

Web appendix: Supplementary materials

Supplementary Methods

Close contact definition of H7N9 case

The close contacts were defined as family members who shared the residence with the patient, social contacts who visited with patients when ill, and health care workers who provided medical services without effective personal protection during the period from one day before his illness onset to isolation in accordance with China's guidelines for prevention and control of human H7N9 virus infection.¹

Hemagglutination inhibition and microneutralization assays

Sera from close contacts were screened with a horse RBC, hemagglutination inhibition (HI) assay against H7N9 virus.² Briefly, sera were pre-treated with receptor destroying enzyme RDE (II) "Seiken" (Denka Seiken Co. Ltd., Tokyo, Japan) and absorbed with 1% horse RBCs to remove nonspecific agglutinins and virus inhibitions. Sera were tested in doubling dilutions starting at 1:10. The titer results are reported as the reciprocal of the highest dilution of serum that inhibited virus-induced hemagglutination of 1% horse RBCs. Sera with a HI titer of $\geq 1:20$ were retested with a microneutralization assay adapted from Rowe³ and tittered in doubling dilutions ranging from 1:10 to 1:1280. Virus neutralization was performed by adding 100 microliters TCID₅₀ of virus to the sera. One hundred microliters of MDCK cells suspension were added to each well and the plate incubated at 37°C with 5% CO₂ for 24 hours. The plate was washed twice with PBS, fixed for 10min with cold 80%

acetone at room temperature. The enzyme-linked immunosorbent assay endpoint titer was expressed as the reciprocal of the highest dilution of serum with optical density (OD) < X, where $X = [(average\ OD\ of\ virus\ control\ wells) + (average\ OD\ of\ cell\ control\ wells)]/2$. The back titration was run in duplicate and was only accepted when both replicates had matching results. A viral isolate from the index patient (A/Quzhou/1/2015/H7N9) and H7N9 antisera purchased from the Harbin Weike Biotechnology Development Company subordinated Harbin Veterinary Research institute, Chinese Academy of Agricultural Sciences (Harbin, China) were used in both HI and MN assays as control sera.

Solid-phase binding assay

Receptor binding specificity was analyzed by a solid-phase direct binding assay biotinylated sialylglycopolymers: 39-sialyllactose-PAA-biotin (39SL-PAA, 39 Neu5Aca2-3Galb1-4Glc) and 69-sialyllactosamine-PAA-biotin (69SLN-PAA, 69 Neu5Aca2-6Galb1-4Glc) (Cat. No. 01-038, 01-039, Glycotech, Gaithersburg, MD) as previously described.⁴ Briefly, serial dilutions (0.039µg/ml, 0.08µg/ml, 0.16µg/ml, 0.3125µg/ml, 0.625µg/ml, 1.25µg/ml, 2.5µg/ml and 5µg/ml) of 3'-sialyllactose-PAA-biotin and 6'-sialyllactosamine-PAA-biotin were prepared in PBS; 100 µl of this dilution was added to each well of the 96-well microtiter plates (Cat. No. costar 30500, Thermo Fisher, MA, USA) and allowed to attach overnight at 4°C. After removal of the glycopolymer solution, the plates were blocked with 0.1 ml of PBS containing 2% bovine serum albumin (BSA) at room temperature for 1 hour.

After washing with ice-cold PBS containing 0.1% Tween 20 (PBST) and cold PBS, the plates were incubated in a solution containing influenza virus (64 HA units in PBS containing 2% BSA) at 4°C overnight. After washing with cold PBST and cold PBS, chicken antisera against A/Quzhou/1/2015/H7N9, A/California/07/2009/H1N1, and A/Chicken/Jiangsu/927/2014/H5N1 viruses were added to each well and the plates were incubated at 4°C for 2 hours. The wells were washed with ice-cold PBST and cold PBS then incubated with HRP-linked goat-anti-chicken antibody (Sigma-Aldrich, www.sigmaaldrich.com) for 2 hours at 4°C. After washing with ice-cold PBST and cold PBS, the plates were incubated 200 µl of TMB substrate (Cat.No.T8665, Sigma-Aldrich) for 20 minutes at room temperature. The reaction was then stopped with 100 µl of 50mM H2SO4 and the optical density was measured at 450 nm.

Table S1. Key molecular characteristics of three H7N9 viruses identified in this study.

Gene	Position	A/Quzhou/1/2015	A/Quzhou/2/2015	A/Chicken/Quzhou/1/2015	Comments
HA	Cleavage	PEIPKGR ↓G	EIPKGR↓G	PEIPKGR↓G	Pathogenic to poultry
	S138A	A	A	A	
	G186V	V	V	V	RBS position, altered receptor specificity
	Q226L	L	L	L	
	G228S	G	G	G	
NA	63–73 deletion	Yes	Yes	Yes	Increased virulence in mice
	R292K	R	R	R	Oseteltamivir and zanamivir resistance
PB2	L89V	V	V	n/a	Mammalian host adaption and increased virulence in mice
	E627K	K	K	n/a	

PB1	H99Y	H	H	n/a	H5 virus transmissible among ferrets
	I368V	V	V	n/a	
M1	N30D	D	D	D	Increased virulence in mice
	T215A	A	A	A	
M2	S31N	N	N	N	Antiviral resistance (amantadine)
NS1	P42S	S	S	S	Increased virulence in mice

n/a=not applicable due the lack sequencing data.

Figure S1. A computed tomography of the index case on 22 February showing

bilateral lobe infiltrates

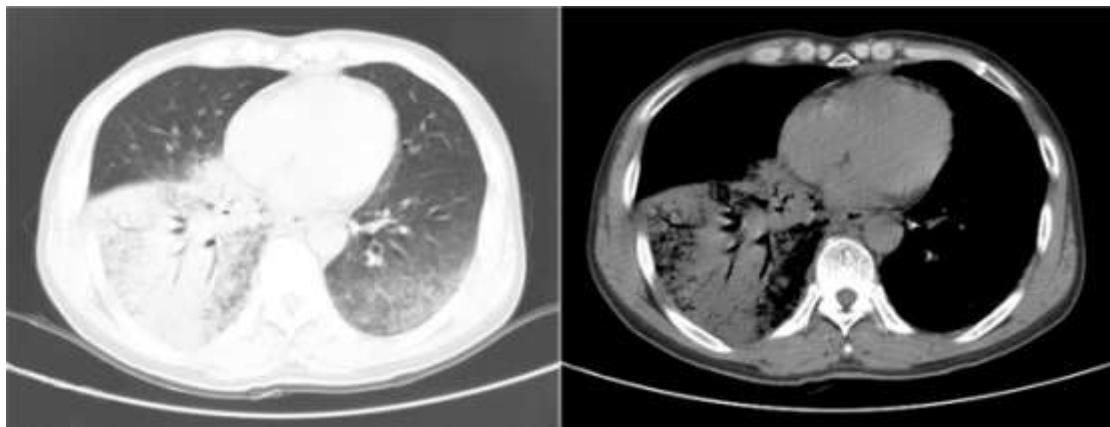


Figure S2. Location of two H7N9 infected patients' home, and the stall at live poultry market where the index case bought two chickens, Quzhou of Zhejiang Province, China, February 2015.

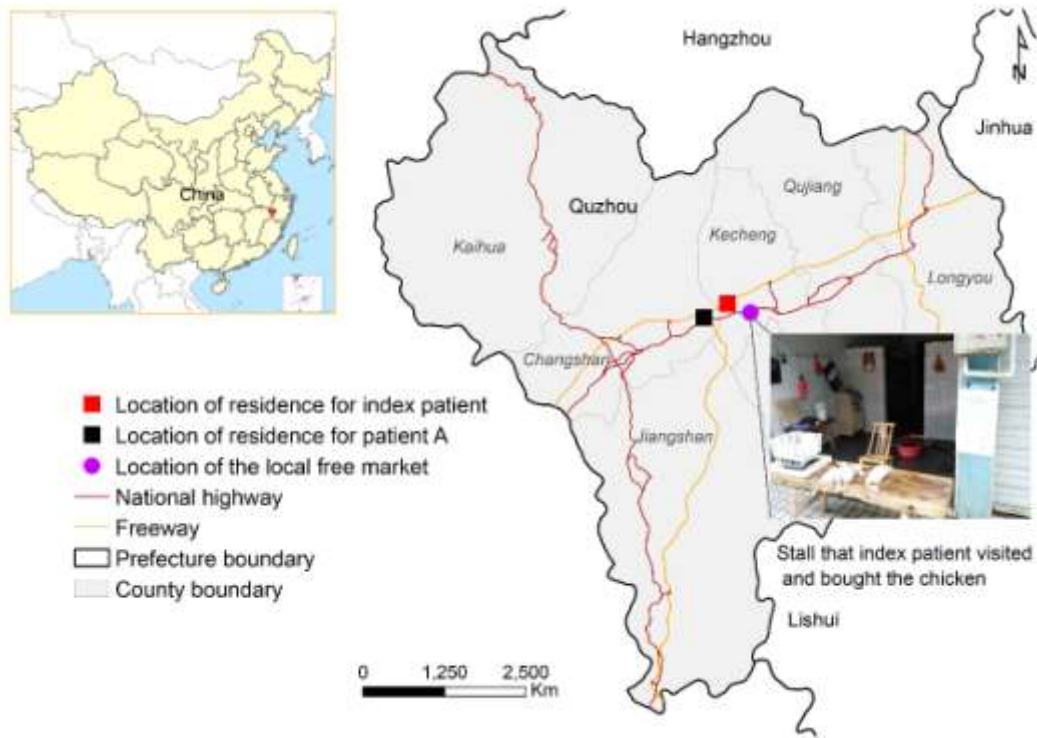


Figure S3. Diagram of the patients' shared hospital ward.

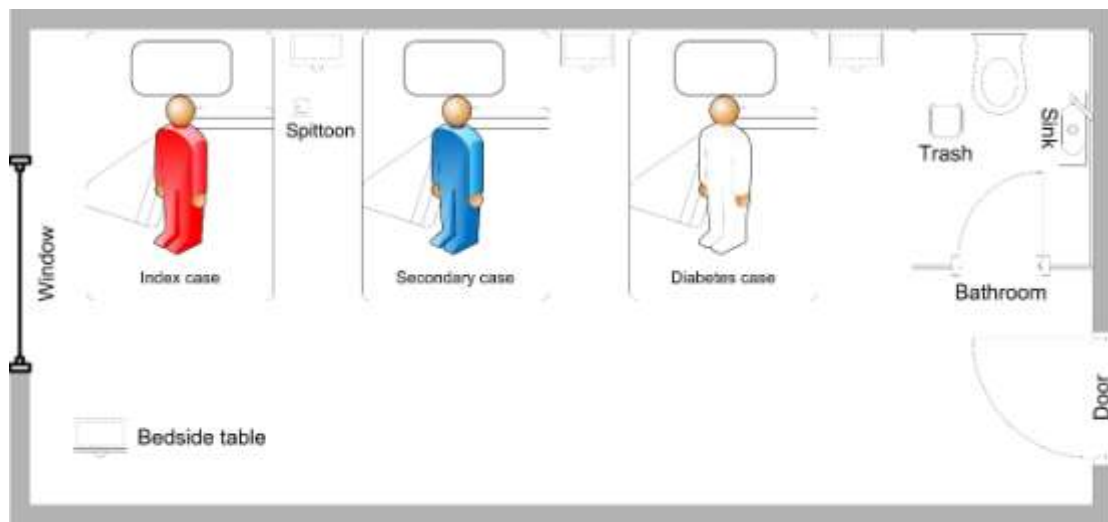
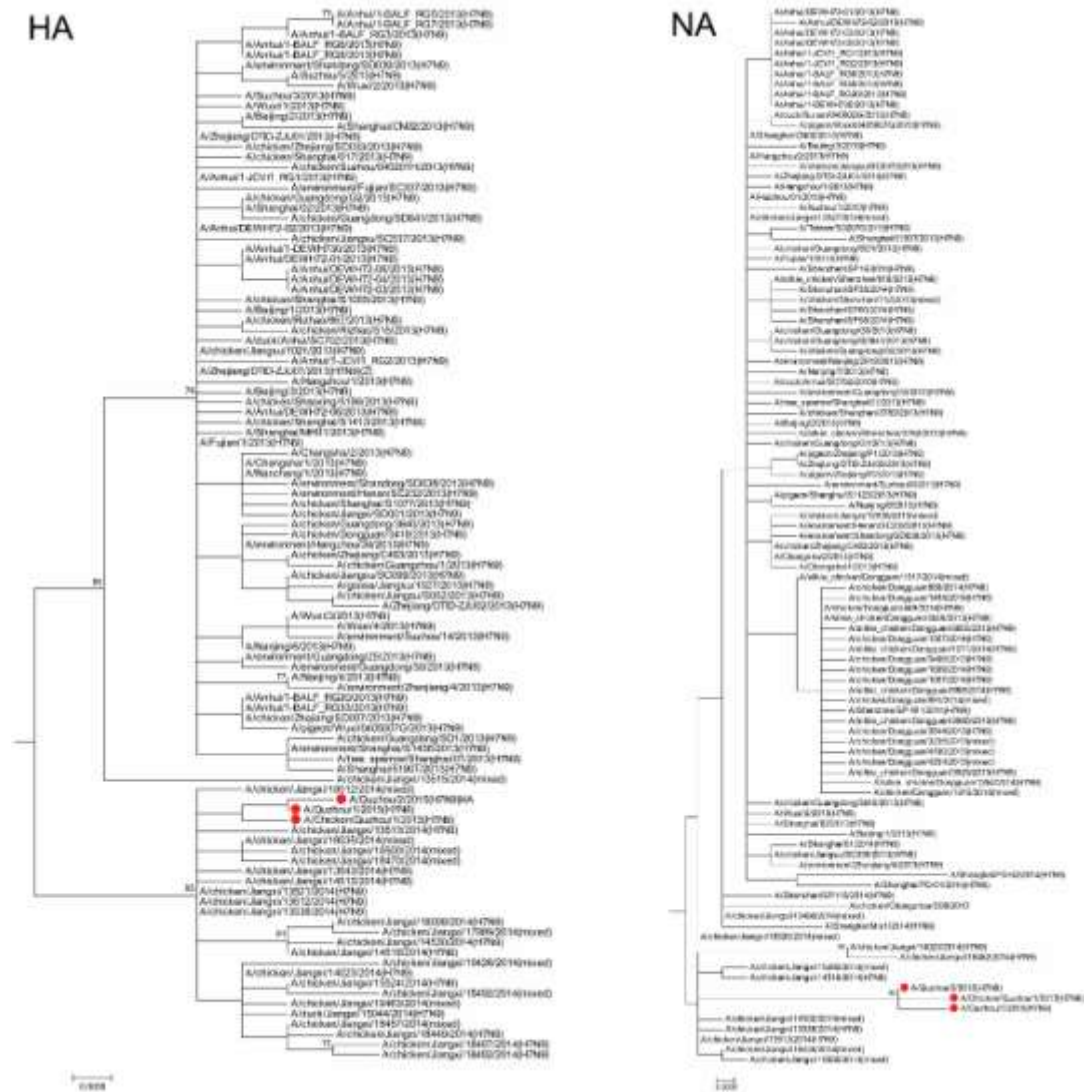
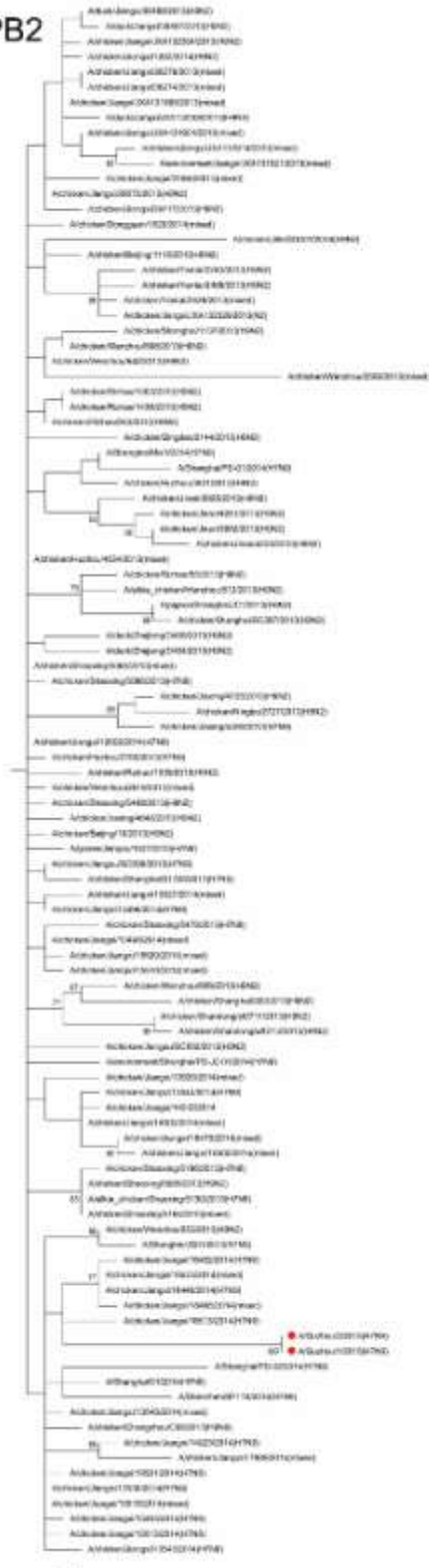


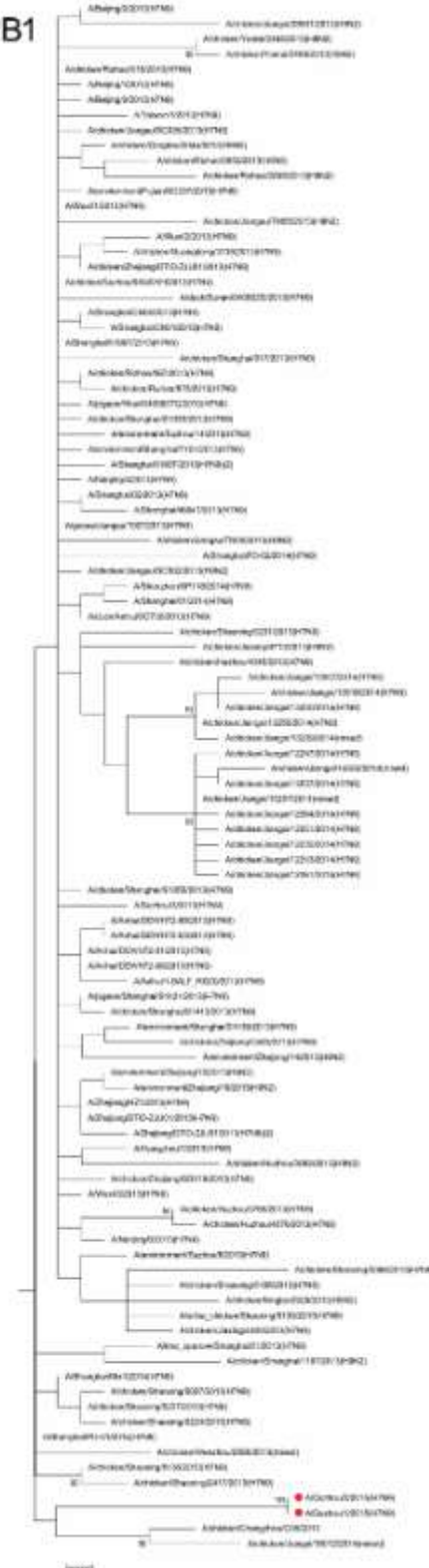
Figure S4. Phylogenetic trees of the H7N9 viruses from the two patients and an environment sample from the live poultry market in Quzhou of Zhejiang Province, China, 2015. Supporting bootstrap values greater than 75 are shown. Scale bars indicates nucleotide substitutions per site. H7N9 viruses isolated or sequenced were market with solid red circles. (HA = haemagglutinin; NA = neuraminidase; PB2= polymerase basic 2; PB1= polymerase basic 1; PA= polymerase acidic; NP= nucleoprotein; M= matrix; NS= nonstructural protein).



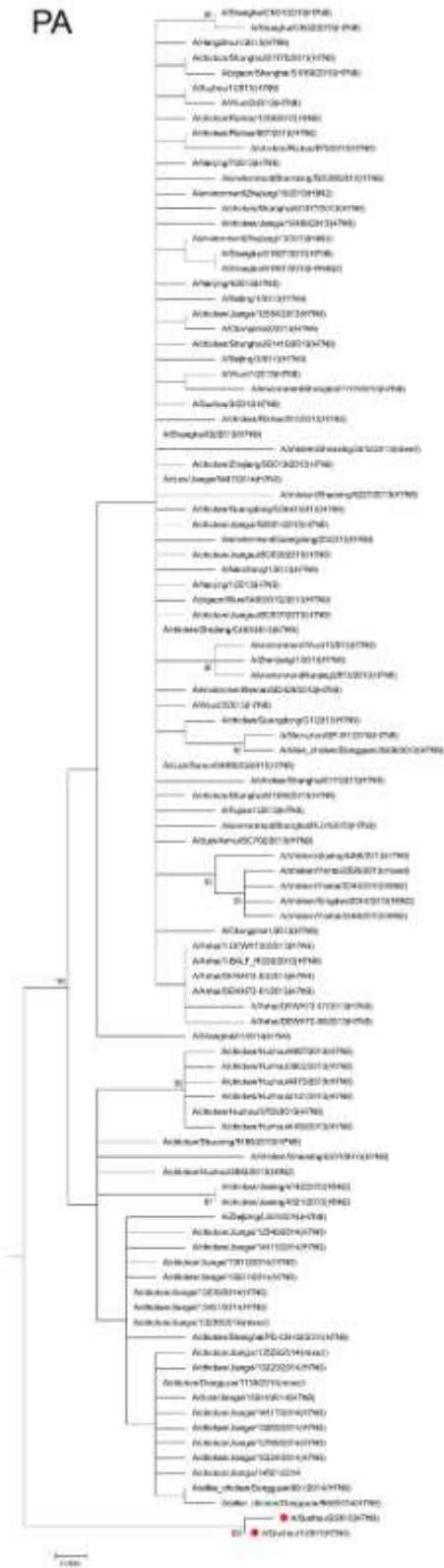
PB2



PB1



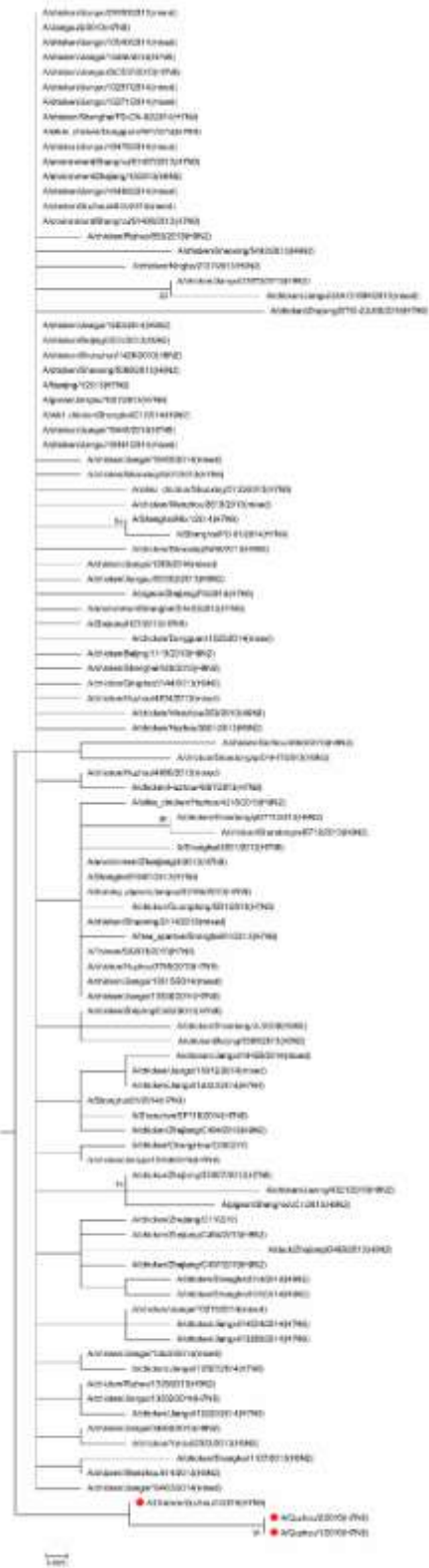
PA



NP



MP



NS

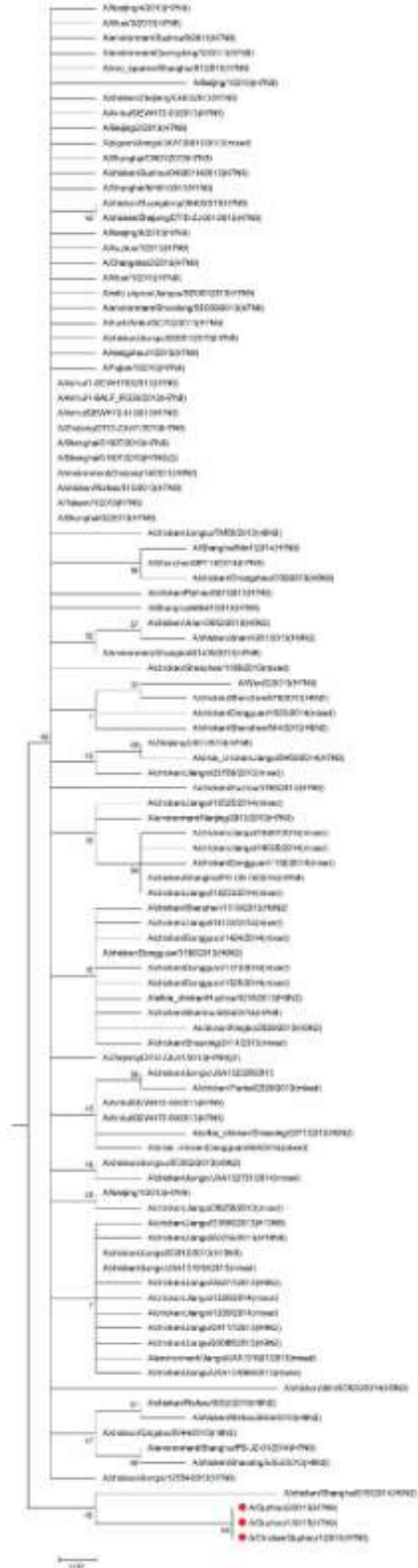


Figure S5. Characterization of receptor-binding properties at virus level.

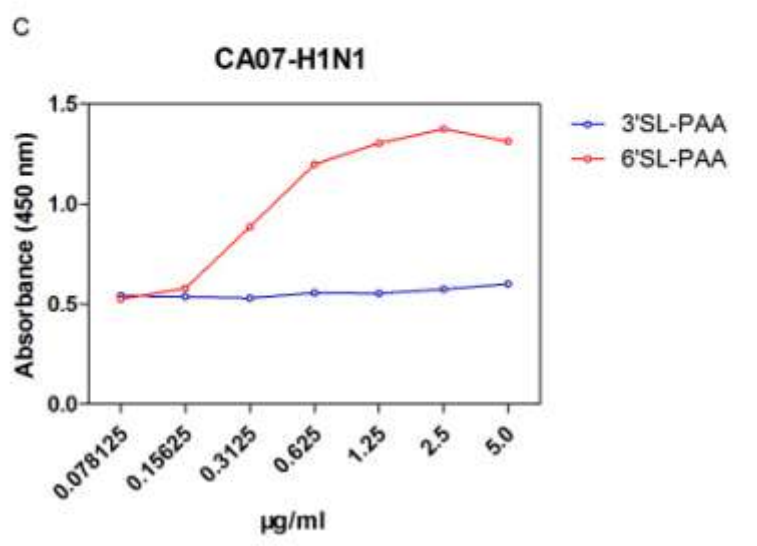
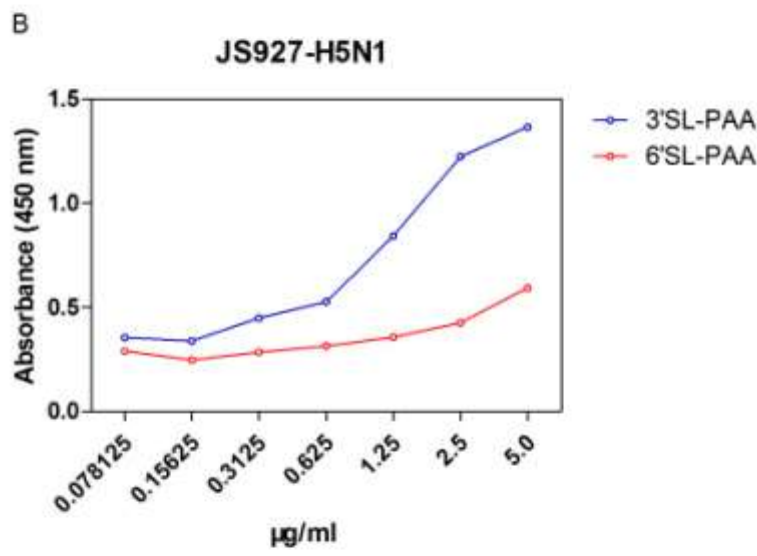
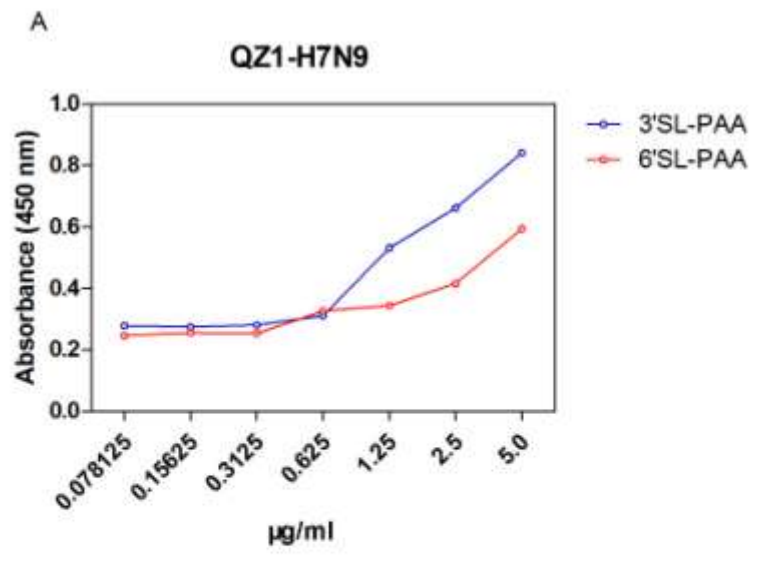
Binding of virus to α 2,3-linked (3'SL-PAA) or α 2,6-linked (6'SL-PAA)

sialylglycan receptors was determined by solid-phase binding assays. (A)

QZ1-H7N9 (A/Quzhou/1/2015/H7N9) virus; (B) CA07-H1N1,

(A/California/07/2009) virus; (C) JS927-H5N1 (A/Chicken/Jiangsu/927/2014)

virus).



Supplementary References

1. China National Health and Family Planning Commission. Chinese guideline for prevention and control for human infection with A(H7N9) avian influenza (2014 edition) [in Chinese]. Third ed, 2014.
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3. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol* 1999;37(4):937-43.
4. Ma MJ, Yang XX, Qian YH, et al. Characterization of a novel reassortant influenza A virus (H2N2) from a domestic duck in Eastern China. *Sci Rep* 2014;4:7588.