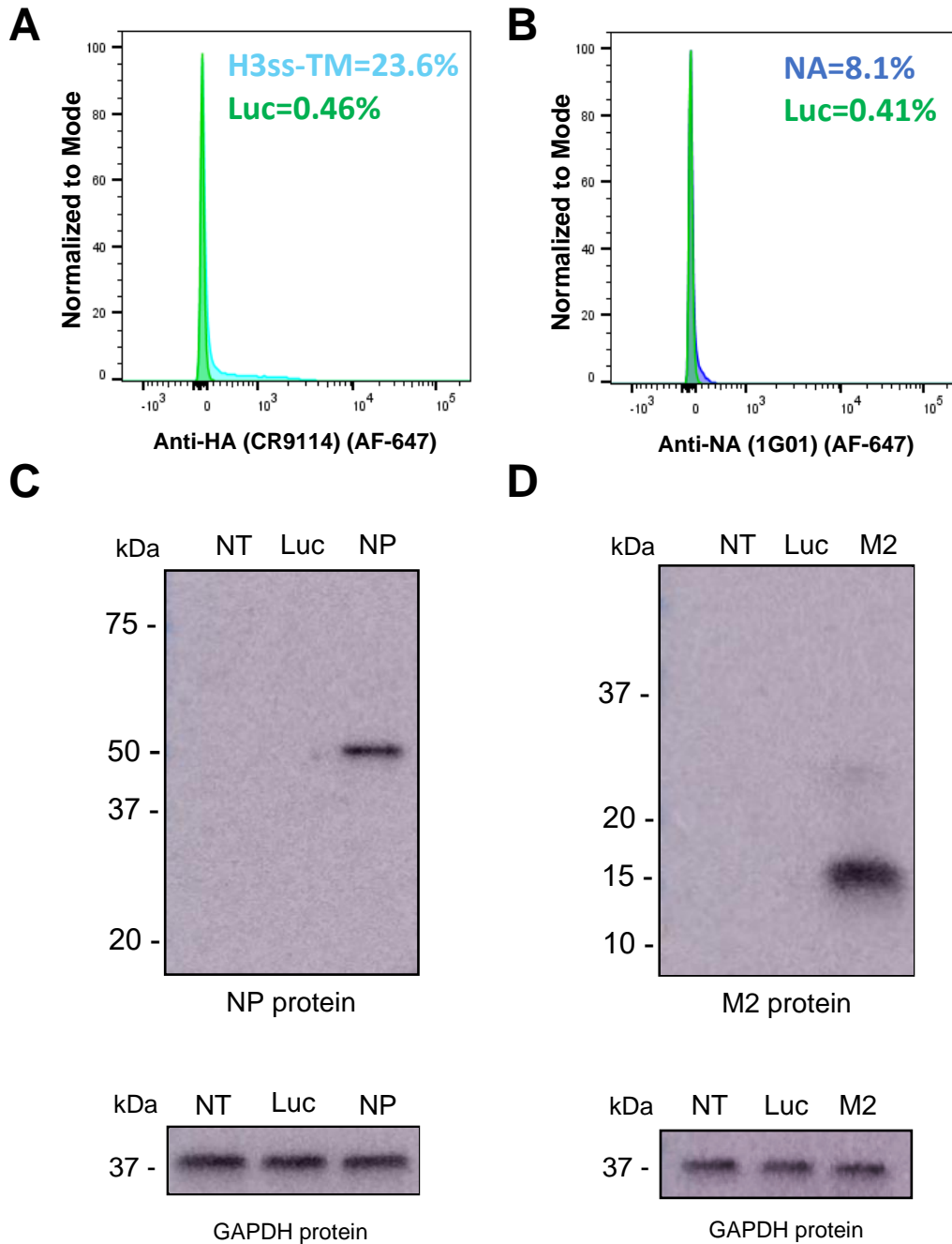
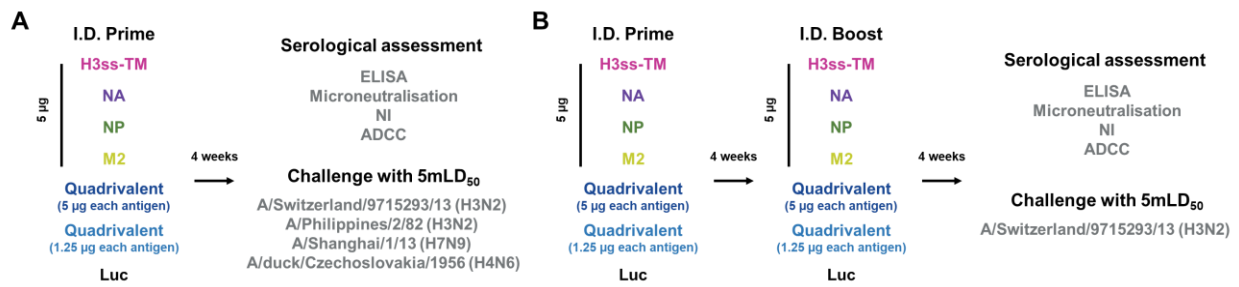


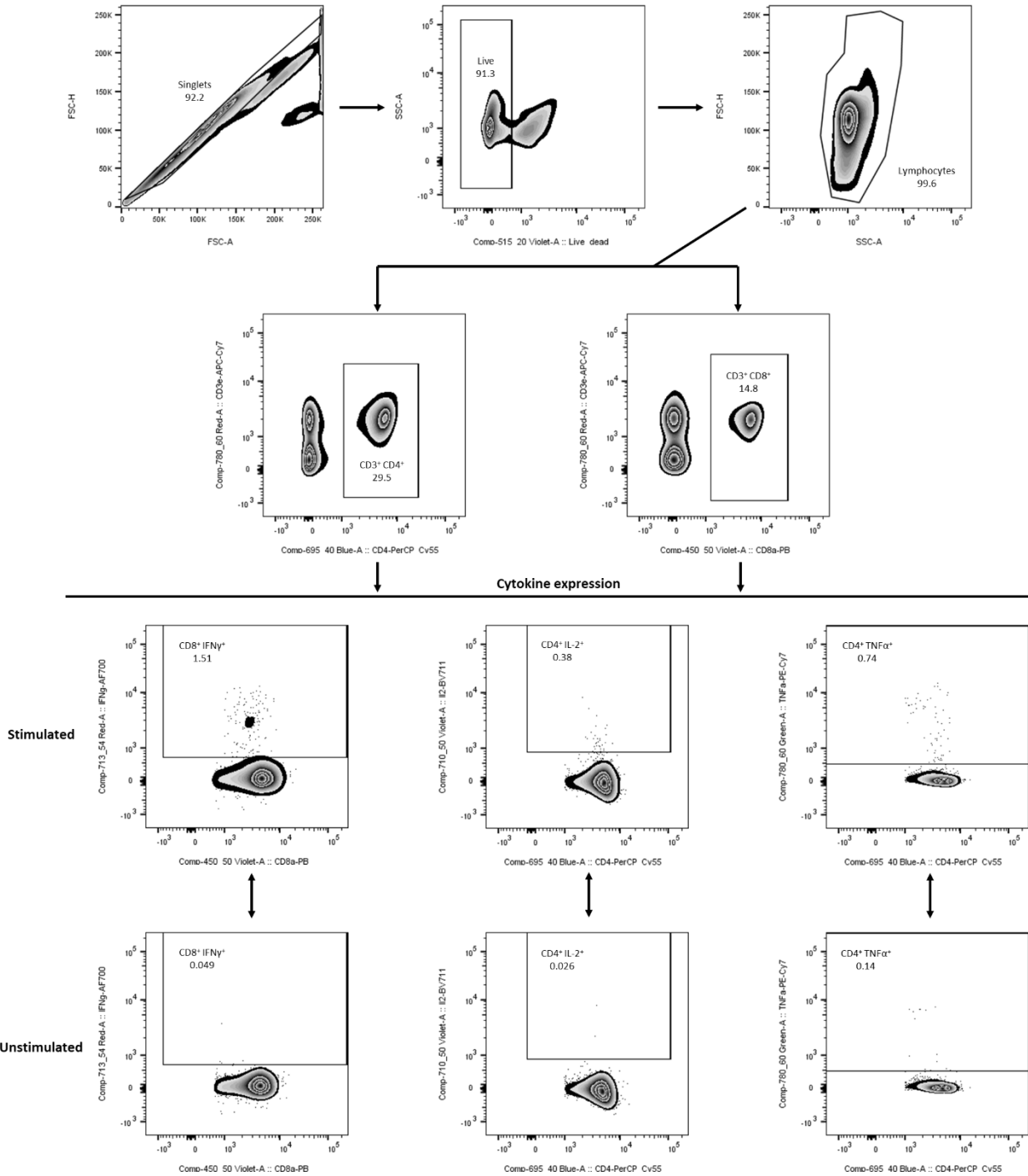
SUPPLEMENTAL INFORMATION



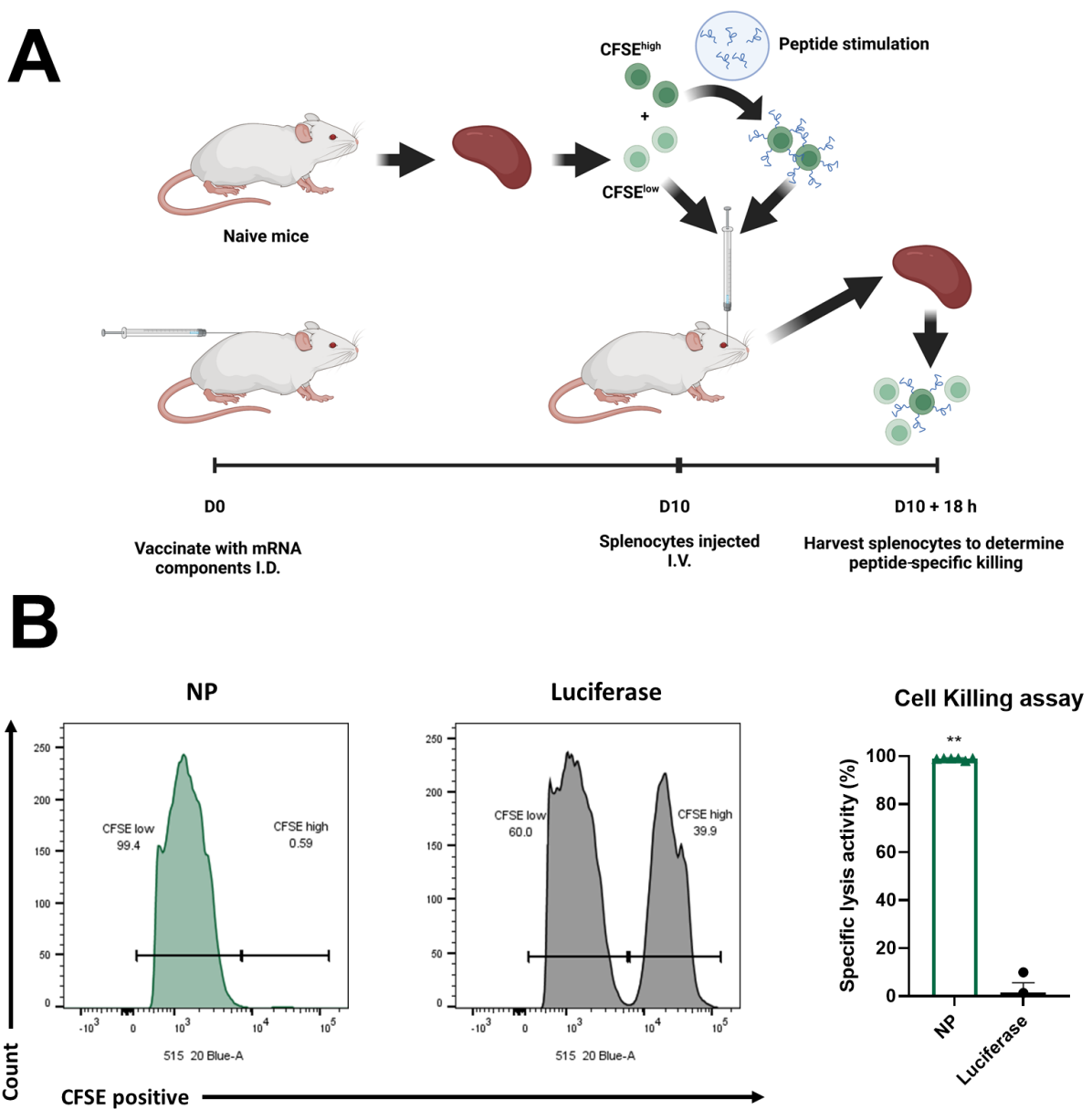
**Fig. S1. Characterization of antigen-encoding mRNAs by flow cytometry or Western blot.** To determine protein expression from the mRNA vaccine components, HEK293T cells were transfected with H3ss-TM (HA)- or NA-encoding mRNAs and protein production was assessed via flow cytometry. Positive binding of the HA- (**A**) and NA- (**B**) specific antibodies relative to Luc control is shown. HEK293T cells were transfected with NP- (**C**) and M2- (**D**) encoding mRNAs and protein production from mRNAs was assessed via Western blotting. A GAPDH-specific antibody was used as a loading control antibody in the Western blot experiments. NT: non-transfected, Luc: firefly luciferase, GAPDH: glyceraldehyde 3-phosphate dehydrogenase



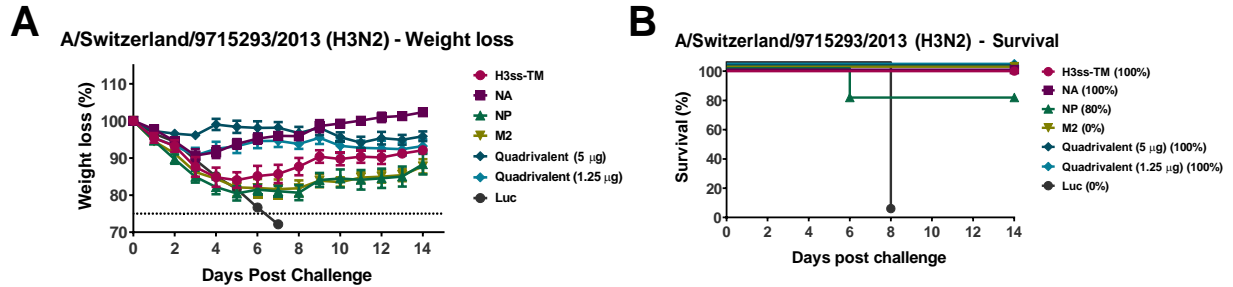
**Fig. S2. Experimental design: vaccines, immunizations, serology and challenge studies.** Mice were intradermally (I.D.) vaccinated with 5 µg of monovalent mRNA-LNP or with 5 µg/antigen or 1.25 µg/antigen of the quadrivalent mRNA-LNP formulation. Four weeks after vaccination, animals were anesthetized and intranasally (I.N.) infected with 5mLD<sub>50</sub> of group 2 influenza viruses. Additionally, mice were bled for serological analysis (ELISA, MNT, NI and ADCC) at this time point (**A**). For prime-boost vaccination studies, mice were vaccinated as described above 4 weeks apart. Four weeks after the second immunization, mice were I.N. challenged with 5mLD<sub>50</sub> of A/Switzerland/9715293/2013 (H3N2) influenza virus. Additionally, animals were bled for serological analysis (ELISA, MNT, NI and ADCC) at this time point (**B**).



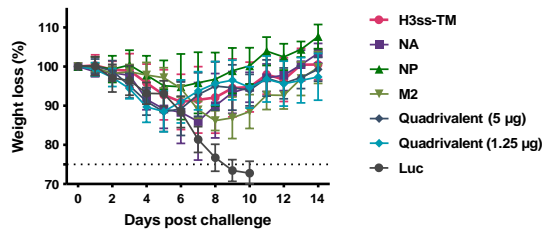
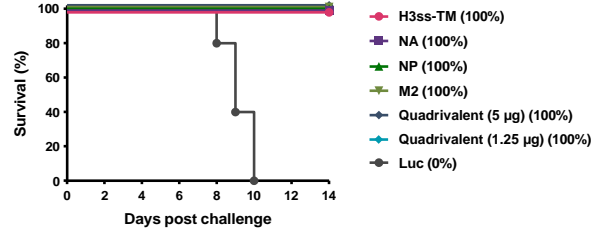
**Fig. S3. Flow cytometric gating strategy for the investigation of T cell responses in influenza A group 2 mRNA-LNP-immunized mice.** Representative flow cytometry plots for unstimulated and peptide-stimulated samples are shown.



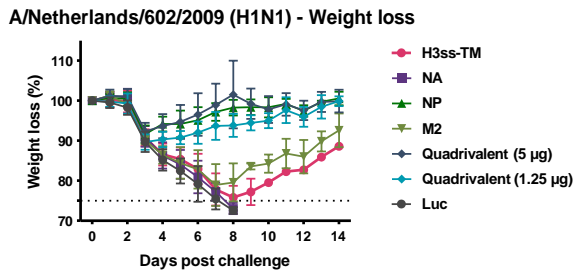
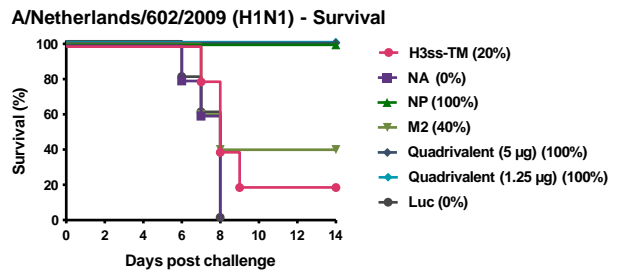
**Fig. S4. Nucleoside-modified NP mRNA-LNP vaccination elicits strong antigen-specific *in vivo* cell killing activity.** Mice were vaccinated I.D. with a single dose of 5  $\mu$ g of NP or Luc mRNA-LNPs and NP-specific killing activity was determined. (A) Schematic illustration of the *in vivo* cytotoxicity assay. (B) Antigen-specific killing activity in mice immunized with NP mRNA-LNPs. Each symbol represents one animal and error is shown as SEM (n = 6 mice per group). Statistical analysis: two-tailed unpaired t-test, \*\* =  $p < 0.01$ .



**Fig. S5. Improved protection afforded to mice vaccinated twice with the quadrivalent mRNA-LNP vaccine.** Mice were I.D. vaccinated twice (4 weeks apart) with monovalent or quadrivalent mRNA-LNPs as described in Figure 1. Four weeks after the second immunization, animals were I.N. challenged with 5mLD<sub>50</sub> of A/Switzerland/9715293/2013 (H3N2) influenza virus and morbidity (A) and mortality (B) were assessed. Survival is also represented in parentheses in panel B. Data are shown as mean and error bars are represented by SD ( $n = 5$  per group).

**A****A/canine/Illinois/41915/2015 (H3N2) - Weight loss****B****A/canine/Illinois/41915/2015 (H3N2) - Survival**

**Fig. S6. Protection afforded to mice against a phylogenetically distant H3N2 influenza virus strain.** Mice were I.D. vaccinated once with the group 2 monovalent or quadrivalent mRNA-LNPs as described in Figure 1. Four weeks after immunization, animals were I.N. challenged with 5mLD<sub>50</sub> of A/canine/Illinois/41915/2015 (H3N2) influenza virus and morbidity (**A**) and mortality (**B**) were assessed. Survival is also represented in parentheses in panel B. Data are shown as mean and error bars are represented by SD ( $n = 5$  per group).

**A****B**

**Fig. S7. Cross-group protection afforded to mice vaccinated once with the quadrivalent mRNA-LNP vaccine.** Mice were I.D. vaccinated once with the group 2 monovalent or quadrivalent mRNA-LNPs as described in Figure 1. Four weeks after immunization, animals were I.N. challenged with 5mLD<sub>50</sub> of A/Netherlands/602/2009 (H1N1) influenza virus and morbidity (**A**) and mortality (**B**) were assessed. Survival is also represented in parentheses in panel B. Data are shown as mean and error bars are represented by SD ( $n = 5$  per group).

Viruses	Vaccine Antigen			
	H3ss-TM	NA	NP	M2
A/Singapore/INFIMH-16-0019/2016 (H3N2)	86.34	100.00	100.00	100.00
A/Switzerland/9715293/2013 (H3N2)	87.67	98.29	91.57	98.97
A/Philippines/2/1982 (H3N2)	88.11	88.70	95.98	94.85
A/canine/Illinois/41915/2015	83.78	79.96		
A/duck/Czechoslovakia/1956 (H4N6)	70.40	45.14		
A/Shanghai/1/2013 (H7N9)	62.78	44.44		
A/Netherlands/602/2009 (H1N1)	49.49	44.54		
A/Puerto Rico/08/1934 (H1N1)			91.77	88.66

**Table S1. Vaccine antigen and challenge strain percent (%) amino acid similarity.** Amino acid sequences from NA and HA vaccine antigens were aligned to proteins from influenza virus strains used in this study (A/Switzerland/9715293/2013 (H3N2), A/Philippines/2/1982 (H3N2), A/canine/Illinois/41915/2015 (H3N2), A/duck/Czechoslovakia/1956 (H4N6), A/Shanghai/1/2013 (H7N9) and A/Netherlands/602/2009 (H1N1)). The NP and M2 vaccine antigens were aligned to the amino acid sequences from A/Switzerland/9715293/2013 (H3N2), A/Puerto Rico/8/1934 (H1N1) and A/Philippines/2/1982 (H3N2). A/Puerto Rico/8/1934 (H1N1) is also included as A/Philippines/2/1982 (H3N2), A/duck/Czechoslovakia/1956 (H4N6) and A/Shanghai/1/2013 (H7N9) contain only the NA and HA of the respective viruses, and were rescued using the A/Puerto Rico/8/1934 backbone. The sequences were aligned using the Clustal Omega 2.1 multiple sequence alignment tool. The percent (%) amino acid identity was determined using the computed percent identity matrix and examined for each challenge virus.