

1 **Replication-competent vesicular stomatitis virus vaccine vector protects against**
2 **SARS-CoV-2-mediated pathogenesis**

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23 **SUMMARY**

24 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused millions of
25 human infections and hundreds of thousands of deaths. Accordingly, an effective vaccine is of
26 critical importance in mitigating coronavirus induced disease 2019 (COVID-19) and curtailing
27 the pandemic. We developed a replication-competent vesicular stomatitis virus (VSV)-based
28 vaccine by introducing a modified form of the SARS-CoV-2 spike gene in place of the native
29 glycoprotein gene (VSV-eGFP-SARS-CoV-2). Immunization of mice with VSV-eGFP-SARS-
30 CoV-2 elicits high titers of antibodies that neutralize SARS-CoV-2 infection and target the
31 receptor binding domain that engages human angiotensin converting enzyme-2 (ACE2). Upon
32 challenge with a human isolate of SARS-CoV-2, mice expressing human ACE2 and immunized
33 with VSV-eGFP-SARS-CoV-2 show profoundly reduced viral infection and inflammation in the
34 lung indicating protection against pneumonia. Finally, passive transfer of sera from VSV-eGFP-
35 SARS-CoV-2-immunized animals protects naïve mice from SARS-CoV-2 challenge. These data
36 support development of VSV-eGFP-SARS-CoV-2 as an attenuated, replication-competent
37 vaccine against SARS-CoV-2.

38

39 INTRODUCTION

40 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense,
41 single-stranded, enveloped RNA virus, is the causative agent of coronavirus disease 2019
42 (COVID-19). Since its outbreak in Wuhan, China in December, 2019, SARS-CoV-2 has infected
43 millions of individuals and caused hundreds of thousands of deaths worldwide. Because of its
44 capacity for human-to-human transmission, including from asymptomatic individuals, SARS-
45 CoV-2 has caused a pandemic, leading to significant political, economic, and social disruption
46 (Bai et al., 2020). Currently, social quarantine, physical distancing, and vigilant hand hygiene
47 are the only effective preventative measures against SARS-CoV-2 infections. Thus, effective
48 countermeasures, particularly vaccines, are urgently needed to curtail the virus spread, limit
49 morbidity and mortality, and end the COVID-19 pandemic.

50 The SARS-CoV-2 spike (S) protein mediates the receptor-binding and membrane fusion
51 steps of viral entry. The S protein also is the primary target of neutralizing antibodies (Baum et
52 al., 2020; Chi et al., 2020; Pinto et al., 2020; Rogers et al., 2020) and can elicit CD4⁺ and CD8⁺
53 T cell responses (Grifoni et al., 2020). Several SARS-CoV-2 vaccine platforms based on the S
54 protein are being developed, including adenovirus-based vectors, inactivated virus formulations,
55 recombinant subunit vaccines, and DNA- and mRNA-based strategies (Amanat and Krammer,
56 2020; Lurie et al., 2020). While several of these vaccines have entered human clinical trials,
57 efficacy data in animals has been published for only a subset of these candidates (Gao et al.,
58 2020; Yu et al., 2020).

59 We recently reported the generation and characterization of a replication-competent,
60 VSV (designated VSV-eGFP-SARS-CoV-2) that expresses a modified form of the SARS-CoV-2
61 spike (Case et al., 2020). We demonstrated that monoclonal antibodies, human sera, and
62 soluble ACE2-Fc potently inhibit VSV-eGFP-SARS-CoV-2 infection in a manner nearly identical
63 to a clinical isolate of SARS-CoV-2. This suggests that chimeric VSV displays the S protein in
64 an antigenic form that resembles native infectious SARS-CoV-2. Because of this data, we

65 hypothesized that a replicating VSV-eGFP-SARS-CoV-2 might serve as an alternative platform
66 for vaccine development. Indeed, an analogous replication-competent recombinant VSV
67 vaccine expressing the Ebola virus (EBOV) glycoprotein protects against lethal EBOV challenge
68 in several animal models (Garbutt et al., 2004; Jones et al., 2005), is safe in
69 immunocompromised nonhuman primates (Geisbert et al., 2008), and was approved for clinical
70 use in humans after successful clinical trials (Henao-Restrepo et al., 2017; Henao-Restrepo et
71 al., 2015). Other live-attenuated recombinant VSV-based vaccines are in pre-clinical
72 development for HIV-1, hantaviruses, filoviruses, arenaviruses, and influenza viruses (Brown et
73 al., 2011; Furuyama et al., 2020; Garbutt et al., 2004; Geisbert et al., 2005; Jones et al., 2005).

74 Here, we determined the immunogenicity and *in vivo* efficacy of VSV-eGFP-SARS-CoV-
75 2 as a vaccine candidate in a mouse model of SARS-CoV-2 pathogenesis. We demonstrate that
76 a single dose of VSV-eGFP-SARS-CoV-2 generates a robust neutralizing antibody response
77 that targets both the SARS-CoV-2 spike protein and the receptor binding domain (RBD) subunit.
78 Upon challenge with infectious SARS-CoV-2, mice immunized with one or two doses of VSV-
79 eGFP-SARS-CoV-2 showed significant decreases in lung and peripheral organ viral loads, pro-
80 inflammatory cytokine responses, and consequent lung disease. VSV-eGFP-SARS-CoV-2-
81 mediated protection likely is due in part to antibodies, as passive transfer of immune sera to
82 naïve mice limits infection after SARS-CoV-2 challenge. This study paves the way for further
83 development of a VSV-vectored SARS CoV-2 vaccine.

84

85 RESULTS

86 **Generation of a VSV-eGFP-SARS-CoV-2 as a vaccine platform.** We previously
87 reported a chimeric, replication-competent VSV expressing the SARS-CoV-2 spike protein as
88 an effective platform for measuring neutralizing antibodies (Case et al., 2020). As replication-
89 competent VSVs are in clinical use as vaccines for emerging RNA viruses or in pre-clinical
90 development (Fathi et al., 2019), we tested whether VSV-eGFP-SARS-CoV-2 could protect
91 mice against SARS-CoV-2.

92 To examine the immune response to VSV-eGFP-SARS-CoV-2, we immunized four-
93 week-old BALB/c mice with 10^6 plaque-forming units (PFU) of VSV-eGFP-SARS-CoV-2 or a
94 control, VSV-eGFP (**Fig 1A**). As murine ACE2 does not serve as a receptor for SARS-CoV-2,
95 we spiked our preparation of VSV-eGFP-SARS-CoV-2 with trace amounts of VSV G to permit a
96 single round of infection, an approach used previously for SARS-CoV (Kapadia et al., 2008). At
97 28 days post-priming, one cohort of animals was boosted with the homologous vaccine. Serum
98 was isolated from all animals at three weeks post priming or boosting, and IgG titers against
99 recombinant SARS-CoV-2 S protein or the RBD were determined by ELISA (**Fig 1B-C**).
100 Immunization with VSV-eGFP-SARS-CoV-2 induced high levels of anti-S and anti-RBD-specific
101 IgG compared to control VSV-eGFP with reciprocal median serum endpoint titers of 3.2×10^5
102 and 2.7×10^6 (anti-S) and 1.1×10^4 and 1.4×10^4 (anti-RBD) for one and two doses of vaccine,
103 respectively.

104 We measured neutralizing antibody titers against SARS-CoV-2 after priming or boosting
105 using a focus-reduction neutralization test (Case et al., 2020). Immunization with a single or
106 two-dose regimen of VSV-eGFP-SARS-CoV-2 induced neutralizing antibodies (median titers of
107 1/59 and 1/5206, respectively) whereas the control VSV-eGFP vaccine did not (**Fig 1D**).
108 Boosting was effective and resulted in a 90-fold increase in neutralizing activity after the second
109 dose of VSV-eGFP-SARS-CoV-2. Collectively, these data suggest that VSV-eGFP-SARS-CoV-

110 2 is immunogenic and elicits high titers of antibodies that neutralize infection and target the RBD
111 of the SARS-CoV-2 S protein.

112 **VSV-eGFP-SARS-CoV-2 protects mice against SARS-CoV-2 infection.** Four weeks
113 after priming or priming and boosting, mice were challenged with SARS-CoV-2 (strain 2019 n-
114 CoV/USA_WA1/2020) after delivery of a replication-defective adenovirus expressing human
115 ACE2 (AdV-hACE2) that enables receptor expression in the lungs (Hassan et al., 2020).
116 Immunized mice were administered 2 mg of anti-Ifnar1 mAb one day prior to intranasal delivery
117 of AdV-hACE2. We administer anti-Ifnar1 antibody to augment virus infection and create a
118 stringent disease model for vaccine protection. Five days later, mice were inoculated with $3 \times$
119 10^5 PFU of SARS-CoV-2 via the intranasal route (**Fig 1A**) and subsequently, we measured viral
120 yield by plaque and RT-qPCR assays. At day 4 post-infection (dpi) infectious virus was not
121 recovered from lungs of mice vaccinated either with one or two doses of VSV-eGFP-SARS-
122 CoV-2 (**Fig 2A**). For mice receiving only one dose of VSV-eGFP-SARS-CoV-2 vaccine, we
123 observed a trend towards decreased levels of viral RNA in the lung, spleen, and heart at 4 dpi
124 and in the lung and spleen at 8 dpi compared to the control VSV-eGFP vaccinated mice (**Fig**
125 **2B-E**). Mice that received two doses of VSV-eGFP-SARS-CoV-2 had significantly lower levels
126 of viral RNA in most tissues examined compared to control VSV-eGFP vaccinated mice (**Fig**
127 **2B-E**). Consistent with our viral RNA measurements, we observed less SARS-CoV-2 RNA by *in*
128 *situ* hybridization in lung tissues of VSV-eGFP-SARS-CoV-2 immunized mice at 4 dpi (**Fig 2F**).
129 Collectively, these data support that immunization with VSV-eGFP-SARS-CoV-2 protects
130 against SARS-CoV-2 infection in mice.

131 **VSV-eGFP-SARS-CoV-2 limits SARS-CoV-2-induced lung inflammation.** Both
132 SARS-CoV and SARS-CoV-2 typically cause severe lung infection and injury that is associated
133 with high levels of pro-inflammatory cytokines and immune cell infiltrates (Gu and Korteweg,
134 2007; Huang et al., 2020). The AdV-hACE2 transduced mouse model of SARS-CoV-2
135 pathogenesis recapitulates several aspects of lung inflammation and coronavirus disease

136 (Hassan et al., 2020). To assess whether VSV-eGFP-SARS-CoV-2 limits virus-induced
137 inflammation, we measured pro-inflammatory cytokine and chemokine mRNA in lung
138 homogenates from vaccinated animals at 4 dpi by RT-qPCR assays (**Fig 3A**). Animals
139 immunized with one or two doses of VSV-eGFP-SARS-CoV-2 had significantly lower levels of
140 pro-inflammatory cytokine and chemokine mRNA compared to VSV-eGFP vaccinated mice.
141 Specifically, type I and III interferons (IFN- β and IFN- λ) were decreased early during infection in
142 both one-dose and two-dose groups of mice immunized with VSV-eGFP-SARS-CoV-2. While
143 there were no detectable differences in IFN- γ or TNF- α levels between groups, IL-6 and IL-1 β
144 were lower at 4 dpi after VSV-eGFP-SARS-CoV-2 vaccination. Similarly, levels of mRNAs
145 encoding chemokines CXCL1, CXCL10, and CXCL11, which recruit immune cells to the lung,
146 were decreased at 4 dpi in VSV-eGFP-SARS-CoV-2 compared to VSV-eGFP immunized mice.

147 To determine the extent of lung pathology in SARS-CoV-2 challenged mice, at 8 dpi, we
148 stained lung sections with hematoxylin and eosin (**Fig 3B**). Lung sections from VSV-eGFP-
149 immunized mice showed immune cell (including neutrophil) infiltration into perivascular,
150 peribronchial, and alveolar locations consistent with viral pneumonia. Lung sections from mice
151 immunized with one dose of VSV-eGFP-SARS-CoV-2 also showed some signs of inflammation.
152 However, mice immunized with two doses of VSV-eGFP-SARS-CoV-2 showed substantially
153 less accumulation of inflammatory cells at the same time point after SARS-CoV-2 infection.
154 These data suggest that immunization with VSV-eGFP-SARS-CoV-2 generates a protective
155 immune response, which limits SARS-CoV-2-induced lung disease in mice. In this model, two
156 sequential immunizations show greater efficacy than a single one.

157 **Vaccine-induced sera limits SARS-CoV-2 infection.** To investigate the contribution of
158 antibodies in vaccine-mediated protection, we performed passive transfer studies. Serum was
159 collected from VSV-eGFP and VSV-eGFP-SARS-CoV-2 vaccinated mice after one or two
160 immunizations. Ten-week-old female BALB/c mice were administered anti-Ifnar1 mAb and AdV-

161 hACE2 as described above to render animals susceptible to SARS-CoV-2. Five days later, 100
162 μ L of pooled immune or control sera was administered by intraperitoneal injection. One day
163 later, mice were inoculated with 3×10^5 PFU of SARS-CoV-2 via the intranasal route (**Fig 4A**).
164 Passive transfer of sera from animals vaccinated with VSV-eGFP-SARS-CoV-2 protected
165 against SARS-CoV-2 infection compared to sera from the VSV-eGFP-immunized mice. At 4 dpi,
166 lungs from animals treated with VSV-eGFP-SARS-CoV-2 immune sera from prime-only and
167 boosted animals showed substantially reduced infectious virus burden (**Fig 4B**). Although not as
168 striking, significant decreases in viral RNA levels also were observed in the lung and spleen of
169 animals receiving VSV-eGFP-SARS-CoV-2 boosted sera compared to the VSV-eGFP sera (**Fig**
170 **4C-D**). Possibly, some of the viral RNA in lung tissue homogenates after passive transfer may
171 represent neutralized virus within cells that has not yet been cleared. Viral RNA levels in the
172 heart of animals given sera from VSV-eGFP-SARS-CoV-2 boosted mice trended toward, but did
173 not reach, statistical significance (**Fig 4E**). No effect was observed in the nasal washes of any
174 treated group (**Fig 4F**), consistent with the results from our vaccinated and challenged animals
175 (**Fig 2E**).

176 To determine the effect of the passive transfer of sera on SARS-CoV-2-mediated
177 inflammation, we assessed the induction of several cytokines in the lung at 4 dpi (**Fig 4G**).
178 Treatment with sera from animals immunized with two doses of VSV-eGFP-SARS-CoV-2 limited
179 induction of some (IFN- β , IFN- λ , and IL-1 β) pro-inflammatory cytokines after SARS-CoV-2
180 challenge. Together, these data suggest that antibodies are a major correlate of VSV-eGFP-
181 SARS-CoV-2-mediated protection against SARS-CoV-2.

182

183 **DISCUSSION**

184 The emergence of SARS-CoV-2 into the human population has caused a global
185 pandemic, resulting in millions of infected individuals and hundreds of thousands of deaths.
186 Despite initial indications that the pandemic had peaked, reopening of countries and renewed
187 human-to-human contact has resulted in a recent surge in case numbers, suggesting that
188 SARS-CoV-2 vaccines will be critical for curtailing the pandemic and resuming normal social
189 interactions. In this study, we tested the efficacy of a replication-competent VSV-eGFP-SARS-
190 CoV-2 vaccine. A single dose of VSV-eGFP-SARS-CoV-2 was sufficient to induce antibodies
191 that neutralize SARS-CoV-2 infection and target the RBD and S protein, and a second dose
192 substantially boosted this response. We then challenged mice with SARS-CoV-2 via the
193 intranasal route and observed a complete loss of recovery of infectious virus in the lung in
194 animals immunized with either one or two doses of VSV-eGFP-SARS-CoV-2. Compared to a
195 single dose, administration of two doses of VSV-eGFP-SARS-CoV-2 elicited greater protection
196 with further diminished viral loads. Immunization with VSV-eGFP-SARS-CoV-2 decreased the
197 induction of several key pro-inflammatory cytokines and protected mice from alveolar
198 inflammation, lung consolidation, and viral pneumonia. We also established an important role for
199 protective antibodies, as passive transfer of immune sera from VSV-eGFP-SARS-CoV-2
200 immunized animals decreased viral burden and inflammation in the lung.

201 Recombinant VSV-based vaccines that encode viral glycoproteins have several
202 advantages as a platform. Whereas DNA plasmid and mRNA-based vaccines have not yet been
203 approved in the United States or elsewhere, Merck's ERVEBO®, a replication-competent VSV
204 expressing the EBOV glycoprotein, is currently in use in humans (Huttner et al., 2015). As a
205 replicating RNA virus, VSV-based vaccines often can be used as single-dose administration and
206 effectively stimulate both humoral and cellular immunity. Recombinant VSV grows efficiently in
207 mammalian cell culture, enabling simple, large-scale production. Advantages of VSV as a
208 vaccine vector also include the lack of homologous recombination and its non-segmented

209 genome structure, which precludes genetic reassortment and enhances its safety profile (Lichty
210 et al., 2004; Roberts et al., 1999). Unlike other virus-based vaccine vectors (Barouch et al.,
211 2004; Casimiro et al., 2003; Santra et al., 2007), there is little preexisting human immunity to
212 VSV as human infections are rare (Roberts et al., 1999) with the exception of some regions of
213 Central America (Cline, 1976) or a limited number of at-risk laboratory workers (Johnson et al.,
214 1966).

215 Several vaccine candidates for SARS-CoV-2 have been tested for immunogenicity. Our
216 VSV-eGFP-SARS-CoV-2 vaccine elicited high levels of inhibitory antibodies with median and
217 mean serum neutralizing titers of greater than 1/5,000. Two doses of VSV-eGFP-SARS-CoV-2
218 induced higher neutralizing titers with more rapid onset than similar dosing of an inactivated
219 SARS-CoV-2 vaccine in the same strain of mice (Gao et al., 2020). Consistent with these
220 results, serum anti-S endpoint titers were higher from mice immunized with two doses of VSV-
221 eGFP-SARS-CoV-2 (1/2,700,000) than the highest two-dose regimen of the inactivated virion
222 vaccine (1/820,000). Two doses of DNA plasmid vaccines encoding variants of the SARS-CoV-
223 2 S protein induced relatively modest neutralizing antibody responses (serum titer of 1/170) in
224 rhesus macaques. Related to this, anti-S titers were approximately 1,000-fold lower after two
225 doses of the optimal DNA vaccine (Yu et al., 2020) when compared to two doses of VSV-eGFP-
226 SARS-CoV-2. In a pre-print study, a single-dose of a chimpanzee adenovirus vectored vaccine
227 encoding SARS-CoV-2 S protein, ChAdOx1 nCoV-19, also produced relatively low levels of
228 serum neutralizing antibodies in mice and NHPs (1/40 to 1/80 in BALB/c and CD1 mice and <
229 1/20 in rhesus macaques). This data corresponded with anti-S1 and anti-S2 mean serum titers
230 of between 1/100 and 1/1,000 in BALB/c mice and anti-S titers of < 1/1,000 in NHPs (DOI:
231 10.1101/2020.05.13.093195). Two doses of a recombinant adenovirus type-5 vectored SARS-
232 CoV-2 vaccine in humans also produced relatively low RBD-binding (1/1,445 at day 28 post-
233 boost) and neutralizing antibody (1/34 at day 28 post-boost) (Zhu et al., 2020). Finally, based on
234 pre-print data (DOI: 10.1101/2020.06.11.145920), BALB/c mice immunized with two 1 µg doses

235 of an mRNA vaccine candidate, mRNA-1273, elicited serum anti-S endpoint titers of 1/100,000.
236 These mice produced mean neutralizing antibodies titers of approximately 1/1,000 and did not
237 show evidence of infectious virus in the lung or nares after SARS-CoV-2 challenge.

238 Even though VSV-eGFP-SARS-CoV-2 is replication-competent and capable of spread, it
239 likely did not do so efficiently in our BALB/c mice because the SARS-CoV-2 spike cannot
240 efficiently utilize murine ACE2 for viral entry (Letko et al., 2020). This likely explains our need for
241 boosting, as the response we observed likely was enabled by the residual small amount of
242 *trans*-complementing VSV G to pseudotype the virions expressing the S protein in a manner
243 similar to VSV-SARS (Kapadia et al., 2008), which effectively limited vaccine virus replication to
244 a single cycle. We anticipate that in animals expressing ACE2 receptors competent for S
245 binding, a single dose of VSV-eGFP-SARS-CoV-2 will be associated with greater
246 immunogenicity, and not require a second immunization for protection. To test this hypothesis,
247 immunization and challenge studies are planned in hACE2 transgenic mice (Bao et al., 2020;
248 Jiang et al., 2020; Sun et al., 2020) as they become widely available, and in hamsters and
249 NHPs.

250 Vaccine safety is a key requirement of any platform. Pathogenicity and immunogenicity
251 of VSV is associated with its native glycoprotein G, which, in turn, determines its pan-tropism
252 (Martinez et al., 2003). Replacing the glycoprotein of VSV with a foreign glycoprotein often
253 results in virus attenuation *in vivo*. Indeed, the vast majority of cases where VSV recombinants
254 express a heterologous viral glycoprotein (e.g., chikungunya virus, H5N1 influenza virus, Lassa
255 virus, lymphocytic choriomeningitis virus, or Ebola virus) and were injected via intracranial route
256 into mice or NHPs, no disease was observed (Mire et al., 2012; Muik et al., 2014; van den Pol et
257 al., 2017; Wollmann et al., 2015). One exception is when VSV expressing the glycoproteins of
258 the highly neurotropic Nipah virus was injected via an intracranial route into adult mice (van den
259 Pol et al., 2017). Should substantial reactogenicity or neuronal infection be observed with VSV-
260 eGFP-SARS-CoV-2, the vaccine could be attenuated further by introducing mutations into the

261 matrix protein (Rabinowitz et al., 1981) or methyltransferase (Li et al., 2006; Ma et al., 2014),
262 rearranging the order of genes (Ball et al., 1999; Wertz et al., 1998), or recoding of the L gene
263 (Wang et al., 2015). The presence of the additional eGFP gene inserted between the leader and
264 N genes also attenuates virus replication in cell culture (Whelan et al., 2000). Further
265 development of a VSV vectored vaccine for SARS-CoV-2 likely will require deletion of eGFP
266 from the genome, which may necessitate additional strategies of attenuation.

267 Future studies are planned to evaluate the durability of VSV-eGFP-SARS-CoV-2 and a
268 variant lacking eGFP in inducing immunity. Other replication-competent VSV-based vaccines
269 such as the rVSV Δ G-ZEBOV-GP have been shown to generate long-lasting immune responses
270 and protection (Kennedy et al., 2017). In addition, we plan to investigate in greater detail the
271 contributions of additional arms of immunity in mediating protection. The robust induction of
272 neutralizing antibodies elicited by one and two doses of VSV-eGFP-SARS-CoV-2 was a
273 correlate of protection, as passive transfer of immune sera reduced viral infection and
274 inflammation in the lung upon SARS-CoV-2 challenge. Nonetheless, it will be important to
275 determine if additional immune responses, particularly CD8⁺ T cells, have an important
276 protective role. Recently, SARS-CoV-2 specific CD4⁺ and CD8⁺ T cells were shown to be
277 present in 100% and 70% of COVID-19 convalescent patients, respectively, with many of the T
278 cells recognizing peptides derived from the S protein (Grifoni et al., 2020). Moreover, additional
279 experiments are planned in aged animals (hACE2-expressing mice, hamsters, and NHPs) to
280 address immunogenicity and protection in this key target population at greater risk for severe
281 COVID-19. Overall, our data show that VSV-eGFP-SARS-CoV-2 can protect against severe
282 SARS-CoV-2 infection and lung disease, supporting its further development as a vaccine.

283

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292

293 **AUTHOR CONTRIBUTIONS**

294 J.B.C. designed experiments, propagated the SARS-CoV-2 stocks, performed VSV
295 immunizations, and SARS-CoV-2 challenge experiments. P.W.R. generated the VSV vaccines.
296 J.B.C., R.E.C., N.M.K., J.M.F., S.S., and E.S.W. performed tissue harvests, histopathological
297 studies, and viral burden analyses. B.T.M. performed in situ hybridization. I.B.H. and B.S.
298 performed ELISAs. S.P.K. and M.J.H. analyzed the tissue sections for histopathology. J.B.C.
299 and R.E.C. performed neutralization assays. L.M.B. generated the VSV-eGFP control. J.B.C.,
300 P.W.R., S.P.J.W., and M.S.D. wrote the initial draft, with the other authors providing editing
301 comments.

302

303 **DECLARATION OF INTERESTS**

304 M.S.D. is a consultant for Inbios, Eli Lilly, Vir Biotechnology, NGM Biopharmaceuticals,
305 and on the Scientific Advisory Board of Moderna. M.J.H. is a member of the Data and Safety
306 Monitoring Board for AstroZeneca and founder of NuPeak Therapeutics. The Diamond
307 laboratory has received funding under sponsored research agreements from Moderna, Vir
308 Biotechnology, and Emergent BioSolutions. The Whelan laboratory has received funding under

309 sponsored research agreements from Vir Biotechnology. S.P.J.W. and P.W.R. have filed a
310 disclosure with Washington University for the recombinant VSV.

311 **FIGURE LEGENDS**

312 **Figure 1. Immunogenicity of VSV-eGFP-SARS-CoV-2.** **A.** Scheme of vaccination and
313 SARS-CoV-2 challenge. **B-D.** Four-week-old female BALB/c mice were immunized with VSV-
314 eGFP or VSV-eGFP-SARS-CoV-2. Some of the immunized mice were boosted with their
315 respective vaccines four weeks after primary vaccination. IgG responses in the sera of
316 vaccinated mice were evaluated three weeks after priming or boosting by ELISA for binding to
317 SARS-CoV-2 S (**B**) or RBD (**C**) or two weeks after priming or boosting by focus reduction
318 neutralization test (FRNT) (**D**) (n = 15 per group; one-way ANOVA with Dunnett's post-test: ****
319 $P < 0.0001$).

320 **Figure 2. VSV-eGFP-SARS-CoV-2 protects mice against SARS-CoV-2 infection.**
321 Three weeks after priming or boosting with VSV-eGFP or VSV-eGFP-SARS-CoV-2, immunized
322 animals were treated with anti-Ifnar1 mAb and one day later, animals were transduced with 2.5
323 $\times 10^8$ PFU of AdV-hACE2 by intranasal administration. Five days later, animals were challenged
324 with 3×10^5 PFU of SARS-CoV-2 via intranasal administration. **A-E.** At 4 or 8 dpi tissues were
325 harvested and viral burden was determined in the lung (**A-B**), spleen (**C**), heart (**D**), and nasal
326 washes (**E**) by plaque (**A**) or RT-qPCR (**B-E**) assay (n = 7-8 mice per group; Kruskal-Wallis test
327 with Dunn's post-test (**A-E**): ns, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P <$
328 0.0001). Dotted lines indicate the limit of detection. **F.** SARS-CoV-2 RNA *in situ* hybridization
329 of lungs of mice vaccinated with VSV-eGFP or VSV-eGFP-SARS-CoV-2 and challenged with
330 SARS-CoV-2 at 4 dpi. Images show low- (left; scale bars, 100 μm), medium- (middle; scale
331 bars, 100 μm), and high-power magnifications (right; scale bars, 10 μm ; representative
332 images from n = 3 per group).

333 **Figure 3. VSV-eGFP-SARS-CoV-2 protects mice from SARS-CoV-2 lung**
334 **inflammation** **A.** Lungs of VSV-eGFP or VSV-eGFP-SARS-CoV-2 immunized mice were

335 evaluated at 4 dpi for cytokine and chemokine expression by RT-qPCR assay. Data are shown
336 as fold-change in gene expression compared to fully naïve, age-matched animals after
337 normalization to *Gapdh* (n = 7-8 per group, Kruskal-Wallis test with Dunn's post-test: * $P < 0.05$,
338 ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). **B.** Hematoxylin and eosin staining of lung
339 sections from immunized mice at 8 dpi with SARS-CoV-2 (3×10^5 PFU). Images show low-
340 (left; scale bars, 250 μm), medium- (middle; scale bars, 50 μm), and high-power
341 magnifications (right; scale bars, 25 μm ; representative images from n = 3 mice per group).

342 **Figure 4. Vaccine-induced sera limits SARS-CoV-2 infection.** **A.** Passive transfer of
343 immune sera and SARS-CoV-2 challenge scheme. Ten-week-old female BALB/c mice were
344 treated with anti-Ifnar1 mAb and one day later, animals were transduced with 2.5×10^8 PFU of
345 AdV-hACE2 by intranasal administration. Four days later, animals were administered 100 μL of
346 pooled immune sera collected from VSV-eGFP or VSV-eGFP-SARS-CoV-2 vaccinated mice
347 after one or two immunizations. One day later, animals were challenged with 3×10^5 PFU of
348 SARS-CoV-2 via intranasal administration. **B-F.** At 4 dpi tissues were harvested and viral
349 burden was determined in the lung (**B-C**), spleen (**D**), heart (**E**), and nasal washes (**F**) by plaque
350 (**B**) or RT-qPCR (**C-F**) assays (n = 7 mice per group; Kruskal-Wallis test with Dunn's post-test
351 (**B-F**): * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$). Dotted lines indicate the limit of detection. **G.**
352 Lungs of mice treated with immune sera were evaluated at 4 dpi for cytokine expression by RT-
353 qPCR assay. Data are shown as fold-change in gene expression compared to naïve, age-
354 matched animals after normalization to *Gapdh* (n = 7 per group, Kruskal-Wallis test with Dunn's
355 post-test: * $P < 0.05$, ** $P < 0.01$).

356

357

358 **STAR METHODS**

359 **RESOURCE AVAILABILITY**

360 **Lead Contact.** Further information and requests for resources and reagents should be
361 directed to and will be fulfilled by the Lead Contact, Michael S. Diamond
362 (diamond@wusm.wustl.edu).

363 **Materials Availability.** All requests for resources and reagents should be directed to
364 and will be fulfilled by the Lead Contact author. This includes mice, antibodies, viruses, and
365 proteins. All reagents will be made available on request after completion of a Materials Transfer
366 Agreement.

367 **Data and code availability.** All data supporting the findings of this study are available
368 within the paper and are available from the corresponding author upon request.

369

370 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

371 **Cells.** BSRT7/5, Vero CCL81, Vero E6, Vero E6-TMPRSS2 (Case et al., 2020), and
372 Vero-furin (Mukherjee et al., 2016) cells were maintained in humidified incubators at 34 or 37°C
373 and 5% CO₂ in DMEM (Corning) supplemented with glucose, L-glutamine, sodium pyruvate,
374 and 10% fetal bovine serum (FBS).

375 **Plasmids.** The S gene of SARS-CoV-2 isolate Wuhan-Hu-1 (GenBank MN908947.3)
376 was cloned into the backbone of the infectious molecular clone of VSV containing eGFP (pVSV-
377 eGFP) as described (Case et al., 2020). pVSV-eGFP was used as previously described, but
378 contains a mutation K535R, the phenotype of which will be described elsewhere. Expression
379 plasmids of VSV N, P, L, and G were previously described (Stanifer et al., 2011; Whelan et al.,
380 1995).

381 **Recombinant VSV.** VSV-eGFP-SARS-CoV-2 and VSV-eGFP were generated and
382 rescued as described previously (Case et al., 2020; Whelan et al., 1995). Briefly, BSRT7/5 cells
383 (Buchholz et al., 1999) were infected with vaccinia virus encoding the bacteriophage T7 RNA

384 polymerase (vTF7-3) (Fuerst et al., 1986) and subsequently transfected with plasmids encoding
385 VSV N, P, L, G, and an antigenome copy of the viral genome under control of the T7 promoter.
386 Rescue supernatants were collected 56 to 72 h post-transfection, clarified by centrifugation (5
387 min at 1,000 x g), and filtered through a 0.22 μ m filter. Virus clones were plaque-purified on
388 Vero CCL81 cells containing 25 μ g/ml of cytosine arabinoside (Sigma-Aldrich) in the agarose
389 overlay, and plaques were amplified on Vero CCL81 cells. All infections for generating stocks
390 were performed at 37°C for 1 h and at 34°C thereafter. Viral supernatants were harvested upon
391 extensive cytopathic effect and clarified of cell debris by centrifugation at 1,000 x g for 5 min.
392 Aliquots were maintained at -80°C.

393 **Mouse experiments.** Animal studies were carried out in accordance with the
394 recommendations in the Guide for the Care and Use of Laboratory Animals of the National
395 Institutes of Health. The protocols were approved by the Institutional Animal Care and Use
396 Committee at the Washington University School of Medicine (assurance number A3381-01).
397 Virus inoculations were performed under anesthesia that was induced and maintained with
398 ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

399 At four weeks of age, female BALB/c mice (Jackson Laboratory, 000651) were
400 immunized with 10^6 PFU of VSV-eGFP-SARS-CoV-2 or VSV-eGFP via the intraperitoneal route.
401 Where indicated, mice were boosted with homologous virus at 4 weeks post-priming. Three
402 weeks post-priming or boosting mice were administered 2 mg of anti-Ifnar1 mAb (MAR1-5A3
403 (Sheehan et al., 2006), Leinco) via intraperitoneal injection. One day later, mice were
404 administered 2.5×10^8 PFU of mouse codon-optimized AdV-hACE2 (Hassan et al., 2020) via
405 intranasal administration. Five days later, vaccinated mice were challenged with 3×10^5 PFU of
406 SARS-CoV-2 via intranasal administration. Passive transfer experiments were conducted as
407 described above but using ten-week-old female BALB/c mice. Pooled immune sera were
408 administered 24 h prior to SARS-CoV-2 challenge. For each immunization (prime or boost),
409 serum from individual mice was collected twice (at days 14 and 22) and pooled.

410

411 **METHOD DETAILS**

412 **Gradient purification of recombinant viruses.** To generate high titer stocks of VSV-
413 eGFP and VSV-eGFP-SARS-CoV-2, viruses were grown on BSRT7/5 cells at an MOI of 3 or 1,
414 respectively. To generate VSV-eGFP-SARS-CoV-2, BSRT7/5 cells were transfected with
415 pCAGGS-VSV-G in Opt-MEM (Gibco) using Lipofectamine 2000 (Invitrogen) and subsequently
416 infected 8 to 12 h later with VSV-eGFP-SARS-CoV-2 at an MOI of 0.01 in DMEM containing 2%
417 FBS and 20 mM HEPES pH 7.7. This VSV G decorated VSV-eGFP-SARS-CoV-2 was titrated
418 by plaque assay and used for a larger scale infection as described above. Cell supernatants
419 were collected after 48 h and clarified by centrifugation at 1,000 x g for 7.5 min. Supernatants
420 were concentrated using a Beckman Optima L-100 XP ultracentrifuge (22,800 RPM x 90 min in
421 a 70Ti fixed-angle rotor). Pellets were resuspended in 100 mM NaCl, 10 mM Tris pH 7.4, 1 mM
422 EDTA (NTE) at 4°C overnight, and virus banded on a 15-45% sucrose-NTE gradient (35,000
423 rpm x 3 h in a SW-41Ti swinging-bucket rotor). Virus was extracted by side puncture of tubes,
424 recovered by ultracentrifugation (22,800 RPM x 90 min in a 70Ti fixed-angle rotor) and
425 resuspended in NTE at 4°C overnight. The VSV-eGFP was purified similarly.

426 **Measurement of viral burden.** Mouse tissues were weighed and homogenized with
427 sterile zirconia beads in a MagNA Lyser instrument (Roche Life Science) in 1 mL of DMEM
428 media supplemented to contain 2% heat-inactivated FBS. Tissue homogenates were clarified by
429 centrifugation at 10,000 rpm for 5 min and stored at -80°C. RNA was extracted using MagMax
430 mirVana Total RNA isolation kit (Thermo Scientific) and a Kingfisher Flex extraction machine
431 (Thermo Scientific). Infectious viral titers in lung homogenates were determined by plaque
432 assays on Vero-furin cells. Viral RNA levels were determined by RT-qPCR as described
433 (Hassan et al., 2020) and normalized to tissue weight.

434 **Cytokine analysis.** Total RNA was isolated from lung homogenates as described above
435 and DNAase treated. cDNA was generated using the HighCapacity cDNA Reverse

436 Transcription kit (Thermo Scientific) with the addition of RNase inhibitor according to the
437 manufacturer's instructions. Cytokine and chemokine expression were determined using
438 TaqMan Fast Universal PCR master mix (Thermo Scientific) with commercially available
439 primer/probe sets specific for *IFN-γ* (IDT: Mm.PT.58.41769240), *IL-6* (Mm.PT.58.10005566), *IL-*
440 *1β* (Mm.PT.58.41616450), *TNF-α* (Mm.PT.58.12575861), *CXCL10* (Mm.PT.58.43575827),
441 *CCL2* (Mm.PT.58.42151692), *CCL5* (Mm.PT.58.43548565), *CXCL11* (Mm.PT.58.10773148.g),
442 *IFN-β* (Mm.PT.58.30132453.g), and *IFNλ-2/3* (Thermo Scientific Mm04204156_gH). All results
443 were normalized to *GAPDH* (Mm.PT.39a.1) levels and the fold-change for each was determined
444 using the $2^{-\Delta\Delta Ct}$ method comparing SARS-CoV-2 infected mice to naïve controls.

445 **Histology and *in situ* hybridization.** Mice were euthanized, and tissues were
446 harvested prior to lung inflation and fixation. The right lung was inflated with approximately 1.2
447 mL of 10% neutral buffered formalin using a 3-mL syringe and catheter inserted into the
448 trachea. To ensure fixation of virus, inflated lungs were kept in a 40-mL suspension of neutral
449 buffered formalin for 7 days before further processing. Tissues were paraffin-embedded and
450 sections were subsequently stained with hematoxylin and eosin. RNA *in situ* hybridization was
451 performed using the RNAscope 2.5 HD Assay (Brown Kit) according to the manufacturer's
452 instructions (Advanced Cell Diagnostics). Briefly, sections were deparaffinized and treated with
453 H₂O₂ and Protease Plus prior to RNA probe hybridization. Probes specifically targeting SARS-
454 CoV-2 S sequence (cat no 848561) were hybridized followed by signal amplification and
455 detection with 3,3'-Diaminobenzidine. Tissues were counterstained with Gill's hematoxylin and
456 an uninfected mouse was stained in parallel and used as a negative control. The lung pathology
457 was evaluated, and representative photomicrographs were taken of stained slides under
458 investigator-blinded conditions. Tissue sections were visualized using a Nikon Eclipse
459 microscope equipped with an Olympus DP71 color camera or a Leica DM6B microscope
460 equipped with a Leica DFC7000T camera.

461 **Neutralization assay.** Serial dilutions of mouse sera were incubated with 10^2 focus-
462 forming units (FFU) of SARS-CoV-2 for 1 h at 37°C. Antibody-virus complexes were added to
463 Vero E6 cell monolayers in 96-well plates and incubated at 37°C for 1 h. Subsequently, cells
464 were overlaid with 1% (w/v) methylcellulose in MEM supplemented with 2% FBS. Plates were
465 harvested 30 h later by removing overlays and fixed with 4% PFA in PBS for 20 min at room
466 temperature. Plates were washed and sequentially incubated with 1 mg/mL of CR3022 (PMID:
467 32245784) anti-S antibody and HRP-conjugated goat anti-human IgG in PBS supplemented
468 with 0.1% saponin and 0.1% bovine serum albumin. SARS-CoV-2-infected cell foci were
469 visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot
470 microanalyzer (Cellular Technologies). Data were processed using Prism software (GraphPad
471 Prism 8.0).

472 **ELISA.** 6-well Maxisorp plates were coated with 2 ug/mL of either SARS-CoV-2 Spike,
473 RBD, NP, or ORF8 proteins in 50 mM Na_2CO_3 (70 μL) overnight at 4°C. Plates were then
474 washed with PBS + 0.05% Tween-20 and blocked with 200 μL 1X PBS + 0.05% Tween-20 +
475 1% BSA + 0.02% sodium azide for 2 h at room temperature (RT). Serum samples were serially
476 diluted (1:3) starting at either 1:100 dilution (day 22 samples) or 1:30 dilution (day 8 post-
477 infection samples) in blocking buffer. Diluted samples were then added to washed plates
478 (50 μL /well) and incubated for 1 h at RT. Bound IgG was detected using HRP-conjugated goat
479 anti-mouse IgG (at 1:2000) or bound IgM was detected using biotin-conjugated anti-mouse IgM
480 (at 1:10000), followed by streptavidin-HRP (at 1:5000). Following a 1 h incubation, washed
481 plates were developed with 50 μL of 1-Step Ultra TMB-ELISA, quenched with 2 M sulfuric acid,
482 and the absorbance was read at 450 nm.

483

484 **QUANTIFICATION AND STATISTICAL ANALYSIS**

485 Statistical significance was assigned when *P* values were < 0.05 using Prism Version 8
486 (GraphPad) and tests are indicated in the relevant Figure legends.

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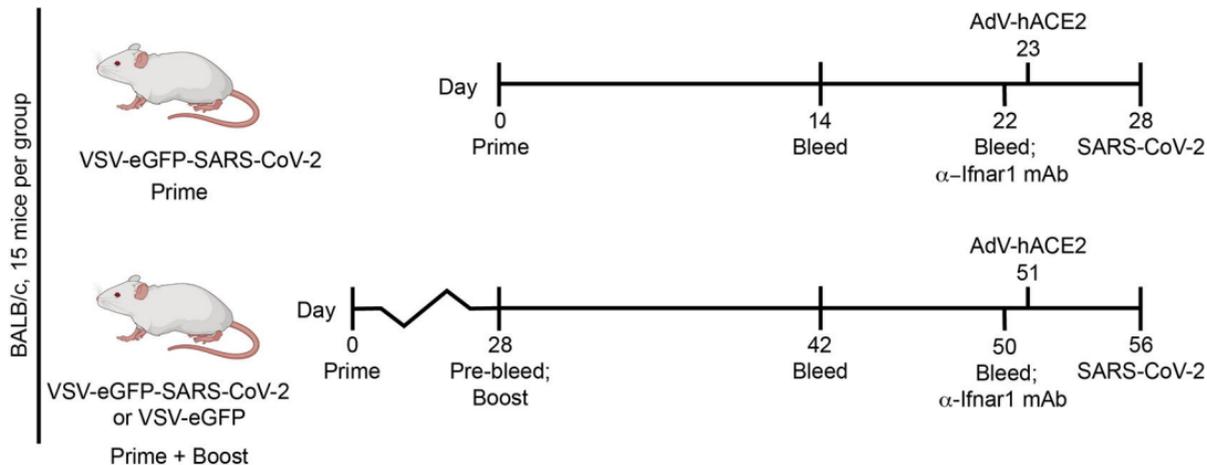
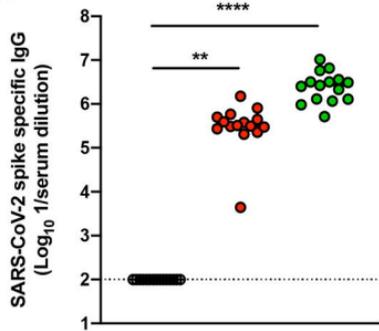
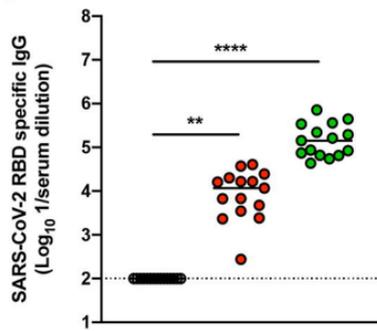
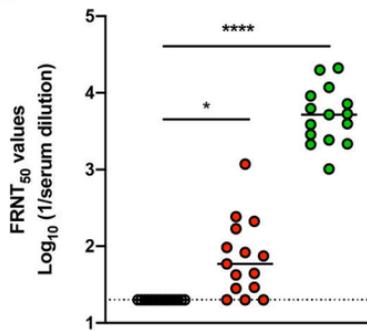
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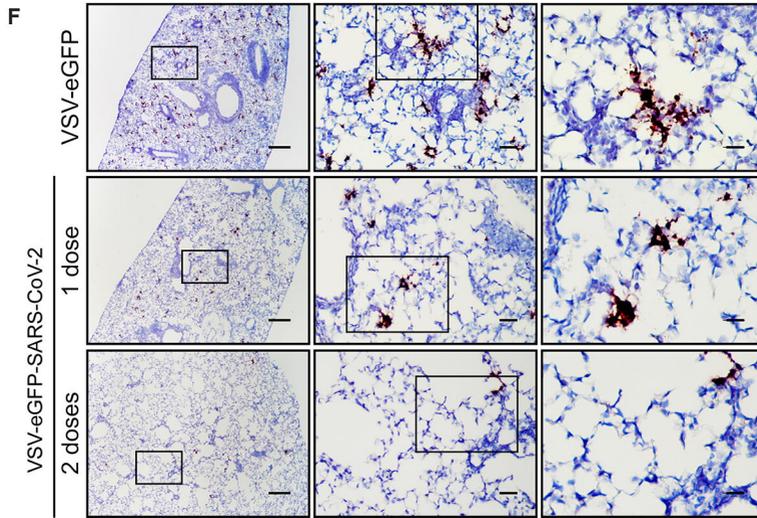
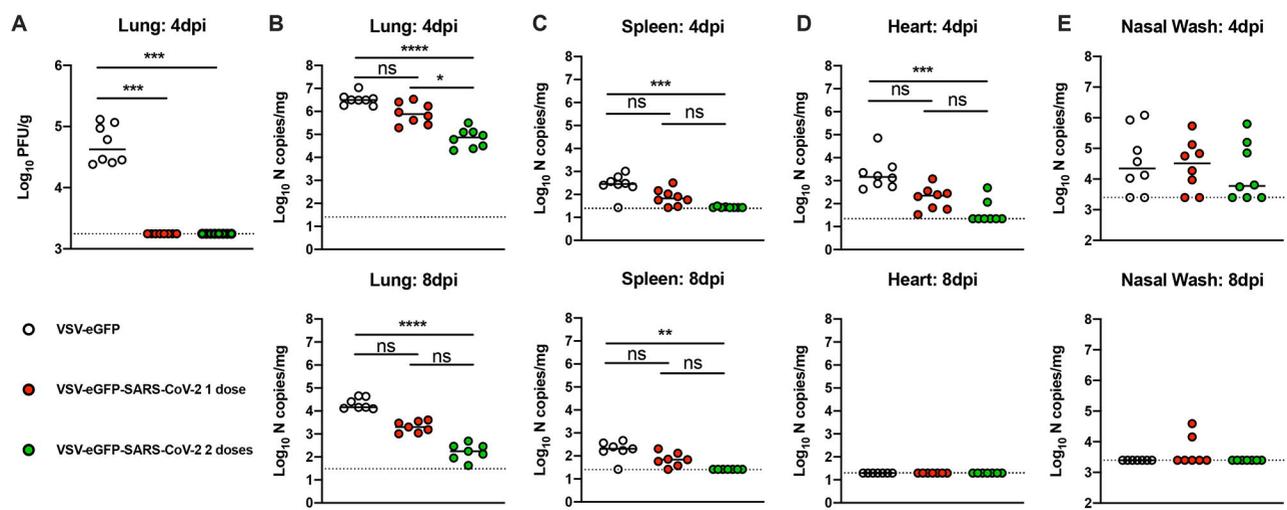
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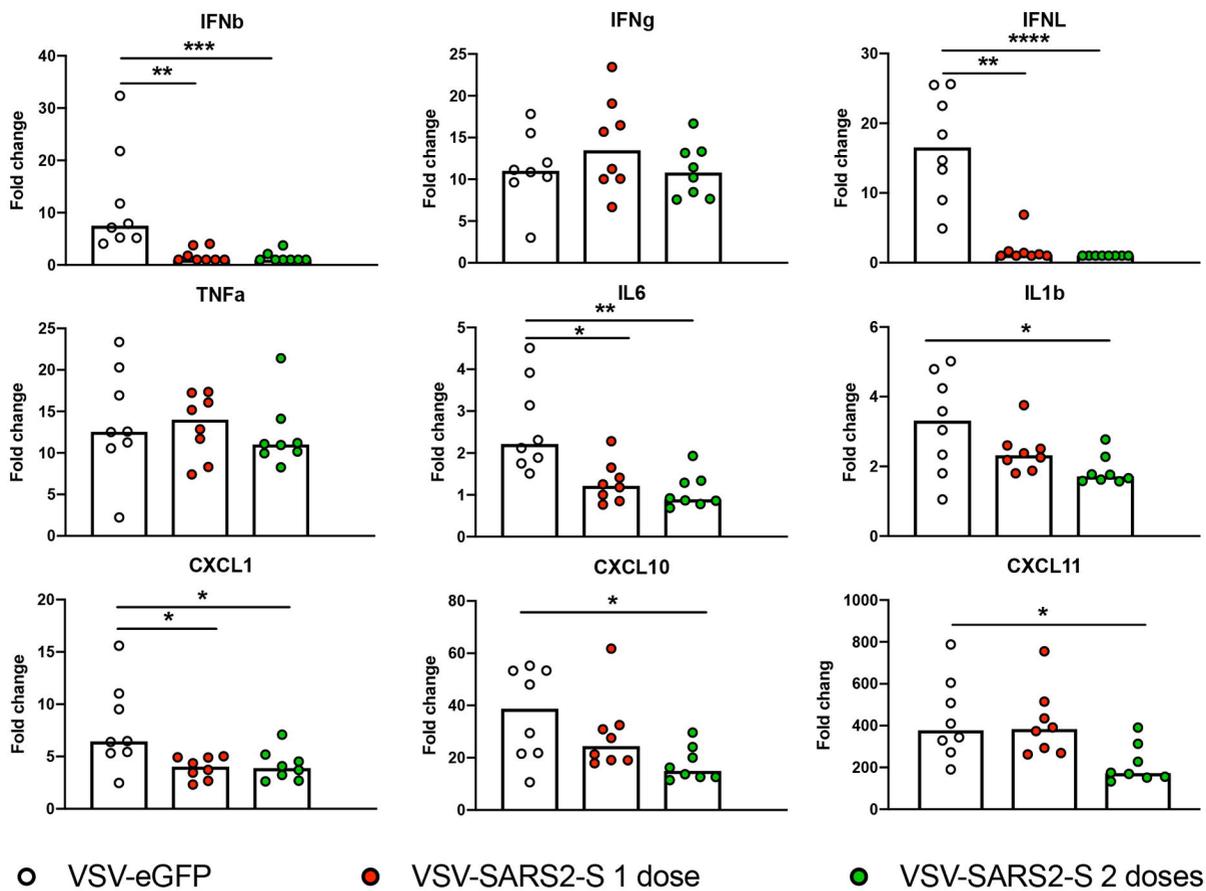
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A**B****Anti-Spike IgG****C****Anti-RBD IgG****D****SARS-CoV-2 neutralization titers**



A**B**