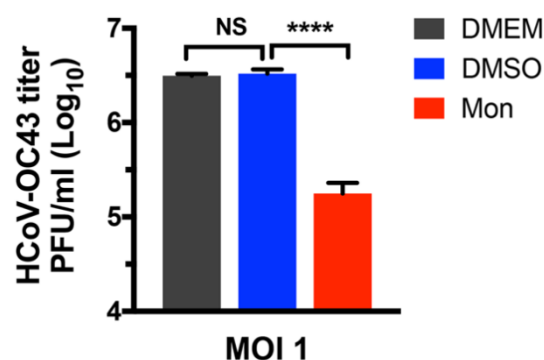
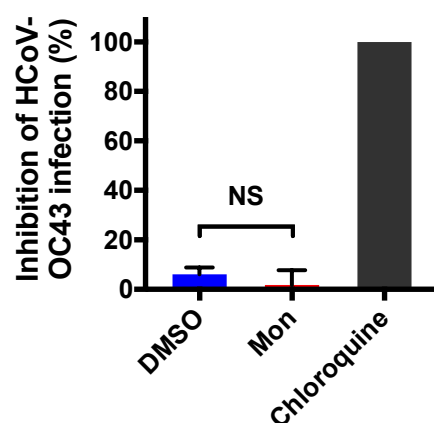


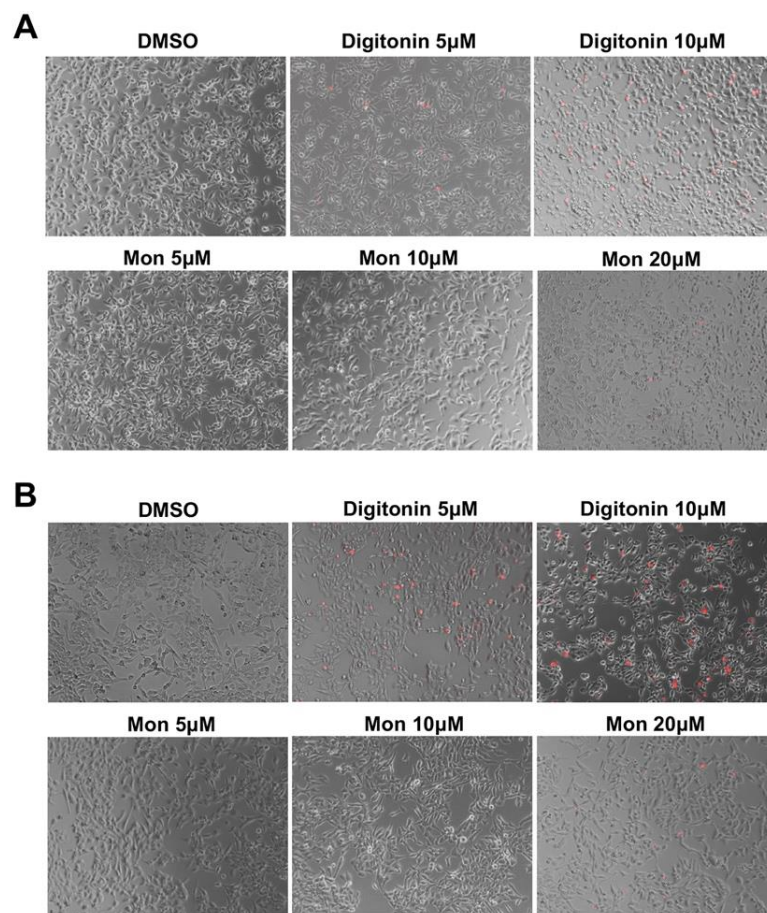
## Montelukast Inhibits HCoV-OC43 Infection as a Viral Inactivator



**Figure S1.** Montelukast (Mon) inhibited the infection of HCoV-OC43 in MRC-5 cells at multiplicity of infection (MOI) of 1. MRC-5 cells ( $2 \times 10^5$ ) were infected with  $2 \times 10^5$  PFU of HCoV-OC43 after viruses were treated with or without 10  $\mu$ M of montelukast for 1 h at 33°C. Then the inoculum was removed from cells after 2 h incubation at 33 °C and fresh medium containing 2% FBS and supplemented with or without 10  $\mu$ M of montelukast was respectively added. The supernatant was collected at 3 days after infection, and the infectious HCoV-OC43 particles were determined by plaque formation assay. Data are presented as means  $\pm$ SD. NS, not significant; \*\*\*\*  $p < 0.0001$ .



**Figure S2.** HCoV-OC43 internalization assay. BHK-21 cells were first infected with 250 PFU of HCoV-OC43 on ice for 1h. After removing the inoculum from cells and washing the cells with fresh DMEM twice, the cells were incubated with or without 10  $\mu$ M of montelukast for 2 h at 33°C to allow virus internalization. Then the plaque assay was performed. Chloroquine was included as positive control. Data are presented as means  $\pm$ SD. NS, not significant.



**Figure S3.** Propidium iodide (PI) staining of RD cells treated with montelukast (Mon) or digitonin. RD cells were seeded in cell culture plates and incubated overnight at 37°C. Cells were treated with Mon (5, 10 and 20 µM) or digitonin (5 and 10 µM) respectively for 2 h (**A**) or 6 h (**B**) at 37°C. After removing the supernatant and washing cells twice with fresh DMEM, 10 µg/ml of PI in DMEM was supplemented for 20 min incubation. Then the cells were observed for PI staining (red puncta) with a fluorescence microscope (100×).