

SHORT COMMUNICATION

A fourth dose of Omicron RBD vaccine enhances broad neutralization against SARS-CoV-2 variants including BA.1 and BA.2 in vaccinated mice

Bing Zhou¹ | Shuo Song¹ | Huimin Guo¹ | Xinrong Zhou¹ | Qing Fan¹ |
Weilong Liu¹ | Lin Cheng¹ | Xiangyang Ge¹ | Bin Ju^{1,2} | Zheng Zhang^{1,2,3}

¹Institute for Hepatology, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital; The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, Shenzhen, Guangdong Province, China

²Guangdong Key Laboratory for Anti-Infection Drug Quality Evaluation, Shenzhen, Guangdong Province, China

³Shenzhen Research Center for Communicable Disease Diagnosis and Treatment of Chinese Academy of Medical Science, Shenzhen, Guangdong Province, China

Correspondence

Bin Ju and Zheng Zhang, Institute for Hepatology, National Clinical Research Center for Infectious Disease, Shenzhen Third People's Hospital; The Second Affiliated Hospital, School of Medicine, Southern University of Science and Technology, 518112, Shenzhen Guangdong Province, China.
Email: jubin2013@163.com and zhangzheng1975@aliyun.com

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Abstract

The SARS-CoV-2 vaccines have been widely used to build an immunologic barrier in the population against the COVID-19 pandemic. However, a newly emerging Omicron variant, including BA.1, BA.1.1, BA.2, and BA.3 sublineages, largely escaped the neutralization of existing neutralizing antibodies (nAbs), even those elicited by three doses of vaccines. Here, we used the Omicron BA.1 RBD as a fourth dose of vaccine to induce potent Omicron-specific nAbs and evaluated the broadly neutralizing activities against SARS-CoV-2 variants. The BA.1-based vaccine was indeed prone to induce a strain-specific antibody response substantially cross-reactive with BA.2 sublineage, and yet triggered broad neutralization against SARS-CoV-2 variants when it was used in the sequential immunization with WT and other variant vaccines. These results demonstrated that the booster of Omicron RBD vaccine could be a rational strategy to enhance the broadly nAb response.

KEYWORDS

neutralization, Omicron variant, RBD vaccine, SARS-CoV-2, sequential immunization

1 | INTRODUCTION

As of March 2022, over 64% of the world population has received at least one dose of SARS-CoV-2 vaccine,¹ contributing to an immunologic barrier against the COVID-19 pandemic. However, with the waning of the titers in neutralizing antibodies (nAbs) and the emerging variants of SARS-CoV-2, the protective efficacies of various

vaccines were significantly decreased.^{2,3} Although several studies showed that further booster vaccination induced potent and broad nAbs against Beta and Delta variants, the newly emerging variant Omicron, including the sublineages of BA.1, BA.1.1, BA.2, and BA.3, largely escaped the neutralization of numerous existing nAbs, even those elicited by three doses of vaccines.⁴⁻⁹ Therefore, it seems that a fourth dose of vaccine may be urgently needed to combat Omicron,

Bing Zhou and Shuo Song contributed equally to this work.

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and the situation highlights the importance of studies on the necessity of replacing the original wild-type (WT) virus or sequences with variants, especially Omicron.

Omicron BA.1 and BA.2 harbor an unprecedented number of mutations in the spike protein, especially 15 and 16 of which appear in the receptor-binding domain (RBD), respectively, including 12 common mutations shared.^{10,11} The variants have been confirmed to have the strongest resistance to neutralization of serum nAbs elicited by natural WT-virus infection or vaccination based on the original virus sequence.^{6,8,11} It is particularly concerning that Omicron also causes widespread antibody evasion, leading to the failure in neutralization by convalescent serum infected with a range of SARS-CoV-2 variants including Alpha, Beta, Gamma, and Delta.¹² These results indicate that Omicron has a distinctive immunogenicity compared with previous variants. On the other hand, several studies showed that the infection with Omicron variant or targeting vaccination induced a relatively strain-specific antibody response with potent neutralizing activity against Omicron but limited cross-immunity to other variants.^{13,14} Overall, to combat Omicron, it seems that another booster shot targeting Omicron should be taken into consideration, following the currently available immunization strategies to enhance the cross antibody titers.

In this study, we used the Omicron BA.1 RBD as a fourth dose of vaccine to induce potent Omicron-specific nAbs and evaluated the broadly neutralizing activities against SARS-CoV-2 variants. The results showed that BA.1 RBD vaccine induced a strain-specific antibody response substantially cross-reactive with BA.2 sublineage and triggered broad neutralization against SARS-CoV-2 variants when it was used in the sequential immunization with WT and other variant vaccines.

2 | MATERIALS AND METHODS

2.1 | Animals, immunization, and serum sample collection

In our previous study, 40 specific pathogen-free female BALB/c mice were randomly divided into eight groups and then immunized intraperitoneally with the SARS-CoV-2 inactivated vaccine (KCONVAC) or RBD subunit vaccines (WT, Beta, or Delta I-P-R-F) at an interval of 2 weeks in different procedures or immunized with aluminum adjuvant which served as a control.¹⁵ To evaluate the immunogenicity of the Omicron RBD as the booster vaccination, all mice were injected with 1 µg of the Omicron BA.1 RBD protein (Sino Biological, 40592-V08H121) at approximately Week 32, which has been previously mixed with a fixed-dose (50 µg per mouse) of alum adjuvant (SERVA, 12261.01) and kept rolling overnight at 4°C to make alum adsorb the immunogen efficiently. No adverse events were observed throughout the experiment. All serum samples were collected before the Omicron boosting and at 1 week post the Omicron vaccination and heat inactivated at 56°C for 30 min before use.

2.2 | SARS-CoV-2 pseudovirus-based neutralization assay

SARS-CoV-2 pseudovirus was generated by cotransfection of HEK-293T cells with a SARS-CoV-2 spike-expressing plasmid and an env-deficient HIV-1 backbone vector (pNL4-3.Luc.R-E-).¹⁶ Two days posttransfection, cell culture containing virus supernatants was collected by centrifugation and stored at -80°C. Detailed sequence information of Omicron spike proteins are listed below, respectively.

SARS-CoV-2 *wild-type* (WT): Wuhan-Hu-1, accession number: NC_045512.

SARS-CoV-2 *Omicron BA.1*: A67V, 69-70del, T95I, G142D, 143-145del, N211I, 212del, 215EPEins, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F.

SARS-CoV-2 *Omicron BA.2*: T19I, L24S, 25-27del, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K.

The neutralization activity of the serum sample was evaluated by incubating the serially diluted serum samples with an equal volume of SARS-CoV-2 pseudovirus at 37°C for 1 h. HEK-293T-hACE2 cells were subsequently added to the all-white 96-well cell culture plate containing virus-serum mixture. After a 48-h incubation, the culture medium was removed, and 100 µl of Bright-Lite Luciferase reagent (Vazyme Biotech) was added to the plates. After a 2-min shock incubation at room temperature (RT), the luminescence of cell plates was measured by using a Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific). The 50% inhibitory dilution (ID₅₀) of each mouse was calculated using GraphPad Prism 8.0 software by the log (inhibitor) versus normalized response-variable slope (four parameters) model. The geometric mean titer (GMT) of each group was calculated as the geometric mean of ID₅₀ of five mice in the group.

3 | RESULTS

Previously, we performed a series of sequential immunization with WT, Beta, or Delta RBD vaccine and inactivated vaccines in mice. Heterologous booster strategies are powerful enough to induce potent and broad neutralization against variants.¹⁵ Here, on the basis of three doses of vaccines, we further explore the enhancing effect of Omicron BA.1 RBD in the titers of nAbs. As shown in Figure 1A, 20 mice were randomly divided into four groups. All mice received two doses of inactivated vaccines at 2-week intervals. Two weeks later, five mice in Group A were boosted with another dose of homologous inactivated vaccine. The rest of 15 mice were immunized with the same doses of heterologous RBD vaccines: WT RBD in Group B, Beta RBD in Group C, and Delta RBD in Group D, respectively. At Week 32, all mice received 1 µg of BA.1 RBD vaccine. One week later, all

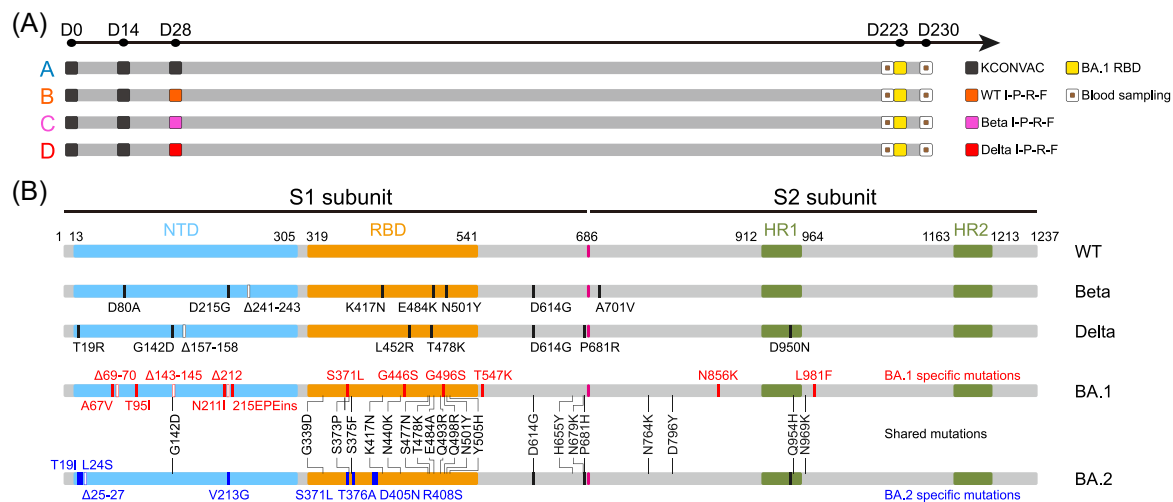


FIGURE 1 Schematic representation of the immunization schedule and the key mutations in the spike protein of SARS-CoV-2 variants used in this study. (A) Schematic representation of the immunization schedule and blood sample collection of mice in four groups (Groups A–D, $n = 5$ in each group). KCONVAC (inactivated vaccine), WT I-P-R-F (WT RBD vaccine), Beta I-P-R-F (Beta RBD vaccine), and Delta I-P-R-F (Delta RBD vaccine) have been used as the former three doses of vaccines in our previous study.¹⁵ The Omicron BA.1 RBD protein was purchased from Sino Biological. (B) Schematic representation of the mutations of SARS-CoV-2 variants including Beta, Delta, BA.1, and BA.2. The strain-specific mutations of Omicron BA.1 and BA.2 were labeled in red and blue, respectively. The shared mutations between BA.1 and BA.2 were shown in red.

serum samples were collected. To test their neutralizing activities against SARS-CoV-2 WT, Beta, Delta, Omicron BA.1, and BA.2 variants, we constructed a series of SARS-CoV-2 pseudoviruses bearing spike proteins of the above strains as shown in Figure 1B. Then the results were acquired by the pseudovirus-based neutralization assay (Supporting Information: Figure S1 and Table S1).

The fourth dose of BA.1 RBD vaccine significantly induced a robust nAb response against BA.1 and BA.2 variants in all mice. The fold changes of enhancement in the GMTs ranged from 5.1- to 14.0-fold for BA.1 and 6.0- to 10.8-fold for BA.2, respectively, in Groups A, B, C, and D (Figure 2). By contrast, the neutralizing activities against other pseudoviruses including WT, Beta, and Delta variants were also increased to some extent, whose fold changes ranged from 1.8- to 5.6-fold, but not as significant as that against Omicron. Similar results were obtained in another study of the rhesus macaque model, showing that the homologous Omicron mRNA booster shot that followed two doses of mRNA-1273 vaccines (the version of the original WT gene) significantly increased the nAb responses to all tested SARS-CoV-2 variants including Omicron.¹⁷

To compare the immune effects of different immunization strategies more clearly, we rearranged these neutralization results by different groups. As shown in Figure 3, the Omicron BA.1 and BA.2 variants were both more resistant to the neutralization of vaccinated mouse serum than WT, Beta, and Delta before the fourth dose of BA.1 RBD vaccine. The GMTs of nAbs against BA.1 and BA.2 were significantly decreased to 696, 1569, 628, 1383 and 253, 1463, 459, 613 in different immunization strategies, respectively. These data indicated that BA.2 had a stronger resistance to neutralization of serum nAbs elicited by vaccination based on WT virus and even some other variants, which was consistent with the

previous report.⁸ After the booster of BA.1 RBD vaccine, vaccinated serum displayed relatively balanced nAb responses against tested SARS-CoV-2 variants. Especially in Group B, sequential immunization with WT RBD vaccine followed by BA.1 RBD vaccine elicited the highest titers of BA.1-specific nAbs, whose declined degrees of neutralization were less than threefold compared with those against WT, Beta, and Delta variants. The boost immunization of BA.1 RBD vaccine in four groups also elicited high titers of nAbs against BA.2 sublineage comparable to BA.1, which suggested a substantial cross-reactive immunity between them.

We are aware that some people are still not fully vaccinated, with a number of people administrated with two or even one shot, regardless of the type of SARS-CoV-2 vaccines. Therefore, we also evaluated the enhancing effect of boosting with the Omicron BA.1 RBD vaccine following one dose (Group E), two doses (Group F) of inactivated vaccines, and heterologous inactivated/WT RBD vaccination (Group G). The results showed that the sequential immunization with Omicron BA.1 RBD vaccine significantly increased the titers of nAbs against BA.1 (ranged from 24.3- to 47.7-fold) and BA.2 (ranged from 13.1- to 41.0-fold), and also obviously promoted the neutralization of vaccinated mouse serum against WT, Beta, and Delta variants in different degrees (Supporting Information: Figure S2, S3, and Table S1).

4 | DISCUSSION

Our study has two limitations. First, due to the limited number of mice, we did not make a head-to-head comparison of the fourth dose of Omicron BA.1 vaccine with BA.2 or other variant vaccines in enhancing the titers of nAbs. Second, long-term monitoring of serum

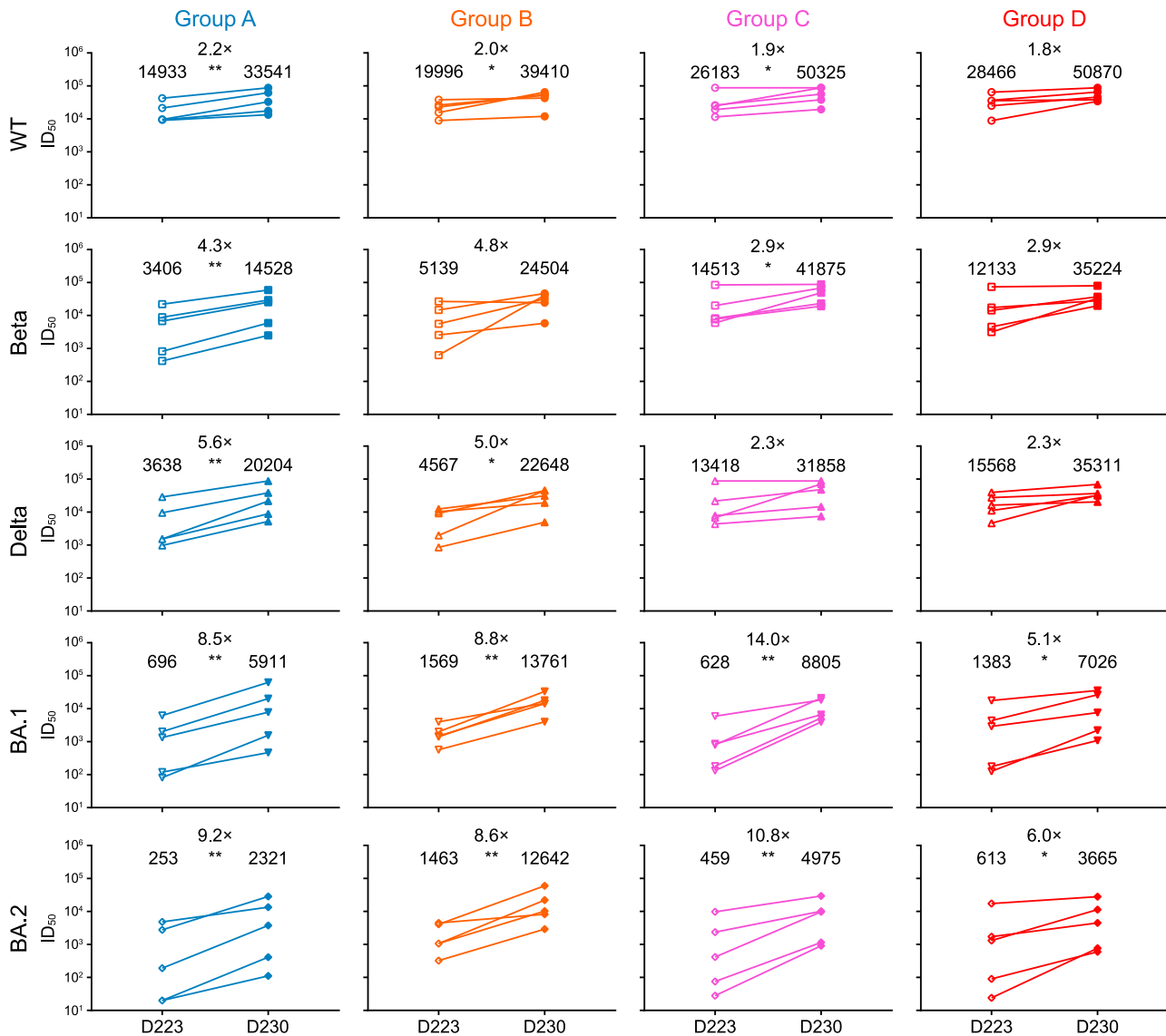


FIGURE 2 Neutralization titers of vaccine-elicited mouse sera against SARS-CoV-2 WT and variants including Beta, Delta, Omicron BA.1 and BA.2. The data are shown for different pseudoviruses. The geometric mean titers (GMTs) and fold changes of serum nAbs between post (D230) and before (D223) the fourth vaccination are calculated and shown on the top. The symbols represent individual mice. Statistical analysis was performed with paired *t*-tests using GraphPad Prism 8.0 software. **p* < 0.05; ***p* < 0.01. "X" indicates fold change.

neutralization will enable to evaluate the duration of the antibody response elicited by the fourth vaccination with Omicron RBD vaccine. Despite the limitations, we evaluated the enhancing effect of heterologous sequential immunization with Omicron RBD vaccine in the titers of nAbs against Omicron and other variants in previously vaccinated mice, which was different from other studies. The SARS-CoV-2 mRNA vaccine has been used as the fourth dose to evaluate its efficacy and protection against the Omicron variant in the clinical trial or in the real world. Either BNT162b2 or mRNA-1273 vaccine administered after three doses of BNT162b2 could induce RBD-specific IgG-binding antibodies and obviously increase the titers of nAbs. By contrast, the antibody levels continued to wane over time in the control group.¹⁸ In a larger study, the researchers revealed the effectiveness of a fourth dose of BNT162b2 vaccine against Omicron

based on the data of more than one-million persons who were 60 years of age or older in Israel. The rates of confirmed SARS-CoV-2 infection and severe COVID-19 were both lower than those after only three doses during the Omicron-dominated period.¹⁹ However, a recent study raised the concern of this homologous booster immunization with the original WT virus sequence. Wang et al. stated that the peak level of RBD-nAbs elicited by a fourth dose of homologous SARS-CoV-2 inactivated vaccine was inferior to that induced by the former three doses and proposed that the next-generation vaccine carrying epitopes of variants would be a future direction for the booster vaccination.²⁰

Given the increased prevalence of Omicron BA.2 in many regions of the world, BA.2-based vaccines seem to be more appropriate to be adopted as booster vaccines. However, our results showed that the

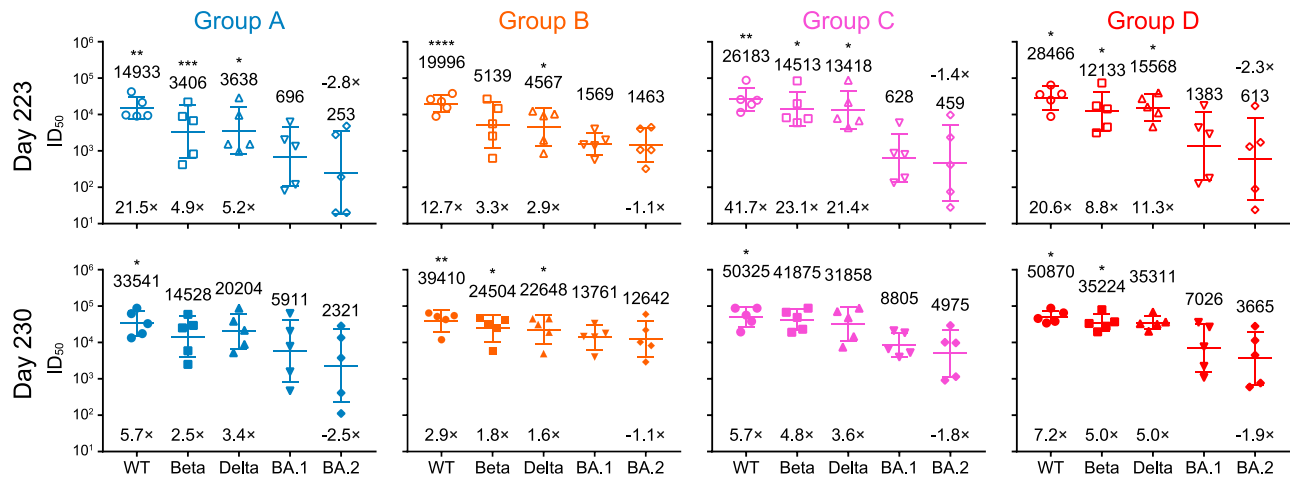


FIGURE 3 Neutralization titers of vaccine-elicited mouse sera against SARS-CoV-2 WT and variants including Beta, Delta, Omicron BA.1, and BA.2. The data are shown for different groups. The GMTs are calculated and shown on the top of each column. The fold changes in GMTs were compared between Omicron BA.1 and WT, Beta, Delta, BA.2, respectively, and written at the bottom or top. The error bars are SDs and the symbols represent individual mice. Statistical analysis was performed with paired *t*-tests using GraphPad Prism 8.0 software. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001. “-” indicates decreased neutralizing activity. “X” indicates fold change.

Omicron BA.1-based vaccine was prone to induce a strain-specific antibody response substantially cross-reactive with BA.2 sublineage, and yet could trigger broad neutralization against SARS-CoV-2 variants when it was used in the sequential immunization with WT and other variant vaccines. The cross-reactive immunity observed between BA.1 and BA.2 sublineages may be attributed to the multiple common mutations shared by them. What's more, no adverse events were observed throughout the experiment, indicating the safety of a fourth dose of Omicron RBD vaccine. In conclusion, the booster of Omicron RBD vaccine could be a rational strategy to enhance the broadly nAb response and to control the current COVID-19 pandemic.

AUTHOR CONTRIBUTIONS

Zheng Zhang is the principal investigator of this study. Zheng Zhang and Bin Ju conceived and designed the study. Bing Zhou and Shuo Song performed all experiments together with assistance from Huimin Guo, Xinrong Zhou, Qing Fan, Weilong Liu, Lin Cheng, and Xiangyang Ge. Zheng Zhang and Bin Ju wrote the manuscript and all authors read and approved this version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

We are happy to share reagents and information in this study upon request.

ETHICS STATEMENT

All animal experiments in mice were approved by the Ethics Committee of Shenzhen Third People's Hospital, China. All procedures were conducted in accordance with animal ethics guidelines and approved protocols to minimize suffering during vaccination and blood collection.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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