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## **SARS-CoV-2 B.1.1.7 and B.1.351 variants of concern induce lethal disease in K18-hACE2 transgenic mice despite convalescent plasma therapy** — [Source link](#)

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1 **SARS-CoV-2 B.1.1.7 and B.1.351 variants of concern induce lethal disease in K18-hACE2**  
2 **transgenic mice despite convalescent plasma therapy**

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26 Keywords: SARS-CoV-2, COVID-19, variants of concern, B.1.1.7, B.1.351, K18-hACE2  
27 transgenic mouse, convalescent plasma

28 **SUMMARY:** SARS-CoV-2 variants of concern (VoCs) are impacting responses to the COVID-19  
29 pandemic. Here we present a comparison of the SARS-CoV-2 USA-WA1/2020 (WA-1) strain with  
30 B.1.1.7 and B.1.351 VoCs and identify significant differences in viral propagation *in vitro* and  
31 pathogenicity *in vivo* using K18-hACE2 transgenic mice. Passive immunization with plasma from  
32 an early pandemic SARS-CoV-2 patient resulted in significant differences in the outcome of VoC-  
33 infected mice. WA-1-infected mice were protected by plasma, B.1.1.7-infected mice were partially  
34 protected, and B.1.351-infected mice were not protected. Serological correlates of disease were  
35 different between VoC-infected mice, with B.1.351 triggering significantly altered cytokine profiles  
36 than other strains. In this study, we defined infectivity and immune responses triggered by VoCs  
37 and observed that early 2020 SARS-CoV-2 human immune plasma was insufficient to protect  
38 against challenge with B.1.1.7 and B.1.351 in the mouse model.

39

40

41 **INTRODUCTION:** The evolution of Severe Acute Respiratory Syndrome CoV-2 (SARS-CoV-2)  
42 VoCs has been a source of escalating epidemiological alarm in the currently ongoing coronavirus  
43 disease 2019 (COVID-19) pandemic. Mutants of SARS-CoV-2 have emerged and are thought to  
44 be more infectious and more lethal than the early 2020 original Wuhan-Hu-1 or USA-WA1/2020  
45 (WA-1) strains (Challen et al., 2021; Korber et al., 2020; Toyoshima et al., 2020). The VoC B.1.1.7,  
46 first identified in the United Kingdom (Rambaut et al., 2020), and B.1.351, first identified in South  
47 Africa (Tegally et al., 2020), are two emerging SARS-CoV-2 VoCs that are rapidly spreading  
48 around the world and exhibit high levels of infectivity and therapeutic resistance (Challen et al.,  
49 2021; Chen et al., 2021; Davies et al., 2021; Galloway et al., 2021; 2021b, 2021a; Wang et al.,  
50 2021). Both VoCs harbor significant evolution in the receptor binding domain (RBD) of the spike  
51 (S) viral glycoprotein (Rambaut et al., 2020; Tegally et al., 2020) that are predicted to impact  
52 binding to the human angiotensin converting enzyme 2 (hACE2) viral receptor and enhance viral  
53 entry to host cells (Bozdaganyan et al., 2021; Laffeber et al., 2021; Ozono et al., 2021; Shah et

54 al., 2020; Tian et al., 2021). In particular, B.1.1.7 contains the D614G, and N501Y, mutations in  
55 the SARS-CoV-2 S RBD which are theorized to increase the ability of the virus to bind to hACE2  
56 (Ozono et al., 2021; Tian et al., 2021). B.1.351 possesses these key mutations in the S RBD, in  
57 addition to the K417N mutation E484K mutation which are not directly implicated in altered viral  
58 transmission and hACE2 binding (Laffeber et al., 2021; Zhou et al., 2021). The culmination of  
59 high infectivity, therapeutic resistance, and key changes in the viral genome suggests that these  
60 VoCs may have an impact on pathogenicity in animal models of SARS-CoV-2. This could have  
61 an impact on evaluating SARS-CoV-2 pathogenesis as well as prophylactic (vaccines) and  
62 therapeutics (antivirals).

63 The K18-hACE2 transgenic mouse model (McCray et al., 2007) of SARS-CoV-2 infection  
64 was established by Perlman and McCray among others in 2020 (Moreau et al., 2020; Oladunni  
65 et al., 2020; Winkler et al., 2020). K18-hACE2 transgenic mice infected with SARS-CoV-2 exhibit  
66 significant morbidity and mortality, viral tropism of the respiratory and central nervous systems,  
67 elevated systemic chemokine and cytokine levels, significant tissue pathologies, and altered  
68 gross clinical measures (Oladunni et al., 2020; Winkler et al., 2020; Yinda et al., 2021; Zheng et  
69 al., 2021). The generation of this mouse model has led to numerous studies of SARS-CoV-2  
70 infection for a variety of purposes including understanding SARS-CoV-2 related immunity, and  
71 therapeutic / vaccine testing (Hassan et al., 2020; Kumari et al., 2020; Liu et al., 2021; Moreau et  
72 al., 2020; Pandey et al., 2020; Sarkar and Guha, 2020; Silvas et al., 2021). As the world  
73 experiences an increase in the number of SARS-CoV-2 VoCs, it is imperative to adapt existing  
74 preclinical animal infection models to these newly emerging VoC. Specifically, it is critical to  
75 understand if the K18-hACE2 transgenic mouse model first, is useful for studying SARS-CoV-2  
76 VoC infection dynamics and second, if it exhibits any differences after challenge with newly  
77 emerged SARS-CoV-2 VoCs. An investigation of these key points will provide context for studies  
78 important for developing new therapeutics and prophylactics as the COVID-19 pandemic  
79 continues and as new VoCs emerge.

80 Clinical studies of therapeutics and vaccines for COVID-19 have been complicated by the  
81 rise of SARS-CoV-2 VoCs. Therapeutic escape by these mutants is documented (Wang et al.,  
82 2021) and requires the development of novel treatment options as well as re-evaluation of existing  
83 ones. One of the first treatment options for COVID-19 was infusion of convalescent plasma (CP).  
84 CP exhibited some beneficial effects early in the pandemic for critically ill patients (Bloch, 2020;  
85 Bloch et al., 2020; Chen et al., 2020), but its utility has recently been called into question  
86 (Casadevall et al., 2021; Cele et al., 2021; NIH, 2021; Zhao and He, 2020). There are speculations  
87 as to the reasons behind this, including that neutralizing antibodies (nAbs) generated against the  
88 original Wuhan-Hu-1 or WA-1 SARS-CoV-2 S RBD may have different affinity to the new VoCs.  
89 As many therapeutics and vaccines have focused on the RBD or S protein of SARS-CoV-2 Wuhan  
90 or WA-1, it was of interest to determine whether early pandemic convalescent plasma containing  
91 neutralizing antibodies against WA-1 SARS-CoV-2 S RBD protects against these VoCs with  
92 mutations in their RBD. Thus, the overall focus of this study was to observe the effects of SARS-  
93 CoV-2 VoCs on the K18-hACE2 transgenic mouse model of COVID-19, and determine whether  
94 these VoCs can evade an early COVID-19 pandemic therapeutic: CP treatment.

95

## 96 **METHODS:**

97 Ethics and biosafety: Human plasma used in this study was obtained under WVU IRB no.  
98 2004976401. Experiments with live SARS-CoV-2 virus were conducted in Biosafety Level 3 (BSL-  
99 3) Texas Biomedical Research Institute (TBRI IBC BSC20-004) or West Virginia University (WVU  
100 IBC 20-09-03). All ABSL-3 animal experiments were conducted under West Virginia University  
101 (WVU) ACUC protocol no. 2009036460.

102

103 Viral growth and in vitro analysis of SARS-CoV-2 replication: SARS-CoV-2 USA-WA-1/2020 (NR-  
104 52281) (WA-1), B.1.1.7 (NR-54000), and B.1.351 (NR-54008) strains were obtained from BEI  
105 Resources and propagated in Vero E6 cells (ATCC-CRL-1586) as previously described (Case et

106 al., 2020; Oladunni et al., 2020). Vero E6 cells for viral titrations (6-well plate,  $10^6$  cells/well) were  
107 infected with serial dilutions of SARS-CoV-2 WA-1, B.1.1.7 or B.1.351 VoCs. At 72 hours post-  
108 infection, cells were fixed overnight with 10% formalin (Sigma HT501128-4L), permeabilized and  
109 immunostained with  $1\mu\text{g}/\text{mL}$  of a SARS-CoV cross-reactive nucleocapsid (N) protein antibody  
110 1C7C7, kindly provided by Dr. Thomas Moran at the Icahn School of Medicine at Mount Sinai.  
111 For viral growth kinetics, Vero E6 cells (6-well plate,  $10^6$  cells/well, triplicates) were infected (MOI  
112 0.01) with SARS-CoV-2 WA-1, B.1.1.7 or B.1.351. At the indicated times after infection (12, 24,  
113 48 and 72 hours), tissue culture samples were collected and titrated by plaque assay as described  
114 previously (Oladunni et al., 2020).

115  
116 Sequencing of SARS-CoV-2 VoCs: SARS-CoV-2 viral RNA from all stocks used for *in vitro*  
117 analyses was deep sequenced according to the method described previously (Ye et al., 2020a).  
118 Briefly, we generated libraries using KAPA RNA HyperPrep Kit (Roche KK8541) with a 45 min  
119 adapter ligation incubation including 6-cycle of PCR with 100 ng RNA and 7 mM adapter  
120 concentration. Samples were sequenced on an Illumina Hiseq X machine. Raw reads were quality  
121 filtered using Trimmomatic v0.39 (Bolger et al., 2014) and mapped to a SARS-CoV-2 reference  
122 genome (Genbank Accession No. MN985325) with Bowtie2 v2.4.1 (Langmead and Salzberg,  
123 2012). Genome coverage was quantified with MosDepth. version 0.2.6 (Pedersen and Quinlan,  
124 2018). We genotyped each sample for low frequency VoCs with LoFreq\* v2.1.3.1 (Wilm et al.,  
125 2012) and filtered sites with allele frequencies less than 20%. SARS-CoV-2 viral RNA from stocks  
126 used for K18-hACE2 transgenic mice infection was deep sequenced and reads were aligned to  
127 the MN908947.3 reference genome using BWA version 0.7.17 (Li and Durbin, 2009) and trimmed  
128 for base-calling quality using iVar version 1.3.1 (Grubaugh et al., 2019) with default parameters.  
129 Consensus sequence and individual mutations relative to the reference genome were determined  
130 using iVar, with a minimum allele frequency of 30% used as a threshold for calling a mutation.  
131 Coverage was computed using samtools mpileup version 1.11 (Li et al., 2009). Lineage was

132 confirmed using pangolin version 2.3.5 and pangoLEARN version 2021-03-16 (O'Toole et al.).  
133 Authentication of the B.1.351 stock was performed using metagenomic sequencing as described  
134 previously (Addetia et al., 2020; Greninger et al., 2017). Viral RNA was treated with Turbo DNase  
135 I (Thermo Fisher). cDNA was generated from random hexamers using SuperScript III reverse  
136 transcriptase, second strand was generated using Sequenase 2.0, and cleaned using 0.8x  
137 Ampure XP beads purification on a SciClone IQ (Perkin Elmer). Sequencing libraries were  
138 generated using two-fifths volumes of Nextera XT on ds-cDNA with 18 cycles of PCR  
139 amplification. Libraries were cleaned using 0.8x Ampure XP beads and pooled equimolarly before  
140 sequencing on an Illumina NovaSeq (1x100bp run). Raw fastq reads were trimmed using  
141 cutadapt (-q 20) (Martin). To interrogate potential resistance alleles, reference-based mapping to  
142 NC\_045512.2 was carried out using our modified Longitudinal Analysis of Viral Alleles (LAVA -  
143 <https://github.com/michellejin/lava>) (Jin et al., 2019) pipeline. LAVA constructs a candidate  
144 reference genome from early passage virus using bwa (Li and Durbin, 2009), removes PCR  
145 duplicates with Picard, calls variants with VarScan (Koboldt et al., 2009, 2012), and converts  
146 these changes into amino acid changes with Annovar (Wang et al., 2010). The genome sequence  
147 for strain B.1.351 is accession number MZ065365 and SRA BioProject PRJNA726258.

148  
149  
150 *Infection of K18-hACE2 transgenic mice with SARS-CoV-2 VoCs and treatment with human*  
151 *plasma*: SARS-CoV-2 VoCs were thawed from -80°C and diluted in infection medium (Dulbecco's  
152 Modified Eagle Medium 4/.5g/L glucose + 2% fetal bovine serum + 1% HEPES + 1%  
153 penicillin/streptomycin at 10,000 units/μg/mL) to a concentration of 10<sup>6</sup> plaque forming units (pfu)  
154 /mL in the WVU BSL-3 high-containment facility. Male and female (Figures 2-3) or male (Figures  
155 4-7) eight-week-old B6.Cg-Tg(K18-hACE2)2PrImn/J mice (Jackson Laboratory 034860) were  
156 anesthetized with a single intraperitoneal dose of ketamine (Patterson Veterinary 07-803-6637,



157 80 mg/kg) + xylazine (Patterson Veterinary 07-808-1947, 8.3 mg/kg) and the 50 $\mu$ L infectious dose  
158 was administered with a pipette intranasally, 25 $\mu$ L per nare. 500 $\mu$ L of convalescent plasma (CP)  
159 or healthy human sera (HHS) with known anti-SARS-CoV-2 IgGs and nAbs (Supplementary  
160 Figure 1) were administered intraperitoneally at this time. Convalescent human plasma was  
161 obtained from a single individual with PCR-confirmed SARS-CoV-2 infection in March 2020 via  
162 WVU IRB no. 2004976401. Mice were monitored until awake and alert.

163  
164 Clinical scoring of SARS-CoV-2 infected mice: Mice were scored daily on a scale encompassing  
165 appearance (score of 0-2), eye health (score of 0-2), respiration (score of 0-2), activity (score of  
166 0-3), weight loss (score of 0-5), and hypothermia (0-2) (Supplementary Figure 1). Appearance  
167 included visual identification of a combination of mild to severe piloerection (0-2) or lack of  
168 grooming (0-2). Eye health scores were defined by observation of squinting (1), prolonged eye  
169 closure not related to sleep (2), or eye discharge (0-2) depending on severity. The maximal  
170 combined score for eye health was 2. Respiration (assessed visually) outside the range of 80-  
171 240 breaths per minute required mandatory euthanasia and scored as 2. Respiration that was  
172 abnormal in regularity was scored as 1. Activity was scored as slow (1), immobile (2), or collapsed  
173 and immobile (3). Weight loss was scored as 0-5% (0), 5-10% (1), 10-15% (2) 15-20% (3), >20%  
174 (4-5). All mice with weight loss greater than 20% were humanely euthanized. Hypothermia was  
175 assessed and scored as not-present (>36.4°C, 0), developing (36.4°C – 35.0°C, 1) or present  
176 (<35.0°C, 2) (Supplementary Figure 1).

177  
178 Euthanasia and necropsy of SARS-CoV-2 infected mice: Euthanasia was conducted by  
179 administering 200  $\mu$ L of pentobarbital (Patterson Veterinary 07-805-9296, 390 mg/kg diluted in  
180 0.9% sterile NaCl) and cardiac puncture. Blood was aliquoted into gold serum separator tubes  
181 (BD 365967) and centrifugated at 15,000 x g for 5 min. Serum was removed and stored in 1.5 mL  
182 tubes at -80°C until needed. Lungs were removed from animals and the right lobes of the lung



183 were homogenized in 1mL of PBS in Miltenyi C tubes (Miltenyi Biotec 130-096-334) using the  
184 m\_lung\_02 program on a Miltenyi gentleMACS tissue dissociator. An aliquot of each lung  
185 homogenate (300 $\mu$ L) was added to 100 $\mu$ L of TRIReagent (Zymo Research R2050-1-200) and  
186 stored at -80°C. Remaining homogenates (300 $\mu$ L) were spun down at 15,000 x *g* and the  
187 supernatants collected. Pellets were frozen at -80°C until use. Brain tissue was removed from  
188 animals and split down the mid-line. The right brain was added to 1mL of PBS in Miltenyi C tubes  
189 and homogenized using the m\_lung\_02 program. An aliquot of each homogenate (500 $\mu$ L) was  
190 added 167 $\mu$ L aliquots of TRIReagent and stored at -80°C until use. Remaining homogenates were  
191 frozen at -80°C until use. To inactivate virus from tissue samples, 1% v/v Triton X-100 (Sigma-  
192 Aldrich T8787) (Winkler et al., 2020) was added to each sample and incubated for 1 hour at room  
193 temperature. Inactivated samples were then removed from the ABSL-3 facility.

194

195 *Evaluating viral copy number in SARS-CoV-2 infected tissues:* RNA from homogenized virus-  
196 inactivated lung and brain tissues of SARS-CoV-2 infected animals was extracted using the  
197 Direct-zol RNA MiniPrep Kit (Zymo Research R2051) following the manufacturer's instructions.  
198 RT-PCR and qPCR were performed by generating a master mix of: 10 $\mu$ L of TaqMan RT-PCR Mix  
199 from the Applied Biosystems TaqMan RNA to CT One Step Kit (Thermo-Fisher Scientific  
200 4392938), 900nM (1.8 $\mu$ L) of (ATGCTGCAATCGTGCTACAA) forward nucleocapsid primer  
201 (Winkler et al., 2020), 900nM (1.8 $\mu$ L) of (GACTGCCGCCTCTGCTC) reverse nucleocapsid primer  
202 (Winkler et al., 2020), 250nM (0.5 $\mu$ L) of TaqMan probe (56-  
203 FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ), 0.5 $\mu$ L of TaqMan RT enzyme from the  
204 Applied Biosystems TaqMan RNA to CT One Step Kit (Thermo-Fisher Scientific 4392938), 100ng  
205 of RNA, and RNase/DNase free water to make a 20 $\mu$ L total reaction volume. Samples were run  
206 in triplicate in Microamp Optical 96-well Fast Reaction Plates (Thermo-Fisher Scientific 4306737)  
207 through the following protocol: reverse transcription at 48°C for 15 minutes, activation of AmpliTaq  
208 Gold DNA polymerase at 95°C for 10 minutes, and 50 cycles of 95°C denaturing for 10 seconds

209 followed by 60°C annealing for 60 seconds. Samples were run on an Applied Biosystems  
210 StepOnePlus Real-Time PCR System. Samples with undetectable virus were assigned a value  
211 of 1. C<sub>T</sub> values and copy numbers were calculated and analyzed in Microsoft Excel and GraphPad  
212 Prism v9.0.0.

213

214 Assessment of human IgGs against WA-1 SARS-CoV-2 S RBD and N: Human IgGs against WA-  
215 1 SARS-CoV-2 S RBD and N were quantified using ELISA as described previously (Horspool et  
216 al., 2021). Briefly, WA-1 S RBD (2µg/mL) or N (1µg/mL) proteins were coated on plates and  
217 blocked with 3% milk in 0.1% Tween 20 +PBS (PBS-T). Plates were washed three times with  
218 PBS-T (200µL) and virus inactivated samples (25µL) from infected mice were added to 100µL of  
219 sample buffer (1% milk + 0.1% Tween 20 diluted in PBS) and serially diluted (5-fold) down the  
220 plates. The final row was left with 100µL of sample buffer as a negative control. Plates were  
221 incubated for 10 minutes at room temperature shaking at 60rpm and subsequently washed four  
222 times with PBS-T (200µL). Secondary antibody (100µL 1:500 anti-human IgG HRP, Invitrogen  
223 31410) was added and plates were incubated for 10 minutes at room temperature shaking at  
224 60rpm. After incubation, plates were washed five times with PBS-T (200µL) and SigmaFAST OPD  
225 (Sigma-Aldrich P9187, 100µL) was added to each well of the plate. OPD development was  
226 stopped with 25µL of 3M hydrochloric acid and plates were read at an absorbance of 492nm on  
227 a Synergy H1 plate-reader. Area under the curve analysis was completed in GraphPad Prism.  
228 Human samples used as a comparison in Supplementary Figure 4 were obtained via WVU IRB  
229 no. 2004976401 as described previously (Horspool et al., 2021).

230

231 Quantification of nAbs against WA-1 SARS-CoV-2 S RBD: An assay to assess nAb levels was  
232 developed using Luminex bead and Magpix technologies. SARS-CoV-2 S RBD (1µg) produced  
233 at WVU as described previously (Horspool et al., 2021) was conjugated to Luminex MagPlex®  
234 Microspheres (MC10012-YY) using the Luminex xMAP antibody coupling kit (Luminex 40-50016)

235 per the manufacturer's instructions. Conjugated beads (50 $\mu$ L containing 2000 beads suspended  
236 in 1x PBS-TBN (Phosphate buffered saline + 0.1% bovine serum albumin + 0.02% Tween 20  
237 +0.05% sodium azide) diluted in de-ionized water from 5x PBS-TBN (Teknova P0211) were  
238 loaded into black non-binding Greiner 96-well plates (Greiner 655900). Human plasma/serum  
239 samples (25 $\mu$ L) were added into 100 $\mu$ L of PBS in the first row of a second black non-binding  
240 plate. Samples were serially diluted (5-fold dilution in PBS) down the plate. The final row contained  
241 PBS as a negative control. Diluted serum samples (50 $\mu$ L) were added to the 96-well plate  
242 containing the beads, creating a total reaction volume of 50 $\mu$ L beads (2000 beads), and 50 $\mu$ L  
243 diluted serum. The plates were covered with foil and shaken at 700rpm for 1 hour at room  
244 temperature. After shaking, beads were pelleted on a 96-well plate magnet and washed two times  
245 for 2 minutes with 200 $\mu$ L of 1x PBS-TBN. Beads were pelleted on the magnet and the wash  
246 solution removed. ACE2-biotin (100 $\mu$ L at 0.25 $\mu$ g/mL, Sino Biological Inc #: 10108-H08H-B) was  
247 added to each well. Plates were covered with foil and shaken at 700rpm for 1 hour at room  
248 temperature. After shaking, beads were pelleted on a 96-well plate magnet and washed two times  
249 for 2 minutes with 200 $\mu$ L of 1x PBS-TBN. Beads were pelleted on the magnet and the wash  
250 solution removed. Streptavidin-phycoerythrin (MOSS INC: SAPE-001) (100 $\mu$ L at 4 $\mu$ g/mL) was  
251 added to each well. Plates were covered with foil and shaken at 700rpm for 30 minutes at room  
252 temperature. After shaking, beads were pelleted on a 96-well plate magnet and washed two times  
253 for 2 minutes with 200 $\mu$ L of 1x PBS-TBN. Beads were resuspended in 100 $\mu$ L of 1x PBS-TBN and  
254 analyzed on a Luminex MagPix. Median fluorescent intensity values were plotted against serum  
255 dilution factor, and a sigmoidal regression line was fitted to the data using GraphPad Prism v9.0.0.  
256 Calculated half maximal inhibitory concentration (IC<sub>50</sub>) values of the sigmoidal curves were plotted  
257 separately as a measure of neutralizing capacity.

258

259 *Cytokine analysis of serum-treated SARS-CoV-2 VoC infected mice:* Virus-inactivated serum

260 samples or lung supernatants from SARS-CoV-2 VoC infected mice were added to a custom 8-

261 plex Mouse Magnetic Luminex Assay (R&D Systems LXSAMSM-08) including IL-6, TNF, IFN- $\gamma$ ,  
262 IL-10, IL-27, IL-1 $\beta$ , IL-2, IL-13, and IL-17 at the recommended dilution factor (2-fold dilution).  
263 Cytokine arrays were read on a Luminex MagPix instrument.

264

265 Flow cytometry of SARS-CoV-2 infected K18-hACE2 transgenic mouse lungs: Lung  
266 homogenates were thawed and pelleted at 1000 x *g* for 5 minutes at 4°C. PharmLyse (BD  
267 Biosciences 555899, 1mL of 1X solution) was added to each sample and homogenates were  
268 incubated for 2 minutes at 37°C. Samples were then pelleted at 1000 x *g* for 5 minutes at 4°C.  
269 Pellets were resuspended in 300 $\mu$ L of PBS + 1% v/v FBS and 150 $\mu$ L were added to 2 $\mu$ L of Mouse  
270 BD F<sub>c</sub> Block (BD Biosciences 553142). Homogenates in F<sub>c</sub> block were incubated for 15 minutes  
271 at 4°C. After blocking, 100 $\mu$ L were transferred into 2 $\mu$ L of antibody cocktail including 0.5 $\mu$ g of:  
272 hamster anti-mouse CD3e BV510: BD Biosciences 563024, rat anti-mouse APC-Cy7: BD  
273 Biosciences 552051, rat anti-mouse CD11b BB515: BD Biosciences 564454, rat anti-mouse  
274 CD8a BD Biosciences 551162, rat anti-mouse CD45 PE 553081, rat anti-mouse Ly6g PerCP-  
275 eFluor710: Thermo-Fisher Scientific 46-9668-82. Samples were incubated with antibody cocktail  
276 for 1 hour at 4°C in the dark. After staining, cells were pelleted at 1000 x *g* for 5 minutes at 4°C.  
277 Pellets were washed in 500 $\mu$ L PBS + 1% FBS (Gibco 10437028) and re-pelleted at 1000 x *g* for  
278 5 minutes at 4°C. Cells were resuspended in 4% paraformaldehyde and fixed for 1 hour at room  
279 temperature. Fixed cells were subsequently pelleted at 1000 x *g* for 5 minutes at 4°C, filtered  
280 through at 100 $\mu$ m mesh filter, resuspended in PBS + 1% FBS and analyzed on a BD LSRFortessa  
281 flow cytometer.

282

283 Statistical analyses: All statistical tests were performed on groups with  $n > 3$  in GraphPad Prism  
284 v9.0.0. To compare two-groups, student's *t*-tests were used. To compare three or more groups,  
285 one-way ANOVA (parametric data) or Kruskal-Wallis (non-parametric data) were used followed  
286 by Tukey's (parametric data) or Dunn's (non-parametric data) multiple comparisons tests. To

287 compare grouped data, two-way ANOVA with no correction was performed followed by Tukey's  
288 multiple comparison test. To assess statistical differences between Kaplan-Meyer curves, Mantel-  
289 Cox log-rank tests were performed.

290

## 291 **RESULTS:**

292 *Analysis of SARS-CoV-2 VoCs in cell culture:* Viral propagation of SARS-CoV-2 VoCs relative to  
293 ancestral SARS-CoV-2 strains is not fully characterized. Vero E6 cells were infected with WA-1,  
294 B.1.1.7, and B.1.351 to investigate whether these VoCs infect cells differently using standard  
295 plaque assays and viral growth kinetics. Plaque morphology of SARS-CoV-2 VoC infected  
296 cultures was distinct (Figure 1A), with B.1.1.7 resulting in a wider and rounder plaque phenotype  
297 relative to WA-1 and B.1.351 infected cells. B.1.351 viral titer was significantly increased 24 hours  
298 post-infection in cell culture but declined to levels comparable to B.1.1.7 and WA-1 over 72 hours  
299 (Figure 1B-F). To determine the genetic background of the SARS-CoV-2 VoCs used in this study,  
300 deep-sequencing was performed, and mutations relative to the WA-1 strain were identified for  
301 B.1.1.7 (Supplementary Figure 2A) and B.1.351 (Supplementary Figure 2B) prior to use in both  
302 *in vitro* and K18-hACE2 experiments. Both strains exhibited mutations associated with widely  
303 propagating B.1.1.7 and B.1.351 SARS-CoV-2 strains (Rambaut et al., 2020; Tegally et al., 2020).  
304 In addition, authentication of B.1.351 stocks used in this study revealed a large deletion in viral  
305 ORF7a (Supplementary Figure 3) that was not previously reported.

306

307 *Clinical disease progression of mice infected with SARS-CoV-2 VoCs:* Enhanced infectivity and  
308 divergent genomes of SARS-CoV-2 VoCs found in humans suggests that infection of pre-clinical  
309 animal models of SARS-CoV-2 by VoCs may be different. Many features of WA-1 SARS-CoV-2  
310 disease progression have previously been described in this model (Hassan et al., 2020; Kumari  
311 et al., 2020; Liu et al., 2021; Moreau et al., 2020; Pandey et al., 2020; Sarkar and Guha, 2020;  
312 Silvas et al., 2021). K18-hACE2 transgenic mice were infected with SARS-CoV-2 VoCs and

313 assessed daily until moribund (Supplementary Figure 1). Physical assessments of mice were  
314 compared using  $10^3$ ,  $10^4$ , and  $10^5$  pfu doses. Temperature (Figure 2A-D) and weight (Figure 2E-  
315 H) were monitored for the duration of the infection. Mice infected with the B.1.1.7 strain exhibit  
316 earlier hypothermia and weight loss relative to either the B.1.351 or WA-1 VoCs (Figure 2A-D).  
317 Hypothermia and weight loss trended higher in the B.1.1.7 VoC infected mice at both  $10^4$  and  $10^5$   
318 pfu doses at five days post-infection (Figure 2A-H). Clinical scores were assigned to mice (scale  
319 described in methods and Supplementary Figure 1) based on their appearance, eye closure,  
320 respiration, activity, hypothermia, and weight loss (Supplementary Figure 1) as described  
321 previously. B.1.1.7 and B.1.351 VoC infected mice exhibited higher cumulative clinical scores  
322 than the WA-1 strain at the  $10^3$  pfu dose (Figure 2I) and individual clinical scores at five days post-  
323 infection increased significantly with viral dose irrespective of the viral strain (Figure 2J). Mice  
324 infected with the B.1.1.7 VoC at  $10^4$  pfu exhibited an increase in clinical score earlier than the  
325 B.1.351 and WA-1 strains at the same dose (Figure 2I-J). At later time-points, both VoC presented  
326 higher clinical scores than the WA-1 at all the challenging doses studied. Importantly, at  $10^3$  pfu  
327 both VoC presented 100% mortality; however, WA-1 presented 60% survival (Figure 3,  
328 Supplementary Figure 4). Mice infected with  $10^4$  and  $10^5$  pfu of each VoC succumbed to infection  
329 (Figure 3). The WA-1 strain exhibited the largest difference in survival between challenge doses,  
330 with approximately 50% of mice recovering from the  $10^3$  pfu dose (Figure 3, Supplementary Figure  
331 4) compared to 0% recovery in B.1.1.7 and B.1.351 challenged groups (Figure 3). Interestingly,  
332 mice infected with the B.1.351 SARS-CoV-2 VoC succumbed to infection around 5 days post-  
333 infection independently of the challenge dose used (Figure 3). These data demonstrate  
334 differences in lethality between VoC doses in the K18-hACE2 transgenic murine host.

335

336 *VoCs escape protection from convalescent plasma:* Our significant differences observed in the  
337 viral genetic sequence, viral replication *in vitro*, and infection *in vivo*, established that SARS-CoV-  
338 2 VoC behave differently during infection driving differential outcome. Given the evidence that

339 SARS-CoV-2 VoCs can resist antibody therapeutics (Wang et al., 2021), we next sought to  
340 investigate if VoCs may bypass protection from antibodies present in convalescent plasma (CP)  
341 from an individual infected with SARS-CoV-2 in March of 2020. K18-hACE2 transgenic mice were  
342 intranasally challenged with a lethal dose of  $10^5$  pfu of SARS-CoV-2 VoCs B.1.1.7, B.1.351, or  
343 WA-1 and subsequently treated with CP obtained from an individual infected in March 2020 (WA-  
344 1), or from non-SARS-CoV-2 exposed healthy human serum (HHS, confirmed by PCR and  
345 serological testing) (Figure 4). IgG levels and nAb data for these sera relative to other serum  
346 samples from SARS-CoV-2 infected, non-infected, and a vaccinated human is provided (see  
347 Supplementary Figure 5). Mice infected with the B.1.1.7 VoC and WA-1 strain exhibited significant  
348 differences in clinical measurements when treated with HHS or CP. Trends in temperature (Figure  
349 4A-C), weight loss (Figure 4E-G), and clinical score (Figure 4I) over time were different between  
350 HHS and CP treated groups. B.1.1.7 and WA-1 exhibited significantly reduced temperature four  
351 days post-infection, B.1.1.7 exhibited significantly reduced weight four days post-infection, and  
352 B.1.1.7 and WA-1 exhibited significantly increased clinical scores four days post-infection when  
353 treated with HHS compared to CP (Figure 4). All WA-1 infected mice had no temperature or weight  
354 loss, and 60% of B.1.1.7 infected mice had no temperature or weight loss when treated with CP  
355 (Figure 4A-H). However, all mice infected with the B.1.351 VoC exhibited observable declines in  
356 temperature and weight regardless of treatment, suggesting that CP does not adequately protect  
357 mice from B.1.351 infection. Survival of mice treated with CP was significantly different than mice  
358 treated with HHS for the B.1.1.7 and WA-1 VoCs (Figure 5A-B). CP protected 100% of mice  
359 against lethal WA-1 infection and 60% of mice against lethal B.1.1.7 infection. However, no  
360 protection was observed in mice infected with the B.1.351 VoC, with 100% of CP-treated infected-  
361 mice reaching morbidity one day later (6 days post-infection) than B.1.351 infected mice treated  
362 with HHS (5 days post-infection). (Figure 5A-B). CP treatment resulted in decreased viral copy  
363 number in the lungs of mice infected with either WA-1 or B.1.1.7, but not mice infected with  
364 B.1.351 (Figure 5C). CP treatment only reduced viral copy number in brain tissue of mice infected



365 with WA-1 and not differences in viral titers were observed in mice infected with B.1.1.7 or B.1.351  
366 (Figure 5D).

367

368 Human and mouse IgG levels in convalescent plasma treated K18-hACE2 transgenic mice

369 infected with SARS-CoV-2 VoCs: To determine the level of IgGs delivered to HHS and CP treated

370 mice, we analyzed whether human anti-SARS-CoV-2 IgGs were present within the lung and sera

371 of animals treated with CP or HHS through the course of infection (Figure 6, Supplementary

372 Figure 6). The data demonstrate that significant quantities of human anti-SARS-CoV-2 IgGs

373 targeting both the S RBD and N proteins were present at two days post-infection (Supplementary

374 Figure 6), in sera (Figure 6A, 6C, 6E, 6G) and lung (Figure 6B, 6D, 6F, 6H) at euthanasia of CP-

375 treated mice relative to HHS-treated mice. Relative quantities of anti-SARS-CoV-2 IgGs were

376 similar across CP groups at all time points. A non-significant decrease of anti-N IgGs was

377 observed in the lungs of CP-treated B.1.351 infected mice (Figure 6H). This observation suggests

378 that there may be a greater prevalence of free N antigen in the lung that is decreasing the anti-N

379 IgG titer. These data demonstrate successful administration of anti-SARS-CoV-2 IgGs to CP-

380 treated mice.

381

382 Immunological response to VoC infection and treatment with convalescent plasma: Next, we

383 assessed cytokine levels in K18-hACE2 transgenic mice infected with SARS-CoV-2 VoCs. Serum

384 from animals at 2 days post-infection and at euthanasia, or supernatants from lung homogenates

385 were tested for the presence of Th1 (TNF, IL-2), Th2 (IL-6, IL-10, IL-13), inflammasome (IL-1 $\beta$ )

386 and regulatory (IL-27) cytokines (Figure 7, Supplementary Figure 7). We assayed these cytokines

387 as many are established as pro-inflammatory mediators that are upregulated during SARS-CoV-

388 2 infection (Hojyo et al., 2020; Horspool et al., 2021; Oladunni et al., 2020; Ye et al., 2020b) or

389 are involved in the response to viral encephalitis (Angioni et al., 2020; Aquino et al., 2021; Fabbri

390 et al., 2017; Oladunni et al., 2020), including K18 hACE2 transgenic mice. CP treatment reduced

391 IL-6, TNF- $\alpha$ , IFN- $\gamma$ , IL-10 and IL-27 in B.1.351 infected mice, and IL-6 in B.1.1.7 two days post  
392 infection (Figure 7A-E). This trend was abrogated or reversed in B.1.351 infected mice at  
393 euthanasia (Figure 7F-J). Limited differences were observed in cytokine expression in the lung,  
394 except decreased TNF- $\alpha$  in the lungs of CP-treated mice infected with WA-1 (Figure 7K-O). Minor  
395 or no differences were detected in IL-1 $\beta$ , IL-2, IL-13, and IL-17 at two days post-infection,  
396 euthanasia, or in the lung (Supplementary Figure 7). To understand the cellular response to  
397 infection, we analyzed T cells and myeloid cells in the lungs of K18-hACE2 transgenic mice  
398 infected with SARS-CoV-2 VoCs. B.1.351 infected mice exhibited significantly increased T-cell  
399 (CD3<sup>+</sup> or CD4<sup>+</sup>) recruitment to the lungs relative to WA-1 infected mice (Supplementary Figure  
400 8A-B). Non-significant trends in CD8<sup>+</sup> T cells and myeloid cells were observed but further studies  
401 defining their function will be required (Supplementary Figure 8C-F).

402  
403 **DISCUSSION:** SARS-CoV-2 VoCs of concern are rapidly evolving and some are exerting  
404 dramatic negative impacts on the currently ongoing COVID-19 pandemic. The goal of this study  
405 was to gain information regarding the infectivity of SARS-CoV-2 VoCs in *in vitro* and *in vivo* using  
406 the validated K18 hACE2 transgenic mouse model of SARS-CoV-2 infection. Our data present a  
407 broad picture suggesting that the VoCs exhibit major differences in pathogenesis, immune  
408 activation, and lethality in the K18-hACE2 transgenic mouse model of infection. B.1.351 replicated  
409 faster in cell culture than B.1.1.7 and WA-1. Both B.1.1.7 and B.1.351 triggered severe clinical  
410 indications of disease at lower infectious doses than the ancestral WA-1 strain in the K18-hACE2  
411 transgenic murine model. Treatment of infected mice with CP from WA-1-infected individuals  
412 (March of 2020, prior to the emergence of B.1.1.7 and B.1.351 VoC) resulted in full protection  
413 only against the WA-1; B.1.1.7 infected mice exhibited partial protection and B.1.351 exhibited no  
414 protection. Both VoCs resulted in significant viral replication in the brain of K18-hACE2 transgenic  
415 mice despite treatment with CP. It is clear by comparing all results from this study (Supplementary  
416 Figure 9) that CP efficacy is significantly reduced against SARS-CoV-2 VoC and that antibodies

417 against ancestral SARS-CoV-2 may not be fully protective against B.1.1.7 and B.1.351. This may  
418 have additional impacts on the efficacy of existing SARS-CoV-2 vaccines that target the S protein  
419 of WA-1 SARS-CoV-2. Ultimately, these results provide a troubling picture of the impact of VoCs  
420 on SARS-CoV-2 pathogenesis and host immunity.

421 SARS-CoV-2 B.1.351 infection resulted in unique phenotypes in this study: increased  
422 replication in cell culture, clinical presentations at low doses of infection, lethality at low infectious  
423 doses, reduced clinical and survival benefits from early (WA-1) CP, increased viral load in lung  
424 and brain tissues, and increased pro-inflammatory cytokines after CP treatment, all supporting  
425 the conclusion that the B.1.351 VoC results in more severe disease than the original WA-1 strain  
426 in this model. Of particular concern is the lack of reduction in viral copy number in all mice treated  
427 with CP infected with B.1.351 relative to WA-1 infected mice. In addition, the severity of viral  
428 burden despite CP treatment was enhanced in B.1.351 infected mice relative to B.1.1.7 infected  
429 mice: 100% of CP-treated B.1.351 infected mice had lung and brain infection relative to 40% of  
430 CP-treated B.1.1.7 infected mice. This demonstrates that B.1.351 infection may harbor more  
431 resistance or tolerance to early pandemic CP than B.1.1.7. It is important to point out the caveat  
432 that we did not investigate antibody dependent enhancement of infection. The observed  
433 differences in our mouse challenge studies may be attributable to the alterations in the viral  
434 B.1.351 S RBD region of the S protein, which may increase affinity for the hACE2 receptor  
435 (Bozdoganyan et al., 2021; Laffebber et al., 2021; Ozono et al., 2021; Shah et al., 2020; Tian et  
436 al., 2021) and could result in increased infectivity and pathogenicity. Although this yet speculative,  
437 sequencing results in Supplementary Figure 2-3 illustrate that many other mutations exist within  
438 the B.1.351 SARS-CoV-2 genome. Mutations within other viral proteins, particularly those  
439 involved in viral replication and genome packaging may provide additional advantages for this  
440 VoC during infection. It is currently unknown what impact the B.1.351 ORF7a deletion we  
441 observed has on pathogenesis, but other studies have suggested limited impacts of similar  
442 ORF7a mutations in cell culture (Chiem et al., 2020; Narayanan et al., 2008; Sims et al., 2005).

443 Dissecting the consequences of these B.1.351 non-S mutations will be useful in determining the  
444 mechanisms behind the increased disease severity observed here and may help better inform  
445 containment of these VoCs, and others emerging in the future.

446         Several additional observations were made in this study, including discovering significant  
447 differences in cytokine production of B.1.351 SARS-CoV-2 VoC infected mice. IL-27 is an  
448 indicator of acute encephalitis and can activate CD8<sup>+</sup> T cells (Angioni et al., 2020; Aquino et al.,  
449 2021; Fabbi et al., 2017) in addition to several other less characterized functions (Awasthi et al.,  
450 2007; Iwasaki et al., 2013; Jung and Robinson, 2014; Kastelein et al., 2007; Seman et al., 2020;  
451 Sugiyama et al., 2008). IL-27 also regulates IL-10 production, the latter acting in an anti-  
452 inflammatory manner by suppressing inflammatory responses (Iyer and Cheng, 2012). IL-27 was  
453 increased in B.1.351 infected mice. B.1.351 challenged mice also had significant viral burden in  
454 the brain, increased CD3<sup>+</sup> T cells in the lung, and non-significantly increased in CD8<sup>+</sup> T cells in  
455 the lung. The combination of these data suggests that IL-27 expression in B.1.351 infected mice  
456 may be linked to more severe encephalitis and potentially higher activation of CD8<sup>+</sup> T cells. HHS-  
457 treated mice infected with B.1.351 exhibited this increase in IL-27 at two days post-infection,  
458 whereas CP-treated mice exhibited this increase only at euthanasia. It is possible that this delay  
459 in increased IL-27 may be beneficial as CP-treated B.1.351 infected mice survive for one  
460 additional day over HHS-treated mice. Mechanistically, this may be due to IL-27 promoting  
461 uncontrolled inflammation (Takeda et al., 2003) and suppression of T<sub>regs</sub> (Cox et al., 2011) which  
462 may increase local and systemic damage. However, IL-27 could also be increased as an  
463 emergency response in response to encephalitic infection and may exert beneficial pro- or anti-  
464 inflammatory effects dependent on the severity of infection. Further studies will be required to  
465 elucidate the true role of IL-27 in this system. In a similar manner, IL-6, TNF, and IFN- $\gamma$  were  
466 increased in B.1.351 infected mice two days post-infection in HHS-treated mice and increased in  
467 CP-treated mice at euthanasia. This delay in induction of pro-inflammatory cytokines may be  
468 beneficial, but ultimately none of these delays appear to protect mice from death.

469           This study also provides additional insights on VoC pathogenicity; however, several  
470 important clarifications must be made when interpreting these results. Firstly, although these  
471 infection models exhibit clear differences in infectivity, infections in cell culture and mice do not  
472 perfectly represent those in humans. In particular, the K18-hACE2 transgenic mouse model is a  
473 lethal disease model, with different viral tropism, which frequently can result in 100% mortality at  
474 low infectious doses as seen in this study and others (Hassan et al., 2020; Kumari et al., 2020;  
475 Liu et al., 2021; Moreau et al., 2020; Oladunni et al., 2020; Pandey et al., 2020; Sarkar and Guha,  
476 2020; Silvas et al., 2021). Human disease can range from mild disease to lethal disease and is  
477 impacted by comorbidities (Elezkurtaj et al., 2021; Mehra et al., 2020; Wu and McGoogan, 2020).  
478 Re-creating this in any animal model is exceptionally challenging. Another complex component is  
479 that it is unknown what the minimum infectious dose of SARS-CoV-2 is for humans, and thus it is  
480 difficult to compare animal models of infection to a real human infection without this basic  
481 information. Secondly, the K18-hACE2 transgenic mouse expresses hACE2 but also retains  
482 expression of murine (m)ACE2. There is speculation that SARS-CoV-2 VoCs can infect wild-type  
483 mice and cause non-lethal disease by binding to mACE2 (Montagutelli et al., 2021); this may  
484 impact the comparisons provided in this study. We have observed similar phenomena in  
485 preliminary studies where some of these VoC are able to infect wild-type mice, contrary to the  
486 situation with WA-1 strain (data not shown). Although this may be true, infection of Vero E6 cells  
487 with B.1.351 (a non-human cell line that is not genetically modified for infection) was significantly  
488 increased 24 hours post-infection. This provides support of the conclusions presented here in  
489 light of the potential for VoCs to bind mACE2, although an effect of mACE2 cannot be excluded.

490           CP as a treatment has been used widely since the onset of the COVID-19 pandemic  
491 (Bloch, 2020; Bloch et al., 2020; Chen et al., 2020), but its efficacy has recently been re-evaluated  
492 under certain conditions (Casadevall et al., 2021; Cele et al., 2021; NIH, 2021; Zhao and He,  
493 2020). It is possible that some of the decreased efficacy of CP in this study (and others) may be  
494 due to SARS-CoV-2 VoCs, and the data presented here support this theory. Other factors

495 including lack of adequate screening for nAbs and lot-to-lot variability of plasma samples may be  
496 the cause of these differences in larger studies of humans treated with CP. The plasma used in  
497 this study was determined to have high levels of IgG antibodies against SARS-CoV-2 S RBD and  
498 N proteins and also exhibited neutralizing activity similar to a vaccinated individual  
499 (Supplementary Figure 4). In this context, this plasma should have high protective capacity, and  
500 indeed protected WA-1 infected mice against severe disease. Consequently, it is concerning that  
501 protection is limited or absent when K18-hACE2 transgenic mice were challenged with B.1.1.7 or  
502 B.1.351 VoCs, respectively. It is important to note that only one CP sample was tested in this  
503 study and was obtained from a naturally infected, not a vaccinated, individual. Therefore, although  
504 there appears to be a lack of protective capacity of this plasma against B.1.351 and partial  
505 protective capacity against B.1.1.7, these data do not provide direct against the efficacy of SARS-  
506 CoV-2 vaccines which stimulate broader immune responses. However, it is likely that the  
507 production of antibodies elicited by existing SARS-CoV-2 vaccines are not fully protective against  
508 VoCs, which warrants caution. Further analyses of these vaccines will be critical in determining  
509 their efficacy against B.1.1.7, B.1.351, new VoCs, or variants of high concern. Continued support  
510 for novel vaccine development against VoCs will be instrumental in providing full protection  
511 against evolving strains of SARS-CoV-2.

512 To summarize, this study provides early insight into differences in SARS-CoV-2 VoC  
513 pathogenicity in K18-hACE2 transgenic mice and the efficacy of CP containing antibodies  
514 targeting WA-1 SARS-CoV-2 against newly emerged SARS-CoV-2 VoCs in the K18-hACE2  
515 transgenic mouse model. This study demonstrates increased disease pathology for mice infected  
516 with B.1.1.7 and B.1.351 VoCs and lack or limited protection from CP in mice infected with B.1.351  
517 and B.1.1.7, respectively. Immunological profiles were different between mice infected with each  
518 VoC, with B.1.351 stimulating greater increases in cytokine levels and potential T-cell recruitment,  
519 most likely due to its more efficient replication as compared to the WA-1 and B.1.1.7 strains.  
520 Overall, these data present a concerning picture of the SARS-CoV-2 VoCs warranting continued

521 caution as the pandemic continues and evolves and suggest the need to update current vaccines  
522 to protect against these newly emerged SARS-CoV-2 VoC.

523

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534

535 **AUTHOR CONTRIBUTIONS:** A.M.H, F.H.D, I.M, J.T, C.Y and L.M.S designed the experiments.  
536 C.Y performed *in vitro* analyses of SARS-CoV-2 VoCs, and J.S., A.G, and R.K.P performed  
537 sequencing. M.T.W and I.M propagated virus for animal experiments. A.M.H, T.Y.W, B.P.R, K.S.L  
538 and F.H.D infected and euthanized animals, dissected organs, and prepared them for analyses.  
539 A.M.H and B.P.R performed ELISA against SARS-CoV-2 antigens and A.M.H and T.Y.W  
540 performed qPCR to determine viral load. A.M.H performed cytokine analyses. A.M.H and T.Y.W  
541 prepared cells for flow cytometry and A.M.H performed flow cytometry and analysis. J.D analyzed  
542 sequence data from viral passages used for infection of K18-hACE2 transgenic mice. A.M.H  
543 analyzed, formatted, and represented data for publication. H.A.C assisted with general revisions.  
544 All authors contributed to the writing and revision of the manuscript.

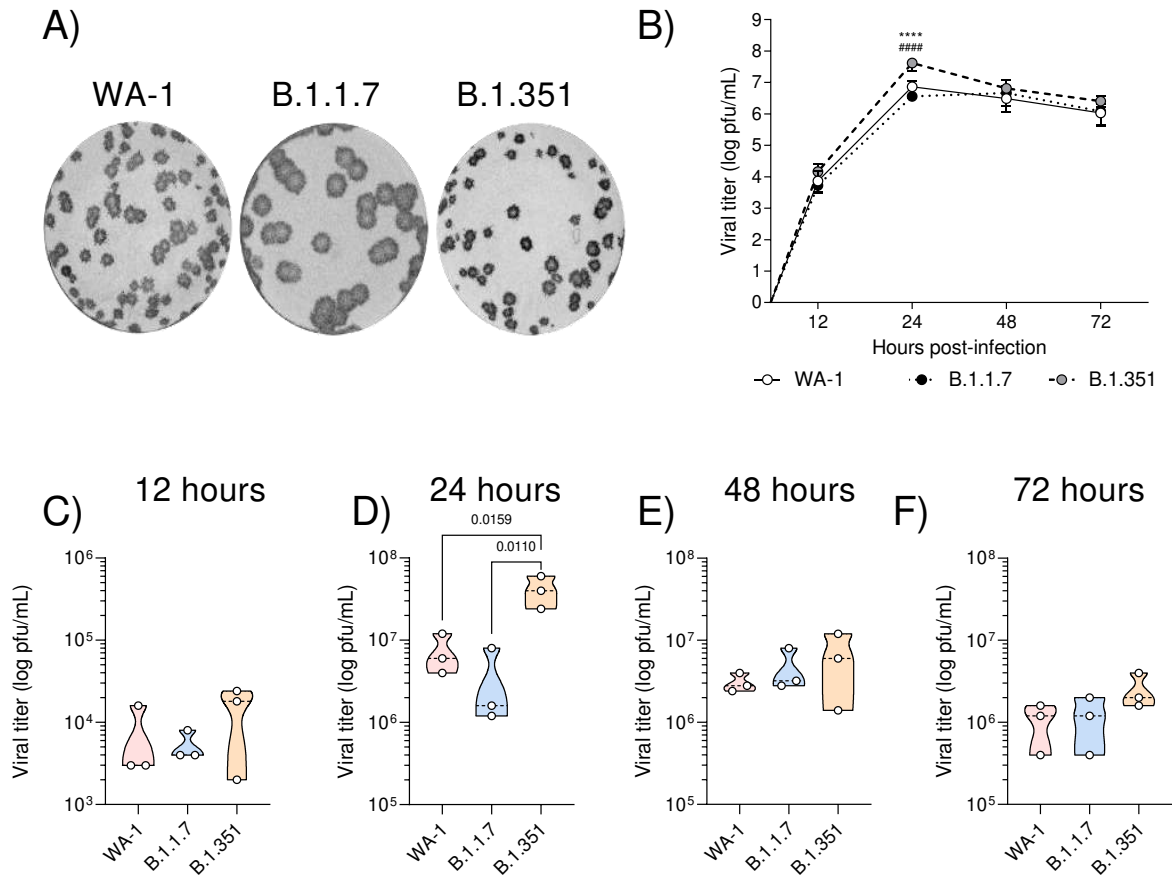
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546 **DECLARATION OF INTERESTS:** The authors declare no competing interests.

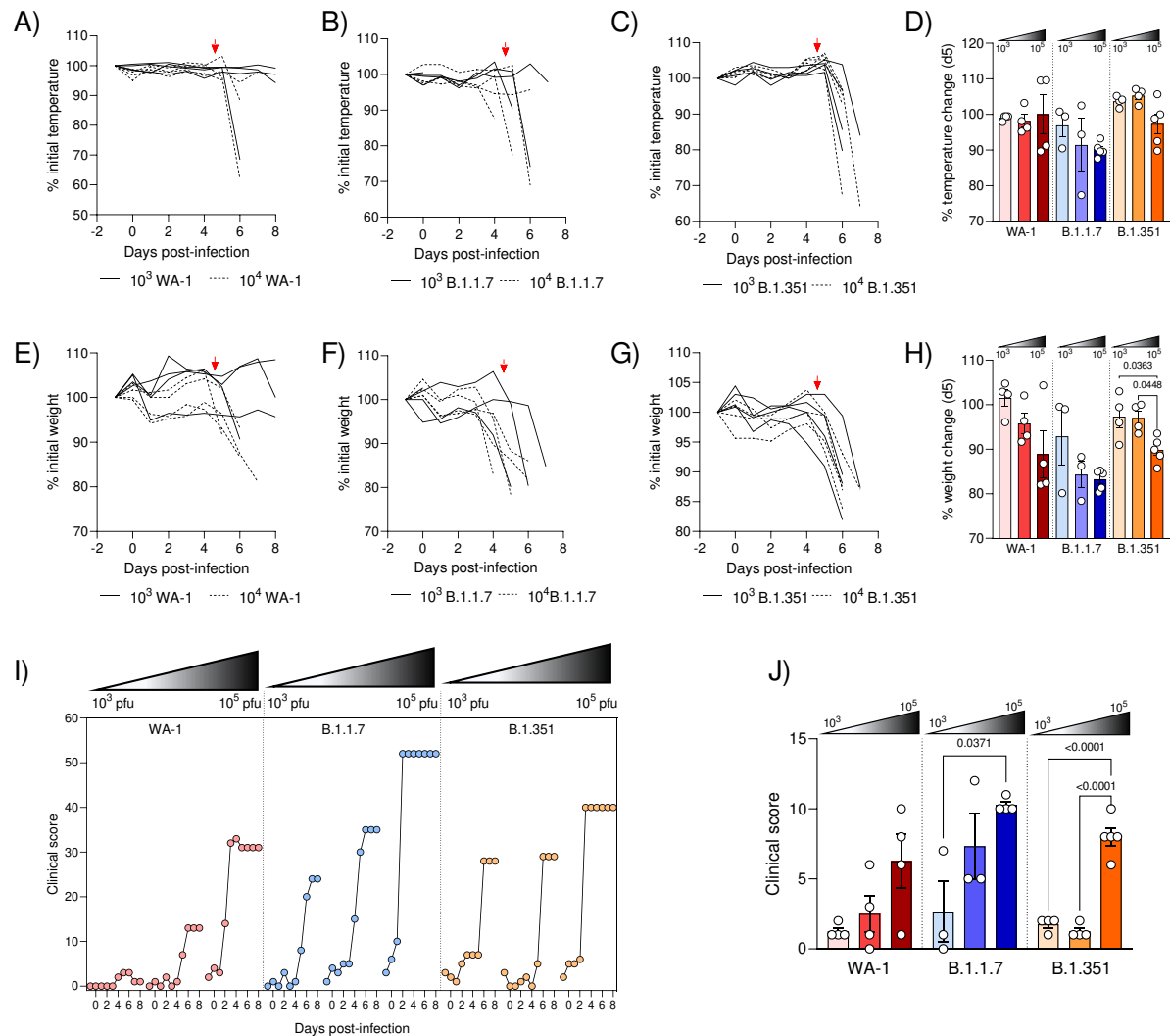
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548 **FIGURES:**



549

550 **Figure 1 | Characterization of SARS-CoV-2 variants *in vitro*:** (A) Plaque morphology of SARS-  
551 CoV-2 WA-1, B.1.1.7 or B.1.351 infected VeroE6 cells. (B) Quantification of viral replication of  
552 SARS-CoV-2 variants in VeroE6 cells over time was quantified. Comparison of viral titers in cell  
553 culture at 12 (C), 24 (D), 48 (E), and 72 (F) hours post-infection. Statistical analysis of viral  
554 replication was completed by two-way ANOVA followed by Tukey's multiple comparison test, or  
555 RM ANOVA followed by Tukey's multiple comparison test. \*\*\*\* =  $P < 0.0001$  relative to WA-1, ####  
556 =  $P < 0.0001$  relative to B.1.1.7.



557

558 **Figure 2 | Establishing clinical endpoints of SARS-CoV-2 VoC infection in K18-hACE2**

559 **transgenic mice:** Mice were infected with 10<sup>3</sup>, 10<sup>4</sup> or 10<sup>5</sup> pfu of SARS-CoV-2 VoCs and were

560 monitored for temperature (A-C), body weight (E-G), and clinical score (I-J) changes over

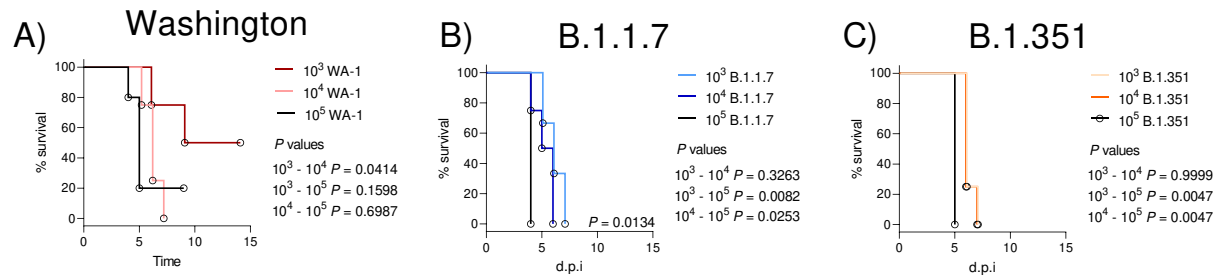
561 infection. Temperature (D), body weight (H), and clinical scores (J) on day 5 post-infection were

562 assessed. If mice were deceased at five days post-infection, their clinical data at time of

563 euthanasia is presented. Arrows indicate day 5 (A-H). Statistical significance was assessed by

564 one-way ANOVA followed by Tukey's multiple comparison test. n > 3 subjects per group.

565



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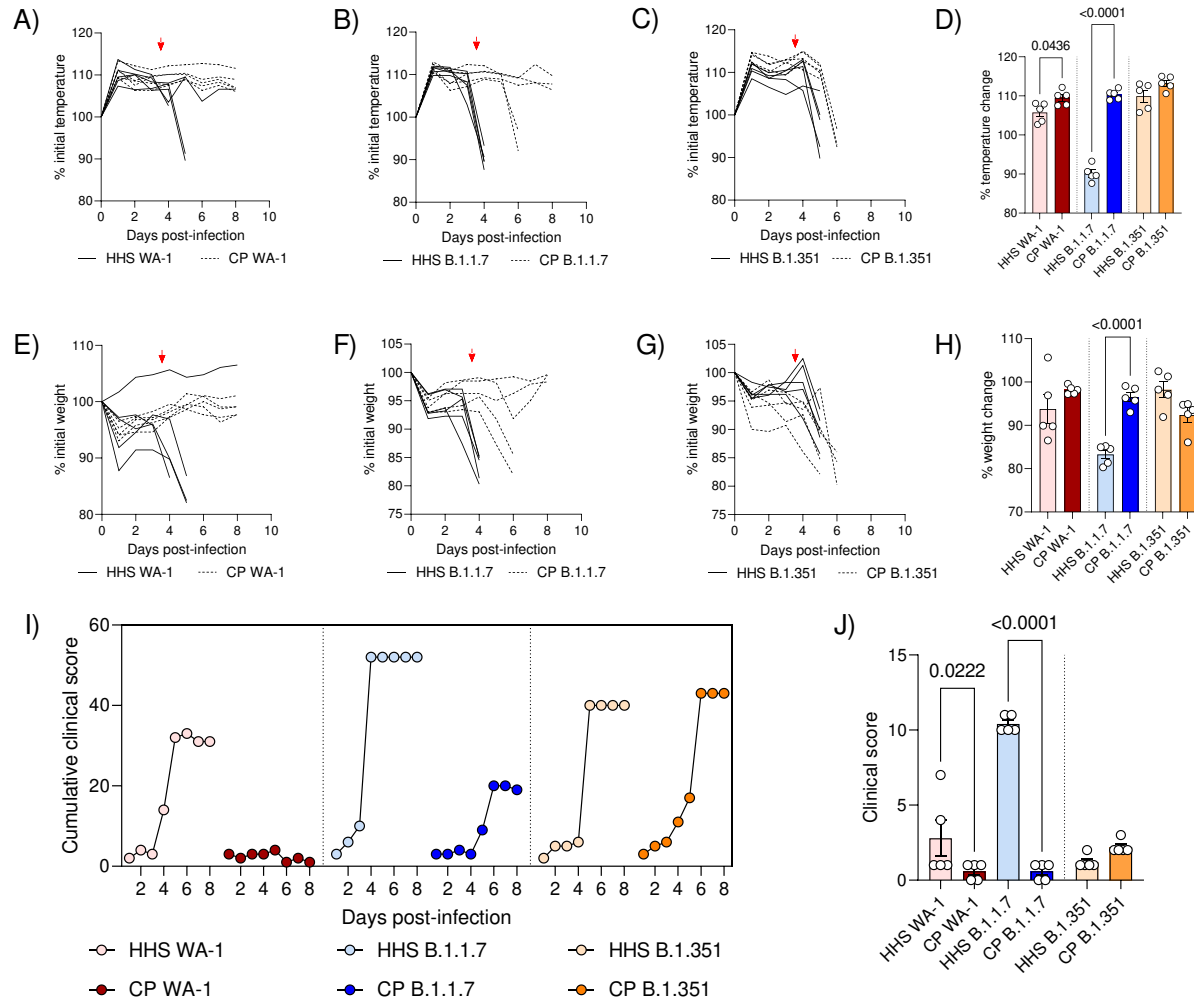
567 **Figure 3 | Impact of dose on survival of K18-hACE2 transgenic mice infected with SARS-**

568 **CoV-2 VoCs:** Kaplan-Meier survival curves of mice infected with WA-1 (A), B.1.1.7 (B), or

569 B.1.351 (C) VoCs at  $10^3$ ,  $10^4$ , and  $10^5$  pfu doses. Statistical significance was assessed by Mantel-

570 Cox tests.  $n = 3$ -5 subjects per group. *P* values for significant differences are reported.

571



572

573 **Figure 4 | Effect of convalescent plasma treatment on SARS-CoV-2 VoC infection in K18-**

574 **hACE2 transgenic mice:** Mice infected with  $10^5$  pfu SARS-CoV-2 VoCs were treated

575 intraperitoneally with 500 $\mu$ L HHS or CP and monitored for temperature (A-C), body weight (E-G)

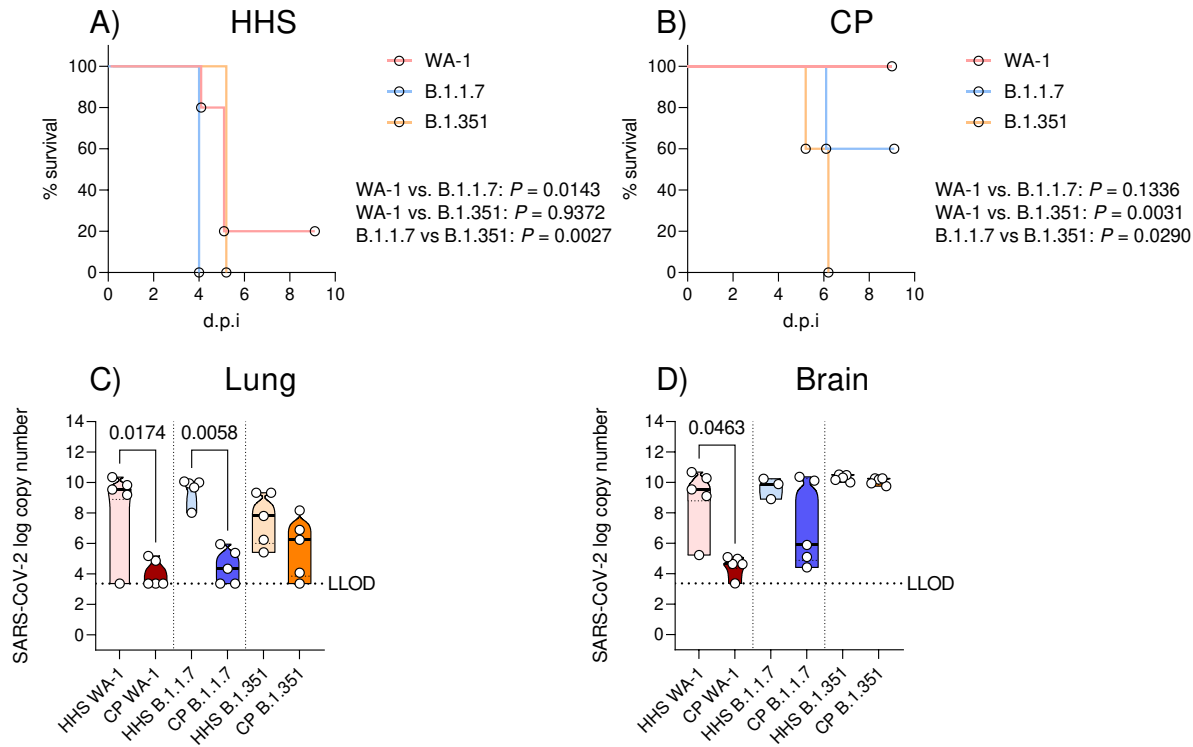
576 and clinical score (I) over the course of infection. Temperatures (D), body weights (H), and clinical

577 scores (J) on day 4 post-infection were assessed. Arrows indicate day 4 (A-H). Statistical

578 significance was assessed by one-way ANOVA followed by Tukey's multiple comparison test. n

579 > 3 subjects per group. P values for significant differences are reported.

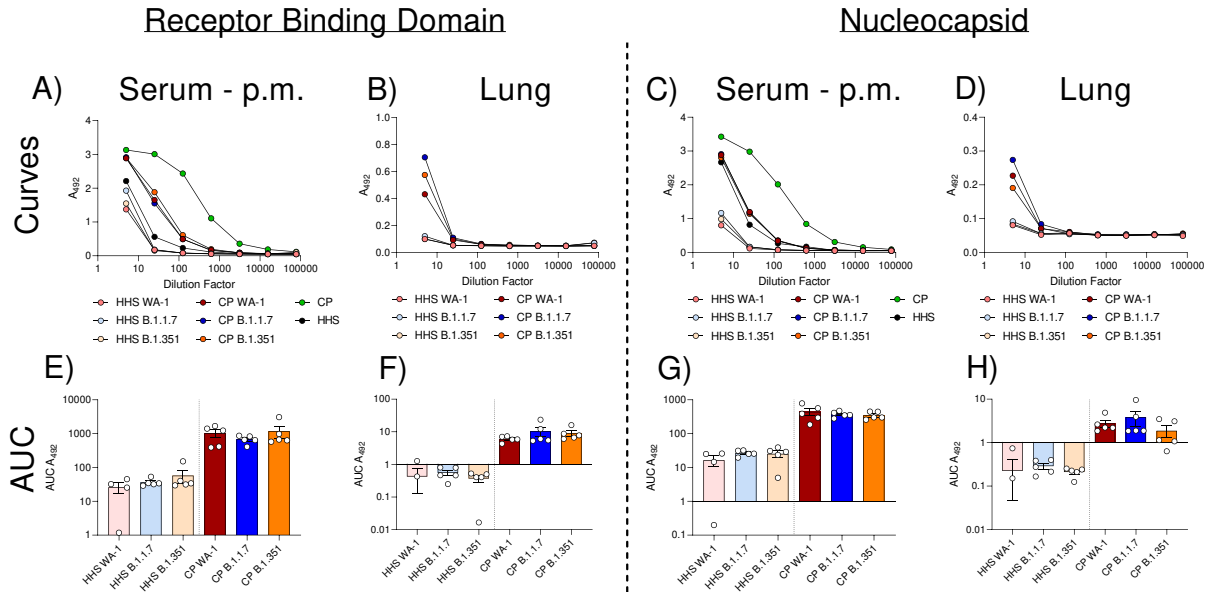
580



581

582 **Figure 5 | Survival and viral infection of serum-treated K18-hACE2 transgenic mice infected**  
583 **with SARS-CoV-2 VoCs:** Kaplan-Meier survival curves of mice infected with B.1.1.7, B.1.351,  
584 or WA-1 treated with HHS (A) or early pandemic SARS-CoV-2 CP (B). Viral copy numbers in the  
585 lung (C) and brain (D) of infected mice. LLOD = lower limit of detection based on a standard curve.  
586 Statistical significance of survival curves was assessed with the Mantel-Cox test. Statistical  
587 significance between viral copy number was assessed by a Kruskal-Wallis test followed by Dunn's  
588 multiple comparisons test.  $n > 3$  subjects per group.  $P$  values for significant differences are  
589 reported.

590



591

592 **Figure 6 | Human anti-SARS-CoV-2 IgGs in serum-treated K18-hACE2 transgenic mice**

593 **infected with SARS-CoV-2 VoCs:** Human antibody (IgG) levels against SARS-CoV-2 antigens.

594 Anti-RBD curves in serum (A) and lung (B) or anti-N curves in serum (C) or lung (D) of infected

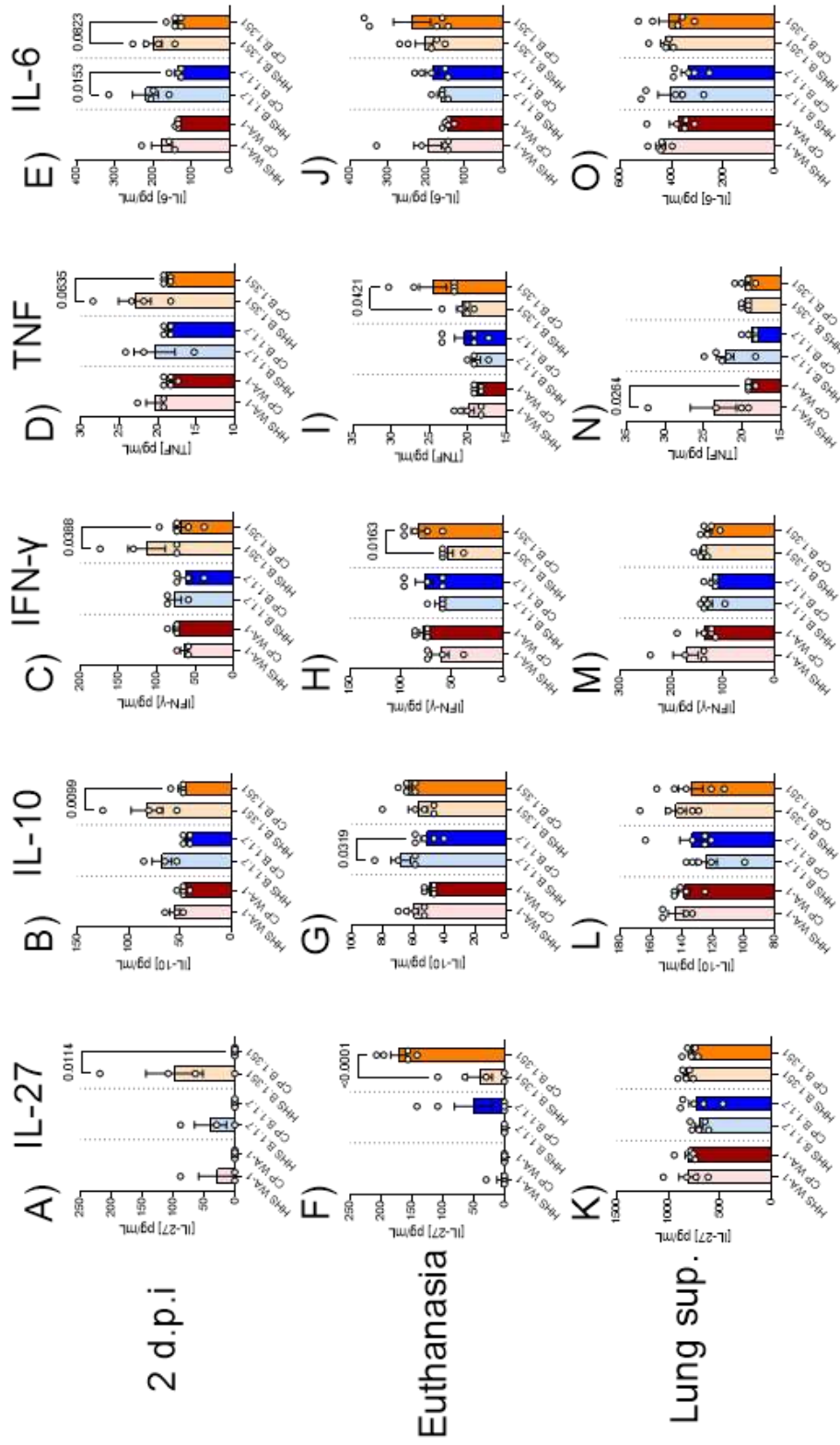
595 mice. Area under the curve (AUC) analyses of anti-RBD IgG levels in the serum (E) or lung (F) of

596 infected mice. AUC analyses of anti-N IgG levels in the serum (G) or lung (H). Statistical

597 significance between AUCs was assessed by a Kruskal-Wallis test followed by Dunn's multiple

598 comparisons test. . n > 3 subjects per group.


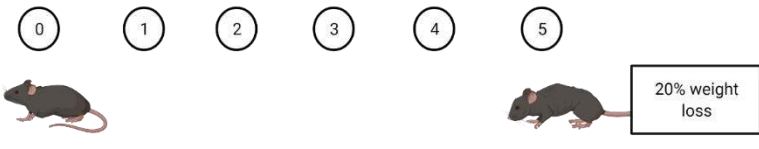









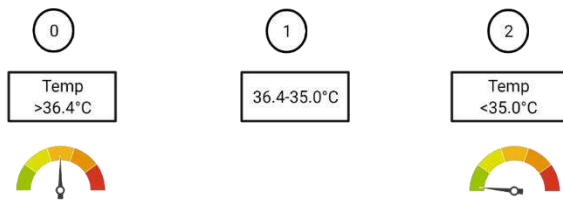
599



**Figure 7 | Cytokine responses in serum-treated K18-hACE2 transgenic mice: IL-6, TNF-α, IFN-γ, IL-10, and IL-27 were quantified in serum two days post infection (A-E), in serum at euthanasia (F-J), or in the lung (K-O). Statistical significance was assessed by one-way ANOVA followed by Tukey's multiple comparison test. n > 3 subjects per group. P values for significant differences are reported.**

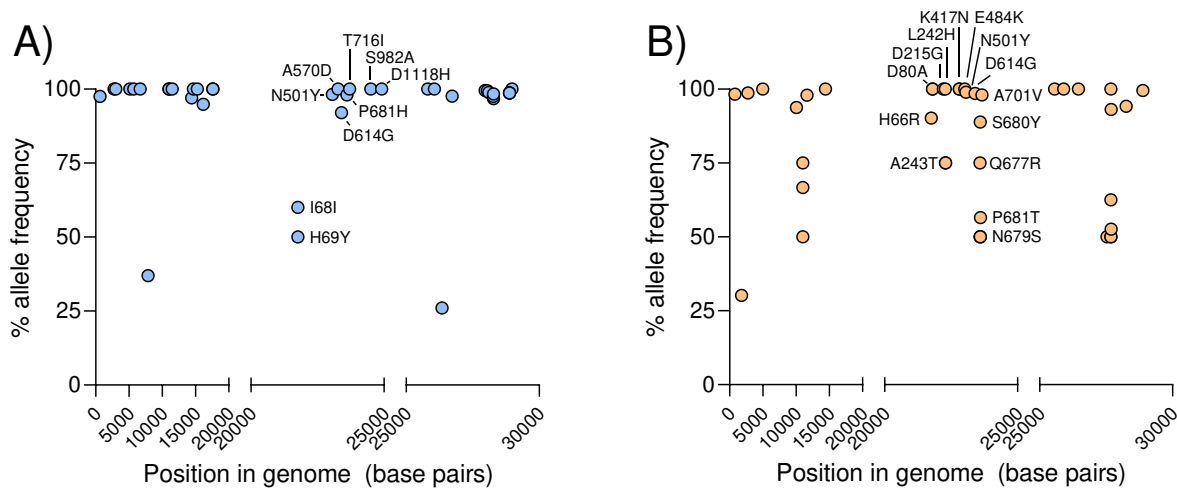


602 **SUPPLEMENTARY FIGURES:**

<b>Weight loss (0-5)</b> 	
<b>Appearance (0-2)</b> 	
<b>Activity (0-3)</b> 	
<b>Eye closure (0-2)</b> 	
<b>Respiration (0-2)</b> 	
<b>Temperature (0-2)</b> 	

603

604 **Supplementary Figure 1 | Clinical scoring system:** K18-hACE2 transgenic mice were  
605 assessed for weight loss, appearance, activity, eye closure, respiration, and temperature and  
606 scored using the metrics provided. Mice with a score of 5 in weight loss or a 2 in respiration were  
607 euthanized.



608

609 **Supplementary Figure 2 | Sequencing of SARS-CoV-2 VoCs in this study: NGS of SARS-**

610 CoV-2 WT and VoCs demonstrates that mutations associated with each variant are present within

611 the B.1.1.7 (A) and B.1.351 (B) genomes. Mutations within the spike protein are labeled.

612

```
ORF7a_Washington_MN985325.1      1  MKIILFLALITLQATCELYHYQECVGRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFS
ORF7aDEL_South_Africa_NR-54008  1  MKIILFLALITLQATCELYHYQECVGRGTTVLLKEPCSSGTYEGNSPFQ.....
```

```
ORF7a_Washington_MN985325.1      61  TQFAFACPDGKVKHVVQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVEITLCFTLKRKT
ORF7aDEL_South_Africa_NR-54008  48  .....IFLIVAAIVEITLCFTLKRKT
```

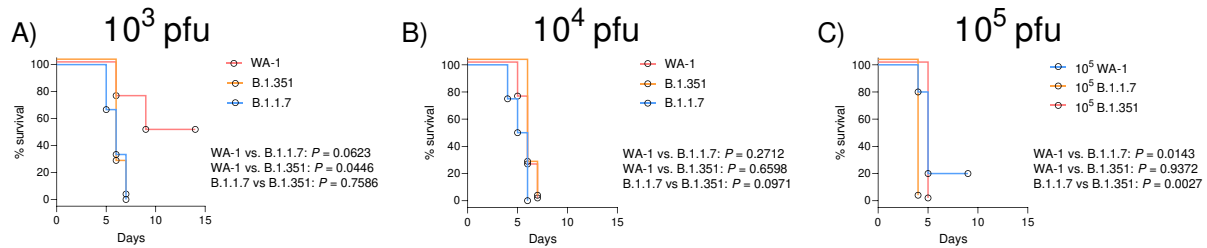
```
ORF7a_Washington_MN985325.1      121  E
ORF7aDEL_South_Africa_NR-54008  69  E
```

613

614 **Supplementary Figure 3 | ORF7a deletion in B.1.351 SARS-CoV-2 VoC in this study:**

615 Annotated deletion within the ORF7a protein of the B.1.351 VoC used in this study.

616



617

618 **Supplementary Figure 4 | Impact of variant on survival of K18-hACE2 transgenic mice**

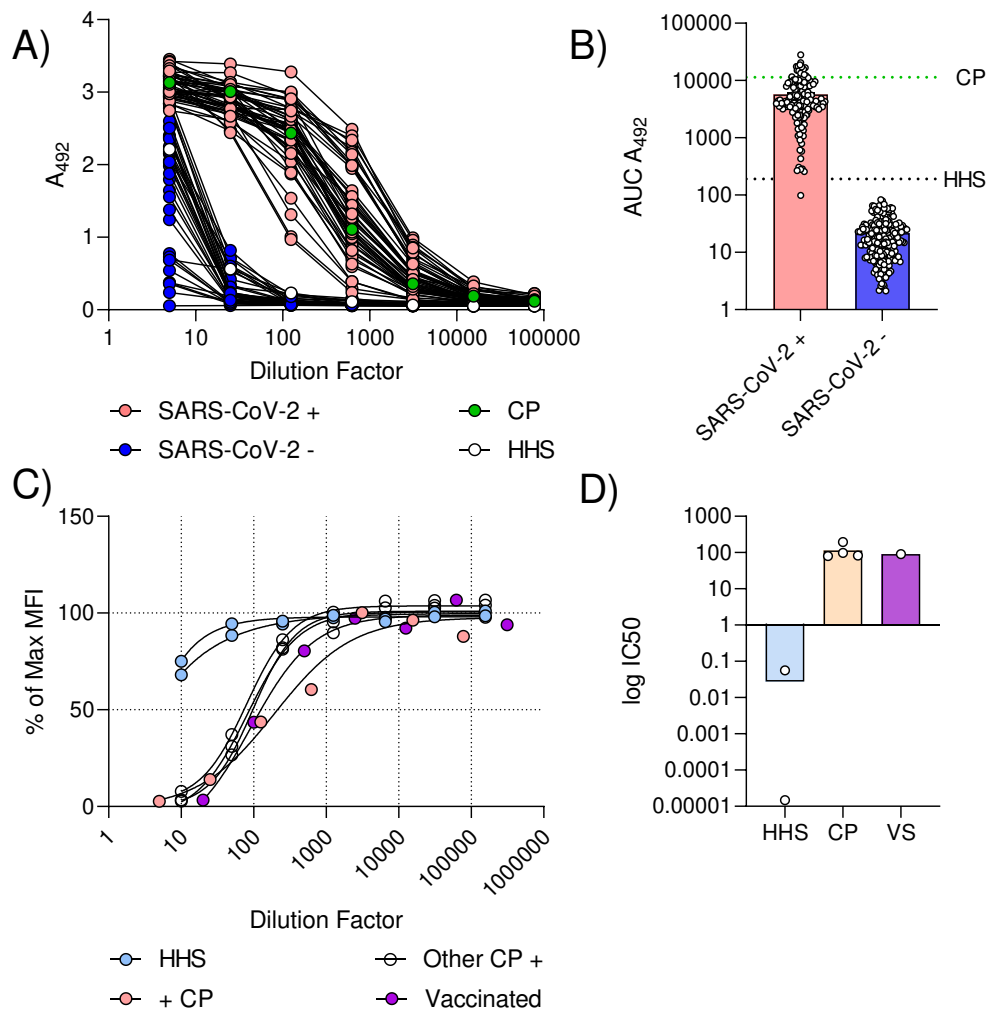
619 **infected with SARS-CoV-2 VoCs :** Kaplan-Meier survival curves of mice infected with  $10^3$  (A),

620  $10^4$  (B), or  $10^5$  (C) pfu doses of WA-1 B.1.1.7, or B.1.351 SARS-CoV-2 VoCs. Statistical

621 significance was assessed by Mantel-Cox tests.  $n > 3$  subjects per group.  $P$  values for significant

622 differences are reported.

623

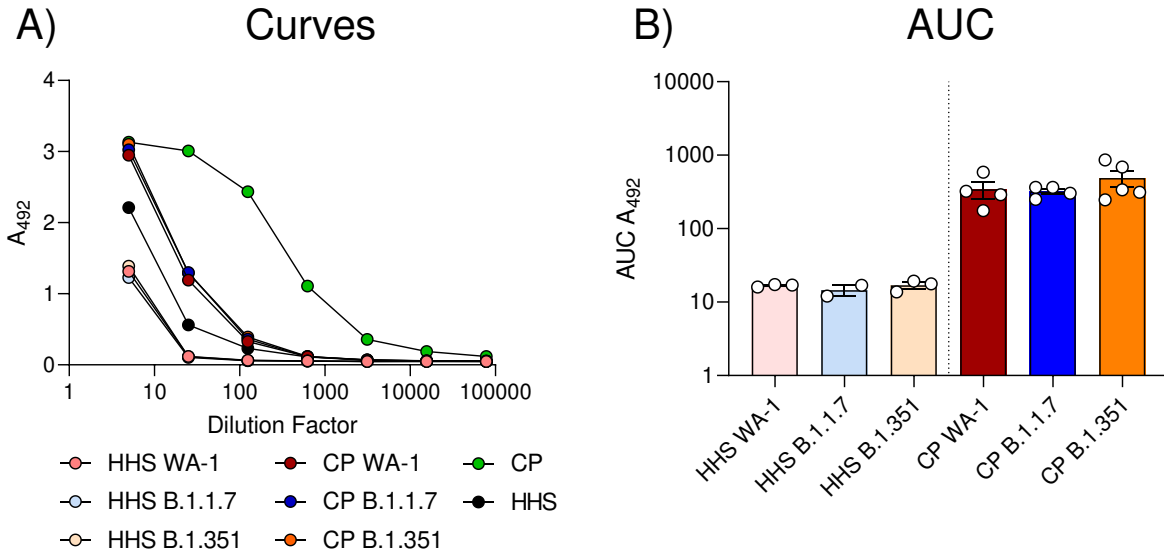


624

625 **Supplementary Figure 5 | Anti-RBD IgG levels and neutralization capacity of HHS and CP:**

626 IgG levels of CP and HHS were assessed using ELISA developed previously. CP (green, dotted  
 627 green line) and HHS (white, dotted black line) ELISA curves (A) and AUCs (B) were compared to  
 628 a subset of SARS-CoV-2+ (red) and SARS-CoV-2- (blue) individuals from a prior study.  
 629 Neutralizing antibody function was assessed with a modified Luminex assay. Neutralization  
 630 curves (C) and IC50 values (D) for nAbs from HHS (blue), CP (red), and a vaccinated individual  
 631 (purple) are provided.

632



633

634 **Supplementary Figure 6 | Human anti-SARS-CoV-2 IgGs two days post-infection in serum-**

635 **treated K18-hACE2 transgenic mice infected with SARS-CoV-2 VoCs: Antibody (IgG) levels**

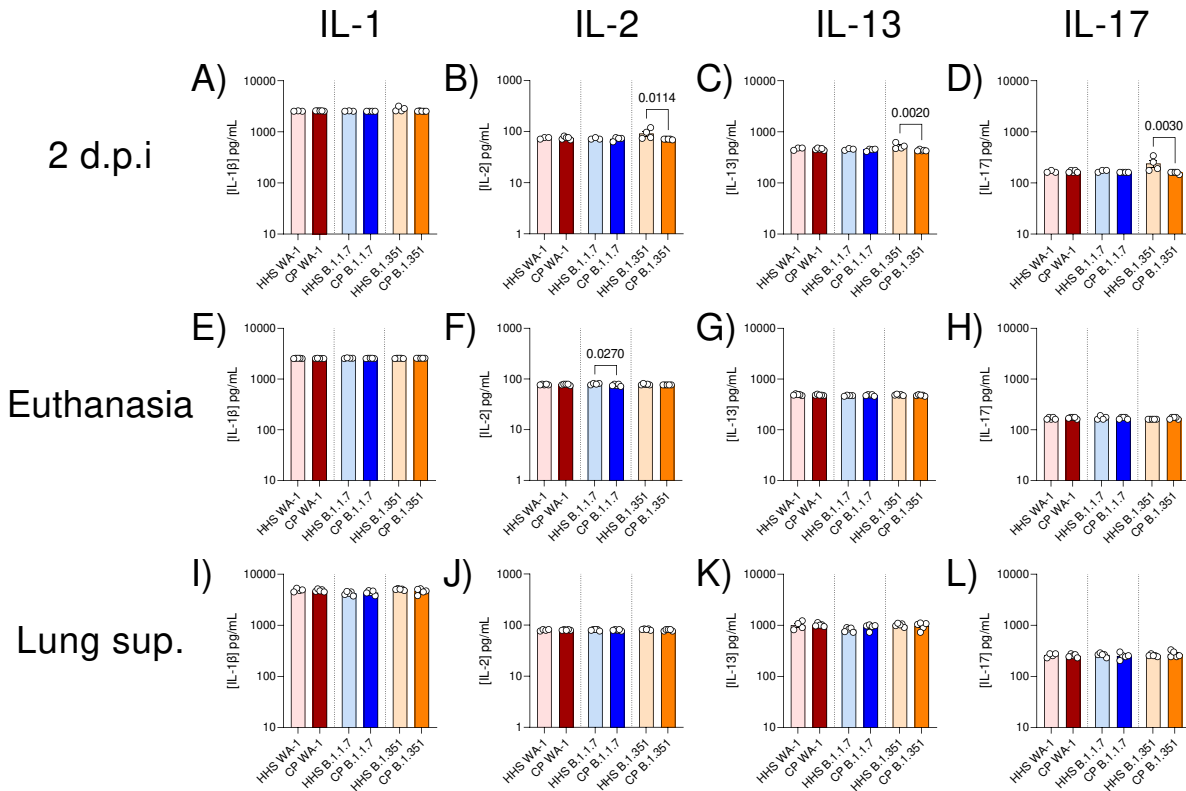
636 against SARS-CoV-2 antigens. Anti-RBD curves in serum (A) and of infected K18-hACE2

637 transgenic mice 2 days post-infection. Area under the curve (AUC) analyses of anti-RBD IgG

638 levels (B). Statistical significance between AUCs was assessed by a Kruskal-Wallis test followed

639 by Dunn's multiple comparisons test. n > 3 subjects per group.

640



641

642 **Supplementary Figure 7 | Minor or no differences in some cytokine levels in K18-hACE2**

643 **transgenic mice treated with serum: IL-1, IL-2, IL-13, and IL-17 were quantified in serum two**

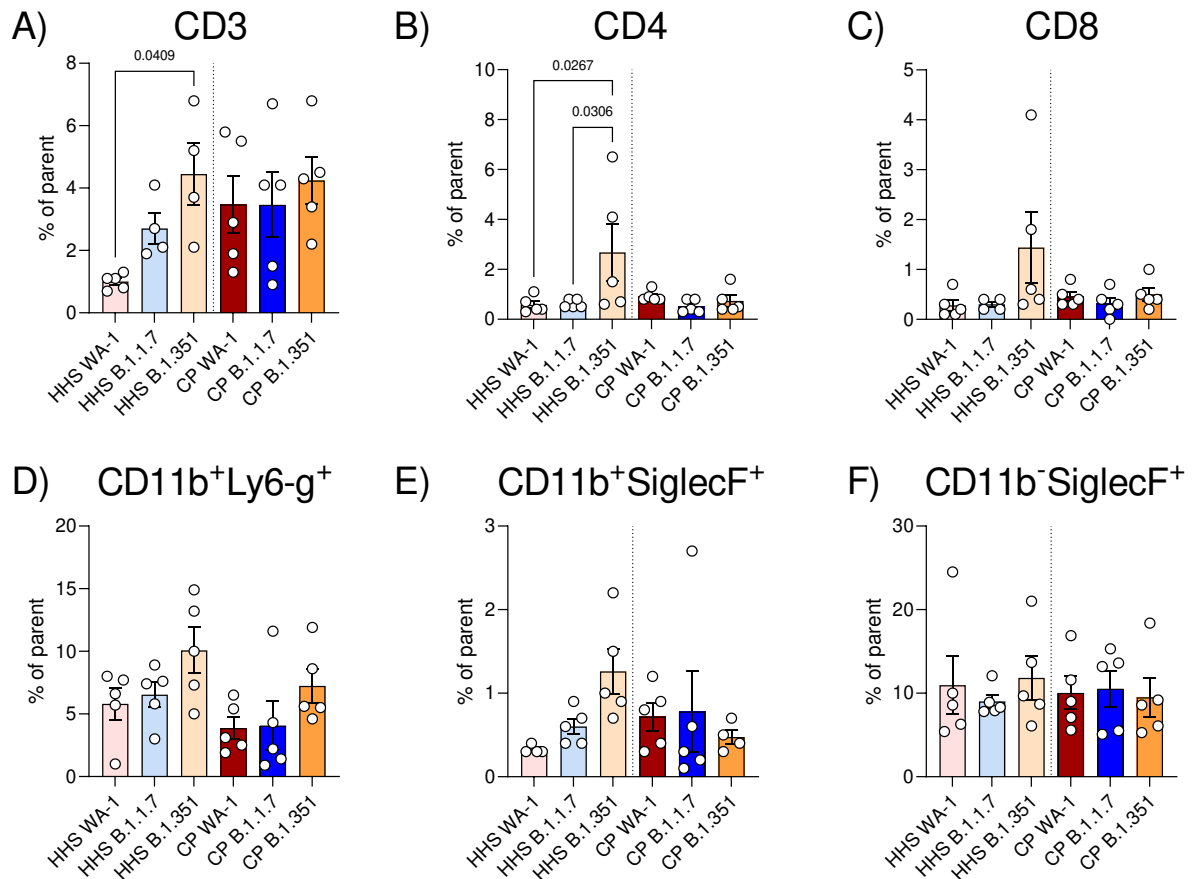
644 **days post infection (A-D), in serum at euthanasia (E-H), or in the lung (I-L). Statistical significance**

645 **was assessed by one-way ANOVA followed by Tukey's multiple comparison test. n > 3 subjects**

646 **per group. P values for significant differences are reported.**

647





648

649 **Supplementary Figure 8 | T cell and myeloid cell populations in lungs of K18-hACE2**

650 **transgenic mice infected with SARS-CoV-2 VoCs: CD3 (A), CD4 (B), and CD8 (C) T cells were**

651 **quantified in lung homogenates from SARS-CoV-2 variant infected K18-hACE2 transgenic mice.**

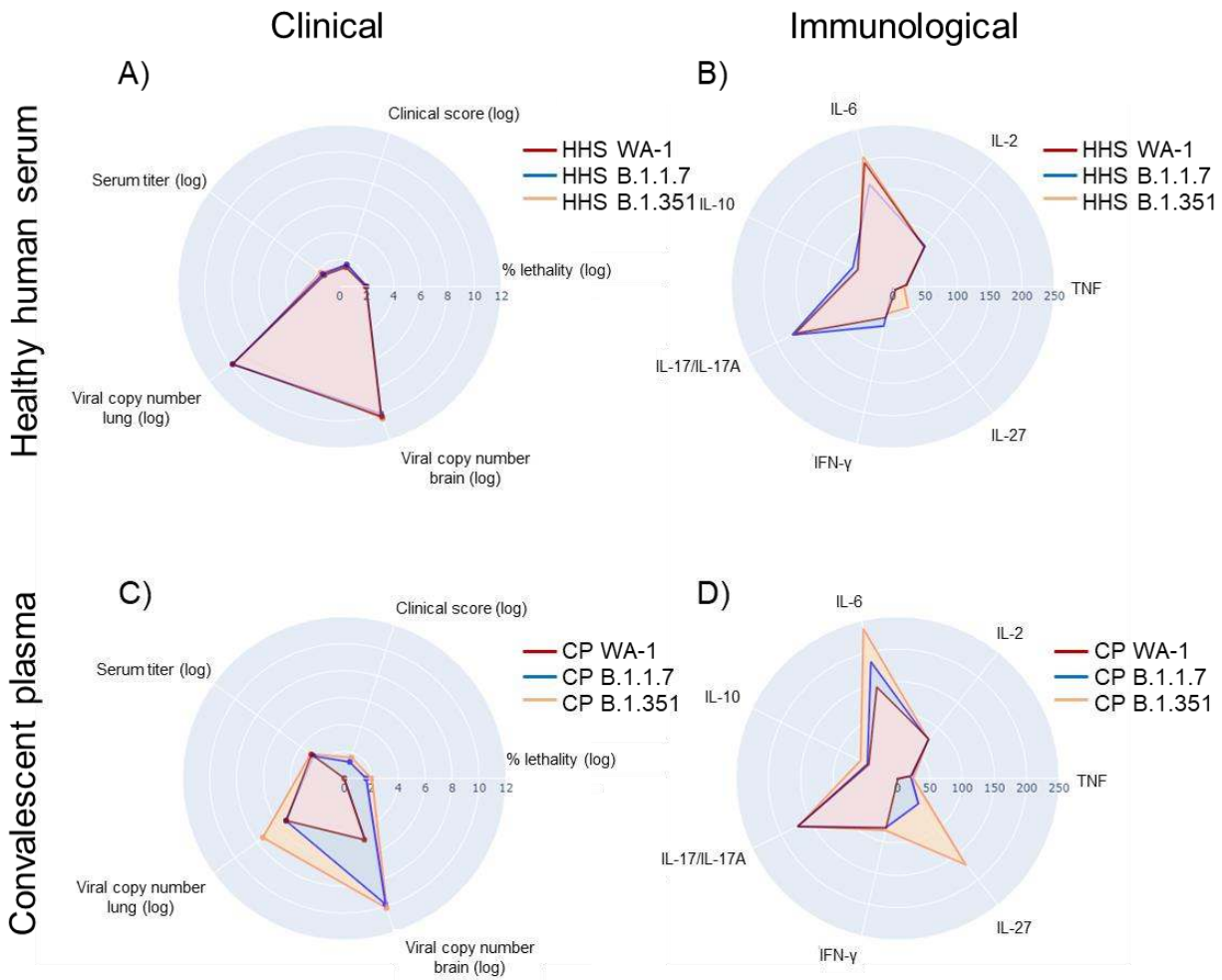
652 **CD11b<sup>+</sup>Ly6-g<sup>+</sup> (D), CD11b<sup>+</sup>SiglecF<sup>+</sup>, and CD11b<sup>-</sup>SiglecF<sup>+</sup> cells myeloid cells were also quantified**

653 **in lung homogenates. Statistical analyses were performed by one-way ANOVA followed by**

654 **Tukey's multiple comparison test. n>3 samples per group. P values of statistically significant**

655 **results are reported.**

656



657

658 **Supplementary Figure 9 | Comparison of clinical and immunological phenotypes of SARS-**

659 **CoV-2 infected K18-hACE2 transgenic mice treated with HHS or CP: Clinical (A) and**

660 **immunological (B) phenotypes of SARS-CoV-2 infected K18-hACE2 transgenic mice treated with**

661 **HHS. Clinical (C) and immunological (D) phenotypes of SARS-CoV-2 infected K18-hACE2**

662 **transgenic mice. Scatterpolar plots were generated in Python using plotly.**

663

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