

## Supporting information

**Table S1. Genetic similarity of H5N8 viruses isolated in 2021 compared to A/whooper swan/Shanxi/4-1/2020 (H5N8) (WS/SX/4-1/2020).**

| Virus              | Identity to WS/SX/4-1/2020 at the nucleotide level (%) |      |      |      |      |      |      |      |
|--------------------|--------------------------------------------------------|------|------|------|------|------|------|------|
|                    | HA                                                     | NA   | PB2  | PB1  | PA   | NP   | M    | NS   |
| WS/SX/4-2/2020     | 100                                                    | 99.9 | 100  | 100  | 100  | 100  | 100  | 99.6 |
| MS/SD/1/2021       | 99.6                                                   | 99.6 | 99.6 | 99.8 | 99.8 | 99.8 | 99.8 | 99.2 |
| GWT/GD/SD004/2021  | 99.5                                                   | 99.4 | 99.6 | 99.6 | 99.6 | 99.7 | 100  | 99.2 |
| WS/SD/SC195/2021   | 96.0                                                   | 95.0 | 95.8 | 94.2 | 95.4 | 95.3 | 97.7 | 95.0 |
| WG/SD/SC196/2021   | 99.6                                                   | 99.9 | 99.8 | 99.5 | 99.8 | 99.7 | 99.9 | 99.3 |
| CC/SD/SC197/2021   | 99.6                                                   | 99.9 | 99.8 | 99.5 | 99.7 | 99.7 | 99.8 | 99.2 |
| WS/SD/SC198/2021   | 99.6                                                   | 99.9 | 99.7 | 99.6 | 99.8 | 99.7 | 99.8 | 99.3 |
| WS/SD/SC199/2021   | 99.6                                                   | 99.9 | 99.7 | 99.5 | 99.8 | 99.7 | 99.9 | 99.3 |
| WS/SD/SC200/2021   | 99.6                                                   | 99.7 | 99.7 | 99.4 | 99.8 | 99.7 | 99.8 | 99.2 |
| BS/BJ/1/2021       | 99.6                                                   | 99.4 | 99.7 | 99.5 | 99.8 | 99.7 | 99.7 | 99.2 |
| WD/JS/SD019/2021   | 99.6                                                   | 99.6 | 99.7 | 99.6 | 99.9 | 99.8 | 99.8 | 99.3 |
| ET/JS/SD021/2021   | 99.7                                                   | 99.6 | 99.7 | 99.7 | 99.9 | 99.8 | 99.8 | 99.2 |
| DK/GX/S10099/2021  | 99.6                                                   | 99.4 | 99.9 | 99.7 | 99.7 | 99.7 | 99.8 | 99.5 |
| DK/GX/S10263/2021  | 99.8                                                   | 99.7 | 99.8 | 99.8 | 99.8 | 99.6 | 99.8 | 99.5 |
| DK/GX/S11043/2021  | 99.7                                                   | 99.6 | 99.8 | 99.8 | 99.9 | 99.6 | 100  | 99.4 |
| WS/SD/SC176/2021   | 99.6                                                   | 99.9 | 99.7 | 99.5 | 99.7 | 99.6 | 99.6 | 99.3 |
| WD/SD/SC177/2021   | 99.7                                                   | 99.8 | 99.7 | 99.4 | 99.7 | 99.4 | 99.7 | 99.3 |
| GB/SD/SC184/2021   | 99.5                                                   | 99.8 | 99.8 | 99.5 | 99.8 | 99.7 | 99.9 | 99.2 |
| WS/SD/SC185/2021   | 99.6                                                   | 99.9 | 99.5 | 99.4 | 99.8 | 99.6 | 99.7 | 99.3 |
| WS/SD/SC188/2021   | 99.7                                                   | 99.9 | 99.7 | 99.4 | 99.8 | 99.6 | 99.7 | 99.3 |
| BBGL/SD/SC189/2021 | 99.6                                                   | 99.8 | 99.8 | 99.5 | 99.9 | 99.7 | 99.9 | 99.3 |
| DK/GD/S1269/2021   | 99.5                                                   | 99.6 | 99.4 | 99.6 | 99.8 | 99.7 | 99.8 | 99.5 |
| DK/JX/S10252/2021  | 99.4                                                   | 99.7 | 99.6 | 99.6 | 99.7 | 99.7 | 99.9 | 99.2 |
| GS/ZJ/S1266/2021   | 99.6                                                   | 99.8 | 99.7 | 99.3 | 99.7 | 99.7 | 99.8 | 99.0 |
| GS/HuN/S11288/2021 | 99.8                                                   | 99.6 | 99.8 | 99.6 | 99.7 | 99.9 | 99.9 | 99.2 |
| GS/JS/S1385/2021   | 99.6                                                   | 99.6 | 99.7 | 99.5 | 99.8 | 99.8 | 99.8 | 99.2 |
| GS/LN/S1266/2021   | 99.2                                                   | 99.5 | 99.6 | 99.3 | 99.6 | 99.7 | 99.6 | 99.2 |
| GS/HeN/S1315/2021  | 99.4                                                   | 99.9 | 99.7 | 99.6 | 99.8 | 99.7 | 99.9 | 98.9 |
| DK/HeN/S1381/2021  | 99.4                                                   | 99.9 | 99.8 | 99.7 | 99.8 | 99.7 | 99.9 | 99.3 |
| DK/HeB/S1070/2021  | 99.3                                                   | 99.7 | 99.6 | 99.5 | 99.8 | 99.7 | 100  | 99.3 |
| DK/GX/S21194/2021  | 99.5                                                   | 99.6 | 99.6 | 99.9 | 99.7 | 99.5 | 99.7 | 99.5 |
| GS/GX/S20601/2021  | 99.5                                                   | 99.7 | 99.8 | 99.9 | 99.7 | 99.5 | 99.8 | 99.2 |
| BHGL/TB/1-1/2021   | 99.6                                                   | 99.6 | 99.6 | 99.6 | 99.9 | 99.8 | 99.7 | 99.5 |
| GB/SN/SD001/2021   | 99.6                                                   | 99.5 | 99.6 | 99.5 | 99.2 | 99.7 | 99.6 | 98.6 |
| GB/NX/SD001/2021   | 99.4                                                   | 99.6 | 99.5 | 99.5 | 99.5 | 99.7 | 99.5 | 98.4 |

7 **Table S2 Amino acids in the H5N8 viruses that contribute to the increased binding to human-type receptors, replication, and virulence in mammals, as well**  
8 **as to resistance to amantadine.**

| Virus             | Amino acids in HA that increase affinity to human-type receptors |       |       |       | Amino acids that increase the replication, virulence, transmission of avian influenza viruses in mammalian hosts, or that increase the resistance to amantadine |       |       |       |       |      |       |      |      |      |       |
|-------------------|------------------------------------------------------------------|-------|-------|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-------|-------|-------|------|-------|------|------|------|-------|
|                   |                                                                  |       |       |       | PB2                                                                                                                                                             |       |       | NP    |       | M1   |       | M2   |      | NS1  |       |
|                   | S137A                                                            | S158N | T160A | T192I | I292V                                                                                                                                                           | K389R | V598T | V286A | M437T | N30D | T215A | V27A | A30S | P42S | I106M |
| WS/SX/4-1/2020    | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WS/SX/4-2/2020    | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| MS/SD/1/2021      | A                                                                | N     | A     | M     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| GWT/GD/SD004/2021 | A                                                                | N     | A     | T     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | A    | A    | S    | M     |
| WS/SD/SC195/2021  | A                                                                | N     | A     | T     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WG/SD/SC196/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| CC/SD/SC197/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WS/SD/SC198/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WS/SD/SC199/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WS/SD/SC200/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| BS/BJ/1/2021      | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WD/JS/SD019/2021  | A                                                                | N     | A     | I     | I                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| ET/JS/SD021/2021  | A                                                                | N     | A     | I     | I                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| DK/GX/S10099/2021 | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| DK/GX/S10263/2021 | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| DK/GX/S11043/2021 | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WS/SD/SC176/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| WD/SD/SC177/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |
| GB/SD/SC184/2021  | A                                                                | N     | A     | I     | V                                                                                                                                                               | R     | T     | A     | T     | D    | A     | V    | A    | S    | M     |

|                    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|--------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WS/SD/SC185/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| WS/SD/SC188/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| BBGL/SD/SC189/2021 | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| DK/GD/S1269/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| DK/JX/S10252/2021  | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GS/ZJ/S1266/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GS/HuN/S11288/2021 | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GS/JS/S1385/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GS/LN/S1266/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GS/HeN/S1315/2021  | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| DK/HeN/S1381/2021  | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| DK/HeB/S1070/2021  | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| DK/GX/S21194/2021  | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GS/GX/S20601/2021  | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| BHGL/TB/1-1/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GB/SN/SD001/2021   | A | N | A | I | V | R | T | A | T | D | A | V | A | S | M |
| GB/NX/SD001/2021   | A | N | A | I | V | R | T | A | T | D | A | V | S | S | M |

[illegible]

#### 2.3.4.4a

Figure S1B

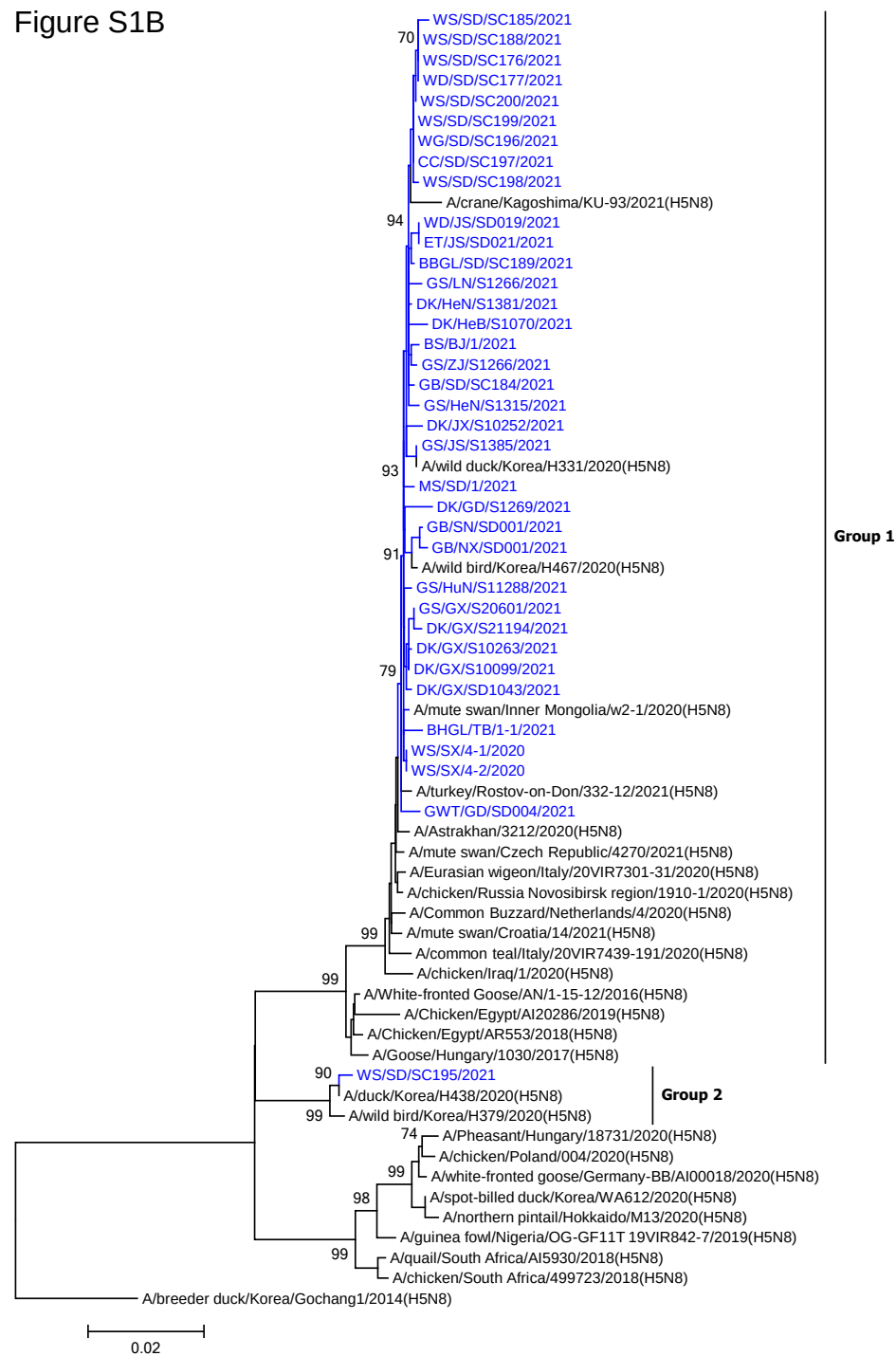


Figure S1C

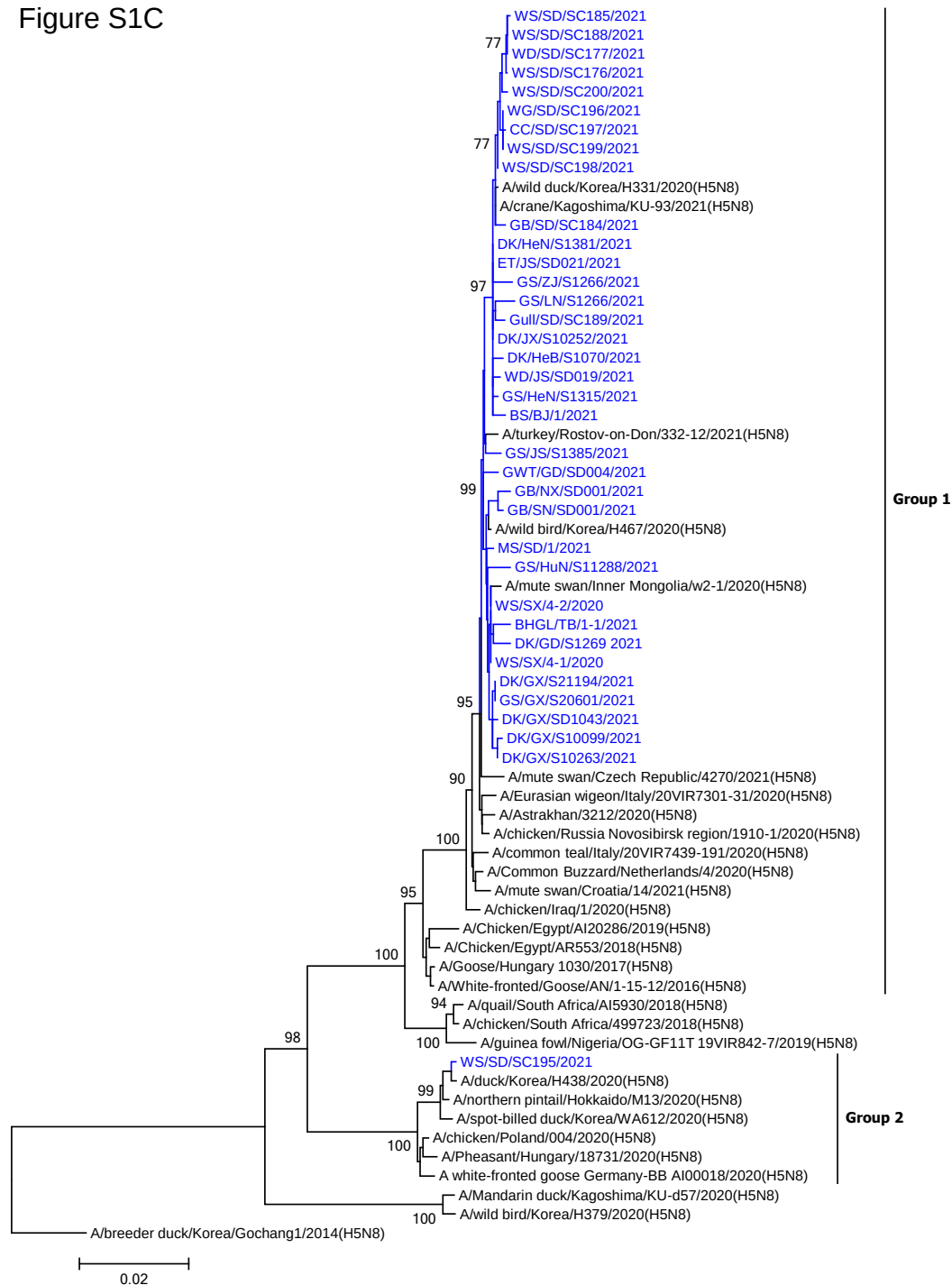
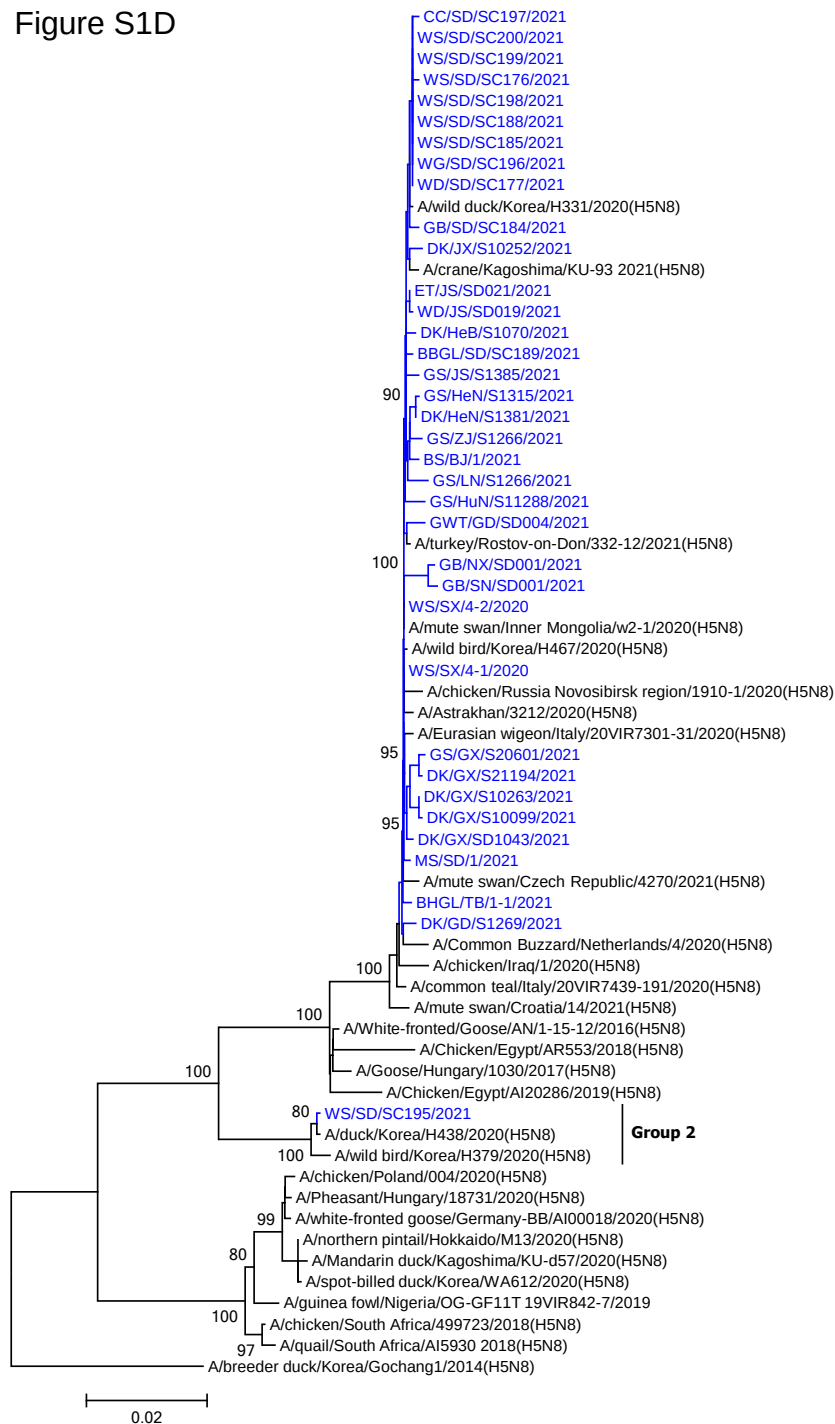


Figure S1D

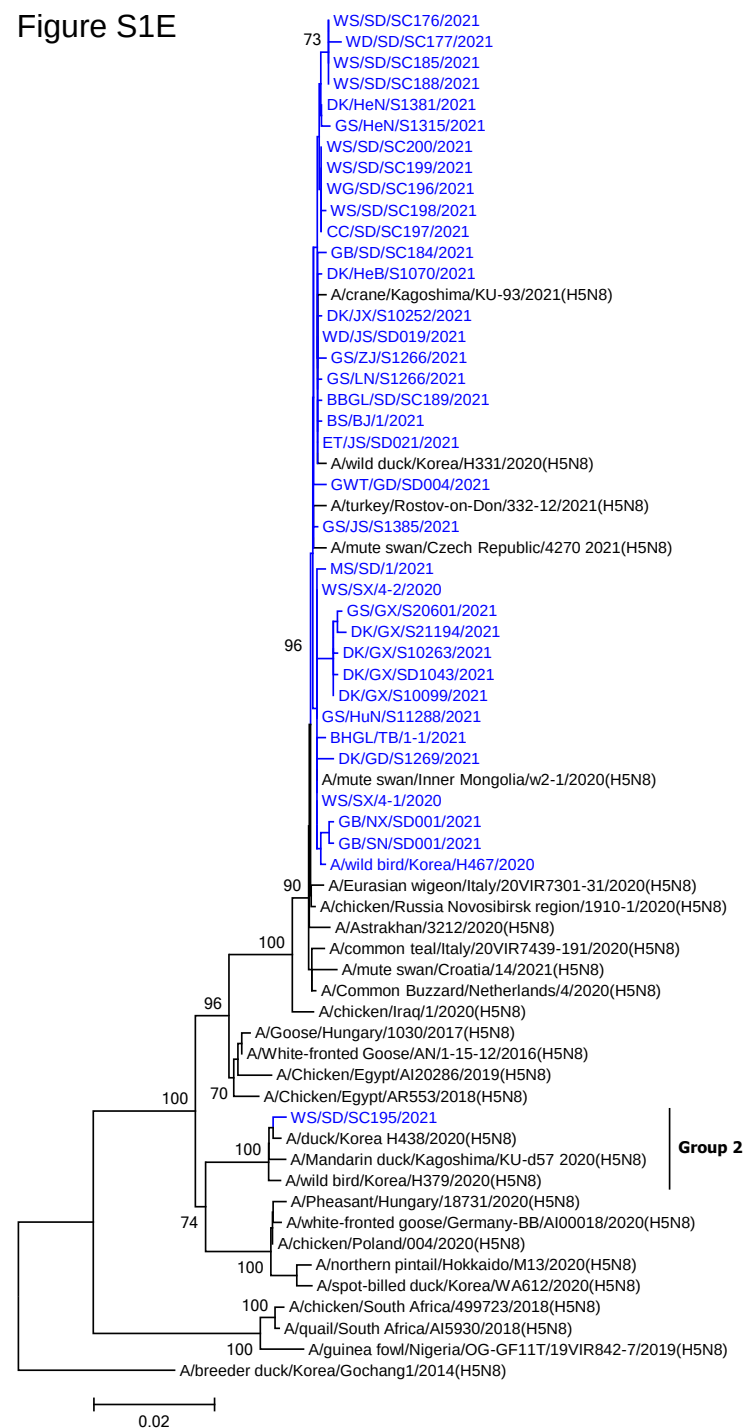


Group 1

Group 2

0.02

Figure S1E



Group 1

Group 2

0.02



Figure S1F

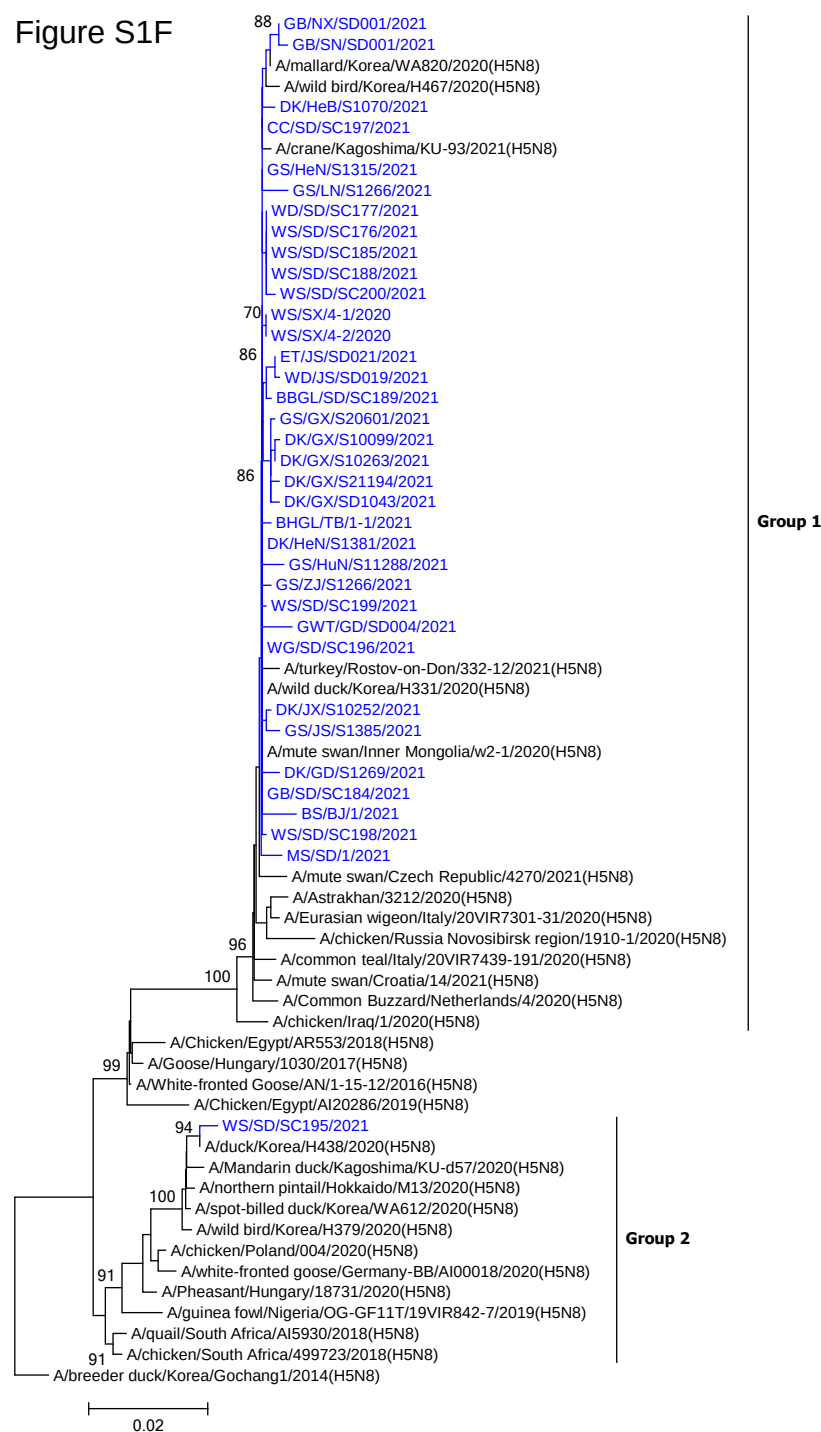


Figure S1G

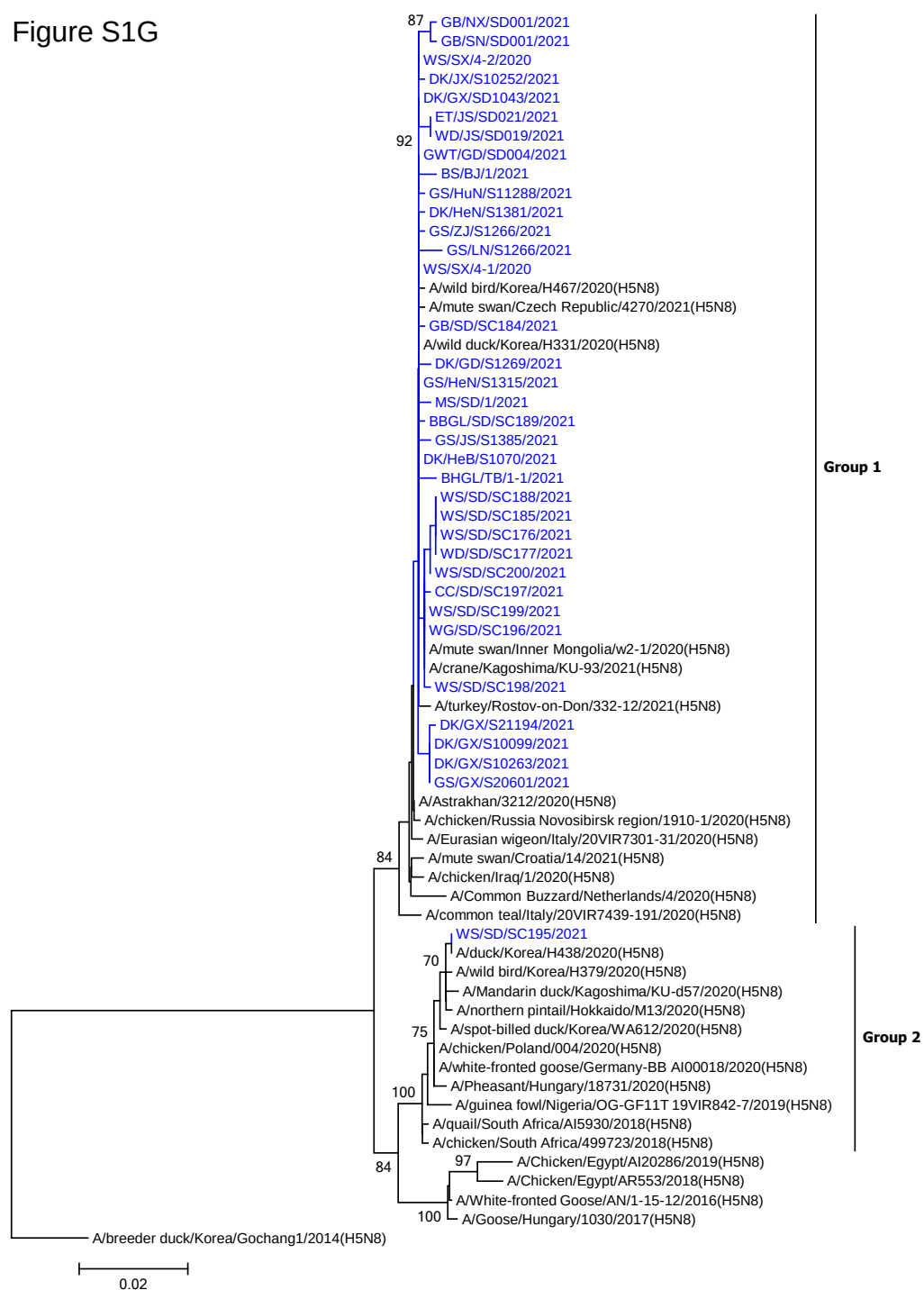
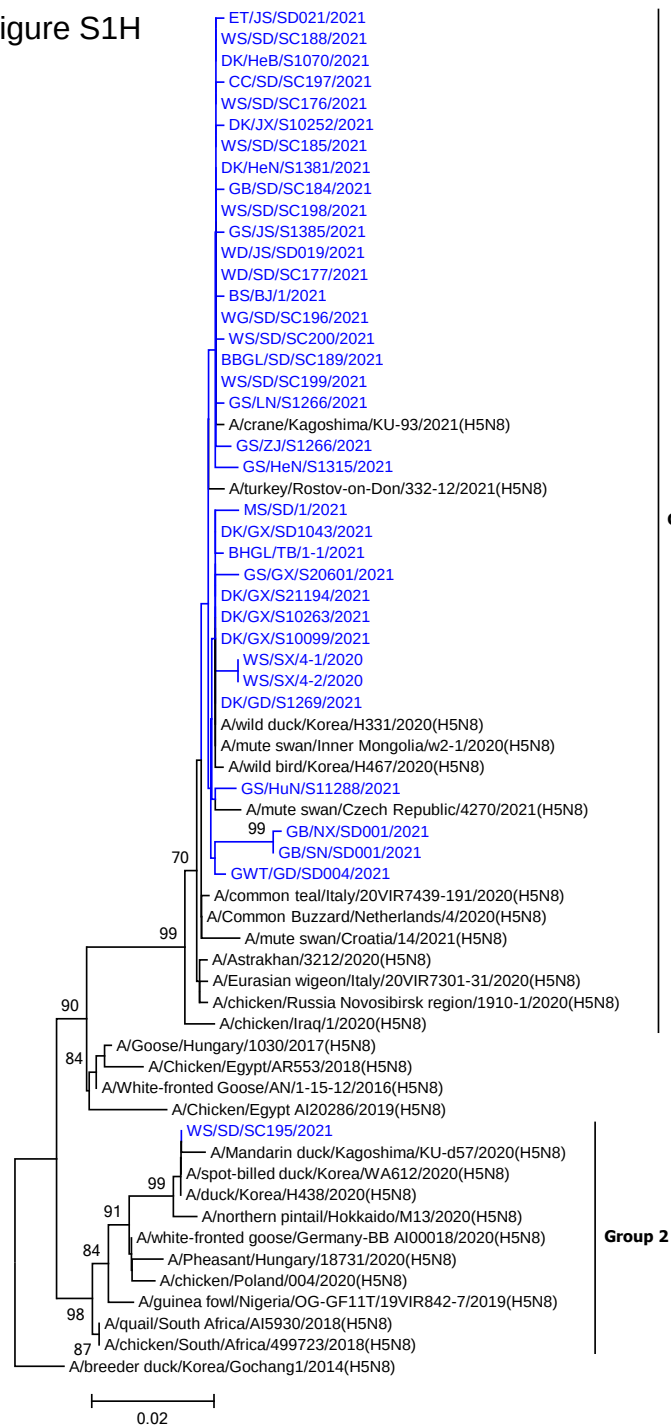


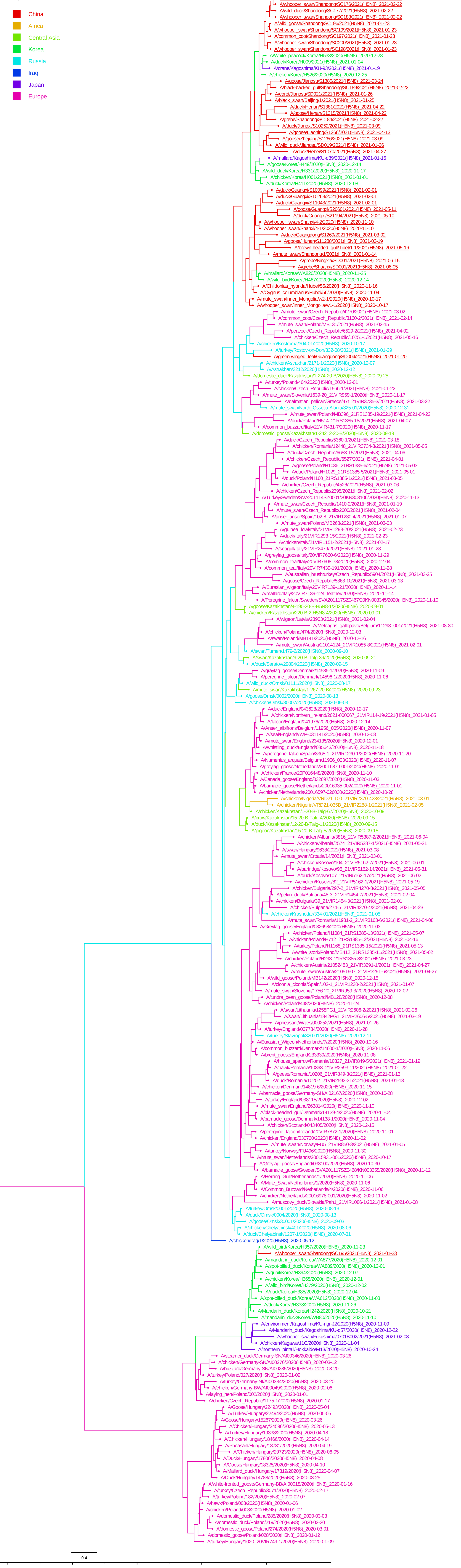
Figure S1H



11 **Figure S1. Phylogenetic analyses of H5N8 viruses.** The trees were generated by neighbor-joining  
12 method using software MEGA 7.0.26 software package. The tree topology was evaluated by 1,000  
13 bootstrap analysis. Viruses with names in black were downloaded from available databases, and viruses  
14 with names in blue were our H5N8 isolates. The phylogenetic tree of HA (A) was rooted to  
15 A/duck/Anhui/1/2006 (H5N1), and the phylogenetic trees of PB2 (B), PB1 (C), PA (D), NP (E), NA (F),  
16 M (G), and NS (H) were rooted to A/breeder duck/Korea/Gochang1/2014(H5N8).



Figure S2





17 **Figure S2. The Bayesian time-resolved phylogenetic tree of HA gene of H5N8 viruses.** The tree was  
18 generated with the HA of the 40 H5N8 viruses detected in China and 202 H5N8 representative viruses  
19 detected in other countries since 2020. The time to the most recent common ancestor and the dates of  
20 divergence are labeled at the left of the tree, which was estimated by using the Bayesian Markov chain  
21 Monte Carlo method in the BEAST v1.10.4 software package.