Supporting Information

Antibodies that engage the hemagglutinin receptor-binding site of influenza B viruses

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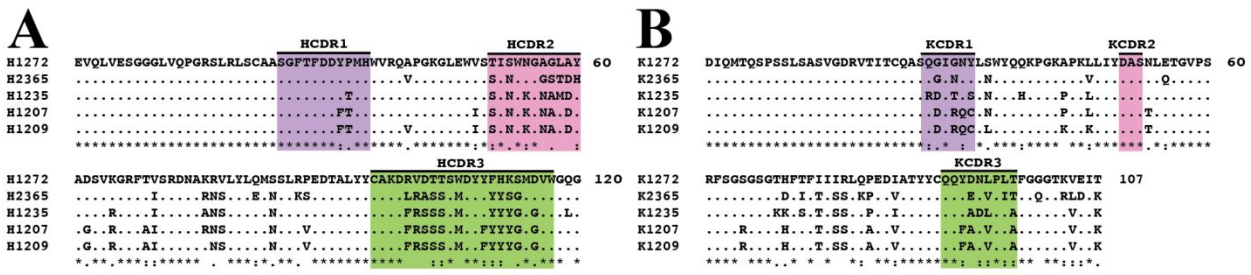

Figure S1. Antibody sequence alignments.

Multiple sequence alignments of antibody variable heavy (A) and kappa light (B) chains. The antibody hypervariable complementarity determining region (CDR) loops are highlighted.

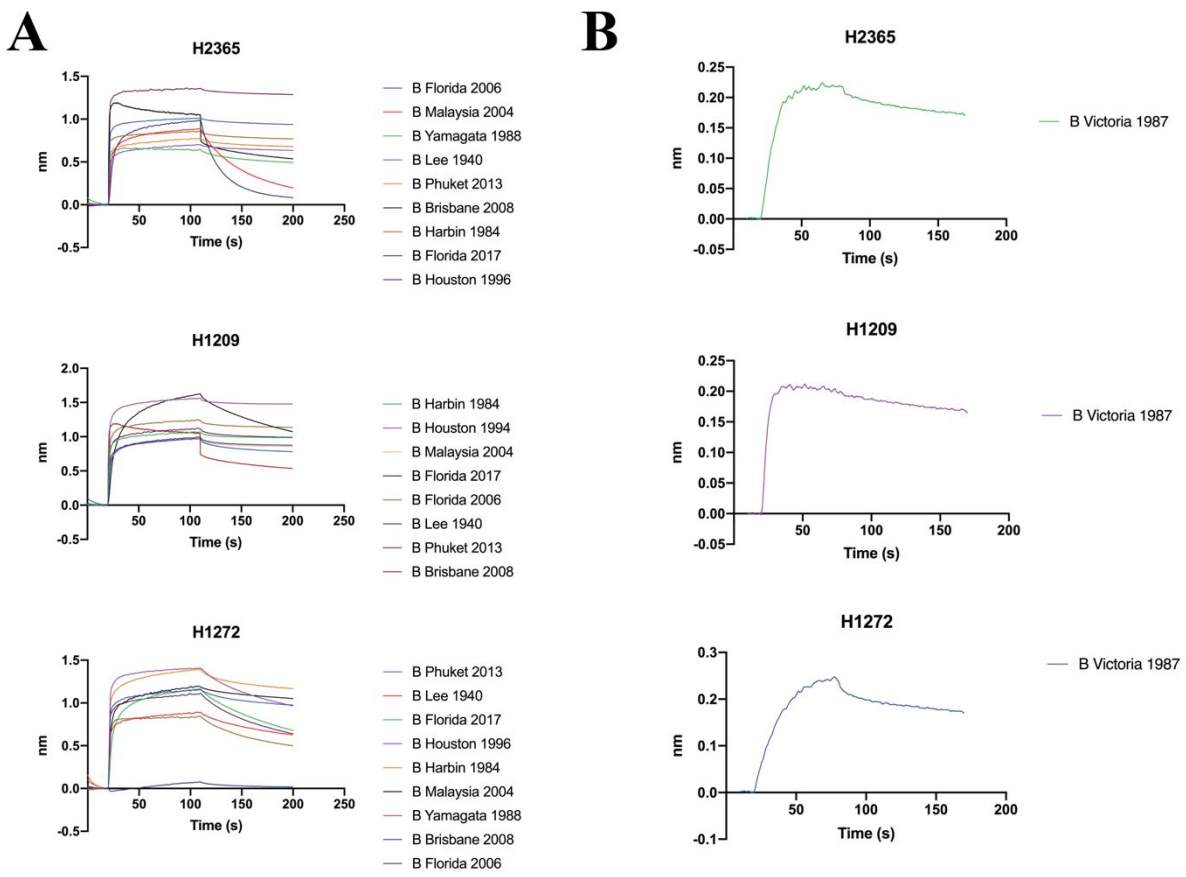

Figure S2. Details of the affinity measurements.

Bio-layer interferometry binding isotherms of Fab:HA interactions. HA was immobilized on a Ni^{2+}-NTA sensor and Fabs were applied in the mobile phase. All measurements were done with Fabs and HA1 head (A) or full-length HA. Dissociation affinity constants (K_Ds) were calculated with a 1:1 binding model using the manufacturer's software.

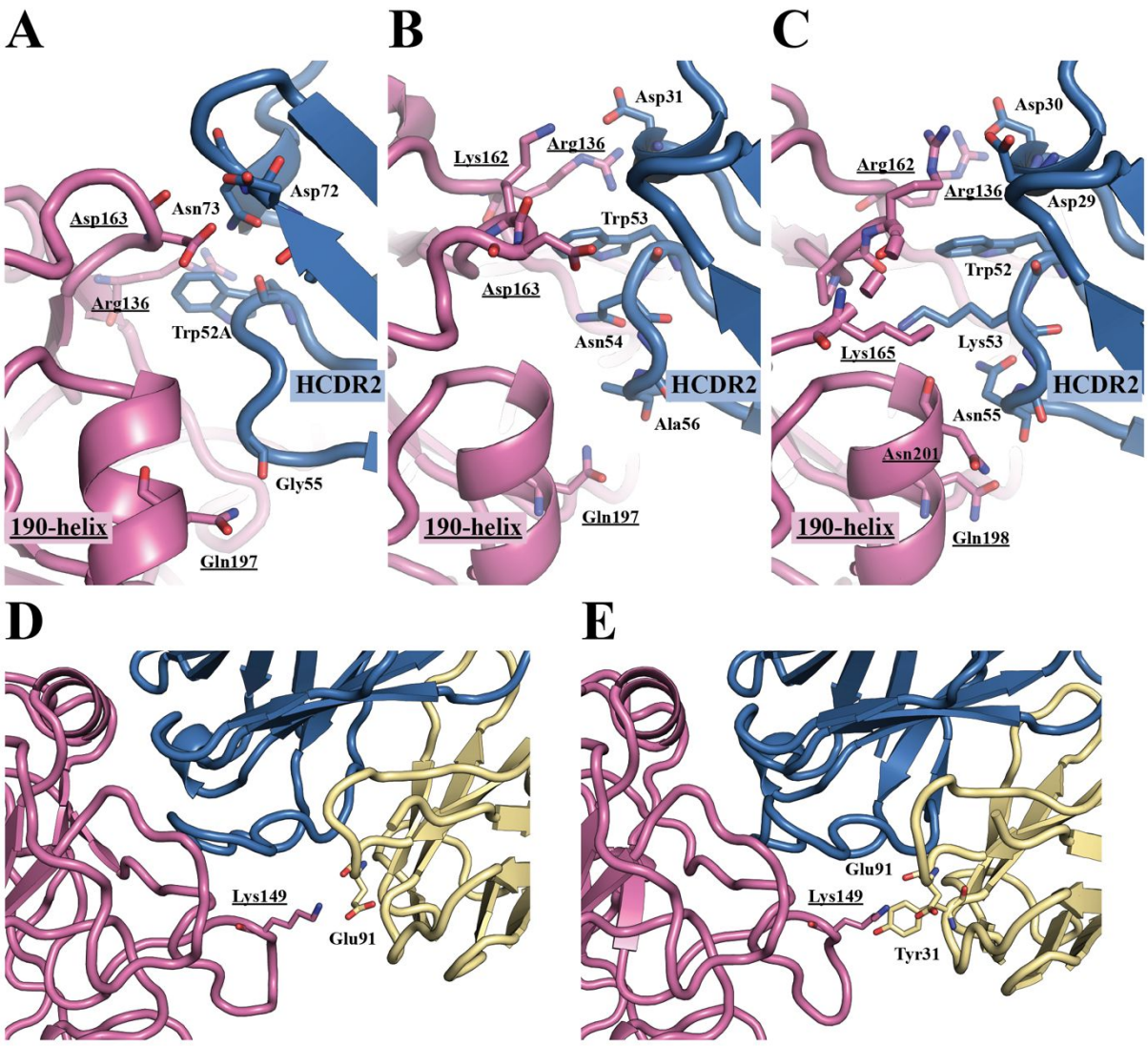

Figure S3. Details of additional molecular interactions at the HA:antibody interface. Additional contacts between the antibody HCDR2 and the HA 190-helix are indicated for the H2365 (A), H1209 (B) and H1272 (C) complexes respectively. Light chain contacts for H2365 and H1272 are shown in (D) and (E), respectively. The electron density map was of lesser quality for the H1209 complex in the 150-loop region of HA and the details of side-chain contacts are omitted. HA residues are underlined.

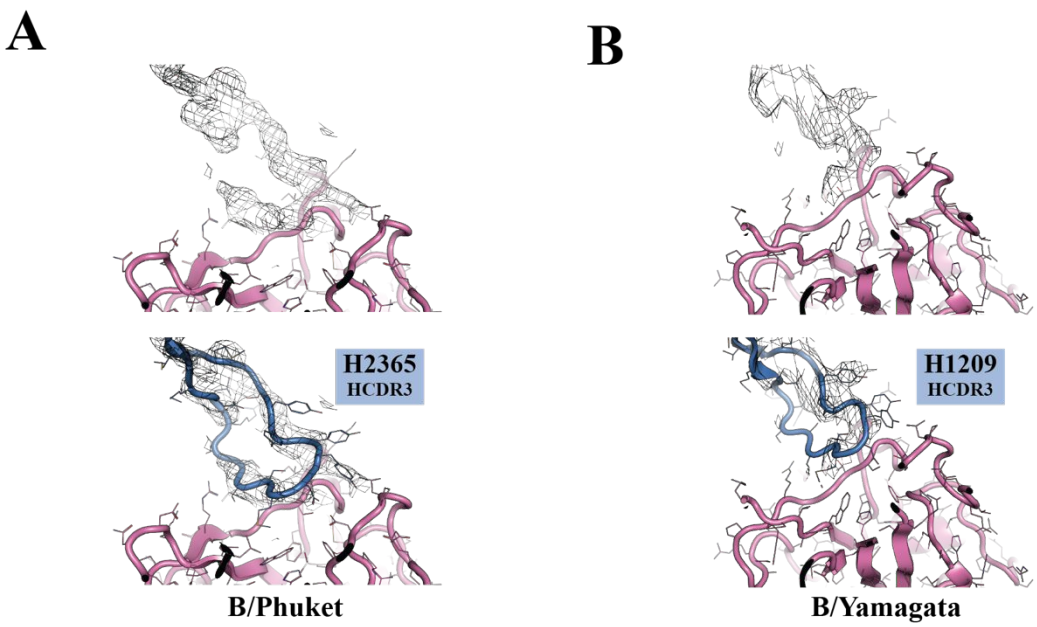

Figure S4. Composite annealed omit maps of the Fab HCDR3.

Composite maps with simulated annealing calculated after refinement of the molecular replacement model and omitting the atoms in the Fab HCDR3 of H2365 (A) and H1209 (B). Maps are contoured at 1 σ.

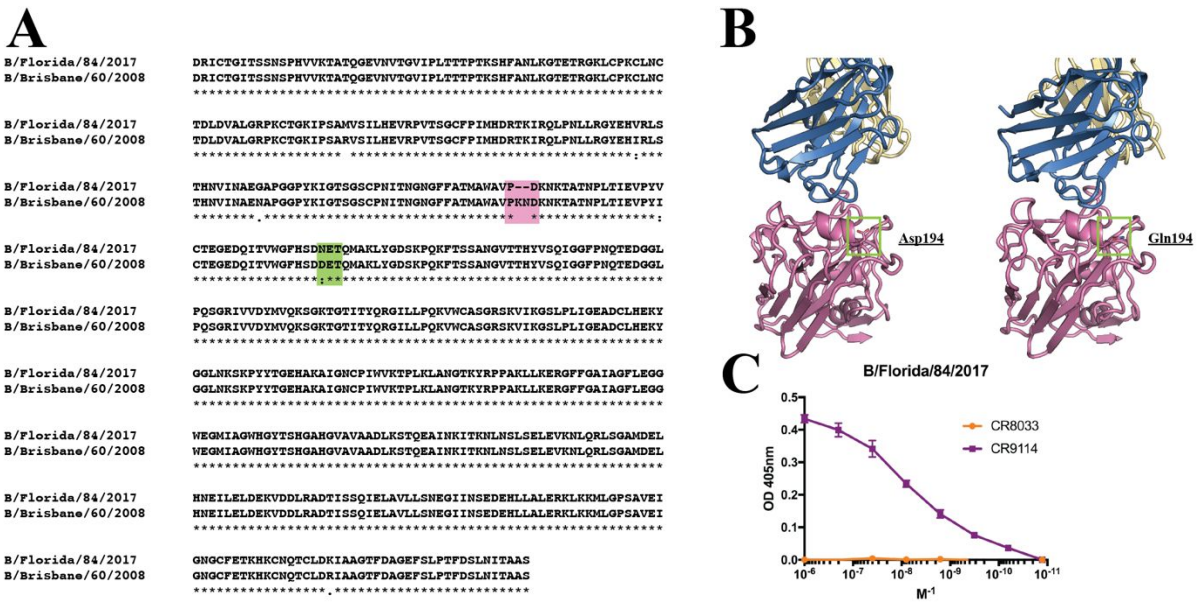

Figure S5. Antigenic evolution of contemporary B Victoria HAs.

Sequence alignment of the contemporary B/Florida/84/2017 and the vaccine strain that the donors received B/Brisbane/60/2008. Noted differences include a 2 amino-acid deletion in the 160-loop (pink) and an N-linked glycosylation site (green). The position of the putative N-linked glycosylation site is shown on the structures of the H2365 and H1272 complexes (B). ELISA reactivity of the contemporary Victoria lineage member B/Florida/84/2017 against two reference Crucell antibodies – an RBS-directed CR8033 and a stem-directed CR9114. Reactivity with CR8033 is lost likely due to the differences highlighted in (A).

Table S1. Data collection and refinement statistics.

	H2365:B/Phuket	H1209:B/Yamagata	H1272:B/Yamagata
Data collection and processing			
Wavelength (Å)	0.97910	0.97918	0.97868
Resolution range (Å)	114.9 - 3.5 (3.62 - 3.5)	49.61 - 4.2 (4.35 - 4.2)	45.6 - 2.6 (2.69 - 2.6)
Space group	P 31	P 61 2 2	P 21 21 21
Unit cell (Å)/°	132.66 132.66 93.13 90 90 120	128.1 128.1 235.28 90 90 120	40.13 77.44 225.65 90 90 90
Total reflections	145358 (14187)	183563 (36009)	179872 (18129)
Unique reflections	23115 (2250)	8923 (1603)	22578 (2229)
Multiplicity	6.3 (6.2)	20.6 (22.5)	8.0 (8.1)
Completeness (%)	99.65 (97.49)	99.7 (100.00)	99.91 (100.00)
Mean I/sigma(I)	5.45 (0.20)	4.64 (0.75)	12.26 (2.78)
Wilson B-factor	180.84	195	31.25
R-merge	0.261 (6.242)	0.8007 (7.489)	0.1782 (0.8381)
R-meas	0.2849 (6.823)	0.8224 (7.66)	0.1905 (0.8961)
CC1/2	0.994 (0.0935)	0.995 (0.294)	0.994 (0.827)
CC*	0.999 (0.413)	0.999 (0.674)	0.998 (0.952)
Refinement			
No. reflections	23046	8861	22570
Rwork/Rfree	0.25/0.30	0.38/0.42	0.23/0.28
RMS (bonds/angles)	0.004/0.71	0.003/0.71	0.005/1.11
Ramachandran favoured/allowed/outliers (%)	91.41/8.13/0.46	94.53/5.31/0.16	96.61/3.24/0.15
Clashscore	14.29	14.74	10.92
Average B-factor	180	193	37

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