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Supplemental Information

Furin Inhibitors Block SARS-CoV-2 Spike Protein Cleavage to Suppress Virus Production and Cytopathic Effects

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Supplemental Information

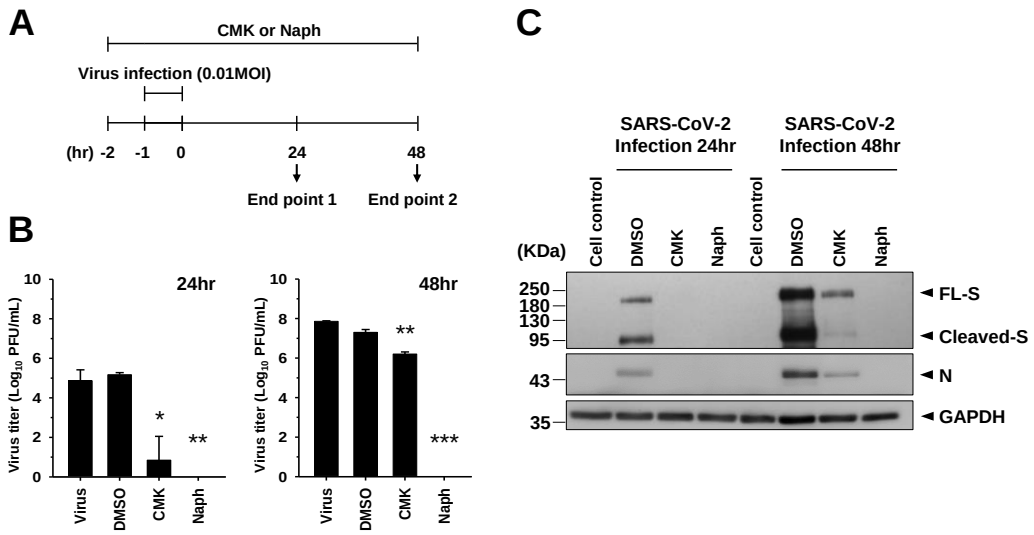


Figure S1. The furin/PC inhibitors CMK and naphthofluorescein reduce viral replication in SARS-CoV-2-infected VeroE6 cells, by multi-step virus growth cycle setup. Related to Figure 3. **(A)** Schematic illustration of inhibitor treatment experiments. **(B)** Plaque assay was performed to determine the PFUs of SARS-CoV-2 virus in the supernatant of VeroE6 cells infected and subjected to inhibitor treatment (CMK 50 μM; naphthofluorescein 15 μM), which were applied 1 hr before infection and maintained in the medium until cell assessment at 24 hr or 48 hr post infection (Mean±SD of triplicate, $P < .05$*; $P < .01$**; $P < .001$***). **(C)** Viral protein in SARS-CoV-2-infected VeroE6 cells treated with CMK or naphthofluorescein were detected by immunoblot analyses.

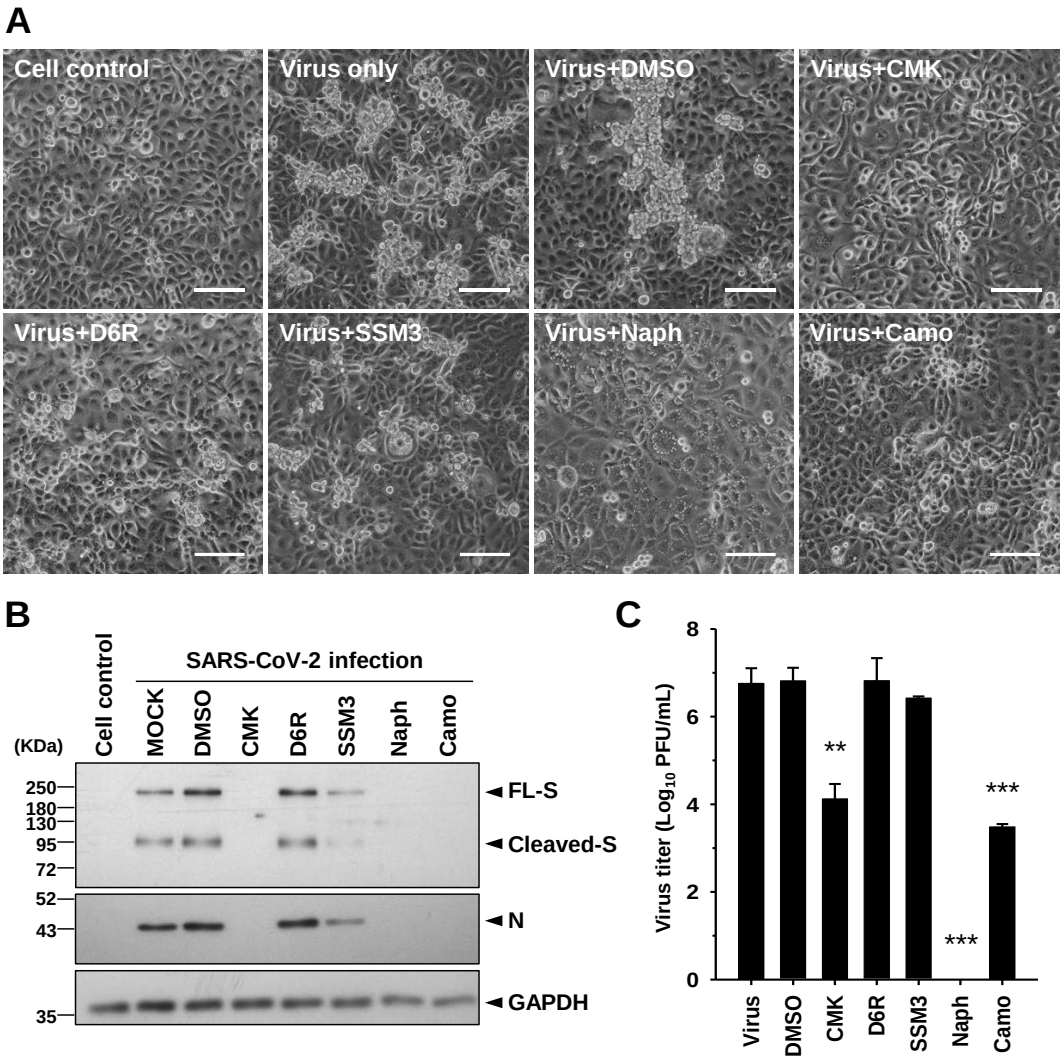


Figure S2. CMK, naphthofluorescein and camostat inhibitors block CPE and viral production in SARS-CoV-2-infected MK2 cells. Related to Figure 3. (A) Microscopic observation of CPE in MK2 cells infected with SARS-CoV-2 (MOI=1) in the absence or presence of CMK (50 μM), D6R (50 μM), SSM3 (25 μM), naphthofluorescein (20 μM)) or camostat (500 μM), which were applied 1 hr before infection and maintained in the medium until cell assessment 24 hr post treatment. Scale bars: 100 μm. **(B)** Immunoblot of lysates from MK2 cells infected with SARS-CoV-2 (MOI=1), which were treated with different inhibitors as described in (A). The immunoblot was probed with anti-spike and anti-nucleocapsid Abs. Full-length (FL) spike proteins, cleaved spike proteins and nucleocapsid (N) proteins are marked as indicated. GAPDH was included as the loading control. **(C)** Plaque assay was performed to determine the PFUs of SARS-CoV-2 virus in the supernatant of MK2 cells infected and subjected to inhibitor treatment as described in (A) (Mean±SD of triplicate, $P < .01$**; $P < .001$***).

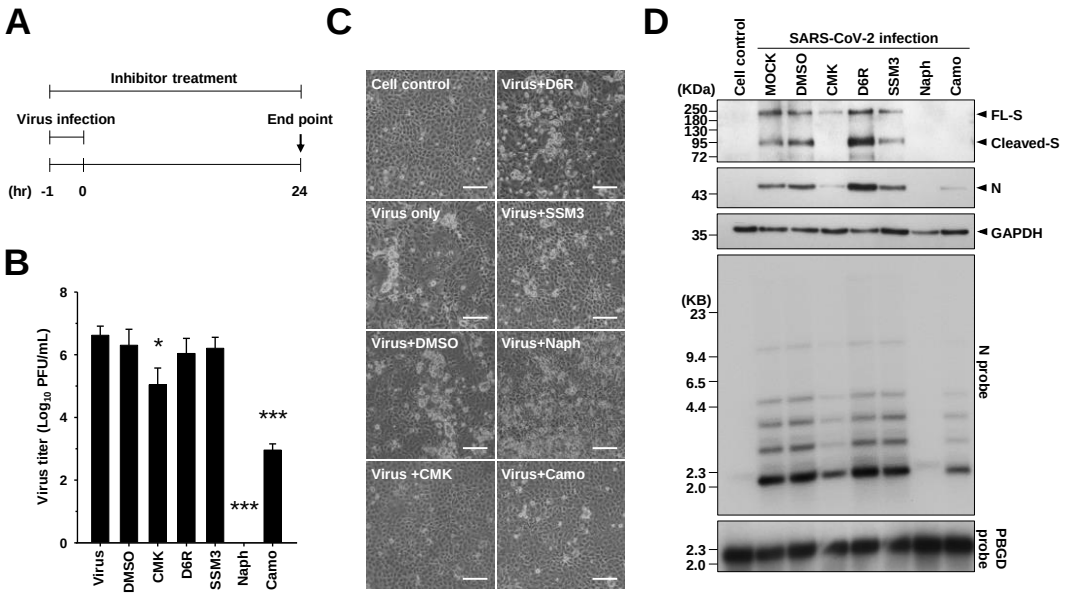


Figure S3. Co-administration of CMK, naphthofluorescein or camostat inhibitors with virus infection decreased viral production and CPE, in association with reduced viral RNA and protein levels in SARS-CoV-2-infected VeroE6 cells. Related to Figure 5. (A) Schematic illustration of inhibitor treatment experiments. **(B)** Plaque assay was performed to determine the PFUs of SARS-CoV-2 virus (MOI=1) in the supernatant of VeroE6 cells infected and subjected to inhibitor treatment (including CMK (50 μM), D6R (50 μM), SSM3 (25 μM), naphthofluorescein (20 μM) or camostat (500 μM)) for 24 hr (Mean±SD of triplicate, $P < .05$*; $P < .001$***). **(C)** Microscopic observation of CPE in VeroE6 cells infected with SARS-CoV-2 and subjected to inhibitor treatment as described in (A), which were assessed at 24 hr post treatment. Scale bars: 100 μm. **(D)** Viral protein and viral RNA in SARS-CoV-2-infected VeroE6 cells treated with indicated inhibitors were detected by immunoblot (upper panels) and northern blot (lower panels) analyses.

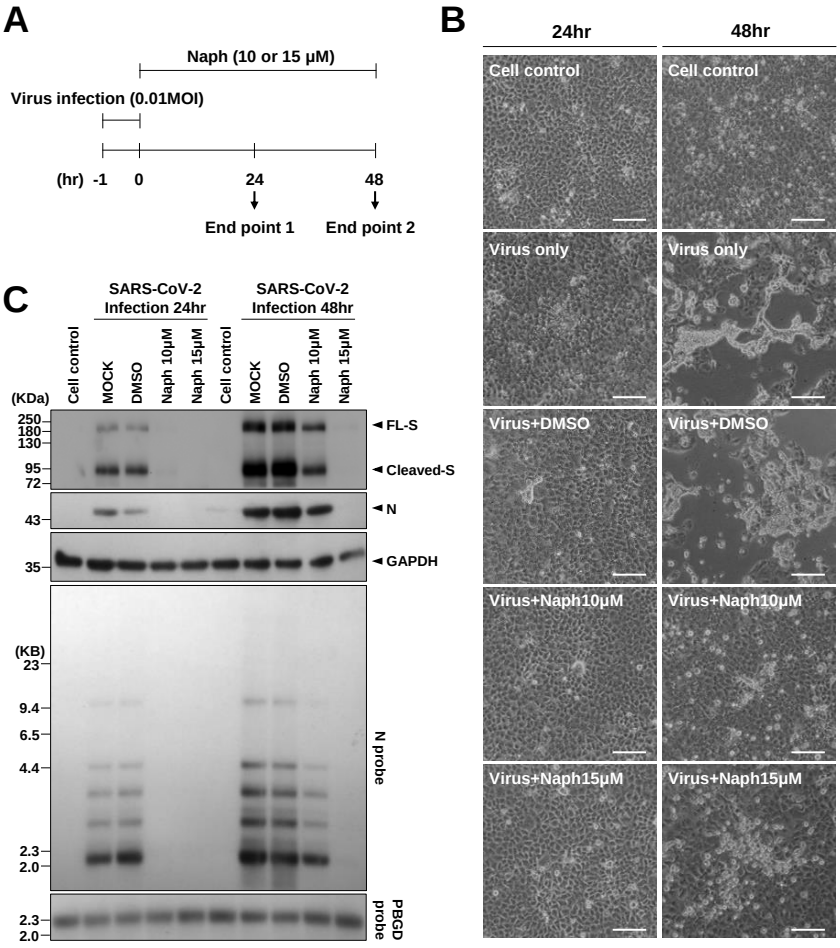
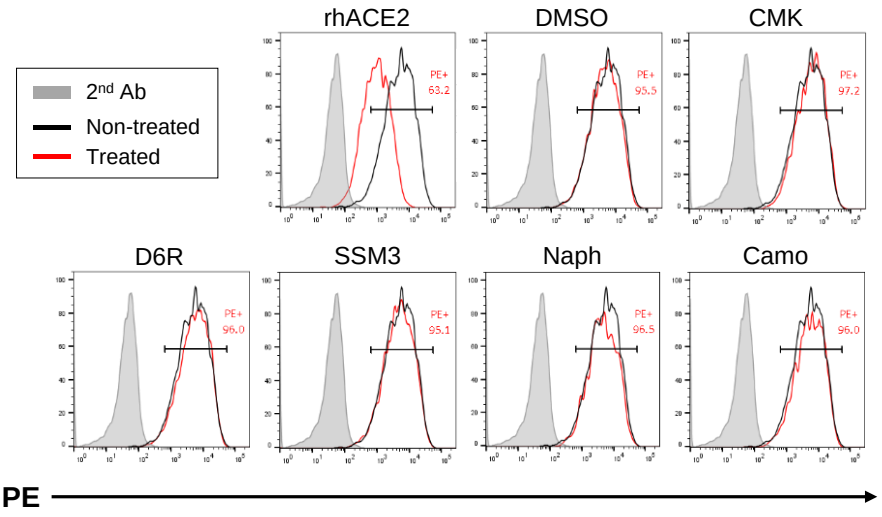


Figure S4. Post-treatment with naphthofluorescein reduced the viral transcription, showing a lower rate of increase in viral RNA transcripts at different time point. Related to Figure 5. (A) Schematic illustration of inhibitor treatment experiments. **(B)** Microscopic observation of CPE in VeroE6 cells infected with SARS-CoV-2 and subjected to inhibitor treatment as described in (A), which were assessed at 24 hr and 48 hr post treatment. Scale bars: 100 μm. **(C)** Viral protein and viral RNA in SARS-CoV-2-infected VeroE6 cells treated with naphthofluorescein (10 μM or 15 μM) were detected by immunoblot (upper panels) and northern blot (lower panels) analyses.

A



B

Group		% of PE+ cells	% of inhibition
Background	2nd Ab only	0.02	
Negative control	Non-treated	95.5	
Positive control	rhACE2	63.2	33.8
Inhibitor treatment	DMSO	95.5	0.0
	CMK 50 μM	97.2	0.0
	D6R 50 μM	96.0	0.0
	SSM3 25 μM	95.1	0.4
	Naph 20 μM	96.5	0.0
	Camo 500 μM	96.0	0.0

Figure S5. Furin/PC and TMPRSS2 inhibitors did not block the binding of the RBD of SARS-CoV-2 spike protein with human ACE2. Related to Figure 5. (A) Histogram of the flow cytometry results of blockage of RBD-hACE2 binding in 293T/hACE2 stable cell lines, including the positive control (recombinant human ACE2, rhACE2) and the ones with treatment of individual inhibitors as indicated. The X-axis indicates the phycoerythrin (PE) signal. The background signals induced by the secondary antibody are represented by the shaded gray lines, the signals for the non-treated groups are represented by the black lines (negative control), and the signals for the inhibitor treated groups are represented by the red lines. Recombinant human ACE2 is added to block the interaction of spike-RBD and hACE2, which is served as positive control for this assay (33.8% inhibition). **(B)** Quantification results for the flow cytometric data shown in (A).