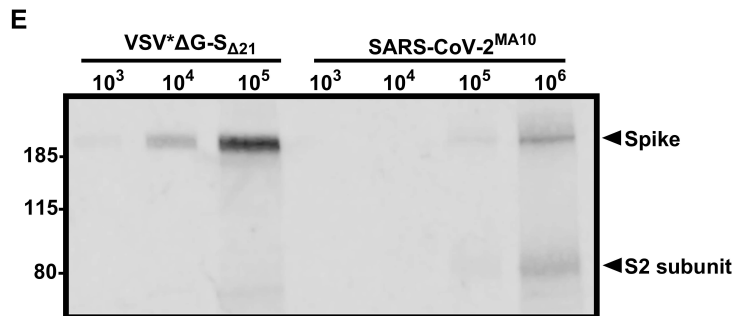
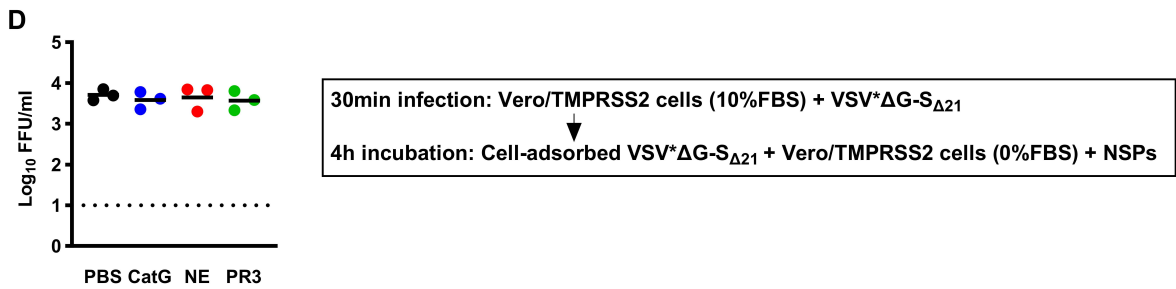
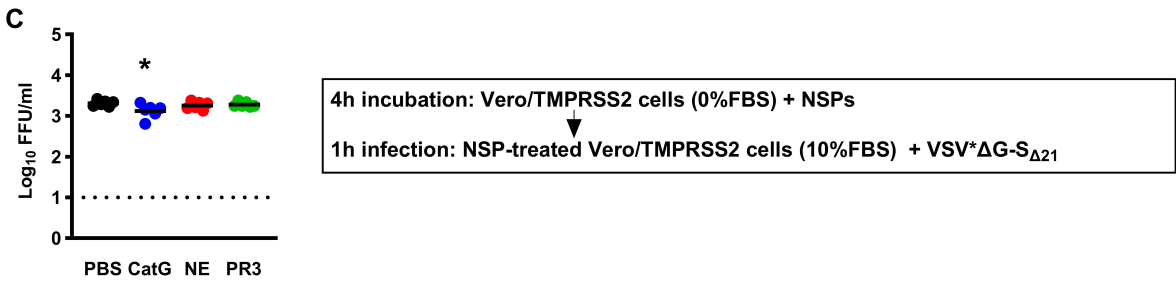
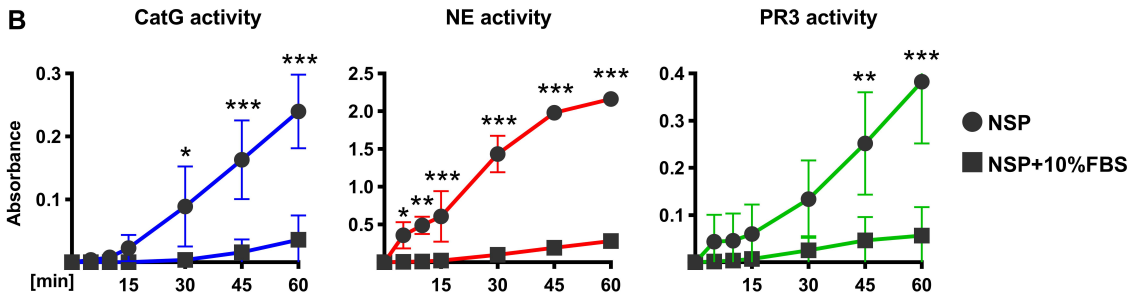
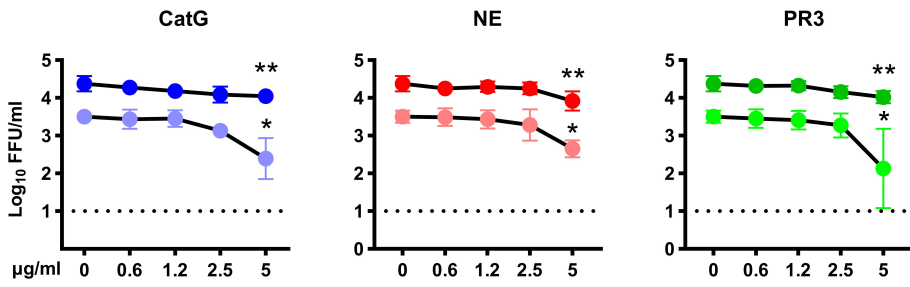


A 4h incubation: High titer (dark) or low titer (light) VSV* Δ G-S Δ 21 + NSPs
 1h infection: NSPs-treated VSV* Δ G-S Δ 21 + Vero/TMPRSS2 (10%FBS)



Supplemental Figure 1. Effects of NSPs on entry of VSV*ΔG-S_{Δ21} in Vero/TMPRSS2 cells.

(A) VSV*ΔG-S_{Δ21} (4×10^4 FFU dark dots; or 4×10^3 FFU light dots) were preincubated with purified NSPs (0.6-5 μg/ml) for 4h at 37°C in PBS. Vero/TMPRSS2 cells in DMEM containing 10% FBS were then inoculated with serial dilutions of NSP-treated VSV*ΔG-S_{Δ21} to determine titers. Data are shown as mean ± SD of 4 independent experiments and were analyzed by one-way ANOVA with Dunnett's multiple comparisons test, comparing the NSP-treated groups to the PBS control; * $P < 0.05$, ** $P < 0.01$. (B) Activity of NSPs (5 μg/ml) in phenol red-free DMEM containing 0% or 10% FBS was determined using specific chromogenic substrates for CatG (Ala-Ala-Pro-Phe), NE or PR3 (Ala-Ala-Pro-Val). Data represent mean ± SD and were analyzed by two-way ANOVA with Sidak's multiple comparisons test, comparing the effect of serum at each time point (n=3); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (C) Vero/TMPRSS2 cells were incubated with NSPs (5 μg/mL) for 4h in FBS-free DMEM. Cells were inoculated with 4×10^3 FFU VSV*ΔG-S_{Δ21} for 1h in DMEM with 10% FBS. Data were analyzed by one-way ANOVA with Dunnett's multiple comparisons test, comparing the NSP-treated groups to the control (n=6); * $P < 0.05$. (D) Titers of cell-adsorbed VSV*ΔG-S_{Δ21} incubated with NSPs. Vero/TMPRSS2 cells were inoculated with 4×10^3 FFU VSV*ΔG-S_{Δ21} for 30 minutes at 37°C, 5% CO₂ allowing virus cell adsorption. Then, medium was removed and FBS-free medium containing purified human CatG, NE, or PR3 at a final concentration of 5 μg/ml was added. Vero/TMPRSS2 cells, cell adsorbed- VSV*ΔG-S_{Δ21} and NSPs were incubated for 4h at 37°C, 5% CO₂. Data were analyzed by one-way ANOVA with Dunett's multiple comparisons test, comparing the NSP-treated groups to the PBS control (n=3). (A-D) Infected cells were detected by fluorescence microscopy and virus titers were calculated and expressed as FFU/ml. (E) Immunoblot of VSV*ΔG-S_{Δ21} and SARS-CoV-2^{MA10} lysates probed with anti-S2 subunit antibody.