

1 **Characteristic and quantifiable COVID-19-like abnormalities in CT- and PET/CT-**
2 **imaged lungs of SARS-CoV-2-infected crab-eating macaques (*Macaca fascicularis*)**

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44 **Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing an**
45 **exponentially increasing number of coronavirus disease 19 (COVID-19) cases**
46 **globally. Prioritization of medical countermeasures for evaluation in randomized**
47 **clinical trials is critically hindered by the lack of COVID-19 animal models that**
48 **enable accurate, quantifiable, and reproducible measurement of COVID-19**
49 **pulmonary disease free from observer bias. We first used serial computed**
50 **tomography (CT) to demonstrate that bilateral intrabronchial instillation of SARS-**
51 **CoV-2 into crab-eating macaques (*Macaca fascicularis*) results in mild-to-moderate**
52 **lung abnormalities qualitatively characteristic of subclinical or mild-to-moderate**
53 **COVID-19 (e.g., ground-glass opacities with or without reticulation, paving, or**
54 **alveolar consolidation, peri-bronchial thickening, linear opacities) at typical**
55 **locations (peripheral>central, posterior and dependent, bilateral, multi-lobar). We**
56 **then used positron emission tomography (PET) analysis to demonstrate increased**
57 **FDG uptake in the CT-defined lung abnormalities and regional lymph nodes.**
58 **PET/CT imaging findings appeared in all macaques as early as 2 days post-**
59 **exposure, variably progressed, and subsequently resolved by 6–12 days post-**
60 **exposure. Finally, we applied operator-independent, semi-automatic quantification**
61 **of the volume and radiodensity of CT abnormalities as a possible primary endpoint**
62 **for immediate and objective efficacy testing of candidate medical countermeasures.**

63

64 The causative agent of human coronavirus disease 2019 (COVID-19), severe acute
65 respiratory syndrome coronavirus 2 (SARS-CoV-2), likely emerged in Wūhàn, Húběi
66 Province, China in November 2019 ([1-3](#)). The virus rapidly spread through the human

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67 population, causing more than 4.2 million infections and almost 300,000 deaths globally
68 by May 14, 2020 (4). Infections result in a wide spectrum of disease ranging from
69 asymptomatic to mild upper respiratory illness to severe pneumonia that can progress to
70 acute respiratory distress syndrome (ARDS) and death despite aggressive supportive care
71 (5). After a short but variable incubation period, most patients with COVID-19 develop
72 self-limiting fever, cough, nonspecific fatigue, and myalgia (6-11). Some patients
73 develop non-productive cough and dyspnea related to lower respiratory tract
74 involvement; particularly in patients of older age or with co-morbidities, this involvement
75 can lead to severe, progressive disease and unfavorable outcomes (5). Well-documented
76 characteristic lung CT findings in humans include ground-glass opacities (GGOs) with or
77 without reticulation, reticulonodular opacities, inter- or intralobular septal paving, or
78 consolidation in a bilateral, lobar to sub-segmental, and peripheral distribution (6-8, 12).
79 Notably, GGOs have been described in patients who are shedding SARS-CoV-2 but do
80 not present with clinical signs (13, 14). Bilateral diffuse alveolar damage, type II
81 pneumocyte hyperplasia, interstitial fibrosis and inflammation, hemorrhage, and edema
82 with syncytia appear to be typical lung histopathological findings seen in a limited human
83 data set that also suggests a high rate of venous thromboembolism (15-19).

84 Currently available rodent/carnivore/tree shrew (20-24) and nonhuman primate
85 (NHP) (11, 25-29) models of SARS-CoV-2 infection do not accurately reflect severe
86 human COVID-19. NHPs, considered an evolutionary proximate for human disease
87 modeling, develop no or only mild clinical disease signs (11, 26-29). In SARS-CoV-2-
88 infected rhesus monkeys (*Macaca mulatta*), quantifiable virus shedding, virus-specific
89 immune responses, and limited histopathologic lesions have been observed (11, 25, 27-

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90 [29](#)). However, in both human disease and animal models, the temporal and mechanistic
91 relationship between viral replication, subsequent immunopathology ([30](#), [31](#)), and clinical
92 disease remains uncertain. Furthermore, in the available NHP models, all of which are
93 sublethal, markers of clinical disease (cage-side scoring, chest X-ray) have been of
94 limited sensitivity. More concerningly, both metrics are subject to observer bias ([32-35](#)).

95 Reliable animal models needed for rapid development and evaluation of candidate
96 medical countermeasures (MCMs) require an unbiased reproducible and quantifiable
97 metric of disease that mirrors key aspects of COVID-19. Based on the rather limited X-
98 ray findings in the lungs of reported NHP models of SARS-CoV-2 infection with either
99 mild or no clinical signs ([11](#), [25](#), [27-29](#)), we turned to high-resolution chest CT and
100 PET/CT to characterize lung abnormalities in infected NHPs toward longitudinal
101 quantitative comparison.

102 We used direct bilateral primary intrabronchial instillation in a 1-day-apart
103 staggered design to expose two groups of three crab-eating macaques (*Macaca*
104 *fascicularis*) to medium (mock group macaques M1–3) or medium including 3.65×10^6
105 pfu/macaque of SARS-CoV-2 (virus group macaques V1–3) (**Supplementary Table 1**).
106 All macaques were observed daily for 11 days prior to exposure (day [D] 0) and for 30
107 days post-exposure. Physical examination scores and blood, conjunctival,
108 nasopharyngeal, oropharyngeal, rectal, fecal, and urine specimens were collected at
109 identical timepoints. Virus-exposed macaques were indistinguishable from mock group
110 macaques during the pre-exposure time period. Two pre-exposure chest CT and whole-
111 body 2-deoxy-2- ^{18}F -fluoro-D-glucose (FDG) PET/CTs and eight post-exposure CTs and
112 three post-exposure PETs were performed at identical timepoints (**Figure 1**). In line with

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113 previously published results ([11](#), [25-29](#)), none of the macaques developed any major
114 clinical abnormalities (including by cage-side assessment and clinical scoring or physical
115 examination) throughout the study and clinical laboratory results were not significantly
116 different between the mock-exposed and virus-exposed groups (**Supplementary Tables**
117 **2–3**). SARS-CoV-2 RNA could not be detected by RT-qPCR in any sample from mock-
118 exposed macaques but was variably present during the early days post-exposure in
119 conjunctival, fecal, nasopharyngeal, oral, and rectal swabs, but never in plasma or urine
120 (**Figure 2a**). Anti-SARS-CoV-2 IgG antibodies were not detectable by ELISA in mock-
121 exposed macaques but were detectable at D10 post-exposure and continued to rise in all
122 virus-exposed macaques to at least D19 (**Figure 2b**). Consistent with ELISA results,
123 fluorescent neutralization titers generated from sera were undetected until D10 and were
124 detected only in virus-exposed macaques (**Figure 2c**). Longitudinal measurement of
125 selected peripheral cytokines revealed between- and within-group differences with
126 marked abnormalities noted in macaque V3, which also had the highest IgG antibody
127 titers (**Supplementary Figure 1**).

128 With the exception of minor and transient abnormalities on baseline imaging, CT
129 scans of all mock-exposed macaques appeared generally normal over the entire study
130 period (**Supplementary Figure 2**). However, all virus group macaques developed lung
131 abnormalities clearly visible by chest CT as early as D2. Qualitatively, the distribution
132 morphology, and duration of abnormalities described a spectrum similar to mild-
133 moderately ill humans with COVID-19. Characteristic CT findings in all virus group
134 macaques included bilateral peripheral GGOs variably in association with intra- or
135 interlobular septal prominence (so-called “crazy paving”), reticular or reticulonodular

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136 opacities, peri-bronchial thickening, subpleural nodules, and, in one macaque, dense
137 alveolar consolidation with air bronchograms (**Figures 3–4a, Videos 1–3,**
138 **Supplementary Figure 3**). Longitudinal serial CT scans showed heterogeneity in the
139 duration and evolution of these abnormalities over the next week from rapid
140 improvement within a few days (macaque V1) to persistence and progression (macaques
141 V2, V3) (**Figure 4a, Supplementary Figure 3**). Nonetheless, by D19, chest CT
142 universally showed complete or nearly complete resolution of lung abnormalities in all
143 virus group macaques. Individual and per-group average radiologist-derived CT scores
144 (adapted from a scoring system generated from human COVID-19 CT images)
145 demonstrate the extent and duration of these qualitative findings (**Figure 5**).

146 Increased FDG uptake detected by PET (**Figure 6, Supplementary Figures 4–5**)
147 corresponded well to the structural changes in the lungs observed by CT, and regional
148 lymph node uptake was seen in all virus group macaques at D2. In macaque V1, FDG
149 uptake decreased in the lungs at D6 but increased in mediastinal lymph nodes, and new
150 FDG uptake was identified in the spleen. The two macaques (V2, V3) with persistent or
151 progressive structural abnormalities on chest CT had variable changes in FDG uptake
152 associated with the structural abnormalities in the lungs (some markedly increased, some
153 improved) with an accompanying marked increase in FDG uptake in regional lymph
154 nodes and spleens on D6. PET scan on D12 revealed normalization of previous areas of
155 increased FDG uptake in the lung parenchyma in all three virus-exposed macaques, and
156 persistent increased FDG uptake in regional lymph nodes and spleen. Mock-exposed
157 macaques did not have similar increased FDG uptake with the exception of transient
158 increased uptake in regional lymph nodes after mock-exposure in a single macaque (M1).

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159 Quantification of the SUV_{max} in selected regions of interest (ROI) in the lung, specific
160 regional lymph nodes, and spleen mapped well to the qualitative findings in both mock-
161 exposed and virus-exposed macaques (**Figure 7**).

162 CT images can be used for quantification of lung abnormalities using measures of
163 volume or radiodensity, i.e., total lung volume (LV); average radiodensity in the total
164 lung volume (LD); hyperdense volume (HV), a volume of lung in which radiodensity
165 (Hounsfield units, HU) is above a pre-defined threshold; and average radiodensity in the
166 hyperdense volume (hyperdensity, HD). Normalized changes from a pre-exposure
167 baseline can be longitudinally measured as the percent change in the volume of lung
168 hyperdensity (PCLH). Toward standardization across lung volumes, PCLH can be also be
169 expressed as a percent of total lung volume (PCLH/LV). Increases in PCLH or PCLH/LV
170 were not seen in the mock-exposed macaques over the entire study (**Figure 8a–d**,
171 **Supplementary Figure 2**). However, post-exposure increases in PCLH or PCLH/LV
172 were noted in all virus group macaques starting at D2, notably with heterogeneity in both
173 the peak and duration of PCLH and PCLH/LV that corresponded well to longitudinal
174 qualitative chest CT observations in individual virus group macaques (**Figure 4b**, **Figure**
175 **8a–d**, **Supplementary Figure 2**). Though both measures captured similar differences
176 between groups, the within-group variability was unsurprisingly less with PCLH/LV
177 versus PCLH. The virus group had significantly higher cumulative PCLH/LV over days
178 0–30 as summarized by the area under the curve (AUC_{0-30} ; $p=0.01$). The AUC_{0-8} for days
179 0–8 showed a similar trend ($p=0.06$), as did the AUC_{0-8} and AUC_{0-30} for the PCLH
180 ($p=0.06$ and $p=0.03$, respectively).

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181 A comparison of PCLH or PCLH/LV (**Figure 8a–d**) and absolute radiodensity
182 (change in HD, change in LD) (**Figure 8e–h**) highlights similarities and differences
183 windowed by these readouts that are particularly apparent as the CT abnormalities
184 evolved in macaque V3. In this macaque, dense consolidation in the left lung reached
185 peak radiodensity at D6, subsequently evolving toward a larger volume of less dense
186 mixed consolidation and GGO at D8 (**Figure 4a**). This progression of findings is
187 captured as an increase in PCLH (**Figure 8a**) and PCLH/LV (**Figure 8c**) from D6–8, but
188 a sharp decline in HD (**Figure 8e**) over the same period.

189 Quantifiable changes in CT lung abnormalities, e.g., AUC of the PCLH/LV curve
190 in an appropriately powered macaque study, could be used to objectively evaluate
191 efficacy of candidate MCMs, including vaccines and therapeutics. Although the
192 described crab-eating macaque model windows only mild to moderate radiographic
193 disease, it captures heterogeneity in both severity and duration of disease; the readout can
194 be similarly applied to any larger animal model of increased severity should they become
195 available in the future. These objective measurements add to semi-quantitative scoring,
196 which is potentially subject to observer bias in experimental settings ([36-39](#)); in our
197 study, semi-quantitative findings include radiologist-derived CT lung scores (**Figure 5**)
198 or mean SUV_{max} measured in operator-selected regions of interest (ROIs) on PET/CT
199 scans (**Figure 7**).

200 Of interest, the disease heterogeneity captured by our imaging readouts is
201 mirrored to some degree in thus-far limited measures of innate or adaptive immunity,
202 namely in ELISA and neutralizing antibody titers and in longitudinal measurements of
203 peripheral cytokines. Conclusions cannot be meaningfully drawn from the small numbers

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204 of measurements taken from only three macaques; nonetheless, between- and within-
205 group differences in cytokines that have been identified as biomarkers of disease, disease
206 severity, and disease outcome in humans ([30](#), [40-44](#)) are observed. Most notable in this
207 regard is a remarkable concentration increase of cytokines associated with cytokine
208 release syndrome (aka “cytokine storm”), such as C-X-C motif chemokine ligand 8
209 (CXCL8), interleukin (IL) 6, IL13, IL15, IL1 receptor antagonist (IL1RN), and tumor
210 necrosis factor (TNF), starting around D6 in the macaque (V3) with the most significant
211 CT and PET/CT abnormalities.

212 A key advantage of quantifiable CT chest imaging readout over serial euthanasia
213 studies, in addition to potentially reduced experimental animal numbers, is the ability not
214 only to evaluate between-group differences, but also to compare severity and duration of
215 disease at higher resolution in single animals and even in isolated parenchymal areas
216 sequentially. This approach can reduce the error inherent in cross-sectional sampling of
217 individual animals at single timepoints. Imaging does, however, introduce its own
218 experimental complexities and limitations. As we aimed to evaluate whether PCLH (or
219 other CT imaging readouts presented in **Figure 8**) could be a useful quantitative readout
220 for radiographic progression in the SARS-CoV-2 infected lung, we chose not to include
221 irradiated inactivated SARS-CoV-2 in the mock inoculum to avoid antigen-induced
222 inflammation and related radiographic changes. For similar reasons, and to avoid
223 artificial dissemination of SARS-CoV-2, we specifically did not perform bronchoalveolar
224 lavage (BAL) to obtain lung samples for downstream cellular, molecular, and virologic
225 analysis ([45](#), [46](#)) and did not perform lung biopsies. The frequency of anesthesia and
226 instrument availability pragmatically limit imaging to carefully chosen timepoints during

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227 a study. In particular, the extended time required to perform PET imaging resulted in
228 logistical limitations of the number of macaques that could be included in the study.
229 Finally, with complete resolution of radiographic abnormalities by the end of the study
230 period, we opted not to euthanize these macaques to be able to perform a re-exposure
231 study in the future. Thus, we cannot correlate radiographic with histopathologic findings.

232 Future studies should extend our initial findings in several directions. First,
233 follow-up confirmation of these pilot results in this model of mild-moderate COVID-19
234 is needed to further establish quantifiable lung CT as a reliable disease readout and to
235 forge imaging-pathologic correlates in macaques euthanized at peak radiographic
236 abnormality. Confirmation should enable proof-of-concept evaluation of whether a
237 candidate MCM will indeed significantly decrease peak or AUC of PCLH or PCLH/LV
238 compared to untreated infected control macaques. Data from additional macaques will be
239 used to confirm the sensitivity and relevance of the AUC_{0-8} and AUC_{0-30} for PCLH or
240 PCLH/LV as robust measures of lung changes from CT evaluation.

241 In parallel, disease severity could possibly be increased in the crab-eating
242 macaque model by optimizing delivery of SARS-CoV-2 to the most vulnerable lung (via
243 aerosol or more distal bronchoscopic delivery), with the ultimate goal of using the CT-
244 quantifiable volume or radiodensity readouts to model the sick hospitalized human.

245 Other groups are already evaluating NHPs of diverse species as possible COVID-
246 19 models. In these models, serial chest CT imaging after intrabronchial instillation of
247 SARS-CoV-2 could be used to establish a meaningful and quantifiable COVID-19-like
248 disease readout that will enable objective evaluation of medical countermeasures and also
249 a comparison of SARS-CoV-2-induced lung abnormalities in different NHP models.

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250

251 **Methods**

252 **Virus**

253 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; *Nidovirales*:
254 *Coronaviridae*: *Sarbecovirus*) isolate 2019-nCoV/USA-WA1-A12/2020 was obtained
255 from the US Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). A
256 master virus stock (designated IRF_0394) was grown under high (biosafety level 3)
257 containment conditions at the IRF-Frederick by inoculating grivet (*Chlorocebus*
258 *aethiops*) Vero cells obtained from the American Type Culture Collection (ATCC;
259 Manassas, VA, USA; #CCL-81) maintained in Dulbecco's Modified Eagle Medium with
260 L-glutamine (DMEM, Lonza, Walkersville, MD, USA) supplemented with 2% heat-
261 inactivated fetal bovine serum (FBS; SAFC Biosciences, Lenexa, KS, USA) at 37°C in a
262 humidified 5% CO₂ atmosphere, harvested after 72 h, and quantified by plaque assay in
263 Vero E6 cells (ATCC #CRL-1586) using a 2.5% Avicel overlay with a 0.2% crystal
264 violet stain at 48 h following a previously published protocol (47). The genomic sequence
265 of the IRF_394 master stock was determined experimentally by two independent
266 amplification approaches: nonspecific DNA amplification (sequence-independent single
267 primer amplification [SISPA]) as described previously (48) and the ARTIC protocol (49),
268 which was designed to amplify overlapping regions of the SARS-CoV-2 reference
269 genome (MN908947.3). Primer information and genomic alignment position are
270 available at [https://github.com/artic-network/artic-](https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019/V1)
271 [ncov2019/tree/master/primer_schemes/nCoV-2019/V1](https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019/V1). PCR products were purified with
272 the MinElute PCR Purification Kit (QIAGEN, Valencia, CA, USA). Libraries were

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273 prepared with the SMARTer PrepX DNA Library Kit (Takara Bio, Mountain View, CA,
274 USA), using the Apollo NGS library prep system (Takara Bio, Mountain View, CA,
275 USA). Libraries were evaluated for quality using the Agilent 2200 TapeStation System
276 (Agilent, Santa Clara, CA, USA). After quantification by qPCR with the KAPA SYBR
277 FAST qPCR Kit (Roche, Pleasanton, CA, USA), libraries were diluted to 2 nM, and
278 sequenced on a MiSeq (Illumina, San Diego, CA, USA). The genomic sequence of
279 IRF_394 was found to be identical to the type sequence of SARS-CoV-2 isolate 2019-
280 nCoV/USA-WA1-A12/2020 (GenBank MT020880.1), and IRF_394 was determined to
281 be devoid of bacterial or viral contaminants.

282

283 **Animals**

284 Six crab-eating (aka cynomolgus) macaques (*Macaca fascicularis* Raffles, 1821) of both
285 sexes, 4–4.5 years old and weighing 3.17–4.62 kg (**Supplementary Table 1**), were
286 obtained from Cambodia via Envigo Captive (Hayward, CA, USA) and housed at the US
287 National Institutes of Health Animal Center (NIHAC; Dickerson, MD, USA) for 3
288 months. All female macaques were on depot medroxyprogesterone acetate (administered
289 intramuscularly, 150 mg/ml) while at NIHAC for several months. The last dose
290 administered was administered approximately one month prior to study start. The
291 macaques were subsequently moved into the maximum (biosafety level 4 [BSL-4])
292 containment laboratory at the IRF-Frederick, a facility accredited by the Association for
293 Assessment and Accreditation of Laboratory Animal Care International (AAALAC).
294 Prior to facility entry, all macaques were serologically screened for herpes B virus,
295 simian immunodeficiency virus (SIV), simian retrovirus, and simian T-lymphotropic

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296 virus (STLV) infection; all macaques tested negative. Macaques also tested negative
297 multiple times for *Mycobacterium tuberculosis* infection. Once in containment, the
298 macaques passed physical exams and routine bloodwork and were confirmed appropriate
299 for study assignment by IRF-Frederick veterinarians. Experimental procedures for this
300 study (protocol “SARS-CoV-2-NHP-064E-1”) were approved by the National Institute of
301 Allergy and Infectious Diseases (NIAID), Division of Clinical Research (DCR), Animal
302 Care and Use Committee (ACUC), and were in compliance with the Animal Welfare Act
303 regulations, Public Health Service policy, and the *Guide for the Care and Use of*
304 *Laboratory Animals 8th Ed.* recommendations. The macaques were singly housed during
305 the 2-week acclimatization to the maximum containment laboratory and the course of the
306 study, and were provided with appropriate enrichment including, but not limited to,
307 polished steel mirrors, durable toys, and food enrichment. Macaques were anesthetized in
308 accordance with maximum containment standard operating procedures prior to all
309 macaque manipulations, including virus exposure, sample collection, and medical
310 imaging. Macaques were observed following anesthesia to ensure complete recovery. All
311 work with NHPs was performed in accordance with the recommendations of the
312 Weatherall Report.

313

314 **Macaque exposures**

315 The macaques were split into 2 groups of 3 animals each (**Supplementary Table 1**).
316 Mock group (M) macaques received 2 ml of DMEM + 2% heat-inactivated FBS into each
317 bronchus by direct bilateral primary post-carinal intrabronchial instillation, followed by a
318 1-ml normal saline flush and then 5 ml air. Virus group (V) macaques were exposed the

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319 same way with each 2-ml instillate containing 9.13×10^5 pfu/ml (i.e., a total exposure dose
320 of 3.65×10^6 pfu) of SARS-CoV-2 followed by 1-ml saline flush and then 5 ml air. All
321 macaques were sedated prior to instillation. Prior to administering anesthesia,
322 glycopyrrolate (0.06 mg/kg) was delivered intramuscularly to reduce saliva secretions.
323 Next, each macaque received 10 mg/kg ketamine and then 35 μ g/kg dexmedetomidine
324 intramuscularly. To reverse anesthesia, 0.15 mg/kg atipamezole was administered
325 intravenously. All macaques were evaluated daily for health and were periodically
326 examined physically, including blood draws, and conjunctival (left and right),
327 nasopharyngeal, oropharyngeal, and rectal swab collection. Stool and urine were also
328 collected on each day swabs were collected. All swabs were collected in 1-ml universal
329 virus transport (UVT) media (BD Biosciences, San Jose, CA, USA).

330

331 **Macaque scoring**

332 Cage-side assessment scoring criteria (**Supplementary Table 4**) were modified from
333 Chertow *et al.* (2016) to include clinical signs relevant to COVID-19 and respiratory rates
334 of crab-eating macaques (9, 50, 51). In addition to cage-side observations, physical exam
335 scoring criteria were implemented to assess clinical conditions on days when macaques
336 were anesthetized (**Supplementary Table 4**). Cage-side and physical exam scoring
337 criteria were developed in collaboration with National Primate Research Centers
338 (NPRCs) to standardize disease assessment and compare disease outcomes between NHP
339 models. Heart rate was not incorporated into the physical exam scores until D2 because
340 heart rate score was determined as beats per minute over baseline. Baseline heart rate was
341 determined as an average over three timepoints, D-11, D-5/D-6, and D0 (except for

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342 macaque V3, for which heart rate was not recorded prior to dexmedetomidine

343 administration).

344

345 **Clinical analysis**

346 Blood samples were collected in Greiner Bio-One Hematology K₃EDTA Evacuated

347 Tubes (Thermo Fisher Scientific). Complete blood counts, including leukocyte

348 differentials and reticulocyte counts (CBC/Diff/Retic), were determined using the Procyte

349 DX (IDEXX Laboratories, Westbrook, ME, USA). The Catalyst One analyzer (IDEXX

350 Laboratories) was used for biochemical analyses of serum samples, which were collected

351 in Greiner Bio-One VACUETTE Z Serum Sep Clot Activator Tubes (Thermo Fisher

352 Scientific). Samples were run on both machines the day of collection shortly following

353 collection such that they were not stored prior to analysis. On D30, an equipment issue

354 with the Procyte DX required that samples from the virus-exposed group be stored at 4°C

355 for about 30 h prior to analysis. For a list of all measured parameters and their values, see

356 **Supplementary Table 3.**

357

358 **Image acquisition**

359 Following sample collection and intubation, macaques were moved to chest computed

360 tomography (CT) or whole-body positron emission tomography combined with CT

361 (PET/CT) imaging. For imaging procedures, each macaque was anesthetized

362 intramuscularly with 15 mg/kg ketamine following 0.06 mg/kg glycopyrrolate

363 intramuscularly. Anesthesia was maintained using a constant rate intravenous infusion of

364 propofol at 0.3 mg/kg/min (except on D-11 when propofol at 0.2 mg/kg/min was used).

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365 Macaques were placed on the scanner's bed in a supine, head-out/feet-in position and
366 connected to a ventilator to facilitate breath holds, and vital signs were monitored
367 throughout the imaging process.

368 *High resolution chest CT*

369 Chest CT scans were performed using the 16-slice CT component of a Gemini TF 16
370 PET/CT (Philips Healthcare, Cleveland, OH, USA) or a Precedence SPECT/CT scanner
371 (Philips Healthcare). Chest CT images were acquired in helical scan mode with the
372 following parameter settings: ultra-high resolution, 140 kVp, 300 mAs/slice, 1 mm
373 thickness, 0.5 mm increment, 0.688 mm pitch, collimation 16x0.75, and 0.75 s rotation.
374 CT image reconstruction used a 512x512 matrix size for a 250-mm transverse field-of-
375 view (FOV), leading to a pixel size of 0.488 mm. Two CT images were produced: one
376 with the standard "B" filter and one with the bone-enhanced "D" filter. No contrast agent
377 was administered. Each macaque underwent a 15–20-s breath-hold during acquisition.
378 The pressure for the breath-hold was maintained at 150 mmH₂O.

379 *Whole-body PET/CT*

380 Whole-body PET/CT scans were performed using a Gemini TF 16 TF PET/CT scanner.
381 Radiotracer (2-deoxy-2-[¹⁸F]-fluoro-D-glucose; FDG) was injected intravenously (0.5
382 mCi/kg FDG, up to 4.0 mCi/scan) and the time of injection was recorded. After high-
383 resolution chest CT imaging with breath-hold session (≈5 min), whole-body CT images
384 were acquired (≈5 min) in helical scan mode with the following parameter settings: high
385 resolution, 140 kVp, 250 mAs/slice, 3 mm thickness, 1.5 mm increment, 0.688 mm pitch,
386 collimation 16x0.75, and 0.5 s rotation. Two CT images were reconstructed from the raw
387 data. An initial CT image was reconstructed into a 600-mm diameter field-of-view

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388 (FOV), resulting in a pixel size of 1.17 mm and a slice spacing of 1.5 mm. This CT image
389 was used to create an attenuation map needed to correct the PET images for photon
390 attenuation. Raw CT data were reconstructed a second time into diagnostic quality CT
391 images by reducing the FOV size to 250 mm, resulting in a pixel size of 0.488 mm with 1
392 mm slice thickness. One CT image was produced with the standard (“B”) filter. No
393 contrast agent was administered, and the macaques breathed freely during the scan.
394 Following whole-body CT scanning, whole-body PET scans covering the macaques’
395 bodies from the top of the head to the middle of the thighs was performed after a 60-min
396 delay. Depending on the size of the NHP, 6 or 7 bed positions (with 50% overlap) were
397 needed for this scan range. Dwell time per bed position was 3 min, resulting in a total
398 duration of 18 or 21 min/scan. PET data were reconstructed into a set of either 300- or
399 342-image slices with 128x128 2-mm-cubic voxels. To ensure quantitative accuracy, all
400 reconstructed PET images were corrected during scans for radioactive decay, uniformity,
401 random coincidences, and attenuation and scattering of PET radiation *in situ*. Lastly,
402 whole-body CT imaging with iopamidol (600 mg iodine/kg) intravenous contrast material
403 were acquired at D-11 and the terminal D30 scans. Each macaque underwent a 30–50-s
404 breath-hold during acquisition. The pressure for the breath-hold was maintained as 150
405 mmH₂O. After completion of imaging, macaques were returned to the clinical team for
406 subsequent procedures.

407

408 **Image evaluation**

409 *Chest CT evaluation*

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410 An adapted semi-quantitative scoring system based on a previously published method
411 ([52](#)) was used to quantitatively estimate the pulmonary involvement of lung
412 abnormalities on the extent of parenchymal lung disease in each lung lobe. The sums of
413 the lobar scores were used to generate total lung summary scores. CT scores ranged from
414 0–5 for each lobe (right upper, right middle, right lower, right accessory, left upper, left
415 middle, and left lower). Lobes were scored as: 0 = no disease involvement, 1 ≤5%, 2 = 5–
416 24%, 3 = 25–49%, 4 = 50–74%, 5 ≥75%, with a maximum total lung score of 35. Score
417 increments of 0.5 were used to indicate improvement (or worsening) in radiodensity
418 when changes in volume were insufficient to change score category. Additional data
419 points included cohort (macaques M1–3 and V1–3), scan date and study day, and number
420 of “lesions”. Types of infiltrates (GGOs, paving, consolidation, and organizing
421 pneumonia) per lobe were given individual 0–5 scores, and the overall predominant type
422 of infiltrate per each lobe and each subject scan were recorded. The summary scores per
423 each lobe and whole lung were used as markers of disease progression. Image analysis
424 was performed by a board-certified radiologist and a research fellow using MIM software
425 version 6.9 (Cleveland, OH, USA).

426 To quantify CT data, the lung field was segmented using a region-growing
427 implementation (MIM software). Entire lung volumes (LV) were measured at each time
428 point (n: at time point n; b: at baseline). A histogram analysis was performed on the voxel
429 intensities (radiodensity in Hounsfield units [HU]) within the segmented lung. Percent
430 change in the volume of hyperdense lung tissue was determined as described previously
431 ([53](#)). Briefly a threshold value was determined for each subject, based on a 5% cutoff in
432 the upper tail of the histogram of lung tissue from the baseline CT scan. Due to an

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433 inability to keep the lungs of the virus group macaques inflated to approximately the
434 same volumes over time, a correction was applied to the 5% PCLH threshold as
435 previously described ([54](#)).

436 As more abnormalities (e.g., GGOs, consolidation) appear during the disease
437 process, a larger volume of tissue will have higher HU values and PCLH as percent
438 change in lung hyperdense volume (HV) from baseline can be expressed as $[(HV_n -$
439 $HV_b)/HV_b]*100$. Then PCLH/LV can be expressed as $[V_n/LV_n]*100$. Change in average
440 lung radiodensity (LD) in the entire lung volume can be $[LD_n - LD_b]$. Change in average
441 hyperdensity in hyperdense volume can be expressed as $[HD_n - HD_b]$. Then, PCLH as
442 percent change in lung hyperdense volume, PCLH as percent of lung volume
443 (PCLH/LV), change in the average radiodensity in the entire lung and change in the
444 averaged hyperdensity in the hyperdense volume were graphed with disease progress. To
445 visualize CT abnormalities in three dimensions (3D), a volume rendering technique was
446 used to create videos. In brief, the lungs and airways were extracted from chest CT
447 images using MIM software. A region growing algorithm was used to segment different
448 classes including normal lung tissue, vessels, airways, and “lesions” with multiple seeds
449 at specific locations to achieve realistic segmentations. 3D volume renderings of the
450 segmentations were generated and animated rotations exhibiting the location and extent
451 of the abnormalities were produced using 3D Slicer 4 software version 4.10.2 ([55](#)).

452

453 *Whole-body PET/CT evaluation*

454 Analysis of imaging data was performed using MIM software. Whole-body CT and PET
455 scans for a given scanning session were co-registered. Regions of interest (ROIs) were

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456 placed manually on the PET scans, and location determined on the co-registered CT
457 scans. These regions included specific lung abnormalities when present, left and right
458 lung when no specific abnormalities were present, mediastinal and hilar lymph nodes,
459 and spleen. Once ROIs were placed, mean FDG maximum standardized uptake values
460 (SUV_{max}) were measured from corrected PET images and averaged SUV_{max} values were
461 graphed longitudinally.

462 The quantitative analysis was correlated with a qualitative evaluation of CT and
463 PET/CT lung pathology over time, performed by a board-certified radiologist.

464

465 **RT-qPCR**

466 RT-qPCR analysis was performed to determine presence of SARS-CoV-2 RNAs in
467 collected specimens. Samples were frozen at -80°C in TRIzol LS (Thermo Fisher
468 Scientific, Wilmington, DE, USA) and thawed on ice. 100 μl of sample were added to
469 5PRIME Phase Lock tubes (Quantabio, Beverly, MA, USA) followed by addition of 20
470 μl of chloroform/tube (Sigma-Aldrich, St. Louis, MO, USA) and inversion by hand 10
471 times. Phase Lock tubes with sample and chloroform were centrifuged at $10,000 \times g$ for 1
472 min at 4°C . Following centrifugation, aqueous phases were removed ($\approx 55 \mu\text{l}/\text{tube}$) and
473 deposited into clean 1.5-ml Eppendorf tubes. 70% ethanol was subsequently added to
474 each tube at a 1:1 ratio (55 μl ethanol), inverted 10 times by hand, and briefly
475 centrifuged. The ethanol/aqueous solution containing extracted RNA was used as input
476 for purification and isolation using the PureLink RNA Mini Kit (Thermo Fisher
477 Scientific) following the manufacturer's instructions. RNA was eluted in 30 μl of water.
478 RT-qPCR was performed using the SuperScript III Platinum One-Step qRT-PCR Kit

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479 (Thermo Fisher Scientific) following the manufacturer's instructions with the following
480 changes: reagent volumes were halved, resulting in 25- μ l final reaction volumes. The N1
481 assay supplied with the 2019-nCoV CDC qPCR Probe Assay (Integrated DNA
482 Technologies [IDT], Coralville, IA, USA) was used (1 μ l/reaction) in lieu of individual
483 primers and probes. 2 μ l of extracted RNA or 2019-nCoV_N_Positive_Control (IDT)
484 were used in each reaction. Samples and controls were run in technical triplicates on a
485 CFX96 Touch Real-Time PCR Detection System (Biorad, Hercules, CA, USA) following
486 the manufacturer's recommendations; a 50°C, 15-min reverse-transcriptase step was used
487 for first strand cDNA synthesis followed by 95°C, 2-min to inactivate the reverse
488 transcriptase, followed by 45 cycles of 95°C, 15 s, 60°C, 30 s.

489

490 **Serology**

491 Serum samples were collected in Greiner Bio-One VACUETTE Z Serum Sep Clot
492 Activator Tubes and frozen at -80°C. Before removal from the maximum containment
493 laboratory, virus in samples was inactivated using a cobalt irradiation source with a target
494 dose of 50 kGy (JLS 484R-2 Cobalt-60 [⁶⁰Co] Irradiator, JLShephard & Associates)
495 following standard inactivation protocols. Serum samples were subsequently heat-
496 inactivated at 56°C for 30 min prior to antibody screening. To determine IgG titers,
497 Immulon 2HB 96-well plates (Thermo Fisher Scientific) were coated with recombinantly
498 expressed SARS-CoV-2 spike S1 subunit (Sino Biological, Wayne, PA, USA) at 0.1
499 μ g/well in 50 μ l/well overnight at 4°C. Plates were washed three times with phosphate-
500 buffered saline (PBS) + 0.1% Tween20 (PBS_T; Sigma-Aldrich) and blocked with
501 ELISA diluent (5% nonfat milk [LabScientific, Danvers, MA, USA] in PBS-T) for 1 h at

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502 37°C. Serum samples were serially diluted 1:2 in a dilution block (1:50 to 1:6,400) with
503 ELISA diluent. After blocking, plates were washed three times with PBS-T and 100
504 µl/well of diluted sample were transferred to the plate. Samples were incubated for 1 h at
505 37°C. Plates were then washed three times with PBS-T, and 100 µl of goat anti-human
506 IgG Fc specific (Jackson ImmunoResearch, West Grove, PA, USA) secondary antibody
507 conjugated to horseradish peroxidase (HRP; diluted 1:20,000 in ELISA diluent) were
508 added to each well. Samples were incubated for 1 h at 37°C and finally washed five times
509 with PBS-T. Plates were developed by adding 100 µl of TMB substrate (Thermo Fisher
510 Scientific) at room temperature for 10 min in the dark. Development was stopped by the
511 addition of 100 µl of Stop Solution (Thermo Fisher Scientific). Plates were read at 450
512 nm with a correction wavelength of 650 nm using a Spectramax Plus 384 (Molecular
513 Devices, San Jose, CA, USA) within 30 min of stopping the reaction. Reciprocal
514 endpoint titers were determined in GraphPad software version 8.4.2 (Prism, La Jolla, CA,
515 USA), using a sigmoidal 4 parameter-logistic fit curve. Endpoint titers were calculated at
516 the point when the curve crossed the ELISA cutoff value. For the S1 subunit IgG ELISA,
517 the cutoff value was determined to be an optical density (OD) of 0.19077, which was
518 determined from control sera collected from twenty-five NHPs sampled prior to the
519 known emergence of SARS-CoV-2. Data are presented as the mean and standard
520 deviation of two independent ELISA runs.

521

522 **Fluorescence neutralization assay**

523 All assays were run with irradiated and heat-inactivated sera. Irradiation and heat-
524 inactivation were performed as described above. Vero E6 cells were seeded at 3×10^4 in

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525 100 μ l DMEM+10% heat-inactivated FBS in 96-well Operetta plates (Greiner Bio-One,
526 Monroe, NC, USA). The following day, a series of six-point dilutions, each 1:2, were
527 performed in duplicate starting with a dilution of 1:20 (1:20, 1:40, 1:60, etc.) in 96-well
528 1.2-ml cluster tubes (Corning Inc, Corning, NY, USA). Then, stock SARS-CoV-2 virus
529 was diluted in serum free media and was added to the sera in each cluster tube at a
530 multiplicity of infection (MOI) of 0.5 using a liquidator, doubling the total volume in
531 each well and further diluting sera 1:2. Thus, the final starting dilution was 1:40. The
532 sera/virus mixtures were then mixed by pipetting up and down with the liquidator.
533 Cluster tubes were next incubated for 1 h at 37°C/5% CO₂. Following incubation, 100
534 μ l/well of each virus/serum mixture were transferred to the Operetta plates from the
535 cluster tubes to yield final volumes of 200 μ /well (100 μ l cell seeding media plus 100 μ l
536 virus/serum mixture). Each set of cluster tubes provided enough material for each sample
537 to be run in duplicate in 2 plates, yielding a total of 4 replicates per sample. The
538 virus/serum mixtures were incubated on plates for 24 h at 37°C/5% CO₂. Subsequently,
539 plates were fixed with 20% neutral buffered formalin (Thermo Fisher Scientific) for 24 h
540 at 4°C. Next, plates were washed with PBS (Thermo Fisher Scientific) and then blocked
541 with 3% bovine serum albumin (BSA) in 1X PBS for 30 min on a rocker. Staining
542 followed, first with the primary antibody and then the secondary antibody. The primary
543 antibody was SARS-CoV-2 Nucleoprotein/NP Antibody, Rabbit mAb (Sino Biological,
544 Chesterbrook, PA, USA) prepared at 1:8,000 in blocking buffer at room temperature.
545 Plates were incubated with primary antibody for 60 min on a rocker. The secondary
546 antibody was goat α -rabbit IgG (H+L), Alexa Fluor 594 Conjugate (Thermo Fisher
547 Scientific) prepared at 1:2,500 in 1X PBS. Plates were incubated with secondary antibody

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548 at room temperature for 30 min on a rocker and in the dark. Between the blocking,
549 primary, and secondary steps, plates were washed three times with 1X PBS. Finally,
550 plates were read on a Operetta High-Content Analysis System (PerkinElmer, Waltham,
551 MA, USA) with at least 4 fields of view of >1,000 cells each. Data were analyzed using
552 Harmony software (PerkinElmer). Half-maximal neutralization titers (NT₅₀) were
553 calculated by averaging the fluorescence intensity in virus control wells and dividing by
554 two. The fluorescence intensity of a sample at each dilution was compared to the NT₅₀
555 values, and the lowest dilution that is equal to or less than the NT₅₀ value was recorded.

556

557 **Cytokine analysis**

558 The MILLIPLEX MAP Non-Human Primate Cytokine Magnetic Bead Panel - Premixed
559 23 Plex – Immunology Multiplex Assay (Millipore, Burlington, MA, USA; #PCYTMG-
560 40K-PX23) was performed on collected plasma samples following the manufacturer's
561 instructions. All reagents were warmed to room temperature prior to addition to assay
562 wells. Quality controls and standards were reconstituted with 250 µl of deionized water
563 and allowed to sit for 10 min prior to use. A four-fold seven-point standard curve was
564 generated by diluting the concentrated stock with assay buffer for each point. Assay
565 buffer alone served as the blank. The plate was first washed with 200 µl of assay buffer
566 and incubated on an orbital shaker at 800 rpm at room temperature for 10 min. Assay
567 buffer was decanted, and plates were inverted on absorbent paper to remove any excess
568 buffer. 25 µl of serum matrix were added to background, control, and standard wells. 25
569 µl of assay buffer was added to sample wells. 25 µl of samples/standards/controls were
570 added to the appropriate wells. Beads were resuspended by vortexing and 25 µl were of

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571 vortexed beads were added to each well. Each plate was sealed and incubated overnight
572 at 4°C on an orbital shaker at 500 rpm. The next day, plates were washed twice using a
573 hand-held magnetic plate holder with 200 µl of wash buffer per well and decanted as
574 previously described. Plates were then incubated on an orbital shaker at 500 rpm at room
575 temperature for 1 h with 25 µl of detection antibody. After incubation, 25 µl of kit-
576 provided streptavidin-phycoerythrin were added directly to each well and incubated at
577 room temperature on an orbital shaker at 500 rpm for 30 min. Plates were washed twice
578 and 150 µl of sheath fluid was added to each well. Plates were read on a Flexmap 3D
579 reader (Luminex, Chicago, IL, USA) within 24 h of completion following assay
580 instructions. The data was exported to Bio-Results Generator version 3.0 and Bio-Plex
581 Manager software version 6.2 (BioRad). Results were graphed using GraphPad software
582 version 8.4.2.

583

584 **Statistical Analysis**

585 Area under the curve (AUC) summaries were calculated using the trapezoidal rule, and
586 compared using Welch's t-tests, using R version 3.6.3.

587

588 **Data Availability**

589 Data from this study were made available publicly as soon as they became available at
590 <https://openresearch.labkey.com/project/Coven/COVID-001/begin.view> to inform the
591 ongoing COVID-19 outbreak response without delay following practices we initiated for
592 NHP studies of Zika virus pathogenesis in 2016 (56).

593

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626

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635

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637

638 **Figure Legends**

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639 **Figure 1 | Study overview.** Three crab-eating macaques (*Macaca fascicularis*) were
640 exposed to $\approx 3.65 \times 10^6$ pfu SARS-CoV-2 each by direct bilateral primary post-carinal
641 intrabronchial instillation and sampled as outlined. A second group of three crab-eating
642 macaques was mock-exposed and sampled in the same manner one day prior to the virus-
643 exposed group (exception: a second baseline CT image was recorded for each group on a
644 single day, D-6 or D-5 (*)).

645

646 **Figure 2 | Detection of SARS-CoV-2 RNA and specific immune responses in SARS-**
647 **CoV-2-inoculated and mock-inoculated macaques. a)** RT-qPCR targeting the SARS-
648 CoV-2 N protein was performed from baseline through D10 in nasopharyngeal swabs for
649 macaques of both groups. **b)** Anti-SARS-CoV-2 S1 subunit IgG ELISA results are
650 expressed as reciprocal endpoint titers over time for both mock group (M) and virus
651 group (V) macaques. **c)** Fluorescence neutralization assays were performed on sera from
652 all macaques on all days. NT₅₀, half-maximal neutralization titer.

653

654 **Figure 3 | COVID-19-like CT abnormalities in the lungs of SARS-CoV-2 inoculated**
655 **macaques V1 (a–b), V2 (c–g), and V3 (h–j).** Distribution of CT scan abnormalities in
656 3D images of SARS-CoV-2-inoculated macaque V1 (**a**), V2 (**c**), and V3 (**h**). Blue:
657 airways; gray: normal lung; red: vessels; yellow: imaging abnormalities. **b)** Selected
658 characteristic abnormalities in macaque V1 include peripheral, peri-bronchial ground-
659 glass opacity (GGO) in the left middle lobe (green inset, top, red arrow) and peripheral
660 GGO with reticulation in the posterior right lower lobe (green inset, bottom, yellow
661 arrow). **d–g)** Selected characteristic abnormalities in macaque V2 include **d)** peri-

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662 bronchial consolidation in the left accessory lobe (blue inset, top, red arrow) and
663 posterior GGO with reticulation in the posterior right lung (blue inset, bottom, red arrow),
664 **e**) bilateral posterior GGO with reticulation (blue inset, top and bottom, red arrows), **f**)
665 GGO with superimposed paving (interlobular septal thickening) in right posterior lung
666 (blue inset, top) and mixed GGO with pleural-based consolidation in left posterior lung
667 (blue inset, bottom), and **g**) pleural-based mixed GGO and consolidation developing on
668 D6 (blue inset, blue arrow). **i–j**) Selected characteristic abnormalities in macaque V3
669 include **i**) GGO with air bronchogram in right posterior lung (orange inset, top, purple
670 arrow), alveolar consolidation with peripheral GGO and air bronchogram in left posterior
671 lung (orange inset, bottom, blue arrow), and **j**) expanding and more dense alveolar
672 consolidation with air bronchogram on D4 (orange inset, blue arrow). Representative 3D-
673 rendered videos of **a**), **c**), and **h**) demonstrating whole-lung pathology are shown in
674 **Videos 1–3**, respectively.

675

676 **Figure 4 | Qualitative and quantitative computed tomography (CT) analysis of**
677 **macaque lungs. a)** Representative axial CT images in three SARS-CoV-2-infected (V)
678 macaques for each indicated study day (D). The grey scale represents radiodensity in
679 Hounsfield units (HU). **b)** Percent change in volume of lung hyperdensity (PCLH)
680 measured over time in the same SARS-CoV-2 inoculated macaques shown in a).
681 Representative axial CT images and PCLH for all study days in both groups, including
682 data for mock group (M) macaques, are shown in **Supplementary Figure 2**. In SARS-
683 CoV-2-inoculated macaques only, selected representative CT images (axial, sagittal, and

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684 coronal views) with detailed radiological descriptions from pre-inoculation baseline to
685 day 19) are shown in **Supplementary Figure 3**.

686

687 **Figure 5 | Radiologist-derived CT lung scores of macaque lungs.** Averaged
688 radiologist-derived CT scores of the entire lungs for individual macaques (**a**) and
689 averaged for mock group (M) and virus group (V) macaques (**b**) over time.

690

691 **Figure 6 | Qualitative positron emission tomography (PET) and FDG uptake**
692 **analysis in macaque lung and regional lymph nodes.** Representative coronal 2-deoxy-
693 2-[¹⁸F]-fluoro-D-glucose (FDG) PET/CT images for each indicated study day (D) from
694 pre-inoculation baseline to 12 days after inoculation. SUV_{max} , maximum standardized
695 FDG uptake value. Selected areas of increased FDG uptake are indicated in lung
696 parenchyma (yellow arrows, blue arrows) and regional lymph nodes (pink arrows). All
697 study days and data for mock group (M) macaques are shown in **Supplementary Figure**
698 **4**. Selected PET/CT images (axial, sagittal, and coronal views) including detailed
699 radiological descriptions are shown in **Supplementary Figure 5**.

700

701 **Figure 7 | Quantitation of 2-deoxy-2-[¹⁸F]-fluoro-D-glucose (FDG) uptake in lung, lymph**
702 **nodes, and spleen. a)** SUV_{max} (mean FDG maximum standardized uptake values) for 3–5
703 selected lung regions of interest (ROIs) with high FDG uptake with tracking of identical
704 regions of interests at all PET/CT timepoints in each macaque and **b)** averaged for mock
705 group (M) and virus group (V) macaques. **c)** SUV_{max} for 2–3 selected lymph node ROIs
706 with high FDG uptake in each macaque and **d)** averaged for (M) and (V) group

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707 macaques. **e**) SUV_{max} for spleen FDG uptake in each macaque and **f**) averaged for (M)
708 and (V) group macaques.

709

710 **Figure 8 | Quantitative analyses of volume and radiodensity of macaque lungs. a–b)**

711 Percent change in volume of lung hyperdensity (PCLH) measured over time for

712 individual macaques (**a**) as in **Figure 4b** and averaged for mock group (M) and virus

713 group (V) macaques (**b**). **c–d**) PCLH standardized as percent of entire overall lung

714 volumes over time (PCLH/LV) for individual macaques (**c**) and averaged for M and V

715 macaques (**d**). **e–f**) Change in the average lung densities (Hounsfield units [HU]) in the

716 entire lung volumes over time for individual macaques (**e**) and averaged for M and V

717 macaques (**f**). **g–h**) Change in average lung hyperdensities (HU) in the lung hyperdense

718 volume over time for individual macaques (**g**) and averaged for M and V macaques (**h**).

719

720 **Videos 1–3 | Qualitative computed tomography (CT) analysis of macaque lungs. 3D**

721 rendering of the lungs of macaque V1 at D2 (**Video 1**), V2 at D4 (**Video 2**), and V3 at D4

722 (**Video 3**) after SARS-CoV-2 exposure. Blue: airways; gray: normal lung; red: vessels;

723 yellow: imaging abnormalities.

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724 **Supplementary Data**

725 **Supplementary Figure 1 | Cytokines.** Cytokien concentration changes measured for
726 individual macaques and averaged for mock group (M) and virus group (V) macaques.
727 Data are represented on logarithmic scales. For graphic representation, values of “0” or
728 “not detected” (below the level of assay sensitivity) were automatically assigned a value
729 = 1.

730

731 **Supplementary Figure 2 | Qualitative and quantitative computed tomography (CT)**
732 **analysis of macaque lungs. a)** Representative axial CT images in three SARS-CoV-2-
733 infected (V) macaques and mock-inoculated (M) macaques for all indicated study days
734 (D). The grey scale represents radiodensity in Hounsfield units (HU). Selected CT images
735 (axial, sagittal, and coronal views) from the SARS-CoV-2 infected macaques with
736 detailed radiological descriptions are shown in **Supplementary Figure 3**. Colored arrows
737 in **Figure 4** and this figure represent regions of interest that are further detailed in figure
738 legends for **Supplementary Figure 3**. **b)** Percent change in lung hyperdensity (PCLH)
739 measured over time in the same macaques shown in **Figure 4**, including also here the
740 mock-infected individual macaques and all study days.

741

742 **Supplementary Figure 3 | Qualitative computed tomography (CT) analysis of**
743 **macaque lungs.**

744

745 **Supplementary Figure 4 | Qualitative positron emission tomography (PET) and**
746 **PET/CT analysis of macaque lungs.** Representative coronal (left panels) and axial

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747 (right panels) 2-deoxy-2-[¹⁸F]-fluoro-D-glucose (FDG) PET/CT images for each
748 indicated study day (D). SUV_{max}, mean FDG maximum standardized uptake values.
749 Selected areas of increased FDG uptake are highlighted in the lung parenchyma (yellow
750 arrows) and regional lymph nodes (pink arrows). Selected merged PET/CT images (axial,
751 sagittal, and coronal views) with detailed radiological descriptions of these areas of
752 interest are shown in **Supplementary Figure 5**.

753

754 **Supplementary Figure 5 | Qualitative positron emission tomography (PET) and**
755 **PET/CT analysis of macaque lungs.**

756

757 **Supplementary Table 1 | Crab-eating macaque (*Macaca fascicularis* Raffles, 1821)**
758 **information.**

759

760 **Supplementary Table 2 | Macaque physical condition/clinical scoring results.**

761

762 **Supplementary Table 3 | Complete blood cell count (CBC/Diff/Retic) and serum**
763 **chemistries.**

764

765 **Supplementary Table 4 |Macaque clinical scoring guide.**

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Figure 1

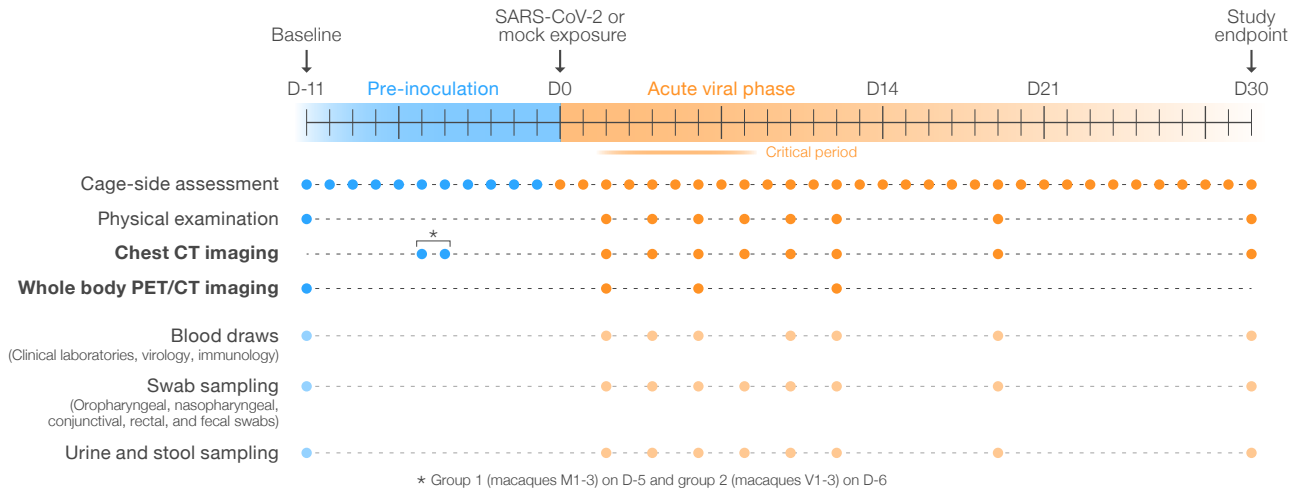


Figure 2

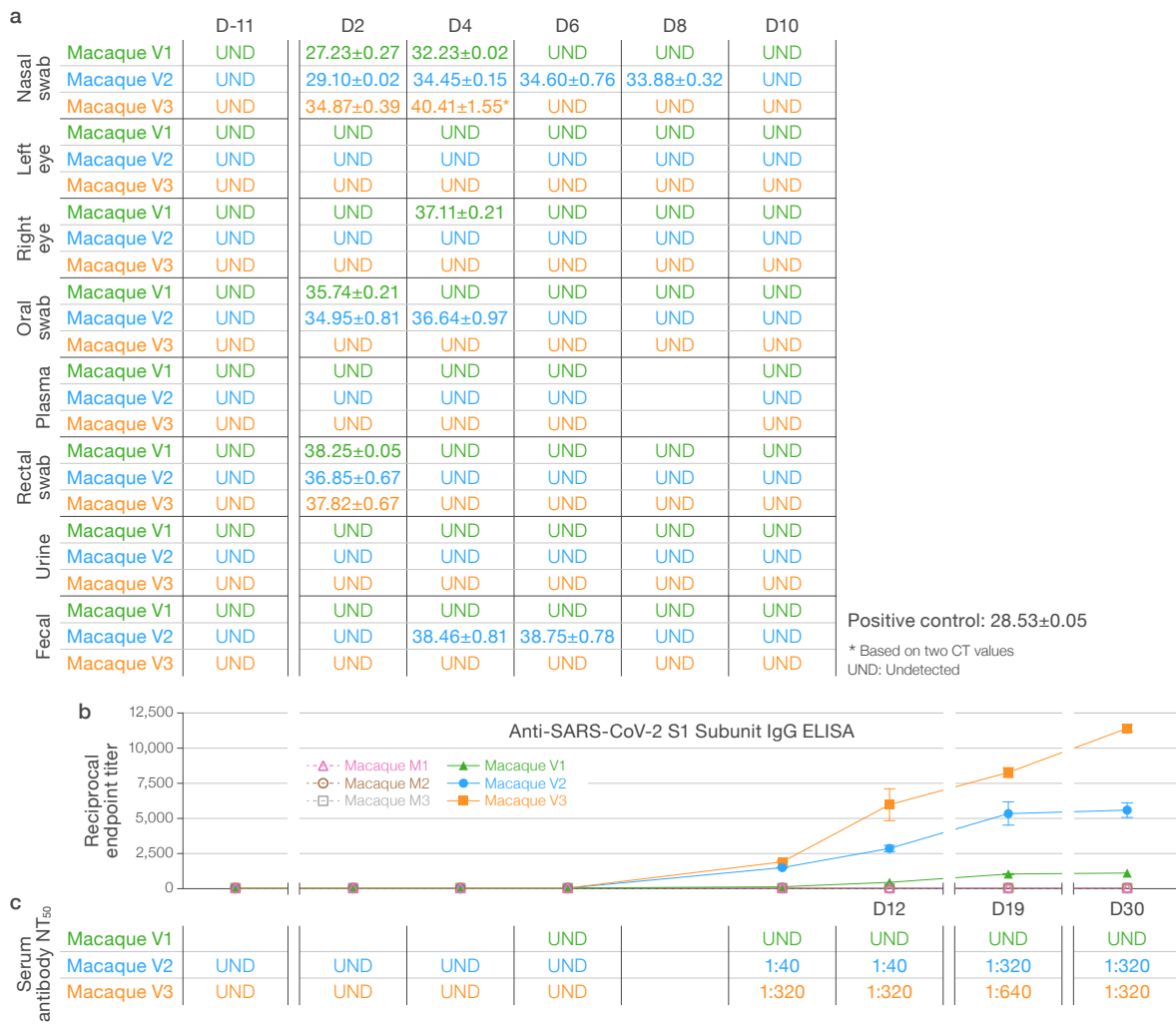


Figure 3

COVID-19-like CT abnormalities in SARS-CoV-2-infected macaques

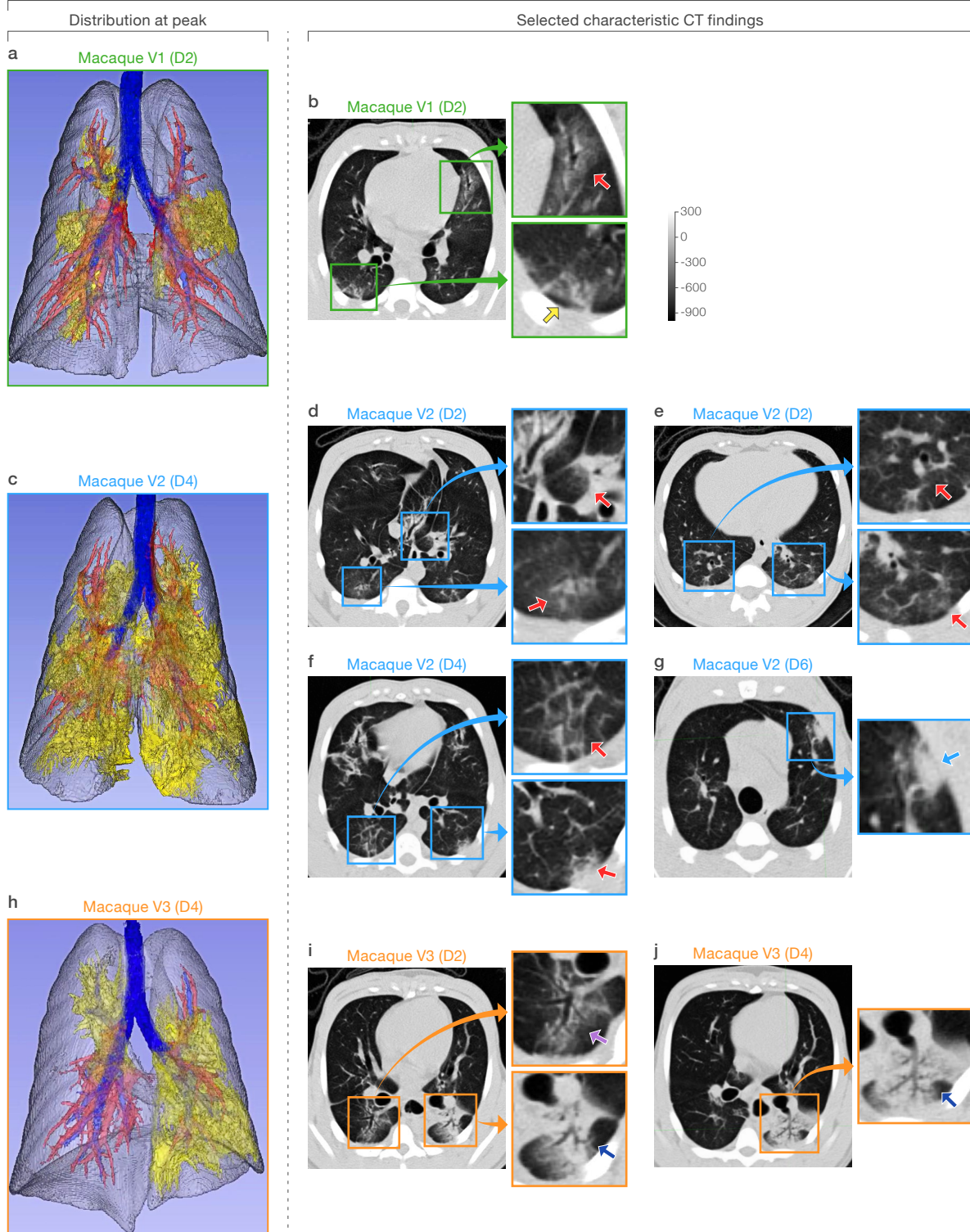


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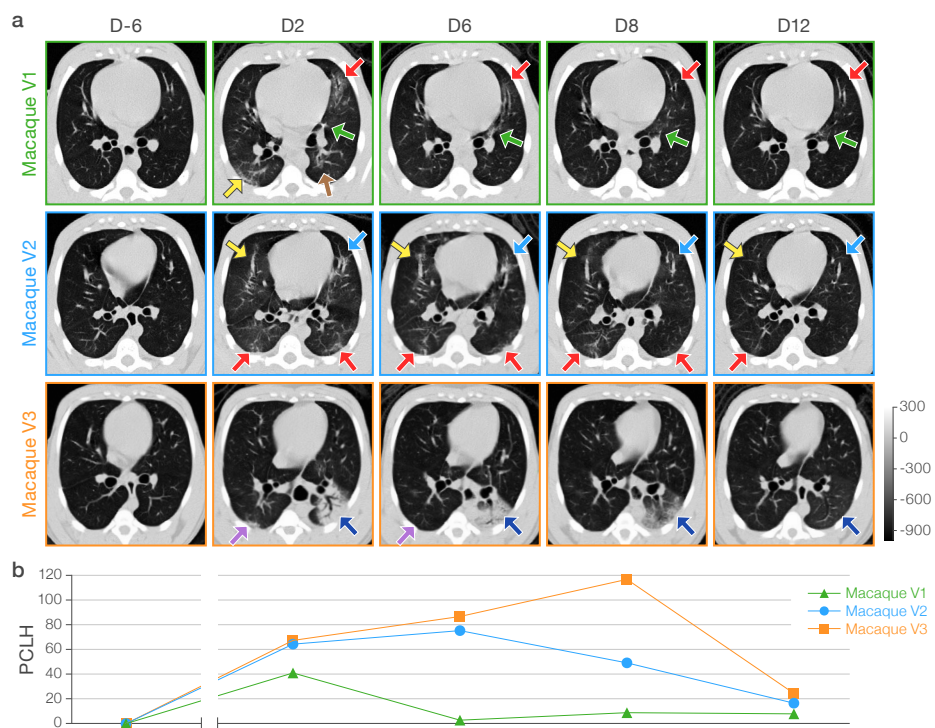


Figure 5

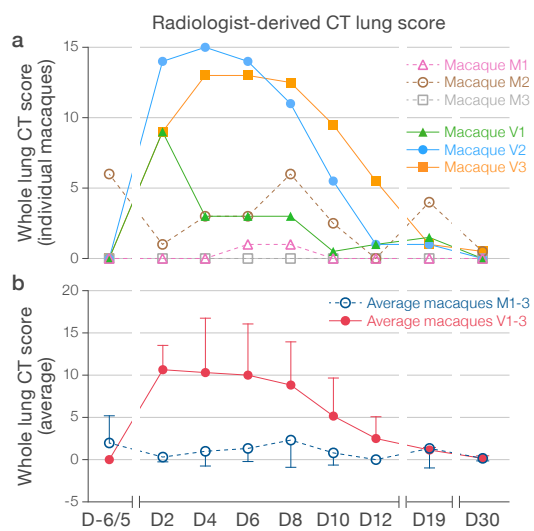


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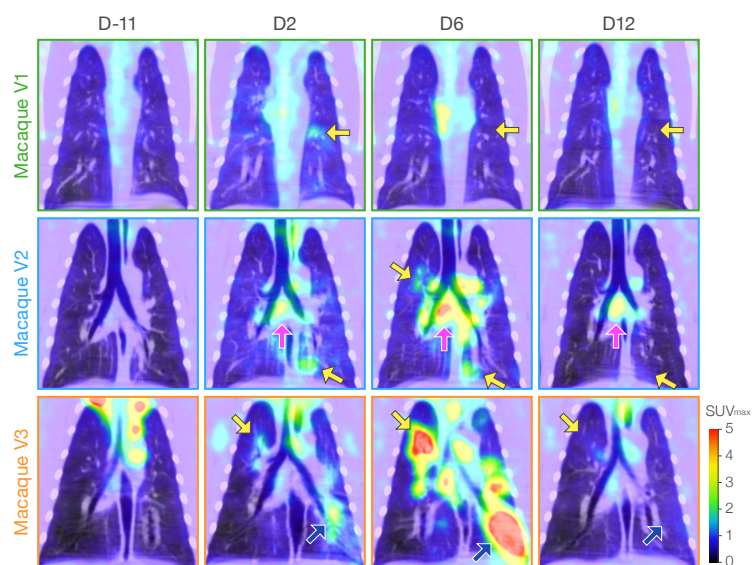


Figure 7

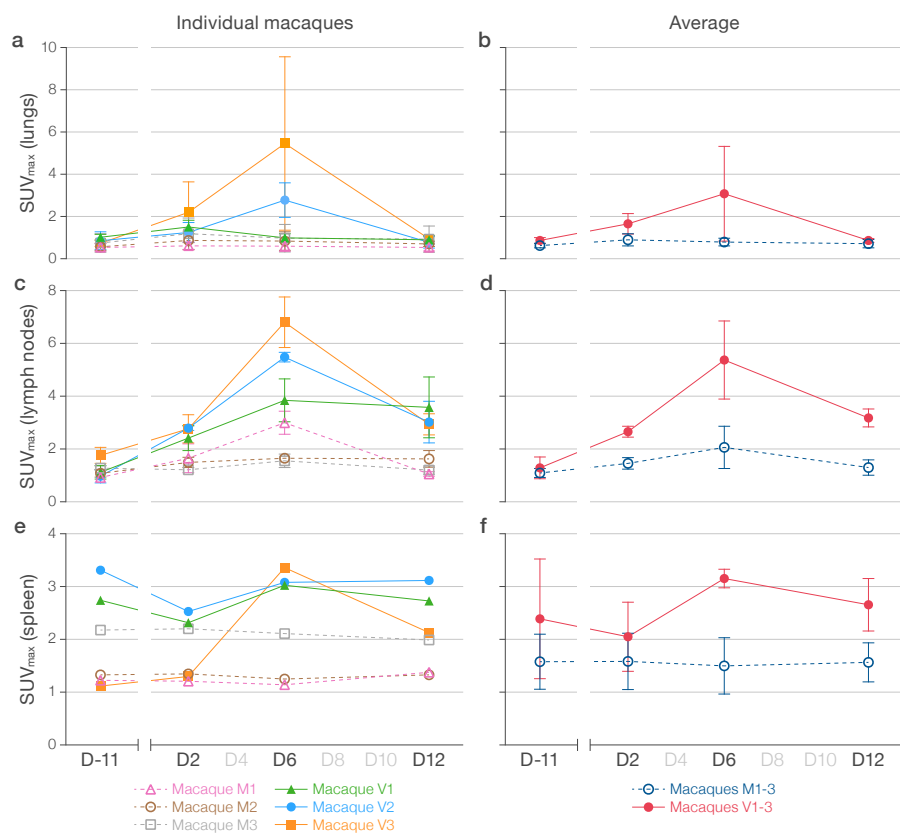


Figure 8

