

Supplemental Material

Supplemental Table S1. Antibodies and detection methods used for immunohistochemistry

Antigen	Antibody (clone)	Antigen retrieval	Antibody dilution, detection method
SARS-CoV NP	pAB rabbit ^a	CB pH 6.0, 98 °C, 20 min	1:6,000, ON, 4 °C EnVision+ (HRP, Rb) ^f
IAV	goat anti-IAV (H1N1; virions) ^b	TE pH 9.0, 96 °C, 30 min	1:200, 60 min, RT Rb anti-goat Ig/HRP ^f
Iba1	pAB rabbit anti-human Iba1 ^c	CB pH 6.0, 98 °C, 20 min	1:750, 60 min, RT HRP EnVision+ (HRP, Rb) ^f
CD3	mAB rabbit anti-mouse CD3 (SP7) ^d	TE pH 9.0, 37 °C, 20 min	1:900, 60 min, 37 °C, OmniMap anti-Rb HRP ⁱ
CD45R	mAB rat anti-mouse CD45R (B220/RA3-6B2) ^e	CB pH 6.0, 98 °C, 20 min	1:800, 60 min, RT EnVision+ (HRP, Rat) ^f
GFAP	pAB rabbit anti-bovine GFAP ^f	CB pH 6.0, 98 °C, 20 min	1:600, 10 min, RT MACH4™ HRP ^k
APP	mAB mouse anti-Alzheimer precursor A4 (pre A4695) fusion protein (22C11) ^g	CB pH 6.0, 98 °C, 20 min	1:6,000, 60 min, RT MACH4™ HRP ^k
ACE2	mAB rabbit anti-human ACE2 (SN0754) ^h	CB pH 6.0, 100 °C, 20 min	1:200, 10 min, RT EnVision+ (HRP, Rb) ^f
Cleaved caspase 3	mAB rabbit anti-cleaved caspase 3 (Asp175, 5A1E) ⁱ	TE pH 9.0, 98 °C, 20 min	1:200, ON, 4 °C EnVision+ (HRP, Rb) ^f

Legend: ACE2 - angiotensin-converting enzyme 2, APP - Alzheimer Precursor Protein A4, GFAP – glial fibrillary acidic protein, IAV – Influenza A virus, mAB – monoclonal antibody; pAB – polyclonal antibody; CB – citrate buffer; ON – overnight; RT – room temperature; Rb – rabbit; SARS-CoV NP – severe acute respiratory syndrome coronavirus nucleocapsid protein; TE – Tris-EDTA buffer;

Commercial providers:

^aRockland Immunochemicals Inc., Limerick, USA

^bMeridian Life Sciences Inc., Memphis, USA

^cWAKO, Osaka, Japan

^dSpring Bioscience Corp., Ventana Medical Systems, Tucson, USA

^eBD Pharmingen, Franklin Lakes, USA

^fAgilent Dako, Glostrup, Denmark

^gMerck Millipore

^hNovus Biologicals, Centennial, USA

ⁱVentana Medical Systems Inc., Tucson, USA

^kBiocare Medical

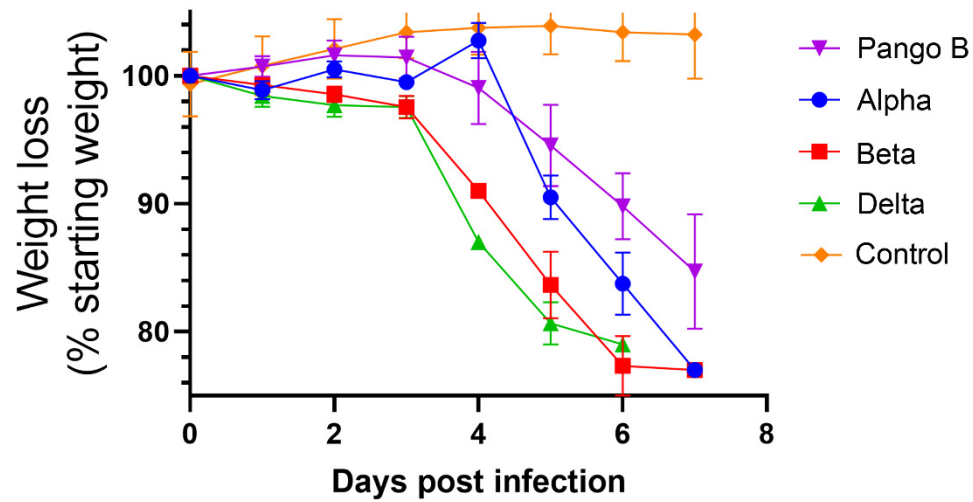


Figure S1. Infection of K18-hACE2 mice with SARS-CoV-2 VOCs leads to substantial weight loss. K18-hACE2 mice were infected intranasally with SARS-CoV-2 Alpha, Beta or Delta variant at 10^3 PFU (n=4 per group). Mice were monitored for weight loss at indicated time points and euthanized at days 5 and 6 post infection due to substantial weight loss (limit: 20% loss) or at the end of the experiment (day 7). The weights of Pango lineage B infected animals (10^4 PFU) from another experiment served for comparison. Data represent the mean value \pm SEM.

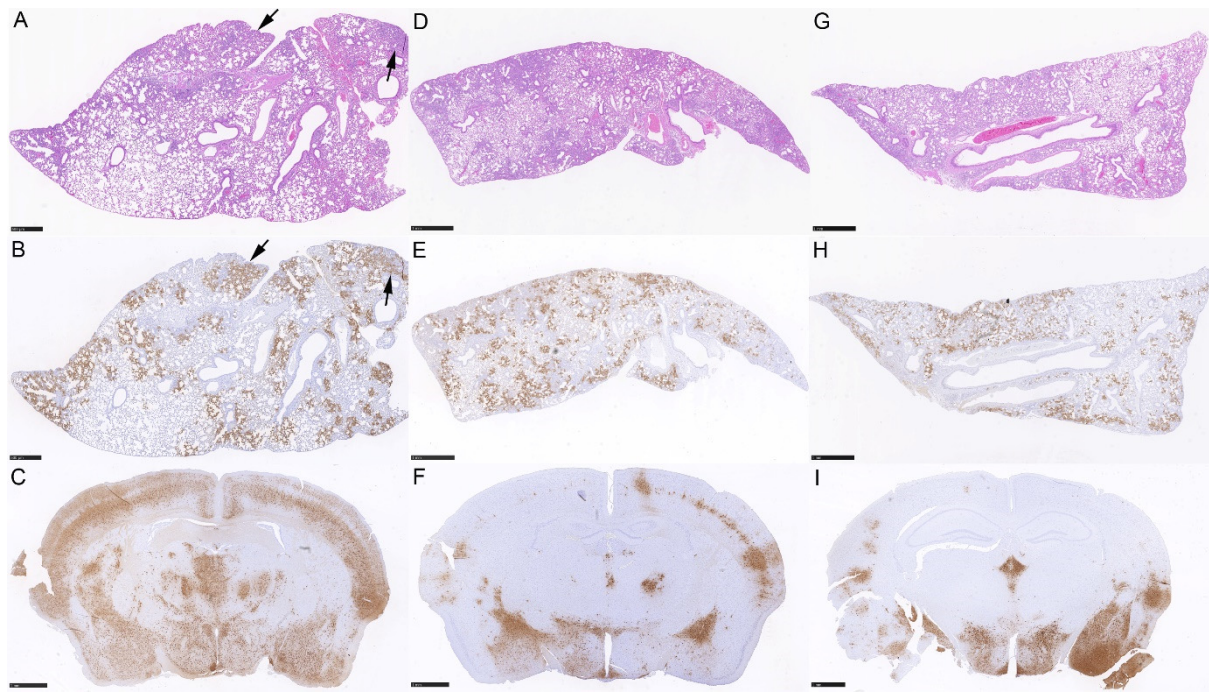


Figure S2. Infection of K18-hACE2 mice with SARS-CoV-2 VOCs leads to extensive pulmonary infection and spreads to the brain.

K18-hACE2 mice were infected intranasally with SARS-CoV-2 Alpha, Beta or Delta variant at 10^3 PFU and euthanized at 5 or 6 days post infection (dpi) due to substantial weight loss (limit: 20% loss). Lungs and brains, overview. **A-C**) Animal infected with Alpha variant and euthanized at 6 dpi. **A, B**) Lung. **A**) Mild multifocal increase in interstitial cellularity (arrows) with activation of type II pneumocytes and occasional degenerate and sloughed off alveolar epithelial cells. **B**) There is extensive viral antigen expression in large patches of unaltered appearing alveoli and in more cell rich areas (arrows). **C**) Brain, coronal section at level of corpus callosum, caudoputamen, thalamus/hypothalamus and frontal cerebral cortex. There is extensive viral antigen expression in neurons in cerebral cortex, thalamic and hypothalamic nuclei as well as piriform area. **D-F**) Animal infected with Beta variant and euthanized at 6 dpi. **D, E**) Lung. **D**) Mild to moderate multifocal increase in interstitial cellularity with activated type II pneumocytes and degenerate and sloughed off alveolar epithelial cells. **E**) There is extensive disseminated viral antigen expression in large patches of unaltered appearing alveoli and in more cell rich areas. **F**) Brain, coronal section at level of corpus callosum, rostral hippocampus, caudoputamen, thalamus/hypothalamus and frontal cerebral cortex. There is multifocal patchy viral antigen expression in neurons in cerebral cortex, amygdala, thalamic/hypothalamic nuclei, piriform area. **G-I**) Animal infected with Delta variant and euthanized at 5 dpi. **G, H**) Lung. **G**) Mild multifocal increase in interstitial cellularity (arrows) with activation of type II pneumocytes and occasional degenerate and sloughed off alveolar epithelial cells. **H**) There is abundant disseminated viral antigen expression in variably sized patches of unaltered appearing alveoli and in more cell rich areas. **I**) Brain, coronal section at corpus callosum, hippocampus, caudoputamen, thalamus/hypothalamus and frontal cerebral cortex. There is multifocal patchy viral antigen expression in neurons in cerebral cortex, amygdala, thalamic/hypothalamic nuclei, piriform area. HE stain (A, D, G). Immunohistochemistry, hematoxylin counterstain (B, C, E, F, H, I).