

## Supplemental material

### **Hemagglutinins of avian influenza viruses are proteolytically activated by TMPRSS2 in human and murine airway cells**

Dorothea Bestle<sup>1\*</sup>, Hannah Limburg<sup>1\*</sup>, Diana Kruhl<sup>1</sup>, Anne Harbig<sup>1</sup>, David A. Stein<sup>2</sup>, Hong Moulton<sup>2</sup>, Mikhail Matrosovich<sup>1</sup>, Elsayed M. Abdelwhab<sup>3</sup>, Jürgen Stech<sup>3</sup>, Eva Böttcher-Friebertshäuser<sup>1#</sup>

1) Institute of Virology, Philipps-University, Marburg, Germany

2) Department of Biomedical Sciences, Oregon State University, Corvallis, USA

3) Institute of Molecular Virology and Cell Biology, Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

\* contributed equally to this work

#Corresponding author: Eva Böttcher-Friebertshäuser

Institute of Virology, Philipps-University Marburg

Hans-Meerwein-Straße 2, 35043 Marburg, Germany

Tel: 0049-6421-2866019

E-mail: friebertshaeuser@staff.uni-marburg.de

Figure S1

```
1 10 20 30 40 50 60
| | | | | | |
1_A/duck/Bavaria/1/1977 (H1N1) -----MEVKLVFLVCTFTALKAD-TICVGYHANNSTDTVDIVLEKQVTVTHSVNLL
2_A/Sentinel Mallard/Germany/S/Ra517K/2007 (H2N5) -----MAIIVLILLFTAVRGD-QICIGYHNSNSTKVKVDILERNVTVTHAQDIL
3_A/duck/Ukraine/1/1963 (H3N8) MKTVIALSYIILCLTFQDPLGNDNSTA-TLCLGHAVPNGITVKTITDDQIEVFNATELV
4_A/Victoria/3/1975 (H3N2) MKTIIALSYSIFCLVFAQDPLGNDNSTATLCLGHAVPNGITVKTITDDQIEVFNATELV
5_A/HongKong/1/68 (H3N2) MKTIIALSYSIFCLAGDPLGNDNSTA-TLCLGHAVPNGITVKTITDDQIEVFNATELV
6_A/Mallard/Germany/1240/1/2007 (H4N6) -----MLSVILFLVVAESSQNYTGNFVTCMGHHAHVANGTVMKTLTDDQEVVTAQELV
7_A/Vietnam/1203/2004 (H5N1) -----MEKIVLLFAIVSLVKSQ-QICIGYHANNSTEQVDIMEKQVTVTHAQDIL
8_A/Turkey/Germany/R617/2007 (H6N2) -----MIAIIVLAILAAGKSD-KICIGYHANNSTTQVDITILEKQVTVTHSVELL
9_A/Anhui/1/2013 (H7N9) -----MNTQILVFAIILPINAD-KICIGHHAHVANGTVMKTLTDDQIEVFNATELV
10_A/Turkey/Ontario/6118/1968 (H8N4) -----MEKFIAMLLASTNAYDRICIGYQSNSTDTVNLIEQNVPTVQIMELV
11_A/chicken/Emirates/R66/2002 (H9N2) -----METISLITILLVVTASNAD-KFCIGHQSTNSTETVDLITETNVPTVTHAKELL
12_A/Quail/Shantou/782 (H9N2) -----METISLITILLVVTASNAD-KICIGHQSTNSTETVDLITETNVPTVTHAKELL
13_A/Turkey/Wisconsin/1/1966 (H9N2) -----METKAIIAALMVTAAANAD-KICIGYQSTNSTETVDLITETNVPTVTHAKELL
14_A/Mallard/Germany/R2075/2007 (H10N7) -----MYKVVVIALVAVGKLDKICLGHAVANGTVMKTLTNEHEEVFNATELV
15_A/DomesticDuck/Germany/R784/2006 (H11N1) -----MKKTLFATIFLCVKAD-EICIGYHNSNSTKVDNIIENNVTSSVELV
16_A/Mallard/Gurijev/263/1982 (H14N3) -----MIALILVALALSHATYQITNGTGNFVTCMGHHAHVANGTVMKTLTDDQIEVFNATELV
17_A/Shearwater/WestAustralia/2576/1979 (H15N9) -----MNTQIVLIVLGLSMVSKDKICLGHAVANGTVMKTLTDDQIEVFNATELV
18_A/black-headedGull/Sweden/5/1999 (H16N3) -----MVIKLVYFLVLLSRYSKADKICIGYLSNNATDVTDLITENGVPVTSVDLV

ENSHNGKLCSLNGIAPLQKGNVAGWILGNPECDDLITANSWSYIIEIENSNGTCTYFG
EKTHNGKLCCLNGIPPELGDGCSAGWLLGNPECDDLITPEWSYIEMKENPRNGCTYFG
QSSSTGKICN-NPHRILDRGACTLIDALLGDPHCDVQNE-TWDLFVRSKAFS-NCYYP
QSSSTGKICN-NPHRILDRGACTLIDALLGDPHCDVQNE-KWDLFVRSKAFS-NCYYP
QSSSTGKICN-NPHRILDRGACTLIDALLGDPHCDVQNE-TWDLFVRSKAFS-NCYYP
ESQNLPELCP-SPLRLIDGQCTDINGALSGPCD-HLNGAENDIETERNAVD-TCYYP
EKKNGKLCCLDGVKPLILRDCSVAGWLLGNPCDFEINVPWSYIEMKANFVNDLCTYFG
ENQKERFCIKLNGKPLDRGCTIEGWLGNPCDGLLGDQSWYIEMKQVTVTHAQDILCTYFG
ERTNIPRIS-KGKRTVLDGQCGLLGTTIPGQDQFLFESA-DLIERREGSD-VCYYP
ETEKHAYCNTLDGAPLELRDCKIEAVIYGNPKDILHLDQGSYIEMKQVTVTHAQDILCTYFG
HTEHNGMLCATLGHPLILDTCTIEGLYGNPCDILLEGREWSYIEMKQVTVTHAQDILCTYFG
HTEHNGMLCATLGHPLILDTCTIEGLYGNPCDILLEGREWSYIEMKQVTVTHAQDILCTYFG
HTEHNGMLCATLGHPLILDTCTIEGLYGNPCDILLEGREWSYIEMKQVTVTHAQDILCTYFG
ESTSLKRLCM-KGRSHKDLGNCHPIGMLIGTACDHLTG-TWDLIERENAIA-VCYYP
ETEHTSGFCSINGKQPSILGDCSFAWLLGNPCDGLLIGKTSYIEMKQVTVTHAQDILCTYFG
ETNHTDELCP-SPLRLIDGQCDLINGALSGPCDR-LQDITWDFIETERTAVD-TCYYP
EITGDKVCT-KGKAVDLGSCGLGTTIPGQDQFLHFEKA-DLIERENSSD-ICYYP
ETNHTGTCYCSLNGIPIHLGDCSFEWLVGNPCATNIREWSYIEMKQVTVTHAQDILCTYFG

EFIDYELREQLSSVXSEKFEIIPKASWNEHETKGVTAACSYS-GASSFYRNLWIT
SFNDYELKHLSSVTHFEKVKILPK-DRW-IQHTTGGSRACVGS-GNPSFRNMLWIT
DIPDYASLRISVASSGTELFITEG--FTW-TGVQNGGSSACKRG-PANGFFSRNLWIT
DVPDYASLRISVASSGTELFITEG--FNW-TGVQNGGSSACKRG-PDGFSSRNLWIT
DVPDYASLRISVASSGTELFITEG--FTW-TGVQNGGSSACKRG-PGSGFFSRNLWIT
DVPDYQSLRISLANNKGFIEFAEE--FQW-NVTKNGKSGACKRA-NVNDFFRNLWIT
DFNDYELKHLSSVTHFEKVKIQIIPK-SWSHSEASLGVSPACYQ-KGSSFFRNLWIT
VLNEVEELKQILGSGSERVFERFEPK-STWAGVDTSSGVTRACPYN-TGSSFYRNLWIT
KFNVEEARQLIRESGGDKKEMG--FTY-SGIRTDGATSAKRRS--GSSFYEMKGLS
SVNLEELRFFSSAASYKRIKIRLFDY-SRW--NVTRSGTSAKCASTGGQSFYRSINWIT
NVNLEELRFFSSAASYKRIKIRLFDY-TIW--NVITYGTSAKCS--DSFYRSMRWIT
NVNLEELRFFSSAASYKRIKIRLFDY-TIW--NVITYGTSAKCS--DSFYRSMRWIT
NVNLEELRFFSSAASYKRIKIRLFDY-TIW--NVITYGTSAKCS--DSFYRSMRWIT
ATVNEEARQLIRESGGDKKEMG--FTY-SGIRTDGATSAKRRS--GSSFYEMKGLS
TLNEEELRKLKFSVLEFSKFEAFTS-NGWAGVNSGAGVTAACKFG-SNSFFRNLWIT
DVPDYQSLRISLASSGTELFITEG--FTW-NGVKVDGSSACKRG-GNPSFRNMLWIT
RFTNEEARQLIRESGGDKKEMG--FTY-SGIRTDGATSAKRRS--VSSFYEMKGLS
ELDNGWELRHLFSVGVNSFRTELISP-NKW--GDILGVTASCRDN-GASSFYRNLWITV

--KKGTSYPKLSKSYTNKQKQEVLIWGVHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KKGSSYFVAKGSYNTSCEQLLIWGIHHPNDEAEQRTLYQNVGTVSSTLNKRS
--KSEAYFVNLVMPNNDNFVKLIWGVHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KSGSTYFVQNVNMPNNDNFVKLIWGVHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KSGSTYFVNLVMPNNDNFVKLIWGVHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KSDGNAYPLQNLTKVNGDYARLYWGVHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KRNSTYPTIKSYNTNQEQLLIWGIHHPNDEAEQRTLYQNVGTVSSTLNKRS
--KTSAAAYFVTKGTYNTRNQPILYWGVHPHTAXEQQSILYQADAYVSGSSKYNRRF
SNTDDAAFPQMTKSYKNTKRSALIVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KKKPDYDFNEAGYVNNEDGDIILFWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--QKSGAYFVQDQYTNNRKSIILFWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--QKSGYFVQDQYTNNRKSIILFWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KKSNSYFPQNAHYNNERENILFVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
SKSEGNFPQNTYRNTDTEHLVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--HQSGTYFVTKRTFNNTKRDVLIWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KATNGYGPINVTKENTGYSYVRLVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
SSMNNQVFPQNLQYRNTKREPALIVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF
--KKNKGYFVIKGQYNTNTRDGLVLIWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF

TPEIARPKVRSQGRMYWYLLDQSDITIFPATGNLIAFWYFALNK-----DSD
VPEIARPKVRSQGRMEFSWVLDLITINFESTGNLIAFWYFALNK-----RGS
IPNIGSRFWRVRSQGRISYIYVIVKPGDVLVNSGNLIAFWYFALNK-----TGK
IPNIGSRFWRVRSQGRISYIYVIVKPGDVLVNSGNLIAFWYFALNK-----TGK
IPNIGSRFWRVRSQGRISYIYVIVKPGDVLVNSGNLIAFWYFALNK-----TGK
VFNIGSRFWRVRSQGRISYIYVIVKPGDVLVNSGNLIAFWYFALNK-----QKQ
VFNIGSRFWRVRSQGRISYIYVIVKPGDVLVNSGNLIAFWYFALNK-----KGD
SPEIARPKVRSQGRIDYVIVKPGDVLVNSGNLIAFWYFALNK-----HNK
VSPGAPRVQVGLSGRIDFHWMLNPNNDVTFSPFNAGIAPDRASFLR-----GK
QPNIGRPLVRSQGRMDYVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF-----ESY
KPVIGRPLVRSQGRINRYVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF-----GSH
KPVIGRPLVRSQGRINRYVWGIHHPHTAXEQQSILYQADAYVSGSSKYNRRF-----VSH
KPVIGRPLVRSQGRIDYVIVKPGDVLVNSGNLIAFWYFALNK-----ESH
VFNIGSRFWRVRSQGRIDYVIVKPGDVLVNSGNLIAFWYFALNK-----GR
IPNIGSRFWRVRSQGRIDYVIVKPGDVLVNSGNLIAFWYFALNK-----VGN
VFNIGSRFWRVRSQGRISYIYVIVKPGDVLVNSGNLIAFWYFALNK-----STK
SPEIARPKVRSQGRIDYVIVKPGDVLVNSGNLIAFWYFALNK-----YGT
ELEIGTR-IGDQGRSWMKLYWHLMPGRIRMFESNGGLIAPRYGYIIEK-----YGT
```

```

1_A/duck/Bavaria/1/1977 (H1N1) SGIITSDAPVH-NCOTRCQTPHAGLNSLPPFQNIHPIITIGCEPKYVSKLRLMATGLRNV
2_A/Sentinel Mallard/Germany/S/Ra517K/2007 (H2N5) SGIMKTEGTLE-NCETKCOQPLGAINITLPPFNIHPLIIGCEPKYVSKLRLMATGLRNV
3_A/duck/Ukraine/1/1963 (H3N8) SSIMRSDAPID-TCISECITPNGSIPNDKPFQNVKTIYGACPKYVSKQNTLKLATGMNRV
4_A/Victoria/3/1975 (H3N2) SSIMRSDAPI-GTCSSECIITPNGSIPNDKPFQNVKTIYGACPKYVSKQNTLKLATGMNRV
5_A/HongKong/1/68 (H3N2) SSIMRSDAPID-TCISECITPNGSIPNDKPFQNVKTIYGACPKYVSKQNTLKLATGMNRV
6_A/Mallard/Germany/1240/1/2007 (H4N6) STILNIAIPI-GSCVCKCHTDKGLSLTTPKPFQNIHPIITIGCEPKYVSKLRLMATGLRNV
7_A/Vietnam/1203/2004 (H5N1) STIMKSELEY-GNCTKCOQPLGAINITLPPFNIHPLIIGCEPKYVSKLRLMATGLRNV
8_A/Turkey/Germany/R617/2007 (H6N2) GAVFKSNLPIE-NCOTRCQTPHAGLNSLPPFQNIHPIITIGCEPKYVSKLRLMATGLRNV
9_A/Anhui/1/2013 (H7N9) SMGIQSGVQVDANCEGCVHSGGTII SNLFPQNIHPIITIGCEPKYVSKLRLMATGLRNV
10_A/Turkey/Ontario/6118/1968 (H8N4) GRIIQNEDIPIGNCTKCOQPLGAINITLPPFNIHPIITIGCEPKYVSKLRLMATGLRNV
11_A/chicken/Emirates/R66/2002 (H9N2) GRILKTDLN-SGNCVVQOQTEKGLNSTLPPFNIHPIITIGCEPKYVSKLRLMATGLRNV
12_A/Quail/Shantou/782 (H9N2) GRILKTDLN-SGNCVVQOQTEKGLNSTLPPFNIHPIITIGCEPKYVSKLRLMATGLRNV
13_A/Turkey/Wisconsin/1/1966 (H9N2) GRILKTDLN-SGNCVVQOQTEKGLNSTLPPFNIHPIITIGCEPKYVSKLRLMATGLRNV
14_A/Mallard/Germany/R2075/2007 (H10N7) GLGIQSDAPIDNNCEKCFWKGGSINLRLPFQNLSPRTVQCPKYVSKLRLMATGLRNV
15_A/DomesticDuck/Germany/R784/2006 (H11N1) GKLFRSELNIE-SCSTKCOQTEGGINLTKSFFNVRHTIGDPCPKYVSKLRLMATGLRNV
16_A/Mallard/Gurijev/263/1982 (H14N3) STVLKSDKRI-GSCTSPCLIDKSGISQDKPFQNVRIAGNCPKYVSKLRLMATGLRNV
17_A/Shearwater/WestAustralia/2576/1979 (H15N9) SLGIQSDAQIDESCEGECYFSGGTINSPLFPQNIHPIITIGCEPKYVSKLRLMATGLRNV
18_A/black-headedGull/Sweden/5/1999 (H16N3) GRIFQSGVRM-AKNCOTRCQTPHAGLNSLPPFQNIHPIITIGCEPKYVSKLRLMATGLRNV
↓
1_A/duck/Bavaria/1/1977 (H1N1) PST-QSRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
2_A/Sentinel Mallard/Germany/S/Ra517K/2007 (H2N5) PQI-ESRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
3_A/duck/Ukraine/1/1963 (H3N8) PEK-QTRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
4_A/Victoria/3/1975 (H3N2) PEK-QTRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
5_A/HongKong/1/68 (H3N2) PEK-QTRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
6_A/Mallard/Germany/1240/1/2007 (H4N6) PEK-ASRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
7_A/Vietnam/1203/2004 (H5N1) PQI-ETRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
8_A/Turkey/Germany/R617/2007 (H6N2) PQI-ENRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
9_A/Anhui/1/2013 (H7N9) PEIPKGRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
10_A/Turkey/Ontario/6118/1968 (H8N4) PSV-EPRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
11_A/chicken/Emirates/R66/2002 (H9N2) PAR-SSRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
12_A/Quail/Shantou/782 (H9N2) PAR-SSRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
13_A/Turkey/Wisconsin/1/1966 (H9N2) PAV-SSRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
14_A/Mallard/Germany/R2075/2007 (H10N7) PEIMQGRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
15_A/DomesticDuck/Germany/R784/2006 (H11N1) PAI-ASRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
16_A/Mallard/Gurijev/263/1982 (H14N3) PGK-QAKGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
17_A/Shearwater/WestAustralia/2576/1979 (H15N9) PEKIRTRGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
18_A/black-headedGull/Sweden/5/1999 (H16N3) PSV-GERGLFGAIAAGFIEGQWGMIDGWYGHQNEQSGSYAADKQSTQNAIDGITNKVN
↓
1_A/duck/Bavaria/1/1977 (H1N1) SVIERMNTQFTAVGHEFNNLERRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
2_A/Sentinel Mallard/Germany/S/Ra517K/2007 (H2N5) SVIERMNTQFVAVGEFSSLEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
3_A/duck/Ukraine/1/1963 (H3N8) RVIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
4_A/Victoria/3/1975 (H3N2) RVIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
5_A/HongKong/1/68 (H3N2) RVIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
6_A/Mallard/Germany/1240/1/2007 (H4N6) RLIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
7_A/Vietnam/1203/2004 (H5N1) STIIDKQNTQFVAVGEFNNLERRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
8_A/Turkey/Germany/R617/2007 (H6N2) STIIDKQNTQFVAVGEFNNLERRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
9_A/Anhui/1/2013 (H7N9) RLIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
10_A/Turkey/Ontario/6118/1968 (H8N4) NIVDRMNTQFVAVGEFNNLERRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
11_A/chicken/Emirates/R66/2002 (H9N2) NIVDRMNTQFVEVNHFEFVEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
12_A/Quail/Shantou/782 (H9N2) NIVDRMNTQFVEVNHFEFVEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
13_A/Turkey/Wisconsin/1/1966 (H9N2) NIVDRMNTQFVEVNHFEFVEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
14_A/Mallard/Germany/R2075/2007 (H10N7) NIVDRMNTQFVEVNHFEFVEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
15_A/DomesticDuck/Germany/R784/2006 (H11N1) NIVDRMNTQFVEVNHFEFVEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
16_A/Mallard/Gurijev/263/1982 (H14N3) RLIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
17_A/Shearwater/WestAustralia/2576/1979 (H15N9) RLIEKTNKQFHEIEKFEVEGRIQDLEKYVEDTKIDLWSYNAELLVLENERITLDFHDS
18_A/black-headedGull/Sweden/5/1999 (H16N3) NIEKRMNTQFVEVNHFEFVEKRIENLNKQMEDGFLDVTWYNAELLVLENERITLDFHDS
↓
1_A/duck/Bavaria/1/1977 (H1N1) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
2_A/Sentinel Mallard/Germany/S/Ra517K/2007 (H2N5) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
3_A/duck/Ukraine/1/1963 (H3N8) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
4_A/Victoria/3/1975 (H3N2) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
5_A/HongKong/1/68 (H3N2) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
6_A/Mallard/Germany/1240/1/2007 (H4N6) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
7_A/Vietnam/1203/2004 (H5N1) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
8_A/Turkey/Germany/R617/2007 (H6N2) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
9_A/Anhui/1/2013 (H7N9) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
10_A/Turkey/Ontario/6118/1968 (H8N4) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
11_A/chicken/Emirates/R66/2002 (H9N2) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
12_A/Quail/Shantou/782 (H9N2) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
13_A/Turkey/Wisconsin/1/1966 (H9N2) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
14_A/Mallard/Germany/R2075/2007 (H10N7) EMLNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
15_A/DomesticDuck/Germany/R784/2006 (H11N1) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
16_A/Mallard/Gurijev/263/1982 (H14N3) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
17_A/Shearwater/WestAustralia/2576/1979 (H15N9) EMNKLFEKTRRQLRENAEDGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
18_A/black-headedGull/Sweden/5/1999 (H16N3) NVRNLYEKVKSQRNNAKEIGNGCFEYHKDCDCEMSEVKNQTYDYPKYSEESKLNREEI
↓
1_A/duck/Bavaria/1/1977 (H1N1) DGVKLESMGV-YQILAIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
2_A/Sentinel Mallard/Germany/S/Ra517K/2007 (H2N5) KGVKLSNMGV-YQILAIY-ATVAGSLSLAIMIAGISLWMSNGSLQCRICI
3_A/duck/Ukraine/1/1963 (H3N8) KGVKLSG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
4_A/Victoria/3/1975 (H3N2) KGVKLSG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
5_A/HongKong/1/68 (H3N2) KGVKLSG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
6_A/Mallard/Germany/1240/1/2007 (H4N6) QGVKLTQ---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
7_A/Vietnam/1203/2004 (H5N1) SGVKLESIGI-YQILSIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
8_A/Turkey/Germany/R617/2007 (H6N2) ESKVLENLGV-YQILAIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
9_A/Anhui/1/2013 (H7N9) DPKVLSG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
10_A/Turkey/Ontario/6118/1968 (H8N4) NGVKLENTI-YKILSIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
11_A/chicken/Emirates/R66/2002 (H9N2) EGVKLESEGT-YKILTIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
12_A/Quail/Shantou/782 (H9N2) EGVKLESEGT-YKILTIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
13_A/Turkey/Wisconsin/1/1966 (H9N2) EGVKLESEGT-YKILTIY-STVASSLVLLVSLGAISEFWMCSNGSLQCRICI
14_A/Mallard/Germany/R2075/2007 (H10N7) NPVKLSG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
15_A/DomesticDuck/Germany/R784/2006 (H11N1) EGVKLSGNGVYKLSIY-SCIASSLVLLVSLGAISEFWMCSNGSLQCRICI
16_A/Mallard/Gurijev/263/1982 (H14N3) NPVLTMG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
17_A/Shearwater/WestAustralia/2576/1979 (H15N9) NPVKLSG---YKDWILWISFAISCLLVCVLLGFLIMWACORGNIRCNICI
18_A/black-headedGull/Sweden/5/1999 (H16N3) EGKIKLSENVYKLSIY-SCIASSLVLLVSLGAISEFWMCSNGSLQCRICI

```

**Figure S1:** Amino acid sequence alignment of all HAs used in this study. The cleavage site is indicated by an arrow. The alignment was generated with Geneious prime.