Legends to Supplementary Figures and Tables

Figure S1. Drugs without antiviral activity against SARS-CoV-2. Cytopathic effect on Vero E6 cells exposed to 200 $TCID_{50}$/ml of SARS-CoV-2 in the presence of increasing concentrations of different tested drugs. Non-linear fit to a variable response curve from one representative experiment out of three with two replicates is shown (red lines), excluding data from drug concentrations with associated toxicity. Cytotoxic effect with the same drug concentrations in the absence of virus is also shown (black lines).

Figure S2. TEM of Vero E6 cells infected with SARS-CoV-2 and effects of OSW-1. Cells infected with SARS-CoV-2 at an MOI of 0.02 PFU/cell were incubated with 3 nM OSW-1 (**A-E**) or 5 nM OSW1 (**F-I**) and prepared for TEM at 48 hpi. (**A**) Overview of a cell with a cluster of DMVs (asterisk). (**B**) Detail of DMVs. (**C-D**) Examples for the variety of complex vacuoles (CV) with viral particles (arrowheads) in their lumen. The insets show higher magnifications of viral particles. (**E**) Viral particles (arrowheads) at the plasma membrane (P). (**F**) Overview of a cell with cluster of DMVs (asterisk). (**G**) Group of DMVs. The arrows mark alterations in their morphology such as protrusions in continuity with the inner membrane. The inset in (**G**) shows a viral particle between the inner and the outer membrane of the DMV. (**H**) Complex vacuole with viral particles in the lumen (arrowheads). The inset shows a viral particle at higher magnification. (**I**) Viral particles (arrowheads) at the plasma membrane (P). N, nucleus. Scale bars, 500 nm in A and F; 200 nm in B-E and G-I.

Figure S3. **TEM of Vero E6 cells infected with SARS-CoV-2 and effects of U18666A**. Cells were infected with SARS-CoV-2 at an MOI of 0.02 PFU/cell, incubated with 1 mM (**A-F**) or 20 mM U18666A (**G-I**) and prepared for TEM at 48 hpi. (**A**) Overview of a cell with a cluster of DMVs (asterisk). (**B**) Group of DMVs with alterations of the inner membrane (arrows). (**C**) Group of altered DMVs. In one of them, two viral particles (black arrowheads) are present between the inner and outer membrane of the DMV. Nearby there is a single membrane vesicle with a viral particle (white arrowhead). (**D**) Group of single membrane vesicles (white arrowheads) with viruses. (**E**) Complex vacuole with altered viral particles (arrowhead) in the lumen. The inset shows a viral particle at higher magnification. (**F**) Viral particles (arrowheads) at the plasma membrane (P). (**G**) Overview of a cell. (**H**) Micro-cluster of DMVs. (**I**) Viral particles (black arrowheads) at the plasma membrane. N, nucleus. Scale bars, 1 µm in A and G; 200 nm in B-F, H and I.

Figure S4. TEM of mock-infected Vero E6 cells. (**A**) Overview of a cell. (**B**) and (**C**) High magnification views of cells showing (**B**) a Golgi complex (G) and lysosomes (L) and (**C**) Mitochondria (M). (**D**) to (**F**) Details of (**D**) Nucleus (N), (**E**) Endoplasmatic reticulum (ER) and (**F**) Plasma membrane (P). Scale bars, 1 µm in A, 200 nm in B-F.

Figure S5. TEM of mock-infected Vero E6 cells treated with OSW-1. Non-infected Vero cells were incubated with 3 nM (A-C) or 5 nM OSW1 (D-F) and prepared for TEM at 48 h

post-incubation**.** (**A**) Overview of a cell. (**B**) and (**C**) High magnification views of cells showing a Golgi complex (G) and lysosomes (L). (**D**) Overview of a cell. (**E**) and (**F**) High magnification views showing a Golgi complex (G) and lysosomes (L). (**F**) Detail of a lysosome (L). N, nucleus. Scale bars, 500 nm in A and D; 200 nm in B, C, E and F.

Figure S6. TEM of mock-infected Vero E6 cells treated with U18666A. Cells were incubated with 1 mM (**A-C**) or 20 mM U18666A (**D-F**) and processed for TEM at 48 h post-incubation**.** (**A**) Overview of a cell. (**B**) and (**C**) High magnification views showing a Golgi complex (G) and a Lysosome (L). (**D**) Overview of a cell. (**E**) Swollen Golgi (G) with an endosomal vacuole (E) nearby. (**F**) Details of an enlarged lysosome (L). N, nucleus. Scale bars, 1 µm in A and D, 200 nm in B, C, E and F.

Figure S7. TEM of Vero E6 cells treated with Phytol and infected or not with SARS-CoV-2. (**A-E**) Cells infected with SARS-CoV-2 at a MOI of 0.02 PFU/cell, incubated with 50 µM phytol and prepared for TEM at 48 hpi. (**A**) Overview of a cell with a cluster of DMVs (asterisk). (**B**) DMV with alteration of the inner membrane (arrow). (**C**) Group of single membrane vesicles with viral particles (white arrowheads). (**D**) Complex vacuole with viral particles in the lumen (arrowheads). The inset shows a viral particle at higher magnification. (**E**) Viral particles (black arrowheads) at the plasma membrane (P). N, nucleus. (**F-I**) Mock-infected cells were incubated with 50 µM phytol and prepared for TEM at 48 h post-incubation. (**F**) Overview of a cell. (**G**) Mitochondria (M). (**H**) Lysosome (L) and glycogen granules (white arrowheads) in the cytosol. (**I**) Lipid droplets (LD). P, plasma membrane. Scale bars, 1 µm in A and F, 200 nm in B-E, and G-I.

Figure S8. TEM of non-infected Vero E6 cells treated with HβCD. Cells were incubated with 0.16 mM (**A-C**) or 20 mM HβCD (**D-F**) and prepared for TEM at 48 h post-incubation. (**A**) Overview of a cell. (**B**) and (**C**) High magnification views showing a Golgi complex (G) and Lysosomes (L). (**D**) Overview of a cell with vacuoles (V). (**E**) Detail of a Golgi complex with swollen cisternae (G) and swollen endoplasmatic reticulum (ER). (**F**) Details of enlarged lysosomes (L). N. nucleus. Scale bars, 1 µm in A and D, 200 nm in B, C, E and F.

Figure S9. Cytotoxicity of drugs tested against SARS-CoV-2. (**A**) Cytotoxicity of indicated drugs at decreasing concentrations in ACE2 expressing HEK-293T cells. At 48 h cells were lysed with the Glo Luciferase system (Promega) and luminescence was measured with a plate reader giving relative light units (RLUs). Cytotoxic concentrations are indicated with an arrow. Mean and standard deviation from one experiment with two replicates is represented. (**B**) Cytotoxicity of indicated drugs at decreasing concentrations on Calu-3. After 24h of adding virus and drugs at the indicated concentrations, cells were washed and drugs were added at the same final concentration for an additional 48h. Then cells were lysed with the Glo Luciferase system (Promega) and luminescence was measured with a plate reader giving relative light units (RLUs). Cytotoxic concentrations are

indicated with an arrow. Mean and standard deviation from one experiment with two replicates is represented. (**C**) Cytotoxicity of indicated cyclodextrins at decreasing concentrations in ACE2 expressing HEK-293T cells. At 48 h cells were lysed with the Glo Luciferase system (Promega) and luminescence was measured with a plate reader giving relative light units (RLUs). Cytotoxic concentrations are indicated with an arrow. Mean and standard deviation from two experiments with two replicates each are represented. (**D**) Cytotoxicity of indicated cyclodextrins at decreasing concentrations on Calu-3. After 24h of adding virus and drugs at the indicated concentrations, cells were washed and drugs were added at the same final concentration for an additional 48h. Then cells were lysed with the Glo Luciferase system (Promega) and luminescence was measured with a plate reader giving relative light units (RLUs). Cytotoxic concentrations are indicated with an arrow. Mean and standard deviation from one experiment with two replicates is represented. Mean and standard deviation from three experiments are represented. (**E**) Cytopathic effect of MβCD, on the apical side or the basal medium, and Remdesivir as control, in the human nasal epithelial model (Mucilair™). At 72h cells were lysed with the Glo Luciferase system (Promega) and luminescence was measured with a plate reader giving relative light units (RLUs). One experiment is represented.

Figure S10. Methyl-β-cyclodextrin nasal application decreases SARS-CoV-2 replication in a hamster model. Hamsters were intranasally administered with or without MβCD at 50 mM and challenged with a replicative competent SARS-CoV-2. Control uninfected animals were also assayed. At 1- or 2-days post-infection, nasal turbinates and lungs collected from euthanized animals were analysed for **(A-B)** viral RNA presence in inverted CTs by qPCR, (**C-D**) by viral infectivity on Vero-E6 cells, (**E-F**) by immunohistochemistry to detect the NP of SAS-CoV-2 and (**G-H**) by histopathological observation in haematoxylin and eosin-stained sections.

Table S1. Vendor origin and concentrations of tested cyclodextrins.

Table S2. Energies for the top-ranked compounds (small molecules) against Mpro, PLPro, NPC1, Spike, and NSP16.

Table S3. Energies for the top-ranked cyclodextrins against the active site and the two allosteric sites of Mpro.

Table S4. Quantification of TEM results. Ultrathin sections of Vero E6 cells infected with SARS-CoV-2 at MOI of 0.02 PFU/cell and 48 hpi and treated in the absence or presence of OSW-1, HβCD, U18666A or Phytol were studied by TEM. The percentage of cells with DMVs, altered DMVs, single-membrane vesicles (SMVs) with viruses, complex vacuoles (CVs) with viruses and extracellular viral particles (VPs) was calculated. A total of 20 cells per condition were included in the quantification. Asterisk (*): very few DMVs per cell.