947	Supplemental Information
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951	Naive human B cells engage the receptor binding domain of SARS-CoV-2.
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980 fig. S1. Design and characterization of SARS-CoV-2 antigens and healthy donor sera 981 **binding.** (A) SARS-CoV-2 RBD in complex with viral receptor, ACE2 shown in blue and grey, 982 respectively (PDB 6M0J). Wild-type RBD with, the receptor binding motif (RBM), shown in 983 orange (left panel). Structural model of the ΔRBM probe designed to abrogate binding to ACE2 984 (right panel). Putative N-linked glycosylation sites engineered onto the RBM are shown in red 985 spheres at amino acid positions 501 and 475. (B) SDS-PAGE gel under reducing (R) and non-986 reducing (NR) conditions for monomeric RBD, RBD-Fc and Δ RBM-Fc. (C) Wildtype RBD, 987 △RBM and single glycan variant binding to ACE2-expressing 293T cells by flow cytometry. Wild-988 type RBD binding shown in blue, glycan variant binding shown in red. Streptavidin-PE was used 989 to detect the relative intensity of antigen binding to cell-surface ACE2. A PBS control (gray) 990 indicates secondary-only staining. (D) Control antibody ELISA binding to RBD and ΔRBM 991 antigens. RBM-specific antibody, B38 (left). Non-RBM-specific control antibody, CR3022 992 (right). (E) ΔRBM and $\Delta 501$ and $\Delta 475$ variants analyzed by SDS-PAGE gel under reducing 993 conditions; wildtype RBD is shown for comparison. (E) SARS-CoV-2 spike (left) and RBD (right) 994 sera ELISA from human subjects 1-8. Sera from a COVID-19 convalescent patient and control 995 antibody, B38, were included as positive controls.





998 fig. S2. PBMC flow cytometry analyses. (A) Representative gating strategy used for FACS of 999 PBMCs pooled from donors 1 and 2. Gating was on naive B cells defined by single living 1000 lymphocytes that were CD19⁺CD3⁻IgD⁺IgG⁻. Sorted cells were RBM-specific as defined by spike-1001 PE⁺/spike-APC⁺/RBD-Fc-BV650⁺/ Δ RBM-Fc-BC650-. Sort gate is denoted by the blue arrow. The 1002 bottom right plot shows CD27 staining of sorted RBM-specific naive B cells. (B) Flow cytometry 1003 showing the sort gate and percentage of RBM-specific B cells for the remaining 6 healthy human 1004 donors. (C) RBM-specific B cell frequency among CD27⁺ and CD27⁻ cells. Each symbol 1005 represents a different donor (n = 8).



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1008 fig. S3. Repertoire comparison, germline identity, and IgG binding by individual donor. (A) Heatmap showing V_H-gene usage of isolated antibodies derived from donors 1-5. Unselected 1009 1010 repertoire gene usage derive from a high-throughput sequencing data set of circulating B cells 1011 across 10 human subjects (46). Heatmap scale represents percent of total paired sequence from 1012 each donor. Divergence from inferred germline gene sequences separated by individual donor for 1013 (B) V_H and (C) V_L . Red bars indicate the median percent values, and each dot represents an 1014 individual paired sequence. (**D**) Heatmap showing IgG binding to RBDs (n = 44) sorted by donor. 1015 (E) ELISA EC₅₀ values for IgGs with detectable SARS-CoV-2 RBD binding (n = 36) against RBM 1016 glycan probes. Red bars indicate the mean EC₅₀ values.





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fig. S4. SARS-CoV-2 RBD-binding kinetics of isolated naive antibodies. (A) Biolayer
interferometry (BLI) binding kinetic analysis of titrated SARS-CoV-2 RBD to immobilized Fabs.
Dotted line at 60 s denotes the start of the dissociation phase. (B) Kinetic and equilibrium constants
for binding to RBD calculated from a 1:1 binding model using a global fit to all curves for each
Fab using vendor supplied software. B38 Fab is used as a positive control.





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TCUN1 2:06		20	30		50	60	70	80	90	100	110	120	130
100V1-2×00	UVULVUSGAEVKKPGAS	SVRVSCRASGTI	FIGTI	MINWVRUAPGUGLEW	MGRINP	115GG INTAUKI	QGKVIMI	RUISISIAIME	LSKLKSDDTAV				
2-4					W	ТМ			V			k	UCKGTTVTVSS
2-15		.R			W	T.D.		V		ARGGSRCSG	GNCYGWAYD		GOGTMITVSS
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C004						Ι.				ASPASRGYS	GYDHGYYYY	MDV	VGKGTTVTVSS
C009		M			W					ARDSPFSAL	GASNDY	k	GOGTLVTVSS
C121					W.S.	V				ARAPLFPTG	VLAGDYYYY	GMDVW	VGQGTTVTVSS
C127					W					ATAHPRRIQ	GVFFLGPGV	V	VGQGTTVTVSS
C212			۷	I	W.S.		W	MT		ARERYFDLG	GMDV	V	VGQGTTVTVSS
CC12.4					W.S.	<mark></mark>	W	V	F	ATESWVYGS	GSYSSGAFD	IV	VGQGTMVTVSS
CC12.5	Ε	I	YS		W.S.	DR.		TT	M	ARGPRYSGT	YFDY	V	VGQGTLVTVSS
CC12.6		I	.s		W.S.	D		T.G	G	ARGPRYSGT	YFDY	V	VGQGTLVTVSS
CC12.7	Ε	TTI	.s	V	W.S.	DA		T.S.V.	.TW.K	ARGPRYSGT	YFDF	V	VGQGTLVTVSS
CC12.8		I	.s	Т	W.S.	D			G	ARGPRYSGT	YFDY	V	VGQGTLVTVSS
CC12.9	Ε	I	.s		W.S.	DN.		G	M	ARGPRYSGT	YFDY	V	VGQGVLVTVSS
CC12.10		I	YSF		W.S.	DAT.		H		ARGPRYSGT	HFDY	V	VGQGTLVTVSS
CC12.11		I	.s	Т	W.S.	D		<u>T</u> V.	G	ARGPRYSGT	YFDY	V	VGQGTLVTVSS
CC12.12		I	YS			DR.		· · · · TT · · · · ·		ARGPRYSGT	YFDY	V	VGQGTLVTVSS
CC12.27		••••••	· I		W		•••••	V		AREMPAAMG	YYYYGMDV	V	VGQGTTVTVSS
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ChC2t1p1_G6	••••••		· .N	1	V.W.HS	L	· · · · · L ·	AP. R		ARASVATIT	DFDY	V	VGQGTLVAVSS
COV2-2050	••••••	•••••	· · D · ·	•••••	· · · · · ·		• • • • • • • •	· · · · · · · · · · · · · · · · · · ·	····!	ARVVVLGYG	RPNNYYDGR	NVWDYW	VGQGTLVTVSS
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CV07-262					W	Т		т.т		ARVGWYDFG	TPGDYYYYY	GMDVW	GOGTTVTVSS
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CV19					w					AREYYYDSS	VYPYYYAM	DVW	VGOGTTVTVSS
CV32					w	DV				AREARDYYG	SGSLDY	V	VGQGTLVTVSS
CV36					W	V			.N	.F.ARDLTTTAG	TDYYYGMDV	V	VGQGTTVTVSS
DH1151			D.		<mark>.</mark>	SV		S		ARDLEDFWS	GYPPLGYAL	DVW	VGQGTTVTVSS
DH1173					W	A	W	N	.RK	FARETSFAIF	GGGGMDV	k	VGQGTTVTVSS
DH1194		.м	D.			S				ARDSSSWRY	NWFDP	V	VGQGTLVTVSS
H4										ARVPYCSST	SCHRDWYF	DLW	VGRGTLVTVSS
REGN10923			.IN	I	W					AIITIFGVV	TWFDP	V	VGQGTLVTVSS
REGN10989		I			W	A	L.	T.V	F	ARGSRYDWN	IQNNWF	DPW	VGQGTLVTVSS
S2M11	E				W	I.SST.		ST		ARAAPFYDF	WSGYSYFDY	V	VGQGTLVTVSS

1030 fig. S5. Structural characterization and analysis. (A) Cryo-EM data processing scheme of 1031 ab090 Fab bound with SARS-CoV-2 spike. See the Methods section for more details. (B) Heavy 1032 chain amino acid sequence alignment of ab090 with IGHV1-2 derived antibodies from convalescent COVID-19 patients. Sequences were obtained from CoV-AbDab (118) and aligned 1033 1034 to the IGHV1-2*06 reference. Residues forming the germline-encoded HCDR1 and HCDR2 motif 1035 contacting the SARS-CoV-2 RBD are highlighted in blue. The single nucleotide polymorphism in the *06 allele at position 50 is highlighted red. The site of the dominant mutation from in vitro 1036 1037 affinity maturation efforts with ab090 is highlighted in green.



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1039 1040 (A) Flow cytometric sorting of diversified single chain variable fragment (scFv) libraries of ab090. 1041 Gates represent the yeast population sorted for subsequent selections. After 2 rounds of enrichment 1042 for wildtype SARS-CoV-2 binding, a "stringent" and "diversity gate were sorted in round 3 1043 indicating the yeast populations sorted for individual colony isolation and sequencing. Alignment 1044 of the V_H sequencing output clones for ab090 (**B**) and ab072 (**C**) with the output frequency of each 1045 mutation from a total of 48 single colonies. (D) Alignment of the V_L sequencing output clones 1046 ab072 with the output frequency of each mutation from a total of 48 single colonies. The V_L output 1047 for ab090 was exclusively parent.