PLGA micro/nanoparticles vaccination elicits non-tumor antigen specific resident memory CD8$^+$ T cells protecting from Hepatocellular Carcinoma.

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Supplementary figures：Figure S1-S6

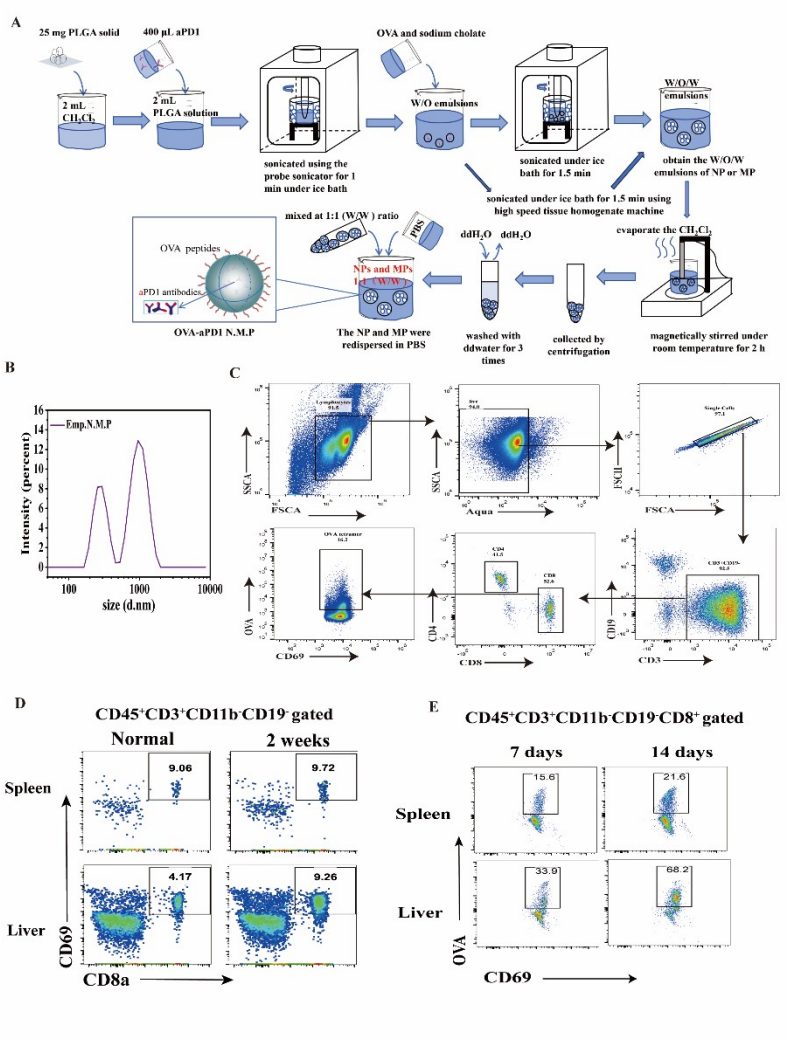

Figure S1. Characterization of particles and the test of CD8$^+$TRM after mouse model established. (A) Preparation method of OVA-aPD1 N.M.P by double emulsification method. **(B)**Size distribution Emp.N.M.P. **(C)** Gating strategy for OVA specific CD8$^+$TRMs. **(D)**Flow cytometry analysis showing the percentage of CD8$^+$TRMs in liver and spleen tissues of mouse after tumor modeling 2 weeks. **(E)**

Flow cytometry analysis showing the percentage of OVA-specific $CD8^+$T cells among $CD3^+CD8^+$T cells in liver and spleen tissues isolated from tumor bearing C57BL/6 mice vaccinated after OVA high dose immunization.

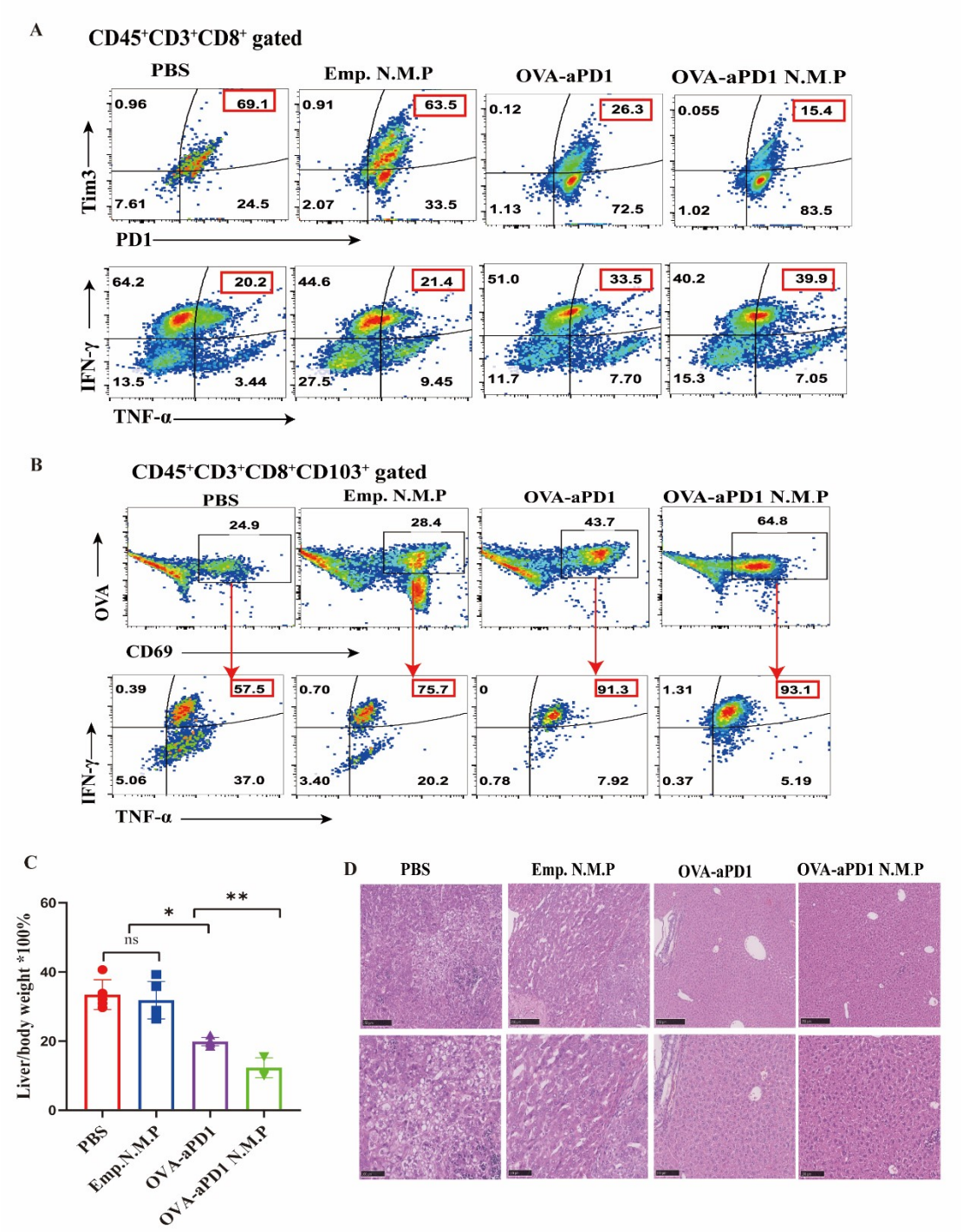

Figure S2. The immune activity $CD8^+$T cells and $CD8^+$TRM after vaccine immunization. (A) The representative flow cytometry plots of intracellular and surface marker staining and gating strategies of $Tim3^+PD\text{-}1^+CD8^+$T cells and $TNF\text{-}\alpha^+IFN\text{-}$

γ⁺CD8⁺T cells. **(B)**The representative flow cytometry plots of OVA tetramer pool, intracellular and surface staining and gating strategies of OVA specific CD8⁺TRMs and TNF-α⁺IFN-γ⁺ OVA specific CD8⁺TRMs. **(C)** The liver-body-weight ratio of different groups, n=5. **(D)** H&E staining was performed to evaluate histopathological changes in livers (images at x100 magnification, scale bar=100μm). Statistical significance was analyzed using two-tailed Student's t-test between two groups, *p<0.05, **p <0.01, ***p<0.001.

A

	OVA-aPD1_N.M.P	PBS
Mean nUMI beforeQC	5659.36651	5346.244164
Mean nGene beforeQC	1522.434938	1391.521535
Mean mito.percent beforeQC	0.054862234	0.063142559
Total cells beforeQC	9806	10239
Mean nUMI afterQC	5567.709478	5463.17754
Mean nGene afterQC	1536.41512	1475.474987
Mean mito.percent afterQC	0.04180712	0.034796295
Total cells afterQC	8082	7756

B

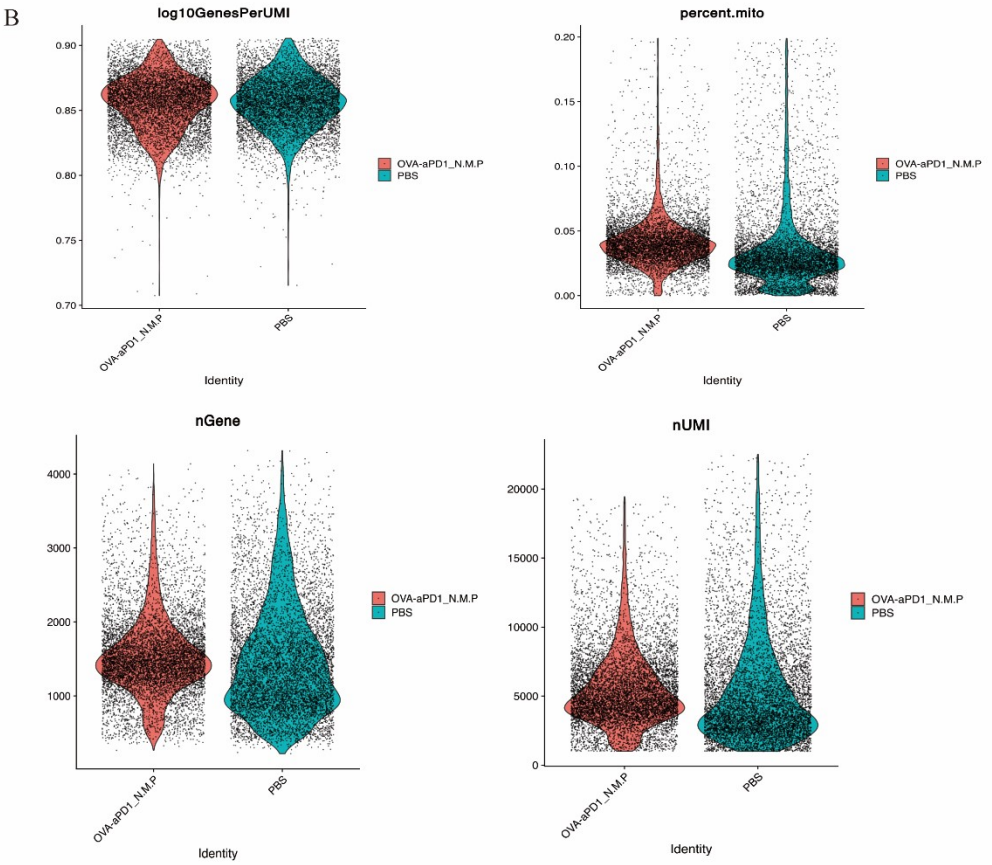

Figure S3. Quality controls for scRNA-seq of mouse liver immune cells. (A) Sequencing parameters of CD45⁺mouse liver immune cells subjected to 10×Genomics scRNA-seq platform. **(B)** Genes number of CD45⁺mouse liver immune cells within each sample.

(C) Mitochondrial genes/all genes (%) in each CD45^{+}mouse liver immune cells transcriptomes of each sample. **(D)** Total genes number identifier counts are shown. **(E)** Unique molecular identifier number per cell for each sample.

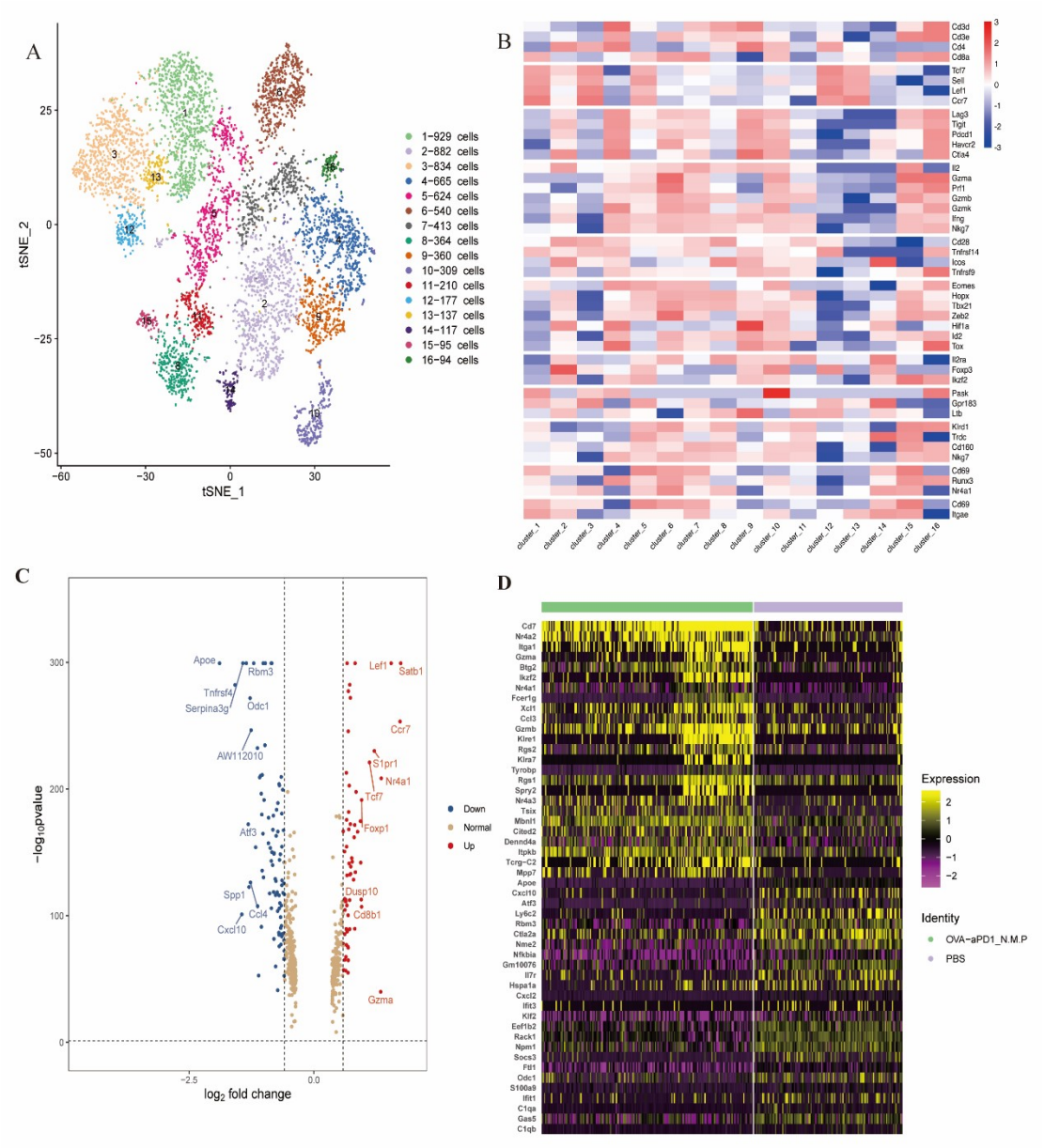

Figure S4. Liver local immune cell changes with vaccination. (A) tSNE map showing liver major immune cell subsets. **(B)** Heatmap showing averaged marker expression level of each cluster. **(C)**Volcano map of differentially expressed genes of T cells, the red points represent upregulated genes selected based on the corrected p-value<0.05 and logFC>1, the blue points represent downregulated genes selected based on the corrected p-value<0.05 and logFC<-1, the brown points represent genes with no significant difference. **(D)** Heatmap displayed the highly expressed marker genes for T

cells.

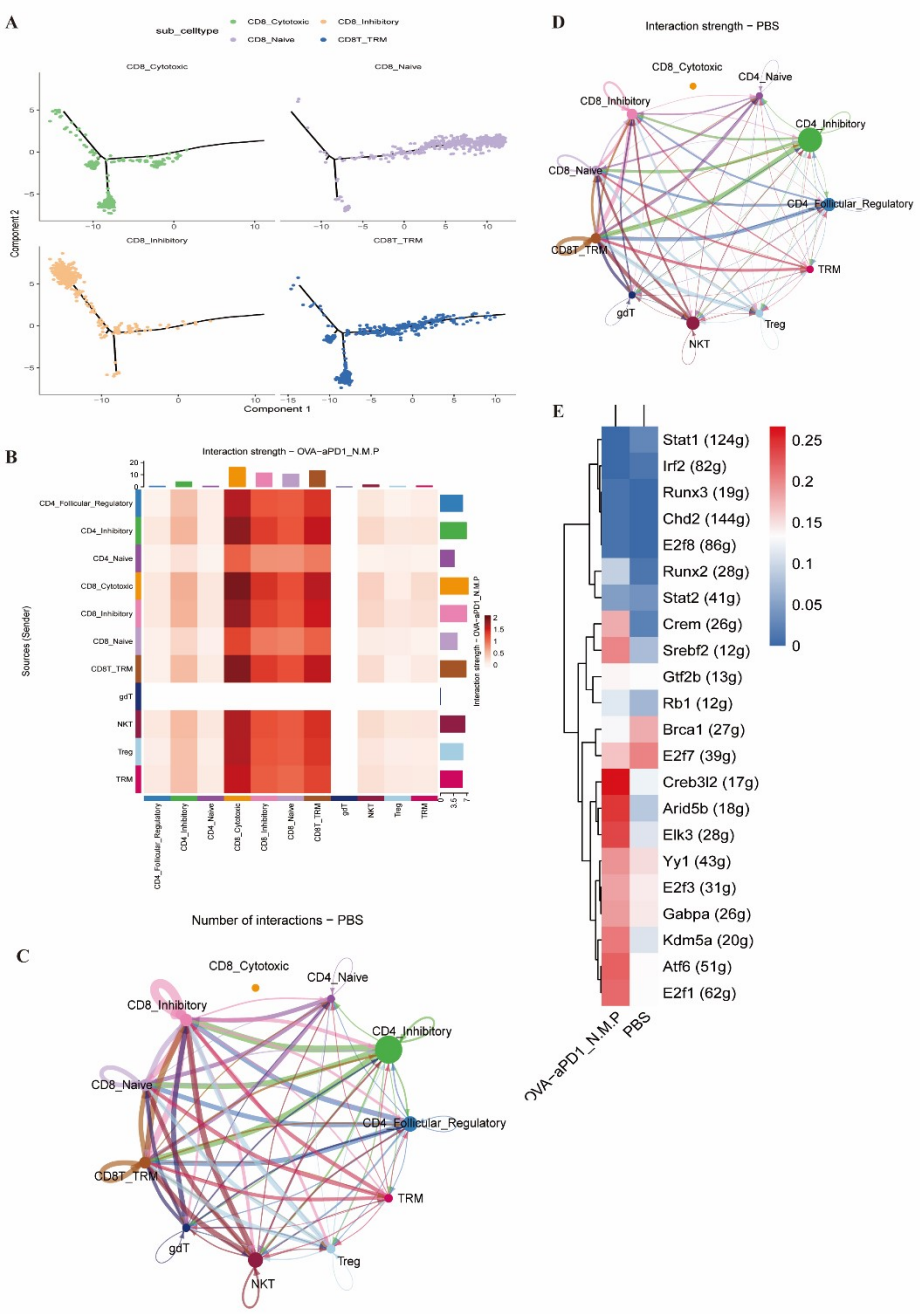

Figure S5. Liver local T cells communication and Transcriptional features. (A) Trajectory analysis revealed T cells subsets of liver tissue with distinct differentiation patterns. **(B)** Graphical comparison of the intercellular communication strength T cells subtypes of OVA-aPD1 N.M.P group. **(C, D)** The differential number **(C)** and strength **(D)** of interactions among T cell subtypes of EMP. N.M.P group. **(E)** Heatmap displayed the highly expressed marker genes for $CD8^{+}$TRMs.