

Supporting Information

for

High-throughput neuraminidase substrate specificity study of human and avian influenza A viruses

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Table S1. The ratio of the natural sialic acid released from the substrates Sia α 2–6Gal β pNP and Sia α 2–3Gal β pNP. ^aRatios higher than 0.41 are shown in bold.

Viral strain	Type	Ratio (1b/1a)^a
sh/HKWF569/07	H3N1	0.26
sh/HKWF216/07	H6N1	0.09
mal/8212/08	H6N1	0.11
mal/8322/08	H6N1	0.09
tel8326/08	H1N1	0.41
sh/HKWF268/07	H6N2	0.25
tel/7972/08	H6N2	0.23
mal/8035/08	H5N2	0.22
wi/HKWF295/07	H6N5	0.10
wi/HKWF541/07	H6N5	0.20
wi/8352/08	H12N5	0.14
dk/7896/08	H4N6	0.22
emu/2092/96	H10N7	0.22
sh/JN950/06	H10N7	0.25
gos/HKWF446/07	H10N7	0.57^c
sh/HKWF848/07	H3N7	0.41
sh/HKWF1021/07	H3N7	0.32
sh/HKWF1128/07	H2N7	0.23
tel/HKWF1111/07	H5N7	0.18
mal/6957/08	H10N7	0.35
dk/K90/05	H6N8	0.06
tel/8204/08	H5N9	0.29
dk/448/78	H9N2	0.35
dk/702/79	H9N2	0.43
Q/A28945/88	H9N2	0.39
Q/29209-1/93	H9N2	0.49
dk/Y280/97	H9N2	0.27
ck/G9/97	H9N2	0.48
pt/2373/98	H9N2	0.22
ck/SF3/99	H9N2	0.36
QA23	H9N2	0.16
Qa23CkA10	H9N2	0.15
<i>HK/2108/03</i>	<i>H9N2</i>	0.46
<i>A/PR8</i>	<i>H1N1</i>	0.53
<i>A/Mem71</i>	<i>H3N1</i>	0.42
<i>A/Udorn72</i>	<i>H3N2</i>	0.64
<i>A/Philips</i>	<i>H3N2</i>	0.53
<i>A/PR8 (purified)</i>	<i>H1N1</i>	0.61
<i>A/Mem71 (purified)</i>	<i>H3N1</i>	0.49

Table S2. Relative activities for α 2–3-linked sialosides^a.

Viral Strain	Type	Substrate Specificity ^b					
		2a	3a	4a	5a	6a	10a
Group 1 NA							
sh/HKWF569/07	H3N1	++++	+	++++	++++	-	-
sh/HKWF216/07	H6N1	++++	-	+++	++++	-	-
mal/8212/08	H6N1	++++	-	+++	++++	-	-
mal/8322/08	H6N1	++++	-	++	+++	-	-
tel8326/08	H1N1	++++	-	+	++++	-	-
<i>A/PR8^c</i>	<i>H1N1</i>	++++	-	++	+++	-	-
<i>A/Mem71^c</i>	<i>H3N1</i>	++++	-	++	+++	-	-
wi/HKWF295/07	H6N5	++++	-	++	+++	-	-
wi/HKWF541/07	H6N5	++++	-	+++	++++	-	+
wi/8352/08	H12N5	++++	-	+++	+++	-	-
dk/K90/05	H6N8	++++	-	+	+++	-	-
Group 2 NA							
sh/HKWF268/07	H6N2	++++	-	+	+++	-	-
tel/7972/08	H6N2	+++	-	-	+++	-	-
dk/448/78	H9N2	++++	-	+	+++	-	-
dk/702/79	H9N2	++++	-	++	++++	-	+
Q/29209-1/93	H9N2	++++	-	++	++++	-	-
pt/2373/98	H9N2	++++	-	-	+++	-	-
ck/SF3/99	H9N2	++++	-	-	+++	-	-
QA23	H9N2	++++	-	-	++	-	-
Qa23CkA10	H9N2	++++	-	-	++	-	-
<i>HK/2108/03^c</i>	<i>H9N2</i>	++++	-	-	+++	-	-
<i>A/Udorn72^c</i>	<i>H3N2</i>	++++	-	-	+++	-	-
<i>A/Philips^c</i>	<i>H3N2</i>	++++	-	-	++	-	-
dk/7896/08	H4N6	++++	-	+	++	-	-
emu/2092/96	H10N7	++++	-	-	+++	+	+
sh/JN950/06	H10N7	++++	-	+	+++	+	+
gos/HKWF446/07	H10N7	++++	+	+++	++++	++	+++
sh/HKWF848/07	H3N7	++++	-	+++	++++	++	++
sh/HKWF1021/07	H3N7	++++	-	+++	++++	+	++
sh/HKWF1128/07	H2N7	++++	-	++	+++	+	+
tel/HKWF1111/07	H5N7	++++	-	+	++	-	+
mal/6957/08	H10N7	++++	-	+	++++	+	+

^aAbsolute values for the percentage hydrolysis of Neu5Ac α 2-3Gal β pNP (compound **1a**) and relative value for all other compounds. Relative value got: Virus neuraminidase activity using Neu5Ac α 2-3Gal β pNP (compound **1a**) as the substrate is considered as 100%. "++++" represents

activity \geq 60%; "+++" represents activity \geq 40% but $<$ 60%; "++" represents activity \geq 20% but $<$ 40%; "+" represents activity \geq 10% but $<$ 20%; "-" represents activity $<$ 10%.

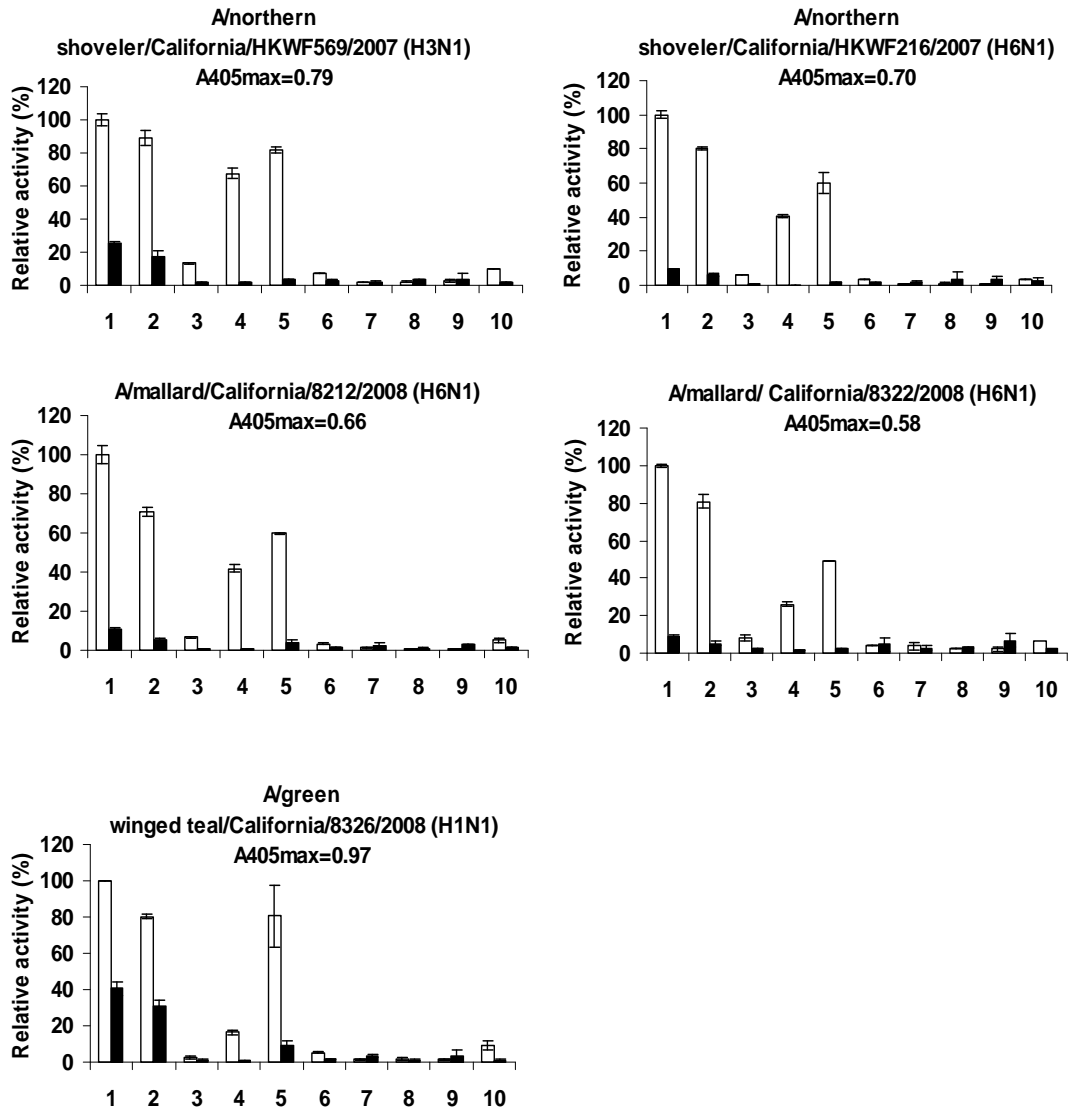
^bThe data for viruses with A405max below 0.2 when using Neu5Ac α 2-3Gal β pNP (**1a**) as the substrate did not listed, including viruses mal/8035/08 (H5N2), tel/8204/08 (H5N9), Q/A28945/88 (H9N2), dk/Y280/97 (H9N2), and ck/G9/97 (H9N2). The data for the relative activity below 10% were not shown.

^cHuman influenza A viruses are shown in italics and NA activities of unpurified human influenza A viruses data were used in this table.

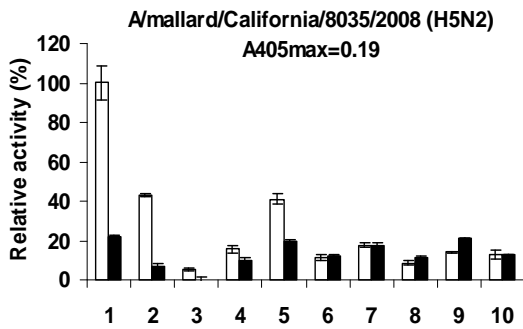
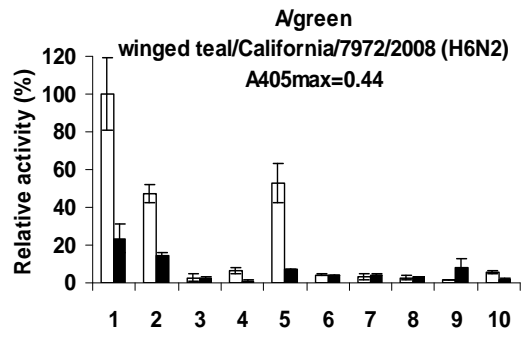
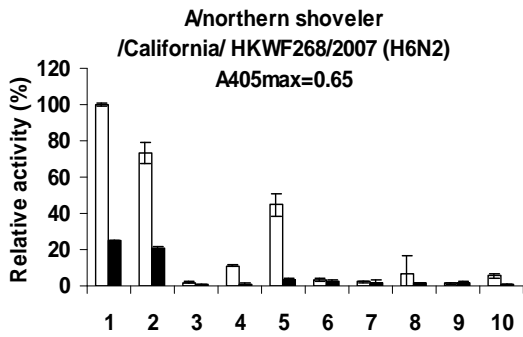
Fig. S1. Substrate specificities of neuraminidases on avian and H9N2 influenza viruses.

A, N1 type (group 1 neuraminidase); B, N2 type (group 2 neuraminidase); C, N5 type (group 1 neuraminidase); D, N7 type (group 2 neuraminidase); E, N6 (group 2 neuraminidase), N8 (group 1 neuraminidase), and N9 (group 1 neuraminidase) types; and F, H9N2 type. Allantoic fluids containing viruses at a titer of 12.8 HAU mL^{-1} were used directly for the assay. Results using $\text{Sia}\alpha 2\text{-3Gal}\beta\text{pNP}$ (**1a-10a**) and $\text{Sia}\alpha 2\text{-6Gal}\beta\text{pNP}$ (**1b-10b**) as substrates were shown in white and black bars respectively. Different numbers (**1-10**) represent sialosides containing different sialic acid structures as shown in Table 2. The sialic acid cleavage activity of viral neuraminidase using $\text{Neu5Ac}\alpha 2\text{-3Gal}\beta\text{pNP}$ as the substrate was plotted as 100% with an assay read out represented by the $A_{405\text{max}}$ shown for each virus.

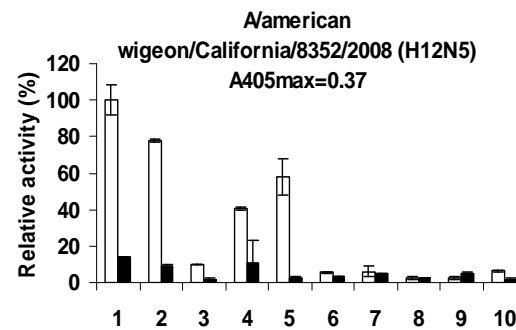
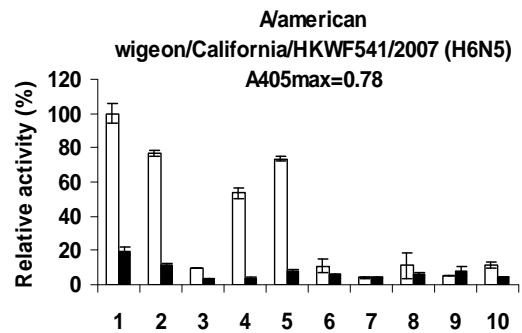
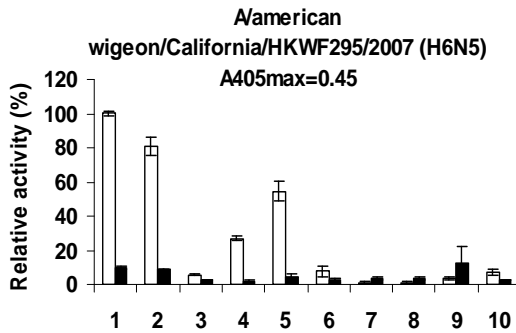
A. N1 Type (group 1 neuraminidase)



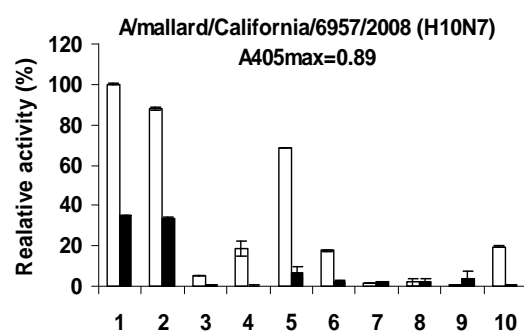
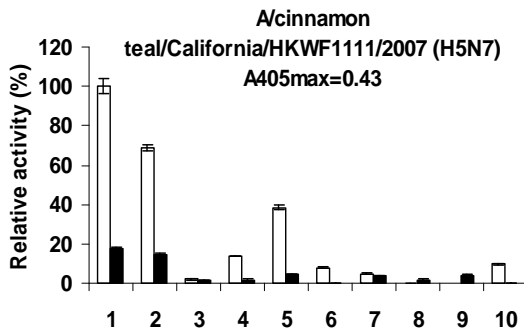
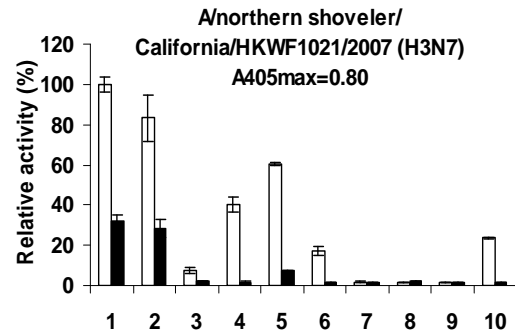
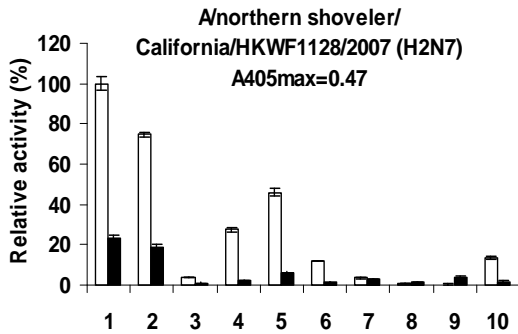
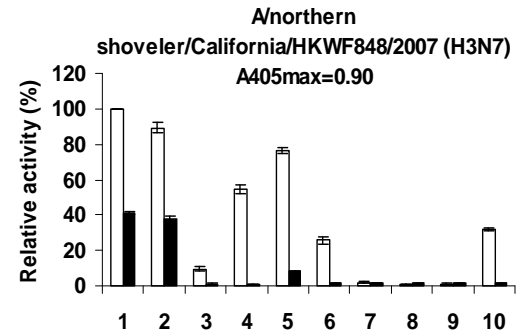
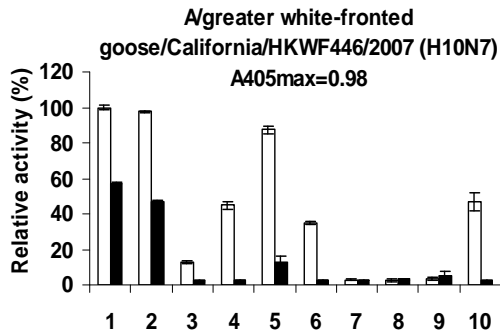
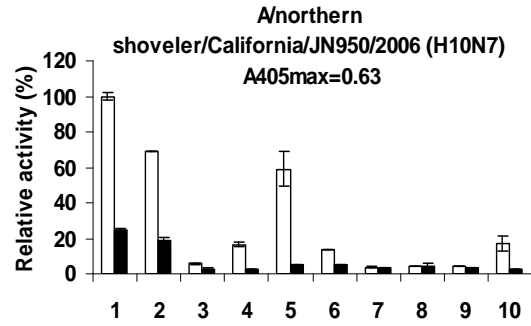
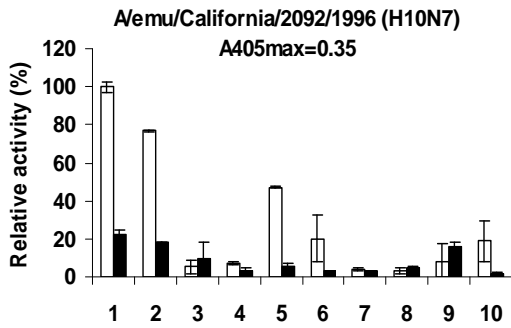
B. N2 Type (group 2 neuraminidase)



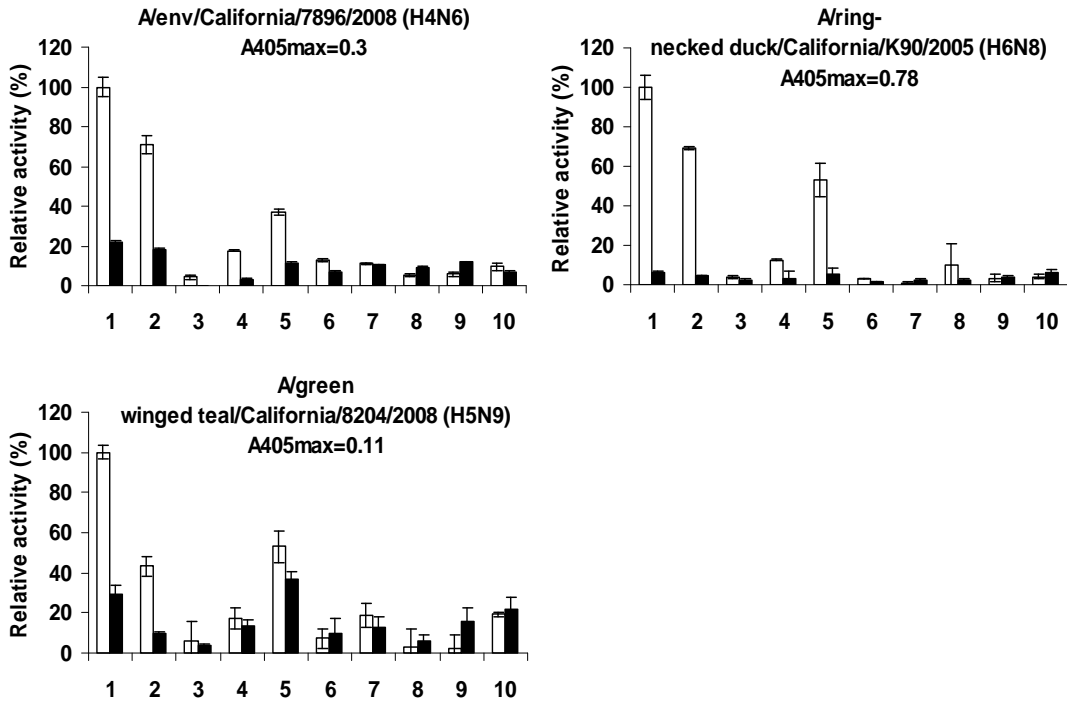
C. N5 Type (group 1 neuraminidase)



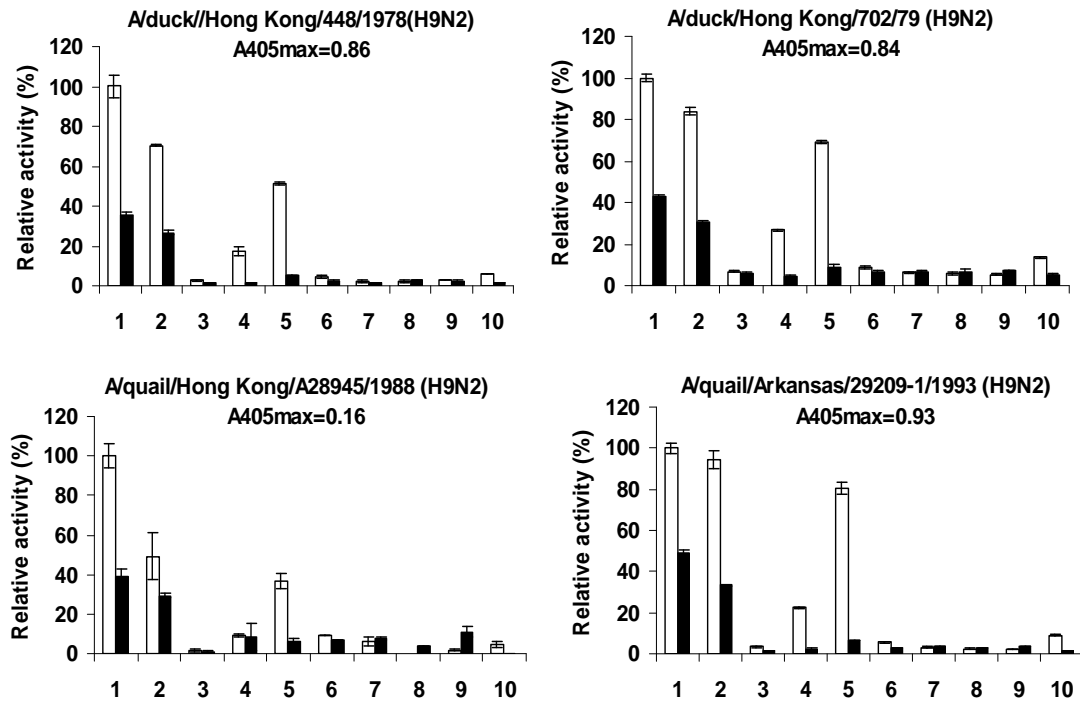
D. N7 Type (group 2 neuraminidase)



E. N6 (group 2 neuraminidase), N8 (group 1 neuraminidase), and N9 (group 1 neuraminidase) Types



F. H9N2 influenza A viruses



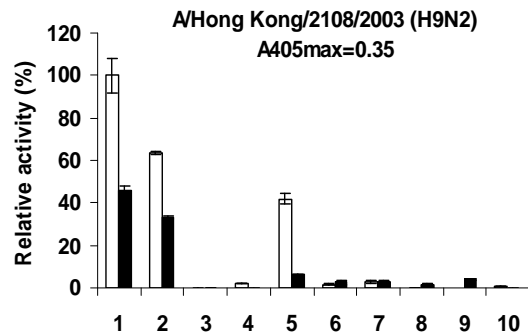
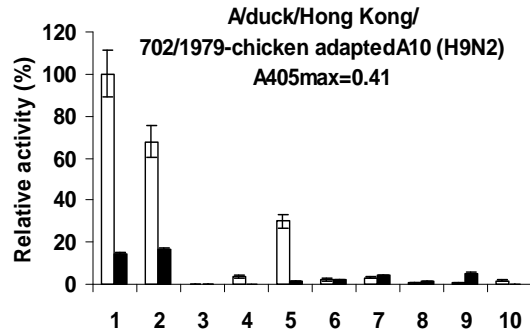
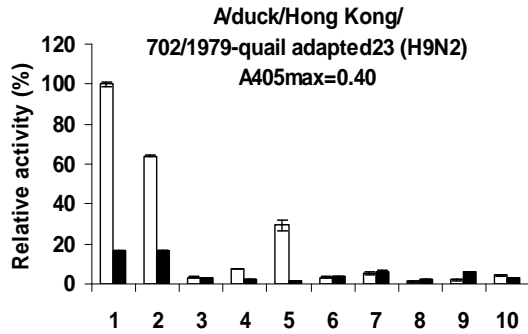
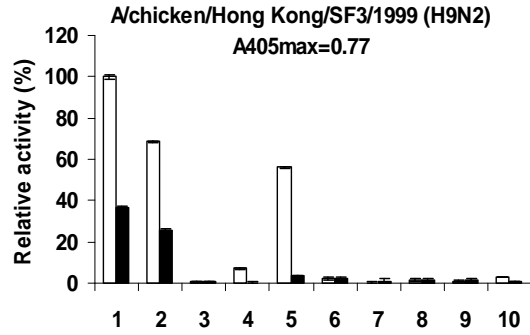
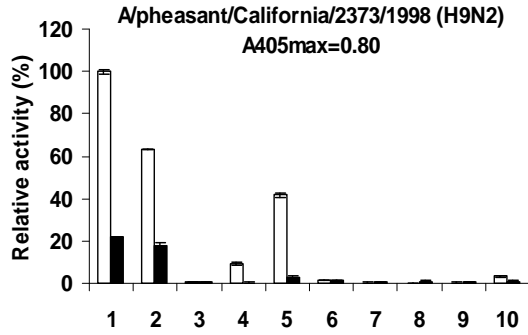
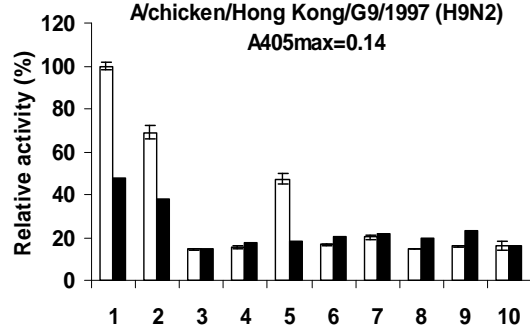
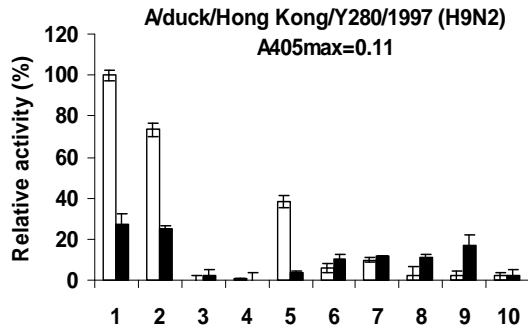
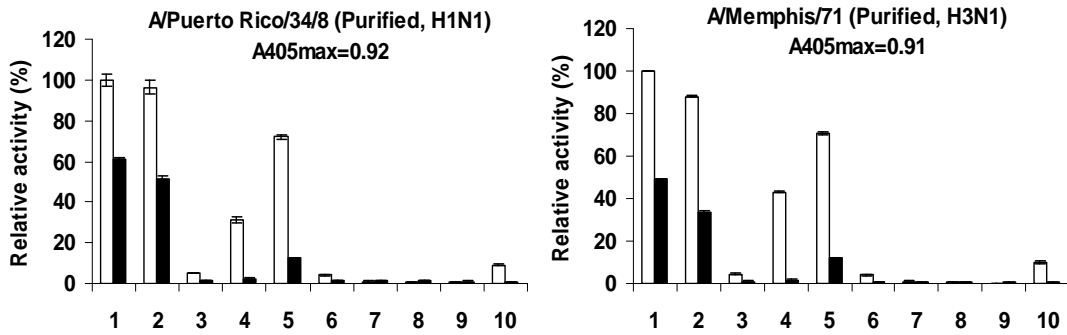


Fig. S2. Substrate specificities of neuraminidases from purified and unpurified human influenza A viruses. The titers of purified viruses were: A/PR8, 1×10^4 HAU mL⁻¹; A/Mem71, 2×10^3 HAU mL⁻¹. The titers of allantoic fluids containing viruses were: A/PR8, 1×10^3 HAU mL⁻¹; A/Mem71, 5×10^2 HAU mL⁻¹; A/Udon, 5×10^3 HAU mL⁻¹; A/Philippines: 1×10^4 HAU mL⁻¹. Results using Sia α 2–3Gal β pNP (**1a–10a**) and Sia α 2–6Gal β pNP (**1b–10b**) as substrates were shown in white and black bars respectively. Different numbers (**1–10**) represent sialosides containing different sialic acid structures as shown in Table 2. The sialic acid cleavage activity of viral neuraminidase using Neu5Ac α 2–3Gal β pNP as the substrate was plotted as 100% with an assay read out represented by the A405max shown for each virus.

A. Purified human virus



B. unpurified human virus

