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Supplementary information

A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial

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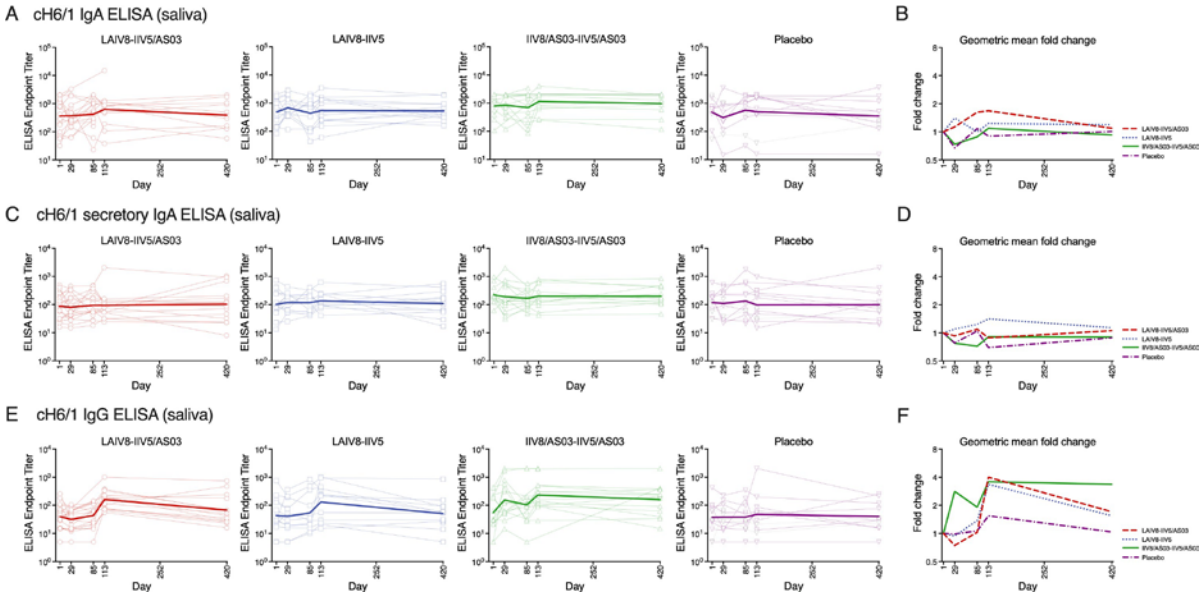
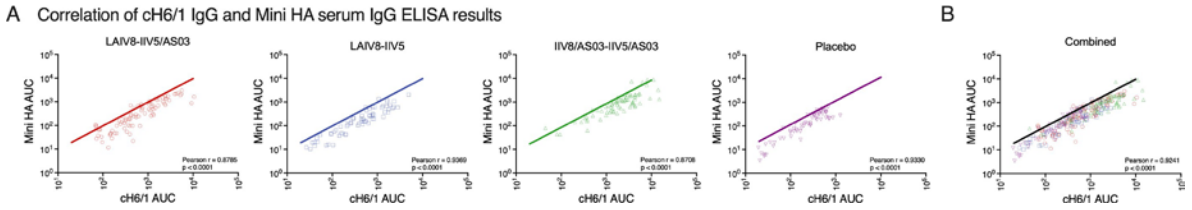
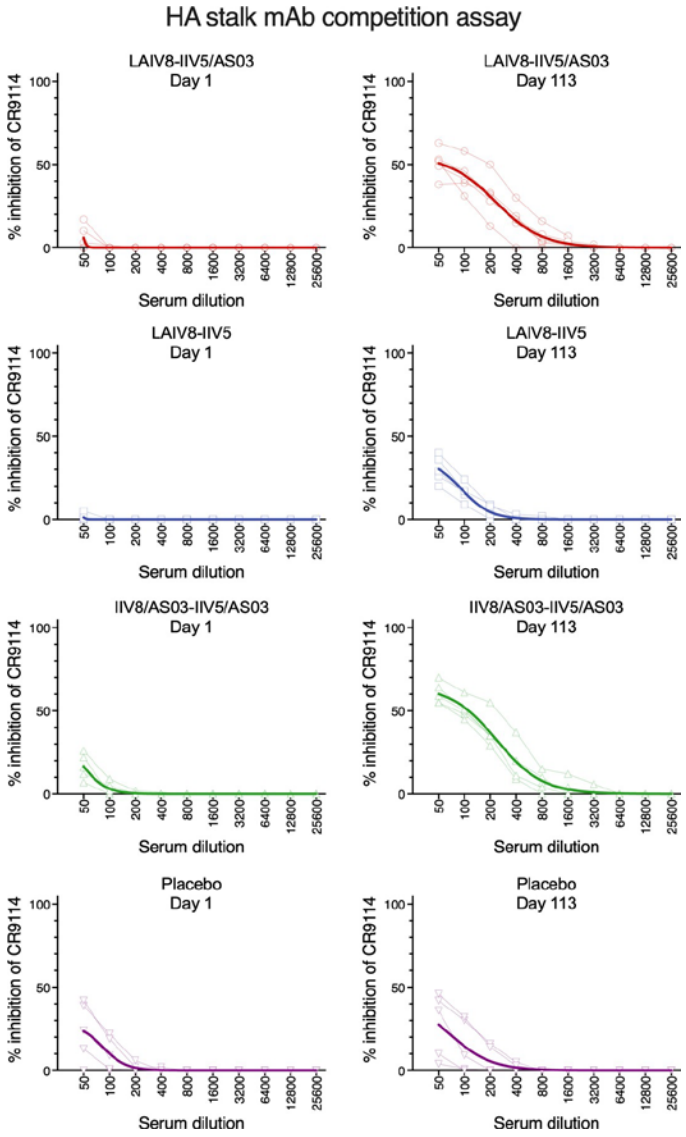
S. Fig. 1: Mucosal anti-stalk antibody titers. Anti-stalk IgA (A), sIgA (C) and IgG (E) antibody titers. Faint lines indicate reactivity of different individuals; bold lines indicate geometric mean titers of the respective groups. Geometric mean fold induction of antibody titers based on data in A, C and E is shown in B, D and F.



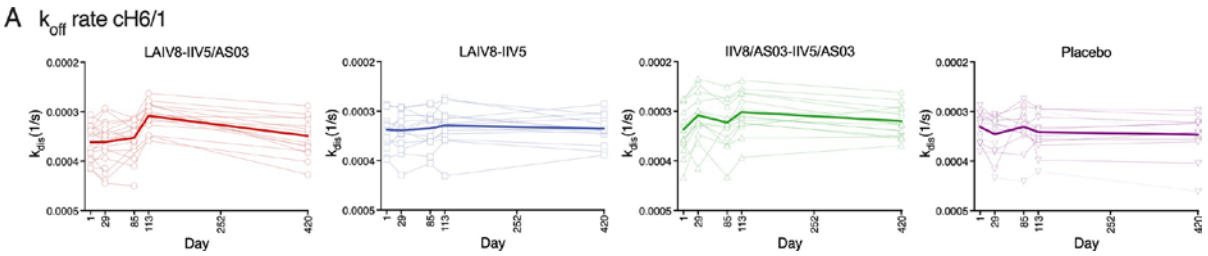
S. Fig. 2: Correlation between high affinity cH6/1 serum IgG ELISA and mini-HA serum IgG ELISA. A shows correlation for the individual groups, B shows pooled correlation. The Pearson r was determined using Graphpad Prism.



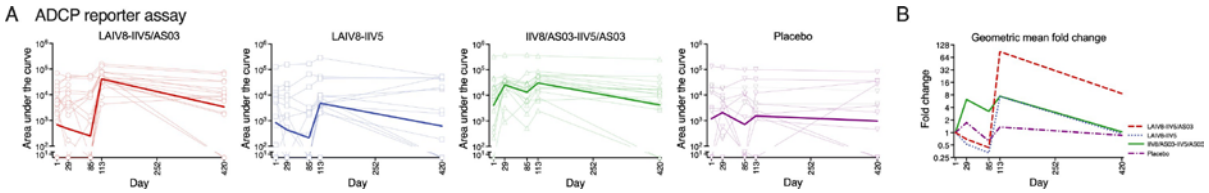
S. Fig. 3: Competition ELISAs against known HA stalk mAb CR9114. The 5 highest cH6/1 IgG antibody inducing individuals from each group were tested for competition with mAb CR9114. Faint lines indicate percent inhibition for different individuals. Bold lines show a nonlinear curve fit of the combined data for each group.



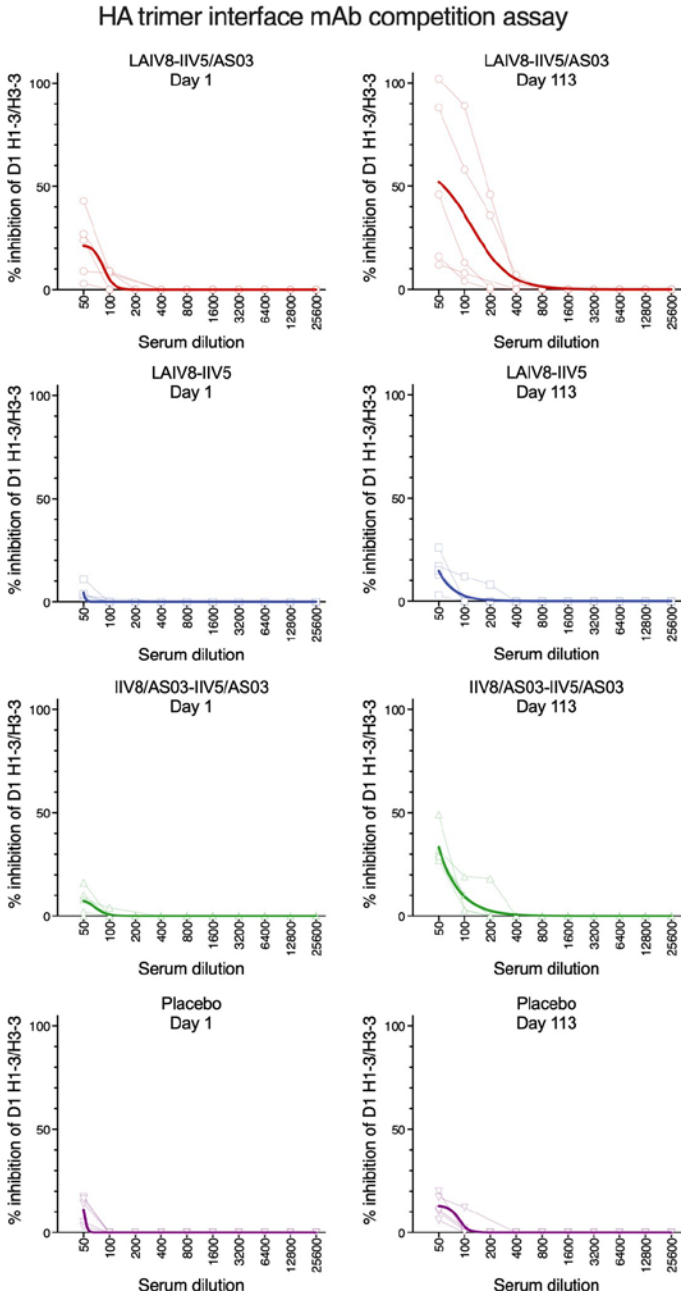
S. Fig. 4: Vaccination increases antibody affinity. K_{off} of sera in the different vaccination groups over time are shown. The K_{off} was measured using cH6/1 HA on an Octet Red.



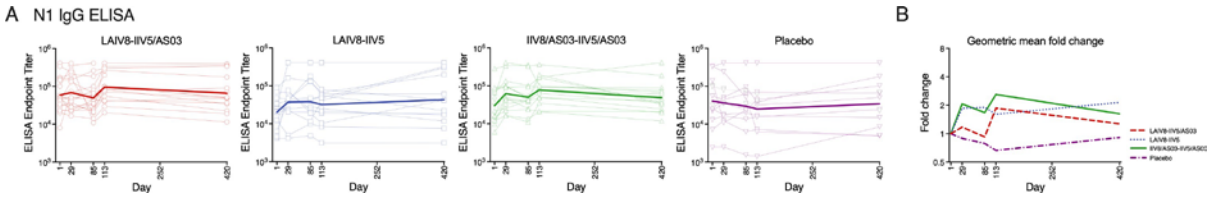
S. Fig. 5: ADCP titers. Serum anti-stalk ADCP activity against in the LAIV8-IIV5/AS03, LAIV8-IIV5, IIV8/AS03-IIV5/AS03 and the combined Placebo groups as measured in a reporter assay. (B) Geometric mean fold induction of ADCP reporter activity based on data from A.



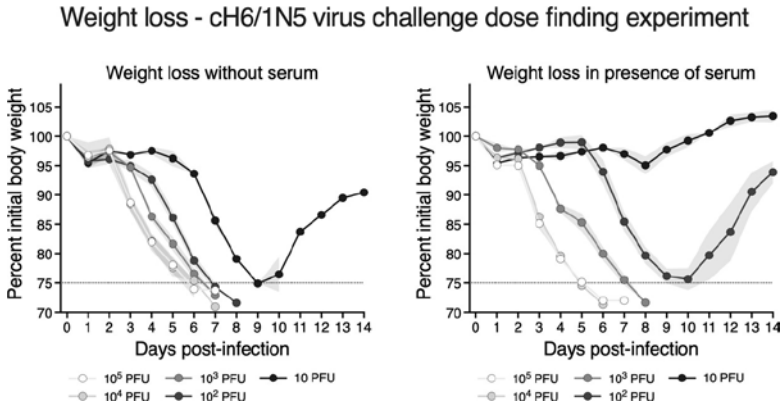
S. Fig. 6: Competition ELISAs against known HA trimer interface mAb D1 H1-3/H3-3. The 5 highest cH6/1 IgG antibody inducing individuals from each group were tested for competition with mAb D1 H1-3/H3-3. Faint lines indicate percent inhibition for different individuals. Bold lines show a nonlinear curve fit of the combined data for each group.



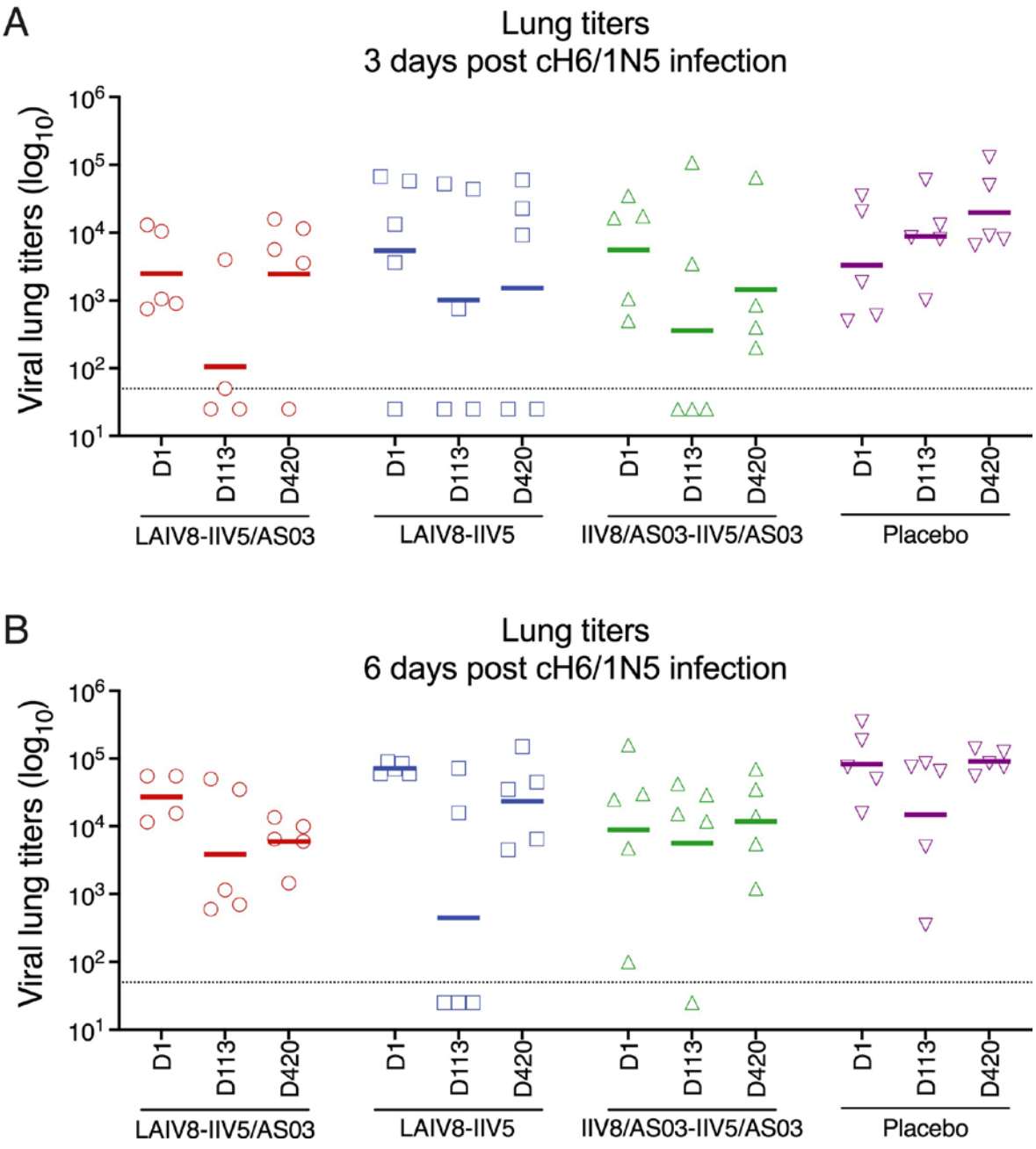
S. Fig. 7: N1 neuraminidase ELISA titers. (A) Faint lines indicate reactivity of different individuals; bold lines indicate geometric mean titers of the respective groups. Geometric mean fold induction of antibody titers based on data in A is shown in B.



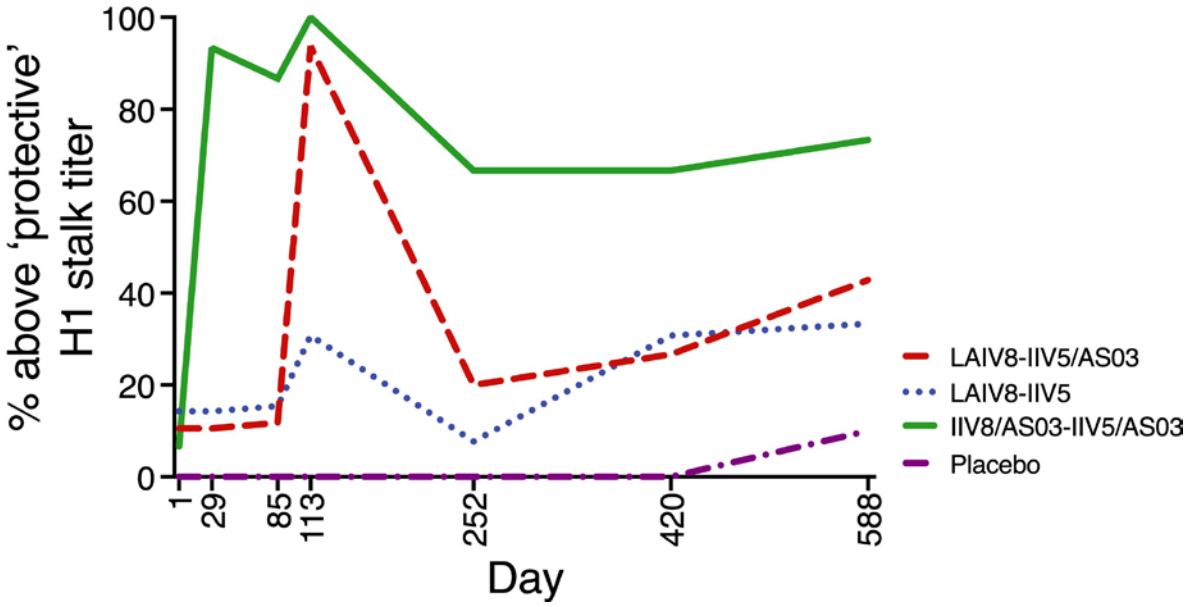
S. Fig. 8: Serum transfer virus challenge dose finding study. Naïve mice (left; n=3/group) and mice receiving pooled commercially available sera (right; n=5/group) were challenged with increasing doses of cH6/1N5 virus (10 PFU-10^5 PFU) to identify a challenge dose that would reliably cause weight loss in mice receiving baseline sera from the clinical trial. Points and connecting lines show the mean and the shaded areas indicate the standard error of the mean.



S. Fig. 9: Viral lung titers after cH6/1N5 challenge. Mice that received pooled serum samples pre- or post-vaccination (days 1, 113 or 420) from each vaccine group were challenged with cH6/1N5 virus. Mice were euthanized either 3 days (**A**) or 6 days (**B**) post infection and lung titers were measured by plaque assay. Each point indicates one mouse (n=4-5/group) and the bars indicate the geometric mean titer.



S. Fig. 10: Percent of individuals with potentially protective H1 stalk titers. Proportion of subjects in each group that have group 1 stalk titers above AUC 1280 which was shown to be protective against H1N1 infection in humans in a previous study.[1]



S. Table 1: Summary of Nasal/Oropharyngeal Shedding of Vaccine Virus by Inpatient Day, Groups 1 and 2 Combined - Safety Population

	PCR Positive for Influenza A[1]		PCR positive for vaccine virus[2]		CPE positive[2]	
Day Post-Prime	**x/n (%)**	**95% CI**	**x/n (%)**	**95% CI**	**x/n (%)**	**95% CI**
Day 2	11/33 (33.3)	18.0, 51.8	5/11 (45.5)	16.7, 76.6	0/11 (0.0)	0.0, 28.5
Day 3	2/33 (6.1)	0.7, 20.2	2/2 (100.0)	15.8, 100.0	0/2 (0.0)	0.0, 84.2
Day 4	0/33 (0.0)	0.0, 10.6	NA	NA	NA	NA
Day 5	3/33 (9.1)	1.9, 24.3	0/3 (0.0)	0.0, 70.8	0/3 (0.0)	0.0, 70.8
Shedding on any day.	15/33 (45.5)	28.1, 63.6	6/15 (40.0)	16.3, 67.7	0/15 (0.0)	0.0, 21.8

x= Number of subjects positive. n= Number of subjects evaluated.
95% CI = 95% Confidence Interval for the proportion (%) positive.
Excludes 3 subjects from Group 3 (placebo), none of whom had viral shedding.

[1] Performed on site for containment unit discharge planning purposes.
[2] Denominator = Subjects who were PCR positive on site for Influenza A.

S. Table 2: Summary of ILI Test Results by Study Group

		Group 1 (N = 19)		Group 2 (N = 14)		Group 3 (N = 3)		Group 4 (N = 15)		Group 5 (N = 10)	
Test Conducted	**Strain/Result**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**	**n (%)**	**95% CI**
Any	Any Test	2 (10.5)	(1.3,33.14)	2 (14.3)	(1.78,42.81)	1 (33.3)	(0.84,90.57)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
Positive for influenza	Any	1 (5.3)	(0.13,26.03)	1 (7.1)	(0.18,33.87)	1 (33.3)	(0.84,90.57)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
	Influenza A	1 (5.3)	(0.13,26.03)	1 (7.1)	(0.18,33.87)	0 (0)	(0,70.76)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
	Influenza A H1	0 (0)	(0,17.65)	0 (0)	(0,23.16)	0 (0)	(0,70.76)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
	Influenza A H3	0 (0)	(0,17.65)	0 (0)	(0,23.16)	0 (0)	(0,70.76)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
	Influenza B	0 (0)	(0,17.65)	0 (0)	(0,23.16)	1 (33.3)	(0.84,90.57)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
Positive for non-influenza pathogens	Yes	2 (10.5)	(1.3,33.14)	1 (7.1)	(0.18,33.87)	0 (0)	(0,70.76)	0 (0)	(0,21.8)	0 (0)	(0,30.85)
	No	2 (10.5)	(1.3,33.14)	5 (35.7)	(12.76,64.86)	1 (33.3)	(0.84,90.57)	0 (0)	(0,21.8)	1 (10)	(0.25,44.5)

N= Number of subjects in the safety population
CI=Exact Confidence Interval
Subject can contribute to multiple influenza type/strain

S. Table 3: Primers and probes used for RT-PCR.

Primers and Probes	Sequence (5'>3')	Ascension Number	Nucleotide Position
H1 Flu (2009 H1N1) Forward Primer	GTG CTA TAA ACA CCA GCC TYC CA	FJ966974	902-924
H1 Flu (2009 H1N1) Reverse Primer	CGG GAT ATT CCT TAA TCC TGT RGC	FJ966974	1017-994
H1 Flu (2009 H1N1) Probe	FAM-CA GAA TAT ACA(T-BHQ1)CC RGT CAC AAT TGG ARA A-Spacer C3	FJ966974	928-957
RNP Forward Primer	AGA TTT GGA CCT GCG AGC G	BC006991.1	309-327
RNP Reverse Primer	GAG CGG CTG TCT CCA CAA GT	BC006991.1	373-354
RNP Probe	FAM-TTC TGA CCT GAA GGC TCT GCG CG-BHQ1	BC006991.1	330-352
cH8/1 HA Forward Primer	GGA GAA ACT TTA AAA ATT AGA ACC		
cH8/1 HA Reverse Primer	GCC ATG GCT CTC CCC TTT GAG C		
cH8/1 HA Probe	FAM-TGG GAA CCT GAT CGC GCC TGA ATT CGG T-BHQ1		

References

1. Ng, S.*, et al.* Novel correlates of protection against pandemic H1N1 influenza A virus infection. *Nat Med* **25**, 962-967 (2019).