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DIA-Vacc supplementary methods
Investigating T-cell SARS-CoV-2 immune response

To explore the cellular SARS-CoV-2 immune response, two independent assays were performed at all time points in subgroups representing all three study groups and both types of vaccine. Hereby, a SARS-CoV-2 specific interferon-γ release assay (Euroimmun-SARS-CoV-2-IGRA for research use only ET 2606-3003 & EQ 6841-9601[1,2]) as well as in-depth immunophenotyping using flow cytometry measurements (as previously described[3]) were applied in about 300 and 140 blood donors, respectively.

The groups for interferon- γ release assay (IGRA) were formed as follows: The analysis of T cells requires the complex isolation of PBMCs. To reach a high viability in the interferon- γ release assay (IGRA) samples should be processed immediately < 24h (established < 6h). To ensure this high sample quality, four centers in the vicinity of the study coordination center were asked about their willingness to participate. The selection took into account that the centers treated a sufficient number of transplanted patients and that both vaccines were represented.

In the additional analysis of deep immunophenotyping three centers collaborated in further blood collection for PBMC isolation. Since flow cytometry measurements are extremely time consuming, fewer patients were collected for FACS analysis compared to IGRA testing. Again, samples from all three groups and both vaccine types were selected. An additional measurement criterion was the sufficient number of PBMCs per probe (min. 10^7/time point and participant) for the planned analyses at least at the time points T0 and T2. Cell counts were performed before cryopreservation and after PBMC isolation. Patients selected for FACS analysis were a subgroup of the IGRA-cohort.

Interferon- γ release assay (IGRA)

Quantitative assessment of IFN-γ release of SARS-CoV-2 specific T cells was performed in a subgroup of the study population. Results ≥ 100 mIU/ml were scored as SARS-CoV-2 specific immune response in the evaluation ELISA, due to infection or vaccination response depending on the time of measurement. Test kit contains several sets of 3 different tubes (BLANK, STIM, TUBE) each. The "BLANK" tube represents the individual interferon-γ background and must be subtracted from the results of the other two tubes. The "STIM" tube, which contains a non-specific mitogen, can be used to check the vitality of the blood sample obtained (positive control). The CoV-2-IGRA "TUBE" is coated on the inside with antigens based on the S1 domain of the spike protein of SARS-CoV-2. Fresh blood (blood collection < 16h, no cryopreservation before incubation) is added to all 3 test tubes and incubated for 20-24h at 37°C. Subsequently, 200 µl plasma each was cryopreserved after centrifugation and measured in the further course using the available ELISA kit. After subtraction of the BLANK value from the results of the STIM tube and the actual test tube, only results confirming sufficient vitality and stimulability of the blood sample were considered (STIM tube after BLANK subtraction > 200 mIU/ml). The following constellations were excluded for further analysis, as not validly assessed: STIM < 200 mIU/ml; BLANK >200 mIU/ml and TUBE < 200 mIU/ml. TUBE values < 0 mIU/ml, after blank subtraction were set to 0 mIU/ml by definition, assuming inconspicuous BLANK and STIM-Tube values.

Flow cytometry data analysis and statistical comparison

Analysis of SARS-CoV-2 Spike protein reactive T cells was performed by flow cytometry as previously described[3]. Briefly, peripheral blood mononuclear cells (PBMC) were isolated from BD CPT™ tubes. CPT tubes contain a combined FICOLL™ and gel density separation system that permanently separates mononuclear cells from other blood cells during centrifugation. Isolated PBMC were stored at -80°C to allow analysis in batches. PBMC were left resting overnight after thawing and afterwards stimulated with SARS-CoV-2-PepTivator peptide-pools solved in water (Miltenyi Biotec). Untreated PBMC were used as negative control to assess unspecific background activation. After 2h of stimulation, Brefeldin-A (Sigma-Aldrich) was added and the stimulation stopped after 16h. Surface- and intracellular-staining for flow cytometry was performed using fixation and permeabilization (ThermoFisher) and antibodies listed in Table S2. Samples were measured on a CytoFlex flow cytometer (Beckman-Coulter).

Flow cytometry data were analyzed using FlowJo version 10.6.2 (BD Biosciences). Single stains and fluorescence-minus-one controls were used for gating. CD4$^+$ T cells expressing CD154 and CD137 and CD8$^+$ T cells expressing CD137 in combination with production of at least one of IL2/IL4/IFNγ/TNFα/GrzB were defined as reactive T cells. Unspecific activation in unstimulated controls was subtracted from stimulated samples to account for SARS-CoV-2-specific activation in the presented frequencies.

Ethic declaration

According to the professional code of conduct for doctors (§15) the clinical trial was submitted to the ethical institutional review boards at Technische Universität Dresden (TU Dresden) responsible for the coordinating investigator (BO-EK-45012021), as well as at the University of Leipzig (046/21-lk) and Saxon Medical Association (Sächsische Landesärztekammer – EK-BR-10/21-1) responsible for further participating trial sites.

Clinical data management and data protection

For creation of the study database the EDC tool REDCap (Research Electronic Data Capture)[4,5], developed and distributed by the Vanderbilt University, has been used. The database has been validated according to the Standard Operating Procedures (SOPs) of the Coordination Centre for Clinical Trials Dresden prior to data capture.

The entered data into the eCRF by the investigator or an authorized member of the study team were systematically checked for completeness, consistency and plausibility by routines implemented in the REDCap database such that discrepancies can be dealt with at data entry. Errors and warnings could be resolved at any time during entry process.

During the whole course of the study, a backup of all data was made on a daily base. Unauthorized access to patient data was prevented by the access concept of the study database, which is based on a strict hierarchy and role model. Any change of data (e.g. when data is changed in the database during query management) is recorded automatically via audit trail within the database.

At the end of the study, once the database was complete and accurate, it has been locked. Thereafter, any changes to the database are possible only by joint written agreement between coordinating investigator, biometrician and data manager.

The Coordination Centre for Clinical Trials Dresden is responsible for implementation of procedures for data collection, storage, protection, retention and destruction. Investigators in the recruiting trial sites initially collected all data. Together with information on the trial, eligible patients and participants were informed about data capture, transmission and analysis processes. Once a participant was eligible and has given his/her informed consent to trial participation and data collection, the investigator has assigned the person a unique patient/participant identification code. This identification code lists are part of the investigator site file and remain at the recruiting site. These lists are the only documents that allow for re-identification of the patients.

Participant data were recorded in pseudonymized form (i.e. without reference to the patient's name) using the identification code. Data capture and processing was in accordance with the applicable law on personal data protection and with the "General Data Protection Regulation" (EC) 2016/679 of the European parliament and of the council.

DIA-Vacc supplementary tables
Table S1: Baseline characteristics of Saxonian Dia-Vacc cohort at study start (T0)

Variable	Category	Medical personnel	Dialysis patients	KTR recipients
Number	evaluable	368	1770	418
Age (years) 64 ± 15.5	**Mean ± SD**	46.2 ± 11.2	67.4 ± 13.9	57.5 ± 13.6
Male Sex	**n / %**	72 / 19.6	1144 / 64.6	275 / 65.8
BMI (kg/m²)	**Mean ± SD**	25.6 ± 4.9	27.7 ± 5.9	26.4 ± 4.7
Cause of end stage renal disease	**n / %**	n.a.	1438 / 81.2	257 / 61.5
Diabetes-Hypertension-Vascular disease	n / %	n.a.	867 / 49	73 / 17.5
Glomerulonephritis-Interstitial nephritis	n / %	n.a.	376 / 21.2	111 / 26.6
Vasculitis	n / %	n.a.	51 / 2.9	11 / 2.6
Polycystic kidney disease	n / %	n.a.	144 / 8.1	62 / 14.8
Unknown	n / %	n.a.	332 / 18.8	161 / 38.5
Drug treated comorbidities	n / %	72 / 19.6	1702 / 96.2	376 / 90
Diabetes mellitus	n / %	8 / 2.2	669 / 37.8	80 / 19.1
Cardiovascular disease	n / %	55 / 14.9	1644 / 92.9	361 / 86.4
Lung disease	n / %	11 / 3	127 / 7.2	28 / 6.7
Liver cirrhosis	n / %	0 / 0	26 / 1.5	4 / 1
Cancer	n / %	3 / 0.8	100 / 5.6	11 / 2.6
None	n / %	296 / 80.4	68 / 3.8	42 / 10
Type of dialysis		n.a.	1770 / 100	n.a.
Hemodialysis	n / %	n.a.	1688 / 95.4	n.a.
Peritonealdialysis	n / %	n.a.	82 / 4.6	n.a.
Time on dialysis (years)	**Mean ± SD**	n.a.	5.8 ± 5.7	6.7 ± 6.7
On transplant waiting list	**n / %**	n.a.	231 / 13.1	n.a.
Time on transplantation (years)	**Mean ± SD**	n.a.	n.a.	9.8 ± 6.8
Previous transplantation	n / %	1 / 0.3	133 / 7.5	68 / 16.3
Hepatitis B vaccination failure	n / %	13 / 3.5	354 / 20	40 / 9.6
Flu vaccination winter 2020/2021	n / %	208 / 56.5	1243 / 70.2	241 / 57.7
On immunosuppressive therapy	n / %	2 / 0.5	87 / 4.9	417 / 99.8
Corticosteroids	n / %	1 / 0.3	55 / 3.1	204 / 48.8
Calcineurin-Inhibitor	n / %	0 / 0	28 / 1.6	366 / 87.6
MMF/MPA	n / %	0 / 0	19 / 1.1	319 / 76.3
mTOR-Inhibitor	n / %	0 / 0	2 / 0.1	65 / 15.6
Belatacept	n / %	0 / 0	2 / 0.1	19 / 4.5
T-cell depleting ab	n / %	0 / 0	0 / 0	0 / 0
B-cell depleting ab	n / %	0 / 0	7 / 0.4	0 / 0
Other	n / %	1 / 0.3	6 / 0.3	5 / 1.2
Vaccination (yes)	n / %	294 / 79.9	1669 / 94.3	416 / 99.5
Type of vaccine	**n / %**			
BNT162b2 mRNA	n / %	110 / 29.9	328 / 18.5	111 / 26.6
mRNA-1273	n / %	175 / 47.6	1341 / 75.8	305 / 73
Previous symptomatic COVID-19 disease	n / %	84 / 22.8	184 / 10.4	12 / 2.9
Post-COVID-19 symptoms	n / %	24 / 6.5	28 / 1.6	4 / 1
Previous asymptomatic COVID-19 disease	n / %	33 / 9	171 / 9.7	20 / 4.8

Table S2: List of antibodies used for surface- and intracellular-staining in the flow cytometry

	Antigen	Fluorophore	Clone	Company
Surface	CD197 (CCR7)	PerCP-Cy5.5	G043H7	Biolegend
	CD185 (CXCR5)	PE/Dazzle-594	J252D4	Biolegend
	CD4	A700	OKT4	Biolegend
	Life/Dead	eFluor780		eBioscience
	CD8	V500	RPA-T8	BD Biosciences
	CD45RA	BV605	HI100.	Biolegend
Intracellular	Granzyme B	FITC	GB11	Biolegend
	IL-2	PE	MQ1-17H12	Biolegend
	CD137 (4-1BB)	PE-Cy7	4B4-1	Biolegend
	CD154 (CD40L)	A647	24-31	Biolegend
	TNFa	eFluor450	MAb11	eBioscience
	IFNg	BV650	4S.B3	Biolegend
	CD3	BV785	OKT3	Biolegend

Table S3: Dialysis patients in the generalized estimating equations (GEE) approach

Risk factor		Log OR (Z-score)	p-value
Sex	Male	Ref.	
	Female	1.294	0·443
Age	per year	0.316	0·865
BMI	per unit	-2.064	0·074
Time on dialysis	per year	-1.580	0·055
Number of comorbidities	per one	-0.373	0·802
Hepatitis B vaccination failure	No	Ref.	
	Yes	0.091	0·956
IS drugs	None	Ref.	
	At least one	6.680	< 0·001
Vaccine type	BNT162b2 mRNA	Ref.	
	mRNA-1273	-3.907	< 0·001

BMI = body mass index; IS = immunosuppressive drugs

Table S4: Transplant patients in the generalized estimating equations (GEE) approach

Risk factor		Log OR (Z-score)	p-value
Sex	Male	Ref.	
	Female	-0.907	0·575
Age	per year	4.663	0·007
BMI	Per unit	-0.437	0·672
Time on transplantation	per year	-3.289	0·004
Number of comorbidities	per one	-0.223	0·799
Hepatitis B vaccination failure	No	Ref.	
	Yes	-1.747	0·072
Number of IS drugs	Per one	4.208	0·001
Vaccine type	BNT162b2 mRNA	Ref.	
	mRNA-1273	-4.641	< 0·001

BMI = body mass index; IS = immunosuppressive drugs

Table S5: Multiple logistic regression analysis for a negative humoral immune response versus immunosuppression for dialysis patients and transplant recipients of the pure vaccination cohort between T0 and T2

Risk factor		OR	95% CI	p-value
Sex	Male	Ref.		
	Female	1.099	[0.748; 1.616]	0.63
Age	per year	1·017	[1·002; 1·031]	0·022
BMI	Per unit	0·971	[0·935; 1·008]	0·126
Group	DP	Ref.		
	KTP	4·572	[2·071; 10·093]	<0·001
Hepatitis B vaccination failure	No	Ref.		
	Yes	0·743	[0·423; 1·302]	0·3
Number of IS drugs	Per one	4·744	[2·868; 7·849]	<0·001
Vaccine type	BNT162b2 mRNA	Ref.		
	mRNA-1273	0·282	[0·187; 0·426]	< 0·001

Ref. = reference category; IS means immunosuppression; T0 = before first vaccination; T2 = 4-5 weeks after booster vaccination.

Table S6: Baseline characteristics of dialysis patients by type of vaccine

Risk factor	BNT162b2 mRNA	mRNA-1273	p-value
Sex	Prop. Male = 36.6%	Prop. Male = 34.5%	0.611
Age	Med = 70, IQR =[61, 79]	Med = 69, IQR =[58; 79]	0.511
BMI	Med = 25.99, IQR =[23, 30.28]	Med = 26.54, IQR =[23.67; 30.48]	0.331
Time on dialysis	Med = 4, IQR =[2,9]	Med = 4, IQR =[2, 7]	0.01
Number of comorbidities	Mean = 1.42, 95%CI = [1.33, 1.50]	Mean = 1.38, 95%CI = [1.34, 1.42]	0.348
Hep B vaccination failure	Failure rate = 27.5%	Failure rate = 20.2%	0.024
Taking IS drugs	8%	4%	0.026

Age in years; BMI = body mass index in kg/m²; Time on dialysis in years; IS = immunosuppressive drugs

Table S7: Baseline characteristics of transplant patients by type of vaccine

Risk factor	BNT162b2 mRNA	mRNA-1273	p-value
Sex	Prop. Male = 33%	Prop. Male = 35%	0.798
Age	Med = 58, IQR = [48; 67]	Med = 60, IQR = [49,67]	0.484
BMI	Med = 25.78, IQR = [23.14;29.11]	Med = 25.61, IQR = [22.99; 28.73]	0.683
Time after transplantation	Med = 8, IQR = [4, 12.5]	Med = 9, IQR = [5; 14]	0.278
Number of comorbidities	Mean = 1.17, 95%CI = [1.05; 1.30]	Mean = 1.15, 95%CI = [1.07; 1.22]	0.710
Hep B vaccination failure	Failure rate = 9%	Failure rate = 9%	1
Number of IS drugs	Mean = 2.29, 95%CI = [2.17; 2.41]	Mean = 2.28, 95%CI = [2.2, 2.35]	0.990

Age in years; BMI = body mass index in kg/m²; Time after transplantation in years; IS = immunosuppressive drugs

DIA-Vacc supplementary figures
Figure S1: DIA-Vacc study schedule

T0: Time of study enrollment

- Participant information

- Participant consent

- Blood sample (1 serum tube approx. 7.5 ml)

- In case of T-cell isolation additional 3 tubes (approx. 25 ml).

- Electronic Case Report Form (eCRF)

Time point	Weeks / Months
T0	Study enrollment/(1st vaccination)
T1	3-4 weeks post 1st vaccination
T2	4-5 weeks post 2nd vaccination

T1-6: Progress time points

- Blood sample (1 serum tube approx. 7.5 ml)

- In case of T-cell isolation additional 3 tubes (approx. 25 ml).

- Electronic Case Report Form (eCRF)

+ if applicable

time

T0 = right before
1st vaccination

T1 = week 3-4 after
1st vaccination

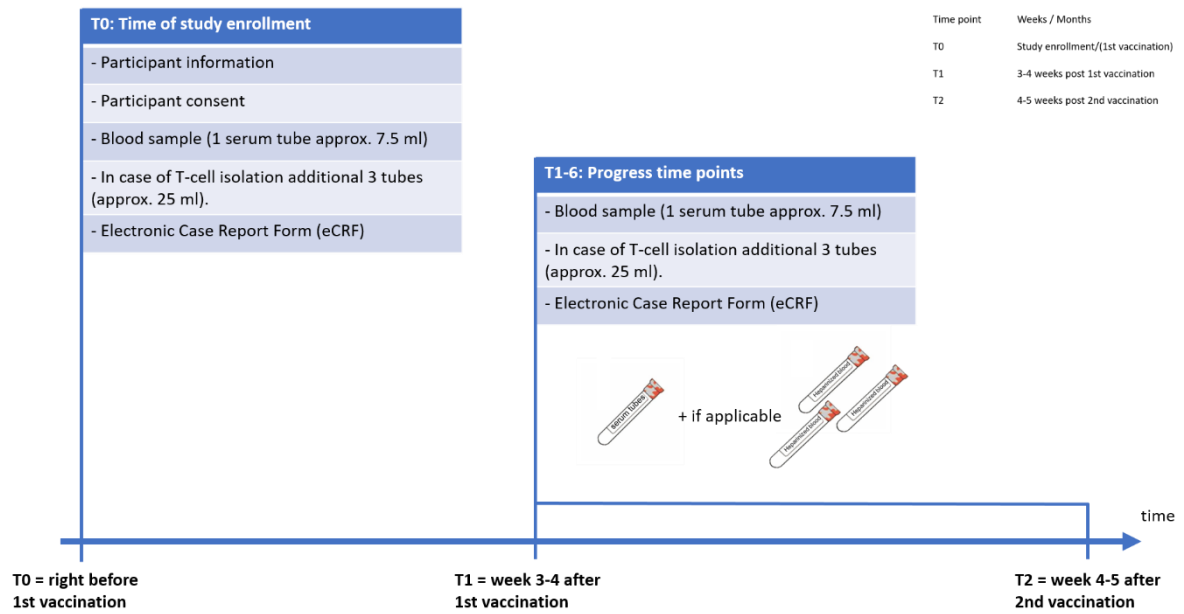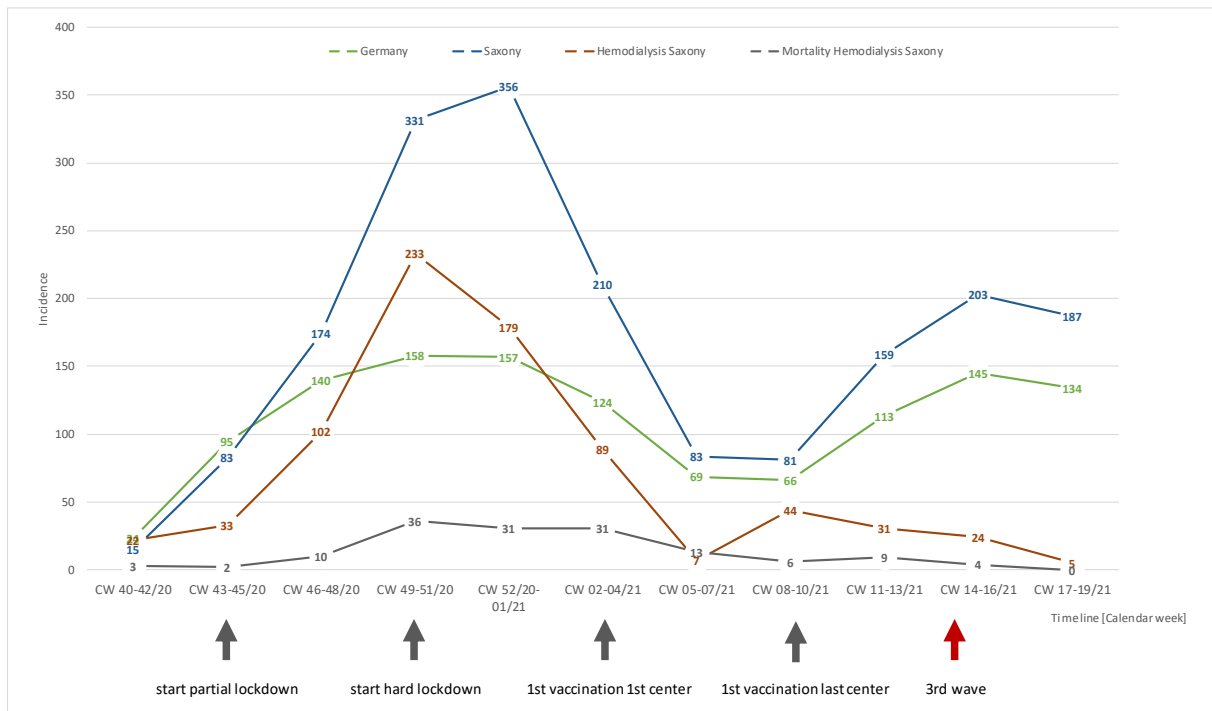**T2 = week 4-5 after**
2nd vaccination

Figure S2: COVID-19 incidences over second and third wave pandemia in the dialysis network of Saxony

Comparison of COVID-19 incidences in medical staff (light gray line) and hemodialysis patients of our dialysis center network in Saxony (red line), the corresponding mortality in hemodialysis patients in Saxony (dark gray line) and the overall incidences in Saxony (blue line) and in Germany (green line)[6]. Plotted at intervals of 3 calendar weeks. The black arrows mark the different phases of the lockdown as well as the vaccination period. The red arrow marks the 3rd wave. Reported on a voluntary, weekly basis by the centers of the dialysis network. The report consisted of cases with positive SARS-CoV-2 detection, non-critically ill patients, patients in inpatient treatment as well as cured and deceased cases. Cases were divided into the categories dialysis patients, transplanted patients, nursing & medical personnel.

Figure S3: Analysis of SARS-CoV-2-reactive CD4+ T-cell helper response by multi-parameter flow cytometry
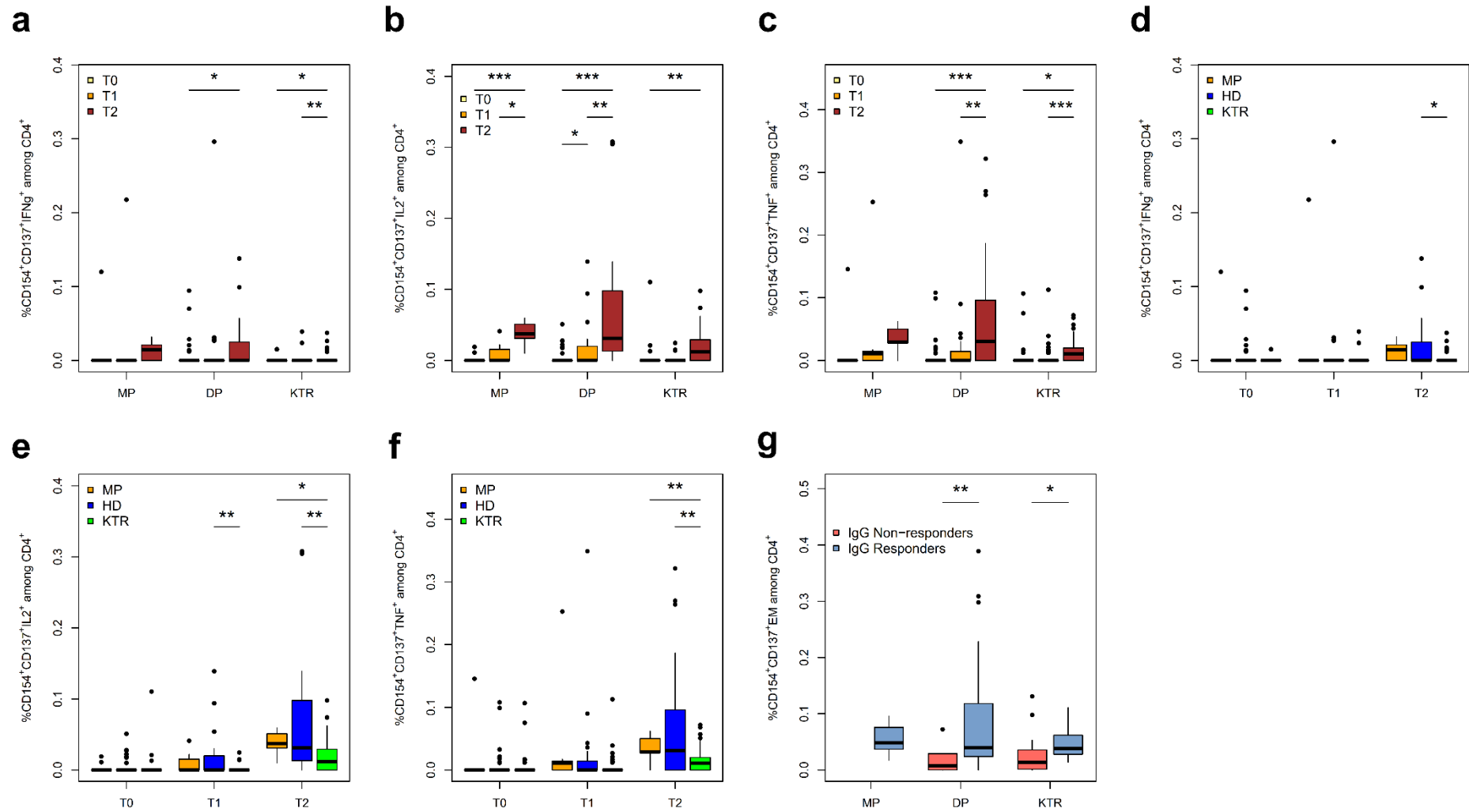

a-c) Kinetics of Spike-reactive cytokine producing CD4+ T helper cells at and following vaccination in different study cohorts. Shown percentages are after correction for background activation.

d-f) Comparison of the Spike-reactive cytokine producing CD4+ T helper cells between the study groups at different study visits. Shown percentages are after correction for background activation.
g) Comparison of SARS-CoV-2 Spike-reactive CD4+ T helper cells with effector memory phenotype in humoral responders and non-responders. The humoral response is defined by IgG serology. No humoral non-responders in MP

Figure S4: Risk factor assessment of individual immunosuppressive drugs regarding humoral vaccination failure at T2 based on elastic net regression
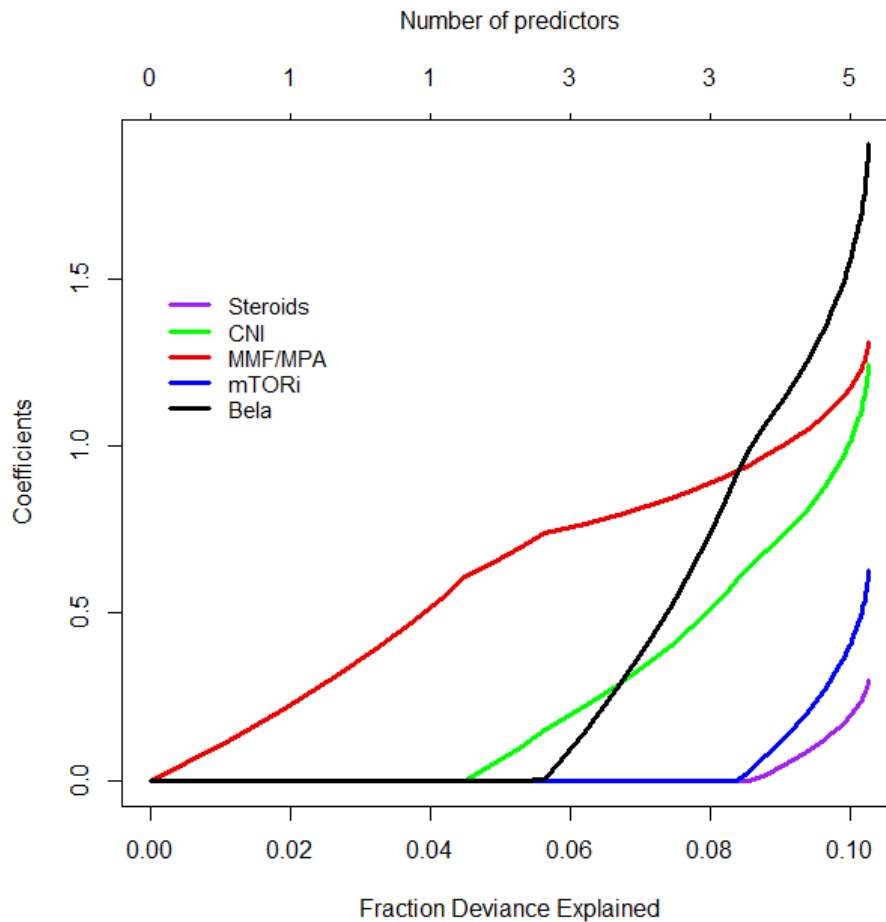
Abbreviation: Steroids = glucocorticosteroids; CNI = calcineurin-inhibitors; MMF/MPA = mycophenolate mofetil or mycophenolic acid; mTORi = mTOR-inhibitors; Bela = belatacept.

Figure S 3 illustrates a stepwise model selection procedure in which predictors are added to a regression model one at a time, to maximize the goodness-of-fit, assessed from the deviance, given the current number of predictors. The slope of each path in Figure S3 changes as a new drug enters the model. According to this plot, MMF/MPA has the strongest explanatory ability as a single predictor, the proportion of explained variability increases after belatacept and CNI are included. Accounting for glucocorticosteroids and mTOR-inhibitors improves the fit, although not considerably.

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