Divergent trajectories of antiviral memory after SARS-Cov-2 infection

Tomic et al

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Table S1 Demographics

		Asymptomatic		Mild		Severe		Total	
Number enrolled		**12**	**% of asymp**	**66**	**% of mild**	**7**	**% of severe**	**85**	**% of total**
Sex	**Female**	11	92%	48	73%	2	29%	61	72%
	Male	1	8%	18	27%	5	71%	24	28%
Age range (at recruitment)	**20-29**	2	17%	21	32%	0	0%	23	27%
	30-39	4	33%	17	26%	0	0%	21	25%
	40-49	2	17%	14	21%	2	29%	18	21%
	50-59	3	25%	14	21%	4	57%	21	25%
	60-69	1	8%	0	0%	1	14%	2	2%
Ethnicity	**Asian**	1	8%	10	15%	1	14%	12	14%
	Black	1	8%	0	0%	1	14%	2	2%
	White	10	83%	48	73%	3	43%	61	72%
	Other	0	0%	4	6%	1	14%	5	6%
	Unknown	0	0%	4	6%	1	14%	5	6%

Table S2. Summary Table of Descriptive Statistics for Humoral Assays

Day	anti-SARS-CoV-2 nucleocapsid tIgG ELISA (ELISA AU)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	3.40 (2.17-4.31)	39	3.64 (1.09-5.84)	0	ND
28	11	2.15 (1.61-3.81)	60	5.58 (3.83-6.5)	0	ND
56	9	2.45 (1.24-3.44)	63	5.16 (3.09-6.21)	0	ND
90	9	1.46 (0.80-2.24)	51	4.39 (2.41-5.68)	0	ND
120	9	1.22 (0.55-1.51)	51	3.39 (1.50-4.91)	0	ND
180	12	0.84 (0.33-1.12)	64	2.03 (0.72-3.66)	0	ND
Day	anti-SARS-CoV-2 spike tIgG ELISA (ELISA AU)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	341.8 (241.0-897.1)	42	122.4 (61.5-754.8)	8	1182.0 (387.7-1947.0)
28	11	427.2 (260.1-753.0)	64	504.1 (196.6-1361.0)	1	2404.0 (2404.0-2404.0)
56	9	424.4 (287.4-788.3)	62	677.9 (236.1-1532.0)	1	1837.0 (1837.0-1837.0)
90	10	229.8 (114.4-404.9)	51	525.4 (178.5-1006.0)	0	ND
120	10	221.0 (115.9-290.2)	53	365.3 (192.9-765.3)	0	ND
180	12	257.7 (134.7-400.4)	65	399.4 (180.5-710.1)	8	1401.0 (903.6-1616.0)
Day	Pseudoneutralising antibodies (IC50)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	226.20 (118.90-701.60)	40	1261.00 (447.90-3813.00)	8	4923.00 (2135.00-30032.00)
28	11	126.10 (53.86-206.00)	64	817.50 (345.00-2954.00)	0	ND
56	8	127.70 (83.88-242.70)	63	441.8 (212.90-913.00)	1	1346.00 (1346.00-1346.00)
90	10	142.60 (80.18-294.00)	51	393.20 (170.20-747.60)	0	ND
120	10	98.96 (71.32-174.50)	51	243.40 (130.80-430.40)	0	ND
180	12	89.76 (52.01-148.70)	65	182.50 (99.29-479.40)	8	765.1 (457.00-1619.00)
Day	anti SARS-CoV-2-spike IgG memory B cell ELISPOT (ASCs per million PBMCs)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	176.70 (108.30-207.50)	10	25.80 (5.85-49.15)	8	49.00 (26.50-131.80)
28	9	231.70 (180.00-477.40)	44	112.50 (30.43-246.30)	0	ND
56	8	145.90 (34.18-260.00)	35	186.70 (126.70-293.30)	0	ND
90	8	196.70 (153.30-211.40)	31	166.70 (95.00-270.00)	0	ND
120	6	227.70 (145.50-311.10)	30	119.00 (77.50-289.00)	0	ND
180	12	134.2 (95.00-173.70)	47	130.00 (58.30-230.00)	8	649.00 (236.00-1111.00)
Day	anti-SARS-CoV-2 spike IgA memory B cell ELISPOT (ASCs per million PBMCs)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	2.50 (0.00-5.00)	10	0.00 (0.00-10.40)	8	5.00 (2.25-56.50)
28	9	0.00 (0.00-7.50)	44	0.00 (0.00-5.00)	0	ND
56	8	0.00 (0.00-5.03)	35	0.00 (0.00-5.00)	0	ND
90	8	0.00 (0.00-0.00)	31	0.00 (0.00-0.00)	0	ND
120	6	0.00 (0.00-8.75)	30	0.00 (0.00-1.25)	0	ND
180	12	0.00 (0.00-1.46)	47	0.00 (0.00-5.00)	8	0.00 (0.00-12.93)

Day	IgG1 (ELISA AU)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	101.50 (18.00-777.50)	10	18.99 (18.00-97.31)	8	1500.00 (1193.00-5106.00)
28	11	18.00 (18.00-612.80)	20	226.9 (54.58-834.30)	1	6835.00 (6835.00-6835.00)
56	9	74.19 (18.00-357.80)	19	338.5 (34.73-826.90)	1	3984.00 (3984.00-3984.00)
90	10	92.07 (18.00-295.90)	15	284.7 (78.74-765.10)	0	ND
120	10	126.40 (18.00-499.60)	15	286 (34.51-818.90)	0	ND
180	12	154.60 (18.00-803.90)	20	459.5 (30.58-941.40)	8	2047.00 (1309.00-3482.00)
Day	IgG2 (OD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	0.123 (0.118-0.245)	10	0.1145 (0.097-0.1728)	8	0.123 (0.098-0.1485)
28	11	0.099 (0.094-0.125)	20	0.1088 (0.099-0.1449)	1	0.148
56	9	0.097 (0.0905-0.1008)	19	0.117 (0.093-0.15)	1	0.155
90	10	0.097 (0.0935-0.1083)	15	0.1 (0.092-0.14)	0	ND
120	10	0.0985 (0.08725-0.1118)	15	0.112 (0.102-0.148)	0	ND
180	12	0.1015 (0.09175-0.1075)	20	0.109 (0.099-0.1295)	8	0.1255 (0.1058-0.1738)
Day	IgG3 (ELISA AU)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	38.08 (27.73-64.09)	10	24.47 (8.00-250.8)	8	254.1 (93.7-553.4)
28	11	18.7 (10.16-49.76)	20	41.99 (16.8-760.4)	1	379.5
56	9	22.76 (10.56-28.34)	19	33.91 (10.59-443.2)	1	605.4
90	10	12.89 (8.00-37.95)	15	32.12 (14.97-256.7)	0	ND
120	10	13.74 (8.00-31.57)	15	29.88 (14.67-217.1)	0	ND
180	12	9.068 (8.00-13.79)	20	17.81 (8.000-65.97)	8	50.39 (19.9-109)
Day	IgG4 (OD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	0.0605 (0.058-0.064)	10	0.06275 (0.058-0.08113)	8	0.082 (0.08075-0.9225)
28	11	0.08 (0.063-0.0835)	20	0.0815 (0.06088-0.08713)	1	0.0795
56	9	0.0845 (0.08225-0.08575)	19	0.08550 (0.0825-0.087)	1	0.0825
90	10	0.08325 (0.08013-0.08888)	15	0.08550 (0.082-0.0895)	0	ND
120	10	0.083 (0.08038-0.09)	15	0.084 (0.0815-0.0885)	0	ND
180	12	0.08125 (0.078-0.086)	20	0.084 (0.07963-0.08888)	8	0.8725 (0.082-0.093)
Day	ADNKA (% CD107a expressing NK cells)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	8.475 (4.188-14.49)	6	13.15 (1.331-19.08)	6	23.91 (20.46-28.12)
28	10	10.76 (3.761-19.68)	17	11.76 (2.77-21.4)	0	ND
56	7	22.54 (12.69-31.59)	18	19.03 (10.2-22.1)	0	ND
90	9	15.25 (7.17-22.02)	13	21.39 (10.62-32.07)	0	ND
120	10	22.62 (14.5-24.4)	15	16.29 (13.79-18.03)	0	ND
180	12	18.54 (8.1-21.44)	20	15.51 (8.6-19.09)	8	23.41 (21.56-25.88)

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Day	ADNP (phagocytic score)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	0	ND	1	0.7071	7	0.2603 (0.1618-0.6023)
28	6	0.2612 (-0.02452-0.41)	12	0.5654 (0.1024-1.154)	1	0.7829
56	9	0.2087 (0.03429-0.487)	17	0.5863 (0.07072-0.9166)	1	0.8277
90	9	0.1695 (-0.01632-0.4668)	13	0.1808 (-0.006721-0.9186)	0	ND
120	8	0.1283 (0.02477-0.3941)	13	0.416 (0.2192-1.087)	0	ND
180	12	0.2889 (0.06631-0.6443)	18	0.685 (0.2207-1.073)	8	0.9643 (0.7522-1.110)
Day	ADMP (phagocytic score)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	0	ND	1	1.104	4	0.8948 (0.4953-1.231)
28	6	0.2983 (0.04294-0.5566)	13	0.5197 (0.1747-1.087)	1	0.7361
56	9	0.3268 (0.1715-0.5015)	18	0.5135 (0.1884-0.8254)	1	1.06
90	9	0.2479 (0.07806-0.6466)	13	0.4937 (0.2748-0.9776)	0	ND
120	8	0.2539 (0.1134-0.4688)	13	0.4637 (0.2604-0.8632)	0	ND
180	11	0.2683 (0.06291-0.4265)	18	0.5556 (0.2570-0.8358)	4	0.8732 (0.6285-0.9976)
Day	ADCD (complement arbitrary units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	9	70.71 (26.23-83.65)	8	44.08 (14.62-706.5)	7	521.1 (496.7-702.8)
28	10	29.58 (23.18-63.61)	19	78.25 949.60-581.1)	1	601.5
56	9	28.83 (16.79-66.81)	19	143.3 (38.75-408.9)	1	459.7
90	9	20.88 (12.92-53.86)	14	75.03 (53.85-225.2)	0	ND
120	10	19.63 (12.38-33.38)	14	84.5 (47.04-148.6)	0	ND
180	10	25.76 (17.16-50.46)	18	65.62 (39.92-131.7)	8	92.56 (68.07-213.7)
Day	MSD-CoV-2-N (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	23874 (10566-63490)	12	22008 (2160-288100)	8	351347 (56110-909112)
28	11	9125 (5550-26640)	59	48980 (11134-150139)	1	759067
56	7	14656 (6139-28209)	22	79304 (25109-122795)	1	1060397
90	9	18875 (6235-24250)	15	66980 (21635-100886)	0	ND
120	10	9668 (4468-18295)	15	41029 (17680-63060)	0	ND
180	11	1450 (800-4315)	65	15270 (4603-34135)	8	66976 (28763-130158)
Day	MSD-CoV-2-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	17508 (13239-35111)	12	2603 (1121-119940)	8	101430 (50745-151061)
28	11	9775 (4675-15650)	59	15934 (6625-58090)	1	172859
56	7	11971 (8063-25715)	22	49465 (12132-103915	1	194617
90	9	10325 (8005-19413)	15	35620 (9060-98421)	0	ND
120	10	11226 (8216-17903)	15	27767 (13731-63222)	0	ND
180	11	5865 (2385-9245)	65	14610 (7848-27165)	8	76010 (50080-83777)

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Day	MSD-CoV-2-RBD (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	5162 (3514-13439)	12	1137 (428.5-42463)	8	28094 (10097-40119)
28	11	2640 (1825-5985)	59	6389 (2260-16863)	1	62130
56	7	4724 (2667-8375)	22	16408 (2052-38674)	1	73667
90	9	4110 (2913-5609)	15	21405 (2225-27426)	0	ND
120	10	4292 (2454-5672)	15	15729 (3969-24675)	0	ND
180	11	1280 (575-3015)	65	5470 (2618-11740)8	8	27437 (19479-35138)
Day	MSD-CoV-1-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	2751 (1765-4642)	12	1469 (558.3-8282)	8	11247 (7049-45153)
28	11	1420 (1140-2640)	59	4827 (930-16094)	1	13838
56	7	2049 (1277-5780)	22	6841 (3024-15560)	1	20930
90	9	1710 (1458-4164)	15	7225(2365-19446)	0	ND
120	10	2338 (1670-3455)	15	5045 (3104-9766)	0	ND
180	11	1840 (435-4870)	65	3895 (1908-7018)	8	15296 (7806-34528)
Day	MSD-MERS-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	540.2 (222.1-8731)	12	1325 (291.2-2613)	8	12607 (6286-24051)
28	11	505(260-1790)	59	1485 (326.2-9838)	1	68698
56	7	741.8 (212.1-5195)	22	2738 (735.7-11182)	1	79785
90	9	565 (282.5-2355)	15	3115 (655-8415)	0	ND
120	10	856.2 (353.2-1256)	15	1875 (650-3528)	0	ND
180	11	856.3 (353.2-1256)	65	1300 (335-5815)	8	3635 (1612-7713)
Day	MSD-229E-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	24102 (13468-48192)	12	41196 (11351-59135)	8	33216 (7191-51864)
28	11	13690 (5670-22120)	59	27464 (11980-46933)	1	45764
56	7	26430 (14338-35753)	22	39582 (26185-71234)	1	76423
90	9	18135 (10473-37915)	15	45493 (34515-74800)	0	ND
120	10	28051 (11973-46341)	15	44060 (33901-73586)	0	ND
180	11	9600 (5190-18660)	65	28410 (17525-47678)	8	37402 (17322-70656)
Day	MSD-HKU1-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	16511 (11681-36256)	12	30221 (6930-50917)	8	60514 (27302-92141)
28	11	10980 (6140-19060)	59	33253 (16048-65480)	1	85830
56	7	13990 (8453-47818)	22	32100 (23770-51565)	1	133025
90	9	12306 (9380-34408)	15	24057 (15220-52627)	0	ND
120	10	11651 (7043-30534)	15	24057 (15220-52627)	0	ND
180	11	6490 (3770-18410)	65	16960 (10873-28140)	8	42123 (14326-62614)

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Day	MSD-NL63-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	5522 (4311-9005)	12	3331 (1781-11318)	8	5252 (2413-10385)
28	11	5505 (2305-7765)	59	3855 (1760-9555)	1	10914
56	7	7673 (6180-11166)	22	7109 (2895-11623)	1	19765
90	9	5759 (5283-11355)	15	8040 (3510-15480)	0	ND
120	10	7819 (3625-11602)	15	8096 (3790-11090)	0	ND
180	11	3780 (2600-5445)	65	4590 (2595-9140)	8	5614 (4137-7214)
Day	MSD-OC43-S (MSD units)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	6	49070 (38844-181033)	12	61023 (26744-131983)	8	168319 (94347-364668)
28	11	44025 (28820-81555)	59	99657 (30725-176897)	1	377370
56	7	48304 (41330-82764)	22	82329 (51781-152439)	1	506545
90	9	54585 (35190-79453)	15	79865 (48805-189773)	0	ND
120	10	44648 (32552-80433)	15	78449 (61302-87133)	0	ND
180	11	29570 (21835-60550)	65	69440 (39608-87133)	8	118990
Day	anti-229E spike IgG memory B cell ELISPOT (ASCs per million PBMCs)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	5 (1.7-6.67)	10	5 (0-11.25)	6	6 (2.25-8.5)
28	2	14.17 (3.33-25)	10	10 (1.693-15)	0	ND
56	0	ND	0	ND	0	ND
90	0	ND	0	ND	0	ND
120	0	ND	0	ND	0	ND
180	11	2.5 (0-7.5)	36	6.67 (1.678-11.7)	5	1.67 (0.0-5.835)
Day	anti- HKU1 spike IgG memory B cell ELISPOT (ASCs per million PBMCs)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	28.3 (5-67.5)	10	187.7 (93.73-300)	6	97 (33.75-199.3)
28	2	85.85 (46.70-125)	9	105 (55-240)	0	ND
56	0	ND	0	ND	0	ND
90	0	ND	0	ND	0	ND
120	0	ND	0	ND	0	ND
180	11	11 (2.5-31.7)	35	16.7 (3.33-40)	5	16.67 (9.585-65.84)
Day	anti-spike NL63 IgG memory B cell ELISPOT (ASCs per million PBMCs)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	6.7 (5-18.3)	10	19.2 (11.28-40.43)	6	9 (0-18)
28	2	84.15 (10-158.3)	10	12.9 (9.575-41.18)	0	ND
56	0	ND	12	12.9 (2.1-22.48)	0	ND
90	0	ND	0	ND	0	ND
120	0	ND	0	ND	0	ND
180	11	10 (1.7-18.3)	24	5 (0.425-15.03)	5	0.00 (0.00-20.84)

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Day	anti-spike OC43 IgG memory B cell ELISPOT (ASCs per million PBMCs)					
	Asymptomatic		Mild		Severe	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
<20	7	43.4 (12.6-98.3)	11	141.7 (83.3-300)	6	141.5(34.5-272.8)
28	2	163.4 971.7-255)	10	160.9 (106.7-313.1)	0	ND
56	0	ND	0	ND	0	ND
90	0	ND	0	ND	0	ND
120	0	ND	0	ND	0	ND
180	11	21.7 (13.3-53.3)	35	26.7 (13.33-51.7)	5	20 (11.67-110.4)

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Table S3 Summary statistics for ex vivo interferon-gamma ELISpot assay

	d28 Median (IQR) N = 70	d28 No. +ve (%)	d90 Median (IQR) N = 64	d90 No. +ve (%)	d180 Median (IQR) N = 78	d180 No. +ve (%)	P value d28 VS d90	Sign.	P value d28 VS d180	Sign.	P value d90 VS d180	Sign.
Total positive pools	373 (201-842)	67/70 (96%)	240 (124-430)	56/63 (89%)	105 (40-218)	61/77 (79%)	0.0006	***	<0.0001	****	0.0002	***
Total spike	180 (71-364)	71/70 (87%)	100 (0-175)	41/63 (65%)	43 (0-103)	46/77 (60%)	0.0016	**	<0.0001	****	0.0262	*
S1	70 (37-171)	56/70 (80%)	50 (23-89)	39/63 (62%)	25 (15-60)	32/77. (40%)	0.0001	***	<0.0001	****	0.0508	ns
S2	83 (27-182)	53/70 (76%)	46 (28-84)	39/63 (62%)	30 (18-58)	33/77. (43%)	0.0027	**	<0.0001	****	0.1646	ns
M	63 (25-160)	47/70 (67%)	33 (15-74)	26/63 (41%)	17 (5-43)	24/77 (31%)	<0.0001	****	<0.0001	****	0.03	*
NP	121 (73-250)	62/70 (89%)	56 (33-95)	44/63 (70%)	40 (20-74)	47/77 (61%)	<0.0001	****	<0.0001	****	0.0343	*
ORF3	15 (5-38)	22/70 (31%)	8 (0-18)	6/63 (8%)	5 (0-13)	2/76 (3%)	0.0028	**	<0.0001	****	0.8959	ns
ORF8	3 (0-15)	6/70 (9%)	3 (0-13)	8/63 (13%)	0 (0-9)	3/76 (4%)	0.7126	ns	0.0368	*	0.5576	ns
NSP3	18 (3-48)	23/70 (33%)	8 (0-32)	15/63 (24%)	5 (0-15)	12/76 (16%)	0.0245	*	<0.0001	****	0.2946	ns
CEFT	488 (209-920)	65/70 (93%)	326 (128-908)	56/63 (89%)	288 (91-823)	66/77 (86%)	0.2034	ns	<0.0001	****	0.0508	ns

Ex vivo IFN-γ ELISpot showing the effector T cell responses to summed SARS-CoV-2 peptide pools spanning spike, accessory and structural proteins (S1, S2, M, NP, ORF 3, ORF8, and NSP3B, summed total of SARS-CoV-2 proteins tested and the CEFT positive control peptides for T cell responses) in 78 individuals 28, 90 and 180 days after SARS-CoV-2 (onset of symptoms for mild cases, PCR positive test for asymptomatic participants). SFC / million PBMCs = spot forming cells per million peripheral blood mononuclear cells, with background (negative control wells) subtracted, and a positive response = final count with background subtracted greater than mean + 2 SD of background. D28, d90 and d180 = days after SARS-CoV-2 diagnosis. Significance of differences between timepoints T1, T2 and T3 calculated by paired Friedman test with Dunn's multiple comparisons test for the n = 57 participants with assays at all three timepoints available.

Supplementary table 4 – Summary of tables for proliferation assay based on disease phenotype

		CD4+ T cells															
		CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180	CD4_ D28	CD4_ D180
	Disease Phenotype	**PHA**	**PHA**	**S1**	**S1**	**S2**	**S2**	**M**	**M**	**NP**	**NP**	**ORF3**	**ORF3**	**ORF8**	**ORF8**	**FECT**	**FECT**
Summary (All)	Numbers tested	56.0	56.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	56.0	56.0	54.0	54.0
	Number positive (>1%)	56.0	56.0	47.0	46.0	45.0	46.0	44.0	32.0	46.0	42.0	30.0	20.0	16.0	14.0	16.0	15.0
	% Positive (>1%)	100.0	100.0	82.5	80.7	78.9	80.7	77.2	56.1	80.7	73.7	52.6	35.1	28.6	25.0	29.6	27.8
Summary (asympto matic)	Numbers tested with peptide pool	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
	Number positive (>1%)	8.0	8.0	4.0	6.0	3.0	6.0	6.0	4.0	4.0	6.0	1.0	4.0	0.0	3.0	1.0	2.0
	% Positive (>1%)	100.0	100.0	50.0	75.0	37.5	75.0	75.0	50.0	50.0	75.0	12.5	50.0	0.0	37.5	12.5	25.0
Summary (Mild)	Numbers tested with peptide pool	48.0	48.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0
	Number positive (>1%)	48.0	48.0	43.0	40.0	42.0	40.0	38.0	28.0	42.0	36.0	29.0	16.0	16.0	11.0	15.0	13.0
	% Positive (>1%)	100.0	100.0	87.8	81.6	85.7	81.6	77.6	57.1	85.7	73.5	59.2	32.7	32.7	22.4	30.6	26.5

		CD8+ T cells															
		CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180	CD8_ D28	CD8_ D180
	Disease Phenotype	**PHA**	**PHA**	**S1**	**S1**	**S2**	**S2**	**M**	**M**	**NP**	**NP**	**ORF3**	**ORF3**	**ORF8**	**ORF8**	**FECT**	**FECT**
Summary (All)	Numbers tested	56.0	56.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	57.0	56.0	56.0	54.0	54.0
	Number positive (>1%)	56.0	56.0	44.0	29.0	39.0	32.0	37.0	19.0	49.0	36.0	21.0	10.0	5.0	5.0	29.0	24.0
	% Positive (>1%)	100.0	100.0	77.2	50.9	68.4	56.1	64.9	33.3	86.0	63.2	36.8	17.5	8.9	8.9	53.7	44.4
Summary (asympto matic)	Numbers tested with peptide pool	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
	Number positive (>1%)	8.0	8.0	2.0	1.0	1.0	2.0	4.0	3.0	5.0	5.0	0.0	3.0	0.0	1.0	2.0	4.0
	% Positive (>1%)	100.0	100.0	25.0	12.5	12.5	25.0	50.0	37.5	62.5	62.5	0.0	37.5	0.0	12.5	25.0	50.0
Summary (Mild)	Numbers tested with peptide pool	48.0	48.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0	49.0
	Number positive (>1%)	48.0	48.0	42.0	28.0	38.0	30.0	33.0	16.0	44.0	31.0	21.0	7.0	5.0	4.0	27.0	20.0
	% Positive (>1%)	100.0	100.0	85.7	57.1	77.6	61.2	67.3	32.7	89.8	63.3	42.9	14.3	10.2	8.2	55.1	40.8

Proliferation assay summary table highlighting the total number of patients in each group as well as percentage of patients that responding (% positive >1%) to any of the different SARS-CoV-2 proteins tested. Data is presents within the separate T cell compartments analysed (CD4+ and CD8+ T cells).

Supplementary table 5 – Summary of tables for proliferation assay based on peptide pool tested

	Median with interquartile range (IQR)							
	CD4				CD8			
	Asymptomatic		Mild		Asymptomatic		Mild	
	Day 28	Day 180	Day 28	Day 180	Day 28	Day 180	Day 28	Day 180
M (%)	2.7 (1.9 - 9.5)	7.6 (1.9 - 20.5)	13.7 (8.4 - 29.6)	7.9 (3.9 - 18.2)	2.7 (1.5 - 3.0)	2.3 (1 - 4)	3.4 (1.6 - 7.9)	2.6 (1.6 - 5.5)
NP (%)	4.2 (2.6 - 16.5)	11.6 (2.9 - 20.1)	12.5 (6.8 - 22.7)	6.7 (2.6 - 21.7)	5.3 (3.7 - 13.5)	5.1 (3.3 - 21.3)	10.2 (5.9 - 22.6)	4.5 (1.8 - 12.1)
ORF3 (%)	1.9 (1.9 - 1.9)	1.9 (1.4 - 4.2)	3.5 (2.5 - 7)	2.9 (1.8 - 5.6)	0	1.9 (1.2 - 2.3)	3.8 (1.7 - 9.2)	4.3 (2 - 7.14)
ORF8 (%)	0	2.6 (2.2 - 2.9)	3.1 (1.6 - 6.4)	2.3 (1.9 - 3.1)	0	3.8 (3.8 - 3.8)	3.7 (2.7 - 13.6)	1.5 (1.1 - 2.0)
Total responses to at least peptide outside spike	87.50%	75%	86%	75.00%	62.50%	75%	91%	71%

Proliferation assay summary table highlighting median responses with IQR to each of the SARS-CoV-2 peptide pool tested in the asymptomatic and mild disease phenotypes. Data is presents within the separate T cell compartments analysed (CD4+ and CD8+ T cells)

Fig S1. Comparison of humoral immune responses in individuals with PCR confirmed SARS-CoV-2 asymptomatic (blue), mild (purple) or severe (red) infection.

Comparison of total IgG specific to the SARS-CoV-2 (A) nucleocapsid and (B) spike glycoprotein, quantification of (C) IgG memory B cells specific to the spike glycoprotein, and (D) pseudoneutralisating antibody titres. Anti-SARS-CoV-2 spike antibody (E-F) isotypes, (G-H) subclasses and (I-L) Fc-mediated effector functions were also compared. See Table S1 for number of individuals evaluated per assay.

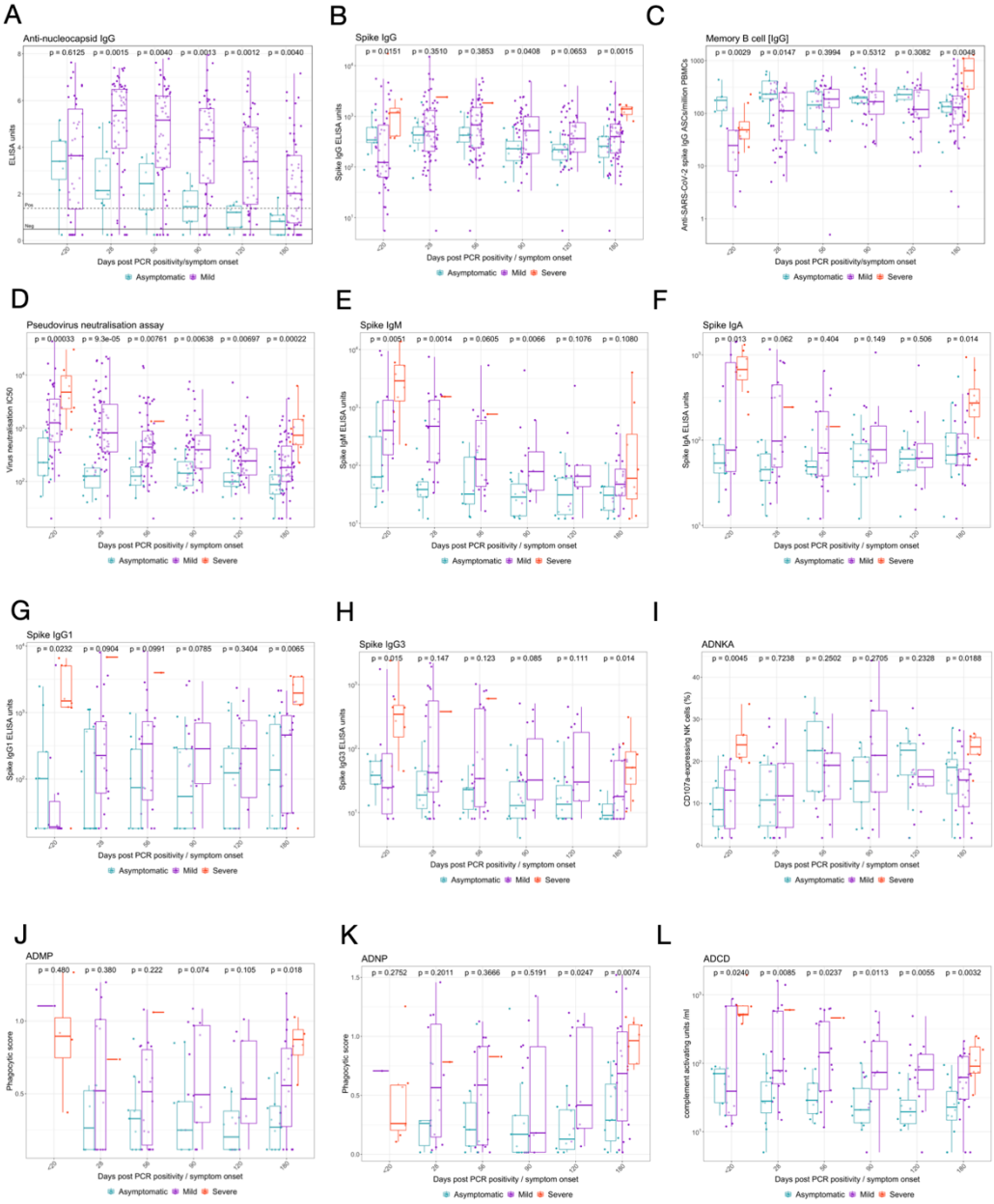


Fig S2. Further characterisation of longitudinal humoral immune responses to SARS-CoV-2 and non-SARS-CoV-2 coronaviruses in individuals with PCR confirmed SARS-CoV-2 asymptomatic (blue), mild (purple) or severe (red) infection.

SARS-CoV-2 spike glycoprotein-specific (A) IgA memory B cells and (B) IgG2 subclass antibodies. Meso Scale Discovery (MSD) multiplexed immunoassay (MIA) platform measurements of antibody levels specific to (C) non-SARS-CoV-2 coronaviruses spike glycoproteins compared across the diseases cohorts at each sampling timepoint, and (D) SARS-CoV-2 spike glycoprotein over time. See Table S1 for number of individuals evaluated per assay.

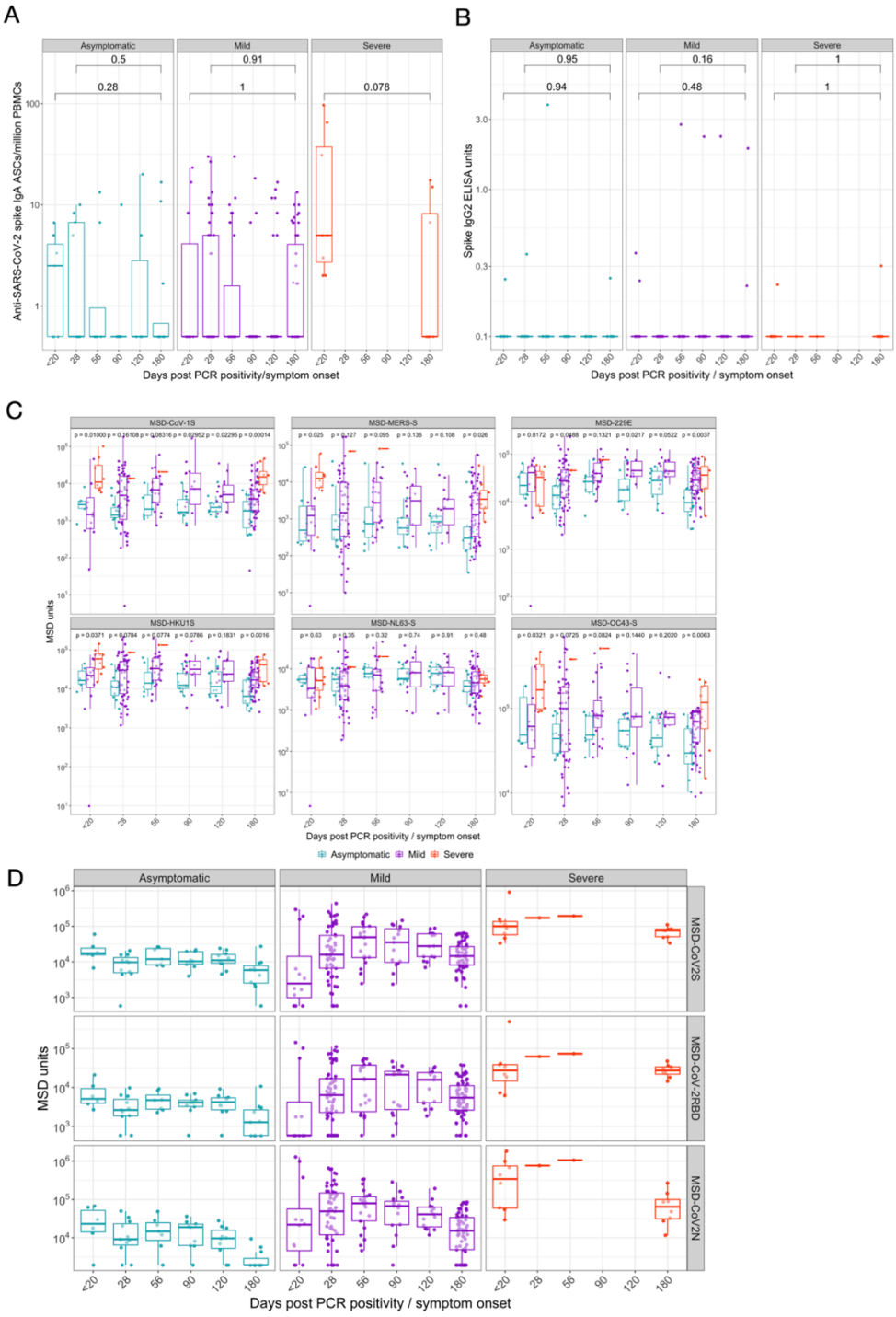


Fig S3. Analysis of T cell responses by clinical disease status, and representative gating strategies

(A) Comparison of *ex vivo* IFN-γ ELISpot responses in healthcare workers post asymptomatic (n=12) vs mild (n=66) SARS-CoV-2 infection. T cell responses to summed SARS-CoV-2 peptide pools spanning spike, accessory and structural proteins (summed total of SARS-CoV-2 proteins, S1, S2, NSP3B, M, NP, ORF 3, ORF8, and the CEFT positive control peptides for T cell responses) at 28, 90 and 180 days post onset of symptoms for mild cases, PCR positive test for asymptomatic participants. Comparison by Mann-Whitney test. **(B) to (D)** PBMC were stimulated with 1ug/ml of peptide pools or 2ug/ml of PHA and 0.2% DMSO for proliferation assay (B and C) or 2ug/mL of peptide pool for ICS assay (D). Representative gating strategy is shown in for T cell proliferation assay (B) . FECT control responses in the proliferation assay is shown in (C) and Representative ICS plots are shown in (D) for both CD4+ and CD8+ T cell responses.

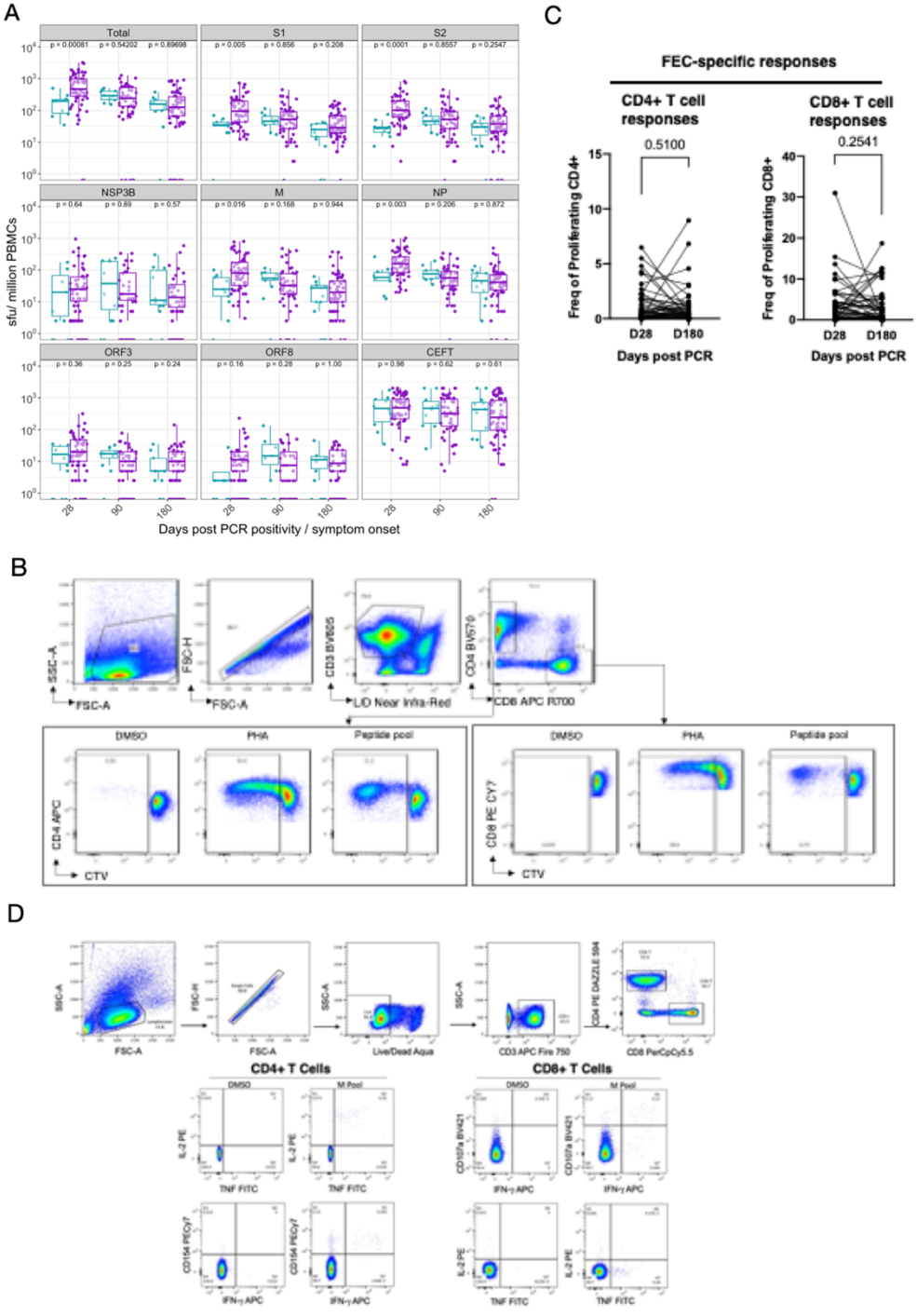


Fig S4 Longitudinal ICS analysis of SARS-CoV-2 T cell responses.
Individuals with ELISpot levels >100 spots/10^6 PBMC for a particular peptide pool at timepoint 2 were studied longitudinally at two timepoints using ICS. A total of n=18 individuals were studied with n=15 providing paired analysis. 1^6 frozen PBMC were stimulated with 2ug/mL of the listed peptide along with 1ug/mL CD28 and CD49d antibodies for 6 hours. Expression levels of IFN-γ, IL-2, and TNF in CD4+ T cells (black) and CD8+ T cells (grey) are shown at two timepoints for S1 **(A)**, S2 **(C)**, M **(E)**, NP **(G)** and NSP3B **(I).** A paired Friedman test was performed with the two-stage step-up method of Benjamini, Kriegar and Yekutieli correction for multiple comparisons. P values <0.1 are shown. Polyfunctional analysis was performed on both timepoints for individuals with sufficient cell populations for polyfunctional analysis. Data for CD4+ T cells are shown at two timepoints for S1 **(B)** (n=7, n=8 timepoints 1 and 2 respectively), S2 **(D)** (n= 5, n=6), M **(F)** (n=4, n=4), and NP pools **(H)** (n= 6, n=5). Data for CD8+ T cells are shown at two timepoints for NSP3B pools **(J)** (n=3, n=5).

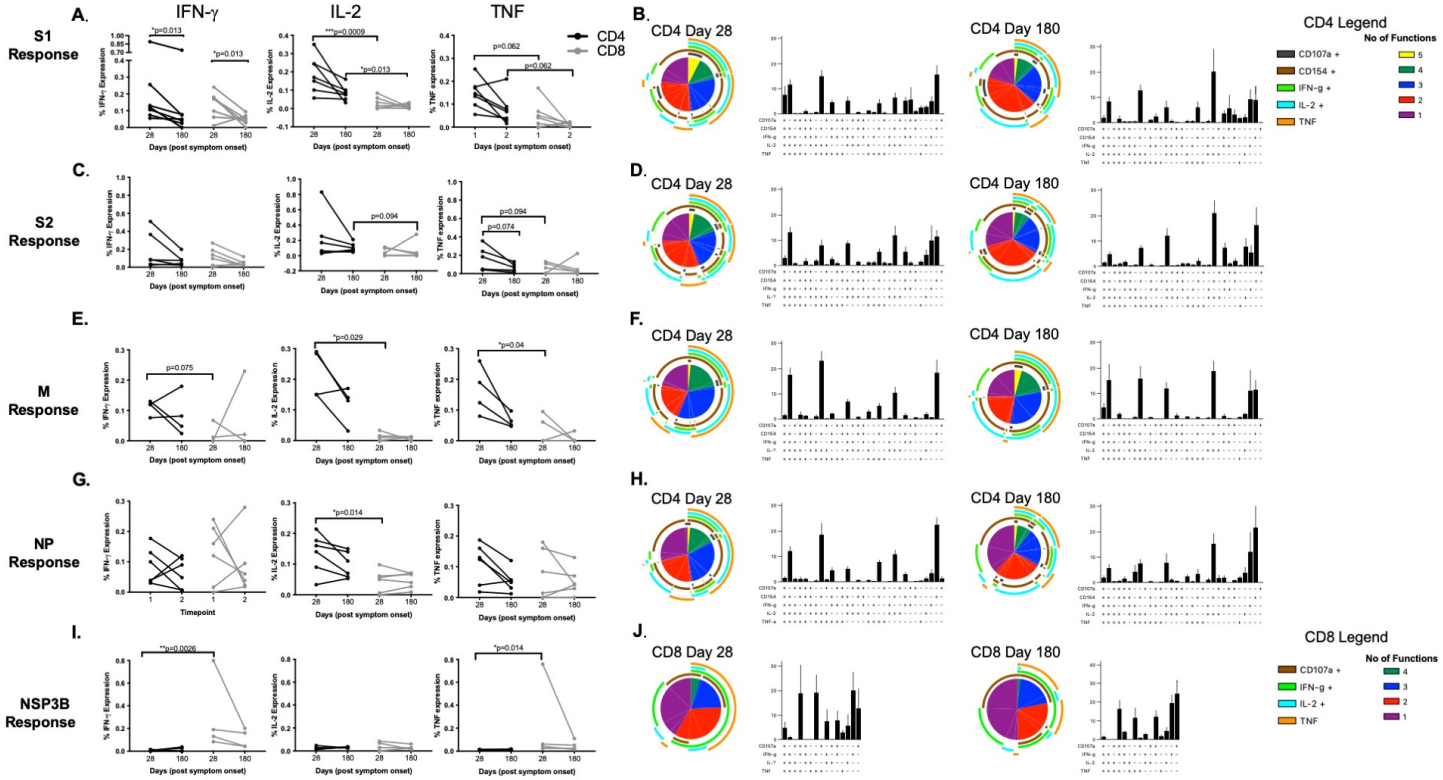
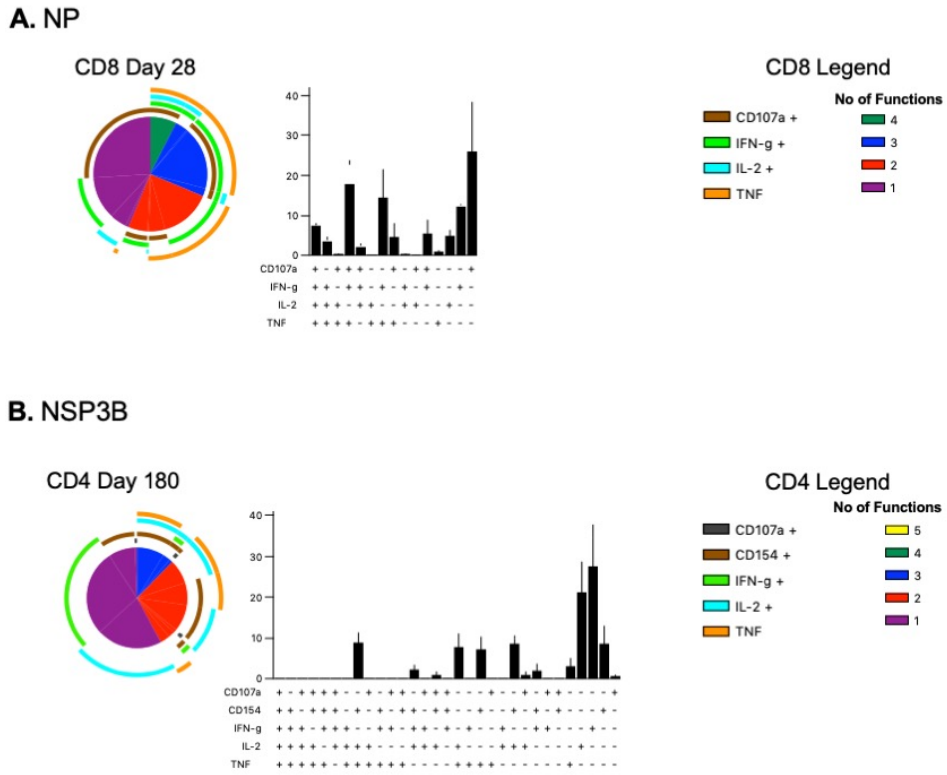


Fig S5. Polyfunctional T cell responses for NP and NSP3B pools.

Polyfunctional analysis was performed as in Supplemental Figure X. **A**) shows CD8+ T cell polyfunctional analysis for the NP pool at day 28 (n=3). **B**) shows CD4+ T cell polyfunctional analysis for the NSP3V pool at day 180 (n=5).



Additional Methods and materials

Peripheral blood mononuclear cells (PBMC) and plasma separation

PBMCs were isolated by density gradient centrifugation using Lymphoprep™ (p=1.077 g/ml, Stem Cell Technologies), washed twice with RPMI 1640 (Sigma, St. Louis, MO, USA) containing 10% heat-inactivated FCS (Sigma), 1mM Pen/Strep (100U/mL) and 2mM L-glutamine (100 ug/mL) (Sigma) or AutoMACS Rinse Buffer and resuspended in R10 or AutoMACs Rinse Buffer and counted using the Guava® ViaCount™ assay on the Muse Cell Analyzer (Luminex Cooperation). PBMCs were frozen and stored in liquid nitrogen. To obtain plasma, the uppermost fraction following the initial Lymphoprep centrifugation above was collected and centrifuged at 2000g for 10 minutes to remove platelets before storage at -80°C.

Serum isolation

Donor blood was collected in a serum-separating tube (SST, Becton Dickinson) which was centrifuged at 2000g for 10 minutes. Serum was removed and stored at -80°C.

IFN-γ Enzyme-Linked immunospot (ELISpot) assay

IFN-γ ELISpot was performed as previously described[1]. In brief, 96-well Multiscreen-I plates (Millipore, UK) were coated for 3 hours with 10 µg/ml clone 1-D1K, Mabtech, AB, Sweden) at room temperature. PBMC were added in duplicate wells at $2x10^5$ cells in 50 µl per well and stimulated with 50 µl of SARS-CoV-2 peptide pools (2ug/ml per peptide). R10 with DMSO (final concentration 0.4%, Sigma) was used as negative control. CMV, EBV, influenza and tetanus antigens (CEFT) peptide pool (2µg/ml, GenScript, Piscataway, NJ, USA were used as positive controls. After 16-18 hours at 37^{o}C PBMC were removed and secreted IFN-γ detected using anti-IFN-γ biotinylated mAb at 1 µg/ml (7-B6-1-biotin, Mabtech) for 2-3 hours, followed by streptavidin alkaline phosphatase at 1 µg/ml for 1-2 hours (SP-3020, Vector Labs). The plates were developed using BCIP/NBT substrate (Pierce) according to the manufacturer's instructions. ELISpot plates were read using an AID ELISpot Reader (v.4.0). Results were reported as spot-forming units (SFU)/10^6 PBMC. Background (mean SFU in negative control wells) was subtracted from antigen stimulated wells to give the final result. Only assays where the background was 50 SFU/10^6 or below were accepted as valid. The cut-off threshold for a positive result was the mean of the negative control well plus 2 times the standard deviation. The lower limit of quantification (LLOQ) for this assay is 2.5 SFU/10^6 PBMC, values below this were assigned a value of 1.

T cell proliferation assay

PBMCs were isolated from blood samples and used fresh or cryopreserved. CellTrace® Violet (CTV, Life Technologies) label was used at a final concentration of 2.5µM to label the cells. Labelling with CTV was done in PBS for 10 minutes at room temperature following which the reaction was stopped using ice-cold fetal bovine serum (FBS). The CTV-labelled PBMC were then plated at 0.25 x 10^6 cells per well of a 96 well round

bottom plate in RPMI supplemented with 10% human blood group type AB serum (Sigma), 1% 1mM Pen/Strep and 1% 2mM L-glutamine and stimulated with peptide pools from SARS-CoV-2 spanning Spike (S1 and S2), M, NP, ORF3 and ORF8, and FEC-T (1µg/ml per peptide). For controls, media containing 0.2% DMSO (Sigma) representing DMSO content in peptide pools was used as a negative control and phytohemagglutinin L (PHA-L, Sigma) at a final concentration of 2ug/ml was used as positive control. Cells were then incubated at 37^{o}C, 5% CO2, 95% humidity for 7 days with hemi-depletion of media on day 4. On day 7, cells were stained for analysis on the flow cytometer. Briefly, PBMC were resuspended in cell staining buffer (Biolegend, San Diego, CA, USA) and incubated for 20min with live/dead near-infrared (Invitrogen, Carlsbad, CA, USA). This was washed off and the cells were incubated with fluorochrome-conjugated primary human-specific antibodies for CD3, CD4 and CD8 in cell staining buffer (Biolegend, San Diego, CA, USA) for 30min at 4^{o}C. This was followed by a wash with cell staining buffer and then fixation with 4% paraformaldehyde (PFA, Sigma). Cells were stored at 4^{o}C in the dark until data acquisition on a MACSQuant 10. Responses above 1% were considered true positive. All data is reported as background subtracted data for each volunteer.

Intracellular cytokine staining

PBMC were thawed and rested overnight in R10 media (1 million cells for peptide stimulation and 500,000 for DMSO and PMA controls) in round bottom plates. Afterwards, cells were stimulated with SARS-CoV-2 peptide pools (2ug/ml), R10 media containing DMSO (0.1%, Sigma) for negative controls and PMA (0.05ug/mL) with ionomycin (0.5ug/mL, Sigma) as a positive control. CD107a BV421 (clone H4A3, BD Biosciences), monensin (Biolegend) and Brefeldin A (MP Biomedicals) were added to cultures at a final concentration of 0.04ug/mL, 0.16uM, and 10ug/mL respectively, and cells were incubated for 6 hours at 37^{o}C, 5% CO_2, 95% humidity. PBMC were then washed with PBS and stained with LIVE/Dead Fixable Aqua stain (Life Technologies) at a 1:400 dilution in PBS and stained for 20 minutes at room temperature. Cells were washed with PBS and resuspended in Cytofix/Cytoperm (BD Biosciences) and incubated for 20 minutes at 4°C. Afterwards, cells were washed twice with BD Perm/Wash buffer (BD Biosciences). Cells were then stained with the following panel of antibodies in Perm/Wash buffer for 20 minutes at room temperature: CD3 APC Fire 750 (clone SK7, Biolegend), CD4 PE Dazzle 594 (clone RPA T4, Biolegend), CD8 PerCpCy5.5 (clone RPA T8, Biolegend), CD154 PE-Cy7 (clone 24-31, Biolegend), IFN-γ APC (clone B27, BD Biosciences), IL-2 PE (clone MQ1-17HI2, Biolegend), TNF-α FITC (clone Mab11, BD Biosciences). Cells were then washed twice with Perm/Wash buffer before resuspending the cells in PBS and running them on a BD LSR II. OneComp compensation beads were used (Life Technologies) as were rainbow fluorescent particles (mid-range intensity) (Biolegend) to calibrate the LSR II before acquisition.

Anti-spike and anti-nucleocapsid total IgG (ELISA/EIA)

Standardised total anti-spike IgG ELISA was performed as described previously[2]. In brief, ELISA plates were coated with 2µg/mL of full-length trimerised SARS-CoV-2 spike glycoprotein protein overnight at 4°C and

blocked with casein in PBS. Plasma samples were diluted in PBS and tested in triplicate. Goat anti-human IgG conjugated to alkaline phosphatase was added as the secondary antibody, and plates were developed using 4-nitrophenyl phosphate in diethanolamine substrate buffer. Plates were read at 405nm, and standardised ELISA units (EU) were determined using a 4-parameter logistic model and various pre-determined control cut-offs (Gen5 v3.09, BioTek). Plate washing in-between each step was undertaken using 0.05% Tween-20 in PBS.Serology for IgG to SARS-CoV-2 nucleocapsid protein was performed using the Abbott Architect i2000 chemiluminescent microparticle immunoassay (Abbott, Maidenhead, UK) and carried out according to manufacturer's instructions using serum. The manufacturer threshold for confirming detection of antibodies is ≥1.40 arbitrary units. Levels between 0.50-1.39 arbitrary units designate equivocal levels (Abbott Diagnostics Product Information Letter PI1060-2020). Values below 0.5 were set to half the LLOQ (i.e. 0.25).

Anti-spike subclass and isotype ELISAs

Both isotype and subclass standardised and OD ELISAs were performed as described previously[3]. In brief, ELISA plates were coated with 5 µg/mL of full-length trimerised SARS-CoV-2 spike protein for overnight incubation at 4°C. Following washing with 0.05% Tween-20 in PBS (PBS/T) plates were blocked with casein in PBS for non-specific binding. In the next step plasma samples were diluted in casein in PBS, as well as positive, negative controls and ten-point standard curve. Plates were incubated for 2h at 37°C with 300 rpm shaking and following washing with PBS/T samples were further incubated with mouse anti-human IgG1 hinge-AP, mouse anti-human IgG3 hinge-AP, goat anti-human IgA-AP and goat anti-human IgM-AP (Southern Biotech) for 1 h at 37 °C with 300 rpm used for detection and the optical density at 405nm was measured until the internal control reached an OD_{405} of 1. For detection of anti-spike IgG2 and IgG4 steps modified as follows: 1) Plates were additionally coated with commercially available human immunoglobulin control (recombinant human IgG2 lambda or recombinant human IgG4 lambda (Bio-Rad)) to serve as internal controls, 2) Mouse anti-human IgG2 Fd-AP or mouse anti-human IgG4 Fc-AP (Southern Biotech) were used, and 3) Optical density at 405 nm was measured using an ELx808 absorbance reader (BioTek) until the immunoglobulin control reached a specified OD405.

Standardised ELISA units (EU) were determined using a 4-parameter logistic model and various pre-determined control cut-offs (Gen5 v3.09, BioTek) while for OD ELISAs negative cut offs were calculated using the formula: mean + 7.858 × standard deviation of the OD405 readings of the pre-pandemic negative-control serum samples, where 7.858 is the standard deviation multiplier with a 99.9% confidence level for n = 5 controls as detailed in [3, 4]. LLOQ were 11, 12, 12, 18, 0.2, 8 and 0.2 EU for total IgG, IgM, IgA, IgG1, IgG2, IgG3 and IgG4, respectively — values below LLOQ were set to half the LLOQ.

MSD Common Cold Coronaviruses

A multiplexed MSD immunoassay (MSD, Rockville, MD) was used to measure the IgG responses to SARS-CoV-2, severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), MERS-CoV and seasonal CoVs (human

coronavirus (HCoV)-OC43, HcoV-HKU1, HcoV-229E, HcoV-NL63). A MULTI-SPOT® 96-well, 10 Spot Plate was coated with three SARS CoV-2 antigens (S, RBD, N), SARS and MERS-CoV spike trimers, as well as spike proteins from seasonal CoV HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-NL63 and bovine serum albumin. Antigens were spotted at 200−400 µg/ml (MSD® Coronavirus Plate 3). Multiplex MSD Assays were performed as per the instructions of the manufacturer. To measure IgG antibodies, 96-well plates were blocked with MSD Blocker A for 30 minutes. Following washing with washing buffer, our samples diluted 1:500-1:5000 in diluent buffer, as well as the reference MSD standard and internal MSD controls were added to the wells. After 2-hour incubation and a washing step, detection antibody (MSD SULFO-TAG™ Anti-Human IgG Antibody, 1/200) was added. Following washing, MSD GOLD™ Read Buffer B was added and plates were read using a MESO® SECTOR S 600 Reader. The standard curve was established by fitting the signals from the standard using a 4-parameter logistic model. Concentrations of samples were determined from the electrochemiluminescence signals by back-fitting to the standard curve. They were multiplied by the dilution factor and expressed in Arbitrary Units/ml. LLOQ were 1160.3, 1169.0 and 3873.5 AU/ml for SARS-CoV-2 spike, SARS-CoV-2 RBD and SARS-CoV-2 NP, respectively — values below the LLOQ were set to half the LLOQ.

Microneutralisation Assay (MNA)

Virus Isolates

Prototype isolate (PANGO lineage B) was Victoria/01/2020 [5] received at Passage(P)3 from Public Health England (PHE) Porton Down (after being supplied by the Doherty Centre Melbourne) in April 2020, passaged in VeroE6/TMPRSS2 cells, used here at P5, and confirmed identical to GenBank MT007544.1, B hCoV-19_Australia_VIC01_2020_ EPI_ ISL_ 406844_ 2020-01-25.

B.1.1.7 (20I/501Y.V1.HMPP1) isolate, H204820430, 2/UK/VUI/1/2020, received in Oxford at P1 from PHE Porton Down in December 2020, passaged in VeroE6/TMPRSS2 cells (NIBSC reference 100978), used here at P4. B.1.351 (20I/501.V2.HV001) isolate was received at P3 from the Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, in Oxford in January 2021, passaged in VeroE6/TMPRSS2 cells (NIBSC reference 100978), used here at P4.

For all isolates, identity was confirmed by deep sequencing at the Wellcome Trust Centre for Human Genetics, University of Oxford.

Virus neutralisation

The microneutralisation assay determines the concentration of antibody that produces a 50% reduction in infectious focus-forming units of authentic SARS-CoV-2 in Vero CCL81 cells. Quadruplicate serial dilutions of serum were preincubated with 100-200 FFU (20 µL) of SARS-CoV-2 for 30 minutes at room temperature. After pre-incubation, 100 µL of Vero CCL81 cells (4.5×10^4) were added and incubated at 37°C, 5% CO_2. After 2 hours, 100 µL of a 1.5% carboxymethyl cellulose-containing overlay was applied to prevent satellite focus formation. Eighteen (B.1.351) or 23 hours (B, B.1.1.7) post-infection, the monolayers were fixed with 4%

paraformaldehyde, permeabilized with 2% Triton X-100 and stained for the nucleocapsid antigen or spike (S) antigen using monoclonal antibodies (mAbs) EY 2A and EY 6A, respectively[6]. After development with a peroxidase-conjugated antibody and TrueBlue peroxidase substrate, infectious foci were enumerated by ELISpot reader. Data were analysed using four-parameter logistic regression (Hill equation) in GraphPad Prism 8.3.

Monogram Bioscience pseudotype neutralisation assay (PseudoNA)

The Monogram Biosciences pseudotype neutralisation assay was performed as described previously[2]. Plasma samples were heat inactivated at 56°C for one hour and diluted in a 9 serial three-fold dilution series starting at 1:40 in cell culture medium. Each sample dilution was mixed with 10^5 relative light units of a lentivirus-based SARS-CoV-2 pseudovirus particle virus. As an internal assay control, an irrelevant pseudotyped virus was also incubated with test samples. The pseudotyped virus and sample mixtures were incubated for one hour at 37°C and HEK 293 ACE2-transfected cells were added to each well, which were then incubated for a further 60-80 hours at 37°C. Luciferase expression was determined, and neutralisation titres are reported as the reciprocal of the plasma dilution conferring 50% inhibition (ID50) of pseudovirus infection. %Inhibition = 100% – (((RLU(Vector+Sample+Diluent) – RLU(Background))/(RLU(Vector+Diluent) – RLU(Background))) x 100%). Inter-assay variation was controlled for by monitoring results acquired from one positive control, one negative control and six patient specimens. The LLOQ for this assay is a titre of 1:40, values below this were set to half LLOQ (i.e. 20).

Spike-specific SARS-CoV-2, OC43, HKU1, 229E and NL63 IgG^+ and IgA^+ B cell memory ELISPOT

PBMCs were adjusted to $2x10^6$ cells/ml in complete media and $2x10^5$ cells/per well were added to a 96-well round bottomed plate with an equal volume of complete media supplemented with 1µg/ml R848 and 10ng/ml of recombinant IL-2, each from the Mabtech Memory B-cell Stimpack. The cells were cultured for 3-3.5 days at 37°C in 5% CO_2. Following polyclonal stimulation, the cells were harvested, washed twice in complete media and counted.

Mabtech flurospot plates were activated with 35% ethanol and coated with the relevant spike glycoprotein (SARS-CoV-2 at 10µg/ml, OC43 at 10µg/ml, NL63 at 15µg/ml, HKU1 at 5µg/ml and 229E at 10µg/ml, all diluted in PBS). Control wells were coated with tetanus toxoid (5µg/ml), capture mAbs anti-human IgG (Mabtech MT91/145) and PBS as a negative control. Following incubation for 16-20 hours at 4°C, the plates were washed five times with PBS and blocked for ≥30 minutes with complete media. The harvested PBMCs were adjusted so that $2x10^5$ cells were added to the spike- and tetanus toxoid-coated, and PBS wells while $2x10^4$ cells were added to the IgG positive control wells. All cells were incubated for ≥16 hours at 37°C, plates were washed five times with PBS and detection mAbs IgG-550 (Mabtech MT78/145) and IgA (Mabtech MT20-490) were diluted 1:500 in 0.5% BSA in PBS and added to plates for two hours at room temperature. Following

five washes in PBS, fluorescent enhancer solution was added to each well for 15 minutes at RT in the dark. Plates were decanted and blotted dry and stored in the dark. Spot forming units were enumerated using AID ELISpot 8.0 software on the AID ELR08IFL reader. The LLOQ for these assays is 1 SFU, values below this were set to half LLOQ (i.e. 0.5).

Antibody-dependent effector functions

The spike-specific antibody-dependent effector functions, natural killer cell activity (ADNKA), neutrophil phagocytosis (ADNP) and monocyte phagocytosis (ADMP) were performed as previously described [3].

Bead coupling for ADNP and ADMP: Red fluorescent (580/605) NeutrAvidin-labeled microspheres (Thermo Fisher, F8775) were freshly coupled to biotinylated SARS-CoV-2 spike protein for each assay. Spike protein (at a concentration of 0.388 µl/ml) was added to the beads at a 3:1 ratio and incubated for 2 h at 37 °C. Beads were washed twice with 0.1% BSA and diluted 100-fold in 0.1% BSA. 10 µl was added to each well in the ADNP and ADMP assays.

ADNP assay

Whole donor blood, collected in sodium heparin tubes, was treated with ammonium–chloride–potassium lysing buffer (Thermo Fisher, A1049201) for 5 minutes followed by centrifugation to collect white blood cells. Cells were washed with DPBS (Sigma, D8537), counted and adjusted to 2.5 × 105 cells per ml in medium consisting of RPMI 1640 medium (Sigma, R5886) supplemented with 100 U ml−1 penicillin–streptomycin (Sigma, P4458) and 20 mmol/L l-glutamine (Sigma, G7513).

Serum diluted 100-fold in RPMI was added to antigen-coupled beads in a 96-well plate and incubated for 2 h at 37 °C. All samples were assayed in duplicate, and each plate contained two QC samples in addition to appropriate negative controls. Wells were washed with DPBS, and 50,000 white blood cells were added to each well followed by a further one hour incubation at 37 °C. Cells were then stained using a cocktail of mouse anti-human CD3 Alexa Fluor 700 (BD Pharmingen, clone UCHT1, nos. 557943 and 9185576; 1:80 dilution), mouse anti-human CD14 APC Cy7 (BD Pharmingen, clone MΦP9, nos. 557831 and 0044497; 1:80 dilution) and mouse anti-human CD66b Pacific Blue (BioLegend; clone G10F5, nos. 305112 and B285068; 1:80 dilution) and incubated for 15 minutes at room temperature in the dark. Following washing and fixation using 4% paraformaldehyde (Santa Cruz Biotechnology, SC-281692), cells were analysed by flow cytometry (BD, Fortessa X20).

Data were analysed with FlowJo (BD; version 10), using a gating strategy to select neutrophils. Neutrophils were gated based on forward and side scatter then doublets excluded. Furthermore, T cells and monocytes were excluded using a double-negative gate for CD3 and CD14. The final neutrophil gate was based on CD66b

positivity, after which bead-positive cells were gated. In all cases, there was a clear separation between positive and negative populations.

Normalized phagocytic scores were calculated by multiplying the percentage of bead-positive cells by the MFI of the events within the bead-positive cell gate and normalizing against a QC sample. As multiple plates were run during an experiment, plates failed if any of the QC sample averages were greater than two standard deviations above the mean of that particular QC across plates. In addition, samples were excluded from further analysis if the replicates showed a coefficient of variation of over 25%. All data are derived from one experiment. The LLOQ for this assay is 0.033, values below this were set to half the LLOQ.

ADMP assay

Human monocytic THP-1 cells (American Type Culture Collection) were grown and maintained using supplier instructions. Serum was diluted 1:4,000 in RPMI, added to antigen-coupled beads in a 96-well plate, and incubated for two hours at 37 °C. All samples were assayed in duplicate, and each plate contained two QC samples in addition to appropriate negative controls. At the end of the two hours incubation period, wells were washed with RPMI and 25,000 THP-1 cells diluted in medium consisting of RPMI 1640 medium (Sigma, R5886) supplemented with 100 U/mL penicillin–streptomycin (Sigma, P4458) and 20 mmol/L l-glutamine (Sigma, G7513) were added to each well. Plates were then incubated for 18 hours at 37 °C. Cells were then washed with PBS and fixed using 4% paraformaldehyde before analysis by flow cytometry (BD, Fortessa X20). Data were analysed with FlowJo (BD, version 10). THP-1 cells were gated based on forward and side scatter to exclude debris then doublets excluded and bead-positive cells gated. There was a clear separation between the positive and negative population.

Normalized phagocytic scores were calculated by multiplying the percentage of bead-positive cells with the MFI of the events within the bead-positive cell gate and normalizing against a QC sample. As multiple plates were run during an experiment, plates failed if any of the QC sample averages were greater than two standard deviations above the mean of that particular QC across plates. In addition, samples were excluded from further analysis if the replicates showed a coefficient of variation of over 25%. All data were derived from one experiment. The LLOQ for this assay is 0.23, values below this were set to half the LLOQ.

ADNKA assay

SARS-CoV-2 spike protein in carbonate/bicarbonate solution (2.5µg/ml) was added to 96-well Nunc MaxiSorp ELISA plates and incubated for 16 hours at 4°C. Plates were washed six times with PBS and blocked with 5% BSA in PBS for one hour at 37°C. Plasma samples were added neat and in duplicate, and plates were incubated for two hours at 37°C. Following another wash step, 10^5 natural killer NK-92 cells expressing human CD16 (PTA-8836 cell line, American Type Culture Collection; described by [7] were added to each well with brefeldin

A (10µg/mL; Sigma Aldrich), GolgiStop (BD Biosciences) and CD107a (1:20 dilution; PE, clone H4A3, BD Biosciences). Plated cells were incubated for five hours at 37°C and then transferred to V-bottom plates, incubated with fixable LIVE/DEAD staining (1:500 dilution; R780, BD Biosciences) and fixed. Data was acquired using a BD Fortessa and percentages of CD107a expressing NK cells relative to control wells with spike protein and blocking buffer only were determined using FlowJo Software (version 10.7.1). To assess inter-assay variation, both a pre-pandemic pool of three donors and a pool of six hospitalised SARS-CoV-2-infected individuals were plated in triplicate on each plate. The LLOQ for this assay is 3.5, values below this were set to half the LLOQ.

Antibody-dependent complement deposition (ADCD) assay

SPHERO^{TM} Carboxyl magnetic blue fluorescent beads (Spherotech, USA) were coupled with SARS-CoV-2 whole spike protein (Lake Pharma, USA) using a two-step Sulpho-NHS/EDC process. Briefly, 5 million beads were washed with 82mM sodium phosphate buffer pH 6.2, prior to activation in the same buffer containing 1.24mg each of N-hydroxysulfosuccinimide and 1-ethyl-3-[3- dimethlyaminopropyl]carbodiimide-HCl). After 20 min activation, the beads were washed in coupling buffer of 50mM 2-(N-morpholino) ethanesulfonic acid (MES) pH 5.0 and resuspended in MES buffer containing 14.5µg antigen for 2 h on a rotational mixer. Finally, beads were washed three times with PBS containing 2% BSA and 0.05% sodium azide, pH7.4, and resuspended in the same buffer overnight. Beads were washed and resuspended in a storage buffer of PBS with 0.05% sodium azide, pH7.4, until use.

Heat-inactivated test serum (3µl, in duplicate) was added to 27µl assay blocking buffer (PBS + 2% BSA:BB) and 10µl taken for serial 3-fold dilutions to give final dilutions of 1:20, 1:60, 1:180, 1:540. 20µl of spike-coated magnetic beads (50 beads per µl) was added, and the mixture incubated at 25^{o}C for 30min with shaking at 900rpm. The beads were washed twice in 200µl wash buffer (BB + 0.05% Tween-20: WB) and then resuspended in 50µl BB containing 10% IgG- and IgM-depleted human plasma, prepared as described previously [8] and incubated at 37^{0}C for 15min with shaking at 900rpm. Beads were next washed twice with 200µl WB and resuspended in 100µl FITC-conjugated rabbit anti-human C3c polyclonal antibody (Abcam, UK) and incubated at room temperature in the dark. After two more washes with 200µl WB, the samples were resuspended in 40µl Hank's Balanced Salt Solution and analysed on the IntelliCyt® iQue Screener PLUS platform (Sartorius, Germany) and ForeCyt®t 8.0 software. For each sample, a minimum of 100 beads were collected and complement activation units (CAU) calculated using a 12-point standard curve of the Anti-SARS-CoV-2 Antibody Diagnostic Calibrant (20/162 NIBSC, UK), with the calibrant standard assigned 1000 CAU. The LLOQ for this assay is 10, values below this were set to half the LLOQ.

Generation of the integrated dataset and data pre-processing

The integrated dataset was generated using the standard extract-transform-load (ETL) procedure, as described [9]. Briefly, primary analysis datasets which included total of 29 csv files across 14 assays and clinical data were merged using donor-specific variable (Donor ID). The outcome of immune response durability was calculated based on the titre of the anti-nucleocapsid specific antibodies measured 6 months post symptoms onset (pso). High responders were determined as individuals who are seropositive 6 months pso, i.e., have anti-N antibody titre ≥ 1.4, while low responders are individuals having anti-N antibody titre below 1.4. The responder status was expressed as a binary value: high responders were given a value of 1, whereas low responders a value of 0. Before the integrative analysis, data was pre-processed using transformation methods available in SIMON knowledge discovery software [10] center (mean subtracted) and scale (standard deviation divided) applied before principal component analysis (PCA), t-distributed stochastic neighbour embedding (t-SNE), hierarchical clustering and SIMON analysis, missing values were imputed based on median values (*medianImpute*) (PCA, t-SNE and hierarchical clustering), features with zero-variance (*zv*) and near-zero-variance (*nzv*) were removed (PCA and SIMON), and finally, highly correlated features with cut-off 0.85 (*corr*) were also removed for the supervised machine learning (ML) analysis using SIMON.

Integrative analysis using unsupervised machine learning analysis

<u>High-dimensional analysis using t-SNE and clustering analysis.</u>

The t-distributed stochastic neighbour embedding (t-SNE) followed by clustering was performed to analyse the pre-processed integrated dataset using SIMON software [10]. Disease severity and timepoint were used as grouping variables, and thus, were excluded from the analysis. T-SNE analysis was performed with 2,000 iterations, a perplexity of 30, and a theta of 0.5. Resulting t-SNE maps were used for cluster analysis using model-based clustering algorithm (*mclust*) with seed number 1337 and 3 clusters allowed [11]. To visualize variation of clinical and immunological features across the t-SNE embedding space, we performed hierarchical clustering on t-SNE maps using Euclidean distance, agglomerative hierarchical clustering with Ward and tightest cluster was ordered first.

<u>Principal component analysis.</u>

Principal component analysis (PCA) was performed on multivariate immunological parameters (continuous variables) with pre-filtering to remove all categorical variables and features with less than 10% of unique values, *i.e.*, any column that has number of unique values less than 10% of total number of observations. Disease severity was used as a grouping variable. Quality of variable representations (cos2), variable correlations and contributions (expressed as percentage) of top 10 variables to first two principal components (PCs) were calculated. The correlation between variables and PCs was used as the coordinates of the variables on the PCs. The observations were represented by their projections, while the variables were represented by their correlations [12].

Correlation analysis.

Pairwise correlations of immunological parameters on all analysed samples were calculated and visualized as a correlogram using a SIMON software. Spearman's rank correlation coefficient was computed and indicated on the correlogram by the heat scale. The significance test of correlation coefficients was performed, and values shown on the correlogram were adjusted for multiple testing using false discovery rate (FDR) correction using the Benjamini-Hochberg correction at the significance threshold FDR < 0.05. Following correlation analysis, correlogram map was fed into agglomerative hierarchical clustering with Ward algorithm and three major clusters were identified.

Hierarchical clustering.

Agglomerative hierarchical clustering was performed on the samples with immunological parameters analysed on day 28 pso and visualized as the dendrogram on heatmap using a SIMON software. Cluster analysis was performed using a set of dissimilarities for the number of samples being clustered. Each sample was assigned to its own cluster, then the algorithm iteratively joined the two most similar clusters, continuing until there was just a single cluster. First, the dissimilarity values were computed (dissimilarity matrix calculated using Euclidean method, these values were then fed into hierarchical clustering using complete linkage agglomeration method and finally, dendrogram was plotted (tightest cluster ordered first).

Integrative analysis using SIMON

To identify early immunological signature at day 28 pso that can predict if the individual will be high or low responder 6 months pso, we performed SIMON (Sequential Iterative Modeling Over Nigh) analysis [13, 14] Joint predictive analysis on all immunological parameters at day 28 pso (excluding clinical data) was performed using 172 ML algorithms. The outcome was seropositivity status determined 6 months pso. Initial data was split into train/test partition (75%/25%) preserving the balanced distribution of the outcome class (seed number 1337). The dataset had 29% missing values, and missing values were removed using multi-set interaction function ('mulset, SIMON software) and 30 resamples were used for the SIMON analysis. The models were evaluated using 10-fold cross-validation on the training sets, and additionally to prevent overfitting on the held-out test sets. The best performing model was built using the Sparse Partial Least Squares (sPLS) algorithm (train AUROC: 0.95 (CI 0.5-1) and test AUROC: 1). In the final step, SIMON calculated the contribution of each feature to the model as variable importance score (scaled to maximum value of 100).

Statistical analyses

Statistical analysis was performed using R (https://www.r-project.org/), and figures were made with R using R package ggplot2[15]. Kruskal-Wallis test —unless otherwise specified — was used for comparison of the disease severity groups. Wilcoxon rank-sum test —unless otherwise specified — was employed to compare

between study time points. A generalised additive mixed model (GAMM) by restricted maximum likelihood (REML) was used to fit the immunological measures (log10 transformed) taken at multiple study time points, using Gaussian process smooth term. Disease severity group was included in the GAMM as a linear predictor and a participant identifier was included as a random effect. The R package *gamm4* was utilised for the GAMM analysis [16]. For ICS cytokine expression analyses, data was prepared using PESTEL v2.0 for formatting and baseline subtraction, followed by export of data to SPICE v6.0 for analysis. Statistical significance was set at $P<0.05$ and all tests were 2-tailed. Machine learning analysis was performed using SIMON software (https://genular.org).

References for Additional Methods

1. Ogbe A, Kronsteiner B, Skelly DT, Pace M, Brown A, Adland E, . . . Oxford Protective TCIfC-CT. T cell assays differentiate clinical and subclinical SARS-CoV-2 infections from cross-reactive antiviral responses. *Nature Communications* **12**, 2055 (2021).

2. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, . . . Oxford CVTG. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* **396**, 467-478 (2020).

3. Barrett JR, Belij-Rammerstorfer S, Dold C, Ewer KJ, Folegatti PM, Gilbride C, . . . Oxford CVTG. Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. *Nat Med* **27**, 279-288 (2021).

4. Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. *J Immunol Methods* **221**, 35-41 (1998).

5. Caly L, Druce J, Roberts J, Bond K, Tran T, Kostecki R, . . . Catton MG. Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. *Med J Aust* **212**, 459-462 (2020).

6. Huang KA, Tan TK, Chen TH, Huang CG, Harvey R, Hussain S, . . . Townsend AR. Breadth and function of antibody response to acute SARS-CoV-2 infection in humans. *PLoS Pathog* **17**, e1009352 (2021).

7. Binyamin L, Alpaugh RK, Hughes TL, Lutz CT, Campbell KS, Weiner LM. Blocking NK cell inhibitory self-recognition promotes antibody-dependent cellular cytotoxicity in a model of anti-lymphoma therapy. *J Immunol* **180**, 6392-6401 (2008).

8. Lesne E, Cavell BE, Freire-Martin I, Persaud R, Alexander F, Taylor S, . . . Gorringe A. Acellular Pertussis Vaccines Induce Anti-pertactin Bactericidal Antibodies Which Drives the Emergence of Pertactin-Negative Strains. *Front Microbiol* **11**, 2108-2108 (2020).

9. Tomic A, Tomic I, Dekker CL, Maecker HT, Davis MM. The FluPRINT dataset, a multidimensional analysis of the influenza vaccine imprint on the immune system. *Sci Data* **6**, 214 (2019).

10. Tomic A, Tomic I, Waldron L, Geistlinger L, Kuhn M, Spreng RL, . . . Davis MM. SIMON: Open-Source Knowledge Discovery Platform. *Patterns (N Y)* **2**, 100178 (2021).

11. Fraley C, Raftery AE. Model-Based Clustering, Discriminant Analysis, and Density Estimation. *Journal of the American Statistical Association* **97**, 611-631.

12. Abdi H, Williams LJ. Principal Component Analysis. *Wires Computational Statistics* **2**, 433-459 (2010).

13. Tomic A, Tomic I, Rosenberg-Hasson Y, Dekker CL, Maecker HT, Davis MM. SIMON, an Automated Machine Learning System, Reveals Immune Signatures of Influenza Vaccine Responses. *Journal of immunology (Baltimore, Md : 1950)* **203**, 749-759 (2019).

14. Tomic A, Tomic I, Waldron L, Geistlinger L, Kuhn M, Spreng RL, . . . Davis MM. SIMON: Open-Source Knowledge Discovery Platform. *Patterns (New York, NY)* **2**, 100178-100178 (2021).

15. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York (2016).

16. Wood S, Scheipl F. gamm4: Generalized Additive Mixed Models using 'mgcv' and 'lme4'. R package version 0.2-6.) (2020).

652 **Peptide Sequences**

Peptide Pool	Peptide ID	Peptide Sequence
NSP3B	ORF1a/1ab_207	DWSYSGQSTQLGIEFLKR
	ORF1a/1ab_208	TQLGIEFLKRGDKSVYY
	ORF1a/1ab_209	LKRGDKSVYYTSNPTTF
	ORF1a/1ab_210	VYYTSNPTTFHLDGEVI
	ORF1a/1ab_211	TTFHLDGEVITFDNLKTL
	ORF1a/1ab_212	VITFDNLKTLLSLREVR
	ORF1a/1ab_213	KTLLSLREVRTIKVFTTV
	ORF1a/1ab_214	VRTIKVFTTVDNINL
	ORF1a/1ab_215	VFTTVDNINLHTQVVDM
	ORF1a/1ab_216	INLHTQVVDMSMTYGQQF
	ORF1a/1ab_217	DMSMTYGQQFGPTYL
	ORF1a/1ab_218	YGQQFGPTYLDGADVTKI
	ORF1a/1ab_219	YLDGADVTKIKPHNSHEG
	ORF1a/1ab_220	KIKPHNSHEGKTFYVL
	ORF1a/1ab_221	SHEGKTFYVLPNDDTLRV
	ORF1a/1ab_222	VLPNDDTLRVEAFEYY
	ORF1a/1ab_223	TLRVEAFEYYHTTDPSFL
	ORF1a/1ab_224	YYHTTDPSFLGRYMSAL
	ORF1a/1ab_225	SFLGRYMSALNHTKKWKY
	ORF1a/1ab_226	ALNHTKKWKYPQVNGL
	ORF1a/1ab_227	KWKYPQVNGLTSIKW
	ORF1a/1ab_228	QVNGLTSIKWADNNCYL
	ORF1a/1ab_229	IKWADNNCYLATALLTL
	ORF1a/1ab_230	CYLATALLTLQQIELKF
	ORF1a/1ab_231	LTLQQIELKFNPPAL
	ORF1a/1ab_232	IELKFNPPALQDAYYRAR
	ORF1a/1ab_233	ALQDAYYRARAGEAANF
	ORF1a/1ab_234	RARAGEAANFCALILAY
	ORF1a/1ab_235	ANFCALILAYCNKTVGEL
	ORF1a/1ab_236	AYCNKTVGELGDVRETM
	ORF1a/1ab_237	GELGDVRETMSYLFQHAN
	ORF1a/1ab_238	TMSYLFQHANLDSCKRVL
	ORF1a/1ab_239	ANLDSCKRVLNVVCK
	ORF1a/1ab_240	CKRVLNVVCKTCGQQQTT
	ORF1a/1ab_241	CKTCGQQQTTLKGVEAVM
	ORF1a/1ab_242	TTLKGVEAVMYMGTLSY
	ORF1a/1ab_243	AVMYMGTLSYEQFKKGV
	ORF1a/1ab_244	LSYEQFKKGVQIPCTCGK
	ORF1a/1ab_245	GVQIPCTCGKQATKYLV
	ORF1a/1ab_246	CGKQATKYLVQQESPFVM
	ORF1a/1ab_247	LVQQESPFVMMSAPPAQY
	ORF1a/1ab_248	VMMSAPPAQYELKHGTF
	ORF1a/1ab_249	AQYELKHGTFTCASEY
	ORF1a/1ab_250	HGTFTCASEYTGNYQCGH
	ORF1a/1ab_251	EYTGNYQCGHYKHITSK
	ORF1a/1ab_252	CGHYKHITSKETLYCI
	ORF1a/1ab_253	ITSKETLYCIDGALLTK
	ORF1a/1ab_254	YCIDGALLTKSSEYKGPI
	ORF1a/1ab_255	TKSSEYKGPITDVFYK
Peptide Pool	Peptide ID	Peptide Sequence

NSP3B continued	ORF1a/1ab_256	KGPITDVFYKENSYTTTI
	ORF1a/1ab_257	YKENSYTTTIKPVTYKL
	ORF1a/1ab_258	TTIKPVTYKLDGVVCTEI
	ORF1a/1ab_259	KLDGVVCTEIDPKLDNYY
	ORF1a/1ab_260	EIDPKLDNYYKKDNSYF
	ORF1a/1ab_261	NYYKKDNSYFTEQPIDLV
	ORF1a/1ab_262	YFTEQPIDLVPNQPY
	ORF1a/1ab_263	PIDLVPNQPYPNASFDNF
	ORF1a/1ab_264	PYPNASFDNFKFVCDNIK
	ORF1a/1ab_265	NFKFVCDNIKFADDLNQL
	ORF1a/1ab_266	IKFADDLNQLTGYKK
	ORF1a/1ab_267	DLNQLTGYKKPASRELKV
	ORF1a/1ab_268	KKPASRELKVTFFPDL
	ORF1a/1ab_269	ELKVTFFPDLNGDVVAI
	ORF1a/1ab_270	PDLNGDVVAIDYKHY
	ORF1a/1ab_271	DVVAIDYKHYTPSFKK
	ORF1a/1ab_272	YKHYTPSFKKGAKLLHK
	ORF1a/1ab_273	FKKGAKLLHKPIVWHV
	ORF1a/1ab_274	LLHKPIVWHVNNATNK
	ORF1a/1ab_275	VWHVNNATNKATYKPNTW
	ORF1a/1ab_276	NKATYKPNTWCIRCLW
	ORF1a/1ab_277	PNTWCIRCLWSTKPV
	ORF1a/1ab_278	IRCLWSTKPVETSNSFDV
	ORF1a/1ab_279	PVETSNSFDVLKSEDAQG
	ORF1a/1ab_280	DVLKSEDAQGMDNLACED
	ORF1a/1ab_281	QGMDNLACEDLKPVSEEV
	ORF1a/1ab_282	EDLKPVSEEVVENPTIQK
	ORF1a/1ab_283	EVVENPTIQKDVLECNVK
	ORF1a/1ab_284	QKDVLECNVKTTEVVGDI
	ORF1a/1ab_285	VKTTEVVGDIILKPANNS
	ORF1a/1ab_286	DIILKPANNSLKITEEV
	ORF1a/1ab_287	NNSLKITEEVGHTDLM
	ORF1a/1ab_288	TEEVGHTDLMAAYVDNSS
	ORF1a/1ab_289	LMAAYVDNSSLTIKK
	ORF1a/1ab_290	VDNSSLTIKKPNELSRVL
	ORF1a/1ab_291	KKPNELSRVLGLKTL
	ORF1a/1ab_292	LSRVLGLKTLATHGLAAV
	ORF1a/1ab_293	TLATHGLAAVNSVPWDTI
	ORF1a/1ab_294	AVNSVPWDTIANYAKPFL
	ORF1a/1ab_295	TIANYAKPFLNKVVSTTT
	ORF1a/1ab_296	FLNKVVSTTTNIVTRCL
	ORF1a/1ab_297	TTTNIVTRCLNRVCTNYM
	ORF1a/1ab_298	CLNRVCTNYMPYFFTLLL
	ORF1a/1ab_299	YMPYFFTLLLQLCTFTR
	ORF1a/1ab_300	LLLQLCTFTRSTNSRIK
	ORF1a/1ab_301	FTRSTNSRIKASMPTTI
	ORF1a/1ab_302	RIKASMPTTIAKNTVKSV
	ORF1a/1ab_303	TIAKNTVKSVGKFCL
	ORF1a/1ab_304	TVKSVGKFCLEASFNYLK
Peptide Pool	Peptide ID	Peptide Sequence
NSP3B continued	ORF1a/1ab_305	CLEASFNYLKSPNFSKLI
	ORF1a/1ab_306	LKSPNFSKLINIIIWFLL

Peptide Pool	Peptide ID	Peptide Sequence
ORF3	ORF3a_1	MDLFMRIFTIGTVTLK
	ORF3a_2	IFTIGTVTLKQGEIK
	ORF3a_3	TVTLKQGEIKDATPSDFV
	ORF3a_4	IKDATPSDFVRATATIPI
	ORF3a_5	FVRATATIPIQASLPFGW
	ORF3a_6	PIQASLPFGWLIVGVALL
	ORF3a_7	GWLIVGVALLAVFQSASK
	ORF3a_8	LLAVFQSASKIITLKKRW
	ORF3a_9	SKIITLKKRWQLALSKGV
	ORF3a_10	RWQLALSKGVHFVCNLLL
	ORF3a_11	GVHFVCNLLLLFVTVY
	ORF3a_12	NLLLLFVTVYSHLLLV
	ORF3a_13	VTVYSHLLLVAAGLEAPF
	ORF3a_14	LVAAGLEAPFLYLYALVY
	ORF3a_15	PFLYLYALVYFLQSINFV
	ORF3a_16	VYFLQSINFVRIIMRLWL
	ORF3a_17	FVRIIMRLWLCWKCRSK
	ORF3a_18	LWLCWKCRSKNPLLY
	ORF3a_19	KCRSKNPLLYDANYFLCW
	ORF3a_20	LYDANYFLCWHTNCYDY
	ORF3a_21	LCWHTNCYDYCIPYNSV
	ORF3a_22	YDYCIPYNSVTSSIVI
	ORF3a_23	YNSVTSSIVITSGDGTTS
	ORF3a_24	VITSGDGTTSPISEHDY
	ORF3a_25	TTSPISEHDYQIGGYTEK
	ORF3a_26	DYQIGGYTEKWESGVK
	ORF3a_27	YTEKWESGVKDCVVLHSY
	ORF3a_28	VKDCVVLHSYFTSDYYQL
	ORF3a_29	SYFTSDYYQLYSTQL
	ORF3a_30	DYYQLYSTQLSTDTGV
	ORF3a_31	STQLSTDTGVEHVTFFIY
	ORF3a_32	GVEHVTFFIYNKIVDEPE
	ORF3a_33	IYNKIVDEPEEHVQIHTI
	ORF3a_34	PEEHVQIHTIDGSSGVV
	ORF3a_35	HTIDGSSGVVNPVMEPIY
	ORF3a_36	VVNPVMEPIYDEPTTTTS
	ORF3a_37	PVMEPIYDEPTTTTSVPL

Peptide Pool	Peptide ID	Peptide Sequence
	M(ORF5)_1	MADSNGTITVEELKKLL
	M(ORF5)_2	ITVEELKKLLEQWNLVI
	M(ORF5)_3	KLLEQWNLVIGFLFLTWI
	M(ORF5)_4	VIGFLFLTWICLLQFAY
	M(ORF5)_5	TWICLLQFAYANRNRFLY

M	M(ORF5)_6	AYANRNRFLYIIKLIFLW
	M(ORF5)_7	LYIIKLIFLWLLWPVTL
	M(ORF5)_8	FLWLLWPVTLACFVLAAV
	M(ORF5)_9	TLACFVLAAVYRINWI
	M(ORF5)_10	LAAVYRINWITGGIAIAM
	M(ORF5)_11	WITGGIAIAMACLVGLMW
	M(ORF5)_12	AMACLVGLMWLSYFIASF
	M(ORF5)_13	MWLSYFIASFRLFARTR
	M(ORF5)_14	ASFRLFARTRSMWSF
	M(ORF5)_15	FARTRSMWSFNPETNILL
	M(ORF5)_16	SFNPETNILLNVPLHGTI
	M(ORF5)_17	LLNVPLHGTILTRPLL
	M(ORF5)_18	HGTILTRPLLESELVI
	M(ORF5)_19	RPLLESELVIGAVILR
	M(ORF5)_20	ELVIGAVILRGHLRI
	M(ORF5)_21	AVILRGHLRIAGHHLGR
	M(ORF5)_22	LRIAGHHLGRCDIKDLPK
	M(ORF5)_23	GRCDIKDLPKEITVATSR
	M(ORF5)_24	PKEITVATSRTLSYYKL
	M(ORF5)_25	TSRTLSYYKLGASQRV
	M(ORF5)_26	YYKLGASQRVAGDSGF
	M(ORF5)_27	SQRVAGDSGFAAYSRYRI
	M(ORF5)_28	GFAAYSRYRIGNYKL
	M(ORF5)_29	SRYRIGNYKLNTDHSSSS
	M(ORF5)_30	KLNTDHSSSSDNIALLV
	M(ORF5)_31	KLNTDHSSSSDNIALLVQ

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Peptide Pool	Peptide ID	Peptide Sequence
ORF8	ORF8_1	MKFLVFLGIITTVAAF
	ORF8_2	LGIITTVAAFHQECSL
	ORF8_3	VAAFHQECSLQSCTQHQP
	ORF8_4	SLQSCTQHQPYVVDDPCP
	ORF8_5	QPYVVDDPCPIHFYSKWY
	ORF8_6	CPIHFYSKWYIRVGARK
	ORF8_7	KWYIRVGARKSAPLIEL
	ORF8_8	ARKSAPLIELCVDEAGSK
	ORF8_9	ELCVDEAGSKSPIQYIDI
	ORF8_10	SKSPIQYIDIGNYTVSCL
	ORF8_11	DIGNYTVSCLPFTINCQE
	ORF8_12	CLPFTINCQEPKLGSLVV
	ORF8_13	QEPKLGSLVVRCSFYEDF
	ORF8_14	VVRCSFYEDFLEYHDVRV
	ORF8_15	FYEDFLEYHDVRVVLDFI
Peptide Pool	Peptide ID	Peptide Sequence
	N(ORF9)_1	MSDNGPQNQRNAPRITF
	N(ORF9)_2	NQRNAPRITFGGPSDSTG
	N(ORF9)_3	TFGGPSDSTGSNQNGER
	N(ORF9)_4	STGSNQNGERSGARSKQR
	N(ORF9)_5	ERSGARSKQRRPQGL
	N(ORF9)_6	RSKQRRPQGLPNNTASWF
	N(ORF9)_7	GLPNNTASWFTALTQHGK

NP	N(ORF9)_8	WFTALTQHGKEDLKFPR
	N(ORF9)_9	HGKEDLKFPRGQGVPI
	N(ORF9)_10	KFPRGQGVPINTNSSPDD
	N(ORF9)_11	PINTNSSPDDQIGYYRR
	N(ORF9)_12	PDDQIGYYRRATRRIR
	N(ORF9)_13	YYRRATRRIRGGDGKMK
	N(ORF9)_14	RIRGGDGKMKDLSPRWYF
	N(ORF9)_15	MKDLSPRWYFYYLGTGPE
	N(ORF9)_16	YFYYLGTGPEAGLPY
	N(ORF9)_17	GTGPEAGLPYGANKDGII
	N(ORF9)_18	PYGANKDGIIWVATEGAL
	N(ORF9)_19	IIWVATEGALNTPKDHI
	N(ORF9)_20	GALNTPKDHIGTRNPANN
	N(ORF9)_21	HIGTRNPANNAAIVLQL
	N(ORF9)_22	ANNAAIVLQLPQGTTLPK
	N(ORF9)_23	QLPQGTTLPKGFYAEGSR
	N(ORF9)_24	PKGFYAEGSRGGSQASSR
	N(ORF9)_25	SRGGSQASSRSSSRSR
	N(ORF9)_26	ASSRSSSRSRNSSRNSTP
	N(ORF9)_27	SRNSSRNSTPGSSRGTSP
	N(ORF9)_28	TPGSSRGTSPARMAGNGG
	N(ORF9)_29	SPARMAGNGGDAALALLL
	N(ORF9)_30	GGDAALALLLLDRLNQL
	N(ORF9)_31	LLLLDRLNQLESKMSGK
	N(ORF9)_32	NQLESKMSGKGQQQQGQT
	N(ORF9)_33	GKGQQQQGQTVTKKSAAE
	N(ORF9)_34	QTVTKKSAAEASKKPRQK
	N(ORF9)_35	AEASKKPRQKRTATKAY
	N(ORF9)_36	RQKRTATKAYNVTQAFGR
	N(ORF9)_37	AYNVTQAFGRRGPEQTQG
	N(ORF9)_38	GRRGPEQTQGNFGDQELI
	N(ORF9)_39	QGNFGDQELIRQGTDYK
	N(ORF9)_40	ELIRQGTDYKHWPQIAQF
	N(ORF9)_41	YKHWPQIAQFAPSASAFF
	N(ORF9)_42	QFAPSASAFFGMSRIGM
	N(ORF9)_43	AFFGMSRIGMEVTPSGTW
	N(ORF9)_44	GMEVTPSGTWLTYTGAIK
	N(ORF9)_45	TWLTYTGAIKLDDKDPNF
	N(ORF9)_46	IKLDDKDPNFKDQVILL
	N(ORF9)_47	PNFKDQVILLNKHIDAYK
	N(ORF9)_48	LLNKHIDAYKTFPPTEPK
	N(ORF9)_49	YKTFPPTEPKKDKKKK
Peptide Pool	Peptide ID	Peptide Sequence
NP continued	N(ORF9)_50	TEPKKDKKKKADETQAL
	N(ORF9)_51	KKKADETQALPQRQKK
	N(ORF9)_52	TQALPQRQKKQQTVTLL
	N(ORF9)_53	QKKQQTVTLLPAADLDDF
	N(ORF9)_54	LLPAADLDDFSKQLQQSM
	N(ORF9)_55	DFSKQLQQSMSSADSTQA

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Peptide Pool	Peptide ID	Peptide Sequence
S1	S_1	MFVFLVLLPLVSSQCVNL
	S_2	PLVSSQCVNLTTRTQL
	S_3	CVNLTTRTQLPPAYTNSF
	S_4	QLPPAYTNSFTRGVYY
	S_5	TNSFTRGVYYPDKVFR
	S_6	GVYYPDKVFRSSVLHSTQ
	S_7	FRSSVLHSTQDLFLPFF
	S_8	STQDLFLPFFSNVTWF
	S_9	LPFFSNVTWFHAIHV
	S_10	NVTWFHAIHVSGTNGTKR
	S_11	HVSGTNGTKRFDNPVLPF
	S_12	KRFDNPVLPFNDGVYF
	S_13	VLPFNDGVYFASTEKSNI
	S_14	YFASTEKSNIIRGWIF
	S_15	KSNIIRGWIFGTTLDSK
	S_16	WIFGTTLDSKTQSLLIV
	S_17	DSKTQSLLIVNNATNVVI
	S_18	IVNNATNVVIKVCEFQF
	S_19	VVIKVCEFQFCNDPFLGV
	S_20	QFCNDPFLGVYYHKNNK
	S_21	LGVYYHKNNKSWMESEFR
	S_22	NKSWMESEFRVYSSANNC
	S_23	FRVYSSANNCTFEYV
	S_24	SANNCTFEYVSQPFLMDL
	S_25	YVSQPFLMDLEGKQGNFK
	S_26	DLEGKQGNFKNLREFVFK
	S_27	FKNLREFVFKNIDGYFKI
	S_28	FKNIDGYFKIYSKHTPI
	S_29	FKIYSKHTPINLVRDL
	S_30	HTPINLVRDLPQGFSAL
	S_31	RDLPQGFSALEPLVDLPI
	S_32	ALEPLVDLPIGINITRF
	S_33	LPIGINITRFQTLLALHR
	S_34	RFQTLLALHRSYLTPGDS
	S_35	HRSYLTPGDSSSGWTAGA
	S_36	DSSSGWTAGAAAYYVGYL
	S_37	GAAAYYVGYLQPRTFLLK
	S_38	YLQPRTFLLKYNENGTI
Peptide Pool	Peptide ID	Peptide Sequence
	S_39	LLKYNENGTITDAVDCAL
	S_40	TITDAVDCALDPLSETK
	S_41	CALDPLSETKCTLKSFTV
	S_42	TKCTLKSFTVEKGIY
	S_43	KSFTVEKGIYQTSNFRV
	S_44	GIYQTSNFRVQPTESIVR
	S_45	RVQPTESIVRFPNITNL
	S_46	IVRFPNITNLCPFGEVF
	S_47	TNLCPFGEVFNATRFASV
	S_48	VFNATRFASVYAWNRKRI
	S_49	SVYAWNRKRISNCVADY
	S_50	KRISNCVADYSVLYNSAS

S1 continued	S_51	DYSVLYNSASFSTFKCY
	S_52	SASFSTFKCYGVSPTKL
	S_53	KCYGVSPTKLNDLCFTNV
	S_54	KLNDLCFTNVYADSFVIR
	S_55	NVYADSFVIRGDEVRQI
	S_56	VIRGDEVRQIAPGQTGKI
	S_57	QIAPGQTGKIADYNYKL
	S_58	GKIADYNYKLPDDFTGCV
	S_59	KLPDDFTGCVIAWNSNNL
	S_60	CVIAWNSNNLDSKVGGNY
	S_61	NLDSKVGGNYNYLYRLFR
	S_62	NYNYLYRLFRKSNLKPF
	S_63	LFRKSNLKPFERDISTEI
	S_64	PFERDISTEIYQAGSTPC
	S_65	EIYQAGSTPCNGVEGF
	S_66	STPCNGVEGFNCYFPL
	S_67	VEGFNCYFPLQSYGF
	S_68	CYFPLQSYGFQPTNGVGY
	S_69	GFQPTNGVGYQPYRVVVL
	S_70	GYQPYRVVVLSFELL
	S_71	RVVVLSFELLHAPATV
	S_72	FELLHAPATVCGPKK
	S_73	APATVCGPKKSTNLVKNK
	S_74	KKSTNLVKNKCVNFNF
	S_75	VKNKCVNFNFNGLTGTGV
	S_76	NFNGLTGTGVLTESNKKF
	S_77	GVLTESNKKFLPFQQFGR
	S_78	KFLPFQQFGRDIADTTDA
	S_79	GRDIADTTDAVRDPQTL
	S_80	TDAVRDPQTLEILDI
	S_81	DPQTLEILDITPCSFGGV
	S_82	DITPCSFGGVSVITPGTN
	S_83	GVSVITPGTNTSNQVAVL
	S_84	TNTSNQVAVLYQDVNCTE
	S_85	VLYQDVNCTEVPVAI
	S_86	VNCTEVPVAIHADQL
	S_87	VPVAIHADQLTPTWRVY
Peptide Pool	Peptide ID	Peptide Sequence
S1 continued	S_88	DQLTPTWRVYSTGSNVF
	S_89	RVYSTGSNVFQTRAGCLI
	S_90	VFQTRAGCLIGAEHV
	S_91	AGCLIGAEHVNNSYECDI
	S_92	HVNNSYECDIPIGAGI
	S_93	ECDIPIGAGICASYQTQT

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Peptide Pool	Peptide ID	Peptide Sequence
	S_94	GICASYQTQTNSPRRAR
	S_95	TQTNSPRRARSVASQSII
	S_96	ARSVASQSIIAYTMSL
	S_97	QSIIAYTMSLGAENSVAY
	S_98	SLGAENSVAYSNNSIAI

S2	S_99	VAYSNNSIAIPTNFTISV
	S_100	AIPTNFTISVTTEILPV
	S_101	ISVTTEILPVSMTKTSV
	S_102	LPVSMTKTSVDCTMYI
	S_103	KTSVDCTMYICGDSTECS
	S_104	YICGDSTECSNLLLQY
	S_105	TECSNLLLQYGSFCTQL
	S_106	LQYGSFCTQLNRALTGI
	S_107	TQLNRALTGIAVEQDK
	S_108	LTGIAVEQDKNTQEVF
	S_109	EQDKNTQEVFAQVKQIYK
	S_110	VFAQVKQIYKTPPIKDF
	S_111	IYKTPPIKDFGGFNFSQI
	S_112	DFGGFNFSQILPDPSK
	S_113	FSQILPDPSKPSKRSFI
	S_114	PSKPSKRSFIEDLLFNKV
	S_115	FIEDLLFNKVTLADAGFI
	S_116	KVTLADAGFIKQYGDCL
	S_117	GFIKQYGDCLGDIAARDL
	S_118	CLGDIAARDLICAQKF
	S_119	ARDLICAQKFNGLTVL
	S_120	AQKFNGLTVLPPLLTDEM
	S_121	VLPPLLTDEMIAQYTSAL
	S_122	EMIAQYTSALLAGTI
	S_123	YTSALLAGTITSGWTF
	S_124	AGTITSGWTFGAGAALQI
	S_125	TFGAGAALQIPFAMQMAY
	S_126	QIPFAMQMAYRFNGIGV
	S_127	MAYRFNGIGVTQNVLY
	S_128	GIGVTQNVLYENQKLI
	S_129	NVLYENQKLIANQFNSAI
	S_130	LIANQFNSAIGKIQDSL
	S_131	SAIGKIQDSLSSTASAL
	S_132	DSLSSTASALGKLQDVV
	S_133	SALGKLQDVVNQNAQAL
Peptide Pool	Peptide ID	Peptide Sequence
	S_134	DVVNQNAQALNTLVKQL
	S_135	QALNTLVKQLSSNFGAI
	S_136	KQLSSNFGAISSVLNDIL
	S_137	AISSVLNDILSRLDKV
	S_138	NDILSRLDKVEAEVQIDR
	S_139	KVEAEVQIDRLITGRL
	S_140	QIDRLITGRLQSLQTYV
	S_141	GRLQSLQTYVTQQLIR
	S_142	QTYVTQQLIRAAEIR
	S_143	QQLIRAAEIRASANL
	S_144	AAEIRASANLAATKM
	S_145	ASANLAATKMSECVL
	S_146	AATKMSECVLGQSKRVDF
	S_147	VLGQSKRVDFCGKGYHLM
	S_148	DFCGKGYHLMSFPQSAPH
	S_149	LMSFPQSAPHGVVFLHV

S2 continued	S_150	APHGVVFLHVTYVPAQEK
	S_151	HVTYVPAQEKNFTTAPAI
	S_152	EKNFTTAPAICHDGKAHF
	S_153	AICHDGKAHFPREGVFV
	S_154	AHFPREGVFVSNGTHWFV
	S_155	FVSNGTHWFVTQRNFY
	S_156	HWFVTQRNFYEPQII
	S_157	QRNFYEPQIITTDNTFV
	S_158	QIITTDNTFVSGNCDVVI
	S_159	FVSGNCDVVIGIVNNTVY
	S_160	VIGIVNNTVYDPLQPEL
	S_161	TVYDPLQPELDSFKEEL
	S_162	PELDSFKEELDKYFK
	S_163	FKEELDKYFKNHTSPDV
	S_164	YFKNHTSPDVDLGDISGI
	S_165	DVDLGDISGINASVVNI
	S_166	SGINASVVNIQKEIDRL
	S_167	VNIQKEIDRLNEVAKNL
	S_168	DRLNEVAKNLNESLIDL
	S_169	KNLNESLIDLQELGKY
	S_170	LIDLQELGKYEQYIKWPW
	S_171	KYEQYIKWPWYIWLGFI
	S_172	WPWYIWLGFIAGLIAIVM
	S_173	FIAGLIAIVMVTIMLCCM
	S_174	VMVTIMLCCMTSCCSCLK
	S_175	CMTSCCSCLKGCCSCGSC
	S_176	LKGCCSCGSCCKFDEDDS
	S_177	SCCKFDEDDSEPVLKGVK
	S_178	FDEDDSEPVLKGVKLHYT

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