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## **REGN-COV2 antibody cocktail prevents and treats SARS-CoV-2 infection in rhesus macaques and hamsters.**

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### **Abstract**

An urgent global quest for effective therapies to prevent and treat COVID-19 disease is ongoing. We previously described REGN-COV2, a cocktail of two potent neutralizing antibodies (REGN10987+REGN10933) targeting non-overlapping epitopes on the SARS-CoV-2 spike protein. In this report, we evaluate the in vivo efficacy of this antibody cocktail in both rhesus macaques and golden hamsters and demonstrate that REGN-COV-2 can greatly reduce virus load in lower and upper airway and decrease virus induced pathological sequelae when administered prophylactically or therapeutically. Our results provide evidence of the therapeutic potential of this antibody cocktail.

## 38 **Introduction**

39 Fully human monoclonal antibodies are a promising class of therapeutics against SARS-CoV-2  
40 infection (Cohen, 2020). To date, multiple studies have described discovery and characterization  
41 of potent neutralizing monoclonal antibodies targeting the spike glycoprotein of SARS-CoV-2  
42 (Baum et al., 2020; Cao et al., 2020; Hansen et al., 2020; Ju et al., 2020; Liu et al., 2020; Pinto et  
43 al., 2020; Robbiani et al., 2020; Wang et al., 2020; Zost et al., 2020). However, evaluation of the  
44 efficacy of these antibodies in vivo is only beginning to emerge, and has largely focused on the  
45 prophylactic setting (Liu et al., 2020; Shi et al., 2020; Zost et al., 2020). Furthermore, as the animal  
46 models of SARS-CoV-2 infection and COVID-19 disease are still being developed, no single  
47 model has emerged as being more relevant for human disease. Indeed, based on the extremely  
48 diverse manifestations of COVID-19 in humans, multiple animal models may be needed to mimic  
49 various settings of human infection. The rhesus macaque model is widely used to assess efficacy  
50 of therapeutics and vaccines and displays a transient and mild course of the disease (Chandrashekar  
51 et al., 2020; Corbett et al., 2020; Deng et al., 2020; Mercado et al., 2020; Munster et al., 2020;  
52 Shan et al., 2020; van Doremalen et al., 2020; Yu et al., 2020). On the contrary, the golden hamster  
53 model manifests a much more severe form of the disease, accompanied by rapid weight loss and  
54 severe lung pathology (Imai et al., 2020; Rogers et al., 2020; Sia et al., 2020).

55  
56 We previously described a cocktail of two fully human antibodies, REGN10933 and REGN10987,  
57 that bind to spike protein, potently neutralize SARS-CoV-2 and were selected as components of  
58 anti-viral antibody cocktail (REGN-COV2) to safeguard against mutational virus escape (Baum et  
59 al., 2020; Hansen et al., 2020). In this study, we utilized two different animal models, rhesus  
60 macaque and golden hamster, that capture the diverse pathology of SARS-CoV-2 infection and  
61 evaluated the in vivo efficacy of this antibody cocktail when used prophylactically or  
62 therapeutically. This assessment allows us to compare performance of the antibodies in diverse  
63 disease settings to more comprehensively understand the mechanisms by which monoclonal  
64 antibody therapies may limit viral load and pathology in infected individuals.

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## 67 **Results**

68 To evaluate the ability of REGN-COV2 to protect rhesus macaques from SARS-CoV-2 infection  
69 we initially assessed the impact of antibody administration prior to virus challenge (NHP Study  
70 #1). Animals were dosed with 50mg/kg of REGN-COV2 (25mg/kg of each antibody) through  
71 intravenous administration and challenged with  $1 \times 10^5$  PFU of virus through intranasal and  
72 intratracheal routes 3 days post mAb dosing. Due to the relatively transient nature of the SARS-  
73 CoV-2 infection in rhesus macaques, the in-life portion of the study was limited to 5 days. To  
74 determine the impact of mAb prophylaxis on viral load in upper and lower airways we collected  
75 nasopharyngeal swabs on a daily basis and bronchoalveolar lavage (BAL) fluid on days 1, 3, and  
76 5 post-challenge (Figure 1A). Both genomic and subgenomic RNA were measured to assess the  
77 impact of mAb prophylaxis on the dynamics of viral replication; while genomic RNA (gRNA)

78 may reflect remaining viral inoculum as well as newly replicating virus, subgenomic RNA  
79 (sgRNA) should only result from newly replicating virus. For placebo-treated animals, the kinetics  
80 of viral load measures was as previously reported, with peak in viral load on day 2 post-challenge,  
81 although the majority of animals were still positive for viral RNA in nasal swabs on day 5; while  
82 the kinetics of gRNA and sgRNA were similar, sgRNA levels were about a hundred-fold lower,  
83 consistent with what others have reported (Chandrashekar et al., 2020; Mercado et al., 2020; Yu  
84 et al., 2020; Zost et al., 2020). For animals receiving REGN-COV2 prophylaxis we observed  
85 markedly accelerated clearance of gRNA with almost complete ablation of sgRNA in the majority  
86 of the animals, showing that REGN-COV2 can almost completely block establishment of virus  
87 infection; this pattern was observed across all measurements in both nasopharyngeal swabs and  
88 BAL compared to placebo animals, demonstrating that mAbs administered prophylactically can  
89 greatly reduce viral load in both upper and lower airways (Figure 1B).

90  
91 A second prophylaxis study (NHP Study #2) was designed to test whether REGN-COV2 could  
92 protect against a 10-fold higher viral inoculum ( $1.05 \times 10^6$  PFU), and compared the 50mg/kg dose  
93 of REGN-COV2 (25mg/kg of each antibody) with a much lower dose (Figure 2A).  
94 Nasopharyngeal and oral swabs were collected and used to measure virus genomic and  
95 subgenomic virus RNA. We observed that 50mg/kg of REGN-COV2 administered 3 days prior to  
96 virus challenge was once again able to minimize virus replication even when animals were  
97 challenged with this 10-fold higher viral challenge (Figure 2B), while the prophylactic effect was  
98 greatly diminished with the 0.3mg/kg dose. Interestingly, in this study we observed increased  
99 impact of mAb treatment on viral load in oral swabs versus nasopharyngeal swabs, potentially  
100 indicating that mAb treatment may impact multiple physiological sources of virus replication  
101 differentially. Additional studies in animal models and humans will be needed to assess whether  
102 this is really the case.

103  
104 Next, we assessed the impact of REGN-COV2 in the treatment setting by dosing animals  
105 challenged with the higher  $1 \times 10^6$  PFU of SARS-CoV-2 virus at 1-day post-infection with  
106 25mg/kg or 150mg/kg of the antibody cocktail (Figure 2A). By day 1 post-challenge the animals  
107 already reached peak viral load as measured by both genomic and subgenomic RNA, mimicking  
108 a likely early treatment clinical scenario of COVID-19 disease, since it has been shown that most  
109 SARS-CoV-2 infected individuals reach peak viral loads relatively early in the disease course and  
110 often prior or just at start of symptom onset (He et al., 2020; Zou et al., 2020). Compared to placebo  
111 treated animals, REGN-COV2 treated animals displayed accelerated viral clearance in both  
112 nasopharyngeal and oral swabs samples, including both genomic and subgenomic RNA samples  
113 (Figure 2C), clearly demonstrating that the monoclonal antibody cocktail can impact virus load  
114 even when administered post infection. Similar to the prophylaxis study, the decrease in viral load  
115 appeared more dramatic in oral swabs versus nasopharyngeal swabs. Both treatment groups  
116 displayed similar kinetics of virus clearance, suggesting that 25mg/kg and 150mg/kg demonstrate  
117 similar efficacy in this study. The treated animals in the 150mg/kg group displayed approximately

118 10-fold higher titers on day 1, at the time of mAb administration, therefore potentially masking  
119 enhanced effect of a higher drug dose. Similar impact of mAb treatment was observed on genomic  
120 and subgenomic RNA for both NP and oral samples, indicating the mAb treatment is directly  
121 limiting viral replication in these animals (Figure 2C).

122

123 The two antibody components of REGN-COV2 were selected to target non-overlapping sites on  
124 the spike protein to prevent selection of escape mutants, which were readily detectable with single  
125 mAb treatment (Baum et al., 2020). To assess whether any signs of putative escape mutants are  
126 observed in an in vivo setting with authentic SARS-CoV-2 virus, we performed RNAseq analysis  
127 on all RNA samples obtained from all animals from the study. Analysis of the spike protein  
128 sequence identified mutations in NHP samples that were not present in the inoculum virus (Figure  
129 S1) further indicating that the virus is actively replicating in these animals. However, we did not  
130 observe any mutations that were unique to treated animals; all identified mutations were either  
131 present in the inoculum or in both treated and placebo animals, indicating that they were likely  
132 selected as part of virus replication in NHPs and were not selected by mAb treatment.

133

134 We next performed pathology analyses of lungs of infected animals. All four placebo monkeys  
135 showed evidence of lung injury characterized in three monkeys by interstitial pneumonia (Figure  
136 2D), with minimal to mild infiltration of mononuclear cells (lymphocytes and macrophages) in the  
137 septa, perivascular space, and/or pleura. In these three animals, the distribution of lesions was  
138 multifocal and involved 2-3 of the 4 lung lobes. Accompanying these changes were alveolar  
139 infiltration of lymphocytes, increased alveolar macrophages, and syncytial cells. Type II  
140 pneumocyte hyperplasia was also observed in occasional alveoli. In the fourth placebo monkey,  
141 lung injury was limited to type II pneumocyte hyperplasia, suggestive of a reparative process  
142 secondary to type I pneumocyte injury. Overall, the histological lesions observed in the placebo  
143 animals were consistent with an acute SARS-CoV-2 infection. In the prophylactic groups, 3 of 4  
144 animals in the low dose (0.3mg/kg) and 1 of 4 animals in the high dose (50mg/kg) groups showed  
145 evidence of interstitial pneumonia (Table S1) that was generally minimal and with fewer  
146 histological features when compared to the placebo group. In the one affected high dose group  
147 animal, only 1 of the 4 lung lobes had a minimal lesion. In the therapeutic treatment groups, 2 of  
148 4 low dose (25mg/kg) and 2 of 4 high dose (150mg/kg) treated animals showed evidence of  
149 interstitial pneumonia. In all affected low and high dose animals, only 1 of 4 lung lobes had lesions.  
150 Finally, there was no test article related toxicities observed at any of the doses tested. In summary,  
151 the incidence of interstitial pneumonia (number of animals as well as number of lung lobes  
152 affected) and the severity were reduced in both prophylactic and therapeutic treatment modalities,  
153 compared to placebo. The analyses demonstrated that prophylactic and therapeutic administration  
154 of REGN-COV2 greatly reduced virus induced pathology in rhesus macaques and showed a clean  
155 safety profile.

156

157 Unlike rhesus macaques which present with a mild clinical course of disease and transient virus  
158 replication when infected with SARS-CoV-2, which may mimic mild human disease, the golden  
159 hamster model is more severe, with animals demonstrating readily observable clinical disease,  
160 including rapid weight loss accompanied by very high viral load in lungs, as well as severe lung  
161 pathology. Thus, this model may more closely mimic more severe disease in humans, although  
162 more extensive characterization of this model and severe human disease is needed to better  
163 understand similarities and differences in pathology. To evaluate the ability of REGN-COV2 to  
164 alter the disease course in this model, we designed a study which evaluated the prophylactic and  
165 treatment efficacy of the antibodies (Figure 3A). Administration of 50, 5 or 0.5mg/kg of REGN-  
166 COV2 2 days before challenge with  $2.3 \times 10^4$  PFU dose of SARS-CoV-2 virus resulted in dramatic  
167 protection from weight loss at all doses. This protection was accompanied by greatly decreased  
168 viral load in the lungs at the end of the study (day 7 post infection) (Figure 3C). Interestingly we  
169 did observe high gRNA and sgRNA levels in the lungs of a few treated animals, however these  
170 individual animals did not show decreased protection from weight loss than the animals with much  
171 lower viral loads. It is possible that mAb treatment may provide additional therapeutic benefit in  
172 this model not directly associated with viral load decrease. Alternatively, it is possible that the  
173 increased detected viral RNA may not necessarily be associated with infectious virus. As viral  
174 replication and lung pathology in the hamster model occur very rapidly, the treatment setting  
175 represents a high bar for demonstrating therapeutic efficacy. We were able to observe therapeutic  
176 benefit in animals treated with 50mg/kg and 5mg/kg doses of REGN-COV2 combination 1-day  
177 post viral challenge (Figure 3B). Taken together the two hamster studies clearly demonstrate that  
178 REGN-COV2 can alter the course of infection in the hamster model of SARS-COV-2 either when  
179 administered prophylactically or therapeutically.

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## 182 **Discussion**

183 In this study, we assessed the in vivo prophylactic and treatment efficacy of the REGN-COV2  
184 mAb cocktail in two animal models, one of mild disease in rhesus macaques and one of severe  
185 disease in golden hamsters. Our results demonstrated that the antibodies are efficacious in both  
186 animal models, as measured by reduced viral load in the upper and lower airways, reduced virus  
187 induced pathology in the rhesus macaque model, and by limited weight loss in the hamster model.

188

189 The ability of REGN-COV2 to almost completely block detection of subgenomic species of  
190 SARS-COV-2 RNA matches or exceeds the effects recently shown in vaccine efficacy studies  
191 using the same animal models (Corbett et al., 2020; Gao et al., 2020; Mercado et al., 2020; Patel  
192 A. et al., 2020; van Doremalen et al., 2020). Additionally, the observed accelerated reduction of  
193 upper airway virus load in rhesus macaques treated with REGN-COV2 contrasts the lack of impact  
194 on viral load in remdesivir treated animals, where reduced viral load could only be observed in  
195 lower airways with no differences in nasal viral RNA levels (Williamson et al., 2020). These  
196 findings highlight the therapeutic potential of REGN-COV2 to both protect from and treat SARS-

197 COV-2 disease. Additionally, the impact of REGN-COV2 prophylaxis on viral RNA levels in  
198 nasopharyngeal and oral swabs may indicate the potential to not only prevent disease in the  
199 exposed individual but also to limit transmission.

200

201 To our knowledge this is the first report demonstrating ability of any therapeutic to limit weight  
202 loss in the treatment setting of SARS-CoV-2 infection in the hamster model, indicating potential  
203 benefit of antibody treatment in the context of a severe infection. Further understanding of both  
204 the hamster and the macaque model and how their disease course and pathology mimics the  
205 breadth of human COVID-19 disease may help to gain more in depth understanding of how mAb  
206 therapeutics may confer clinical benefit.

207

208 Importantly, in our studies we did not observe any signs of increased viral load and/or worsening  
209 of pathology in presence of antibodies at either high or low doses in either animal model. Potential  
210 for antibody mediated enhancement of disease (ADE) is a serious concern for antibody-based  
211 therapeutics and vaccines. And although a recent report showed ability of some anti-spike mAbs  
212 to mediate pseudovirus entry into FcγR expressing cell lines, these data do not address whether  
213 similar behavior would be observed with authentic SARS-CoV-2 virus and primary immune cells  
214 (Wang S. et al., 2020). Our results are consistent with no evidence of enhanced disease in clinical  
215 studies assessing convalescent plasma therapy (Li et al., 2020).

216

217 In conclusion, our data provide evidence that REGN-COV2 based therapy may offer clinical  
218 benefit in both prevention and treatment settings of COVID-19 disease, where it is currently being  
219 evaluated (clinicaltrials.gov NCT04426695, NCT04425629 and NCT 04452318).

220

## 221 **Methods**

222

### 223 **Studies conducted at BIOQUAL (NHP Study #1 and Hamster Study):**

#### 224 **Ethics Statement and Animal Exposure**

225 Animal research was conducted under BIOQUAL Institute Institutional Animal Care and Use  
226 Committee (IACUC)-approved protocols, 20-070P (hamster study) and 20-069P (NHP study) in  
227 compliance with the Animal Welfare Act and other federal statutes and regulations relating to  
228 animals and experiments involving animals. BIOQUAL is accredited by the Association for  
229 Assessment and Accreditation of Laboratory Animal Care International and adheres to principles  
230 stated in the Guide for the Care and Use of Laboratory Animals, National Research Council.  
231 Animals were monitored at least twice daily, and enrichment included commercial toys and food  
232 supplements. Prior to all blood collections, animals were anesthetized using Ketamine (NHPs) or  
233 Ketamine/Xylazine (hamsters). At the end of the study, animals were euthanized with an  
234 intravenous (NHPs) or intraperitoneal (hamsters) overdose of sodium pentobarbital.

235

#### 236 **Rhesus macaque study:**

237 A total of 12 naïve rhesus macaques of Indian origin (purpose bred, *Macaca mulatta*) were used  
238 in the study. Animals were distributed to treatment groups based on age distribution. Antibodies  
239 or saline were administered through intravenous infusion. Animals were challenged with  $1.1 \times 10^5$   
240 PFU (USA-WA1/2020 (NR-52281; BEI Resources) total dose of virus divided between intranasal  
241 and intratracheal routes. Virus was administered using a 3mL syringe to drop-wise instill 1 mL by  
242 the intranasal (IN) route (0.5 mL in each nare) and using a French rubber tube, administer 1 mL  
243 via the intratracheal (IT) route. Viral titers were collected by nasal swabs (2x Copan flocced per  
244 animal, placed into one vial each with 1mL PBS) and bronchioalveolar lavage (BAL) using 10 mL  
245 saline via a rubber feeding tube. Collected swabs and BAL aliquots were stored at  $-80^\circ\text{C}$  until viral  
246 load analysis.

247

#### 248 **Hamster study:**

249 A total of 50 golden hamsters, male and female, 6-8 weeks old were used in the study. Animals  
250 were weighed prior to the start of the study. The animals were monitored twice daily for signs of  
251 COVID-19 disease (ruffled fur, hunched posture, labored breathing, a.o.) during the study period.  
252 Body weights were measured once daily during the study period. Antibodies were dosed through  
253 intraperitoneal (IP) injection. Animals were challenged with  $2.3 \times 10^4$  PFU of (USA-WA1/2020  
254 (NR-52281; BEI Resources) by administration of 0.05mL of viral inoculum dropwise into each  
255 nostril. Tissues were sampled for viral load assays by collecting two small pieces (0.1-0.2 gram  
256 each) from the lung (total of 4 pieces, 2 per tissue). Tissues were stored at  $-80^\circ\text{C}$  until viral load  
257 analysis.

258

#### 259 **Cells and Virus**

260 Vero E6 cells (ATCC, catalog number CRL 1586) were grown in Dulbecco's modified essential  
261 media (DMEM; Gibco) with 10% heat-inactivated fetal bovine serum (FBS; Gibco) at 37°C with  
262 5% CO<sub>2</sub>. SARS-CoV-2 (P4) isolate USA-WA1/2020 (BEI resources NR-52281, GenBank  
263 accession number MN985325.1) was used to generate the animal exposure stock (P5). The stock  
264 was generated by infecting Vero E6 cells at an MOI of 0.002 in DMEM containing 2% FBS; viral  
265 supernatant was harvested at four days post infection. The stock has been confirmed to be SARS-  
266 CoV-2 via deep sequencing and confirmed to be free of adventitious agents. The viral titer was  
267 determined to be  $2.3 \times 10^5$  PFU/mL.

268

### 269 **Quantitative RT-PCR Assay for SARS-CoV-2 RNA**

270 The amounts of RNA copies per mL bodily fluid or per gram tissue were determined using  
271 a qRT-PCR assay. The qRT-PCR assay utilized primers and a probe specifically designed  
272 to amplify and bind to a conserved region of nucleocapsid gene of coronavirus. The signal  
273 was compared to a known standard curve and calculated to give copies per mL. For the  
274 qRT-PCR assay, viral RNA was first isolated from nasal wash using the Qiagen MinElute  
275 virus spin kit (cat. no. 57704). For tissues it was extracted with RNA-STAT 60 (Tel-  
276 test" B")/ chloroform, precipitated and resuspended in RNase-free water. To generate a  
277 control for the amplification reaction, RNA was isolated from the applicable SARS-CoV-  
278 2 stock using the same procedure. qPCR assay was performed with Applied Biosystems  
279 7500 Sequence detector and amplified using the following program: 48°C for 30 minutes,  
280 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, and 1 minute at 55°C.  
281 The number of copies of RNA per mL was calculated by extrapolation from the standard  
282 curve and multiplying by the reciprocal of 0.2 mL extraction volume. This gives a practical  
283 range of 50 to  $5 \times 10^8$  RNA copies per mL for nasal washes or per gram of tissue.

284 Primers/probe sequences:

285 2019-nCoV\_N1-F :5'-GAC CCC AAA ATC AGC GAA AT-3'

286 2019-nCoV\_N1-R: 5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'

287 2019-nCoV\_N1-P: 5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'

288

### 289 **Quantitative RT-PCR Assay for SARS-CoV-2 subgenomic RNA**

290 SARS-CoV-2 E gene subgenomic mRNA (sgRNA or sgmRNA) was assessed by RT-PCR using  
291 primers and probes as previously described(Chandrashekar et al., 2020). Briefly, to generate a  
292 standard curve, the SARS-CoV-2E gene sgRNA was cloned into a pcDNA3.1 expression plasmid;  
293 this insert was transcribed using an AmpliCap-Max T7 High Yield MessageMaker Kit (Cellscript)  
294 to obtain RNA for standards. Prior to RT-PCR, samples collected from challenged animals or  
295 standards were reverse-transcribed using Superscript III VILO (Invitrogen) according to the  
296 manufacturer's instructions. A Taqman custom gene expression assay (ThermoFisher Scientific)  
297 was designed using the sequences targeting the E gene sgRNA20. Reactions were carried out on a  
298 QuantStudio 6 and 7 Flex Real-Time PCR System (Applied Biosystems) according to the  
299 manufacturer's specifications. Standard curves were used to calculate sgRNA in copies per ml or



300 per swab; the quantitative assay sensitivity was 50 copies per ml or per swab. This gives a practical  
301 range of 50 to  $5 \times 10^7$  RNA copies per mL for nasal washes, and for tissues the viral loads are  
302 given per gram.

303 Subgenomic RNA Primers:

304 SG-F: CGATCTTG TAGATCTG TTCCTCAAACGAAC

305 SG-R: ATATTGCAGCAGTACGCACACACA

306 PROBE: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ

307

308

309 **Studies conducted at Texas Biomedical Research Institute (NHP Study #2):**

310 **Ethics Statement and Nonhuman Primate Exposure**

311 Animal research was conducted under Texas Biomedical Research Institute Institutional Animal  
312 Care and Use Committee (IACUC)-approved protocol (1721MM) in compliance with the Animal  
313 Welfare Act and other federal statutes and regulations relating to animals and experiments  
314 involving animals. Texas Biomedical Research Institute is accredited by the Association for  
315 Assessment and Accreditation of Laboratory Animal Care International and adheres to principles  
316 stated in the Guide for the Care and Use of Laboratory Animals, National Research Council.  
317 Animals were monitored at least twice daily and enrichment included commercial toys and food  
318 supplements. Prior to all blood collections, animals were anesthetized using Telazol (Zoetis Inc.,  
319 Parsippany-Troy Hills, NJ, USA). At the end of the study, animals were euthanized with an  
320 intravenous overdose of sodium pentobarbital.

321 **Animal challenge**

322 Twenty-four (24) rhesus macaques (13 female and 11 males) were used in this study, and randomly  
323 assigned to one of six groups. Animals were obtained from the Southwest National Primate  
324 Research Center (SNPRC) colony and were between 2.5 and 6 years of age and approximately 3  
325 to 10 kg at the time of study enrollment. On Study Day 0, each NHP was exposed at ABSL-4 with  
326 a targeted dose of  $1.05 \times 10^6$  PFU of SARS-CoV-2 in a total volume of 500  $\mu$ l ( $5.25 \times 10^5$  PFU in  
327 250  $\mu$ l via intranasal route and  $5.25 \times 10^5$  PFU in 250  $\mu$ l via intratracheal route). Intranasal delivery  
328 was via a mucosal atomization device (Teleflex Intranasal Mucosal Atomization Device LMA  
329 MAD Nasal Device), which allows for IN delivery of atomized particles 30 - 100 microns in size,  
330 which model droplet transmission. Mucosal atomization devices have been developed for safe and  
331 efficient drug delivery to administer drugs that are United States Food and Drug Administration  
332 (U.S. FDA) approved for IN delivery. Intratracheal delivery used a Tracheal Mucosal Atomization  
333 Device (Teleflex Laryngo-Tracheal Mucosal Atomization Device LMA MADGIC). Animals were  
334 exposed in ascending order based on Texas Biomed animal ID in order to minimize timing bias  
335 for treatment administration. On Day -3 relative to exposure, prophylactic group animals were  
336 sedated and received treatment. On Day 1 (post virus exposure), therapeutic group animals were  
337 sedated and received treatment. Treatment was administered via intravenous injection over the  
338 course of approximately 90 seconds.

339

## 340 **Cells and Virus**

341 Vero E6 cells (VERO C1008, catalog number NR-596, BEI resources) were grown in Dulbecco's  
342 modified essential media (DMEM; Gibco) with 10% heat-inactivated fetal bovine serum (FBS;  
343 Gibco) at 37°C with 5% CO<sub>2</sub>. SARS-CoV-2 isolate USA-WA1/2020 (BEI resources NR-52281,  
344 GenBank accession number MN985325.1) was used to generate the animal exposure stock. A  
345 fourth cell-culture passage (P4) of SARS-CoV-2 was obtained from in 2020 and propagated at  
346 Texas Biomedical Research Institute. The fourth cell-culture passage (P4) stock virus obtained  
347 from BEI was passaged one time to generate a master stock by infecting Vero E6 cells at a  
348 multiplicity of infection (MOI) of approximately 0.001 in DMEM containing 2% FBS; viral  
349 supernatant was harvested at 3 days post infection. The P5 stock was used to generate the exposure  
350 stock by infecting Vero E6 cells at an MOI of 0.02 in DMEM containing 2% FBS; viral supernatant  
351 was harvested at three days post infection. The stock has been confirmed to be SARS-CoV-2 via  
352 deep sequencing and confirmed to be free of adventitious agents. The viral titer was determined to  
353 be 2.1 x 10<sup>6</sup> PFU/mL.

354

## 355 **RNA extraction for viral load determination via RT-qPCR**

356 Samples were inactivated using TRIzol LS Isolation Reagent (Invitrogen): 250 µL of test sample  
357 were mixed with 750 µL TRIzol LS. Inactivation controls were prepared with each batch of  
358 samples. Prior to extraction, 1 x 10<sup>3</sup> pfu of MS2 phage (Escherichia coli bacteriophage MS2,  
359 ATCC) was added to each sample to assess extraction efficiency RNA extraction was performed  
360 using the EpMotion M5073c Liquid Handler (Eppendorf) and the NucleoMag Pathogen kit  
361 (Macherey-Nagel). Extraction controls were prepared with each batch of samples. After  
362 processing, the presence of the eluate was confirmed and the extracted RNA was stored at -  
363 80°C±10°C.

364

## 365 **Determination of Viral load via RT-qPCR**

366 5 µL RNA sample was taken to duplex RT-qPCR reaction detecting both SARS-CoV-2 and MS2  
367 phage. Two assays were used to assess SARS-CoV-2 present in the samples. The CDC-developed  
368 2019-nCoV\_N1 assay was used to target a region of the N gene. SARS-CoV-2\_N1 probe  
369 (ACCCCGCATTACGTTTGGTGGACC) is labeled with 6-FAM fluorescent dye. The forward  
370 primer sequence is: GACCCCAAATCAGCGAAAT, and the reverse primer sequence is:  
371 TCTGGTTACTGCCAGTTGAATCTG. A secondary qPCR assay to measure subgenomic RNA  
372 was also performed to target a region of E (Envelope)(Corman et al., 2020; Wolfel et al., 2020)  
373 The probe is also labeled with 6-FAM fluorescent dye (ACACTAGCCATCCTTACTGCGC  
374 TTCG). The forward primer sequence is: CGATCTCTTGATAGATCTGTTCTC, and the reverse  
375 primer sequence is: ATATTGCAGCAGTACGCACACA. The MS2 probe is labeled with VIC  
376 fluorescent dye. Both assays used the TaqPath™ 1-Step RT-qPCR Master Mix, CG  
377 (ThermoFisher) and were performed on a QuantStudio 3 instrument (Applied Biosystems).  
378 QuantStudio Design and Analysis Software (Applied Biosystems) was used to run and analyze the  
379 results. Cycling parameters were set as follows: Hold stage 2 min at 25°C, 15 min at 50°C, 2 min

380 at 95°C. PCR stage: 45 cycles (N1 assay) or 40 cycles (E assay) of 3 sec at 95°C, 30 sec at 60°C.  
381 The average Ct value for MS2 phage was calculated for all processed samples and SARS-CoV-2  
382 quantification only performed in samples in which the MS2 Ct value was lower than Average MS2  
383 + 5%.

384

### 385 **Histopathology**

386 Necropsies were conducted by BSL-4 personnel in accordance with SOP Texas Biomed 916 and  
387 selected tissue samples (tracheobronchial lymph node, nasal cavity, trachea, heart, liver, spleen,  
388 kidney, and all 4 right lung lobes) were collected. Tissues were fixed by immersion in 10% neutral-  
389 buffered formalin for a minimum of fourteen days, then trimmed, routinely processed, and  
390 embedded in paraffin. Sections of the paraffin-embedded tissues were cut at 5 µm thick, and  
391 histology slides were deparaffinized, stained with hematoxylin and eosin (H&E), cover slipped,  
392 and labeled. Slides were blindly evaluated by a board-certified veterinary pathologist.

393

### 394 **Virus RNA Sequencing**

395 10 ul of RNA combined with 25 ng Human Universal Reference RNA (Agilent) was purified by  
396 PureBeads (Roche Sequencing). cDNA synthesis was performed using SuperScript™ IV First-  
397 Strand Synthesis System (Thermal Fisher) following vendor's protocol. Then one half of cDNA  
398 (10 ul) was used to generate libraries using Swift Normalase™ Amplicon Panel (SNAP) SARS-  
399 CoV-2 Panel (Swift Biosciences) following vendor's protocol. Sequencing was run on NextSeq  
400 (Illumina) by multiplexed paired-read run with 2X150 cycles.

401

### 402 **RNAseq data analysis**

403 RNAseq analysis was perform using Array Studio software package platform (Omicsoft). Quality  
404 of paired-end RNA Illumina reads was assessed using the “raw data QC of RNA-Seq data suite”.  
405 Minimum and maximum read length, total nucleotide number, and GC% were calculated. Overall  
406 quality report was generated summarizing the quality of all reads in each sample, along each base  
407 pair. Swift amplicon bulk RNA-seq reads were aligned to the SARS-COV-2 reference genome  
408 Wuhan-Hu-1 (MN908947) using Omicsoft Sequence Aligner (OSA) version 4. The alignments  
409 were sorted by read name, and primers were clipped by the complementary Swiftbiosciences  
410 primerclip software (v0.3.8) (<https://github.com/swiftbiosciences/primerclip>). Reads were  
411 trimmed by quality score using default parameters (when aligner encountered nucleotide in the  
412 read with a quality score of 2 or less, it trimmed the remainder of the read). OSA outputs were  
413 analyzed and annotated using Summarize Variant Data and Annotate Variant Data packages  
414 (Omicsoft). The rest of the analysis focused on the genome section encoding the Spike protein.  
415 Using custom scripts, target coverage was summarized for each sample and SNPs calling was  
416 calculated. The frequency of viral mutations inferred from the sequencing reads were calculated if  
417 mutated reads were higher than 1% relative to total number reads.

418

419 **Acknowledgments:** The following reagent was deposited by the Centers for Disease Control and  
420 Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2,  
421 Isolate USA-WA1/2020, NR-52281.

422 **Funding:** A portion of this project has been funded in whole or in part with Federal funds from  
423 the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness  
424 and Response; Biomedical Advanced Research and Development Authority, under OT number:  
425 HHSO100201700020C.

426 **Author contributions:** A.B., N.S, A.J.M, G.D.Y., C.A.K. conceptualized and designed  
427 experiments. Y.G.G, J.D., E.C., H.S., C.B., B.K., O.G., E.D., L.P., M.P., A.C., R.B., V.A., J.G.,  
428 T.T., performed experiments and A.B., R.C., D.A., A.O, K.A., R.C., M.G., H.A., M.G.L., M.A.,  
429 G.D.Y., C.A.K. analyzed data. R.C., K.L., N.N., M.N., Y.W. prepared sequencing libraries and  
430 performed bioinformatics analysis A.B. and C.A.K. wrote the paper. C.A.K. acquired funding.

431 **Competing interests:** Regeneron authors own options and/or stock of the company. This work  
432 has been described in one or more pending provisional patent applications. N.S, A.J.M., G.D.Y.  
433 and C.A.K. are officers of Regeneron.

434

## 435 References

- 436
- 437 Baum, A., Fulton, B.O., Wloga, E., Copin, R., Pascal, K.E., Russo, V., Giordano, S., Lanza, K.,  
438 Negron, N., Ni, M., *et al.* (2020). Antibody cocktail to SARS-CoV-2 spike protein prevents rapid  
439 mutational escape seen with individual antibodies. *Science* (New York, NY).
- 440 Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C.,  
441 *et al.* (2020). Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-  
442 Throughput Single-Cell Sequencing of Convalescent Patients' B Cells. *Cell* *182*, 73-84 e16.
- 443 Chandrashekar, A., Liu, J., Martinot, A.J., McMahan, K., Mercado, N.B., Peter, L., Tostanoski,  
444 L.H., Yu, J., Maliga, Z., Nekorchuk, M., *et al.* (2020). SARS-CoV-2 infection protects against  
445 rechallenge in rhesus macaques. *Science* (New York, NY).
- 446 Cohen, J. (2020). The race is on for antibodies that stop the new coronavirus. *Science* (New  
447 York, NY) *368*, 564-565.
- 448 Corbett, K.S., Flynn, B., Foulds, K.E., Francica, J.R., Boyoglu-Barnum, S., Werner, A.P., Flach,  
449 B., O'Connell, S., Bock, K.W., Minai, M., *et al.* (2020). Evaluation of the mRNA-1273 Vaccine  
450 against SARS-CoV-2 in Nonhuman Primates. *N Engl J Med*.
- 451 Corman, V.M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D.K., Bleicker, T.,  
452 Brunink, S., Schneider, J., Schmidt, M.L., *et al.* (2020). Detection of 2019 novel coronavirus  
453 (2019-nCoV) by real-time RT-PCR. *Euro Surveill* *25*.
- 454 Deng, W., Bao, L., Liu, J., Xiao, C., Liu, J., Xue, J., Lv, Q., Qi, F., Gao, H., Yu, P., *et al.* (2020).  
455 Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. *Science*  
456 (New York, NY).
- 457 Gao, Q., Bao, L., Mao, H., Wang, L., Xu, K., Yang, M., Li, Y., Zhu, L., Wang, N., Lv, Z., *et al.*  
458 (2020). Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* (New York,  
459 NY) *369*, 77-81.
- 460 Hansen, J., Baum, A., Pascal, K.E., Russo, V., Giordano, S., Wloga, E., Fulton, B.O., Yan, Y.,  
461 Koon, K., Patel, K., *et al.* (2020). Studies in humanized mice and convalescent humans yield a  
462 SARS-CoV-2 antibody cocktail. *Science* (New York, NY).
- 463 He, X., Lau, E.H.Y., Wu, P., Deng, X., Wang, J., Hao, X., Lau, Y.C., Wong, J.Y., Guan, Y., Tan,  
464 X., *et al.* (2020). Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat*  
465 *Med* *26*, 672-675.
- 466 Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P.J., Nakajima, N.,  
467 Watanabe, T., Ujie, M., Takahashi, K., Ito, M., *et al.* (2020). Syrian hamsters as a small animal  
468 model for SARS-CoV-2 infection and countermeasure development. *Proc Natl Acad Sci U S A*  
469 *117*, 16587-16595.
- 470 Ju, B., Zhang, Q., Ge, J., Wang, R., Sun, J., Ge, X., Yu, J., Shan, S., Zhou, B., Song, S., *et al.*  
471 (2020). Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature*.
- 472 Li, L., Zhang, W., Hu, Y., Tong, X., Zheng, S., Yang, J., Kong, Y., Ren, L., Wei, Q., Mei, H., *et*  
473 *al.* (2020). Effect of Convalescent Plasma Therapy on Time to Clinical Improvement in Patients  
474 With Severe and Life-threatening COVID-19: A Randomized Clinical Trial. *JAMA*.

475 Liu, L., Wang, P., Nair, M.S., Yu, J., Rapp, M., Wang, Q., Luo, Y., Chan, J.F., Sahi, V.,  
476 Figueroa, A., *et al.* (2020). Potent neutralizing antibodies directed to multiple epitopes on SARS-  
477 CoV-2 spike. *Nature*.

478 Mercado, N.B., Zahn, R., Wegmann, F., Loos, C., Chandrashekar, A., Yu, J., Liu, J., Peter, L.,  
479 McMahan, K., Tostanoski, L.H., *et al.* (2020). Single-shot Ad26 vaccine protects against SARS-  
480 CoV-2 in rhesus macaques. *Nature*.

481 Munster, V.J., Feldmann, F., Williamson, B.N., van Doremalen, N., Perez-Perez, L., Schulz, J.,  
482 Meade-White, K., Okumura, A., Callison, J., Brumbaugh, B., *et al.* (2020). Respiratory disease  
483 in rhesus macaques inoculated with SARS-CoV-2. *Nature*.

484 Patel A., Walters J., Reuschel E., Katherine Schultheis, Elizabeth Parzych, Ebony N. Gary, Igor  
485 Maricic, and Mansi Purwar, Z.E., Susanne N. Walker, Diana Guimet, Pratik Bhojnarwala,  
486 Arthur Doan, Ziyang Xu, Dustin Elwood, Sophia M. Reeder, Laurent Pessaint, Kevin Y. Kim,  
487 Anthony Cook, Neethu Chokkalingam, Brad Finneyfrock, Edgar Tello-Ruiz, Alan Dodson, Jihae  
488 Choi, Alison Generotti, John Harrison, Nicholas J. Tursi, Viviane M. Andrade, Yaya Dia, Faraz  
489 I. Zaidi, Hanne Andersen, Mark G. Lewis, Kar Muthumani, J Joseph Kim, Daniel W. Kulp,  
490 Laurent M. Humeau, Stephanie Ramos, Trevor R.F. Smith, David B. Weiner, Kate E. Broderick  
491 (2020). Intradermal-delivered DNA vaccine provides anamnestic protection in a rhesus macaque  
492 SARS-CoV-2 challenge model. *bioRxiv*. <https://doi.org/10.1101/2020.07.28.225649>

493 Pinto, D., Park, Y.J., Beltramello, M., Walls, A.C., Tortorici, M.A., Bianchi, S., Jaconi, S.,  
494 Culap, K., Zatta, F., De Marco, A., *et al.* (2020). Cross-neutralization of SARS-CoV-2 by a  
495 human monoclonal SARS-CoV antibody. *Nature* 583, 290-295.

496 Robbiani, D.F., Gaebler, C., Muecksch, F., Lorenzi, J.C.C., Wang, Z., Cho, A., Agudelo, M.,  
497 Barnes, C.O., Gazumyan, A., Finkin, S., *et al.* (2020). Convergent antibody responses to SARS-  
498 CoV-2 in convalescent individuals. *Nature*.

499 Rogers, T.F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W.T., Limbo, O., Smith, C., Song,  
500 G., Woehl, J., *et al.* (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and  
501 protection from disease in a small animal model. *Science* (New York, NY).

502 Shan, C., Yao, Y.F., Yang, X.L., Zhou, Y.W., Gao, G., Peng, Y., Yang, L., Hu, X., Xiong, J.,  
503 Jiang, R.D., *et al.* (2020). Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in  
504 Rhesus macaques. *Cell Res*.

505 Shi, R., Shan, C., Duan, X., Chen, Z., Liu, P., Song, J., Song, T., Bi, X., Han, C., Wu, L., *et al.*  
506 (2020). A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature*.

507 Sia, S.F., Yan, L.M., Chin, A.W.H., Fung, K., Choy, K.T., Wong, A.Y.L., Kaewpreedee, P.,  
508 Perera, R., Poon, L.L.M., Nicholls, J.M., *et al.* (2020). Pathogenesis and transmission of SARS-  
509 CoV-2 in golden hamsters. *Nature* 583, 834-838.

510 van Doremalen, N., Lambe, T., Spencer, A., Belij-Rammerstorfer, S., Purushotham, J.N., Port,  
511 J.R., Avanzato, V.A., Bushmaker, T., Flaxman, A., Ulaszewska, M., *et al.* (2020). ChAdOx1  
512 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature*.

513 Wang, C., Li, W., Drabek, D., Okba, N.M.A., van Haperen, R., Osterhaus, A., van Kuppeveld,  
514 F.J.M., Haagmans, B.L., Grosveld, F., and Bosch, B.J. (2020). A human monoclonal antibody  
515 blocking SARS-CoV-2 infection. *Nat Commun* 11, 2251.

516 Wang S., Yun Peng, Rongjun Wang, Shasha jiao, Min Wang, Weijin Huang, Chao Shan, Wen  
517 Jiang, and Zepeng Li, C.G., Ben Chen, Xue Hu, Yanfeng Yao, Juan Min, Huajun Zhang, Ying  
518 Chen, Ge Gao, Peipei Tang, Gang Li, An Wang, Lan Wang, Shuo Chen, Xun Gui, Jinchao  
519 Zhang, Zhiming Yuan, Datao Liu (2020). An antibody-dependent enhancement (ADE) activity  
520 eliminated neutralizing antibody with potent prophylactic and therapeutic efficacy against  
521 SARS-CoV-2 in rhesus monkeys. *bioRxiv*. <https://doi.org/10.1101/2020.07.26.222257>

522 Williamson, B.N., Feldmann, F., Schwarz, B., Meade-White, K., Porter, D.P., Schulz, J., van  
523 Doremalen, N., Leighton, I., Yinda, C.K., Perez-Perez, L., *et al.* (2020). Clinical benefit of  
524 remdesivir in rhesus macaques infected with SARS-CoV-2. *Nature*.

525 Wolfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Muller, M.A., Niemeyer,  
526 D., Jones, T.C., Vollmar, P., Rothe, C., *et al.* (2020). Virological assessment of hospitalized  
527 patients with COVID-2019. *Nature* 581, 465-469.

528 Yu, J., Tostanoski, L.H., Peter, L., Mercado, N.B., McMahan, K., Mahrokhian, S.H., Nkolola,  
529 J.P., Liu, J., Li, Z., Chandrashekar, A., *et al.* (2020). DNA vaccine protection against SARS-  
530 CoV-2 in rhesus macaques. *Science* (New York, NY).

531 Zost, S.J., Gilchuk, P., Chen, R.E., Case, J.B., Reidy, J.X., Trivette, A., Nargi, R.S., Sutton, R.E.,  
532 Suryadevara, N., Chen, E.C., *et al.* (2020). Rapid isolation and profiling of a diverse panel of  
533 human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med*.

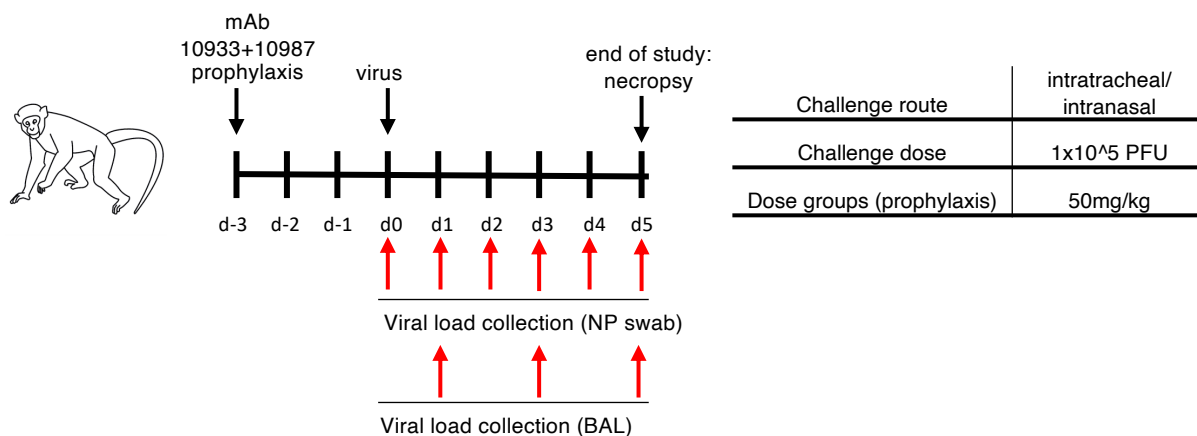
534 Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., Yu, J., Kang, M., Song, Y., Xia,  
535 J., *et al.* (2020). SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients.  
536 *N Engl J Med* 382, 1177-1179.

537

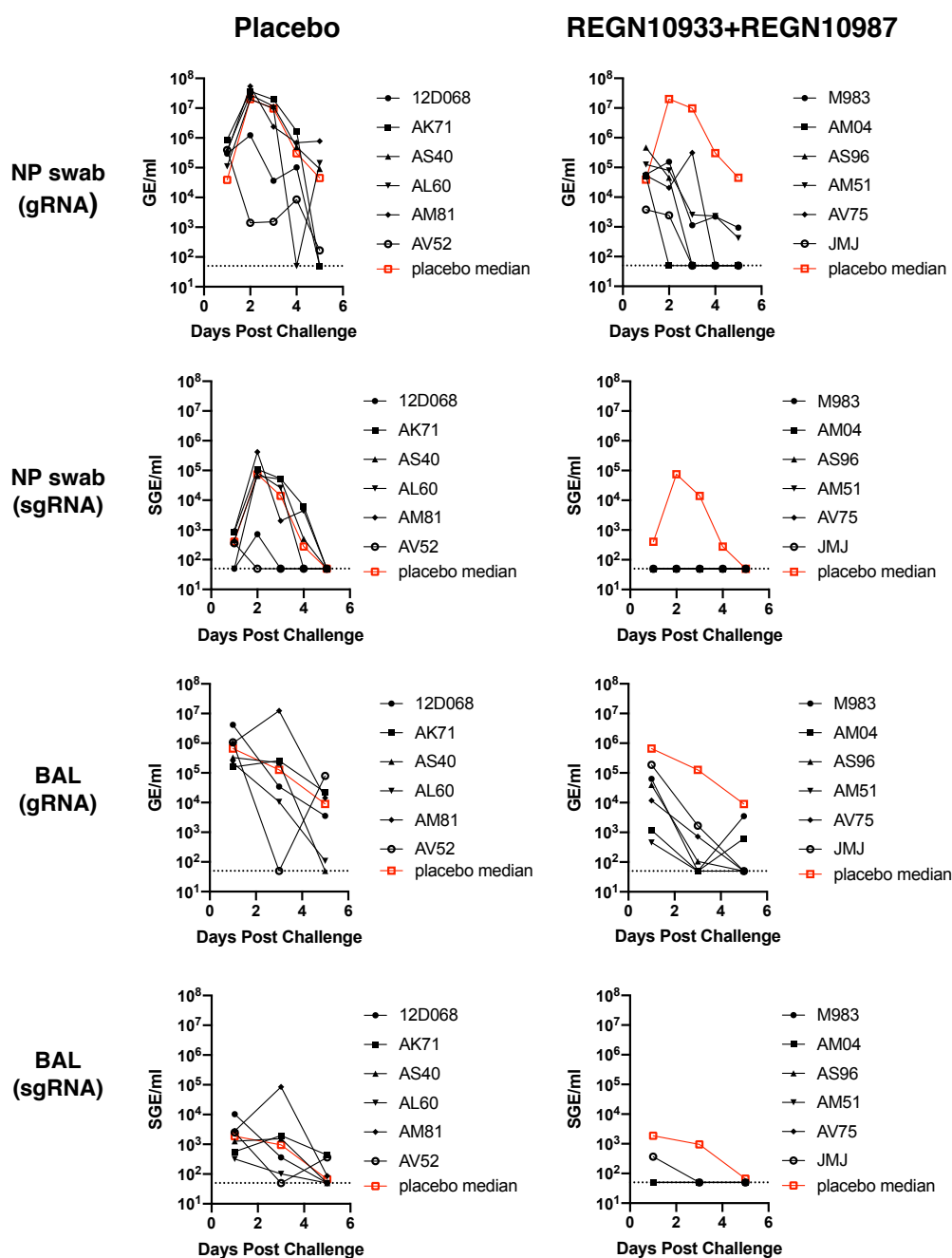
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# Figure 1.

**A**



**B**

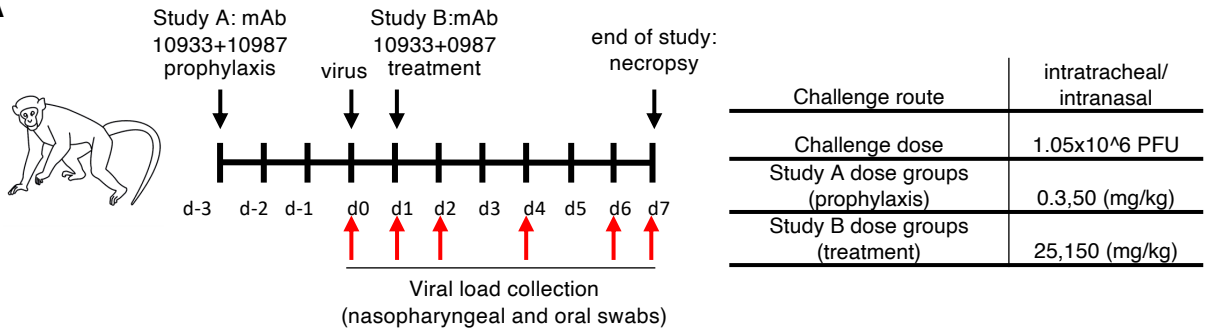


**Figure 1. Prophylactic efficacy of REGN-COV2 in the rhesus macaque model of SARS-CoV-2 infection (NHP Study #1)** (A) Overview of study design. (B) Impact of REGN-COV2 prophylaxis on viral genomic RNA (gRNA) and subgenomic RNA (sgRNA) in nasopharyngeal swabs and bronchioalveolar lavage (BAL) fluid.

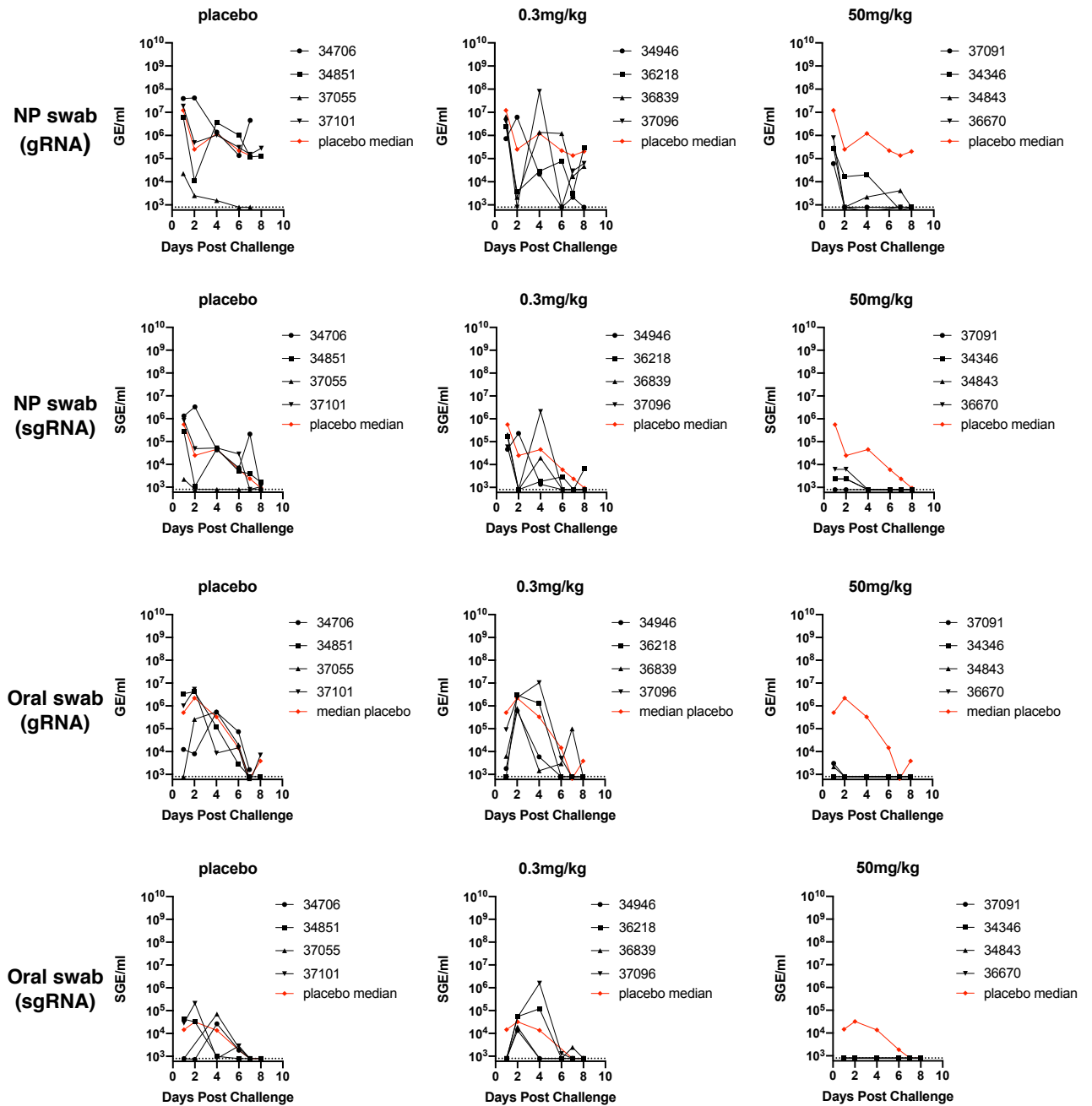


# Figure 2

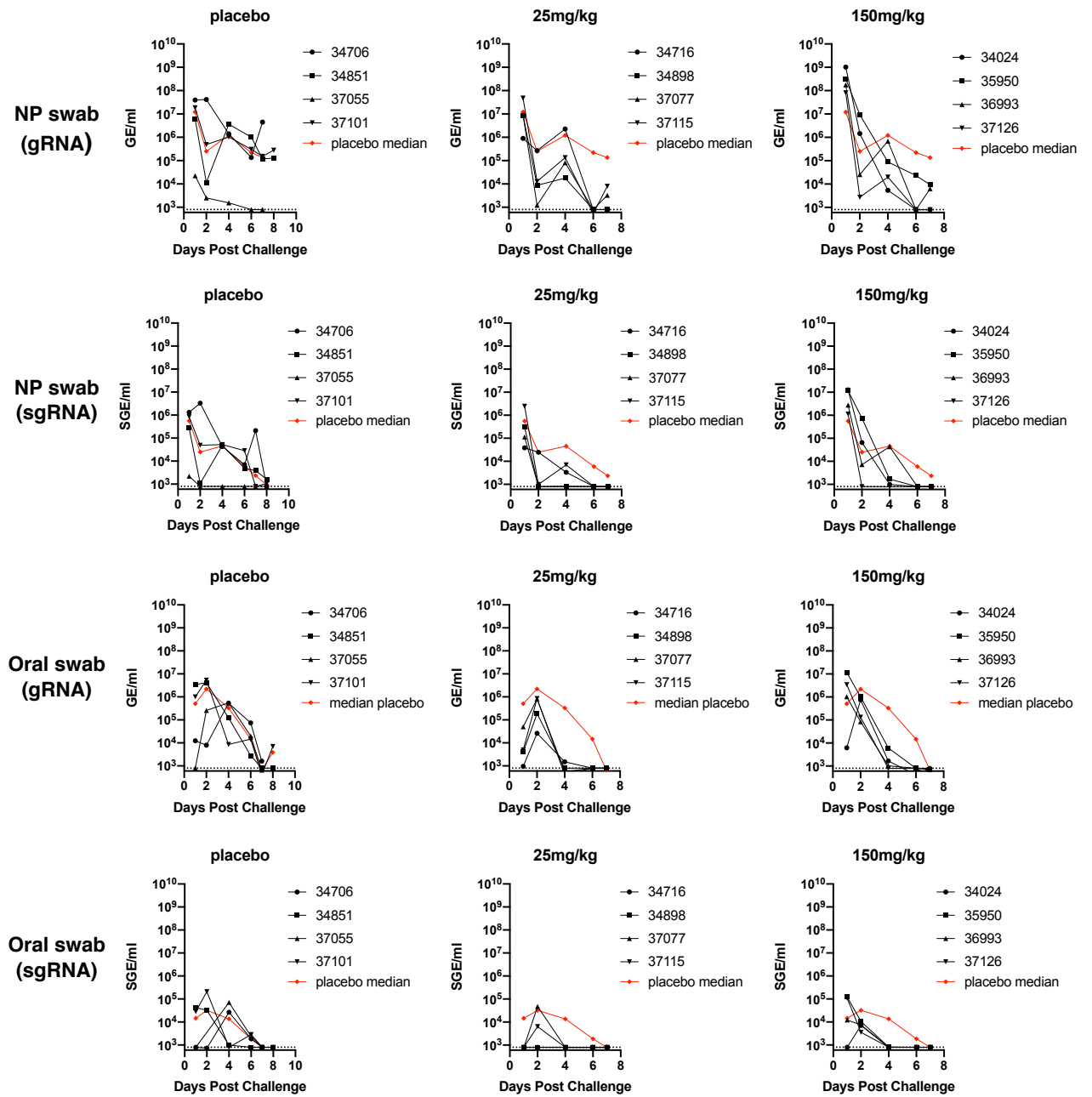
**A**



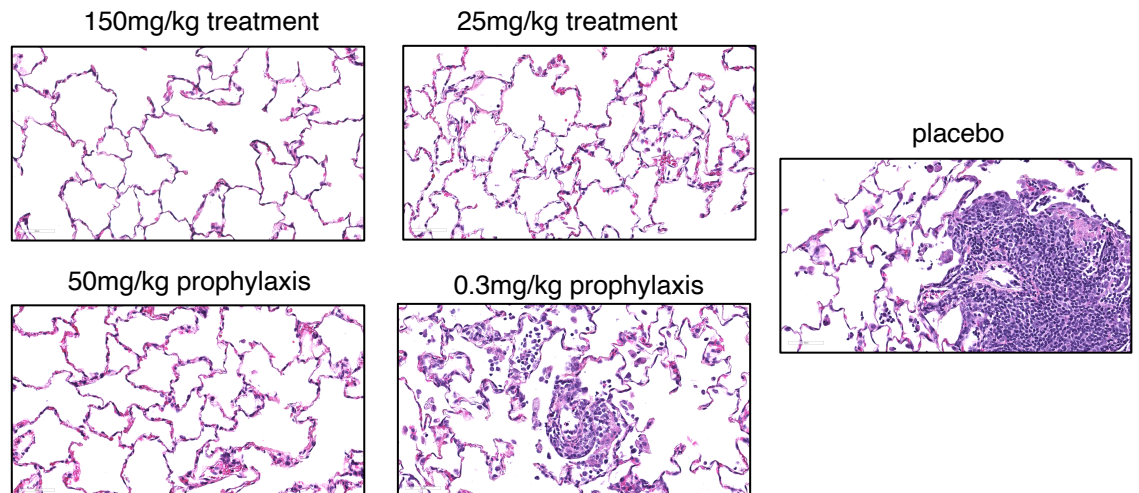
**B**



C

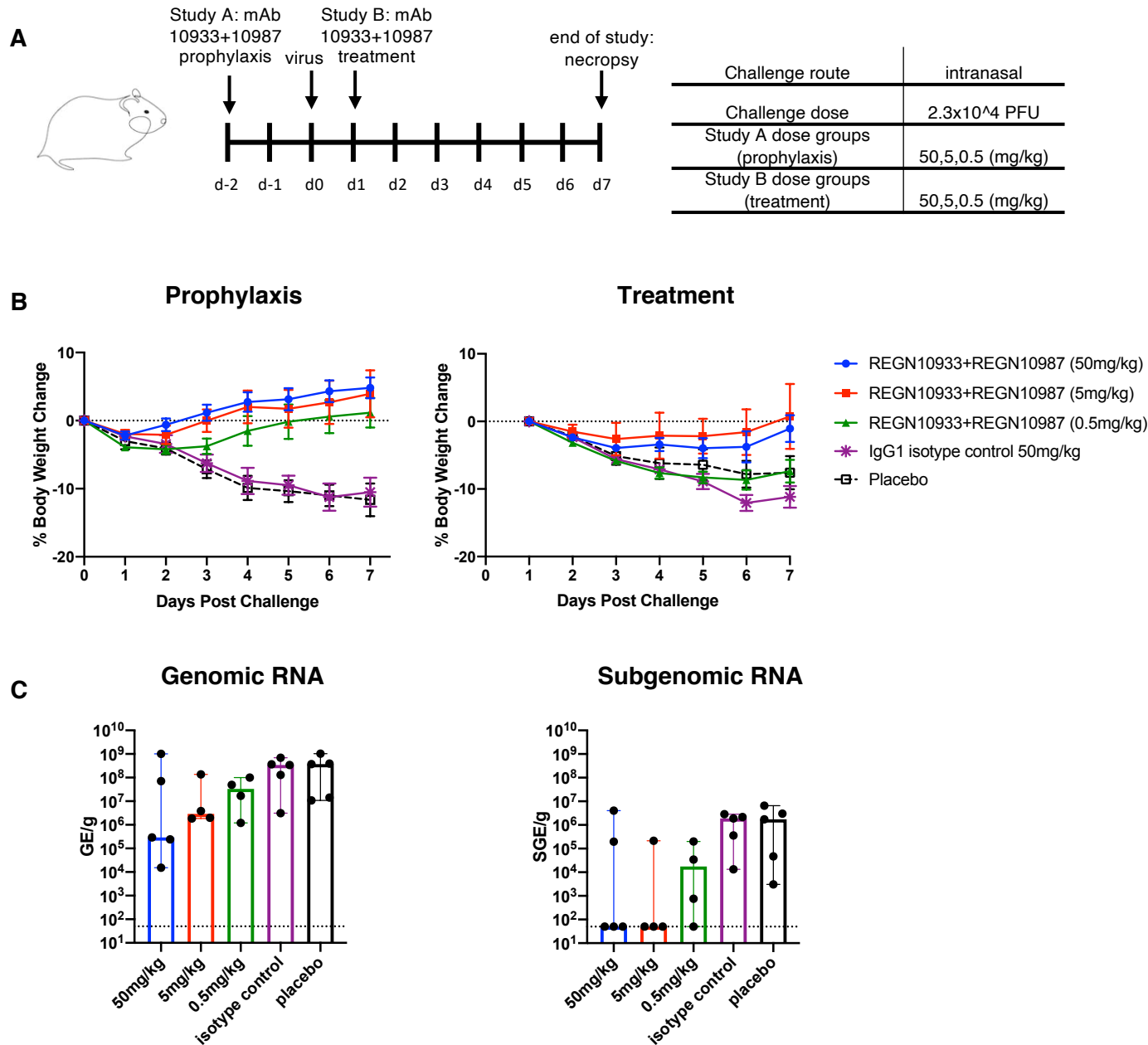


D



**Figure 2. Prophylactic and therapeutic efficacy of REGN-COV2 in the rhesus macaque model of SARS-CoV-2 infection (NHP Study #2)** (A) Overview of study design. (B) Impact of REGN-COV2 prophylaxis on viral genomic RNA (gRNA) and subgenomic RNA (sgRNA) in nasopharyngeal swabs and oral swabs. (C) Impact of REGN-COV2 treatment on viral genomic RNA (gRNA) and subgenomic RNA (sgRNA) in nasopharyngeal swabs and oral swabs. (D) representative images of histopathology in lungs of treated and placebo animals.

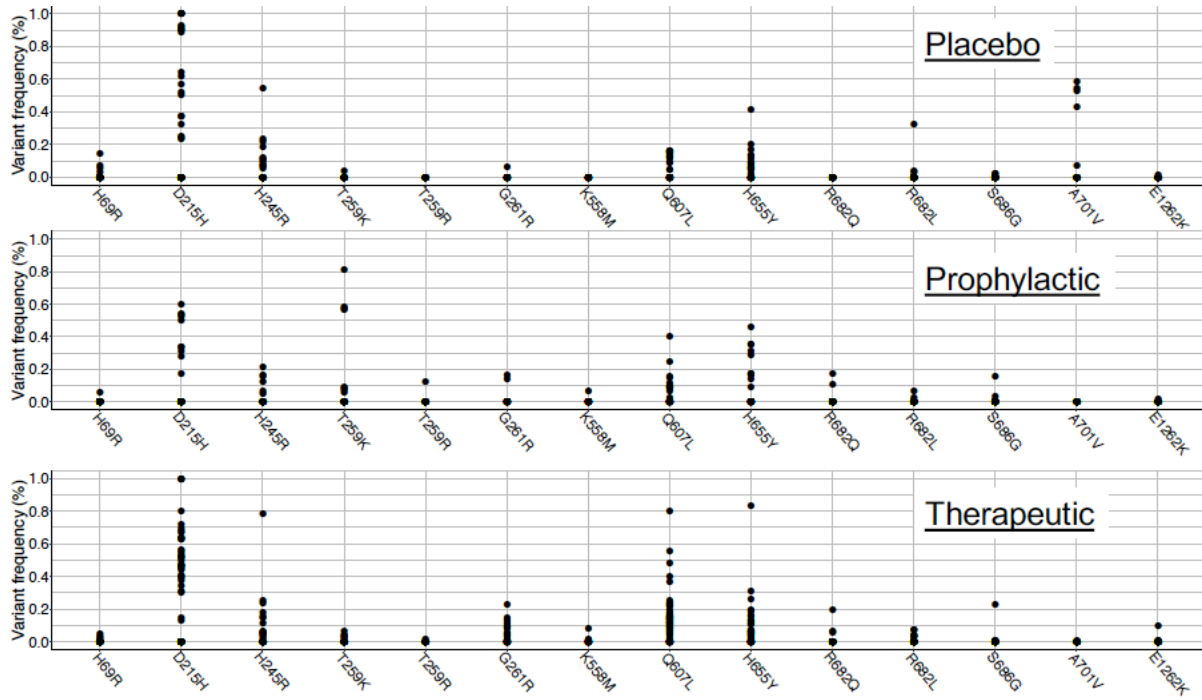
Figure 3.



**Figure 3. Efficacy of REGN-COV2 in treatment and prophylaxis in the golden Syrian hamster model of SARS-CoV-2 infection. (A) Study design overview, (B) Impact of REGN-COV2 on weight loss in prophylaxis and treatment, (C) Impact of REGN-COV-2 prophylaxis on levels of gRNA and sgRNA in hamster lungs (7dpi).**

Figure S1.

A



B

Genomic position	21768	22205	22296	22338	22338	22343	23235	23382	23525	23607	23607	23618	23664	25346
Reference nucleotide	A	G	A	C	C	G	A	A	C	G	G	A	C	G
Variant nucleotide	G	C	G	A	G	C	T	T	T	A	T	G	T	A
Gene name	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike	Spike
Gene position	206	643	734	776	776	781	1673	1820	1963	2045	2045	2056	2102	3784
Mutation nature	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP	nSNP
Amino acid position	69	215	245	259	259	261	558	607	655	682	682	686	701	1262
Reference amino acid	H	D	H	T	T	G	K	Q	H	R	R	S	A	E
Variant amino acid	R	H	R	K	R	R	M	L	Y	Q	L	G	V	K
Inoculum	1%	39%	1%	1%	0%	1%	0%	14%	3%	0%	0%	2%	0%	0%
Placebo group	0% - 14%	0% - 100%	0% - 55%	0% - 4%	0%	0% - 6%	0%	0% - 16%	0% - 41%	0%	0% - 32%	0% - 3%	0% - 58%	0% - 1%
Prophylactic group	0% - 6%	0% - 60%	0% - 21%	0% - 81%	0% - 12%	0% - 16%	0% - 7%	0% - 40%	0% - 46%	0% - 17%	0% - 6%	0% - 16%	0%	0% - 2%
Therapeutic group	0% - 5%	0% - 100%	0% - 79%	0% - 7%	0% - 1%	0% - 23%	0% - 8%	0% - 80%	0% - 83%	0% - 20%	0% - 7%	0% - 23%	0% - 1%	0% - 10%

**Figure S1. RNAseq analysis of viral RNA from NHP study #2.** (A) Virus RNA was sequenced and RNAseq analysis was performed to identify amino acid changes relative to virus inoculum sequence. The graph shows the frequencies of all amino acid changes identified in the spike protein across all virus sequences. Each dot represents the frequency of the corresponding amino acid change in a specific virus sample. Samples are grouped based on treatment regiment: isotype control (Placebo), therapeutic antibodies administered prior (Prophylactic) or following (Treatment) viral challenge. (B) Detailed genomic information on all amino acid changes identified within the spike protein sequence across all samples. For each sample, the frequency of all mutations has been calculated. These frequencies are shown as percentage of the virus population with the amino acid change in the input virus or as range of frequency percentages (lowest to highest %) in the virus populations isolated from the placebo, prophylactic and therapeutic groups.

Table S1.

Group	placebo				Prophylaxis								Treatment							
					0.3 mg/kg				50 mg/kg				25 mg/kg				150 mg/kg			
Animal No.	34851	37101	34706	37055	34946	36218	36839	37096	34346	34843	36670	37091	34716	34898	37077	37115	34024	35950	36993	37126
No of lobes examined	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
No of lobes with inflammation	2	1	0	3	2	0	2	3	1	0	0	0	0	1	1	0	1	0	1	0
Inflammation																				
Septa	1	1	0	1	1	0	1	1	1	0	0	0	0	1	1	0	1	0	2	0
Alveoli	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	0	0	0	1	0
Perivascular	1	1	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Pleura	0	1	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0
Syncytial cells	0	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0
Hyperplasia, Type II cells	0	1	1	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0
Increased alveolar macrophages	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	1

**Table S1. Pathology analysis in rhesus macaque lungs (NHP Study #2).** Pathology scores in individual animals treated with either REGN-COV-2 or placebo.

Severity score of lesions: Minimal (1); Mild (2); Moderate (3); Severe (4)