

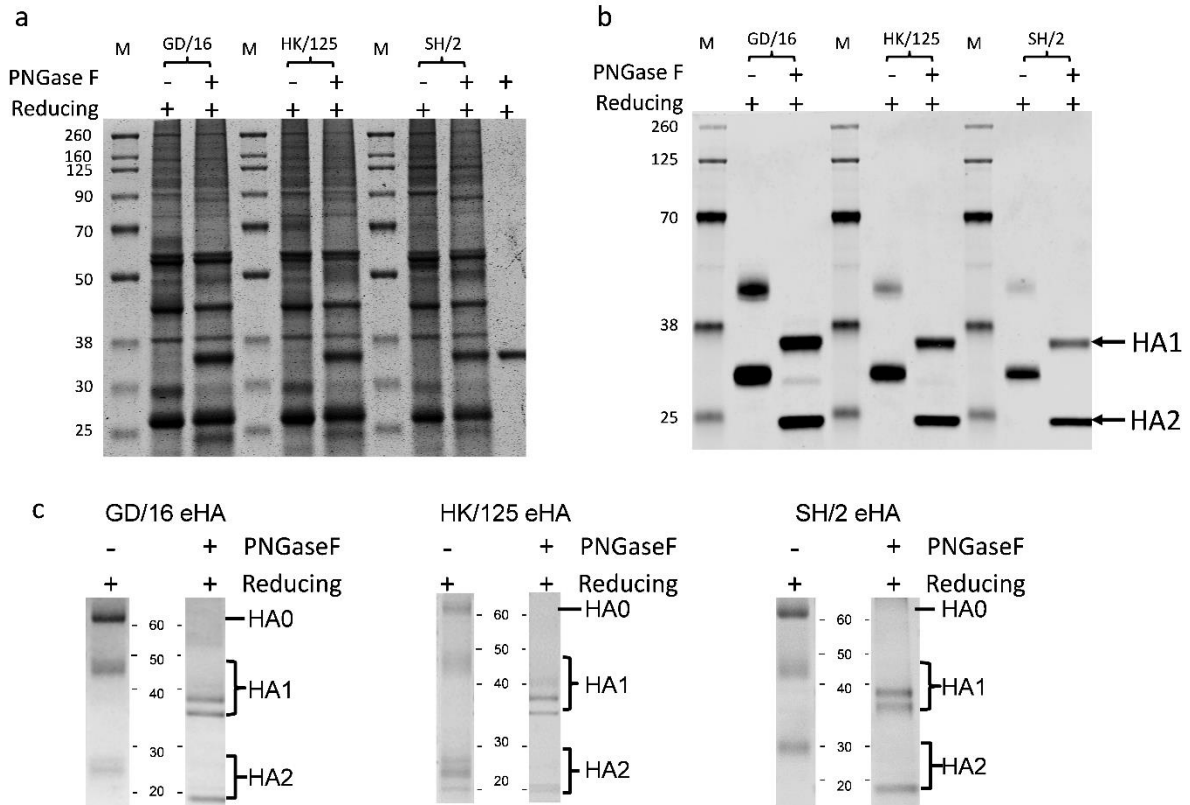
**Highly pathogenic avian influenza A/Guangdong/17SF003/2016 of the 5<sup>th</sup> wave is immunogenic and induces broad cross-protection against antigenically divergent H7N9 viruses**

Peter Radvak<sup>1</sup>, Martina Kosikova<sup>1</sup>, Yuan-Chia Kuo<sup>1</sup>, Xing Li<sup>1</sup>, Richard Garner<sup>1</sup>, Falko Schmeisser<sup>2</sup>, Ivan Kosik<sup>3</sup>, Zhiping Ye<sup>1</sup>, Jerry P. Weir<sup>2</sup>, Jonathan W. Yewdell<sup>3</sup>, Hang Xie<sup>1\*</sup>

<sup>1</sup>Laboratory of Pediatric and Respiratory Viral Diseases, <sup>2</sup>Laboratory of DNA Viruses, Division of Viral Products, Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, United States Food and Drug Administration, Silver Spring, Maryland, USA;

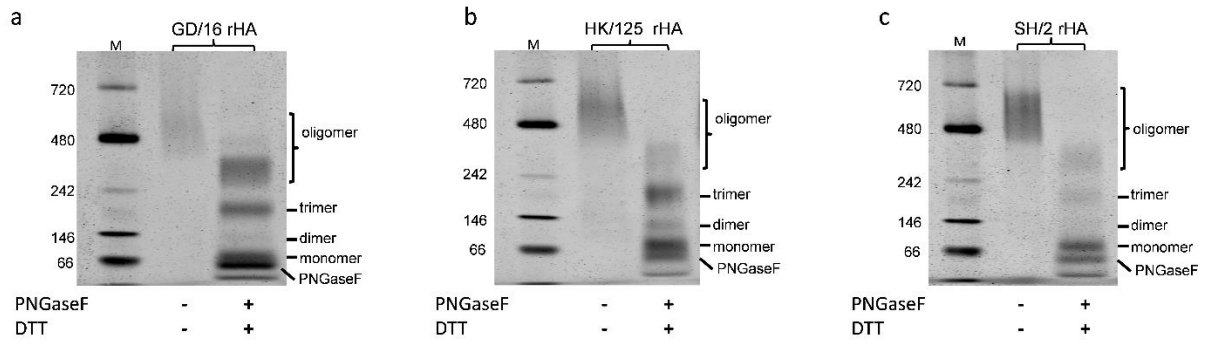
<sup>3</sup>Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.

**Supplemental figures and legends**

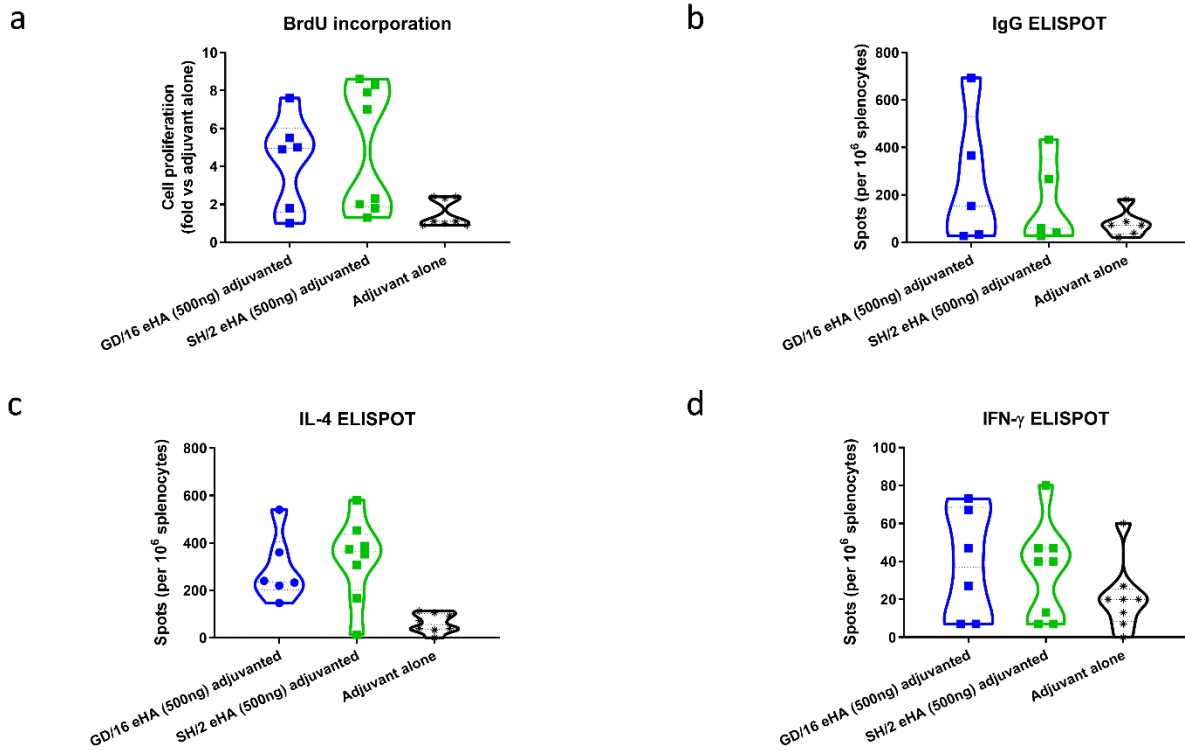


**Supplemental Figure 1. SDS-PAGE analysis of HA extracted from H7N9 candidate vaccine virus.** HAs extracted (eHAs) from H7N9 candidate vaccine viruses (CVVs)

A/Guangdong/17SF003/2016 (GD/16), A/Hong Kong/125/2017 (HK/125) or A/Shanghai/2/2013 (SH/2) were purified. (a) H7N9 CVVs with or without deglycosylation using PNGase F; (b) HA-specific western blot of H7N9 CVVs; (c) Purified H7 eHAs with or without deglycosylation using PNGase F.



**Supplemental Figure 2. Native PAGE analysis of Recombinant H7 HA.** Recombinant HA (rHA) from H7N9 viruses (a) A/Guangdong/17SF003/2016 (GD/16), (b) A/Hong Kong/125/2017 (HK/125) or (c) A/Shanghai/2/2013 (SH/2) was subjected to native PAGE with or without deglycosylation using PNGase F.



**Supplemental Figure 3. In vitro proliferation and ELISPOT after immunization.**

Splenocytes were harvested from mice immunized with adjuvanted HA extracted (eHAs) from H7N9 A/Shanghai/2/2013 (SH/2), A/Hong Kong/125/2017 (HK/125), or A/Guangdong/17SF003/2016 (GD/16) and were restimulated in vitro with purified H7N9 candidate vaccine viruses or recombinant HAs (rHAs) for proliferation and ELISPOTs. (a) Cell proliferation; (b) IgG ELISPOT; (c) IL-4 ELISPOT; (d) IFN- $\gamma$  ELISPOT. n=5-8 replicates/group.