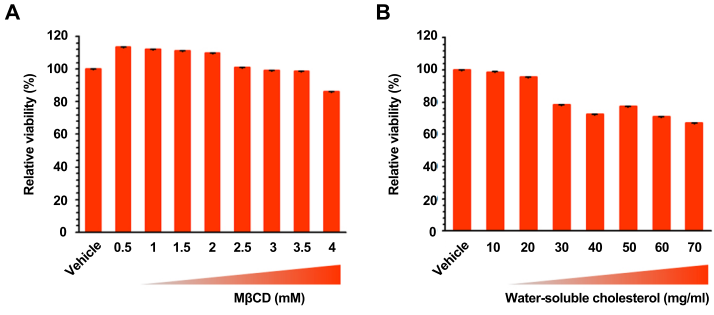
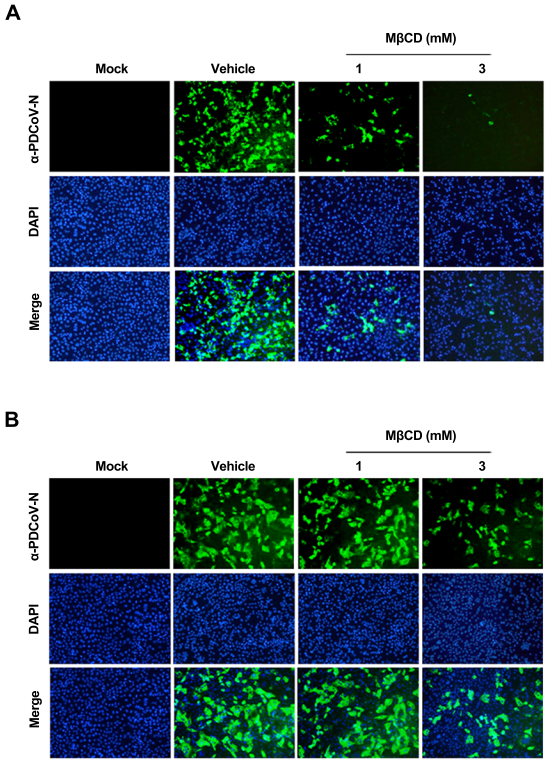
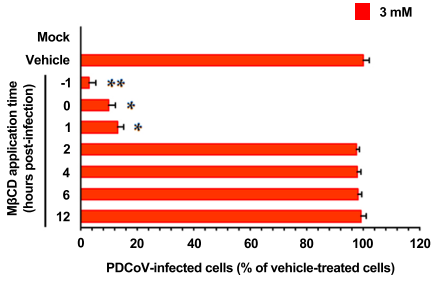


Supplementary Fig. 1. ST cells were incubated with various concentrations of MβCD (A) or water-soluble cholesterol (B) for 24 h prior to the MTT assay, and the cytotoxic effects of MβCD and cholesterol were determined by the MTT assay.



Supplementary Fig. 2. Effects of cellular (A) or viral (B) cholesterol depletion on the replication of PDCoV. (A) ST cells were preincubated with MβCD at the indicated concentrations for 1 h prior to infection and were mock infected or infected with PDCoV at an MOI of 1. Virus-infected cells were further maintained in the presence of vehicle or MβCD. (B) PDCoV was treated with MβCD at the indicated concentrations for 1 h and ultracentrifuged. The purified viral suspensions were used to infect ST cells, and the virus-infected cells were subjected to IFA. For immunostaining, infected cells were fixed at 12 hpi and incubated with MAb against the PDCoV N protein, followed by incubation with Alexa-green-conjugated goat anti-mouse secondary antibody. The cells were then counterstained with DAPI and examined using a fluorescent microscope at 200× magnification.



Supplementary Fig. 3. Effects of cellular cholesterol sequestration on virus propagation at early time points post-infection. ST cells were pretreated with MβCD and were mock-infected or infected with PDCoV. At 24 hpi, the virus-infected cells were fixed, and viral infectivity was determined by measuring the percentage of cells expressing PDCoV N proteins using FACS analysis. The data are presented as the mean values of three independent experiments, and error bars represent standard deviations. *, $P = 0.001–0.05$; **, $P < 0.001$.