Supporting Information

Polycationic HA/CpG Nanoparticles Induce Cross-Protective Influenza Immunity in Mice

Chunhong Dong, Ye Wang, Wandi Zhu, Yao Ma, Joo Kim, Lai Wei, Gilbert X. Gonzalez, and Bao-Zhong Wang*

Center for Inflammation, Immunity & Infection, Georgia State University Institute for Biomedical Sciences, Atlanta, Georgia 30303, USA

*Bao-Zhong Wang

Email: bwang23@gsu.edu

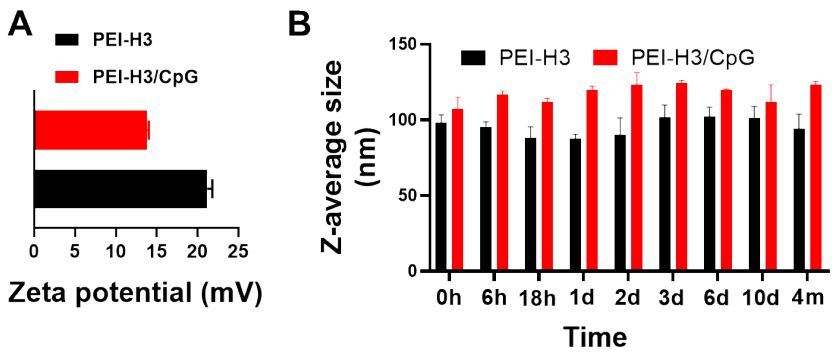

Figure S1. (A) Nanoparticle Zeta potential determined by Malvern Zetasizer. (B) Nanoparticle size stability at 4 ℃.

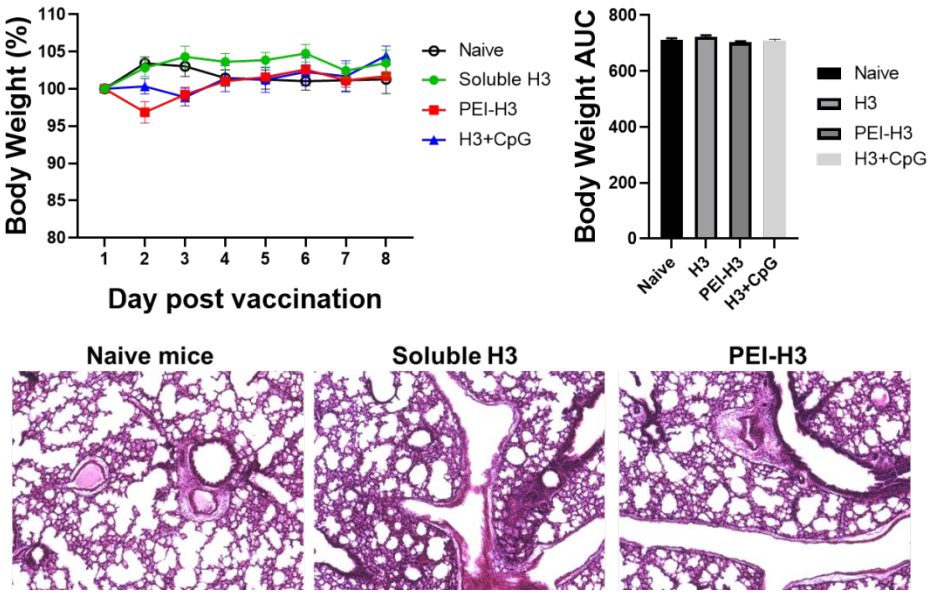

Figure S2. Mouse body weight changes and inflammatory cell infiltration in lungs 7 days post-vaccination.

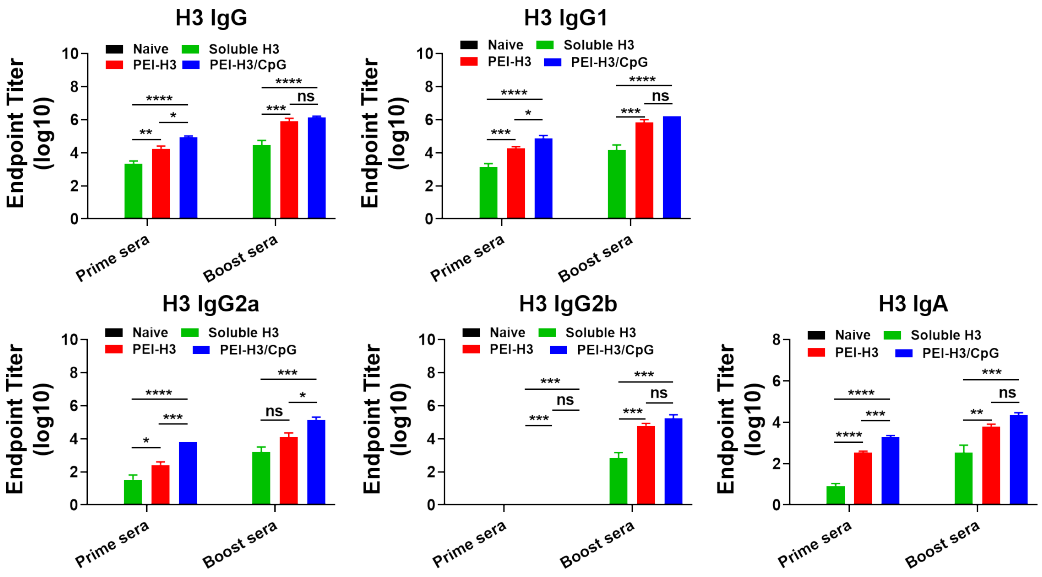

Figure S3. H3-specific IgG and IgA endpoint titers in mouse immune sera.

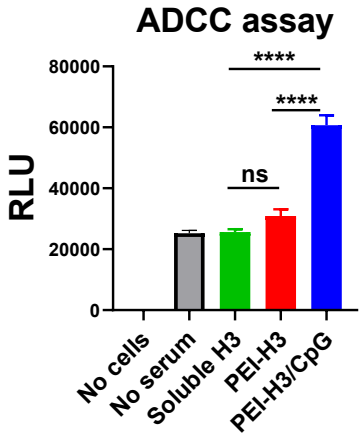

Figure S4. ADCC assay results for immune sera at a 1:250 dilution.

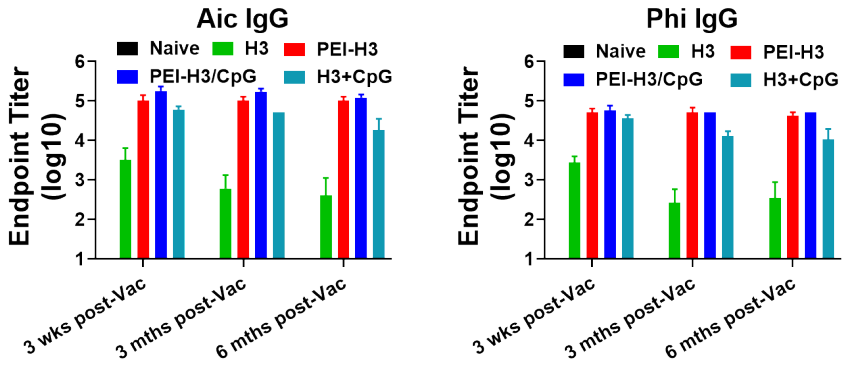

Figure S5. Long-term serum antibody titers against Aic and Phi viruses over six months post-boosting immunization.

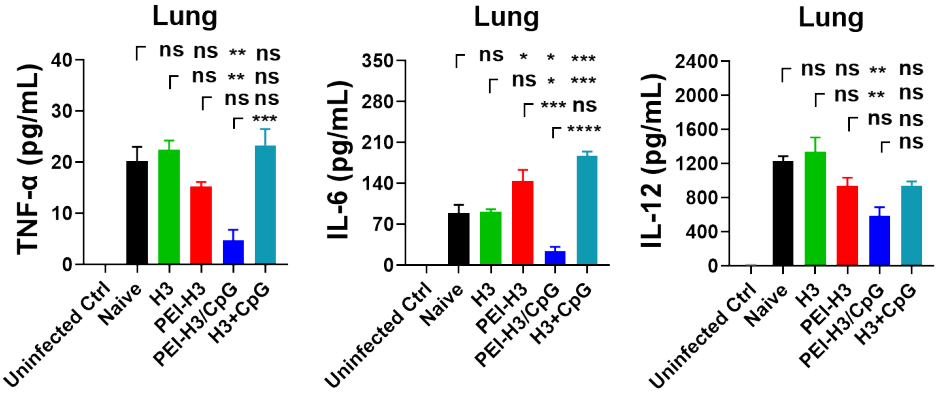

Figure S6. Inflammatory cytokine (TNF-α, IL-6, and IL-12) levels in lung supernatants of infected mice.

Table S1. Comparative analysis of the hemagglutinin (HA) amino acid sequences between influenza A/Aichi/2/1968 and A/Philippines/2/1982 or A/Wisconsin/15/2009 strains.

(A) Amino acid sequence of influenza A virus (A/Aichi/2/1968) HA	MKTIIALSYIFCLPLGQDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNAT ELVQSSSTGKICNNPHRILDGIDCTLIDALLGDPHCDVFQNETWDLFVERSKAFS NCYPYDVPDYASLRSLVASSGTLEFITEGFTWTGVTQNGGSNACKRGPGSGFF SRLNWLTKSGSTYPVLNVTMPNNDNFDKLYIWGIHHPSTNQEQTSLYVQASGR VTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINSNGNLIAPRGY FKMRTGKSSIMRSDAPIDTCISECITPNGSIPNDKPFQNVNKITYGACPKYVKQN TLKLATGMRNVPEKQTRGLFGAIAGFIENGWEGMIDGWYGFRHQNSEGTGQA ADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDL WSYNAELLVALENQHTIDLTDSEMNKLFEKTRRQLRENAEEMGNGCFKIYHKC DNACIESIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCV VLLGFIMWACQRGNIRCNICI
(B) Amino acid sequence of influenza A virus (A/Philippines/2/1982) HA	MKTIIALSYMFCLVFAQNLPGNDNSTATLCLGHHAVPNGTLVKTITNDQIEVTNAT ELVQSSSTGRICDSPHRILDGKNCTLIDALLGDPHCDGFQNEKWDLFVERSKAF SNCYPYDVPDYASLRSLVASSGTLEFINEGFNWTGVTQSGGSYTCKRGSNNSF FSRLNWLYESESKYPVLNVTMPNNGKFDKLYIWGIHHPSTDKEQTNLYIRASGR VTVSTKRSQQTVIPNIGSRPWVRGLSSRISIYWTIVKPGDILLINSTGNLIAPRGYF KIRTGKSSIMRSDAPIGTCSSECITPNGSIPNDKPFQNVNKITYGACPRYVKQNTL KLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGTGQAAD LKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWS YNAELLVALENQHTIDLTDSEMNKLFEKTRKQLRENAEDMGNGCFKIYHKCDNA CIGSIRNGTYDHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLL GFIMWACQKGNIRCNICI
(C) Amino acid sequence of influenza A virus (A/Wisconsin/15/2009) HA	MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATE LVQSSSTGEICDSPHQILDGKNCTLIDALLGDPQCDGFQNKKWDLFVERSKAYS NCYPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSACIRRSKNSFF SRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIFPYAQASGRI TVSTKRSQQTAIPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKI RSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLK LATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSEGRGQAADL KSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSY NAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNAC IGSIRNGTYAHDVYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVALLG FIMWACQKGNIRCNICI
Difference between A and B	8.48%, as determined by the NCBI protein BLAST tool.
Difference between A and C	13.43%, as determined by the NCBI protein BLAST tool.