

# COVA1-18 neutralizing antibody protects against SARS-CoV-2 in three preclinical models

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# 1 **COVA1-18 neutralizing antibody protects against SARS-CoV-2 in three** 2 **preclinical models**

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50

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52

53 **Abstract**

54 One year into the Coronavirus Disease 2019 (COVID-19) pandemic caused by Severe Acute  
55 Respiratory Syndrome coronavirus 2 (SARS-CoV-2), effective treatments are still needed<sup>1-3</sup>.  
56 Monoclonal antibodies, given alone or as part of a therapeutic cocktail, have shown promising  
57 results in patients, raising the hope that they could play an important role in preventing clinical  
58 deterioration in severely ill or in exposed, high risk individuals<sup>4-6</sup>. Here, we evaluated the  
59 prophylactic and therapeutic effect of COVA1-18 *in vivo*, a neutralizing antibody isolated from  
60 a convalescent patient<sup>7</sup> and highly potent against the B.1.1.7. isolate<sup>8,9</sup>. In both prophylactic  
61 and therapeutic settings, SARS-CoV-2 remained undetectable in the lungs of COVA1-18  
62 treated hACE2 mice. Therapeutic treatment also caused a dramatic reduction in viral loads in  
63 the lungs of Syrian hamsters. When administered at 10 mg kg<sup>-1</sup> one day prior to a high dose  
64 SARS-CoV-2 challenge in cynomolgus macaques, COVA1-18 had a very strong antiviral  
65 activity in the upper respiratory compartments with an estimated reduction in viral infectivity  
66 of more than 95%, and prevented lymphopenia and extensive lung lesions. Modelling and  
67 experimental findings demonstrate that COVA1-18 has a strong antiviral activity in three  
68 different preclinical models and could be a valuable candidate for further clinical evaluation.

## 69 **Main text**

70 Across the world, the Coronavirus Disease 19 (COVID-19) pandemic caused by severe acute  
71 respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to escalate<sup>10</sup>. Despite the  
72 progressive rollout of vaccines, there remains an urgent need for both curative and preventive  
73 measures, especially in individuals with high risk. Monoclonal neutralizing antibodies (NABs),  
74 isolated from convalescent COVID-19 patients, are one of the most promising approaches and  
75 two NAb-based products have already received an emergency use authorization by the FDA.  
76 Although their clinical efficacy remains to be fully assessed<sup>4-6</sup>, their capability to reduce viral  
77 loads<sup>4,5</sup> shows sufficient promise that such an approach could be effective if the treatment is  
78 administered early enough.

79

80 We and others have previously isolated and characterized several highly potent monoclonal  
81 NABs with half-maximum inhibitory concentration (IC<sub>50</sub>) values in the picomolar range<sup>7,11-14</sup>,  
82 with the majority of these targeting the receptor binding domain (RBD) on the S1 subunit of  
83 the S protein. We previously identified COVA1-18, an RBD-specific monoclonal Ab, as one  
84 of the most potent NAB *in vivo*<sup>7</sup>. Using three different experimental models as well as  
85 mathematical modeling, we demonstrate that its rapid and extensive biodistribution is  
86 associated with a very potent antiviral effect, and make it a promising candidate for clinical  
87 evaluation, both as a prophylactic or therapeutic treatment of COVID-19.

88

### 89 **COVA1-18 *in vitro* potency is dependent on avidity**

90 To advance our earlier *in vitro* results<sup>7</sup> on COVA1-18 and allow for better comparability with  
91 other studies, we used two new pseudovirus assays, one using lentiviral pseudotypes with an  
92 ACE2-expressing 293T cell line<sup>15</sup>, and one using VSV-pseudotypes with Vero E6 cells<sup>16</sup>, to  
93 confirm the potency of COVA1-18. With these assays, we confirmed the remarkable potency  
94 of COVA1-18 IgG which inhibited lentiviral SARS-CoV-2 pseudovirus with an IC<sub>50</sub> of 0.8 ng  
95 ml<sup>-1</sup> (5.2 pM) and VSV-based pseudovirus with an IC<sub>50</sub> of 9 ng ml<sup>-1</sup> (60 pM) (**Extended Data**  
96 **Fig. 1a, Extended Data Table 1**). These results were corroborated in multiple independent labs  
97 and COVA1-18 was also equipotent against the D614G variant (**Extended Data Table 1**) that  
98 now dominates worldwide<sup>17-21</sup> as well as the recently emerged B.1.1.7 variant that includes the  
99 N501Y mutation<sup>8,9</sup>.

100



101 COVA1-18 bound strongly to SARS-CoV-2 S protein and showed no cross-reactivity with S  
102 proteins of SARS-CoV, MERS-CoV and common cold coronaviruses HKU1-CoV, 229E-CoV  
103 and NL63-CoV (Extended Data Fig. 1b)<sup>7</sup>. Biolayer interferometry experiments showed that  
104 COVA1-18 IgG bound to soluble SARS-CoV-2 S protein with an apparent dissociation  
105 constant ( $K_D$ ) of 5 nM, and its affinity for RBD was similar at 7 nM (Fig. 1a, Extended Data  
106 Fig. 1c, d, Extended Data Table 1). Its Fab displayed a 12-fold weaker binding to RBD  
107 compared to IgG (84 nM), with the difference mainly caused by a faster Fab off-rate (Fig. 1a,  
108 Extended Data Table 1), also observed in a different assay setting (Extended Data Fig. 1d).  
109 With an  $IC_{50}$  of 199 ng ml<sup>-1</sup>, the COVA1-18 Fab was 237-fold less potent at neutralizing SARS-  
110 CoV-2 pseudovirus, showing that the IgG avidity effect is important for COVA1-18  
111 neutralization potency (Extended Data Fig. 1a, Extended Data Table 1).

112

### 113 **COVA1-18 inhibits viral replication in rodents**

114 We sought to evaluate whether COVA1-18 could control SARS-CoV-2 viral infection in a  
115 previously described Ad5-hACE2 mouse model<sup>22,23</sup> using a 10 mg kg<sup>-1</sup> dose. COVA1-18  
116 administered intraperitoneally 24 h prior to, or after a SARS-CoV-2 challenge with 10<sup>4</sup> plaque  
117 forming units (PFU) was fully protective with no detectable viral replication in the lungs (Fig.  
118 1b, c). We then tested the efficacy of COVA1-18 in the golden Syrian hamster model (n = 5  
119 per group), which is naturally susceptible to SARS-CoV-2 and develop severe pneumonia upon  
120 infection<sup>24</sup>. We evaluated the effect on lung viral loads of 10 mg kg<sup>-1</sup> of COVA1-18 given 24  
121 h after a 10<sup>5</sup> PFU intranasal challenge (Fig. 1b, d). At 3 days post-infection (d.p.i.), 3/5 animals  
122 had high serum neutralization while 2/5 animals had low neutralization activity (Extended Data  
123 Fig. 1e). On day 3, the COVA1-18 treated group had significantly lower median lung viral  
124 titers compared to the control group (3.5 vs 6.7 log<sub>10</sub> PFU g<sup>-1</sup>, respectively,  $p < 0.01$ ) with lowest  
125 viral titers in the higher serum neutralizers (Fig. 1d).

126

### 127 **COVA1-18 PrEP prevents infection in NHP**

128 We evaluated the potential of COVA1-18 to prevent SARS-CoV-2 infection in cynomolgus  
129 macaques in a pre-exposure prophylaxis (PrEP) study. The animals were treated intravenously  
130 24 h prior to viral challenge with a dose of 10 mg kg<sup>-1</sup> of COVA1-18 (Fig. 2a). Treated and  
131 control animals (n = 5 per group) were challenged on day 0 with 10<sup>6</sup> PFU of SARS-CoV-2 via  
132 combined intranasal and intratracheal routes using an experimental protocol developed  
133 previously<sup>25,26</sup>. On the day of challenge, the mean COVA1-18 serum concentration was 109 ±  
134 2.7 µg ml<sup>-1</sup> (Fig. 2b, Extended Data Fig. 2a), and 4/5 animals had serum neutralization activity

135 while no neutralization activity was observed in the control group (Extended Data Fig. 2b-d).  
136 COVA-18 was also detected in all respiratory tract samples and rectal samples (Fig. 2c-e,  
137 Extended Data Fig. 3a-c), and represented on average 1.5% and 1.2% of the total IgG in heat-  
138 inactivated content in the nasopharyngeal and tracheal mucosae, respectively. These levels  
139 remained constant throughout the study period and similar levels were detected at 3 d.p.i. in  
140 bronchoalveolar lavages (BAL) and saliva (Fig. 2e-f). As SARS-CoV-2 can cause damage to  
141 non-respiratory organs, we performed a pharmacokinetic study on two additional macaques to  
142 characterize the COVA1-18 distribution within the first 24 h (Extended Data Fig. 3d-f).  
143 COVA1-18 was found in all organs studied, including the lungs, at concentrations of 4 to 22  
144 ng mg<sup>-1</sup> of tissue, except for the brain where concentrations were substantially lower (250 pg  
145 mg<sup>-1</sup> of tissue). Altogether, these data showed that COVA1-18 administered intravenously was  
146 rapidly and efficiently distributed to the natural sites of infection as well as to organs affected  
147 by COVID-19 pathology.

148

149 Following viral challenge, control animals showed similar genomic (g)RNA and subgenomic  
150 (sg)RNA levels and kinetics as previously described<sup>25,26</sup> with median peak viral loads (VL) of  
151 6.4 and 6.2 log<sub>10</sub> copies per ml at 1-2 d.p.i. in the nasopharyngeal and tracheal swabs,  
152 respectively (Fig. 3a and Extended Fig. 4a). Active viral replication, as assessed by sgRNA  
153 levels, peaked at 1-2 d.p.i. in nasopharyngeal and tracheal swabs with median values of 4.6 and  
154 4.0 log<sub>10</sub> copies per ml, respectively (Fig. 3b and Extended Fig. 4b). At 3 d.p.i., viral loads  
155 were detected in the BAL with a median value of 4.9 log<sub>10</sub> copies per ml of gRNA and 3.2 log<sub>10</sub>  
156 copies per ml of sgRNA, including 3 animals with no detectable sgRNA.

157

158 In comparison, treated animals had a reduction of 2.2 and 3.4 log<sub>10</sub> median gRNA VL in  
159 tracheal swabs on days 1 and 2 (both  $p < 0.01$  to controls), and had undetectable VL after day 4  
160 (Fig. 3a and Extended Fig. 4a). The difference was also evident in nasopharyngeal swabs, with  
161 treated animals having a reduction of 1.5 and 2.2 log<sub>10</sub> gRNA VL on days 1 and 2 (both  $p < 0.01$   
162 to controls). By day 4, 4/5 treated animals had undetectable gRNA in the nasopharyngeal swabs  
163 while one animal (MF7) remained positive with a low residual gRNA signal up to 7 d.p.i..  
164 COVA1-18 treatment dramatically hindered viral replication in the upper respiratory tract as  
165 evidenced by the absence of detectable sgRNA in the nasopharyngeal and tracheal swabs for  
166 all treated animals with the exception of animal (MF9) that showed a low signal at 1 d.p.i. only  
167 in the tracheal swabs (Fig. 3b and Extended Fig. 4b). Therefore, in the treated group, most  
168 upper respiratory tract gRNA VL likely represents the progressive elimination of the challenge

169 inoculum, and does not result from active replication. The gRNA and sgRNA loads in BAL  
170 were also lower in COVA1-18 recipients compared to controls but the difference did not reach  
171 statistical significance (Fig. 3a, b, Extended Fig. 4c). Overall, these results demonstrate that a  
172 10 mg kg<sup>-1</sup> dose of COVA1-18 PrEP dramatically reduced the acquisition and/or early spread  
173 of SARS-CoV-2 in the different respiratory compartments.

174

175 Analysis of lung lesions by chest computed tomography (CT) showed that all treated animals  
176 had few and small lung lesions as recorded by low CT scores at 3 d.p.i. while 2/5 controls  
177 showed mild pulmonary lesions characterized by non-extended ground-glass opacities (GGOs)  
178 with scores superior to 5, consistent with what was observed in historic controls (Fig. 3c)<sup>25</sup>. In  
179 addition, we observed that all control animals were lymphopenic at 2 d.p.i., consistent with  
180 previous studies<sup>25,26</sup>, while all treated animals had normal lymphocyte counts throughout the  
181 study ( $p < 0.01$  for the comparison) (Fig. 3d, Extended Fig. 4d).

182

183 One concern about SARS-CoV-2 vaccines and NAb treatments is the possible generation of  
184 suboptimal concentrations of NAb in individuals, which could foster viral escape<sup>27</sup>. The  
185 COVA1-18 treatment resulted in enrichment of subclonal variations in N and ORF1ab, but no  
186 treatment-induced escape mutations were detected in the *S* gene when applying standard  
187 quality filters (Extended Data Fig. 5).

188

### 189 **Prediction models refine COVA1-18 dosage**

190 Next, we used a viral dynamic model previously developed in the same SARS-CoV-2 NHP  
191 experimental model<sup>28</sup> to evaluate the level of protection conferred by COVA1-18. The model  
192 considers a target cell limited infection in both nasopharyngeal and tracheal compartments. In  
193 addition to the previously developed model, we assumed that sgRNA was a proxy for the total  
194 number of non-productively and productively infected cells (see supplementary methods) and  
195 we further assumed that COVA1-18 plasma drug concentrations over time, noted  $C(t)$ , was the  
196 driver of drug efficacy. We modeled the changes in  $C(t)$  using a standard first order absorption  
197 and elimination model, which led estimated half-life of COVA1-18 in plasma of 12.6 days  
198 (Extended Data Fig. 6b). We assumed that COVA1-18 reduces infectivity rate in both tracheal  
199 and nasopharyngeal compartments with an efficacy, noted  $\eta(t)$ , determined by the following  
200 model  $\eta(t) = \frac{C(t)}{C(t) + EC_{50}}$ , where  $EC_{50}$  is the plasma COVA1-18 concentrations corresponding to  
201 a 50% reduction of viral infectivity. The model fitted the viral kinetics well in all animals (Fig.

202 4a, Extended Data Fig. 6a, Extended Data Table 2). In treated animals,  $EC_{50}$  was estimated to  
203 2.2 and 0.053  $\mu\text{g ml}^{-1}$  in the nasopharynx and trachea, respectively, which is roughly 50 and  
204 2000 times lower than the plasma drug concentrations of 109.7  $\mu\text{g ml}^{-1}$  observed on the day of  
205 infection (see above). Thus these results confirm that the efficacy of COVA1-18 was very high,  
206 with efficacies above 95% and 99.9% in nasopharyngeal and tracheal compartments on the day  
207 of infection, respectively (Fig. 4a, Extended Data Fig. 6a). Given the long half-life of the drug,  
208 this efficacy could be maintained over time, and we estimated that the mean individual efficacy  
209 of the COVA1-18 in the first 10 days following infection ranged between 96.67% and 97.50%  
210 in the nasopharynx and between 99.91% and 99.94% in the trachea (Extended Data Table 3).

211

212 Next, we used our model to investigate changes in experimental conditions, such as COVA1-  
213 18 dose being administered at a lower dose and/or after the viral challenge (see methods). In  
214 all scenarios considered, a dose of 5  $\text{mg kg}^{-1}$  was determined to provide nearly similar results  
215 than 10  $\text{mg kg}^{-1}$  (Fig. 4b, c, Extended Data Fig. 7). A dose of 1  $\text{mg kg}^{-1}$  could be sufficient to  
216 prevent active viral replication as long as treatment is given prior to infection, but might be  
217 insufficient in a therapeutic setting. However, this dose could be relevant if lower doses of  
218 virus were used for infection, such as  $10^4$  or  $10^5$  PFU (Extended Data Fig. 6c-f).

219

## 220 Discussion

221 Despite the recent approval of several SARS-CoV-2 vaccines by health authorities, the slow  
222 roll-out of vaccination campaigns will not result in resolution of the pandemic in the immediate  
223 future. Furthermore, the emergence of viral escape mutants may lead to reduced vaccine  
224 efficacy, and some individuals, such as immunocompromised patients or the elderly, may not  
225 mount adequate protective immune responses to vaccination. Thus, there is an urgent need to  
226 develop effective therapeutics, in particular for individuals with high risk of severe disease.  
227 Pre-clinical and clinical studies to evaluate SARS-CoV-2 NAb for prophylaxis and/or  
228 treatment and such studies have supported the implementation of several NAb candidates and  
229 NAb cocktails for emergency use<sup>22,29-35</sup>. However, the narrow efficacy range of FDA-approved  
230 NAb<sup>4-6</sup>, together with rapidly spreading new variants complicate treatment strategies<sup>36-39</sup>,  
231 highlights the need for additional treatment options, including potent NAb such as COVA1-  
232 18.

233 In hACE2-expressing mice and golden Syrian hamsters, COVA1-18 showed remarkable  
234 control of SARS-CoV-2 infection. These promising results were confirmed in NHPs, with  
235 COVA1-18 given one day prior to infection achieving nearly complete protection in the upper  
236 respiratory tract in cynomolgus macaques. Using a viral dynamic model, we estimated that  
237 COVA1-18 reduced viral infectivity by >95% and 99.9% in nasopharyngeal and tracheal  
238 compartments, respectively. The robustness of these results are reinforced by the high  
239 challenge dose that we used, which was 10 to 100-fold higher than in other NHP studies  
240 evaluating NABs for PrEP against SARS-CoV-2<sup>29-34</sup>. In fact, the model allowed us to predict,  
241 without using additional animals, that protection could be achieved with lower doses of 5 mg  
242 kg<sup>-1</sup> and 1 mg kg<sup>-1</sup> with an inoculum dose of 10<sup>5</sup> or 10<sup>4</sup> PFU, both in prophylactic and  
243 therapeutic settings (Extended Data Fig. 6, Extended Data Fig. 7).

244

245 How do these levels of efficacy greater than 95% translate into clinical efficacy? In previous  
246 work, we estimated that achieving 90% efficacy would be sufficient to confer a high level of  
247 protection against infection acquisition if treatment can be administered prophylactically or  
248 just after a high-risk contact<sup>40</sup>. In hospitalized patients, where viral load kinetics after admission  
249 is associated with the risk of death, we estimated that administration of treatment with an  
250 efficacy higher than 90% could reduce the time to viral clearance by more than 3 days in  
251 patients over 65 years of age, which could translate into significantly lower rates of mortality  
252 in this population<sup>41</sup>. Altogether, the results obtained here in a NHP model suggest that COVA1-  
253 18 could be a valuable candidate for clinical evaluation.

254

255 A relevant concern is that these results may be jeopardized by the increasing prevalence of  
256 mutant strains, which could reduce the sensitivity to NABs. While escape mutations can arise  
257 following single NAb treatment as recently demonstrated<sup>31,42</sup>, COVA1-18 did not select for S  
258 protein escape mutants when evaluated as PrEP in NHP. Importantly, studies have determined  
259 that COVA1-18 retains high potency against the B.1.1.7 variant, which includes the N501Y  
260 mutation<sup>8,9</sup>. However, as it is derived from IGHV3-66, it will likely lose potency against  
261 variants harboring the E484K mutation (*i.e.* the B.1.351 and B.1.1.28 lineages), as recently  
262 shown for convalescent plasma and many NABs<sup>37,38</sup>. This highlights the necessity of using  
263 NABs cocktails targeting distinct epitopes. In addition, the half-life of COVA1-18 can be  
264 extended by incorporating the LS or YTE<sup>43</sup> mutations which can further reduce the protective  
265 dose required and reduce the cost of treatment.

266

267 In conclusion, our COVA1-18 *in vitro* data translated into a powerful protective drug in three  
268 preclinical models to prevent SARS-CoV-2 replication. Together with our prediction model,  
269 these data showed that COVA1-18 could be used in patients at low doses either to prevent  
270 infection or to reduce viral loads in a therapeutic setting, with a potential greater impact in  
271 high-risk patients. The high *in vivo* efficacy of COVA1-18 and its demonstrated potency  
272 against the B.1.1.7. isolate also suggests it is a great candidate for a NAb cocktail.

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364

## 365 **Methods**

### 366 **IgG, Fab and soluble viral protein expression**

367 COVA1-18 was isolated from a participant in the “COVID-19 Specific Antibodies” (COSCA)  
368 study as described<sup>7</sup>. The COSCA study was conducted at the Amsterdam University Medical  
369 Centre, location AMC, the Netherlands and approved by the local ethical committee of the  
370 AMC (NL 73281.018.20). COVA1-18 IgG was produced in HEK293F suspension cells as  
371 previously described<sup>7</sup>. COVA1-18 His-tagged Fab was produced in ExpiCHO cells as  
372 previously described<sup>44</sup>. Spike and RBD proteins were produced and purified as previously  
373 described<sup>7</sup>.

374

### 375 **Bio-layer interferometry**

376 The affinity of COVA1-18 IgG and His-tagged Fab versions were determined using Ni-NTA  
377 biosensors (ForteBio) onto which 20  $\mu\text{g ml}^{-1}$  of SARS-CoV-2 RBD in running buffer (PBS,  
378 0.02% Tween-20, 0.1% BSA) was loaded for 300 s as previously described<sup>44</sup>. The association  
379 rate and dissociation step were assessed over a 120 s step each. Serially diluted IgG (50, 100,  
380 200 and 400 nM) and Fab (100, 200, 400 and 800 nM) were tested and an anti-HIV-1 His-  
381 tagged Fab at 800 nM in running buffer was included as negative control.  $K_{\text{DS}}$  were determined  
382 using ForteBio Octet CFR software using a 1:2 fitting model for IgGs and a 1:1 fitting model  
383 for Fabs. The apparent affinity of COVA1-18 IgG to the SARS-CoV-2 S trimer was determined  
384 as described above except that 20  $\mu\text{g ml}^{-1}$  SARS-CoV-2 S 2P Fld His protein was loaded  
385 instead of RBD. The COVA1-18 IgG avidity effect was further evaluated by titrating the loaded  
386 SARS-CoV-2 RBD (5, 1, 0.2 and 0.04  $\mu\text{g ml}^{-1}$ ). An additional loading step using His-tagged  
387 HIV-1 gp41 was performed to minimize background binding of His-tagged Fabs to the  
388 biosensor and both the COVA1-18 IgG and Fab concentrations were set at 250 nM. All other  
389 steps were performed as described above.

390

### 391 **Ni-NTA-capture ELISA**

392 SARS-CoV-2, SARS-CoV, MERS, HKU1, 229E and NL63 S His-tagged proteins were loaded  
393 at 2  $\mu\text{g ml}^{-1}$  in TBS/2% skimmed milk (100  $\mu\text{l/well}$ ) on 96-well Ni-NTA plates (Qiagen) for 2  
394 h at room temperature (RT). Three-fold serially diluted COVA NAb were then added onto the  
395 plates for 2 h at RT followed by the addition goat anti-human IgG-HRP (Jackson  
396 Immunoresearch) secondary Ab (1:3000) for 1 h at RT. The plates were developed for 3 min

397 using TMB solution then stopped, optical densities measured at 450 nm on a spectrophotometer  
398 and data graphed using GraphPad Prism software (v8.3.0).

399

#### 400 **Detection of human IgG in NHP fluid**

401 Detection of COVA1-18 in NHP samples determined by ELISA using a protocol adapted from  
402 others<sup>30</sup>. Briefly, half area high binding 96-well plates (Greiner Bio-One) were coated  
403 overnight with goat anti-Human IgG H+L (monkey pre-adsorbed) at 1  $\mu\text{g ml}^{-1}$  in PBS. The  
404 plates were then blocked in casein buffer (Thermo Scientific) for 2 h at RT. Serum and mucosal  
405 samples were serially diluted and loaded onto the plates as well as serially diluted COVA1-18  
406 as the standard. Following a 1 h RT incubation, goat anti-Human IgG (monkey adsorbed)-HRP  
407 secondary antibody (Southern Biotech) was added for serum samples (1:4000). For mucosal  
408 samples, goat anti-Human IgG (monkey adsorbed)-BIOT (Southern Biotech) was added at  
409 1:10000 dilution. After 1 h RT incubation, serum sample plates were ready for development.  
410 For mucosal samples, an additional 1 h incubation with poly-HRP40 (Fitzgerald) (1:10000)  
411 was necessary. The plates were then developed for 5 min, the optical densities measured at 450  
412 nm on a spectrophotometer and raw data exported and analyzed using Microsoft Excel and  
413 GraphPad Prism (v8.3.0) software. The COVA1-18 concentration in a specific sample was  
414 determined by interpolating OD values from dilutions that fell into the linear range of the  
415 standard curve of the matching ELISA plate.

416

#### 417 **Cynomolgus monkey IgG ELISA**

418 Half area high binding 96-well plates were coated overnight (4 °C) with goat anti-Human IgG  
419  $\lambda$  and goat anti-Human IgG  $\kappa$  (Southern Biotech), 1:2000 (each) in PBS, 50  $\mu\text{l/well}$ . The plates  
420 were washed (1X TBS – 0,05% Tween20) and block for 2 h at RT with 50  $\mu\text{l/well}$  casein buffer.  
421 Serially diluted mucosal and serum samples were loaded onto the plates. Serially diluted  
422 polyclonal cynomolgus IgG (Molecular Innovations) was used as standard. Following a 1 h  
423 incubation at RT, mouse anti-Monkey IgG Fc-BIOT (Southern Biotech) was loaded onto the  
424 plate (1:50000). After 1 h at RT, poly-HRP40 was added (1:10000) and the plates incubated  
425 for 1 h. Finally, the plates were washed 5 times, developed for 5 min, and analysed as described  
426 above.

427

#### 428 **Pseudovirus neutralization assay**

429 Neutralization assays were performed using SARS-CoV-2 S-pseudotyped HIV-1 virus and  
430 HEK293T hACE2 cells as described previously<sup>15</sup>. In brief, pseudotyped virus was produced

431 by co-transfecting expression plasmids of SARS-CoV-2 $\Delta$ 19 S proteins (GenBank MT449663.1)  
432 with an HIV backbone expressing NanoLuc luciferase (pHIV-1<sub>NL4-3</sub>  $\Delta$ Env-NanoLuc) in  
433 HEK293T cells (ATCC, CRL-11268). After 2 days, the cell culture supernatants containing  
434 SARS-CoV-2 S-pseudotyped HIV-1 viruses were harvested and stored at -80 °C. HEK293T  
435 hACE2 cells were seeded 20,000 cells/well in a flat-bottom 96-well plates one day prior to the  
436 start of the neutralization assay. COVA1-18 IgG and His<sub>6</sub>-tagged Fab as well as heat-  
437 inactivated serum samples were serially diluted in 3-fold steps using cell culture medium and  
438 then mixed with pseudotyped virus in a 1:1 ratio and incubated for 1 h at 37 °C. The mixtures  
439 were then added to the HEK293T hACE2 cells in a 1:1 medium to mixture ratio. The final  
440 starting concentration for IgGs was 20  $\mu$ g ml<sup>-1</sup> and 13.33  $\mu$ g ml<sup>-1</sup> for Fab. The cells were then  
441 incubated at 37 °C for 48 h followed by one PBS wash and lysis buffer addition. The luciferase  
442 activity in the cell lysates was measured using the Nano-Glo Luciferase Assay System  
443 (Promega) and GloMax Discover microplate reader. Relative luminescence units (RLU) were  
444 normalized to those from positive control wells where cells were infected with SARS-CoV-2  
445 pseudovirus without IgG, Fab or serum. The inhibitory concentration (IC<sub>50</sub>) and neutralization  
446 titers (ID<sub>50</sub>) were determined as the IgG/Fab concentration or serum dilution at which  
447 infectivity was inhibited by 50%.

448  
449 Pseudotyped Vesicular Stomatitis Virus (VSV $\Delta$ G) particles displaying SARS-CoV-2 $\Delta$ 19 S and  
450 containing a luciferase reporter were used as previously described<sup>16</sup>. Two-fold dilution series  
451 of COVA1-18 were prepared in complete medium, pseudotyped virus added and the mixture  
452 incubated for 1 h at 37 °C. The virus-antibody mixtures were then loaded onto plates seeded  
453 with Vero E6 cells 24 h prior this step. Following a 20 h incubation 37 °C, the luciferase  
454 substrate was added to lysed cells and RLU determined and analyzed as described above.

455

#### 456 **Ethics and biosafety statement**

457 Female golden Syrian hamsters, aged 6-7 weeks, were housed in the ABSL-4 facility of the  
458 Galveston National Laboratory. The animal protocol # 2004049 was approved by the  
459 Institutional Animal Care and Use Committee (IACUC) of the University of Texas Medical  
460 Branch at Galveston (UTMB).

461

462 The mouse experimental study was approved by the Icahn School of Medicine at Mount Sinai  
463 Institutional Animal Care and Use Committee (IACUC-2017-0170 and IACUC-2017-0330).

464

465 Male and female cynomolgus macaques (*Macaca fascicularis*), aged 3-6 years and originating  
466 from Mauritian AAALAC certified breeding centers were used in this study. All animals were  
467 housed in IDMIT infrastructure facilities (CEA, Fontenay-aux-roses), under BSL-2 and BSL-  
468 3 containment when necessary (Animal facility authorization #D92-032-02, Préfecture des  
469 Hauts de Seine, France) and in compliance with European Directive 2010/63/EU, the French  
470 regulations and the Standards for Human Care and Use of Laboratory Animals, of the Office  
471 for Laboratory Animal Welfare (OLAW, assurance number #A5826-01, US). The protocols  
472 were approved by the institutional ethical committee “Comité d’Ethique en Expérimentation  
473 Animale du Commissariat à l’Energie Atomique et aux Energies Alternatives” (CEtEA #44)  
474 under statement number A20-011. The study was authorized by the “Research, Innovation and  
475 Education Ministry” under registration number APAFIS#24434-2020030216532863.

476

#### 477 **Ethics committee**

478 All information on the ethics committee is available at [https://cache.media.enseignementsup-  
479 recherche.gouv.fr/file/utilisation\\_des\\_animaux\\_fins\\_scientifiques/22/1/comiteethiqueea17\\_ju  
480 in2013\\_257221.pdf](https://cache.media.enseignementsup-recherche.gouv.fr/file/utilisation_des_animaux_fins_scientifiques/22/1/comiteethiqueea17_ju_in2013_257221.pdf)

481

#### 482 **Viruses and cells**

483 For the macaques studies, SARS-CoV-2 virus (hCoV-19/France/ IDF0372/2020 strain) was  
484 isolated by the National Reference Center for Respiratory Viruses (Institut Pasteur, Paris,  
485 France) as previously described<sup>45</sup> and produced by two passages on Vero E6 cells in DMEM  
486 (Dulbecco’s Modified Eagles Medium) without FBS, supplemented with 1% P/S (penicillin at  
487 10,000 U ml<sup>-1</sup> and streptomycin at 10,000 µg ml<sup>-1</sup>) and 1 µg ml<sup>-1</sup> TPCK-trypsin at 37 °C in a  
488 humidified CO<sub>2</sub> incubator and titrated on Vero E6 cells. Whole genome sequencing was  
489 performed as described<sup>45</sup> with no modifications observed compared with the initial specimen  
490 and sequences were deposited after assembly on the GISAID EpiCoV platform under accession  
491 number ID EPI\_ISL\_406596. Sequencing analysis revealed two clonal mutations, one in the *S*  
492 gene (22661G>T : V367F, non-synonymous) and one in the *ORF3a* gene (26144G>T : G251V,  
493 non-synonymous), which were already present in the challenge inoculum.

494

#### 495 **Animals and study design**

496 Seven week old female Balb/cJ mice (Jackson Laboratories Bar Harbor, ME) were anesthetized  
497 before being administered with 2.5 x 10<sup>8</sup> PFU of human adenovirus type 5 encoding the human  
498 angiotensin converting enzyme-2 receptor (Ad5-hACE2) 5-days prior to challenge with SARS-

499 CoV-2, as previously described<sup>22,23</sup>. Animals were transferred to the BSL-3 facility where two  
500 groups of n = 5 mice per group received 10 mg kg<sup>-1</sup> of COVA1-18 intraperitoneally 24 h prior  
501 to, or post-infection with 10<sup>4</sup> PFU SARS-CoV-2 in 50 µl PBS. A control group of n = 3 mice  
502 received 50 µl PBS. Mice were euthanized 3 d.p.i. and lungs harvested to quantify viral lung  
503 titers. Lungs were homogenized in PBS using a Beadblaster Microtube homogenizer  
504 (Benchmark Scientific). SARS-CoV-2 plaque assay was performed on 10-fold serial dilutions  
505 of lung homogenates prepared in 0.2% bovine serum albumin (BSA) in PBS that were plated  
506 onto a Vero E6 cells monolayer and incubated with shaking for 1 h. Inoculum was removed  
507 and plates were overlaid with Minimal Essential Media (MEM) containing 2% FBS/0.05%  
508 oxoid agar and incubated for 72 h at 37°C. Plates were fixed with 4% formaldehyde overnight,  
509 stained with a mAb cocktail composed of SARS-CoV-2 spike and SARS-CoV-2 nucleoprotein  
510 (Center for Therapeutic Antibody Discovery; NP1C7C7) followed by anti-Mouse IgG-HRP  
511 (Abcam ab6823) and developed using KPL TrueBlue peroxidase substrate (Seracare; 5510-  
512 0030).

513

514 Golden Syrian hamsters were randomly assigned to two groups of n = 5 and microchipped 24  
515 h before SARS-CoV-2 challenge. On the day of challenge, hamsters were anesthetized with  
516 ketamine/xylazine and challenged by the intranasal route with 10<sup>5</sup> PFU of SARS-CoV-2  
517 diluted in sterile PBS in the total volume 100 µl. Body weight and body temperature were  
518 measured each day, starting at day 0. Twenty four hours post-challenge, hamsters were treated  
519 with 10 mg kg<sup>-1</sup> of COVA1-18 diluted in 0.5 ml of sterile PBS via the intraperitoneal route.  
520 The control group of animals received an equal volume of sterile PBS via the intraperitoneal  
521 route. All animals were euthanized 72 h post-infection with an overdose of anesthetic  
522 (isoflurane or ketamine/xylazine) followed by bilateral thoracotomy, and terminal blood and  
523 lungs were collected at necropsy. Right lungs were frozen in 5 ml L-15 Leibowitz medium  
524 (Gibco) with 10% FBS. Tissue sections were homogenized in bead beater tubes, weighed, and  
525 supernatants were titrated per standard protocol. Briefly, of 10-fold dilutions of supernatants at  
526 100 µl per well were placed atop of Vero-E6 monolayers in 96-well plates, the plates were  
527 incubated for 1 h, supernatants were replaced by methyl cellulose overlay, incubated for 3 days  
528 at 5% CO<sub>2</sub> and 37 °C. The plates were fixed with formalin, removed from BSL-4 according the  
529 approved protocol, and plaques counted to determine the viral titers.

530

531 Ten female cynomolgus macaques were randomly assigned between the control and treated  
532 groups to evaluate the efficacy of COVA1-18 prophylaxis. The treated group (n = 5) received

533 one bolus dose of COVA-18 human IgG1 monoclonal antibody (10 mg kg<sup>-1</sup>) by the intravenous  
534 route in the saphenous vein one day prior to challenge, while control animals (n = 5) received  
535 no treatment. All animals were then exposed to a total dose of 10<sup>6</sup> PFU of SARS-CoV-2  
536 (BetaCoV/France/IDF/0372/2020; passaged twice in VeroE6 cells) *via* the combination of  
537 intranasal and intratracheal routes (day 0), using atropine (0.04 mg kg<sup>-1</sup>) for pre-medication  
538 and ketamine (5 mg kg<sup>-1</sup>) with medetomidine (0.05 mg kg<sup>-1</sup>) for anesthesia. Animals were  
539 observed daily and clinical exams were performed at baseline, daily for one week, and then  
540 twice weekly, on anaesthetized animals using ketamine (5 mg kg<sup>-1</sup>) and medetomidine (0.05  
541 mg kg<sup>-1</sup>). Body weight and rectal temperature were recorded and blood, as well as  
542 nasopharyngeal, tracheal and rectal swabs, were collected. Broncho-alveolar lavages (BAL)  
543 were performed using 50 ml sterile saline on 3 d.p.i. Chest CT was performed at 3 d.p.i. in  
544 anesthetized animals using tiletamine (4 mg kg<sup>-1</sup>) and zolazepam (4 mg kg<sup>-1</sup>). Blood cell counts,  
545 haemoglobin and haematocrit were determined from EDTA blood using a DHX800 analyzer  
546 (Beckman Coulter).

547

548 One male and one female cynomolgus macaques received the treatment as described above for  
549 the pharmacokinetic and pharmacodynamics (PK/PD) study. Blood was sampled before and 2,  
550 4, 6 and 24 h post-treatment. Saliva, nasopharyngeal and tracheal fluids were sampled before  
551 and 24 h post-treatment. Twenty-four hours post-treatment, animals were euthanized and their  
552 lungs, heart, kidney, liver, spleen, trachea and brain were sampled, rinsed with PBS and around  
553 100 mg of tissue was homogenized in 500 µl of PBS with a Precellys and stored at -80°C.

554

### 555 **Virus quantification in NHP samples**

556 Upper respiratory (nasopharyngeal and tracheal) and rectal specimens were collected with  
557 swabs (Viral Transport Medium, CDC, DSR-052-01). Tracheal swabs were performed by  
558 insertion of the swab above the tip of the epiglottis into the upper trachea at approximately 1.5  
559 cm of the epiglottis. All specimens were stored between 2°C and 8°C until analysis by RT-  
560 qPCR with a plasmid standard concentration range containing an RdRp gene fragment  
561 including the RdRp-IP4 RT-PCR target sequence. SARS-CoV-2 E gene subgenomic mRNA  
562 (sgRNA) levels were assessed by RT-qPCR using primers and probes previously described<sup>46,47</sup>:  
563 leader-specific primer sgLeadSARSCoV2-F CGATCTCTTG TAGATCTGTTCTC, E-  
564 Sarbeco-R primer ATATTGCAGCAGTACGCACACA and E-Sarbeco probe HEX-  
565 AACTAGCCATCCTTACTGCGCTTCG-BHQ1. The protocol describing the procedure for  
566 the detection of SARS-CoV-2 is available on the WHO website

567 ([https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-](https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2)  
568 [detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6\\_2](https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2)).

569

### 570 **Chest CT and image analysis**

571 Lung images were acquired using a computed tomography (CT) system (Vereos-Ingenuity,  
572 Philips) as previously described<sup>25,26</sup>. Lesions were defined as ground glass opacity, crazy-  
573 paving pattern, consolidation or pleural thickening as previously described<sup>32,48</sup>. Lesions and  
574 scoring were assessed in each lung lobe blindly and independently by two persons and the final  
575 results were established by consensus. Overall CT scores include the lesion type (scored from  
576 0 to 3) and lesion volume (scored from 0 to 4) summed for each lobe as previously  
577 described<sup>25,26</sup>.

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### 579 **Viral sequencing**

580 10 RNA samples from nasopharyngeal swabs at day 3 post-exposure were selected for  
581 sequencing along with the inoculum. cDNA and multiplex PCR reactions were prepared  
582 following the ARTIC SARS-CoV-2 sequencing protocol v2<sup>49</sup>. V3 primer scheme  
583 (<https://github.com/artic-network/primer-schemes/tree/master/nCoV-2019/V3>) was used to  
584 perform the multiplex PCR for SARS-CoV-2. All samples were run for 35 cycles in the two  
585 multiplex PCRs. Pooled and cleaned PCR reactions were quantified using Qubit<sup>TM</sup>  
586 fluorometer (Invitrogen). The Ligation Sequencing kit (SQK-LSK109; Oxford Nanopore  
587 Technologies) was used to prepare the library following the manufacturer's protocol ("PCR  
588 tiling of COVID-19 virus", release F; Oxford Nanopore Technologies). Twenty-four samples  
589 were multiplexed using Native Barcoding Expansion 1-12 and Native Barcoding Expansion  
590 13-24 kits (EXP-NBD104 and EXP-NBD114; Oxford Nanopore Technologies). Two libraries  
591 of 24 samples were prepared independently and quantified by Qubit<sup>TM</sup> fluorometer  
592 (Invitrogen). After the quality control, two R9.4 flowcells (FLO-MIN106; Oxford Nanopore  
593 Technologies) were primed as described in the manufacturer's protocol and loaded with 45 and  
594 32 ng of library. Sequencing was performed on a GridION (Oxford Nanopore Technologies)  
595 for 72h with high-accuracy Guppy basecalling (v3.2.10). After sequencing, demultiplexing was  
596 performed using Guppy v4.0.14 with the option `--require_barcodes_both_ends` to ensure high  
597 quality demultiplexing. Reads were then filtered by Nanoplot v1.28.1 based on length and  
598 quality to select high quality reads. Then, reads were aligned on the SARS-CoV-2 reference  
599 genome NC\_045512.2 using minimap2 v2.17. Primary alignments were filtered based on reads  
600 length alignment and reads identity. Reads were basecalled and demultiplexed with Guppy



601 4.0.14. The potential clonal and subclonal variants were detected with a custom pipeline based  
602 on ARTIC network workflow. Longshot v0.4.1 was used for variant detection. The potential  
603 subclonal variants were manually curated by comparing the generated VCF files and visual  
604 inspection of the alignments in IGV browser.

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#### 606 **Statistical analysis**

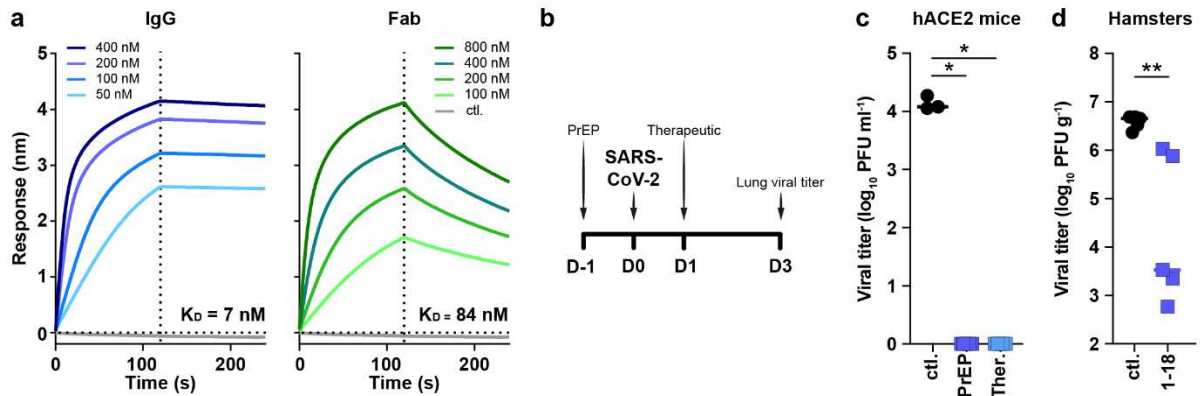
607 Statistical analysis of Syrian hamsters and hACE2 mice lung viral titers as well as for NHP  
608 gRNA and sgRNA were carried out using Mann-Whitney unpaired t-test in GraphPad Prism  
609 software (v8.3.0).

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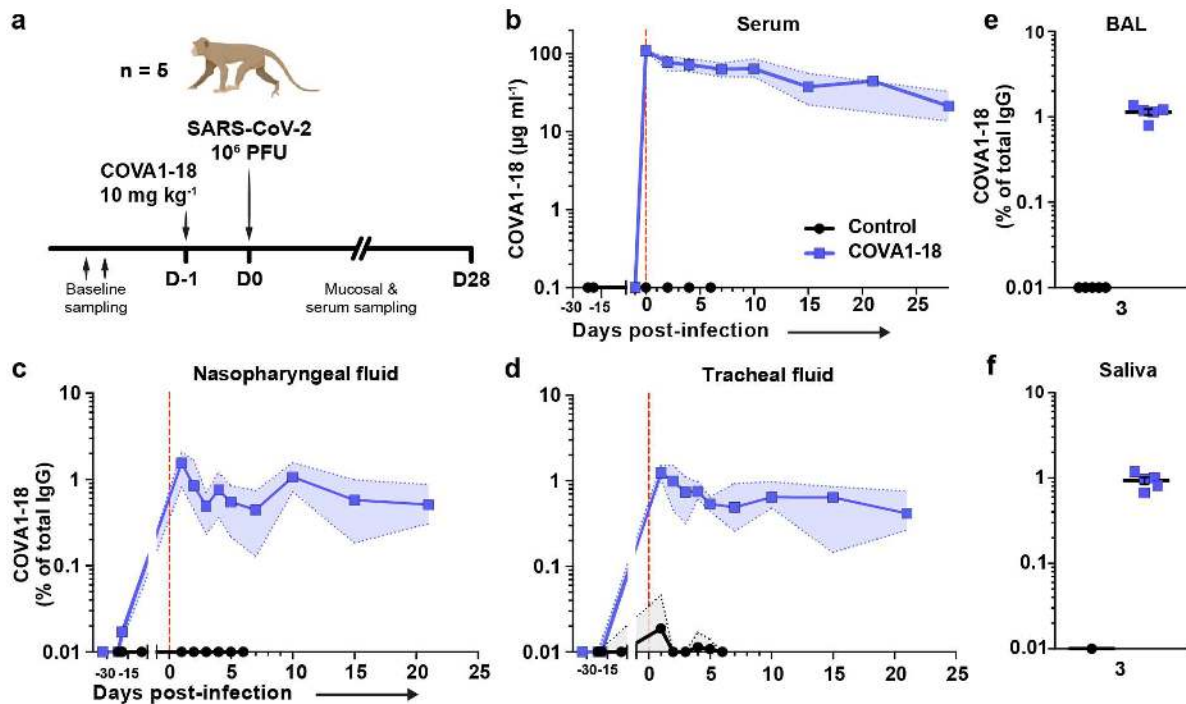
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**Figure 1. COVA1-18 avidity and SARS-CoV-2 protection in rodents.** (a) Bi-layer interferometry sensorgrams comparing COVA1-18 IgG and Fab binding to RBD.  $K_D$ s are indicated. Representative of 3 independent experiments. (b) Study design with  $n = 5$  per group, except mouse control group ( $n = 3$ ). Hamsters were infected with  $10^5$  PFU on day 0 and treated on day 1. Mice received COVA1-18 24 h prior to or after exposure to  $10^4$  PFU. Lung viral titers at 3 d.p.i. are shown for mice (c) and hamsters (d). Bars indicate medians. Mann-Whitney unpaired t-test,  $p$  values:  $* < 0.05$ ,  $** < 0.01$ . Ctl., control group; PrEP, pre-exposure prophylaxis; Ther., therapeutic.



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654 **Figure 2. COVA1-18 serum and mucosal pharmacokinetic in infected cynomolgus**

655 **macaques. (a)** Study design. Two groups of  $n = 5$  were exposed to  $10^6$  PFU of SARS-CoV-2

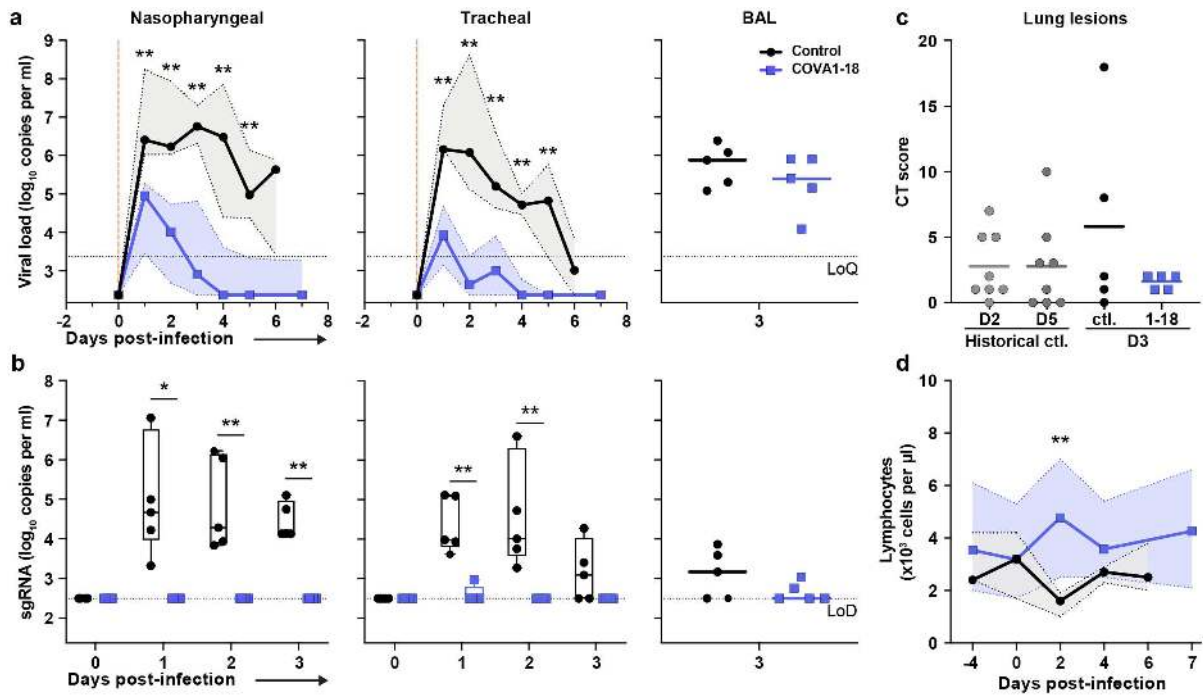
656 (intranasal and intratracheal routes). Treated animals received  $10 \text{ mg kg}^{-1}$  of COVA1-18 1 day

657 before challenge. **(b)** COVA1-18 serum concentration (mean with range). COVA1-18

658 concentration reported as percent of total cynomolgus IgG in heat-inactivated **(c)**

659 nasopharyngeal fluid, **(d)** tracheal fluid (means with range), **(e)** bronchoalveolar lavage (BAL)

and **(f)** saliva (means  $\pm$  SEMs). The red dashed line indicates challenge day.



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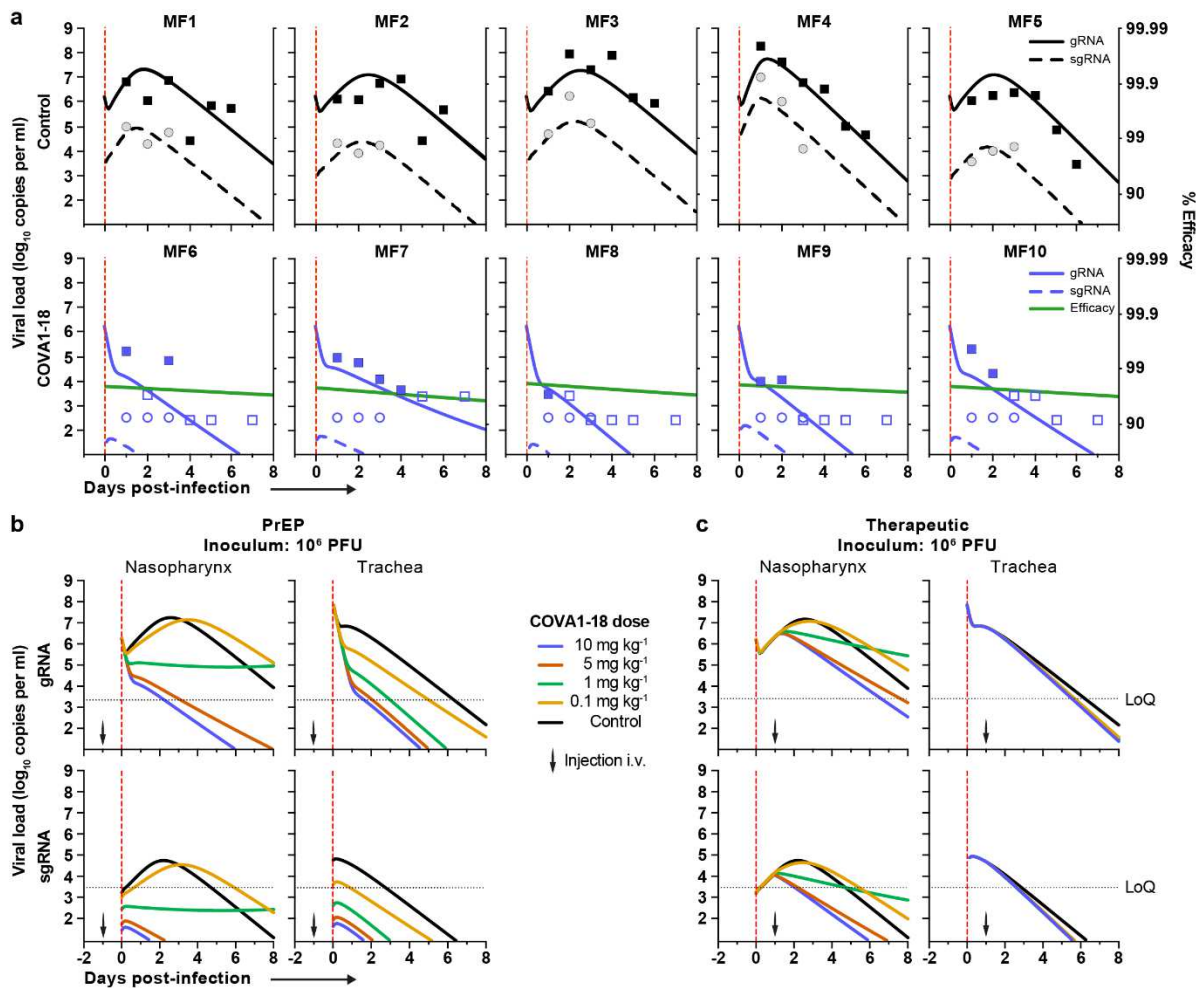
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**Figure 3. COVA1-18 pre-exposure prophylaxis protects cynomolgus monkeys against SARS-CoV-2 challenge and clinical symptoms.** (a) Genomic (g)RNA and (b) subgenomic (sg)RNA loads determined by PCR in nasopharyngeal fluids (left), tracheal fluids (middle) and bronchoalveolar lavages (BAL) (right). Medians with range are indicated for fluids and bars represent medians for BAL. (c) Chest CT scores were determined at 3 d.p.i. and at 2 or 5 d.p.i. for historical controls. (d) Absolute lymphocyte count in the blood (mean with range). Mann-Whitney unpaired t-test, *p* values: \* < 0.05, \*\* < 0.01. Ctl., control group; LoD, limit of detection; LoQ, limit of quantification.



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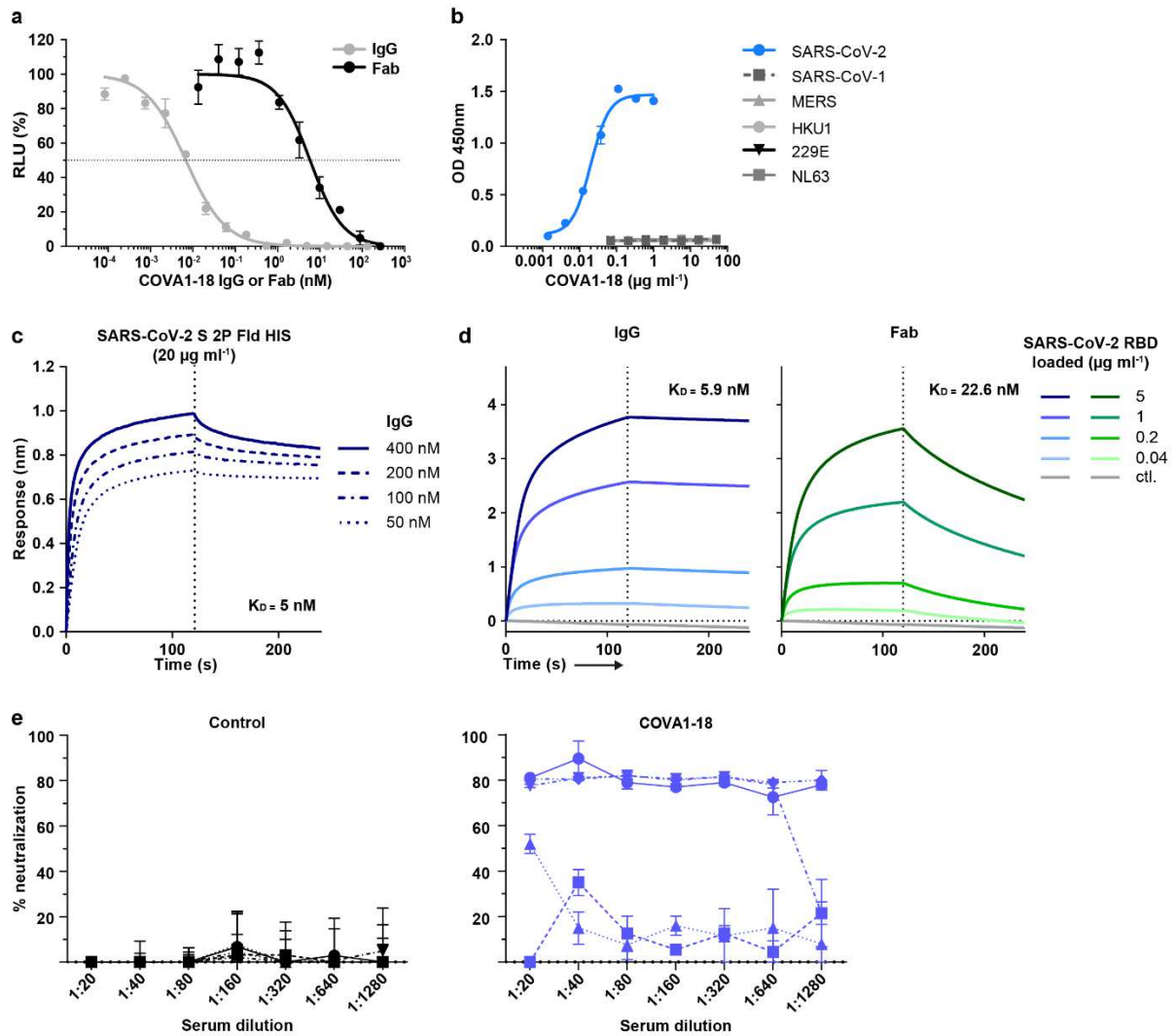
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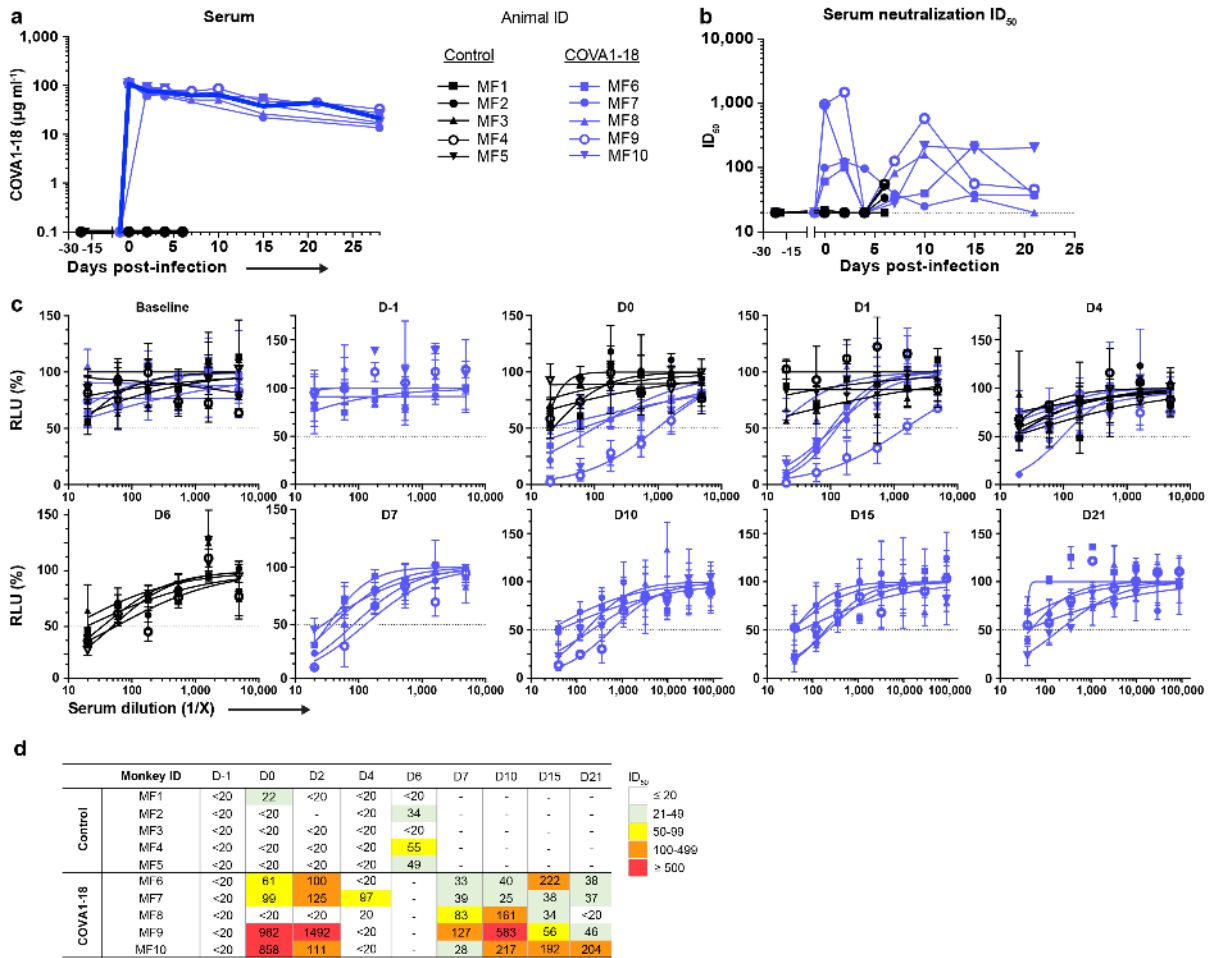
**Figure 4. Modeling of viral dynamics and treatment efficacy.** (a) Individual prediction of the nasopharyngeal gRNA and sgRNA in control (top) and treated animals (bottom) with individual efficacy prediction indicated (green line). The dashed red line indicates the time of infection. gRNA (squares) and sgRNA (circles) data are indicated as plain (above LoQ) or open (below LoQ). (b) Model predictions of gRNA and sgRNA dynamics with 4 doses of COVA1-18 given 24 h prior to challenge (arrow). (c) Simulation as in (b) with COVA1-18 given 24 h post-infection. Black dotted lines indicate LoQ (limit of quantification); i.v., intravenous; PFU, plaque forming units.



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**Extended Data Figure 1. COVA1-18 IgG and Fab neutralization, cross-reactivity, binding kinetic and Syrian hamster serum neutralization.** (a) IgG (grey) and Fab (black) pseudotype particle neutralization curves for COVA1-18. Representative of  $n \geq 4$  independent experiments. (b) Antigen specificity of COVA1-18 was assessed by ELISA against the soluble S protein derived from different human coronaviruses. (c) BLI sensorgrams of COVA1-18 binding to immobilized soluble SARS-CoV-2 S protein. Representative of  $n \geq 2$  independent experiments. (d) BLI sensorgrams of COVA1-18 binding to SARS-CoV-2 RBD loaded onto the sensor chip at various concentrations ( $n = 1$ ). (e) Serum neutralization potency at 3 d.p.i. in Syrian hamsters for the control group (left) and COVA1-18 treated group ( $n = 5$  animals per group).

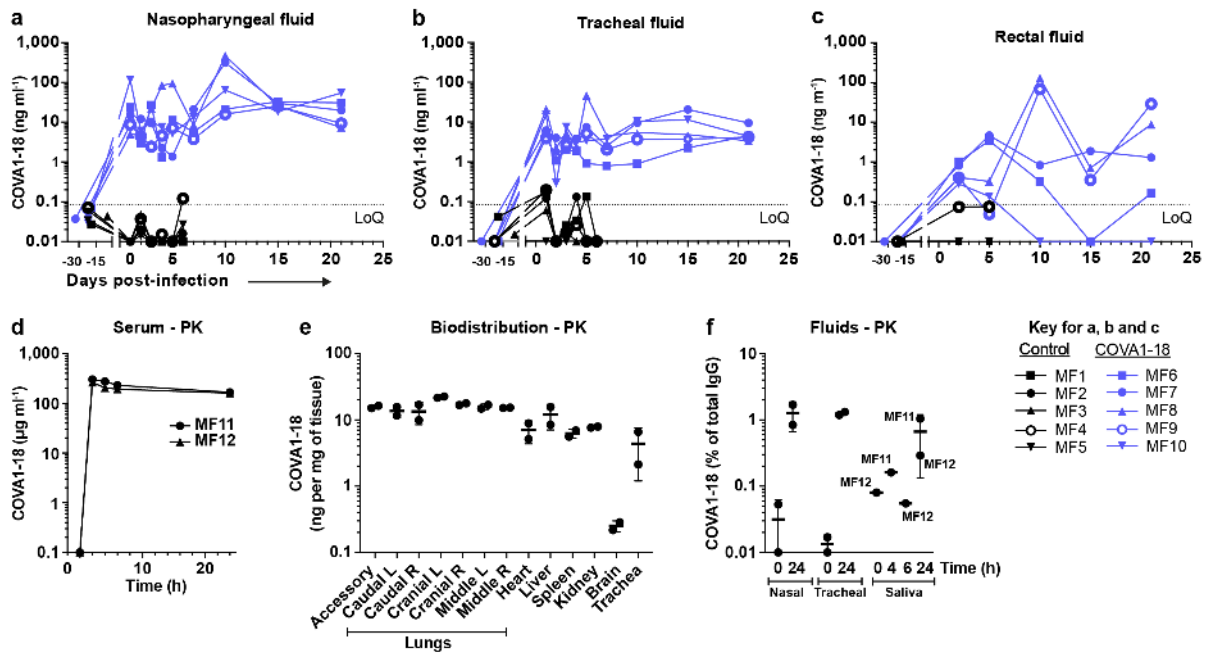




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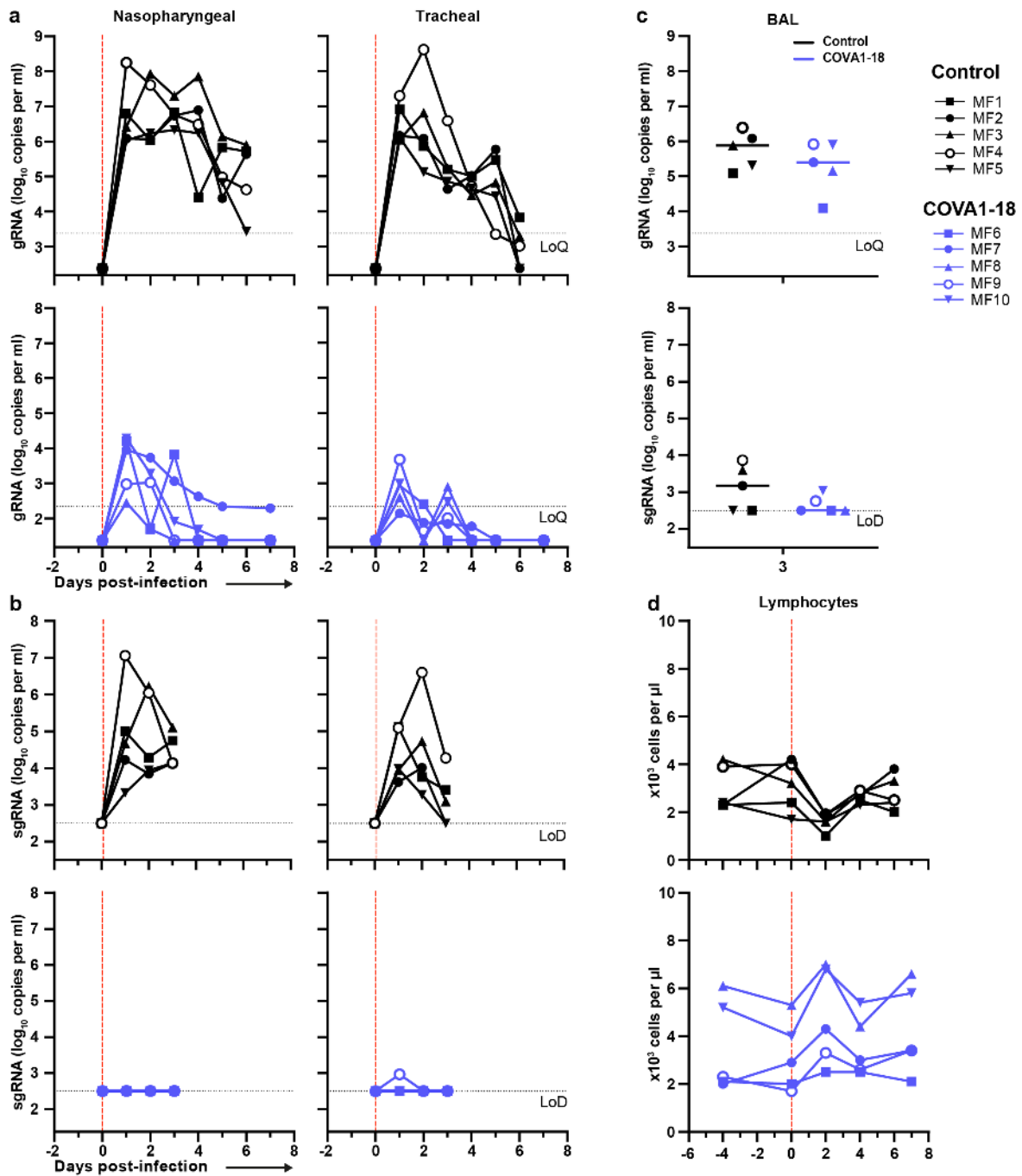
**Extended Data Figure 2. Serum and mucosal pharmacokinetics of COVA1-18 in treated macaques (1/2).** (a) Serum COVA1-18 concentration for each animal. The mean COVA-18 concentration for each group is indicated by a thick blue line (treated animals) and a thick black line (control). (b) Individual serum neutralization ID<sub>50</sub>. (c) Serum neutralization curve for each animal at the indicated day post-treatment. (d) Individual serum neutralization ID<sub>50</sub> with titer range indicated as ID<sub>50</sub> 21-49 in green, 50-99 in yellow, 100-499 in orange, >500 red.





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**Extended Data Figure 3. Serum and mucosal pharmacokinetics of COVA1-18 in treated macaques (2/2).** The COVA1-18 concentrations measured in nasopharyngeal (a), tracheal (b) and rectal (c) fluids by ELISA are reported for each animal in both groups. (d) Serum COVA1-18 concentration from two animals injected with  $10 \text{ mg kg}^{-1}$  of COVA1-18 and sampled at 0, 2, 4, 6 and 24 h for a pharmacokinetic (PK) study. (e) The two macaques were euthanized at 24 h post-treatment and organs analyzed to assess the biodistribution of COVA1-18. The concentration of COVA1-18 was normalized to the weight of each sample for every organ. (f) COVA1-18 was measured in fluid samples of the PK study animals and normalized to the total cynomolgus IgG content for each sample. LoQ, limit of quantification.



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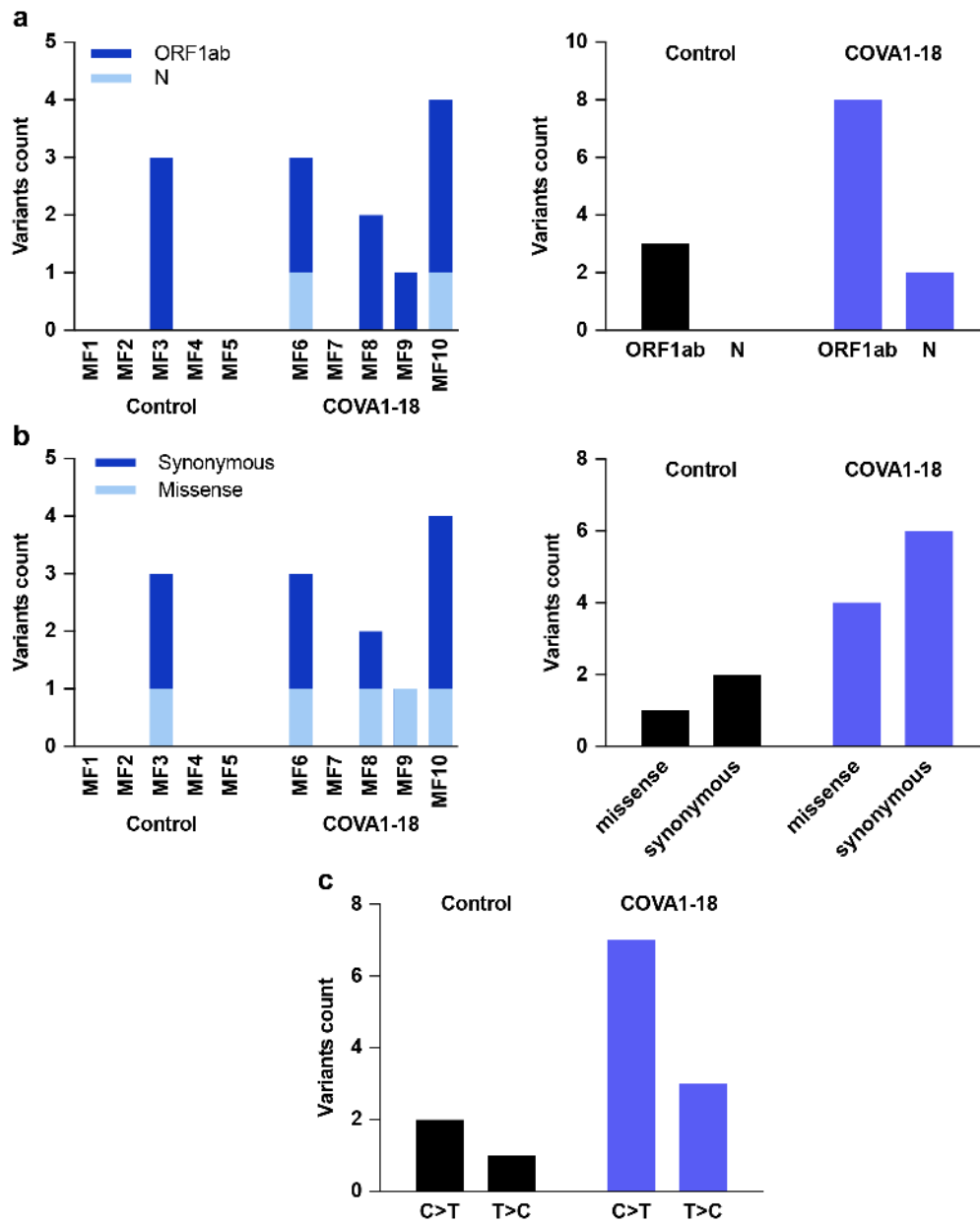
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**Extended Data Figure 4. COVA1-18 pre-exposure prophylaxis protects cynomolgus monkeys against SARS-CoV-2 challenge and clinical symptoms. (a)** Genomic (g)RNA and **(b)** subgenomic (sg)RNA loads determined by PCR in nasopharyngeal fluids (left) and tracheal fluids (right) of control (top) and treated (bottom) animals. **(c)** gRNA (top) and sgRNA (bottom) in the bronchoalveolar lavages (BAL) at day 3 post-infection. **(d)** Absolute lymphocyte count in the blood of control (top) and treated (bottom) animals. LoD, limit of detection; LoQ, limit of quantification.



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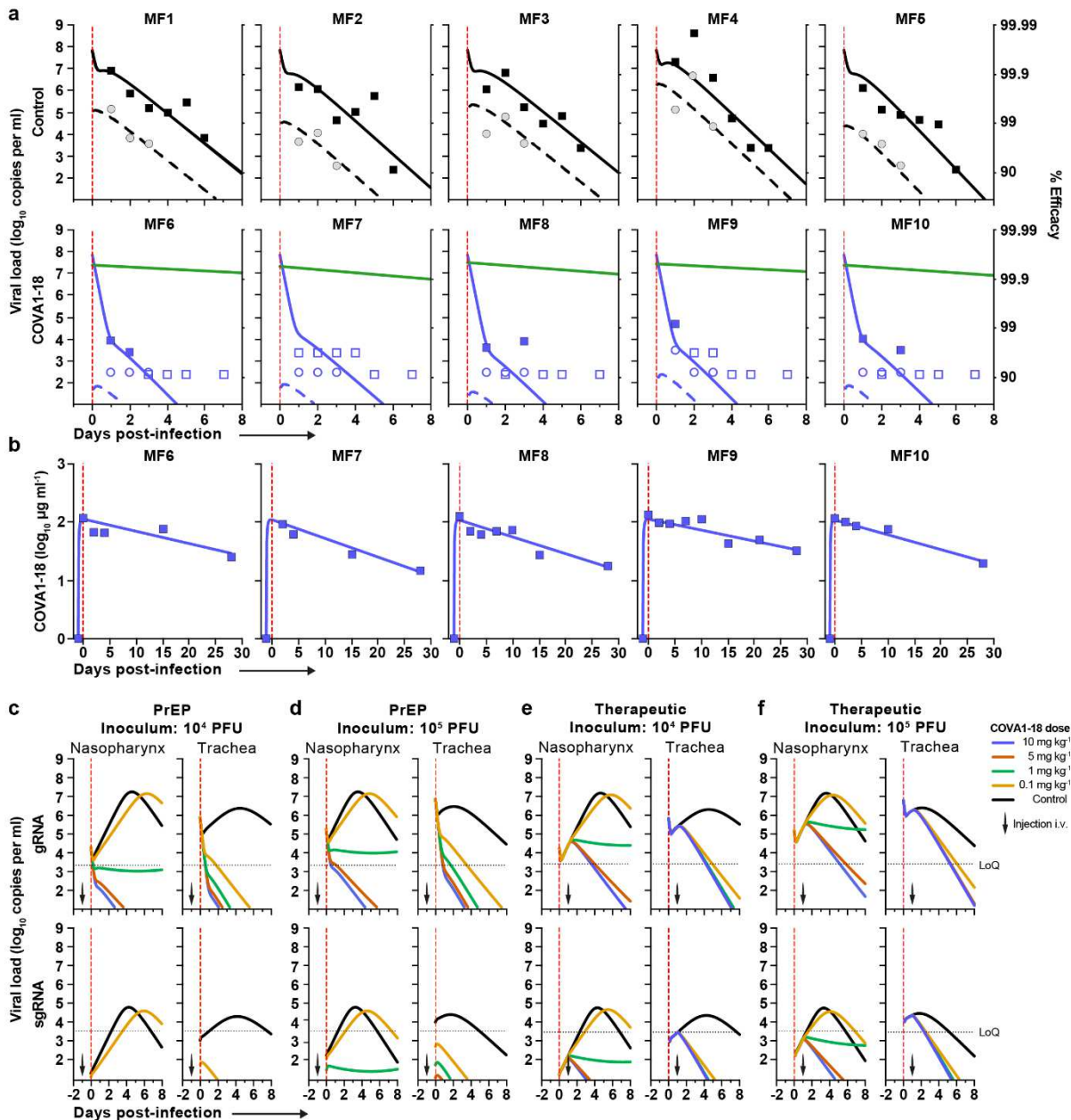
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**Extended Data Figure 5. Sequences in treated and exposed NHP.** Viral population sequences in the nasopharyngeal swabs at day 3 were analyzed by Next Generation Sequencing. **(a)** Variants count detected in the N and ORF1ab genes for each individual (left) and cumulative variants count for each gene in the control and COVA1-18 treated groups (right). **(b)** Individual (left) and cumulative (right) synonymous and missense variants count for the control and treated groups. **(c)** Nucleotide substitution observed by type for both groups.



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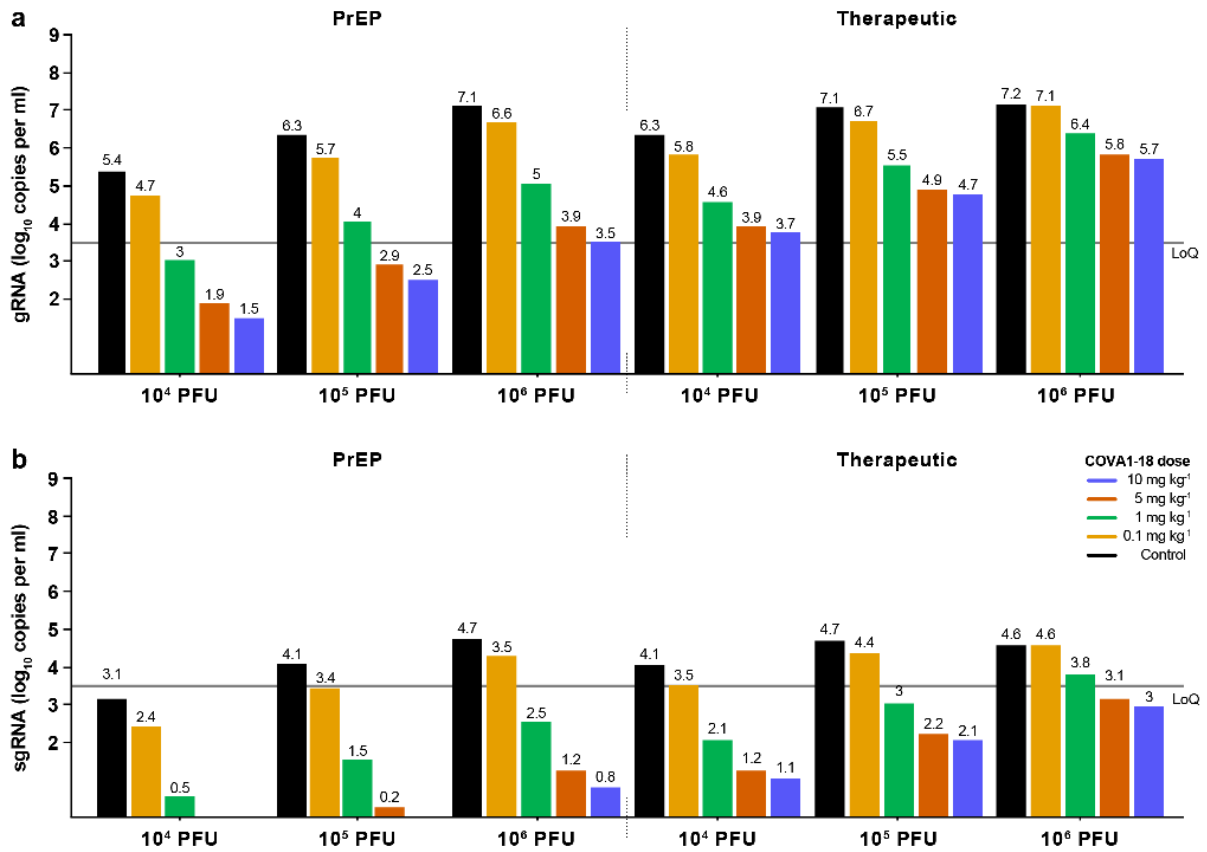
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**Extended Data Figure 6. Modeling of viral dynamics and treatment efficacy (1/2).** (a) Individual prediction of the tracheal gRNA and sgRNA in control (top) and treated animals (bottom) with individual efficacy prediction indicated (green line). The dashed red line indicates the time of viral infection. gRNA (squares) and sgRNA (circles) data are indicated as plain (above LoQ) or open (below LoQ). (b) Individual prediction of the COVA1-18 plasma concentration. (c-d) Simulation of the predicted gRNA (top) and sgRNA (bottom) viral loads in the nasopharynx and trachea for a  $10^4$  and  $10^5$  PFU challenge dose according to the dose of COVA1-18 given 24 h prior challenge (arrow). (e-f) Simulation as in (c) with COVA1-18 given 24 h post-infection. Black dotted lines indicate the limit of quantification (LoQ). i.v., intravenous; PFU, plaque forming units. PrEP, Pre-Exposure Prophylaxis.



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**Extended Data Figure 7. Modeling of viral dynamics and treatment efficacy (2/2).** Simulation of the predicted gRNA (top) and sgRNA (bottom) viral loads in the nasopharynx, according to the dose of COVA1-18 received and the dose of virus received. Left: Pre-Exposure Prophylaxis (PrEP) treatment at -1 d.p.i., viral load measured at 2 d.p.i.; Right: Therapeutic treatment at 1 d.p.i., viral load measured at 3 d.p.i. Black: control; yellow: 0.1 mg kg<sup>-1</sup>; green: 1 mg kg<sup>-1</sup>; orange: 5 mg kg<sup>-1</sup>; blue: 10 mg kg<sup>-1</sup>. LoQ, limit of quantification.

740 **Extended Data Table 1. BLI and neutralization potency of IgG vs Fab in HEK293T**  
 741 **hACE2 cells.** AMC and Duke neutralization assays use lentiviral pseudotyped particles and  
 742 HEK293T hACE2 cells. Nexelis neutralization assay uses VSVΔG pseudotyped particles and  
 743 Vero E6 cells. BLI, biolayer interferometry; RBD, receptor binding domain.

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		IC <sub>50</sub>				BLI						
		AMC (n ≥ 4)		Duke (n = 1)	Duke D614G (n = 1)	Nexelis (n = 1)	RBD loaded (n = 3)			Soluble S loaded (n = 3)		
		ng ml <sup>-1</sup>	pM	ng ml <sup>-1</sup>			K <sub>D</sub> (nM)	K <sub>a</sub> (M <sup>-1</sup> s <sup>-1</sup> )	K <sub>d</sub> (s <sup>-1</sup> )	K <sub>D</sub> (nM)	K <sub>a</sub> (M <sup>-1</sup> s <sup>-1</sup> )	K <sub>d</sub> (s <sup>-1</sup> )
1-18	IgG	0.8	5.6	9.0	7.0	9.0	7.0	1.7E+05	1.3E-03	5.0	3.7E+05	1.9E-03
	Fab	199.0	3968.0	N/A	N/A	N/A	84.1	5.0E+04	4.1E-03	N/A	N/A	N/A

745 **Extended Data Table 2. Parameter estimates of the viral dynamic model.** RSE: relative  
 746 standard error

Parameters	Unit	Description	Fixed effect (RSE%)	Sd of random effect (RSE%)
$\beta_N$	ml per copies per day	Virion infectivity	$2.14 \times 10^{-4}$	0.378
$\beta_T$	ml per copies per day		$1.68 \times 10^{-3}$	
$p_N$	copies d <sup>-1</sup>	Viral production	$3.32 \times 10^4$	0.552 (99.3)
$p_T$	copies d <sup>-1</sup>		$1.12 \times 10^4$	
$f$		Scaling factor for the subgenomic RNA	6.98 (66.7)	1.53 (36)
$EC_{50N}$	$\mu\text{g ml}^{-1}$	Concentration required to block infectivity by 50%	2.2	0.366 (133)
$EC_{50T}$	$\mu\text{g ml}^{-1}$		0.053	
$EC_{90N}$	$\mu\text{g ml}^{-1}$	Concentration required to block infectivity by 90%	19.8	
$EC_{90T}$	$\mu\text{g ml}^{-1}$		0.48	
$\delta$	d <sup>-1</sup>	Baseline clearance rate of productively infected cells	1.88	0.172
$ka$	d <sup>-1</sup>	Absorption rate	4.45	
$k$	d <sup>-1</sup>	Elimination rate	0.0549 (13.3)	0.225 (44.5)
$V$	ml kg <sup>-1</sup>	Volume of distribution	88.7 (6.56)	
$D$	mg kg <sup>-1</sup>	Administered dose of COVA1-18	10	

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748 **Extended Data Table 3. Mean individual efficacy of the COVA1-18 for each individual in**  
749 **both compartments (calculated over the first 10 days of administration).**  
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		MF6	MF7	MF8	MF9	MF10
Mean efficacy (%)	Nasopharynx	97.34	96.67	97.37	97.50	97.16
	Trachea	99.93	99.91	99.93	99.94	99.93

751



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797

798 **Author contributions:**

799 P.M. and Y.A. conceived, designed, performed experiments, analysed data, managed the  
800 project and wrote the manuscript (original draft). A.M. conceived and developed the predictive  
801 model, and wrote the manuscript. R.M., N.D.B. performed, supervised and analysed macaque  
802 experiments. N.A.K. designed, performed and analysed the hamster experiment. M.S.  
803 designed, performed and analysed the mouse experiment. A.W.F. performed the mouse  
804 experiment. J.L.S. produced antibodies and performed ELISAs. A.G. contributed to the  
805 predictive model development. J.A.B., M.P., C.M., M.O.T., N.S.A. and L.G. performed  
806 neutralization assays. V.Ch., S.D. and A.I. performed sequencing, analysed and interpreted the  
807 data. A.J.R. analysed the hamster histology data. S.J. and R.R. performed the mouse  
808 experiment. T.G.C., P.J.M.B., T.P.L.B., J.v.S., M.B., M.J.v.B., H.L. and M.Y. produced  
809 proteins. C.E.M. contributed to the hamster experiment. V.Co. contributed to performing and  
810 supervising macaque studies. T.N. and J.L. contributed to the macaque experiments and  
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820 manuscript.

821

822 **Competing interests:**

823 Amsterdam UMC filed a patent application on SARS-CoV-2 monoclonal antibody COVA1-  
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829

830 **Additional information:**

831 Supplementary information is available for this paper.

832 The data that support the findings of this study are available from the corresponding author  
833 upon reasonable request.

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# Figures

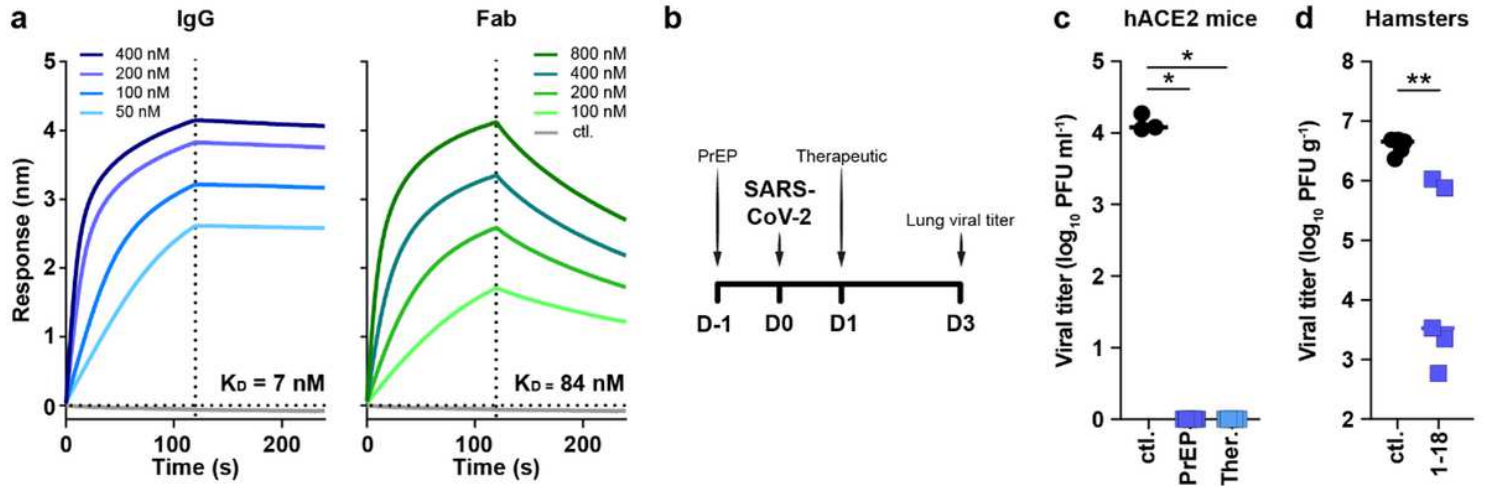
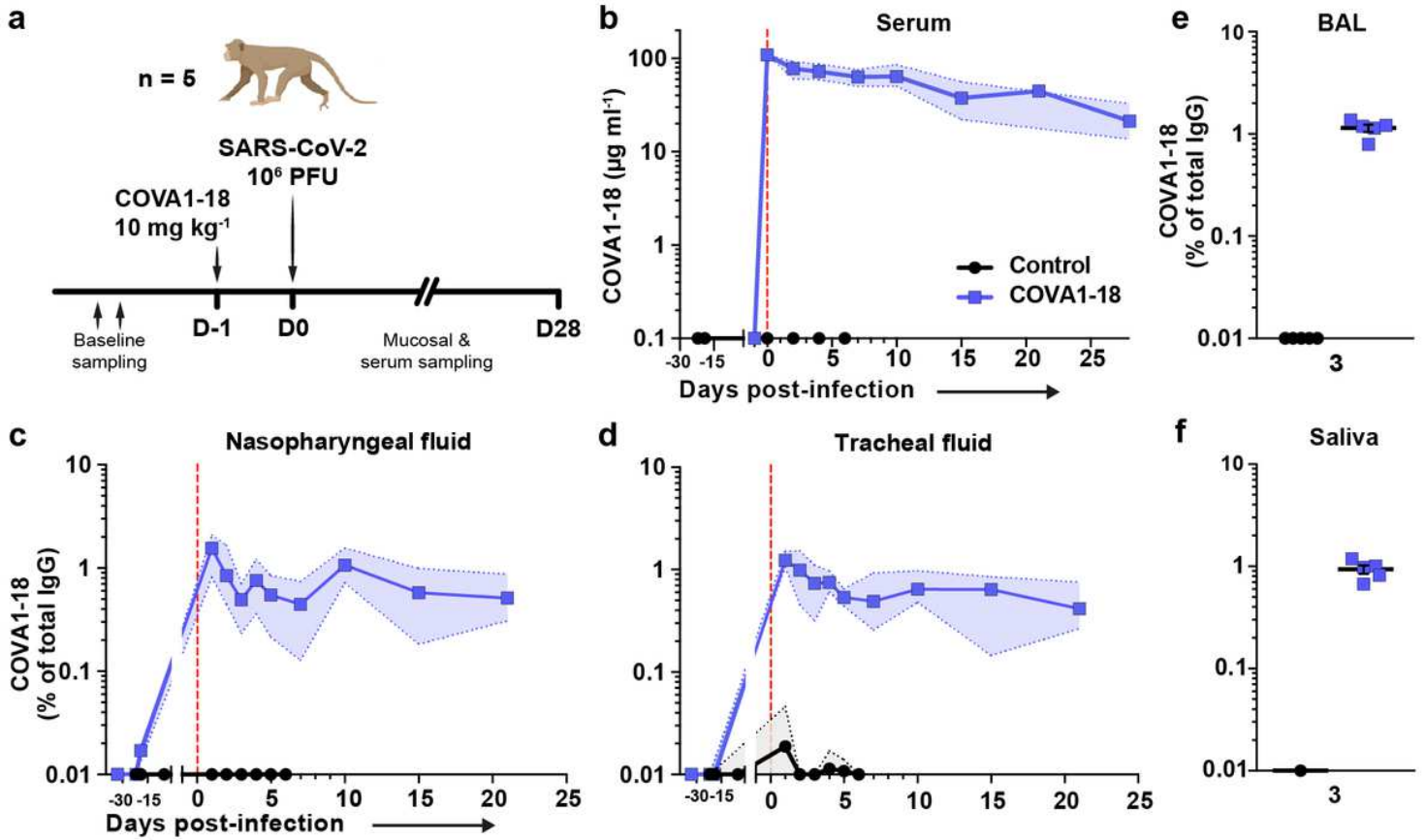


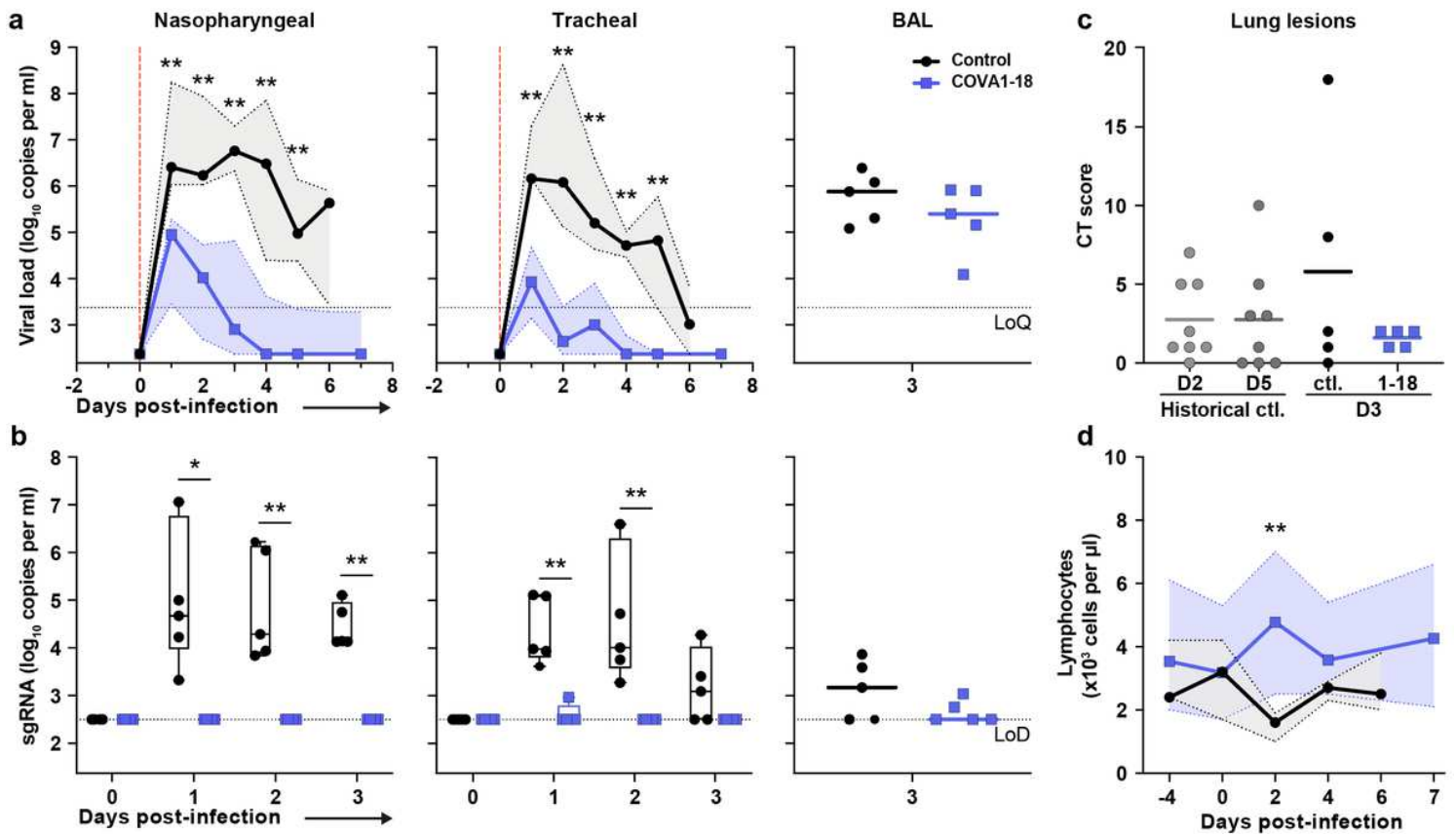
Figure 1

COVA1-18 avidity and SARS-CoV-2 protection in rodents. (a) Bi-layer interferometry sensorgrams comparing COVA1-18 IgG and Fab binding to RBD.  $K_D$ s are indicated. Representative of 3 independent experiments. (b) Study design with  $n = 5$  per group, except mouse control group ( $n = 3$ ). Hamsters were infected with 105 PFU on day 0 and treated on day 1. Mice received COVA1-18 24 h prior to or after exposure to 104 PFU. Lung viral titers at 3 d.p.i. are shown for mice (c) and hamsters (d). Bars indicate medians. Mann-Whitney unpaired t-test, p values: \* $<0.05$ , \*\* $<0.01$ . Ctl., control group; PrEP, pre-exposure prophylaxis; Ther., therapeutic.



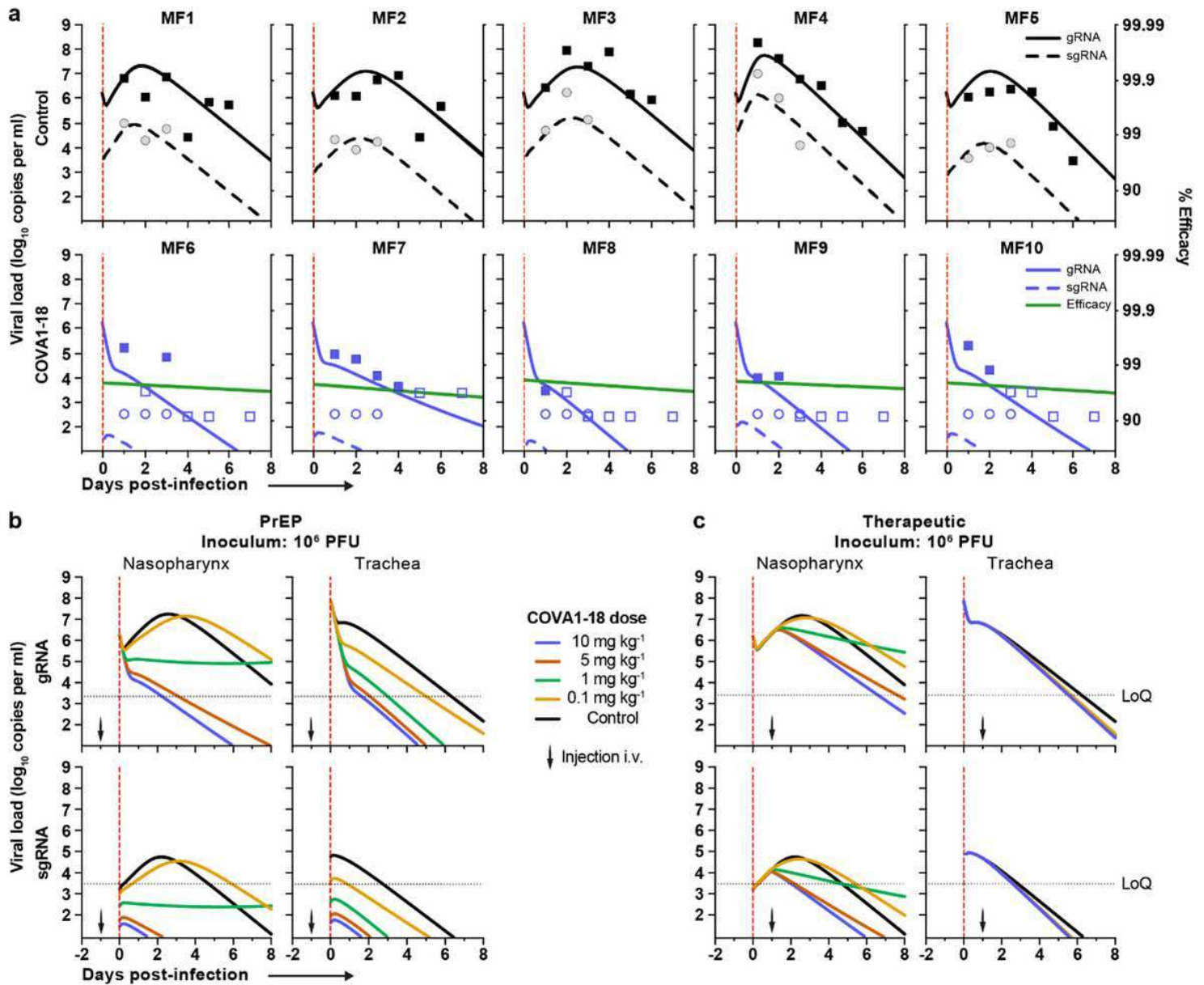
**Figure 2**

COVA1-18 serum and mucosal pharmacokinetic in infected cynomolgus macaques. (a) Study design. Two groups of n = 5 were exposed to 10<sup>6</sup> PFU of SARS-CoV-2 (intranasal and intratracheal routes). Treated animals received 10 mg kg<sup>-1</sup> of COVA1-18 1 day before challenge. (b) COVA1-18 serum concentration (mean with range). COVA1-18 concentration reported as percent of total cynomolgus IgG in heat-inactivated (c) nasopharyngeal fluid, (d) tracheal fluid (means with range), (e) bronchoalveolar lavage (BAL) and (f) saliva (means ± SEMs). The red dashed line indicates challenge day.



**Figure 3**

COVA1-18 pre-exposure prophylaxis protects cynomolgus monkeys against SARS-CoV-2 challenge and clinical symptoms. (a) Genomic (g)RNA and (b) subgenomic (sg)RNA loads determined by PCR in nasopharyngeal fluids (left), tracheal fluids (middle) and bronchoalveolar lavages (BAL) (right). Medians with range are indicated for fluids and bars represent medians for BAL. (c) Chest CT scores were determined at 3 d.p.i. and at 2 or 5 d.p.i for historical controls. (d) Absolute lymphocyte count in the blood (mean with range). Mann-Whitney unpaired t-test, p values: \* < 0.05, \*\* < 0.01. Ctl., control group; LoD, limit of detection; LoQ, limit of quantification.



**Figure 4**

Modeling of viral dynamics and treatment efficacy. (a) Individual prediction of the nasopharyngeal gRNA and sgRNA in control (top) and treated animals (bottom) with individual efficacy prediction indicated (green line). The dashed red line indicates the time of infection. gRNA (squares) and sgRNA (circles) data are indicated as plain (above LoQ) or open (below LoQ). (b) Model predictions of gRNA and sgRNA dynamics with 4 doses of COVA1-18 given 24 h prior to challenge (arrow). (c) Simulation as in (b) with COVA1-18 given 24 h post-infection. Black dotted lines indicate LoQ (limit of quantification); i.v., intravenous; PFU, plaque forming units.

## Supplementary Files

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