Supplemental Materials

Supplemental Methods

Recombinant virus construction and purification. Mutant viruses were constructed using a reverse genetics system in a similar manner as previously described (1). Briefly, classically described epitopes of pdm09-like H1 (A/Michigan/45/2015) were substituted with the corresponding potential epitopes of H5 (A/Vietnam/1203/2004), and/or H13 (A/black headed gull/Sweden/1/1999). Constructed ambisense DNA plasmids were cloned and transfected into human embryonic kidney 293T cells (ATCC) with a 7-segment plasmid encoding the essential viral proteins and virus-like RNA of PR8. Scraped cells and supernatants were injected into 8-10 day old embryonated chicken eggs (Charles River Laboratories) for viral rescue at 37^{o}C for 48 hours. Viruses were plaque purified on Madin-Darby Canine Kidney (MDCK) cells (ATCC) grown in Dulbecco's Modified Eagles Medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Hyclone) and penicillin-streptomycin mix (100 units/ml of penicillin and 100ug/ml of streptomycin, Gibco). Individual plaques were picked and injected into embryonated eggs, and viral RNAs were extracted from the allantoic fluids and HA segments were Sanger sequenced.

Animal sample collection and seroconversion. Six- to eight-week old female BALB/c mice (n=10, Jackson Laboratories), five- to six-week-old female Hartley guinea pigs (n=4, Charles River Laboratories), and circulating H1N1 influenza virus seronegative-confirmed four- to five-month-old male Fitch ferrets (n=3, Triple F Farms, Sayre, PA) were anesthetized and intranasally infected with 1x10^5 PFU of A/Michigan/45/2015 (H1N1) virus per animal. Triple F Farms claims that their ferrets are 'Flu-Free'. Four weeks post infection samples were collected and animals were euthanized. Antisera from two ferrets immunized independently were harvested three weeks post infection. Samples were then *Vibrio cholerae* receptor-destroying enzyme (Denka Seiken, Chuo-ku, Tokyo, Japan) treated for use in HI assays as described previously (2).

Human sample collection and treatment. See supplemental methods. Eighteen individuals provided informed consented and donated blood before or on the day of seasonal influenza vaccination as well as four-eight weeks later. Plasma samples were stored at -80^{o}C until use. Each 100 µl human plasma sample was heat treated at 56^{o}C for 30 minutes. Samples were then *Vibrio cholerae* receptor-destroying enzyme (Denka Seiken, Chuo-ku, Tokyo, Japan) treated for use in HI assays as described previously (2).

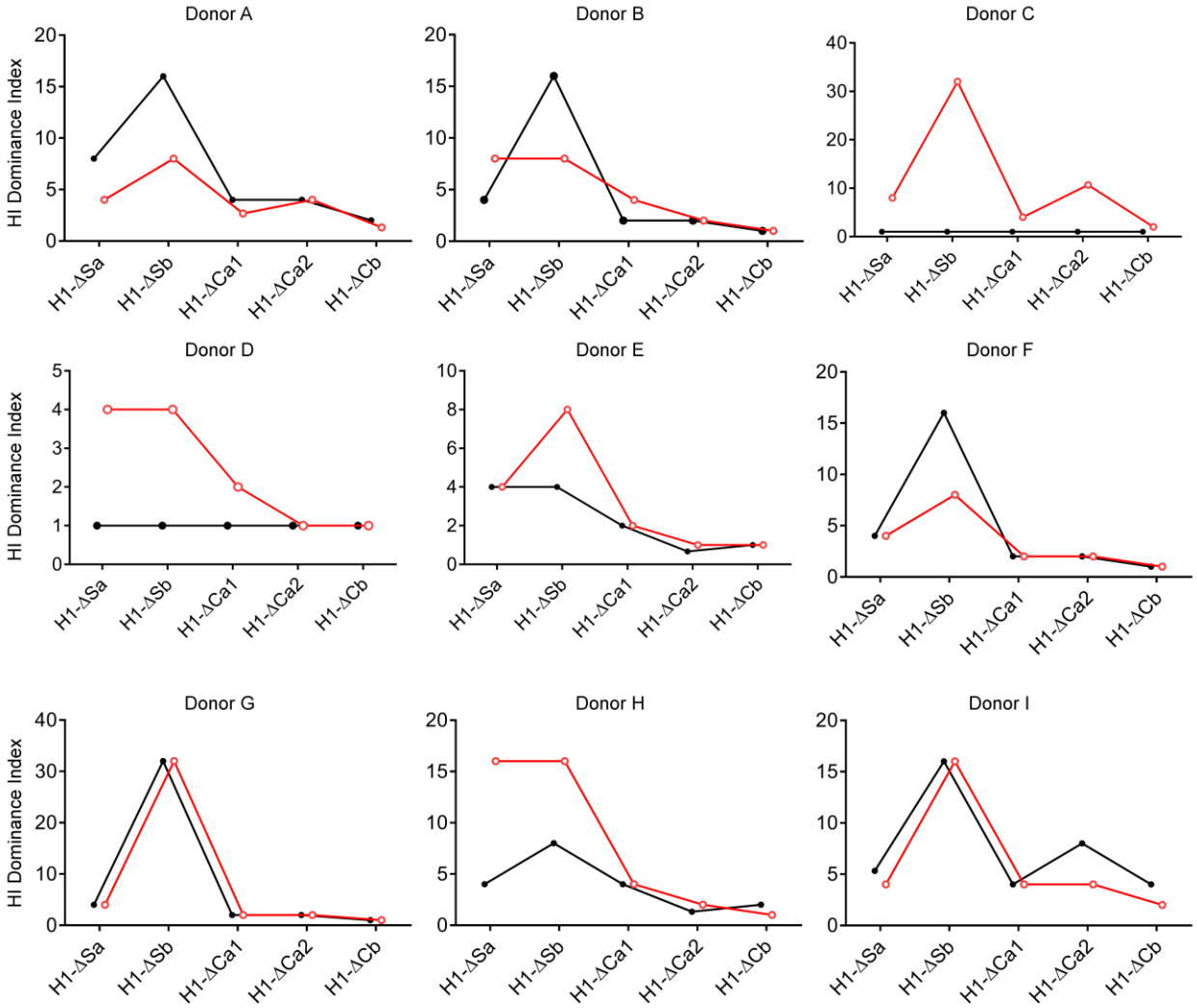
Hemagglutination inhibition (HI) assay. Chicken red blood cells (Lampire) were washed in Phosphate Buffered Saline (PBS) and resuspended at a concentration of 0.5% hematocrit. Receptor-destroying enzyme treated human samples that resulted in a 10-fold dilution were further serially diluted 1:2 in 25µl volumes across a 96-well V-bottom plate. Allantoic fluid containing wild-type or mutant H1 viruses was diluted to eight HA-units and then incubated in equal volumes to antisera (25µl each) for 30 minutes at 25^{o}C. Chicken red blood cells were then added and HI titers were visually determined. All samples were tested in duplicates.

Antigenic cartography. Antigenic map construction was performed as described previously (3). Briefly, modified multi-dimensional scaling methods arrange point distances between antisera and viruses based on HI titers. The map displayed in this study was generated from Acmacs Web Cherry (https://acmacs-web.antigenic-cartography.org).

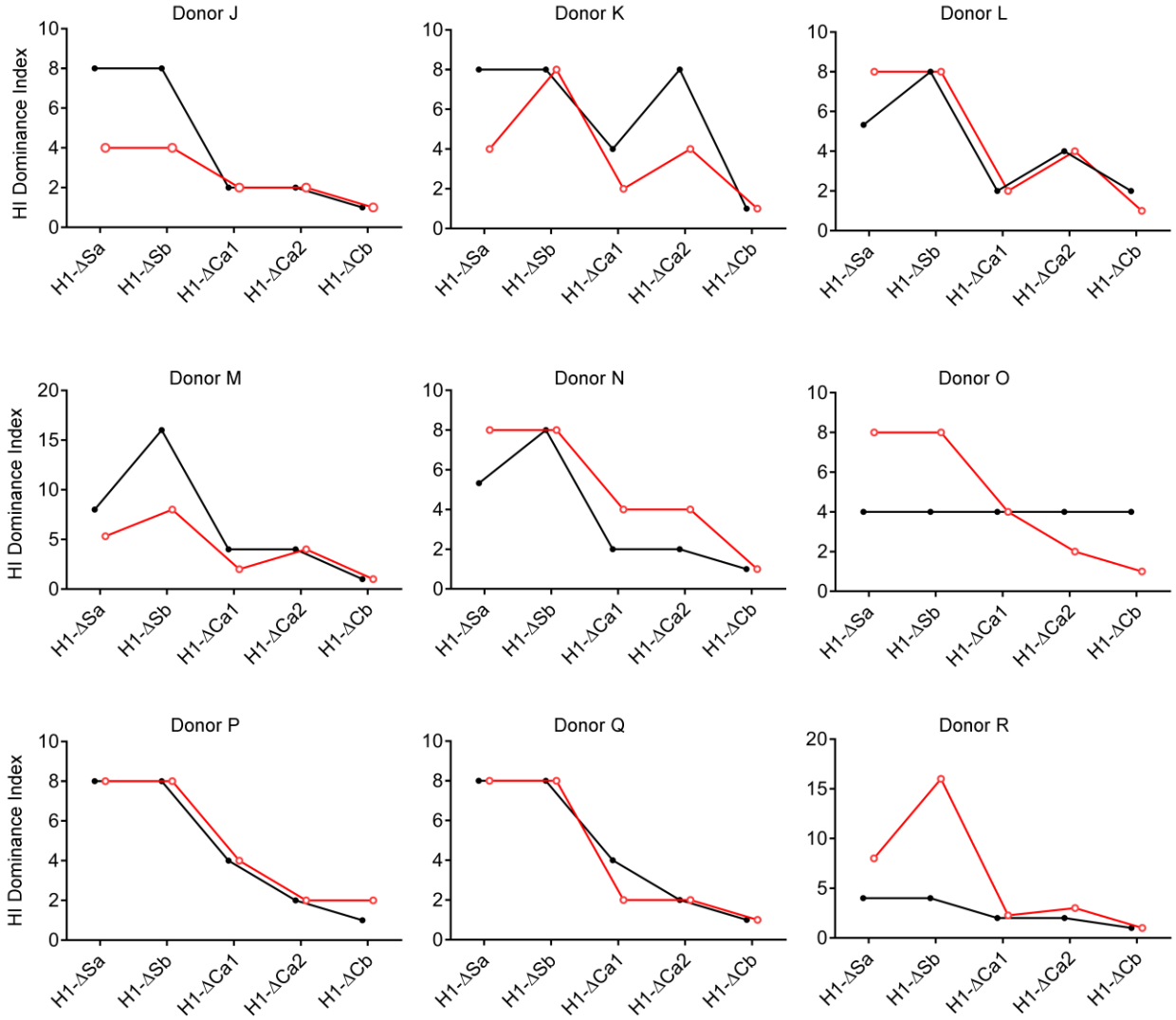
Supplemental Table 1. Demographics of human donors and respective seasonal vaccinations. Donors varied in sex, age, sample collection times, and the type of seasonal vaccines that they received.

Donor ID	Sample collection pre-vaccination (Days)	Sample collection post-vaccination (Days)	Sex (F/M)	Age group (Years)	Seasonal vaccine received (2017-2018)
A	0	49	F	50-59	Flucelvax
B	5	43	M	30-39	Flucelvax
C	0	41	M	>60	Fluzone High-Dose
D	1	36	F	>60	Fluzone High-Dose
E	0	33	F	40-49	Flucelvax
F	260	42	F	50-59	Flucelvax
G	275	57	M	40-49	Flucelvax
H	270	52	M	50-59	Flucelvax
I	12	28	M	30-39	Fluarix Quadrivalent
J	0	29	M	>60	Fluvirin
K	0	29	M	50-59	Fluarix Quadrivalent
L	3	28	M	40-49	Fluarix Quadrivalent
M	3	28	F	30-39	Fluzone Quadrivalent
N	0	28	M	20-29	Fluarix Quadrivalent
O	9	28	F	40-49	Fluarix Quadrivalent
P	2	27	M	30-39	Flublok Quadrivalent
Q	0	28	F	30-39	Fluarix Quadrivalent
R	0	28	M	30-39	Fluzone Quadrivalent

Supplemental Figure 1. Diversity of human HI profiles. HI profiles showing HI dominance indices of mutant viruses were plotted for each antiserum (pre- and post- vaccination: black and red, respectively). Plots for donors A through I are on this page. Plots for donors J through R are on following page.

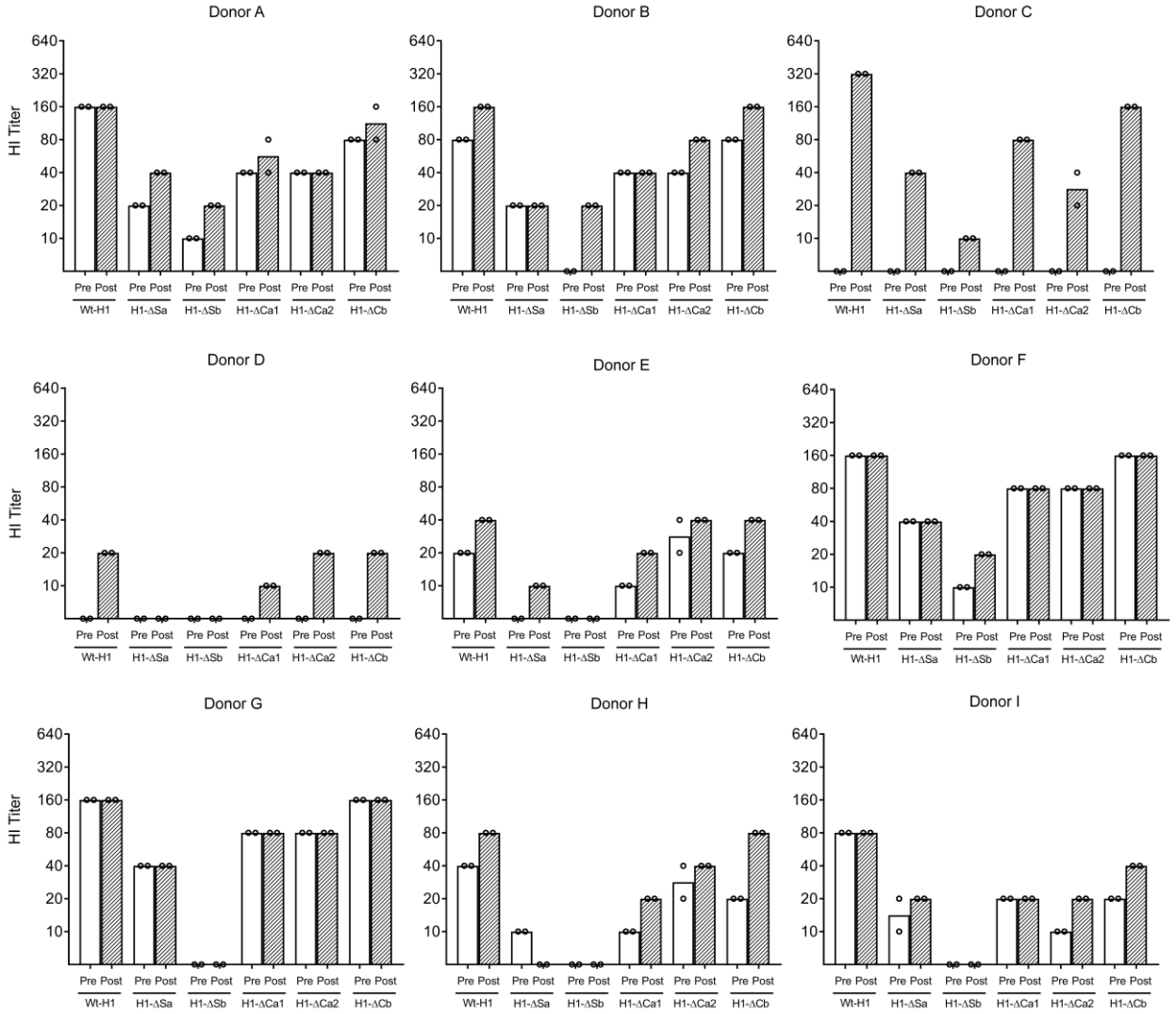


Supplemental Figure 1. Diversity of human HI profiles. Continued from previous page. Plots for donors J through R are displayed below.

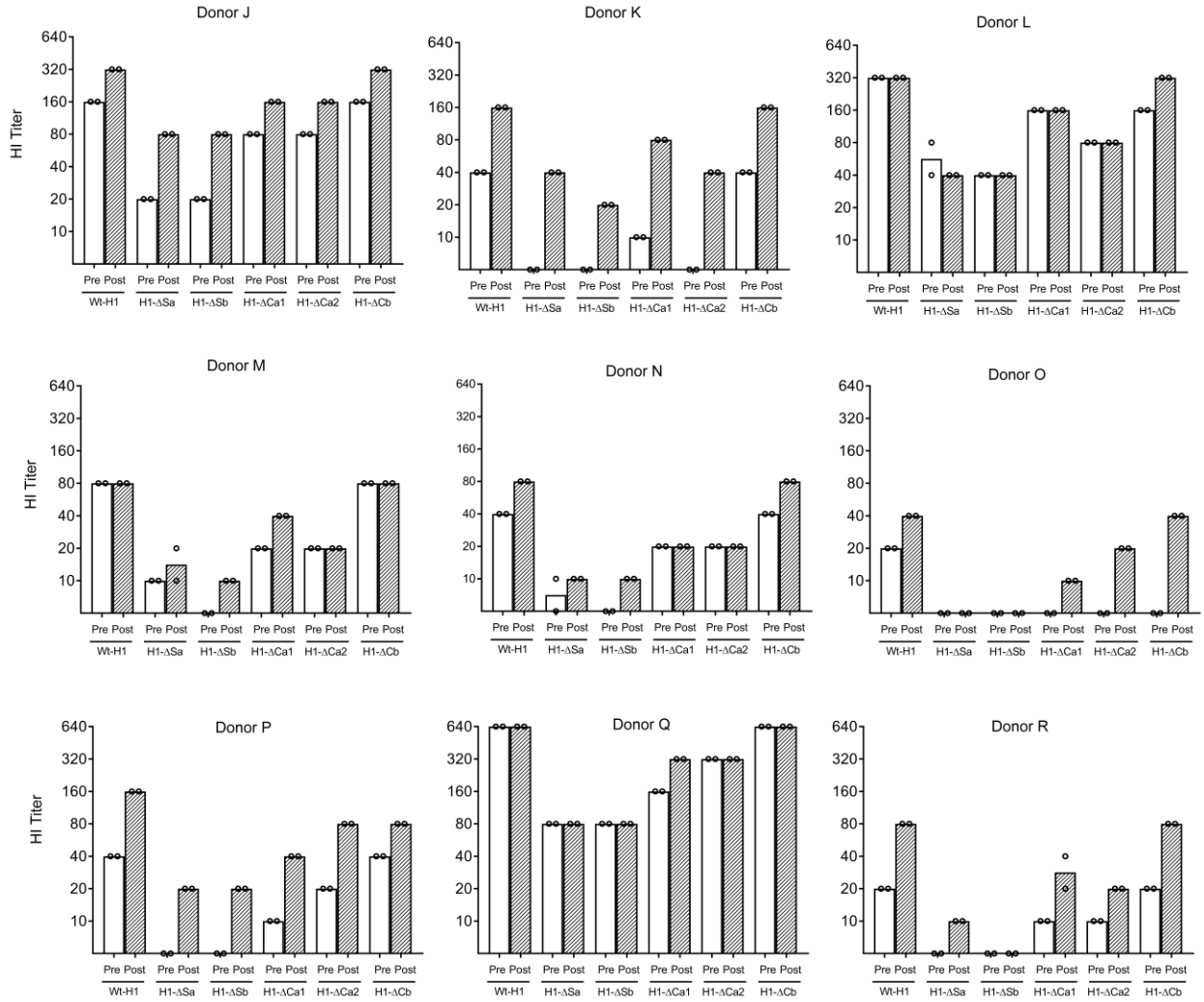


Supplemental Figure 2. Hemagglutination inhibition (HI) titers of antisera from human volunteers. HI titers were measured for antisera from human donors (pre- and post- vaccination: white and gray, respectively) tested against wild-type H1 virus and the panel of mutant viruses. Dots (o) represent single measurements of an individual's serum against the indicated viruses. Bars represent the geometric mean of the two HI titers. Plots for donors A through I are on the next page. Plots for donors J through R are on following page.

Supplemental Figure 2. Continued from previous page. Plots for donors A through I displayed below.



Supplemental Figure 2. Continued from previous page. Plots for donors J through R displayed below.



Supplemental References

1. Chen CJ, Ermler ME, Tan GS, Krammer F, Palese P, Hai R. Influenza A Viruses Expressing Intra- or Intergroup Chimeric Hemagglutinins. *Journal of virology.* 2016;90(7):3789-3793.
2. Robinson RQ, Dowdle. WR. Influenza viruses. In: Lennette EH, Schmidt NJ, eds. *Diagnostic procedures for viral and rickettsial infections.* Fourth ed. New York: American Public Health Association, Inc.; 1969:414-433.
3. Smith DJ, Lapedes AS, de Jong JC, et al. Mapping the antigenic and genetic evolution of influenza virus. *Science.* 2004;305(5682):371-376.