

Table S1. Amino acids substitutions of the H10 viruses that contribute to the enhanced replication, pathogenicity, and transmissibility in mammals.

Viruses	HA (H3 Number)				PB2			PB1		PA		M1		NS1
	Q226L	G228S	N379T	Cleavage site	G309D	E627K	D701N	K328N	S375N	E382D	A515T	N30D	T215A	P42S
BTG/W1496/20	Q	G	T	VPEVVQG.GLF	D	E	D	N	N	D	T	D	A	A
SW/W3875/20	Q	G	T	VPEVVQG.GLF	D	E	D	N	N	D	T	D	A	S
SW/W3917/20	Q	G	T	VPEVVQG.GLF	D	E	D	N	N	D	T	D	A	S
SW/W4047/20	Q	G	T	VPEVVQG.GLF	G	E	D	N	N	D	T	D	A	S
SW/W4074/20	Q	G	T	VPEVVQG.GLF	D	E	D	N	N	D	T	D	A	S
SW/W4322/20	Q	G	T	VPEVVQG.GLF	D	E	D	N	N	D	T	D	A	S
EC/W4446/20	Q	G	T	VPEVVQG.GLF	D	E	D	N	N	E	T	D	A	S

Table S2. Cross-reactive hemagglutinin inhibition (HI) antibody titers of the H10viruses with different chicken antisera.

viruses	HI antibody titer of chicken antiserum <sup>a</sup>		
	BTG/1496/20	SW/3875/20	EC/4446/20
BTG/1496/20 H10N8	<b>128</b> <sup>c</sup>	64	32
SW/3875/20 H10N8	32	<b>256</b>	64
EC/4446/20 H10N4	32	128	<b>128</b>
SW/4322/20 H10N4	128	128	64

<sup>a</sup> The antisera were generated by infecting SPF chickens with the H10 viruses, as indicated in this table.

<sup>c</sup> The homologous titer is shown in bold.

Table S3. Seroconversion of the chickens and ducks inoculated or contacted with H10N4 and H10N8 influenza viruses

Animals	Viruses	Seroconversion: positive/total <sup>a</sup> (HI antibody titer)					
		Inoculated group (dpi <sup>b</sup> )			Contact group (dpc <sup>c</sup> )		
		10	15	21	10	15	21
Chicken	BTG/1496/20	3/3 (64, 64, 256)	3/3 (64, 64, 256)	3/3 (64, 128, 128)	3/3 (16, 64, 128)	3/3 (32, 64, 64)	3/3 (128, 128, 128)
	SW/3875/20	3/3 (128, 256, 256)	3/3 (128, 128, 256)	3/3 (128, 128, 256)	3/3 (16, 16, 64)	3/3 (32, 64, 64)	3/3 (64, 64, 128)
	EC/4446/20	3/3 (64, 128, 128)	3/3 (32, 32, 64)	3/3 (128, 128, 128)	1/3 (- <sup>d</sup> , -, 16)	1/3 (-, -, 64)	1/3 (-, -, 128)
Duck	BTG/1496/20	3/3 (16, 16, 32)	3/3 (16, 16, 16)	3/3 (8, 8, 8)	3/3 (8, 8, 16)	3/3 (16, 16, 32)	3/3 (4, 16, 16)
	SW/3875/20	3/3 (32, 32, 64)	3/3 (16, 32, 128)	3/3 (16, 32, 32)	3/3 (8, 16, 16)	3/3 (8, 32, 32)	2/3 (-, 2, 8)
	EC/4446/20	3/3 (32, 64, 64)	3/3 (32, 32, 64)	3/3 (16, 32, 32)	3/3 (8, 8, 16)	3/3 (8, 8, 16)	3/3 (2, 2, 4)

<sup>a</sup> The chicken or the duck serum was collected on days 10, 15, and 21 p.i. The HI antibody titers were detected by HI assay. The three H10 viruses were diluted to 4 HAU and then reacted with the chicken or the duck serum, e.g., the BTG/1496/20 was diluted to 4 HAU and then reacted with the chicken and duck serum of the BTG/1496/20 group, respectively.

<sup>b</sup> days post-inoculation.

<sup>c</sup> days post-contact.

<sup>d</sup> The HI antibody titer was negative.

Fig S1 A

PB2

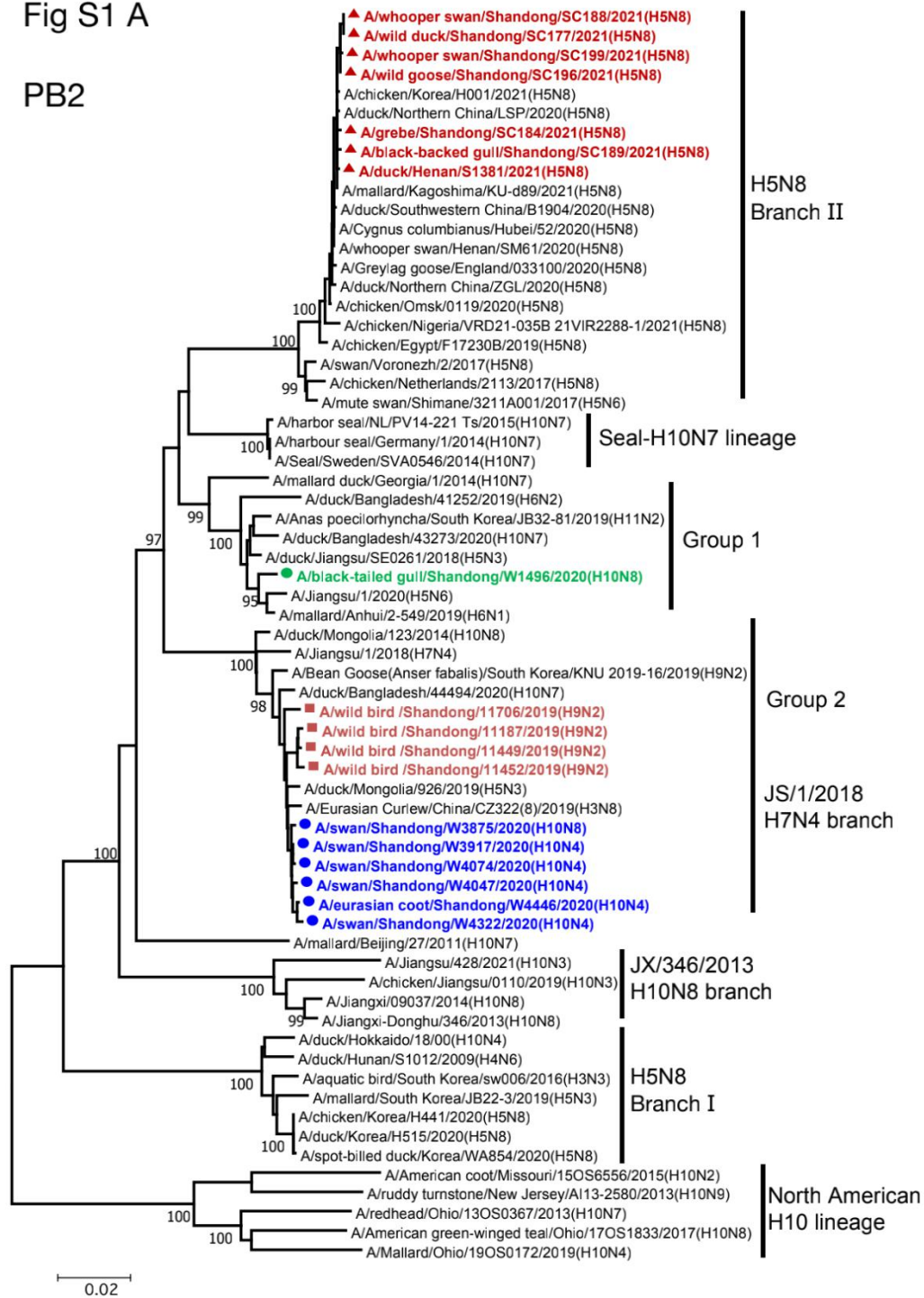


Fig S1 B

PB1

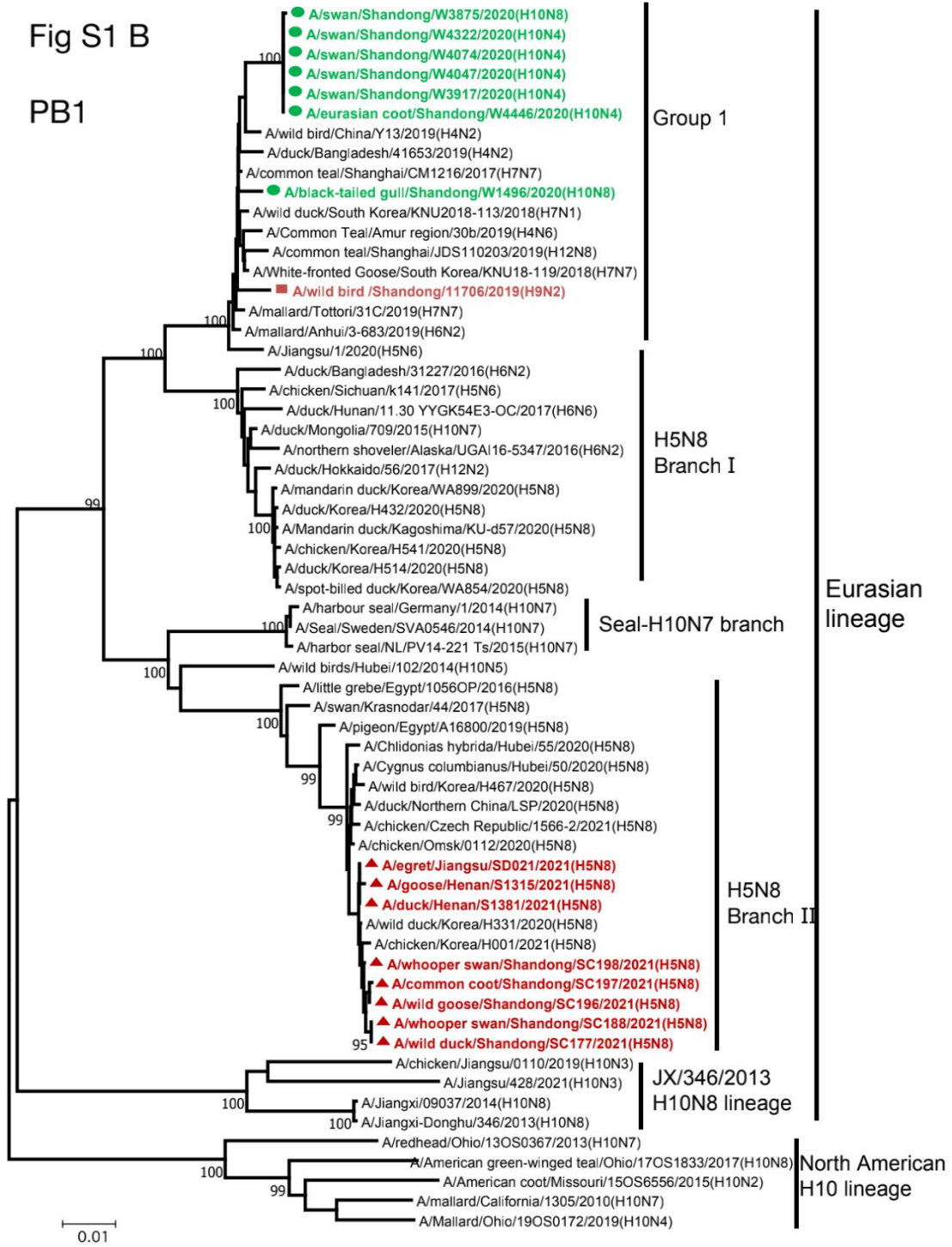


Fig S1 C

PA

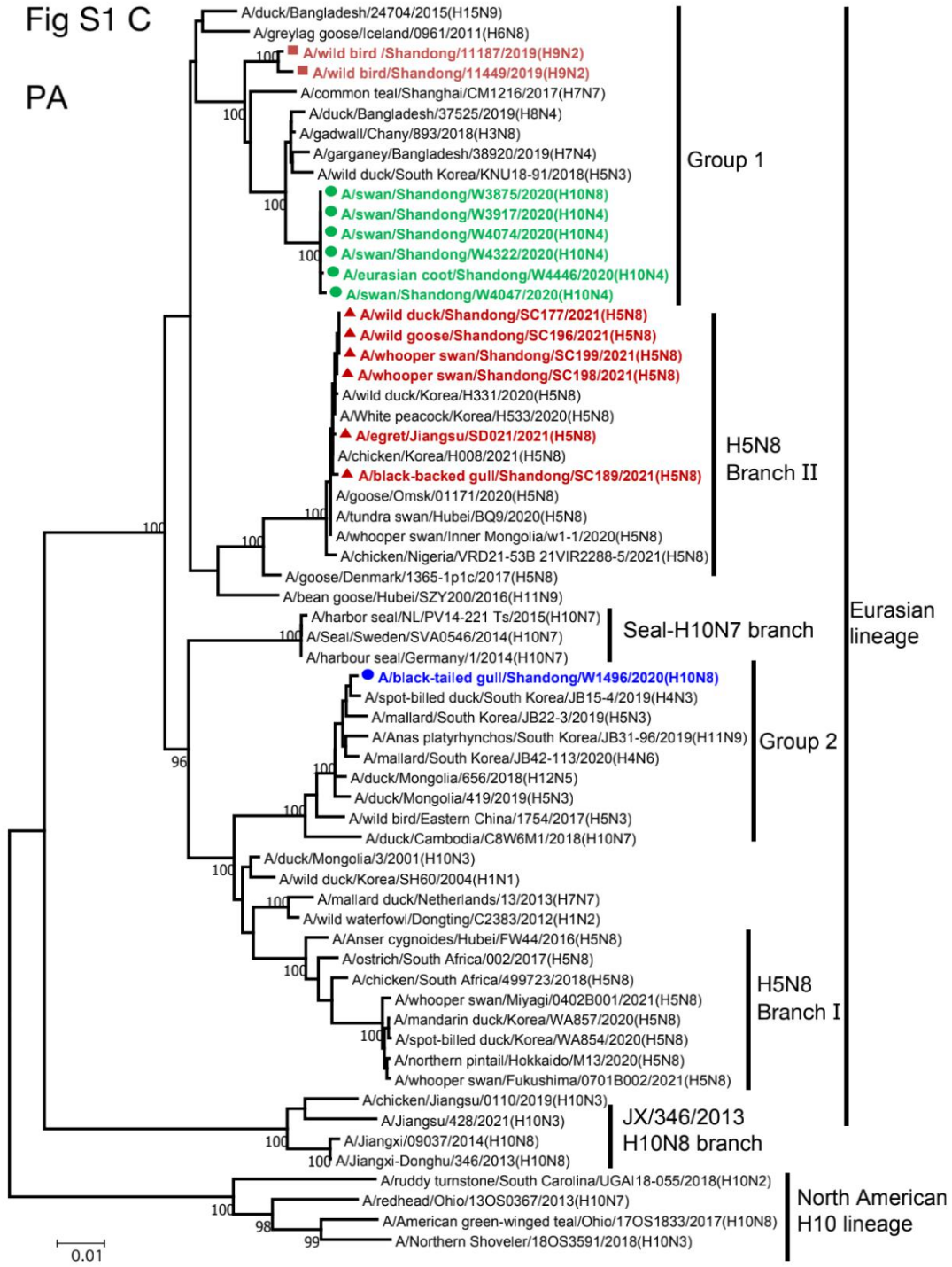


Fig S1 D

NP

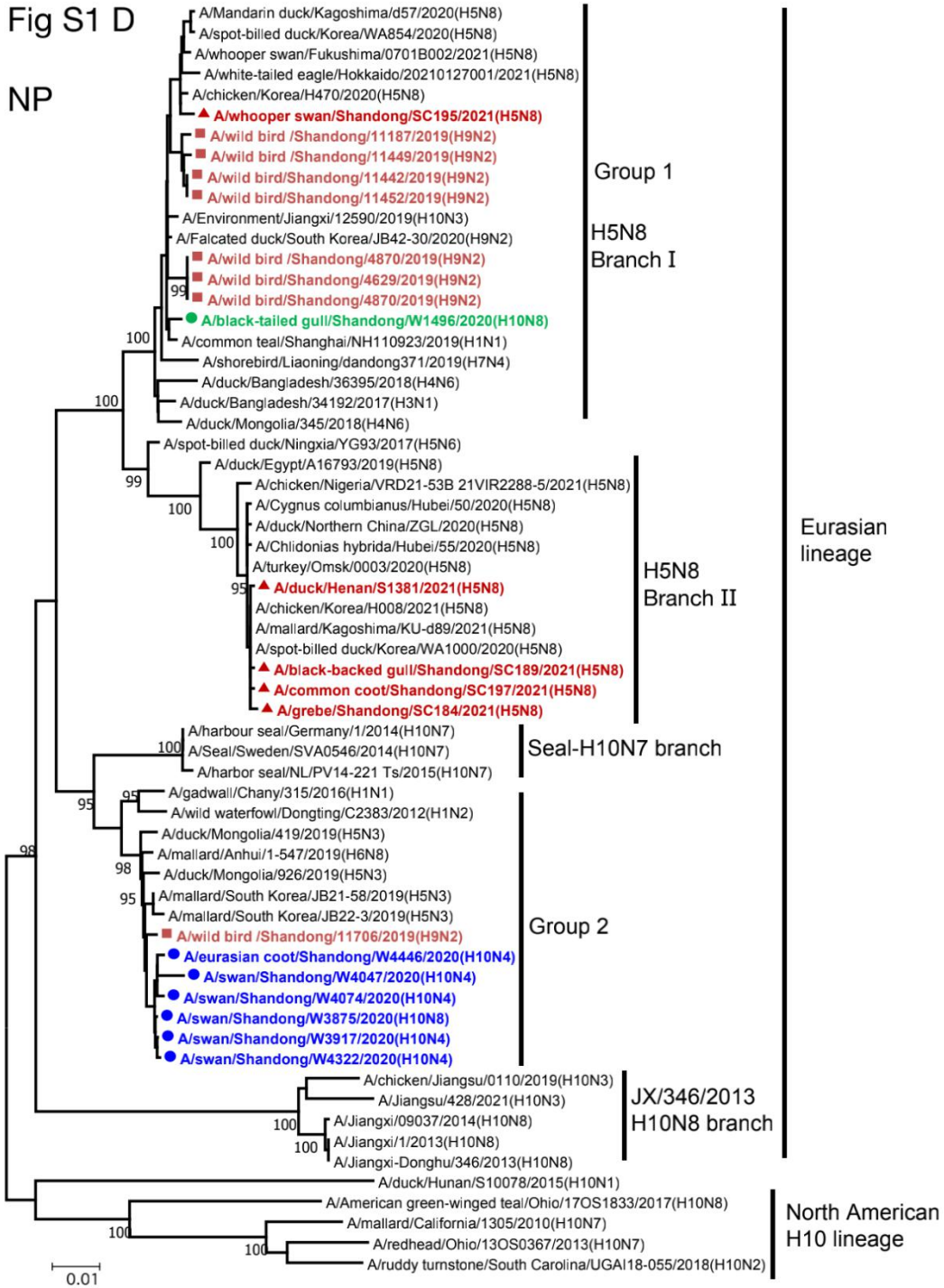
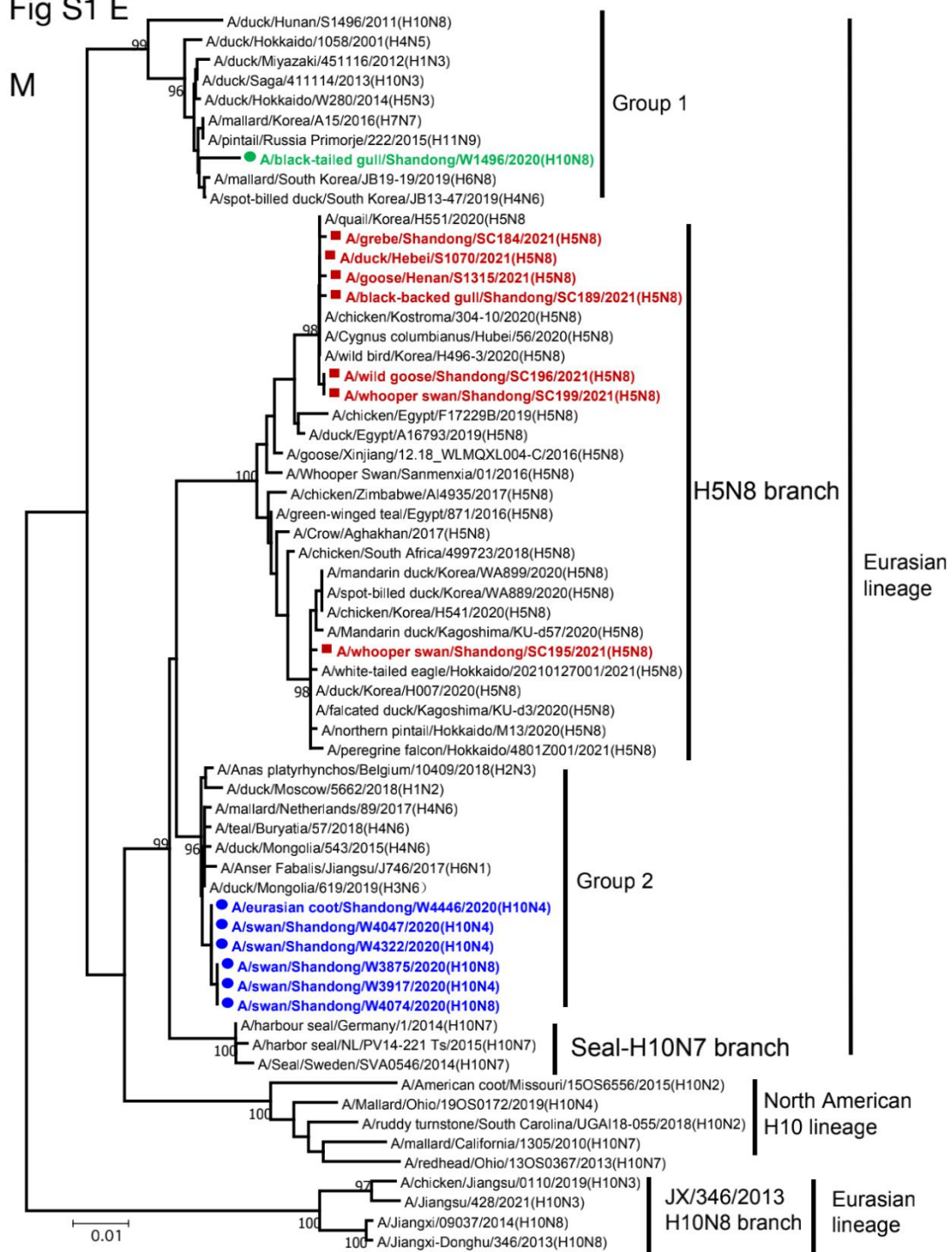
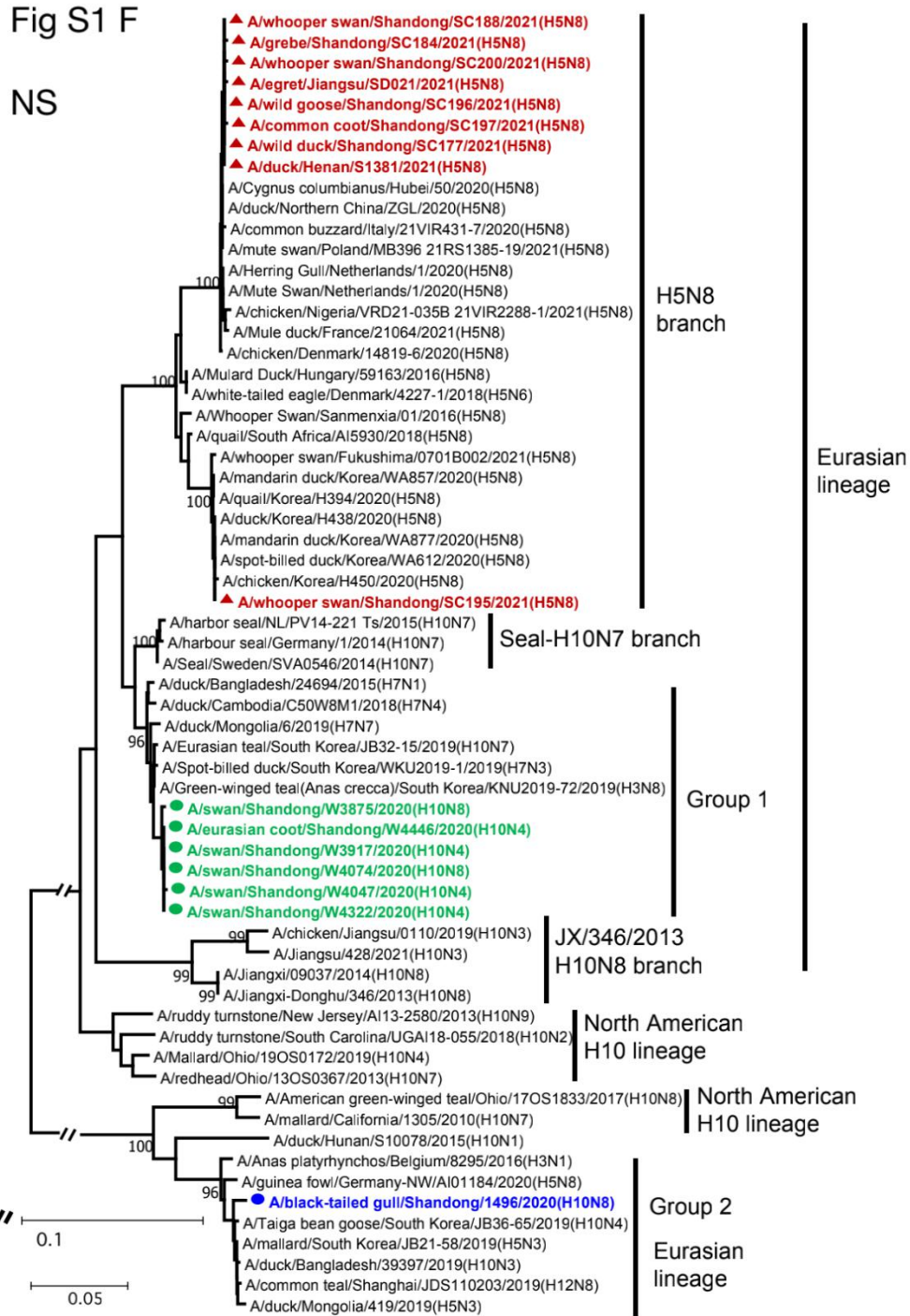


Fig S1 E

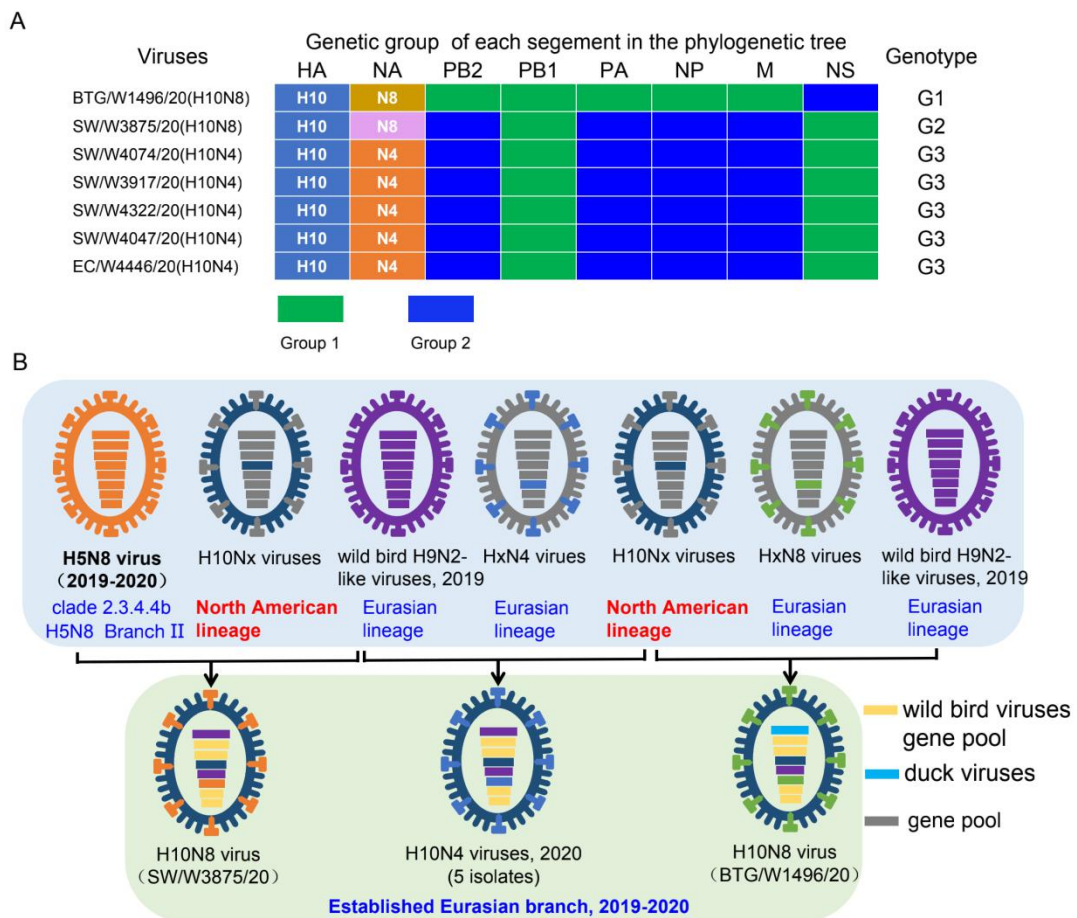




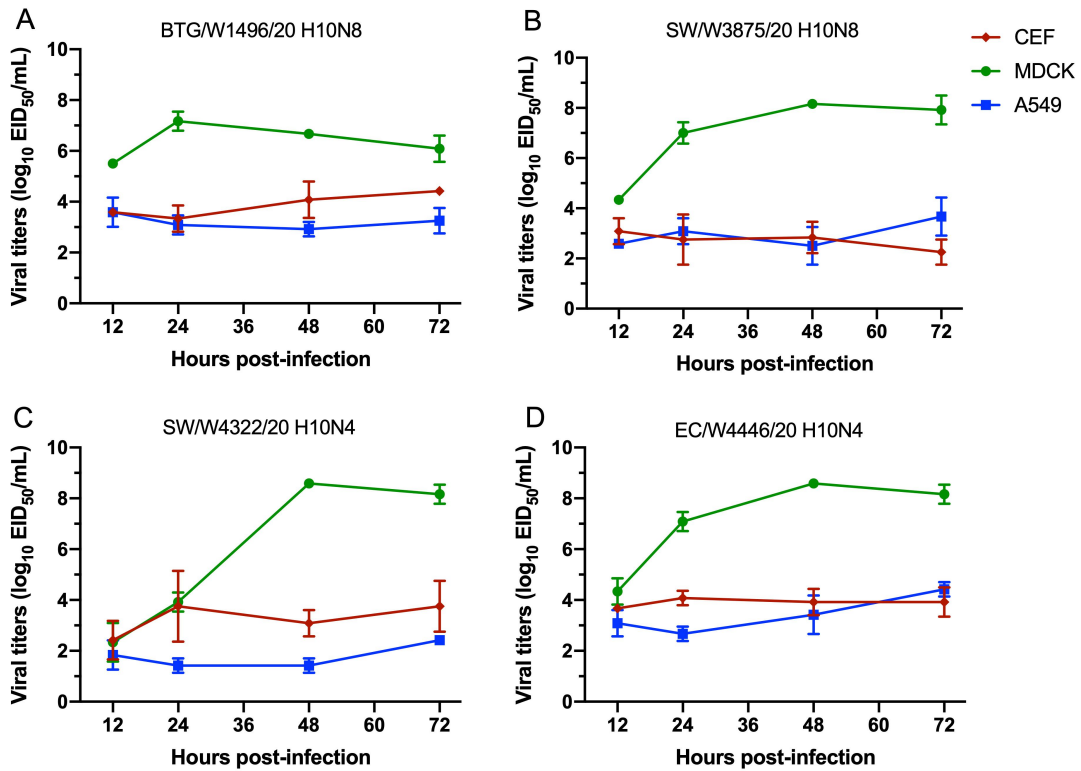


**Figure S1. Phylogenetic diagram of PB2 (A), PB1 (B), PA (C), NP (D), M (E), and NS (F) genes of H10N4, and H10N8 viruses.** The phylogenetic tree of the six internal genes were constructed by MEGA 7.0 with Neighbor-Joining method. The sequence name colored in green and blue were the viruses isolated in this study, and the sequences name in red were the H5N8 or H9N2 viruses isolated in our previous study. The sequence name with black were downloaded from the database.

Figure S2



**Figure S2. Genotypes and reassortment of H10N4 and H10N8 viruses. (A) Genotypes of H10N4 and H10N8 viruses.** Each gene segment of the virus was divided into different groups according to their genetic divergence in the phylogenetic tree. (B) Reassortment of H10N4 and H10N8 viruses in wild birds.



**Figure S3. Growth kinetics of H10N4 and H10N8 viruses in chicken and mammal cells.** Virus growth curves in MDCK, A549, and CEF cells. The dashed lines indicate the lower limit of detection. Data shown are mean  $\pm$  SD for three independent experiments, and significance was assessed with a two-tailed unpaired Student's t-test.