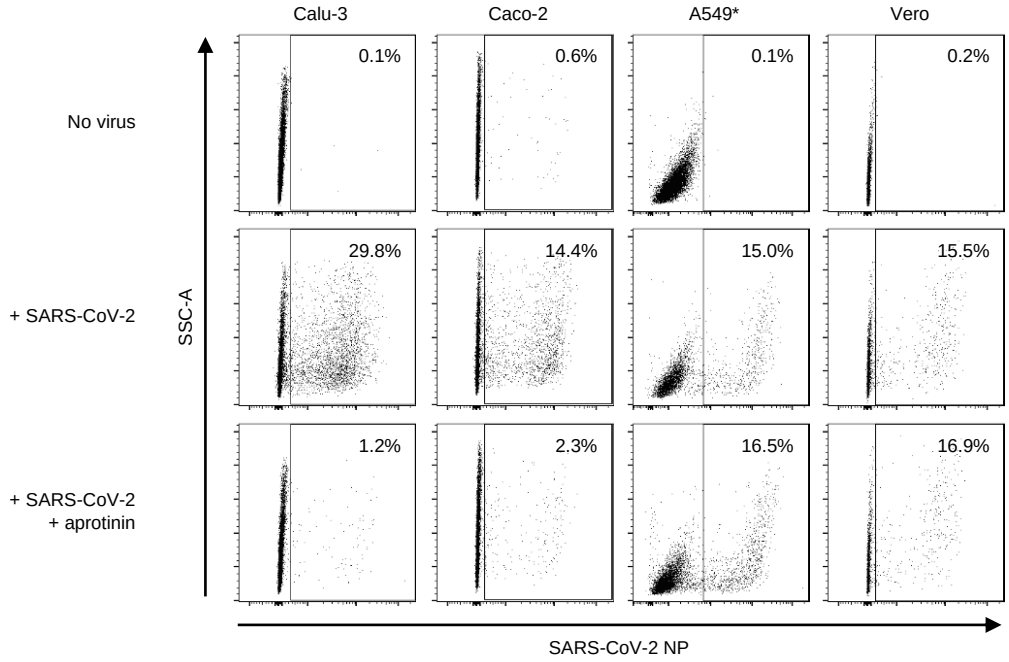
Supplemental data figures



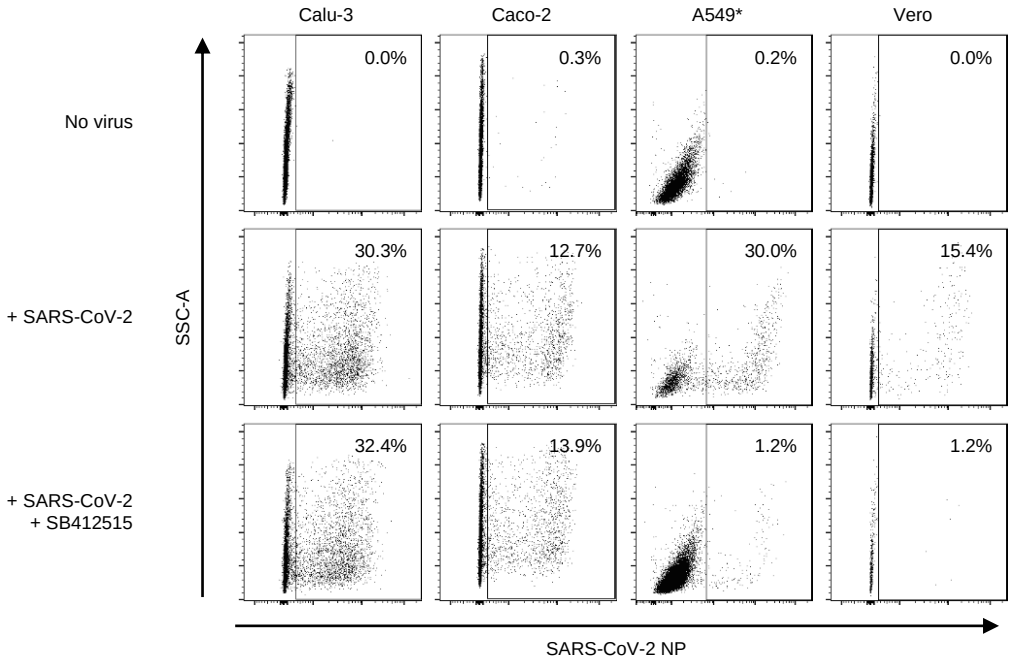
Appendix Figure S1. Kinetic of SARS-CoV-2 infection. Infection of Caco-2 and Vero cells was monitored over 24 h using the same flow cytometry-based assay used for the experiment shown in Fig. 1E. Infection is given as the percentage of SARS-CoV-2 NP-positive cells (percent of total cells). n = 3.

Data information: Results are representative of 3 independent experiments and expressed as mean ± SEM from 3 biological replicates.



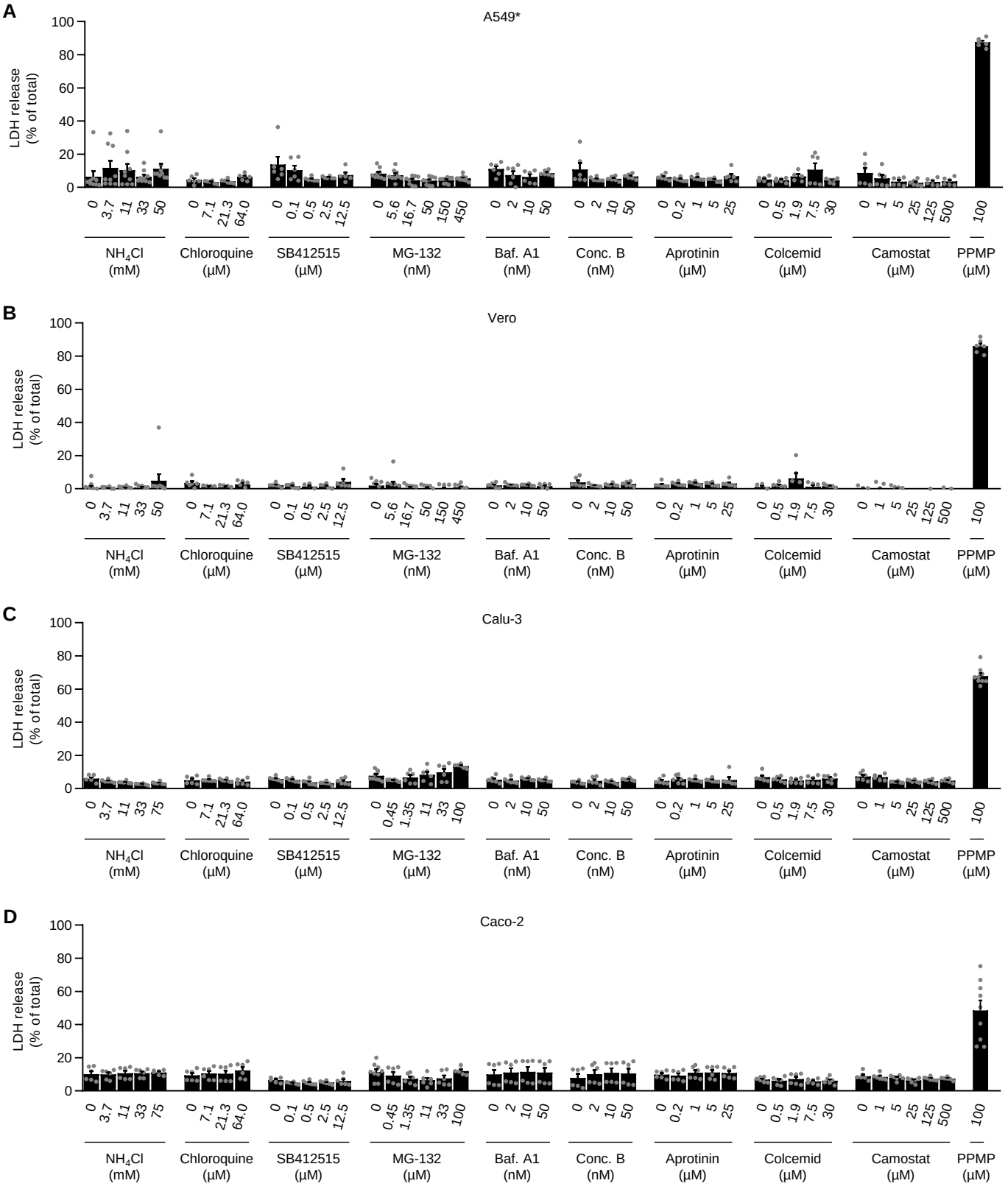
Appendix Figure S2. Aprotinin inhibits SARS-CoV-2 infection in TMPRSS2+ cells. Calu-3, Caco-2, A549*, and Vero cells were pretreated with aprotinin at 5 µM, and subsequently infected with SARS-CoV-2 at MOIs of 0.3, 0.4, 0.2, and 0.3, respectively, in the continuous presence of the drug. Infected cells were quantified by flow cytometry as described in Fig. 1*D*.

Data information: data are representative of many independent experiments.



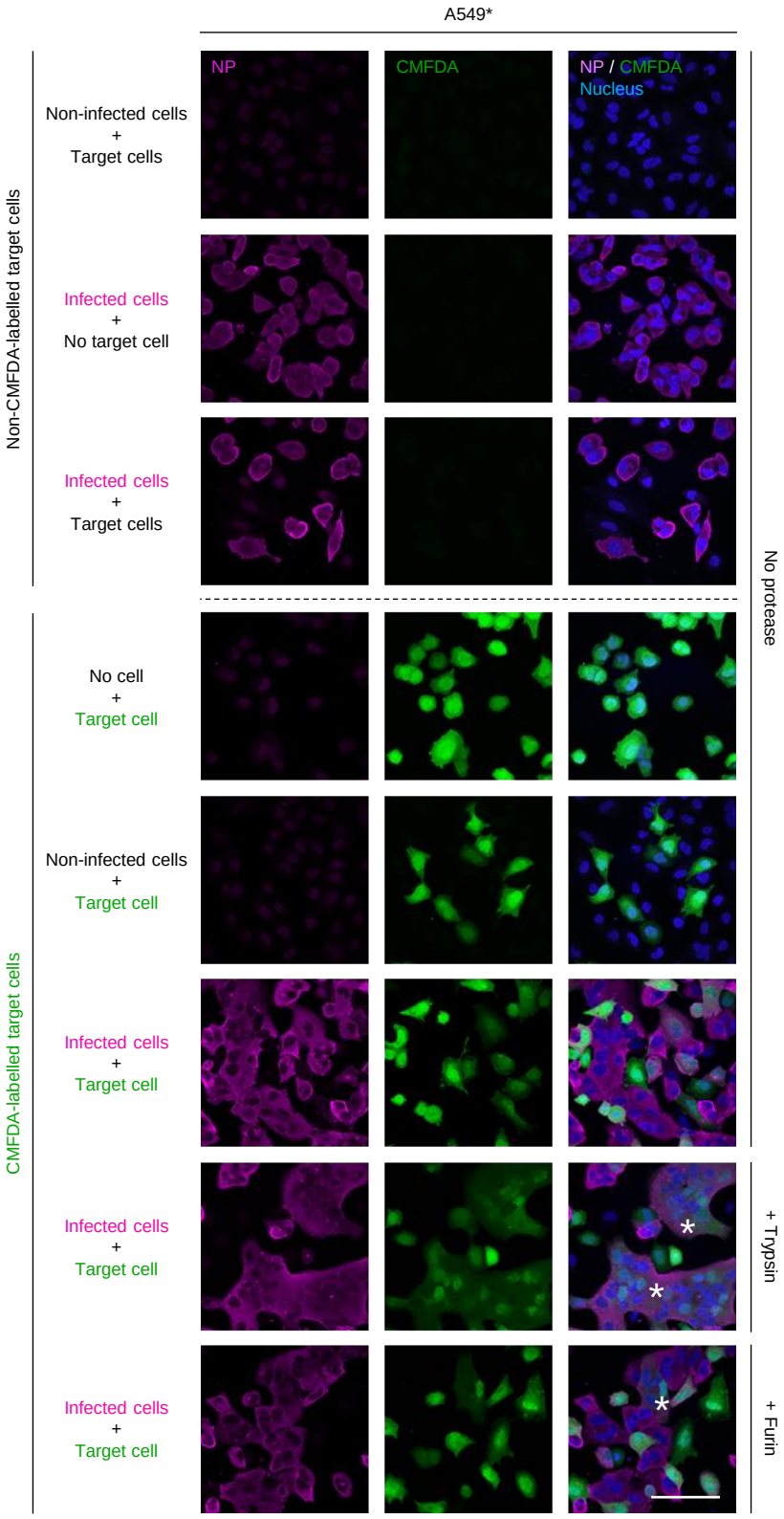
Appendix Figure S3. SB412515 impairs SARS-CoV-2 infection in TMPRSS2-cells. Calu-3, Caco-2, A549*, and Vero cells were pretreated with SB412515 at 2.5 µM, and subsequently infected with SARS-CoV-2 at MOIs of 0.3, 0.4, 0.2, and 0.3, respectively, in the continuous presence of the drug. Infected cells were quantified by flow cytometry as described in Fig. 1*D*.

Data information: data are representative of many independent experiments.



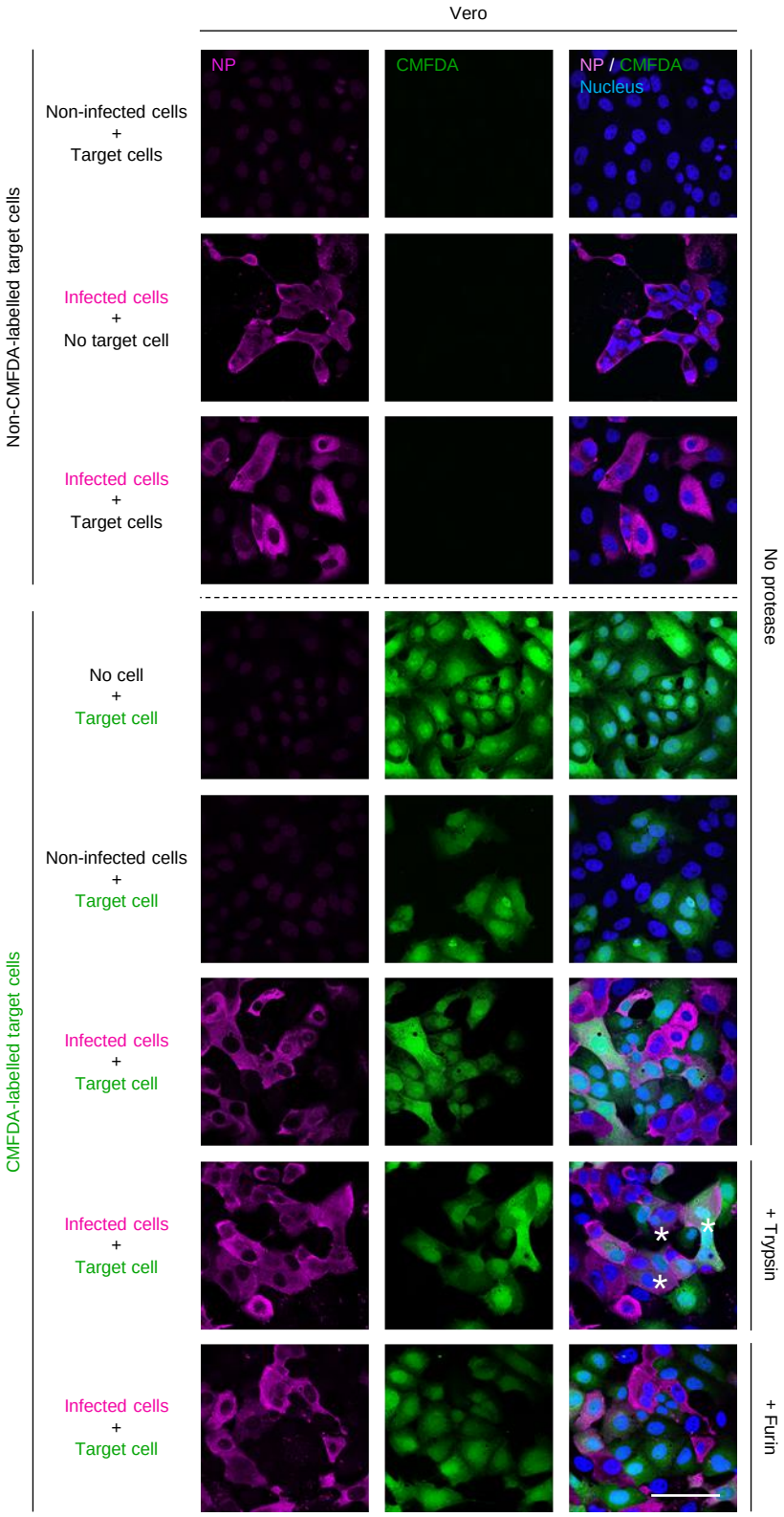
Appendix Figure S4. Drug cytotoxicity in A549*, Vero, Calu-3, and Caco-2 cells. (*A* to *D*) Assessment of cytotoxicity of drugs in the range of concentrations applied to A549*, Vero, Calu-3, and Caco-2 cells for 9 h at 37°C using the CytoTox96 Non-Radioactive Cytotoxicity colorimetric assay (Promega). PPMP, a ceramide analog that impairs ceramide maturation and cell membrane integrity, was used as a positive control. Values were normalized to those of untreated cells after lysis, which corresponds to the maximum possible release of lactate dehydrogenase into the extracellular medium. Baf. A1, bafilomycin A1; Conc. B, concanamycin B. n = 5-9.

Data information: data are expressed as mean ± SEM from 2 independent experiments.



Appendix Figure S5. SARS-CoV-2-mediated cell-cell fusion in A549* cells. A549* cells were first infected with SARS-CoV-2 at an MOI of ~0.1 for 24 h and cocultured for 5 h along with target cells, which were not infected with SARS-CoV-2 but had been prestained with CMFDA, a cytosolic green dye. Cells were subsequently treated with trypsin or furin for 5 min at 37°C and left to coculture for an additional hour at 37°C. After fixation, nuclei were stained with Hoechst (blue), and infected cells were subjected to immunofluorescence staining against SARS-CoV-2 nucleoprotein (magenta). Samples were imaged by confocal fluorescence microscopy. White stars indicate syncytia with at least four nuclei. **Scale bar: 100 μm.**

Data information: images are representative of 3 independent experiments.



Appendix Figure S6. SARS-CoV-2-mediated cell-cell fusion in Vero cells. Vero cells were first infected with SARS-CoV-2 at an MOI of ~0.2 for 24 h and cocultured for 5 h along with target cells, which were not infected with SARS-CoV-2 but had been prestained with CMFDA, a cytosolic green dye. Cells were subsequently treated with trypsin or furin for 5 min at 37°C and left to coculture for an additional hour at 37°C. After fixation, nuclei were stained with Hoechst (blue), and infected cells were subjected to immunofluorescence staining against SARS-CoV-2 nucleoprotein (magenta). Samples were imaged by confocal fluorescence microscopy. White stars indicate syncytia with at least four nuclei. **Scale bar: 100 μm.**

Data information: images are representative of 3 independent experiments.