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Supplemental information

A boost with SARS-CoV-2 BNT162b2 mRNA vaccine elicits strong humoral responses independently of the interval between the first two doses

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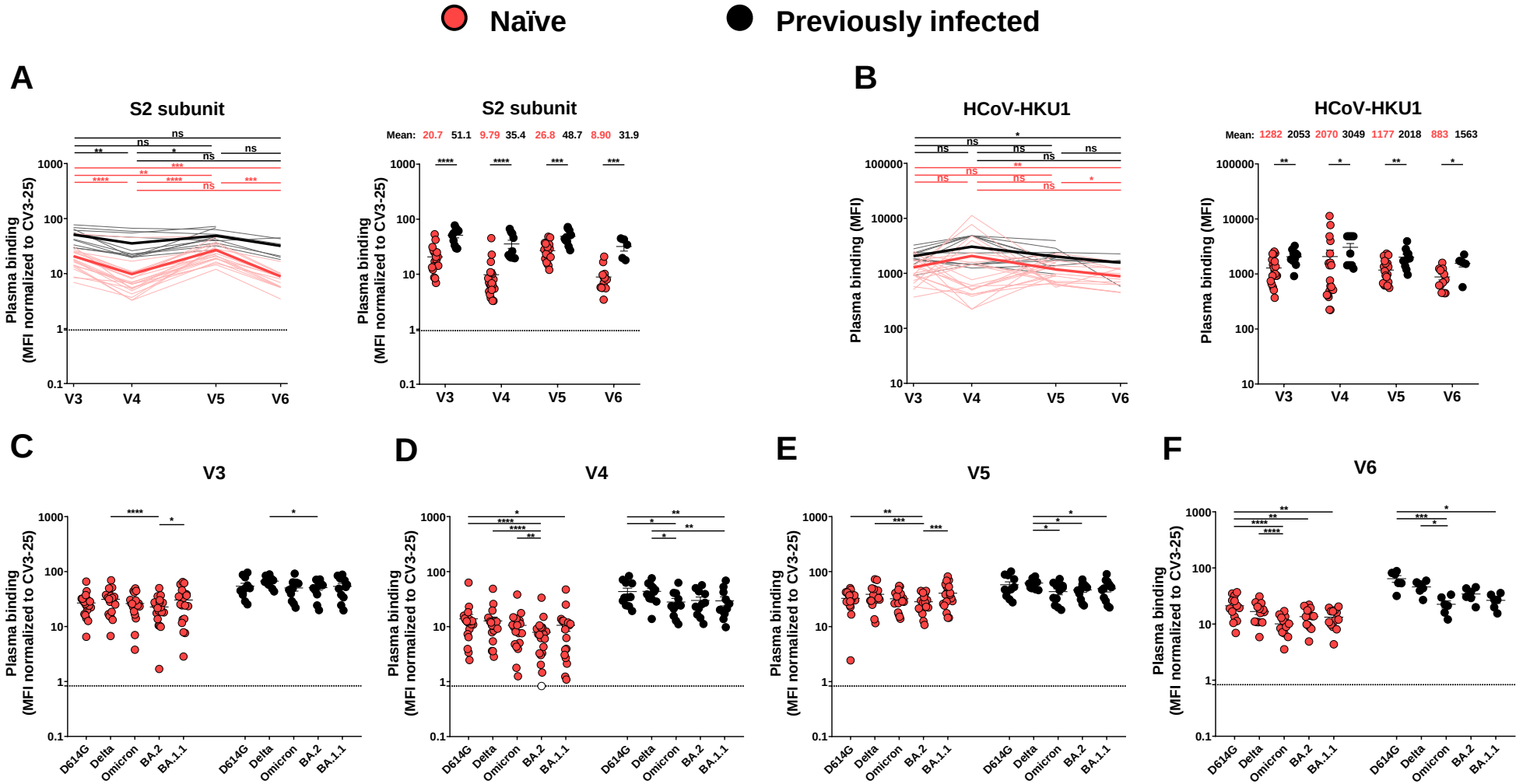

Figure S1 : Recognition of SARS-CoV-2 Spike variants and HCoV-HKU1 S by plasma from naïve and PI donors, Related to Figure 1. 293T cells were transfected with the S2 subunit (**A**) or the indicated full-length S from different SARS-CoV-2 variants (**C-E**) or the HCoV-HKU1 S (**B**) and stained with the CV3-25 Ab or with plasma from naive or PI donors collected at V3, V4, V5 and V6 and analyzed by flow cytometry. The values represent the MFI (**B**) or the MFI normalized by CV3-25 Ab binding (**A**, **C-E**). (**A-B**) Left panel: Each curve represents the values obtained with the plasma of one donor at every time point. Mean of each group is represented by a bold line. Right panel: Plasma samples were grouped in different time points (V3, V4, V5 and V6). (**C-F**) Binding of plasma collected at V3 (**C**), V4 (**D**), V5 (**E**) and V6 (**F**). Naïve and PI donors are represented by red and black points respectively, undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns, non-significant). For naïve donors, n=20 at V3, V4, V5 and n=13 at V6 and for previously infected donors n=11 at V3, V4, V5 and n=6 at V6 .

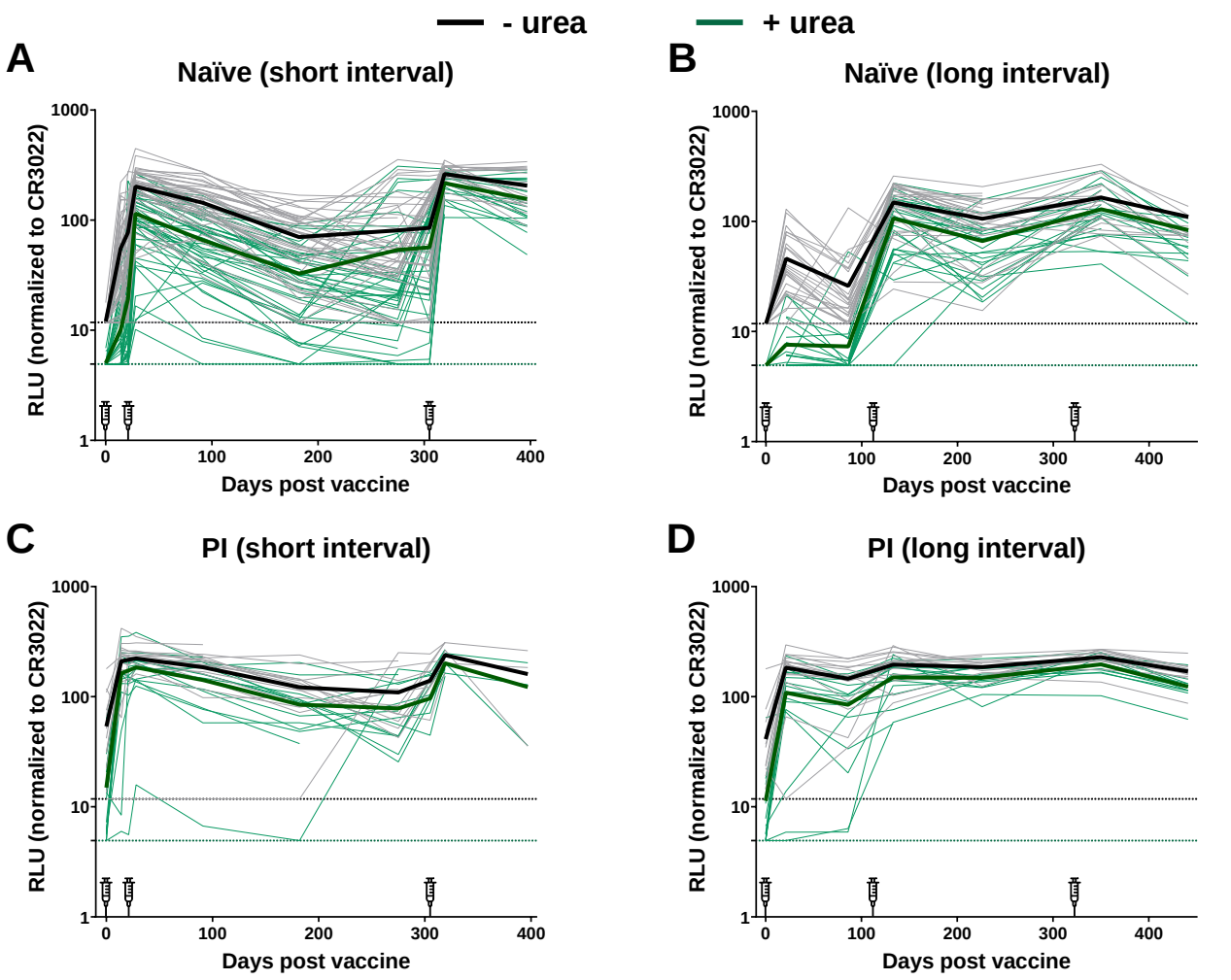

Figure S2 : Comparison of the detection of RBD specific antibodies between ELISA and stringent ELISA in SARS-CoV-2 naïve and previously infected individuals vaccinated with a short or a long interval, Related to Figure 4. (**A-D**) Indirect ELISA was performed by incubating plasma samples from naïve (**A-B**) and PI (**C-D**) vaccinated donors after a short (**A, C**) or a long (**B, D**) interval with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated anti-human IgG. Relative light unit (RLU) values obtained were normalized to the signal obtained with the anti-RBD CR3022 mAb present in each plate. For ELISA (black curves), all the wash steps were made with washing buffer and for stringent ELISA (green curves), the wash steps were supplemented with 8M of urea. Each curve represents the normalized RLUs obtained with the plasma of one donor at every time point. Mean of each group is represented by a bold line. Limits of detection are plotted. For naïve donors n=46 for the short interval and n=30 for the long interval and for previously infected donors n=16 for the short interval and n=15 for the long interval.

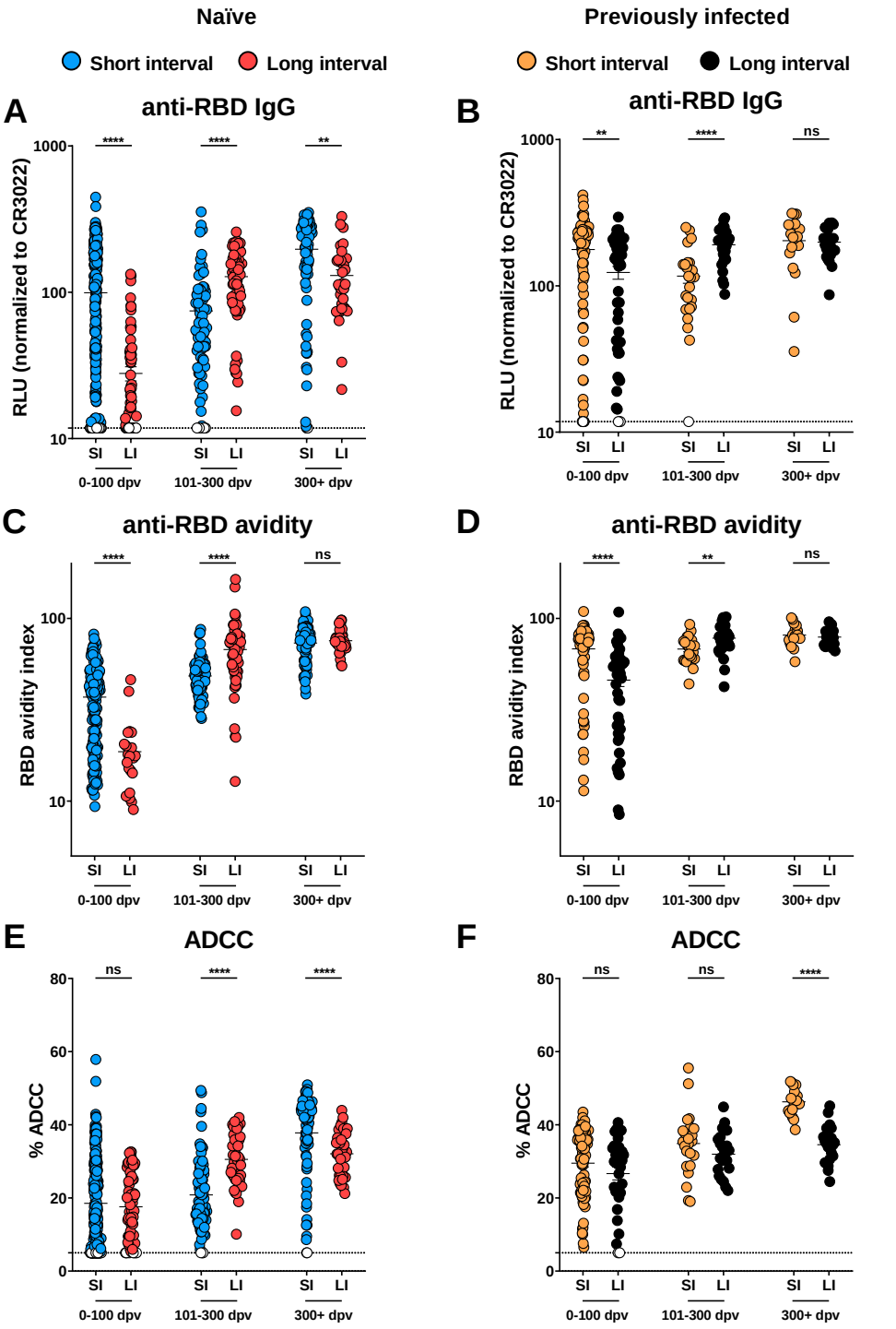

Figure S3 : Humoral responses in SARS-CoV-2 naïve and previously-infected individuals vaccinated with a short or a long interval, Related to Figure 4. Humoral responses were measured in plasma samples collected 0-100 days post-vaccination (dpv), 101-300 dpv or 300+ dpv in naïve or PI donors that received a short or a long dose interval. (**A-B**) Indirect ELISA was performed by incubating plasma samples with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated anti-human IgG. RLU values obtained were normalized to the signal obtained with the anti-RBD CR3022 mAb present in each plate. (**C-D**) Indirect ELISA and stringent ELISA was performed by incubating plasma samples with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated anti-human IgG. RBD avidity index corresponded to the value obtained with the stringent ELISA divided by that obtained with the ELISA. (**E-F**) CEM.NKr parental cells were mixed at a 1:1 ratio with CEM.NKr-S cells and were used as target cells. PBMCs from uninfected donors were used as effector cells in a FACS-based ADCC assay.Naïve and PI donors vaccinated with the SI are represented by blue and yellow lines respectively and naïve and PI donors vaccinated with the LI are represented by red and black lines respectively. Undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM (**p < 0.01; ****p < 0.0001; ns, non-significant)**.**

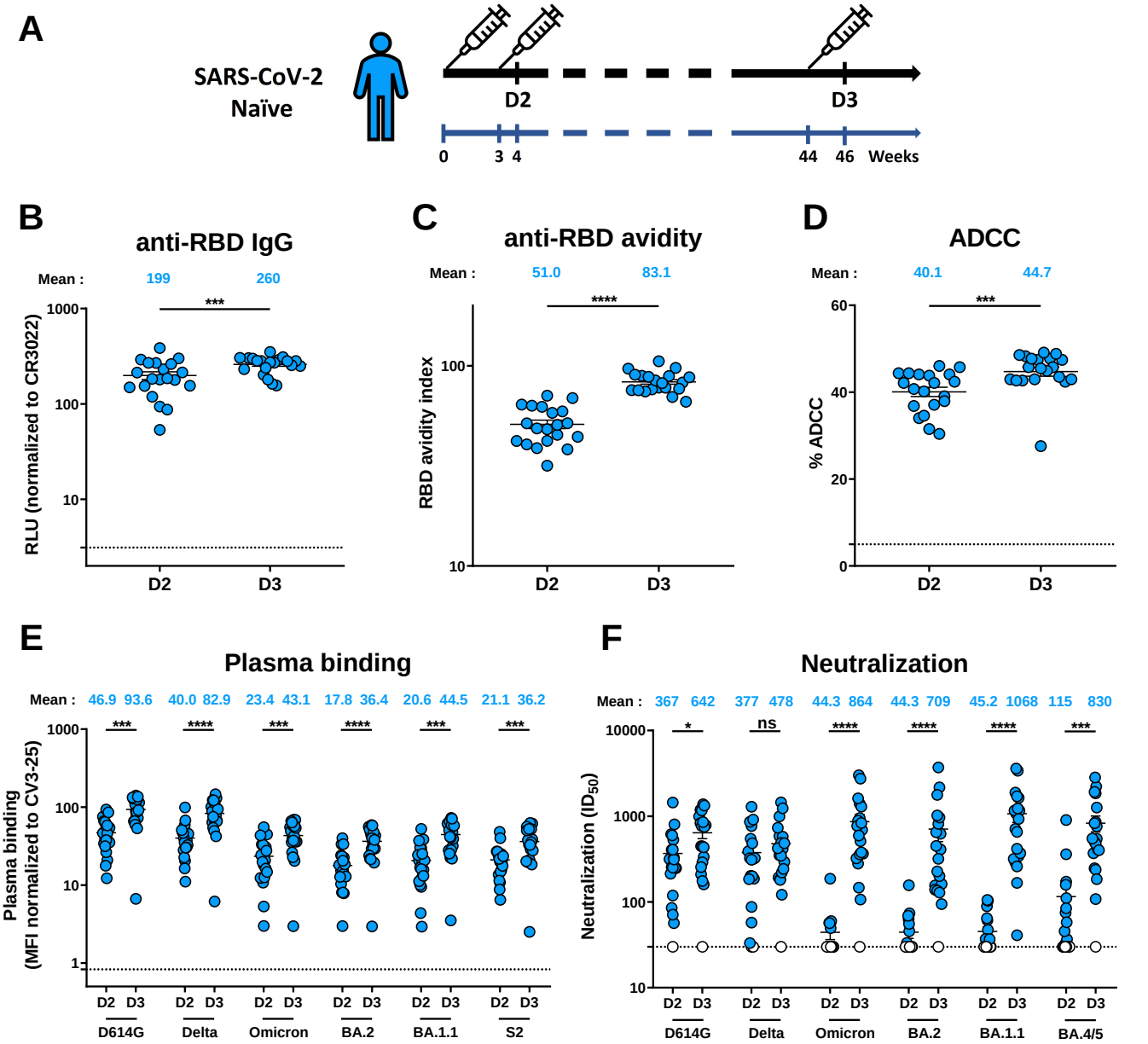

Figure S4 : Humoral responses in SARS-CoV-2 naïve individuals that received a short dose interval, Related to Figure 4. (**A**) SARS-CoV-2 vaccine cohort design. (**B-F**) Humoral responses were measured in plasma samples collected after the second (D2) and the third dose (D3) from naïve donors that received a short dose interval. (**B**) Indirect ELISA was performed by incubating plasma samples with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated anti-human IgG. RLU values obtained were normalized to the signal obtained with the anti-RBD CR3022 mAb present in each plate. (**C**) Indirect ELISA and stringent ELISA was performed by incubating plasma samples with recombinant SARS-CoV-2 RBD protein. Anti-RBD Ab binding was detected using HRP-conjugated anti-human IgG. RBD avidity index corresponded to the value obtained with the stringent ELISA divided by that obtained with the ELISA. (**D**) CEM.NKr parental cells were mixed at a 1:1 ratio with CEM.NKr-S cells and were used as target cells. PBMCs from uninfected donors were used as effector cells in a FACS-based ADCC assay. (**E**) 293T cells were transfected with the indicated full-length S or the S2 subunit and stained with the CV3-25 Ab or with plasma and analyzed by flow cytometry. The values represent the MFI normalized by CV3-25 Ab binding.(**F**) Neutralizing activity was measured by incubating pseudoviruses bearing SARS-CoV-2 S glycoproteins with serial dilutions of plasma for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half maximal inhibitory serum dilution (ID_{50}) values were determined using a normalized non-linear regression using GraphPad Prism software. Undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; ns, non-significant). n = 20.

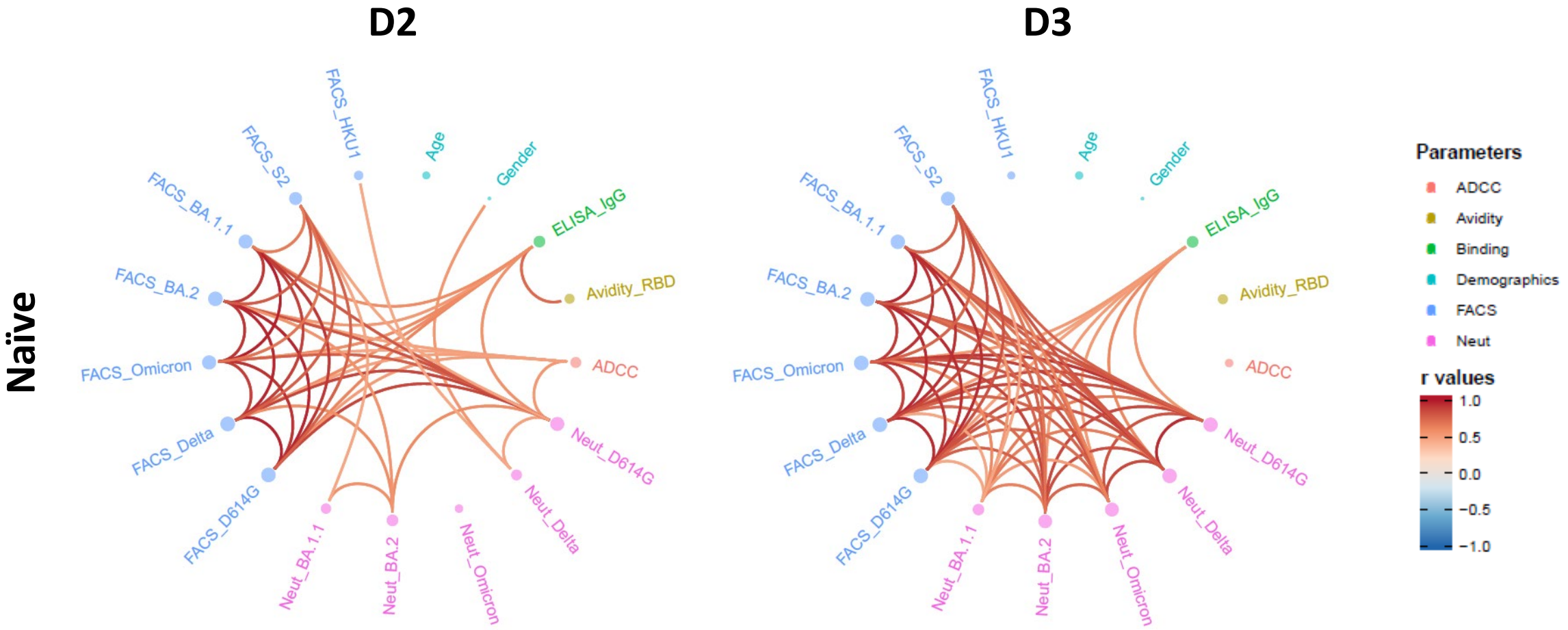

Figure S5 : Mesh correlations of humoral response parameters after the second and the third dose of mRNA vaccine with the short interval regimen, Related to Figure 4. Edge bundling correlation plots where red and blue edges represent positive and negative correlations between connected parameters, respectively. Only significant correlations ($p < 0.05$, Spearman rank test) are displayed. Nodes are color coded based on the grouping of parameters according to the legend. Node size corresponds to the degree of relatedness of correlations. Edge bundling plots are shown for correlation analyses using two different datasets; i.e., SARS-CoV-2 naïve individuals vaccinated with the short interval at D2 and D3 respectively. n=20.

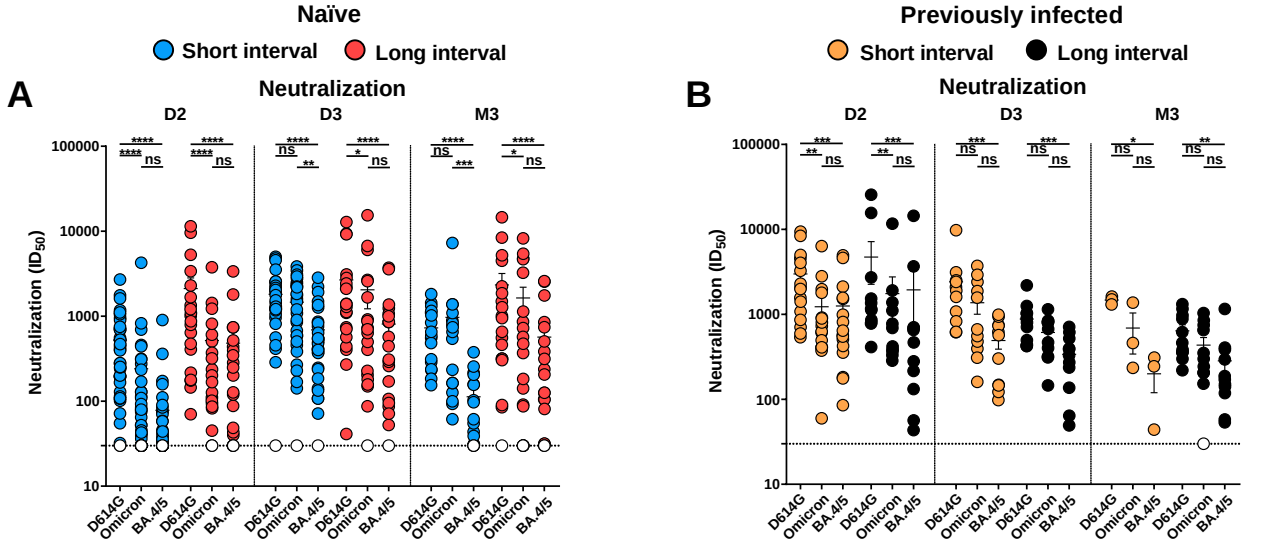

Figure S6 : Neutralization activities in SARS-CoV-2 naïve and previously infected individuals that received a short or a long dose interval, Related to Figure 5. (**A-B**) Neutralizing activity was measured by incubating pseudoviruses bearing SARS-CoV-2 S glycoproteins, with serial dilutions of plasma for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half maximal inhibitory serum dilution (ID50) values were determined using a normalized non-linear regression using GraphPad Prism software. (**A-B**) Plasma samples were grouped in different time points (D2: 1 (SI) or 3 (LI) weeks after the second dose, D3: 2 (SI) or 4 (LI) weeks after the third dose and M3: 3 (SI) or 4 (LI) months after the third dose. Naïve and PI donors vaccinated with the SI are represented by blue and yellow points respectively and naïve and PI donors vaccinated with the LI are represented by red and black points respectively. Undetectable measures are represented as white symbols, and limits of detection are plotted. Error bars indicate means ± SEM. (* $p < 0.05$; ** $P < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, non-significant). For naïve donors vaccinated with the SI, n= 45 at D2, n=33 at D3 and n=18 at M3. For naïve donors vaccinated with the LI, n= 20 at D2, D3 and M3. For PI donors vaccinated with the SI, n= 16 at D2, n=11 at D3 and n=3 at M3. For PI donors vaccinated with the LI, n= 11 at D2, D3 and M3.