Supplementary Material

Table. S1. Monoclonal antibodies used in this study

Antibody name	Specificity	Reference
C7G6-IgM	HA (RBS[1])	/[3]
C7G6-IgG	HA (RBS[1])	/[3]
C3G10-IgM	HA (RBS[1])	/[3]
C3G10-IgG	HA (RBS[1])	/[3]
C10H10-IgM	HA (RBS[1])	/[3]
C10H10-IgG	HA (RBS[1])	/[3]
C11B10	HA (RBS[1])	/[3]
C12G6	HA (RBS[1])	[21]
CR8033-like	HA (RBS[1])	[18]
CR8071-like	HA (VE[2])	[18]
CR9114-like-IgM	HA (Stem)	[18]
CR9114-like-IgG	HA (Stem)	[18]
5A7-like	HA1 (C terminal)	[19]
46B8-like	HA (VE[2])	[20]

[1]RBS: receptor binding site
[2]VE: vestigial esterase domain
[3]antibody generated in this study

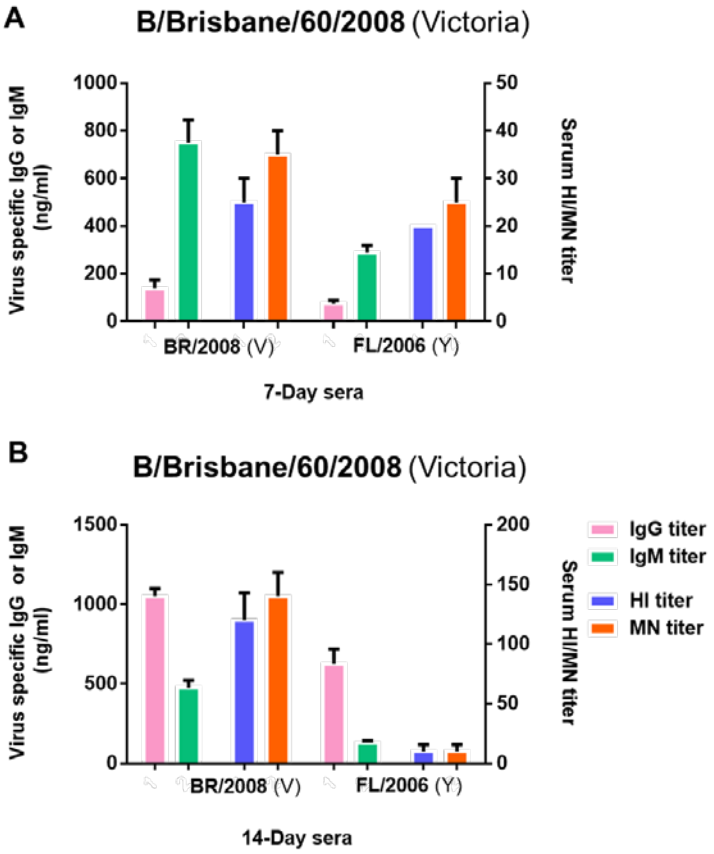

Fig. S1. Characterization of 7-day and 14-day anti-influenza sera following intranasal immunization with Victoria lineage strain of influenza B virus. Shown are data for serum total IgG titers, serum total IgM titers, serum HI titers and serum MN titers of BR/2008-immunized sera against two representative influenza B viruses, FL/2006 and BR/2008, analyzed in parallel. Recombinant HA proteins of FL/2006 and BR/2008 were used as ELISA plate-coating antigens. IgG and IgM titers were determined with quantitative ELISA and are expressed in ng/ml. Bars represent averages and standard errors.

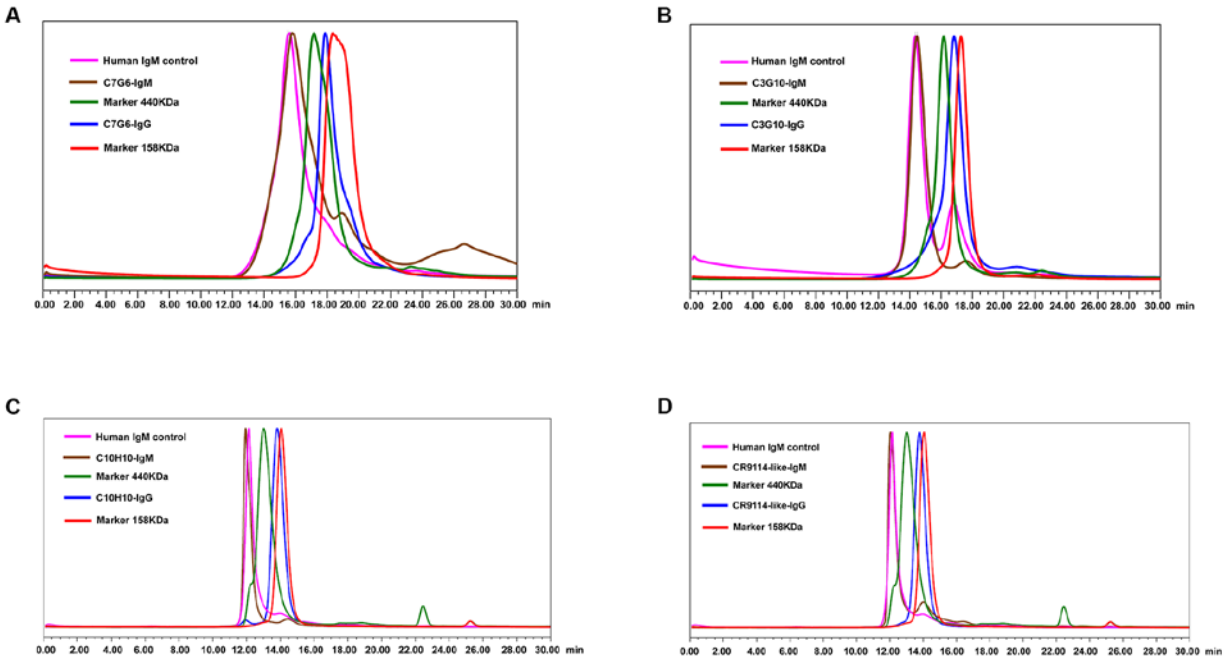

Fig. S2. HPLC analysis of the IgM and IgG subtypes of C7G6 (A), C3G10 (B), C10H10 (C) and CR9114-like (D). Purified human IgM and protein standards (440 kDa and 158 kDa) were used as controls.

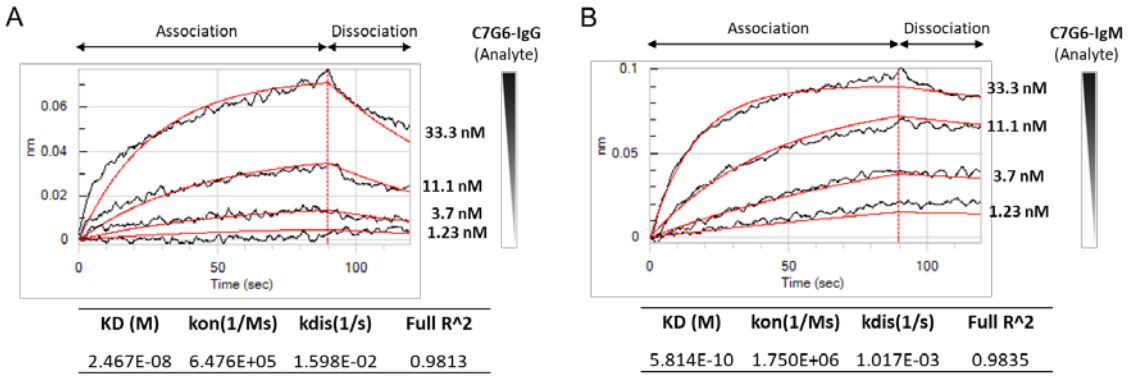

Fig. S3. Binding kinetics of C7G6-IgG and C7G6-IgM to B/Florida/4/2006 HA protein were measured using bio-layer interferometry (BLI). Biotin-B/Florida/4/2006 HA protein was immobilized on Streptavidin (SA) Biosensor tips and incubated with antibodies at concentrations ranging from 1.23 nM to 33.3 nM. (**A**) The affinity of C7G6-IgG to B/Florida/4/2006 HA is 24.67 nM and (**B**) the affinity of C7G6-IgM to B/Florida/4/2006 HA is 0.58 nM.

	Virus strains	MN-IC50 (µg/ml)		
		C7G6-IgM-pen	C7G6-IgM-mono	C7G6-IgG
Ancestral	B/Lee/1940	4.42	>50	>50
	B/Taiwan/2/1962	0.55	8.84	17.68
	B/Singapore/3/1964	0.55	35.36	>50
Yamagata	B/Florida/4/2006	0.28	2.21	1.84
	B/Victoria/507/2007-like	0.73	2.21	2.21
	B/Brisbane/3/2007-like	0.28	1.84	1.84
	B/Massachusetts/02/2012-like	0.28	>50	23.57
	B/Singapore/Gp414/2014-like	0.55	17.68	17.68
	B/California/10/2016-like	0.55	35.36	8.84
Victoria	B/Hong Kong/330/2001	0.28	2.21	2.21
	B/Malaysia/2506/2004	0.55	4.42	8.84
	B/Taiwan/94/2005-like	0.55	35.36	17.68
	B/New York/1093/2006-like	0.46	17.68	17.68
	B/Brisbane/60/2008	0.73	17.68	35.36
	B/Brisbane/33/2008	1.1	35.36	23.57
	B/Hong Kong/537/2009-like	2.21	35.36	35.36
	B/Rhode Island/01/2012-like	1.1	>50	17.68
	B/New York/1352/2012-like	1.84	35.36	29.47
	B/Washington/07/2014-like	1.84	35.36	35.36
	B/California/11/2016-like	1.1	>50	>50
H1N1	A/California/04/2009	>50		>50

Fig. S4. *In vitro* neutralization activities (IC_{50} values) of pentameric and monomeric forms of the C7G6 antibody. Fifty percent inhibitory concentrations (IC_{50}) of C7G6-IgM-pen (pentameric form), C7G6-IgM-mono (monomeric form) and C7G6-IgG against representative strains from the three influenza B lineages and an H1N1 subtype influenza A virus were determined by performing microneutralization assays. The values are representative of three independent experiments and reported in µg/ml; one representative dataset is shown. The values below 50µg/ml are color-filled; red shades=strong reactivity; yellow shades=moderate reactivity; green shades=weak reactivity; no reactivity is indicated by >50.

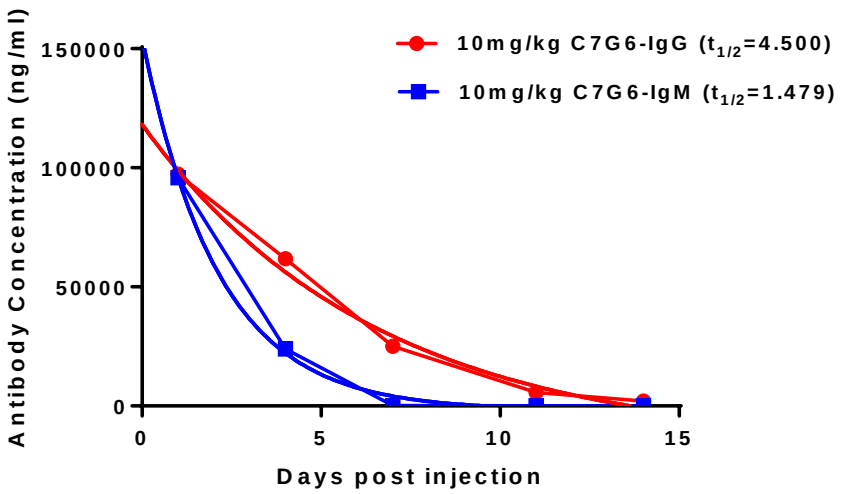

Fig. S5. Serum antibody concentrations after C7G6-IgG or C7G6-IgM treatment. Antibody half-lives were measured in 6- to 8-week-old female BALB/c mice injected intravenously (i.v.) with a single dose of purified antibodies (C7G6-IgG or C7G6-IgM) at a concentration of 10 mg/kg. The mice were bled on days 1, 4, 7, 11, and 14 and serum antibody levels measured by ELISA. The $t_{1/2}$ of the elimination phase was determined with a one-phase exponential decay model using data points between days 1 and 14 post-injection. This experiment was repeated three times; one representative dataset is shown. n = 5/group.

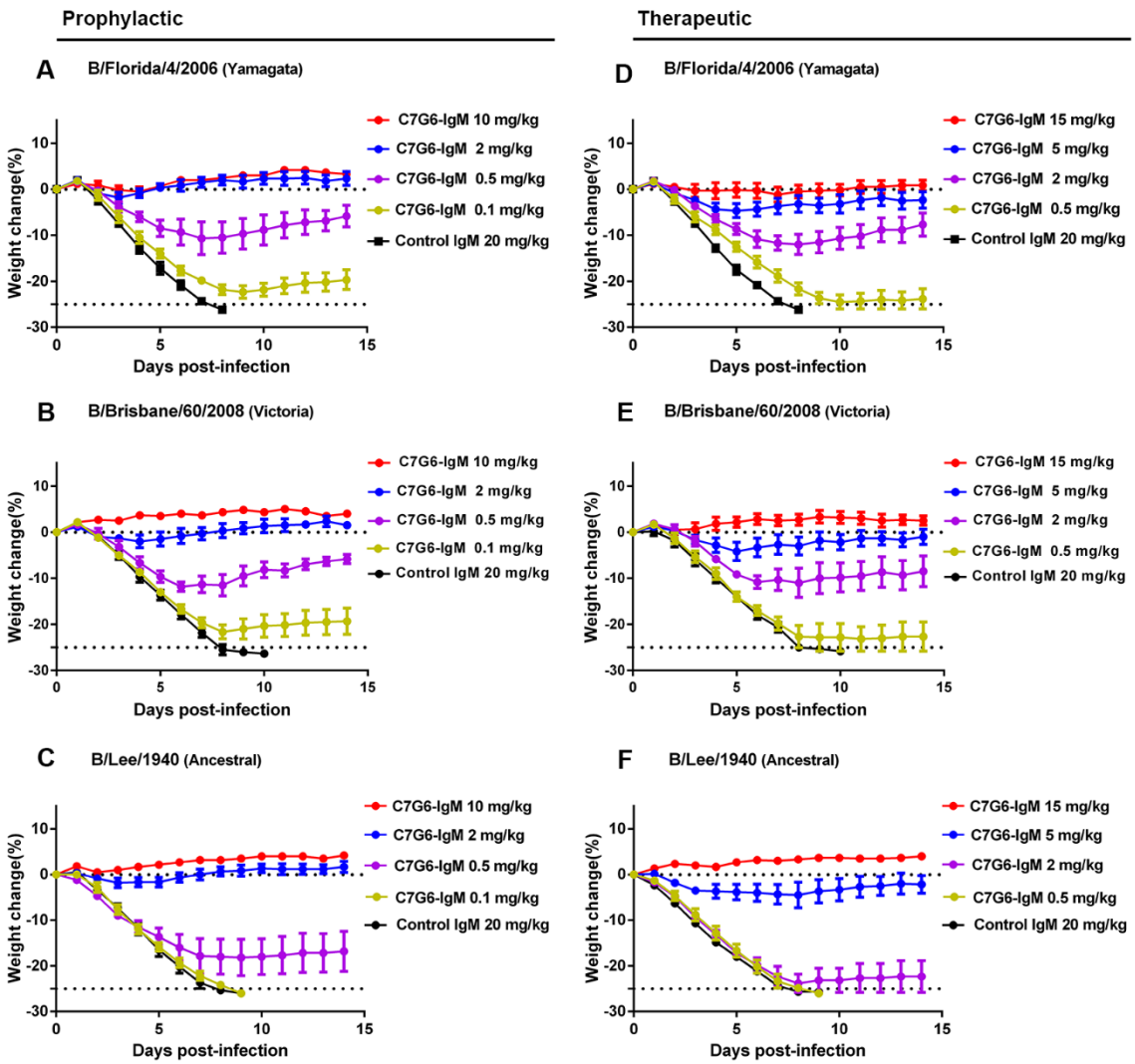

Fig. S6. Body weight change curves of mice treated with C7G6-IgM before or after challenge with influenza B viruses. (**A** to **C**) The prophylactic efficacy of C7G6-IgM against lethal challenge with mouse-adapted B/Florida/4/2006 (Yamagata) (A), B/Brisbane/60/2008 (Victoria) (B) or B/Lee/1940 (Ancestral) (C) viruses. Shown are the weight changes of BALB/c mice (n=6/group) treated with 10, 2, 0.5 or 0.1 mg/kg C7G6-IgM or 20 mg/kg control IgM one day prior to lethal challenge (25 MLD_{50}) via intranasal inoculation (at day 0). (**D** to **F**) For the therapeutic groups, weight change curves are shown for mice that received different doses of C7G6-IgM or 20 mg/kg control IgM one day after lethal challenge with 25 MLD_{50} of mouse-adapted B/Florida/4/2006 (D), B/Brisbane/60/2008 (E) or B/Lee/1940 (F) virus. The mice were infected and subsequently treated with 15, 5, 2 or 0.5 mg/kg C7G6-IgM. This experiment was repeated three times; one representative dataset is shown. The body weight curves represent the mean ± 95% confidence interval of the mean. If a mouse died or was euthanized during the study, the last observed body weight was carried forward until all the mice in the group had died. The control IgM is C5G6-IgM (a chimeric mAb against 2009 pandemic H1N1 influenza A viruses) [18].

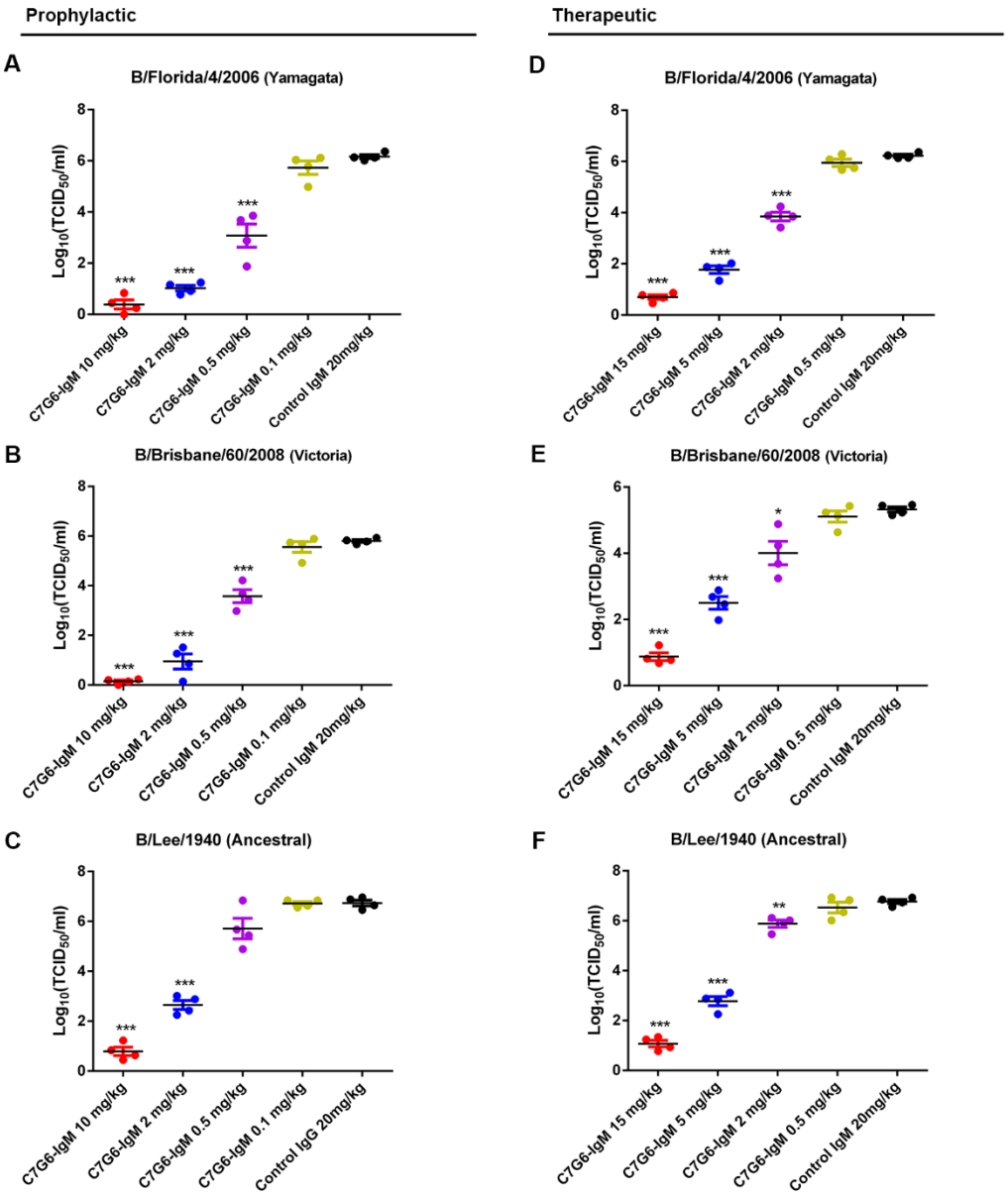

Fig. S7. Analysis of lung virus titers from mice treated with C7G6-IgM. (**A** to **C**) Mice (n=4 per group) were treated with C7G6-IgM at the indicated doses 1 day before lethal challenge with mouse-adapted B/Florida/4/2006 (Yamagata) (A), B/Brisbane/60/2008 (Victoria) (B) or B/Lee/1940 (Ancestral) (C) virus. (**D** to **F**) Mice (n=4 per group) were treated with C7G6-IgM at the indicated doses 1 day after lethal challenge with mouse-adapted B/Florida/4/2006 (Yamagata) (D), B/Brisbane/60/2008 (Victoria) (E) or B/Lee/1940 (Ancestral) (F) virus. Lungs were collected for determination of virus titers 4 days after infection. The values are representative of three independent experiments; one representative dataset is shown. Black bars are mean values and error bars represent SE. The t-test was used to assess the significance of lung viral titers. *P < 0.05, **P < 0.01 and ***P < 0.001, compared to the control IgM-treated group. $TCID_{50}$, median tissue culture infectious dose. The control IgM is C5G6-IgM [18].

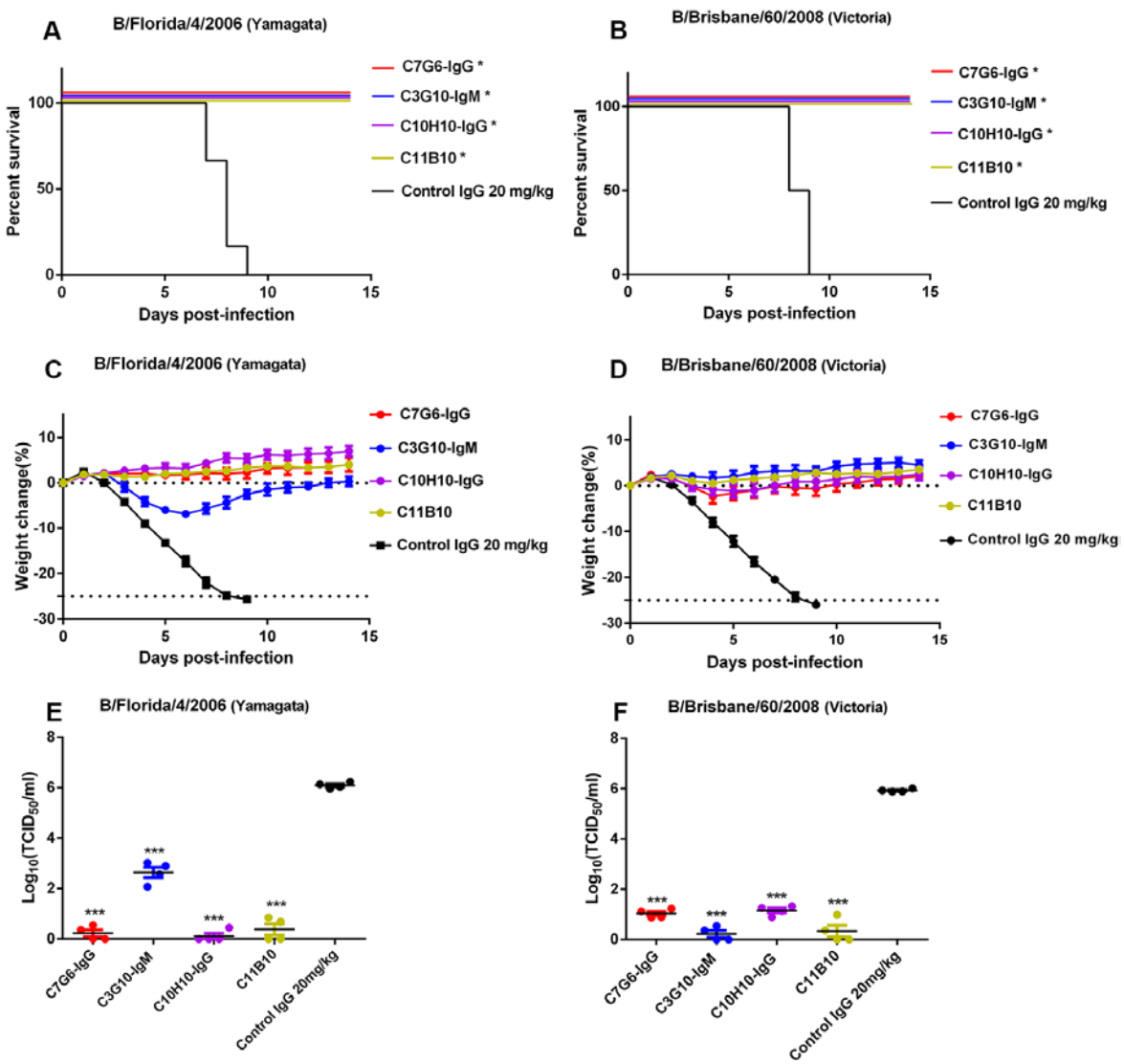

Fig. S8. *In vivo* protective efficacy of C7G6-IgG, C3G10-IgM, C10H10-IgG and C11B10 in mice. (**A** to **F**) Survival curves (A and B), body weight change (C and D), and lung viral titers (E and F) for BALB/c mice (n = 6 per group for A to D and n=4 per group for E and F) treated intraperitoneally with the indicated antibodies (10 mg/kg), 24 hours after lethal challenge with 25 MLD_{50} of MA-B/Florida/4/2006 or MA-B/Brisbane/60/2008. Lungs were collected for determination of virus titers on day 4 after infection. This experiment was repeated three times, and one representative dataset is shown. The black bars indicate mean values. The body weight curves represent mean ± 95% confidence interval of the mean. If a mouse died or was euthanized during the study, the last observed body weight was carried forward until all the mice in the group had died. For (A) and (B), statistical analysis was performed by log-rank test. For (E) and (F), statistical analysis was performed by t-test. *P < 0.05 and ***P < 0.001, compared to the control IgG–treated group. The control IgG is C5G6 [18].

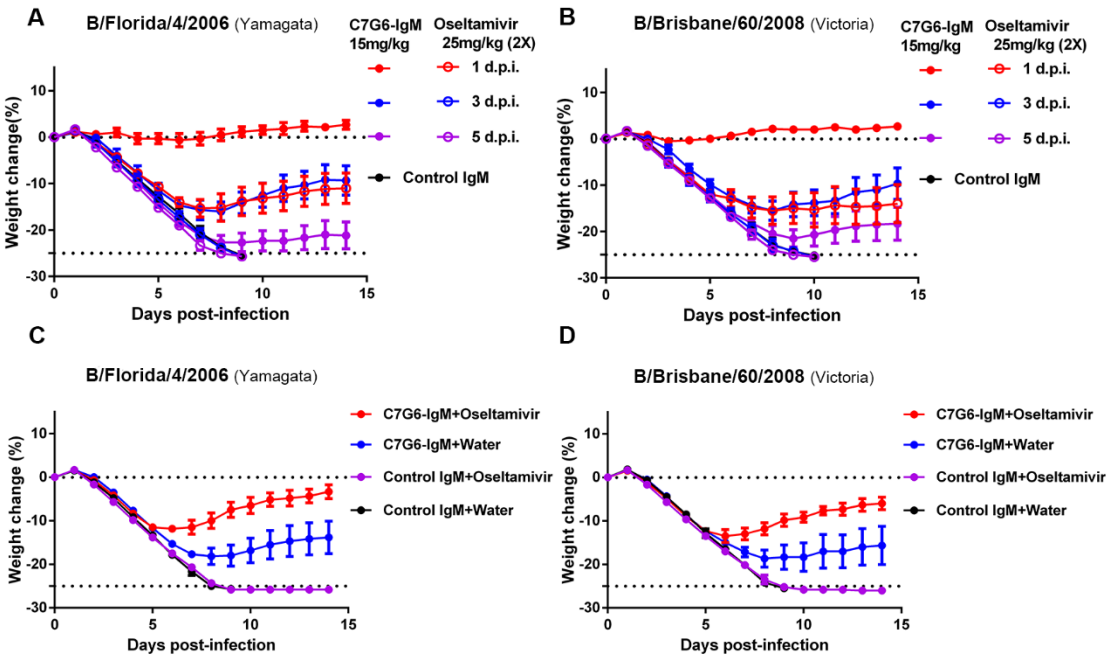

Fig. S9. Comparison and combination of therapeutic effects of C7G6-IgM and oseltamivir against influenza B infection in mice. (**A** and **B**) Weight loss curves of BALB/c mice (n = 6 per group) that received 15 mg/kg C7G6-IgM (closed symbols) or 25 mg/kg oseltamivir (open symbols) or 25mg/kg C5G6-IgM on the indicated day following intranasal infection with 25 MLD_{50} of MA-B/Florida/4/2006 (A) or MA-B/Brisbane/60/2008 (B). (**C** and **D**) Body weight changes in BALB/c mice (n = 6 per group) that received a single treatment of C7G6-IgM or a control IgM (C5G6-IgM) intraperitoneally at 5 mg/kg, oseltamivir orally at 25 mg/kg twice a day for 4 days, or a combined treatment of C7G6-IgM and oseltamivir, starting from 2 days after intranasal infection with 25 MLD_{50} of MA-B/Florida/4/2006 (C) or MA-B/Brisbane/60/2008 (D). The values are representative of three independent experiments; one representative dataset is shown. The body weight curves represent mean±95% confidence interval of the mean. If a mouse died or was euthanized during the study, the last observed body weight was carried forward until all the mice in the group had died. d.p.i.: days post-infection.

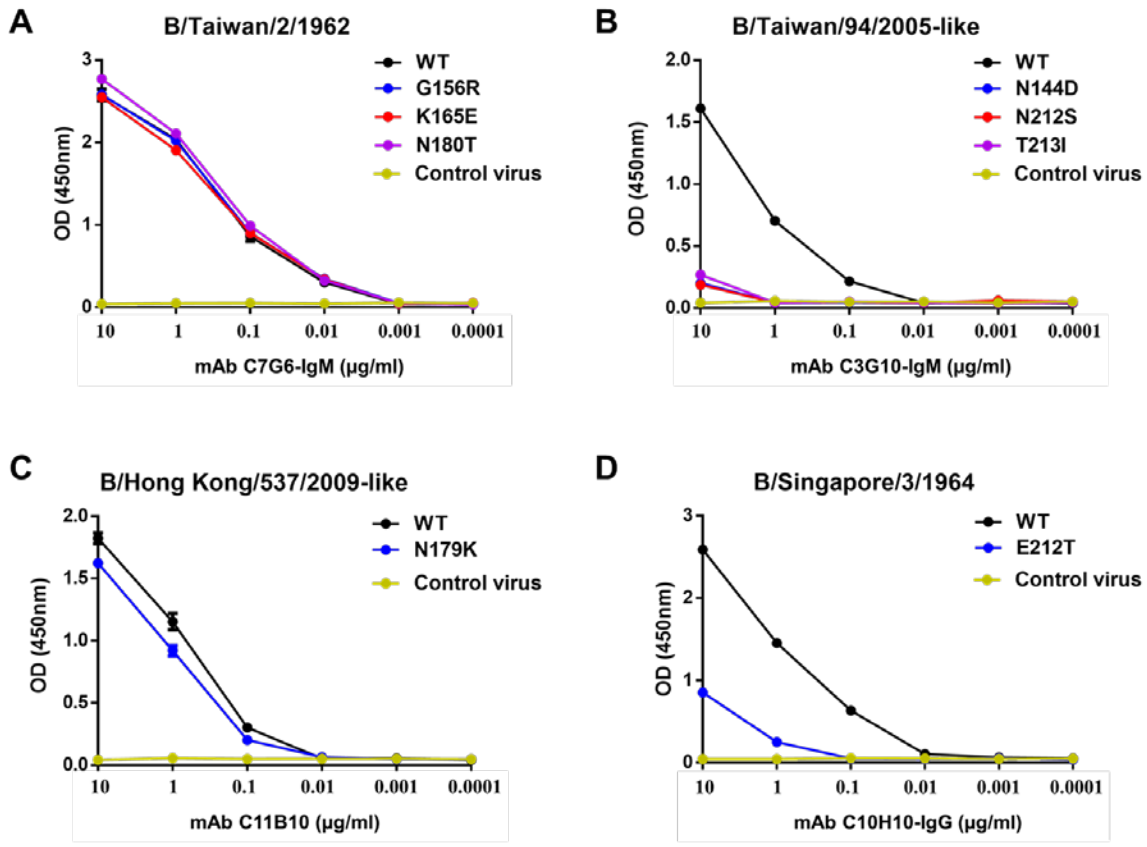

Fig. S10. Reactivity of C7G6-IgM, C3G10-IgM, C11B10 or C10H10-IgG with WT or corresponding escape mutant viruses in ELISA. The indicated concentrations of C7G6-IgM, C3G10-IgM, C11B10 or C10H10-IgG were reacted with purified WT or their respective escape mutants, or the control virus, A/California/04/2009 in ELISA.

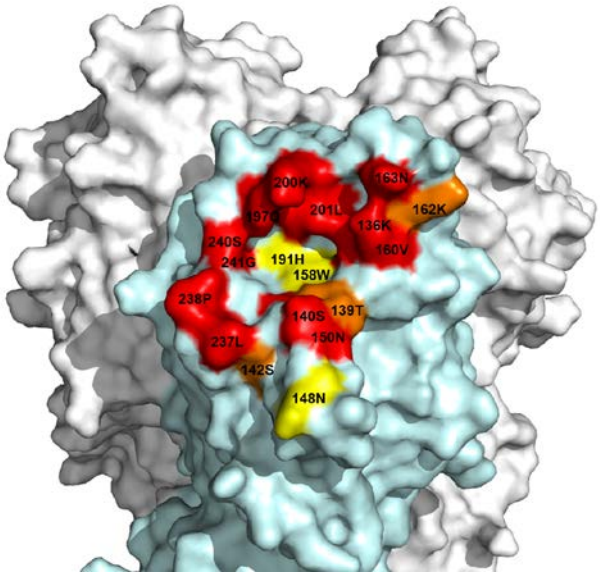

Fig. S11. Comparison of the published CR8033 epitope with the CR8033 epitope predicted using a molecular docking method. Red=common contact residues using the two methods, yellow=contact residues unique to predicted CR8033 epitope; orange=contact residues unique to published CR8033 epitope.

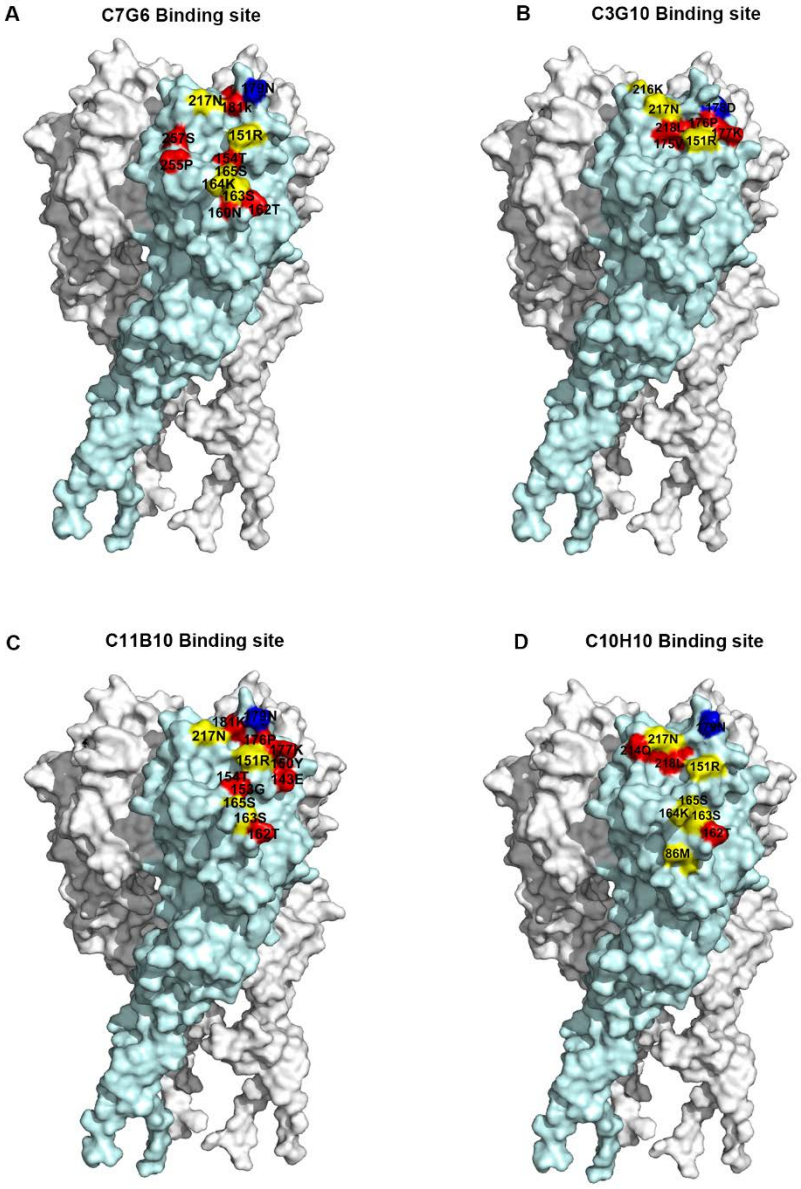

Fig. S12. Conservation analysis of the neutralizing epitopes recognized by C7G6 (A), C3G10 (B), C11B10 (C) and C10H10 (D). Conservation analysis of the epitopes of the indicated antibodies on the HA trimer model of B/Florida/4/2006, using DS Visualizer 1.7. One HA protomer of the HA trimer is colored in cyan, whereas the other two protomers are colored in gray. The residues are colored according to the conservation of contact residues across all available influenza B virus sequences: red, more than 97% conserved; blue, 84 to 97% conserved; yellow, 48 to 84% conserved. Residue numbers are shown in black.

Residue	C7G6-IgM Frequency (%)		Residue	C3G10-IgM Frequency (%)		Residue	C11B10 Frequency (%)		Residue	C10H10-IgG Frequency (%)
K151	50.9		K144	58.2		E143	97.4	97.4	K86	52.4
R151	47.1	100	N144	37.2	97.0	Y150	100	100	M86	47.4
I151	1.8		T144	1.5		K151	50.9		K151	50.9
T151	0.15		K151	50.9		R151	47.1	100	R151	47.1
T154	100	100	R151	47.1	98.2	I151	1.8		I151	1.8
G156	99.4	99.4	T151	0.15		T151	0.15		T162	99.9
N160	100	100	V175	99.8	100	G153	100	100	S163	52.6
T162	99.9	99.9	I175	0.15		T154	100	100	N63	47.4
S163	52.6	100	R176	100	100	T162	99.9	99.9	G164	52.4
N163	47.4		K177	99.9	99.9	S163	52.6	100	K164	44.7
G164	52.4		D178	92.4	95.9	N163	47.4		R164	2.9
K164	44.7	100	E178	3.5		N165	52.3		N165	52.3
R164	2.9		N211	94.5		S165	30.03	99.7	S165	30.0
N165	52.3		D211	1.8	97.6	I165	17.1		I165	17.1
S165	30.0		S211	1.2		K165	0.3		K165	0.3
I165	17.1	99.6	T213	88.6	91.5	R176	100	100	N179	84.8
K165	0.3		A213	2.9		K177	99.85	99.85	K179	14.2
N179	84.8		A216	48.9		N179	84.8	98.9	E212	52.0
K179	14.2	99.7	K216	47.4	99.4	K179	14.2		K212	48.0
D179	0.76		V216	3.05		K181	100	100	Q214	100
N180	81.2	98.6	K217	52.0		K217	52.0		K217	52.0
Y180	17.4		N217	33.7	99.2	N217	33.7	99.9	N217	33.7
K181	100	100	S217	13.6		S217	13.6		S217	13.6
K217	52.0		L218	100	100	T217	0.6		T217	0.6
N217	33.7								L218	100
S217	13.6	100								
T217	0.6									
I217	0.15									

Fig. S13. Frequency of potential C7G6-IgM, C3G10-IgM, C11B10 and C10H10-IgG interacting residues. A multiple sequence alignment of 2000 full-length influenza B HA sequences from the NCBI database was used to assess genetic diversity and to calculate the frequencies of potential C7G6-IgM and C3G10-IgM interacting residues. All of the residues listed were found in the HA proteins of influenza B viruses, all of which can be neutralized by the indicated antibodies.

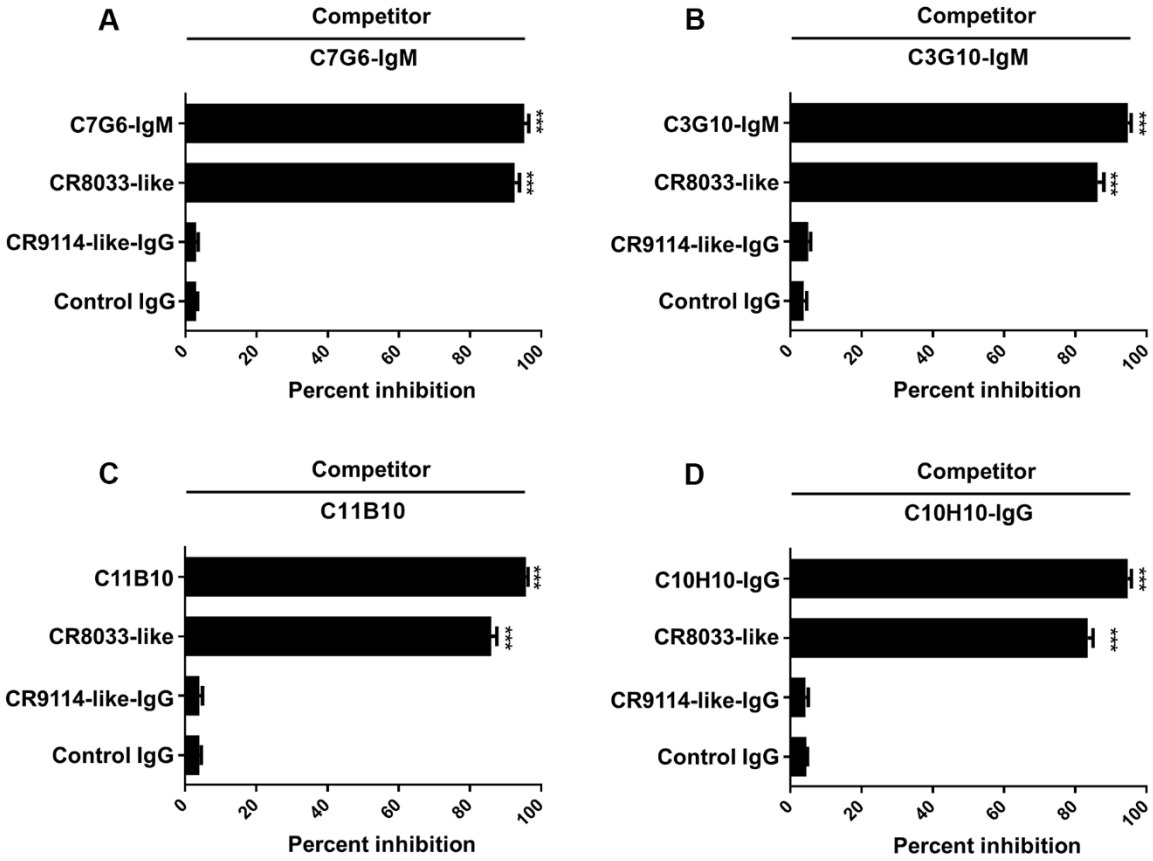

Fig. S14. Determination of epitopes of C7G6-IgM, C3G10-IgM, C11B10 and C10H10-IgG. Determination of epitopes of C7G6-IgM (**A**), C3G10-IgM (**B**), C11B10 (**C**) and C10H10-IgG (**D**) using a competition ELISA test with two representative bnAbs (CR8033-like and CR9114-like-IgG). C7G6-IgM, C3G10-IgM, C11B10 or C10H10-IgG was used as a competitor, and C5G6 was used as a negative control. This experiment was repeated three times; one representative dataset is shown. Bars represent averages and standard errors. ***P < 0.001, compared to the control group.

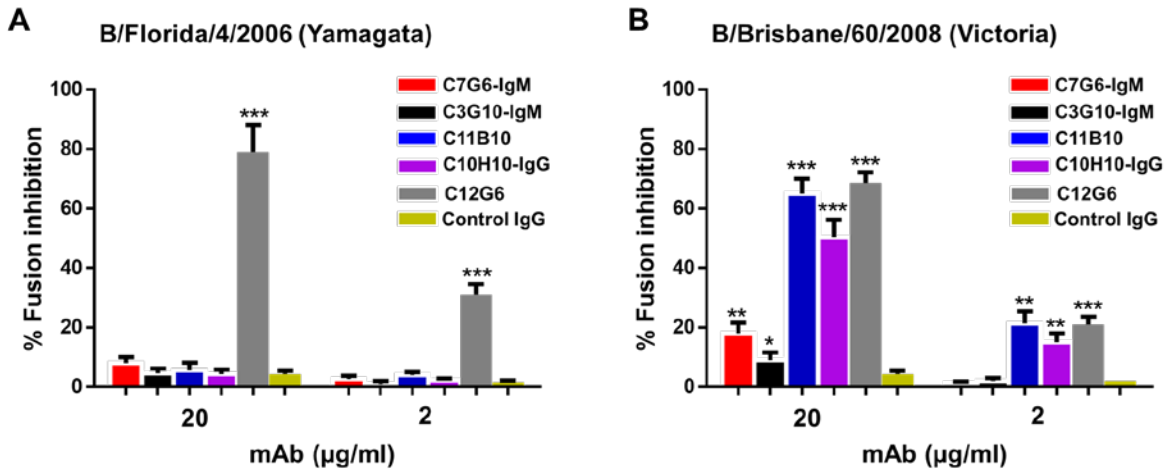

Fig. S15. Red blood cell fusion assay. Fusion inhibition assay using C7G6-IgM, C3G10-IgM, C11B10, C10H10-IgG, C12G6 (positive control antibody) or control IgG antibody (C5G6) incubated with (**A**) B/Florida/4/2006 or (**B**) B/Brisbane/60/2008 virus and human red blood cells and exposed to low pH to induce viral fusion. Percent fusion inhibition was calculated based on the release of NADPH into the supernatant (absorbance at 340 nm). This experiment was repeated three times, and one representative dataset is shown. Bars represent averages and standard errors. *: P<0.05, **: P<0.01, ***: P<0.001, compared to the control IgG group.