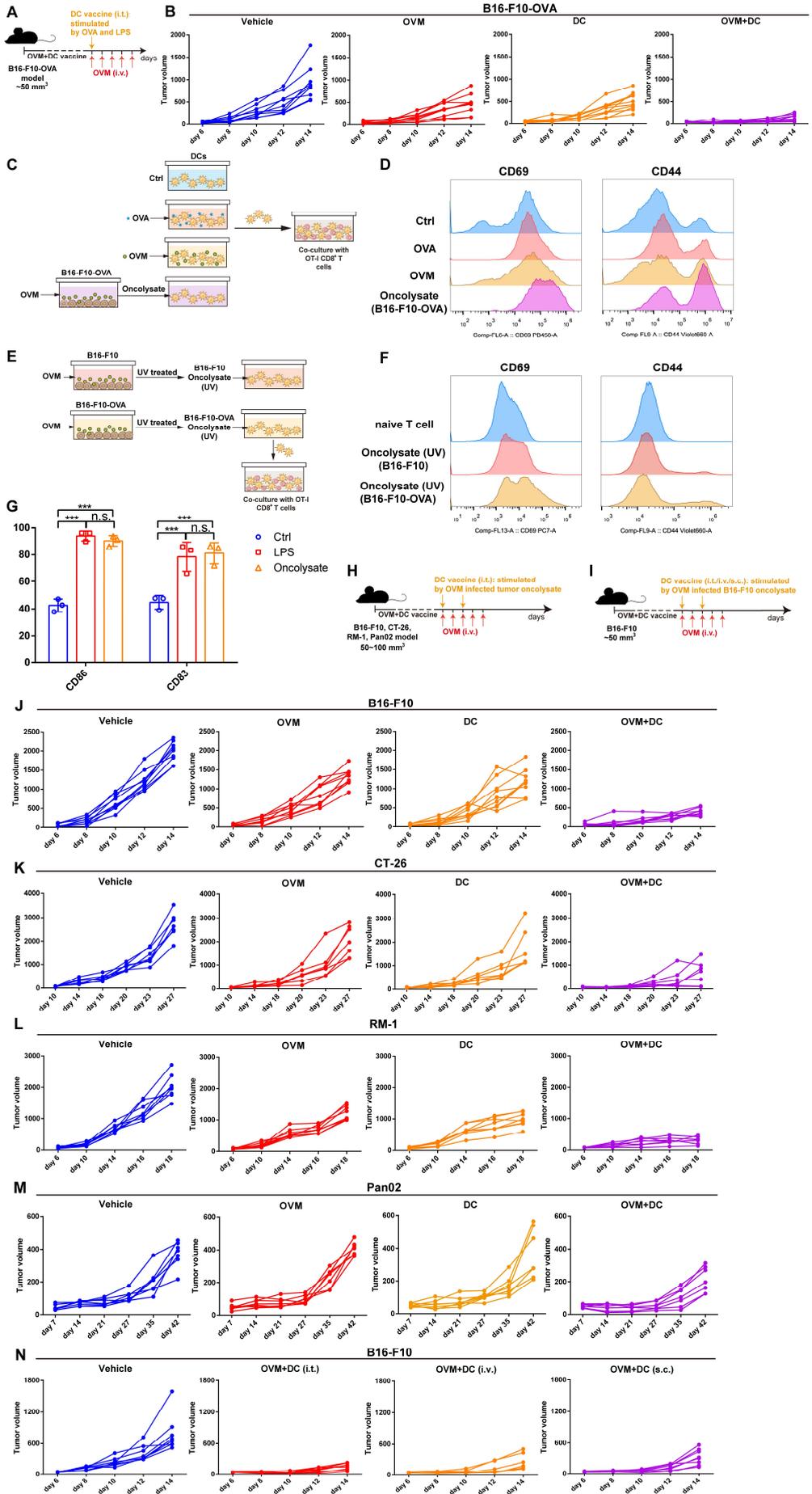


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**Supplemental information**

**Oncolytic virus M1 functions as a bifunctional  
checkpoint inhibitor to enhance  
the antitumor activity of DC vaccine**

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**Figure S1. OVM infection releases tumor-associated antigens and enhances the efficacy of DC vaccines in various tumor models, related to Figure 1.**

A-B.) Schematic diagram (A) and tumor growth curves for each mouse (B) in the experimental results shown in Figure 1A.

C-D.) Diagrammatic sketch (C) and the representative histogram of CD69 and CD44 (D) in Figure 1B.

E-F.) Diagrammatic sketch (E) and the representative histogram of CD69 and CD44 (F) in Figure 1C.

G.) The expressions changes of CD86 and CD83 on the CD11c<sup>+</sup> DCs stimulated with vehicle (Ctrl), LPS (500 ng/mL) or B16-F10-derived oncolysate (1 mL) for 24 h. n=3.

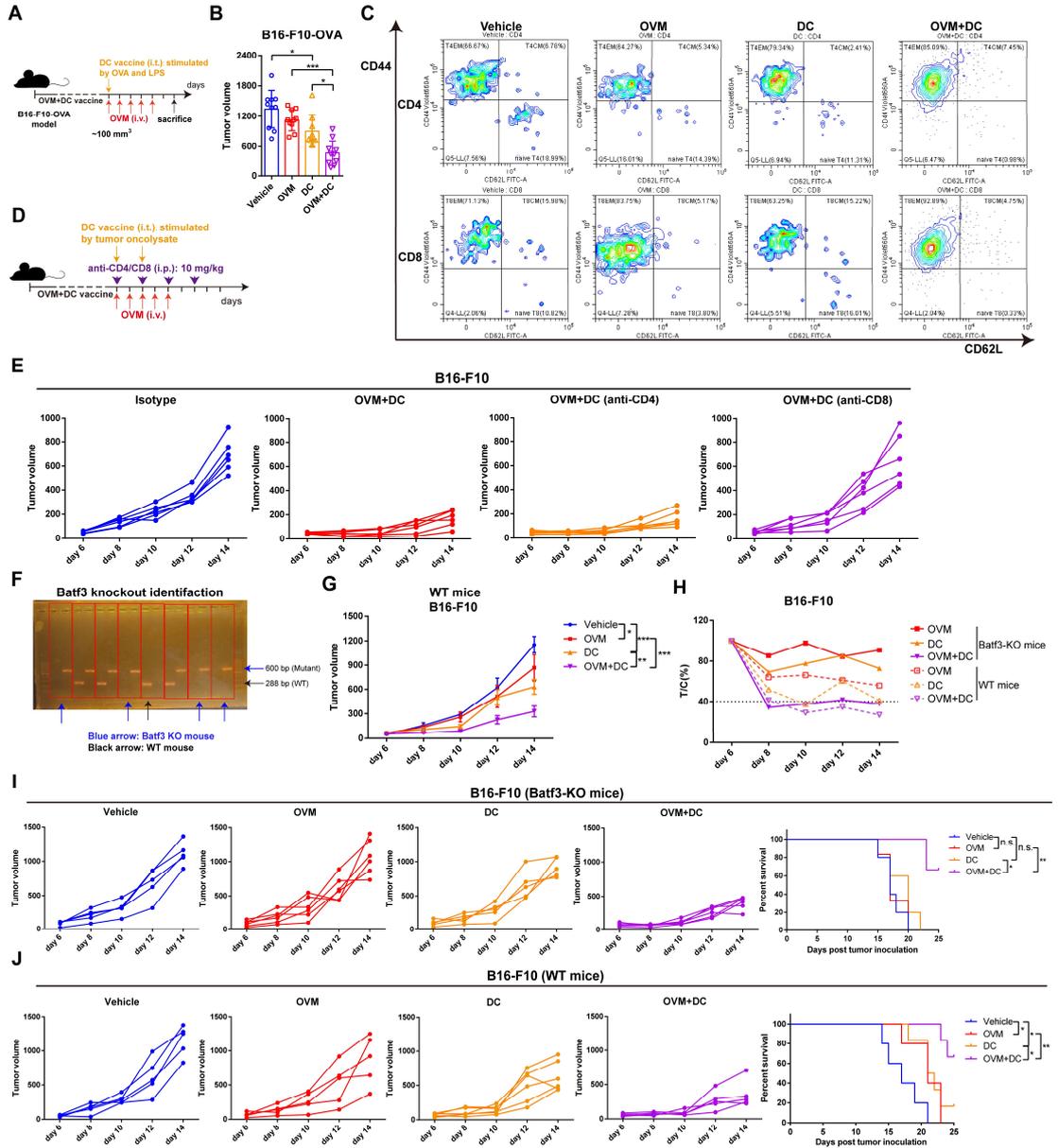
H-I.) Schematic diagram of Figure 1D-G (B) and Figure 1H (C).

J-N.) Tumor growth curves for each mouse in the experimental results shown in Figure D-H, respectively.

one-way ANOVA was used to determine the significance of differences between groups.

All data are presented as the means  $\pm$  SDs.

*n.s.*, not significant; \* $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .



**Figure S2. Combination of OVM and DC vaccine strongly enhances systemic antitumor response, and this efficacy remains unaffected by cDC1s, related to Figure 2.**

A.) Schematic diagram of Figure 2A-R.

B.) The tumor volumes on the second day after the last administration. n=9. Data are presented as the means ± SDs.

C.) Representative plot for the proportion of CD4<sup>+</sup> CD62L<sup>+</sup> T<sub>CM</sub> cells and CD4<sup>+</sup> CD62L<sup>-</sup> T<sub>EM</sub> cells among CD4<sup>+</sup> and CD8<sup>+</sup> T cells, respectively.

D-E.) Schematic diagram of the treatment regimen (D) and tumor growth curves for each mouse (E) in Figure 2S.

F.) Genotype identification of Batf3-KO mice.

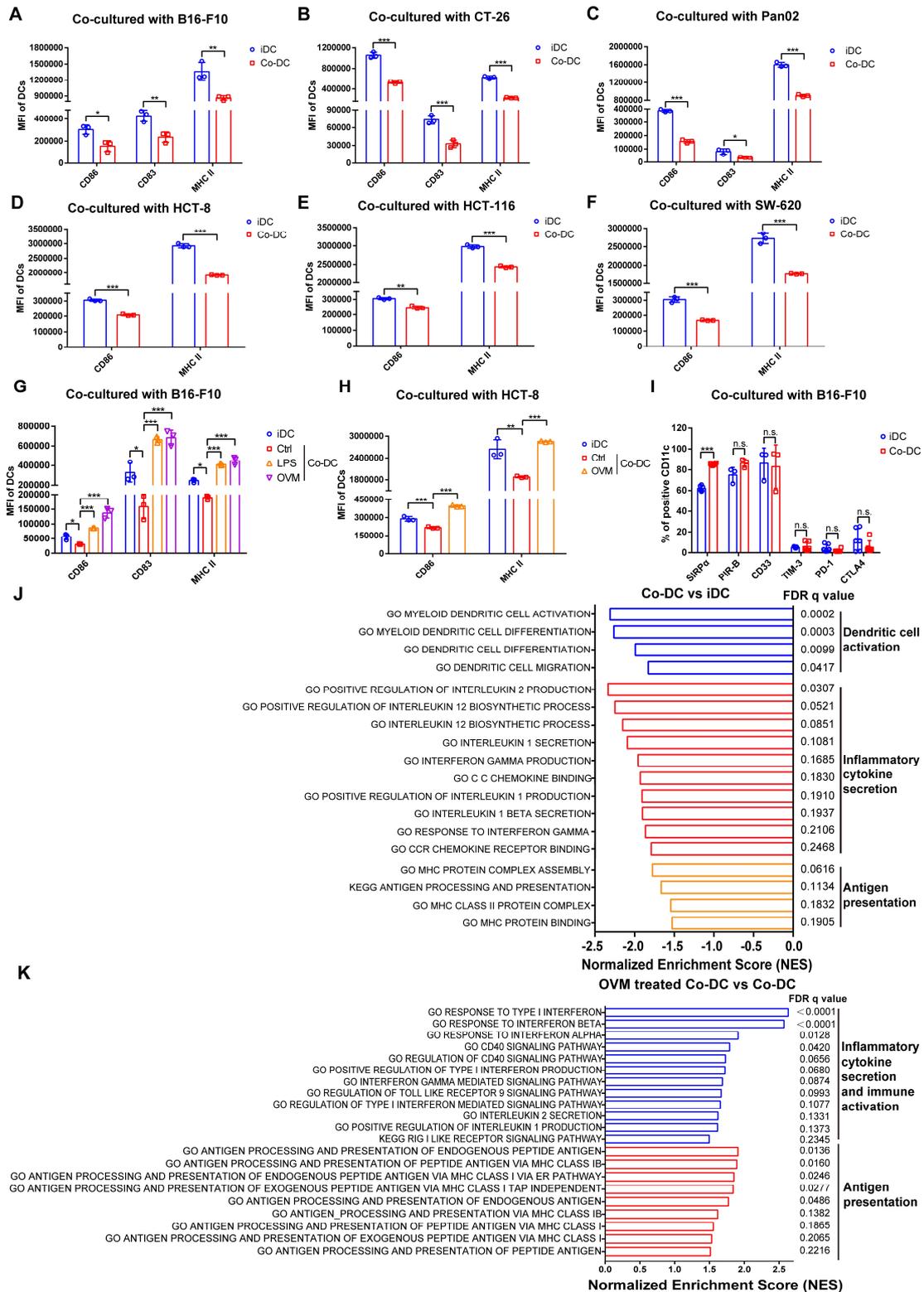
G-J.) Batf3-KO mice and their WT littermates were implanted subcutaneously in the right flank with B16-F10 cells on day 0 and received corresponding treatments on day 7. WT mice: n=5 in vehicle and OVM treated group. n=6 in DC vaccine group and n=7 in OVM plus DC vaccine group.

G.) Tumor growth curves of WT mice. *p* values were determined by one-way ANOVA at the final time point as indicated in the graphs.

H.) The T/C% values showing the relative tumor growth rate in every group compared with the vehicle group in Batf3-KO and WT mice.

I-J.) Tumor growth curves for each mouse and Kaplan–Meier survival curves in the Figure 2T (I) and Figure S2G (J), respectively. *p* values were determined by the log-rank test.

*n.s.*, not significant; \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001.



**Figure S3. OVM reactivates the function of DCs inhibited by tumor cells, related to Figure 3.**

A-C.) The MFI of CD86, CD83 and MHC II in mouse CD11c<sup>+</sup> DCs after cocultured at a 3:1 ratio with B16-F10 cells (A), CT-26 cells (B) for 48 h or with Pan02 cells for 72 h (C).

D-F.) The expression of CD86 and MHC II in human CD11c<sup>+</sup> DCs after cocultured at a 3:1 ratio with HCT-8 cells (D), HCT-116 cells (E) or SW-620 cells for 24 h (F).

G.) The MFI changes of CD86, CD83 and MHC II on mouse CD11c<sup>+</sup> DCs cocultured with B16-F10 cells for 24 h and stimulated with control (Ctrl), LPS (1 µg/ml, as a positive control) or OVM (1 MOI) for another 24 h.

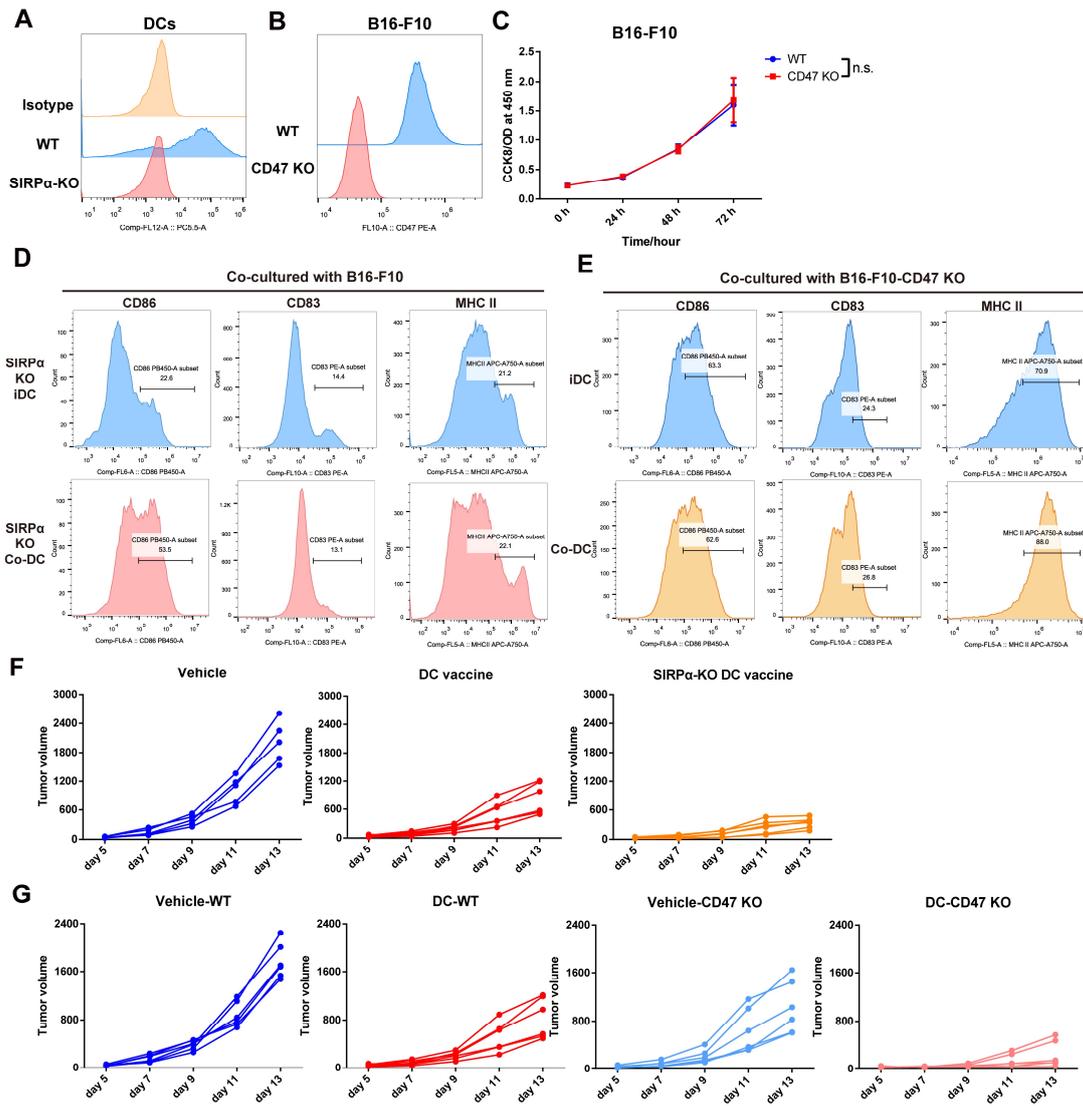
H.) The expressions changes of CD86 and MHC II on human DCs cocultured with HCT-8 cells for 24 h and stimulated with vehicle (Ctrl) or OVM (1 MOI) for another 24 h.

I.) The ratio changes of checkpoints on DCs after cocultured with B16-F10 for 48 h.

n=3 in every group. All data are presented as the means ± SDs. *n.s.*, not significant; \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001.

J.) GSEA pathways enriched with downregulated genes in cocultured DCs (co-DCs) compared to untreated BMDCs (iDCs).

K.) Pathways enriched with upregulated genes in OVM-treated Co-DCs compared to Co-DCs. *p* < 0.05 and false discovery rate q value (FDR-q) < 0.25.



**Figure S4. SIRP $\alpha$ -CD47 knockout cancels the inhibition of DCs by tumor cells, related to Figure 5.**

A-B.) SIRP $\alpha$ -KO (A) or B16-F10-CD47 KO (B) verification.

C.) Growth curves of B16-F10-WT and B16-F10-CD47 KO tumor cells *in vitro*. Cell viability at every time point was evaluated by a CCK-8 assay. n=3. one-way ANOVA was used to determine the significance of differences between groups. Data are presented as the means  $\pm$  SDs.

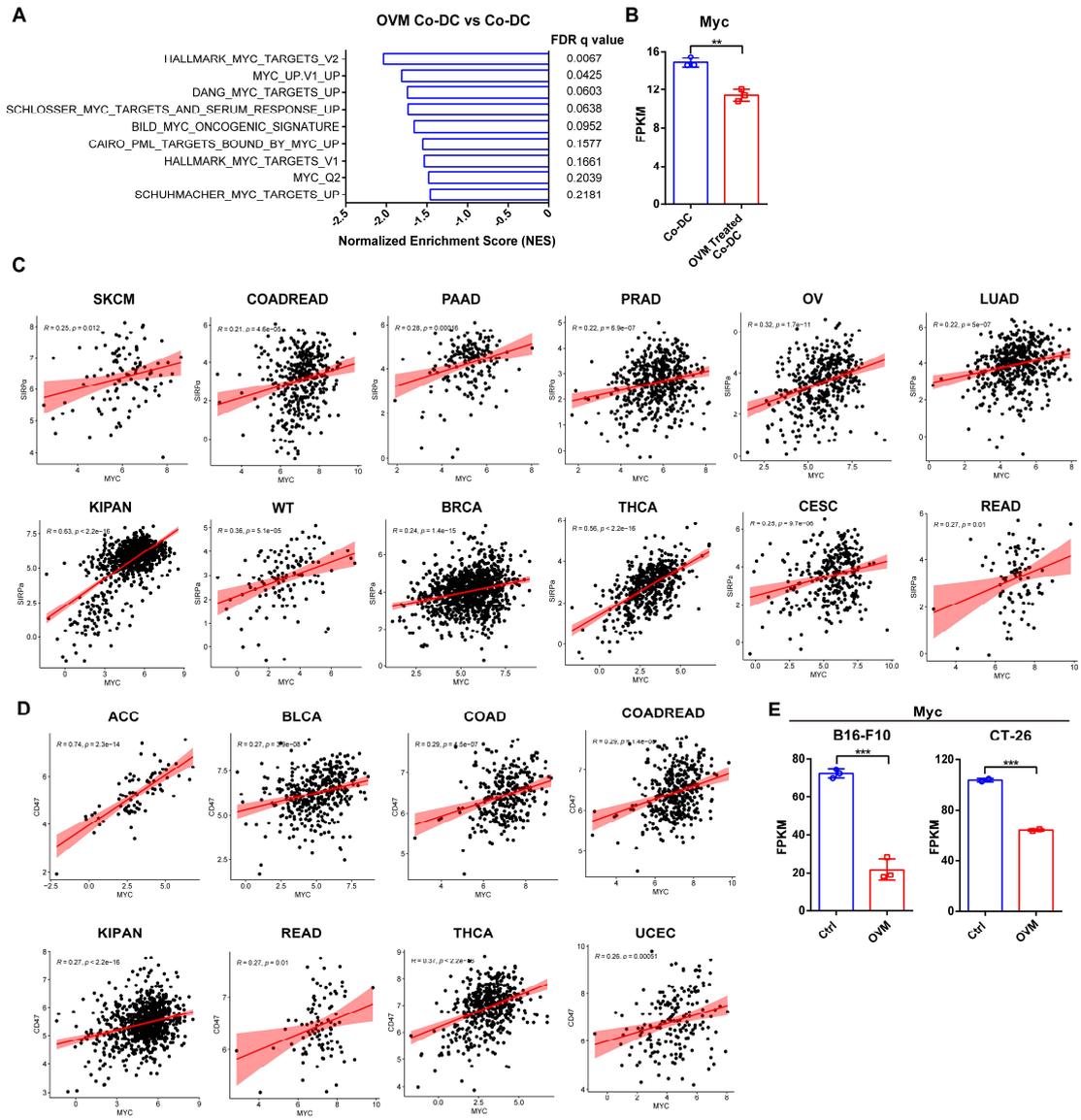
D.) Representative histograms of CD86, CD83 and MHC II in SIRP $\alpha$ -KO DCs or WT-DCs after cocultured with B16-F10.

E.) The representative histograms of CD86, CD83 and MHC II expressed in untreated iDCs and Co-DCs cocultured with B16-F10-CD47 KO.

F-G.) Tumor growth curves for each mouse, as described in the experimental results

shown in Figure 3K (F) and Figure 3N (G), respectively. n=6 in every group.

*n.s.*, not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .



**Figure S5. MYC may be the potential transcription factor for OVM to regulate both SIRPα and CD47 expression, related to Figure 6.**

A.) Gene sets changes related to MYC regulation in OVM-treated Co-DCs compared to Co-DCs.

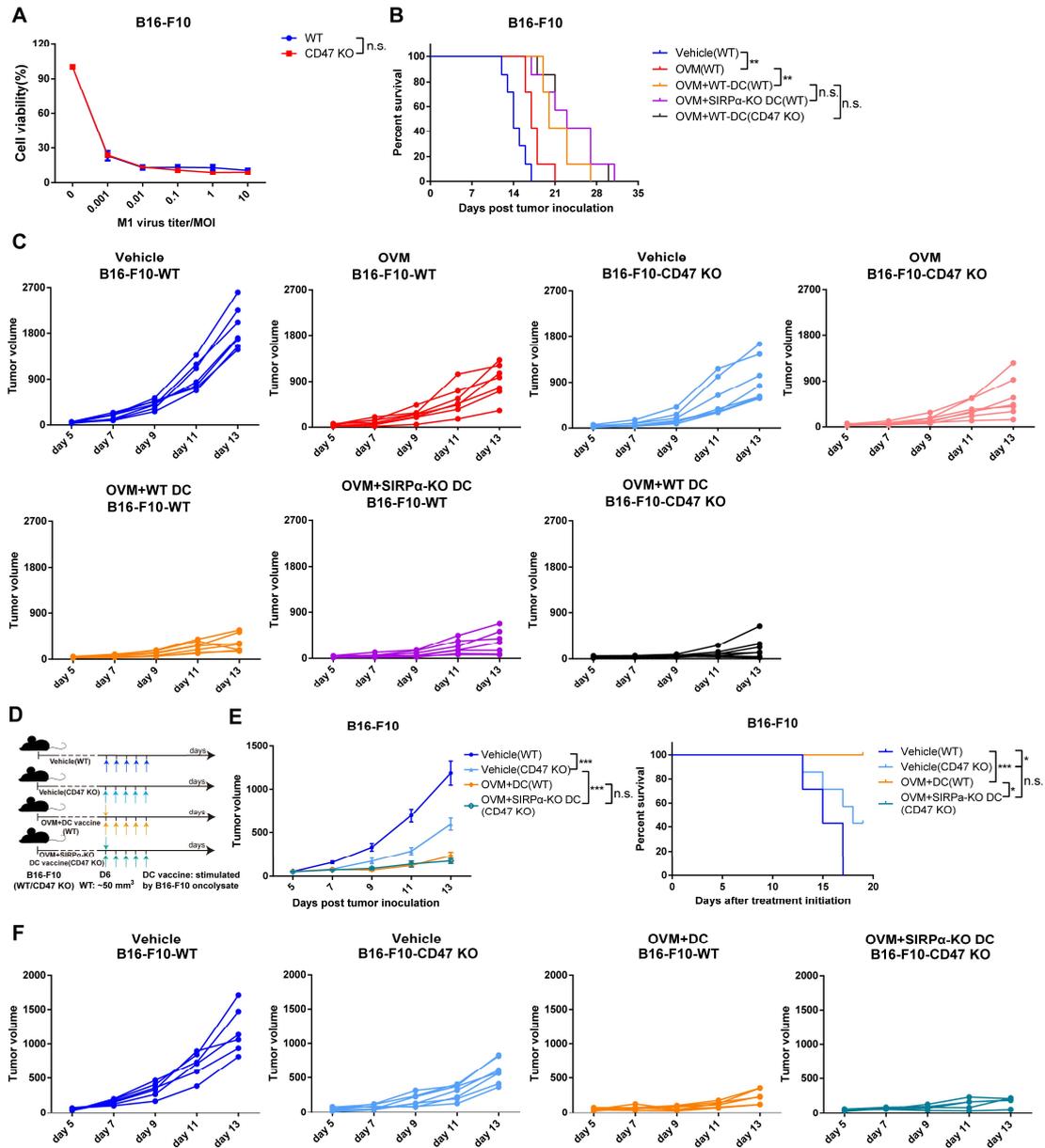
B.) Myc expression changes in B16-F10 cocultured Co-DCs and OVM-treated Co-DCs.

C-D.) Correlation analyses reveal associations between MYC and SIRPα expression (C), as well as between MYC and CD47 expression (D). SKCM: Skin Cutaneous Melanoma; COADREAD: Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma; PAAD: Pancreatic adenocarcinoma; PRAD: Prostate adenocarcinoma; KIPAN: Pan-kidney cohort (including Kidney Chromophobe, Kidney renal clear cell carcinoma and Kidney renal

papillary cell carcinoma); WT: High-Risk Wilms Tumor; BRCA: Breast invasive carcinoma; THCA: Thyroid carcinoma; OV: Ovarian serous cystadenocarcinoma; LUAD: Lung adenocarcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; READ: Rectum adenocarcinoma. ACC: Adrenocortical carcinoma; BLCA: Bladder Urothelial Carcinoma; COAD: Colon adenocarcinoma; UCEC: Uterine Corpus Endometrial Carcinoma.

E.) Myc expression changes in OVM-infected B16-F10 (0.5 MOI) or CT-26 (1 MOI) tumor cells.

*n.s.*, not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .



**Figure S6. OVM enhances the therapeutic effect of DC vaccine by blocking the interaction of SIRP $\alpha$ -CD47, related to Figure 6.**

A.) Cell viability assays were performed on B16-F10-WT and B16-F10-CD47 KO tumor cells 24 h after exposure to OVM.  $n=3$ . The data are shown as the means  $\pm$  SDs.

B-C.) The Kaplan–Meier survival curves (B) and tumor growth curves for each group (C) are linked to Figure 6I.  $p$  values were determined by the log-rank test.

D-F.) C57BL/6J mice were implanted subcutaneously in the right flank with B16-F10-WT or B16-F10-CD47 KO cells on day 0. On day 6, B16-F10-WT tumor-bearing C57BL/6J mice were treated with vehicle or OVM plus B16-F10 oncolysate-stimulated DC vaccine, and B16-F10-CD47 KO tumor-bearing C57BL/6J mice were treated vehicle or OVM plus

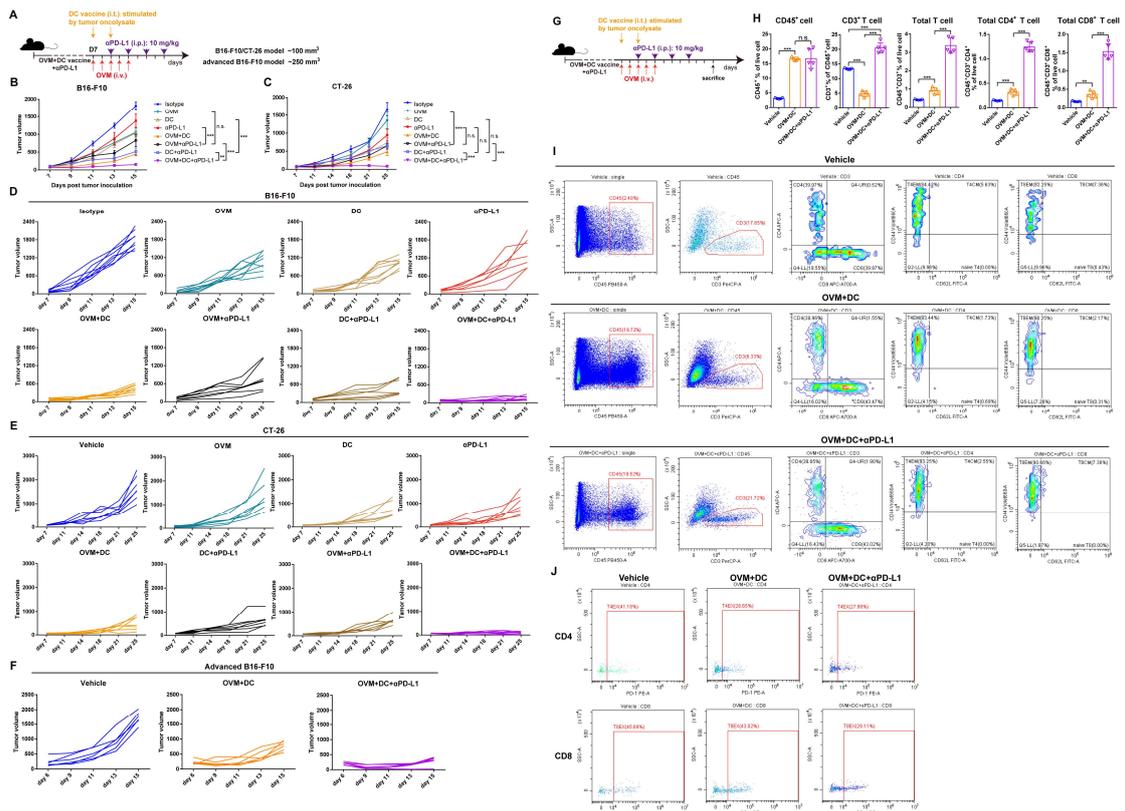
DC vaccine.

D.) Schematic diagram of the treatment regimen of B16-F10-WT and B16-F10-CD47 KO tumor-bearing C57BL/6J mice.  $n=6$ .

E.) Tumor growth curves and the Kaplan–Meier survival curves.  $p$  values were determined by one-way ANOVA at the final time point and the log-rank test, respectively.

F.) Tumor growth curves for each mouse in every group.

*n.s.*, not significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .



**Figure S7. PD-L1 blockade increases the efficacy of the combination of DC vaccine and OVM, related to Figure 7.**

A-F.) C57BL/6J or Balb/c mice were implanted subcutaneously in the right flank with B16-F10 or CT-26 cells on day 0 and treated with the indicated drugs.

A.) Schematic diagram of tumor inoculation and treatment in Figure 7D-F.

B-C.) Tumor growth curves in all groups are linked to Figure 7D (B) and E (C).

D-F.) Tumor growth curves for each mouse in every group derived from Figure 7D (D), E (E) and F (F).

G-J.) C57BL/6J mice were implanted subcutaneously in the right flank with B16-F10 cells and treated with corresponding therapy. Mice were sacrificed on the 3<sup>rd</sup> day after the last administration of αPD-L1 treatment, and infiltrated immune cells in the TME were analyzed by flow cytometry. n=5 in every group.

G.) Schematic diagram of the treatment regimen.

H.) The proportion of CD45<sup>+</sup> cells among live cells, CD3<sup>+</sup> T cells among CD45<sup>+</sup> cells, CD3<sup>+</sup> T cells among live cells, CD4<sup>+</sup> and CD8<sup>+</sup> T cells among live cells, respectively.

I.) Representative plot for the proportion of CD45, CD3, CD4, CD8, CD44 and CD62L,

respectively.

J.) Representative plot for the proportion of PD-1<sup>+</sup> among CD4<sup>+</sup> and CD8<sup>+</sup> T cells.

*p* values were determined by one-way ANOVA. *n.s.*, not significant; \**p* < 0.05; \*\**p* < 0.01;

\*\*\**p* < 0.001.