Cell Host & Microbe

Supplemental Figures and Tables

Gilchuk IM, Bangaru S, Gilchuk P, RP, Kose N, Bombardi RG, Thornburg NJ, Creech B, Edwards KM, Li S, Turner HL, Yu W, Zhu X, Wilson IA, Ward AB, Crowe JE Jr.

Influenza H7N9 virus neuraminidase-specific human monoclonal antibodies inhibit viral egress and protect from lethal influenza infection in mice

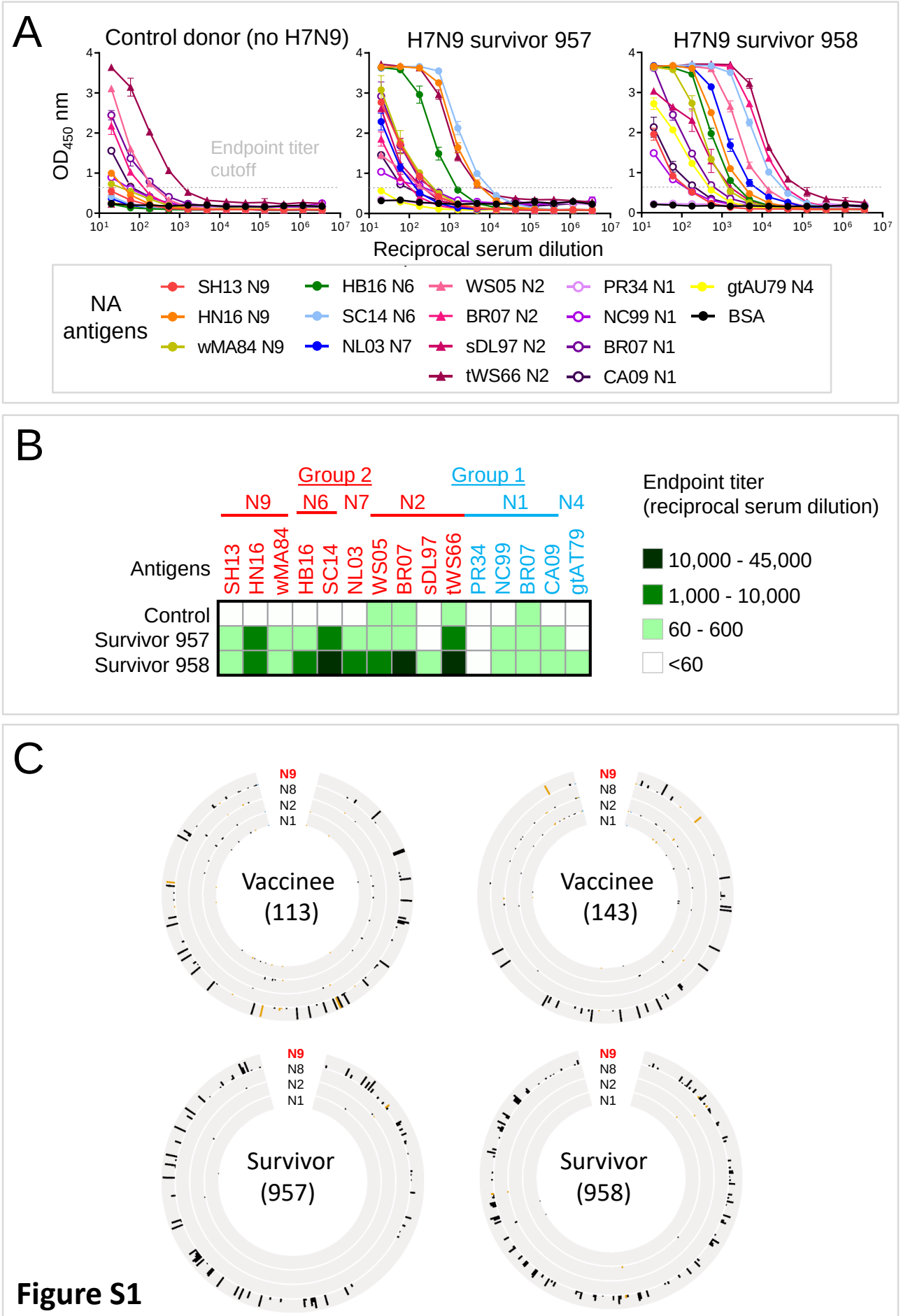

A
Control donor (no H7N9)
H7N9 survivor 957
H7N9 survivor 958
OD450 nm
Endpoint titer cutoff
Reciprocal serum dilution
NA antigens
SH13 N9
HN16 N9
wMA84 N9
HB16 N6
SC14 N6
NL03 N7
WS05 N2
BR07 N2
sDL97 N2
tWS66 N2
PR34 N1
NC99 N1
BR07 N1
CA09 N1
gtAU79 N4
BSA
B
Group 2
Group 1
N9
N6
N7
N2
N1
N4
Antigens
SH13
HN16
wMA84
HB16
SC14
NL03
WS05
BR07
sDL97
tWS66
PR34
NC99
BR07
CA09
gtAT79
Control
Survivor 957
Survivor 958
Endpoint titer
(reciprocal serum dilution)
10,000 - 45,000
1,000 - 10,000
60 - 600
<60
C
N9
N8
N2
N1
Vaccinee (113)
Vaccinee (143)
Survivor (957)
Survivor (958)

Figure S1

Figure S1. Broad reactivity of serum and subtype-specific anti-NA responses by individual mAbs from LCL cultures. Related to Figure 1.

Cross-reactivity of serum samples from two survivors and negative control serum (donor without exposure history to H7N9) (**A, B**) and LCLs (**C**) were assessed by ELISA using for binding using indicated NA antigens.

(**A**) Binding curves of diluted serum. Data shown as mean ± SD of assay triplicates.

(**B**) Heatmap of cross-reactive activity of serum samples from (**A**).

(**C**) Circos plots of antibody cross-reactivity measured in LCL supernatants for four donors. NA antigens include SH13 N9, 2009(H1N1)pdm N1, A/Wisconsin/67/2005 (H3N2) N2, and A/Pennsylvania/1/2007 (H3N8) N8. Each circle has 384 positions, with each representing one well of a 384-well plate. Each ring represents binding to one indicated NA protein. The height of the bar represents OD 405 nm. Black, gold, aqua or coral bars indicate that a well contained IgG that bound to 1, 2, 3, or 4 NA proteins, respectively.

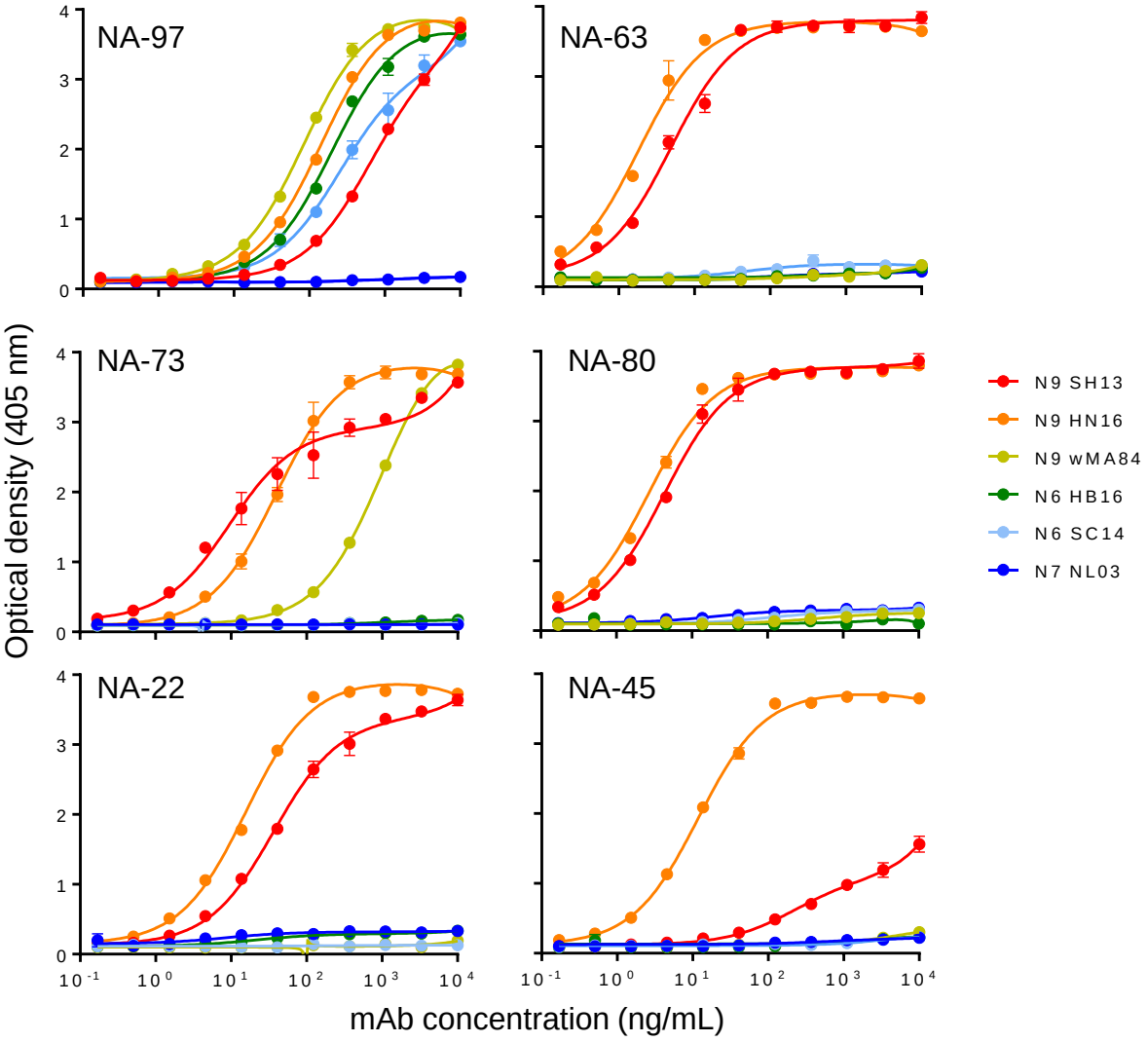


Figure S2. Cross-reactivity of human mAbs to N9 NA. Related to Figure 2.

Cross-reactivity of representative mAbs to different NA antigens was assessed by ELISA using purified recombinant NA antigens at a concentration of 2 μg/mL. Data represent one of two independent experiments, shown as mean ± SD of assay triplicates.

Figure S2

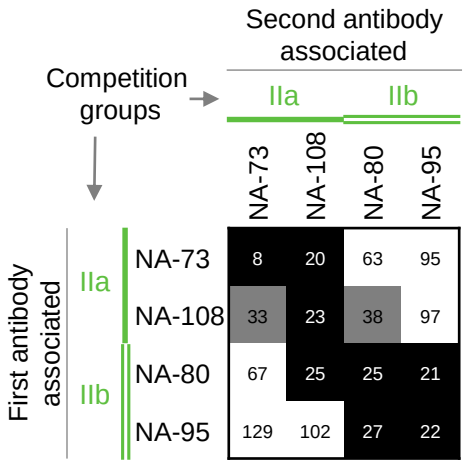


Figure S3. MAbs from competition-binding Group II can be segregated further into subgroups IIa and IIb. Related to Figure 4.

Four mAbs from competition-binding Group II were assessed surface plasmon resonance using a Biacore 8K instrument. MAbs were judged to compete for the same site if maximum binding of second antibody was reduced to ≤ 39% of its uncompeted binding (shown in black boxes). The mAbs were considered non-competing if maximum binding of second mAb was ≥ 61% of its uncompeted binding (shown in white boxes). Grey boxes indicate an intermediate phenotype (competition between 40% and 60% of uncompeted binding). Green lines and Roman numerals indicate inferred competition-binding groups.

Figure S3

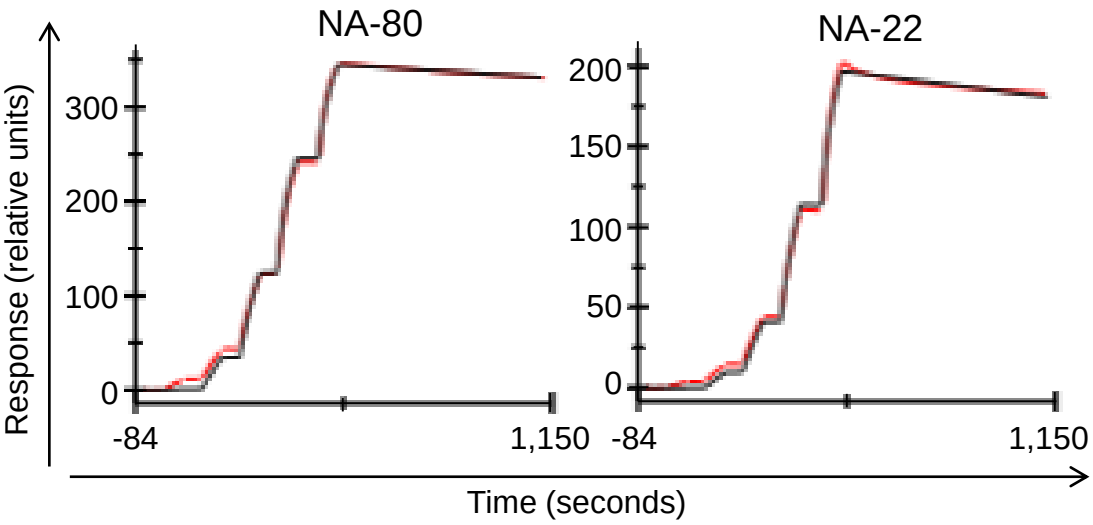
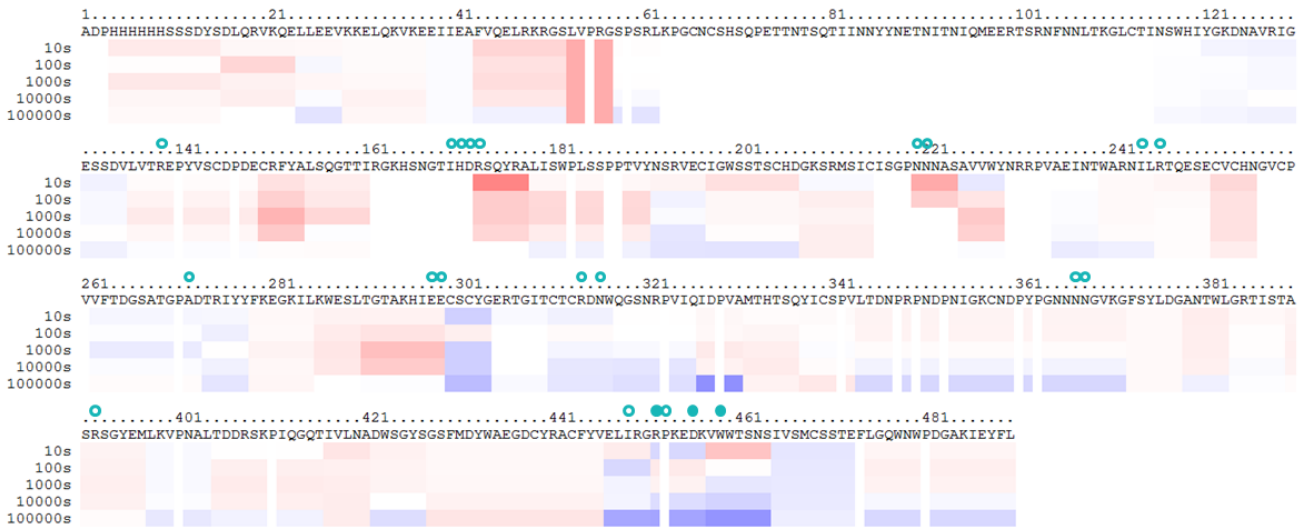


Figure S4. Surface plasmon resonance sensorgrams of human NA-80 and NA-22 mAbs to N9 NA. Related to Figure 4.

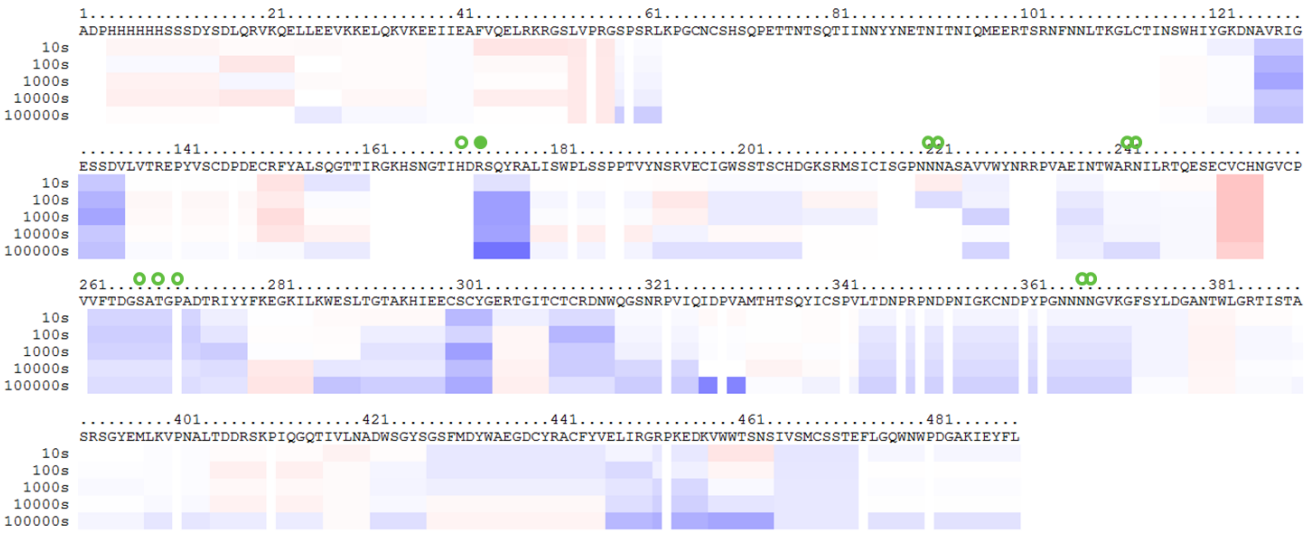
Binding affinity of anti-N9 mAb to N9 NA was measured using a Biacore 8K instrument (GE Healthcare Life Sciences). IgGs were captured on a Protein G sensor chip (GE Healthcare Life Sciences) with final surface densities of ~245 to 400 relative units. Kinetic measurements were performed using a single-cycle kinetics experimental setup, by injecting a 0.156 µg/mL solution of IgG, and then bound with four-fold serial dilutions of N9 NA (starting from a 100 nM concentration). Dissociation data for IgGs were collected for 10 min. Binding data were globally fit to a bivalent analyte model using the Biacore 8K control software. This analysis determined the kinetic rate constants (Kon, Koff), from which the apparent KD then was calculated as Koff/Kon as shown in Figure 4B. Dissociation percent indicates the percent of IgG dissociated from antigen at time of dissociation step, according to RU values.

Figure S4

NA-45 mAb influence on N9 NA deuteration level



NA-73 mAb influence on N9 NA deuteration level



NA-63 mAb influence on N9 NA deuteration level

Figure S5

Figure S5. Hydrogen deuterium exchange mass spectrometry reveals changes in deuteration level for N9 NA incubated with mAbs NA-45, NA-73, or NA-63 compared to N9 NA alone. Related to Figure 5.

Each horizontal row indicates a separate time point. The percentage of deuterium level change is indicated as a color gradient: blue for decrease, red for increase of deuteration level. Circles indicate amino acid residues within the epitope determined by crystallography or cryo-EM reconstruction of N9-Fab complex structures for mAbs NA-45 (cyan), NA-73 (green), or NA-63 (orange) (see Zhu et al., 2019). Solid circles indicate amino acid residues with decreased deuteration level within the epitope upon mAb binding to N9 NA.

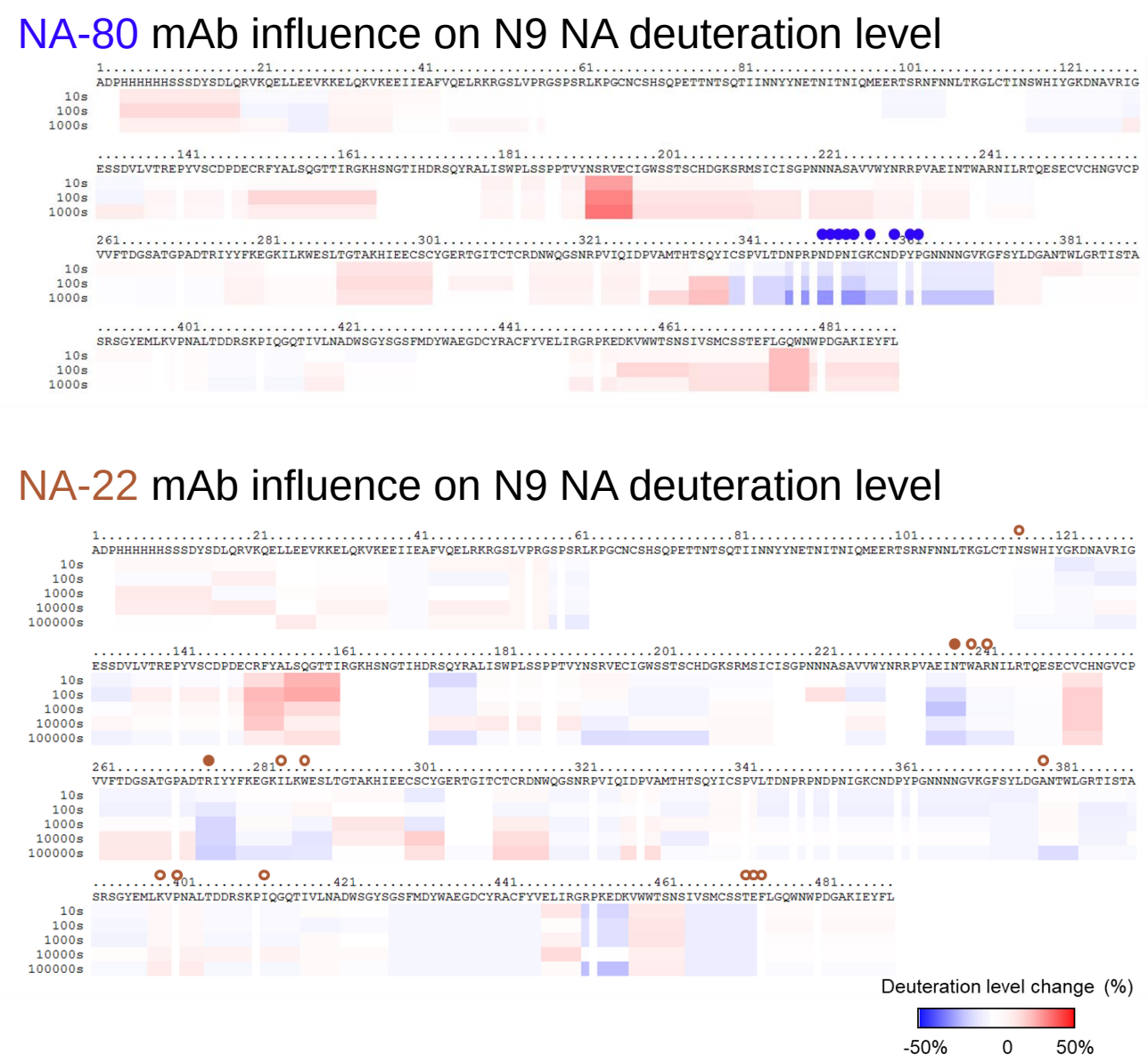
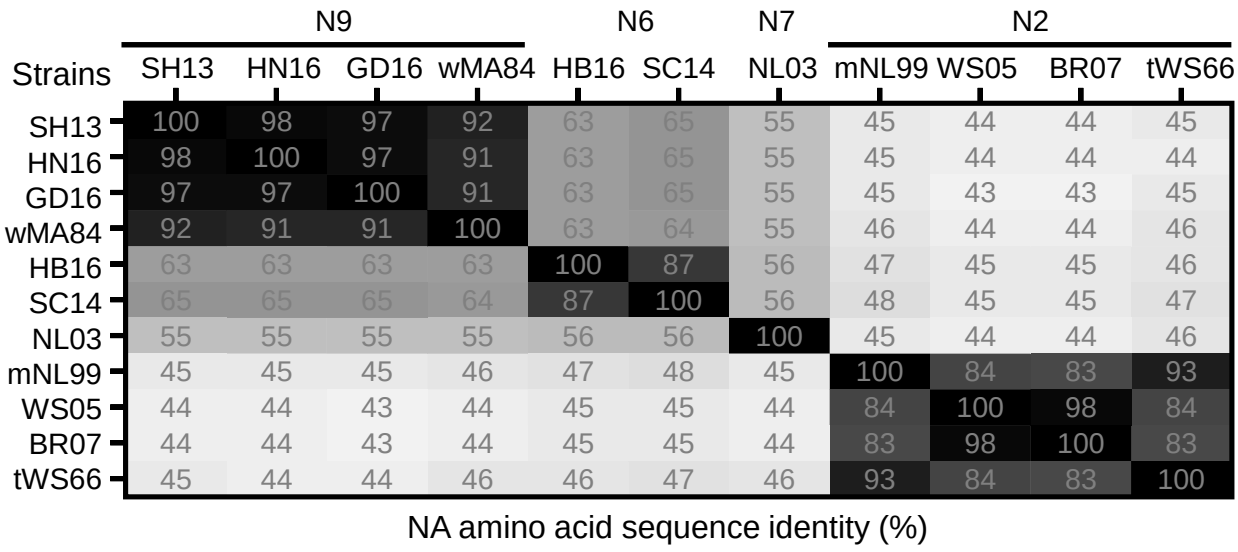


Figure S6. Hydrogen deuterium exchange mass spectrometry reveals changes in deuteration level for N9 NA incubated with mAbs NA-80 or NA-22 compared to N9 NA alone. Related to Figure 5.

Each horizontal row indicates a separate time point. The percentage of deuterium level change is indicated as a color gradient: blue for decrease, red for increase of deuteration level. Circles indicate amino acid residues within the epitope determined by crystallography or cryo-EM reconstruction of N9-Fab complex structures for mAbs NA-80 (blue) or NA-22 (brown) (see Zhu et al., 2019). Solid circles indicate amino acid residues with decreased deuteration level within the epitope upon mAb binding to N9 NA.

Figure S6

A



B

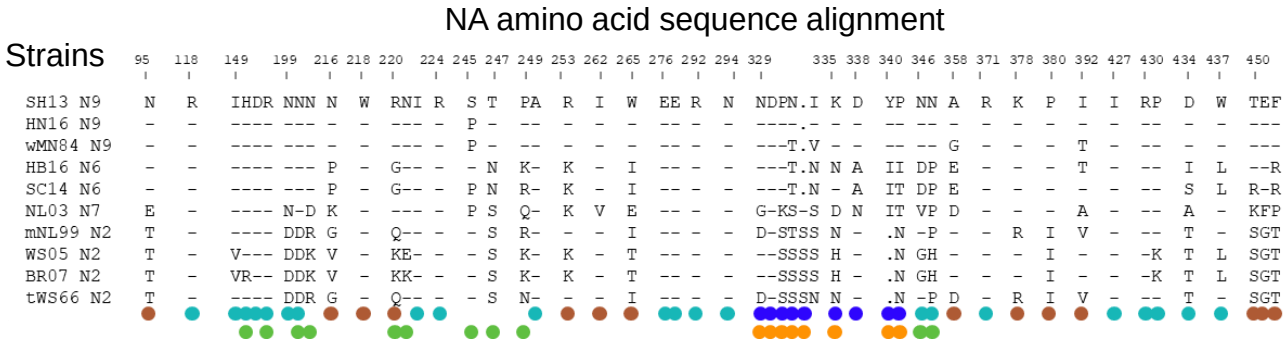


Figure S7. Conservation of NA sequences within identified epitopes for five characterized anti-N9 NAs mAbs. Related to Figure 5

NA amino acid sequences for N9, N6, N7 and N2 NAs are shown.

(A) Heatmap showing amino acid identity for indicated pairwise comparisons of NA proteins.

(B) Alignment of NAs amino acid sequences within epitopes of NA-45, NA-73, NA-63, NA-80 and NA-22 mAbs. Circles indicate amino acid residues within the epitope determined by crystallography or cryo-EM reconstruction of N9-Fab complex structures for mAbs NA-45 (cyan), NA-73 (green), NA-63 (orange), NA-80 (blue) or NA-22 (brown) as described (Zhu et al., 2019).

Figure S7

SUPPLEMENTAL TABLES

Table S1. Generation of human B cell hybridomas from PBMCs of H7N9-immunized subjects or from subjects who had a history of naturally acquired H7N9 infection, Related to Figure 1

Donor	Vaccine formulation (hemagglutinin amount, μg + adjuvant)		Vaccine or infection	Time post-exposure when blood was taken	Frequency of H7N9 reactive EBV-transformed lymphoblastoid cell lines (LCLs) (%)		Number of individual N9-reactive hybridomas generated from sample
	prime vaccination	boost vaccination			H7 HA reactive	N9 NA reactive	
231	15 + AS03	15 + AS03	Monovalent inactivated H7N9 A/Shanghai/02/2013 vaccine	Day 42 (21 days after the boost vaccination)	1.33	1.45	0
113	15 + AS03	15 + AS03			1.41	0.81	13
169	15 +AS03	15			2.21	0.59	0
166	3.75 + AS03	3.75 + AS03			0.63	0.46	0
143	7.5 + AS03	7.5 + AS03			0.70	0.32	3
155	7.5 + AS03	7.5 + AS03			0.03	0.88	0
100	15 + AS03	15 + AS03			0.08	0.74	0
220	15	15 +AS03			0.00	0.64	0
148	15 + MF59	15 + AS03			0.10	0.51	0
076	15 + MF59	15 + MF59			0.05	0.4	0
051	15 + AS03	15 + MF59			0.08	0.34	0
069	15 + AS03	15 + MF59			0.10	0.32	0
201	15 + MF59	15 + AS03			2.57	0.00	0
212	45	45			1.51	0.00	0
196	15 + MF59	15 + MF59			1.37	0.05	0
198	7.5 + AS03	7.5 + AS03			0.87	0.00	0
211	15 + MF59	15 + AS03			0.61	0.00	0
158	15 +AS03	15			0.08	0.15	0
238	15 + MF59	15 + MF59			0.09	0.11	0
174	3.75 + AS03	3.75 + AS03			0.02	0.10	0
079	45	45			0.02	0.05	0
193	7.5 + AS03	7.5 + AS03			0.21	0.04	0
072	7.5 + AS03	7.5 + AS03			0.16	0.06	0
135	15	15			0.09	0.00	0
067	15	15			0.04	0.00	0
177	15 + AS03	15			0.01	0.00	0
225	15 + AS03	15			0.01	0.00	0
101	15 + AS03	15 + AS03			0.00	0.00	0
130	45	45			0.00	0.00	0
141	15	15 +AS03			0.00	0.00	0
957	Not applicable		H7N9 (A/British Columbia/1/2015) infection	~11 months	0.38	0.56	11
958					1.45	0.86	8

Table S2. Sequence diversity of antibody variable genes encoding N9-specific mAbs, Related to Figure 1

Donor	mAb	Heavy chain variable gene sequence						Light chain variable gene sequence				
		V_H gene	V_H region nucleotide % homology to V_H gene	D_H gene	J_H gene	HCDR3 amino acids (aa)	CDR3 length (aa)	V_L gene	V_L region nucleotide % homology to V_L gene	J_L gene	LCDR3 amino acids (aa)	CDR3 length (aa)
143	NA-3	3-9	98.7	6-19	3	AKDKGSSDWFPAFDI	15	1-44	98.9	3	AAWDDSLNGPV	11
	NA-4	3-30-3	98.6	6-19	3	ARDPLPVSSGWYRDDAFDI	19	ND	ND	ND	ND	ND
	NA-5	3-30	98.6	3-22	6	ARGPSLTYYYYSSGQTSYGMDV	22	2-14	97.6	3	SSYTSSSTWV	10
113	NA-16	3-48	100	6-13	4	ARDRGYSNSWYTDY	14	3-20	99.6	3	QQYGSSP	7
	NA-17	ND	ND	ND	ND	ND	ND	3-15	94.9	2	QQYNQWPYT	9
	NA-22*	3-30	99.3	3-22	4	AKDKRFITMILVGPFDY	17	2-14	97.2	1	SSYTSSTTNV	10
	NA-37	3-15	97.9	3-9	4	TTDGLGLVWFGEGY	14	1-44	99.3	3	AAWDDSLNGWV	11
	NA-45	4-39	98.9	1-26	5	ARDPGLEWELSVLSNWFDP	19	1-51	98.9	1	GTWDTSLSAYV	11
	NA-55	3-53	98.9	2-15	5	ARSPYCDGGSCLPT	14	ND	ND	ND	ND	ND
	NA-63	3-7	98.9	6-13	4	ASSTAAEFFDY	11	1-39 or 1D-39	97.1	2	QQSYSTPLYT	10
	NA-69*	3-30	99.3	3-22	4	AKDKRFITMLVVGPFDY	17	2-14	98.3	1	SSYTSSSTNV	10
	NA-73	4-38-2	95.1	2-2	6	ARDPSFWSSTSRTSPYYYGMDV	22	1-44	98.9	3	AAWDDNLNGWV	11
	NA-77	1-69 or 1-69D	99.6	6-19	6	ARGGIAVAYTALNPTYYYYYMDV	23	2D-29	99.7	3	MQIIQLPPGFT	11
	NA-80	1-18	98.6	6-19	4	ARVIPGTAVDYFDY	14	1-39 or 1D-39	97.5	3	QQSYSAPFT	9
	NA-81	5-10-1	98.7	3-10	2	ARHVRPNWYFDL	12	3-15	98.2	1	QQFNNWPLT	9
	NA-86	4-61	98.3	6-13	6	ARAPIVAADYGMDV	14	1-39 or 1D-39	96.8	2	QQSYNTLMYT	10
957	NA-89	3-30	96.5	5-24	6	ARDALQLRWLYYYYMDV	17	3-11	99.3	3	GTWDSSLSAGV	11
	NA-93	3-30	95.8	3-3	6	ARDLFFRSNYYFMDV	15	1-51	97.5	3	GTWDSSLSAWV	11
	NA-95	3-21	95.1	6-13	3	AMTYLAETVDAFDI	14	ND	ND	ND	ND	ND
	NA-97	3-30	92.7	3-22	4	ARDLKRYYYDTSGSDLNDF	19	3-11	98.6	3	QQRSNWLLT	9
	NA-108	4-61	95.5	3-22	3	ARDLYYYDSSAFYTAFDI	18	3-15	97.8	2	QQYNDWPSYT	10
	NA-110	3-53	95.8	3-22	4	ARSNGYYKRYFDY	13	1-33 or 1D-33	ND	3	QQYHNLPTH	9
	NA-111	3-33	92.4	5-12	4	AKDLLWLRSIIGCPDV	16	1-51	95.1	3	GTWDSSLSAWV	11
	NA-121	4-31	95.9	4-17	1	ARGPTEEFFHQ	11	1-39 or 1D-39	94.9	3	QQSYSPPVT	9
	NA-126	1-18	96.2	3-3	4	ARDHRFLKWLLFPPLDY	17	1-47	97.5	3	AAWDDSLSAWM	11
	NA-127	1-18	94.1	2-2	5	ARDRIVCNKTSCYMPRWTPYWFDP	24	3-11	97.8	4	QQRSNRPLT	9
	NA-144	7-4-1	97.9	3-3	3	AGSQAGFFLGAFDI	14	3-20	98.6	1	QQYGSSPPT	9
958	NA-148	3-30	95.5	3-9	6	ARILNLLRSVDWAMGDYGMDV	21	1-51	96.1	3	GTWDSSLGAWV	11
	NA-152	ND	ND	ND	ND	ND	ND	1-39 or 1D-39	97.5	1	QQSFSSPLT	9
	NA-157	4-59	91.9	3-10	4	ARDVGGPYYFDY	12	1-39 or 1D-39	94.6	4	QQSYNTPLT	9
	NA-164	5-51	97.2	3-9	5	ARLTPRLVIMGGNWFDP	17	1-39 or 1D-39	98.9	3	QQSYTTPRFT	10
	NA-171	1-69	92.0	6-19	4	ARNFYPGYLQY	16	ND	ND	ND	ND	ND
	NA-175	1-69	95.5	6-19	3	AKEACGGDCYSNYFHY	12	2-8	92.4	1	CSLAGSNNFV	10
	NA-177	ND	ND	ND	ND	ARVPCGGDCYSGYLQH	ND	3-20	98.2	4	QQYGSSPT	8
	NA-181	1-46	99.6	D4-17	5	ARVGAVRIAAAAPDY	15	ND	ND	ND	ND	ND

ND - not determined
* - indicates clonally related mAbs

Table S3. Cross-reactivity of serum samples from H7N9 survivors and control donor, Related to Figure S1

Donor	Binding, Endpoint titer (reciprocal serum dilution)													
	NA antigens from Group 2									NA antigens from Group 1				
	N9			N6		N7	N2				N1			N4
	SH13	HN16	wMA84	HB16	SC14	NL03	WS05	BR07	sDL97	tWS66	PR34	NC99	BR07	CA09	grAT79
Control	20	20	20	<	<	<	180	60	<	1620	<	60	180	60	<
957	180	4860	180	1620	4860	60	180	60	180	4860	<	60	180	60	<
958	60	1620	540	1620	14580	4860	4860	14580	540	43740	<	60	540	180	180

The > symbol indicates endpoint titer less than 1/20

Table S4. Cross-reactivity of anti-N9 mAbs, Related to Figure 2

Donor	mAb	Binding, EC_{50} (ng/mL)																		
		NA antigens from Group 2													NA antigens from Group 1					
		N9					N6		N7	N2					N1				N4	N8
		SH13	SH13 E119V	SH13 R292K	HN16	wMA84	HB16	SC14	NL03	mNL99	WS05	BR07	sDL97	tWS66	PR34	NC99	BR07	CA09	gtAT79	ePS07
957	NA-97	865	110	121	133	76	189	332	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-171	2	2	3	3	2	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-95	5	3	3	5	3	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-108	12	3	3	3	36	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-127	4	4	6	7	36	>	>	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-175	27	7	8	12	12	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-144	6	3	2	3	2	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-110	11	11	12	12	12	>	>	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-164	8	7	8	10	17	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-16	7	9	10	8	15	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-37	114	3	4	5	6	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-111	30	31	26	22	90	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-73	15	5	6	37	885	>	>	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-157	27	12	12	10	11	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-89	10	12	14	9	77	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-86	6	5	5	6	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-80	4	2	3	3	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-55	6	5	5	6	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-77	14	10	9	8	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-93	9	8	7	10	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-148	9	11	12	11	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-17	17	14	15	20	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-126	32	20	22	24	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-63	10	3	2	2	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
143	NA-3	11	5	4	4	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-5	10	8	7	5	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-69	20	7	5	4	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-81	17	25	19	13	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-22	42	16	14	15	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
143	NA-4	86	55	38	24	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
113	NA-45*	>	>	>	11	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-181	>	623	321	235	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
	NA-177	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
957	NA-121	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>	>
958	NA-152	>	ND	ND	ND	ND	ND	ND	>	>	>	>	>	>	>	>	>	>	>	>

ND indicates not determined. The > symbol indicates binding was not detected when tested at concentrations as high as 10 μg/mL

* NA-45 binds SH13 N9 NA but EC_{50} value is impossible to define since concentration response curve do not reach maximum at tested concentrations. See Figure S1.

Table S5. Neutralizing activity of anti-N9 mAbs, Related to Figure 3

Donor	mAb	HAI	Egress inhibition assay	ELLA NI assay	NA-Fluor NI assay
		Effective endpoint antibody concentration (nM)		IC_{50} (nM)	IC_{50} (nM)
957	NA-108	ND	ND	0.11	0.54
113	NA-73	>	0.21	0.21	3
113	NA-80	>	<0.03	0.08	>
957	NA-95	>	<0.03	0.19	>
113	NA-63	>	0.09	0.40	>
113	NA-77	>	0.27	0.62	>
957	NA-127	>	0.27	0.63	>
113	NA-45*	>	0.27	0.79	>
958	NA-171	>	0.21	2.72	>
113	NA-55	>	0.45	0.28	>
113	NA-69	>	0.81	0.38	>
143	NA-5	>	0.81	0.47	>
113	NA-22	>	0.81	0.63	>
113	NA-17	>	0.81	0.86	>
143	NA-3	>	1.36	1.01	>
958	NA-181	>	1.37	1.72	>
113	NA-86	>	1.37	1.73	>
143	NA-4	>	2.47	0.79	>
957	NA-93	>	1.92	2.31	>
958	NA-157	>	2.47	4.16	>
957	NA-89	>	1.92	4.64	>
957	NA-110	>	2.47	20.59	>
958	NA-148	>	17.3	1.17	>
957	NA-126	>	12.3	1.31	>
957	NA-121	ND	ND	2.33	>
113	NA-16	>	0.45	>	>
958	NA-164	>	2.47	>	>
113	NA-81	>	2.47	>	>
957	NA-111	ND	ND	9.84	>
	NA-97	>	22	66.67	>
958	NA-175	>	>	>	>
957	NA-144	>	>	>	>
113	NA-37	>	>	>	>
958	NA-177	>	>	>	>
958	NA-152	ND	ND	ND	ND
Controls	Zanamivir	ND	7.33	7.56	1.93
	rCR9114	>	>	ND	ND
	H7.167	0.27	ND	ND	ND
	2D22	>	>	>	>

> indicates that activity was not detected when tested at concentrations as high as 20 μg/mL
ND indicates not determined
* NA-45 inhibits A/Shanghai/02/2013 H7N9 NA activity but IC_{50} value is impossible to define since concentration response curve do not reach minimum at tested concentrations. See Figure 3C.