Supplementary Methods

Viruses, cell lines and mice

The A/Netherlands/602/2009 (Neth09) H1N1 virus were grown in 10-d-old specific-pathogen-free embryonated chicken eggs (Charles River Laboratories, Wilmington, MA). MDCK, A549, and 293T cells were maintained in DMEM (Life Technologies, Carlsbad, CA) supplemented with 10% FBS (Sigma, St. Louis, MO), 100 units per ml of penicillin and 100 µg/ml of streptomycin (Life Technologies). C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). *Fcer1g*$^{-/-}$ (1), and FcRα-null (2) mice on the C57BL/6 genetic background have been previously described. All mice were maintained in a specific-pathogen-free facility at the Rockefeller University, all infections were performed in a BSL-2 facility at the Rockefeller University, and all studies were approved by the Rockefeller University Institutional Animal Care and Use Committee. Female mice 6–10 weeks of age were used in all experiments.

Antibodies

To generate the mAb constructs tested in this manuscript, the heavy chain variable regions of FI6, 2G02, 4G05, 1F05, 1A01, 1A05, 4G01, 3C02, and 3C05 were cloned in-frame into mammalian expression vectors with mouse IgG2a, DA265 mutant, or mouse kappa Fc backbones. Antibodies were produced by transient transfection of 293T cells and subsequent protein G purification from culture supernatants, as described (3). Alkaline-phosphatase-conjugated or phycoerythrin-conjugated anti-mouse IgG (Cat. #0102-04) and anti-mouse kappa (Cat. #1050-04) were from SouthernBiotech (Birmingham, AL).

ELISA and antibody binding assays

To compare the binding characteristics of mAb mutants to HA, ELISA plates (Thermo Fisher Scientific, Waltham, MA) were coated with purified Cal09 virus (provided by Florian Krammer, Icahn School of Medicine at Mt. Sinai), recombinant Cal09 HA (Life Science Technologies), 2014-2015 trivalent influenza vaccine (Novartis, Rockefeller University Hospital Pharmacy),

recombinant Cal09 HA1 head domain (Life Technologies), or recombinant chimeric HA ((4); H5 head, H1 stalk; provided by Florian Krammer) overnight at 4 °C, washed with PBS with 0.1% Tween 20, blocked with 1% BSA (Sigma- Aldrich, St. Louis, MO) in PBS and incubated with mAb diluted in PBS plus 1% BSA. Plates were incubated with the appropriate secondary antibody conjugated to alkaline phosphatase and developed with *p*-nitrophenyl phosphate (PNPP, Thermo Fisher Scientific).

To test mAb binding to cell-surface HA, A549 cells were infected with Neth09 virus at an MOI of 1. After 4 hours, the infected A549 cells were incubated with various concentrations of IgG2a mAbs for 30 min, were washed three times, and were incubated with phycoerythrin-conjugated anti-mouse IgG antibody before flow cytometry analysis.

Plaque-reduction neutralization assay

In vitro neutralization assays were performed as described (5). Various dilutions of each mAb were pre-incubated with 60 to 80 PFU of virus for 1 h at room temperature on a shaker. The virus and mAb mixture was then used to infect a monolayer of MDCK cells in duplicate in a six-well plate that was incubated at 37 °C for 1 h with intermittent rocking every 5-10 min. Without washing off the inoculum, an agar overlay that was supplemented with corresponding mAb dilutions was added to each well. Two days later, the monolayer was fixed with 4% paraformaldehyde in PBS for 30 min, and plaques were counted using crystal violet staining.

In vivo viral challenge experiments.

Mice (6-10 weeks old) received various doses of mAb via i.p. injection (in 200 µl) 2 h before being anesthetized with a ketamine (75 mg/kg)/xylazine (15 mg/kg) mixture and receiving an intranasal infection with 5 mLD_{50} of Neth09 virus in 30 µl of PBS (5). All mice were monitored daily, and their weights were recorded for 14 d. Death was determined by a 20% body weight

loss threshold that was authorized by the Rockefeller University Institutional Animal Care and Use Committee.

References

1. Takai T, Li M, Sylvestre D, Clynes R, and Ravetch JV. FcR gamma chain deletion results in pleiotrophic effector cell defects. *Cell.* 1994;76(3):519-29.
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3. Li F, and Ravetch JV. Inhibitory Fcgamma receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. *Science.* 2011;333(6045):1030-4.
4. Krammer F, Pica N, Hai R, Margine I, and Palese P. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. *Journal of virology.* 2013;87(12):6542-50.
5. DiLillo DJ, Tan GS, Palese P, and Ravetch JV. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcgammaR interactions for protection against influenza virus in vivo. *Nature medicine.* 2014;20(2):143-51.

Supplementary Table 1. mAb Characteristics

Patient ID	mAb Name	Original Reference	Immunization
n/a	FI6	1	2009 pandemic H1N1 infection
n/a	6F12	2	Murine sequential immunization with HA
045	045-051310 2B06 (2B06)	3	2009/10 pandemic H1N1 vaccine*
SFV	SFV005-2G02 (2G02)	4	2009/10 pandemic H1N1 vaccine*
SF70	SF70-1F02 (1F02)	5	2009 pandemic H1N1 infection
n/a	7B2	2	Murine immunization with HA
EM	EM-4C04 (4C04)	5	2009 pandemic H1N1 infection
n/a	PY102	6	Murine immunization with HA
SFV	SFV019-4G05 (4G05)	n.p.	2009/10 pandemic H1N1 vaccine*
019	019-10117 1F05 (1F05)	n.p.	2009/10 pandemic H1N1 vaccine*
045	045-051310 1A01 (1A01)	n.p.	2009/10 pandemic H1N1 vaccine*
047	047-051310 1A05 (1A05)	n.p.	2009/10 pandemic H1N1 vaccine*
051	051-051310 4G01 (4G01)	n.p.	2009/10 pandemic H1N1 vaccine*
EM	EM-3C02 (3C02)	5	2009 pandemic H1N1 infection
1000	1000-3C05 (3C05)	5	2009 pandemic H1N1 infection

n.a., not applicable; n.p., not published; *, monovalent vaccine

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Supplementary Table 2. mAb Characteristics

Name	VH	VH CDR3	Original Isotype
FI6	VH3-30	AKDSQLRSLLYFEWLSQGYFDY	huIgG1
6F12	msVH1-4	ARGRQFGLSGAMDY	msIgG2b
2B06	VH1-18	ARDHVQGEVSIYYYAMDV	huIgG1
2G02	VH1-18	ARDRRDLLTGSLGDY	huIgA1
1F02	VH1-69	ARGPYYYGNSHLDF	huIgA1
7B2	msVH1-82	VRNDYYAMDY	msIgG2a
4C04	VH3-21	ARAGLGTVDLRWGGAFDH	huIgG1
PY102	msVH5-6-5	GRVRHYGYGMDY	msIgG1
4G05	VH3-74	ARDHDYGDYRGNAYDI	huIgG1
1F05	VH3-48	VGDDVDVEGYYGLNV	huIgG1
1A01	VH4-31	ARAAKESLCIGGSCDSNYEHYGLDV	huIgG1
1A05	VH1-02	AKDTTHTDCSDGGCYWENFDC	huIgG1
4G01	VH3-07	ARDFQERLLDTLTGCYDY	huIgA1
3C02	VH3-33	ARDSEGSGWYFDY	huIgG1
3C05	VH4-34	ARGRGGYATYYYYYYVDV	huIgG1

Supplementary Table 3. mAb Binding Characterstics

Name	Binding Reactivity (ELISA)	
	Subtype Specificity	Strains Tested
FI6	Group 1 and 2	See reference 1
6F12	Pan-H1	PR8, USSR77, TX91, NC99, Bris07, Cal09
2B06	Group 1 and 2	Wisc05, Uru07, Perth09, Vict11, rhea93, Neth03, Canada04, Shanghai13, Jalisco12, Cal09, SI06, NC99, SC18, H5Viet04
2G02	Group 1 and 2	Cal09, H1Brisb07, NC99, SI06, H5Viet04, H3Uru07, Wisc05, Perth09
1F02	H1 and H5	Cal09, H1Brisb07, NC99, SI06, H5Indo05
7B2	Cal09-Specific	Cal09
4C04	Cal09-Specific	Cal09
PY102	PR8-Specific	PR8
4G05	Pan-H1	Cal09, H1Brisb07, NC99, SI06
1F05	H1 and H3	Cal09, SI06, Uru07, Perth09, Wisc05
1A01	Pan-H1	Cal09, H1Brisb07, NC99, SI06
1A05	Pan-H1	Cal09, H1Brisb07, NC99, SI06
4G01	Pan-H1	Cal09, H1Brisb07, NC99, SI06
3C02	Cal09-Specific	Cal09
3C05	Pan-N1	Cal09, H1Brisb07

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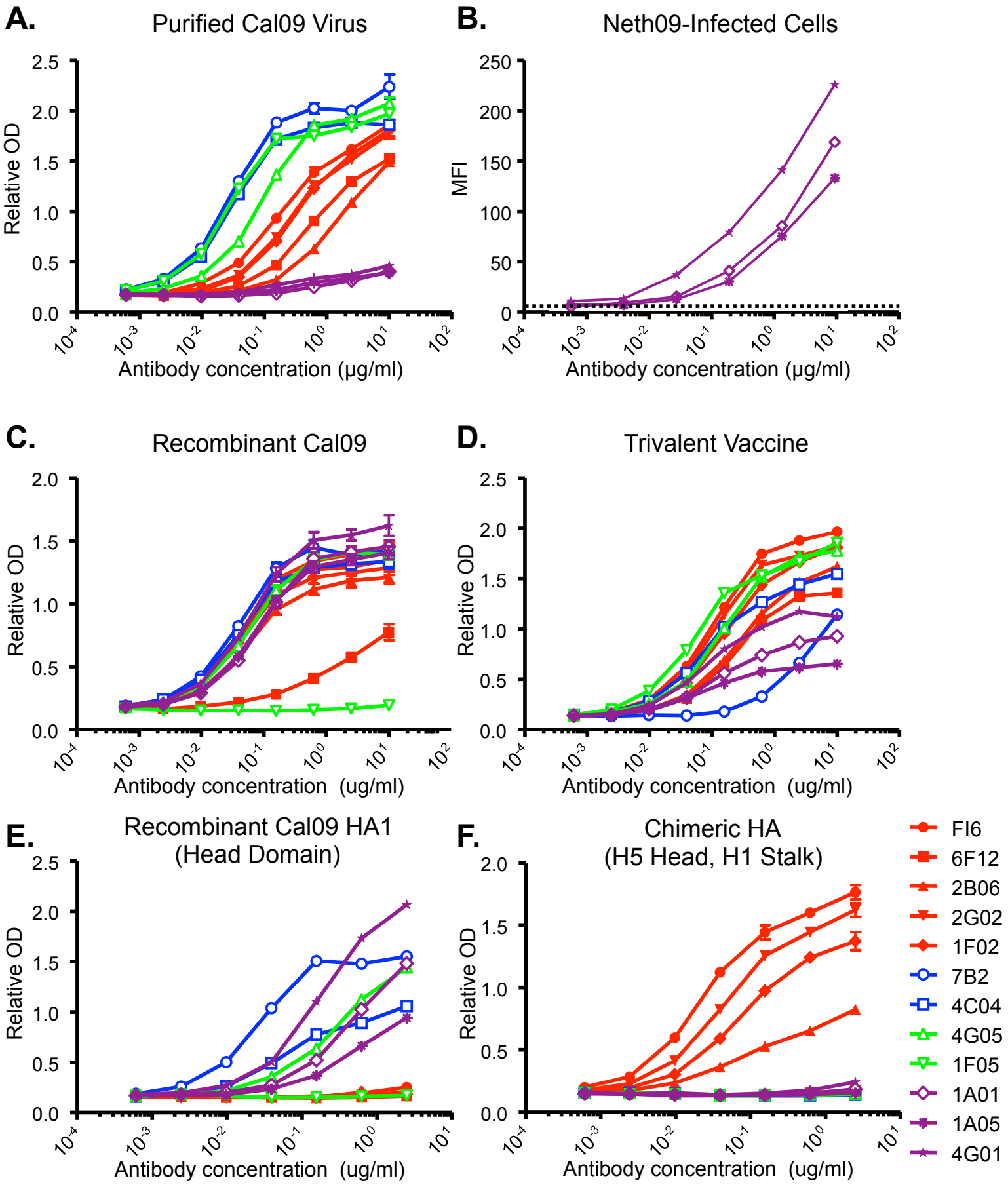
Supplementary Table 4. mAb Neutralization Characteristics

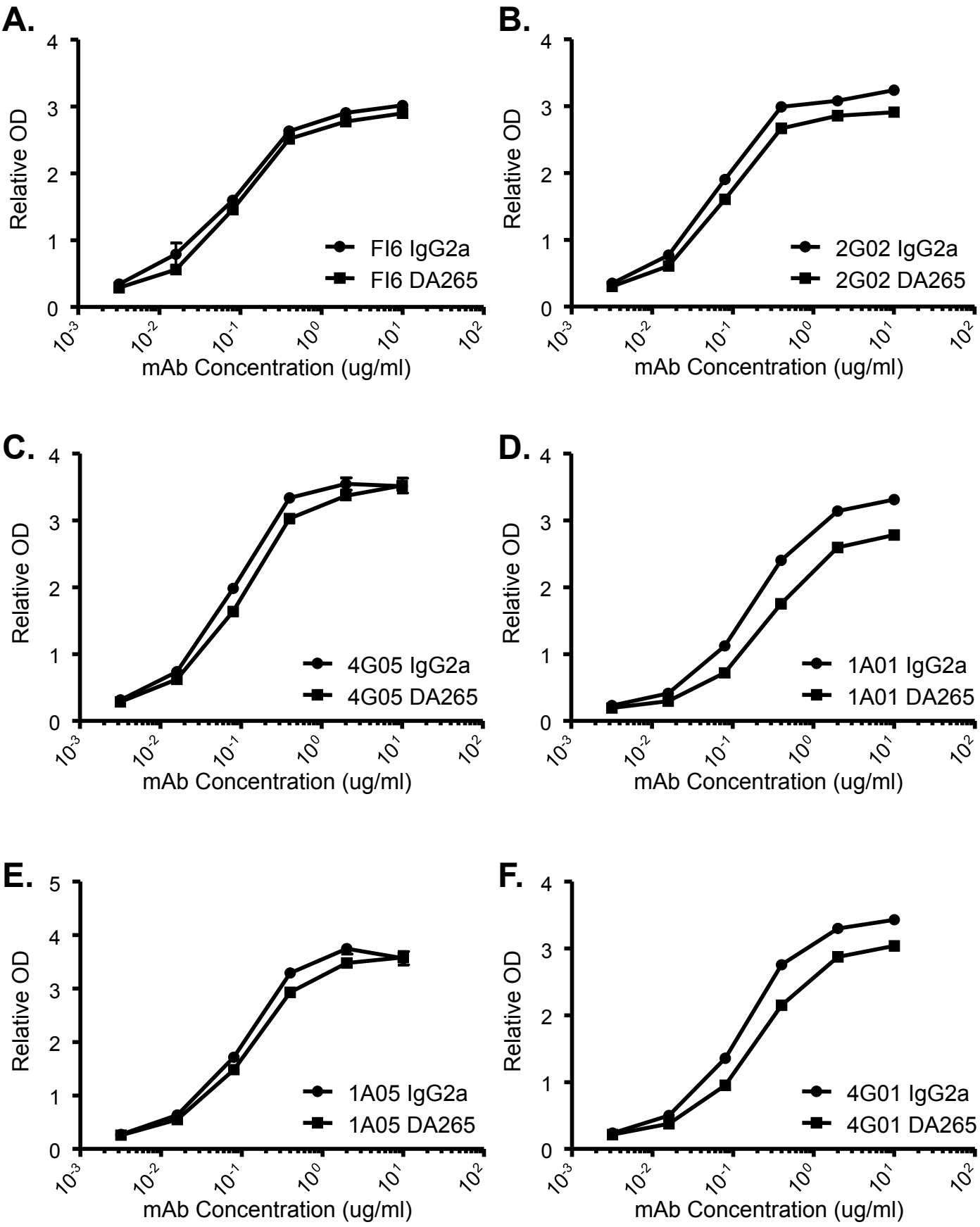
Name	HAI	In vitro neutralization		
		Positive?	Subtypes Neutralized	Strains Tested
FI6	-	+	H1, H3, H5, H7	See reference 1
6F12	-	+	H1	PR8, sw30, USSR77, TX91, NC99, Cal09
2B06	-	+	H1, H3, H7	Wisc05, Uru07, Perth09, Vict11, Cal09, Shanghai13, Jalisco12
2G02	-	+	H1, H3	Cal09, SI06
1F02	-	+	H1, H5	PR8, FM, NC99, SI06, H1Brisb07, Cal09
7B2	+	+	H1	Cal09
4C04	+	+	H1	Cal09
PY102	+	+	H1	PR8
4G05	+	+	H1	Cal09
1F05	+	+	H1, H3	Cal09
1A01	-	-	-	-
1A05	-	-	-	-
4G01	-	-	-	-
3C02	-	+	N1	Cal09
3C05	-	+	N1	Cal09

1. Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, Vachieri SG, Pinna D, Minola A, Vanzetta F, Silacci C, Fernandez-Rodriguez BM, Agatic G, Bianchi S, Giacchetto-Sasselli I, Calder L, Sallusto F, Collins P, Haire LF, Temperton N, Langedijk JP, Skehel JJ, Lanzavecchia A. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. Science. 2011 Aug 12;333(6044):850-6.

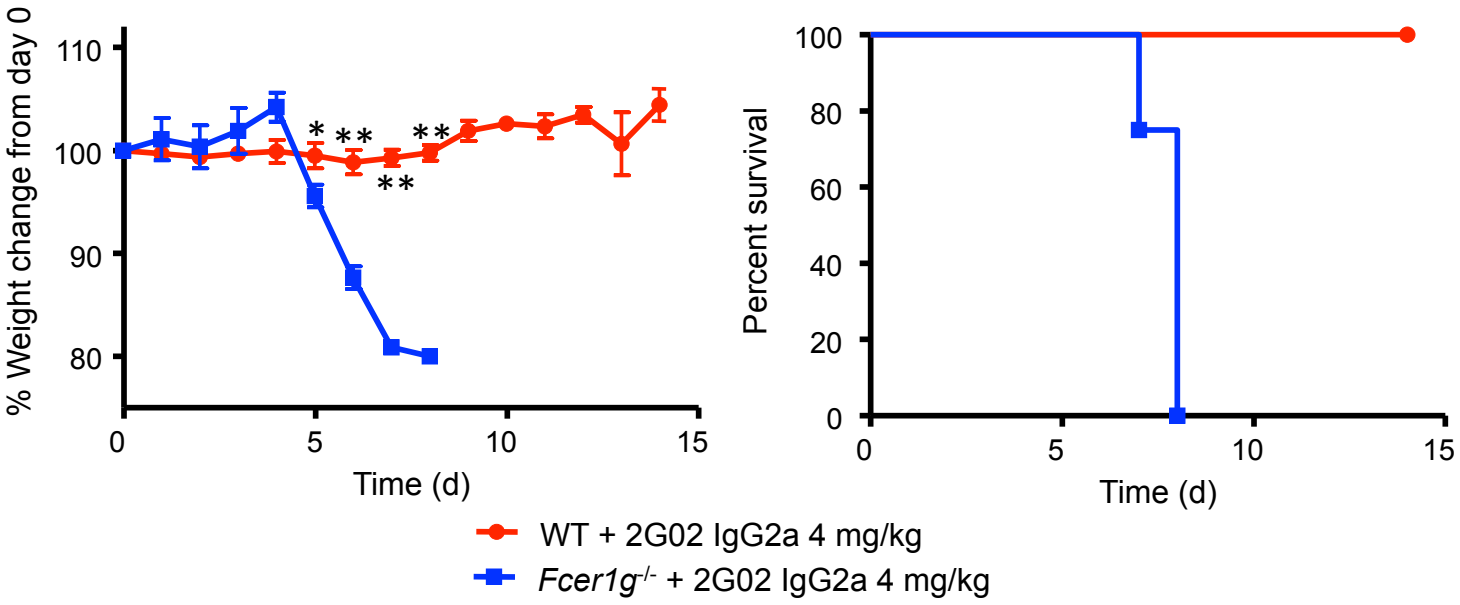


Supplementary Figure 1. Anti-HA mAb binding characteristics. (**A-F**) Broadly-neutralizing anti-stalk (red lines), strain-specific anti-head (blue lines), broadly-neutralizing anti-head (green lines), and non-neutralizing anti-head (purple lines) mAb binding to purified Cal09

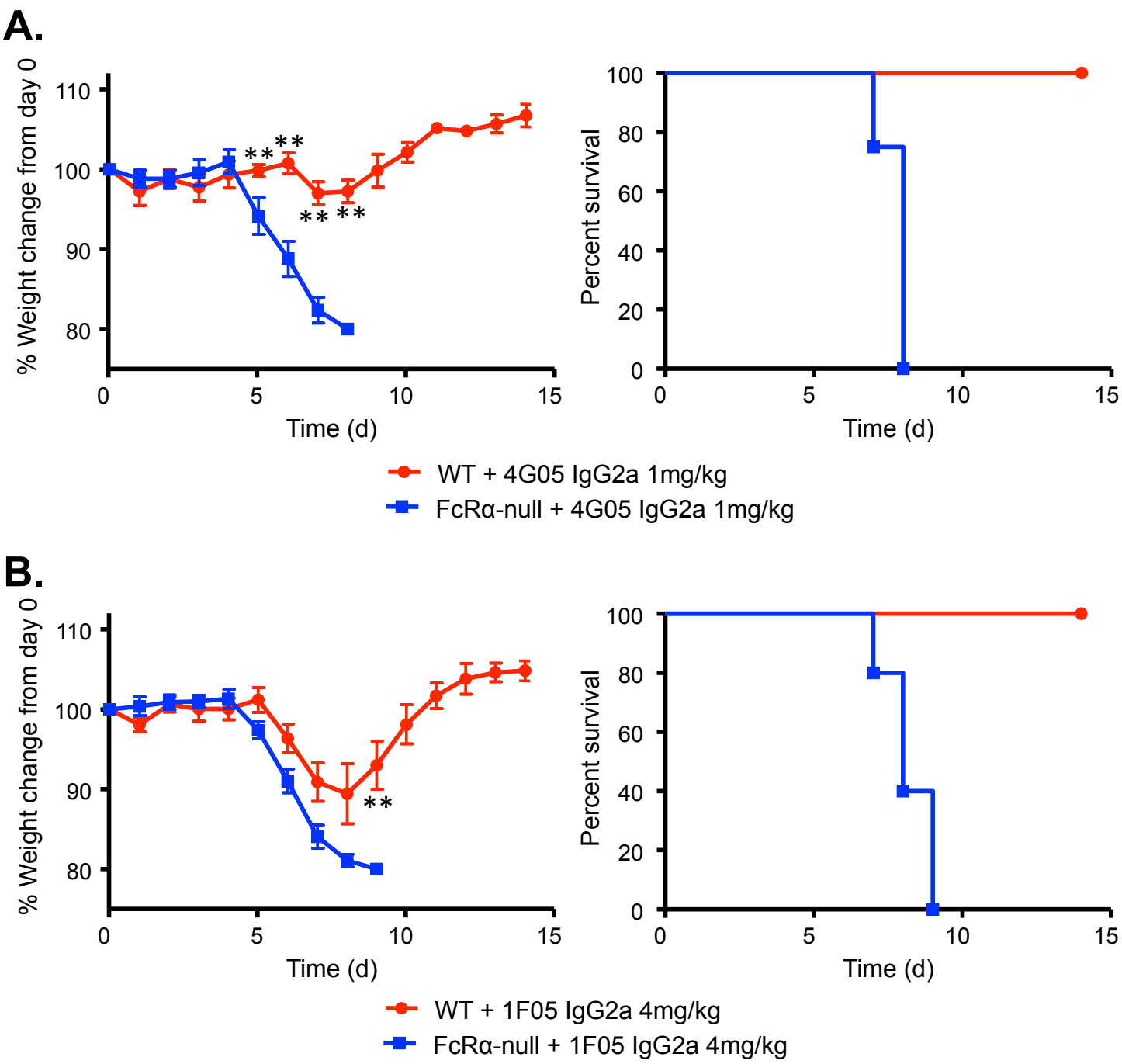
Supplementary Figure 1 (continued)
Virus (A), Neth09-infected A549 cells (B), recombinant Cal09 H1N1 protein (C), 2014-2015 trivalent vaccine (D), recombinant Cal09 HA1 head domain protein (E), or chimeric H5-head/H1-stalk HA protein (F) by ELISA assay (A, C, D, E, F) or flow cytometry analysis (B). Values represent mean (± SEM) relative OD values or mean fluorescence intensities (MFI) from triplicate (A, C, F) or duplicate (E) samples. Dashed line in (B) represents the level of detection in the flow cytometry assay as determined by mean MFI of samples stained with secondary antibody alone.



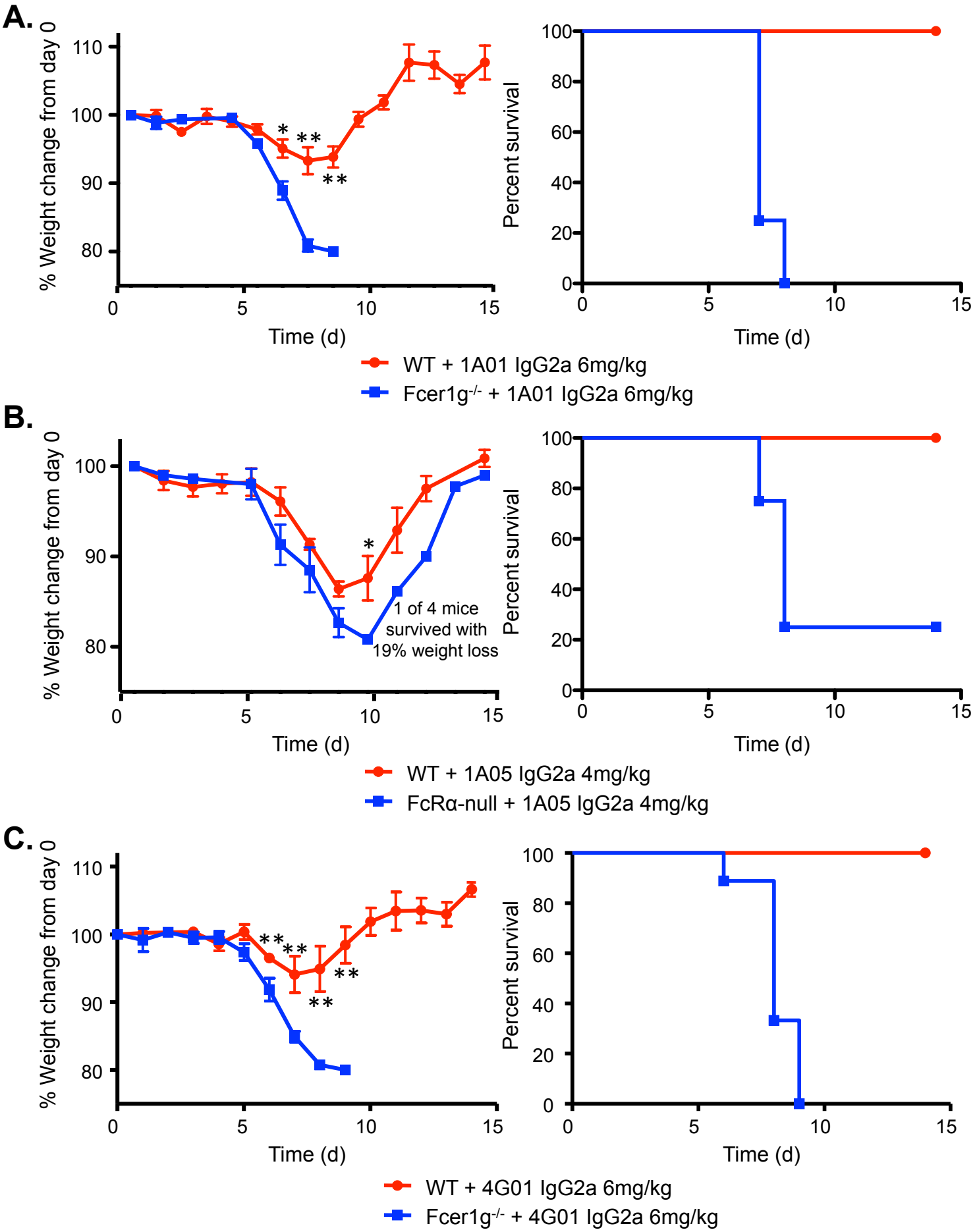
Supplementary Figure 2. IgG2a and DA265 mutant antibodies bind HA similarly. Mouse IgG2a (circles) and DA265 mutant (squares) mAbs were diluted as indicated and tested for binding to recombinant Cal09 HA by ELISA. Values represent mean (± SEM) relative OD values from duplicate wells.



Supplementary Figure 3. Broadly-neutralizing anti-HA stalk mAb requires Fc-FcR interactions to mediate protection in vivo. Wild type (WT) mice or *Fcer1g*$^{-/-}$ mice were treated with 4 mg/kg mouse IgG2a 2G02 mAb 2 hours before intranasal infection with 5 mLD50 of Neth09 virus. Values represent mean (±SEM) % weight change compared to day 0 (left panel) or % survival (right panel). n≥4 mice per group. Significant differences (student's t-test) between *Fcer1g*$^{-/-}$ mice and WT mice receiving mAb are shown: *, $p<0.05$; **, $p<0.001$.

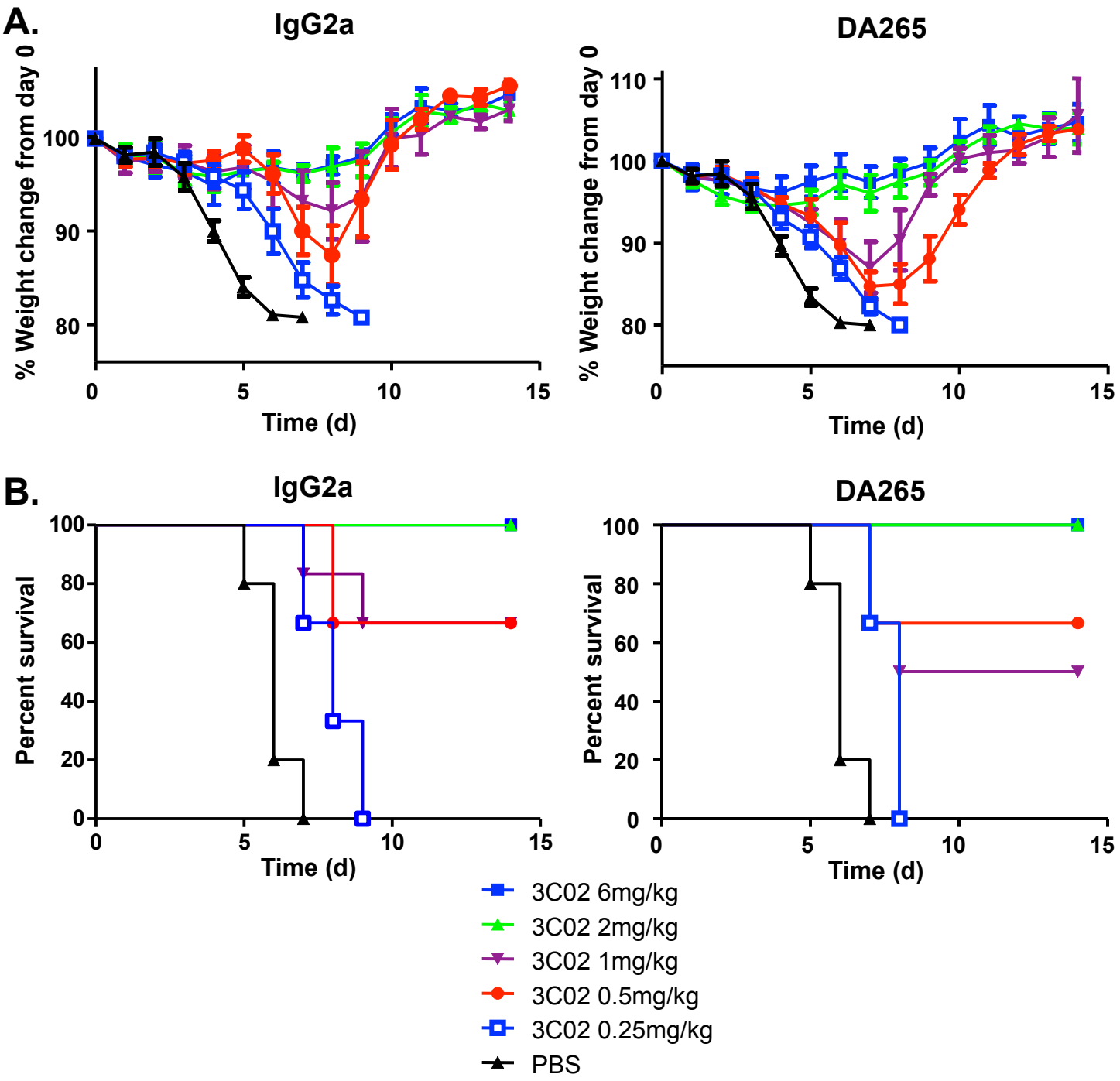


Supplementary Figure 4. Broadly-neutralizing anti-HA head mAbs require Fc-FcR interactions to mediate protection in vivo. Wild type (WT) mice or FcRα-null mice were treated with 1 mg/kg mouse IgG2a 4G05 mAb (**A**) or 4 mg/kg mouse IgG2a 1F05 mAb (**B**) 2 hours before intranasal infection with 5 mLD50 of Neth09 virus. Values represent mean (±SEM) % weight change compared to day 0 (left panel) or % survival (right panel). n≥4 mice per group. Significant differences (student's t-test) between FcRα-null mice and WT mice receiving mAb are shown: *, $p<0.05$; **, $p<0.001$.



Supplementary Figure 5. Pan-H1, non-neutralizing anti-HA head mAbs require Fc-FcR interactions to mediate protection in vivo. Wild type (WT) mice or *Fcer1g*$^{-/-}$ mice were treated with 6 mg/kg 1A01 (**A**), 4 mg/kg 1A05 (**B**), or 6 mg/kg 4G01 (**C**) mAb (IgG2a isotype)

Supplementary Figure 5 (continued)
2 hours before intranasal infection with 5 mLD50 of Neth09 virus. Values represent mean (±SEM) % weight change compared to day 0 (left panel) or % survival (right panel). n≥4 mice per group. Significant differences (student's t-test) between FcRα-null mice and WT mice receiving mAb are shown: *, $p<0.05$; **, $p<0.001$.



Supplementary Figure 6. Strain-specific, neutralizing anti-NA mAb does not require Fc-FcR interactions to mediate protection in vivo. Wild type mice were treated with the indicated doses of mouse IgG2a or DA265 mutant 3C02 mAb, or PBS, 2 hours before intranasal infection with 5 mLD50 of Neth09 virus. Values represent mean (±SEM) % weight change compared to day 0 (top panels) or % survival (bottom panels). n=4-6 mice per group.