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Supplementary Materials for

An influenza hemagglutinin stem nanoparticle vaccine induces cross-group 1 neutralizing antibodies in healthy adults

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

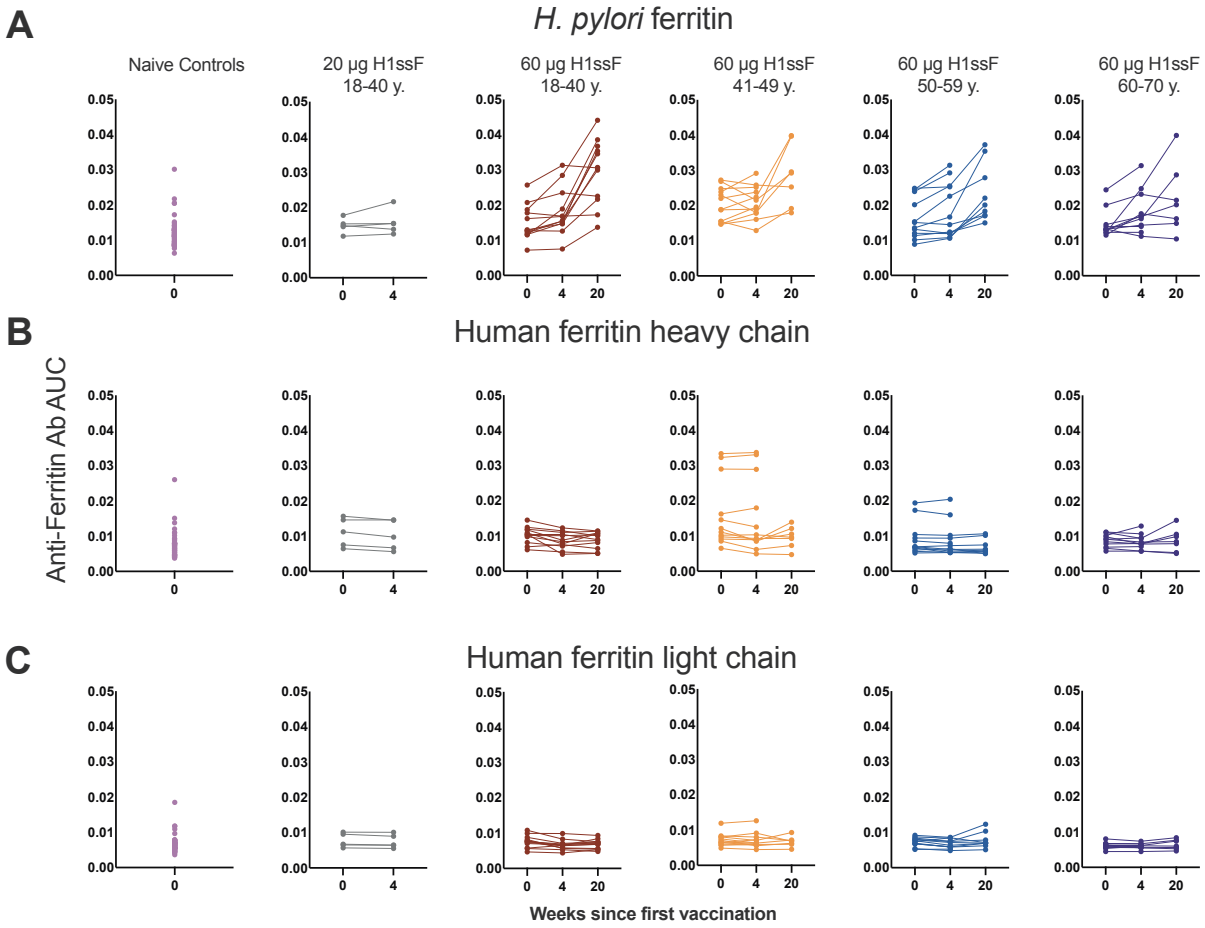

Fig. S1. Vaccination with H1ssF elicits antibodies against *H. pylori* ferritin. (**A to C**) Antibodies binding *H. pylori* ferritin (A), human ferritin heavy chain (B) and human ferritin light chain (C) were assessed by enzyme-linked immunosorbent assay (ELISA). Antibody responses from 47 human naïve control samples are included in the first column alongside responses from the trial participants stratified by dose and age (column headers). ss, stabilized stem; Ab, antibody; AUC, area under the curve.

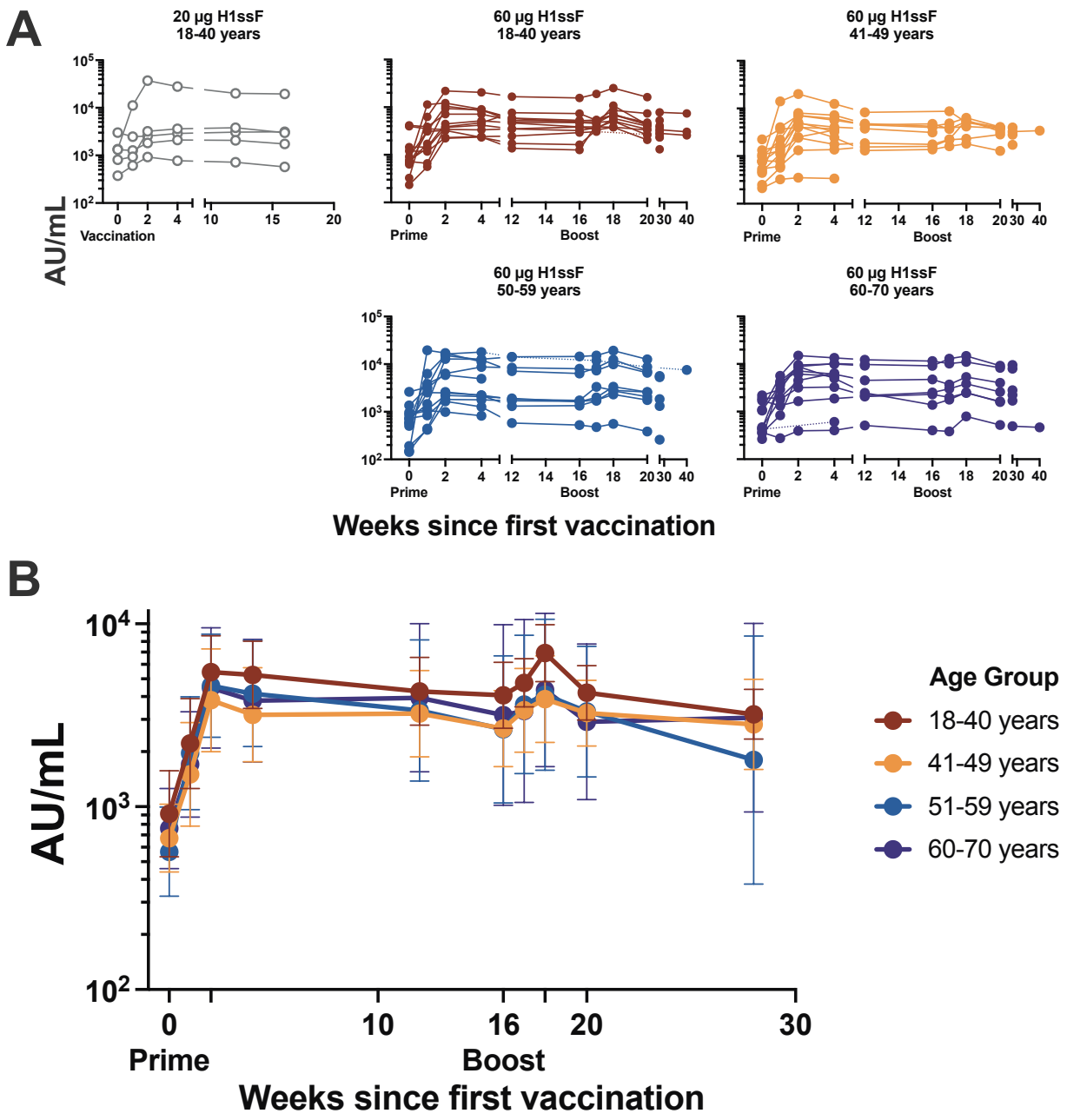

Fig. S2. H1ssF vaccination elicits antibodies that bind to the H1 stem. (**A and B**) H1ss binding antibody concentrations of individuals (A) or age groups (B) against H1 (A/New Caledonia/20/1999) stem were measured by electrochemiluminescence immunoassay (ECLIA) and are reported as arbitrary unit (AU)/mL. In (A), samples from the same individual are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed. In (B), geometric means and 95% confidence intervals (CIs) are presented stratified by age. Additional ticks in (B) denote primary immunogenicity endpoints at week 2 and 18. Exact numbers of participant samples analyzed at each time point are listed in table S4.

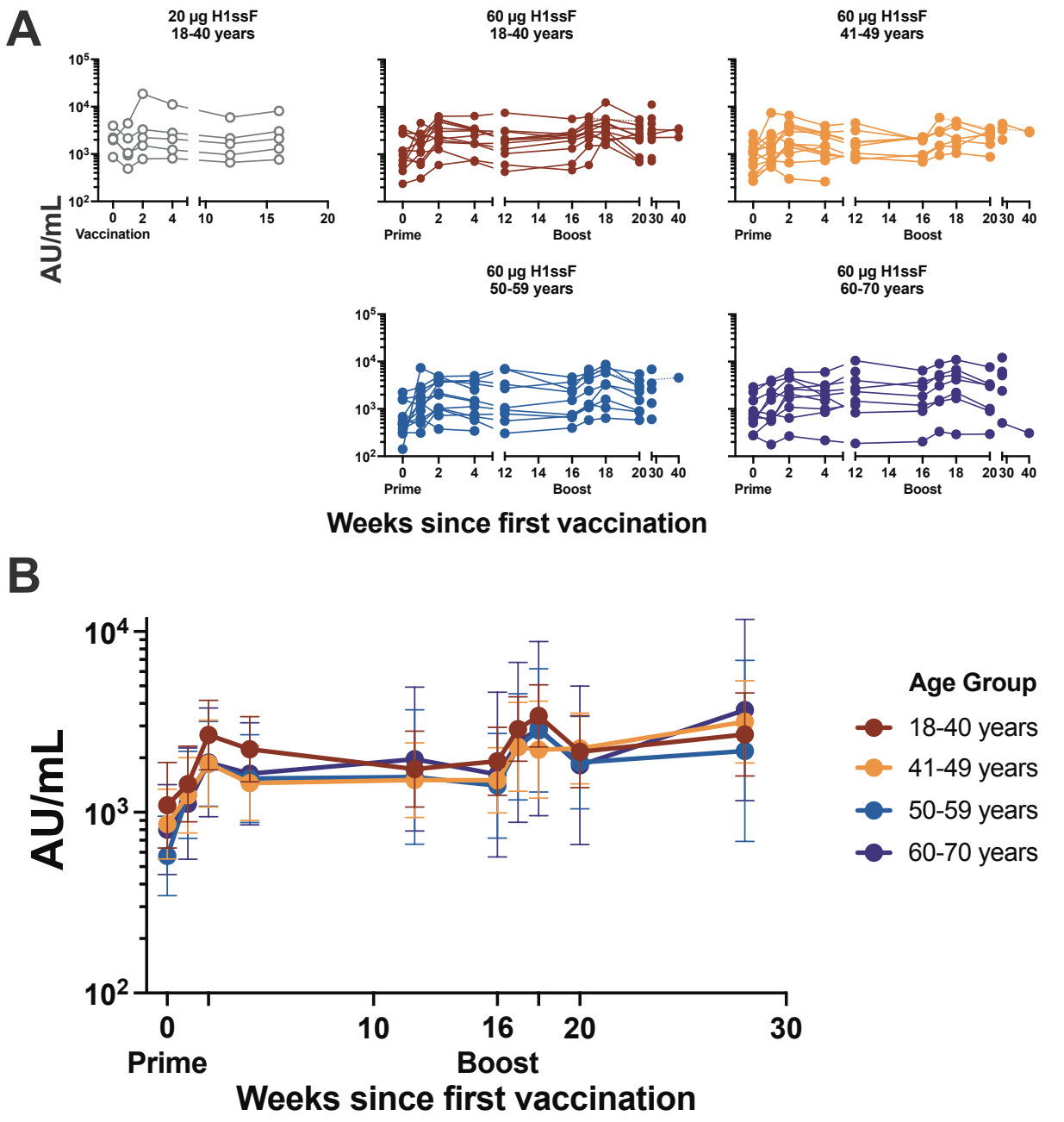

Fig. S3. H1ssF vaccination elicits antibodies that bind to full-length H1. (**A and B**) H1FL binding antibody concentrations of individuals (A) or age groups (B) against full-length H1 (A/New Caledonia/20/1999) were measured by ECLIA and are reported as AU/mL. In (A), Samples from the same individual are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed. In (B), geometric means and 95% CIs stratified by age. Additional ticks in (B) denote primary immunogenicity endpoints at week 2 and 18. Exact numbers of participant samples analyzed at each time point are listed in table S4.

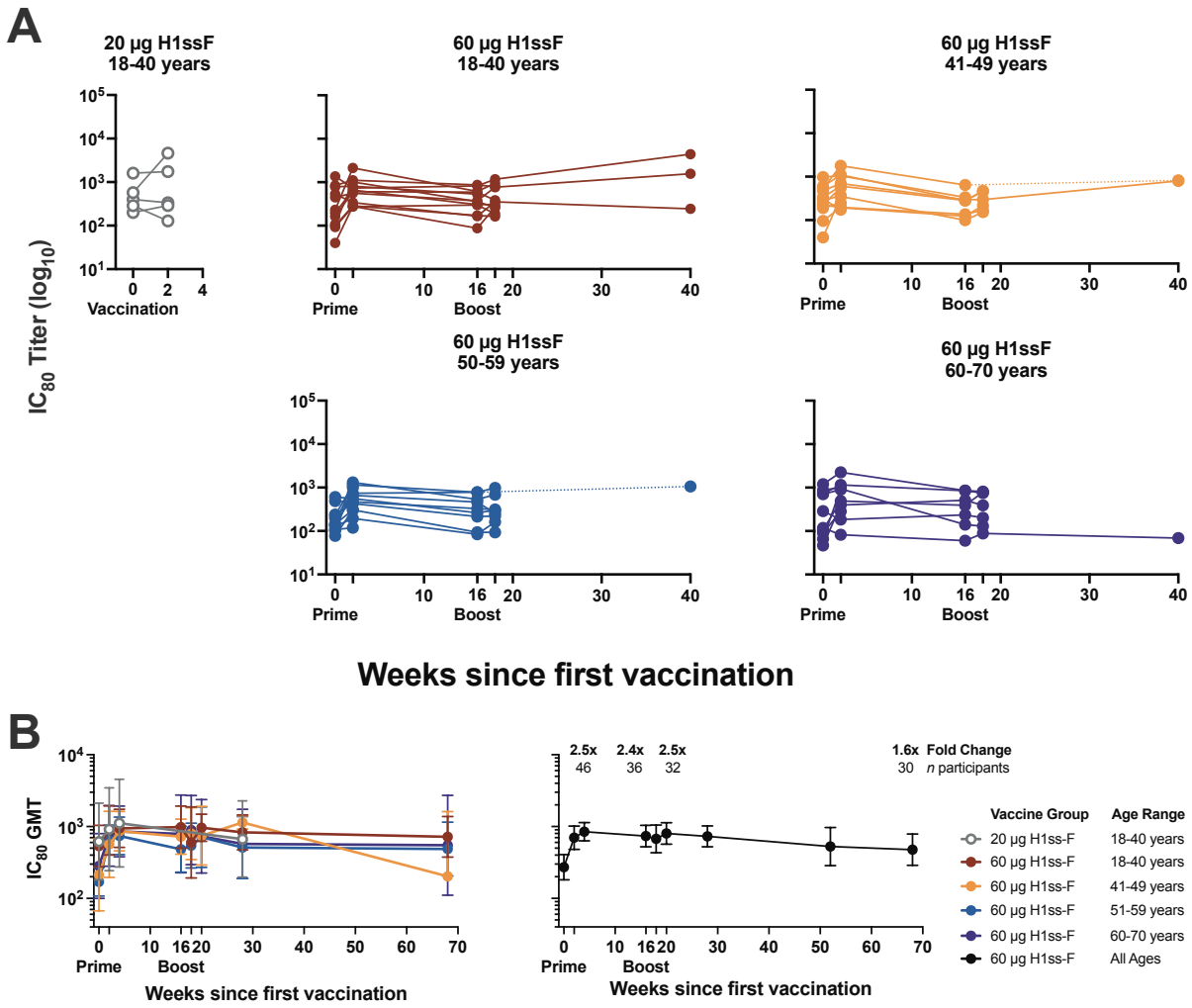

Fig. S4. H1ssF vaccination elicits H1 neutralizing antibodies. (**A and B**) Neutralizing antibody titers (IC_{80}) against H1 A/New Caledonia/20/1999 were determined by microneutralization assay (A) and pseudotyped lentiviral neutralization assay (B). In (A), individual participants' neutralizing antibody titers are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed. In (B), geometric mean neutralization antibody titers (IC_{80} GMT) and 95% CIs are shown stratified by dose and age in the left panel, and for all 60 µg dose recipients in the right panel. Fold changes over baseline and number of participants evaluated are indicated in the right panel at weeks 2, 16, 18, and 68. Additional ticks denote primary immunogenicity endpoints at week 2 and 18. Exact numbers of participant samples analyzed at each time point are listed in table S4.

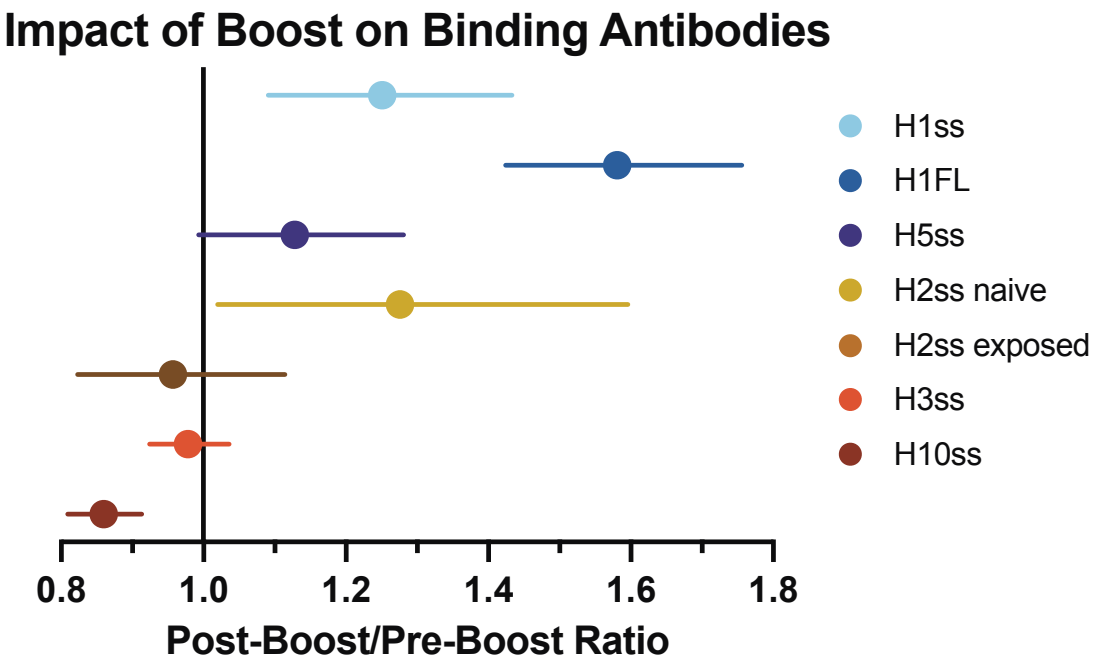

Fig. S5. The H1ssF boost improved the magnitude of the antibody response against H1 influenza hemagglutinin. For 60 µg dose recipients, week 20 and week 18 ECLIA binding concentration geometric mean values were averaged together and divided by the average of the week 12 and 16 geometric mean values. The geometric mean ratio of the post-boost over the pre-boost timepoints is graphed along with 95% CIs of the ratio. The post-boost binding antibody concentrations were significantly different than pre-boost binding antibody concentrations for H1ss (p=0.002), H1FL (p<0.001), and H2ss naïve (p=0.034). Only two participants had a non-H1ssF influenza exposure (one vaccination, one influenza infection) between weeks 12 and 20.

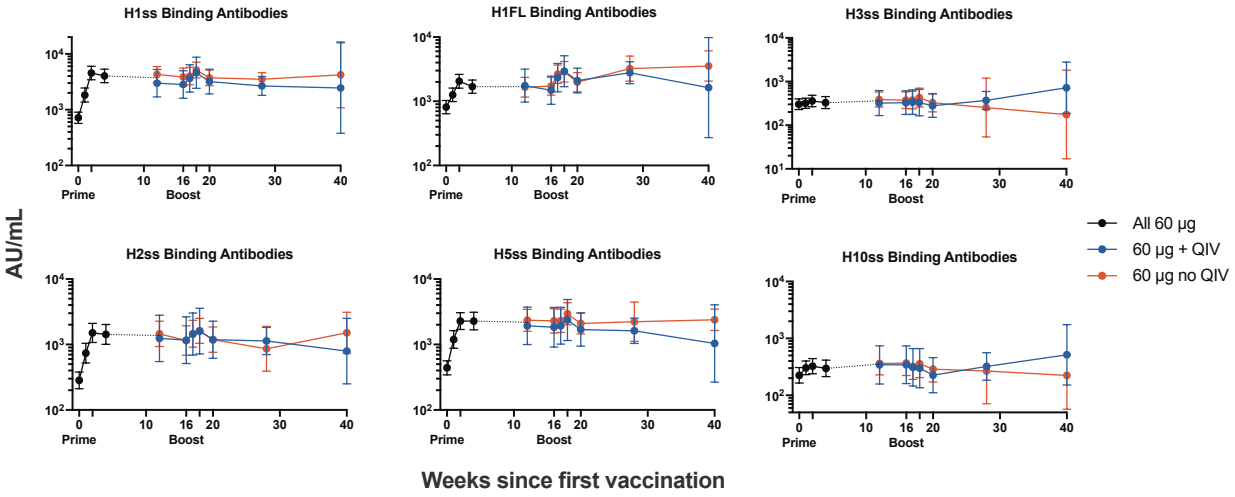

Fig. S6. Receipt of seasonal quadrivalent influenza vaccine (QIV) during the trial did not impact binding antibody responses to the H1ssF vaccine. Geometric mean binding antibody concentrations and 95% CIs are shown for the 60 µg dose recipients' serum stratified by receipt of QIV immunization. All participants' samples were assigned to the "+ QIV" group following the date of their first QIV immunization during the trial and thereafter. No significant differences were observed between participants based on receipt of QIV for any antigen. Exact numbers of participant samples analyzed at each time point are listed in table S4.

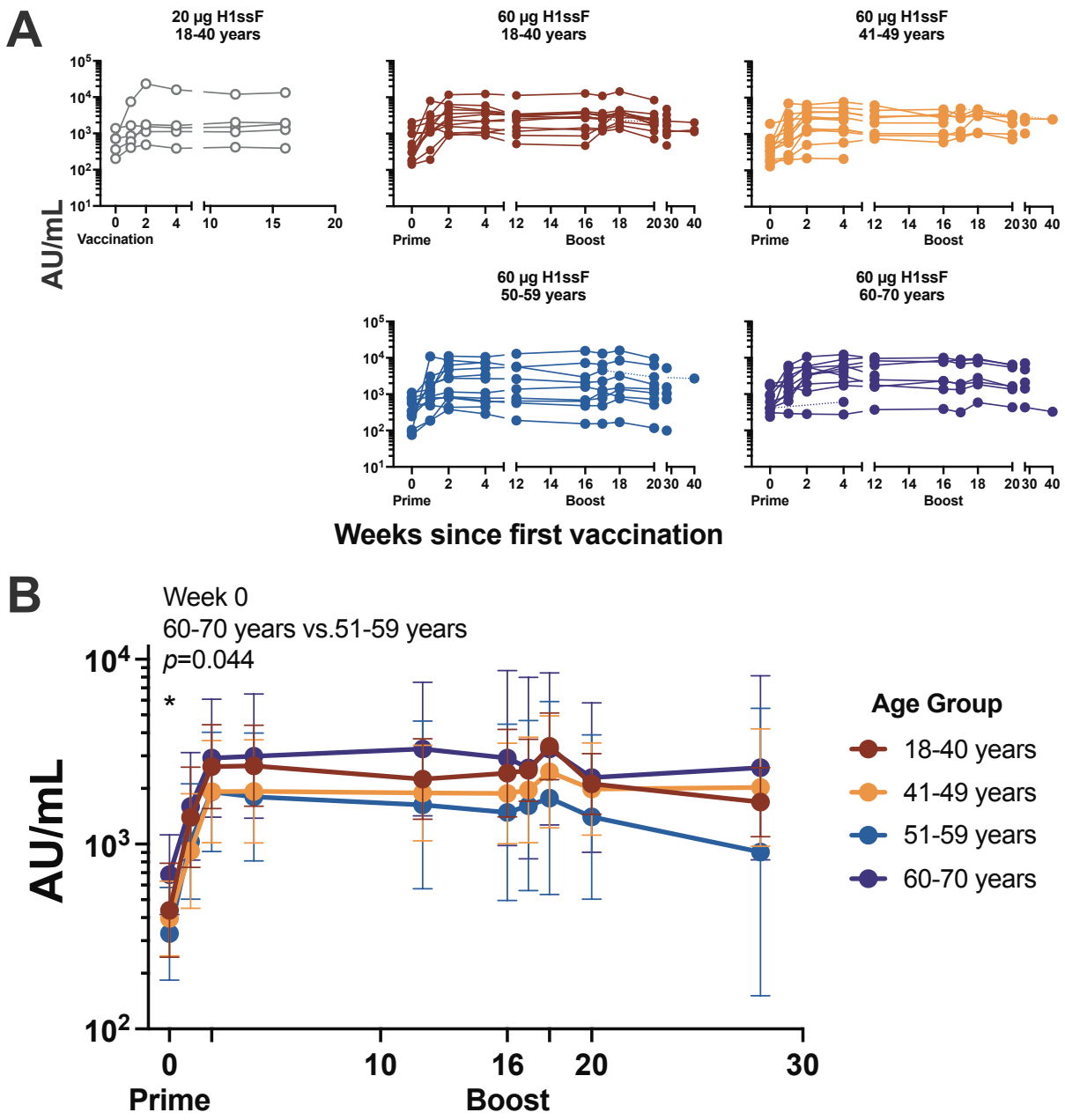

Fig. S7. H1ssF vaccination elicits antibodies that bind to the H5 stem. (**A and B**) H5ss binding antibody concentrations of individuals (A) or age groups (B) against H5 (A/Indonesia/5/2005) stem were measured by ECLIA and are reported as AU/mL. In (A), Samples from the same individual are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed. In (B), geometric means and 95% CIs stratified by age. Additional ticks in (B) denote primary immunogenicity endpoints at week 2 and 18. Two-sample *t* tests were used to compare binding antibody

concentrations between groups. Exact numbers of participant samples analyzed at each time point are listed in table S4.

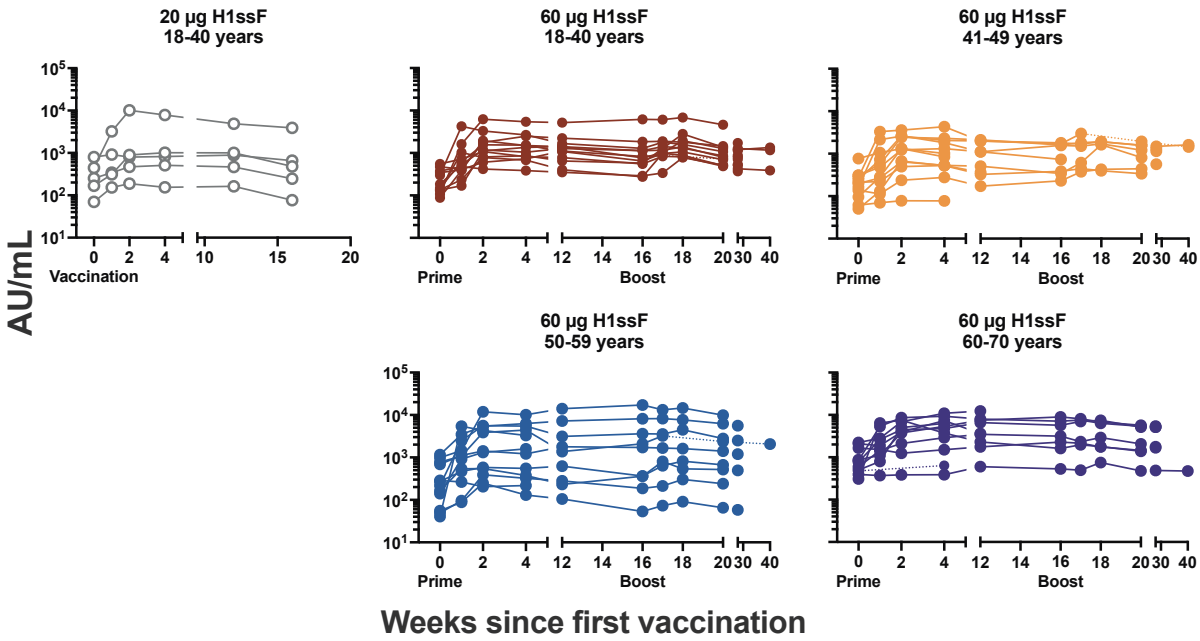

Fig. S8.H1ssF vaccination elicits antibodies that bind to the H2 stem. H2ss binding antibody concentrations of individuals against H2 (A/Singapore/1/1957) stem were measured by ECLIA and reported by AU/mL. Samples from the same individual are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed.

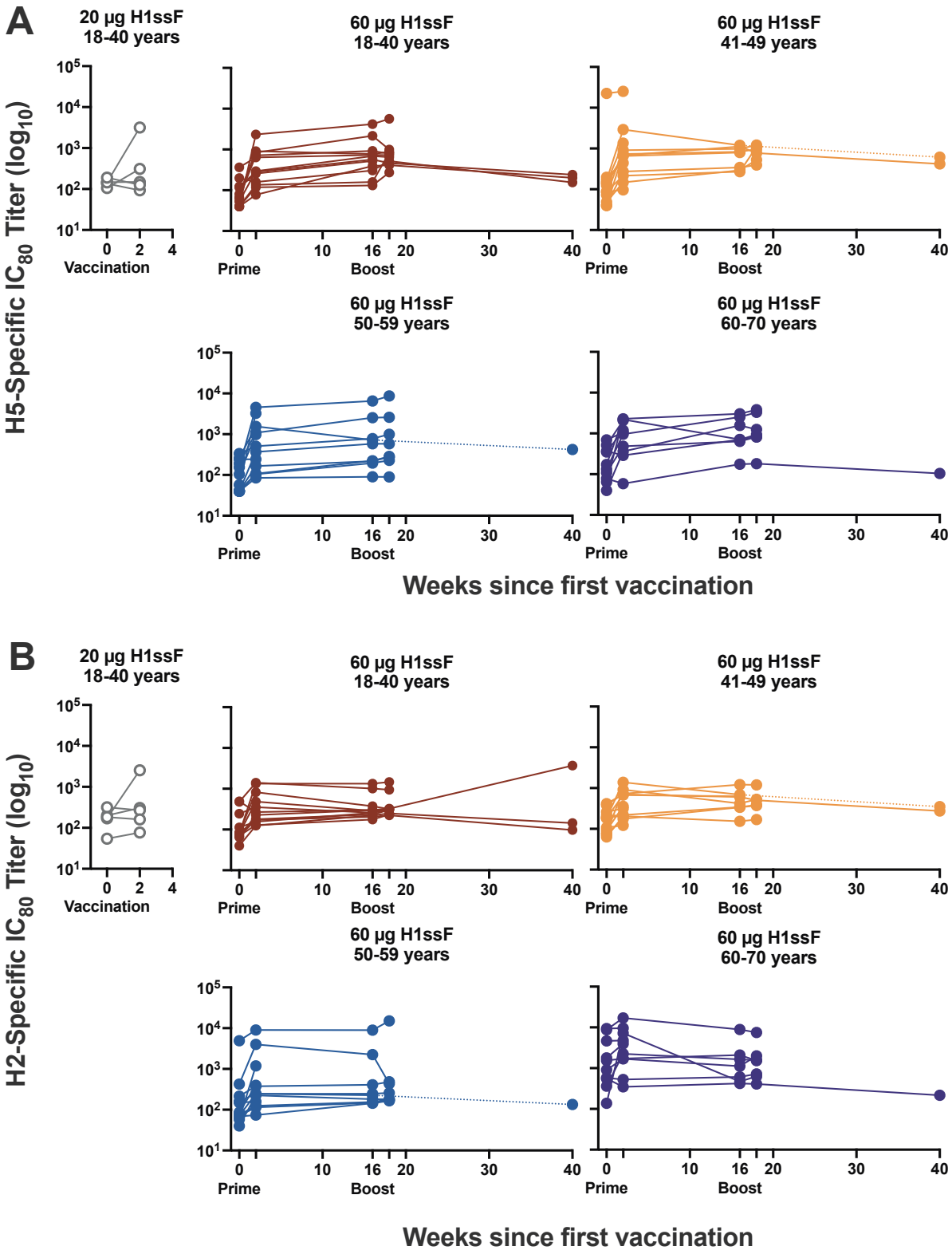

Fig. S9. H1ssF vaccination elicits heterologous H5 and H2 neutralizing antibodies. (**A and B**) A/Indonesia/5/2005 (A) or H2 A/Singapore/1/1957 (B) neutralizing antibody titers (IC_{80}) were determined by microneutralization assay. Individual participants'

neutralizing antibody titers are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed. Additional ticks denote primary immunogenicity endpoints at week 2 and 18.

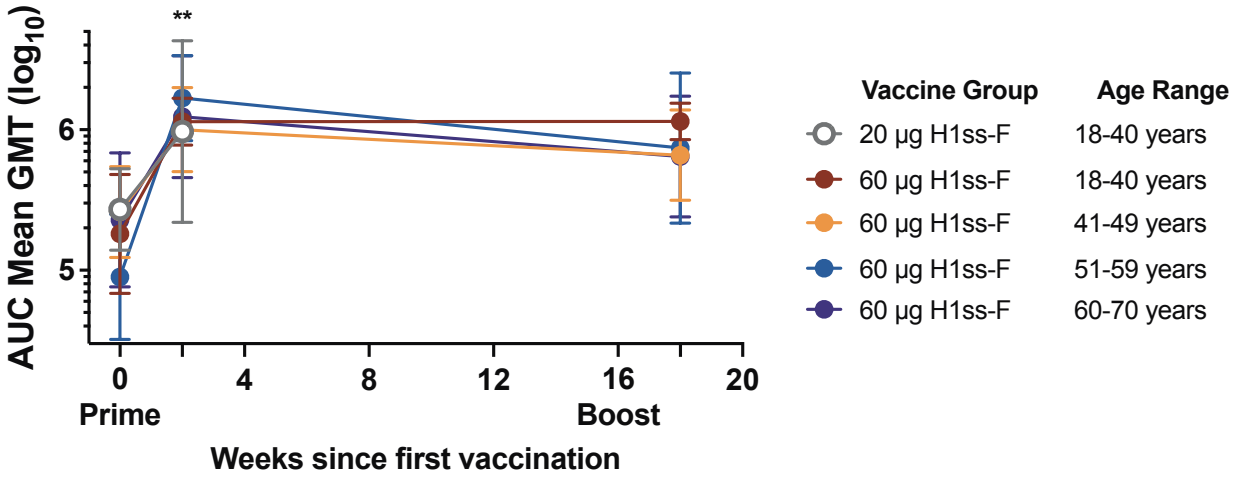

Fig. S10. H1 stem ADCC activity increased after H1ssF vaccination. Geometric mean AUC and 95% CI values of ADCC activity are shown for 60 µg dose recipients' serum at baseline, week two, and week 18 after H1ssF vaccination. Data are stratified by age group**. **** indicates a significant difference from baseline to week 2 across all 60 µg dose recipients and age-stratified subgroups. For all 60 µg recipients, the 18-40 and 51-59 years age groups, $p<0.001$. For 41-49 years, $p=0.002$. For 60-70 years, $p=0.01$. No significant difference was observed between week 2 and week 18. Exact numbers of participant samples analyzed at each time point are listed in table S4.

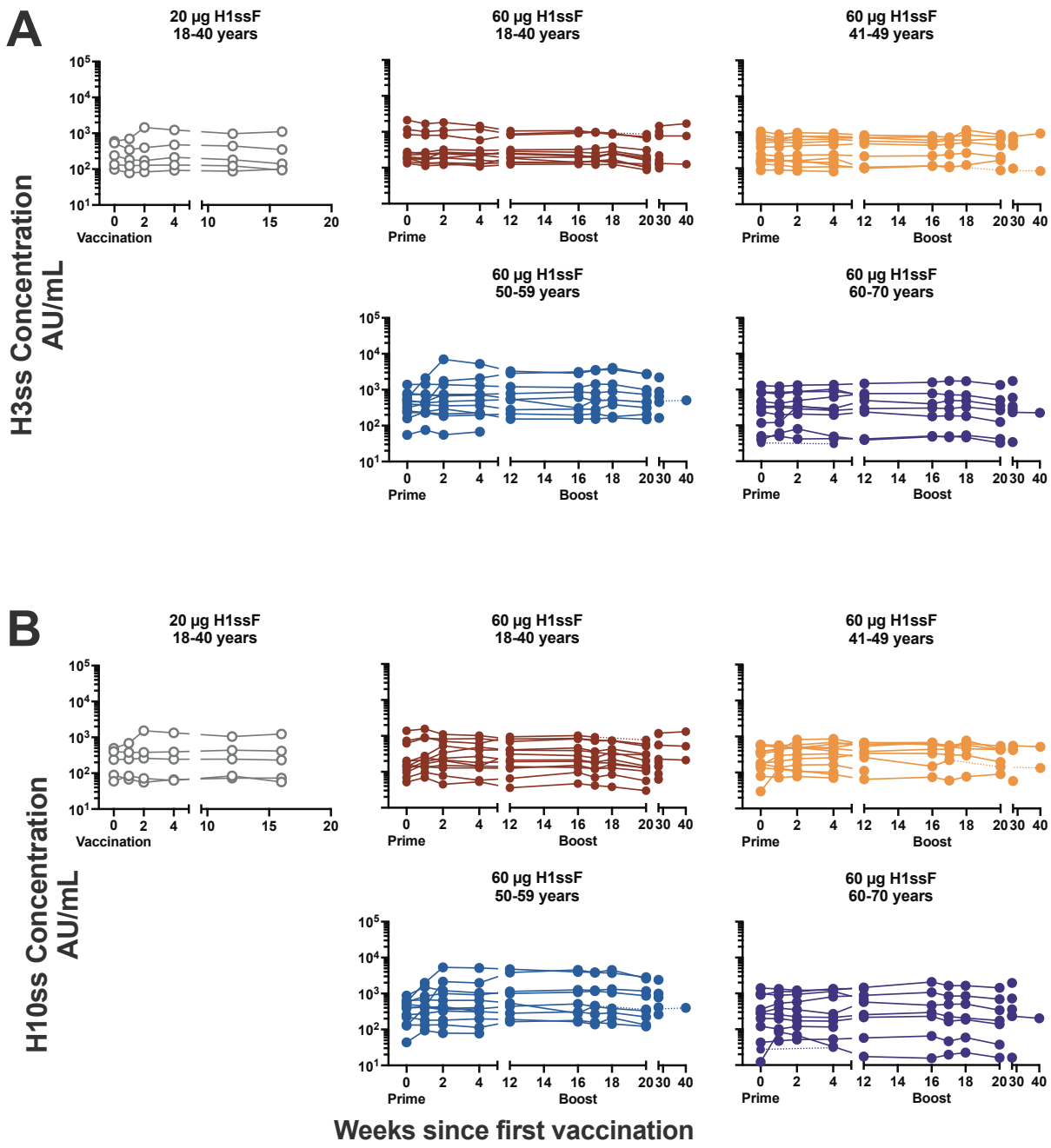

Fig. S11. H1ssF vaccination induced little to no binding antibodies against group 2 influenza subtypes. (**A and B**) H3ss (A/Finland/486/2004) (A) and H10ss (A/Jiangxi-Donghu/346/2013) (B) binding antibody concentrations of individuals were measured by ECLIA and reported by AU/mL. Samples from the same individual are connected longitudinally by lines; these lines are dotted where intervening sampling dates were missed.

Table S1. The impact of the COVID-19 pandemic on study visits. In-person clinic follow-up visits were interrupted beginning in March 2020, due to the COVID-19 pandemic; instead, follow-up visits were conducted by phone. This impacted all participants for at least one study visit, resulting in missed sample collections. Certain in-person visits were conducted, with accompanying sample collections, at PI discretion.

Group (Number in Group)	Number of participants that did not receive boost	Phone calls conducted for safety signals (Week: no.)	In-clinic visit conducted per PI request (Week: no.)
20 µg H1ssF 18-40 years (*n*=5)	**0**	Week 40: 1 Week 52: 4	 Week 52: 1
60 µg H1ssF 18-40 years (*n*=12)	**0**	Week 28: 1 Week 40: 9 Week 52: 10 Week 68: 1	 Week 52: 2 Week 68: 10
60 µg H1ssF 41-49 years (*n*=12)	**4**	Week 12: 3 Week 16, 17: 4 Week 18: 5 Week 20: 4 Week 28: 7 Week 40: 5 Week 52: 3	 Week 20: 1 Week 40: 5 Week 52: 9 Week 68: 8
60 µg H1ssF 50-59 years (*n*=12)	**3**	Week 16, 17 : 3 Week 18: 4 Week 20: 3 Week 28: 7 Week 40: 8 Week 52: 5 Week 68: 1	 Week 20: 1 Week 40: 4 Week 52: 7 Week 68: 8
60 µg H1ssF 60-70 years (*n*=11)	**4**	Week 2, 3: 1 Week 12: 2 Week 16-28: 4 Week 40: 6 Week 52: 5 Week 68: 1	Week 4: 1 Week 28: 1 Week 40: 4 Week 52: 6 Week 68: 6

Table S2. Adverse events attributed to vaccination. [a]These two events apply to the same participant.

Group (Number in Group)	Days Post-Product Administration	Adverse Event	SAE	Severity	Duration of Event	Resolution
60 µg H1ssF 18-40 years (*n*=12)	0 days post-prime	Vivid Dreams[a]	No	Mild	4 days	Resolved without sequelae
	14 days post-prime	Lymphopenia[a]	No	Mild	14 days	Resolved without sequelae
60 µg H1ssF 50-59 years (*n*=12)	14 days post-prime	Neutropenia	No	Moderate	14 days	Resolved without sequelae

Table S3. Confirmed influenza virus infections after study product administration. [a]Binding ECLIA concentrations or Microneutralization IC_{80} titers as of the last tested sample prior detection of the influenza-like illness. These are provided as a reference, but do not indicate the serum antibody titers at time of influenza-like illness. AE, adverse event; SAE, Severe Adverse Event. Severity: grade 2: some interference with activity not requiring medical intervention; grade 3: prevented from daily activity and required some type of medical intervention, not including hospitalization. For the grade 3 infection described here, the participant presented to an urgent care facility and was prescribed Tamiflu.

Group (Number in Group)	Days since Product Administration	Pathogen Identified	AE/ SAE	Severity	Duration of Illness	H1 FL Binding ECLIA AU/mL[a]	H1 Neutralization Titer[a]	Days since last serum sampling
60 µg H1ssF 50-59 years (*n*=12)	23 days after boost	H1	No	2	7 days	3336	222	9
	62 days after prime	H1	No	3	5 days	2529	994	34

Table S4. Number of participants' samples analyzed at each time point for the 60 µg dose groups. [a]Weeks 0 and 16 represent the vaccine time points for the trial. [b]One 60-70 year old participant's week 0 (baseline) sample was omitted from the microneutralization and antibody-dependent cellular cytotoxicity (ADCC) assays because no post-vaccination microneutralization timepoints (weeks 2, 16, 18, 40) or ADCC timepoints (week 2 and 18) were available for the participant. The same participant's sample was unable to be converted to AU/mL in the ECLIA results for the H1FL antigen and was excluded from the analysis of that antigen (affecting weeks 0 and 4). [c]n/a indicates not applicable because samples from week 52 or 68 were not stratified by H2 exposure.

Age Group	Weeks since first vaccination												
	0[a]	**1**	**2**	**4**	**12**	**16[a]**	**17**	**18**	**20**	**28**	**40**	**52**	**68**
18-40 years	12	12	12	12	12	12[b]	12	11	12	11	3	2	10
41-49 years	12	12	12	12	9	8	7	6	7	4	2	5	6
51-59 years	12	12	12	12	9	9	9	8	9	5	1	4	8
60-70 years	11[d]	10	10	11	9	7	7	7	7	6	1	2	6
H2 naïve	27	27	27	27	23	22	21	18	21	15	6	n/a[e]	n/a
H2 exposed	20	19	19	20	16	14	14	14	14	11	1	n/a	n/a
All 60 µg	47[d]	46	46	47	39	36	35	32	35	26	7	13	30

Table S5. **Percent and number of participants receiving licensed seasonal quadrivalent influenza vaccines (QIV) during the study**. [a]Four 60 µg H1ssF recipients received two vaccines in the post-boost follow-up period, *n*=1 in the 18-30 years age group, *n*=1 in the 51-59 years age group, and *n*=2 in the 60-70 years age group. These participants are counted once each during the post-boost period in this table. [b]QIV Timing is the span of weeks (rounded to the nearest week) following prime (Pre-Boost) and boost (Post-Boost) during which the participants received a QIV vaccine. In post-boost column, when a large gap separated participants' QIV timing, this gap was noted.

Vaccine Group	**Pre-Boost (%, *n*)** QIV Timing[b]	**Post-Boost[a] (%, *n*)** QIV Timing	**Both (%, *n*)**	**Total Participants (%, *n*)**
20 µg H1ssF 18-40 years	(60%, *n*=3/5) 12-33 weeks	N/A	0	(60%, *n*=3/5)
60 µg H1ssF 18-40 years	(33%, *n*=4/12) 4-9 weeks	(67%, *n*=8/12) 4-12, 40-53 weeks	(17%, *n*=2/12)	(83%, *n*=10/12)
60 µg H1ssF 41-49 years	(17%, *n*=2/12) 4-5 weeks	(92%, *n*=11/12) 2-7, 33-42 weeks	(8%, *n*=1/12)	(100%, *n*=12/12)
60 µg H1ssF 51-59 years	(33%, *n*=4/12) 6-9 weeks	(83%, *n*=10/12) 4, 30-52 weeks	(33%, *n*=4/12)	(83%, *n*=10/12)
60 µg H1ssF 60-70 years	(36%, *n*=4/11) 4-10 weeks	(91%, *n*=10/11) 4-12, 26-51 weeks	(36%, *n*=4/11)	(91%, *n*=10/11)
		Total QIV Vaccination Rate During Study:		(87%, *n*=45/52)

Table S6. Geometric mean microneutralization assay titers for H3 and H10 viruses.

	H3 Geometric Mean Titers (95% CI)		H10 Geometric Mean Titers (95% CI)	
Vaccine and Age Group	Week 0	Week 2	Week 0	Week 2
20 µg H1ssF 18-40 years	840 (209, 3376)	717 (247, 2081)	178 (50, 627)	236 (63, 889)
60 µg H1ssF 18-40 years	1047 (397, 2758)	1010 (390, 2620)	175 (101, 302)	157 (90, 272)
60 µg H1ssF 41-49 years	772 (372, 1603)	668 (308, 1450)	242 (140, 419)	249 (155, 401)
60 µg H1ssF 51-59 years	341 (100, 1160)	345 (97, 1226)	327 (170, 630)	325 (171, 618)
60 µg H1ssF 60-70 years	776 (198, 3036)	741 (198, 2780)	305 (161, 577)	291 (145, 581)

Data file S1. CONSORT Checklist
Data file S2. Raw, individual-level data for experiments where n<20.