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Supplemental information

Bioluminescence imaging reveals enhanced SARS-CoV-2 clearance in mice with combinatorial regimens

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Supplementary Figures:

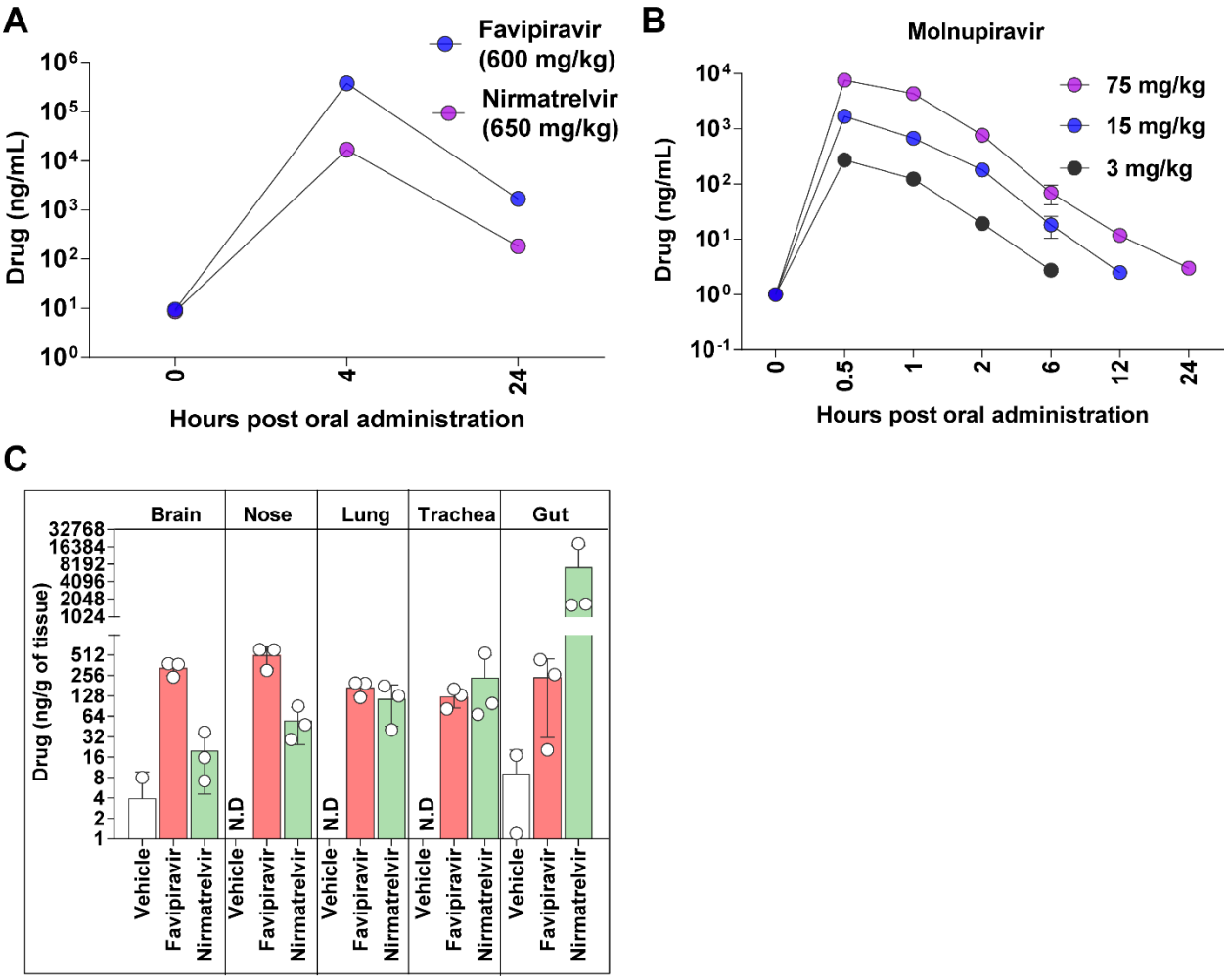

Figure S1. Pharmacokinetics of Antiviral Drugs used in the Study, Related to Figure 1

(A, B) Temporal concentrations (ng/ml) of indicated drugs in the serum. Favipiravir (600 mg/kg b.w, i.p.) and nirmatrelvir (650 mg/kg b.w., oral) were administered to K18-hACE2 mice and serum samples were collected at 0, 4 and 24 h post-treatment in A. Molnupiravir (3 mg/kg, 15 mg/kg and 75 mg/kg b.w, oral) was administered to BALB/c mice and serum samples were collected at 0.5, 1, 2, 6, 12 and 24 h post-treatment in B).

(C) Drug concentrations (ng/g of tissue) in indicated clarified tissue homogenates for an experiment as in A at 24 h post-treatment following necropsy.

b.w.: body weight

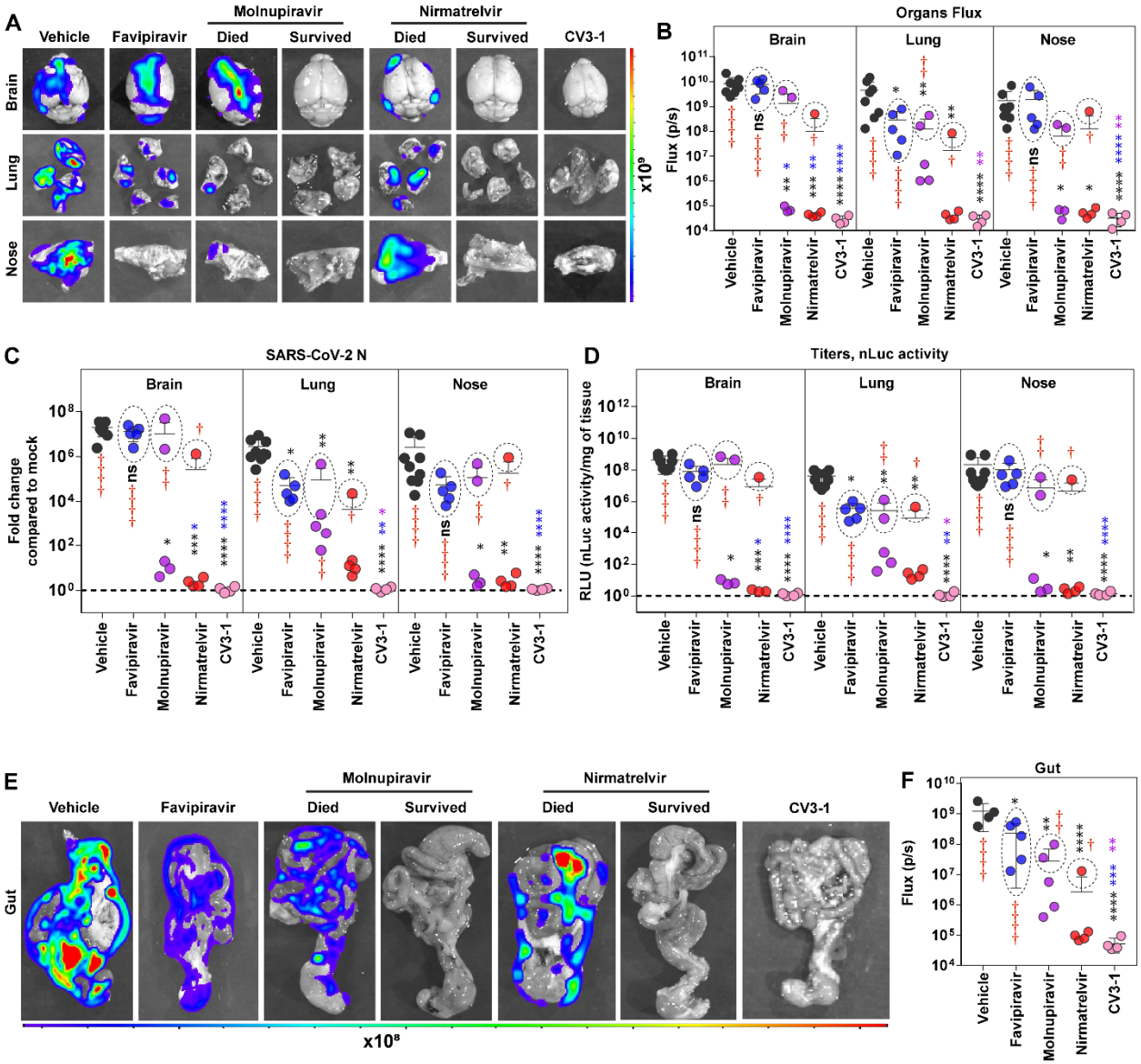

Figure S2. *In Vivo* Efficacy of Antiviral Drugs and Neutralizing Antibody Against Delta VOC, Related to Figure 1.

(A-B) *Ex vivo* imaging of indicated organs and quantification of nLuc signal as flux (photons/sec) after necropsy for an experiment shown in Figure 1A. Organs from dead and surviving mice are shown separately for clarity.

(C) Fold changes in nucleocapsid (N) mRNA expression in brain, lung, and nasal cavity. Data were normalized to *Gapdh* mRNA expression in the same sample and that in non-infected mice after necropsy when mice died from infection or at 14 or 22 dpi for surviving mice.

(D) Viral loads (nLuc activity/mg) in indicated organs from mice under specified treatment regimens evaluated using Vero E6 cells as targets when mice died from infection or at 14 or 22 dpi for surviving mice.

(E, F) *Ex vivo* imaging of gut and quantification of nLuc signal as flux (photons/sec) after necropsy at the time of death (see Figure 1F) for an experiment shown in Figure 1A.

Scale bars in (A) and (E) denote radiance (photons/s/cm^2/steradian). Grouped data in (B-D), were analyzed by 2-way ANOVA followed by Tukey's multiple comparison tests. The data in (F) was analyzed by one-way ANOVA followed by Kruskal-Wallis' test. Statistical significance for group comparisons to Vehicle are shown in black, with favipiravir are shown in blue, with molnupiravir are shown in purple, with nirmatrelvir are shown as red and with CV3-1 are shown as pink. $*$, $p < 0.05$; $**$, $p < 0.01$; ns not significant; Mean values ± SD are depicted. Dotted circles with red cross signs at indicated time points are used to demarcate mice that succumbed to infection.

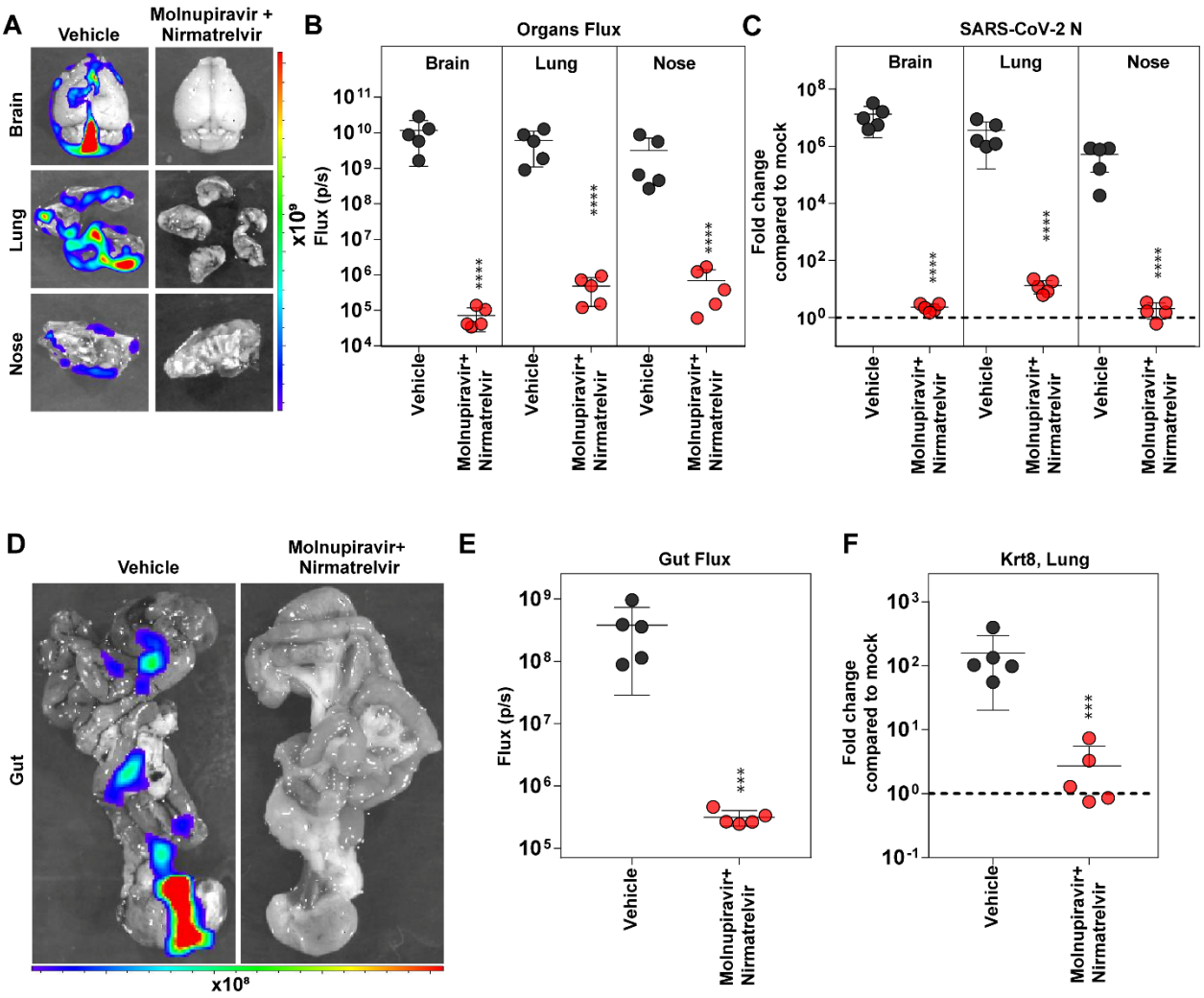

Figure S3. *In Vivo* Efficacy of Molnupiravir Combined with Nirmatrelvir Against Delta VOC, Related to Figure 2.

(A-B) *Ex vivo* imaging of indicated organs and quantification of nLuc signal as flux (photons/sec) after necropsy for an experiment shown in Figure 2A.

(C) Fold changes in nucleocapsid (N) mRNA expression in brain, lung, and nasal cavity upon death or at 14 dpi in the surviving animals. Data were normalized to *Gapdh* mRNA in the same sample and that in non-infected mice after necropsy.

(D, E) *Ex vivo* imaging of gut and quantification of nLuc signal as flux (photons/sec) after necropsy at the time of death (see Figure 2F) for an experiment shown in Figure 2A.

Scale bars in (A) and (D) denote radiance (photons/s/cm^2/steradian). Grouped data in (B-D), were analyzed by 2-way ANOVA followed by Tukey's multiple comparison tests. The data in (F) was

analyzed by no parametric Mann-Whitney test. $*$, $p < 0.05$; $**$, $p < 0.01$; ns not significant; Mean values ± SD are depicted.

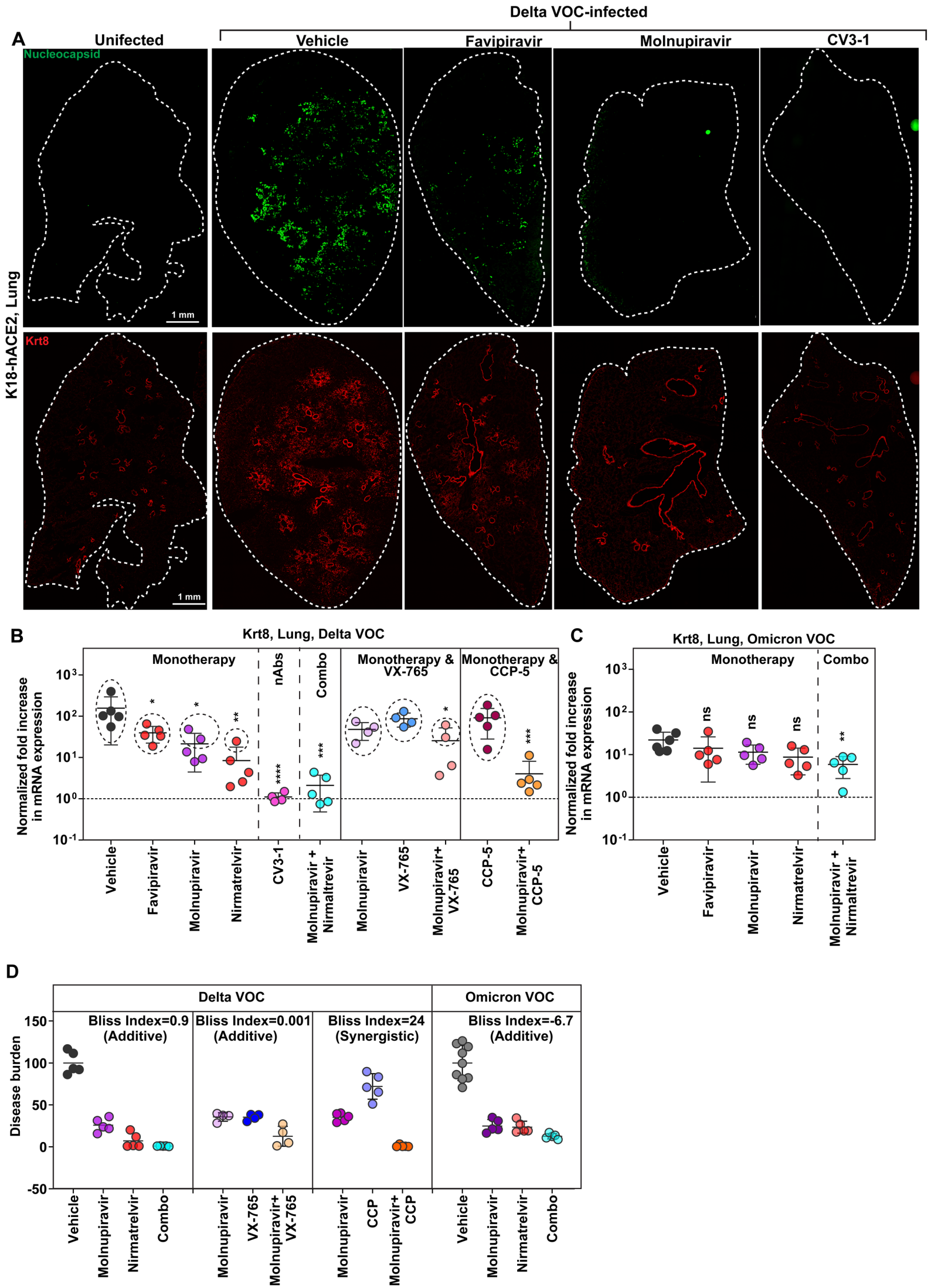
Delta VOC-infected
A
Unifected
Vehicle
Favipiravir
Molnupiravir
CV3-1
Nucleocapsid
K18-hACE2, Lung
1 mm
Krt8
1 mm
B
Krt8, Lung, Delta VOC
Monotherapy
nAbs
Combo
Monotherapy & VX-765
Monotherapy & CCP-5
Normalized fold increase in mRNA expression
Vehicle
Favipiravir
Molnupiravir
Nirmatrelvir
CV3-1
Molnupiravir + Nirmaltrevir
Molnupiravir
VX-765
Molnupiravir+ VX-765
CCP-5
Molnupiravir+ CCP-5
C
Krt8, Lung, Omicron VOC
Monotherapy
Combo
Normalized fold increase in mRNA expression
ns
**
Vehicle
Favipiravir
Molnupiravir
Nirmatrelvir
Molnupiravir + Nirmaltrevir
D
Delta VOC
Omicron VOC
Bliss Index=0.9 (Additive)
Bliss Index=0.001 (Additive)
Bliss Index=24 (Synergistic)
Bliss Index=-6.7 (Additive)
Disease burden
Vehicle
Molnupiravir
Nirmatrelvir
Combo
Molnupiravir
VX-765
Molnupiravir+ VX-765
Molnupiravir
CCP
Molnupiravir+ CCP
Vehicle
Molnupiravir
Nirmatrelvir
Combo

Figure S4. Therapeutic Interventions Using Drug Monotherapy or Evaluated Combinations Reduced SARS-CoV-2 VOC-Mediated Infection and Krt8 Expression, a Marker for Lung Repair/Injury, Related to Figures 1-6.

(A) Images of lung cryosections from uninfected or Delta VOC-challenged K18-hCE2 mice that were treated as indicated and immunostained with antibody to SARS-CoV-2 N conjugated with Alexa Fluor™ 488 to detect the extent of residual virus-infected cells under indicated treatment regimens after necropsy for an experiment as in Figure 1A. Cytokeratin 8 (Krt8) expression was monitored with antibodies to mouse Krt8 followed by detection using secondary antibody conjugated to Alexa Fluor™ 568 for an experiment as in Figure 1A is shown in lower panel. Enhanced Krt8 staining of injured alveolar epithelial cells due to Delta VOC infection in the areas surrounding the bronchiolar epithelium can be clearly seen in vehicle and favipiravir-treated mouse lung compared to predominant staining of bronchial epithelial cells in CV3-1 nAb and molnupiravir (survived) treated conditions. Scale bar: 1 mm

(B, C) Summary of fold changes in *Krt8* mRNA expression in the lung of mice infected with Delta or Omicron nLuc reporter VOCs under specified therapeutic interventions at the time of death or 14-22 dpi for surviving animals described in the study. Data were normalized to *Gapdh* mRNA expression in the same sample and that in non-infected mice after necropsy. Each data point represents one mouse. Dotted circles are used to denote mice that succumbed to infection.

(D) Summary of multiparametric disease burden (see Method details) and Bliss index estimation for indicated treatment regimens against Delta and Omicron VOC. Bliss score of -10 to 10 suggest additive drug interaction whereas a score above 10 suggest synergy.

Grouped data in (A-B), were analyzed by 2-way ANOVA followed by Tukey's multiple comparison tests for determining statistical significance to vehicle-treated mice. $*$, $p < 0.05$; $**$, $p < 0.01$; ns not significant; Mean values ± SD are depicted.

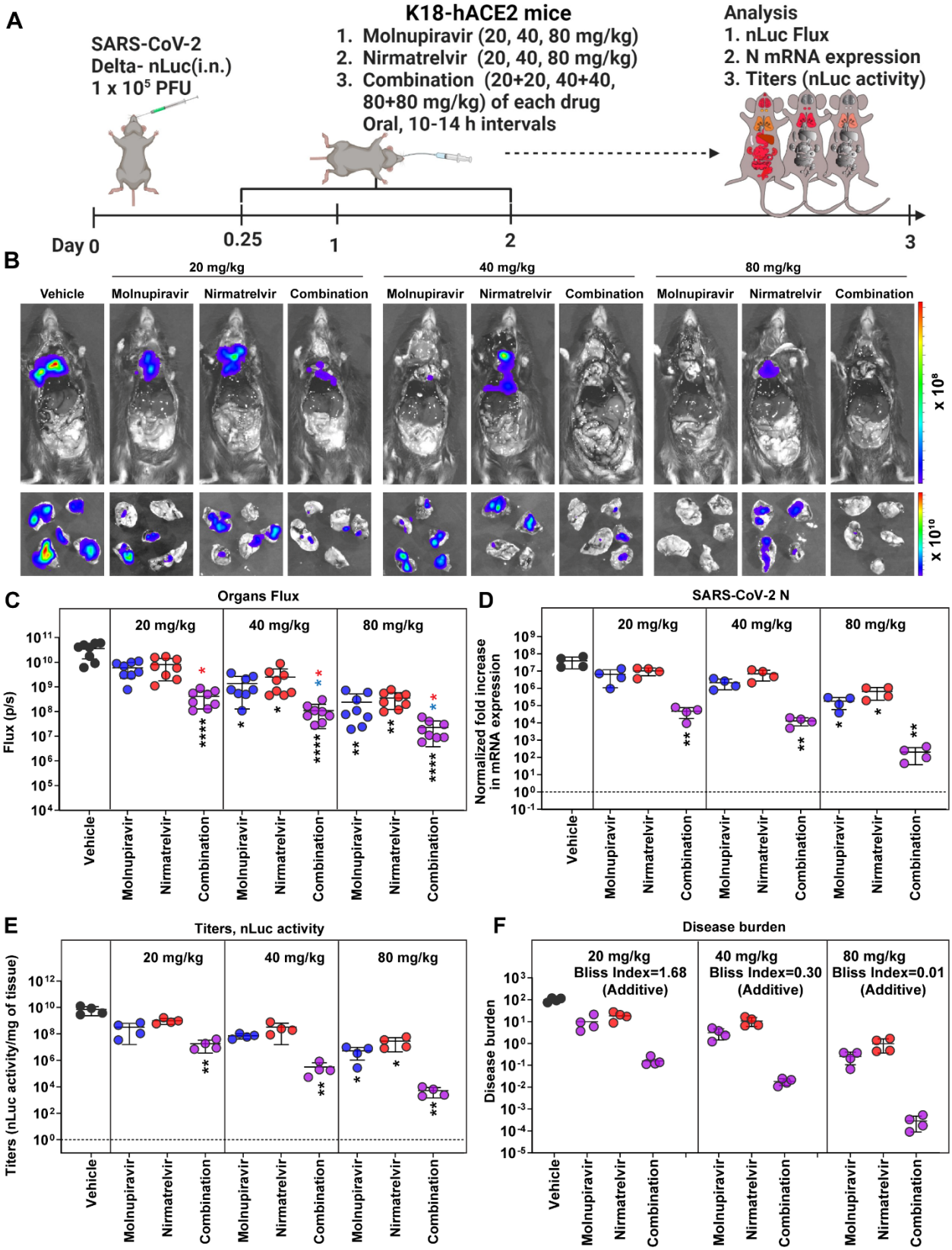
A
SARS-CoV-2
Delta- nLuc(i.n.)
1 x 10^5 PFU
K18-hACE2 mice
1. Molnupiravir (20, 40, 80 mg/kg)
2. Nirmatrelvir (20, 40, 80 mg/kg)
3. Combination (20+20, 40+40, 80+80 mg/kg) of each drug
Oral, 10-14 h intervals
Analysis
1. nLuc Flux
2. N mRNA expression
3. Titers (nLuc activity)
Day 0
0.25
1
2
3
B
20 mg/kg
40 mg/kg
80 mg/kg
Vehicle
Molnupiravir
Nirmatrelvir
Combination
x 10^8
x 10^10
C
Organs Flux
Flux (p/s)
D
SARS-CoV-2 N
Normalized fold increase in mRNA expression
E
Titers, nLuc activity
Titers (nLuc activity/mg of tissue)
F
Disease burden
Disease burden
20 mg/kg Bliss Index=1.68 (Additive)
40 mg/kg Bliss Index=0.30 (Additive)
80 mg/kg Bliss Index=0.01 (Additive)

Figure S5. Dose-Response Analysis Shows Enhanced *In vivo* Efficacy of DAA Combinations in Comparison to Monotherapy, Related to Figure 2.

(A) Experimental design to monitor the therapeutic effectiveness of specified DAAs and their combinations based on varying dosages. K18-hACE2 mice (n = 2 per dose) challenged intranasally (i.n.) with 1 x 10^5 PFU of reporter SARS-CoV-2-Delta-nLuc VOC were treated with indicated concentration of molnupiravir, nirmatrelvir or their combinations via oral gavage (oral) under a therapeutic regimen starting at 0.25 dpi. All drugs except were given twice a day (BID) for 2 days and the mice were sacrificed at 3 dpi. Vehicle-treated (Vehicle) mice (n =2) were used as control.

(B-C) BLI of mice and lung lobes under indicated treatment regimens for experiment as in A and quantification of nLuc signal as flux (photons/sec) after necropsy. Scale bars denote radiance (photons/s/cm^2/steradian).

(D) Fold changes in nucleocapsid (N) mRNA expression in individual lung lobes at 3 dpi. Data were normalized to *Gapdh* mRNA in the same sample and that in non-infected mice after necropsy.

(E) Estimated viral loads (nLuc activity/mg) in the lung homogenates from mice under specified treatment regimens using Vero E6 cells as targets.

(F) Summary of disease burden computed based on viral loads analyses [N mRNA expression and titers (nLuc activity); see method details] and Bliss index scores for drug combinations. Bliss score of -10 to 10 suggest additive drug interaction whereas a score above 10 suggest synergy.

Each data point represents individual lung lobe. Grouped data in (B-D), were analyzed by non-parametric t-test followed by Kruskal-Wallis comparison tests for determining statistical significance to vehicle-treated mice. $*$, $p < 0.05$; $**$, $p < 0.01$; ns not significant; Mean values ± SD are depicted.

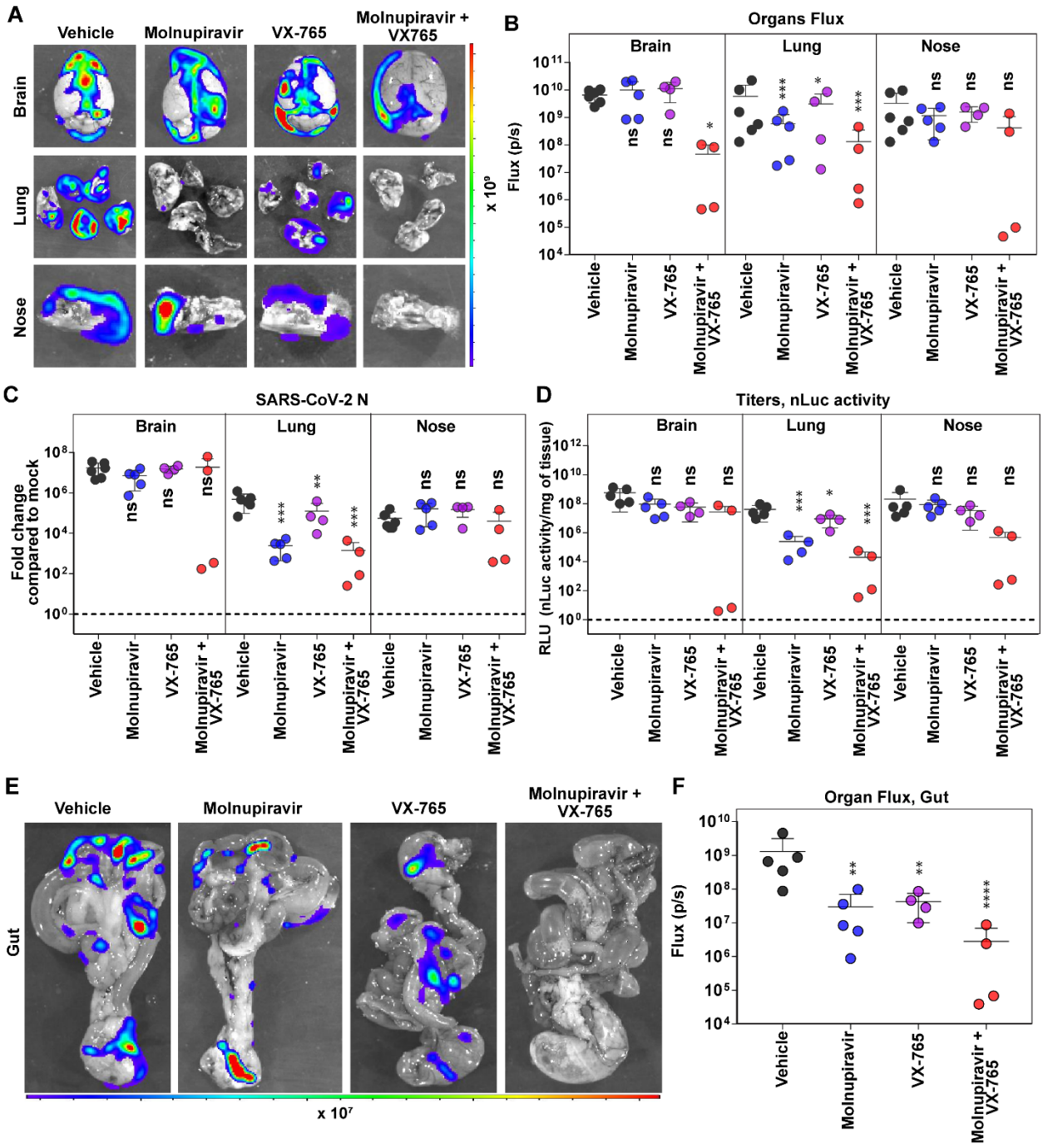

Figure S6. *In Vivo* Efficacy of Molnupiravir Combined with VX-765 Against Delta VOC, Related to Figure 5.

(A-B) *Ex vivo* imaging of organs from mice under indicated treatment regimens and quantification of nLuc signal as flux (photons/sec) after necropsy for an experiment shown in Figure 5A.

(C) Fold changes in nucleocapsid (N) mRNA expression in brain, lung, and nasal cavity tissues upon death or at 14 dpi in the surviving animals. Data were normalized to *Gapdh* mRNA in the same sample and that in non-infected mice after necropsy.

(D) Viral loads (nLuc activity/mg) in tissues from mice under specified treatment regimens using Vero E6 cells as targets upon death or at 14 dpi for surviving mice.
(E, F) *Ex vivo* imaging of gut and quantification of nLuc signal as flux (photons/sec) after necropsy at the time of death (see Figure 5F) or at 14 dpi for surviving mice for an experiment shown in Figure 5A.
Scale bars in (A) and (E) denote radiance (photons/s/cm^2/steradian). Grouped data in (B-D), were analyzed by 2-way ANOVA followed by Tukey's multiple comparison tests. The data in (F) was analyzed by one-way ANOVA followed by Kruskal-Wallis' test. Statistical significance for group comparisons to Vehicle are shown in black, with molnupiravir are shown in blue, with VX-765 are shown in purple and with molnupiravir combined with VX-765 are shown as red. $*$, $p < 0.05$; $**$, $p < 0.01$; $***$, $p < 0.001$; $****$, $p < 0.0001$; ns not significant; Mean values ± SD are depicted.

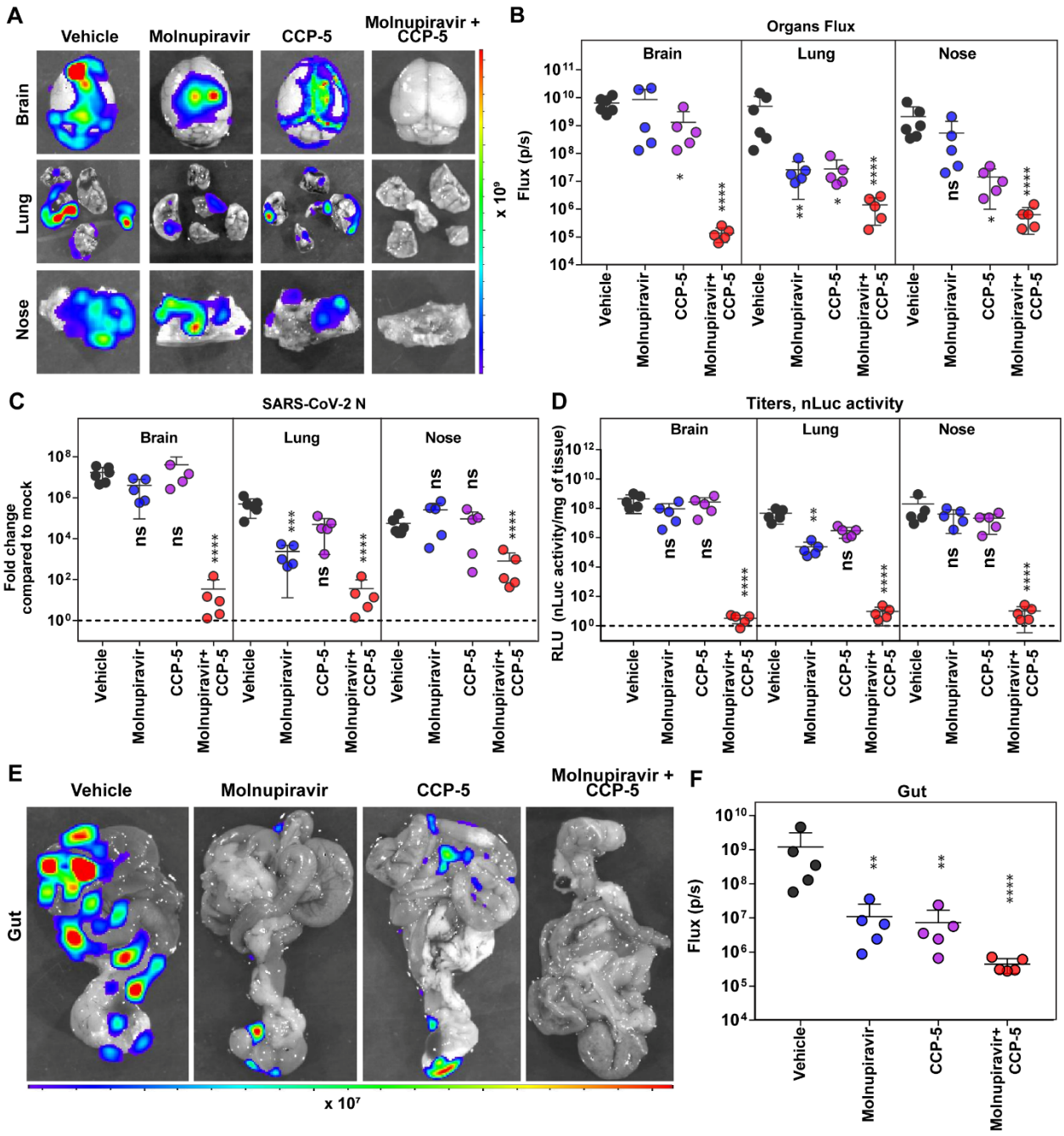

Figure S7 *In Vivo* Efficacy of Molnupiravir Combined with CCP-5 Against Delta VOC, Related to Figure 6.

(A-B) *Ex vivo* imaging of organs from mice under indicated treatment regimens and quantification of nLuc signal as flux (photons/sec) after necropsy for an experiment shown in Figure 6A.

(C) Fold changes in nucleocapsid (N) mRNA expression in brain, lung, and nasal cavity upon death or at 14 dpi in the surviving animals. Data were normalized to *Gapdh* mRNA in the same sample and that in non-infected mice after necropsy.

(D) Viral loads (nLuc activity/mg) in tissues from mice under specified treatment conditions using Vero E6 cells as targets upon death or at 14 dpi for surviving mice.
(E, F) *Ex vivo* imaging of gut and quantification of nLuc signal as flux (photons/sec) after necropsy at the time of death or at 14 dpi for surviving mice (see Figure 6F) for an experiment shown in Figure 6A.
Scale bars in (A) and (E) denote radiance (photons/s/cm^2/steradian). Grouped data in (B-D), were analyzed by 2-way ANOVA followed by Tukey's multiple comparison tests. The data in (F) was analyzed by one-way ANOVA followed by Kruskal-Wallis' test. Statistical significance for group comparisons to Vehicle are shown in black, with molnupiravir are shown in blue, with CCP-5 are shown in purple and with molnupiravir with CCP-5 are shown as red. $*$, $p < 0.05$; $**$, $p < 0.01$; $***$, $p < 0.001$; $****$, $p < 0.0001$; ns not significant; Mean values ± SD are depicted.

Table S1. Summary of In Vivo Efficacy Analyses for Antiviral Therapeutic Regimens Evaluated in K18-hACE2 Mice Against SARS-CoV-2 Delta and Omicron VOC, Related to Figures 1-6.

Drugs	Delta VOC						Omicron VOC				
	Dosage	Mortality	Titers in lung (Fold change) N mRNA	Titers in lung (Fold change) nLuc activity	*Cxcl10* (Fold change)	Krt8 in Lung (Fold change)	Dosage	Titers in lung (Fold change) N mRNA	Titers in lung (Fold change) nLuc activity)	*Cxcl10* (Fold change)	*Krt8* in Lung (Fold change)
Vehicle	Solvent	100% Death 6-7 dpi	1	1	1	1	Solvent	1	1	1	1
Favipiravir 600 mg/kg, i.p.	0.25-6 dpi every 10-14 h	100% Delayed death 8 dpi	99.82	54.53	12.66	3.98	0.25-2 dpi every 10-14 h	5.79	18.13	3.30	1.62
Molnupiravir 250 mg/kg, oral	0.25-3 dpi every 10-14 h	100% Delayed death 8-9 dpi	160.36	29.88	1.91	3.27	0.25-2 dpi every 10-14 h	342.94	798.53	2.29	2.01
Molnupiravir 250 mg/kg, oral	0.25-6 dpi every 10-14 h	40% Delayed death 10-11 dpi	140.04	1113.89	5.08	7.33	-		-	-	
Nirmatrelvir 650 mg/kg, oral	0.25-6 dpi every 10-14 h	25 % Delayed death 8 dpi	418.89	613.12	8.32	18.71	0.25-2 dpi every 10-14 h	311.33	3490.11	1.91	2.62
Molnupiravir 250 mg/kg, oral + Nirmatrelvir 650 mg/kg, oral	0.25-6 dpi every 10-14 h	0 %	26261526	207059	44.56	74.67	0.25-2 dpi every 10-14 h	891.28	18952.50	6.40	3.90
CV3-1 (nAb)	1 dpi	0 %	31921475	2429366	206.00	142.37	-	-	-	-	-
VX-765 inhibitor	0-6 dpi alternate days	100% Delayed death 8 dpi	4.30	21.70	25.51	1.80	-	-	-	-	-
Molnupiravir 250 mg/kg, oral + VX-765 inhibitor	0.25-3 dpi every 10-14 h + 0-6 dpi alternate days	50% Delayed death 10-11 dpi	1914.45	1935.89	42.96	6.18	-	-	-	-	-
Convalescent Plasma, 1ml, i.p.	1 dpi	100% Delayed death 8-9 dpi	12.89	21.75	2.57	1.72	-	-	-	-	-
Molnupiravir 250 mg/kg, oral + convalescent plasma, 1ml, i.p.	0.25-3 dpi every 10-14 h + 1 dpi	0%	3975120	76004	59.68	39.99	-	-	-	-	-