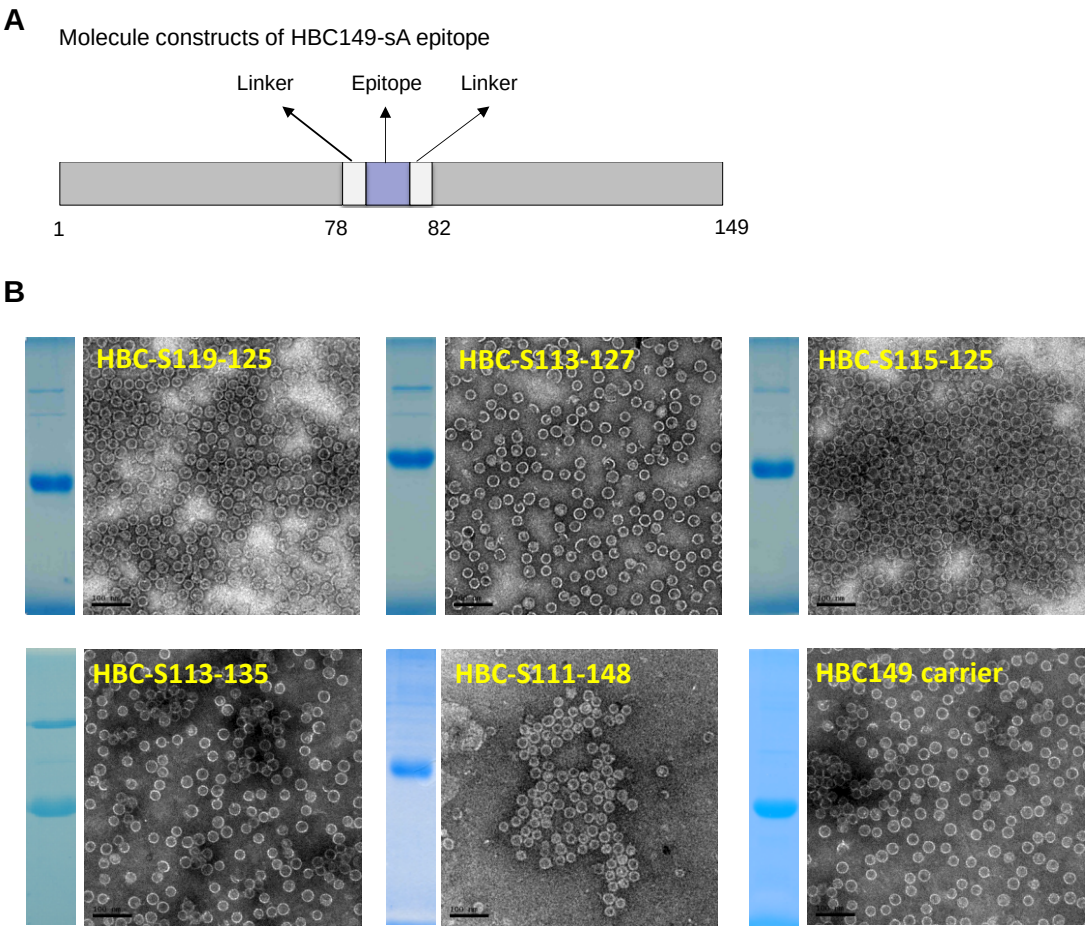
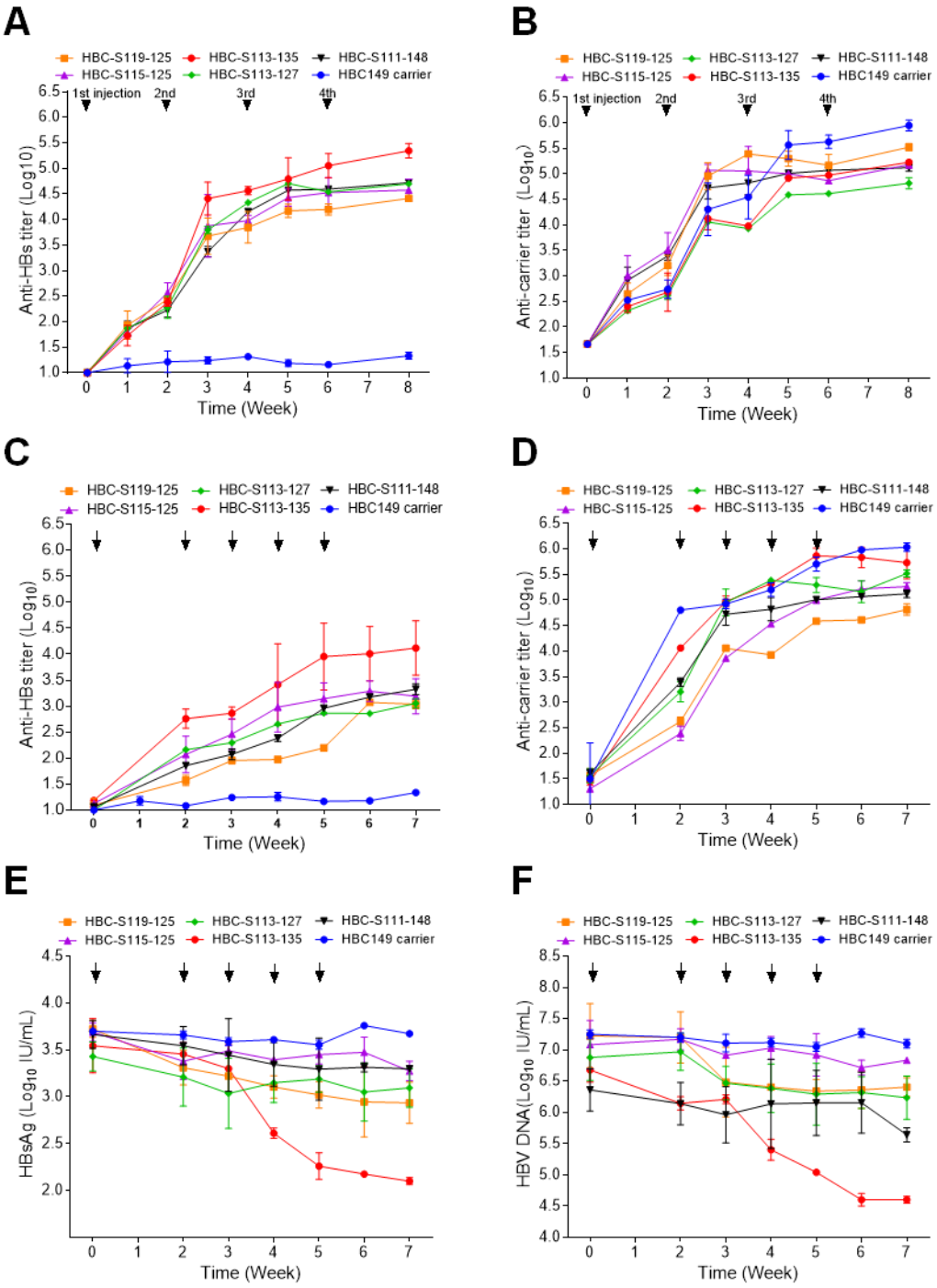
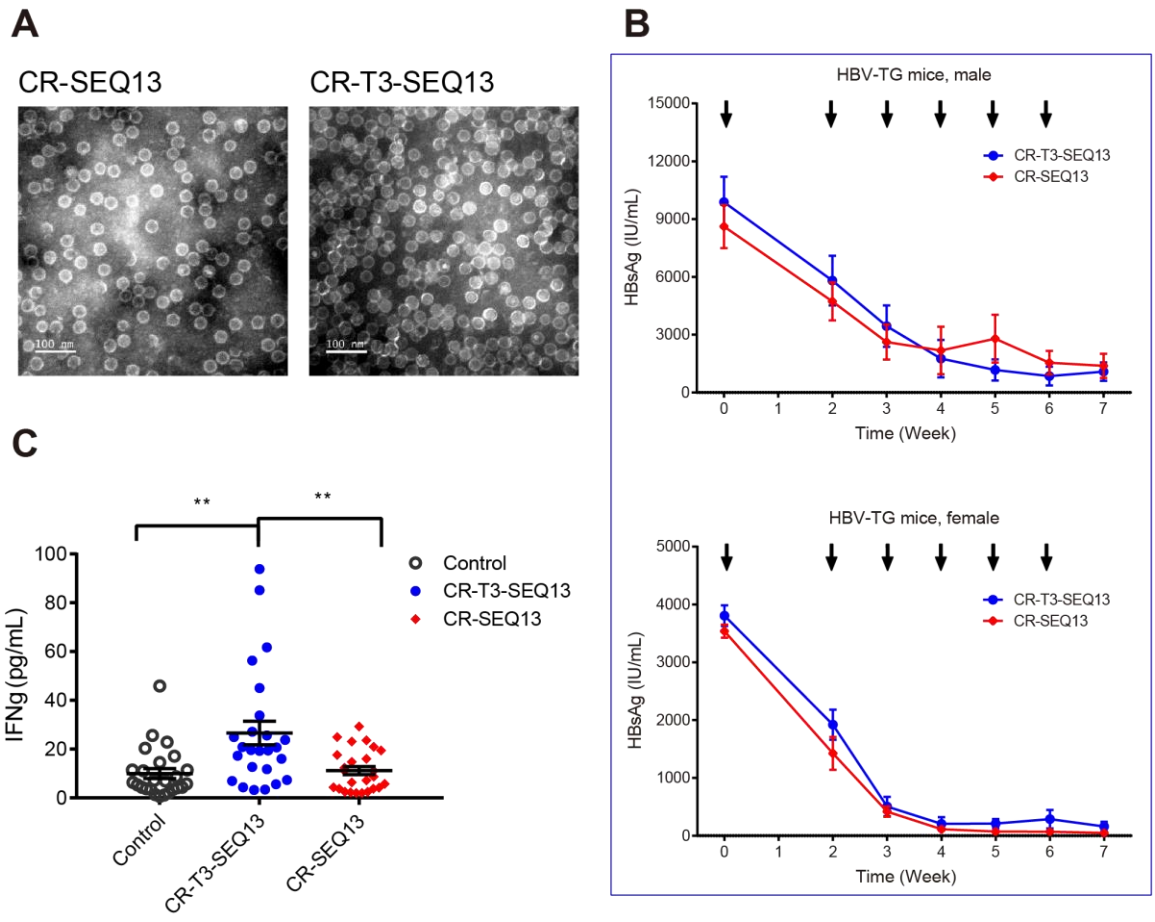


Supplemental figure 1. Chimeric Virus-like particles (VLPs) displaying sA-epitopes. (A) Schematic constructs of HBC149-sA epitope, sA-epitopes with two-sided linkers (GGGGSGGGGS) are inserted into HBC149 between aa78 and aa82 of HBcAg. (B) SDS-PAGE analysis of purified recombinant proteins and EM picture of recombinant VLPs, including HBC149 carrier displaying HBsAg-aa119-125, HBsAg-aa113-127, HBsAg-aa115-125, HBsAg-aa113-135, HBsAg-aa111-148 and carrier control.



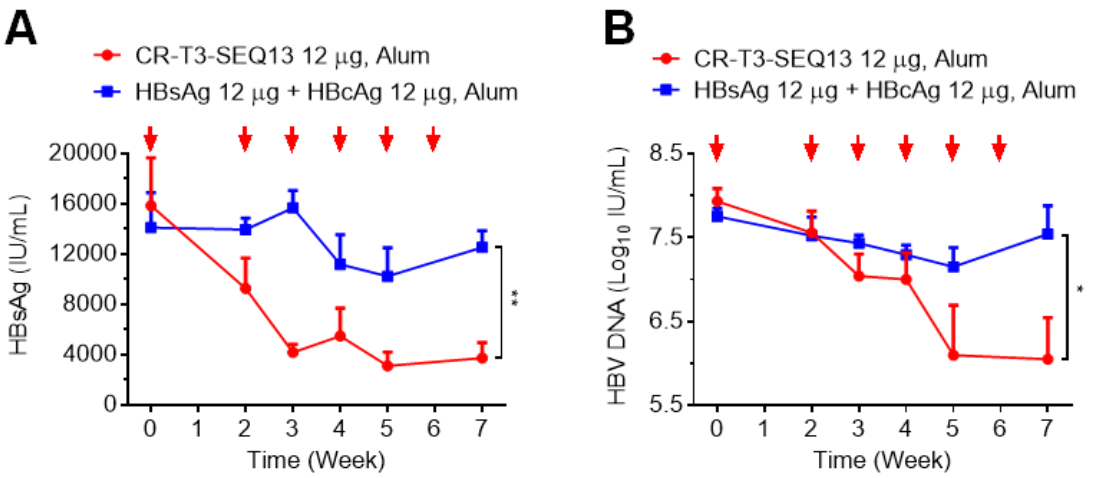
Supplemental figure 2. Evaluation of immunogenicity and antiviral effects of VLPs displaying peptides containing HBsAg-aa119-125. Kinetics of (A) Anti-HBs and (B) Anti-carrier antibody titers in BALB/c mice immunized with sA epitope based vaccine, four doses of vaccine were injected at 0, 2, 4 and 6

week, respectively. Kinetics of (C) Anti-HBs and (D) Anti-carrier antibody titers in HBV-Tg mice treated with sA epitope based vaccine, 5 doses of vaccine were injected at week 0, 2, 3, 4 and 5, respectively. Dynamic change of the levels of (E) HBsAg and (F) HBV DNA in HBV-Tg mice vaccinated with VLPs displaying peptides containing HBsAg-aa119-125. The data were expressed as the mean±SEM, N=4.

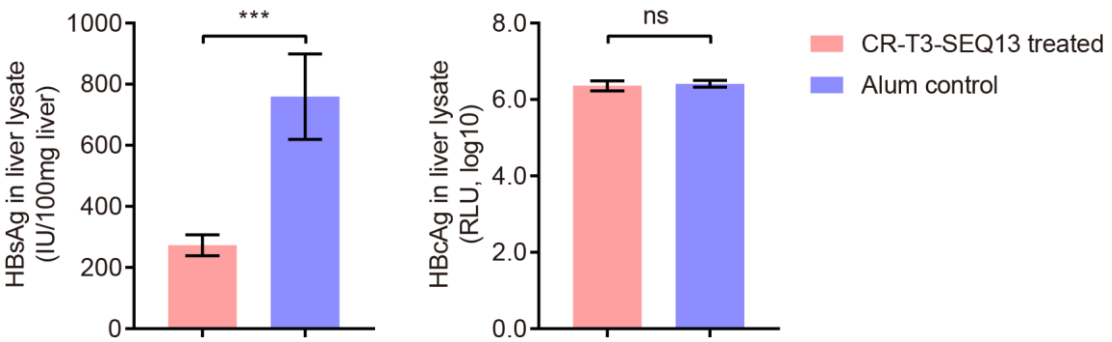


Supplemental figure 3. Comparison of characteristics and function of CR-T3-SEQ13 and CR-SEQ13. (A) Negative staining electron microscopy picture of recombinant VLPs of CR-T3-SEQ13 and CR-SEQ13. (B) Dynamic change of HBsAg levels in HBV-Tg mice (N=4, up right panel for male, lower right panel for female) treated with CR-T3-SEQ13 and CR-SEQ13 by the same dosage and schedule, respectively. (C) Comparison of whole-blood interferon gamma responses to CR-T3-SEQ13, CR-SEQ13 and negative buffer control. Whole blood were derived from 25 chronic hepatitis B patients, IFNγ release assay (IGRA) was performed as described in methods. The data were expressed as the mean±SEM. Two-sided, unpaired t-test was used. Statistical significance

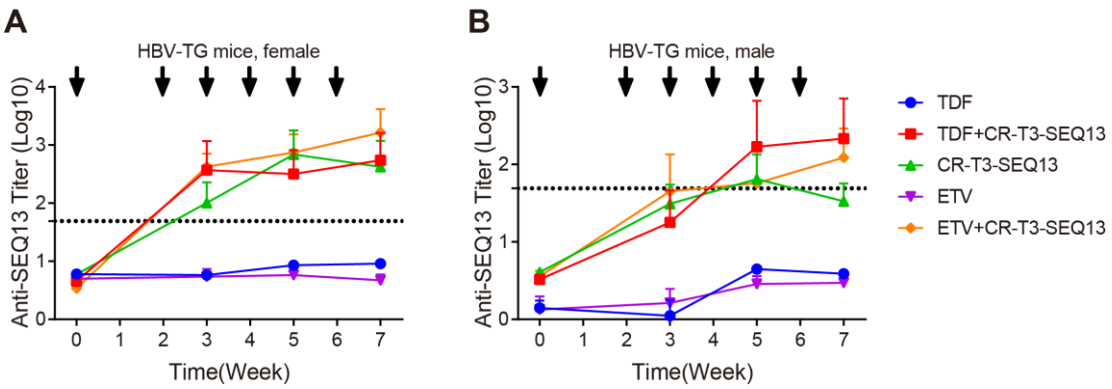
was defined as *P < 0.05; **P < 0.01.



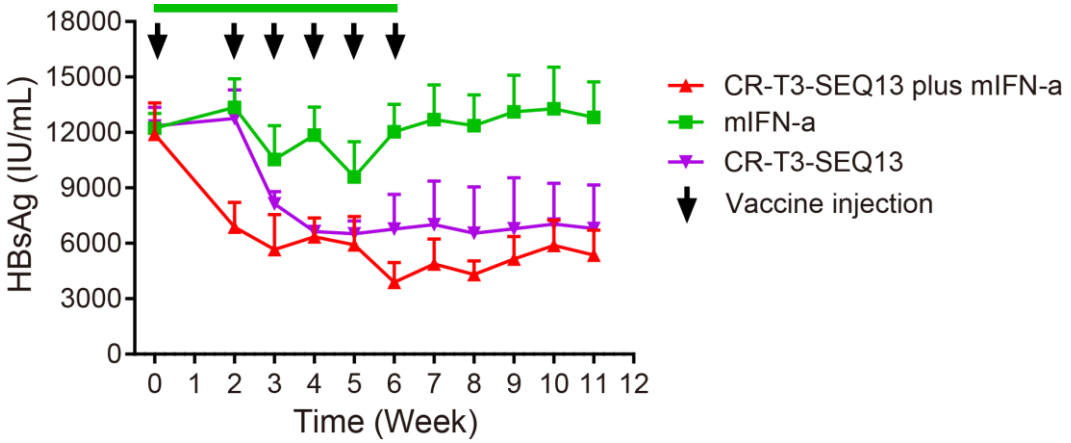
Supplemental figure 4. Comparison of antiviral effects of CR-T3-SEQ13 vaccine and HBsAg & HBcAg vaccine. Dynamic change of (A) HBsAg and (B) HBV DNA levels in HBV-Tg mice (N=4) treated with CR-T3-SEQ13 vaccine or HBsAg & HBcAg vaccine. The data were expressed as the mean±SEM. Two-sided, unpaired t-test was used. Statistical significance was defined as *P < 0.05; **P < 0.01. Recombinant HBsAg derived from CHO cells HBcAg derived from E.Coli were purchased from Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.



Supplemental figure 5. Quantitative analyses for HBsAg (left panel) and HBcAg (right panel) in the specimens of liver tissue lysates derived from HBV-Tg mice (Genotype A, N=4) that after CR-T3-SEQ13 treatment. Mice were sacrificed at week 8 after treatment. The data were expressed as the mean±SEM. p Values were calculated using a two-sided unpaired t test, and ***indicated $p<0.001$.



Supplemental figure 6. Kinetics of anti-SEQ13 antibody titers in (A) Male and (B) Female HBV-Tg mice. HBV transgenic mice were treated with TDF alone, ETV alone, CR-T3-SEQ13 alone, CR-T3-SEQ13plus ETV and CR-T3-SEQ13 plus TDF. In the combinational therapy group, two weeks after oral administration of the analogues, the CR-T3-SEQ13 was injected at Week 0, 2, 3, 4, 5, 6. Data are presented as mean ± SEM, N=4. The arrow refers to injection of CR-T3-SEQ13. ETV=Entecavir; TDF= Tenofovir disoproxil fumarate.

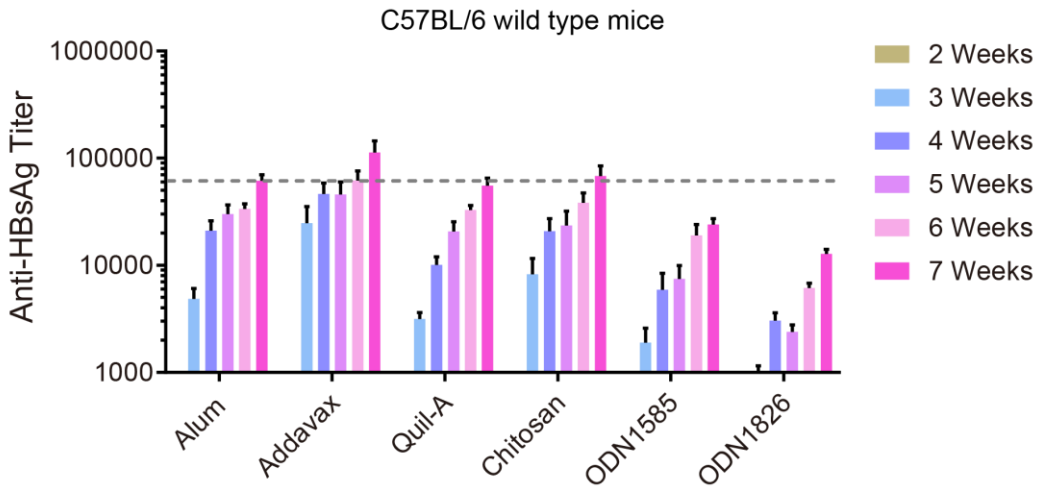


Supplemental figure 7. Dynamics change of serum HBsAg levels in male HBV-Tg mice treated with mIFN-a alone, CR-T3-SEQ13 alone and CR-T3-SEQ13 plus mIFN-a. In the mIFN-a group and combinational therapy group, 300000 unit/dose of mIFN-a was given by subcutaneous injection every two days between week0-6; in the CR-T3-SEQ13 group and combinational therapy group, CR-T3-SEQ13 was injected at week 0, 2, 3, 4, 5, 6. Data are presented as mean ± SEM, N=4. The arrow refers to injection of CR-T3-SEQ13 and the green band refers to mIFN-a.

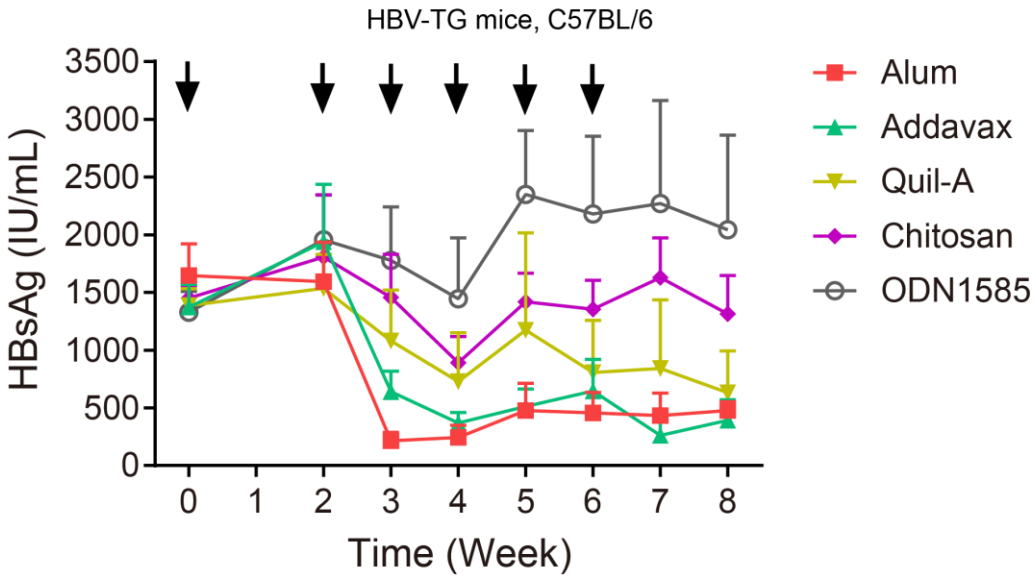
A

Adjuvants	Function description	Dosage
Alum adjuvant	Aluminium phosphate Th2 response	126 µg/dose
AddaVax™	Squalene-Oil-in-water Th1/Th2 response	75 µL/dose
Quil-A® adjuvant	Saponin vaccine adjuvant	15 µg/dose
Chitosan VacciGrade™	NLRP3 inflammasome inducer - Deacetylated derivative of chitin Th1/Th2 response	100 µg/dose
ODN 1585 VacciGrade™	TLR9 agonist - CpG ODN, class A (murine) Th1 response	20 µg/dose
ODN 1826 VacciGrade™	TLR9 agonist - CpG ODN, class B (murine) Th1 response	20 µg/dose

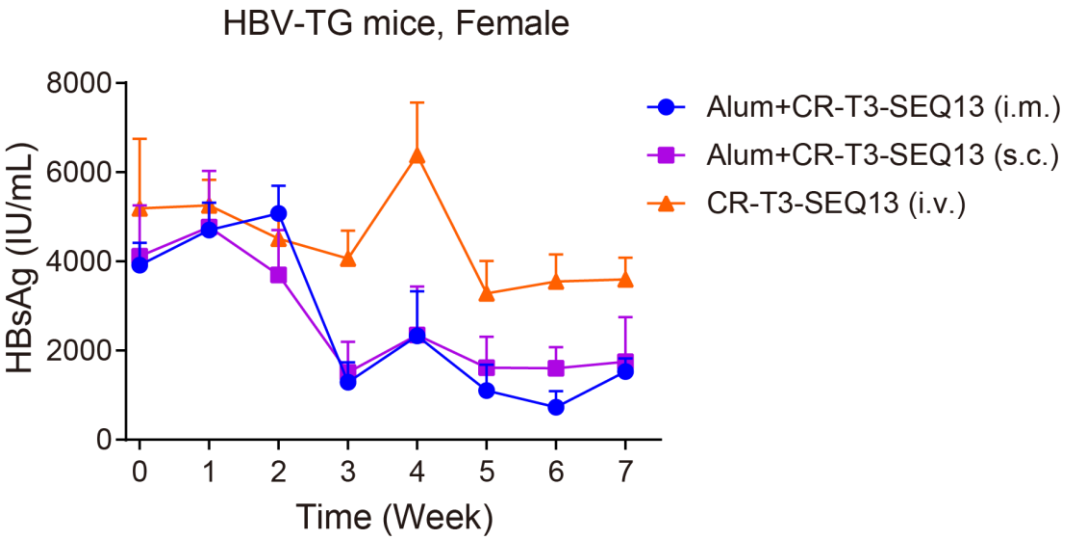
B



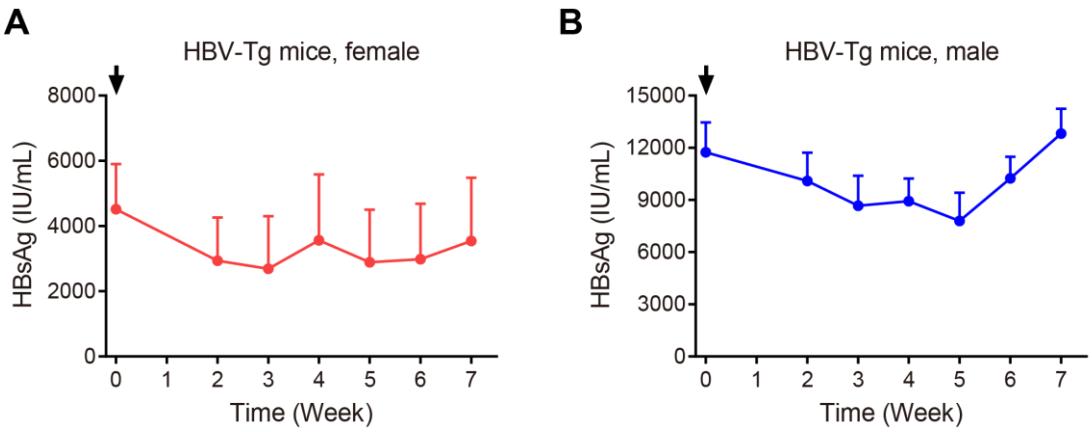
C



Supplemental figure 8. Comparison of the effects of CR-T3-SEQ13 vaccine formulations containing different adjuvants (A) Brief information of adjuvants used in this study, except for the alum adjuvant was prepared in our lab, the other five adjuvants were purchased from InvivoGen. (B) Comparison of the anti-HBsAg antibody titer induced CR-T3-SEQ13 formulated with different adjuvants. Each dose of formulation contain 1.2 μg of CR-T3-SEQ13 antigen and determined dose of adjuvant that listed in the table A. C57BL/6 mice (N=7) were immunized with 6 doses of vaccine at week 0, 2, 3, 4, 5 and 6, respectively. (C) Dynamics of HBsAg levels in female HBV-Tg mice treated by CR-T3-SEQ13 formulated with different adjuvants. Each dose of formulation contain 12 μg of CR-T3-SEQ13 antigen and determined dose of adjuvant that listed in the table A. HBV-Tg mice mice (N=4) were immunized with 6 doses of vaccine at week 0, 2, 3, 4, 5 and 6, respectively. The data were expressed as the mean±SEM.



Supplemental figure 9. Comparison of the effects of CR-T3-SEQ13 vaccine by different delivery routes. Dynamic change of HBsAg levels in female HBV-Tg mice (Genotype A, N=4) treated with CR-T3-SEQ13 by intramuscular injection, subcutaneous injection and intravenous injection. The data were expressed as the mean±SEM.



Supplemental figure 10. Dynamic change of HBsAg levels in HBV-Tg mice (Genotype A, N=4) treated by single dose of CR-T3-SEQ13 vaccine. The data were expressed as the mean±SEM.