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ORIGINAL ARTICLE

Boosting with Omicron-specific mRNA vaccine or historical SARS-CoV-2 vaccines elicits discriminating immune responses against Omicron variants

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Abstract Booster vaccinations are highly recommended in combating the SARS-CoV-2 Omicron variant and its subvariants. However, the optimal booster vaccination strategies and related immune mechanisms with different prior vaccinations are under-revealed. In this study, we systematically evaluated the immune responses in mice and hamsters with different prime-boost regimens before their protective efficacies against Omicron were detected. We found that boosting with Ad5-nCoV, S_{WT}-2P or $S_{Omicron}$-6P induced significantly higher levels of neutralization activities against Omicron variants than

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Commercial vaccines;
Booster vaccination;
Immune response

CoronaVac and ZF2001 by eliciting stronger germinal center (GC) responses. Specifically, $S_{Omicron}$-6P induced even stronger antibody responses against Omicron variants in CoronaVac and Ad5-nCoV-primed animals than non-Omicron-specific vaccines but with limited differences as compared to Ad5-nCoV and S_{WT}-2P. In addition, boosting with a specific vaccine has the potential to remodel the existing immune profiles. These findings indicated that adenovirus-vectored vaccines and mRNA vaccines would be more effective than other types of vaccines as booster shots in combating Omicron infections. Moreover, the protective efficacies of the vaccines in booster vaccinations are highly related to GC reactions in secondary lymphatic organs. In summary, these findings provide timely important information on prime-boost regimens and future vaccine design.

1. Introduction

To date, coronavirus disease 2019 (COVID-19) has caused more than 768 million infections, including 6.9 million deaths, as recorded by the World Health Organization (WHO) (https://covid19.who.int). As the virus evolves, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with mutations are escalating[1-3]. A new SARS-CoV-2 variant, B.1.1.529 (namely, Omicron BA.1), reported in late 2021, has emerged and replaced the previous dominant variants[4,5]. More than 30 mutations were characterized in the spike protein of Omicron variants, resulting in more favorable spike protein binding to angiotensin-converting enzyme 2 (ACE2) on human cells. The mutations endowed the virus with high transmissibility and immune evasion[6-9]. Since then, the Omicron lineage has continued to evolve with additional or different mutations (including BA.2, BA.4, BA.2.75, and BA.5)[10-13]. The continued evolution of increased transmissible SARS-CoV-2 variants greatly jeopardizes the protective efficacy conferred by historical vaccines targeting the wild-type (WT) strain of SARS-CoV-2 and causes repeat infections around the world that contributes additional risks of death[14-16]. To rescue the lost protective efficacy of prior vaccines against Omicron and its sublineages, updated vaccines or booster doses are highly needed[17-21]. Although booster vaccinations have been studied by some scientists[22,23], systematic comparisons of the differences in immune responses elicited by these immunization regimes (including Omicron-specific vaccines) and the protective efficacies of different booster vaccines, which could guide future vaccine design and vaccination strategy determination, are under investigation.

A set of vaccines against COVID-19 of different vaccine technologies were approved or admitted for emergency use authorization (EUA), most of which were based on the original WT strain (especially the spike protein) of SARS-CoV-2[24]. Among the COVID-19 vaccines, mRNA vaccines (mRNA-1273 and BNT162b2), inactivated whole-virus vaccines (BBIBP-CorV and CoronaVac), adenovirus-vectored vaccines (Ad26.COV2. S, ChAdOx1 nCoV-19, and Ad5-nCoV), and adjuvanted protein subunit vaccine (ZF2001) are most widely used in the world[25-32]. The use of these vaccines significantly reduced the number of infections, hospitalizations, severe cases, and deaths[33]. Nonetheless, the highly transmissible Omicron and its sublineages significantly escaped the antibody response elicited by prior vaccinations, which threatens the protective efficacy[34,35].

Here, we systematically compared the humoral and cellular immune responses in mice and hamsters, in addition to protective activities against Omicron variants in hamsters after different booster shots. In inactivated vaccine (CoronaVac)-primed, adjuvanted protein subunit vaccine (ZF2001)-primed, and adenovirus-vectored vaccine (Ad5-nCoV)-primed animals, booster vaccinations with CoronaVac, ZF2001, Ad5-nCoV, WT mRNA vaccine (S_{WT}-2P, based on the full-length protein sequence of WT SARS-CoV-2 virus), and Omicron-specific mRNA vaccine ($S_{Omicron}$-6P, based on the full-length protein sequence of SARS-CoV-2 Omicron BA.1 virus) were carried out[36-38]. After the primary vaccination series, boosting with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P elicited significantly stronger germinal center (GC) reactions in secondary lymphatic organs than that elicited by CoronaVac and ZF2001 and conferred broad immunologic protection against the virus. Among these vaccines, boosting with Ad5-nCoV induced the highest levels of follicular helper T (TFH) cells, GC B cells, plastic $CD38^+GL7^+$ B cells, and class-switched $IgG1^+$/$IgG2a^+$ B cells but the lowest levels of $CD4^+$ and $CD8^+$ T cells in secondary lymphatic organs. Additionally, a booster vaccination could also remodel the immune system and elicit a different biased immune response. More importantly, our data suggest the optimal prime-boost strategies against SARS-CoV-2 in different regimens and highlight the importance of GC reactions in booster vaccinations for generating potent protective immune responses.

2. Materials and methods

2.1. Experimental design

This study aimed to provide optimal booster vaccination strategies for populations with different regimen cohorts and reveal the underlying mechanisms. We systematically compared the protective efficacies and immune responses in mice and hamsters by different booster shots (Fig. 1). Animals were first immunized with inactivated whole-virus vaccine (CoronaVac), adjuvanted protein subunit vaccine (ZF2001), or adenovirus-vectored vaccine (Ad5-nCoV), which are widely used in humans, according to the regulations and established guidelines. Ten weeks or more than ten weeks after the last vaccinations, homologous and heterologous booster vaccinations with CoronaVac, ZF2001, Ad5-nCoV, WT mRNA vaccine (S_{WT}-2P), and Omicron BA.1-specific mRNA vaccine ($S_{Omicron}$-6P) were carried out. The binding antibody responses against WT and BA.1 and neutralizing antibody responses against BA.1 and BA.5 were evaluated after booster vaccinations. The corresponding immune responses were also analyzed by flow cytometry and ELISPOT. The underlying correlations of neutralizing activities and GC reactions were assessed by using

Spearman's rank coefficient. In Syrian hamster models, the protective efficacies were further performed after different booster shots. Mouse experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of the USTC (USTCACUC25010122049). Syrian hamster studies were approved by the Animal Ethics Committee of the Wuhan Institute of Biological Products (WIBP) (WIBP-AII382020001). The animal experiments with SARS-CoV-2 challenge were conducted in the biosafety level 3 (BSL-3) facility in Wuhan Institute of Virology, Chinese Academy of Sciences.

2.2. Vaccines

The inactivated vaccine, CoronaVac, was kindly provided by Sinovac Life Sciences. The protein subunit vaccine ZF2001 was kindly provided by Anhui Zhifei Longcom Biologic Pharmacy (China). The adenovirus-vectored vaccine, Ad5-nCoV, was kindly provided by Beijing Institute of Biotechnology (China). WT mRNA vaccine (S_{WT}-2P) and Omicron BA.1-matched mRNA vaccine ($S_{Omicron}$-6P) were designed based on the specific sequences of spike proteins and produced as previously described[36-38]. Briefly, S_{WT}-2P, with the same sequence as BNT162b2 RNA, was developed based on the spike protein sequence of ancestral SARS-CoV-2 with two beneficial proline substitutions (K986P and V987P), and $S_{Omicron}$-6P encodes the BA.1 spike protein with six beneficial proline substitutions (F817P, A892P, A899P, A942P, K986P, and V987P). *In vitro* transcribed and purified mRNA was subsequently introduced into lipid nanoparticles (LNPs) for further use.

2.3. Animal immunizations

Two animal models were used in this study, *i.e.*, BALB/c mice and Syrian hamsters. The dosages applied in the animals were set as ten percent of the corresponding human doses. For CoronaVac, ZF2001, and Ad5-nCoV, 50 μL doses of the vaccine were applied to animals. For S_{WT}-2P and $S_{Omicron}$-6P, 3 μg mRNA in 50 μL PBS was applied to mice and hamsters.

For the CoronaVac-primed group, female BALB/c mice and hamsters were primed with two doses of CoronaVac *via* the intramuscular route on Day 0 and Day 21 and boosted with CoronaVac, ZF2001, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P on Day 188. For the ZF2001-primed group, female BALB/c mice and hamsters were immunized intramuscularly with a primary series over a 14-day interval with three doses of ZF2001. On Day 190, animals were boosted with ZF2001, CoronaVac, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P. For the Ad5-nCoV-primed group, mice and hamsters were immunized with a single dose of Ad5-nCoV and boosted with CoronaVac, ZF2001, S_{WT}-2P, or $S_{Omicron}$-6P after 71 days. Serum samples harvested from mice and hamsters were tested for binding and neutralizing antibodies before (data not shown) and after booster vaccinations. All hamsters were further evaluated for protective efficacies by challenge with 1×10^4 PFU authentic Omicron BA.1 at 9 days post booster immunizations.

2.4. Binding antibody titer determination

Binding antibody responses in mice and hamsters after different booster immunizations were determined by an enzyme-linked immunosorbent assay (ELISA). ELISA plates (ThermoFisher) were precoated overnight with 100 μg/mL trimer spike proteins (WT or BA.1) (Sino Biological) in PBS, followed by blocking with 5% skim milk in PBST. Heat-inactivated sera were diluted (starting at 1:100), added to each well of the ELISA plates and incubated for 1 h at room temperature. After incubation, HRP-conjugated goat anti-mouse and goat anti-hamster IgG antibodies were added to the plate and incubated for another 1 h at room temperature. Next, the plates were developed with TMB substrate (Beyotime) and halted with a stop solution (Beyotime). The absorbance was measured at 450 nm by a microplate reader (SpectraMax iD5, Molecular Devices).

To calculate the binding antibody titers, set the initial serum dilution factor as *m*, followed by gradient dilutions at a factor of *n*, resulting in dilution factors of *m*, *nm*, *nnm*, *nnnm*, and so on. We perform a total of 7 gradient dilutions, labeled from 1 to 7 on the *X*-axis, with the corresponding OD values measured by ELISA plotted on the *Y*-axis. We select 3 to 4 data points that show a good linear relationship to establish a linear equation. Assuming the slope and intercept of this equation are *a* and *b* respectively, we use the following formula: $(c \times 2.1-a)/b$, where *c* is the OD value of the blank control and 2.1 is the threshold constant. Solving for *d*, we then substitute *d* into the formula: *POWER*(*n*, *d*−1)**m*, to calculate the binding antibody titer.

2.5. Neutralizing antibody titer determination

Circulating neutralizing antibody titers against authentic BA.1 and BA.5 were measured by a plaque reduction neutralization test (PRNT). Vero E6 cells were seeded in 24-well plates at a density of 1×10^5 cells per well and incubated for 16 h at 37 °C. Heat-inactivated sera were diluted 4-fold with DMEM in a volume of 200 μL and incubated with the Omicron BA.1 and BA.5 viruses (120 PFU in 200 μL DMEM) for 1 h at 37 °C. Virus mixed with 200 μL DMEM served as the mock control. An antibody-virus mixture of 250 μL was added to a 24-well culture plate. After infection at 37 °C for 1 h, the supernatant was discarded, replaced with medium containing 1% methylcellulose and incubated for 5 days. The plates were further fixed with 8% paraformaldehyde and stained with 1% crystal violet.

The neutralizing antibody titers in the tested samples were determined by counting the number of plaques. The infection inhibition rates of each sample were calculated according to Eq. (1):

$$\text{Inhibition rate}\,(\%) = [1-(\text{Average number of plaques in sample} / \text{Average number of plaques in virus control})] \times 100 \quad (1)$$

Based on the inhibition rate results, the VNT_{50} of each sample was calculated using the Reed−Muench method with GraphPad Prism software. The parameters were set to "log(inhibitor) *vs.* normalized response - variable slope least squares fit".

2.6. Flow cytometry

For T/B-cell phenotyping, lymph nodes (LNs) and spleens were harvested from different immunized mice and minced and lysed for single-cell suspensions. Single cells were incubated with anti-CD16/32 at 4 °C for 15 min prior to staining for extracellular antigens with fluorescent dye-conjugated antibodies in PBS buffer at 4 °C for 30 min. After staining, the cells were washed and acquired by using the CytoFLEX LX flow cytometer, and the data were analyzed with the associated CytoFLEX LX software.

For ICS, 5×10^6 splenocytes were *ex vivo* restimulated with overlapping peptide pools spanning full-length spike protein (Sino

Biological) for 12 h at 5% CO_2 and 37 °C. The restimulated cells were stained for extracellular antigens as described for T/B-cell phenotyping. Then, the cells were fixed and permeabilized using a Foxp3/Transcription Factor Staining Buffer Set (Invitrogen). Permeabilized cells were next stained for intracellular antigens with fluorescent dye-conjugated antibodies in Perm Buffer. Furthermore, the cells were acquired, and data were analyzed as described above.

2.7. Analysis of viral titers by plaque assay

To determine viral titers in hamsters, the infected animals were sacrificed at 3 days post infection, and the nasal turbinates, tracheas, and lungs were harvested and ground in 1 mL DMEM. Vero E6 cells were seeded in 24-well plates at a density of 1×10^5 cells per well and incubated for 16 h at 37 °C. The supernatants of collected tissues were diluted 10-fold and added to a 24-well culture plate. After infection at 37 °C for 1 h, the supernatant was discarded, and the cells were overlaid with medium containing 1% methylcellulose and incubated for 5 days at 37 °C. Later, the viral titers were determined by performing crystal violet staining and calculated by using GraphPad Prism 8.0 software.

2.8. Analysis of viral RNA loads by RT-qPCR assay

To determine viral RNA loads in hamsters, viral RNA was extracted from nasal turbinates, tracheas, and lungs using a Virus DNA/RNA Extraction Kit 2.0 (Vazyme). The quantification of viral RNA was tested using a HiScript® II One Step RT-qPCR SYBR® Green Kit (Vazyme Biotech) with the primers ORF1a/b-F (5′-CCCTGTGGGTTTTACACTTAA-3′) and ORF1a/b-R (5′-ACGATTGTGCATCAGCTGA-3′). Serial dilutions of the SARS-CoV-2 ORF1ab gene control plasmid was tested to generate standard curves.

2.9. Statistical analysis

Flow cytometric data were analyzed using Beckman CytoFlex software. All statistical analyses and relative graphs were performed using GraphPad Prism version 8.0. Data are displayed as the mean ± standard error of mean (SEM). Analysis of variance (ANOVA) or *t* test was used to determine statistical significance among different groups ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$).

3. Results and discussions

3.1. Study design

Animals were primed with three commercial vaccines, CoronaVac, ZF2001, and Ad5-nCoV, *via* the intramuscular route and boosted with homologous as well as heterologous CoronaVac, ZF2001, Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P 5−6 months later (10 weeks later for Ad5-nCoV primed groups). The dosages of different vaccines in the study were set as one-tenth of the human dose. For CoronaVac-primed animals, female BALB/c mice and Syrian hamsters were first immunized intramuscularly with two doses of PBS or CoronaVac at a 21-day interval (Fig. 1A). After a 167-day rest period, booster vaccination with PBS, CoronaVac, ZF2001, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P was carried out. Serum samples were harvested from mice and hamsters on Days 0 (before immunization), 21 (before immunization), 28, 35, 188 (before immunization), and 195. On Day 197, mice were sacrificed for immune response analysis, and hamsters were challenged intranasally with 1×10^4 plaque-forming units (PFU) authentic Omicron BA.1. For ZF2001-primed animals, mice and hamsters were immunized over a 14-day interval with three doses of PBS or ZF2001 and boosted with PBS, ZF2001, CoronaVac, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P on Day 190 (Fig. 1B). Serum samples were collected at the indicated time points. Subsequently, immune response analysis and Omicron challenge were carried out 9 days post booster shots. Ad5-nCoV-primed animals were immunized with one dose of PBS or Ad5-nCoV, followed by boosting with PBS, CoronaVac, ZF2001, S_{WT}-2P, or $S_{Omicron}$-6P on Day 71 (Fig. 1C). Serum samples were collected from mice and hamsters at the indicated time points. On Day 80, the mice were euthanized to evaluate the induced immune responses, and the hamsters were challenged for protection analysis. Sera were evaluated both for spike-specific binding antibody responses and neutralizing activities against Omicron variants (part of the data was not presented). In addition, all challenged hamsters were sacrificed and analyzed for viral loads in different tissues 3 days post infection.

3.2. SARS-CoV-2 spike-specific antibody responses in mice and hamsters after different booster vaccinations

The IgG responses against spike proteins of the WT strain and variant Omicron BA.1 were investigated in the serum of mice and hamsters before and after different boosters. For CoronaVac-primed animals, boosting with the homologous vaccine CoronaVac elicited the lowest level of binding antibodies against spike proteins (WT and BA.1) compared with heterologous shots (Fig. 2A−D and Supporting Information Fig. S1). However, mRNA vaccines (S_{WT}-2P and $S_{Omicron}$-6P) as heterologous boosters induced the highest levels of IgG responses among the five groups. These two boosters induced a similar level of WT and Omicron-specific antibody response. In addition, Ad5-nCoV, as an adenovirus vectored vaccine, also elicited high levels of IgG responses against both WT and BA.1 spike proteins. For ZF2001-primed mice, compared with the heterologous CoronaVac booster, boosting with homologous ZF2001 produced higher levels of IgG antibodies against WT and BA.1 spike proteins, although no significant differences were observed (Fig. 2E and F). Among the five boosters, Ad5-nCoV induced the highest and second-highest levels of binding antibodies against WT and BA.1 spike proteins. Moreover, the Omicron BA.1-specific mRNA vaccine, $S_{Omicron}$-6P, induced the highest levels of anti-spike (WT) IgG antibody and anti-spike (BA.1) IgG antibody responses in mice. However, the specific humoral immune responses were severely disrupted in the ZF2001 (adjuvanted RBD subunit vaccine)-primed hamsters (Fig. 2G and H, and Supporting Information Fig. S2). Specifically, the IgG antibody titers ranged from 100 (lower limit of detection, LLOD) to 1060 against the WT spike protein and 143 to 2543 against the BA.1 spike protein in ZF2001-primed hamsters after different booster vaccinations. Nonetheless, booster shots with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P elicited more robust IgG antibody responses against WT and BA.1 spike proteins than CoronaVac and ZF2001. The low immunogenicity of RBD-based vaccines in Syrian hamsters has been reported, but the potential mechanisms are under revealed[39]. Furthermore, we evaluated the spike protein-specific antibody responses in Ad5-nCoV-primed animals after booster

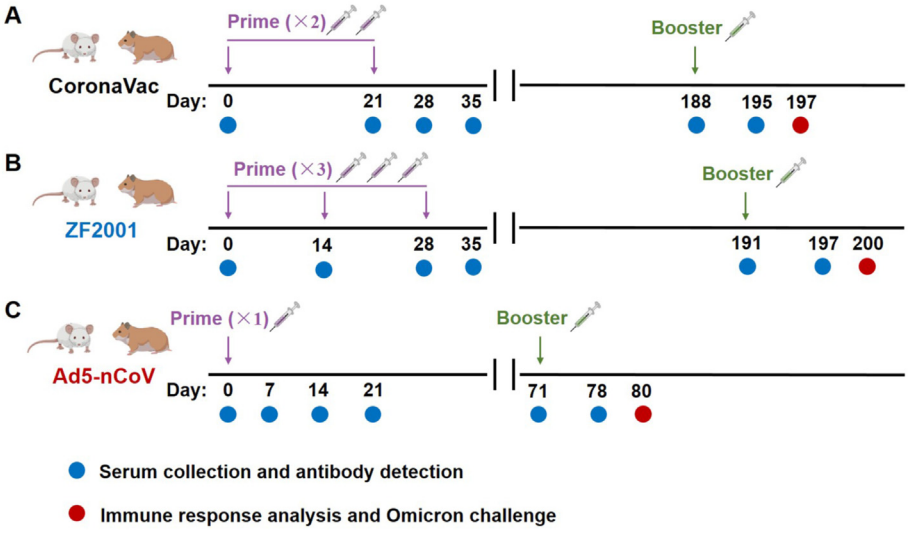

Figure 1 The overall schedule of the study. (A−C) Timeline of immunization, serum collection, immune response, and challenge in mice and hamsters. Purple arrows indicate the prime immunizations, green arrows indicate the booster immunizations, blue dots indicate the serum collections, and red dots indicate the immune response analysis and the virus challenge.

vaccinations. As shown in Fig. 2I−L and Supporting Information Fig. S3, among the boosters, boosting with SOmicron-6P induced the highest levels of IgG antibodies against WT and BA.1 spike proteins in both animal models. In addition, boosting with S_{WT}-2P also induced high levels of binding antibody responses against the spike proteins. Overall, after full comparisons, it seems that boosting CoronaVac, ZF2001, or Ad5-nCoV-primed animals with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P instead of CoronaVac or ZF2001 would elicit a significantly higher level of humoral immune responses against both WT SARS-CoV-2 and variant Omicron BA.1.

3.3. Neutralizing antibody responses against authentic Omicron BA.1 and BA.5 after different booster vaccinations

Functional antibodies in blood circulation are neutralizing antibodies that can block the entry of live viruses into host cells. Given that the new infections rising worldwide are caused by Omicron and its subvariants, we compared the levels of neutralizing antibody responses against live Omicron BA.1 and BA.5 after different booster vaccinations. For CoronaVac-primed animals, consistent with the results in Fig. 2, boosting with CoronaVac or ZF2001 elicited the lowest levels of neutralizing antibody responses against live Omicron BA.1 and BA.5 (Fig. 3A−D and Supporting Information Figs. S4−S6). Comparatively, Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P, as heterologous booster shots, induced highly potent neutralizing antibodies against Omicron and its variants in mice and hamsters. In particular, the Omicron BA.1-specific mRNA vaccine $S_{Omicron}$-6P showed the highest neutralization titers. Next, we assessed the neutralizing activities in ZF2001-primed animals. For mice, ZF2001 as a homologous booster vaccination exhibited the lowest neutralizing activities against Omicron variants, which differed from the data in anti-spike IgG titers (Fig. 3E and F, and Supporting Information Fig. S7). We observed approximately 2-fold higher neutralization titers against BA.1 and BA.5 in CoronaVac-boosted mice than in ZF2001-boosted mice (Fig. 3E and F). Moreover, the other three heterologous booster vaccines, Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P, induced significantly higher levels of neutralizing antibodies against BA.1 and BA.5 than CoronaVac and ZF2001. Interestingly, Ad5-nCoV and S_{WT}-2P exhibited even better performances than the BA.1-specific mRNA vaccine in combating Omicron infections. However, except for two animals in the S_{WT}-2P shot group, the neutralizing activities disappeared in all ZF2001-primed hamsters after booster vaccinations (Fig. 3G and H, Supporting Information Figs. S8 and S9). Furthermore, the neutralizing activities in Ad5-nCoV-primed animals were studied after different booster shots. $S_{Omicron}$-6P induced the highest neutralization titers against BA.1 but not BA.5 in both mice and hamsters, while ZF2001, S_{WT}-2P, and this BA.1-specific vaccine exhibited similar abilities in combating BA.5 (Fig. 3I−L, Supporting Information Figs. S10−S12). CoronaVac induced the lowest levels of neutralizing antibody responses against BA.1 in mice but higher levels of neutralizing antibodies than ZF2001 in hamsters. However, we observed no obvious neutralizing activities against BA.5 in Ad5-nCoV-primed hamsters after all these booster vaccinations. Overall, the data in Fig. 3 shows that animals primed with Ad5-nCoV instead of CoronaVac or ZF2001 elicited higher levels of neutralizing antibody responses against Omicron infections after booster vaccinations.

3.4. Immune responses to different prime-boost vaccinations in inactivated whole virus vaccine-primed mice

In addition to antibodies, we analyzed the immune responses in inactivated whole virus vaccine (CoronaVac)-primed mice with different booster strategies. T/B-cell responses in LNs and spleen were determined by flow cytometry on Day 9 after booster vaccination. The gating strategy for flow cytometry analysis can be found in Supporting Information Fig. S13. We observed a significant reduction in T cells in LNs in the CoronaVac + Ad5-nCoV group, specifically total T cells ($CD3^+$) and $CD4^+$ and $CD8^+$ T cells (Supporting Information Fig. S14A−S14C). In contrast to that of T cells, the proportion of B cells in the LNs of this group was robustly increased after booster vaccination (Fig. S14D). The groups CoronaVac boosted with mRNA vaccines

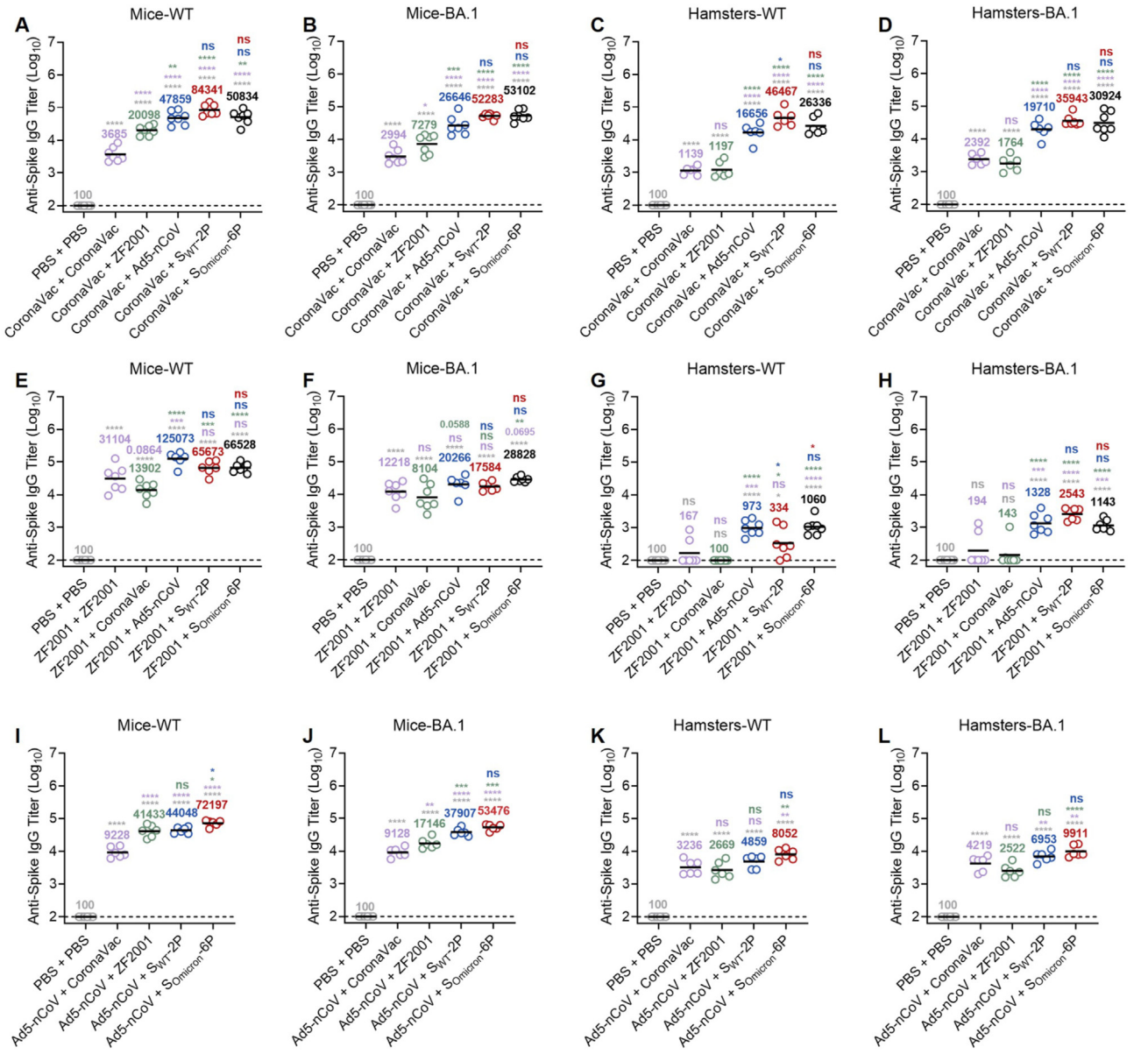

Figure 2 Binding antibody responses against spike proteins of wild-type SARS-CoV-2 (WT) and variant Omicron BA.1 in mice and hamsters after different booster vaccinations. The anti-spike IgG antibody titers in mice and hamsters were measured by an enzyme-linked immunosorbent assay (ELISA). (A−D) Serum IgG responses in CoronaVac-primed mice (A, B) and hamsters (C, D) after booster vaccinations against WT or BA.1 spike proteins. (E−H) IgG titers in ZF2001-primed mice (E, F) and hamsters (G, H) after booster vaccinations against WT or BA.1 spike proteins. (I−L) Binding antibody responses in Ad5-nCoV-primed mice (I, J) and hamsters (K, L) after booster vaccinations against WT or BA.1 spike proteins. All statistics were calculated using unpaired one-way ANOVA with multiple comparison tests. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; ns, not significant. The values of the geometric mean titers (GMTs) of each group are displayed in the graph.

(including CoronaVac + S_{WT}-2P and CoronaVac + $S_{Omicron}$-6P) exhibited no significant changes in T-cell proportions but elevated B-cell ratios (Fig. S14A−S14D). In the spleen, compared to the PBS group, no significant fluctuations in T-cell frequencies were observed after CoronaVac + Ad5-nCoV immunization (Fig. S14E−S14G). However, boosting with Ad5-nCoV induced a lower level of B cells in the spleen (Fig. S14H). Compared to the Ad5-nCoV-boosted vaccination, the other four vaccines (including CoronaVac, ZF2001, S_{WT}-2P, and $S_{Omicron}$-6P) induced a notable increase in T cells in the spleen, particularly $CD4^+$ T cells. Among the four vaccines, the mRNA vaccines, similar to Ad5-nCoV, also decreased the B-cell ratios in the spleen. Overall, it seems that in both LNs and spleen, the levels of T cells are opposite to the levels of B cells after booster vaccinations. We speculated that the discriminating immune responses were attributed to the spatiotemporal differences in lymphocyte recirculation and homing and proliferation after vaccination with different types of vaccines[40]. The exact mechanisms and potential effects are being investigated.

Next, we studied the subcellular population changes of functional T/B cells in both LNs and spleen after different booster shots. Among the five vaccines, Ad5-nCoV induced the highest levels of TFH cells, which are essential for GC reactions, GC B cells (the source of the high affinity and class-switched

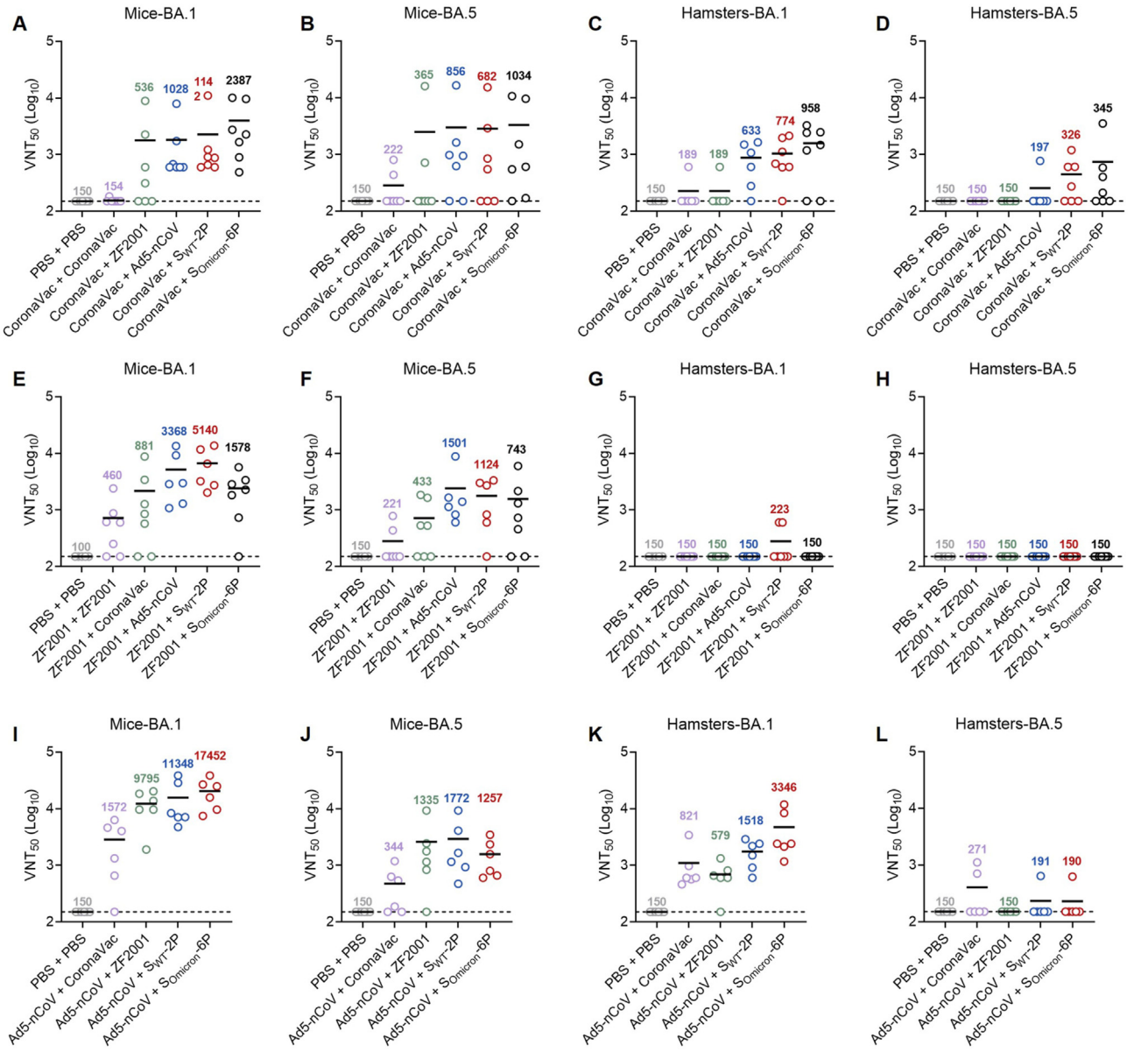

Figure 3 Neutralizing antibody responses against live Omicron BA.1 and BA.5 in mice and hamsters after different booster vaccinations. The collected sera after different booster vaccinations were further evaluated for neutralizing activities by a plaque reduction neutralization test (PRNT). (A−D) The 50% virus-neutralization titers (VNT_{50}) in CoronaVac-primed mice (A, B) and hamsters (C, D) after booster vaccinations against authentic SARS-CoV-2 Omicron BA.1 and BA.5. (E−H) VNT_{50} in ZF2001-primed mice (E, F) and hamsters (G, H) after booster vaccinations against BA.1 and BA.5. (I−L) VNT_{50} in Ad5-nCoV-primed mice (I, J) and hamsters (K, L) after booster vaccinations against BA.1 and BA.5. The GMTs of each group are displayed in the graph.

antibodies), plastic $CD38^+GL7^+$ B cells (the multipotent precursors having the potential to differentiate into memory B cells and later GC B cells), and class-switched $IgG1^+/IgG2a^+$ B cells (produce specific antibodies to neutralize antigens) in both LNs and spleen (Fig. 4A−H)[40-44]. The two types of mRNA vaccines exhibited a similar (but weaker than Ad5-nCoV, and $S_{Omicron}$-6P seems better than S_{WT}-2P) ability to elicit these immune responses in the LNs and spleen. Boosting with ZF2001, a kind of aluminum-adjuvanted protein subunit vaccine, elicited a slight elevation of the percentages of these cells (elicited a weak immune response). However, the inactivated vaccine, CoronaVac, as a homologous vaccine, built up the lowest level of functional immune responses compared to the other four heterologous vaccines. These findings were consistent with the different levels of humoral immune response in different vaccine groups in Figs. 2 and 3.

In addition to humoral immune responses, T-cell-mediated immunity also contributes to protection against re-exposure to the virus by secreting active cytokines. For intracellular cytokine staining (ICS) of T cells, splenocytes were *ex vivo* restimulated with the spike peptide pool and analyzed by flow cytometry. Boosting CoronaVac with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P instead of CoronaVac and ZF2001 induced a higher fraction of $CD8^+$ and $CD4^+$ T cells that secreted the type 1 intracellular cytokine IL-2 (Fig. S14I and S14J). However, except for $S_{Omicron}$-6P, the other four vaccines did not increase the expression of INF-γ (type 1 intracellular cytokine) in $CD8^+$ or $CD4^+$ T cells

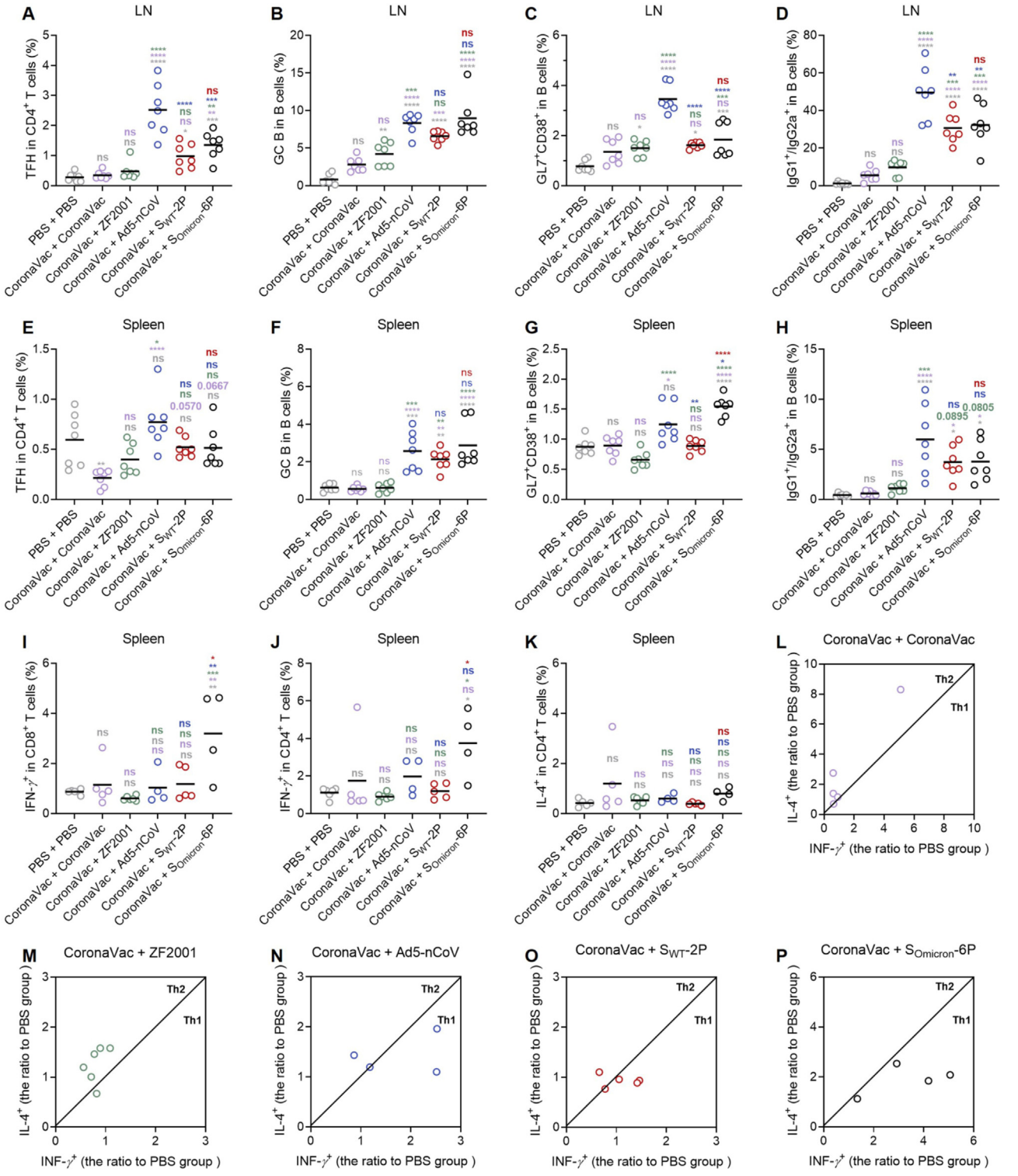

Figure 4 T-cell and B-cell responses in CoronaVac-primed mice after boosting with CoronaVac, ZF2001, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P. Nine days postboost, all mice were sacrificed, and the spleen and draining lymph nodes (LNs) (popliteal and inguinal lymph nodes) were harvested for T/B-cell analysis, as described in Fig. 1A. $n = 7$ for all groups except for specific statements. (A–D) Flow cytometric analysis of follicular helper T (TFH) cells (A), germinal center B (GC B) cells (B), plastic $CD38^+GL7^+$ B-cell precursors (C), and class-switched $IgG1^+$/$IgG2a^+$ B cells in LNs (D). (E–H) Frequencies of TFH cells (E), germinal center B cells (F), plastic $CD38^+GL7^+$ B-cell precursors (G), and class-switched $IgG1^+$/$IgG2a^+$ B cells in the spleen (H). (I–P) For T-cell intracellular cytokine staining (ICS), splenocytes were restimulated with the spike peptide pool for 12 h and then stained with fluorescence-conjugated antibodies and analyzed by flow cytometry ($n = 4$–6). The expression of interferon-γ (IFN-γ) (I, J) among $CD4^+$ (I) and $CD8^+$ (J) T cells in the spleen. Frequency of interleukin-4 (IL-4)-positive $CD4^+$ T cells in the spleen (K). Scatter plots showing the correlations of IFN-γ- and IL-4-secreting cells by flow cytometry (L–P). The groups contained

(Fig. 4I and J). There were no significant fluctuations in $CD4^+$ T cells that secreted the type 2 intracellular cytokine IL-4 after different vaccinations (Fig. 4K)[45]. Furthermore, we evaluated the balance of Th1 and Th2 responses in different vaccinated mice. We found that CoronaVac boosted with itself or ZF2001 induced a low-level but Th2-biased immune response, while CoronaVac boosted with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P induced a Th1-biased immune response (Fig. 4L−P). Although the sample sizes of some groups are small due to an operating loss, these results are still instructive.

3.5. *Immune responses to different prime-boost vaccinations in adjuvanted protein vaccine-primed mice*

In adjuvanted protein vaccine (ZF2001)-primed mouse models, we investigated the immune responses after different booster vaccinations by flow cytometry. Similar to the data shown in CoronaVac-primed mice, boosting with Ad5-nCoV significantly decreased the frequencies of $CD4^+$ T cells and increased the frequencies of B cells in LNs but not in spleen Supporting Information Fig. S15). No significant differences in the T-cell frequencies were observed among the PBS + PBS, ZF2001 + ZF2001, ZF2001 + CoronaVac, ZF2001 + S_{WT}-2P, and ZF2001 + $S_{Omicron}$-6P groups. The B-cell frequencies in LNs of the ZF2001 + S_{WT}-2P and ZF2001 + $S_{Omicron}$-6P groups were increased compared with the control group. However, these changes in immune cell frequencies were attenuated in the spleen. The lower frequencies of $CD4^+$ T cells corresponded to the higher frequencies of B cells or the opposite, which has been revealed in the above results.

We next determined the frequency and phenotype of T/B cells in mice after different vaccinations. Interestingly, in ZF2001-primed mice, among the five vaccines (including ZF2001, CoronaVac, Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P) as the booster shots, Ad5-nCoV induced almost the highest frequencies of TFH cells, GC B cells, plastic $CD38^+GL7^+$ B cells, and class-switched $IgG1^+/IgG2a^+$ B cells in both LNs and spleen, which were already observed in the CoronaVac-primed mice (Fig. 5A−H). Compared with ZF2001, CoronaVac, as a heterologous vaccine, exhibited no advantages in eliciting these beneficial immune responses. Boosting with ZF2001 or CoronaVac induced no increased frequencies of the functional immune cells compared with the control group. However, boosting with mRNA vaccines, S_{WT}-2P or $S_{Omicron}$-6P, significantly increased the frequencies of the above-mentioned immune cell populations, although they were weaker than those in the Ad5-nCoV-boosted group.

In addition to quantitative analysis of the frequency and phenotype of T/B cells, we also characterized the cytokine profiles at the single-cell level. Boosting ZF2001 with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P instead of ZF2001 and CoronaVac induced a higher fraction of $CD8^+$ and $CD4^+$ T cells that secreted the type 1 intracellular cytokine interferon-γ (IFN-γ) (Fig. 5I and J). There were no significant differences in the secretion of interleukin-4 (IL-4) among the six groups (Fig. 5K). Based on the intracellular cytokine-secreting data, we analyzed the balance of Th1 and Th2 responses in mice under different vaccinations. Four doses of the protein subunit vaccine, ZF2001, elicited a Th1/Th2-balanced cellular immune response in mice (Fig. 5L)[46]. Three doses of ZF2001 boosted with a dose of CoronaVac elicited low levels of both Th1 and Th2 cellular immune responses in mice (Fig. 5M). However, boosting ZF2001 with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P resulted in a Th1-biased immune response in mice (Fig. 5N−P). Taken together, in both inactivated vaccine (CoronaVac) and protein subunit vaccine (ZF2001)-primed mice, boosting with the adenovirus type 5 vectored vaccine and mRNA vaccines rather than the CoronaVac or ZF2001 vaccines induced higher levels of functional immune responses, and the cellular immune responses were Th1-biased.

3.6. *Immune responses to heterologous prime-boost vaccinations in adenovirus-vectored vaccine-primed mice*

In the adenovirus-vectored vaccine (Ad5-nCoV)-primed mouse models, we studied the immune responses after different booster vaccinations. Except for $S_{Omicron}$-6P booster vaccination inducing increased percentages of $CD8^+$ T cells in the spleen, Ad5-nCoV boosted with CoronaVac, S_{WT}-2P, or $S_{Omicron}$-6P exhibited nondistinctive changes in T cells and B cells in the LNs and spleen compared with the control group (Supporting Information Fig. S16). Ad5-nCoV + ZF2001 treatment induced a significantly decreased level of $CD4^+$ T cells and an increased level of B cells in the spleen compared with the other groups, although this effect was not evident in LNs. Similar to the results in inactivated vaccine-primed animals, the lower levels of $CD4^+$ T cells corresponded to higher levels of B cells in Ad5-nCoV-primed mice after booster vaccination.

The T/B-cell phenotyping of Ad5-nCoV-primed mice after boosting with different vaccines was also analyzed. The frequencies of TFH cells in LNs significantly increased in the Ad5-nCoV + CoronaVac and Ad5-nCoV + ZF2001 groups, especially the latter group (Fig. 6A). However, the frequencies of GC B cells in LNs did not increase in the Ad5-nCoV + CoronaVac or Ad5-nCoV + ZF2001 groups but increased in the mRNA vaccine-boosted groups (Fig. 6B). The same effects were also observed for TFH cells, GC B cells, plasma B cells (the long-lived mediators of lasting humoral immunity), and plasmablast B cells (the rapidly produced and short-lived effector cells of the early antibody response) in the spleen (Fig. 6C−F)[47]. However, the mechanisms of this phenomenon remain to be further elucidated.

We next studied the intracellular cytokine-producing abilities of mice in different groups. The expression of IFN-γ and IL-4 was determined by an enzyme-linked immunospot (ELISPOT) assay. All mice after booster vaccination induced increased IFN-γ, and the mRNA vaccines as the booster shots induced the highest levels of IFN-γ (Fig. 6G and Supporting Information Fig. S17). In contrast to IFN-γ, no significant differences in IL-4 secretion were found among these groups (Fig. 6H and Fig. S17). Taken together, in Ad5-nCoV-primed mice, CoronaVac induced the lowest level of immune responses among the four vaccines, while mRNA vaccines (including S_{WT}-2P and $S_{Omicron}$-6P) elicited significantly increased humoral and cellular immune responses. Furthermore, we analyzed Th1- or Th2-biased immune responses in different groups. We found that all groups exhibited a strong Th1-biased immune response, which indicated that priming with Ad5-nCoV induces a Th1-biased immune response that is irrelevant to the booster vaccines (Fig. 6I−L).

mice immunized with two doses of CoronaVac and then boosted with CoronaVac (L), ZF2001 (M), Ad5-nCoV(N), S_{WT}-2P (O), or $S_{Omicron}$-6P (P). All statistics were calculated using unpaired one-way ANOVA with multiple comparison tests. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; ns, not significant.

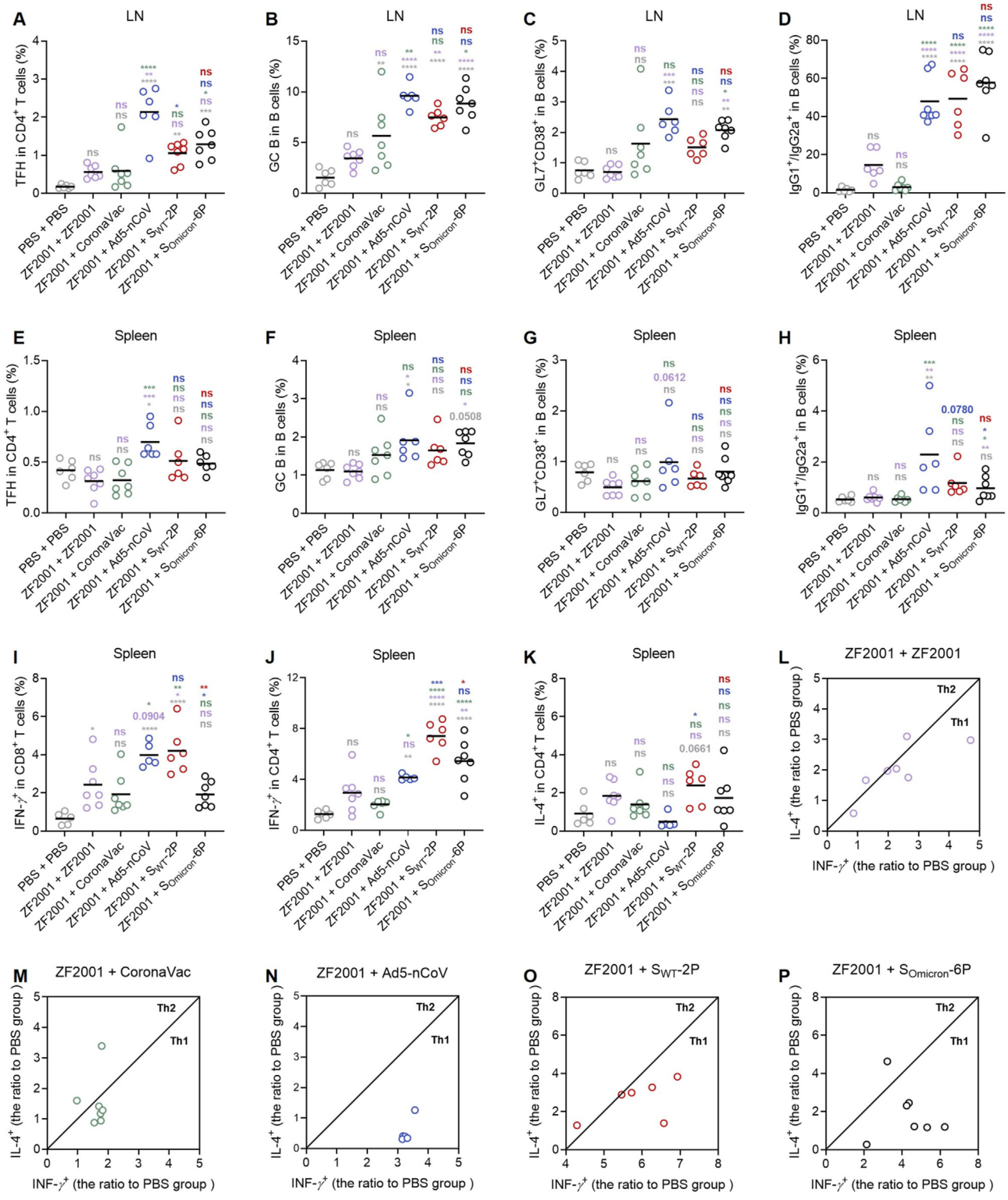

Figure 5 T-cell and B-cell responses in ZF2001-primed mice after boosting with ZF2001, CoronaVac, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P. On Day 200, all groups of mice were sacrificed for T/B-cell analysis, as described in Fig. 1B. $n = 6-7$ per group. (A–D) Frequencies of TFH cells (A), GC B cells (B), plastic $CD38^+GL7^+$ B-cell precursors (C), and class-switched $IgG1^+/IgG2a^+$ B cells (D) in LNs. (E–H) Frequencies of TFH cells (E), GC B cells (F), plastic $CD38^+GL7^+$ B-cell precursors (G), and class-switched $IgG1^+/IgG2a^+$ B cells (H) in the spleen. (I–P) For T-cell ICS, splenocytes were restimulated with the spike (S) peptide pool for 12 h and then stained with fluorescence-conjugated antibodies and analyzed by flow cytometry. The expression of IFN-γ (I, J) among $CD4^+$ (I) and $CD8^+$ (J) T cells in the spleen. Frequency of IL-4-positive $CD4^+$ T cells in the spleen (K). Scatter plots showing the correlations of IFN-γ- and IL-4-secreting cells by flow cytometry (L–P). The groups contained mice immunized with three doses of ZF2001 and then boosted with ZF2001 (L), CoronaVac (M), Ad5-nCoV (N), S_{WT}-2P (O), or $S_{Omicron}$-6P (P). All

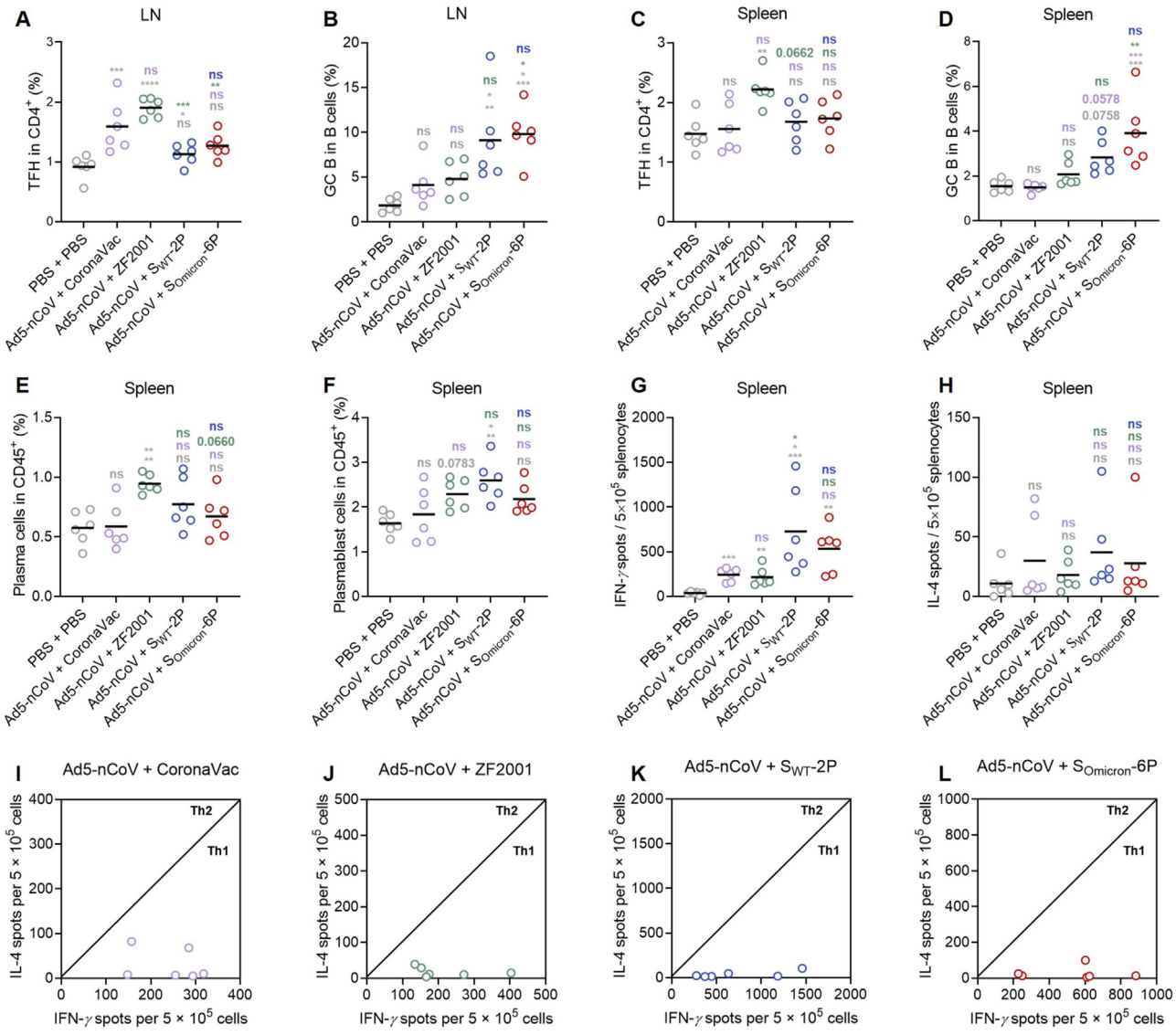

Figure 6 T-cell and B-cell responses in Ad5-nCoV-primed mice after boosting with CoronaVac, ZF2001, S_{WT}-2P, or $S_{Omicron}$-6P. Nine days after the booster shot, all mice were sacrificed for T/B-cell analysis, as described in Fig. 1C. $n = 6$ per group. (A, B) Frequencies of TFH cells (A), and GC B cells (B) in LNs. (C−F) Frequencies of TFH cells (C), GC B cells (D), plasma B cells (E), and plasmablast B cells (F) in the spleen. (G, H) The expression levels of IFN-γ (G) and IL-4 (H) in splenocytes stimulated by the spike peptide pool were measured by enzyme-linked immunospot (ELISPOT) assay. (I−L) Scatter plots showing the correlations of IFN-γ- and IL-4-secreting cells by ELISPOT assay. The groups contained mice immunized with a single dose of Ad5-nCoV and then boosted with CoronaVac (I), ZF2001 (J), S_{WT}-2P (K), or $S_{Omicron}$-6P (L). All statistics were calculated using unpaired one-way ANOVA with multiple comparison tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; ns, not significant.

3.7. Booster vaccinations enhance potent GC reactions in secondary lymphatic organs associated with neutralizing antibody production

After characterizing the neutralizing activities and functional immune responses in the three vaccine-primed animals, we further investigated the potential relationships. Class-switched $IgG1^+/IgG2a^+$ B cells have the potential to generate high-affinity neutralizing antibodies. As shown in Fig. 7A−D, the neutralizing antibody titers against Omicron BA.1 and BA.5 were strongly associated with $IgG1^+/IgG2a^+$ B cells in LNs, while they showed a weaker correlation in spleen. As reported, B cells undergo class switch recombination within the GC to ultimately produce high-affinity, class-switched antibodies[48]. We thus hypothesized the existence of an association between neutralizing responses and GC B cells. As anticipated, the anti-BA.1 and BA.5 neutralizing activities correlated with GC B cells in the secondary lymphatic organs, especially in LNs (Fig. 7E−H). In addition, GC responses

statistics were calculated using unpaired one-way ANOVA with multiple comparison tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; ns, not significant.

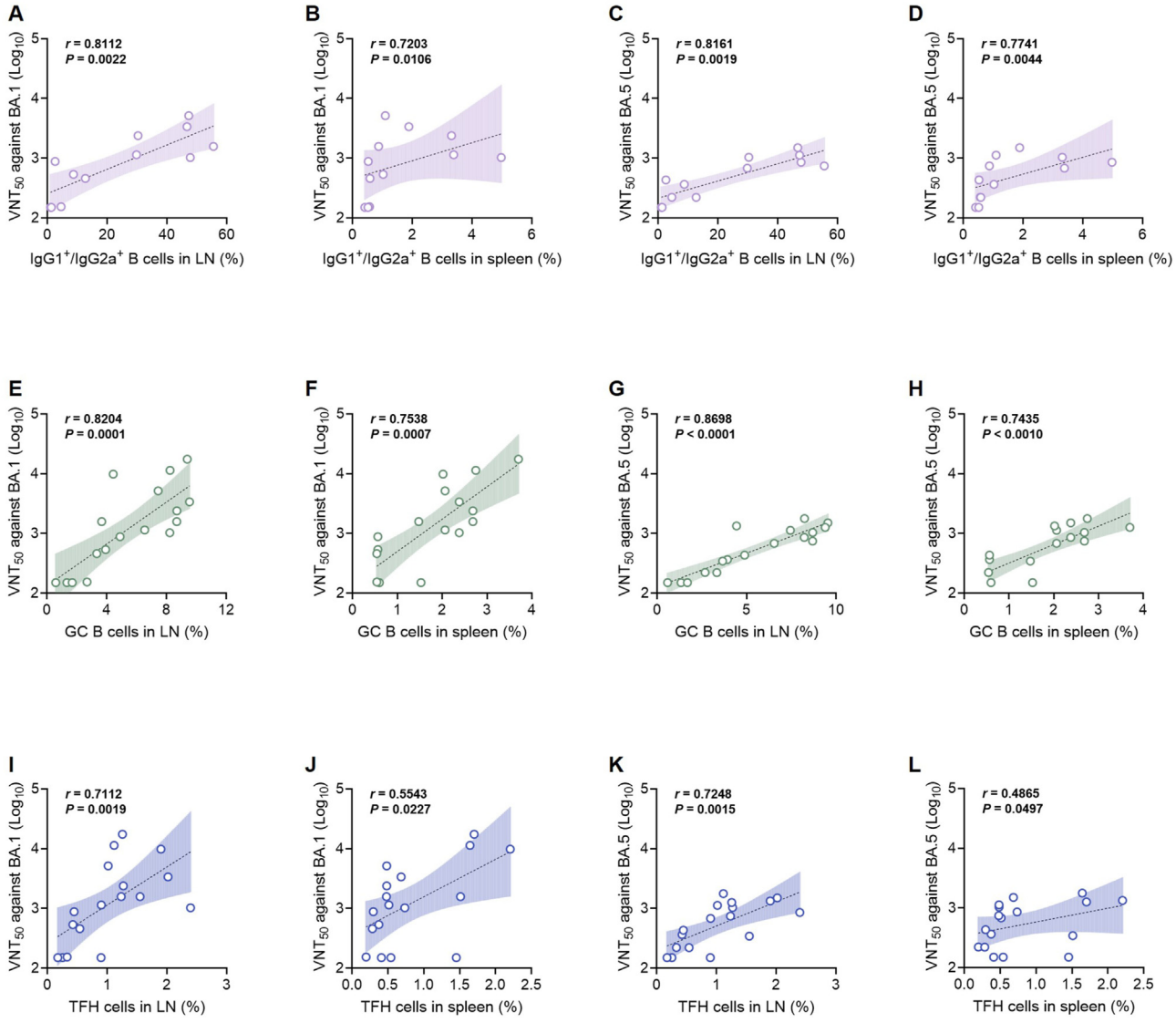

Figure 7 Correlations of neutralizing antibody activities and functional immune cells. For all correlations, the data of mice from the whole-virus neutralization assay in Fig. 3 and flow cytometric analysis in Figs. 4−6 were used. (A−D) Spearman correlations of neutralization titers against BA.1 and class-switched $IgG1^+/IgG2a^+$ B cells in LNs (A) and spleen (B), and neutralization titers against BA.5 and class-switched $IgG1^+/IgG2a^+$ B cells in LNs (C) and spleen (D). (E−H) Spearman correlations of neutralization titers against BA.1 and GC B cells in LNs (E) and spleen (F), and neutralization titers against BA.5 and GC B cells in LNs (G) and spleen (H). (I−L) Spearman correlations of neutralization titers against BA.1 and TFH cells in LNs (I) and spleen (J), and neutralization titers against BA.5 and TFH cells in LNs (K) and spleen (L). Every hollow dot represents a geometric mean value of a group of data. Spearman's correlation coefficients (r) and P values (P) are shown in the graphs.

are tightly regulated by specialized TFH cells through the delivery of costimulatory molecules and cytokines to GC B cells[41,49]. We observed positive correlations between neutralizing titers (against BA.1 and BA.5) and TFH cells in the LNs and spleen (Fig. 7I−L). Interestingly, the LNs showed stronger correlations of neutralization titers and switched $IgG1^+/IgG2a^+$ B cells, GC B cells, and TFH than the spleens. Furthermore, we directly evaluated the correlations of these functional immune cells that are involved in GC reactions. We found that switched $IgG1^+/IgG2a^+$ B cells, GC B cells, and TFH cells correlated strongly with each other in LNs but showed weaker associations in the spleen (Supporting Information Fig. S18).

Taken together, these data indicate that booster vaccination-elicited GC responses play an important role in producing high-quality neutralizing antibodies, and the switched $IgG1^+/IgG2a^+$ B cells and GC B cells are tightly regulated by TFH cells within the GC. Boosting prior vaccinations with a booster vaccine that can effectively reactivate a cyclic program of GC functions would significantly facilitate long-lasting immunologic protection against SARS-CoV-2 infection.

3.8. Protective efficacies in hamsters after receiving different booster vaccinations

After characterizing the humoral and cellular immune responses, we further investigated the protection efficacies in the hamster models. To simulate the acute COVID-19 infection after booster immunization, Syrian hamsters in different groups were

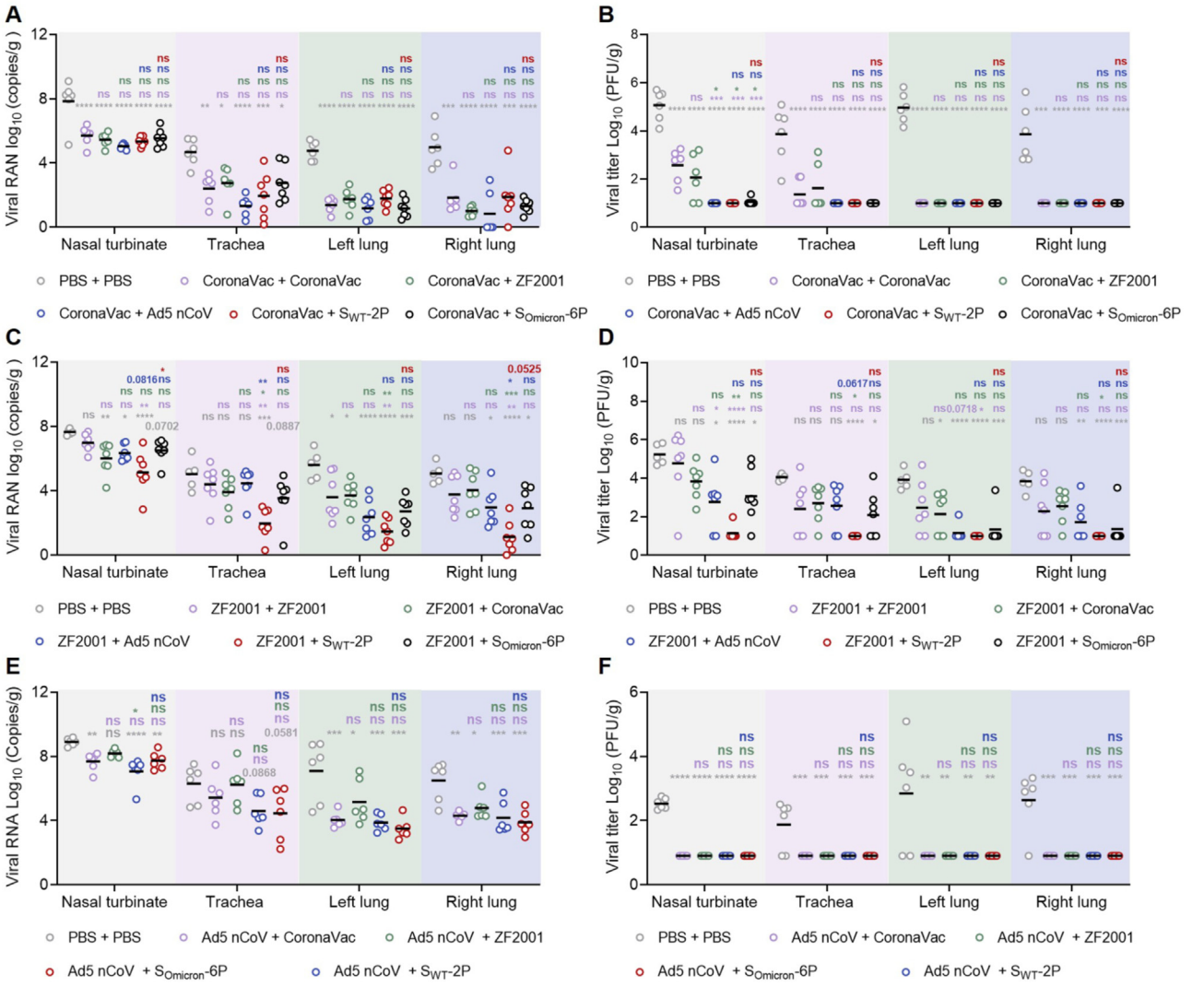

Figure 8 The protective efficacies of three commercial vaccines after boosting with homologous or heterologous vaccines in hamster models. (A, B) Female hamsters were primed with two doses of CoronaVac, followed by boosting with CoronaVac, ZF2001, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P, as described in Fig. 1A. Viral RNA loads in the nasal turbinate, trachea and both lungs were determined by quantitative reverse transcription-polymerase chain reaction (RT-qPCR) (A). Live viral loads in hamster tissues were quantified by using a plaque assay (B). (C, D) Hamsters were primed with three doses of ZF2001 followed by boosting with ZF2001, CoronaVac, Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P, as described in Fig. 1B. RT-qPCR analysis of the viral loads in hamster tissues (C). Plaque assay determines the live viral loads in hamster tissues (D). (E, F) Hamsters were primed with a single dose of Ad5-nCoV followed by boosting with CoronaVac, ZF2001, S_{WT}-2P, or $S_{Omicron}$-6P, as described in Fig. 1C. Viral RNA loads were determined by RT-qPCR (E). Viral load tests measured the amount of live virus in hamster tissues (F). All statistics were calculated using unpaired one-way ANOVA with multiple comparison tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; ns, not significant.

challenged with 1×10^4 plaque-forming units (PFU) of authentic Omicron BA.1. Nine days after booster shots and analyzed for viral RNA loads and viral titers 3 days post infection. For inactivated vaccine (CoronaVac)-primed hamsters, the viral RNA loads and live viral titers in the nasal turbinate, trachea and both lungs of all vaccine-boosted animals were significantly decreased compared with those of the PBS control (Fig. 8A and B). However, there were no significant differences in the viral RNA loads of the five different booster vaccinations. In contrast, a certain amount of live virus was detected in the nasal turbinate and trachea of the CoronaVac and ZF2001-boosted hamsters instead of the Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P groups. No live virus was detected in either lung of any of the boosted hamsters. The differences in protective efficacy among different booster vaccines were not significant enough, which could be attributed to the reduced pathogenicity of BA.1. Nonetheless, these data indicated that a WT-based inactivated vaccine boosted with a WT-based adenovirus vectored vaccine or mRNA or BA.1-specific mRNA vaccine could provide more robust protection against Omicron BA.1 than a WT inactivated vaccine and an adjuvanted protein subunit vaccine. We next evaluated the protective efficacies in ZF2001 (a tandem-repeat dimeric RBD protein)-primed hamsters after different booster immunizations. Consistent with the IgG and neutralization titers revealed above, we observed weak protective efficacies in these animals (Fig. 8C and D). These data suggest that further studies are needed to evaluate the immunogenicity of

RBD-based vaccines in other animal models[39]. Nonetheless, Ad5-nCoV and mRNA vaccines, especially S_{WT}-2P, were more effective than CoronaVac and ZF2001 in combating BA.1 infection. For Ad5-nCoV-primed hamsters, all heterologous booster vaccinations significantly reduced the viral RNA loads, and no live virus was detected in hamster tissues 3 days post infection (Fig. 8E and F).

Furthermore, the lungs of hamsters after challenge were also evaluated by hematoxylin-eosin staining (H&E) for detecting damage and inflammatory changes. All hamsters in PBS groups displayed pathology with immune cell infiltration, airway space thickening, mild alveolar congestion, and interstitial edema. In contrast, hamsters boosted with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P did not develop lung pathology and while those receiving booster immunizations with CoronaVac or ZF2001 exhibited minor to moderate pathology (Supporting Information Figs. S19−S21). However, no significant fluctuations in body weight were observed among the different immunization groups of hamsters (Supporting Information Fig. S22).

Taken together, Ad5-nCoV, S_{WT}-2P and $S_{Omicron}$-6P exhibited greater potential than CoronaVac and ZF2001 as booster vaccines in protecting against Omicron infections. However, the differences in the protective efficacies of Ad5-nCoV, S_{WT}-2P and $S_{Omicron}$-6P in the live virus challenge experiments are indistinguishable.

4. Conclusions

Omicron and its subvariants, with numerous mutations and deletions in the spike proteins, escape the immune protection conferred by WT-based vaccinations or previous infections. It has resulted in a rapid spread in vaccinated or unvaccinated populations worldwide. Moreover, many people around the world are experiencing SARS-CoV-2 Omicron reinfections, which contributed additional risks of death[50]. Additional vaccination or updated vaccines are highly needed to conquer the loss in protective efficacies against Omicron variants by authorized vaccines based on the WT strain. Consequently, pressing scientific questions are raised in the vaccine booster field: Among the SARS-CoV-2 vaccines of different types, which ones are the optimal booster vaccination strategies? What are the potential mechanisms and the differences in the humoral and cellular immune responses by the different vaccines? In this study, we performed a systematic comparison in two animal models with different prime-boost regimens, including inactivated whole-virus vaccine (CoronaVac), aluminum hydroxide-adjuvanted RBD protein vaccine (ZF2001), adenovirus-vectored vaccine (Ad5-nCoV), WT mRNA vaccine (S_{WT}-2P), and Omicron BA.1-specific mRNA vaccine ($S_{Omicron}$-6P). Compared with homologous immunizations, heterologous boosters elicited significantly stronger humoral immune responses against WT SARS-CoV-2, Omicron BA.1, and BA.5[24,51-53]. Among the five SARS-CoV-2 vaccines, we found that Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P as heterologous immunizations induced high levels of neutralizing antibodies against the Omicron variants, especially $S_{Omicron}$-6P, which showed many advantages. Interestingly, booster immunizations did not exhibit immunogenicity in RBD protein vaccine-primed hamsters, with undetectable neutralization activities, which have been reported previously, but the mechanisms are unknown.

In-depth studies were conducted by assessing the humoral and cellular immune responses with flow cytometry and ELISPOT. We observed significantly stronger GC responses in mice that have been characterized by high levels of neutralization activities against Omicron variants. Specifically, boosting with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P induced markedly elevated frequencies of TFH cells, class-switched $IgG1^+$/$IgG2a^+$ B cells, and GC B cells in secondary lymphatic organs, especially in LNs. Prolonged antigen availability presented by antigen-presenting cells (APCs) and intrinsic TFH cell adjuvanticity of the vaccine systems that encode SARS-CoV-2 antigens could be the two potential explanations[41,54,55]. Furthermore, the study indicated the important role of GC responses in booster vaccinations, which meant that a good GC reaction inducer could be a good booster by enhanced immunologic protection efficacy. It seems that Th1-biased immune responses would be more protective in SARS-CoV-2 vaccine immunizations[56]. Consistent with that, a specific booster vaccination that can remodel the existing immune profiles and modify to a Th1-biased immune response showed better performance in the study.

In the live virus challenge studies, consistent to the above results, boosting with Ad5-nCoV, S_{WT}-2P, or $S_{Omicron}$-6P exhibited the best protective efficacies against Omicron BA.1 infection in hamsters. No live virus was detected in the tissues of hamsters receiving these boosters. These results indicated the advantages of Ad5-nCoV, S_{WT}-2P, and $S_{Omicron}$-6P as booster vaccinations in protecting against Omicron variants. However, there are some limitations to our study. (1) We chose some historical vaccines as typical types of vaccines, but we did not conclude that the same types of vaccines were generated by different producers in the animal experiments. (2) SARS-CoV-2 Omicron evolved a number of subvariants (such as XBB and BQ.1.1) with increased transmissibility and immunity escape potential compared to the historical BA.1 strain, but the neutralizing activities and protective efficacies against these viruses after different booster vaccinations were not determined due to the lack of viral strains. (3) No significant differences were observed in the BA.1 challenge studies between Omicron-specific ($S_{Omicron}$-6P) and non-Omicron-specific vaccines (Ad5-nCoV and S_{WT}-2P) when used as boosters. We propose that vaccines expressing the spike protein *in vivo* can induce stronger specific GC responses and cross-reactive memory T/B cell-mediated immune responses against Omicron infections. In addition, the results could be partly attributed to the reduced pathogenicity of Omicron variants. Utilizing a higher infection dose of BA.1 and a nonhuman primate (NHP) model could provide more effective insights. Further studies exploring the specific underlying mechanisms are warranted.

Taken together, our studies in mice and hamsters suggest optimal prime-boost strategies for combating SARS-CoV-2 Omicron infections. More importantly, the protective efficacies of the vaccines in booster vaccinations are highly related to GC reactions in secondary lymphatic organs. This study coupled with other vaccine efforts could have impacts on the design of the next generation of vaccines against coronavirus and other pathogens.

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Author contributions

Yi Wu: Writing − original draft, Methodology, Investigation, Data curation, Conceptualization. Xiaoying Jia: Investigation, Formal analysis, Data curation. Namei Wu: Methodology, Investigation, Formal analysis, Data curation. Xinghai Zhang: Methodology, Investigation, Data curation. Yan Wu: Investigation, Data curation. Yang Liu: Methodology, Investigation. Minmin Zhou: Validation, Methodology. Yanqiong Shen: Methodology, Investigation. Entao Li: Methodology. Wei Wang: Writing − review & editing, Resources, Methodology, Funding acquisition. Jiaming Lan: Writing − review & editing, Supervision, Resources, Funding acquisition. Yucai Wang: Writing − review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. Sandra Chiu: Writing − review & editing, Writing − original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Conflicts of interest

Yucai Wang is the inventor on pending patent applications related to the Omicron mRNA vaccine. The other authors declare no competing interest.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2024.12.030.

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