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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: Ravussin A, Robertson AH, Wolf A-S, et al. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study. *Lancet Healthy Longev* 2023; **4:** e188–99.

(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

SUPPLEMENTARY MATERIAL

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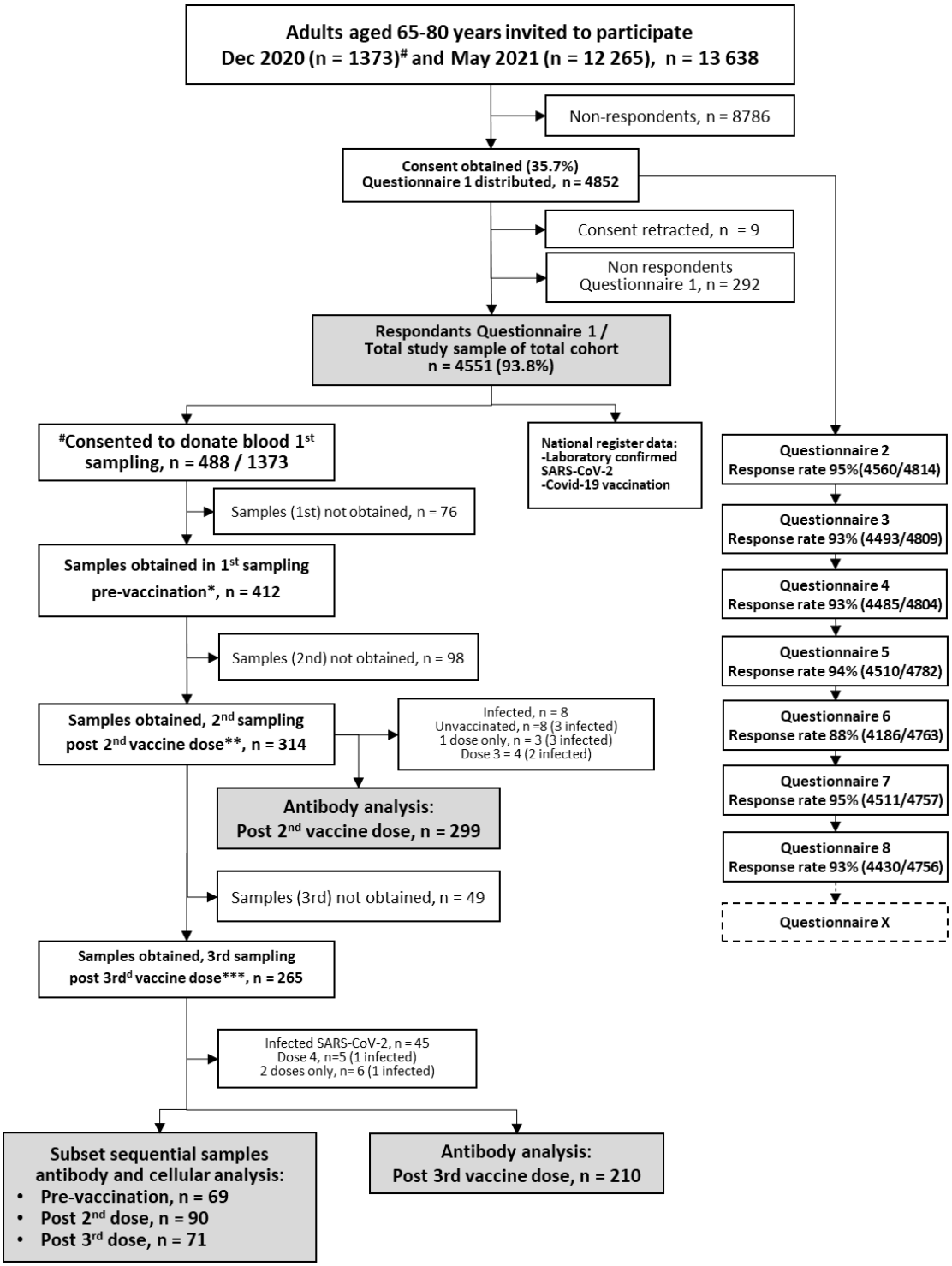
(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Methods. IFN-γ quantification

Supernatants were collected from additional T cell cultures after 72h for IFN-γ quantification; sandwich ELISAs were performed by coating plates with 1µg/ml IFN-γ capture antibody overnight and blocking for 1h before incubation for 2h with samples at 1:5 dilution. Standard curves were made with 2-fold serial dilutions of recombinant IFN-γ in duplicate starting at 500pg/ml. Plates were further incubated with 0.5µg/ml biotinylated mouse anti-human antibody for 1h and 1:5000 diluted streptavidin-horseradish peroxidase (HRP) for 30min. Binding was visualized with 3,3′,5,5′-tetramethylbenzidine (TMB). Optical density was measured at 405nm with a EL808 plate reader (Agilent).

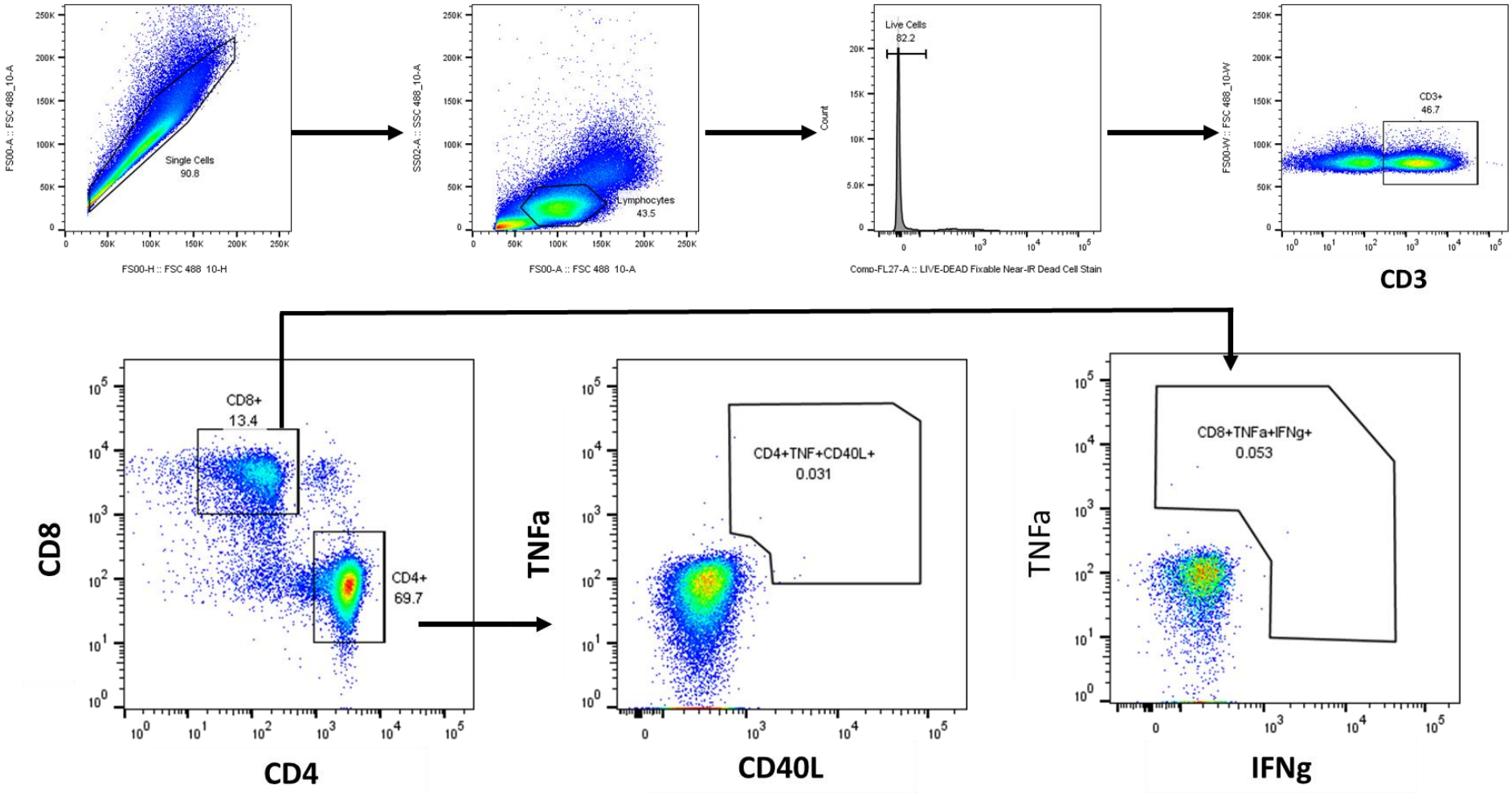
(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Figure 1. Flow chart of recruitment of participants and study samples in the Senior cohort. Individuals were recruited in two rounds in December 2020 and May 2021. All participants who consented to participation in December 2020 (488/1373) were immediately invited to donate blood samples (#). Time of sampling corresponding to the period prior to vaccination (*) of healthy older adults, relative to the national vaccination campaign, and with a second dose (**) and third dose (***) respectively. Note that the denominator of the response rates decreases slightly from questionnaire 2 through to 8 due to loss to follow-up.



(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

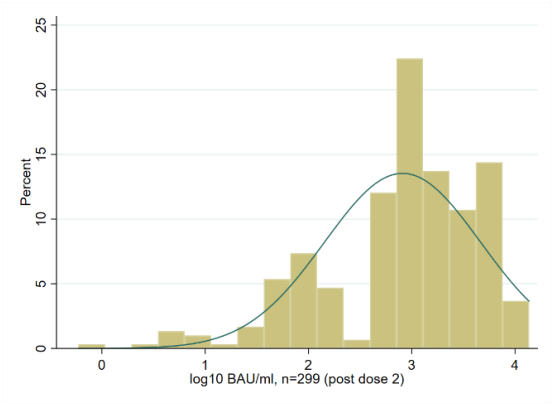
Supplementary Figure 2. Flow gating strategy for identification of activated CD4$^+$ and CD8$^+$ T cells.



(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Figure 3. Histograms of log-transformed immunological outcomes in the Senior cohort. A) IgG anti-RBD antibody levels after dose 2, log10 transformed BAU/ml (n=299), B) RBD antibody levels after dose 3, log10 transformed BAU/ml (n=210), C) Normalised log10 CD4% spike responses after dose 2 (n=90), D) Normalised log10 CD8% spike responses after dose 2 (n=90), D) and E) Normalised log10 CD4% spike responses after dose 3 (n=71), F) Normalised log10 CD8% spike responses after dose 3 (n=71)

A)



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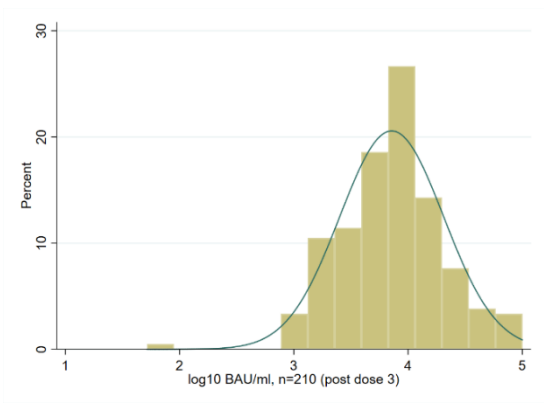

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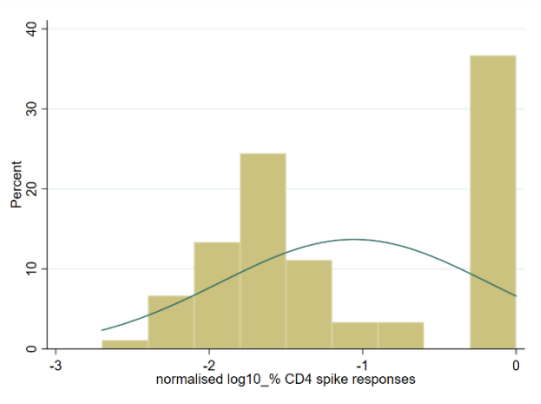

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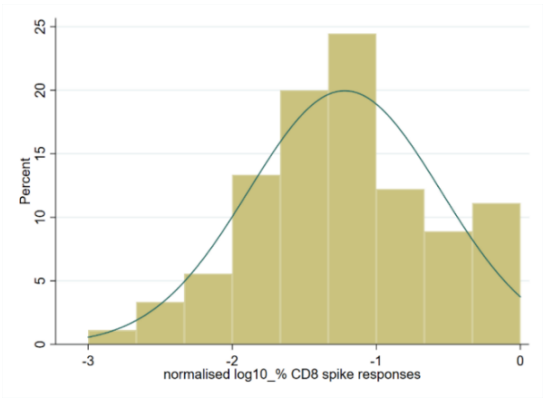
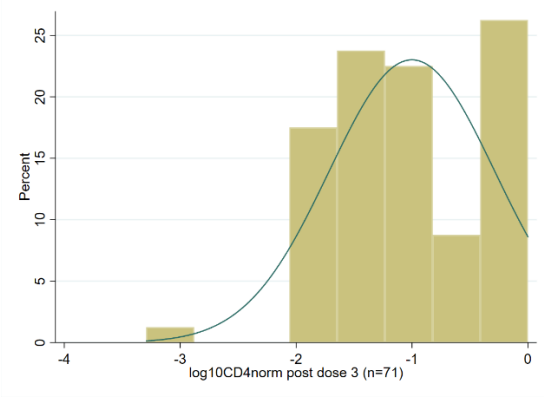
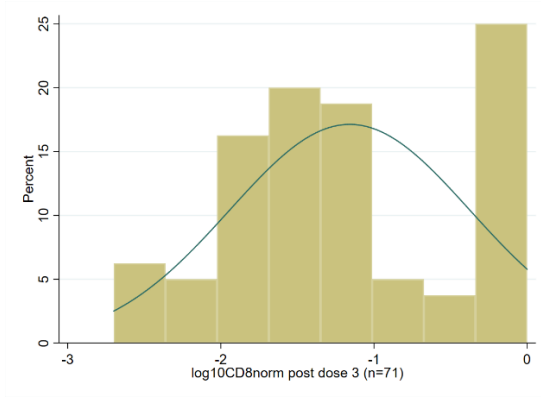


E)

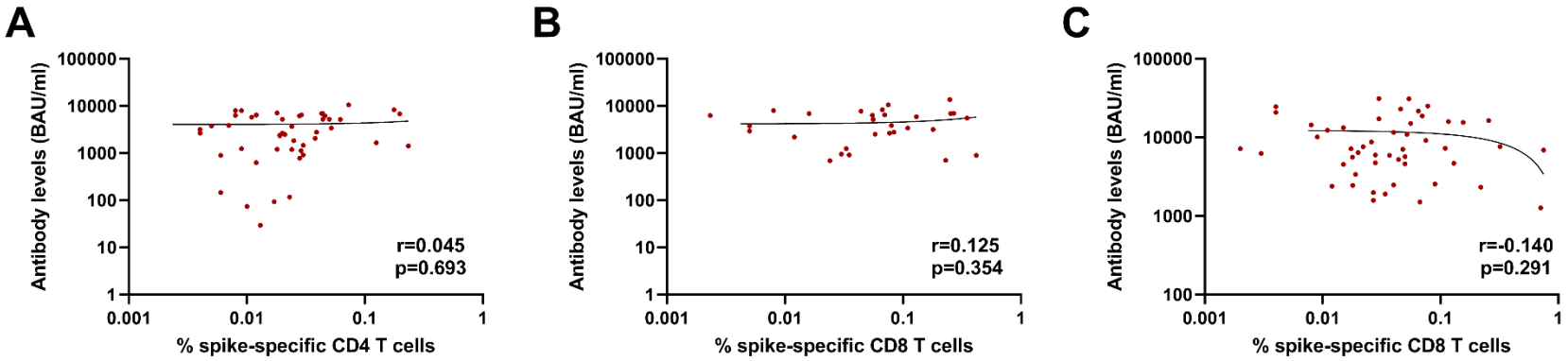


F)



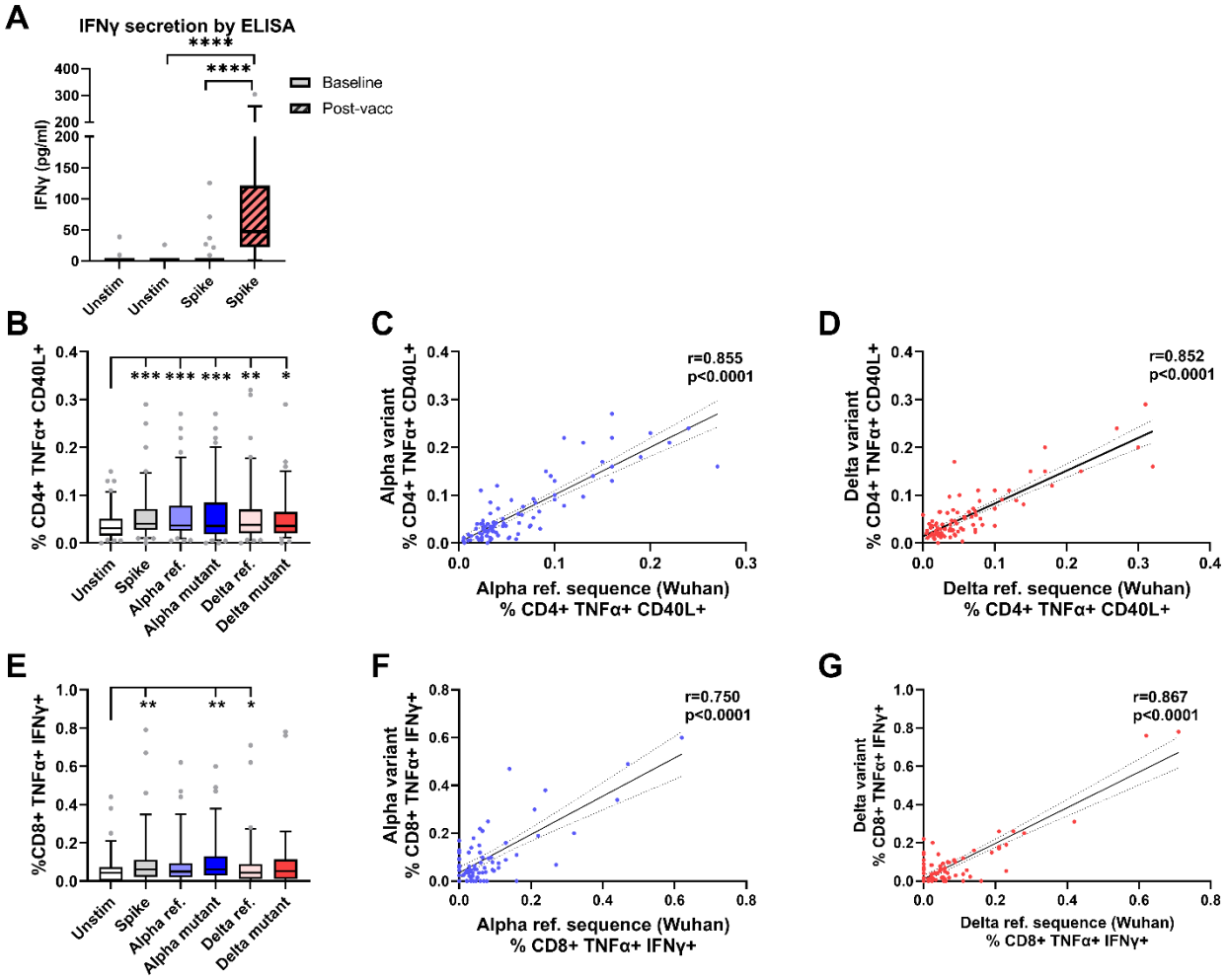
(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Figure 4. Correlation between antibody and CD4 and CD8 T cell responses after 2 or 3 vaccine doses. Antibody levels (BAU/ml) did not correlate with CD4+ (A) or CD8+ (B) T cell responses after 2 vaccine doses, or with CD8+ T cell responses after 3 vaccine doses (C). Correlations calculated by Pearson correlation coefficients, r and p values shown on plots (all non-significant).



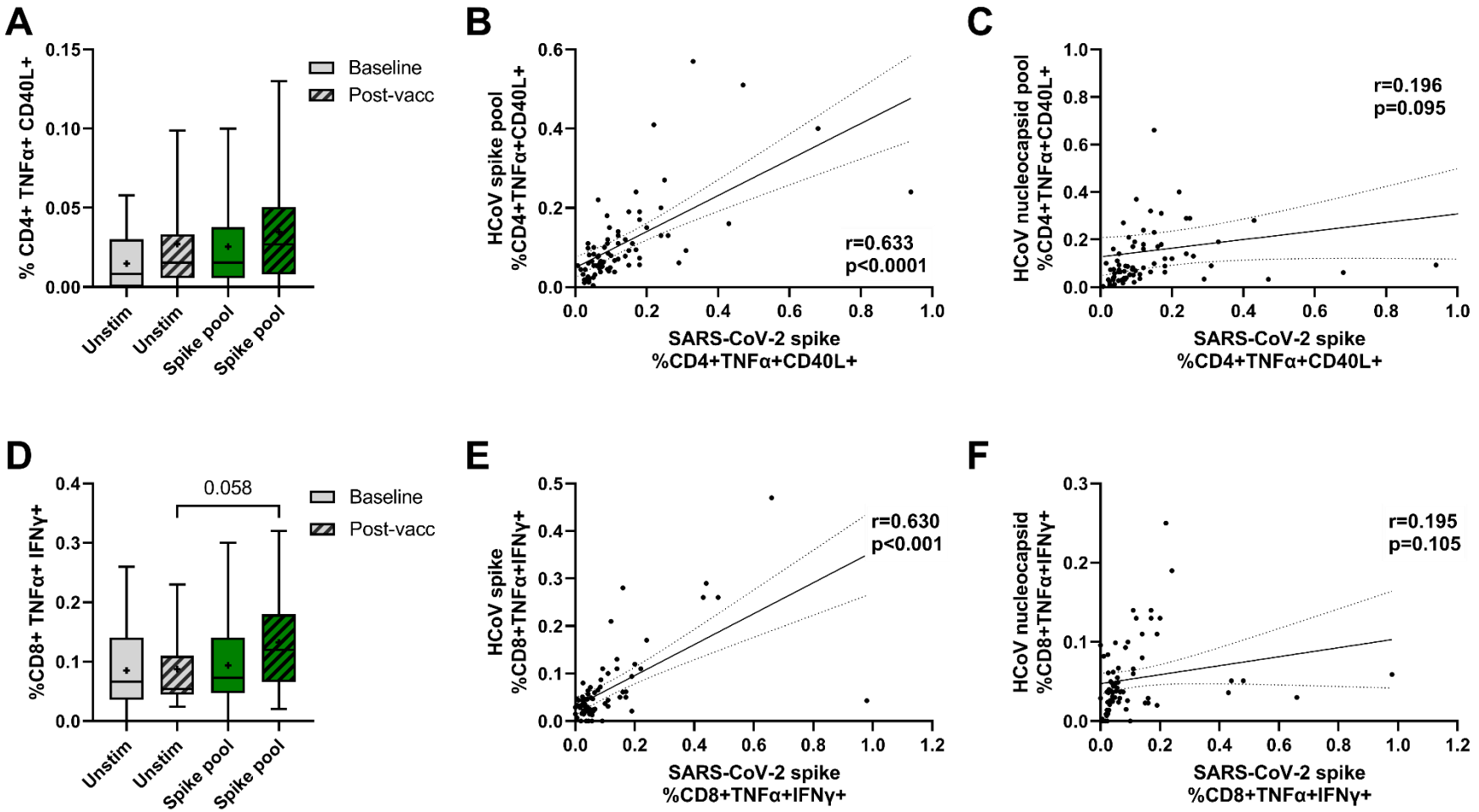
(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Figure 5. T cell responses after 2 vaccine doses. (A) IFN-γ levels in cell supernatants after 72hrs of PBMC stimulation with SARS-CoV-2 spike peptides before (empty) and after (striated) two vaccine doses. $CD4^+$ (B-D) and $CD8^+$ (E-G) T cell responses after 2 vaccine doses to the Alpha and Delta VOCs. All comparisons (B and E) are between the unstimulated control and the spike, VOC reference, and VOC variant peptides (results indicated by asterisks). T cell responses to the VOC mutated and reference sequences correlated strongly for both Alpha (C and F) and Delta (D and G) VOCs. Box-and-whisker plots show the median, IQR, and 5th and 95th percentiles; all individuals are plotted as dots. R and p values are indicated on each correlation plot. * p<0.05, ** p<0.01, *** p<0.001.



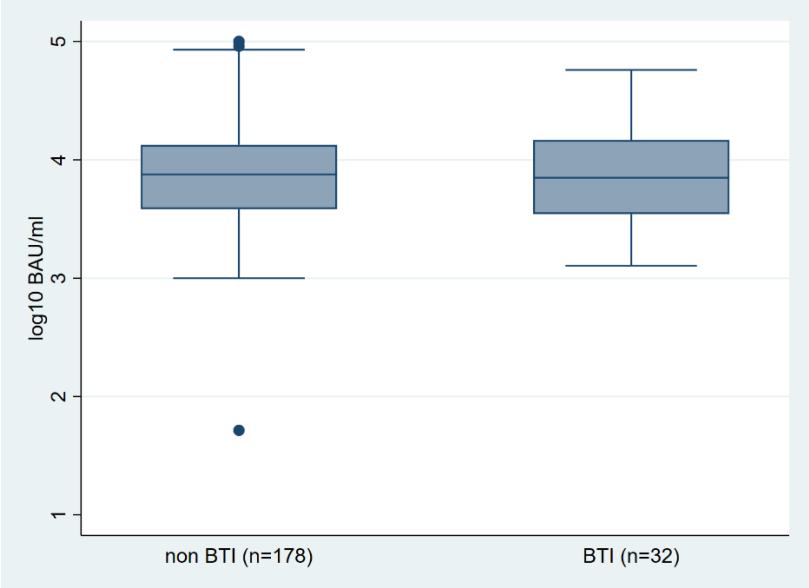
(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Figure 6: Cross reactive T cell responses to seasonal coronaviruses show correlation with SARS-CoV-2 spike responses and are enhanced after vaccination. (A) $CD4^+$ and (D) $CD8^+$ T cell responses to pooled HCoV spike peptides (green) before (empty boxes) and after (striated boxes) 2 vaccine doses. SARS-CoV-2 WT spike-specific responses correlate with HCoV spike-specific responses for both $CD4^+$ (B) and $CD8^+$ (E) T cells but not with HCoV nucleocapsid-specific responses (C and F).



(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Figure 7: Box plot of anti-RBD antibody levels according to breakthrough infections (BTIs) after the third dose.



(Ravussin, Robertson and Wolf et al 2023. Determinants of humoral and cellular immune responses to three doses of mRNA SARS-CoV-2 vaccines in older adults: a longitudinal cohort study).

Supplementary Table 1. Additional characteristics of Senior cohort participants. Characteristics are shown for the total cohort sample (N=4 551), and subsets for antibody (N=299) and cellular analysis (N =90) respectively

Characteristic	**Study sample N= 4551[a]**	**Antibody subset N=299 [b]**	**Cellular subset, N= 90 [c]**
Alcohol intake, amount (n=4275/ n=267/ n=83)[d]			
Per time at weekends, > 4 units, n (%)	847 (19·8)	59 (22·1)	18 (21·7)
Per time at weekends, ≤ 4 units, n (%)	3155 (73·8)	202 (75·7)	60 (72·3)
Missing, n(%)	273 (6·4)	12 (4·0)	5 (5·6)
Per time on weekdays, > 4 units, n (%)	90 (2·1)	7 (2·6)	0 (0)
Per time on weekdays, ≤ 4 units, n (%)	3912 (91·5)	256 (95·9)	77 (92·8)
	273 (6·4)	12 (4·0)	6 (7·2)
Alcohol intake, frequency			
> 3 times per week, n (%)	1022 (22·5)	73 (24·4)	15 (16·7)
2-3 times per week, n (%)	1463 (32·1)	95 (31·8)	33 (36·7)
< 2 times per week, n (%)	1548 (34·0)	99 (33·1)	30 (33·3)
Never, n (%)	276 (6·1)	20 (6·7)	7 (7·8)
Missing, n (%)	242 (5·3)	12 (4)	5 (5·6)
Smoking			
Daily smoker, n (%)	221 (4·9)	9 (3·0)	2 (2·2)
Occasional smoker, n (%)	135 (3·0)	7 (2·3)	2 (2·2)
Missing, n (%)	232 (5·1)	11 (3·7)	4 (4·4)
Frailty index			
1) (*Unintentional weight loss*) In the last year, have you lost more than 10 pounds unintentionally (i.e., not due to dieting or exercise)? yes=1, no=0			
Yes, n (%)	137 (3·0)	9 (3·0)	1 (1·1)
Missing, n (%)	68 (1·5)	4 (1·3)	2 (2·2)
2) (*Low activity*) We would like to know about the type and amount of physical activity you do in your daily life. How often do you engage in activities that require a low or moderate level of energy such as gardening, cleaning the car, or going for a walk?			
1-3 times a month/hardly ever/never = 1, n (%)	299 (6·6)	16 (5·4)	4 (4·4)
≥ once a week = 0, n (%)	4 232 (93·0)	283 (94·6)	86 (95·6)
Missing, n (%)	20 (0·4)	0 (0)	0 (0)
3) (*Exhaustion*) During the past month have you had too little energy to do things/activities you enjoy doing? yes=1, no=0			
Yes, n (%)	815 (17·9)	51 (17·1)	11 (12·2)
Missing, n (%)	26 (0·6)	0 (0)	0 (0)
Global Activity Limitation Index (GALI)			
Do you experience limitations in daily activities due to chronic illness, health condition or limited function?			
No, n (%)	3947 (86·7)	261 (87·3)	83 (92·2)
Yes, n (%)	584 (12·8)	34 (11·4)	6 (6·7)
Missing, n (%)	20 (0·4)	4 (1·3)	1 (1·1)
If yes, have these limitations lasted for 6 months of longer? (n = 584 / n=34 / n=6)			
No, n (%)	89 (15·2)	5 (14·7)	0 (0)

Yes, n (%)	489 (83·7)	29 (85·3)	6 (100·0)
Missing, n (%)	6 (1·0)	0 (0)	0 (0)
If yes, do you experience severe limitation or moderate limitation? *(n=489 / n=29 / n=6)*			
Severe limitation, n (%)	131 (26·8)	11 (37·9)	2 (33·3)
Moderate limitation, n (%)	352 (72·0)	18 (62·1)	4 (66·7)
Missing, n (%)	6 (1·2)	0 (0)	0 (0)
Index: Limitation min. 6 months, 3 categories			
No limitation, n (%)	4036 (88·7)	266 (89·0)	83 (92·2)
Moderate limitation, n (%)	352 (7·7)	18 (6·0)	4 (4·4)
Severe limitation, n (%)	131 (2·9)	11 (3·7)	2 (2·2)
Missing, n(%)	32 (0·7)	4 (1·3)	1 (1·1)
Physical Exercise (≥10 min. per day)			
≥2 times per week, n (%)	3793 (83·3)	256 (85·6)	76 (84·4)
<2 times per week, n (%)	526 (11·6)	31 (10·4)	10 (11·1)
In detail:			
How often do you exercise in your spare time? By exercise we mean for e.g. going for a walk, ski-ing, swimming, training, at least 10 minutes per day. Include any training to and from work. On average.			
Never, n (%)	125 (2·7)	2 (0·7)	2 (2·2)
Less than 1 time per week, n (%)	401 (8·8)	29 (9·7)	8 (8·9)
2-3 times per week, n (%)	1 599 (35·1)	103 (34·4)	33 (36·7)
4-5 times per week, n (%)	1 049 (23·0)	69 (23·1)	17 (18·9)
Every day, n (%)	1 145 (25·2)	84 (28·1)	26 (28·9)
Missing, n (%)	232 (5·1)	12 (4·0)	4 (4·4)
(*If more than "never", n =4194 / n=285 / n=84)* How hard do you exercise?			
Don't cause sweating or breathlessness, n (%)	2 069 (49·3)	140 (49·1)	30 (35·7)
Cause sweating and/or breathlessness, n (%)	2 013 (48·0)	139 (48·8)	54 (64·3)
Complete exhaustion, n (%)	90 (2·1)	4 (1·4)	0 (0)
Missing, n (%)	22 (0·5)		0 (0)
Living situation			
Living alone, n (%)	1 400 (30·8)	98 (32·8)	27 (30·0)
Missing, n (%)	229 (5·0)	11 (3·7)	2 (2·2)
Housing *(multiple options possible)*			
Apartment, n (%)	2 784 (61·2)	185 (61·9)	59 (65·6)
Detached house, n (%)	985 (21·6)	62 (20·7)	16 (17·8)
Terraced house, n (%)	479 (10·5)	29 (9·7)	9 (10·0)
Long-term care facility (LTCF), n (%)	6 (0·1)	0 (0)	0 (0)
Multi-generational home, n (%)	70 (1·5)	6 (2·0)	2 (2·2)
Other types of housing, n (%)	50 (1·1)	8 (2·7)	4 (4·4)
Missing, n (%)	229 (5·0)	12 (4·0)	2 (2·2)
Higher education, n (%)			
< High school, n (%)	275 (6·0)	23 (7·7)	6 (6·7)
High school, n (%)	639 (14·0)	47 (15·7)	18 (20·0)
College ≤4 years, n (%)	1409 (31·0)	93 (31·1)	26 (28·9)
>4 years college, n (%)	1458 (32·0)	87 (29·1)	24 (26·7)
Other, n (%)	233 (5·1)	19 (6·4)	5 (5·6)

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Missing, n (%)	537 (11·8)	30 (10·0)	11 (12·2)
Current work situation *(multiple options possible)*			
Retired, n (%)	3674 (80·7)	257 (86·0)	79 (87·8)
Still working/partially retired, n (%)	849 (18·7)	50 (16·7)	15 (16·7)
Fully retired, n (%)	3674 (80·7)	236 (78·9)	79 (87·8)
Rehabilitation/disabled/sick leave, n (%)	55 (1·2)	3 (1·0)	0 (0)
Employed in public sector, n (%)	234 (5·1)	13 (4·3)	5 (5·6)
Employed in private sector, n (%)	327 (7·2)	12 (4·0)	5 (5·6)
Self-employed, n (%)	263 (5·8)	23 (7·7)	5 (5·6)
Freelancer, n (%)	63 (1·4)	2 (0·7)	0 (0)
Voluntary work/charity, n (%)	75 (1·6)	9 (3·0)	3 (3·3)
Stay at home (unpaid), n (%)	16 (0·4)	1 (0·3)	0 (0)
Other unpaid work, n (%)	20 (0·4)	0 (0)	0 (0)
Missing, n (%)	266 (5·8)	10 (3·3	2 (2·2)
Chronic diseases/ conditions[e]			
1 disease/ condition, n (%)	1438 (31·6)	97 (32·4)	32 (35·6)
2 diseases/ conditions, n (%)	513 (11·3)	33 (11·0)	11 (12·2)
3 diseases/ conditions, n (%)	161 (3·5)	5 (1·7)	1 (1·1)
4 diseases/ conditions, n (%)	50 (1·1)	4 (1·3)	0 (0)
5 diseases/ conditions, n (%)	16 (0·4)	0 (0)	0 (0)
≥ 6 diseases/ conditions, n (%)	6 (0·1)	0 (0)	0 (0)
"Any other condition"[f]	1046 (23·0)	55 (18·4)	13 (14·4)
Missing, n (%)	31 (0·7)	12 (4·0)	1 (1·1)
At least 1 chronic disease – excluding previous cancer	1775 (39·0)	118 (39·5)	32 (35·6)
Missing, n (%)	108 (2·4)	6 (2·0)	1 (1·1)
Fracture in past 5 years[k]			
Yes, n (%)	493 (10·8)	29 (9·7)	6 (6·7)
Missing, n (%)	14 (0·3)	1 (0·3)	0 (0)

[a]percentages shown as proportion of N= 4551 unless otherwise stated
[b]percentages shown as proportion of N= 299 unless otherwise stated
[c]percentages shown as proportion of N= 90 unless otherwise stated
[d]n based on participants who answered they drink alcohol
[e]asthma, other chronic lung disease, diabetes, myocardial infarction, heart condition or cardiovascular disease, stroke (anytime), chronic liver disease, chronic kidney disease, neurological disease, cancer (anytime), immunosuppressed. Note that high blood pressure is not included as a chronic disease/condition
[f]open question in Q1 following the specified list of conditions [e]

(Ravussin, Robertson and Wolf et al).

Supplementary Table 2. Reagents for cell stimulation and flow cytometry analysis

Stimulation condition	**Product name**	**Catalogue number**	**Company**
Spike WT	PepTivator SARS-CoV-2 Prot_S	130-126-700	Miltenyi Biotec
Alpha VOC	PepTivator SARS-CoV-2 Prot_S B.1.1.7 Reference Pool	130-127-841	Miltenyi Biotec
	PepTivator SARS-CoV-2 Prot_S B.1.1.7 Mutation Pool	130-127-844	Miltenyi Biotec
Delta VOC	PepTivator SARS-CoV-2 Prot_S B.1.617.2 Reference Pool	130-128-761	Miltenyi Biotec
	PepTivator SARS-CoV-2 Prot_S B.1.617.2 Mutation Pool	130-128-763	Miltenyi Biotec
Omicron VOC	PepTivator SARS-CoV-2 Prot_S B.1.1.529/BA.1 Reference Pool	130-129-927	Miltenyi Biotec
	PepTivator SARS-CoV-2 Prot_S B.1.1.529/BA.1 Mutation Pool	130-129-928	Miltenyi Biotec
HCoV spike pool	PepMix HCoV-OC43 (Spike Glycoprotein)	PM-OC43-S-2	JPT Peptide Technologies
	PepMix HCoV-NL63 (Spike Glycoprotein)	PM-NL63-S-1	JPT Peptide Technologies
	PepMix HCoV-HKU1 (Spike Glycoprotein)	PM-HKU1-S-1	JPT Peptide Technologies
HCoV nucleocapsid pool	PepMix HCoV-OC43 (NCAP)	PM-OC43-NCAP-1	JPT Peptide Technologies
	PepMix HCoV-NL63 (NCAP)	PM-NL63-NCAP-1	JPT Peptide Technologies
	PepMix HCoV-HKU1 (NCAP)	PM-HKU1-NCAP-1	JPT Peptide Technologies
CMV	PepTivator CMV pp65	130-093-435	Miltenyi Biotec
CytoStim	CytoStim, human	130-092-172	Miltenyi Biotec
Flow cytometry staining			
Surface antibodies	CD3-Brilliant Violet 605	563219	Beckton Dickinson
	CD4-eFluor450	48-0047-42	ThermoFisher Scientific
	CD8-AlexaFluor 488	53-0086-42	ThermoFisher Scientific
	Fixable Live/Dead Near-IR	L34976A	Invitrogen
Intracellular antibodies	CD40L-Brilliant Violet 510	310830	BioLegend
	IFNg-Brilliant Violet 711	502540	BioLegend
	TNFa-Phycoerythrin (PE)	559321	Beckton Dickinson
	CD69-Allophycocyanin (APC)	555533	Beckton Dickinson
Sandwich ELISA	Mouse anti-human recombinant IFNg capture antibody	M700A	ThermoFisher Scientific
	Human IFNg Recombinant Protein	2983-19-65	ThermoFisher Scientific
	Biotinylated mouse anti-human IFNg	M701B	ThermoFisher Scientific
	Streptavidin horseradish peroxidase	7105-05	AH Diagnostics

(Ravussin, Robertson and Wolf et al).

Supplementary Table 3. **Median anti-RBD antibody levels (BAU/ml) post 2nd covid-19 vaccine dose in the Senior cohort (N=299), and median regression analysis to explore factors influencing antibody levels.** p-values <0·05 are shown in bold.

		Study sample N=299*		Anti-spike antibody levels	Univariate median regression		Multivariate median regression**	
EXPOSURE/ FACTOR	**Category**	**n**	**%**	**Median, BAU/ml (IQR)**	**Coefficient (95%CI) *****	**p-value**	**Coefficient(95%CI) *****	**p-value**
CATEGORICAL VARIABLES								
Age categories (years)	65-70	126	42 %	1662 (800, 4318)	**Ref**		Ref	
	71-75	109	36 %	765 (159, 2015)	**-939 (-1390, -487)**	**0·000**	-314 (-947, 319)	0·330
	76-80	94	31 %	757 (162, 1794)	**-929 (-1459, -400)**	**0·001**	-131 (-879, 617)	0·731
Sex	Female	152	51 %	1123 (654, 2973)	Ref		Ref	
	Male	147	49 %	908 (173, 2685)	-215 (-563, 132)	0·223	-168 (-711, 375)	0·543
Vaccine type, dose 2	BNT-162b2	212	71 %	813 (149, 1979)	**Ref**		**Ref**	
	mRNA-1273	87	29 %	2019 (866, 5752)	**1200 (670, 1729)**	**0·000**	**1494 (889, 2098)**	**0·000**
Chronic condition	No	148	50 %	1105 (650, 3860)	Ref		Ref	
	Yes	146	50 %	900 (200, 2400)	-208 (-642, 226)	0·347	-219 (-777, 338)	0·439
Asthma/ chronic lung condition	No	265	90 %	1064 (580, 2797)	Ref		Ref	
	Yes	31	10 %	819 (609, 2447)	-245 (-934, 445)	0·486	-219 (-1177, 740)	0·654
Chronic heart/ cardiovascular disease	No	266	89 %	980 (600, 2934)	Ref		Ref	
	Yes	32	11 %	1132 (369, 1949)	206 (-470, 882)	0·549	593 (-271, 1457)	0·178
Hypertension	No	193	65 %	1099 (597, 3012)	Ref		Ref	
	Yes	104	35 %	906 (606, 2241)	-191 (-647, 265)	0·411	-160 (-765, 445)	0·604
Diabetes	No	276	92 %	1015 (612, 2866)	Ref		Ref	
	Yes	23	8 %	775 (89, 1912)	-249 (-1060, 562)	0·546	360 (-633, 1353)	0·476
Cancer (anytime)	No	248	83 %	1083 (641, 2973)	Ref		Ref	
	Yes	50	17 %	703 (173, 2154)	-385 (-944, 174)	0·176	-33 (-728, 662)	0·926
Immunosuppressed	No	286	96 %	1043 (600, 2797)	Ref		Ref	
	Yes	11	4 %	881 (29, 2685)	-181 (-1318, 956)	0·754	-226 (-1651, 1199)	0·755
Obesity (≥ 30.0 kg/m^2)	No	255	89 %	984 (612, 2797)	Ref		Ref	
	Yes	33	11 %	1123 (196, 3500)	140 (-608, 887)	0·713	-125 (-968, 719)	0·771
Frailty Index	Not frail	230	78 %	1102 (612, 3178)	Ref		Ref	
	Prefrail/frail	64	22 %	860 (162, 2099)	-240 (-742, 262)	0·348	-195 (-809, 418)	0·532

(Ravussin, Robertson and Wolf et al).

Global Activity Limitation Index	Not limited Limited	266 29	90 % 10 %	1015 (597, 2934) 1075 (676, 2388)	Ref 51 (-645, 747)	 0·885	Ref -485 (-1340, 371)	 0·266
CONTINUOUS VARIABLES	**Unit**	**n**			**Coefficient**	**p-value**	**Coefficient**	**p-value**
Age	Per year	299		NA[#]	**Ref (per year)** **-68 (-126, -11)**	 **0·020**	Ref (per year) -17 (-92, 58)	 0·657
Time since vaccination	Per day	299		NA	**Ref (per day)** **-18 (-23, -13)**	 **0·000**	**Ref (per day)** **-21 (-26, -16)**	 **0·000**
BMI	Per kg/m^2	295		NA	Ref (per kg/m^2) 8 (-51, 66)	 0·793	Ref (per kg/m2) 17 (-54, 87)	 0·638

* for some factors data was missing for up to 11 participants. These were excluded in the analysis.
** adjusted for age (continuous variable), time since vaccination (continuous variable), sex and vaccine type (BNT-162b2 vs mRNA-1273), (unless the covariate was the exposure variable).
*** interpretation: the coefficient is the difference in the median BAU/ml titre between the groups being compared, or per unit (continuous variables).
#not applicable

(Ravussin, Robertson and Wolf et al).

Supplementary Table 4. Median CD4$^+$ spike responses (%) post 2nd covid-19 vaccine dose in the Senior cohort (N=90), and median regression analysis to explore factors that influence the response.

		Study sample* N=90	CD4spike, % cells	Univariate median regression		Multivariate median regression **	
EXPOSURE/ FACTOR	**Category**	**n (%)**	**Median % (IQR)**	**Coefficient (95%CI) *****	**p-value**	**Coefficient (95%CI) *****	**p-value**
CATEGORICAL VARIABLES							
Age categories (years)	65-70	44 (48·9%)	0·007 (0, 0·019)	Ref (per cat)		Ref	
	71-75	30 (33·3%)	0·019 (0, 0·03)	0·013 (0·001, 0·025)	0·04	0·015 (0·001, 0·029)	0·03
	76-80	16 (17·8%)	0·013 (0, 0·022)	0·003 (-0·012, 0·018)	0·70	0·009 (-0·007, 0·025)	0·27
Sex	Female	44 (48·9%)	0·006 (0, 0·028)	Ref		Ref	
	Male	46 (51·1%)	0·012 (0, 0·024)	0·006 (-0·005, 0·017)	0·30	0·006 (-0·005, 0·017)	0·27
Vaccine type, dose 2	BNT-162b2	59 (65·6%)	0·012 (0, 0·025)	Ref		Ref	
	mRNA-1273	31 (34·4%)	0·008 (0, 0·028)	-0·004 (-0·017, 0·009)	0·53	-0·003 (-0·014, 0·009)	0·67
Chronic condition	No	45 (50·6%)	0·009 (0, 0·024)	Ref		Ref	
	Yes	44 (49·4%)	0·011 (0, 0·017)	0·002 (-0·008, 0·012)	0·70	0·003 (-0·008, 0·013)	0·61
Asthma/chronic lung condition	No	79 (89·8%)	0·01 (0, 0·024)	Ref		Ref	
	Yes	9 (10·2%)	0·006 (0, 0·028)	-0·004 (-0·021, 0·013)	0·65	0·003 (-0·015, 0·021)	0·76
Chronic heart/ cardiovascular condition	No	81 (91%)	0·009 (0, 0·025)	Ref		Ref	
	Yes	8 (9%)	0·022 (0·01, 0·034)	0·015 (-0·004, 0·034)	0·12	0·008 (-0·011, 0·027)	0·41
Hypertension	No	58 (64·4%)	0·01 (0, 0·028)	Ref		Ref	
	Yes	32 (35·6%)	0·012 (0, 0·026)	0·002 (-0·010, 0·014)	0·74	-0·001 (-0·012, 0·009)	0·81
Diabetes	No	86 (95·6%)	0·011 (0, 0·028)	Ref		Ref	
	Yes	4 (4·4%)	0·003 (0·001, 0·012)	-0·007 (-0·034, 0·020)	0·61	-0·004 (-0·031, 0·024)	0·80
Cancer (anytime)	No	72 (80·9%)	0·009 (0, 0·024)	Ref		Ref	
	Yes	17 (19·1%)	0·012 (0, 0·043)	0·003 (-0·012, 0·018)	0·69	0·001 (-0·014, 0·017)	0·88
Immunosuppression	No	86 (95·6%)	0·011 (0, 0·028)	Ref		Ref	
	Yes	4 (4·4%)	0·007 (0, 0·017)	0·002 (-0·026, 0·030)	0·89	0·009 (-0·020, 0·038)	0·54
Obesity (≥ 30.0 kg/m^2)	No	75 (86·2%)	0·01 (0, 0·026)	Ref		Ref	
	Yes	12 (13·8%)	0·011 (0, 0·027)	0·002 (-0·013, 0·017)	0·79	-0·001 (-0·018, 0·016)	0·88

(Ravussin, Robertson and Wolf et al).

Frailty Index	Not frail Prefrail/frail	73 (83%) 15 (17%)	0·008 (0, 0·028) 0·011 (0, 0·024)	Ref 0·003 (-0·012, 0·018)	 0·69	Ref 0·004 (-0·009, 0·018)	 0·53
Global Activity Limitation Index	Not limited Limited	83 (93·3%) 6 (6·7%)	0·011 (0, 0·028) 0·003 (0, 0·026)	Ref -0·007 (-0·030, 0·016)	 0·55	Ref -0·003 (-0·022, 0·017)	 0·80
CONTINUOUS VARIABLES	**Unit**	**n**		**Coefficient**	**p-value**	**Coefficient**	**p-value**
Age	Per year	90		Ref 0·001 (-0·000, 0·003)	 0·09	Ref 0·001 (-0·001, 0·002)	 0·50
Time since vaccination	Per day	90		Ref 0 ·000 (-0·000, 0·000)	 0·56	Ref 0 ·000 (-0·000, 0·000)	 0·60
BMI	Per kg/m^2	87		Ref 0.000 (-0.002, 0.001)	 0.82	Ref 0 .000 (-0.002, 0.001)	 0.76

*for some factors, data was missing for 1, 2 or 3 participants. These were excluded in the analysis.

** adjusted for age (continuous variable), sex and vaccine type (BNT-162b2 vs mRNA-1273), unless the covariate was the exposure variable.

***interpretation: the coefficient is the difference in the median % response between the groups being compared, or per unit (continuous variables).

(Ravussin, Robertson and Wolf et al).

Supplementary Table 5. Median CD8$^+$ spike responses (%) post 2nd covid-19 vaccine dose in the Senior cohort (N=90), and median regression analysis to explore factors that influence the response.

		Study sample* n=90	CD8spike, % cells	Univariate median regression		Multivariate median regression**	
EXPOSURE/ FACTOR	**Category**	**n (%)**	**Median % (IQR)**	**Coefficient (95%CI) *****	**p-value**	**Coefficient (95%CI) *****	**p-value**
CATEGORICAL VARIABLES							
Age categories (years)	65-70	44 (48·9%)	0·043 (0·009, 0·093)	Ref		Ref	
	71-75	30 (33·3%)	0·048 (0·023, 0·084)	0·005 (-0·029, 0·039)	0·77	0·007 (-0·029, 0·043)	0·70
	76-80	16 (17·8%)	0·025 (0·007, 0·119)	-0·019 (-0·061, 0·023)	0·38	-0·016 (-0·058, 0·026)	0·45
Sex	Female	44 (48·9%)	0·039 (0·018, 0·093)	Ref		Ref	
	Male	46 (51·1%)	0·043 (0·008, 0·089)	0·000 (-0·030, 0·030)	1·00	0·000 (-0·030, 0·031)	0·99
Vaccine type, dose 2	BNT-162b2	59 (65·6%)	0·042 (0·016, 0·090)	Ref		Ref	
	mRNA-1273	31 (34·4%)	0·044 (0·010, 0·080)	0·002 (-0·032, 0·036)	0·91	0·000 (-0·030, 0·031)	0·99
Chronic condition	No	45 (50·6%)	0·044 (0·015, 0·084)	Ref		Ref	
	Yes	44 (49·4%)	0·05 (0·014, 0·093)	0·013 (-0·020, 0·046)	0·44	0·011 (-0·021, 0·043)	0·49
Asthma/chronic lung condition	No	79 (89·8%)	0·042 (0·014, 0·089)	Ref		Ref	
	Yes	9 (10·2%)	0·077 (0·020, 0·119)	0·035 (-0·018, 0·088)	0·20	0·044 (-0·011, 0·099)	0·11
Chronic heart /cardiovascular condition	No	81 (91%)	0·044 (0·014, 0·089)	Ref		Ref	
	Yes	8 (9%)	0·021 (0·010, 0·159)	-0·023 (-0·076, 0·030)	0·39	-0·019 (-0·072, 0·035)	0·49
Hypertension	No	58 (64·4%)	0·038 (0·010, 0·077)	Ref		Ref	
	Yes	32 (35·6%)	0·062 (0·021, 0·145)	0·027 (-0·010, 0·063)	0·14	0·024 (-0·012, 0·060)	0·19
Diabetes	No	86 (95·6%)	0·043 (0·014, 0·089)	Ref		Ref	
	Yes	4 (4·4%)	0·05 (0·034, 0·119)	0·014 (-0·060, 0·088)	0·71	0·011 (-0·062, 0·085)	0·76
Cancer (anytime)	No	72 (80·9%)	0·043 (0·013, 0·090)	Ref		Ref	
	Yes	17 (19·1%)	0·062 (0·020, 0·089)	0·018 (-0·022, 0·058)	0·38	0·025 (-0·013, 0·062)	0·20
Immunosuppression	No	86 (95·6%)	0·043 (0·014, 0·089)	Ref		Ref	
	Yes	4 (4·4%)	0·049 (0·001, 0·108)	0·052 (-0·020, 0·124)	0·16	0·059 (-0·016, 0·133)	0·12
Obesity (≥ 30.0 kg/m^2)	No	75 (86·2%)	0·046 (0·014, 0·096)	Ref		Ref	
	Yes	12 (13·8%)	0·034 (0·014, 0·064)	-0·011 (-0·059, 0·037)	0·65	-0·014 (-0·059, 0·031)	0·54

(Ravussin, Robertson and Wolf et al).

Frailty Index	Not frail	73 (83%)	0·044 (0·014, 0·090)	Ref		Ref	
	Prefrail/frail	15 (17%)	0·026 (0·006, 0·077)	-0·018 (-0·062, 0·026)	0·42	-0·013 (-0·052, 0·027)	0·53
Global Activity Limitation Index	Not limited	83 (93·3%)	0·044 (0·014, 0·089)	Ref		Ref	
	Limited	6 (6·7%)	0·038 (0·020, 0·090)	0·005 (-0·057, 0·067)	0·87	-0·005 (-0·065, 0·056)	0·88
CONTINUOUS VARIABLES	**Unit**	**n**		**Coefficient (95CI%)**	**p-value**	**Coefficient (95CI%)**	**p-value**
Age	Per year	90		Ref		Ref	
				0·000 (-0·004, 0·004)	0·90	0·001 (-0·003, 0·005)	0·70
Time since vaccination	Per day	90		Ref		Ref	
				0·000 (-0·001, 0·001)	0·83	0·000 (-0·001, 0·001)	0·53
BMI	Per kg/m^2	87		Ref		Ref	
				-0·001 (-0·005, 0·003)	0·53	-0·001 (-0·005, 0·002)	0·48

*for some factors, data was missing for 1, 2 or 3 participants. These were excluded in the analysis.

** adjusted for age (continuous variable), sex and vaccine type (BNT-162b2 vs mRNA-1273), unless the covariate was the exposure variable.

***interpretation: the coefficient is the difference in the median % response between the groups being compared, or per unit (continuous variables).

(Ravussin, Robertson and Wolf et al).

Supplementary Table 6. Median CD8$^+$ spike responses (%) post 3rd covid-19 vaccine dose in the Senior cohort (N=71), and median regression analysis to explore factors that influence the response.

		Study sample n=71*	CD8spike % cells	Univariate median regression		Multivariate median regression**	
EXPOSURE/ FACTOR	**Category**	**n (%)**	**Median % (IQR)**	**Coefficient (95CI%) *****	**p-value**	**Coefficient (95CI%) *****	**p-value**
CATEGORICAL VARIABLES							
Age categories (years)	65-70	36 (50·7%)	0·027 (0·004, 0·066)	Ref		Ref	
	71-75	23 (32·4%)	0·022 (0·000, 0·040)	-0·005 (-0·035, 0·026)	0·75	0·002 (-0·029, 0·032)	0·92
	76-80	12 (16·9%)	0·04 (0·009, 0·100)	0·019 (-0·019, 0·057)	0·32	0·021 (-0·016, 0·059)	0·26
Sex	Female	34 (47·9%)	0·017 (0·002, 0·054)	Ref		Ref	
	Male	37 (52·1%)	0·03 (0·010, 0·050)	0·013 (-0·010, 0·035)	0·26	0·012 (-0·008, 0·033)	0·23
Vaccine type, dose 3	BNT-162b2	52 (73·2%)	0·027 (0·003, 0·054)	Ref		Ref****	
	mRNA-1273	19 (26·8%)	0·031 (0·017, 0·054)	0·004 (-0·021, 0·029)	0·75	0·006 (-0·016, 0·029)	0·56
Vaccine type, combinations	BNT-162b2 x3	43 (60·6%)	0·026 (0·002, 0·052)	Ref		Ref	
	mRNA-1273 x3	12 (16·9%)	0·026 (0·018, 0·049)	0·005 (-0·028, 0·038)	0·76	0·005 (-0·025, 0·036)	0·74
	BNT-162b2 x2 +mRNA-1273	7 (9·9%)	0·040 (0·000, 0·067)	0·014 (-0·027, 0·055)	0·50	0·011 (-0·024, 0·045)	0·54
	mRNA-1273x2 +BNT-162b2	9 (12·7%)	0·03 (0·004, 0·065)	0·004 (-0·033, 0·041)	0·83	0·001 (-0·030, 0·032)	0·97
Chronic condition	No	40 (56·3%)	0·017 (0·003, 0·046)	Ref		Ref	
	Yes	31 (43·7%)	0·037 (0·006, 0·090)	0·02 (-0·005, 0·042)	0·11	0·009 (-0·012, 0·030)	0·41
Asthma/ chronic lung condition	No	64 (91·4%)	0·025 (0·004, 0·051)	Ref		Ref	
	Yes	6 (8·6%)	0·037 (0·026, 0·220)	0·01 (-0·043, 0·063)	0·71	0·019 (-0·032, 0·071)	0·46
Chronic heart/ cardiovascular condition	No	64 (91·4%)	0·024 (0·004, 0·052)	Ref		Ref	
	Yes	6 (8·6%)	0·049 (0·030, 0·710)	0·026 (-0·065, 0·117)	0·57	0·011 (-0·079, 0·100)	0·81
Hypertension	No	43 (60·6%)	0·019 (0·004, 0·070)	Ref		Ref	
	Yes	28 (39·4%)	0·029 (0·005, 0·045)	0·011 (-0·016, 0·038)	0·43	0·002 (-0·022, 0·025)	0·88
Diabetes	No	68 (95·8%)	0·028 (0·004, 0·053)	Ref		Ref	
	Yes	3 (4·2%)	0·027 (0·006, 0·257)	-0·001 (-0·070, 0·068)	0·98	0·008 (-0·055, 0·070)	0·81
Cancer (anytime)	No	57 (80·3%)	0·027 (0·006, 0·050)	Ref		Ref	
	Yes	14 (19·7%)	0·028 (0·000, 0·067)	0·003 (-0·027, 0·033)	0·84	0·004 (-0·025, 0·032)	0·80

(Ravussin, Robertson and Wolf et al).

Immunosuppression	No Yes	69 (97·2%) 2 (2·8%)	0·027 (0·004, 0·054) 0·02 (0·000, 0·040)	Ref 0·013 (-0·057, 0·083)	0·71	Ref 0·005 (-0·064, 0·074)	0·88
Obesity (≥ 30.0 kg/m^2)	No Yes	61 (87·1%) 9 (12·9%)	0·028 (0·004, 0·054) 0·026 (0·003, 0·037)	Ref -0·002 (-0·034, 0·030)	0·90	Ref -0·001 (-0·033, 0·031)	0·97
Frailty Index	Not frail Prefrail/frail	57 (82·6%) 12 (17·4%)	0·026 (0·004, 0·052) 0·028 (0·005, 0·046)	Ref 0·002 (-0·028, 0·032)	0·90	Ref 0·000 (-0·027, 0·027)	0·98
Global Activity Limitation Index	Not limited Limited	65 (92·9%) 5 (7·1%)	0·028 (0·004, 0·054) 0·006 (0·000, 0·027)	Ref -0·022 (-0·064, 0·020)	0·30	Ref -0·007 (-0·050, 0·037)	0·76
CONTINUOUS VARIABLES	**Unit**	**n**		**Coefficient (95CI%)**	**p-value**	**Coefficient (95CI%)**	**p-value**
Age	Per year	71		Ref 0·002 (-0·001, 0·005)	0·20	Ref 0·002 (-0·001, 0·005)	0·24
Time since vaccination	Per day	71		Ref 0·000 (-0·001, 0·000)	0·59	Ref 0·000 (-0·001, 0·000)	0·37
BMI	Per kg/m^2	70		Ref 0·000 (-0·003, 0·003)	1·00	Ref 0·000 (-0·003, 0·003)	0·97

* for some factors, data was missing for up to 2 participants.

** adjusted for age (continuous variable), time since vaccination (continuous variable), sex and vaccine combination of the three doses (unless the covariate was the exposure variable).

*** interpretation: the coefficient is the difference in the median % response between the groups being compared, or per unit (continuous variables).

**** adjusted for age (continuous variable), time since vaccination (continuous variable) and sex.

(Ravussin, Robertson and Wolf et al).

Supplementary Data on validity and completeness of questionnaire data on vaccination and infection

The proportion of participants with self-reported primary COVID-19 vaccination also registered in the national vaccination registry, SYSVAK, was high (validity of 99·8%). Most participants registered in SYSVAK with two doses, had self-reported vaccination (completeness, 99·7%). There were 79/4551 cases of SARS-CoV-2 infection registered in the national infection registry, MSIS, by the end of June 2021 (when PCR testing and reporting was still mandatory). All self-reported cases (n=75) were also registered in MSIS.