Antibody and T cell subsets analysis unveils an immune profile heterogeneity mediating long-term responses in individuals vaccinated against SARS-CoV-2

Running Title: Determination of COVID-19 antibody persistence correlates

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Footnotes

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Abstract

Background. Based on the fact that COVID-19 is still spreading despite vaccine worldwide administration, there is an imperative need to understand the underlying mechanisms of vaccine-induced inter-individual immune response variations.

Methods. We compared humoral and cellular immune responses in 127 individuals vaccinated with either BNT162b2, mRNA-1273 or ChAdOx1-nCoV-19 vaccine.

Results. We found that both mRNA vaccines induced faster and stronger humoral responses as assessed by high Spike- and RBD-specific antibody titers and neutralizing efficacy in comparison to ChAdOx1-nCoV-19 vaccine. At 7 months post vaccination, a decreasing trend in humoral responses was observed, irrespective of the vaccine administered. Correlation analysis between anti-S1 IgG and IFNγ production unveiled a heterogeneous immune profile among BNT162b2-vaccinated individuals. Specifically, vaccination in the high-responder group induced sizable populations of polyfunctional memory $CD4^+$ T_H1 cells, follicular helper T cells (T_{FH}) and T cells with features of stemness along with high neutralizing antibody production that persisted up to 7 months.. In contrast, low responders were characterized by loss or significantly reduced antibody titers and memory T cells and a considerably lower capacity for IL-2 and IFNγ production.

Conclusions. We identified that long-term humoral responses correlate with the individual's ability to produce antigen-specific persistent memory T cell populations.

Keywords. COVID-19; vaccines; immune response; antibodies; central memory T cells; stem cell memory T cells; cytokines; high and low responders

BACKGROUND

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of coronavirus disease (COVID)-19, has spread worldwide during the last two years. As of March 2022, SARS-CoV-2 has infected more than 400 million people and caused about 6 million deaths globally [1]. To achieve a sustainable containment of the pandemic, several vaccines against SARS-CoV-2 have been developed, with mRNA vaccines, namely BNT162b2 and mRNA-1273, being the first approved and administered since December 2020, followed by ChAdOx1-nCoV-19, an adenoviral vectored vaccine. These vaccines conferred protection against COVID-19, with mRNA vaccines having demonstrated higher efficacy and a good safety profile in clinical trials [2-4].

The concentration of produced antibodies against the spike (S) protein or the receptor-binding domain (RBD) and the titers of neutralizing antibodies that prevent binding of SARS-CoV-2 to the angiotensin-converting enzyme 2 (ACE2) receptor, are key measures for evaluating vaccine effectiveness [5-7]. Despite the marked decrease of anti-SARS-CoV-2 antibody levels over time, recent studies have shown that vaccine competency remains high for up to six months after initial vaccination [8, 9].

Although older ages have been associated with lower antibody responses [10, 11], there is a subgroup of fully vaccinated young individuals that fails to mount a strong and durable neutralizing antibody response, with no evidence of underlying factors associated with reduced antibody production [12, 13]. Thus, it seems that, besides age and comorbidities, the effectiveness of vaccination depends on factors such as preexisting immunity to the pathogen(s), sex, but also on several unidentified genetic and immune-related factors that impact on antibody response variation. There is evidence that both humoral and cellular immune responses are needed to achieve a robust and persistent protective immunity against SARS-CoV-2 [14-18]. However, the interplay between the two arms of adaptive immunity is complex and their investigation and correlation is difficult to

assess. To date, no direct comparison between long-term persistence of humoral and cellular responses persistence has been reported.

Thus, we compared antibody responses in vaccine recipients after the first and second dose of BNT162b2, mRNA-1273 and ChAdOx1-nCoV-19, by conducting a real-life population-based study in Greece. Additionally, assessment of antibody and IFNγ levels at 7 months post vaccination revealed a heterogeneous immune response profile among individuals vaccinated with BNT162b2. Thus, samples obtained from high and low responders were used to identify specific T cell subsets which likely relate to long-term immunity, with ultimate goal to identify signatures that can predict the successful outcome of vaccination among individuals.

METHODS

Study population and ethics declaration

The present study is a longitudinal study including administrative and laboratory staff of the Hellenic Pasteur Institute (HPI), as well as their family members. Inclusion criteria comprised vaccination against COVID-19, age of 18 years or older, and willingness and ability to provide informed consent. Enrolled participants completed a baseline survey questionnaire on demographic data, clinical profile, previous COVID-19 exposure and vaccine side effects. During the study, participants were weekly subjected to SARS-CoV-2 oropharyngeal swab tests to detect infection. All participants were assigned unique randomization numbers that remained unchanged throughout the study. The study complies with the Declaration of Helsinki and the design of the protocol was approved by the Review Board of the HPI (Ref. No.: 7345/23.06.2021) and the Research Protocol Approval Committee of the Department of Biology, NKUA (Ref. No.: 01/21.01.2021).

Study design

Sera samples were collected at six time points, i.e., prior or within 2 days after the first dose (T_0); 20, 30 or 90 days after BNT162b2, mRNA-1273 and ChAdOx-nCoV-19 first vaccination, respectively (T_1); and 20 days after the second dose, irrespectively of the vaccine used (T_2). Additional samples were collected at 3 (T_3) and 7 months (T_4) after the second dose and two weeks after the third dose (T_5) given at 5 up to 10 months after the second shot. At T_0, T_2 and T_4, whole blood was also collected for PBMCs isolation, whereas at T_4 and T_5 whole blood was obtained for cytokine quantitation.

Detection of anti-SARS-CoV-2 antibodies

Sera samples were tested for anti-S1 IgG, anti-S1 IgA, and anti-NCP IgG antibody responses using commercial ELISA kits from EuroImmun (EUROIMMUN, Lubeck, Germany). cPass™ SARS-CoV-2 Nabs Detection Kit (Genscript, Piscataway, NJ, USA) and SARS-CoV-2-NeutraLISA (EUROIMMUN) were used for detection of neutralizing antibodies. Anti-Spike-RBD IgG, IgG1, IgG2, IgG3 and IgG4 antibodies were measured using a custom ELISA described in Supplementary data.

Interferonγ release assay (IGRA)

T-cell responses against SARS-CoV-2 were assessed at T_4 and T_5, using an interferon γ (IFNγ) release assay (IGRA; EUROIMMUN), according to the manufacturer's instructions as described in Supplementary Data. Values > 200 mIU/mL of IFNγ were considered reactive.

Cytokine measurements and flow cytometry

The remaining supernatants from IGRA assay at T_4 were analyzed by Milliplex® MAP Kit using the Human Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore Corporation, Billerica, MA,

USA) for interleukin (IL)-2, IL-5, IL-13 and tumor necrosis factor (TNF)α according to the manufacturer's instructions. Analysis was performed using Luminex® 200^{TM} and data were analyzed using the xPONENT® software. PBMCs isolated from selected vaccinees at $T_{0,}$ T_2 and T_4 were used for assessment of vaccine-induced T cell immune responses by flow cytometry (Supplementary Data).

Statistical analysis

The effect size calculated on G-power analysis estimated a minimum group of n=120 with a significance level of 0.05 and a power of 95% covering the BNT162b2 vaccine. For data and statistical analyses, GraphPad Prism 6.0 was used. Unless specified otherwise, for reporting averaged results, median values were calculated, as data contained many outliers and skewed distributions. Tests for statistically significant differences in continuous variables between groups were mainly performed via Mann-Whitney U test, unless otherwise specified and adjusted P values are displayed. Pairwise correlations were assessed using Spearman's rank-order correlation.

RESULTS

Characteristics of the study cohort

A total of 127 participants were included in the final analysis (Supplementary Table 1). Among enrolled individuals, 64.57% were females and 35.43% males with a mean age of 46.10 ± 13.38 years. In general, 67.72% of participants were self-reported as healthy, while 32.28% reported at least one comorbidity and 37.01% mentioned outpatient self-medication. Among them, 16.53% had an autoimmune disease, followed by asthma (8.66%) and arterial hypertension (5.51%) (Table 1). Comparing the number of total adverse events after the first and the second dose of each vaccine,

reactogenicity after the second dose was significantly higher in individuals receiving BNT162b2 and mRNA-1273, while the opposite pattern was observed for individuals receiving the ChAdOx1-nCoV-19 vaccine (Supplementary Table 2). The vast majority of symptoms were mild to moderate in terms of severity, coinciding with published reports on vaccine safety [19].

Comparative kinetic analysis of humoral responses among the different vaccines

Primary vaccination with BNT162b2 induced detectable anti-S1 IgG, anti-RBD IgG and anti-S1IgA antibody responses in 94.2% of vaccine recipients (ratio >1.1), while 66.7% exhibited an intermediate neutralization activity of 44.55% (>35%) (Figure 1). Boosting with the second dose (T_2) led to the enhancement of all antibodies tested in 98% of the participants and to an impressive increase of neutralization activity (Figure 1). At T_3, all antibodies remained at high levels despite a slight decline, and a similar trend was recorded for neutralizing antibodies (Figure 1). Eventually, at T_4, anti-S1 IgG and anti-S1 IgA decreased by about 2-fold with a median neutralization activity of 45.30% (Figure 1). Among study participants, 2 were previously infected with SARS-CoV-2 and their produced antibodies after the first dose were in similar levels to those detected at T_2 in the rest of the population that remained stably elevated up to 7 months (T_4). On the contrary, severely immunocompromised individuals did not mount a humoral immune response at any time point.

mRNA-1273 vaccinated individuals displayed an impressively uniform and homogeneous pattern compared to BNT162b2 (Figure 1). Specifically, at T_1, 100% of participants produced high anti-S1 IgG, anti-RBD IgG and anti-S1 IgA levels and 75% developed a strong neutralizing activity. Moreover, at T_2, all antibody levels, as well as median neutralizing activity, significantly increased, plateaued till T_3, and slightly declined at T_4 with only one participant turning marginally negative (Figure 1).

ChAdOx1-nCoV-19 vaccine recipients exhibited a quite different profile from both mRNA-vaccinated participants. Specifically, 37.5% of participants were negative for anti-S1 IgG antibodies

at T_1, while 62.5% of anti-S1-positive participants did not develop neutralizing antibodies before the second dose (Figure 1), revealing a slow antibody production independent of age or sex (Supplementary Figure 2). The booster shot enhanced anti-S1 IgG and anti-RBD IgG antibody levels in all participants with only 70.6% being capable of neutralization with a median value of 66.0% (Figure 1). The same effect was observed at T_3 followed by significant reduction at T_4 with 22.2% of participants being negative for anti-S1 IgG and 66.7% for neutralizing antibodies (Figure 1). Importantly, ChAdOx1-nCoV-19 vaccinees were found negative for anti-S1 IgA antibodies (Figure 1).

Overall, ChAdOx1-nCoV-19 was less effective as compared to the mRNA vaccines, whereas mRNA-1273 being slightly advantageous over BNT162b2 in terms of antibody production. However, in all cases, results showed strong correlations between all antibody measurements at all time-points (T_1-T_4) (Supplementary Figure 3). Age-associated differences in neutralization activity were detected for BNT162b2 after the first and second vaccinations (Supplementary Figure 4), whereas, despite the limited sample size, sex-associated differences were identified in ChAdOx1-nCoV-19 vaccine recipients, with females being less responsive compared to males (Supplementary Figure 5).

Evaluation of S1-specific IFNγ responses induced by the three vaccines

Based on the fact that at 7 months after the second vaccination (T_4), a notable number of vaccine recipients exhibited a significant decline of neutralizing antibodies, we determined the presence of S1-specific cellular immune responses via assessing the production of IFNγ. We found that 94.5% of BNT162b2 and 91.6% of mRNA-1273 vaccine recipients produced IFNγ above threshold and at similar levels, with a median production of 1086.0 mUI/mL and 1357.0 mUI/mL, respectively (Figure 2A). Regarding ChAdOx1-nCoV-19, 100% of recipients produced 2-fold lower levels of IFNγ compared to those detected in mRNA vaccine recipients (Figure 2A). Using Spearman's test,

no correlation was found between secreted IFNγ and neutralizing antibody levels for all tested vaccines. PCA mapping using the 7-month post-vaccination data revealed a rather heterogenic response among BNT162b2 recipients in contrast to mRNA-1273 and ChAdOx1-nCoV-19 vaccine recipients that showed a more homogenous distribution based on anti-S1 IgG production, neutralization activity and IFNγ secretion (Figure 3B and Table 2).

Comparative analysis of the underlying cellular responses

In order to identify the factors that are related with the distinct immunological profiles detected in the BNT162b2-vaccinated individuals, the two groups found at the extremes of antibody and cytokine responses, i.e., high (HH) and low (LL) (Figure 2B) were selected for the determination of phenotypic traits and cytokine expression patterns. It must be noted that the size and median age of the two groups were similar. Convalescent individuals or participants on immunosuppressive medication were excluded, since their immune responses would be biased by infection or medication. Moreover, anti-NCP IgG antibody detection in HH group throughout study period excluded any asymptomatic infection (Supplementary Figure 6).

To investigate the cellular immune responses in depth, intracellular cytokine staining (ICS) was performed in PBMCs isolated at T_2 and T_4 from 6 individuals from each group after stimulation with Spike N-terminal S1 and C-terminal S2. After the boost dose, all individuals responded to S1 and S2 stimulation with ICS^+CD4^+ and ICS^+CD8^+ T cells (Figure 3A and Supplementary Figure 8A). Importantly, HH group acquired higher frequencies of $CD4^+IFN\gamma^+$ and $CD4^+IL\text{-}2^+$ as compared to LL group against S1 (Figure 3A). Also, $CD4^+IFN\gamma^+$ remained largely unaffected followed by $CD4^+TNF\alpha^+$ T cells increase at T_4 (Figure 3A). No differences were found regarding ICS^+CD8^+ T cells among HH and LL groups, which were maintained to 80% of HH and LL responders at T_4 (Figure 3A). Nonetheless, the responses against S2 were equal among two groups (Supplementary Figure 8A). Multifunctional analysis revealed that 80% of HH individuals contained significantly enhanced frequencies of $IFN\gamma^+TNF\alpha^+$, $IFN\gamma^+IL\text{-}2^+$ and $TNF\alpha^+IL\text{-}2^+$ T cells against S1 with the latter

two subgroups remaining detectable at T_4. Regarding $CD8^+$ T cells, LL group contained higher frequencies of $IFN\gamma^+TNF\alpha^+$ T cells against S1 as well as $CD107a^+IL\text{-}2^+$ and $CD107a^+TNF\alpha^+$ against S1 and S2 that remained stable till T_4 (Figure 3A and Supplementary Figure 9).

Detection of major T cell subsets revealed that pre-vaccination responses were undetectable in the majority of individuals though some of them having low frequencies of antigen-specific T cells mainly against S2 domain irrespective of their vaccination group that may be attributed to cross-reactive cells from prior seasonal coronavirus infection (Supplementary Figure 10). Vaccination induced S1- and S2-specific T follicular helper (T_{FH}) cells in 83.3% of HH and LL individuals and 100% and 83.3% of HH and LL, respectively. Importantly, S1-specific T_{FH} were still detected in 83% of vaccinees of HH group in contrast to 16.7% of LL group at T_4, whereas S2-specific T_{FH} were maintained up to 7 months in both groups (Figure 3D and Supplementary Figure 8C). Regarding memory T cell subsets, it was found that effector memory (EM) cells against S1 and S2 dominated $CD4^+$ and $CD8^+$ T cells subsets in both HH and LL groups with similar frequencies at T_2 and showed an increasing tendency of their numbers at T_4. Regarding central memory populations (CM), only HH group exhibited high frequencies in $CD4^+$ T cell subset when stimulated with S1 which remained stable till T_4 (Figure 3C and Supplementary Figure 8C). Importantly, a significant number of $CD4^+$ and $CD8^+$ T cells with stem cell memory (T_{SCM}) phenotype was detected in HH group at T_2, specific for S1 and S2 and higher than that observed in LL group. Those cell subsets were preserved at significant numbers at T_4 (Figure 3C and Supplementary Figure 8C).

High responders are characterized by an overall T_H1-type cytokine secretion profile

Eventually, T_H1 versus T_H2-type cytokine secretion profile was determined via detection of IL-2, TNFα, IL-5 and IL-13 levels after stimulation of whole blood with Spike S1 domain. The HH group responded to S1 peptide restimulation by producing 3-fold higher IFNγ and 10-fold higher IL-2 compared to the LL group (Figure 4A and Table 3). Surprisingly, no differences were detected in

TNFα levels between high and low responders, as well as in IL-5 which in most cases was marginally detectable. On the contrary, IL-13 levels were significantly higher (4.5-fold) in the HH group compared to the LL group (Figure 4B and Table 3). Spearman's correlation analyses between individual S1-specific cellular immune responses revealed a positive significant correlation between IFNγ and IL-2, IFNγ and IL-13, IL-13 and IL-2 as well as IFNγ and TNFα (Supplementary Figure 11). In parallel, anti-RBD IgG subclasses analysis revealed a superior production of IgG1 antibodies in HH group relative to T_H1 profile (Figure 4C). Spearman's correlation analyses considering all parameters of vaccine-induced immune responses at T_4 revealed that a significant association between antibody levels and S1-specifc T_{FH} cells and $CD4^+$ T_{SCM} cells. (Figure 4D). Moreover, correlation analysis between T cell subsets at T_2 and antibody responses at T_4 reveled a strong association of antibodies with S1-specific memory $CD4^+$ T cells, providing an indicator of long-term humoral immunity (Figure 4D).

Assessment of antibody responses along with IFNγ production in the HH and LL groups, two weeks after the third dose (T_5) showed that the LL group exhibited a 2.1-fold increase in median anti-S1 IgG titer and a 4.4-fold increase in median neutralization activity (median value 97.1%), reaching the levels detected in the HH group (Figure 4E). Regarding cellular immune responses, the LL group responded to S1 peptide restimulation by producing 4.6-fold increased levels of IFNγ as compared with those detected at T_4, reaching the levels detected in HH group (Figure 4E).

DISCUSSION

The development of multiple vaccines is one of the key pillars of humanity's eventual success against the COVID-19 pandemic. Authorized vaccines, despite their differences in technology used, provide significant protection against SARS-CoV-2 infection [2-4]. Vaccination-induced neutralizing antibodies are considered vital correlates of protection, since they have been constantly associated with prevention of symptomatic disease [5, 7]. In accordance with previous reports [20, 21], our

findings showed that BNT162b2 and mRNA-1273 effectively mobilized robust humoral immune responses in healthy, as well as in convalescent recipients readily after the first dose, in contrast to ChAdOx1-nCoV-19 that required two doses. In general, irrespective of the vaccine administered, antibody responses were maintained for up to 7 months, with only one documented symptomatic infection, suggesting that neutralizing titers can also be used as surrogate markers of vaccine efficacy. Previous studies showed both anti-S1 IgG and neutralizing antibody persistence for at least 6 months following BNT162b2 and mRNA-1273 vaccination [21-23]. In our cohort, antibody levels exhibited a gradual decrease at 3 months after the second dose, with 30% of participants having lost their neutralization activity at 7 months in all vaccines tested (T_4 in Figure 1). Likewise, several groups have reported a drop in antibody titers along with a marked decrease in neutralizing capacity in the long term [22, 24-26]. Nevertheless, in our study the reduction observed at T_4 was age-independent, in contrast to the documented inverse relationship between age and neutralizing responses after the first dose of BNT162b2 and mRNA-1273 [10, 27]. Specifically, we found a striking inter-individual variation in the amplitude and nature of the humoral response explained only in part by age, sex, previous exposure, and drug treatments.

In many cases, waning of antibodies in peripheral blood does not necessarily associate with the absence of specific protection against SARS-CoV-2, since it has been demonstrated that virus-specific memory B cells persist for more than 240 days after COVID-19 symptom onset [28, 29]. Memory B cell activation and eventual antibody production are supported by the presence of antigen-specific cell responses which are not necessarily dependent on follicular T cells [30, 31]. Thus, the generation of adequate antigen-specific T cell responses aids memory B cell activation and, eventually, antibody production. This is similar to the responses induced by the hepatitis B vaccine, where no cases of acute hepatitis B or chronic antigen carriage have been reported, despite the failure of the vaccine to generate strong antibody response even after the booster dose [32, 33]. Evaluation of SARS-CoV-2-specific cell-mediated immune responses, unveiled a high heterogeneity in their

magnitude among BNT162b2-vaccinated participants, irrelevant to anti-S1 IgG and neutralizing efficacy, in contrast to mRNA-1273 and ChAdOx1-nCoV-19 vaccine recipients, as also previously reported [22]. Such a dichotomy of unimpaired vaccine-specific humoral and cellular responses has been reported in tick borne encephalitis, hepatitis B and smallpox vaccination, in which cases efficacy depended not only on the vaccine-antigen but also on the genetic predisposition of vaccinated individuals [34, 35].

This heterogeneity prompted us to group BNT162b2-vaccinated participants with similar immune responses in two "immune extreme" phenotypes, i.e., high or low levels of both humoral and cellular responses, further designated as high and low responders, respectively. Main characteristics of high responders were the significantly increased numbers of S1-specific $CD4^+$ T_{CM} and T_{SCM} with a multifunctional profile, as well as T_{FH} cells that were maintained up to 7 months post vaccination that in many cases those populations guarantee vaccination success, since both are associated with superior pathogen control via establishment of T_{SCM}-mediated long-lived immunity [36-38]. .. This is verified in our study by the significant correlation found between all memory populations and the neutralizing activity of anti-S1-specific antibodies. Indeed, several groups have shown that extensive IgG class-switching is probably instructed by vaccine-induced T_H1-polarized $CD4^+$ T cell responses [39-41]. Moreover, the importance of high IL-2 levels which indirectly stimulate B cells via T_H cells differentiation was also evidenced in vaccinated or COVID-19 as well as in SARS-CoV-1 convalescent individuals [42-45].

Our most interesting finding was that about 30% of high responders were capable of producing significantly high levels of IL-13, a signature-cytokine produced by T_H2 cells [46]. *In vitro* data have shown that IL-13 induces the proliferation and differentiation of human B cells [47] and this is in agreement with the high neutralizing activity of high responders detected at 7 months post vaccination. Despite the fact that data regarding COVID-19 so far have linked IL-13 production with disease severity [48], a most recent study on vaccine–induced immune responses in

immunocompromised and healthy individuals, revealed that booster vaccinations induced memory T cell populations able to produce not only T_H1 skewed cytokines, but also high levels of IL-13 [49]. The supportive role of IL-13 in B cell activation and eventually in antibody production was further supported in our study by the significant increase of humoral immune responses along with IFNγ production, detected in IL-13-high responders after the third dose of the vaccine. Similarly, high responders to hepatitis B vaccination were capable of producing high levels of IL-13 after antigenic stimulation, which were significantly correlated with plasma IgG levels, suggesting that the levels of IL-13 are involved in the determination of antigen-specific memory B cell number [50].

In conclusion, by using "immune extreme" phenotypes we were able to provide a deeper insight into vaccine responses by explaining and characterizing inter-individual differences in both antibody and cellular responses. Specifically, we demonstrated that the induction of high numbers of antigen-specific T_{FH} and $CD4^+$ T cell memory populations, able to produce high levels of IL-2, IFNγ and in some cases of IL-13, are positively correlated with increased and sustained long-term antibody responses.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Figure Legends

Figure 1. Kinetic analysis of antibody responses and neutralization activity after vaccination with BNT162b2, mRNA-1273 and ChAdOx1-nCoV-19. Serum samples from BNT162b2-, mRNA-1273- and ChAdOx1-nCoV-19-vaccinated individuals were collected at 20, 30 and 90 days post the priming dose (T_1), respectively. Serum was also collected at 20 days (T_2), 3 months (T_3) and 7 months (T_4) after the second dose, irrespectively of the administered vaccine. (A) Average anti-Spike IgG titers (Ratio), anti-RBD IgG (OD_{450}), anti-Spike IgA titers (Ratio) and neutralization activity (% inhibition). (B) Individual values of anti-Spike IgG, anti-RBD IgG, anti-Spike IgA and neutralization activity. In (A) SD values are not shown for clarity. In (B) each dot represents one participant. Horizontal lines indicate median values. Comparison between groups was performed by multiple two-paired student's t test and statistical significance was assessed using Holm-Sidak method.* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Figure 2. (A) IFNγ production, assessed via the IGRA, in whole blood cells from vaccinated participants at 7 months after the second dose. Boxes show median and 25th-75th percentiles; whiskers show range; error bands represent 95% confidence limits. The statistical difference between the three vaccines is calculated using two-sided Mann-Whitney rank-sum test. (B) Principal component analysis (PCA) of antibody and IFNγ responses that were analyzed in vaccinated participants at 7 months after the second dose.

Figure 3. Analysis of S1-specific $CD4^+$ and $CD8^+$ T cell subsets in low (LL) and high (HH) responders at 20 days (T_2) and 7 months (T_4) post second vaccination. (A) Frequencies of S2-specific IFNγ, IL-2 and TNFα-producing $CD4^+$ and $CD8^+$ T cells subsets. (B) Polyfunctional analysis and relative distribution of single or multiple cytokine responses in $CD4^+$ and $CD8^+$ T cells subsets. (C)

Frequencies of central memory (CM; $CD45RO^{+}CD62L^{+}CCR7^{+}CD95^{+}$), effector memory (EM; $CD45RO^{+}CD62L^{-}CCR7^{-}CD95^{+}$) and stem cell memory (SCM; $CD45RO^{-}CD62L^{+}CCR7^{+}CD95^{+}$) in $CD4^{+}$ and $CD8^{+}$ T cells. (D) Frequencies of follicular helper (FH; $CD4^{+}CXCR5^{+}$) T cells. Each dot represents one participant. Horizontal lines indicate mean values. The statistical difference between the two groups is calculated using two-sided Mann-Whitney rank-sum test. * $P<0.05$, **** $P<0.0001$.

Figure 4. Assessment of IFNγ (A**)** and IL-2, TNFα, IL-5 and IL-13 (B) production after S1-peptide re-stimulation of whole blood cells and (C) anti-RBD IgG1, IgG2, IgG3 and IgG4 subclasses in sera obtained from BNT162b2-vaccinated participants in the LL and HH groups. Boxes show median and 25th-75th percentiles; whiskers show range; error bands represent 95% confidence limits. Statistical differences between groups are calculated using two-sided Mann-Whitney rank-sum test.* $P<0.05$, **** $P<0.0001$. (D) Correlation matrix heatmap of S1- and S2-specific humoral and cellular immune responses tested. Spearman correlation co-efficient is shown. (E) Immune responses of high (HH) and low (LL) responders after the third dose of the BNT126b2 vaccine. Comparison of anti-S1 IgG antibodies levels (ratio), neutralizing activity (% inhibition) and IFNγ levels after S1-peptide restimulation of whole blood cells at 7 months post second vaccination (T_4) and two weeks post third vaccination (T_5) are shown. Lines connect samples from the same individual. Statistical differences between the two time points are calculated using two-sided Mann-Whitney rank-sum test.

Table 1. Key baseline demographic and clinical characteristics of study participants.

Variable	*BNT162b2* (N =102)	*mRNA-1273* (N = 14)	*ChAdOx1-S* (N = 11)	TOTAL (N = 127)
	frequency (%)	frequency (%)	frequency (%)	frequency (%)
Sex				
Female	72/102 (70.59)	4/14 (28.6)	6/11 (54.55)	82/127 (64.57)
Male	30/102 (29.41)	10/14 (71.4)	5/11 (45.45)	45/127 (35.43)
Age Group Range				
Young adults total (18 – 30 years) (31 – 40 years)	37/102 (36.28) 18/102 (17.65) 19/102 (18.63)	2/14 (14.3) 2/14 (14.3) 0/14 (0.0)	4/11 (36.4) 1/11 (9.1) 3/11 (27.3)	43/127 (33.86) 21/127 (16.54) 22/127 (17.32)
Middle aged total (41 – 50 years) (51 – 60 years)	51/102 (50.0) 32/102 (31.37) 19/102 (18.63)	7/14 (50.0) 3/14 (21.4) 4/14 (28.6)	1/11 (9.1) 1/11 (9.1) 0/11 (0.0)	59/127 (46.46) 36/127 (28.35) 23/127 (18.11)
Old adults (> 60)	14/102 (13.72)	5/14 (35.7)	6/11 (54.5)	25/127 (19.68)
Mean age (years)	45.22 ± 12.6	53.1 ± 16.0	50.5 ± 14.0	46.10 ± 13.38

Mean bodyweight (kg)	73.76 ± 19.9	81.2 ± 11.6	81.4 ± 16.8	74.36 ± 19.14
Mean Height (cm)	169.37 ± 8.8	173.6 ± 7.2	173.5 ± 10.1	170.37 ± 8.86
Mean BMI (kg/m^2)	25.45 ± 5.6	26.9 ± 3.0	26.9 ± 4.3	25.44 ± 5.26
Comorbidity				
Asthma	10/102 (9.80)	0/14 (0.00)	1/11 (9.09)	11/127 (8.66)
Arterial hypertension	6/102 (5.88)	1/14 (7.14)	0/11 (0.00)	7/127 (5.51)
Autoimmune disease	19/102 (18.63)	0/14 (0.00)	2/11 (18.18)	21/127
Other	10/102 (9.80)	0/14 (0.00)	1/11 (9.09)	(16.53)
				11/127 (8.66)
Covid-19 infection				
Past infection	1/102 (0.98)	0/14 (0.00)	0/11 (0.00)	1/127 (0.79)
Post infection	8/102 (7.84)	0/14 (0.00)	0/11 (0.00)	8/127 (6.30)
Blood type				
0	31/102 (30.39)	6/14 (42.86)	0/11 (0.00)	37/127
A	41/102 (40.20)	6/14 (42.86)	7/11 (63.64)	(29.13)
B	8/102 (7.84)	1/14 (7.14)	0/11 (0.00)	54/127
AB	2/102 (1.96)	0/14 (0.00)	0/11 (0.00)	(42.52)
Unknown	20/102 (19.61)	1/14 (7.14)	4/11 (36.36)	9/127 (7.09)
				2/127 (1.57)
				25/127
				(19.69)
Rhesus (Rh)				
Negative	12/102 (11.76)	0/14 (0.00)	0/11 (0.00)	12/127 (9.45)
Positive	68/102 (66.67)	12/14 (85.71)	8/11 (72.73)	88/127
Unknown	22/102 (21.57)	2/14 (14.29)	3/11 (27.27)	(69.29)

				27/127 (21.26)
Other vaccines in the past year				
Influenza Pneumococcal HSV	44/102 (43.14) 12/102 (11.76) 0/102 (0.00)	5/14 (35.71) 3/14 (21.43) 0/14 (0.00)	5/11 (45.45) 2/11 (18.18) 0/11 (0.00)	54/127 (42.52) 17/127 (13.39) 0/127 (0.00)
Outpatient self-medicated				
Yes No	40/102 (39.22) 62/102 (60.78)	2/14 (14.29) 12/14 (85.71)	5/11 (45.45) 6/11 (54.55)	47/127 (37.01) 80/127 (62.99)

1

2

Table 2. Long term Spike S1-specific humoral and cellular immune responses in individuals vaccinated with BNT162b2.

Variable[a]	High antibody - High IFNγ (HH) group	Low antibody – High IFNγ (LH) group	High antibody – Low IFNγ (HL) group	Low antibody - Low IFNγ (LL) group
Neutralization activity (% inhibition)[b]	62.2 (53.2 – 74.1)	23.9 (15.3 – 33.7)	58.3 (49.2 – 75.8)	24.2 (15.3 – 32.9)
IFNγ (mUI/mL)[c]	1632.0 (1343.0 – 2117.0)	1815.0 (1503.0 – 2226.0)	635.5 (308.6 – 720.2)	578.9 (339.5 – 956.8)

[a]Data are median values (Interquartile ranges).

[b]A value of < 30% inhibition is considered negative.

[c]A value of < 200mUI/mL is considered negative.

Table 3. Levels of secreted cytokines in the HH and LL groups of individuals vaccinated with BNT162b2.

Variable[a]	High antibody - High IFNγ (HH) group	Low antibody – Low IFNγ (LL) group
IFNγ (mUI/mL)	1632.0 (1343.0 – 2117.0)	578.9 (339.5 - 956.8)
IL-2 (pg/mL)	83.3 (39.8 – 125.4)	8.1 (3.5 – 21.3)
TNFα (pg/mL)	154.1 (64.2 – 802.5)	104.6 (17.6 – 606.3)
IL-5 (pg/mL)	0.6 (0.3 – 1.2)	0.3 (0 – 1.6)
IL-13 (pg/mL)	8.9 (0 – 75.7)	1.9 (0 – 8.4)

[a]Data are median values (Interquartile ranges). Cytokines are defined as the participant–specific S1-stimulated responses minus the unstimulated response.

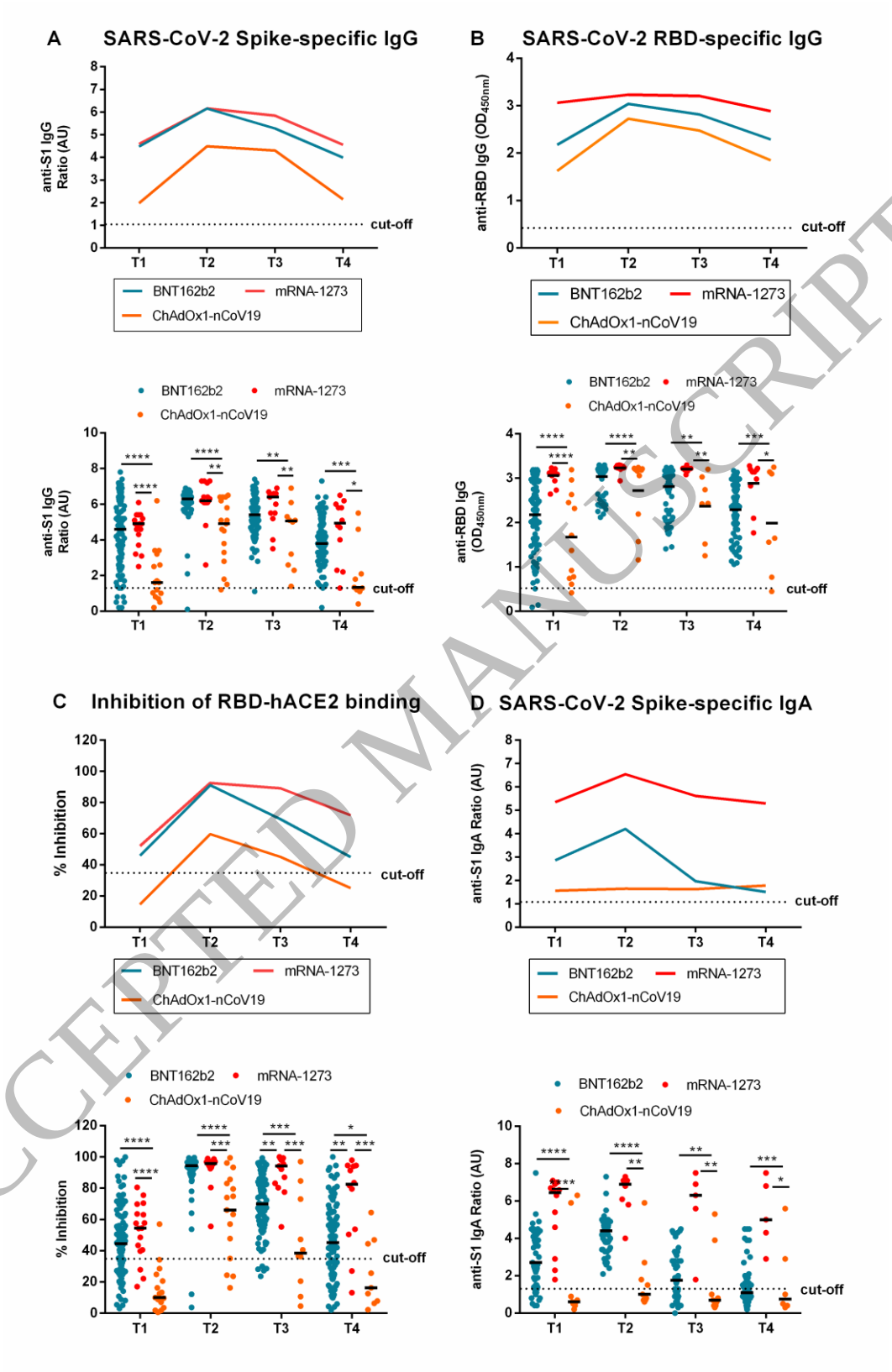

Figure 1

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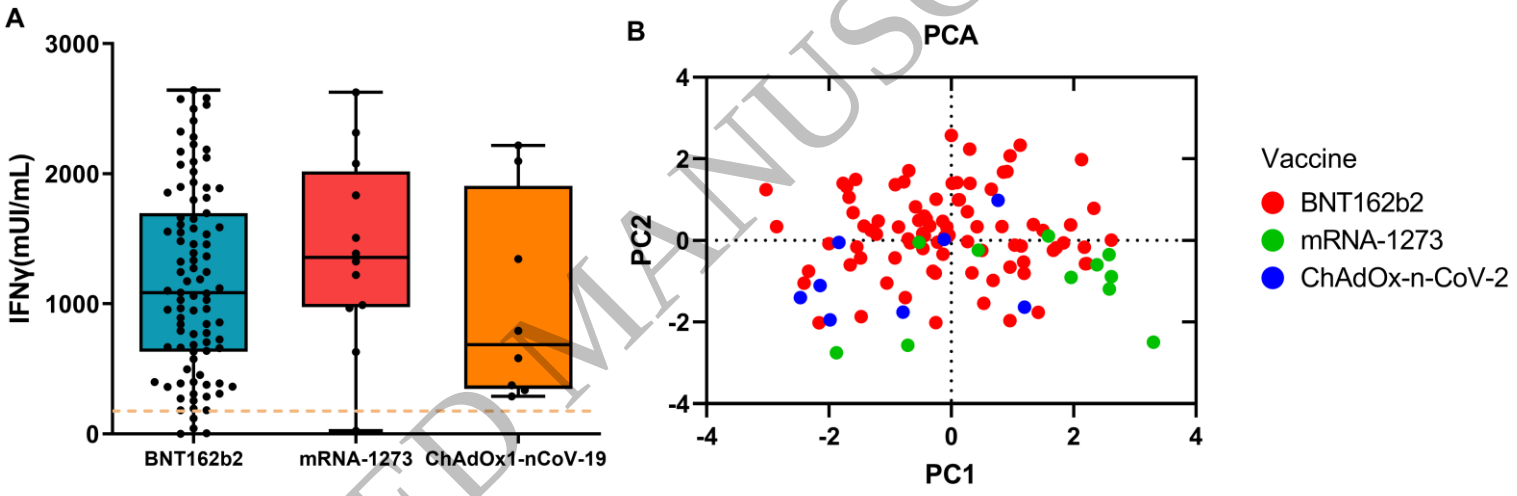

Figure 2
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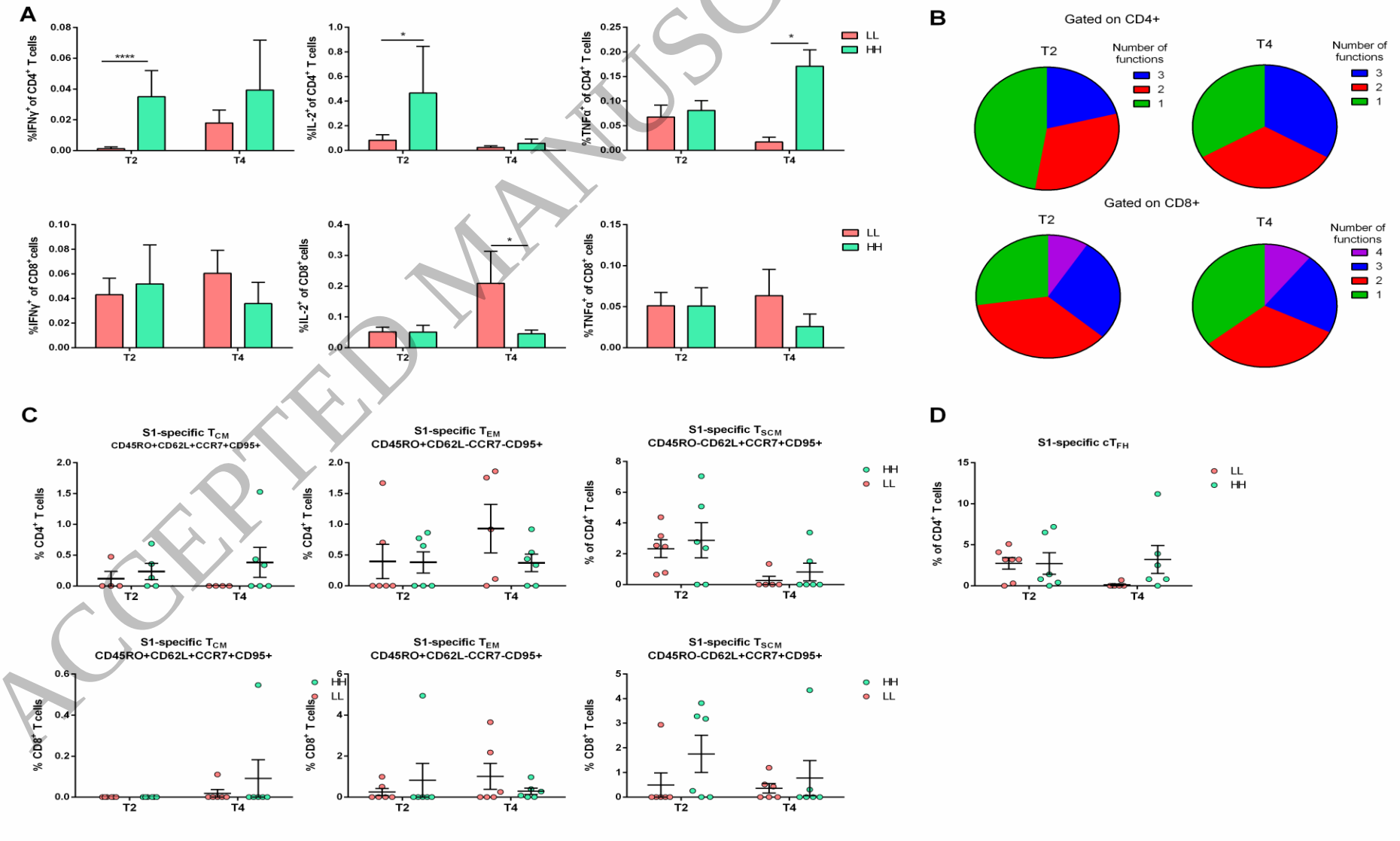

Figure 3
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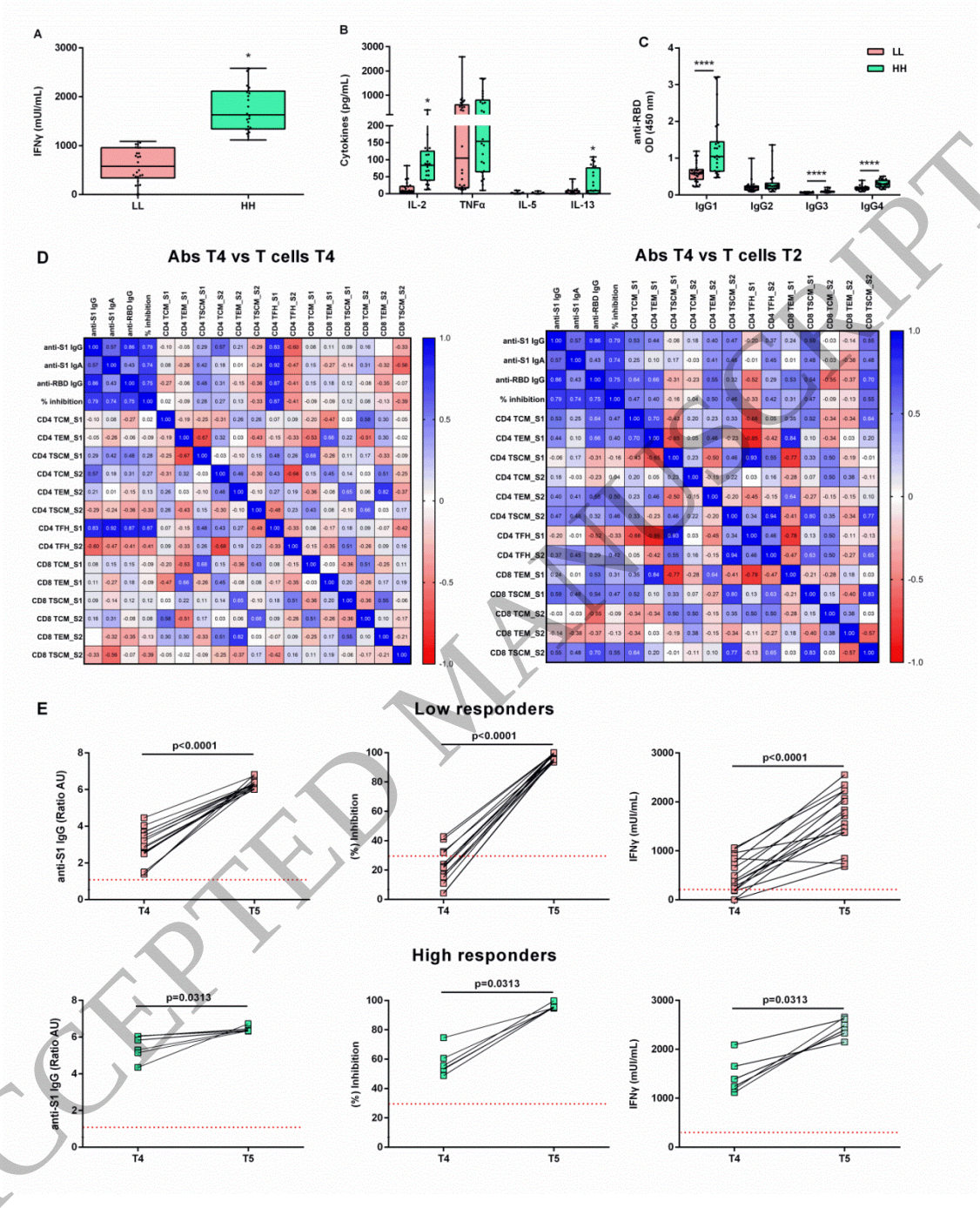

Figure 4

203x248 mm (x DPI)