

1 A valid protective immune response elicited in rhesus macaques by an  
2 inactivated vaccine is capable of defending against SARS-CoV-2 infection

3

4 Hongbo Chen<sup>1, #</sup>, Zhongping Xie<sup>1, #</sup>, Runxiang Long<sup>1, #</sup>, Shengtao Fan<sup>1, #</sup>, Heng Li<sup>1, #</sup>,  
5 Zhanlong He<sup>1, #</sup>, Kanwei Xu<sup>5, #</sup>, Yun Liao<sup>1</sup>, Lichun Wang<sup>1</sup>, Ying Zhang<sup>1</sup>, Xueqi Li<sup>1</sup>, Xingqi  
6 Dong<sup>2</sup>, Tangwei Mou<sup>1</sup>, Xiaofang Zhou<sup>3</sup>, Yaoyun Yang<sup>1</sup>, Lei Guo<sup>1</sup>, Jianbo Yang<sup>1</sup>, Huiwen  
7 Zheng<sup>1</sup>, Xingli Xu<sup>1</sup>, Jing Li<sup>1</sup>, Yan Liang<sup>1</sup>, Dandan Li<sup>1</sup>, Zhimei Zhao<sup>1</sup>, Chao Hong<sup>1</sup>, Heng  
8 Zhao<sup>1</sup>, Guorun Jiang<sup>1</sup>, Yanchun Che<sup>1</sup>, Fengmei Yang<sup>1</sup>, Yunguang Hu<sup>1</sup>, Xi Wang<sup>1</sup>, Jing  
9 Pu<sup>1</sup>, Kaili Ma<sup>1</sup>, Lin Wang<sup>4</sup>, Chen Chen<sup>1</sup>, Weiguo Duan<sup>1</sup>, Dong Shen<sup>1</sup>, Hongling Zhao<sup>1</sup>,  
10 Ruiju Jiang<sup>1</sup>, Xinjiang Deng<sup>1</sup>, Yan Li<sup>1</sup>, Hailian Zhu<sup>1</sup>, Jian Zhou<sup>1</sup>, Li Yu<sup>1</sup>, Mingjue Xu<sup>1</sup>,  
11 Huijuan Yang<sup>1</sup>, Li Yi<sup>1</sup>, Zhenxin Zhou<sup>1</sup>, Jiafang Yang<sup>1</sup>, Nan Duan<sup>1</sup>, Huan Yang<sup>1</sup>, Wangli  
12 Zhao<sup>1</sup>, Wei Yang<sup>1</sup>, Changgui Li<sup>5, \*</sup>, Longding Liu<sup>1, \*</sup>, Qihan Li<sup>1, \*</sup>

13

14 1, Institute of Medical Biology, Chinese Academy of Medicine Science & Peking Union

15 Medical College, Yunnan Key Laboratory of Vaccine Research and Development on

16 Severe Infectious Diseases, Kunming, 650118, China

17 2, Yunnan Provincial Infectious Disease Hospital, Kunming, 650301, China

18 3, Yunnan Center for disease control and prevention, Kunming, 650034, China

19 4, Kunming Third People's Hospital, Kunming, 650041, China

20 5, National Institute of Food and Drug Control, Beijing, 100050, China

21

22 **Running title:** SARS-CoV-2 inactivated vaccine protected rhesus macaques against

23 infection

24

25 <sup>#</sup> Equal contributors

26 <sup>\*</sup> Correspondence authors: Qihan Li (liqihan@imbcams.com.cn), Longding Liu

27 (liuld@imbcams.com.cn) and Changgui Li (changguili@aliyun.com).

28 Institute of Medical Biology, Chinese Academy of Medicine Sciences and Peking

29 Union Medical College, Yunnan Key Laboratory of Vaccine Research and

30 Development on Severe Infectious Diseases. No. 935 Jiaolong Road, Kunming, Yunnan,

31 China, 650118. Tel: 86-871-68335905. Fax: 86-871-68334483.

32

33 **Abstract**

34 With the relatively serious global epidemic outbreak of SARS-CoV-2 infection, public  
35 concerns focus on not only clinical therapeutic measures and public quarantine for  
36 this disease but also the development of vaccines. The technical design of our  
37 SARS-CoV-2 inactivated vaccine provides a viral antigen that enables the exposure of  
38 more than one structural protein based upon the antibody composition of COVID-19  
39 patients' convalescent serum. This design led to valid immunity with increasing  
40 neutralizing antibody titers and a CTL response detected post-immunization of this  
41 vaccine by two injections in rhesus macaques. Further, this elicited  
42 immunoprotection in macaques enables not only to restrain completely viral  
43 replication in tissues of immunized animals, compared to the adjuvant control and  
44 those immunized by an RBD peptide vaccine, but also to significantly alleviate  
45 inflammatory lesion in lung tissues in histo-pathologic detection, compared to the  
46 adjuvant control with developed interstitial pneumonia. The data obtained from  
47 these macaques immunized with the inactivated vaccine or RBD peptide vaccine  
48 suggest that immunity with a clinically protective effect against SARS-CoV-2 infection  
49 should include not only specific neutralizing antibodies but also specific CTL  
50 responses against at least the S and N antigens.

51 **Introduction**

52 Since the end of last year, a new species of coronavirus, a contagious agent  
53 capable of causing acute and severe respiratory infection and pneumonia through  
54 airborne transmission, has rapidly caused global public concern and even popular  
55 panic (1-3); the specific coronavirus was named SARS-CoV-2 by the World Health  
56 Organization (WHO) (4). Updated data have reported more than 3 million infection  
57 cases in the adult age group (5), with a death rate of approximately 2-10%, mostly in  
58 the elderly population (6, 7). With its high pathogenicity and infectivity and its spread  
59 in 200 countries and areas in a short time (8-10), not only multiple measures for  
60 clinical treatment and cure and public isolation but also vaccine development are  
61 urgently needed, and vaccine development could play a proactive role in controlling  
62 this epidemic (11). However, as SARS-CoV-2 is a new viral agent with an unknown  
63 infection mechanism and an unclear interaction with the immune system, vaccine  
64 development should first answer some basic questions, including characterization of  
65 antigenic component of this virus and its immunogenicity; validation of the  
66 protective immunity elicited by the viral antigen via certain immune processes; and  
67 whether the process of eliciting immunity to a viral antigen may be associated with  
68 immunopathogenesis during viral infection, similar to many concerns related to  
69 antibody-dependent enhancement (ADE) (12, 13). Based on these considerations,  
70 the work here raised the following inferential idea: the fact that SARS-CoV-2 infects  
71 cells through its spike (S) protein undergoing membrane binding with angiotensin  
72 converting enzyme 2 (ACE2) molecules in the cell membrane indicates the S protein

73 is a major viral antigen that elicits a neutralizing antibody response (14, 15), while  
74 the nucleocapsid (N) protein, the other major structural component, acts as an  
75 antigenic stimulator of the innate immune response through its recognition as a  
76 pathogen-associated molecule pattern (PAMP) by cellular pattern recognition  
77 receptors (PRRs) in epithelial cells (16, 17). If this is the case, the N antigen should be  
78 significant in the study of viral vaccines, and the antibodies against the N protein may  
79 play a role in the antiviral immunity expected in vaccine development. Logically,  
80 these N-specific antibodies should be considered with the S-specific antibodies to be  
81 related to ADEs, which might exist in SARS-CoV-2-infected individuals (12). Our work  
82 describes a SARS-CoV-2 inactivated vaccine developed based on the above deduction  
83 and suggests a characterized immune response capable of defending against viral  
84 attack in rhesus macaques immunized with this vaccine, while the possible ADEs  
85 induced by existing antibodies against the S and N proteins are negated, as indicated  
86 by pathological observation and immunological detection. Conclusively, the  
87 inactivated vaccine is identified as having application potential.

88

## 89 **Results**

### 90 **The antigenicity of the SARS-CoV-2 inactivated vaccine interacting with** 91 **convalescent serum from COVID-19 patients**

92 Previous studies of viral inactivated vaccines have indicated that the capacity of  
93 vaccines to elicit valid antiviral immunity in immunized individuals depends upon the  
94 viral antigenicity that is required to have viral antigenic components and structures

95 displayed to the immune system of the body during the natural infectious process  
96 (18-20). With little knowledge about SARS-CoV-2, our inactivated vaccine created  
97 using a specific inactivating process was developed and investigated for its  
98 antigenicity by studying its interaction with convalescent serum, which was inferred  
99 to reflect, to some extent, the valid immune reactions of recovered individuals  
100 against viral infection. The results of a series of experiments were helpful in  
101 developing this vaccine. First, dozens of convalescent serum samples showed a  
102 neutralizing effect on the seed virus of the vaccine with varied titers of 1:16-256 (Fig.  
103 1a), which suggests a potential quantized responsiveness of immunity leading to  
104 recovery in infected individuals. Furthermore, 2D electrophoresis and immune  
105 blotting using the convalescent serum suggested that not only the S protein but also  
106 the N protein and other proteins in this inactivated vaccine were recognized by the  
107 serum, which was more extensive recognition than that observed by blotting using a  
108 mAb against the S or N protein (Fig. 1b), and importantly, the anti-N antibody  
109 appeared to be an ascendant component. This result suggests that convalescent  
110 serum contains more antiviral antibodies than simply neutralizing antibodies and  
111 that these additional antibodies may play multiple functions in antiviral immunity.  
112 Our work using ELISA plates coated with the purified S or N protein or whole virions  
113 of the inactivated vaccine detected the convalescent serum and showed an  
114 interesting relationship between the anti-S, anti-N and anti-whole virion antibodies  
115 in the serum (Fig. 1c). The design of our inactivated vaccine based on this  
116 relationship allowed exposure of the N and S antigens, which was determined by

117 visible electron microscopy observation using convalescent serum, mAb-S or mAb-N;  
118 the results showed similar interactions of the virion composing the inactivated  
119 vaccine and these antibodies (Fig. 1d).

120

121 **Immunization of rhesus macaques with the SARS-CoV-2 inactivated vaccine elicits**  
122 **effective immunity with indexes of humoral and cellular reactions**

123 Based on immunological studies of the SARS-CoV-2 inactivated vaccine in mice, our  
124 work basically integrated the GMTs of neutralizing antibodies observed in the  
125 convalescent serum from COVID-19 patients and those detected in the serum from  
126 mice immunized with the vaccine at various doses (Supplemental Fig. 1). The  
127 immune dose of 100 EU was determined to elicit neutralizing antibody titers in the  
128 range of 1:16-64 through the intramuscular route with two inoculations on days 0  
129 and 14 (Supplemental Fig. 1). Furthermore, 3 doses of 200, 100 or 20 EU were used  
130 to immunize groups A (4 macaques), B (3 macaques) and C (3 macaques),  
131 respectively, followed by a booster immunization on the 14<sup>th</sup> day, while 10 macaques  
132 were used as adjuvant controls. The immunological evaluation of these immunized  
133 macaques on day 7 after the 2<sup>nd</sup> inoculation indicated increasing neutralizing  
134 antibody titers (Fig. 2a), with GMTs of 107.6, 25.4 and 2 found in the 3 dose groups,  
135 respectively, and titers lower than 1:4 were observed in 2 macaques in the low-dose  
136 group. This result suggests a dose-dependent relationship for vaccine immunization.  
137 However, ELISA analysis with plates coated with the S or N protein indicated no  
138 obvious difference in trends of the titers of anti-S and anti-N antibodies (Fig. 2b).

139 Furthermore, ELISPOT analysis of IFN- $\gamma$  specificity also showed a positive cytotoxic T  
140 lymphocyte (CTL) response with no dose difference after stimulation with the S or N  
141 antigen (Fig. 2c). These results seem to suggest that the neutralizing antibodies  
142 against the S antigen showing a dose-dependent effect related to vaccine  
143 immunization are one of the components elicited in the humoral immune reaction.

144

145 **The integrated immune response elicited by the vaccine is capable of restraining**  
146 **viral replication in the respiratory and alimentary tracts of challenged macaques**

147 Based on the data obtained from above immunological detection of the SARS-CoV-2  
148 inactivated vaccine in rhesus macaques, we designed a viral challenge test involving  
149 10 macaques immunized with the vaccine as described above to identify the  
150 immunoprotective effect of the vaccine (Fig. 3a). The other 10 macaques were used  
151 as adjuvant controls for observation of clinical manifestations and viral shedding, and  
152 one challenged macaque was sacrificed under anesthesia at each time point.

153 Another 2 macaques, which were immunized with a ferritin-fused peptide from the  
154 RBD region of the S protein expressed in CHO cells twice with inoculations on days 0  
155 and 14 via the intramuscular route, and their neutralizing antibody titers reached  
156 1:16-32, but no anti-N antibody set-up (Supplemental Fig. 2) was used as a parallel  
157 control. All animals were challenged with wild-type virus with dose of  $2 \times 10^5$   
158 CCID<sub>50</sub>/each animal via the nasal route. Following viral challenge, monitoring of the  
159 body temperature; the viral load in pharyngeal secretions, nasal secretions, and anal  
160 swabs; and viremia was performed each day. The results suggested that no obvious

161 fluctuation in body temperature was observed in any immunized macaques,  
162 including the two immunized with the peptide vaccine, compared with the 10  
163 adjuvant control animals (Fig. 3b). The detection of viral loads indicated that viral  
164 shedding occurred in the nasal cavity of the macaques immunized with the  
165 inactivated vaccine, which is the site of virus challenge and the viral load decreased  
166 from 36 copies/100  $\mu$ l -  $3 \times 10^3$  copies/100  $\mu$ l on day 1 to less than 50 copies/100  $\mu$ l  
167 on day 2 and was maintained at that level through day 15 post infection (Fig. 3c),  
168 while the viral loads in pharyngeal and anal swabs were lower than 50 copies/100  $\mu$ l  
169 (Fig. 3c). Values of  $10^3$  -  $10^4$  copies/100  $\mu$ l and higher were found in the pharyngeal,  
170 nasal and anal swabs of the adjuvant control macaques for at least 8-9 days (Fig. 3c).  
171 In the analysis of the peripheral blood, no positive result was found in the inactivated  
172 vaccine group, but a peak value was observed on days 5-7 in the positive control  
173 group (Fig. 3c). Interestingly, the two macaques immunized with the peptide vaccine  
174 had neutralizing antibody titers of 1:16-32 and showed values higher than  $10^4$  and  
175  $10^3$  copies/100  $\mu$ l in nasal and anal swabs, respectively, on days 3-5 and 6-8 (Fig. 3c).  
176 These results suggest not only that the inactivated vaccine elicits a valid  
177 immunoprotective effect but also that an integrated immune response rather than  
178 neutralizing antibodies alone may be needed to restrain viral replication in the  
179 respiratory and/or alimentary tracts.

180

181 **The immunity induced by the inactivated vaccine can disrupt viral proliferation in**  
182 **organs and largely alleviate pathological damage to tissues**

183 Generally, the experimental index of immunoprotective effects on animal models for  
184 the study of inactivated virus vaccines includes viral replication and the virus-induced  
185 damage to tissues of vaccine-immunized animals during challenge tests with a  
186 wild-type virus (21, 22). Our work here also focused on the detection of the viral  
187 loads in tissues and pathological lesions in these tissues in immunized macaques,  
188 which were sacrificed under anesthesia on day 3, 5, 7, 9, or 15 post viral challenge.  
189 All tissue samples collected from these challenged animals, including adjuvant  
190 control monkeys and the monkeys immunized with the RBD peptide vaccine, were  
191 used for the detection of viral loads and pathological observation. q-RT-PCR results  
192 suggested that almost all samples from various tissues of the animals in the  
193 inactivated vaccine immunization group showed values lower than 50 copies/100 mg  
194 and only those in spleens were higher than 100 copies/100 mg (Fig. 4a). Macaque No.  
195 19177, which was immunized with the RBD peptide vaccine and sacrificed under  
196 anesthesia on day 9, was found to have 635, 2200 and 140 copies/100 mg in the  
197 intestine, cervical lymph nodes and spleen, respectively (Fig. 4a); and macaque No.  
198 19295, which was in the same group and sacrificed under anesthesia on day 15, was  
199 found to have 288,670 copies/100 mg in the cerebellum (Fig. 4a). However, the  
200 evaluation of adjuvant control animals showed a trend toward increasing viral loads  
201 in various organs during the observation period (Fig. 4a). In the pathological  
202 detection at 5 time points, the lung tissues of 3 groups of macaques immunized with  
203 the inactivated vaccine showed slight and nonspecific inflammatory reactions,  
204 including a few local aggregations of inflammatory cells, inflammatory exudation in

205 some alveoli and bronchioles, slight hyperplasia of the epithelial tissue in a few  
206 alveolar tissues and slight congestive reactions (Fig. 4b). The two macaques  
207 immunized with the RBD peptide vaccine presented similar changes (Fig. 4b), while  
208 the macaques in the adjuvant control group presented more severe inflammatory  
209 reactions, which were recognized as interstitial pneumonia based upon the  
210 histopathologic observation (Fig. 4b). These results suggest that the immunity  
211 elicited by the inactivated vaccine can restrain viral proliferation in various tissues in  
212 immunized individuals and alleviate pathological lesions in tissues caused by viral  
213 replication. Interestingly, elimination of the challenge virus by this immunity in  
214 macaques did not seem to be due to the existence of neutralizing antibodies. Two  
215 macaques immunized with the inactivated vaccine with a neutralizing antibody titer  
216 lower than 1:4 were capable of completely restraining viral replication *in vivo*, but  
217 the other two immunized with the RBD peptide vaccine had a titer of 1:16-32 and  
218 were not capable of completely eliminating viral replication.

219

220 **The immune response elicited in macaques by the inactivated vaccine presents an**

221 **immunologically dynamic process**

222 Previous reports on COVID-19 suggest it to be an acute and severe inflammatory  
223 disease of the respiratory system (1, 23) and lead to the deduction that the  
224 pathological mechanism may be due to not only viral infection but also a process of  
225 excessive immune reaction, especially ADE, induced by the virus (12). In this case,  
226 the study of the SARS-CoV-2 inactivated vaccine in an animal model should be

227 focused on the immunologically dynamic process associated with the elicitation of  
228 specific antiviral immunity through monitoring various indexes of the immune cell  
229 population, which should be maintained in a stable dynamic state (24). Our work  
230 detected altered proportions of different immune cells in the total population of  
231 PBMCs after viral challenge in macaques immunized with the inactivated vaccine.  
232 The results suggested that compared to that of the adjuvant control animals (Fig. 5a),  
233 the dynamic alteration in the PBMC population in all animals immunized with  
234 inactivated vaccine included increases in the proportions of T cells, NK cells, T cells  
235 with IFN- $\gamma$  specificity and Treg cells (Fig. 5a, b), in which upregulation of the  
236 percentages of IFN- $\gamma$ -specific T cells and Treg cells was observed on days 9 and 5 post  
237 viral challenge, respectively (Fig. 5b). Furthermore, the detection of some  
238 inflammatory factors, including IL-2, IL-4, IL-5, IL-6 and TNF- $\alpha$ , in the serum of all  
239 animals indicated that viral challenge was capable of slightly upregulating IL-2 and  
240 IL-5 levels in the inactivated vaccine group (Fig. 5c). These results suggest not only  
241 the dynamic activation of the immune system induced in macaques immunized with  
242 the inactivated vaccine.

243

## 244 **Discussion**

245 The global public health crisis induced by SARS-CoV-2, which leads to acute and  
246 severe infectious respiratory disease and/or pneumonia, is driving the rapid  
247 development of a suitable vaccine (3, 25). Facing this emergency requirement, a  
248 basic scientific question that first needs to be addressed is the antigen composition

249 capable of eliciting effective immunity in a vaccine; however, no clear antigenic  
250 analysis of different viral structural proteins is available. We investigated the  
251 interaction of our inactivated vaccine and convalescent serum from COVID-19  
252 patients based on the deduction that the recovery of a patient means his/her  
253 immune system was capable of controlling and/or eliminating the virus, and  
254 convalescent serum may contain antibodies effective in this process. The data from  
255 this study suggest that anti-S and anti-N antibodies may play similar roles in  
256 convalescent serum and that both types of antibodies, but especially anti-N  
257 antibodies, show a strong relationship with the general antibody response to the  
258 virus. This result led to our technical design of an inactivated vaccine capable of  
259 exposing the S protein and N protein upon immunization into animals. In the viral  
260 challenge test with macaques, this vaccine elicited a specific immune response with a  
261 neutralizing antibody titer that increased depending on the dose and specific CTL  
262 response against the S-antigen, N-antigen and virion. The ELISA confirmed that both  
263 anti-S and anti-N antibodies were elicited in these animals, which is different from  
264 the serum of animals immunized with the RBD peptide vaccine containing only anti-S  
265 antibodies that showed neutralizing activity of 1:16-32. Usually, neutralizing  
266 antibodies are capable of blocking the binding of a virus to receptors and are an  
267 index indicative of effective antiviral immunity (26, 27). In our study of the  
268 inactivated vaccine, an immunoprotective effect against viral challenge was observed  
269 in macaques immunized with high and medium doses of the inactivated vaccine,  
270 which were associated with increasing neutralizing antibody titers, while similar

271 protective effects were observed in low-dose immunized animals with a neutralizing  
272 antibody GMT of 2 and a CTL response similar to those of the high- and  
273 medium-dose groups. Interestingly, the immunity elicited in macaques immunized  
274 with the RBD peptide vaccine was unable to completely restrain viral proliferation in  
275 some tissues, even though the animals possessed neutralizing antibodies with titer of  
276 1:16-32. Two animals were found to have high viral shedding in the nasopharynx  
277 and/or alimentary tract during later times of viral infection with slightly more serious  
278 pathological inflammatory reactions in the lungs than animals in the high- and  
279 medium-dose groups treated with inactivated vaccine immunization. These results  
280 suggest that the immunity elicited by our inactivated vaccine can provide systematic  
281 immune protection against viral infection not only by producing upregulation of  
282 neutralizing antibody titers but also by functioning through the CTL response  
283 associated with increased anti-N antibody levels. This conclusion that integrated  
284 immunity involving antibodies against various viral proteins elicited by an inactivated  
285 vaccine is needed for the effective prevention of SARS-CoV-2 infection, at least in  
286 macaques, is supported by observation of macaques immunized with the RBD  
287 peptide vaccine. All data obtained here may lead to a logical analysis of the  
288 interaction of SARS-CoV-2 with the immune system, which indicates that the N  
289 protein may play an important role in the activation of innate immunity in epithelial  
290 cells to further the specific antiviral immune response via the N protein being a  
291 major viral antigen that interacts with PRRs in cells, while the S protein greatly  
292 impacts the elicitation of neutralizing antibodies and interacts with PRRs to a lesser

293 degree than the N protein. In this case, a valid systematic immunoprotective  
294 response against SARS-CoV-2 infection should include at least anti-S and anti-N  
295 antibodies, and innate immunity may contribute largely to this response and should  
296 be considered in vaccine development. The technical design of our inactivated  
297 vaccine enables N and S antigen exposure and elicits antibodies against both viral  
298 proteins, which indicates that this vaccine is of practicable significance. However, the  
299 observation of immunized macaques with a low titer of neutralizing antibodies due  
300 to the very low antigen amount does not suggest that ADE exists. Based on these  
301 data, this vaccine was permitted to enter clinical trial by CFDA with approval number  
302 of 2020L00020.

303

304 **Methods**

305 ***Virus and cells***

306 The SARS-CoV-2 virus used was isolated from the respiratory secretions of an adult  
307 male patient at Yunnan Hospital of Infectious Diseases in Kunming in January 2020.  
308 The virus proliferated in Vero cells (ATCC, Manassas, USA) and was purified by plaque  
309 cloning. The cloned virus was identified via genomic sequencing and named KMS-1  
310 (GenBank No: MT226610.1). Vero cells were cultured in DMEM (Corning, NY, USA)  
311 containing 5% fetal bovine serum (FCS; HyClone, Logan, USA).

312

313 ***Viral titration***

314 All experimental procedures were performed under BSL-3 laboratory conditions.

315 Virus samples were serially diluted 10-fold with serum-free DMEM (Corning, NY, USA).  
316 Different dilutions of the virus were added to a 96-well plate. Each dilution (100  $\mu$ l  
317 per well) was added to 8 parallel wells. Then, 100  $\mu$ l of Vero cell suspension was  
318 added to each well at a concentration of  $2.5 \times 10^5$  cells/ml. After the plate was  
319 incubated at 37°C in 5% CO<sub>2</sub> for 7 days, the cytopathic effect (CPE) was observed and  
320 assessed with an inverted microscope (Nikon, Tokyo, Japan).

321

322 ***Inactivated vaccine***

323 The SARS-CoV-2 inactivated vaccine was developed by the Institute of Medical  
324 Biology (IMB), Chinese Academy of Medical Sciences (CAMS). Briefly, the virus seed  
325 strain for the vaccine was inoculated into Vero cells, which was provided by WHO  
326 with batch number of UCC91-02 of main seed for vaccine production, and obtained  
327 the verification approval number of 201501 from National Institute for Food and  
328 Drug Control (NIFDC) of China, in BSL-3 environment. The viral harvest was  
329 inactivated by formaldehyde in rate of 1:4000 for 48 hours, which was found enable  
330 to break viral membrane. After this inactivation, the chromatograph process using  
331 Core-700 gel medium was performed for the first purification. Further, viral antigenic  
332 component collected was concentrated for the second inactivated with  
333 beta-propiolactone in rate of 1:2000 destructing viral genomic structure. After the  
334 second purification by the same medium, the vaccine stock was evaluated with  
335 various quality indexes including antigen content, immunogenicity, sterility and  
336 residues test etc. The viral antigen content was measured via ELISA assay, in which,

337 the antibodies against S, N protein and whole virion were used to coat plate for the  
338 detections of each antigenic component. A solution of the inactivated virus vaccine  
339 stock was then emulsified in 0.5 mg/ml Al(OH)<sub>3</sub> adjuvant, constituting the final  
340 vaccine.

341

342 ***Ethics***

343 Human --- Convalescent serum samples were collected from patients diagnosed with  
344 new coronavirus pneumonia at Yunnan Hospital of Infectious Diseases, Kunming  
345 Third People's Hospital and CDC of Xianyang city, Hubei province, with the patients  
346 providing informed consent. The protocols were reviewed and approved by the  
347 Experimental Management Association of the IMB, CAMS (approval number: DWSP  
348 202003 004).

349 Animal --- The animal experiment was designed and performed according to the  
350 principles in the "Guide for the Care and Use of Laboratory Animals" and in  
351 "Guidance for Experimental Animal Welfare and Ethical Treatment". The protocols  
352 were reviewed and approved by the Experimental Animal Management Association  
353 of the IMB, CAMS (approval number: DWSP 202003 005). All animals were fully  
354 under the care of veterinarians at the IMB, CAMS.

355

356 ***Animals***

357 Mouse --- Four-week-old female BALB/c mice (Beijing Vital River Laboratory Animal  
358 Technologies Co. Ltd, Beijing, China) were housed in a laboratory (ABSL-3) within the

359 specific pathogen-free facility at the IMB, CAMS. Mice were anesthetized with  
360 inhaled 2% isoflurane for all procedures, with every effort made to minimize  
361 suffering.

362 Monkey --- Rhesus monkeys (age 1.5-2 years) were bred and fed pellets (IMB, CAMS,  
363 China) and fresh fruits in a laboratory (ABSL-3) at the IMB, CAMS.

364

365 ***Immunization***

366 Mouse --- Mice were randomly divided into four groups, intramuscularly immunized  
367 with the vaccine at 200 ELISA units (EU; viral antigen concentration determined by  
368 ELISA), 100 EU or 20 EU or with Al(OH)<sub>3</sub> adjuvant alone, which was used as a control,  
369 on days 0 and 14. Blood samples were collected for neutralization assays on day 7  
370 after the booster injection.

371 Monkey --- Rhesus monkeys were randomly divided into four groups intramuscularly  
372 immunized with the vaccine at 200 EU (N=4), 100 EU (N=3) or 20 EU (N=3) or with  
373 Al(OH)<sub>3</sub> adjuvant alone (N=10) as a control on days 0 and 14. The monkeys were  
374 tested for neutralizing antibodies and IFN- $\gamma$ -secreting cells in blood samples taken on  
375 day 7 after the booster injection.

376

377 ***Viral challenge***

378 SARS-CoV-2 infection ( $2 \times 10^5$  CCID<sub>50</sub>/monkey) via nasal spray was performed under  
379 ABSL-3 laboratory conditions for the immunized macaques by inactivated vaccine  
380 and adjuvant control group. As a parallel control, two monkeys immunized with a

381 peptide vaccine (Supplemental Fig. 2) were also infected with the patient-derived live  
382 SARS-CoV-2 strain. All animals were monitored daily for clinical signs. Pharyngeal  
383 secretion, nasal secretion, anal swab and blood samples were obtained every day  
384 after infection. Blood was collected under appropriate anesthesia to alleviate pain  
385 and minimize suffering.

386 In the 200 EU-dose vaccine group, a monkey was euthanized on days 3, 5, 7 and 9  
387 postinfection (dpi). In the 100 EU-dose vaccine group, a monkey was euthanized on  
388 days 3, 7 and 15 dpi. In the 20 EU-dose vaccine group, a monkey was euthanized on  
389 days 5, 9 and 15 dpi. In the adjuvant control group, a monkey was euthanized at each  
390 time point (days 3, 5, 7, 9 and 15 dpi). In the RBD peptide vaccine group, a monkey  
391 was euthanized on days 9 and 15 dpi. The blood and organs of the euthanized  
392 monkeys were obtained for various experiments. The remaining 5 macaques in the  
393 adjuvant control groups were used for observation of clinical manifestations and viral  
394 shedding.

395

396 ***2D protein electrophoresis-Western blot analysis***

397 Purified virus samples were resuspended in 2D lysis buffer (8 M urea, 2 M thiourea, 4%  
398 CHAPS, 100 mM DTT, and 2% IPG buffer). Total protein was quantified using the  
399 PlusOne 2D Quant Kit (GE Healthcare Europe GmbH, Freiburg, Germany) according to  
400 the manufacturer's instructions. Approximately 200 µg of protein were first  
401 separated based on their pI using immobilized linear gradient strips (ImmobilineTM  
402 DryStrip, Amersham Biosciences Europe GmbH, Freiburg, Germany) covering the pH

403 range 4-7 and then separated by 12% SDS-PAGE. Proteins were transferred to a  
404 polyvinylidene fluoride membrane. The transferred membrane was blocked in 5%  
405 bovine serum albumin (BSA)-Tris-buffered saline/Tween 20 (Tris-HCl, 100 mM, pH 7.5;  
406 NaCl, 0.9%; and Tween-20, 0.2%) and treated with convalescent serum, anti-S (Snio  
407 Biological, Beijing, China), anti-N antibody (Snio Biological) and horseradish  
408 peroxidase (HRP)-labeled secondary antibody (Abcam, MA, USA) to visualize the  
409 proteins according to the standard protocol of the enhanced chemiluminescence  
410 (ECL) reagent.

411

412 ***Neutralizing antibody test***

413 Heat-inactivated serum was diluted and coincubated with live virus (100  
414 IgCCID<sub>50</sub>/well) for 2 h at 37°C, followed by addition of Vero cells (10<sup>5</sup>/mL) of 100 µl to  
415 the mixture. Then, the plates were incubated at 37°C in 5% CO<sub>2</sub> for 7 days. The CPEs  
416 were observed and assessed with an inverted microscope (Nikon) to determine the  
417 neutralizing antibody titer of the serum. The geometric mean titers (GMTs) of  
418 neutralizing antibodies were measured.

419 .

420 ***ELISA***

421 The S protein (Sanyou Biopharmaceuticals Co., Ltd., Shanghai, China), the N protein  
422 (Sanyou Biopharmaceuticals Co., Ltd.) and purified viral antigen were used to coat  
423 separate wells of 96-well ELISA plates (Corning, NY, USA) at a concentration of 5  
424 µg/well and incubated at 4°C overnight. Then, the plates were blocked with 5%

425 BSA-phosphate-buffered saline (PBS), incubated with serum samples, and visualized  
426 with an HRP-conjugated antibody (Abcam, MA, USA) and TMB substrate (Solarbio,  
427 Beijing, China) according to previously described methods (28). The absorbance of  
428 each well at 450 nm was measured using an ELISA plate reader (Gene Company,  
429 Beijing, China), and the following equation was used: resulting OD = (experimental  
430 well OD) – (mock well OD). The standards used were commercial antibodies at  
431 known concentrations (Sino Biological). Antibody concentrations were calculated  
432 according to a standard curve.

433

434 ***ELISPOT***

435 An ELISPOT assay was performed with the Monkey IFN- $\gamma$  ELISPOT Kit (Mabtech,  
436 Cincinnati, OH, USA) according to the manufacturer's protocol. Briefly, peripheral  
437 blood mononuclear cells (PBMCs) were isolated from the blood by lymphocyte  
438 isolation (Ficoll-Paque PREMIUM; GE Healthcare, Piscataway, NJ, USA) and plated in  
439 duplicate wells. Three different stimulators, purified SARS-CoV-2 antigen,  
440 recombinant S-protein (Sanyou Biopharmaceuticals Co., Ltd.) and recombinant  
441 N-protein (Sanyou Biopharmaceuticals Co., Ltd.), were added to separate wells. The  
442 positive control was phytohemagglutinin (PHA). The plate was incubated at 37°C for  
443 24 h, after which time the cells were removed, and the spots were developed. The  
444 colored spots were counted with an ELISPOT reader (CTL, Shaker Heights, OH, USA).

445

446 ***Electron microscopy***

447 Purified inactivated SARS-CoV-2 preparations were coincubated with convalescent  
448 serum, a monoclonal antibody (mAb) against S (mAb-S) or N (mAb-N) (Solarbio,  
449 Beijing, China) at 37°C for 24 h, stained with 1% phosphotungstic acid and observed  
450 by transmission electron microscopy (Hitachi, Kyoto, Japan).

451

452 ***Quantitation of the viral load by q-RT-PCR***

453 Total RNA was extracted from blood and tissue samples from experimental monkeys  
454 with TRIzol reagent (Tiangeng, Beijing, China). According to the protocol, q-RT-PCR  
455 was performed using the One Step PrimeScript™ RT-PCR Kit (Perfect Real Time;  
456 TaKaRa). The primers used for q-RT-PCR were selected to be specific for the E and  
457 ORF1ab sequences in the SARS-CoV-2 genome (Table 1). Viral copies were quantified  
458 according to vitro-synthesized RNA, and the quantity was expressed as a relative  
459 copy number, determined by the equation  $[(\mu\text{g of RNA}/\mu\text{l}) / (\text{molecular weight})] \times$   
460 Avogadro's number = viral copy number/ $\mu\text{l}$  (29).

461

462 ***Histopathological examinations***

463 The organs of experimental monkeys were fixed in 10% formalin, embedded in  
464 paraffin, sliced into 4- $\mu\text{m}$  sections and stained with hematoxylin and eosin (H&E).  
465 Morphology was assessed with an inverted microscope (Nikon).

466

467 ***Immune cell populations***

468 PBMCs were isolated from monkeys by lymphocyte isolation (Ficoll-Paque PREMIUM;

469 GE Healthcare). Anti-CD3, anti-CD20 and anti-CD16 antibodies were added to the  
470 PBMCs. The mixtures were incubated at room temperature (RT) for 30 min in the  
471 dark. Reagents for red blood cell lysis (BD) and membrane permeabilization (BD)  
472 were added in sequence. After washing with PBS twice, the cells were resuspended  
473 in PBS and detected using a flow cytometer (BD, USA). T cells (CD3<sup>+</sup>), B cells (CD20<sup>+</sup>)  
474 and NK cells (CD16<sup>+</sup>) were evaluated. Furthermore, the T cells were typed into T  
475 helper (Th) 1, Th2, regulatory T (Treg) and Th17 cells. The PBMCs were coincubated  
476 with an anti-CD4 antibody (BD) at RT for 30 min. Red blood cell lysis (BD) and  
477 membrane permeabilization (BD) reagents were added. After incubating with the red  
478 blood cell lysis and membrane permeabilization reagents and washing with PBS twice,  
479 the cells were stained with anti-FOXP3, anti-IL-4, anti-IFN- $\gamma$  and anti-IL-17A  
480 antibodies for 30 min at RT and then washed once. The percentages of immune cells  
481 were detected using a flow cytometer (BD). Th1 cells (CD4<sup>+</sup>/IFN- $\gamma$ <sup>+</sup>), Th2 cells  
482 (CD4<sup>+</sup>/IL-4<sup>+</sup>), Treg cells (CD4<sup>+</sup>/FOXP3<sup>+</sup>) and Th17 cells (CD4<sup>+</sup>/IL-17A<sup>+</sup>) were assessed.

483

484 ***Cytokine assay***

485 The levels of IL-2, IL-4, IL-5, IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the serum of experimental  
486 monkeys were detected with the Non-Human Primate (NHP) Th1/Th2 Cytokine Kit  
487 (BD) according to the manufacturer's protocol. Briefly, serum was added into a tube  
488 containing detection beads. Then, the PE detection reagent was added, and the  
489 mixtures were incubated at RT for 2 h in the dark. After washing, the beads were  
490 resuspended in wash buffer and detected using a flow cytometer (BD). The levels of

491 these cytokines were calculated according to a standard curve.

492

493 ***Statistical analysis***

494 Data are shown as the mean and standard deviation. GraphPad Prism software (San  
495 Diego, CA, USA) and STATA (Version 15.0; STATA Corp., College Station, TX, USA) were  
496 used for statistical analyses. The association of antibodies against S, N and virus  
497 (WVN) was Spearman rank correlation analysis.

498

499 **Author contributions**

500 QHL, LDL and CGL conceived and designed the experiments; HBC, ZPX, RXL, STF, HL,  
501 ZLH, KWX, YLiao, LCW, XQL, TWM, YYY, LG, JBY, HWZ, XLX, JL, YLiang, DDL, HZ, GRJ,  
502 FMY, YGH, XW, CC, XQD, YLi, HLZ, JZ, HJY, JFY and WLZ performed the experiments;  
503 QHL, LDL, YZ and ZMZ analyzed the data; XQD, XFZ, CH, YCC, JP, KLM, LW, WGD, DS,  
504 HLZ, RJJ, LY, MJX, LYi, ZXZ, ND, HYang and WY contributed reagents/materials/analysis  
505 tools; and QHL wrote the paper.

506

507 **Acknowledgments**

508 This work was supported by grant of National Key R&D Program of China  
509 (2020YFC0849700) and Major science and technology special projects of Yunnan  
510 Province.

511

512 **Conflicts of Interest**

513 All authors have completed the Unified Competing Interest form and declare that  
514 they have no financial and non-financial competing interests. The corresponding  
515 authors had full access to all the data generated in the present study and assume full  
516 responsibility for the final submission of this manuscript for publication.

517

518 **Reference**

- 519 1. Bulut, C., and Kato, Y. 2020. Epidemiology of COVID-19. *Turk J Med Sci* 50:563-570.
- 520 2. Asadi-Pooya, A.A., and Simani, L. 2020. Central nervous system manifestations of COVID-19: A  
521 systematic review. *J Neurol Sci* 413:116832.
- 522 3. Jee, Y. 2020. WHO International Health Regulations Emergency Committee for the COVID-19  
523 outbreak. *Epidemiol Health* 42:e2020013.
- 524 4. Gabutti, G., d'Anchera, E., Sandri, F., Savio, M., and Stefanati, A. 2020. Coronavirus: Update  
525 Related to the Current Outbreak of COVID-19. *Infect Dis Ther*:1-13.
- 526 5. Johns\_Hopkins\_University. 2020. COVID-19 Dashboard by the Center for Systems Science and  
527 Engineering (CSSE) at Johns Hopkins University (JHU).
- 528 6. Tezer, H., and Bedir Demirdag, T. 2020. Novel coronavirus disease (COVID-19) in children. *Turk  
529 J Med Sci* 50:592-603.
- 530 7. Palacios Cruz, M., Santos, E., Velazquez Cervantes, M.A., and Leon Juarez, M. 2020. COVID-19,  
531 a worldwide public health emergency. *Rev Clin Esp*.
- 532 8. Setiati, S., and Azwar, M.K. 2020. COVID-19 and Indonesia. *Acta Med Indones* 52:84-89.
- 533 9. Bahl, P., Doolan, C., de Silva, C., Chughtai, A.A., Bourouiba, L., and MacIntyre, C.R. 2020.  
534 Airborne or droplet precautions for health workers treating COVID-19? *J Infect Dis*.
- 535 10. People's\_Daily. 2020. Over 2.93 million cases worldwide, over 960,000 cases in the United  
536 States.
- 537 11. Ahn, D.G., Shin, H.J., Kim, M.H., Lee, S., Kim, H.S., Myoung, J., Kim, B.T., and Kim, S.J. 2020.  
538 Current Status of Epidemiology, Diagnosis, Therapeutics, and Vaccines for Novel Coronavirus  
539 Disease 2019 (COVID-19). *J Microbiol Biotechnol* 30:313-324.
- 540 12. Tetro, J.A. 2020. Is COVID-19 receiving ADE from other coronaviruses? *Microbes Infect*  
541 22:72-73.
- 542 13. Sharma, A. 2020. It is too soon to attribute ADE to COVID-19. *Microbes Infect*.
- 543 14. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens,  
544 T.S., Herrler, G., Wu, N.H., Nitsche, A., et al. 2020. SARS-CoV-2 Cell Entry Depends on ACE2  
545 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 181:271-280 e278.
- 546 15. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., et al.  
547 2020. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2  
548 receptor. *Nature*.
- 549 16. Kumar, H., Kawai, T., and Akira, S. 2011. Pathogen recognition by the innate immune system.  
550 *Int Rev Immunol* 30:16-34.

551 17. Wolf, A.J., and Underhill, D.M. 2018. Peptidoglycan recognition by the innate immune system.  
552 *Nat Rev Immunol* 18:243-254.

553 18. Tang, G., Yin, W., Cao, Y., Tan, L., Wu, S., Cao, Y., Fu, X., Yan, J., and Jiang, X. 2018.  
554 Immunogenicity of sequential inactivated and oral poliovirus vaccines (OPV) versus  
555 inactivated poliovirus vaccine (IPV) alone in healthy infants: A systematic review and  
556 meta-analysis. *Hum Vaccin Immunother* 14:2636-2643.

557 19. Esparza, J. 2013. A tale of two vaccines: lessons from polio that could inform the  
558 development of an HIV vaccine. *Aids* 27:1-5.

559 20. Plotkin, S.A. 2009. Vaccines: the fourth century. *Clin Vaccine Immunol* 16:1709-1719.

560 21. Riese, P., Trittel, S., Schulze, K., and Guzman, C.A. 2015. Rodents as pre-clinical models for  
561 predicting vaccine performance in humans. *Expert Rev Vaccines* 14:1213-1225.

562 22. Tuero, I., and Robert-Guroff, M. 2014. Challenges in mucosal HIV vaccine development:  
563 lessons from non-human primate models. *Viruses* 6:3129-3158.

564 23. Tufan, A., Avanoglu Guler, A., and Matucci-Cerinic, M. 2020. COVID-19, immune system  
565 response, hyperinflammation and repurposing antirheumatic drugs. *Turk J Med Sci*  
566 50:620-632.

567 24. Enjuanes, L., Zuniga, S., Castano-Rodriguez, C., Gutierrez-Alvarez, J., Canton, J., and Sola, I.  
568 2016. Molecular Basis of Coronavirus Virulence and Vaccine Development. *Adv Virus Res*  
569 96:245-286.

570 25. Singhal, T. 2020. A Review of Coronavirus Disease-2019 (COVID-19). *Indian J Pediatr*  
571 87:281-286.

572 26. VanBlargan, L.A., Goo, L., and Pierson, T.C. 2016. Deconstructing the Antiviral  
573 Neutralizing-Antibody Response: Implications for Vaccine Development and Immunity.  
574 *Microbiol Mol Biol Rev* 80:989-1010.

575 27. Zinkernagel, R.M., LaMarre, A., Ciurea, A., Hunziker, L., Ochsenbein, A.F., McCoy, K.D., Fehr, T.,  
576 Bachmann, M.F., Kalinke, U., and Hengartner, H. 2001. Neutralizing antiviral antibody  
577 responses. *Adv Immunol* 79:1-53.

578 28. Zhang, X., Jiang, Q., Xu, X., Wang, Y., Liu, L., Lian, Y., Li, H., Wang, L., Zhang, Y., Jiang, G., et al.  
579 2018. Immune mechanisms induced by an HSV-1 mutant strain: Discrepancy analysis of the  
580 immune system gene profile in comparison with a wild-type strain. *Vaccine* 36:2394-2402.

581 29. Liu, L., Zhao, H., Zhang, Y., Wang, J., Che, Y., Dong, C., Zhang, X., Na, R., Shi, H., Jiang, L., et al.  
582 2011. Neonatal rhesus monkey is a potential animal model for studying pathogenesis of EV71  
583 infection. *Virology* 412:91-100.

584

585

586

**Table 1 The primers of qRT-PCR to detect SARS-CoV-2**

Name	Sequence (5'-3')
COVID-19 Ef	ACAGGTACGTTAATAGTTAATAGCGT
COVID-19 Er	ATATTGCAGCAGTACGCACACA
E-probe	FAM-ACACTAGCCATCCTTACTGCGCTTCG-TAMR3
COVID-19 orf1f	CCCTGTGGGTTTACACTAA
COVID-19 orf1r	ACGATTGTGCATCAGCTGA
ORF-probe	FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-TAMR3

## Figure legends

### **Figure 1. The SARS-CoV-2 inactivated vaccine showed more than one antigenic component recognized by convalescent serum derived from COVID-19 patients**

- a. The convalescent serum from patients (N=71) could identify and neutralize the virus strain prepared for the SARS-CoV-2 inactivated vaccine. Geometric mean ± SD.
- b. 2D electrophoresis (upper left) and immune blotting showed the convalescent serum (upper right), mAb-S (lower left) and mAb-N (lower right) could identify the viral proteins in the SARS-CoV-2 inactivated vaccine .
- c. Correlation analysis of the convalescent serum from patients (N=71) identified the S and N proteins and whole virion (WVN) by Spearman rank correlation analysis. The antibody concentrations were tested and calculated according to a standard curve determined by ELISA.
- d. The convalescent serum (left), mAb-S (middle) and mAb-N (right) could identify and enrich the virion, as determined by visible electron microscopy.

### **Figure 2. The SARS-CoV-2 inactivated vaccine elicited humoral and CTL immune responses in rhesus monkeys**

- a. Different doses of the inactivated vaccine induced neutralizing antibodies in rhesus monkeys (N=10). The GMT values for all monkeys in the Al(OH)<sub>3</sub> adjuvant control group (N=10) were <2 (GMT=1 in the picture). Geometric mean ± SD.
- b. Different doses of the inactivated vaccine elicited antibodies against the S and N

proteins and whole virion (WVN) in rhesus monkeys. The antibody concentrations were tested and calculated according to a standard curve determined by ELISA. Geometric mean  $\pm$  SD.

c. Different doses of the inactivated vaccine elicited IFN- $\gamma$ -specific immune responses against the S and N proteins and whole virion (WVN) in rhesus monkeys. PBMCs were incubated for 24 h in the presence of a stimulus. Mean  $\pm$  SD.

Samples were obtained on day 7 post 2<sup>nd</sup> inoculation.

**Figure 3. The integrated immune response elicited by the inactivated vaccine can restrain viral replication in the respiratory and/or alimentary tracts**

a. The design of the immunity and viral challenge protocol.

b. Monitoring of the body temperature of rhesus monkeys infected by SARS-CoV-2.

The normal range from 37.5 to 39.5°C (refer to the normal monkeys (N=10) monitored during the same period) is indicated with dotted lines. Inactivated vaccine (200EU, 100EU and 20EU; N=10), RBD peptide vaccine (N=2) or Al(OH)<sub>3</sub> adjuvant (control; N=10). Mean  $\pm$  SD.

c. The viral loads of pharyngeal secretions, nasal secretions, anal swabs and blood from monkeys immunized with the inactivated vaccine (200EU, 100EU and 20EU; N=10), RBD peptide vaccine (N=2) or Al(OH)<sub>3</sub> adjuvant (control; N=10) after infection with live virus. Negative is the copies less than 10 (dotted lines). Mean  $\pm$  SD.

**Figure 4. The integrated immune response elicited by the inactivated vaccine can eliminate viruses and alleviate the pathological damage induced by the challenge virus**

- a. The viral loads of various organs from monkeys immunized with the inactivated vaccine (200EU, 100EU and 20EU; N=10), RBD peptide vaccine (N=2) or Al(OH)<sub>3</sub> adjuvant (control; N=5) after infection with live virus. Negative is the copies less than 10 (dotted lines). Mean  $\pm$  SD. There are no data on day 15 of the 200EU group, on days 5 and 9 of the 100EU group, on days 3 and 7 of the 20EU group, and on days 3, 5, and 7 of the RBD group.
- b. Typical pathological changes in the lungs of rhesus monkeys immunized with the inactivated vaccine (200EU, 100EU and 20EU; N=10), RBD peptide vaccine (N=2) or Al(OH)<sub>3</sub> adjuvant (control; N=5) and infected with live virus. Congestion (black arrow), infiltration of inflammatory cells (green arrow) and edema (blue arrow). Samples were obtained at 7 dpi (200EU), 7 dpi (100EU), 9 dpi (20EU), 9 dpi (RBD vaccine) and 7 dpi (control).

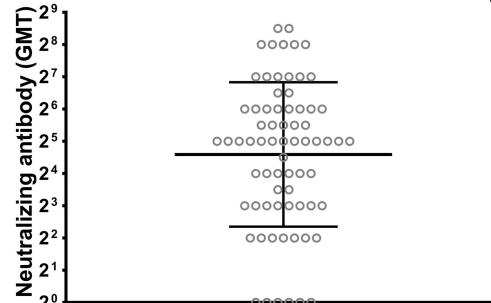
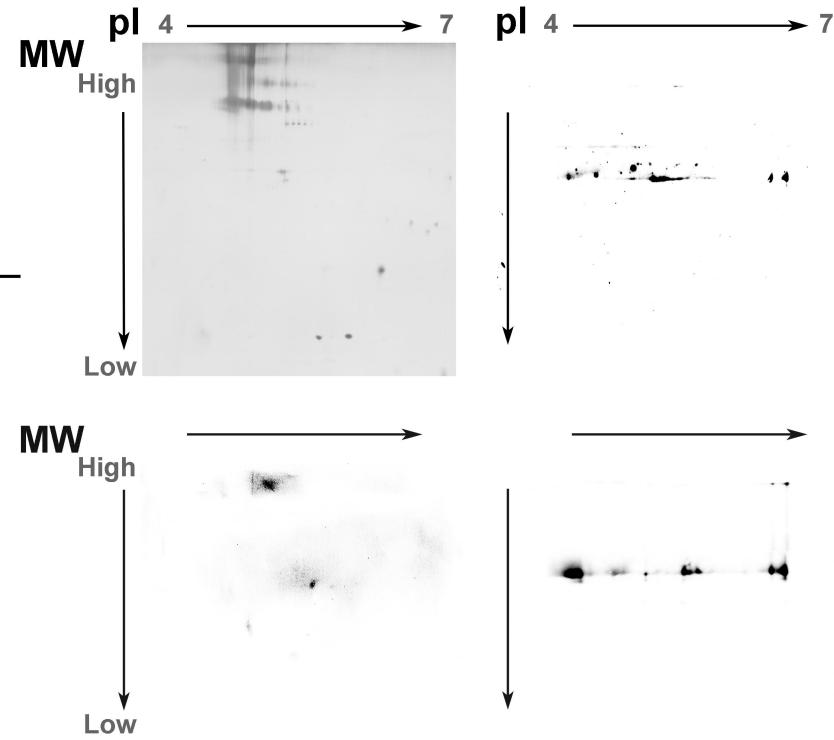
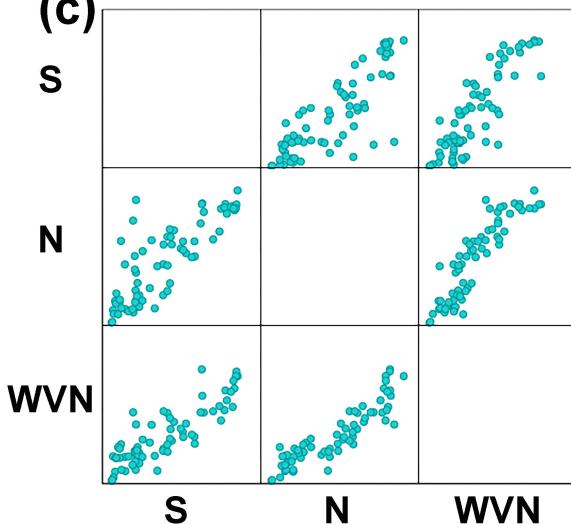
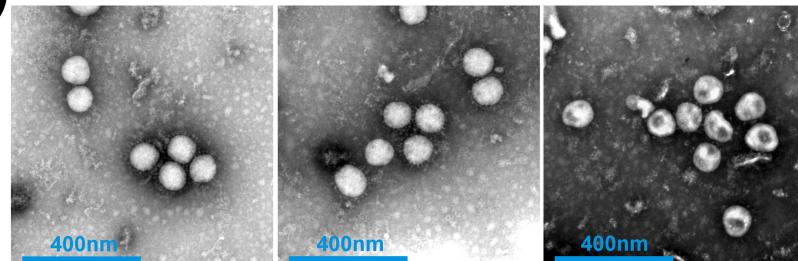
**Figure 5. The dynamic immune reaction process is maintained in macaques immunized with the inactivated vaccine during viral challenge**

- a. The percentages of T, NK and B cells in PBMCs or lung from rhesus monkeys immunized with the inactivated vaccine (N=10) or Al(OH)<sub>3</sub> adjuvant (control; N=5) during viral challenge. Mean  $\pm$  SD.

b. The percentages of Th1, Th2, Th17 and Treg cells in PBMCs or lung from rhesus monkeys immunized with the inactivated vaccine (N=10) or Al(OH)<sub>3</sub> adjuvant (control; N=5) during viral challenge. Mean ± SD.

c. IL-2, IL-4, IL-5, IL-6 and TNF- $\alpha$  levels in the serum of rhesus monkeys immunized with the inactivated vaccine (N=10) or Al(OH)<sub>3</sub> adjuvant (control; N=5) during viral challenge. Mean ± SD.

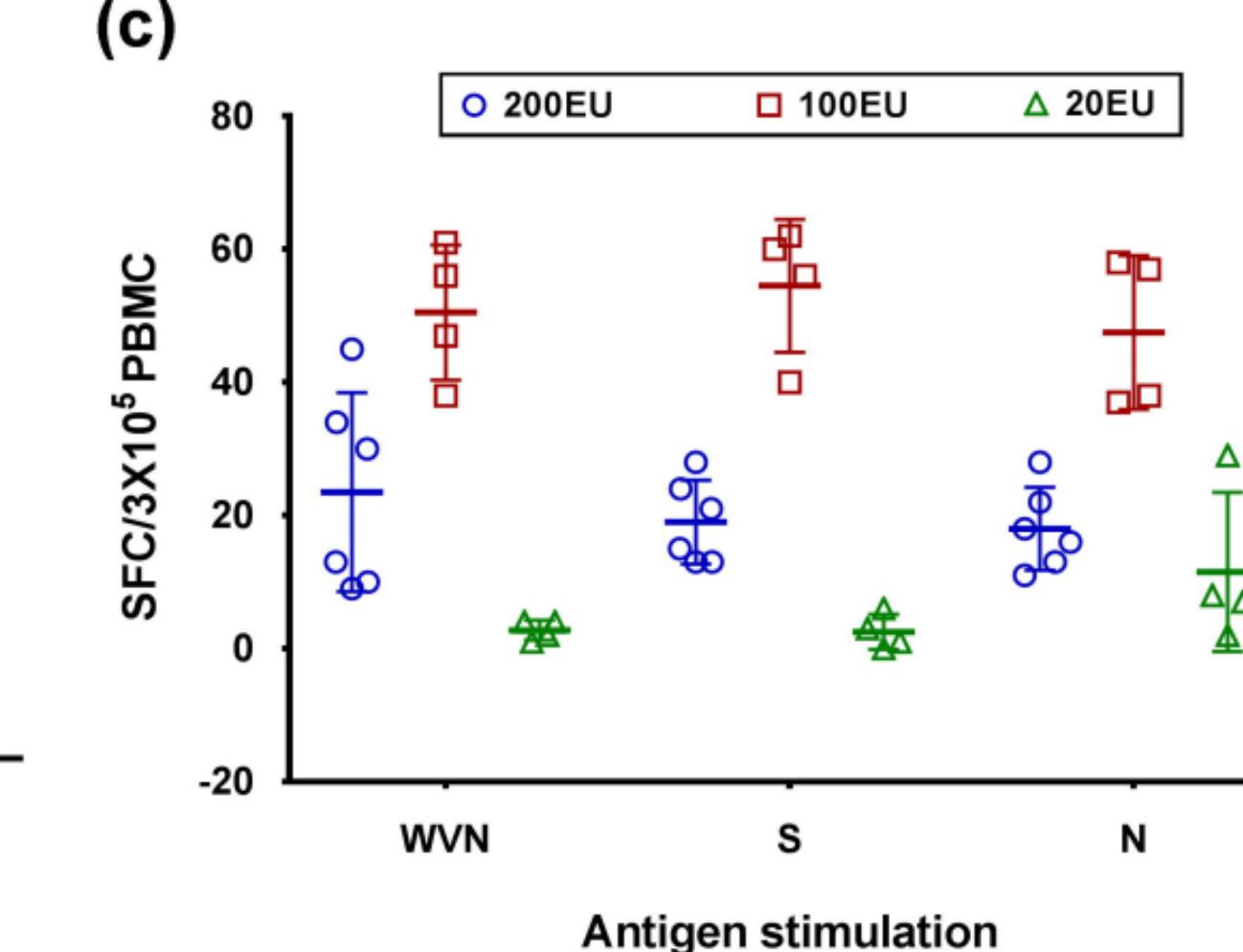
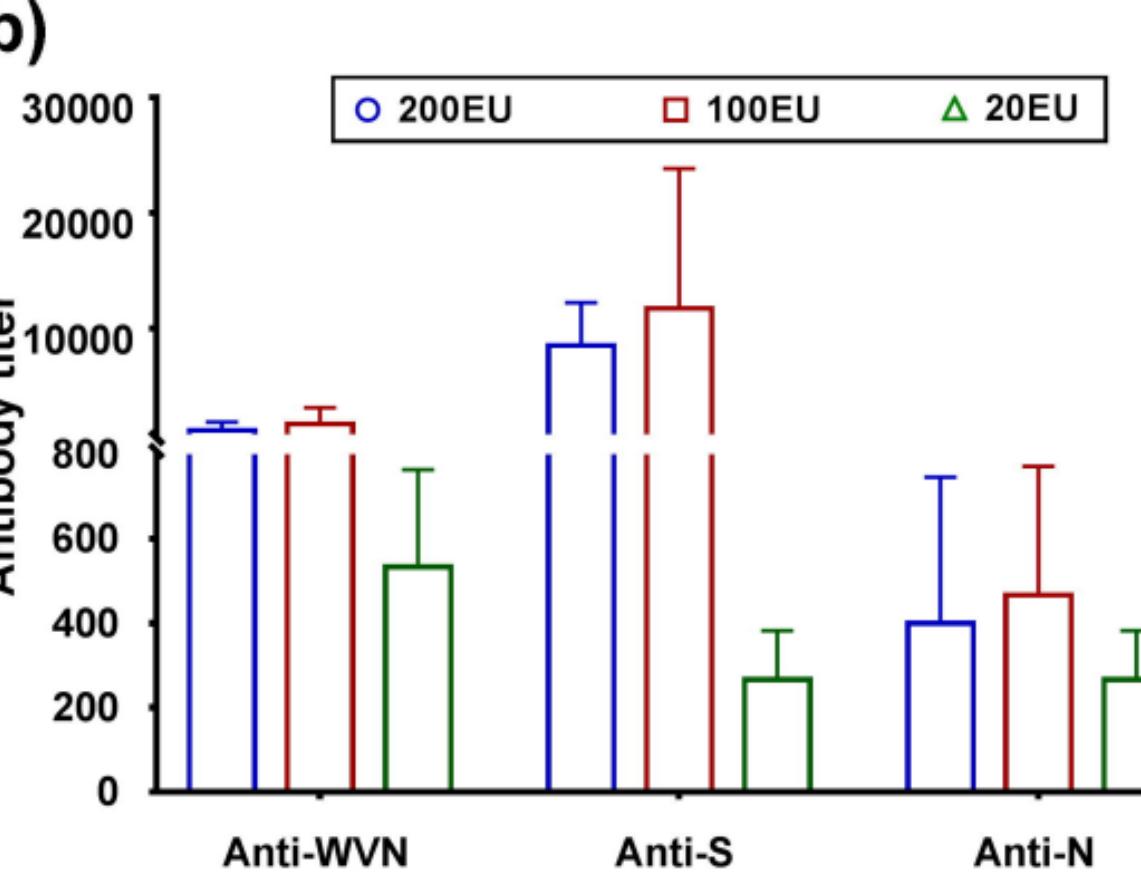
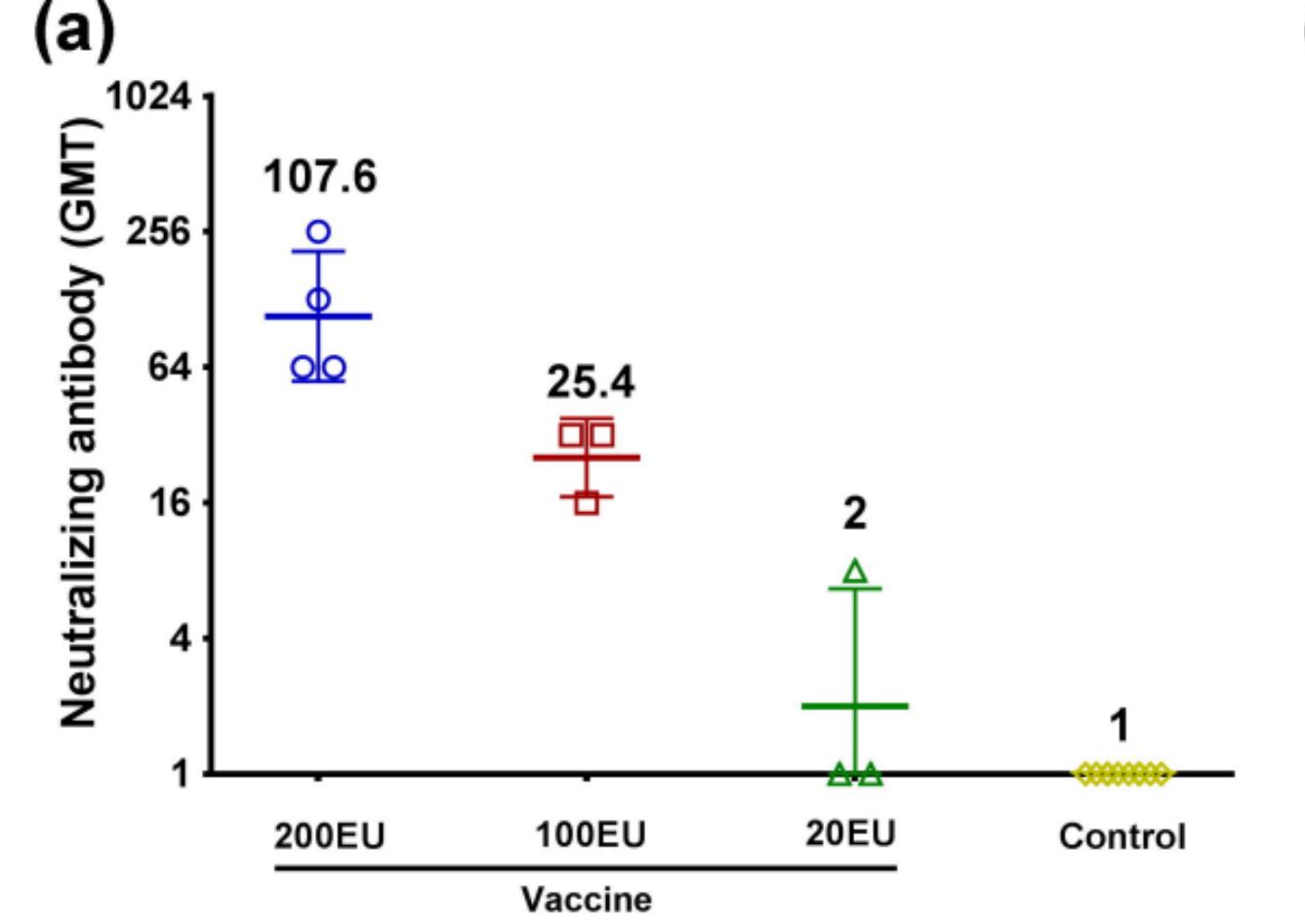
The normal range (refer to the normal monkeys (N=10) monitored during the same period) is indicated with dotted lines.

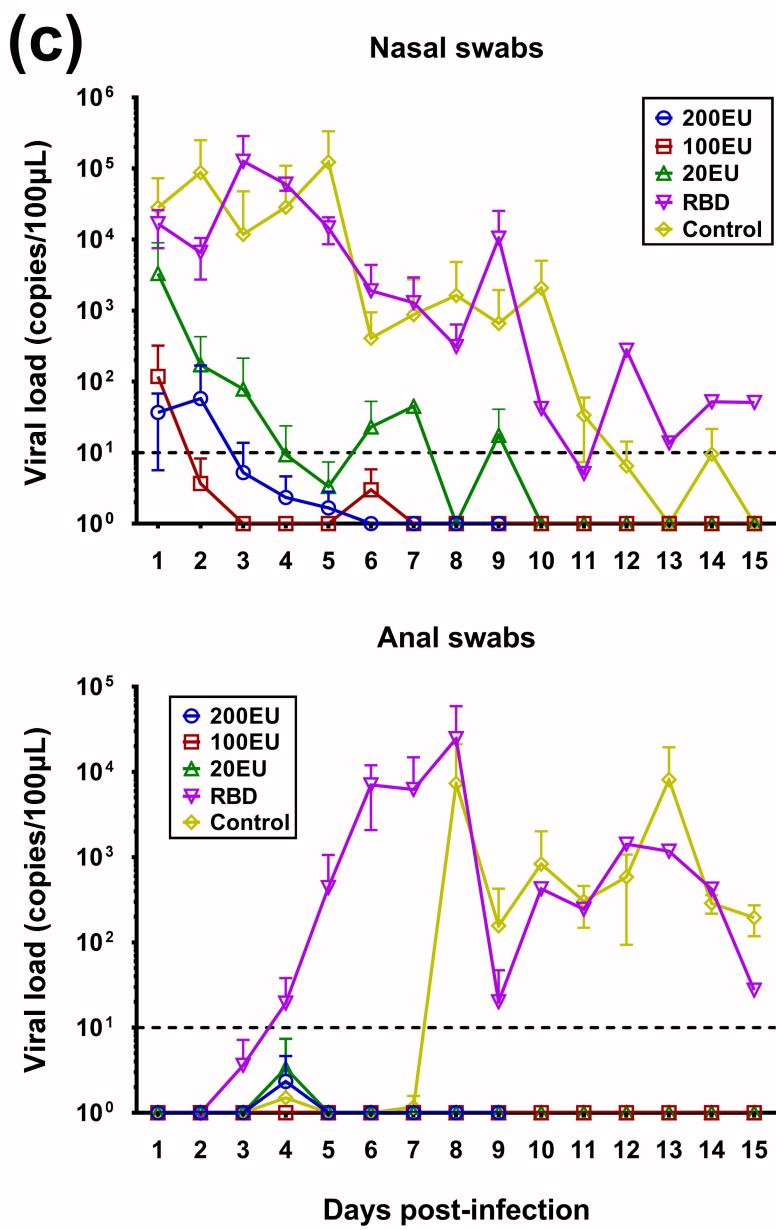
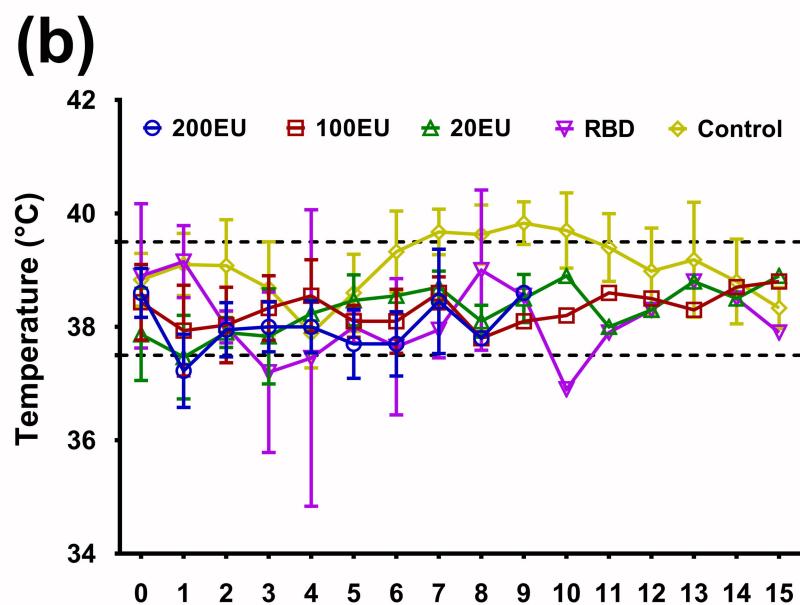
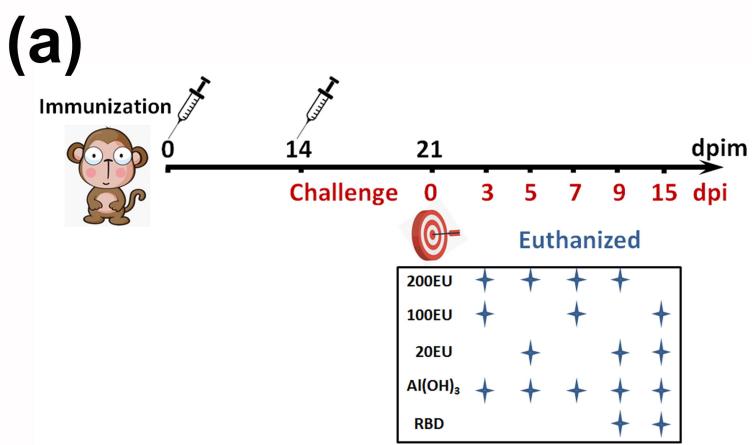
**(a)****(b)****(c)****(d)**

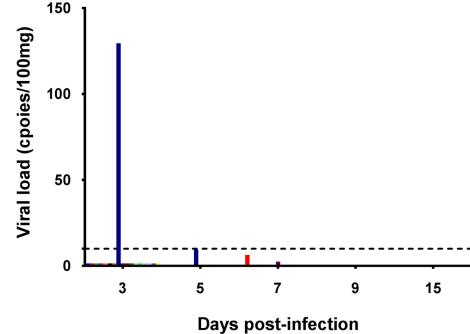
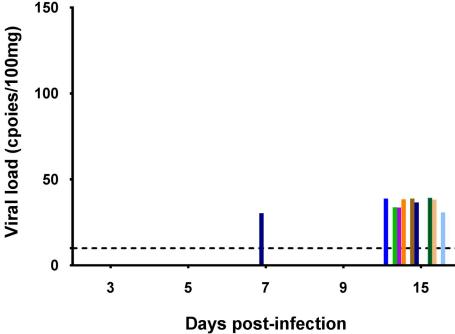
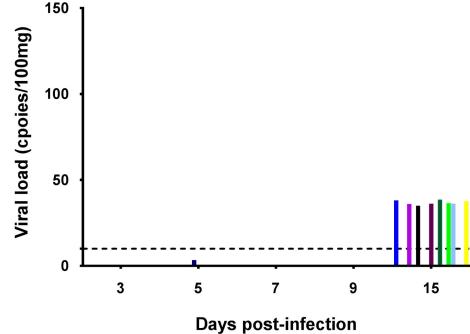
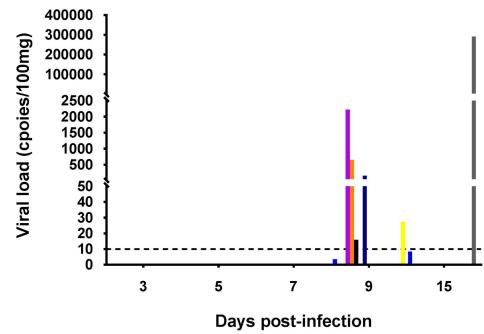
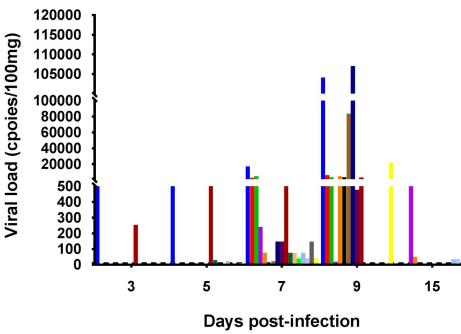
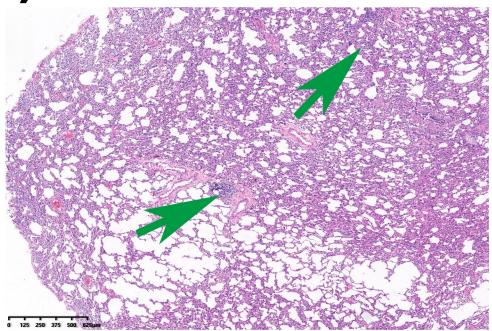
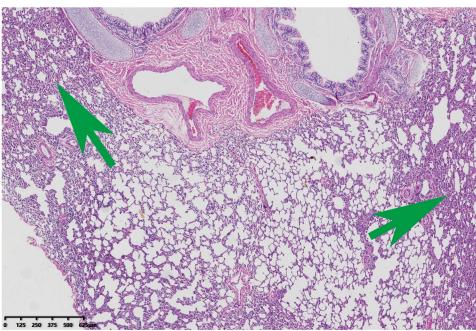
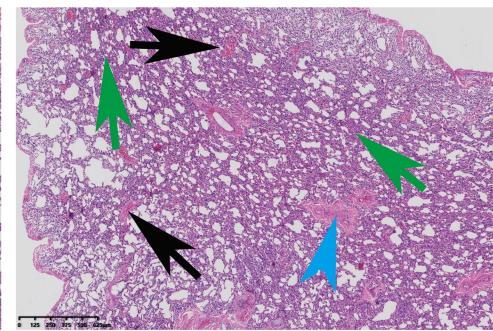
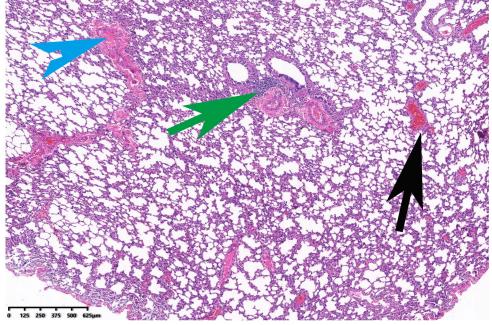
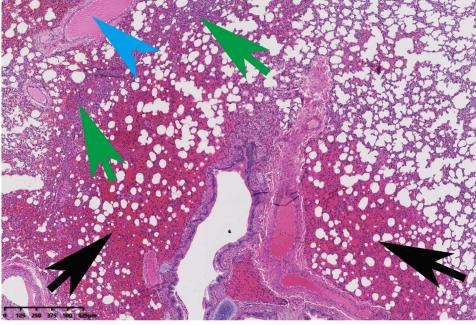
Spearman rank correlation analysis of S antibody, N antibody and total antibody in human convalescent serum.

	Anti-S		Anti-N		Total	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Anti-S	1.00	-				
Anti-N	0.810	<0.001	1.00	-		
Total	0.848	<0.001	0.912	<0.001	1.00	-

Note: The closer the absolute value of *r* is to 1, the higher the degree of correlation.





**(a)****200EU****100EU****20EU****RBD****Control****(b)****200EU****100EU****20EU****RBD****Control**

- Trachea
- Pulmonary lymph nodes
- Tracheal lymph node
- Cervical lymph nodes
- Small intestine
- Kidney
- Liver
- Spleen
- Heart
- Lung
- Brain
- Cervical spinal cord
- Thoracic spinal cord
- Lumbar spinal cord
- Midbrain
- Cerebellum
- Reproductive system

