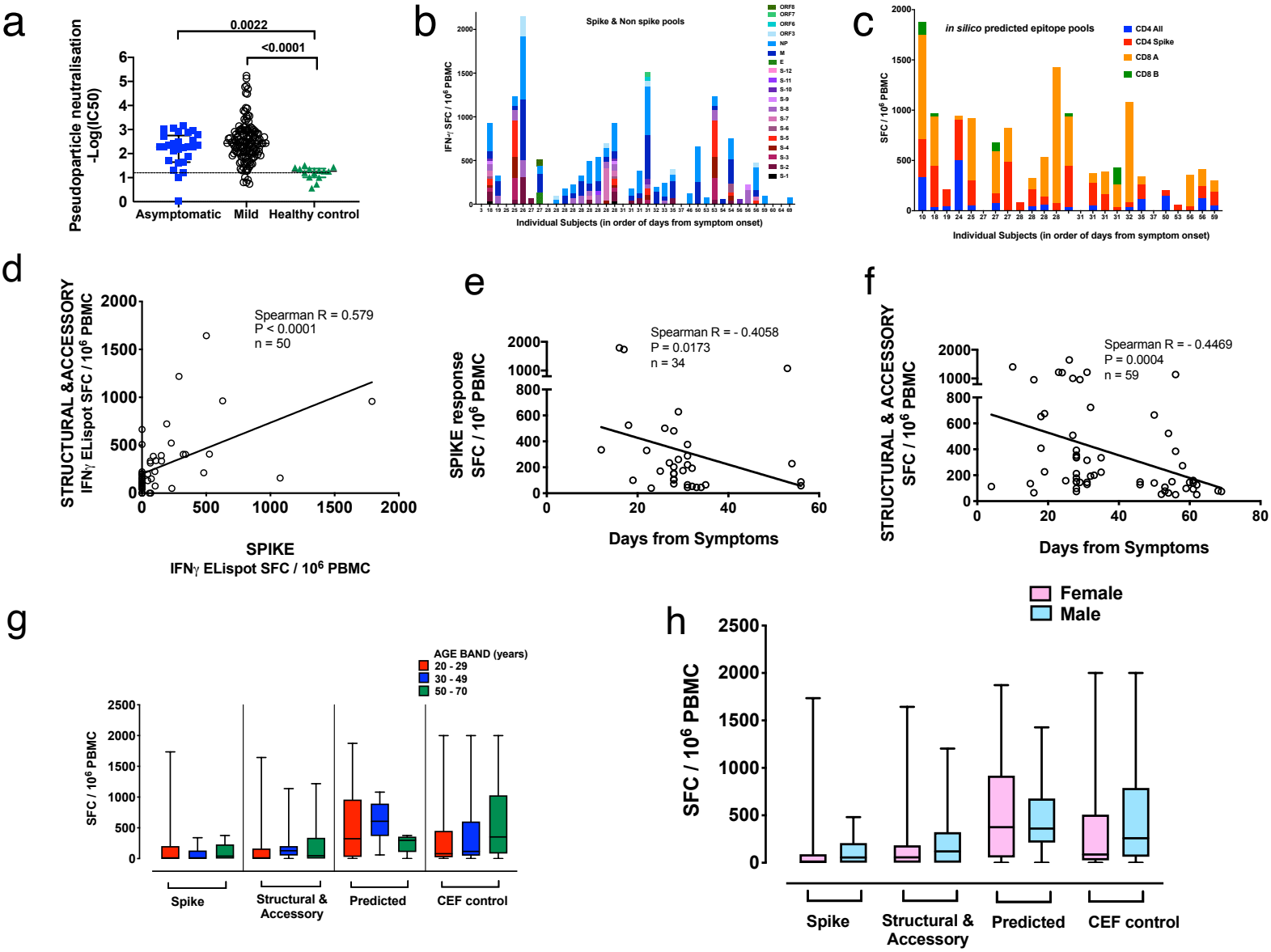
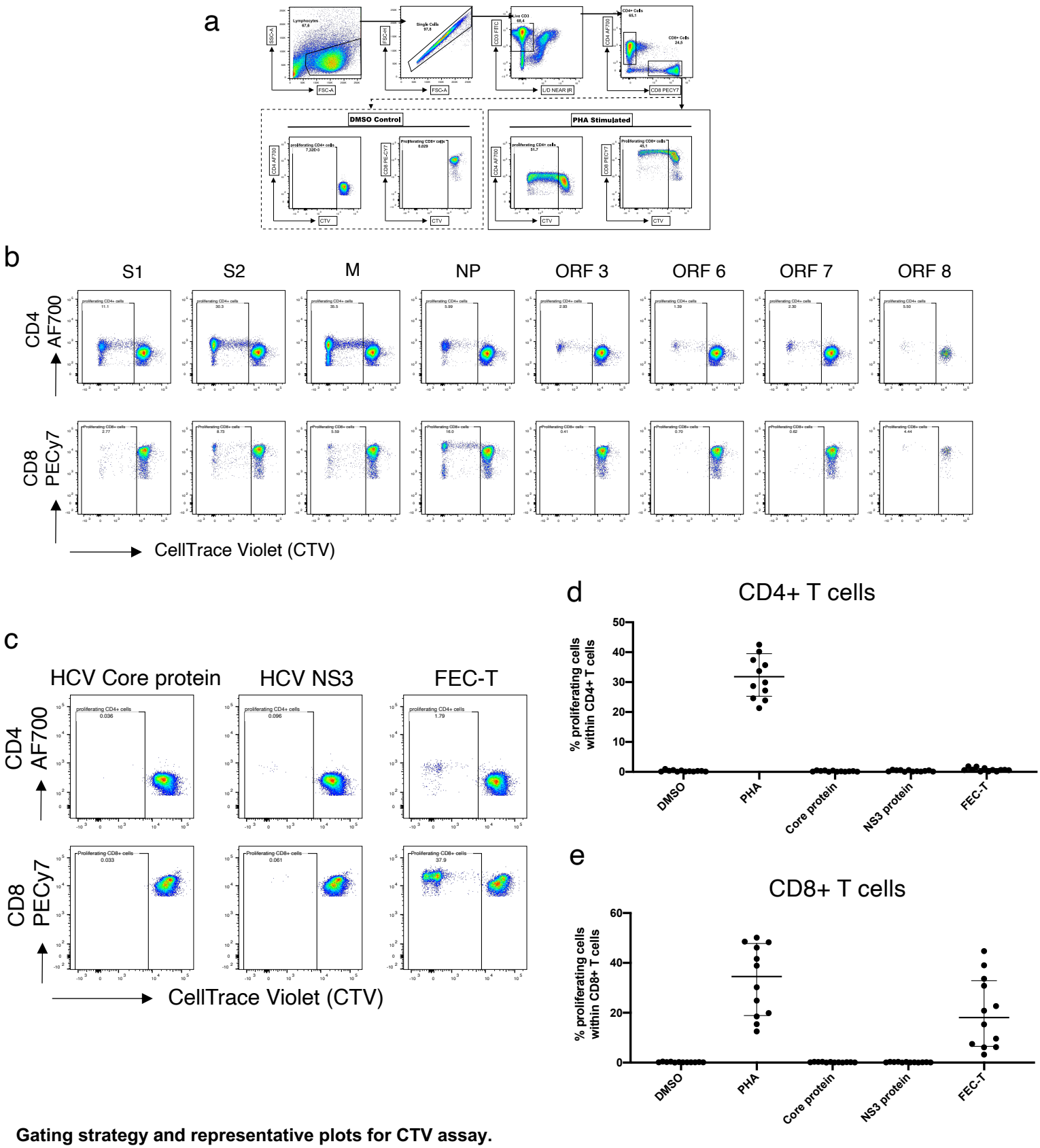
Supplementary figure 1



Detailed SARS-CoV-2 specific Immune Response.

a) Neutralising antibody responses in a pseudoparticle assay in 30 asymptomatic and 146 mildly symptomatic healthcare workers (HCWs) with PCR-confirmed SARS-CoV-2 infection, and 14 seronegative controls. *Ex vivo* IFN-γ ELISpot showing the magnitude and breadth of effector T cell responses to b) SARS-CoV-2 spike peptide pools and M, NP, and accessory proteins ORF 3, ORF6, ORF7 and ORF8 (n=38) and c) *in silico* predicted pools[10] in individual HCWs convalescent with mildly symptomatic SARS-CoV-2 infection (n=25). X axis shows number of days from onset of symptoms (not to scale), with blank columns representing zero response in the individual tested at that time-point. d) Correlation between summed *ex vivo* IFN-γ ELISpot responses to spike protein and to structural and accessory proteins (n=50). Correlation between days from symptom onset and summed *ex vivo* IFN-γ ELISpot responses to e) spike protein (n=34) and f) structural and accessory proteins (n=59). *Ex vivo* IFN-γ ELISpot responses to summed SARS-CoV-2 peptide pools spanning spike, accessory and structural proteins (E, M, NP, ORF 3, ORF6, ORF7 and ORF8), *in silico* predicted pools[10] and the CEF T cell control panel by g) age band in years (20-29 years n=41, 30-49 years n=43, 50-70years n=27), and by h) sex (female n=74, male n=37). SFC/10^6 PBMC = spot forming cells per million peripheral blood mononuclear cells, with background subtracted. Correlation was performed via Spearman's rank correlation coefficient and comparison of three groups for age by Kruskal-Wallis one-way ANOVA and two groups for sex by Mann-Whitney U test. ns = not significant, * = <0.05, ** = <0.01, *** = < 0.001 and **** = <0.0001.

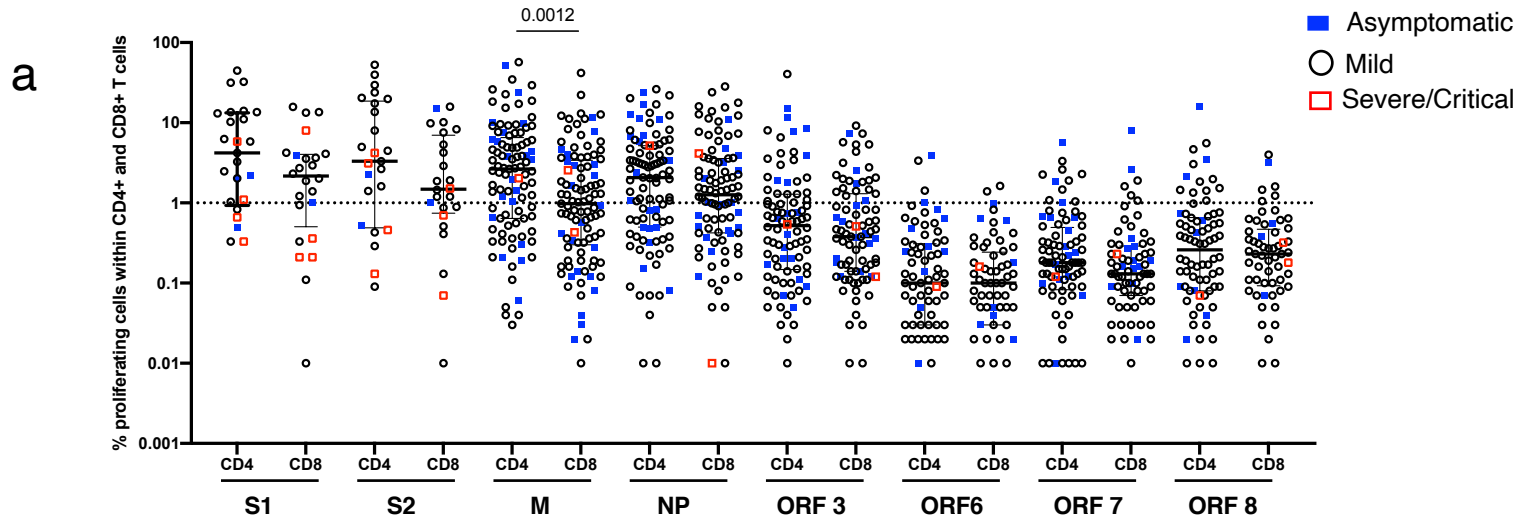
Supplementary figure 2

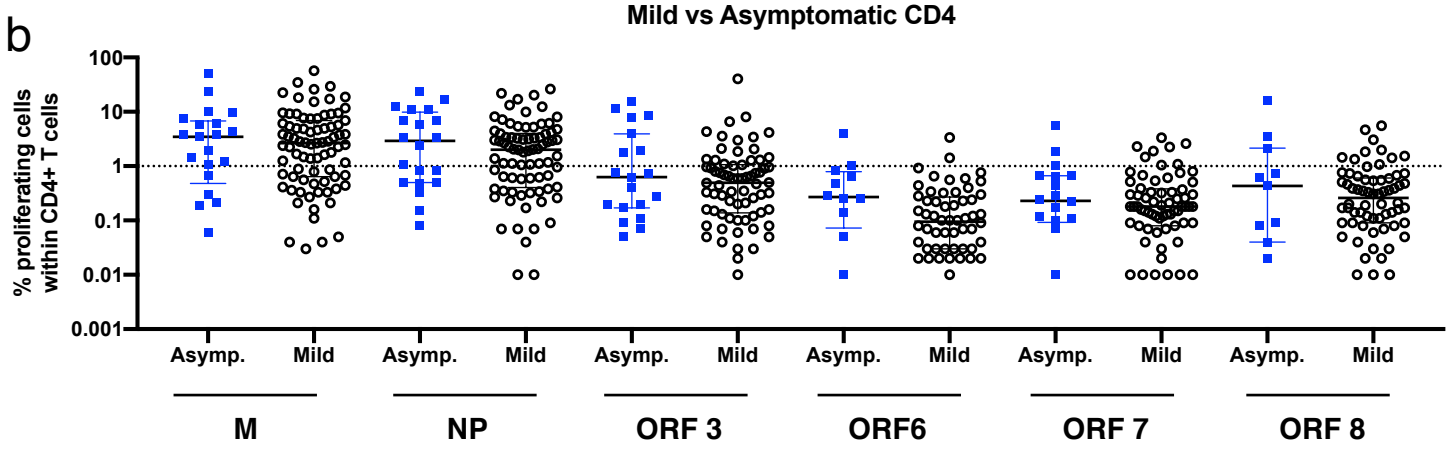


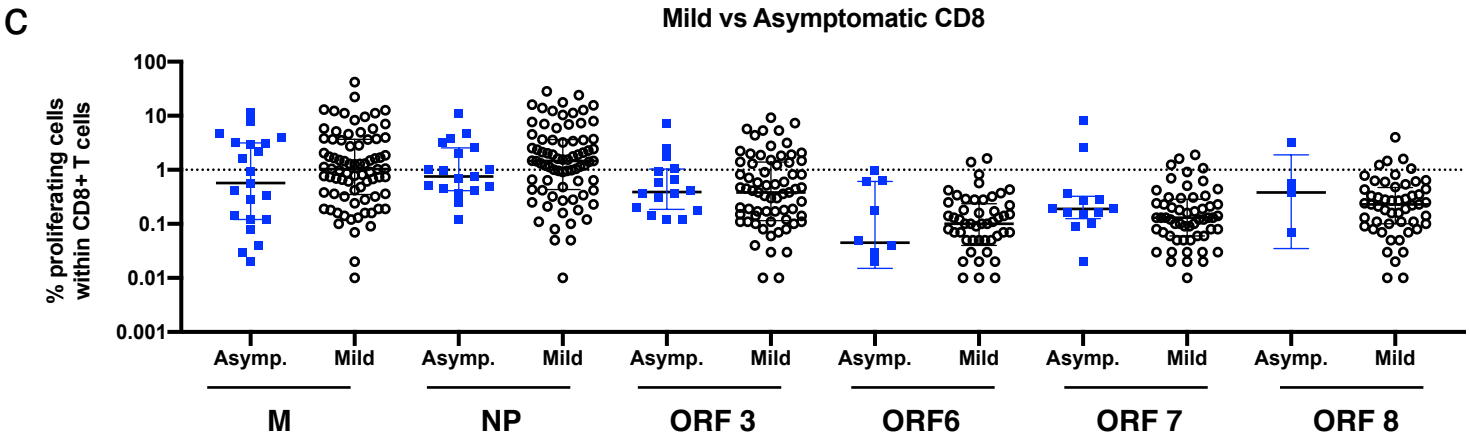
Gating strategy and representative plots for CTV assay.

a) Gating strategy used for the identification of proliferating CD4+ and CD8+ T cells as analysed in figures 3(a, b, c,), 6(a, b, c, d, f, g), 7(b, c, d, e, f, g), supplementary figure 2(d, e), supplementary figure 3 and supplementary figure 6. b) Representative plots for gating peptide pool specific proliferative responses. c) Representative plots for validation of specificity of proliferation assay using HCV seronegative samples d) quantification of HCV and FEC-T specific protein response in CD4+ and e) CD8+ T cells. PHA is used as positive control. All data have been background subtracted. Each datapoint represents a single volunteer and plots show median with error bars indicating +/- IQR. n=11 volunteers.

Supplementary figure 3





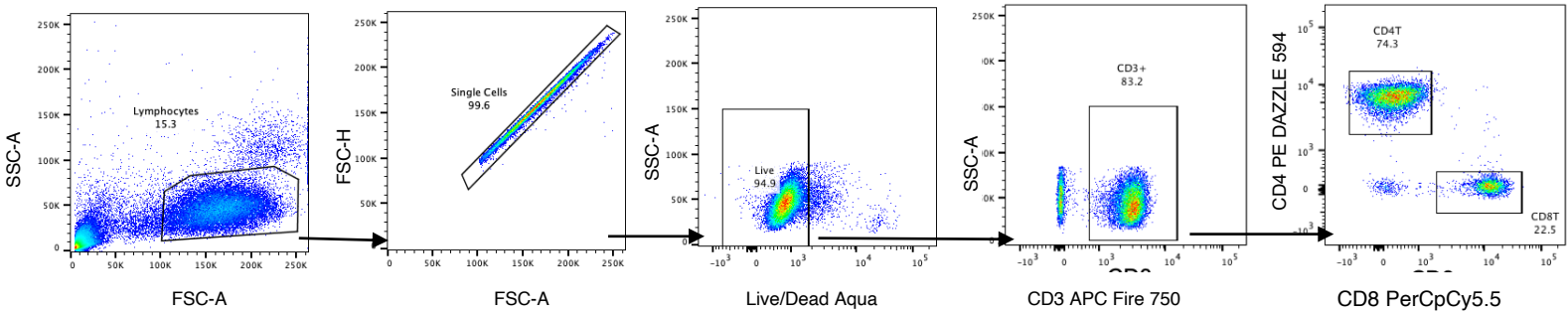


Comparative analysis of the magnitude of proliferative responses.

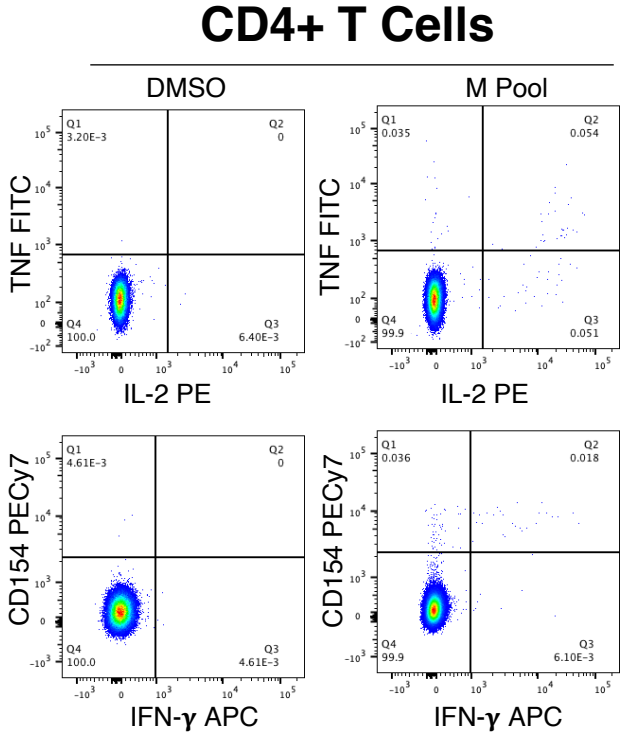
a) CD4+ and CD8+ T cells. b) mild but symptomatic and asymptomatic convalescent HCWs within the CD4+ T cell population and c) mild but symptomatic and asymptomatic convalescent HCWs within the CD8+ T cell population. Number of volunteers: asymptomatic = 23, mild = 88, severe = 4, critical = 2. Each datapoint represents a single volunteer and plots show median with error bars indicating +/- IQR. Comparison of two groups was done by Mann-Whitney U test. Where indicated, ns = not significant, * = <0.05, ** = <0,01, *** = < 0.001 and **** = <0.0001

Supplementary figure 4

a



b



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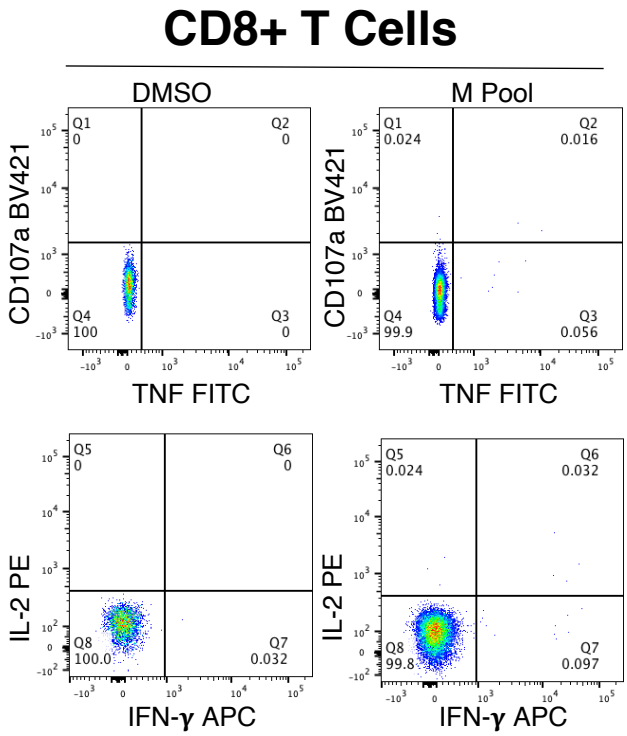
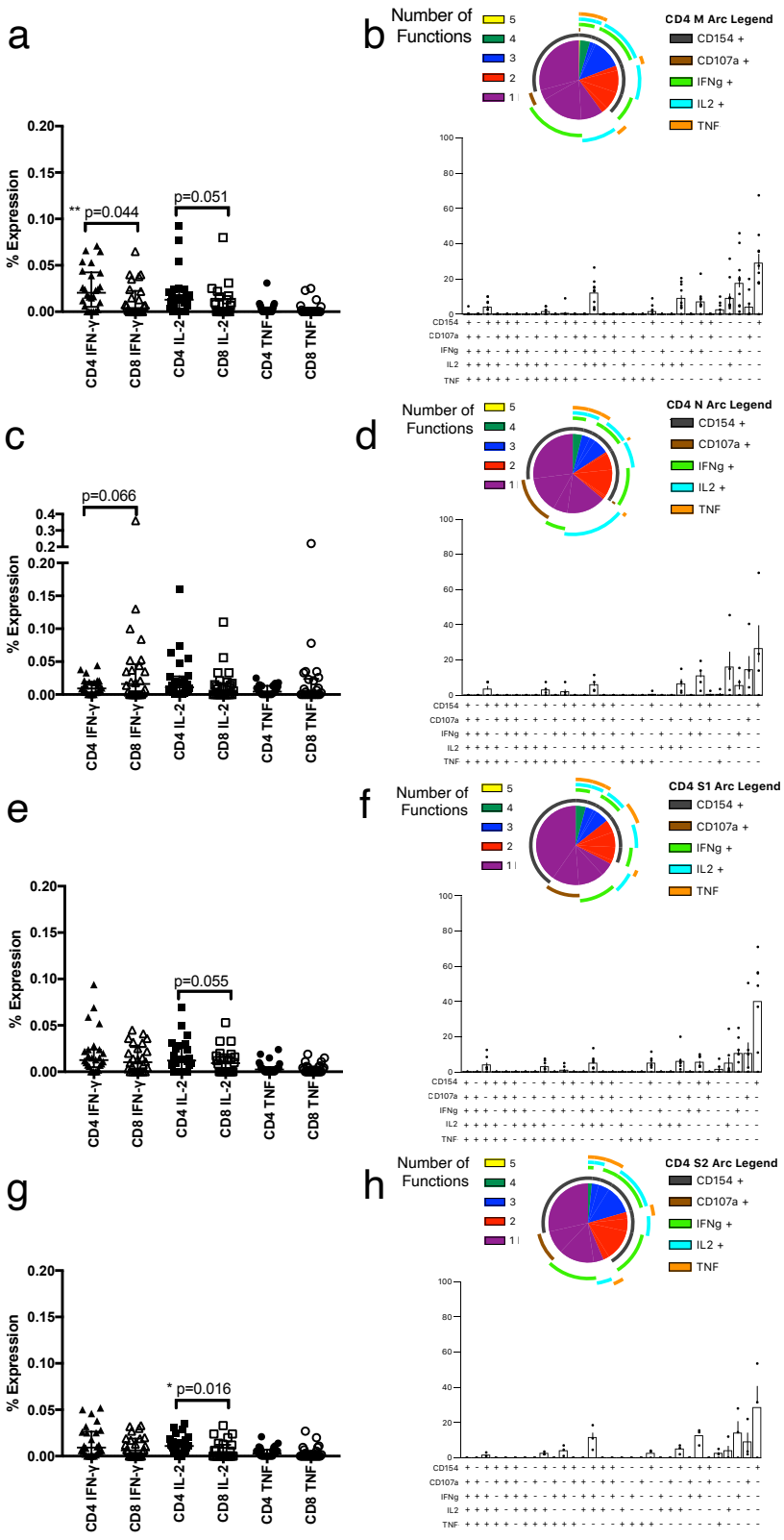


Representative ICS plots

PBMC were stimulated with 2ug/mL of the indicated peptide pool or DMSO control. The gating strategy for CD4+ and CD8+ T cells is shown in (a). Representative plots for gated CD4+ T cells are shown in (b) and gated CD8+ T cells in (c). Gating strategy was used for analysis of figures 4 and supplementary figure 5

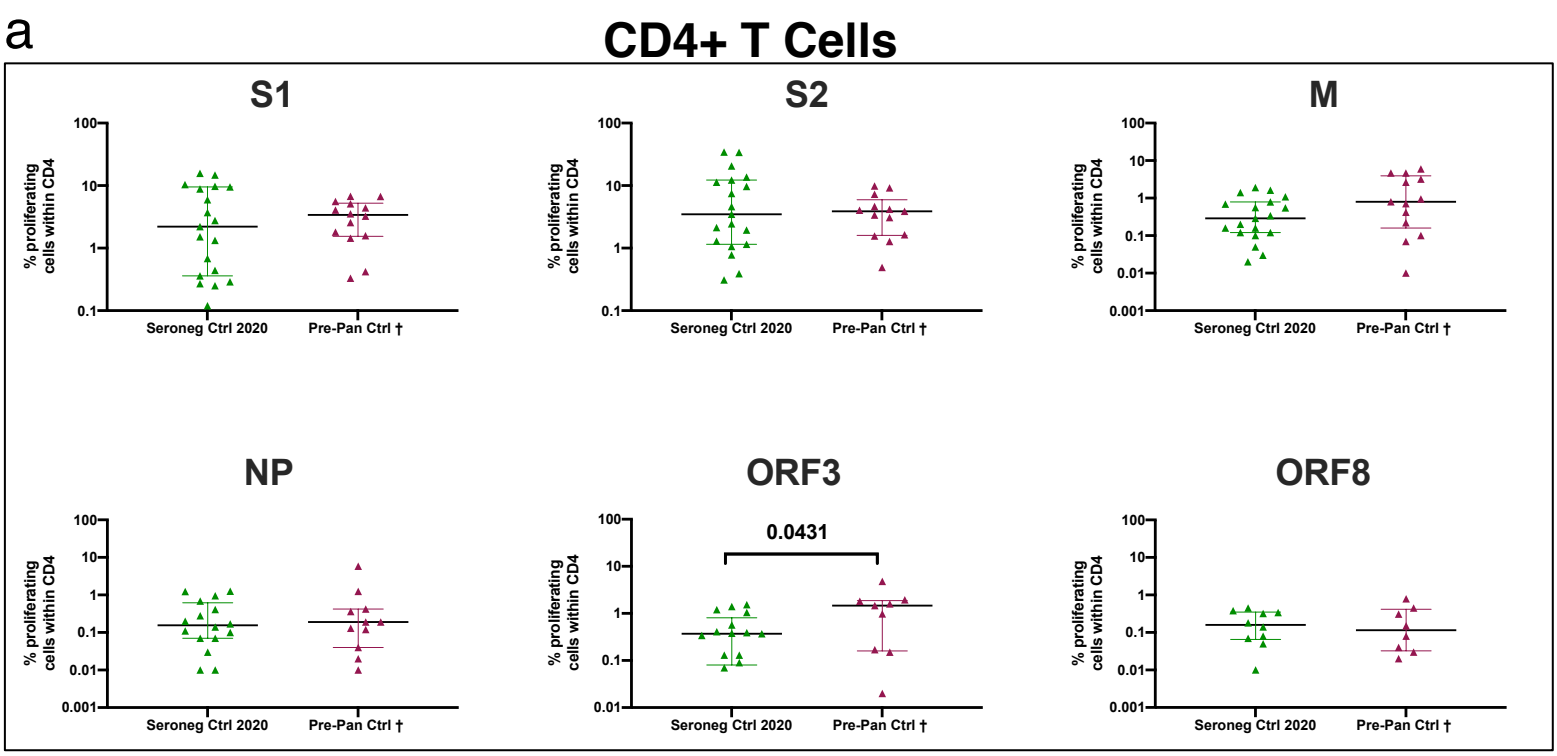
Supplementary figure 5

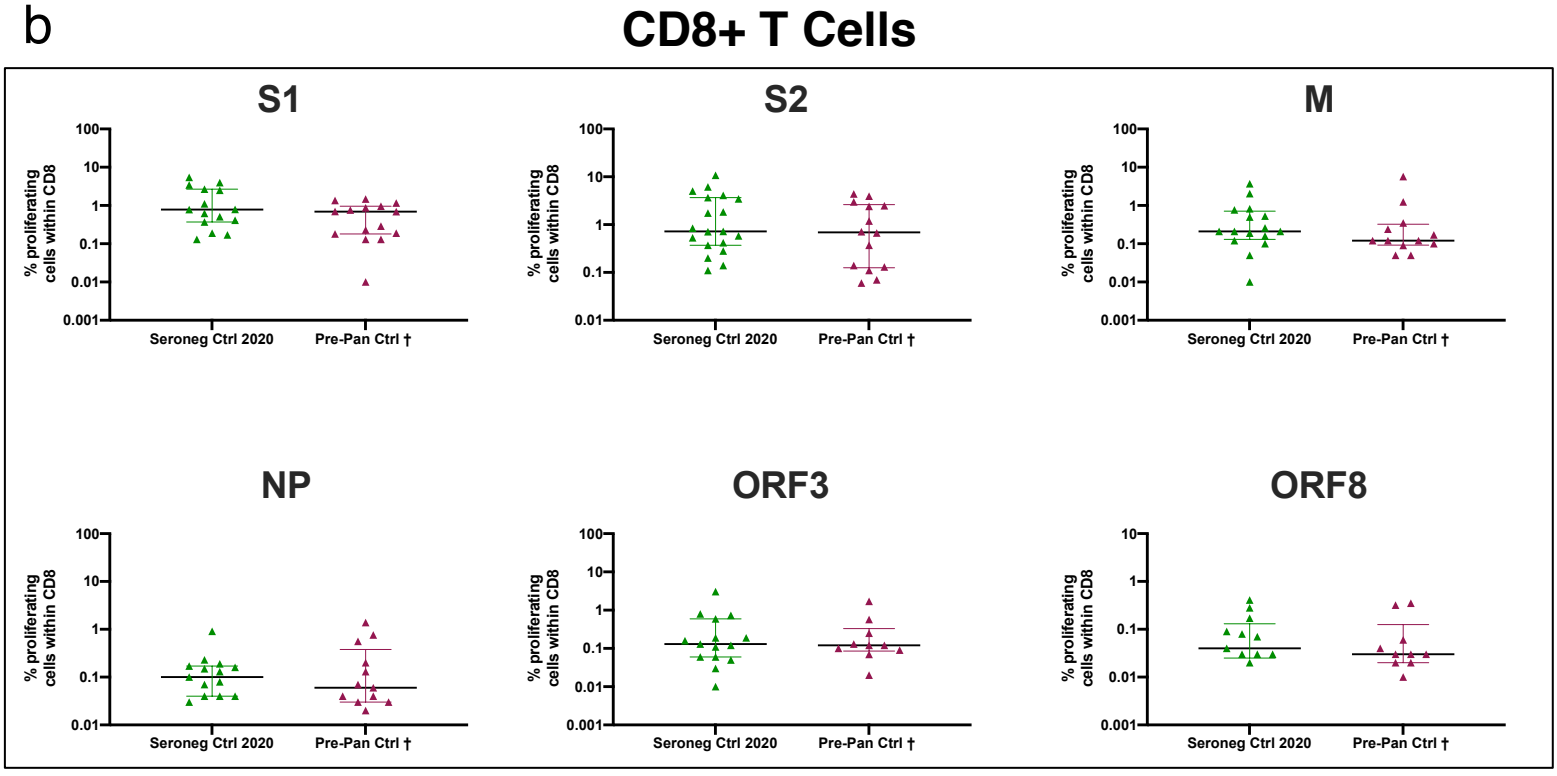


ICS responses in CD4+ and CD8+ T cells for Spike Pools.

ICS was performed as in Figure 4 on n=26 individuals PCR+ for SARS-CoV-2. Expression levels of IFN-γ, IL-2 and TNF in CD4+ and CD8+ T cells are shown for the peptide pools M in (a), NP in (c), S1 in (e), and S2 in (g). Bars represent median +/- IQR. Statistics were performed using two-tailed Wilcoxon matched-pairs signed rank test between each cytokine in CD4+ vs CD8+ T cells. Polyfunctionality was then assessed as in Figure 4. Multiple cytokine expression is shown for CD4+ T cells for M pools in (b) n=10, NP pools in (d) n=4, S1 pools in (f) n=6, and S2 pools in (h) n=3.

Supplementary figure 6

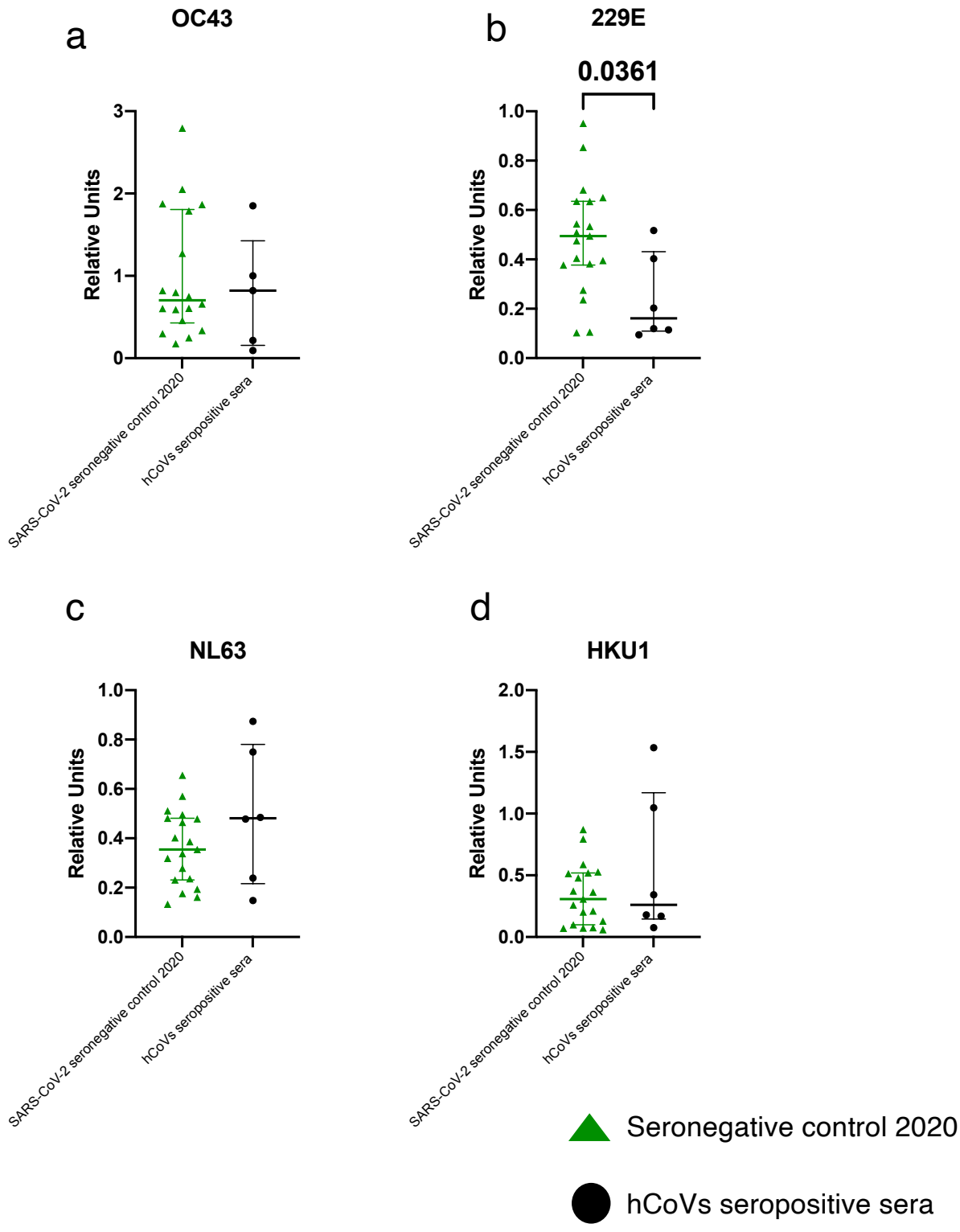




Proliferative responses in seronegative samples from 2020 and pre-pandemic.

Comparative analysis of the magnitude of proliferative responses in SARS-COV-2 seronegative controls from 2020 (n=22) analysed from fresh PBMCs and cryopreserved pre-pandemic seronegative controls (n=15) in a) CD4+ and b) CD8+ T cells. Each datapoint represents a single volunteer and plots show median with error bars indicating +/- IQR. Comparison of two groups was done by two-tailed Mann-Whitney U test. Where indicated, ns = not significant, * = <0.05, ** = <0,01, *** = < 0.001 and **** = <0.0001

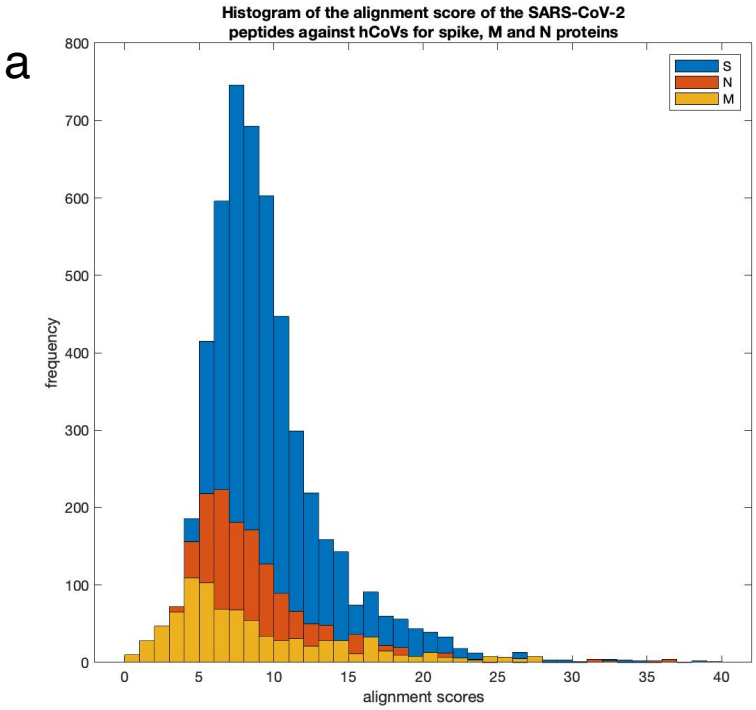
Supplementary figure 7

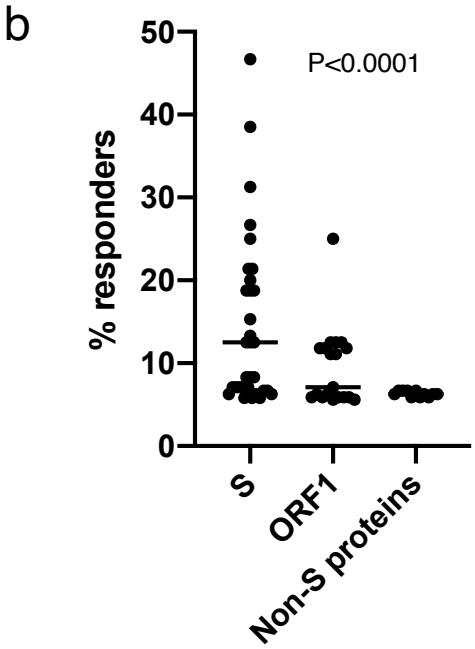


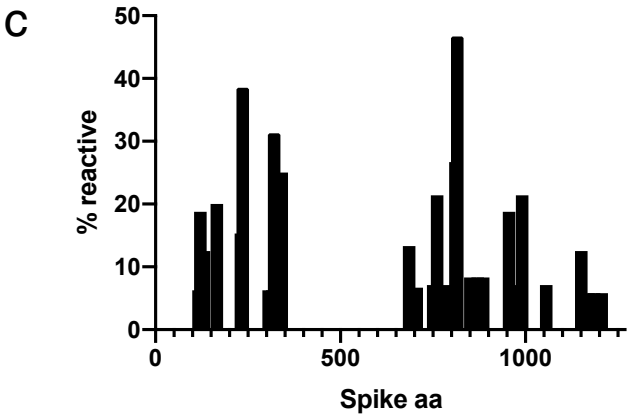
Serologic responses to circulating HCoVs in seronegative individuals.

The sera from 19 SARS-CoV-2 seronegative individuals used in the T cell assays (with a pool of 6 seropositives for comparison) were assessed for reactivity against spike proteins derived from the four major circulating coronaviruses – a) OC43, b) 229E, c) NL63 and d) HKU1) as indicated. Each datapoint represents a single volunteer and plots show median with error bars indicating +/- IQR. Comparison of two groups was done by two-tailed Mann-Whitney U test. Where indicated, ns = not significant, * = <0.05, ** = <0,01, *** = < 0.001 and **** = <0.0001

Supplementary figure 8







Analyses of crossreactivity of S1 and S2 vs other antigens from SARS-CoV-2

a) *Cross reactivity of S vs M and NP at a peptide level.* The SARS-CoV-2 spike, M and NP proteins were divided into 9-mer peptides (with overlap of 8 amino acids). These peptides were then aligned against the homologues proteins of the four species of human coronaviruses (OC43, NL63, 229E and HKU1,) to find the equivalent peptide. The alignment score was recorded for each peptide (higher values indicated higher conservation between SARS-CoV-2 peptide and the equivalent peptide in the HCoVs). The median alignment score for each of spike, NP and M proteins was 8.5, 7.5 and 6.5 respectively which indicates that on average SARS-CoV-2 spike protein peptides are more conserved relative to the HCoVs than M and NP proteins (Mann-Whitney U-test p-values: S vs. NP < 2.2×10^{-16}, S vs. M < 2.2×10^{-16}). Additionally focusing on the top 5% most homologous peptides (alignment score > 18.5) across the three proteins, we observed that 61%, 20% and 19% of the peptides were in S, NP and M proteins respectively. The histogram of these alignment scores is plotted for each of the spike, M and NP proteins. b) *Crossreactivity of S vs non-S peptides derived from published datasets.* Samples from the SARS-CoV-2 seronegative population from Matues et al[21] were tested against pools totalling 61 peptides selected for maximal crossreactivity derived from S and non-S regions of SARS-CoV-2. b) The % responders to these peptides is shown, indicating more consistent reactivity against S compared to non-S (ORF1 or other structural proteins) p<0.0001 by two-tailed Welch's ANOVA. c) data from b) plotted across S to demonstrate responses are against both S1 and S2 regions of spike.

Supplementary Table 1

Group	N	Sex F/M (%)	Age - median (IQR)	Time from PCR - median (IQR)	Time from symptoms - median, (IQR)
Asymptomatic	33	26/7 (79/21%)	36 (27-47)	6 (5-28)	n/a
Mild	126	92/34 (73/27%)	34.5 (27-48)	29 (24-54)	34.5 (27-57)
Severe & Critical	9	3/6 (33/67%)	52 (41-69)	18 (9-24)	23 (13-29)
TOTAL PCR-confirmed SARS-CoV-2 infection	168				
Healthy controls: Contemporaneous (Oxford)	30	13/17 (43/57%)	37 (33-47)	n/a	n/a
Healthy controls: Contemporaneous (Sheffield)	13	7/6 (54/46%)	39 (32-46)	n/a	n/a
Healthy controls: Pre-Pandemic (Oxford) *	19	6/4 (60/40%)	25 (21-38)	n/a	n/a
Healthy controls: Pre-Pandemic (Liverpool)	48	35/13 (73/27%)	26 (22-38.5)	n/a	n/a
PCR Negative Inpatients	9	4/5 (44/56%)	74 (47-79)	n/a	n/a
TOTAL Unexposed controls	119				
Highly Exposed - PCR & Antibody negative	10	3/7 (30/70%)	31 (25-34)	n/a	n/a
P value (ANOVA)		0.002	<0.0001	<0.0001	0.03

*missing demographic data for 9 samples

Clinical Information for Patients used in the Study

Supplementary Table 2

Antigen stimulants	Spike			Structural & Accessory Proteins			Predicted Epitopes		
Group	**N**	**Median (IQR) SFC/10^6**	**Responder (%)**	**N**	**Median (IQR) SFC/10^6**	**Responder (%)**	**N**	**Median (IQR) SFC/10^6**	**Responder (%)**
Asymptomatic SARS-CoV-2 infection	22	0 (0-0)	4/22 (18%)	23	43 (0-148)	12/23 (52%)	2	28 (0-55)	1/2 (50%)
Mild SARS-CoV-2 infection	46	49 (0-207)	27/46 (59%)	73	108 (0-325)	47/73 (64%)	24	205 (0-381)	21/24 (88%)
Severe / critical SARS-CoV-2 infection	7	97 (0-919)	3/7 (43%)	7	113 (0-223)	6/7 (86%)	3	130 (0-1780)	2/3 (67%)
Healthy seronegative controls	22	0 (0-0)	0/22 (0%)	23	0 (0-0)	1/23 (4%)	5	0 (0-43)	1/5 (20%)
Hospitalised PCR negative	7	0 (0-0)	0/7 (0%)	6	0 (0-0)	0/6 (0%)	5	0 (0-0)	0/5 (0%)

Ex vivo interferon-gamma ELISpot Responses by Group

Supplementary Table 3

	Dunn's multiple comparisons test	Summary of P value	Adjusted P Value
SPIKE	Control vs. Asymptomatic	ns	>0.9999
	Control vs. Mild HCW	****	<0.0001
	Control vs. Severe/Critical	ns	0.0777
	Control vs. Negative inpatient	ns	>0.9999
	Asymptomatic vs. Mild HCW	*	0.0276
	Asymptomatic vs. Severe/Critical	ns	0.6870
	Asymptomatic vs. Negative inpatient	ns	>0.9999
	Mild HCW vs. Severe/Critical	ns	>0.9999
	Mild HCW vs. Negative inpatient	*	0.0394
	Severe/Critical vs. Negative inpatient	ns	0.2678
STRUCTURAL & ACCESSORY	Control vs. Asymptomatic	ns	0.0984
	Control vs. Mild HCW	****	<0.0001
	Control vs. Severe/Critical	***	0.0003
	Control vs. Negative inpatient	ns	>0.9999
	Asymptomatic vs. Mild HCW	ns	0.8993
	Asymptomatic vs. Severe/Critical	ns	0.1755
	Asymptomatic vs. Negative inpatient	ns	0.7317
	Mild HCW vs. Severe/Critical	ns	>0.9999
	Mild HCW vs. Negative inpatient	*	0.0386
	Severe/Critical vs. Negative inpatient	**	0.0090
PREDICTED	Control vs. Asymptomatic	ns	>0.9999
	Control vs. Mild HCW	*	0.0410
	Control vs. Severe/Critical	ns	>0.9999
	Control vs. Negative inpatient	ns	>0.9999
	Asymptomatic vs. Mild HCW	ns	0.8620
	Asymptomatic vs. Severe/Critical	ns	>0.9999
	Asymptomatic vs. Negative inpatient	ns	>0.9999
	Mild HCW vs. Severe/Critical	ns	>0.9999
	Mild HCW vs. Negative inpatient	*	0.0114
	Severe/Critical vs. Negative inpatient	ns	0.8517
CEF POSITIVE CONTROL	Control vs. Asymptomatic	ns	>0.9999
	Control vs. Mild HCW	ns	0.7163
	Control vs. Severe/Critical	ns	0.7528
	Control vs. Negative inpatient	ns	>0.9999
	Asymptomatic vs. Mild HCW	ns	0.1164
	Asymptomatic vs. Severe/Critical	ns	0.3007
	Asymptomatic vs. Negative inpatient	ns	0.5417
	Mild HCW vs. Severe/Critical	ns	>0.9999
	Mild HCW vs. Negative inpatient	ns	>0.9999
	Severe/Critical vs. Negative inpatient	ns	>0.9999

Kruskal Wallis test with Dunn's multiple comparison on magnitude of *ex vivo* interferon-gamma ELISpot responses

Supplementary Table 4

Dunn's multiple comparisons test	Summary of P value	Adjusted P Value
DMSO vs. S1	****	<0.0001
DMSO vs. S2	****	<0.0001
DMSO vs. M	****	<0.0001
DMSO vs. NP	****	<0.0001
DMSO vs. ORF 3	****	<0.0001
DMSO vs. ORF 6	ns	>0.9999
DMSO vs. ORF 7	ns	0.0669
DMSO vs. ORF 8	**	0.0054
S1 vs. S2	ns	>0.9999
S1 vs. M	ns	>0.9999
S1 vs. NP	ns	>0.9999
S1 vs. ORF 3	***	0.001
S1 vs. ORF 6	****	<0.0001
S1 vs. ORF 7	****	<0.0001
S1 vs. ORF 8	****	<0.0001
S2 vs. M	ns	>0.9999
S2 vs. NP	ns	>0.9999
S2 vs. ORF 3	**	0.0095
S2 vs. ORF 6	****	<0.0001
S2 vs. ORF 7	****	<0.0001
S2 vs. ORF 8	****	<0.0001
M vs. NP	ns	>0.9999
M vs. ORF 3	****	<0.0001
M vs. ORF 6	****	<0.0001
M vs. ORF 7	****	<0.0001
M vs. ORF 8	****	<0.0001
NP vs. ORF 3	***	0.0009
NP vs. ORF 6	****	<0.0001
NP vs. ORF 7	****	<0.0001
NP vs. ORF 8	****	<0.0001
ORF 3 vs. ORF 6	***	0.0007
ORF 3 vs. ORF 7	ns	0.1732
ORF 3 vs. ORF 8	ns	>0.9999
ORF 6 vs. ORF 7	ns	>0.9999
ORF 6 vs. ORF 8	ns	>0.9999
ORF 7 vs. ORF 8	ns	>0.9999

Kruskal Wallis test with Dunn's multiple comparison on frequency of proliferative CD4+ T cells

Supplementary Table 5

Dunn's multiple comparisons test	Summary of P value	Adjusted P Value
DMSO vs. S1	****	<0.0001
DMSO vs. S2	****	<0.0001
DMSO vs. M	****	<0.0001
DMSO vs. NP	****	<0.0001
DMSO vs. ORF 3	****	<0.0001
DMSO vs. ORF 6	ns	>0.9999
DMSO vs. ORF 7	ns	>0.9999
DMSO vs. ORF 8	ns	0.7346
S1 vs. S2	ns	>0.9999
S1 vs. M	ns	>0.9999
S1 vs. NP	ns	>0.9999
S1 vs. ORF 3	*	0.0291
S1 vs. ORF 6	****	<0.0001
S1 vs. ORF 7	****	<0.0001
S1 vs. ORF 8	****	<0.0001
S2 vs. M	ns	>0.9999
S2 vs. NP	ns	>0.9999
S2 vs. ORF 3	ns	0.0842
S2 vs. ORF 6	****	<0.0001
S2 vs. ORF 7	****	<0.0001
S2 vs. ORF 8	***	0.0001
M vs. NP	ns	>0.9999
M vs. ORF 3	*	0.0178
M vs. ORF 6	****	<0.0001
M vs. ORF 7	****	<0.0001
M vs. ORF 8	****	<0.0001
NP vs. ORF 3	**	0.0018
NP vs. ORF 6	****	<0.0001
NP vs. ORF 7	****	<0.0001
NP vs. ORF 8	****	<0.0001
ORF 3 vs. ORF 6	***	0.0004
ORF 3 vs. ORF 7	*	0.0268
ORF 3 vs. ORF 8	ns	0.3482
ORF 6 vs. ORF 7	ns	>0.9999
ORF 6 vs. ORF 8	ns	>0.9999
ORF 7 vs. ORF 8	ns	>0.9999

Kruskal Wallis test with Dunn's multiple comparison on frequency of proliferative CD8+ T cells

Supplementary Table 6

			CD4+ T cells								CD8+ T cells											
ID	Anti-spike_IgG	Total ELISpot	S1	S2	M	N	ORF 3	ORF 6	ORF 7	ORF 8	S1	S2	M	N	ORF 3	ORF 6	ORF 7	ORF 8	Symptoms	Sex	Age	symptoms to pcr test(days)	Days since symptom onset
1†	4	318	48.30	19.60	8.40	0.00	2.97	0.00	0.00	0.00	4.18	2.13	3.89	0.00	0.00	0.00	0.00	0.00	myalgia, cough, night sweats	m	40	6	107
2	1	1018	0.00	3.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	cough, fatigue	m	23	61	111
3	1	55	0.00	15.18	4.84	6.90	0.00	0.00	0.00	0.00	0.00	1.45	1.16	1.36	0.00	0.00	0.00	0.00	sore throat, cough	m	33	56	118
4†	1	35	2.37	6.85	0.00	17.28	0.00	0.00	1.71	0.00	1.07	1.69	0.00	1.79	0.00	0.00	1.22	0.00	fatigue, fever, shortness of breath	f	29	2	83
5†	1	1208	10.15	37.55	24.45	1.84	0.00	0.00	6.53	0.00	1.20	3.17	2.14	0.00	0.00	0.00	1.23	0.00	sore throat, fatigue, headache	f	24	1	82
6†	1	90	30.49	15.59	13.09	7.19	2.48	17.29	0.00	25.79	1.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	night sweats, fever, sore throat, anorexia, myalgia	f	34	55	120
7	1	8	Not Tested								Not Tested								fever, cough, sore throat, malaise, altered sense of taste	m	33	36	126
8	1	105	0.00	15.08	1.19	0.00	2.13	1.82	4.10	0.00	0.00	1.71	0.00	0.00	1.11	0.00	0.00	0.00	cough, fever	m	34	128	140
9	1	90	0.00	0.00	1.31	0.00	1.77	0.00	0.00	1.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	fever, chills, cough	m	29	36	134
10	1	67.5	Not Tested								Not Tested								fever, lethergy, dizziness, fatigue	m	25	51	143

† PBMC samples from these volunteers were analysed from cryopreserved samples.

Clinical information and ELISpot and proliferation assay responses for highly exposed doctors used in this study

Supplementary Table 7

	S	M	NP
OC43	AOL02453	AAT84365	AGT51654
NL63	AIW52836	ALJ53434	AAS58181
229E	AIW52754	AFR79253	AFR79254
HKU1	ABD75513	ABD75500	AGT17773

Accession ID for consensus sequence for seasonal coronaviruses

Other members of the Oxford Immunology Network Covid-19 Response T cell Consortium

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7. Radcliff Department of Medicine, University of Oxford, UK
8. Target Discovery Institute, University of Oxford, UK
9. Thames Valley and South Midlands Clinical Research Network
10. Wellcome Centre for Human Genetics, University of Oxford, UK
11. Worthing Hospital, Worthing, UK