SUPPLEMENTARY MATERIAL

Supplementary Table 1. HA DNA and protein sequences. In the DNA sequences, 15 bp overhangs used for cloning are shown in bold lowercase letters. In the mosaic HAs, sequences that have been altered compared to the H3 backbone sequence are highlighted in color-code depending on the antigenic site; red, site A; cyan, site B; magenta, site C; green, site D; yellow, site E. Adaptive mutations that occurred after viral rescue of the mH10/3 virus are highlighted in grey and in lowercase; the same adaptive mutations except the one located in site C were incorporated into the mH14/3 sequence. In the chimeric HAs, sequences changed to H10 or H14 are shown in lowercase letters, and one adaptive mutation in cH10/3 is highlighted in grey.

Construct	Sequence
mH10/3 DNA	**ccgaagttgggggggg**AGCAAAAGCAGGGGATAATTCTATTAACCATGAAGACTATCATTG CTTTGAGCTACATTCTATGTCTGGTTTTCGCTCAAAAAATTCCTGGAAATGACAATAGCA CGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCA CGAATGACCGAATTGAAGTcACTAATGCTACTGAGCTGGTTGAGAGTACAGGCAcAAACA GATTATGTATGAAACCTCATCAGATCCTTGATGGAGGCAACTGCCATCTAATAGATGCTC TATTGGGAGACCCTCAGTGTGATGGCTTTCAAAATAAGATGTGGGACCTTTTTGTTGAAC GAAGCAAAGCCTACAGCAGCTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGT CACTAGTTGCCTCATCCGGCACACTGGAGTTcAACAAcGAAAGCTTCAATTGGACTGGAG TCACTCAAAACGGAACAAGTTCTGCTTGCATGAGGAATGGAGGGAATAGCTTCTTTAGTA GATTAAATTGGTTGACCCACTTAAACCAAAAATACCCAGCATTGAACGTGACTATGCCAA ACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACCAAG ACCAAATCTTCCCGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCTCCACTTACC AACAAaCTGTAATCCCAAATATCGGATCTAGACCCAGAATAAGGAATATCCCTAGCAGAA TAAGCATCTATTGGACAATAGTAAAACCGGGAGACATACTTTTGATTcACAGCACAGGGA ATCTAATTGCTCCTAGGGGTTACTTCAAATTACGAATTGGGAGGAGCTCAATAATGAGAT CAGATGCACCAATAGACAATAATTGTGAGTCCAAATGCATCACTCCAAATGGAAGCATTC CCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATG TTAAGCATAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTA GAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATG GTTGGTACGGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAA GCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAAAACCA ACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGAAGAATTCAGGACC TTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTG TTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTG AAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAA TATACCACAAATGTGACAATGCCTGCATAGGATCAATAAGAAATGGAACTTATGACCACA ATGTGTACAGGGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCTGAAGT CAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTTGTG TTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAGGGCAACATTAGGTGCAACATTT GCATTTGAGTGCATTAATTAAAAACACCCTTGTTTCTACT**aataacccggcggcc**
mH10/3 protein	MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVE STGTNRLCMKPHQILDGGNCHLIDALLGDPQCDGFQNKMWDLFVERSKAYSSCYPYDVPD YASLRSLVASSGTLEFNNESFNWTGVTQNGTSSACMRNGGNSFFSRLNWLTHLNQKYPAL NVTMPNNEQFDKLYIWGVHHPGTDQDQIFPYAQSSGRITVSTSTYQQTVIPNIGSRPRIR NIPSRISIYWTIVKPGDILLIHSTGNLIAPRGYFKLRIGRSSIMRSDAPIDNNCESKCIT PNGSIPNDKPFQNVNRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGW EGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVE GRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMG NGCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVELKSGYKDWILWISFAIS CFLLCVALLGFIMWACQKGNIRCNICI

mH14/3 DNA	**ccgaagttgggggggAGCAAAAGCAGGGGATAATTCTATTAACCATGAAGACTATCATTG** CTTTGAGCTACATTCTATGTCTGGTTTTCGCTCAAAAAATTCCTGGAAATGACAATAGCA CGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCA CGAATGACCGAATTGAAGTcACTAATGCTACTGAGCTGGTTGAGACGAACCACACTGATG AACTGTGCCCAAGCCCTCATCAGATCCTTGATGGACAAGACTGCGACCTAATAGATGCTC TATTGGGAGACCCTCAGTGTGATGGCTTTCAAAATAAGACTTGGGACCTTTTTGTTGAAC GAAGCAAAGCCTACAGCAGCTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGT CACTAGTTGCCTCATCCGGCACACTGGAGTTcAACAAcGAAAGCTTCAATTGGACTGGAG TCACTCAAAACGGAACAAGTTCTGCTTGCTTGAGGGGCGGTCGCAACAGCTTCTTTAGTA GATTAAATTGGTTGACCCACTTAAACGGAAAATACCCAGCATTGAACGTGACTATGCCAA ACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAATG ACCAAATCTTCCCGTATGCTCAATCATCAGGAAGAATCACAGTATCTACCCGCTCGGACC AACAAaCTGTAATCCCAAATATCGGATCTAGACCCAGAATAAGGAATATCCCTAGCAGAA TAAGCATCTATTGGACAATAGTAAAACCGGGAGACATACTTTTGATTcACAGCACAGGGA ATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAAAGGGAAGAGCTCAATAATGAGAT CAGATGCAAGGATTGGGTCATGCACAAGCCCTTGCATCACTCCAAATGGAAGCATTCCCA ATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTA AGCATAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAG GCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTT GGTACGGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCA CTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAAAACCAACG AGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGAAGAATTCAGGACCTTG AGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGTTG CCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAA AAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAAAATAT ACCACAAATGTGACAATGCCTGCATAGGATCAATAAGAAATGGAACTTATGACCACAATG TGTACAGGGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCTGAAGTCAG GGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTTGTGTTG CTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAGGGCAACATTAGGTGCAACATTTGCA TTTGAGTGCATTAATTAAAAACACCCTTGTTTCTACT**aataacccggcggcc**
mH14/3 protein	MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVE TNHTDELCPSPHQILDGQDCDLIDALLGDPQCDGFQNKTWDLFVERSKAYSSCYPYDVPD YASLRSLVASSGTLEFNNESFNWTGVTQNGTSSACLRGGRNSFFSRLNWLTHLNGKYPAL NVTMPNNEQFDKLYIWGVHHPGTDNDQIFPYAQSSGRITVSTRSDQQTVIPNIGSRPRIR NIPSRISIYWTIVKPGDILLIHSTGNLIAPRGYFKIRKGKSSIMRSDARIGSCTSPCITP NGSIPNDKPFQNVNRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENGWE GMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEG RIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGN GCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVELKSGYKDWILWISFAISC FLLCVALLGFIMWACQKGNIRCNICI
cH10/3 DNA	**ccgaagttgggggggAGCAAAAGCAGGGGATAATTCTATTAACCATGAAGACTATCATTG** CTTTGAGCTACATTCTATGTCTGGTTTTCGCTCAAAAAATTCCTGGAAATGACAATAGCA CGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCA CGAATGACCGAATTGAAGTTACTAATGCTACTGAGCTGGTTgagagtacagtcataaaca gattatgtatgaaaggaagaaaacataaagacctgggcaactgccatccaatagggatgc taatagggactccagcttgtgatctgcaccttacagggatgtgggacactctcattgaac gagagaatgctattgcttactgctaccctggagctactgtaaatgtagaagcactaaggc agaagataatggagagtggagggatcaacaagataagcactggcttcacttatggatctt ccataaactcggccgggaccactagagcgtgcatgaggaatggagggaatagcttttatg cagagcttaagtggctggtatcaaagagcaaaggacaaaacttccctcagaccacgaaca cttacagaaatacagacacggctgaacacctcataatgtggggaattcatcacccttcta gcactcaagagaagaatgatctatatggaacacaatcactgtccatatcagtcgggagtt ccacttaccggaacaattttgttccggttgttggagcaagacctcaggtcaatggacaaa gtggcagaattgattttcactggacactagtacagccaggtgacaacatcaccttctcac acaatgggggcctgatagcaccgagccgagttagcaaattaattgggaggggattgggaa

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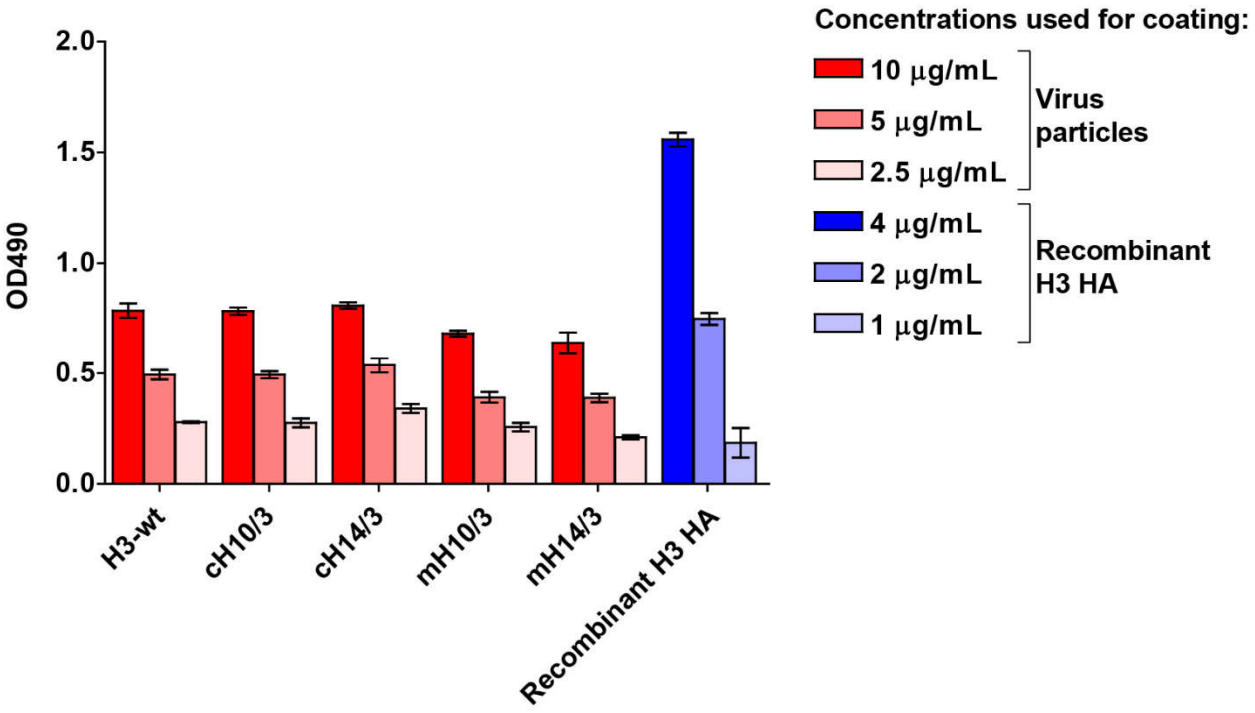
cH10/3 protein	MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVe stginrlcmkgrkhkdlgnchpigmligtpacdlhltgmwdtlierenaiaycypgatvn vealrqkimesgginkistgftygssinsagttracmrnggnsfyaelkwlvskskgqnf pqttntyrntdtaehlimwgihhpsstqekndlygtqslsisvgsstyrnnfvpvvgarp qvngqsgridfhwtlvqpgdnitfshnggliapsrvskligrglgiqsdapidnnceskC ITPNGSIPNDKPFQNVNRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIEN GWEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSE VEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAED MGNGCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVELKSGYKDWILWISFA ISCFLLCVALLGFIMWACQKGNIRCNICI
cH14/3 DNA	**ccgaagttgggggggg**AGCAAAAGCAGGGGATAATTCTATTAACCATGAAGACTATCATTG CTTTGAGCTACATTCTATGTCTGGTTTTCGCTCAAAAAATTCCTGGAAATGACAATAGCA CGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCA CGAATGACCGAATTGAAGTTACTAATGCTACTGAGCTGGTTgagacgaaccacactgatg aactgtgcccaagccccttgaagcttgtcgacgggcaagactgcgacctcatcaatggtg cattggggagtccaggctgtgaccgtttgcaggacaccacttgggatgtcttcattgaaa ggcccactgcagtagacacatgttatccattcgacgtcccagattaccagagtctcagaa gcatcctagcaagcagtgggagtttggagttcatcgccgaacaattcacctggaatggtg tcaaagttgacggatcaagcagtgcttgtttgaggggcggtcgcaacagcttcttctccc gactaaactggctaaccaaagcaacaaatggaaactatggacctattaacgtcactaaag aaaatacgggctcttatgtcaggctctatctctggggagtgcatcacccatcaagcgata atgagcaaacggatctctacaaggtggcaacagggagagtaacagtatctacccgctcgg accaaatcagtattgttcccaatataggaagtagaccgagggtaaggaatcagagcggca ggataagcatctactggaccctagtaaacccaggggactccatcattttcaacagtattg ggaatttgattgcaccaagaggccactacaaaataagcaaatctactaagagcacagtgc ttaaaagtgacaaaaggattgggtcatgcacaagccctTGCATCACTCCAAATGGAAGCA TTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGAT ATGTTAAGCATAGCACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAA CTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGG ATGGTTGGTACGGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCA AAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAAAA CCAACGAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGAAGAATTCAGG ACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTC TTGTTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGT TTGAAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCA AAATATACCACAAATGTGACAATGCCTGCATAGGATCAATAAGAAATGGAACTTATGACC ACAATGTGTACAGGGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCTGA AGTCAGGGTACAAAGATTGGATCCTATGGATTTCCTTTGCCATATCATGTTTTTTGCTTT GTGTTGCTTTGTTGGGGTTCATCATGTGGGCCTGCCAAAAGGGCAACATTAGGTGCAACA

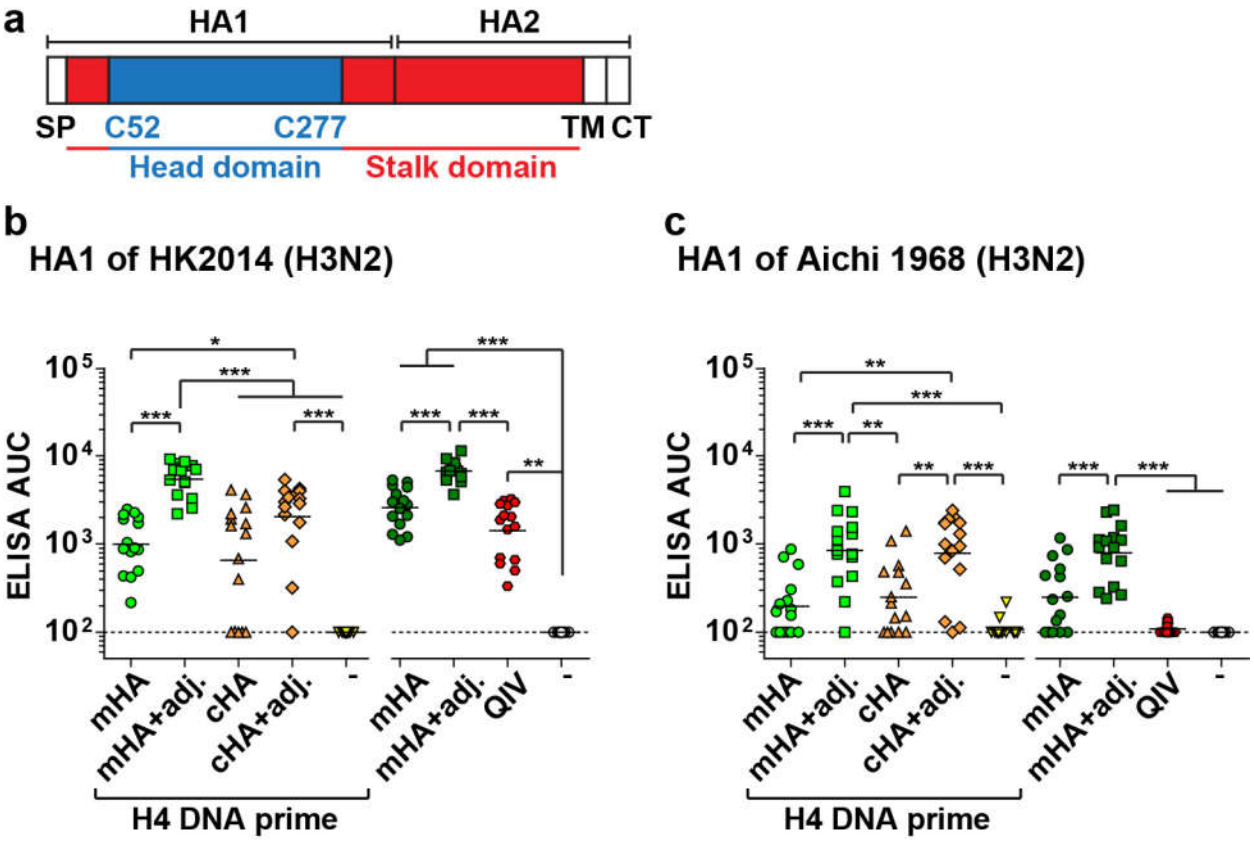
	TTTGCATTTGAGTGCATTAATTAAAAACACCCTTGTTTCTACT**aataacccggcggcc**
cH14/3 protein	MKTIIALSYILCLVFAQKIPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATELVe tnhtdelcpsplklvdgqdcdlingalgspgcdrlqdttwdvfierptavdtcypfdvpd yqslrsilassgslefiaeqftwngvkvdgsssaclrggrnsffsrlnwltkatngnygp invtkentgsyvrlylwgvhhpssdneqtdlykvatgrvtvstrsdqisivpnigsrprv rnqsgrisiywtlvnpgdsiifnsignliaprghykiskstkstvlksdkrigsctspCI TPNGSIPNDKPFQNVNRITYGACPRYVKHSTLKLATGMRNVPEKQTRGIFGAIAGFIENG WEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEV EGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDM GNGCFKIYHKCDNACIGSIRNGTYDHNVYRDEALNNRFQIKGVELKSGYKDWILWISFAI SCFLLCVALLGFIMWACQKGNIRCNICI

Supplementary Table 2. Primers used in this study.

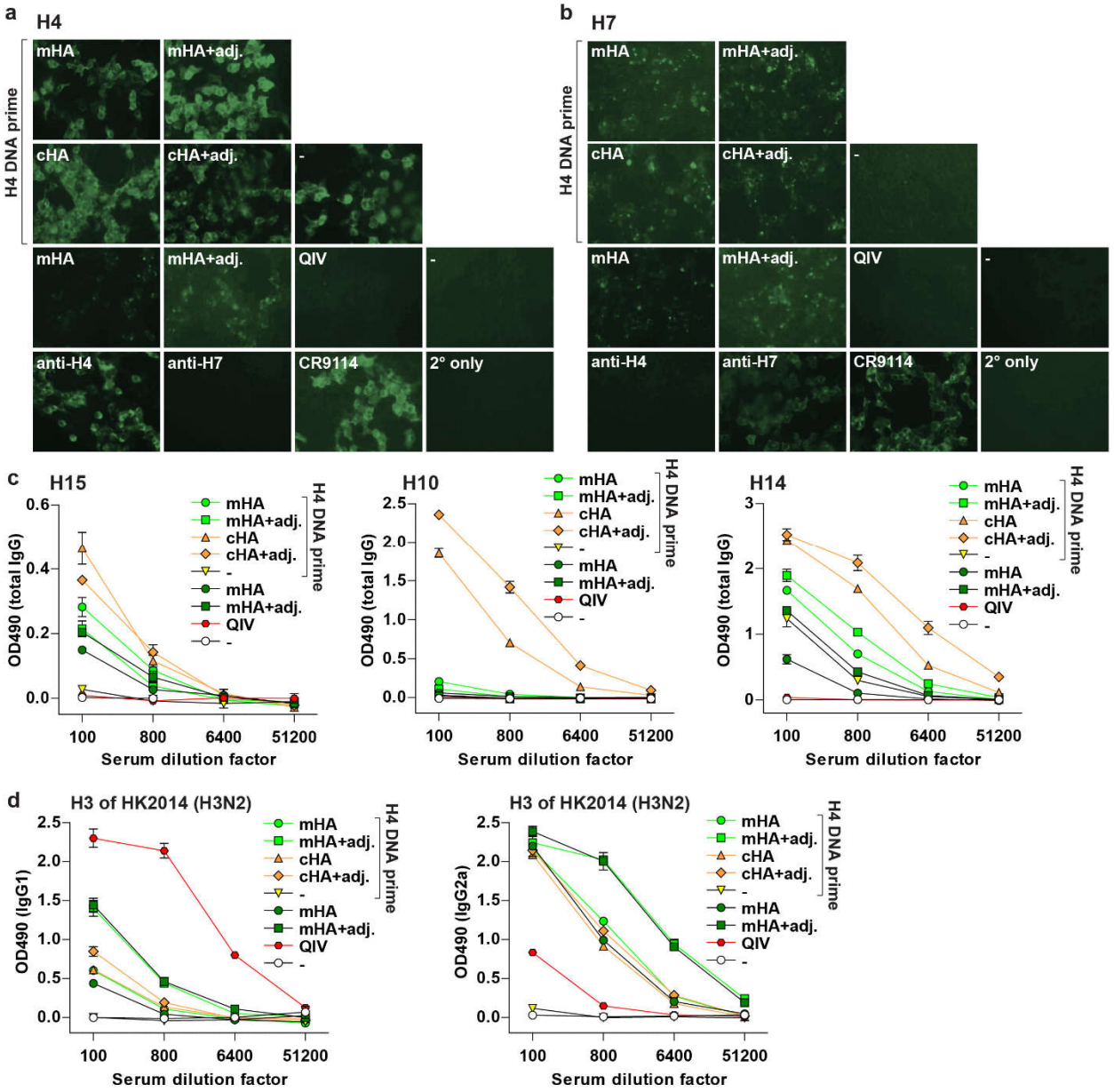
Primer	Purpose	Sequence (5’ to 3’)
pDZ_forward	Sequencing of plasmids	TACAGCTCCTGGGCAACGTGCTGG
pDZ_reverse	Sequencing of plasmids	AGGTGTCCGTGTCGCGCGTCGCC
H3_forward	Sequencing of PCR fragments	GGGAGCAAAAGCAGGGGATAATTC
H3_internal	Sequencing of plasmids and PCR fragments	TACCCAGCATTGAACGTGAC
H3_reverse	Sequencing of PCR fragments	GGGTTATTAGTAGAAACAAGGGTGTTTTTAATTAATG



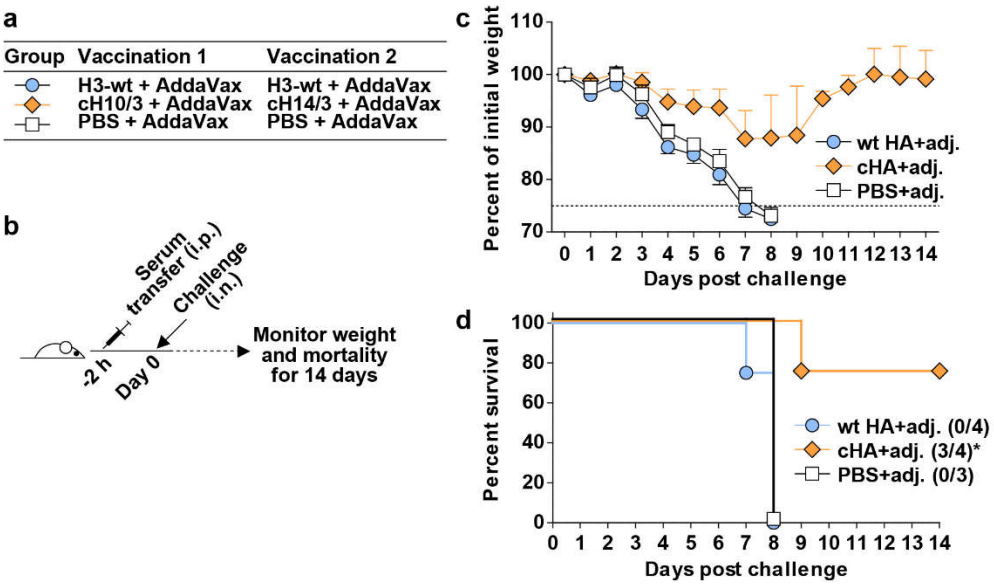
Supplementary Figure 1. Characterization of the hemagglutinin content of the different recombinant viruses, as determined by ELISA. Inactivated virus particles were diluted in phosphate-buffered saline (PBS) to the indicated concentrations and coated overnight onto ELISA plates. Recombinant H3 HA of HK2014 virus (H3N2) diluted in PBS was coated as a control. The next day, monoclonal antibody CR9114[30,31] was used to quantify the HA protein. The bars represent the mean ± SD of measurements in triplicates.



Supplementary Figure 2. Serum antibody responses of vaccinated mice determined by ELISA**.** **a** Schematic representation of the HA protein. The globular head domain is located between residues C52 and C277 (H3 numbering) indicated blue. The stalk domain comprises the remaining portions of HA1 and HA2 subunits. SP, signal peptide; TM, transmembrane domain; CT, cytoplasmic tail. The HA1 proteins used in panels **b** and **c** comprise the HA1 region without signal peptide and carry a C-terminal hexahistidine tag. **b,c** IgG responses against recombinant HA1 proteins from the A/Hong Kong/4801/2014 (panel **b**) and A/Aichi/2/1968 (panel **c**) H3N2 viruses depicted as area under the curve (AUC). Data points represent sera of individual mice (15 per group), horizontal bars the geometric mean values. The dashed lines indicate the limit of detection (AUC=100), signals below this threshold were set to 100. Statistical significance was determined using Bonferroni-corrected ANOVA with **P*≤0.05, ***P*≤0.01, ****P*≤0.001. The mouse groups and immunization regime are explained in **Fig. 2** of the main text.



Supplementary Figure 3. Detection of serum IgG against various group 2 HAs. **a,b** Immunofluorescence microscopy. 293T cells transfected with plasmids expressing H4 HA of A/duck/Czechoslovakia/1956 (H4N6) (**a**) or H7 HA of A/Hunan/02285/2017 (H7N9) (**b**) were incubated with pooled sera of 15 mice per group diluted 1:50 or mAbs at 10 µg/mL. Binding was visualized with fluorescence-labeled secondary antibodies. Anti-H4 and anti-H7 mAbs were produced in-house, CR9114 is a pan anti-HA stalk antibody[30,31]. **c** ELISA-inferred IgG responses of pooled sera against trimeric recombinant H15 HA from A/shearwater/West Australia/2576/1979 (H15N9), trimeric recombinant H10 HA from A/Jiangxi-Donghu/346-1/2013 (H10N8) and trimeric recombinant H14 HA from A/mallard/Gurjev/263/1982 (H14N5). **d** ELISA-inferred IgG1 and IgG2a responses of pooled sera against trimeric recombinant H3 HA from HK2014 (H3N2) virus. Data points in **c** and **d** represent the mean ± SD of pooled sera from 15 mice measured in triplicate



Supplementary Figure 4. Serum transfer and virus challenge studies in mice. A Mice (n=10) were immunized intramuscularly either twice with inactivated virus carrying wildtype H3 protein of the HK2014 virus (H3-wt), or with inactivated cH10/3 virus followed by cH14/3 virus, or twice with PBS. All immunizations were in three-week intervals in the presence of AddaVax adjuvant, and viruses were administered at a dose of 10 µg per immunization. Sera were obtained four weeks after the second vaccination, pooled, and used for serum transfer experiments displayed in panels **b-d**. **b** Mice (n=3-4) received 200 µL of pooled sera intraperitoneally (i.p.) and were challenged intranasally (i.n.) with 5 mLD_{50} of X-31 (a reassortant virus with the HA and NA of A/Hong Kong/1/1968 and the internal proteins of PR8). Weight (panel **c**) and survival (panel **d**) were observed for 14 days post-infection. The weight curves show the mean with SD. In the survival plot, the proportion of surviving animals in each group is shown in parentheses and statistical significance was inferred by log rank Mantel-Cox tests against the PBS group with *P≤0.05.