# A Single Dose of BNT162b2 mRNA Vaccine Induces Airway Immunity in SARS-CoV-2 Naive and recovered COVID-19 subjects

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**Abbreviations** : recovered COVID-19 subjects (RCS), Nasal Epithelial Lining Fluid (NELF), Antibody Secreting Cells (ASCs), Receptor Binding Domain (RBD), angiotensin-converting enzyme 2 (ACE2), mucosal-associated lymphoid tissue (MALT).

**Abstract**

**Background:** Mucosal antibodies can prevent virus entry and replication in mucosal epithelial cells and hence virus shedding. Parenteral booster injection of a vaccine against a mucosal pathogen promotes stronger mucosal immune responses following prior mucosal infection compared to injections of a parenteral vaccine in a mucosally naive subject. We investigated whether this was also the case for the BNT162b2 COVID-19 mRNA vaccine.

**Methods**: Twenty recovered COVID-19 subjects (RCS) and 23 SARS-CoV-2 naive subjects were vaccinated with respectively one and two doses of the BNT162b2 COVID-19 vaccine. Nasal Epithelial Lining Fluid (NELF) and plasma were collected before and after vaccination and assessed for Immunoglobulin (Ig)G and IgA antibody levels to Spike and for their ability to neutralize binding of Spike to ACE-2 receptor. Blood was analyzed one week after vaccination for the number of Spike-specific Antibody Secreting Cells (ASCs) with a mucosal tropism.

**Results**: All RCS had both nasal and blood SARS-CoV-2 specific antibodies at least 90 days after initial diagnosis. In RCS, a single dose of vaccine amplified pre-existing Spike-specific IgG and IgA antibody responses in both NELF and blood against both vaccine homologous and variant strains, including delta. These responses were associated with Spike-specific IgG and IgA ASCs with a mucosal tropism in blood. Nasal IgA and IgG antibody responses were lower in magnitude in SARS-CoV-2 naive subjects after two vaccine doses compared to RCS after one dose.

***Conclusion***: Mucosal immune response to the SARS-CoV-2 Spike protein is higher in RCS after a single vaccine dose compared to SARS-CoV-2 naive subjects after two doses.

***Keywords:*** mucosal immunity; secretory antibodies; SARS-CoV-2; COVID-19 mRNA vaccine

## Introduction

Amid the surge of variants of concern, most COVID-19 vaccines are highly effective against hospitalization and death caused by a variety of SARS-CoV-2 strains (1-3). Early studies performed before the emergence of the delta variant showed that individuals who were fully vaccinated with an mRNA vaccine were less likely than unvaccinated persons to be infected with SARS-CoV-2 or to transmit it to others (4, 5). Likewise, studies have shown that vaccination reduces the risk of infection with the delta variant. Nonetheless, fully vaccinated individuals with breakthrough infections still transmit SARS-CoV-2, including to fully vaccinated contacts (6). Therefore, the risk of breakthrough infection in fully vaccinated people cannot be eliminated as long as there is continued transmission of the virus.

Protection from severe forms of COVID-19 in fully vaccinated adults is mediated at least in part by SARS-CoV-2-specific antibodies as demonstrated by the transfer of plasma from recovered COVID-19 subjects (RCS) to recently infected subjects (7). However, the immune mechanisms that prevent carriage and shedding of SARS-CoV-2 remain to be elucidated. Lessons drawn from other mucosal pathogens suggest that mucosal antibodies and especially secretory immunoglobulin A (sIgA) can efficiently block transmission of respiratory viruses (8). Relevant to this issue, SARS-CoV-2-specific humoral responses are dominated by IgA and peripheral expansion of IgA ASCs with a mucosal homing potential occurs shortly after disease onset (9). Further, sIgA are substantially more potent than bone marrow-derived serum monomeric IgA and IgG at neutralizing SARS-CoV-2 (10). Spike-specific IgA induced by mRNA COVID-19 vaccines are detected in SARS-CoV-2 naive individuals in plasma (10), milk (12), saliva (13) and nasal fluids (14) as early as two weeks after vaccination and for up to 6 months.

A dominant concept in vaccinology is that mucosal immunity is more efficiently induced by mucosal, e.g. nasal or oral, administration of vaccines than by parenteral injection, and that mucosal immune memory wanes more rapidly than systemic immune memory (11, 12). On a related topic, we and others have shown that a single booster injection of inactivated poliovirus vaccine boosted mucosal immune responses and reduced virus shedding in individuals previously vaccinated with an oral live polio vaccine (13). Building on these results, we hypothesized that immunization by parenteral injection with the BNT162b2 COVID-19 vaccine might be

more effective at inducing airway mucosal immunity in RCS compared to SARS-CoV-2 naive individuals.

## Methods

*Study design and participants*

Forty-three otherwise healthy subjects (23 SARS-CoV-2 naive and 20 RCS) were included in a prospective monocentric longitudinal study between April 14, 2021 and June 15, 2021 at the Nice University Hospital (Nice, France). Participants who recovered from a SARS-CoV-2 infection had experienced mild to moderate COVID-19 182 ± 85 days (median 170 days; min 73 days; max 383 days) before inclusion. Among these, 19 had been infected with the ancestral SARS-CoV-2 and one with the alpha variant. While all subjects received one injection of the BNT162b2 mRNA COVID-19 vaccine on day 0, only SARS-CoV-2 naive individuals received a second injection of this vaccine on day 21, as recommended by the French National Health Authority (14). Blood was collected on day 0, 7 and 21 from all subjects, and NELF were collected on day 0 and 21 from all subjects and on day 28 and 42 for SARS-CoV-2 naive subjects (Supplementary Figure 1). All samples were centralized and stored in our Biobank (15). All subjects signed an informed consent to participate in this work. The study was approved by the CPP Sud Méditerranée V ethics committee. ClinicalTrial.gov identifier: NCT04418206.

*Nasal epithelial lining fluid (NELF)*

Merocel® Standard Dressing Hydroxylated polyvinyl acetate nasal packs (Medtronic, Minneapolis, USA), were inserted between the nasal septum and the inferior turbinate (16), allowed to swell for 3 to 6 minutes, and gently retrieved prior to being placed into a 50 ml Falcon tube (Dustcher, Bernolsheim, France) containing 2 ml of saline solution. NELF was extracted by straining the swollen pack and then aliquoted and frozen at -70°C.

*SARS-CoV-2-specific IgG and IgA*

We used standardized assays that were selected by Operation Warp Speed as standard binding assays for immunogenicity assessments in several Phase II/III COVID 19 vaccine trials (17). In both plasma and NELF, we measured IgA and IgG levels to the SARS-CoV-2 Spike full-length protein and its Receptor Binding Domain (RBD) using the V-PLEX® SARS-CoV-2 Panel 2 (IgA) and V-PLEX® SARS-CoV-2 Panel 2 (IgG) multiplex serology assays (MSD, Maryland, US). In NELF, and

because of inter-individual variability in the concentration of proteins, we normalized in each sample the level of Spike- and RBD-specific IgG (or IgA) to the level of total IgG (or IgA) measured using the V-PLEX® Isotyping Panel 1 Human/NHP Kit. Plasma and NELF were diluted 100-fold and 10-fold respectively before being analyzed by immunoassay. Data were acquired on the V-PLEX® Sector Imager 2400 plate reader and analyzed using Discovery Workbench 3·0 software. Serial 4-fold dilutions of the standards were run to generate a 7-standard curve, and the diluent alone was used as a blank. The Lower Limit of Detection of anti-Spike IgG, anti-RBD IgG, anti-Spike IgA, and anti-RBD IgA were 0.44, 3.84, 9.52 and 11.00 WHO/NIBSC International Standard Units per mL (BAU/mL) respectively.

*Binding inhibition assay*

Plasma and NELF were assessed for antibodies inhibiting the binding of a soluble angiotensin-converting enzyme 2 (ACE2) receptor to the SARS-CoV-2 RBD derived from the Wuhan strain and from its B.1.1.7 (alpha), B.1.351 (beta) , B.1.617.2 (delta), P.1 (gamma) and B.1.617.1 (kappa), using the V-PLEX® SARS-CoV-2 Panel 13 ACE2 multiplex assay (MSD) (18). In this assay, plasma dilutions are added to the wells of a microtitration plate coated with the SARS-CoV-2 Spike RBD derived from the Wuhan strain and its variants. After washing, a human ACE2 protein conjugated to electroluminescent SULFO-TAG is added to the wells. After washing, light emitting bound ACE2 is measured with an MSD chemiluminescence reader. Plasma and NELF were diluted 100- and 10-fold respectively before being assessed for IgA and IgG. Data were acquired on the V-PLEX® Sector Imager 2400 plate reader and analyzed using the Discovery Workbench 3·0 software (MSD). For standard, serial 4-fold dilutions of the standards were run to generate a 7-standard concentration set, and the diluent alone was used as a blank. The percentage of inhibition was calculated according to the manufacturer's instructions.

*ELISPOT assay*

Anti-coagulated venous blood was collected before and 7 days after vaccination with the BNT162b2 COVID-19 mRNA to measure the frequency of vaccine-specific antibody-secreting cells (ASCs). Such cells are the circulating precursors of terminally differentiated tissue plasma cells, and appear in blood within a few days after antigen/vaccine exposure, peaking around day 7 and exiting from the circulation where they are no longer detectable after 2 weeks. While all ASCs express CD38, a subpopulation co-express tissue-specific homing receptors, such as the integrin

□4β7 which preferentially directs these ASCs to mucosal sites. Thus, the frequency and characteristics of blood ASCs provide a very early estimate of the nature and intensity of a humoral immune response to any vaccine. Here, we used a modified ELISPOT assay to measure the proportion of ASCs producing Spike-specific IgG and IgA with a mucosal tropism (19). Briefly, we incubated 5 ml of blood cells with magnetic beads (Dynabeads Pan Mouse IgG, Invitrogen) coated with monoclonal antibodies (mAbs) to either CD38 (clone HB-7, Biolegend) or β7 integrin (clone FIB504, BD Bioscience). We then applied a magnetic field to obtain cell suspensions enriched for either $CD38^+$ or $\beta7^+$ cells. To detect ASCs secreting IgG or IgA to Spike, we coated the wells of ELISPOT with purified Spike (Sino Biological Europe GmbH) or control antigens (i.e. Bovine Serum Albumin, BSA). After incubation of ASC-cell suspensions for 3 hours at 37°C, wells were extensively washed with PBS-EDTA and PBS-Tween 20. Next, a mixture of goat antibodies to human IgA and IgG, respectively labelled with alkaline phosphatase and horseradish peroxidase (Southern Biotech) was added to the wells. Zones of solid phase-bound secreted IgA and IgG antibodies were visualized by stepwise incubation with corresponding enzyme chromogen substrates. After drying, plates were scanned and blue (IgA) and red (IgG) spots enumerated using an automated ELISPOT reader. The frequencies of total CD38+ and β7+ immunoglobulin-secreting cells (ISCs) were measured in wells previously coated with anti-kappa and anti-lambda light chains and detected exactly as above.

*Statistical analyses*

Data are presented as geometric means and 95% confidence intervals of geometric means with a standard error of the mean (SEM) and medians with interquartile ranges (IQR) for quantitative variables, or as numbers and percentages for categorical variables. Comparison between pre- and post-vaccination was performed with the Mann Whitney non parametric test using GraphPad Prism 9·0 (GraphPad Software, Inc., San Diego, CA). Differences were considered significant when the p-value was < 0·05.

**Results**

We enrolled SARS-CoV-2 naive seronegative subjects (n=23) and RCS (n=20) who had been infected with SARS-CoV-2 before the emergence of the delta variant and. Subjects in the two groups did not differ in age, gender, Body Mass Index (BMI), and

total number of blood leukocytes, lymphocytes and neutrophils (Supplementary Table 1).

Before vaccination, IgA and IgG to Spike and RBD were readily detectable in both the NELF (Figure 1) and plasma (Supplementary Figure 2) of RCS, i.e. at least 3 months after recovery from COVID-19 disease. Likewise, NELF and plasma antibodies inhibited the binding of the Spike protein from the Wuhan, alpha and delta strain to its ACE-2 receptor (Figure 2). Binding inhibition activity was also manifest in plasma against all variants tested (Supplementary Figure 3).

We then compared IgG and IgA mucosal and blood responses to Spike and RBD in RCS having received one vaccine dose and in SARS-CoV-2 naive individuals given two doses. As for NELF, an IgG response to Spike and RBD was observed in all subjects with these antibodies being 5- to 20-fold more abundant in RCS than in SARS-CoV-2 naive individuals (Figure 1). In contrast, Spike-specific IgA antibodies were only detected in some subjects in each group with a higher proportion of responders among RCS compared to SARS-CoV-2 naive subjects (Figure 1). Further, NELF antibodies of RCS were more potent than those of SARS-CoV-2 naive individuals at inhibiting the binding of the Wuhan and gamma variant Spike protein to ACE-2 (Figure 2). As for plasma, IgG and IgA responses were similar in the two groups both in terms of proportion of responders (Supplementary Figure 2), IgG and IgA levels among responders (Supplementary Figure 2), and ability of plasma antibodies to block the binding of Spike to ACE-2 (Supplementary Figure 3).

IgA antibodies in NELF are produced by a subpopulation of $CD38^+$ ASCs that migrate to the nasal mucosa where they release dimeric IgA (19). Because the IgA responses in NELF were different between RCS and SARS-CoV-2 naive individuals, we hypothesized that subjects from these two groups differed with regard to frequencies of SARS-CoV2-reactive blood IgA- ASCs, and more specifically in the proportion of IgA- ASCs with a mucosal tropism. To test this, we used a modified antigen-specific ELISPOT assay to measure the number of ASCs producing Spike-specific IgA or IgG in blood after prior enrichment of CD38-expressing cells. Because mucosal ASCs preferentially express tissue specific cell surface homing markers such as the integrin β7, we also partitioned ASCs expressing β7. ASCs producing Spike-specific IgA or IgG were neither detected before vaccination nor in SARS-CoV-2 naive individuals 7 days after the first vaccine dose (not shown). In contrast, these cells were readily detected after enrichment for $CD38^+$ and $\beta7^+$ cells in both RCS

after a single vaccine dose and in SARS-CoV-2 naive subjects after two doses. The proportion of $\beta7^+$ cells among $CD38^+$ was higher in the first group compared to the second one, indicating that prior mucosal exposure through natural infection by SARS-CoV2 led to pronounced expansion of mucosal memory B cells after recall by a single injection of BNT162b2 vaccine (Figure 3).

## Discussion

In keeping with our initial hypothesis, we found that the levels of Spike-specific IgG and IgA in NELF, the proportion of responders and the ability of nasal antibodies to inhibit the binding of Spike to ACE-2 were higher among RCS given a single dose of BNT162b2 mRNA vaccine than in SARS-CoV-2 naive individuals after receiving two doses. Further, the proportion of ASCs with a mucosal tropism was higher in RCS than in naive subjects.

Which underlying mechanisms explain this difference? While primary infection with a mucosal pathogen induces the activation of naive B cells in mucosal-associated lymphoid tissue (MALT), parental vaccination induces B cell activation in regional lymph nodes (LN). In both cases, activated B cells proliferate and differentiate into either ASCs or memory B cells. After homing to mucosal sites, ASCs produce IgA or IgG, which are actively transported through epithelial cells by polymeric immunoglobulin receptor (pIgR)- and neonatal Fc receptor (FcRn)-mediated transcytosis, respectively. Although blood derived antibodies can transudate into mucosal, including nasal, secretions, especially during inflammatory conditions, this process is generally transient. ASCs homing to selective sites is controlled by differential co-expression of adhesion molecules and chemokine receptors, which depends on molecular cues that are governed by the local tissue microenvironment where initial antigen priming occurs. For example, ASCs that have been primed in MALTs are instructed to home to mucosal tissues, a process determined by the coordinated expression of chemokine receptors such as CCR9 and CCR20, and integrins such as α4β7 and α4β1. As for memory B cells, they position themselves strategically in secondary lymphoid organs, become tissue-resident at the site of infection or vaccination, or patrol as recirculating cells. During a secondary immune response, memory B cells are induced to differentiate into ASCs the homing tropism of which is determined by the environment in which they have been primed. Based on these findings, we propose the following scenario. Infection with SARS-CoV-2 or parenteral injection of BNT162b2 COVID-19 vaccine induces

the activation of naive Spike-specific B cells and their differentiation into memory B cells in nasal-associated lymphoid tissue and regional LN, respectively. In RCS, the injection of a single dose of the BNT162b2 COVID-19 vaccine activates systemic and mucosal memory B cells generated by prior SARSCoV2 infection. This leads to expansion and differentiation of ASC precursors among which a contingent of ASCs have preferential tropism to mucosal tissues. In contrast, in vaccine-primed SARS-CoV-2 naive individuals, the injection of a second vaccine dose induces B cells to differentiate into ASCs which are less prone to home to the mucosa. In line with this hypothesis, we found that the proportion of ASCs with mucosal tropism was higher in RCS after a single vaccine dose compared to SARS-CoV-2 naive individuals after two doses.

Infection with SARS-CoV-2 induces the production of RBD-specific IgG and IgA that inhibit the binding of Spike to ACE-2. In keeping with this, plasma and NELF from RCS before vaccination displayed higher receptor binding inhibition activity than those from SARS-CoV-2 naive individuals. This was observed for the Wuhan parental strain and variants including delta. Vaccination further increased receptor binding inhibitory activity in RCS compared to vaccine-primed individuals. Of note, receptor binding inhibitory antibody activity in NELF from RCS after primary BNT162b2 vaccination was higher than in SARS-CoV-2 naive individuals after vaccine priming and boosting. Since subjects from these two groups exhibited similar amounts of RBD-specific IgG and IgA in plasma, this observation indirectly suggests that the inhibitory activity of antibodies detected in NELF was likely the result of locally produced mucosal antibodies rather than from mere transudation of systemic antibodies. The observation that IgA and IgG RBD-specific antibody levels in plasma did not appear to differ between the two groups while receptor binding inhibitory antibody activity was higher in RCS could have resulted from competition by higher and saturating concentrations of receptor-binding but non-inhibitory antibodies in these convalescent subjects and with the assay used.

The present study has several limitations. First, our study sample consisted of a small cohort of RCS and SARS-CoV-2 naive individuals, and only 11 SARS-CoV-2 naive subjects were sampled after the second vaccine dose. Therefore, our results need to be validated with a larger and independent cohort. Second, the results showing the impact of vaccination on binding inhibition by nasal antibodies should be taken with caution because of the inherently high inter-individual variability in the

levels of total IgG and IgA in nasal secretions. Third, RCS were sampled three weeks after vaccination but not at later time points. Therefore, it is possible that nasal levels of SARS-CoV-2-specific IgA could have dropped after three weeks. Fourth, we have compared immune responses in SARS-CoV-2 naive subjects 42 days after priming with those in vaccinated RCS who had been exposed to SARS-CoV-2 three months or longer before vaccination. Finally, while secretory IgA antibodies have been demonstrated to prevent virus shedding in other infectious diseases, this has not been demonstrated after COVID infection or vaccination.

Few studies have documented mucosal immunity in the upper airway mucosa in COVID-19 individuals or after vaccination. For example, higher nasal Spike-specific antibody levels were associated with lower viral load, and that spike-specific mucosal antibodies were associated with the resolution of systemic, but not respiratory symptoms (20). Also, the BNT162b2 mRNA vaccine induced the production of nasal and salivary Spike-specific IgA, and this mucosal humoral immune response was stronger after the injection of the second vaccine dose compared to RCS (21). Together with these latter findings, and given the exceptionally potent SARS-CoV-2 neutralizing properties of secretory antibodies and particularly secretory IgA (22), the present study indicates that sIgA antibodies should be given special attention as potential correlates or surrogates of immune protection for candidate SARS CoV-2 vaccines.

While a few SARS-CoV-2 mucosal vaccines are being tested (23), none has been approved yet. Therefore, we could not directly compare a mucosal prime-parenteral to a parenteral prime-parenteral boost regimen to enhance COVID-19 vaccine immunogenicity. We therefore compared the impact of a parenteral boost in subjects who had been primed by SARS-CoV-2 infection and in SARS-CoV-2 naive individuals after parenteral priming. Our results provide a rationale for evaluating the effectiveness and programmatic utility of immunization strategies based on mucosal/systemic prime-boost of COVID-19 mucosal vaccines in development for controlling infection and transmission of SARS-CoV-2.

**Contributors**

CZ, NG and CHM designed the study. NG and CHM wrote the clinical protocol. PH supervised the preparation and storage of biological samples. CHM, CM, SL OG, JBE, JBO, SL, OG and EM, included subjects and collected informed consent. CM and EM collected blood samples and NELF. VT and MA prepared and stored

biological samples. EM, PB and CZ performed experiments. ME, VH, TS, AS provided advice. EM and NG analyzed data. CZ and NG wrote the manuscript. All authors revised the manuscript and gave their final approval.

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**Potential Conflicts of Interest**

V.H. reports receiving payment for serving on the BMS advisory board for Mesothelioma and Immunotherapy. M.I. reports grants from Fondation ARC paid to their institution and honoraria from the University of Saskatchewan Canada and Yoyal College of Physicians and Surgeons of Canada. J.Be. reports honoraria from Astra Zeneca. C.Cz. received honoraria for serving as a scientific adviser to Altimmune Inc. S.L. reports honoraria from Boehringer Ingelheim, Chiesi, and Zambon; and took part in an Astra Zeneca advisory board.

**Data availability statement**

The data that support the findings of this study are available from NG.

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## Legends to Tables and Figures

### Figure 1: SARS-CoV2-specific IgG and IgA in NELF

NELF of SARS-CoV-2 naive (filled dots) and RCS (empty dots) subjects were analyzed for IgG (upper panel) and IgA (lower panel) to Spike (left panel) and RBD (right panel) before vaccination (pre vacc.) and 21 days after two and one vaccine dose respectively. Data in individual subjects are expressed as the ratio between the levels of antigen-specific IgG (or IgA) and the levels of total IgG (or IgA). Medians with the interquartile range are shown. The mean + 2 SD normalized level of IgG and IgA in SARS-CoV-2 naive subjects before vaccination is indicated by a dotted line. In each group, levels of IgG and IgA to the indicated antigens before and after vaccination were compared using a two-tailed Wilcoxon-Mann-Whitney test. Of note, the normalized mean levels of anti-Spike IgG and IgA in NELF of SARS-CoV-2 naive individuals 3 weeks after they have received the first vaccine dose were 352.7 AU/ml for anti-Spike IgG, 39.3 AU/ml for anti-Spike IgA 107.4 7 AU/ml for anti-RBD IgG and 48.4 7 AU/ml for anti-RBD IgA. n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$. Of note,

### Figure 2: Inhibition of binding of Spike to ACE-2 by NELF antibodies

NELF of SARS-CoV-2 naive (filled dots) and convalescent (empty dots) subjects before (pre vacc.) and after (post vacc.) vaccination were analyzed for the ability to inhibit the binding of the Spike protein of the Wuhan SARS-CoV-2 reference strain and its indicated variants to ACE-2. Percentage of inhibition in individual subjects are shown. The percentage of inhibition of binding in SARS-CoV-2 naive and RCS before and after vaccination were compared using a two-tailed Wilcoxon-Mann-Whitney test. n.s., not significant; *, $p < 0.05$; **, $p < 0.01$.

### Figure 3: Proportion of ASCs with mucosal tropism

Blood of SARS-CoV-2 naive subjects drawn on day 28 and from RCS drawn on day 7 were analyzed for the number of Spike-specific IgA- and IgG-secreting cells in individual subjects after enrichment of $CD38^+$ and $\beta7^+$ cells. The proportion of $\beta7^+$ ASCs in the two groups were compared using a two-tailed Wilcoxon-Mann-Whitney test. *, $p < 0.05$.

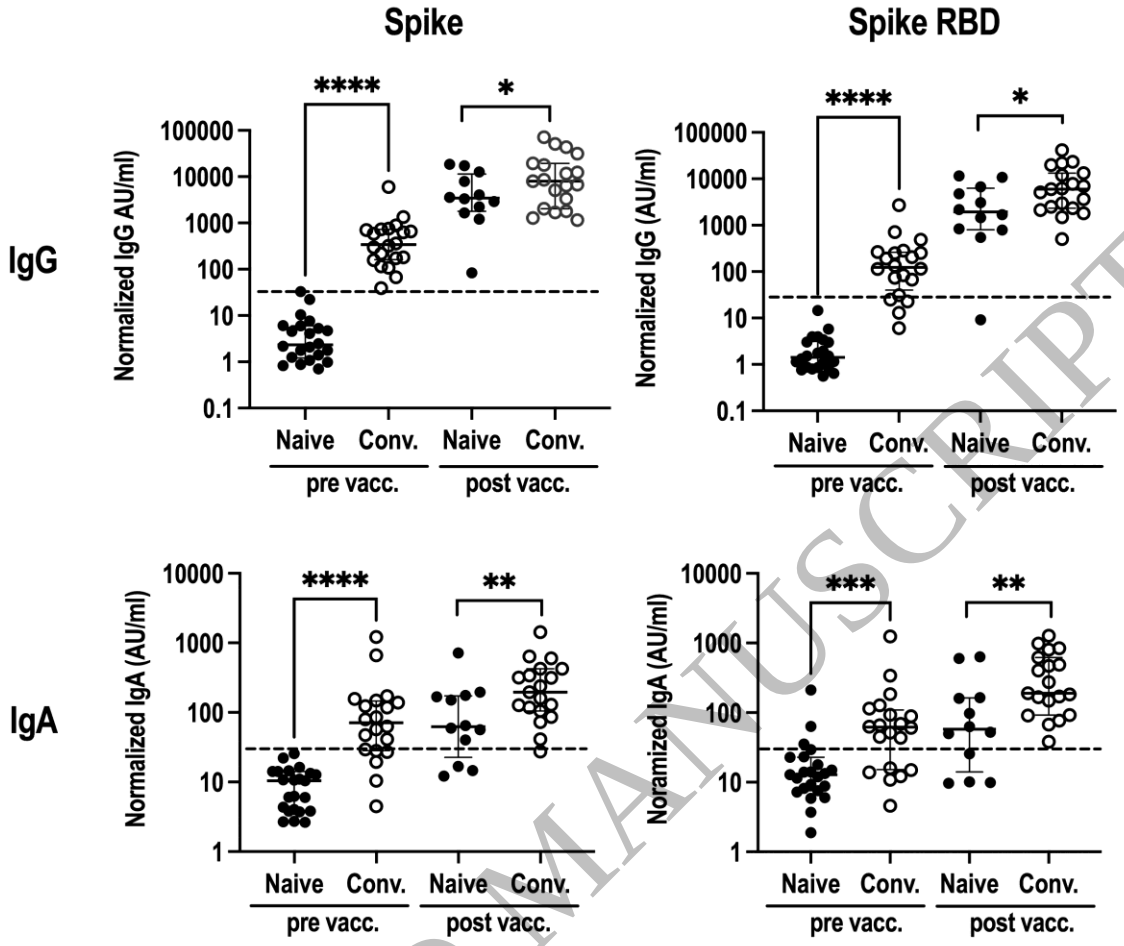


Figure 1
160x130 mm (.41 x  DPI)

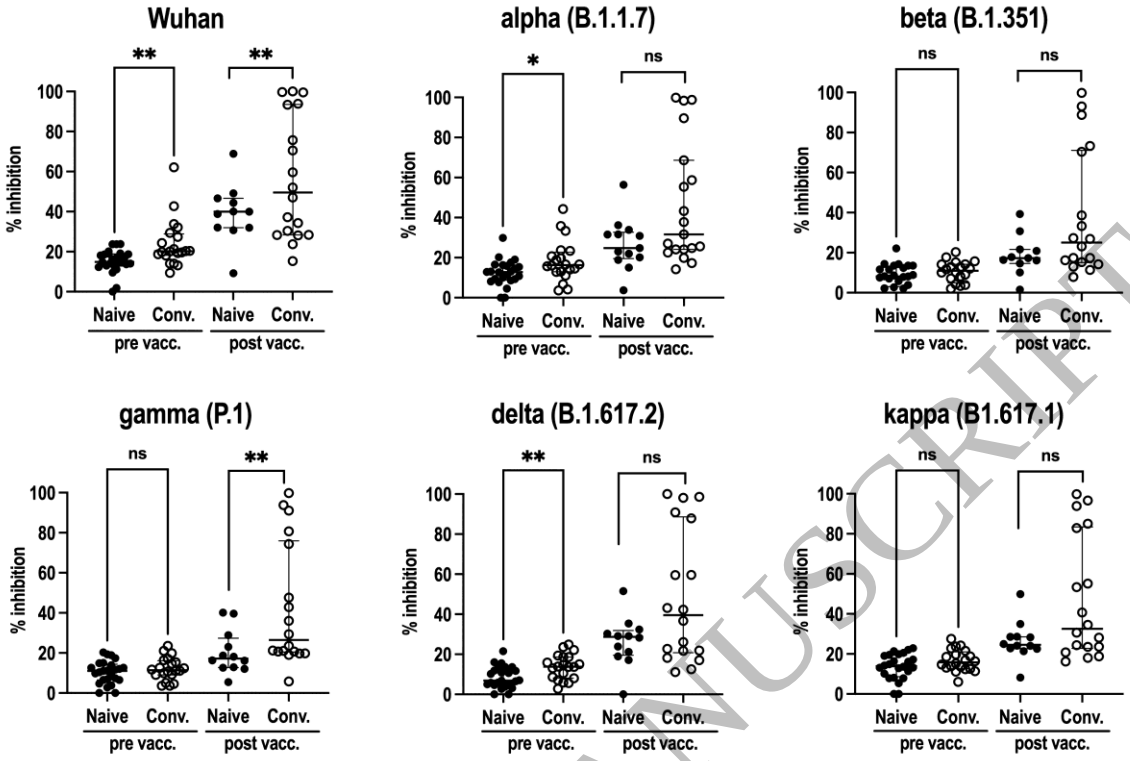


**Figure 2**
**160x106 mm (.41 x DPI)**

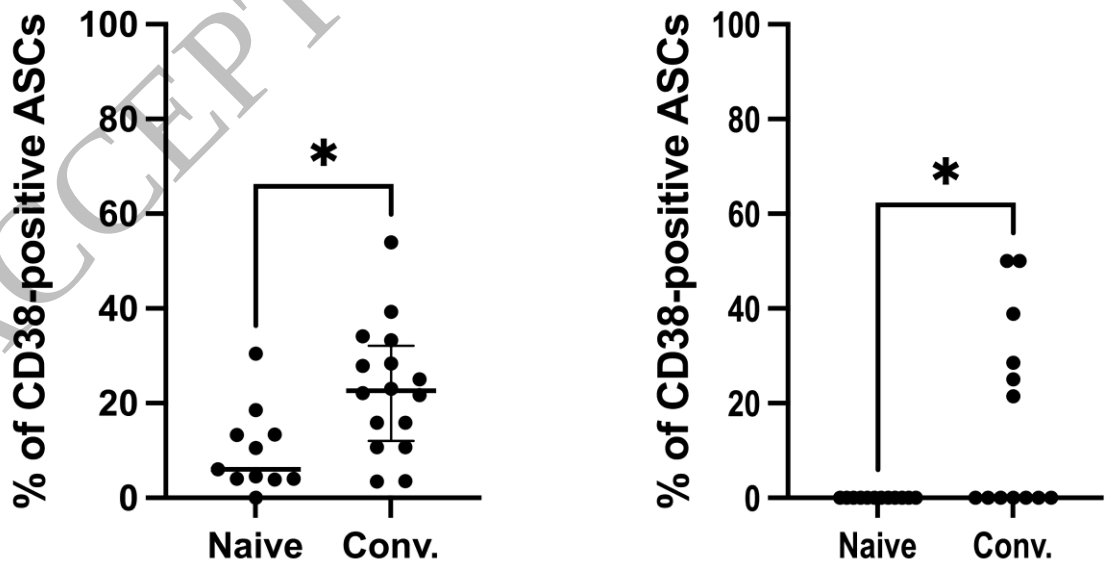


**Figure 3**
**160x84 mm (.41 x DPI)**