

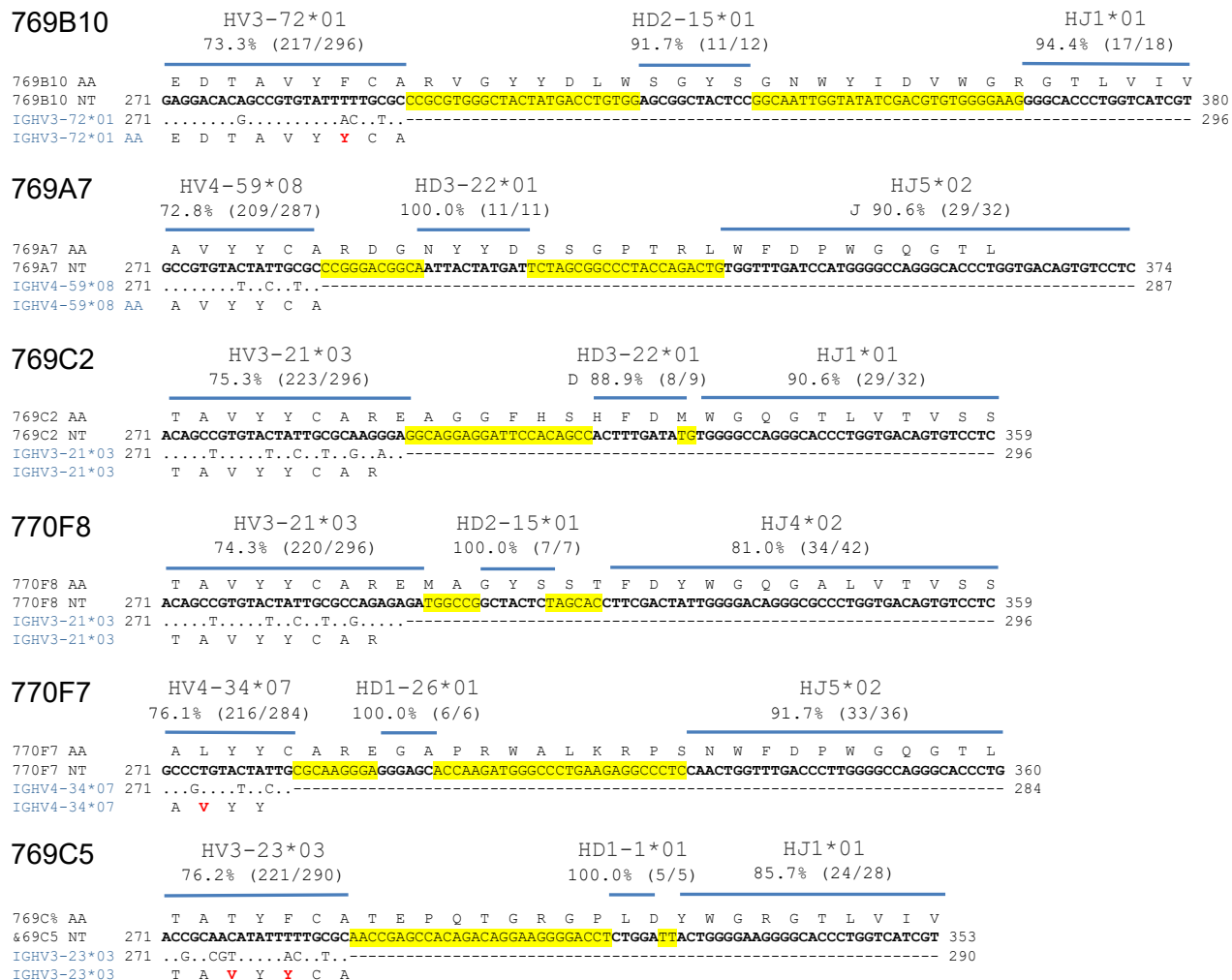
Figure S1. Characterization of EBV gH/gL-targeting antibodies (related to Figure 1)

(A) Flow cytometry plots of antibody sort using labeled EBV gH/gL recombinant protein.

(B,C) Neutralization of EBV infection in B cells and epithelial cells by mAbs.

(D,E) Inhibition of B cell and epithelial cell fusion by isolated mAbs.

A



B

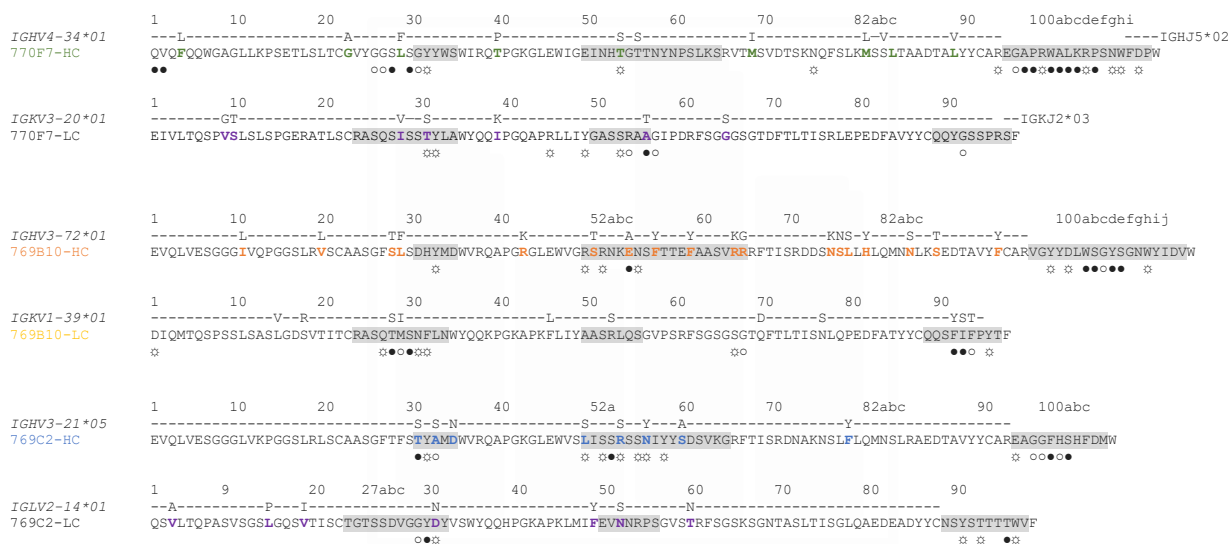


Figure S2 Immunoglobulin gene recombination analysis of EBV gH/gL neutralizing antibodies (related to Figure 1-5).
 (A) Sequence alignment of EBV gH/gL-targeting antibody CDR3 recombination region with their respective germline genes.
 (B) Sequence alignment of EBV gH/gL-targeting antibodies and their respective precursor germline genes. Antibody residues that interact with EBV gH/gL are indicated (open circles denote antibody main chain-only contacts, open circles with rays denote antibody side-chain-only contacts and filled circles denote both main-chain and side-chain contacts).

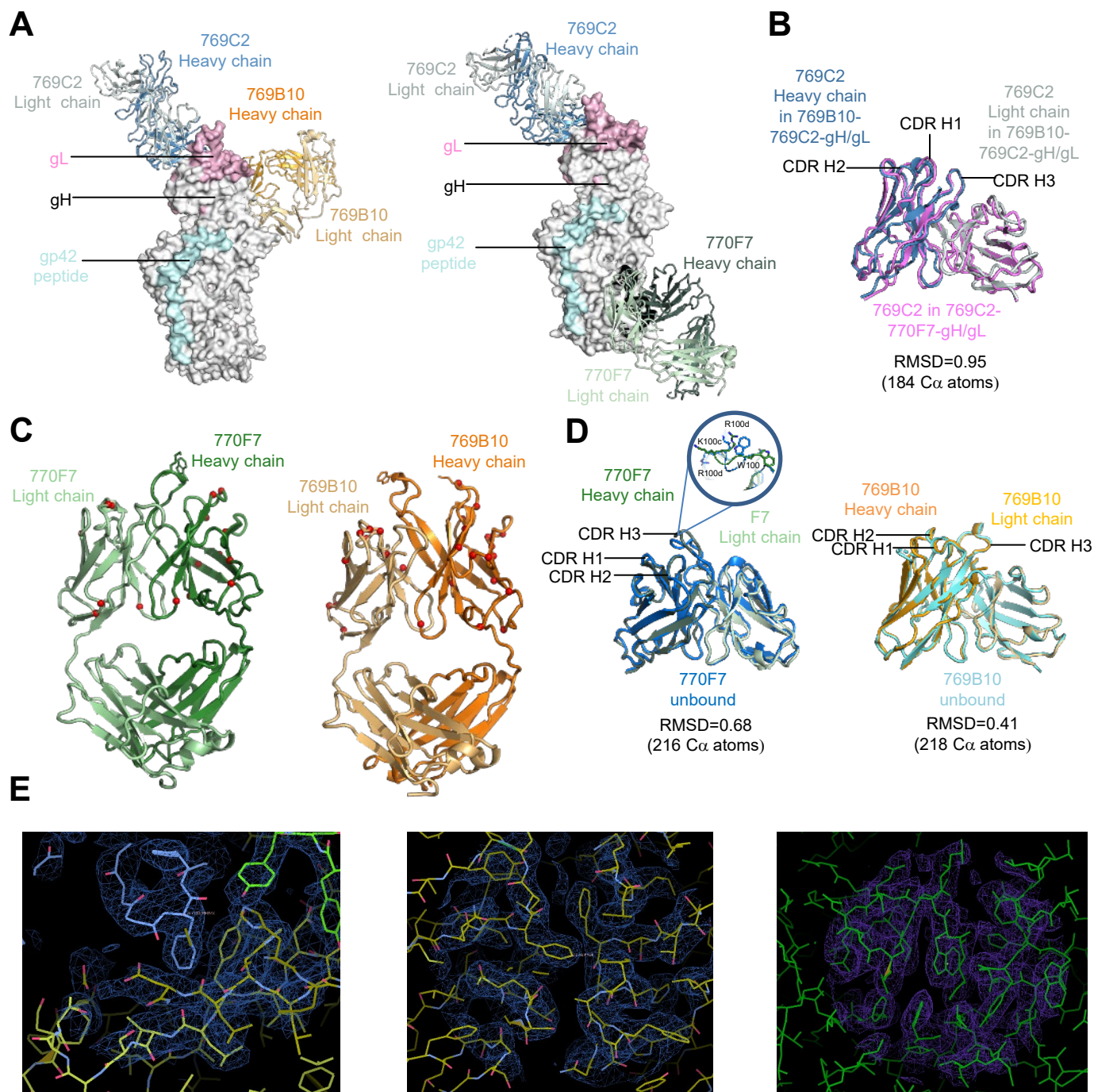


Figure S3. Crystal structures of EBV gH/gL-targeting antibodies (related to Figures 2-5)

(A) Crystal asymmetric unit of the two EBV gH/gL/gp42p antibody complexes. (Left) 769C2 and 769B10 Fabs in complex and (right) 769C2 and 770F7 in complex with EBV gH/gL/gp42 respectively.

(B) Overlay of 769C2 Fv region from the two crystal structures.

(C) Crystal structure of 770F7 Fab (left) and 769B10 (right).

(D) Overlay of the Fv regions of 770F7 and 769B10 from bound and unbound structures. A zoom-in view of the 770F7 CDR H3 region with select residues shown in stick representation.

(E) Representative electron density images of the mAb-gH/gL interfaces. *Left*, 769C2 interface with EBV gH/gL centered on 769C2 CDR H3 Phe99 (Kabat numbering); *Center*, 769B10 interface with EBV gH/gL centered on 769B10 CDR L3 Phe92; *Right*, 770F7 interface with EBV gH/gL centered on 770F7 CDR H3 Trp100. Electron density 2Fo-Fc map is contoured at 1 σ for all panels.

Figure S4

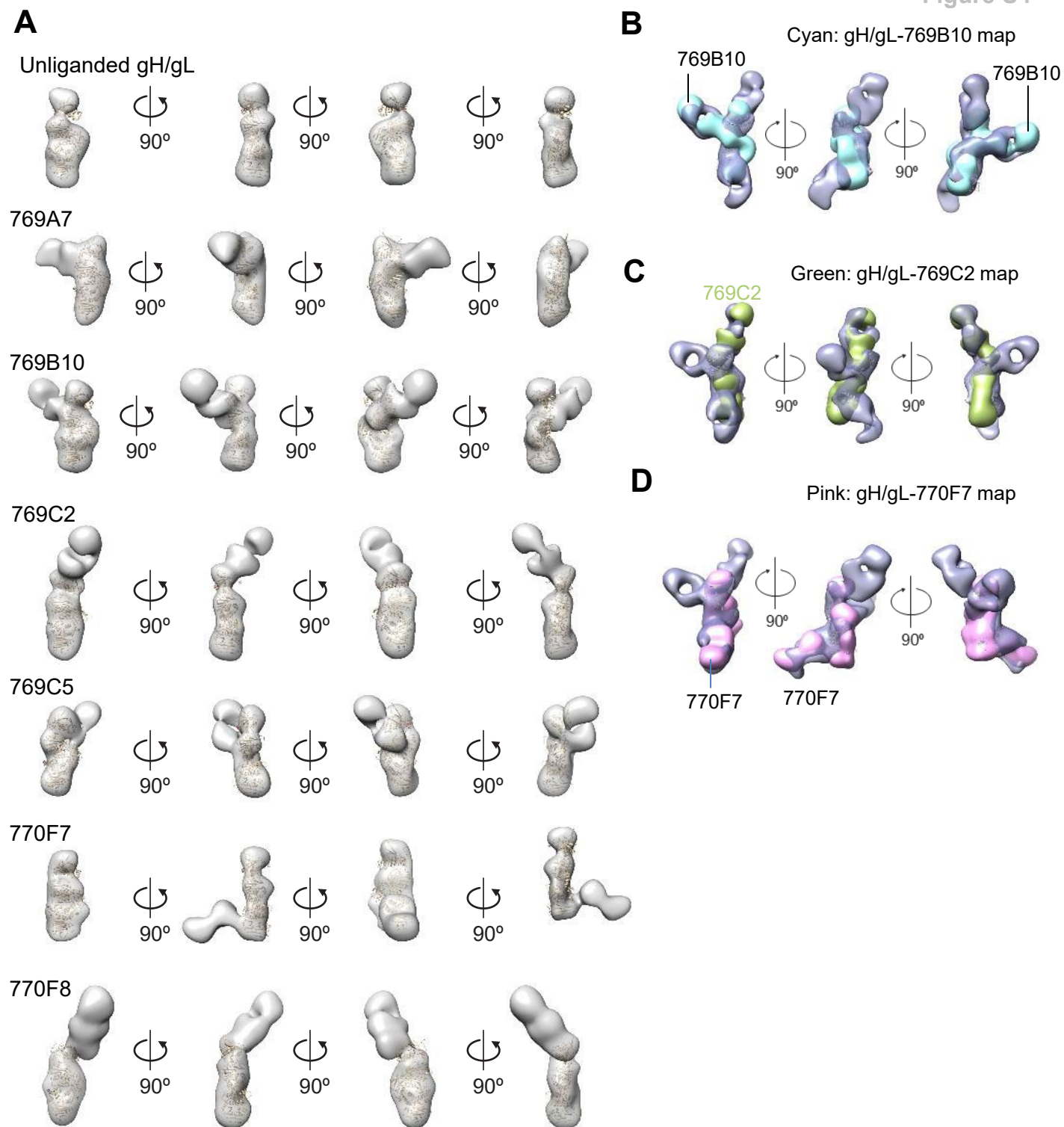


Figure S4. Negative Stain EM 3D reconstruction of EBV gH/gL-targeting human antibodies (related to Figures 2-4)

EBV gH/gL structure model is docked to the negative-stain EM maps.

(A) Negative-stain EM maps of gH/gL/gp42p molecule unliganded or in complex with single mAb Fab molecules 769A7, 769B10, 769C2, 769C5, 770F7, and 770F8.

(B-D) Comparison of the binary complexes from (A) for 769B10 (B), 769C2 (C), and 770F7 (D) were compared to a gH/gL/gp42p complex with 769B10, 769C2 and 770F7 Fabs bound.

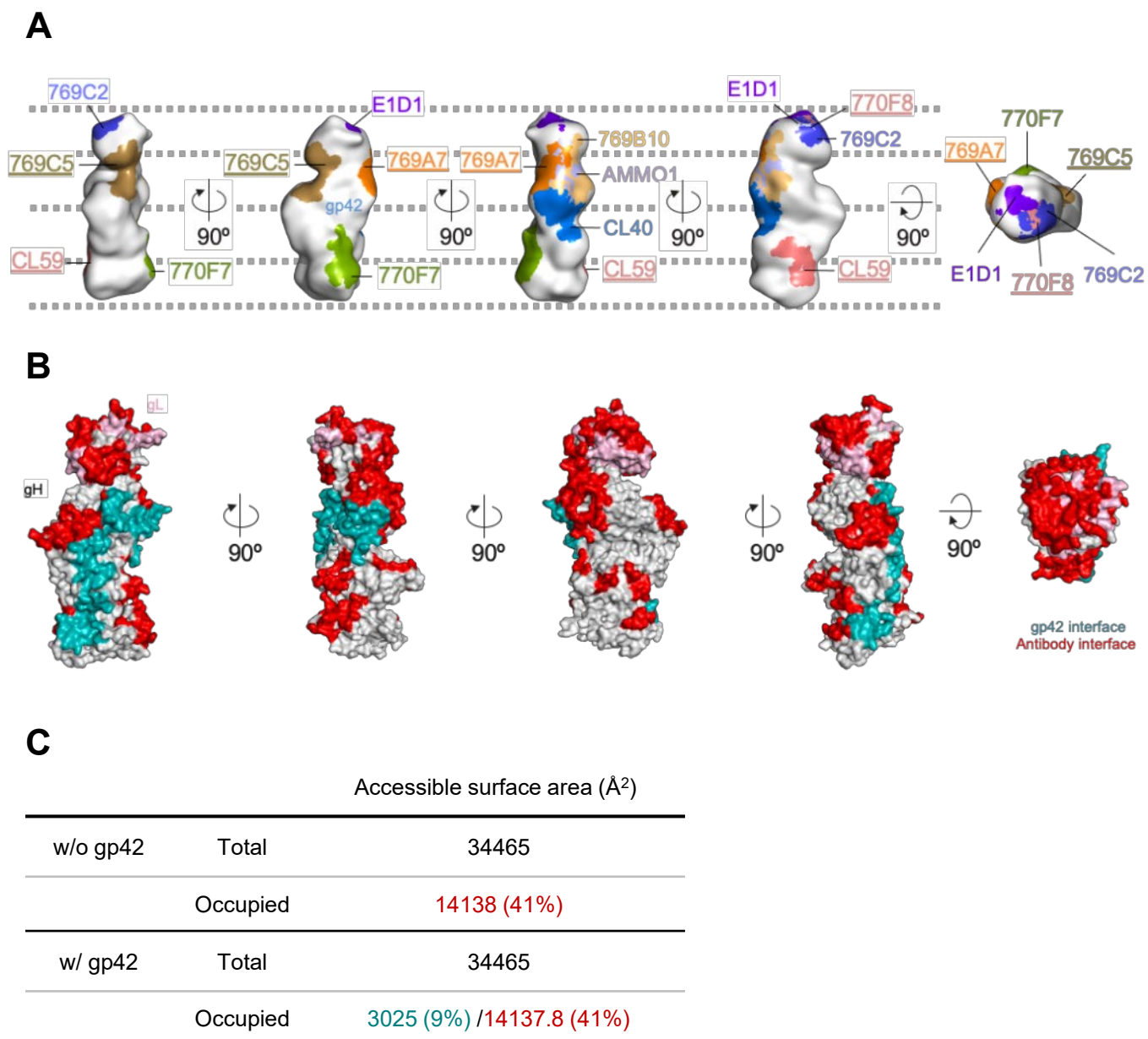


Figure S5 Antigenic sites of vulnerability on the EBV gH/gL/gp42 fusion machinery (related to Figure 6)

(A) EBV gH/gL is shown in surface representation with specific mAb epitopes indicated. Multiple rotation views are shown. EBV gH domains are indicated by dotted lines.

(B) EBV gH/gL is shown in surface representation with all mAb epitopes colored in red and gp42 domain interface colored in teal

(C) EBV gH/gL accessible surface area is shown with area bound by antibodies or gp42 indicated.

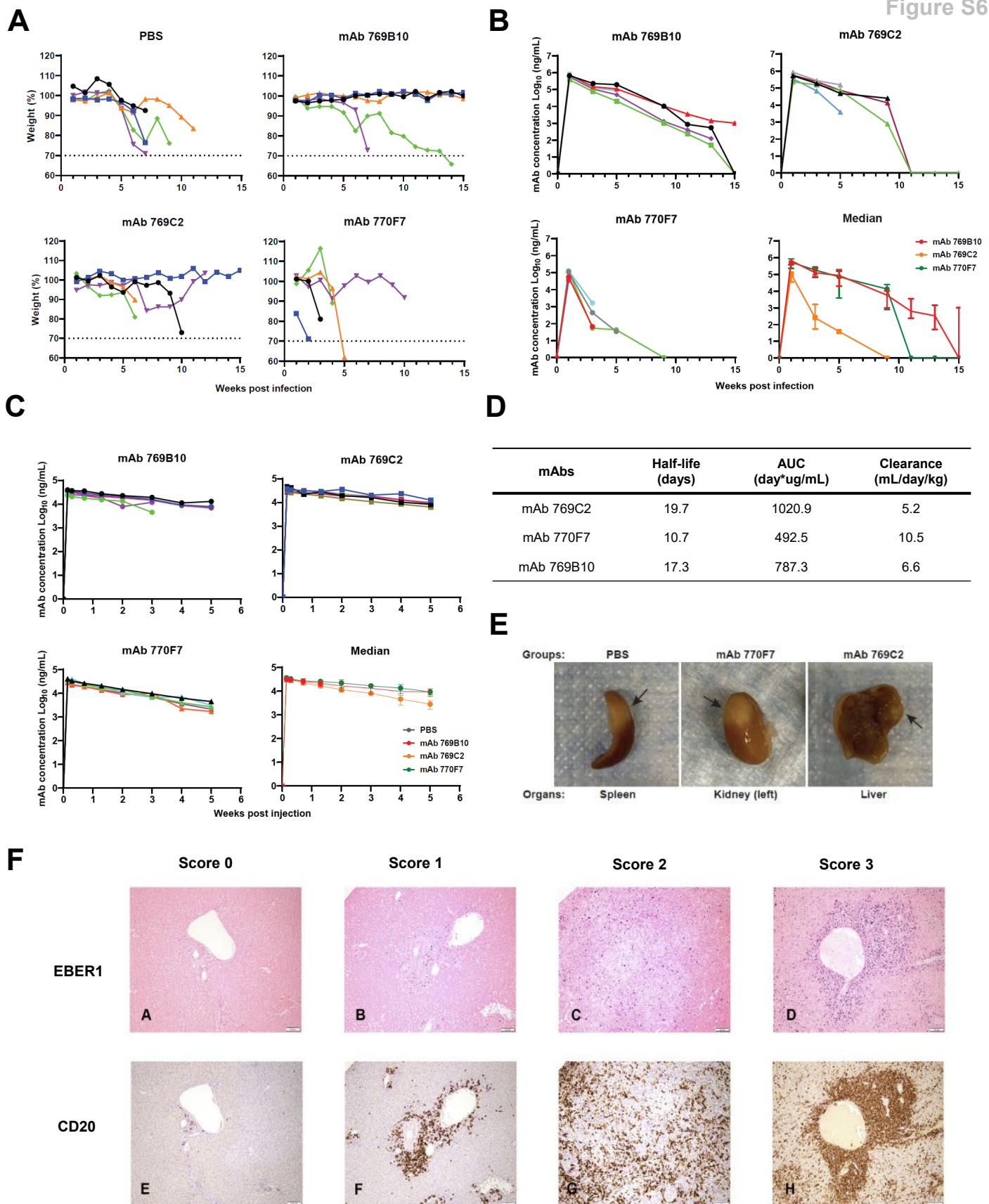


Figure S6. Weight loss, antibody levels, and EBV lesions in humanized mice (related to Figure 7)

(A) Percentage of weight in mice relative to the weight (set as 100%) before EBV challenge throughout the study were plotted. Each line indicates one mouse. The dotted line indicates the endpoint which is 30% weight loss.

(B) Concentration of gH/gL mAbs in the plasma of individual humanized mice at various times after EBV challenge were measured in duplicate by ELISA. The median and standard errors of gH/gL mAb concentrations for each group of 5 humanized mice are also shown.

(C) gH/gL mAb concentrations in the serum at various days after a single injection of mAb in individual human FcRn transgenic mice were quantified by ELISA assay to gH/gL in duplicate. The median and standard errors for each group of 5 mice are also shown.

(D) Half-life, area under the curve (AUC), and clearance rate of each mAb in each group of human FcRn transgenic mice was calculated using WinNonLin software.

(E) Macroscopic lesions observed on the surface of the spleen (left), kidney (middle), and liver (right) of humanized mice challenged with EBV.

(F) EBV EBER staining with eosin counterstain (upper panels) and CD20 staining (lower panels) in mice with lymphomas. Scores for EBV RNA signal in the upper panels are 0 (panel A), 1+ (panel B), 2+ (panel C), and 3+ (panel D). The lower panels show CD20 staining for the corresponding tissues in the upper panels.

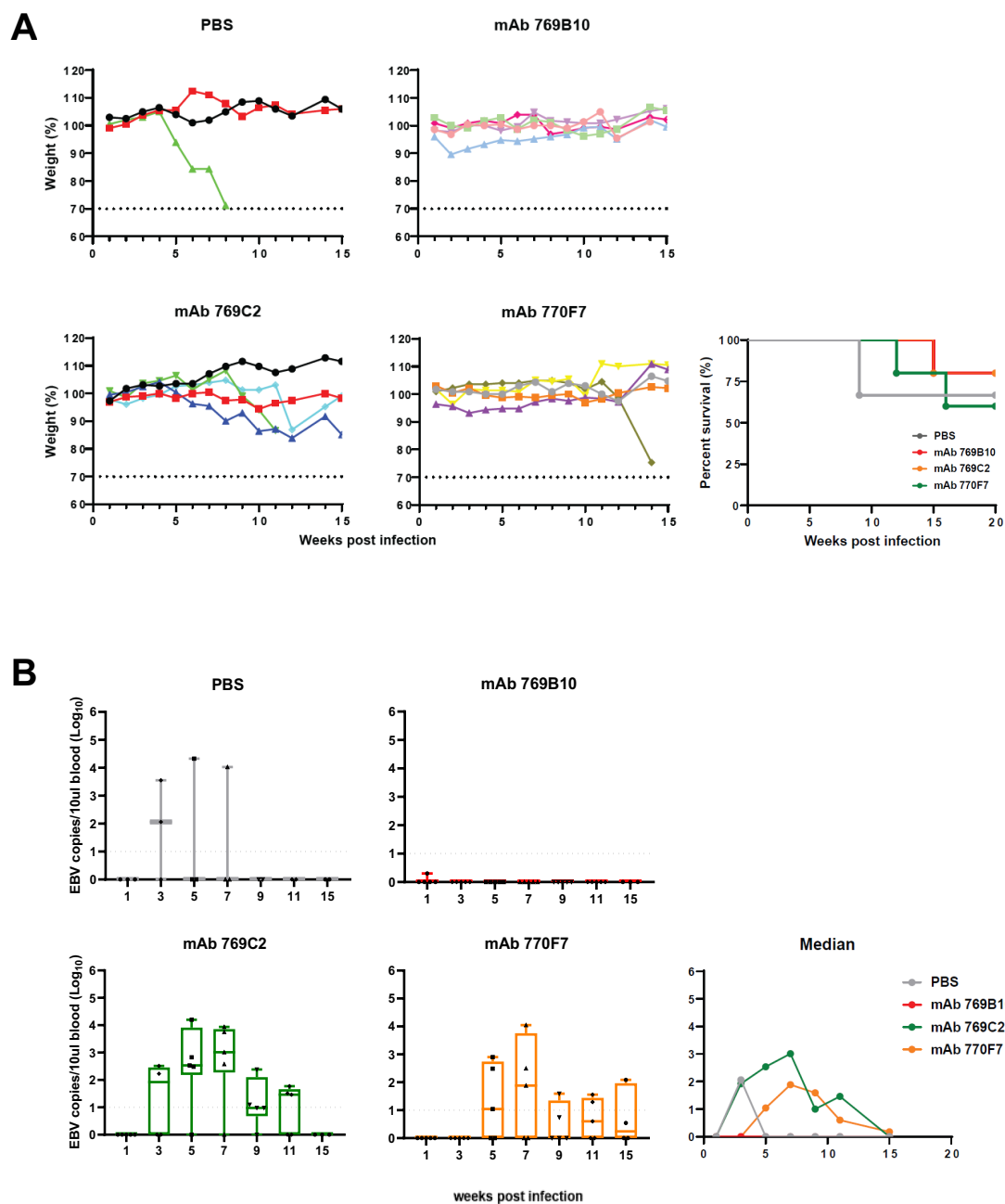


Figure S7. Repeat experiment for passive transfer of gH/gL mAbs in humanized mice and challenge with EBV (related to Figure 7)

(A) Survival rates of mice given six doses of gH/gL mAbs 769C2, 770F7, 769B10, or PBS intraperitoneally and challenged intravenously with EBV after the second dose of mAb.

(B) EBV DNA copies in the blood quantified by real-time qPCR in mice from panel A. Each dot represents one mouse, and the dotted line indicates the detection limit of the assay. Whiskers indicates minimum and maximum data points and box represents upper and lower quartiles; the horizontal line in the box is the median. Median EBV DNA copies in the blood were calculated.

Table S1. Crystal Data Collection and Refinement Statistics (related to Figures 2-4)

	EBV gH/gL/gp42p + 769C2 Fab + 769B10 Fab	EBV gH/gL/gp42p + 769C2 Fab + 770F7 Fab	769B10 Fab	770F7 Fab
Crystallization condition	0.1M Bis-Tris pH6.5, 20% PEG3350, 7.5% Glycerol, 0.7% Dioxane	0.1M HEPES pH7.4, 24% PEG3350, 7.5% Glycerol.	0.1M Tris-HCl pH8.0, 18% PEG5000MME, 0.2M NaCl, 2.5% Glycerol	0.1M HEPES pH7.5, 0.1M CaCl ₂ , 25% PEG3350, 4% isopropanol
Data collection				
Space group	P 2 ₁	P 2 ₁	P 2 ₁	C 2
Cell dimensions				
a, b, c (Å)	90.7, 130.4, 93.6	89.3, 107.7, 110.7	56.2, 67.1, 65.0	187.2, 40.2, 135.2
α, β, γ (°)	90, 104.6, 90	90, 93.1, 90	90, 107.6, 90	90, 110.5, 90
Resolution (Å)	50.0 – 3.3 (3.6-3.5, 3.5-3.4, 3.2-3.3)	50.0-3.0 (3.1-3.0)	41.8-2.2 (2.3-2.2)	50.0-2.4 (2.5-2.4)
R _{sym}	0.303 (1.115, 1.267, 1.382)	0.203 (2.325)	0.189 (0.498)	0.191 (0.962)
I / σI	5.5 (1.21, 1.03, 0.94)	7.1 (0.87)	5.3 (1.92)	8.7 (1.07)
Reflections (total/unique)	136898/41284	275778/40453	48517/22877	160636/36186
Completeness (%)	88.0 (77.8, 68.4, 57.2)	89.7 (66.6)	89.7 (88.6)	95.4 (84.3)
Redundancy	4.5 (3.7, 3.4, 3.0)	6.8 (6.6)	2.4 (1.4)	4.5 (2.5)
CC _(1/2)	0.65 (0.35)	0.99 (0.77)	0.82 (0.72)	0.85 (0.522)
R _{pim}	0.127 (0.78)	0.078 (0.88)	0.109 (0.34)	0.089 (0.55)
Refinement				
Resolution (Å)	48.6-3.3 (3.4-3.3)	50-3.3 (3.3-3.0)	41.9-2.2 (2.3-2.2)	50.0-2.4 (2.5-2.4)
No. reflections	31330 (205)	36540 (2692)	22877(1928)	67674(5342)
R _{work} / R _{free} *	25.3/29.7	24.5/30.2	23.2/26.7	20.5/25.8
Ramachandran				
favored/allowed/outliers	91.2/8.6/0.2	90.3/9.3/0.4	95.0/4.8/0.2	96.3/3.4/0.3
B-Factor				
Protein/water	54.1/ n.a.	46.6/ 50.5	30.8/ 35.6	37.11/ 42.7
R.m.s deviations				
Bond lengths (Å)	0.002	0.009	0.003	0.002
Bond angles (°)	0.584	0.675	0.532	0.600
PDB ID	7S07	7S1B	7S08	7S0J
Deposition code	D_1000259334	D_1000259335	D_1000259333	D_1000259217

Values in parentheses are shown for the highest-resolution data shells.

n.a.: not applicable

* Rfree was calculated using ~5% randomly selected reflections.

Table S2A. 769B10 antibody interface with EBV gH (chain A), gL (chain B) (related to Figure 2)

	769B10	Glycoprotein	Distance(Å)
Hydrogen bonds	H:TYR 33[OH]	A:SER 79[O]	3.51
	H:ARG 52[NH1]	A:SER 79[O]	2.35
	H:ASN 53[OD1]	A:THR 81[N]	3.21
	H:ARG 64[NH1]	B:GLU 121[OE2]	3.58
	H:ARG 65[NH2]	B:ASP 57[O]	2.90
	H:ARG 65[NH2]	B:ASP 57[OD2]	2.41
	H:TYR 97[OH]	A:SER 79[OG]	2.90
	H:TRP 100a[O]	A:LEU 246[N]	3.14
	H:TRP 100a[O]	A:LYS 247[N]	3.01
	H:TYR 100d[O]	A:THR 78[OG1]	2.15
	H:TYR 100d[O]	A:GLY 80[N]	3.59
	H:TYR 100d[O]	A:SER 79[N]	2.94
	H:SER 100e[O]	A:SER 79[OG]	3.33
	H:SER 100e[N]	A:SER 79[OG]	3.61
	H:SER 100e[OG]	A:TYR 235[OH]	2.74
	H:SER 100e[OG]	A:TYR 76[OH]	2.58
Salt Bridge	L:SER 67[OG]	A:ASN 240[ND2]	3.54
	L:SER 30[OG]	A:ASN 240[OD1]	3.16
	H:ARG 64[NH1]	B:GLU 121[OE2]	3.58
	H:ARG 65[NE]	B:ASP 122[OD2]	3.79
	H:ARG 65[NH2]	B:ASP 57[OD2]	2.41
	L:ASP 1[OD1]	A:LYS 73[NZ]	3.60

H: 769B10 heavy chain; L: 769B10 light chain; A: gH; B: gL

Table S2B. 769C2 interface with EBV gH (chain A), gL (chain B) (related to Figure 3)

	769C2	Glycoprotein	Distance (Å)
Hydrogen bonds	X:SER 30[O]	B:GLN 54[NE2]	3.80
	X:SER 52[OG]	A:GLU 30[O]	2.99
	X:SER 52a[N]	A:GLU 30[O]	3.57
	Y:GLY 31[O]	A:LYS 24[NZ]	2.61
	Y:TYR 32[OH]	A:THR 37[N]	3.57
	Y:TYR 32[OH]	A:HIS 35[O]	3.62
	Y:TYR 32[OH]	B:HIS 103[NE2]	3.11
	Y:ASP 33[OD2]	B:LYS 68[NZ]	3.41
	Y:TYR 93[OH]	A:SER 34[OG]	2.33
	Y:TYR 93[OH]	A:HIS 35[O]	3.48
Salt Bridge	Y:ASP 33[OD2]	B:LYS 68[NZ]	3.41

X: 769C2 heavy chain; Y: 769C2 light chain; A: gH; B: gL

Table S2C. 770F7 interface with EBV gH (chain A) (related to Figure 4)

	770F7	Glycoprotein	Distance (Å)
Hydrogen bonds	H:SER 28[N]	A:ASP 536[O]	3.00
	H:TYR 32[OH]	A:LYS 533[O]	3.57
	H:TYR 32[OH]	A:VAL 535[O]	2.48
	H:ARG 94[NH2]	A:LYS 533[O]	3.87
	H:ARG 94[NH2]	A:VAL 535[O]	3.70
	H:ARG 94[NH2]	A:ASP 536[OD1]	3.45
	H:TRP 100[NE1]	A:TYR 532[OH]	3.38
	H:ALA 100A[N]	A:ASP 525[OD2]	3.53
	H:LEU 100B[N]	A:GLU 522[OE2]	3.90
	H:LYS 100C[N]	A:GLU 522[OE2]	3.44
	H:ARG 100D[NH1]	A:ASP 525[OD1]	2.24
	H:ARG 100D[NH2]	A:LEU 432[O]	3.76
	H:ARG 100D[NH2]	A:ARG 433[O]	3.84
	H:ARG 100D[NH1]	A:GLU 522[O]	3.64
	H:ARG 100D[NH2]	A:GLU 522[O]	3.65
	H:ARG 100D[NH2]	A:LEU 432[O]	3.76
	Salt Bridge	L:TYR 32[OH]	A:ASN 435[O]
L:TYR 32[OH]		A:ASN 435[OD1]	3.61
L:TYR 49[OH]		A:LYS 533[NZ]	3.17
H:ARG 100D[NH1]		A:ASP 525[OD1]	2.24
H:ARG 100D[NH1]		A:ASP 525[OD2]	3.23
H:ARG 94[NH2]		A:ASP 536[OD1]	3.45

H: 769C2 heavy chain; L: 769C2 light chain; A: gH

Table S3A. Buried surface area of 769B10 antibody in complex with gH/gL/gp42p (related to Figure 2)

	B10 antibody residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
gH	H:TYR 33	H	1.2	0.3
	H:ARG 50		17.5	5.4
	H:ARG 52	H	57.4	30.4
	H:GLU 52c		120.5	43.7
	H:ASN 53	H	39.2	23.8
	H:TYR 97	H	38.7	6.1
	H:ASP 99		56.6	4.6
	H:LEU 100		83.99	17.2
	H:TRP 100a	H	216.1	123.9
	H:SER 100b		65.5	50.3
	H:GLY 100c		29.9	29.0
	H:TYR 100d	H	81.8	77.2
	H:SER 100e	H	105.4	73.4
	H:ASN 100g		12.7	0.9
H:TRP 100h		80.4	3.8	
gH	L:ASP 1	S	123.8	30.8
	L:GLN 27		84.9	11.3
	L:THR 28		89.5	51.6
	L:MET 29		2.2	2.2
	L:SER 30	H	53.3	49.7
	L:ASN 31		52.3	22.1
	L:PHE 32		81.5	34.4
	L:SER 67		76.3	6.7
	L:GLY 68		22.3	0.8
	L:PHE 92		97.8	79.2
	L:LEU 93		46.6	39.7
	L:PHE 94		157.4	3.4
	L:TYR 96		120.3	25.8
gL	H:GLU 58		86.4	19.7
	H:ALA 61		87.2	17.6
	H:ARG 64	HS	129.6	92.0
	H:ARG 65	HS	232.0	69.9
gL	L:PHE 94		157.4	27.7
	L:PRO 95		72.0	2.0

Bond Type: H: Hydrogen bond; S: Salt bridge

mAb chain: H: 769B10 heavy chain; L: 769B10 light chain

Table S3B. Buried surface area of gH/gL in complex with 769B10 (related to Figure 2)

	gH/gL residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
Heavy chain	A:TYR 76	H	30.4	20.8
	A:LYS 77		46.5	0.3
	A:THR 78	H	29.8	18.0
	A:SER 79	H	128.2	104.8
	A:GLY 80	H	39.6	32.4
	A:THR 81	H	52.6	31.0
	A:LEU 82		94.8	85.0
	A:GLY 83		57.8	25.2
	A:SER 218		19.1	12.2
	A:ILE 228		12.5	0.2
	A:TYR 235		64.4	23.5
	A:HIS 239		89.7	4.1
	A:VAL 243		83.6	38.2
	A:PRO 244		36.5	15.9
	A:ASN 245		78.5	29.1
A:LEU 246	H	42.2	26.1	
A:LYS 247	H	101.8	26.0	
Light chain	A:LYS 73	S	133.9	29.8
	A:TYR 76		30.4	9.5
	A:LYS 77		46.4	3.7
	A:SER 79		128.2	23.4
	A:PHE 232		91.0	72.0
	A:VAL 233		89.4	29.7
	A:TYR 235		64.4	22.9
	A:ALA 236	H	58.8	58.8
	A:ASN 237		53.1	3.7
	A:HIS 239		89.7	57.8
A:ASN 240		122.7	38.6	
Heavy chain	B:ASP 57	H	119.6	26.0
	B:GLY 58		14.5	2.5
	B:SER 119		32.7	1.6
	B:GLU 121	HS	150.7	46.9
	B:ASP 122	S	113.5	81.8
	B:LEU 123	H	97.6	11.2
Light chain	B:PHE 124		259.2	28.4
	B:GLU 121		150.7	29.4

H: Hydrogen bond; S: Salt bridge; A: gH; B: gL

Table S4A. Buried surface area of mAb 769C2 in complex with gH/gL/gp42p (related to Figure 3)

	769C2 residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
Heavy chain	A:HIS 26		128.2	22.7
	A:LEU 27		78.0	3.0
	A:ASP 28		115.3	43.0
	A:ILE 29		88.9	44.3
	A:GLU 30		159.2	79.9
	A:GLY 31		68.3	68.3
	A:HIS 32		132.8	80.6
	A:ALA 33		45.1	42.4
	A:SER 34		17.9	0.5
	A:HIS 35		120.3	32.6
A:TYR 36		114.1	0.8	
Light chain	A:LYS 24	H	104.0	36.8
	A:HIS 26		128.2	22.3
	A:HIS 32		132.8	52.2
	A:ALA 33		45.1	2.7
	A:SER 34	H	17.9	14.0
	A:HIS 35	H	120.3	83.6
	A:TYR 36		114.1	10.3
A:THR 37	H	76.0	19.2	
Heavy chain	B:TYR 49		121.5	50.5
	B:LEU 50		59.9	14.2
	B:VAL 51		40.9	12.5
	B:GLN 54	H	166.6	58.3
	B:GLU 106		118.4	13.5
B:THR 110		72.5	2.8	
Light chain	B:TYR 49		121.5	17.3
	B:LYS 68	HS	182.6	29.6
	B:HIS 103	H	143.8	58.5
	B:GLU 106		118.4	8.1

H: Hydrogen bond; S: Salt bridge; A: gH; B: gL

Table S4B. Buried surface area of gH/gL/gp42p in complex with 769C2 (related to Figure 3)

	769C2 residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
gH	X:SER 30		31.9	1.0
	X:THR 31		98.0	23.2
	X:TYR 32		62.0	1.3
	X:ALA 33		16.7	16.7
	X:LEU 50		32.7	16.3
	X:SER 52	H	22.7	22.4
	X:SER 52a	H	28.6	24.6
	X:ARG 53		165.3	2.3
	X:SER 55		74.5	6.2
	X:ASN 56		83.4	26.0
	X:TYR 58		119.5	34.9
	X:GLU 95		48.7	31.9
	X:GLY 97		24.6	17.9
	X:GLY 98		81.6	39.6
	X:PHE 99		208.3	94.6
X:HIS 100		116.6	7.3	
X:SER 100a		117.3	29.2	
gH	Y:GLY 31	H	44.6	23.7
	Y:TYR 32	H	98.2	79.4
	Y:TYR 34		140.4	55.3
	Y:TYR 93	H	100.1	82.9
	Y:THR 95		85.1	7.2
	Y:THR 98		86.3	22.1
	Y:TRP 99		174.4	21.2
gL	X:SER 30	H	31.9	4.8
	X:THR 31		98.0	10.6
	X:SER 52a		28.6	4.0
	X:ARG 53		165.3	26.0
	X:TYR 58		119.5	14.7
	X:PHE 99		208.3	89.7
gL	Y:TYR 32	H	98.2	18.9
	Y:ASP 33	HS	97.8	28.5
	Y:TYR 34		140.4	18.5
	Y:VAL 53		36.9	2.4
	Y:TYR 93		100.0	4.0
	Y:THR 95		85.0	47.5

H: Hydrogen bond; S: Salt bridge; X: 769C2 heavy chain; Y: 769C2 light chain

Table S5A. Buried surface area of 770F7 antibody in complex with EBV gH (related to Figure 4)

	770F7 residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
gH	H:GLN 1		172.6	67.1
	H:VAL 2		18.9	16.9
	H:GLY 26		79.5	50.8
	H:GLY 27		32.1	31.5
	H:SER 28	H	58.0	49.9
	H:SER 30		46.6	34.7
	H:GLY 31		3.3	1.2
	H:TYR 32	H	46.2	46.0
	H:THR 54		106.2	11.2
	H:ASN 76		43.8	10.6
	H:ARG 94	HS	30.9	36.8
	H:GLY 96		15.0	22.6
	H:ALA 97		12.7	19.4
	H:PRO 98		63.3	75.7
	H:TRP 100	H	122.4	164.3
	H:ALA 100A	H	46.4	46.4
	H:LEU 100B	H	15.2	131.4
	H:LYS 100C	H	33.0	190.2
	H:ARG 100D	HS	121.7	140.3
	H:PRO 100E		35.9	103.7
H:ASN 100G		15.0	43.5	
H:TRP 100H		22.7	157.0	
gH	L:THR 31		91.0	16.9
	L:TYR 32		101.1	35.6
	L:ARG 45		127.6	11.2
	L:TYR 49	H	111.7	39.1
	L:SER 53		44.8	5.9
	L:ARG 54		105.1	1.7
	L:ALA 56		106.7	65.3
	L:GLY 57		79.2	29.9
	L:ILE 58		22.3	1.0

Bond type: H: Hydrogen bond; S: Salt bridge
mAb chain: H: 769C2 heavy chain; L: 769C2 light chain

Table S5B. Buried surface area of 770F7 antibody in complex with gH (related to Figure 4)

	gH/gL residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
Heavy chain	A:LEU 432	H	7.1	2.1
	A:ARG 433	H	88.8	7.8
	A:LEU 434	H	48.8	1.4
	A:ASN 435		122.8	42.1
	A:SER 519		54.8	33.1
	A:GLU 520		144.0	59.3
	A:GLU 522	H	108.0	71.1
	A:ASP 525	HS	50.6	41.9
	A:TRP 527		171.9	117.8
	A:TYR 532	H	39.4	32.5
	A:LYS 533	H	136.9	81.7
	A:VAL 535	H	7.6	6.9
	A:ASP 536	HS	82.7	81.3
	A:ARG 537		126.2	60.3
	A:LEU 538		79.1	71.8
	A:ASP 539		118.4	41.6
	A:GLU 559		75.2	26.4
	A:ARG 561		53.6	26.2
	A:GLN 589		189.0	9.6
	A:GLY 590		33.2	10.5
	A:ALA 591		34.4	8.8
Light chain	A:ASN 435		122.8	17.9
	A:VAL 436		120.1	23.6
	A:TRP 527		171.9	3.6
	A:LYS 533	H	136.9	33.1
	A:LYS 586		149.4	20.7
	A:CYS 587		41.6	16.9
	A:SER 588		45.3	6.2
		A:GLN 589		189.0

H: Hydrogen bond; S: Salt bridge; A: gH