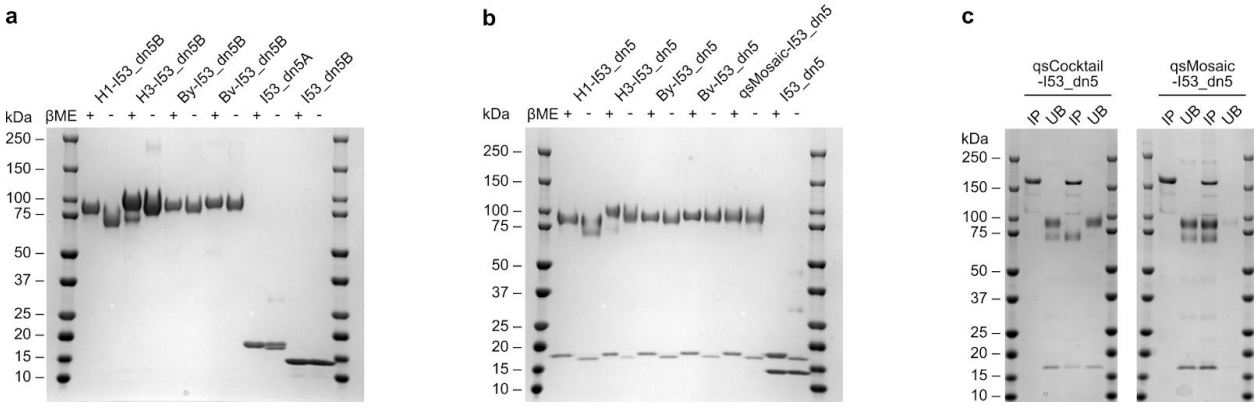
Supplementary information for

Quadrivalent influenza nanoparticle vaccines induce broad protection

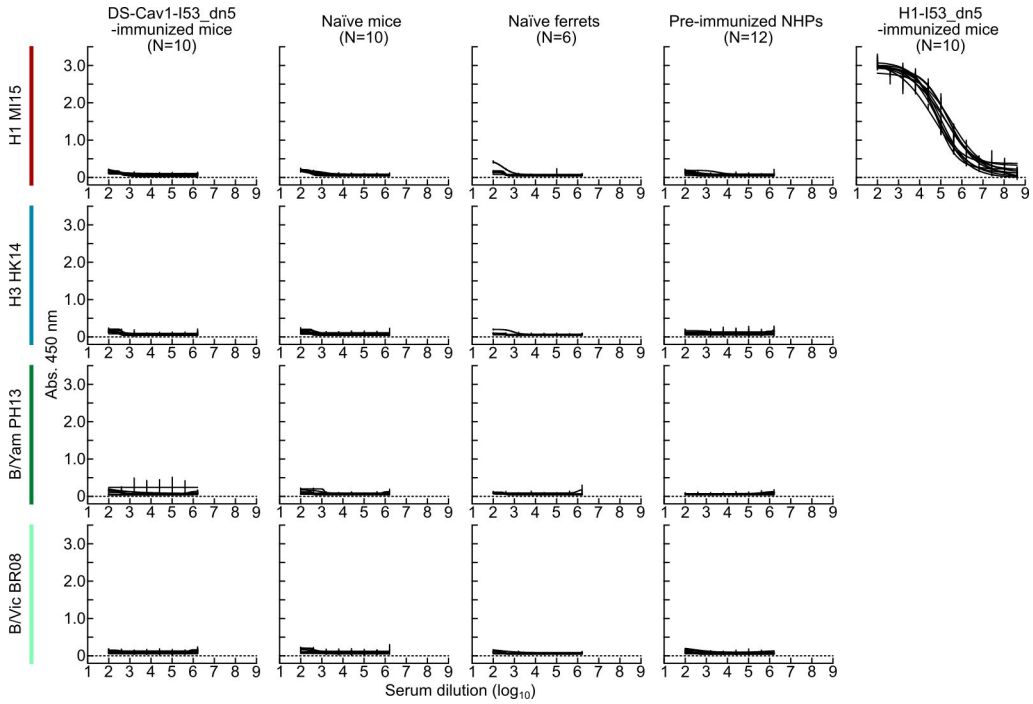
Seyhan Boyoglu-Barnum[1,8], Daniel Ellis[2,3,4,8], Rebecca A. Gillespie[1,9], Geoffrey B. Hutchinson[1,9], Young-Jun Park[3], Syed M. Moin[1], Oliver J. Acton[3,7], Rashmi Ravichandran[2,3], Mike Murphy[2,3], Deleah Pettie[2,3], Nick Matheson[2,3], Lauren Carter[2,3], Adrian Creanga[1], Michael J. Watson[5], Sally Kephart[5], Sila Ataca[1], John R. Vaile[1], George Ueda[2,3], Michelle C. Crank[1], Lance Stewart[2,3], Kelly K. Lee[5], Miklos Guttman[5], David Baker[2,3,6], John R. Mascola[1], David Veesler[3], Barney S. Graham[1,*], Neil P. King[2,3,10,*], Masaru Kanekiyo[1,10,*]

[1]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, United States.
[2]Institute for Protein Design, University of Washington, Seattle, WA 98195, United States.
[3]Department of Biochemistry, University of Washington, Seattle, WA 98195, United States.
[4]Graduate Program in Molecular and Cellular Biology, University of Washington, Seattle, WA 98195, United States.
[5]Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195, United States.
[6]Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, United States.
[7]Present address: Macromolecular Structure Laboratory, The Francis Crick Institute, London, United Kingdom.
[8]These authors contributed equally: Seyhan Boyoglu-Barnum and Daniel Ellis.
[9]These authors contributed equally: Rebecca A. Gillespie and Geoffrey B. Hutchinson.
[10]These authors contributed equally: Neil P. King and Masaru Kanekiyo.
*email: bgraham@nih.gov (B.S.G.); neil@ipd.uw.edu (N.P.K.); kanekiyom@nih.gov (M.K.)

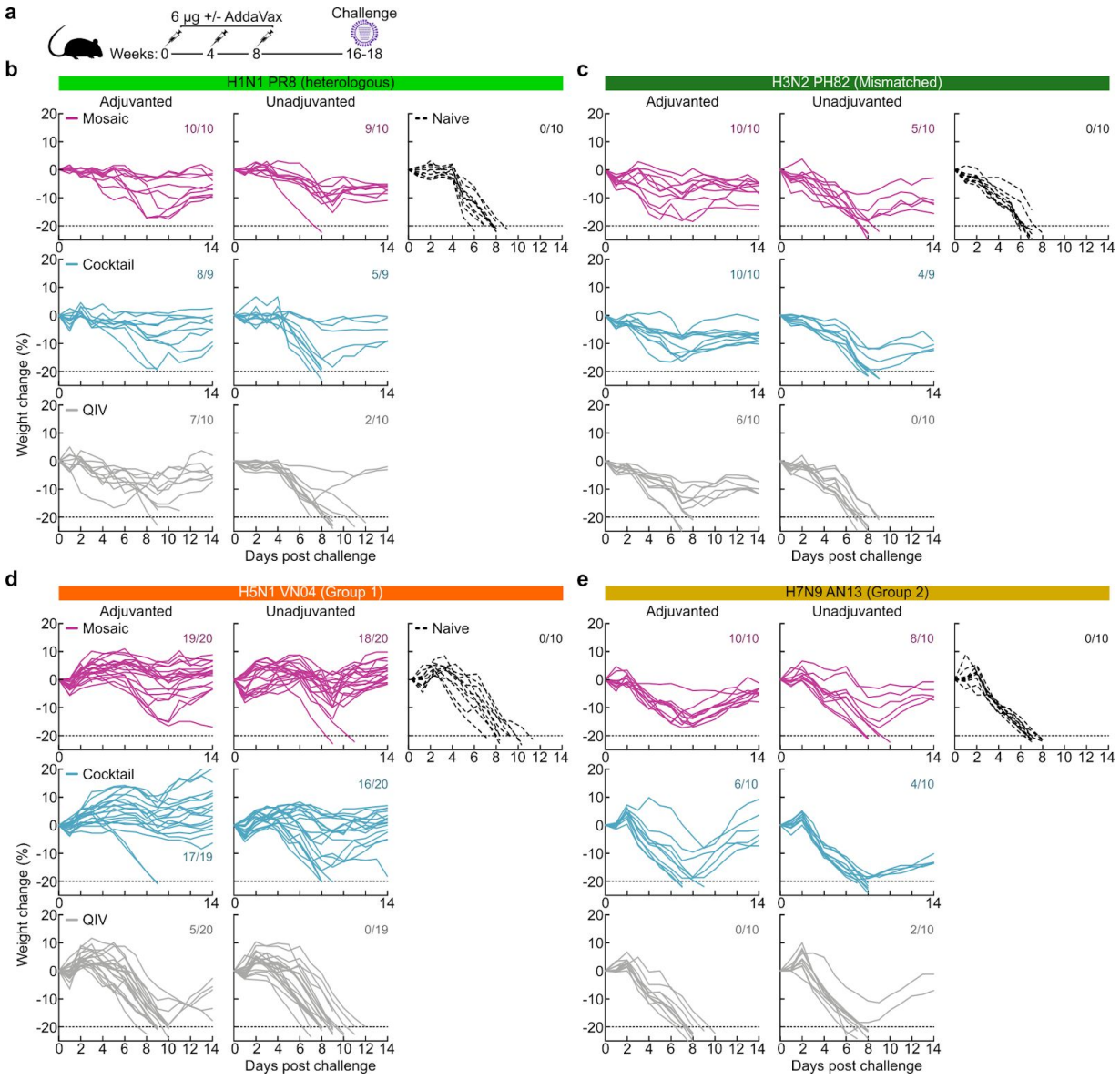
Table of contents



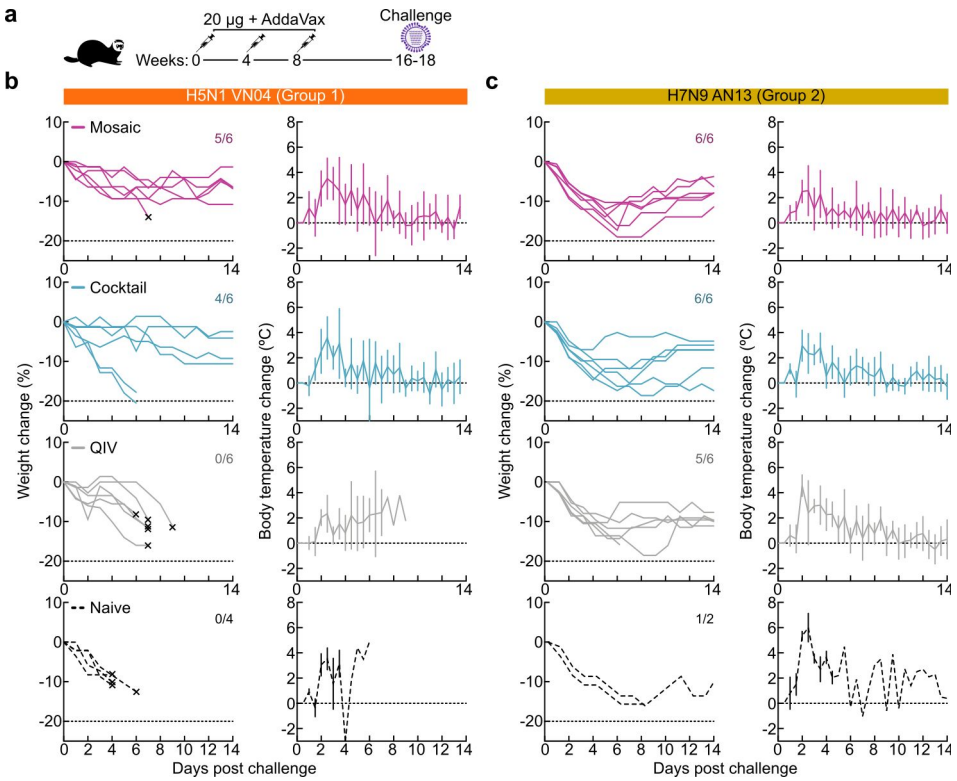
Supplementary Fig. 1 | Uncropped SDS-PAGE of H1-I53_dn5B fusions, purified nanoparticles and immunoprecipitations. a, Uncropped SDS-PAGE gel from Extended Data Fig. 1b. **b,** Uncropped SDS-PAGE gel from Extended Data Fig. 1d. **c,** Uncropped SDS-PAGE gel of qsCocktail-I53_dn5 and qsMosaic-I53_dn5 immunoprecipitations from Fig. 1d. IP, immunoprecipitated; UB, unbound.



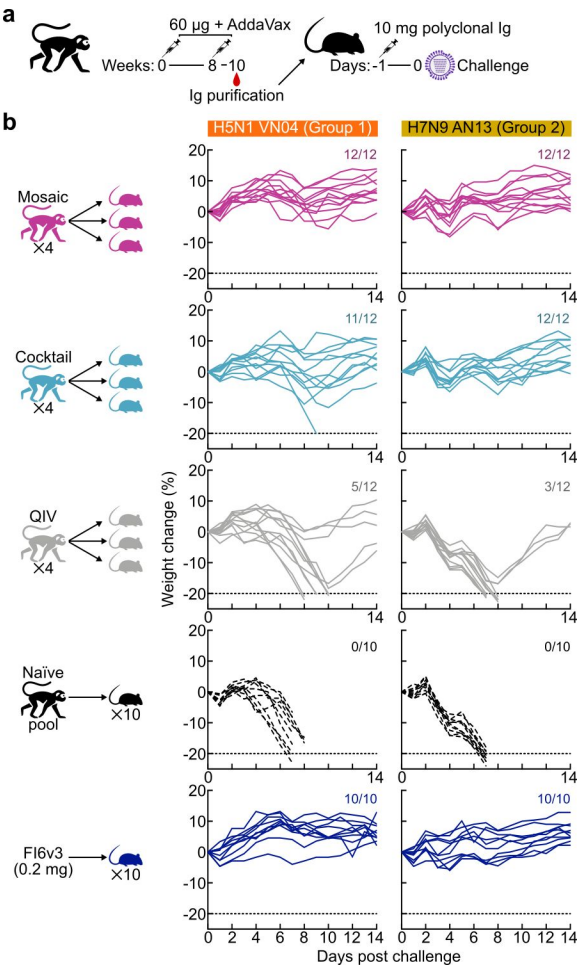
Supplementary Fig. 2 | ELISA binding curves of serum samples obtained from unimmunized, PBS- or irrelevant antigen-immunized animals. Mice and ferrets were immunized intramuscularly with PBS, DS-Cav1-I53_dn5, or H1-I53_dn5 plus AddaVax. NHP samples taken prior to immunization with our vaccines. Groups of BALB/cJ mice (*N* = 10), Finch ferrets (*N* = 6), and rhesus macaques (*N* = 12) were used. ELISA binding curves for HAs of H1 MI15, H3 HK14, B/Yam PH13 and B/Vic BR08 are shown. All animal experiments except for NHP were performed at least twice and representative data are shown.



Supplementary Fig. 3 | Body weight changes for mouse challenge studies. a, Experimental scheme of mouse challenge studies. Changes in body weight after heterologous H1N1 (**b**), mismatched H3N2 (**c**), heterosubtypic H5N1 (**d**) or H7N9 (**e**) virus infection in immunized BALB/cJ mice (*N* =10; *N* = 9 for qsCocktail-I53_dn5 groups in **b** and unadjuvanted qsCocktail-I53_dn5 group in **c**; *N* = 19 for adjuvanted qsCocktail-I53_dn5 and unadjuvanted QIV group in **d**; and *N* = 20 for all other groups in panel **d**). Viruses were given intranasally on day 0 and mice were monitored for 14 days post infection. Body weight curves are plotted for each individual animal. Animals suffered >20% of body weight loss from day 0 were humanely euthanized.



Supplementary Fig. 4 | Body weight and temperature changes for ferret challenge studies. a, Experimental scheme of ferret challenge studies. Body weight (left) and temperature (right) changes after heterosubtypic H5N1 (**b**) or H7N9 (**c**) virus infection in immunized ferrets. Viruses were given intranasally on day 0 and animals were monitored for 14 days post infection. Changes in body weight and temperature are plotted for each individual animal. Predetermined humane endpoints include >20% of body weight loss from day 0 or other symptoms such as neurological abnormalities and moribound state. X symbols indicate animals euthanized based on the symptom-based humane endpoints. These animals exhibited one or more of the following symptoms: neurological abnormalities, bilateral hind leg paresis or paralysis, one-sided head tilt and side rolling, and moribound state. One animal was found dead.



Supplementary Fig. 5 | Body weight changes for mouse challenge studies after passive transfer of hyper immunoglobulins. a, Experimental scheme of the study. Immunoglobulins were purified from immunized NHPs and given to recipient BALB/cAnNHsd mice (*N* = 3 per NHP; *N* = 12 per immunization group). Control groups of mice (*N* = 10) were given either immunoglobulins obtained prior to immunization (Naïve pool) or anti-HA stem monoclonal antibody FI6v3. **b,** Changes in body weight after heterosubtypic H5N1 (left) or H7N9 (right) virus infection in recipient mice. Immunoglobulins were given intraperitoneally at day -1. Viruses were given intranasally on day 0 and mice were monitored for 14 days post infection. Body weight curves are plotted for each individual animal. Animals suffered >20% of body weight loss from day 0 were humanely euthanized.

Supplementary Table 1 | Amino acid sequences for novel proteins used in this study.

Non-antigen-bearing nanoparticle components

>I53_dn5A
MGKYDGSKLRIGILHARWNAEIILALVLGALKRLQEFGVKRENIIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIIPIG
VLIKGSTMHFEYICDSTTHQLMKLNFELGIPVIFGVLTCLTDEQAEARAGLIEGKMHNHGEDWGAAAVEMATKFNLE
HHHHHH

>I53_dn5B
MEEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWY
NLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQGSLEHHHHHH

HA-I53_dn5B trimers

>H1-I53_dn5B: A/Michigan/45/2015 HA 1-676 Y98F no lkr dn5B.SA.WELQ-H
MKAILVVLLYTFTTANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAGWI
LGNPECESLSTASSWSYIVETSNSDNGTCFPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAACP
HAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFVGTSRYSKKFKPE
IATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSL
PFQNIHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADLK
STQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYHDSNVKN
LYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVSAEEAELAYLLGELAYK
LGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAI
EYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>H3-I53_dn5B: A/Hong Kong/4801/2014 HA 1-676 Y98F GG lkr dn5B.SA.WELQ-H
MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVQNSSIGEICDSPHQILDGENC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCFPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSAC
IRRSSSSFFSRLNWLTHLNYTYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPN
IGSRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDKPFQNV
NRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQ
AAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEK
TKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVGGSAEEAELAYLLGELAYKLG
EYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAIEY
YRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>B/Yam-I53_dn5B: B/Phuket/3073/2013 HA 1-674 no lkr dn5B.SA.WELQ-H
MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTDL
DVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEKIRLSTQNVIDAEKAPGGPYRLGTS
GSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSS
ANGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIG
EADCLHEEYGGLNKSKPYYTGKHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAG
WHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIEL
AVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAAS
AEEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWY
NLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>B/Vic-I53_dn5B: B/Brisbane/60/2008 HA 1-674 no lkr dn5B.SA.WELQ-H
MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDL
DVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSG
SCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEA

DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGW
HGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELA
VLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASA
EEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWYNL
GNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>SI16-I53_dn5B: A/Singapore/INFIMH-16-0019/2016 HA 1-676 Y98F GG lkr dn5B.SA.WELQ-H
MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVQNSSIGEICDSPHQILDGENC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCFPYDVPDYASLRSLVASSGTLEFKNESFNWTGVTQNGTSSAC
IRGSSSSFFSRLNWLTHLNYTYPALNVTMPNKEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPN
IGSRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDKPFQNV
NRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQ
AAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRVQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFE
KTKKQLRENAEDMGNGCFKIYHKCDNACIESIRNETYDHNVYRDEALNNRFQIKGVGGSAEEAELAYLLGELAYKL
GEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAIE
YYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>CO17-I53_dn5B: B/Colorado/06/2017 HA 1-672 no lkr dn5B.SA.WELQ-H
MKAIIVLLMVVTSSADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDL
DVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHVRLSTHNVINAEGAPGGPYKIGTS
GSCPNITNGNGFFATMAWAVPDKNKTATNPLTIEVPYVCTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEA
DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGW
HGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELA
VLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDKIAAGTFDAGEFSLPTFDSLNITAASA
EEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAEAWYNL
GNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

HA-1na0C3_int2 (non-assembling) trimers

>H1-1na0C3_int2: A/Michigan/45/2015 HA 1-676 Y98F no lkr 1na0C3_int2.SA.WELQ-H
MKAILVVLLYTFTTANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAGWI
LGNPECESLSTASSWSYIVETSNSDNGTCFPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAACP
HAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFVGTSRYSKKFKPE
IATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSL
PFQNIHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADLK
STQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYHDSNVKN
LYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVSAEEAELAYLLGELAYK
LGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGDYDEAIEYYQKALELDPNNAEAWYNLGNAYYKQGDYDEAI
EYYQKALELDPNNAEAKQNLGNAKQKQGGGWELQHHHHHH

>H3-1na0C3_int2: A/Hong Kong/4801/2014 HA 1-676 Y98F GG lkr 1na0C3_int2.SA.WELQ-H
MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVQNSSIGEICDSPHQILDGENC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCFPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSAC
IRRSSSSFFSRLNWLTHLNYTYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPN
IGSRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDKPFQNV
NRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQ
AAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEK
TKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVGGSAEEAELAYLLGELAYKLG
EYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGDYDEAIEYYQKALELDPNNAEAWYNLGNAYYKQGDYDEAIEY
YQKALELDPNNAEAKQNLGNAKQKQGGGWELQHHHHHH

>B/Yam-1na0C3_int2: B/Phuket/3073/2013 HA 1-674 no lkr 1na0C3_int2.SA.WELQ-H
MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTDL
DVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEKIRLSTQNVIDAEKAPGGPYRLGTS
GSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSS
ANGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIG
EADCLHEEYGGLNKSKPYYTGKHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAG
WHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIEL
AVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAAS
AEEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGDYDEAIEYYQKALELDPNNAEAWY
NLGNAYYKQGDYDEAIEYYQKALELDPNNAEAKQNLGNAKQKQGGGWELQHHHHHH

>B/Vic-1na0C3_int2: B/Brisbane/60/2008 HA 1-674 no lkr 1na0C3_int2.SA.WELQ-H
MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDL
DVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSG
SCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEA
DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGW
HGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELA
VLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASA
EEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQGDYDEAIEYYQKALELDPNNAEAWYNL
GNAYYKQGDYDEAIEYYQKALELDPNNAEAKQNLGNAKQKQGGGWELQHHHHHH

HA-foldon trimers (ELISA antigens and EMPEM)

>H1 MI15: A/Michigan/45/2015 HA 1-676 Y98F FAH
MKAILVVLLYTFTTANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAGWI
LGNPECESLSTASSWSYIVETSNSDNGTCFPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKGVTAACP
HAGAKSFYKNLIWLVKKGNSYPKLNQSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFVGTSRYSKKFKPE
IATRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFTMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSL
PFQNIHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADLK
STQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLVLLENERTLDYHDSNVKN
LYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKIDGVGSGYIPEAPRDGQAYV
RKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H3 HK14: A/Hong Kong/4801/2014 HA 1-676 Y98F FAH
MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVQNSSIGEICDSPHQILDGENC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCFPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSAC
IRRSSSSFFSRLNWLTHLNYTYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPN
IGSRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDKPFQNV
NRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQ
AAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEK
TKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVGSGYIPEAPRDGQAYVRKDG
EWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>B/Yam PH13: B/Phuket/3073/2013 HA 1-676 FAH
MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSYFANLKGTRTRGKLCPDCLNCTDL
DVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEKIRLSTQNVIDAEKAPGGPYRLGTS
GSCPNATSKIGFFATMAWAVPKDNYKNATNPLTVEVPYICTEGEDQITVWGFHSDNKTQMKSLYGDSNPQKFTSS
ANGVTTHYVSQIGDFPDQTEDGGLPQSGRIVVDYMMQKPGKTGTIVYQRGVLLPQKVWCASGRSKVIKGSLPLIG
EADCLHEEYGGLNKSKPYYTGKHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAG
WHGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIEL
AVLLSNEGIINSEDEHLLALERKLKKMLGPSAVDIGNGCFETKHKCNQTCLDRIAAGTFNAGEFSLPTFDSLNITAAS
LGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>B/Vic BR08: B/Brisbane/60/2008 HA 1-676 FAH
MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDL
DVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSG
SCPNITNGNGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEA
DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGW
HGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELA
VLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFDAGEFSLPTFDSLNITAASL
GSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H5 IN05: A/Indonesia/05/2005 HA 1-676 Y98F delF FAH
MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDCSVAGWLL
GNPMCDEFINVPEWSYIVEKANPTNDLCFPGSFNDYEELKHLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYLG
SPSFFRNVVWLIKKNSTYPTIKKSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRS
KVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNI
HPLTIGECPKYVKSNRLVLATGLRNSPQRESRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADKESTQ
KAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNL
YDKVRLQLRDNAKELGNGCFEFYHKCDNECMESIRNGTYNYPQYSEEARLKREEISGVGSGYIPEAPRDGQAYVR
KDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H6 TW13: A/Taiwan/2/2013 HA 1-676 Y98F FAH
MIAIVVIAILASAGKSDKICIGYHANNSTTQVDTLLEKNVTVTHSVELLENQKEKRFCKIMNKAPLDLKDCTIEGWILGN
PKCDLLLGDQSWSYIVERPNAQNGICFPGVLNELEELKAFIGSGERVERFEMFPKSTWAGVDTSRGVTNACPSYTI
DSSFYRNLVWIVKTDSATYPVIKGTYNNTGTQPILYFWGVHHPLDTTVQDNLYGSGDKYVRMGTESMNFAKSPEIA
ARPAVNGQRSRIDYYWSVLRPGETLNVESNGNLIAPWYAYKFVSTNKKGAVFKSDLPIENCDATCQTITGVLRTNK
TFQNVSPLWIGECPKYVKSESLRLATGLRNVPQIATRGIFGAIAGFIEGGWTGMIDGWYGYHHENSQGSGYAADR
ESTQKAIDGITNKVNSIINKMNTQFEAVDHEFSNLERRIGNLNKRMEDGFLDVWTYNAELLVLLENERTLDLHDANV
KNLYEKVKSQLRDNANDLGNGCFEFWHKCDNECMESVKNGTYDYPKYQKESKLNRQGIESVGSGYIPEAPRDGQ
AYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H7 AN13: A/Anhui/1/2013 HA 1-676 Y98F FAH
MNTQILVFALIAIIPTNADKICLGHHAVSNGTKVNTLTERGVEVVNATETVERTNIPRICSKGKRTVDLGQCGLLGTIT
GPPQCDQFLEFSADLIIERREGSDVCFPGKFVNEEALRQILRESGGIDKEAMGFTYSGIRTNGATSACRRSGSSFY
AEMKWLLSNTDNAAFPQMTKSYKNTRKSPALIVWGIHHSVSTAEQTKLYGSGNKLVTVGSSNYQQSFVPSPGARP
QVNGLSGRIDFHWLMLNPNDTVTFSFNGAFIAPDRASFLRGKSMGIQSGVQVDANCEGDCYHSGGTIISNLPFQNI
DSRAVGKCPRYVKQRSLLLATGMKNVPEIPKGRGLFGAIAGFIENGWEGLIDGWYGFRHQNAQGEGTAADYKSTQ
SAIDQITGKLNRLIEKTNQQFELIDNEFNEVEKQIGNVINWTRDSITEVWSYNAELLVAMENQHTIDLADSEMDKLYE
RVKRQLRENAEEDGTGCFEIFHKCDDDCMASIRNNTYDHSKYREEAMQNRIQIDPVGSGYIPEAPRDGQAYVRKD
GEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H10 JD13: A/Jiangxi Donghu/346/2013 HA 1-676 Y98F FAH
MYKIVVIIALLGAVKGLDKICLGHHAVANGTIVKTLTNEQEEVTNATETVESTGINRLCMKGRKHKDLGNCHPIGMLIG
TPACDLHLTGMWDTLIERENAIAYCFPGATVNVEALRQKIMESGGINKISTGFTYGSSINSAGTTRACMRNGGNSFY
AELKWLVSKSKGQNFPQTTNTYRNTDTAEHLIMWGIHHPSSTQEKNDLYGTQSLSISVGSSTYRNNFVPVVGARP
QVNGQSGRIDFHWTLVQPGDNITFSHNGGLIAPSRVSKLIGRGLGIQSDAPIDNNCESKCFWRGGSINTRLPFQNL
SPRTVGQCPKYVNRRSLMLATGMRNVPELIQGRGLFGAIAGFLENGWEGMVDGWYGFRHQNAQGTGQAADYKS
TQAAIDQITGKLNRLVEKTNTEFESIESEFSEIEHQIGNVINWTKDSITDIWTYQAELLVAMENQHTIDMADSEMLNLY
ERVRKQLRQNAEEDGKGCFEIYHACDDSCMESIRNNTYDHSQYREEALLNRLNINPVGSGYIPEAPRDGQAYVRK
DGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H1 stem: A/California/04/2009 stabilized stem FAH
MKAILVVLLYTFATANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLGSGLRLATGLRNIPQRETRGLFGAIAGFI
EGGWTGMVDGWYGYHHQNEQGSGYAADLKSTQNAIDEITNMVNSVIEKMGSGGSGTDLAELLVLLLNQWTLLYH

DSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREEIDGSGYIPEAPRDG
QAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H3 stem: A/Finland/486/2004 stabilized stem FAH
MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVFPGCGVLKLATGMRNVPEK
QTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGIGQAADLKSTQAAINQINGMVNRVIALMAQGGPDCYLAELL
VALLNQHVIDLTDSEMRKLFERTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKG
GPGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>H3 SG16: A/Singapore/INFIMH-16-0019/2016 HA 1-676 Y98F FAH
MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVQNSSIGEICDSPHQILDGENC
TLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCFPYDVPDYASLRSLVASSGTLEFKNESFNWTGVTQNGTSSAC
IRGSSSSFFSRLNWLTHLNYTYPALNVTMPNKEQFDKLYIWGVHHPGTDKDQIFLYAQSSGRITVSTKRSQQAVIPN
IGSRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDKPFQNV
NRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADLKSTQ
AAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRVQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFE
KTKKQLRENAEDMGNGCFKIYHKCDNACIESIRNETYDHNVYRDEALNNRFQIKGVGSGYIPEAPRDGQAYVRKD
GEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>B/Vic CO17: B/Colorado/06/2017 HA 1-676 FAH
MKAIIVLLMVVTSSADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTETRGKLCPKCLNCTDL
DVALGRPKCTGKIPSARVSILHEVRPVTSGCFPIMHDRTKIRQLPNLLRGYEHVRLSTHNVINAEGAPGGPYKIGTS
GSCPNITNGNGFFATMAWAVPDKNKTATNPLTIEVPYVCTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSA
NGVTTHYVSQIGGFPNQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEA
DCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGW
HGYTSHGAHGVAVAADLKSTQEAINKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDLRADTISSQIELA
VLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDKIAAGTFDAGEFSLPTFDSLNITAASL
GSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

Appended sequences including the foldon trimerization domain, WELQut protease recognition sites, Avi tags, and hexa-histidine tags are underlined.

Supplementary Table 2 | Cryo-EM data collection, refinement and validation statistics.

	H1-I53_dn5 nanoparticle (EMD-22935)	H1 HA/MI15 Localized reconstruction (EMD-22937; PDB 7KNA)	H5 bound to 3 polyclonal Fabs (EMD-22940)	H5 bound to 2 polyclonal Fabs (EMD-22939)	H5 bound to 1 polyclonal Fab (EMD-22938)
Data collection and processing					
Magnification	130,000	130,000	130,000	130,000	130,000
Voltage (kV)	300	300	300	300	300
Electron exposure (e^{-}/Å^2)	70	70	70	70	70
Defocus range (μm)	0.5-3.0	0.5-3.0	0.5-3.0	0.5-3.0	0.5-3.0
Pixel size (Å)	0.525	0.525	0.525	0.525	0.525
Symmetry imposed	I	C3	C1	C1	C1
Initial particle images (no.)	22,563	397,700 (sym-exp)	115,722	115,722	115,722
Final particle images (no.)	19,885	148,916	36,783	19,849	22,211
Map resolution (Å)	6.6	3.3	3.6	4.1	4.0
FSC threshold	0.143	0.143	0.143	0.143	0.143
Refinement					
Model resolution (Å)		3.4			
FSC threshold		0.5			
Map sharpening *B* factor (Å^2)		-174			
Model composition					
Nonhydrogen atoms		11,593			
Protein residues		1,449			
Ligands		24 (NAG)			
B factors (Å^2)					
Protein		37.5			
Ligand		44.2			
R.m.s. deviations					
Bond lengths (Å)		0.015			
Bond angles (°)		1.4			
Validation					
MolProbity score		0.66			
Clashscore		0.4			
Poor rotamers (%)		0.5			
Ramachandran plot					
Favored (%)		97.91			
Allowed (%)		1.88			
Disallowed (%)		0.21			

Supplementary Table 3 | Peptides used in label-free quantitation of HA content in mosaic nanoparticles and data for post-SEC quantitation of HA content in qsMosaic-I53_dn5 (related to Extended Data Fig. 3).

HA con-struct	Unique peptide sequence[a]	Standard equimolar mix (area)	qsMosaic-I53_dn5 post-SEC (area)	Relative ratio (qsMosaic-I53_dn5 /standard)	Average relative ratio and standard deviation	qsMosaic-I53_dn5 post-SEC composition[b]
H1	NLIWLVK	1.07×10^{7}	5.35×10^{6}	0.502	0.558 +/- 0.028	21.2 +/- 2.8 %
	GVTAACPHAGAK	1.44×10^{6}	9.59×10^{5}	0.668		
	KFKPEIATRPK	1.18×10^{6}	6.22×10^{5}	0.529		
	FKPEIATRPK	2.46×10^{6}	1.32×10^{6}	0.536		
H3	KWDLFVERSK +WDLFVERSK[c]	5.88×10^{6}	4.87×10^{6}	0.827	0.720 +/- 0.030	27.3 +/- 3.0 %
	SSIMRSDAPIGK[d]	1.05×10^{7}	7.45×10^{6}	0.707		
	LATGMRNVPEK[d]	6.24×10^{6}	3.99×10^{6}	0.638		
	EFSEVEGRIQDLEK[e]	8.62×10^{6}	6.11×10^{6}	0.709		
B/Yam	IRLSTQNVIDAEK	5.44×10^{6}	3.77×10^{6}	0.693	0.632 +/- 0.012	24.0 +/- 1.6 %
	SLYGDSNPQK	5.01×10^{6}	3.11×10^{6}	0.621		
	SYFANLK	6.73×10^{6}	4.00×10^{6}	0.595		
	IRQLPNLLRGYEK	1.30×10^{7}	8.07×10^{6}	0.620		
B/Vic	GSLPLIGEADCLHEK	7.38×10^{6}	5.19×10^{6}	0.703	0.727 +/- 0.013	27.6 +/- 1.3 %
	LYGDSKPQK	4.92×10^{5}	3.60×10^{5}	0.731		
	TGTITYQRGILLPQK	6.72×10^{6}	4.70×10^{6}	0.700		
	VWCASGRSK	1.99×10^{6}	1.54×10^{6}	0.773		

[a]Only abundant peptides that were unique to each specific HA were included.

[b]Normalized to the total sum of the average relative ratios.

[c]A high degree of missed cleavage was observed for this peptide and the sum of the two peptides was used for the calculations.

[d]The oxidized methionine forms were also included in these calculations.

[e]The pyro-glutamic acid form of the peptide was also included in these calculations.

Supplementary Table 4 | Viruses used in HAI, microneutralization, and challenge experiments.

Subtype	Virus	Short name	Type of experiment	Figure
H1N1	A/Michigan/45/2015	MI15; 2015	HAI	2a; ED 4e; ED 5c
			Microneutralization	2b; 3a; ED 4e; ED 5d; 5b; ED 7f; ED 10
	A/Boston/YGA-01050/2012	2012	Microneutralization	3a; ED 6
	A/California/07/2009	2009	Microneutralization	3a; ED 6
	A/Weiss/1943	1943	Microneutralization	3a; ED 6
	A/Malaysia/1954	1954	Microneutralization	3a; ED 6
	A/USSR/90/1977	1977	Microneutralization	3a; ED 6
	A/Memphis/4/1987	1987	Microneutralization	3a; ED 6
	A/New York/146/2000	2000	Microneutralization	3a; ED 6
	A/New Caledonia/20/1999	1999	Microneutralization	3a; ED 6
	A/Solomon Islands/03/2006	2006	Microneutralization	3a; ED 6
	A/Puerto Rico/8/1934	PR8	Challenge (mice)	3b; S3b
			Pseudovirus neutralization	3b
H3N2	A/Hong Kong/4801/2014	HK14; 2014	HAI	2a
			Microneutralization	2b; 3a; ED 4e; ED 7f
	A/Switzerland/9715293/2013	2013	Microneutralization	3a; ED 6
	A/Texas/50/2012	2012	Microneutralization	3a; ED 6
	A/Victoria/361/2011	2011	Microneutralization	3a; ED 6
	A/Brisbane/10/2007	2007	Microneutralization	3a; ED 6
	A/Wisconsin/67/2005	2005	Microneutralization	3a; ED 6
	A/Fujian/411/2002	2002	Microneutralization	3a; ED 6
	A/Moscow/10/1999	1999	Microneutralization	3a; ED 6
	A/Shangdong/9/1993	1993	Microneutralization	3a; ED 6
	A/Philippines/2/1982	PH82	Challenge (mice)	3c; S3c
			Pseudovirus neutralization	3c
	A/Singapore/INFIMH-16-0019/2016	SG16	Microneutralization	ED 5d; ED 10
H5N1	A/Vietnam/1203/2004	VN04	Challenge (mice, ferrets)	4d–f; S3d; S4b; S5b
			Microneutralization	5b
H7N9	A/Anhui/1/2013	AN13	Challenge (mice, ferrets)	4d–f; S3e; S4c
B/Yam	B/Phuket/3073/2013	PH13	HAI	2a; ED 4e; ED 5c
			Microneutralization	2b; ED 4e; ED 5d; ED 7f; ED 10
B/Vic	B/Colorado/06/2017	CO17	HAI	2a; ED 4e; ED 5c
			Microneutralization	2b; ED 5d; ED 7f; ED 10