

## Supplementary information

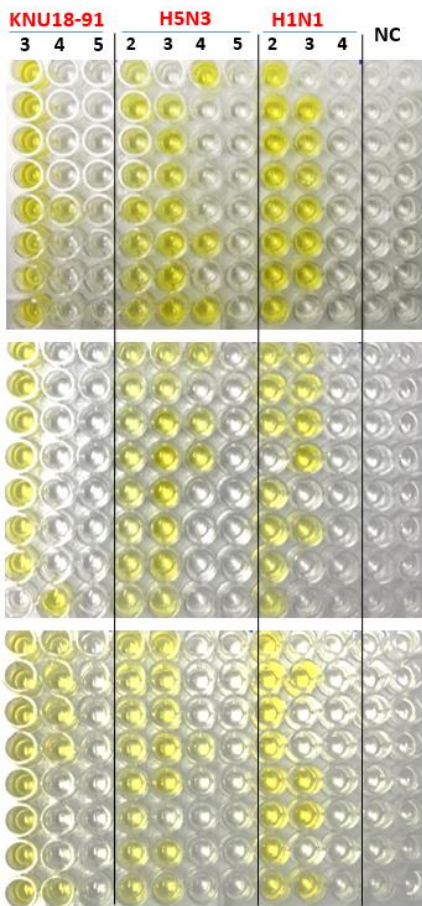
Emergence of novel reassortant H5N3 avian influenza viruses in Korean Mallard ducks in 2018

Seon-Ju Yeo\*, Vui Thi Hoang\*, Duong Tuan Bao\*<sup>1</sup>, Ngoc Minh Nguyen<sup>1</sup>, Hien Thi Tuong<sup>1</sup>, Mudsser Azam, Haan Woo Sung§, Hyun Park§

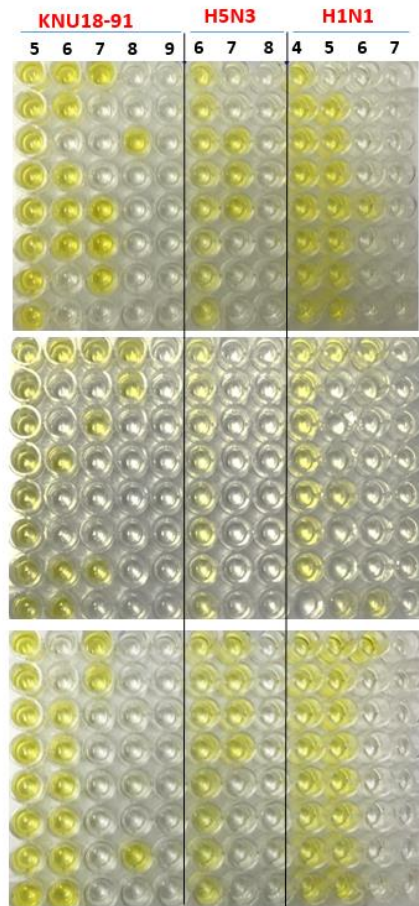
Table S1. Detailed NGS analysis of KNU18-91 isolate

Reference					Sequenced Sample								R_ORF			S_ORF		
Segment#	gene name	desc.	Accession_ID	ref bp	# of Pre-processed reads	# of Influenza Virus extracted reads	# of non-Influenza Virus reads	Virus reads %	#M_Reads	Unique Matches (%)	S_Con_bp	%Cov. (S/R)	Length	S_position	E_position	Length	S_position	E_position
1	PB2	A/greater white-fronted goose/Netherlands/5/2010/H5N3	KX979214	2,341	19369890	735257	18634633	0.03946	24,228	4.0	2,353	98.97	2,307	1	2,307	2,088	228	2,315
2	PB1, PB1-F2		KX979754	2,341					11,483	1.9	2,315	98.89	2,274	25	2,298	2,274	18	2,291
3	PA, PA-X		KX977828	2,233					19,656	3.3	2,226	99.69	2,157	19	2,175	2,157	12	2,168
4	HA		KX978851	1,767					95,971	16.00	1,764	99.94	1,704	20	1,723	1,704	17	1,720
5	NP		KX978800	1,565					170,535	28.40	1,595	98.40	1,542	1	1,542	1,548	21	1,568
6	NA		KX979439	1,453					11,718	2.00	1,419	97.87	1,428	1	1,428	1,416	3	1,418
7	M2, M1		KX978221	1,027					249,453	41.60	1,029	99.03	762	23	784	786	1	786
8	NEP, NS1		KX977601	890					427	0.10	839	93.26	717	3	719	564	30	593

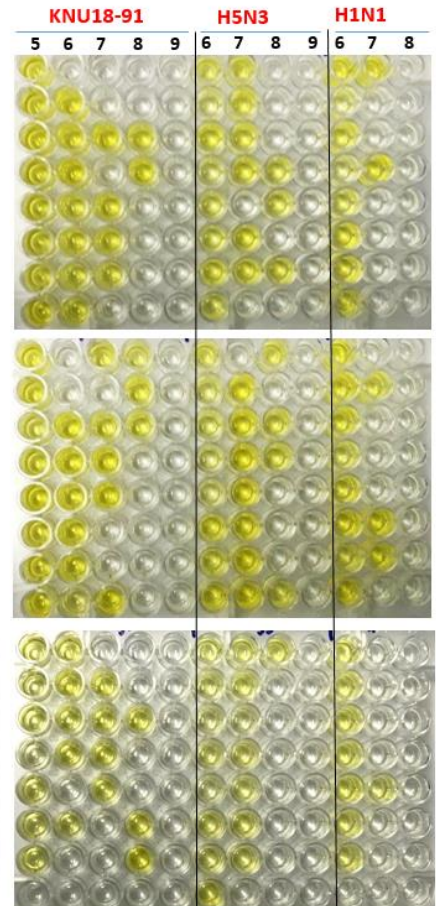
a. 12hpi



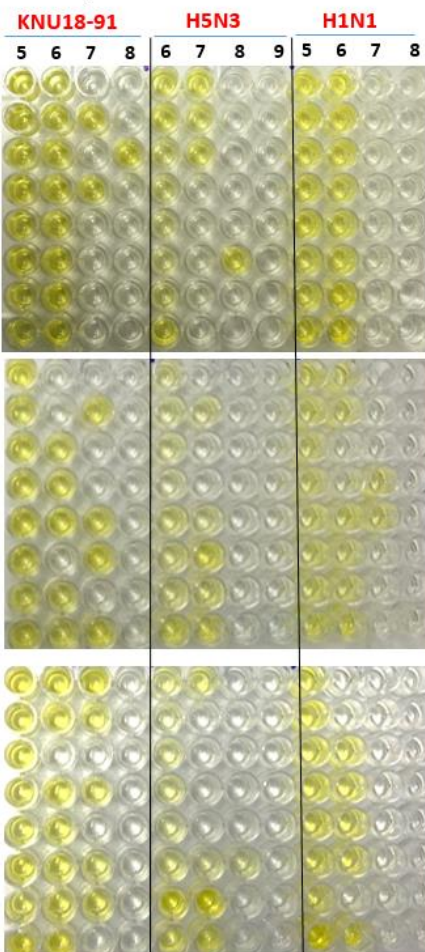
b. 24hpi



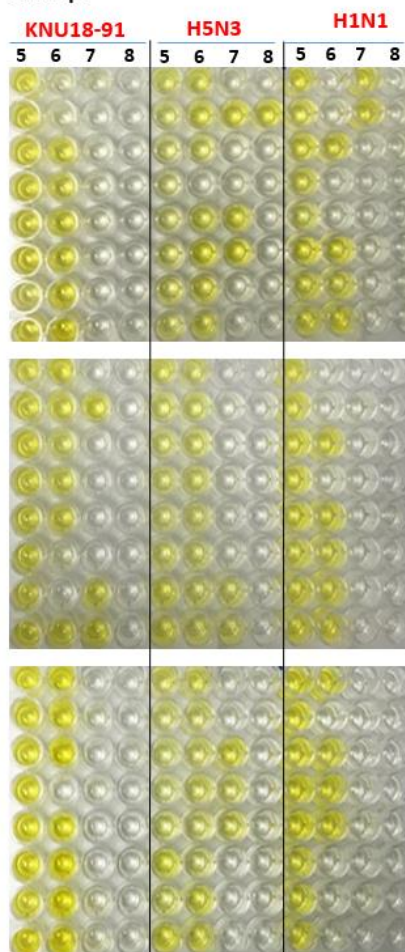
c. 36hpi



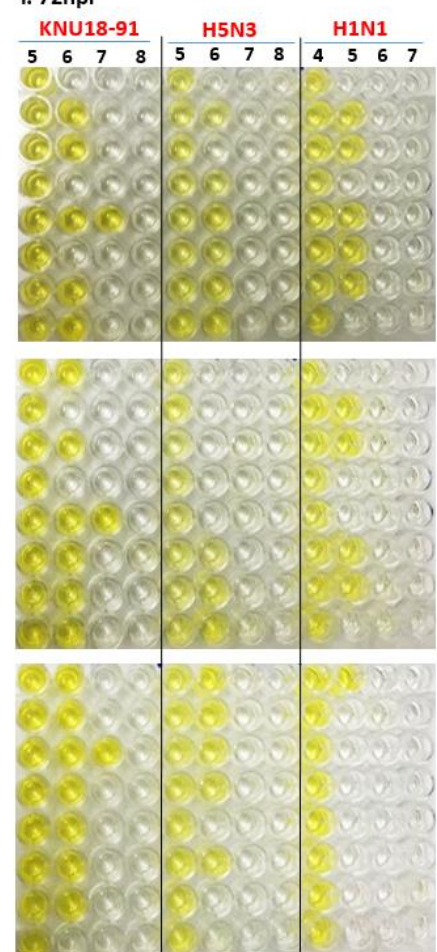
d. 48hpi



e. 60hpi



f. 72hpi



**Fig. S1. Raw ELISA data to conduct TCID<sub>50</sub> assay.** KNU18-91, H5N3, H1N1 were infected to MDCK cells at MOI=0.01, Infected supernatants were collected at each 12hpi for 72 hours. Serial 10-fold dilutions of three different viruses' supernatant at different hpi. KNU18-91 (a-f), H5N3 (g-l), and H1N1 (m-r) were infected MDCK for 3 dpi., cell monolayers were fixed with 80% acetone and then blocked with 5% non-fat milk. Cells were washed with PBST 3 times and incubated with 0.1 µg/well of anti-influenza nucleoprotein (Medix Biochemica, Finland) at 37°C, 1 hour. Wash cells by PBST 3 times, add secondary Ab in the form of horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (Abcam, Cambridge, UK), incubated at 37°C, 1 hour. Cell then were washed with PBS-T for five times to remove nonspecific binding and 100 µL of 3,3',5,5'-tetra methyl benzidine (Sigma-Aldrich) substrate solution was added, it was followed by 100 µL of H<sub>2</sub>SO<sub>4</sub> 0.18M solution to stop reaction.

3: 10<sup>3</sup>- fold dilution of stock;

4: 10<sup>4</sup>- fold dilution of stock;

5: 10<sup>5</sup>- fold dilution of stock;

6: 10<sup>6</sup>- fold dilution of stock;

7: 10<sup>7</sup>- fold dilution of stock;

8: 10<sup>8</sup>- fold dilution of stock;

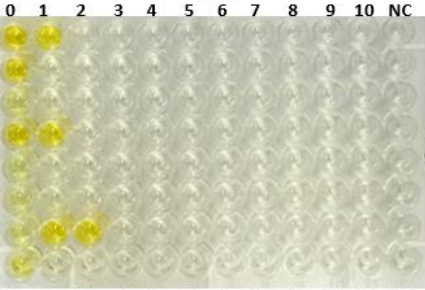
9: 10<sup>9</sup>- fold dilution of stock;

10: 10<sup>10</sup>- fold dilution of stock;

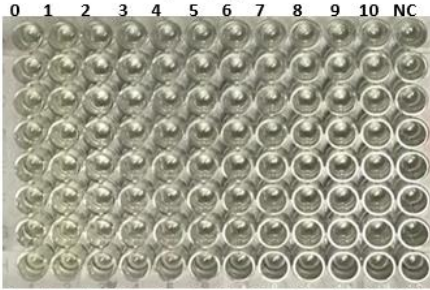
NC: negative control (uninfected virus);

a.

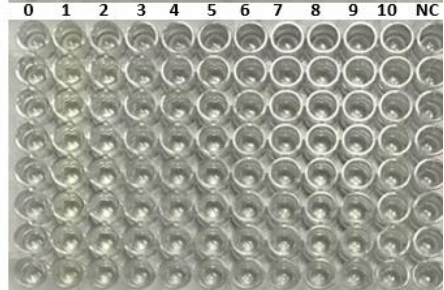
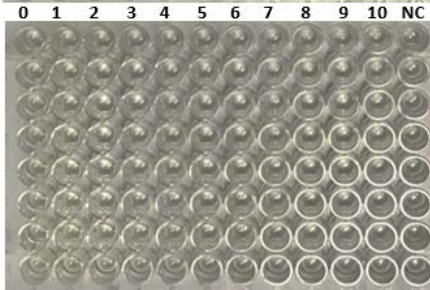
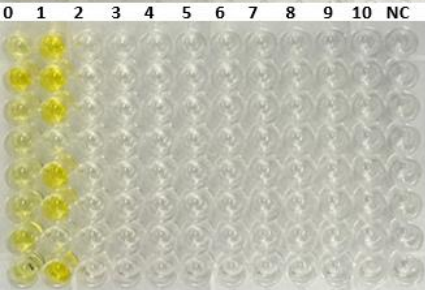
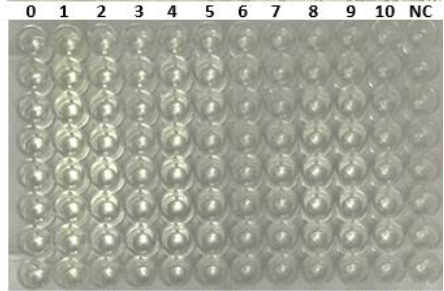
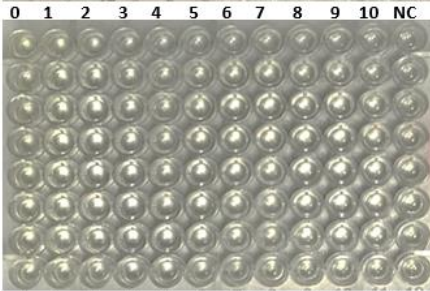
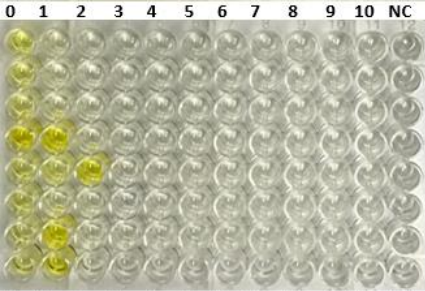
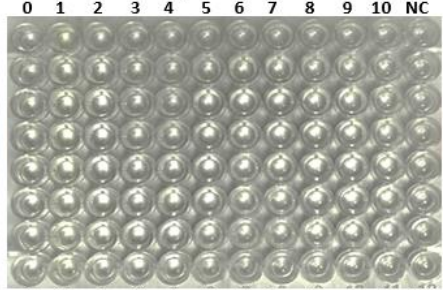
KNU18-91 (3dpi)



KNU18-91 (6dpi)

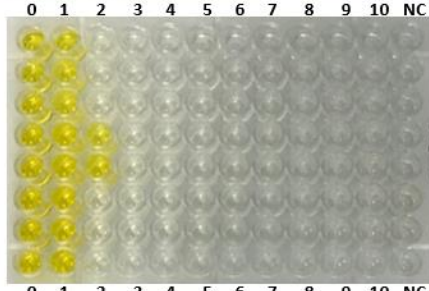


KNU18-91 (14dpi)

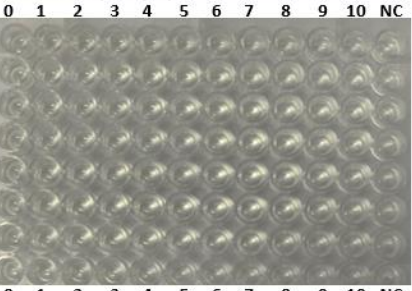


b.

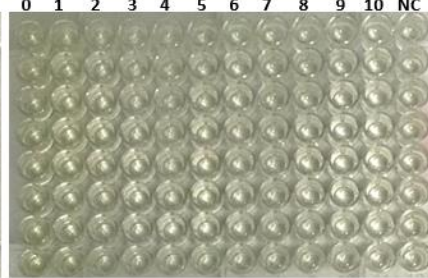
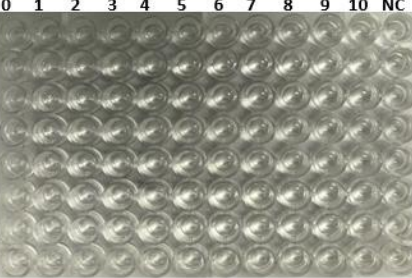
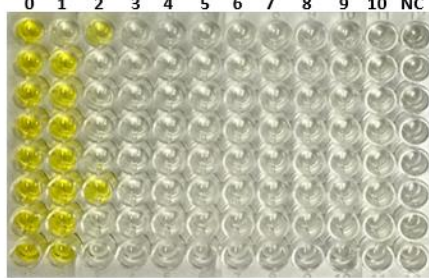
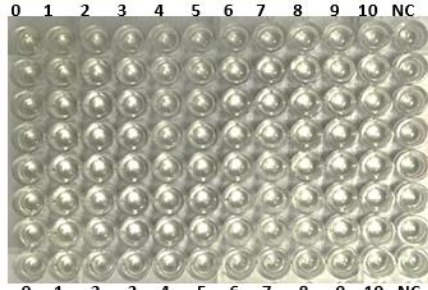
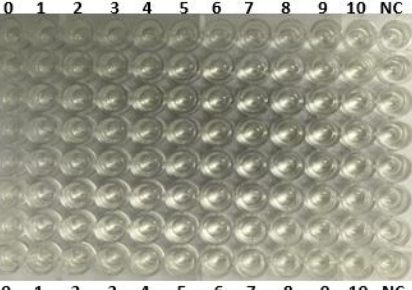
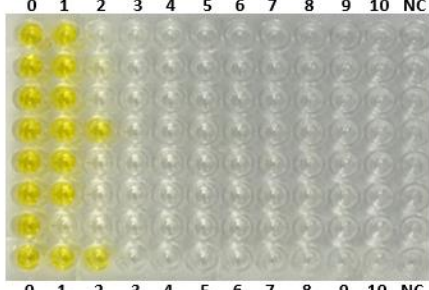
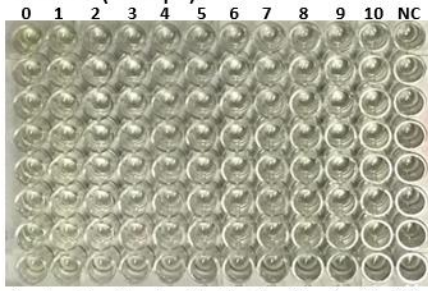
H5N3 (3dpi)



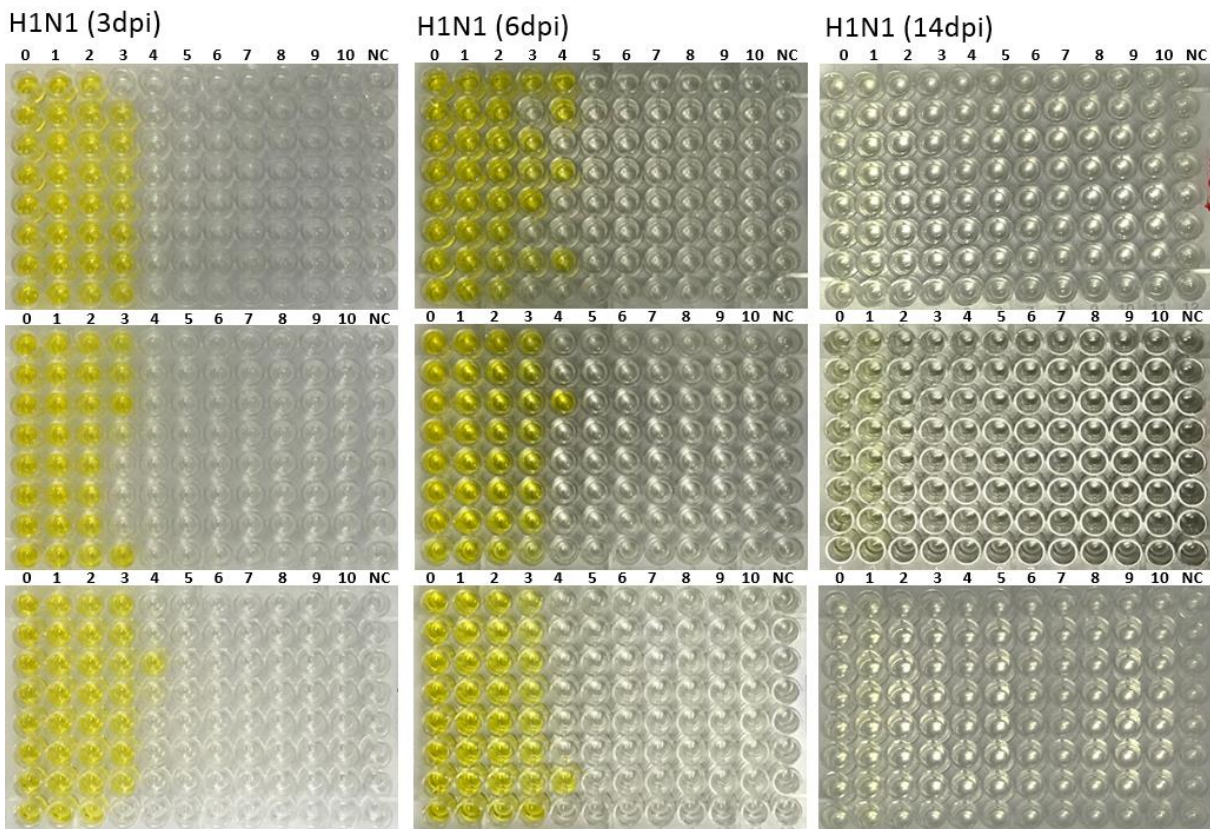
H5N3 (6dpi)



H5N3 (14dpi)



c.



**Fig. S2. Raw ELISA data to conduct TCID<sub>50</sub> assay to measure virus titer in lung.** After 3, 6, 14 dpi of intranasal challenge of viruses into female Balb/c mice 8 weeks old (initial intranasal virus = 10<sup>4</sup> EID<sub>50</sub>/mouse). Mice lung were collected and virus titer (TCID<sub>50</sub>) was conducted by ELISA method (n=3)

0: 10<sup>0</sup>- fold dilution of stock;

1: 10<sup>1</sup>- fold dilution of stock;

2: 10<sup>2</sup>- fold dilution of stock;

3: 10<sup>3</sup>- fold dilution of stock;

4: 10<sup>4</sup>- fold dilution of stock;

5: 10<sup>5</sup>- fold dilution of stock;

6: 10<sup>6</sup>- fold dilution of stock;

7: 10<sup>7</sup>- fold dilution of stock;

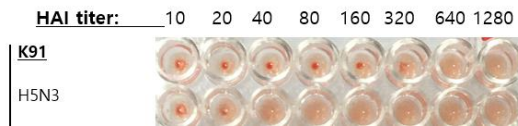
8: 10<sup>8</sup>- fold dilution of stock;

9: 10<sup>9</sup>- fold dilution of stock;

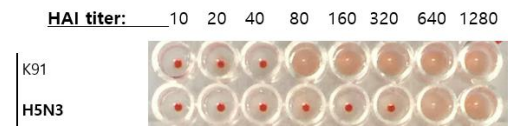
10: 10<sup>10</sup>- fold dilution of stock;

NC: negative control (uninfected virus);

**Antigen:** K91 virus



**Antigen:** SYG06/2006 (H5N3)virus



**Fig. S3. Hemagglutination assay.** The mouse antisera were treated with RDE and then serially diluted with PBS, mixed with 4 hemagglutination units of a virus, and incubated for 30 min at room temperature. The HI result was obtained from the highest serum sample dilution that inhibited hemagglutination.