

1 **Pathogenic and transcriptomic differences of emerging SARS-CoV-2 variants in**
2 **the Syrian golden hamster model**

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20

21 **Abstract**

22 Following the discovery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and
23 its rapid spread throughout the world, new viral variants of concern (VOC) have emerged. There
24 is a critical need to understand the impact of the emerging variants on host response and
25 disease dynamics to facilitate the development of vaccines and therapeutics. Syrian golden
26 hamsters are the leading small animal model that recapitulates key aspects of severe
27 coronavirus disease 2019 (COVID-19). In this study, we show that intranasal inoculation of
28 SARS-CoV-2 into hamsters with the ancestral virus (nCoV-WA1-2020) or VOC first identified in
29 the United Kingdom (B.1.1.7) and South Africa (B.1.351) led to similar gross and histopathologic
30 pulmonary lesions. Although differences in viral genomic copy numbers were noted in the lungs
31 and oral swabs of challenged animals, infectious titers in the lungs were comparable. Antibody
32 neutralization capacities varied, dependent on the original challenge virus and cross-variant
33 protective capacity. Transcriptional profiling indicated significant induction of antiviral
34 pathways in response to all three challenges with a more robust inflammatory signature in
35 response to B.1.1.7. Furthermore, no additional mutations in the spike protein were detected
36 at peak disease. In conclusion, the emerging VOC showed distinct humoral responses and
37 transcriptional profiles in the hamster model compared to the ancestral virus.

38

39

40 **Keywords**

41 COVID-19, Severe acute respiratory syndrome coronavirus-2, variants of concern, pathogenesis,
42 interstitial pneumonia, animal model

43 **Introduction**

44 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has emerged as a novel, highly
45 infectious respiratory CoV and the causative agent of CoV disease 2019 (COVID-19)¹. First
46 described in the city of Wuhan in Hubei province of China, SARS-CoV-2 is a member of the
47 *Coronaviridae* family, which possess large, non-segmented RNA genomes¹. High levels of
48 transmission, especially in regions with low vaccination rates, facilitate the emergence of
49 mutations that improve viral fitness. SARS-CoV-2 variants of concern (VOC) are defined as
50 variants that have one or more mutations that confer worrisome epidemiologic, immunologic,
51 or pathogenic properties². Several SARS-CoV-2 VOC have emerged such as B.1.1.7 first reported
52 in the United Kingdom (UK), which is associated with increased transmission compared to the
53 ancestral virus reported from Washington, USA in early 2020³. This variant acquired over 20
54 mutations including N501Y within the spike (S) protein that increased binding affinity to the
55 angiotensin converting enzyme 2 (ACE2) receptor^{4,5}. In addition, the S protein of the B.1.1.7
56 variant has a deletion of amino acids 69 and 70 which has been shown to increase viral escape
57 in immunocompromised individuals^{6,7}. VOC B.1.351 was originally reported in South Africa (SA)
58 and harbors similar mutations in S compared to B.1.1.7 as well as the K417N and E484K
59 substitutions that may decrease the efficacy of existing vaccines⁸⁻¹². Other variants more
60 recently reported in the United States (B.1.427, B1.429) also harbor mutations in S (e.g., N501Y)
61 that have been associated with reductions in neutralizing antibody titers¹³.

62
63 There is an urgent need to understand the effect of new mutations within VOC on the host
64 immune response to facilitate the development of vaccines and therapeutics. In this study, we
65 compared pathologic features of and immune responses to the original virus (ancestral), and
66 the later B.1.1.7 and B.1.351 variants in the well-established Syrian golden hamster model of
67 severe COVID-19¹⁴. Specifically, we longitudinally assessed viral replication, histopathological
68 changes, development of humoral immunity and humoral cross-reactivity amongst VOC.
69 Additionally, we employed RNA-seq and digital cell quantification of lung homogenates to
70 determine differences in transcriptomic signatures and to infer changes in immune cell subsets.
71 We identified similar histopathological changes, levels of infectious virus, and antibody titers

72 among all infections. However, transcriptional responses and the capacity to cross-neutralize
73 SARS-CoV-2 was VOC-dependent. Collectively, these data demonstrate that mutations within
74 SARS-CoV-2 modulate host defense pathways.

75

76 **Results**

77 *Gross lung pathology*

78 Syrian golden hamsters were separated into three cohorts (n=15 per cohort) and challenged
79 intranasally (IN) with 10^5 TCID₅₀ of one of three different SARS-CoV-2 variants: ancestral (nCoV-
80 WA1-2020), B.1.1.7, and B.135. Five uninfected animals served as negative controls. Scheduled
81 necropsies were performed at 4, 14, and 28 days post-challenge (DPC) for all groups to capture
82 peak disease and convalescence (Fig. S1A). Peak weight loss was achieved amongst all three
83 groups 7 DPC, however, no significant difference in body weight changes occurred over the first
84 10 DPC for any of the infections (Fig. S1B). Gross pulmonary lesions were observed in all
85 infected hamsters at 4 DPC (Fig. S1D). Lungs harvested 4 DPC showed multifocal to locally
86 extensive areas of red to purple coloration (consistent with consolidation) disseminated
87 throughout all lung lobes. Additionally, lungs generally failed to collapse indicating interstitial
88 disease. Lung samples harvested 14 and 28 DPC had either no gross lesions or limited, small,
89 multifocal areas of consolidation and/or congestion. Analysis of histopathology samples
90 demonstrated evidence of interstitial pneumonia on 4 and 14 DPC in all groups (Fig. S1C).

91

92 *Histopathology and immunohistochemistry of hamster lungs*

93 Pulmonary pathology consistent with previously described coronavirus respiratory disease was
94 observed at 4 DPC in lung samples from hamsters infected with each virus (Fig. 1)¹⁵. Five
95 uninfected animals served as negative controls (Fig. 1A, E, I). Foci of interstitial pneumonia and
96 bronchiolitis were observed throughout all evaluated lung lobes of infected hamsters. Minimal
97 to mild bronchiolitis characterized by individual epithelial cell necrosis, epithelial cell basophilia
98 and hyperplasia and rare syncytial cell formation was observed throughout all variants (Fig. 1 B-
99 D). Interstitial pneumonia varying in percent of lung involvement and moderate to severe

100 severity was observed within each animal regardless of the variant. Interstitial pneumonia at 4
101 DPC was defined by expansion of alveolar septa by edema fluid, leukocyte infiltration and fibrin,
102 with leukocyte spillover into adjacent alveolar spaces and in severe cases, complete loss of
103 pulmonary architecture (Fig. 1 F-H). Tracheitis characterized by neutrophilic influx and epithelial
104 cell necrosis was observed in all evaluated sections of trachea in each animal at 4 DPC.
105 Immunohistochemical analysis showed immunoreactivity to an antibody specific to SARS-CoV-2
106 within bronchiolar epithelia, type I and type II pneumocytes and macrophages in lungs of all
107 hamsters regardless of the viral variant (Fig. 1 J-L).

108 At 14 and 28 DPC pulmonary pathology was similar in lungs of hamsters infected with all viruses
109 (Fig. S1C, D). Foci of persistent type II pneumocyte hyperplasia with occasional apical cilia
110 formation (alveolar bronchiolization) adjacent to terminal bronchioles was observed
111 throughout all lung lobes. Frequently, foci of alveolar bronchiolization entrapped low to
112 moderate numbers of foamy macrophages. Antigen was not detected by immunohistochemical
113 evaluation for any viral variant at either 14 or 28 DPC.

114

115 *Viral burden*

116 Total viral RNA copy numbers and infectious viral titers were quantified in lungs of challenged
117 animals at the three time points mentioned above (Fig. 2A-C). There was no difference in viral
118 RNA copy numbers amongst challenged groups at 4 DPC (Fig. 2A). However, there was
119 significantly more viral RNA at 14 DPC in the B.1.1.7-challenged group compared to the
120 ancestral and B.1.351 groups. At 28 DPC there were significantly more viral RNA copies in the
121 lungs of ancestral-challenged hamsters than the B.1.1.7 group (Fig. 2A). We also assessed sub-
122 genomic viral RNA (sgRNA) as a surrogate of active viral replication^{16,17}. Levels of lung sgRNA
123 peaked at 4 DPC and were comparable among the three variants (Fig. 2B). In contrast, we
124 observed a significant difference in sgRNA among the groups at 14 DPC. Specifically, B.1.1.7-
125 infected hamsters exhibited the highest residual sgRNA present compared to the ancestral and
126 B.1.351 groups (Fig. 2B). The B.1.351 group also had significantly higher sgRNA levels compared
127 to the ancestral group (Fig. 2B). However, infectious viral titers were only detected 4 DPC in
128 lungs in all hamsters (Fig. 2C).

129 Oral viral shedding and viremia were evaluated at the time of necropsy. Infection with the
130 B.1.1.7 VOC resulted in significantly more oral viral shedding than the B.1.351 variant at 4 and
131 14 DPC (Fig. 2D). Viremia peaked at 4 DPC and was comparable amongst all infections (Fig. 2E).
132 Profiling the viral genomes recovered from the lungs of infected hamsters at 4 DPC revealed no
133 changes in the viral sequences in ancestral and B.1.1.7-infected animals compared to the
134 reference genomes (Table 1). However, we identified three mutations in all B.1.351-infected
135 animals, including two nonsynonymous mutations in 5' UTR (T201C) and nsp3 (G172C), and one
136 synonymous mutation in nsp3 (G5942G). A single B.1.351-infected animal presented with an
137 additional mutation (L3892F) in nsp3 (Table 1). No mutations in S were detected.

138

139 *Humoral immune responses post-challenge*

140 We utilized standard ELISA methods to determine the SARS-CoV-2 S-specific IgG responses, and
141 S receptor-binding domain (RBD)-specific IgG responses. There was no difference in the S-
142 specific IgG titers at either 14 or 28 DPC amongst the groups (Fig. 3A). Similarly, no difference
143 was determined in the RBD-specific IgG titers at 14 DPC (Fig. 3B). However, at 28 DPC the RBD-
144 specific IgG titer was significantly higher in animals challenged with B.1.351 compared to
145 B.1.1.7 (Fig. 3B).

146 Next, we assessed the functionality of the humoral response by neutralization assay, not only
147 against the homologous challenge virus, but also against the other two variants to determine
148 cross-reactivity generated from the primary infection. Hamsters challenged with the ancestral
149 virus exhibited comparable neutralizing titers against the homologous challenge variant
150 (ancestral) and the B.1.1.7 variant at 14 and 28 DPC but lower titers against the B.1.351 variant
151 at both timepoints assessed (Fig. 3C). In contrast, hamsters challenged with the B.1.1.7 or the
152 B.1.351 variant each exhibited significantly higher neutralizing titers against their homologous
153 challenge virus at 14 DPC compared to variants to which they were not exposed (Fig. 3D, E). This
154 difference persisted for the B.1.351-infected animals at 28 DPC when comparing anti-B.1.351
155 and anti-B.1.1.7 titers (Fig. 3E). Moreover, the overall neutralization titers against the B.1.351
156 variant were 1-2 logs lower than the other two variants regardless of homologous or
157 heterologous assessment.

158

159 *COVs elicit unique transcriptional responses in the lungs*

160 To elucidate differences in the host responses to VOC, we profiled the transcriptional responses
161 in lung tissues obtained at peak viral loads (4 DPC) (Fig. 4, S2). Principal component analysis
162 (PCA) revealed distinct separation between uninfected and uninfected animals (Fig. S2A), with
163 the B.1.1.7 variant infection resulting in the most distinct transcriptional profile and the largest
164 number of differentially expressed genes (DEGs) (n=1,277) while infection with B.1.351 resulted
165 in the smallest number of DEGs (n=395) (Fig. 4A-C). Most DEGs were upregulated following
166 infection with all three viruses (Fig. 4A-C). A core of 291 DEGs was shared by all variants and an
167 additional ~270 DEGs were shared only between B.1.1.7- and ancestral-infected hamsters (Fig.
168 4D).

169 We performed functional enrichment of DEGs in order to determine their biological relevance.
170 DEGs induced by all three viral infections enriched to Gene Ontology (GO) terms associated
171 with antiviral immunity (e.g., “response to virus”), immune cell recruitment (e.g., “leukocyte
172 chemotaxis”) and mobilization of adaptive immunity (e.g., “lymphocyte activation”, “B cell-
173 mediated immunity”) (Fig. 4E). DEGs enriching to “response to virus” and common to all three
174 infections play roles in type I interferon (IFN) signaling (e.g., *IRF7*, *IRF9*, *STAT1/2*), nucleic acid
175 detection (e.g., *DDX60*, *DHX58*) and the antiviral response (e.g., *ISG15*, *MX1*, *RSAD2*, *SAMHD1*)
176 (Fig. S2B). These DEGs were upregulated following infection with all three variants, particularly
177 B.1.1.7. DEGs enriching to this GO term and upregulated following infection by the ancestral
178 and B.1.1.7 variants only were part of T cell activation pathways (e.g., *IFNG*, *IL12RB1*, *TBX21*,
179 *XCL1*) (Fig. S2B).

180 Other DEGs that were upregulated following infection with all three variants enriched to GO
181 term “blood vessel development”. These genes are involved in angiogenesis (e.g., *ANGPTL2*,
182 *ANGPTL4*, *ADM2*, *HOX1*), apoptosis (e.g., *BAK1*, *FASLG*), tissue remodeling (e.g., *CHI3L1*,
183 *MMP19*), and leukocyte chemotaxis (e.g., *CCL11*, *CCL2*, *CXCL10*, *CXCL17*) (Fig. S2C). Regulators
184 of angiogenesis, like *SOX4* and *KDR*, and genes involved in tissue remodeling (e.g., *ADAM12*,
185 *SHH*) were downregulated only in infections with the B.1.1.7 and ancestral virus (Fig. S2C).

186 Shared DEGs that enriched to GO term “lymphocyte activation” included genes important for B

187 cell maturation (e.g., *AIRE*, *CD27*, *CD38*, *ICOS*, *TNFSF13B*) as well as negative regulation of T cell
188 responses (e.g., *CD274*, *CTLA4*, *FOXP3*, *IDO1*, *PDCDC1*, *PDCD1LG2*) (Fig. S2E). DEGs shared
189 between B.1.1.7- and ancestral-infected hamsters were important for T cell activation (e.g.,
190 *PRKCQ*, *TNFSF9*), cytotoxic responses (e.g., *KLRK1*, *PRF1*) myeloid cell activation (e.g., *IFNG*,
191 *SLAMF1*, *CD177*, *CXCL6*) and IL-6 production (e.g., *TLR1*, *IL-6*, *IL18RAP*, *C3AR1*, *C1QA*) (Fig. 4E,
192 S2D).

193 We next analyzed DEGs unique to each infection to understand infection-specific
194 transcriptional responses (Fig. 5). The largest group of unique DEGs was detected following
195 B.1.1.7 infection (n=648). These unique DEGs enriched to GO terms reflecting tissue remodeling
196 (e.g., “response to growth factor”, “tissue morphogenesis”) (Fig. 5A). Most DEGs in these GO
197 terms are downregulated and associated with angiogenesis (e.g., *ENG*, *JCAD*, *PDFGB*, *VEGFD*)
198 and lung development (e.g., *FZD1*, *SOX17*, *TMEM100*, *VANGL2*), while a smaller upregulated
199 portion was associated with cell death (e.g., *APAF1*, *CASP3*), and protein degradation (e.g.,
200 *CASP3*, *DAB2*, *SFRP1*). Other DEG enriched to GO terms associated with host defense (e.g.,
201 “adaptive immune response”) and cell recruitment (e.g., “chemotaxis”) were identified. Most of
202 these DEGs were upregulated and are important for antigen presentation (e.g., *CD74*, *HLA-DRA*,
203 *B2M*) and natural killer (NK) cell-mediated immunity (e.g., *CD84*, *IL12A*) (Fig. 5B-D). Notable
204 DEGs unique to infection with B.1.351 play a role in cell morphogenesis (e.g., *ACTA2*, *ACTC1*,
205 *FGF1*), myeloid cell differentiation (e.g., *CAV3*, *PDE1B*, *TFRC*), and response to injury (e.g.
206 *COL4A3*, *MPL*, *TSPAN*) (Fig. 5E). Downregulated DEGs unique to infection with the ancestral
207 strain encoded components of cellular respiration (e.g., *MT-CO3*, *MT-ND1*) and mediators of cell
208 adhesion (e.g., *IKF26B*, *VIT*) (Fig. 5F).

209

210 *Digital cell quantification in hamster lungs*

211 Since Syrian golden hamsters lack adequate reagents for immunophenotyping, we performed
212 digital cell quantification (DCQ) to predict changes in immune cell populations using the IRIS
213 immune cell database¹⁸. Changes in gene expression were predicted to be associated with
214 increased frequencies of activated NK cells, activated dendritic cells (DCs), and neutrophils after
215 ancestral and B.1.1.7 infection (Fig. S3A). In contrast, B.1.351 infection was associated with a

216 decrease in NK cells and monocytes (Fig. S3B). Reduced frequencies of B cells were predicted
217 for all infections, while increases in Th1 and Th2 CD4+ T cells were only predicted after B.1.1.7
218 infection (Fig. S3B).

219

220 **Discussion**

221 Over the last several months a number of SARS-CoV-2 VOC have emerged. These VOC are
222 associated with increased transmissibility and enhanced viral fitness due to mutations in S.
223 Several studies have shown that the N501Y mutation harbored in both the B.1.1.7 and the
224 B.1.351 variants utilized here increases ACE2 binding and enhances transmission
225 capabilities^{4,5,19}. The K417N and E484K mutations introduced into the S of the B.1.351 enhances
226 the ability to evade pre-existing humoral responses^{3,7,10,20-22}. A comparative study of viral
227 pathogenesis of VOC has recently been conducted in the hamster model²³. The study measured
228 the viral burden, histopathology, and select cytokine gene expression induced by VOC
229 compared to the prototypic Wuhan-Hu-1 isolate and an isolate harboring the secondary D614G
230 mutation in S. The study showed no significant differences in viral burden and histopathologic
231 findings in the hamster lungs at 4 DPC, but enhanced expression of cytokine genes was
232 described in hamsters infected with the B.1.1.7 variant.²³ However, longitudinal analysis of the
233 host response to VOC and the degree of cross-protection is lacking. Therefore, in this study, we
234 sought to evaluate the longitudinal impact of these VOC on the host immune and
235 transcriptional responses.

236 Syrian golden hamsters were chosen for this study as they are highly susceptible to infection
237 and were found to have high viral replication in the lungs. Hamsters were infected IN with the
238 ancestral, B.1.1.7 or B.1.351 variants. Challenged hamsters displayed moderate weight loss
239 lethargy, rapid breathing, and ruffled fur, but were able to clinically recover by 14 DPC as
240 previously described^{14,24,25}. As recently reported, no discernable differences in gross pathology
241 or lung viral burden were noted among all three groups²⁶. However, B.1.1.7 sgRNA persisted
242 longer in the hamster lungs. Analysis of the viral genomes recovered post-infection showed no
243 changes in the ancestral- and B.1.1.7-infected hamsters; however, we detected three mutations
244 in all B.1.351-infected animals. The two nonsynonymous mutations occurred in nsp3 and

245 ORF3a, both of which have been implicated in evasion of type I IFN^{27,28}. A second mutation in
246 nsp3 was also identified in a single B.1.351-infected animal. The implication of these mutations
247 remains to be elucidated.

248 Analysis of the humoral response revealed that the overall IgG response of the infected
249 hamsters did not result in robust differences amongst the variants; however, the neutralization
250 cross-protection depended on the variant the hamster was initially exposed to. Specifically,
251 infection with B.1.1.7 results in the widest breath of neutralization activity despite comparable
252 binding antibody titers. This phenomenon was most noticeable at 14 DPC, and was still evident
253 28 DPC when the humoral response is more mature. Moreover, the overall neutralization
254 activity, regardless of initial challenge virus, against B.1.351 is much lower than the other two
255 variants, suggesting that B.1.351 may have indeed an enhanced ability to evade humoral
256 immune responses. The overall IgG response of the infected hamsters did not result in robust
257 differences amongst the variants; however, the neutralization cross-protection depended on
258 the variant the hamster was initially exposed to. Our data demonstrates that early in the
259 humoral response (14 DPC) antibodies induced by B.1.1.7 infection show an increased
260 crossreactivity compared to the other variants tested. By 28 DPC, when the humoral response
261 is more mature, this differences is less prominent, but the trend remains the same. This
262 observation suggests that the timing of the antibody response could affect the crossreactivity
263 potential. Notably, the neutralization capacity of crossreactive antibodies and homologous
264 antibodies against B.1.351 is much lower than that of the other two variants tested. This
265 observation is reflective of previous studies that attribute increased antibody evasion to this
266 VOC^{3,4,8-10,12,21,22}, demonstrating that the hamster model reflects the differences in humoral
267 responses and effectivity of prior immunity seen in clinical cases²⁹.

268 A significant challenge when using the hamster model is the lack of reagents to analyze cellular
269 immune responses³⁰⁻³⁶. Therefore, we employed transcriptomic analysis to elucidate
270 differences in the host responses to VOC compared to the ancestral variant in the lungs of
271 hamster 4 DPC, as has been done for other studies³⁷⁻³⁹. Our transcriptional analysis of lung
272 tissues at peak infection identified distinct, but also overlapping transcriptional signatures for
273 each variant. All infections exhibited gene expression patterns associated with innate antiviral

274 responses, notably type I IFN signaling, mobilization of lymphocytes, and apoptosis⁴⁰⁻⁴³. The
275 type I IFN response is critical for rapid control of viral infection⁴⁴. However, dysregulated innate
276 immune and type I IFN responses can result in tissue damage and oxidative stress as noted in
277 other viral infections, including influenza virus and Ebola virus in addition to severe COVID-
278 19^{40,41,45,46}. Our data differs from those reported in studies where a suppressed IFN response in
279 the peripheral blood, the bronchoalveolar lavage, and lungs obtained at autopsy from
280 individuals with severe COVID-19⁴⁷⁻⁵². A potential explanation for this difference is the fact that
281 we profiled the lungs during the peak of viral replication and virus-induced pathology (4 DPC)
282 while clinical cases rarely present viral antigen at the time of death, rather immune
283 dysregulation and coagulation abnormalities are the cause of death⁵³⁻⁵⁵. Additionally, the Syrian
284 golden hamster model does not mimic severe COVID-19 interstitial pneumonia in that clinical
285 symptomology is less severe and none of the animals in this model succumb to disease.
286 Interestingly, transcriptional inflammatory indicators were particularly heightened following
287 infection with B.1.1.7 and least severe following infection with B.1.351. Expression of several
288 inflammatory and complement genes were only upregulated following infection with B.1.1.7
289 and ancestral variants, while *NFκB1* was upregulated only following infection with B.1.1.7^{56,57}.
290 *In vitro* and *in vivo* NFκB-driven inflammatory responses have been previously associated with
291 severe COVID-19^{48,50,58,59}. Additionally, NK cell activation was evident by higher expression of
292 cytolytic molecules (e.g., *PRF1*). This inflammatory damage facilitates immune cell influx,
293 including inflammatory cells like neutrophils, which we predicted to increase in all infections⁴⁸.
294 Moreover, significant increases in IL-2-stimulated NK cells was also predicted following
295 infection with the ancestral and B.1.1.7 variants. Expression of canonical T cell regulatory and
296 exhaustion markers like *CTLA4*, *CD274* (PD-L1), and *FOXP3* suggests compensatory mechanisms
297 to reduce tissue damage.
298 Transcriptional changes were also predicted to result in significant B cell loss in the lungs
299 following infection with all three viruses. Previous studies indicate that B cell lymphopenia does
300 not preclude robust antibody responses⁶⁰⁻⁶². This re-distribution could indicate B cell migration
301 to lymphoid tissue for priming. Indeed, significant neutralizing and binding antibody titers were
302 detected following all three infection, albeit lower following infection with B.1.135.

303 Furthermore, we detected a large number of DEGs related to tissue morphogenesis and
304 angiogenesis in all infections^{63,64}. Microvascular injury can further exacerbate inflammation-
305 driven lung fibrosis⁶⁵. Additionally, genes that play a role in tissue repair were downregulated
306 following infection with the B.1.351 and ancestral variants.

307 In this study we describe the pathogenesis of the SARS-CoV-2 variants and the development of
308 crossreactive neutralizing antibodies. To our knowledge this is the first study performing a
309 comparative and longitudinal analysis of the antibody response after SARS-CoV-2 VOC infection.
310 Our data show that infection with the B.1.1.7 VOC results in a broader antibody response
311 compared to infection with B.1.35 VOC. This broader response could be in part mediated by the
312 more robust transcriptional response elicited by this variant that includes a larger induction of
313 antiviral and inflammatory pathways. Future experiments should assess transcriptional changes
314 beyond 4 DPC to determine the kinetics of the host response at this critical site. Moreover,
315 additional studies should investigate the mechanisms by which the mutations detected in the
316 B.1.35 VOC lead to reduced neutralization potential.

317

318

319 **Methods**

320 *Ethics statement*

321 All infectious work with SARS-CoV-2 was performed in the containment laboratories at the
322 Rocky Mountain Laboratories (RML), Division of Intramural Research, National Institute of
323 Allergy and Infectious Diseases, National Institutes of Health. RML is an institution accredited by
324 the Association for Assessment and Accreditation of Laboratory Animal Care International
325 (AAALAC). All procedures followed standard operating procedures (SOPs) approved by the RML
326 Institutional Biosafety Committee (IBC)⁶⁶. Animal work was performed in strict accordance with
327 the recommendations described in the Guide for the Care and Use of Laboratory Animals of the
328 National Institute of Health, the Office of Animal Welfare and the Animal Welfare Act, United
329 States Department of Agriculture. The studies were approved by the RML Animal Care and Use
330 Committee (ACUC). Procedures were conducted in animals anesthetized by trained personnel

331 under the supervision of veterinary staff. All efforts were made to ameliorate animal welfare
332 and minimize animal suffering; food and water were available *ad libitum*.

333

334 *Cells and Viruses*

335 VeroE6 cells were grown at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM)
336 (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS) (Wisent Inc., St. Bruno,
337 Canada), 2 mM L-glutamine (Thermo Fisher Scientific, Waltham, MA), 50 U/mL penicillin
338 (Thermo Fisher Scientific), and 50 µg/mL streptomycin (Thermo Fisher Scientific). SARS-CoV-2
339 ancestral isolate nCoV-WA1-2020 (MN985325.1)⁶⁷, SARS-CoV-2 isolate B.1.351 (hCoV-19/South
340 African/KRISP-K005325/2020), or SARS-CoV-2 isolate B.1.1.7
341 (hCoV_19/England/204820464/2020) were used for the neutralizing antibody assays. The
342 following reagent was obtained through BEI Resources, NIAID, NIH: Severe Acute Respiratory
343 Syndrome-Related Coronavirus 2, Isolate hCoV-19/England/204820464/20200, NR-54000,
344 contributed by Bassam Hallis. SARS-CoV-2 B. 1.351 was obtained with contributions from Dr.
345 Tulio de Oliveira and Dr. Alex Sigal (Nelson R Mandela School of Medicine, UKZN). All viruses
346 were grown and titered on Vero E6 cells, and sequence confirmed.

347

348 *Animal study*

349 Fifty female Syrian golden hamsters (5-8 weeks of age) were used in this study¹⁴. Five animals
350 were used as uninfected controls; three study cohorts for challenge with the ancestral virus and
351 variants B1.1.7 and B.1.351 consisted of 15 hamsters each. On day 0, hamsters were infected
352 with SARS-CoV-2 as previously described¹⁴. On 4, 14 and 28 DPC, 5 hamsters per group were
353 euthanized for sample collection.

354

355 *RNA extraction and RT-qPCR*

356 RNA from blood and oral swab samples was extracted using the QIAamp Viral RNA Mini Kit
357 (QIAGEN) according to manufacturer specifications. Lung tissue, a maximum of 30 mg each, was
358 processed and RNA was extracted using the RNeasy Mini Kit (QIAGEN) according to
359 manufacturer specifications. One step RT-qPCR for genomic viral RNA was performed using

360 specific primer-probe sets and the QuantiFast Probe RT-PCR +ROX Vial Kit (QIAGEN), in the
361 Rotor-Gene Q (QIAGEN) as described previously⁶⁸. Five μ L of each RNA extract were run
362 alongside dilutions of SARS-CoV-2 standards with a known concentration of RNA copies.

363

364 *Enzyme-linked immunosorbent assay*

365 Serum samples from SARS-CoV-2-infected animals were inactivated by gamma-irradiation and
366 used in BSL2 according to IBC-approved SOPs. NUNC Maxisorp Immuno plates were coated with
367 50 μ l of 1 μ g/mL of recombinant SARS-CoV-2 S (S1+S2) antigen at 4°C overnight and then
368 washed three times with PBS containing 0.05% Tween 20 (PBST). The plates were blocked with
369 3% skim milk in PBS for 1 hour at room temperature, followed by three additional washes with
370 PBST. The plates were incubated with 50 μ l of serial dilutions of the samples in PBS containing
371 1% skim milk for 1 hour at room temperature. After three washes with PBST, the bound
372 antibodies were labeled using 50 μ l of 1:2,500 peroxidase anti-hamster IgG (H+L) (SeraCare Life
373 Sciences) diluted in 1% skim milk in PBST. After incubation for 1 hour at room temperature and
374 three washes with PBST, 50 μ l of KPL ABTS peroxidase substrate solution mix (SeraCare Life
375 Sciences) was added to each well, and the mixture was incubated for 30 min at room
376 temperature. The optical density (OD) at 405 nm was measured using a GloMax[®] explorer
377 (Promega). The OD values were normalized to the baseline samples obtained with naïve
378 hamster serum and the cutoff value was set as the mean OD plus standard deviation of the
379 blank.

380

381 *Virus neutralization assay*

382 The day before this assay, VeroE6 cells were seeded in 96-well plates. Serum samples were
383 heat-inactivated for 30 min at 56°C, and 2-fold serial dilutions were prepared in DMEM with 2%
384 FBS. Next, 100 TCID₅₀ of SARS-CoV-2 were added and the mixture was incubated for 1 hour at
385 37°C and 5% CO₂. Finally, media was removed from cells and the mixture was added to VeroE6
386 cells and incubated at 37°C and 5% CO₂ for 6 days. Then the cytopathic effect (CPE) was
387 documented, and the virus neutralization titer was expressed as the reciprocal value of the
388 highest dilution of the serum which inhibited virus replication (no CPE).

389

390 *Histology and immunohistochemistry*

391 Necropsies and tissue sampling were performed according to IBC-approved SOPs. Tissues were
392 fixed in 10% neutral buffered formalin with two changes, for a minimum of 7 days. Tissues were
393 placed in cassettes and processed with a Sakura VIP-6 Tissue Tek, on a 12-hour automated
394 schedule, using a graded series of ethanol, xylene, and ParaPlast Extra. Embedded tissues are
395 sectioned at 5 μ m and dried overnight at 42°C prior to staining. Specific anti-CoV
396 immunoreactivity was detected using Sino Biological Inc. SARS-CoV/SARS-CoV-2 nucleocapsid
397 antibody (Sino Biological cat#40143-MM05) at a 1:1000 dilution. The secondary antibody was
398 the Vector Laboratories ImPress VR anti-mouse IgG polymer (cat# MP-7422). The tissues were
399 then processed for immunohistochemistry using the Discovery Ultra automated stainer
400 (Ventana Medical Systems) with a ChromoMap DAB kit (Roche Tissue Diagnostics cat#760–
401 159). All tissue slides were evaluated by a board-certified veterinary pathologist and a
402 pathology scored was assigned based on the following observations; 0= no pathology, 1=
403 minimal, 2= mild, 3= moderate, 4= severe (Fig. S1C).

404

405 *cDNA library construction and sequencing*

406 Quality and quantity of RNA lung samples at 4 DPC were determined using an Agilent 2100
407 Bioanalyzer. cDNA libraries were constructed using the NEB Next Ultra II Direction RNA Library
408 Prep Kit (Thermo Fischer). RNA was treated with RNase H and DNase I following depletion of
409 ribosomal RNA (rRNA). Adapters were ligated to cDNA products and the subsequent ~300 base
410 pair (bp) amplicons were PCR-amplified and selected by size exclusion. cDNA libraries were
411 assessed for quality and quantity prior to 150 bp single-end sequencing using the Illumina
412 NovaSeq platform.

413

414 *RNA-Seq Bioinformatic analysis*

415 Preliminary data analysis was performed with RNA-Seq workflow module of systemPipeR,
416 developed by Backman *et. al*⁶⁹. RNA-Seq reads were demultiplexed, quality-filtered and
417 trimmed using Trim Galore (average Phred score cut-off of 30, minimum length of 50 bp).

418 FastQC was used to generate quality reports. Hisat2 was used to align reads to the reference
419 genome *Mesocricetus auratus* (*Mesocricetus_auratus.MesAur1.0.dna.toplevel.fa*) and the
420 *Mesocricetus_auratus.MesAur1.0.103.gtf* file was used for annotation. Raw expression values
421 (gene-level read counts) were generated using the `summarizeOverlaps` function and normalized
422 (read per kilobase of transcript per million mapped reads, rpkm) using the `edgeR` package.
423 Statistical analysis with `edgeR` was used to determine differentially expressed genes (DEGs)
424 meeting the following criteria: genes with median rpkm of ≥ 1 , a false discovery rate (FDR)
425 corrected p-value ≤ 0.05 and a \log_2 fold change ≥ 1 compared to control tissues.
426 Functional enrichment of DEGs was performed using Metascape to identify relevant GO
427 terms⁷⁰. Digital cell quantification was performed using ImmQuant with the IRIS database.
428 Heatmaps, bubbleplots, Venn diagrams and violin plots were generated using R packages
429 `ggplot2` and `VennDiagrams`. Graphs were generated using GraphPad Prism software (version 8).

430

431 *SARS-CoV-2 viral genome library construction and sequencing*

432 Enrichment of SARS-CoV-2 was performed using the Qiagen QIASeq SARS-CoV-2 Primer Panel
433 (V.2). Libraries were constructed from resulting SARS-CoV-2 amplicons using the Qiagen QIASeq
434 FX DNA Library preparation kit. Briefly, adapters were ligated to cDNA products and the ~300
435 bp amplicons were minimally PCR-amplified. cDNA libraries were assessed for quality and
436 quantity prior to 150 bp paired-end sequencing using the Illumina HiSeq platform (≥ 1 M reads
437 per sample).

438

439 *SARS-CoV-2 viral genome assembly and bioinformatic analysis*

440 Reads were demultiplexed and quality-filtered using Trim Galore (average Phred score cut-off
441 of 30, minimum length 100 bp). FastQC was used to generate quality reports. `MaskPrimers.py`
442 from the `pRESTO` R package was used to remove primers prior to alignment to the SARS-CoV-2
443 genome using `BWA-mem` software version 0.7.17. The following reference genomes were used
444 for Ancestral, B.1.1.7 and B.1.351 variants: `WA_MN985325.1`, `EPI_ISL_683466`, and
445 `EPI_ISL_6786156`. All genomes had greater than 95% coverage and 10X depth. Single nucleotide
446 polymorphisms and amino acid changes were identified using `CorGAT`.

447

448 *Statistical analyses*

449 All statistical analysis was performed in Prism 8 (GraphPad). Two-tailed Mann-Whitney test was
450 conducted to compare differences between groups for data in Figs. 2, 3 and S1. Statistical
451 significance was determined using one-way ANOVA with multiple comparisons for the
452 bioinformatic analysis with comparisons made among variant- and control-challenged animals
453 Statistically significant differences are indicated as follows: $p < 0.0001$ (****), $p < 0.001$ (***),
454 $p < 0.01$ (**) and $p < 0.05$ (*).

455

456

457 **Data Availability**

458 All transcriptomic sequencing data are accessible at BioProject PRJNAXXXX upon publication.

459

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462 Technology Branch (all NIAID) for their efforts to obtain and characterize the SARS-CoV-2
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464 studies, and Anita Mora (NIAID) for assistance generating the pathology figures.

465

466

467 **Author contributions**

468 A.M. conceived the idea and secured funding. K.L.O., I.M, and A.M. designed the studies. K.L.O.,
469 C.S.C., K.S., T.G., P.F., and A.M. conducted the animal studies, processed the samples and
470 acquired the data. A.N.P. performed transcriptomics analysis and viral genome sequencing.
471 K.L.O., A.N.P., C.S.C., I.M., and A.M. analyzed and interpreted the data. K.L.O., A.N.P., I.M., and
472 A.M. prepared the manuscript with input from all authors. All authors approved the
473 manuscript.

474

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479

480 **Competing interest**

481 The authors declare no conflicts of interest.

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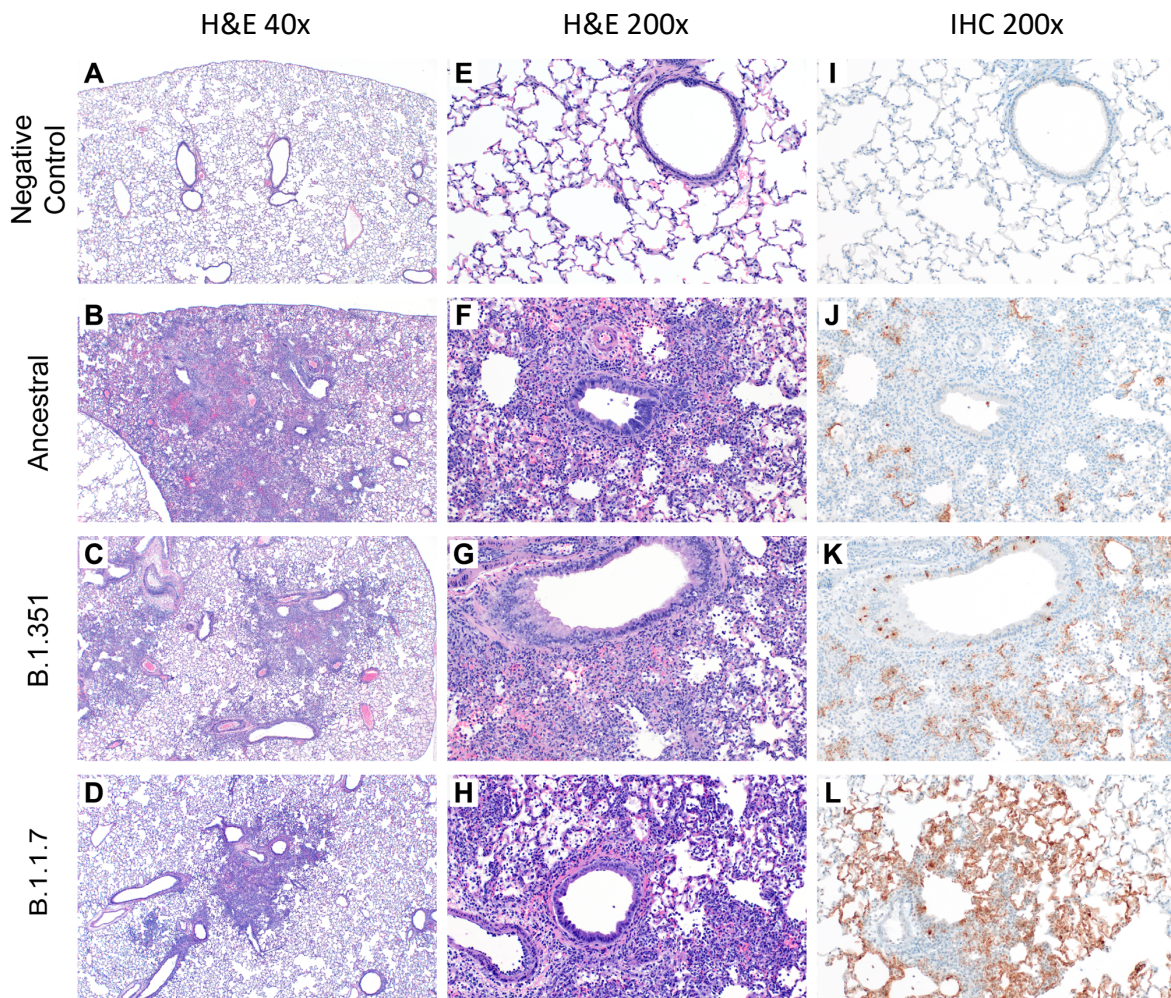
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500 **Fig. 1. Histopathology and Immunohistochemistry of hamster lungs. (A-H)** Representative H&E

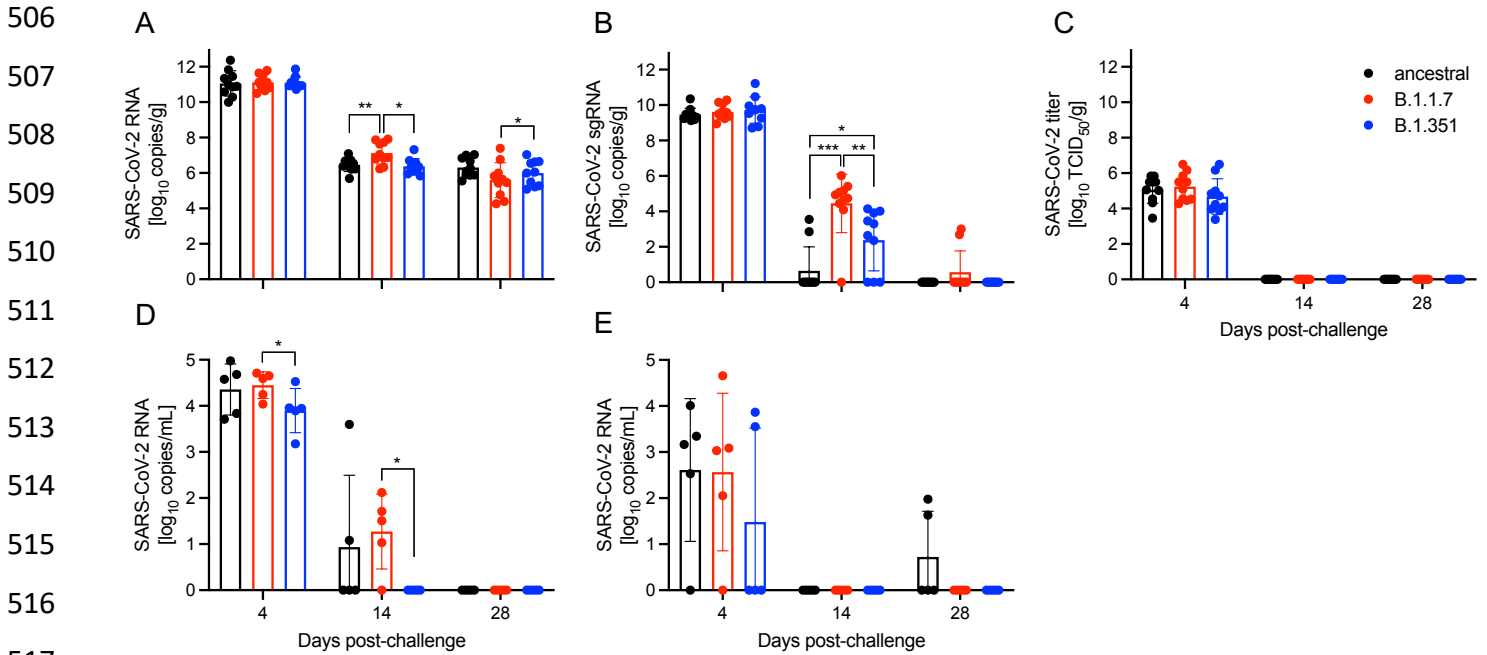
501 images of lungs of hamsters infected with 10^5 TCID₅₀ of ancestral, B.1.1.7, and B.1.351 variants

502 at 4 days post-challenge (DPC). Foci of interstitial pneumonia and bronchiolitis were observed

503 throughout all evaluated lung lobes of infected hamsters. **(I-L)** Immunohistochemistry (IHC)

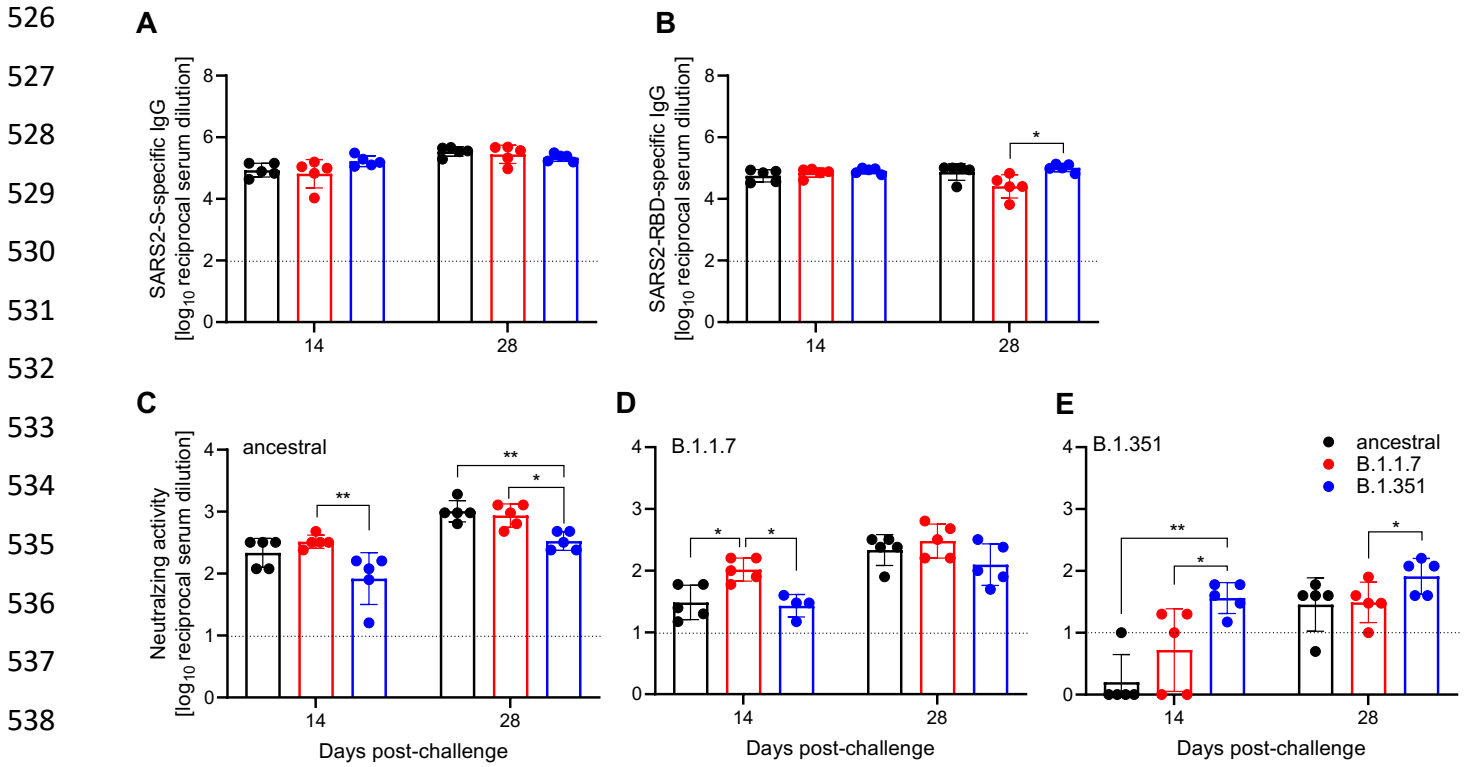
504 detected SARS-CoV-2 nucleocapsid staining in the lungs of all infected hamsters.

505



519 **Fig. 2. SARS-CoV-2 burden in lungs, oral swabs and blood. (A)** Total SARS-CoV-2-specific RNA
 520 and **(B)** total SARS-CoV-2-specific sub-genomic RNA (sgRNA) in the lungs of challenged animals
 521 at 4, 14, and 28 days post-challenge (DPC). **(C)** Infectious SARS-CoV-2 titer in the lungs of
 522 infected hamsters. Total SARS-CoV-2-specific RNA in the **(D)** oral swabs and **(E)** blood of
 523 infected hamsters at the time of euthanasia. Geometric mean and standard deviation (SD) are
 524 depicted; statistical significance is indicated ***p < 0.001, **p < 0.01 and *p < 0.05.

525



541 **Fig. 3. Humoral immune response in challenged hamsters.** Serum samples were collected at 14
542 and 28 DPC and by ELISA. **(A)** Total SARS-CoV-2 S-specific IgG and **(B)** RBD-specific IgG are
543 shown. Cross-variant and homologous neutralization was assessed against **(C)** ancestral, **(D)**
544 B.1.1.7, and **(E)** B.1.351 viruses. Line indicates limit of detection. Geometric mean and SD are
545 depicted; statistical significance is indicated $**p < 0.01$ and $*p < 0.05$.

546

576

577 **Fig. 4. SARS-CoV-2 variants induce distinct transcriptional changes.** Volcano plot of global gene

578 expression changes at 4 DPC with SARS-CoV-2 **(A)** ancestral, **(B)** B.1.1.7 or **(C)** B.1.351 variants.

579 Downregulated and upregulated differentially expressed genes (DEGs; average RPKM ≥ 1) are

580 colored blue and red, respectively. Exemplary genes are labeled. **(D)** Venn diagram of DEGs

581 determined in panels A-C. **(E)** Functional enrichment of DEGs determined following each

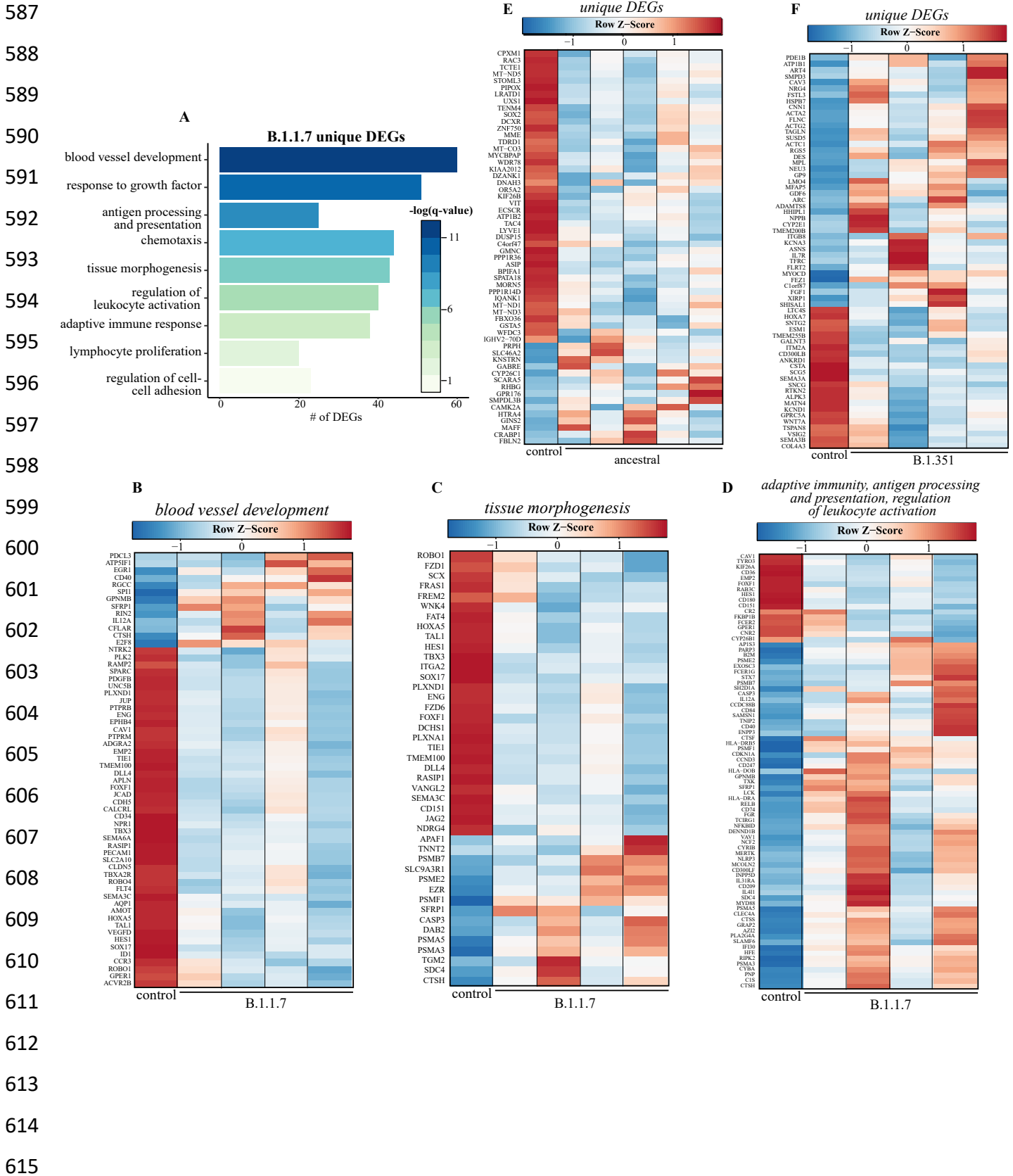
582 infection in panels A-C. Color intensity represents statistical significance as the negative log of

583 the FDR-adjusted p-value $[-\log(q\text{-value})]$, with range of colors based on the GO terms with the

584 lowest and highest statistical value for all GO terms present. Blank boxes indicate no statistical

585 significance. Numbers of DEGs enriching to each GO term are noted in each box.

586



616 **Fig. 5. Transcriptional response unique to B.1.1.7 variant suggests distinct host responses.**
617 **(A)** Functional enrichment of DEGs unique to B.1.1.7 variant infection at 4 DPC (n=684).
618 Horizontal bars represent the number of genes enriching to each GO term with color intensity
619 representing the negative log of the FDR-adjusted p-value [-log(q-value)]. Range of colors based
620 on the GO terms with the lowest and highest -log(q-value) values. Heatmaps representing
621 B.1.1.7 variant unique DEGs enriching to GO terms from panel A: **(B)** “blood vessel
622 development”, **(C)** “tissue morphogenesis” and **(D)** adaptive terms “adaptive immunity”,
623 “antigen processing and presentation”, and “regulation of leukocyte activation.” Heatmaps of
624 DEGs unique to **(E)** Ancestral (n=58) and **(F)** B.1.351 variants (n=64) at 4 DPC. Columns of all
625 heatmaps represent the average rpkm of controls and rpkm of a single variant-infected animal.
626 Range of colors per each heatmap is based on scaled and centered rpkm values of the
627 represented DEGs. Red represents upregulation; blue, downregulation.

628

629

630 **Table 1. Genome comparison of SARS-CoV-2 variants**

Variant of concern	Amino acid changes (# of animals affected, 4 DPC)
ancestral	none detected
B.1.1.7	none detected
B.1.351	5' UTR, T201C (5/5) nsp3, L3892F (1/5) nsp3, G5942G (5/5) ORF3a, G172C (5/5)

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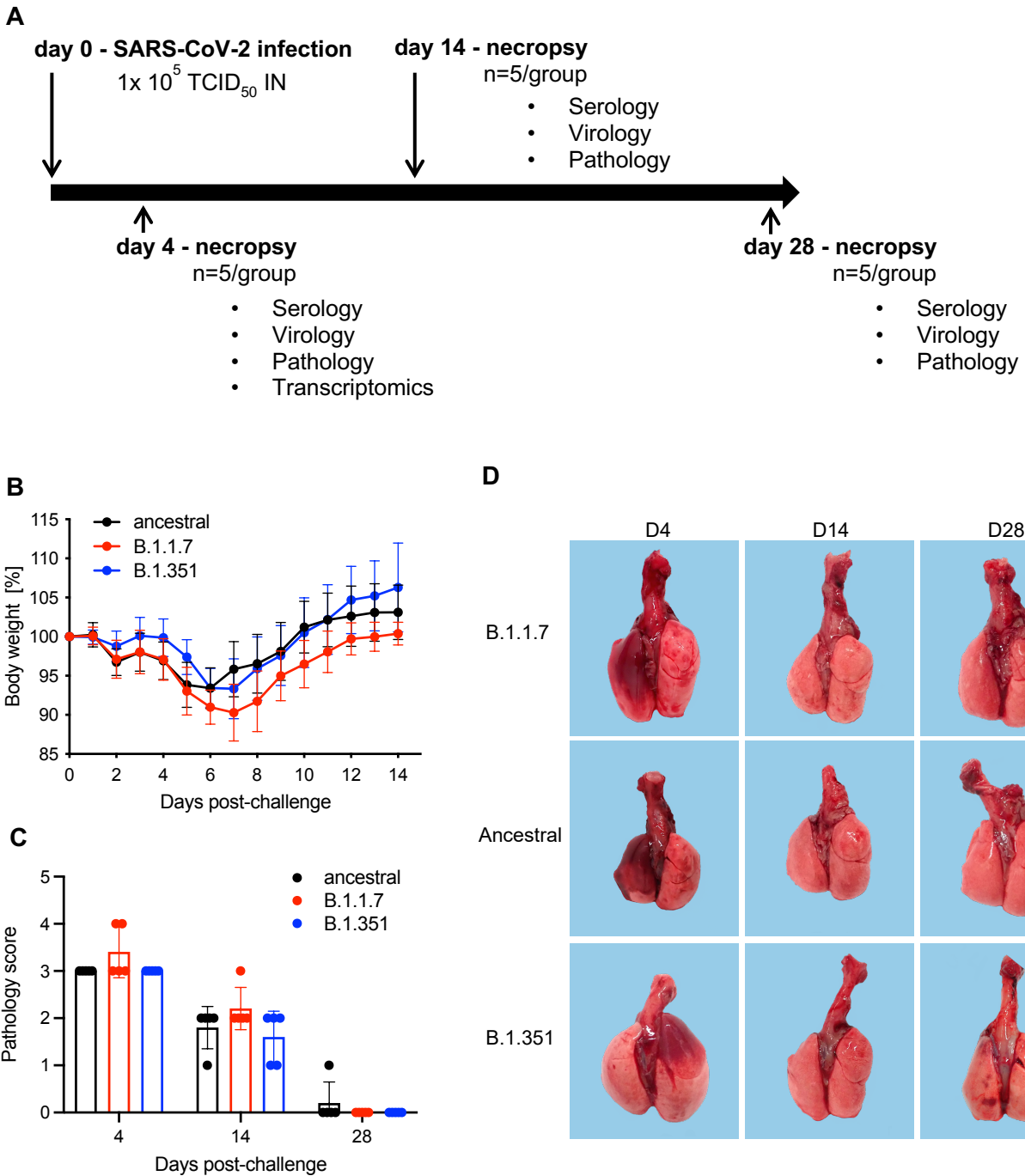


Figure S1. Study outline, body weight changes and pathology of infected hamsters. (A) Schematic study outline. (B) Body weight changes in hamsters (n=10/group). (C) Evidence of interstitial pneumonia was recorded in histopathology samples. (D) Representative pictures of hamster lungs with lesions during disease progression. Gross lung images at day (D) 4 , 14 and 28 post-challenge.

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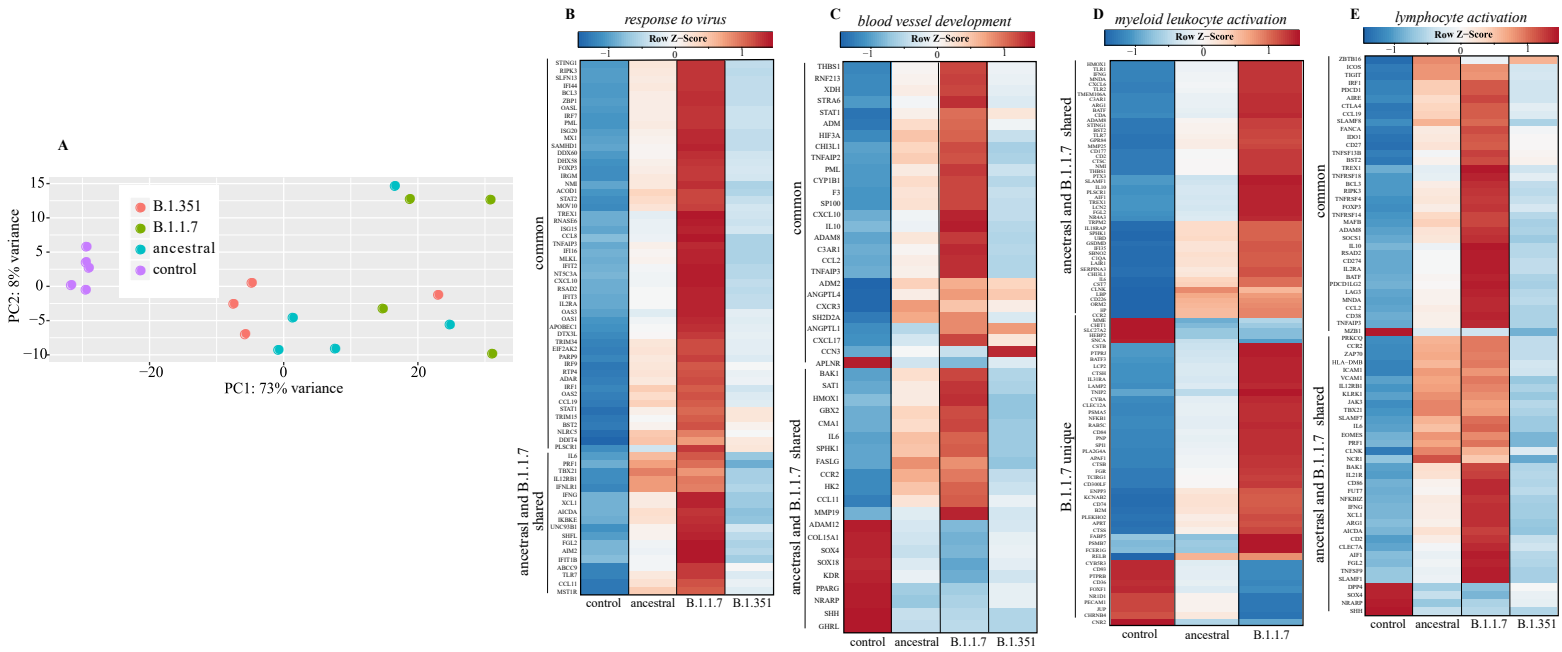


Figure S2. SARS-CoV-2 variants induce distinct transcriptional changes. (A) Principal component analysis of control (n=5) animals and infected animals 4 DPC with ancestral (n=5), B.1.1.7 (n=4) or B.1.351 (n=4) variants. Heatmaps representing DEGs enriching to GO terms from Fig. 5E including (B) “response to virus”, (C) “blood vessel development”, (D) “myeloid leukocyte activation” and (E) lymphocyte activation.” DEGs are either shared among all variant infections or between ancestral and B.1.1.7 variant infections. Columns of all heatmaps represent the average rpkms. Range of colors per each heatmap is based on scaled and centered rpkms values of the represented DEGs. Red represents upregulation; blue, downregulation.

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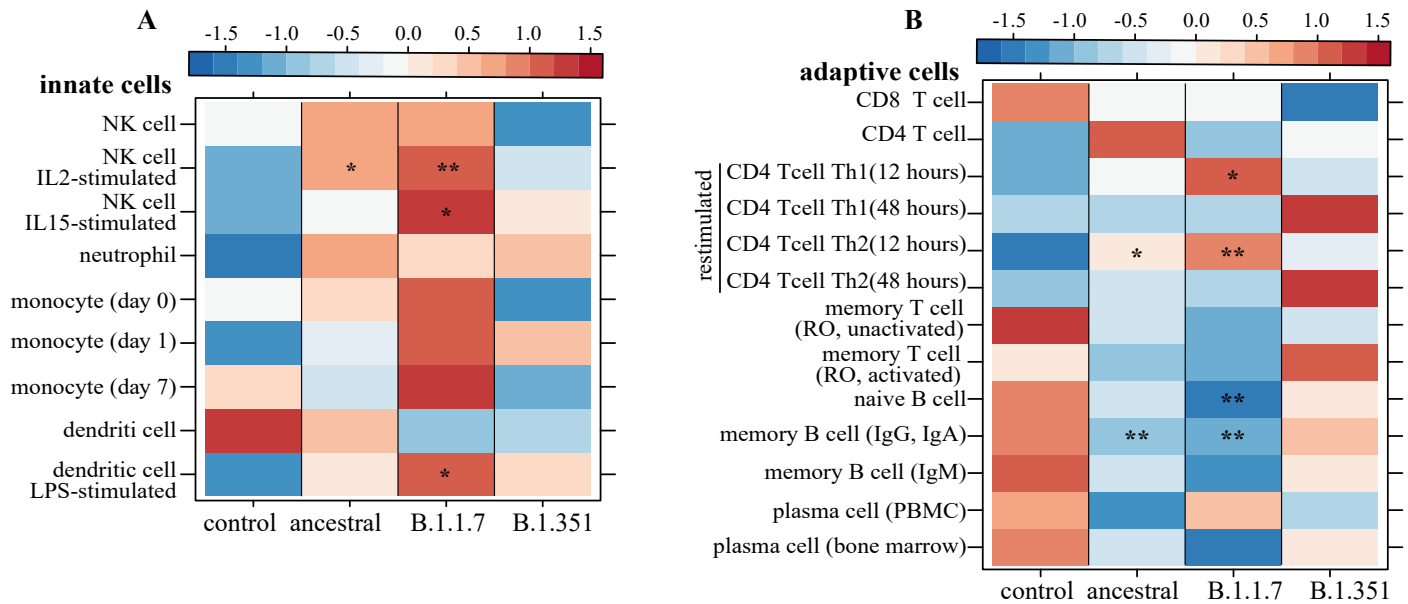


Figure S3. Digital cell quantification in hamster lungs. Heatmaps representing relative changes in (A) innate and (B) adaptive immune cell frequencies using ImmQuant with IRIS database. Each column represents the average relative expression level of the given immune cell. Range of colors per each heatmap is based on scaled and centered relative expression values. Red represents upregulation; blue represents downregulation. Statistical significance is indicated ** $p < 0.01$ and * $p < 0.05$.

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