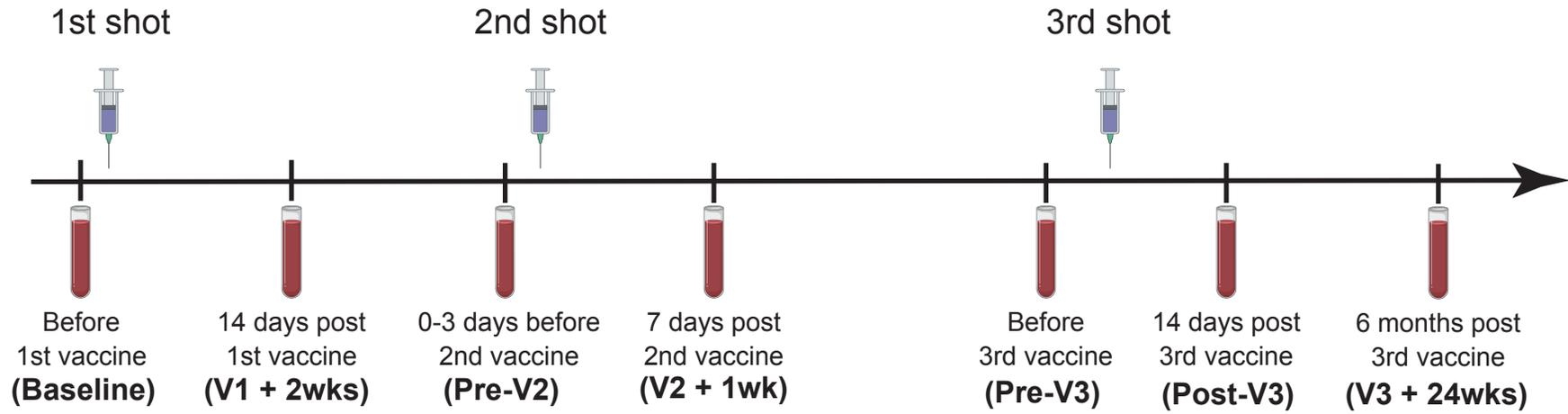


# Supple Fig. 1

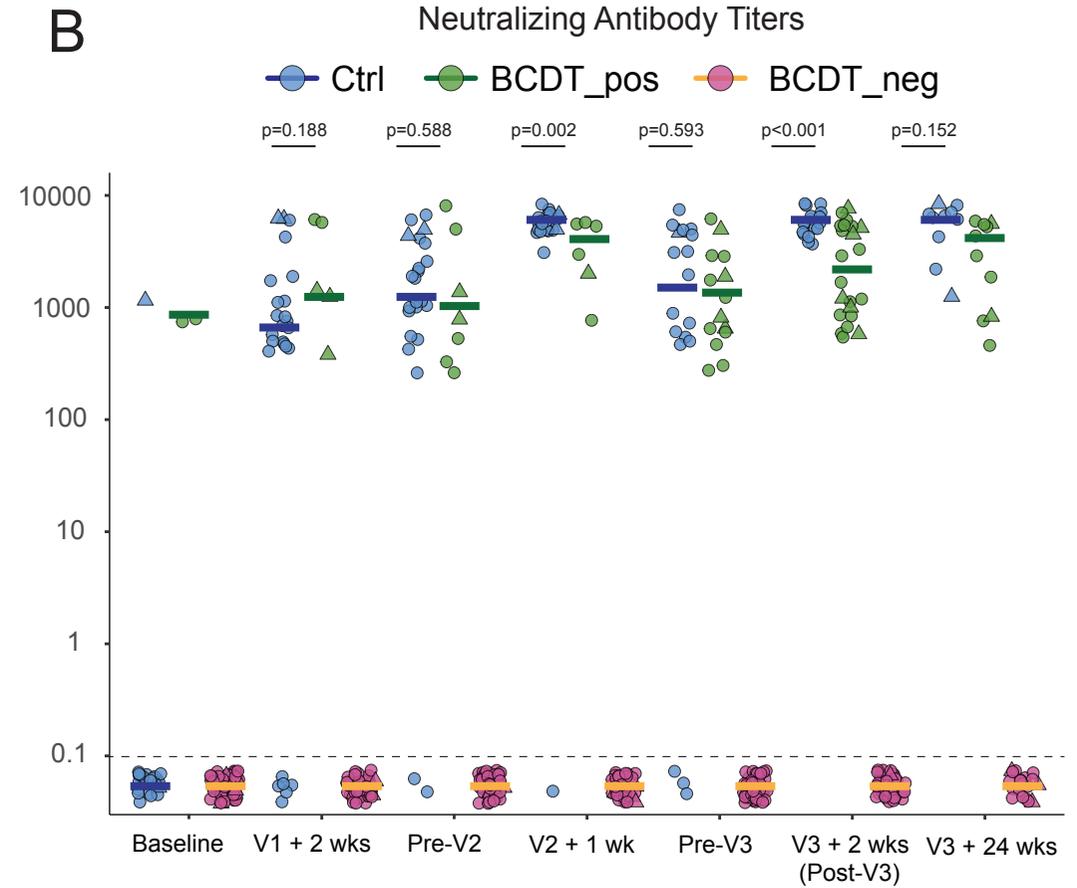
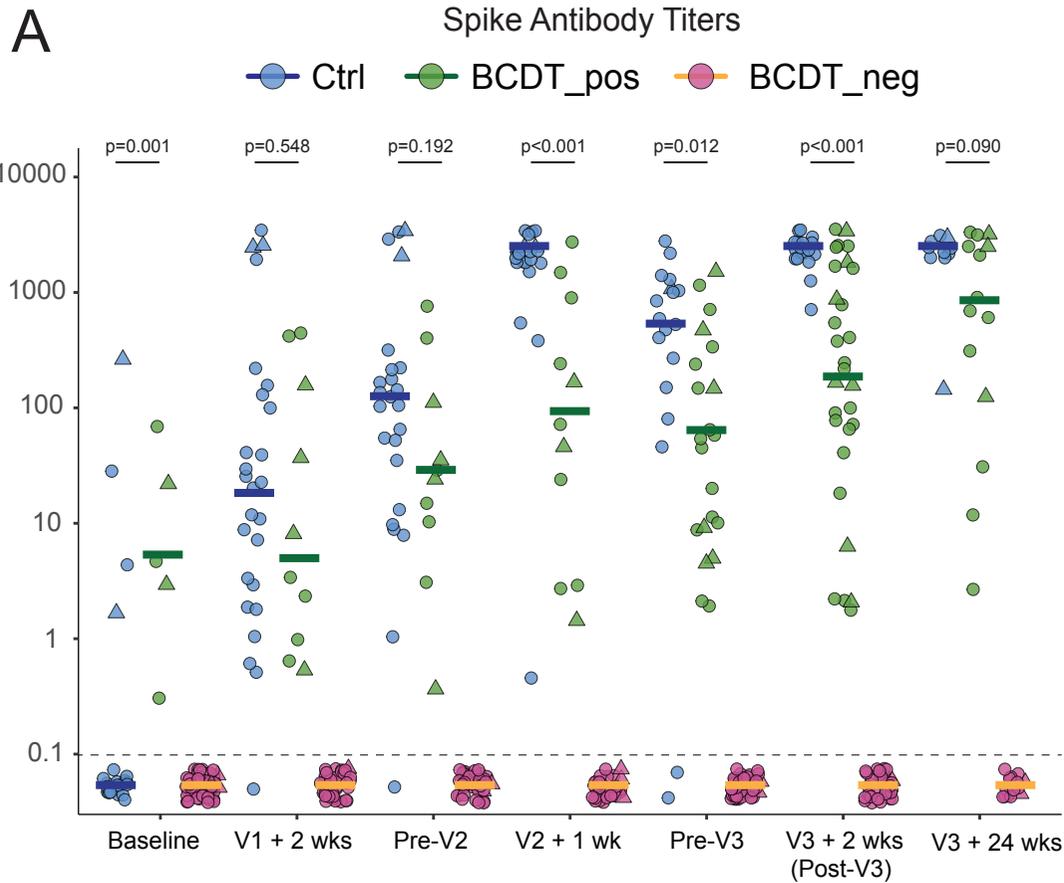


|              |              |           |           |           |           |           |           |           |
|--------------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|              | <b>Total</b> | <b>75</b> | <b>76</b> | <b>71</b> | <b>64</b> | <b>85</b> | <b>82</b> | <b>39</b> |
| <b>Sera</b>  | <b>Ctrl</b>  | <b>22</b> | <b>26</b> | <b>24</b> | <b>22</b> | <b>18</b> | <b>18</b> | <b>10</b> |
|              | [ HD ]       | 12        | 14        | 13        | 11        | 9         | 9         | 6         |
|              | [ DC ]       | 10        | 12        | 11        | 11        | 9         | 9         | 4         |
|              | <b>BCDT</b>  | <b>53</b> | <b>50</b> | <b>47</b> | <b>42</b> | <b>67</b> | <b>64</b> | <b>29</b> |
|              | <b>Total</b> | <b>40</b> | —         | —         | —         | <b>58</b> | <b>58</b> | —         |
| <b>PBMCs</b> | <b>Ctrl</b>  | <b>17</b> | —         | —         | —         | <b>10</b> | <b>10</b> | —         |
|              | [ HD ]       | 8         | —         | —         | —         | 7         | 7         | —         |
|              | [ DC ]       | 9         | —         | —         | —         | 3         | 3         | —         |
|              | <b>BCDT</b>  | <b>23</b> | —         | —         | —         | <b>48</b> | <b>48</b> | —         |

## Supplemental Figure 1. Experimental study design

Longitudinal study design and time points collected pre- and post-SARS-CoV-2 mRNA vaccination for controls and patients with anti-CD20 mAbs. Total sample numbers at each timepoint were denoted and numbers in each group are shown. Sera were evaluated for antibodies and proteomics, and PBMCs were evaluated for spike-specific T cells and B cells. Ctrl; control subjects, BCDT; patients with B cell depletion therapy, HC; healthy controls, DC; disease controls

# Supple Fig. 2



**C** Multivariate logistic regression for presence of anti-spike-antibodies among BCDT

|   | OR (95% CI)        | p-value |
|---|--------------------|---------|
| Age                                     | 1.04 (1.00, 1.07)  | 0.07    |
| Gender, M vs F                          | 0.56 (0.16, 1.89)  | 0.35    |
| BMI                                     | 1.04 (0.98, 1.11)  | 0.17    |
| Time from last $\alpha$ CD20 Abs to V3  | 1.00 (0.98, 1.02)  | 0.79    |
| Prior cycles of $\alpha$ CD20 Abs       | 0.82 (0.68, 0.97)  | 0.03    |
| Previous COVID-19 infections, Yes vs No | 4.96 (1.32, 18.56) | 0.02    |

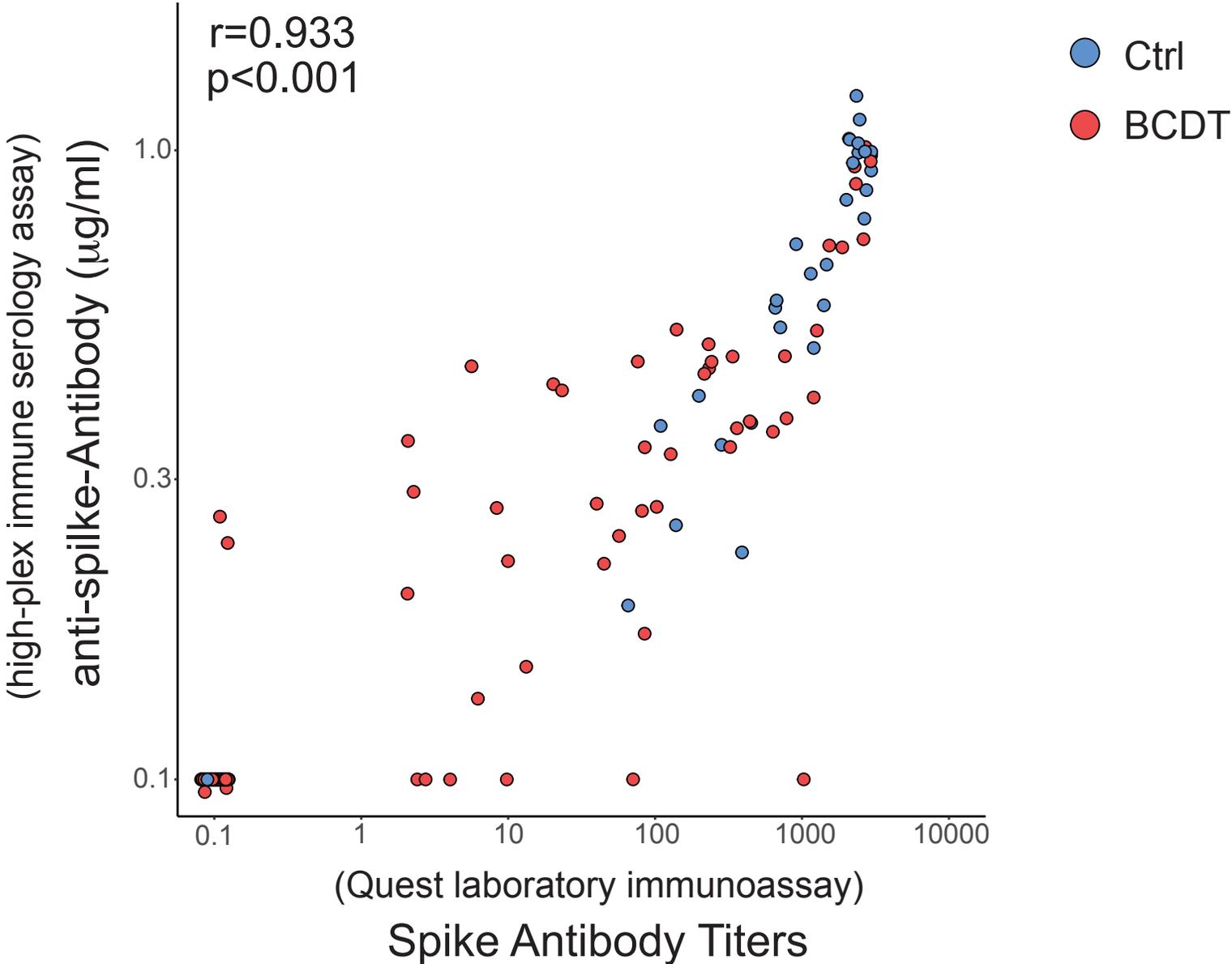
**D** Multivariate logistic regression for presence of anti-neutralizing-antibodies among BCDT

|   | OR (95% CI)        | p-value |
|---|--------------------|---------|
| Age                                     | 1.03 (0.99, 1.07)  | 0.13    |
| Gender, M vs F                          | 0.53 (0.14, 2.05)  | 0.36    |
| BMI                                     | 1.03 (0.97, 1.10)  | 0.37    |
| Time from last $\alpha$ CD20 Abs to V3  | 1.00 (0.98, 1.03)  | 0.75    |
| Prior cycles of $\alpha$ CD20 Abs       | 0.79 (0.64, 0.96)  | 0.02    |
| Previous COVID-19 infections, Yes vs No | 4.39 (1.24, 15.49) | 0.02    |

**Supplemental Figure 2. Humoral vaccine responses among patients with B cell depletion**

**A and B**, Dot plots of anti-SARS-CoV-2 spike antibody titers (**A**) and neutralizing antibody titers (**B**) were evaluated from pre 1st vaccine (Baseline) to 6 months post 3rd vaccine (V3 + 24 weeks) among controls (Ctrl), SARS-CoV-2-seropositive patients with B cell depleted (B cell depleted\_pos) and seronegative patients with B cell depleted (B cell depleted\_neg). Circles represent participants without documented COVID-19 infections and triangles show those with a known infection at each time point. The median is marked by a horizontal line. The dotted line denotes the threshold of detection. Data were evaluated by two-tailed independent samples t-test. **C and D**, Multivariate logistic regression models predicting positive anti-spike antibody titers (**C**) or anti-neutralizing antibody titers (**D**) in B cell depleted subjects. Odds ratio (OR) and 95% confidence intervals (95% CI) are shown.

# Supple Fig. 3

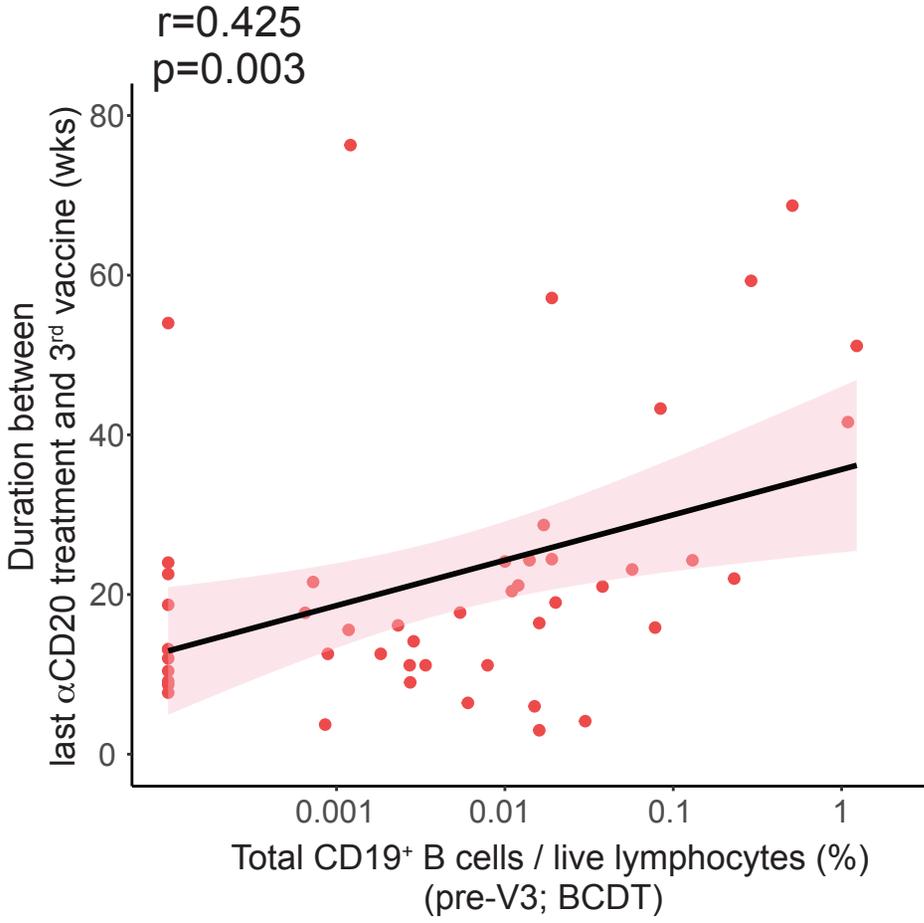


### Supplemental Figure 3. Validation of our high-plex immune-serology assay

Correlation between anti-SARS-CoV-2 spike antibody titers from commercial immunoassays and the concentration (µg/ml) of anti-SARS-CoV-2 spike antibodies from our high-plex immune-serology assays. 156 samples (31 from controls and 125 from subjects with B cell depletion) were applied and correlation statistics were performed by two-tailed Spearman's rank correlation test.

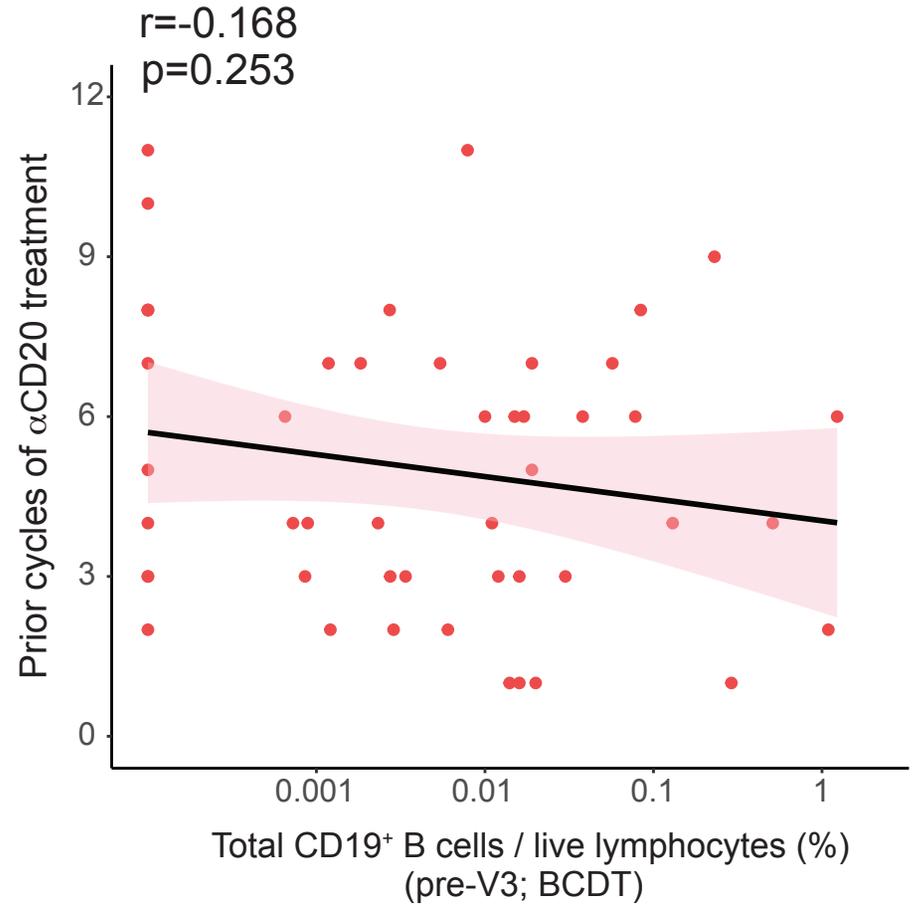
# Supple Fig. 4

A



● BCDT

B



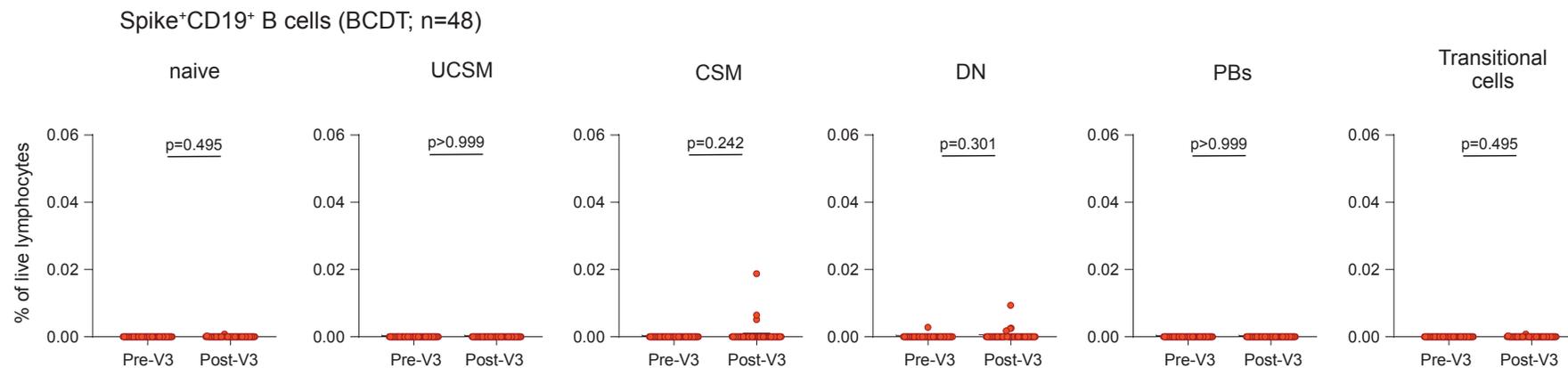
● BCDT

## Supplemental Figure 4. Correlations between total CD19+ B cells in blood and clinical information

**A and B**, Correlation between total CD19+ B cells at pre-V3 and the duration between last cycles of anti-CD20 antibodies and 3<sup>rd</sup> vaccine (weeks) (**A**) or prior cycles of anti-CD20 antibodies at pre-V3 (**B**). Samples at pre-V3 of B cell depleted subjects (n=48) were applied. Linear regression is shown with 95% confidence interval (pink area) and correlation statistics by two-tailed Spearman's rank correlation test were performed.

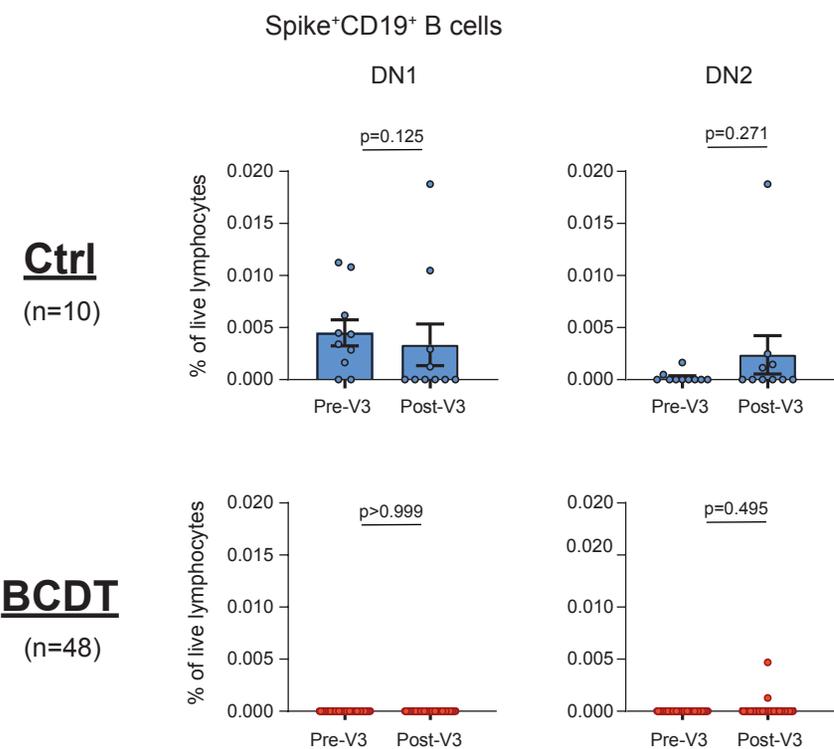
# Supple Fig. 5

**A**



**BCDT**

**B**



**Ctrl**

(n=10)

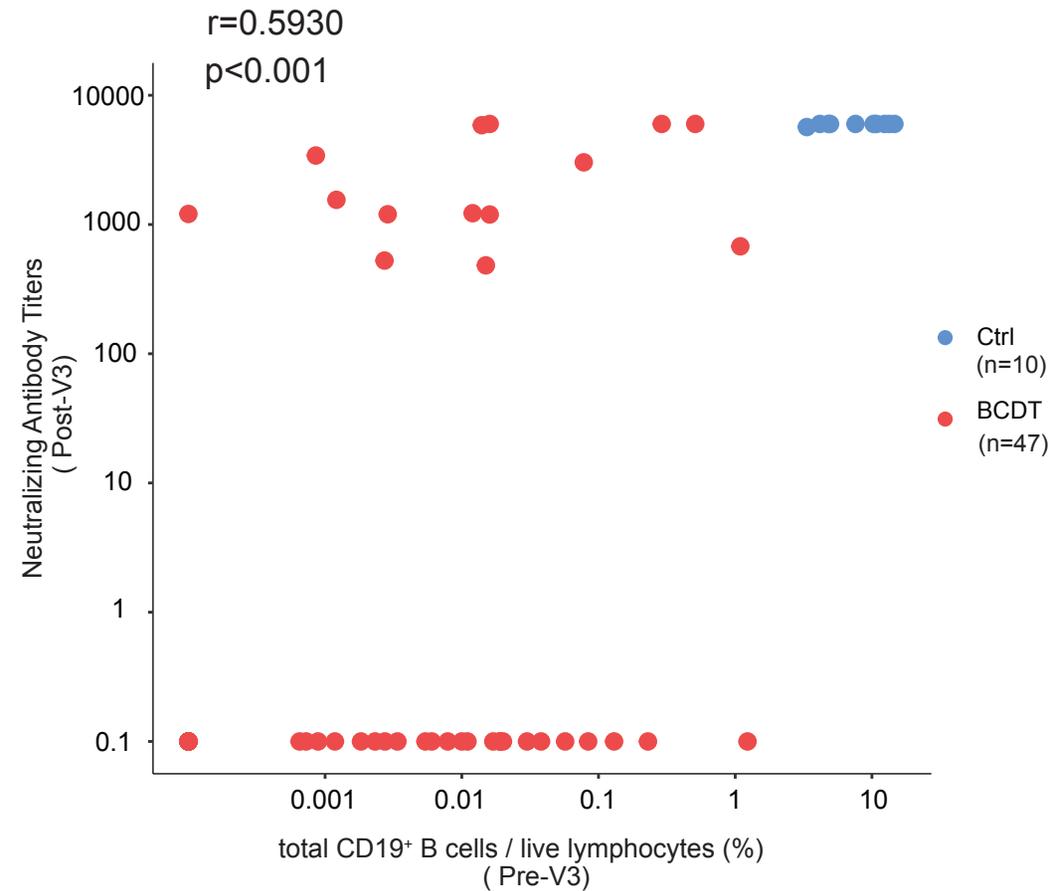
**BCDT**

(n=48)

## Supplemental Figure 5. Subsets of spike-specific B cells

**A**, The proportions of spike-specific B cells between pre-V3 and post-V3 in B cell depleted patients (n=48). UCSM; IgD+CD27+ unclass-switched memory B cells, CSM; IgD-CD27+ class-switched memory B cells, DN; IgD-CD27- double-negative B cells. **B** The proportions of DN cells between pre-V3 and post-V3. DN1; IgD-CD27-CD11c-CXCR5+ double-negative 1 cells, DN2; IgD-CD27-CD11c+CXCR5- double-negative 2 cells.

# Supple Fig. 6



● BCDT (n=47)

|  | Neut. Ab Titer > 0<br>(Post-V3) | Neut. Ab Titer = 0<br>(Post-V3) | p-value             |
|--|---------------------------------|---------------------------------|---------------------|
| n  | 14                              | 33                              |                     |
| Age (yrs), Mean (SD)                                 | 49 (15.7)                       | 48 (15.0)                       | p=0.93              |
| Sex, n(%)  | M: 2 (14.3)<br>F: 12 (85.7)     | M: 5 (15.2)<br>F: 28 (84.8)     | p=1.00 <sup>§</sup> |
| BMI (kg/m <sup>2</sup> )<br>Mean (SD)                | 28.5 (7.6)                      | 27.8 (6.0)                      | p=0.99              |
| Prior Cycles of αCD20<br>Mean (SD)                   | 3.6 (2.7)                       | 5.4 (2.3)                       | p=0.01*             |
| Time from αCD20 to 3rd shot<br>(wks), Median (Range) | 18.8 (3.0 - 76.3)               | 17.7 (4.1 - 57.1)               | p=0.46              |

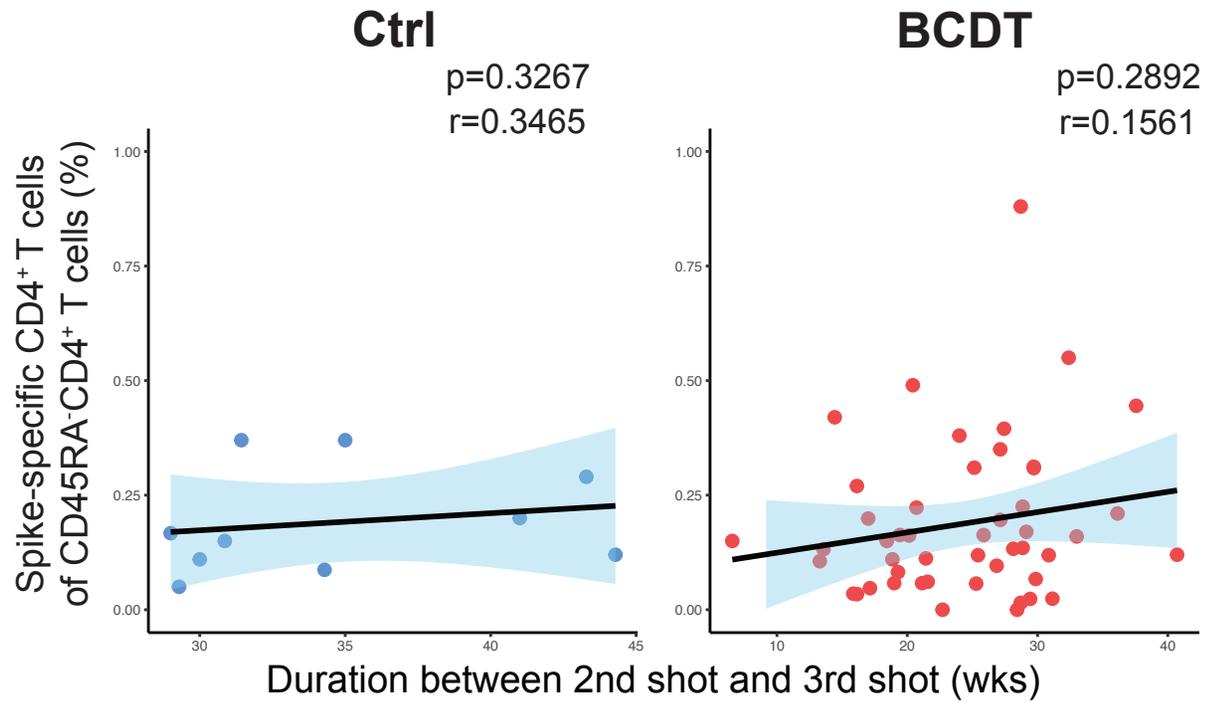
## Supplemental Figure 6. Relationships between clinical characteristics and neutralizing antibodies

The proportion of CD19+ B cells at the pre-V3 timepoint was correlated with neutralizing antibody titers at the post-V3 timepoint in B cell depleted subjects (n=47). Correlation was performed using two-tailed Spearman's rank correlation test (left). Clinical information between neutralizing antibody producers (titers > 0 at post-V3; n=14) and antibody non-producers (titers = 0 at post-V3; n=33) after B cell depletion is shown (right). §denotes Fisher exact test; other p-values were calculated using two-tailed independent samples t-test. \*denotes statistical significance (p<0.05).

# Supple Fig. 7

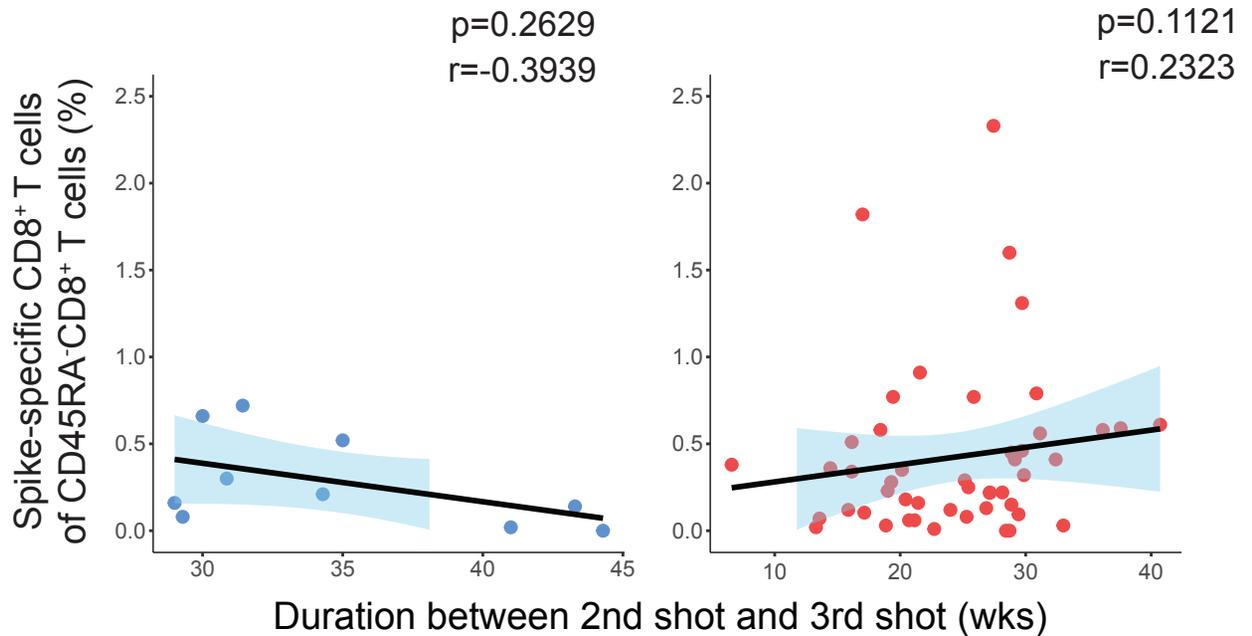
## A

### Spike-CD4<sup>+</sup> T cells



## B

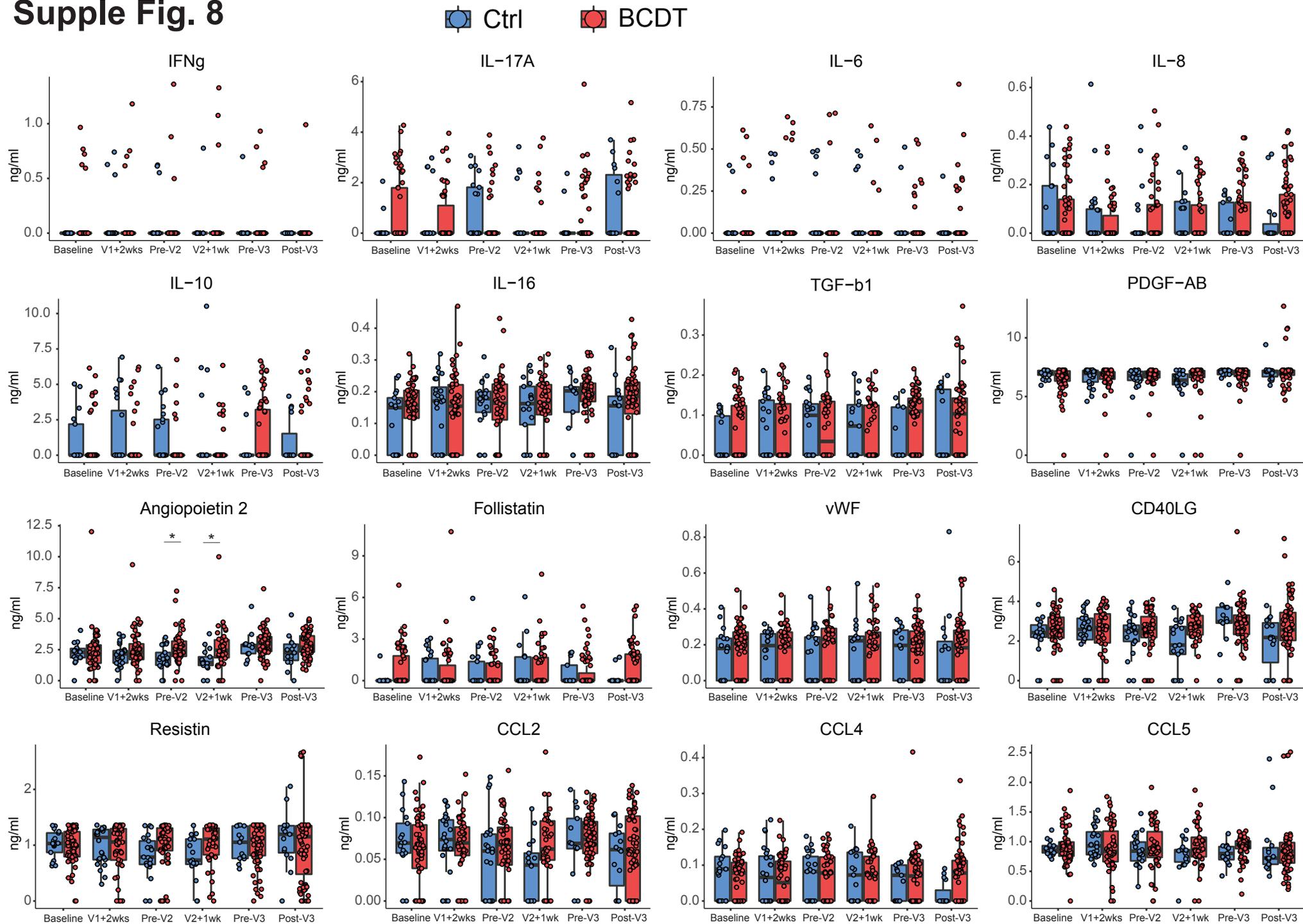
### Spike-CD8<sup>+</sup> T cells



### Supplemental Figure 7. Relationships between time to third vaccine and spike-specific T cell responses

**A and B**, The proportion of spike-specific CD4<sup>+</sup> T cells (**A**) or CD8<sup>+</sup> T cells (**B**) at the pre-V3 timepoint was correlated with the duration between 2nd and 3rd vaccines (Ctrl: n=10, BCDT: n=48). Linear regression is shown with 95% confidence interval (sky blue area). Correlation was performed using two-tailed Spearman's rank correlation test. Ctrl: control, BCDT: B cell depletion therapy.

# Supple Fig. 8



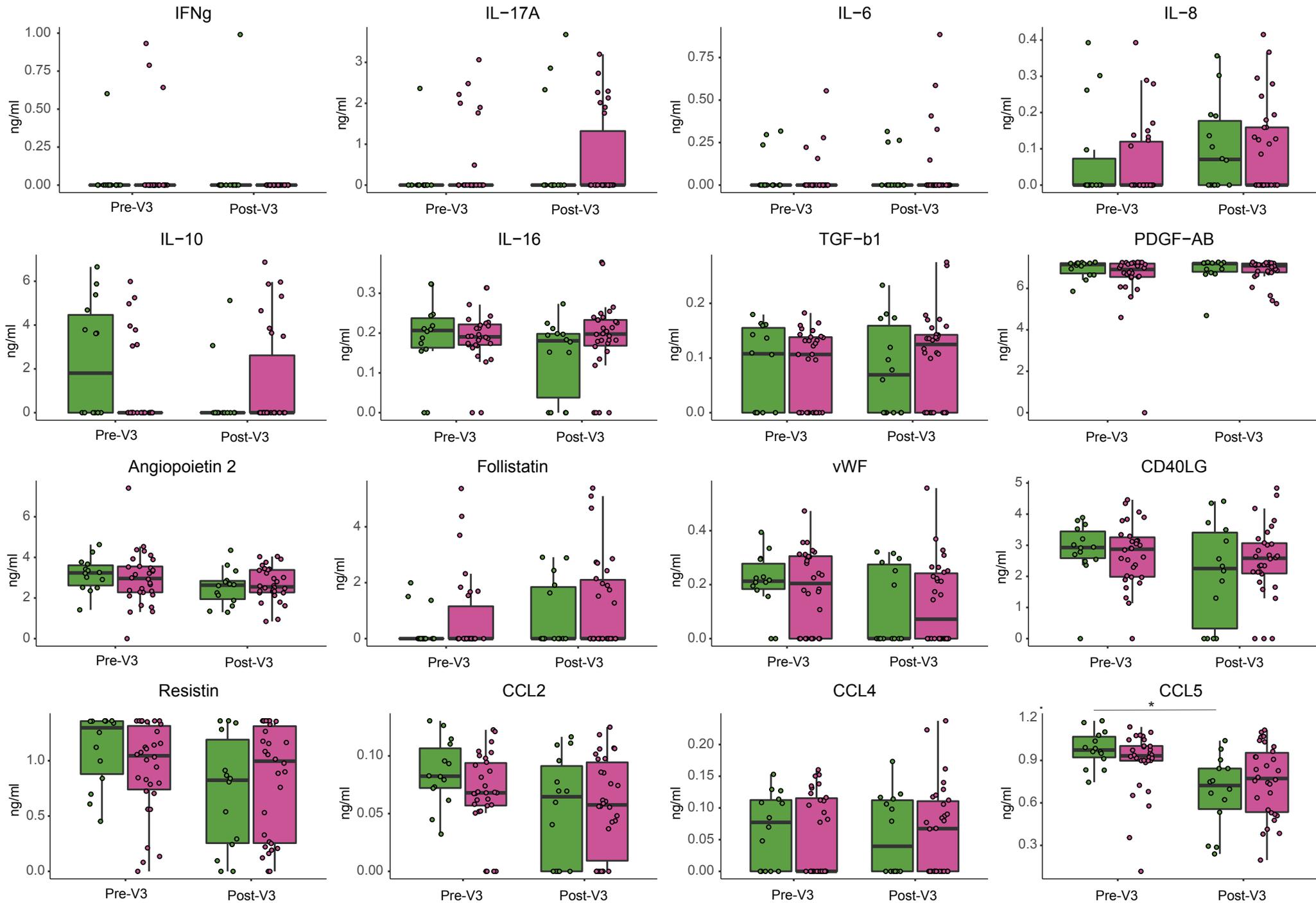
**Supplemental Figure 8. Longitudinal proteomics data between controls and patients with B cell depletion therapy**

The concentrations of each protein were evaluated cross-sectionally between controls and B cell depletion. A total of 432 samples were evaluated and numbers at each time point were as follows; Baseline (control; n=17, B cell depleted; n=57), V1+2 weeks (control; n=24, B cell depleted; n=50), Pre-V2 (control; n=21, B cell depleted; n=46), V2+1week (control; n=17, B cell depleted; n=41), Pre-V3 (control; n=13, B cell depleted; n=67), Post-V3 (control; n=15, B cell depleted; n=64).

# Supple Fig. 9

● Increased spike-Abs post-V3 (n=14)

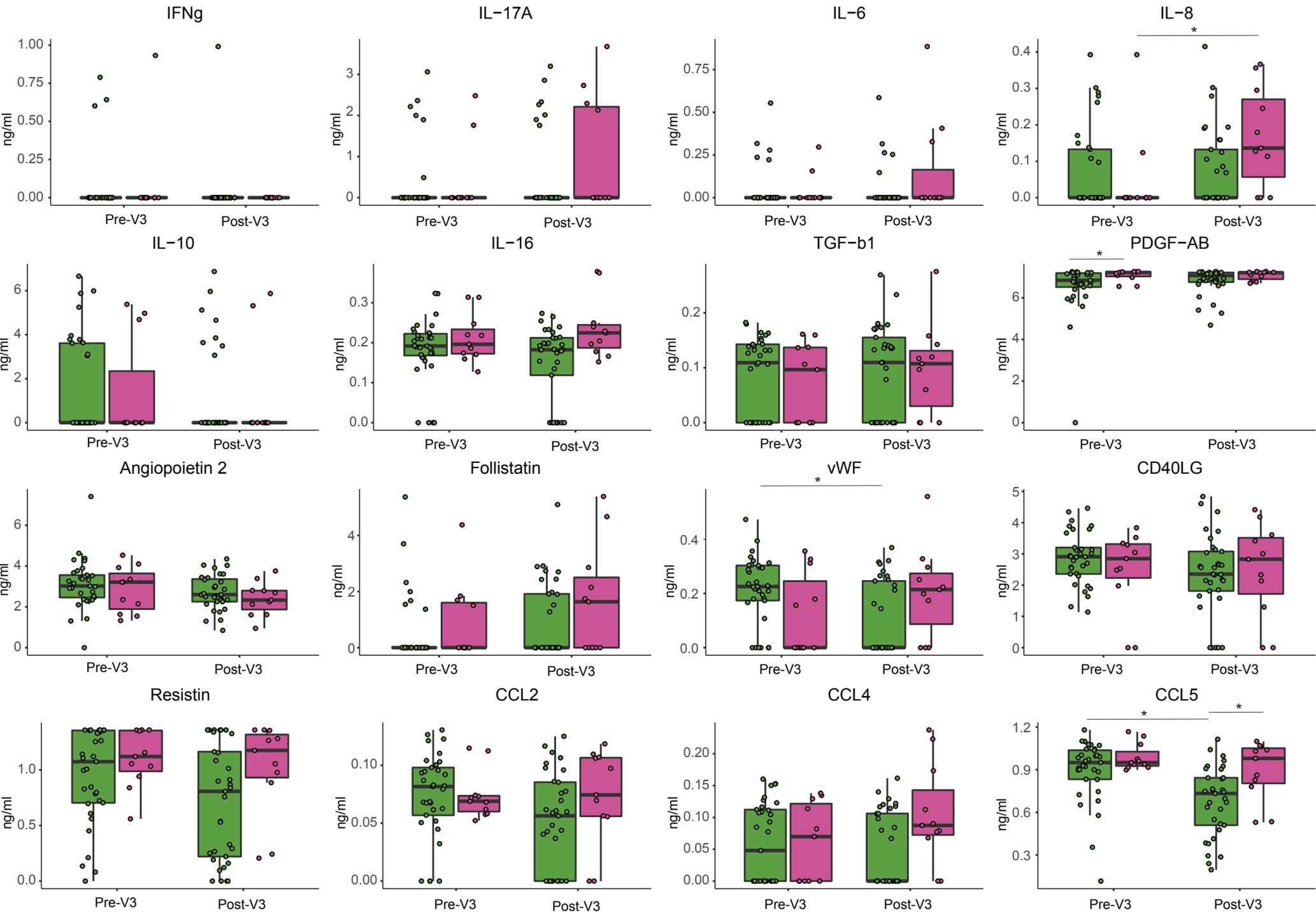
● Non-increased spike-Abs post-V3 (n=30)



## Supplemental Figure 9. The relationship between humoral immune responses and proteomics in B cell depletion

The concentrations of each protein pre- and post-V3 were evaluated between B cell depleted subjects with increased anti-spike antibody titers post-V3 (n=14) and subjects with no increase (n=30). Independent samples t-tests with Bonferroni correction were performed ( $p < 0.0125$  as significant).

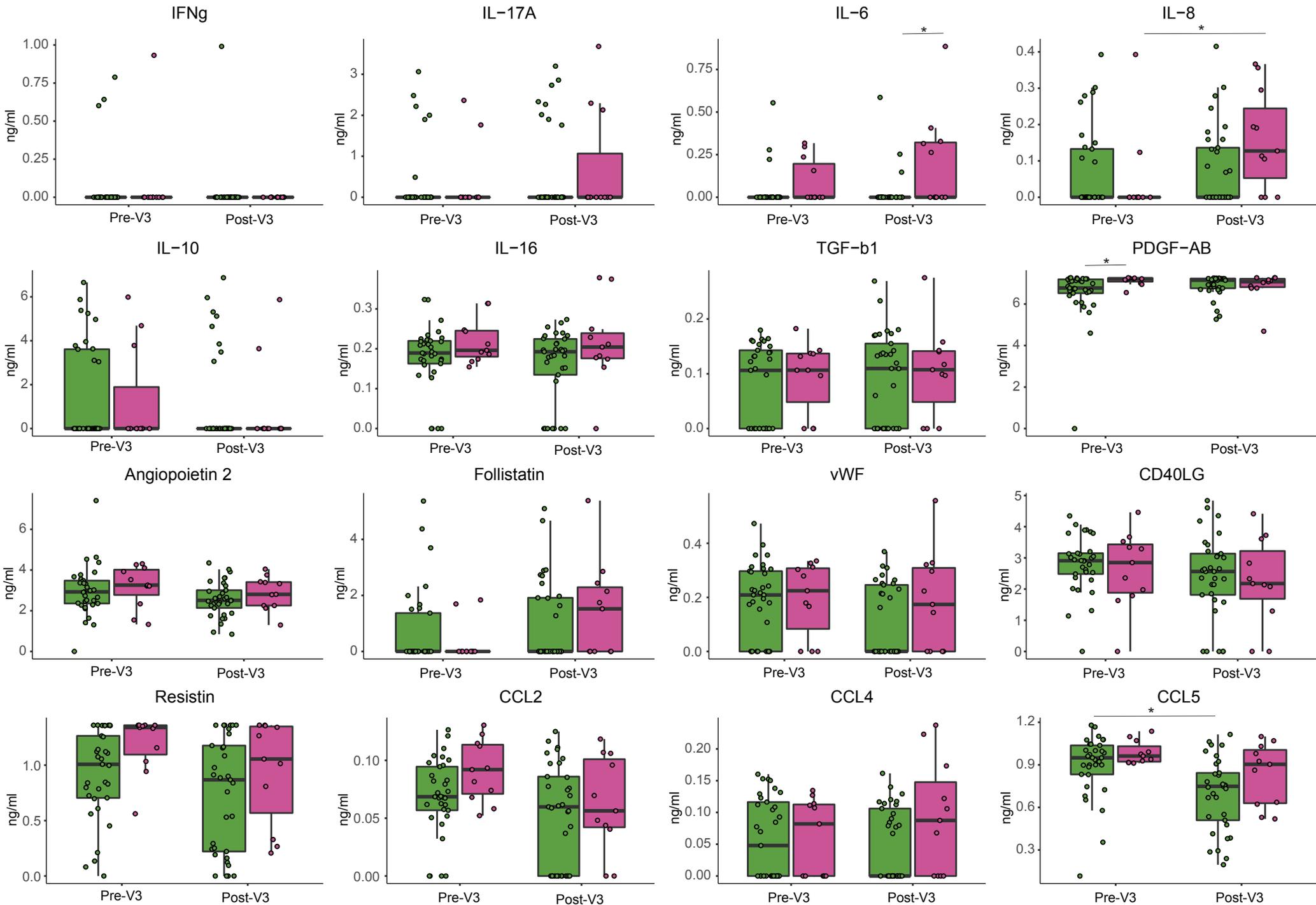
**Supple Fig. 10** ■ Increased spike-specific CD4<sup>+</sup>T post-V3 (n=33) ■ Non-increased spike-specific CD4<sup>+</sup>T post-V3 (n=11)



**Supplemental Figure 10. The relationship between spike-specific CD4<sup>+</sup> T cell responses and proteomics in B cell depleted patients**

The concentrations of each protein pre- and post-V3 were evaluated between B cell depleted subjects with increased spike-specific CD4<sup>+</sup> T cells post-V3 (n=33) and subjects with no increase (n=11). Independent samples t-tests with Bonferroni correction were performed (p < 0.0125 as significant).

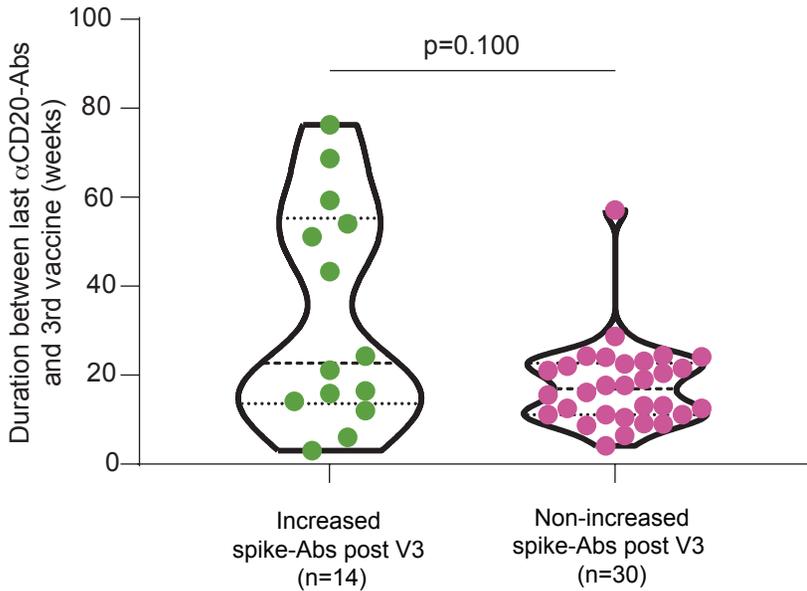
**Supple Fig. 11** ■ Increased spike-specific CD8<sup>+</sup>T post-V3 (n=33) ■ Non-increased spike-specific CD8<sup>+</sup>T post-V3 (n=11)



**Supplemental Figure 11. The relationship between spike-specific CD8<sup>+</sup> T cell responses and proteomics in B cell depleted patients**

The concentrations of each protein pre- and post-V3 were evaluated between B cell depleted subjects with increased spike-specific CD8<sup>+</sup> T cells post-V3 (n=33) and subjects with no increase (n=11). Independent samples t-tests with Bonferroni correction were performed (p<0.0125 as significant).

# Supple Fig. 12



**Supplemental Figure 12. The duration between last  $\alpha$ CD20-Abs and 3rd vaccine in B cell depleted patients**

Duration between last anti-CD20 antibodies and 3rd vaccine in B cell depleted subjects with increased anti-spike antibodies (n=14) and without an increase (n=30). Two-tailed independent samples t-test was performed.