Method

Reagents

Endotoxin-free ovalbumin (OVA) was purchased from Invitrogen. BCA kit was purchased from Beyotime Biotechnology. Branched 60 kDa polyethyleneimine (PEI), beta-mercaptoethanol, collagenase IV and DNase were obtained from Sigma. Easter-terminated 50:50 PLGA polymer (RG 505) was purchased from EVONIK. Lipopolysaccharide (LPS), carboxyfluorescein diacetate succinimidylester (CFSE), protease and phosphatase inhibitor cocktail, Dynabeads® mouse T-activator CD3/CD28, 1'-dioctadecyl-3,3,3',3-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD) and Hoechst 33342 were obtained from Thermo Fisher Scientific. Ficoll-Paque™ PREMIUM was purchased from GE Healthcare. Murine recombinant GM-CSF and IL-4 were purchased from Peprotech. $CD8^+$ T Cell Isolation MACS Kit was purchased from Miltenyi Biotec. Antibodies for western blotting were purchased from Cell Signaling Technology. All other reagents were purchased from domestic suppliers and used as received.

Preparation of PLGA nanoparticles

Nanoparticulate cores were prepared through nanoprecipitation of RG505 PLGA polymer (0.66-0.74 dL/g). The choice of this chemical composition was depended on the biocompatibility and biodegradability as well as the feasibility of nanoparticle preparation and subsequent coating[1, 2]. Firstly, PLGA polymer was dissolved in acetone at a concentration of 1 mg/ml. In order to label with fluorescence, 2 μg/ml of DiD was

added. A volume of 1 ml mixture prepared above was added dropwise into 3 ml nanopure water and then stirred in air for 30 minutes. A vacuum aspirator was employed to concentrate the solution to a final volume of 1 ml. The obtained nanoparticle solution was ready for further use.

Cell culture

B16-OVA, Hepa 1-6 and TC-1 cells were cultured in complete medium (DMEM, 10% fetal bovine serum, 1% penicillin-streptomycin) at 37 °C in 5% CO_2. Dendritic cells were generated by culturing bone marrow cells collected from the femurs and tibiae of 8-10 weeks old male C57BL/6J mice in RPMI 1640 supplemented with 10% FBS, 1% penicillin-streptomycin, 20 ng/ml GM-CSF, and 10 ng/ml IL-4. Culture medium was half replaced and replenished every 2 days.

Animals

All the animal procedures complied with the guidelines of the Shanghai Medical Experimental Animal Care. Animal protocols were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine. Male C57BL/6 mice (6-8 weeks old) were obtained from Jiesijie Laboratory Animal Center (Shanghai, China).

Preparation of cell membranes

To obtain the plasma membranes, collected cells were separately suspended in a hypotonic lysing buffer consisting of 25 mM sucrose, 10 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM KCl, 2 mM phenylmethylsulfonyl fluoride (PMSF) and 1 × protease and phosphatase inhibitor cocktail on ice for 30 minutes. The entire lysate was administered in a condition of 20 passes pounding up and down using a dounce homogenizer with a tight-fitting pestle. The supernatant was harvested after 3, 200 × *g* for 5 minutes and the pellet was resuspended in hypotonic lysing buffer and treated with another 20 passes. This process was repeated for another 5 times to ensure that the pellet was free of intact cells. The supernatants were pooled and centrifuged at 15000 rpm for 30 minutes at 4 °C. The collected membranes were dispersed in PBS on ice and washed for three time at 4 °C. The samples were measured by a BCA assay. RBC membranes were isolated exactly following a previously published study[3]. The whole blood was collected from male ICR mice (6-8 w) and centrifuged at 800 × *g* for 5 minutes at 4 °C to remove the serum and buffy coat. The purified RBCs were suspended in 0.25 × PBS in an ice bath for 20 minutes and then centrifuged at 800 × *g* for 5 minutes to remove the hemoglobin. The obtained RBC membranes were suspended in PBS and ready for further use.

Preparation of cell membrane-coated nanoparticles

To prepare cell membrane-coated nanoparticles, the obtained cancer cell or DC membranes were mixed with PLGA nanoparticulate cores at a membrane protein to nanoparticle weight ratio of 1:1. The mixture was physically extruded through a 400 nm polycarbonate membrane for 11 passes. The extrusion was subsequently repeated

by using a 200 nm polycarbonate membrane. The resulting cell membrane-coated nanoparticles were measured by dynamic light scattering (DLS) for the characterization of particle size and zeta potential. The stabilities of these nanoparticles in water and 1 × PBS were also monitored by DLS for 14 days. Transmission electron microscopy (HT7700 TEM, HITACHI) imaging was carried out to obtain their morphological information.

Western blotting

Identification of antigen OVA associated with B16-OVA was performed via western blotting. Lysed B16-OVA cells and CCNPs were measured by BCA assay to determine protein loading. Then, 40 μl of each sample was loaded to western blot, separated via 12% SDS-PAGE, and electrotransferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). After blocking with 5% skim milk, the samples were incubated with anti-ovalbumin antibody (181688, Abcam). Mouse antibody for GAPDH was purchased from Zsbio (Beijing, China). Horseradish peroxidase-conjugated antibody (Biolegend) was used as secondary staining. The membranes were observed in a gel-imaging instrument (Aplegen, Omega Fluor Plus).

Antigen presentation of BMDCs

An amount of 1×10^6 non-adherent BMDCs was incubated with 40 μg OVA, 40 μg OVA complexed with 10 μg PEI or CCNPs with 40 μg membrane proteins for 18 hours and harvested for further use. BCA kit was used to determine the total protein of B16-

OVA membranes. In order to detect efficiency of antigen presentation, CCNPs with different amounts of membrane proteins including 40, 60, 80, 120, and 160 μg were incubated with BMDCs. The OVA 257-267 exhibited on the MHC-I on cell surface was bound with anti-SIINFEKL/H-2Kb and analyzed by flow cytometry (CytoFLEX, Beckman Coulter). The gp100 peptide presented on cell surface was bound with anti-melanoma gp100 and dylight 488 goat anti-rabbit IgG [H+L], and analyzed by flow cytometry (CytoFLEX, Beckman Coulter).

Interaction between BNs and T cells

In accordance with the manufacturer's protocol of $CD8^+$ T Cell Isolation MACS Kit, primary $CD8^+$ T cells were isolated from mouse spleen. DiD-labeled Ns, RBC-Ns, DCNs and BDCNs were separately incubated with $CD8^+$ T cells with a concentration of 0.2 mg/ml at 4 °C for 1-hour. After staining with Hoechst 33342, the cells were observed under confocal laser scanning microscopy (Leica TSC SP8). In addition, flow cytometry was employed to quantify the binding between BDCNs and $CD8^+$ T cells.

In vitro cross-priming of T cells

$CD8^+$ T cells were stained with carboxy fluorescein succinimidyl ester (CFSE, eBioscience) and diluted to 2.5×10^6 cells/ml in 96-well cell culture plate for assessing the degree of T cell proliferation. BDCNs or HDCNs were directly incubated with T cells at a ratio of 10:1 (T cells/DCs used for coating) at 37 °C. After incubation for 3 days, T cells were harvested and analyzed by flow cytometry. To assess antigen-

specific presentation, T cells were collected and stained with anti-CD16/CD32 (eBioscience, Catalog: MA5-18012), anti-CD8-FITC (Biolegend, Catalog: D271-4), H-2Kb OVA Tetramer-SIINFEKL-APC (MBL International, Catalog: TS-5001-2C) and H-2D^{b} gp100 Tetramer-EGSRNQDWL-PE (MBL International, Catalog:TS-M546-1). The percentages of OVA-specific or gp100-specific T cells were calculated by flow cytometry.

Antigen-specific T cell generation in mimic tumor environment

CD8^{+} T cells were isolated from primary splenocytes extracted from C57BL/6J mice using CD8^{+} T cell isolation MACS Kit, seeded onto 12-well cell culture plate at a density of 10^{6} cells per well. DCs or BDCNs with equivalent numbers were cocultured with T cells with a ratio of 1:10 in presence of 10 mM lactic acid at 37 °C for 3 days. Then, T cells were collected and stained with anti-CD16/CD32 (eBioscience, Catalog: MA5-18012), anti-CD8-APC (Biolegend, Catalog: 100712) and H-2K^{b} OVA Tetramer-SIINFEKL-APC (MBL International, Catalog: TS-5001-2C). The percentage of OVA-specific T cells was analyzed by FACS.

Effects of storage condition on DCs and BDCNs

Before coculturing with CD8^{+} T cells, DCs and BDCNs were stored at 4 °C for 24 hours in order to study the influence of storage condition on DCs and BDCNs. After incubation for 3 days, the percentage of OVA-specific T cells was collected and analyzed by flow cytometry.

***In vivo* immune responses induced by BNs**

Six-eight weeks old male C57BL/6 mice were set up in five groups and separately injected with 100 μl PBS, Ns alone (equivalent PLGA to BNs), and 10 mg ml^{-1} of BNs or DCNs intravenously through tail vein every 5 days for 3 times. Five days post the last injection, the spleen of the treated mice was harvested and homogenized to analyze the frequency of $CD3^+CD8^+$ T, $Ki67^+$ in $CD3^+$, IFN-γ^+ in $CD3^+$, mean fluorescence intensities of CD80 and CD86 in $CD11c^+$ cells by flow cytometry. In addition, OVA-tetramer and gp100-tetramer staining assay was performed to evaluate the antigen-specific T cells.

Biodistribution

An amount of 5×10^6 B16-OVA cells were inoculated at the right hind leg to study the biodistribution of BDCNs. The mice were intravenously injected with 150 μl BDCNs which were loaded with 250 μg DiD. All the mice were imaged by in vivo imaging system (Caliper Life Sciences, USA) at the indicated time points and sacrificed for analysis at the predetermined time intervals. The liver, spleen, lung, heart, kidney, and tumor were collected in cell culture dishes and imaged accordingly.

Tumor-specific immunity elicited by BNs in tumor-bearing mice

Six-eight weeks old male C57BL/6 mice were subcutaneously inoculated on day 0 with 3×10^6 B16-OVA (at the right hind leg) or Hepa 1-6 (the right flank). Mice were daily

administered through tail vein with 100 μl PBS, Ns alone (equivalent PLGA to BNs), and 10 mg ml^{-1} of DCNs, ODCNs, or BDCNs for 7 days since day 8 (the tumor size reached 50~100 mm^3) post-inoculation. The tumor volume was measured every other day using a digital caliper and calculated as 0.5 × length × $width^2$ by blinded researchers. When tumor size of the control group exceeding 2000 mm^3, all mice were sacrificed. Peripheral blood, spleen and tumor were collected for analyzing the activation of immune cells and expression of cytokines. The serum samples were tested by ELISA. Tumor tissue was excised and sectioned for H&E and immunofluorescence staining.

Cross validation experiments

Six-eight weeks old male C57BL/6 mice were subcutaneously inoculated on day 0 with 3×10^6 B16-OVA or TC-1 cells at the right flank. B16-OVA tumor-bearing mice were administered with 100 μl PBS and 10 mg/ml of BDCNs or TDCNs. On the other hand, TC-1 tumor-bearing mice were administered with 100 μl PBS, 10 mg/ml of TDCNs or BDCNs. BDCNs or TDCNs were daily injected for 5 days since the tumor size reached 50~100 mm^3. Both tumor size and bodyweight were recorded every other day. Mice were sacrificed once tumor size reaching 2000 mm^3 or bodyweight dropping by 30 weight%.

Antitumor responses and therapeutic efficacy after combination with αPD-1

Six-eight weeks old male C57BL/6 mice were subcutaneously inoculated on day 0 with 3×10^6 B16-OVA or Hepa 1-6 cells at the right hind leg and the right flank, respectively.

Five doses of 100 μl BDCNs or HDCNs (10 mg/ml) were daily injected once the tumor size reached 50~100 mm^3. Meantime, 100 μg of aPD-1 (RMP1-14, BioXCell, intraperitoneal injection) were administered at day 8, 9, 10, 12 and 14, respectively. Equivalent free αPD-1 was used as a control. The tumor volume was measured every other day and the mice were sacrificed once tumor size of the control group exceeding 2000 mm^3. Peripheral blood, spleen and tumor were collected for further analyses including the activation of immune cells, expression of cytokines, ELISA as well as H&E and immunofluorescence staining. To investigate survival rates, mice were similarly treated and sacrificed once tumor size reaching 2000 mm^3 or bodyweight dropping by 30 weight%.

Flow cytometric analysis

The maturation and antigen-presentation of DCs was examined by staining with fluorescence-conjugated antibodies including anti-CD11c-FITC (Biolegend, Catalog 117306), anti-CD80-PE (eBioscience, Catalog 12-0801-85), anti-CD86-APC (Biolegend, Catalog 105012), anti-MHC Class II-PE (eBioscience, Catalog 12-5321-83), mouse IgG1 kappa isotype control APC (Catalog 17-4714-41), anti-OVA257-264 (SIINFEKL) peptide bound to H-2Kb (eBioscience, Catalog 17-5743-82), anti-melanoma gp100 [EP4863(2)] (Abcam, Catalog 137078), and Dylight 488 goat anti-rabbit IgG [H+L] (Multisciences, Catalog 70-GAR4882). To analyze T cell subsets in the spleen and tumor of the treated mice, single cell suspensions prepared from these samples were examined by flow cytometry. Following primary antibodies were used:

APC anti-mouse CD3 (Biolegend, Catalog 100236), Percp/Cyanine 5.5 anti-mouse CD4 (Biolegend, Catalog 100434), anti-mouse CD4 (eBioscience, Catalog 11-0041-85), PE anti-mouse CD8a (Biolegend, Catalog 100707), anti-Mo/Rt Ki67 (eBioscience, Catalog 11-5698-82), PE/Cyanine7 anti-mouse IFN-γ (Biolegend, Catalog 505826), anti-CD8 (mouse) mAb-FITC (MBL, Catalog D271-4), T-select H-2kb OVA Tetramer-SIINFEKL-APC (MBL, Catalog TS-5001-2C), H-2Db gp100 Tetramer-EGSRNQDWL-PE (MBL, Catalog TS-M546-1), anti-Mo CD16/32 (eBioscience, Catalog 14-0161-81), PerCP/Cyanine 5.5 anti-mouse CD3 (Biolegend, Catalog 100218), PE-Cy7 anti-mouse CD25 (BD, Catalog 552880), anti-CD25-Alexa Fluor 700 (eBioscience, Catalog 56-0251-82), anti-Foxp3-PE (eBioscience, Catalog 12-4771-82), and PE/Cyanine7 anti-mouse CD45 (Biolegend, Catalog 103114). In addition, permeabilization buffer 10 × (Invitrogen, Catalog 00-8333-56), fixation/perm diluent (Invitrogen, Catalog 00-5223-56), and fixation/permeabilization concentrate (Invitrogen, Catalog 00-5123-43) were used. Data were acquired on a CytoFLEX flow cytometry and analyzed using CytExpert (Beckman Coulter) and FlowJo (TreeStar) software.

Statistical analysis.

The data are shown as means ± standard deviation. Statistical analysis was performed using Excel (Microsoft), Prism 8.0 (GraphPad) and origin 2015. Data was analyzed via One-way ANOVA with Dunnett's post-hoc test. Survival rates were analyzed using the log-rank test.

References

1. Qiangzhe Zhang et al. Cellular nanosponges inhibit SARS-CoV-2 infectivity. *Nano Lett* **20**, 5570-5574 (2020).

2. Audrey Arrighi et al. Development of PLGA microparticles with high immunoglobulin G-loaded levels and sustained-release properties obtained by spray-drying a water-in-oil emulsion. *Int J Pharm* **566**, 291-298 (2019).

3. Hu CM et al. Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 10980-10985 (2011).

Table S1. Weight ratios of cell membrane/PLGA nanoparticles, hydrodynamic particle sizes and zeta potentials of CCNPs, DCNs, and RBCNs measured by DLS.

	Membrane : PLGA (w/w)	Size (nm)	Zeta potential
CCNPs	2	139.1 ± 6.1	-31.6 ± 1.3
DCNs	2	100.4 ± 2.2	-13.7 ± 0.8
RBCNs	2	91.2 ± 1.1	-11.2 ± 1.1

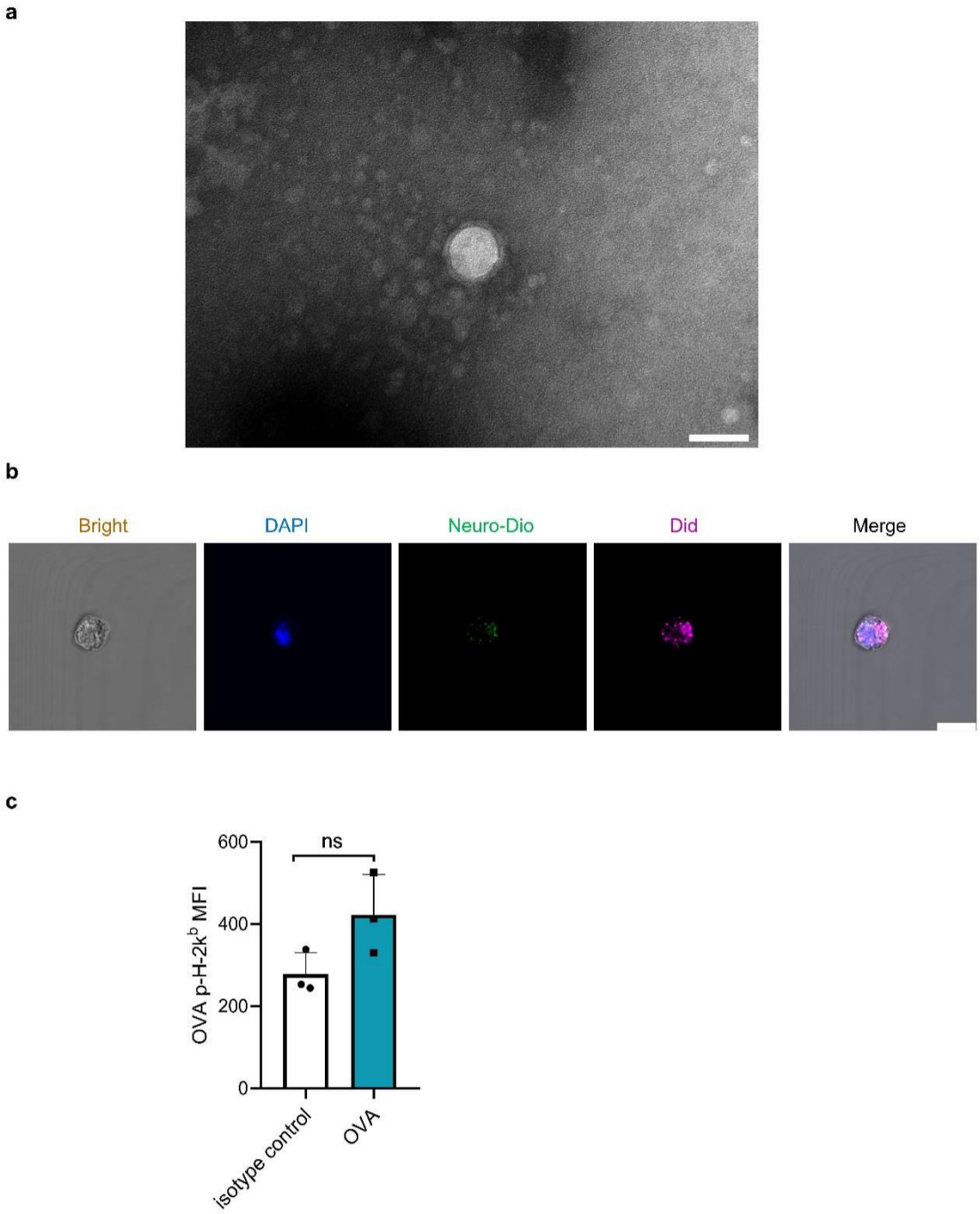

Figure S1. (a) A typical TEM image of CCNPs negatively stained with uranyl acetate. Scale bar: 100 nm. (b) Confocal images of BMDCs after incubation with cancer cell membrane-coated PLGA nanoparticles. Cell nucleus was stained with DAPI (blue channel). PLGA cores were loaded with DiD (red channel) and their coating membranes were labeled with Neuro-Dio (green channel). Scale bar: 10 μm. (c) Mean fluorescence intensity of BMDCs pulsed with OVA. An amount of 40 μg free OVA was added into 1×10^6 BMDCs, which were incubated at 37 ℃ for 18 hours.

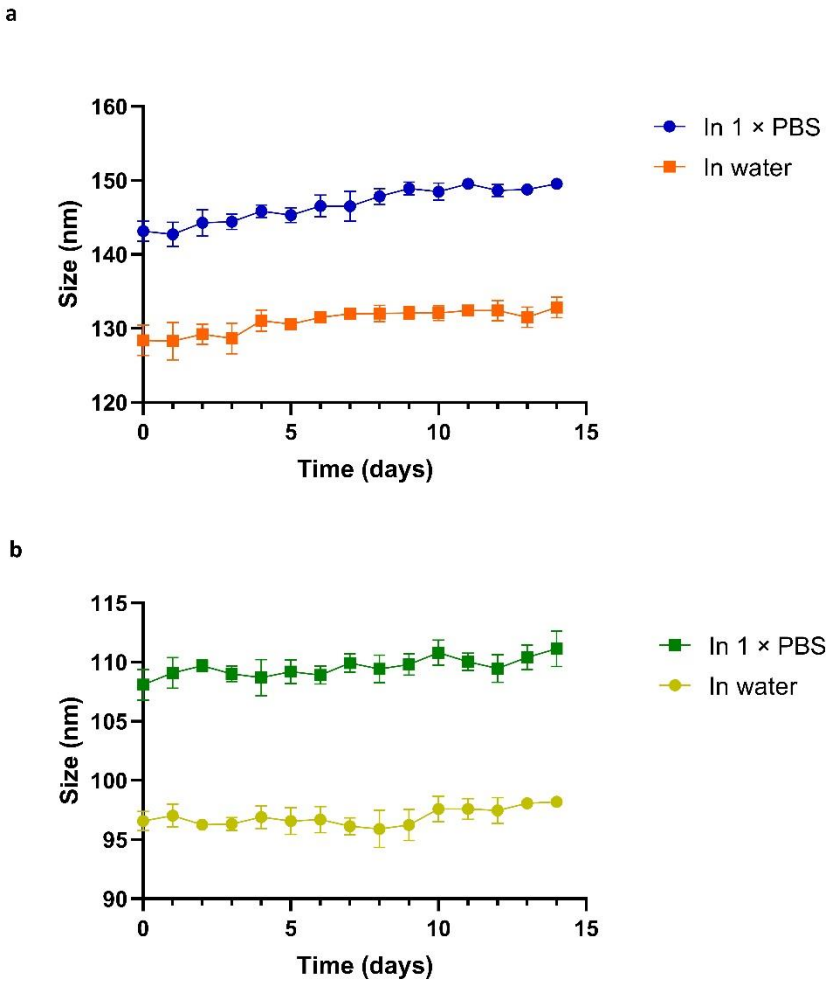

Figure S2. Stabilities of (a) CCNPs and (b) BDCNs in 1 × PBS and pure water determined by DLS (n = 3).

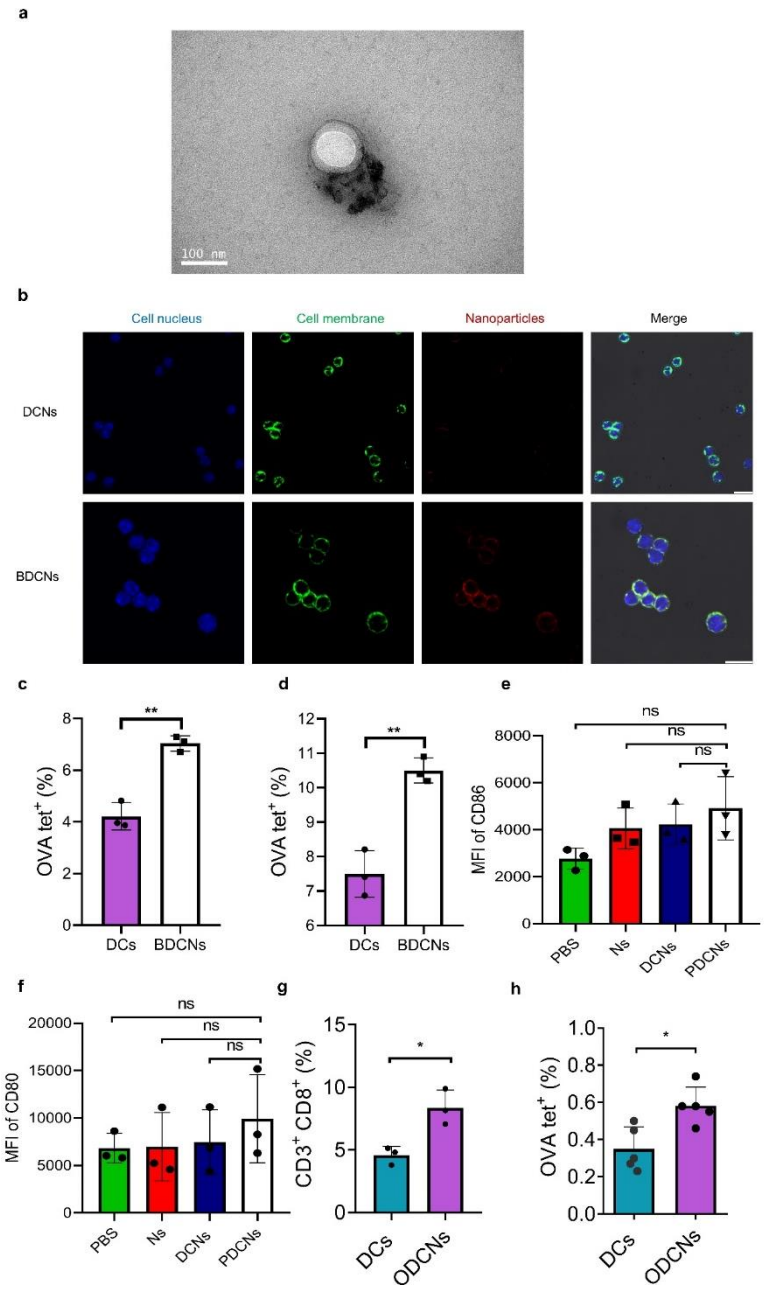

Figure S3. (a) TEM images of BDCNs negatively stained with uranyl acetate. Scale bar: 100 nm. (b) Representative confocal images of primary $CD8^+$ T cells after incubation with DCNs and BDCNs at 4 ℃ for 1-hour, respectively. DCNs indicates PLGA nanoparticles coated with cell membranes extracted from DCs without pre-pulsing with CCNPs. Cell nucleus, cell membrane and PLGA nanoparticles were separately stained with Hochest (blue channel), Neuro-DiO (green channel), and DiD (red channel). Scale bar: 10 μm. Frequencies of OVA tet^+ $CD8^+$ T cells after culturing DCs or BDCNs (c) with 10 mM lactic acid for 3 days and (d) at 4 ℃ for 24 hours. Mean fluorescence intensity of co-stimulatory markers (e) CD86 and (f) CD80 of DCs in the spleen tissue sampled from mice after treatment with 3 doses at day 0, 5, and 10. Frequencies of (g) $CD3^+CD8^+$ and (h) OVA tet^+ in peripheral blood sampled from mice on day 15. Mice were vaccinated with 3 doses of DCs or ODCNs on day 0, 5, and 10, respectively.

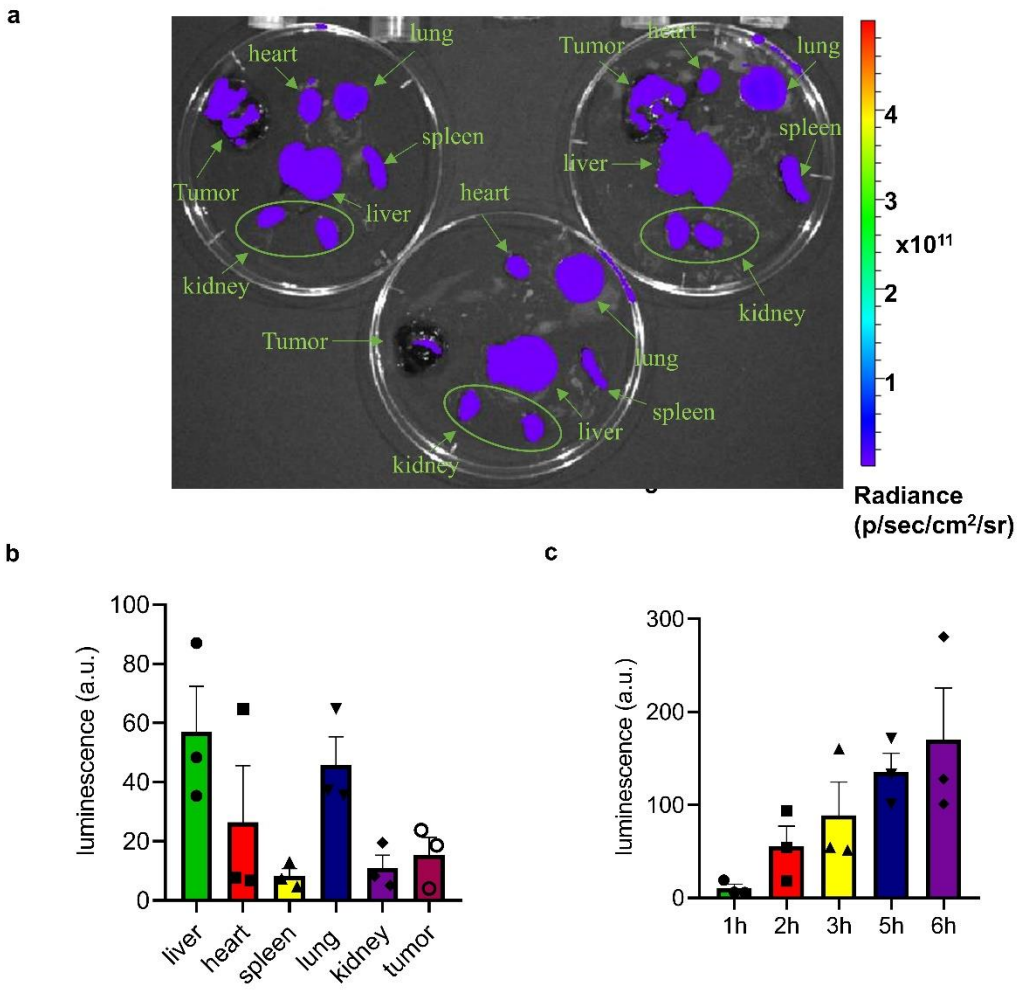

Figure S4. Biodistribution of BDCNs in B16-OVA tumor-bearing mice after intravenous injection of BDCNs. (a) IVIS images of the sampled major organs including liver, spleen, lung, heart, kidney, and tumor 6 hours post-injection. (b) Quantification of the intensity of fluorescence signals from the major organs. (c) Relationship between the intensity of fluorescence signals from tumor and time post-injection. Error bars represent the standard deviation (n = 3).

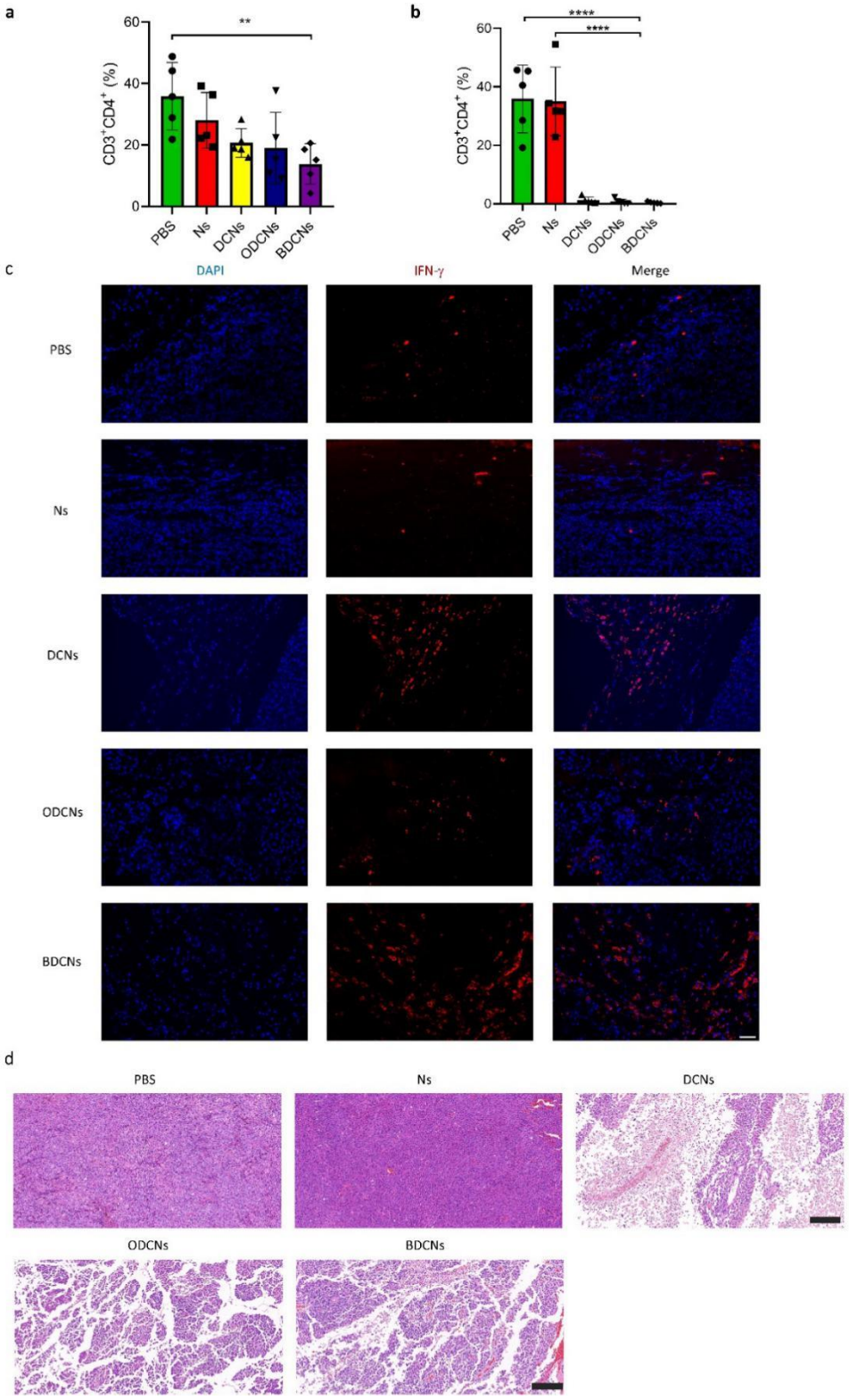

Figure S5. The percentages of $CD3^+CD4^+$ in (a) the spleen and (b) tumor site in the B16-OVA model. (c) IFN-γ (red channel) of the tumor sections. Scale bar: 100 μm. (d) H&E staining of the tumor sections. Scale bar: 100 μm. B16-OVA tumor-bearing mice were intravenously administrated with PBS, Ns, DCNs, ODCNs and BDCNs at predetermined time points and sacrificed after the volume of the tumor in the control group exceeded 2000 mm^3.

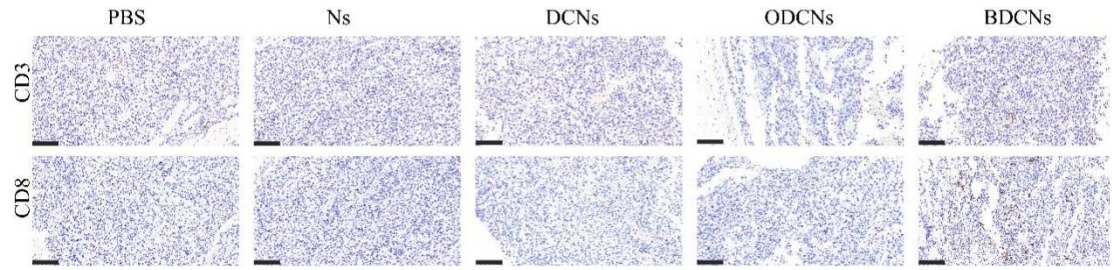

Figure S6. **IHC data in tumor site in the B16-OVA model.** B16-OVA tumor-bearing mice were vaccinated with PBS, Ns, DCNs, ODCNs and BDCNs and sacrificed after the volume of tumor in the control group exceeded 2000 mm^3. Scale bar: 100 μm.

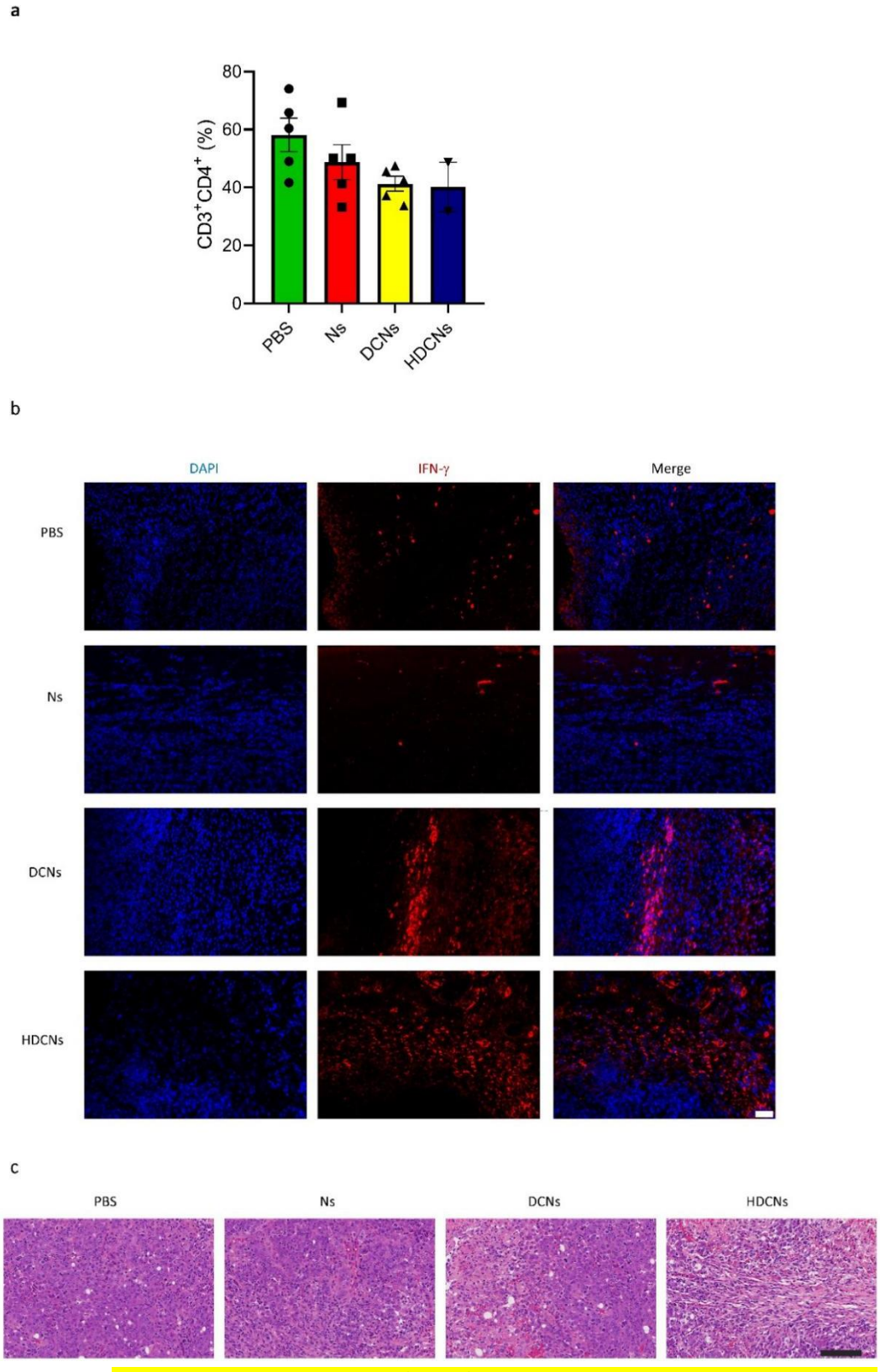

Figure S7. (a) The percentage of $CD3^+CD4^+$ in tumor site in the Hepa 1-6 model. (b) IFN-γ (red channel) and (c) H&E staining of the tumor sections. Scale bar: 100 μm. Hepa 1-6 tumor-bearing mice were intravenously administrated with PBS, Ns, DCNs and HDCNs at predetermined time points and sacrificed after the volume of the tumor in the control group exceeded 2000 mm^3.

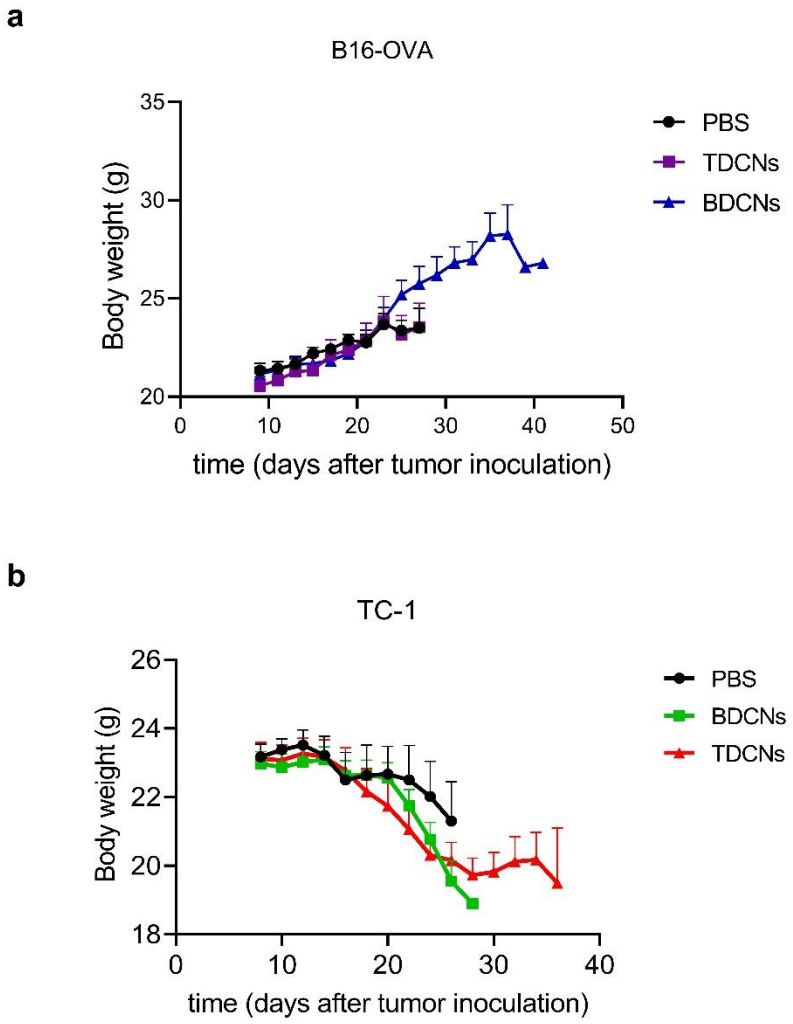

Figure S8. Variation of the bodyweights of B16-OVA and TC-1 tumor-bearing mice after treatment.

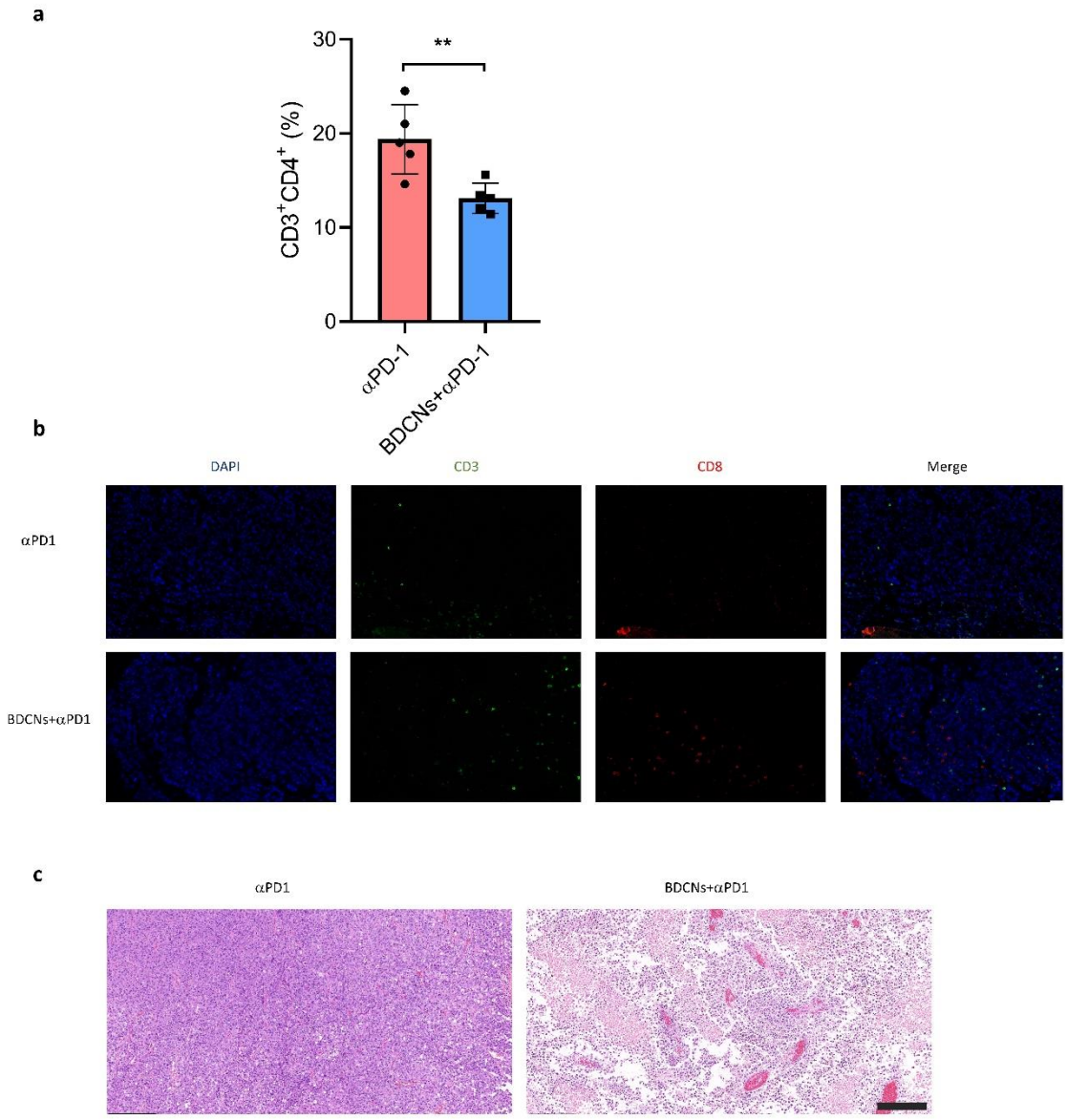

Figure S9. Combination of BDCNs with αPD-1 in B16-OVA tumor-bearing mice. (a) The percentage of $CD3^+CD4^+$ in the spleen. (b) $CD3^+$ T cells (green channel) and $CD8^+$ T cells (red channel) in the tumor sections. Scale bar: 50 μm. (c) H&E staining of the tumor sections. Scale bar: 200 μm.