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## **Supplemental Methods**

### **Humoral response evaluation**

Humoral response was evaluated by SARS-CoV-2 serology using the Abbott Architect SARS COV-2 IgG II quant Assay (Abbott Laboratories, USA) for IgG anti-RBD Spike (S) SARS-CoV-2 detection and quantification. The lower limit of detection was 21 AU/mL (an undetectable response was considered as null when comparing titers) and the upper limit was 40 000 AU/mL. When the result was above this limit, a 1/5 dilution was performed to quantify the anti-S titers.

Additionally, IgG anti-N SARS-CoV-2 were measured using SARS COV-2 IgG anti-N (Abbott Laboratories, USA), according to manufacturer instructions, with a positive result considered when index Ig(S) (S/C) was  $\geq 1.40$ . We considered an IgG(N) S/C of  $\geq 1.4$  as denoting seropositive status due to prior SARS-CoV-2 exposure as mRNA vaccines only encodes for the Spike protein (S) of the virus.

### **Recombinant protein purification**

#### *Construct design*

The SARS-CoV-2 Receptor Binding Domain (RBD) was cloned in pcDNA3.1(+) encompassing the Spike (S) residues 331-528, and it was flanked by an N-terminal IgK signal peptide and a C-terminal Thrombin cleavage site followed by Hisx8, Strep and Avi tags.

#### *Protein expression and purification*

The plasmid coding for recombinant protein was transiently transfected in Expi293F<sup>TM</sup> cells (Thermo Fischer) using FectoPRO<sup>®</sup> DNA transfection reagent (Polyplus), according to the manufacturer's instructions. The cells were incubated at 37 °C for 5 days and then the culture was centrifuged, and the supernatant was concentrated. The proteins were purified from the supernatant by affinity chromatography using His-Trap<sup>TM</sup> Excel columns (Cytiva) (SARS-CoV-2 RBD). A final step of size-exclusion chromatography (SEC) in PBS was also performed, using Superdex200 10/300 (Cytiva).

### **Flow cytometry**

PBMCs were isolated from venous blood samples via standard density gradient centrifugation and used after cryopreservation at -150°C. Cells were thawed using RPMI-1640 (Gibco) 10% FBS (Gibco), washed twice and incubated with 5 µg of the SARS-CoV-2 His-tagged RBD protein in 100 µL of PBS (Gibco) 2% FBS during 20 min on ice. Cells were washed and resuspended in the same conditions, then the fluorochrome-conjugated antibody cocktail including the 2 anti-His antibodies was added at pre-titrated concentrations for 20 min at 4°C (see Table S2) and viable cells were identified using a LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific). Samples were acquired using a LSR Fortessa (BD Biosciences). Detailed reagent and antibody references are provided in Supplementary Table 1. Detailed gating strategies for individual markers are depicted in supplemental Figure S2.

### **Statistical analysis**

Ordinary One-way ANOVA and Kruskal-Wallis test were used to compare continuous variables as appropriate (indicated in Figures). Wilcoxon matched-pairs signed rank test was used for longitudinal analysis. A *P*-value ≤ 0.05 was considered statistically significant. Statistical analyses involved use of GraphPad Prism 9.0 (La Jolla, CA, USA).

**Supplemental Table 1.** Conjugated antibodies used in flow-cytometry.

<b>Antibody</b>	<b>Source</b>	<b>Clone, catalog number and RRID</b>
CD3 APC-H7	Biologend	Clone : UCHT1; Cat#300425; RRID : AB_830754
CD14 APC-H7	BD Bioscience	Clone : M φP9; Cat#561709; RRID : AB_1645464
CD19 PE-CF594	BD Bioscience	Clone : HIB19; Cat# 562321; RRID : AB_11154408
CD38 Percp Cy 5,5	BD Bioscience	Clone : HIT2; Cat# 551400; RRID AB_394184
CD27 BV421	Biologend	Clone : M-T271; Cat# 356417; RRID: AB_2562598
CD11c BV605	BD Bioscience	Clone : S-HCL-3; Cat#744436; RRID : AB_2742232
IgD FITC	Life technologies	Clone : Polyclonal; Cat# H15501; RRID : AB_2536563
His Tag APC	Biologend	Clone : J095G46; Cat# 362605; RRID:AB_2563634
His Tag PE	Biologend	Clone : J095G46; Cat# 362603; RRID:AB_2715818
CD71 BV785	Biologend	Clone : CY1G4; Cat# 334111; RRID:AB_2563118
CD21 BV711	BD Bioscience	Clone : B-ly4; Cat#563163 ; RRID:AB_2741028

**Supplemental Table 2.** Median values of Anti-SARS-CoV-2 IgG titer and Frequency of SARS-CoV-2 RBD-specific memory B cells ( % of CD27 + IgD-) in Virus naïve healthy controls, dialysis patients after two doses and three doses

	<b>Healthy controls n=23</b>	<b>Dialysis patients (2 doses) n=21</b>	<b>Dialysis patients (3 doses) n=26</b>
<b>Anti-SARS-CoV-2 IgG titer (A.U), median (IQR)</b>	6230 [3793-11975] (n=23)	3039 [1506-5432] (n=20)	7906 [3377-26136] (n=25)
<b>Frequency of SARS-CoV-2 RBD-specific memory B cells , (% of CD27 + IgD-)</b>	0.07 [0.03-014] (n=23)	0.06 [0.035-19] (n=21)	0.33 [0.215-1.245] (n=26)

**Supplemental Table 3.** Median values of Anti-SARS-CoV-2 IgG titer and Frequency of SARS-CoV-2 RBD-specific memory B cells (% of CD27 + IgD-) in SARS-CoV-2 recovered healthy controls, dialysis patients after two doses and three doses

	<b>Healthy controls n=26</b>	<b>Dialysis patients (2 doses) n=20</b>	<b>Dialysis patients (3 doses) n=13</b>
<b>Anti-SARS-CoV-2 IgG titer (A.U), median (IQR)</b>	18865[12287- 30095] (n=26)	65092[39538- 142481] (n=20)	38417 [18692- 75475] (n=13)
<b>Frequency of SARS-CoV-2 RBD-specific memory B cells , (% of CD27 + IgD-)</b>	0.49 [0.28-0.965] (n=25)	0.83 [0.43-1.865] (n=17)	0.63 [0.16-1.07] (n=13)

**Supplemental Table 4.** Baseline characteristics of Naive hemodialysis patients according to number of vaccine doses

	<b>All (n=33)</b>	<b>Patients with 2 doses (n=7)</b>	<b>Patients with 3 doses (n=26)</b>	<b>p value</b>
<b>Male, n (%)</b>	23 (70)	6 (86)	17 (65)	0.397
<b>Age (years), median (IQR)</b>	71 [64-78]	76 [70-81]	70 [61-76]	0.210
<b>Comorbidities</b>				
<b>Diabetes mellitus, n (%)</b>	16 (48)	3 (43)	13 (50)	>0.99
<b>Hypertension, n (%)</b>	31 (94)	7 (100.0)	24 (96)	>0.99
<b>Immunosuppression, n (%)</b>	5 (15)	1 (15)	4 (15)	>0.99

**Supplemental Table 5.** Baseline characteristics of SARS-Cov2 recovered hemodialysis patients according to number of vaccine doses

	<b>All (n =26)</b>	<b>Patients with 2 doses (n =13)</b>	<b>Patients with 3 doses (n=13)</b>	<b>p value</b>
<b>Male, n (%)</b>	17 (5)	10 (77)	7 (54)	0.411
<b>Age (years), median (IQR)</b>	70[64-74]	69 [66-73]	70 [60-78]	0.659
<b>Comorbidities</b>				
<b>Diabetes mellitus, n (%)</b>	11 (42)	7 (54)	4 (31)	0.318
<b>Hypertension, n (%)</b>	25 (96)	13 (100.0)	12 (92)	>0.99
<b>Immunosuppression, n (%)</b>	2 (8)	1 (8)	1 (8)	>0.99



### **Supplemental Figure 1. Cohort design**

Virus-naive (n=23) or SARS-CoV-2 recovered (n=26) vaccinated patients with respectively 2 doses and 1 dose of BNT162b2 mRNA vaccine were compared to virus-naive (n= 33) or SARS-CoV-2 recovered (n= 26) dialysis patients after 2 or 3 doses of BNT162b2 mRNA vaccine.

### **Supplemental Figure 2. Gating strategies**

**A.** Flow cytometric gating strategies for the analysis of major B cell populations from PBMCs of patients.

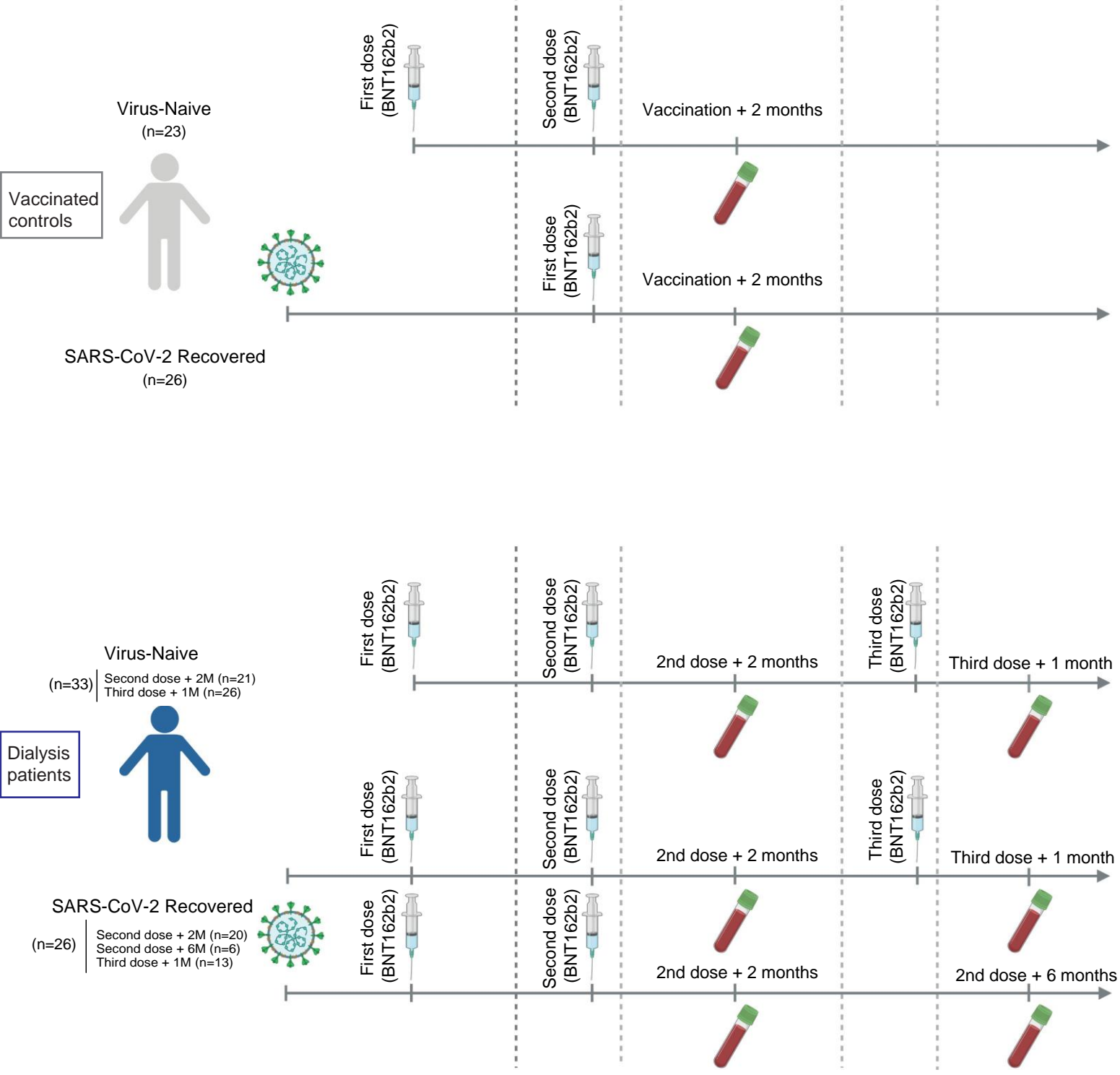
Lymphocytes were first gated based on morphology, before exclusion of doublets, dead cells and CD3/CD14 cells. CD19<sup>+</sup> cells were next gated before exclusion of CD38<sup>hi</sup> plasma cells. CD38<sup>int/-</sup> cells were then divided in four quadrants using CD27 and IgD. Upper left quadrant defines memory B cells (MBCs), lower left quadrant double-negative (DN), upper right quadrant CD27<sup>+</sup>IgD<sup>+</sup> cells (MZB) and lower right quadrant naive B cells (excluding CD38<sup>hi</sup> transitional). SARS-CoV-2 RBD-specific B cells were then analyzed within the B cell population of interest using a double-staining strategy with anti-His antibodies of a His-tagged SARS-CoV-2 RBD. **B.** Gating strategy for CD71<sup>+</sup> MBCs, within the IgD<sup>-</sup>CD27<sup>+</sup> gate **C.** Gating strategy for DN1 and DN2 B cells, within the IgD<sup>-</sup>CD27<sup>-</sup> gate

### **Supplemental Figure 3. Humoral and Memory B Cell response to SARS-CoV-2 RBD, in SARS-CoV-2-recovered dialysis patients after 2 mRNA vaccine doses according to baseline characteristics**

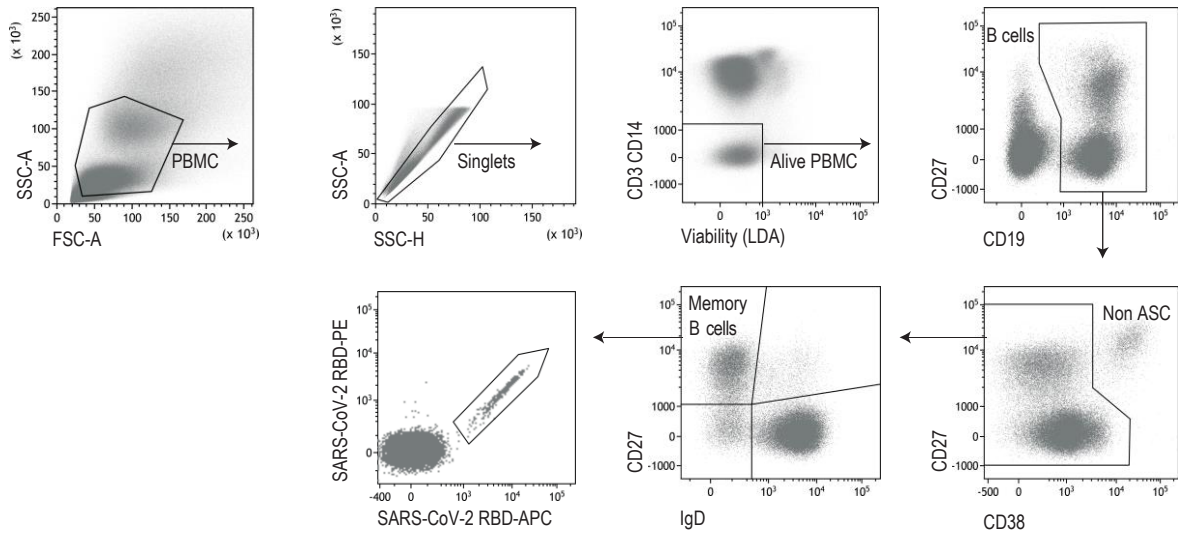
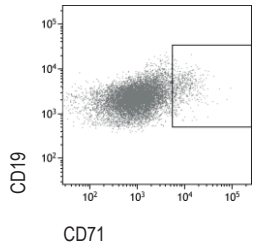
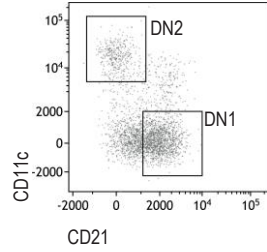
Anti-SARS-CoV-2 RBD serum IgG titers measured by ELISA and frequencies of SARS-CoV-2 RBD-specific cells in live CD19<sup>+</sup>IgD<sup>-</sup>CD27<sup>+</sup>CD38<sup>int/-</sup> memory B cells according to **A.** timing of COVID-19 episode from 1<sup>st</sup> dose injection (cut-off: 4 months) (COVID-19 < 4months from 1<sup>st</sup> dose n=6; COVID-19 > 4 months from 1<sup>st</sup> dose n=8) **B.** age at vaccine injection (Age < 70 yrs n=9; Age > 70 yrs n=11) and **C** diabetes status (No diabetes n=10; Diabetes n=10) Mann-Whitney test \*P < 0.05. Bars indicate median ± IQR. Values equal to zero were converted to 0.01 for logarithmic scale

**Supplemental Figure 4. Humoral and Memory B Cell response to SARS-CoV-2 RBD in SARS- CoV-2 naïve dialysis patients after 2 mRNA vaccine doses according to baseline characteristics**

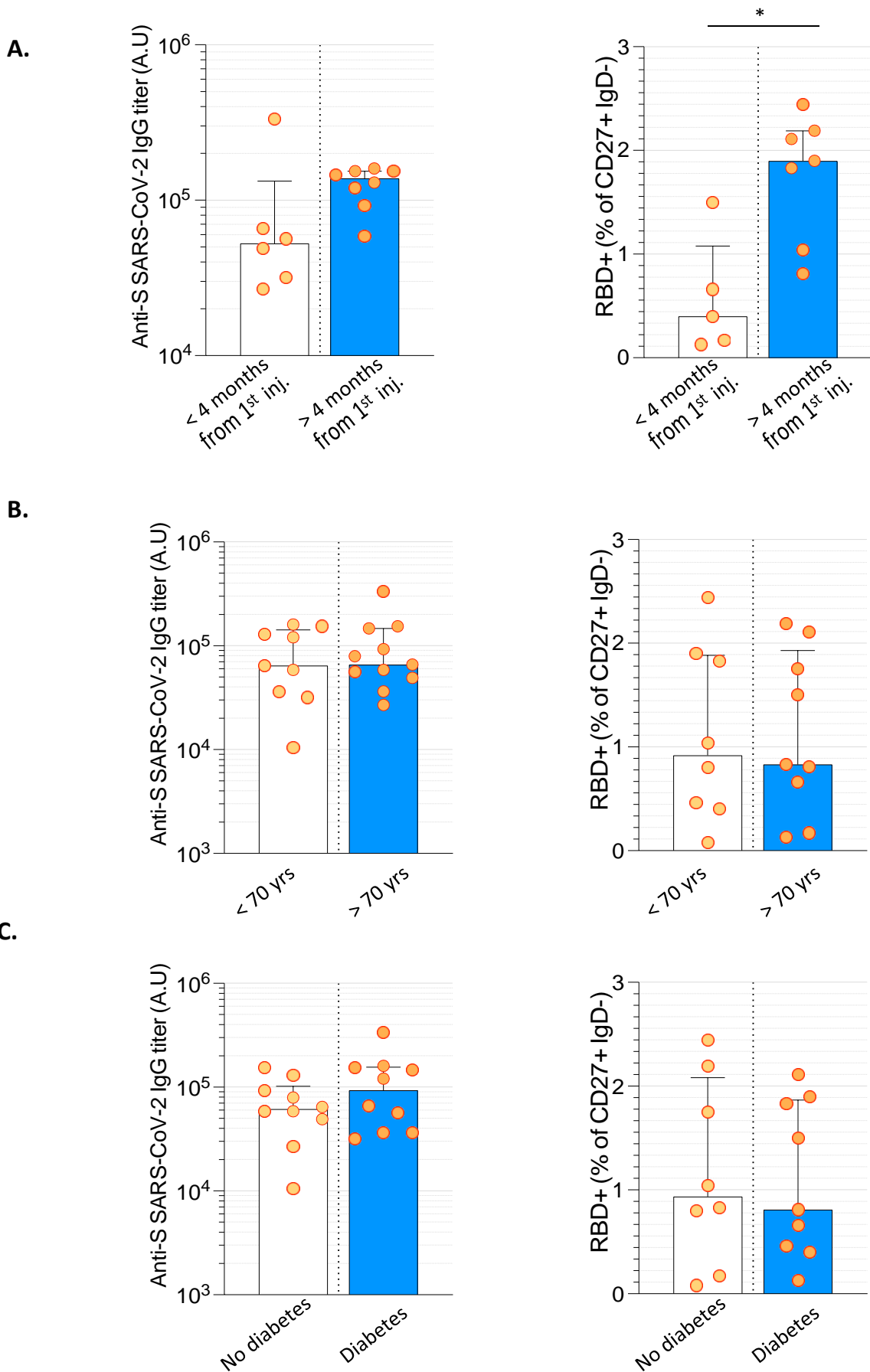
Anti-SARS-CoV-2 RBD serum IgG titers measured by ELISA and frequencies of SARS-CoV-2 RBD-specific cells in live CD19+IgD-CD27+CD38int/- memory B cells according to **A.** age at vaccine injection (Age < 70 yrs n=9; Age > 70 yrs n=11) and **B** diabetes status (No diabetes n=10; Diabetes n=10) Mann-Whitney test \*P < 0.05. Bars indicate median  $\pm$  IQR. Values equal to zero were converted to 0.01 for logarithmic scale



Supplemental Figure 1. Cohort design

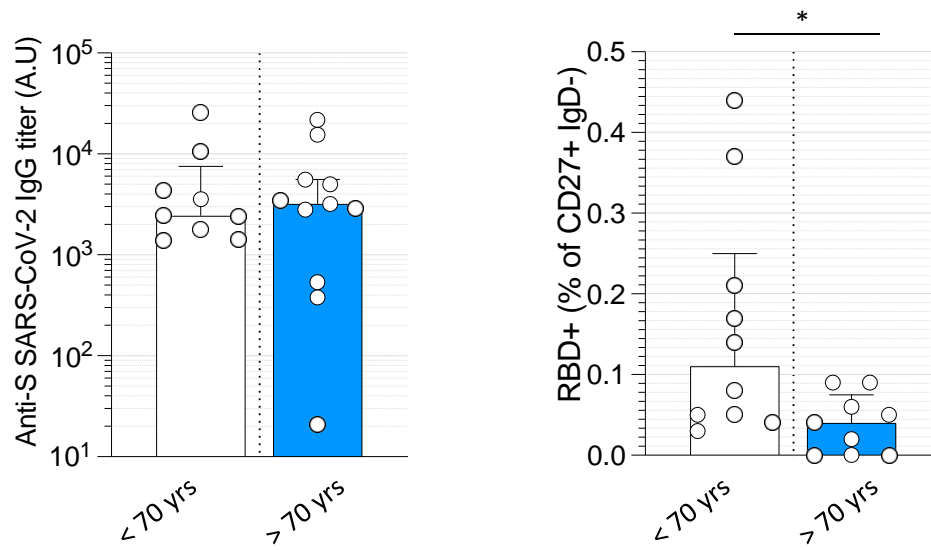
**A****B****C**

Supplemental Figure 2. Gating strategies

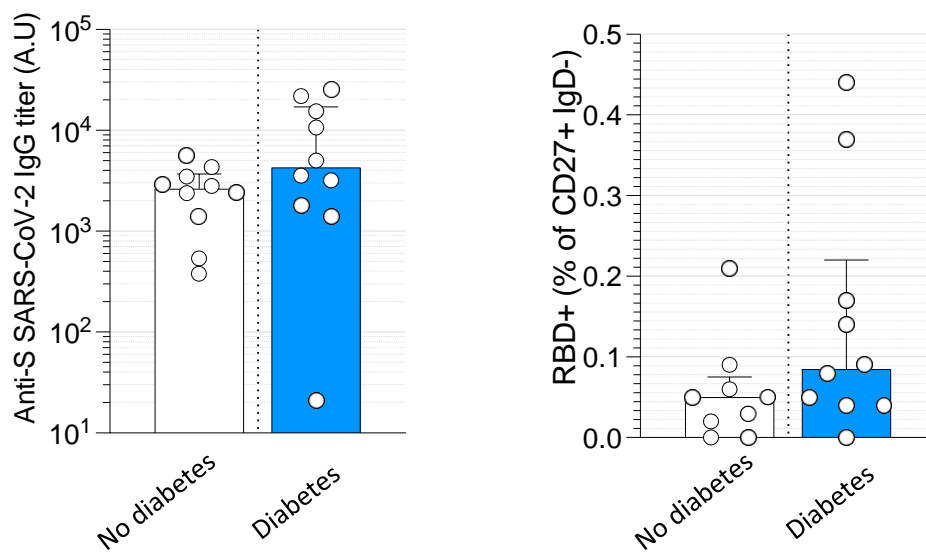


Supplemental Figure 3. Humoral and Memory B Cell response to SARS-CoV-2 RBD, in SARS-CoV-2-recovered dialysis patients after 2 mRNA vaccine doses according to baseline characteristics

**A.**



**B.**



Supplemental Figure 4. Humoral and Memory B Cell response to SARS-CoV-2 RBD in SARS-CoV-2 naïve dialysis patients after 2 mRNA vaccine doses according to baseline characteristics.