Supplementary Information for
A potent and protective human neutralizing antibody targeting a novel vulnerable site of Epstein-Barr Virus

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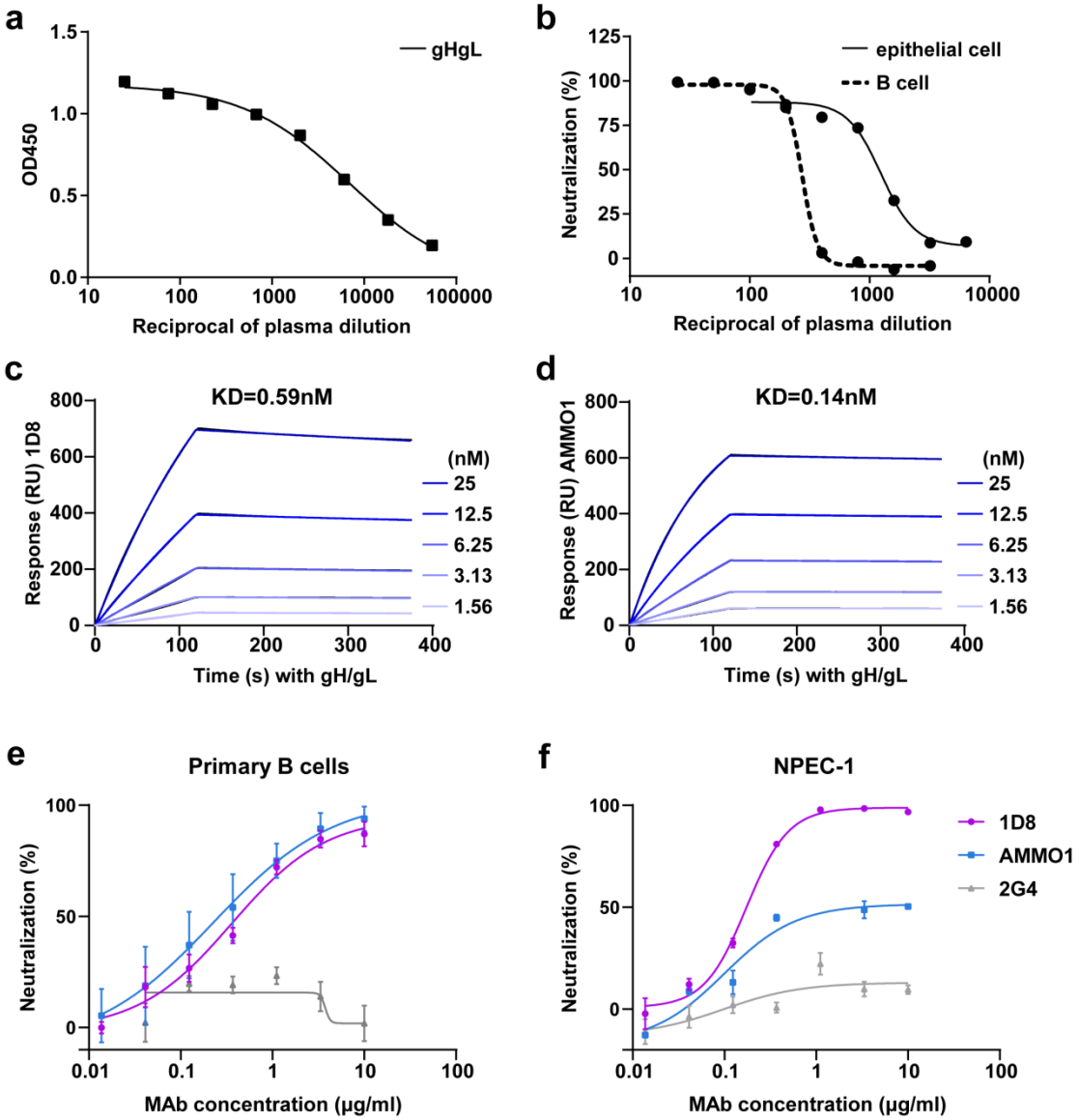
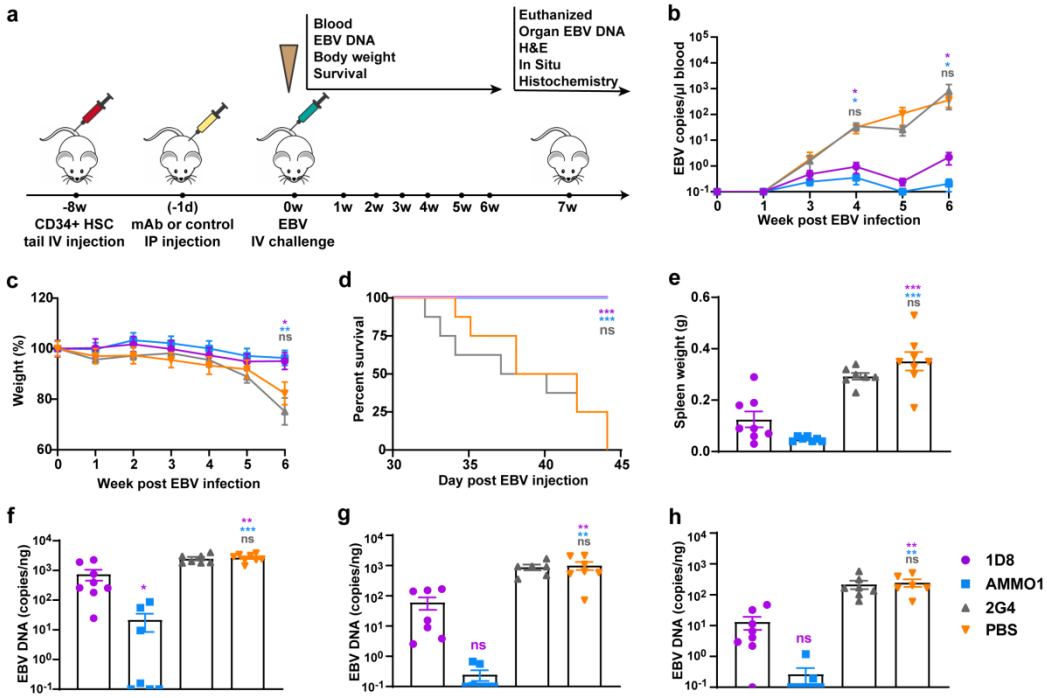
Supplementary Figures

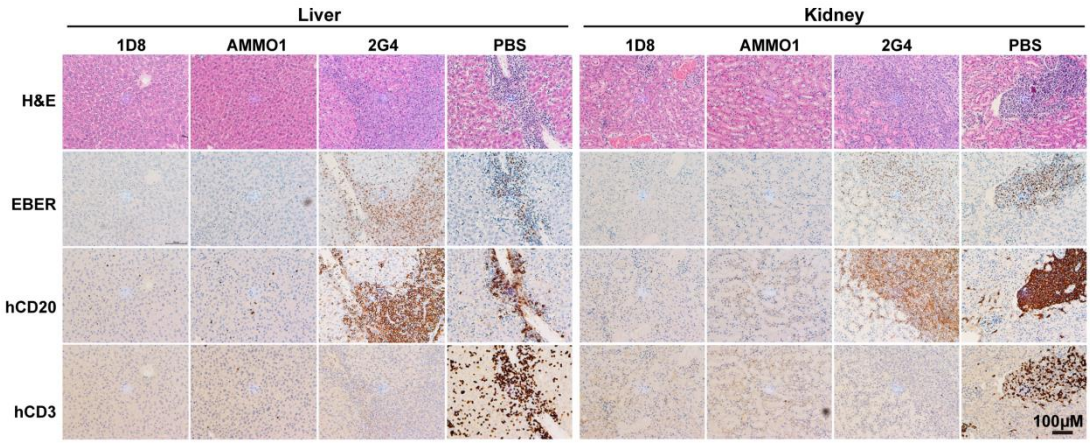


Supplementary Figure 1. Plasma binding and neutralizing activities from donor 27. (**a**) Plasma binding activities to gH/gL measured by ELISA. (**b**) Plasma neutralizing activities against EBV infection of Raji B cells and HNE1 epithelial cells. Binding activity of 1D8 (**c**) or AMMO1 (**d**) to gH/gL measured by SPR. (**e**) Neutralizing activities of 1D8, AMMO1 and 2G4 against EBV infection of primary B cells and (**f**) Bmi1-immortalized nasopharyngeal epithelial cell line (NPEC1-Bmi1). The data shown is means ± SEM from three replicates. Source

data are provided as a Source Data File.

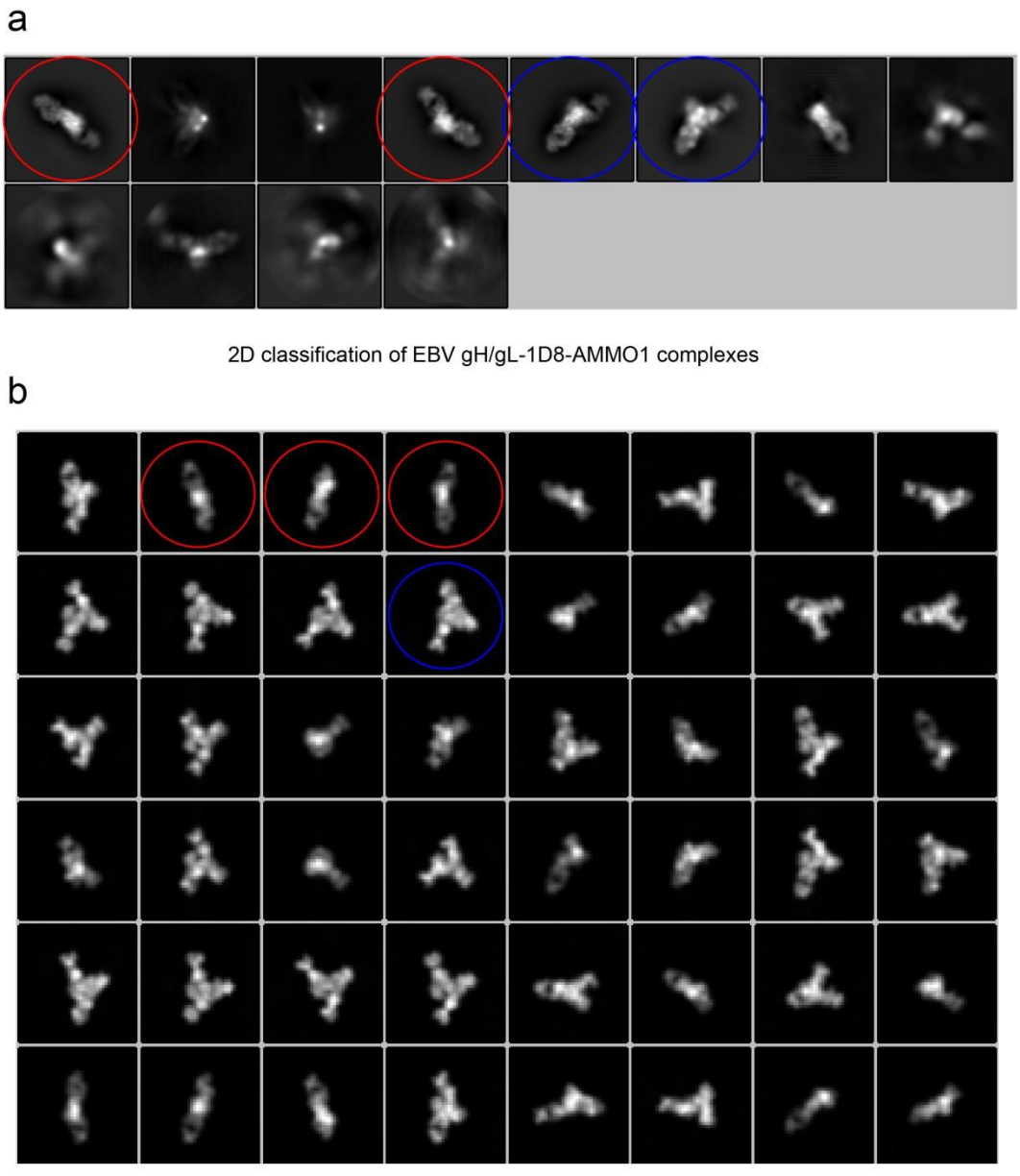


Supplementary Figure 2. Protective effect of single dose of 1D8 on fatal EBV attack in humanized mice (**a**) Timeline for engrafting CD34+ human hematopoietic stem cells (HSC), antibody administration, viral challenge, and monitoring for various biological and clinical outcomes. 400 μg of 1D8 (n=8), positive control AMMO1 (n=7), negative control 2G4 (n=7), or PBS (n=8) were administered to the humanized mice via intraperitoneal injection 24 h prior to intravenous challenge with Akata EBV. (**b**) EBV DNA in the peripheral blood, (**c**) body weight, and (**d**) survival were monitored weekly. On week 7 post infection, (**e**) spleen weight (**f**) virus titers in spleen, (**g**) liver, (**h**) kidney were analyzed. All data are presented as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, no significant, two-tailed unpaired Student's *t*-test. (**b**) 1D8 vs PBS in 4w *p=0.038, AMMO1 vs PBS in 4w *p=0.048, 1D8 vs PBS in 6w *p=0.011, AMMO1 vs PBS in 6w *p=0.017; (**c**) *p=0.016, **p=0.009; (**d**) ***$p < 0.001$, log-rank test (Mantel-Cox); (**e**) 1D8 vs PBS ***p=0.0003, AMMO1 vs PBS ***$p < 0.001$; (**f**) **p=0.0018, ***$p < 0.001$; *p=0.0417; (**g**) 1D8 vs PBS **p=0.005, AMMO1 vs PBS **p=0.0057; (**h**) 1D8 vs PBS **p=0.0017, AMMO1 vs PBS **p=0.0022. Source data are provided as a Source Data File.

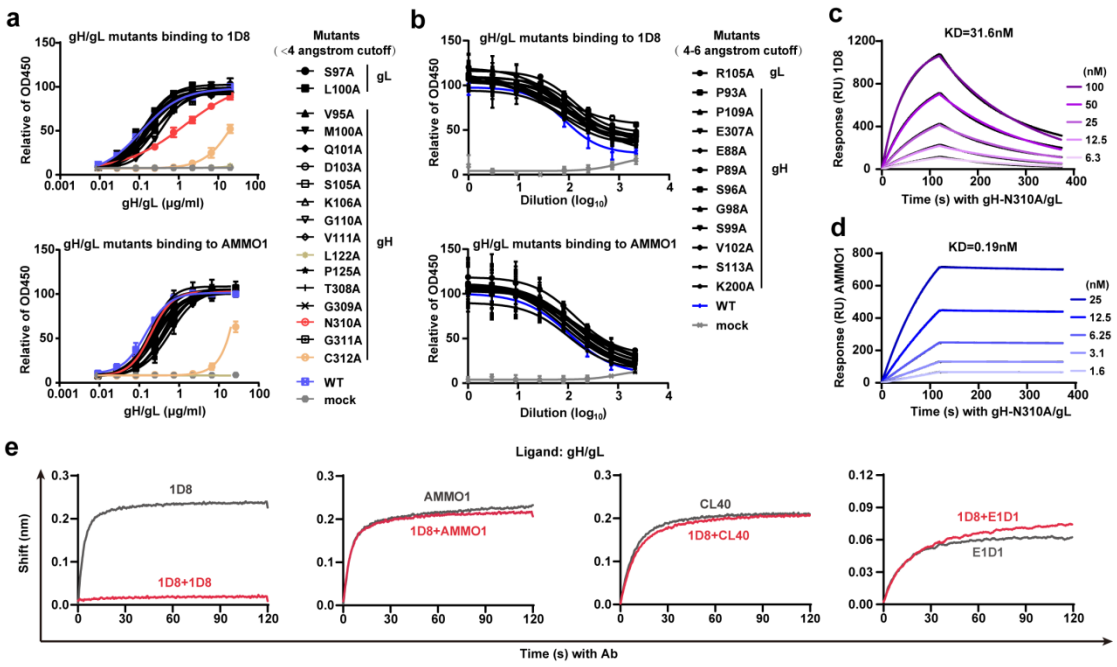


Supplementary Figure 3. 1D8 reduces viral replication and tissue damages in liver and kidney of mice.

Hepatic and renal sections stained for hematoxylin and eosin (H&E), human CD20 (hCD20), human CD3 (hCD3), and EBV encoded RNA (EBER) at necropsy. Scale bar of 100 μm is shown. Each image is representative of a group of 7-8 mice.



Supplementary Figure 4. The 2D classification results of gH/gL-1D8-AMMO1 ternary complex and the projection of merged gH/gL-1D8-AMMO1. (a) The 2D classification results of gH/gL-1D8-AMMO1 ternary complex. (b) The projection of merged gH/gL-1D8-AMMO1 model. Circles of the same color indicate projections of the same or similar orientation.



Supplementary Figure 5. Binding of 1D8 to gH/gL mutants and its competition with other gH/gL antibodies.

1D8 and AMMO1 binding to various gH/gL mutants measured by ELISA, determined by using a 4 angstrom (a) and 4-6 angstrom (b) distance cutoff. ELISA was performed in duplicate wells, and the data shown are means with SEM from two replicates. Binding activity of 1D8 (**c**) and AMMO1 (**d**) to gH-N310A/gL mutant measured by SPR. (**e**) Competitive binding of 1D8 with AMMO1, CL40 or E1D1 to gH/gL measured by BLI. Source data are provided as a Source Data File.

Supplementary Tables

Supplementary Table 1. Neutralization potency of monoclonal antibodies.

Mab	IC_{50} (μg/ml)			
	HNE1	Raji	Primary B cells	NPEC1-Bmi1
1D8	0.123	0.238	0.361	0.173
2A6	0.745	1.320	-	-
AMMO1	0.127	0.318	0.227	~10
2G4	NA	NA	NA	NA

Supplementary Table 2. Kinetic Analysis of Antibodies Binding to gH/gL Measured by SPR.

Ligand	Anylate	kon (1/Ms) $\times 10^{5}$	koff (1/s) $\times 10^{-4}$	KD (nM)
1D8	gH/gL S97A	1.33	1.36	1.02
1D8	gH/gL L100A	1.07	1.55	1.44
1D8	gH V95A/gL	1.30	2.31	1.77
1D8	gH M100A/gL	1.51	1.96	1.30
1D8	gH Q101A/gL	0.95	1.95	2.03
1D8	gH D103A/gL	0.72	0.69	0.95
1D8	gH S105A/gL	0.90	0.97	1.08
1D8	gH K106A/gL	1.26	1.09	0.86
1D8	gH G110A/gL	2.66	0.26	0.10
1D8	gH V111A/gL	1.11	1.48	1.34
1D8	gH P125A/gL	0.71	2.00	2.79
1D8	gH T308A/gL	1.04	1.01	0.97
1D8	gH G309A/gL	0.95	0.95	1.00
1D8	gH N310A/gL	1.75	55.20	31.60
1D8	gH G311A/gL	0.78	2.05	2.62
1D8	gH/gL WT	2.45	1.46	0.59
AMMO1	gH N310A/gL	4.97	0.96	0.19
AMMO1	gH/gL WT	5.77	0.85	0.14

Supplementary Table 3. Data collection and refinement statistics.

	EBV gH/gL-1D8
Data collection	
Space group	$P4_12_12$
Cell dimensions	
a, *b*, *c* (Å)	212.865, 212.865, 598.128
α, β, γ, (°)	90, 90, 90
Resolution (Å)	50.03-4.201(4.351-4.201) *
R_{sym} or R_{merge}	0.131 (1.261)
I / sI	8 (1.4)
Completeness (%)	99.34 (98.99)
Redundancy	8.1 (8.2)
Refinement	
Resolution (Å)	50.03-4.201
No. reflections	100335
R_{work} / R_{free}	25.30/28.33
No. atoms	
Protein	36304
B-factors	
Protein	153.13
R.m.s. deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.81
Ramachandran plot (%)	
Favored	93.35%
Allowed	6.54%
outlier	0.11%

One crystal was used.

*Values in parentheses are for highest-resolution shell.