Supporting Information

Galli et al. 10.1073/pnas.0903181106

SI Text

Eligibility Criteria. Subjects were excluded if they had known allergy to eggs, antibiotics, or any vaccine; immunosuppression as a result of disease or treatment; any medical condition that would interfere with vaccine responses; pregnancy or refusal to use reliable contraception during the study; receipt of blood products in the preceding 3 months; any other vaccination or experimental drug use during the previous 4 weeks; use of antibiotics within previous 7 days; or fever higher than 38 °C in the previous 3 days. Women had a negative urine pregnancy test before any immunization. All participants had to provide written informed consent.

Vaccine Preparation. The seed virus, reassortant A/Vietnam/1194/2004/NIBRG-14, was supplied by the United Kingdom National Institute for Biological Standards and Controls and is recommended as a pandemic vaccine strain by the EU CHMP. The virus was propagated on embryonated hens' eggs, and vaccine was produced by Novartis Vaccines and Diagnostics Srl by standard processes used for inter-pandemic vaccines. Hemagglutinin content was determined by single radial immunodiffusion. Each vaccine dose was formulated as 0.5 ml in a prefilled monodose syringe and contained 7.5 μg H5 hemagglutinin, 9.75 mg squalene, 1.175 mg polysorbate 80, 1.175 mg sorbitan trioleate, sodium citrate dehydrate, and citric acid monohydrate.

Vaccination of Subjects. Participants recorded local symptoms (erythema, swelling, induration, pain, ecchymosis) by diameter of reaction, systemic symptoms (chills, malaise, myalgia, arthralgia, nausea, headache, sweating, fatigue), temperature, and analgesic use on self-completed diaries for 7 days. Systemic symptoms were graded as none, mild (if symptoms did not interfere with normal activities), moderate (if symptoms resulted in some interference with normal activities), or severe (if symptoms prevented subjects from engaging in daily activities). Screening for additional reactogenicity conducted when serum was collected and at each postvaccination visit.

Enumeration of H5N1-Specific Memory B Cells. Peripheral mononuclear blood cells were isolated from heparinized whole blood obtained on days 0, 21, 42, and 202. Parallel negative control cultures were run in medium without mitogens. On day 10, supernatants were collected separately and kept at –20 °C until tested in ELISA for their content in H5N1-specific and total IgG. Wells displaying an OD $\geq$ 0.4 (total-IgG) or an OD $\geq$ 0.45 (H5N1-IgG) at 405 nm were considered positive. Frequencies of H5N1-IgG-secreting cell precursors were expressed as percentages of the total IgG secreting cell precursors measured.

Statistical Analysis. Percentages of participants who reported postvaccination reactions were based on the most severe response reported. The overall frequency of local or systemic reactions between priming vaccination groups was compared by Fisher's exact test. GMTs of antibody and 95% CI were calculated by taking the exponential of the least square means and of the upper and lower limits of associated 95% CI of $\log_{10}$ transformed titers, which were obtained from an analysis of variance with 1 factor for the priming vaccination group. P-values for comparisons between priming groups also were obtained from an ANOVA with 1 factor for the priming vaccination group. For each priming vaccination group, 95% CI for seroprotection or seroconversion was calculated according to Clopper-Pearson. Generally confidence intervals and p-values were not adjusted for multiplicity. P-values $\leq$ 0.05 were regarded as significant. In addition to GMT, other end points were based on HI and SRH CHMP criteria. For the analysis of H5N1-IgG MBC response, comparisons between groups were done by 1-factor ANOVA (WinSTAT™ for Excel 2002, Microsoft). Regression analysis was applied to the whole dataset to determine the existence, at any point before or after vaccination, of a linear correlation between $\log_{10}$ transformed frequency of H5N1-IgG MBC and $\log_{10}$ transformed neutralization titers (WinSTAT™ for Excel 2002).

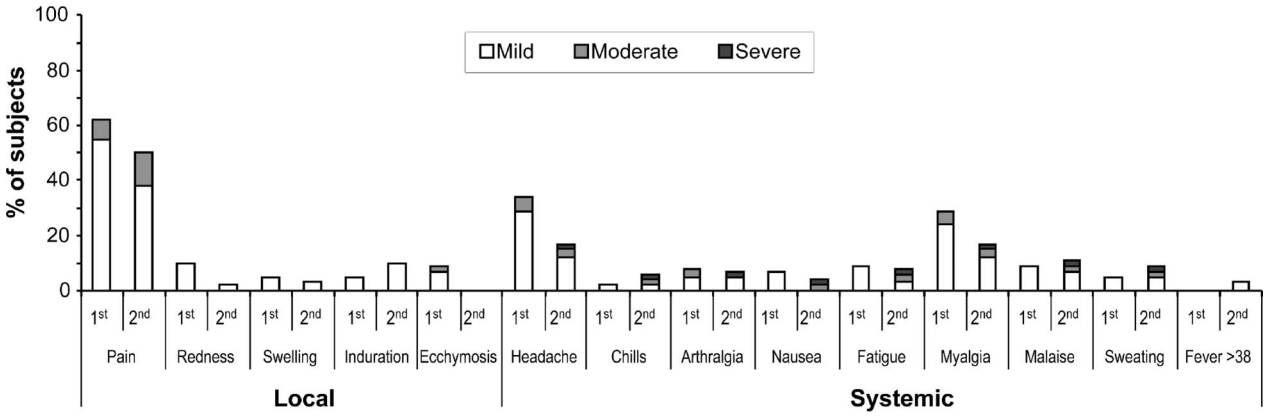

Fig. S1. Incidence of solicited local and systemic adverse reactions after MF59-adjuvanted A/Vietnam/1194/2004/NIBRG-14 vaccine. All reactions were self-limiting and graded as mild or moderate except for 1 subject in the MF59-primed group who experienced self-limiting gastroenteritis with associated symptoms that were graded as severe after receipt of the second vaccine dose. Her family concurrently experienced similar symptoms, and these symptoms were not considered vaccine related.

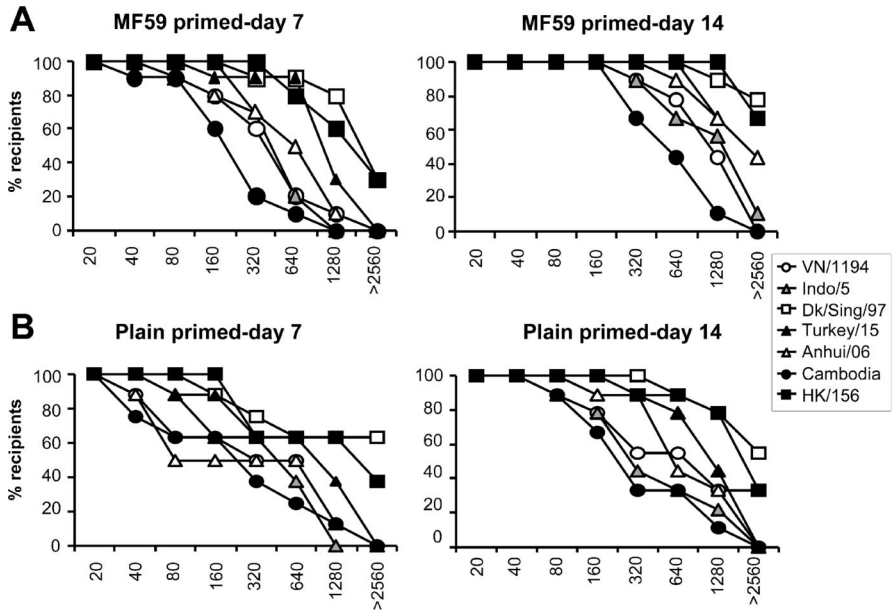

Fig. S2. Reverse cumulative distribution curves of neutralizing antibody responses after booster vaccine in (*A*) MF59-H5-primed and (*B*) plain-H5-primed groups. The percentage of recipients achieving neutralization titer is based on the total number of samples available. Viruses used: A/Vietnam/1194/2004 (clade 1) A/Indonesia/5/2005 (clade 2.1), A/duck/Singapore/97 (clade 0), A/Turkey/15/2006 (clade 2.2), A/Anhui/1/2005 (clade 2.3), A/Cambodia/R0405050/2007 (clade 1), and A/Hong Kong/156/97 (clade 0).

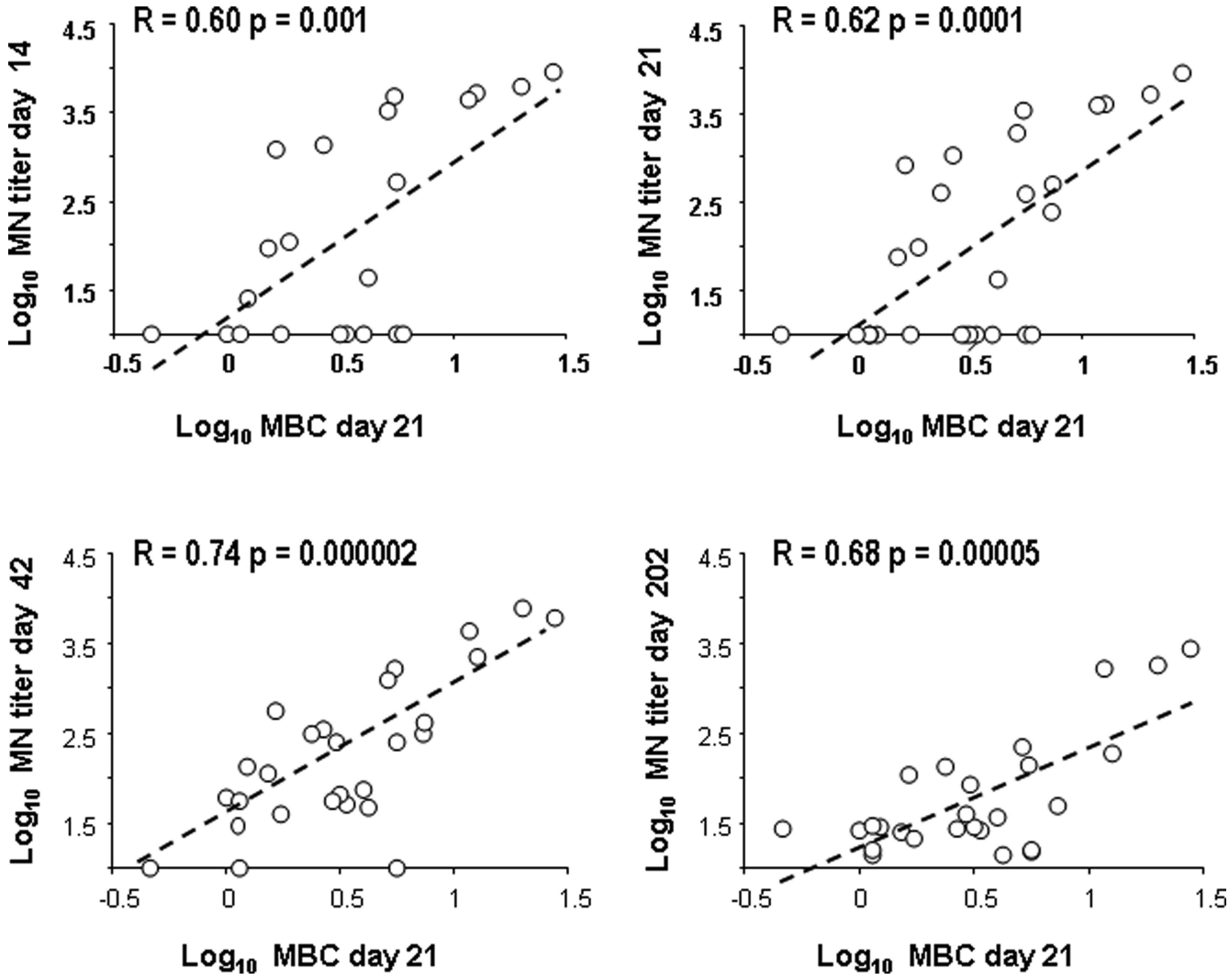

Fig. S3. Linear correlation between memory B cell (MBC) and neutralizing antibody responses to A/Vietnam/1194/2004. Shown are dot plots of paired H5N1-IgG MBC frequency (log_{10} transformed) at day 21 and neutralizing titers (log_{10}) at day 14. ρ and p-values for simple linear regression between paired data within the whole dataset are indicated above each plot.

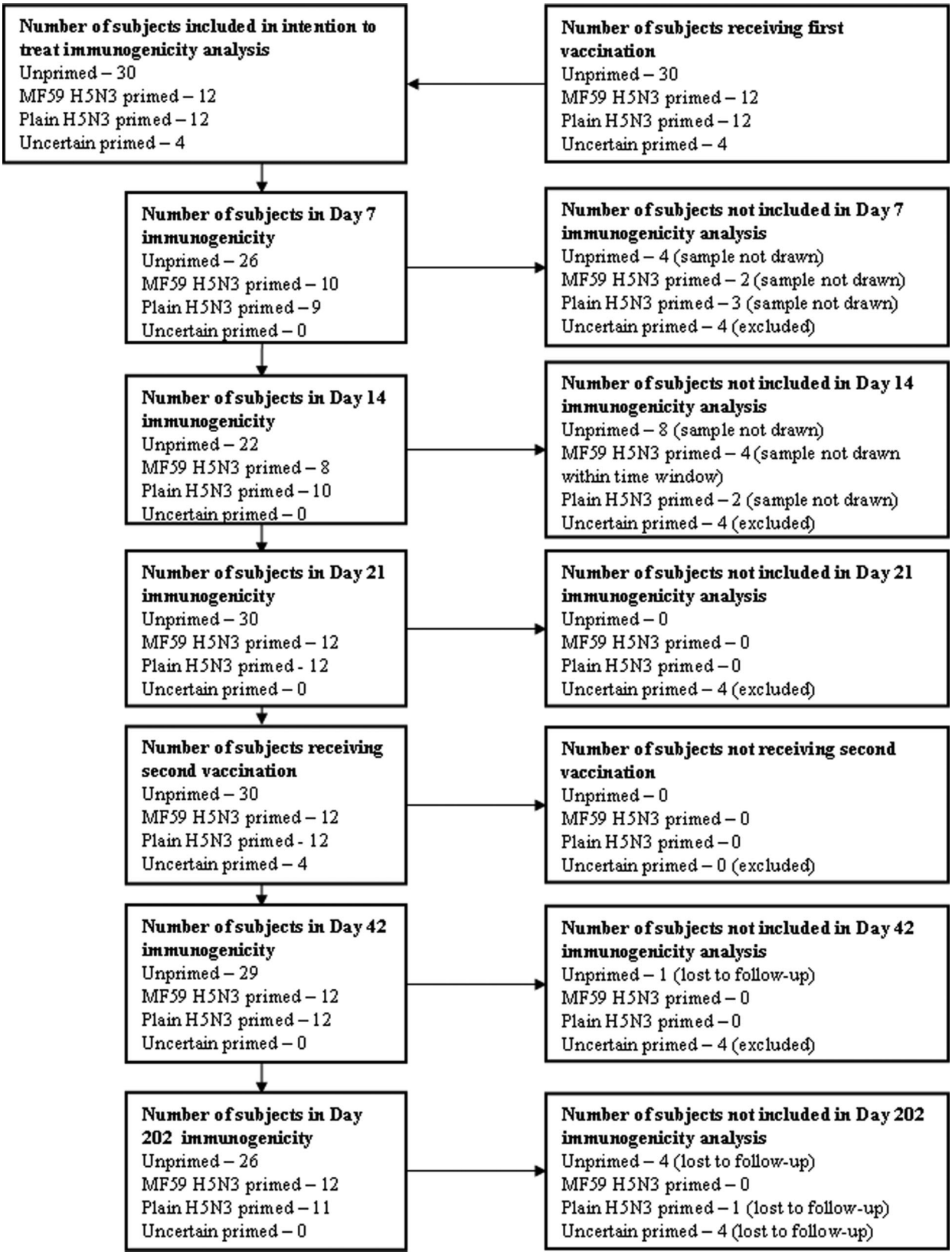

Fig. S4. Trial profile.

Table S1. Serological responses to reassortant A/turkey/Turkey/1/2005/NIBRG-23 vaccine strain

	Mean Geometric Increase in Antibody: Ratio to Day 0 GMT (95% Confidence Interval)			Frequency of Seroconversions (95% Confidence Interval)			Seroprotection Rate (95% Confidence Interval)		
Group	Unprimed $N = 30$	MF59-H5 $N = 12$	Plain-H5 $N = 12$	Unprimed $N = 30$	MF59-H5 $N = 12$	Plain-H5 $N = 12$	Unprimed $N = 30$	MF59-H5 $N = 12$	Plain-H5 $N = 12$
Hemagglutination-inhibition (HI) responses									
Day 7	1.2 (0.7–2.0)	21* (8.9–50)	12* (4.7–29)	8% (1–25)	80%* (44–97)	56%* (21–86)	8% (1–25)	90%* (55–100)	56%, (21–86)
Day 14	1.4 (0.8–2.5)	56* (21–147)	28* (12–66)	14% (3–35)	88%* (47–100)	80%* (44–97)	14% (3–35)	100%* (63–100)	80%* (44–97)
Day 21	1.6 (1.0–2.5)	52* (26–104)	25* (12–49)	13% (4–31)	92%* (62–100)	83%* (52–98)	13% (4–31)	100%* (74–100)	83%* (52–98)
Day 42	2.0 (1.2–3.2)	45* (21–96)	16* (7.8–3.21)	21% (8–40)	92%* (62–100)	75%* (43–95)	21% (8–40)	100%* (74–100)	75%* (43–95)
Single Radial Hemolysis (SRH) responses									
Day 7	1.9 (1.3–2.8)	11* (6.2–20)	8.3* (4.5–15)	15% (4–35)	90%* (55–100)	67%* (30–93)	15% (4–35)	90%* (55–100)	78%* (40–97)
Day 14	3.3* (2.3–4.7)	18* (9.8–33)	11* (6.4–19)	32% (14–55)	100%* (63–100)	80%* (44–97)	32% (14–55)	100%* (63–100)	90%* (55–100)
Day 21	3.5* (2.6–4.7)	17* (10–27)	11* (6.9–18)	30% (15–49)	100%* (74–100)	83%* (52–98)	30% (15–49)	100%* (74–100)	92%* (62–100)
Day 42	7.7* (6.1–9.7)	17* (12–25)	12* (8.2–17)	86%* (68–96)	100%* (74–100)	92%* (62–100)	90%* (73–98)	100%* (74–100)	100%* (74–100)
Microneutralization antibody (MN) responses									
Day 7	1 (0.6–1.6)	48 (23–99)	21 (9.9–46)	0% (0–13)	90% (55–100)	78% (40–97)	0% (0–13)	90% (55–100)	78% (40–97)
Day 14	1.2 (0.8–1.9)	204 (102, 409)	54 (29–100)	9% (1–29)	100% (63–100)	100% (69–100)	9% (1–29)	100% (63–100)	100% (69–100)
Day 21	1.2 (0.8–1.6)	133 (80–222)	41 (25–68)	7% (1–22)	100% (74–100)	92% (62–100)	7% (1–22)	100% (74–100)	92% (62–100)
Day 42	1.6 (1.1–2.3)	102 (59–176)	29 (17–50)	10% (2–27)	100% (74–100)	100% (74–100)	10% (2–27)	100% (74–100)	100% (74–100)

GMT = geometric mean titer.

Seroconversion for HI and MN is defined as a 4-fold or greater increase in antibody titer. Seroconversion for SRH is defined as a 50% or greater increase in area. Seroprotection for HI is defined as a titer of 1:32 or greater. Seroprotection for SRH is defined as a titer of 25 mm^2 or greater. Seroprotection for MN is defined as a titer of 1:40 or greater. EU Committee for Medicinal Products for Human Use (CHMP) criteria for HI and SRH responses (in persons age 18–60 years) are mean geometric increase > 2.5, seroconversion rate $> 40\%$; seroprotection rate $> 70\%$. There are no equivalent MN criteria. Figures marked by asterisks fulfill CHMP criteria for inter-pandemic vaccines.

Table S2. Demographics of study participants

Characteristic	Vaccine group and number of subjects		
	Unprimed $n = 30$	MF59 H5 primed $n = 12$	Plain H5 primed $n = 12$
No. of doses and dose content (μg) of priming H5N3 vaccine			
None	30 (100%)	0 (0%)	0 (0%)
2 doses	0 (0%)	5 (42%)	7 (58%)
3 doses	0 (0%)	7 (58%)	5 (42%)
Ethnicity			
White	20 (67%)	11 (92%)	12 (100%)
Asian	10 (33%)	1 (8%)	0 (0%)
Female sex	17 (57%)	7 (58%)	9 (75%)
Median age in years (range)	36.5 (23–60)	33 (28–47)	36 (26–47)
Median weight in kg (range)	72 (49–105)	72 (56–106)	71 (50–122)