SARS-CoV-2 BA.4/5 Spike recognition and neutralization elicited after the third dose of mRNA vaccine

Alexandra Tauzin[1,2], Debashree Chatterjee[1], Katrina Dionne[1,2], Gabrielle Gendron-Lepage[1], Halima Medjahed[1], Yuxia Bo[3] Josée Perreault[4] Guillaume Goyette[1], Laurie Gokool[1], Pascale Arlotto[1], Chantal Morrisseau[1], Cécile Tremblay[1,2], Valérie Martel-Laferrière[1,2], Daniel E. Kaufmann[1,5], Gaston De Serres[6], Inès Levade[7], Marceline Côté[3], Renée Bazin[4] and Andrés Finzi[1,2,8,*]

[1]Centre de Recherche du CHUM, Montreal, QC, H2X 0A9 Canada
[2]Département de Microbiologie, Infectiologie et Immunologie, Université de Montréal, Montreal, QC, H2X 0A9, Canada
[3]Department of Biochemistry, Microbiology and Immunology, and Centre for Infection, Immunity, and Inflammation, University of Ottawa, Ottawa, ON K1H 8M5, Canada
[4]Héma-Québec, Affaires Médicales et Innovation, Quebec, QC G1V 5C3, Canada
[5]Département de Médecine, Université de Montréal, Montreal, QC, H3T 1J4, Canada
[6]Institut National de Santé Publique du Québec, Quebec, QC, H2P 1E2, Canada
[7]Laboratoire de Santé Publique du Québec, Institut Nationale de Santé Publique du Québec, Sainte-Anne-de-Bellevue, QC H9X 3R5, Canada.

[8]Lead contact
*Correspondence: andres.finzi@umontreal.ca (A.F.)

SUMMARY

The SARS-CoV-2 Omicron BA.4 and BA.5 subvariants have recently emerged, with BA.5 becoming the dominant circulating strain in many countries. Both variants share the same Spike glycoprotein sequence which contains a large number of mutations, raising concerns about vaccine efficacy. In this study, we evaluated the ability of plasma from a cohort of individuals that received three doses of mRNA vaccine to recognize and neutralize the BA.4/5 Spike. We observed that BA.4/5 Spike is markedly less recognized and neutralized compared to the D614G and Omicron BA.2 Spike variants. Individuals who have been infected before or after vaccination present better humoral responses than SARS-CoV-2 naïve vaccinated individuals, thus indicating that hybrid immunity generates better humoral responses against this subvariant.

Keywords: Coronavirus, COVID-19, SARS-CoV-2, Third mRNA vaccine dose, Spike glycoproteins, Humoral responses, Neutralization, BA.4/5

INTRODUCTION

The SARS-CoV-2 Omicron variant BA.1 emerged at the end of 2021 and rapidly became the dominant circulating strain in the world (Viana et al., 2022; WHO). Since its emergence, several sublineages of Omicron rapidly replaced the BA.1 variant due to higher transmission rates. BA.2 became the dominant circulating strain in spring 2022 (CDC, 2022; Elliott et al., 2022), and currently the BA.4 and BA.5 variants [sharing the same mutations in their Spike (S) glycoproteins, named BA.4/5 S in the manuscript], are becoming the dominant circulating strains in several countries (Mohapatra et al., 2022; PHO, 2022; Tegally et al., 2022; Yamasoba et al., 2022).

It was previously shown that poor humoral responses against BA.1 and BA.2 variants were observed after two doses of mRNA vaccine (Muik et al., 2022; Nemet et al., 2022; Yu et al., 2022). We and other reported that an extended interval between the first two doses of mRNA vaccine led to strong humoral responses to several variants of concern (VOCs) including BA.1 and BA.2 after the second dose of mRNA vaccine (Chatterjee et al., 2022; Payne et al., 2021; Tauzin et al., 2022a). However, a third dose of mRNA vaccine led to an increase of humoral responses against these Omicron variants, regardless of the interval between doses (Kurhade et al., 2022; Muik et al., 2022; Tauzin et al., 2022a; Yu et al., 2022). Previous studies also reported that breakthrough infection (BTI) in vaccinated people induced strong neutralizing Abs against VOCs, including BA.1 (Kitchin et al., 2022; Miyamoto et al., 2022). However, recent studies have shown that BA.4/5 appears to be more resistant than BA.1 and BA.2 to vaccination and monoclonal antibodies (Abs) (Qu et al., 2022; Tuekprakhon et al., 2022; Wang et al., 2022).

In this study, we analyzed the ability of plasma from vaccinated individuals to recognize and neutralize pseudoviral particles bearing the BA.4/5 Spike four weeks (median [range]: 30 days [20–44 days]) and four months (median [range]: 121 days [92–135 days]) after the third dose of mRNA vaccine. This study was conducted in a cohort of individuals who received their first two doses with a 16-weeks extended interval (median [range]: 110 days [54–146 days]) and their third

dose seven months after the second dose (median [range]: 211 days [151-235 days]). The cohort included 15 naïve individuals who were never infected with SARS-CoV-2, 15 previously infected (PI) individuals who were infected during the first wave of COVID-19 in early 2020 (before the advent of the alpha variant and other VOCs) and before vaccination, and 15 BTI individuals who were infected after vaccination. All BTI individuals were infected between mid-December 2021 and May 2022, when almost only Omicron variants (BA.1 and BA.2) were circulating in Quebec. Basic demographic characteristics of the cohorts and detailed vaccination time points are summarized in Table 1 and Figure 1A.

RESULTS

Recognition of SARS-CoV-2 Spike variants by plasma from vaccinated individuals

We first measured the ability of plasma to recognize the SARS-CoV-2 D614G, BA.2 and BA.4/5 S in vaccinated naïve, PI and BTI individuals four weeks and four months after the third dose of mRNA vaccine. Spike expression levels of VOCs were normalized to the signal obtained with the conformationally independent anti-S2 neutralizing CV3-25 antibody (Li et al., 2022; Prévost et al., 2021; Ullah et al., 2021) that efficiently recognized all these VOCs Spikes (Figure S1). Four weeks after the third dose of mRNA vaccine, we observed that plasma from PI individuals recognized more efficiently the D614G S than naïve individuals (Figure 1B). We also observed that BTI individuals recognized the D614G S as efficiently as the PI individuals. Four months after the third dose, the level of recognition of the D614G S decreased for the three groups but with a more significant reduction in the naïve group. The same pattern of recognition was observed with the BA.2 S (Figure 1C). In contrast, for the BA.4/5 S, naïve and BTI had the same level of recognition four weeks after the third dose, and this level was significantly lower than for PI individuals (Figure 1D). Four months after the third dose, we observed a significant decrease of the recognition for naïve and PI individuals. In contrast, for the BTI group, the level of

recognition remained stable and reached the same level than for the PI group. With the exception of PI individuals four months after the third dose, we observed that the BA.4/5 S was always significantly less recognized than the D614G and BA.2 S at both time points (Figure 1E-F).

Neutralizing activity of the vaccine-elicited antibodies.

We also evaluated the neutralizing activity against pseudoviral particles bearing these Spikes in the three groups. In agreement with the pattern of S recognition, PI individuals neutralized more efficiently the three variants than naïve individuals four weeks after the third dose (Figure 2A-C). For the BTI group, the level of neutralizing Abs was intermediate between the two other groups, but a significant difference was only observed with PI individuals and the BA.4/5 S. Four months after the third dose, we did not observe significant differences between PI and BTI individuals. In contrast, the naïve group neutralized less efficiently the D614G, BA.2 and BA.4/5 S (Figure 2A-C). Four weeks after the third dose, no significant difference in the level of neutralization was measured between the D614G and BA.2 S for the three groups (Figure 2D). In contrast, the BA.4/5 S was significantly more resistant to neutralization than the D614G S in all groups. Four months after the third dose, weak or no neutralizing activity against BA.2 and BA.4/5 S was detected in most naïve individuals (Figure 2B-C, E). For BTI and PI individuals, although neutralizing activity was higher than in naïve individuals, the BA.4/5 S was also significantly less neutralized than the D614G and the BA.2 S (Figure 2B-C, E).

DISCUSSION

More than two years after its emergence, and although an important proportion of the world population has received several doses of vaccine, the SARS-CoV-2 variants continue to circulate globally. In recent months, new sub-variants of Omicron emerged, carrying increasing numbers of mutations making them more transmissible and resistant to vaccination and monoclonal antibodies treatment (Kurhade et al., 2022; Tuekprakhon et al., 2022; Yamasoba et al., 2022). In agreement with this, we observed that the BA.4/5 S was less efficiently recognized and neutralized than the D614G and the BA.2 S by plasma from individuals who received three doses of mRNA vaccine.

Several studies reported that poor neutralizing activity against VOCs was observed after two doses of mRNA vaccine, but a third dose strongly improved this response (Gruell et al., 2022; Muik et al., 2022; Tauzin et al., 2022a). However, when the second dose of vaccine was administered with an extended 16-weeks interval, higher humoral responses against VOCs (including BA.1 and BA.2) were observed after the second dose of vaccine (Chatterjee et al., 2022), that were not increased by a booster dose (Tauzin et al., 2022a). Therefore, there is no evidence that additional doses of the original SARS-CoV-2 vaccines after the third dose will result in increased responses against VOCs.

The Omicron variants spread more easily in vaccinated individuals than pre-Omicron variants (Garrett et al., 2022; Sun et al., 2022). Interestingly, it was recently shown that previous infection with an Omicron variant prevents reinfection more efficiently than previous infection with a pre-Omicron variant (Altarawneh et al., 2022; Carazo et al., 2022), thus suggesting that new vaccines based on Omicron variants may generate humoral responses more likely to control Omicron sub-variants.

It was previously shown that hybrid immunity due to SARS-CoV-2 infection followed by vaccination confers stronger immune responses than vaccination alone (Carazo et al., 2022; Goel

et al., 2021; Nayrac et al., 2022; Tauzin et al., 2022a). Here we observed that individuals with BTI had the same level of S recognition and neutralization than individuals previously infected supporting the concept that hybrid protection is similar whatever the order of infection and vaccination. However, the durability of these responses remains unknown.

In conclusion, virus recognition and neutralizing activity induced by current mRNA vaccine are low against Omicron subvariants, rapidly decline over 4 months in naïve individuals, and will likely decrease further with future SARS-CoV-2 evolution. There is a need to rapidly develop new generations of vaccines that will elicit broader and less labile protection.

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AUTHOR CONTRIBUTIONS

A.T. and A.F. conceived the study. A.T., D.C., K.D., G.G.L., H.M., G.G, J.P., Y.B., and A.F. performed, analyzed, and interpreted the experiments. A.T. performed statistical analysis. G.G.L., H.M., G.G., M.C, and A.F. contributed unique reagents. L.G., P.A., C.M., C.T., and V.M.-L. collected and provided clinical samples. R.B., G.D.S., D.E.K. and I.L. provided scientific input related to VOCs and vaccine efficacy. A.T. and A.F. wrote the manuscript with inputs from others. Every author has read, edited, and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Recognition of SARS-CoV-2 Spike variants by plasma from naïve, BTI and PI individuals after the third dose of mRNA vaccine.

(**A**) SARS-CoV-2 vaccine cohort design. The yellow box represents the period under study. (**B-F**) 293T cells were transfected with the indicated full-length S from different SARS-CoV-2 variants and stained with the CV3-25 mAb or with plasma from naïve, BTI or PI individuals collected 4 weeks or 4 months after the third dose of mRNA vaccine and analyzed by flow cytometry. The values represent the MFI normalized by CV3-25 Ab binding. (**B-D**) Plasma samples were grouped in two different time points (4 weeks and 4 months). (**E-F**) Binding of plasma collected at 4 weeks (**E**) and 4 months (**F**) post vaccination were measured. Naïve, BTI and PI individuals are represented by red, yellow and black points respectively, undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, non-significant). For all groups, n=15.

Figure 2. Neutralization activity of SARS-CoV-2 Spike variants by plasma from naïve, BTI and PI individuals after the third dose of mRNA vaccine.

(**A-E**) Neutralization activity was measured by incubating pseudoviruses bearing SARS-CoV-2 S glycoproteins, with serial dilutions of plasma for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half maximal inhibitory serum dilution (ID_{50}) values were determined using a normalized non-linear regression using GraphPad Prism software. (**A-C**) Plasma samples were grouped in two different time points (4 weeks and 4 months). (**D-E**) Neutralization activity of plasma collected at 4 weeks (**D**) and 4 months (**E**) post vaccination were measured. Naïve, BTI and PI individuals are represented by red, yellow and black points respectively, undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars

indicate means ± SEM. (* P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns, non-significant). For all groups, n=15.

Table 1. Characteristics of the vaccinated SARS-CoV-2 cohorts

		Entire cohort	Naïve	Breakthrough infection[b]	Previously infected
Number		45	15	15	15
Age		51 (24-67)	54 (24-67)	43 (30-64)	48 (29-65)
Gender	**Male (n)**	17	4	5	8
	Female (n)	28	11	10	7
Days between symptom onset and the 1st dose [a]		N/A	N/A	N/A	288 (166-321)
Days between the 1st and 2nd dose [a]		110 (54-146)	109 (65-120)	110 (54-113)	112 (90-146)
Days between the 2nd and 3rd dose [a]		211 (151-235)	210 (184-227)	215 (151-224)	219 (187-235)
Days between the 3rd dose and 4W		30 (20-44)	32 (21-37)	28 (20-38)	33 (24-44)
Days between the 3rd dose and 4M		121 (92-135)	124 (105-135)	121 (92-131)	119 (111-127)

[a] Values displayed are medians, with ranges in parentheses. Continuous variables were compared by using Kruskal-Wallis tests. p<0.05 was considered statistically significant for all analyses. No statistical differences were found for any of the parameter tested between the different groups. [b] All Breakthrough infection individuals were infected between mid-December 2021 and May 2022, when almost only Omicron variants (BA.1 and BA.2) were circulating in Quebec.

STAR METHODS

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Andrés Finzi (andres.finzi@umontreal.ca).

Materials availability

All unique reagents generated during this study are available from the Lead contact without restriction.

Data and code availability

- All data reported in this paper will be shared by the lead contact (andres.finzi@umontreal.ca) upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact (andres.finzi@umontreal.ca) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics Statement

All work was conducted in accordance with the Declaration of Helsinki in terms of informed consent and approval by an appropriate institutional board. Blood samples were obtained from donors who consented to participate in this research project at CHUM (19.381). Plasmas were isolated by centrifugation and Ficoll gradient, and samples stored at -80°C until use.

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237 **Human subjects**

238 The study was conducted in 15 SARS-CoV-2 naïve individuals (4 males and 11 females; age
239 range: 24-67 years), 15 SARS-CoV-2 breakthrough infection individuals (5 males and 10 females;
240 age range: 30-64 years) infected after the second or third dose of mRNA vaccine, and 15 SARS-
241 CoV-2 previously infected individuals (8 males and 7 females; age range: 29-65 years) infected
242 before vaccination during the first wave of COVID-19 in march-may 2020. This information is
243 presented in table 1. No specific criteria such as number of patients (sample size), gender, clinical
244 or demographic were used for inclusion, beyond PCR confirmed SARS-CoV-2 infection in adults
245 before vaccination for PI group, PCR confirmed SARS-CoV-2 infection or anti-N positive in adults
246 after vaccination for BTI group and no detection of Abs recognizing the N protein for naïve
247 individuals.

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249 **Plasma and antibodies**

250 Plasmas were isolated by centrifugation with Ficoll gradient, heat-inactivated for 1 hour at
251 56°C and stored at -80°C until use in subsequent experiments. Healthy donor's plasmas, collected
252 before the pandemic, were used as negative controls in flow cytometry assays (data not shown).
253 The conformationally independent S2-specific monoclonal antibody CV3-25 was used as a
254 positive control and to normalize Spike expression in our flow cytometry assays, as described
255 (Gong et al., 2021; Jennewein et al., 2021; Prévost et al., 2021; Tauzin et al., 2022b). Alexa Fluor-
256 647-conjugated goat anti-human Abs able to detect all Ig isotypes (anti-human IgM+IgG+IgA;
257 Jackson ImmunoResearch Laboratories) were used as secondary Abs to detect plasma binding
258 in flow cytometry experiments.

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Cell lines

293T human embryonic kidney cells (obtained from ATCC) were maintained at 37°C under 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) (Wisent) containing 5% fetal bovine serum (FBS) (VWR) and 100 µg/ml of penicillin-streptomycin (Wisent). 293T-ACE2 cell line was previously reported (Prévost et al., 2020).

METHOD DETAILS

Plasmids

The plasmids encoding the SARS-CoV-2 Spike variants D614G and BA.2 (T19I, LPPA24S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K) were previously described (Beaudoin-Bussières et al., 2020; Gong et al., 2021; Tauzin et al., 2022a). The plasmids encoding the BA.4/5 Spike was generated by overlapping PCR using the BA.2 SARS-CoV-2 Spike gene as a template and cloned in pCAGGS. The BA.4/5 Spike sequence shows the following amino acid changes compared to the BA.2 Spike sequence: L452R, H69-, V70-, F486V, R493Q. All constructs were verified by Sanger sequencing.

Cell surface staining and flow cytometry analysis

293T were transfected with full-length SARS-CoV-2 Spikes and a green fluorescent protein (GFP) expressor (pIRES2-eGFP; Clontech) using the calcium-phosphate method. Two days post-transfection, Spike-expressing 293T cells were stained with the CV3-25 Ab (5 µg/mL) as control or plasma from naïve, BTI or PI individuals (1:250 dilution) for 45 min at 37°C. AlexaFluor-647-conjugated goat anti-human IgG (1/1000 dilution) were used as secondary Abs. The percentage of Spike-expressing cells (GFP + cells) was determined by gating the living cell population based on viability dye staining (Aqua Vivid, Invitrogen). Samples were acquired on a LSR II cytometer (BD Biosciences), and data analysis was performed using FlowJo v10.7.1 (Tree Star). The

conformationally-independent anti-S2 antibody CV3-25 was used to normalize Spike expression, as reported (Gong et al., 2021; Li et al., 2022; Prévost et al., 2021; Ullah et al., 2021). CV3-25 was shown to be effective against all Spike variants (Figure S1). The Median Fluorescence intensities (MFI) obtained with plasma were normalized to the MFI obtained with CV3-25 and presented as percentage of CV3-25 binding.

Virus neutralization assay

To produce SARS-CoV-2 pseudoviruses, 293T cells were transfected with the lentiviral vector pNL4.3 R-E− Luc (NIH AIDS Reagent Program) and a plasmid encoding for the indicated S glycoprotein (D614G, BA.2 or BA.4/5) at a ratio of 10:1. Two days post-transfection, cell supernatants were harvested and stored at −80°C until use. For the neutralization assay, 293T-ACE2 target cells were seeded at a density of 1×10^4 cells/well in 96-well luminometer-compatible tissue culture plates (PerkinElmer) 24h before infection. Pseudoviral particles were incubated with several plasma dilutions (1/50; 1/250; 1/1250; 1/6250; 1/31250) for 1h at 37°C and were then added to the target cells followed by incubation for 48h at 37°C. Cells were lysed by the addition of 30 µL of passive lysis buffer (Promega) followed by one freeze-thaw cycle. An LB942 TriStar luminometer (Berthold Technologies) was used to measure the luciferase activity of each well after the addition of 100 µL of luciferin buffer (15mM $MgSO_4$, 15mM KH_2PO_4 [pH 7.8], 1mM ATP, and 1mM dithiothreitol) and 50 µL of 1mM d-luciferin potassium salt (Prolume). The neutralization half-maximal inhibitory dilution (ID_{50}) represents the plasma dilution to inhibit 50% of the infection of 293T-ACE2 cells by pseudoviruses.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

Symbols represent biologically independent samples from SARS-CoV-2 naïve, BTI or PI individuals. Statistics were analyzed using GraphPad Prism version 8.0.1 (GraphPad, San Diego,

CA). Every dataset was tested for statistical normality and this information was used to apply the appropriate (parametric or nonparametric) statistical test. p values < 0.05 were considered significant; significance values are indicated as $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{***}P<0.0001$, ns, non-significant.

REFERENCES

Altarawneh, H.N., Chemaitelly, H., Ayoub, H.H., Hasan, M.R., Coyle, P., Yassine, H.M., Al-Khatib, H.A., Benslimane, F.M., Al-Kanaani, Z., Al-Kuwari, E., et al. (2022). Protection of SARS-CoV-2 natural infection against reinfection with the Omicron BA.4 or BA.5 subvariants. 2022.07.11.22277448. https://doi.org/10.1101/2022.07.11.22277448.

Beaudoin-Bussières, G., Laumaea, A., Anand, S.P., Prévost, J., Gasser, R., Goyette, G., Medjahed, H., Perreault, J., Tremblay, T., Lewin, A., et al. (2020). Decline of Humoral Responses against SARS-CoV-2 Spike in Convalescent Individuals. MBio *11*. https://doi.org/10.1128/mBio.02590-20.

Carazo, S., Skowronski, D.M., Brisson, M., Sauvageau, C., Brousseau, N., Gilca, R., Ouakki, M., Barkati, S., Fafard, J., Talbot, D., et al. (2022). Protection against Omicron re-infection conferred by prior heterologous SARS-CoV-2 infection, with and without mRNA vaccination. 2022.04.29.22274455. https://doi.org/10.1101/2022.04.29.22274455.

CDC (2022). COVID Data Tracker Weekly Review, https://www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/past-reports/04222022.html.

Chatterjee, D., Tauzin, A., Marchitto, L., Gong, S.Y., Boutin, M., Bourassa, C., Beaudoin-Bussières, G., Bo, Y., Ding, S., Laumaea, A., et al. (2022). SARS-CoV-2 Omicron Spike recognition by plasma from individuals receiving BNT162b2 mRNA vaccination with a 16-week interval between doses. Cell Rep. 110429. https://doi.org/10.1016/j.celrep.2022.110429.

Elliott, P., Eales, O., Steyn, N., Tang, D., Bodinier, B., Wang, H., Elliott, J., Whitaker, M., Atchison, C., Diggle, P.J., et al. (2022). Twin peaks: The Omicron SARS-CoV-2 BA.1 and BA.2 epidemics in England. Science *376*, eabq4411. https://doi.org/10.1126/science.abq4411.

Garrett, N., Tapley, A., Andriesen, J., Seocharan, I., Fisher, L.H., Bunts, L., Espy, N., Wallis, C.L., Randhawa, A.K., Ketter, N., et al. (2022). High Rate of Asymptomatic Carriage Associated with Variant Strain Omicron. MedRxiv 2021.12.20.21268130. https://doi.org/10.1101/2021.12.20.21268130.

Goel, R.R., Apostolidis, S.A., Painter, M.M., Mathew, D., Pattekar, A., Kuthuru, O., Gouma, S., Hicks, P., Meng, W., Rosenfeld, A.M., et al. (2021). Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals following mRNA vaccination. Sci. Immunol. *6*. https://doi.org/10.1126/sciimmunol.abi6950.

Gong, S.Y., Chatterjee, D., Richard, J., Prévost, J., Tauzin, A., Gasser, R., Bo, Y., Vézina, D., Goyette, G., Gendron-Lepage, G., et al. (2021). Contribution of single mutations to selected SARS-CoV-2 emerging variants spike antigenicity. Virology *563*, 134–145. https://doi.org/10.1016/j.virol.2021.09.001.

Gruell, H., Vanshylla, K., Tober-Lau, P., Hillus, D., Schommers, P., Lehmann, C., Kurth, F., Sander, L.E., and Klein, F. (2022). mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. Nat. Med. 1–4. https://doi.org/10.1038/s41591-021-01676-0.

Jennewein, M.F., MacCamy, A.J., Akins, N.R., Feng, J., Homad, L.J., Hurlburt, N.K., Seydoux, E., Wan, Y.-H., Stuart, A.B., Edara, V.V., et al. (2021). Isolation and characterization of cross-

neutralizing coronavirus antibodies from COVID-19+ subjects. Cell Rep. *36*, 109353. https://doi.org/10.1016/j.celrep.2021.109353.

Kitchin, D., Richardson, S.I., van der Mescht, M.A., Motlou, T., Mzindle, N., Moyo-Gwete, T., Makhado, Z., Ayres, F., Manamela, N.P., Spencer, H., et al. (2022). Ad26.COV2.S breakthrough infections induce high titers of neutralizing antibodies against Omicron and other SARS-CoV-2 variants of concern. Cell Rep. Med. *3*, 100535. https://doi.org/10.1016/j.xcrm.2022.100535.

Kurhade, C., Zou, J., Xia, H., Cai, H., Yang, Q., Cutler, M., Cooper, D., Muik, A., Jansen, K.U., Xie, X., et al. (2022). Neutralization of Omicron BA.1, BA.2, and BA.3 SARS-CoV-2 by 3 doses of BNT162b2 vaccine. Nat. Commun. *13*, 3602. https://doi.org/10.1038/s41467-022-30681-1.

Li, W., Chen, Y., Prévost, J., Ullah, I., Lu, M., Gong, S.Y., Tauzin, A., Gasser, R., Vézina, D., Anand, S.P., et al. (2022). Structural basis and mode of action for two broadly neutralizing antibodies against SARS-CoV-2 emerging variants of concern. Cell Rep. *38*, 110210. https://doi.org/10.1016/j.celrep.2021.110210.

Miyamoto, S., Arashiro, T., Adachi, Y., Moriyama, S., Kinoshita, H., Kanno, T., Saito, S., Katano, H., Iida, S., Ainai, A., et al. (2022). Vaccination-infection interval determines cross-neutralization potency to SARS-CoV-2 Omicron after breakthrough infection by other variants. Med S2666634022000897. https://doi.org/10.1016/j.medj.2022.02.006.

Mohapatra, R.K., Kandi, V., Sarangi, A.K., Verma, S., Tuli, H.S., Chakraborty, S., Chakraborty, C., and Dhama, K. (2022). The recently emerged BA.4 and BA.5 lineages of Omicron and their global health concerns amid the ongoing wave of COVID-19 pandemic – Correspondence. Int. J. Surg. Lond. Engl. *103*, 106698. https://doi.org/10.1016/j.ijsu.2022.106698.

Muik, A., Lui, B.G., Wallisch, A.-K., Bacher, M., Mühl, J., Reinholz, J., Ozhelvaci, O., Beckmann, N., Güimil Garcia, R. de la C., Poran, A., et al. (2022). Neutralization of SARS-CoV-2 Omicron by BNT162b2 mRNA vaccine–elicited human sera. Science *375*, 678–680. https://doi.org/10.1126/science.abn7591.

Nayrac, M., Dubé, M., Sannier, G., Nicolas, A., Marchitto, L., Tastet, O., Tauzin, A., Brassard, N., Lima-Barbosa, R., Beaudoin-Bussières, G., et al. (2022). Temporal associations of B and T cell immunity with robust vaccine responsiveness in a 16-week interval BNT162b2 regimen. Cell Rep. *0*. https://doi.org/10.1016/j.celrep.2022.111013.

Nemet, I., Kliker, L., Lustig, Y., Zuckerman, N., Erster, O., Cohen, C., Kreiss, Y., Alroy-Preis, S., Regev-Yochay, G., Mendelson, E., et al. (2022). Third BNT162b2 Vaccination Neutralization of SARS-CoV-2 Omicron Infection. N. Engl. J. Med. *386*, 492–494. https://doi.org/10.1056/NEJMc2119358.

Payne, R.P., Longet, S., Austin, J.A., Skelly, D.T., Dejnirattisai, W., Adele, S., Meardon, N., Faustini, S., Al-Taei, S., Moore, S.C., et al. (2021). Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. Cell *184*, 5699-5714.e11. https://doi.org/10.1016/j.cell.2021.10.011.

PHO (2022). Public Health Ontario : SARS-CoV-2 Omicron Variant Sub-Lineages BA.4 and BA.5: Evidence and Risk Assessment, chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.publichealthontario.ca/-

/media/Documents/nCoV/voc/2022/07/evidence-brief-ba4-ba5-risk-assessment-jul-8.pdf?sc_lang=en.

Prévost, J., Gasser, R., Beaudoin-Bussières, G., Richard, J., Duerr, R., Laumaea, A., Anand, S.P., Goyette, G., Benlarbi, M., Ding, S., et al. (2020). Cross-Sectional Evaluation of Humoral Responses against SARS-CoV-2 Spike. Cell Rep. Med. *1*, 100126. https://doi.org/10.1016/j.xcrm.2020.100126.

Prévost, J., Richard, J., Gasser, R., Ding, S., Fage, C., Anand, S.P., Adam, D., Vergara, N.G., Tauzin, A., Benlarbi, M., et al. (2021). Impact of temperature on the affinity of SARS-CoV-2 Spike glycoprotein for host ACE2. J. Biol. Chem. 101151. https://doi.org/10.1016/j.jbc.2021.101151.

Qu, P., Faraone, J., Evans, J.P., Zou, X., Zheng, Y.-M., Carlin, C., Bednash, J.S., Lozanski, G., Mallampalli, R.K., Saif, L.J., et al. (2022). Neutralization of the SARS-CoV-2 Omicron BA.4/5 and BA.2.12.1 Subvariants. N. Engl. J. Med. *386*, 2526–2528. https://doi.org/10.1056/NEJMc2206725.

Sun, K., Tempia, S., Kleynhans, J., von Gottberg, A., McMorrow, M.L., Wolter, N., Bhiman, J.N., Moyes, J., du Plessis, M., Carrim, M., et al. (2022). SARS-CoV-2 transmission, persistence of immunity, and estimates of Omicron's impact in South African population cohorts. Sci. Transl. Med. *0*, eabo7081. https://doi.org/10.1126/scitranslmed.abo7081.

Tauzin, A., Gong, S.Y., Painter, M.M., Goel, R.R., Chatterjee, D., Beaudoin-Bussières, G., Marchitto, L., Boutin, M., Laumaea, A., Okeny, J., et al. (2022a). A boost with SARS-CoV-2 BNT162b2 mRNA vaccine elicits strong humoral responses independently of the interval between the first two doses. 2022.04.18.22273967. https://doi.org/10.1101/2022.04.18.22273967.

Tauzin, A., Gong, S.Y., Beaudoin-Bussières, G., Vézina, D., Gasser, R., Nault, L., Marchitto, L., Benlarbi, M., Chatterjee, D., Nayrac, M., et al. (2022b). Strong humoral immune responses against SARS-CoV-2 Spike after BNT162b2 mRNA vaccination with a 16-week interval between doses. Cell Host Microbe *30*, 97-109.e5. https://doi.org/10.1016/j.chom.2021.12.004.

Tegally, H., Moir, M., Everatt, J., Giovanetti, M., Scheepers, C., Wilkinson, E., Subramoney, K., Makatini, Z., Moyo, S., Amoako, D.G., et al. (2022). Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. Nat. Med. 1–1. https://doi.org/10.1038/s41591-022-01911-2.

Tuekprakhon, A., Nutalai, R., Dijokaite-Guraliuc, A., Zhou, D., Ginn, H.M., Selvaraj, M., Liu, C., Mentzer, A.J., Supasa, P., Duyvesteyn, H.M.E., et al. (2022). Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum. Cell *185*, 2422-2433.e13. https://doi.org/10.1016/j.cell.2022.06.005.

Ullah, I., Prévost, J., Ladinsky, M.S., Stone, H., Lu, M., Anand, S.P., Beaudoin-Bussières, G., Symmes, K., Benlarbi, M., Ding, S., et al. (2021). Live imaging of SARS-CoV-2 infection in mice reveals that neutralizing antibodies require Fc function for optimal efficacy. Immunity S1074-7613(21)00347-2. https://doi.org/10.1016/j.immuni.2021.08.015.

Viana, R., Moyo, S., Amoako, D.G., Tegally, H., Scheepers, C., Althaus, C.L., Anyaneji, U.J., Bester, P.A., Boni, M.F., Chand, M., et al. (2022). Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature 1–10. https://doi.org/10.1038/s41586-022-04411-y.

438 Wang, Q., Guo, Y., Iketani, S., Nair, M.S., Li, Z., Mohri, H., Wang, M., Yu, J., Bowen, A.D., Chang,
439 J.Y., et al. (2022). Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, &
440 BA.5. Nature 1–3. https://doi.org/10.1038/s41586-022-05053-w.

441 WHO Update on Omicron, https://www.who.int/news/item/28-11-2021-update-on-omicron.

442 Yamasoba, D., Kosugi, Y., Kimura, I., Fujita, S., Uriu, K., Ito, J., and Sato, K. (2022). Neutralisation
443 sensitivity of SARS-CoV-2 omicron subvariants to therapeutic monoclonal antibodies. Lancet
444 Infect. Dis. *22*, 942–943. https://doi.org/10.1016/S1473-3099(22)00365-6.

445 Yu, J., Collier, A.Y., Rowe, M., Mardas, F., Ventura, J.D., Wan, H., Miller, J., Powers, O., Chung,
446 B., Siamatu, M., et al. (2022). Neutralization of the SARS-CoV-2 Omicron BA.1 and BA.2 Variants.
447 N. Engl. J. Med. *386*, 1579–1580. https://doi.org/10.1056/NEJMc2201849.

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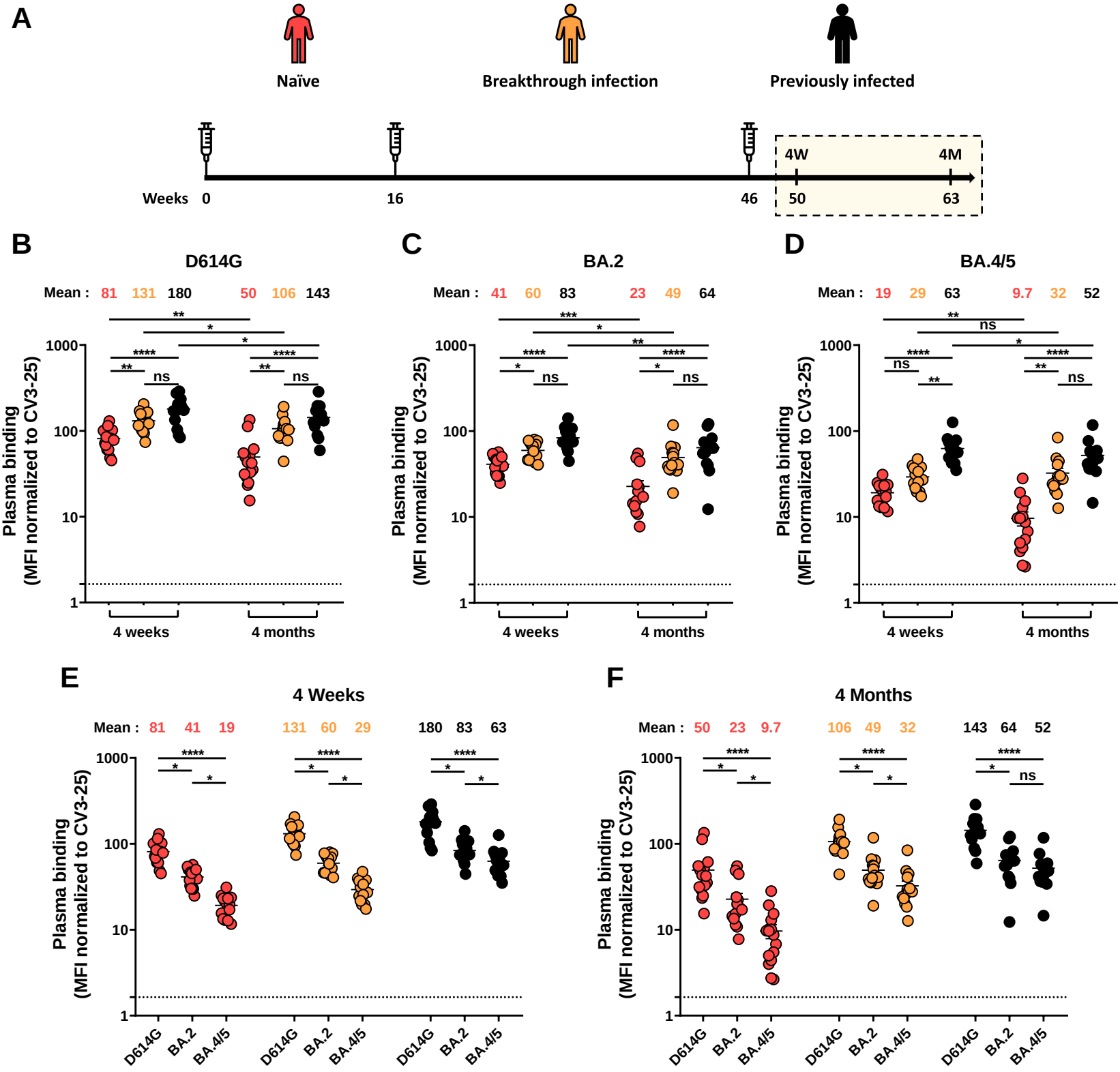
A
Naïve
Breakthrough infection
Previously infected
Weeks 0
16
46
50
63
4W
4M
B
D614G
Mean : 81 131 180 50 106 143
C
BA.2
Mean : 41 60 83 23 49 64
D
BA.4/5
Mean : 19 29 63 9.7 32 52
Plasma binding
(MFI normalized to CV3-25)
4 weeks
4 months
E
4 Weeks
Mean : 81 41 19 131 60 29 180 83 63
F
4 Months
Mean : 50 23 9.7 106 49 32 143 64 52
D614G
BA.2
BA.4/5

Figure 1

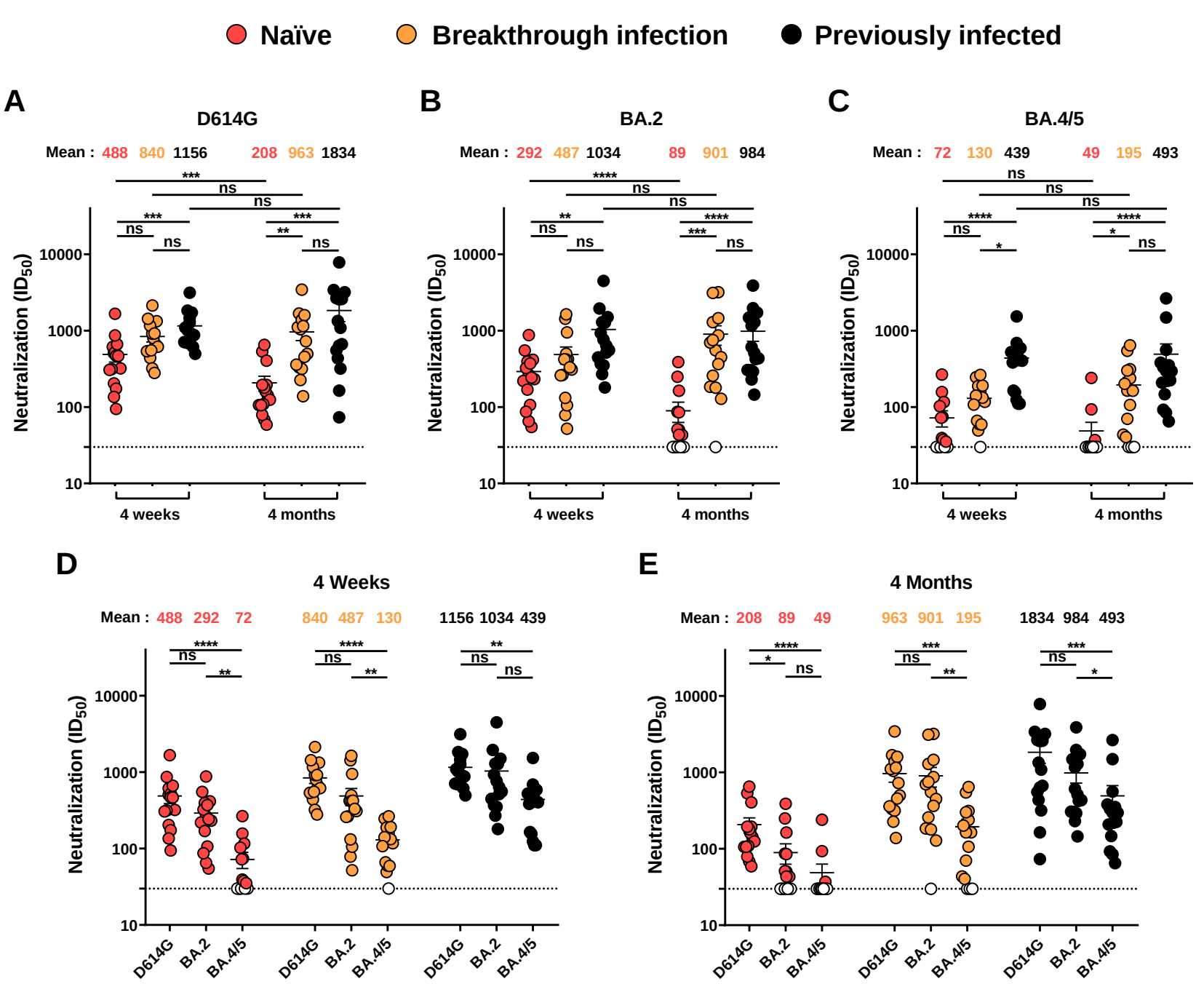
Naïve
Breakthrough infection
Previously infected
A
D614G
Mean : 488 840 1156 208 963 1834
B
BA.2
Mean : 292 487 1034 89 901 984
C
BA.4/5
Mean : 72 130 439 49 195 493
Neutralization (ID50)
4 weeks
4 months
D
4 Weeks
Mean : 488 292 72 840 487 130 1156 1034 439
E
4 Months
Mean : 208 89 49 963 901 195 1834 984 493
D614G
BA.2
BA.4/5

Figure 2

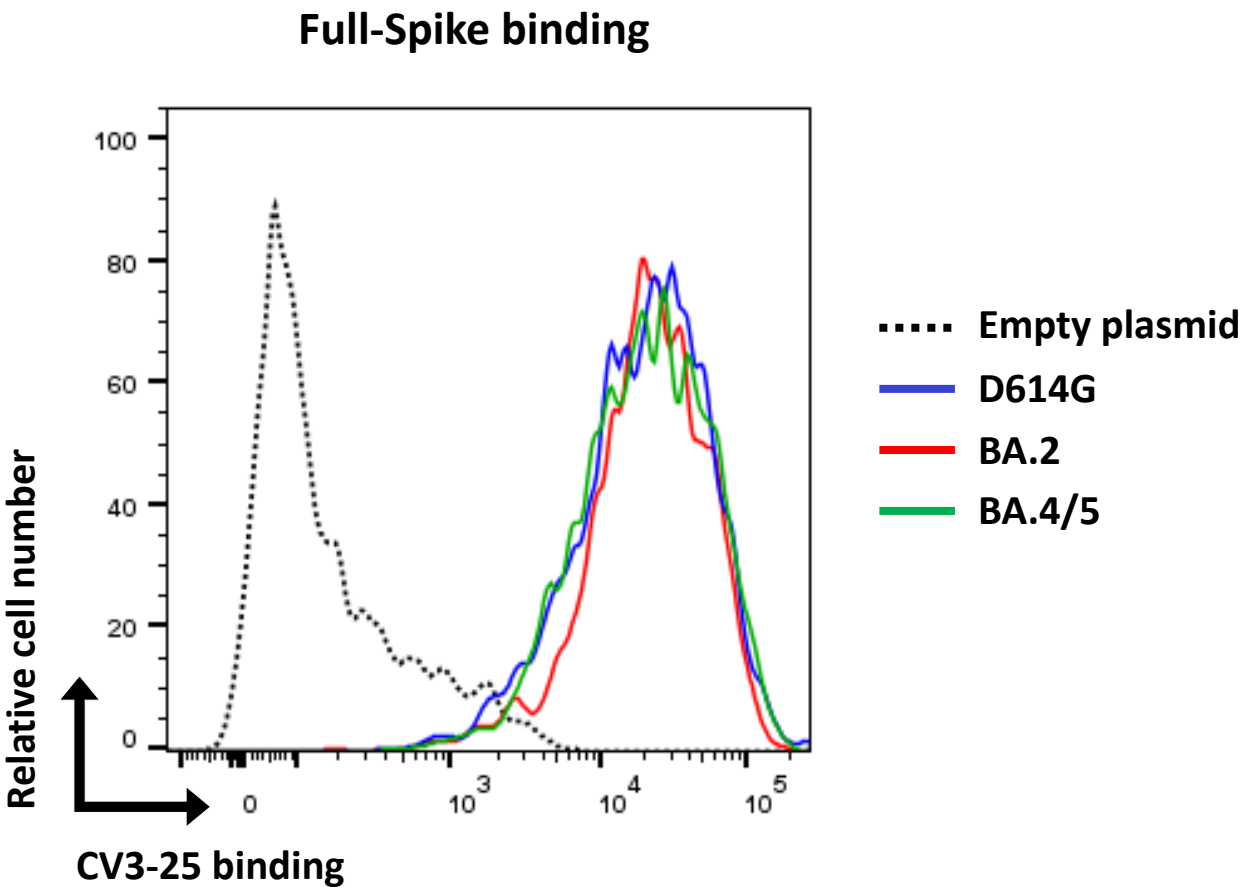

Figure S1. Recognition of different VOCs Spikes by the anti-S2 CV3-25 antibody, Related to Figure1.

293T cells were transfected with the full-length Spikes from different VOCs (D614G, BA.2 and BA.4/5), stained with the CV3-25 mAb and analyzed by flow cytometry.