

# 1 **Supplementary Information**

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## 3 **Bioinspired nanoplatform potentiates sonodynamic immunotherapy by** 4 **remodeling the antioxidant tumor microenvironment and activating** 5 **STING pathway**

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## 1 **Materials and Methods**

2 Hydrogenated Soybean Phosphatidylcholine (HSPC), 1,2-distearoyl-sn-glycero-3-  
3 phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000), Cholesterol were  
4 purchased from RuiXi Biochemical Co., Ltd; Diethyldithiocarbamic acid silver salt (Ag(DDTC), 98%)  
5 was bought from Aladdin Industrial Co., Ltd.; Manganese(II)2,4-pentanedionate, Octadecene (ODE,  
6 95%), Methylene Blue were purchased from Macklin Biochemical Co., Ltd; ROSGreen™ H<sub>2</sub>O<sub>2</sub> probe  
7 was purchased from Maokang Co., Ltd; 2,2,6,6-tetramethylpiperidine (TEMP, 95%), 5,5-dimethyl-1-  
8 pyrroline N-oxide (DMPO) and 2,7-dichlorofluorescein diacetate (DCFH-DA, 97%) were purchased  
9 from Sigma-Aldrich Co., Ltd; The following reagents were procured from Beyotime Biotechnology  
10 Co., Ltd: Singlet Oxygen Sensor Green (SOSG), Mitochondrial Membrane Potential Assay Kit (JC-  
11 1), Reactive Oxygen Species Assay Kit, and the Membrane and Cytosol Protein Extraction Kit. The  
12 FITC Annexin V Apoptosis Detection Kit was sourced from BD Biosciences. All antibodies for flow  
13 cytometry were obtained from BioLegend. All chemicals were of analytical grade and used without  
14 further purification.

### 15 **Determination of Encapsulation Efficiency of Mn<sub>3</sub>O<sub>4</sub> NPs and Ag<sub>2</sub>S QDs in Mn<sub>3</sub>O<sub>4</sub>/QD@LM**

16 The encapsulation efficiencies (EE) of Mn<sub>3</sub>O<sub>4</sub> NPs and Ag<sub>2</sub>S QDs were determined by ICP-MS. After  
17 synthesizing Mn<sub>3</sub>O<sub>4</sub>/QD@LM, 500 μL of the product was digested with concentrated nitric acid-  
18 perchloric acid. Excess acid was evaporated, the solution was fixed to volume with 2% dilute nitric  
19 acid, filtered through a 0.22 μm membrane, and detected by ICP-MS.

20 Based on the detected Mn<sup>2+</sup> and Ag<sup>+</sup> concentrations, the mass of these ions in the 500 μL sample  
21 was calculated, and then converted to the total mass in the entire product volume. Using the relative  
22 molecular masses of Mn<sub>3</sub>O<sub>4</sub> (190.89 g/mol) and Ag<sub>2</sub>S (247.80 g/mol), the total Mn<sup>2+</sup> and Ag<sup>+</sup> masses

1 were converted to the actual mass of encapsulated Mn<sub>3</sub>O<sub>4</sub> NPs and Ag<sub>2</sub>S QDs, respectively. EE was  
2 calculated with the formula:

$$3 \quad EE (\%) = \frac{M_{encap}}{M_{feed}}$$

4  $M_{encap}$  = mass of Mn<sub>3</sub>O<sub>4</sub> NPs or Ag<sub>2</sub>S QDs encapsulated in the liposomes

5  $M_{feed}$  = initial feeding mass of Mn<sub>3</sub>O<sub>4</sub> NPs or Ag<sub>2</sub>S QDs during the synthesis process.

### 6 **Evaluation of the Catalase-like Activity of Mn<sub>3</sub>O<sub>4</sub>/QD@LM at the Cellular Level**

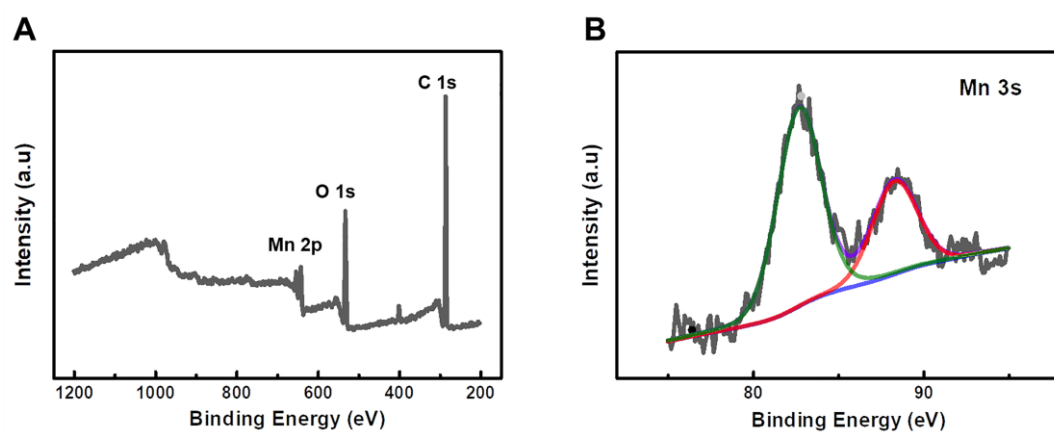
7 The intracellular oxygen levels in CT26 cells were quantitatively assessed using the oxygen-sensitive  
8 fluorescent probe [Ru(dpp)<sub>3</sub>]Cl<sub>2</sub> to evaluate the oxygen-modulating capacity of the nanoparticles under  
9 both normoxic and simulated hypoxic conditions. CT26 cells were seeded in 6-well plates at a density  
10 of  $5 \times 10^5$  cells per well and cultured until reaching 80% confluence. Two experimental conditions  
11 were established. The normoxic group was maintained under standard culture conditions. The hypoxic  
12 group was treated with deferoxamine (DFO; 30  $\mu$ M) and covered with a 3 mm layer of liquid paraffin  
13 to establish a combined chemical-physical hypoxia model.

14 Different types of nanoparticles (Control, QD@LM, Mn<sub>3</sub>O<sub>4</sub>/QD@LM) were added to their  
15 respective wells, and the cells were incubated for another 6 h. After treatment, the culture medium was  
16 removed, and the cells were washed three times with pre-cooled PBS. Then, 2 mL of serum-free  
17 medium containing 30  $\mu$ M [Ru(dpp)<sub>3</sub>]Cl<sub>2</sub> probe was added to each well, followed by incubation in the  
18 dark for 30 min. After removing the probe-containing medium, the cells were washed three times with  
19 pre-cooled PBS. Fluorescence images were acquired using a fluorescence microscope under consistent  
20 exposure settings, with multiple randomly selected fields captured per well.

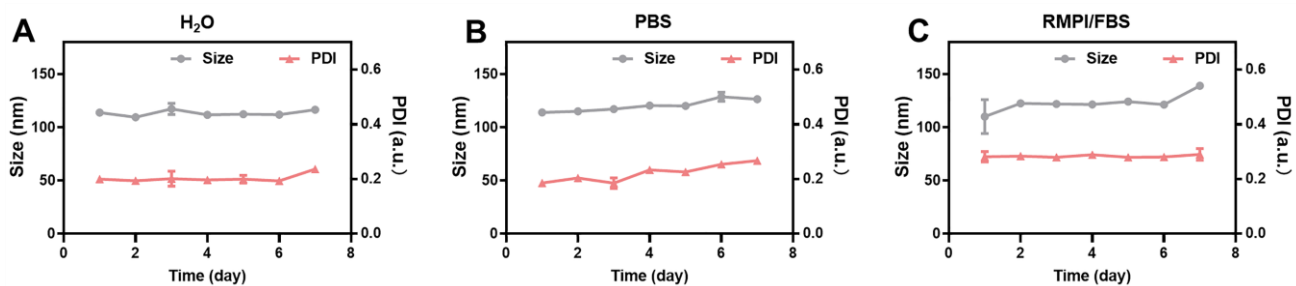
### 21 **Evaluation of the Glutathione Peroxidase-like Activity of Mn<sub>3</sub>O<sub>4</sub>/QD@LM at the Cellular Level**

1 The intracellular GSH level in CT26 cells was measured using a commercial GSH quantification kit.  
2 CT26 cells were seeded in 6-well plates at a density of  $5 \times 10^5$  cells per well and cultured until they  
3 reached 80% confluence. The cells were then treated with different types of nanoparticles (Control,  
4 QD@LM,  $Mn_3O_4$ /QD@LM) for 6 h. After incubation, the culture medium was removed, and the cells  
5 were collected and disrupted by ultrasonication. GSH quantification was performed according to the  
6 manufacturer's instructions. Briefly, 100  $\mu$ L of cell lysate was mixed with an equal volume of Reagent  
7 1, and the mixture was centrifuged at 3500 rpm for 10 min. The supernatant was collected for analysis.  
8 The GSH standard provided in the kit was diluted to various concentrations. Then, 100  $\mu$ L of  
9 precipitant was added to the blank well, 100  $\mu$ L of standard solution was added to the standard wells,  
10 and 100  $\mu$ L of the supernatant was added to the sample wells. Subsequently, 25  $\mu$ L of reagent 2 and  
11 100  $\mu$ L of reagent 3 were added to each well. After incubating in the dark for 10 min, the absorbance  
12 at 405 nm was measured.

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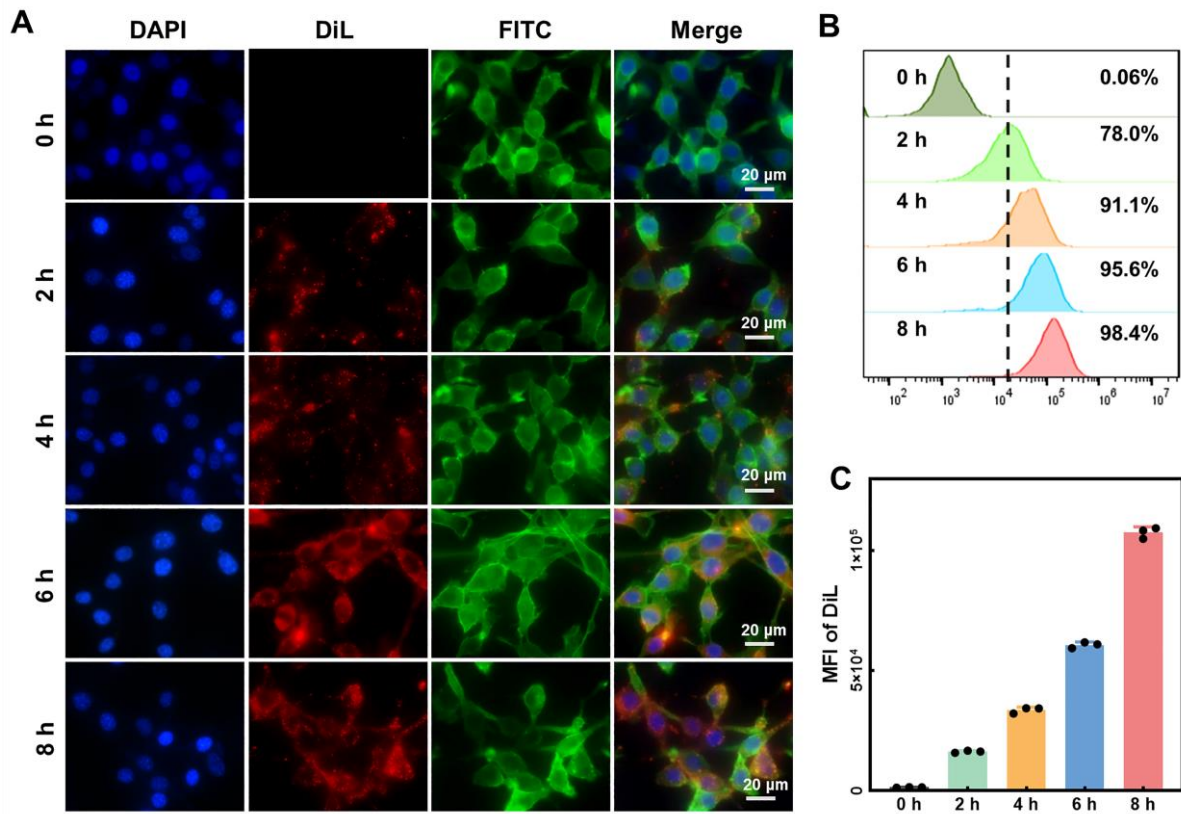
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2 **Figure S1. XPS spectra of Mn<sub>3</sub>O<sub>4</sub> NPs.** (A) XPS spectra for the C 1s peak of Mn<sub>3</sub>O<sub>4</sub> NPs. (B) XPS  
3 spectra for the Mn 3s peak of Mn<sub>3</sub>O<sub>4</sub> NPs.  
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 2 **Figure S2 Stability of Mn<sub>3</sub>O<sub>4</sub>/QD@LM.** Changes in hydrated particle size and polydispersity index  
 3 (PDI) over time of Mn<sub>3</sub>O<sub>4</sub>/QD@LM in water (A), PBS (B), and RPMI 1640 medium with 10% FBS.  
 4 (n = 3). Data are presented as mean ± SD

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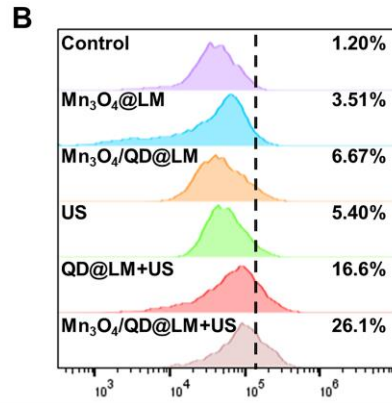
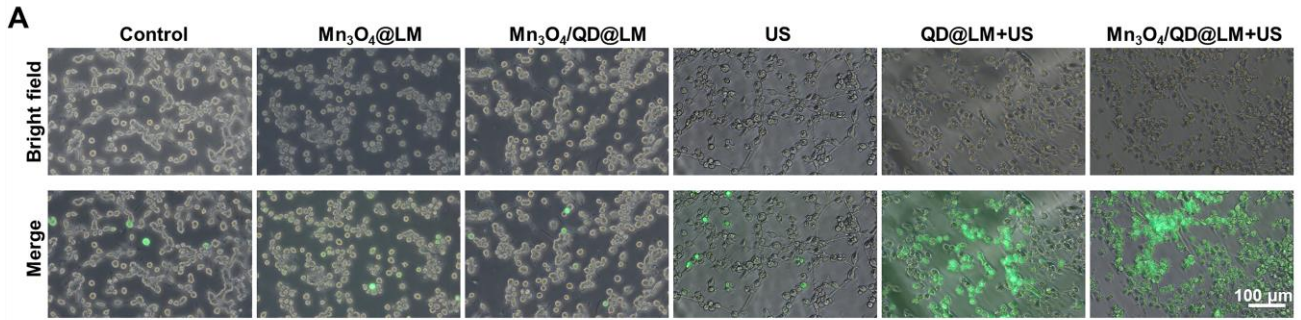


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2 **Figure S3. Uptake of DiL@LM by CT26 cells after different incubation times.** (A) Fluorescence  
 3 images of CT26 cells after 0 h, 2 h, 4 h, and 8 h of co-incubation with DiL@LM. (B) Flow cytometric  
 4 quantification of DiL<sup>+</sup> CT26 cells after incubation with DiL@LM for different durations. (C) Mean  
 5 fluorescence intensity of CT26 cells after incubation with DiL@LM for different times. (n = 3). Data  
 6 are presented as mean ± SD

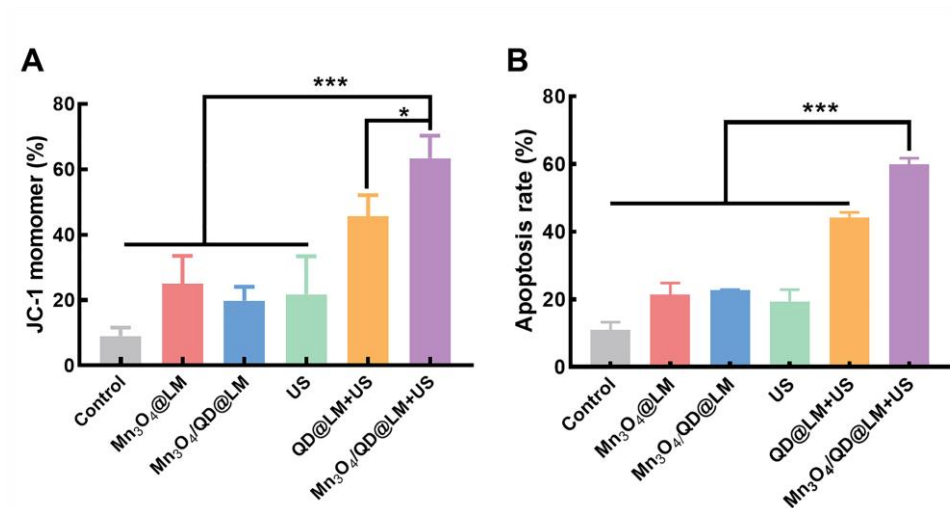
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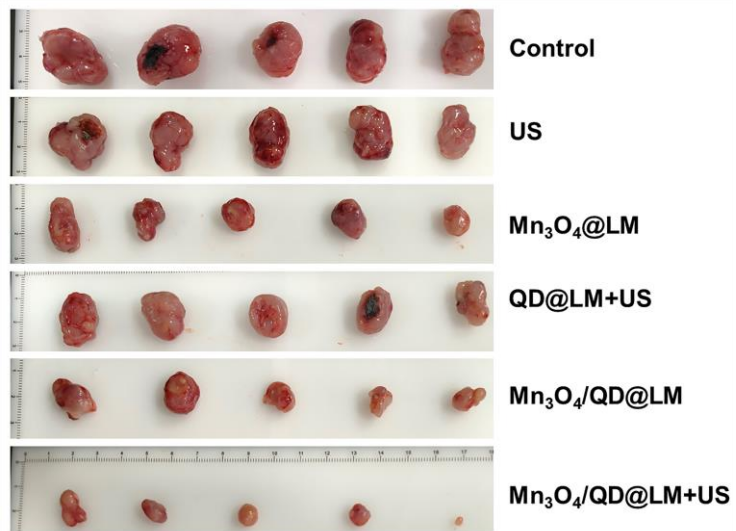
**Figure S4.** (A) Brightfield and fluorescence merge images of DCFH-DA-stained CT26 cells following different treatments. (B) Flow cytometric analysis of ROS levels in CT26 cells after different treatments.



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2 **Figure S5. The relative quantification results of MMP assay, and apoptosis assay.** (A) Quantitative  
 3 analysis of MMP in CT26 cells measured by flow cytometry. (n = 3) (B) Quantitative analysis of  
 4 apoptosis in CT26 cells measured by flow cytometry. (n = 3) Data are presented as mean ± SD.  
 5 Statistical comparisons were performed by one-way ANOVA. Statistically significant difference: \**p* <  
 6 0.05, \*\**p* < 0.01, and \*\*\**p* < 0.001.

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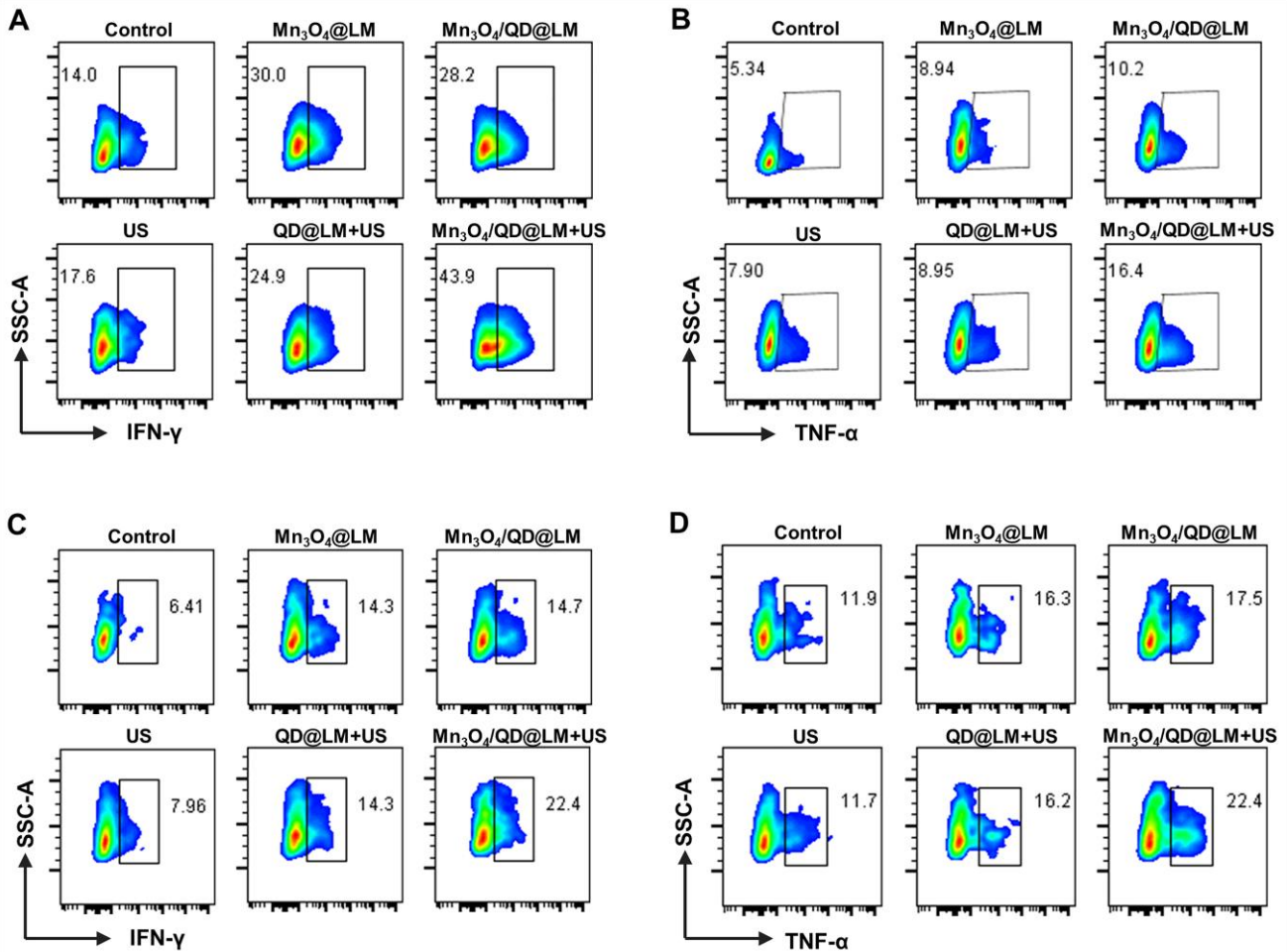


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2 **Figure S6.** Photograph of excised tumors from mice in different treatment groups.

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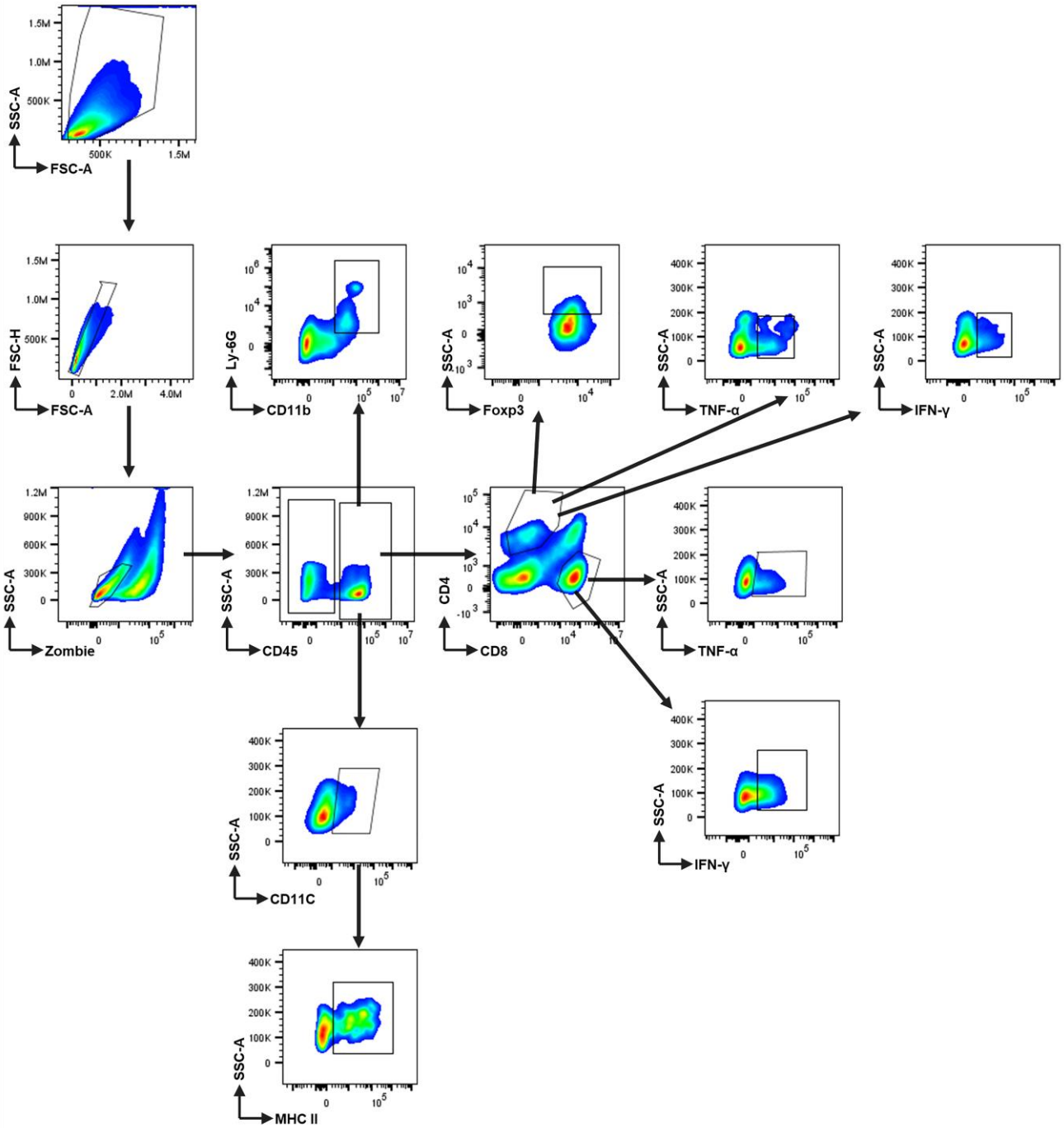


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2 **Figure S7. Cytokine levels in tumor tissues of mice in different treatment groups (A)**  
 3 **Representative flow cytometry scatter plots of CD8<sup>+</sup> IFN- $\gamma$ <sup>+</sup> T cells in tumor tissues from different**  
 4 **treatment groups. (B) Representative flow cytometry scatter plots of CD8<sup>+</sup>TNF- $\alpha$ <sup>+</sup> T cells in tumor**  
 5 **tissues from different treatment groups. (C) Representative flow cytometry scatter plots of CD4<sup>+</sup> IFN-**  
 6  **$\gamma$ <sup>+</sup> T cells in tumor tissues from different treatment groups. (D) Representative flow cytometry scatter**  
 7 **plots of CD4<sup>+</sup>TNF- $\alpha$ <sup>+</sup> T cells in tumor tissues from different treatment groups.**

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**Figure S8. Gating strategy for flow cytometric analysis of mouse tumor tissues.**