

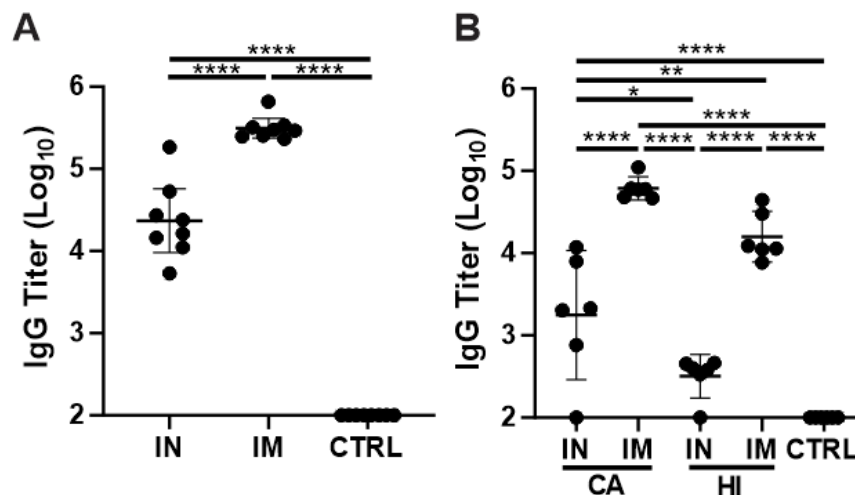
Supplemental Information:

Respiratory vaccination with hemagglutinin nanoliposomes protects mice from homologous and heterologous strains of influenza virus

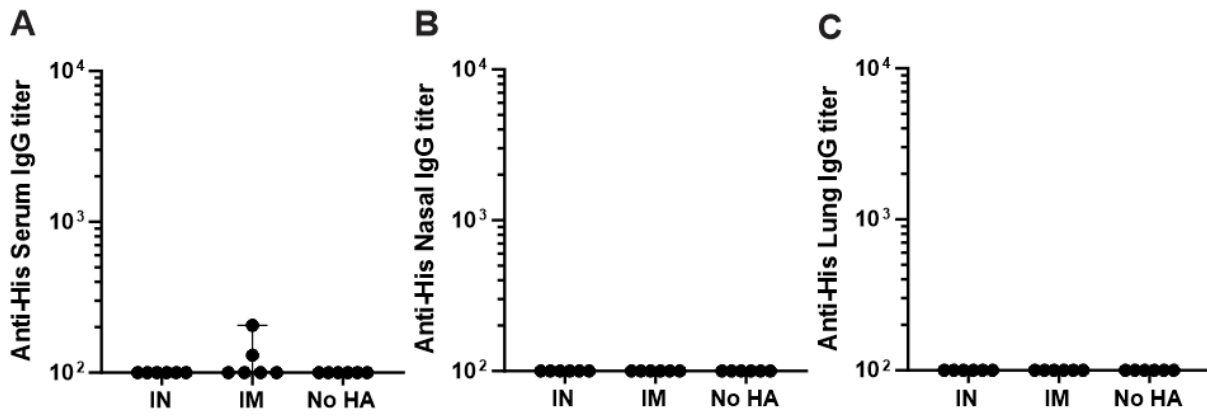
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Antigen Protein Sequence Comparison	A/Ca/Illinois/11613/2015 (H3N2)	A/Hong Kong/1/1968 (H3N2)	A/Hawaii/70/2019pdm (H1N1)	A/California/04/2009pdm (H1N1)
A/Ca/Illinois/11613/2015 (H3N2)	100.0%	92.50%	50.19%	46.75%
A/Hong Kong/1/1968 (H3N2)	92.50%	100.0%	44.23%	45.24%
A/Hawaii/70/2019pdm (H1N1)	50.19%	44.23%	100.0%	94.66%
A/California/04/2009pdm (H1N1)	46.75%	45.24%	94.66%	100.0%

Supplemental Table 1. HA protein sequence homology of influenza antigens in challenge. Comparisons of the amino acid residue sequences of utilized influenza HA antigens as assessed by Protein BLAST. Protein sequences were compiled from International Reagent Resource (IRR) catalogue and Global Initiative on Sharing Avian Influenza Data (GISAID) database.



Supplemental Figure 1. IgG antibody titer in mouse serum prior to mouse challenge study. In addition to antibody serum analysis performed in Figure 4A, IgG titers were analyzed in subsequent challenge studies to quantify antibody response to each vaccine. (A) IgG titers for mice vaccinated with A/Ca/Illinois/11613/2015 (H3N2) for heterologous challenge and (B) either A/California/04/2009pdm (H1N1) or A/Hawaii/70/2019pdm (H1N1) were measured by ELISA assay. Statistical analysis was performed with one-way ANOVA with Tukey's multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.



Supplemental Figure 2. Anti-his-tag antibody titer. Mice were vaccinated with 100 ng of HA trimers from A/Ca/Illinois/11613/2015 H3N2 bound to CoPoP/PHAD® liposomes following a schedule of prime and boost at 14 days and then assessed for anti-his-tag IgG antibodies by ELISA two weeks later. Serum, nasal lavage, and lung homogenates were analyzed, and significant antibody titers against his-tag were not observed in any sample groups.