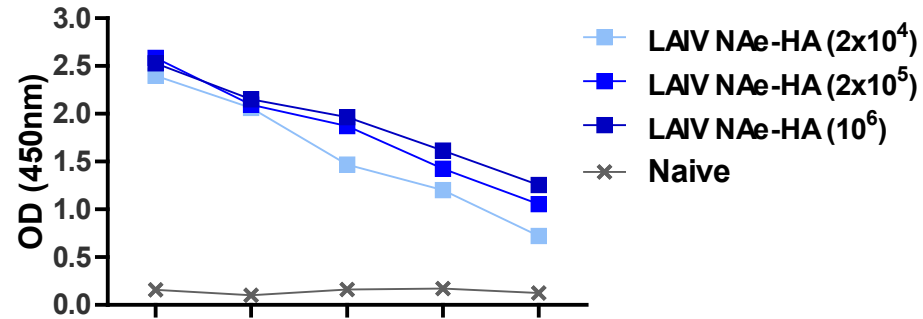
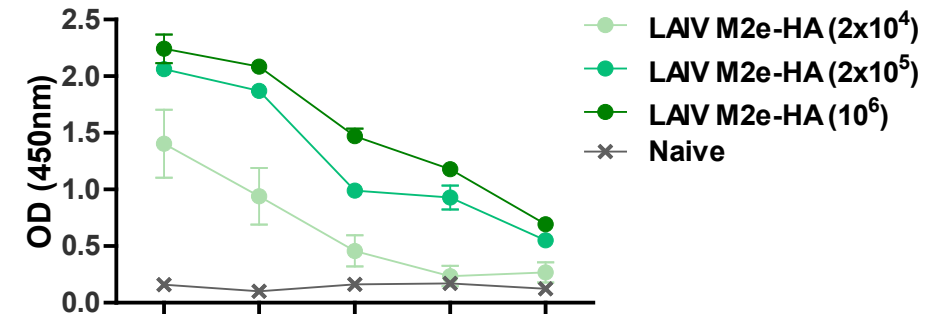
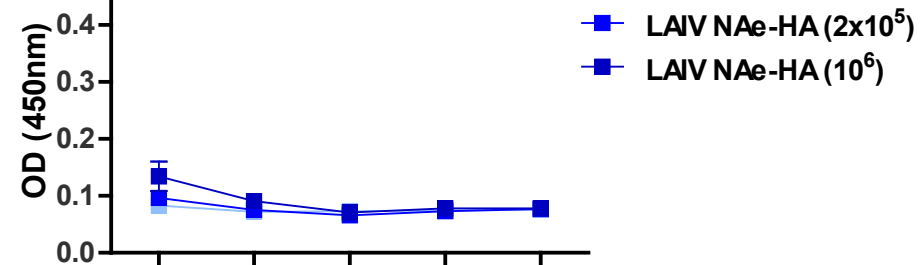
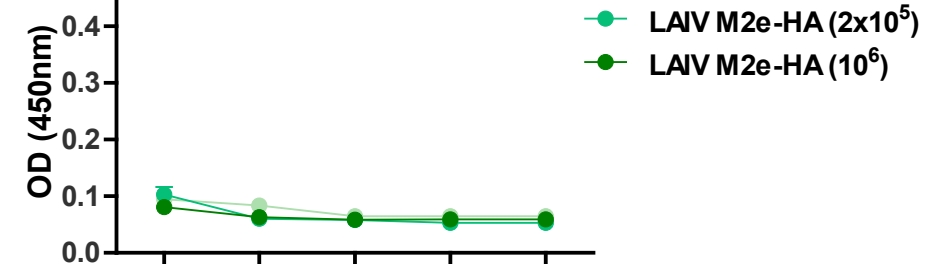
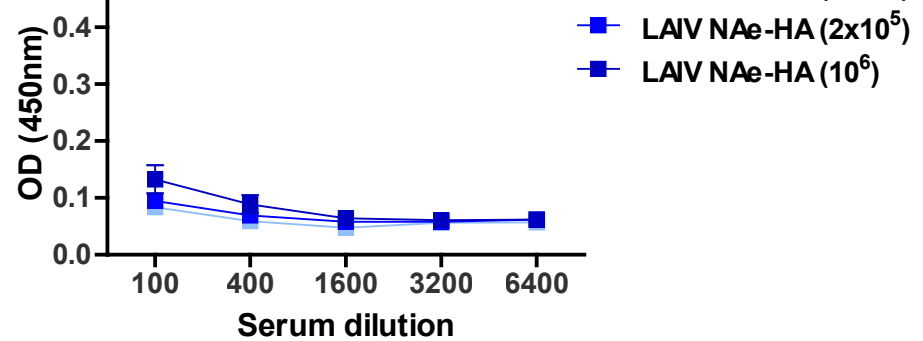
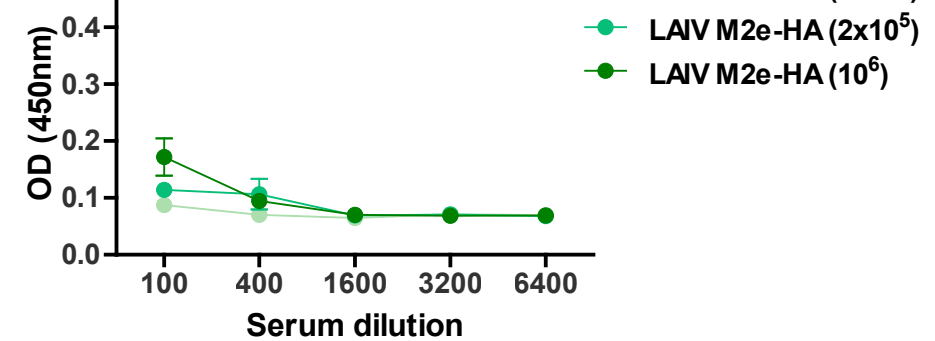
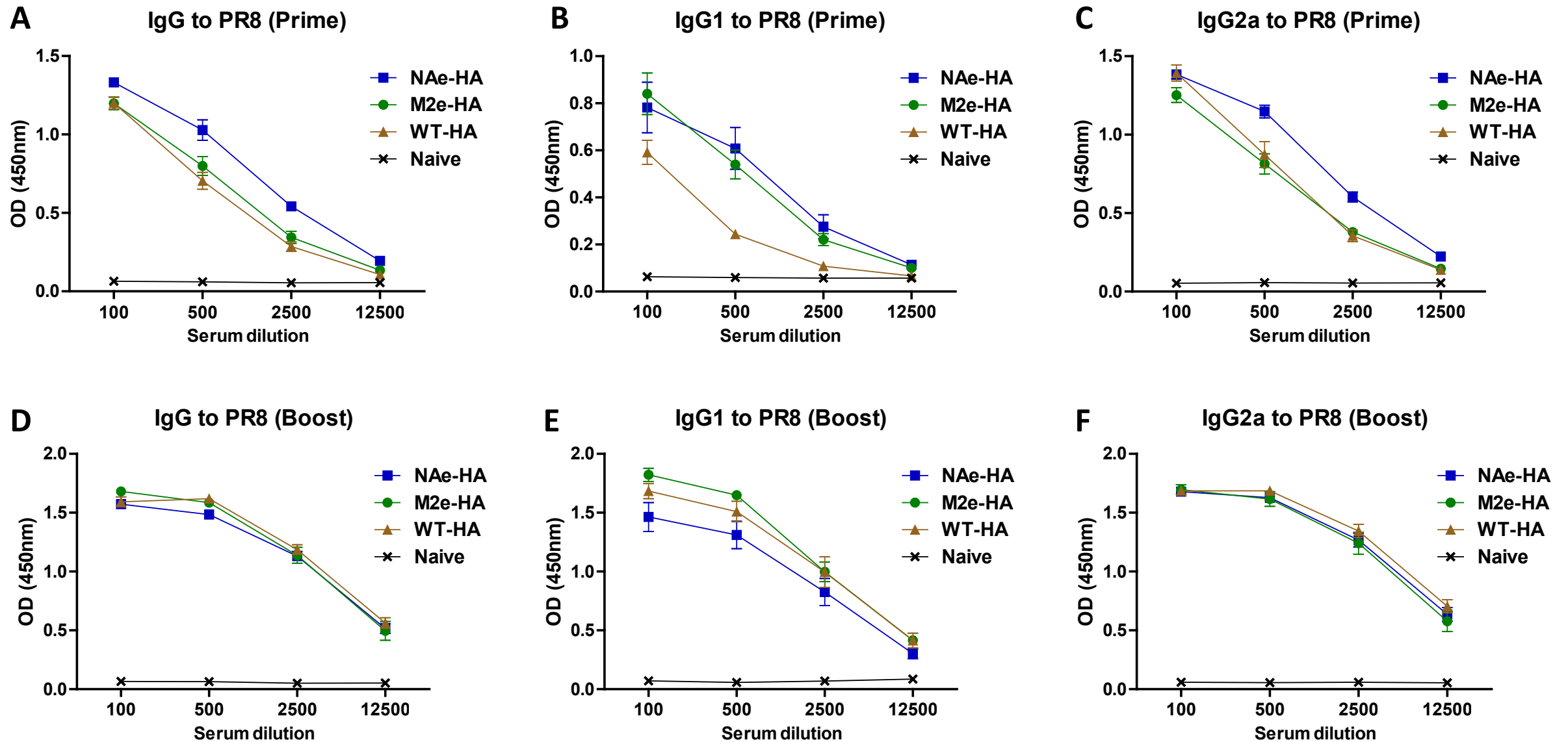


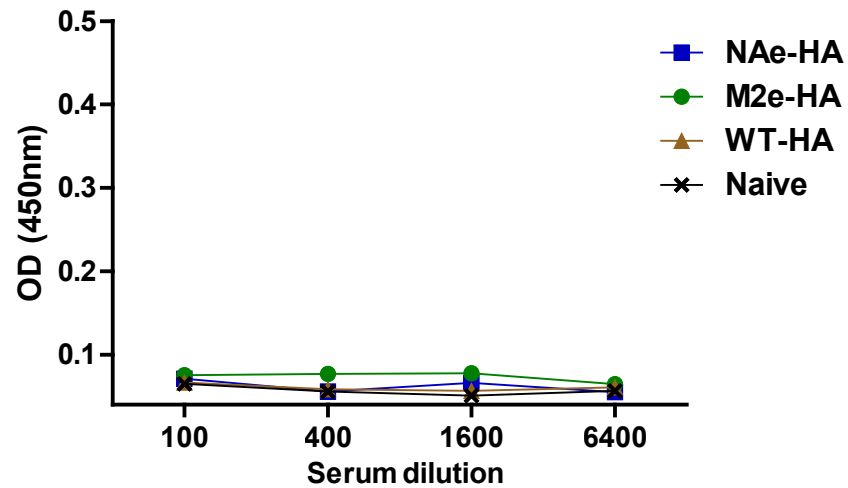
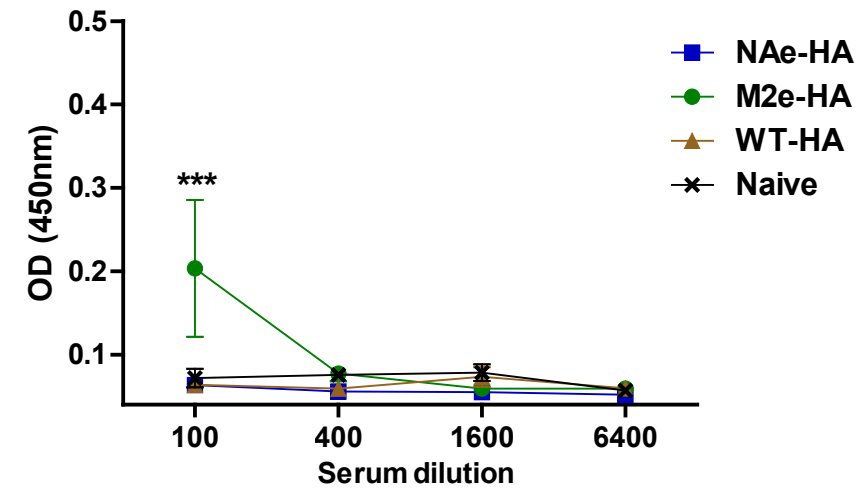
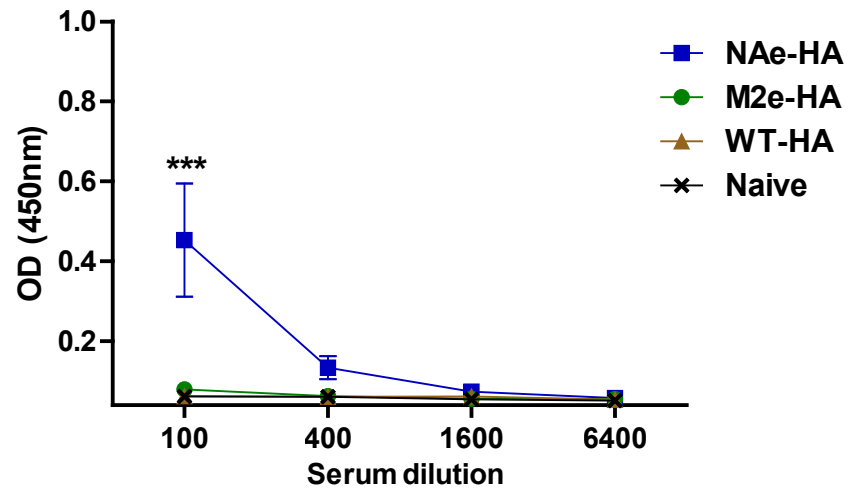
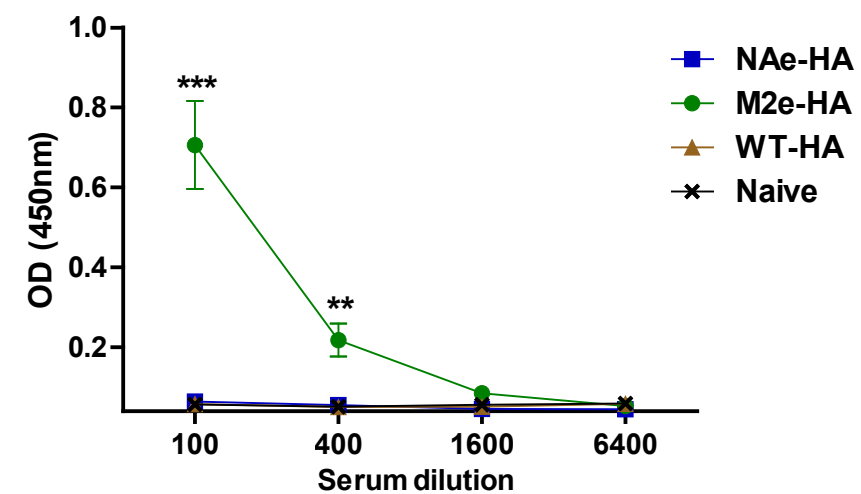
Supplementary Figure S1. Live recombinant chimeric HA A/PR8 influenza viruses display attenuated phenotypes in mice. Six to eight weeks old female BALB/c mice (N=3 per group) were intranasally (IN) inoculated with 2×10^4 , 2×10^5 , 1×10^6 EID₅₀ of attenuated recombinant NAe-HA, -M2e HA, -WT-HA A/PR8 viruses, or a control WT pathogenic backbone A/PR8 influenza virus. Body weight changes were monitored daily for 14 days to assess the pathogenicity of attenuated recombinant A/PR8 viruses. (A-C) Body weight changes of mice after inoculation with attenuated recombinant NAe-HA, -M2e-HA, WT-HA A/PR8 influenza viruses. (D) Body weight changes of mice after inoculation with pathogenic backbone WT A/PR8 virus (WT HA-PR8).

A IgG to PR8 (Prime)**B IgG to PR8 (Prime)****C IgG to NAe (Prime)****D IgG to NAe (Prime)****E IgG to M2e (Prime)****F IgG to M2e (Prime)**

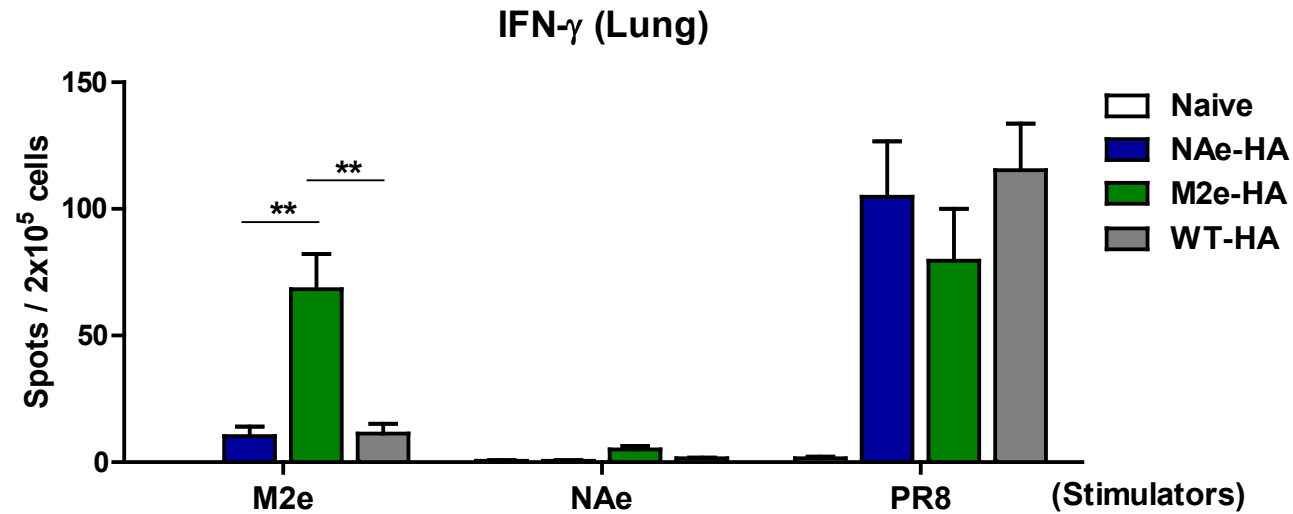
Supplementary Figure S2. IgG antibody responses after prime inoculation with attenuated recombinant A/PR8 influenza viruses containing chimeric NAe-HA or -M2e-HA. BALB/c mice were IN inoculated with indicated EID₅₀ of attenuated chimeric NAe-HA or -M2e-HA A/PR8 influenza viruses. Sera were collected at 3 weeks after prime immunization. Antibody responses specific for A/PR8 virus, NAe or M2e peptides were determined by ELISA. IgG antibody response to A/PR8 virus (A and B), NAe peptide (C and D), and M2e peptide (E and F) in prime sera.



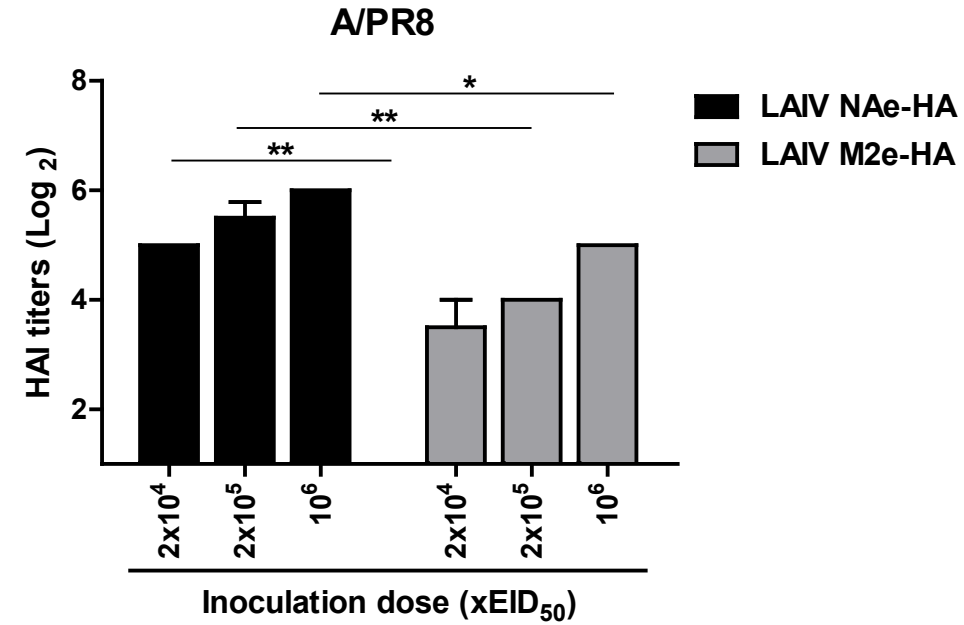
Supplementary Figure S3. Virus specific IgG antibody responses after immunization with inactivated recombinant A/PR8 influenza viruses containing chimeric HA. BALB/c mice were IM immunized with 10 μ g of inactivated recombinant A/PR8 influenza viruses (NAe-HA, M2e-HA, WT-HA A/PR8). Sera were collected at 3 weeks after prime and boost immunization. IgG antibody responses specific for A/PR8 virus was determined by ELISA. Total IgG, IgG1, IgG2a isotype antibodies specific for A/PR8 virus in prime sera (A-C) and boost sera (D-F).

A IgG to NAe (Prime)**B** IgG to M2e (Prime)**C** IgG to NAe (Boost)**D** IgG to M2e (Boost)

Supplementary Figure S4. NAe or M2e-specific IgG antibody responses after immunization with inactivated recombinant A/PR8 influenza viruses. Sera were collected at 3 weeks after prime and boost IM immunization of BALB/c mice with 10 μ g of inactivated recombinant influenza viruses (NAe-HA, M2e-HA, WT-HA A/PR8). IgG antibody responses specific for NAe or M2e peptides were determined by ELISA. Total IgG antibody in prime sera (A and B) and boost sera (C and D). Statistical significance was determined by using two-way ANOVA test. Data are representative of individual animal out of two independent experiments. Error bars indicate the means \pm SEM. ***, $p < 0.001$, **, $p < 0.01$.



Supplementary Figure S5. Comparison of T cell responses secreting IFN- γ upon stimulation with various antigens in lung cells from vaccinated mice. The lung cells were harvested from vaccinated mice at day 6 after challenge with A/Phil/H3N2 ($8 \times LD_{50}$). The cells were stimulated with NAe, M2e peptide ($5 \mu\text{g/ml}$), and A/PR8 virus antigen ($5 \mu\text{g/ml}$) and then the IFN- γ positive cell spots were determined by ELISpot assay. Antigens (M2e, NAe, A/PR8)-specific IFN- γ secreting cell spots. Statistical significance was determined by using two-way ANOVA test. Data are representative of individual animal out of two independent experiments. Error bars indicate the means \pm SEM. **: $p < 0.01$.



Supplementary Figure S6. Hemagglutination inhibition titer after intranasal inoculation of live attenuated recombinant chimeric HA A/PR8 influenza viruses. HAI titers to A/PR8 influenza virus were determined after intranasal prime inoculation as described in figure 4. Statistical significance was determined by using two-way ANOVA. Error bars indicate the means \pm SEM. **: $p < 0.01$. *: $p < 0.05$.