Supporting information for

A single-injection vaccine providing protection against two HPV types

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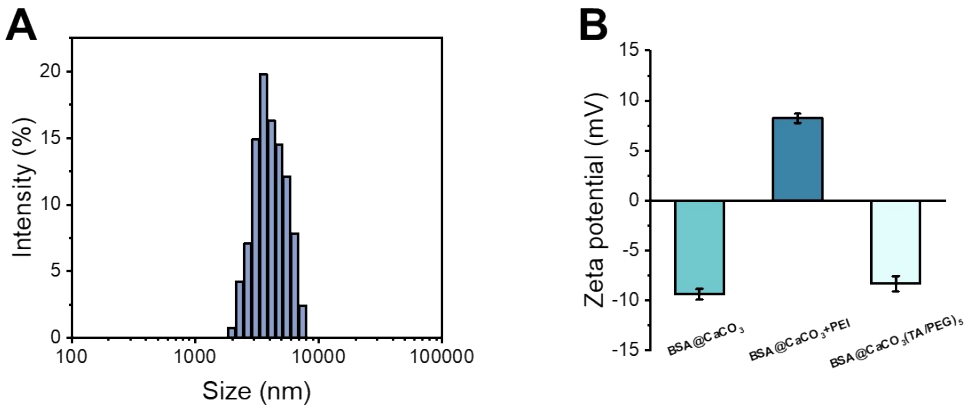

Fig. S1. (A) Size distribution of BSA@$CaCO_3$ determined by DLS. (B) Zeta potential of BSA@$CaCO_3$ microspheres, the same microspheres deposited sequentially with a PEI layer and a 5 bilayer TA/PEG film.

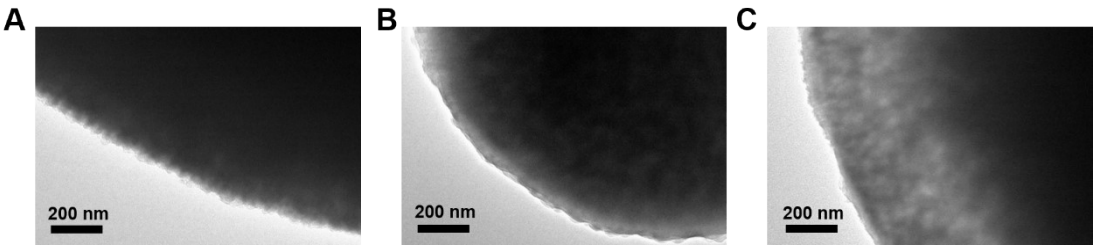

Fig. S2. TEM images of bare BSA@$CaCO_3$ microsphere (A), BSA@$CaCO_3$ microsphere coated with 5 (B) and 50 bilayers of TA/PEG LBL film (C).

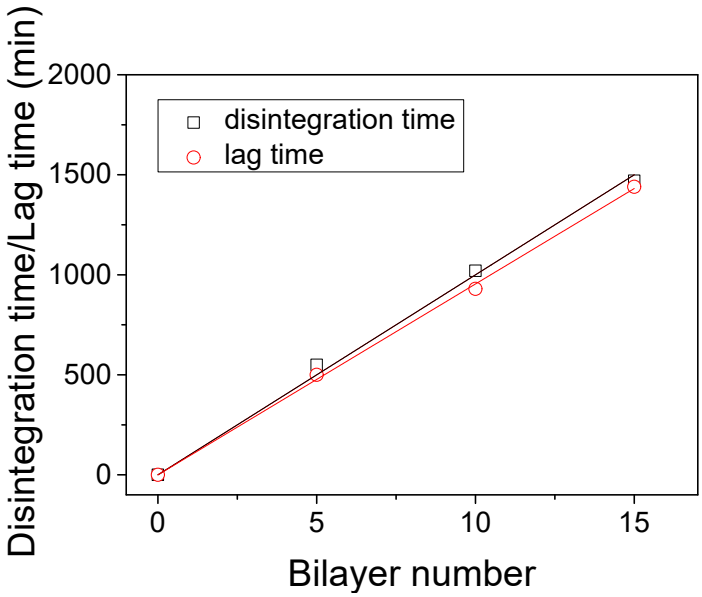

Fig. S3. Disintegration time of TA/PEG films (□) and lag time of FITC-BSA release from the $CaCO_3$ microspheres coated with TA/PEG films (○) as a function of the bilayer number of the TA/PEG films.

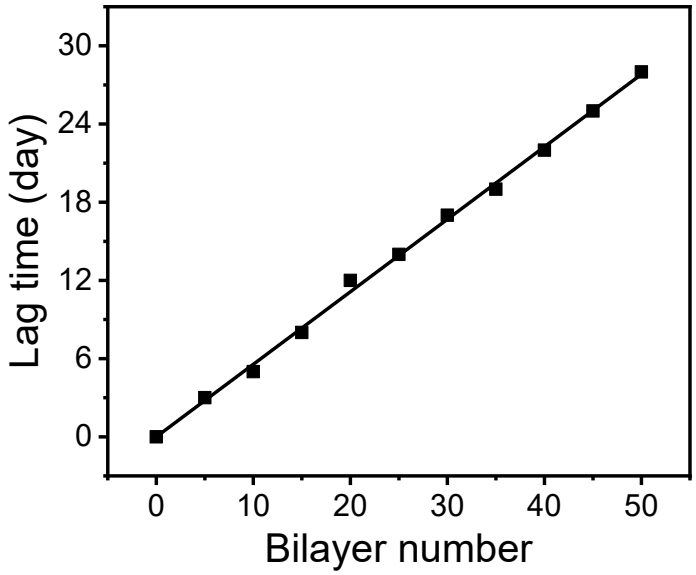

Fig. S4. In vivo release lag time of FITC-BSA from the $CaCO_3$ microspheres coated with TA/PEG films as a function of the bilayer number of the TA/PEG films.

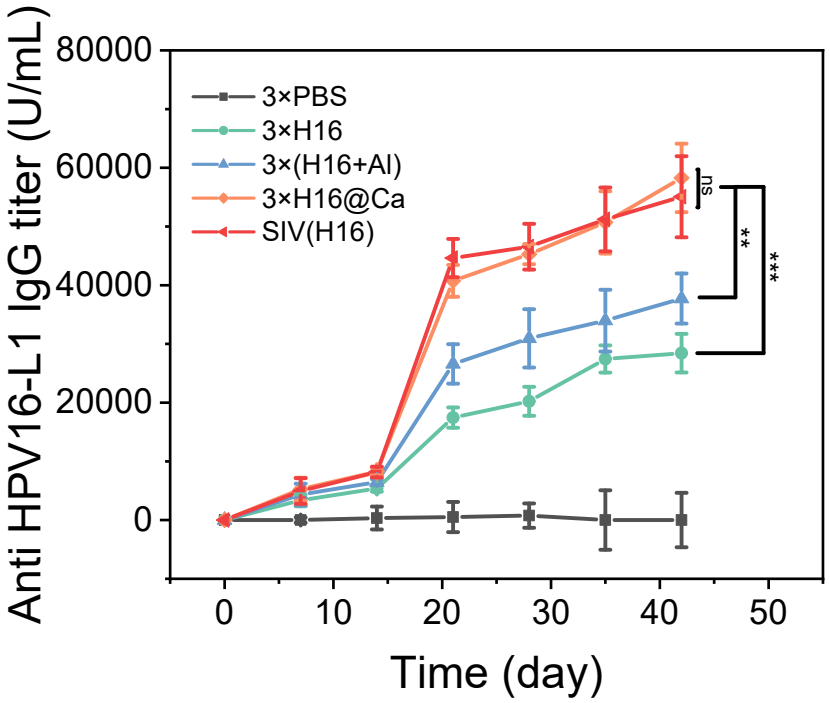

Fig. S5. Anti HPV16-L1 IgG titer produced in sera of the immunized mice.

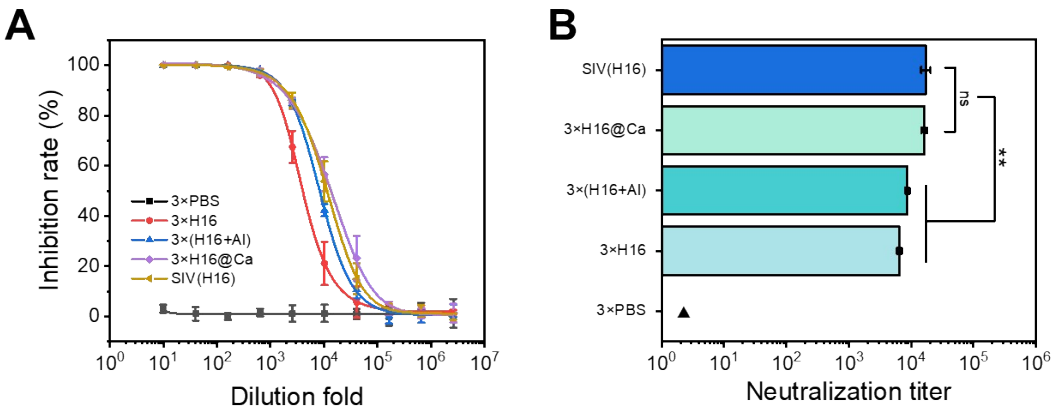

Fig. S6. (A) The inhibition rate of HPV16 pseudovirus by serially diluted mice sera. The sera were collected 35 days after initial injection . (B) The HPV16 pseudovirus neutralization titers of the sera collected 35 days after initial injection. The solid triangle refers to no detectable neutralization reaction with HPV16 pseudovirus.

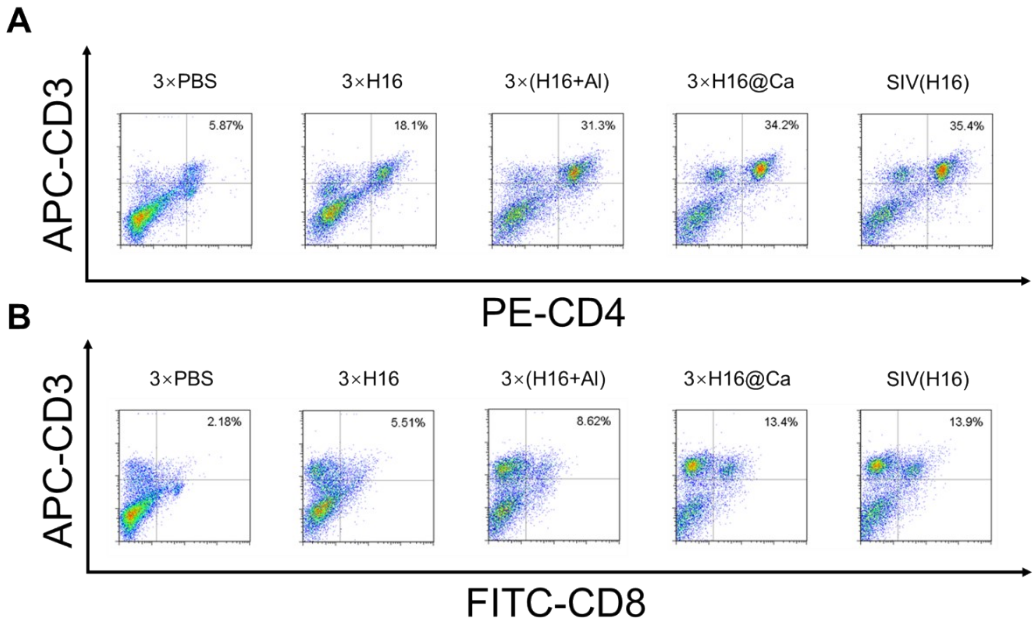

Fig. S7. Flow cytometric analysis to detect the percentage of CD3+CD4+ (A) and CD3+CD8+ (B) in splenocytes collected from the immunized mice after 72 h re-stimulation with HPV16-L1.

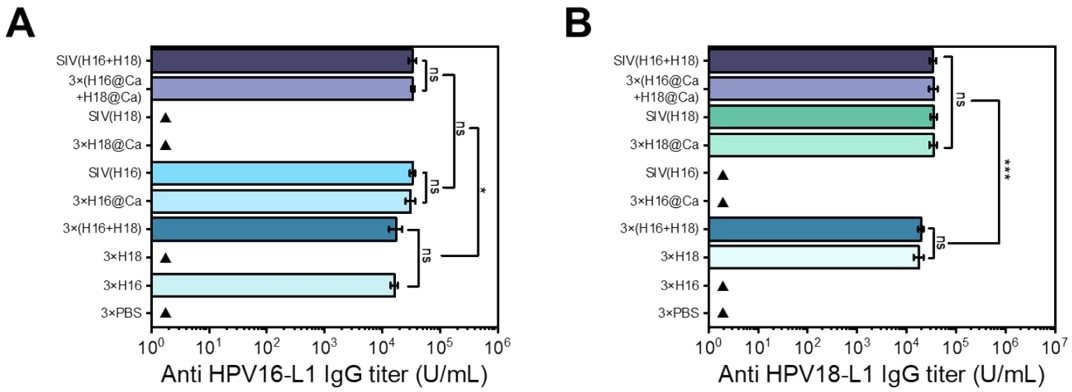

Fig. S8. Anti HPV16-L1 IgG titer (A) and anti HPV18-L1 IgG titer (B) produced in sera 35 days after initial injection. The solid triangle refers to no detectable antibody against HPV16-L1 or HPV18-L1.

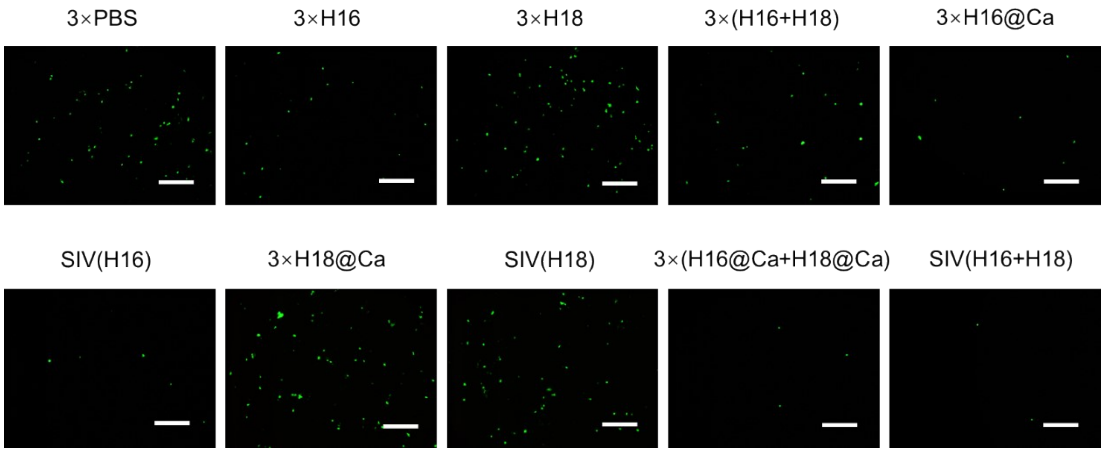

Fig. S9. Fluorescent images of 239FT cells after incubation with a mixture of HPV16 pseudovirus and diluted sera (1:2560) from immunized mice. The sera were collected 42 days after the initial injection. The scale bar is 50 μm.

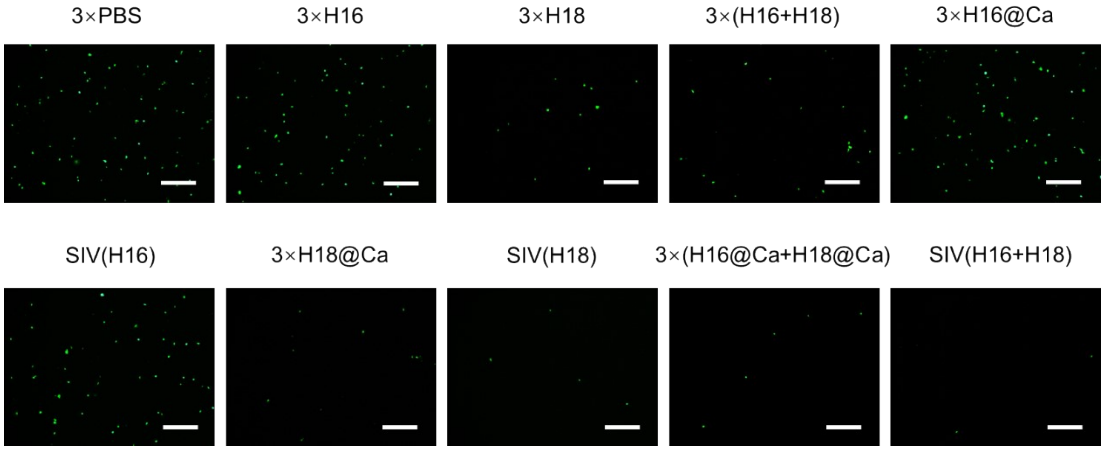

Fig. S10. Fluorescent images of 239FT cells after incubation with a mixture of HPV18 pseudovirus and diluted sera (1:2560) from immunized mice. The sera were collected 42 days after the initial injection. The scale bar is 50 μm.

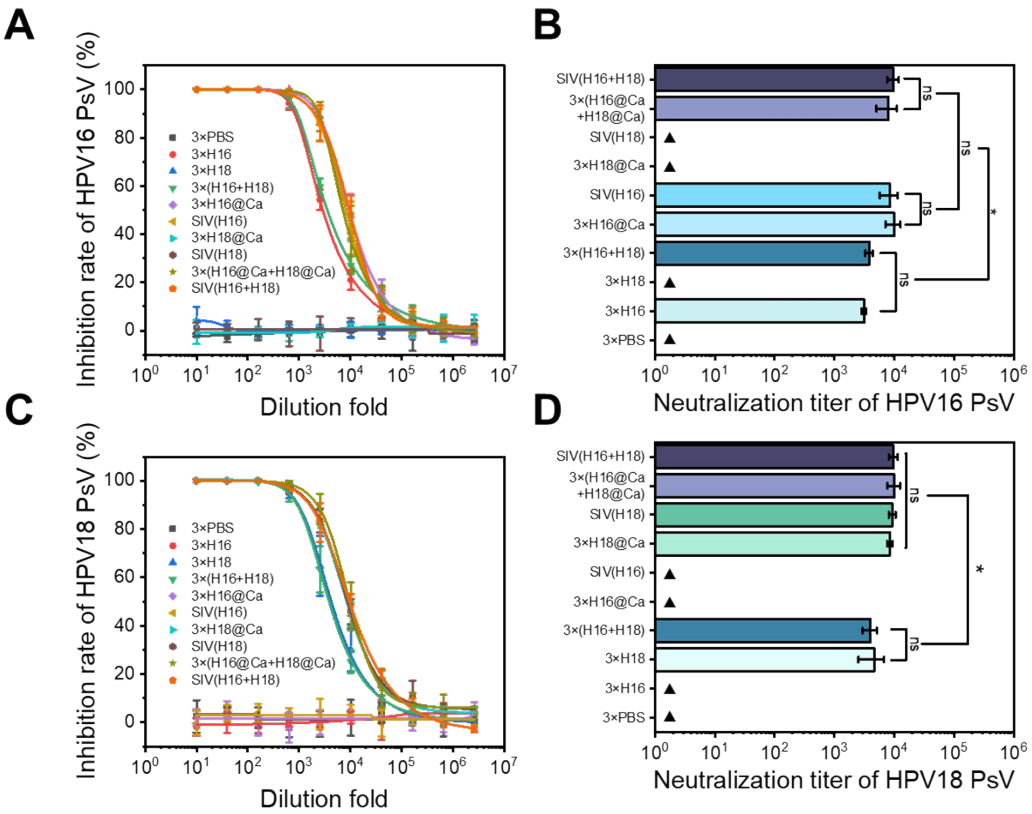

Fig. S11. (A, C) The inhibition rate of HPV16 pseudovirus (A) or HPV18 pseudovirus (B) by serially diluted mice sera. (B, D) Neutralizing antibody titers against HPV 16 (B) or HPV18 (D) of the sera. The sera were collected 35 days after initial injection.

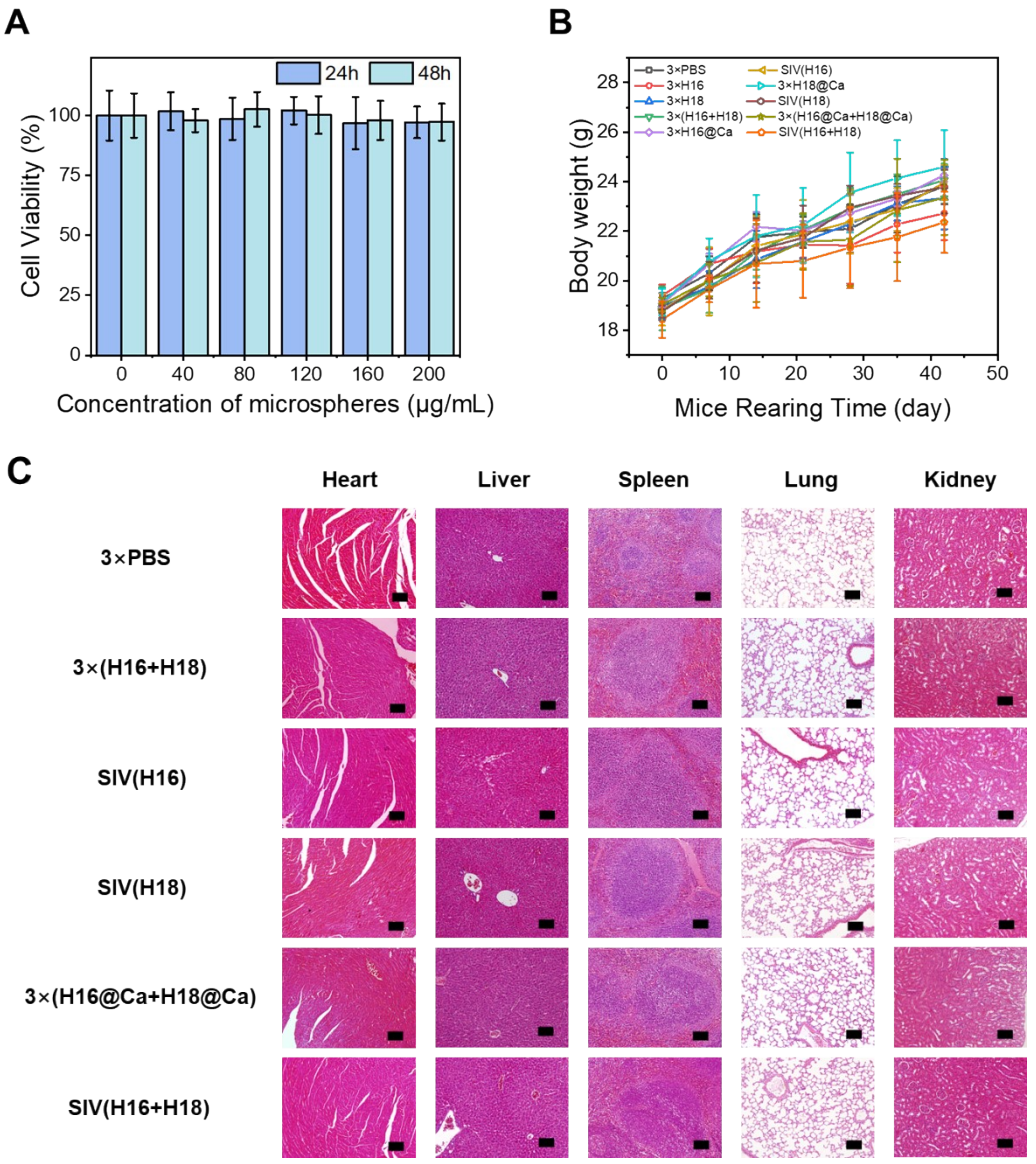

Fig. S12. (A) Viability of BMDCs cocultured with various concentration of HPV16@$CaCO_3$(TA/PEG)$_{50}$ and HPV18@$CaCO_3$(TA/PEG)$_{50}$ measured by CCK-8 assay. (B) Body weight change of mice during immunization process. (C) H&E stained tissues of major organs. The mice were sacrificed 6 weeks after first injection. The scale bar is 100 μm.

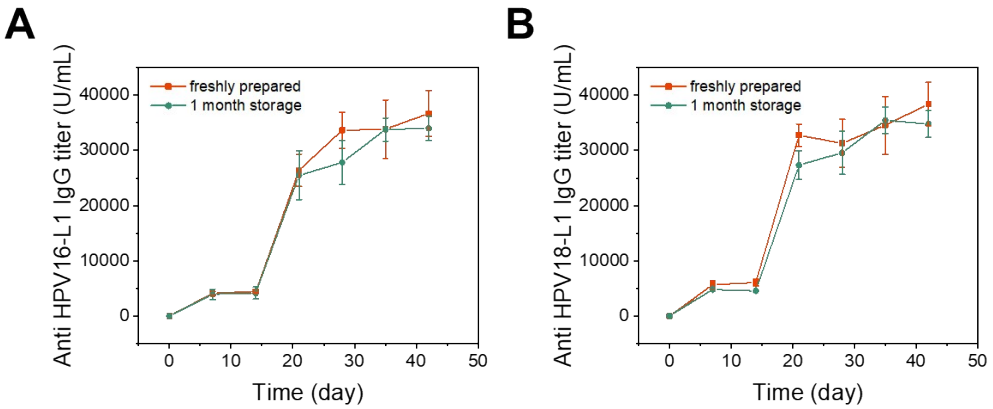

Fig. S13. Anti HPV16-L1 IgG titer (A) and anti-HPV18-L1 IgG titer produced in sera of the immunized mice. The mice were immunized with freshly prepared SIV(H16+H18) or SIV(H16+H18) after 1 month storage under ambient conditions.

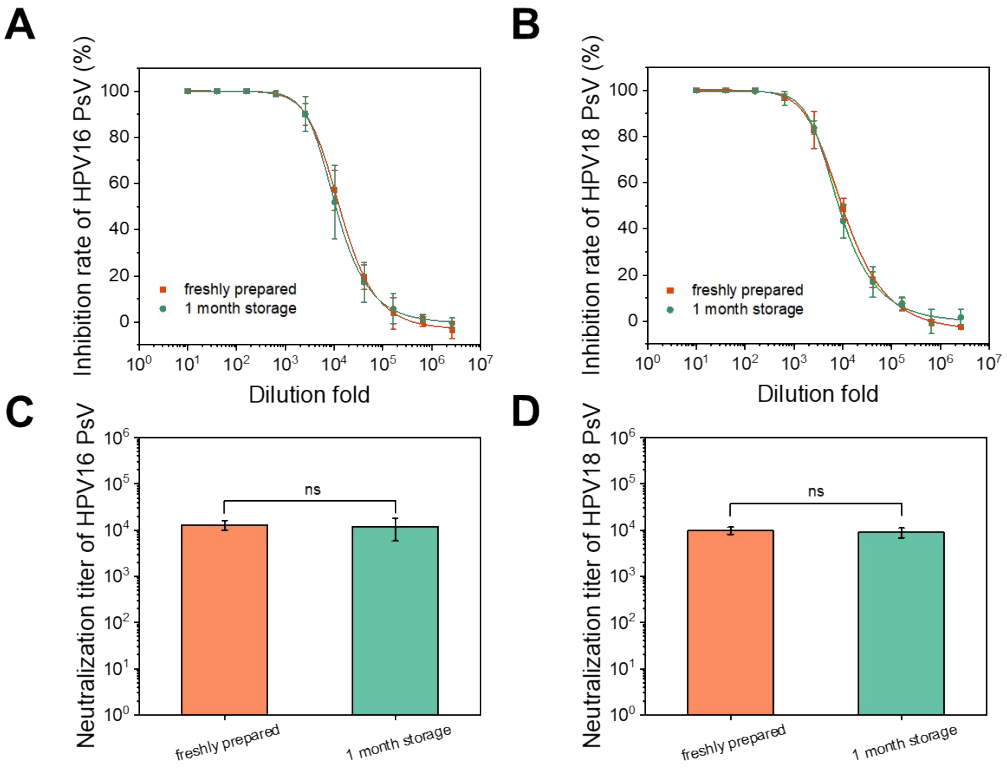

Fig. S14. (A, B) The inhibition rate of HPV16 pseudovirus (A) and HPV18 pseudovirus (B) by serially diluted mice sera. (C, D) Neutralizing antibody titers against HPV 16 (C) and HPV 18 (D). The mice were immunized with freshly prepared SIV(H16+H18) or SIV(H16+H18) after 1 month storage under ambient conditions. The sera were collected 42 days after immunization.