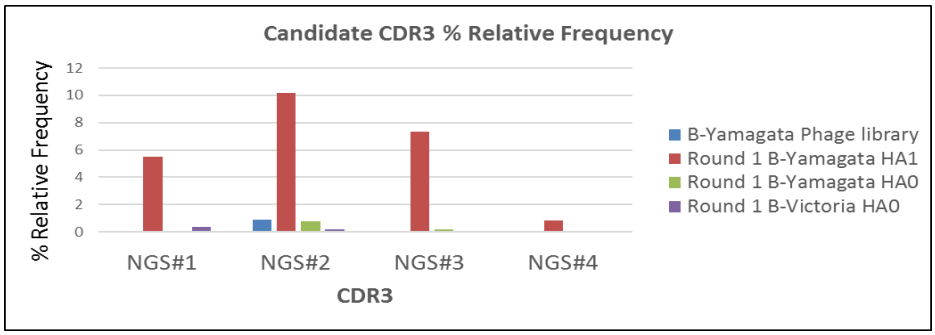
A

	CDR3 copy number			
Candidate CDR3's	B-Yamagata Phage library	Round 1 B-Yamagata HA1	Round 1 B-Yamagata HA0	Round 1 B-Victoria HA0
NGS#1 AAASLCSFSSNDYFY	15	1666	0	135
NGS#2 ATGCPRNGSYYHTGY	243	3078	276	70
NGS#3 ADDCSGPVWGS	10	2213	68	19
NGS#4 TTDWSTYCDLGPRKYNK	1	244	6	3
Total CDR3s	**26504**	**30270**	**35037**	**40633**

B

	CDR3 % relative frequency			
Candidate CDR3's	B-Yamagata Phage library	Round 1 B-Yamagata HA1	Round 1 B-Yamagata HA0	Round 1 B-Victoria HA0
NGS#1 AAASLCSFSSNDYFY	0.057	5.504	0.000	0.332
NGS#2 ATGCPRNGSYYHTGY	0.917	10.168	0.788	0.172
NGS#3 ADDCSGPVWGS	0.038	7.311	0.194	0.047
NGS#4 TTDWSTYCDLGPRKYNK	0.004	0.806	0.017	0.007

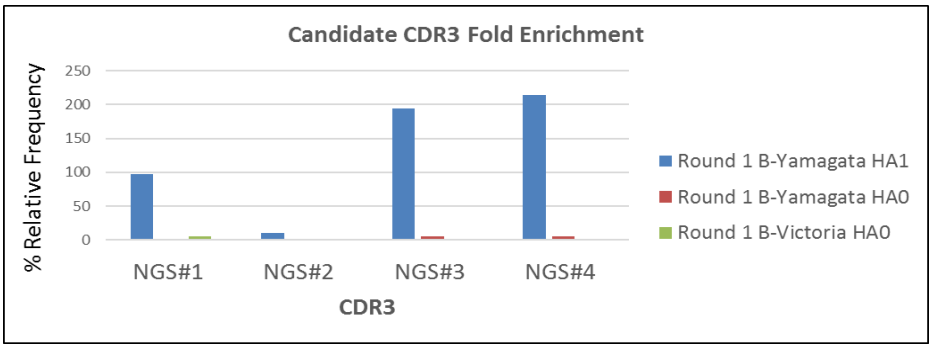
C



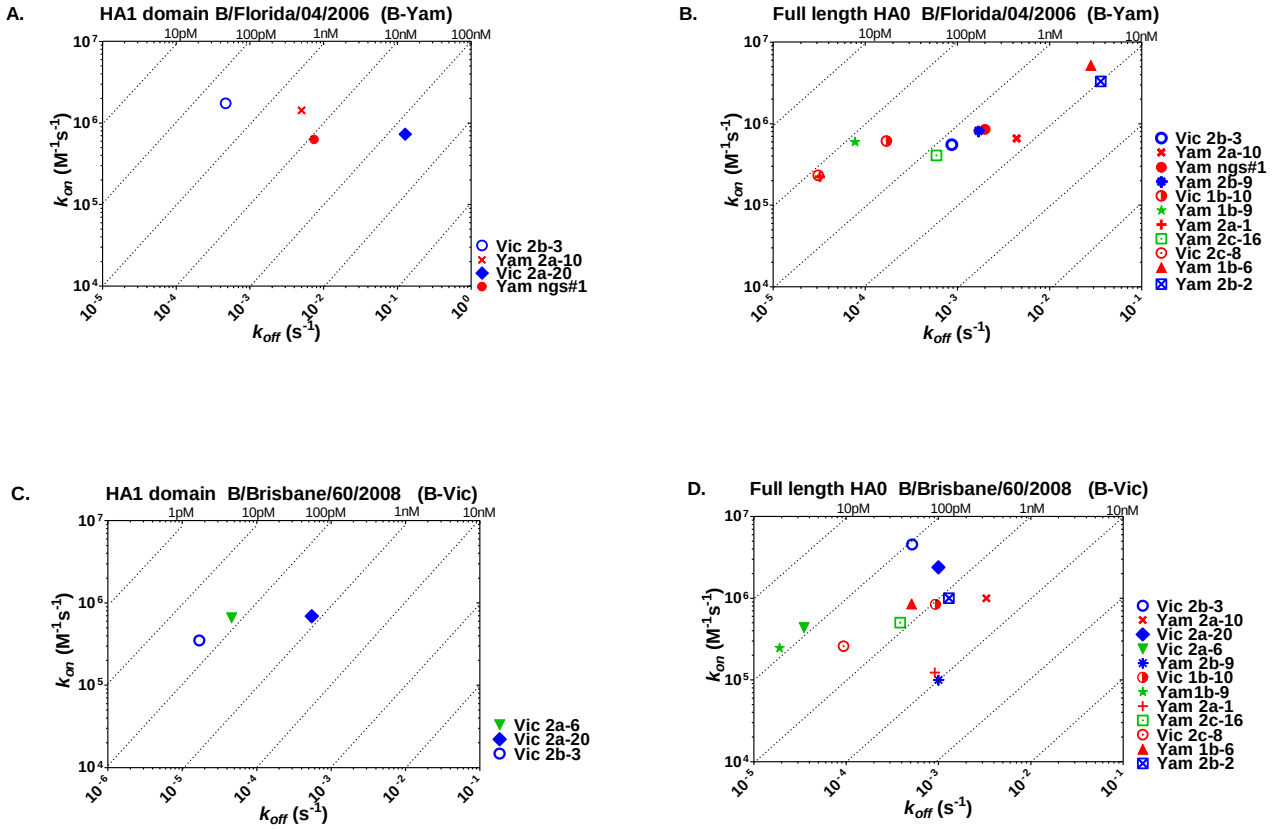
D

	CDR3 fold enrichment			
Candidate CDR3's	B-Yamagata Phage library	Round 1 B-Yamagata HA1	Round 1 B-Yamagata HA0	Round 1 B-Victoria HA0
NGS#1 AAASLCSFSSNDYFY	NA	97.248	0.000	5.870
NGS#2 ATGCPRNGSYYHTGY	NA	11.091	0.859	0.188
NGS#3 ADDCSGPVWGS	NA	193.767	5.144	1.239
NGS#4 TTDWSTYCDLGPRKYNK	NA	213.643	5.000	1.957

E



Supplementary Figure S1. Isolation of B-Yamagata lineage specific nanobodies using NGS. High copy number CDR3's using antibody mining toolbox (A) were normalised for each sequencing run and presented as % Relative Frequency (B, C). Fold increase in CDR3 frequencies were then calculated from a CDR3 frequency in the unselected phage library and the same CDR3's frequency after selection on the HA1 domain or on full length HA0 (D, E).



Supplementary Figure S2. Grouping as head or stem specific binding using SPR. The kinetic binding constants (k_{on} *and* k_{off}) of the panel of nanobodies were determined using SPR and single cycle kinetics [22]. Data are presented as rate plots with iso-affinity diagonals (RAPID) where the diagonals (dotted lines) are connecting the points of equal dissociation constant (K_D). Affinity on (A) B-Yamagata head domain (HA1) of B/Florida/04/2006, (B) full length HA0 of B/Florida/04/2006, (C) B-Victoria head domain (HA1) of B/Brisbane/60/2008 and (D) full length HA0 of B/Brisbane/60/2008 is shown. Fitting was with single cycle kinetics and a 1:1 Langmuir fitting model using BIAevaluation™ software. Equilibrium dissociation constants are given in Table 3.