Expanded View Figures

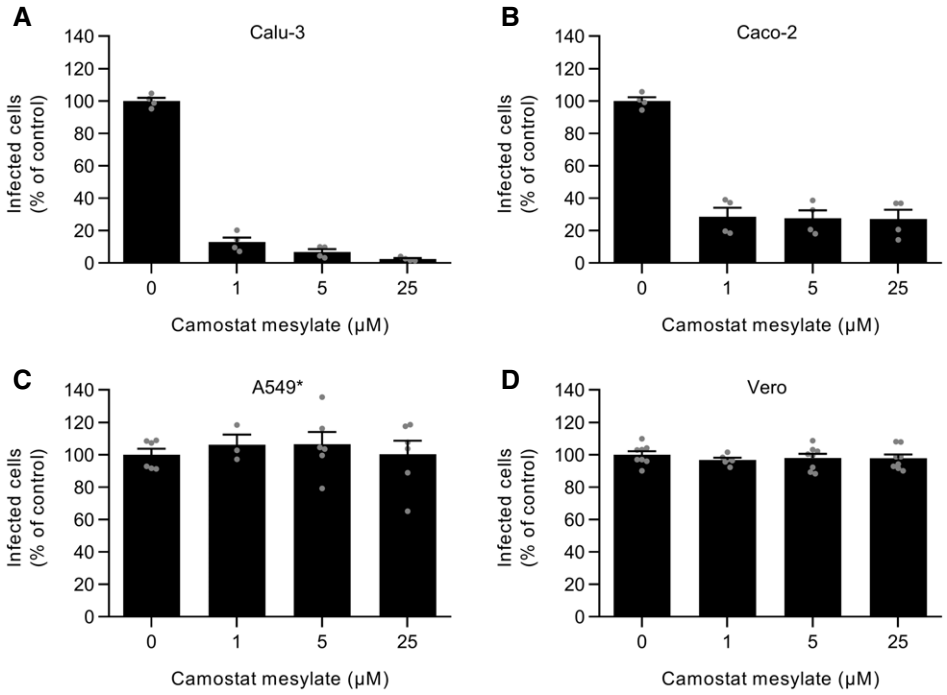

Figure EV1. Camostat mesylate blocks SARS-CoV-2 infection in TMPRSS2+ cells.

A–D Calu-3, Caco-2, A549*, and Vero cells were pretreated with camostat mesylate, which selectively inhibits TMPRSS2, at the indicated concentrations and subsequently infected with SARS-CoV-2 (MOI ~0.3, 0.4, 0.2, and 0.3, respectively) in the continuous presence of the drug. The proportion of infected cells was quantified by flow cytometry as described in Fig 1D, and data were normalized to that from control samples where camostat mesylate had been omitted. *n* (A and B) = 4 and *n* (C and D) = 3–8.

Data information: Data are expressed as mean ± SEM from 2 to 3 independent experiments.
Source data are available online for this figure.

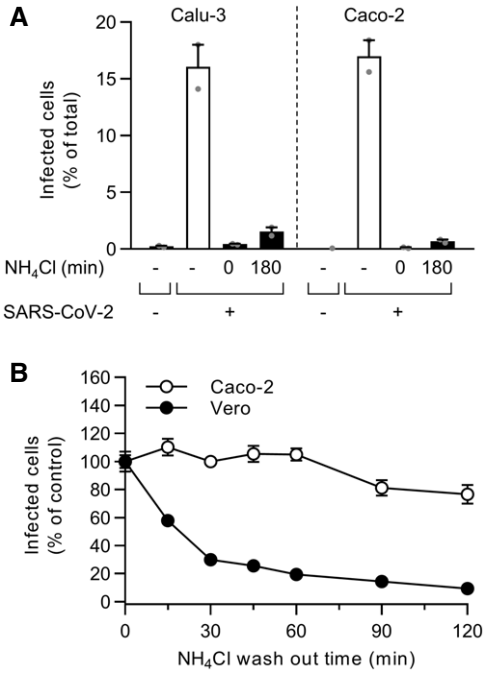

Figure EV2. NH_4Cl interferes with SARS-CoV-2 replication in Calu-3 and Caco-2 cells.

A The controls of Fig 3E are shown here. Briefly, binding of SARS-CoV-2 to Calu-3 or Caco-2 cells (MOI ~0.6 and 0.5) was synchronized on ice for 90 min. Subsequently, cells were rapidly shifted to 37°C to allow penetration. NH_4Cl (75 mM) was added 3 hpi to neutralize endosomal pH. Infection was analyzed by flow cytometry, and raw data are shown. $n = 1–2$.

B After the synchronization of SARS-CoV-2 binding to Caco-2 and Vero cells (MOI ~0.5 and 0.3, respectively) on ice for 90 min, NH_4Cl (50 mM) was added and then washed out at the indicated time points to allow endosomal acidification and the acid-dependent step of SARS-CoV-2 infectious penetration. Infection was analyzed by flow cytometry, and values were normalized to those from samples for which NH_4Cl was removed at t0. $n = 2$.

Data information: Results are representative of two independent experiments and expressed as mean ± SEM from two biological replicates.
Source data are available online for this figure.

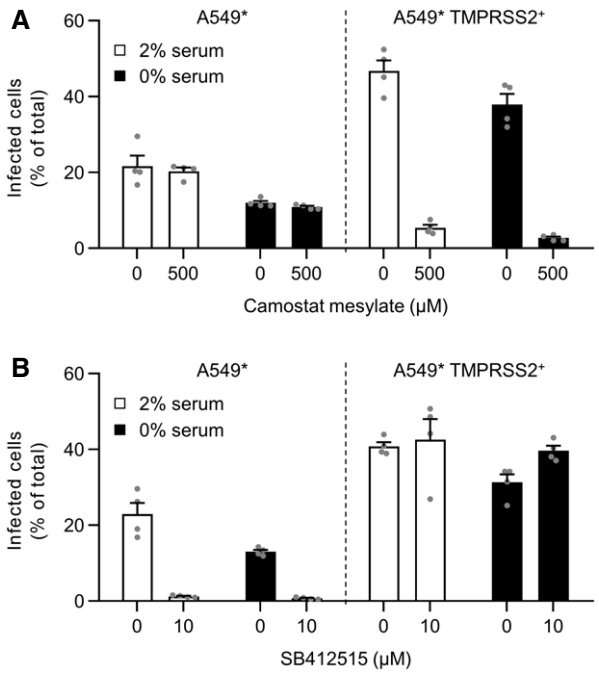

Figure EV3. The presence of serum during biosynthesis does not influence SARS-CoV-2 infectious entry pathways.

A, B A549* cells expressing or lacking TMPRSS2 were pretreated with camostat mesylate at 500 μM (A) or SB412515 at 10 μM (B) and subsequently infected with SARS-CoV-2 (MOI of 0.2) produced in the presence or absence of serum. Infection was performed in the continuous presence of the drugs. Infected cells were quantified by flow cytometry as described in Fig 1D, and data were normalized to samples where inhibitors had been omitted. $n = 4$.

Data information: Data are expressed as mean ± SEM from two independent experiments.
Source data are available online for this figure.

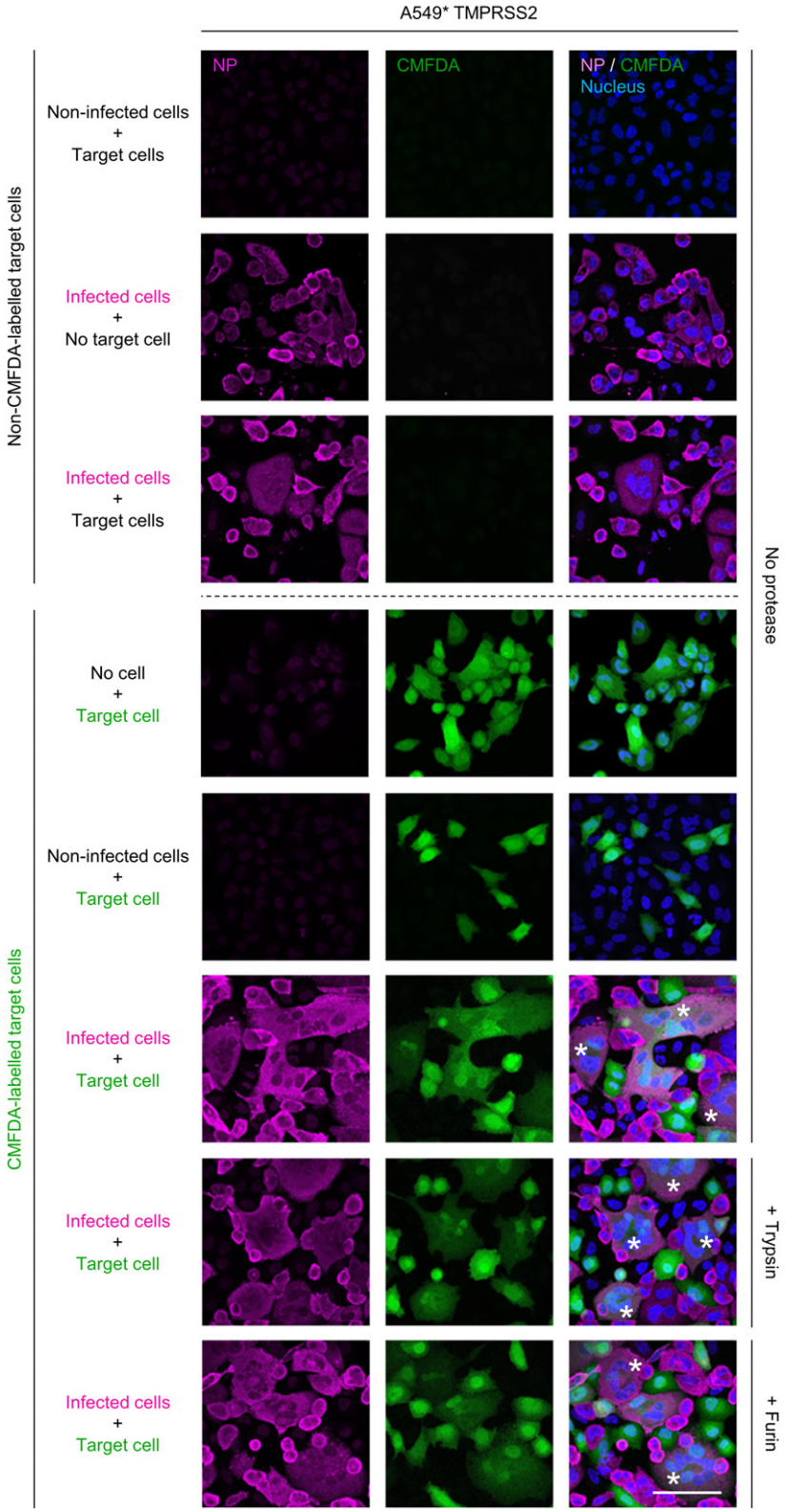

Figure EV4. SARS-CoV-2-mediated cell–cell fusion in TMPRSS2+ A549* cells.

TMPRSS2+ 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 three independent experiments.