THE LANCET
Respiratory Medicine

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Lustig Y, Sapir E, Regev-Yochay G, et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir Med* 2021; published online July 2. http://dx.doi.org/10.1016/S2213-2600(21)00220-4.

Supplementary information:

Methods:

Computer-based questionnaire

	Question	Answer1	Answer1
1	What is your date of birth?		
2	What is your gender?	male	female
3	What is your weight in cm?		
4	What is your weight in kg?		
5	Did you perform an IgG assay before receiving the first dose of the vaccine?	Yes	No
6	Was your last IgG assay above 1.1?	Yes	No
7	Have you recovered from Covid-19?	Yes	No
8	Do you have high blood pressure disease?	Yes	No
9	Do you have dyslipidemia?	Yes	No
10	Do you have autoimmune disease?	Yes	No
11	Do you have diabetes?	Yes	No
12	Do you have heart disease?	Yes	No
13	Do you have lung disease?	Yes	No
14	Do you have coagulation disorder?	Yes	No
15	Are you immunosuppressed?	Yes	No
16	Have you ever had a serious allergic reaction (anaphylaxis) that required immediate treatment?	Yes	No
17	Do you have liver disease?	Yes	No
18	Do you have kidney disease?	Yes	No
19	Are you pregnant?	Yes	No

Immunosuppression included organ transplantation, biologic therapy, chemotherapy, steroids and splenectomy. An allergic disease was defined as at least one evet of a severe allergic reaction (anaphylaxis) that required immediate treatment. The questionnaire was reviewed and approved by the Institutional review board of the Sheba Medical Center**.**

PCR testing

Hospital personnel were tested in several scenarios: upon every symptom suspected to be COVID-19, following exposure to a positive COVID-19 contact (hospital or community contacts), as part of a "return to work" protocol during the end of isolation period following exposure or disease.

For quantitative RealTime-PCR (qRT-PCR), nasopharyngeal swabs were placed in 3mL of universal transport medium (UTM) or viral transport medium (VTM). Test was performed according to manufacturers' instructions on various platforms: Allplex™ 2019-nCoV (Seegene, S. Korea), NeuMoDx™ SARS-CoV-2 assay (NeuMoDx™ Molecular, Ann Arbor, Michigan), Xpert®, Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA).

Antibody detection testing

Samples from vaccinated HCW were tested using the following immunoassays: The access SARS-CoV-2 RBD IgG assay (Beckman-Coulter, CA, U.S.A.) commercial test was conducted according to manufacturer's instructions with one modification – based on a national validation study[1] which determined the utility and limitations for SARS-CoV-2 diagnosis the cut off was lowered to 0.62. IgM and IgA RBD- based ELISA was performed as described previously[2]. Briefly, a 96 well microtiter Polysorb plate (Nunc, Thermo, Denmark) coated overnight with 1ug/ml of RBD antigen was blocked with 5% skimmed milk at 25ºC for 60 minutes and human serum samples (diluted 1:100 with 3% skimmed milk) were added to antigen coated wells. Following incubation at 25ºC for 120 minutes and incubation for 60 min after the addition of horseradish peroxidase (HRP)-conjugated isotype specific antibody (anti-human IgA HRP conjugate (Abcam, MA, USA, product number: ab7383) (diluted 1:2000) or goat anti-human IgM HRP conjugate (Jackson

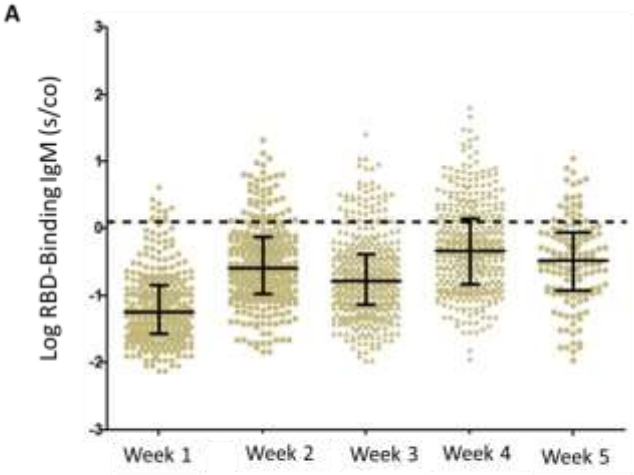
ImmunoResearch, PA, USA Code: 109-035-129) (diluted 1:20000)) TMB substrate was added followed by stop solution (1M HCl) and the OD of each well was measured at 450nm. ELISA index value below 1.1 was considered negative, and equal or above 1.1, positive.

SARS-CoV-2 Pseudovirus (psSARS-2) Neutralization Assay

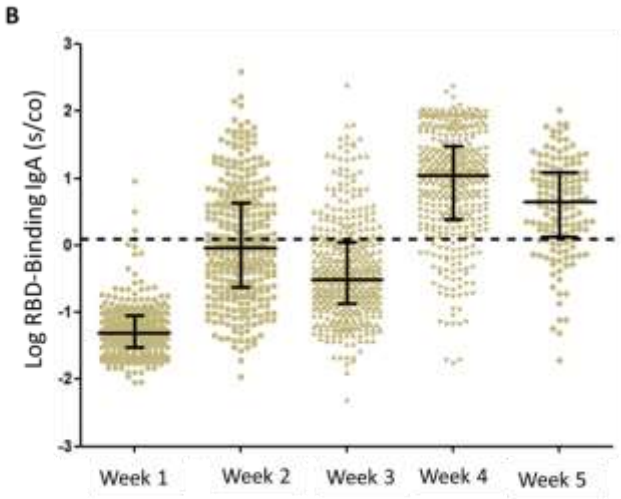
SARS-CoV-2 Pseudo-virus (psSARS-2) Neutralization Assay was performed using a propagation-competent VSV-spike similar to the one previously published[3] which was kindly provided by Gert Zimmer, University of Bern, Switzerland and shown to be highly correlative to authentic SARS-CoV-2 virus micro-neutralization assay. . Following titration, 100 focus forming units (ffu) of psSARS-2 were incubated with 2 fold serial dilution of heat inactivated (56°C for 30 min) tested sera. After incubation for 60 min at 37°C, virus/serum mixture was transferred to Vero E6 cells that have been grown to confluency in 96-well plates and incubated for 90 min at 37°C. After the addition of 1% methyl cellulose in dulbecco's modified eagle's medium (DMEM) with 2% of fetal bovine serum (FBS), plates were incubated for 24hr and 50% plaque reduction titer was calculated by counting green fluorescent foci using a fluorescence microscope (EVOS M5000, Invitrogen). Sera not capable of reducing viral replication by 50% at 1 to 16 dilution were considered borderline and not capable of reducing viral replication by 50% at 1 to 8, non-neutralizing. For clear presentation non- neutralizing samples were marked as a titer of 2. A typical neutralization dilution curve is shown in supplementary Figure 2.

Supplementary Figure 1. Quantitation of antibodies following BNT162b2 vaccination. (A) Immunoglobulin M (IgM). (B) Immunoglobulin A (IgA). Antibodies were tested at weeks 1-2 following the 1st vaccination dose, at week 3 with the administration of the 2nd vaccination dose, and at weeks 4-5 which refers to the 1-2 weeks

following the 2nd vaccination dose, respectively. The dotted black line indicates limit of positive antibodies level. The black line indicates median and IQR. RBD= Receptor Binding Domain. S/CO=sample/cutoff ratio. NeutAb= Neutralizing antibodies**.**

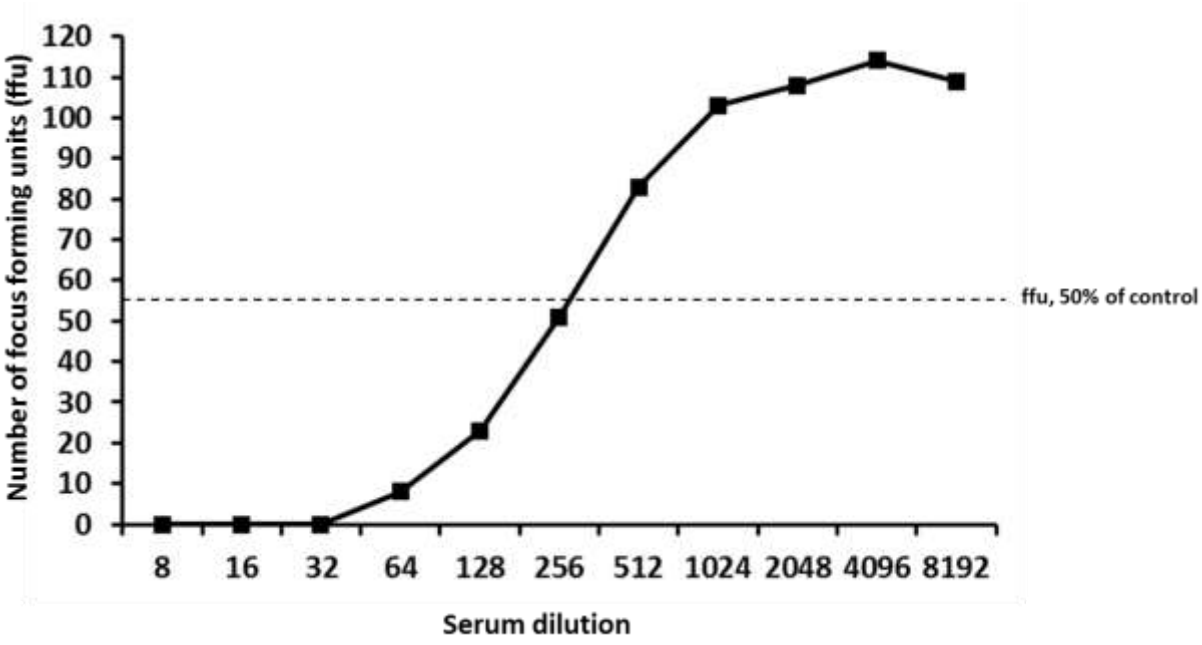


N	315	321	397	393	137
IgM>0.095 (%)	12 (3.8)	51 (15.9)	49 (12.3)	109 (27.7)	23 (16.8)
Median	-1.24	-0.58	-0.78	-0.33	-0.48

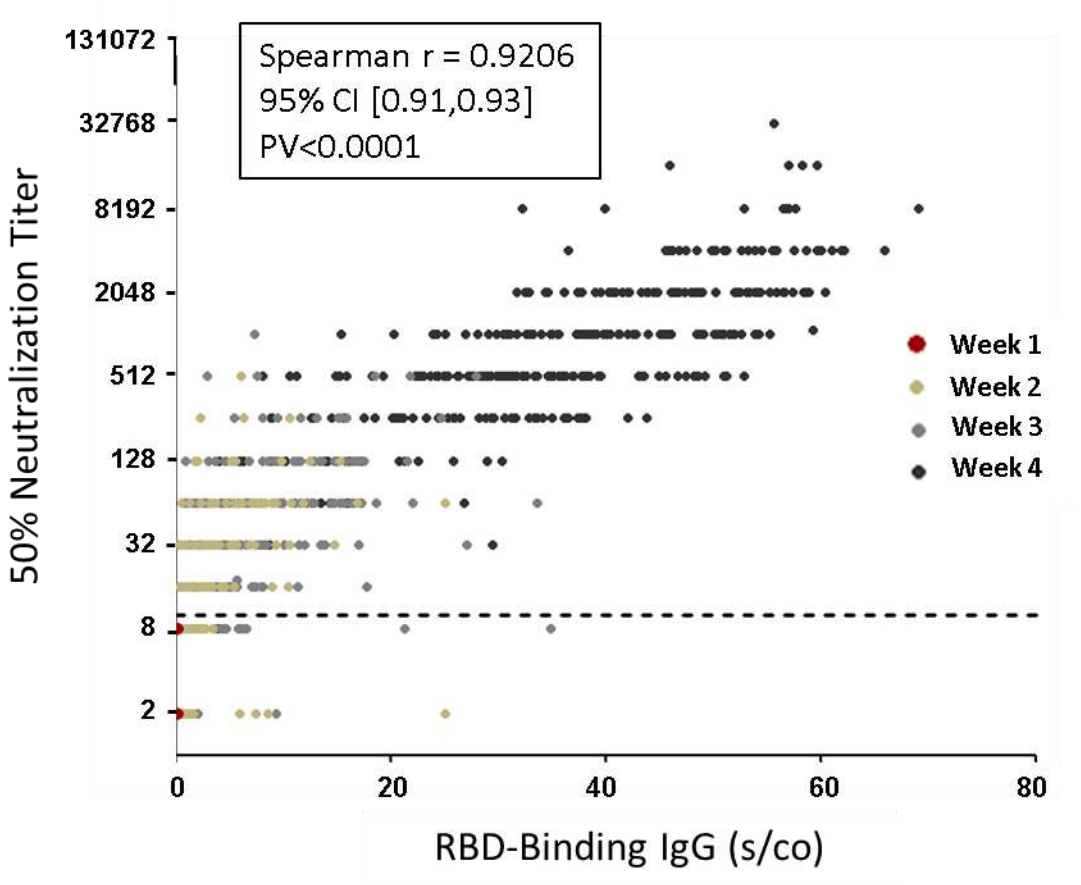


N	315	321	396	385	137
IgA>0.095 (%)	4 (1.27)	139 (43.3)	90 (22.7)	326 (84.7)	103 (71.2)
Median	-1.31	-0.04	-0.51	1.05	0.65

Supplementary Figure 2. A typical neutralization dilution curve. Following psSARS-2 neutralization assay with sera from control or vaccinated individuals, the number of green fluorescent foci in the control well was counted and 50% ffu was determined (dotted line). The number of ffu in each dilution of vaccinated sera was counted and a dilution of 256 was determined to be the last dilution to reduce viral replication by more than 50%.


Number of focus forming units (ffu)
120
110
100
90
80
70
60
50
40
30
20
10
0
8
16
32
64
128
256
512
1024
2048
4096
8192
Serum dilution
ffu, 50% of control

Supplementary Figure 3: Correlation of IgG and Neutralizing antibodies. The correlation was analyzed along four weeks following vaccination: 1-2 weeks following the first vaccine dose, week three with the administration of the second vaccine dose, and week four refers to the week following the second vaccine dose. IgG= immunoglobulin G. RBD= Receptor Binding Domain. S/CO=sample/cutoff ratio



Supplementary Table 1: Multivariate linear regression analysis of predictors of IgG and neutralizing antibodies levels following vaccination

Variable	IgG levels (week 5†) (N=1467)			Neutralization levels (week 4†) (N=366)		
	Number of individuals (%)	Ratio of mean (95% CI)	P	Number of individuals (%)	Ratio of mean (95% CI)	P
Female	998 (68.0)	Ref.		289 (78.9)	Ref.	
Male	469 (32.0)	0.84 (0.80, 0.89)	<0.0001	77 (21.1)	0.52 (0.35, 0.77)	0.001
Age 18-45.99	697 (47.5)	Ref.	<0.0001¥	155 (42.3)	Ref.	<0.0001¥
Age 46-65.99	660 (45.0)	0.85 (0.81, 0.90)	<0.0001	145 (39.6)	0.57 (0.41, 0.8)	0.001
Age ≥66	110 (7.5)	0.64 (0.58, 0.71)	<0.0001	66 (18.0)	0.26 (0.16, 0.41)	<0.0001
BMI<25	738 (50.3)	Ref.	0.1590¥	203 (55.5)	Ref.	0.0170¥
BMI 25-29.99	485 (33.1)	1.05 (0.99-1.11)	0.079	108 (29.5)	0.76 (0.53-1.08)	0.131
BMI ≥ 30	244 (16.6)	1.05 (0.98-1.13)	0.188	55 (15)	0.52 (0.33-0.83)	0.0057
No hypertension	1297 (88.4)	Ref.		313 (85.5)	Ref.	
Hypertension	170 (11.6)	0.90 (0.82-0.98)	0.012	53 (14.5)	1.60 (0.99-2.57)	0.054
No autoimmune disease	1393 (95.0)	Ref.		322 (78.0)	Ref.	
Autoimmune disease	74 (5.0)	0.82 (0.73-0.92)	0.0008	44 (12.0)	0.76 (0.47-1.21)	0.244
No diabetes	1383 (94.3)	Ref.		338 (92.3)	Ref.	
Diabetes	84 (5.7)	0.88 (0.79-0.98)	0.23	28 (7.7)	0.58 (0.32-1.06)	0.076
No lung disease	1428 (97.3)	Ref.		348 (95.1)	Ref.	
Lung disease	39 (2.7)	1.09 (0.93-1.26)	0.2935	18 (4.9)	0.79 (0.39-1.61)	0.512
No heart disease	1422 (96.9)	Ref.		349 (95.4)	Ref.	
Heart disease	45 (3.1)	0.86 (0.75-0.99)	0.049	17 (4.6)	0.60 (0.28-1.29)	0.192
No immunosuppression	1455 (99.2)	Ref.		NA	NA	NA
Immunosuppression‡	12 (0.82)	0.44 (0.33-0.58)	<0.0001	NA	NA	NA

†Weeks 4 and 5 refer to weeks 1-2 following the 2nd vaccination dose, respectively. ‡Immunosuppression included organ transplantation, biologic therapy, chemotherapy, steroids and splenectomy. ¥Global P value. Immunoglobulin G=IgG. CI=confidence interval. BMI=Body-mass index**.** NA=not available.

Bibliography

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2. Indenbaum V, Koren R, Katz-Likvornik S, Yitzchaki M, Halpern O, Regev-Yochay G, et al. Testing IgG antibodies against the RBD of SARS-CoV-2 is sufficient and necessary for COVID-19 diagnosis. PLoS One. 2020; 15(11): e0241164.

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