

Figure S1 Preparation and verification of Ad-AURKA or Ad-CDK7 vaccine. (A) Plaques represent the success of packaging Ads, encompassing Ad-Ctrl, Ad-AURKA, and Ad-CDK7. (B) The presence of successfully packaged Ad-AURKA/CDK7 in the virus supernatant was verified through virus gene PCR. (C, D). Western blot confirmed the expression of Ad-AURKA or Ad-CDK7 proteins.

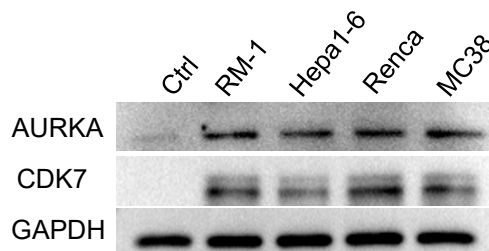


Figure S2 Expression of AURKA and CDK7 in various tumor cell lines. The expression of AURKA or CDK7 was detected by Western blot in normal muscle tissue (Ctrl), RM-1, Hepa1-6, Renca, and MC38 cell lines.

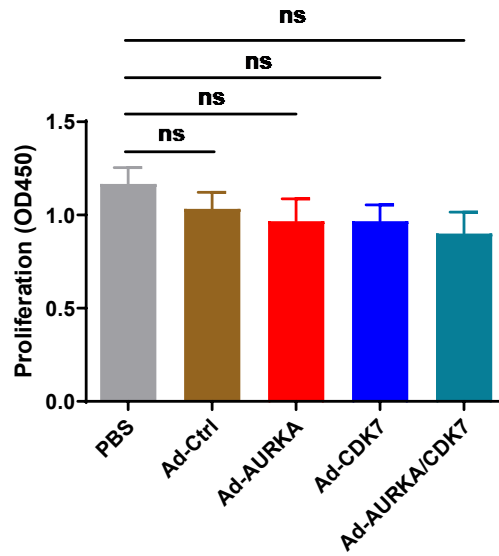


Figure S3 The proliferation of Renca cells infected with Ad vaccine in vitro. Renca cells were plated into 96-well plates at a density of 3×10^3 cells/well, and infected with Ad-Ctrl, Ad-AURKA, Ad-CDK7, and Ad-AURKA/CDK7 respectively, PBS as a control. The infected cells were cultured at 37 °C for 72 h. CCK-8 kit was used to measure the cell proliferation. Data are shown as means \pm SD. The different significance was no significant (ns).

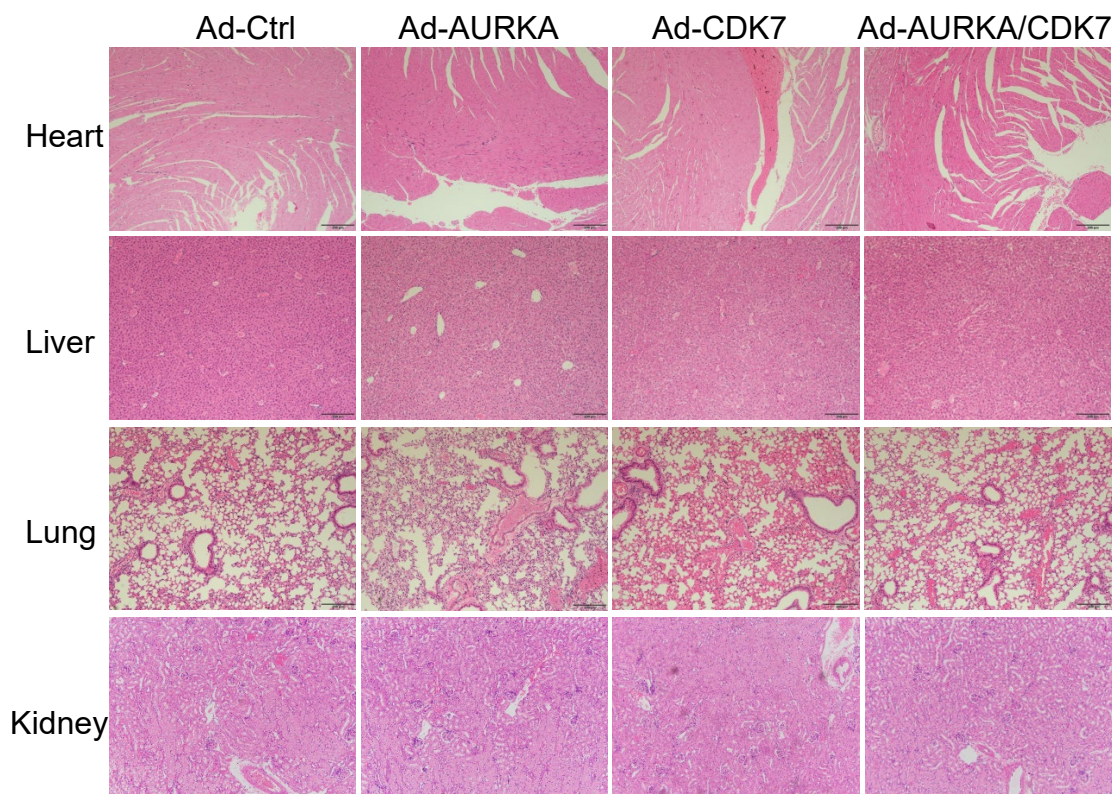


Figure S4 The safety of the Ad-AURKA/CDK7 vaccine verified by HE staining. The safety and absence of toxic side effects associated with the Ad-AURKA/CDK7 vaccine were evaluated in the heart, liver, lung, and kidney tissues using HE staining.

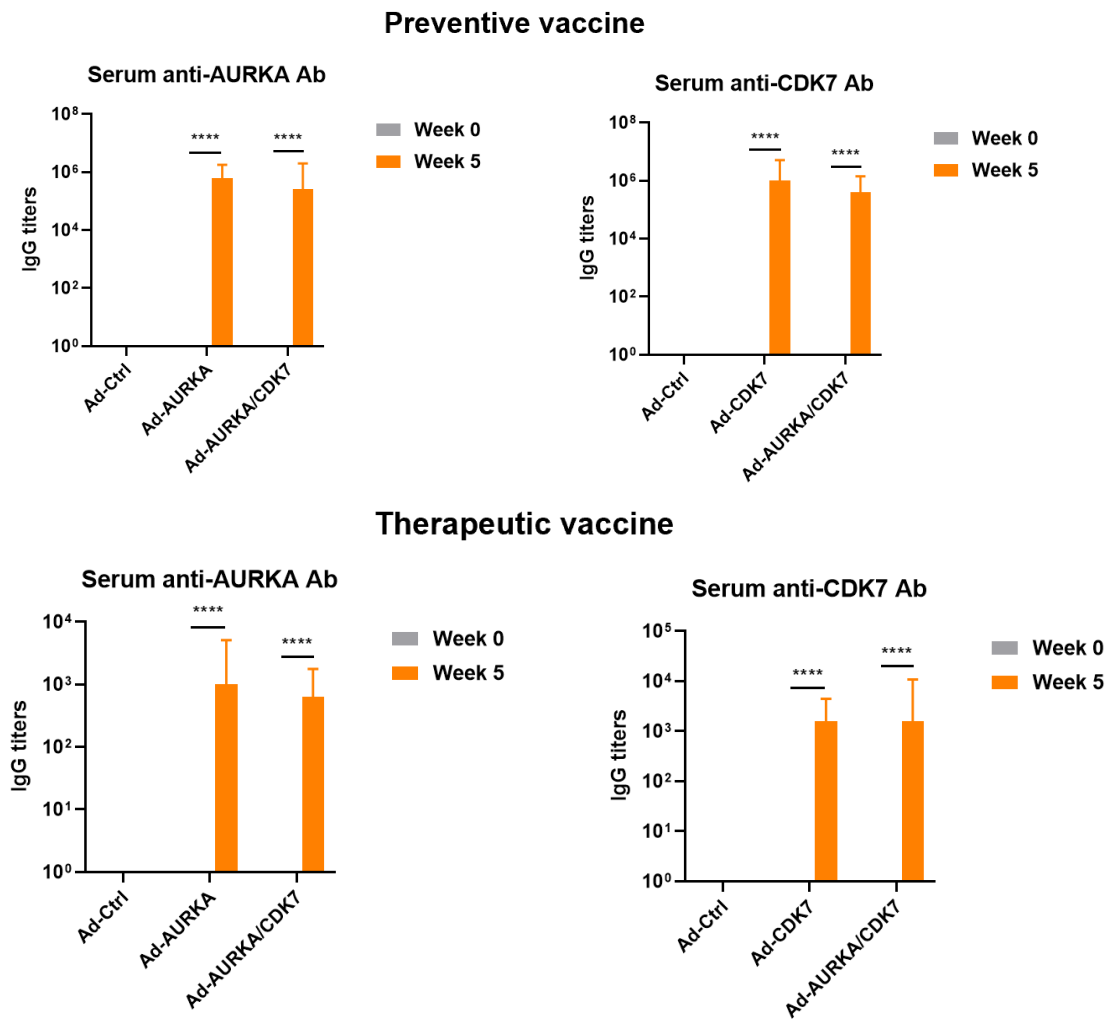


Figure S5 Serum IgG antibodies induced by Ad vaccines. Mice were immunized 3 times with preventive or therapeutic vaccines at 10-day intervals (on days 0, 10, 20). Serum IgG antibodies against AURKA or CDK7 were detected by ELISA. AURKA or CDK7 antibody titers were determined at week 0 or week 5; Data are presented as the mean \pm SD (n = 5). The statistical significance levels were set as ****P < 0.0001.

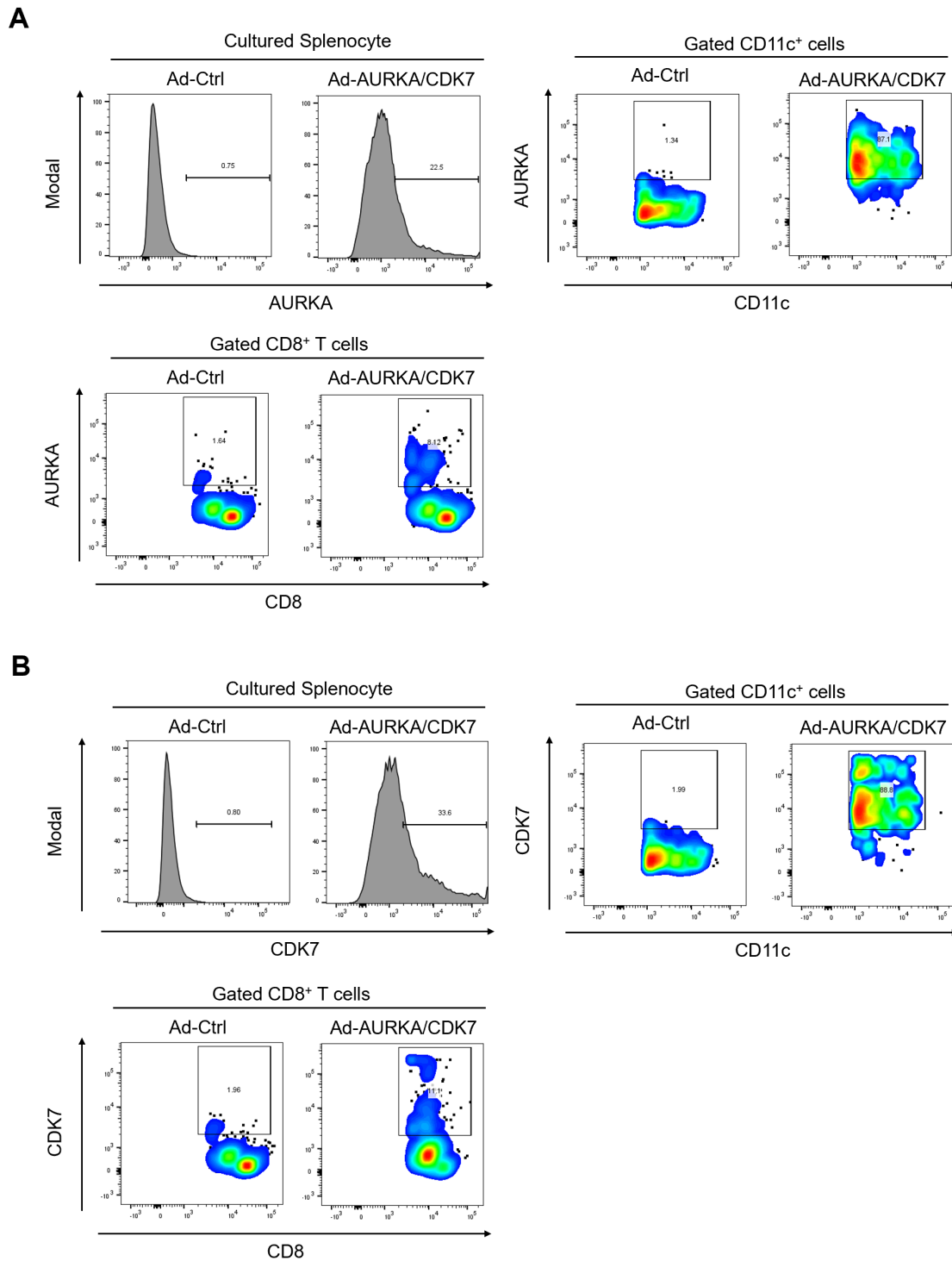


Figure S6 The expression of AURKA and CDK7 in infected splenocytes, DCs, and CD8⁺ T cells with Ad-AURKA/CDK7 vaccine in vitro. Lymphocytes were cultured in the medium containing IL-2 (50 U/ml) infected with Ad-AURKA/CDK7 and then inoculated on 48-well flat plates at a density of $1 \times$

10⁶ cells per well. The plates were placed in a constant temperature incubator at 37 °C for 3 days. The expression of AURKA and CDK7 in infected splenocytes, DCs, and CD8⁺ T cells was analyzed by flow cytometry.