Supplementary Information for :

Symptomatology during previous SARS-CoV-2 infection and serostatus before vaccination influence the immunogenicity of BNT162b2 COVID-19 mRNA vaccine

AUTHORS

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SUPPLEMENTAL TABLES

Supplemental Table 1. Characteristics of naïve and recovered HCWs included in assays.

	Number (%) of naïve individuals (n=14)	Number (%) of recovered HCWs (n=55)
Demographics		
Sex		
Male	5 (36)	20 (36)
Female	9 (64)	35 (64)
Age (y)		
Mean ± SD	43 ± 9	41.8 ± 12.3
Median [Min, Max]	41 [27, 57]	44.6 [19.4, 67.1]
Ethnicity		
Caucasian	13 (93)	45 (82)
Middle Eastern	0 (0)	1 (2)
Latin American	0 (0)	2 (4)
Asian	1 (7)	4 (7)
Black or African American	0 (0)	3 (5)
Symptomatology at initial infection		
Asymptomatic / WHO Score of 1	n/a	19 (35)
Symptomatic / WHO Score of 2-3	n/a	30 (54)
Symptomatic / WHO Score of 4-5	n/a	6 (11)
Time since initial infection at enrollment (m)		
Mean ± SD	n/a	5.3 ± 1.9
Median [Min, Max]	n/a	5.3 [0.7, 9.3]
Serology Status at Enrollment		
Seronegative	n/a	24 (44)
Seropositive	n/a	31 (56)

Abbreviations : SD, Standard Deviation; y, years; m, months.

Supplemental Table 2. Intervals for assessment of naïve individuals and HCWs who recovered from previous asymptomatic or symptomatic SARS-CoV-2 infection and were vaccinated with BNT162b2 mRNA vaccine.

	Number (%) of naïve individuals (n=14)	Number (%) of asymptomatic HCWs (n=19)	Number (%) of symptomatic HCWs (n=36)
Vaccination received			
Pfizer BioNTech	14 (100)	9 (100)	15 (100)
Time since initial infection at first analysis (prior to vaccination; m)			
Mean ± SD	n/a	6.8 ± 2.3	7.1 ± 2.2
Median [Min, Max]	n/a	7.1 [0.7, 10.5]	7.3 [3.2, 13.1]
Time since initial infection at first vaccine dose (m)			
Mean ± SD	n/a	8.4 ± 2.4	8.4 ± 2.3
Median [Min, Max]	n/a	8.7 [0.8, 12.5]	8.5 [3.3, 13.1]
Time since first dose at analysis (d)			
Mean ± SD	29.1 ± 2.1	47.4 ± 29.2	43.8 ± 23.0
Median [Min, Max]	28 [27, 33]	41 [10, 93]	34.5 [10, 91]
Time since initial infection at second vaccine dose (m)			
Mean ± SD	n/a	12.4 ± 1.8	12.4 ± 2.9
Median [Min, Max]	n/a	12.5 [7.3, 15]	12.7 [5, 16.4]
Time since second dose at analysis (d)			
Mean ± SD	35.2 ± 4.9	51.3 ± 31.8	60.6 ± 29.4
Median [Min, Max]	34 [26.45]	44 [13, 121]	58 [23, 116]
Time between first and second vaccine dose (w)[#]			
Mean ± SD	9.5 ± 0.8	15.3 ± 3.1	16.8 ± 6.7
Median [Min, Max]	9.6 [8.0, 11.3]	15.7 [8.6, 23]	16.1 [3, 32.3]

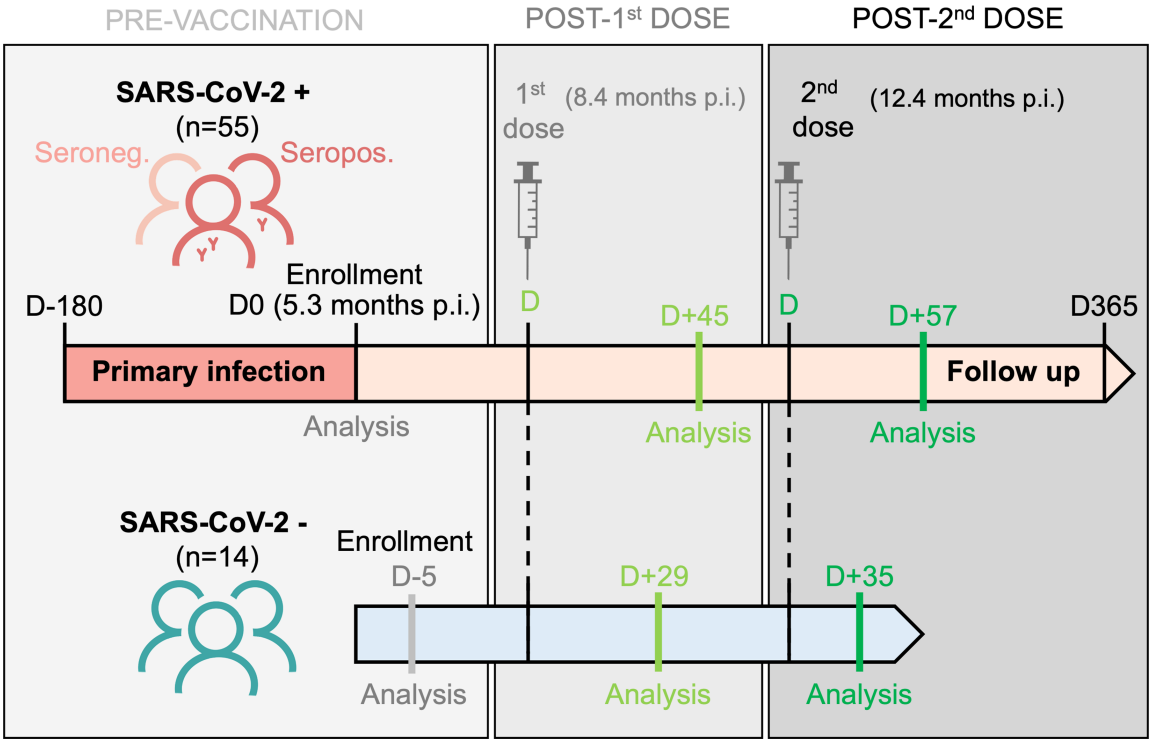
[#]: $p < 0.0001$

Abbreviations : SD, Standard Deviation; w, weeks; m, months; d, days.

Supplemental Table 3. Antibodies used for flow cytometry analysis.

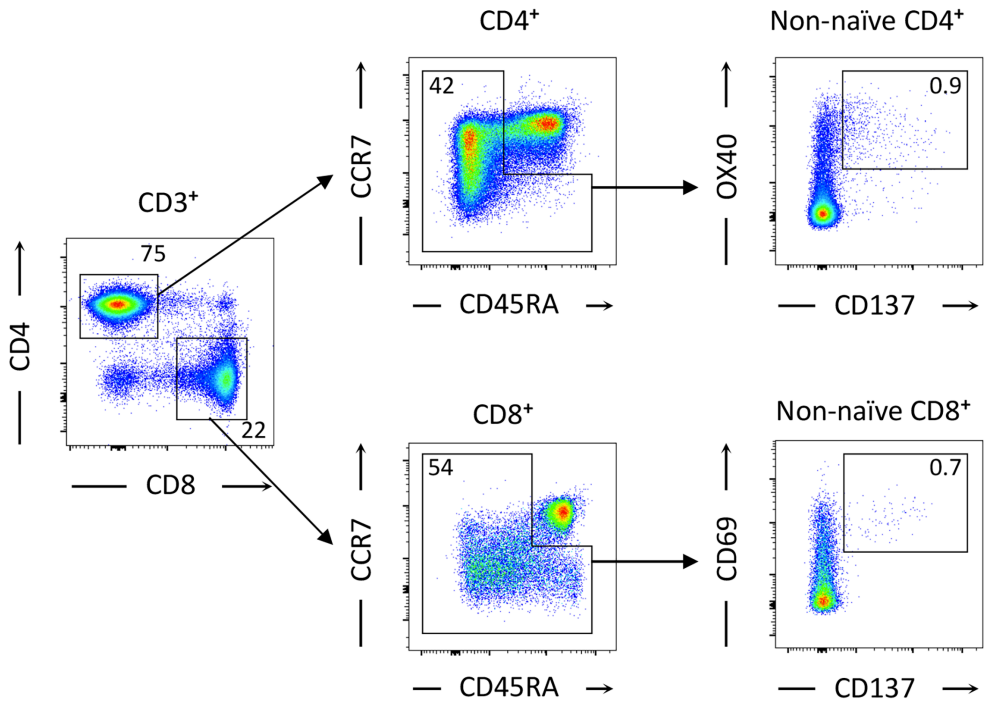
Antibodies	Company	Clone	Cat#
BUV395 Mouse Anti-Human CD3	BD	SK7	564001
V500 Mouse Anti-Human CD4	BD	RPA-T4	560768
AF700 Mouse Anti-Human CD8	BD	RPA-T8	557945
BUV737 Mouse Anti-Human CCR7	BD	2-L1-A	749676
eF450 Mouse Anti-Human CD45-RA	Invitrogen	HI100	48-0458-42
APC Mouse Anti-Human CD137	BioLegend	4B4-1	309810
PE-Cy7 Mouse Anti-Human OX40	BioLegend	Ber-ACT35	350012
BV785 Mouse Anti-Human CD25	BioLegend	BC96	302638
PE Mouse Anti-Human CD69	BD	FN50	555531

Figure S1. Timeline of blood collection for humoral and cellular analyses of vaccinated HCWs who recovered from SARS-CoV-2 infection or individuals naïve to SARS-CoV-2 before vaccination.



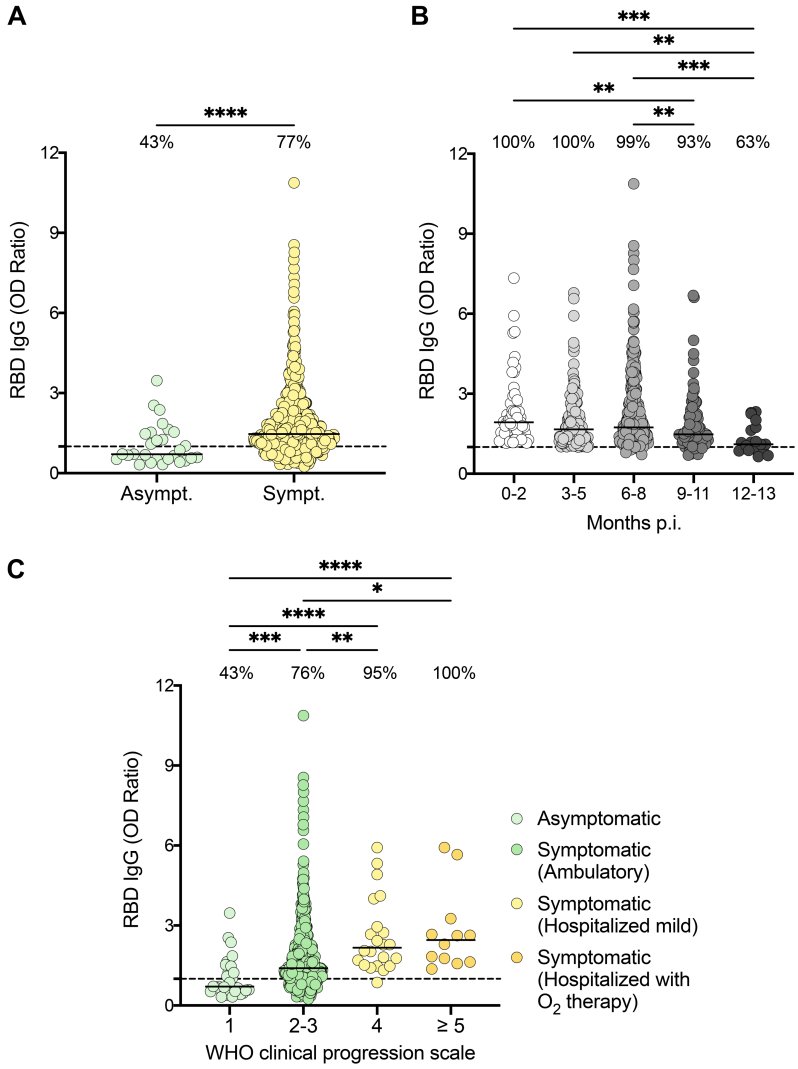
Blood samples for humoral and cell-mediated immunity were collected at enrollment (D0), around D+45 after the first dose and around D+57 after the second dose of vaccine for seronegative (Seroneg.) and seropositive (Seropos.) HCWs recovered from SARS-CoV-2 infection (SARS-CoV-2 +, n=55). For 14 naïve participants (SARS-CoV-2 -), blood samples were collected before their first dose of vaccine (D-5/D0), around D+29 after the first dose and around D+35 after the second dose.

Figure S2. Gating strategy for AIM assays and representative dot plots.



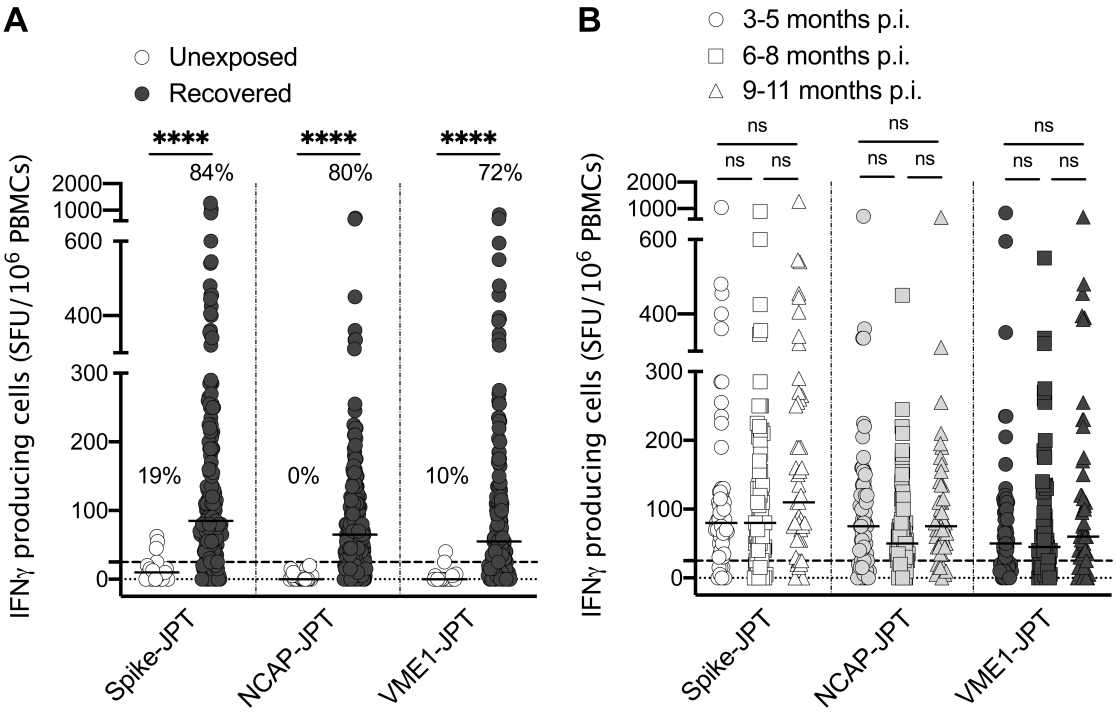
Gating used to identify SARS-CoV-2–specific $CD4^+$ ($OX40^+CD137^+$) and $CD8^+$ ($CD69^+CD137^+$) T cells in recovered HCWs from SARS-CoV-2 infection or naïve to the infection vaccinees after stimulation of PBMCs with CD4-S mega pool of peptides from the Spike glycoprotein. The percentage of events is indicated within AIM^+ (double-positive) quadrant.

Figure S3. The strength of the humoral response in recovered HCWs from SARS-CoV-2 infection is correlated with symptomatology during infection.



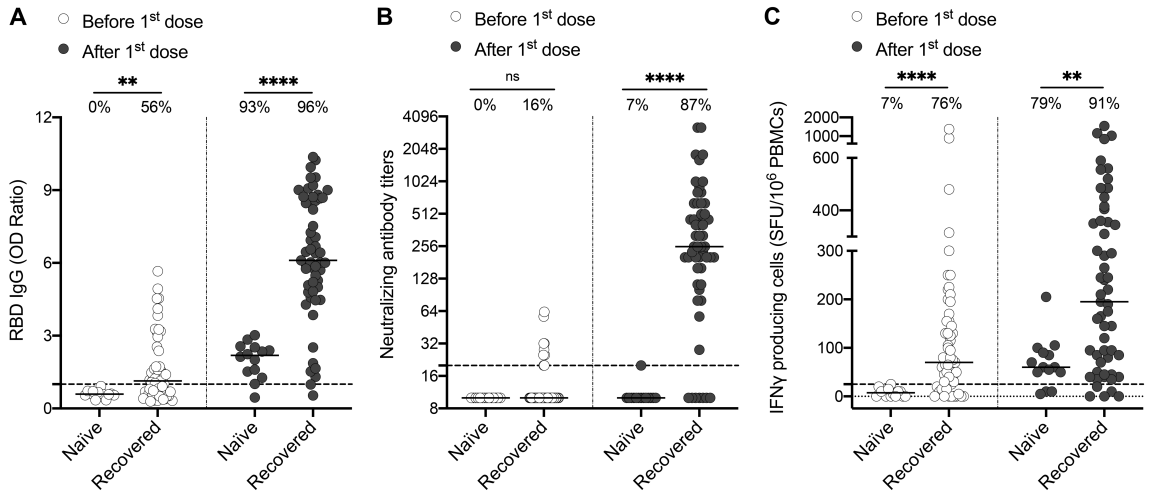
(**A**) SARS-CoV-2 Spike RBD–specific binding IgG levels assessed by ELISA for 569 recovered HCWs classified based on their symptomatology upon infection. (**B**) Kinetic of RBD-binding IgG levels over one-year post infection in participants who were seropositive at enrollment. (**C**) SARS-CoV-2 Spike RBD–specific binding IgG levels assessed by ELISA for 569 recovered HCWs classified according to WHO clinical progression scale. (**A**) Asymptomatic (Asympt.): n=28, Symptomatic (Sympt.): n=541. (**B**) 0-2 months: n=57, 3-5: n=175, 6-8: n=297, 9-11: n=108, 12-13: n=19. (**C**) WHO clinical progression scale 1: n=28, 2-3: n=507, 4: n=22, ≥5: n=12. The black dashed lines indicate the positive threshold value, and the percentage of seropositive participants is indicated for each subgroup. Horizontal bars indicate the median of each group. Statistical significance was assessed by Mann-Whitney (**A**) and Kruskal-Wallis (**B-C**) tests. *P <.05; **P <.01; ***P <.001; ****P <.0001.

Figure S4. Recovered HCWs from SARS-CoV-2 infection maintain strong and stable SARS-CoV-2-specific cellular memory up to 11 months after infection.



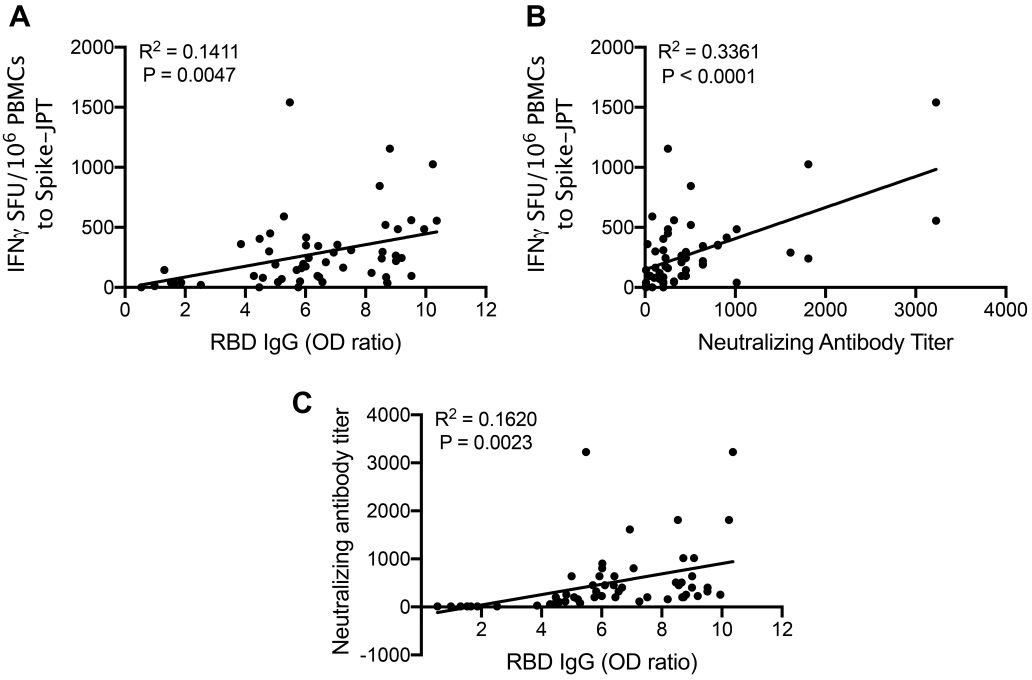
(**A-B**) IFN-γ producing cells per million in response to SARS-CoV-2 Spike glycoprotein, nucleocapsid protein (NCAP) and membrane protein (VME1) mega pools of peptides assessed by ELISpot for naïve (white circles) (n=21) and recovered individuals (black circles) (n=200) at enrollment (**A**) and kinetic of the response one-year post infection (3-5 months p.i.: n=63, 6-8 months p.i.: n=70, 9-11 months p.i.: n=48) (**B**). Black dashed lines indicate the positive threshold value, and the percentage of individuals with responses above positive threshold value is indicated for each subgroup and time point. Horizontal bars indicate the median of each group. Statistical significance was assessed by Mann-Whitney (**A**) and Kruskal-Wallis (**B**) tests. Not significant (ns) P >.05; ****P <.0001.

Figure S5. Recovered HCWs develop much stronger SARS-CoV-2-specific humoral and cellular immune responses than naïve individuals after one dose of vaccine.



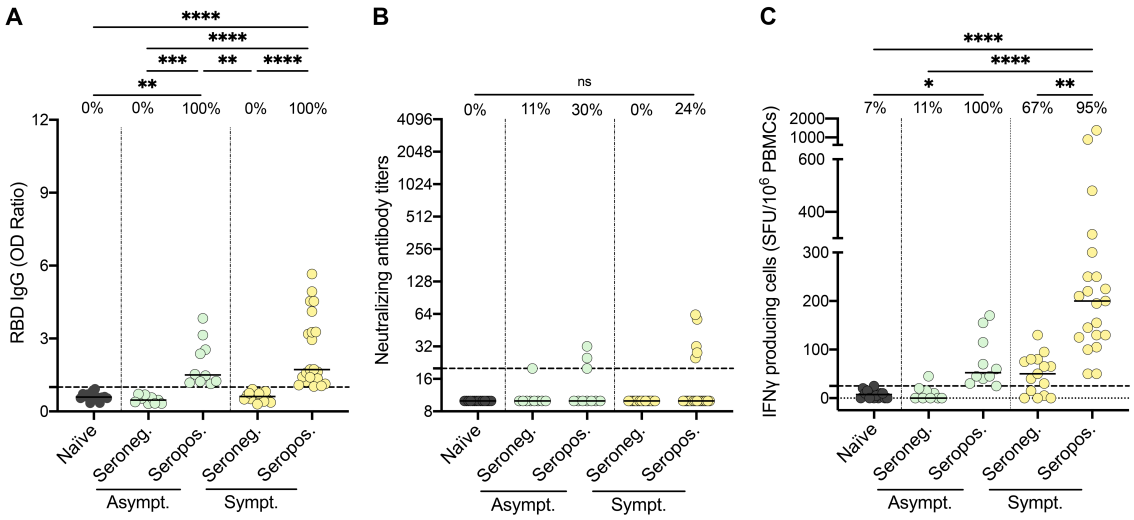
(**A-C**) SARS-CoV-2 Spike RBD–specific binding IgG levels assessed by ELISA (**A**), SARS-CoV-2 neutralizing antibody titers (**B**), IFN-γ producing cells per million in response to SARS-CoV-2 spike glycoprotein peptides stimulation (**C**) assessed by ELISpot for naïve (n=14) and recovered HCWs (n=55) before (white circles) and after one dose of vaccine (black circles). Black dashed lines indicate the positive threshold value, and the percentage of individuals with responses above positive threshold value is indicated for each subgroup and time point. Horizontal bars indicate the median of each group. Statistical significance was assessed by Mann-Whitney tests. Not significant (ns) P >.05; **P <.01; ****P <.0001.

Figure S6. Humoral and cellular responses developed after one vaccine dose are correlated in HCWs who recovered from SARS-CoV-2 infection.



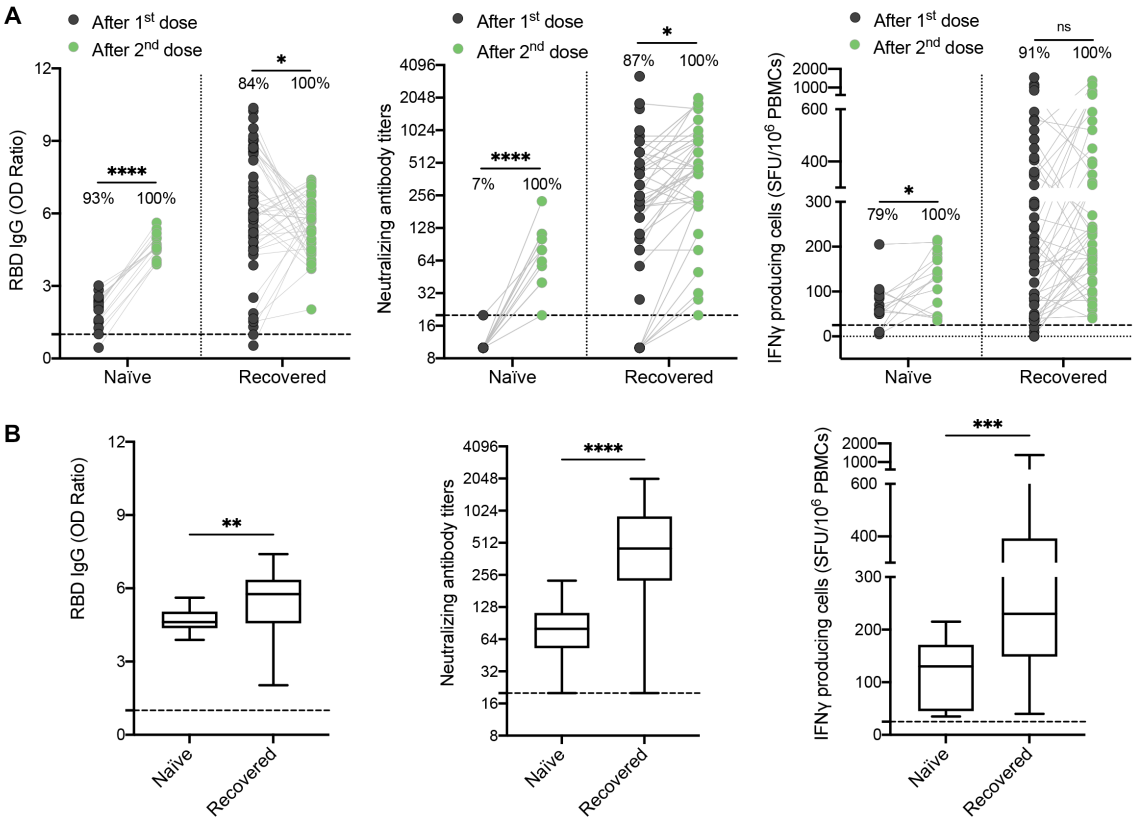
(**A-C**) Correlation between IFN-γ secreting cells per million in response to SARS-CoV-2 Spike glycoprotein peptides and SARS-CoV-2 Spike RBD–specific binding IgG levels (**A**), IFN-γ secreting cells per million in response to SARS-CoV-2 Spike glycoprotein peptides and SARS-CoV-2 neutralizing antibody titers (**B)**, SARS-CoV-2 neutralizing antibody titers and SARS-CoV-2 Spike RBD–specific binding IgG levels (**C**) in recovered HCWs from SARS-CoV-2 infection after one dose of vaccine (n=67). Correlation was assessed by Pearson correlation coefficient. Coefficient R^2 and P-values are shown.

Figure S7. Previously infected but seronegative individuals show limited humoral and cellular response before vaccination.



(**A-C**) SARS-CoV-2 Spike RBD–specific binding IgG levels assessed by ELISA (**A**), SARS-CoV-2 neutralizing antibody titers (**B**), IFN-γ secreting cells per million in response to SARS-CoV-2 Spike glycoprotein peptides stimulation (**C**) assessed by ELISpot for seronegative (Seroneg.) (n=18) and seropositive (Seropos.) (n=37) recovered HCWs after natural infection. Asymptomatic recovered HCWs (Asympt.) are represented with green circles (n=17) and symptomatic HCWs (Sympt.) with yellow circles (n=38). Naïve individuals are shown as negative control (black circles). Black dashed lines indicate the positive threshold value, and the percentage of HCWs with responses above positive threshold value is indicated for each subgroup. Horizontal bars indicate the median of each group. Statistical significance was assessed by Kruskal-Wallis tests. Not significant (ns) P >.05; *P <.05; **P <.01; ***P <.001; ****P <.0001.

Figure S8. Previously infected individuals develop stronger humoral and cellular responses to mRNA vaccination than naïve subjects.



(**A**) SARS-CoV-2 Spike RBD–specific binding IgG levels assessed by ELISA (left panel), SARS-CoV-2 neutralizing antibody titers (middle panel), IFN-γ secreting cells per million in response to SARS-CoV-2 Spike glycoprotein peptides stimulation (right panel) assessed by ELISpot for naïve (n=14) and recovered individuals (n=55) after one dose (black circles) and two doses of vaccine (green circles). (**B**) SARS-CoV-2 Spike RBD–specific binding IgG levels assessed by ELISA (left panel), SARS-CoV-2 neutralizing antibody titers (middle panel), IFN-γ secreting cells per million in response to SARS-CoV-2 Spike glycoprotein peptides stimulation (right panel) assessed by ELISpot for naïve (n=14) and recovered individuals (n=42) after two doses of vaccine. Black dashed lines indicate the positive threshold value, and the percentage of individuals with responses above positive threshold value is indicated for each time point. (**B**) Boxes delimitate the IQR 25th – 75th percentiles and the median are identified by the horizontal line in each box. Error bars indicate the minimal and maximal values. Statistical significance was assessed by Mann-Whitney tests. Not significant (ns) P >.05; *P <.05; **P <.01; ***P <.001; ****P <.0001.