vaccines

MDPI

Supplementary Material

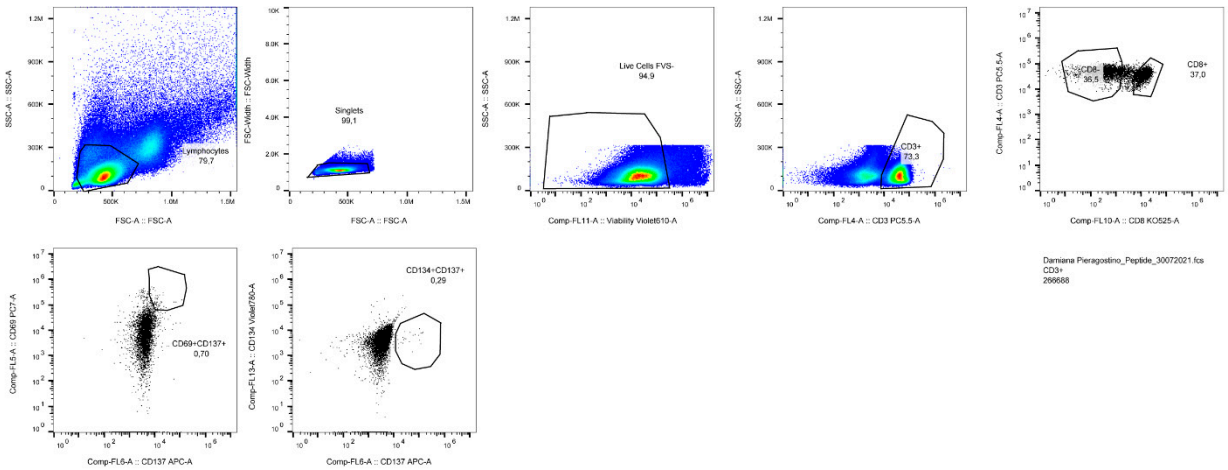

Figure S1. Gating strategy for SARS-CoV-2 S-reactive T cell identification. Lymphocytes were first selected in a forward scatter (FSC) area versus side scatter (SSCA) area dot-plot. Aggregates were next excluded (FSC-A/FSC-width [W] plot) and live cells were identified. CD3+ T-cells were then gated (CD3/SSCA dot-plot). T-cells were split into CD8- (CD4+ cells) and CD8+ subtypes (CD8/CD3). TCR-dependent activation induced markers (AIM) from a representative donor are also represented for CD8+ T cells (CD137/CD69 dot-plot) and CD4+ T cells (CD137/CD134 dot-plot).

Table S1: Clinical Details of enrolled patients

Subjecs		Number of subjects for IgG	Number of Subjects for Memory		Follow up	Age of IgG levels	Age of Follow-Up subjects
CTR		20	15			45,95 ± 19,13	
T1		40	15		5	36,57 ± 10,32	42,2 ± 5,54
T2		40	12		5	36,57 ± 10,32	42,2 ± 5,54

Table S2: Reagent List for Flow Cytometry Analyses

Specifity	Clone	Fluorochrome	Amount *per* test	Type staining	Catalogue Number
CD197	2-L1-A	PE	5 µl	Surface	566742
CD3	SK7	PerCP-Cy5.5	20 µl	Surface	332771
CD69	FN50	PE-Cy7	5 µl	Surface	561928
CD45RA	HI100	APC-H7	5 µl	Surface	560674
CD8	SK1	V500-C	5 µl	Surface	647457

FVS 575V	-	-	1 µl	Surface	565694
CD134	L106	BV768	5 µl	Intracellular	744746
CD137	4B4-1	APC	20 µl	Intracellular	550890
IFNg	B27	FITC	20 µl	Intracellular	552887
TNFa	MAb11	Alexa Fluor™700	5 µl	Intracellular	557996
IL-2	5344.111	BV711	5 µl	Intracellular	563946
R-phycoerythrin (PE); peridinin chlorophyll protein-cyanine 5.5 (PerCP-Cy 5.5); PE-Cyanine 7 (Cy7); Allophycocyanin-Hilite®7 (APC-H7); Fixable Viability Stain (FVS); Brilliant Violet (BV); Allophycocyanin (APC); Fluorescein isothiocyanate (FITC). All the reagents listed in the table are from BD Bdiosciences.					

Figure S2: Spike specific cytokine production within (T1) and after (T2) two months from the second dose BNT162b2 administration. Cytokines (interferon [IFN]-γ, CD40L, tumour necrosis factor [TNF]-α and interleukin [IL]-2) were individually analysed for each subset (CD4+ and CD8+), in a representative BNT162b2 vaccinated donor at two different time points after the second dose administration, using the corresponding DMSO control to assess and subtract the background.

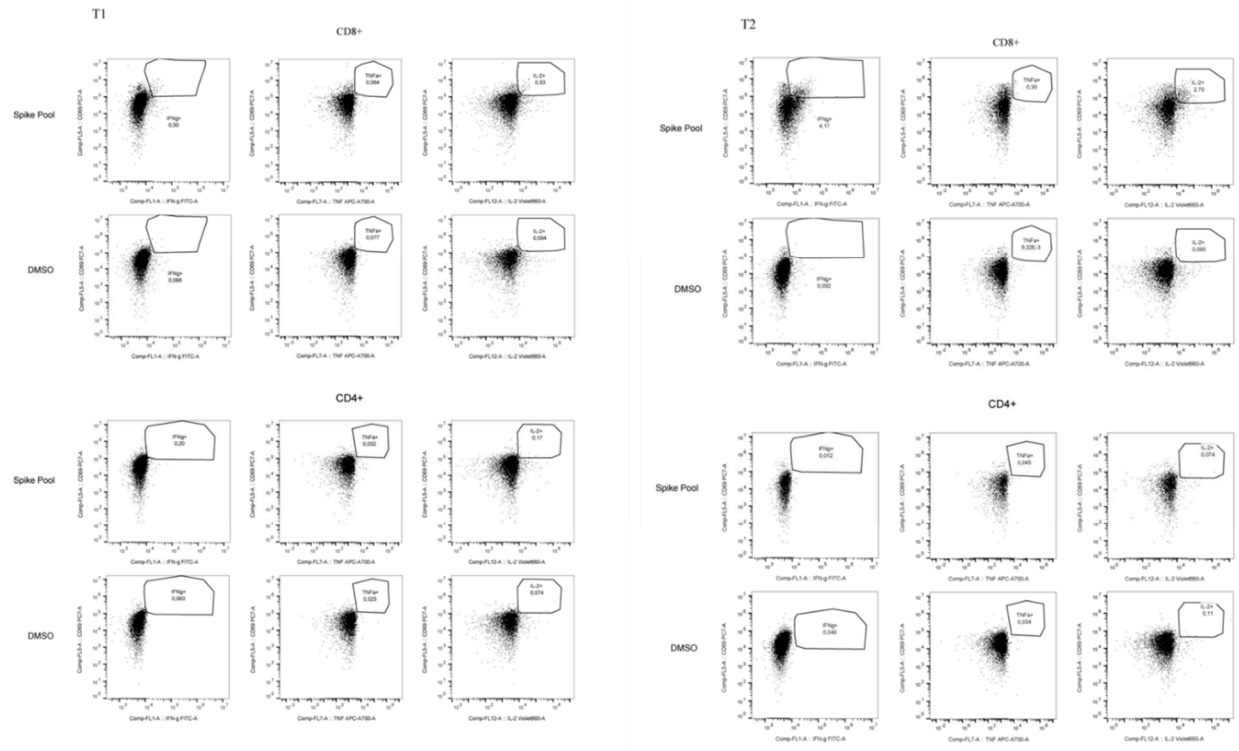

Figure S3: CD4+ memory T-cell frequencies analysed at two different time points: within (T1) and after (T2) two months from the second dose BNT162b2 administration.

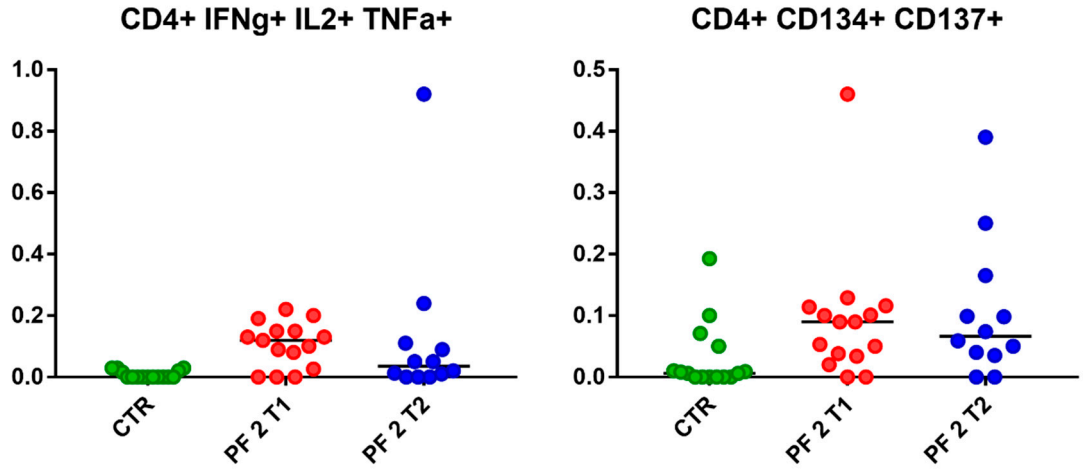

Table S3 Mean of measured biomarkers

Table S3	Levels of IgG	Age of Memory subjects	Frequencies CD8+IFNg+ IL2+ TNFa+	Frequencies CD8+CD137+CD69+	Frequencies CD8+IFNg+ IL2+ TNFa+ Follow-Up subjects	Frequencies CD8+CD137+CD69+
CTR	0,2185 ± 0,0631	46,07 ± 11,73	0,224 ± 0,384	0,038294 ± 0,071532		
VACC T1	66,23375 ± 22,10569	44,13 ± 9,3	0,3497 ± 0,373	0,0636 ± 0,07877	0,5132 ± 0,5540	0,1054 ± 0,1044
VACC T2	8,819 ± 6,58186	40,75 ± 6,94	1,47625 ± 1,5757	0,38883 ± 0,41877	2,2816 ± 2,0848	0,552 ± 0,4663

Table S4: Correlation analysis between age of the subjects and their immunological responses in terms of antibody levels and CD8+ cells frequencies

	Age	Age	Age
	vs.	vs.	vs.
	CD8+IFNg+ IL2+ TNFa+	CD8+CD137+CD69+	IgG at time of collection
95% confidence interval	-0,4763 to 0,195	-0,5475 to 0,1006	-0,3996 to 0,3325
R squared	0,02526	0,06321	0,001502
P value			
P (two-tailed)	0,3770	0,1581	0,8418
P value summary	ns	ns	ns