Structure and receptor binding preferences of recombinant human A(H3N2) virus hemagglutinins

Hua Yang1, Paul J. Carney1, Jessie C. Chang1, , Zhu Guo1, Julie M. Villanueva1, James Stevens1\*

1 Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.

**SUPPLEMENTARY TABLES AND FIGURES**

**Supplementary Table S1** Glycan array differences between the A(H3N2) recHAs analyzed in this study. The leftmost column shows the glycan number plotted on the graphs in Figures 5 and 7. Different categories of glycans on the array are color coded in the leftmost column as follows: no color, sialic acid; blue, α2-3 sialosides; red, α2-6 sialosides, violet, mixed α2-3/ α2-6 biantennaries; green, N-glycolylneuraminic acid-containing glycans; brown, α2-8-linked sialosides; yellow, β2-6-linked and 9-O-acetylated sialic acids; gray, asialo glycans. The structures of the glycans are shown in column 2. Results for each HA are listed by year and exact HAs analyzed are listed in Table 1 of the manuscript. Significant binding of samples to glycans was qualitatively estimated based on the relative strength of the signal for the data. Symbols: +, relative fluorescence intensity (RFI) of 3,001 to 9,999; ++, RFI of 10,000 to 19,999; +++, RFI of >20,000; nb, no binding or relative fluorescence intensity of <3,000.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **#** | **Structure** | **1968** | **1973** | **1975** | **1979** | **1986** | **1987** | **1992** | **1994** | **1995** | **1997** | **1999** | **2003** | **2004** | **2005** | **2007** | **2009** | **2011** |  | **Panama99 WT** | **Panama99 130-loop** | **Panama99 150-loop** | **Panama99 190-helix** | **Panama99 220-loop** | **Wyo03 WT** |
| 1 | Neu5Acα | nb | nb | nb | nb | ++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 2 | Neu5Acα | nb | + | nb | +++ | +++ | +++ | + | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 3 | Neu5Acβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 4 | Neu5Acα2-3(6-O-Su)Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | + | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 5 | Neu5Acα2-3Galβ1-3[6OSO3]GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 6 | Neu5Acα2-3Galβ1-4[6OSO3]GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | +++ | +++ | nb | nb | nb | nb | nb | nb | nb |  | + | nb | nb | nb | nb | nb |
| 7 | Neu5Acα2-3Galβ1-4(Fucα1-3)[6OSO3]GlcNAcβ-propyl-NH2 | nb | nb | nb | nb | nb | nb | nb | nb | +++ | +++ | nb | nb | nb | nb | nb | nb | nb |  | + | nb | nb | nb | nb | nb |
| 8 | Neu5Acα2-3Galβ1-3[6OSO3]GlcNAc-propyl-NH2 | nb | nb | nb | nb | nb | nb | nb | nb | +++ | +++ | nb | nb | nb | nb | nb | nb | nb |  | + | nb | nb | nb | nb | nb |
| 9 | Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4)GlcNAcβ | nb | nb | nb | nb | nb | nb | + | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 10 | Neu5Acα2-3Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα | nb | nb | nb | nb | nb | nb | +++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 11 | Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ | nb | + | ++ | nb | nb | nb | +++ | nb | nb | nb | ++ | nb | nb | nb | nb | nb | nb |  | + | +++ | nb | nb | nb | nb |
| 12 | Neu5Acα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[Neu5Acα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ | nb | nb | nb | nb | nb | nb | +++ | nb | nb | nb | + | nb | nb | nb | nb | nb | nb |  | nb | ++ | nb | nb | nb | nb |
| 13 | Neu5Acα2-3Galβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | + | nb | nb | nb | nb |
| 14 | Neu5Acα2-3Galβ1-3GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 15 | Neu5Acα2-3Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 16 | Neu5Acα2-3Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 17 | Neu5Acα2-3Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 18 | Neu5Acα2-3Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 19 | Neu5Acα2-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 20 | Neu5Acα2-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 21 | Neu5Acα2-3GalNAcβ1-4GlcNAcβ | nb | +++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 22 | Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 23 | Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 24 | Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 25 | Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 26 | Neu5Acα2-3Galβ1-3GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 27 | Galβ1-3(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6)GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 28 | Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 29 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 30 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 31 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 32 | Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAcβ1-3Galβ1-4[Fucα1-3]GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 33 | Neu5Acα2-3Galβ1-3[Fucα1-3]GlcNAcβ1-3Galβ1-4[Fucα1-3]GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 34 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 35 | Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 36 | Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 37 | Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 38 | Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 39 | Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 40 | Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 41 | Neu5Acα2-6Galβ1-4[6OSO3]GlcNAcβ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | nb | +++ | +++ | +++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | + | ++ | nb | + |
| 42 | Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ | nb | ++ | nb | + | +++ | +++ | nb | nb | nb | + | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 43 | Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ | + | +++ | + | +++ | +++ | +++ | +++ | nb | ++ | ++ | ++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | nb | nb | nb |
| 44 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]Manβ1-4GlcNAcβ1-4GlcNAcβ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | +++ | nb | nb |
| 45 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6]-Manβ1-4GlcNAcβ1-4GlcNAcβ | ++ | +++ | + | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | +++ |  | +++ | +++ | +++ | +++ | +++ | +++ |
| 46 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6]GalNAca | +++ | +++ | +++ | ++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ | +++ | + | nb | nb | nb | nb |  | +++ | +++ | ++ | +++ | +++ | +++ |
| 47 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3[Neu5Acα2-6Galβ1-4GlcNAcβ1-6]GalNAca | ++ | +++ | +++ | ++ | +++ | +++ | +++ | ++ | ++ | ++ | +++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | nb | nb | nb |
| 48 | Neu5Acα2-6GalNAcα | nb | nb | nb | ++ | +++ | +++ | nb | nb | + | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 49 | Neu5Acα2-6Galβ | nb | nb | nb | + | ++ | ++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 50 | Neu5Acα2-6Galβ1-4Glcβ | nb | +++ | ++ | +++ | ++ | +++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 51 | Neu5Acα2-6Galβ1-4Glcβ | nb | ++ | nb | +++ | +++ | +++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 52 | Neu5Acα2-6Galβ1-4GlcNAcβ | nb | +++ | nb | +++ | +++ | ++ | + | nb | + | + | +++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | nb | nb | nb |
| 53 | Neu5Acα2-6Galβ1-4GlcNAcβ | + | +++ | nb | +++ | +++ | +++ | + | nb | ++ | ++ | +++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | + | nb | nb |
| 54 | Neu5Acα2-6GalNAcβ1-4GlcNAcβ | + | +++ | +++ | +++ | +++ | +++ | + | nb | ++ | + | ++ | nb | nb | nb | nb | nb | nb |  | ++ | ++ | nb | nb | nb | nb |
| 55 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3GalNAcα | +++ | +++ | nb | + | +++ | ++ | ++ | nb | ++ | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 56 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ | + | +++ | ++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ | + | nb | nb | nb | + |  | +++ | +++ | +++ | +++ | +++ | +++ |
| 57 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3GalNAcα | nb | ++ | nb | ++ | +++ | +++ | + | + | + | +++ | +++ | +++ | nb | nb | nb | nb | ++ |  | +++ | +++ | + | +++ | +++ | +++ |
| 58 | Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ | ++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |  | +++ | +++ | +++ | +++ | +++ | +++ |
| 59 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ | ++ | +++ | ++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |  | +++ | +++ | +++ | +++ | +++ | +++ |
| 60 | Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glcβ-Sp10 | nb | +++ | nb | nb | +++ | nb | +++ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 61 | Neu5Acα2-6[Galβ1-3]GalNAca | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 62 | Neu5Acα2-6Galβ1-4GlcNAcβ1-6[Galβ1-3]GalNAca | + | ++ | ++ | + | ++ | +++ | +++ | nb | +++ | ++ | +++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | nb | nb | nb |
| 63 | Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6[Galβ1-3]GalNAca | +++ | +++ | ++ | ++ | +++ | +++ | +++ | ++ | ++ | ++ | +++ | +++ | nb | nb | nb | nb | + |  | +++ | +++ | +++ | +++ | +++ | +++ |
| 64 | Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ | ++ | +++ | + | +++ | +++ | +++ | +++ | nb | ++ | ++ | ++ | nb | nb | nb | nb | nb | nb |  | +++ | +++ | nb | nb | nb | nb |
| 65 | Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ | + | +++ | ++ | ++ | +++ | +++ | +++ | nb | + | ++ | +++ | nb | nb | nb | nb | nb | nb |  | ++ | +++ | nb | nb | nb | nb |
| 66 | Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα | nb | nb | nb | nb | nb | nb | + | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 67 | Neu5Acα2-3(Neu5Acα2-6)GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 68 | Neu5Gcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 69 | Neu5Gcα2-3Galβ1-3(Fucα1-4)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 70 | Neu5Gcα2-3Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 71 | Neu5Gcα2-3Galβ1-4(Fucα1-3)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 72 | Neu5Gcα2-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 73 | Neu5Gcα2-6GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 74 | Neu5Gcα2-6Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 75 | Neu5Acα2-8Neu5Acα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 76 | Neu5Acα2-8Neu5Acα2-8Neu5Acα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 77 | Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 78 | Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 79 | Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 80 | Neu5Acα2-8Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 81 | Neu5Acα2-8Neu5Acβ-Sp17 | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 82 | Neu5Acα2-8Neu5Acα2-8Neu5Acβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 83 | Neu5Acβ2-6GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 84 | Neu5Acβ2-6Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 85 | Neu5Gcβ2-6Galβ1-4GlcNAc | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 86 | Galβ1-3(Neu5Acβ2-6)GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 87 | [9NAc]Neu5Acα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 88 | [9NAc]Neu5Acα2-6Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | +++ | nb | nb |
| 89 | Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 90 | Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 91 | Galβ1-4GlcNAcβ1-2Manα1-3[Galβ1-4GlcNAcβ1-2Manα1-6]Manβ1-4GlcNAcβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 92 | GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 93 | GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 94 | Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 95 | Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |
| 96 | Galβ1-3GalNAcα | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb | nb |  | nb | nb | nb | nb | nb | nb |

**Fig S1.** Consensus sequence alignment of A(H3N2) virus HAs by year. The human H3N2 HA gene sequences from GISAID ([www.gisaid.org](http://www.gisaid.org)) were downloaded and a consensus sequence was generated for each year. The alignment is annotated with potential glycosylation sites (highlighted green). In addition, the alignment includes three additional lines to annotate each residue if it contributes to; the surface of the HA trimer (srf); the HA1 antigenic sites (AS) and the receptor binding site (RBS). The Structures reported here were used to assess residue srf and RBS contributions, while antigenic sites were based on previous publications (29-32).

10 20 30 40 50 60 70 80 90 100

1968 QDLPGNDNST ATLCLGHHAV PNGTLVKTIT DDQIEVTNAT ELVQSSSTGK ICNNPHRILD GIDCTLIDAL LGDPHCDVFQ NETWDLFVER SKAFSNCYPY

1969 .......... .......... .......... N......... .......... .......... ..N....... .......... D......... ..........

1970 .......... .......... .......... N......... .......... .......... ..N....... .......... D......... ..........

1971 .......... .......... .......... N......... .......... .......... .......... .......G.. .......... ..........

1972 ..F....... .......... .......... N......... .......... .......... .......... .......G.. .......... ..........

1973 ..F....... .......... .......... N......... .......... .......... .......... .......G.. .......... ..........

1974 .......... .......... .......... N......... .......... .......... ..N....... .......G.. ..K....... ..........

1975 .......... .......... .......... N......... .......... .......... ..N....... .......G.. ..K....... ..........

1976 .......... .......... .......... N......... .......... ..D....... ..N....... .......G.. ..K....... ..........

1977 .N........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1978 .N........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1979 .N........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1980 .N........ .......... .......... N......... .........R ..DS...... .KN...V... .......G.. ..K....... ..........

1981 .N........ .......... .......... N......... .........R ..DS...... .KN...V... .......G.. ..K....... ..........

1982 .N........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1983 .N........ .......... .......... N......... .......A.R ..DS...... .KN....... .......G.. ..K....... ..........

1984 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1985 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1986 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ..........

1987 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ...Y......

1988 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. ..K....... ...Y......

1989 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1990 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1991 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1992 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1993 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1994 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1995 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1996 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1997 .K........ .......... .......... N......... .........R ..DS...... .KN....... .......G.. .KE....... ...Y......

1998 .K........ .......... .......... N......... .........R ..DS...... .EN....... .......G.. .KE....... ...Y......

1999 .K........ .......... .......... N......... .........R ..DS..Q... .EN....... .......G.. .KE....... ...Y......

2000 .K..V..... .......... .......... N.H....... .........R ..DS..Q... .EN....... .......G.. .KE....... .T.Y......

2001 .K........ .......... .......... N......... .........G ..DS..Q... .EN....... .......G.. .KE....... ...Y......

2002 .K........ .......... .......... N......... .........R ..DS..Q... .EN....... .......G.. .KE....... ...Y......

2003 .K........ .......... ....I..... N......... .........G ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2004 .K........ .......... ....I..... N......... .........G ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2005 .K........ .......... ....I..... N......... .........G ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2006 .K........ .......... ....I..... N......... .........G ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2007 .K........ .......... ....I..... N......... .........E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2008 .K........ .......... ....I..... N......... .........E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2009 .K........ .......... ....I..... N......... .........E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2010 .K........ .......... ....I..... N......... .........E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2011 .K........ .......... ....I..... N......... .........E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2012 .K........ .......... ....I..... N......... .........E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

2013 .K........ .......... ....I..... N.R....... ....N..I.E ..DS..Q... .EN....... ....Q..G.. .KK....... ...Y......

srf ssssssssss ss.s...sss sssssss... sssss.ssss s..sssssss ..ssssssss .ss....... ...ss.ssss sss.s....s ss.sss.s..

AS .......... .......... .......... .......... ........CC ..CC...... .......... .......... .......... ..........

RBS .......... .......... .......... .......... .......... .......... .......... .......... .......... .......R.. 110 120 130 140 150 160 170 180 190 200

1968 DVPDYASLRS LVASSGTLEF ITEGFTWTGV TQNGGSNACK RGPGSGFFSR LNWLTKSGST YPVLNVTMPN NDNFDKLYIW GVHHPSTNQE QTSLYVQASG

1969 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1970 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1971 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1972 .......... .......... .N........ .......... ...D...... ....Y..... .......... .......... .......D.. ..........

1973 .......... .......... .N........ .......... ...D...... ....Y..... .......... .......... .......D.. ..........

1974 .......... .......... .N...N.... .......... ...D...... ....Y..... .......... .......... .......D.. ..N.......

1975 .......... .......... .N...N.... .......... ...D...... ....Y..... .......... .......... .......DK. ..N.......

1976 .......... .......... .N...N.... ......S... ...DN..... ....Y..... ...Q...... ...S...... .......DK. ..D.......

1977 .......... .......... .N...N.... ......Y... ...DN..... ....Y..E.. .......... .......... .......DK. ..N.......

1978 .......... .......... .N...N.... ......Y... ...DNS.... ....Y..E.. .......... .......... .......DK. ..N.......

1979 .......... .......... .N...N.... ..S...Y... ..SDNS.... ....YE.E.K .......... .G........ .......DK. ..N...R...

1980 .......... .......... .N.S.N.... ..S...Y... ..SDNS.... ....YE.E.K .......... .G........ .......DK. ..N...R...

1981 .......... .......... .N...N.... ..S...Y... ..SDNS.... ....YE.E.K .......... .G........ .......DK. ..N...R...

1982 .......... .......... .N...N.... ..S...Y... ..SDNS.... ....YE.E.K ..A....... .G........ .......DK. ..N...R...

1983 .......... .......... .N...N.... ..S...Y... ..S.NS.... ....YE.E.K ..A....... .G........ .......DK. ..N...R...

1984 .......... .......... .N...N.... ..S...Y... ..SVNS.... ....YE.E.K .......... .GK....... .......DK. ..N...R...

1985 .......... .......... .N...N.... ..S...Y... ..SVNS.... ....YE.E.K ..A....... .GK....... .......DK. ..N...R...

1986 .......... .......... .N...N.... ..S...Y... ..SVNS.... ....YE.EYK ..A....... .GK....... .......EK. ..N...R...

1987 .......... .......... .N.D.N.... ..S...Y... ..SVNS.... ....HE.EYK ..A....... .GK....... .......DR. ..N...R...

1988 .......... .......... .N.D.N.... ..S...Y... ..SVNS.... ....HE.EYK ..A....... .GK....... .......DR. ..N...R...

1989 .......... .......... .N.D.N.... A.S...Y... ..SVNS.... ....HE.EYK ..A....... .GK....... .......DR. ..N...R...

1990 .......... .......... .N.D.N.... A.S...Y... ..SVNS.... ....HE.EYK ..A....... .GK....... .......DR. ......R...

1991 .......... .......... .N.D.N.... A.S.E.Y... ..SVKS.... ....HE.DYK ..A....... .GK....... .......DR. ......R...

1992 .......... .......... .N.D.N.... A.S.E.Y... ..SVKS.... ....HE.EYK ..A....... .GK....... .......DR. ......R...

1993 .......... .......... .N.D.N.... A.D.K.Y... ..SVNS.... ....H.LEYK ..A....... .GK....... .......DSD ......R...

1994 .......... .......... .N.D.N.... A.D.K.Y... ..SVNS.... ....H.LEYK ..A....... .GK....... .......DSD ......R...

1995 .......... .......... TN...N.... A.D.K.Y... ..SVNS.... ....H.LEYK ..A....... ..K....... .......DSD ..........

1996 .......... .......... TN.S.N.... A...T.Y... ..SVKS.... ....H.LEYK ..A....... ..K....... .......DSD ..........

1997 .......... .......... NN.S.N.... A...T.Y... .RSVKS.... ....H.LEYK ..A....... ..K....... .......DSD ..........

1998 .......... .......... NN.S.N.... A...T.Y... .RSIKS.... ....HQLKYK ..A....... ..K....... .......DSD .....A....

1999 .......... .......... NN.S.N.... A...T.S... .RSIKS.... ....HQLKYK ..A....... .EK....... .......DSD .I...A....

2000 .......... .......... NN.S.N.... A...T.S... .RSIKS.... ....HQLKYK ..A....... .EK....... .......DSD .I...A....

2001 .......... .......... NN.S.N.... A...T.S... .RSNKS.... ....HQLKYK ..A....... .EK....... .......DSD .I...A....

2002 .....V.... .......... NN.S.N.... A...T.S... .RSDKS.... ....HQLKYK ..A....... .EK....... .....G.DSD .I...A....

2003 .......... .......... NN.S.N.... ....T.S... .RSNKS.... .....HLKYK ..A....... .EK....... .....G.DSD .I...A....

2004 .......... .......... NN.S.N.... ....T.S... .RSNNS.... .....HLKFK ..A....... .EK....... .....G.DND .I...A....

2005 .......... .......... NN.S.N.... ....T.S... .RSNNS.... .....HLKFK ..A....... .EK....... .....G.DND .I...A....

2006 .......... .......... NN.S.N.... ....T.S... .RSNNS.... .....HLKFK ..A....... .EK....... .....G.DND .IF..A....

2007 .......... .......... NN.S.N.... ....T.S..I .RSNNS.... .....HLKFK ..A....... .EK....... .....G.DND .IF..A....

2008 .......... .......... NN.S.N.... ....T.S..I .RSNNS.... .....HLKFK ..A....... .EQ....... .....G.DND .IF..A....

2009 .......... .......... NN.S.N.... ....T.S..I .RSNNS.... .....HLNFK ..A....... .EQ....... .....G.DKD .IF..A....

2010 .......... .......... NN.S.N.... ....T.S..I .RSNNS.... .....HLNFK ..A....... .EQ....... .....G.DKD .IF..A....

2011 .......... .......... NN.S.N.... ....T.S..I .RSNNS.... .....HLNFK ..A....... .EQ....... .....G.DKD .IF..A....

2012 .......... .......... NN.S.N.... ....T.S..I .RSN.S.... .....HLNFK ..A....... .EQ....... .....G.DKD .IF..A.S..

2013 .......... .......... NN.S.N.A.. ....T.S..I ..SN.S.... .....HLNFK ..A....... .EQ....... .....G.DKD .IF..A.S..

srf .......... ....s...ss ssssssssss ssssssss.s sssssss.ss ..s.ssssss sssssssss. ssssss.... ..s..sssss .sss.ssss.

AS .......... .......... AAAAAAAAAA AAAAAAAA.A AAAAAA.... ....BBBBBB ......D... .......... .....BBBBB BBBB.BBBB.

RBS .......... .......... .........R RRRRRRRRR. ...RRR.... ..RRRRRRR. .......... .......... ..RRRRRRRR RRRRRR....

210 220 230 240 250 260 270 280 290 300

1968 RVTVSTRRSQ QTIIPNIGSR PWVRGLSSRI SIYWTIVKPG DVLVINSNGN LIAPRGYFKM RTGKSSIMRS DAPIDTCISE CITPNGSIPN DKPFQNVNKI

1969 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1970 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1971 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1972 ......K... .......... .......... .......... .I........ .......... .......... ....G..... .......... ..........

1973 ......K... .......... .......... .......... .I........ .......... .......... ....G..... .......... ..........

1974 ......K... .......... .......... .......... .I........ .......... .......... ....G..... .......... ..........

1975 ......K... ......V... .......... .......... .I........ .......... .......... ....G..S.. .......... ..........

1976 K.....K... ..V...V... .........V .......... .I........ .......... .......... ....G..S.. .......... ..........

1977 ......K... ......V... .......... .......... .I.L...... .........I .......... ....G..S.. .......... ..........

1978 ......K... ......V... .......... .......... .I.L...... .........I .......... ....G..S.. .......... ..........

1979 ......K... .......... .......... .......... .I.L...... .........I .......... ....G..S.. .......... ..........

1980 ......K... .......... .......... .......... .I.L...... .........I .......... ....G..S.. .......... ..........

1981 ......K... .......... .......... .......... .I.L...... .........I .......... ....G..S.. .......... ..........

1982 ......K... ..V.....P. .......... .......... .I.L...S.. .........I .......... ....G..S.. .......... ..........

1983 ......K... ..V.....P. .......... .......... .I.L...S.. .........I .......... ....G..S.. .......... ..........

1984 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ..........

1985 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ..........

1986 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ..........

1987 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ..........

1988 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ..........

1989 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ........R.

1990 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ........R.

1991 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ........R.

1992 ......K... ..V....... .......... .......... .I.L...T.. .........I .......... ....G..S.. .......... ........R.

1993 ......K... ..V....... .......... .......... .I.L...T.. .........I .N........ ....GN.S.. .......... ........R.

1994 ......K... ..V.....F. .......... .......... .I.L...T.. .........I .N........ ....GN.S.. .......... ........R.

1995 ......K... ..V....... .....I.... .......... .I.L...T.. .........I .N........ ....GN.N.. .......... ........R.

1996 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ ....GN.N.. .......... ........R.

1997 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ ....GN.N.. .......... ........R.

1998 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

1999 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2000 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ N...GK.N.. .......... ........R.

2001 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2002 ......K... ..V....... .....V.... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2003 .I....K... ..V....... .R..DV.... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2004 .I....K... ..V....... .R..DIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2005 .I....K... ..V....... .R..DIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2006 .I....K... ..V....... .R..NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2007 .I....K... ..V....... .R..NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2008 .I....K... ..V....... .R..NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2009 .I....K... ..V....... .R..NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2010 .I....K... .AV....... .R..NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2011 .I....K... .AV....... .R..NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2012 .I....K... .AV....... .RI.NIP... .......... .I.L...T.. .........I .S........ ....GK.N.. .......... ........R.

2013 .I....K... .AV....... .RI.NIP... .......... .I.L...T.. .........I .S........ ....GK.K.. .......... ........R.

srf s.....ss.. .......sss sssssssss. .......sss .s.s.s.s.. ....s.s.ss sssss...s. s.ssssss.s ...ss...ss ss...ssss.

AS DDDDDDDD.. ...D.DDDDD .DDDDDD... .......... .D........ .......... .........C CCCCCC.C.. .......... ..........

RBS .......... ........RR RRRRRRRR.. .......... .......... .......... .......... .......... .......... .......... 310 320 330 340 350 360 370 380 390 400

1968 TYGACPKYVK QNTLKLATGM RNVPEKQTRG LFGAIAGFIE NGWEGMIDGW YGFRHQNSEG TGQAADLKST QAAIDQINGK LNRVIEKTNE KFHQIEKEFS

1969 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1970 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1971 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1972 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1973 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1974 .......... .......... .......... I......... .......... .......... .......... .......... .......... ..........

1975 .......... .......... .......... I......... .......... .......... .......... .......... .......... ..........

1976 .......... .......... .......... I......... .......... .......... .......... .......... .......... ..........

1977 .......... .......... .......... I......... .......... .......... .......... .......... .......... ..........

1978 .......... .......... .......... I......... .......... .......... .......... .......... .......... ..........

1979 .......... .......... .......... I......... ......V... .......... .......... .......... .......... ..........

1980 .......... .......... .......... I......... ......V... .......... .......... .......... .......... ..........

1981 .......... .......... .......... I......... ......V... .......... .......... .......... .......... ..........

1982 .......... .......... .......... I......... ......V... .......... .......... .......... .......... ..........

1983 .......... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1984 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1985 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1986 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1987 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1988 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1989 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1990 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1991 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1992 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1993 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1994 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1995 ......R... .......... .......... I......... ......V... .......... .......... .......... ...L...... ..........

1996 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L...... ..........

1997 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L...... ..........

1998 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L...... ..........

1999 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L...... ..........

2000 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L...... ..........

2001 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L...... ..........

2002 ......R... .......... .......... I......... ......M... .......... .......... ....N..... ...L...... ..........

2003 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L.G.... ..........

2004 ......R... .......... .......... I......... ......V... .......... .......... ....N..... ...L.G.... ..........

2005 ......R... .......... .......... I......... ......V... .......... I......... ....N..... ...L.G.... ..........

2006 ......R... .......... .......... I......... ......V... .......... I......... ....N..... ...L.G.... ..........

2007 ......R... .......... .......... I......... ......V... .......... I......... .......... ...L.G.... ..........

2008 ......R... .......... .......... I......... ......V... .......... R......... .......... ...L.G.... ..........

2009 ......R... .......... .......... I......... ......V... .......... R......... .......... ...L.G.... ..........

2010 ......R... .......... .......... I......... ......V... .......... R......... .......... ...L.G.... ..........

2011 ......R... .......... .......... I......... ......V... .......... R......... .......... ...L.G.... ..........

2012 ......R... .S........ .......... I......... ......V... .......... R......... .......... ...L.G.... ..........

2013 ......R... .S........ .......... I......... ......V... .......... R......... .......... ...L.G.... ..........

srf .......s.s sss.s..s.. sssssssss. .....ss..s s..sssssss ..ssssssss ssssssss.s ss.ssssss. sss.ssss.s .s.....sss

AS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

RBS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

410 420 430 440 450 460 470 480 490 500

1968 EVEGRIQDLE KYVEDTKIDL WSYNAELLVA LENQHTIDLT DSEMNKLFEK TRRQLRENAE DMGNGCFKIY HKCDNACIES IRNGTYDHDV YRDEALNNRF

1969 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1970 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1971 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

1972 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1973 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1974 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1975 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1976 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1977 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1978 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1979 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1980 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1981 .......... .......... .......... .......... .......... .......... .......... ........G. .......... ..........

1982 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1983 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1984 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1985 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1986 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1987 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1988 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1989 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1990 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1991 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1992 .......... .......... .......... .......... .......... ..K....... .......... ........G. .......... ..........

1993 .......... .......... .......... .......... .........R ..K....... .......... ........G. .......... ..........

1994 .......... .......... .....G.... .......... .........R ..K....... .......... ........G. .......... ..........

1995 .......... .......... .......... .......... .........R ..K....... .......... ........G. .......... ..........

1996 .......... .......... .......... .......... .........R ..K....... .......... ........G. .......... ..........

1997 .......... .......... .......... .......... .........R ..K....... .......... ........G. .......... ..........

1998 .......... .......... .......... .......... .........R ..K....... .......... ........G. .......... ..........

1999 .......... .......... .......... .......... .........R ..K....... .......... ........G. .......... ..........

2000 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2001 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2002 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2003 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2004 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2005 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2006 .......... .......... .......... .......... .........R .KK....... .......... ........G. .......... ..........

2007 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

2008 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

2009 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

2010 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

2011 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

2012 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

2013 .......... .......... .......... .......... .......... .KK....... .......... ........G. .......... ..........

srf .......... .......... .......... .......... ..s..ss..s ..ss...... .s.s.s.s.. .s.sss..ss .sssssssss s.....ss..

AS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

RBS .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... 510 520 530 540 550

1968 QIKGVELKSG YKDWILWISF AISCFLLCVV LLGFIMWACQ RGNIRCNICI

1969 .......... .......... .......... .......... ..........

1970 .......... .......... .......... .......... ..........

1971 .......... .......... .......... .......... ..........

1972 .......... .......... .......... .......... K.........

1973 .......... .......... .......... .......... K.........

1974 .......... .......... .......... .......... K.........

1975 .......... .......... .......... .......... K.........

1976 .......... .......... .......... .......... K.........

1977 .......... .......... .......... .......... K.........

1978 .......... .......... .......... .......... K.........

1979 .......... .......... .......... .......... K.........

1980 .......... .......... .......... .......... K.........

1981 .......... .......... .......... .......... K.........

1982 .......... .......... .......... .......... K.........

1983 .......... .......... .......... .......... K.........

1984 .......... .......... .......... .......... K.........

1985 .......... .......... .......... .......... K.........

1986 .......... .......... .......... .......... K.........

1987 .......... .......... .......... .......... K.........

1988 .......... .......... .......... .......... K.........

1989 .......... .......... .......... .......... K.........

1990 .......... .......... .......... .......... K.........

1991 .......... .......... .......... .......... K.........

1992 .......... .......... .......... .......... K.........

1993 .......... .......... .......... .......... K.........

1994 .......... .......... .......... .......... K.........

1995 .......... .......... .......... .......... K.........

1996 .......... .......... .......... .......... K.........

1997 .......... .......... .......... .......... K.........

1998 .......... .......... .......... .......... K.........

1999 .......... .......... .......... .......... K.........

2000 .......... .......... .......... .......... K.........

2001 .......... .......... .......... .......... K.........

2002 .......... .......... ........I. .......... K.........

2003 .......... .......... .........A .......... K.........

2004 .......... .......... .........A .......... K.........

2005 .......... .......... .........A .......... K.........

2006 .......... .......... .........A .......... K.........

2007 .......... .......... .........A .......... K.........

2008 .......... .......... .........A .......... K.........

2009 .......... .......... .........A .......... K.........

2010 .......... .......... .........A .......... K.........

2011 .......... .......... .........A .......... K.........

2012 .......... .......... .........A .......... K.........

2013 .......... .......... .........A .......... K.........

srf s.s------- ---------- ---------- ---------- ----------

AS .......... .......... .......... .......... ..........

RBS .......... .......... .......... .......... ..........

**Fig S2.** Sequence alignment of A(H3N2) recHAs used in this study. The alignment is annotated as described in Figure S1 for surface residues (srf), antigenic sites (AS) and receptor binding site resdiues (RBS). At the end of each line the total number of potential glycosylation sites is listed.

1 10 20 30 40 50 60 70 80 90

. | | | | | | | | | |

HK68 QDLPGNDN-S TATLCLGHHA VPNGTLVKTI TDDQIEVTNA TELVQSSSTG KICNNPHRIL DGIDCTLIDA LLGDPHCDVF QNETWDLFVE RSKAFSNCYP

PtChalmers73 ..F.....-. .......... .......... .N........ .......... .......... ...N...... ........G. .......... ..........

Victoria75 ........N. .......... .......... .N........ .......... .......... ...N...... ........G. ...K...... ..........

Bangkok79 .N......-. .......... .......... .N........ .......... R..DS..... ..KN...... ........G. ...K...... ..........

Leningrad86 .K......-. .......... .......... .N........ .......... R..DS..... ..KN...... ........G. ...K....I. ..........

Shanghai87 .K......-. .......... .......... .N........ .......... R..DS..... ..KN...... ........G. ...K...... ....Y.....

Beijing92 .K......-. .......... .......... .N........ .......... R..DS..... ..KN...... ........G. ..KE...... ....Y.....

Johannesburg94 .K......-. .......... .......... .N........ .......P.. R..DS..... ..KN...... ........G. ..KE...... ....Y.....

Nanchang95 .K......-. .......... .......... .N........ .......... R..DS..... ..KN...... ........G. ..KE...... ....Y.....

Sydney97 .KI.....-. .......... .......... .N........ .......... R..DS..... ..EN...... ........G. ..KE...... ....Y.....

Panama99 .K......-. .......... .S........ .N........ .......... R..DS..Q.. ..EN...... ........G. ..KE...... ....Y.....

Wyoming03 .K......-. .......... .....I.... .N........ .......... G..DS..Q.. ..EN...... .....Q..G. ..KK...... ....Y.....

California04 .K......-. .......... .....I.... .N........ .......... G..DS..Q.. ..EN...... .....Q..G. ..KK...... ....Y.....

Hiroshima05 .K......-. .......... .....I.... .N........ .......... G..DS..Q.. ..EN...... .....Q..G. ..KK...... ....Y.....

Brisbane07 .K......-. .......... .....I.... .N........ .......... E..DS..Q.. ..EN...... .....Q..G. ..KK...... ....Y.....

Perth09 .K......-. .......... .....I.... .N........ .......... E..DS..Q.. ..KN...... .....Q..G. ..KK...... ....Y.....

Victoria11 .K......-. .......... .....I.... .N........ .....N..I. E..DS..Q.. ..EN...... .....Q..G. ..KK...... ....Y.....

srf ssssssssss ss.s...sss sssssss... sssss.ssss s..sssssss ..ssssssss .ss....... ...ss.ssss sss.s....s ss.sss.s..

AS .......... .......... .......... .......... ........CC ..CC...... .......... .......... .......... ..........

RBS .......... .......... .......... .......... .......... .......... .......... .......... .......... .......R..

100 110 120 130 140 150 160 170 180 190

. | | | | | | | | | |

HK68 YDVPDYASLR SLVASSGTLE FITEGFTWTG VTQNGGSNAC KRGPGSGFFS RLNWLTKSGS TYPVLNVTMP NNDNFDKLYI WGVHHPSTNQ EQTSLYVQAS

PtChalmers73 .......... .......... ..N....... .......... ....D..... .....Y.... A......... .......... ........D. ...N......

Victoria75 .......... .......... ..N...N... .......S.. ....D..... .....Y.... ....Q..... ....S..... ........DK ...N......

Bangkok79 .......... .......... ..N...N... ...S...Y.. ...SDNS... .....YE.E. K......... ..G....... ........DK ...N...R..

Leningrad86 .......... .......... ..N...N... ...S...YT. ...SVNS... .....YE.EY K..A...... ..GK...... ........EK ...N...R..

Shanghai87 .......... .......... ..N.D.N... ...S...Y.. ...SVNS... .....HE.EY K..A...... ..GK...... ........DR ...K...R..

Beijing92 .......... .......... ..N.D.N... .A.D...Y.. ...SVNS... .....H..EY K..A...... ...K...... ........DR D......R..

Johannesburg94 .......... .......... ..N.N.N... .A.D.K.Y.. ...SVNS... .....H.LEY K..A...... ..GK...... ........DS D......R..

Nanchang95 .......... .......... .TN...N... .A.D.T.Y.. ...SVKS... .....H.LEY K..A...... ...K...... ........DS V.........

Sydney97 .......... .......... .NN.S.N... .A...T.Y.. ..SSIKS... .....HQLKY K..A...... ...K...... ........DS D...I.A...

Panama99 .......... .......... .NN.S.N... .A...T.S.. ..RSNKS... .....HQLKY K..A...... ..EK...... ........DS D.I.I.A...

Wyoming03 .......... .......... .NN.S.N.A. .....T.S.. ..RSNKS... ......HLKY K..A...... ..EK...... ......G.DS D.I...A...

California04 .......... .......... .NN.S.N... .....T.SS. ..RSNNS... ......HLKF K..A...... ..EK...... ......G..N D.I...T...

Hiroshima05 .......... .......... .NN.S.N... .....T.S.. ..RSNNS... ......QLKF K..A.K.... ..EK...... ......V.DN D.IF..A...

Brisbane07 .......... .......... .NN.S.N... .....T.S.. I.RSNNS... ......HLKF K..A...... ..EK...... ......G.DN D.IF..A...

Perth09 .......... .......... .NN.S.N... .....T.S.. I.RSKNS... ......HLNF K..A...... ..EQ...... ......G.DK D.IF..A...

Victoria11 .......... .......... .NN.S.N... .....T.S.. I.RSNNS... ......HLNF K..A...... ..EQ...... ......G.DK D.IF..A.S.

srf .......... ....s...ss ssssssssss ssssssss.s sssssss.ss ..s.ssssss sssssssss. ssssss.... ..s..sssss .sss.ssss.

AS .......... .......... AAAAAAAAAA AAAAAAAA.A AAAAAA.... ....BBBBBB ......D... .......... .....BBBBB BBBB.BBBB.

RBS .......... .......... .........R RRRRRRRRR. ...RRR.... ..RRRRRRR. .......... .......... ..RRRRRRRR RRRRRR....

. 200 210 220 230 240 250 260 270 280 290

. | | | | | | | | | |

HK68 GRVTVSTRRS QQTIIPNIGS RPWVRGLSSR ISIYWTIVKP GDVLVINSNG NLIAPRGYFK MRTGKSSIMR SDAPIDTCIS ECITPNGSIP NDKPFQNVNK

PtChalmers73 .......K.. .......... .......... .......... ..I....... .......... .......... .....G.... .......... ..........

Victoria75 .K.....K.. .......V.. .......... .......... ..I....... .......... .......... .....G..S. .......... ..........

Bangkok79 .......K.. .......... .......... .......... ..I.L..... .......... I......... .....G..S. .......... ..........

Leningrad86 .......K.. ...V...... .......... .......... ..I.L...T. .......... I......... .....G..S. .......... ..........

Shanghai87 .......K.. ...V...... .......... .......... ..I.L...T. .......... I......... .....G..S. .......... ..........

Beijing92 .......K.. ...V...... .......... .......... ..I.L...T. .......... I......... .....G..S. .......... .........R

Johannesburg94 .......K.. ...V..D..Y ......Q... .......... ..I.L...T. .......... I.N....... .....GN.S. .......... .........R

Nanchang95 .......K.. ...V...... ......I... .......... ..I.L...T. .......... I.S....... .....GN.N. .......... .........R

Sydney97 .......K.. ...V...... ......I... ...H...... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

Panama99 .......K.. ...V...... S.....V... .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

Wyoming03 ..I....K.. ...V...... ..R..DV... .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

California04 ..I....K.. ...V...... ..R..DIP.. .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

Hiroshima05 ..I....K.. ...V...... ..R..NIP.. .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

Brisbane07 ..I....K.. ...V...... ..R..NIP.. .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

Perth09 ..I....K.. ...VS..... ..R..NIP.. .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

Victoria11 ..I....K.. ..AV...... ..RI.NIP.. .......... ..I.L...T. .......... I.S....... .....GK.N. .......... .........R

srf s.....ss.. .......sss sssssssss. .......sss .s.s.s.s.. ....s.s.ss sssss...s. s.ssssss.s ...ss...ss ss...ssss.

AS DDDDDDDD.. ...D.DDDDD .DDDDDD... .......... .D........ .......... .........C CCCCCC.C.. .......... ..........

RBS .......... ........RR RRRRRRRR.. .......... .......... .......... .......... .......... .......... ..........

. 300 310 320 330 340 350 360 370 380 390

. | | | | | | | | | |

HK68 ITYGACPKYV KQNTLKLATG MRNVPEKQTR GLFGAIAGFI ENGWEGMIDG WYGFRHQNSE GTGQAADLKS TQAAIDQING KLNRVIEKTN EKFHQIEKEF

PtChalmers73 .......... .......... .......... .I........ .......... .......... .......... .......... .......... ..........

Victoria75 .......... .......... .......... .I........ .......... .......... .......... .......... .......... ..........

Bangkok79 .......... .......... .......... .I........ .......V.. .......... .......... .......... .......... ..........

Leningrad86 .......R.. .......... .......... .I........ .......V.. .......... .......... .......... ....L..... ..........

Shanghai87 .......R.. .......... .......... .I........ .......V.. .......... .......... .......... ....L..... ..........

Beijing92 .......R.. .......... .......... .I........ .......V.. .......... .......... .......... ....L..... ..........

Johannesburg94 .......R.. .......... .......... .I........ .......V.. .......... .......... .......... ....L..... ..........

Nanchang95 .......R.. .......... .......... .I........ .......V.. .......... .......... .....N.... ....L..... ...P......

Sydney97 .......R.. .......... .......... .I........ .......V.. .......... .......... .....N.... ....L..... ..........

Panama99 .......R.. .......... .......... .I........ .......V.. .......... .......... .....N.... ....L..... ..........

Wyoming03 .......R.. .......... .......... .I........ .......V.. .......... .......... .....N.... ....L.G... ..........

California04 .......R.. .......... .......... .I........ .......V.. .......... .I........ .....N.... ....L.G... ..........

Hiroshima05 .......R.. .......... .......... .I........ .......V.. .......... .I........ .....N.... ....L.G... ..........

Brisbane07 .......R.. .......... .......... .I........ .......V.. .......... .I........ .......... ....L.G... ..........

Perth09 .......R.. .......... .......... .I........ .......V.. .......... .R........ .......... ....L.G... ..........

Victoria11 .......R.. ..S....... .......... .I........ .......V.. .......... .R........ .......... ....L.G... ..........

srf .......s.s sss.s..s.. sssssssss. .....ss..s s..sssssss ..ssssssss ssssssss.s ss.ssssss. sss.ssss.s .s.....sss

AS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

RBS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

. 400 410 420 430 440 450 460 470 480 490

. | | | | | | | | | |

HK68 SEVEGRIQDL EKYVEDTKID LWSYNAELLV ALENQHTIDL TDSEMNKLFE KTRRQLRENA EDMGNGCFKI YHKCDNACIE SIRNGTYDHD VYRDEALNNR

PtChalmers73 .......... .......... .......... .......... .......... .......... .......... .........G .......... ..........

Victoria75 .......... .......... .......... .......... .......... .......... .......... .........G .......... ..........

Bangkok79 .......... .......... .......... .......... .......... .......... .......... .........G .......... ..........

Leningrad86 .......... .......... .......... .....Y.... .......... ...K...... .......... .........G .......... ..........

Shanghai87 .......... .......... .......... .......... .......... ...K...... .......... .........G .......... ..........

Beijing92 .......... .......... .......... .......... .......... ...K...... .......... .........G .......... ..........

Johannesburg94 .......... .......... .......... .......... .......... R..K...... .......... .........G .......... ..........

Nanchang95 .......... .......... .......... .......... .......... R..K...... .......... .........G .......... ..........

Sydney97 .......... .......... .......... .......... .......... R..K...... .......... .........G .......... ..........

Panama99 .......... .......... .......... .......... .......... R.KK...... .......... .........G .......... ..........

Wyoming03 .......... .......... .......... .......... .......... R.KK...... .......... .......... .......... ..........

California04 .......... .......... .......... .......... .......... R.KK...... .......... .........G .......... ..........

Hiroshima05 .......... ......I... .......... .......... .......... R.KK...... .......... .........G .......... ..........

Brisbane07 .......... .......... .......... .......... .......... ..KK...... .......... .........G .......... ..........

Perth09 .......... .......... .......... .......... .......... ..KK...... .......... .........G .......... ..........

Victoria11 .......... .......... .......... .......... .......... ..KK...... .......... .........G .......... ..........

srf .......... .......... .......... .......... ..s..ss..s ..ss...... .s.s.s.s.. .s.sss..ss .sssssssss s.....ss..

AS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

RBS .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

500

| CHO

HK68 FQIK 7

PtChalmers73 .... 8

Victoria75 .... 8

Bangkok79 .... 8

Leningrad86 .... 9

Shanghai87 .... 9

Beijing92 .... 9

Johannesburg94 .... 10

Nanchang95 .... 9

Sydney97 .... 11

Panama99 .... 12

Wyoming03 .... 11

California04 .... 12

Hiroshima05 .... 11

Brisbane07 .... 12

Perth09 .... 11

Victoria11 .... 13

srf s.s-

AS ....

RBS ....

**Fig S3.** Consensus sequence alignment of the A(H3N2) virus RBS by year. Figure S1 was truncated to highlight only those residues in and around the receptor binding site. RBS residues that overlap onto antigenic sites (AS) are colored red for AS-A, yellow for AS-B, and blue for AS-D.

**98 131 140 144 146 153 159 183 196 219 228**

**| | | | | | | | | | |**

1968 Y TQNGGSNA-K GSG WLTKSGS H-PSTNQEQTSLYV SRPWVRGLSS

1969 . ........-K ... ....... .-............ ..........

1970 . ........-K ... ....... .-............ ..........

1971 . ........-K ... ....... .-............ ..........

1972 . ........-K D.. ..Y.... .-...D........ ..........

1973 . ........-K D.. ..Y.... .-...D........ ..........

1974 . ........-K D.. ..Y.... .-...D....N... ..........

1975 . ........-K DN. ..Y.... .-...D....N... ..........

1976 . ......S.-K DN. ..Y.... .-...DK...D... ..........

1977 . ......Y.-K DN. ..Y..E. .-...DK...N... ..........

1978 . ......Y.-K DNS ..Y..E. .-...DK...N... ..........

1979 . ..S...Y.-K DNS ..YE.E. .-...DK...N... ..........

1980 . ..S...Y.-K DNS ..YE.E. .-...DK...N... ..........

1981 . ..S...Y.-K DNS ..YE.E. .-...DK...N... ..........

1982 . ..S...Y.-K DNS ..YE.E. .-...DK...N... ..........

1983 . ..S...Y.-K VNS ..YE.E. .-...DK...N... ..........

1984 . ..S...Y.-K VNS ..YE.E. .-...DK...N... ..........

1985 . ..S...Y.-K VNS ..YE.E. .-...DK...N... ..........

1986 . ..S...Y.-K VNS ..YE.EY .-...DK...N... ..........

1987 . ..S...Y.-K VNS ..HE.EY .-...DR...N... ..........

1988 . ..S...Y.-K VNS ..HE.EY .-...DR...N... ..........

1989 . A.S...Y.-K VNS ..HE.EY .-...DR...N... ..........

1990 . A.S...Y.-K VNS ..HE.EY .-...DR...N... ..........

1991 . A.S.E.Y.-K VKS ..HE.DY .-...DR....... ..........

1992 . A.S.E.Y.-K VKS ..HE.EY .-...DR....... ..........

1993 . A.D.K.Y.-K VNS ..H.LEY .-...DSD...... ..........

1994 . A.D.K.Y.-K VNS ..H.LEY .-...DSD...... F.........

1995 . A.D.K.Y.-K VNS ..H.LEY .-...DSD...... .......I..

1996 . A.D.T.Y.-K VKS ..H.LEY .-...DSD...... .......V..

1997 . A...T.Y.-K VKS ..H.LEY .-...DSD...... .......V..

1998 . A...T.Y.-K IKS ..HQLKY .-...DSD.....A .......V..

1999 . A...T.S.-K IKS ..HQLKY .-...DSD.I...A .......V..

2000 . A...T.S.-K IKS ..HQLKY .-...DSD.I...A .......V..

2001 . A...T.S.-K NKS ..HQLKY .-...DSD.I...A .......V..

2002 . A...T.S.-K DKS ..HQLKY .-.G.DSD.I...A .......V..

2003 . ....T.S.-K NKS ...HLKY .-.G.DSD.I...A ...R..DV..

2004 . ....T.S.-K NNS ...HLKF .-.G.DND.I...A ...R..DIP.

2005 . ....T.S.-K NNS ...HLKF .-.G.DND.I...A ...R..DIP.

2006 . ....T.S.-K NNS ...HLKF .-.G.DND.IF..A ...R..NIP.

2007 . ....T.S.-I NNS ...HLKF .-.G.DND.IF..A ...R..NIP.

2008 . ....T.S.-I NNS ...HLKF .-.G.DND.IF..A ...R..NIP.

2009 . ....T.S.-I NNS ...HLNF .-.G.DKD.IF..A ...R..NIP.

2010 . ....T.S.-I NNS ...HLNF .-.G.DKD.IF..A ...R..NIP.

2011 . ....T.S.-I NNS ...HLNF .-.G.DKD.IF..A ...R..NIP.

2012 . ....T.S.-I NNS ...HLNF .-.G.DKD.IF..A ...RI.NIP.

2013 . ....T.S.-I N.S ...HLNF .-.G.DKD.IF..A ...RI.NIP.

**Fig S4.** Movie highlighting how the A(H3N2) RBS has changed in shape and charge since 1968. RBS residues colored red are negatively charged, white neutral and blue positively charged. Asn residues that make up a potential glycosylation site are colored orange