Impaired humoral but substantial cellular immune response to variants of concern B1.1.7 and B.1.351 in hemodialysis patients after vaccination with BNT162b2

Constantin J. Thieme[1], Arturo Blazquez-Navarro[1,2], Lema Safi[2], Sviatlana Kaliszczyk[2], Krystallenia Paniskaki[3], Isabel E. Neumann[1], Kita Schmidt[1], Mara Stockhausen[2], Jan Hörstrup[4], Ocan Cinkilic[5], Linus Flitsch-Kiefner[6], Toni L. Meister[7], Corinna Marheinecke[7], Stephanie Pfaender[7], Eike Steinmann[7], Felix S. Seibert[2], Ulrik Stervbo[2], Timm H. Westhoff[2]*, Toralf Roch[1,2]*, Nina Babel[1,2]*

[1]*Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin Center for Advanced Therapies, Augustenburger Platz 1, 13353 Berlin, Germany*

[2]*Center for Translational Medicine and Immune Diagnostics Laboratory, Medical Department I, Marien Hospital Herne, University Hospital of the Ruhr-University Bochum, Hölkeskampring 40, 44625 Herne, Germany*

[3]*Department of Infectious Diseases, West German Centre of Infectious Diseases, University Hospital Essen, University Duisburg-Essen, Germany*

[4]*KfH Kuratorium für Dialyse und Nierentransplantation e.V., Berlin, Germany*

[5]*Dialyse Schwerte, Schwerte, Germany*

[6]*KfH Kuratorium für Dialyse und Nierentransplantation e.V., Hagen, Germany*

[7]*Department of Molecular and Medical Virology, Ruhr-University Bochum, Germany*

*equal contribution

Corresponding author:
Prof. Nina Babel
Center for Translational Medicine, Medical Department I, Marien Hospital Herne, University Hospital of the Ruhr-University Bochum, Hölkeskampring 40, 44625 Herne, Germany and
Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin-Brandenburg Center for Regenerative Therapies, Augustenburger Platz 1, 13353 Berlin, Germany

email: nina.babel@elisabethgruppe.de; nina.babel@charite.de

Table S1: Patient characteristics		
No. of patients	34	
Age in years; Median (range)	77.5 (43-96)	
Gender	20 male (59%) / 14 female (41%)	
Medical history		
Previous COVID-19 infection	0 (0%)	
Vaccinated with BioNTech/Pfizer BNTb162b	34 (100%)	
Time since vaccination in days; Median (range)	42 (16-83)	
Hemodialysis	34 (100%)	
Immunosuppressive medication	0 (0%)	
Causes of renal failure (multiple causes per patient possible)		
Diabetic nephropathy	16 (47%)	
Hypertensive kidney disease	12 (35%)	
Glomerulonephritis including vasculitis	4 (12%)	
Other	6 (18%)	
Not specified	1 (3%)	
Neutralizing antibody responses in relation to age		
S-WT neutralizing antibodies +	n=19; Median age 79, range 43-92	P=0.4832
S-WT neutralizing antibodies -	n=15; Median age 76, range 64-96	
S-B.1.1.7 neutralizing antibodies +	n=12; Median age 78, range 43-90	P=0.3332
S-B.1.1.7 neutralizing antibodies -	n=22; Median age 77, range 59-96	
S-B.1.351 neutralizing antibodies +	n=9; Median age 82, range 43-90	P=0.6770
S-B.1.351 neutralizing antibodies -	n=25; Median age 76, range 59-96	
Statistical comparison was performed using two-tailed unpaired t test		

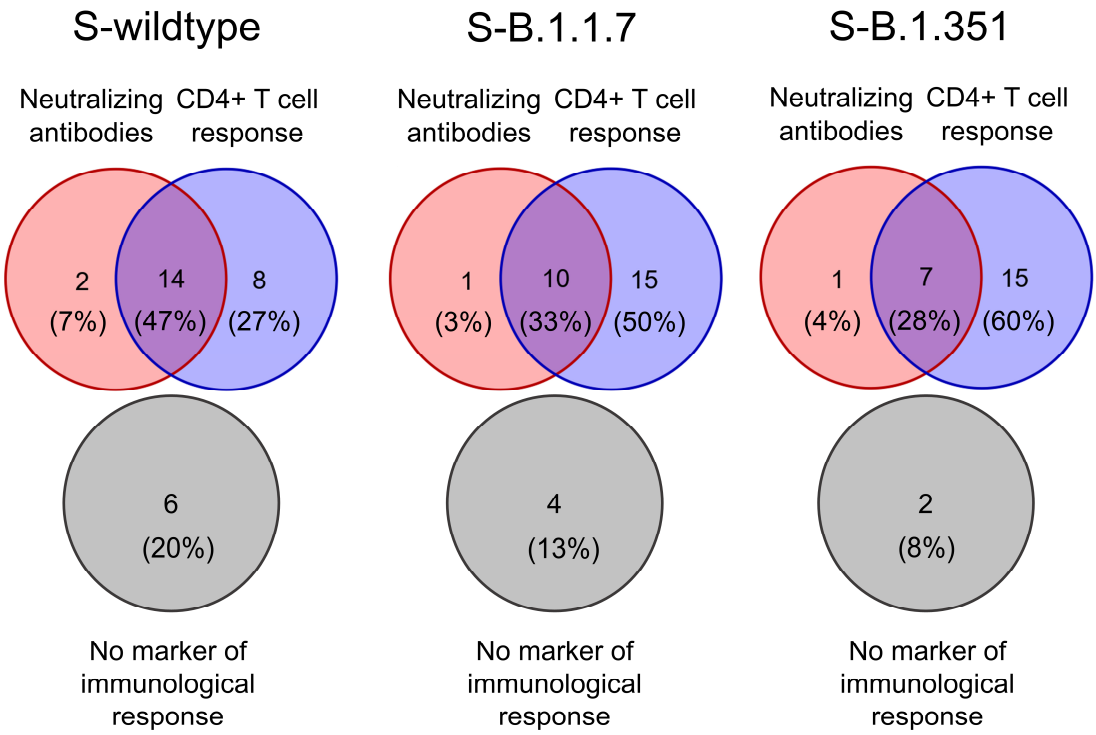

Fig. S1: Venn diagrams for hemodialysis patients with surrogate markers of immunity at least two weeks after two doses of SARS-CoV-2 vaccination BNTb162b

Number and percentage of hemodialysis patients with neutralizing serum antibodies and/or positive CD4+ T cell responses (stimulation index >3) against SARS-CoV-2 spike of wildtype (S-WT, n=30) and variants of concern B.1.1.7 (n=30) and B.1.351 (n=25) or no immunological surrogate marker of protection.

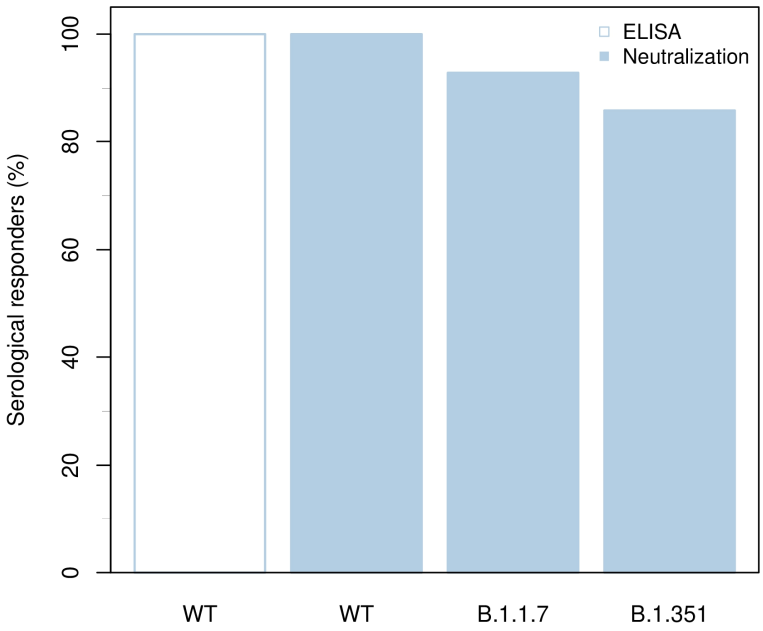

Figure S2: Humoral immunity of healthy donors at least two weeks after two doses of SARS-CoV-2 vaccination BNTb162b

Young healthy donors (n=14) were included at least two weeks after complete vaccination with BioNTech/Pfizer vaccine BNTb162b as positive controls for SARS-CoV-2 spike-protein binding antibodies assessed ELISA and for the neutralization assay of different SARS-CoV-2 strains wildtype virus hCoV-19, VOC B.1.1.7, or VOC B.1351.

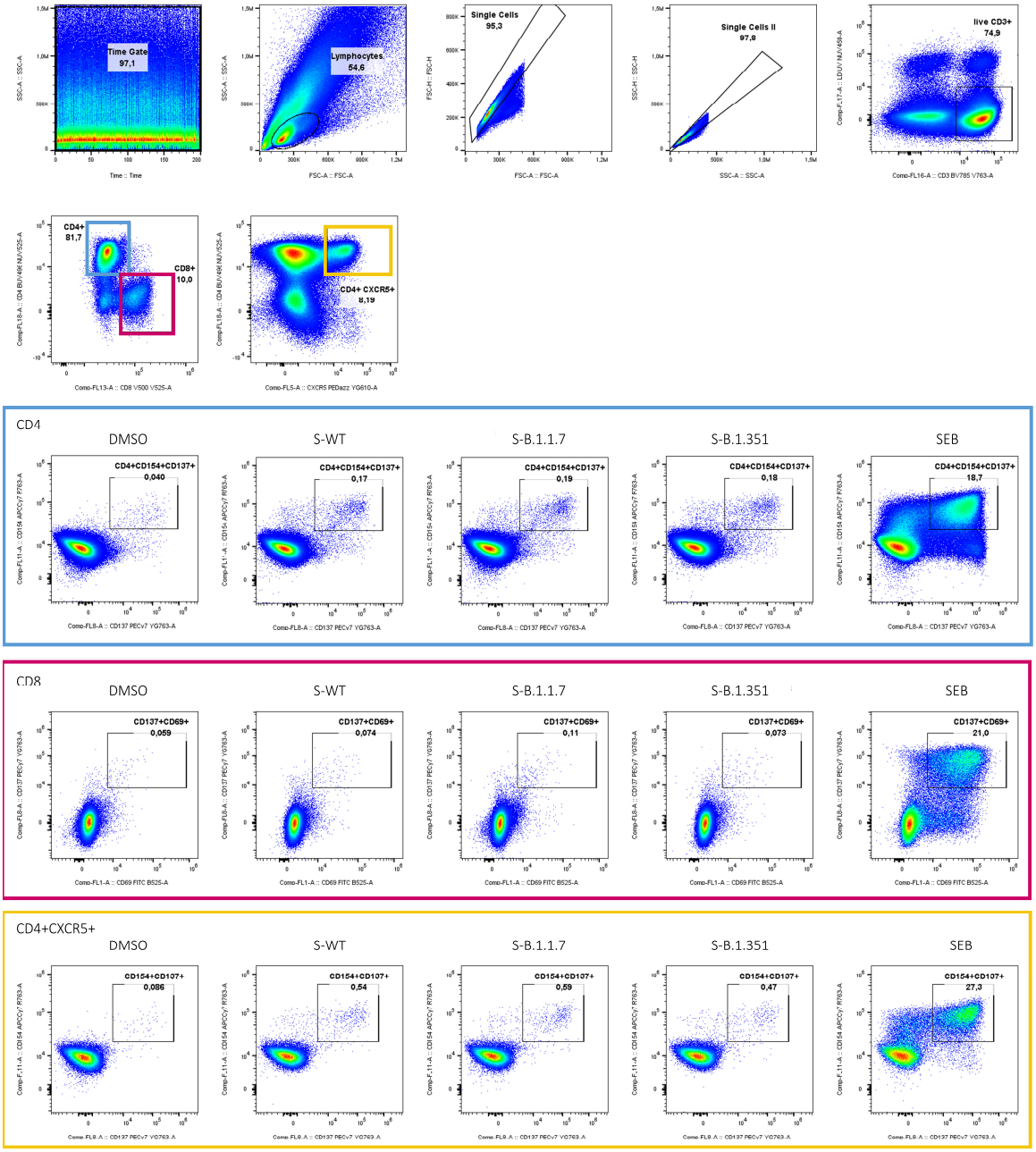

Fig. S3: Representative gating strategy for the analysis of activated CD4+, CD4+ CXCR5+, and CD8+ T cells

PBMC were stimulated overnight with overlapping peptide pools of the SARS-CoV-2 strains wildtype (WT), B.1.1.7, and B.1.351. After 2h, brefeldin A was added to inhibit release of cytokines. Analysis was performed by flow cytometry.

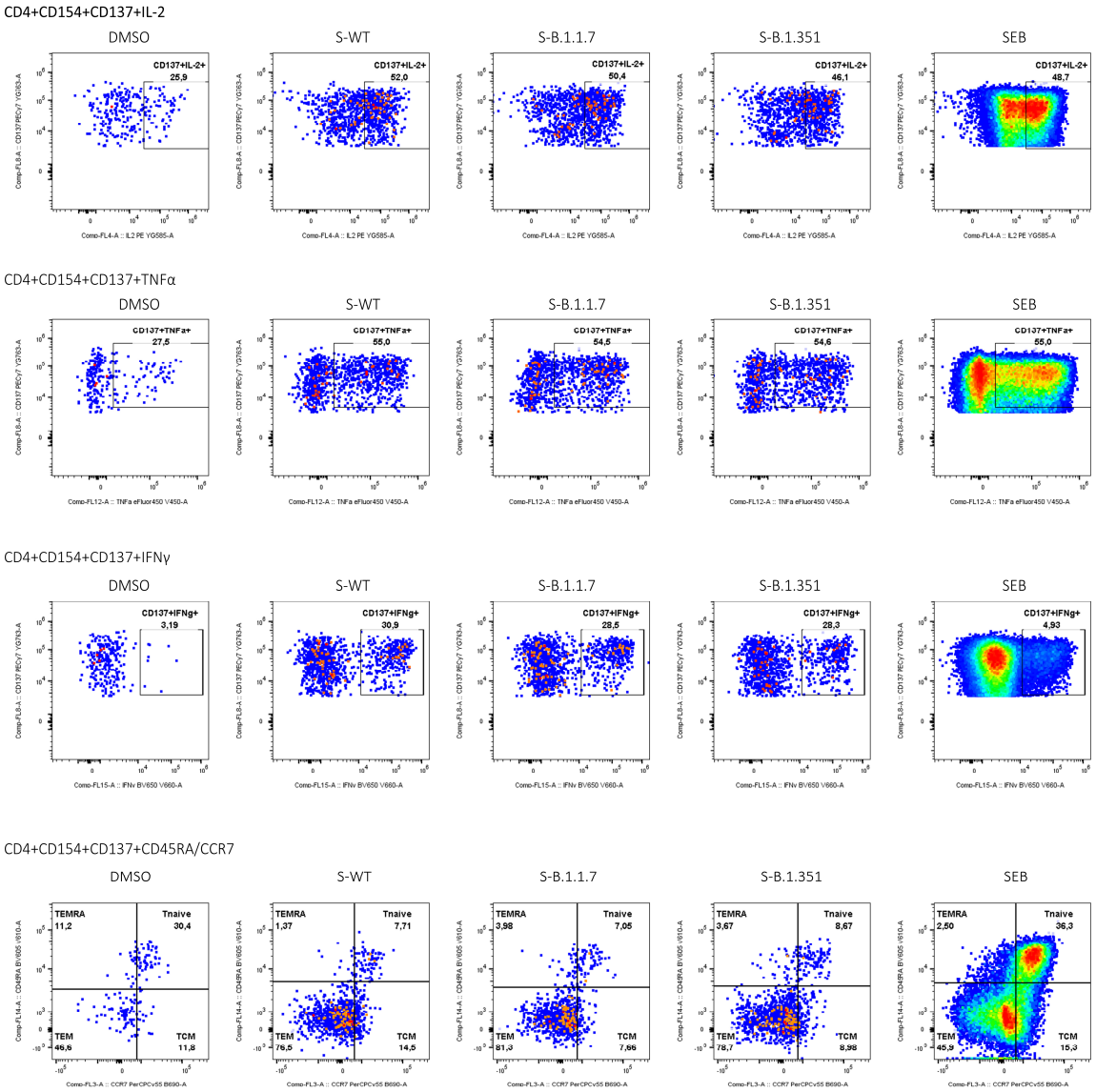

Figure S4: Representative gating strategy for the analysis of cytokines IL-2, TNFα, and IFNγ producing activated CD4+ T cells

PBMC were stimulated overnight with overlapping peptide pools of the SARS-CoV-2 strains wildtype (WT), B.1.1.7, and B.1.351. After 2h, brefeldin A was added to inhibit release of cytokines. Analysis was performed by flow cytometry.

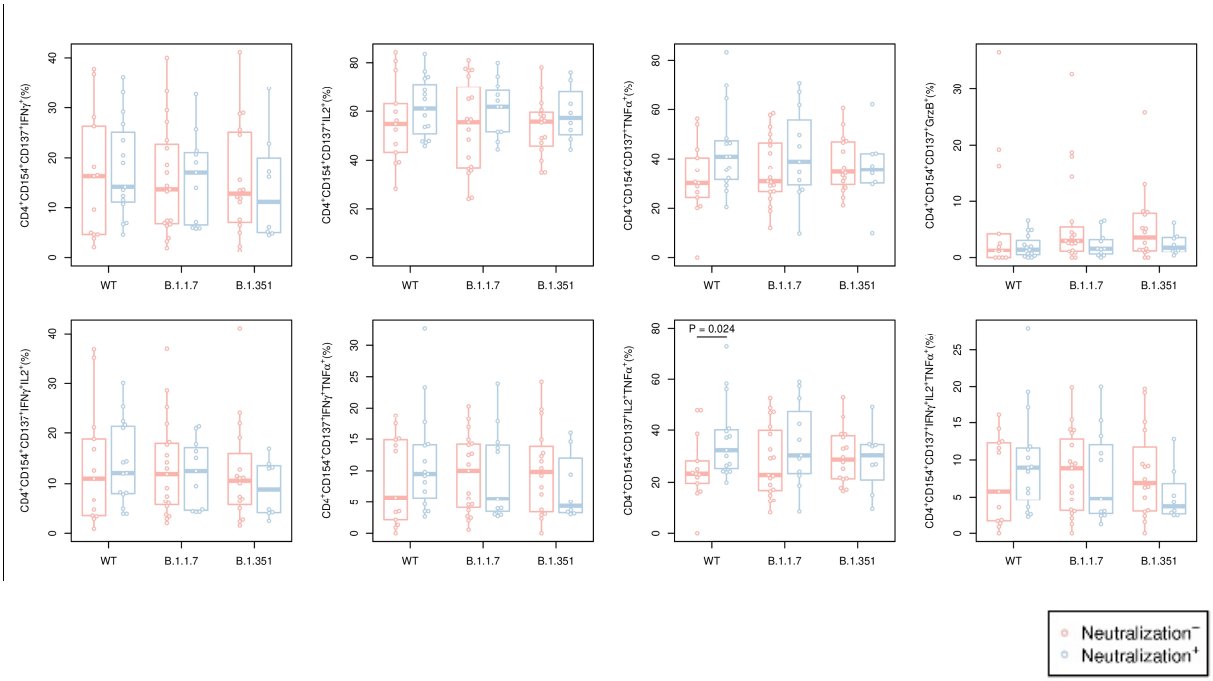

Figure S5: Frequency of cytokine producing cells among activated (CD154 and CD137+) CD4+ CD3+ T cells in humoral responders and non-responders

Hemodialysis patients (DP, n=30) were included at least two weeks after complete vaccination with BioNTech/Pfizer vaccine BNTb162b. DP were categorized as neutralization + or neutralization- according to neutralizing antibody titres in neutralization assays with the SARS-CoV-2 strains wildtype (WT), B.1.1.7, and B.1.351. Expression of cytokines interferon γ (IFNγ), tumor necrosis factor α (TNFα), and interleukin (IL-)2 as well as effector molecule Granzyme B (GrzB) were analyzed among activated CD4+ T cells by intracellular staining and flow cytometry after stimulation with overlapping peptide pools spanning the entire sequence of the spike protein of the three included SARS-CoV-2 strains (n=15 WT, n=11 B.1.1.7, and n=8 B.1.351 for responders; n=13 WT, n=19 B.1.1.7, and n=16 B.1.3.5.1 for non-responders; samples with less than 0.01% activated CD4+ T cells were excluded from the analysis). Polyfunctional T cells producing two or three of the cytokines IFNγ, TNFα, and IL-2 were analyzed by Boolean gating. 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. Comparisons were performed only between samples stimulated with the same strain. Differences between two groups are analyzed using the unpaired, two-tailed Mann-Whitney U test. P values below 0.050 were considered significant.

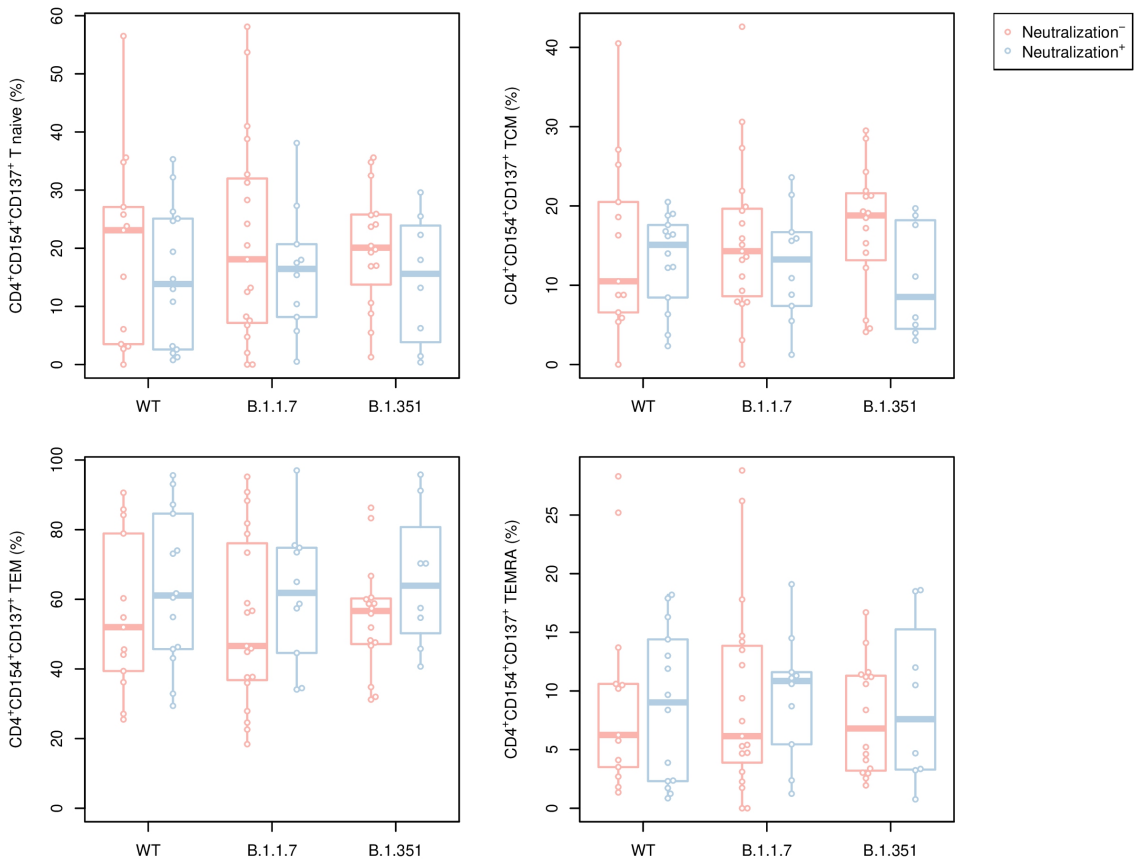

Figure S6: Frequency of memory phentoypes among activated (CD154 and CD137+) CD4+ CD3+ T cells in humoral responders and non-responders

Hemodialysis patients (DP) were included at least two weeks after complete vaccination with BioNTech/Pfizer vaccine BNTb162b. DP were categorized as neutralization + or neutralization- according to neutralizing antibody titres in neutralization assays with the SARS-CoV-2 strains wildtype (WT), B.1.1.7, and B.1.351. Naïve T cells (TNaive), central memory (TCM), effector memory (TEM), and effector memory reexpressing CD45RA (TEMRA) were analyzed among activated CD4+ T cells by flow cytometry after stimulation with overlapping peptide pools spanning the entire sequence of the spike protein of the three included SARS-CoV-2 strains (n=14 WT, n=10 B.1.1.7, and n=8 B.1.351 in responders; n=13 WT, n=19 B.1.1.7, and n=16 B.1.3.5.1 in non-responders; samples with less than 0.01% activated CD4+ T cells were excluded from the analysis, two samples were excluded due to technical reasons). 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. Comparisons were performed only between samples stimulated with the same strain. Differences between two groups are analyzed using the unpaired, two-tailed Mann-Whitney U test. P values below 0.050 were considered significant.

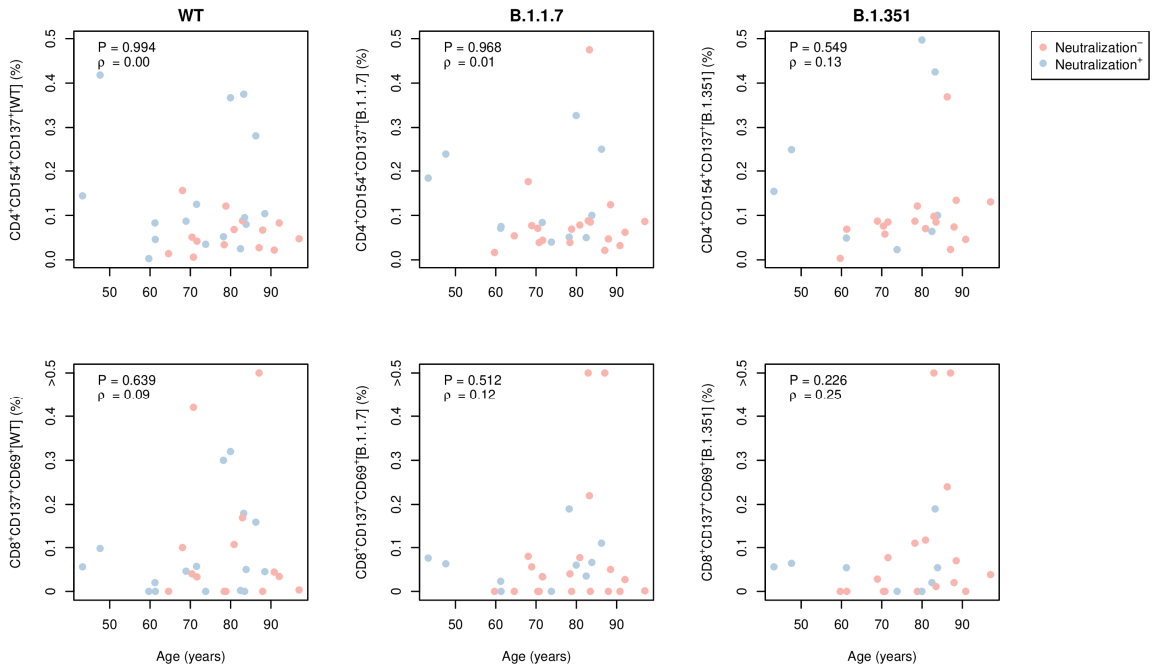

Figure S7: Influence of age on SARS-CoV-2 reactive T cell responses
Correlation analysis of SARS-CoV-2 specific CD4+ (top) and CD8+ (bottom) T cells versus age in hemodialysis patients (DP) who were included at least two weeks after complete vaccination with BioNTech/Pfizer vaccine BNTb162b. The frequencies of CD4 and CD8 T cells reactive to the WT (n=30), B.1.1.7 (n=30), and B.1.351 (n=25) S-protein were correlated with the patient age by Spearman's rank correlation co-efficient. No significant correlation could be observed.

Supplementary Methods

Study population and design

For this study, 34 hemodialysis patients and 14 healthy volunteers were recruited and vaccinated two times with BNTb162b mRNA. Analysis was done in median 42 days post-vaccination (range 16-83 days) in DP. The median age of DP was 77.5 years (range 43-96 years). Of the 34 participants, 20 (59%) were of male and 14 (41%) of female gender. The clinical characteristics of DP patients are summarized in Table S1.
The study was approved by the ethical committee of the Ruhr-University Bochum (20-6886). Written informed consent was obtained from all participants.

SARS-CoV-2 IgG antibody titers

Peripheral blood was collected in S-Monovette Z-Gel and serum was prepared according to the manufacturer's instructions (Sarstedt). SARS-CoV-2 IgG levels were analyzed in serum using the SARS-CoV-2 IgG kit (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions. Briefly, serum samples from patients requiring maintenance hemodialysis (n=70) or healthy individuals (n=10) were diluted 1:100 and added to plates coated with recombinant SARS-CoV-2 S-WT antigen. Bound SARS-CoV-2 S1 protein-specific IgG was detected by an HRP-conjugated anti-human IgG. The absorbance was read on a microplate reader at 450 nm with reference at 620 nm and evaluated as the ratio of the absorbance of the sample to the absorbance of the internal standard.

SARS-CoV-2 Wild Type, B.1.1.7 and B.1.351 neutralization assay

For the neutralization assay VeroE6 cells (kindly provided by C. Drosten and M. Müller) cultured in Dulbecco's Modified Eagle's Medium (DMEM, supplemented with 10 % (v/v) fetal calf serum (FCS), 1 % (v/v) non-essential amino acids, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2 mM L-Glutamine) were seeded at 1×104 cells/well one day prior the experiment.
Patient sera were heat-inactivated for 30 min at 56 °C and serially diluted 1:2 in a 96 well plate with an initial dilution of 1:10 in quadruplicates. Subsequently, 200 TCID50 of either wildtype virus hCoV-19/Germany/BY-Bochum-1/2020 (GISAID accession ID: EPI_ISL_1118929), VOC B.1.1.7 RKI-0026_B.1.1.7 (GISAID accession ID: EPI_ISL_751799), or VOC B.1351 RKI-0029_B.1.351 (GISAID accession ID: EPI_ISL_803957) was added to each well and incubated for 1 h at 37 °C. The virus-serum suspension was then transferred onto the seeded VeroE6 cells and incubated for 72 h at 37 °C. After the incubation period, the cells were stained with crystal violet following an optical analysis with respect to cytopathic effects (CPE). Based on CPE the 50% neutralization capacity (ND50 value) was determined by non-linear regression.

Preparation of PBMCs and stimulation with overlapping peptide pools from SARS-COV-2 S-protein VOC

Peripheral blood mononuclear cells (PBMCs) were isolated and stimulated as previously described.[1] In brief, PBMC were prepared from whole blood by gradient centrifugation. Cells were stimulated with 15mer overlapping peptides pools (OPP) from SARS-CoV-2 S-WT, B1.1.7, and B.1.351 S-Proteins with an overlap of 11 amino acids (JPT, Germany).

SARS-CoV-2 overlapping peptide pools (JPT, Germany) were used containing overlapping peptides of Spike wildtype (Wuhan type), B.1.1.7, and B.1.351. All Peptide pools were dissolved according to the manufacturer's instructions in DMSO and used at a concentration of 1 µg/ml. 2.5x106 PBMCs were plated for each condition in 96-UWell Plates in 200µl 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 h. As a positive control, cells were stimulated with SEB (1 µg/ml, Sigma Aldrich). After 2 h Brefeldin A (1µg/ml, Sigma Aldrich, USA) was added. T cells stimulated with SARS-CoV-2 OPP were stained as previously described.[1]
All antibodies used for staining were purchased from BioLegend, USA unless otherwise noted: Surface staining: Live-dead Fixable Blue Dead Cell Stain Kit (ThermoFisher, USA), CCR7 (CD197)-PerCP-Cy5.5; clone: G043H7, CD45RA-BV605; clone: HI100. CXCR5-PE-Dazzle594, clone: J252D4; CD3-BV785, clone: OKT3. Intracellular staining: CD4-BUV496, clone: SK3 (leu3a) (BD Biosciences, USA); CD8-V500; clone: RPA-T8 (BD Biosciences, USA); Granzyme B-AL700; clone: R712 (BD Biosciences, USA); IL-2-PE; clone: MQ1-17H12; CD137 (4-1BB)-PE-Cy7; clone: 4B4-1; CD154 (CD40L)-APC/Cy7; clone: 24-31, TNFa-eFluor450; clone: MAb11 (eBioscience, USA), IFNγ-BV650; clone: 4S.B3; CD69-FITC, clone: FN50 (BD Biosciences, USA).
After staining all samples were immediately acquired on a CytoFlex LX flow cytometer (Beckman Coulter, USA). As a result of low cell counts after thawing, SARS-CoV-2 S-WT, S-B.1.1.7, and S-B.1.351-reactive T cells could be measured in 30, 30, and 25 out of 34 DP, respectively. Antigen-reactive responses were considered as positive if the specific response after stimulation exceeded the background activation 3 times (stimulation index SI >3).[1] For activated cells that are presented as frequency of CD4+, CD8+, or CD4+ CXCR5+ T cells, non-specific background in DMSO control was subtracted.

Statistical Analysis

Flow cytometry data was analyzed using FlowJo version 10.7.1 (BD Biosciences, USA). Statistical analysis was performed using R, version 4.0.3. 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. The applied statistical tests are two-sided. Comparisons were performed only between samples stimulated with the same strain. Differences between two groups are analyzed using the unpaired, two-tailed Mann-Whitney U test. P values below 0.050 were considered significant. No adjustment for multiple testing was performed, as part of an exploratory study.[2]

Supplementary References

1. Thieme CJ, Anft M, Paniskaki K, et al. Robust T Cell Response Toward Spike, Membrane, and Nucleocapsid SARS-CoV-2 Proteins Is Not Associated with Recovery in Critical COVID-19 Patients. *Cell Reports Med*. 2020;1(6):100092. doi:10.1016/j.xcrm.2020.100092
2. Bender R, Lange S. Adjusting for multiple testing - When and how? *J Clin Epidemiol*. 2001;54(4):343-349. doi:10.1016/S0895-4356(00)00314-0