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Supplemental information

Molecular basis for antiviral activity of two

pediatric neutralizing antibodies

targeting SARS-CoV-2 Spike RBD

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Analyte	Immobilized ligand	Fitting mode	<i>k_a</i> (M⁻¹s⁻¹)	<i>k_d</i> (s⁻¹)	K _D (pM)	K_{D} reduction fold compared to RBD _{WT}		
EH3 lgG	RBD _{WT}	1:1	1.01 x 10⁵	6.09 x 10 ⁻⁷	6.06	1		
	RBD _{E484K}	1:1	1.11 x 10⁵	4.46 x 10 ⁻⁷	4.04	0.67		
EH8 lgG	RBD _{WT}	1:1	2.11 x 10⁵	3.55 x 10 ⁻⁷	1.68	1		
	RBD _{E484K}	1:1	1.83 x 10⁵	6.01 x 10 ⁻⁷	3.29	1.96		

Table S1. Summary of biolayer interferometry kinetic constants. Related to Table 1 and Figure S2C.

	EH3-RBD	EH8-RBD				
Data collection						
Wavelength, Å	0.979	0.979				
Resolution range, Å	34.38 - 2.65 (2.745 - 2.65)	46.91 - 2.49 (2.58 - 2.49)				
Space group	P 2 ₁ 2 2 ₁	P 2 ₁ 2 2 ₁				
Unit cell parameter						
a, b, c, Å	86.7, 103.1, 112.3	81.8, 100.7, 106.0				
$\alpha, \beta, \gamma, \circ$	90.0, 90.0, 90.0	90.0, 90.0, 90.0				
Redundancy	6.6	18.8				
Completeness, %	99.21 (98.85)	99.20 (92.82)				
Mean I/sigma(I)	10.37 (2.28)	30.40 (2.67)				
R _{merge} ^a	0.190 (0.720)	0.144 (1.037)				
R _{pim} ^b	0.079 (0.301)	0.034 (0.259)				
$CC_{1/2}^{c}$	0.933 (0.772)	0.996 (0.877)				
Wilson B _{factor} , (1/Å ²) ^d	39.62	57.01				
Refinement						
R _{work} ^e	0.179 (0.245)	0.244 (0.425)				
R_{free}^{f}	0.221 (0.305)	0.270 (0.431)				
Resolution, Å	34.38 - 2.65	46.91 - 2.49				
# of non-hydrogen atoms						
proteins						
ligands	4968	4829				
water	144	102				
Overall B $(Å^2)$	1++	102				
proteins	45.13	62 54				
ligands	90.70	96.86				
water	42.48	49.96				
RMS (bond lengths) Å	0.004	0.005				
RMS (bond angles) °	0.74	0.89				
Rivis (bond angles),	0.74	0.07				
Favored %	97.11	96.96				
Allowed %	273	3.04				
Authors %	0.16	0.00				
PDR ID	7111.1	7111.0				

Table S2. Data collection and model refinement statistics. Related to Figure 3.

Statistics for the highest-resolution shell are shown in parentheses.

 ${}^{a}R_{merge} = \sum |I - \langle I \rangle |/\sum I$, where *I* is the observed intensity and $\langle I \rangle$ is the average intensity obtained from multiple observations of symmetry-related reflections after rejections ${}^{b}R_{pim} = as$ defined in [45].

 $^{\circ}CC_{1/2}$ = as defined by Karplus and Diederichs [46]

^dWilson B_{factor} as calculated in [47] ^e $R = \sum ||F_o|| - ||F_c|| / \sum ||F_o||$, where F_o and F_c are the observed and calculated structure factors, respectively.

^fR_{free} = as defined by Brünger [48]

⁹Calculated with MolProbity [49].



Figure S1. Gating strategy for the isolation of RBD-specific mAbs. Related to Figure 1.

Flow cytometry gates to identify RBD-specific B cells from PBMCs of a pediatric patient (Patient 12). RBD-specific B cells were identified according to cell morphology by light-scatter parameters and excluding doublets cells while gating on live cells (7-AAD-). Cells were then gated based on lineage markers: CD3- (T cell marker), CD14- (monocyte marker) and CD19+ (B cell marker). Cells were further gated for isotypic expression: IgD- (naïve) and IgG+ (class-switched). Finally, RBD-specific IgG+ B cells were identified using a dual staining with fluorescent RBD probes (RBD-AF647 and RBD-AF488). The nine RBD-specific B cells were sorted as single cells to clone their BCR sequence.



Figure S2. Binding affinity of RBDs of SARS-CoV-2 VOCs to EH3 and EH8. Related to Table 1.

(A-B) SPR kinetic measurement of SARS-CoV-2 RBDs binding to immobilized (A) EH3 IgG and (B) EH8 IgG. Either IgGs (~250-350 RU) were immobilized on a Protein A chip and 2-fold dilutions of SARS-CoV-2 RBDs of WT and 6 VOCs (6.25-200nM) were injected as flow analytes. The experimental sensorgrams are colored in indicated colors and the 1:1 Langmuir fitting are in grey. RBD_{mini} is the segment of residue 329-527 and otherwise are residue 319-537. The detailed SPR kinetic constants are listed in **Table 1**. (**C**) Binding kinetics between SARS-CoV-2 RBD (WT or E484K) and EH3 or EH8 mAbs assessed by biolayer interferometry. Biosensors loaded with RBD proteins were soaked in two-fold dilution series of indicated mAbs (50 nM–3.125 nM) at 25°C. Raw data are shown in blue and 1:1 binding model is shown in red. The detailed biolayer interferometry kinetic constants are listed in **Table S1**.



Figure S3. The ability of RBD-specific mAbs to induce S1 shedding is epitope-dependent. Related to Figure 4.

S1 shedding was evaluated by transfection of 293T cells to express SARS-CoV-2 S D614G followed by radiolabeling using 35S-methionine/cysteine mix in presence of RBD-specific mAbs (CV3-1, EH3, EH8), ACE2-Fc, S2-specific CV3-25, or two FDA-approved RBD-specific mAbs (Casirivimab and Imdevimab). This was followed by immunoprecipitation of cell lysates and supernatant with CV3-25 and a rabbit antiserum raised against SARS-CoV-2 RBD produced in-house. Samples were analyzed by SDS-PAGE and autoradiography.

A	1	0 20	3.0	4.0	50	152 60		70	8082abc	90	100abcc	lefahijklmno	110
Heavy chain	-		Ĩ				Í	Ĩ		Ĩ	IUUUUUU	ierginr j krimio	110
EH3 CoV11 CV30 COV32-04 COV0X2-04 BD-629 CC12.3 P5A-3A1 CC12.3 P5A-3A1 CC12.3 C1A-F10 C1A-C2 C1A-F10 C1A-C2 COV0X-150 P22A-1D1 COV0X-150 P2A-1D1 COV0X-269 C105 P4A1 LY-CoV481 LY-CoV481 LY-CoV481 LY-CoV481 COVA2-39 C144	EVQLVESG EVQLVESG QVQLVESG QVQLVESG EVQLVESG EVQLVESG EVQLVESG EVQLVESG EVQLVESG EVQLVESG QVQLVESG QVQLVESG QVQLVESG EVQLVE	GLVQPGGSLRLSC GLIQPGGSLRLSC	- t	H TYMT WVRQAPG TYMS VVRQAPG TYMS VVRQAPG	KGLEWVS X KGLEWVS X	±-+ UISGGGTFYJ UISGGGTFYJ UISGGGTFYJ UISGGGTFYJ UISGGGTFYJ UISGGGTFYJ UISGGTTYJ UISGGTTYJ UISGGTTYJ UISGGTTYJ UISGGTTYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTFYJ UISGGSTYJ UISGGSTYJ UISGGTYJ UISGGSTYJ UISGGSTYJ UISGGSTYJ UISGGTYJ UISGGGTYJ UISGGSTYJ UISGGTYJ	ADSVRG R ADSVKG R	FT I SRONSKNTI FT I SRONSKNTI	LY LQMNSLRAED LY LQMNSLRAED LY LQMNSLRAED LY LQMNSLRAED LY LQMNSLGAED LY LQMNSLRAED LY LQMNSLRAED	+ TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR TAVYYCAR	++ DIEWAGAF DIEVAGAI DLDVSGGM DLDVSGGM DLDVSGGM DYGDYYF DYGDYYF DYGDYYF DYGDYYF DYGDYYF DYGDYYF DYGYYGL DRDYYGM DECSGDM DECSGDM DECSGDM DECSGSM DECSGST SPYGG SPYGG SPYGG		I WGQGTLVTVSS Y WGQGTLVTVSS V WGQGTLVTVSS V WGQGTLVTVSS Y WGQGTLVTVSS S WGQGTLVTVSS S WGQGTLVTVSS S WGQGTLVTVSS S WGQGTLVTVSS S WGQGTLVTVSS S WGQGTLVTVSS S WGQGTLVTVSS MGQGTLVTSS MGQGTLVTVSS MGQGTLVTSS MGQTL MGQGTLVTS
	Light chain	10 	20 	27a 30 		40 I	50 	60 	70 	80 	90 9 	5ab 100	107
IGKV3-20 - IGKV1-9 -	EH3 CoV11 CvV30 CoVa2-04 CoVox-222 BD-629 Cc12.1 Cc12.1 Cl02 ClA-22 ClA-F10 ClA-C2 ClA-F10 ClA-C2 CiA-F10 CiA-C2 CiA-F10 CoVox-150 P22A-1D1 CoVox-158 CoVox-269 Cl05 P4A1 LY-Cov481 LY-Cov481 LY-Cov481 LY-Cov481 CoVa2-39 Cl44	EEVLTQSPGTLS EIVLTQSPGTLS EIVLTQSPGTLS EIVLTQSPGTLS EIVLTQSPGTLS EIVLTQSPGTLS EIVLTQSPGTLS EIVLTQSPGTLS DIQLTQSPSLS DIQLTQSPSSLS DIQLTQSPSSLS DIQLTQSPSSLS DIQLTQSPSSLS DIVMTQSPSSLS DIVMTQSPSSLS DIQMTQSPSSUS DIQMTQSPSSUS DIQMTQSPSSUS OSAL-TQPSVS CSAL-TQPSVS CSAL-TQPSVS CSAL-TQPSVS CSAL-TQPSVS	LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC LSPGERATLSC ASVGDRVTITC ASVGDRVTITC ASVGDRVTITC ASVGDRVTITC ASVGDRVTITC ASVGDRVTITC ASVGDRVTITC ASVGDRVTITC GSPGQSITISC GSPGQSITISC GSPGQSITISC GSPGQSITISC	RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCSV-SS RASCGI	+ + SULA WYCG SYLA	XFGQAPRLLI XFGQAPRLLI XFGQAPRLLI XFGQAPRLLI XFGQAPRLLI XFGQAPRLLI XFGQAPRLLI XFGQAPRLLI XFGGAPRLLI XFGGAPRLLI XFGGAPRLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI XFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI YFGGAPKLLI	Y GASSRI Y GASSRI Y GASSRI Y GASSRI Y GASSRI Y GASSRI Y GASSRI Y ASTLI Y ASSLI Y ASSLI Y ASSLI Y GSSRI Y GSSRI Y GSSRI Y COSC Y COSC	GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS AT GIPDRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS GVPSRFSGS	GSGTDFTLTIS GSGTASLATTS	LLEPEDSAV LLEPEDFAV LLEPEDFAV LLEPEDFAV LLEPEDFAV LLEPEDFAV LLEPEDFAV LLEPEDFAV LLEPEDFAT LLEPEDFAT LLEPEDFAT LLEPEDFAT LLEPEDFAT LLQPEDFAT LLQPEDFAT LLQPEDFAT LLQPEDFAT LLQPEDFAT LLQPEDFAT LLQPEDFAT LLQPEDFAT	-± TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQYGSS TrC QQISSY TrC QQLSSY TrC QQSSE TrC QSSTSS TrC SSYTSS CDR		LEIK LEIK LEIK VDIK VDIK VEIK ZEIK LEIK LEIK LEIK LEIK VDIK LEIK VDIK LVL LEVL LEVL LEVL JTVL 4
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Heavy chain	1	0 20 	50	40 	5	 		1		90	TODa	Dederghij	TTO
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Light chain	1	I 20	27abc 30 	u 40 I I		50	60 	70	80 	90 95 I I	a 100	106a 	
EH8 Beta-44 BG7-15 CV07-250 S309	QSALTQ-PAS QSVVTQ-PAS EIVLTQSPGT QSALTQ-PPS EIVLTQSPGT	SVSGSPGQSITISC SVSGSPGQSITISC FLSLSPGERATLSC SASGSPGQSVTISC FLSLSPGERATLSC FWR1	TGTSSDVGS TGTSSDVGS RASQSVSC TGTSSDLGA RASQTVS CDRL1	YNLVS WYQQHE YNLVS WYQQHE STFLA WYQQKE YHFVT WYQQKE STSLA WYQQKE	OKAPKFMI GKAPKLMI GQAPRLLI GKAPKVMI GQAPRLLI WR2	Y EGTKRPS Y AGSKRPS S GASSRAT Y GVRKRPS Y GASSRAT CDRL2-	GVSNRFSG GVSNRFSG GIPDRFSG GVPDRFSG GIPDRFSG	SKSGNTASLTI SKSGNTASLTI SGSGTDFTLTI SKSGNTASLTV SGSGTDFTLTI FWR3	SGLQAEDEADYY SGLQAEDEADYY SRLEPEDFAVYY SGLQDEDEADYY SRLEPEDFAVYY	C CSYAGNS C CSYAGSS C QQYGSSF C SSYAGNN C QQHDTS- CDRI	TWV FGGGTI TWV FGGGTI -PT FGQGTI DFV FGGGTI -LT FGGGTI FWF	KLTVL KLEIK KLEIK KVEIK R4	
		RBD contact	ing residues	Hyd	rogen-bond	led/salt-bridge	ed residue	s: (+) side chair	n (-) main chai	n (±) bot	h side chain a	and main chain	

Figure S4. V_H and V_L sequence alignments of EH3 and EH8 to other structurally available RBD-specific mAbs. Related to Figure 4.

(A) Alignments of EH3 and other 23 IGHV3-53 encoded Abs. (B) Alignments of EH8 and other 4 IGHV1-18 Abs. The RBD-contacting residues (BSA>0) in EH3-RBD and EH8-RBD crystal structures are shaded in green. Residues involved in salt-bridges or H-bonds to the RBD are marked above the sequence with (+) for the side chain, (-) for the main chain and (±) for both.