SUPPLEMENTAL DATA

Structure and receptor binding of the hemagglutinin from a human H6N1 influenza virus

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                                             10        20        30        40        50
Consensus sequence             MIAIIVIAILAAAGKSDKICIGYHANNSTTQVDTILEKNVTVTHSVELLENQKEERFCKI
A/Taiwan/2/2013                --------------PG..................L...................K.....
A/Chicken/Taiwan/A2837/2013    ....V......S......................L...................K.....
A/chicken/Taiwan/TC135/2010    --------------...R................L..........I....S...N...N.
A/chicken/Taiwan/ch1006/04     --------------....................L..........I........K.....
A/chicken/Taiwan/0305/04       --------------..............................................
A/chicken/Taiwan/0107/02       --------------....................L...............S...K.....
A/chicken/Taiwan/2838N/00      --------------..............................................
A/chicken/Taiwan/SP1/00        --------------...............................I..............
A/chicken/Taiwan/0705/99       --------------..............................................
A/chicken/Taiwan/G2/87         --------------..............................................
A/chicken/Taiwan/0222/02       ...........S......................L...................K.....
A/chicken/Taiwan/67/2013       ....V......S......................L...................K.....
A/chicken/Taiwan/G23/87        ...........TT...............................................
A/chicken/Taiwan/ch1006/04     ...........ST.....................L..........I........K.....
A/duck/Guangxi/3333/2006       ..............R.............................................
A/duck/Guangxi/GXd-5/2010      ...F........T.E...............K.............................
A/duck/Hong Kong/202/77        .....I..V..T................................................
A/duck/Hong Kong/3461/99       .....I......................................................
A/duck/Korea/S17/03            .....I.....S...........................I....................
A/duck/Taiwan/0526/72          ...........TT.........F.....................................
A/gray teal/Australia/1/1979   .....IF..V.STS...............................I....S.....L.RV
A/mallard/California/6695/2009 ........M..T..R.............................................
A/mallard/Jiangxi/7787/2003    ..............R.............................................
A/mallard/Sweden/30/2005       ............................................................

                                    60        70        80         90        100       110
Consensus sequence             LNKAPLDLRGCTIEGWILGNPQCDLLLGDQSWSYIVERPTAQNGICYPGVLNEVEELKAL
A/Taiwan/2/2013                M.......KD...........K.................N.............L.....F
A/Chicken/Taiwan/A2837/2013    M.......KD...........K.................N.............L.....F
A/chicken/Taiwan/TC135/2010    M........D...........K......................................
A/chicken/Taiwan/ch1006/04     M........D...........K.................N....................
A/chicken/Taiwan/0305/04       M..S.....E...........K...........................T..........
A/chicken/Taiwan/0107/02       M........D...........K......................................
A/chicken/Taiwan/2838N/00      MK.G.....E...........K......................................
A/chicken/Taiwan/SP1/00        M..S.....E...........K...........................A..........
A/chicken/Taiwan/0705/99       M..S.....E...........K...........................A..........
A/chicken/Taiwan/G2/87         ............................................................
A/chicken/Taiwan/0222/02       M........D...........K..I.....N.............................
A/chicken/Taiwan/67/2013       M.......KD...........K.................N.........A...L.....F
A/chicken/Taiwan/G23/87        ............................................................
A/chicken/Taiwan/ch1006/04     M........D...........K.................N....................
A/duck/Guangxi/3333/2006       ........GV....................K...................A.........
A/duck/Guangxi/GXd-5/2010      S........D..L........R.GI..A...........N.R.......I..GA......
A/duck/Hong Kong/202/77        .................................................I..........
A/duck/Hong Kong/3461/99       .................................................A.......R..
A/duck/Korea/S17/03            ........S............R....I.................................
A/duck/Taiwan/0526/72          ............................................................
A/gray teal/Australia/1/1979   ........K...............I..............G..H......A...L......
A/mallard/California/6695/2009 .................................................A..........
A/mallard/Jiangxi/7787/2003    .................................................A..........
A/mallard/Sweden/30/2005       ............................................................

                                     120         130      140        150       160
Consensus sequence             IGSGERVERFEMFPKSTWAGVDTSSGVTNACPSYTSGSSFYRNLLWIIKTKSAAYPVIKG
A/Taiwan/2/2013                ........................R..........ID.......V..V..D..T......
A/Chicken/Taiwan/A2837/2013    ........................R..........ID.......V..V..D..T......
A/chicken/Taiwan/TC135/2010    ........................R..........ID.......V..V..E.........
A/chicken/Taiwan/ch1006/04     ........................R..........ID.......V..V..E.........
A/chicken/Taiwan/0305/04       .....G.................N.........D.I........V.....N.........
A/chicken/Taiwan/0107/02       ........................R........S.ID.......V..V..E.........
A/chicken/Taiwan/2838N/00      .E......................R........D.ID.............G..E......
A/chicken/Taiwan/SP1/00        ...................................I........V.....N......N..
A/chicken/Taiwan/0705/99       ...................................I........V.....N.........
A/chicken/Taiwan/G2/87         ............................K...-.N...............G.........
A/chicken/Taiwan/0222/02       ........................R..........ID.......V..V..E.........
A/chicken/Taiwan/67/2013       ........................R..........ID.......V..V..D..T......
A/chicken/Taiwan/G23/87        ............................K...-.N...............G.........
A/chicken/Taiwan/ch1006/04     ........................R..........ID.......V..V..E.........
A/duck/Guangxi/3333/2006       ..................T.........K...-.H.........................
A/duck/Guangxi/GXd-5/2010      .......................N...SS...-LGN.P.S.........L..SE....R.
A/duck/Hong Kong/202/77        ............................K...-.N...........M..P..........
A/duck/Hong Kong/3461/99       ............................R...-.H..............P..........
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A/duck/Korea/S17/03            ..................K....N....K...-.........S.................
A/duck/Taiwan/0526/72          ............................K...-.N...............G.........
A/gray teal/Australia/1/1979   ...R...Q..........T....G....K..S-.N.......................R.
A/mallard/California/6695/2009 ..................T....N....R...-.S.........................
A/mallard/Jiangxi/7787/2003    ..................T.........K...-.N.........................
A/mallard/Sweden/30/2005       ............................K...-.N.........................

                                170       180       190       200       210       220
Consensus sequence             TYNNTGNQPILYFWGVHHPPDTNEQNTLYGSGDRYVRMGTESMNFAKSPEIAARPAVNGQ
A/Taiwan/2/2013                ......T............L..TV.DN......K..........................
A/Chicken/Taiwan/A2837/2013    ......T...............TV.DN......K..........................
A/chicken/Taiwan/TC135/2010    ......................TV.DN.................................
A/chicken/Taiwan/ch1006/04     ......................TV.DN.................................
A/chicken/Taiwan/0305/04       ....................S.GL.ES.........................E..V....
A/chicken/Taiwan/0107/02       ......................TV.DN.................................
A/chicken/Taiwan/2838N/00      ......................MV....................................
A/chicken/Taiwan/SP1/00        ....................N.GL.D..........................E..I....
A/chicken/Taiwan/0705/99       ....................N.GV.D......E.......D...........E..V....
A/chicken/Taiwan/G2/87         ..........................K.................................
A/chicken/Taiwan/0222/02       ......................TV.D..................................
A/chicken/Taiwan/67/2013       ......T...............TV.DN......K..........................
A/chicken/Taiwan/G23/87        ..........................K.................................
A/chicken/Taiwan/ch1006/04     ......................TV.DN.................................
A/duck/Guangxi/3333/2006       ..S.................................K..........G............
A/duck/Guangxi/GXd-5/2010      .F....DKS...........V.T..EA.....N.............R.............
A/duck/Hong Kong/202/77        ......S.....................................................
A/duck/Hong Kong/3461/99       ..S...................S............I.........V..............
A/duck/Korea/S17/03            ..S.P..........................................G............
A/duck/Taiwan/0526/72          ..........................K.................................
A/gray teal/Australia/1/1979   ......S.S...................................................
A/mallard/California/6695/2009 ............................................................
A/mallard/Jiangxi/7787/2003    ............................................................
A/mallard/Sweden/30/2005       ............................................................

                                230       240       250       260         270       280
Consensus sequence             RGRIDYYWSVLKPGETLNVESNGNLIAPWYAYKFVSTNKKGAVFKSNLPIENCDATCQTI
A/Taiwan/2/2013                .S.........R..................................D.............
A/Chicken/Taiwan/A2837/2013    .S.........R..................................D.............
A/chicken/Taiwan/TC135/2010    .S..........................................................
A/chicken/Taiwan/ch1006/04     .S............................................D.............
A/chicken/Taiwan/0305/04       .............................H..............................
A/chicken/Taiwan/0107/02       .S..........................................................
A/chicken/Taiwan/2838N/00      .S..........................................................
A/chicken/Taiwan/SP1/00        ....G.............A.........................................
A/chicken/Taiwan/0705/99       ............................................................
A/chicken/Taiwan/G2/87         ......................................S.........S......K....
A/chicken/Taiwan/0222/02       .S..........................................................
A/chicken/Taiwan/67/2013       .S.........R........................K.........D.............
A/chicken/Taiwan/G23/87        ............................................................
A/chicken/Taiwan/ch1006/04     .S............................................D.............
A/duck/Guangxi/3333/2006       ..................................I...N.....................
A/duck/Guangxi/GXd-5/2010      ......F..I......................R..NKDSN..I.R..............T
A/duck/Hong Kong/202/77        ..................................F...N.....................
A/duck/Hong Kong/3461/99       ......................................D.....................
A/duck/Korea/S17/03            ................................R.....N....................T
A/duck/Taiwan/0526/72          ......................................S.........S......K....
A/gray teal/Australia/1/1979   .........I........................I.S.N............D..TA...V
A/mallard/California/6695/2009 ......................................N.....................
A/mallard/Jiangxi/7787/2003    ......................................N.....................
A/mallard/Sweden/30/2005       ................................R.....N.R..........S........

                                 290        300       310       320         1       10
Consensus sequence             AGVLRTNKTFQNVSPLWIGECPKYVKSESLRLATGLRNVPQIETRGLFGAIAGFIEGGWT
A/Taiwan/2/2013                T.........................................A...I.............
A/Chicken/Taiwan/A2837/2013    ....K.....................................A...I.............
A/chicken/Taiwan/TC135/2010    ...........................K.............V....I.............
A/chicken/Taiwan/ch1006/04     ..........................................A...I.............
A/chicken/Taiwan/0305/04       V.....................................I.......I.............
A/chicken/Taiwan/0107/02       ..............................................I.............
A/chicken/Taiwan/2838N/00      E.............................................ILR...........
A/chicken/Taiwan/SP1/00        ...................K..................I...K...I.............
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A/chicken/Taiwan/0705/99       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . I . . . K . . . I . . . . . . . . . . . . .
A/chicken/Taiwan/G2/87         . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0222/02       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . I . . . . . . . . . . . . .
A/chicken/Taiwan/67/2013       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . I . . . . . . . . . . . . .
A/chicken/Taiwan/G23/87        . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . I . . . K . . . I . . . . . . . . . . . . .
A/chicken/Taiwan/ch1006/04     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . A . . . I . . . . . . . . . . . . .
A/duck/Guangxi/3333/2006       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Guangxi/GXd-5/2010      E . . I . . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Hong Kong/202/77        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Hong Kong/3461/99       . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Korea/S17/03            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . P . . . . . . . . . . . . . . . .
A/duck/Taiwan/0526/72          . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/gray teal/Australia/1/1979   . . A . . . . . . . . . . . . . . . . . . . . . . . . D . . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . .
A/mallard/California/6695/2009 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Jiangxi/7787/2003    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Sweden/30/2005       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .


                                     20                  30                  40                  50                  60                  70
Consensus sequence             G M I D G W Y G Y H H E N S Q G S G Y A A D R E S T Q K A I D G I T N K V N S I I D K M N T Q F E A V D H E F S N L E R
A/Taiwan/2/2013                . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/Chicken/Taiwan/A2837/2013    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/TC135/2010    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . T . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/ch1006/04     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0305/04       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0107/02       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/2838N/00      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/SP1/00        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0705/99       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/G2/87         . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0222/02       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/67/2013       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/G23/87        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/ch1006/04     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . .
A/duck/Guangxi/3333/2006       . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Guangxi/GXd-5/2010      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . . . . .
A/duck/Hong Kong/202/77        . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Hong Kong/3461/99       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Korea/S17/03            . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Taiwan/0526/72          . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/gray teal/Australia/1/1979   . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . E . . . . . . . .
A/mallard/California/6695/2009 . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Jiangxi/7787/2003    . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Sweden/30/2005       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .


                                        80                    90                 100                 110                  120                130
Consensus sequence             R I D N L N K R M E D G F L D V W T Y N A E L L V L L E N E R T L D L H D A N V K N L Y E K V K S Q L R D N A N D L G N
A/Taiwan/2/2013                . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/Chicken/Taiwan/A2837/2013    . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/TC135/2010    . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/ch1006/04     . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0305/04       . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0107/02       . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/2838N/00      . . G . . . . . . . . . . . E . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/SP1/00        . . G . . . . . . . . . . . . . . . . . . . . . . . . . D . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0705/99       . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/G2/87         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/0222/02       . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . .
A/chicken/Taiwan/67/2013       . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/G23/87        . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/chicken/Taiwan/ch1006/04     . . G . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Guangxi/3333/2006       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Guangxi/GXd-5/2010      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . H . . . R . . . . . . . . . . . .
A/duck/Hong Kong/202/77        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Hong Kong/3461/99       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . M . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Korea/S17/03            . . . . . . . . X . . . . . . . . . . . . . . . . . . . . . . . . . X . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Taiwan/0526/72          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . Q . . . . . . . . . . . . . . . . . . . . . . . . .
A/gray teal/Australia/1/1979   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . K . . . .
A/mallard/California/6695/2009 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Jiangxi/7787/2003    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Sweden/30/2005       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
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                                    140                 150                 160                 170
Consensus sequence            G C F E F W H K C D N E C I E S V K N G T Y D Y P K Y Q D E S K L N R Q E I E
A/Taiwan/2/2013               . . . . . . . . . . . . . M . . . . . . . . . . . . . . K . . . . . . . G . .
A/Chicken/Taiwan/A2837/2013   . . . . . . . . . . . . . M . . . . . . . . . . . . . . K . . . . . . . G . .
A/chicken/Taiwan/TC135/2010   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . R . .
A/chicken/Taiwan/ch1006/04    . . . . . . . . . . . . . . . . . . . . . . . . . . . . E . . . . . . . G . .
A/chicken/Taiwan/0305/04      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . K V .
A/chicken/Taiwan/0107/02      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . .
A/chicken/Taiwan/2838N/00     . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . .
A/chicken/Taiwan/SP1/00       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . H K . .
A/chicken/Taiwan/0705/99      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . H K . .
A/chicken/Taiwan/G2/87        . . . . . . . . . . . . . . . . . . . . . . . . . . . . N . . . . . . . . . .
A/chicken/Taiwan/0222/02      . . . . . . . . . . . . . . . . . E . . . . . . . . . . . . . . . . . . G . .
A/chicken/Taiwan/67/2013      . . . . . . . . . . . . . M . . . . . . . . . . . . . . K . . . . . . . G . .
A/chicken/Taiwan/G23/87       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . H K . .
A/chicken/Taiwan/ch1006/04    . . . . . . . . . . . . . . . . . . . . . . . . . . . . E . . . . . . . G . .
A/duck/Guangxi/3333/2006      . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . . . . . . . . .
A/duck/Guangxi/GXd-5/2010     . . . . . . . . . . . . . M . . . . . . . . . . . . . . T . . R . . . . K . .
A/duck/Hong Kong/202/77       . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Hong Kong/3461/99      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Korea/S17/03           . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/duck/Taiwan/0526/72         . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/gray teal/Australia/1/1979  . . . . . . . . . . D . . . N . . . . . . . . . . . . . . . . . . . . . . . D
A/mallard/California/6695/2009. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Jiangxi/7787/2003   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
A/mallard/Sweden/30/2005      . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
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B

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                                  10        20        30        40        50
H6N1_A/Taiwan/2/2013              KSDKICIGYHANNSTTQVDTLLEKNVTVTHSVELLENQKEKRFCKIMNKA
H1N1_A/Brevig_Mission/1/1918      NADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDSHNGKLCKLKGIA
H1N1_A/California/04/2009         NADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVA
H2N2_A/Singapore/1/1957           RGDQICIGYHANNSTEKVDTILERNVTVTHAKDILEKTHNGKLCKLNGIP
H2N2_A/Japan/305/1957             RGDQICIGYHANNSTEKVDTNLERNVTVTHAKDILEKTHNGKLCKLNGIP
H3N2_A/Hong_Kong/1/1968           STATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGKICNNP-HR
H5N1_A/Vietnam/CL12/2004          KSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVK
H5N1_A/Indonesia/5/2005           KSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVK
H7N9_A/Shanghai/02/2013           NADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKG-KR
H7N9_A/Hangzhou/1/2013            NADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKG-KR

                                   60        70        80         90        100
H6N1_A/Taiwan/2/2013              PLDLKDCTIEGWILGNPKCDLLLGDQSWSYIVERPNAQNGICYPGVLNEL
H1N1_A/Brevig_Mission/1/1918      PLQLGKCNIAGWLLGNPECDLLLTASSWSYIVETSNSENGTCYPGDFIDY
H1N1_A/California/04/2009         PLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDY
H2N2_A/Singapore/1/1957           PLELGDCSIAGWLLGNPECDRLLSVPEWSYIMEKENPRDGLCYPGSFNDY
H2N2_A/Japan/305/1957             PLELGDCSIAGWLLGNPECDRLLSVPEWSYIMEKENPRDGLCYPGSFNDY
H3N2_A/Hong_Kong/1/1968           ILDGIDCTLIDALLGDPHCDVFQ-NETWDLFVERSKAFSN-CYPYDVPDY
H5N1_A/Vietnam/CL12/2004          PLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPVNDLCYPGDFDDY
H5N1_A/Indonesia/5/2005           PLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDY
H7N9_A/Shanghai/02/2013           TVDLGQCGLLGTITGPPQCDQFL-EFSADLIIERREGSDV-CYPGKFVNE
H7N9_A/Hangzhou/1/2013            TVDLGQCGLLGTITGPPQCDQFL-EFSADLIIERREGSDV-CYPGKFVNE

                                    110       120          130        140        150
H6N1_A/Taiwan/2/2013              EELKAFIGSGERVERFEMFP-KSTWAGVDTSRGVTNACPSYTIDSSFYRN
H1N1_A/Brevig_Mission/1/1918      EELREQLSSVSSFEKFEIFPKTSSWPNHETTKGVTAACSYAGA-SSFYRN
H1N1_A/California/04/2009         EELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAACPHAGA-KSFYKN
H2N2_A/Singapore/1/1957           EELKHLLSSVKHFEKVKILP-KDRWTQH-TTTGGSRACAVSGN-PSFFRN
H2N2_A/Japan/305/1957             EELKHLLSSVKHFEKVKILP-KDRWTQH-TTTGGSRACAVSGN-PSFFRN
H3N2_A/Hong_Kong/1/1968           ASLRSLVASSGTLEFITEGF---TWTGV-TQNGGSNACKRGPG-SGFFSR
H5N1_A/Vietnam/CL12/2004          EELKHLLSRINHFEKIQIIP-KSSWSSHEASLGVSSACPYQGK-SSFFRN
H5N1_A/Indonesia/5/2005           EELKHLLSRINHFEKIQIIP-KSSWSDHEASSGVSSACPYLGS-PSFFRN
H7N9_A/Shanghai/02/2013           EALRQILRESGGIDKEAMGF---TYSGI-RTNGATSACRR-SG-SSFYAE
H7N9_A/Hangzhou/1/2013            EALRQILRESGGIDKEAMGF---TYSGI-RTNGATSACRR-SG-SSFYAE

                                            160       170       180       190
H6N1_A/Taiwan/2/2013              LVWIVKTDS--ATYPVIKGTYNNTGTQPILYFWGVHHPLDTTVQDNLYGS
H1N1_A/Brevig_Mission/1/1918      LLWLTKK-G--SSYPKLSKSYVNNKGKEVLVLWGVHHPPTGTDQQSLYQN
H1N1_A/California/04/2009         LIWLVKK-G--NSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSLYQN
H2N2_A/Singapore/1/1957           MVWLTEK-G--SNYPVAKGSYNNTSGEQMLIIWGVHHPNDEKEQRTLYQN
H2N2_A/Japan/305/1957             MVWLTKE-G--SDYPVAKGSYNNTSGEQMLIIWGVHHPIDETEQRTLYQN
H3N2_A/Hong_Kong/1/1968           LNWLTKS-G--STYPVLNVTMPNNDNFDKLYIWGVHHPSTNQEQTSLYVQ
H5N1_A/Vietnam/CL12/2004          VVWLIKK-N--STYPTIKRSYNNTNQEDLLVMWGIHHPNDAAEQTKLYQN
H5N1_A/Indonesia/5/2005           VVWLIKK-N--STYPTIKKSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQN
H7N9_A/Shanghai/02/2013           MKWLLSN-TDNAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGS
H7N9_A/Hangzhou/1/2013            MKWLLSN-TDNAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGS

                                  200       210       220       230       240
H6N1_A/Taiwan/2/2013              GDKYVRMGTESMNFAKSPEIAARPAVNGQRSRIDYYWSVLRPGETLNVES
H1N1_A/Brevig_Mission/1/1918      ADAYVSVGSSKYNRRFTPEIAARPKVRDQAGRMNYYWTLLEPGDTITFEA
H1N1_A/California/04/2009         ADTYVFVGSSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEA
H2N2_A/Singapore/1/1957           VGTYVSVGTSTLNKRSTPDIATRPKVNGLGSRMEFSWTLLDMWDTINFES
H2N2_A/Japan/305/1957             VGTYVSVGTSTLNKRSTPEIATRPKVNGQGGRMEFSWTLLDMWDTINFES
H3N2_A/Hong_Kong/1/1968           ASGRVTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINS
H5N1_A/Vietnam/CL12/2004          PTTYISVGTSTLNQRLVPRIATRSKVNGQSGRMEFFWTILKPNDAINFES
H5N1_A/Indonesia/5/2005           PTTYISIGTSTLNQRLVPKIATRSKVNGQSGRMEFFWTILKPNDAINFES
H7N9_A/Shanghai/02/2013           GNKLVTVGSSNYQQSFVPSPGARPQVNGLSGRIDFHWLMLNPNDTVTFSF
H7N9_A/Hangzhou/1/2013            GNKLVTVGSSNYQQSFVPSPGARPQVNGISGRIDFHWLMLNPNDTVTFSF

                                  250       260         270        280       290
H6N1_A/Taiwan/2/2013              NGNLIAPWYAYKFVSTNKKGAVFKSDLPIEN-CDATCQTITGVLRTNKTF
H1N1_A/Brevig_Mission/1/1918      TGNLIAPWYAFALNRG-SGSGIITSDAPVHD-CNTKCQTPHGAINSSLPF
H1N1_A/California/04/2009         TGNLVVPRYAFAMERN-AGSGIIISDTPVHD-CNTTCQTPKGAINTSLPF
H2N2_A/Singapore/1/1957           TGNLIAPEYGFKISKR-GSSGIMKTEGTLEN-CETKCQTPLGAINTTLPF
H2N2_A/Japan/305/1957             TGNLIAPEYGFKISKR-GSSGIMKTEGTLEN-CETKCQTPLGAINTTLPF
H3N2_A/Hong_Kong/1/1968           NGNLIAPRGYFKMRTG--KSSIMRSDAPIDT-CISECITPNGSIPNDKPF
H5N1_A/Vietnam/CL12/2004          NGNFIAPEYAYKIVKK-GDSTIMKSELEYGN-CNTKCQTPMGAINSSMPF
H5N1_A/Indonesia/5/2005           NGNFIAPEYAYKIVKK-GDSAIMKSELEYGN-CNTKCQTPMGAINSSMPF
H7N9_A/Shanghai/02/2013           NGAFIAPDRASFLRGK---SMGIQSGVQVDANCEGDCYHSGGTIISNLPF
H7N9_A/Hangzhou/1/2013            NGAFIAPDRASFLRGK---SMGIQSGVQVDANCEGDCYHSGGTIISNLPF
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                                     300       310       320             1       10
H6N1_A/Taiwan/2/2013              QNVSPLWIGECPKYVKSESLRLATGLRNVPQI-ATR---GIFGAIAGFIE
H1N1_A/Brevig_Mission/1/1918      QNIHPVTIGECPKYVRSTKLRMATGLRNIPSI-QSR---GLFGAIAGFIE
H1N1_A/California/04/2009         QNIHPITIGKCPKYVKSTKLRLATGLRNIPSI-QSR---GLFGAIAGFIE
H2N2_A/Singapore/1/1957           HNVHPLTIGECPKYVKSEKLVLATGLRNVPQI-ESR---GLFGAIAGFIE
H2N2_A/Japan/305/1957             HNVHPLTIGECPKYVKSEKLVLATGLRNVPQI-ESR---GLFGAIAGFIE
H3N2_A/Hong_Kong/1/1968           QNVNKITYGACPKYVKQNTLKLATGMRNVPEK-QTR---GLFGAIAGFIE
H5N1_A/Vietnam/CL12/2004          HNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRRKKRGLFGAIAGFIE
H5N1_A/Indonesia/5/2005           HNIHPLTIGECPKYVKSNRLVLATGLRNSPQRESRRKKRGLFGAIAGFIE
H7N9_A/Shanghai/02/2013           QNIDSRAVGKCPRYVKQRSLLLATGMKNVPEI-PKGR--GLFGAIAGFIE
H7N9_A/Hangzhou/1/2013            QNIDSRAVGKCPRYVKQRSLLLATGMKNVPEI-PKGR--GLFGAIAGFIE

                                         20        30        40        50        60
H6N1_A/Taiwan/2/2013              GGWTGMIDGWYGYHHENSQGSGYAADRESTQKAIDGITNKVNSIINKMNT
H1N1_A/Brevig_Mission/1/1918      GGWTGMIDGWYGYHHQNEQGSGYAADQKSTQNAIDGITNKVNSVIEKMNT
H1N1_A/California/04/2009         GGWTGMVDGWYGYHHQNEQGSGYAADLKSTQNAIDEITNKVNSVIEKMNT
H2N2_A/Singapore/1/1957           GGWQGMIDGWYGYHHSNDQGSGYAADKESTQKAFDGITNKVNSVIEKMNT
H2N2_A/Japan/305/1957             GGWQGMVDGWYGYHHSNDQGSGYAADKESTQKAFDGITNKVNSVIEKMNT
H3N2_A/Hong_Kong/1/1968           NGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNE
H5N1_A/Vietnam/CL12/2004          GGWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNT
H5N1_A/Indonesia/5/2005           GGWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNT
H7N9_A/Shanghai/02/2013           NGWEGLIDGWYGFRHQNAQGEGTAADYKSTQSAIDQITGKLNRLIEKTNQ
H7N9_A/Hangzhou/1/2013            NGWEGLIDGWYGFRHQNAQGEGTAADYKSTQSAIDQITGKLNRLIEKTNQ

                                         70        80        90       100       110
H6N1_A/Taiwan/2/2013              QFEAVDHEFSNLERRIGNLNKRMEDGFLDVWTYNAELLVLLENERTLDLH
H1N1_A/Brevig_Mission/1/1918      QFTAVGKEFNNLERRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDFH
H1N1_A/California/04/2009         QFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYH
H2N2_A/Singapore/1/1957           QFEAVGKEFSNLERRLENLNKKMEDGFLDVWTYNAELLVLMENERTLDFH
H2N2_A/Japan/305/1957             QFEAVGKEFGNLERRLENLNKRMEDGFLDVWTYNAELLVLMENERTLDFH
H3N2_A/Hong_Kong/1/1968           KFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLT
H5N1_A/Vietnam/CL12/2004          QFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFH
H5N1_A/Indonesia/5/2005           QFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFH
H7N9_A/Shanghai/02/2013           QFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAELLVAMENQHTIDLA
H7N9_A/Hangzhou/1/2013            QFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAELLVAMENQHTIDLA

                                        120       130       140       150       160
H6N1_A/Taiwan/2/2013              DANVKNLYEKVKSQLRDNANDLGNGCFEFWHKCDNECMESVKNGTYDYPK
H1N1_A/Brevig_Mission/1/1918      DSNVRNLYEKVKSQLKNNAKEIGNGCFEFYHKCDDACMESVRNGTYDYPK
H1N1_A/California/04/2009         DSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPK
H2N2_A/Singapore/1/1957           DSNVKNLYDKVRMQLRDNVKELGNGCFEFYHKCDDECMNSVKNGTYDYPK
H2N2_A/Japan/305/1957             DSNVKNLYDKVRMQLRDNVKELGNGCFEFYHKCDDECMNSVKNGTYDYPK
H3N2_A/Hong_Kong/1/1968           DSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNGTYDHDV
H5N1_A/Vietnam/CL12/2004          DSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQ
H5N1_A/Indonesia/5/2005           DSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESIRNGTYNYPQ
H7N9_A/Shanghai/02/2013           DSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSK
H7N9_A/Hangzhou/1/2013            DSEMDKLYERVKRQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSK

                                        170
H6N1_A/Taiwan/2/2013              YQKESKLNRQGIESVK
H1N1_A/Brevig_Mission/1/1918      YSEESKLNREEIDGVK
H1N1_A/California/04/2009         YSEEAKLNREEIDGVK
H2N2_A/Singapore/1/1957           YEEESKLNRNEIKGVK
H2N2_A/Japan/305/1957             YEEESKLNRNEIKGVK
H3N2_A/Hong_Kong/1/1968           YRDEALNNRFQIKGVE
H5N1_A/Vietnam/CL12/2004          YSEEARLKREEISGVK
H5N1_A/Indonesia/5/2005           YSEEARLKREEISGVK
H7N9_A/Shanghai/02/2013           YREEAMQNRIQIDPVK
H7N9_A/Hangzhou/1/2013            YREEAMQNRIQIDPVK
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Figure S1, related to Figure 1. **Sequence Alignment of Taiwan2 H6 HA with Other H6 HAs and with HA Subtypes that Have Infected Humans.**

(A) Sequence alignment of representative full-length H6N1 HA sequences deposited in NCBI influenza virus database. The Taiwan 2 HA sequence is marked in blue. Residues that contribute to receptor binding are highlighted in bold (H3 numbering). (B) Alignment of the sequence of H6 HA of the Taiwan2 virus isolated from a human (in blue) with sequences of HAs of human pandemic viruses (H1N1, H2N2 and H3N2) and avian zoonotic viruses H5N1 and H7N9. Residues that contribute to receptor binding are highlighted in bold (H3 numbering). The alignment scores are shown in Table S2.

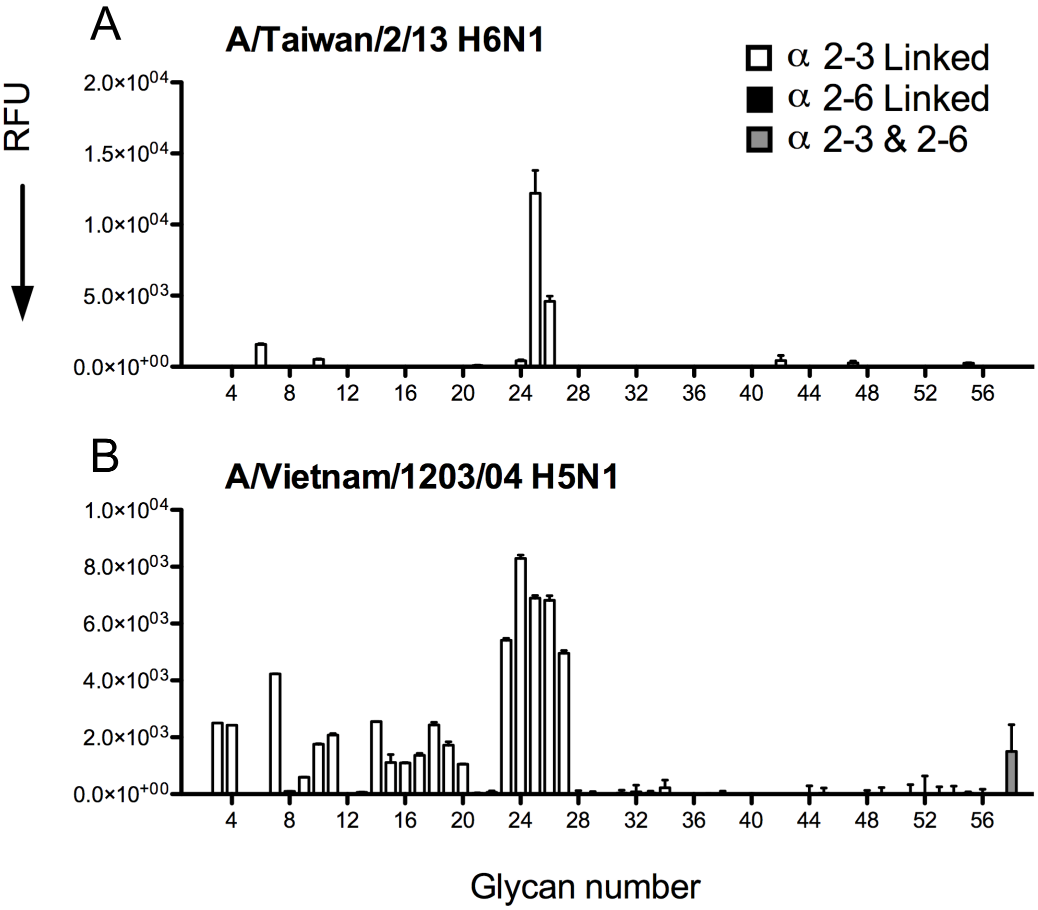

Figure S2, related to Figure 2. Receptor Binding Specificity of Taiwan2 H6N1 HA
(A-B) Glycan microarray analysis of recombinant Taiwan2 H6 (A) and A/Vietnam/1203/04 H5N1 (B) HAs, expressed in mammalian cells, indicates specific binding to α2-3 sialosides. The mean signal and standard error were calculated from six independent replicates on the array. Nonsialylated control glycans are glycans 1 and 2 on the x axis, α2-3 linked sialosides are in white bars (glycans 3 to 35), α2-6 linked sialosides in black bars (glycans 36 to 56), and mixed biantennary glycans containing both α2-3 and α2-6–linked sialylated glycans are in gray bars (glycans 57 and 58). Glycans imprinted on the array are listed in Table S3.

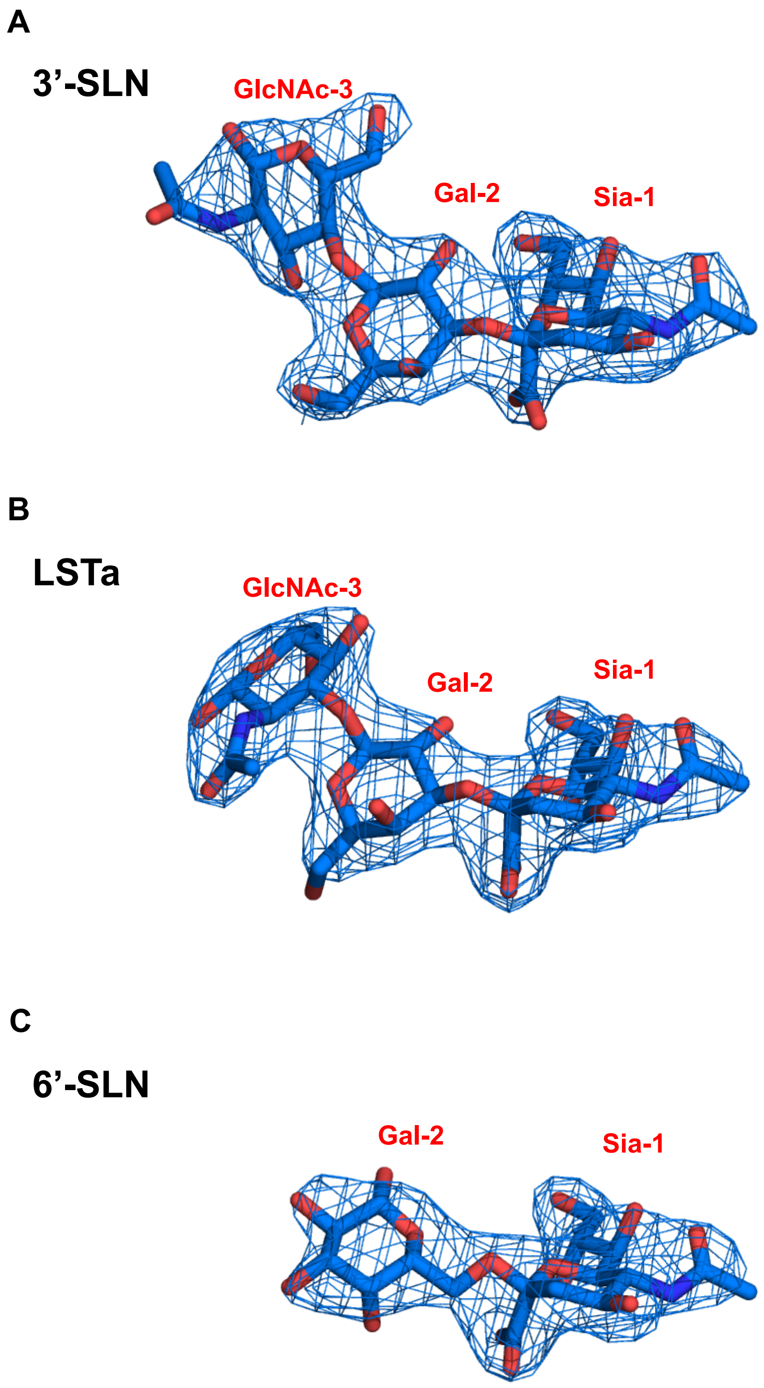

Figure S3, related to Figures 3 and 4. Electron Density Maps for the Receptor Analogs in Taiwan2 H6 HA Crystal Structures
(A-C) Electron density maps (2Fo-Fc) of avian receptor analogues 3’-SLN (A) and LSTa (B) and human receptor analog 6’-SLN (C) in crystal structures of Taiwan2 H6 wild type HA. Density corresponding only to the receptor analog is shown at a 1σ level.

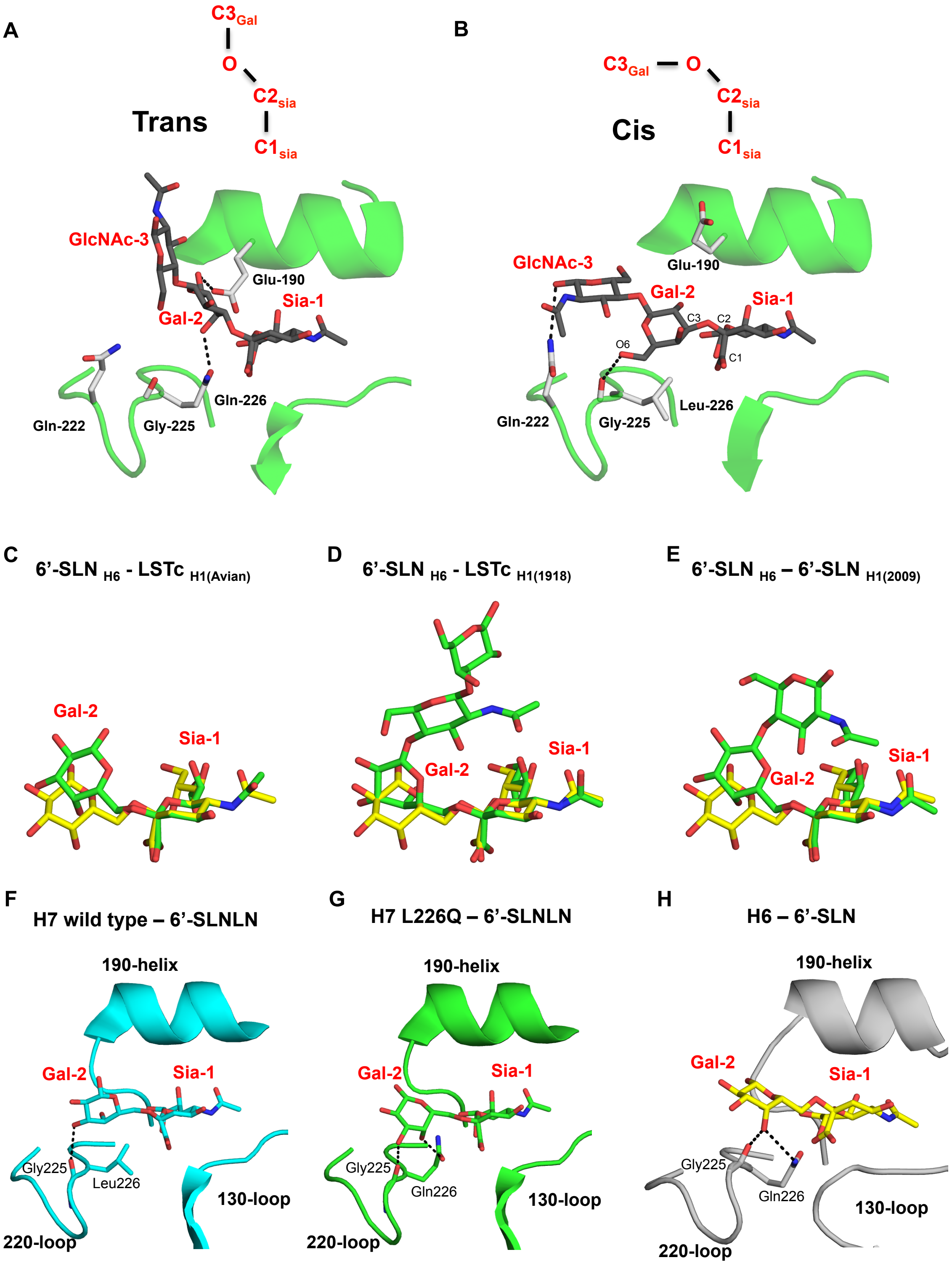
A
C3Gal
O
C2sia
C1sia
Trans
GlcNAc-3
Glu-190
Sia-1
Gal-2
Gln-226
Gln-222
Gly-225
B
C3Gal
O
C2sia
C1sia
Cis
GlcNAc-3
Glu-190
Gal-2
Sia-1
C3
C2
O6
C1
Leu-226
Gln-222
Gly-225
C
6'-SLN H6 - LSTc H1(Avian)
Gal-2
Sia-1
D
6'-SLN H6 - LSTc H1(1918)
Sia-1
Gal-2
E
6'-SLN H6 – 6'-SLN H1(2009)
Sia-1
Gal-2
F
H7 wild type – 6'-SLNLN
190-helix
Gal-2
Sia-1
Gly225
Leu226
130-loop
220-loop
G
H7 L226Q – 6'-SLNLN
190-helix
Gal-2
Sia-1
Gly225
Gln226
130-loop
220-loop
H
H6 – 6'-SLN
190-helix
Gal-2
Sia-1
Gly225
Gln226
130-loop
220-loop

Figure S4, related to Figures 3 and 4. Conformation of Avian and Human Receptor Analogs in HA Structures

(A and B) Binding of avian receptor analog 3′-SLN to the RBS of the H7 HA subtype in both cis and trans conformations with respect to the Sia-Gal linkage. Avian analogs (3′-SLN and LSTa) can bind to the RBS in two conformations, trans (A, PDB 4BSI) and cis (B, PDB 4BSD), resulting in two modes of binding. (A) In the trans conformation, Gal-2 hydrogen bonds with the side chains of Gln226 and Glu190. (B) In the cis conformation, Gal-2 hydrogen bonds to the main chain of Gly225 and makes hydrophobic interactions with the Leu226 side chain. In addition, GlcNAc-3 hydrogen bonds with Gln222. The H7 complexes are used as representative of the two different binding conformations of avian receptors in the HA RBS.

(C-E) Superposition of 6′-SLN receptor analog from the H6 HA complex (yellow) compared to LSTc or 6′-SLN human analogs from structures with HAs from (A) avian H1N1 (PDB 3HTQ), (B) 1918 pandemic H1N1 (PDB 2WRG) and (C) 2009 pandemic H1N1 (PDB 3UBN), which are colored in green as representative of other HA subtypes. The superposition indicates conformational changes arising from rotation around the linkage between Sia-1 and Gal-2 (phi angle changes from ~60-70 ° in H1 structures to ~120 ° for H6). The superposition was done on Sia-1.

(F-H) Structures of complexes of AH H7N9 wild type HA (F, cyan, PDB entry 4KON) and AH H7N9 L226Q mutant HA (G, green, PDB entry 4LKK) with the human analog 6′-SLNLN (yellow) indicating changes in the linkage of Sia-1 and Gal-2 (from 63° in the wild type to 21° in the mutant). In the mutant complex, Gal-2 hydrogen bonds with Gly225 and Gln226 similar to the interaction between 6′-SLN Gal-2 and H6 HA (F).

Table S1, related to Figure 1. Data Collection and Refinement Statistics for H6 HA and Glycan Complexes

Data collection	Apo	3′-SLN	LSTa	6′-SLN
Beamline	SSRL11-1	APS-23	APS-23	APS-23
Wavelength (Å)	0.97945	1.03320	1.03320	1.03320
Space Group	C2	C2	C2	C2
Unit cell parameters (Å)	a=184.4, b=98.9, c=132.8 β=126.1°	a=185.5, b=99.2, c=133.4 β=126.3°	a=185.7, b=99.2, c=133.4 β=126.4°	a=185.5, b=98.9, c=133.6 β=126.6°
Resolution range (Å)	50.00-2.50 (2.54-2.50)[a]	50.00-2.35 (2.39-2.35)	50.00-2.45 (2.49-2.45)	50.00-2.25 (2.29-2.25)
Observations	240,934	238,063	249,206	290,644
Unique reflections	66,521 (3186)	78,465 (3652)	71,296 (3296)	90,738 (4375)
Completeness (%)	98.3 (94.4)	98.2 (92.7)	99.1 (93.0)	97.5 (94.9)
I/σ(I)	11.9 (1.4)	19.7 (1.4)	16.6 (2.1)	21.2 (3.6)
$CC_{1/2}$	0.78	0.73	0.77	0.87
R_{sym}[b]	0.11 (0.53)	0.12 (0.75)	0.12 (0.51)	0.10 (0.37)
R_{pim}[c]	0.06 (0.28)	0.06 (0.44)	0.06 (0.29)	0.05 (0.20)
Redundancy	3.6 (3.2)	3.0 (2.3)	3.5 (2.9)	3.2 (3.1)
Refinement Statistics				
Resolution (Å)	50.00-2.50 (2.54-2.50)	50.00-2.35 (2.39-2.35)	50.00-2.45 (2.49-2.45)	50.00-2.25 (2.29-2.25)
No. reflections	66,484	78,319	71,268	90,698
R_{cryst}[d]/R_{free}[e]	0.207/0.257	0.230/0.274	0.210/0.253	0.208/0.253
No. atoms				
Protein	11,897	11,909	11,894	11,903
Carbohydrate	84	130	130	116
Water	247	111	140	422
Wilson B (Å^2)	45.2	55.3	42.2	33.5
Average B value (Å^2)				
Overall	41.7	63.5	47.3	40.4
Ligand		78.5	74.3	69.5
R.m.s deviation from ideal geometry				
Bond length (Å)	0.016	0.015	0.011	0.011
Bond angle (º)	1.5	1.5	1.6	1.6
Ramachandran Plot (%) [f]				
Favored	97.3	96.7	96.3	97.0
Outliers	0.1	0	0	0
PDB ID	4XKD	4XKE	4XKF	4XKG

[a] Parentheses refer to outer shell statistics.

[b] $R_{sym} = \Sigma_{hkl} \Sigma_i | I_{hkl,i} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \Sigma_i I_{hkl,i}$, where $I_{hkl,i}$ is the scaled intensity of the i^{th} measurement of reflection h, k, l, and $\langle I_{hkl} \rangle$ is the average intensity for that reflection.

[c] $R_{pim} = \Sigma_{hkl} (1/(n-1))^{1/2} \Sigma_i | I_{hkl,i} - \langle I_{hkl} \rangle | / \Sigma_{hkl} \Sigma_i I_{hkl,i}$, where n is the redundancy.

[d] $R_{cryst} = \Sigma_{hkl} | F_o - F_c | / \Sigma_{hkl} | F_o | \times 100$, where F_o and F_c are the observed and calculated structures factors.

[e] R_{free} was calculated as for R_{cryst}, but on a test set of 5% of the data excluded from refinement.

[f] Calculated using MolProbity (Davis et al., 2007)

Table S2, related to Figure 1. Comparison of H6 HA with Other Subtypes

HA strain name	PDB code	RMSD values of C_α atoms (Å)				Alignment score (%)[b]
		Monomer	HA1	HA2	RBS subdomain[a]	
H1N1_A/California/04/2009	3AL4	1.1 (480)[c]	1.0 (319)	0.5 (162)	0.6 (142)	58
H2N2_A/Japan/305/1957	3KU5	0.8 (479)	0.8 (310)	0.5 (169)	0.7 (134)	60
H3N2_A/Hong_Kong/1/1968	4FNK	2.4 (464)	1.6 (300)	0.9 (163)	0.8 (121)	42
H5N1_A/Vietnam/CL17/2004	2FKO	1.3 (490)	1.5 (390)	0.8 (151)	0.8 (145)	59
H7N9_A/Shanghai/02/2013	4N5J	2.5 (480)	2.0 (312)	1.0 (167)	0.8 (126)	42

[a] The receptor binding subdomain (RBS subdomain) is defined as residues 117-265 of the HA1 subunit (H3 numbering).

[b] Calculated for the HA sequence (Figure S1) using ClustalW2 (ENBL-EBI) (Larkin et al., 2007).

[c] Number in parentheses is the number of residues included in the comparison.

Table S3, related to Figure 2. Glycans Imprinted on the Microarray. Purple diamonds represent NeuAc, yellow circles represent Gal, blue circles represent Glc, green circles represent Man, yellow squares represent GalNAc, blue squares represent GlcNAc, red triangles represent Fuc and light blue diamonds represent NeuGc.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Structural Determination of the H6 HA. Diffraction data were collected at the Advanced Photon Source (APS) and at the Stanford Synchrotron Radiation Lightsource (SSRL). Data were integrated and scaled using HKL2000 (Otwinowski and Minor, 1997). The initial H6 apo structure was solved by molecular replacement method using Phaser (McCoy et al., 2005) with an H1 HA structure (PDB entry 4M4Y) as a search model. The H6 HA apo structure was then used as the starting model for structure determination of the H6 HA-glycan complex structures. Structure refinement was carried out in Phenix (Adams et al., 2002) and model building with COOT (Emsley and Cowtan, 2004). Final data collection and refinement statistics are summarized in Table S1.

Expression and Purification of H6 and H5 HAs in Baculovirus Expression System for Glycan Microarray Analyses. The HA expression was similar to that used for the crystallization experiments. The expressed HA0s were purified through a His-tag affinity purification step and dialyzed against 20 mM Tris-HCl, 50 mM NaCl, pH 8.0 overnight at 4 ^{o}C. Proteins were concentrated to 1 mg/ml prior to binding assays.

Expression and Purification of H1, H5 and H6 HAs from a Mammalian Expression System for Glycan Microarray Analyses. Codon-optimized H1, H5 and H6 encoding cDNAs (Genscript, USA) of A/Kentucky/07 (Accession; CY028163), A/Vietnam/1203/04 (Accession; EF541403) and A/Taiwan/2/13 (Accession; EPI459855) were cloned into the pCD5 expression as described previously (Xu et al., 2013). The HA proteins were expressed in HEK293S GnTI$^{-/-}$ cells and purified from the cell culture supernatants as described previously (de Vries et al., 2010).

Glycan Microarray Analysis of HAs Expressed in Insect and Mammalian Cells. Purified, soluble trimeric HA was pre-complexed with horseradish peroxidase (HRP)-linked anti-Strep-tag mouse antibody and with Alexa647-linked anti-mouse IgG (4:2:1 molar ratio) prior to incubation for 15 min on ice in 100 µl PBS-T, and incubated on the array surface in a humidified chamber for 90 minutes. Slides were subsequently washed by successive rinses with PBS-T, PBS, and deionized H_2O. Washed arrays were dried by centrifugation and immediately scanned for FITC signal on a Perkin-Elmer ProScanArray Express confocal microarray scanner. Fluorescent signal intensity was measured using Imagene (Biodiscovery) and mean intensity minus mean background was calculated and graphed using MS Excel. For each glycan, the mean signal intensity is calculated from 6 replicates spots. The highest and lowest signals of the 6 replicates are removed and the remaining 4 replicates are used to calculate the mean signal, standard deviation (SD), and standard error measurement (SEM). Bar graphs represent the averaged mean signal minus background for each glycan sample and error bars are the SEM value. A list of glycans on the microarray is included in Table S3.

REFERENCES

Adams, P.D., Grosse-Kunstleve, R.W., Hung, L.W., Ioerger, T.R., McCoy, A.J., Moriarty, N.W., Read, R.J., Sacchettini, J.C., Sauter, N.K., and Terwilliger, T.C. (2002). PHENIX: building new software for automated crystallographic structure determination. Acta Crystallogr. D Biol. Crystallogr. *58*, 1948-1954.

Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall, W.B., 3rd, Snoeyink, J., Richardson, J.S.*, et al.* (2007). MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. *35*, W375-383.

de Vries, R.P., de Vries, E., Bosch, B.J., de Groot, R.J., Rottier, P.J., and de Haan, C.A. (2010). The influenza A virus hemagglutinin glycosylation state affects receptor-binding specificity. Virology *403*, 17-25.

Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta. Crystallogr. D Biol. Crystallogr. *60*, 2126-2132.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R.*, et al.* (2007). Clustal W and Clustal X version 2.0. Bioinformatics *23*, 2947-2948.

McCoy, A.J., Grosse-Kunstleve, R.W., Storoni, L.C., and Read, R.J. (2005). Likelihood-enhanced fast translation functions. Acta Crystallogr. D Biol. Crystallogr. *61*, 458-464.

Otwinowski, Z., and Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. Meth. Enzymol. *276*, 307-326.

Stevens, J., Blixt, O., Tumpey, T.M., Taubenberger, J.K., Paulson, J.C., and Wilson, I.A. (2006b). Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. Science *312*, 404-410.

Xu, R., de Vries, R.P., Zhu, X., Nycholat, C.M., McBride, R., Yu, W., Paulson, J.C., and Wilson, I.A. (2013). Preferential recognition of avian-like receptors in human influenza A H7N9 viruses. Science *342*, 1230-1235.