OMTN, Volume *35*

Supplemental information

Inhibition of SARS-CoV-2 growth in the lungs of mice by a peptide-conjugated morpholino oligomer targeting viral RNA

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Table S1. Bioinformatic analysis of sequence agreement* between 5'END-2 PPMO and SARS-CoV-2 target in Omicron genomes from an 18 month period from April 2022-October 2023.

SARS-CoV-2 Sequence collection dates	Number of sequences analyzed	% with perfect agreement between 5'END-2 and SARS-CoV-2	% with 1 mismatch between 5'END-2 and SARS-CoV-2	% with 2 mismatches between 5'END-2 and SARS-CoV-2	% with 3 mismatches between 5'END-2 and SARS-CoV-2	% with 4 or more mismatches between 5'END-2 and SARS-CoV-2
April 2022-Sept 2022	185,638	98.08	1.5	0.18	0.1	0.13
October 2022-March 2023	95,430	98.25	1.33	0.15	0.09	0.19
April 2023-October 2023	40,585	96.85	2.36	0.26	0.03	0.5

*Based on Wuhan-Hu-1, GenBank NC_045512

Fig.S1

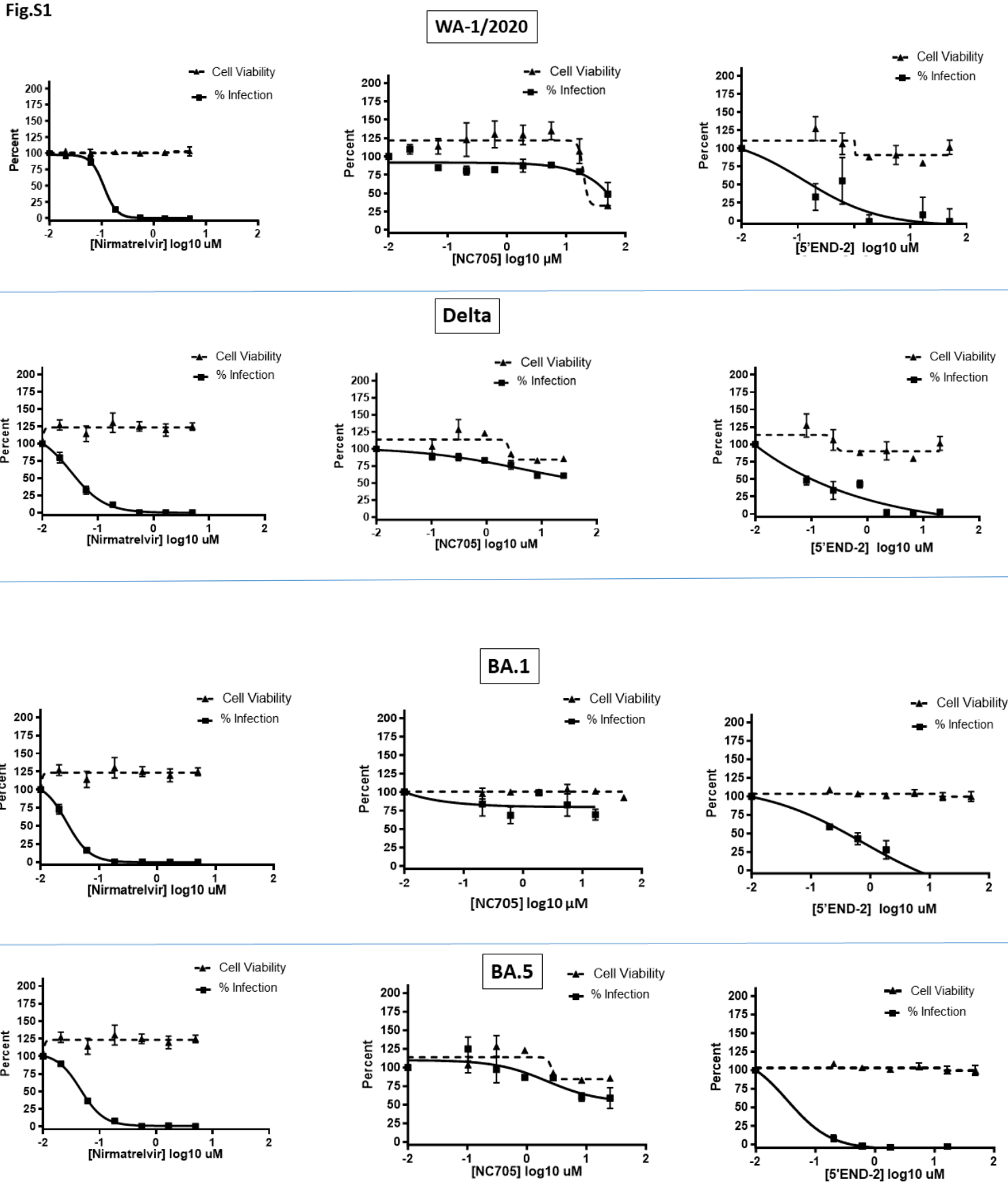
WA-1/2020
Cell Viability
% Infection
Percent
[Nirmatrelvir] log10 uM
[NC705] log10 µM
[5'END-2] log10 uM
Delta
[Nirmatrelvir] log10 uM
[NC705] log10 uM
[5'END-2] log10 uM
BA.1
[Nirmatrelvir] log10 uM
[NC705] log10 µM
[5'END-2] log10 uM
BA.5
[Nirmatrelvir] log10 uM
[NC705] log10 uM
[5'END-2] log10 uM

Fig.S1 con't

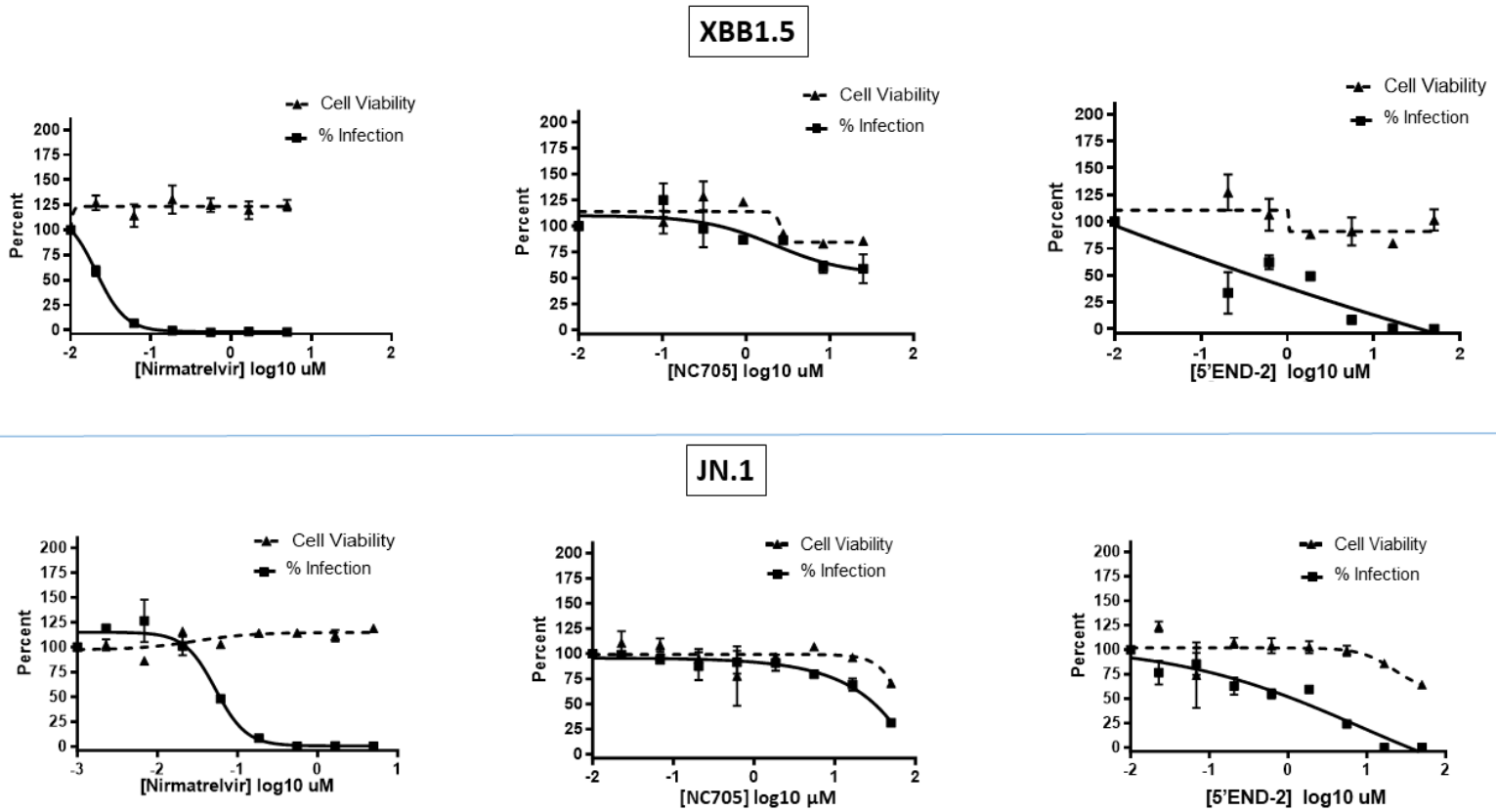

Figure S1. PPMO 5'END-2 inhibition of SARS-CoV-2 growth in HeLa-Ace2 cells is dose responsive and non-cytotoxic. Nirmetralvir and PPMOs NC705 and 5'END-2 were titrated in uninfected cells, to determine their affect on cell viability (as a percentage difference compared to PBS-treated cells) and, at the same set of concentrations, on cells infected with six (indicated) SARS-CoV-2 variants to determine antiviral activity (as a percentage difference compared to PBS-treated cells). See Materials and Methods for detailed information on the experimental conditions and protocol. The curves were fitted with a nonlinear regression model to determine EC_{50}, EC_{90} and IC_{50} concentrations using GraphPad Prism version 10.0.0. Error bars denote mean ± SD of 3 independent replicates.

Figure S2

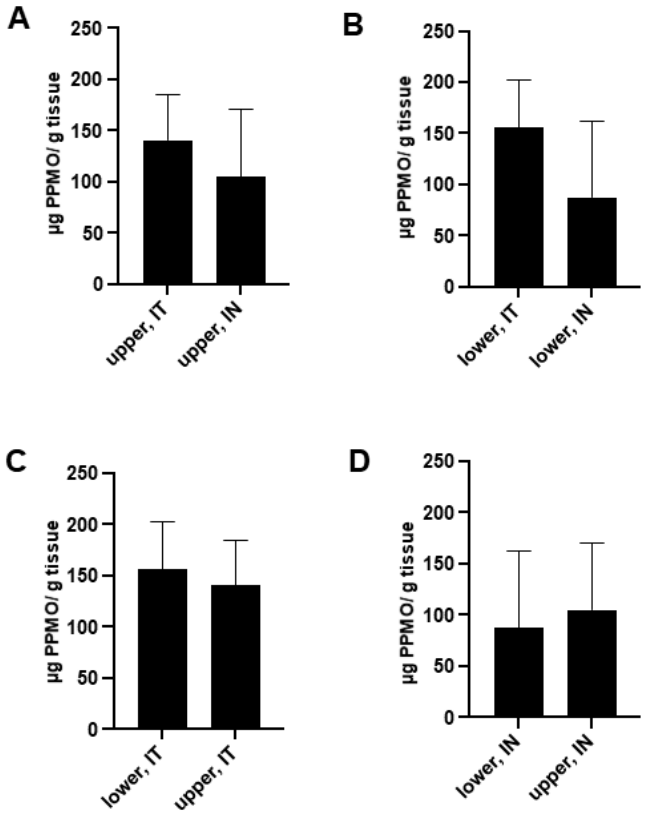

Figure S2. PPMO-lissamine concentration in mouse lung 24 hours following intratracheal/intranasal instillation. Fluorescence quantitation of PPMO-lissamine in mouse lungs after direct administration to the airway. A single treatment of PPMO-lissamine at 10mg/kg was administered via intratracheal (IT) or intranasal (IN) instillation to K18-ACE2 mice. Total fluorescence (represented as micrograms PPMO per gram of lung tissue) was measured in upper and lower lungs 24 hrs after the treatment, as described in Materials and Methods. Comparison of intratracheal and intranasal routes in (A) upper and (B) lower lung; comparison of upper and lower lung for (C) intratracheal and (D) intranasal routes. Mean with standard deviation shown are shown (n=5). Data were analyzed using unpaired two-tailed Student's t-tests, but did not achieve statistical significance.