Supplementary Methods

Study population and design

A total of 40 hemodialysis patients (HDP) have been analysed after SARS-CoV-2 vaccination. The analyses were performed 4-6 weeks following the third doses (HDP3x; n=40) in all patients. HDP who received a fourth doses (HDP4x; n=19) have been analysed 4-6 weeks thereafter and HDP who received only three doses ($HDP3x_{T12}$; n=21) after 12 weeks. All but the third vaccination in HDP4x group was performed by COVID-19 mRNA vaccine (Comirnaty, Biontech/Pfizer). The third dose in HDP4x group was performed by COVID-19 Johnson&Johnson vaccine. Demographics and clinical characteristics of patients are shown in Supplementary Table S1.

Preparations of PBMC

Peripheral blood was collected in S-Monovette K3 EDTA blood collection tubes (Sarstedt, Germany). Collected blood was pre-diluted in PBS/BSA (Gibco, USA) at a 1:1 ratio and underlaid with 15 mL Ficoll-Paque Plus (GE Healthcare, USA). Tubes were centrifuged at 800 g for 20 minutes at room temperature. Isolated PBMCs were washed twice with PBS/BSA and stored at -80°C until use as previously described.

Stimulation with SARS-CoV-2 overlapping Peptide Pool

Isolated PBMC were stimulated with SARS-CoV-2 PepTivator (Miltenyi Biotec, Germany) overlapping peptide pools (OPP) containing 15-mer sequences with 11 amino acids overlap of whole S glycoprotein (WT) or mutated regions in the surface or S glycoprotein of the SARS-CoV-2 lineage B.1.1.529 (omicron). Peptide pools were dissolved per manufacturer's instructions and used at a concentration of 1 µg/ml. $2.5x10^6$ PBMC were thawed and plated for each condition in 96-UWell Plates in RPMI media (Life Technologies, USA), supplemented with 1% Penicillin-Streptomycin-Glutamine (Sigma Aldrich, USA), and 10% FCS (PAN-Biotech, Germany) and were stimulated or left untreated as a control for 16 hours. As a positive control, cells were stimulated with SEB (1 µg/ml, Sigma Aldrich, USA) and negative control was with vehicle (a medium to dissolve peptide pools). After 2 hours, Brefeldin A (5 µg/ml, Sigma Aldrich, USA) was added. As previously applied by our groups and others, antigen-specific responses were considered positive after the non-specific background was subtracted, and more than 0.001% or at least 15 positive cells were detectable. Negative values were set to zero.

Antibodies

All antibodies are from BioLegend, USA unless otherwise noted: CD4-A700; clone: OKT4, CD8-V500; clone: RPA-T8 (BD Biosciences, USA), CD137 (4-1BB)-PE-Cy7; clone: 4B4-1, CD154 (CD40L)-A647; clone: 24-31, CD3-BV785; clone: OKT3. Fixable Viability Dye eFluor 780 (eBioscience, USA) was used for live/dead discrimination.

Flow Cytometry

T cells stimulated with SARS-Cov-2 OPP were stained with optimal concentrations of antibodies for 10 minutes at room temperature in the dark. All samples were immediately acquired on a CytoFlex flow cytometer (Beckman Coulter, USA). Quality control was performed daily using the recommended CytoFlex Daily QC Fluorospheres (Beckman Coulter, USA). No modification to the compensation matrices was required throughout the study. The Gating Strategy to identify SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T cells is shown in Supplementary Figure S2.

SARS-CoV-2 IgG antibody titers

SARS-CoV-2 IgG antibodies were detected using SARS-CoV-2 IgG kit (EUROIMMUN, Lübeck, Germany). The test was performed according to the manufacturer's instructions. Briefly, peripheral blood was collected in S-Monovette Z Gel (Sarstedt, Germany). Serum samples were diluted 1:100 and added to plates coated with SARS-CoV-2 S1 antigen (wildtype). Anti-human IgG conjugated with horseradish peroxidase (HRP), was used to detect bound SARS-CoV-2 S1-specific IgG antibodies. The absorbance was detected at 450 nm with reference at 620 nm.

SARS-CoV-2 neutralizing antibodies

Neutralizing antibodies were analysed using a propagation-incompetent VSV*DG (firefly luciferase) pseudovirus system bearing SARS-CoV-2 S-protein (wild type and omicron) in the envelope. Serial dilutions of serum samples were diluted with the pseudovirus system prior to the infection of Vero E6 cells employing pseudovirus. After 18 hours incubation in Dulbecco's minimal essential medium (supplemented with 10% FCS and non-essential amino acids, all Life Technologies, Switzerland) the firefly luciferase reporter activity was determined. The 50% neutralization dose was determined as the reciprocal antibody dilution causing 50% inhibition of the calculated luciferase reporter.

Statistical analysis

FlowJo software 10.8.1 (BD Bioscience, USA) was used for analysing flow cytometry data. Statistical analysis was performed using R 4.0.4. Box plots depict the median, first and third quartile of a variable; the maximum length of the whiskers corresponds to 1.5 times the interquartile range. Groups were compared using two-sided, unpaired Mann-Whitney-U-Test; except for (C), where Wilcoxon signed-rank paired test was employed; the correlation (D) was evaluated employing the Pearson correlation coefficient. P-values ≤ 0.050 were defined significant.

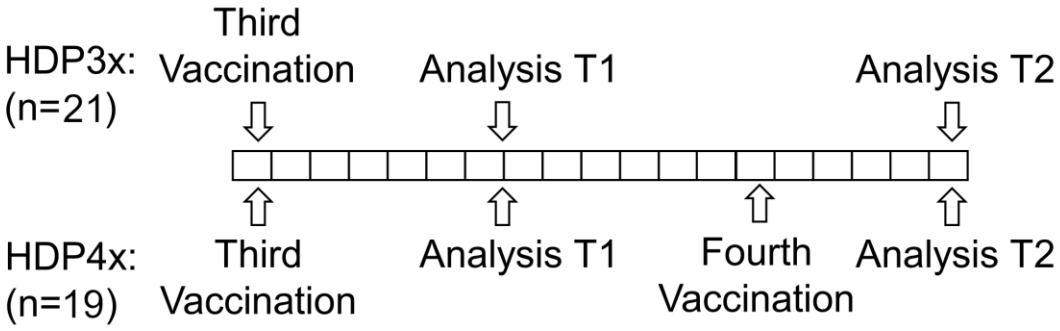
Study approval

The study was approved by the ethical committee of the Ruhr-University Bochum (20-6886). Written consent was obtained from all participants.

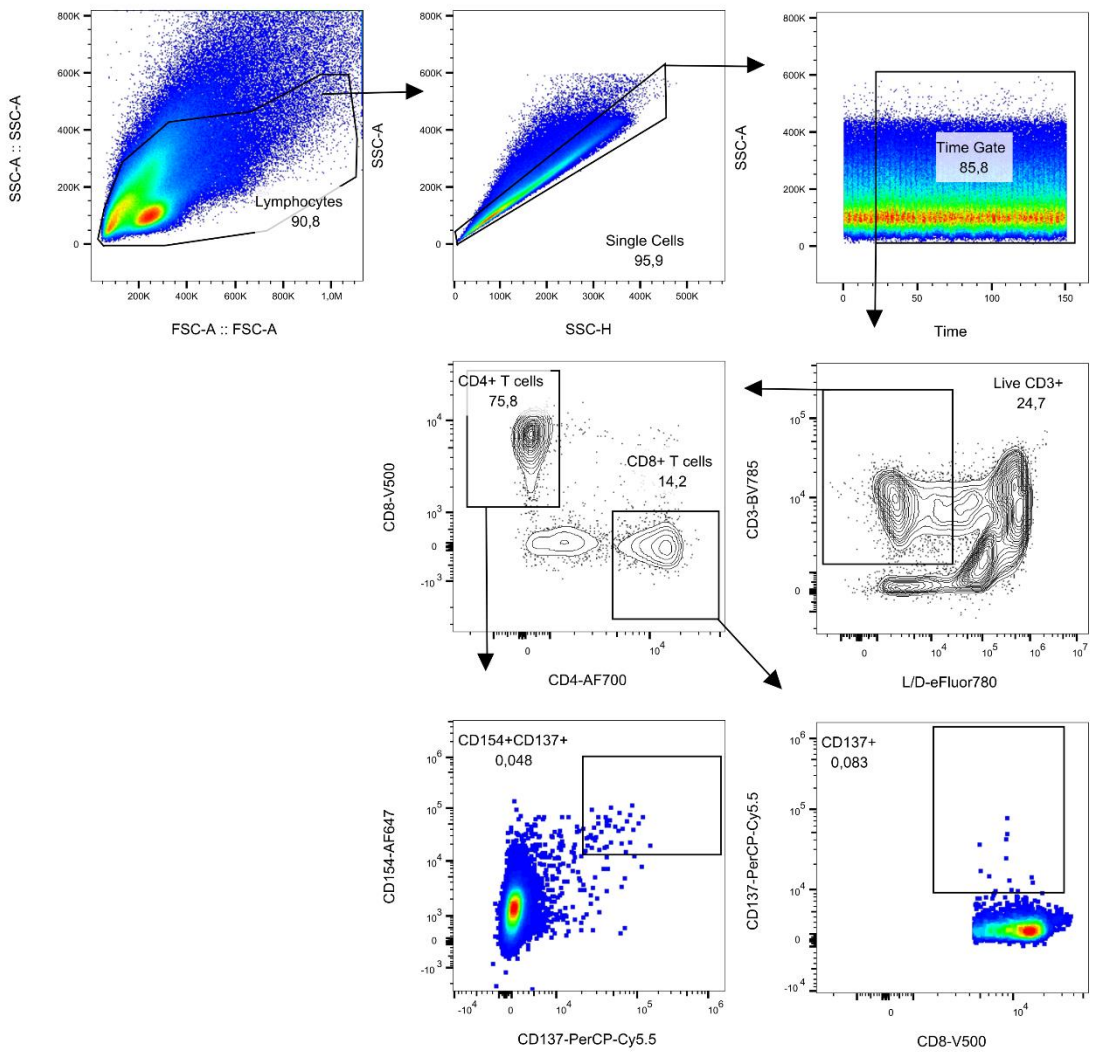
Supplementary Table S1. Study population

	HDP3x	HDP4x	P-Value
Patients [n]	21	19	
Sex [female]	8	7	1.000[§]
Age [years, mean±SD]	72.0 [±14.7]	63.8 [±18.4]	0.15[#]
Time on Dialysis therapy until third Vaccine doses [months, mean±SD]	41.2 [±38.1]	58.1 [± 35.0]	0.17[#]
Pre-existing diseases			
Diabetes [n]	10	9	1.000[§]
Hypertension [n]	18	18	1.000[§]
Cancer [n]	0	6	**0.008**[§]

[§] Unpaired two-tailed Fishers exact test, [#] Unpaired two-tailed t-test



Supplementary Figure S1: Schematic illustration of the course of vaccination. The analyses were performed 4-6 weeks following the third doses in HDP3x and HDP4x (T1, respectively). Patients who received an additional dose after 6-8 weeks were analyzed 4-6 weeks thereafter (HDP4x, T2) and patients who received only three doses were analysed after 12 weeks (HDP3x, T2).



Supplementary Figure S2: Gating strategy to identify SARS-CoV-2 S-protein reactive T cells among $CD4^+$ T and $CD8^+$ T cells. Isolated PBMC were stimulated with SARS-CoV-2 spike protein OPPs or left untreated for 16 h. Lymphocytes were gated based on side and forward scatter profile, doublets were excluded and living T cells were identified as $CD3^+$ and Live/Dead-Marker negative cells, expressing either CD4 or CD8. Antigen specific T cells have been identified as $CD4^+CD154^+CD137^+$ T-helper cells or $CD8^+CD137^+$ cytotoxic T cells.