Supporting Information

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SI Materials and Methods

Air Sampling. To determine the quantity and size of virus-laden particles in air, NIOSH BC 251 samplers capable of collecting aerosols at different size ranges (>4, 1–4, and <1 μm) at 3.5 L/min were applied to sample air from the donor and recipient chamber via preinstalled ports at the roof of the chambers. Two NIOSH samplers were installed at the recipient chamber, and one NIOSH sampler was installed at the donor chamber. The two NIOSH samplers at the recipient chamber each sampled air for 4 h; the first one was turned on during the setup of the chambers, and the airflow before the impactor was measured and calibrated to 30 L/min. After 4 h, the second NIOSH sampler was turned on while the first NIOSH sampler was turned off, with the airflow at the impactor remaining at 30 L/min. The NIOSH sampler installed at the donor chamber did not affect the airflow at the impactor and was turned on to sample air for 4 h during the second half of the 8-h exposure. The quantity of virus-laden particles was assessed by determining influenza virus M gene copies by quantitative real-time RT-PCR (1). The lower limit of the linear range of quantification was 476 copies per cubic meter.

An APS (TSI) was used to characterize the total particle distribution inside the donor chamber before (1 d before inoculation) and after influenza inoculation (at 2 dpi). During the sampling, two ferrets were placed inside the donor chamber, the air was drawn from the chamber at 25 L/min by a vacuum pump, and the APS sampled air at 5 L/min. The particle-size distribution was reported as particle counts per liter of air during the 1-min sampling for aerodynamic diameters in the range of 0.5–20 μm. The particle count was monitored for 30 min each time.

Cells and Viruses. Madin–Darby canine kidney (MDCK) cells and HEK 293T cells were obtained from the American Type Culture Collection and were maintained in MEM with 10% FCS and Opti-MEM (Invitrogen) with 5% FCS, respectively. Normal human bronchial epithelial primary cells were purchased from MatTek and cultured in an air–liquid interface system (2). The WT and recombinant A/California/04/09 viruses (CA04; H1N1) and the recombinant A/Wuhan/359/95(Rg-WH359; H3N2) viruses were propagated as described previously (2, 3). The swine influenza virus, A/swine/Kansas/00246/2004 (KS246; H1N2) was provided by Amy Vincent (US Department of Agriculture, Washington, DC), and the eight genes were amplified by RT-PCR and cloned into pHW2000 plasmid to generate recombinant viruses as described previously (4). All recombinant viruses were propagated in MDCK cells for two or three passages at an MOI of 0.001 PFU per cell to prepare virus stocks.

Replication Kinetics in Differentiated HAE Cells. Replication kinetics of the virus in HAE cells was determined at an MOI of 0.01 PFU/cell. Cells were prewashed with warm PBS solution before infection and were infected via apical sides with 200 μL of diluted viruses. After 1 h incubation at 37 °C, excess or unbound viruses were removed. The apical and basolateral surfaces of the Transwell inserts were washed once with PBS solution before replenishment with fresh medium and incubation at 37 °C. The virus was collected at 2, 12, 24, 48, and 72 h postinfection by adding 200 μL medium onto the apical surface followed by equilibration for 20 min at 37 °C. Samples were stored at 80 °C and titrated in MDCK cells to determine viral load (in PFU per milliliter).

Infectivity. The $TCID_{50}$ was determined by incubating serial half-log dilutions of viruses in MDCK cells at 37 °C for 72 h. A hemagglutination assay was performed to determine the endpoint of infection, and the $TCID_{50}$ was calculated by the Reed–Muench method (5). Plaque assays were performed by incubating 1 mL of serial-diluted samples in confluent MDCK cells in six-well plates. After 1 h incubation at 37 °C, inocula were removed and overlaid with infection medium (MEM with 0.3% BSA) containing 1% agarose. After incubation at 37 °C for 3 d, cells were fixed overnight with 4% formaldehyde in PBS solution followed by staining with 0.2% crystal violet.

Glycan Array Analysis. CA04 and KS246 viruses propagated in MDCK cells were concentrated by using Amicon Ultra-15 Centrifugal Filter Units (100 kDa). The glycans were synthesized and dotted at the Genomics Research Center at Academia Sinica (Taiwan). Viruses were inactivated by 0.025% formaldehyde before glycan array analysis. Inactivated viruses were diluted to 16 HA titers by using 0.5% turkey red blood cells and were incubated on the array slide for 1 h, followed by incubation with anti-H1 monoclonal antibodies (provided by Elena Govorkova, St. Jude Children's Research Hospital) and Alexa Fluor 647-labeled goat anti-mouse IgG (A-21235; Thermo Fisher). The fluorescence signal was acquired by using the NimbleGen MS 200 Microarray Scanner.

NA Kinetics. NA kinetics were determined using the fluorogenic substrate MUNANA at final concentrations of 0–1,000 μM at 37 °C in a final volume of 50 μL. All viruses were diluted to 10^6 PFU/mL. The fluorescence of released 4-methylumbelliferone was measured every 68 s for 68 min by using a FLUOstar Optima reader (BMG Labtech), with excitation and emission wavelengths of 355 and 460 nm, respectively. Enzyme kinetics data were fitted to the Michaelis–Menten equation by using nonlinear regression (GraphPad Prism) to determine K_m and V_{max} of substrate conversion.

Transmission EM. MDCK cells were infected at an MOI of 2 PFU/cell. At 14 h postinfection, samples were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer overnight at 4 °C. The samples were then prepared and imaged at the EM unit at the University of Hong Kong. The cells infected with influenza viruses were imaged with a Philips CM100 transmission electron microscope.

Statistical Analysis. A Kruskal–Wallis test followed by the Dunn's multiple-comparison posttest were applied to compare differences between multiple groups of independent variables (e.g., quantity of virus-laden particles detected in air, viral titers in nasal washes, AUC, maximal weight loss) The lengths of virions formed by recombinant viruses in MDCK cells were analyzed by one-way ANOVA. Differences were considered significant at $P < 0.05$. Analyses were performed by using SPSS software (IBM).

1. Zhou J, et al. (2016) Isolation of H5N6, H7N9 and H9N2 avian influenza A viruses from air sampled at live poultry markets in China, 2014 and 2015. *Euro Surveill* 21, 10.2807/1560-7917.ES.2016.21.35.30331.
2. Yen HL, et al. (2011) Hemagglutinin-neuraminidase balance confers respiratory-droplet transmissibility of the pandemic H1N1 influenza virus in ferrets. *Proc Natl Acad Sci USA* 108:14264–14269.
3. Wong DD, et al. (2012) Comparable fitness and transmissibility between oseltamivir-resistant pandemic 2009 and seasonal H1N1 influenza viruses with the H275Y neuraminidase mutation. *J Virol* 86:10558–10570.
4. Hoffmann E, Neumann G, Kawaoka Y, Hobom G, Webster RG (2000) A DNA transfection system for generation of influenza A virus from eight plasmids. *Proc Natl Acad Sci USA* 97:6108–6113.
5. Reed LJ, Muench H (1938) A simple method of estimating fifty percent endpoints. *Am J Hyg* 27:493–497.

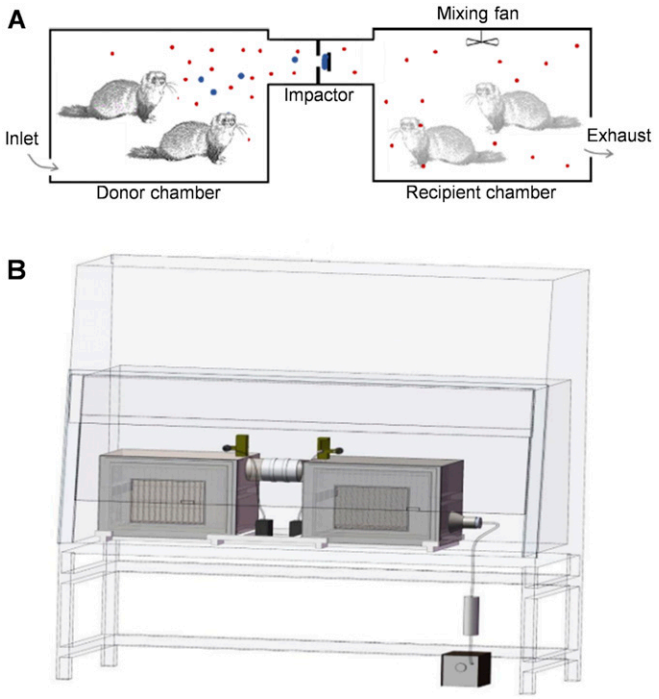

Fig. S1. Establishment of an exposure chamber able to separate influenza virus-laden particles into specific size ranges with the application of impactors. (*A*) Schematic representation of the donor and recipient chambers with an impactor installed in between. Unidirectional flow is created by a vacuum pump that draws air from the donor chamber into the recipient chamber. A mixing fan ensured even distribution of the particles within the recipient chamber. (*B*) Schematic representation of the exposure chamber set up inside a 6-ft class II BSC.

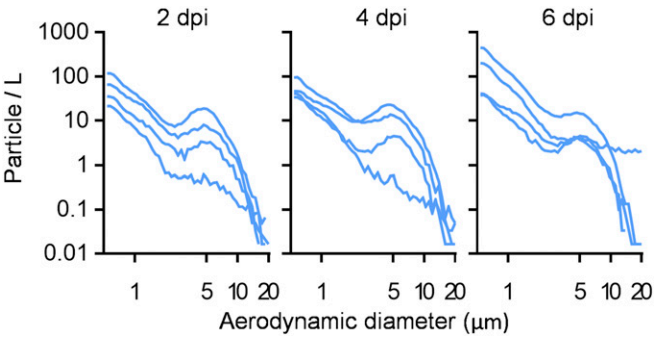

Fig. S2. The size distribution of total particles released from CA04-inoculated donor ferrets at 2 dpi, 4 dpi, and 6 dpi. The total particle size distribution was analyzed by an APS with two donors placed inside the donor chamber. The particle-size distribution was reported as particle counts per liter of air during the 1-min sampling for aerodynamic diameters in the range of 0.5–20 μm. The particle count was monitored for 30 min each time.

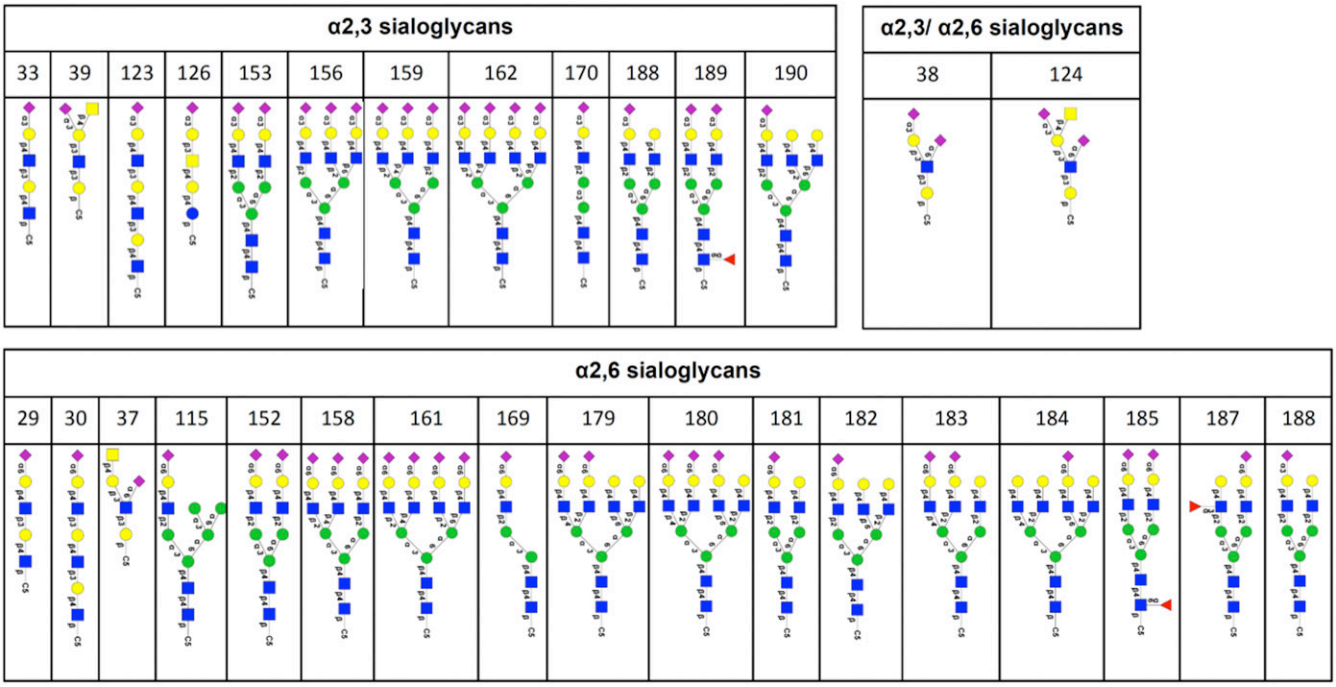

Fig. S3. N-linked glycan structures used for glycan array analysis.