Fig S1

Fig S1. (A) HEK293T cells were transiently transfected with the vector expressing V5-FLAG-Nsp10, 14, 16, or empty vector. Protein expression was analyzed by protein immunoblotting. **(B)** HEK293T cells transfected with the vector expressing V5-FLAG-Nsp10, 14, 16, or empty vector along with ISRE-driven firefly luciferase and TK-driven renilla luciferase reporter vectors were un-treated or treated with IFN-α. **(C)** HEK293T cells transfected with the vector expressing V5-FLAG-Nsp10, 14, 16, or empty vector along with NF-κB-driven firefly luciferase and TK-driven renilla luciferase reporter vectors were un-treated or treated with TNF-α. Luciferase activity (firefly/renilla) in these cells was measured and normalized to the empty vector. Results were calculated from 3 technical repeats and presented as mean +/- standard error of the mean (SEM). (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; by one-way ANOVA and Tukey's multiple comparison test).
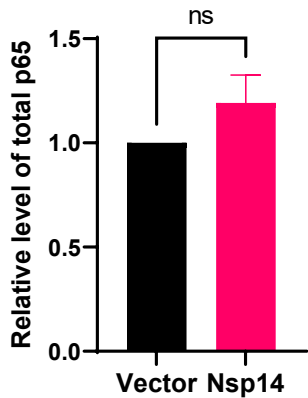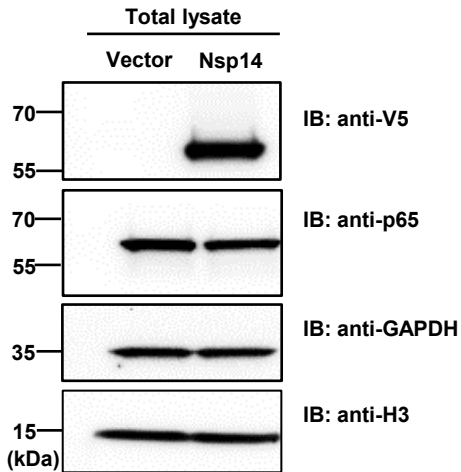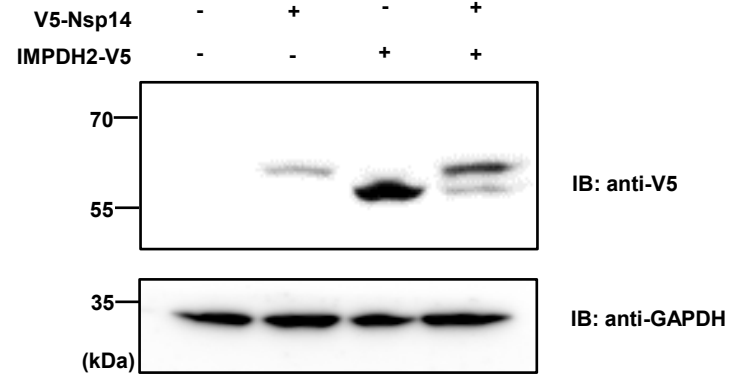
Fig S2. NF-κB p65 in the total lysate of HEK293T cells transfected with the vector expressing V5-FLAG-Nsp14 or empty vector was analyzed by protein immunoblotting. Histone H3 was used as the nuclear marker. The intensity of the p65 protein band was quantified and normalized to the empty vector. (ns: not significant)

Fig S3

A

V5-Nsp14	-	+	-	+
IMPDH2-V5	-	-	+	+

70 —

55 —

IB: anti-V5

35 —

IB: anti-GAPDH

(kDa)

B

NF-κB Luc (RLU)

***

****

Vector, Nsp14, IMPDH2, Nsp14+IMPDH2

TNF-α

NF-κB Luc (RLU)
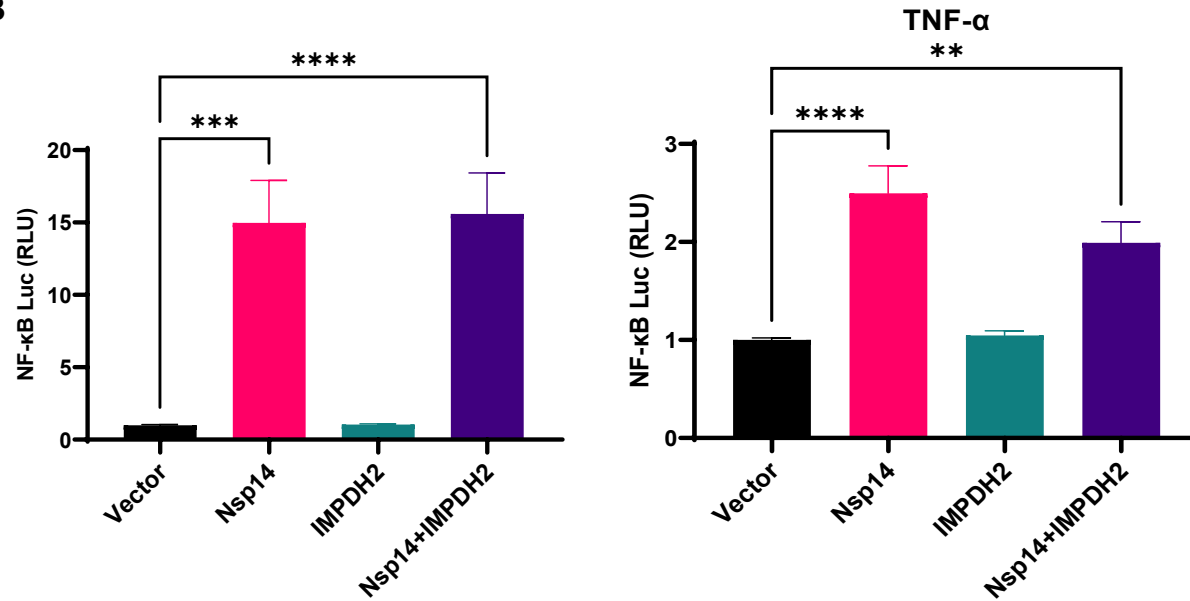
****

**

Vector, Nsp14, IMPDH2, Nsp14+IMPDH2

Fig S3. (A) HEK293T cells were transiently transfected with the vector expressing V5-Nsp14 or V5-IMPDH2, alone or together. Protein expression was analyzed by immunoblotting. **(B)** Cells in **(A)** were further transfected with NF-κB-driven firefly luciferase and TK-driven renilla luciferase reporter vectors without or with TNFα stimulation. Luciferase activity (firefly/renilla) was measured and normalized to the empty vector. Results were calculated from 3 independent experiments and presented as mean +/- standard error of the mean (SEM). (** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; by one-way ANOVA and Tukey's multiple comparison test)
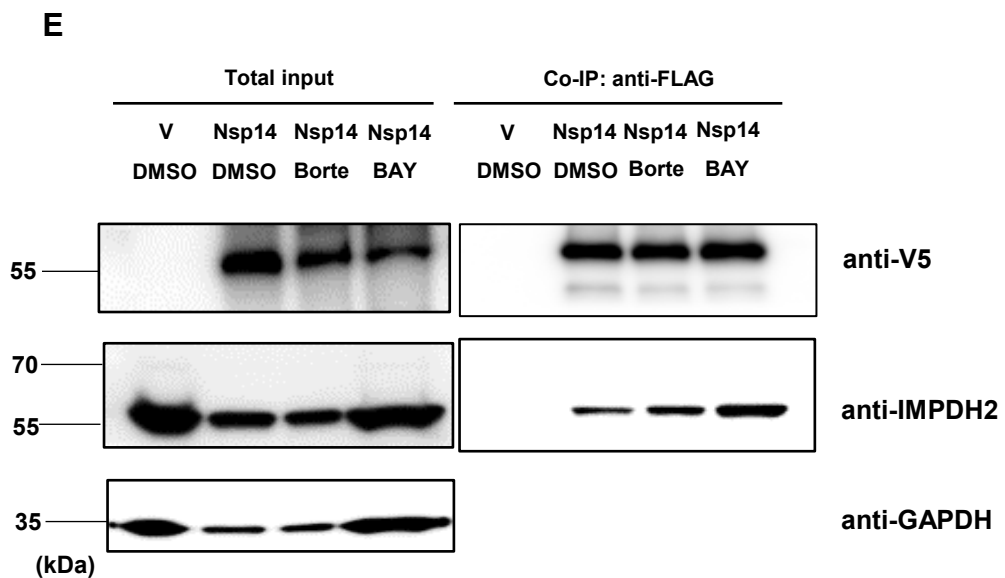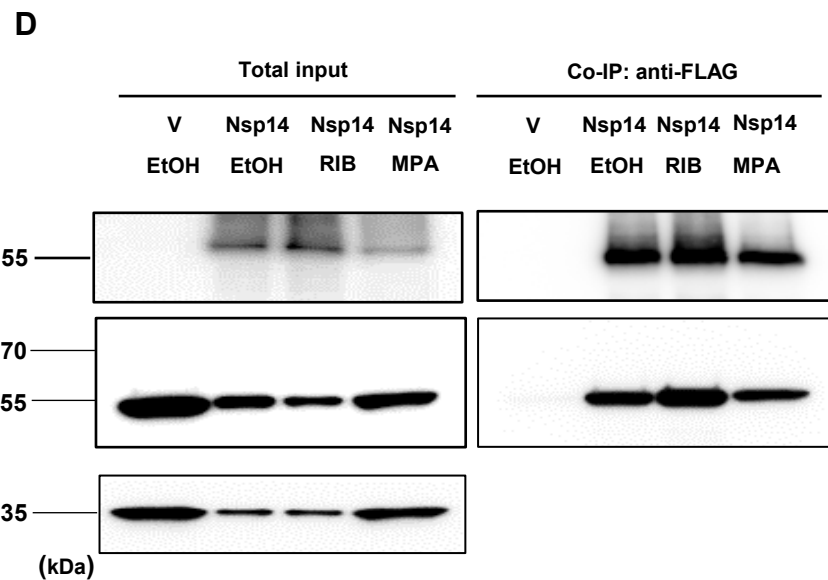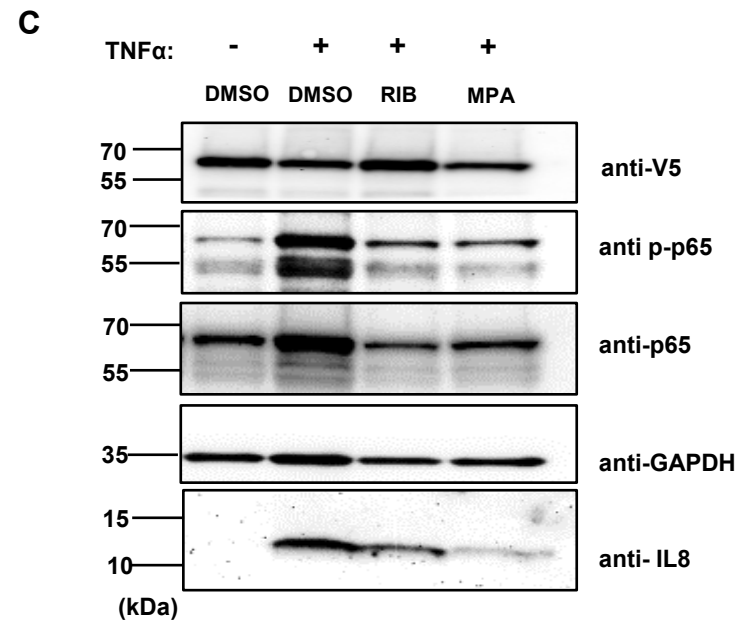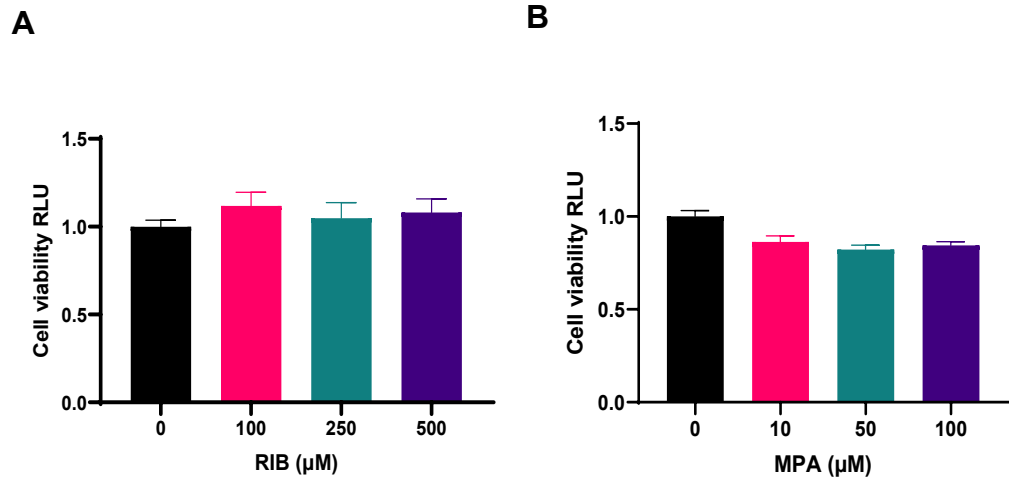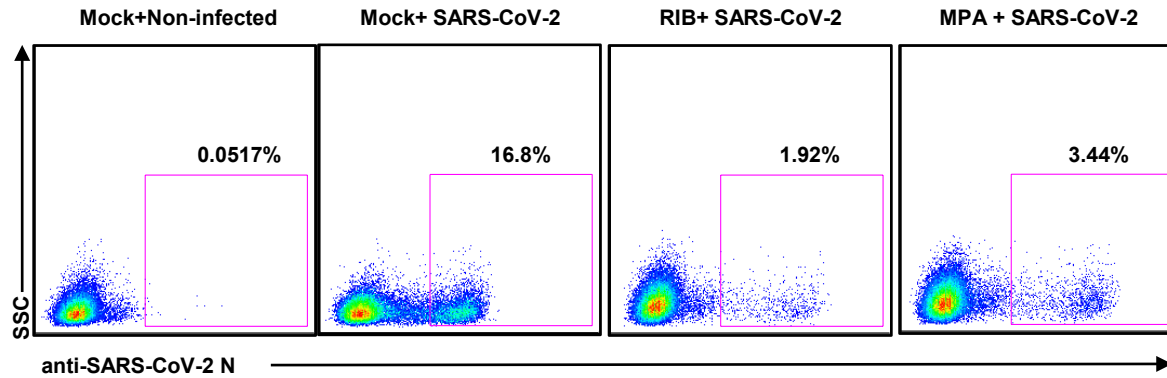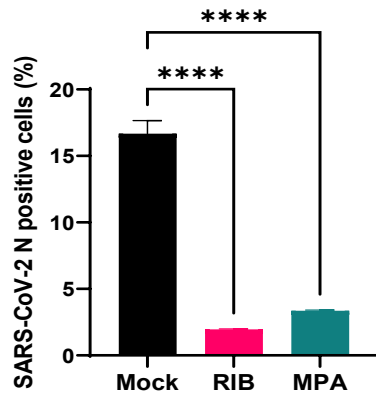
Fig S4

Fig S4. (A, B) Viability of HEK293T cells treated with RIB (**A**) or MPA (**B**) for 24h at increasing doses was measured by CellTiter-Glo®. (**C**) A549 cells were transfected with the FLAG-V5-Nsp14 vector, followed with the treatment of IMDPH2 inhibitors (RIB 500 µM; MPA 100 µM) along with TNF-α stimulation for 24h. Cells were harvested and lysed for protein immunoblotting. (**D, E**) HEK293T cells were transiently transfected with the empty vector or FLAG-V5-Nsp14 vector, along with treatment of IMPDH2 inhibitors (**D**, RIB 500 µM, MPA 100 µM) or NF-κB inhibitors (**E,** bortezomib (Borte) 10 µM, BAY 11-7082 (BAY) 10 µM) for 24h. Cell lysates were prepared and subjected to protein co-IP assays using an anti-FLAG antibody. Precipitated protein samples were analyzed by immunoblotting using anti-V5 and anti-IMPDH2 antibodies.
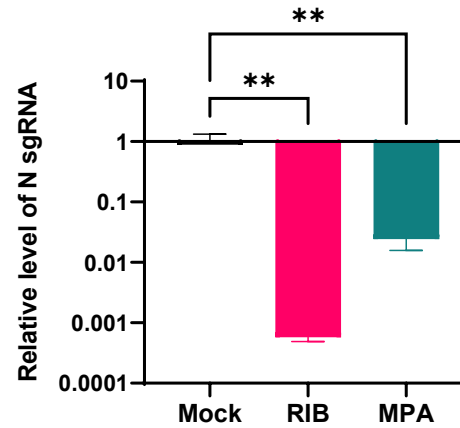
A HEK293T-ACE2

Fig S5

Mock+Non-infected | Mock+ SARS-CoV-2 | RIB+ SARS-CoV-2 | MPA + SARS-CoV-2

0.0517% | 16.8% | 1.92% | 3.44%

SSC / anti-SARS-CoV-2 N

B

SARS-CoV-2 N positive cells (%)

Mock, RIB, MPA

**** ****

C

Relative level of N sgRNA

Mock, RIB, MPA

** **

D

Vero E6, BAY 11-7082

EC_{50} = 20.86 µM
CC_{50} = 18.77 µM
SI < 1

Log$_{10}$ antiviral concentration (µM)

Percent viral infection /Cell viability

A549-ACE2, BAY 11-7082

Percent viral infection
Percent cell viability

EC_{50} = 20.34 µM
CC_{50} = 29.94 µM
SI = 1.47

Log$_{10}$ antiviral concentration (µM)

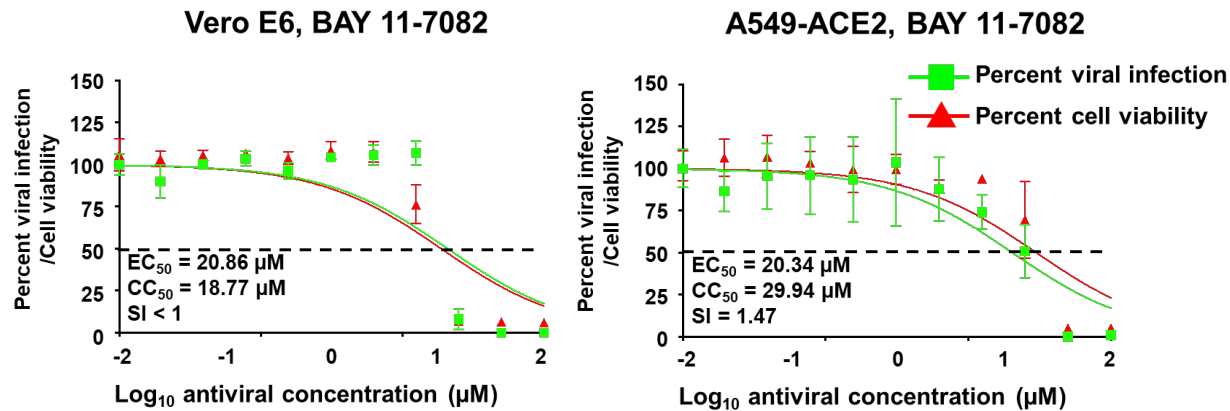Percent viral infection /Cell viability

Fig S5. HEK293T-ACE2 cells were treated with RIB (500 µM), or MPA (100 µM), or mock, and simultaneously infected with SARS-Cov-2 viruses for 24 h. The SARS-CoV-2 infection was detected by intracellular staining of SARS-CoV-2 N protein (**A**). The percentage of SARS-CoV-2 N protein-positive cells was calculated (**B**). Cells were harvested for RNA extraction, and N protein sgRNA was analyzed and normalized to the mock treatment (**C**). The results were calculated from 3 technical repeats and presented as mean +/- standard error of the mean (SEM). (** $p < 0.01$; **** $p < 0.001$; by one-way ANOVA and Tukey's multiple comparison test) (**D**) Vero E6 or A549-ACE2 cells were briefly infected with SARS-CoV-2 (~100 PFU/well), followed by treatment of BAY 11-7082. At 24 hpi, the cells were subjected to PRMNT assay at four biological replicates. Results were calculated as mean +/- standard deviation (SD). The dotted line indicates the 50% inhibition. Selectivity index (SI) is presented as CC_{50}/EC_{50}.