Title:

Three-dose vaccination-induced immune responses protect against SARS-CoV-2 Omicron-BA.2

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Summary

The ongoing outbreak of SARS-CoV-2 Omicron BA.2 infections in Hong Kong, the world model city of universal masking, has resulted in a major public health crisis. In this study, we investigate public servants who had been vaccinated with two dose (82.7%) or three dose (14%) of either CoronaVac (CorV) or BNT162b2 (BNT). During the BA.2 outbreak, 29.3% vaccinees were infected. Three-dose vaccination provided protection with lower incidence rates of breakthrough infections (2×BNT 49.2% vs 3×BNT 16.6%, $p<0.0001$; 2×CorV 48.6% vs 3×CoV 20.6%, $p=0.003$). The third heterologous vaccination showed the lowest incidence (2×CorV+1×BNT 6.3%). Although BA.2 conferred the highest neutralization resistance compared with variants of concern tested, the third dose vaccination-activated spike-specific memory B and Omicron cross-reactive T cell responses contributed to reduced frequencies of breakthrough infection and disease severity. Our results have implications to timely boost vaccination and immune responses likely required for vaccine-mediated protection against Omicron BA.2 pandemic.

Keywords

SARS-CoV-2; The third dose vaccination; Omicron; BA.2; Breakthrough infection; BNT162b2; CoronaVac; Neutralizing antibody; T cell response

Introduction

To fight the ongoing SARS-CoV-2 pandemic, over 10 billion doses of COVID-19 vaccines under emergency use authorization (EUA) have been administered globally, which has reduced the rates of hospitalization, disease severity and death significantly (Baden et al., 2021; Polack et al., 2020; Tanriover et al., 2021; Voysey et al., 2021; Xia et al., 2021). Unfortunately, the emergence of variants of concern (VOCs), especially the Omicron variants, have threatened the vaccine efficacy substantially (Abu-Raddad et al., 2021). We recently reported that significantly waned anti-Omicron neutralizing antibody and T cell responses especially among CoronaVac-vaccinees might pose a risk to vaccine-breakthrough infections in Hong Kong (Peng et al., 2022a). Although the third heterologous BNT162b2 vaccination after 2-dose CoronaVac generated high neutralizing antibody responses against ancestral and Omicron BA.1 than the third homologous CoronaVac booster (Cheng et al., 2022a; Perez-Then et al., 2022), vaccine efficacy and corelates of immune protection against

the major circulating Omicron BA.2 remains to be investigated (Cheng et al., 2022b; Sette and Crotty, 2021; Zhou et al., 2020).

Results

In our survey of 7247 public servants who work for Hong Kong government in January 2022 right before the Omicron BA.2 outbreak, 5995 (82.7%) and 1012 (14%) study subjects had received two and three doses of vaccinations, respectively, resulting in an overall vaccination rate of 96.7%. During the recent fifth wave of COVID-19 in Hong Kong since the end of January 2022 (Cheng et al., 2022b), 481 (6.6%) subjects joined our follow-up study. These subjects had received 2-dose BNT162b2 (2×BNT, n=169), 3-dose BNT162b2 (3×BNT, n=175), 2-dose CoronaVac (2×CorV, n=37), 3-dose CoronaVac (3×CorV, n=68) or a heterologous booster dose of BNT162b2 after two prior doses of CoronaVac (2×CorV+1×BNT, n=32) (Table 1). Among these 481 subjects, a total of 141 (141/482, 29.3%) BA.2 infections were confirmed by governmental reverse transcriptase-polymerase chain reaction (RT-PCR) or lateral flow-based rapid antigen test (RAT) during the study period. Gender difference in infection was not observed. Patients in 2×BNT were relatively younger than 3×BNT (2×BNT vs 3×BNT: median 32 years vs median 40 years, p<0.0001), likely indicating the hesitation for taking the third dose BNT162b2 among younger people. Patients received either two dose or three dose of BNT162b2 were significantly younger than subjects received CoronaVac (2×CorV vs 2×BNT: median 45.5 years vs median 32 years, p<0.0001; 3×CorV vs 3×BNT: median 49 years vs median 40 years, p=0.045) (Table 1 and Table S1), in line with elder people's preference of taking CoronaVac with less side effects. Moreover, shorter median interval between latest vaccination and symptom onset was noticed for 3×BNT than 3×CorV and for 2×BNT and 2×CorV (2×BNT vs 3×BNT: median 227 days vs median 45 days, p<0.0001; 2×CorV vs 3×CorV: median 224 days vs median 53.5 days, p<0.0001), respectively (Table 1 and Table S1).

Infections were found in both 2×BNT and 2×CorV groups with comparable incidence rates of 49.2% (78/169) and 48.6% (18/37) (p=0.783), respectively. The third dose vaccination groups, however, showed significantly lower incident rates of 16.6% (3×BNT, p<0.0001), 20.6% (3×CorV, p=0.003) compared with 2×BNT and 2×CorV groups, respectively. The third heterologous BNT162b2 vaccination group (2×CorV+1×BNT) exhibited the lowest incident rate of 6.3% compared with the 2×CorV group (p<0.0001). No statistical significance was found in incident rates between any 3 dose groups (Table 1 and Table S1). Notably, most infected subjects developed mild disease presenting three major symptoms including fever, cough and/or sore throat. Asymptotic infections were only found in 2×BNT and 3×BNT

groups with low frequencies of 3.8% (3/78) and 3.4% (1/29), respectively (Table 1). The hospitalization rate was lower for 3×BNT (3.4%) than that of 3×CorV (21.4%) patients. One median day shorter illness was observed in 2×BNT (7 days) and 3×BNT (7 days) than those of 2×CorV (8 days) and 3×CorV (8 days) without statistical significance. There was no significant difference in terms of duration time for viral antigen conversion to negativity between any groups (Table 1 and Table S1). These results suggested that the third dose vaccination by both BNT162b2 and CoronaVac reduced the incident rate of BA.2 infection and the third dose of BNT162b2 vaccination achieved a slightly lower hospitalization rate and sickness duration compared with the third CoronaVac.

To characterize the third dose vaccination-induced immune responses, we were able to obtain 92 blood samples donated by subjects in the same cohort including 42 from 3×BNT, 29 from 3×CorV and 21 from 2×CorV+1×BNT at median 23, 55 and 47 days after the last vaccination, respectively, on January 27, 2022 (Table S2). Considering that memory B cell responses contribute to long-term immunological protection against COVID-19, we measured the frequency of spike (S)-specific B cells (gated on $CD19^+$ IgG^+ IgD^- cells) after the third dose vaccination (Figure 1A). We found that the third dose of BNT162b2, either 3×BNT (mean 2.78%) or 2×CorV+1×BNT (mean 1.33%), induced significant higher frequency of S-specific B cells than 3×CorV (mean 0.35%) (Figure 1B). Significant boost effect of S-specific B cells was not observed by the third dose of CoronaVac (Figure 1C). Moreover, S-specific B cells elicited by the third dose of BNT162b2 reached the peak around 4-6 weeks and lasted for 3 months with a higher mean frequency than that of 3×CorV (Figure 1D). Further phenotypical analysis (Figure 1E) showed that the third dose of BNT162b2 resulted in elevated frequency of activated memory B cells (AM, $CD21^-CD27^+$) compared with 2×BNT or 2×CorV whereas the third dose of CoronaVac enhanced the frequency of resting memory (RM) B cells (Figure 1F). The frequency of AM reached the peak at 4 weeks after the third booster and subsequently declined, accompanied by proportional increase of RM, in both 3×BNT and 2×CorV+1×BNT groups whereas AM remained unchanged in the 3×CorV group around two months (Figure 1G). These results demonstrated that S-specific memory B cells were predominantly activated by the third dose of BNT162b2 but insignificantly by the third dose of CoronaVac.

We then measured the titer and breadth of neutralizing antibodies (NAbs) against a full panel of current SARS-CoV-2 VOCs including D614G, Alpha, Beta, Delta and three Omicron variants (BA.1, BA.1.1 and BA.2) using the pseudovirus assay as we previously described (Peng et al., 2022a). We included data from subjects who

previously received 2×BNT or 2×CorV at the activation (0-4 weeks) and memory (4-12 weeks) periods were used for comparison (Peng et al., 2022a). In line with significantly higher frequencies of S-specific B cells, both 3×BNT- and 2×CorV+1×BNT-vaccinees displayed significantly stronger geometric mean 50% neutralizing titers (GMT) than 3×CorV against all variants tested (Figure 1H). The overall fold of neutralization resistance followed the order of Alpha < Beta < Delta < BA.1 < BA.2 in all three vaccine groups. Omicron BA.2 was the most resistant variant with 4.67-, 4.74- and 6.49-fold reductions in 3×BNT, 3×CorV and 2×CorV+1×BNT groups, respectively (Figure S1A). Moreover, based on the magnitude of geometric mean titer (GMT), 59% of 3×BNT and 48% of 2×CorV+1×BNT vaccinees had high neutralization activity (>1280) against D614G whereas none of 3×CorV vaccinees showed similar activities (Figure S1B). For BA.2, neither 3×BNT nor 2×CorV+1×BNT vaccinees had high neutralization activity, but 40% of 3×BNT and 29% of 2×CorV+1×BNT vaccinees still had medium neutralization activity (321-1280). Strikingly, 66% of 3×CorV vaccinees showed undetectable neutralization antibodies against BA.2. Furthermore, the third dose of BNT162b2 induced significant higher NAb titers against all VOCs in 3×BNT and 2×CorV+1×BNT groups compared to the 2-dose groups at both 0-4 weeks (activation) and >4 weeks (memory) after vaccination (Table S3). In contrast to weak boost effects by the third dose of CoronaVac in the 3×CorV group, 10.1-12.9-fold and 10.9-15.5-fold enhancements against Omicron variants at activation and memory phases were observed after the third heterologous BNT162b2 (2×CorV+1×BNT), like the boost effects in the 3×BNT group (Table S3). Besides significantly increased NAb titers, the responder rates of anti-Omicron variants raised from 33% to 96%, from 0% to 78% and from 0% to 100% at 0-4 weeks, and from 0% to 100%, from 0% to 50% and from 0% to 100% at >4 weeks in 3×BNT, 3×CorV and 2×CorV+1×BNT groups, respectively, post the last vaccination. Consistently, BA.2 exhibited the most resistant profile to the boost effect, especially in 3×CorV (Table S4). These results demonstrated that the third heterologous BNT162b2 vaccination in 2×CorV+1×BNT made significant improvement not only bringing the anti-Omicron responder rate to 100% but also enhancing NAb titers close to 3×BNT at both 0-4 and >4 weeks (Table S3 and Table S4).

T cell responses may play an important role in control of SARS-CoV-2 infection (Peng et al., 2022b; Sette and Crotty, 2021; Zhou et al., 2020), CD4 and CD8 T cell responses to viral Spike and nucleocapsid protein (NP) were determined by measuring intracellular IFN-γ, TNF-α and IL-2 (Figure 2A and 2E). Since many amino acid mutations were found in Omicron S, we measured ancestral and Omicron S-specific T

cell responses in parallel. Significantly higher mean frequencies of S-specific IFN-γ^+ CD4 T cells were found in 3×BNT (ancestral: 0.069% and Omicron: 0.079%) than those in 3×CorV (ancestral: 0.024% and Omicron: 0.026%) and in 2×CorV+1×BNT (ancestral: 0.034% and Omicron: 0.030%) (Figure 2B). No significant differences of S-specific IFN-γ^+ and polyfunctional CD4 T cells were found between ancestral and Omicron (Figure 2B and 2C). There were also no significant differences between 2×BNT and 3×BNT, between 2×CorV and 3×CorV, and between 2×CorV and 2×CorV+1×BNT at both activation (Figure 2D, left) and memory periods (Figure 2D, right). In addition, significantly higher mean frequencies of S-specific IFN-γ^+ CD8 T cells were found in 3×BNT (ancestral: 0.082% and Omicron: 0.096%) than those in 3×CorV (ancestral: 0.017% and Omicron: 0.015%) and in 2×CorV+1×BNT (ancestral: 0.021% and Omicron: 0.013%) (Figure 2F). The frequency of S-specific polyfunctional CD8 T cells were relatively higher in 3×BNT than those in 3×CorV and 2×CorV+1×BNT (Figure 2G). Significant increase of S-specific IFN-γ^+ CD8 T cells was not observed in 3×BNT than that in 2×BNT at acute (Figure 2H, left) but observed at the memory period (Figure 2H, right). CoronaVac, however, did not show similar activities. Besides spike, weak nucleocapsid protein (NP)-specific IFN-γ^+ CD4 and CD8 T cells were observed in 3 groups although more CD4 T cell responders (67%) were found in 3×CorV (Figure S2), indicating the pre-existing of cross-reactive NP-specific T cell responses in unexposed donors (Le Bert et al., 2020). Considering that S-specific circulating T follicular helper cells (cTFH, CD45RA$^-$ CXCR5$^+$CD4$^+$) are associated with potent NAb responses (Juno et al., 2020), we found that the frequency of IFN-γ^+ cTFH cells were low with mean 0.032-0.047%, 0.01-0.022% and 0.017-0.059% in 3×BNT, 3×CorV and 2xBNT+1xCorV groups, respectively (Figure S3A-S3B). However, the responder rate was higher in 3×BNT and 2×BNT+1×CorV than that of 3×CorV (7-10%) (Figure S3B). These results indicated that the third dose of BNT162b2 vaccination significantly improved S-specific IFN-γ^+, polyfunctional and memory T cells in 3×BNT but not in 2×CorV+1×BNT and 3×CorV.

Immune correlation analysis was subsequently conducted for 23 antigen-specific measurements together with gender, age, time interval between second and third vaccinations, sampling time after third dose of vaccination and infection. Age and gender did not show significant correlation with any immune responses in either 3×BNT or 2×CorV+1×BNT groups (Figure 3A-3C) but they were positively correlated with S-specific B and T cell responses in the 3×CorV group (Figure 3B). Consistent with the kinetics of AM proportion, S-specific AM correlated negatively with the time after third dose of vaccination in the 2×CorV+1×BNT group (Figure

3C). Positive correlations between S-specific B cells and NAbs were observed in both 3×BNT and 2×CorV+1×BNT groups while the RM was positively associated with NAbs in the 3×CorV group (Figure 3A-3C, green rectangle). Consistently, significant positive correlations were found in NAbs titers against all 7 viral variants (Figure 3A-3C, purple triangles). Since the third dose vaccination by BNT162b2 or CoronaVac did not improve S-specific CD4 T cell responses among 2×CorV vaccinees, positive correlations among S-specific CD4 T cells, S-specific B cells and NAbs were limited to the 3×BNT group (Figure 3A, red rectangle). However, positive correlations between S-specific cTFH cells and NAbs were observed in 3×BNT and 2×CorV+1×BNT, but not in 3×CorV (Figure 3A-3C, yellow rectangles). Of note, in the 3×BNT group, Omicron S-specific CD4 T cell and cTFH responses exhibited stronger correlation with S-specific B cell and the broadly NAbs than those with ancestral S-specific CD4 T cell and cTFH responses (Figure 3A, yellow rectangle).

We then combined all three groups for overall analysis (Figure 3D). Strong positive correlations were consistently found in NAbs titers against all 7 viral variants (Figure 3D, purple triangle). Both age and S-specific RM B cells were negatively correlated with NAb activity (Figure 3D, purple rectangle) whereas S-specific AM B cells were positively correlated with neutralizing activity (Figure 3D, green rectangle). Moreover, the frequency of S-specific AM B cells was significantly lower in infected vaccinees than uninfected vaccinees before vaccine-breakthrough infection (Figure 3E) whereas the anti-BA.2 NAb titer did not achieve statistical significance (Figure 3F). Notably, few vaccinees (2/13, 15.4%) with NAb titer higher than 1:320 became infected. We further analyzed the relationships between immune responses and clinical characteristics among our study subjects who were subsequently infected by BA.2 (Figure 3G). NAb titer was negatively correlated with hospitalization rate (Figure 3G, purple rectangle), indicating the importance of NAb in reducing COVID-19 severity. Age was positively correlated with viral negative conversion time, suggesting a longer viral clearance time among older patients (Figure 3G, black square). Notably, IFN-γ^+ CD4 T cells were negatively associated with age and viral negative conversion time (Figure 3G, red squares). In addition, hospitalization was negatively correlated with the interval between second and third dose of vaccinations and with the interval between third dose of vaccination and symptom onset, likely suggesting the importance of the optimal timing for the third dose vaccination (Figure 3G, black rectangle). These results demonstrated that third dose vaccination-induced NAbs and T cell response contributed to reducing risk of severe clinical outcomes after infection.

Discussion

Clinical trials have demonstrated that a third heterologous booster vaccination by EUA SARS-CoV-2 mRNA vaccines (BNT162b2 and mRNA-1273) increased neutralizing antibody titer accompanied by better prevention and lower disease severity than the initial two doses with BBIP-CorV or CoronaVac during the Gamma and Delta epidemics (Accorsi et al., 2022; Cerqueira-Silva et al., 2022; Costa Clemens et al., 2022; Moghnieh et al., 2021; Zeng et al., 2021). After the emergence of the Omicron variants, some cohort studies reported that Omicron BA.1 infection was associated with milder disease and shorter duration of clinical symptoms than Delta infection (Davies et al., 2022; Houhamdi et al., 2022; Jassat et al., 2022; Kim et al., 2022; Maslo et al., 2022; Menni et al., 2022; Wolter et al., 2022). The third vaccination was helpful in reducing the infection and hospitalization rates during the Delta and Omicron BA.1 prevalence in other countries (Accorsi et al., 2022; Thompson et al., 2022; Yoon et al., 2022). Till now, the association between immune responses induced by the third vaccination and Omicron BA.2 breakthrough infection remains unknown. In this study, we investigated immune responses of vaccinees after they received the third vaccination right before the explosive fifth wave of SARS-CoV-2 epidemic caused by Omicron BA.2 in Hong Kong (Cheng et al., 2022b). We also followed up the infection status and clinical outcomes of our study subjects during the wave period. We found that the third dose of either BNT162b2 or CoronaVac led to significantly lower infection rates than those who received the standard 2-dose vaccination regimen, particularly in the heterologous 2×CorV+1×BNT group. Furthermore, the third BNT162b2 resulted in significantly higher rates of asymptomatic and lower rates of hospitalization than 3×CorV group. Our findings, therefore, provided critical knowledge on understanding the role of third vaccination-induced immune responses in protection against the globally spreading Omicron BA.2 infections.

Omicron BA.2 has higher transmissibility and immune evasion than Omicron BA.1 (Barnes et al., 2020; Sho Iketani et al., 2022), explaining its rapid spread in Hong Kong and other places (Lyngse et al., 2022; Yamasoba et al., 2022). Since the end of January 2022, BA.2 has quickly dominated the fifth wave of SARS-CoV-2 epidemic in Hong Kong, where the universal masking policy remains unchanged, with a shorter doubling time of 1.28 days than 1.6-2.8 days of BA.1 (Cheng et al., 2022b). BA.2 shares 21 mutations in the S protein with BA.1. Although Q496S and N501Y mutations are missing in the BA.2 S-BRD domain, unique S371F, T376A, D405N and R408S mutations have been found (Sho Iketani et al., 2022). Due to these mutations, we and others (Sho Iketani et al., 2022; Yu et al., 2022) demonstrated that NAb titers against BA.2 showed 0.97-1.18 and 1.14-1.42 time lower than those against BA.1 at

0-4 weeks and >4 weeks after third vaccination by BNT162b2 or CoronaVac. Also, we consistently found that BA.2 confers the highest NAb resistance compared with other VOCs including BA.1 and BA.1.1. While 58-71% and 29-40% BNT162b2 booster recipients had low (IC_{50}: 20-320) and median (IC_{50}: 321-1280) NAbs against BA.2, 66% CoronaVac booster recipients had undetectable (IC_{50}<20) NAbs. Surprisingly, although the third BNT162b2 vaccination boosted higher anti-BA.2 NAb titer and responder rate as well as more S-specific T cell responses than the third CoronaVac, there was no significant difference in incidence of breakthrough infections between 3×BNT and 3×CorV. To this end, we believe that, although low amounts of NAbs and S-specific T cell responses were observed among 3×CorV vaccinees, the prior two doses of CoronaVac likely primed both B and T cell memory responses well. This hypothesis was then supported by the significantly elevated NAbs and S-specific T cell responses among 2×CorV+1×BNT vaccines after they received the third vaccination with BNT162b2. Moreover, comparable neutralizing activity were reported in patients with breakthrough infection after two doses of inactivated vaccines and individuals who received a third heterologous vaccination with AZD1222 (Suntronwong et al., 2022), suggesting that breakthrough infection recalled and boosted the antibody responses-induced by inactivated vaccines. It is, therefore, conceivable that BA.2 infection might also activate similar immune responses among 2×CorV vaccinees for protection. Such BA.2 infection-mediated immune activation might be even more profound among 3×CorV vaccinees, resulting in significantly reduced incidence and hospitalization rates compared with 2×CorV vaccinees. Therefore, when all vaccinees were analyzed together, we found that S-specific activated memory B cell subset was a significant factor in preventing BA.2 infection because these AM B cells could differentiate into long-lived plasma cells (Lau et al., 2017) and are associated with expansion of memory B cells, and re-establishment of B cell memory after the third vaccination (Goel et al., 2022; Muecksch et al., 2022). Moreover, T cell responses could be another protective factor because they may recognize mutated viral variants without significantly reduced potency (Scully et al., 2017). We found that both BNT162b2 and CoronaVac-induced T cell responses cross-reacted to Omicron S peptides with comparable activities to ancestral S (Gao et al., 2022; Keeton et al., 2022). Since S-specific T cells are associate with control and clearance of the ongoing infection (Sette and Crotty, 2021), potent T cell responses correlated with fewer hospitalization among patients who received the third vaccination.

Limitations of the study

There are some limitations in our study. First, we were unable to obtain blood samples

from our subjects after they became infected and quarantined. We, therefore, could not determine the B and T cell activation post BA.2 infection. Nevertheless, vaccine breakthrough infections often recall rapid NAbs and T responses against various VOCs, including Omicron (Collier et al., 2022; Suntronwong et al., 2022; Zhou et al., 2022). Second, most of our infected vaccinees were confirmed infection by self-RAT, thus the effect of different vaccine regimens on controlling viral loads was not determined. It remains necessary to compare the dynamics and magnitudes of recalled immune responses among vaccinees with BA.2 breakthrough infection patients in the future.

In summary, we report that 3×BNT and 3×CorV provided better protection against SARS-COV-2 BA.2 than 2×BNT and 2×CorV. High frequencies of S-specific activated memory B cells and cross-reactive T cell responses induced by the third vaccination are critical for protection and illness reduction during the Omicron BA.2 breakthrough infection.

STAR Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagent should be directed to and will be fulfilled by the Lead Contact, Zhiwei Chen (zchenai@hku.hk).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The study did not generate any unique dataset or code.

EXPERIMENTAL MODELS AND SUBJECT DETAILS

MATERIALS AND METHODS

Human subjects

This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (Ref No. UW 21-452). A total of 481 participants were recruited in this study. Written informed consent and questionnaire of vaccination and infection were obtained from these subjects. Patients provided the information of symptom onset date, type of symptoms, hospitalization, duration of illness and the date of viral negative conversion as summarized in Table 1. Peripheral blood mononuclear cells (PBMCs) from 92 participants who had third vaccination were isolated from fresh blood samples before SARS-CoV-2 infection using Ficoll-Paque density gradient centrifugation in our BSL-3 laboratory at the same day of blood collection. The majority of purified PBMCs were used for immune cell phenotyping whereas plasma samples were subjected to antibody testing. The rest of the cells were cryopreserved in freezing medium (Synth-a-Freeze Cryopreservation Medium, ThermoFisher Scientific) at 5×10^6 cells/mL at −150°C.

Pseudotyped viral neutralization assay

To determine the neutralizing activity of subject' plasma, plasma was inactivated at 56°C for 30 min prior to a pseudotyped viral entry assay. In brief, different SARS-CoV-2 pseudotyped viruses were generated through co-transfection of 293T cells with 2 plasmids, pSARS-CoV-2 S and pNL4-3Luc_Env_Vpr, carrying the optimized SARS-CoV-2 S gene and a human immunodeficiency virus type 1 backbone, respectively. At 48 h post-transfection, viral supernatant was collected and frozen at −150°C. Serially diluted plasma samples (from 1:20 to 1:14580) were incubated with 200 $TCID_{50}$ of pseudovirus at 37°C for 1 h. The plasma-virus mixtures were then added into pre-seeded HEK293T-hACE2 cells. After 48 h, infected cells were lysed, and luciferase activity was measured using Luciferase Assay System kits (Promega) in a Victor3-1420 Multilabel Counter (PerkinElmer). The 50% inhibitory concentrations (IC_{50}) of each plasma specimen were calculated to reflect anti-SARS-CoV-2 potency.

Antigen-specific B cells

To characterize the SARS-CoV-2 Spike-specific B cells, PBMCs from each vaccinee were first stained with an antibody cocktail contained dead cell dye (Zombie aquae), CD3-Pacific Blue, CD14-Pacific Blue, CD56-Pacific Blue, CD19-BV785, IgD-BV605, IgG-PE, CD27-BV711, CD21-PE/Cy7, CD38-Percp/Cy5.5, CD11C-APC/Fire750 and His-tag Spike protein. Cells were then washed with FACS buffer (PBS with 2% FBS) and further stained with the secondary antibodies including APC anti-His and DyLight 488 anti-his antibodies. Stained cells were acquired by

FACSAriaIII Flow Cytometer (BD Biosciences) and analyzed with FlowJo software (v10.6) (BD Bioscience).

Intracellular cytokine staining (ICS)

To measure antigen-specific T cell responses, PBMCs were stimulated with 2 μg/mL Spike peptide pool (15-mer overlapping by 11) from SARS-CoV-2 ancestral or Omicron variant, or 2 μg/mL nucleocapsid protein (NP) peptide pool in the presence of 0.5 μg/mL anti-CD28 and anti-CD49d mAbs (Biolegend). Cells were incubated at 37°C for 9 hours and BFA was added at 3 h post incubation, as previously described (Zhou et al., 2020). PMA/ionomycin stimulation was included as positive control. Cells were then washed with staining buffer (PBS containing 2% FBS) and stained with mAbs against surface markers, including dead cell dye (Zombie aqua), CD3-Pacific Blue, CD4-Percp/Cy5.5, CD8-APC/Fire750, CD45RA-BV711, CCR7-BV785, CXCR5-APC, CCR6-BV605. For intracellular staining, cells were fixed and permeabilized with BD Cytofix/Cytoperm (BD Biosciences) prior to staining with the mAbs against IFN-γ-PE, TNF-α-AF488 and IL-2-PE-Cy7. Stained cells were acquired by FACSAriaIII Flow Cytometer (BD Biosciences) and analyzed with FlowJo software (v10.6) (BD Bioscience). Results were subtracted from percentage of unstimulated control.

Correlation plots and heatmap visualizations

Correlograms plotting the Spearman rank correlation coefficient (r), between all parameter pairs were generated with the corrplot package (v0.84) (Wei and Sikmo, 2017) running under R (v3.6.1) in Rstudio (1.1.456). Spearman rank two-tailed P values were calculated using corr.test (psych v1.8.12) and graphed (ggplot2 v3.1.1) based on *p<0.05, **p<0.01, ***p<0.001.

Statistical analysis

Statistical analysis was performed using PRISM 8.0. For between-group or multiple-group categorical values comparison, two-sided chi-square tests or fisher's exact tests were used. Unpaired Student's t tests were used to compare group means between two groups only. A P-value <0.05 was considered significant.

Supplemental information

The supplemental information includes 4 Tables and 3 Figures.

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Author contributions: Z.C. and R.Z. conceived and designed the study. R.Z. and Z.C. designed experiments, analyzed data, and wrote the manuscript. Z.C., R.Z. X.L., Y.-C.K., H.-Y.K., I.F.-N.H., and K.-Y.Y coordinated donor recruitment and specimen collection. R.Z., N.L., H.H., D.Y., Q.P. prepared the clinical sample. R.Z., N.L. and H.H. performed the flow cytometry analysis. R.Z., N.L. and D.Y. performed pseudoviral neutralization assay. Z.D. did the correlation analysis.

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Figure legends

Figure 1. Activation of spike-specific memory B cells by the third vaccination. (**A**) Representative flow cytometry plots showing staining patterns of SARS-CoV-2 Spike probes on memory B cells (IgD^- IgG^+ $CD19^+$). (**B**) Quantified results depict the percentage of $Spike^+$ B cells in overall 3-dose vaccination recipients. (**C**) Comparisons of $Spike^+$ B cell frequency between 2-dose and 3-dose samples collected within 4 weeks after vaccinations. (**D**) Cross-sectional analysis of Spike-specific B cells by time after third dose vaccination. The connection lines indicate the mean

value. (**E**) Phenotypes of Spike-specific B cells were defined by using CD21 and CD27 markers. (**F**) Pie chart showed the proportion of activated (AM), tissue-like (TLM) memory, intermediate memory (IM) and resting-memory (RM) B cells. (**G**) Cross-sectional analysis of the percentage of AM (upper) and RM (bottom) in Spike-specific B cells by time after third vaccination. The connection lines indicate the mean value. (**H**) Neutralizing antibody titers (IC_{50} represents serum dilution required to achieve 50% virus neutralization) against seven SARS-CoV-2 strains were measured by pseudovirus-based assay among 3×BNT (orange), 3×CorV (blue) and 2×CorV+1×BNT vaccinees (purple) after the third dose vaccination. Numbers under the x-axis indicate the responder rates (IC_{50}>20 termed 'responder'). Each symbol represents an individual donor with a line indicating the mean of each group. Statistics were generated by using 2-tailed Student's t test. *p<0.05; **p<0.01; ***p<0.001.
See also Figure S1 and Table S2-S4.

Figure 2. Spike-specific CD4 and CD8 T cell responses. PBMCs were stimulated with the Spike peptide pools from ancestral or Omicron SARS-CoV-2 prior to intracellular cytokine staining assay. Representative flow cytometry plots showing single positive of IFN-γ^+ or TNF-α^+ or IL-2^+ as well as the polyfunctional cells with ≥2 cytokines among $CD4^+$ (**A**) and $CD8^+$ (**E**) T cells. Paired analysis of the frequencies of IFN-γ-producing $CD4^+$ (**B**) and $CD8^+$ (**F**) T cells as well as the frequencies of polyfunctional $CD4^+$ (**C**) and $CD8^+$ (**G**) T cells to ancestral (open dots) or Omicron (solid dots) Spike among the 3×BNT (orange), 3×CorV (blue) and 2×CorV+1×BNT (purple) vaccinees. The mean frequencies were depicted under the x-axis. The frequencies of IFN-γ-producing $CD4^+$ (**D**) and $CD8^+$ (**H**) T cell to ancestral Spike among 2×BNT, 3×BNT, 2×CorV, 3×CorV and 2×CorV+1×BNT vaccinees at 0-4 weeks (left) and >4 weeks (right) periods after vaccinations. Undetected (UD): % of IFN-γ^+ cells<0.00781%. The green lines in **B**, **C**, **F**, **G** indicate the change of mean responses to ancestral and Omicron Spike. The responses are depicted as the background-subtracted percentage of S-specific T cells (Background subtraction refers to the subtraction of the values of the negative control sample from the peptide-stimulated sample). The responder rates were depicted on the top of dots (% of IFN-γ^+ cells>0.00781% termed 'responder' after subtracted from percentage of unstimulated control). Each symbol represents an individual donor with a line indicating the mean of each group. Statistics were generated by using 2-tailed Student's t test. Ns: no significance, $^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.
See also Figure S2 and S3.

Figure 3. Associations among humoral, cellular immune response and

breakthrough infection features. Correlogram of immune responses among 3×BNT (**A**), 3×CorV (**B**), 2×CorV+1×BNT (**C**) and overall (**D**) vaccinees. Comparison of AM^+ B cell frequency on Spike-specific B cells (**E**) and neutralizing titer against BA.2 (**F**) between uninfected and infected vaccinees. Uninfected vaccinees, infected 3×BNT vaccinees, infected 3×CorV vaccinees and infected 2×CorV+1×BNT vaccinees were presented as grey, orange, blue and purple dots, respectively. Statistics were generated by using 2-tailed Student's t test. $^*p<0.05$. (**G**) Correlogram of clinical characteristics and immune responses among patients. Spearman rank order correlation values (r) are shown from red (-1.0) to blue (1.0); r values are indicated by color and square size. p values are indicated by white asterisks. The green rectangles denote SARS-CoV-2 Spike-specific B cell features, the purple triangle and rectangles denote anti-SARS-CoV-2 variants' neutralizing antibody features, the red rectangles denote the SARS-CoV-2 Spike-specific CD4 T cell features, the yellow rectangle denotes the SARS-CoV-2 Spike-specific cTFH features and the black rectangles denotes clinical characteristic features.

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Table 1. Demographic characteristics of breakthrough infection among 481 vaccinees

Vaccinations	2xBNT (n=169)	3xBNT (n=175)	2xCorV (n=37)	3xCorV (n=68)	2xCorV+1xBNT (n=32)
Infection rate % (No. patient/Total No.)	49.2% (78/169)	16.6% (29/175)	48.6% (18/37)	20.6% (14/68)	6.3% (2/32)
Patients					
Age, year (ranges in parentheses)	32 (24-58)	40 (27-60)	45.5 (24-64)	49 (20-62)	47.5 (37-58)
Gender					
Male (% of all participants)	60 (48.8%)	20 (16.7%)	11 (47.8%)	9 (20%)	2 (7.1%)
Female (% of all participants)	18 (39.1%)	9 (16.4%)	7 (50%)	5 (21.7%)	0 (0%)
Median interval days between latest vaccination and symptom onset (ranges in parentheses)	227 (140-332)	45 (0-111)	224 (4-341)	53.5 (1-109)	25.5 (10-41)
Asymptomatic rate % (No. Asymptomatic patient/No. total patient)	3.8% (3/78)	3.4% (1/29)	0 % (0/18)	0% (0/14)	0% (0/2)
Disease severity	Mild	Mild	Mild	Mild	Mild
Number of symptoms (ranges in parentheses)	4 (0-6)	3 (0-5)	3 (1-6)	3 (1-5)	3.5 (3-5)
Presentation to hospital % (No. patients presenting to hospital/No. total patient)	19.2% (15/78)	3.4% (1/29)	22.2% (4/18)	21.4% (3/14)	50% (1/2)
Duration of illness, days (ranges in parentheses)	7 (0-19)	7 (0-19)	8 (6-21)	8 (2-14)	9.5 (2-17)
The interval days between symptom onset and two negative RAT	8 (1-20)	9 (4-18)	8 (6-12)	9 (3-14)	8 (5-11)

Values displayed are medians, with ranges in parentheses

See also Table S1.

Figure 1

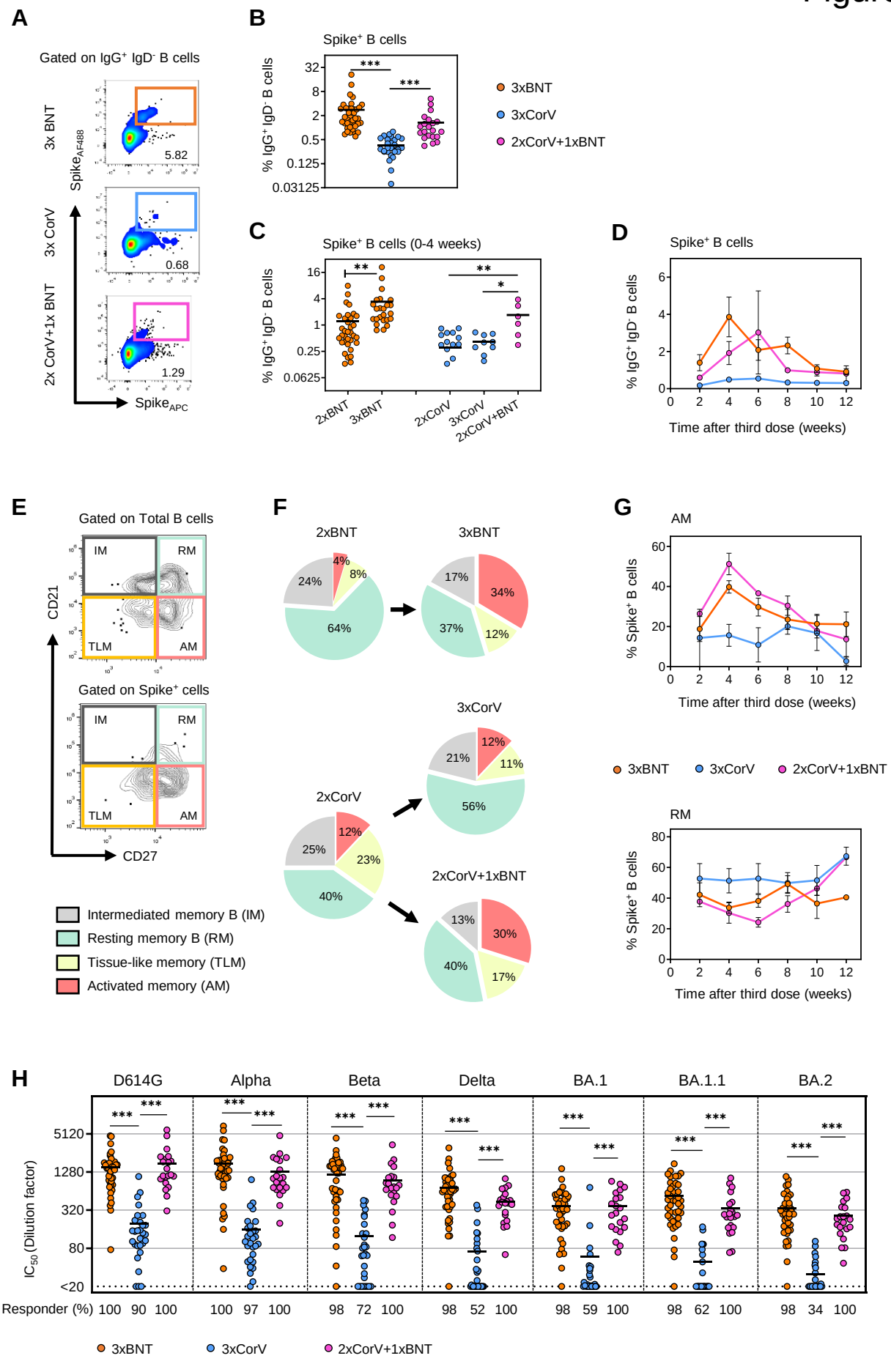


Figure 2

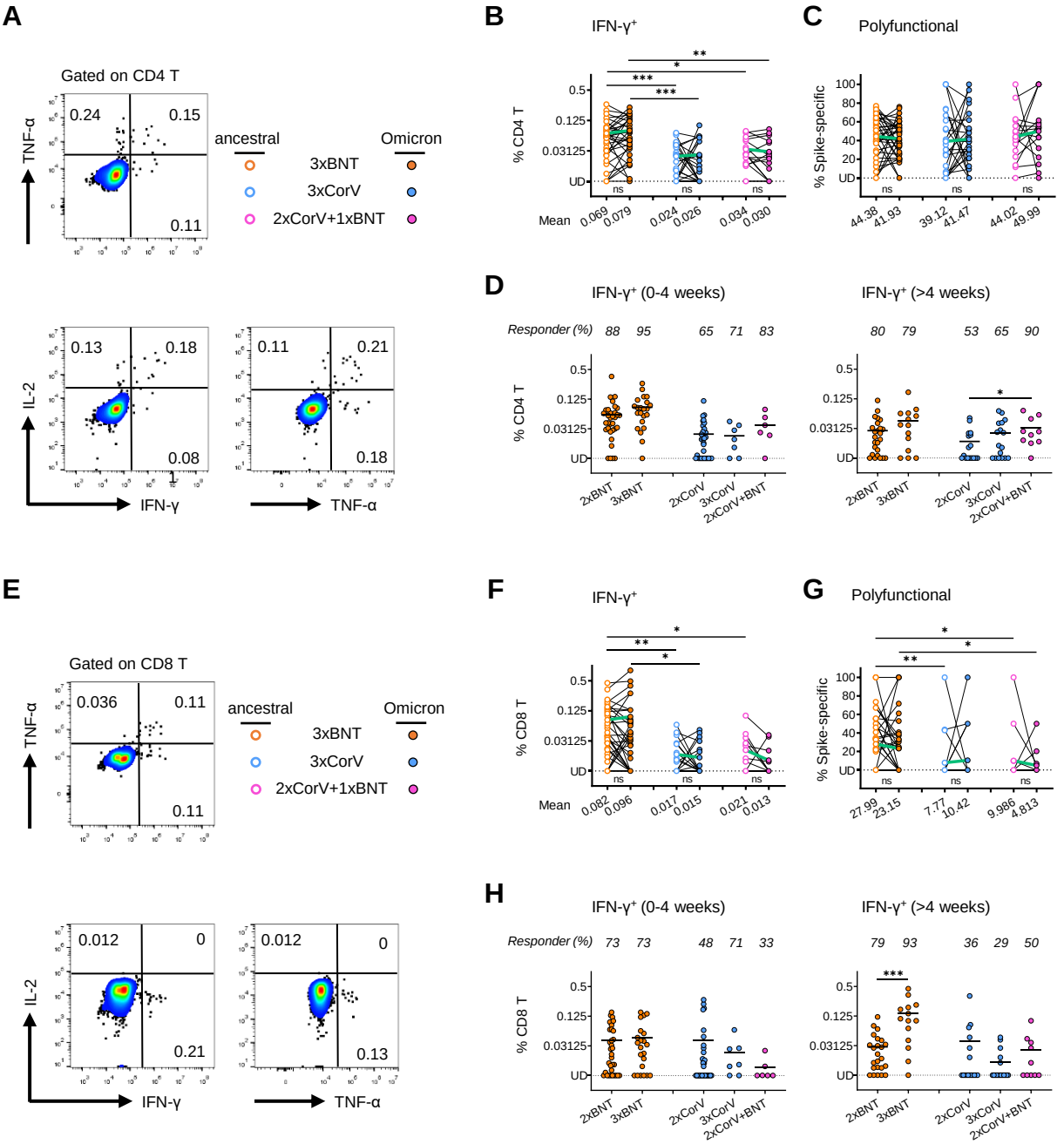
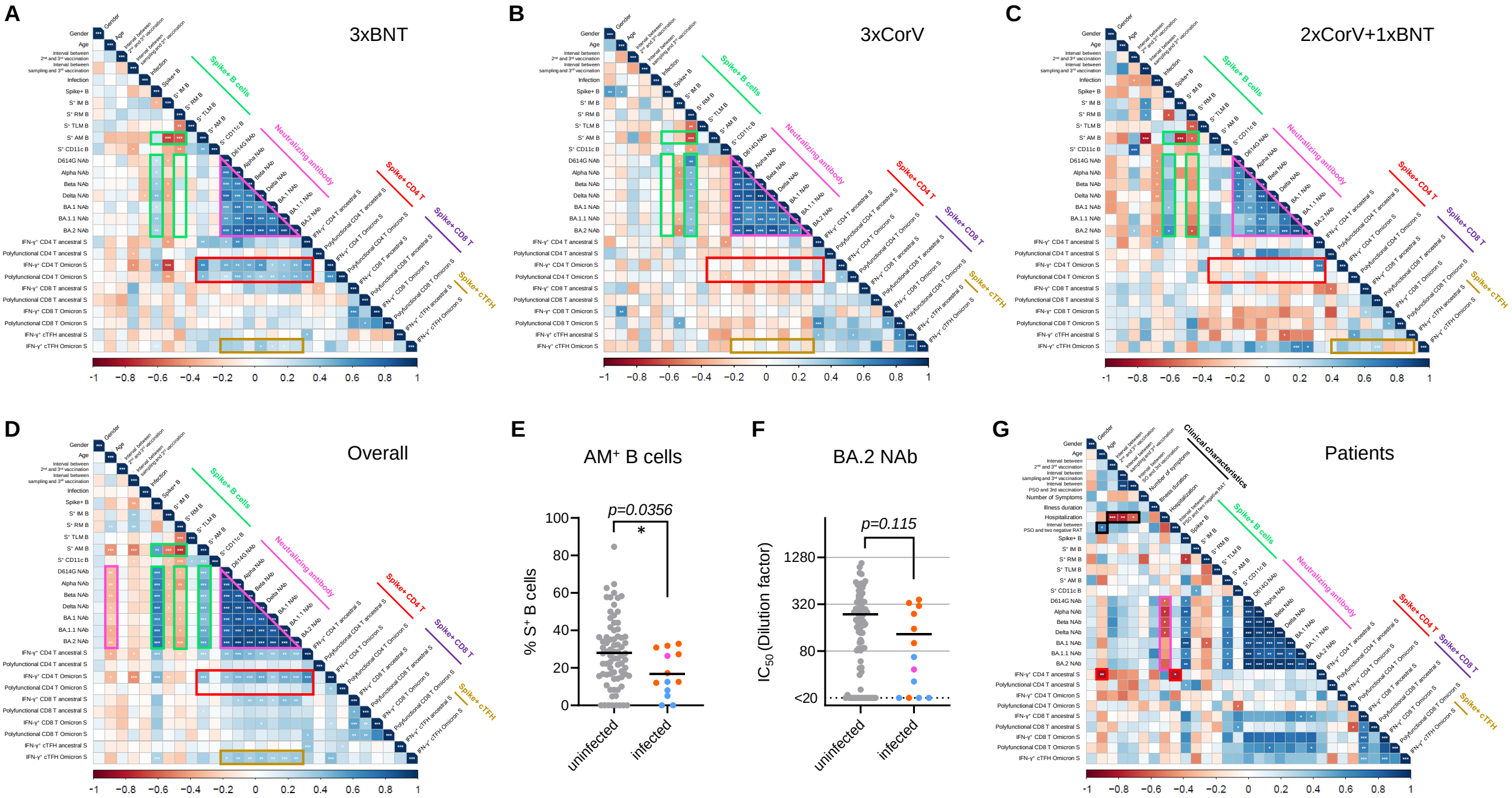


Figure 3


A
3xBNT
B
3xCorV
C
2xCorV+1xBNT
D
Overall
E
AM+ B cells
p=0.0356
% S+ B cells
uninfected
infected
F
BA.2 NAb
p=0.115
IC50 (Dilution factor)
1280
320
80
<20
uninfected
infected
G
Patients
Clinical characteristics
Spike+ B cells
Neutralizing antibody
Spike+ CD4 T
Spike+ CD8 T
Spike+ cTFH
-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1