Supplementary Information

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Supplementary Results

Chemokine/cytokine levels in Anhui/1- or Dk/GM466- infected macaques

At 3 dpi, the levels of proinflammatory cytokines such as IL-1β, IL-6, TNFα, and IL-18 were upregulated in the lungs of animals infected with Anhui/1 or Dk/GM466 (Figure S9). By 6 dpi, inflammatory responses were suppressed, but IFNγ levels were elevated in the lungs of Anhui/1-infected macaques, indicating T cell-mediated antiviral responses. To analyze systemic host responses, we assessed cytokine concentrations in the serum of infected animals. The levels of IL-6, monocyte chemoattractant protein-1 (MCP-1: CCL2), IL-15, IL-10, and IL1Ra were transiently increased at 1 dpi, particularly after infection with Anhui/1 (Figure S9). Thus, Anhui/1 appears to induce strong inflammatory responses both systemically and at the site of virus infection.

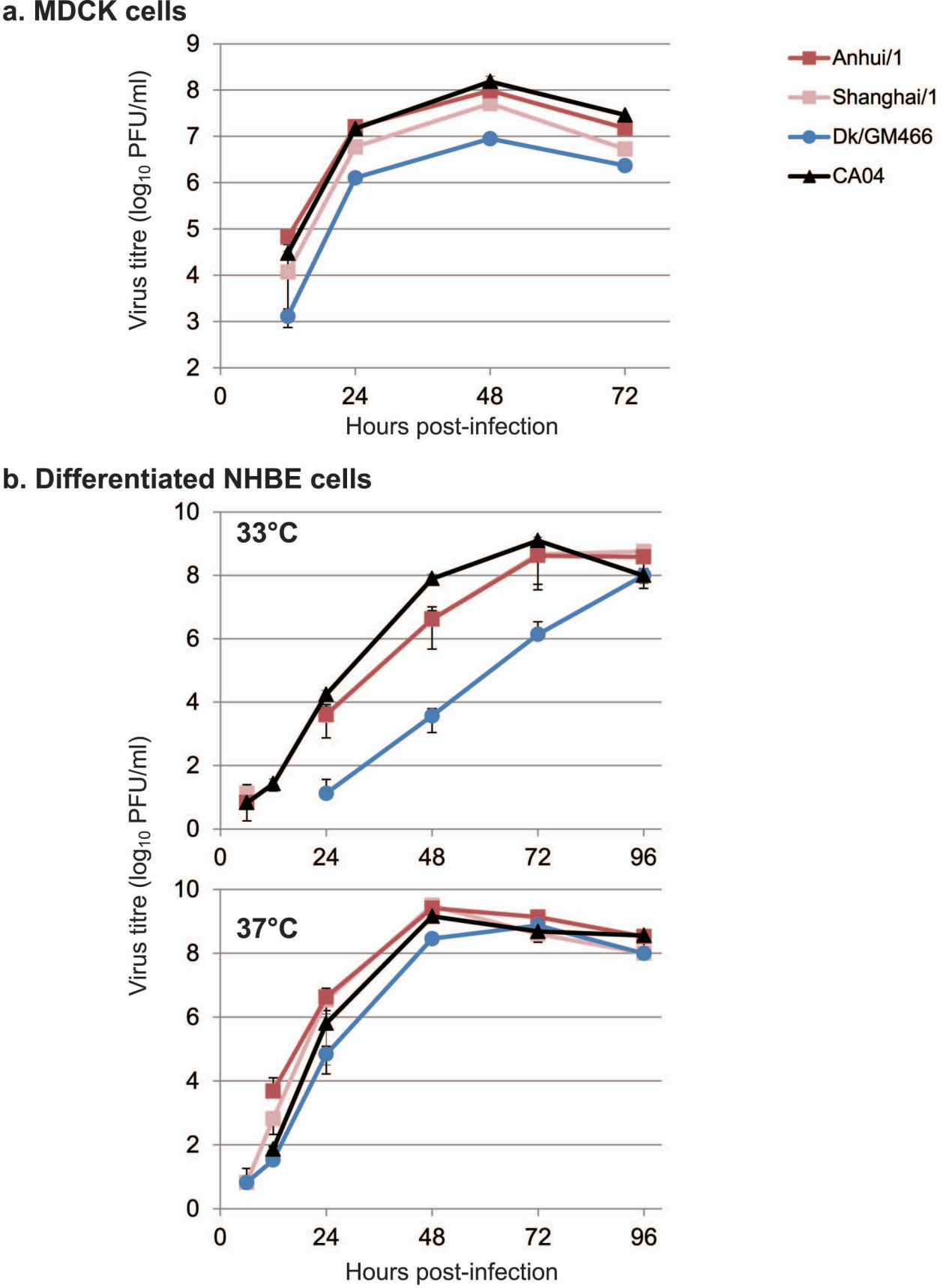
Infection of miniature pigs with Anhui/1 or Dk/GM466 viruses

Pigs occupy a unique role in influenza virus ecology because they may serve as "mixing vessels" in which human and avian influenza viruses reassort. In fact, the 2009 H1N1 pandemic influenza virus likely emerged from reassortment in pigs. Specific-pathogen-free miniature pigs intranasally infected with 10^7 PFU of Anhui/1 or Dk/GM466 showed no signs of disease, consistent with a lack of efficient virus replication (Tables S10, S11). Mild pathological changes were observed throughout the lung except in the right upper lobules, where focal severe alveolar damage was observed (Figure S11). Viral antigen was detected in the tracheal, bronchial, bronchiolar, and alveolar epithelia of Anhui/1-infected pigs; few viral antigens were detected in Dk/GM466-infected pigs. Apparently, infections with Anhui/1 and

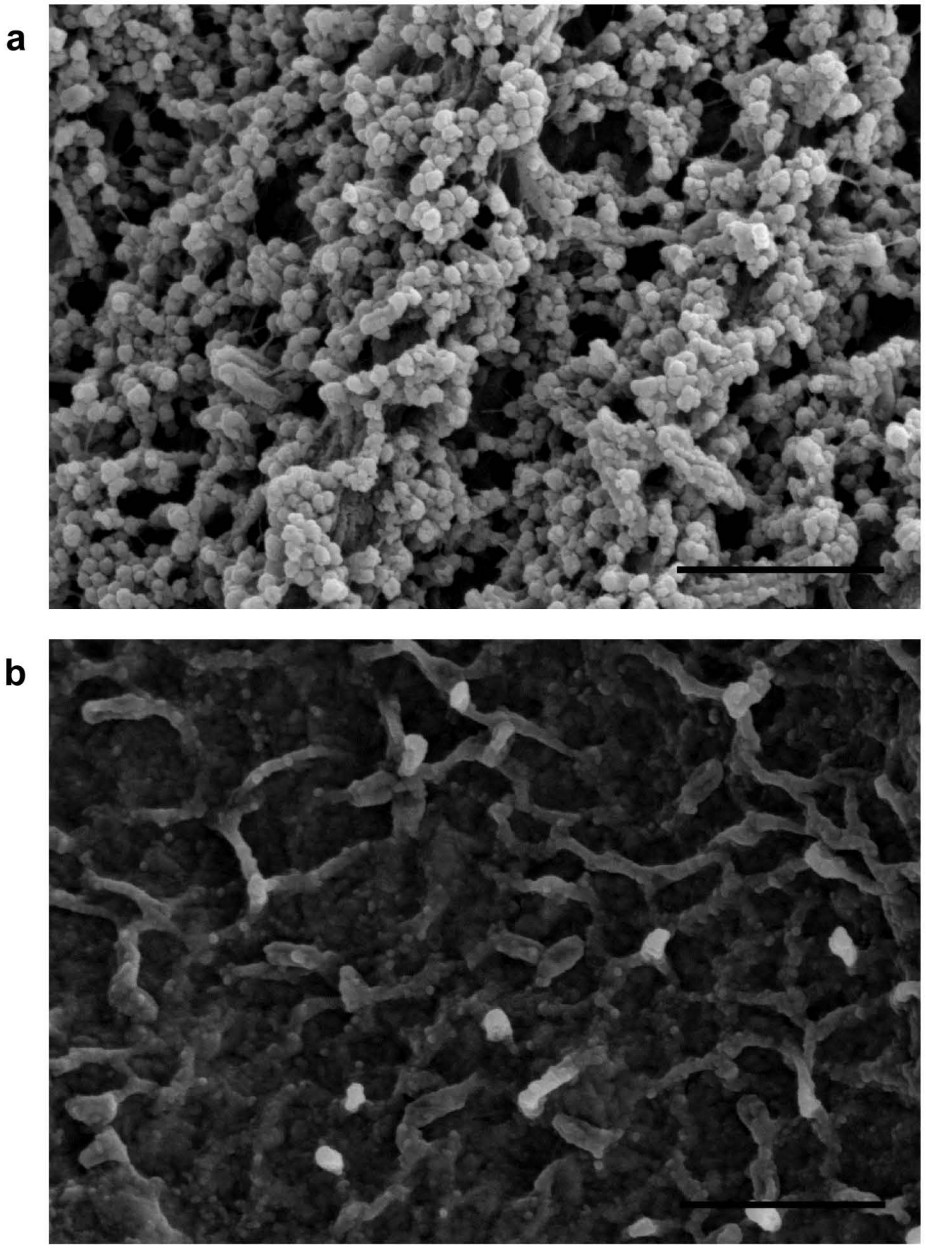
Dk/GM466, but also with pandemic 2009 H1N1 CA04, seasonal H1N1 (A/Kawasaki/UTK-4/2009), and avian H5N1 viruses do not cause severe disease in pigs.

Infection of chickens and quail with Anhui/1 or Dk/GM466 viruses

A(H7N9) viruses have been isolated from poultry in live bird markets in Eastern China[1]. Chickens and quails infected with Anhui/1 or Dk/GM466 did not display signs of disease. Higher titres of virus were recovered from tracheal than from cloacal swabs for both viruses in quails and chickens. Virus titres were higher in quail than in chicken samples. However, both viruses were recovered from only a limited number of organs (Tables S12–15). These virological findings and the lack of multiple basic amino acids at the HA cleavage site – a hallmark of highly pathogenic avian influenza viruses such as the currently circulating avian H5N1 viruses – clearly establish A(H7N9) viruses as low pathogenic avian influenza viruses. The lack of detectable disease symptoms in poultry greatly hampers the detection of these viruses through routine surveillance.

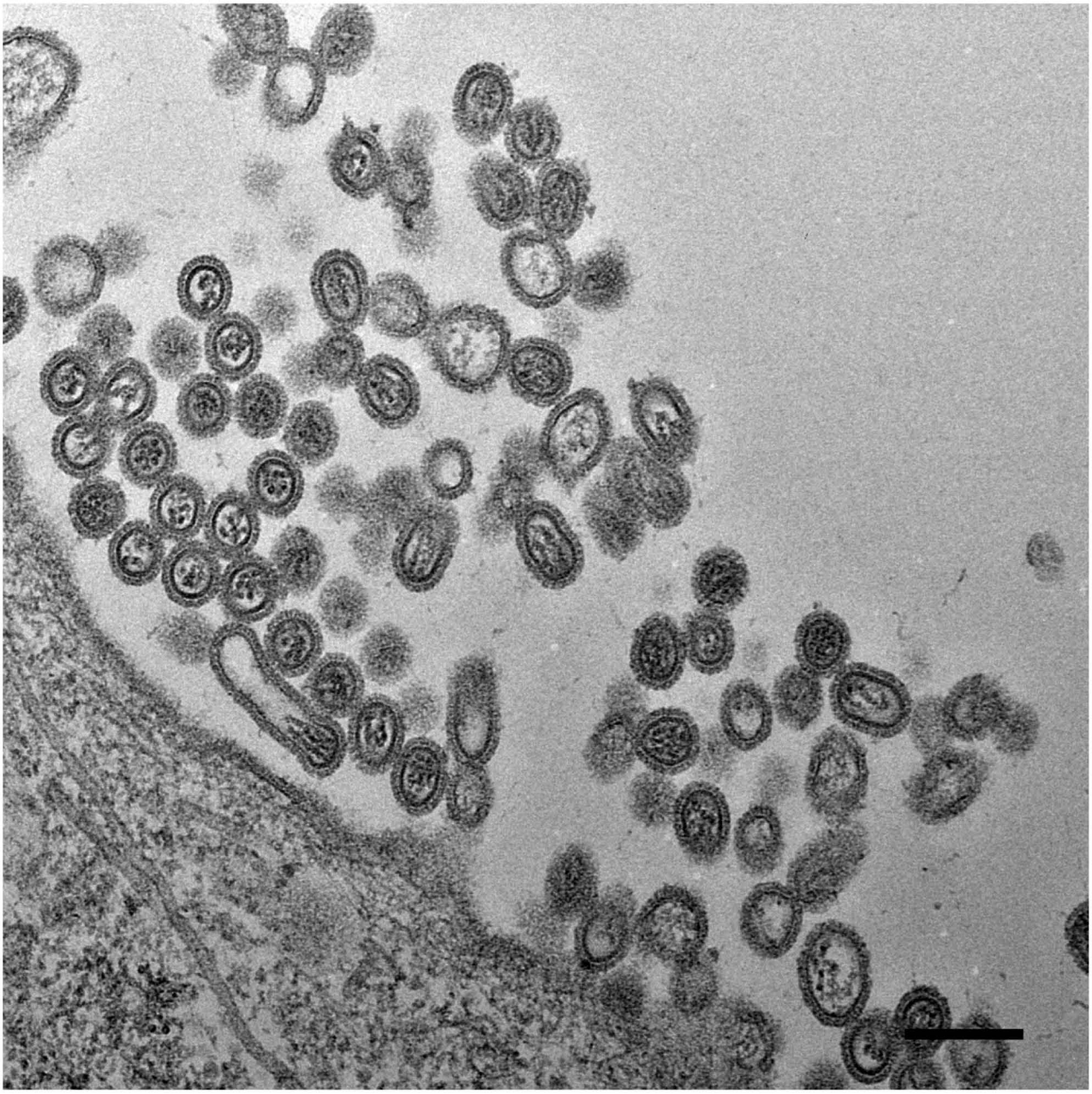


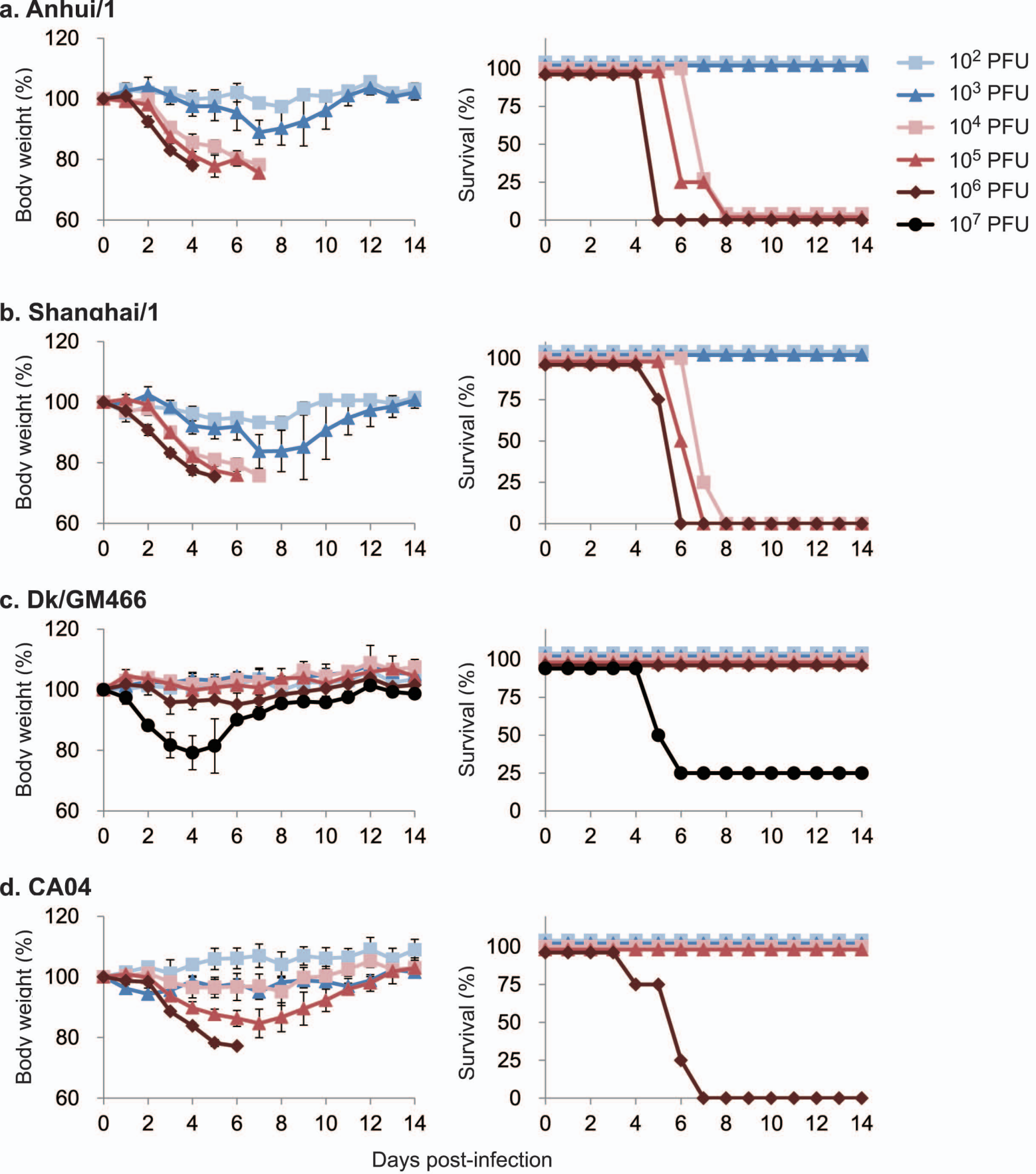
Supplementary Figure S1. Growth kinetics of viruses in cells. MDCK (**a**) or differentiated NHBE (**b**) cells were infected with Anhui/1 (red), Shanghai/1 (light red), Dk/GM466 (blue), or CA04 (black) at an MOI of 0.01 (MDCK) or 0.001 (differentiated NHBE), respectively. The supernatants of infected cells were harvested at the indicated times and virus titres were determined by plaque assays in MDCK cells. Error bars indicate standard deviations from three independent experiments.



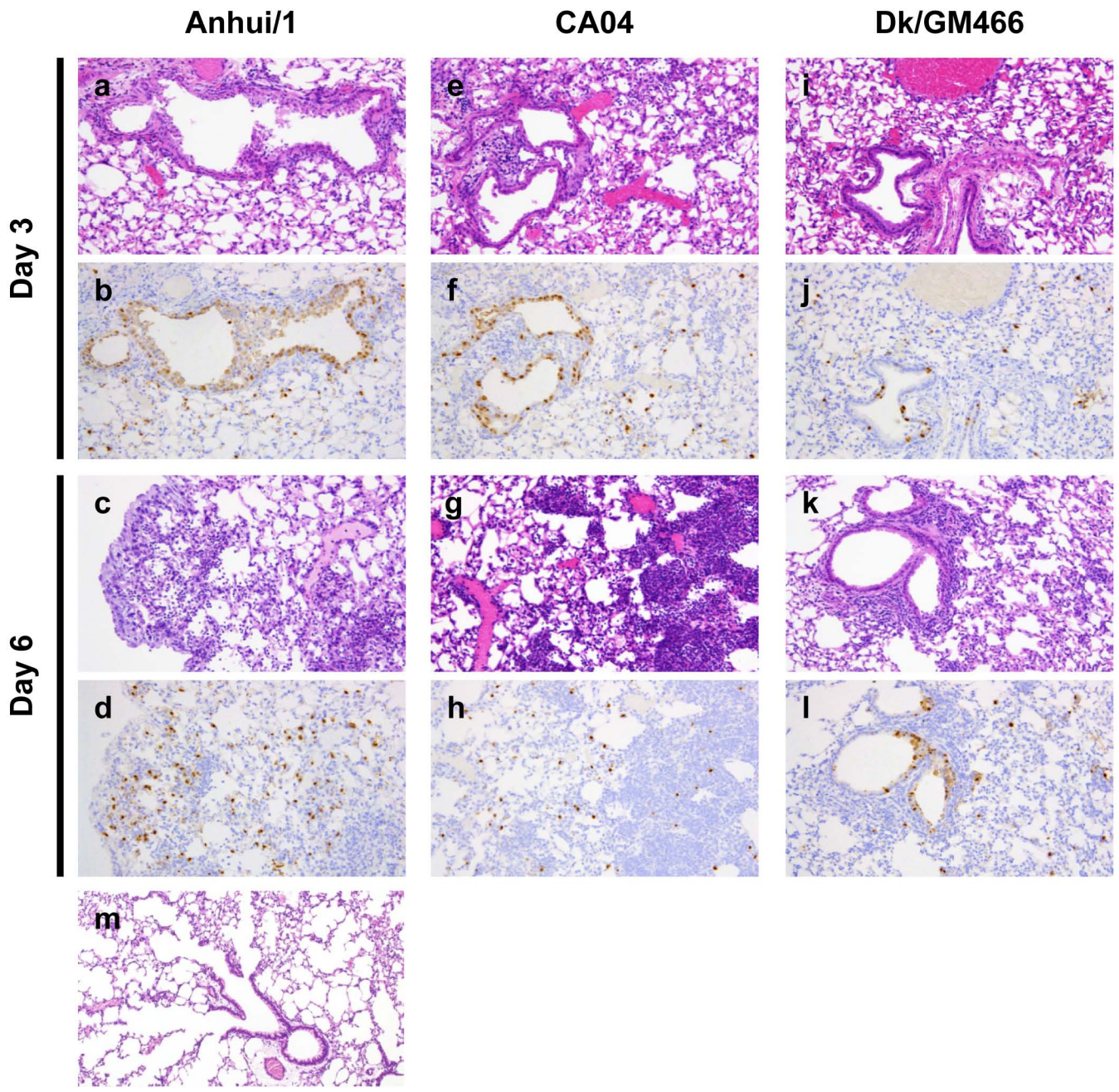
Supplementary Figure S2. Morphology of budding Anhui/1 virions. Cells of the allantoic membrane of Anhui/1-infected (**a**) and mock-infected (**b**) embryonated chicken eggs were observed by means of scanning electron microscopy. Bars, 1 μm. Virus-infected cells of the allantoic membrane were examined by using transmission electron microscopy (**c**). Bar, 200 nm.

c

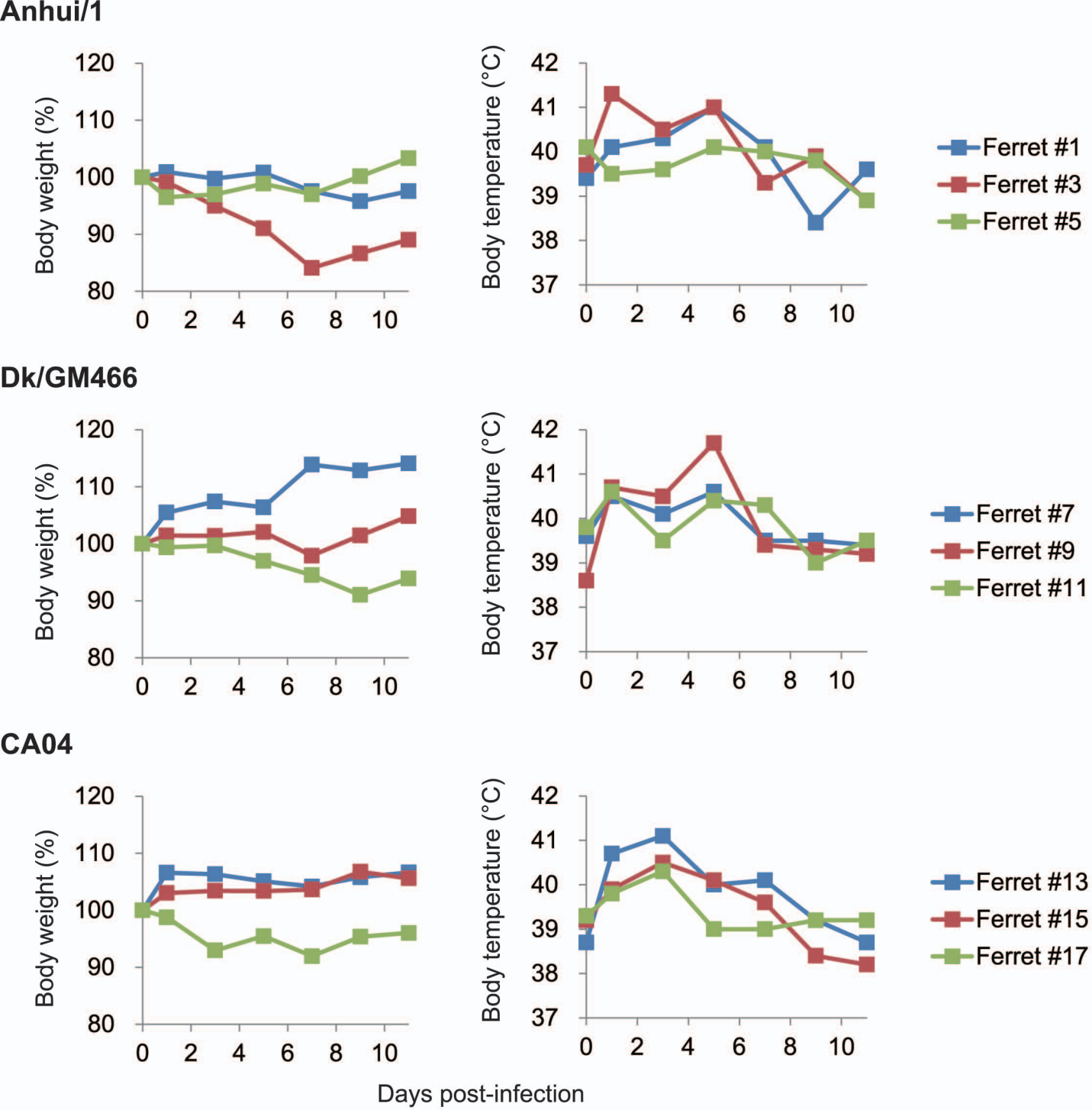




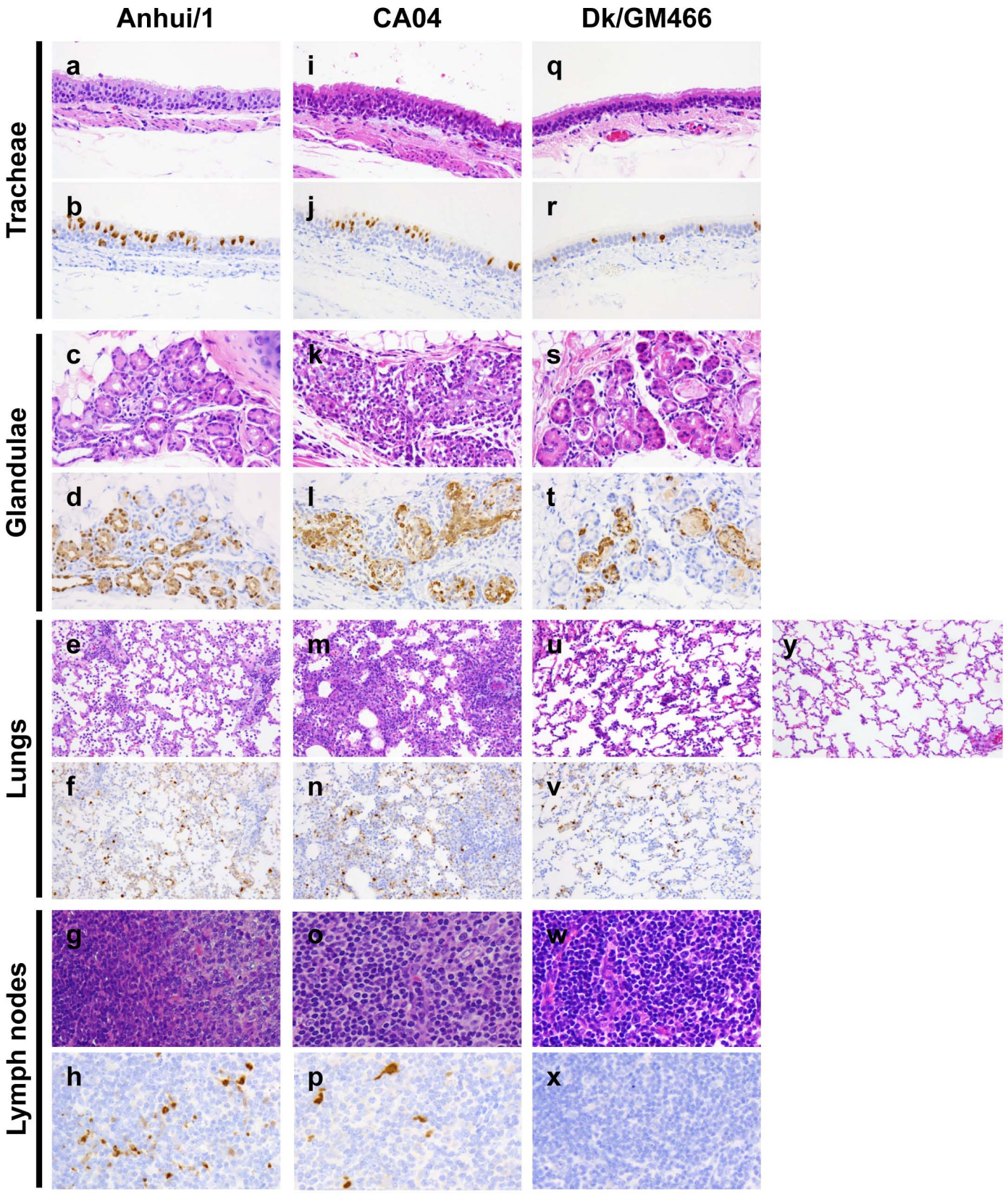
Supplementary Figure S3. Pathogenicity in mice. Four mice per group were intranasally inoculated with 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 PFU (each in 50 µl; 107 PFU applies only to Dk/GM466) of Anhui/1 (**a**), Shanghai/1 (**b**), Dk/GM466 (**c**), or CA04 (**d**). Body weight (left panels) and survival (right panels) were monitored daily. The values for body weights are means ± SD from live mice.



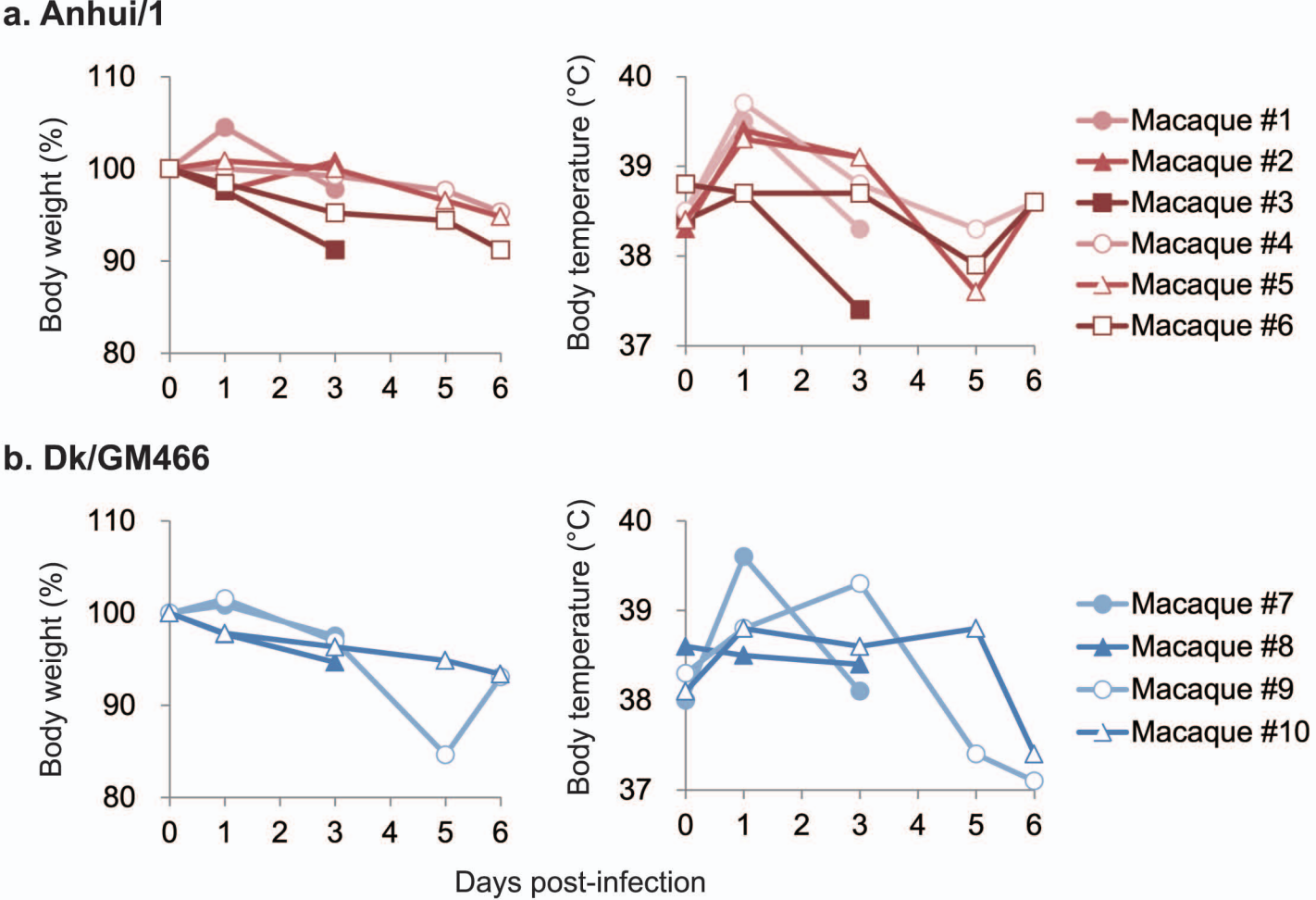
Supplementary Figure S4. Pathological findings in infected mice. Shown are representative pathological findings in the lungs of mice infected with Anhui/1 (**a–d**), CA04 (**e–h**), or Dk/GM466 (**i–l**) at 3 (**a, b, e, f, i, j**) and 6 (**c, d, g, h, k, l**) dpi with hematoxylin-eosin (HE) staining (**a, c, e, g, i, k**) and immunohistochemistry for influenza viral antigen (NP) (**b, d, f, h, j, l**).



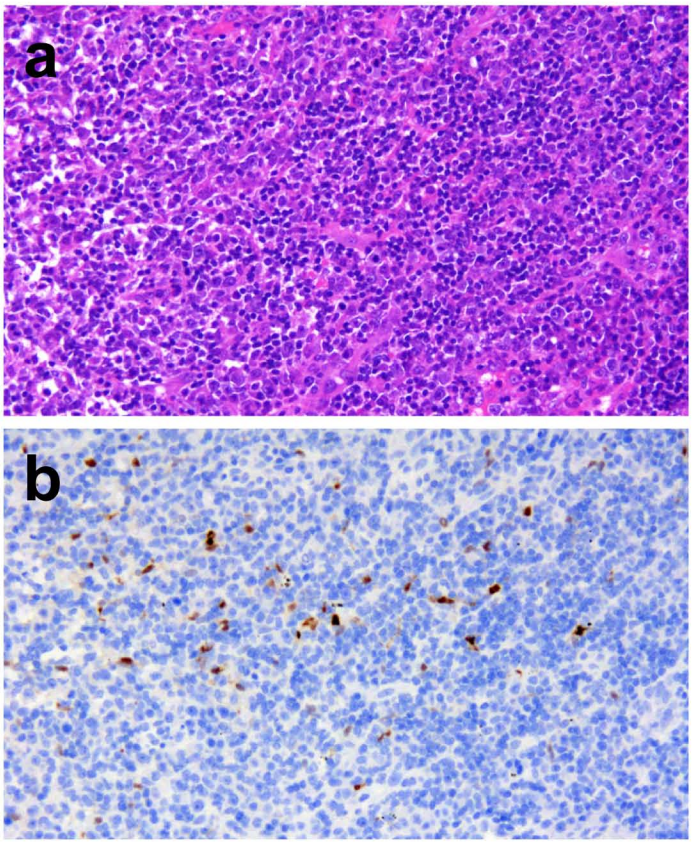
Supplementary Figure S5. Body weights and temperatures of ferrets. Three ferrets per group were intranasally inoculated with 5 x 10^5 PFU (0.5 ml) of Anhui/1, Dk/GM466, or CA04. Body weights (left panels) and temperatures (right panels) were measured at 1, 3, 5, 7, 9, and 11 dpi.



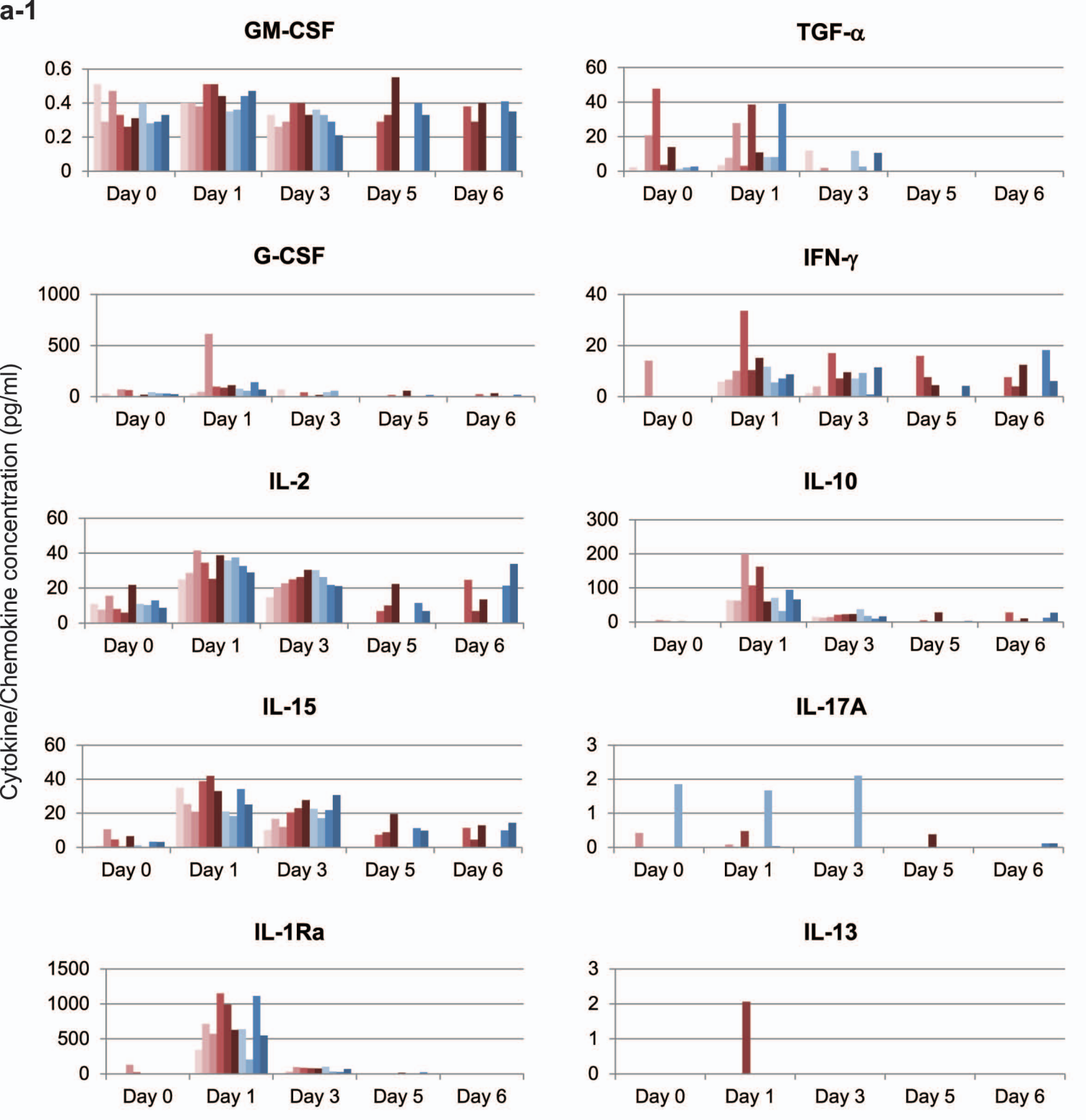
Supplementary Figure S6. Pathological findings in infected ferrets. Shown are representative pathological findings in the tracheae (**a, b, i, j, g, r**), glandulae (**c, d, k, l, s, t**), lungs (**e, f, m, n, u, v**), and mediastinum lymph nodes (**g, h, o, p, w, x)** of ferrets infected with Anhui/1 (**a–h**), CA04 **(i–p**), or Dk/GM466 (**q–x**) at 3 dpi with HE staining (**a, c, e, g, i, k, m, o, q, s, u, w**) or with immunohistochemistry for influenza viral antigen (**b, d, f, h, j, l, n, p, r, t, v, x**).



Supplementary Figure S7. Body weights and temperatures of infected cynomolgus macaques. Six and four macaques were inoculated with 6.7 x 10^7 PFU (total volume: 6.7 ml) of Anhui/1 (**a**) or Dk/GM466 (**b**), respectively, through multiple routes (see Supplementary Materials and Methods). Body weights (left panels) and temperatures (right panels) were measured at 0, 1, 3, 5, and 6 dpi.

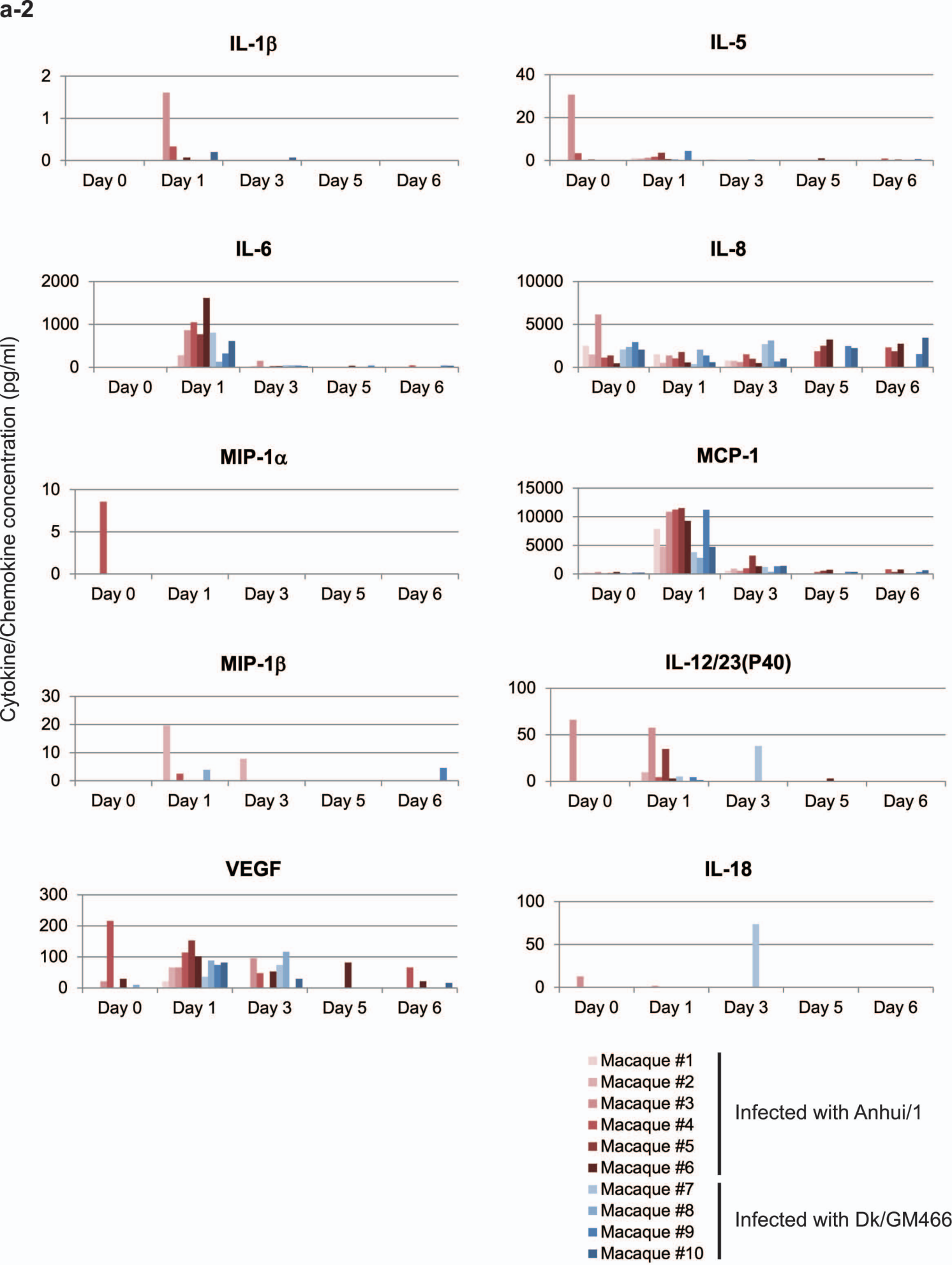


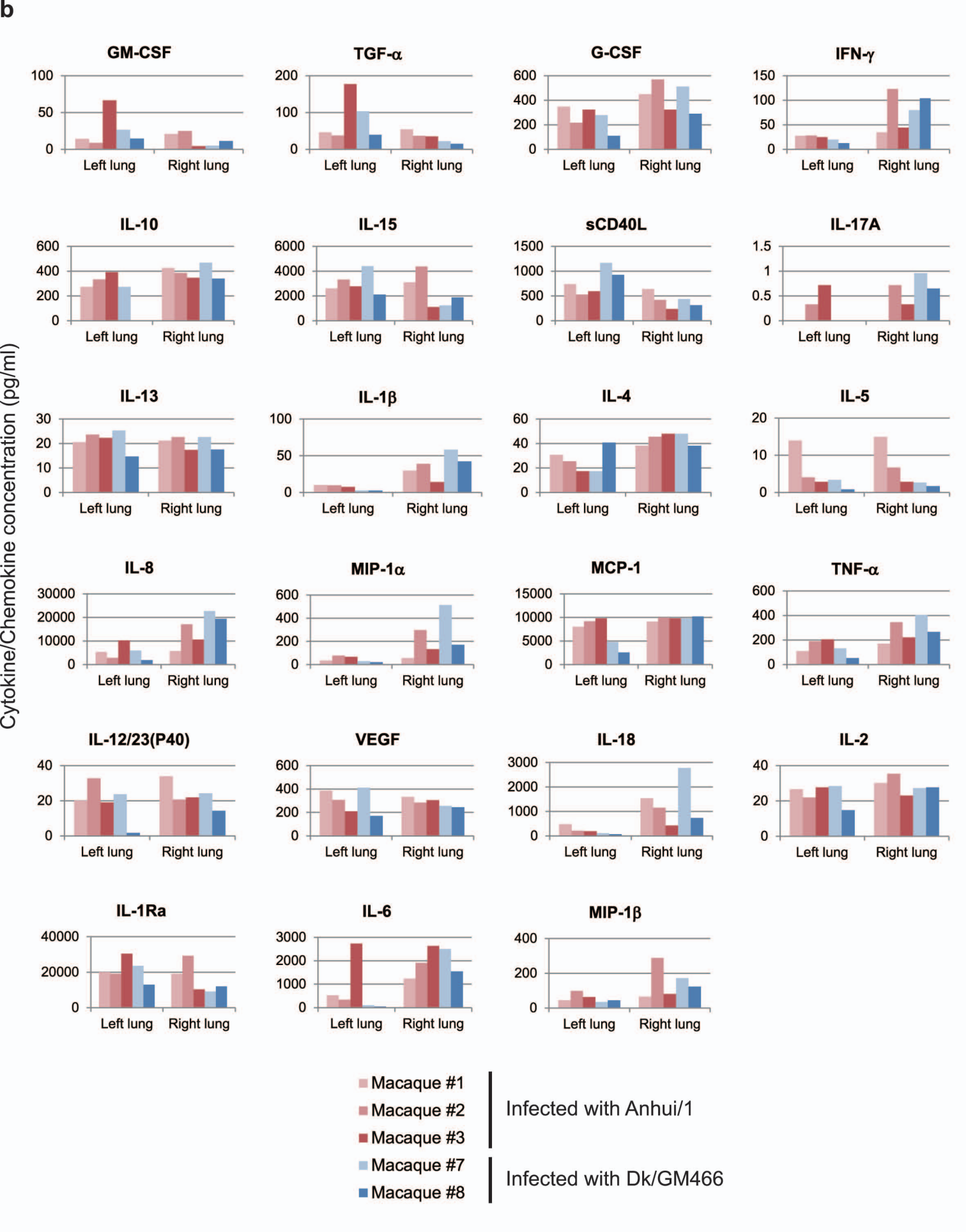
Supplementary Figure S8. Pathological findings in the mediastinum lymph nodes of Anhui/1-infected macaque at 6 dpi. Viral antigen-positive cells were detected in the mediastinal lymph node section without prominent pathological change. HE staining (**a**) and immunohistochemistry for influenza viral antigen (**b**).

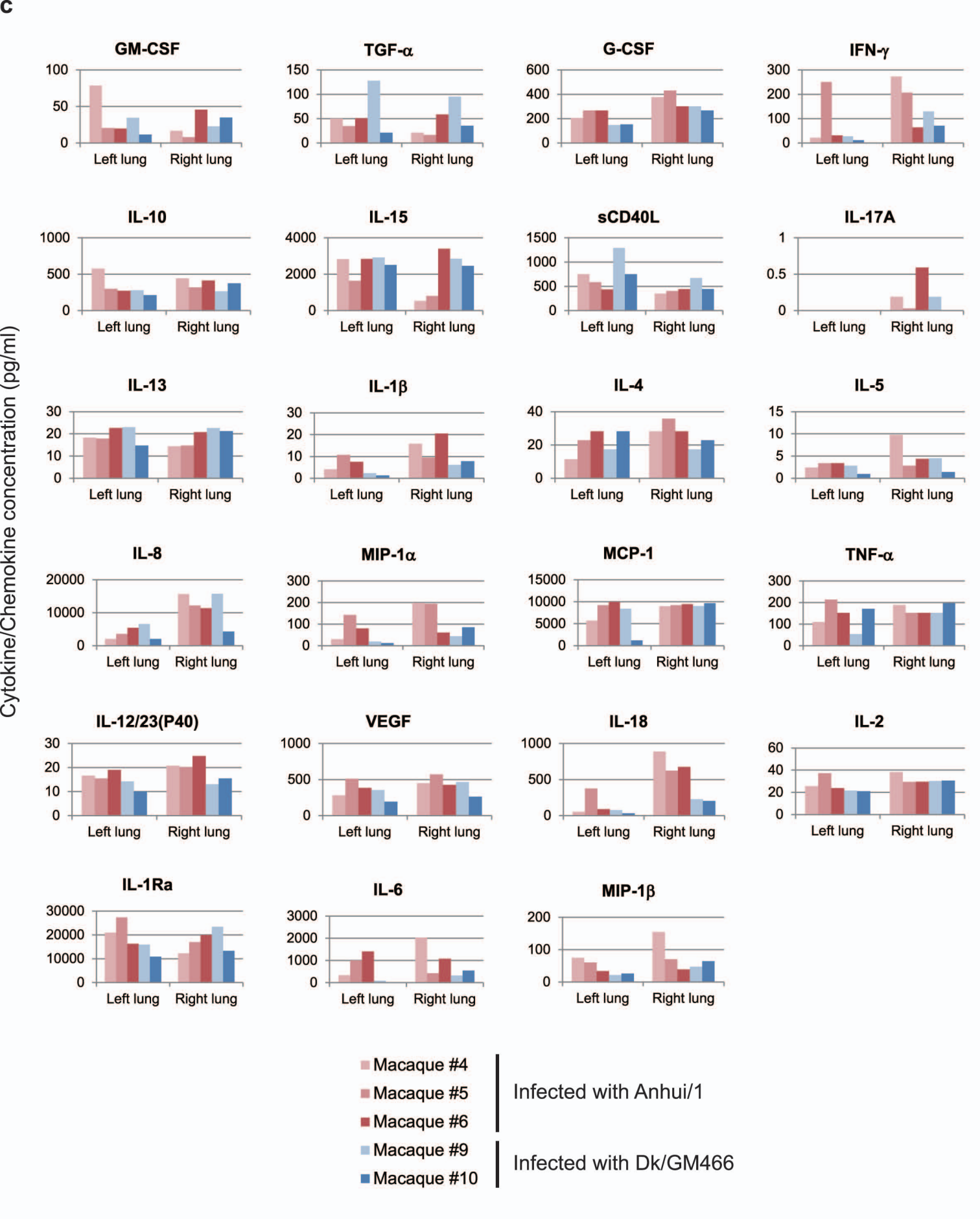


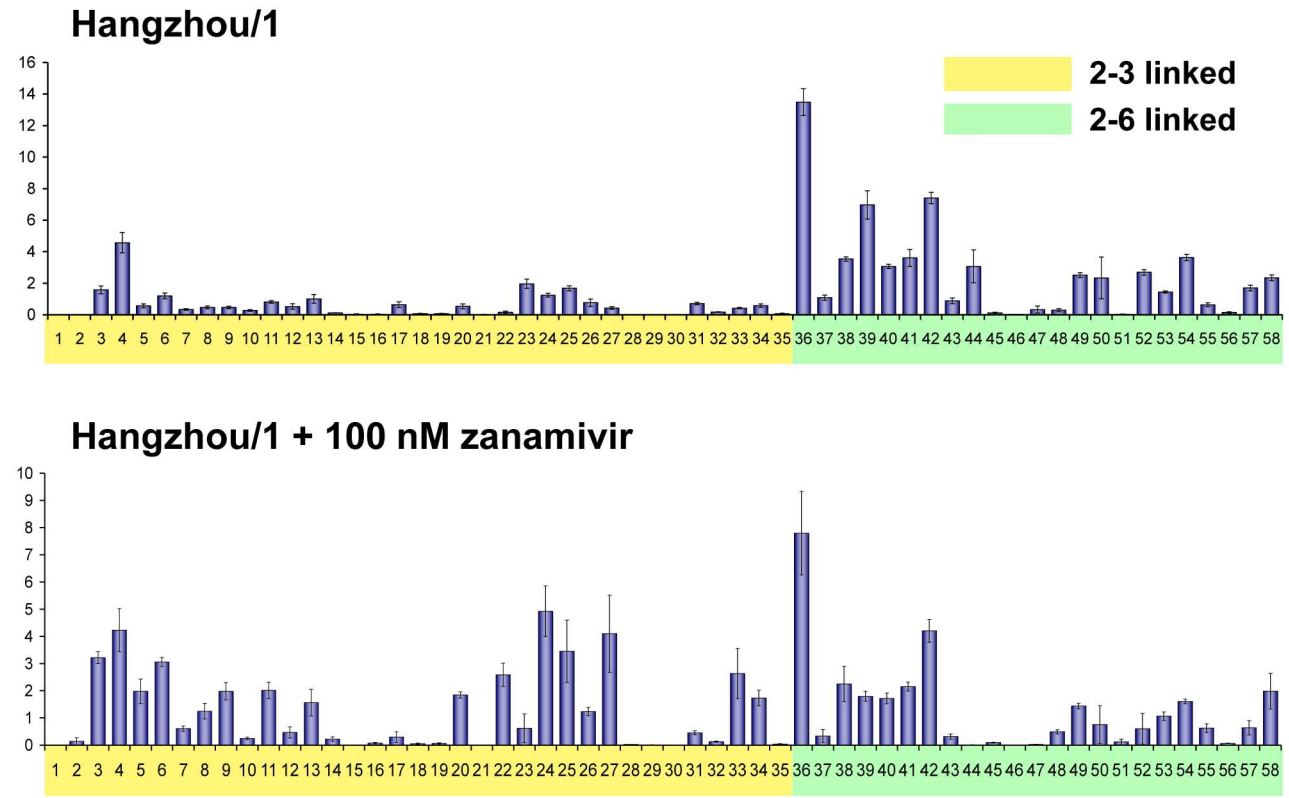
Supplementary Figure S9. Pro-inflammatory cytokine/chemokine responses in the lungs of infected cynomolgus macaques. The concentrations of various cytokines/chemokines in the serum of infected cynomolgus macaques at 0, 1, 3, 5, and 6 dpi (**a**) and their lungs (left and right lobes independently) at 3 (**b**) and 6 (**c**) dpi were measured by means of protein array analysis with the MILLIPLEX MAP Non-human Primate Cytokine/Chemokine Panel–Premixed 23-Plex (Merck Millipore).

a-2

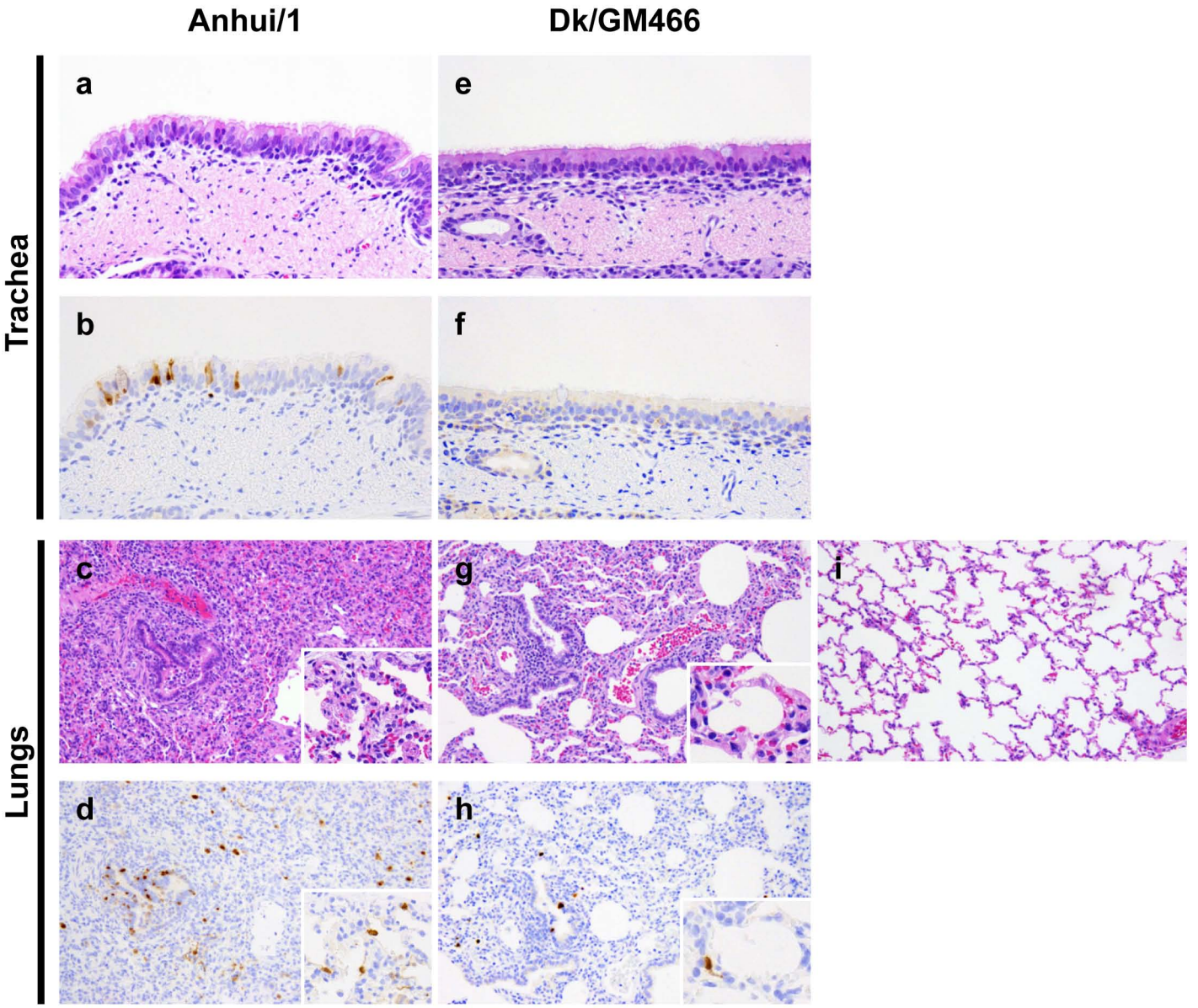

IL-1β
IL-5
IL-6
IL-8
MIP-1α
MCP-1
MIP-1β
IL-12/23(P40)
VEGF
IL-18
Day 0
Day 1
Day 3
Day 5
Day 6
Cytokine/Chemokine concentration (pg/ml)
Macaque #1
Macaque #2
Macaque #3
Macaque #4
Macaque #5
Macaque #6
Infected with Anhui/1
Macaque #7
Macaque #8
Macaque #9
Macaque #10
Infected with Dk/GM466


b
GM-CSF
TGF-α
G-CSF
IFN-γ
IL-10
IL-15
sCD40L
IL-17A
IL-13
IL-1β
IL-4
IL-5
IL-8
MIP-1α
MCP-1
TNF-α
IL-12/23(P40)
VEGF
IL-18
IL-2
IL-1Ra
IL-6
MIP-1β
Left lung
Right lung
Cytokine/Chemokine concentration (pg/ml)
Macaque #1
Macaque #2
Macaque #3
Macaque #7
Macaque #8
Infected with Anhui/1
Infected with Dk/GM466


c
Cytokine/Chemokine concentration (pg/ml)
GM-CSF
TGF-α
G-CSF
IFN-γ
IL-10
IL-15
sCD40L
IL-17A
IL-13
IL-1β
IL-4
IL-5
IL-8
MIP-1α
MCP-1
TNF-α
IL-12/23(P40)
VEGF
IL-18
IL-2
IL-1Ra
IL-6
MIP-1β
Left lung
Right lung
Macaque #4
Macaque #5
Macaque #6
Macaque #9
Macaque #10
Infected with Anhui/1
Infected with Dk/GM466



Supplementary Figure S10. Effect of neuraminidase activity on glycan array specificity of recombinant Hangzhou/1 virus. Glycan array analysis of a recombinant Hangzhou/1 virus (possessing the Hangzhou/1 HA and Anhui/1 NA genes with the remaining genes from PR8) was performed at 256 HAU/ml as described in Figure 3 in the absence (top) or presence (bottom) of 100 nM zanamivir during the adsorption step. Glycans on the array are shown in Table S9, except that glycan 1 is missing (value of 0).



Supplementary Figure S11. Pathological findings in infected pigs. Shown are representative pathological findings in the trachea (**a, b, e, f**) and lungs (**c, d, g, h**) of miniature pigs infected with Anhui/1 (**a–d**) or Dk/GM466 (**e–h**) at 3 dpi with HE staining (**a, c, e, g**) or immunohistochemistry for influenza viral antigen (**b, f, d, h**).

Supplementary Tables

Supplementary Table S1. Virus titres in organs of infected mice[a].

Inoculum titre	Virus		Virus titres (mean log_{10} PFU ± SD/g) in:	
			Nasal turbinates	Lungs
10^4 PFU	Anhui/1	Day 3	6.23±0.55	7.92±0.02*
		Day 6	5.67±0.46	6.26±0.34
	Dk/GM466	Day 3	5.82±1.36	5.90±0.40
		Day 6	4.58±0.21	5.97±0.09
	CA04	Day 3	6.42±0.14	7.55±0.40*
		Day 6	5.05±0.25	6.32±0.16
10^6 PFU	Anhui/1	Day 3	6.70±0.09	7.20±0.18*
		Day 6	6.10±0.46	6.87±0.13*
	Dk/GM466	Day 3	6.35±0.31	6.25±0.23
		Day 6	5.07±0.77	5.31±0.09
	CA04	Day 3	7.40±0.27*	7.36±0.14*
		Day 6	6.70±0.29	6.20±0.23*
	Shanghai/1	Day 3	6.64±0.27	7.27±0.24*
		Day 6	6.50±0.59	6.18±0.40

[a]BALB/c mice were intranasally infected with 10^4 or 10^6 PFU (50 µl each) of virus. Three animals from each group were euthanized at 3 and 6 days post-infection for virus titration. The statistical significance of differences in virus titres of Dk/GM466-infected mice and the others were assessed by use of the Welch's *t*-test with Bonferroni's correction (asterisk, $p<0.05$).

Supplementary Table S2. Virus titres in organs of infected ferrets[a].

	Virus titres (mean log_{10} PFU ± SD/g) of animals infected with:							
	Anhui/1		Shanghai/1		Dk/GM466		CA04	
	Day 3	Day 6	Day 3	Day 6	Day 3	Day 6	Day 3	Day 6
Nasal turbinate	7.1±0.3	5.4±1.6	6.9±0.2	5.0±0.6	7.2±0.2	6.2±0.4	7.9±0.4	-[b]
Trachea	6.9±0.7	4.4±0.6	6.9±0.5	4.9±0.6	5.5±0.6	5.0	6.7±0.1	-
Lung	3.0, 3.5	-	2.2	5.5	3.1, 2.3	-	-	3.4
Spleen	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-
Brain	3	-	2.1	-	-	-	-	-
Intestines	-	2.3	-	-	-	-	-	-
Liver	-	2.5	-	-	-	-	2.0	-
Feces	-	5.3, 2.7	-	3.5	3.1	-	3.6	-

[a]Ferrets were intranasally infected with 10^6 PFU (0.5 ml) of virus. Three ferrets from each group were euthanized at 3 and 6 dpi for virus titration. Replication of virus in spleen and kidneys was also examined; no virus was detected in these organs. Individual titers were recorded when virus was not recovered from all three ferrets.

[b]–, virus not detected (detection limit: 1.3 log_{10} PFU/g).

Supplementary Table S3. Virus titres in respiratory swabs from infected cynomolgus macaques[a].

	Virus titres (log_{10} PFU/g) of animals infected with:										
		Anhui/1						Dk/GM466			
animal ID		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Nasal swab	Day 1	6.48	4.71	4.00	4.36	6.08	5.26	4.57	1.95	4.18	3.51
	Day 3	3.58	5.34	2.26	3.15	2.78	2.58	2.40	3.59	2.85	1.95
	Day 5				4.70	3.20	4.15			5.18	3.73
	Day 6				-	3.5	-			5.26	4.53
Tracheal swab	Day 1	3.60	3.26	4.28	4.45	5.56	5.48	4.36	2.90	2.76	3.30
	Day 3	2.04	2.61	-[b]	1.48	1.85	2.00	-	2.00	1.48	-
	Day 5				3.36	1.48	3.11			2.57	3.73
	Day 6				3.04	5.00	6.51			-	3.30

[a]Cynomolgus macaques were inoculated with 6.7 x 10^7 PFU of virus (6.7 ml) through multiple routes. Nasal and tracheal swabs were collected every other day for virus titration.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

Diagonal lines: not applicable, animals were euthanized at 3 days post-infection.

Supplementary Table S4. Virus titres in organs of infected cynomolgus macaques[a].

	Virus titres (log_{10} PFU/g) of animals infected with:									
	Anhui/1						Dk/GM466			
	Day 3			Day 6			Day 3		Day 6	
Animal ID	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Nasal turbinates	5.5	6.8	6.9	3.1	4.8	5.9	4.6	6.2	6.7	5.6
Tonsils	2.1	4.1	2.2	4.3	5.8	5.4	3.1	3.5	6.5	5.6
Trachea	4.9	4.2	3.7	4.7	-[b]	5.6	3.5	4.5	6.6	4.9
Bronchus (right)	4.6	3.3	3.8	-	2.9	7.5	3.8	4.3	4.1	6.6
Bronchus (left)	-	4.4	4.3	3.3	-	7.4	5.4	3.6	2.9	3.5
Lung (upper right)	-	4.4	3.9	2.7	3.7	4.2	4.3	3.5	4.9	-
Lung (middle right)	3.3	4.5	5.2	3	3.4	5.1	-	3.9	4.1	-
Lung (lower right)	-	5.5	5.4	2.1	2.7	5.1	5.1	5.2	6.4	-
Lung (upper left)	3.3	2.5	4.8	-	3.8	4.8	-	4.0	5.7	-
Lung (middle left)	-	4	3.4	-	3.8	3.9	4.1	3.4	2.5	-
Lung (lower left)	3.9	5.4	5.6	-	3.3	4.8	3.9	4.8	3.7	-
Brain (frontal)	-	2.4	-	-	-	2.5	-	-	-	1.9
Brain (parietal)	-	2.4	-	-	-	-	-	-	-	-
Brain (temporal)	-	2.6	-	-	-	-	-	-	-	-
Brain (occipital)	-	-	-	-	-	2.4	-	-	-	1.9
Brain (cerebellum)	-	-	-	-	-	2.0	-	-	-	1.9
Brain (brain stem)	-	2.3	-	-	-	2.6	-	-	-	-
Olfactory bulb	-	2.9	-	-	-	3.6	-	-	-	2.5
Mediastinal LNs	2.5	-	3.3	-	-	-	2.6	-	3.3	2.4
Heart	2.4	-	-	-		3.6	-	-	-	2.0
Kidney	-	-	-	-	-	4.3	-	-	-	-
Duodenum	-	-	-	-	-	2.6	4.3	-	-	-
Rectum	-	-	-	-	-	3.4	-	-	-	-

[a]Cynomolgus macaques were inoculated with 6.7 x 10^7 PFU of virus (6.7 ml) through multiple routes. Three and two macaques per group were euthanized on days 3 and 6 post-infection for virus titration. No virus was recovered from the spleen or liver of any animals.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/g). LN, lymph node.

Supplementary Table S5. Ferret HI titres.

Virus			Animal ID	Virus titres (log_{10} PFU/ml) in nasal washes						Seroconversion (HI titre)[c]
				Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	
Anhui/1	Pair 1	*i*	#1	7.09	5.82	5.10	-[b]	-	-	320
		c	#2	-	-	-	-	-	-	<10
	Pair 2	*i*	#3	7.05	5.62	5.72	3.00	-	-	320
		c	#4	-	-	-	-	-	-	<10
	Pair 3	*i*	#5	7.51	5.62	4.83	-	-	-	320
		c	#6	-	3.89	6.11	4.78	-	-	640
Dk/GM 466	Pair 4	*i*	#7	4.04	5.26	5.18	-	-	-	80
		c	#8	-	-	-	-	-	-	<10
	Pair 5	*i*	#9	5.64	5.26	4.72	-	-	-	160
		c	#10	-	-	-	-	-	-	<10
	Pair 6	*i*	#11	4.68	5.15	5.09	2.91	-	-	320
		c	#12	-	-	-	-	-	-	<10
CA04	Pair 7	*i*	#13	6.78	4.64	4.58	-	-	-	1280
		c	#14	-	5.85	4.51	-	-	-	640
	Pair 8	*i*	#15	6.79	5.19	5.38	-	-	-	1280
		c	#16	-	4.91	4.94	-	-	-	1280
	Pair 9	*i*	#17	7.36	5.05	5.41	-	-	-	160
		c	#18	-	7.13	3.96	3.60	-	-	640

[a]For each pair of ferrets, one animal was intranasally inoculated with 5 x 10^6 PFU of virus (0.5 ml) (inoculated ferret, *i*) and one day later, a naïve ferret was placed in an adjacent cage (contact ferret, *c*). Nasal washes were collected from inoculated and contact ferrets every other day for virus titration.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

[c]Haemagglutination Inhibition (HI) assays were carried out with homologous virus and turkey red blood cells.

Supplementary Table S6. Differences in amino acid sequence between the reference virus and virus recovered from contact ferret.

Segment	Amino acid sequence and position (nucleotide sequence and position)					
	Reference[a]	Stock virus[b]	Virus isolated from infected ferret[c]	Virus isolated from contact ferret[d]		
				Day 3	Day 5	Day 7
PB2	72E (243G)	72E (243G)	72E (243G)	72E (243A)	72E (243A)	72E (243A)
	426Q (1305G)	426Q (1305G)	426Q (1305G)	426Q (1305A)	426Q (1305A)	426Q (1305A)
	659N (2004C)	659N (2004C)	659N (2004C)	659N (2004T)	659N (2004T)	659N (2004T)
PB1	629N (1901C)	629N (1901C)	629N (1901C)	629N (1901T)	629N (1901T)	629N (1901T)
PA	110Y (354T)	110Y (354T)	110Y (354T)	110Y (354C)	110Y (354C)	110Y (354C)
HA[e]	71T (257C)	71T (257C)	71T (257C)	71T/I (257C/T[f])	71I (257T)	71I (257T)
	131R (437G)	131R (437G)	131R (437G)	131R/K (437G/A[f])	131K (437A)	131K (437A)
	132N (442A)	132N/D (442A/G)	132N/D (442A/G)	132N (442A)	132N (442A)	132N (442A)
	135A (448G)	135A/T (448G/A[f])	135A/T (448G/A[f])	135A/T (448G/A[f])	135T (448A)	135T (448A)
	159-160N[g] (520A)	159-160N/D (520A/G[f])	159-160N/D (520A/G [f])	159-160N (520A)	159-160N (520A)	159-160N (520A)
NA	10T (47C)	10T/I (47C/T)	10T/I (47C/T)	10T (47C)	10T (47C)	10T (47C)
	27A (97G)	27A (97G)	27A (97G)	27A/T (97G/A[f])	27T (97A)	27T (97A)
	228Q (687G)	228Q (687G)	228Q (687G)	228Q (687G/A[f])	228Q (687A)	228Q (687A)
	412D (1239C)	412D (1239C)	412D (1239C)	412D (1239C/T[f])	412D (1239T)	412D (1239T)

[a]The Anhui/1 reference sequence was obtained from the GISAID database (GISAID accession number EPI439507).

[b]The stock of egg-grown Anhui/1 virus was sequenced.

[c]We sequenced virus isolated from the infected ferret from which virus transmitted to the contact ferret.

[d]Viruses isolated from contact ferrets in the transmission experiment were sequenced.

[e]Amino acid positions of HA are based on H3 HA numbering.

[f]Indicates a mixed population.

[g]H7 HA possesses an amino acid insertion relative to H3 HA, which was used as a reference for numbering.

Supplementary Table S7. Amino acid sequences of HA clones of the egg-grown virus stock of Anhui/1 and viruses recovered from the contact ferrets[a].

		Amino acid position (H3 numbering)					Number of HA clones	
		71	131	132	135	159/160[b]		
Reference[c]		T	R	N	A	N		
Stock virus[d]								
	Combination 1	T	R	D	A	D	18/39	
	Combination 2	T	R	N	A	D	6/39	
	Combination 3	T	R	N	T	N	8/39	
	Combination 4	T	R	N	A	N	5/39	
	Combination 5	I	K	N	T	N	2/39	
Virus recovered from the contact ferret[e]							3 days post-contact	5 days post-contact
	Combination 1	I	K	N	T	N	19/44	17/17
	Combination 2	T	R	N	A	N	17/44	0/17
	Combination 3	I	R	N	A	N	4/44	0/17
	Combination 4	T	K	N	T	N	3/44	0/17
	Combination 5	I	K	N	A	N	1/44	0/17

[a]Amino acid positions are based on H3 HA numbering.
[b] H7 HA possesses an amino acid insertion relative to H3 HA, which was used as a reference for numbering.
[c]The Anhui/1 reference sequence was obtained from the GISAID database (GISAID accession number EPI439507).
[d]The stock of egg-grown Anhui/1 virus was sequenced.
[e]Sequences of viruses recovered from the contact ferret in the transmission experiment.

Supplementary Table S8. Comparison of the amino acid sequences of Anhui/1, Shanghai/1, and Hangzhou/1 HAs.

Amino acid position[a]	Anhui/1	Shanghai/1	Hangzhou/1
138	A	S	A
174	S	N	S
186	V	G	V
221	P	T	P
226	L	Q	I
276	D	N	N
283	H	Y	H
400	N	T	N
531	V	A	V

[a]Positions at which the amino acid sequences of Anhui/1, Shanghai/1, and Hangzhou/1 HA differ.

Supplementary Table S9. List of glycans used for arrays.

Glycan #	Name
1	Galβ(1-4)GlcNAcβ-ethyl-NH2
2	Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
3	NeuAcα(2-3)Galβ(1-4)6-O-sulfo-GlcNAcβ-propyl-NH_2
4	NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]6-O-sulfo-GlcNAcβ-propyl-NH2
5	NeuAcα(2-3)6-O-sulfo-Galβ(1-4)GlcNAcβ-ethyl-NH2
6	NeuAcα(2-3)6-O-sulfo-Galβ(1-4)[Fucα(1-3)]GlcNAcβ-propyl-NH2
7	NeuAcα(2-3)Galβ(1-3)6-O-sulfo-GlcNAcβ-propyl-NH2
8	NeuAcα(2-3)Galβ(1-4)Glcβ-ethyl-NH_2
9	NeuAcα(2-3)Galβ(1-4)GlcNAcβ-ethyl-NH_2
10	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ-ethyl-NH_2
11	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ-ethyl-NH2
12	NeuAcα(2-3)GalNAcβ(1-4)GlcNAcβ-ethyl-NH_2
13	NeuAcα(2-3)Galβ(1-3)GlcNAcβ-ethyl-NH_2
14	NeuAcα(2-3)Galβ(1-3)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ-ethyl-NH_2
15	NeuAcα(2-3)Galβ(1-3)GlcNAcβ(1-3)Galβ(1-3)GlcNAcβ-ethyl-NH_2
16	NeuAcα(2-3)Galβ(1-3)GalNAcβ(1-3)Galα(1-4)Galβ(1-4)Glcβ-ethyl-NH_2
17	NeuAcα(2-3)Galβ(1-3)GalNAcα-Thr-NH_2
18	NeuAcα(2-3)Galβ(1-3)[GlcNAcβ(1-6)]GalNAcα-Thr-NH_2
19	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-6)[Galβ(1-3)]GalNAcα-Thr-NH_2
20	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-6)[Galβ(1-3)]GalNAcα-Thr-NH_2
21	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)GalNAcα-Thr-NH_2
22	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)GalNAcα-Thr-NH_2
23	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)[NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-6)]GalNAcα-Thr-NH_2
24	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)[NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-6)]GalNAcα-Thr-NH_2
25	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
26	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
27	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
28	NeuAcα(2-3)[GalNAcβ(1-4)]Galβ(1-4)GlcNAcβ-ethyl-NH_2
29	NeuAcα(2-3)[GalNAcβ(1-4)]Galβ(1-4)Glcβ-ethyl-NH_2
30	Galβ(1-3)GalNAcβ(1-4)[NeuAcα(2-3)]Galβ(1-4)Glcβ-ethyl-NH_2
31	NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ-propyl-NH_2
32	NeuAcα(2-3)Galβ(1-3)[Fucα(1-4)]GlcNAcβ(1-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ-ethyl-NH_2
33	NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ(1-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ-ethyl-NH_2
34	NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ(1-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ(1-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ-ethyl-NH_2
35	NeuGcα(2-3)Galβ(1-4)GlcNAcβ-ethyl-NH_2
36	NeuAcα(2-6)Galβ(1-4)6-O-sulfo-GlcNAcβ-propyl-NH_2
37	NeuAcα(2-6)Galβ(1-4)Glcβ-ethyl-NH_2
38	NeuAcα(2-6)Galβ(1-4)GlcNAcβ-ethyl-NH_2
39	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ-ethyl-NH_2
40	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ-ethyl-NH_2
41	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)[NeuAcα(2-6)]Galβ(1-4)GlcNAcβ-ethyl-NH2
42	NeuAcα(2-6)GalNAcβ(1-4)GlcNAcβ-ethyl-NH_2

43	NeuAcα(2-6)[Galβ(1-3)]GalNAcα-Thr-NH_2
44	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-6)[Galβ(1-3)]GalNAcα-Thr-NH_2
45	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-6)[Galβ(1-3)]GalNAcα-Thr-NH_2
46	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)GalNAcα-Thr-NH_2
47	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)GalNAcα-Thr-NH_2
48	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-6)]GalNAcα-Thr-NH_2
49	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-6)]GalNAcα-Thr-NH_2
50	Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
51	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
52	GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
53	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
54	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
55	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
56	NeuGcα(2-6)Galβ(1-4)GlcNAcβ-ethyl-NH_2
57	NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2
58	NeuAcα(2-6)Galβ(1-4)GlcNAcβ(1-2)Manα(1-3)[NeuAcα(2-3)Galβ(1-4)GlcNAcβ(1-2)Manα(1-6)]Manβ(1-4)GlcNAcβ(1-4)GlcNAcβ-Asn-NH_2

Supplementary Table S10. Virus titres in organs of infected miniature pigs[a].

	Virus titres (log_{10} PFU/g) of animals infected with:					
	Anhui/1				Dk/GM466	
	Day 3		Day 6		Day 3	
Animal ID	#1	#2	#3	#4	#5	#6
Nasal turbinates	-[b]	5.2	3.6	1.9	-	-
Oro/nasopharynx	-	-	-	-	-	-
Tonsils	-	-	-	-	-	-
Trachea	2.5	-	4.1	-	-	--
Bronchus (right)	1.8	-	-	-	-	-
Bronchus (left)	-	-	3.5	-	2.7	-
Lung (upper right)	-	-	5.0	-	6.0	4.5
Lung (middle right)	-	-	-	-	-	-
Lung (lower right)	-	-	-	-	-	-
Lung (upper left)	-	-	-	-	-	-
Lung (middle left)	-	-	-	-	-	-
Lung (lower left)	-	-	-	-	-	-

[a]Specific-pathogen-free miniature pigs were intranasally infected with 10^6 PFU (1 ml) of virus. For virus titration, two animals from each group were euthanized at 3 dpi; the remaining two Anhui/1-inoculated pigs were euthanized at 6 dpi. No virus was recovered from mediastinal lymph nodes, heart, spleen, kidneys, liver, duodenum, ileum, jejunum, rectum, mesenteric lymph nodes, bladder, cerebrum, brain stem, feces, or urine.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/g).

Supplementary Table S11. Virus titres in respiratory swabs from infected miniature pigs[a].

	Virus titres (log_{10}PFU/g) in swab samples collected from animals infected with:					
	Anhui/1				Dk/GM466	
Animal ID	#1	#2	#3	#4	#5	#6
Day 1	-	-	3.08	-	-	-
Day 2	1.00	2.18	3.70	1.78	-	-
Day 3	1.30	1.95	3.11	-	-	-
Day 4			4.18	-		
Day 5			4.08	2.60		
Day 6			3.00	2.49		

[a]Specific-pathogen-free miniature pigs were intranasally infected with 10^6 PFU (1 ml) of virus. Nasal swabs were collected every day for 6 days for virus titration.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

Diagonal lines: not applicable, animals were euthanized at 3 days post-infection.

Supplementary Table S12. Virus titres in organs of infected quails[a].

	Virus organ titres (log_{10}PFU/g) of animals infected with:											
	Anhui/1						Dk/GM466					
	Day 3			Day 6			Day 3			Day 6		
Animal ID	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Trachea	1.4	8.1	7.2	-[b]	-	8.1	7.2	6.9	7.4	-	-	-
Lung	-	2.6	2.6	-	-	6.3	-	-	4.7	-	-	-
Ileum	-	-	-	-	-	4.0	-	-	-	-	-	-
Cecum	-	-	-	3	-	-	-	-	-	-	-	-
Colon	-	-	-	-	-	-	-	-	-	-	-	-
Brain	-	2.7	-	-	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	2.9	-	-	5.4	-	-	-
Liver	-	-	-	-	-	3.3	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-	-	-

[a]Quails were intranasally infected with 2 x 10^6 PFU (0.2 ml) of virus.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

Supplementary Table S13. Virus titres in swabs from infected quails[a].

		Virus titres (log_{10}PFU/g) in swab samples collected from animals infected with:											
		Anhui/1						Dk/GM466					
Animal ID		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Tracheal swab	Day 1	5.2	4.3	3.3	4.2	5.1	4.4	3.5	3.2	4.3	3.6	5.2	4.7
	Day 2	2.6	4.5	4.2	4.5	5.4	3.9	4.1	4.3	4.4	2.0	3.4	4.0
	Day 3	4.6	5.5	4.7	4.2	3.5	4.7	4.0	4.7	4.6	2.0	5.7	4.6
	Day 4				3.9	3.5	4.9				2.9	4.7	5.1
	Day 5				-[b]	-	4.2				1.3	1.5	3.1
	Day 6				-	-	6.0				-	-	-
Cloacal swab	Day 1	-	-	-	-	-	-	-	-	-	-	-	-
	Day 2	-	-	-	-	-	-	-	-	-	-	-	-
	Day 3	-	-	-	-	-	-	-	-	-	-	-	-
	Day 4				-	-	5.2				-	-	-
	Day 5				-	-	5.0				-	-	-
	Day 6				-	3.4	-				-	-	-

[a]Quails were intranasally infected with 2 x 10^6 PFU (0.2 ml) of virus. Tracheal and cloacal swabs were collected every day for 6 days for virus titration.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

Diagonal lines: not applicable, animals were euthanized at 3 days post-infection.

Supplementary Table S14. Virus titres in organs of infected chickens[a].

	Virus organ titres (log_{10}PFU/g) of animals infected with:											
	Anhui/1						Dk/GM466					
	Day 3			Day 6			Day 3			Day 6		
Animal ID	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Trachea	0.7[b]	3.2	5.5	-[c]	-	-	3.9	-	-	-	-	-
Lung	-[b]	-	3.5	-	-	-	-	-	2.0	-	-	-
Ileum	-	-	-	-	-	-	-	-	4.4	-	-	-
Cecum	-	-	-	-	-	-	-	2.2	-	-	-	1.9
Colon	-	-	-	-	-	-	-	-	4.6	-	-	-
Brain	-	-	-	-	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	5.4	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	2.0	-	-	-

[a]Chickens were intranasally infected with 2 x 10^6 PFU (0.2 ml) of virus.

[b]The detection limit for tracheal and lung samples collected from animal #1 is 0.4 log_{10} PFU/ml.

[c]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

Supplementary Table S15. Virus titres in swabs from infected chickens[a].

		Virus titres (log_{10}PFU/g) in swab samples collected from animals infected with:											
		Anhui/1						Dk/GM466					
Animal ID		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Tracheal swab	Day 1	-	1.0	2.0	2.2	1.3	1.0	2.5	2.0	2.4	1.3	1.3	3.8
	Day 2	2.5	2.5	4.6	3.3	-	3.3	2.4	-	1.3	-	-	1.0
	Day 3	5.3	-	5.4	-	-	2.6	1.0	-	-	-	1.0	-
	Day 4				-	-	2.6				1.3	-	1.0
	Day 5				-	-	-				-	1.5	-
	Day 6				-	-	-				-	-	-
Cloacal swab	Day 1	-	-	-	-	-	-	-	-	-	-	-	-
	Day 2	-	-	-	-	-	-	-	-	-	-	-	-
	Day 3	-	-	-	-	-	-	-	-	1.9	-	-	-
	Day 4				-	-	-				-	-	-
	Day 5				-	-	-				-	-	3.4
	Day 6				-	-	-				-	-	2.7

[a]Chickens were intranasally infected with 2 x 10^6 PFU (0.2 ml) of virus. Tracheal and cloacal swabs were collected every day for virus titration.

[b]-, virus not detected (detection limit: 1.3 log_{10} PFU/ml).

Diagonal lines: not applicable, animals were euthanized at 3 days post-infection.

Supplementary Table S16. *In vitro* virus sensitivity to NA inhibitors.

	IC_{50} value[a]			
	Anhui/1	Shanghai/1	Shanghai/1-NA-292K[b]	Shanghai/1-NA-292R[b]
Oseltamivir carboxylate[c]	0.49	0.47	5403.50	0.16
Zanamivir	0.65	1.32	15.50	0.32
Laninamivir[d]	0.83	0.93	12.93	0.53
Peramivir	0.09	0.22	245.80	0.06

[a]IC_{50} value: mean nM of duplicate reactions.

[b]Plaque-purified Shanghai/1 with either NA292K or NA292R.

[c]Oseltamivir carboxylate is the active form of oseltamivir.

[d]Laninamivir is the active form of laninamivir octanoate.

Supplementary Table S17. Effect of different ratios of NA-292R and NA292K variants on *in vitro* sensitivity to NA inhibitors.

Oseltamivir-sensitive virus (%)[b]	IC_{50} value[a]			
	Oseltamivir carboxylate[c]	Zanamivir	Laninamivir[d]	Peramivir
0	5403.50	15.50	12.93	245.80
10	3394.88	6.56	4.21	27.69
20	130.33	3.87	2.47	8.54
30	6.57	1.87	1.40	0.89
40	0.77	1.17	1.25	0.07
50	0.28	0.81	0.79	0.05
60	0.23	0.56	0.76	0.04
70	0.18	0.44	0.59	0.04
80	0.15	0.40	0.56	0.05
90	0.15	0.31	0.56	0.06
100	0.16	0.32	0.53	0.06

[a]IC_{50} value: mean nM of duplicate reactions.

[b]Shanghai/1-NA292R and Shanghai/1-NA292K were mixed at different ratios (based on PFU) and tested for sensitivity to antiviral compounds.

[c]Oseltamivir carboxylate is the active form of oseltamivir.

[d]Laninamivir is the active form of laninamivir octanoate.

Supplementary Table S18. Antigenic characterization of H7 viruses by use of monoclonal and polyclonal antibodies.

Virus	Mouse monoclonal antibody against:									Goat polyclonal antibody against HA from Netherlands/219/03 (H7N7)
	HA from A/Seal/Mass/1/88 (H7N7)			HA from A/Netherlands/219/03 (H7N7)		HA from A/FPV/Rostock/1934 (H7N1)				
	46/6	46/2	55/3	B1275m	B1273m	127-10023	10H9	9A9	1H11	NR-9226
A/Ruddy turnstone/DE/2372/88 (H7N7)	4000	2000	8000	-	-	-	-	-	-	2000
A/Turkey/Oregon/71 (H7N3)	4000	2000	4000	-	-	-	-	2000	-	4000
A/Turkey/PV/1/81 (H7N7)	-[b]	-	-	-	-	-	-	-	-	-
A/Ruddy Turnstone/DE/2770/87 (H7N5)	-	-	-	-	-	-	-	-	-	-
A/African starling/England/983/79 (H7N1)	4000	2000	8000	-	-	-	-	-	-	2000
A/Seal/Massachusetts/1/80 (H7N7)	4000	2000	8000	-	-	-	-	-	-	2000
A/Equine/Prague/1/56 (H7N7)	-	-	-	1000	-	-	-	-	-	2000
A/Duck/Hong Kong/301/78 (H7N2)	-	-	8000	-	-	-	-	-	-	1000
A/Mallard/Italy/229/05 (H7N7)	4000	2000	8000	-	-	-	-	1000	-	4000
A/Anhui/1/2013 (H7N9) Egg stock	2000	-	16000	-	2000	-	-	1000	-	4000
A/Anhui/1/2013 (H7N9) MDCK stock	4000	1000	>32000	-	16000	1000	1000	8000	-	4000
A/Shanghai/1/2013 HA/PR8	4000	-	8000	-	-	-	-	-	-	4000
A/Hangzhou/1/2013 HA/PR8	4000	1000	16000	-	2000	-		1000	-	4000

[a]HI Two-fold serial dilutions of antibodies were mixed with the amount of virus equivalent to 8 haemagglutination units in 96-well U-bottom microtitre plates, followed by incubation at room temperature for 30 min. After an equal volume of 0.5% chicken red blood cells was added, the mixtures were gently mixed, and then incubated at room temperature for a further 45 min. HI titres were determined as the inverse of the highest antibody dilution that inhibited the haemagglutination.

[b]-, virus not detected (detection limit: 1,000 HI).

Supplementary References

1. Liu, X. *et al.* Poor responses to oseltamivir treatment in a patient with influenza A (H7N9) virus infection. *Emerg Microbes & Infections* 2, e27 (2013).