Effectiveness of Whole, Inactivated, Low Pathogenicity Influenza A(H7N9) Vaccine against Antigenically Distinct, Highly Pathogenic H7N9 Virus

Masato Hatta, Gongxun Zhong, Shiho Chiba, Tiago J.S. Lopes, Gabriele Neumann, Yoshihiro Kawaoka

The recent emergence of highly pathogenic influenza A(H7N9) variants poses a great risk to humans. We show that ferrets vaccinated with low pathogenicity H7N9 virus vaccine do not develop severe symptoms after infection with an antigenically distinct, highly pathogenic H7N9 virus. These results demonstrate the protective benefits of this H7N9 vaccine.

Low pathogenicity influenza A(H7N9) viruses, which cause mild or asymptomatic disease in poultry, have caused ≥1,564 human infections since March 2013, with a case-fatality rate of ≈40% (*1*–*5*). Recently, highly pathogenic H7N9 viruses, characterized by multiple basic amino acids at the cleavage site of their hemagglutinin (HA) protein, have emerged. More than 750 cases of human H7N9 infections in 2017 (*6*) and the emergence of highly pathogenic H7N9 viruses emphasize the need for effective vaccines against low pathogenicity and highly pathogenic H7N9 viruses. We examined whether a World Health Organization (WHO) candidate vaccine based on a low pathogenicity H7N9 influenza virus would protect ferrets against an antigenically distinct, highly pathogenic H7N9 influenza virus.

The Study

We generated a recombinant virus (HK125–HYPR8) that possesses the HA and neuraminidase (NA) genes of a low pathogenicity WHO-recommended H7N9 candidate vaccine virus (A/Hong Kong/125/2017 [*7*]) and the remaining genes from a high-yield A/Puerto Rico/8/34 (PR8) vaccine backbone virus (*8*). The HK125–HYPR8 virus was inactivated with β-propiolactone and purified through sucrose gradient ultracentrifugation.

We vaccinated 5-month-old female ferrets (6 per group) that were serologically negative for currently circulating human influenza viruses with 15 μg of HA of inactivated whole HK125–HYPR8 virions without adjuvant (Group 1) or mixed at a 1:1 ratio with AddaVax adjuvant (InvivoGen, San Diego, CA, USA), a squalene-based oil-in-water nanoemulsion similar to MF59 (*9*) (group 2); control animals received phosphate-buffered saline (group 3) or adjuvant (group 4) (Figure 1, panel A). All animals were vaccinated intramuscularly in both hind legs twice, 28 days apart.

Twenty-eight days after the second immunization, we intranasally challenged ferrets with 10^6 PFUs of highly pathogenic H7N9 rGD/3-NA294R virus (a neuraminidase inhibitor–sensitive subpopulation of highly pathogenic A/Guangdong/17SF003/2016 H7N9 virus) (*10*). These vaccine and challenge viruses belong to the Yangtze River Delta lineage of H7N9 viruses, which is responsible for recent infections of humans with highly pathogenic H7N9 viruses (*6*). However, A/Hong Kong/125/2017 and the A/Guangdong/17SF003/2016 challenge virus differ antigenically (*11*) (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/24/10/18-0403-Techapp1.pdf).

We monitored clinical signs, body weight, and body temperature daily for 14 days and collected throat and nasal swab specimens every day until day 7 postchallenge. On day 4 postchallenge, we euthanized 3 ferrets from each group and collected organs (lung, trachea, nasal turbinates, olfactory bulbs, and brain tissues pooled from anterior and posterior brain sections) for virus titration. We conducted statistical analysis of hemagglutinin inhibition (HI) titers, virus titers in swab and organ samples, and bodyweight and temperature changes among groups (online Technical Appendix Tables 2–21). We defined statistical significance as $p<0.05$.

After 1 immunization, HI titers were significantly lower in the ferrets immunized with nonadjuvanted HK125–HYPR8 vaccine than in those immunized with AddaVax-adjuvanted HK125–HYPR8 vaccine ($p = 0.038$; Figure 1, panel B; online Technical Appendix Table 2); however, after 2 immunizations, ferrets vaccinated with or without adjuvant (groups 1 and 2) developed high HI titers against HK125–HYPR8 virus. Vaccination with HK125–PR8 vaccine did not elicit measurable HI titers against the rGD/3-NA294R challenge virus after the first immunization but elicited reasonably high titers after the second immunization (Figure 1, panel B). After challenge with highly pathogenic H7N9 virus,

Author affiliations: University of Wisconsin–Madison, Madison, Wisconsin, USA (M. Hatta, G. Zhong, S. Chiba, T.J.S. Lopes, G. Neumann, Y. Kawaoka); University of Tokyo, Tokyo, Japan (Y. Kawaoka)

DOI: https://doi.org/10.3201/eid2410.180403

nonvaccinated ferrets (groups 3 and 4) became lethargic, experienced diarrhea, and lost appetite and bodyweight on days 2–6 postinfection (online Technical Appendix Figure), whereas vaccinated ferrets showed no noticeable symptoms. In addition, nonvaccinated ferrets demonstrated statistically higher body temperature than vaccinated ferrets on days 1, 2, 3, 5, and 6 postchallenge (online Technical Appendix Figure, Table 5). One ferret in group 3 and 2 ferrets in group 4 had to be euthanized on days 6–8 postinfection (Figure 1, panel C) because of severe symptoms (neurologic signs or inability to remain upright). In contrast, none of the vaccinated ferrets had any symptoms, indicating a protective effect of the low pathogenicity H7N9 vaccine against the challenge virus.

Analysis of throat and nasal swab samples demonstrated replication of highly pathogenic challenge virus in all ferrets (Figure 2, panel A). However, virus titers started to decline in vaccinated ferrets by day 3 postchallenge, and the infection was resolved by day 5 postchallenge; in contrast, nonvaccinated ferrets continued to shed high titers of challenge virus 4–7 days postchallenge. The virus titers in nasal swab samples on days 1, 3, 4, 5, 6, and 7 postchallenge and those in throat swab samples on days 1–7 postchallenge from nonvaccinated ferrets were significantly higher than those in vaccinated ferrets (online Technical Appendix Table 10). Thus, vaccination with HK125–HYPR8 virus led to reduced replication of the challenge virus in the upper respiratory tract of infected ferrets.

On day 4 postinfection, we euthanized 3 animals per group and determined virus titers in organs. We also assessed virus titers in organs of ferrets that were euthanized because of severe disease symptoms. In nonvaccinated ferrets, we detected high titers of virus in respiratory organs; in addition, we recovered virus from the olfactory bulbs or pooled samples from anterior and posterior sections of

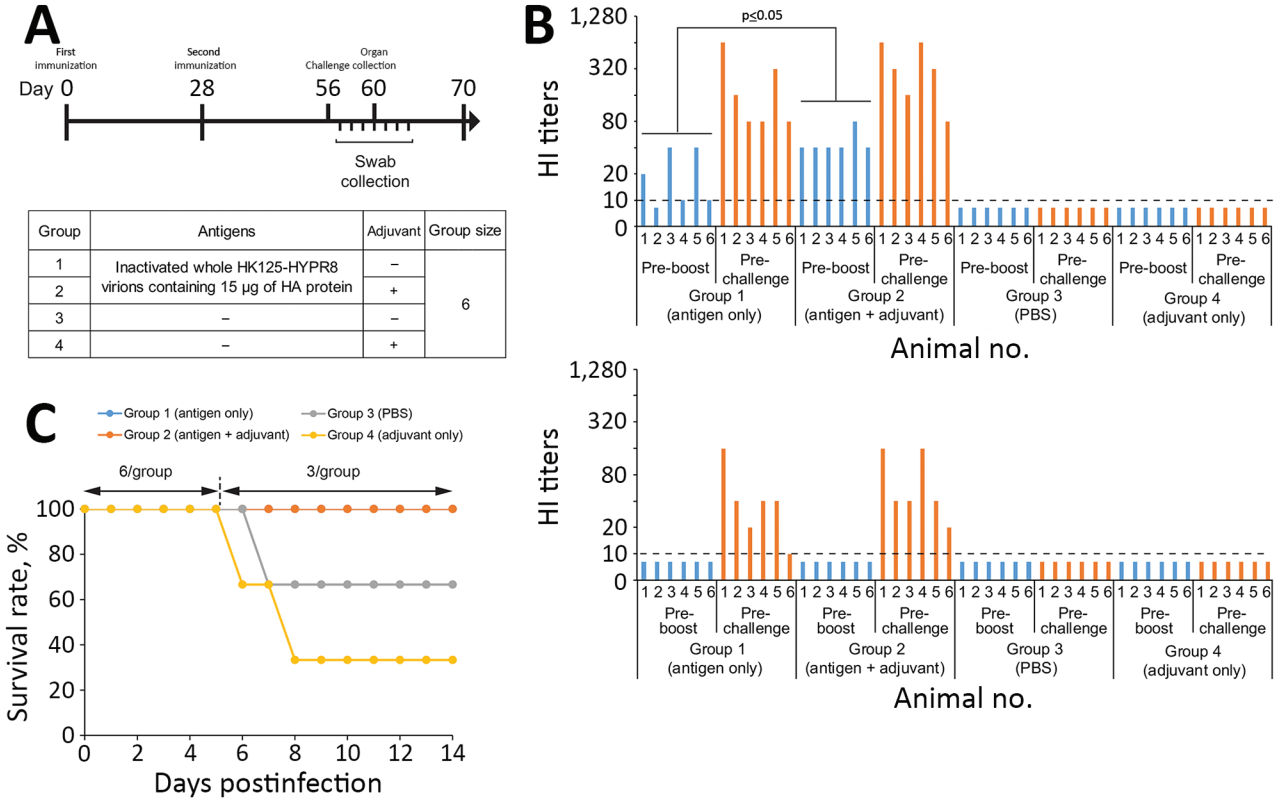


Figure 1. Study design, HI titers after vaccination, and survival rates of vaccinated and nonvaccinated ferrets challenged with highly pathogenic influenza A(H7N9) virus. A) Study design. Six ferrets per group were immunized with inactivated whole HK125–HYPR8 virions containing 15 μg of HA protein without (group 1) or with adjuvant (group 2); control animals were vaccinated with PBS (group 3) or adjuvant (group 4). Animals were vaccinated intramuscularly twice 28 days apart. Twenty-eight days after the second immunization, ferrets were challenged with highly pathogenic H7N9 rGD/3-NA294R virus. Throat and nasal swab specimens were collected on days 1–7 postchallenge; 3 animals per group were euthanized on day 4 postchallenge to assess virus titers in organs. B) HI titers after vaccination. HI assays were performed against HK125–HYPR8 (upper panel) and rGD/3-NA294R (lower panel) with ferret sera collected before the second immunization (preboost) and before challenge (prechallenge). Statistical significance was determined as described in the online Technical Appendix (https://wwwnc.cdc.gov/EID/article/24/10/18-0403-Techapp1.pdf). C) Survival rates. Survival was monitored for 14 days after challenge. Because 3 ferrets were euthanized on day 4 postchallenge for organ sampling, the survival rate was calculated on the basis of a group size of n = 3 thereafter. HA, hemagglutinin; HI, hemagglutination inhibition; PBS, phosphate-buffered saline.

the brains of 7 of the 9 animals tested (Figure 2, panel B). In vaccinated ferrets, we detected virus in the nasal turbinates of 4 of 6 animals and in the olfactory bulbs of 2 of 6 animals. We recovered no virus from the tracheas, lungs, or pooled samples from anterior and posterior brain sections (Figure 2, panel B), indicating that vaccination with HK125–HYPR8 prevented challenge virus replication in the lower respiratory organs.

Conclusions

We report the effectiveness of a whole, inactivated, low pathogenicity H7N9 vaccine against an antigenically distinct, highly pathogenic H7N9 virus in a ferret model. Vaccination prevented challenge virus replication in the lower respiratory organs, led to faster virus clearance in the upper respiratory organs, and prevented severe disease and death in ferrets, although the HI titers against the rGD/3-NA294R

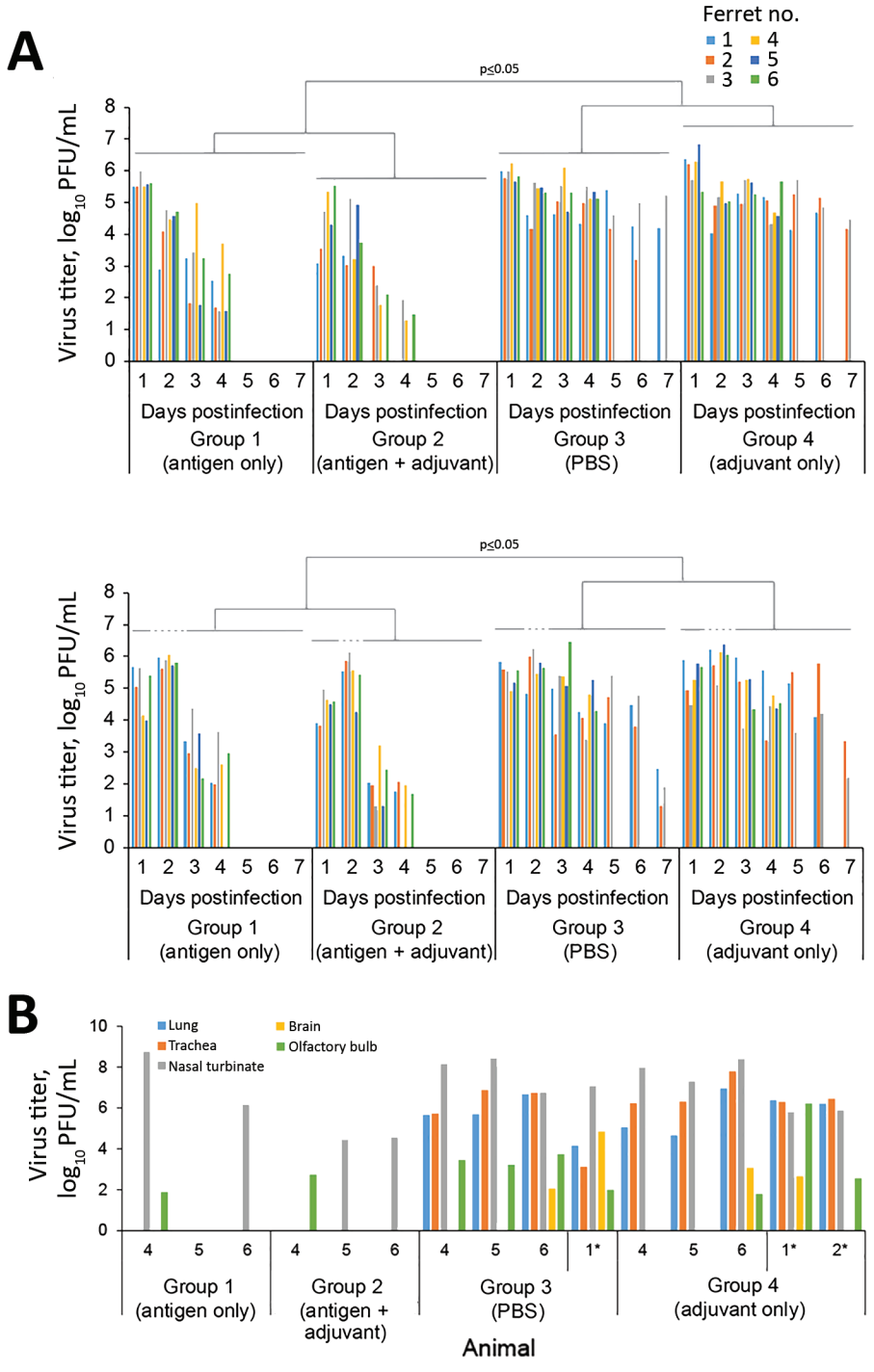


Figure 2. Virus titers in throat and nasal swab specimens and in the organs of vaccinated and nonvaccinated ferrets challenged with highly pathogenic influenza A(H7N9) virus. A) Virus titers in swab samples. Throat and nasal swabs were collected on days 1–7 postchallenge. Virus titers were determined based on plaque assays in MDCK cells. Statistical significance was determined as described in the online Technical Appendix (https://wwwnc.cdc.gov/EID/article/24/10/18-0403-Techapp1.pdf). B) Three ferrets from each group were euthanized on day 4 postchallenge for virus titration in the indicated organs. We also assessed virus titers in organs of ferrets that were euthanized because of severe symptoms (*). Virus titers were determined based on plaque assays in MDCK cells. Numbers along baseline indicate animal number. PBS, phosphate-buffered saline.

challenge virus were lower than those against the HK125–HYPR8 vaccine virus. Statistical analyses demonstrated that HI titers against the HK125–HYPR8 vaccine virus after the first immunization were significantly higher ($p = 0.038$) in animals immunized with adjuvanted vaccine compared with animals immunized with nonadjuvanted vaccine (Figure 1, panel B; online Technical Appendix Table 2). Bodyweight changes after challenge were significantly milder ($p = 0.0132–0.0489$ on days 4–10, 12, and 13) in ferrets immunized with adjuvanted vaccine than in those vaccinated with nonadjuvanted vaccine. In addition, virus titers in nasal swabs on days 3 and 4 postchallenge ($p = 0.0052$ on day 3; $p = 0.0163$ on day 4) and in throat swabs on days 1, 3, and 4 ($p = 0.0047$ on day 1; $p = 0.0003$ on days 3 and 4) in ferrets immunized with nonadjuvanted vaccine were significantly higher than in those ferrets immunized with adjuvanted vaccine (online Technical Appendix Tables 9, 11), suggesting superior efficacy with Addavax.

Previously, WHO selected several low pathogenicity H7N9 candidate vaccine viruses, including A/Hong Kong/125/2017 (*7*). With the emergence of highly pathogenic H7N9 viruses that are antigenically distinct from previously circulating H7N9 viruses, WHO has updated its recommendations, and a candidate vaccine virus for highly pathogenic H7N9 viruses is now available (*12*). We tested whether in the event of a large-scale outbreak of highly pathogenic H7N9 viruses, candidate vaccine viruses to antigenically distinct H7N9 viruses might serve as a first line of defense. Our results in ferrets indicate the potential of a whole, inactivated vaccine based on a low pathogenicity H7N9 virus to prevent severe disease with fatal outcome after infection with an antigenically distinct, highly pathogenic H7N9 virus.

Acknowledgments

We thank Susan Watson for scientific editing and Alexander Karasin, Kelly E. Moore, Zachary Najacht, and Backiyalakshmi Ammayappan Venkatachalam for technical assistance. We thank personnel from the Research Animal Resources Center and the Charmany Instructional Facility, University of Wisconsin–Madison, for animal care and technical support.

This work was supported by the National Institute of Allergy and Infectious Diseases–funded Center for Research on Influenza Pathogenesis (grant no. HHSN272201400008C), the Japan Initiative for Global Research Network on Infectious Diseases of the Japan Agency for Medical Research and Development (AMED), AMED's Leading Advanced Projects for Medical Innovation, AMED's Research Program on Emerging and Re-emerging Infectious Diseases, and by Grants-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Science, Sports, and Technology of Japan (grant nos. 16H06429, 16K21723, and 16H06434).

About the Author

Dr. Hatta is a senior scientist at the Influenza Research Institute at the University of Wisconsin–Madison. His research focuses on identifying the molecular determinants of influenza virus pathogenicity, with particular emphasis on the pathogenicity of highly pathogenic influenza viruses.

References

1. Centers for Disease Control and Prevention (CDC). Asian lineage avian influenza A (H7N9) virus [cited 2018 Feb 28]. https://www.cdc.gov/flu/avianflu/h7n9-virus.htm
2. Shen Y, Lu H. Global concern regarding the fifth epidemic of human infection with avian influenza A (H7N9) virus in China. Biosci Trends. 2017;11:120–1. http://dx.doi.org/10.5582/bst.2017.01040
3 Wang X, Jiang H, Wu P, Uyeki TM, Feng L, Lai S, et al. Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland China, 2013–17: an epidemiological study of laboratory-confirmed case series. Lancet Infect Dis. 2017;17:822–32. http://dx.doi.org/10.1016/S1473-3099(17)30323-7
4. Zhou L, Ren R, Yang L, Bao C, Wu J, Wang D, et al. Sudden increase in human infection with avian influenza A(H7N9) virus in China, September–December 2016. Western Pac Surveill Response J. 2017;8:6–14. http://dx.doi.org/10.5365/wpsar.2017.8.1.001
5. Iuliano AD, Jang Y, Jones J, Davis CT, Wentworth DE, Uyeki TM, et al. Increase in human infections with avian influenza A(H7N9) virus during the fifth epidemic—China, October 2016–February 2017. MMWR Morb Mortal Wkly Rep. 2017;66:254–5. http://dx.doi.org/10.15585/mmwr.mm6609e2
6. Centre for Health Protection (Hong Kong). Avian influenza report, volume 13, number 42 [cited 2017 Dec 29]. http://www.chp.gov.hk/files/pdf/2017_avian_influenza_report_vol13_wk42.pdf
7. World Health Organization. Summary of status of development and availability of avian influenza A(H7N9) candidate vaccine viruses and potency testing reagents [cited 2017 Sep 28]. http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_a_h7n9_cvv_20170928.pdf?ua=1
8. Ping J, Lopes TJ, Nidom CA, Ghedin E, Macken CA, Fitch A, et al. Development of high-yield influenza A virus vaccine viruses. Nat Commun. 2015;6:8148. http://dx.doi.org/10.1038/ncomms9148
9. Ott G, Radhakrishnan R, Fang J-H, Hora M. The adjuvant MF59: a 10-year perspective. In: O'Hagan DT, editor. Vaccine adjuvants: preparation methods and research protocols. Totowa (NJ): Springer New York; 2000. p. 211–28.
10. Imai M, Watanabe T, Kiso M, Nakajima N, Yamayoshi S, Iwatsuki-Horimoto K, et al. A highly pathogenic avian H7N9 influenza virus isolated from a human is lethal in some ferrets infected via respiratory droplets. Cell Host Microbe. 2017;22:615–626.e8. http://dx.doi.org/10.1016/j.chom.2017.09.008
11. Zoonotic influenza viruses: antigenic and genetic characteristics and development of candidate vaccine viruses for pandemic preparedness [in French]. Wkly Epidemiol Rec. 2017;92:129–44.
12. World Health Organization. Summary of status of development and availability of avian influenza A(H7N9) candidate vaccine viruses and potency testing reagents [cited 2018 Mar 5]. http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_a_h7n9_cvv_20180305.pdf?ua=1

Address for correspondence: Yoshihiro Kawaoka, University of Wisconsin–Madison, Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, 575 Science Dr, Madison, WI 53711, USA; email: yoshihiro.kawaoka@wisc.edu

Article DOI: https://doi.org/10.3201/eid2410.180403

Whole, Inactivated, Low Pathogenicity Influenza A(H7N9) Vaccine against Antigenically Distinct, Highly Pathogenic H7N9

Technical Appendix

Supplementary Methods

Cells

Madin-Darby canine kidney (MDCK) cells (obtained from ATCC) were maintained in Eagle's minimal essential medium (MEM) containing 5% newborn calf serum and antibiotics. Human embryonic kidney 293T cells (obtained from ATCC) were propagated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) with antibiotics. All cells were maintained at 37°C with 5% CO_2 unless otherwise stated.

Virus and Reverse Genetics

The sequences of the haemagglutinin (HA) and neuraminidase (NA) genes of a low pathogenic WHO-recommended H7N9 candidate vaccine virus (A/Hong Kong/125/2017, H7N9) (*1*) were obtained from GenBank (accession numbers: CY235363 and CY235364, respectively). Based on the obtained sequences, the HA and NA genes were oligo-synthesized by SGI-DNA (La Jolla, CA) and cloned into a plasmid for viral RNA production (pPolI vector) (*2*). Plasmid-based reverse genetics for generating HK125-HYPR8 virus possessing the HA and NA genes of A/Hong Kong/125/2017 and the remaining genes from our high-yield A/Puerto Rico/8/34 (PR8) vaccine backbone virus was performed as previously described (*2*,*3*). At 48 h post-transfection, culture supernatants were collected and inoculated to MDCK cells for virus propagation. The virus stock was sequenced to confirm the absence of unwanted mutations.

Vaccine Preparation

The HK125-HYPR8 virus was propagated in 10-day-old embryonated chicken eggs. The viruses in the allantoic fluids were inactivated with 0.1% β-propiolactone (final concentration) at 4°C overnight and then purified through ultracentrifugation by using a linear 20%–50% (w/v) sucrose gradient. The HA amount of purified virus was calculated based on the intensities of the viral protein bands separated on a 4%–12% (wt/vol) NuPAGE Bis-Tris gel (Thermo Fisher Scientific) and the amount of total viral proteins was determined by using a Pierce BCA Protein assay kit (Thermo Fisher Scientific).

Animal Experiments

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Wisconsin-Madison, which also approved the protocol used (protocol numbers V00806). The facilities where this research was conducted are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Ferret Vaccine-Challenge Experiment

Five-month-old female ferrets (Triple F Farms), which were serologically negative by hemagglutination inhibition assay for currently circulating human influenza viruses, were used in this study. Six ferrets per group were vaccinated with 15 μg of HA of inactivated whole HK125-HYPR8 virions without adjuvant (Group 1) or mixed at a 1:1 ratio with AddaVax adjuvant (InvivoGen) (Group 2); control animals received PBS (Group 3) or adjuvant (Group 4) (Figure 1, panel A). All animals were vaccinated intramuscularly in both hind legs twice 28 days apart.

Twenty-eight days after the second immunization, ferrets were intranasally challenged with 10^6 PFUs (PFU) of highly pathogenic H7N9 rGD/3-NA294R virus (a neuraminidase inhibitor-sensitive subpopulation of highly pathogenic A/Guangdong/17SF003/2016 H7N9 virus) (*4*). Clinical signs, bodyweight, and body temperature were monitored daily for 14 days. Throat and nasal swabs were collected every day until day 7 post-challenge. On day 4 post-challenge, three ferrets from each group were euthanized and organs (lung, trachea, nasal turbinates, olfactory bulbs, and brain tissues pooled from anterior and posterior brain sections) were collected for virus titration.

Hemagglutination Inhibition (HI) Assay

To detect hemagglutination inhibition (HI) activity (https://www.cdc.gov/flu/professionals/laboratory/antigenic.htm), serum samples were treated with receptor-destroying enzyme (RDE; Denka Seiken Co., Ltd) at 37°C for 16–20 h, followed by RDE inactivation at 56°C for 30–60 min. The treated sera were serially diluted 2-fold with PBS in 96-well U-bottom microtiter plates (Thermo Scientific, Rochester, New York, USA) and mixed with the amount of virus equivalent to eight hemagglutination units, followed by incubation at room temperature (25°C) for 30 min. After 50 μL of 0.5% turkey red blood cells was added to the mixtures, they were gently mixed and incubated at room temperature for a further 45 min. HI titers are expressed as the inverse of the highest antibody dilution that inhibited hemagglutination.

Statistical Analysis

Body temperature, bodyweight, nasal, and throat swabs were analyzed using a linear mixed model, with the groups and time as fixed effects, and the animals as random effects.

The commands lmer, lsmeans, and cld were used for the analysis, and all groups were compared to each other (pairwise). The *p*-values were adjusted using Holm's method. For the comparison of the HI titers, we used two-tailed unpaired t-tests, and adjusted the *p*-values using Holm's method. The virus titers from the organs were analyzed using one-way ANOVA, followed by Tukey's post-hoc test.

Biosafety and Biosecurity

All recombinant DNA protocols were approved by the University of Wisconsin-Madison's Institutional Biosafety Committee after risk assessments were conducted by the Office of Biologic Safety. In addition, the University of Wisconsin-Madison Biosecurity Task Force regularly reviews the research program and ongoing activities of the laboratory. The task force has a diverse skill set and provides support in the areas of biosafety, facilities, compliance, security, and health. Members of the Biosecurity Task Force are in frequent contact with the principal investigator and laboratory personnel to provide oversight and assure biosecurity. All experiments with live highly pathogenic H7N9 virus were performed in biosafety level 3 agricultural (BSL-3Ag) laboratories at the University of Wisconsin-Madison approved for such

use by the Centers for Disease Control and Prevention (CDC) and Animal and Plant Health Inspection Service (APHIS). Staff working in BSL-3Ag wear disposable overalls and powered air-purifying respirators.

The BSL-3Ag facility at University of Wisconsin-Madison was designed to exceed the standards outlined in *Biosafety in Microbiological and Biomedical Laboratories* (5th edition; http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf). Features include controlled access, entry/exit through a shower change room, effluent decontamination, negative air-pressure, double-door autoclaves, gas decontamination ports, HEPA-filtered supply and double-HEPA-filtered exhaust air, double-gasketed watertight and airtight seals, and airtight dampers on all ductwork. The structure is pressure-decay tested regularly. The University of Wisconsin-Madison facility has a dedicated alarm system that monitors all building controls (~500 possible alerts). Redundancies and emergency resources are built into the facility, including two air handlers, two compressors, two filters wherever filters are needed, two effluent sterilization tanks, two power feeds to the building, an emergency generator in case of a power failure, and other physical containment measures in the facility that operate without power. Biosecurity monitoring of the facility is ongoing. All personnel undergo Select Agent security risk assessment by the United States Criminal Justice Information Services Division and complete rigorous biosafety, BSL-3, and Select Agent training before participating in BSL-3-level experiments. Refresher training, including drills and review of emergency plans, is scheduled on a regular basis. The principal investigator participates in training sessions and emphasizes compliance to maintain safe operations and a responsible research environment. The laboratory occupational health plan is in compliance with the University of Wisconsin-Madison Occupational Health Program. Select Agent virus inventory, secured behind two physical barriers, is checked monthly and documentation is submitted to the University of Wisconsin-Madison Select Agent Program Manager. Virus inventory is submitted 1–2 times per year to the file holder in the Division of Select Agents and Toxins at the CDC. The research program, procedures, occupational health plan, documentation, security, and facilities are reviewed annually by the University of Wisconsin-Madison Responsible Official and at regular intervals by the CDC and the APHIS as part of the University of Wisconsin-Madison Select Agent Program.

References

1. World Health Organization (WHO). Summary of status of development and availability of avian influenza A(H7N9) candidate vaccine viruses and potency testing reagents [cited 2017 Sep 28]. http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_a_h7n9_cvv_20170928.pdf?ua=1

2. Neumann G, Watanabe T, Ito H, Watanabe S, Goto H, Gao P, et al. Generation of influenza A viruses entirely from cloned cDNAs. Proc Natl Acad Sci U S A. 1999;96:9345–50. PubMed http://dx.doi.org/10.1073/pnas.96.16.9345

3. Ping J, Lopes TJ, Nidom CA, Ghedin E, Macken CA, Fitch A, et al. Development of high-yield influenza A virus vaccine viruses. Nat Commun. 2015;6:8148. PubMed http://dx.doi.org/10.1038/ncomms9148

4. Imai M, Watanabe T, Kiso M, Nakajima N, Yamayoshi S, Iwatsuki-Horimoto K, et al. A Highly pathogenic avian H7N9 influenza virus isolated from a human is lethal in some ferrets infected via respiratory droplets. Cell Host Microbe. 2017;22:615–626.e8. PubMed http://dx.doi.org/10.1016/j.chom.2017.09.008

Technical Appendix Table 1. Antigenic differences among H7 viruses by hemagglutination inhibition assays

	Hemagglutination inhibition (HI) titers*							
	Monoclonal antibodies against HA from						Antisera against	
	A/seal/Massachusetts/1/80 (H7N7)			A/Anhui/1/2013 (H7N9)			A/Netherlands/219/03 (H7N7)	HA and NA from A/Hong Kong/125/2017
Virus	46/6	55/3	58/2	2–20–20	3–7-19	19–17–20	NR-9226	3,031
H7N9								
HK125-HYPR8 (HA and NA from A/Hong Kong/125/2017)	6,400	12,800	3,200	1,600	1,600	1,600	2,560	**640**
A/Guangdong/17SF003/2016	100	3,200	800	400	400	400	40	80
A/Anhui/1/2013	3,200	25,600	3,200	**6,400**	**12,800**	**6,400**	1,280	640
H7N7								
A/seal/Massachusetts/ 1/1980	**6,400**	**12,800**	**3,200**	800	800	800	640	40

*HI titers are described as the inverse of the highest antibody dilution that inhibited hemagglutination. Values obtained with homologous antibodies are shown in bold. Monoclonal antibodies against the HA proteins of A/seal/Massachusetts/1/80 (H7N7) and A/Anhui/1/2013 (H7N9) viruses, and ferret antisera against A/Hong Kong/125/2017 were generated in our laboratory. Goat antiserum against A/Netherlands/219/03 (H7N7) was obtained from BEI Resources.

Technical Appendix Table 2. Statistical analysis of HI titers of groups 1 and 2 against HK125-HYPR8 in Figure 1, panel B (Upper panel).

A	B	Stage	*P* value
Group 1	Group 2	Pre-boost	0.0380
Group 1	Group 2	Pre-challenge	0.3381

The two groups listed in columns A and B were compared.
Cyan: Values in column B are significantly higher than those in column A.

Technical Appendix Table 3. Statistical analysis of HI titers of groups 1 and 2 against rGD/3-NA294R in Figure 1, panel B (Lower panel).

A	B	Stage	*P* value
Group 1	Group 2	Pre-boost	N.A.
Group 1	Group 2	Pre-challenge	0.4871

The two groups listed in columns A and B were compared.
N.A.: not applicable

Technical Appendix Table 4. Statistical analyses of body temperature changes in the Technical Appendix Figure (Comparison of the indicated groups)

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Group 4	Group 2	0	0.1000	0.2670	0.7898
		1	0.4500	1.2015	0.2314
		2	1.1667	3.1151	0.0022
		3	1.3167	3.5156	0.0006
		4	–0.0667	–0.1780	0.8590
		5	1.0333	1.9510	0.0529
		6	0.6667	1.2587	0.2100
		7	–0.4115	–0.6944	0.4885
		8	–0.4777	–0.6366	0.5253
		9	0.7223	0.9624	0.3373
		10	1.5889	2.1173	0.0358
		11	0.6556	0.8736	0.3837
		12	–0.7111	–0.9475	0.3448
		13	0.3556	0.4739	0.6363
		14	–0.2444	–0.3257	0.7451
Group 4	Group 1	0	0.3167	0.8455	0.3991
		1	0.9833	2.6256	0.0095
		2	1.4833	3.9606	0.0001
		3	1.2167	3.2486	0.0014
		4	0.5333	1.4240	0.1565
		5	1.1333	2.1398	0.0339
		6	1.2667	2.3915	0.0180
		7	0.3218	0.5430	0.5879
		8	0.0889	0.1185	0.9058
		9	0.5889	0.7848	0.4338
		10	0.6556	0.8736	0.3837
		11	1.3223	1.7620	0.0800
		12	–0.5111	–0.6810	0.4969
		13	–0.1111	–0.1480	0.8825
		14	–0.1777	–0.2368	0.8131
Group 4	Group 3	0	0.1333	0.3560	0.7223
		1	–0.9333	–2.4921	0.0138
		2	0.7333	1.9581	0.0520
		3	–0.0500	–0.1335	0.8940
		4	–0.5833	–1.5575	0.1214
		5	–1.0333	–1.9510	0.0529
		6	–1.0000	–1.8880	0.0609
		7	–0.8782	–1.4818	0.1404
		8	–0.9495	–1.1937	0.2344
		9	0.2005	0.2520	0.8014
		10	1.1005	1.3834	0.1685
		11	0.9505	1.1949	0.2340
		12	–0.5495	–0.6908	0.4907
		13	–0.4995	–0.6280	0.5309
		14	–0.5495	–0.6908	0.4907
Group 2	Group 1	0	0.2167	0.5785	0.5638
		1	0.5333	1.4240	0.1565
		2	0.3167	0.8455	0.3991
		3	–0.1000	–0.2670	0.7898
		4	0.6000	1.6020	0.1112

A	B	Days post-challenge	Estimate	t-ratio	*P* value
		5	0.1000	0.1888	0.8505
		6	0.6000	1.1328	0.2590
		7	0.7333	1.3846	0.1682
		8	0.5667	1.0699	0.2863
		9	–0.1333	–0.2517	0.8016
		10	–0.9333	–1.7622	0.0800
		11	0.6667	1.2587	0.2100
		12	0.2000	0.3776	0.7062
		13	–0.4667	–0.8811	0.3796
		14	0.0667	0.1259	0.9000
Group 2	Group 3	0	0.0333	0.0890	0.9292
		1	–1.3833	–3.6936	0.0003
		2	–0.4333	–1.1570	0.2490
		3	–1.3667	–3.6491	0.0004
		4	–0.5167	–1.3795	0.1697
		5	–2.0667	–3.9019	0.0001
		6	–1.6667	–3.1467	0.0020
		7	–0.4667	–0.8811	0.3796
		8	–0.4718	–0.7961	0.4272
		9	–0.5218	–0.8805	0.3800
		10	–0.4885	–0.8243	0.4111
		11	0.2949	0.4975	0.6195
		12	0.1615	0.2725	0.7856
		13	–0.8551	–1.4430	0.1510
		14	–0.3051	–0.5149	0.6074
Group 1	Group 3	0	–0.1833	–0.4895	0.6252
		1	–1.9167	–5.1177	0.0000
		2	–0.7500	–2.0026	0.0470
		3	–1.2667	–3.3821	0.0009
		4	–1.1167	–2.9816	0.0033
		5	–2.1667	–4.0907	0.0001
		6	–2.2667	–4.2795	0.0000
		7	–1.2000	–2.2656	0.0249
		8	–1.0385	–1.7523	0.0817
		9	–0.3885	–0.6555	0.5131
		10	0.4449	0.7506	0.4540
		11	–0.3718	–0.6274	0.5313
		12	–0.0385	–0.0649	0.9483
		13	–0.3885	–0.6555	0.5131
		14	–0.3718	–0.6274	0.5313

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
Cyan: Values in columns B are significantly higher than those in column A.

Technical Appendix Table 5. Statistical analyses of body temperature changes in the Technical Appendix Figure [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Non-vaccinated	Vaccinated	0	0.1417	0.5182	0.6050
Non-vaccinated	Vaccinated	1	1.1833	4.3285	0.0000
Non-vaccinated	Vaccinated	2	0.9583	3.5055	0.0006
Non-vaccinated	Vaccinated	3	1.2917	4.7248	0.0000
Non-vaccinated	Vaccinated	4	0.5250	1.9204	0.0564
Non-vaccinated	Vaccinated	5	1.6000	4.1384	0.0001
Non-vaccinated	Vaccinated	6	1.4667	3.7936	0.0002
Non-vaccinated	Vaccinated	7	0.4690	1.1559	0.2492
Non-vaccinated	Vaccinated	8	0.4463	0.9404	0.3483
Non-vaccinated	Vaccinated	9	0.5296	1.1160	0.2659
Non-vaccinated	Vaccinated	10	0.3963	0.8350	0.4048
Non-vaccinated	Vaccinated	11	0.3629	0.7648	0.4454
Non-vaccinated	Vaccinated	12	–0.2371	–0.4995	0.6180
Non-vaccinated	Vaccinated	13	0.4629	0.9755	0.3306
Non-vaccinated	Vaccinated	14	0.1629	0.3433	0.7317

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.

Technical Appendix Table 6. Statistical analyses of bodyweight changes in the Technical Appendix Figure (Comparison of the indicated groups)

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Group 4	Group 2	0	0.0000	0.0000	1.0000
		1	–2.8454	–1.8434	0.0672
		2	–4.7353	–3.0678	0.0025
		3	–7.8214	–5.0671	0.0000
		4	–10.5902	–6.8609	0.0000
		5	–13.9604	–6.3952	0.0000
		6	–17.0433	–7.8075	0.0000
		7	–17.9853	–7.3576	0.0000
		8	–19.9715	–8.1702	0.0000
		9	–19.7374	–6.3707	0.0000
		10	–16.9340	–5.4658	0.0000
		11	–17.7547	–5.7307	0.0000
		12	–15.9514	–5.1487	0.0000
		13	–16.7998	–5.4225	0.0000
		14	–21.7251	–7.0123	0.0000
Group 4	Group 1	0	0.0000	0.0000	1.0000
		1	–1.4601	–0.9459	0.3456
		2	–3.5500	–2.2999	0.0228
		3	–5.8525	–3.7915	0.0002
		4	–7.3417	–4.7563	0.0000
		5	–8.9101	–4.0817	0.0001
		6	–12.1794	–5.5793	0.0000
		7	–12.5117	–5.1184	0.0000
		8	–15.1942	–6.2158	0.0000
		9	–14.7183	–4.7507	0.0000
		10	–12.5474	–4.0500	0.0001
		11	–14.5611	–4.6999	0.0000
		12	–10.6810	–3.4475	0.0007
		13	–12.4662	–4.0237	0.0001
		14	–18.4718	–5.9622	0.0000
Group 4	Group 3	0	0.0000	0.0000	1.0000
		1	0.5914	0.3831	0.7022
		2	–0.6286	–0.4072	0.6844
		3	–0.6640	–0.4302	0.6677
		4	–0.4278	–0.2772	0.7820
		5	–2.5794	–1.1816	0.2392
		6	–2.9783	–1.3643	0.1744
		7	–1.6499	–0.6750	0.5007
		8	–6.1377	–2.2957	0.0230
		9	–9.0080	–2.7447	0.0068
		10	–5.0677	–1.5441	0.1246
		11	–8.9753	–2.7348	0.0070
		12	–4.3746	–1.3329	0.1845
		13	–5.4998	–1.6758	0.0958
		14	–11.3311	–3.4526	0.0007
Group 2	Group 1	0	0.0000	0.0000	1.0000
		1	1.3853	0.8975	0.3709
		2	1.1853	0.7679	0.4437
		3	1.9689	1.2755	0.2040
		4	3.2486	2.1046	0.0369
		5	5.0503	2.3135	0.0220
		6	4.8640	2.2282	0.0273
		7	5.4736	2.5075	0.0132
		8	4.7773	2.1885	0.0301
		9	5.0191	2.2992	0.0228
		10	4.3866	2.0095	0.0462
		11	3.1936	1.4630	0.1455
		12	5.2704	2.4143	0.0169
		13	4.3337	1.9852	0.0489
		14	3.2533	1.4903	0.1382
Group 2	Group 3	0	0.0000	0.0000	1.0000
		1	3.4368	2.2265	0.0274
		2	4.1067	2.6605	0.0086

A	B	Days post-challenge	Estimate	t-ratio	*P* value
		3	7.1574	4.6369	0.0000
		4	10.1624	6.5837	0.0000
		5	11.3811	5.2136	0.0000
		6	14.0650	6.4432	0.0000
		7	16.3354	7.4832	0.0000
		8	13.8338	5.6593	0.0000
		9	10.7294	4.3893	0.0000
		10	11.8663	4.8544	0.0000
		11	8.7794	3.5916	0.0004
		12	11.5768	4.7360	0.0000
		13	11.3001	4.6228	0.0000
		14	10.3940	4.2521	0.0000
Group 1	Group 3	0	0.0000	0.0000	1.0000
		1	2.0515	1.3290	0.1858
		2	2.9215	1.8927	0.0603
		3	5.1885	3.3614	0.0010
		4	6.9138	4.4791	0.0000
		5	6.3307	2.9001	0.0043
		6	9.2011	4.2150	0.0000
		7	10.8617	4.9757	0.0000
		8	9.0565	3.7049	0.0003
		9	5.7103	2.3360	0.0208
		10	7.4798	3.0599	0.0026
		11	5.5858	2.2851	0.0237
		12	6.3065	2.5799	0.0108
		13	6.9664	2.8499	0.0050
		14	7.1407	2.9212	0.0040

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
Cyan: Values in columns B are significantly higher than those in column A.

Technical Appendix Table 7. Statistical analyses of bodyweight changes in the Technical Appendix Figure [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Non-vaccinated	Vaccinated	0	0.0000	0.0000	1.0000
Non-vaccinated	Vaccinated	1	–2.4485	–2.2821	0.0237
Non-vaccinated	Vaccinated	2	–3.8284	–3.5683	0.0005
Non-vaccinated	Vaccinated	3	–6.5050	–6.0630	0.0000
Non-vaccinated	Vaccinated	4	–8.7520	–8.1574	0.0000
Non-vaccinated	Vaccinated	5	–10.1456	–6.6866	0.0000
Non-vaccinated	Vaccinated	6	–13.1222	–8.6484	0.0000
Non-vaccinated	Vaccinated	7	–13.7356	–8.6156	0.0000
Non-vaccinated	Vaccinated	8	–14.4331	–8.4747	0.0000
Non-vaccinated	Vaccinated	9	–11.5925	–6.1986	0.0000
Non-vaccinated	Vaccinated	10	–11.7323	–6.2734	0.0000
Non-vaccinated	Vaccinated	11	–10.5443	–5.6382	0.0000
Non-vaccinated	Vaccinated	12	–10.7698	–5.7587	0.0000
Non-vaccinated	Vaccinated	13	–11.3365	–6.0617	0.0000
Non-vaccinated	Vaccinated	14	–12.9144	–6.9055	0.0000

The two groups listed in columns A and B were compared.
Cyan: Values in columns B are significantly higher than those in column A.

Technical Appendix Table 8. Statistical analyses of nasal swab titers in Figure 2, panel A (Comparison of the indicated groups)

Group A	Group B	Days post-challenge	Estimate	t-ratio	*P* value
Group 4	Group 2	1	0.9364	2.3933	0.0185
		2	0.4640	1.1860	0.2384
		3	2.9255	7.4770	0.0000
		4	3.2619	8.3367	0.0000
		5	4.7591	8.6007	0.0000
		6	4.7011	8.4959	0.0000
		7	2.7733	4.4827	0.0000
Group 4	Group 1	1	0.3571	0.9128	0.3635
		2	0.0909	0.2324	0.8167
		3	1.8088	4.6230	0.0000
		4	2.3067	5.8954	0.0000
		5	4.7591	8.6007	0.0000
		6	4.7011	8.4959	0.0000

Group A	Group B	Days post-challenge	Estimate	t-ratio	*P* value
		7	2.7733	4.4827	0.0000
Group 4	Group 3	1	–0.0891	–0.2276	0.8204
		2	0.2704	0.6911	0.4910
		3	–0.1784	–0.4560	0.6494
		4	0.1618	0.4135	0.6801
		5	0.0813	0.1470	0.8834
		6	0.3496	0.6318	0.5289
		7	0.8748	1.4140	0.1604
Group 2	Group 1	1	–0.5793	–1.4805	0.1418
		2	–0.3731	–0.9536	0.3425
		3	–1.1167	–2.8540	0.0052
		4	–0.9552	–2.4413	0.0163
		5	0.0000	0.0000	1.0000
		6	0.0000	0.0000	1.0000
		7	0.0000	0.0000	1.0000
Group 2	Group 3	1	–1.0255	–2.6209	0.0101
		2	–0.1936	–0.4949	0.6218
		3	–3.1040	–7.9330	0.0000
		4	–3.1002	–7.9233	0.0000
		5	–4.6778	–8.4537	0.0000
		6	–4.3515	–7.8641	0.0000
		7	–1.8985	–3.4310	0.0009
Group 1	Group 3	1	–0.4462	–1.1404	0.2568
		2	0.1795	0.4587	0.6474
		3	–1.9873	–5.0789	0.0000
		4	–2.1449	–5.4820	0.0000
		5	–4.6778	–8.4537	0.0000
		6	–4.3515	–7.8641	0.0000
		7	–1.8985	–3.4310	0.0009

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
Cyan: Values in columns B are significantly higher than those in column A.

Technical Appendix Table 9. Statistical analyses of nasal swab titers in Figure 2, panel A [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Non-vaccinated	Vaccinated	1	0.6913	2.5238	0.0131
Non-vaccinated	Vaccinated	2	0.1423	0.5194	0.6045
Non-vaccinated	Vaccinated	3	2.4564	8.9678	0.0000
Non-vaccinated	Vaccinated	4	2.7034	9.8697	0.0000
Non-vaccinated	Vaccinated	5	4.7185	12.1807	0.0000
Non-vaccinated	Vaccinated	6	4.5263	11.6847	0.0000
Non-vaccinated	Vaccinated	7	2.2603	5.5538	0.0000

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.

Technical Appendix Table 10. Statistical analyses of throat swab titers in Figure 2, panel A (Comparison of the indicated groups)

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Group 4	Group 2	1	1.7086	4.1442	0.0001
		2	1.0755	2.6085	0.0105
		3	3.8785	9.4070	0.0000
		4	4.1299	10.0168	0.0000
		5	5.0419	8.6470	0.0000
		6	4.8938	8.3930	0.0000
		7	4.2523	6.5030	0.0000
Group 4	Group 1	1	0.5150	1.2492	0.2146
		2	0.7191	1.7441	0.0843
		3	2.3366	5.6672	0.0000
		4	2.6004	6.3071	0.0000
		5	5.0419	8.6470	0.0000
		6	4.8938	8.3930	0.0000
		7	4.2523	6.5030	0.0000
Group 4	Group 3	1	0.2125	0.5153	0.6075
		2	–0.1370	–0.3324	0.7403
		3	0.2038	0.4942	0.6223
		4	–0.1453	–0.3525	0.7252

A	B	Days post-challenge	Estimate	t-ratio	*P* value
		5	0.3165	0.5429	0.5885
		6	0.7407	1.2703	0.2070
		7	1.1074	1.6936	0.0935
Group 2	Group 1	1	−1.1936	−2.8950	0.0047
		2	−0.3564	−0.8644	0.3895
		3	−1.5419	−3.7398	0.0003
		4	−1.5295	−3.7097	0.0003
		5	0.0000	0.0000	1.0000
		6	0.0000	0.0000	1.0000
		7	0.0000	0.0000	1.0000
Group 2	Group 3	1	−1.4962	−3.6289	0.0005
		2	−1.2125	−2.9409	0.0041
		3	−3.6747	−8.9128	0.0000
		4	−4.2752	−10.3693	0.0000
		5	−4.7254	−8.1041	0.0000
		6	−4.1531	−7.1227	0.0000
		7	−3.1449	−5.3935	0.0000
Group 1	Group 3	1	−0.3026	−0.7339	0.4648
		2	−0.8561	−2.0765	0.0405
		3	−2.1328	−5.1729	0.0000
		4	−2.7457	−6.6595	0.0000
		5	−4.7254	−8.1041	0.0000
		6	−4.1531	−7.1227	0.0000
		7	−3.1449	−5.3935	0.0000

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
Cyan: Values in columns B are significantly higher than those in column A.

Technical Appendix Table 11. Statistical analyses of throat swab titers in Figure 2, panel A [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	Days post-challenge	Estimate	t-ratio	*P* value
Non-vaccinated	Vaccinated	1	1.0056	3.1990	0.0018
Non-vaccinated	Vaccinated	2	0.9658	3.0723	0.0027
Non-vaccinated	Vaccinated	3	3.0057	9.5613	0.0000
Non-vaccinated	Vaccinated	4	3.4378	10.9361	0.0000
Non-vaccinated	Vaccinated	5	4.8836	10.9852	0.0000
Non-vaccinated	Vaccinated	6	4.5235	10.1750	0.0000
Non-vaccinated	Vaccinated	7	3.5427	7.5786	0.0000

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.

Technical Appendix Table 12. Statistical analyses of brain titers in Figure 2, panel B (Comparison of the indicated groups)

A	B	LWR	UPR	Difference	Adjusted *P* value
Group 2	Group 4	−3.8181	1.7667	−1.0257	0.6569
Group 1	Group 4	−3.8181	1.7667	−1.0257	0.6569
Group 3	Group 4	−3.1334	2.4514	−0.3410	0.9783
Group 1	Group 2	−2.7924	2.7924	0.0000	1.0000
Group 3	Group 2	−2.1078	3.4770	0.6846	0.8592
Group 3	Group 1	−2.1078	3.4770	0.6846	0.8592

The two groups listed in columns A and B were compared.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 13. Statistical analyses of brain titers in Figure 2, panel B [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	LWR	UPR	Difference	Adjusted *P* value
Vaccinated	Non-vaccinated	−2.0956	0.3853	−0.8551	0.1556

The two groups listed in columns A and B were compared.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 14. Statistical analyses of lung titers in Figure 2, panel B (Comparison of the indicated groups)

A	B	LWR	UPR	Difference	Adjusted *P* value
Group 2	Group 4	−7.3317	−3.7885	−5.5601	0.0000
Group 1	Group 4	−7.3317	−3.7885	−5.5601	0.0000
Group 3	Group 4	−1.3220	2.2212	0.4496	0.8469
Group 1	Group 2	−1.7716	1.7716	0.0000	1.0000
Group 3	Group 2	4.2381	7.7813	6.0097	0.0000

Group 3	Group 1	4.2381	7.7813	6.0097	0.0000

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
Cyan: Values in columns B are significantly higher than those in column A.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 15. Statistical analyses of lung titers in Figure 2, panel B [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	LWR	UPR	Difference	Adjusted *P* value
Vaccinated	Non-vaccinated	−6.5960	−4.9737	−5.7849	0.0000

The two groups listed in columns A and B were compared.
Cyan: Values in columns B are significantly higher than those in column A.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 16. Statistical analyses of nasal turbinate titers in Figure 2, panel B (Comparison of the indicated groups)

A	B	LWR	UPR	Difference	Adjusted *P* value
Group 2	Group 4	−11.8235	2.0552	−4.8841	0.1885
Group 1	Group 4	−9.8570	4.0217	−2.9177	0.5621
Group 3	Group 4	−7.0473	6.8314	−0.1079	1.0000
Group 1	Group 2	−4.9729	8.9058	1.9664	0.8017
Group 3	Group 2	−2.1632	11.7155	4.7762	0.2017
Group 3	Group 1	−4.1296	9.7491	2.8097	0.5896

The two groups listed in columns A and B were compared.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 17. Statistical analyses of nasal turbinate titers in Figure 2, panel B [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	LWR	UPR	Difference	Adjusted *P* value
Vaccinated	Non-vaccinated	−7.0544	−0.6395	−3.8469	0.0234

The two groups listed in columns A and B were compared.
Cyan: Values in columns B are significantly higher than those in column A.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 18. Statistical analyses of olfactory bulb titers in Figure 2, panel B (Comparison of the indicated groups)

A	B	LWR	UPR	Difference	Adjusted *P* value
Group 2	Group 4	−2.5603	3.1929	0.3163	0.9839
Group 1	Group 4	−2.8465	2.9067	0.0301	1.0000
Group 3	Group 4	0.0017	5.7549	2.8783	0.0499
Group 1	Group 2	−3.1628	2.5904	−0.2862	0.9880
Group 3	Group 2	−0.3146	5.4386	2.5620	0.0820
Group 3	Group 1	−0.0284	5.7248	2.8482	0.0523

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 19. Statistical analyses of olfactory bulb titers in Figure 2, panel B [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	LWR	UPR	Difference	Adjusted *P* value
Vaccinated	Non-vaccinated	−3.1841	0.6521	−1.2660	0.1722

The two groups listed in columns A and B were compared.
LWR: Lower confidence interval
UPR: Upper confidence interval

Technical Appendix Table 20. Statistical analyses of tracheal titers in Figure 2, panel B (Comparison of the indicated groups)

A	B	LWR	UPR	Difference	Adjusted *P* value
Group 2	Group 4	−8.1933	−5.3690	−6.7811	0.0000
Group 1	Group 4	−8.1933	−5.3690	−6.7811	0.0000
Group 3	Group 4	−1.7448	1.0795	−0.3326	0.8724
Group 1	Group 2	−1.4121	1.4121	0.0000	1.0000
Group 3	Group 2	5.0363	7.8606	6.4485	0.0000
Group 3	Group 1	5.0363	7.8606	6.4485	0.0000

The two groups listed in columns A and B were compared.
Orange: Values in columns A are significantly higher than those in column B.
Cyan: Values in columns B are significantly higher than those in column A.
LWR: Lower confidence interval

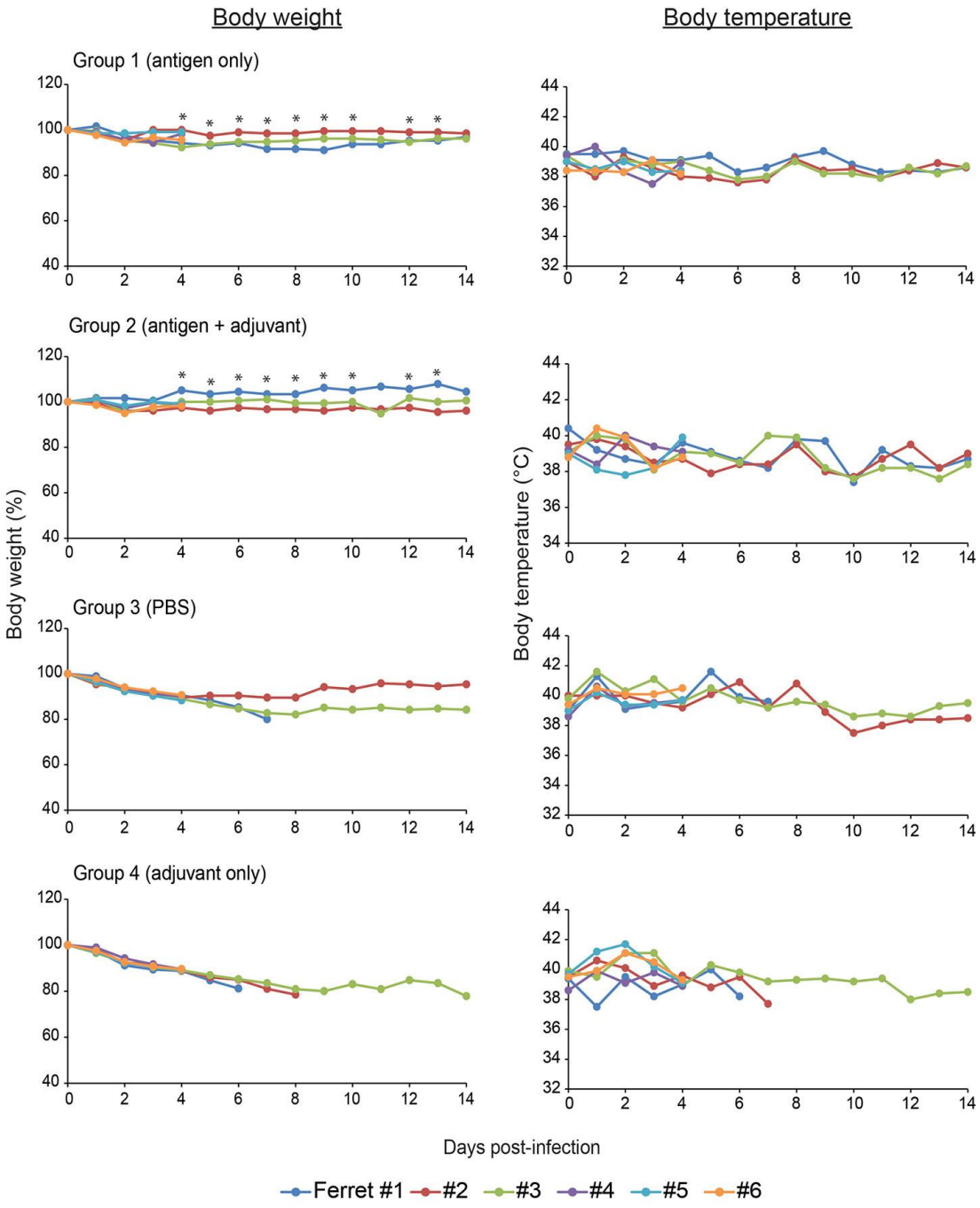
A	B	LWR	UPR	Difference	Adjusted *P* value

UPR: Upper confidence interval

Technical Appendix Table 21. Statistical analyses of tracheal titers in Figure 2, panel B [Comparison of vaccinated (groups 1 and 2 merged) and unvaccinated (groups 3 and 4 merged) groups].

A	B	LWR	UPR	Difference	Adjusted *P* value
Vaccinated	Non-vaccinated	−7.2579	−5.9717	−6.6148	0.0000

The two groups listed in columns A and B were compared.
Cyan: Values in columns B are significantly higher than those in column A.
LWR: Lower confidence interval
UPR: Upper confidence interval



Technical Appendix Figure. Bodyweight and temperature changes in vaccinated and non-vaccinated ferrets challenged with highly pathogenic H7N9 virus. Six ferrets per group were challenged intranasally with 10^6 PFU of highly pathogenic H7N9 rGD/3-NA294R virus; bodyweight and temperature were monitored daily for 14 days. Ferrets #4 – #6 in each group were euthanized on day 4 post-challenge for organ sampling. Ferret #1 in group 3, and ferrets #1 and #2 in group 4 were euthanized on days 7, 6, and 8 post-challenge, respectively, due to severe symptoms. Statistically significant differences in bodyweight changes between ferrets in Groups 1 and 2 are marked (*); *, $p \leq 0.05$.