

Supplementary Fig. 1. Rg-H5N1 specific antibody levels and protection after rgH5N1 infection in the immunized C57BL/6 mice. C57BL/6 mice (n=5-6) were intramuscularly immunized two times with 3 μ g of split virus vaccine (sCal, A/California/2009 H1N1 virus) alone, sCal plus MPL 0.5 μ g + CpG 2 μ g, or MPL 0.5 μ g + CpG 2 μ g only. (A-C) After boost immunization, the immune sera were taken to measure rgH5N1 specific antibody levels. (D-E) The immunized mice were infected with rgH5N1 (1.5xLD₅₀), and body weight changes and survival rates were monitored for 14 days. All data were shown in mean ± standard deviation (SD).

A. Prime



Supplementary Fig. 2. Levels of serum IgG and IgG isotype antibodies recognizing the vaccine strain in BALB/c mice after vaccination. BALB/c mice (n=5) were intramuscularly immunized with 0.3 μ g of split virus vaccine (sCal, A/California/2009 H1N1 virus) alone or in the presence of adjuvants two times at a 3-week interval. The doses of adjuvants were as follows: MPL 0.5 μ g, CpG 2 μ g, or MPL 0.5 μ g + CpG 2 μ g. The immune sera were taken 2 weeks after each immunization (prime and boost) and A/Cal H1N1 virus-specific antibody levels were measured by ELISA. All data were shown in mean ± standard deviation (SD).



Supplementary Fig. 3. Neuraminidase inhibition (NAI) assay with the immune sera of Balb/c mice. (A) NAI titers against homologous A/Cal H1N1 virus. (B) NAI titers against heterosubtypic rgH5N1 virus. Percentages of inhibitions were based on the virus only treated wells. All data were shown in mean ± standard deviation (SD).



Supplementary Fig. 4. Influenza nucleoprotein (NP)-specific CD8 memory T cells in the immunized Balb/c mice after rgH5N1 infection. Bronchoalveolar lavage (BAL) was collected at day 7 post infection and BAL cells were stained with NP-tetramer to determine antigen-specific T cell responses. CD3⁺CD8⁺CD44⁺NP_{tet}⁺ cells were shown. All data were shown in mean \pm standard deviation (SD).