Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. N Engl J Med. DOI: 10.1056/NEJMoa2114583

Supplementary Materials

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Supplementary Methods S1 - 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).

Supplementary Method S2- Inclusion criteria for selecting the neutralizing antibody subgroup

1. Age ≥65
2. Body mass index ≥30
3. Pregnancy
4. Allergy
5. Hypertension
6. Diabetes
7. Dyslipidemia
8. Heart disease
9. Lung disease
10. Kidney disease
11. Liver disease
12. Autoimmune disease
13. Immunosuppression

Additionally, 50% of healthy health care workers were randomly selected for the neutralizing antibody subgroup.

Supplementary Methods S3- antibody detection

SARS-CoV-2 IgG Assay

Samples from vaccinated health care workers (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.

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[2] 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 8 dilution or below were considered non-neutralizing. For clear presentation non- neutralizing samples were marked as a titer of 2.

Supplementary Method S4 – IgG Linear Mixed Model

We modeled the natural log-transformed IgG in a mixed effects linear model with subject level random effects for both intercept and slope across time (measured in days). After inspection of the data, we decided to fit the slope only from 30 days after full vaccination onwards. IgG tests before 30 days were used to estimate the "maximum" IgG attained. First, we fitted a model that included days from the second vaccination (day 30 was defined as day 0 in the model), age, sex, age-sex interaction, time (slope), interaction of time with age and sex as fixed effects, and subject-level random intercept and slope. A 3-way interaction of time with age with sex was included and found to be significant, owing to a faster decline in IgG levels among older men. This we call our basic model.

Next, we added different comorbidities into the model: BMI (<30, 30+), immunosuppression, autoimmune disease, number of specific comorbidities (0, 1 or ≥2), and their interaction with time. We maintained all main effects and the age, sex, and time interactions found previously. Of the comorbidity with time interactions, we retained in the model only the significant interactions (a p-value of 0.01 was used, following a Bonferroni adjustment for the 5 multiple comparisons), and thus only the interaction between BMI with time was retained. This we call our comorbidity model

Supplementary Method S5 – Neutralizing antibody Linear Mixed Model

We modeled log-transformed neutralizing antibody (NeutAb) using a linear mixed effects model similar to the model used for the IgG analysis. The model included subject-level random effects for both intercept and slope across time. We found that NeutAb kinetics over time were different from IgG kinetics, and modeled them accordingly. The decline started from 30 days after the second vaccination as in the IgG data (see Supplementary Methods 4). However, this decline lasted only up to ~70 days after the second vaccination, and then stabilized to a much slower decline. The rate of this slower decline did not significantly differ across age groups or sex.

The change point at 70 days was chosen based on inspection of the fit without the change point, and examining AIC for models with a change point at 60, 70, 80 and 90 days. The AIC of the basic fitted models (with no comorbidities) with 60, 70, 80, 90 and 100 days were 15530, 15529, 15527, 15526 and 15526 respectively. Thus the best fits were seen with change point at 60 or 70 days. We chose 70 days on the basis of visual comparisons of predicted with observed values. Therefore, we included in the model a common fixed-effects slope from day 70 to day 180. Other main effects and interactions, including interaction between fixed-effect factors with time in the period from day 30 to day 70, were the same as in the model for IgG levels.

The analysis of comorbidities was performed as in the IgG analysis. A significant interaction of ≥2 specific comorbidities with time (between 30 to 70 days) was found.

The estimated probabilities and 95%CI of being below various NeutAb thresholds at 180 days after second vaccination for different covariate profiles were estimated using computer simulations, based on the estimated parameters from the final model. For each of the personal profiles in Table 2 (gender x age group x {no comorbidities, BMI 30+ but no other comorbidities, immunosuppressed but no other comorbidities}), a set of 2000 pairs of random intercepts and slopes were first generated from a binormal distribution with zero mean and covariance matrix as estimated from the linear mixed model. The parameters of the fixed effect terms in the mixed model were then drawn from their estimated joint normal distribution and were used to estimate the mean log antibody level at 180 days. To this mean

was added the random intercept plus 180 times the random slope for each pair, yielding 2000 values of the log antibody level at 180 days for the given personal profile. The probability of falling below a certain threshold was then estimated as the proportion of the 2000 values that were less than the threshold. This process was repeated 1000 times. The probabilities reported in Table 2 are the means over the 1000 repeats, and the confidence intervals are based on the distribution of the 1000 values obtained.

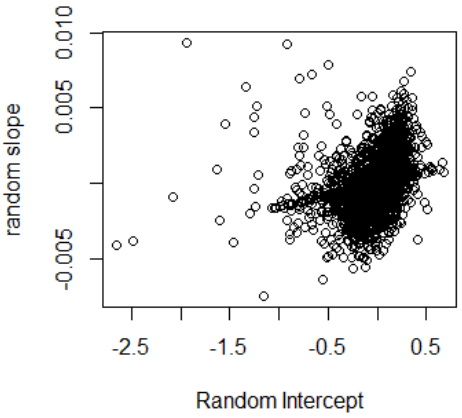
Supplementary Results S1 – IgG Linear Mixed Model

Basic Model (output from the R statistical package)

Random effects:
Formula: ~1 + days30 | ID
Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	0.61143929	(Intr)
days30	0.00498175	0.17
Residual	0.26112124	

The dependent variable is log IgG level.
The Intercept row provides the SD of the random subject peak levels.
The days30 row provides the SD of the random subject slopes with time.
The Corr column gives the correlation between peak level and slope across subjects.
The graph below is a scatter plot of slope versus peak level



Fixed effects: ligg ~ agec * male * days30

	Value	Std.Error	DF	t-value	p-value
(Intercept)	3.567585	0.01610265	9862	221.55258	0.0000
agec45-64	-0.21305	0.02291731	4862	-9.29645	0.0000
agec65+	-0.515976	0.0386125	4862	-13.36292	0.0000
male	-0.082746	0.03115104	4862	-2.65628	0.0079
days30	-0.02154	0.00021134	9862	-101.92107	0.0000
agec45-64:male	-0.072187	0.04570284	4862	-1.57949	0.1143
agec65+:male	-0.273893	0.06522785	4862	-4.19902	0.0000
agec45-64:days30	0.000572	0.00028183	9862	2.03015	0.0424
agec65+:days30	0.001751	0.00052335	9862	3.34496	0.0008
male:days30	0.001647	0.00045862	9862	3.59134	0.0003
agec45-64:male:days30	-0.001964	0.00061557	9862	-3.19025	0.0014
agec65+:male:days30	-0.003779	0.00090073	9862	-4.19561	0.0000

The references for the categorical variables are as follows:
agec45-64 and agec65+ have reference group <45y;
male has reference group female.
days30 is a continuous variable equal to zero for days 0 to 30, and then
equal to time - 30 for days 30 onwards.
The : symbol indicates interaction.

Comorbidity model (output from the R statistical package)

Random effects

Formula: ~1 + days30 | ID
Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	0.565444200	(Intr)
days30	0.004964874	0.171
Residual	0.254156686	

Fixed Effects

ligg ~ agec * male * days30 + bmi30 * days30 + ndis + autoim + imunosup

	Value	Std.Error	DF	t-value	p-value
(Intercept)	3.569736	0.0176611	9167	202.1237	0.0000
agec45-64	-0.212518	0.0241982	3748	-8.78238	0.0000
agec65+	-0.40201	0.0479047	3748	-8.39188	0.0000
male	-0.111148	0.0331988	3748	-3.34795	0.0008
days30	-0.021429	0.0002239	9167	-95.71713	0.0000
bmi30	0.046484	0.0276194	3748	1.68302	0.0925
ndis1	0.018674	0.029121	3748	0.64126	0.5214
ndis2+	-0.196936	0.0441415	3748	-4.46148	0.0000
autoim	-0.132353	0.041835	3748	-3.16369	0.0016
imunosup	-1.054757	0.0974591	3748	-10.82256	0.0000
agec45-64:male	-0.018491	0.0476245	3748	-0.38826	0.6978
agec65+:male	-0.245425	0.0777133	3748	-3.15808	0.0016
agec45-64:days30	0.000836	0.0002931	9167	2.85067	0.0044
agec65+:days30	0.002095	0.0005596	9167	3.74453	0.0002
male:days30	0.001648	0.000475	9167	3.46843	0.0005
days30:bmi30	-0.00152	0.0003343	9167	-4.5464	0.0000
agec45-64:male:days30	-0.001951	0.0006363	9167	-3.06669	0.0022
agec65+:male:days30	-0.004407	0.000958	9167	-4.60002	0.0000

The meaning and reference groups for the following categorical variables are as follows:
bmi30: BMI = 30+; reference group: BMI <30;
ndis1 and ndis2+: number of comorbidities =1 and ≥2 respectively; reference group: 0 comorbidities;
autoim: autoimmune disease; reference group: no autoimmune disease;
imunosup: immunosuppressed person; reference group: no immunosuppression.

Further exploration of BMI and its relation with IgG and NeutAb levels:

We divided BMI into four subgroups (<25, 25-30, 30-35, ≥35) instead of <30, ≥30) that was used in the main analysis. We then ran the comorbidity model again with this new categorization of BMI. Results are shown below.

Random effects:
Formula: ~1 + days30 | ID
Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	0.245603697	(Intr)
days30	0.002152254	0.174
Residual	0.109767396	

Fixed effects: ligg ~ agec * male * days30 + bmi * days30 + ndis + autoim + imunosup

	Value	Std .Error	DF	t-value	p-value
(Intercept)	1.5474	0.0081	9161	191.0341	0.0000
agec45-64	-0.0938	0.0105	3754	-8.9037	0.0000
agec65+	-0.1764	0.0209	3754	-8.4598	0.0000
male	-0.0504	0.0145	3754	-3.4771	0.0005
days30	-0.0093	0.0001	9161	-90.3257	0.0000
bmi[25,30)	0.0116	0.0099	3754	1.1704	0.2419
bmi[30,35)	0.0277	0.0141	3754	1.9667	0.0493
bmi[35,100)	0.0206	0.0222	3754	0.9282	0.3533
ndis1	0.0069	0.0127	3754	0.5443	0.5862
ndis2+	-0.0896	0.0192	3754	-4.6696	0.0000
autoim	-0.0576	0.0182	3754	-3.1731	0.0015
imunosup	-0.457	0.0423	3754	-10.8017	0.0000
agec45-64:male	-0.0079	0.0207	3754	-0.3811	0.7031
agec65+:male	-0.1054	0.0337	3754	-3.1256	0.0018
agec45-64:days30	0.0004	0.0001	9161	3.113	0.0019
agec65+:days30	0.001	0.0002	9161	3.9222	0.0001
male:days30	0.0008	0.0002	9161	3.6653	0.0002
days30:bmi[25,30)	-0.0001	0.0001	9161	-1.0361	0.3002
days30:bmi[30,35)	-0.0006	0.0002	9161	-3.2918	0.001
days30:bmi[35,100)	-0.0012	0.0003	9161	-4.2144	0.0000
agec45-64:male:days30	-0.009	0.0003	9161	-3.1837	0.0015
agec65+:male:days30	-0.0019	0.0004	9161	-46728	0.0000

The meaning and reference groups for the following categorical variables are as follows:
bmi[25,30): BMI ≥ 25 and <30; reference group: BMI <25;
bmi[30,35): BMI ≥ 30 and <35; reference group: BMI <25;
bmi[35,100): BMI ≥ 35; reference group: BMI <25.
The meaning of the other variables and their reference groups is explained in the footnotes to the models displayed in the sections preceding this one.

Supplementary Results S2 – Neutralizing Antibody Linear Mixed Model

Basic Model (output from the R statistical package)

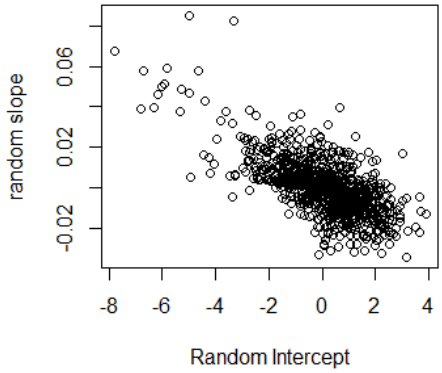
Random effects:

Random effects:
Formula: ~1 + days30 | ID
Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	1.71763680	(Intr)
days30	0.02269346	-0.63
Residual	0.98029484	

The dependent variable is log NeutAb level.
The Intercept row provides the SD of the random subject peak levels.
The days30 row provides the SD of the random subject slopes with time.
The Corr column gives the correlation between peak level and slope across subjects.

The graph below is a scatter plot of slope versus peak level



Fixed effects: lneut ~ days30 * agec * male + days70

	Value	Std.Error	DF	t-value	p-value
(Intercept)	9.959674	0.1135957	3253	87.67647	0.0000
days30	-0.056054	0.0025549	3253	-21.93945	0.0000
agec45-64	-1.01018	0.1586674	1263	-6.36665	0.0000
agec65+	-0.929223	0.1757313	1263	-5.28775	0.0000
male	-0.889713	0.2865281	1263	-3.10515	0.0019
days70	-0.003583	0.0005981	3253	-5.99013	0.0000
days30:agec45-64	0.012351	0.0033593	3253	3.67664	0.0002
days30:agec65+	0.00499	0.0038554	3253	1.29431	0.1957
days30:male	0.014811	0.0059393	3253	2.49369	0.0127
agec45-64:male	0.4299	0.3995388	1263	1.07599	0.2821
agec65+:male	0.124735	0.359353	1263	0.34711	0.7286
days30:agec45-64:male	-0.023051	0.00854	3253	-2.69918	0.007
days30:agec65+:male	-0.019046	0.0076748	3253	-2.48166	0.0131

The references for the categorical variables are as follows:
agec45-64 and agec65+ have reference group <45y; male has reference group female.
days30 is a continuous variable equal to zero for days 0 to 30, and then equal to time - 30 for days 30 -70, and then equal to 40 for days 70 onwards.
Days70 is a continuous variable equal to zero for days 0 to 70, and then equal to time - 70 for days 70 onwards.
The : symbol indicates interaction.

Comorbidity model (output from the R statistical package)

Random effects:

Formula: ~1 + days30 | ID

Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	1.68095284	(Intr)
days30	0.02290468	-0.632
Residual	0.97881590	

Fixed effects:

lneut ~ days30 * agec * male + days70 + bmi30 + ndis * days30 + autoim + imunosup

	Value	Std.Error	DF	t-value	p-value
(Intercept)	9.921351	0.121149	3110	81.894	0.0000
days30	-0.05729	0.002636	3110	-21.735	0.0000
agec45-64	-1.0095	0.163409	1128	-6.17776	0.0000
agec65+	-0.78398	0.200859	1128	-3.90312	0.0001
male	-0.91286	0.297237	1128	-3.07113	0.0022
days70	-0.0036	0.000609	3110	-5.90941	0.0000
bmi30	0.388413	0.104767	1128	3.70741	0.0002
ndis1	-0.18439	0.167655	1128	-1.0998	0.2717
ndis2+	-0.76741	0.219079	1128	-3.5029	0.0005
autoim	0.190596	0.146375	1128	1.30211	0.1931
imunosup	-1.71742	0.301595	1128	-5.69447	0.0000
days30:agec45-64	0.011803	0.003495	3110	3.37703	0.0007
days30:agec65+	0.003393	0.004279	3110	0.79277	0.428
days30:male	0.014891	0.006216	3110	2.39583	0.0166
agec45-64:male	0.567702	0.408549	1128	1.38956	0.1649
agec65+:male	0.256511	0.386597	1128	0.66351	0.5071
days30:ndis1	0.004141	0.003599	3110	1.15047	0.25
days30:ndis2+	0.014825	0.004718	3110	3.14209	0.0017
days30:agec45-64:male	-0.02386	0.008801	3110	-2.71059	0.0068
days30:agec65+:male	-0.02037	0.00816	3110	-2.49666	0.0126

The meaning and reference groups for the following categorical variables are as follows:
bmi30: BMI = 30+; reference group: BMI <30;
ndis1 and ndis2: number of comorbidities =1 and ≥2 respectively; reference group: 0 comorbidities;
autoim: autoimmune disease; reference group: no autoimmune disease;
imunosup: immunosuppressed person; reference group: no immunosuppression.

Further exploration of BMI and its relation with IgG and NeutAb levels:

We divided BMI into four subgroups (<25, 25-30, 30-35, ≥35) instead of <30, ≥30) that was used in the main analysis. We then ran the comorbidity model again with this new categorization of BMI. Results are shown below.

Random effects:
Formula: ~1 + days30 | ID
Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	1.68341425	(Intr)
days30	0.02298689	-0.638
Residual	0.97239137	

Fixed effects:
lneut ~ days30 * agec * male + days70 + bmi + ndis * days30 + autoim + imunosup

	Value	Std.Error	DF	t-value	p-value
(Intercept)	9.837	0.1256	3106	78.3068	0.0000
days30	-0.0574	0.0026	3106	-21.8367	0.0000
agec45-64	-1.0285	0.1636	1126	-6.288	0.0000
agec65+	-0.8265	0.2015	1126	-4.102	0.0000
male	-0.9175	0.2973	1126	-3.0866	0.0021
days70	-0.0037	0.0006	3106	-6.1558	0.0000
bmi[25,30)	0.2835	0.1115	1126	2.5437	0.0111
bmi[30,35)	0.5221	0.1238	1126	4.2167	0.0000
bmi[35,100)	0.455	0.1793	1126	2.5375	0.0113
ndis1	-0.1947	0.1677	1126	-1.1613	0.2457
ndis2+	-0.8087	0.2199	1126	-3.6771	0.0002
autoim	0.1889	0.1459	1126	1.2946	0.1957
imunosup	-1.6897	0.3008	1126	-5.6177	0.0000
days30:agec45-64	0.0121	0.0035	3106	3.4626	0.0005
days30:agec65+	0.0038	0.0043	3106	0.8858	0.3758
days30:male	0.0153	0.0062	3106	2.463	0.0138
agec45-64:male	0.5428	0.4088	1126	1.3278	0.1845
agec65+:male	0.2274	0.3869	1126	0.5877	0.5569
days30:ndis1	0.0041	0.0036	3106	1.1454	0.2521
days30:ndis2+	0.0149	0.0047	3106	3.1724	0.0015
days30:agec45-64:male	-0.0243	0.0088	3106	-2.7637	0.0057
days30:agec65+:male	-0.0208	0.0081	3106	-2.5599	0.0105

The meaning and reference groups for the following categorical variables are as follows:
bmi[25,30): BMI ≥ 25 and <30; reference group: BMI <25;
bmi[30,35): BMI ≥ 30 and <35; reference group: BMI <25;
bmi[35,100): BMI ≥ 35; reference group: BMI <25.
The meaning of the other variables and their reference groups is explained in the footnotes to the models displayed in the sections preceding this one.

Supplementary Table S1 – Baseline characteristics of the study population

Variable	Whole study population (N=4,868)	NeutAb subgroup* (N=1,269)
Gender, n (%)		
Female	3558 (73.09)	959 (75.57)
Male	1310 (26.91)	310 (24.43)
Age, mean (+/-std)	46.90 (+/-13.69)	52.70 (+/-14.23)
Age, n (%)		
18-44.99	2241 (46.04)	398 (31.36)
45-64.99	2072 (42.56)	527 (41.53)
65+	555 (11.40)	344 (27.11)
BMI, mean (+/-std)	25.51 (+/-4.62)	26.86 +/-5.28)
BMI, n (%)		
<25	1948 (51.71)	488 (42.84)
25-29.99	1216 (32.28)	325 (28.53)
30+	446 (11.84)	326 (28.62)
Sector, n (%)		
Physician	909 (18.67)	246 (19.39)
Nurse	1391 (28.57)	282 (22.22)
Para	1161 (23.85)	349 (27.50)
Administration	1407 (28.90)	392 (30.89)
Comorbidity, n (%)		
Pregnancy	31 (0.64)	23 (1.81)
Allergy	77 (2.00)	46 (3.98)
Hypertension	411 (10.79)	206 (17.93)
Diabetes	209 (5.49)	102 (8.88)
Dyslipidemia	244 (6.41)	119 (10.36)
Heart disease	104 (2.73)	51 (4.44)
Lung disease	124 (3.26)	48 (4.18)
Kidney disease	21 (0.55)	7 (0.61)
Coagulation disease	68 (1.79)	25 (2.18)
Liver disease	26 (0.68)	12 (1.04)
Autoimmune disease	242 (6.36)	137 (11.92)
Immunosuppression	41 (1.08)	28 (2.44)
Specific comorbidities, n (%)**		
0	3001 (78.81)	785 (61.86)
1	568 (14.92)	233 (18.36)
≥2	239 (6.28)	131 (10.32)

*NeutAb subgroup was defined as HCW with as at least one NeutAb assays during study follow-up. **Specific comorbidities included the following: hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease and liver disease. BMI=Body mass index; NeutAb=neutralizing antibodies

Supplementary Table S2 - Computer-based questionnaire

	Question	Answer1	Answer2
1	What is your date of birth?		
2	What is your gender?	Male	Female
3	What is your current height in m?		
4	What is your current weight in kg?		
5	Did you perform an IgG assay before receiving the first dose of the vaccine?	Yes	No
6	Do you have high blood pressure disease (systolic blood pressure above 140) treated with medication?	Yes	No
7	Do you have dyslipidemia (total cholesterol above 200 or LDL cholesterol above 160) treated with medication?	Yes	No
9	Do you have autoimmune disease treated with medication?	Yes	No
10	Do you have diabetes (HbA1C>6.5 or fasting blood sugar>126) treated with medication?	Yes	No
11	Do you have heart disease treated with medication?	Yes	No
12	Do you have lung disease as asthma, COPD, lung fibrosis treated with medication/s?	Yes	No
13	Do you have any coagulation disorder resulting in hemorrhage or thrombosis treated with medication?	Yes	No
14	Are you immunosuppressed (organ transplantation, biologic therapy, chemotherapy, steroids, splenectomy, or HIV)?	Yes	No
15	Have you ever had a serious allergic reaction (anaphylaxis) that required immediate treatment?	Yes	No
16	Do you have liver disease as cirrhosis, hepatitis, liver cancer, metabolic disorder?	Yes	No
17	Do you have kidney disease (creatinine>1.2 or GFR<60) treated with medication?	Yes	No
18	Are you pregnant (confirmed by a beta HCG blood test and ultrasound fetal heartbeats detection)?	Yes	No

The questionnaire was reviewed and approved by the Institutional review board of the Sheba Medical Center**.**

IgG=Immunoglobulin G; BMI=Body mass index; Kg=kilogram. M=meter; LDL=low-density lipoproteins; HbA1C=hemoglobin A1C; COPD= chronic obstructive pulmonary disease; HIV=human immunodeficiency; GFR=Glomerular filtration rate.

Supplementary Table S3- Variable Definitions

Variable	Values	Definitions	Timing
Outcomes			
IgG at the peak period	Continuous (S/CO)	SARS-CoV-2 Receptor Binding Domain (RBD) Immunoglobulin G (IgG) assay (Beckman-Coulter, CA, U.S.A.)	During the peak period (days 4-30 after the second vaccination)
NeutAb at the peak period	Continuous (50% titer)	SARS-CoV-2 Pseudo-virus (psSARS-2) Neutralization Assay	During the peak period (days 4-30 after the second vaccination)
IgG in the EoS	Continuous (S/CO)	SARS-CoV-2 Receptor Binding Domain (RBD) Immunoglobulin G (IgG) assay (Beckman-Coulter, CA, U.S.A.)	At the end of the study (day 175 after the second vaccination)
IgG and NeutAb in the EoS	Continuous (50% titer)	SARS-CoV-2 Pseudo-virus (psSARS-2) Neutralization Assay	At the end of the study (day 175 after the second vaccination)
Variables			
Sex	Female/male	As defined in SMC' files	Current
Age	Continuous (years)	As defined in SMC' files	At second vaccine dose
BMI	Categorical: <25, 25-29.99, ≥30	BMI was calculated by weight (kg)/(height (m))^2 according to the HCW answer to the questionnaire.	At second vaccine dose
Blood pressure disease	0/1	According to the HCW answer to the questionnaire: defined as systolic blood pressure above 140 treated with medication	At second vaccine dose
Dyslipidemia	0/1	According to the HCW answer to the questionnaire: defined as total cholesterol above 200 or LDL cholesterol above 160 treated with medication	At second vaccine dose
Autoimmune disease	0/1	According to the HCW answer to the questionnaire: defined as known autoimmune	At second vaccine dose

		disease treated with medication	
Diabetes	0/1	According to the HCW answer to the questionnaire: defined as HbA1C>6.5 or fasting blood sugar>126 treated with medication	At second vaccine dose
Heart disease	0/1	According to the HCW answer to the questionnaire: defined as known heart disease treated with medication	At second vaccine dose
Lung disease	0/1	According to the HCW answer to the questionnaire: defined as defined as known lung disease treated with medication	At second vaccine dose
Coagulation disorder	0/1	According to the HCW answer to the questionnaire: defined as known hemorrhage or thrombosis disease treated with medication	At second vaccine dose
Immunosuppressed	0/1	According to the HCW answer to the questionnaire: defined as organ transplantation, biologic therapy, chemotherapy, steroids, splenectomy, or HIV	At second vaccine dose
Allergy	0/1	According to the HCW answer to the questionnaire: defined as a serious allergic reaction (anaphylaxis) that required immediate treatment	During the life
Liver disease	0/1	According to the HCW answer to the questionnaire: defined as cirrhosis, hepatitis, liver cancer, metabolic disorder	At second vaccine dose
Kidney disease	0/1	According to the HCW answer to the questionnaire: defined as creatinine>1.2 or GFR<60) treated with medication	At second vaccine dose
Pregnancy	0/1	According to the HCW answer to the questionnaire:	At second vaccine dose

		defined as confirmed pregnancy by a beta HCG blood test and ultrasound fetal heartbeats detection	
Specific comorbidities	Categorical: 0, 1, ≥2	Count of comorbidities that were with significant lower antibodies titers compared to healthy people during the first 5 weeks after the first vaccine dose[3]: • Hypertension • Diabetes • Dyslipidemia • Heart disease • Lung disease • Kidney disease • Liver disease	At second vaccine dose

Abbreviations: NeutAb= neutralizing antibodies; EoS=end of study; IgG=Immunoglobulin G; S/CO=sample cutoff ratio; SARS-CoV-2=severe acute respiratory syndrome; BMI=Body mass index; Kg=kilogram. M=meter; HCW=health care worker; LDL=low-density lipoproteins; HIV=human immunodeficiency; GFR=Glomerular filtration rate; HbA1C=hemoglobin A1C.

Supplementary Table S4 – Distribution of demographic and comorbidity according to test period of IgG

Variable	P0 (N=3991)	P1 (N=2690)	P2 (N=1829)	P3 (N=1732)	P4 (N=1606)	P5 (N=1518)	P6 (N=1370)
Number of days after the 2nd vaccine, mean (+/-std)	11.19 (+/-3.27)	27.88 (+/-4.19)	56.40 (+/-3.79)	85.08 (+/-4.37)	112.85 (+/-4.33)	140.96 (+/-4.74)	167.89 (+/-3.91)
Gender, n (%)							
Female	2960 (74.17)	2028 (75.39)	1442 (78.84)	1380 (79.68)	1258 (78.33)	1194 (78.66)	1046 (76.35)
Male	1031 (25.83)	662 (24.61)	387 (21.16)	352 (20.32)	348 (21.67)	324 (21.34)	324 (23.65)
Age, mean (+/-std)							
Age, n (%)							
18-44.99	1798 (45.05)	1183 (43.98)	760 (41.55)	688 (39.72)	636 (39.60)	573 (37.75	483 (35.26)
45-64.99	1754 (43.95)	1232 (45.80)	894 (48.88)	874 (50.46)	816 (50.81)	795 (52.37)	747 (54.53)
65+	439 (11.00)	275 (10.22)	175 (9.57)	170 (9.82)	154 (9.59)	150 (9.88)	140 (10.22)
BMI, mean (+/-std)							
BMI, n (%)							
<25	1645 (51.83)	1204 (51.94)	877 (52.61)	846 (53.61)	785 (52.47)	735 (52.16)	669 (51.66)
25-29.99	1024 (32.26)	740 (31.92)	525 (31.49)	486 (30.80)	478 (31.95)	442 (31.37)	410 (31.66)
30+	505 (15.91)	374 (16.13)	265 (15.90)	246 (15.59)	233 (15.57)	232 (16.47)	216 (16.68)
Sector, n (%)							
Physician	732 (18.34)	480 (17.84)	302 (16.51)	273 (15.76)	260 (16.19)	245 (16.14)	240 (17.52)
Nurse	1131 (28.34)	720 (26.77)	435 (23.78)	411 (23.73)	380 (23.66)	358 (23.58)	328 (23.94)
Para	1000 (25.06)	699 (25.99)	555 (30.34)	521 (30.08)	505 (31.44)	468 (30.83)	396 (28.91)
Administration	1128 (28.26)	791 (29.41)	537 (29.36	527 (30.43)	461 (28.70)	447 (29.45)	406 (29.64)
Comorbidity, n (%)							
Pregnancy	25 (0.63)	23 (0.86)	19 (1.04)	12 (0.69)	8 (0.50)	5 (0.3)	2 (0.15)
Allergy	64 (1.97)	56 (2.38)	42 (2.49)	45 (2.81)	40 (2.65)	39 (2.73)	13 (0.99)
Hypertension	348 (10.84)	259 (11.07)	185 (11.01)	191 (12.00)	188 (12.49)	178 (12.54)	171 (13.09)
Diabetes	168 (5.23)	120 (5.13)	82 (4.88)	83 (5.21)	77 (5.12)	79 (5.57)	75 (5.74)
Dyslipidemia	212 (6.60)	150 (6.41)	113 (6.73)	105 (6.60)	108 (7.18)	99 (6.98)	104 (7.96)
Heart disease	93 (2.90)	57 (2.44)	46 (2.74)	40 (2.51)	39 (2.59)	38 (2.68)	35 (2.68)
Lung disease	106 (3.30)	84 (3.59)	62 (3.69)	59 (3.71)	60 (3.99)	59 (4.16)	52 (3.98)
Kidney disease	20 (0.62)	15 (0.64)	8 (0.48)	8 (0.50)	10 (0.66)	9 (0.63)	7 (0.54)
Coagulation disease	59 (1.84)	46 (1.97)	35 (2.08)	29 (1.82)	31 (2.06)	28 (1.97)	25 (1.91)
Liver disease	22 (0.69)	15 (0.64)	14 (0.83)	11 (0.69)	7 (0.47)	9 (0.63)	11 (0.84)
Autoimmune disease	211 (6.57)	157 (6.71)	118 (7.02)	107 (6.72)	99 (6.58)	97 (6.84)	80 (6.13)
Immunosuppression	38 (1.18)	28 (1.20)	24 (1.43)	21 (1.32)	21 (1.40)	14 (0.99)	13 (1.00)
Specific comorbidities*, n (%)							
No disease	2525 (78.66)	1842 (78.75)	1319 (78.51)	1240 (77.89)	1161 (77.14)	1093 (77.03)	993 (76.03)
One disease	481 (14.98)	351 (15.01)	251 (14.94)	249 (15.64)	237 (15.75)	221 (15.57)	211 (16.16)
≥2 diseases	204 (6.36)	146 (6.24)	110 (6.55)	103 (6.47)	107 (7.11)	105 (7.40)	102 (7.81)

*Specific comorbidities included the following: hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease, and liver disease. Abbreviations: P=period. BMI =Body mass index.

Supplementary Table S5 – Distribution of demographic and comorbidity according to test period of neutralizing antibody

Variable	P0 (N=681)	P1 (N=622)	P2 (N=724)	P3 (N=559)	P4 (N=700)	P5 (N= 721)	P6 (N=520)
Number of days after the 2nd vaccine, mean (+/-std)	9.20 (+/-3.26)	28.20 (+/-3.26)	56.36 (+/-3.33)	83.87 (+/-2.72)	112.91 (+/-4.08)	140.82 (+/-4.32)	167 (+/-2.92)
Gender, n (%)							
Female	518 (76.06)	484 (77.81)	578 (79.83)	426 (76.21)	533 (76.47)	556 (77.12)	378 (73.11)
Male	163 (23.94)	138 (22.19)	146 (20.17)	133 (23.79)	164 (23.53)	165 (22.88)	139 (26.89)
Age, mean (+/-std)	54.06 (+/-15.61)	51.94 (+/-14.9)	51.07 (+/-13.34)	52.38 (+/-12.65)	51.86 (+/-12.76)	51.77 (+/-12.56)	52.19 (+/-12.25)
Age, n (%)							
18-44.99	222 (32.6)	222 (35.69)	255 (35.22)	163 (29.16)	216 (30.99)	223 (30.93)	154 (29.79)
45-64.99	192 (28.19)	219 (35.21)	320 (44.2)	276 (49.37)	349 (50.07)	363 (50.35)	270 (52.22)
65+	267 (39.21)	181 (29.1)	149 (20.58)	120 (21.47)	132 (18.94)	135 (18.72)	93 (17.99)
BMI, mean (+/-std)	25.65 (+/-4.74)	25.45 (+/-4.72)	27.18 (+/-5.47)	26.82 (+/-5.24)	26.6 (+/-5.24)	26.83 (+/-5.3)	26.77 (+/-5.09)
BMI, n (%)							
<25	299 (50.34)	303 (52.79)	283 (41.07)	236 (43.54)	299 (44.96)	298 (43.5)	223 (44.16)
25-29.99	198 (33.33)	188 (32.75)	178 (25.83)	144 (26.57)	184 (27.67)	184 (26.86)	134 (26.53)
30+	97 (16.33)	83 (14.46)	228 (33.09)	162 (29.89)	182 (27.37)	203 (29.64)	148 (29.31)
Sector, n (%)							
Physician	139 (20.41)	129 (20.74)	135 (18.65)	103 (18.43)	121 (17.36)	120 (16.64)	95 (18.38)
Nurse	121 (17.77)	134 (21.54)	155 (21.41)	131 (23.43)	158 (22.67)	161 (22.33)	123 (23.79)
Para	205 (30.1)	194 (31.19)	214 (29.56)	157 (28.09)	218 (31.28)	231 (32.04)	147 (28.43)
Administration	216 (31.72)	165 (26.53)	220 (30.39)	168 (30.05)	200 (28.69)	209 (28.99)	152 (29.4)
Comorbidity, n (%)							
Pregnancy	12 (1.76)	15 (2.41)	9 (1.24)	2 (0.36)	2 (0.29)	5 (0.69)	1 (0.19)
Allergy	20 (3.3)	33 (5.67)	29 (4.16)	9 (1.64)	16 (2.37)	22 (3.17)	8 (1.56)
Hypertension	108 (18.03)	96 (16.55)	120 (17.27)	97 (17.73)	122 (18.15)	129 (18.67)	97 (19.02)
Diabetes	47 (7.85)	38 (6.55)	58 (8.35)	49 (8.96)	55 (8.18)	61 (8.83)	46 (9.02)
Dyslipidemia	63 (10.52)	59 (10.17)	69 (9.93)	57 (10.42)	65 (9.67)	66 (9.55)	55 (10.78)
Heart disease	30 (5.01)	21 (3.62)	28 (4.03)	22 (4.02)	25 (3.72)	24 (3.47)	18 (3.53)
Lung disease	23 (3.84)	27 (4.66)	33 (4.75)	22 (4.02)	31 (4.61)	33 (4.78)	23 (4.51)
Kidney disease	6 (1)	4 (0.69)	2 (0.29)	2 (0.37)	4 (0.6)	3 (0.43)	2 (0.39)
Coagulation disease	13 (2.17)	16 (2.76)	15 (2.16)	12 (2.19)	15 (2.23)	15 (2.17)	10 (1.96)
Liver disease	5 (0.83)	4 (0.69)	10 (1.44)	7 (1.28)	6 (0.89)	8 (1.16)	8 (1.57)
Autoimmune disease	71 (11.85)	91 (15.69)	87 (12.52)	55 (10.05)	64 (9.52)	74 (10.71)	54 (10.59)
Immunosuppression	22 (3.67)	20 (3.45)	19 (2.73)	10 (1.83)	14 (2.08)	13 (1.88)	8 (1.57)
Specific comorbidities*, n (%)							
No disease	419 (69.95)	418 (72.07)	478 (68.78)	371 (67.82)	462 (68.75)	473 (68.45)	341 (66.86)
One disease	114 (19.03)	101 (17.41)	140 (20.14)	118 (21.57)	137 (20.39)	140 (20.26)	111 (21.76)
≥2 diseases	66 (11.02)	61 (10.52)	77 (11.08)	58 (10.6)	73 (10.86)	78 (11.29)	58 (11.37)

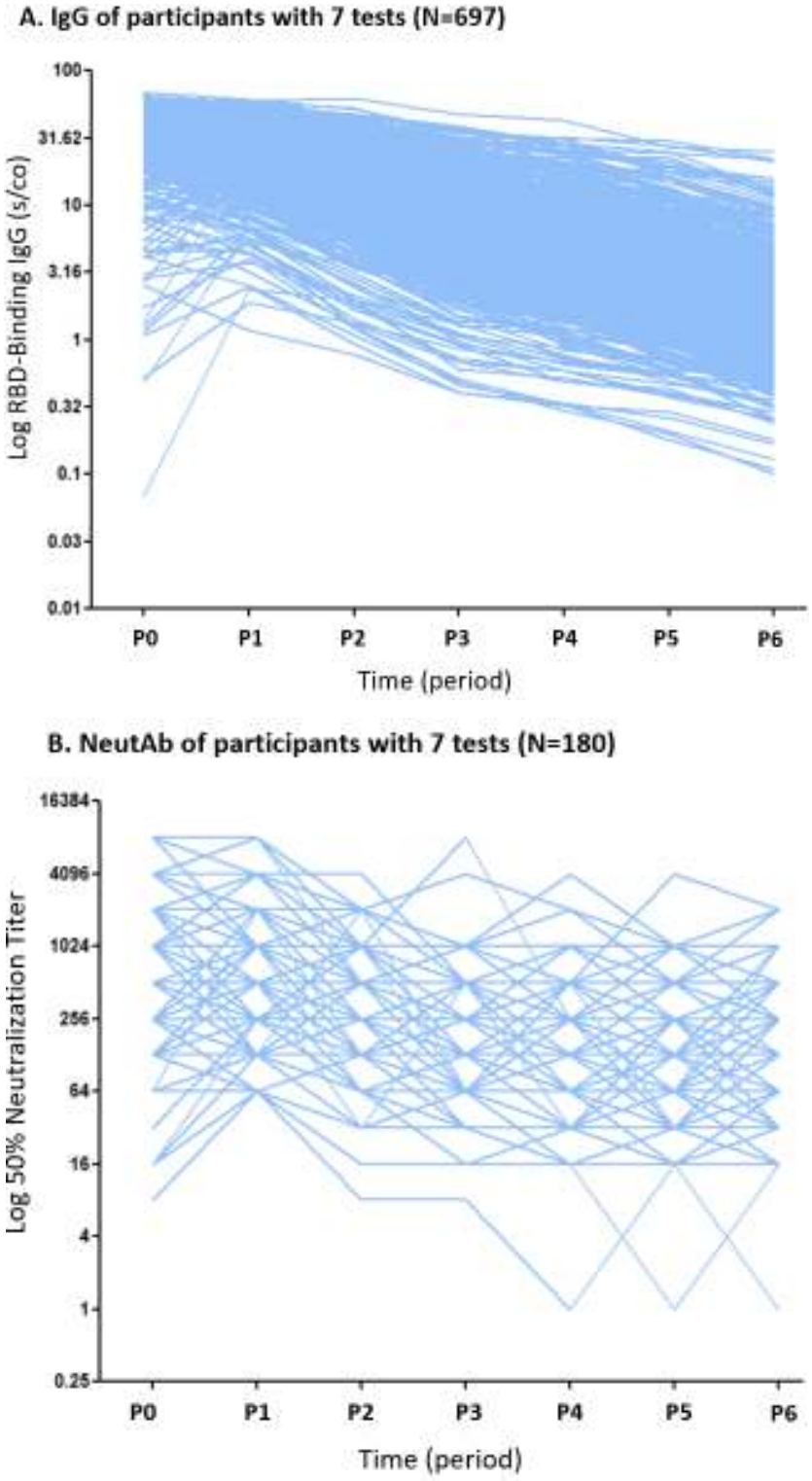
*Specific comorbidities included the following: hypertension, diabetes, dyslipidemia, heart disease, lung disease, kidney disease, and liver disease. Abbreviations: P=period. BMI =Body mass index.

Supplementary Table S6: Expected and observed GMTs of IgG and neutralizing antibody in each period

	Baseline: Days 4-17	P1: Days 18-42	P2: Days 43-70	P3: Days 71-98	P4: Days 99-126	P5: Days 127-154	P6: Days 155-175
N of IgG tests	3991	2690	1829	1732	1606	1518	1370
IgG, period observed GMT (95%CI), S/CO	32.9 (32.2-33.6)	25.5 (24.8-26.2)	15.1 (14.6-15.7)	7.6 (7.3-7.9)	4.8 (4.6-5.0)	2.9 (2.8-3.0)	1.8 (1.7-1.8)
IgG, mid-period expected GMT (95% CI), S/CO	29.3 (28.7-29.8)	29.3 (28.7-29.8)	16.9 (16.6-17.3)	9.4 (9.2-9.6)	5.2 (5.1-5.4)	2.9 (2.8-3.0)	1.6 (1.5-1.7)
N of Neutralizing antibody tests	681	622	724	559	697	721	517
Neutralizing antibody, period observed GMT (95%CI), 50% titer	685.5 (608.5-772.1)	497.5 (447.5-553.2)	254.4 (232.2-278.6)	141.0 (128.7-154.4)	138.7 (127.0-151.6)	115.0 (106.0-124.9)	113.3 (102.9-124.7)
Neutralizing antibody, mid-period expected GMT (95% CI), 50% titer	557.1 (510.8-607.7)	557.1 (510.8-607.7)	229.5 (214.7-245.4)	142.4 (132.2-153.4)	138.0 (128.7-148.0)	128.4 (120.6-136.7)	119.4 (112.0-127.3)

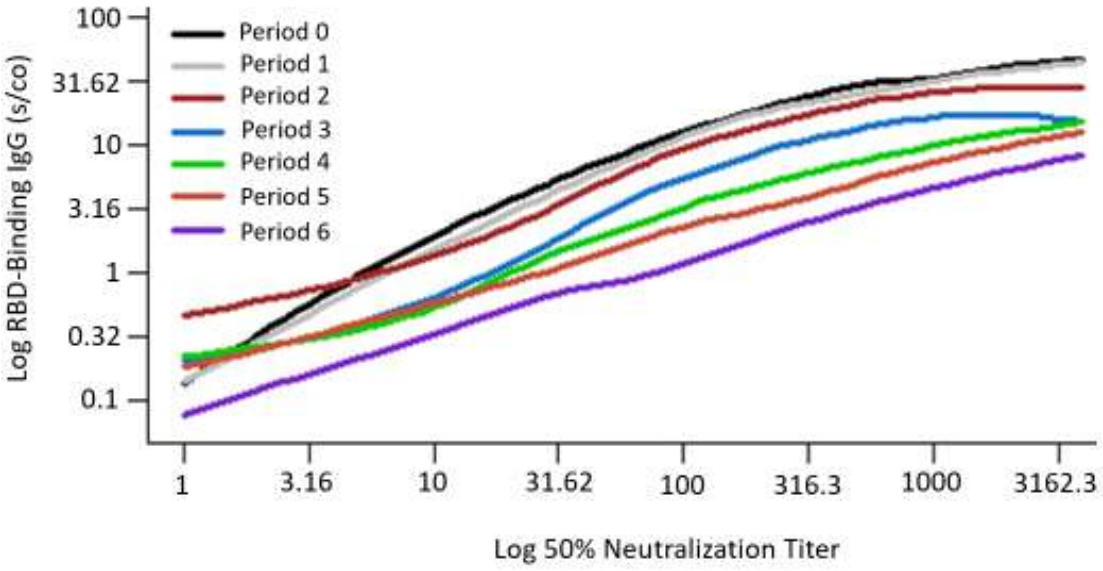
S/CO=sample-to-cutoff ratio. P=period.

Supplementary Figure S1: Monthly trajectory antibodies of participants with repeated tests



Legend: Each line represents the monthly trajectory of participants with repeated IgG or NeutAb tests. NeutAb=neutralizing antibody. RBD=receptor binding domain. s/co=sample-to-cutoff ratio. P=period

Supplementary Figure S2: Monthly correlation between IgG and neutralizing antibody



	N	Spearman's correlation coefficient	P-value
P0	676	0.749	<0.001
P1	621	0.727	<0.001
P2	723	0.720	<0.001
P3	558	0.749	<0.001
P4	698	0.693	<0.001
P5	720	0.684	<0.001
P6	520	0.691	<0.001

Legend: Each line was obtained by local polynomial regression and represents the association between IgG and NeutAb of each period. NeutAb=neutralizing antibody. RBD=receptor binding domain. s/co=sample-to-cutoff ratio. P=period.

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Supplementary References

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