Supplementary Figures



Supplementary Figure 1. *In vitro* antigen-specific assay for lenti-HA and lenti-AFP. Day-7 established ectopic HCC mice were treated with lenti-HA, lenti-AFP or lentivirus containing empty vectors $(3x10^7 \text{ copies})$ per week for 3 weeks subcutaneously. Mixed splenic T lymphocytes were harvested from treated HCC mice 3 days after last injection. (A) Measurement of IFN- γ and IL-2 in supernatants of mixed splenic T lymphocytes from different treatment groups stimulated with control peptide (Con), AFP₂₁₂ and AFP₄₉₉ for 72 hrs. (B) Cytolysis rates against murine Hepa1-6 cells with effector T cells (activated splenic T lymphocytes) at the E: T ratio of 10:1 (n=5, **P<0.01). N.s denotes not significant. Two-tailed test was used for

statistical analysis.



Supplementary Figure 2. Measurement of IFN- γ and IL-2 (A) or IL-10 and TGF- β (B) in tumor lysates from ectopic HCC mice treated with lenti-HA or lenti-AFP (3x10⁷ copies) (n=5, *P<0.05; **P<0. 01). N.s denotes not significant. Two-tailed t test was used for statistical analysis and all the experiments were repeated twice (two repeated experiments yielded similar results and thus one representative result was shown).



Supplementary Figure 3. Examination on tumor microenvironment and cumulative immune responses in orthotopic HCC mice treated with lentiviral vaccines weekly for 3 weeks. Measurement of IFN- γ and IL-2 (A) or IL-10 and TGF- β (B) in tumor tissues from orthotopic HCC mice treated with lenti-HA or lenti-AFP 3 days after last injection (n=5, *P<0.05; **P<0.01). Measurement of IL-2 and IFN- γ (C) or IL-10 and TGF- β (D) in blood from orthotopic HCC mice treated with lentiviral vaccines at different time-points (n=5, *P<0.05; **P<0.01). Blood was collected from orthotopic HCC mice 3 days after each injection via retro-orbital bleeding. (E) Flow cytometric analysis of CD11c+ and CD103⁺CD11b⁻ DCs in inguinal lymph nodes from orthotopic C57BL/6 HCC mice treated with PBS, lenti-AFP or lenti-HA 3 days after last injection (n=5, **P<0.01). Two-tailed t test was used for statistical analysis and all the experiments were repeated twice (two repeated experiments yielded similar results and thus one representative result was shown).





Supplementary Figure 4. Characterization of DENA-induced autochthonous HCC mice. (**A**) Morphological examination of micronodules in DENA-induced autochthonous HCC mice at 7.5 months after induction. Arrowheads point to the micronodules. (**B**) Western blot analysis for detecting the expression of AFP in tumor tissues from DENA-induced autochthonous HCC mice. Total protein (30 μg) was loaded and GAPDH was used as a loading control.



Supplementary Figure 5. Examination on immune microenvironment in DENA-induced autochthonous HCC mice treated with lentiviral vaccines weekly for 3 weeks. Measurement of IFN- γ and IL-2 (A) or IL-10 and TGF- β (B) in blood from autochthonous HCC mice treated with lenti-HA or lenti-AFP 3 days after last injection. Measurement of IFN- γ and IL-2 (C) or IL-10 and TGF- β (D) in tumor tissues from autochthonous HCC mice treated with lenti-HA or lenti-AFP 3 days after last ast injection (n=5, **P<0.01). Two-tailed t test was used for statistical analysis.





Supplementary Figure 6. Analysis of immune cells in inguinal lymph nodes from treated autochthonous HCC mice and *in vitro* activity assay. (A) Flow cytometric analysis of immune cells in inguinal lymph nodes (LN) from HCC mice treated with lenti-HA, lenti-AFP or PBS weekly for 3 weeks (n=5, **P<0.01). (B) Flow cytrometric analysis of surface markers and co-stimulatory molecules on CD103⁺CD11b⁻ DCs from treated HCC mice. (C) Cytolysis assay for murine Hepa1-6 cells with effector T cells at the Effector: Target (E: T) ratio of 10:1 (n=5, **P<0.001). Two-tailed t test was used for statistical analysis.

Supplementary Figure 7



Supplementary Figure 7. Western blot analysis for detecting the expression of AFP in different cell lysates. DC_{AFP} was used as a control. Total protein (30 µg) was loaded and GAPDH was used as a loading control.



Supplementary Figure 8. *In vitro* antigen-specific assay for human lenti-HA or lenti-AFP. (A) Measurement of IL-2 and IFN- γ in supernatants of human lymphocytes primed by lentivirus-transduced PMDCs, followed by further stimulation with recombinant AFP loaded PMDCs (PMDC_{AFP}) or unpulsed PMDCs (PMDC), with ELISA (n=5, *P<0.05; **P<0.01). (B) Cytolysis assay for recombinant human AFP loaded PMDCs with lymphocytes primed by lenti-HA or lenti-AFP transduced PMDCs at the E: T (Effector: Target) ratio of 10:1 (n=5). N.s refers to not significant. Two-tailed t test was used for statistical analysis.