Supplementary Data

Reduced humoral response three months following BNT162b2 vaccination in SARS-CoV-2 uninfected residents of long-term care facilities

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Supplementary material and methods

Determination of anti-SARS-CoV-2 antibodies

Nunc MaxiSorp ELISA plates (Sigma-Aldrich, Cat# M9410-1CS) were coated overnight at 4ºC with 50ng/mL of capture antibody (anti-6xHis antibody, clone HIS.H8; ThermoFisher Scientific; Cat# MA1-21315) at 2 μg/mL in PBS. After washing, plates were blocked for two hours at room temperature using PBS/1% of bovine serum albumin (BSA, Miltenyi biotech, Cat# 130-091-376). Then, 50 μL of the following SARS-CoV-2 derived antigens diluted in in blocking buffer were added: Spike (S2) (0.9 μg/mL, Cat# 40590- V08B), receptor binding domain (RBD, Cat# 40592- V08B) (0.3 μg/mL) or nucleocapsid protein (NP, 40588- V08B) (1 μg/mL) (Sino Biologicals) and incubated overnight at 4ºC. Each plasma sample was evaluated in duplicated at dilution ranging from 1/100- 1/50000 in blocking buffer for each antigen. Diluted samples were incubated at room temperature for one hour. Antigen free wells were also assayed in parallel for each sample in the same plate to evaluate sample background. Serial dilutions of a positive plasma sample were used as standard. A pool of 10 SARS-CoV-2 negative plasma samples, collected before June 2019, were included as negative control. The following reagents were used as secondary antibodies: HRP conjugated (Fab)2 Goat anti-human IgG (Fc specific, Cat# 109-036- 098) (1/20,000), Goat anti-human IgM (1/10,000, Cat# 109-036-129), and Goat anti-human IgA (alpha chain specific, Cat# 109-036-011) (1/10,000) (all from Jackson Immunoresearch). Secondary antibodies were incubated for 30 minutes at room temperature. After washing, plates were revealed using o-Phenylenediamine dihydrochloride (OPD, Sigma Aldrich, Cat #P8787) and the enzymatic reaction was stopped with 4N of H_2SO_4 (Sigma Aldrich). The signal was analysed as the optical density (OD) at 492 nm with noise correction at 620 nm. The specific signal for each antigen was calculated after subtracting the background signal obtained for each sample in antigen-free wells. Values are plotted into the standard curve. Standard curve was calculated by plotting and fitting the log of standard dilution (in arbitrary units) vs. response to a 4-parameter equation in Prism 8.4.3 (GraphPad Software).

Pseudovirus neutralization assay.

SARS-CoV-2.SctΔ19 WH1 and B.1.617.2/Delta were generated (Geneart) from the full protein sequence of the original SARS-Cov-2 isolate Wuhan-Hu-1 (WH1) and the Delta variant (B.1.617.2) spike sequences respectively, with the deletion of the last 19 amino acids in C-

terminal [1], human-codon optimized and inserted into pcDNA3.1(+). HIV reporter pseudoviruses expressing SARS-CoV-2 S protein and Luciferase were generated using the defective HIV plasmid pNL4-3.Luc.R-.E- obtained from the NIH AIDS Reagent Program [2]. Expi293F cells were transfected using ExpiFectamine293 Reagent (Thermo Fisher Scientific) with pNL4-3.Luc.R-.E- and SARS-CoV-2.SctΔ19 (WH1, B.1.617.2/Delta), at an 8:1 ratio, respectively. Control pseudoviruses were obtained by replacing the S protein expression plasmid with a VSV-G protein expression plasmid as reported [3]. Supernatants were harvested 48 h after transfection, filtered at 0.45 µm, frozen and titrated on HEK293T cells overexpressing WT human ACE-2 (Integral Molecular). Neutralization assays were performed in duplicate. Briefly, in Nunc 96-well cell culture plates (Thermo Fisher Scientific, Cat #165305), 200 TCID50 of pseudovirus were preincubated with three-fold serial dilutions (1/60–1/14,580) of heat-inactivated plasma samples for 1 h at 37 ◦C. Then, 2×10^4 HEK293T/hACE2 cells treated with DEAE-Dextran (Sigma-Aldrich, Cat# D9885) were added. Results were read after 48 h using the EnSight Multimode Plate Reader and BriteLite Plus Luciferase reagent (Perkin Elmer, Waltham, MA, USA, Cat# 6066761). The values were normalized, and the ID50 (the reciprocal dilution inhibiting 50% of the infection) was calculated by plotting and fitting the log of plasma dilution vs. response to a 4-parameter equation in Prism 8.4.3 (GraphPad Software). This neutralization assay had been previously validated in a large subset of samples [4,5]. The lower limit of detection was 60 and the upper limit was 14,580 (reciprocal dilution).

References:

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[2] Connor RI, Chen BK, Choe S, Landau NR. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology 1995;206:935–44. https://doi.org/10.1006/viro.1995.1016.

[3] Sánchez-Palomino S, Massanella M, Carrillo J, García A, García F, González N, et al. A cell-to-cell HIV transfer assay identifies humoral responses with broad neutralization activity. Vaccine 2011;29. https://doi.org/10.1016/j.vaccine.2011.05.016.

[4] Trinité B, Tarrés-Freixas F, Rodon J, Pradenas E, Urrea V, Marfil S, et al. SARS-CoV-2 infection elicits a rapid neutralizing antibody response that correlates with disease severity. Sci Rep 2021;11. https://doi.org/10.1038/s41598-021-81862-9.

[5] Pradenas E, Trinité B, Urrea V, Marfil S, Tarrés-Freixas F, Ortiz R, et al. Clinical course impacts early kinetics and long-term magnitude and amplitude of SARS-CoV-2 neutralizing antibodies beyond one year after infection Edwards. MedRxiv 2021. https://doi.org/10.1101/2021.08.12.21261921.

Supplementary Table 1. Univariate and Multivariate analysis of Neutralization (WH1 virus) post-vaccine.

A. Infected before vaccination

	Univariate			Multivariate		
	Estimated	Std error	*P-value*	Estimated	Std error	*P-value*
Hospitalization	0.32	0.194	*0.10*	0.343	0.19	*0.07*
Sex (Ref. female)	0.156	0.089	*0.08*	0.196	0.089	***0.03***
Age	0.003	0.002	*0.11*	0.003	0.002	*0.07*
AGM level[1]	0.056	0.035	*0.11*			

[1]AGM: Stratum of Adjusted Morbidity ranging from 1 to 4 according to the number of comorbidities and their need for health care

B. Uninfected

	Univariate		
	Estimated	Std error	P-value
Sex (Ref. female)	-0.119	0.185	*0.527*
Age	-0.014	0.003	***<0.0001***
AGM level[1]	-0.145	0.073	*0.06*

[1]AGM: Stratum of Adjusted Morbidity ranging from 1 to 4 according to the number of comorbidities and their need for health care

Supplementary Table 2. Univariate and Multivariate analysis of neutralization (Delta variant) post-vaccination.

A. Infected before vaccination

	Univariate			Multivariate		
	Estimated	Std error	*P-value*	Estimated	Std error	*P-value*
Hospitalization	0.496	0.237	***0.04***	0.441	0.217	***0.04***
Sex (ref. female)	0.12	0.111	*0.87*			
Age	0.009	0.002	***<0.0001***	0.009	0.002	***<0.0001***
AGM level[1]	0.18	0.04	***<0.0001***			

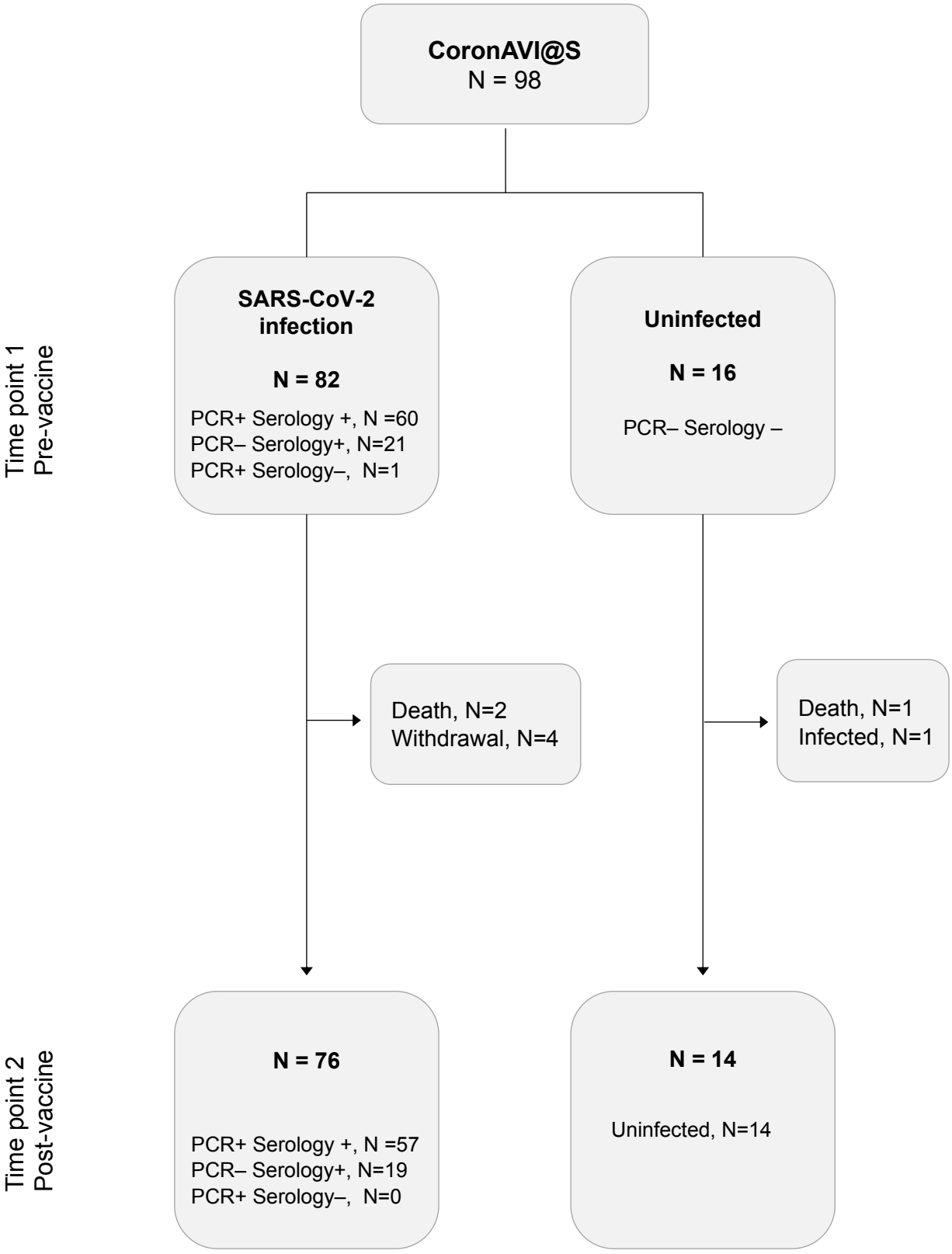
[1]AGM: Stratum of Adjusted Morbidity ranging from 1 to 4 according to the number of comorbidities and their need for health care

B. Uninfected

	Univariate		
	Estimated	Std error	P-value
Sex	0.014	0.122	*0.907*
Age	-0.008	0.002	***<0.0001***
AGM level[1]	-0.081	0.049	*0.106*

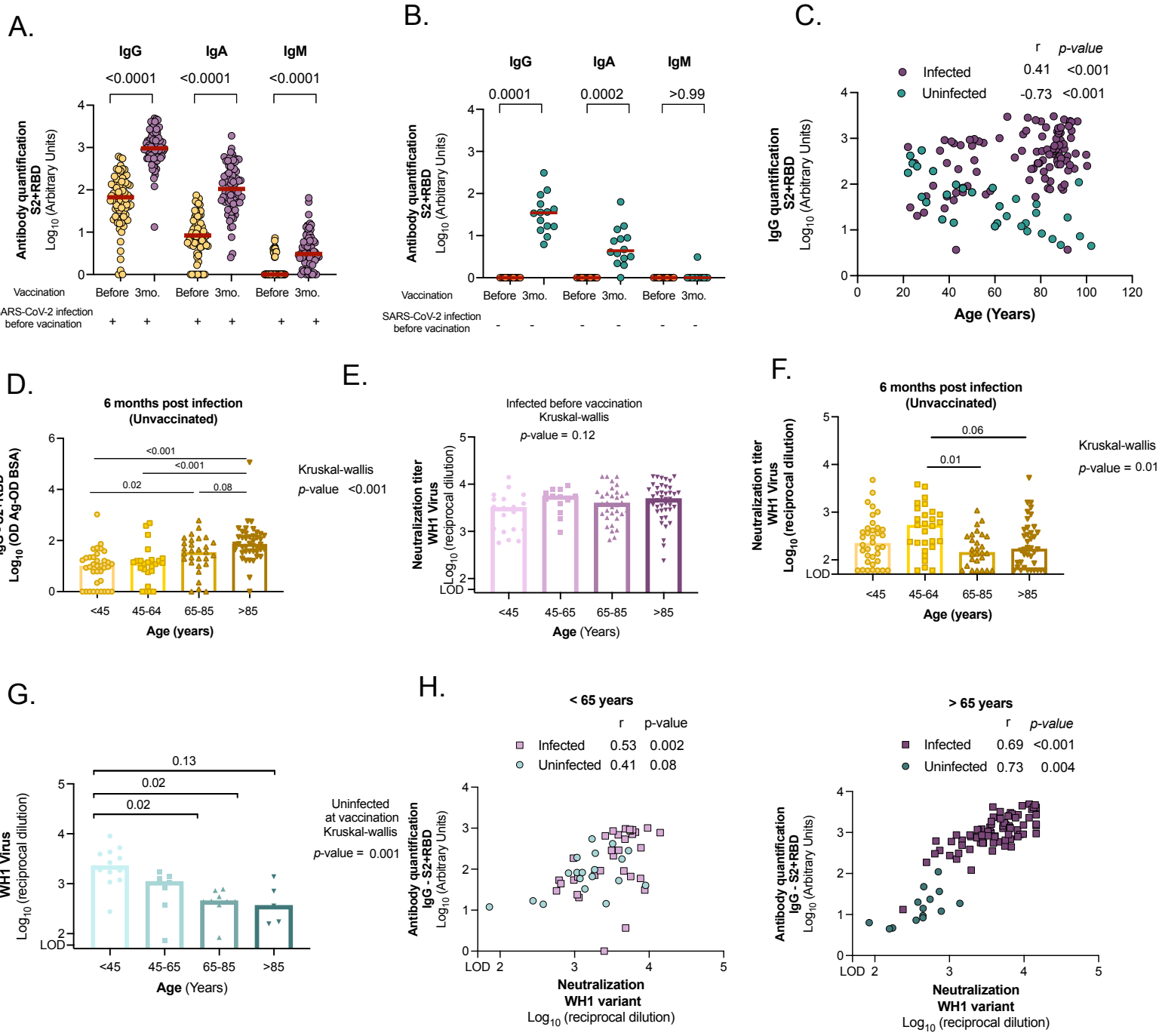
[1]AGM: Stratum of Adjusted Morbidity ranging from 1 to 4 according to the number of comorbidities and their need for health care

Supplementary Figure 1: Flowchart Coronavi@s study



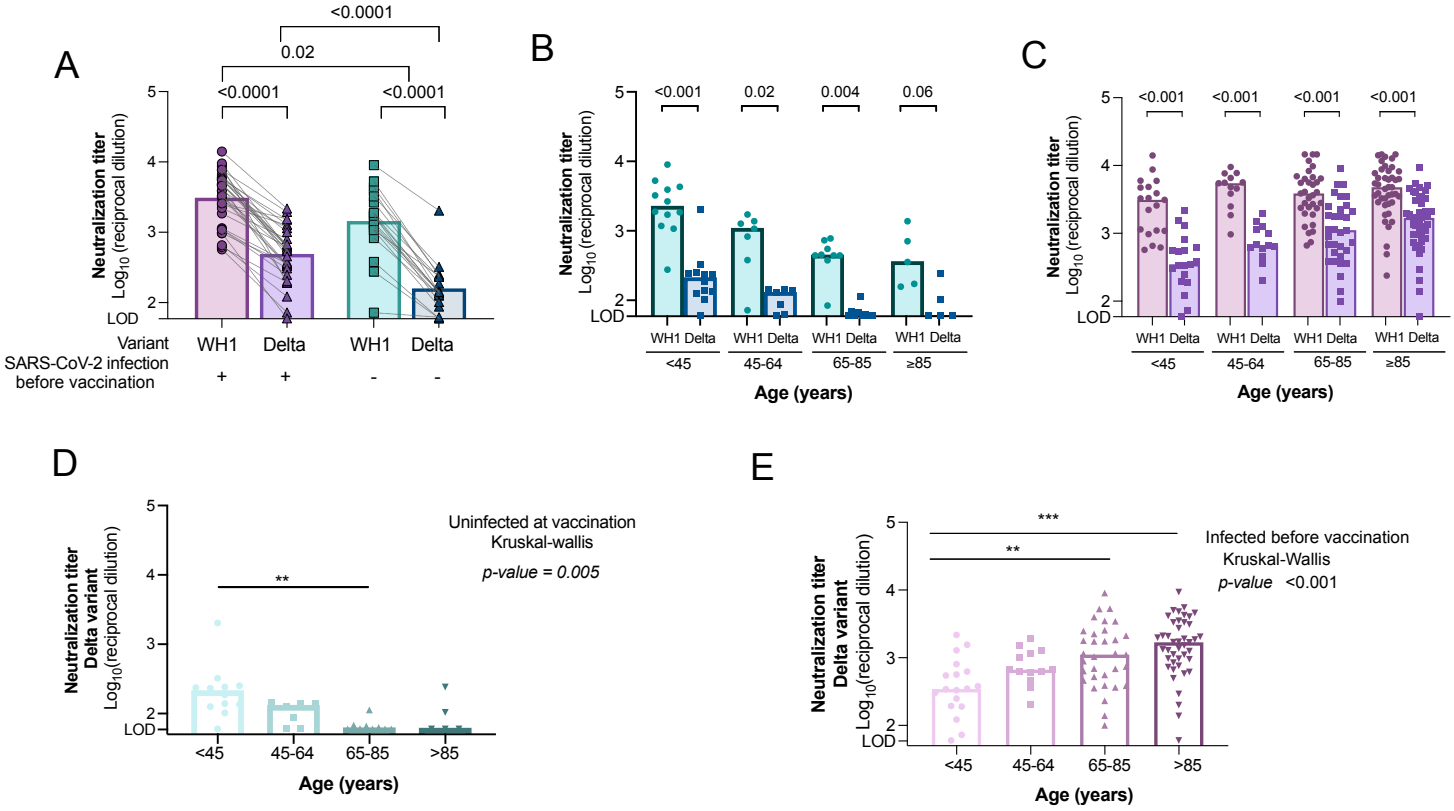
Supplementary Figure 1: Flowchart Coronavi@s study, including 98 older adults living in LTCF. Number of samples analysed at each time point are indicated.

Supplementary Figure 2:Comparison of humoral response and neutralizing activity between uninfected and infected individuals at different ages before and after three months from BNT162b2 mRNA Covid-19 vaccine.



Supplementary Figure 2: Comparison of humoral response and neutralizing activity between uninfected and infected individuals at different ages before and after three months from BNT162b2 mRNA Covid-19 vaccine. Levels of specific SARS-CoV-2 immunoglobulins (IgG, IgA and IgM) against S2+RBD proteins quantified in plasma from infected (**Panel A**) and uninfected (**Panel B**) residents by ELISA, before and after vaccination. **Panel C**: Correlation of the levels of specific SARS-CoV2 IgG antibodies (S2+RBD) after vaccination with age in participants infected and uninfected. **Panel D**: SARS-CoV-2 specific IgG antibody levels (against S2+RBD proteins) after 6 months from infection (and before vaccination) across ages in infected participants. Plasma neutralizing activity against WH1 variant across ages in infected (**Panel E**) or uninfected (**Panel G**) after vaccination. **Panel F**: Plasma neutralizing activity against WH1 variant across ages in participants after six months from symptoms onset (and before vaccination). **Panel H**: Correlation of the levels of specific SARS-CoV2 IgG antibodies (S2+RBD) after vaccination with neutralization titers in younger and older adults. Median values are indicated. P-values were obtained from Mann–Whitney test for comparison between groups (**Panel F**), Wilcoxon for paired tests (**Panel A and B**), Kruskal–Wallis test for each group (**Panel D, E, F, and G**). Correlation coefficient and p-values were obtained from Spearman correlation in Panels C and H. In all panels, uninfected and infected individuals at vaccination are indicated in turquoise and purple, respectively.

Supplementary Figure 3: Neutralizing activity against B.1.617.2/Delta variant between uninfected and infected individuals at different ages after three months from BNT162b2 mRNA Covid-19 vaccine.



Supplementary Figure 3: Neutralizing activity against B.1.617.2/Delta variant between uninfected and infected individuals at different ages after three months from BNT162b2 mRNA Covid-19 vaccine. **Panel A:** Comparison of plasma neutralization capacity after vaccination against WH1 virus (original) and Delta variant in infected and uninfected younger individuals. Comparison of plasma neutralization capacity after vaccination against WH1 virus (original) and Delta variant across ages in uninfected (**Panel B**) and infected individuals (**Panel C**). Neutralizing activity against Delta variant after vaccination across ages in uninfected (**Panel D**) and infected (**Panel E**). In all panels, median values are indicated and P-values were obtained from Mann-Whitney test (**Panel A**) and Wilcolxon for paired tests (**Panel B and C**) and Kruskal–Wallis test for each group (**Panel D and E**).