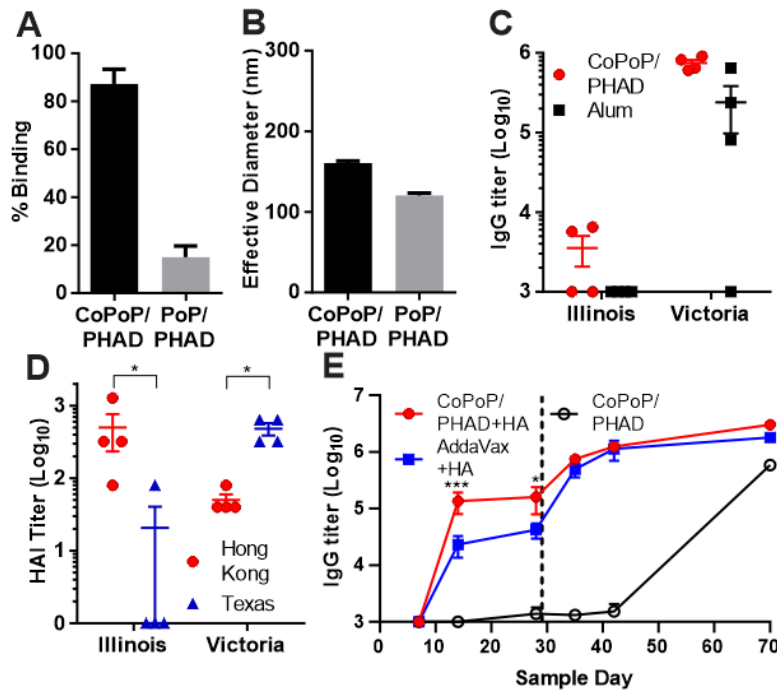


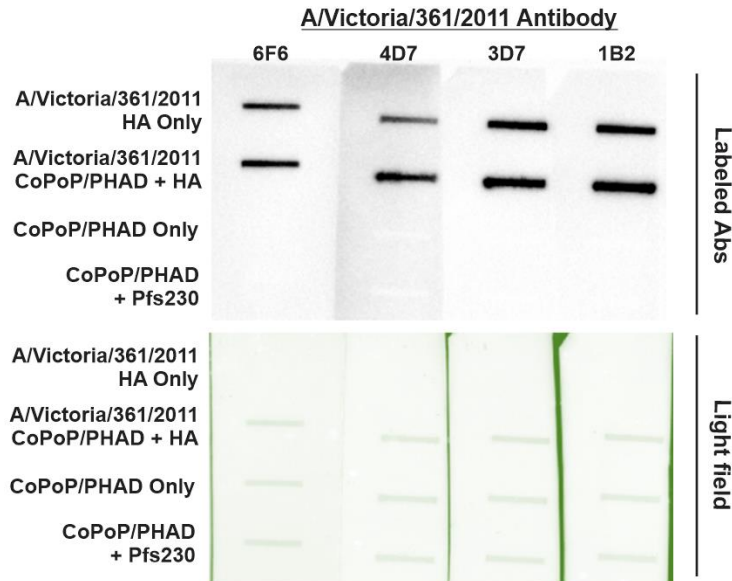
Supplemental Information:

A liposome-displayed hemagglutinin vaccine platform protects mice and ferrets from heterologous influenza virus challenge

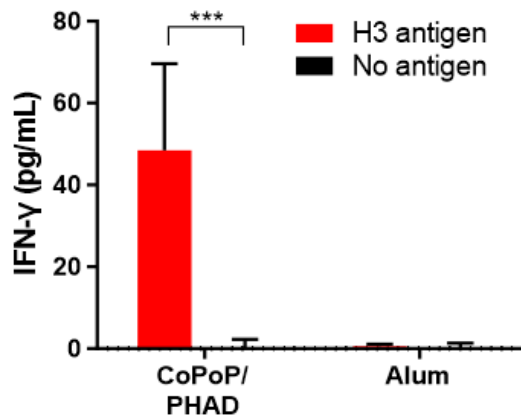
Zachary R. Sia, Xuedan He, Ali Zhang, Jann C. Ang, Shuai Shao, Amal Seffouh, Wei-Chiao Huang, Michael R. D'Agostino, Amir Teimouri Dereshgi, Suryaprakash Sambhara, Joaquin Ortega, Hanne Anderson, Matthew S. Miller, Bruce A. Davidson, Jonathan F. Lovell



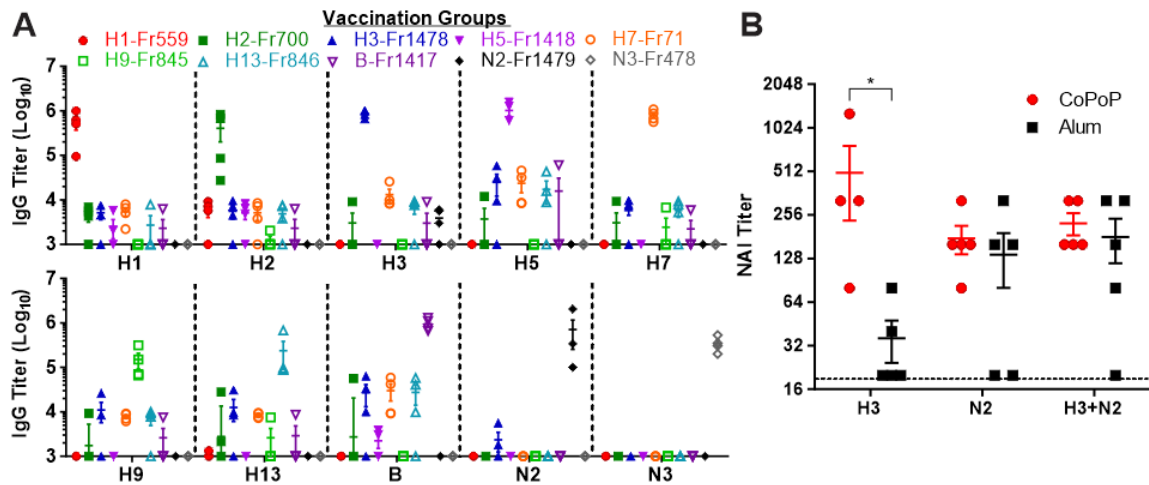
Supplemental Figure 1. H3 antigen for ferret vaccination. (A) H3 from Victoria strain achieves CoPoP binding consistent with H3 from Illinois strain. (B) Average particle size also remains consistent with a homogenous solution without aggregates. (A-B) Represent data as mean \pm s.d. from $n = 3$ samples. (C) IgG ELISA assay was used to compare heterologous strain-antibody binding potential between H3 strains. Plates were coated using the antigen from the Victoria strain then serum from mice vaccinated with the heterologous Hong Kong or homologous Victoria HA were introduced. Antibodies against Hong Kong strain show little binding affinity for HA antigen of Victoria strain. (D) CoPoP formulation with H3 from Victoria strain resulted in significantly higher HA inhibition of A/Texas/50/2012 virus than H3 from Hong Kong strains. (E) IgG titers in the serum of challenged ferrets was assessed at time points before and after the challenge virus inoculation (date indicated by dotted line). All vaccinations in mice performed with 100 ng doses of antigen. (C, D) Statistical analysis by unpaired t tests, (E) by one-way ANOVA of CoPoP/PHAD+HA against AddaVax+HA, $n = 6$. IgG and HAI titers expressed as mean \pm s.e.m (*indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.005$).



Supplemental Figure 2. Slot blot for A/Victoria/361/2011 HA using HA monoclonal antibodies. Monoclonal antibodies against A/Victoria/361/2011 from clones 6F6, 4D7, 3D7, and 1B2 were tested against both soluble or liposome-bound HA antigen. Qualitative assessment of band weight indicates that labeled antibody binding between soluble antigen and liposome-bound antigen is comparable across four binding epitopes, and liposomes lacking or bearing irrelevant antigens do not bind with these antibodies. Light field images confirm the presence of CoPoP/PHAD liposomes in the imaged samples. Pfs230 served as an irrelevant control antigen.



Supplemental Figure 3. IFN-γ measured in splenocytes. Mice vaccinated with CoPoP/PHAD + His-tagged H3 antigen from A/canine/Illinois/11613/2015 H3N2 yielded observable IFN-γ with splenocyte restimulation with H3, while no IFN-γ was detected in splenocytes from mice vaccinated with H3 and alum. A immunization dose of 100 ng of antigen was used. (***) indicates p < 0.005).



Supplemental Figure 4. Additional multiplex data. (A) When nanoliposomes were loaded with 50 ng of a single antigen type, the resulting antibody response showed strong binding (average greater than 10^5) to only the matched antigen, with a maximum heterologous binding titer of less than 10^4 . (B) Assessment of NAI by ELLA assay yielded detectable NA inhibitory titers in each sample group, with CoPoP/PHAD yielding detectable titers in all sample groups. IgG and NAI titers expressed as mean \pm s.e.m (*indicates $p < 0.05$)

Supplemental Table 1: Head sequence identity of various trimeric HA antigens and challenge strains.

Head Region - % Protein Sequence Identity									Challenge Strain Identity	
H3 Antigen	Hiroshima	Brisbane	Hawaii	Perth	Wisconsin	Victoria	Switzerland	Illinois	Hong Kong/1968	Texas/2012
Hiroshima/2005		98.23	95.58	95.58	81.42	95.58	92.92	76.11	77.43	95.13
Brisbane/2007	98.23		97.35	97.35	82.3	97.35	94.69	76.11	77.43	96.02
Hawaii/2009	95.58	97.35		99.12	82.74	97.35	94.69	76.11	77.43	96.02
Perth/2009	95.58	97.35	99.12		82.74	97.35	94.69	75.66	76.99	96.02
Wisconsin/2010	81.42	82.3	82.74	82.74		81.42	80.53	75.22	76.99	80.09
Victoria/2011	95.58	97.35	97.35	97.35	81.42		97.35	74.78	76.11	98.23
Switzerland/2013	92.92	94.69	94.69	94.69	80.53	97.35		74.78	76.11	96.02
Illinois/2015	76.11	76.11	76.11	75.66	75.22	74.78	74.78		91.59	74.34

Supplemental Table 2: Stalk sequence identity of various trimeric HA antigens and challenge strains.

Stalk Region - % Protein Sequence Identity									Challenge Strain Identity	
H3 Antigen	Hiroshima	Brisbane	Hawaii	Perth	Wisconsin	Victoria	Switzerland	Illinois	Hong Kong/1968	Texas/2012
Hiroshima/2005		98.23	98.2	97.87	94.24	97.12	96.04	89.21	93.5	96.04
Brisbane/2007	98.23		99.64	99.29	93.88	98.56	97.48	90.29	94.58	97.48

Hawaii/2009	98.2	99.64		100	93.88	98.92	97.84	90.29	94.58	98.19
Perth/2009	97.87	99.29	100		93.88	98.92	97.84	90.29	94.58	98.19
Wisconsin/2010	94.24	93.88	93.88	93.88		92.81	91.73	90.65	94.22	92.06
Victoria/2011	97.12	98.56	98.92	98.92	92.81		98.92	89.93	93.5	99.28
Switzerland/2013	96.04	97.48	97.84	97.84	91.73	98.92		89.93	92.78	99.64
Illinois/2015	89.21	90.29	90.29	90.29	90.65	89.93	89.93		94.22	89.53

Supplemental Table 3: Statistical analysis of challenge studies shown in Figure 3.

Figure 3A Comparisons	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Tukey's multiple comparisons (p values)								
CoPoP/PHAD vs. CoPoP	0.9987	0.9317	0.7951	0.2214	*0.0144	*0.0317	0.1149	0.2375
CoPoP/PHAD vs. Alum	0.953	0.5343	0.4953	0.073	***0.0012	***0.0028	*0.0119	*0.0326
CoPoP/PHAD vs. ISA720	0.9823	0.402	0.3424	*0.0159	***0.0002	***0.0003	***0.0016	***0.0037
CoPoP vs. Alum	0.9823	0.8678	0.944	0.8528	0.7023	0.6951	0.6956	0.7235
CoPoP vs. ISA720	0.9963	0.7507	0.8187	0.2995	0.2153	0.1911	0.2227	0.2094
Alum vs. ISA720	0.9987	0.9954	0.9875	0.6985	0.7886	0.7565	0.8054	0.7596
Dunnett's multiple comparisons (p values)								
no vaccine vs. CoPoP/PHAD	0.7231	0.1257	0.9694	*0.022	*0.0243	*0.0106	***0.0036	***0.0007
no vaccine vs. CoPoP	0.6291	0.3299	0.972	0.6535	0.9121	>0.9999	0.6362	0.0999
no vaccine vs. Alum	0.4112	0.7791	0.7142	0.9972	0.2402	0.615	0.9999	0.6296
no vaccine vs. ISA720	0.4966	0.8975	0.5105	0.7563	*0.0335	0.1131	0.66	>0.9999

Supplemental Table 4: Statistical analysis of challenge studies shown in Figure 4.

Figure 4A Comparisons	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Tukey's multiple comparisons (p values)														
2000ng in AddaVax vs. 200ng in AddaVax	0.9313	0.983	>0.9999	>0.9999	0.9985	0.9996	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	0.9972	0.9993	0.9366
2000ng in AddaVax vs. 200ng in CoPoP	>0.9999	0.9967	0.8645	0.682	0.3156	0.0932	*0.0445	0.0645	0.0511	0.0521	*0.0158	*0.088	0.1348	0.1286
2000ng in AddaVax vs. 20ng in CoPoP	0.5396	0.6273	0.7297	0.8804	0.865	0.6489	0.6135	0.6625	0.5235	0.5805	0.3174	0.2879	0.3275	0.5211
2000ng in AddaVax vs. 2ng in CoPoP	0.962	>0.9999	0.9984	>0.9999	0.9989	0.9896	0.8879	0.783	0.5688	0.614	0.2064	0.3833	0.3379	0.574
200ng in AddaVax vs. 200ng in CoPoP	0.8885	0.8558	0.8058	0.564	0.1218	*0.0334	*0.0174	*0.0468	*0.0411	*0.0349	*0.0132	0.1244	0.1588	0.1671
200ng in AddaVax vs. 20ng in CoPoP	0.1234	0.2562	0.6554	0.791	0.5952	0.4036	0.4182	0.6233	0.5112	0.5179	0.3224	0.3979	0.3902	0.5799
200ng in AddaVax vs. 2ng in CoPoP	0.5105	0.9604	0.9943	0.9992	0.9606	0.9261	0.7458	0.7549	0.5591	0.553	0.2044	0.517	0.4025	0.6185
200ng in CoPoP vs. 20ng in CoPoP	0.6182	0.8794	0.9998	0.9987	0.8974	0.7566	0.5676	0.6233	0.6898	0.6272	0.4652	0.9477	0.9772	0.7206
200ng in CoPoP vs. 2ng in CoPoP	0.9818	0.9994	0.9771	0.7721	0.4596	0.2228	0.27	0.4876	0.6422	0.5917	0.6382	0.8787	0.9737	0.7206
20ng in CoPoP vs. 2ng in CoPoP	0.9442	0.7153	0.9171	0.9338	0.965	0.9188	0.9933	>0.9999	>0.9999	>0.9999	0.9983	0.9995	>0.9999	0.9366
Dunnett's multiple comparisons (p values)														
Control vs. 2000ng in AddaVax	0.5112	0.9674	0.9997	0.9916	0.8973	0.7792	0.4118	0.0952	*0.0258	**0.0042				
Control vs. 200ng in AddaVax	0.1062	0.605	0.9965	0.9996	0.9929	0.916	0.4963	0.0699	*0.0163	**0.0031				
Control vs. 200ng in CoPoP	0.5974	0.9999	0.8263	0.2371	*0.0224	**0.0024	***0.0002	****<.0001	****<.0001	****<.0001				
Control vs. 20ng in CoPoP	0.9972	0.7797	0.6532	0.4336	0.1917	0.0528	*0.0123	**0.0025	***0.0005	***0.0002				
Control vs. 2ng in CoPoP	0.9674	0.9909	0.9997	0.9666	0.6242	0.3319	*0.0423	**0.0041	**0.0006	***0.0002				