

1 **Rapid protection from COVID-19 in nonhuman primates vaccinated intramuscularly but not**
2 **intranasally with a single dose of a recombinant vaccine**

3

4 One sentence summary

5 VSV vaccine protects NHPs from COVID-19 in 10 days

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23

24 **Abstract**

25 The ongoing pandemic of Coronavirus disease 2019 (COVID-19) continues to exert a significant
26 burden on health care systems worldwide. With limited treatments available, vaccination remains an
27 effective strategy to counter transmission of severe acute respiratory syndrome coronavirus 2 (SARS-
28 CoV-2). Recent discussions concerning vaccination strategies have focused on identifying vaccine
29 platforms, number of doses, route of administration, and time to reach peak immunity against SARS-
30 CoV-2. Here, we generated a single dose, fast-acting vesicular stomatitis virus-based vaccine derived
31 from the licensed Ebola virus (EBOV) vaccine rVSV-ZEBOV, expressing the SARS-CoV-2 spike protein and
32 the EBOV glycoprotein (VSV-SARS2-EBOV). Rhesus macaques vaccinated intramuscularly (IM) with a
33 single dose of VSV-SARS2-EBOV were protected within 10 days and did not show signs of COVID-19
34 pneumonia. In contrast, intranasal (IN) vaccination resulted in limited immunogenicity and enhanced
35 COVID-19 pneumonia compared to control animals. While IM and IN vaccination both induced
36 neutralizing antibody titers, only IM vaccination resulted in a significant cellular immune response. RNA
37 sequencing data bolstered these results by revealing robust activation of the innate and adaptive
38 immune transcriptional signatures in the lungs of IM-vaccinated animals only. Overall, the data
39 demonstrates that VSV-SARS2-EBOV is a potent single-dose COVID-19 vaccine candidate that offers
40 rapid protection based on the protective efficacy observed in our study.

41

42 Introduction

43 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense, single-
44 stranded RNA virus first isolated from a patient with severe respiratory illness in Wuhan, China (1).
45 SARS-CoV-2 infection manifests as a clinical syndrome termed Coronavirus disease 2019 (COVID-19),
46 which can lead to respiratory failure (2). In addition to respiratory distress, other clinical manifestations
47 associated with SARS-CoV-2 infection include cardiac pathology, gastrointestinal disease, coagulopathy,
48 and hyperinflammatory syndrome (3-5). Patients with an increased risk of severe clinical manifestation
49 include the elderly, immunocompromised, and individuals with co-morbidities (obesity, diabetes,
50 hypertension etc.)(6). Virtually every country has been affected with almost one hundred million
51 infections to date and an estimated case fatality rate of ~2% (<https://coronavirus.jhu.edu/map.html>).
52 The widespread morbidity, mortality, and socioeconomic impact of COVID-19 emphasize the urgent
53 need for the development and deployment of countermeasures, including vaccines.

54 The COVID-19 pandemic has made the development of a vaccine a global priority (7-9). An ideal
55 vaccine candidate is safe, effective, fast-acting, rapidly deployable, and requires only a single
56 immunization. Most of the current vaccine candidates encode the trimeric SARS-CoV-2 spike (S) protein
57 as the primary antigen. S is essential for SARS-CoV-2 infectivity since it binds the angiotensin-converting
58 enzyme 2 (ACE2) receptor and promotes viral-cell membrane fusion (10). It is also the main target for
59 virus neutralization (11). The route of vaccination can greatly influence the local immune environment at
60 the vaccination and infection site. Recently, the comparison of intramuscular (IM) and intranasal (IN)
61 vaccination of mice with a chimpanzee adenoviral vector-based vaccine revealed an increase in
62 stimulation of local mucosal immunity and generation of antigen-specific IgA and lung resident T cells
63 after IN vaccination. The local mucosal immunity was improved by the generation of antigen-specific IgA
64 and lung resident T cell generation after IN vaccination (12). Prior to progression to human clinical trials,
65 several COVID-19 vaccine candidates were IM administered to nonhuman primates (NHPs) to evaluate
66 their efficacy (13-16).

67 The recombinant vesicular stomatitis virus (VSV) vaccine platform has previously been utilized in
68 vaccines against multiple viral pathogens, such as Ebola virus (EBOV), Marburg, Nipah, and Lassa viruses
69 (17-19). VSV-based vaccines elicit a robust and rapid immune response to the encoded antigen(s) after a
70 single immunization (20). The time to immunity has been demonstrated to be within 7-10 days for a
71 number of pathogens in preclinical and clinical studies, greatly reducing the time needed between
72 vaccination and protection (21-24). Multiple routes of VSV-based vaccination, such as IM and IN, have

73 been shown to be efficacious (21, 22, 25). Furthermore, the general population is predominantly
74 seronegative for VSV, circumventing pre-existing immunity neutralizing the vaccine virus (20). These
75 unique attributes - robust immune stimulation and time to immunity - make this an attractive vaccine
76 platform for SARS-CoV-2. However, the immunogenicity and efficacy of an IM- or IN-administered
77 COVID-19 VSV-based vaccine has not been tested in the NHP model.

78 In the present study, we developed a VSV-based vector expressing the SARS-CoV-2 S in
79 combination with the EBOV glycoprotein (GP). We utilized the NHP challenge model and compared the
80 vaccine efficacy with a shorter time to challenge in tandem with comparing the optimal route of
81 immunization. We demonstrate that IM-vaccinated NHPs developed no to mild lesions of COVID-19 with
82 variable immunopathology, whereas IN vaccination resulted immune-enhanced disease with interstitial
83 pneumonia in NHPs. IM vaccination resulted in robust and rapid humoral and cellular immune responses
84 while with IN vaccination did not. Transcriptional analysis of the lungs supports our immunological
85 findings by revealing greater expression of innate and adaptive immune genes in the IM vaccination
86 group.

87

88 **Results**

89 *Vaccine construction and characterization*

90 The VSV-backbone encoding the EBOV Kikwit GP, rVSV-ZEBOV, was used as a parental vector to
91 construct this COVID-19 vaccine. Therefore, we generated a VSV construct co-expressing the EBOV GP
92 and SARS-CoV-2 S (VSV-SARS2-EBOV) by the adding the full-length codon-optimized SARS-CoV-2 S
93 upstream of the EBOV GP into the existing VSV vector (Fig. S1A). The construct was recovered from
94 plasmid following previously established protocols (26). Expression of both antigens, SARS-CoV-2 S and
95 EBOV GP, was confirmed by Western blot analysis of the VSV particles in cell supernatant (Fig. S1B).
96 Next, we performed viral growth kinetics. VSV-SARS2-EBOV replicated with similar kinetics and had
97 comparable endpoint titers to the parental VSV-EBOV in Vero E6 cells (Fig. S1C), which does not impact
98 potential vaccine production.

99

100 *Efficacy in nonhuman primates (NHPs)*

101 We demonstrated previously that the parental VSV-EBOV is a fast-acting, IM-administered
102 vaccine (21); that was confirmed in human phase 3 clinical trials (24). Therefore, we set out to analyze
103 the fast-acting potential of a single dose of this VSV-based COVID-19 vaccine in rhesus macaques. Unlike

104 EBOV disease (EVD), COVID-19 is a respiratory disease. However, a previously published study
105 demonstrated that mucosal immunization with VSV-EBOV protected the NHPs from EVD (25). To
106 determine the efficacy of mucosal vaccination against COVID-19 in NHPs, we compared the efficacy of
107 IM and IN vaccination in the rhesus macaque model (27). Groups of 6 NHPs were either IN- or IM-
108 vaccinated with VSV-SARS2-EBOV while control animals received a single dose of VSV-EBOV IN (n=2) or
109 IM (n=2)(Fig. S1D). All NHPs were observed for potential adverse effects, particularly after IN vaccination
110 as this is not the standard route of administration for this vaccine platform, but no clinical changes were
111 noted. After 10 days, all NHPs were challenged with SARS-CoV-2 as previously described (27). On days
112 post challenge (dpc) 0, 1, 3, 5, and 7, a clinical exam including thoracic radiographs and nasal swab
113 collection was performed; in addition, the dpc 3 exam included a bronchoalveolar lavage (BAL). On dpc
114 7, all NHPs were euthanized and samples were collected for analysis. None of the vaccinated animals
115 displayed clinical signs of disease after challenge.

116 We determined differences in total SARS-CoV-2 RNA and subgenomic (sg) RNA in the nasal
117 swabs of the animals throughout the study. Interestingly, IN vaccination resulted in significantly lower
118 levels of nasal viral RNA on dpc 1 compared to IM indicating better local control of virus replication (Fig.
119 1A). However, on dpc 3 only the total SARS-CoV-2-specific RNA levels were significantly different (Fig.
120 1A). In contrast, both total and sg RNA levels in the BAL were significantly lower for the IM vaccination
121 group compared to IN-vaccinated and control groups (Fig. 1B). This finding is supported by our
122 observation that the IN-vaccinated NHPs had more lung infiltrates compared to the IM and control
123 groups at the time of euthanasia (Fig. 1C). Additionally, only IN-vaccinated NHPs exhibited lung lesions
124 (Fig. S2). This was accompanied by a significant reduction of total RNA and sgRNA in IM-vaccinated, but
125 not IN-vaccinated NHPs compared to controls (Fig. 1D). The comparison of RNA levels in individual lung
126 lobes and other examined tissue samples did not reveal any significant differences (Fig. S3).

127

128 *Histopathology in NHPs*

129 Histopathologic analysis of the collected lung samples revealed pulmonary pathology consistent
130 with the previously described rhesus macaque model of SARS-CoV-2 infection in the control group
131 regardless of IM or IN administration of the control vaccine (Fig. 2A,D)(27). In the IM vaccination group
132 pulmonary lesions consisted of low to moderate numbers of eosinophils multifocally infiltrating
133 bronchiolar mucosa, excess mucus accumulation in the lumen of bronchi and bronchioles, and profound
134 perivascular lymphocytic cuffing (interpreted as immune pathology) disseminated throughout all lung
135 lobes (Fig. 2B,E). In combination, these lesions are suggestive of a localized hypersensitivity response.

136 Hypersensitivity lesion location mirrored that of what has previously been described in this NHP model
137 of COVID-19 (27), with a high proportion of lesions located at the periphery of the lung and increased
138 lesion severity in lower lung lobes. Limited evidence of type I pneumocyte damage was present in rare
139 foci and was characterized by lining of alveoli by type II pneumocytes and a scant amount of
140 proteinaceous fluid within alveolar spaces (Fig. 2B,E). Histopathologic lesions in the IN vaccination group
141 mirrored the enhanced gross lesion severity and histologically consisted of an immune-enhanced
142 disease with evidence of classic moderate to severe SARS-CoV-2 pulmonary pathology and moderate
143 hypersensitivity response (Fig. 2C,F). The hypersensitivity response was similar to that observed in the
144 IM-vaccinated group, but more severe with the addition of eosinophil spillover into bronchiolar lumen
145 and moderate numbers of alveolar spaces. SARS-CoV-2 nucleoprotein immunoreactivity was observed in
146 type I pneumocytes and macrophages of both the control and IN groups, but not in the IM vaccination
147 group (Fig. 2G-I).

148

149 *Immune responses in NHPs*

150 We next analyzed the peripheral humoral response. IgG responses to the full-length SARS-CoV-2
151 S, the S receptor binding domain (RBD), and the EBOV GP were determined following vaccination and
152 challenge (Fig. 3). We demonstrated that the IM-vaccinated NHPs attained significantly higher SARS-
153 CoV-2 S-specific IgG starting 10 days after vaccination and following challenge compared to IN-
154 vaccinated NHPs and controls (Fig. 3A). Similarly, the IgG response to the SARS-CoV-2 S RBD was higher
155 at day 10 post vaccination (0 dpc) in the IM group compared to the IN and controls (Fig. 3B). Analysis of
156 the IgG subclasses in serum on dpc 0 and 7 showed that both vaccination routes resulted in
157 predominantly IgG1, IgG2 and IgG3 antibodies with no significant difference between the groups (Fig.
158 3C). Only IgG1 increased after challenge in both vaccine groups significantly compared to controls (Fig.
159 3C). Interestingly, on dpc 7 both IgG2 and IgG3 antibodies were largely absent in sera from IN-
160 vaccinated NHPs (Fig. 3C). IgG responses specific to EBOV GP support the finding that IM vaccination
161 appears more immunogenic compared to IN even though the data are only significantly different on day
162 0 and day 3 (Fig. S4A). Measurable SARS-CoV-2 neutralizing titers were detected as early as 10 days
163 following vaccination for the IM group, and 11 days for the IN group (Fig. 3D). On day 3, significantly
164 higher titers were observed comparing IM-vaccinated NHPs to controls only (Fig. 3D). At the time of
165 euthanasia, the neutralizing titers in IM- and IN-vaccinated NHPs were comparable but significantly
166 higher than those observed in control animals (Fig. 3D)

167 Next, we investigated the humoral responses in the BAL obtained on dpc 3. We detected SARS-
168 CoV-2 S-specific IgG in 3 of the 6 NHPs in the IM vaccinated group but only in 1 of 6 NHPs in the IN group
169 (Fig. 3E). Only the IM-vaccinated NHP with the highest titer of SARS-CoV-2 S-specific IgG had anti-SARS-
170 CoV-2 S RBD IgG (Fig. 3E). SARS-CoV-2 S-specific IgA was not detected in any of the BAL samples (Fig. 3E).
171 In contrast to serum, no IgG1 was detected; however, IgG2 and IgG3 were readily detected in several
172 IM-vaccinated NHPs (Fig. 3F). Unexpectedly, IM vaccination resulted in higher humoral responses in the
173 lung to all antigens including EBOV GP (Fig. S4B).

174 We analyzed the peripheral cellular response even though T cell responses have shown to play
175 only a limited role mediating protection using the VSV-EBOV vaccine (28, 29). Peripheral blood
176 mononuclear cells (PBMCs) were stimulated with a peptide-pool spanning the entire length of the SARS-
177 CoV-2 S and the antigen-specific T cells were identified using intracellular cytokine staining (Fig.4A,B).
178 While there was minimal cytokine production by CD4⁺ T cells, a significant increase in the CD69
179 activation marker was seen in IM-vaccinated NHPs on 0 and 7 dpc relative to controls (Fig. 4A). Similarly,
180 a significantly higher portion of the CD8⁺ T cells from the IM group produced granzyme B on dpc 0 and 7
181 compared to controls and IN groups (Fig. 4B). Interestingly, numbers of IL-2⁺ CD4⁺ and CD8⁺ T cells were
182 significantly lower in the IM group on dpc 0, and comparable to IN and control groups by dpc 7 (Fig.
183 4A,B). Additionally, a greater number of granzyme B⁺ NK cells was measured on dpc 0 and 7 in IM-
184 vaccinated animals compared to IN-vaccinated and control animals (Fig. 4C).

185 Finally, we monitored levels of systemic and BAL cytokines and chemokines. We found a
186 significant increase in MCP-1 on dpc 1 and 3 in IN-vaccinated animals compared to IM-vaccinated
187 animals. A similar trend was observed for IL-18 on dpc 3 and 5 (Fig. S5A). Levels of MIP-1 β were
188 significantly lower at all dpcs in IM-vaccinated animals compared to the control and IN-vaccinated
189 animals (Fig. S5A). Analysis of cytokine and chemokine levels in the BAL revealed that MCP-1 levels were
190 significantly decreased in IN-vaccinated NHPs (Fig. S5B). All other investigated cytokines did not show
191 significant differences.

192

193 *Transcriptional analysis of BAL samples*

194 To better understand the molecular underpinnings of differential vaccine responses, we profiled
195 the host transcriptional response in the BAL (dpc 3) samples. Principal component analysis (PCA) of BAL
196 samples indicated a distinct separation of uninfected/naïve (historical data) and the three challenged
197 groups, with no clear distinction between the challenged groups (Fig. S6A). Therefore, the samples from
198 control, IM- or IN-vaccinated animals were compared to the uninfected samples. Over 1,000

199 differentially expressed genes (DEGs) were detected in the challenged groups compared to the
200 uninfected animals, with most DEGs being upregulated (Fig. S6B). The majority of DEGs were shared
201 amongst the three challenged groups (Fig. 5A). Functional enrichment showed that upregulated and
202 downregulated DEGs shared by all three challenged groups play a role in regulating cell structure (e.g.,
203 “actin cytoskeleton organization”) and innate immunity (e.g., “positive regulation of cytokine
204 production”, “myeloid leukocyte activation”) (Fig. 5B). However, only upregulated DEGs enriched to
205 gene ontology (GO) terms associated with adaptive immunity (e.g., “lymphocyte activation”, “T cell
206 differentiation”)(Fig. 5B).

207 Further analysis of shared DEGs showed that genes involved in pro-inflammatory pathways (e.g.,
208 *RELB*, *MFHAS1*, *IL12RB1*, *TNFSF4*, *TRAF2*, *C5AR1*, and *TOLLIP*) were more highly expressed in the control
209 group compared to IM and IN groups (Fig. S6C, D). A second cluster of inflammation-related genes were
210 induced to a greater extent in the IN group (*IFT88*, *IL12B*, *CLEC9*, and *IL27RA*), which is in line with the
211 greater inflammatory response observed in these animals. On the other hand, DEGs implicated in T and
212 B cell-mediated immunity (e.g., *LCP1*, *PRKCB*) were induced to a greater extent in IM-vaccinated NHPs
213 (Fig. 5C). Genes enriching to GO term “myeloid cell activation/neutrophil downregulation” were
214 suppressed to a greater extent in the IM cohort, consistent with the lower inflammation profile
215 observed in this group (Fig. S6E).

216 We next analyzed vaccine-specific transcriptional responses in the BAL samples of the IN and IM
217 groups to elucidate unique molecular responses (Fig. 5D-G). DEGs unique to the IN group enriched to GO
218 terms related to organelle dynamics, such as “organelle localization” as well as antiviral immunity
219 (“human papillomavirus infection”) (Fig. 5D). Genes important for vesicular mobilization (*RAB8A* and
220 *RABD3D*) as well as antiviral *HERC5* were more highly expressed in IN-vaccinated NHPs whereas those
221 associated with signaling (e.g., *AKT2*, *PDE4D*) were more down-regulated (Fig. 5E) compared to IM-
222 vaccinated NHPs. DEGs uniquely upregulated in the IM group have roles in protein synthesis and folding
223 (e.g., *TRMT10B*, *TRNAU1AP*, *HSPB1*), cell proliferation (e.g., *KITLF*, *TM4SF19*) and T cell activation (e.g.,
224 *CRACR2A*, *SH2D2A*) (Fig. 5G).

225 Due to limited sample availability, we were unable to perform phenotyping of immune cells in
226 the BAL. Therefore, we performed *in silico* flow cytometry to infer changes in cell frequencies based on
227 the transcriptional landscape (Fig. S6F). This analysis predicted significant increases in the levels of
228 monocytes, NK cells and stimulated CD4 Th2 cells while frequencies of naïve & plasma B cell, CD4⁺ &
229 CD8⁺ T cells, stimulated dendritic cells and neutrophils were predicted to decline for all three challenged

230 groups. On the other hand, plasma cells and monocytes were predicted to be induced to a lower
231 magnitude in controls compared to IM and IN groups (Fig. S6F).

232

233 *Transcriptional analysis of lung samples*

234 As described for BAL samples, we observed a clear distinction between lung samples of
235 uninfected/naïve compared to vaccinated and challenged NHPs with no clear separation between the
236 three challenged groups, therefore, we employed the same strategy as described above for BAL (Fig.
237 S7A). A robust transcriptional response to SARS-CoV-2 infection was evident in all three vaccinated
238 groups compared to naïve animals (Fig. S7B) with the majority of DEGs shared among the three groups
239 (Fig. 6A). Downregulated DEGs shared by all three vaccinated groups enriched to GO terms primarily
240 involved in innate immunity (e.g., “regulation of innate immune response”, “antigen processing and
241 presentation”), cellular stress (e.g., “coagulation”, “response to decreased oxygen levels”, “wound
242 healing”) and cell cycle (e.g., “regulation of cell cycle process”) (Fig. 6B). Genes playing roles in protein
243 folding and turnover (*CALR* and *CTSF*), immune activation (e.g., *LYN* and *ADA*) coagulation (e.g., *PLAT*,
244 *SIRT2*, *FERMT3*), fluid homeostasis (e.g., *ADM*, *SERPINA5*) and cell morphogenesis (e.g., *NOTCH4*, *TEK*)
245 were more suppressed in the IN group (Fig. 6C and S7C). Shared upregulated DEGs enriched to GO terms
246 reflecting innate immune processes (e.g., “regulated exocytosis”, “myeloid leukocyte activation”,
247 “neutrophil degranulation”), as well as cell migration (e.g., “chemotaxis”) and extracellular structural
248 dynamics (e.g., “extracellular structural organization”, “cell projection morphogenesis”) (Fig. 6B).
249 Overall, a large portion of these genes were induced to a greater extent in the IN group, notably those
250 that play a role in neutrophil activation (e.g., *GRN*, *AZU1*, *CR1*), cell metabolism (e.g., *ALDOC*, *PGM1*,
251 *IMPDH1*), chemotaxis (e.g., *CCL20*, *CCL8*, *CCL13*, *ICAM3*), and extracellular matrix remodeling (e.g.,
252 *MMP25*, *MMP16*) (Fig. 6D, S7D). Additionally, genes important for angiogenesis and apoptosis were
253 more upregulated in the IN group (e.g., *VEGFD*, *PRKD1*, *SHC1*, *DAB2IP*) (Fig. S7E).

254 We next analyzed vaccine route-specific DEGs to identify differences in the molecular responses
255 to vaccination and challenge (Fig. 6E-H). Enrichment of the 161 DEGs unique to the IN group revealed
256 enrichment to GO terms suggesting tissue injury (e.g., “lung development”), metabolism (e.g., “protein
257 modification by small protein removal”), and signaling (e.g., “calcium-mediated signaling”) (Fig. 6F).
258 Notably, downregulated DEGs in this group include components of the mitochondrial cellular respiration
259 complex (e.g., *MT-CYB*, *MT-CO2*) and cellular homeostasis (e.g., *SKIV2L*, *PDE6C*, *SKIV2L*, *SHROOM4*) (Fig.
260 6F).

261 In contrast, the 226 DEGs unique to the IM group enriched to GO terms related to cellular
262 defense (e.g., type I interferon signaling pathway”, “TLR9 signaling”, “autophagy”), morphogenesis (e.g.,
263 “epithelial cell morphogenesis”) and membrane dynamics (e.g., “protein localization to membrane”,)
264 (Fig. 6G). The higher expression of DEGs related to the type I interferon response (e.g., *CDC37*, *USP18*,
265 *IFI6*) and ribosome assembly (e.g., *MRRF*, *OGFOD1*, *FARSA*) in the IM and control groups suggests
266 greater mobilization of host defense processes (Fig. 6H). Additionally, genes associated with cilia
267 formation (e.g., *INPP5E*, *TMEM231*, *PPFIBP2*) and regulation of inflammation (e.g., *GLOD5*, *ITIH5*, *TIFAB*)
268 were highly expressed in the IM group in line with reduced damage (Fig. 6H). *In silico* flow cytometry
269 analysis indicated that these transcriptional changes were consistent with increases in the levels of
270 neutrophils, monocytes, NK cells, dendritic cells and naïve lymphocytes in all the infected animals while
271 frequencies of activated monocytes were predicted to decrease relative to tissue from naïve animals
272 (Fig. S7F).

273

274 Discussion

275 Many vaccine platforms have been utilized to develop a COVID-19 vaccine quickly (13-16), with
276 several already approved for human use within a year of SARS-CoV-2 emergence. However, many of
277 these vaccines require 2 doses to elicit protection and are delivered IM rather than the site of infection.
278 Therefore, we developed a single dose, fast-acting VSV-based vaccine against COVID-19, which is based
279 on the rVSV-ZEBOV vaccine approved by the US Food and Drug administration (FDA) and the European
280 Medicines Agency (EMA) for human use. Additionally, we compared the protective efficacy of IM and IN
281 delivery in the rhesus macaque model (27).

282 A single dose IM-, but not IN-delivered vaccine protected NHPs from COVID-19 pneumonia
283 within 10 days post vaccination. This short time to immunity is a tremendous advantage and highlights
284 its potential to be used rapidly during a public health crisis, particularly in emergency situations when
285 many people were exposed at once. Interestingly, IM vaccination resulted in superior immune response
286 compared to IN as evidenced by the significantly higher SARS-CoV-2-S-specific antibody titers and lower
287 viral loads in this group. None of the animals in this study showed overt clinical signs of disease
288 regardless of their vaccination status. However, histological examination of the lung tissue revealed
289 immunopathology that was most significant in IN-vaccinated animals. The observed immunopathology
290 was not consistent with a classic hypersensitivity response or immune-enhanced disease as the lesions
291 were limited to the periphery of lung lobes and almost exclusively observed in lower lung lobes as

292 previously reported for SARS-CoV-2 infection (27). Importantly, IM-vaccinated animals did not develop
293 signs of interstitial pneumonia, nor could we detect SARS-CoV-2 antigen in the lungs. Indeed, at the time
294 of euthanasia, lung lesions were apparent in animals from the IN and controls groups, but not in the IM
295 group. This is surprising given mucosal vaccination for other respiratory pathogens has been
296 demonstrated to be superior or comparable to IM (30, 31). It possible that the immunopathology
297 observed in the vaccinated animals is due to the short duration between vaccination and challenge (10
298 days) and that it might also occur with other SARS-CoV-2 vaccine platforms, as this change was clinically
299 silent in our model.

300 In line with the observations above, the transcriptional analysis of lung samples showed a
301 divergence of antiviral states between the IN, IM and control groups. IN vaccination induced
302 transcriptional changes enriched to cell metabolism, apoptosis, angiogenesis, and neutrophil activation
303 processes. Some notable DEGs include genes associated with neutrophil activation and the formation of
304 azurophil granules (e.g. *GRN*, *CR1*, and *AZU1*). While neutrophils play a role in protecting the host,
305 sustained neutrophil activation has been shown to directly correlate to more severe COVID-19 cases
306 (32). In contrast, IM vaccination induced transcriptional changes playing a role in cilia formation,
307 inflammation regulation, and type I IFN. A majority of these genes are responsible for the regulation and
308 control of early innate inflammation such as *USP18* which disrupts the JAK-STAT pathway downstream
309 of the IFN receptor (33), and *TIFAB* which inhibits the activation of the NF κ B pathway (34). Collectively
310 these findings support the significant decrease in virus replication between the IN and IM vaccination
311 groups. Transcriptional analysis of acute BAL samples also demonstrated a divergence of antiviral states
312 evidenced by T cell differentiation genes upregulated after IM vaccination compared to innate antiviral
313 posttranslational modifications after IN vaccination. While variable immunopathology was observed in
314 all VSV-SARS2-EBOV-vaccinated NHPs regardless of the route, transcriptional analysis of the BAL
315 demonstrated an upregulation of antiviral genes such as *HERC5* in the IN group only, in line with
316 enhanced viral loads in this group. *HERC5* is responsible for the production of *HECT*-type E3 protein
317 ligase, a facilitator of the ISG conjugation system of interferon stimulated gene 15 (35) possibly
318 contributing to the enhanced immunopathology observed in the IN group.

319 Since the importance of a cellular immune response has been recently highlighted in COVID-19
320 patients (36), we assessed development of both innate as well as the adaptive cellular responses
321 following each vaccination strategy. Our analysis showed a higher frequency of granzyme B⁺ NK cells
322 after IM vaccination. While there was minimal antigen-specific cytokine production from the CD4⁺ T
323 cells, a significant increase in the early activation marker, CD69, was observed on 0 and 7 dpc. In

324 addition, an increase in the IL-2 production was only observed 7 dpc, indicative of the priming of
325 cytotoxic CD8⁺ T cells (37). Transcriptional analysis revealed that IM vaccination induced upregulation of
326 *CRACR2A* and *SH2D2a*. *SH2D2a* encodes a T cell-specific adaptor protein, which facilitates the formation
327 and maintenance of the immunological synapse between the antigen presenting cell and the T cell
328 receptor allowing for a more robust antigen-specific stimulation (38). *CRACR2A* also has a role in the
329 maintenance of the immunological synapse and promotes downstream signaling, which results in an
330 increased Th1 response and Th17 effector functions which is supported by the significant decrease of IL-
331 2 on 0 dpc (39).

332 Serum cytokine analyses demonstrated a significant increase in IL-18 and innate chemokines,
333 MCP-1 and MIP-1 β , circulating in animals that presented with severe pathology. The increased
334 expression of IL-18 could indicate a priming of the infiltrating immune cells in the lungs to a more
335 proinflammatory state that would result in the observed tissue destruction. However, we did not
336 observe an increase of IL-18 in BAL samples from 3 dpc. The most striking observation was the
337 significant downregulation of MIP-1 β on all dpc measured in IM-vaccinated animals. MIP-1 β and MCP-1
338 have previously been demonstrated to be indicators of severe COVID-19 pathogenesis by transcriptomic
339 profiling of human patients (40). A decrease in MIP-1 β and MCP-1 could contribute to the lack of
340 immune cell infiltration in the lungs of the IM group. Predicted *in silico* flow cytometry data from BAL on
341 3 dpc showed a decrease of naïve and plasma B cells, CD4⁺ and CD8⁺ T cells, stimulated dendritic cells
342 and neutrophils supporting this hypothesis. Furthermore, *in silico* flow cytometry from the lungs on 7
343 dpc were indicative of a decrease in activated monocytes for all SARS-CoV-2-infected NHPs. Further
344 immune cell characterization from BAL and within the lungs is needed to expand upon our results and
345 confirm this hypothesis.

346 In summary, in this study we generated a potent single-dose, fast-acting vaccine for COVID-19.
347 This vaccine grows to high titers like the parent rVSV-ZEBOV vector and to higher titers compared to a
348 VSV vaccine expressing the SARS-CoV-2 S alone (41)(unpublished data). Several important questions
349 remain to be addressed in future studies. An extension of the time between vaccination and challenge
350 might overcome the observed difference in protection between the vaccination routes and might
351 eliminate the signs of immunopathology. This aspect will be investigated in future studies in conjunction
352 with assessing the durability of SARS-CoV-2-specific immunity and a possible dose reduction of the
353 vaccine as has been described for the rVSV-ZEBOV, the parental vaccine (42). The rVSV-ZEBOV has been
354 shown to elicit a durable humoral response, which lasts for at least 2 years in humans (43). We will also
355 investigate the addition of another SARS-CoV-2 antigen into the vaccine to promote a stronger T cell

356 response as these responses are typically longer lasting. Furthermore, we will analyze if pre-existing
357 immunity to EBOV could impact the immunogenicity of this bivalent vaccine. For now, the VSV-SARS2-
358 EBOV vaccine presents a vaccine with a high potential as a boosting option after the already approved
359 mRNA-based vaccine because the VSV-SARS2-EBOV elicits primarily a humoral response in contrast to
360 the predominantly T cell-driven immune response after mRNA vaccination (16).

361

362 **Materials and Methods**

363 *Ethics statement*

364 All infectious work with SARS-CoV-2 was performed in the high containment laboratories at the
365 Rocky Mountain Laboratories (RML), Division of Intramural Research, National Institute of Allergy and
366 Infectious Diseases, National Institutes of Health. RML is an institution accredited by the Association for
367 Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All procedures
368 followed standard operating procedures (SOPs) approved by the RML Institutional Biosafety Committee
369 (IBC). Animal work was performed in strict accordance with the recommendations described in the
370 Guide for the Care and Use of Laboratory Animals of the National Institute of Health, the Office of
371 Animal Welfare and the Animal Welfare Act, United States Department of Agriculture. The studies were
372 approved by the RML Animal Care and Use Committee (ACUC). Procedures were conducted in animals
373 anesthetized by trained personnel under the supervision of veterinary staff. All efforts were made to
374 ameliorate animal welfare and minimize animal suffering in accordance with the Weatherall report on
375 the use of nonhuman primates in research

376 (<https://royalsociety.org/policy/publications/2006/weatherall-report/>). Animals were housed in
377 adjoining individual primate cages that enabled social interactions, under controlled conditions of
378 humidity, temperature, and light (12 hours light - dark cycles). Food and water were available *ad libitum*.
379 Animals were monitored and fed commercial monkey chow, treats, and fruit at least twice a day by
380 trained personnel. Environmental enrichment consisted of commercial toys, music, video and social
381 interaction.

382

383 *Animal study*

384 Sixteen female rhesus macaques (3.5-10 years of age; 4.5-10kg, Indian-origin) were used in this
385 study. The NHPs were randomly selected for two vaccine groups (n=6) and one control group (n=4). On

386 day-10 NHPs received a single vaccine dose of 1×10^7 PFU of VSV-SARS2-EBOV by the IM (injection caudal
387 thigh) or IN route (dropping vaccine into each nostril). Control animals received the same dose of a
388 control vaccine (VSV-EBOV) by the IM (n=2) or IN (n=2) route (Fig. S1D). On day 0, animals were
389 challenged with SARS-CoV-2 as previously described (27). On day 0, 1, 3, 5 and 7 after challenge a clinical
390 exam was performed including thoracic radiograph and nasal swab collection. The day 3 exam included
391 bronchoalveolar lavage (BAL) using 10 ml sterile saline. On day 7, all animals were euthanized for sample
392 collection.

393

394 *Cells and Viruses*

395 Huh7 and VeroE6 cells were grown at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium
396 (DMEM) (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS) (Wisent Inc., St. Bruno,
397 Canada), 2 mM L-glutamine (Thermo Fisher Scientific, Waltham, MA), 50 U/mL penicillin (Thermo Fisher
398 Scientific), and 50 µg/mL streptomycin (Thermo Fisher Scientific). BHK-T7 (baby hamster kidney) cells
399 expressing T7 polymerase were grown at 37°C and 5% CO₂ in minimum essential medium (MEM)
400 (Thermo Fisher Scientific) containing 10% tryptose phosphate broth (Thermo Fisher Scientific), 5% FBS, 2
401 mM L-glutamine, 50 U/mL penicillin, and 50 µg/mL streptomycin. SARS-CoV-2 isolate nCoV-WA1-2020
402 (MN985325.1) (44) was used for the animal challenge studies and neutralization test.

403

404 *Generation of VSV-based vaccine candidates*

405 The SARS-CoV-2 S ORF was PCR-amplified from an expression plasmid encoding the codon-
406 optimized (human) gene based on GenBank accession number MN908947 which was kindly provided by
407 Vincent Munster (NIAID). Full-length SARS-CoV-2 S was cloned into the pATX-VSV-EBOV plasmid
408 upstream of the EBOV-Kikwit GP resulting in VSV-SARS2-EBOV (Fig. S1A) following a previously
409 successful strategy (45). The replication-competent recombinant VSV was recovered in BHK-T7 cells as
410 described previously (26). VSV-SARS2-EBOV was propagated on Huh7 cells. The complete sequence of
411 the virus was confirmed by Sanger sequencing. The titer of the virus stock was quantified using standard
412 plaque assay on VeroE6 cells.

413

414 *Growth kinetics*

415 VeroE6 cells were grown to confluency in a 12-well plate and infected in triplicate with VSVwt,
416 VSV-EBOV, or VSV-SARS2-EBOV at a multiplicity of infection of 0.01. After 1 h incubation at 37°C, cells
417 were washed three times with plain DMEM, and covered with DMEM containing 2% FBS. Supernatant

418 samples were collected at 0, 6, 12, 24, 48, 72, and 96 hours post infection and stored at -80°C . The titer
419 of the supernatant samples was determined performing TCID₅₀ assay on VeroE6 cells as previously
420 described (26).

421

422 *Western blot analysis*

423 Supernatant samples containing VSV were mixed 1:1 with sodium dodecyl sulfate-
424 polyacrylamide (SDS) gel electrophoresis sample buffer containing 20% β -mercaptoethanol and heated
425 to 99°C for 10 min. SDS-PAGE and transfer to Trans-Blot polyvinylidene difluoride membranes (Bio-Rad
426 Laboratories) of all samples was performed as described elsewhere (22). Protein detection was
427 performed using anti-SARS-CoV-2 S RBD (1:1000; Sino Biological) or anti-EBOV GP (ZGP 12/1.1, 1 $\mu\text{g}/\text{ml}$;
428 kindly provided by Ayato Takada, Hokkaido University, Japan) or anti-VSV M (23H12, 1:1000; Kerast
429 Inc.). After horse-radish peroxidase (HRP)-labeled secondary antibody staining using either anti-mouse
430 IgG (1:10,000) or anti-rabbit IgG (1:5000) (Jackson ImmunoResearch), the blots were imaged using the
431 SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific) and an iBright™ CL1500
432 Imaging System (Thermo Fisher Scientific).

433

434 *RNA extraction and RT-qPCR*

435 Blood, BAL fluid, and nasal swabs were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN)
436 according to manufacturer specifications. Tissues, a maximum of 30 mg each, were processed and
437 extracted using the RNeasy Mini Kit (QIAGEN) according to manufacturer specifications. One step RT-
438 qPCR for both genomic and subgenomic viral RNA was performed using specific primer-probe sets and
439 the QuantiFast Probe RT-PCR +ROX Vial Kit (QIAGEN), in the Rotor-Gene Q (QIAGEN) as described
440 previously (13). Five μL of each RNA extraction were run alongside dilutions of SARS-CoV-2 standards
441 with a known concentration of RNA copies.

442

443 *Enzyme-linked immunosorbent assay*

444 Serum and BAL samples from SARS-CoV-2-infected animals were inactivated by γ -irradiation and
445 used in BSL2 according to IBC-approved SOPs. NUNC Maxisorp Immuno plates were coated with 50 μL of
446 1 $\mu\text{g}/\text{mL}$ of recombinant SARS-CoV-2 spike (S1+S2), SARS-CoV-2 RBD (Sino Biological) or EBOV GP
447 antigen at 4°C overnight and then washed three times with phosphate buffer saline containing 0.05%
448 Tween 20 (PBST). The plates were blocked with 3% skim milk in PBS for 3 hours at room temperature,
449 followed by three additional washes with PBST. The plates were incubated with 50 μL of serial dilutions

450 of the samples in PBS containing 1% skim milk for 1 hour at room temperature. After 3 washes with
451 PBST, the bound antibodies were labeled using 50 μ l of 1:2,500 horse-radish peroxidase (HRP)-labeled
452 anti-monkey IgG (H+L) (SeraCare Life Sciences) diluted in 1% skim milk in PBST. For the IgG subclass
453 ELISAs the plates were incubated with samples at 4 °C overnight. After three washes with PBST, 50 μ l of
454 1 μ g/mL Anti-rhesus IgG1 [ena], IgG2 [dio], IgG3 [tria], or IgG4 [tessera] (NHPRR) diluted in 1% skim milk
455 in PBST was added and incubated for 1 h at room temperature. After 3 washes with PBST, the bound
456 antibodies were labeled using 50 μ l of 1:10,000 HRP-labeled anti-mouse IgG (H+L) (SeraCare Life
457 Sciences) diluted in 1% skim milk in PBST. For all ELISAs, after incubation for 1 h at room temperature
458 and 3 washes with PBST, 50 μ l of KPL ABTS peroxidase substrate solution mix (SeraCare Life Sciences)
459 was added to each well, and the mixture was incubated for 30 min at room temperature. The optical
460 density (OD) at 405 nm was measured using a GloMax[®] explorer (Promega). The OD values were
461 normalized to the baseline samples obtained on day -10 and the cutoff value was set as the mean OD
462 plus standard deviation of the blank.

463

464 *Virus neutralization assay*

465 The day before this assay, VeroE6 cells were seeded in 96-well plates. Serum samples were
466 heat-inactivated for 30 min at 56°C, and 2-fold serial dilutions were prepared in DMEM with 2% FBS.
467 Next, 100 TCID₅₀ of SARS-CoV-2 were added and the mixture was incubated for 1 hour at 37°C and 5%
468 CO₂. Finally, media was removed from cells and the mixture was added to VeroE6 cells and incubated at
469 37°C and 5% CO₂ for 6 days. Then the CPE was documented, and the virus neutralization titer was
470 expressed as the reciprocal value of the highest dilution of the serum which inhibited virus replication
471 (no CPE).

472

473 *Flow cytometry*

474 Rhesus macaque PBMCs were isolated from ethylene diamine tetracetic acid (EDTA) whole blood
475 by overlay on a Histopaque[®]-1077 density cushion and separated according to manufacturers'
476 instructions. Isolated PBMCs were resuspended in FBS with 10% DMSO and frozen at -80°C until
477 analysis. For analysis of T cell intracellular cytokine production, cells were stimulated for 6 hours with
478 1 μ g/ml SARS-CoV-S peptide pool, media, cell stimulation cocktail (containing PMA-Ionomycin,
479 Biolegend), or Lassa virus (LASV) GP peptide pool together with 5 μ g/ml Brefeldin A (Biolegend).
480 Following surface staining with Live/Dead-APCCy7, CD3-FITC, CD4-Alexa700, CD8-PeTexas Red, CD56-
481 BV421 and CD69-PeCy7, cells were fixed with 4% paraformaldehyde (PFA) and stained intracellularly

482 with IFN γ -BV605, IL-4-APC, IL-2-PerCPCy5.5 diluted in perm-wash buffer (Biolegend). For analysis of NK
483 cell intracellular cytokine production, cells were stimulated as described above. Following surface
484 staining with Live/Dead-APCCy7, CD3-FITC, CD4-PerCPCy5.5, CD8-PeTexas Red, CD16-Alexa700, and
485 CD56-BV421, cells were fixed with 4% PFA and stained intracellularly with granzyme B-APC.
486 Sample acquisition was performed on a Cytoflex-S (Beckman Coulter) and data analyzed in FlowJo V10
487 (TreeStar). Antigen specific T cells were identified by gating on Live/Dead negative, doublet negative
488 (SSC-H vs SSC-A), CD3⁺, CD56⁻, CD4⁺ or CD8⁺ cells and cytokine positive. Three NK cell sub-populations
489 were identified by gating on Live/Dead negative, doublet negative (SSC-H vs SSC-A), CD3⁺, CD56⁻, and
490 CD8⁺ or CD16⁺ or CD8⁺CD16⁺ double positive. Cytokine responses for each sub-population were
491 identified by gating on the population then granzyme B⁺ cells. Cytokine positive responses are presented
492 after subtraction of the background responses detected in the LASV GP peptide stimulated samples.

493

494 *Cytokine analysis*

495 Macaque serum and BAL samples were inactivated by γ -irradiation and removed from the high
496 containment laboratory according to IBC-approved SOPs. Samples were then diluted 1:2 in serum matrix
497 for analysis with Milliplex Non-Human Primate Magnetic Bead Panel as per manufacturer's instructions
498 (Millipore Corporation). Concentrations for each cytokine were determined for all samples using the Bio-
499 Plex 200 system (BioRad Laboratories Inc.).

500

501 *Histology and immunohistochemistry*

502 Necropsies and tissue sampling were performed according to IBC-approved SOPs. Lungs were
503 perfused with 10% formalin and processed for histologic review. Harvested tissues were fixed for eight
504 days in 10% neutral-buffered formalin, embedded in paraffin, processed using a VIP-6 Tissue Tek (Sakura
505 Finetek, USA) tissue processor, and embedded in Ultratrain paraffin polymer (Cancer Diagnostics,
506 Durham, NC). Samples were sectioned at 5 μ m, dried overnight at 42 °C, and resulting slides were
507 stained with hematoxylin and eosin. Specific anti-CoV immunoreactivity was detected using an in-house
508 SARS-CoV-2 nucleocapsid protein (U864YFA140-4/CB2093) rabbit antibody (Genscript) at a 1:1000
509 dilution. The IHC assay was carried out on a Discovery ULTRA automated staining instrument (Roche
510 Tissue Diagnostics) with a Discovery ChromoMap DAB (Ventana Medical Systems) kit. All tissue slides
511 were evaluated by a board-certified veterinary pathologist. Sections taken at 3 levels from each lung
512 lobe, totally 18 sections, were evaluated for each animal; a representative lesion from each group was
513 selected for Fig. 3.

514

515 *Library construction and sequencing*

516 Quality and quantity of RNA from BAL and lower left lung (LLL) were determined using an
517 Agilent 2100 Bioanalyzer. cDNA libraries were constructed using the NEB Next Ultra II Direction RNA
518 Library Prep Kit (Thermo Fischer). RNA was treated with RNase H and DNase I following depletion of
519 ribosomal RNA (rRNA). Adapters were ligated to cDNA products and the subsequent ~300 base pair (bp)
520 amplicons were PCR-amplified and selected by size exclusion. cDNA libraries were assessed for quality
521 and quantity prior to 150 bp single-end sequencing using the Illumina NovaSeq platform.

522

523 *Bioinformatic analysis*

524 Preliminary data analysis was performed with RNA-Seq workflow module of systemPipeR,
525 developed by Backman *et. al* (46). RNA-Seq reads were demultiplexed, quality-filtered and trimmed
526 using Trim Galore (average Phred score cut-off of 30, minimum length of 50 bp). FastQC was used to
527 generate quality reports. Hisat2 was used to align reads to the reference genome *Macaca mulatta*
528 (*Macaca_mulatta.Mmul_8.0.1.dna.toplevel.fa*) and the *Macaca_mulatta.Mmul_8.0.1.97.gtf* was used
529 for annotation. For viral read quantification, RNA-Seq reads were separately aligned to the severe acute
530 respiratory syndrome coronavirus 2 Wuhan isolate genome (NC_045512.2) and the
531 GCF_009858895.2_ASM985889v3_genomic.gff annotation file was used. Raw expression values (gene-
532 level read counts) were generated using the summarizeOverlaps function and normalized (read per
533 kilobase of transcript per million mapped reads, rpkm) using the edgeR package. Statistical analysis with
534 edgeR was used to determine differentially expressed genes (DEGs) meeting the following criteria: genes
535 with median rpkm of ≥ 5 , a false discovery rate (FDR) corrected p-value ≤ 0.05 and a \log_2 fold change ≥ 1
536 compared to uninfected tissues. The number of total viral reads was determined as the total number of
537 normalized read counts mapping to all viral genes.

538 Functional enrichment of DEGs was performed using Metascape to identify relevant Gene
539 Ontology (GO) biological process terms and KEGG pathways. *In silico* flow cytometry was performed
540 using ImmQuant with the IRIS database. Heatmaps, bubbleplots, Venn diagrams and violin plots were
541 generated using R packages ggplot2 and VennDiagrams. GO network plots were generated in Cytoscape
542 (Version 3.5.1). Graphs were generated using GraphPad Prism software (version 8).

543

544 *Statistical analyses*

545 All statistical analysis was performed in Prism 8 (GraphPad). The *in vitro* growth kinetics of
546 recombinant VSVs (Fig. S1C) was examined using two-way ANOVA with Tukey's multiple comparisons to
547 evaluate statistical significance at all timepoints. Bioinformatics data were analyzed using one-way
548 ANOVA with multiple comparisons, comparisons were made to either uninfected animals or control-
549 vaccinated animals. Two-tailed Mann-Whitney's rank or Wilcoxon tests were conducted to compare
550 differences between groups for all other data. A Bonferroni correction was used to control for type I
551 error rate where required. Statistically significant differences are indicated as follows: $p < 0.0001$ (****),
552 $p < 0.001$ (***), $p < 0.01$ (**) and $p < 0.05$ (*).

553

554 **Acknowledgements**

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556 Mora (NIAID) for assistance generating the pathology figures.

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559 the National Center for Research Resources and the National Center for Advancing Translational
560 Sciences, NIH, through grant UL1 TR001414 awarded to I.M.

561

562 **Author contributions**

563 A.M. conceived the idea and secured funding. W.F. and A.M. designed the studies. W.F., K.S., A.J.G., F.F.,
564 A.O., T.G., J.L., P.W.H., T.T., C.S.C., K.L.O., and A.M. conducted the studies, processed the samples and
565 acquired the data. A.N.P. and A.J. performed the transcriptomics work. W.F., K.S., A.N.P., C.S.C., I.M.,
566 K.L.O., and A.M. analyzed and interpreted the data. W.F., I.M., K.L.O., and A.M. prepared the
567 manuscript. All authors approved the manuscript.

568

569 **Competing interest**

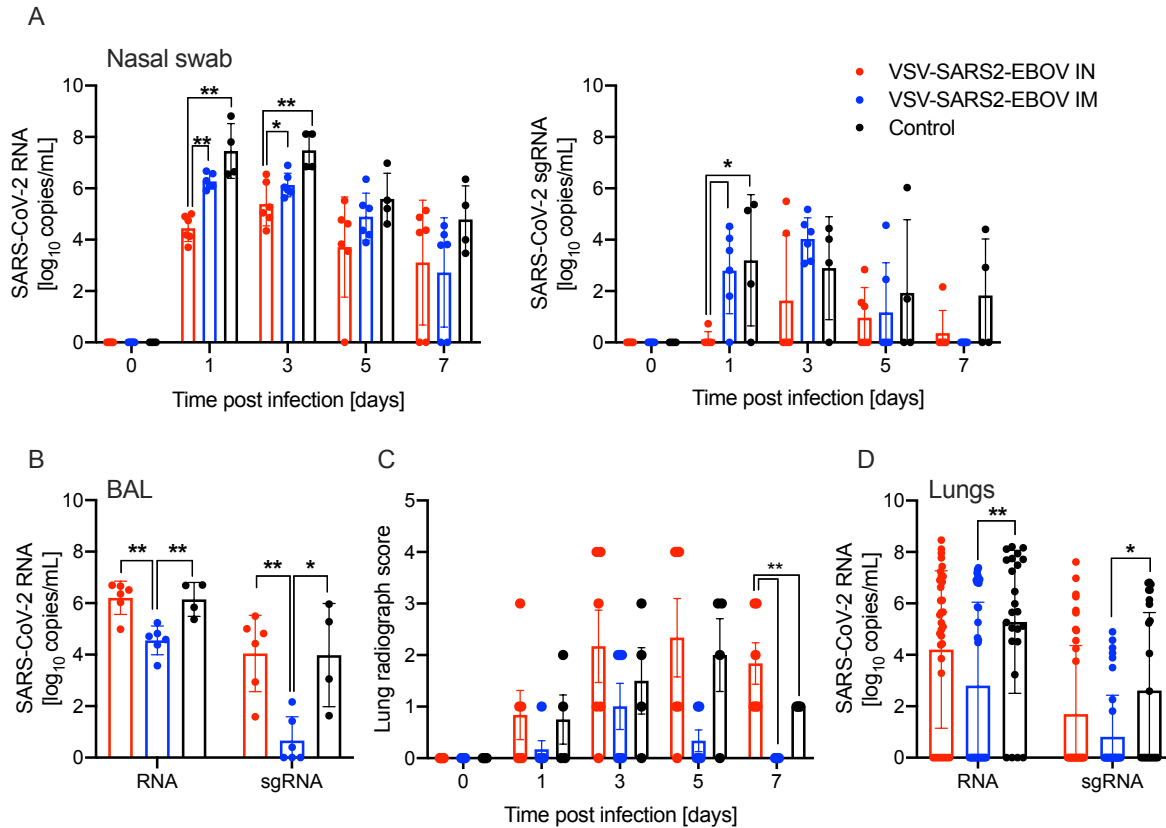
570 The authors declare no conflicts of interest.

571

572 **Data availability**

573 All data is available in the manuscript or the supplementary materials.

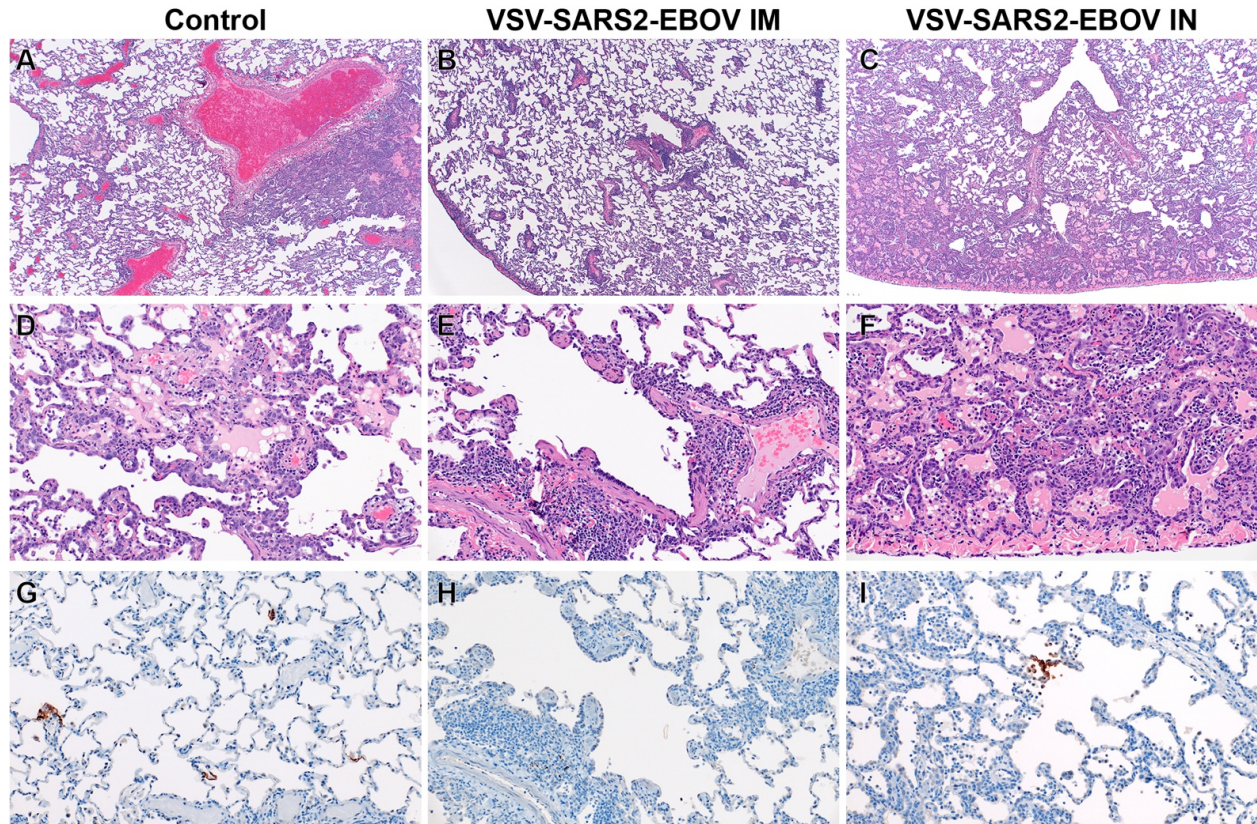
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575
 576 **Figure 1. SARS-CoV-2 loads in vaccinated NHPs.** Groups of 6 NHPs were IN or IM vaccinated with a
 577 single dose of VSV-SARS2-EBOV; 4 control animals received the VSV-EBOV. **(A)** Total SARS-CoV-2-specific
 578 RNA (left panel) and subgenomic (sg) RNA (right panel) in nasal swabs collected from NHPs. **(B)** Total
 579 SARS-CoV-2-specific RNA and sgRNA in bronchoalveolar lavage (BAL) samples collected on day 3. **(C)**
 580 Lung radiograph scores after challenge. Mean and standard deviation (SD) are shown. **(D)** Total SARS-
 581 CoV-2-specific RNA and sgRNA in lung samples collected on day 7. **(A, B, D)** Geometric mean and
 582 geometric SD are depicted. Statistical significance is indicated.

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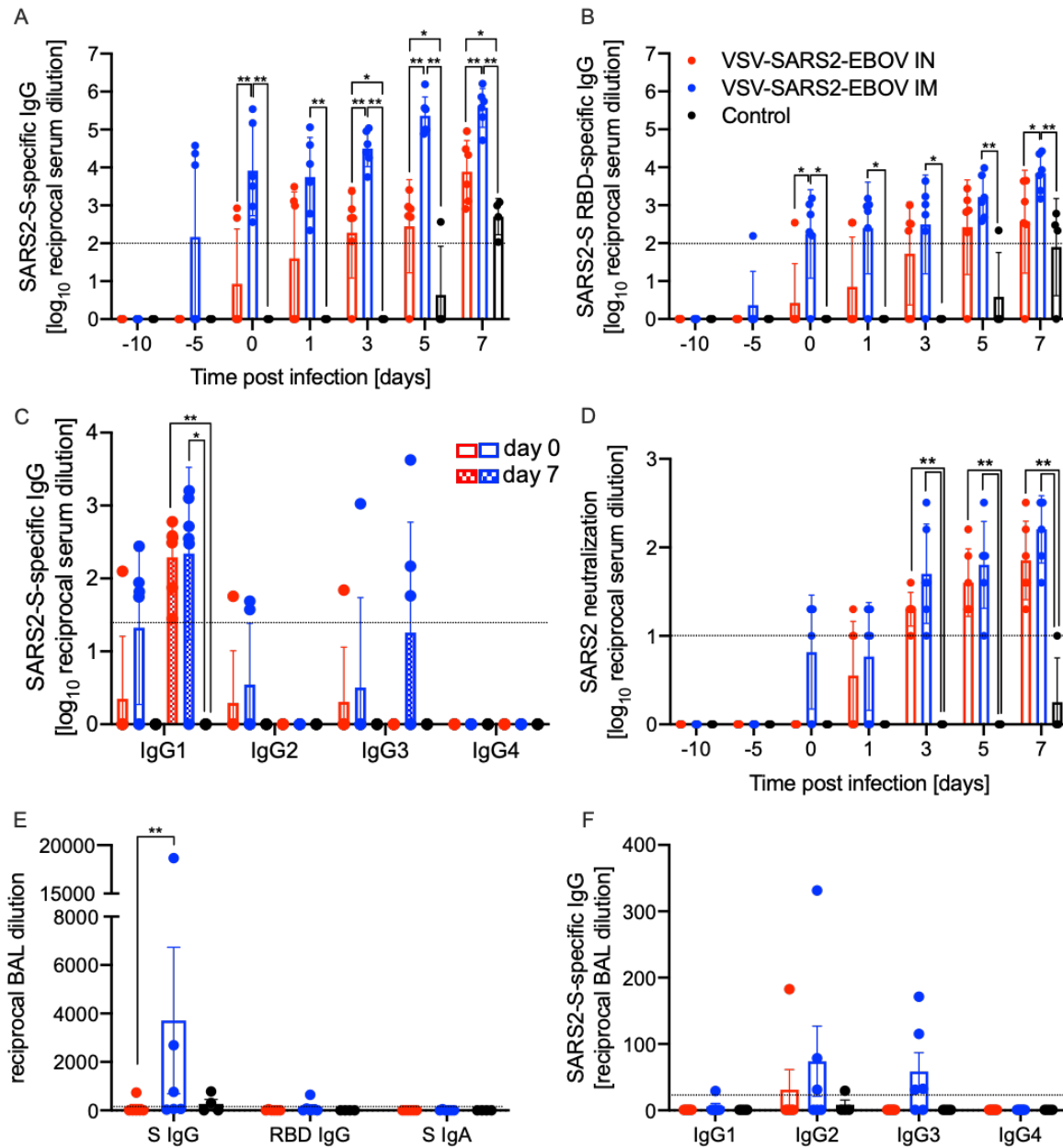


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587 **Figure 2. Histopathology and Immunohistochemistry of NHP lungs.** (A) Pulmonary lesions depicting
588 typical coronavirus respiratory pathology including locally extensive regions of bronchioalveolar
589 pneumonia and proteinaceous fluid accumulation in adjacent alveoli (40x, H&E). (B) Disseminated
590 immunopathology with prominent perivascular lymphocytic cuffing and multifocal involvement at
591 terminal airways (40x, H&E). (C) IN vaccination shows pulmonary pathology characterized by a
592 combination of interstitial pneumonia and immunopathology (40x, H&E). (D) Foci of interstitial
593 pneumonia are characterized by prominent type II pneumocyte hyperplasia, leukocyte infiltration and
594 expansion of alveolar septa and accumulation of low numbers of macrophages, neutrophils and
595 proteinaceous fluid in alveolar spaces (200x, H&E). (E) Terminal airways and medium to small caliber
596 blood vessels are cuffed by moderate numbers of lymphocytes with scattered eosinophils (200x, H&E).
597 (F) Foci of interstitial pneumonia show pronounced type II pneumocyte hyperplasia, thickening of
598 alveolar septa by an infiltration of leukocytes and leukocyte spillover into adjacent alveolar spaces with
599 moderate numbers of alveolar eosinophils noted and multifocal fibrin mats filling alveolar spaces (200x,
600 H&E). (G) Low numbers of type I pneumocytes in regions lacking pathology are immunoreactive for
601 SARS-CoV-2 antibody (200x, immunohistochemistry (IHC)). (H) SARS-CoV-2-specific immunoreactivity
602 was not observed in evaluated sections of the IM vaccinated group (200x, IHC). (I) Low numbers of type I
603 pneumocytes and alveolar macrophages are immunoreactive for SARS-CoV-2 in select foci of interstitial
604 pneumonia (200x, IHC).

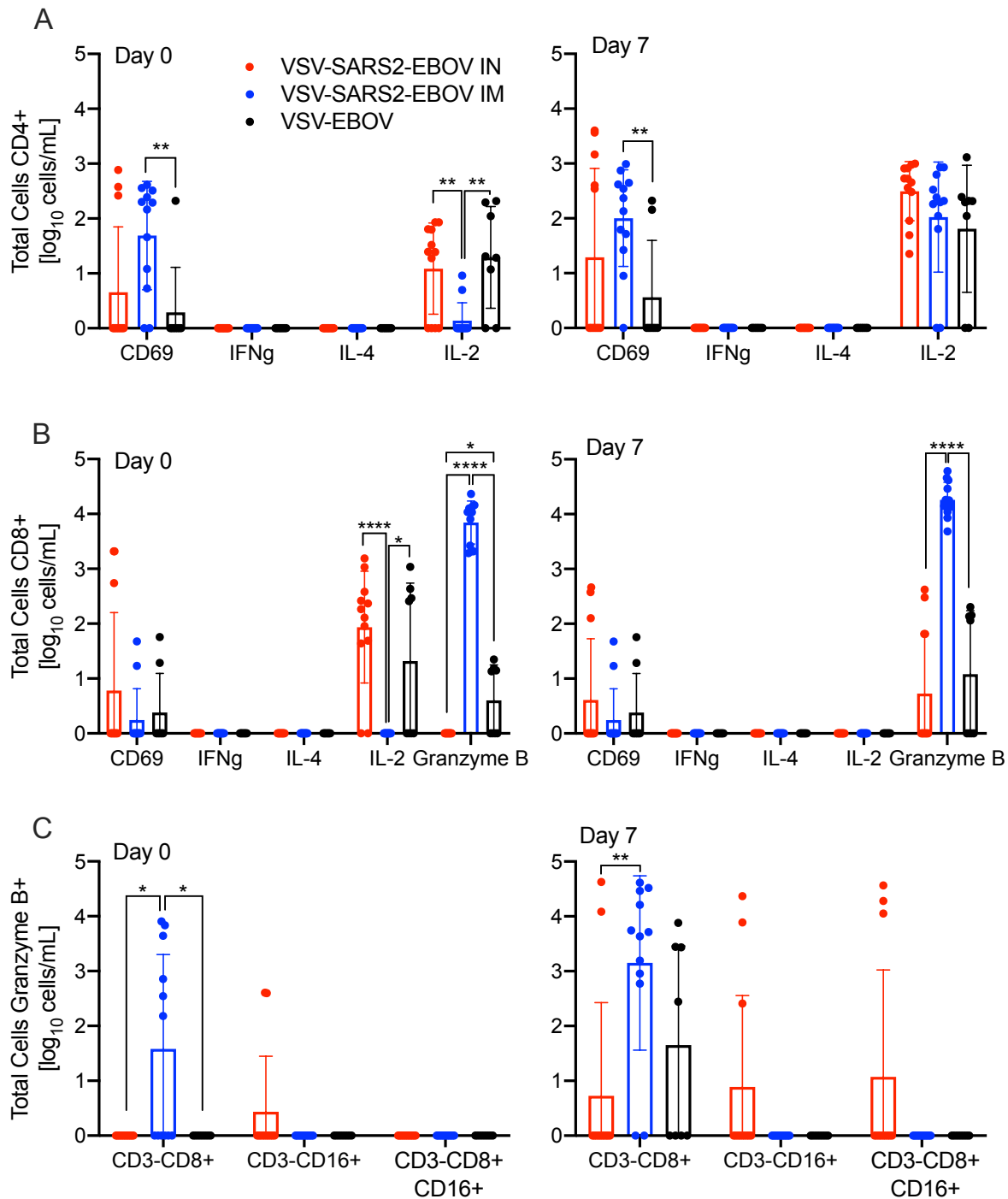
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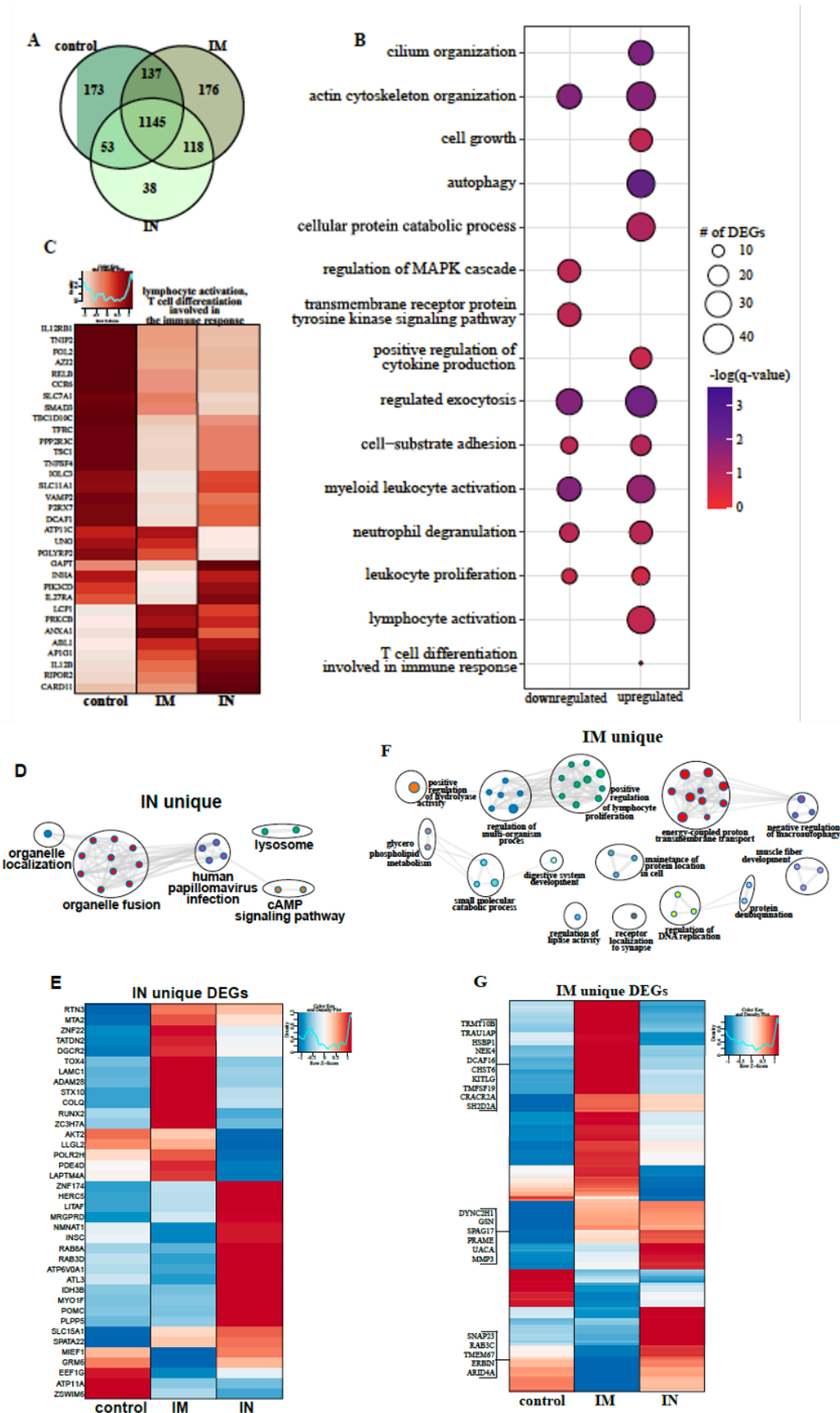


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Figure 3. Humoral immune responses in NHPs. Serum samples collected throughout the study from all NHPs were examined for (A) SARS-CoV-2 S-specific IgG, (B) SARS-CoV-2 S receptor binding domain (RBD)-specific IgG or (C) IgG subclasses specific to SARS-CoV-2 S by ELISA. (D) Neutralizing titers to SARS-CoV-2 were determined. (E) Bronchoalveolar lavage (BAL) samples were analyzed for SARS-CoV-2 S-specific IgG (S IgG) or IgA (S IgA), and SARS CoV-2 S RBD-specific IgG (RBD IgG) by ELISA. (A-D) Geometric mean and geometric standard deviation (SD) are depicted. (F) IgG subclasses specific to SARS-CoV-2 S in BAL samples were analyzed by ELISA. (E, F) Mean and SD are depicted. Statistical significance is indicated.



619
 620 **Figure 4. Peripheral cellular immune response post challenge. (A)** CD4⁺ T cells and from PBMCs were
 621 stained for expression of early activation marker CD69 and intracellular cytokine staining (ICS) for IFN γ ,
 622 IL-4, and IL-2 on day 0 and 7 post challenge. **(B)** CD8⁺ T cells from PBMCs were phenotyped for
 623 expression of early activation marker CD69 and ICS for IFN γ , IL-4, IL-2, and granzyme B on day 0 and 7
 624 post challenge. **(C)** NK cell subpopulations were stained for the expression of granzyme B on day 0 and 7
 625 post challenge. Data was measured in duplicate for all animals. Geometric mean and SD are depicted.
 626 Statistical significance is indicated.



628 **Figure 5. BAL RNA-sequencing. (A)** Venn diagram of differentially expressed genes (DEGs) expressed 3
629 days post challenge with SARS-CoV-2. Animals either received a control, intramuscular (IM) or intranasal
630 (IN) vaccination. **(B)** Bubbleplot representing functional enrichment of DEGs shared by all infected
631 groups at 3 days post challenge. Color intensity of each bubble represents the negative log of p-value
632 and the relative size of each bubble represents the number of DEGs belonging to the specified Gene
633 Ontology (GO) term. **(C)** Heatmap representing shared upregulated DEGs enriching to GO terms
634 “lymphocyte activation” and “T cell differentiation involved in the immune response.” Expression is
635 represented as the normalized rpkm, where each column represents the median rpkm of the given
636 group. Range of colors is based on scale and centered rpkm values of the represented DEGs. GO term
637 network depicting functional enrichment of DEGs unique to **(D)** IN and **(F)** IM using Mediascape. Color-
638 coded clustered nodes correspond to one GO term or KEGG pathway. Node size represents the number
639 of DEGs associated with the indicated term or pathway. Gray lines represent shared interactions
640 between terms/pathways, with density and number indicating the strength of connections between
641 closely related terms/pathways. Heatmaps representing DEGs unique to **(E)** IN and **(G)** IM. Exemplar
642 DEGs are annotated. Red represents upregulation, blue presents downregulation. Each column
643 represents the median rpkm of the given group. For all heatmaps, range of colors is based on scale and
644 centered rpkm values of the represented DEGs.
645
646

648 **Figure 6. Lung RNA-Sequencing. (A)** Venn diagram of differentially expressed genes (DEGs) expressed 3
649 days post challenge with SARS-CoV-2. Animals either received a control, intramuscular (IM) or intranasal
650 (IN) vaccination. **(B)** Bubbleplot representing functional enrichment of DEGs shared by all infected
651 groups at 7 days post challenge. Color intensity of each bubble represents the negative log of p-value
652 and the relative size of each bubble represents the number of DEGs belonging to the specified Gene
653 Ontology (GO) term. Heatmaps representing shared GO terms **(C)** “regulation of innate immune
654 response”, “antigen processing and presentation of exogenous antigen” for downregulated DEGs; and
655 **(D)** “regulated exocytosis”, “myeloid leukocyte activation” and “neutrophil degranulation” for
656 upregulated DEGs. Expression is represented as the normalized rpkm, where each column represents
657 the median rpkm of the given group. Range of colors is based on scale and centered rpkm values of the
658 represented DEGs. GO term network depicting functional enrichment of DEGs unique to **(E)** IN and **(G)**
659 IM using Mediascape. Color-coded clustered nodes correspond to one GO term or KEGG pathway. Node
660 size represents the number of DEGs associated with the indicated term or pathway. Gray lines represent
661 shared interactions between terms/pathways, with density and number indicating the strength of
662 connections between closely related terms/pathways. Heatmaps representing DEGs unique to **(F)** IN and
663 **(H)** IM. Exemplar DEGs are annotated. Red represents upregulation, blue presents downregulation. Each
664 column represents the median rpkm of the given group. For all heatmaps, range of colors is based on
665 scale and centered rpkm values of the represented DEGs.
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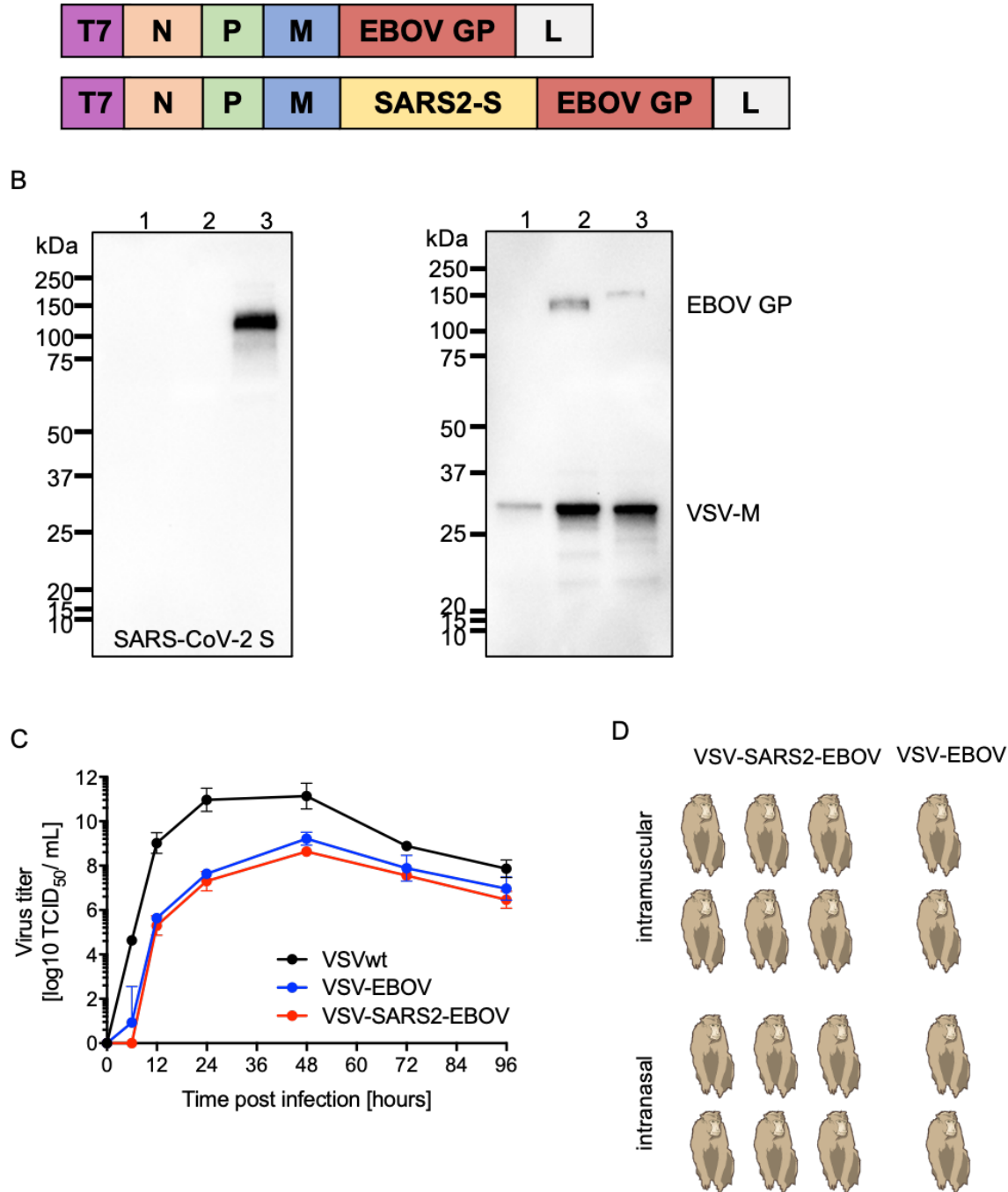


Figure S1. Schematic and characterization of VSV-based vaccines. (A) Schematic illustrating vaccine vector design. T7 promotor; N nucleoprotein; P phosphoprotein; M matrix protein; EBOV GP Ebola virus glycoprotein; L RNA-dependent RNA polymerase; SARS2-S SARS-CoV-2 S. **(B)** Western blot analysis of cell supernatant samples containing VSV vaccines probed for SARS-CoV-2 S (left), VSV M (middle) or EBOV GP (right). 1 VSV wildtype (VSVwt); 2 VSV- EBOV; 3 VSV- SARS2-EBOV. **(C)** Viral growth kinetics on VeroE6 cells. Geometric mean and SD are depicted. Results are not statistically significant. **(D)** Schematic outline of the rhesus macaque study.

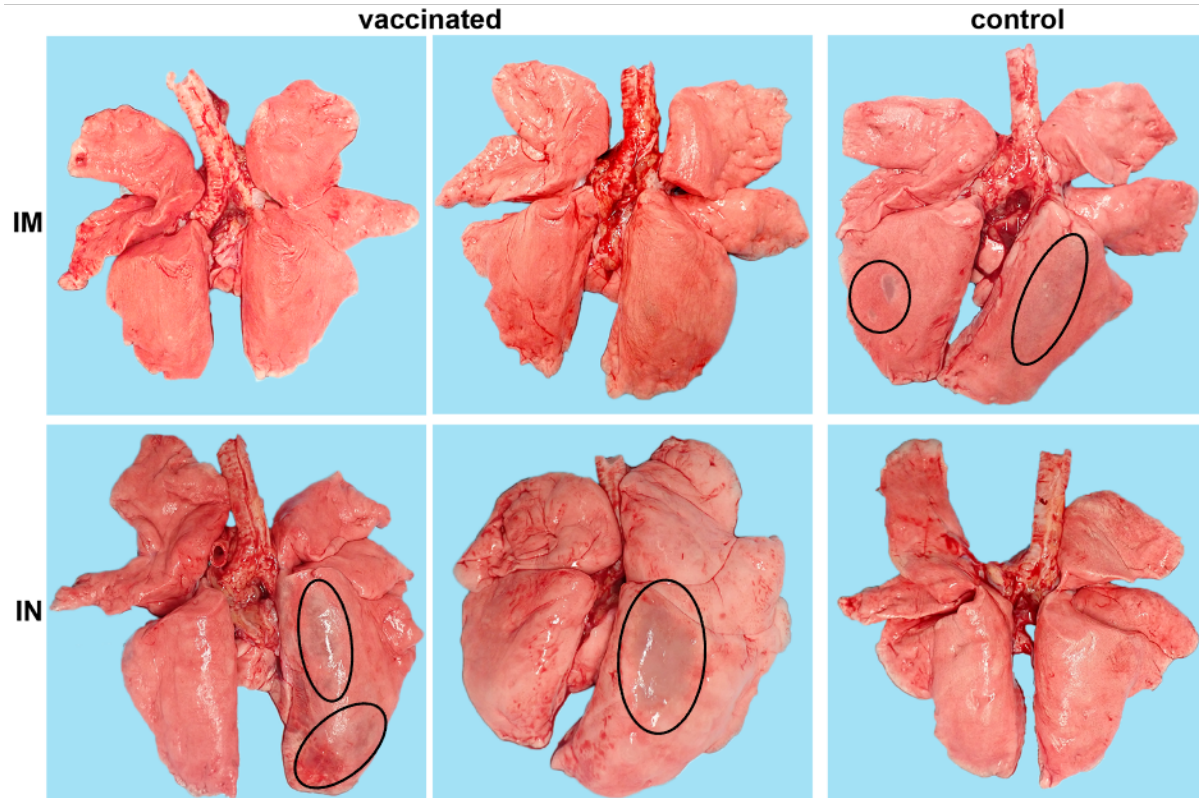


Figure S2. Lung gross pathology of NHPs. Representative pictures of NHP lungs with lesions on day 7 are shown. IM intramuscular vaccination; IN intranasal vaccination. Lesions are circled.

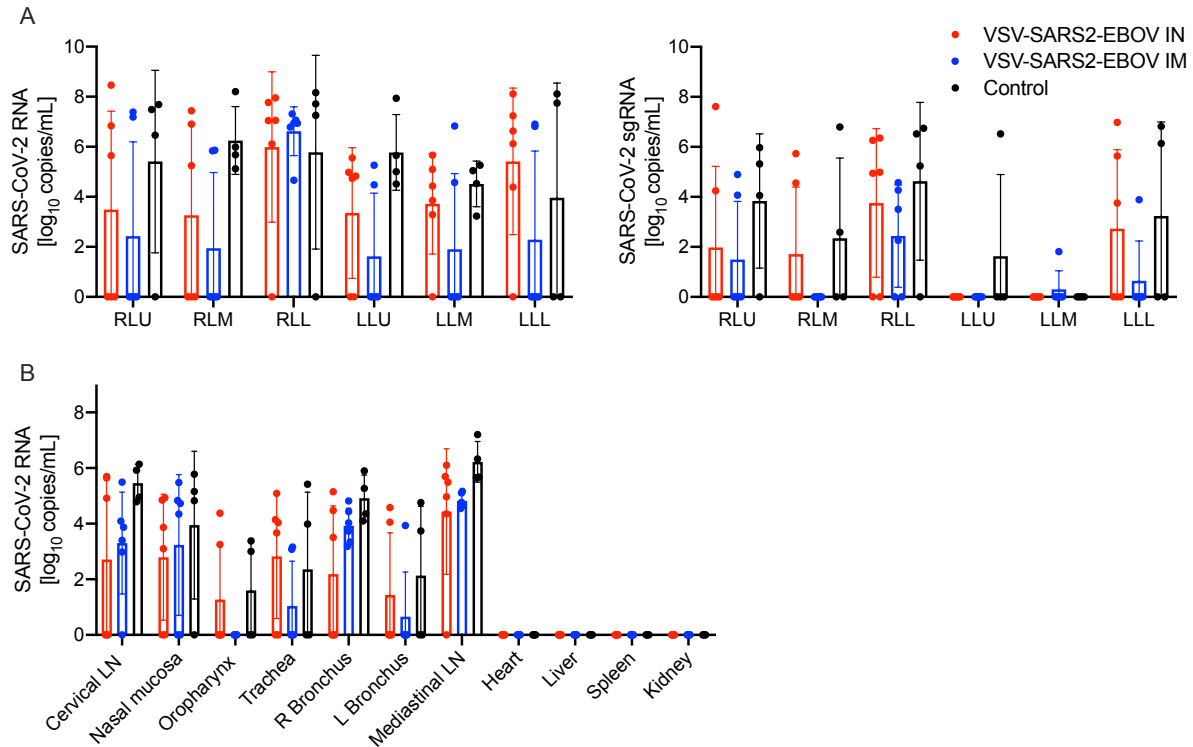


Figure S3. Virus load in NHP tissue samples on day 7. (A) Total SARS-CoV-2-specific RNA (left panel) and subgenomic (sg) RNA (right panel) in lung samples collected from NHPs. RLU right lobe upper; RLM right lobe middle; RLL right lobe lower; LLU left lobe upper; LLM left lobe middle; LLL left lobe lower. **(B)** Total SARS-CoV-2-specific RNA in tissue samples (right panel) from NHPs. LN lymph node; R right; L left.

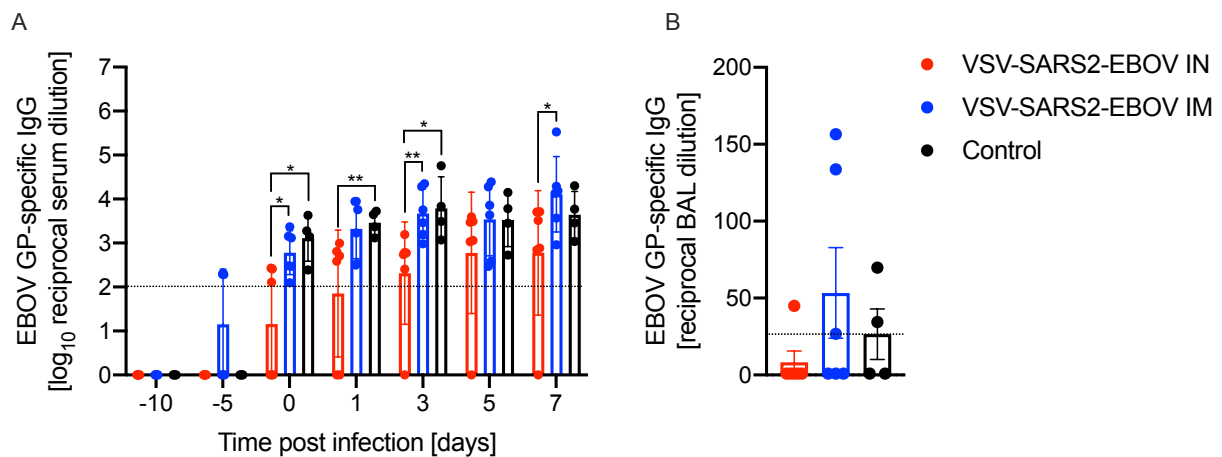


Figure S4. EBOV GP-specific antibodies in NHPs after vaccination and challenge. Bronchoalveolar lavage (BAL) samples collected on day 3 and serum samples collected throughout the study were analyzed by ELISA for EBOV GP-specific IgG. Statistical significance is indicated.

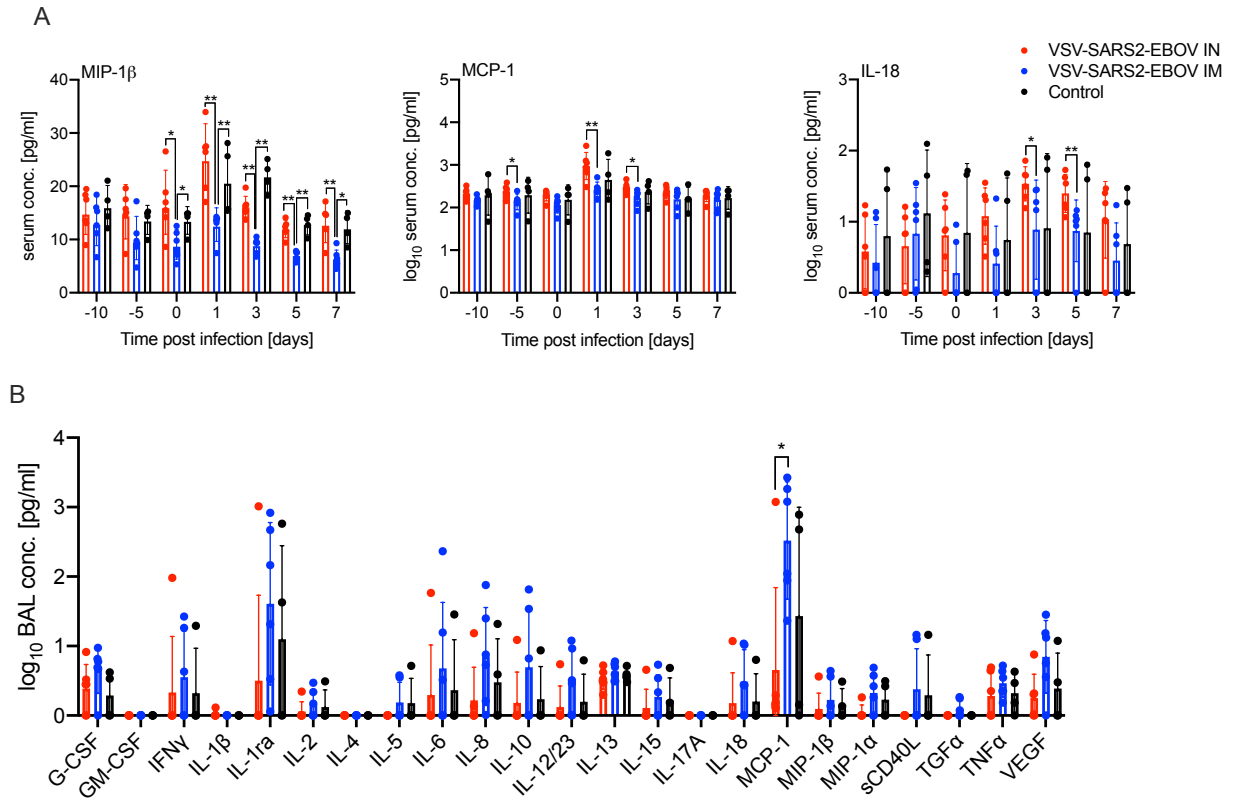


Figure S5. Serum cytokine levels in NHPs after vaccination and challenge. (A) Serum samples collected throughout the study and **(B)** bronchoalveolar lavage (BAL) samples collected on day 3 were analyzed. Statistical significance is indicated.

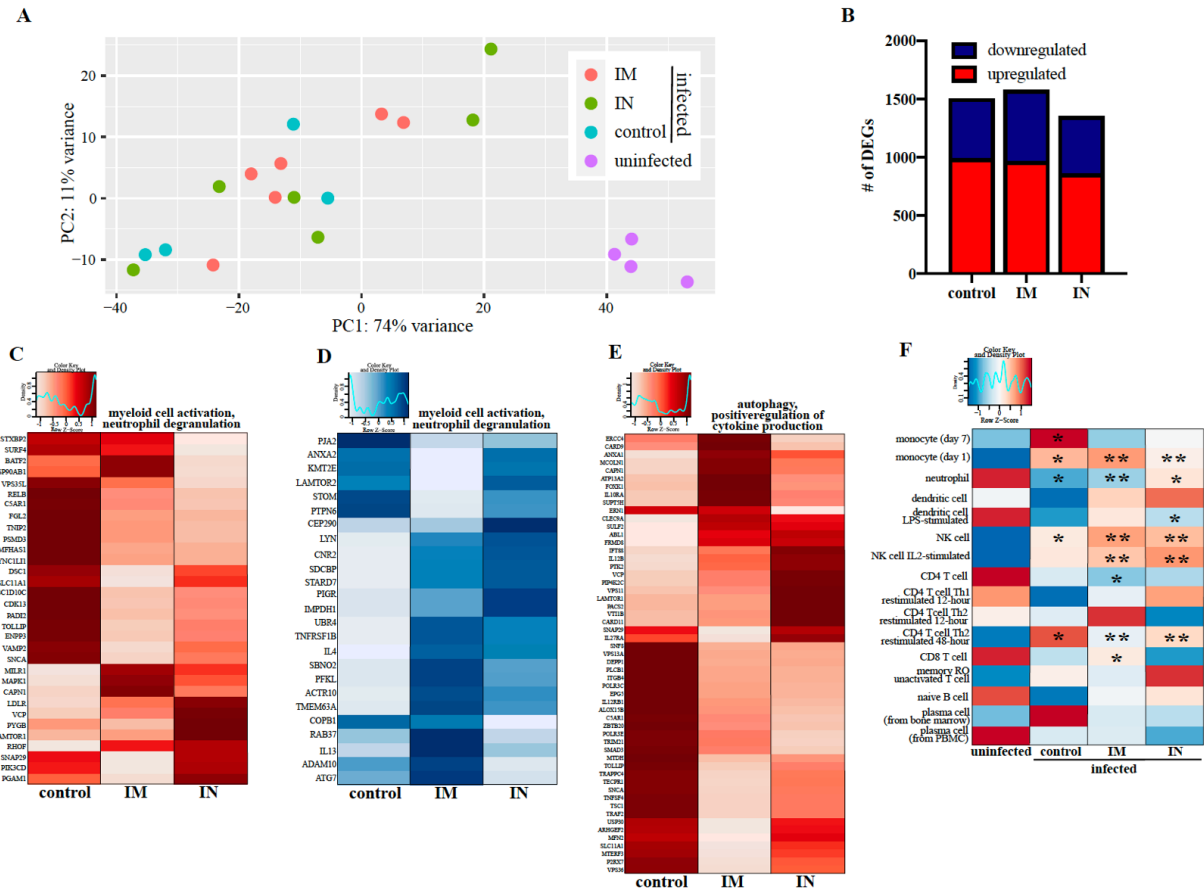


Figure S6. BAL RNA-sequencing. (A) Principal component analysis of bronchoalveolar lavage (BAL) samples from uninfected animals and vaccinated animals 3 days post challenge (control, intramuscular (IM) or intranasal (IN) vaccination). (B) Down- and up-regulated differentially expressed genes (DEGs). Heatmaps representing DEGs shared by all infected groups and enriching to Gene Ontology (GO) terms “myeloid cell activation” and “neutrophil degranulation” for (C) upregulated DEGs and (D) downregulated DEGs, and (E) “autophagy” and “positive regulation of cytokine production” for upregulated DEGs. Each column represents the median rpk of the given group. Range of colors is based on scale and centered rpk values of the represented DEGs. Red represents upregulated DEGs; blue represents downregulated DEGs. (F) *In silico* flow cytometry using ImmQuant IRIS database comparing challenged groups to uninfected controls. Red represents upregulation; blue represents downregulation. Each column represents the average relative predicted frequency of the given cell type. P-values are calculated relative to the uninfected animals. Statistical significance is indicated. For all heatmaps, range of colors is based on scale and centered rpk values of the represented DEGs.

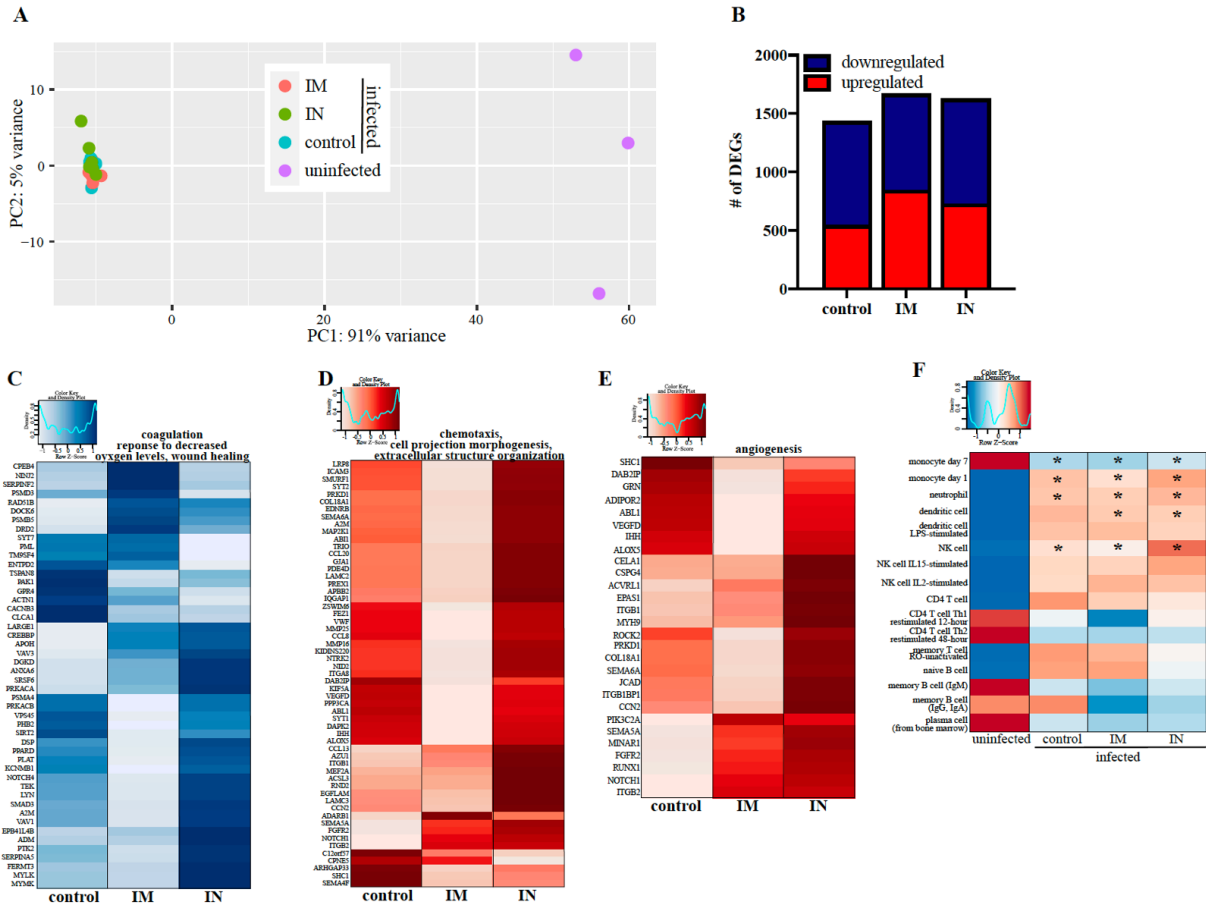


Figure S7. Lung RNA-sequencing. (A) Principal component analysis of lower left lung (LLL) samples from uninfected animals and vaccinated animals 7 days post challenge (control, intramuscular (IM) or intranasal (IN) vaccination). (B) Down- and up-regulated differentially expressed genes (DEGs). Heatmaps representing DEGs shared by all infected groups and enriching to Gene Ontology (GO) terms (C) “coagulation”, “response not decreased oxygen levels” and “wound healing” for downregulated DEG; and (D) “chemotaxis”, “cell projection morphogenesis” and “extracellular structure organization” and (E) “angiogenesis” for upregulated DEGs. Each column represents the median rpkms of the given group. Range of colors is based on scale and centered rpkms values of the represented DEGs. Red scale represents upregulated DEGs; blue scale represents downregulated DEGs. (F) *In silico* flow cytometry using ImmQuant IRIS database comparing challenged groups to uninfected controls. Red represents upregulation; blue represents downregulation. Each column represents the average relative predicted frequency of the given cell type. P-values are calculated relative to the uninfected animals. Statistical significance is indicated. For all heatmaps, range of colors is based on scale and centered rpkms values of the represented DEGs.

671 **References**

- 672 1. P. Zhou *et al.*, A pneumonia outbreak associated with a new coronavirus of probable bat
673 origin. *Nature* **579**, 270-273 (2020).
- 674 2. W. J. Guan *et al.*, Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J*
675 *Med* **382**, 1708-1720 (2020).
- 676 3. E. W. Cheung *et al.*, Multisystem Inflammatory Syndrome Related to COVID-19 in
677 Previously Healthy Children and Adolescents in New York City. *JAMA* **324**, 294-296
678 (2020).
- 679 4. R. Mao *et al.*, Manifestations and prognosis of gastrointestinal and liver involvement in
680 patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol*
681 *Hepatol* **5**, 667-678 (2020).
- 682 5. D. Wichmann *et al.*, Autopsy Findings and Venous Thromboembolism in Patients With
683 COVID-19: A Prospective Cohort Study. *Ann Intern Med* **173**, 268-277 (2020).
- 684 6. F. Zhou *et al.*, Clinical course and risk factors for mortality of adult inpatients with
685 COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* **395**, 1054-1062 (2020).
- 686 7. F. Wu *et al.*, A new coronavirus associated with human respiratory disease in China.
687 *Nature* **579**, 265-269 (2020).
- 688 8. M. L. Holshue *et al.*, First Case of 2019 Novel Coronavirus in the United States. *N Engl J*
689 *Med* **382**, 929-936 (2020).
- 690 9. Q. Li *et al.*, Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected
691 Pneumonia. *N Engl J Med* **382**, 1199-1207 (2020).
- 692 10. M. Letko, A. Marzi, V. Munster, Functional assessment of cell entry and receptor usage
693 for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol* **5**, 562-569 (2020).
- 694 11. B. Ju *et al.*, Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* **584**,
695 115-119 (2020).
- 696 12. A. O. Hassan *et al.*, A Single-Dose Intranasal ChAd Vaccine Protects Upper and Lower
697 Respiratory Tracts against SARS-CoV-2. *Cell* **183**, 169-184 e113 (2020).
- 698 13. N. van Doremalen *et al.*, ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in
699 rhesus macaques. *Nature* **586**, 578-582 (2020).
- 700 14. J. Yu *et al.*, DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science* **369**,
701 806-811 (2020).
- 702 15. N. B. Mercado *et al.*, Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus
703 macaques. *Nature* **586**, 583-588 (2020).
- 704 16. K. S. Corbett *et al.*, Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in
705 Nonhuman Primates. *N Engl J Med* **383**, 1544-1555 (2020).
- 706 17. A. Marzi, H. Feldmann, T. W. Geisbert, D. Falzarano, Vesicular Stomatitis Virus-Based
707 Vaccines for Prophylaxis and Treatment of Filovirus Infections. *J Bioterror Biodef* **S1**,
708 (2011).
- 709 18. C. E. Mire *et al.*, Use of Single-Injection Recombinant Vesicular Stomatitis Virus Vaccine
710 to Protect Nonhuman Primates Against Lethal Nipah Virus Disease. *Emerg Infect Dis* **25**,
711 1144-1152 (2019).
- 712 19. D. Safronetz *et al.*, A recombinant vesicular stomatitis virus-based Lassa fever vaccine
713 protects guinea pigs and macaques against challenge with geographically and
714 genetically distinct Lassa viruses. *PLoS Negl Trop Dis* **9**, e0003736 (2015).

- 715 20. A. Fathi, C. Dahlke, M. M. Addo, Recombinant vesicular stomatitis virus vector vaccines
716 for WHO blueprint priority pathogens. *Hum Vaccin Immunother* **15**, 2269-2285 (2019).
- 717 21. A. Marzi *et al.*, EBOLA VACCINE. VSV-EBOV rapidly protects macaques against infection
718 with the 2014/15 Ebola virus outbreak strain. *Science* **349**, 739-742 (2015).
- 719 22. W. Furuyama *et al.*, A single dose of a vesicular stomatitis virus-based influenza vaccine
720 confers rapid protection against H5 viruses from different clades. *NPJ Vaccines* **5**, 4
721 (2020).
- 722 23. K. S. Brown, D. Safronetz, A. Marzi, H. Ebihara, H. Feldmann, Vesicular stomatitis virus-
723 based vaccine protects hamsters against lethal challenge with Andes virus. *J Virol* **85**,
724 12781-12791 (2011).
- 725 24. A. M. Henao-Restrepo *et al.*, Efficacy and effectiveness of an rVSV-vectored vaccine in
726 preventing Ebola virus disease: final results from the Guinea ring vaccination, open-
727 label, cluster-randomised trial (Ebola Ca Suffit!). *Lancet* **389**, 505-518 (2017).
- 728 25. X. Qiu *et al.*, Mucosal immunization of cynomolgus macaques with the
729 VSVDeltaG/ZEBOVGP vaccine stimulates strong ebola GP-specific immune responses.
730 *PLoS One* **4**, e5547 (2009).
- 731 26. J. Emanuel *et al.*, A VSV-based Zika virus vaccine protects mice from lethal challenge. *Sci*
732 *Rep* **8**, 11043 (2018).
- 733 27. V. J. Munster *et al.*, Respiratory disease in rhesus macaques inoculated with SARS-CoV-
734 2. *Nature* **585**, 268-272 (2020).
- 735 28. A. Marzi *et al.*, Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection
736 against lethal Ebola virus challenge in nonhuman primates. *Proc Natl Acad Sci U S A* **110**,
737 1893-1898 (2013).
- 738 29. A. R. Menicucci *et al.*, Transcriptome Analysis of Circulating Immune Cell Subsets
739 Highlight the Role of Monocytes in Zaire Ebola Virus Makona Pathogenesis. *Front*
740 *Immunol* **8**, 1372 (2017).
- 741 30. J. Wang *et al.*, Single mucosal, but not parenteral, immunization with recombinant
742 adenoviral-based vaccine provides potent protection from pulmonary tuberculosis. *J*
743 *Immunol* **173**, 6357-6365 (2004).
- 744 31. M. R. Neutra, P. A. Kozlowski, Mucosal vaccines: the promise and the challenge. *Nat Rev*
745 *Immunol* **6**, 148-158 (2006).
- 746 32. L. H. A. Cavalcante-Silva *et al.*, Neutrophils and COVID-19: The road so far. *Int*
747 *Immunopharmacol* **90**, 107233 (2020).
- 748 33. V. Francois-Newton *et al.*, USP18-based negative feedback control is induced by type I
749 and type III interferons and specifically inactivates interferon alpha response. *PLoS One*
750 **6**, e22200 (2011).
- 751 34. T. Matsumura *et al.*, TIFAB inhibits TIFA, TRAF-interacting protein with a forkhead-
752 associated domain. *Biochem Biophys Res Commun* **317**, 230-234 (2004).
- 753 35. J. J. Wong, Y. F. Pung, N. S. Sze, K. C. Chin, HERC5 is an IFN-induced HECT-type E3 protein
754 ligase that mediates type I IFN-induced ISGylation of protein targets. *Proc Natl Acad Sci*
755 *U S A* **103**, 10735-10740 (2006).
- 756 36. Y. Peng *et al.*, Broad and strong memory CD4(+) and CD8(+) T cells induced by SARS-CoV-
757 2 in UK convalescent individuals following COVID-19. *Nat Immunol* **21**, 1336-1345
758 (2020).

- 759 37. O. Boyman, J. Sprent, The role of interleukin-2 during homeostasis and activation of the
760 immune system. *Nat Rev Immunol* **12**, 180-190 (2012).
- 761 38. G. Abrahamsen, V. Sundvold-Gjerstad, M. Habtamu, B. Bogen, A. Spurkland, Polarity of
762 CD4+ T cells towards the antigen presenting cell is regulated by the Lck adapter TSAd.
763 *Sci Rep* **8**, 13319 (2018).
- 764 39. J. S. Woo *et al.*, CRACR2A-Mediated TCR Signaling Promotes Local Effector Th1 and Th17
765 Responses. *J Immunol* **201**, 1174-1185 (2018).
- 766 40. Y. Xiong *et al.*, Transcriptomic characteristics of bronchoalveolar lavage fluid and
767 peripheral blood mononuclear cells in COVID-19 patients. *Emerg Microbes Infect* **9**, 761-
768 770 (2020).
- 769 41. J. B. Case *et al.*, Replication-Competent Vesicular Stomatitis Virus Vaccine Vector
770 Protects against SARS-CoV-2-Mediated Pathogenesis in Mice. *Cell Host Microbe* **28**, 465-
771 474 e464 (2020).
- 772 42. A. Marzi *et al.*, Single low-dose VSV-EBOV vaccination protects cynomolgus macaques
773 from lethal Ebola challenge. *EBioMedicine* **49**, 223-231 (2019).
- 774 43. A. Huttner *et al.*, Determinants of antibody persistence across doses and continents
775 after single-dose rVSV-ZEBOV vaccination for Ebola virus disease: an observational
776 cohort study. *Lancet Infect Dis* **18**, 738-748 (2018).
- 777 44. J. Harcourt *et al.*, Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with
778 Coronavirus Disease, United States. *Emerg Infect Dis* **26**, 1266-1273 (2020).
- 779 45. Y. Tsuda *et al.*, Protective efficacy of a bivalent recombinant vesicular stomatitis virus
780 vaccine in the Syrian hamster model of lethal Ebola virus infection. *J Infect Dis* **204 Suppl**
781 **3**, S1090-1097 (2011).
- 782 46. H. B. TW, T. Girke, systemPipeR: NGS workflow and report generation environment.
783 *BMC Bioinformatics* **17**, 388 (2016).