Supplementary data to:

Contemporary human H3N2 influenza A viruses require a low threshold of suitable glycan receptors for efficient infection

Running head: Recent H3N2 influenza requires a low threshold of receptors

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Supplementary data includes: Figs. S1-S8 and Tables SI-SXIII

Supplementary figures

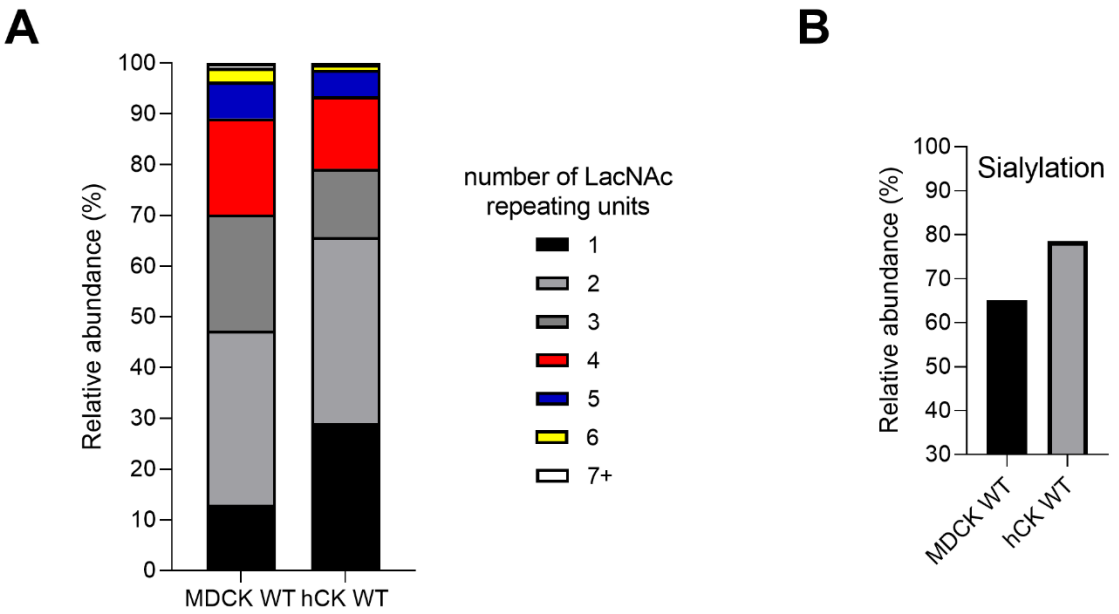


Fig S1. Meta-analysis of the MALDI-TOF-MS analysis of assigned peaks of *N*-glycans of (Byrd-Leotis, L., Jia, N., et al. 2022) (**A**) The data in Supplementary Table 1 of (Byrd-Leotis, L., Jia, N., et al. 2022) was analyzed to investigate the number of LacNAc repeating units of the detected *N*-glycans. Analysis was done similar to the analysis presented in Fig. 3B. (**B**) The data from (Byrd-Leotis, L., Jia, N., et al. 2022) was further analyzed to investigate the relative abundance of glycans (30-100%) with at least one Sia. The percentages were calculated as a percentage of the total abundance of glycans with at least one LacNAc repeating unit (the glycans shown in Fig. S1A).

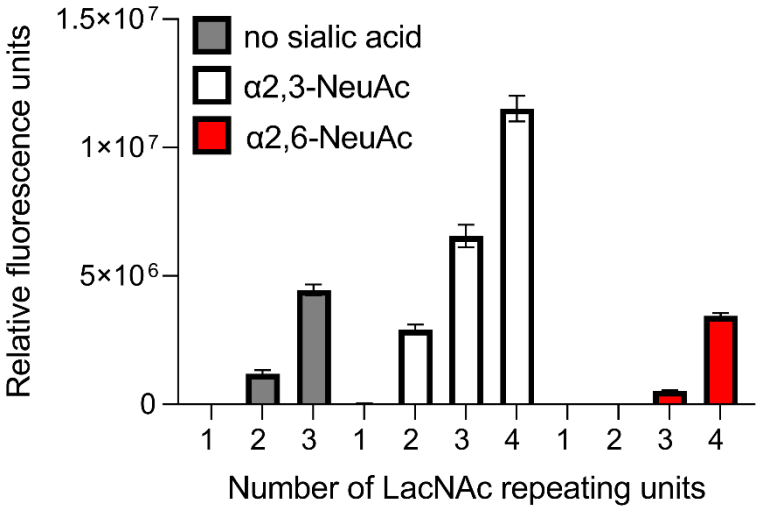


Fig S2. Binding specificity of *Lycopersicon esculentum* lectin on the glycan microarray

The binding of the *Lycopersicon esculentum* lectin (LEL) to symmetric bi-antennary *N*-glycans with 1, 2, 3, or 4 consecutive LacNAc repeating units terminating in no sialic acid, α2,3-linked NeuAc, or α2,6-linked NeuAc was investigated. Six replicates were performed simultaneously, after which the highest and lowest replicates were removed, and the mean and standard deviation were calculated over the four remaining replicates. The glycan array was performed as described earlier (Broszeit, F., Tzarum, N., et al. 2019).

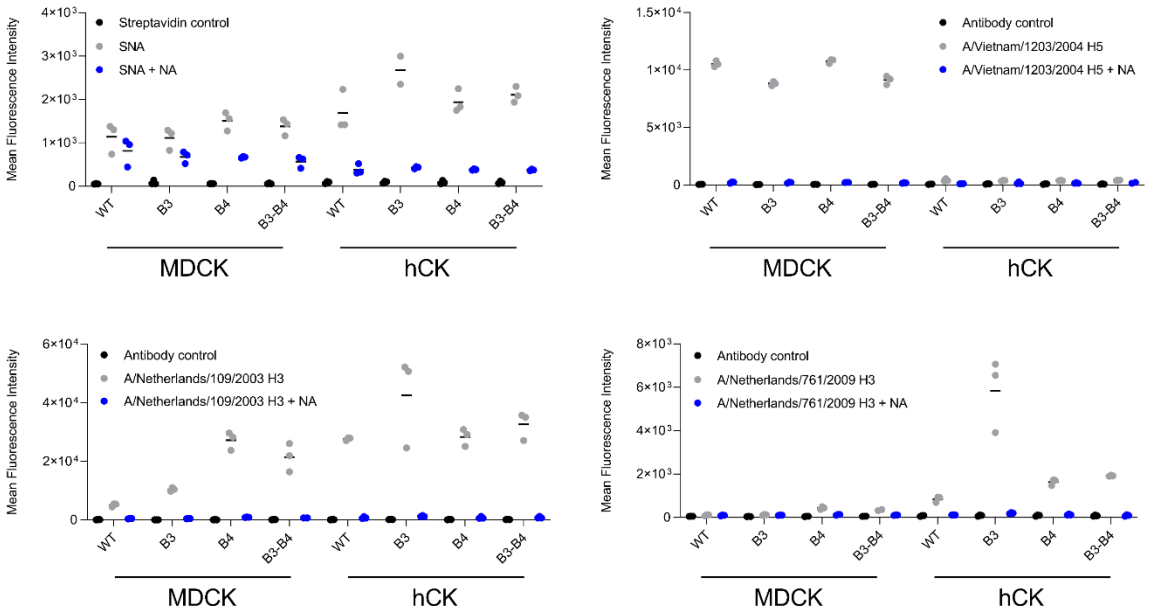


Fig S3. Flow cytometry with neuraminidase-treated B3GNT2/B4GALT1 knock-in MDCK and hCK cells

Binding of the lectin SNA and HAs A/Vietnam/1203/2004 H5, A/Netherlands/109/2003 H3, and A/Netherlands/761/2009 H3 with and without neuraminidase (NA) were measured using flow cytometry. The gating strategy as indicated in Fig. 2A was used. Triplicate measurements were performed, and the mean and all individual measurements are shown.

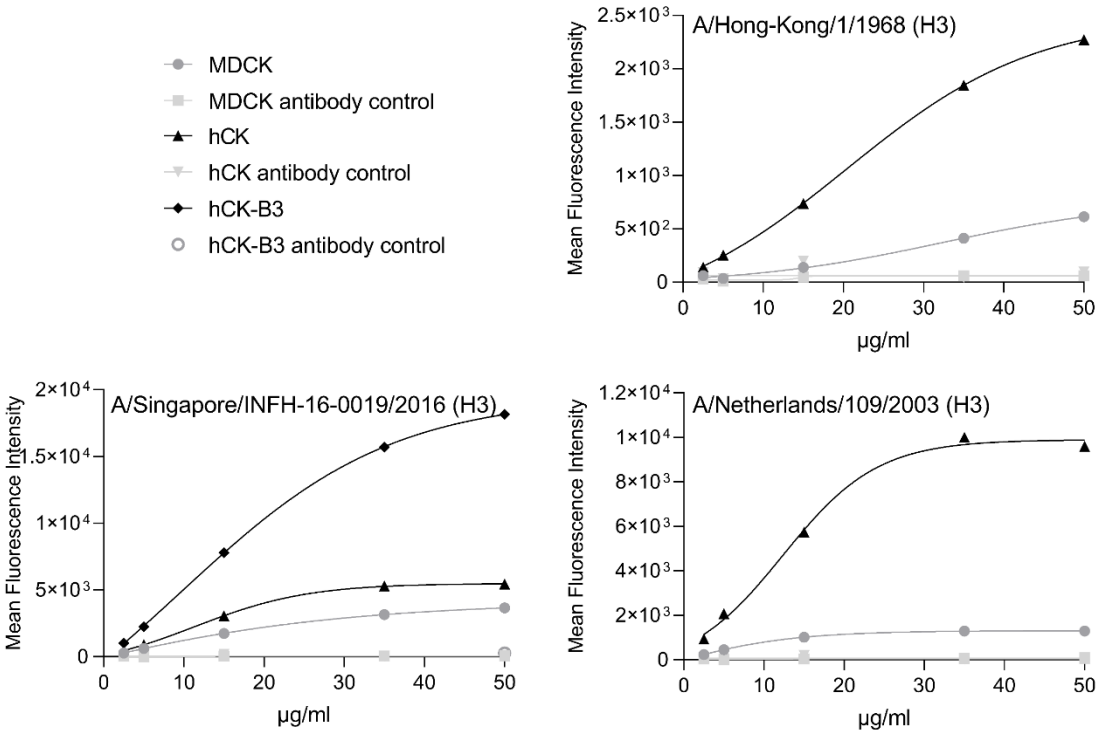


Fig S4. Titration of influenza hemagglutinins in flow cytometry

Flow cytometric titrations of the H3 HAs of A/Hong-Kong/1/1968, A/Singapore/INFH-16-0019/2016, and A/Netherlands/109/2003 in MDCK and hCK cells, including controls in the presence of just precomplexing controls were performed. For A/Singapore/INFH-16-0019/2016, also hCK-B3GNT2 cells were used. The gating strategy as described in Fig. 2A was used.

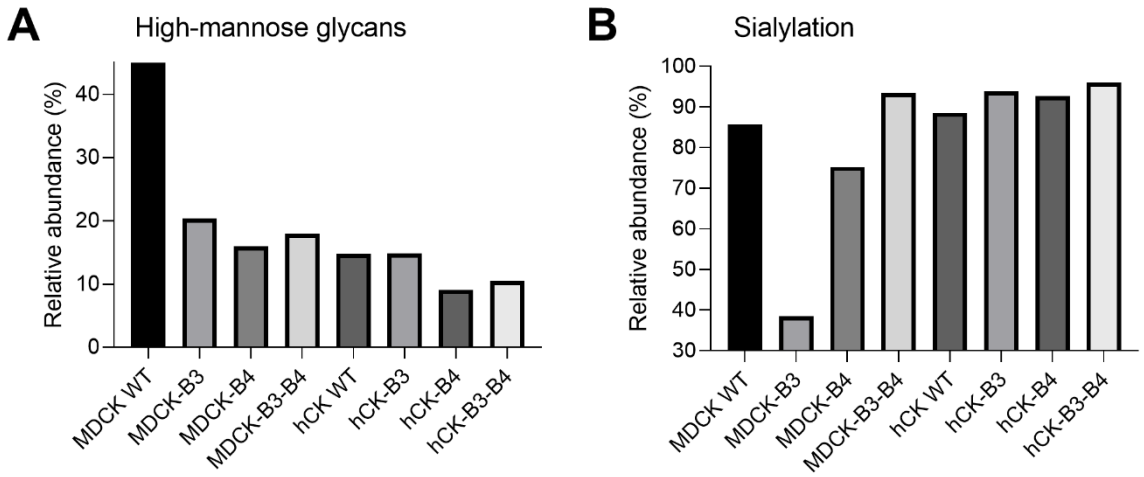


Fig S5. Relative abundance of high-mannose glycans and sialylation on WT and B3GNT2/B4GALT1 knock-in MDCK and hCK cells

The *N*-glycans from WT and B3GNT2/B4GALT1 knock-in MDCK and hCK cells were measured using HILIC-IMS-QTOF positive mode mass spectrometry. (**A**) The relative abundance of high-mannose glycans was calculated as a percentage of all detected *N*-glycans (see Table SII-IX). (**B**) The relative abundance of glycans (30-100%) with at least one Sia was calculated as a percentage of the total abundance of glycans with at least one LacNAc repeating unit (the glycans shown in Fig. 3B).

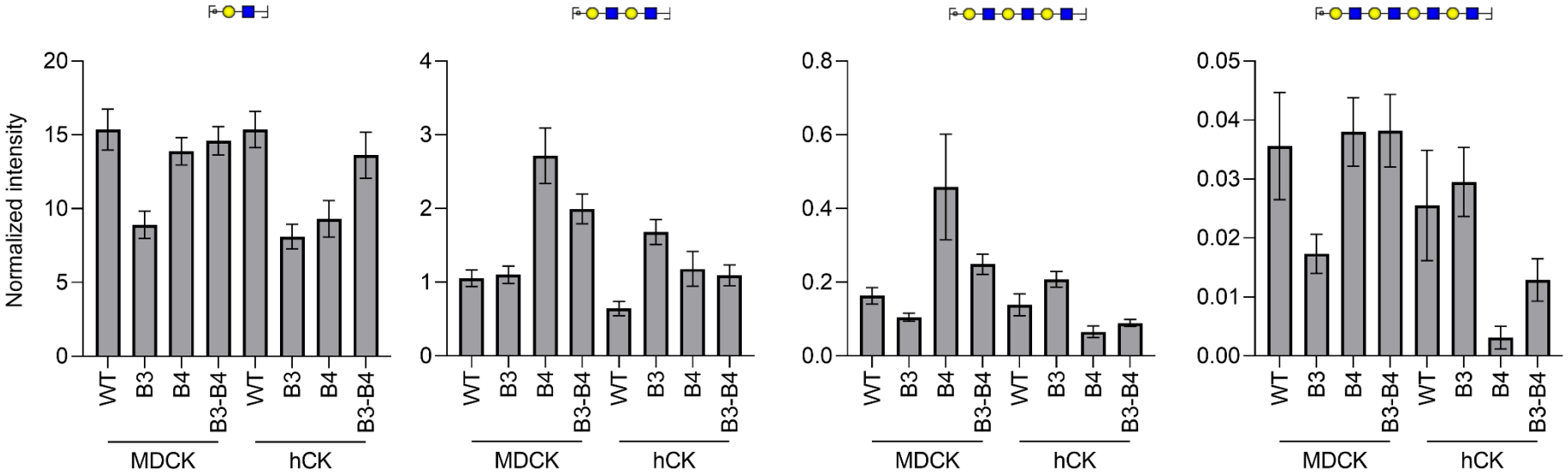


Fig S6. Normalized intensity of (repeating) LacNAc oxonium ions in MS/MS analysis

The *N*-glycans from WT and B3GNT2/B4GALT1 knock-in MDCK and hCK cells were analyzed by MS/MS. Analysis of the *N*-glycans was additionally performed by LC-MS/MS, followed by analysis of the glycan oxonium ions (Table SX). (Repeating) LacNAc oxonium ions with masses (from left to right) 366.1395, 731.2717, 1096,4039, and 1461.5361 were identified and the amounts detected were normalized to the core fragments. Mean and standard errors (n=3) are shown.

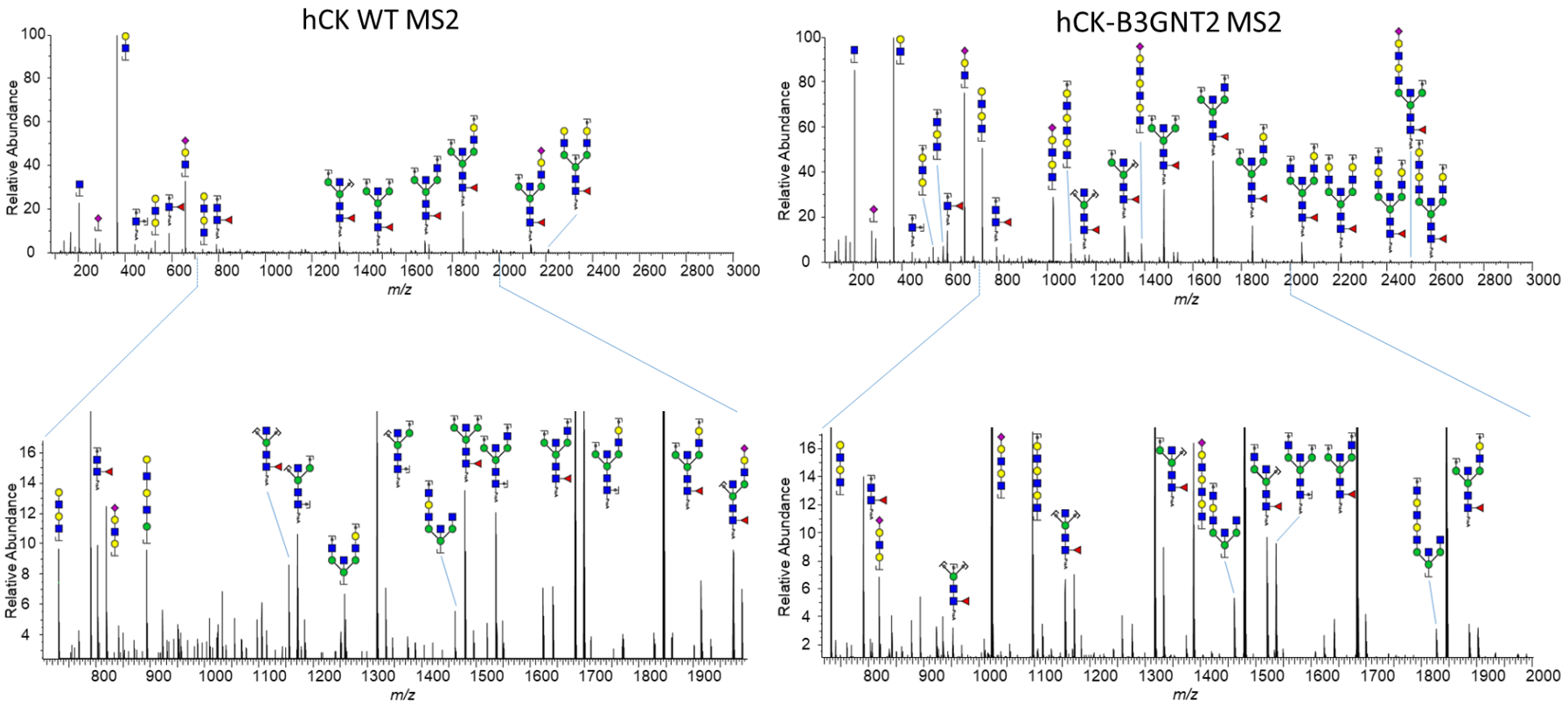
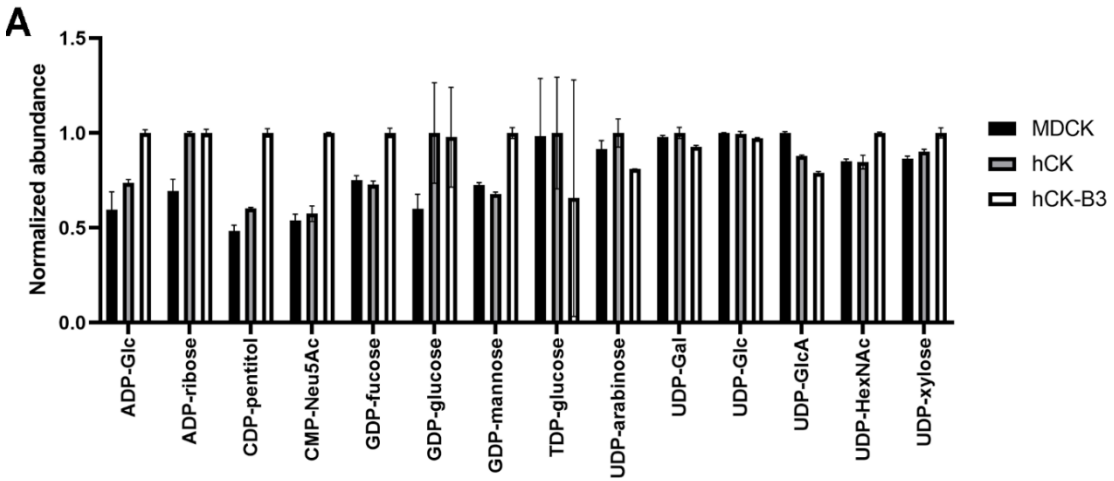


Fig S7. Annotated MS/MS spectra of selected *N*-glycans of hCK WT and hCK-B3GNT2 cells

Higher-energy collisional dissociation fragmentation at 15% NCE showed the presence of multiple oxonium ions that indicate repeating LacNAc units as well as bisection. The glycan cartoons are mostly compositional, since our LC-MS/MS method lacks the ability to give structure specific information.

A



B

	ADP-Glc	ADP-ribose	CDP-pentitol	CMP-Neu5Ac	GDP-fucose	GDP-glucose	GDP-mannose
MDCK	4.7E-04	5.9E-02	8.4E-01	1.4E+00	4.7E-01	6.8E-03	6.6E-01
hCK	2.8E-04	4.1E-02	4.0E-01	7.6E-01	3.6E-01	4.2E-03	4.8E-01
hCK-B3	3.5E-04	5.9E-02	5.0E-01	8.1E-01	3.4E-01	7.0E-03	4.5E-01

	TDP-glucose	UDP-arabinose	UDP-Gal	UDP-Glc	UDP-GlcA	UDP-HexNAc	UDP-xylose
MDCK	1.1E-03	1.0E-01	1.7E+01	6.7E+01	9.6E-01	9.9E+00	1.5E+00
hCK	1.6E-03	1.2E-01	1.8E+01	6.9E+01	1.2E+00	8.5E+00	1.3E+00
hCK-B3	1.6E-03	1.3E-01	1.8E+01	6.9E+01	1.1E+00	8.4E+00	1.3E+00

Fig S8. Sugar nucleotide analysis of MDCK, hCK, and hCK-B3GNT2 cells

The sugar nucleotides in the lysate of MDCK, hCK, and hCK-B3GNT2 cells were analyzed by mass spectrometry (n=2). (**A**) The normalized abundance of all measured sugar nucleotides is shown. Normalization was performed on the cell line with the highest amount of each sugar nucleotide. (**B**) Details of all analyzed sugar nucleotides, normalized over the sum of all measured nucleotide sugars.

Supplementary tables

Table SI. Specificities of used lectins and viral proteins

Lectin/viral protein	Receptor specificity	Reference
Sambucus nigra agglutinin (SNA)	α2,6-linked Sias	(Shibuya, N., Goldstein, I.J., et al. 1987)
Lycopersicon esculentum lectin (LEL)	Elongated glycans, prefers α2,3-linked Sias over α2,6-linked Sias	(Sweeney, J.G., Liang, J., et al. 2018), this work
Erythrina cristagalli lectin (ECA)	Terminal galactose, thus glycans lacking Sia capping	(Broszeit, F., Tzarum, N., et al. 2019)
γCoV/AvCoV/guinea fowl/France/14032 /2014 NTD	Elongated glycans	(Bouwman, K.M., Delpont, M., et al. 2019)
A/Vietnam/1203/2004 H5	Terminal α2,3-linked Sias	(Broszeit, F., Tzarum, N., et al. 2019, Spruit, C.M., Nemanichvili, N., et al. 2021, Spruit, C.M., Zhu, X., et al. 2022)
A/Puerto-Rico/8/1934 H1	Terminal α2,6-linked Sias	(Nemanichvili, N., Tomris, I., et al. 2019)
A/Hong-Kong/1/1968 H3	Terminal α2,6-linked Sias, does not require multiple consecutive LacNAc, but strong preference for glycans with three or four consecutive LacNAc repeating units.	(Peng, W., de Vries, R.P., et al. 2017)
A/Netherlands/109/2003 H3	Terminal α2,6-linked Sias, binds glycans with both two and three, but not one, consecutive LacNAc repeating units.	(Broszeit, F., van Beek, R.J., et al. 2021)
A/Netherlands/761/2009 H3		
A/Hong-Kong/480/2014 H3 (3C.2a)	Exact specificity unknown. In general, contemporary H3 IAVs are known to bind terminal α2,6-linked Sias presented on glycans with multiple consecutive LacNAc repeating units	See references in introduction
A/Netherlands/354/2016 H3		
A/Singapore/INFH-16-0019/2016 H3 (3C.2a)		
A/Kansas/14/2017 H3 (3C.3a)		
A/Netherlands/00010/2019 H3 (3C.2a)		

Supplementary tables presented in the attached excel file

Table SII. Relative abundance of *N*-glycans of MDCK WT cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SIII. Relative abundance of *N*-glycans of MDCK-B3GNT2 cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SIV. Relative abundance of *N*-glycans of MDCK-B4GALT1 cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SV. Relative abundance of *N*-glycans of MDCK-B3GNT2-B4GALT1 cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SVI. Relative abundance of *N*-glycans of hCK WT cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SVII. Relative abundance of *N*-glycans of hCK-B3GNT2 cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SVIII. Relative abundance of *N*-glycans of hCK-B4GALT1 cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SIX. Relative abundance of *N*-glycans of hCK-B3GNT2-B4GALT1 cells measured using HILIC-IMS-QTOF positive mode mass spectrometry

Table SX. List of oxonium ions of *m/z* 120-2000 [M+H]+ that were investigated in the MS/MS analysis

Supplementary tables

Table SXI. Primers used for the generation of the transfer plasmids. In brackets is indicated which segment is amplified, and the other part of the name indicates the overhang. The overhang is marked in bold in the sequence.

Primer	Sequence	Used in plasmid
B4GALT1-(pCF)-fwd	**GGACACCGAGCT**ACGCGTTAAGTCGACAATC	pCF-B3GNT2-B4GALT1 pCF-B4GALT1
ss-(pCF)-rev	**CCGAAGCCTCAT**CGGTCCAGGATTCTCTTC	pCF-B3GNT2-B4GALT1 pCF-B3GNT2 pCF-B4GALT1
pCF-(ss)-fwd	**GAATCCTGGACCG**ATGAGGCTTCGGGAGCCG	pCF-B3GNT2-B4GALT1 pCF-B3GNT2 pCF-B4GALT1
B3GNT2-(ss)-rev	**CTTTTCCATTTTT**CTGCAGCGGTGTGGAGAC	pCF-B3GNT2-B4GALT1 pCF-B3GNT2
ss-(B3GNT2)-fwd	**ACACCGCTGCAG**AAAAATGGAAAAGGGGAAG	pCF-B3GNT2-B4GALT1 pCF-B3GNT2
T2A-(B3GNT2)-rev	**CCCCTGCCCTCTCTAGAGGG**GCATTTTAAATGAGCACTCTGCAAC	pCF-B3GNT2-B4GALT1
B3GNT2-(T2A)-fwd	**GTTGCAGAGTGCTCATTTAAAATGC**CCCTCTAGAGAGGGCAGGGGAAG	pCF-B3GNT2-B4GALT1
ss-(T2A)-rev	**CTCAGGAGCGGCTCCCGAAGCCTCAT**CTCGAGTGGGCCGGGATTTTCC	pCF-B3GNT2-B4GALT1
T2A-(ss)-fwd	**GGAAAATCCCGGCCCACTCGAGATGAG**GCTTCGGGAGCCGCTCCTGAG	pCF-B3GNT2-B4GALT1
B4GALT1-(ss)-rev	**CACTGTTCGAGCC**CTGCAGCGGTGTGGAGAC	pCF-B3GNT2-B4GALT1 pCF-B4GALT1
ss-(B4GALT1)-fwd	**GGTCGGAGTCTCCACACCGCTGCAG**GGCTCGAACAGTGCCGCCGCCATC	pCF-B3GNT2-B4GALT1 pCF-B4GALT1
pCF-(B4GALT1)-rev	**GAGGTTGATTGTCGACTTAACGCGT**AGCTCGGTGTCCCGATGTCCACTG	pCF-B3GNT2-B4GALT1 pCF-B4GALT1
B3GNT2-(pCF)-fwd	**TTTAAAATGC**ACGCGTTAAGTCGACAATC	pCF-B3GNT2
pCF-(B3GNT2)-rev	**CTTAACGCGT**GCATTTTAAATGAGCACTC	pCF-B3GNT2

Table SXII. Primers used in the RT-qPCR experiments

Primer	Sequence
B4GALT1-fw	GACGTGGACCTCATTCCAA
B4GALT1-rev	CCCAATAATTATTAGGAAATCCATTGAT
B3GNT2-fw	GACGTTTATACTGGAATGTGCC
B3GNT2-rev	CATCTCTTGAGGTTTTCTACTATG
ST6GAL1-fw	GATCATGACGCAGTCCTGAG
ST6GAL1-rev	GGTCCCATACAATTAGGATTCC
GAPDH-fw	GTCGGAGTGAACGGATTTG
GAPDH-rev	GGAATTTGCCGTGGGTAG

Table SXIII. Codon optimized nucleotide sequences of viral proteins. The (codon optimized) sequences (synthesized by Genscript) were cloned into addgene plasmid #182546 using the underlined NheI and PacI restriction sites.

Viral protein	Nucleotide sequence
γCoV/AvCoV/guinea fowl/France/14032/ 2014 NTD (codon optimized)	GCTAGCAGTCCCCTCCATCTGTAACAATGTCACTGTCGGAAGCGGGTCATGTATCTCATGCCTGTCAATCCCAGCCTATGCCAACGGCCAGAACGGCGGCGAGGCCCTGGACTTCTAC TCCCCAGATGTGATGCGGCCCCAGGACGGAGCCTATATCCAGTCTGGCTACTATGAGCCAGTGTTCACAGGCTGCTTTAACCAGACCAATCAGACAGATCCCACCTGTAGGAACGGCC TGTACAAGGGCACCCCTGGCAATCTGTCTATCCCAGGCGGCTTCCTGAACAAGTACGAGGCCTTCGGCATCATGTTTTATTGGGGCCTGGACACAGTGCTGACCAGCGGACGGGACC CCACCTTCAACCTGACCCTGGGCAATTTCTTTGTGAACTCTAAGAATTTCACAGGCTTTCCAAAGGTGAAGAGCGTGATCTTCATCAACACCGGCGACATCTTTGTGAATGGCGCCCTG CTGGGCAAGTACAACCTGAATTTCACAAACAACCTGGTCATCTGGCTGGCCCAGTGCGTGGGCACCATGAAGGTGGTGGTGCTGAAGCAGTCTAATGCCCTGGTGCAGTTCAGCGCC GGCAACGTGGTGGCCTTTGAGCCATGCACAGGCGATACCATCCTGAATAAGCTGCACTGTTCCTACCAGCAGTTCAACTTTTCTACAGGCTTCTATGACATCGATACCTTTGTGCCCGT GAGCTCCAATCTGACATACCTGCCCTATCCTGACCTGAAGGATAACACCGGCCAGCAGGTGTACGACTTTTATGTGGCCCTGAGGGGAGCAGAGGTGCACTACAACTTAATTAA
A/Vietnam/1203/ 2004 H5 (not codon optimized)	GCTAGCAGATCAGATTTGCATTGGTTACCATGCAAACAACTCGACAGAGCAGGTTGACACAATAATGGAAAAGAACGTTACTGTTACACATGCCCAAGACATACTGGAAAAGACACA CAATGGGAAGCTCTGCGATCTAGATGGAGTGAAGCCTCTAATTTTGAGAGATTGTAGTGTAGCTGGATGGCTCCTCGGAAACCCAATGTGTGACGAATTCATCAATGTGCCGGAATG GTCTTACATAGTGGAGAAGGCCAATCCAGTCAATGACCTCTGTTACCCAGGGGATTTCAATGACTATGAAGAATTGAAACACCTATTGAGCAGAATAAACCATTTTGAGAAAATTCAG ATCATCCCCAAAAGTTCTTGGTCCAGTCATGAAGCCTCATTGGGGGTGAGCTCAGCATGTCCATACCAGGGAAAGTCCTCCTTTTTCAGAAATGTGGTATGGCTTATCAAAAAGAACA GTACATACCCAACAATAAAGAGGAGCTACAATAATACCAACCAAGAAGATCTTTTGGTACTGTGGGGGATTCACCATCCTAATGATGCGGCAGAGCAGACAAAGCTCTATCAAAACC CAACCACCTATATTTCCGTTGGGACATCTACACTAAACCAGAGATTGGTACCAAGAATAGCTACTAGATCCAAAGTAAACGGGCAAAGTGGAAGGATGGAGTTCTTCTGGACAATTTT AAAACCGAATGATGCAATCAACTTCGAGAGTAATGGAAATTTCATTGCTCCAGAATATGCATACAAAATTGTCAAGAAAGGGGACTCAACAATTATGAAAAGTGAATTGGAATATGG TAACTGCAACACCAAGTGTCAAACTCCAATGGGGGCGATAAACTCTAGCATGCCATTCCACAATATACACCCTCTCACCATCGGGGAATGCCCCAAATATGTGAAATCAAACAGATTA GTCCTTGCGACTGGGCTCAGAAATAGCCCTCAACGAGAGACGCGAGGATTATTTGGAGCTATAGCAGGTTTTATAGAGGGAGGATGGCAGGGAATGGTAGATGGTTGGTATGGGTA CCACCATAGCAACGAGCAGGGGAGTGGGTACGCTGCAGACAAAGAATCCACTCAAAAGGCAATAGATGGAGTCACCAATAAGGTCAACTCGATTATTGACAAAATGAACACTCAGT TTGAGGCCGTTGGAAGGGAATTTAACAACTTAGAAAGGAGAATAGAGAATTTAAACAAGAAGATGGAAGACGGGTTCCTAGATGTCTGGACTTATAATGCTGAACTTCTAGTTCTCA TGGAAAACGAGAGAACTCTAGACTTTCATGACTCAAATGTCAAGAACCTTTACGACAAGGTCCGACTACAGCTTAGGGATAATGCAAAGGAGCTGGGTAACGGTTGTTTCGAGTTCT ATCATAAATGTGATAATGAATGTATGGAAAGTGTAAGAAACGGAACGTATGACTACCCGCAGTATTCAGAAGAAGCAAGACTAAAAAGAGAGGAAATAAGTGGAGTAAAACTCGAG TTAATTAA
A/Puerto-Rico/8/1934 H1 (codon optimized)	GCTAGCAGACACAATCTGCATTGGCTACCACGCCAACAATAGCACTGACACCGTGGATACTGTGCTGGAGAAGAACGTGACAGTGACTCACAGCGTGAACCTGCTGGAAGACTCCCA TAATGGGAAGCTGTGCAGGCTGAAAGGCATCGCCCCTCTGCAGCTGGGAAAGTGCAACATTGCTGGATGGCTGCTGGGGAATCCTGAGTGTGATCCCCTGCTGCCTGTGAGATCTTG GAGTTACATCGTGGAGACCCCAAACTCTGAAAATGGCATCTGCTACCCCGGAGACTTTATTGATTATGAGGAACTGCGGGAGCAGCTGAGCAGCGTGAGCAGCTTCGAGAGGTTCGA AATTTTCCCAAAAGAAAGCTCCTGGCCCAACCACAATACCACAAAGGGCGTGACTGCCGCTTGTTCCCATGCCGGAAAATCTAGTTTCTACCGGAACCTGCTGTGGCTGACCGAGAAG GAAGGCTCTTACCCTAAGCTGAAAAACAGTTACGTGAATAAGAAAGGGAAGGAGGTGCTGGTGCTGTGGGGCATCCACCATCCAAGCAACTCCAAAGATCAGCAGAATATCTACCA GAACGAAAATGCCTATGTGAGCGTGGTGACATCCAACTACAATAGGAGGTTCACCCCTGAGATTGCTGAAAGACCTAAGGTGCGCGACCAGGCTGGAAGAATGAACTACTATTGGA CTCTGCTGAAACCTGGGGATACCATCATTTTCGAGGCCAACGGCAATCTGATTGCTCCACGGTATGCCTTTGCTCTGTCTCGCGGGTTCGGCAGTGGAATCATTACATCTAACGCCAGT ATGCACGAGTGCAATACCAAGTGTCAGACACCCCTGGGCGCTATCAACAGCTCCCTGCCCTTCCAGAATATCCATCCTGTGACCATTGGAGAGTGCCCAAAATACGTGAGGAGCGCCA AGCTGAGAATGGTGACAGGGCTGAGGAACATCCCCAGCATTCAGTCCAGAGGCCTGTTTGGAGCCATCGCTGGATTCATTGAGGGCGGATGGACAGGGATGATCGACGGGTGGTA CGGCTATCACCATCAGAACGAACAGGGGAGCGGCTATGCCGCTGATCAGAAGTCCACCCAGAACGCCATCAATGGCATTACAAACAAAGTGAATTCCGTGATCGAGAAGATGAACAT TCAGTTTACTGCTGTGGGAAAGGAATTCAATAAGCTGGAGAAACGGATGGAAAACCTGAATAAGAAAGTGGACGATGGGTTTCTGGACATCTGGACTTACAACGCCGAGCTGCTGG TGCTGCTGGAGAATGAACGCACCCTGGACTTCCACGATTCTAACGTGAAGAATCTGTATGAAAAGGTGAAAAGTCAGCTGAAGAACAATGCTAAAGAGATCGGAAACGGGTGTTTC

	GAGTTCTACCATAAGTGCGACAACGAGTGTATGGAATCCGTGAGGAATGGCACCTACGATTATCCTAAATATTCTGAAGAGAGTAAGCTGAATAGAGAGAAAGTGGATGGAGTGAA GCTCGAGTTAATTAA
A/Hong-Kong/1/1968 H3 (codon optimized)	GCTAGCACAGGACCTGCCCGGCAACGATAATTCCACCGCCACCCTGTGCCTGGGACACCACGCAGTGCCTAATGGCACCCTGGTGAAGACCATCACAGACGATCAGATCGAGGTGAC CAACGCCACAGAGCTGGTGCAGAGCTCCTCTACCGGCAAGATCTGCAACAATCCTCACCGGATCCTGGACGGCATCGATTGTACACTGATCGACGCACTGCTGGGCGACCCACACTG CGACGTGTTCCAGAATGAGACCTGGGATCTGTTCGTGGAGAGGTCCAAGGCCTTTTCTAACTGTTACCCCTATGACGTGCCTGATTACGCCTCTCTGCGCAGCCTGGTGGCCAGCTCC GGCACCCTGGAGTTCATCACAGAGGGCTTTACCTGGACAGGCGTGACCCAGAATGGAGGATCCAACGCATGCAAGAGGGGACCAGGAAGCGGCTTCTTTTCCAGACTGAACTGGCT GACCAAGAGCGGCTCCACATACCCCGTGCTGAATGTGACCATGCCTAACAACGACAACTTCGATAAGCTGTACATCTGGGGCGTGCACCACCCTTCTACCAATCAGGAGCAGACATCC CTGTACGTGCAGGCCTCTGGAAGGGTGACCGTGTCTACACGGAGAAGCCAGCAGACCATCATCCCTAACATCGGCTCCCGGCCATGGGTGAGAGGCATGTCTAGCAGAATCTCTATC TATTGGACAATCGTGAAGCCTGGCGACGTGCTGGTCATCAACTCTAATGGCAACCTGATCGCCCCAAGGGGCTACTTCAAGATGCGCACCGGCAAGTCCTCTATCATGAGGAGCGAC GCCCCAATCGATACCTGCATCAGCGAGTGTATCACACCCAATGGCTCCATCCCAAACGATAAGCCCTTTCAGAATGTGAACAAGATCACCTACGGCGCCTGTCCAAAGTATGTGAAGC AGAATACCCTGAAGCTGGCCACAGGCATGAGGAACGTGCCCGAGAAGCAGACACGCGGCCTGTTCGGAGCAATCGCAGGCTTTATCGAGAATGGCTGGGAGGGCATGATCGACGG CTGGTATGGCTTCCGCCACCAGAACTCTGAGGGAACCGGACAGGCAGCAGACCTGAAGAGCACACAGGCAGCCATCGATCAGATCAATGGCAAGCTGAACAGAGTGATCGAGAAG ACCAATGAGAAGTTCCACCAGATCGAGAAGGAGTTTTCCGAGGTGGAGGGCAGAATCCAGGATCTGGAGAAGTACGTGGAGGACACAAAGATCGATCTGTGGAGCTATAATGCCG AGCTGCTGGTGGCCCTGGAGAACCAGCACACCATCGACCTGACAGATTCCGAGATGAACAAGCTGTTCGAGAAGACCAGGCGCCAGCTGAGGGAGAATGCCGAGGACATGGGCAA CGGCTGCTTTAAGATCTACCACAAGTGCGATAATGCCTGTATCGAGAGCATCCGCAACGGCACATACGACCACGACGTGTACCGGGACGAGGCCCTGAACAATAGATTTCAGATCAA GGGCGTGGAGCTGAAGCTCGAGTTAATTAA
A/Netherlands/109/ 2003 H3 (not codon optimized)	GCTAGCACAAAAGCTTCCCGGAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACGAATGACCAAATTGAAGTTAC TAATGCTACTGAGCTGGTTCAGAGTTCCTCAACAGGTGGAATATGCAACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGT GATGGATTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGCAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGG CACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAGAATGGAACAAGCTCTGCTTGCAAAAGGAGATCTAATAAAAGTTTCTTTAGTAGATTGAATTGGTTGACC CACTTAAAATACAAATACCCAGCATTGAACGTGACTATGCCAAACAATGAAAAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAGTGACCAAATCAGCCTAT ATGCTCAAGCATCAGGAAGAATCACAGTCTCTACCAAAAGAAGCCAACAAACTGTAATCCCGAATATCGGATCTAGACCCAGGGTAAGGGATATCTCCAGCAGAATAAGCATCTATT GGACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTCGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAATAATGAGATCAGATGCAC CCATTGGCAAATGCAATTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAACCATTTCAAAATGTAAACAGGATCACATATGGGGCCTGTCCCAGATATGTTAAGCAAAA CACTCTGAAATTGGCAACAGGGATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATCGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGACGGTTGGT ACGGTTTCAGGCATCAAAATTCTGAGGGCACAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCAACCAAATCAATGGGAAACTGAATAGGTTAATCGGGAAAACAAAC GAGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGGAGAATTCAGGACCTCGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTT GTTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAGAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTC AAAATATACCACAAATGTGACAATGCCTGCATAGAGTCAATCAGAAATGGAACTTATGACCATGATGTATACAGAGATGAAGCATTAAACAACCGGTTCCAGATCAAAGGTGTTGAG CTGAAGTTAATTAA
A/Netherlands/761/ 2009 H3 (not codon optimized)	GCTAGCACAAAAACTTCCTGGAAATGACAACAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATAACGAATGACCAAATTGAAGTTAC TAATGCTACTGAGCTGGTTCAGAGTTCCTCAACAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGT GATGGCTTCCAAAATAAGAAATGGGACCTTTTTGTTGAACGCAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAATTGCCTCATCCGG CACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAATGGAACAAGCTCTGCTTGCATAAGGAGATCTAATAACAGTTTCTTTAGTAGATTAAATTGGTTGACCC ACTTAAGATTCAAGTACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAATGACCAAATCTTCCTGTA TGCTCAAGCATCAGGAAGAATCACAGTCTCTACCAAAAGAAGCCAACAAACTGTAATCCCGAATATCGGATCTAGACCCAGAGTAAGGAATATCCCTAGCAGAATAAGCATCTATTGG ACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAATAATGAGATCAGATGCACCC ATTGGCAAATGCAATTCTGAATGCATCACACCAAATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCAAAACA CTCTGAAATTGGCAACAGGGATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATCGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTAC GGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATAGATTGATCGGAAAAACCAACGA

	GAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTCGAAGGGAGAATTCAGGACCTTGAGAAATATGTTGAGGACACCAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGT TGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCAA AATATACCACAAATGTGACAATGCCTGCATAGGATCAATCAGGAATGGAACTTATGACCACGATGTATACAGAGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCT GAAGTTAATTAA
A/Hong-Kong/480/2014 H3 (3C.2a) (codon optimized)	GCTAGCTCAAAAAATTCCTGGAAATGACAATAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACGAATGACCGAATTGAAGTTAC TAATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGT GATGGCTTTCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGG CACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAGATCTAGTAGTAGTTTCTTTAGTAGATTAAATTGGTTGACC CACTTAAACTACACATACCCAGCATTGAACGTGACTATGCCAAACAATGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGT ATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGTAATCCCAAATATCGGATCTAGACCCAGAATAAGGGATATCCCTAGCAGAATAAGCATCTATTG GACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAATAATGAGATCAGATGCACC CATTGGCAAATGCAAGTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCATAGC ACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTA CGGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAAAACCAACG AGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGAAGAATTCAGGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTG TTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGCAATGGTTGTTTCA AAATATACCACAAATGTGACAATGCCTGCATAGGATCAATAAGAAATGGAACTTATGACCACAATGTGTACAGGGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGC TGAAGTTAATTAA
A/Netherlands/354/2016 H3 (not codon optimized)	GCTAGCACAAAAAATTCCTGGAAATGACAATAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACAAATGACCGAATTGAAGTTAC TAATGCTACTGAGCTGGTTCAGAATTCCTCAATAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAAAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGT GATGGCTTTCAAAATAAGAAATGGGACCNTTTTGTTGAACGAAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGG CACACTGGAGTTTAACAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGAAATCTAGTAGTAGTTTCTTTAGTAGATTAAATTGGTTGACCC ACTTAAAATACACATACCCAGCATTGAACGTGACTGTGCCAAACAATGAACAATTTGACAAATTGTACATTTNGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGTA TGCTCGATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGTAATCCCAAATATCGGATCTAGACCCAGAATAAGGGATATCCCTAGCAGAATAAGCATCTATTGG ACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAATAATGAGATCAGATGCACCC ATTGGCAAATGCAAGTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCATAGCA CTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTAC GGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATCGATTGATCGGGAAAACCAACGA GAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGAAGAATTCAGGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTGT TGCCCTGGAGAATCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGAAATGGTTGTTTCAA AATATACCACAAATGTGACAATGACTGCATAGGATCAATAAGAAATGGAACTTATGACCACAATGTGTACAGGGATGAAGCATTAAACAACCGGTTCCAGATCAAGGGAGTTGAGCT GAAGTTAATTAA
A/Singapore/INFH-16-0019/2016 H3 (3C.2a) (not codon optimized)	GCTAGCACAAAAAATTCCTGGAAATGACAATAGCACGGCAACGCTGTGCCTTGGGCACCATGCAGTACCAAACGGAACGATAGTGAAAACAATCACAAATGACCGAATTGAAGTTAC TAATGCTACTGAGTTGGTTCAGAATTCCTCAATAGGTGAAATATGCGACAGTCCTCATCAGATCCTTGATGGAGAGAACTGCACACTAATAGATGCTCTATTGGGAGACCCTCAGTGT GATGGCTTTCAAAATAAGAAATGGGACCTTTTTGTTGAACGAAGCAAAGCCTACAGCAACTGTTACCCTTATGATGTGCCGGATTATGCCTCCCTTAGGTCACTAGTTGCCTCATCCGG CACACTGGAGTTTAAAAATGAAAGCTTCAATTGGACTGGAGTCACTCAAAACGGAACAAGTTCTGCTTGCATAAGGGGATCTAGTAGTAGTTTCTTTAGTAGATTAAATTGGTTGACC CACTTAAACTACACATATCCAGCATTGAACGTGACTATGCCAAACAAGGAACAATTTGACAAATTGTACATTTGGGGGGTTCACCACCCGGGTACGGACAAGGACCAAATCTTCCTGT ATGCTCAATCATCAGGAAGAATCACAGTATCTACCAAAAGAAGCCAACAAGCTGTAATCCCAAATATCGGATCTAGACCCAGAATAAGGGATATCCCTAGCAGAATAAGCATCTATTG GACAATAGTAAAACCGGGAGACATACTTTTGATTAACAGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAAATACGAAGTGGGAAAAGCTCAATAATGAGATCAGATGCACC CATTGGCAAATGCAAGTCTGAATGCATCACTCCAAATGGAAGCATTCCCAATGACAAACCATTCCAAAATGTAAACAGGATCACATACGGGGCCTGTCCCAGATATGTTAAGCATAGC

	ACTCTGAAATTGGCAACAGGAATGCGAAATGTACCAGAGAAACAAACTAGAGGCATATTTGGCGCAATAGCGGGTTTCATAGAAAATGGTTGGGAGGGAATGGTGGATGGTTGGTA CGGTTTCAGGCATCAAAATTCTGAGGGAAGAGGACAAGCAGCAGATCTCAAAAGCACTCAAGCAGCAATCGATCAAATCAATGGGAAGCTGAATAGGTTGATCGGAAAAACCAACG AGAAATTCCATCAGATTGAAAAAGAATTCTCAGAAGTAGAAGGAAGAGTTCAAGACCTTGAGAAATATGTTGAGGACACTAAAATAGATCTCTGGTCATACAACGCGGAGCTTCTTG TTGCCCTGGAGAACCAACATACAATTGATCTAACTGACTCAGAAATGAACAAACTGTTTGAAAAAACAAAGAAGCAACTGAGGGAAAATGCTGAGGATATGGGAAATGGTTGTTTCA AAATATACCACAAATGTGACAATGCCTGCATAGAATCAATAAGAAATGAAACTTATGACCACAATGTGTACAGGGATGAAGCATTGAACAACCGGTTCCAGATCAAGGGAGTTGAGC TGAAGTTAATTAA
A/Kansas/14/2017 H3 (3C.3a) (codon optimized)	GCTAGCTCAGAAGCTGCCTGGCAACGACAATTCTACCGCCACCCTGTGCCTGGGACACCACGCAGTGCCAAATGGCACCATCGTGAAGACCATCACAAACGATAGAATCGAGGTGAC CAATGCCACAGAGCTGGTGCAGAACAGCTCCATCGGCGAGATCTGCGACAGCCCACACCAGATCCTGGATGGCGAGAACTGTACACTGATCGACGCACTGCTGGGCGACCCACAGT GCGATGGCTTCCAGAATAAGAAGTGGGATCTGTTTGTGGAGAGATCTAAGGCCTACAGCAACTGTTACCCCTATGACGTGCCTGATTATGCCTCCCTGAGGTCTCTGGTGGCCTCTAG CGGCACCCTGGAGTTCAACAATGAGAGCTTTAATTGGGCCGGCGTGACCCAGAACGGCACATCCTCTAGCTGCATCCGCGGCAGCAAATCCTCTTTCTTTTCCCGGCTGAACTGGCTG ACTCACCTGAATTCCAAGTACCCTGCCCTGAACGTGACAATGCCAAACAATGAGCAGTTCGACAAGCTGTATATCTGGGGCGTGCACCACCCTGGCACAGACAAGGATCAGATCTCTC TGTACGCACAGAGCTCCGGAAGGATCACCGTGTCCACAAAGCGGTCTCAGCAGGCCGTGATCCCTAACATCGGCTCCCGGCCAAGAATCAGGGACATCCCCAGCCGCATCTCCATCT ATTGGACCATCGTGAAGCCAGGCGATATCCTGCTGATCAACTCTACAGGCAATCTGATCGCCCCCAGAGGCTACTTCAAGATCAGGTCTGGCAAGTCTAGCATCATGCGGAGCGACG CCCCCATCGGCAAGTGCAAGTCCGAGTGTATCACCCCTAACGGCTCTATCCCAAATGATAAGCCCTTTCAGAACGTGAATAGAATCACATACGGCGCCTGTCCCAGATATGTGAAGCA GTCCACCCTGAAGCTGGCCACAGGCATGAGGAACGTGCCTGAGAGGCAGACCAGGGGAATCTTCGGAGCAATCGCCGGCTTTATCGAGAATGGCTGGGAGGGCATGGTGGACGGC TGGTATGGCTTCAGACACCAGAACAGCGAGGGAAGGGGACAGGCAGCAGACCTGAAGTCCACCCAGGCCGCCATCGATCAGATCAACGGCAAGCTGAATCGCCTGATCGGCAAGA CAAATGAGAAGTTCCACCAGATCGAGAAGGAGTTTTCCGAGGTGGAGGGCCGGATCCAGGATCTGGAGAAGTACGTGGAGGACACCAAGATCGATCTGTGGTCTTATAATGCCGAG CTGCTGGTGGCCCTGGAGAACCAGCACACCATCGACCTGACAGATAGCGAGATGAACAAGCTGTTCGAGAAGACCAAGAAGCAGCTGAGAGAGAACGCCGAGGACATGGGCAATG GCTGCTTTAAGATCTACCACAAGTGCGATAATGCCTGTATCGGCAGCATCAGGAACGGCACATACGACCACGACGTGTACCGCGACGAGGCCCTGAACAATCGGTTTCAGATCAAGG GCGTGGAGCTGAAGTTAATTAA
A/Netherlands/00010 /2019 H3 (3C.2a) (codon optimized)	GCTAGCTCAGAAGATCCCTGGCAACGACAATTCCACCGCCACCCTGTGCCTGGGACACCACGCAGTGCCAAACGGCACCATCGTGAAGACCATCACAAACGATAGAATCGAGGTGAC CAATGCCACAGAGCTGGTGCAGAACAGCTCCATCGGCGAGATCTGCAATTCTCCACACCAGATCCTGGACGGCGGCAACTGTACACTGATCGATGCACTGCTGGGCGACCCACAGTG CGATGGATTCCAGAATAAGAAGTGGGACCTGTTTGTGGAGCGGAGCAGAGCCTACTCCAACTGTTACCCCTATGACGTGCCTGATTATGCCTCTCTGAGGAGCCTGGTGGCCTCTAGC GGCACCCTGGAGTTCAAGAACGAGAGCTTTAATTGGGCCGGCGTGACACAGAATGGCAAGTCCTCTGCCTGCATCAGAGGCAGCTCCTCTAGCTTCTTTTCCAGGCTGAACTGGCTG ACCCACCTGAATTACACATATCCTGCCCTGAACGTGACCATGCCAAATAAGGAGCAGTTCGACAAGCTGTACATCTGGGGCGTGCACCACCCTGGCACAGACAAGGATCAGATCTTTC TGTATGCCCAGTCCTCTGGCCGCATCACCGTGTCTACAAAGCGGAGCCAGCAGGCCGTGATCCCTAACATCGGCTCTAGGCCAAGGATCAGGGACATCCCATCCAGGATCTCTATCTA CTGGACCATCGTGAAGCCAGGCGATATCCTGCTGATCAACAGCACAGGCAATCTGATCGCCCCCAGAGGCTATTTCAAGATCAGGTCCGGCAAGAGCTCCATCATGCGGTCTGACGC CCCCATCGGCAAGTGCAAGAGCGAGTGTATCACCCCTAACGGCTCCATCCCAAATGATAAGCCCTTTCAGAACGTGAATAGAATCACATACGGCGCCTGTCCCAGATATGTGAAGCAG AGCACCCTGAAGCTGGCCACAGGCATGCGCAATGTGCCTGAGAAGCAGACCAGGGGAATCTTCGGAGCAATCGCAGGCTTTATCGAGAATGGCTGGGAGGGCATGGTGGACGGCT GGTACGGCTTCAGACACCAGAACTCTGAGGGAAGGGGACAGGCAGCAGACCTGAAGAGCACCCAGGCCGCCATCGATCAGATCAACGGCAAGCTGAATCGCCTGATCGGCAAGAC AAACGAGAAGTTCCACCAGATCGAGAAGGAGTTTAGCGAGGTGGAGGGAAGGGTGCAGGATCTGGAGAAGTACGTGGAGGACACCAAGATCGATCTGTGGTCCTATAATGCCGAG CTGCTGGTGGCCCTGGAGAACCAGCACACCATCGACCTGACAGATTCTGAGATGAATAAGCTGTTCGAGAAGACCAAGAAGCAGCTGAGAGAGAACGCCGAGGACATGGGCAATG GCTGCTTTAAGATCTACCACAAGTGCGATAACGCCTGTATCGGCTCCATCAGGAACGAGACATACGACCACAACGTGTACCGCGATGAGGCCCTGAACAATCGGTTTCAGATCAAGG GCGTGGAGCTGAAGTTAATTAA

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