Supplementary information

Caveolin-mediated cytosolic delivery of spike nanoparticle enhances antitumor immunity of neoantigen vaccine for hepatocellular carcinoma

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**Table S1.** Encapsulation efficiency and loading capacity of NeoAgs and ODN-1826 in V-scVLPs and SPS, respectively.

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<th>scVLPs</th>
<th>NeoAgs</th>
<th>ODN-1826</th>
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<td>Entrapment efficiency</td>
<td>91.8%</td>
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<td>Loading efficiency</td>
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<th>SPS</th>
<th>NeoAgs</th>
<th>ODN-1826</th>
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<td></td>
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<td>Entrapment efficiency</td>
<td>63.1%</td>
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<td>Loading efficiency</td>
<td>23.98%</td>
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**Figure. S1.** N\textsubscript{2} sorption isotherm for scVLPs and DFT pore size distribution for scVLPs.
Figure. S2. The concentration of NeoAgs from scVLPs (FAM-NeoAgs) was quantified according to the linear fit ($Y = 43.79x - 15.687$, $R^2 = 0.9996$) of fluorescence intensity at 520 nm, the correlation curve was prepared from 4.0–20.0 μg/mL.

Figure. S3. The concentration of ODN-1826 from scVLPs was quantified according to the linear fit ($Y = 238.22x - 23.613$, $R^2 = 0.9955$) of fluorescence intensity at 570 nm, the correlation curve was prepared from 0.5–2.5 μg/mL.
Figure S4. TEM image of SPS.

Figure S5. The loading percentage of NeoAgs and ODN-1826 in SPS or scVLPs, respectively.
Figure. S6. PCCs of Cy5 labeled scVLPs with lysosomes at different incubation times, (n=3). The statistical analysis was performed with ANOVA analysis, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, (n = 3). Data are presented as mean ± SD.
Figure. S7. Western blot analysis of NF-κB in BMDCs after co-incubation with V-scVLPs for 48 h, and PBS treated cell were used as control.

Figure. S8. Identify the maturation of BMDCs after receiving different treatment through FACS with staining anti-CD11c-APC, anti-CD80-PE, and anti-CD86-PE-Cy7 antibodies.
Figure. S9. Identify the maturation of BMDCs after receiving different treatment through FACS with staining anti-CD11c-APC, anti-CD40-FITC antibodies.
Figure. S10. Identify the population of T cells after receiving different treatment through FACS with staining anti-CD3-APC, anti-CD8-PE antibodies.
**Figure. S11.** Identify the activation of T cells after receiving different treatment through FACS with staining anti-CD3-APC, anti-CD8-PE and anti-CD69-FITC antibodies.

**Figure. S12.** Representative fluorescence intensity of FAMNeoAgs positive cells gated on DCs in lymph node after 12h of Neo/ODN and scVLPs injection.
Figure. S13. Identify the maturation of DCs in LNs after receiving different treatment through FACS with staining anti-CD11c-APC, and anti-80-PE and anti-86-PECy7 antibodies.
Figure. S14. Identify the maturation of DCs in LNs after receiving different treatment through FACS with staining anti-CD11c-APC, and anti-MHC-I-PE antibodies.

Figure. S15. Identify the CD8^+T cells in spleen after receiving different treatment through FACS with staining anti-CD3-APC and anti-CD8-PE antibodies.
Figure. S16. Bioluminescence imaging of mice at the 7, 14, 21, 28, 35, 42, 49 and 56 days before and after receiving different treatments as indicated, n = 7.
Figure S17. Identify the CD3⁺CD4⁺CD44⁺CD62L⁺T cells in the spleen after receiving different treatments through FACS with staining anti-CD3-APC, anti-CD4-FITC antibodies, anti-CD44-PE-Cy7 and anti-CD62-PerCP-Cy5.5 antibodies.

Figure S18. Identify the CD3⁺CD8⁺CD44⁺CD62L⁺T cells in the spleen after receiving different treatments through FACS with staining anti-CD3-APC, anti-CD8-PE antibodies, anti-CD44-PE-Cy7 and anti-CD62-PerCP-Cy5.5 antibodies.
Figure. S19. Identify the CD3^+CD4^+T cells in the spleen after receiving different treatments through FACS with staining anti-CD3-APC and anti-CD4-FITC antibodies.
Figure. S20. Identify the CD8^+T cells in tumor after receiving different treatment through FACS with staining anti-CD3-APC and anti-CD8-PE antibodies.

Figure. S21. Identify different types of IFN-γ^+CD8^+CD3^+T cells in tumors after receiving different treatments through FACS with staining anti-CD3-APC, anti-CD8-PE, IFN-γ-PE-Cy7 antibodies.
Figure S22. Bioluminescence imaging of mice at the day 0, 14, 21, 28, 35, 42 and 49 after receiving different treatments as indicated, n = 7.
Figure. S23. Identify the different types of TIM-3⁺CD8⁺CD3⁺T cells in tumors after receiving different treatments by FCM with staining anti-CD3-APC, anti-CD8-PE, TIM-3-FITC antibodies.

Figure. S24. The percentage of CD3⁺CD8⁺TIM-3⁺T cells in orthotopic tumors after receiving different treatment as indicated, n = 5. Statistical analysis was performed with T test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Data are expressed as mean ± SD.
Figure. S25. Bioluminescence imaging of mice at the day 0, 14, 21, 28, 35,42 and 49 before and after receiving different treatments as indicated, n = 7.
Figure. S26. The H&E staining of liver and lung tissues after receiving different treatments at the 49th day.

T represents tumor tissues, L was liver tissues, and Lu was lung tissues.

Figure. S27. Identify the different types of CD3^+CD4^+CD25^+Foxp3^+T cells in tumors after receiving different treatments by FCM with staining anti-CD3-APC, anti-CD8-PE, TIM-3-FITC antibodies.
Figure. S28. Identify the different types of CD3⁺CD8⁺CD69⁺ T cells in tumors after receiving different treatments by FCM with staining anti-CD3-APC, anti-CD8-PE, CD69-FITC antibodies.

Figure. S29. Identify the different types of CD3⁺CD8⁺IFN-γ⁺ T cells in tumors after receiving different treatments by FCM with staining anti-CD3-APC, anti-CD8-PE, IFN-γ-PE-Cy7 antibodies.

Figure. S30. Identify the different types of CD8⁺41BB⁺ T cells in tumors after receiving different treatments by FCM with staining anti-CD8-PE, 41BB-FITC antibodies.
Figure S31. Bioluminescence imaging of mice at the day 0, 10, 15 and photographs of orthotopic liver tumors and their H&E staining that excised from mice after inoculation Hepa1-6 tumor cells for 10 days, n = 7.
Figure. S32. Bioluminescence imaging of mice at the day 27, 34, 41, 48, 55, 62 and 69 after rechallenge as indicated, n = 7.
Figure. S33. The H&E staining of liver tissues and tumors after receiving different treatments at the day 69. T represents tumor tissues, L was liver tissues.
**Figure. S34.** Weight loss of Hepa1-6 tumor-bearing mice during different treatments as indicated. Data are presented as mean ± SD (n = 7).
Figure. S35. Biochemical analysis (aspartate aminotransferase, AST; alanine aminotransferase, ALT; creatinine, UREA, Serum urea; CREA; total bilirubin, TBIL; thyroglobulin, TG; Alkaline phosphatase, CK; Glucose, GLU) in healthy mice at 3rd and 14th day after s.c. injection of V-scVLPs. Data are presented as mean ± SD (n = 3). The statistical analysis was performed with ANOVA analysis.
Figure. S36. H&E imaging of major organs (heart, spleen, lung and kidney) from healthy mice after s.c. injection with V-scVLPs for 3 or 14 days later.