Supplemental Figures and Tables

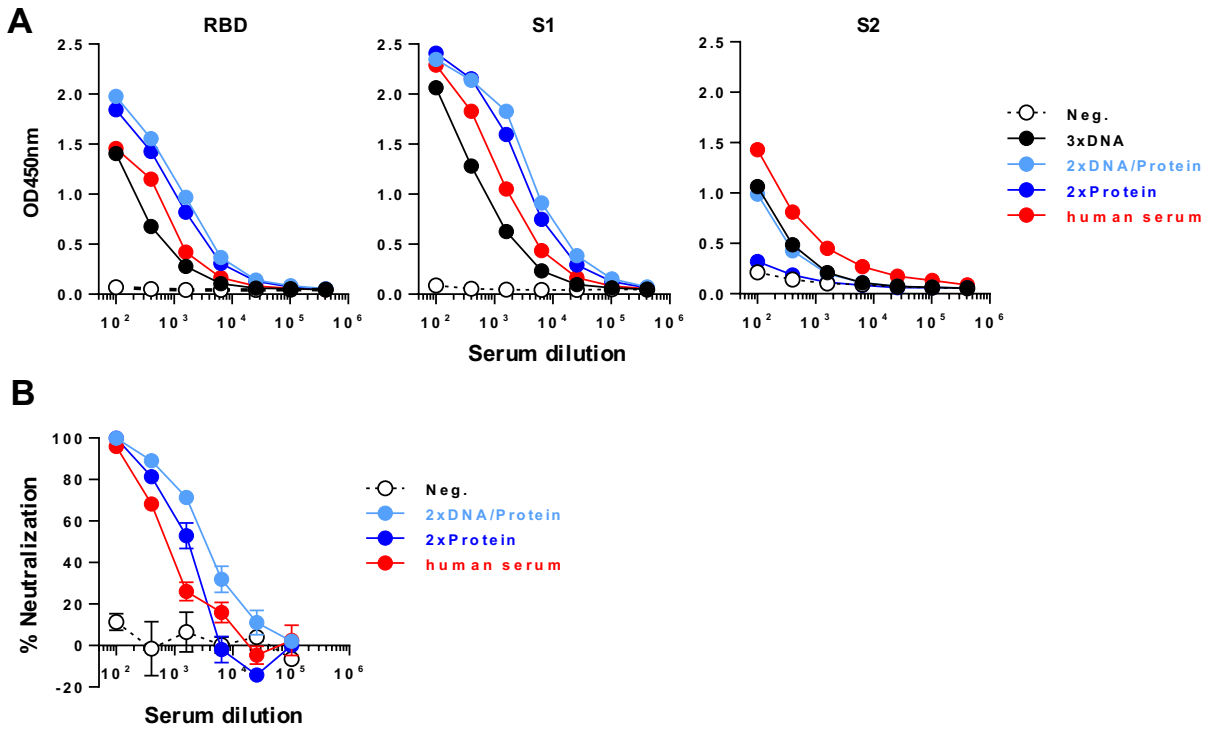
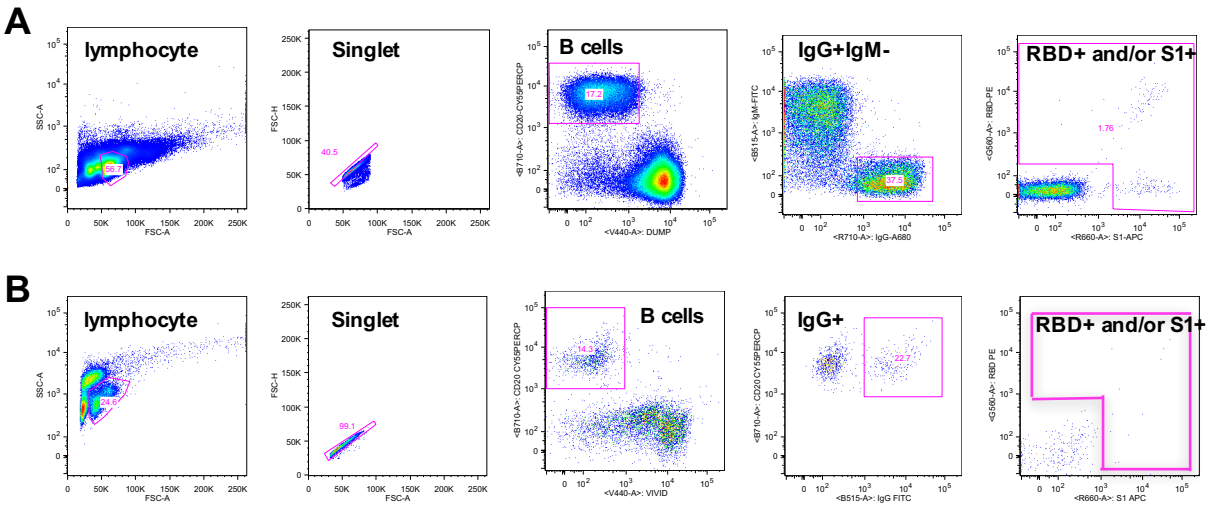


Fig. S1. Binding of antibodies from immunized NHPs and MERS patient to S1 and S2 subunits of MERS-CoV S. (A) Binding to MERS-CoV S proteins. Sera from a human donor (three weeks after illness onset) and NHPs immunized with MERS-CoV S DNA three times (3xDNA), primed with S DNA two times and boosted with S1 protein plus Ribi adjuvant (2xDNA/Protein), or primed and boosted with S1 protein plus Ribi adjuvant (2xProtein) (20) were assayed by ELISA for binding to soluble MERS-CoV S proteins, RBD, S1 and S2. **(B)** Neutralization activity. Sera from the human donor and immunized NHPs as described in **Fig. S1A** were assessed for neutralizing activity against MERS-CoV EMC S pseudotyped lentivirus particles. Percent neutralization at different serum dilutions is shown. Data represent the mean of triplicate replicates with standard errors.



C

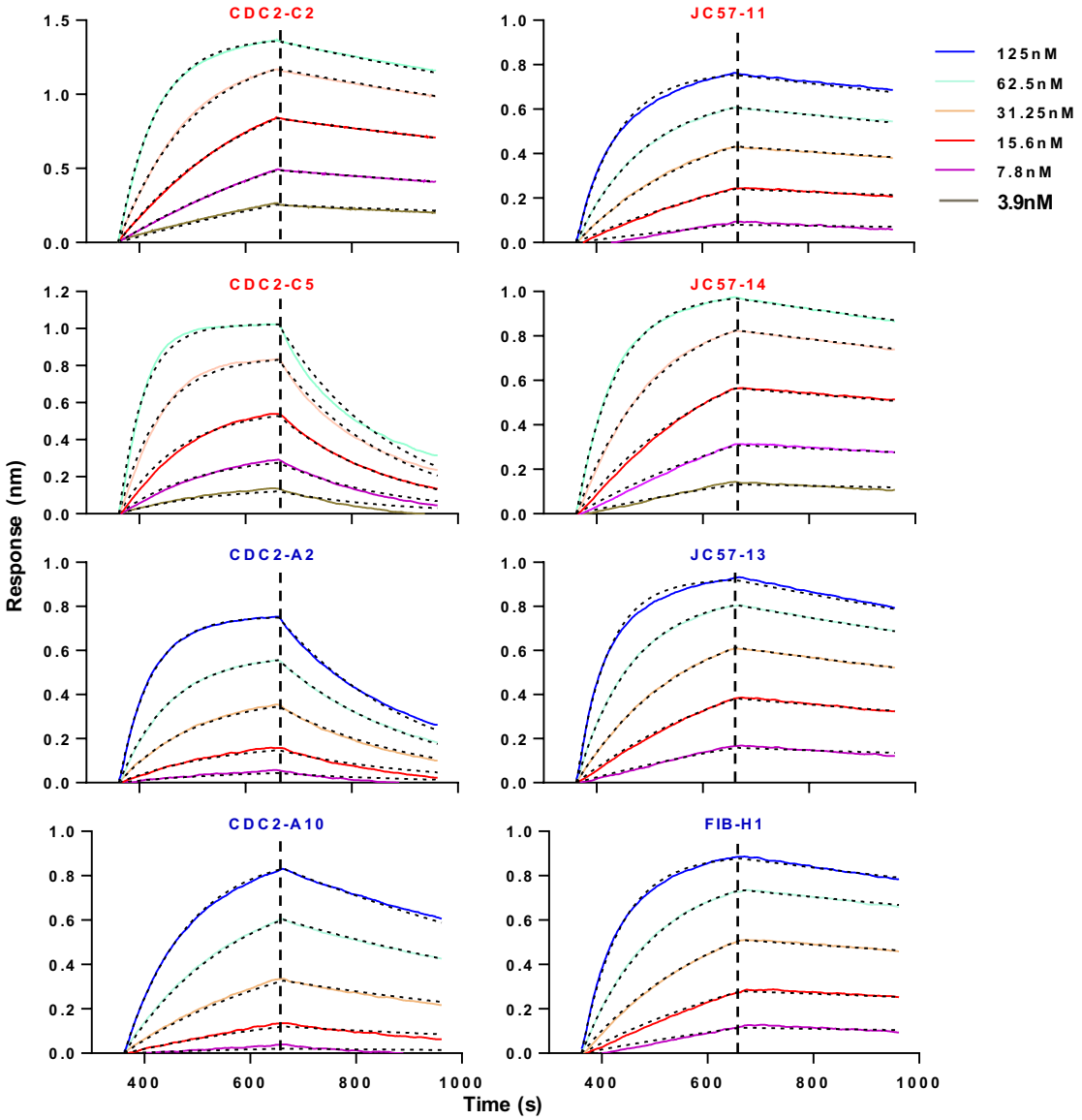
mAb ID	Rhesus or human heavy chain V gene	% Divergence (nt) from germline	CDRH3 Sequence (length)	Rhesus or human light chain V gene	% Divergence (nt) from germline	CDRL3 sequence (length)
JC57-11	Macmul IGHV4-2*01F	9%	CAREPFKYCSGGVCYAHKDNSLDVW (23)	Macmul IGKV3-9*01	5%	CYQHSSGYTF (8)
JC57-14	Macmul IGHV4-2*01F	3%	CAKTYSGTFDYW (10)	Macmul IGKV1-20*01	9%	CQQYNNSPYSF (9)
JC57-13	Macmul IGHV4-2*01F	7%	CARLLPLGGGYCFDYW (14)	Macmul IGKV2S17*01	5%	CMQSVEYPFTF (9)
FIB-H1	Macmul IGHV1-1*01F	17%*	CAASSGSYGVSSLDVW (14)	Macmul IGLV11-1*01F	1%	CQVYDSSANWVF (10)
CDC2-C2	IGHV1-69*14F	5%	CAREGGHQGYCSGGSCYDFDYW (20)	IGKV2-28*01 F	1%	CMQALQTPAF (8)
CDC2-C5	IGHV4-39*01F	0%	CASLLRPLIYCSGGSCTDYW (18)	IGLV2-14*01 F	1%	CSSYTSNITLVF (10)
CDC2-A2	IGHV3-11*01F	1%	CARVGLGSGWYDWFDPW (15)	IGLV1-40*01 F	1%	CQSYDSSLSGVVF (11)
CDC2-A10	IGHV1-69*01 F or IGHV1-69D*01 F	3%	CARGSRSSSSAEYFQHW (15)	IGLV2-11*01 F	1%	CCSYAGSYTLEVVF (12)

*including deletions

Fig. S2. Isolation of MERS-CoV RBD- and/or S1-specific B cells. (A) and **(B)** Gating strategy for isolating memory B cells from rhesus macaques (A) and a human donor (B). Macaque and human PBMCs were stained with a viability marker and CD3/CD4/CD8/CD14 antibodies to exclude T cells and monocytes, antibodies to CD20/IgG/IgM (for NHP) or CD20/IgG (for human), and MRES-CoV S1 and RBD probes to select MERS-CoV specific memory B cells. RBD+ and/or S1+ single B cells were sorted into 96-well plates for isolation of mAbs. **(C)** Genetic characteristics of mAbs. The top four mAbs were isolated from rhesus macaques, while the bottom four mAbs were from a human donor. The heavy/light chain genes, % divergence relative to

germline sequence and CDR3 lengths were analyzed with IMGT/V-QUEST website tool (http://www.imgt.org/IMGT_vquest/share/textes/).

A



B

mAbs	Binding Kinetics		
	$K_{on}(Ms^{-1})$	$K_{off}(s^{-1})$	$K_D(M)$
CDC2-C2	2.16E+05	5.73E-04	2.65E-09
CDC2-C5	2.65E+05	4.66E-03	1.75E-08
JC57-11	1.01E+05	3.63E-04	3.61E-09
JC57-14	2.10E+05	3.56E-04	1.70E-09
CDC2-A2	1.04E+05	3.85E-03	3.70E-08
CDC2-A10	5.75E+04	1.16E-03	2.01E-08
JC57-13	1.45E+05	5.34E-04	3.68E-09
FIB-H1	1.12E+05	3.19E-04	2.86E-09

Fig. S3. Octet biosensorgrams of MERS-CoV S1 molecules binding to mAbs. (A) Human and macaque mAbs were loaded onto anti-human IgG Fc (AHC) probes, and association with MERS-CoV S1 protein was allowed to proceed for 300 s, followed by dissociation for 300 s. Responses were measured in nm using an Octet HTX instrument. The dashed lines represent the best fit of the kinetic data to a 1:1 binding model. All experiments were carried out at 30°C in PBS (pH 7.4) supplemented with 1% BSA to minimize non-specific binding. The dotted vertical line indicates the beginning of dissociation, and the legend indicates MERS-CoV S1 antigen concentrations. **(B)** Binding kinetics in K_{on} (Ms^{-1}), K_{off} (S^{-1}) and K_D (M).

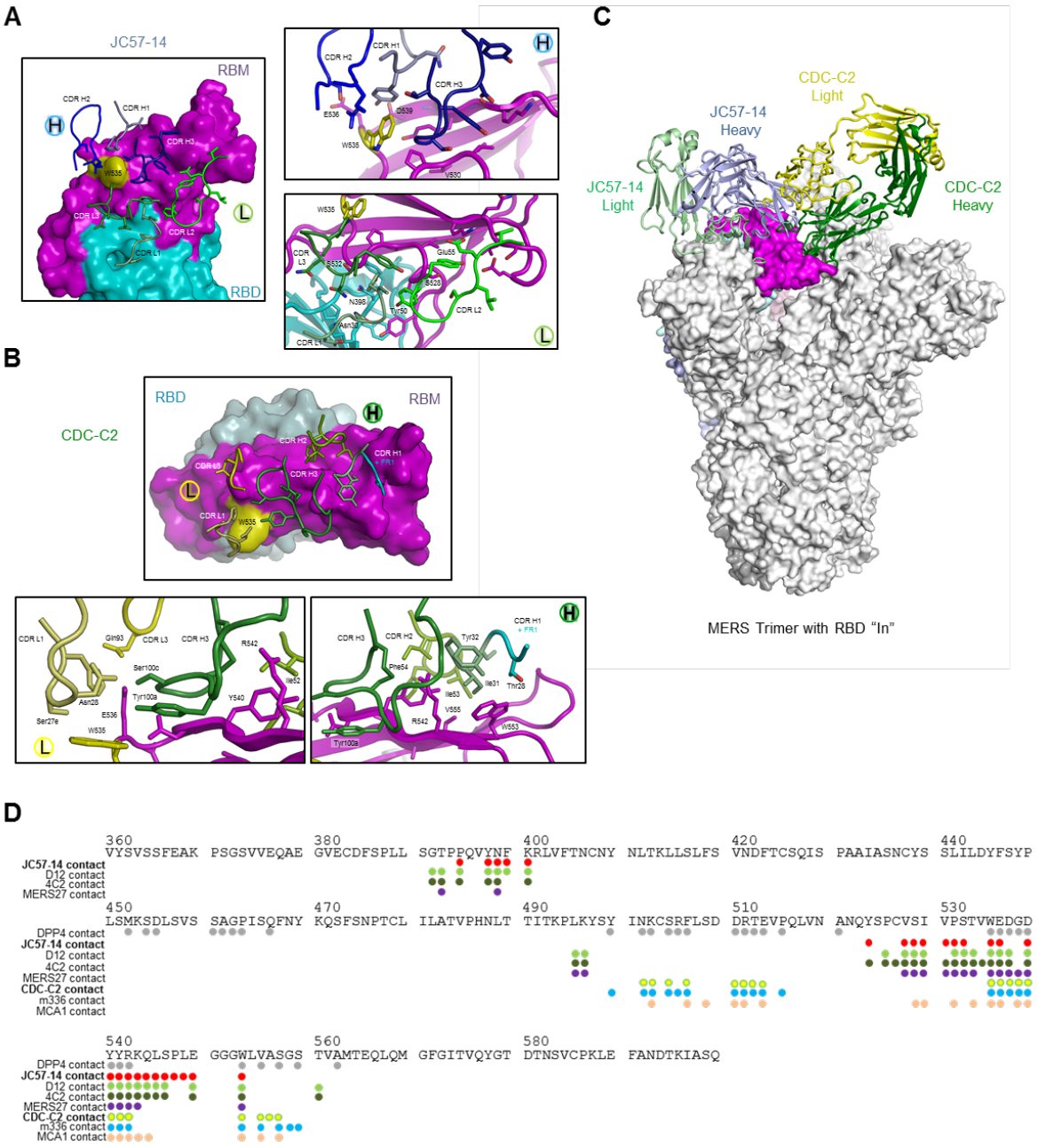


Fig. S4. Structural analysis of MERS RBD-targeting antibodies. **(A)** Crystal structure of JC57-14 in complex with MERS RBD. The MERS RBD is shown in surface representation with antibody CDRs shown in ribbon representation. The antibody-antigen interactions are displayed in two panels centered around the heavy (H) and light (L) chains of JC57-14. The W535 residues is highlighted in yellow as a reference marker, with interacting residue side chains shown in stick representation. **(B)** Crystal

structure of CDC-C2 in complex with MERS *England1* RBD. The RBD is shown in surface representation with interacting CDRs shown in ribbon representation (CDR L2 is not shown). The antibody paratope is displayed in two panels focused on the light chain and CDR H3 interactions (L) and the CDR H1, and CDR H2 interactions (H). **(C)** JC57-14 and CDC-C2 antibodies are modeled on the MERS Spike trimer molecule using an RBD molecule that is located in the "closed" conformation with both antibodies having significant clashes with adjacent protomers of the MERS Spike trimer. **(D)** The amino acid sequence of the MERS *England1* RBD is shown with residues bound by the DPP4 receptor and neutralizing antibodies indicated below the sequence with a circle.

Fig. S5. IC50 Neutralization titers to MERS-CoV EMC and its mutants for Figure 4a

Virus \ mAbs	D12	JC57-14	CDC2-C5	CDC2-C2	JC57-11	F11
EMC	0.017	0.004	0.056	0.001	0.006	0.212
EMC_L506F	0.008	0.007	0.029	0.005	0.020	**0.004**
EMC_D509G	0.013	0.004	0.085	0.025	0.009	>10
EMC_D509R	0.033	0.005	0.088	0.449	0.016	>10
EMC_T512A	0.012	0.004	0.060	0.017	0.010	0.914
EMC_S532P	2.927	0.011	0.072	0.002	0.005	0.172
EMC_V534A	4.737	0.370	>10	0.017	0.032	1.508
EMC_W535R	>10	>10	>10	0.008	0.005	0.185
EMC_E536R	>10	0.044	>10	0.013	0.020	0.454
EMC_D539R	>10	0.900	>10	>10	>10	0.133
EMC_Y540H	0.012	0.004	0.057	0.521	0.405	**0.013**
EMC_R542G	0.010	0.005	0.078	>10	0.102	0.552
EMC_P547S	0.018	0.011	0.064	0.004	0.011	1.237

Fully neutralization-resistant **Neutralization activity decreased >10-fold**

Fig. S5. mAb IC50 neutralization titers to MERS-CoV native and mutant S-pseudotyped lentiviruses. Supporting data for **Fig. 4A**. Six mAbs display a total of four unique neutralization patterns, which are color-coded in blue, red, black and purple

Competitor mAbs	Analyte mAbs										
	D12	JC57-14	CDC2-C5	CDC2-C2	JC57-11	F11	G2	JC57-13	FIB-H1	CDC2-A2	CDC2-A10
D12	100.43	100.58	102.71	100.12	100.34	3.84	6.42				
JC57-14	103.82	102.79	105.15	105.62	101.29	23.89	6.16				
CDC2-C5	103.13	100.32	101.46	103.42	100.74	11.55	16.32				
CDC2-C2	102.26	102.22	104.00	98.44	100.15	104.00	-8.92				
JC57-11	105.94	105.41	108.76	107.42	104.57	109.65	0.40				
F11	-8.09	-4.47	4.34	95.53	95.17	99.89	-10.67				
G2				14.51			103.44	108.72	106.09	47.66	51.41
JC57-13				42.87			100.80	115.65	112.59	36.91	52.73
FIB-H1				22.64			83.82	107.76	105.78	42.45	24.63
CDC2-A2				30.89			27.54	7.15	26.74	85.08	11.18
CDC2-A10				27.85			15.29	11.09	15.00	5.97	102.71

Fig. S6. Competition binding of mAbs by Octet. Competitor mAbs, RBD-specific (in red) and S1-specific (in blue), were tested for binding to His-tagged S1 protein using an anti-penta-his biosensor. Saturating concentrations of analyte mAbs were determined prior to competition assay. Competing and analyte mAbs were sequentially bound to S1 protein loaded onto the biosensor. Percent inhibition of analyte-mAb binding by competing mAbs >90% is highlighted. Percent inhibition of analyte-mAb binding by competing mAbs as measured by ELISA is shown in **Fig. 5**.

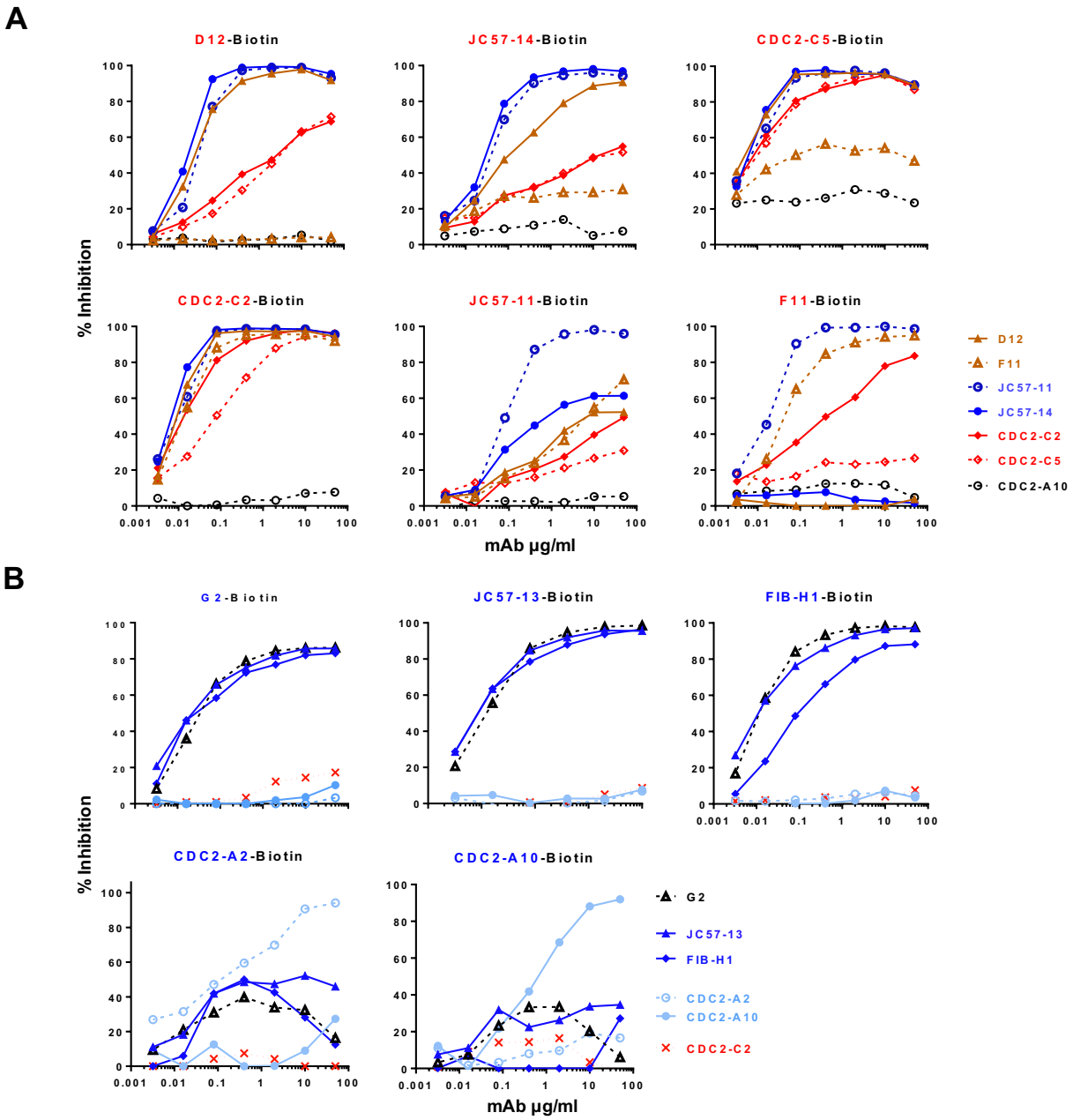


Fig. S7. Competition binding curves of RBD- and S1-specific mAbs by ELISA.

Percent inhibition for RBD-specific mAbs in red **(A)** and S1-specific mAbs in blue **(B).** F11, D12 and G2 were isolated from immunized mice; JC57-11, JC57-14 and JC57-13 from immunized NHP; and CDC2-C2, CDC2-C5, CDC2-A2 and CDC2-A10 from a human MERS survivor. Summary of percent competitive binding is shown in **Fig. 5**.

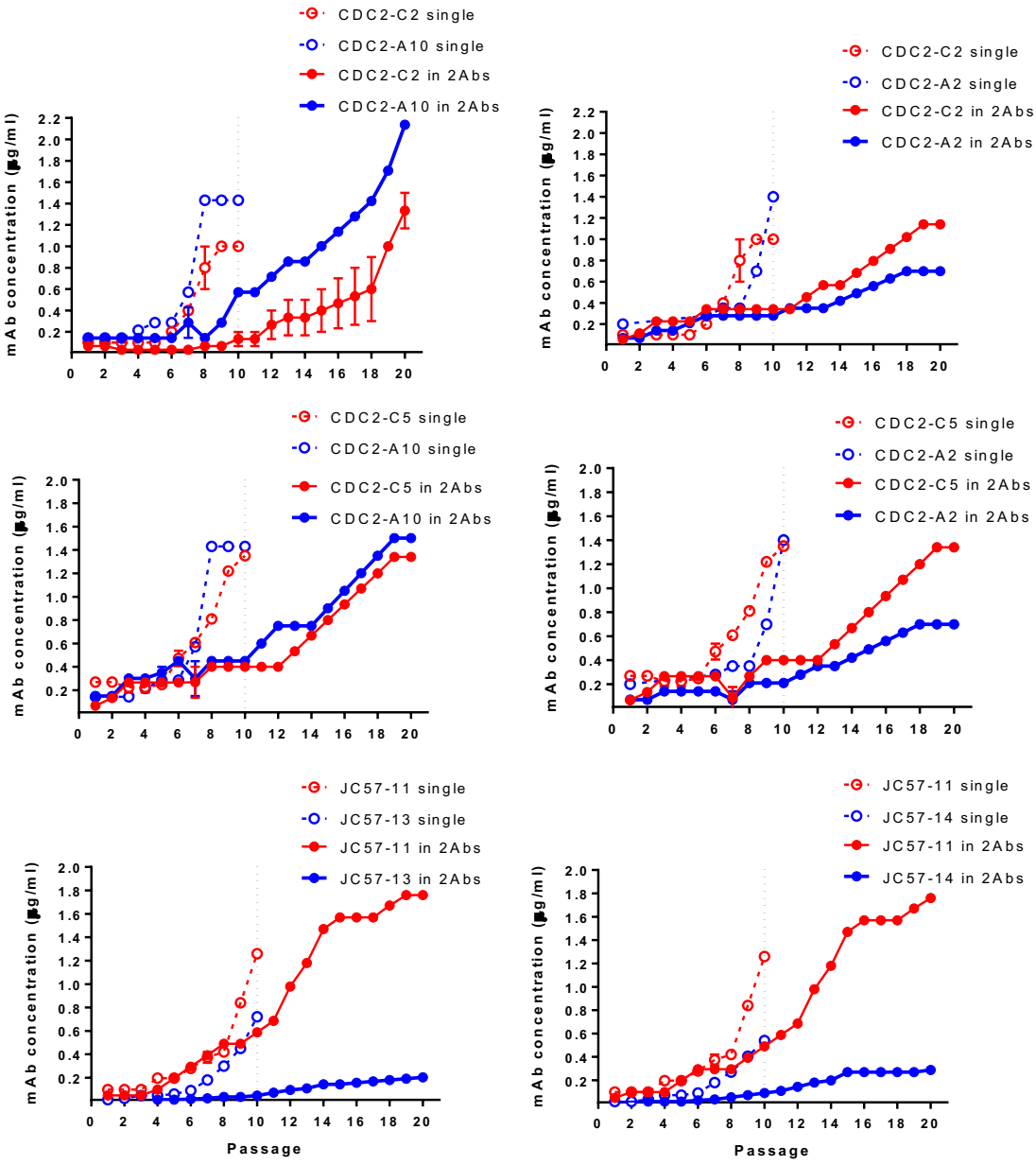


Fig. S8. mAb concentrations used for selection of escape mutations. MERS-CoV EMC/2012 was propagated in the presence of one or two mAbs. Cultures were carried for 10 and 20 serial passages under single- and double-mAb selection, respectively. The combinations of two mAbs, either RBD-specific plus S1-specific (CDC2-C2 + CDC2-A10, CDC2-C2 + CDC2-A2, CDC2-C5 + CDC2-A10, CDC2-C5 + CDC2-A2 and

JC57-11 + JC57-13) or two RBD-specific (JC57-11 + JC57-14) slowed viral adaptation to the presence of antibody, necessitating a shallower mAb concentration ramp and extended passaging to obtain escape-mutant viruses capable of efficient replication.

Table S1. Crystallographic data collection and refinement statistics.
Values in parentheses are for highest-resolution shell. n/a: not applicable.

	JC57-14 Fab	JC57-14 and *England1* RBD	CDC2-C2 and *England1* RBD
Data collection			
Growth condition	0.09 M CHES pH 9.5, 18% PEG 8,000	0.23 M ammonium sulfate, 22% PEG 8,000	0.1 M Tris pH 6.5, 10% 2-Propanol, and 10% PEG 3350
Cryoprotectant	Mother liquor + 22% ethylene glycol	Mother liquor + 22% ethylene glycol	Mother liquor + 20% ethylene glycol
Space group	$P2_1$	$C222_1$	$P2_12_12_1$
Cell constants			
a, *b*, *c* (Å)	83.7, 129.8, 84.0	81.6, 148.1, 259.0	60.82, 166.85, 184.82
α, β, γ (°)	90.0, 110.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	1.00	1.00	0.9792
Resolution (Å)	50.0-2.00 (2.07-2.00)	50.0-3.30 (3.42-3.30)	47.65-2.10 (2.14-2.10)
R_{merge}	12.0	14.6	0.128 (1.223)
No. reflections	269,908	100,857	677,925 (33,386)
No. unique reflections	103,552	21,926	107,760 (5202)
$I / \sigma I$	7.71 (2.12)	9.96 (1.64)	8.3 (2.2)
Completeness (%)	90.8 (62.8)	91.7 (54.4)	97.7 (97.3)
Redundancy	2.8 (1.9)	4.7 (2.9)	6.3 (6.4)
Refinement			
Resolution (Å)	41.28-1.99	42.44-3.32	47.65-2.10
R_{work} / R_{free} (%)	17.9 / 21.3	22.0 / 27.0	19.1 / 22.8
No. atoms			
Protein	12951	9695	9519
Ligand/ion	0	0	42
Water	927	0	719
B-factors			
Protein	40.7	84.0	42.5
Ligand/ion	n/a	n/a	74.9
Water	45.0	n/a	46.3
R.m.s. deviations			
Bond lengths (Å)	0.004	0.002	0.005
Bond angles (°)	0.788	0.589	0.730
Ramachandran			
Preferred regions (%)	97.03	94.74	98.6
Allowed regions (%)	2.97	5.26	1.4
Outliers (%)	0.00	0.00	0.00

Table S1. Crystallographic data collection and refinement statistics.

Table S2. Antibody interactions with MERS *England1* RBD.

	JC57-14 antibody	MERS RBD	Distance (Å)
Hydrogen bonds			
	H:TYR33 [OH]	R:GLU536 [N]	3.90
	H:TYR33 [OH]	R:ASP539 [OD2]	2.66
	L:ASN30 [ND2]	R:ASN398 [O]	3.16
	L:TYR32 [OH]	R:ASN398 [ND2]	2.90
	L:TYR32 [OH]	R:VAL530 [O]	3.69
	L:TYR49 [OH]	R:GLU549 [OE1]	3.08
	L:THR56 [OG1]	R:LYS543 [NZ]	3.31
	L:ASN92 [O]	R:SER532 [OG]	2.16
	L:THR56 [OG1]	R:GLN544 [O]	3.60
Salt bridges			
	L:ASP28 [OD2]	R:LYS400 [NZ]	3.59
	L:GLU55 [OE2]	R:LYS543 [NZ]	2.94

	CDC-C2 antibody	MERS RBD	Distance (Å)
Hydrogen bonds			
	H:THR28 [OG1]	B:ASP510 [OD1]	3.28
	H:THR28 [OG1]	B:TRP553 [OD2]	3.09
	H:ILE31 [O]	B:TYR540 [OH]	2.72
	H:ILE31 [O]	B:ARG542 [NH1]	3.30
	H:TYR33 [OH]	B:ASP539 [OD2]	2.66
	H:ILE53 [O]	B:LYS502 [NZ]	2.92
	H:LYS73 [NZ]	B:ARG511 [O]	2.87
	H:SER74 [O]	B:ARG511 [NH2]	3.05
	H:GLU95 [OE1]	B:ARG542 [NH2]	2.58
	H:GLU95 [OE2]	B:ARG542 [NH1]	2.78
	H:GLN99 [O]	B:ARG542 [NH2]	3.55
	H:CYS100B [N]	B:TYR540 [O]	2.89
	H:CYS100B [O]	B:TYR540 [N]	2.95
	H:SER100C [OG]	B:ASP539 [OD1]	3.67
	H:SER100C [OG]	B:GLU536 [OE1]	2.86
	H:GLY100D [N]	B:ASP539 [OD1]	2.60
	H:GLY100D [N]	B:GLU536 [OE1]	3.62
	L:HIS27D [NE2]	B:GLU536 [OE1]	2.93
	L:GLN93 [NE2]	B:GLU536 [OE2]	3.29
Salt bridges			
	H:GLU95 [OE1]	B;ARG542 [NH1]	3.24
	H:GLU95 [OE2]	B;ARG542 [NH1]	2.77
	H:GLU95 [OE1]	B;ARG542 [NH2]	2.58
	H:GLU95 [OE2]	B;ARG542 [NH2]	3.69
	L:HIS27D [NE2]	B:GLU536 [OE1]	2.93

Table S2. Antibody interactions with MERS *England1* RBD.

Table S3. Buried surface area of JC57-14 antibody in complex with MERS *England1* RBD.

JC57-14 antibody			
Residue	Bond Type	Accessible Surface Area (Å^2)	Buried Surface Area (Å^2)
H:GLN 1		176.9	3.4
H:SER 31		95.6	3.8
H:ASN 32		39.1	19.7
H:TYR 33	H	94.9	76.6
H:ARG 50		75.7	49.2
H:SER 56		52.1	33.0
H:LYS 95		18.6	4.5
H:THR 96		6.2	3.5
H:TYR 97		135.7	104.1
H:SER 98		92.4	65.9
H:GLY 99		49.5	11.0
H:ASP 102		63.2	21.6
H:TYR 103		95.0	21.9
L:ASP 28		102.1	27.1
L:ASN 30*	H	88.2	75.0
L:TYR 32	H	81.0	75.0
L:TYR 49	H	107.3	76.4
L:TYR 50*		99.0	69.9
L:SER 53		44.2	9.3
L:LEU 54		77.6	20.8
L:GLU 55*	S	51.4	24.5
L:THR 56*	H	136.9	67.5
L:GLY 57		74.1	6.9
L:VAL 58		19.9	1.8
L:TYR 91		67.7	13.9
L:ASN 92	H	53.7	27.4
L:TYR 96		129.5	29.3
MERS *England1* RBD			
Residue	Bond Type	Accessible Surface Area (Å^2)	Buried Surface Area (Å^2)
R:PRO 394		12.8	2.0
R:TYR 397		1.4	1.4
R:ASN 398	H	35.8	33.9
R:PHE 399		2.4	2.4
R:LYS 400	S	99.9	63.5
R:TYR 523		86.6	14.3
R:VAL 527		50.1	36.4
R:SER 528		95.4	84.8
R:ILE 529		27.2	26.2
R:VAL 530	H	11.8	11.8
R:PRO 531		27.2	27.2
R:SER 532	H	83.7	68.7
R:TRP 535		182.1	157.8
R:GLU 536	H	118.8	36.6
R:ASP 539	H	39.2	19.3
R:TYR 540		132.1	12.5
R:TYR 541		25.9	24.1
R:ARG 542		153.4	39.8
R:LYS 543	HS	118.1	118.1
R:GLN 544	H	97.0	22.3
R:LEU 545		23.0	6.4
R:SER 546		37.3	36.8
R:PRO 547		119.2	5.37
R:LEU 548		155.9	18.2
R:GLU 549	H	87.6	32.7
R:TRP 553		88.4	3.3

Residues that form hydrogen bonds and salt bridges are indicated.
* light chain contact residues mutated from germline during somatic mutation.

Table S3. Buried surface area of JC57-14 antibody in complex with MERS *England1* RBD.

Table S4. Buried surface area of CDC2-C2 antibody in complex with MERS *England1* RBD.

CDC-C2 antibody			
Residue	Bond Type	Accessible Surface Area (Å^2)	Buried Surface Area (Å^2)
H:GLY 27		73.1	7.5
H:THR 28	H	80.8	68.4
H:PHE 29		25.6	1.5
H:SER 30		18.5	16.2
H:ILE 31*	H	85.6	85.6
H:TYR 32		47.0	25.9
H:ILE 52		14.9	7.5
H:ILE 53	H	112.2	101.8
H:PHE 54		155.2	94.1
H:LYS 73	H	160.6	76.1
H:SER 74	H	92.9	26.1
H:THR 75		63.6	0.8
H:SER 76		28.0	9.5
H:GLU 95	S	6.3	6.2
H:GLY 97		22.2	6.6
H:GLN 99	H	88.0	10.2
H:GLY 100		87.4	19.5
H:TYR 100A		162.6	96.0
H:CYS 100B	H	39.8	39.6
H:SER 100C	H	79.0	21.0
H:GLY 100D	H	93.3	56.3
H:GLY 100E		40.8	25.3
L:HIS 27D	HS	78.3	45.6
L:SER 27E		113.9	26.6
L:ASN 28		80.0	33.0
L:GLN 93	H	154.8	23.1
MERS *England1* RBD			
Residue	Bond Type	Accessible Surface Area (Å^2)	Buried Surface Area (Å^2)
B:ASN 501		46.4	1.4
B:LYS 502	H	80.5	45.0
B:SER 504		1.3	0.7
B:PHE 506		67.4	65.5
B:ASP 510	H	83.9	34.0
B:ARG 511	H	197.8	94.1
B:THR 512		55.8	0.2
B:GLU 513		86.1	28.6
B:TRP 535		212.1	106.0
B:GLU 536	H	116.0	84.3
B:ASP 537		87.5	7.3
B:GLY 538		46.4	23.2
B:ASP 539	H	32.3	32.3
B:TYR 540	H	129.4	129.2
B:TYR 541		31.5	21.0
B:ARG 542	HS	161.3	138.5
B:TRP 553		91.3	64.3
B:VAL 555		26.6	26.6
B:ALA 556		0.8	0.8
B:SER 557		17.9	13.6

Residues that form hydrogen bonds and salt bridges are indicated.
* Contact residue mutated from germline during somatic mutation.

Table S4. Buried surface area of CDC2-C2 antibody in complex with MERS *England1* RBD.

Table S5. Structurally characterized antibody contact region and source.

Antibody	PDB ID	Heavy chain area (Å^2)	Light chain area (Å^2)	Source
JC57-14		404	520	Non-human primate - vaccine
D12	4ZPT	485	523	Mouse - vaccine
4C2	5DO2	508	505	Mouse - vaccine
MERS27	4ZS6	402	283	Yeast display scFv library
CDC2-C2		794	130	Human - infection
m336	4XAK	770	110	Naïve IgM antibody library
MCA1	5GMQ	827	120	Human - infection

Table S5. Structurally characterized antibody contact region and source.