

	H3 HK-68	gHA ^{cRBS}	gHA ^{RBS}	H1 SI-06
HC19	+++	~300-fold*	~4-fold	-
HC45	+++	~300-fold	>1,000-fold	-
FI6	+++	~200-fold	~150-fold	+++
CH67	-	-	-	+++

Figure S1. Loss of reactivity to diagnostic antibodies by glycan addition. Related to Figure 1. Antibodies, HC19 (RBS-directed), HC45 (vestigial esterase domain-directed) and FI6 (stem-directed) were assayed for binding to wildtype H3 HK-68, gHA^{cRBS}, gHA^{RBS} using a Luminex-based assay. +++ indicates strong binding and loss of antibody binding is reported as a fold change in MFI in reference to H3 HK-68. (-) indicates no binding. H1 SI-06 and CH67 (RBS-directed antibody) was used as additional controls.

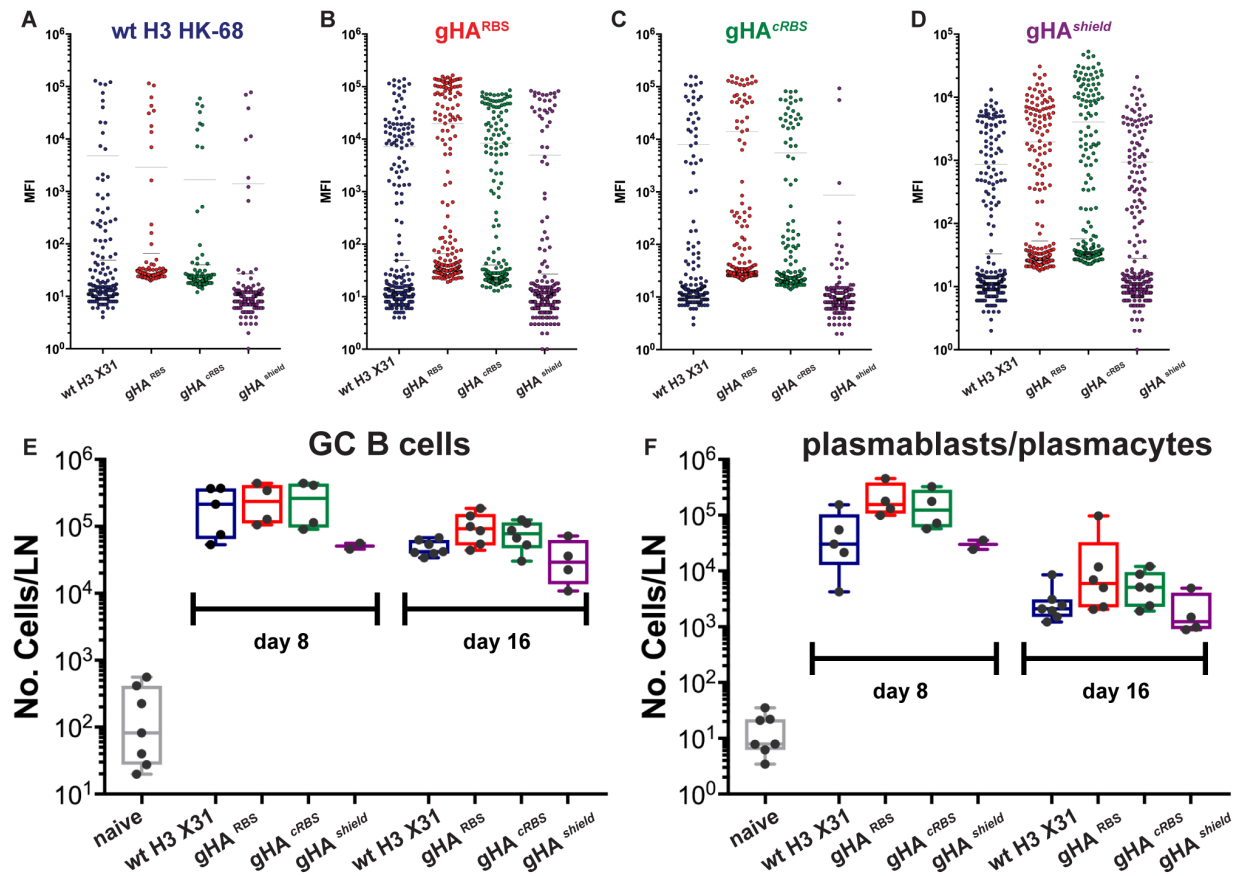


Figure S2. Germinal center and plasma cell responses are unaffected by HA glycosylation. Related to Figure 2. (A-D) Single GC B cells were sorted from the popliteal lymph nodes of immunized B6 mice and cultured with NB-21.2D9 feeder cells. Individual single-cell culture supernatants from mice immunized with (A) wt H3 HK-68 (blue), (B) gHA^{RBS} (red), (C) gHA^{cRBS} (green), and (D) gHA^{shield} (purple) were harvested and screened for binding in a Luminex-based assay. (E-F) Popliteal lymph nodes from mice immunized with wt H3 HK-68 (blue), gHA^{RBS} (red), gHA^{cRBS} (green), and gHA^{shield} (purple) were harvested 8 or 16 days post-immunization and the number of (E) GC B cells (GL-7⁺B220^{hi}CD38^{lo}IgD⁻CD93⁻CD138⁻) and (F) plasmablasts/-cytes (B220^{lo}CD138^{hi}) quantified. Naïve mice (n=7) and H3 H3X31 (n=5, 7), gHA^{RBS} (n=4, 6), gHA^{cRBS} (n=4, 6) and gHA^{shield} (n=2, 4); n = number of mice used for day 8, 16 analyses, respectively. Each circle in E-F represents one lymph node (LN) per mouse.

A

mAb	V _H gene	CDR H3 Sequence	wt HK-68	gHA ^{cRBS}	gHA ^{shield}
FL-1146	IGHV1-9	CARSFQATSFAMDYW			
FL-1151	IGHV1-9	CARSFQATSFAMDYW			
FL-1134	IGHV1-9	CARSFQATSYAMDYW			
FL-1136	IGHV1-9	CARSFQATSFAMDYW			
FL-1144	IGHV1-9	CSRSFQATSFAMDYW			
FL-1137	IGHV1-9	CTRSFQATSFAMDYW			
FL-1141	IGHV1-9	CARSFQATSFAMDYW			
FL-1152	IGHV1-9	CSRSFQATSFAMDYW			
FL-1132	IGHV1-9	CARSFQATSFAMDYW			
FL-982	IGHV1-9	CARSHYGSSFAMDCW			
FL-1140	IGHV1-9	CARSFQATSFAMDYW			
FL-1138	IGHV1-9	CARSFQATSFAMDYW			
FL-1156	IGHV1-9	CARSFQATSFAMDYW			
FL-1153	IGHV1-9	CARSFQATSFAMDYW			
FL-993	IGHV1-9	CARSHYGCNFMADYW			
FL-1012	IGHV1-9	CARSHYGSSFAMDFW			
FL-1157	IGHV1-9	CARSFQATSFAMDYW			
FL-985	IGHV1-9	CARSHYDSSFAMDYW			
FL-1150	IGHV1-9	CARSFQATSFAMDYW			
FL-1149	IGHV1-9	CSRSFQATSFAMDYW			
FL-1025	IGHV1-9	CARSHYGSTTFMDYW			
FL-995	IGHV1-9	CARSHYGSSFAMDYW			
FL-1000	IGHV1-9	CARSHYGSTFAMDYW			
FL-977	IGHV1-9	CARSHYGSCFAMDYW			
FL-1143	IGHV1-9	CARSGIPYFAMDYW			
FL-1147	IGHV1-9	CARSFQATSFAMDYW			
FL-1026	IGHV1-9	CARSHYGSTFAMDYW			
FL-1023	IGHV1-9	CARSHYGSSFAMDNDW			
FL-979	IGHV1-9	CARSHYGSTFAMDYW			
FL-1007	IGHV1-9	CARSHYGSTFAMDYW			
FL-989	IGHV1-9	CARSHYGSSFAMDYW			
FL-1158	IGHV1-9	CARSPQSTSFAMDYW			
FL-1010	IGHV1-9	CARSHYGSTFAMDYW			
FL-984	IGHV1-9	CATSHYGSSFAMDCW			
FL-1155	IGHV1-9	CARSFQATSFALDYW			
FL-1016	IGHV1-9	CARSHYGCSFAMDYW			
FL-1002	IGHV1-9	CALWLHDLFDYW			
FL-1021	IGHV1-9	CARSHYGSSFAMDYW			

B

mAb	V _H gene	CDR H3 Sequence	wt HK-68	gHA ^{RBS}	gHA ^{shield}
FL-1050	IGHV5-9-1	CTRDSDYYGSRVWFAYW			
FL-1041	IGHV5-9-1	CTRVGDYDAWFAYW			
FL-1056	IGHV5-9-1	CTRVGEYDAWFAYW			
FL-1066	IGHV5-9-1	CTRDSDYYGSRVWFAYW			
FL-1081	IGHV5-9-1	CTRVGDYDAWFAYW			
FL-1030	IGHV1-69	CARNYGSSYGYFDVW			
FL-1086	IGHV1-69	CARNYGSSYGYFDVW			
FL-1042	IGHV1-69	CARNYGSTYGYFDVW			
FL-1087	IGHV1-69	CARNYGSSYGYFDVW			
FL-1102	IGHV1-69	CARNYGSTYGYFDVW			
FL-1035	IGHV1-69	CARNYGSSYGYFDVW			
FL-1065	IGHV1-69	CTRNYGSSYGYFDVW			
FL-1098	IGHV1-69	CARNYGSTYGYFDVW			
FL-1058	IGHV1-69	CARNYGSSYGYFDVW			
FL-1060	IGHV1-69	CARNYGSTYGYFDVW			
FL-1039	IGHV1-69	CARNYGSTYGYFDVW			
FL-1034	IGHV1-69	CARNYGSSYGYFDVW			
FL-1029	IGHV1-69	CARNYGSSYGYFDVW			
FL-1036	IGHV1-69	CARNYGSSYGYFDVW			
FL-1040	IGHV1-69	CVRNYGSTYGYFDVW			
FL-1071	IGHV1-69	CARNYGSSYGYFDVW			
FL-1093	IGHV1-69	CARNYGSSYGYFDVW			
FL-1037	IGHV1-69	CARNYGSSYGYFDVW			
FL-1073	IGHV1-69	CARNYGSSYGYFDVW			
FL-1072	IGHV1-69	CARNYGSSYGYFDVW			
FL-1103	IGHV1-69	CARNFGSAYGYFDVW			
FL-1052	IGHV1-69	CARNYGSSYGYFDVW			
FL-1092	IGHV1-69	CARNYGTSYGYFDVW			
FL-1099	IGHV1-69	CARNYGSTYGYFDVW			
FL-1076	IGHV1-69	CARNYGSTYGYFDVW			

MFI
> 100,001
10,001 - 100,000
1,001 - 10,000
101-1,000
< Threshold

Figure S3. Single-cell cultures reveal differences in binding of B cells elicited by gHAs. Related to Figure 3. Antibodies from (A) gHA^{cRBS} and (B) gHA^{RBS} immunizations were tested for reactivity to wildtype HA, gHA^{cRBS} or gHA^{RBS} and gHA^{shield} using a Luminex-based assay. A heat map for reactivity is shown based on MFI. The CDR H3 sequences are listed.

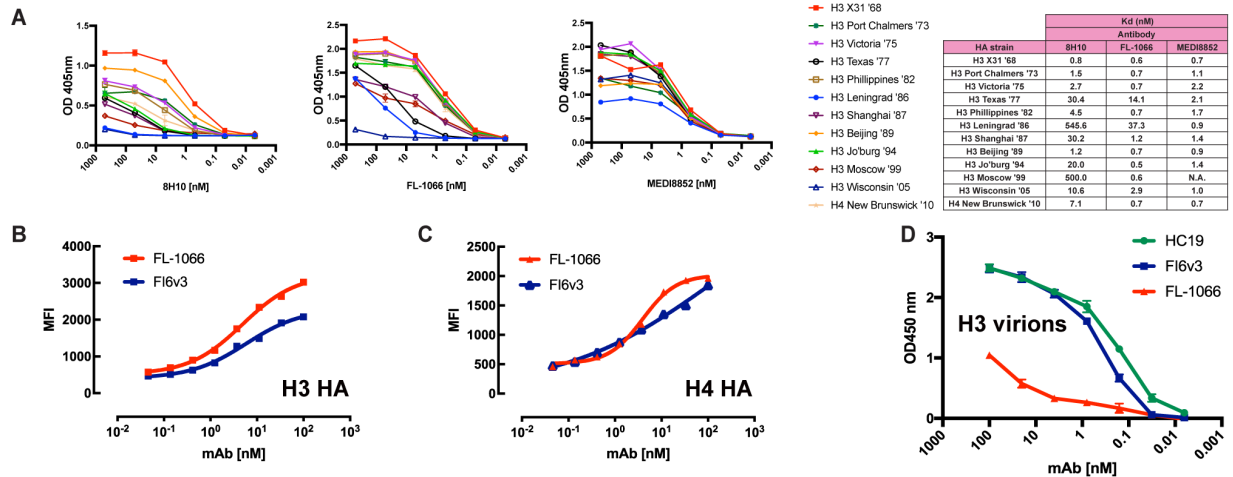


Figure S4. Reactivity of V_H -restricted antibodies and breadth of a V_H 5-9-1 antibody. Related to Figure 4 and STAR Methods. (A) Reactivity of 8H10 (left), FL-1066 (middle) and MEDI8852 (right) to a panel of historical H3 influenza HAs as measured by ELISA. Estimated affinity dissociation constants (K_d) for each HA are reported. (B-C) Binding of FL-1066 and FI6v3 mAbs to cell surface-expressed (B) H3 Aichi 1968 (X31) and (C) wildtype H4 New Brunswick 2010. (D) Binding of FL-1066, HC19 and FI6v3 mAbs to intact H3N2 Aichi 1968 (X31) virions as measured by ELISA. (Error bars represent the standard deviation of the mean and calculated in GraphPad Prism.)

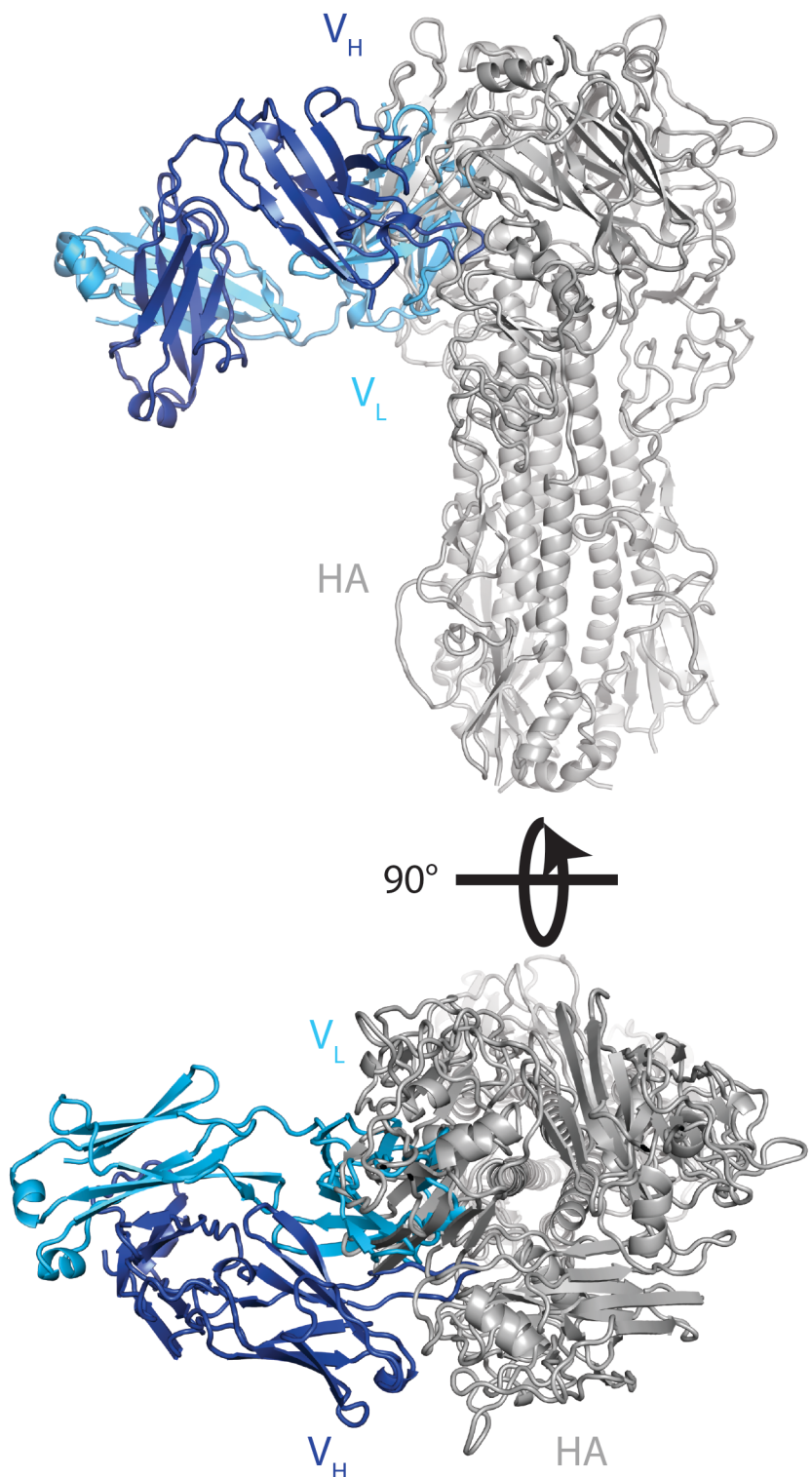


Figure S5. Structural superposition of a V_n 5-9-1 Fab complex illustrates the occluded nature of the HA epitope. Related to Figure 5. Crystal structure of the 8H10 Fab complex (this study) was superposed to the H3 HK-68 HA trimer (PDB ID 1HA0).

Crystal	8H10-complex	FL-1056-complex	FL-1066-complex
Wavelength (Å)	0.978	0.978	0.978
Resolution range (Å)	46.12 - 3.5 (3.625 - 3.5)	44.37 - 3.0 (3.107 - 3.0)	44.33 - 3.0 (3.108 - 3.0)
Space group	P 3 ₁ 2 1	P 3 ₁	P 3 ₁
Unit cell (Å) (°)	124.00 124.00 90.09 90 90 120	153.19 153.19 94.16 90 90 120	153.56 153.56 91.81 90 90 120
Multiplicity	5.2 (4.9)	6.4 (6.5)	6.4 (6.5)
Completeness (%)	98.47 (99.12)	99.97 (99.98)	99.80 (99.61)
Mean I/sigma(I)	6.1 (1.32)	9.29 (1.45)	7.37 (1.02)
Wilson B-factor	114.6	51.9	55.7
R _{int}	0.174 (1.2)	0.284 (1.68)	0.349 (2.50)
Reflections used in refinement	10239 (1018)	49440 (4948)	48387 (4876)
Reflections used for R _{int}	540	2396	2303
R _{int} /R _{int}	0.23/0.28	0.204/0.24	0.25/0.28
Number of atoms	5043	16356	16381
Protein residues	654	2148	2143
RMS bonds(Å)/angles(°)	0.01/1.19	0.006/1.00	0.01/1.37
Ramachandran favored/allowed/outliers (%)	93.4/6/0.6	92/8/0	92.5/7/0.5
Clashscore	19.46	13.41	28.5
Average B-factor	134.99	55.30	55.3
macromolecules	134.95	55.30	52.2
ligands	151.37		65.6

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

Table S1. Crystallographic data and refinement statistics. Related to Figure 5.