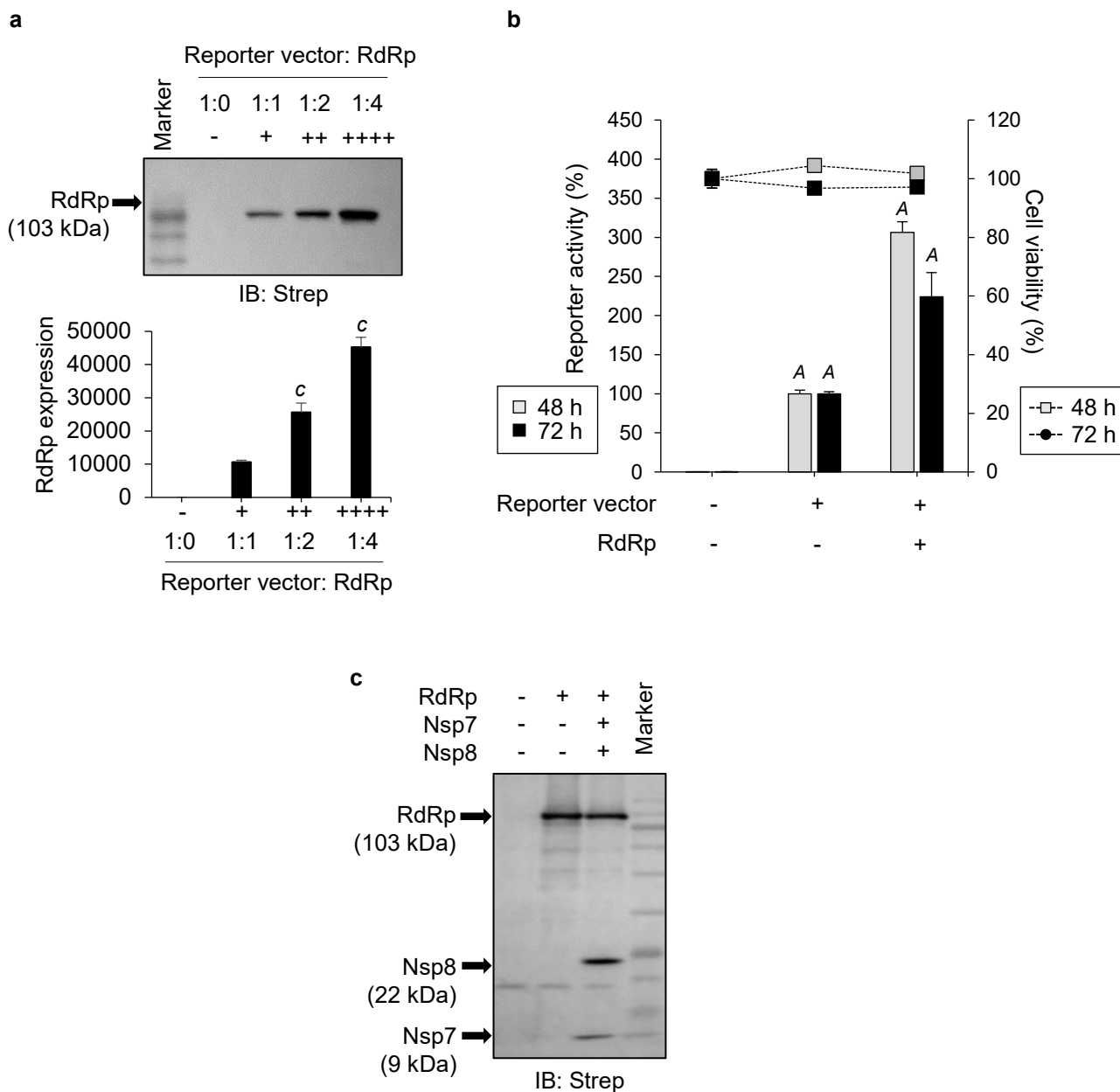
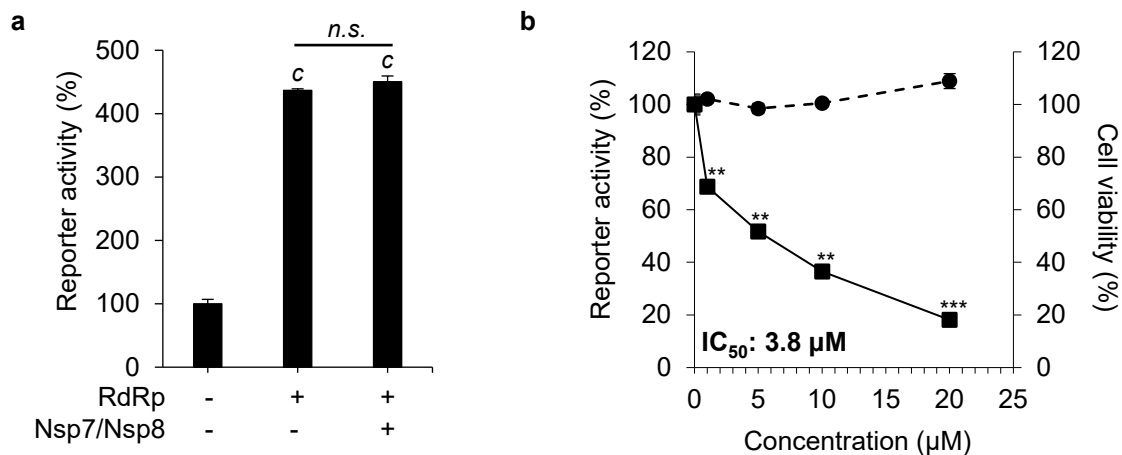
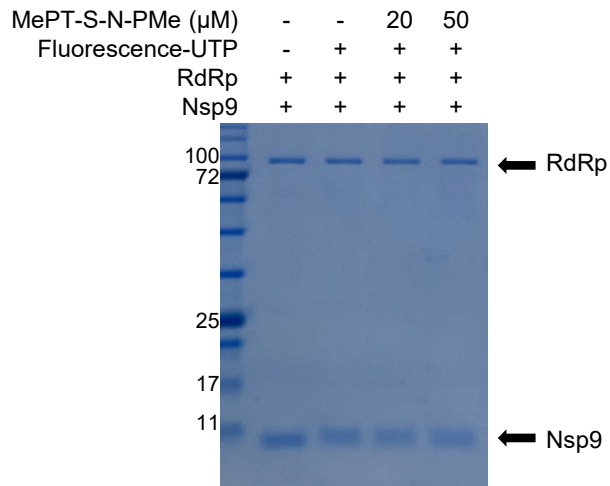




Supplementary Figure S1. Expression of Strep-tagged SARS-CoV-2 RdRp, Nsp7, and Nsp8 (a) HEK 293 cells were transfected with varying ratios of reporter and Strep-tagged RdRp, and subsequently incubated for 48 h. The protein expressions were subsequently validated through immunoblotting using an anti-Strep antibody. The immunoblotting bands were quantified using ImageJ based on three independent experiments conducted in triplicate. The results are presented as the band intensity relative to reporter expressed group. (b) HEK 293 cells were transfected with reporter and Strep-tagged RdRp and cultured for 48 and 72 h. Reporter activity was determined using a reporter assay kit, and cytotoxicity was assessed using Ez-Cytox. Results are expressed as a percentage of the group without reporter vector and RdRp. Luciferase activity is represented as a bar graph, and cell viability is represented as a dotted line. (c) Strep-tagged RdRp, Nsp7, and Nsp8 were transfected into HEK 293 cells and incubated for 48 h. Protein expression levels were determined by immunoblotting analysis using strep antibody. $p < 0.001$ compared to the RdRp-reporter expression group. All data are based on three independent experiments performed in triplicate. $^4p < 0.001$ compared to the group without reporter vector and RdRp.

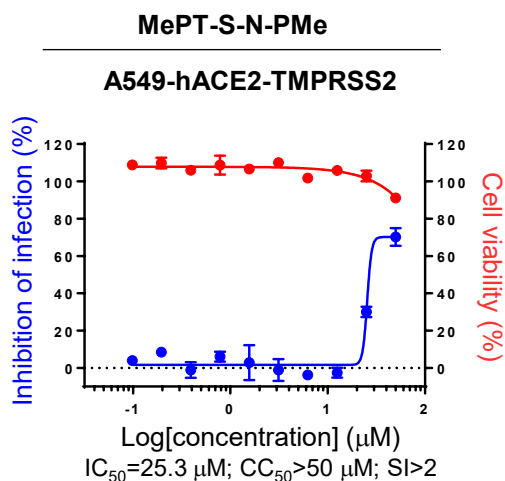


Supplementary Figure S2. Evaluation of the inhibitory effect of MePT-S-N-PMe on SARS-CoV-2 RdRp activity in A549 cell line. (a) The activity of reporter was compared between single expression of RdRp and co-expression of Nsp7, Nsp8, and RdRp in A549 cells transfected with reporter. The results are presented as the percentage relative to reporter expressed group (negative control group). (b) A549 cells expressing reporter and RdRp were treated with MePT-S-N-PMe (1, 5, 10, and 20 μM) for 24 h. The inhibition rate of RdRp activity for MePT-S-N-PMe were determined using a reporter assay kit, and cytotoxicity was assessed using Ez-Cytox. reporter activity and cell viability are indicated by solid and dotted lines, respectively. The results are presented as the percentage relative to reporter and RdRp co-expressed group (positive control group). All data are based on three independent experiments performed in triplicate. $p < 0.001$ compared to the reporter expression group; $**p < 0.01$ and $***p < 0.001$ compared to the reporter and RdRp co-expressed group; *n.s.*, non-significance.

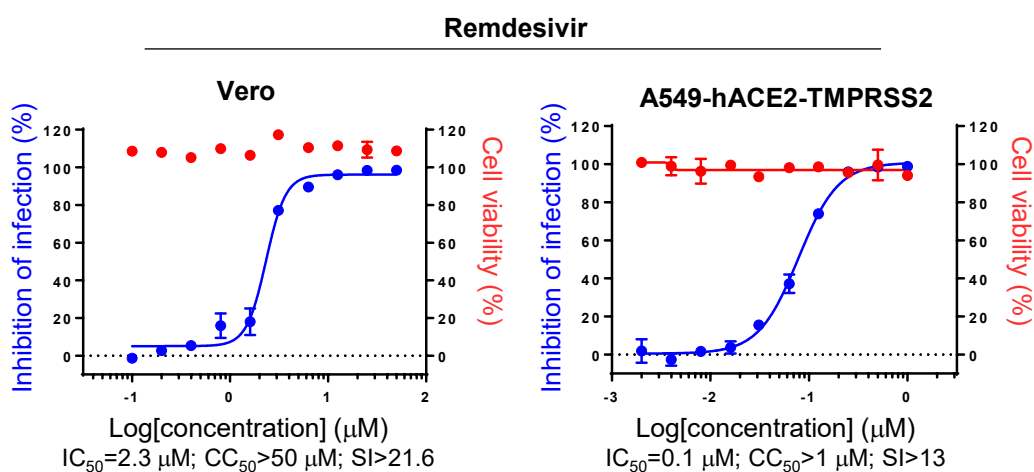


Supplementary Figure S3. The protein expression of SARS-CoV-2 RdRp and Nsp9 in NMPylation assay. Nsp9 (12 kDa) and RdRp (88-98 kDa) were separated on 12% SDS-PAGE gel and stained with Coomassie Brilliant Blue solution.

a



b



Supplementary Figure S4. Inhibition of SARS-CoV-2 replication by MePT-S-N-PMe and remdesivir in Vero and A549-hACE2-TMPRSS2 cells. (a) A549-hACE2-TMPRSS2 cells were treated with various concentrations of MePT-S-N-PMe or (b) Vero cells and A549-hACE2-TMPRSS2 cells were treated with remdesivir as a positive control. Following compound treatment, cells were inoculated with authentic SARS-CoV-2 viruses. In Vero cells, after 24 h, cells were fixed with 4% paraformaldehyde and immunostained for SARS-CoV-2. In A549-hACE2-TMPRSS2 cells, luciferase activity and cell viability were measured after 24 h of incubation at 37°C using the Nano-Glo® Luciferase assay and CellTiter-Glo Luminescent cell viability assay, respectively, with signal detection by a VICTOR Nivo™ multilabel plate reader. The extent of SARS-CoV-2 infection (blue) and cell viability (red) were quantified relative to the untreated control group and are presented as percentage values in the graph. Data represent the mean $\pm$ standard deviation from duplicate experiments.