



Figure S1. Immunofluorescence assay to detect serum antibodies against reporter GFP protein. After the first injection, HHS-L group generated highest level of anti-GFP antibodies, and the level was maintained throughout the experimental duration. In SSH-L group, sequential inoculation of HAdV5-GFluc significantly increased the level of serum antibody against GFP. After the third injection, there was no significant difference in serum antibody level between groups. ** $p < 0.01$, *** $P < 0.001$. The experimental procedure was described below.

A plasmid carrying CMV promoter-controlled GFP gene (pLEGFP-C1, CLONTECH) was used to transfect 293 cells. The next day, the cells were detached by trypsin treatment, dispersed into single cells, seeded in 96-well plates and cultivated for another 24 hours. The percentage of GFP+ cells is approximately 42%. Cells were fixed in 100 μ l 4% polyformaldehyde in PBS per well at room temperature for 20 minutes, washed with PBS for 3 times, penetrated by 0.2% Triton X-100 at room temperature for 10 minutes, washed with PBS for 3 times, blocked with PBS plus 1% BSA at room temperature for 30 minutes, incubated with 1:50 diluted mouse sera at room temperature for 2 hours (50 μ l per well, in duplicate), washed with PBST for 3 times (250 μ l per well), incubated with 1:100 diluted TRITC-conjugated goat anti-mouse IgG (Cat. ZF-0313, ZSGB-BIO, Beijing, China) at room temperature for 1 hour and washed with PBST for 3 times. Cells with red fluorescence signal were photographed by using a high-throughput image analyzer (Countstar Castor, Alit Biotech, Shanghai, China). The fluorescence intensity per well was calculated by using Fiji image processing package (<http://fiji.sc/>). Briefly, open image > image|color| split channels > using the red channel image > oval tool to select the whole well > edit | clear outside > image | adjust | threshold > analyze | set measurements (select intensity density) > analyze | measure (the steps were recorded as a Macro programming and used to analyzed images of other wells). The fluorescence intensity was log-transformed and normalized by subtracting the mean value of negative sera from PBS mock-infected mice.