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Supplemental information

Enhanced virulence and waning vaccine-elicited antibodies account for breakthrough infections caused by SARS-CoV-2 delta and beyond

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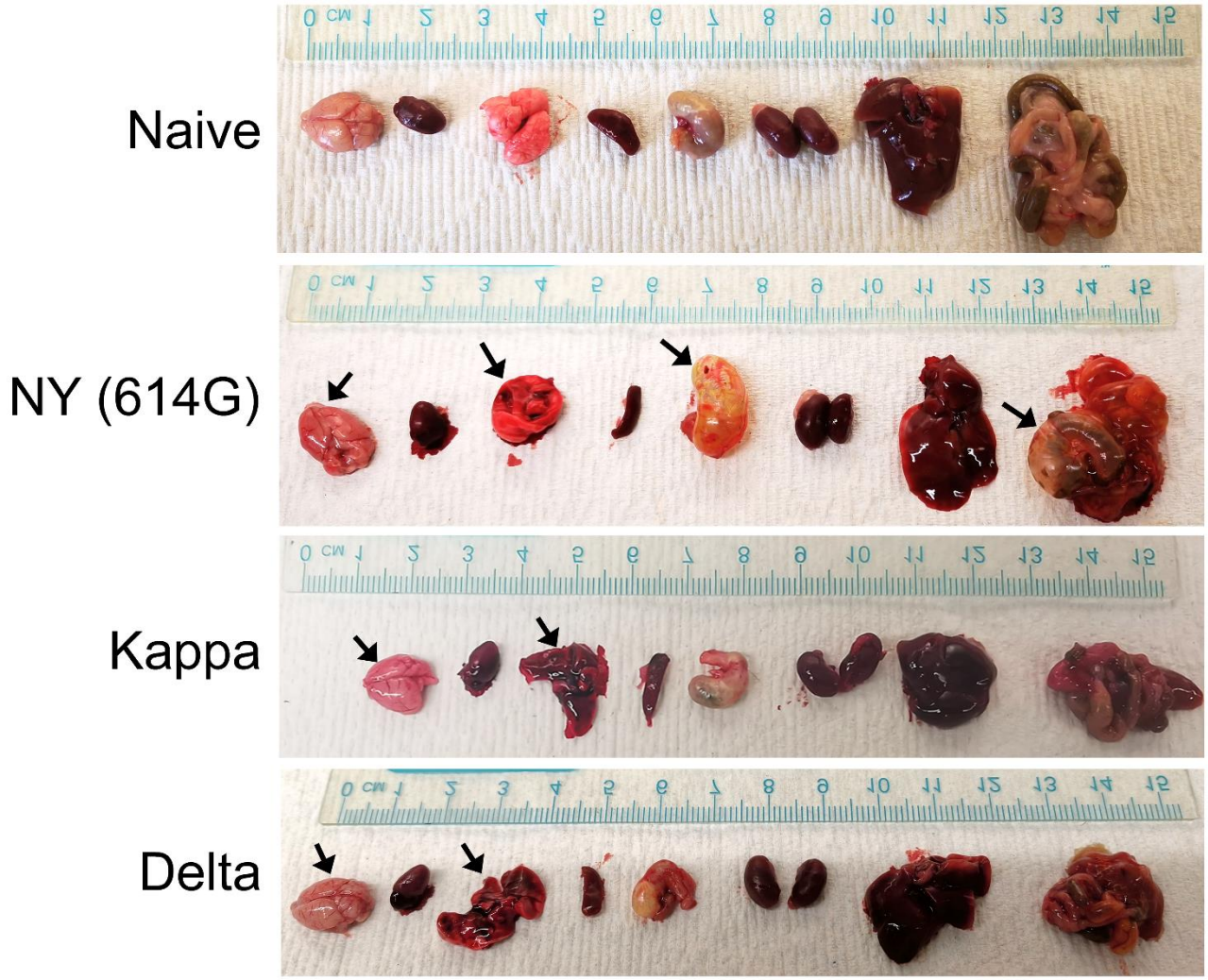
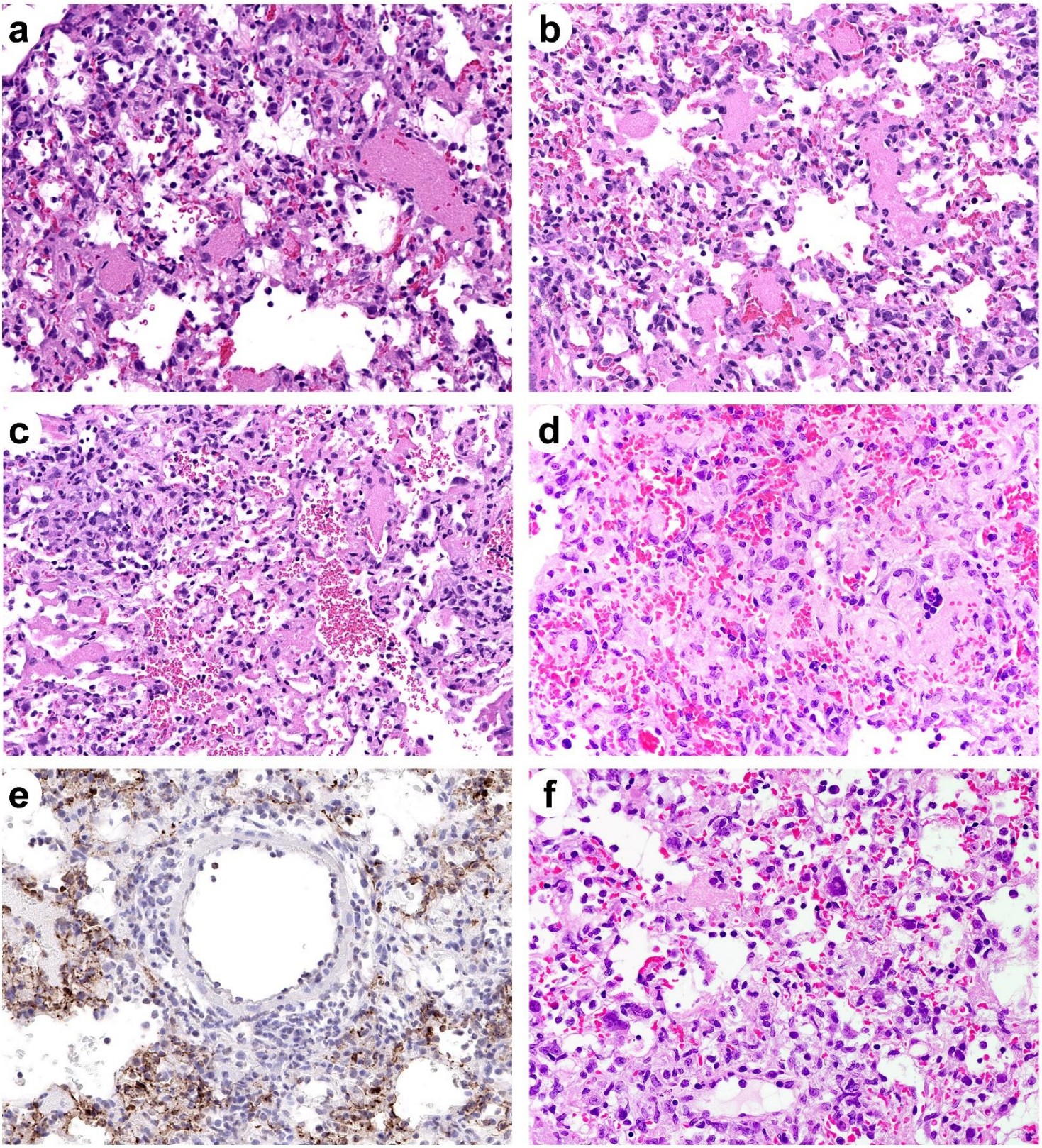
Supplemental Figure S1



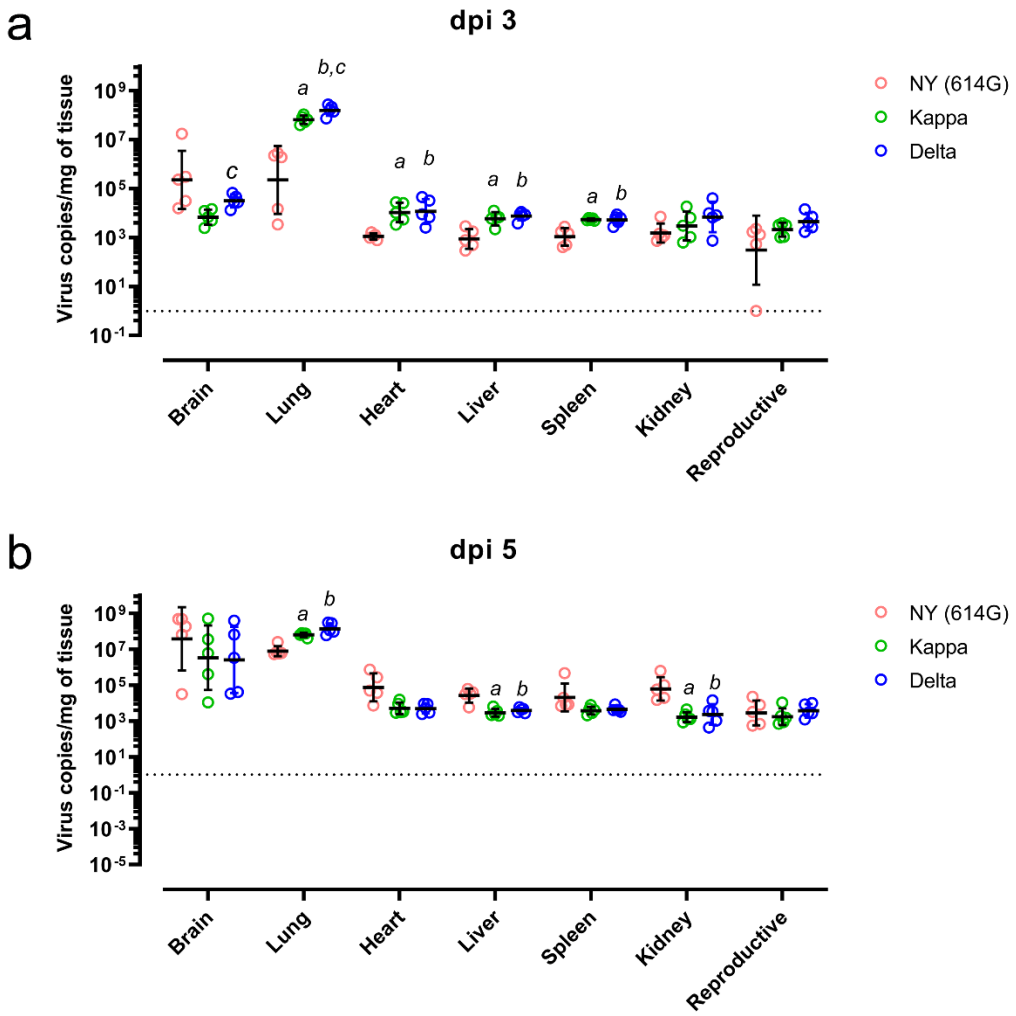
Supplemental Figure S1. Representative images of gross lesions observed in K18-hACE2 transgenic mice infected with SARS-CoV-2 and variants (Related to Figure 3). Adult K18-hACE2 transgenic mice were infected intranasally with New York-PV09158/2020 (NY (614G)) at 10^3 $TCID_{50}$/mouse, or Kappa or Delta variant at 10^2 $TCID_{50}$/mouse in an ABSL-3 biocontainment. Infected mice were humanely euthanized after reaching the moribund stage. Representative images of gross lesions (arrows) in internal organs (n= 6-7 mice/virus, female vs male at 1:1 ratio) are shown.

Supplemental Figure S2



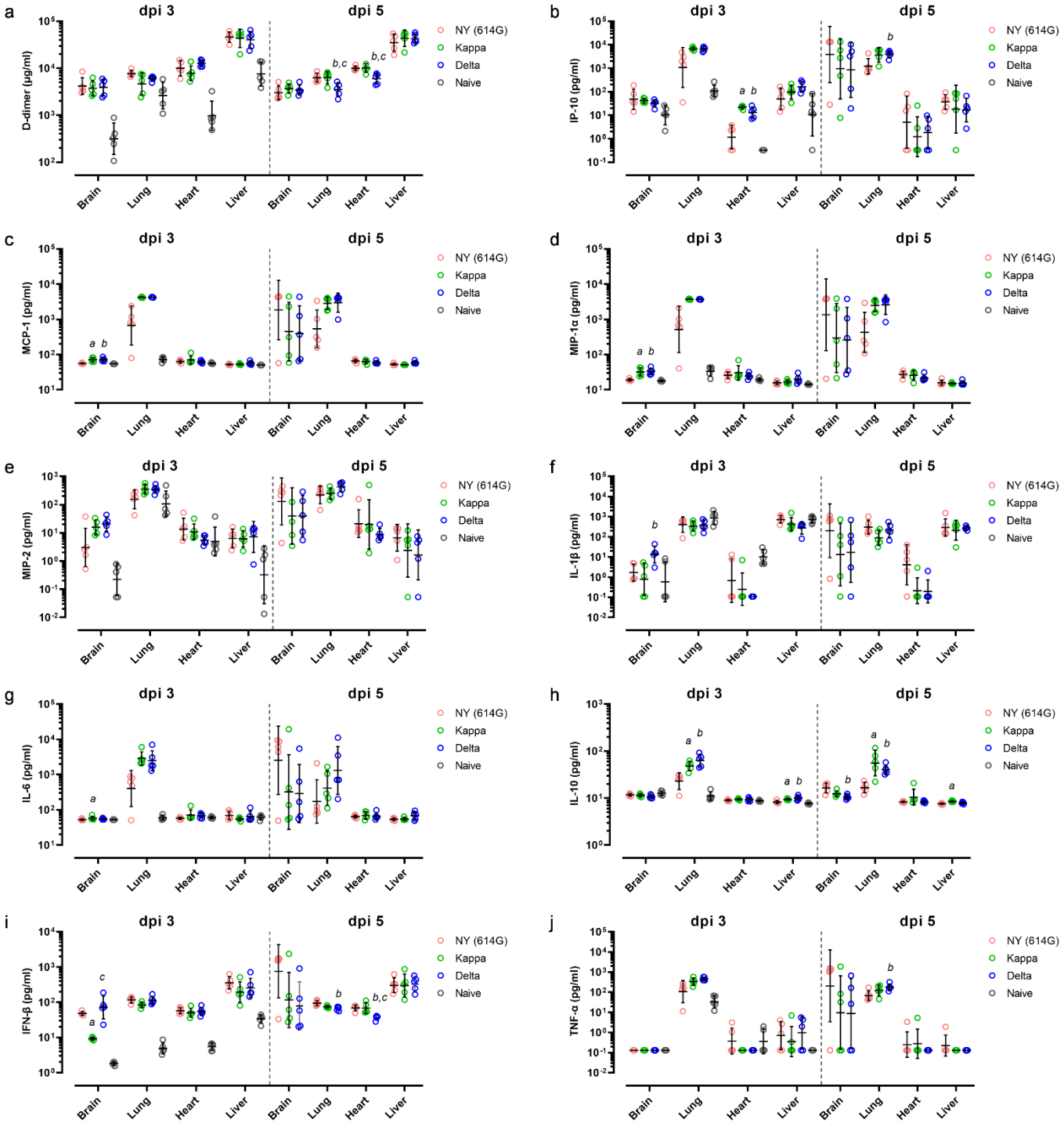
Supplemental Figure S2. Representative lung lesions of K18-hACE2 transgenic mice infected with Kappa (B.1.617.1) or Delta (B.1.617.2) variants (Related to Figure 3). Adult K18-hACE2 transgenic mice were infected intranasally with Kappa or Delta variant at 10^2 $TCID_{50}$/mouse in an ABSL-3 biocontainment. Infected mice were euthanized after reaching the moribund stage. Representative lung lesions from 6 mice/virus (female vs male at 1:1 ratio) processed are shown. (a & b) Micro-thrombi-like lesions in alveolar capillaries of mice infected with Delta (a) or Kappa (b) variant; (c & d) Breakdown of thrombotic alveolar capillaries (c) and alveolar hemorrhage from alveolar capillary rupture (d) in lungs of mice infected with Kappa variant; (e) Perivascular cuffing and mild vasculitis with no viral antigen present in endothelium of Delta-infected mouse; (f) Multinucleated macrophages in alveoli of Delta-infected mouse.

Supplemental Figure S3



Supplemental Figure S3. Viral loads in various organs of K18-hACE2 transgenic mice after infection with SARS-CoV-2 and variants (Related to Figure 4). Naïve K18-hACE2 mice of both sexes (1:1 ratio, n=5 mice/group) were infected intranasally with New York-PV09158/2020 (NY (614G)) (10^3 $TCID_{50}$/mouse), Kappa (10^2 $TCID_{50}$/mouse) or Delta (10^2 $TCID_{50}$/mouse). Viral nucleocapsid (N) gene in brain, heart, lung, liver, spleen, kidney, and reproductive organs (ovary or testis) on 3 and 5 days post infection (dpi) were measured by RT-qPCR. Individual viral titers are shown with short lines indicating geometric mean ± geometric SD of 5 mice/group. Dotted horizontal line indicates the limit of detection. *a* (Kappa vs NY (614G)) and *b* (Delta vs NY (614G)) indicate $p < 0.05$ by two-way mixed ANOVA after viral loads were log transformed.

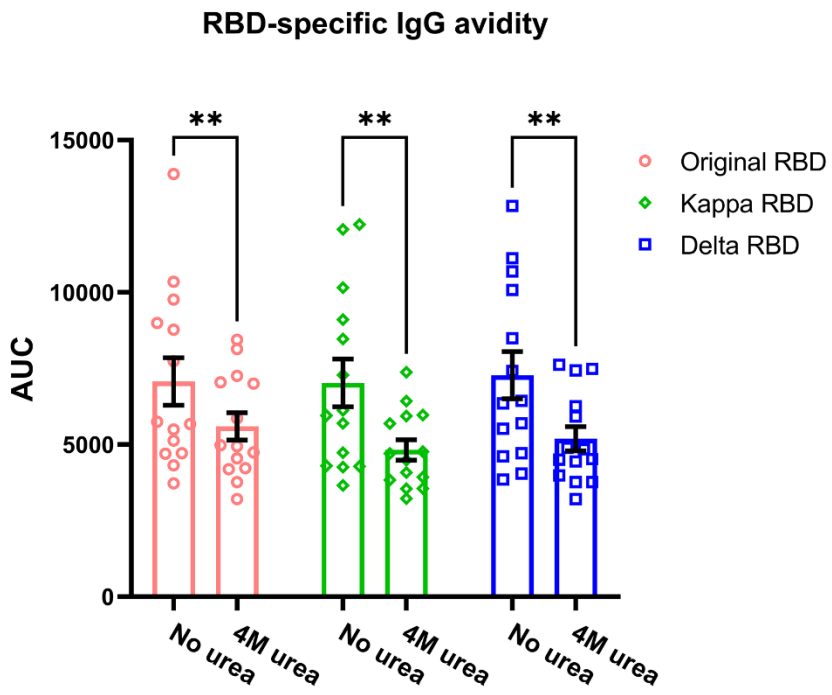
Supplemental Figure S4



Supplemental Figure S4. Tissue-specific D-dimer and cytokines following infection of SARS-CoV-2 and variants (Related to Figure 4). Naïve K18-hACE2 mice of both sexes (1:1 ratio, n=5 mice/virus/group) were infected intranasally with New York-PV09158/2020 (NY (614G)) (10^3 $TCID_{50}$/mouse), Kappa (10^2 $TCID_{50}$/mouse) or Delta (10^2 $TCID_{50}$/mouse). D-dimer and cytokines in brain,

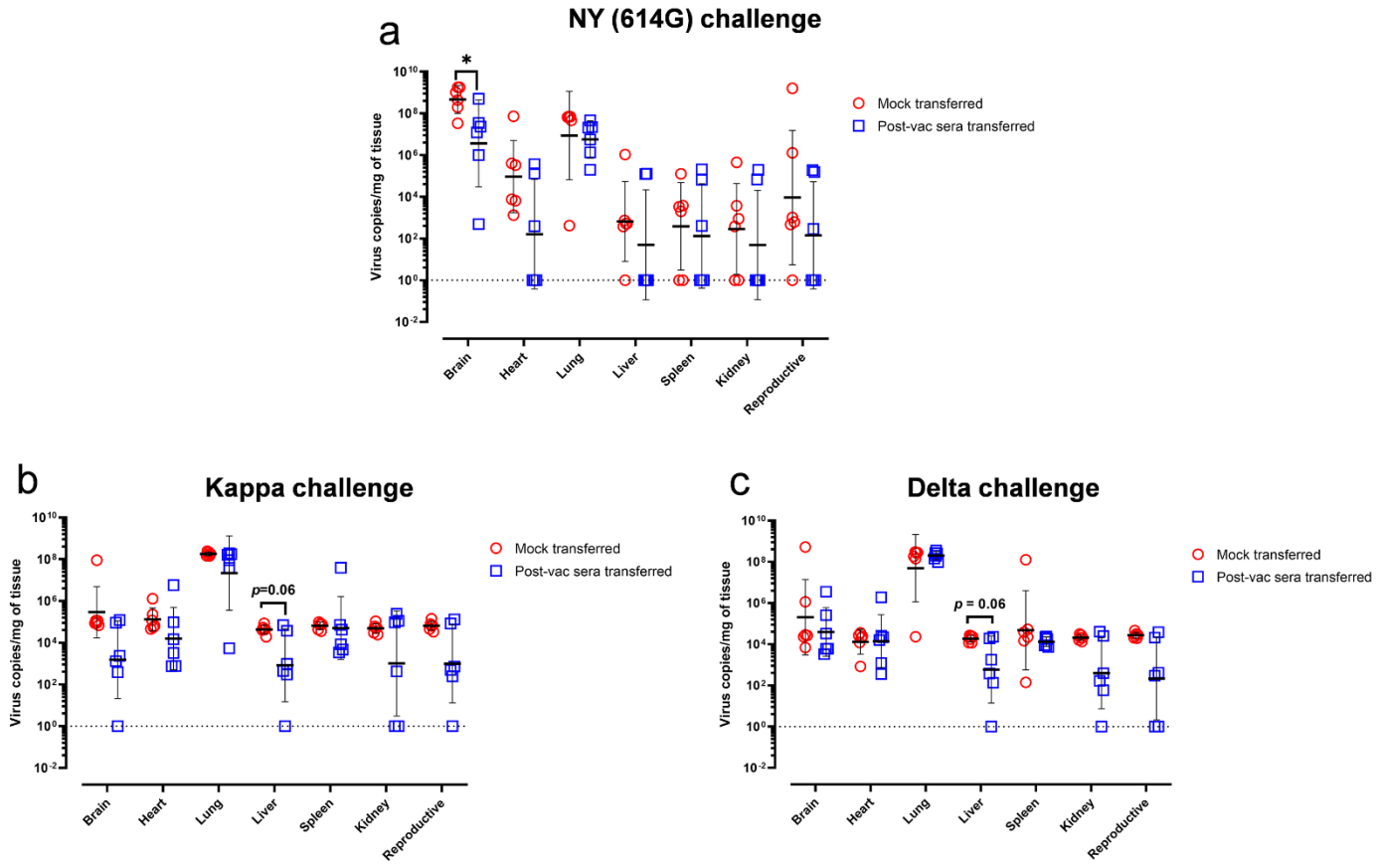
heart, lung and liver on 3 and 5 days post infection (dpi) were determined. (a) D-dimer; (b) IP-10; (c) MCP-1; (d) MIP-1α; (e) MIP-2; (f) IL-1β; (g) IL-6; (h) IL-10; (i) IFN-β and (j) TNF-α. D-dimer and cytokines of individual mice are shown with short lines indicating geometric mean ± geometric SD of 5 mice/group. *a* (Kappa vs NY (614G)), *b* (Delta vs NY (614G)), and *c* (Delta vs Kappa) indicate $p < 0.05$ for the same organ at the same time point by two-way mixed ANOVA.

Supplemental Figure S5



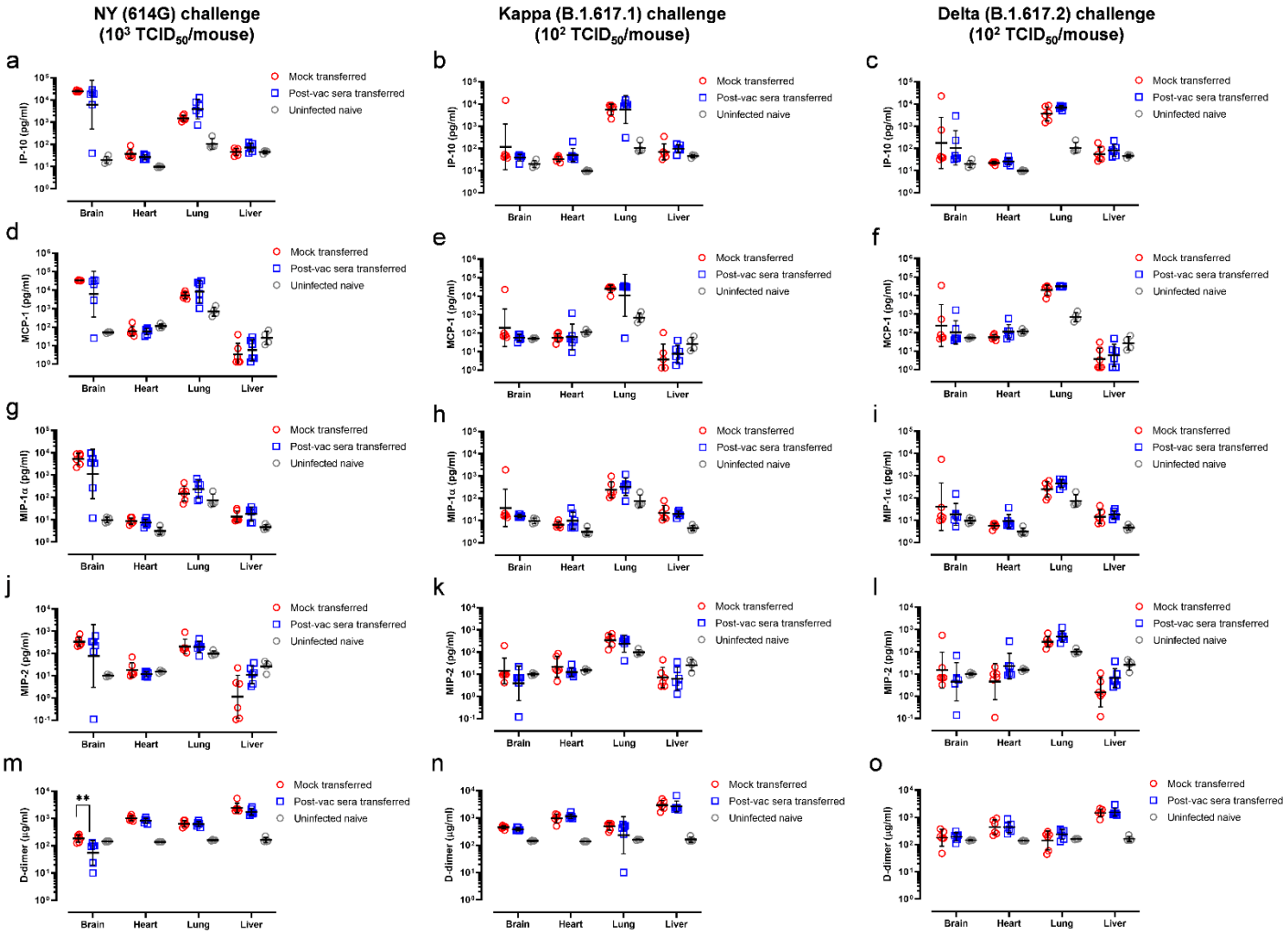
Supplemental Figure S5. RBD-specific IgG avidity (Related to Figure 5). Human sera collected at approximately 1 month after the 2nd dose of COVID-19 mRNA vaccination were tested in serial dilutions against original RBD, Kappa or Delta RBD with or without 4M urea. Bound IgG was reported as Area under curve (AUC). Individual AUC are shown with bars indicating mean ± s.e.m. (n= 14 subjects). ** indicates *p* <0.01 vs no urea treatment by Two-way ANOVA.

Supplemental Figure S6



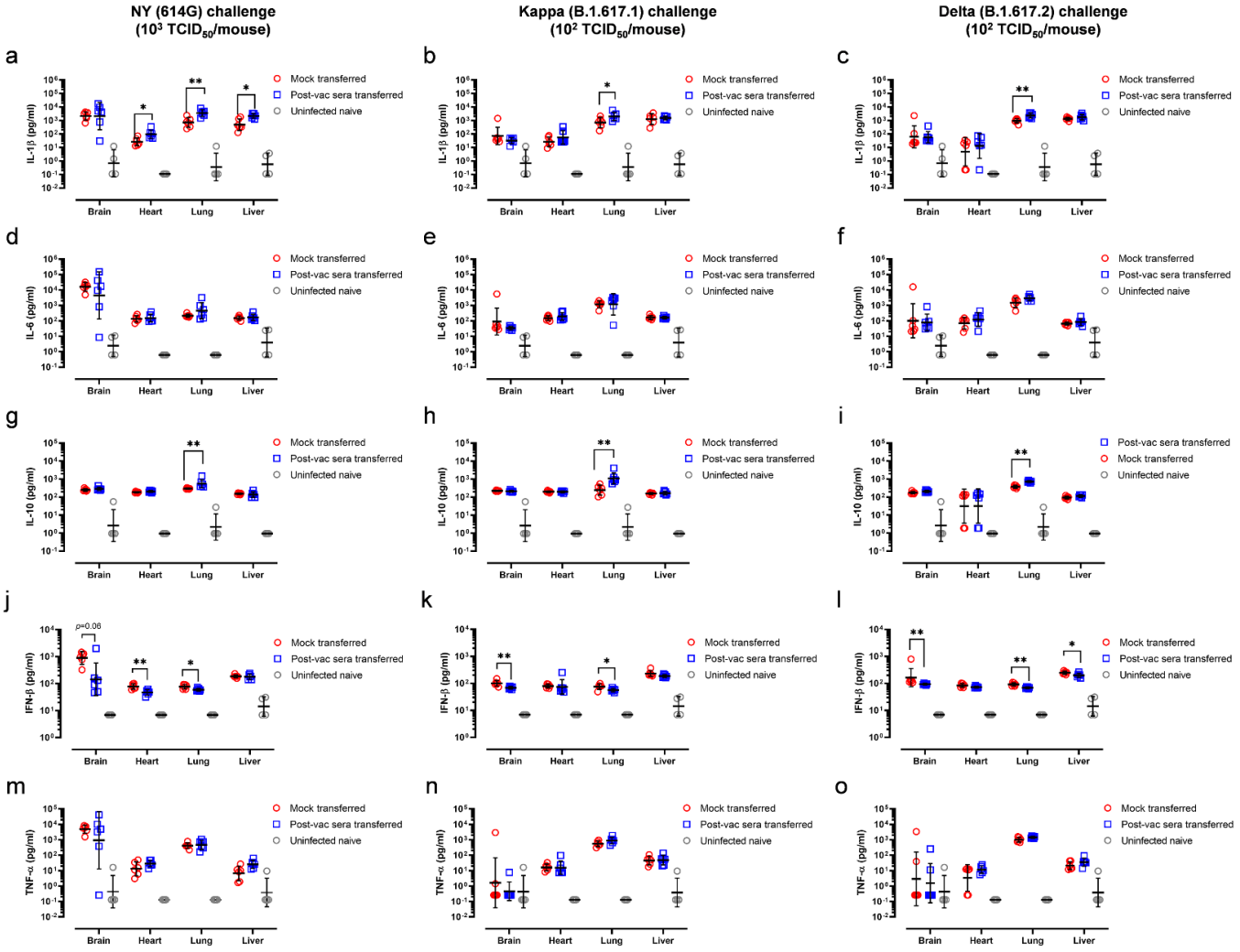
Supplemental Figure S6. Viral loads in various organs of K18-hACE2 transgenic mice after challenges with SARS-CoV-2 and variants (Related to Figure 7). Naïve K18-hACE2 mice of both sexes (1:1 ratio, n=6 mice/group) were passively transferred with 0.2 ml/mouse of pooled 1-month human COVID-19 post-vaccination sera (post-vac transferred) via intraperitoneal route. Naïve K18-hACE2 mice receiving 0.2 ml/mouse of PBS intraperitoneally served as controls (mock transferred). Two hours after passive transfer, recipient mice were challenged intranasally with (a) New York-PV09158/2020 (NY (614G)) (10^3 $TCID_{50}$/mouse), (b) Kappa (10^2 $TCID_{50}$/mouse) or (c) Delta (10^2 $TCID_{50}$/mouse). Viral nucleocapsid (N) gene in brain, heart, lung, liver, spleen, kidney, and reproductive organs (ovary or testis) at 5 days post infection were measured by RT-qPCR. Individual viral titers are shown with short lines indicating geometric mean ± geometric SD of 6 mice/group. Dotted horizontal line indicates the limit of detection. *$p < 0.05$ vs mock transferred by Mann-Whitney test.

Supplemental Figure S7



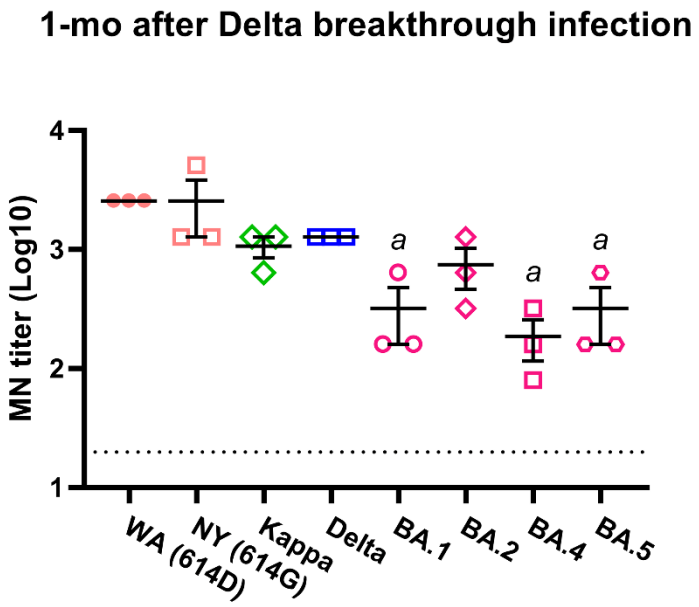
Supplemental Figure S7. Tissue-specific D-dimer and chemokines in K18-hACE2 transgenic mice after challenges of SARS-CoV-2 and variants (Related to Figure 9). Naïve K18-hACE2 mice of both sexes (1:1 ratio, n=6 mice/group) were passively transferred with 0.2 ml/mouse of pooled 1-month human COVID-19 post-vaccination sera (post-vac transferred) via intraperitoneal route. Naïve K18-hACE2 mice receiving 0.2 ml/mouse of PBS intraperitoneally served as controls (mock transferred). Two hours after passive transfer, recipient mice were challenged intranasally with New York-PV09158/2020 (NY (614G)) (10^3 $TCID_{50}$/mouse), Kappa (10^2 $TCID_{50}$/mouse) or Delta (10^2 $TCID_{50}$/mouse). D-dimer and Chemokines in brain, heart, lung and liver at 5 days post infection were determined. (a-c) IP-10; (d-f) MCP-1; (g-i) MIP-1α; (j-l) MIP-2; (m-o) D-dimer. Tissue-specific D-dimer or chemokine levels of individual mice are shown with short lines indicating geometric mean ± geometric SD of 6 mice/group. ** $p < 0.01$ vs mock transferred by Mann-Whitney test.

Supplemental Figure S8



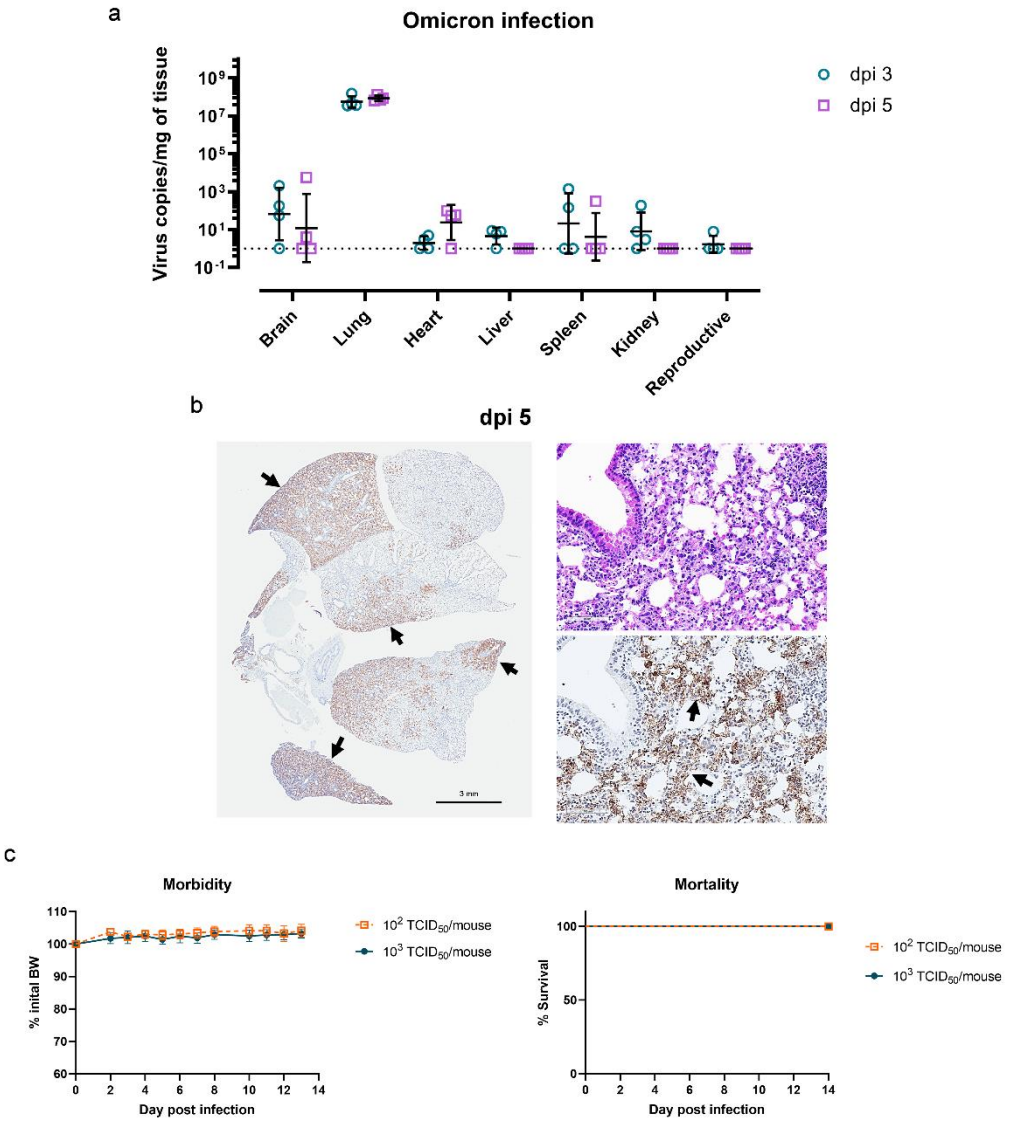
Supplemental Figure S8. Tissue-specific proinflammatory cytokines in K18-hACE2 transgenic mice after challenges of SARS-CoV-2 and variants (Related to Figure 9). Naïve K18-hACE2 mice of both sexes (1:1 ratio, n=6 mice/group) were passively transferred with 0.2 ml/mouse of pooled 1-month human COVID-19 post-vaccination sera (post-vac transferred) via intraperitoneal route. Naïve K18-hACE2 mice receiving 0.2 ml/mouse of PBS intraperitoneally served as controls (mock transferred). Two hours after passive transfer, recipient mice were challenged intranasally with New York-PV09158/2020 (NY (614G)) (10^3 $TCID_{50}$/mouse), Kappa (10^2 $TCID_{50}$/mouse) or Delta (10^2 $TCID_{50}$/mouse). Proinflammatory cytokines in brain, heart, lung, and liver at 5 days post infection were determined. (a-c) IL-1β; (d-f) IL-6; (g-i) IL-10; (j-l) IFN-β; (m-o) TNF-α. Tissue-specific cytokines of individual mice are shown with short lines indicating geometric mean ± geometric SD of 6 mice/group. * $p < 0.05$ & ** $p < 0.01$ vs mock transferred by Mann-Whitney test.

Supplemental Figure S9



Supplemental Figure S9. In vitro neutralization of human convalescent sera after the Delta breakthrough infection (Related to Figure 10). Three fully vaccinated donors (1 with Moderna and 2 with Pfizer) provided convalescent sera at approximately 1 month (mo) after the Delta breakthrough infection. The microneutralization (MN) titers against live USA-WA1/2020 (WA (614D)), New York-PV09158/2020 (NY (614G)), Kappa, Delta and emerging Omicron subvariants (BA.1, BA.2, BA.4 and BA.5) were determined (mean ± s.e.m., n= 3 donors/group). MN titers were log transformed before One-way ANOVA with nonparametric test. *a* indicates $p < 0.05$ vs WA (614D). Dashed lines indicate the lowest serum dilutions tested.

Supplemental Figure S10



Supplemental Figure S10. Tissue-specific viral loads, lung pathology, morbidity and mortality of K18-hACE2 mice infected with Omicron (Related to Figure 4 and Figure 6). Naïve K18-hACE2 mice of both sexes (1:1 ratio, n=4 mice/time point/group) were infected intranasally with Omicron BA.1 (10^3 $TCID_{50}$/mouse). Viral nucleocapsid (N) gene in brain, heart, lung, liver, spleen, kidney, and reproductive organs (ovary or testis) on 3 and 5 days post infection (dpi) were measured by RT-qPCR. (a) Tissue-specific viral loads with short lines indicating geometric mean ± geometric SD of 4 mice/time point/group. Dotted horizontal line indicates the limit of detection. (b) Representative images of mouse lungs infected with Omicron BA.1 variant on dpi 5 following immunohistochemical (IHC) staining (left and lower right panels) and hematoxylin and eosin (H&E) staining (upper right panel). Brownish staining indicates positive SARS CoV2 antigen staining (arrows). (c) Morbidity and mortality of mice after Omicron BA.1 infection at 10^2 or 10^3 $TCID_{50}$/mouse (n=4 mice/dose/group).

Supplemental Table S1: Demographics of study participants (Related to STAR Methods).

				Available Sera			
				Post 2nd dose		Post 3rd dose	Post Delta breakthrough infection
Donor	Age	Sex	Vaccine	1 month	5 months	1 month	1 month
1	Adult	Female	Moderna	Yes	Yes	-	Yes
2	Adult	Female	Pfizer	Yes	Yes	-	Yes
3	Adult	Female	Moderna	Yes	Yes	Yes	-
4	Adult	Male	Moderna	Yes	Yes	Yes	-
5	Adult	Female	Moderna	Yes	Yes	-	-
6	Adult	Female	Moderna	Yes	Yes	-	-
7	Adult	Male	Pfizer	Yes	Yes	-	Yes
8	Adult	Male	Pfizer	Yes	Yes	Yes	-
9	Adult	Male	Pfizer	Yes	-	-	-
10	Adult	Male	Pfizer	Yes	-	-	-
11	Adult	Female	Moderna	Yes	Yes	Yes	-
12	Adult	Female	Moderna	Yes	-	-	-
13	Adult	Male	Moderna	Yes	-	-	-
14	Adult	Female	Pfizer	Yes	Yes	Yes	-

-: not available.