# Cross-reactive antibody responses to coronaviruses elicited by SARS-CoV-2 infection or vaccination

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**Abstract**

Background The newly emerged SARS-CoV-2 possesses shared antigenic epitopes with other human coronaviruses. We investigated if COVID-19 vaccination or SARS-CoV-2 infection may boost cross-reactive antibodies to other human coronaviruses. Methods Pre- and post-vaccination sera from SARS-CoV-2 naïve healthy subjects who received three doses of the mRNA vaccine (BioNTech, BNT) or the inactivated vaccine (CoronaVac, CV) were used to monitor the level of cross-reactive antibodies raised against other human coronaviruses by enzyme-linked immunosorbent assay. In comparison, convalescent sera from COVID-19 patients with or without prior vaccination history were also tested. Pseudoparticle neutralization assay was performed to detect neutralization antibody against MERS-CoV. Results Among SARS-CoV-2 infection naïve subjects, BNT or CV significantly increased the anti-S2 antibodies against Betacoronaviruses (OC43 and MERS-CoV) but not Alphacoronaviruses (229E). The pre-vaccination antibody response to the common cold human coronaviruses did not negatively impact the post-vaccination antibody response to SARS-CoV-2. Cross-reactive antibodies that binds to the S2 protein of MERS-CoV were similarly detected from the convalescent sera of COVID-19 patients with or without vaccination history. However, these anti-S2 antibodies do not possess neutralizing activity in MERS-CoV pseudoparticle neutralisation tests. Conclusions Our results suggest that SARS-CoV-2 infection or vaccination may potentially modulate population immune landscape against previously exposed or novel human coronaviruses. The findings have implications for future sero-epidemiological studies on MERS-CoV.

Original Article

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1. The authors declare no conflict of interest.
2. Data is available upon request from the corresponding author.
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4. The study was approved by the institutional review board of the Hong Kong West Cluster of the Hospital Authority of Hong Kong (Reference No.: UW20-169) and the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (Reference No.: 2020.229).
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**Background**

The newly emerged SARS-CoV-2 possesses shared antigenic epitopes with other human coronaviruses. We investigated if COVID-19 vaccination or SARS-CoV-2 infection may boost cross-reactive antibodies to other human coronaviruses.

**Methods**

Pre- and post-vaccination sera from SARS-CoV-2 naïve healthy subjects who received three doses of the mRNA vaccine (BioNTech, BNT) or the inactivated vaccine (CoronaVac, CV) were used to monitor the level of cross-reactive antibodies raised against other human coronaviruses by enzyme-linked immunosorbent assay. In comparison, convalescent sera from COVID-19 patients with or without prior vaccination history were also tested. Pseudoparticle neutralization assay was performed to detect neutralization antibody against MERS-CoV.

**Results**

Among SARS-CoV-2 infection naïve subjects, BNT or CV significantly increased the anti-S2 antibodies against Betacoronaviruses (OC43 and MERS-CoV) but not Alphacoronaviruses (229E). The pre-vaccination antibody response to the common cold human coronaviruses did not negatively impact the post-vaccination antibody response to SARS-CoV-2. Cross-reactive antibodies that binds to the S2 protein of MERS-CoV were similarly detected from the convalescent sera of COVID-19 patients with or without vaccination history. However, these anti-S2 antibodies do not possess neutralizing activity in MERS-CoV pseudoparticle neutralisation tests.

**Conclusions**

Our results suggest that SARS-CoV-2 infection or vaccination may potentially modulate population immune landscape against previously exposed or novel human coronaviruses. The findings have implications for future sero-epidemiological studies on MERS-CoV.

## INTRODUCTION

SARS-CoV-2 is a newly emerged human coronavirus (HCoV) that has rapidly swept through the globe and resulted in significant public health and socioeconomic loss. As a member of the genus Betacoronavirus, SARS-CoV-2 possesses shared epitopes with other HCoVs including the common cold Alphacoronaviruses (229E, NL63) and Betacoronaviruses (OC43, HKU1), as well as two newly emerged Betacoronaviruses, SARS-CoV-1 in 2002 and MERS-CoV in 2012 (1, 2). Prior exposures to common cold HCoVs may provide cross-protective humoral or cell-mediated immunity. However, previous studies showed conflicting results on whether pre-existing antibodies towards common cold HCoVs provide cross-protection against SARS-CoV-2 infection or severe disease outcomes (3, 4, 5, 6). On the other hand, pre-existing memory T cells that are likely elicited in response to common cold CoVs have been consistently associated with cross-protection among SARS-CoV-2-exposed healthcare workers or household contacts (7, 8).

Since 2020, 7.7 billion SARS-CoV-2 infected and re-infected cases have been reported to WHO and 13.5 billion doses of COVID-19 vaccines have been administered globally. The extensive exposure to SARS-

CoV-2 through infection and immunization may substantially affect the population immune landscape and susceptibility to other HCoVs. The pre-existing immunity to common cold HCoVs may potentially result in back-boosting of antibodies against the conserved S2 epitopes upon SARS-CoV-2 infection or vaccination as reported previously (9, 10, 11, 12, 13). In addition, de novo antibody response against SARS-CoV-2 may also cross-react with other HCoVs (10, 14). A better understanding on the cross-reactive antibody responses may provide guidance on the development of pan-coronavirus vaccines.

While the spike-encoding mRNA and the whole-virion inactivated vaccines have been the main COVID-19 vaccines administered globally to date, most studies so far have focused on the effect of mRNA vaccines. The mRNA vaccines adopted the pre-fusion conformation of the spike protein while the pre-fusion and post-fusion conformations have been reported for the inactivated vaccines (15). Inactivated vaccines also contain additional SARS-CoV-2 structural proteins that may stimulate humoral or cell-mediated immunity. Using pre- and post- vaccination sera, we firstly compared the cross-reactive antibody responses against different HCoVs elicited by mRNA and inactivated COVID-19 vaccines in individuals previously infection-naïve for SARS-CoV-2. We also separately investigated cross-reactive antibody response in convalescent sera of SARS-CoV-2 patients with or without prior vaccination history.

## METHODS

**Study design** . Sera were collected from healthy subjects enrolled in a longitudinal study for monitoring population immunity to SARS-CoV-2 infection and vaccination in Hong Kong. The study was approved by the institutional review board of the Hong Kong West Cluster of the Hospital Authority of Hong Kong (Reference No.: UW20-169) and the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (Reference No.: 2020.229). Enrolled participants were followed up every 6 months for sera collection and self-reported SARS-CoV-2 infections that have been RT-PCR confirmed. Pre- and post-vaccination sera were collected from age-matched (27-73 years old) individuals who received 3-doses of BioNTech (BNT) (n=20) or CoronaVac (CV) (n = 21) in 2021-2022. The pre-vaccination sera were collected on the day of receiving the first dose of the BNT or CV vaccine, and the post-vaccination sera were collected 4-8 week after receiving the third dose of the vaccine. The convalescent sera from SARS-CoV-2 infected subjects without (n=20) or with vaccination history (n=20 each for BNT and CV) were collected from study participants after self-reported SARS-CoV-2 infections. The archived pre-pandemic sera in 2019 from healthy blood donors (n=20) were used as controls.

**Enzyme-linked immunosorbent assay (ELISA).** MaxiSorp 96-well plates (Thermo Fisher) were coated with 0.1 μg recombinant spike [S1, S2, or full-length S (S1+S2), as indicated] or nucleocapsid proteins of OC43, 229E, SARS-CoV-2, or MERS-CoV (Sino Biological) per well overnight at 4°C. The plates were washed with PBST (PBS containing 0.05% Tween 20) and blocked with blocking buffer (5% non-fat milk in PBST) for 2 hours. Human sera were heat treated at 56°C for 30 minutes and were serially 3-fold diluted from 1:100 to 1:2700 with blocking buffer. Diluted sera (100 μL/ well) were added in duplicate to the plate and incubated for 1 hour, followed by detection using 1:10000 diluted HRP-conjugated goat anti-human IgG secondary antibody (100 μL/ well). TMB substrate (100 μL/ well) (Thermo Scientific) was added to the plate for colorimetric signal formation for 10 minutes and stopped by adding 50 μl/well of 2M sulphuric acid. Plates were read at wavelength of 450 nm for absorbance (OD 450nm). In each ELISA plate, the mean OD 450nm from wells without human sera (n=8 per plate) was calculated as the background. The area under the curve (AUC) were calculated for each serially diluted sera after subtracting the background.

**MERS-CoV spike pseudoparticle neutralisation tests (ppNT).**Luciferase expressing HlV/MERS-RBD pseudoparticles (5 ng of p24) were pre-incubated with serially diluted sera at 4°C for 30 minutes and added to Vero E6 cells in triplicates. Infection was determined by quantifying the firefly luciferase activity at 2 days post-infection (Promega Corporation,) using the Microbeta luminometer (PerkinElmer). The highest serum dilution that gave [?]90% reduction of the maximal luciferase activity (eg. in the absence of antibody) was regarded as the ppNT antibody titre (16).

**Statistical analysis** . The difference of grouped AUC of pre- and post-vaccination against each antigen

within vaccination group was analysed with the Wilcoxon test. The individual AUC difference between pre- and post-vaccination of the same individual was calculated and compared with the AUC difference between two vaccination groups with the Mann-Whitney test. Correlation between AUC ratio of samples against SARS-CoV-2 versus other Human Coronaviruses were analysed using Spearman's rank correlation. The statistical significance of all statistical tests was set at $p < 0.05$.

## RESULTS

**COVID-19 vaccination increased cross-reactive antibody responses against other Betacoronaviruses.** We first compared the pre- and post- COVID-19 vaccination antibody responses towards various HCoVs among individuals who have not been previously infected with SARS-CoV-2. Both BNT (Figure 1A) and CV (Figure 1B) vaccinees showed significant increase of antibody AUC against the spike protein of Betacoronaviruses after vaccination, including SARS-CoV-2 (S1+S2), MERS-CoV (S2), OC43 (S2). In contrast, the post-vaccination sera showed a modest, but significant decrease in median antibody AUC against Alphacoronavirus 229E (S2), possible suggesting a waning antibody response over time. Only CV vaccinees showed significantly increased antibody AUC against the nucleocapsid (N) protein of SARS-CoV-2, MERS-CoV and 229E (Figure 1B). Comparing the BNT and CV vaccination responses (Figure 1C), BNT induced significantly greater S-binding antibody for SARS-CoV-2 (S1+S2) and MERS-CoV (S2), while CV induced significantly greater N-binding antibodies for SARS-CoV-2, MERS-CoV, OC43, and 229E.

Pre-vaccination sera showed high baseline binding antibodies for OC43 and 229E (Figure 1A & 1B). As the baseline antibody titres towards common cold HCoVs may affect the post-exposure antibody response against SARS-CoV-2 (5), we analysed the correlation between the pre-vaccination antibody levels against other HCoVs versus the post-vaccination antibody response against SARS-CoV-2. For BNT vaccinees, their post-vaccination antibody AUC against S of SARS-CoV-2 was not affected by the pre-vaccination antibody against OC43 or 229E (Figure 2A). For CV vaccinees, their post-vaccination antibody AUC against S of SARS-CoV-2 was marginally correlated with the pre-vaccination antibody against 229E-S1+S2 (Spearman's $\rho$=0.46, $p<0.05$) and 229E-S2 (Spearman's $\rho$=0.44, $p<0.05$). The post-vaccination antibody AUC against the N protein of SARS-CoV-2 did not correlate with the pre-vaccination antibody responses against the N proteins of OC43 or 229E. These results suggest that the de novo antibody response against SARS-CoV-2 after vaccination was not negatively affected by the baseline antibody responses against previously exposed HCoV.

The BNT vaccinees' post-vaccination antibody AUC against S of SARS-CoV-2 was positively correlated with the post-vaccination anti-MERS-CoV-S2 (Spearman's $\rho$=0.63, $p<0.01$) and anti-OC43-S1+S2 (Spearman's $\rho$=0.57, $p<0.01$) responses (Figure 2B). For CV vaccinees, the post-vaccination antibody AUC against S of SARS-CoV-2 was positively correlated with anti-OC43-S1+S2 response (Spearman's $\rho$=0.47, $p<0.05$), and their post-vaccination antibody AUC against N of SARS-CoV-2 was positively correlated with anti-MERS-N response (Spearman's $\rho$=0.73, $p<0.001$) (Figure 2B). Taken together, both the mRNA or inactivated COVID-19 vaccines may boost antibody responses against conserved epitopes shared with previously exposed (OC43) or novel (MERS-CoV) Betacoronaviruses.

**SARS-CoV-2 infection increased cross-reactive antibody responses against MERS-CoV.** We further investigated if SARS-CoV-2 infection may similarly boost cross-reactive antibody response against MERS-CoV S and N proteins (Figure 3A). Convalescent sera from SARS-CoV-2 patients without a history of COVID-19 vaccination (n=20), those who have been infected and vaccinated (n=20), and those who were vaccinated and had a breakthrough infection (n=20) were compared with pre-pandemic sera collected from healthy adults in 2019 (n=20). Compared to the pre-pandemic sera (using the mean+3SD AUC value as threshold), 41 out of 60 (71.7%) convalescent sera showed increased antibody against MERS-CoV S2, while 6 of 60 (10%) showed increased antibody against MERS-CoV S1. In regard to the antibody response to the S2 protein of MERS-CoV-2, those who were vaccinated followed by a breakthrough infection generally showed higher AUC than those who were infected without vaccination history, although the differences were not significant. In addition, those who were infected followed by BNT vaccination showed greater anti-S2 antibody response than those who were infected followed by CV vaccination, suggesting that BNT

vaccination may better expand the breath of antibody response than CV vaccination among those who were infected. Compared to the pre-pandemic sera, increase in the anti-N protein antibody AUC was also observed from those who were infected followed by CV vaccination or those who were CV vaccinated followed by breakthrough infection.

**Cross-reactive antibody against MERS-CoV S2 were non-neutralizing.** Pseudoparticle neutralization test (ppNT) was used to evaluate if the cross-reactive anti-S2 antibodies possess neutralizing activity against MERS-CoV (Figure 3B). None, except one subject who was vaccinated with BNT followed by SARS-CoV-2 infection, showed neutralizing antibody at 1:10 dilution using ppNT assay. Despite of non-neutralizing, sera from subjects with CV vaccination followed by infection (mean ± SD % inhibition = 44.6 ± 27.8) showed greater inhibition against MERS-CoV than the sera of pre-pandemic controls (15.3 ± 16.9) (Kruskal-Wallis test, p=0.0435). Taken together, these results suggest that the majority of the cross-reactive anti-MERS-CoV-S2 antibodies detected after vaccination or infection were non-neutralizing.

## DISCUSSION

Majority of the global population have been exposed to SARS-CoV-2 through infection or vaccination to date. As SARS-CoV-2 share common epitopes with other HCoVs, it is anticipated that SARS-CoV-2 exposure may boost cross-reactive antibodies towards other HCoV. Using the pre- and post- vaccination sera of uninfected subjects, we show that both the mRNA vaccine (BNT) and the inactivated vaccine (CV) increased cross-reactive antibodies against the S2 protein of the two Betacoronaviruses, OC43 and MERS-CoV, but not Alphacoronavirus 229E. CV vaccination further boosted anti-N protein antibodies against MERS-CoV and 229E. The antibody response against S2 protein of MERS-CoV were also detected from 41 out of 60 (71.7%) convalescent sera of SARS-CoV-2 patients with or without COVID-19 vaccination history. Our results are in line with a recent study that detected high prevalence of cross-reactive antibodies to spike proteins of viruses in the*Orthocoronavirinae* among the post-COVID-19 population (17). Taken together, these results suggest that SARS-CoV-2 exposure may modulate population antibody response towards other human coronaviruses.

The high level pre-existing antibodies against OC43 and 229E generated from prior infection or vaccination have been shown to impact on the de novo humoral responses against SARS-CoV-2 (5). Among our study subjects, no negative impact was observed in the correlation analysis between pre-vaccination antibody levels against OC43 or 229E and the post-vaccination antibody levels against SARS-CoV-2. Due to various public health and social measures implemented in Hong Kong during COVID-19 pandemic, the activity of various respiratory viruses have been reduced, which may limit recent exposure of our study population to common cold HCoVs. As co-circulation of SARS-CoV-2 and other HCoVs is anticipated, follow up studies are needed to understand how pre-existing immunity may shape the antibody landscapes of various HCoVs.

Both COVID-19 vaccines back-boosted antibodies against the S2 domain of OC43, which aligned with the results reported from previous studies (9, 10, 11, 12, 13). Furthermore, the de novo antibody response to SARS-CoV-2 generated after vaccination or infection were cross-reactive with S2 of MERS-CoV. The conserved region on S2 stem-helix domains across Betacoronaviruses may explain the high cross reactivity of anti-S2 antibodies (18), while other studies identified cross-reactive neutralizing antibody that targets S2 region (19, 20, 21, 22). By comparing the anti-MERS-CoV antibodies from infected subjects with or without vaccination history, we noted that those who have been vaccinated, followed by a SARS-CoV-2 breakthrough infection generally showed higher anti-MERS S2 AUC than those who were infected without vaccination history. These study subjects were vaccinated with the prototype virus followed by infection with the BA.2 Omicron variant in 2022. In addition, higher anti-MERS S2 AUC was detected from those infected followed by BNT vaccination compared to those who were infected without vaccination or those infected followed by CV vaccination. These results suggest that BNT vaccine may stimulate broader antibody response than CV among those who were previously infected. Taken together, these findings have implications for future sero-epidemiological studies on MERS-CoV. While binding antibody responses to MERS-CoV S1 is still likely to be specific for MERS-CoV infection, binding antibody to MERS-CoV S2 should no longer be considered as a specific marker for MERS-CoV infection.

Using the ppNT assay that specifically detect neutralizing antibodies targeting the receptor binding domain (RBD) of MERS-CoV, we showed that the anti-MERS-CoV antibodies were non-neutralizing. However, other neutralising mechanisms such as inhibition of fusion peptide cannot be ruled out (19). Further studies are needed to evaluate if these cross-reactive antibodies possess Fc-mediated effector functions and if they confer protection in vivo.

## ACKNOWLEDGMENTS

This study was supported by the HKU URC Seed Funding for Strategic Interdisciplinary Research Scheme 2021/22, Contract (U01AI151810) of NIAID, National Institutes of Health, USA, and the Theme-based Research Scheme (#T11-705/21-N) of the Research Grants Council of the Hong Kong SAR Government, China.

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## FIGURE LEGENDS

**Figure 1. COVID-19 vaccination increased cross-reactive antibody responses against other Betacoronaviruses.** (A) Pre- and post- vaccination antibody responses of BNT vaccinees (n=20) to the S and N proteins of different human coronaviruses, Wilcoxon test was used to compare the pre and post-vaccination antibody response. (B) Pre- and post- vaccination antibody responses of CV vaccinees (n=21) to the S and N proteins of different human coronaviruses, Wilcoxon test was used to compare the pre and post-vaccination antibody response. (C) Differences between BNT and CV vaccinees in the post-vaccination antibody responses against the S and N proteins of different human coronaviruses, Mann-Whitney test was used to compare antibody responses of BNT and CV vaccinees.

**Figure 2. Correlation between post-vaccination antibody response against SARS-CoV-2 versus antibody response against other human coronaviruses.** (A) Post-vaccination antibody response against SARS-CoV-2 versus pre-vaccination antibody responses against other HCoVs. (B) Post-vaccination antibody response against SARS-CoV-2 versus post- vaccination antibody responses against other HCoVs. The Spearman coefficient were shown, * $p<0.05$, ** $p<0.01$.

**Figure 3. SARS-CoV-2 infection increased cross-reactive antibody responses against MERS-CoV.** (A) Antibody responses against MERS-CoV S1, S2, or N proteins detected from sera of healthy donors collected in 2019 prior to the COVID-19 pandemic (control sera, n=20), subjects infected with SARS-CoV-2 without vaccination history (infected, n=20), subjects infected with SARS-CoV-2 followed by 2-doses CV vaccination (infected + CV, n=10), subjects infected with SARS-CoV-2 followed by 2-doses BNT vaccination (infected + BNT, n=10), subjects vaccinated with 2-doses of CV followed by SARS-CoV-2 infection (CV + infected, n=10), subjects vaccinated with 2-doses of BNT followed by SARS-CoV-2 infection (BNT + infected, n=10). The threshold AUC values (mean+3SD) determined from the pre-pandemic sera

were shown in dotted lines. (B) Neutralizing antibody response against MERS-CoV were determined using the ppNT assay. In addition to the pre-pandemic sera and convalescent sera tested above, we also determined neutralizing antibody responses against MERS-CoV among those who have been vaccinated with 3-doses of BNT (n=20) or CV (n=20) without a history of SARS-CoV-2 infection. Sera were diluted at 1:10 dilution and any sample with [?] 90% inhibition was considered positive.

**Figure 1.**

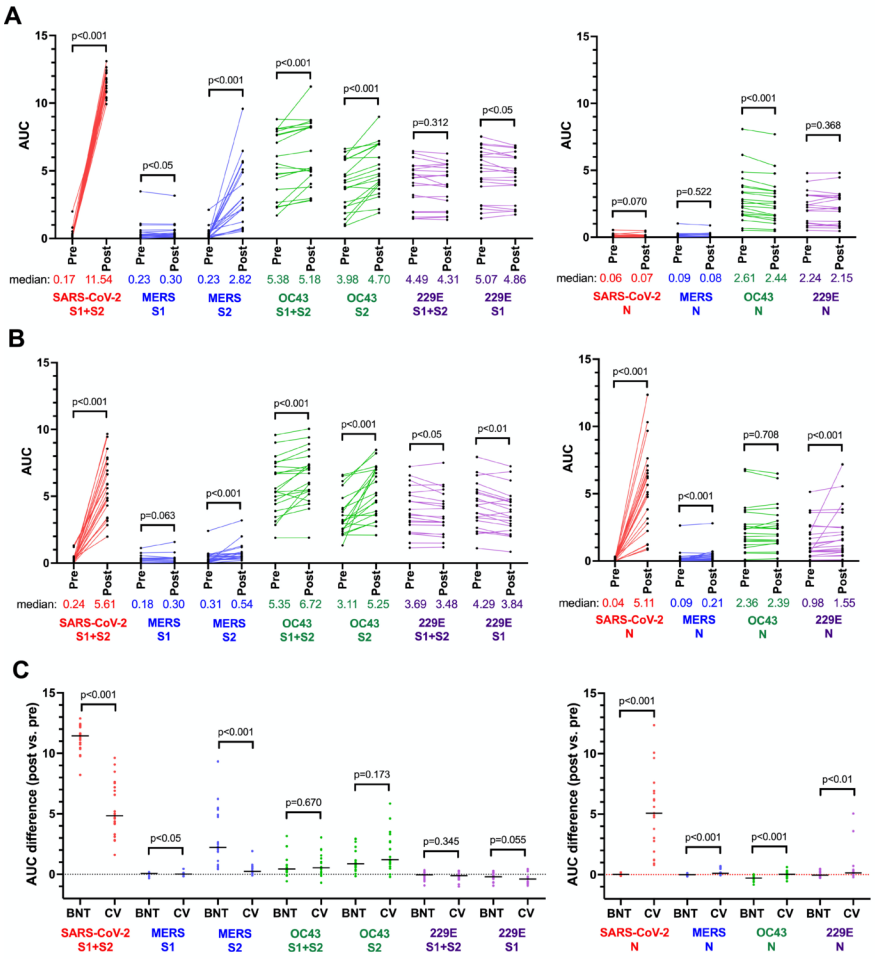


**Figure 2.**

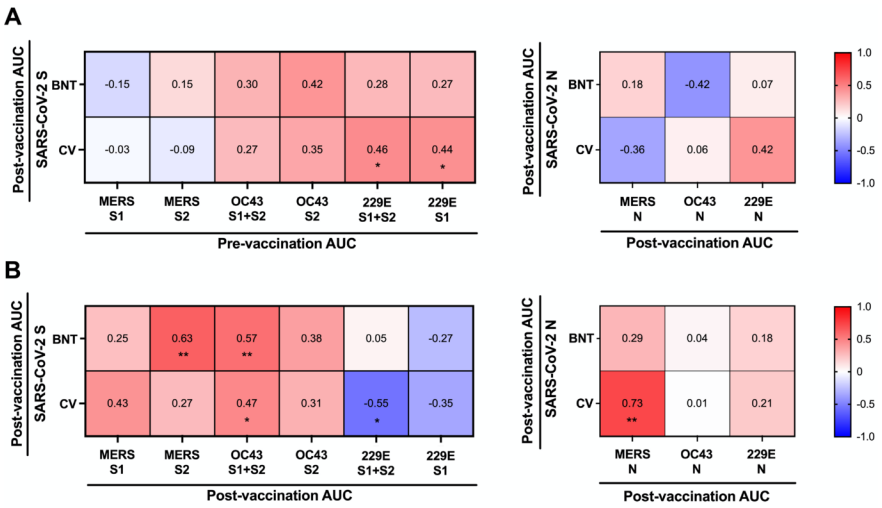


**Figure 3.**