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Supplementary appendix

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Supplementary Materials

Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study s

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Methods

Patient cohort used in this study

The duration of the study was 30 January 2020 to 14 August 2020 inclusive. While case definitions evolved throughout this period as part of enhanced surveillance in line with national public health policy, almost all patients with respiratory illnesses in Singapore are tested for COVID-19 with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) polymerase chain reaction (PCR). All respiratory samples were collected by trained healthcare providers. Additionally, a large number of asymptomatic individuals were tested, namely close contacts of confirmed cases and individuals living in congregate settings (namely migrant worker dormitories where large outbreaks occurred). As a result, confirmed diagnoses in Singapore included a large proportion of mild and asymptomatic cases. During this period of the outbreak, all patients with confirmed COVID-19 infection were hospitalised for initial evaluation, regardless of illness severity. There was no target sample size for the study, and patients were invited to participate in the prospective cohort study on a best-effort basis. However, not all patients could be approached due to resource limitations at the peak of the outbreak.

SARS recalls were recruited between February to September in 2020. The inclusion criterion was previous confirmed SARS coronavirus infection and hospitalisation in Tan Tock Seng Hospital. SARS recall cases had to be asymptomatic at the time of enrolment but did not need to test negative for SARS-CoV-

2. However, there was a very limited circulation of SARS-CoV-2 in the community in Singapore at the time these subjects were recruited into our study, and hence it is highly unlikely that they were exposed to SARS-CoV-2.

Surrogate virus neutralisation test (sVNT) for SARS-CoV-2 and SARS-CoV

Briefly, 50 µl of hACE2 protein (Genscript) diluted to a final concentration of 2 µg/ml in 100 mM carbonate buffer was coated on MaxiSORP plates (Nunc) overnight at 4°C, followed by blocking with OptEIA assay diluent (BD). An equal volume of 3 ng of HRP-SARS-CoV-2 Receptor Binding Domain (RBD) protein (Genscript) or HRP-SARS-CoV RBD protein (Genscript) was then mixed with test sera diluted to 1:10 and incubated at 37°C for 1 h. 50 µl of pre-incubated sera/HRP-RBD mixture were next added to each hACE2 well and further incubated for 1 h at room temperature. Unbound HRP-RBD was washed off with copious volumes of PBST buffer and colourimetric signal developed using 3,3',5,5'-tetramethylbenzidine (TMB) (Invitrogen). TMB stop solution was added to stop the chromogenic reaction and the absorbance readings were acquired using Cytation 5 microplate reader (BioTek) at 450 nm and 570 nm. A 30% cut-off is used in the current study to ensure the high stringency of the assay specificity.

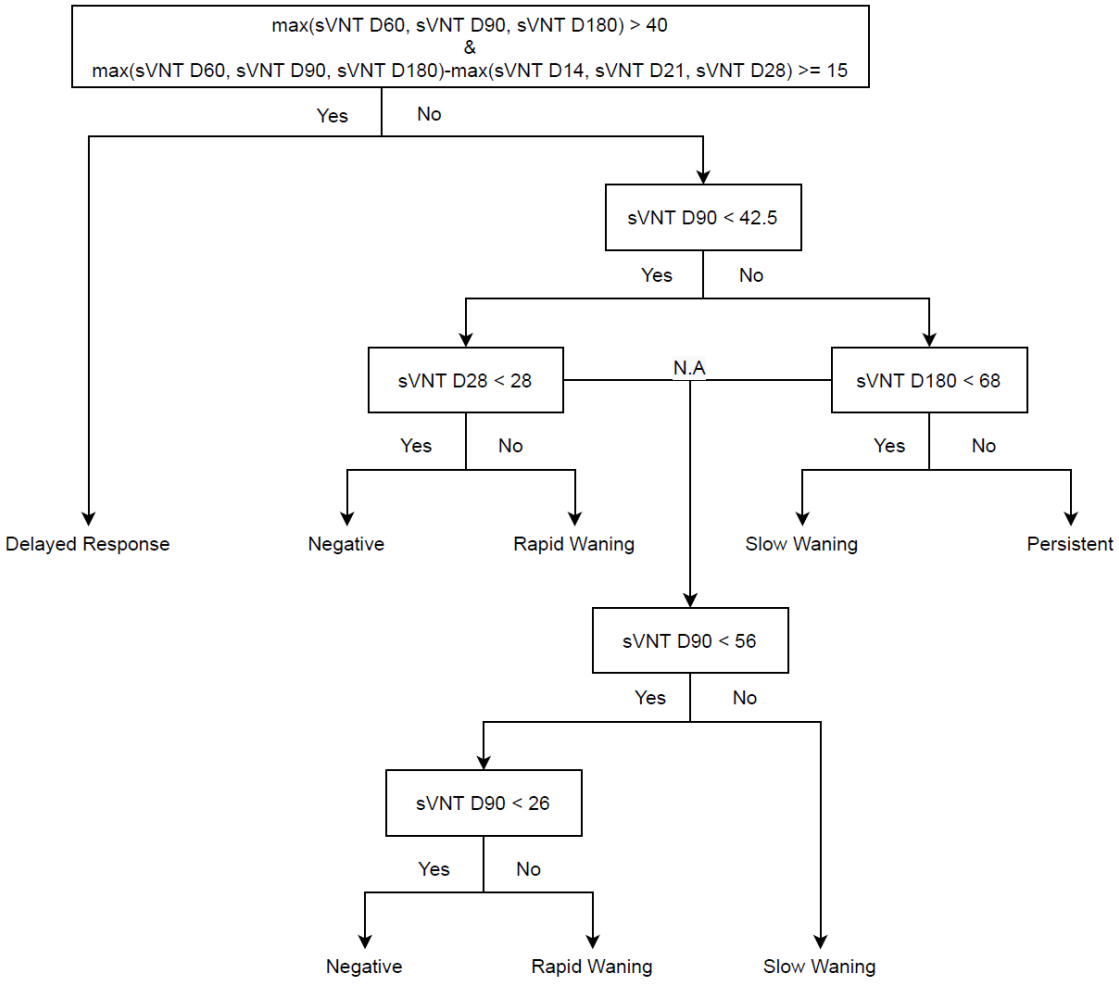
Determination of antibody avidity by ELISA

Indirect RBD ELISA was used to measure the avidity of anti-RBD antibodies using quadruplicate wells. RBD proteins were diluted to a final concentration of 2 µg/ml in carbonate buffer and coated on MaxiSORP plates overnight at 4°C, followed by blocking with OptEIA assay diluent (BD). Test sera were diluted to 1:100, added to ELISA plates and incubated at 37°C for 1 h. After the primary antibody was washed off with five washes of PBST buffer, 50 µl of 6 M urea was added to duplicate wells and incubated at room temperature for 10 min. After five washes of PBST buffer, 1:10000 of HRP-anti-human IgG (Santa Cruz) was added and further incubated at 37°C for 30 min. The ELISA wells were washed with PBST buffer and the colourimetric signal was developed as described in the sVNT methodology above. Antibody avidity was calculated as a fraction of signal intensity change before and after urea treatment.

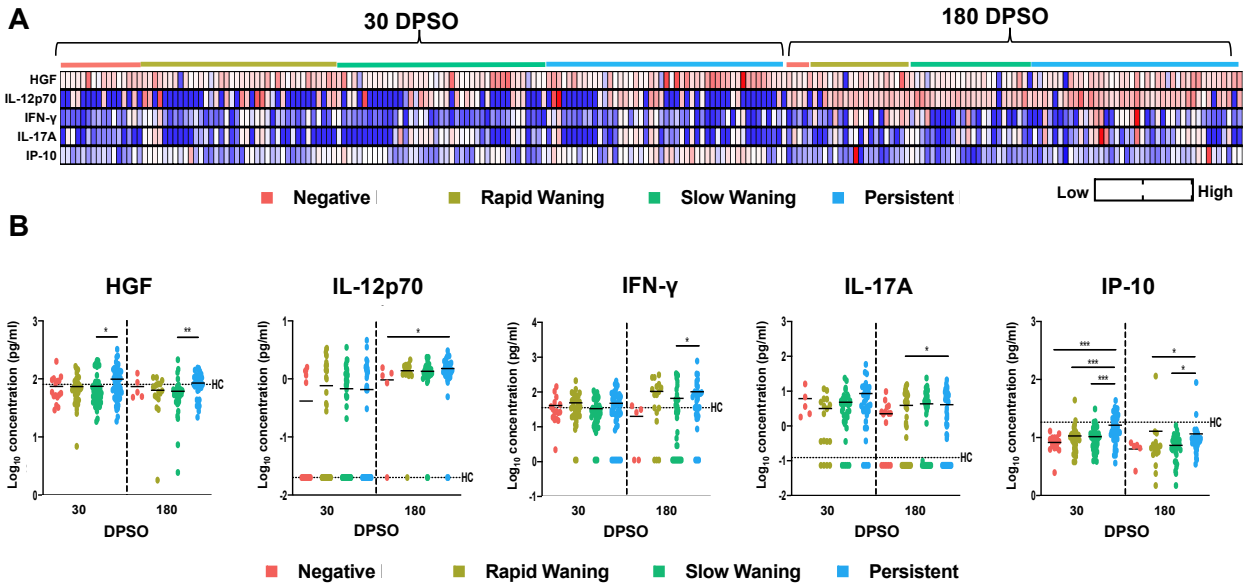
Multiplex microbead-based immunoassay

The Cytokine/Chemokine/Growth Factor 45-plex Human ProcartaPlex™ Panel 1 panel includes granulocyte-macrophage colony-stimulating factor (GM-CSF), epidermal growth factor (EGF), brain-

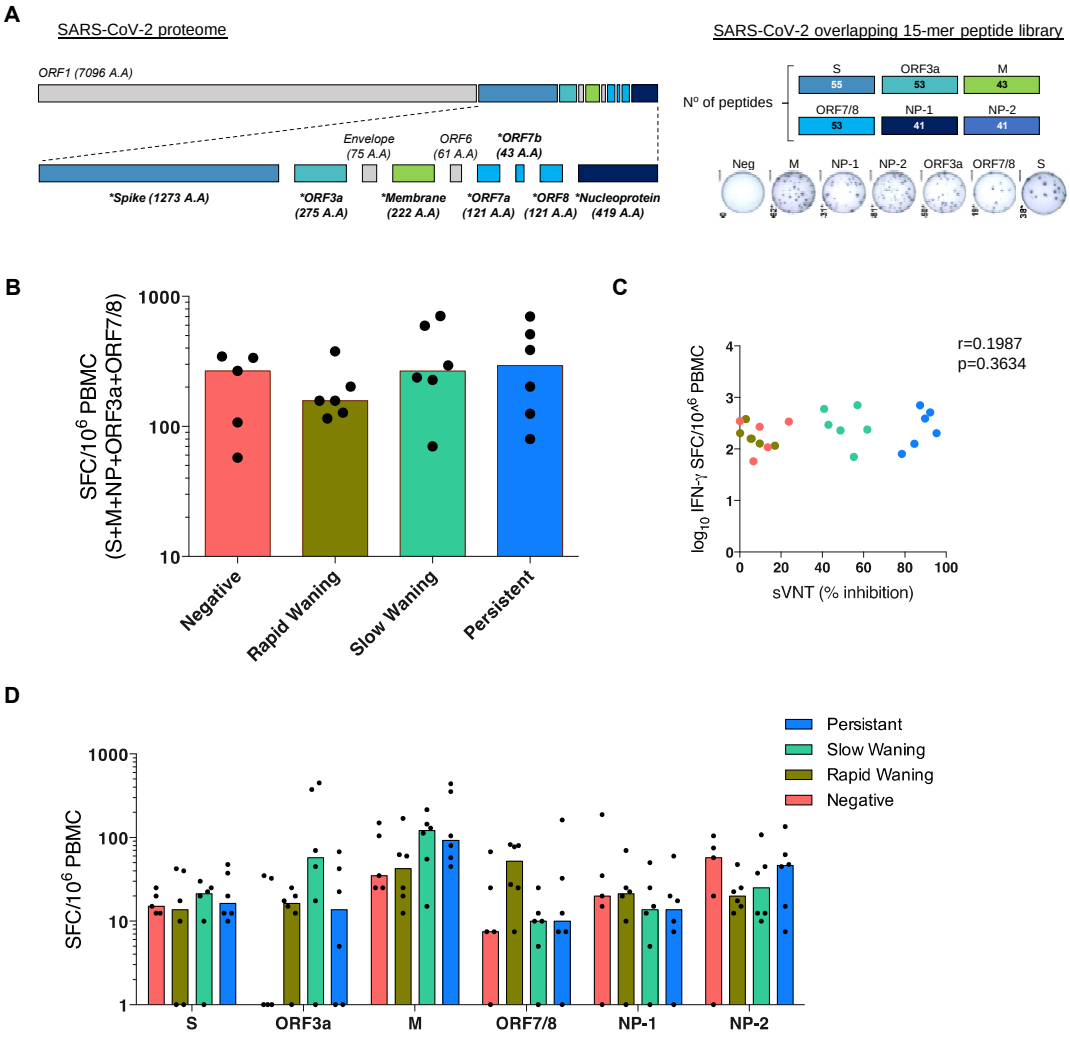
derived neurotropic factor, beta-nerve growth factor (bNGF), basic fibroblast growth factor (FGF-2), hepatocyte growth factor (HGF), monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1α, MIP-1β, RANTES (regulated on activation, normal T cell expressed and secreted), chemokine (C-X-C motif) ligand (CXCL) 1 (GRO-α), stromal cell-derived factor 1 (SDF-1α), interferon (IFN) gamma-induced protein 10 (IP-10), eotaxin, IFN-α, IFN-γ, interleukin (IL)-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, leukemia inhibitory factor (LIF), stem cell factor (SCF), tumor necrosis factor (TNF-α), TNF-β, vascular endothelial growth factors A and D (VEGF-A, VEGF-D), platelet derived growth factor (PDGF-BB), and placental growth factor (PLGF-1). Standards and plasma from COVID-19 patients and healthy controls were incubated with fluorescent-coded magnetic beads pre-coated with respective antibodies in a black 96-well clear-bottom plate overnight at 4˚C. After incubation, plates were washed 5 times with wash buffer (PBS with 1% BSA (Capricorn Scientific) and 0.01% Tween (Promega). Sample-antibody-bead complexes were incubated with biotinylated detection antibodies for 1 hour and washed 5 times with wash buffer. Subsequently, Streptavidin-PE was added and incubated for another 30 mins. Plates were washed 5 times again before sample-antibody-bead complexes were re-suspended in sheath fluid for acquisition on the FLEXMAP® 3D (Luminex) using xPONENT® 4.0 (Luminex) software. Internal control samples were included in each Luminex assay to remove any potential plate effects. A correction factor was obtained from the readout differences observed from the internal control samples across the multiple assays and this correction factor was then used to normalise all the samples. Standard curves were generated with a 5-PL (5-parameter logistic) algorithm, reporting values for mean fluorescence intensity (MFI) and concentration data. The concentrations were logarithmically transformed to ensure normality.



Supp Figure 1. Decision tree to classify the same grouping according to NAb at different timepoints. N.A: 90 dpso data was used to further classify samples if 28 dpso or 180 dpso data was unavailable.



Supp Figure 2. Plasma immune mediator levels of COVID-19 patients grouped by SARS-CoV-2 neutralising antibody responses. Concentrations of 45 immune mediators were quantified using a 45-plex microbead-based immunoassay. (A) Heatmap of immune mediator levels in plasma samples of patients with different dynamics of SARS-CoV-2 neutralising antibody responses at 180 days post symptom onset. Each colour represents the relative concentration of a particular analyte. Blue and red indicate low and high concentration, respectively. (B) Profiles of significant immune mediators of COVID-19 patients across four groupings of SARS-CoV-2 neutralising antibody responses are illustrated as scatter plots. Immune mediator levels in plasma fraction samples from 30 days (Negative, n=16; Rapid Waning, n=37; Slow Waning, n=41; Persistent, n=48) and 180 days (Negative, n=5; Rapid Waning, n=17; Slow Waning, n=34; Persistent, n=33) post symptom onset were compared among the patients with different dynamics of neutralising antibody responses. Kruskal-Wallis with Dunn's post hoc tests were performed which discerned the differences between the groups (*P < 0.05; **P < 0.01; *P < 0.001). Immune mediator levels for healthy controls (n = 23) are indicated by the black dotted line. Patient samples with concentration out of measurement range are presented as the value of logarithm transformation of Limit of Quantification.



Supp Figure 3. Analysis of SARS-CoV-2 specific T-cell responses. (A) SARS-CoV-2 proteome organisation; analysed proteins are coloured and marked by *. 15-mer peptides overlapping by 10 amino acids were split into pools covering ORF3a, membrane (M), ORF7 and ORF8 combined, nucleoprotein (NP1, NP-2) and selected 15-mers covering the more T cell-immunogenic regions of Spike (S). T cell reactivity was tested by ex-vivo IFNγ-ELISpot. (B) Combined frequency of IFNγ-secreting cells (SFC) reactive to the peptide pools of S, ORF3a, M, ORF7/8, NP-1 and NP-2 per 106 PBMC in COVID-19 convalescents 180 days post symptom onset. Patients are divided based on their profile of neutralising antibodies: Negative (n=5), Rapid Waning (n=6), Slow Waning (n=6), Persistent (n=6). (C) The magnitude of combined SARS-CoV-2-specific T cell responses to all measured peptide pools was correlated with the level of inhibition by virus neutralising antibodies a 477 t day 180 post symptom onset. Spearman correlation, p-value two-tailed. (D) Frequency of IFNγ-secreting cells (SFU) reactive to the individual peptide pools of S, ORF3a, M, ORF7/8, NP-1 and NP-2 per 106 PBMC in COVID-19 convalescents at 180 days post symptom onset.

Supplementary Table 1. Summary of sample details

Sample#	Sampling time points (days post symptom onset)	NAb dynamics Grouping (1=Negative; 2=Rapid Waning; 3=Slow Waning; 4=Persistent; 5=Delayed Response)	Observed NAb longevity (days)	Predicted NAb longevity (days)	Predicted NAb longevity confidence intervals (days)	Representative samples used for cytokine studies (Y=Yes)	Representative samples used for T-cell immunity studies (Y=Yes)
S2-1	16, 24, 32, 88, 177	1	NA	NA	NA	Y	Y
S2-2	19, 60, 92, 167	1	NA	NA	NA	Y	Y
S2-3	17, 31, 97	1	19	NA	NA	Y	
S2-4	62, 104, 193	1	NA	NA	NA	Y	Y
S2-5	16, 30, 184	1	NA	NA	NA	Y	Y
S2-6	71, 111, 182	1	NA	NA	NA		
S2-7	14, 29, 91, 171	1	NA	NA	NA	Y	
S2-8	60, 95, 166	1	NA	NA	NA	Y	
S2-9	33, 165	1	NA	NA	NA	Y	
S2-10	15, 31, 99, 171	1	NA	NA	NA	Y	
S2-11	32, 90	1	NA	NA	NA	Y	
S2-12	29, 94	1	NA	NA	NA	Y	
S2-13	28, 99, 169	1	NA	NA	NA	Y	
S2-14	15, 43, 92	1	NA	NA	NA	Y	
S2-15	35, 92, 161	1	NA	NA	NA	Y	
S2-16	31, 85	1	NA	NA	NA		
S2-17	14, 29, 87	1	NA	NA	NA	Y	
S2-18	15, 31, 89	1	NA	NA	NA	Y	
S2-19	14, 28, 88	1	NA	NA	NA	Y	
S2-20	15, 22, 28, 90	2	NA	130	109-150	Y	
S2-21	28, 90, 180	2	49	50	0-334	Y	
S2-22	28, 90	2	NA	96	NA	Y	
S2-23	28, 90	2	NA	109	NA		
S2-24	14, 28, 90, 180	2	75	113	0-231	Y	
S2-25	59, 140	2	139	139	NA	Y	

S2-26	22, 30, 148	2	125	123	103-143	Y	
S2-27	14, 21, 28, 95, 181	2	68	95	39-152	Y	Y
S2-28	27, 181	2	120	120	NA	Y	Y
S2-29	16, 21, 30, 93, 179	2	100	115	89-142	Y	Y
S2-30	21, 28, 86	2	31	40	0-118		
S2-31	28, 93, 169	2	55	58	0-206	Y	Y
S2-32	14, 21, 34, 92, 182	2	140	142	110-173	Y	
S2-33	31, 86	2	NA	95	NA	Y	
S2-34	24, 105, 175	2	105	118	0-244	Y	Y
S2-35	57, 102, 179	2	135	139	44-234	Y	
S2-36	30, 93	2	64	65	NA	Y	
S2-37	7, 14, 27, 90, 168	2	76	72	12-132	Y	Y
S2-38	21, 28,100	2	NA	136	123-149	Y	
S2-39	33, 91, 180	2	130	131	128-135	Y	
S2-40	32, 90	2	NA	96	NA	Y	
S2-41	46, 97, 167	2	61	61	0-187	Y	
S2-42	32, 97, 180	2	78	75	0-159	Y	
S2-43	33, 101	2	NA	148	NA	Y	
S2-44	69, 102, 180	2	95	113	0-333	Y	
S2-45	64, 106	2	NA	139	NA		
S2-46	28, 97, 169	2	130	132	0-272	Y	
S2-47	52, 99	2	NA	130	NA	Y	
S2-48	30, 89, 169	2	100	99	67-131	Y	
S2-49	28, 89, 166	2	45	41	0-260	Y	
S2-50	34, 91, 173	2	76	93	0-334	Y	
S2-51	17, 39, 89, 180	2	55	68	4-133		
S2-52	43, 94, 170	2	142	142	4-281	Y	
S2-53	9, 16, 28, 97	2	NA	88	0-198	Y	
S2-54	31, 91, 168	2	89	125	0-553	Y	
S2-55	14, 28, 91, 162	2	78	75	36-113	Y	
S2-56	44, 87	2	85	84	NA		
S2-57	14, 28, 91	2	88	84	0-274	Y	
S2-58	28, 91	2	NA	114	NA	Y	
S2-59	16, 28, 77	2	27	42	0-187	Y	
S2-60	18, 28, 88	2	26	35	0-153	Y	
S2-61	14, 28, 79	2	50	49	3-96	Y	

S2-62	14, 29, 81	2	78	78	0-270	Y	
S2-63	20, 28, 78	2	NA	94	77-111	Y	
S2-64	28, 90, 180	3	NA	217	0-537	Y	Y
S2-65	14, 28, 90, 180	3	NA	261	229-294	Y	
S2-66	23, 31, 102, 180	3	NA	171	0-419	Y	
S2-67	23, 31, 102, 180	3	NA	288	200-376	Y	
S2-68	13, 20, 27, 90, 188	3	NA	231	47-416	Y	
S2-69	14, 22, 29, 92, 182	3	NA	178	98-259	Y	
S2-70	18, 25, 95, 180	3	NA	215	0-522	Y	Y
S2-71	18, 25, 32, 110, 193	3	NA	197	76-318	Y	
S2-72	19, 26, 96, 180	3	178	162	86-238	Y	
S2-73	19, 25, 92, 179	3	NA	184	46-321	Y	
S2-74	17, 23, 30, 92, 179	3	NA	308	197-419	Y	Y
S2-75	34, 92, 183	3	NA	160	0-773	Y	
S2-76	15, 31, 89, 180	3	NA	248	0-513	Y	Y
S2-77	20, 29, 91, 180	3	166	158	94-222	Y	
S2-78	17, 29, 86, 179	3	160	156	66-245	Y	
S2-79	16, 31, 106, 181	3	NA	190	170-210	Y	
S2-80	27, 92, 187	3	NA	336	0-1104	Y	
S2-81	33, 91, 190	3	165	163	95-231	Y	
S2-82	35, 86, 192	3	115	146	0-719	Y	
S2-83	20, 33, 96, 210	3	155	159	32-285	Y	
S2-84	16, 28, 89	3	NA	201	0-491	Y	
S2-85	35, 106, 181	3	NA	233	0-574	Y	Y
S2-86	18, 31, 97, 180	3	NA	307	212-402	Y	
S2-87	31, 86, 184	3	NA	209	0-817	Y	
S2-88	46, 103, 208	3	NA	174	0-1112	Y	

S2-89	44, 99, 172	3	NA	277	0-825	Y	
S2-90	32, 95, 180	3	130	150	0-449	Y	
S2-91	54, 106, 183	3	NA	168	0-547	Y	
S2-92	43, 99, 175	3	166	163	0-333	Y	
S2-93	28, 98, 177	3	NA	275	0-940	Y	Y
S2-94	15, 29, 177	3	NA	321	46-595	Y	
S2-95	20, 43, 103, 180	3	165	149	56-242	Y	
S2-96	33, 89, 180	3	NA	259	0-678	Y	
S2-97	31, 120, 175	3	NA	320	0-802	Y	
S2-98	28, 97, 187	3	NA	228	151-305	Y	
S2-99	72, 94, 168	3	NA	211	16-407		
S2-100	64, 91, 182	3	NA	212	7-416		
S2-101	17, 30, 90	3	NA	201	98-304	Y	
S2-102	31, 86	3	NA	244	NA	Y	
S2-103	29, 91, 168	3	NA	202	43-362	Y	
S2-104	28, 93, 170	3	160	160	0-550	Y	
S2-105	9, 16, 28, 97	3	NA	164	125-202	Y	
S2-106	36, 93, 167	3	NA	175	0-508	Y	
S2-107	35, 90, 168	3	NA	146	0-638	Y	
S2-108	23, 90	3	NA	320	NA		
S2-109	29, 87	3	NA	178	NA		
S2-110	28, 90, 180	4	NA	574	0-1928	Y	
S2-111	28, 90, 180	4	NA	1516	0-7466	Y	
S2-112	14, 28, 90, 180	4	NA	737	225-1250	Y	
S2-113	7, 14, 28, 90, 180	4	NA	693	174-1213		
S2-114	14, 28, 90, 180	4	NA	360	209-511	Y	
S2-115	20, 27, 94, 180, 187	4	NA	464	347-581	Y	
S2-116	20, 28, 91	4	NA	NA	NA		
S2-117	14, 21, 28, 96, 181	4	NA	931	407-1456	Y	
S2-118	11, 17, 24, 90	4	NA	343	74-613		
S2-119	16, 23, 33, 93, 170	4	NA	1704	155-3253	Y	Y
S2-120	14, 21, 34, 90, 184	4	NA	514	381-647	Y	
S2-121	12, 19, 31, 88, 183	4	NA	686	0-2699	Y	

S2-122	22, 32, 90, 180, 207	4	NA	375	327-423	Y	
S2-123	20, 28, 90, 180	4	NA	348	0-3437	Y	
S2-124	29, 86, 178	4	NA	538	101-974	Y	
S2-125	30, 93, 185	4	NA	361	0-1583	Y	
S2-126	7, 15, 27, 96, 179	4	NA	867	162-1572	Y	Y
S2-127	15, 28, 96, 167	4	NA	1663	0-4733	Y	
S2-128	16, 33, 97, 180	4	NA	530	140-921	Y	
S2-129	18, 32, 99, 183	4	NA	368	0-1395	Y	
S2-130	16, 25, 99, 175	4	NA	651	400-902	Y	Y
S2-131	32, 100, 179	4	NA	1884	0-18628	Y	Y
S2-132	15, 30, 107, 178	4	NA	500	0-1135	Y	
S2-133	59, 120, 181	4	NA	326	0-1735	Y	
S2-134	33, 110, 167	4	NA	14881	1-119570	Y	
S2-135	27, 103, 183	4	NA	2186	0-4988	Y	Y
S2-136	26, 46, 101, 179	4	NA	431	0-875	Y	
S2-137	16, 29, 101, 182	4	NA	474	202-745	Y	
S2-138	29, 114, 178	4	NA	NA	NA	Y	
S2-139	20, 34, 100, 178	4	NA	2161	0-10142	Y	
S2-140	19, 32, 98, 179	4	NA	416	387-444	Y	
S2-141	34, 98, 180	4	NA	4287	0-69054	Y	
S2-142	28, 98, 182	4	NA	4358	0-24274	Y	
S2-143	16, 29, 93, 180	4	NA	NA	NA	Y	
S2-144	17, 28, 90, 168	4	NA	3629	2456-4801	Y	Y
S2-145	48, 85, 167	4	NA	1384	0-5427	Y	
S2-146	30, 93, 179	4	NA	NA	NA	Y	
S2-147	30, 90, 180	4	NA	580	0-2620	Y	

S2-148	32, 91, 180	4	NA	8728	0-123605	Y	
S2-149	13, 27, 91, 176	4	NA	NA	NA	Y	
S2-150	29, 92, 179	4	NA	2682	0-11263	Y	
S2-151	28, 90, 178	4	NA	2172	0-18620	Y	
S2-152	19, 30, 91, 180	4	NA	520	0-1098	Y	
S2-153	71, 104, 167	4	NA	501	411-592		
S2-154	78, 179	4	NA	5497	NA		
S2-155	55, 103, 167	4	NA	364	0-1420		
S2-156	28, 91, 167	4	NA	956	0-8969	Y	
S2-157	42, 92, 175	4	NA	546	0-2389	Y	
S2-158	30, 89, 164	4	NA	566	0-3457		
S2-159	43, 93, 168	4	NA	NA	NA	Y	
S2-160	14, 28, 87	4	NA	NA	NA	Y	
S2-161	25, 92	4	NA	548	NA	Y	
S2-162	15, 23, 31, 92, 180	5	NA	NA	NA		
S2-163	57, 100, 167	5	NA	NA	NA		
S2-164	14, 28, 104, 182	5	NA	NA	NA		

Supplementary Table 2. Level of NAb determined by sVNT for SARS-CoV

Sample group	Sample #	% inhibition
SARS patients recalled in 2020 (17 years after initial infection)	S1-1	96.92
	S1-2	81.92
	S1-3	75.83
	S1-4	99.46
	S1-5	51.08
	S1-6	94.66
	S1-7	83.72
	S1-8	80.96
	S1-9	82.76
	S1-10	27.56
	S1-11	99.60
	S1-12	72.10
	S1-13	98.41
	S1-14	99.07
	S1-15	84.39
	S1-16	99.07
	S1-17	86.77
	S1-18	21.19
	S1-19	85.89
	S1-20	82.50
COVID-19 patients sampled in 2020	S2-165	-6.38
	S2-166	-1.81
	S2-167	2.83
	S2-168	-0.86
	S2-169	6.82
	S2-170	11.39
Healthy controls sampled before December 2019	HC-1	11.60
	HC-2	15.59
	HC-3	18.72
	HC-4	25.86
	HC-5	-4.10
	HC-6	1.86
	HC-7	4.49
	HC-8	8.76
	HC-9	9.32
	HC-10	18.42

Supplementary Table 3. Clinical characteristics of cohort subset selected for T cell analysis

Patient characteristics	**T cell subset (n = 23)**
Age, years	52 (25 – 72)
Sex, female	10 (43.5%)
Ethnicity	
Chinese	20 (87.0%)
Malay	2 (8.7%)
South Asian (Indian / Bangladeshi)	0 (0.0%)
Others	1 (4.3%)
Any comorbidity	12 (52.2%)
Diabetes	4 (17.4%)
Hypertension	9 (39.1%)

Supplementary Table 4. SARS-CoV-2 overlapping peptide library used in T cell assays

NP-1 peptide pool		**NP-2 peptide pool**	
Amino Acid Sequence	**Amino Acid Position**	**Amino Acid Sequence**	**Amino Acid Position**
MSDNGPQNQRNAPRI	1-15	SPARMAGNGGDAALA	206-220
PQNQRNAPRITFGGP	6-20	AGNGGDAALALLLLD	211-225
NAPRITFGGPSDSTG	11-25	DAALALLLLDRLNQL	216-230
TFGGPSDSTGSNQNG	16-30	LLLLDRLNQLESKMS	221-235
SDSTGSNQNGERSGA	21-35	RLNQLESKMSGKGQQ	226-240
SNQNGERSGARSKQR	26-40	ESKMSGKGQQQQGQT	231-245
ERSGARSKQRRPQGL	31-45	GKGQQQQGQTVTKKS	236-250
RSKQRRPQGLPNNTA	36-50	QQGQTVTKKSAAEAS	241-255
RPQGLPNNTASWFTA	41-55	VTKKSAAEASKKPRQ	246-260
PNNTASWFTALTQHG	46-60	AAEASKKPRQKRTAT	251-265
SWFTALTQHGKEDLK	51-65	KKPRQKRTATKAYNV	256-270
LTQHGKEDLKFPRGQ	56-70	KRTATKAYNVTQAFG	261-275
KEDLKFPRGQGVPIN	61-75	KAYNVTQAFGRRGPE	266-280
FPRGQGVPINTNSSP	66-80	TQAFGRRGPEQTQGN	271-285
GVPINTNSSPDDQIG	71-85	RRGPEQTQGNFGDQE	276-290
TNSSPDDQIGYYRRA	76-90	QTQGNFGDQELIRQG	281-295
DDQIGYYRRATRRIR	81-95	FGDQELIRQGTDYKH	286-300
YYRRATRRIRGGDGK	86-100	LIRQGTDYKHWPQIA	291-305
TRRIRGGDGKMKDLS	91-105	TDYKHWPQIAQFAPS	296-310
GGDGKMKDLSPRWYF	96-110	WPQIAQFAPSASAFF	301-315
MKDLSPRWYFYYLGT	101-115	QFAPSASAFFGMSRI	306-320
PRWYFYYLGTGPEAG	106-120	ASAFFGMSRIGMEVT	311-325
YYLGTGPEAGLPYGA	111-125	GMSRIGMEVTPSGTW	316-330
GPEAGLPYGANKDGI	116-130	GMEVTPSGTWLTYTG	321-335
LPYGANKDGIIWVAT	121-135	PSGTWLTYTGAIKLD	326-340
NKDGIIWVATEGALN	126-140	LTYTGAIKLDDKDPN	331-345
IWVATEGALNTPKDH	131-145	AIKLDDKDPNFKDQV	336-350
EGALNTPKDHIGTRN	136-150	DKDPNFKDQVILLNK	341-355
TPKDHIGTRNPANNA	141-155	FKDQVILLNKHIDAY	346-360
IGTRNPANNAAIVLQ	146-160	ILLNKHIDAYKTFPP	351-365
PANNAAIVLQLPQGT	151-165	HIDAYKTFPPTEPKK	356-370
AIVLQLPQGTTLPKG	156-170	KTFPPTEPKKDKKKK	361-375
LPQGTTLPKGFYAEG	161-175	TEPKKDKKKKADETQ	366-380
TLPKGFYAEGSRGGS	166-180	DKKKKADETQALPQR	371-385
FYAEGSRGGSQASSR	171-185	ADETQALPQRQKKQQ	376-390

SRGGSQASSRSSSRS	176-190	ALPQRQKKQQTVTLL	381-395
QASSRSSSRSRNSSR	181-195	QKKQQTVTLLPAADL	386-400
SSSRSRNSSRNSTPG	186-200	TVTLLPAADLDDFSK	391-405
RNSSRNSTPGSSRGT	191-205	PAADLDDFSKQLQQS	396-410
NSTPGSSRGTSPARM	196-210	DDFSKQLQQSMSSAD	401-415
SSRGTSPARMAGNGG	201-215	QLQQSMSSADSTQA	406-419

M peptide pool		**ORF3a peptide pool**	
Amino Acid Sequence	**Amino Acid Position**	**Amino Acid Sequence**	**Amino Acid Position**
MADSNGTITVEELKK	1-15	MDLFMRIFTIGTVTL	1-15
GTITVEELKKLLEQW	6-20	RIFTIGTVTLKQGEI	6-20
EELKKLLEQWNLVIG	11-25	GTVTLKQGEIKDATP	11-25
LLEQWNLVIGFLFLT	16-30	KQGEIKDATPSDFVR	16-30
NLVIGFLFLTWICLL	21-35	KDATPSDFVRATATI	21-35
FLFLTWICLLQFAYA	26-40	SDFVRATATIPIQAS	26-40
WICLLQFAYANRNRF	31-45	ATATIPIQASLPFGW	31-45
QFAYANRNRFLYIIK	36-50	PIQASLPFGWLIVGV	36-50
NRNRFLYIIKLIFLW	41-55	LPFGWLIVGVALLAV	41-55
LYIIKLIFLWLLWPV	46-60	LIVGVALLAVFQSAS	46-60
LIFLWLLWPVTLACF	51-65	ALLAVFQSASKIITL	51-65
LLWPVTLACFVLAAV	56-70	FQSASKIITLKKRWQ	56-70
TLACFVLAAVYRINW	61-75	KIITLKKRWQLALSK	61-75
VLAAVYRINWITGGI	66-80	KKRWQLALSKGVHFV	66-80
YRINWITGGIAIAMA	71-85	LALSKGVHFVCNLLL	71-85
ITGGIAIAMACLVGL	76-90	GVHFVCNLLLLFVTV	76-90
AIAMACLVGLMWLSY	81-95	CNLLLLFVTVYSHLL	81-95
CLVGLMWLSYFIASF	86-100	LFVTVYSHLLLVAAG	86-100
MWLSYFIASFRLFAR	91-105	YSHLLLVAAGLEAPF	91-105
FIASFRLFARTRSMW	96-110	LVAAGLEAPFLYLYA	96-110
RLFARTRSMWSFNPE	101-115	LEAPFLYLYALVYFL	101-115
TRSMWSFNPETNILL	106-120	LYLYALVYFLQSINF	106-120
SFNPETNILLNVPLH	111-125	LVYFLQSINFVRIIM	111-125
TNILLNVPLHGTILT	116-130	QSINFVRIIMRLWLC	116-130
NVPLHGTILTRPLLE	121-135	VRIIMRLWLCWKCRS	121-135
GTILTRPLLESELVI	126-140	RLWLCWKCRSKNPLL	126-140
RPLLESELVIGAVIL	131-145	WKCRSKNPLLYDANY	131-145
SELVIGAVILRGHLR	136-150	KNPLLYDANYFLCWH	136-150
GAVILRGHLRIAGHH	141-155	YDANYFLCWHTNCYD	141-155

RGHLRIAGHHLGRCD	146-160	FLCWHTNCYDYCIPY	146-160
IAGHHLGRCDIKDLP	151-165	TNCYDYCIPYNSVTS	151-165
LGRCDIKDLPKEITV	156-170	YCIPYNSVTSSIVIT	156-170
IKDLPKEITVATSRT	161-175	NSVTSSIVITSGDGT	161-175
KEITVATSRTLSYYK	166-180	SIVITSGDGTTSPIS	166-180
ATSRTLSYYKLGASQ	171-185	SGDGTTSPISEHDYQ	171-185
LSYYKLGASQRVAGD	176-190	TSPISEHDYQIGGYT	176-190
LGASQRVAGDSGFAA	181-195	EHDYQIGGYTEKWES	181-195
RVAGDSGFAAYSRYR	186-200	IGGYTEKWESGVKDC	186-200
SGFAAYSRYRIGNYK	191-205	EKWESGVKDCVVLHS	191-205
YSRYRIGNYKLNTDH	196-210	GVKDCVVLHSYFTSD	196-210
IGNYKLNTDHSSSSD	201-215	VVLHSYFTSDYYQLY	201-215
LNTDHSSSSDNIALL	206-220	YFTSDYYQLYSTQLS	206-220
SSSSDNIALLVQ	211-222	YYQLYSTQLSTDTGV	211-225
		STQLSTDTGVEHVTF	216-230
		TDTGVEHVTFFIYNK	221-235
		EHVTFFIYNKIVDEP	226-240
		FIYNKIVDEPEEHVQ	231-245
		IVDEPEEHVQIHTID	236-250
		EEHVQIHTIDGSSGV	241-255
		IHTIDGSSGVVNPVM	246-260
		GSSGVVNPVMEPIYD	251-265
		VNPVMEPIYDEPTTT	256-270
		EPIYDEPTTTTSVPL	261-275

S peptide pool		**ORF7/8 peptide pool**	
Amino Acid Sequence	**Amino Acid Position**	**Amino Acid Sequence**	**Amino Acid Position**
IRGWIFGTTLDSKTQ	101-115	MKIILFLALITLATC	1-15
FGTTLDSKTQSLLIV	106-120	FLALITLATCELYHY	6-20
CTFEYVSQPFLMDLE	166-180	TLATCELYHYQECVR	11-25
VSQPFLMDLEGKQGN	171-185	ELYHYQECVRGTTVL	16-30
TRFQTLLALHRSYLT	236-250	QECVRGTTVLLKEPC	21-35
LLALHRSYLTPGDSS	241-255	GTTVLLKEPCSSGTY	26-40
RSYLTPGDSSSGWTA	246-260	LKEPCSSGTYEGNSP	31-45
CALDPLSETKCTLKS	291-305	SSGTYEGNSPFHPLA	36-50
LSETKCTLKSFTVEK	296-310	EGNSPFHPLADNKFA	41-55
CTLKSFTVEKGIYQT	301-315	FHPLADNKFALTCFS	46-60
FTVEKGIYQTSNFRV	306-320	DNKFALTCFSTQFAF	51-65

GIYQTSNFRVQPTES	311-325	LTCFSTQFAFACPDG	56-70
SASFSTFKCYGVSPT	371-385	TQFAFACPDGVKHVY	61-75
TFKCYGVSPTKLNDL	376-390	ACPDGVKHVYQLRAR	66-80
YNYKLPDDFTGCVIA	421-435	VKHVYQLRARSVSPK	71-85
WNSNNLDSKVGGNYN	436-450	QLRARSVSPKLFIRQ	76-90
LDSKVGGNYNYLYRL	441-455	SVSPKLFIRQEEVQE	81-95
GGNYNYLYRLFRKSN	446-460	LFIRQEEVQELYSPI	86-100
YLYRLFRKSNLKPFE	451-465	EEVQELYSPIFLIVA	91-105
FRKSNLKPFERDIST	456-470	LYSPIFLIVAAIVFI	96-110
LKPFERDISTEIYQA	461-475	FLIVAAIVFITLCFT	101-115
GPKKSTNLVKNKCVN	526-540	AIVFITLCFTLKRKT	106-120
TNLVKNKCVNFNFNG	531-545	TLCFTLKRKTE	111-121
FNFNGLTGTGVLTES	541-555	MIELSLIDFYLCFLA	1-15
LTGTGVLTESNKKFL	546-560	VLIMLIIFWFSLELQ	21-35
RAGCLIGAEHVNNSY	646-660	IIFWFSLELQDHNET	26-40
IGAEHVNNSYECDIP	651-665	SLELQDHNETCHA	31-43
SVASQSIIAYTMSLG	686-700	MKFLVFLGIITTVAA	1-15
SIIAYTMSLGAENSV	691-705	FLGIITTVAAFHQEC	6-20
TMSLGAENSVAYSNN	696-710	TTVAAFHQECSLQSC	11-25
STECSNLLLQYGSFC	746-760	FHQECSLQSCTQHQP	16-30
NLLLQYGSFCTQLNR	751-765	SLQSCTQHQPYVVDD	21-35
KNTQEVFAQVKQIYK	776-790	TQHQPYVVDDPCPIH	26-40
VFAQVKQIYKTPPIK	781-795	YVVDDPCPIHFYSKW	31-45
KQIYKTPPIKDFGGF	786-800	PCPIHFYSKWYIRVG	36-50
TPPIKDFGGFNFSQI	791-805	FYSKWYIRVGARKSA	41-55
NFSQILPDPSKPSKR	801-815	YIRVGARKSAPLIEL	46-60
AGFIKQYGDCLGDIA	831-845	ARKSAPLIELCVDEA	51-65
QYGDCLGDIAARDLI	836-850	PLIELCVDEAGSKSP	56-70
GAALQIPFAMQMAYR	891-905	CVDEAGSKSPIQYID	61-75
QMAYRFNGIGVTQNV	901-915	GSKSPIQYIDIGNYT	66-80
FNGIGVTQNVLYENQ	906-920	IQYIDIGNYTVSCLP	71-85
DSLSSTASALGKLQD	936-950	IGNYTVSCLPFTINC	76-90
TASALGKLQDVVNQN	941-955	VSCLPFTINCQEPKL	81-95
AQALNTLVKQLSSNF	956-970	FTINCQEPKLGSLVV	86-100
VLNDILSRLDKVEAE	976-990	QEPKLGSLVVRCSFY	91-105
LITGRLQSLQTYVTQ	996-1010	GSLVVRCSFYEDFLE	96-110
QLIRAAEIRASANLA	1011-1025	RCSFYEDFLEYHDVR	101-115
AEIRASANLAATKMS	1016-1030	EDFLEYHDVRVVLDF	106-120
APHGVVFLHVTYVPA	1056-1070	YHDVRVVLDFI	111-121

HWFVTQRNFYEPQII	1101-1115		
KEIDRLNEVAKNLNE	1181-1195		
LNEVAKNLNESLIDL	1186-1200		
KNLNESLIDLQELGK	1191-1205		
IWLGFIAGLIAIVMV	1216-1230		