The magnitude and functionality of SARS-CoV-2 reactive cellular and humoral immunity in transplant population is similar to the general population despite immunosuppression

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Supplementary Tables

Table S1: Fluorochrome coupled antibodies and fluorescent dye for analysis of SARS-CoV-2 reactive T cells

Antibodies or fluorescent dye	Fluorochrome	Source	Identifier
Fixable Viability-Dye	eFluor780	eBioscience	Cat#: 65-0865-14
anti CCR7 (clone G043H7)	PerCP-Cy5.5	BioLegend	Cat#: 353220, RRID: AB_10916121
anti CD4 (clone OKT4)	A700	BioLegend	Cat#: 317426, RRID: AB_571943
anti CD8 (clone RPA-T8)	V500	BD Biosciences	Cat#: 560775, RRID: n/a
anti CD45RA (clone HI100)	BV605	BioLegend	Cat#: 304134, RRID: AB_2563814
anti Granzyme B (clone GB11)	FITC	BioLegend	Cat#: 515403, RRID: AB_2114575
anti IL2 (clone MQ1-17H12)	PE	BioLegend	Cat#: 500307, RRID: AB_315094
anti IL4 (clone MP4-25D2)	PE-Dazzle594	BioLegend	Cat#: 500832, RRID: AB_2564036
anti CD137 (4-1BB) (clone 4B4-1)	PE-Cy7	BioLegend	Cat#: 309818, RRID: AB_2207741
anti CD154 (CD40L) (clone 24-31)	A647	BioLegend	Cat#: 310818, RRID: AB_492970
anti TNFα (clone MAb11)	eFluor450	eBioscience	Cat#: 48-7349-42, RRID: AB_2043889
anti IFNγ (clone 4S.B3)	BV650	BioLegend	Cat#: 502538, RRID: AB_2563608
anti CD3 (clone OKT3)	BV785	BioLegend	Cat#: 317330, RRID: AB_2563507

Table S2: Clinical characteristics, COVID-19 treatment, and monitoring strategy of transplant (Tx) patients and non-Tx-patients

Variable	Tx	Non-Tx	P-value
No. patients (%)	10 (27.8%)	26 (72.2%)	-
Demographic characteristics			
Gender, male/female (%)	6/4 (60.0%/40.0%)	16/10 (61.5%/38.5%)	ns
Age (years), median [IQR]	55 [41-61]	69 [58-82]	0.006
Initial COVID-19 severity			
Moderate	8 (80.0%)	10 (38.0%)	ns
Severe	1 (10.0%)	7 (27.0%)	
Critical	1 (10.0%)	9 (35.0%)	
Maximal COVID-19 severity in follow up			
Moderate	7 (70.0%)	6 (23.1%)	0.043
Severe	1 (10.0%)	10 (38.5%)	
Critical	2 (20.0%)	10 (38.5%)	
Monitoring scheme			
First measurement (days after first positive PCR), median [IQR]	4 [2-6]	3 [1-9]	ns
No. measurements per patient, median [IQR]	3 [2-3]	2 [2-3]	ns
Mean time of follow-up measurements (days after first positive PCR), median [IQR]	9 [8-10]	11 [5-23]	ns
Chest CT abnormalities			
Bilateral ground-glass opacity (%)	3 (30.0%)	5 (19.2%)	ns
Acute respiratory distress syndrome (%)	1 (10.0%)	10 (38.5%)	ns
Treatments			
Antibiotics (%)	6 (60.0%)	23 (88.5%)	ns
Anticoagulants (%)	9 (90.0%)	11 (42.3%)	0.022
Remdesivir (%)	1 (10.0%)	0 (0.0%)	ns
Plasma therapy (%)	2 (20.0%)	0 (0.0%)	ns
Admission to intensive care unit (%)	2 (20.0%)	11 (42.3%)	ns
Mechanical ventilation (%)	1 (10.0%)	10 (38.5%)	ns

Supplementary Figures

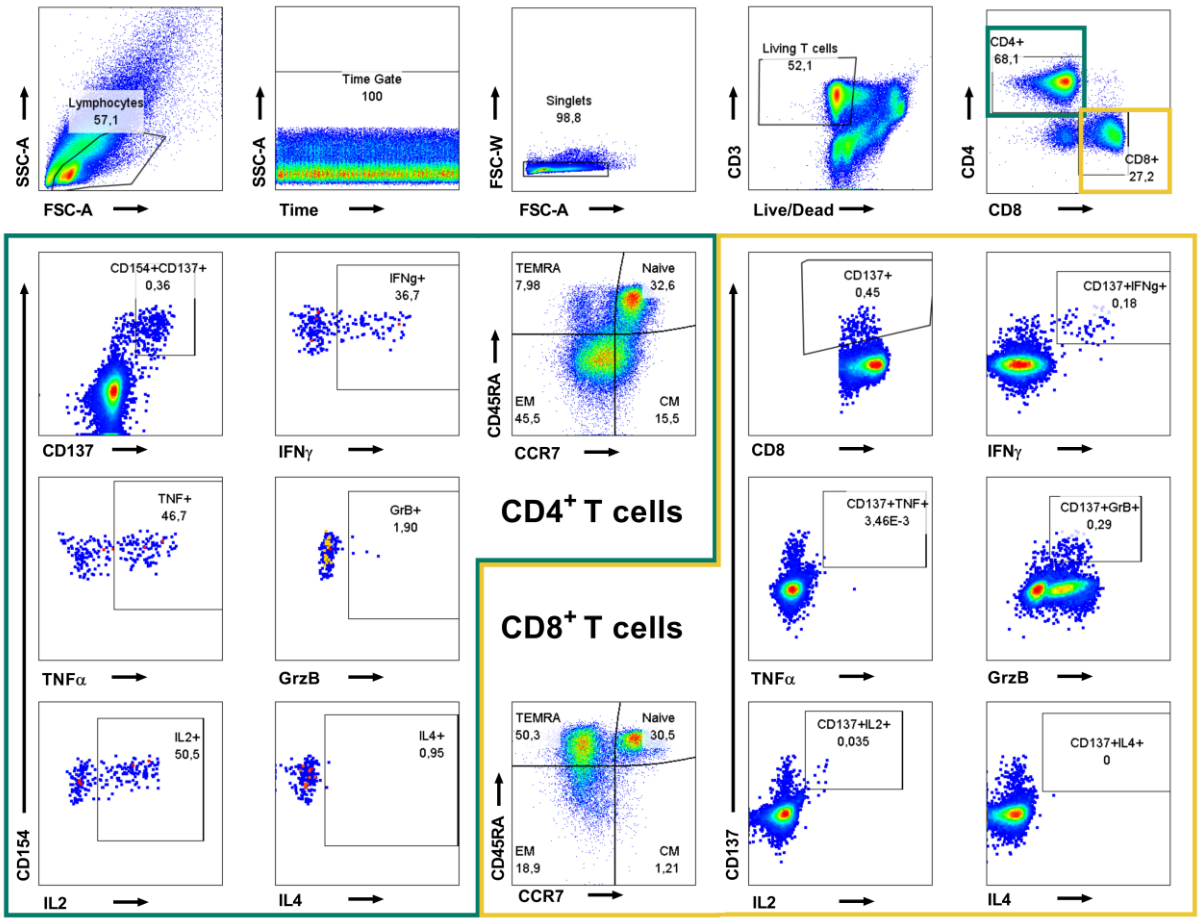

Figure S1: Flow cytometry gating strategy for identification and quantification of SARS-CoV-2-reactive CD4+ and CD8+ T cells.

PBMC were stimulated for 16h with SARS-CoV-2 overlapping peptide pools. After 2h Brefeldin A was added to the culture to block secretion of cytokines and effector molecules. Living single lymphocytes were analyzed for expression of CD3, CD4, and CD8. $CD4^+$ T cells (green box) were analyzed for the expression of CD154 and CD137. $CD8^+$ T cells (yellow box) were analyzed for expression of CD137. Both $CD4^+$ and $CD8^+$ T cells were further analyzed for the production of cytokines interferon γ (IFNγ), tumor necrosis factor α (TNFα), interleukin (IL)-2, IL4, and effector molecule granzyme B (GrB). Memory phenotypes were characterized by expression of C-C chemokine receptor type 7 (CCR7) and CD45-RA. Plots show pseudocolor plots. For better visibility SARS-CoV-2 specific T cells are shown as large dots. Representative example of 10 transplant and 26 non-transplant COVID-19 patients.

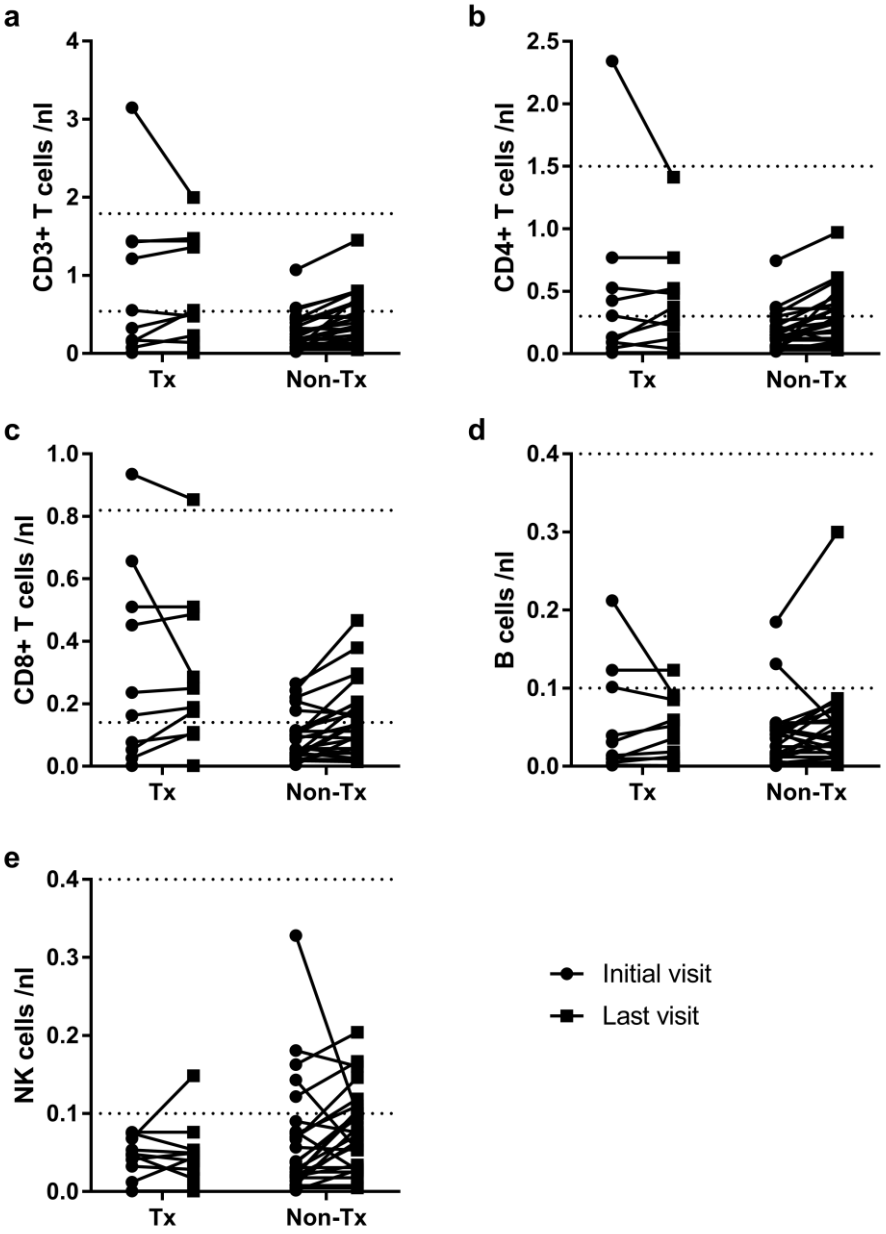

Figure S2: Absolute counts of lymphocyte subsets in transplanted (Tx) and non-Tx COVID-19 patients

EDTA-treated blood samples of Tx- (n=10) and Non-Tx- (n=26) COVID-19 patients were collected at the study inclusion and in follow-up. Absolute counts are expressed as cells per nanoliter (nl) of $CD3^+$ (T cells) (**a**), $CD4^+$ $CD3^+$ (**b**), $CD8^+$ $CD3^+$ (**c**), $CD19^+$ $CD3^-$ (B cells) (**d**), and $CD56^+$ $CD3^-$ (NK cells) (**e**) SSC^{low} $CD45^+$ lymphocytes. Normal distribution was assessed using D'Agostino-Pearson-omnibus-normality-test. Statistical comparison between Tx- and Non-Tx-patient samples was done with Mann-Whitney-U-test. Dotted lines represent minimum and maximum reference values.

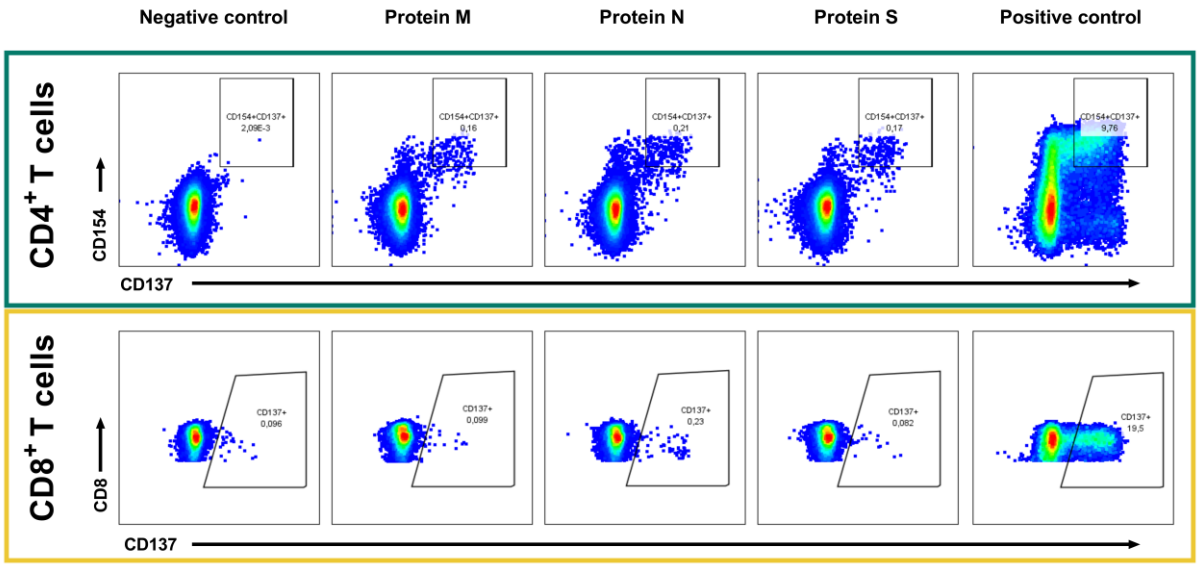

Figure S3: Activation marker expression of T cells stimulated with SARS-CoV-2 peptide pools and controls.

PBMC were stimulated for 16h with SARS-CoV-2 M-, N-, or S-protein overlapping peptide pools or left untreated (negative control) or stimulated with staphylococcal enterotoxin B (positive control). After 2h Brefeldin A was added to the culture to block secretion of cytokines and effector molecules. $CD4^+$ T cells (green box) were analyzed for the expression of activation markers CD154 and CD137. $CD8^+$ T cells (yellow box) were analyzed for expression of CD137. Plots show pseudocolor plots with large dots. Representative example of 10 transplant and 26 non-transplant COVID-19 patients.

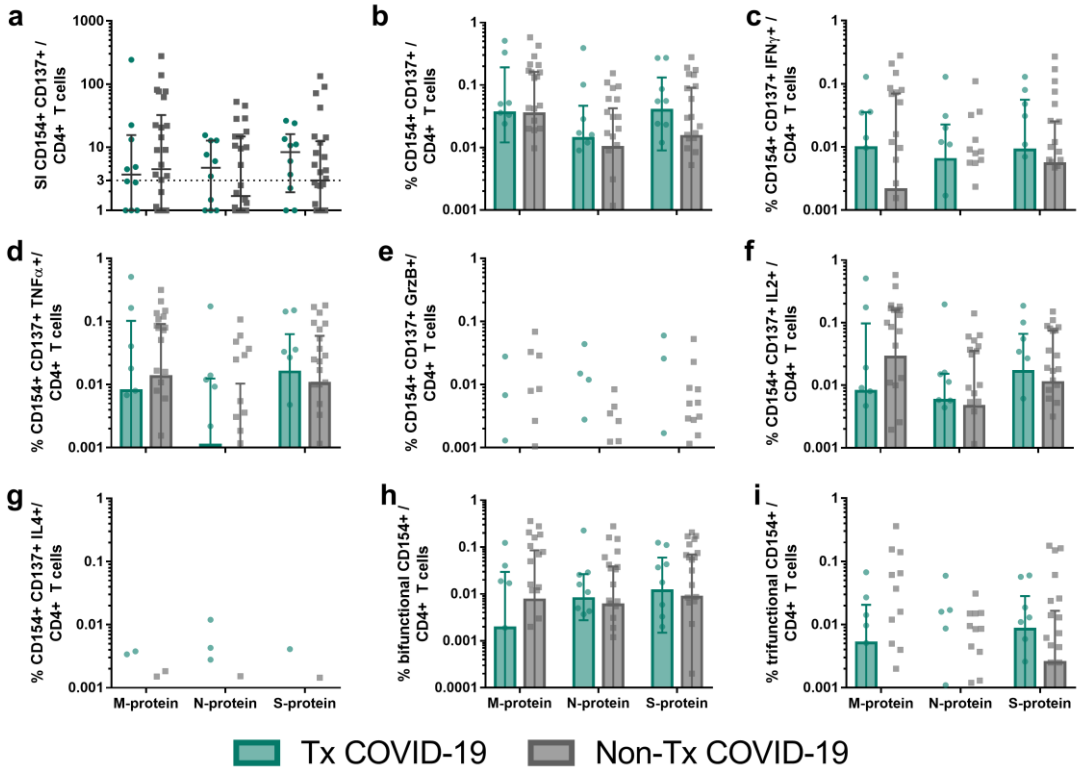

Figure S4: Characterization of SARS-CoV-2-reactive CD4⁺ T cells in transplant and non-transplant COVID-19 patients early after COVID-19 diagnosis.

Blood samples of 10 transplant (Tx) patients and 26 control (non-Tx) patients suffering from acute COVID-19 were collected early after COVID-19 diagnosis and stimulated with SARS-CoV-2 membrane (M)-, nucleocapsid (N)-, and spike (S)-protein and analyzed by flow cytometry. In one Tx-patient, only N- and S-protein reactive T cells could be analyzed due to the limited amount of collected blood and lymphopenia.

a Stimulation index (SI) of activation markers CD154 and CD137 expressing CD4⁺ T cells (SARS-CoV-2 specific CD4⁺ T cells). SI was calculated by dividing the measured T cell subset response by the respective response in the negative control. Values above 3 are considered above detection limit. For patients with multiple samples, the maximal response was calculated. Scatter plot with line at median and interquartile range.

b-i Frequencies of SARS-CoV-2 specific CD4⁺ T cells (**b**) and SARS-CoV-2 specific CD4⁺ T cells expressing IFNγ (**c**), TNFα (**d**), GrzB (**e**), IL2 (**f**), or IL4 (**g**) as well as bifunctional (**h**) and trifunctional (**i**) CD154⁺ CD4⁺ T cells. Bi- and trifunctional T cells were calculated by Boolean gating of production of IFNγ, TNFα, GrzB, IL2, and IL4. Negative controls were subtracted from specifically stimulated samples to exclude unspecific activation.

Statistical comparison was done with Mann-Whitney-U-test and controlled by multivariate analysis for the influence of transplantation status and age. $P<0.05$ was considered significant.

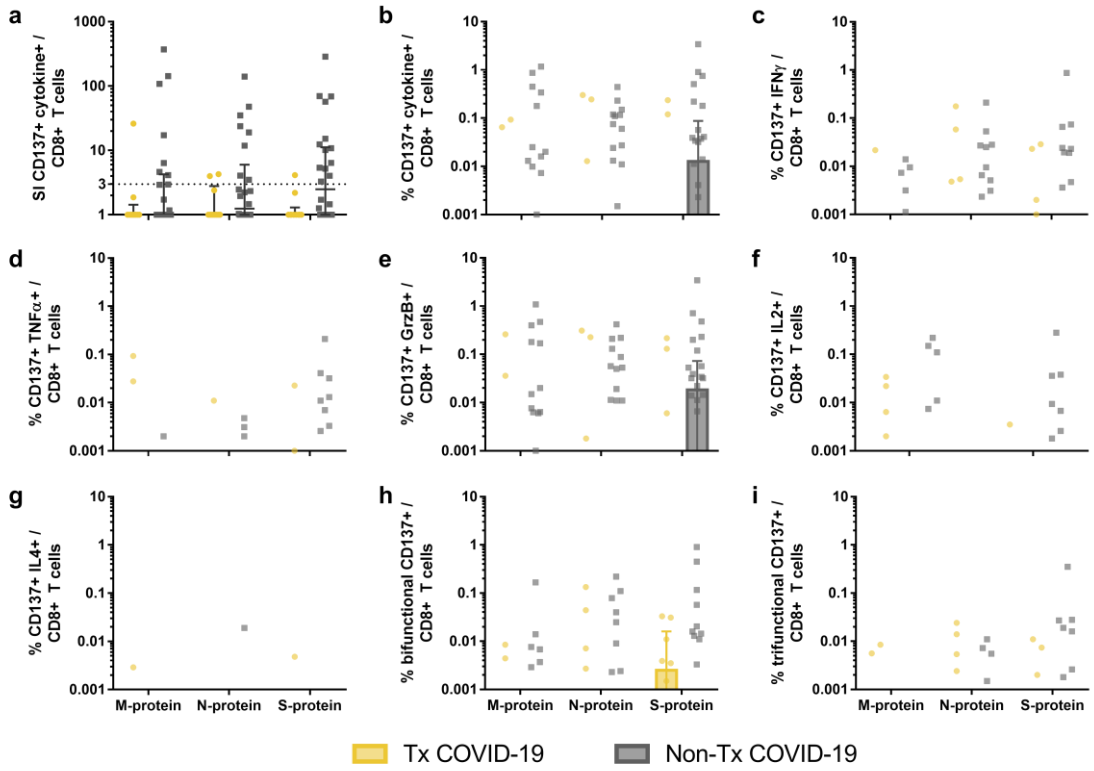

Figure S5: Characterization of SARS-CoV-2-reactive CD8⁺ T cells in transplant and non-transplant COVID-19 patients early after COVID-19 diagnosis.

Blood samples of 10 transplant (Tx) patients and 26 control (non-Tx) patients suffering from acute COVID-19 were collected early after COVID-19 diagnosis and stimulated with SARS-CoV-2 membrane (M)-, nucleocapsid (N)-, and spike (S)-protein and analyzed by flow cytometry. In one Tx-patient, only N- and S-protein reactive T cells could be analyzed due to the limited amount of collected blood and lymphopenia.

a Stimulation index (SI) of activation marker CD137 and at least one of the cytokines IFNγ, TNFα, IL2, IL4, or effector molecule GrB expressing CD8⁺ T cells (SARS-CoV-2 specific CD8⁺ T cells). SI was calculated by dividing the measured T cell subset response by the respective response in the negative control. Values above 3 are considered detectable. For patients with multiple samples, the maximal response was calculated. Scatter plot with line at median and interquartile range.

b-i Frequencies of SARS-CoV-2 specific (CD137⁺ cytokine⁺) CD8⁺ T cells (**b**) and CD137⁺ CD8⁺ T cells expressing IFNγ (**c**), TNFα (**d**), GrzB (**e**), IL2 (**f**), or IL4 (**g**) as well as bifunctional (**h**) and trifunctional (**i**) CD137⁺ CD8⁺ T cells. Bi- and trifunctional T cells were calculated by Boolean gating of production of IFNγ, TNFα, GrzB, IL2, and IL4. Negative controls were subtracted from specifically stimulated samples to exclude unspecific activation.

Statistical comparison was done with Mann-Whitney-U-test and controlled by multivariate analysis for the influence of transplantation status and age. $P<0.05$ was considered significant.