Supporting Information for

ORIGINAL ARTICLE

Immunostimulatory gene therapy combined with checkpoint blockade reshapes tumor microenvironment and enhances ovarian cancer immunotherapy

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Cells and animals

ID8 cells were purchased from ATCC (USA) and cultured in DMEM medium supplemented with 10% FBS, and 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were kept in the incubator with an atmosphere of 37°C and 5% CO_2. Female C57/BL6 mice at 6-8 weeks were obtained from Huafukang Biotechnology Co., Ltd. (Beijing, China). The animal experiments of the study were all conducted according to the guidelines of the Animal Experimental Ethics Committee of the State Key Laboratory of Biotherapy (SKLB), Sichuan University.

Intracellular trafficking

To track the intracellular delivery behavior of pDNA by F-DPC carrier, ID8 cells were planked in a dish with a glass bottom. The pIL-12 that pre-labeled with nucleic acid dye YOYO™-1 (Invitrogen, USA) was incorporated into F-DPC nanoparticles and transfected into the ID8 cells, followed by the staining with Lyso-tracker dye and nuclei dye Hoechst 33342. Then, the stained ID8 cells were continuously observed under a confocal laser scanning microscopy (LSM880, ZEISS, Germany) for analyzing the endosomal escape capability and relocation of F-DPC/pIL-12.

Gel retardation experiment

To detect the gene encapsulation capability of nanocomposite, the pIL-12 nanoparticles and F-DPC/iPDL-1 at different mass ratios (1:0, 1:12.5, 1:25, 1:50) were gently mixed to form complex and loaded onto a 1% agarose gel (Invitrogen Corp, Carlsbad, CA, U.S.). The gel electrophoresis was conducted in pH 7.4 Tris-acetate (TAE) running buffer containing GelRed at 120 V for 25 min. The gel image was digitally photographed using a gel documentation system (Bio-Rad Laboratories, USA).

Tumor cells apoptosis in vitro

For validating the hypothesis that the PDL-1 expression of tumor cells was upregulated after the stimulated lymphocytes supernatant treatment, tumor killing assay and tumor apoptotic study were performed in the study. Tumor cells pre-labeled with CFSE (150347-59-4, Meilunbio, Dalian, China) were seeded in the 12-well plates. After 24h incubation, the tumor cells were transfected as described before, or treated with iPDL-1 (1 μmol/L), followed by the addition of murine

spleen-derived lymphocytes at a ratio of 1:15. The tumor killing effect was observed with a light microscope (Olympus, Japan). The cells were collected and stained with anti-PDL-1 antibody or PE-Annexin V after 48 h incubation, and ultimately analyzed by flow cytometry to identify the expression level of PDL-1 and the apoptosis of tumor cells.

Supplementary figures and figure legend:

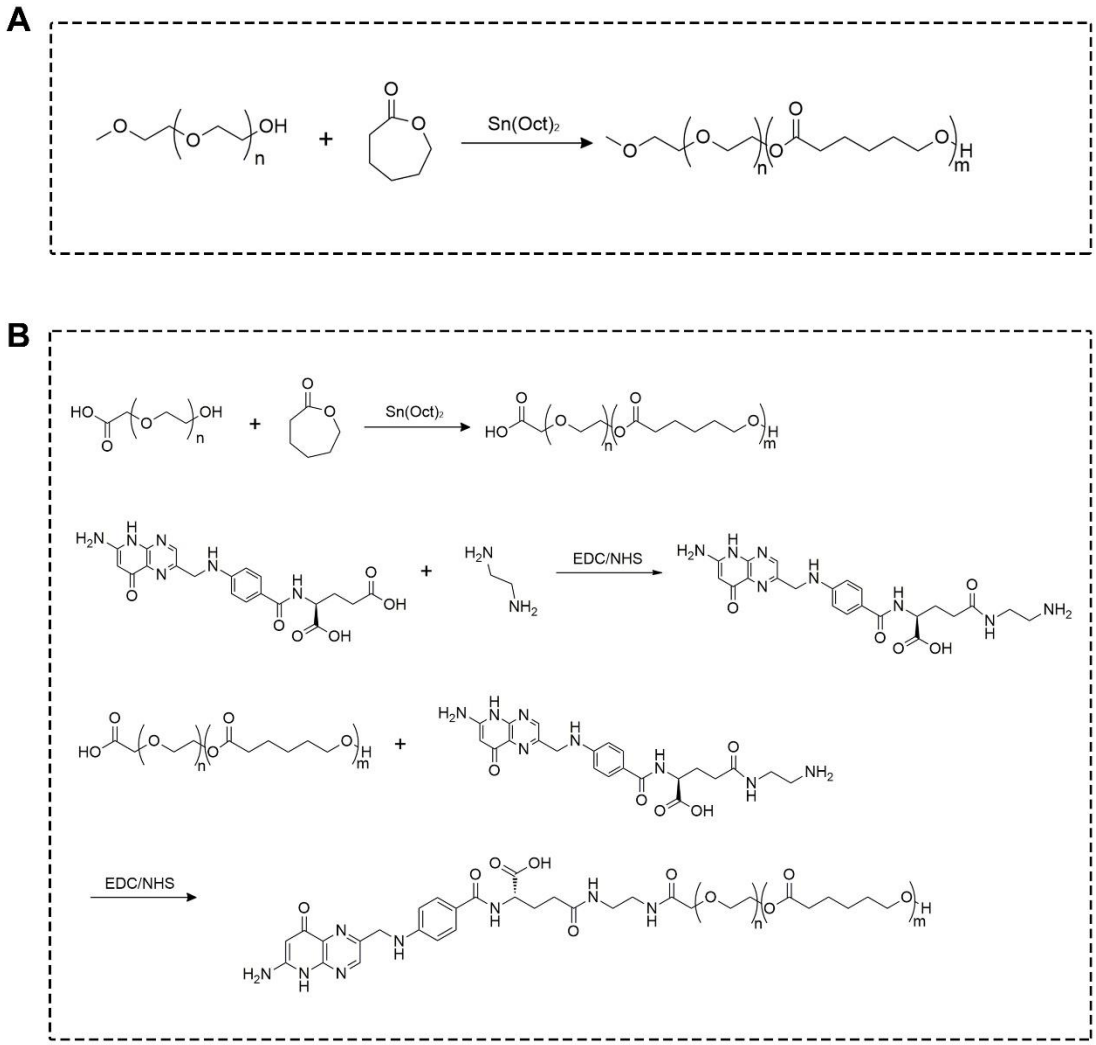

Figure S1. Synthetic routes of PEG-PCL (A) and FA-PEG-PCL (B) copolymers.

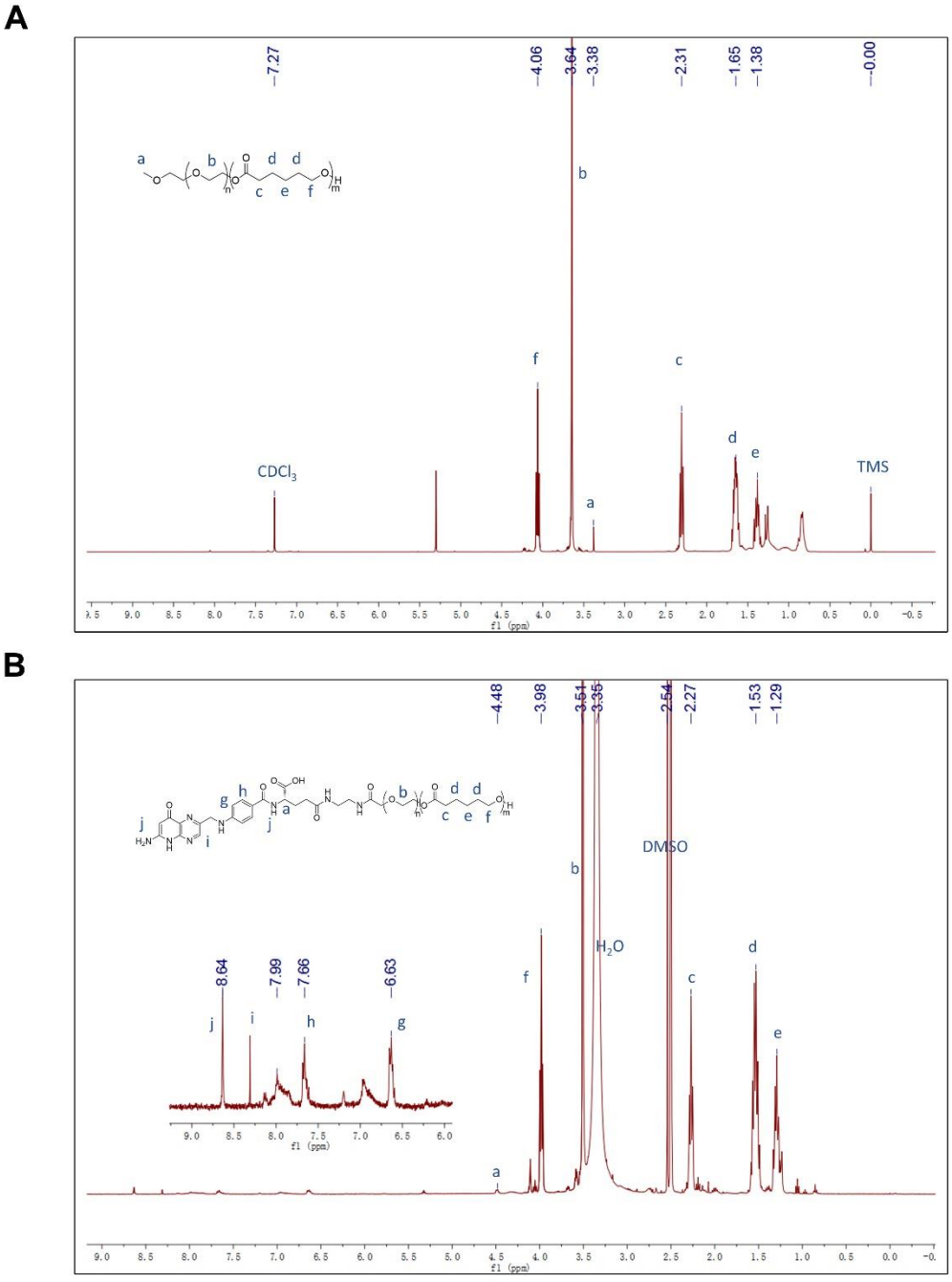

Figure S2. ^{1}H NMR spectrums of PEG-PCL and FA-PEG-PCL copolymers.

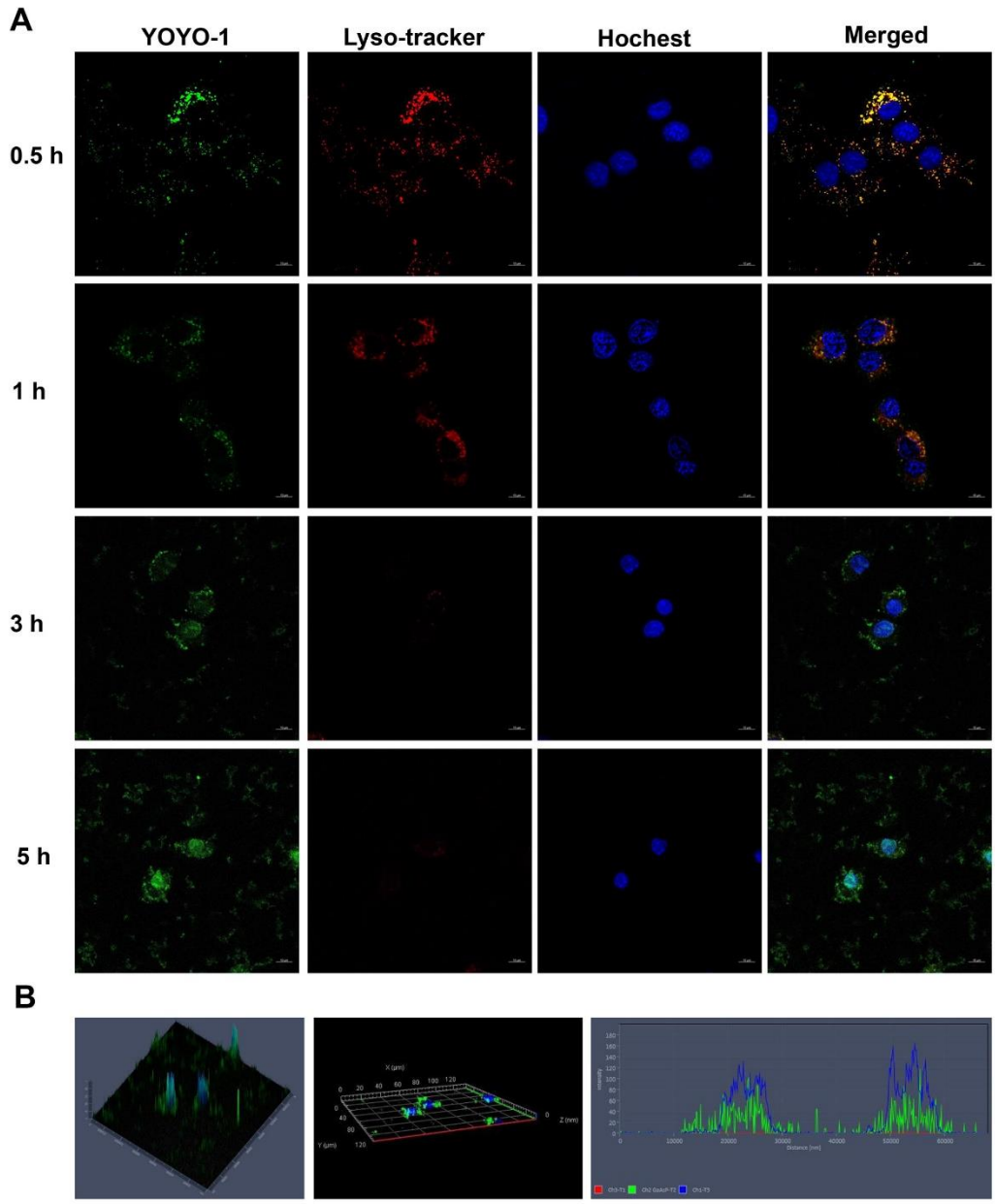

Figure S3. Confocal images of ID8 cells treated with F-DPC/pIL-12/iPDL-1 complexes. pIL-12 was labeled with YOYO-1, lysosomes were stained with Lyso-tracker and the nuclei were stained with Hoechst 33342. (A) Images were taken at 0.5, 1, 3 and 5 h, respectively (scale bar: 10 μm). (B) 3-dimensional images and analysis of fluorescence intensity in cross-section of ID8 cells at 5 h after treatment.

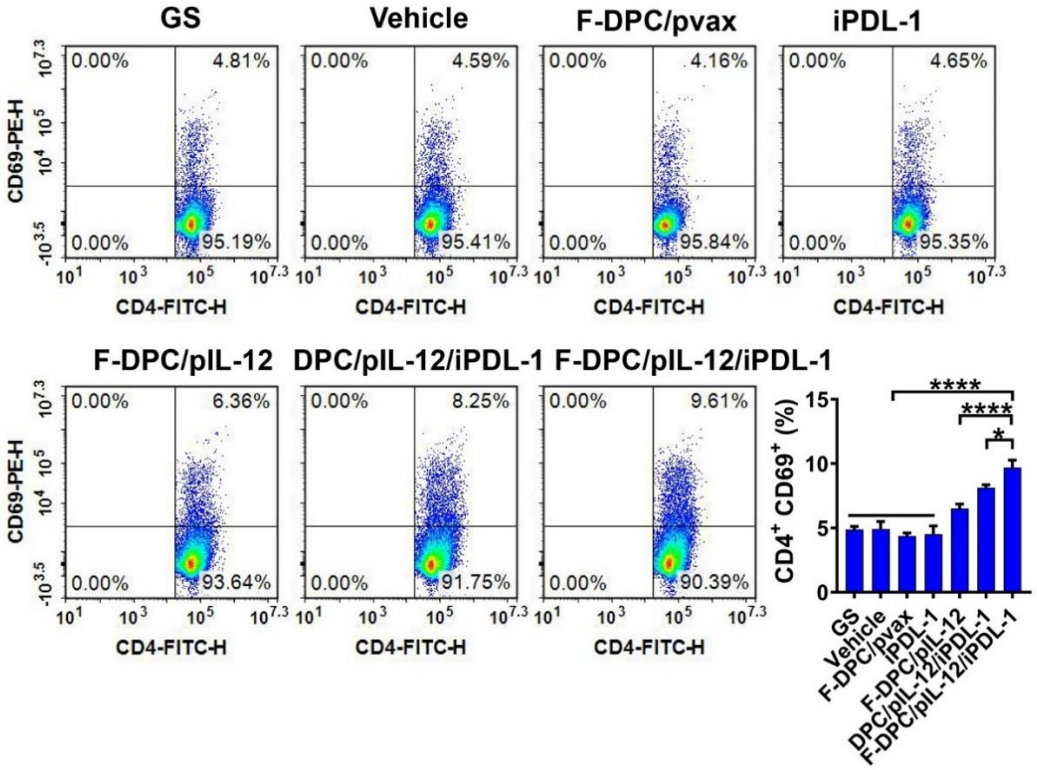

Figure S4. The subsets of $CD4^{+}CD69^{+}$ lymphocytes in the peritoneal lavage fluid of mice with abdominal metastasis of ovarian cancer were measured by flow cytometry. (n = 3, *$P < 0.05$, ****$P < 0.0001$).

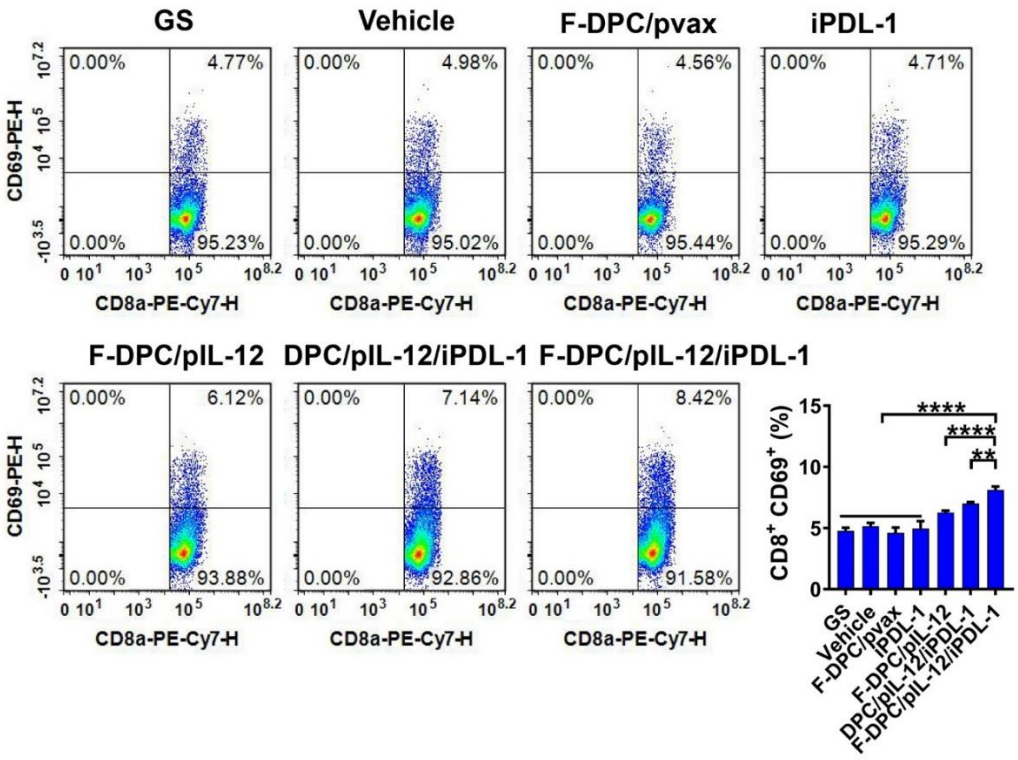

Figure S5. The subsets of $CD8^{+}CD69^{+}$ lymphocytes in the peritoneal lavage fluid of mice with abdominal metastasis of ovarian cancer were measured by flow cytometry. (n = 3, $^{**}P < 0.01$, $^{****}P < 0.0001$).

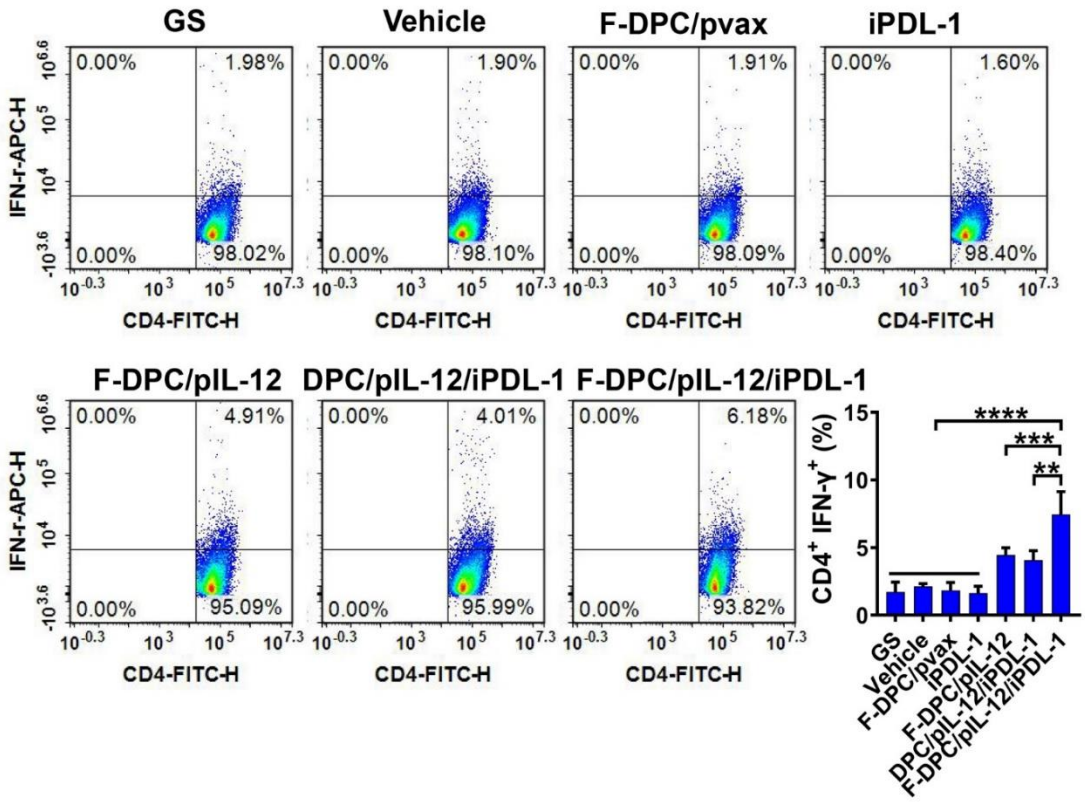

Figure S6. The subsets of $CD4^{+}IFN\text{-}\gamma^{+}$ lymphocytes in the peritoneal lavage fluid of mice with abdominal metastasis of ovarian cancer were measured by flow cytometry. (n = 3, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$**).**

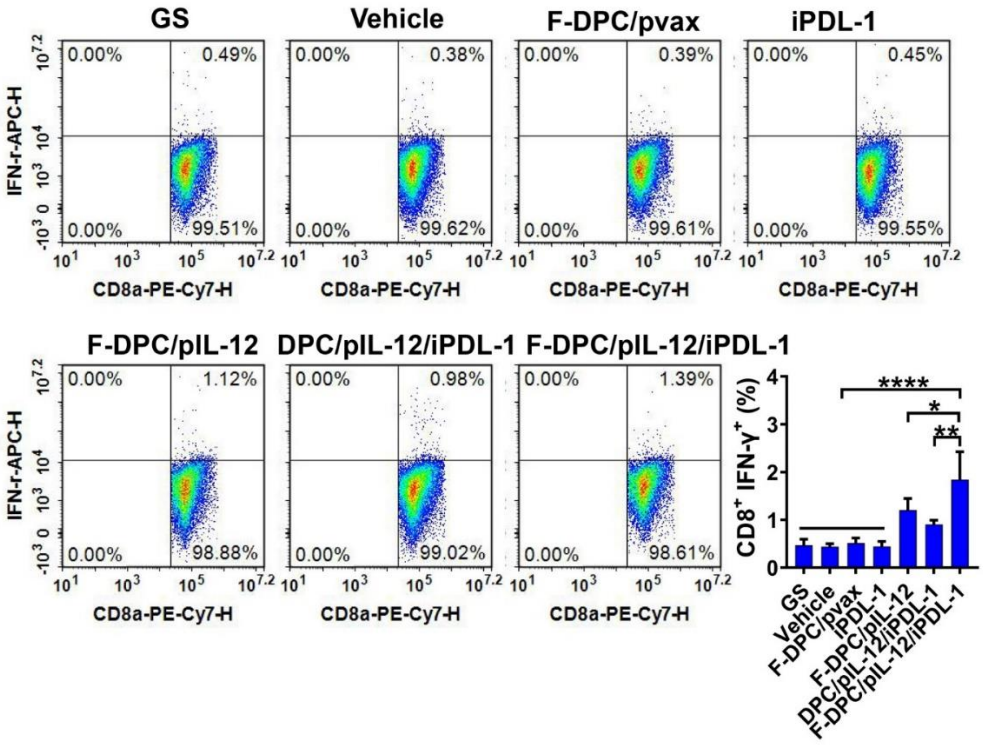

Figure S7. The subsets of $CD8^{+}IFN\text{-}\gamma^{+}$ lymphocytes in the peritoneal lavage fluid of mice with abdominal metastasis of ovarian cancer were measured by flow cytometry. (n = 3, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001).

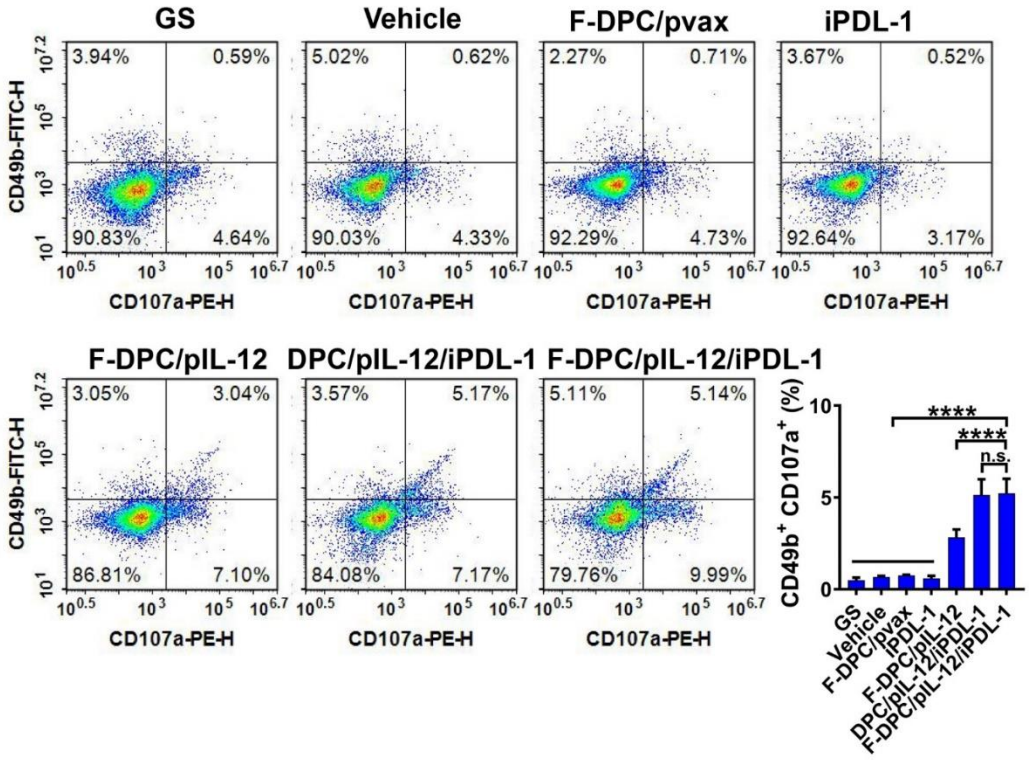

Figure S8. The proportion of NK cells in ascites was evaluated by the flow cytometry. (n = 3, ****$P < 0.0001$).

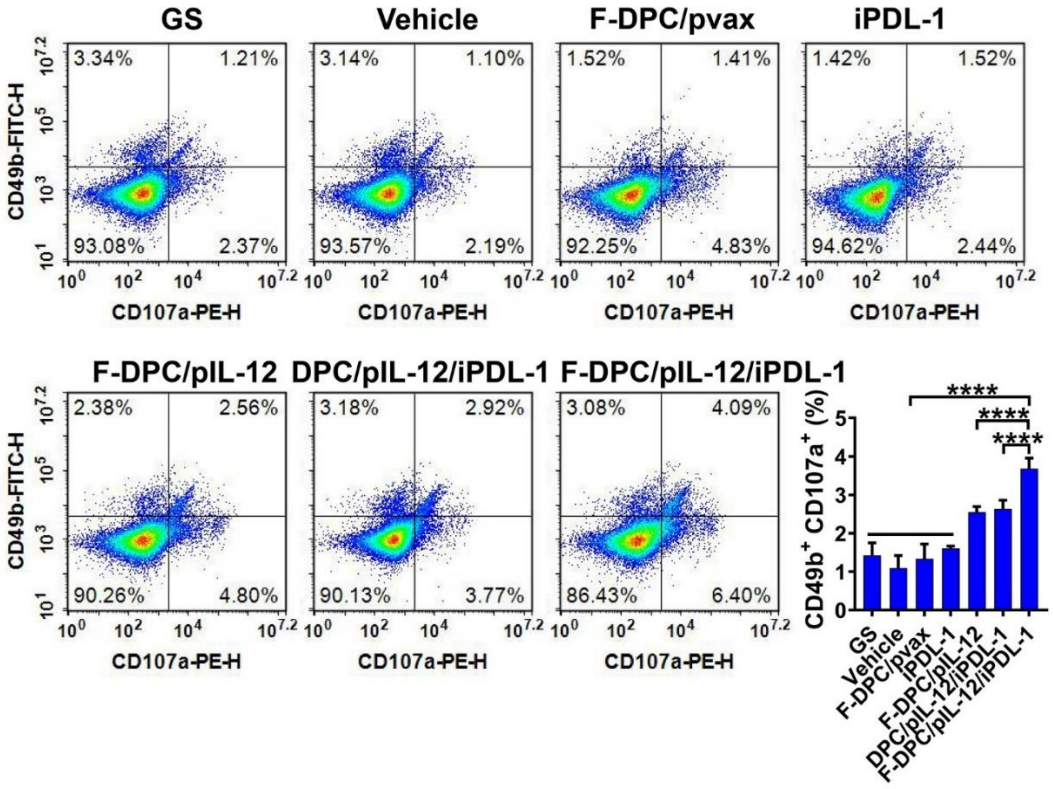

Figure S9. The proportion of NK cells in spleen was evaluated by the flow cytometry (n = 3, ****$P < 0.0001$).

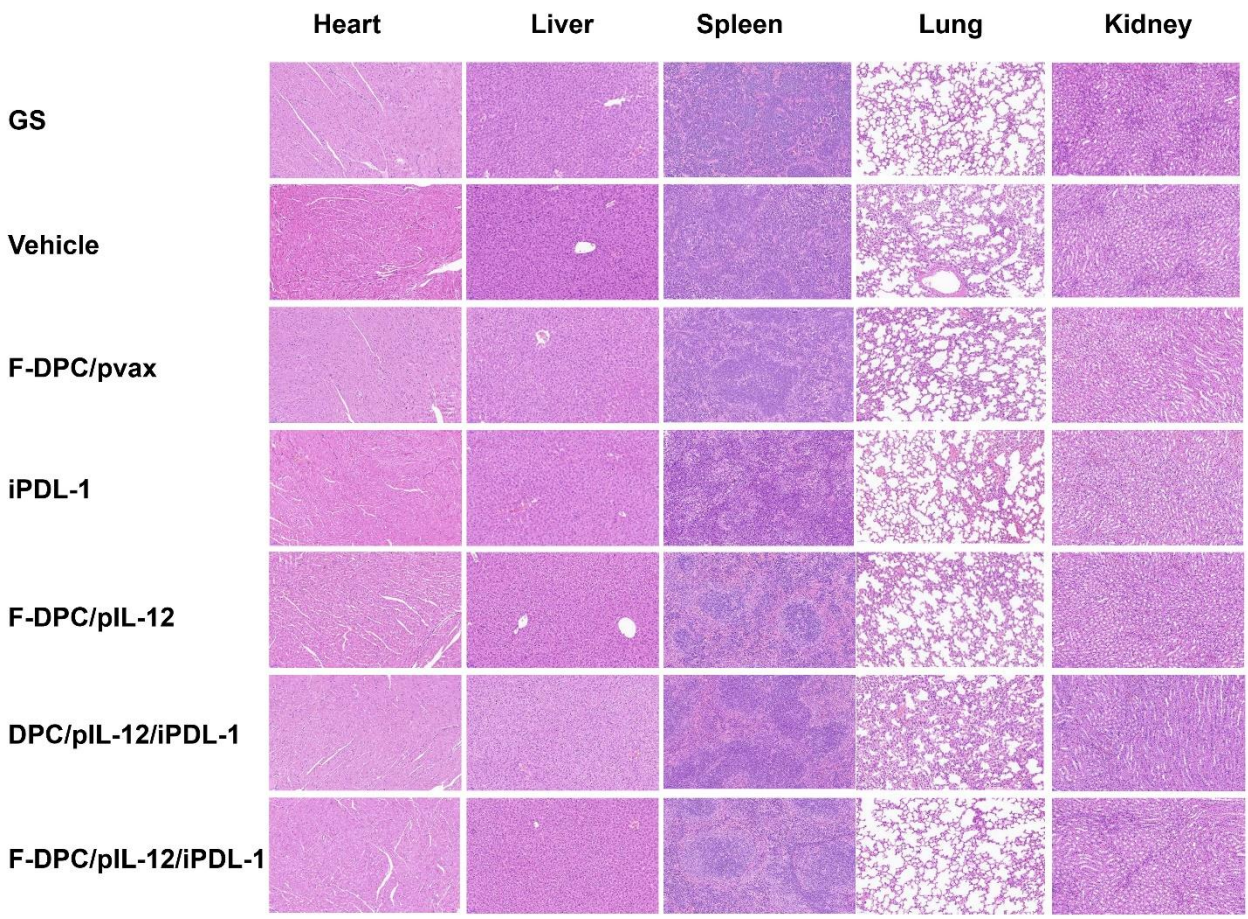

Figure S10. Vital organs of heart, liver, spleen, lung, and kidney were collected from mice after treatment and stained with H&E staining for histological examination.

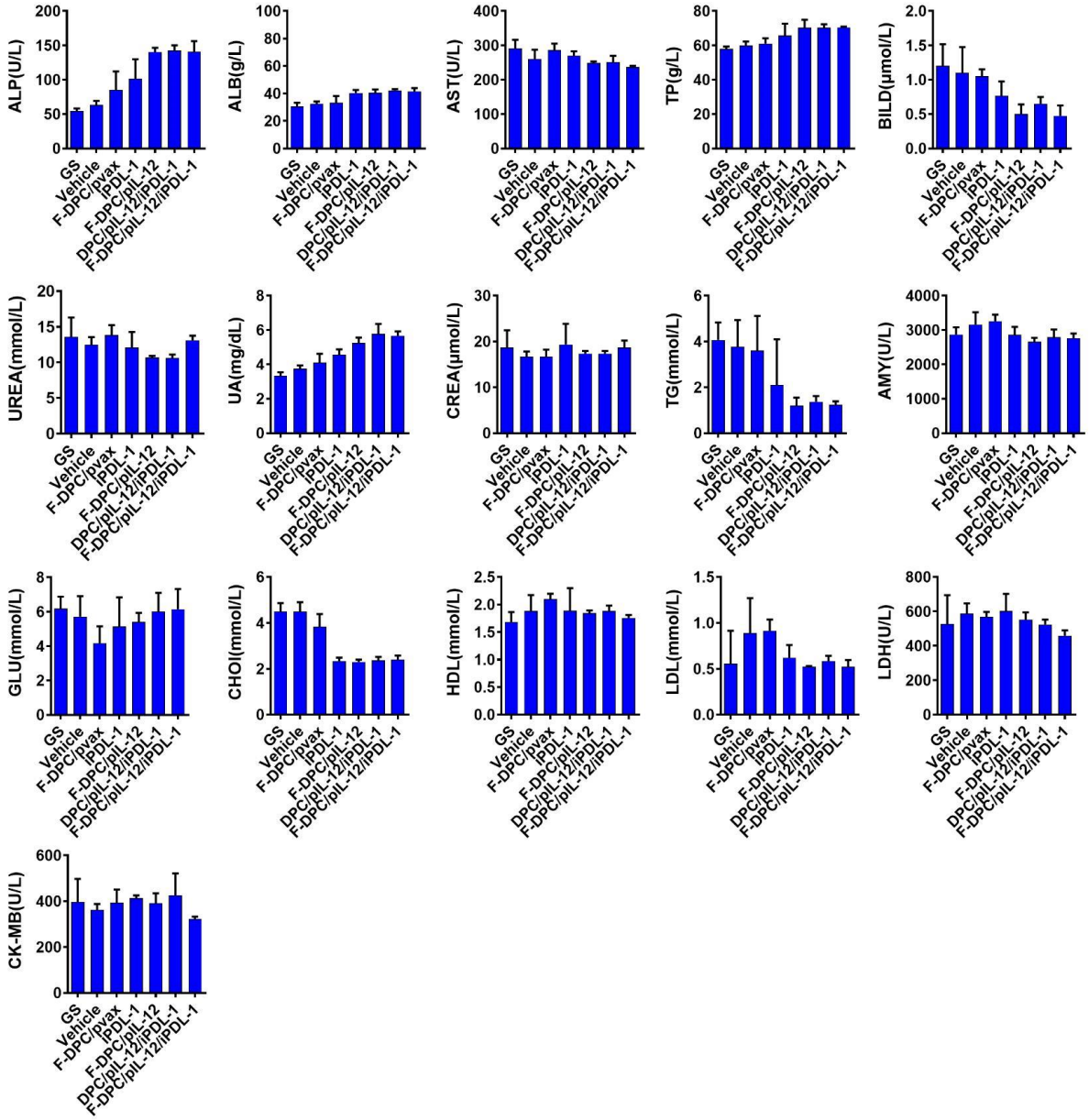

Figure S11. Serological biochemical analysis. Abbreviations: ALP, alkaline phosphatase; ALB, albumin; AST, aspartate aminotransferase; TP, total protein; BILD, bilirubin direct; UREA, urea; UA, uric acid; CREA, creatinine; TG, triglycerides; AMY, amylase; GLU, glucose; CHO, total cholesterol; HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB.