Supplementary Information

Methods

Cell culture infection assay

VeroE6/TMPRSS2 cells, a VeroE6 cell clone overexpressing the transmembrane protease, serine 2 (TMPRSS2), were cultured in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal bovine serum (FBS) (Sigma), 100 units/mL penicillin, 100 μg/mL streptomycin, 10 mM HEPES (pH 7.4), and 1 mg/mL G418 (Nacalai) at 37˚C in 5% CO2 (Ohashi et al., 2021).

SARS-CoV-2 was investigated in a biosafety level 3 (BSL3) room. Through viral inoculation, we used WK-521 (Wuhan strain, EPI_ISL_408667), QK002 (Alpha, EPI_ISL_768526), TY7-501 (Gamma, EPI_ISL_833366), TY11-927 (Delta, EPI_ISL_2158617), TY38-873 (Omicron BA.1, EPI_ISL_7418017), and TY40-385 (Omicron BA.2, EPI_ISL_9595859) strains, isolated from COVID-19 patients and registered in GISAID. Viral infectious titers were measured by inoculating VeroE6/TMPRSS2 cells with a 10-fold serial dilution of the virus followed by measuring cytopathology to calculate $TCID_{50}$/ml (Ohashi et al., 2021). Infection assay was conducted by inoculating VeroE6/TMPRSS2 cells with each SARS-CoV-2 strain at an MOI of 0.003 for 1 h, followed by washing out free viruses and culturing cells with fresh medium. A medium with 2% FBS without G418 was used during the infection assay. Antibodies and antiviral drugs were treated for 1 h during virus inoculation and 24 h after inoculation. At 24 h postinfection, the culture supernatant was recovered to isolate RNA using MagMax Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher Scientific) and quantify SARS-CoV-2 RNA by real time RT-PCR with a one-step qRT-PCR kit (THUNDERBIRD Probe One-step qRT-PCR kit, TOYOBO) using 5'- ACAGGTACGTTAATAGTTAATAGCGT-3', 5'-ATATTGCAGCAGTACGCACACA-3', and 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-TAMRA-3'. Simultaneously, cell viability was quantified by fixing the cells with 4% paraformaldehyde and staining with DAPI to count the number of cells using a high content imaging system ImageXpress (Molecular Devices).

Statistics

Statistical significance was determined by using Student's t test. P-values < 0.05 were considered significant. Where applicable, P-values are indicated as $*P < 0.05$ or $**P < 0.01$.

Supplementary Figure

Fig. S1

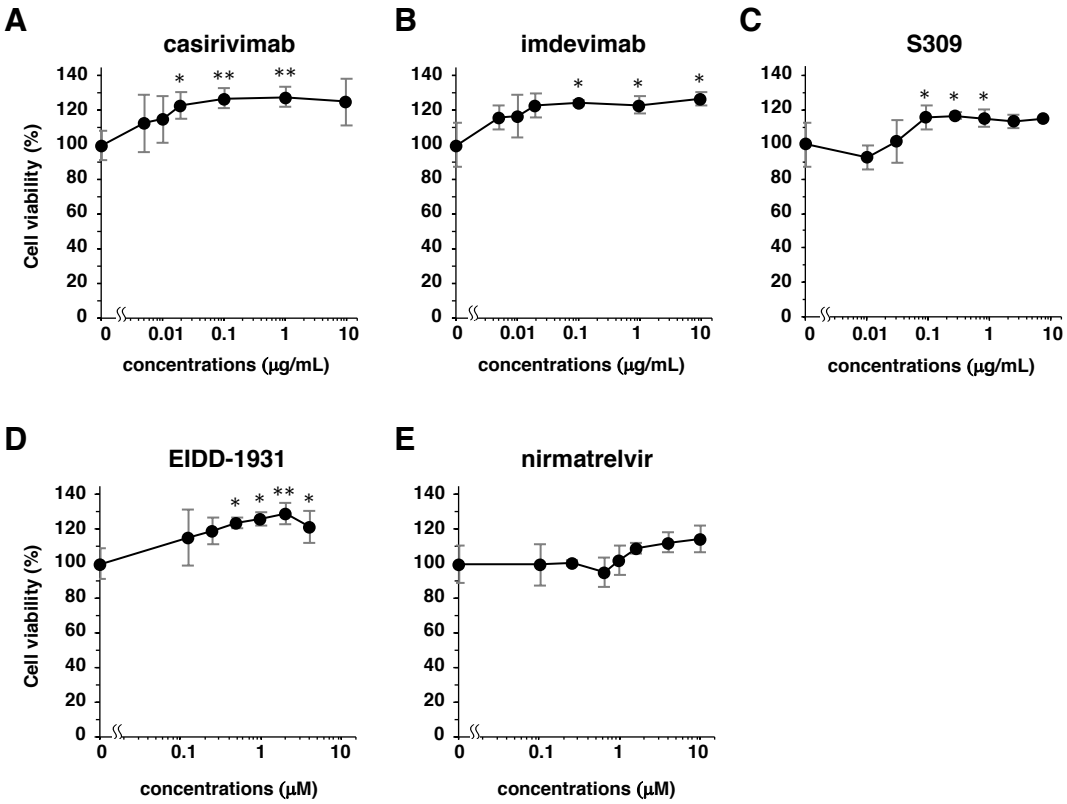

Fig. S1. Cell viability upon treatment with neutralizing antibodies and antiviral drugs. VeroE6/TMPRSS2 cells were inoculated with SARS-CoV-2 at an MOI of 0.003 in the presence of indicated antibodies or drugs at indicated concentrations for 1 h. The cells were then washed out and were cultured in a medium supplemented with the indicated concentrations of antibodies or drugs. After 24 h, cells were subsequently fixed with 4% paraformaldehyde and stained with DAPI, followed by counting using a high content imaging system ImageXpress Micro Confocal (Molecular Devices) to quantify cell viability. Data are presented as mean ± SD across the three replicate experiments. Relative values are shown as percentages of cell viability to the control cells that were incubated without antibodies/drugs. Statistical significance was determined by using Student's t test (∗p <0.05, ∗∗p <0.01).

Fig. S2

casirivimab

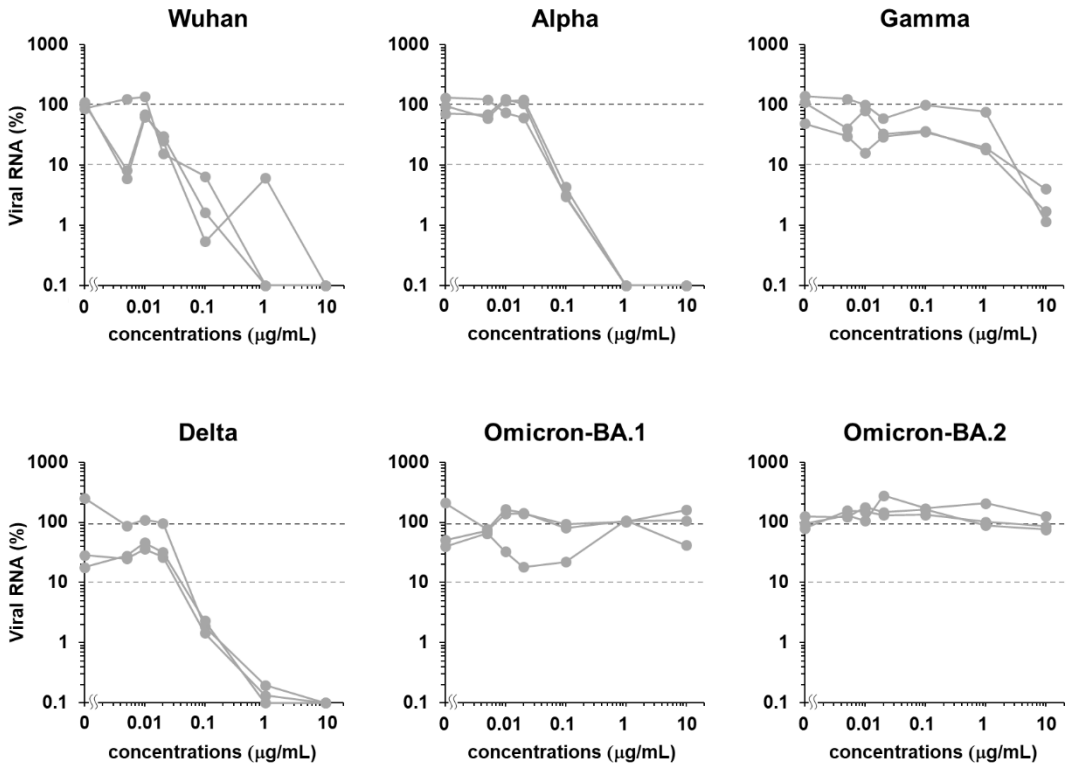

Figure. S2. Dose-response curves of casirivimab for SARS-CoV-2 variants (Wuhan, alpha, gamma, delta, omicron BA.1, and omicron BA.2). Secreted viral RNA into the supernatant from infected VeroE6/TMPRSS2 cells at 24 hours after virus inoculation was quantified and plotted in gray against drug concentration. Values less than 0.1% are shown as 0.1% in these graphs.

Fig. S3

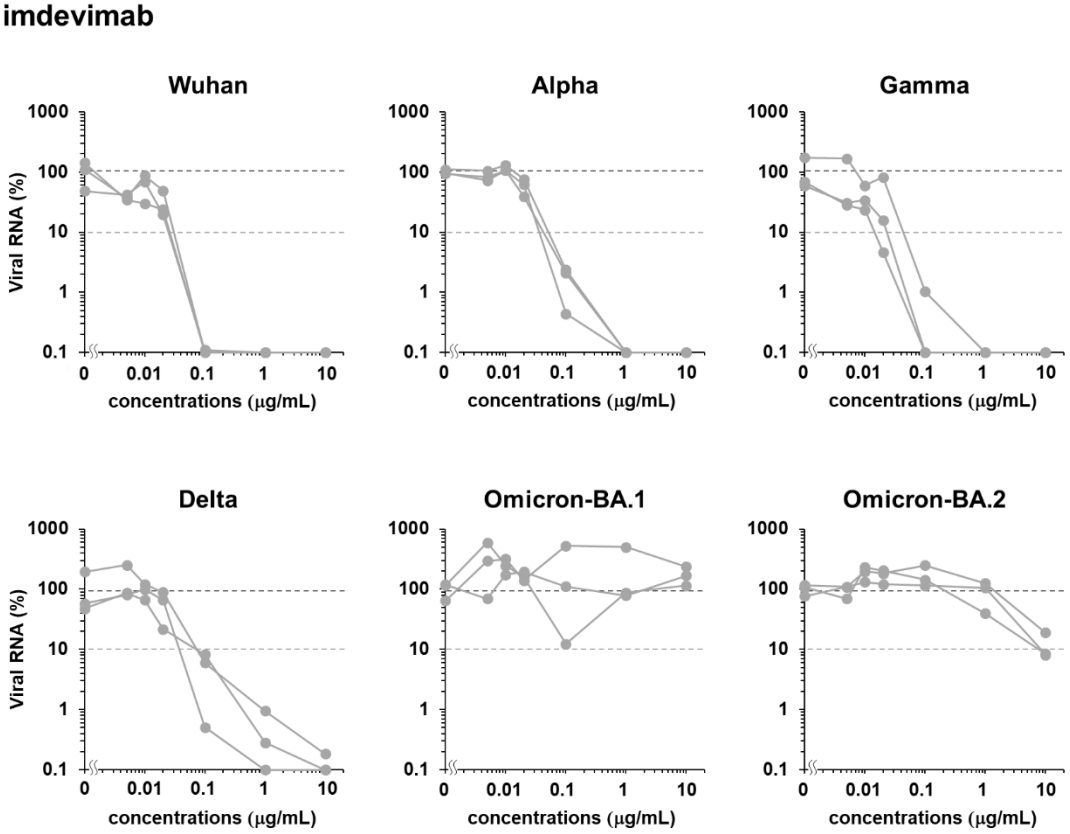

Figure. S3. Dose-response curves of imdevimab for SARS-CoV-2 variants (Wuhan, alpha, gamma, delta, omicron BA.1, and omicron BA.2). Secreted viral RNA into the supernatant from infected VeroE6/TMPRSS2 cells at 24 hours after virus inoculation was quantified and plotted in gray against drug concentration. Values less than 0.1% are shown as 0.1% in these graphs.

Fig. S4

S309

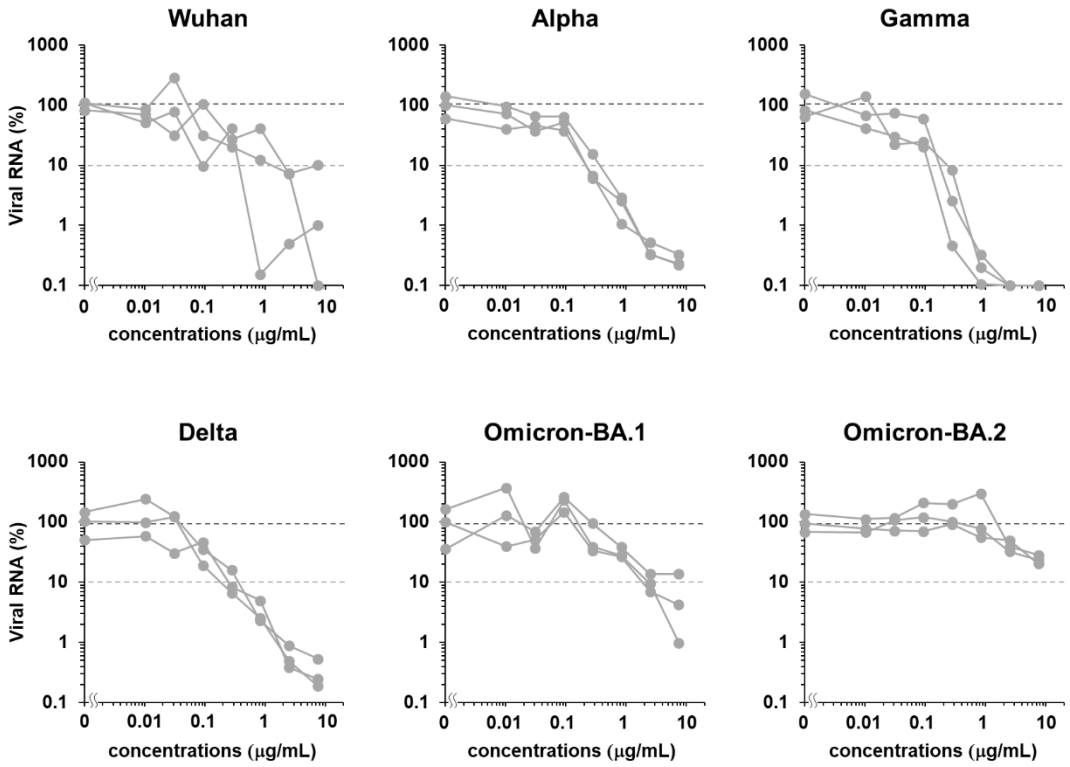

Figure. S4. Dose-response curves of S309 for SARS-CoV-2 variants (Wuhan, alpha, gamma, delta, omicron BA.1, and omicron BA.2). Secreted viral RNA into the supernatant from infected VeroE6/TMPRSS2 cells at 24 hours after virus inoculation was quantified and plotted in gray against drug concentration. Values less than 0.1% are shown as 0.1% in these graphs.

Fig. S5

EIDD-1931

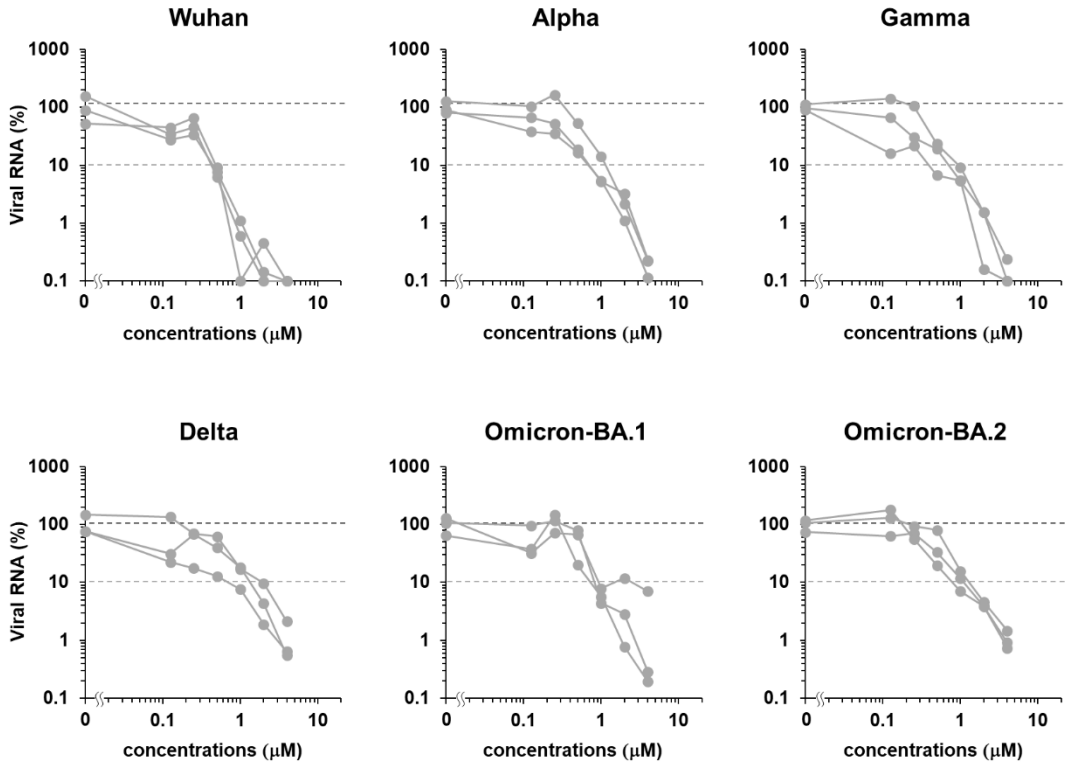

Figure. S5. Dose-response curves of EIDD-1931 for SARS-CoV-2 variants (Wuhan, alpha, gamma, delta, omicron BA.1, and omicron BA.2). Secreted viral RNA into the supernatant from infected VeroE6/TMPRSS2 cells at 24 hours after virus inoculation was quantified and plotted in gray against drug concentration. Values less than 0.1% are shown as 0.1% in these graphs.

Fig. S6

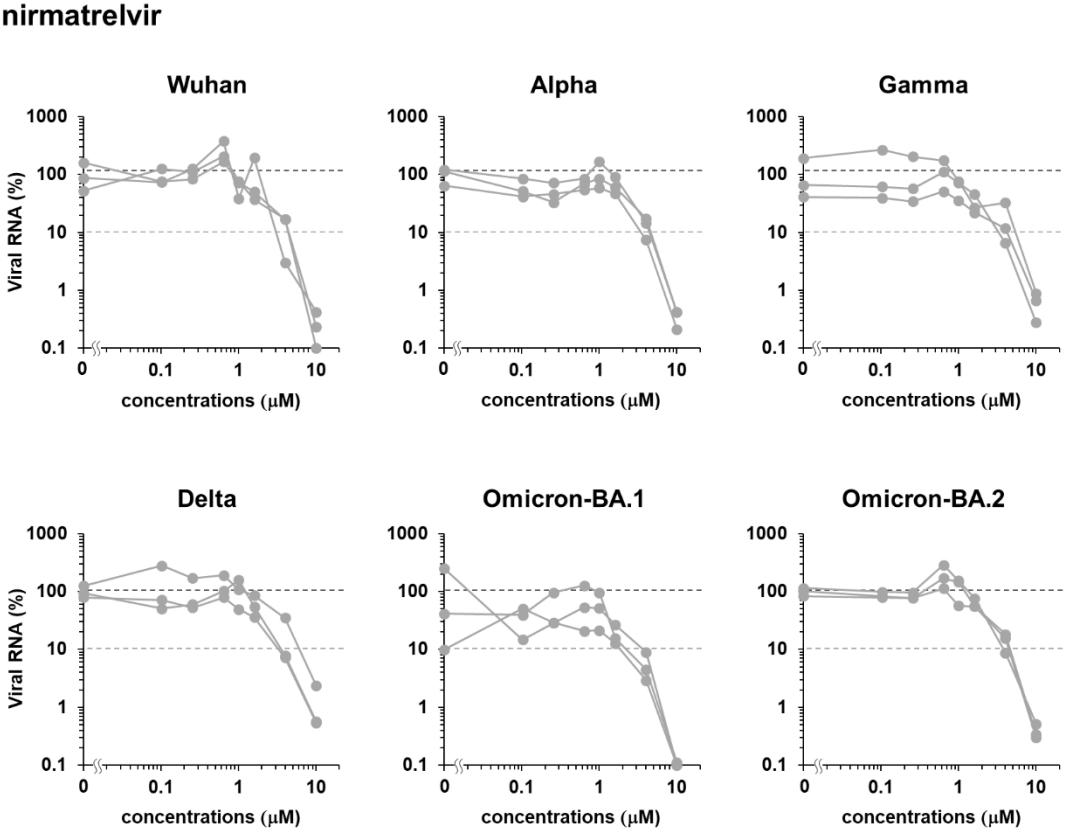

Figure. S6. Dose-response curves of nirmatrelvir for SARS-CoV-2 variants (Wuhan, alpha, gamma, delta, omicron BA.1, and omicron BA.2). Secreted viral RNA into the supernatant from infected VeroE6/TMPRSS2 cells at 24 hours after virus inoculation was quantified and plotted in gray against drug concentration. Values less than 0.1% are shown as 0.1% in these graphs.

Supplementary Table

Table. S1. Drug concentrations in patients

Drug	C_{max} (unit)	AUC (unit)	Molecular mass (unit)
casirivimab	192 (μg/mL)	2580 (day*μg/mL)[*1]	145230 (g/mol)
imdevimab	198 (μg/mL)	1990 (day*μg/mL)[*1]	144140 (g/mol)
S309	143 (μg/mL)	1410 (day*μg/mL)[*2]	149000 (g/mol)
EIDD-1931	2.97 (μg/mL)	602.0 (day*μM)[*3]	329.31 (g/mol)
nirmatrelvir	2.21 (μg/mL)	1105 (day*μM)[*4]	499.54 (g/mol)

[*1] AUC_{inf}, 600 mg subcutaneous injection
[*2] $AUC_{D1\text{-}29}$, sotrovimab 500 mg intravenous infusion
[*3] $AUC_{0\text{-}12h}$, 800 mg oral administration in patients
[*4] AUC_{inf}, 300 mg with ritonavir (100 mg) oral administration in healthy subjects

The pharmacokinetics of these antibodies/drugs are available in reference articles (2-5).

References

1. Ohashi, H., Watashi, K., Saso, W., Shionoya, K., Iwanami, S., Hirokawa, T., Shirai, T., Kanaya, S., Ito, Y., Kim, K.S., et al. (2021). Potential anti-COVID-19 agents, cepharanthine and nelfinavir, and their usage for combination treatment. iScience 24, 102367. doi: 10.1016/j.isci.2021.102367

2. Food and Drug Administration (2021) Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of REGEN-COV (casirivimab and imdevimab). https://www.fda.gov/media/145611/download

3. Food and Drug Administration (2022) Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Sotrovimab. https://www.fda.gov/media/149534/download

4. Food and Drug Administration (2022) Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Molnupiravir. https://www.fda.gov/media/155054/download

5. Food and Drug Administration (2021) Fact Sheet for Health Care Providers Emergency Use Authorization (EUA) of Paxlovid. https://www.fda.gov/media/155050/download