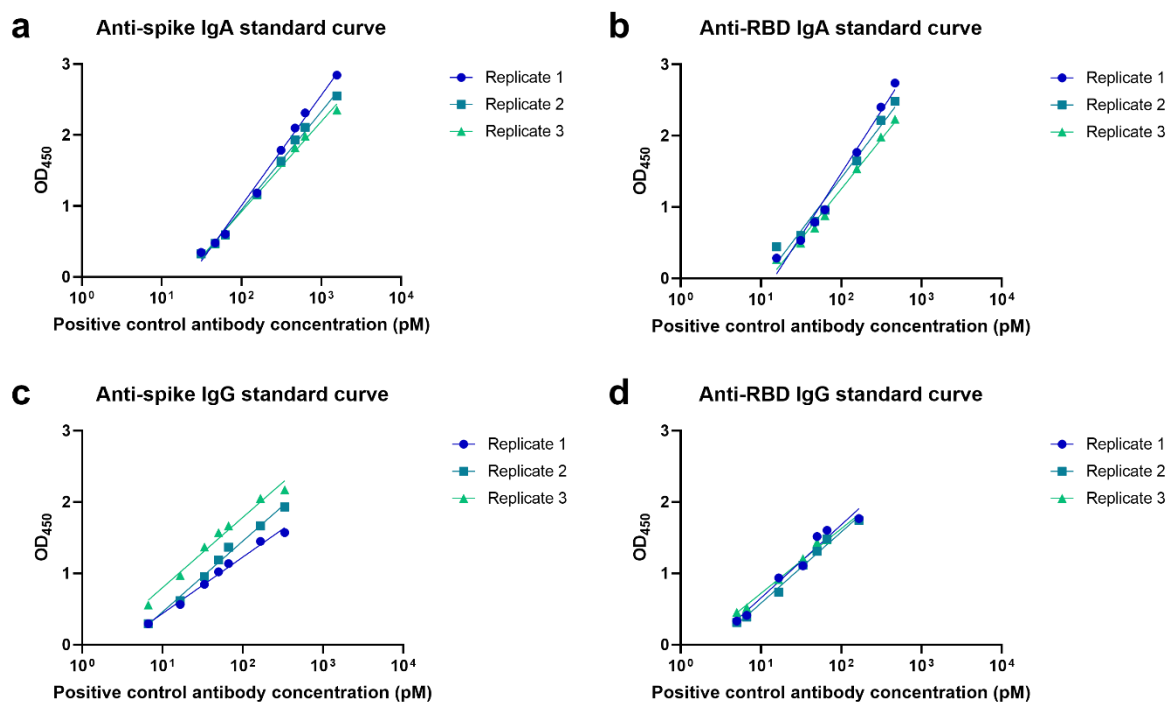# Supplementary Information
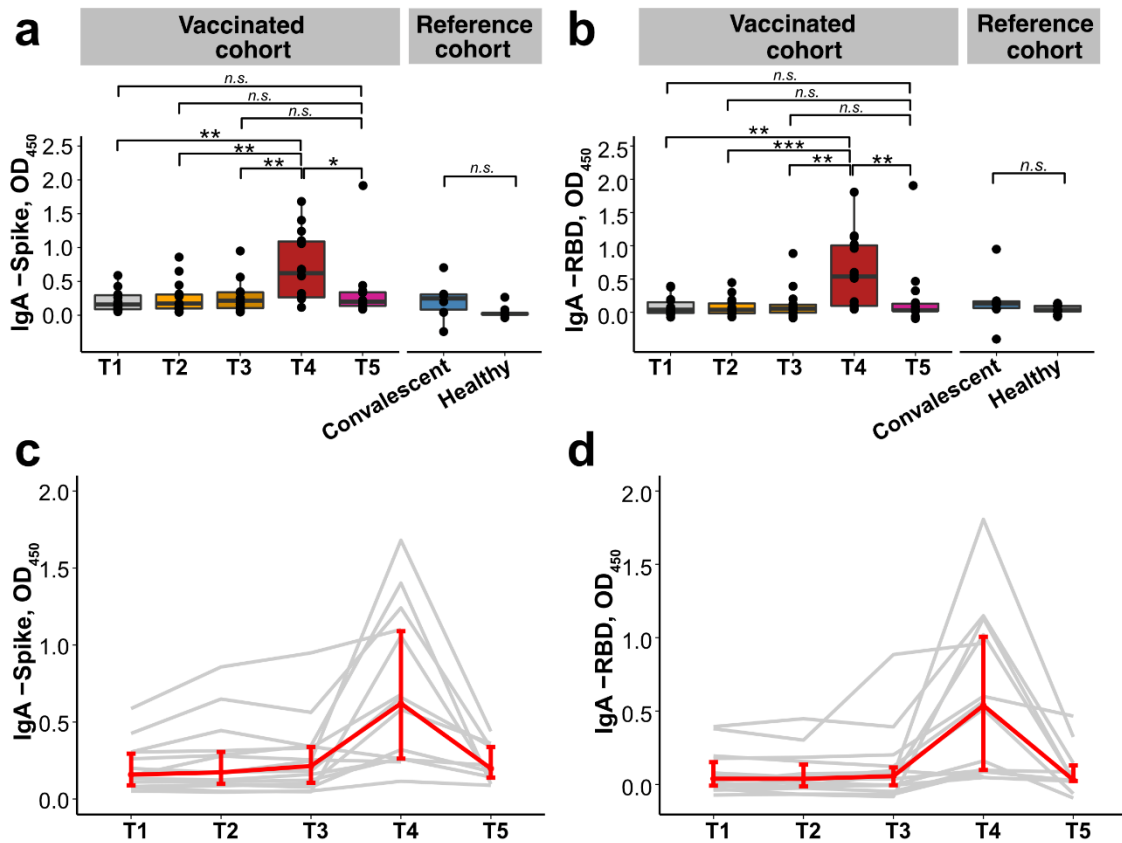
## Supplementary Figures



**Supplementary Figure 1. Standard curves used in quantitative ELISA.** A human monoclonal antibody binding to both full spike and RBD of SARS-CoV-2 was recombinantly expressed as human IgA1 and IgG1. Concentrations of **(a)** anti-spike IgA, **(b)** anti-RBD IgA, **(c)** anti-spike IgG, and **(d)** anti-RBD IgG antibodies in human milk samples were estimated from interpolation of the standard curves constructed using known concentrations of this antibody (in pM[a]).

Abbreviations: RBD, receptor-binding domain; IgA1, immunoglobulin A1; IgG1, immunoglobulin G1; pM, picomolar; $OD_{450}$, optical density measured at a wavelength of 450 nanometer.

[a] SI conversion factors: To convert concentration from pM to M, multiply values by $10^{12}$.

**Supplementary Figure 2. SARS-CoV-2 antigen-specific IgA levels in human milk samples.** $OD_{450}$ values were measured for **(a)** anti-spike and **(b)** anti-RBD IgA antibodies in 10-fold diluted human milk samples collected from vaccinated women (n = 14) at T1-T4 timepoints and (n = 10) at T5 pre- and post- vaccination. Statistics were calculated with Kruskal-Wallis test with Dunn's post-test, *P<.05, **P<.01,***P<.001, n.s., non-significant. The same results are shown as line plots in **(c)** and **(d)** for visualization of antibody response from each individual. Each line in grey represents data from one individual and the median ± IQR is represented in red.

Abbreviations: $OD_{450}$, optical density measured at a wavelength of 450 nanometer; RBD, receptor-binding domain; IgA, immunoglobulin A; IQR, interquartile range; T1, pre-vaccination; T2, 1-3 days after dose one of BNT162b2 vaccine; T3, 7-10 days after dose one of BNT162b2 vaccine; T4, 3-7 days after dose two of BNT162b2 vaccine; T5, 4-6 weeks after dose two of BNT162b2 vaccine.
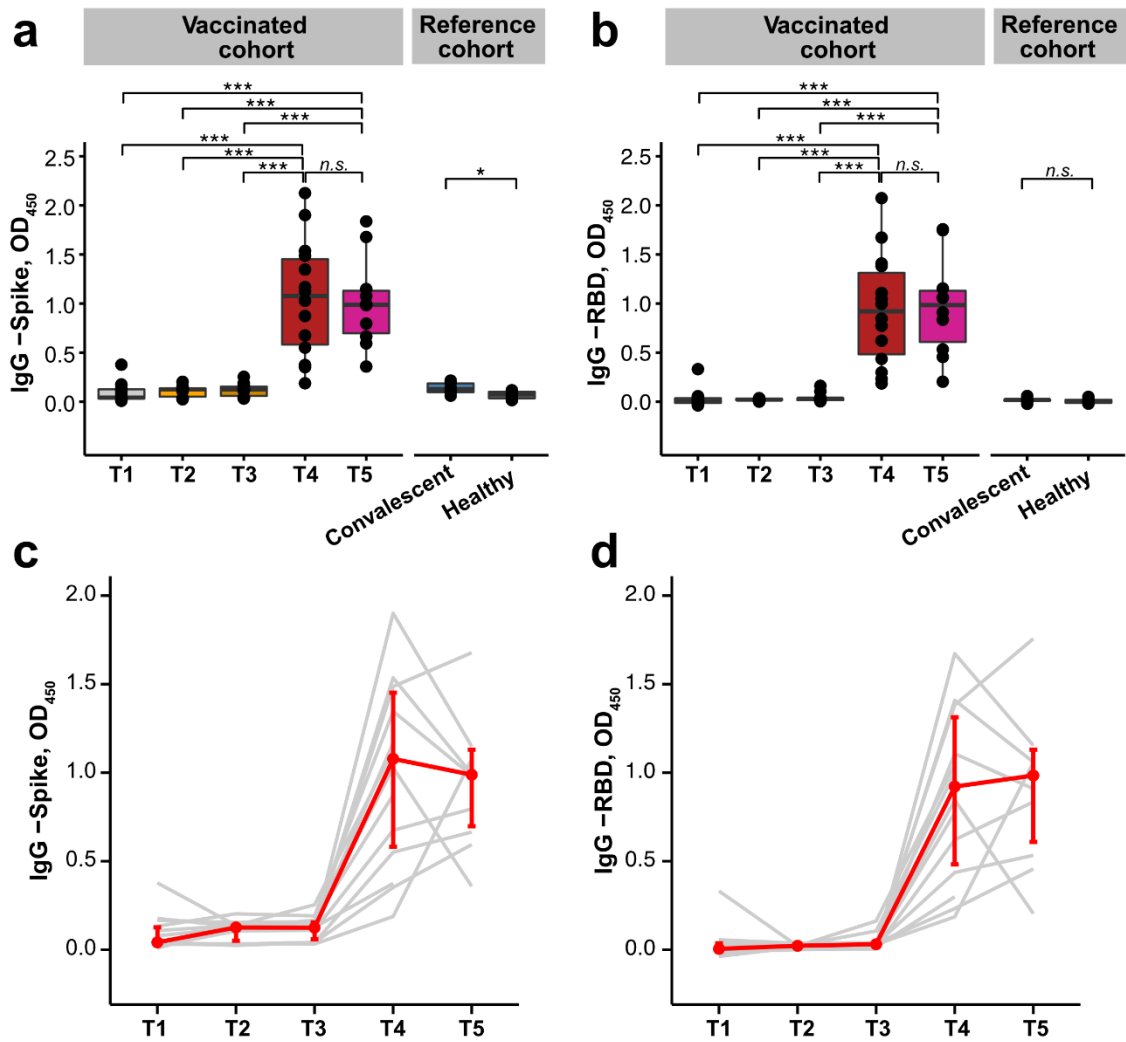
**Supplementary Figure 3. SARS-CoV-2 antigen-specific IgG levels in human milk samples.** $OD_{450}$ values were measured for **(a)** anti-spike and **(b)** anti-RBD IgG antibodies in 10-fold diluted human milk samples collected from vaccinated women (n = 14) at T1-T4 timepoints and (n = 10) at T5 pre- and post- vaccination. Statistics were calculated with Kruskal-Wallis test with Dunn's post-test, *P<.05, **P<.01,***P<.001, n.s., non-significant. The same results are shown as line plots in **(c)** and **(d)** for visualization of antibody response from each individual. Each line in grey represents data from one individual and the median ± IQR is represented in red.

Abbreviations: $OD_{450}$, optical density measured at a wavelength of 450 nanometer; RBD, receptor-binding domain; IgG, immunoglobulin G; IQR, interquartile range; T1, pre-vaccination; T2, 1-3 days after dose one of BNT162b2 vaccine; T3, 7-10 days after dose one

of BNT162b2 vaccine; T4, 3-7 days after dose two of BNT162b2 vaccine; T5, 4-6 weeks after dose two of BNT162b2 vaccine.
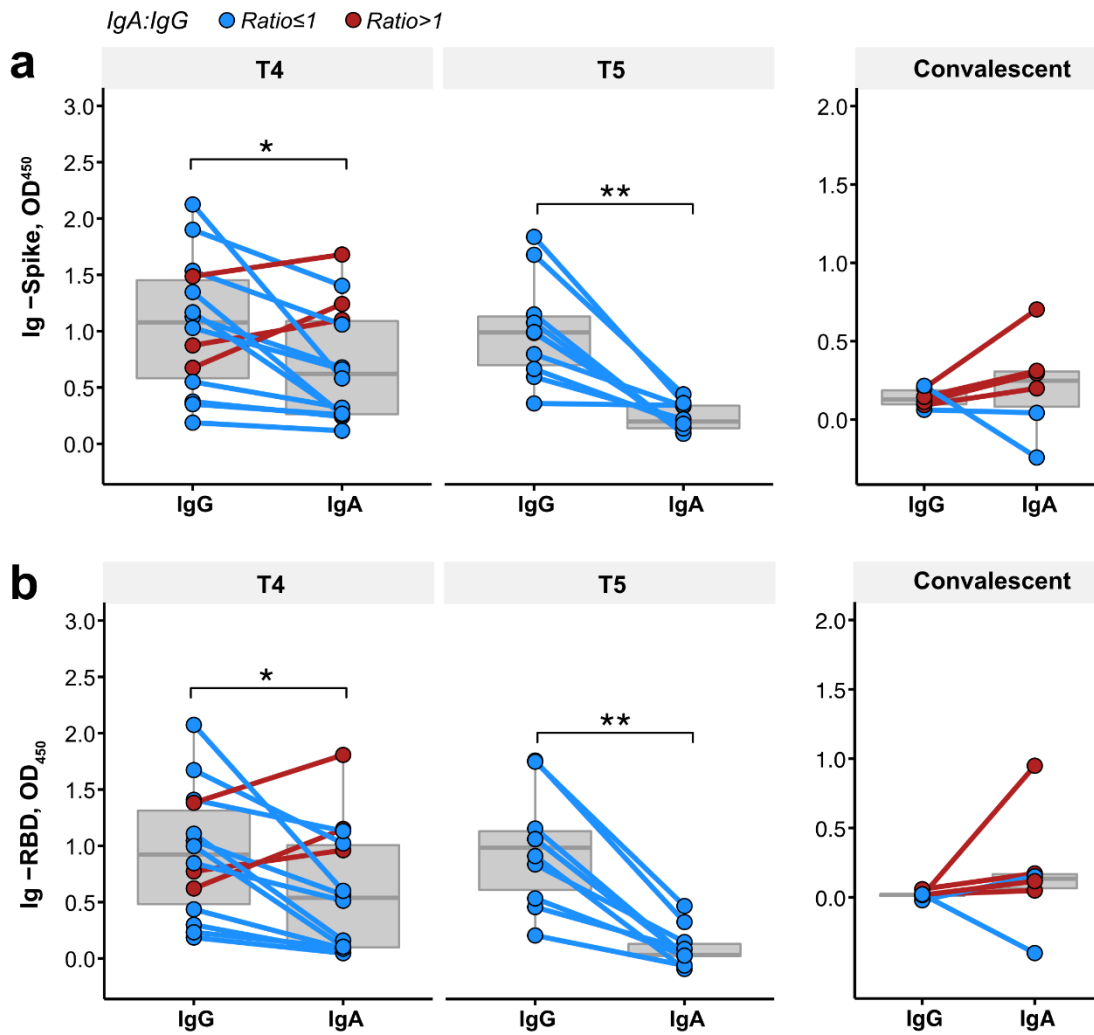
**Supplementary Figure 4. Cross-comparison of SARS-CoV-2 antigen-specific IgA and IgG levels in human milk samples. (a)** $OD_{450}$ values were measured for vaccinated women at T4 (n = 14) and T5 (n = 10) or in convalescent women (right, n = 6) and show anti-spike in 10-fold diluted human milk samples. **(b)** $OD_{450}$ values were measured for vaccinated women at T4 (n = 14) and T5 (n = 10) or in convalescent women (right, n = 6) and show anti-RBD in 10-fold diluted human milk samples. Each solid line represents one individual, and is colored to whether the IgA to IgA ratio was decreased (blue), or increased (red). Ratios were calculated by dividing the concentration of IgA over IgG antibodies. P values were calculated with Wilcoxon signed rank exact test, *P<0.05, **P<0.01.
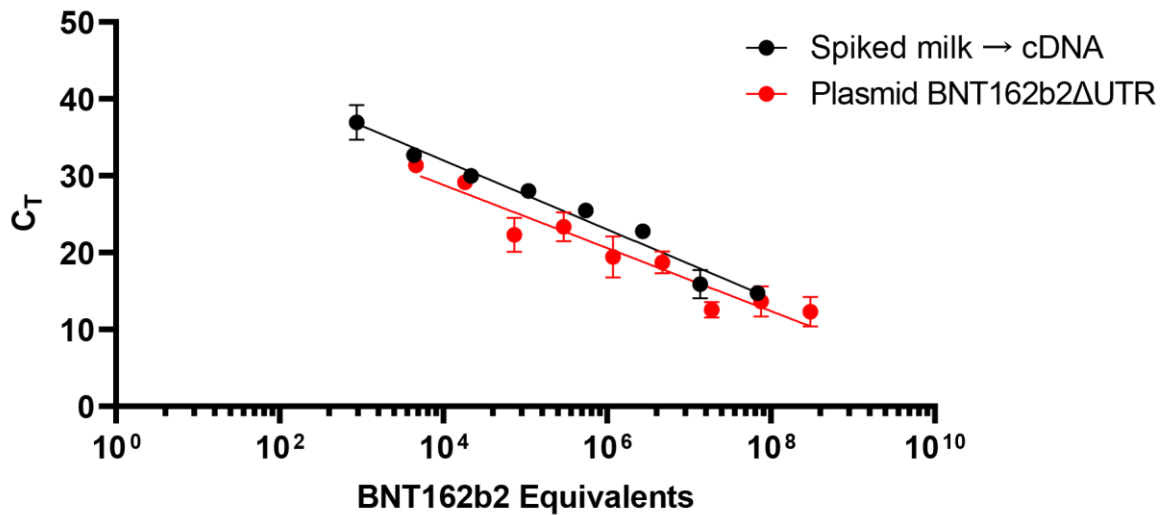
Abbreviations: $OD_{450}$, optical density measured at a wavelength of 450 nanometer; RBD,

receptor-binding domain; IgA, immunoglobulin A; IgG, immunoglobulin G; T4, 3-7 days

after dose two of BNT162b2 vaccine; T5, 4-6 weeks after dose two of BNT162b2 vaccine.

**Supplementary Figure 5. Standard curves using cDNA generated from BNT162b2-spiked human milk versus pTwist-BNT162b2ΔUTR plasmid.** One BNT162b2 equivalent is defined as a single-stranded polynucleotide molecule that contains a linear sequence that binds either the forward or reverse primer. Prism analysis of the two regression lines revealed that their slopes/gradients were not significantly different ($P = .42$). Data represented as the mean ± SEM, n ≥ 3 technical replicates.

Abbreviations: cDNA, complementary DNA; $C_T$, cycle threshold.

**Supplementary Table 1: Demographic and clinical details of infants born to mothers in convalescent and control cohorts**

| Demographic and clinical details of infants | Convalescent cohort (n = 6) | Control cohort (n = 9) |
|---|---|---|
| Mode of delivery | 6 Vaginal | 6 Vaginal |
| | | 3 Caesarean section |
| Gender of infants | 5 Female | 6 Female |
| Infant birth weight, mean [SD], kg | 3.59 [0.24] | 3.11 [0.61] |
| Infant birth length, mean [SD], cm | 50.8 [1.6] | 50.5 [2.2] |
| Infant birth occipital frontal circumference, mean [SD], cm | 34.6 [0.9] | 22.3 [1.2] |
| Any immediate postnatal complications | 5 None | 8 None |
| | 1 Transient tachypnoea of the newborn | 1 Exaggerated physiological jaundice |

**Supplementary Table 2: Clinical details of antenatal COVID-19 and perinatal findings for convalescent cohort (n = 6)**

| Clinical details of antenatal COVID-19 and perinatal findings | |
|---|---|
| COVID-19 diagnosis gestation | 2 First trimester<br><br>1 Second trimester<br><br>3 Third trimester |
| Length of time between COVID-19 diagnosis and delivery, mean [SD], days | 136 [80] |
| Severity of COVID-19 | 1 Asymptomatic<br><br>4 Mild<br><br>1 Moderate |
| Time between first positive PCR and first negative PCR, median (range), days | 23 (17 - 39) |
| SARS-CoV-2 PCR from perinatal samples (nasopharyngeal, vaginal, umbilical cord and placental swabs, maternal blood, cord blood, amniotic fluid, human milk) | All negative |
| Placental / umbilical cord histology | 2 Normal<br><br>3 Abnormal<br><br>1 Not done |
| Abbreviations: COVID-19, coronavirus disease of 2019; PCR, polymerase chain reaction | |

# Supplementary Spreadsheet 1

**Spike (pM)**

|     | T1 | T2 | T3 | T4 |     | T1 | T2 | T3 | T4 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| T2 | 0.348759784 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.490751122 | 0.804224959 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **7.77426E-05** | **0.002576096** | **0.001105746** | NA | T4 | *** | ** | ** | NA |
| T5 | **0.006952115** | **0.065216457** | **0.038447045** | *0.3639543* | T5 | ** | **0.06521646** | * | *n.s.* |

**RBD (pM)**

|     | T1 | T2 | T3 | T4 |     | T1 | T2 | T3 | T4 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| T2 | 0.682652666 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.964745325 | 0.715375337 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **0.005933693** | **0.001576234** | **0.005179982** | NA | T4 | ** | ** | ** | NA |
| T5 | 0.99356138 | 0.714996495 | 0.974249714 | **0.01174336** | T5 | *n.s.* | *n.s.* | *n.s.* | * |

**Spike (OD450)**

|     | T1 | T2 | T3 | T4 |     | T1 | T2 | T3 | T4 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| T2 | 0.686483005 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.561350337 | 0.859347762 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **0.001159137** | **0.004439516** | **0.007632421** | NA | T4 | ** | ** | ** | NA |
| T5 | 0.448153305 | 0.696512151 | 0.819437778 | **0.0273007** | T5 | *n.s.* | *n.s.* | *n.s.* | * |

**RBD (OD450)**

|     | T1 | T2 | T3 | T4 |     | T1 | T2 | T3 | T4 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| T2 | 0.867085576 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.832369848 | 0.70466953 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **0.00130751** | **0.00072042** | **0.002676285** | NA | T4 | ** | *** | ** | NA |
| T5 | 0.9863765 | 0.865124289 | 0.860179423 | **0.00353201** | T5 | *n.s.* | *n.s.* | *n.s.* | ** |

Kruskal-wallis with Dunn's post test

| * | p<0.05 |
| --- | --- |
| ** | p<0.01 |
| *** | p<0.001 |
| n.s. | p>0.05 |

|  | p value |  |
|---|---|---|
| **IgA - Spike (pM)** | 0.02850533 | * |
| **IgA - RBD (pM)** | 0.3541089 | *n.s.* |
| **IgA - Spike (OD450)** | **0.08791209** | *n.s.* |
| **IgA - RBD (OD450)** | 0.1134865 | *n.s.* |
| | | |
| **IgG - Spike (pM)** | 0.7650872 | *n.s.* |
| **IgG - RBD (pM)** | 0.5286713 | *n.s.* |
| **IgG - Spike (OD450)** | 0.03596404 | * |
| **IgG - RBD (OD450)** | NA | *n.s.* |

Mann Whitney test comparing convalescent and

| | |
|---|---|
| * | p<0.05 |
| ** | p<0.01 |
| *** | p<0.001 |
| n.s. | p>0.05 |

**Spike (OD45)**

| | T1 | T2 | T3 | T4 | | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|
| T2 | 0.53510723 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.31767005 | 0.70466659 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **4.52E-07** | **9.63E-06** | **5.20E-05** | NA | T4 | *** | *** | *** | NA |
| T5 | **5.09E-06** | **6.48E-05** | **0.0002635** | *0.9641586* | T5 | *** | *** | *** | *n.s.* |

**RBD (OD450)**

| | T1 | T2 | T3 | T4 | | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|
| T2 | 0.62952891 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.24939125 | 0.50321534 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **6.00E-07** | **6.52E-06** | **0.00012332** | NA | T4 | *** | *** | *** | NA |
| T5 | **2.60E-06** | **2.05E-05** | **0.00026358** | *0.8856655* | T5 | *** | *** | *** | *n.s.* |

**Spike (pM)**

| | T1 | T2 | T3 | T4 | | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|
| T2 | 0.50662712 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.31538257 | 0.73393707 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **3.24E-07** | **8.80E-06** | **4.05E-05** | NA | T4 | *** | *** | *** | NA |
| T5 | **3.28E-07** | **6.78E-06** | **2.79E-05** | *0.6577786* | T5 | *** | *** | *** | *n.s.* |

**RBD (pM)**

| | T1 | T2 | T3 | T4 | | T1 | T2 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|
| T2 | 0.77952923 | NA | NA | NA | T2 | *n.s.* | NA | NA | NA |
| T3 | 0.77952923 | 1 | NA | NA | T3 | *n.s.* | *n.s.* | NA | NA |
| T4 | **6.12E-07** | **1.38E-07** | **1.38E-07** | NA | T4 | *** | *** | *** | NA |
| T5 | **2.44E-05** | **7.62E-06** | **7.62E-06** | *0.7393407* | T5 | *** | *** | *** | *n.s.* |

Kruskal-wallis with Dunn's post test

| | |
|---|---|
| * | p<0.05 |
| ** | p<0.01 |
| *** | p<0.001 |
| n.s. | p>0.05 |

**Spike (pM)**

| | | |
|---|---|---|
| T4 | 0.2676 | *n.s.* |
| T5 | 0.1602 | *n.s.* |
| Convalescent | 0.1814 | *n.s.* |

**RBD (pM)**

| | | |
|---|---|---|
| T4 | 1 | *n.s.* |
| T5 | 0.01285 | * |
| Convalescent | 0.3711 | *n.s.* |

**Spike (OD450)**

| | | |
|---|---|---|
| T4 | 0.04187 | * |
| T5 | 0.001953 | ** |
| Convalescent | 0.4375 | *n.s.* |

**RBD (OD450)**

| | | |
|---|---|---|
| T4 | 0.04944 | * |
| T5 | 0.001953 | ** |
| Convalescent | 0.3125 | *n.s.* |

Wilcox signed rank exact test comparing

| | |
|---|---|
| * | p<0.05 |
| ** | p<0.01 |
| *** | p<0.001 |
| n.s. | p>0.05 |

# Supplementary Spreadsheet 2

**IgA - Spike,pM**

| Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|
| T1 | 14 | 144.9365193 | 111.220315 | 0 | 218.153724 | 218.153724 |
| T2 | 14 | 255.2765672 | 234.473256 | 27.0021855 | 341.867559 | 314.865373 |
| T3 | 14 | 234.8559409 | 117.857385 | 27.102247 | 284.795295 | 257.693048 |
| T4 | 14 | 1499.672907 | 826.835075 | 571.860637 | 1470.26792 | 898.407281 |
| T5 | 10 | 476.8536117 | 498.571931 | 387.887983 | 635.426631 | 247.538648 |

**IgA - RBD,pM**

| Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|
| T1 | 14 | 46.969842 | 0 | 0 | 59.5702703 | 59.5702703 |
| T2 | 14 | 39.58761936 | 0 | 0 | 38.6442398 | 38.6442398 |
| T3 | 14 | 57.30281571 | 0 | 0 | 52.6867318 | 52.6867318 |
| T4 | 14 | 463.3496039 | 281.515753 | 13.4201603 | 555.751253 | 542.331093 |
| T5 | 10 | 74.3794641 | 0 | 0 | 124.887315 | 124.887315 |

**IgA - Spike, OD450**

| Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|
| T1 | 14 | 0.209347619 | 0.1585 | 0.08824167 | 0.29425 | 0.20600833 |
| T2 | 14 | 0.263254762 | 0.173 | 0.09880833 | 0.306 | 0.20719167 |
| T3 | 14 | 0.269661905 | 0.21433333 | 0.10611667 | 0.33866667 | 0.23255 |
| T4 | 14 | 0.704704762 | 0.62081667 | 0.26266667 | 1.09025 | 0.82758333 |
| T5 | 10 | 0.233683333 | 0.19815 | 0.1384 | 0.338475 | 0.200075 |

**IgA - RBD,OD450**

| Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|
| T1 | 14 | 0.088419048 | 0.03866667 | -0.00915 | 0.15194167 | 0.16109167 |
| T2 | 14 | 0.079647619 | 0.03788333 | -0.0136167 | 0.13508333 | 0.1487 |
| T3 | 14 | 0.122345238 | 0.05616667 | -0.005975 | 0.11575 | 0.121725 |
| T4 | 14 | 0.592426191 | 0.53866667 | 0.0981 | 1.00616667 | 0.90806667 |
| T5 | 10 | 0.09707 | 0.03021667 | 0.02248333 | 0.13036667 | 0.10788333 |

**IgG - Spike,pM**

| | Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|---|
| 1 | T1 | 14 | 2.319225286 | 0 | 0 | 0 | 0 |
| 2 | T2 | 14 | 14.62996807 | 0 | 0 | 20.0292023 | 20.0292023 |
| 3 | T3 | 14 | 21.05971486 | 0 | 0 | 33.742577 | 33.742577 |
| 4 | T4 | 14 | 915.3158191 | 392.378148 | 144.46501 | 1000.73262 | 856.267606 |
| 5 | T5 | 10 | 1422.407878 | 657.405417 | 264.878147 | 1227.69151 | 962.813361 |

**IgG - RBD,pM**

| | Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|---|
| 1 | T1 | 14 | 3.214945143 | 0 | 0 | 0 | 0 |
| 2 | T2 | 14 | 0 | 0 | 0 | 0 | 0 |
| 3 | T3 | 14 | 0 | 0 | 0 | 0 | 0 |
| 4 | T4 | 14 | 373.0752676 | 188.458432 | 68.7087688 | 501.146096 | 432.437327 |
| 5 | T5 | 10 | 286.0967628 | 184.11296 | 89.8310483 | 250.409877 | 160.578829 |

**IgG - Spike, OD450**

| | Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|---|
| 1 | T1 | 14 | 0.091647619 | 0.04171667 | 0.03235 | 0.12641667 | 0.09406667 |
| 2 | T2 | 14 | 0.106328571 | 0.12585 | 0.05123333 | 0.13575833 | 0.084525 |
| 3 | T3 | 14 | 0.119116667 | 0.12486667 | 0.058775 | 0.1544 | 0.095625 |
| 4 | T4 | 14 | 1.052276191 | 1.07816667 | 0.58171667 | 1.45166667 | 0.86995 |
| 5 | T5 | 10 | 1.012836667 | 0.98831667 | 0.69756667 | 1.130275 | 0.43270833 |

**IgG - RBD,OD450**

| | Timepoint | N | Mean | Median | Q1 | Q3 | IQR |
|---|---|---|---|---|---|---|---|
| 1 | T1 | 14 | 0.030611905 | 0.00516667 | -0.0118333 | 0.03589167 | 0.047725 |
| 2 | T2 | 14 | 0.021652381 | 0.0222 | 0.01603333 | 0.03005833 | 0.014025 |
| 3 | T3 | 14 | 0.04025 | 0.03108333 | 0.01589167 | 0.03633333 | 0.02044167 |
| 4 | T4 | 14 | 0.934266667 | 0.9215 | 0.48248333 | 1.31333333 | 0.83085 |
| 5 | T5 | 10 | 0.971336667 | 0.98383333 | 0.60866667 | 1.13031667 | 0.52165 |