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Supplemental information

Impact of SARS-CoV-2 variants on the total $CD4^+$ and $CD8^+$ T cell reactivity in infected or vaccinated individuals

Alison Tarke, John Sidney, Nils Methot, Esther Dawen Yu, Yun Zhang, Jennifer M. Dan, Benjamin Goodwin, Paul Rubiro, Aaron Sutherland, Eric Wang, April Frazier, Sydney I. Ramirez, Stephen A. Rawlings, Davey M. Smith, Ricardo da Silva Antunes, Bjoern Peters, Richard H. Scheuermann, Daniela Weiskopf, Shane Crotty, Alba Grifoni, and Alessandro Sette

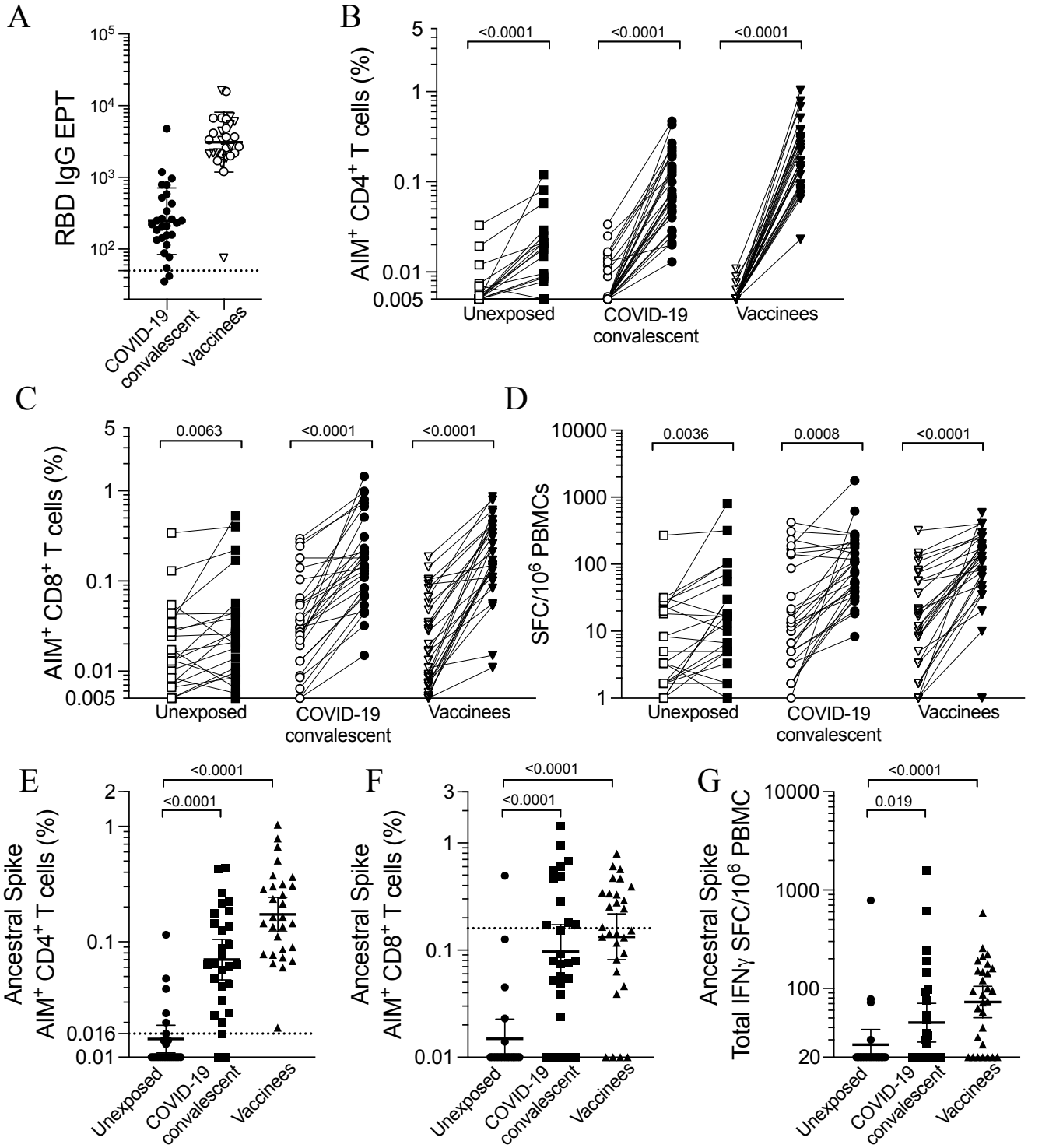

Fig. S1. SARS-COV-2 serology, DMSO controls, and ancestral Spike MP responses for all the cohorts analyzed in this study, **related to Figures 1, 2 and 3 and Table 1.**

(**A**) Spike RBD serology in COVID-19 convalescents (n=28, filled circles) and COVID-19 vaccinees (Pfizer/BioNTech BNT162b2 (n=14, open triangles) and Moderna COVID-19 vaccines (n=15, open circles)). Unexposed donors (n=23, not plotted) were seronegative or collected prior to the emergence of SARS-CoV-2. (**B-C**) AIM$^+$ CD4$^+$ (**B**) or CD8$^+$ (**C**) T cells stimulated with DMSO or the ancestral S MP at 1ug/mL. (**D**) IFNγ SFC per million PBMC stimulated with DMSO or the ancestral S MP at 1ug/mL.

(**E-F**) AIM$^+$ CD4$^+$ (**E**) or CD8$^+$ (**F**) T cells stimulated with the ancestral S MP at 1ug/mL. Dotted black lines indicate the threshold of positivity calculated based on median +2 SD of the DMSO controls (>80). Data is plotted after background subtraction and SI>2. (**G**) IFNγ SFC per million PBMC stimulated with the ancestral S MP at 1ug/mL. Data is plotted after background subtraction, SI>2, and p value <0.05 by Poisson or T test.

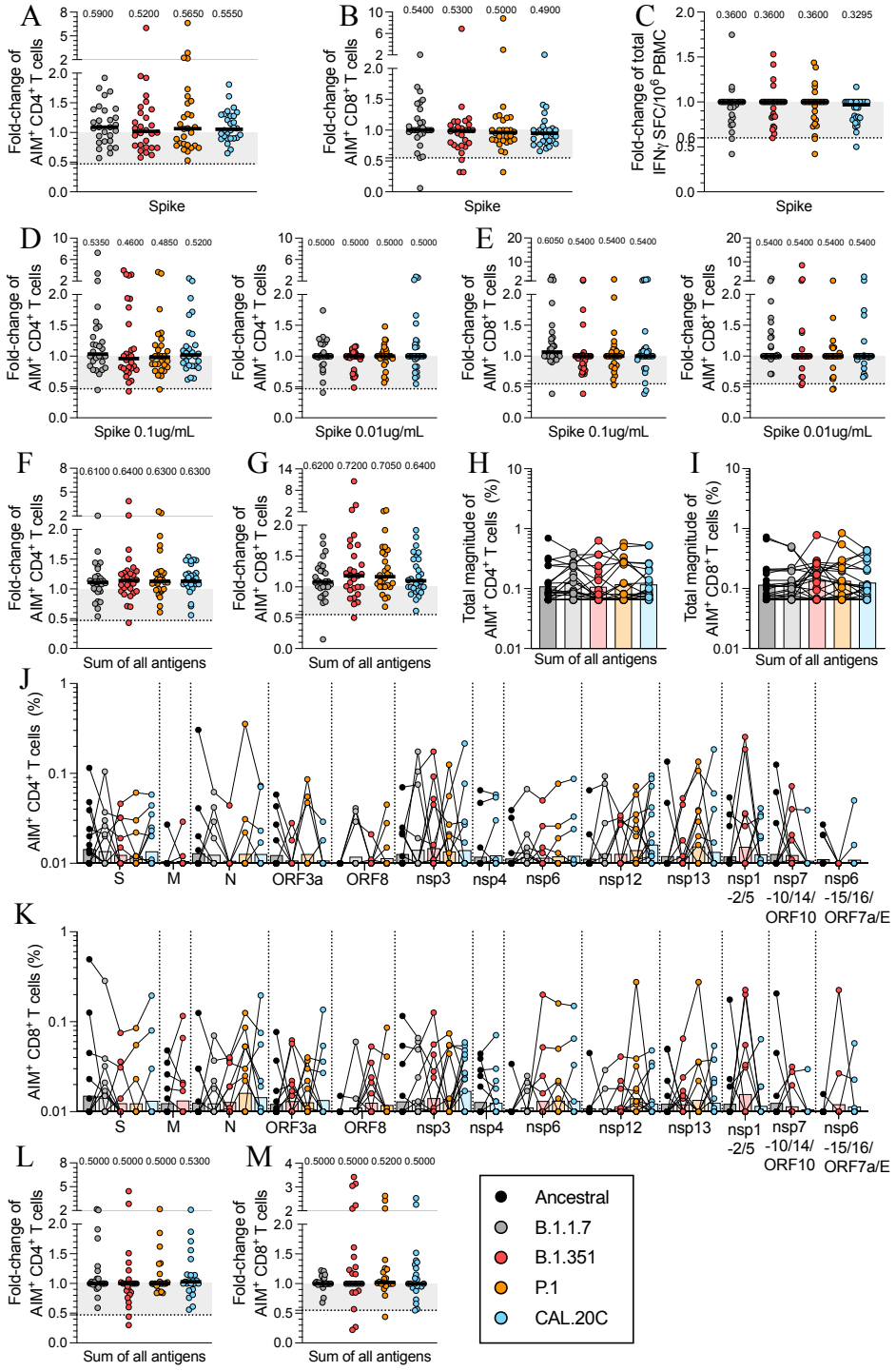

Fig. S2. Fold-change analyses of convalescent COVID-19 and unexposed donor responses to variant MPs, Related to Figures 1 and 2.

(**A-G**) Convalescent COVID-19 (n = 28) and (**H-M**) unexposed (n = 23) donors were stimulated with SARS-CoV-2 MPs corresponding to the ancestral reference strain (black) and the B.1.1.7 (grey), B.1.351 (red), P.1 (orange) and CAL.20C (light blue) SARS-CoV-2 variants. (**A-B**) Fold-change of AIM$^+$ CD4$^+$ (**A**) and CD8$^+$ (**B**) T cells with S MPs at 1ug/mL. (**C**) Fold-change of total IFNγ SFC/10^6 PBMC with S MPs at 1ug/mL. (**D-E**) Fold-change of AIM$^+$ CD4$^+$ (**D**) and CD8$^+$ (**E**) T cells with Spike MPs at 0.1 and 0.01 ug/mL. (**F-G**) Fold-change of the sum of AIM$^+$ CD4$^+$ (**F**) and CD8$^+$ (**G**) T cells for all SARS-CoV-2 antigens. (**H-I**) Percentages of AIM$^+$ (OX40$^+$CD137$^+$) CD4$^+$ T cells (**H**) and AIM$^+$ (CD69$^+$CD137$^+$) CD8$^+$ T cells (**I**) for the total reactivity. (**J-K**) Percentages of AIM$^+$ (OX40$^+$CD137$^+$) CD4$^+$ T cells (**J**) and AIM$^+$ (CD69$^+$CD137$^+$) CD8$^+$ T cells (**K**) for each MP. Bars represent the geometric mean. (**L-M**) Fold-change of the sum of AIM$^+$ CD4$^+$ (**L**) and CD8$^+$ (**M**) T cells for all SARS-CoV-2 antigens in unexposed donors. p values listed at the top of graphs correspond to the discrepancy and were calculated by one sample Wilcoxon Signed Rank test compared to the lower bound fold change threshold of 0.47 for CD4 AIM$^+$, 0.55 for CD8 AIM$^+$, and 0.6 for IFNγ SFC/10^6 PBMC.

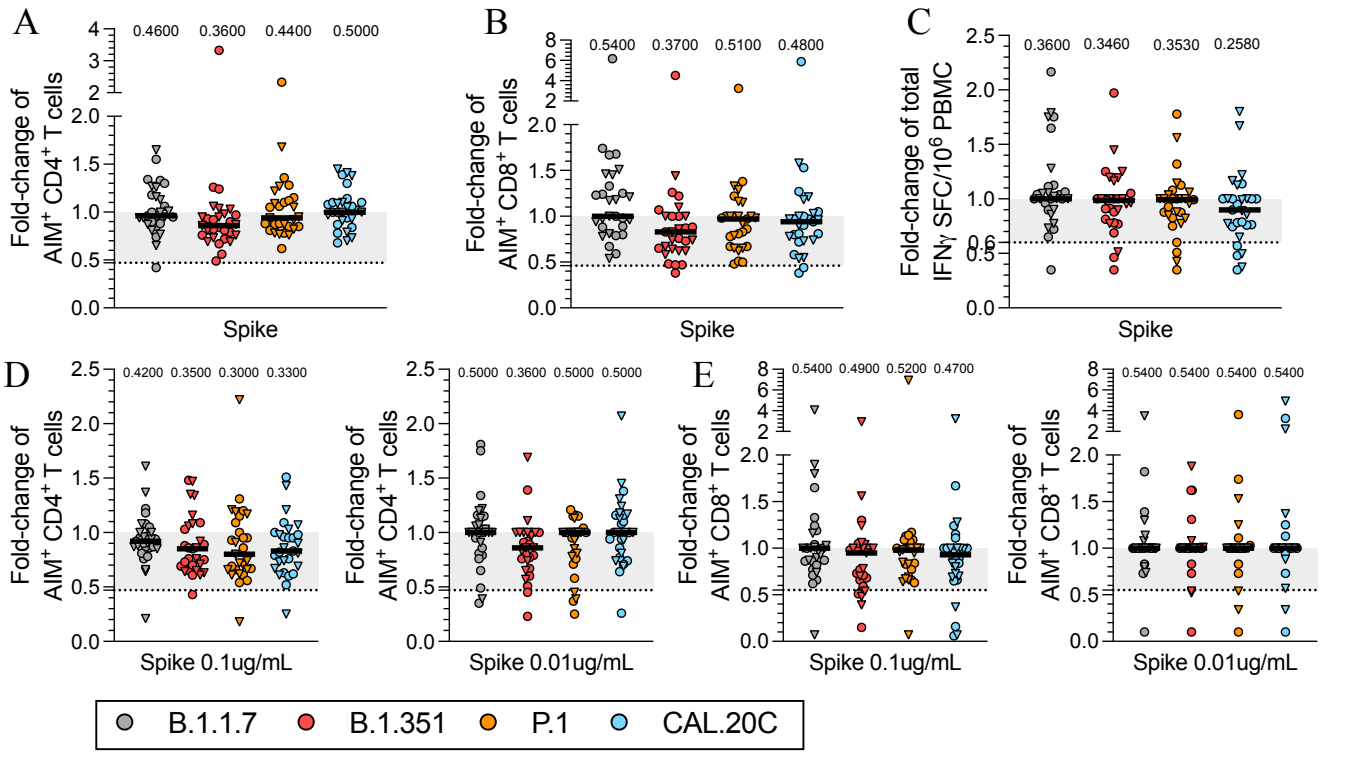

Figure S3. Fold-change analyses of COVID-19 vaccinees tested with the variant MPs, related to Figure 3.
PBMCs of Pfizer/BioNTech BNT162b2 (n=14, triangles) and Moderna COVID-19 vaccines (n=15, circles) were stimulated with the Spike MPs corresponding to the ancestral reference strain (black) and the B.1.1.7 (grey), B.1.351 (red), P.1 (orange) and CAL.20C (light blue) SARS-CoV-2 variants. (**A-B**) Fold-change of AIM$^+$ CD4$^+$ (**A**) and CD8$^+$ (**B**) T cells with Spike MPs at 1ug/mL. (**C**) Fold-change of total IFNγ SFC/10^6 PBMC with Spike MPs at 1ug/mL. (**D-E**) Fold-change of AIM$^+$ CD4$^+$ (**D**) and CD8$^+$ (**E**) T cells with Spike MPs at 0.1 and 0.01 ug/mL. p values listed at the top of graph correspond to the discrepancy and were calculated by one sample Wilcoxon Signed Rank test compared to the lower bound fold change threshold of 0.47 for CD4 AIM$^+$, 0.55 for CD8 AIM$^+$, and 0.6 for IFNγ SFC/10^6 PBMC.

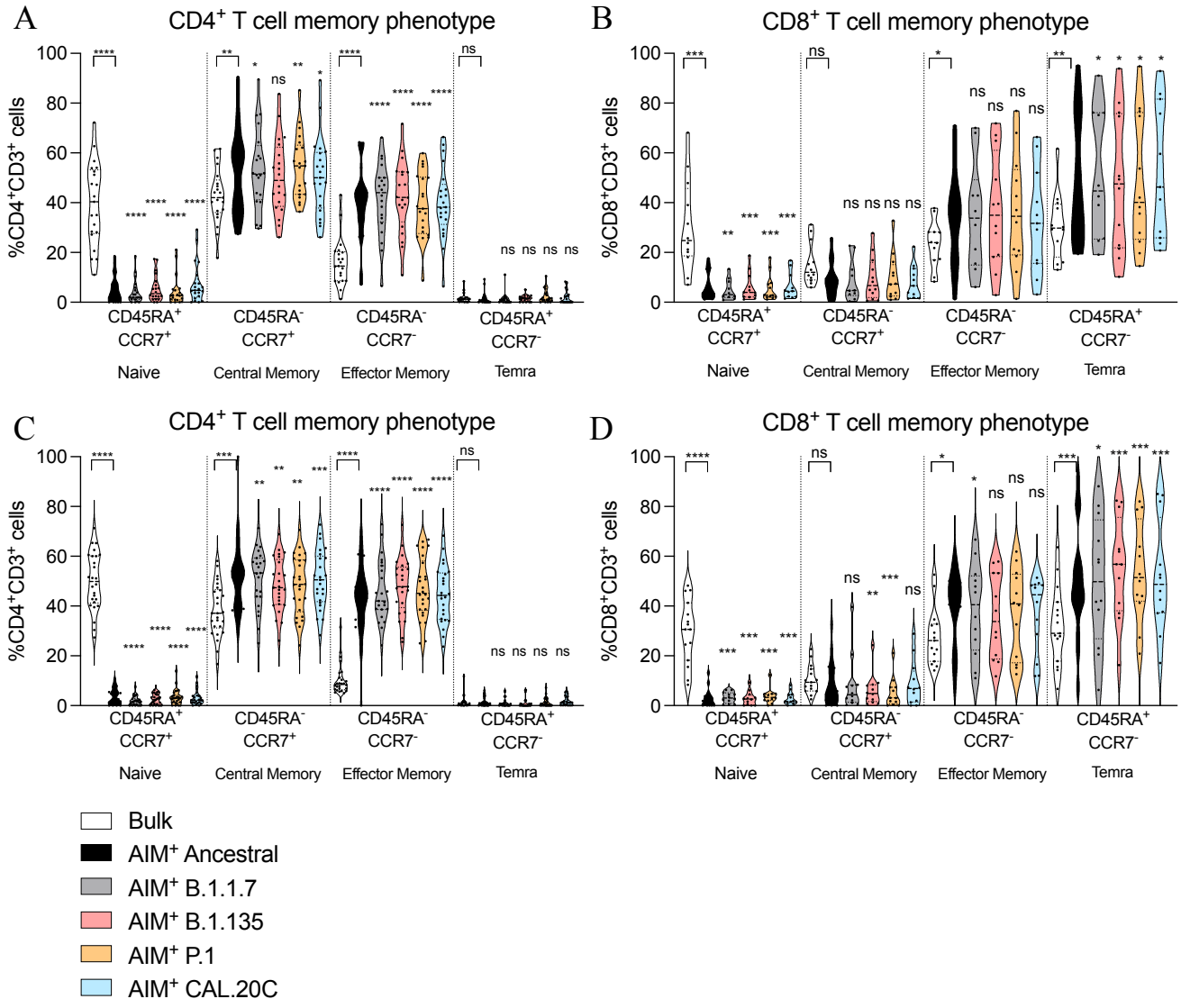

Figure S4. Memory phenotype of COVID-19 convalescent and vaccinated donors, related to Figures 1, 2, and 3.
(**A-B**) The memory phenotype of COVID-19 convalescent donors (n = 28) was analyzed to determine the makeup of naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and terminally differentiated effector memory ($CD45RA^+CCR7^-$) in the bulk and SARS-CoV-2 AIM^+ populations for $CD4^+$ (**A**) and $CD8^+$ (**B**) T cells. (**C-D**) The memory phenotype was also analyzed for the COVID-19 vaccinees (n = 29) for $CD4^+$ (**C**) and $CD8^+$ (**D**) T cells. The violin plots represent the bulk $CD4^+$ or $CD8^+$ T cell populations (white) or the subsets of AIM^+ T cells positive for the ancestral reference strain (black) and the B.1.1.7 (grey), B.1.351 (red), P.1 (orange) and CAL.20C (light blue) SARS-CoV-2 variants. p values were calculated comparing the bulk population to the AIM^+ populations separately for each variant by two-tailed Wilcoxon test.

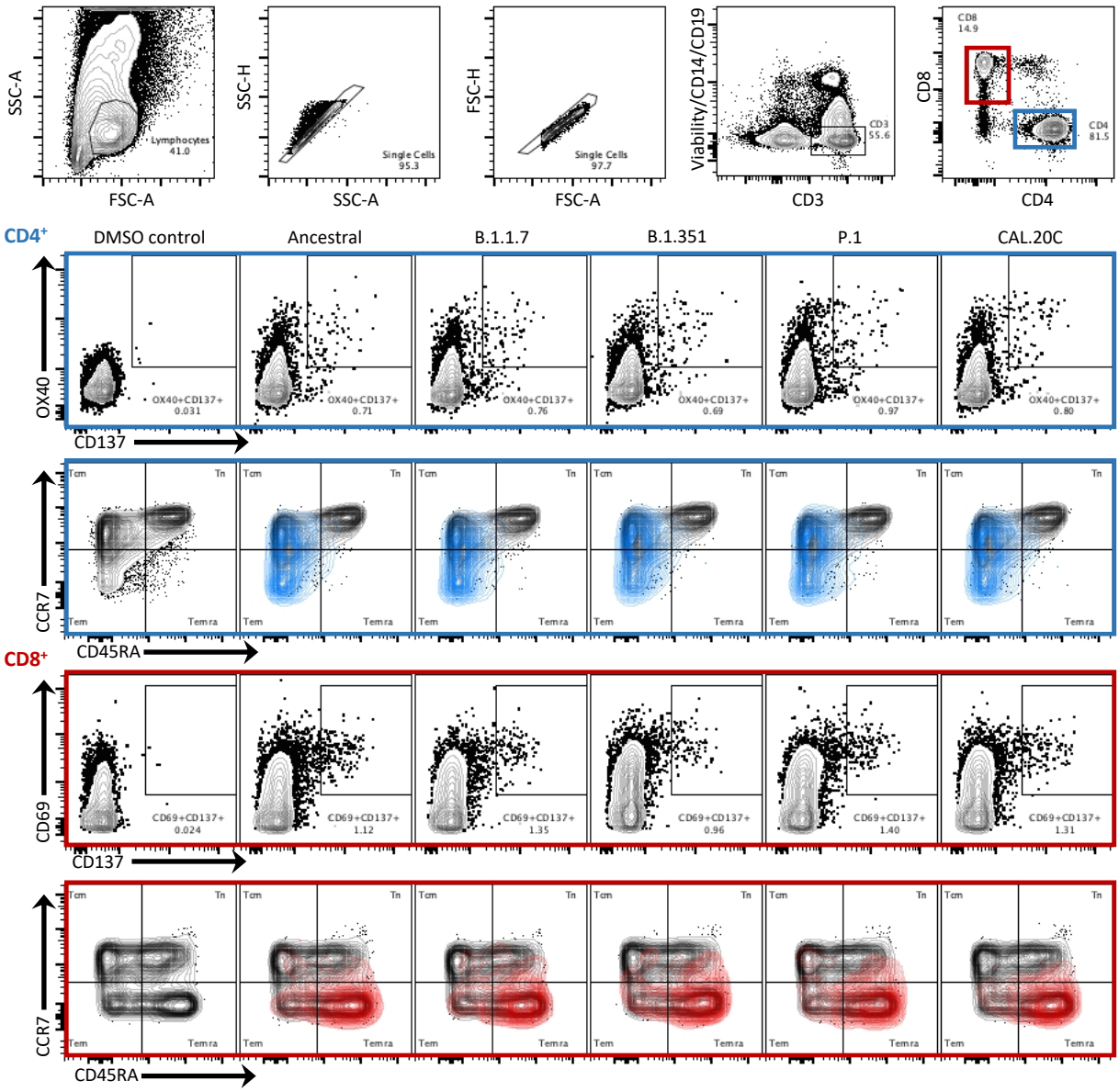

Figure S5. Gating strategy for AIM assay and memory phenotyping, related to Figures 1, 2, and 3.
Representative graphs illustrating the gating strategy used in the flow cytometry AIM assays in order to define antigen-specific $CD4^+$ (outlined in blue) and $CD8^+$ (outlined in red) T cells by the expression of $OX40^+CD137^+$ and $CD69^+CD137^+$, respectively. The memory compartment was analyzed by gating naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and terminally differentiated effector memory ($CD45RA^+CCR7^-$) cells on the bulk $CD4^+$ and $CD8^+$ T cells as well as the AIM^+ subsets, overlaid in blue and red, respectively. These graphs depict one of the COVID-19 convalescent donors from this study tested with each of the VOCs S MP and are representative of the gating strategy utilized with all donors tested.

Table S1. Related to Figures 1, 2, 3 and 4. List of amino acid positions and relative

Protein	Amino acid position	Ancestral (Wu)	B.1.1.7 (UK)	B.1.351 (SA)	P.1. (BR)	CAL.20C (CA)
S	13	S				I
S	18	L		F	F	
S	20	T			N	
S	26	P			S	
S	69	H	Del			
S	70	V	Del			
S	80	D		A		
S	138	D			Y	
S	145	Y	Del			
S	152	W				C
S	190	R			S	
S	215	D		G/H		
S	241	L		Del		
S	242	L		Del		
S	243	A		Del		
S	417	K		N	T	
S	452	L				R
S	484	E		K	K	
S	501	N	Y	Y	Y	
S	570	A	D			
S	614	D	G	G	G	G
S	655	H			Y	
S	681	P	H			
S	701	A		V		
S	716	T	I			
S	938	L				F
S	982	S	A			
S	1027	T			I	
S	1118	D	H			
S	1176	V			F	
S	1191	K				N
M	162	K		N		
N	3	D	L			
N	13	P		S		
N	32	R		H		
N	80	P			R	
N	203	R	K		K	K
N	204	G	R		R	R
N	205	T		I		I
N	212	G		C		
N	234	M				I
N	235	S	F			
E	71	P		L		
ORF3a	57	Q		H		H
ORF3a	131	W		L		
ORF3a	171	S		L		
ORF3a	253	S			P	
ORF7a	93	V		F		

ORF8	27	-	Stop			
ORF8	92	E			K	
ORF8	121	I		L		
nsp1	109	P		S		
nsp2	85	T		I		I
nsp2	339	G		S		
nsp2	366	S		T		
nsp2	427	Q		H		
nsp2	563	E		D		
nsp3	183	T	I			
nsp3	186	T			A	
nsp3	370	S			L	
nsp3	778	P				S
nsp3	837	K		N		
nsp3	890	A	D			
nsp3	926	C		S		
nsp3	977	K			Q	
nsp3	1180	T		I		
nsp3	1412	I	T			
nsp3	1778	N		S		
nsp4	395	S				T
nsp5	90	K		R		
nsp5	193	A		V		
nsp6	106	S	Del	Del	Del	
nsp6	107	G	Del	Del	Del	
nsp6	108	F	Del	Del	Del	
nsp6	125	L				F
nsp6	135	G		S		
nsp6	149	V		F		
nsp6	167	L				F
nsp9	65	I				V
nsp10	105	N		K		
nsp12	323	P	L	L	L	L
nsp13	53	P				L
nsp13	209	V				F
nsp13	260	D				Y
nsp13	341	E			D	
nsp13	588	T		I		
nsp14	177	L		F		
nsp14	326	F				L
nsp14	328	V		F		
nsp15	91	D				Y

Table S2. Related to Figures 1, 2, 3 and 4. List of mutated peptides with respect to the ancestral Wuhan strain in the different variants studied.

Protein	Aminoacid position (Start)	Aminoacid position (End)	SARS-CoV-2 strain	Sequence
nsp3	173	187	B.1.1.7	QDGSEDNQTTIIQTI
nsp3	178	192	B.1.1.7	DNQTTIIQTIVEVQP
nsp3	183	197	B.1.1.7	IIQTIVEVQPQLEME
nsp3	878	892	B.1.1.7	QDAYYRARAGEADNF
nsp3	883	897	B.1.1.7	RARAGEADNFCALIL
nsp3	888	902	B.1.1.7	EADNFCALILAYCNK
nsp3	1398	1412	B.1.1.7	NYLKSPNFSKLINIT
nsp3	1403	1417	B.1.1.7	PNFSKLINITIWFLL
nsp3	1408	1422	B.1.1.7	LINITIWFLLLSVCL
nsp6	92	106	B.1.1.7	MRIMTWLDMVDTSLK
nsp6	97	111	B.1.1.7	WLDMVDTSLKLKDCV
nsp6	102	116	B.1.1.7	DTSLKLKDCVMYASA
nsp6	107	121	B.1.1.7	KLKDCVMYASAVVLL
nsp12	309	323	B.1.1.7	HCANFNVLFSTVFPL
nsp12	314	328	B.1.1.7	NVLFSTVFPLTSFGP
nsp12	319	333	B.1.1.7	TVFPLTSFGPLVRKI
N	1	15	B.1.1.7	MSLNGPQNQRNAPRI
N	191	205	B.1.1.7	RNSSRNSTPGSSKRT
N	196	210	B.1.1.7	NSTPGSSKRTSPARM
N	201	215	B.1.1.7	SSKRTSPARMAGNGG
N	221	235	B.1.1.7	LLLLDRLNQLESKMF
N	226	240	B.1.1.7	RLNQLESKMFGKGQQ
N	231	245	B.1.1.7	ESKMFGKGQQQQGQT
ORF8	41	55	B.1.1.7	FYSKWYIRVGAIKSA
ORF8	46	60	B.1.1.7	YIRVGAIKSAPLIEL
ORF8	51	65	B.1.1.7	AIKSAPLIELCVDEA
ORF8	61	75	B.1.1.7	CVDEAGSKSPIQCID
ORF8	66	80	B.1.1.7	GSKSPIQCIDIGNYT
ORF8	71	85	B.1.1.7	IQCIDIGNYTVSCLP
S	56	70	B.1.1.7	LPFFSNVTWFHAISG
S	61	75	B.1.1.7	NVTWFHAISGTNGTK
S	66	80	B.1.1.7	HAISGTNGTKRFDNP
S	131	145	B.1.1.7	CEFQFCNDPFLGVYH
S	136	150	B.1.1.7	CNDPFLGVYHKNNKS
S	141	155	B.1.1.7	LGVYHKNNKSWMESE
S	491	505	B.1.1.7	PLQSYGFQPTYGVGY
S	496	510	B.1.1.7	GFQPTYGVGYQPYRV
S	501	515	B.1.1.7	YGVGYQPYRVVVLSF
S	556	570	B.1.1.7	NKKFLPFQQFGRDID
S	561	575	B.1.1.7	PFQQFGRDIDDTTDA
S	566	580	B.1.1.7	GRDIDDTTDAVRDPQ
S	601	615	B.1.1.7	GTNTSNQVAVLYQGV
S	606	620	B.1.1.7	NQVAVLYQGVNCTEV
S	611	625	B.1.1.7	LYQGVNCTEVPVAIH
S	671	685	B.1.1.7	CASYQTQTNSHRRAR
S	676	690	B.1.1.7	TQTNSHRRARSVASQ
S	681	695	B.1.1.7	HRRARSVASQSIIAY
S	706	720	B.1.1.7	AYSNNSIAIPINFTI
S	711	725	B.1.1.7	SIAIPINFTISVTTE
S	716	730	B.1.1.7	INFTISVTTEILPVS
S	971	985	B.1.1.7	GAISSVLNDILARLD
S	976	990	B.1.1.7	VLNDILARLDKVEAE
S	981	995	B.1.1.7	LARLDKVEAEVQIDR
S	1106	1120	B.1.1.7	QRNFYEPQIITTHNT
S	1111	1125	B.1.1.7	EPQIITTHNTFVSGN
S	1116	1130	B.1.1.7	TTHNTFVSGNCDVVI
N	1	15	B.1.351	MSDNGPQNQRNASRI
N	6	20	B.1.351	PQNQRNASRITFGGP
N	11	25	B.1.351	NASRITFGGPSDSTG
N	21	35	B.1.351	SDSTGSNQNGEHSGA
N	26	40	B.1.351	SNQNGEHSGARSKQR
N	31	45	B.1.351	EHSGARSKQRRPQGL
N	191	205	B.1.351	RNSSRNSTPGSSRGI
N	196	210	B.1.351	NSTPGSSRGISPARM
N	201	215	B.1.351	SSRGISPARMACNGG
N	206	220	B.1.351	SPARMACNGGDAALA
N	211	225	B.1.351	ACNGGDAALALLLLD
ORF8	107	121	B.1.351	DFLEYHDVRVVLDFL
ORF7a	81	95	B.1.351	SVSPKLFIRQEEFQE
ORF7a	86	100	B.1.351	LFIRQEEFQELYSPI
ORF7a	91	105	B.1.351	EEFQELYSPIFLIVA
M	151	165	B.1.351	IAGHHLGRCDINDLP
M	156	170	B.1.351	LGRCDINDLPKEITV
M	161	175	B.1.351	INDLPKEITVATSRT
ORF3a	46	60	B.1.351	LIVGVALLAVFHSAS
ORF3a	51	65	B.1.351	ALLAVFHSASKIITL
ORF3a	56	70	B.1.351	FHSASKIITLKKRWQ
ORF3a	121	135	B.1.351	VRIIMRLWLCLKCRS
ORF3a	126	140	B.1.351	RLWLCLKCRSKNPLL
ORF3a	131	145	B.1.351	LKCRSKNPLLYDANY
ORF3a	161	175	B.1.351	NSVTSSIVITLGDGT
ORF3a	166	180	B.1.351	SIVITLGDGTTSPIS
ORF3a	171	185	B.1.351	LGDGTTSPISEHDYQ
nsp1	96	110	B.1.351	QYGRSGETLGVLVSH
nsp1	101	115	B.1.351	GETLGVLVSHVGEIP
nsp1	106	120	B.1.351	VLVSHVGEIPVAYRK
nsp5	183	197	B.1.351	GPFVDRQTAQVAGTD
nsp5	188	202	B.1.351	RQTAQVAGTDTTITV
nsp5	193	207	B.1.351	VAGTDTTITVNVLAW
nsp10	93	107	B.1.351	KGKYVQIPTTCAKDP
nsp10	98	112	B.1.351	QIPTTCAKDPVGFTL
nsp10	103	117	B.1.351	CAKDPVGFTLKNTVC
nsp12	309	323	B.1.351	HCANFNVLFSTVFPL
nsp12	314	328	B.1.351	NVLFSTVFPLTSFGP
nsp12	319	333	B.1.351	TVFPLTSFGPLVRKI

nsp14	316	330	B.1.351	VVKAALLADKFPFLH
nsp14	321	335	B.1.351	LLADKFPFLHDIGNP
nsp14	326	340	B.1.351	FPFLHDIGNPKAIKC
S	6	20	B.1.351	VLLPLVSSQCVNFTT
S	11	25	B.1.351	VSSQCVNFTTRTQLP
S	16	30	B.1.351	VNFTTRTQLPPAYTN
S	66	80	B.1.351	HAIHVSGTNGTKRFA
S	71	85	B.1.351	SGTNGTKRFANPVLP
S	76	90	B.1.351	TKRFANPVLPFNDGV
S	201	215	B.1.351	FKIYSKHTPINLVRH
S	201	215	B.1.351	FKIYSKHTPINLVRG
S	206	220	B.1.351	KHTPINLVRHLPQGF
S	206	220	B.1.351	KHTPINLVRGLPQGF
S	211	225	B.1.351	NLVRHLPQGFSALEP
S	211	225	B.1.351	NLVRGLPQGFSALEP
E	61	75	B.1.351	RVKNLNSSRVLDLLV
nsp2	71	85	B.1.351	LQTPFEIKLAKKFDI
nsp2	76	90	B.1.351	EIKLAKKFDIFNGEC
nsp2	81	95	B.1.351	KKFDIFNGECPNFVF
nsp2	326	340	B.1.351	CGNFKVTKGKAKKSA
nsp2	331	345	B.1.351	VTKGKAKKSAWNIGE
nsp2	336	350	B.1.351	AKKSAWNIGEQKSIL
nsp2	356	370	B.1.351	FASEAARVVRTIFSR
nsp2	361	375	B.1.351	ARVVRTIFSRTLETA
nsp2	366	380	B.1.351	TIFSRTLETAQNSVR
nsp2	416	430	B.1.351	VVMAYITGGVVHLTS
nsp2	421	435	B.1.351	ITGGVVHLTSQWLTN
nsp2	426	440	B.1.351	VHLTSQWLTNIFGTV
nsp2	551	565	B.1.351	MPLKAPKEIIFLDGE
nsp2	556	570	B.1.351	PKEIIFLDGETLPTE
nsp2	561	575	B.1.351	FLDGETLPTEVLTEE
nsp3	823	837	B.1.351	SFLGRYMSALNHTKN
nsp3	828	842	B.1.351	YMSALNHTKNWKYPQ
nsp3	833	847	B.1.351	NHTKNWKYPQVNGLT
nsp3	1168	1182	B.1.351	LHKPIVWHVNNAINK
nsp3	1173	1187	B.1.351	VWHVNNAINKATYKP
nsp3	1178	1192	B.1.351	NAINKATYKPNTWCI
nsp3	1768	1782	B.1.351	VAVKMFDAYVSTFSS
nsp3	1773	1787	B.1.351	FDAYVSTFSSTFNVP
nsp3	1778	1792	B.1.351	STFSSTFNVPMEKLK
nsp13	577	591	B.1.351	SDRDLYDKLQFISLE
nsp13	582	596	B.1.351	YDKLQFISLEIPRRN
nsp13	587	601	B.1.351	FISLEIPRRNVATLQ
nsp6	92	106	B.1.351	MRIMTWLDMVDTSLK
nsp6	97	111	B.1.351	WLDMVDTSLKLKDCV
nsp6	102	116	B.1.351	DTSLKLKDCVMYASA
nsp6	107	121	B.1.351	LKDCVMYASAVVLLI
nsp6	122	136	B.1.351	LLILMTARTVYDDSA
nsp6	127	141	B.1.351	TARTVYDDSARRVWT
nsp6	132	146	B.1.351	YDDSARRVWTLMNVL
nsp6	137	151	B.1.351	RRVWTLMNVLTLFYK
nsp6	142	156	B.1.351	LMNVLTLFYKVYYGN
nsp6	147	161	B.1.351	TLFYKVYYGNALDQA
S	231	245	B.1.351	IGINITRFQTLHRSY
S	236	250	B.1.351	TRFQTLHRSYLTPGD
S	241	255	B.1.351	LHRSYLTPGDSSSGW
S	406	420	B.1.351	EVRQIAPGQTGNIAD
S	411	425	B.1.351	APGQTGNIADYNYKL
S	416	430	B.1.351	GNIADYNYKLPDDFT
S	476	490	B.1.351	EIYQAGSTPCNGVKG
S	481	495	B.1.351	GSTPCNGVKGFNCYF
S	486	500	B.1.351	NGVKGFNCYFPLQSY
S	491	505	B.1.351	PLQSYGFQPTYGVGY
S	496	510	B.1.351	GFQPTYGVGYQPYRV
S	501	515	B.1.351	YGVGYQPYRVVVLSF
S	606	620	B.1.351	GTNTSNQVAVLYQGV
S	611	625	B.1.351	NQVAVLYQGVNCTEV
S	616	630	B.1.351	LYQGVNCTEVPVAIH
S	691	705	B.1.351	SIIAYTMSLGVENSV
S	696	710	B.1.351	TMSLGVENSVAYSNN
S	701	715	B.1.351	VENSVAYSNNSIAIP
N	66	80	P.1.	FPRGQGVPINTNSSR
N	71	85	P.1.	GVPINTNSSRDDQIG
N	76	90	P.1.	TNSSRDDQIGYYRRA
N	191	205	P.1.	RNSSRNSTPGSSKRT
N	196	210	P.1.	NSTPGSSKRTSPARM
N	201	215	P.1.	SSKRTSPARMAGNGG
ORF8	81	95	P.1.	VSCLPFTINCQKPKL
ORF8	86	100	P.1.	FTINCQKPKLGSLVV
ORF8	91	105	P.1.	QKPKLGSLVVRCSFY
ORF3a	241	255	P.1.	EEHVQIHTIDGSPGV
ORF3a	246	260	P.1.	IHTIDGSPGVVNPVM
ORF3a	251	265	P.1.	GSPGVVNPVMEPIYD
nsp12	309	323	P.1.	HCANFNVLFSTVFPL
nsp12	314	328	P.1.	NVLFSTVFPLTSFGP
nsp12	319	333	P.1.	TVFPLTSFGPLVRKI
nsp13	327	341	P.1.	IDKCSRIIPARARVD
nsp13	332	346	P.1.	RIIPARARVDCFDKF
nsp13	337	351	P.1.	RARVDCFDKFKVNST
S	6	20	P.1.	VLLPLVSSQCVNFTN
S	11	25	P.1.	VSSQCVNFTNRTQLP
S	16	30	P.1.	VNFTNRTQLPSAYTN
S	21	35	P.1.	RTQLPSAYTNSFTRG
S	26	40	P.1.	SAYTNSFTRGVYYPD
S	126	140	P.1.	VVIKVCEFQFCNYPF
S	131	145	P.1.	CEFQFCNYPFLGVYY
S	136	150	P.1.	CNYPFLGVYYHKNNK
S	176	190	P.1.	LMDLEGKQGNFKNLS
S	181	195	P.1.	GKQGNFKNLSEFVFK

S	186	200	P.1.	FKNLSEFVFKNIDGY
S	406	420	P.1.	EVRQIAPGQTGTIAD
S	411	425	P.1.	APGQTGTIADYNYKL
S	416	430	P.1.	GTIADYNYKLPDDFT
S	471	485	P.1.	EIYQAGSTPCNGVKG
S	476	490	P.1.	GSTPCNGVKGFNCYF
S	481	495	P.1.	NGVKGFNCYFPLQSY
S	491	505	P.1.	PLQSYGFQPTYGVGY
S	496	510	P.1.	GFQPTYGVGYQPYRV
S	501	515	P.1.	YGVGYQPYRVVVLSF
S	601	615	P.1.	GTNTSNQVAVLYQGV
S	606	620	P.1.	NQVAVLYQGVNCTEV
S	611	625	P.1.	LYQGVNCTEVPVAIH
S	641	655	P.1.	NVFQTRAGCLIGAEY
S	646	660	P.1.	RAGCLIGAEYVNNSY
S	651	665	P.1.	IGAEYVNNSYECDIP
S	1016	1030	P.1.	AEIRASANLAAIKMS
S	1021	1035	P.1.	SANLAAIKMSECVLG
S	1026	1040	P.1.	AIKMSECVLGQSKRV
S	1166	1180	P.1.	LGDISGINASFVNIQ
S	1171	1185	P.1.	GINASFVNIQKEIDR
S	1176	1190	P.1.	FVNIQKEIDRLNEVA
nsp3	173	187	P.1.	QDGSEDNQTTTIQAI
nsp3	178	192	P.1.	DNQTTTIQAIVEVQP
nsp3	183	197	P.1.	TIQAIVEVQPQLEME
nsp3	358	372	P.1.	AVFDKNLYDKLVLSF
nsp3	363	377	P.1.	NLYDKLVLSFLEMKS
nsp3	368	382	P.1.	LVLSFLEMKSEKQVE
nsp3	963	977	P.1.	KGVQIPCTCGKQATQ
nsp3	968	982	P.1.	PCTCGKQATQYLVQQ
nsp3	973	987	P.1.	KQATQYLVQQESPFV
nsp6	92	106	P.1.	MRIMTWLDMVDTSLK
nsp6	97	111	P.1.	WLDMVDTSLKLKDCV
nsp6	102	116	P.1.	DTSLKLKDCVMYASA
nsp6	107	121	P.1.	LKDCVMYASAVVLLI
N	191	205	CAL.20C	RNSSRNSTPGSSKRI
N	196	210	CAL.20C	NSTPGSSKRISPARM
N	201	215	CAL.20C	SSKRISPARMAGNGG
N	221	235	CAL.20C	LLLLDRLNQLESKIS
N	226	240	CAL.20C	RLNQLESKISGKGQQ
N	231	245	CAL.20C	ESKISGKGQQQQGQT
nsp2	71	85	CAL.20C	LQTPFEIKLAKKFDI
nsp2	76	90	CAL.20C	EIKLAKKFDIFNGEC
nsp2	81	95	CAL.20C	KKFDIFNGECPNFVF
nsp3	768	782	CAL.20C	MSMTYGQQFGSTYLD
nsp3	773	787	CAL.20C	GQQFGSTYLDGADVT
nsp3	778	792	CAL.20C	STYLDGADVTKIKPH
nsp4	383	397	CAL.20C	ICISTKHFYWFFTNY
nsp4	388	402	CAL.20C	KHFYWFFTNYLKRRV
nsp4	393	407	CAL.20C	FFTNYLKRRVVFNGV
nsp6	112	126	CAL.20C	DCVMYASAVVLLIFM
nsp6	117	131	CAL.20C	ASAVVLLIFMTARTV
nsp6	122	136	CAL.20C	LLIFMTARTVYDDGA
nsp6	157	171	CAL.20C	ALDQAISMWAFIISV
nsp6	162	176	CAL.20C	ISMWAFIISVTSNYS
nsp6	167	181	CAL.20C	FIISVTSNYSGVVTT
nsp9	51	65	CAL.20C	LKWARFPKSDGTGTV
nsp9	56	70	CAL.20C	FPKSDGTGTVYTELE
nsp9	61	75	CAL.20C	GTGTVYTELEPPCRF
nsp12	309	323	CAL.20C	HCANFNVLFSTVFPL
nsp12	314	328	CAL.20C	NVLFSTVFPLTSFGP
nsp12	319	333	CAL.20C	TVFPLTSFGPLVRKI
nsp13	42	56	CAL.20C	VLSVNPYVCNAPGCD
nsp13	47	61	CAL.20C	PYVCNAPGCDVTDVT
nsp13	52	66	CAL.20C	APGCDVTDVTQLYLG
nsp13	197	211	CAL.20C	EYTFEKGDYGDAFVY
nsp13	202	216	CAL.20C	KGDYGDAFVYRGTTT
nsp13	207	221	CAL.20C	DAFVYRGTTTYKLNV
nsp13	247	261	CAL.20C	VRITGLYPTLNISYE
nsp13	252	266	CAL.20C	LYPTLNISYEFSSNV
nsp13	257	271	CAL.20C	NISYEFSSNVANYQK
nsp14	316	330	CAL.20C	VVKAALLADKLPVLH
nsp14	321	335	CAL.20C	LLADKLPVLHDIGNP
nsp14	326	340	CAL.20C	LPVLHDIGNPKAIKC
nsp15	79	93	CAL.20C	IAANTVIWDYKRYAP
nsp15	84	98	CAL.20C	VIWDYKRYAPAHIST
nsp15	89	103	CAL.20C	KRYAPAHISTIGVCS
ORF3a	46	60	CAL.20C	LIVGVALLAVFHSAS
ORF3a	51	65	CAL.20C	ALLAVFHSASKIITL
ORF3a	56	70	CAL.20C	FHSASKIITLKKRWQ
S	1	15	CAL.20C	MFVFLVLLPLVSIQC
S	6	20	CAL.20C	VLLPLVSIQCVNLTT
S	11	25	CAL.20C	VSIQCVNLTTRTQLP
S	141	155	CAL.20C	LGVYYHKNNKSCMES
S	146	160	CAL.20C	HKNNKSCMESEFRVY
S	151	165	CAL.20C	SCMESEFRVYSSANN
S	441	455	CAL.20C	LDSKVGGNYNYRYRL
S	446	460	CAL.20C	GGNYNYRYRLFRKSN
S	451	465	CAL.20C	YRYRLFRKSNLKPFE
S	601	615	CAL.20C	GTNTSNQVAVLYQGV
S	606	620	CAL.20C	NQVAVLYQGVNCTEV
S	611	625	CAL.20C	LYQGVNCTEVPVAIH
S	926	940	CAL.20C	QFNSAIGKIQDSFSS
S	931	945	CAL.20C	IGKIQDSFSSTASAL
S	936	950	CAL.20C	DSFSSTASALGKLQD
S	1181	1195	CAL.20C	KEIDRLNEVANNLNE
S	1186	1200	CAL.20C	LNEVANNLNESLIDL
S	1191	1205	CAL.20C	NNLNESLIDLQELGK

Table S3. Related to Figures 1, 2, and 3. Technical replicates for the AIM and FluoroSPOT assays testing multiple donors in repeated assays with the same SARS-CoV-2 MPs.

AIM+ CD4+

Donor	MP 1	MP 1	MP 1	MP 1	MP 1	MP 2	MP 2	MP 2	MP 3	MP 3	MP 3
4801	0.381	0.2316	0.125	0.1312	0.1412	0.231	0.1616		0.171	0.0626	
4837	0.50351	0.1145	0.165	0.135		0.22351	0.2745		0.15351	0.0575	
4842	0.14784	0.1443	0.16674			0.15784	0.0943	0.09474	0.13784	0.2243	0.11674
4853	0.13846	0.039				0.02846			0.12846	0.067	
4863	0.53843	0.71211				0.25843	0.46211		0.14843		
4866	0.17948	0.106				0.05548			0.07248		
4867	0.36489	0.335	0.064	0.22567		0.16489			0.11489	0.071	
4868	1.18466	1.1895	0.40587			0.22466			0.18466		
4878	0.16885	0.0455	0.158			0.09885			0.09185		
4882	0.12956	0.6415	0.104			0.04556			0.08756		
4963	0.324	0.3052	0.29933			0.244	0.2052	0.22933	0.194	0.1252	0
4972	0.318	0.5635				0.568	0.2935		0.278	0.2435	
4945	0.27203	0.2833				0.12203	0.1233		0.25203	0.1833	
4973	0.10415	0.1414	0.08613			0.12415	0.1014	0.08313	0.13415	0.0684	0.08713
5329	0.047	0.082				0.017	0.135		0.046	0.021	
5328	0.5267	0.7097				0.5067	0.7497		0.0867	0	
5476	0.022	0.125				0.012	0.053		0.014	0.064	
5475	0.067	0.091				0.019	0		0.082	0.079	
5402	0	0.035				0.028	0.115		0.016	0	
5380	0.057	0.059				0.012	0.034		0.032	0.023	
5220	0.055	0.05				0.013	0.025		0	0	
5221	0.024	0.041				0	0		0	0.012	
5451	0.032	0.023				0	0		0	0.015	

AIM+ CD8+

Donor	MP 1	MP 1	MP 1	MP 1	MP 1	MP 2	MP 2	MP 2	MP 3	MP 3	MP 3
4801	0.12	0.192	0.073	0.053	0.061	0.093	0.122		0.068	0.054	
4837	0.06983	0.1295	0.125	0.034		0.03683	0.1095		0.03283	0.1295	
4842	0.021	0.095	0			0.062	0.028	0	0.02	0.059	0
4853	0.26699	0.1905				0.02599			0.01499	0	
4863	0.875	1.203				0.445	0.743		0.145		
4866	0.08938	0.09212				0.02838			0.03638		
4867	0.366	0.615	0.285	0.51933		0.116			0.031	0.075	
4868	1.799	1.556	0.3175			0.05			0.068		
4878	0.211	0	0.0735			0.111			0.131		
4882	0.04636	0.3925	0.072			0			0.02036		
4963	0.214	0.1365	0			0.104	0.0665	0	0.214	0.0865	0
4972	0.397	0.1435				0.337	0.1135		0.187	0.1335	
4945	0.478	0.045				0.128	0		0.818	0.564	
4973	0.037	0.0657	0.09541			0.037	0.0447	0.03341	0.025	0	0
5329	0.0937	0.2943				0.1737	0.4443		0	0	
5328	1.7597	1.8747				1.5197	1.6847		0.0797	0	
5476	0.0313	0.084				0.0133	0		0.0153	0.049	
5475	0	0				0	0		0.1017	0.2187	
5402	0	0				0	0		0	0	
5380	0.1117	0.1463				0	0		0.0617	0	
5220	0	0				0	0		0	0	
5221	0	0.057				0	0.021		0	0	
5451	0	0				0	0		0	0	

Fluorospot

Donor	MP 4	MP 4	MP 5	MP 5
4270	382	554	167	200
4245	38		310	300
4271	780	1081	43	48
4246	541	504	109	102
4272	528	620	756	1019
4260	109	76	760	1766
4265	831	1024	170	140
4261	374	385	57	72
4278	484	973	47	76
4288	1074	1989	198	139
4266	82	121	459	649
4277	770	1425	24	27
4292	103	90	107	174
4292			126	118
4264			87	123
4244			318	336

Donor	MP 6	MP 6	MP 6	MP 6
Donor0009	63	48	50	319
Donor0010	108	46	52	36
Donor0011	79	58	64	87
Donor0012	263	387	222	145
Donor0014	27	49	35	21
Donor0016	134	175	155	142
Donor0017	144	129	164	166
Donor0018	146	73	157	176
Donor0019	108	251	162	266
Donor0020	63	68	99	54
Donor0021	19	30	18	21
Donor0022	19	8	6	9
Donor0023	88	108	117	107
Donor0024	76	90	87	84
Donor0025	142	226	238	216
Donor0026	77	70	87	
Donor0027	385	451	51	77
Donor0028	51	45	38	51
Donor0029	38	50	23	9
Donor0030	23	22	38	8
Donor0031	38	8	4	10

Table S4. Related to Figure 4. Effect of mutations on CD8 epitope HLA class I binding capacity

Origin	Ancestral reference sequence	Protein	Start	Mutation[a]	Mutated sequence[b]	HLA restriction	WT (IC_{50} nM)[c]	Mutant (IC_{50} nM)	Fold difference	Effect[d]
B.1.1.7	HVSGTNGTK	S	69	HV69-70 del	HAISGTNGTK	A*68:01	55	44	0.8	Neutral
B.1.1.7	GVYYHKNNK	S	142	Y145 del	FLGVYHKNNK	A*03:01	28	1078	39	Decrease
B.1.1.7	YYHKNNKSW	S	144	Y145 del	VYHKNNKSW	A*24:02	117	308	2.6	Decrease
B.1.1.7	YGFQPTNGV	S	495	N501Y	YGFQPTYGV	B*51:01	3488	3541	1.0	Neutral
B.1.1.7	YQDVNCTEV	S	612	D614G	YQGVNCTEV	A*02:06	18	57	3.2	Decrease
B.1.1.7	QTNSPRRAR	S	677	P681H	QTNSHRRAR	A*31:01	35	33	0.94	Neutral
B.1.1.7	SPRRARSV	S	680	P681H	SHRRARSV	B*08:01	429	2449	5.7	Decrease
B.1.1.7	NSIAIPTNF	S	710	T716I	NSIAIPINF	B*57:01	1335	968	0.73	Neutral
B.1.1.7	IAIPTNFTI	S	712	T716I	IAIPINFTI	B*51:01	209	189	0.90	Neutral
B.1.1.7	IAIPTNFTI	S	712	T716I	IAIPINFTI	B*53:01	396	266	0.67	Neutral
B.1.1.7	IPTNFTISV	S	714	T716I	IPINFTISV	B*07:02	188	168	0.89	Neutral
B.1.1.7	IPTNFTISV	S	714	T716I	IPINFTISV	B*51:01	156	94	0.60	Neutral
B.1.1.7	SVLNDILSR	S	975	S982A	SVLNDILAR	A*68:01	109	92	0.84	Neutral
B.1.1.7	KLINIIIWF	nsp3	1407	I1412T	KLINITIWF	A*32:01	161	48	0.30	Increase
B.1.1.7	STVFPPTSF	nsp12	318	P323L	STVFPLTSF	B*57:01	1583	637	0.40	Increase
B.1.351	RFDNPVLPF	S	78	D80A	RFANPVLPF	A*24:02	458	34	0.075	Increase
B.1.351	FDNPVLPFNDGVYF	S	79	D80A	FANPVLPFNDGVYF	B*35:01	65	65	1.0	Neutral
B.1.351	TPINLVRDL	S	208	D215G	TPINLVRGL	B*07:02	213	119	0.56	Neutral
B.1.351	TPINLVRDL	S	208	D215H	TPINLVRHL	B*07:02	213	199	0.93	Neutral
B.1.351	QIAPGQTGK	S	409	K417N	QIAPGQTGN	A*68:01	137	27998	204	Decrease
B.1.351	YGFQPTNGV	S	495	N501Y	YGFQPTYGV	B*51:01	3488	3541	1.0	Neutral
B.1.351	YQDVNCTEV	S	612	D614G	YQGVNCTEV	A*02:06	18	57	3.2	Decrease
B.1.351	YTMSLGAENSVAY	S	695	A701V	YTMSLGVENSVAY	A*26:01	184	253	1.4	Neutral
B.1.351	LGAENSVAY	S	699	A701V	LGVENSVAY	B*35:01	19	21	1.1	Neutral
B.1.351	GPQNQRNAPRITF	N	5	K17N	GPQNQRNASRITF	B*07:02	640	696	1.1	Neutral
B.1.351	QSASKIITL	ORF3a	57	Q57H	HSASKIITL	B*08:01	1788	573	0.32	Increase
B.1.351	MSALNHTKK	nsp3	829	K837N	MSALNHTKN	A*30:01	102	7035	69	Decrease
B.1.351	MSALNHTKKW	nsp3	829	K837N	MSALNHTKNW	B*57:01	16	14	0.88	Neutral
B.1.351	SALNHTKKW	nsp3	830	K837N	SALNHTKNW	B*57:01	111	93	0.84	Neutral
B.1.351	STVFPPTSF	nsp12	318	P323L	STVFPLTSF	B*57:01	1583	637	0.40	Increase
P.1.	LPPAYTNSF	S	24	P26S	LPSAYTNSF	B*07:02	294	51	0.17	Increase
P.1.	LPPAYTNSF	S	24	P26S	LPSAYTNSF	B*35:01	44	4.1	0.093	Increase
P.1.	LPPAYTNSF	S	24	P26S	LPSAYTNSF	B*53:01	366	18	0.049	Increase
P.1.	QIAPGQTGK	S	409	K417T	QIAPGQTGT	A*68:01	137	20478	149	Decrease
P.1.	YGFQPTNGV	S	495	N501Y	YGFQPTYGV	B*51:01	3488	3541	1.0	Neutral
P.1.	YQDVNCTEV	S	612	D614G	YQGVNCTEV	A*02:06	18	57	3.2	Decrease
P.1.	AEHVNNSY	S	653	H655Y	AEYVNNSY	B*44:02	1038	904	0.87	Neutral
P.1.	AEHVNNSY	S	653	H655Y	AEYVNNSY	B*44:03	1020	577	0.57	Neutral
P.1.	RASANLAATK	S	1019	L1027I	RASANLAAIK	A*03:01	85	99	1.2	Neutral
P.1.	NASVVNIQK	S	1173	V1176F	NASFVNIQK	A*68:01	13	6.2	0.48	Increase
P.1.	NTNSSPDDQIGYY	N	75	P80R	NTNSSRDDQIGYY	A*01:01	44	44	1.0	Neutral
P.1.	SPDDQIGYY	N	79	P80R	SRDDQIGYY	B*35:01	101	17781	175	Decrease
P.1.	LYDKLVSSF	nsp3	364	S370L	LYDKLVLSF	A*24:02	77	70	0.91	Neutral
P.1.	STVFPPTSF	nsp12	318	P323L	STVFPLTSF	B*57:01	1583	637	0.40	Increase
CAL.20C	LPLVSSQCV	S	8	S13I	LPLVSIQCV	B*51:01	402	272	0.68	Neutral
CAL.20C	YYHKNNKSW	S	144	W152C	YYHKNNKSC	A*24:02	117	11134	95	Decrease
CAL.20C	SWMESEFRVY	S	151	W152C	SCMESEFRVY	A*29:02	49	980	20	Decrease
CAL.20C	KVGGNYNYLY	S	444	L452R	KVGGNYNYRY	A*29:02	101	505	5.0	Decrease
CAL.20C	VGGNYNYLY	S	445	L452R	VGGNYNYRY	A*29:02	94	519	5.5	Decrease
CAL.20C	NYNYLYRLF	S	448	L452R	NYNYRYRLF	A*24:02	21	108	5.1	Decrease
CAL.20C	YNYLYRLFR	S	449	L452R	YNYRYRLFR	A*31:01	16	12	0.75	Neutral
CAL.20C	YQDVNCTEV	S	612	D614G	YQGVNCTEV	A*02:06	18	57	3.2	Decrease
CAL.20C	QSASKIITL	ORF3a	57	Q57H	HSASKIITL	B*08:01	1788	573	0.32	Increase
CAL.20C	WFFSNYLKR	nsp4	392	S395T	WFFTNYLKR	A*31:01	70	98	1.4	Neutral
CAL.20C	STVFPPTSF	nsp12	318	P323L	STVFPLTSF	B*57:01	1583	637	0.40	Increase

[a]Mutation noted as ancestral residue-position-variant residue. Del refers to deletion of the corresponding residue.

[b]For deletion mutants, the peptide sequence shown represents the variant encompassing the same region that has the highest predicted binding affinity for the corresponding restricting allele.

[c]Indicates predicted IC_{50} for the corresponding reported restricting allele. Predictions were performed using the NetMHCpan BA 4.1 algorithm, hosted by the IEDB.

[d]Increase/decrease in affinity defined by a two-fold difference in predicted IC_{50} nM.