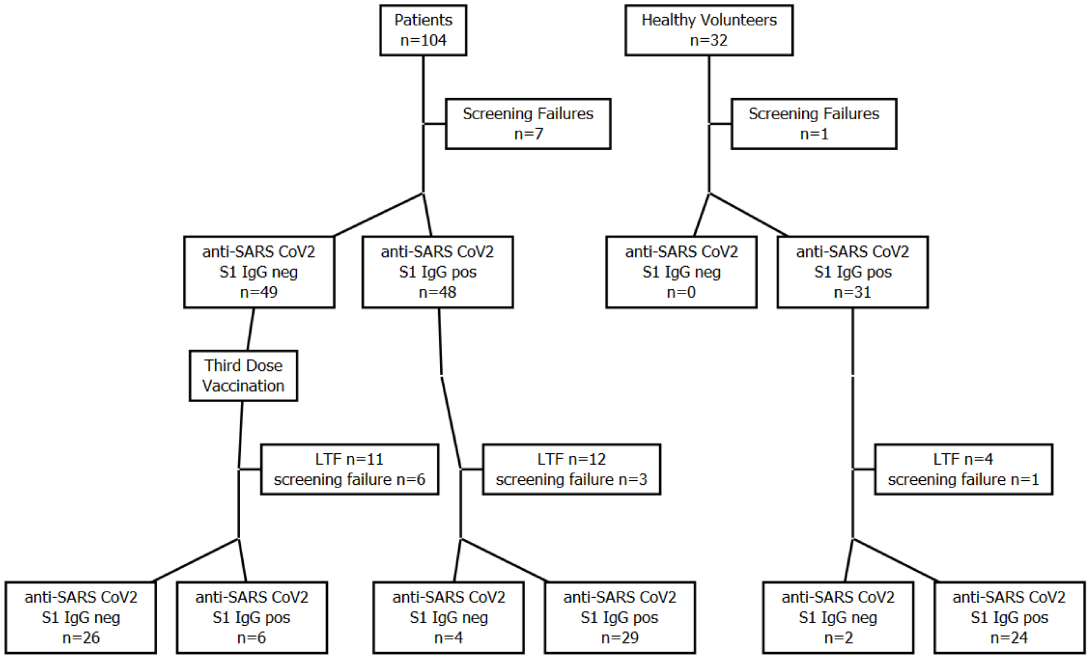
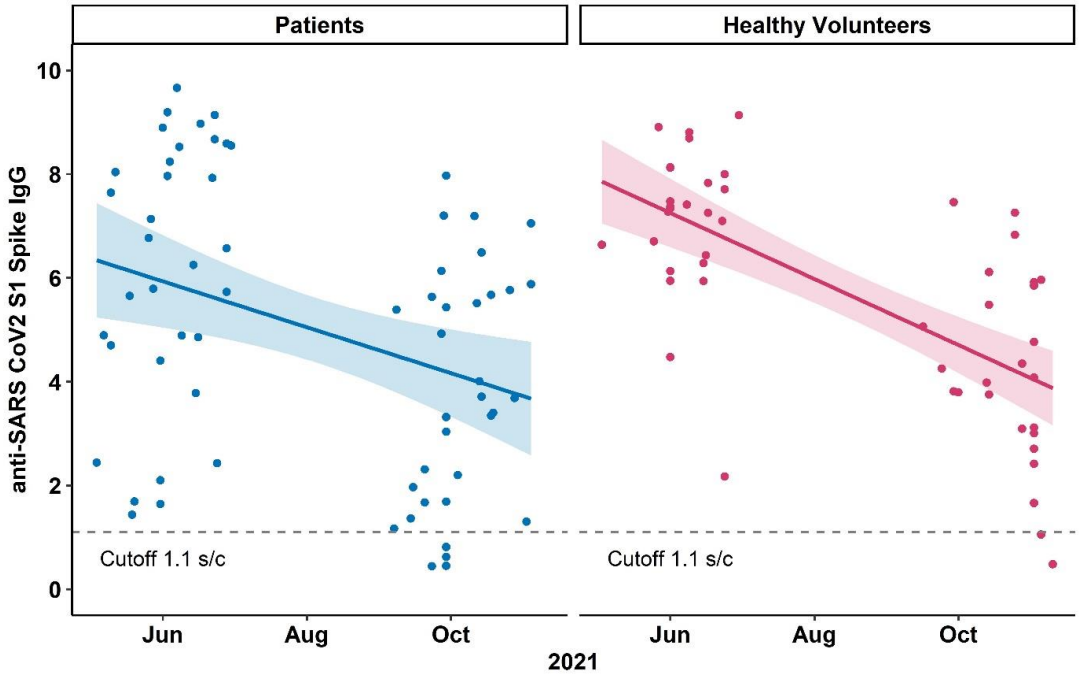
Trajectories of humoral and cellular immunity and responses to a third dose of mRNA vaccines against SARS-CoV-2 in patients with a history of anti-CD20 therapy

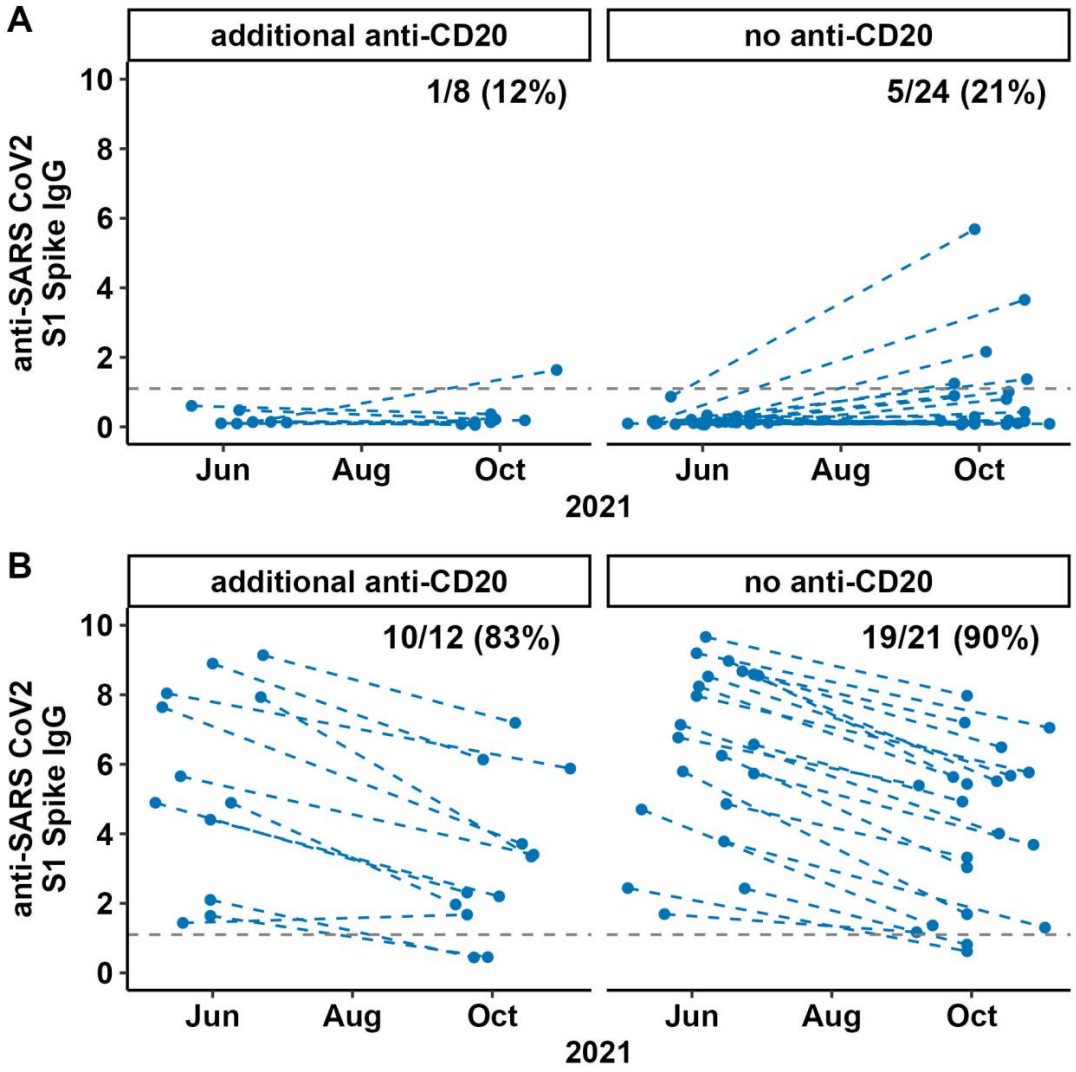
Supplement



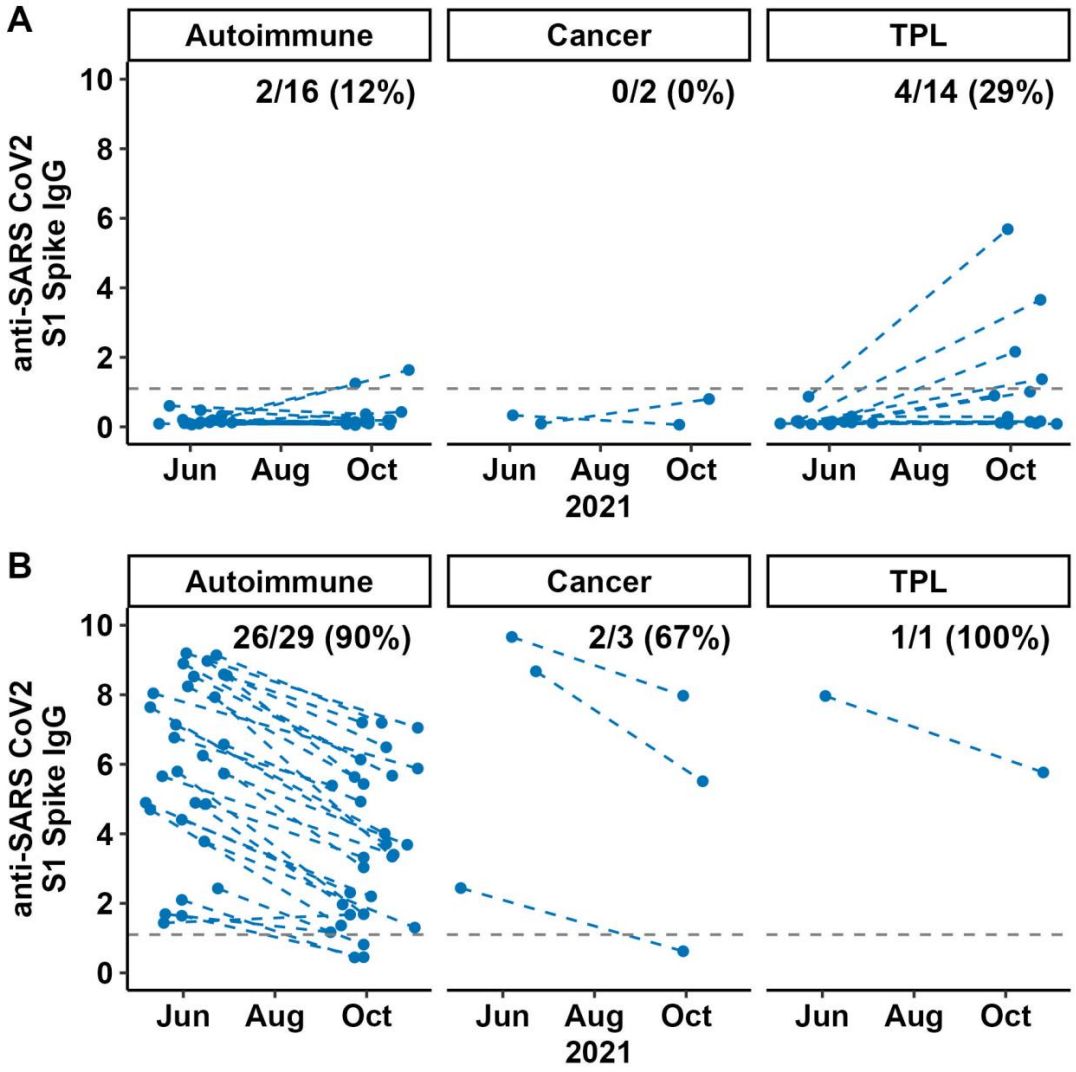
Supplementary Figure 1: Flowchart of screening and grouping of patients and healthy volunteers in the RituxiVac study. LTF: lost to follow-up, pos=positive, neg=negative



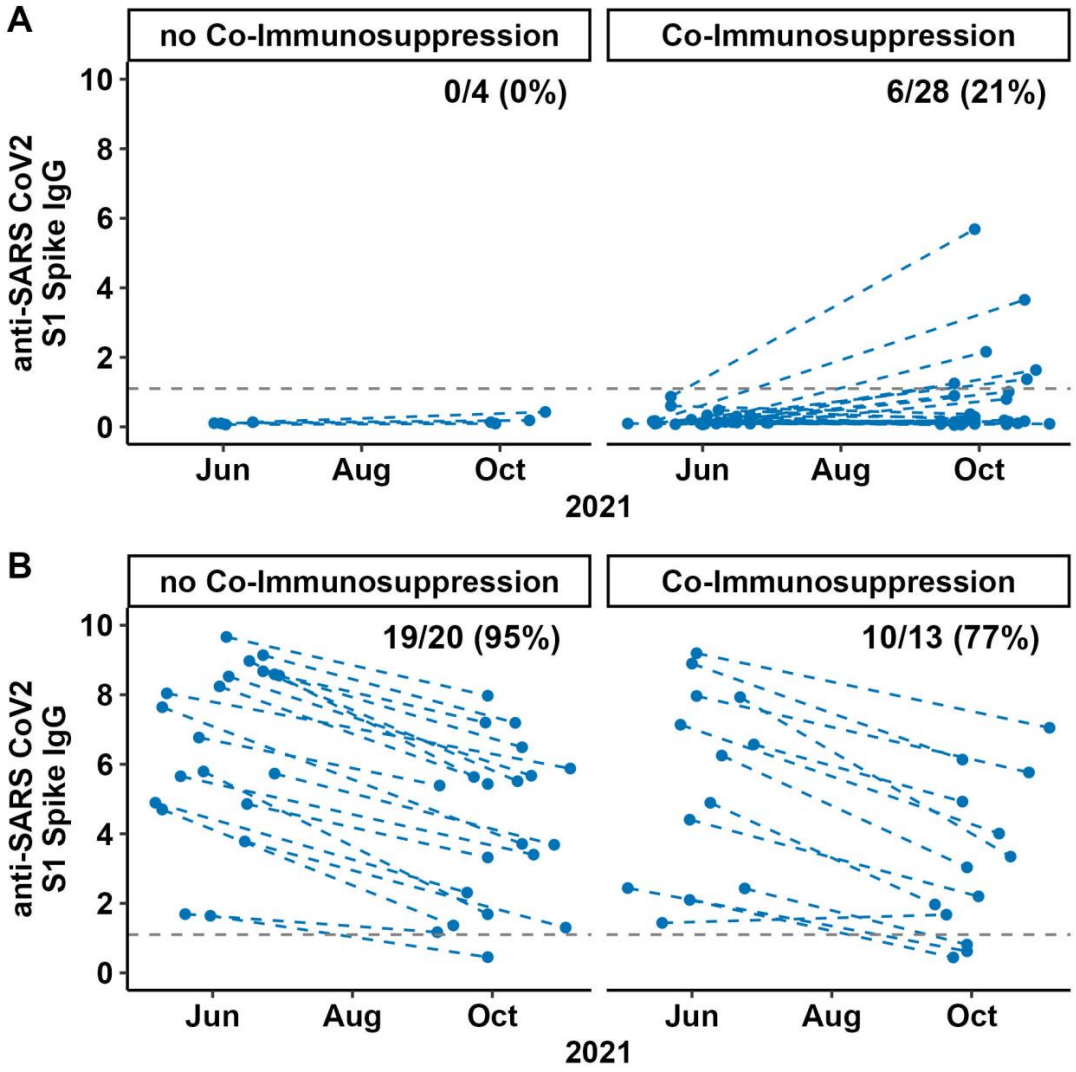
Supplementary Figure 2: Linear regression between anti-SARS-CoV-2 S1 IgG concentrations and date of study visit. The shaded area represents the 95% CI for the regression line. Points denote individual values. Each point represents one study visit. The dotted line denotes the cut-off anti-SARS-CoV-2 S1-IgG value of 1.1 (signal to cutoff ratio).



Supplementary Figure 3: anti-SARS-CoV-2 S1 Spike IgG levels at study visit 1 and 2 in patients with a third dose vaccination (A, top panel) and patients with a humoral response after two dosese (A, bottom panel) in patients with (left) and without (right) anti-CD20 treatment since second vaccination dose. A: p>0.9; B: p=0.6 (Fisher's Exact test). Each point represents one study visit. Intra-individual values are connected with dashed lines, the later one representing the post-3rd vaccination visit (A), or the follow-up visit after two vaccines (B). The dotted grey line denotes the cut-off anti-SARS-CoV-2 S1-IgG value of 1.1 (signal to cutoff ratio).



Supplementary Figure 4: anti-SARS-CoV-2 S1 Spike IgG levels at study visit 1 and 2 in patients with a third dose vaccination (A, top panel) and patients with a humoral response after two doses (A, bottom panel) dependent on the underlying disease. A: p=0.6; B: p=0.4 (Fisher's Exact test). Each point represents one study visit. Intra-individual values are connected with dashed lines, the later one representing the post-3rd vaccination visit (A), or the follow-up visit after two vaccines (B). The dotted grey line denotes the cut-off anti-SARS-CoV-2 S1-IgG value of 1.1 (signal to cutoff ratio).



Supplementary Figure 5. Anti-SARS-CoV-2 S1 Spike IgG levels at study visit 1 and 2 in patients with a third dose vaccination (A, top panel) and patients with a humoral response after two doses (A, bottom panel) dependent on co-immunosuppressive medication (antimetabolites, calcineurin inhibitors, steroids, biologicals, chemotherapy). A: p=0.6; B: P=0.3 (Fisher's Exact test). Each point represents one study visit. Intra-individual values are connected with dashed lines, the later one representing the post-3rd vaccination visit (A), or the follow-up visit after two vaccines (B). The dotted grey line denotes the cut-off anti-SARS-CoV-2 S1-IgG value of 1.1 (signal to cutoff ratio).

Supplementary Table 1: anti-CD20 treatment and vaccination history in patients stratified for underlying disease.

	Autoimmune	Cancer	TPL	Overall
	n=45	n=5	n=15	n=65
Cumulative anti-CD20 dose before 2nd vaccine (g)	3.0 (2.0, 5.0)	2.8 (2.5, 4.3)	0.6 (0.6, 0.6)	2.3 (1.0, 4.0)
Anti-CD20 doses since 2nd vaccine (g)	1.0 (0.5, 1.0)	-	-	1.0 (0.5, 1.0)
Time between 2nd vaccine to anti-CD20 therapy during follow-up (months)	5.05 (4.82, 5.43)	-	-	5.05 (4.82, 5.43)
Time from last anti-CD20 to 3rd vaccine (months)	0.84 (0.66, 0.85)	-	-	0.84 (0.66, 0.85)
Patients having received anti-CD20 treatments during follow-up (n, %)	20/45 (44%)	0/5 (0%)	0/15 (0%)	20/65 (31%)

Supplementary Table 2: Bivariate analyses of anti-SARS-CoV-2 IgG persistence and third dose humoral response for sets of clinical or laboratory variables important for immune competence in patients with a history of anti-CD20 therapy as independent variables. Positive indicates anti-SARS-CoV-2 IgG above 1.1 (s/c ratio) at V2 visit. Data are presented as median (interquartile range) or counts (frequency of group).

	Two-Dose humoral responders			**Two-Dose humoral non-responders**		
	Loss of anti-S1 IgG in follow-up	**Persistence of anti-S1 IgG in follow-up**		**No humoral response to 3rd dose**	**Humoral response to 3rd dose**	
	n=4	**n=29**	**p-value**	**n=26**	**n=6**	**p-value**
Male sex (%)	1 (25%)	14 (48%)	0.6	14 (54%)	4 (67%)	0.7
Median age (years)	76 (70, 79)	66 (53, 70)	0.2	66 (54, 72)	58 (43, 71)	0.5
Immunosuppression (%)	3 (75%)	10 (34%)	0.3	22 (85%)	6 (100%)	0.6
Vaccine (BioNTech/Pfizer) (%)	3 (75%)	14 (48%)	0.6	19 (73%)	5 (83%)	>0.9
Cumulative dose anti-CD20 (g)	3.63 (2.75, 4.45)	3.50 (2.40, 5.35)	0.8	2.00 (0.74, 3.45)	0.65 (0.65, 1.66)	0.13
CD4 cells (cells/µl)	748 (720, 1,126)	949 (658, 1,048)	0.9	624 (350, 905)	638 (427, 814)	>0.9
CD19 cells (cells/µl)	34 (26, 45)	51 (24, 176)	0.5	3 (0, 21)	52 (15, 92)	0.052
V1 anti-SARS-CoV-2 S1 IgG	2.26 (1.98, 2.43)	7.14 (4.89, 8.55)	0.002	0.12 (0.09, 0.15)	0.16 (0.14, 0.29)	0.069
V1 IFNγ release (mU/ml)	0.15 (0.05, 0.42)	0.25 (0.13, 0.70)	0.5	0.01 (0.00, 0.14)	0.02 (0.01, 0.10)	0.5