Supporting Information for

Manganese-Phenolic Networks Platform Amplifying STING Activation to Potentiate MRI Guided Cancer Chemo-/Chemodynamic/Immune Therapy

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1. Methods and materials

Preparation of PD nanoparticles: Prior to the fabrication of DOX-loaded PEG-PLGA nanoparticles,

we first prepared hydrophobic Doxorubicin. Briefly, 300 μL triethylamine was gradually mixed with an aqueous solution of 10 mL DOX•HCl (10 mg/mL) and stirred overnight. The resultant solution was collected through centrifugation (9000 rpm, 10 min) and rinsed 5 times with ultrapure water. The sediment was freeze-dried to obtain hydrophobic DOX. Next, PEG-PLGA-DOX (PD) nanocomplexes were prepared with the nano precipitation method. The resultant DOX was used to dissolve in DMSO (10 mg/mL) whereas PEG_{5k}-$PLGA_{11.7k}$ was used to dissolve in DMSO (10 mg/ml). The mixture of 100 μL DOX and 1 mL PEG_{5k}-$PLGA_{11.7k}$ in DMSO was added gradually into 5 mL ultrapure water and stirred for 1 h. This mixture was dialyzed overnight to obtain 8 mL solution. The solution was then centrifuged (5000 rpm, 5 min) to form free DOX.

Preparation of TMPD Nanocomplexes: The TMPD nanocomplexes were prepared using a simple one-step assembly method. 40 μL of the tannic acid (40 mg/mL) solution and 20 μL of $MnCl_2 \cdot 4H_2O$ (19.8 mg/mL) were added into 8 ml PD nanocomplexes solution while vortexing. The pH of the solution was adjusted to 8.0 by addition of 1 mL Tris-HCl buffer solution (10 mM) while vortexing.

Detection of ·OH: The $NaHCO_3$ buffer (25 mM) with H_2O_2 (8.0 mM), MB (10 μg/mL), and TMPD ($[Mn^{2+}]$ = 0.5 mM) were incubated to evaluate ·OH generation. The mixture solution was stayed for 30 min at 37°C, then recorded the UV−vis absorbance spectrum of the solutions and the change in absorbance of MB at 665 nm. TMPD nanoparticles were added in DMEM medium and measured nanoparticle size for continuous 7 days.

Cellular uptake: 1×10^5 4T1 cells per well were planted in 24-well plates for 24 h. Then the nanoparticle containing PD, TMP and TMPD ([DOX] = 10 μg/mL) was used to replace the medium and further incubated for 1, 2, 4, 6 h, respectively. Subsequently, 4T1 cells were analyzed by flow cytometry (BD Accuri C6Plus, USA). 5×10^4 4T1 cells were cultured on confocal dishes. After

treating with PD, TMP and TMPD for 4 h, the cells were washed out and stained with DAPI (1 μg/mL). Finally, the cells were analyzed with confocal laser scanning microscope (CLSM) (ZEISS LSM 880 with Airyscan, Germany).

MTT assay: 1×10^4 4T1 cells per well were planted in 96-well plates for 24 h, and administrated with a gradient concentration of different nanoparticles including PD, TMP, TMPD ([DOX] = 30, 15, 7.5, 3.75, 1.825 μg/mL，[Mn] = 7.5, 3.75, 1.88, 0.94, 0.47 μg/mL) for 24 h. Subsequently, the drugs were cleaned and then the cells were administrated with medium containing 10% CCK8 reagent for 1 h. Finally, the solution's absorption was recorded at 450 nm.

Apoptosis assay: 1×10^5 4T1 cells per well were cultured in 24-well plates and treated with PD, TMP, TMPD ([DOX] = 10 μg/mL, $[Mn^{2+}]$ = 2.5 μg/mL) for 24 h. Then the cells were examined with Annexin V-APC/DAPI Apoptosis kit, and analyzed by flow cytometry (FACSCelesta, BD).

Intracellular ROS detection: 5×10^4 4T1 cells were planted in 24-well plates for the night. The cells were administrated with TMPD ([DOX] = 10 μg/mL, $[Mn^{2+}]$ = 2.5 μg/mL) while TMP and PD served as the control treatments at same concentrations for 4 h. After remove the drugs, the cells were dyed with DAPI (1 μg/mL) for 10 minutes and then washed. After that, they were dyed with DCFH-DA (10 μM) for 20 minutes and washed. The generated DCF was captured under a Fluorescence microscope (Olympus IX73, Japan) based on the green fluorescence. The same method was used to quantify the fluorescence of cells by flow cytometry.

Verification of ICD: 4T1 cells were cultured in 24-well plates for the night and administrated with PD, TMP and TMPD nanoparticles for 24 h. Subsequently, the cells were collected and stained with anti-Calreticulin antibody for 45 min and examined with flow cytometry. The supernatant was collected for the examination of HMGB-1 secretion utilizing a HMGB-1 ELISA kit. 5×10^4 4T1 cells

were cultured on confocal dishes and treated with PBS, PD, TMP and TMPD nanoparticles for 4 h. The cells were washed out and dyed with DAPI (1 μg/mL) for 10 min and anti-Calreticulin antibody for 45 min. Finally, the cells were captured with CLSM. 4T1 cells were cultured in 24-well plates for the night and administrated with PD, TMP and TMPD nanoparticles for 24 h. Then the cells stained with ds DNA Marker (HYB331-01) and DAPI to verify that ds DNA leaked into cytoplasm.

BMDCs maturation in vitro *and secretion of IFN-β*: Bone marrow derived dendritic cells (BMDCs) were separated from femurs and tibias of 6-weeks Balb/c mice. The cells were planted in 24-well plates involving 1 mL medium with 10% inactivated FBS, 10 ng/mL IL-4, 20 ng/mL GM-CSF, and 1% penicillin-streptomycin per well. The medium was half-changed each 2 days. Meanwhile, 5×10^5 4T1 cells were cultured in 12-well plates overnight. The cells were treated with PD, TMP and TMPD nanoparticles for 12 h and then replaced with fresh medium to treat for 12 h. Subsequently, the supernatant of tumor cells was used to co-cultured with BMDCs on day 6. One day later, the BMDCs were dyed with antibodies Including DAPI, anti-Percp-Cy5.5-CD3, anti-FITC-CD80 and anti-APC/Cy7-CD86 antibodies for flow cytometry analysis. The supernatant was used for measurement of IFN-*β* concentration by ELISA kits.

Western blotting: 4T1 cells treated with PBS, PD, TMP and TMPD formulations for 24 h, then the supernatant was raised and treated with BMDCs for 4 h. Then BMDCs were obtained and treated with the radioimmunoprecipitation assay (RIPA) buffer to lyse the cells for 30 min. Subsequently, the cell lysate was centrifuged in the presence of phosphatase and protease inhibitor cocktail at 12000 rpm for 20 min. The supernatant was then collected and subjected processed with the Pierce BCA Protein Assay Kit to quantify the concentration of proteins. The samples were separated from SDS-PAGE gel transferred on the nitro cellulose membrane. After that, the samples were blocked and

incubated with TBK1/NAK (D1B4) Rabbit mAb, IRF-3 (D83B9) Rabbit mAb, phospho-TBK1/NAK (Ser172) XP Rabbit mAb and phospho-IRF3 (S396) Rabbit mAb overnight at 4℃. This was treated with HRP-conjugated goat anti-rabbit secondary antibody, then administrated with ECL Substrate. The results were processed using ImageJ (GE Amersham Imager 600, USA).

Determination of in vivo *antitumor efficacy:* 1×10^6 4T1 cells were orthotopically implanted on the female Balb/c mice's mammary fat pad (6 weeks old). These mice were divided randomly into 4 groups and administrated with PD, TMP, TMPD (4 mg/kg DOX, 1 mg/kg Mn) and PBS (n = 6 per group) when tumors grew to ~ 100 mm^3. Tumor volume and mice's body weight were measured every two days and then calculated by following equation: volume = length × $width^2$ × 0.5. On the 22^{nd} day, mice were sacrificed to collect tumors and main organs for flow cytometric analysis and H&E staining. The harvested major organs and tumor were steeped in 4% paraformaldehyde and imbedded in paraffin. Subsequently, the paraffins were sliced and stained with TdT-mediated dUTP Nick-End Labeling (TUNEL) fluorescence and hematoxylin and eosin (H&E). In addition, sera were collected for blood biochemical testing.

The therapeutic effect of TMPD combined with aCTLA-4 was also evaluated. The 4T1 tumor was orthotopically implanted for 10 days. Then mice were separated into 4 groups randomly and administered with PBS, TMPD, aCTLA-4, and the combination of TMPD and aCTLA-4. On the 18^{th} day, the tumors were harvested for further flow cytometric analysis. Meanwhile, the mice lungs were harvested for H&E staining assay.

Flow cytometric analysis: To examine DCs maturation, we harvested the tumor draining lymph nodes (TDLNs) and ground them to obtain lymph cells. These cells were dyed with anti CD45-BV510, anti CD3-PE, anti CD86-APC/Cy7 antibodies, then subjected to flow cytometric analysis. To examine

$CD8^+$T cells infiltration level, the extracted tumors were ground and digested with a digestion solution (40 μg/mL DNase I, collagenase type IV, and 40 μg/mL hyaluronidase). They were treated with 40% Percoll medium to obtain immune cells through discontinuous density gradient centrifugal method. The single immune cells were stained with anti CD45-BV510, anti CD3-FITC, anti CD8-PE and analyzed by flow cytometry. To analyze the frequency of Treg cells, lymphocytes in tumor were dyed with anti CD45-BV510, anti CD3-FITC, anti APC/Cy7-CD4, and anti Foxp3-BV421 antibodies. The MDSCs ($CD45^+CD11b^+Gr\text{-}1^+$) in tumor tissue cell suspensions were dyed with anti CD45-BV510, anti CD11b-BV421, and anti Gr-1-FITC antibodies, and subjected to flow cytometric analysis. To analyze T_{EM} cells' frequency in spleen, lymphocytes were dyed with anti CD45-BV510, anti CD3-BV421, anti CD8-PE, and anti CD62L-FITC, anti CD44-APC antibodies according to the manufacturer's protocols and analyzed by flow cytometry (BD LSRFortessa instruments, BD Biosciences).

T_1-weighted MR relaxometry: Different concentrations of TMPD solution was added to the EP tubes. The 3.0 T MRI scanner (Verio, Siemens, Germany) was used for the T_1 mapping MR scan. The solution was scanned as the following parameters: TE: 12 ms, TR: 600 ms, slice thickness: 3 mm, flip angle: 3°, 18° to obtain the T_1 relaxivity.

In vivo *MR imaging:* The 3.0 T MRI scanner (Verio, Siemens, Germany) equipped with a soft coil was used to examine 4T1-bearing mice (n = 3). T_1-weighted images were scanned before and after intravenous administration of TMPD solutions with a dose of 1.5 mg/kg Mn^{2+} of mice body weight. Parameters used in the measurements were: repetition time (TR)/echo time (TE), 600/12 ms; matrix, 256 × 256; slice thickness, 1.5 mm; field of view, 50 mm. After that, we analyzed the T_1-weighted images with the built-in workstation. Finally, the signals collected from upper limb muscle and tumor

site which performed from the same coronal muscle sections were used for statistical analysis.

Statistical Analysis: Data are expressed by mean ± standard deviation. Student's t-test (two-tailed) was utilized to compare two groups' mean values. whereas one-way ANOVA with Tukey's multiple comparisons was used to compare multiple groups. Statistically significant levels were considered as follows; *n.s.*: no significant difference, $P > 0.05$, ⊠$P < 0.05$, ⊠⊠$P < 0.01$, ⊠⊠⊠$P < 0.001$, and ⊠⊠⊠⊠$P < 0.0001$, GraphPad Prism v8.03.10 (GraphPad Software, San Diego, CA) was used to calculate P values.

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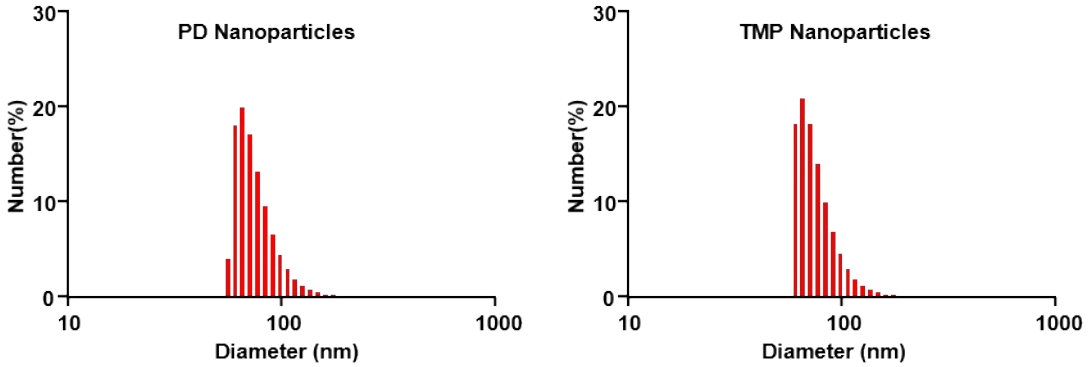

Figure S1. Size distribution s of PD and TMP.

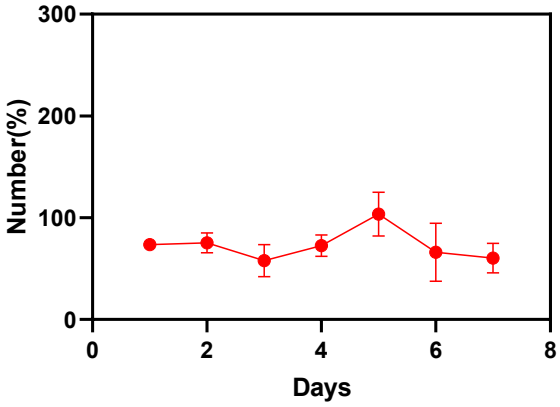

Figure S2. The stability of TMPD nanoparticles in DMEM medium for 7 days

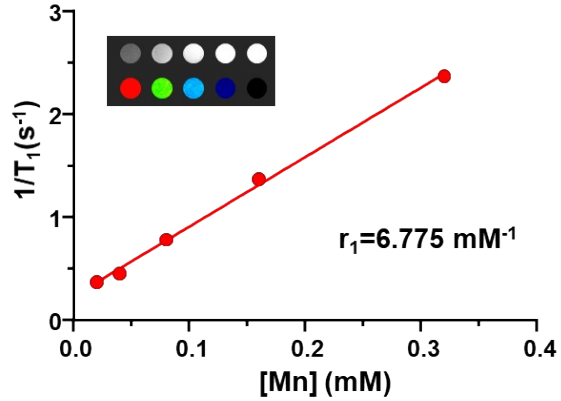

Figure S3. In *vit*ro T_1-weighted MR images and T_1-map images of TMPD.

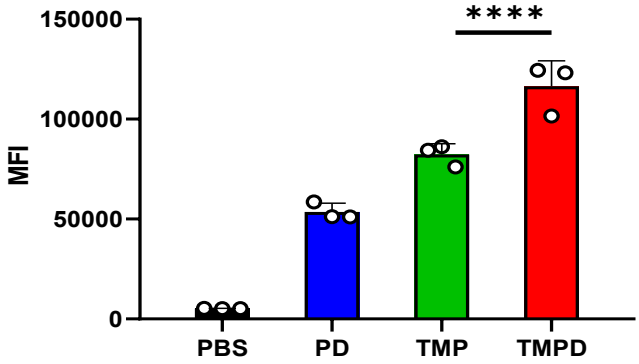

Figure S4. ROS generation analysed via flow cytometry.

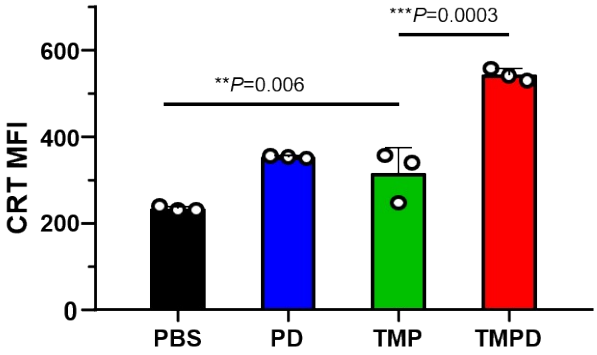

Figure S5. Corresponding quantitative CRT expression measured by flow cytometry (n=3).

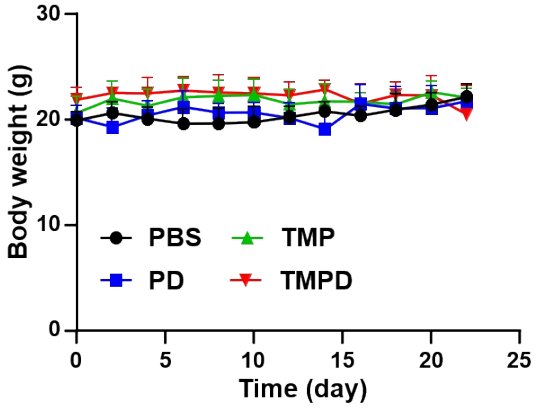

Figure S6. Body weight of mice in different groups.

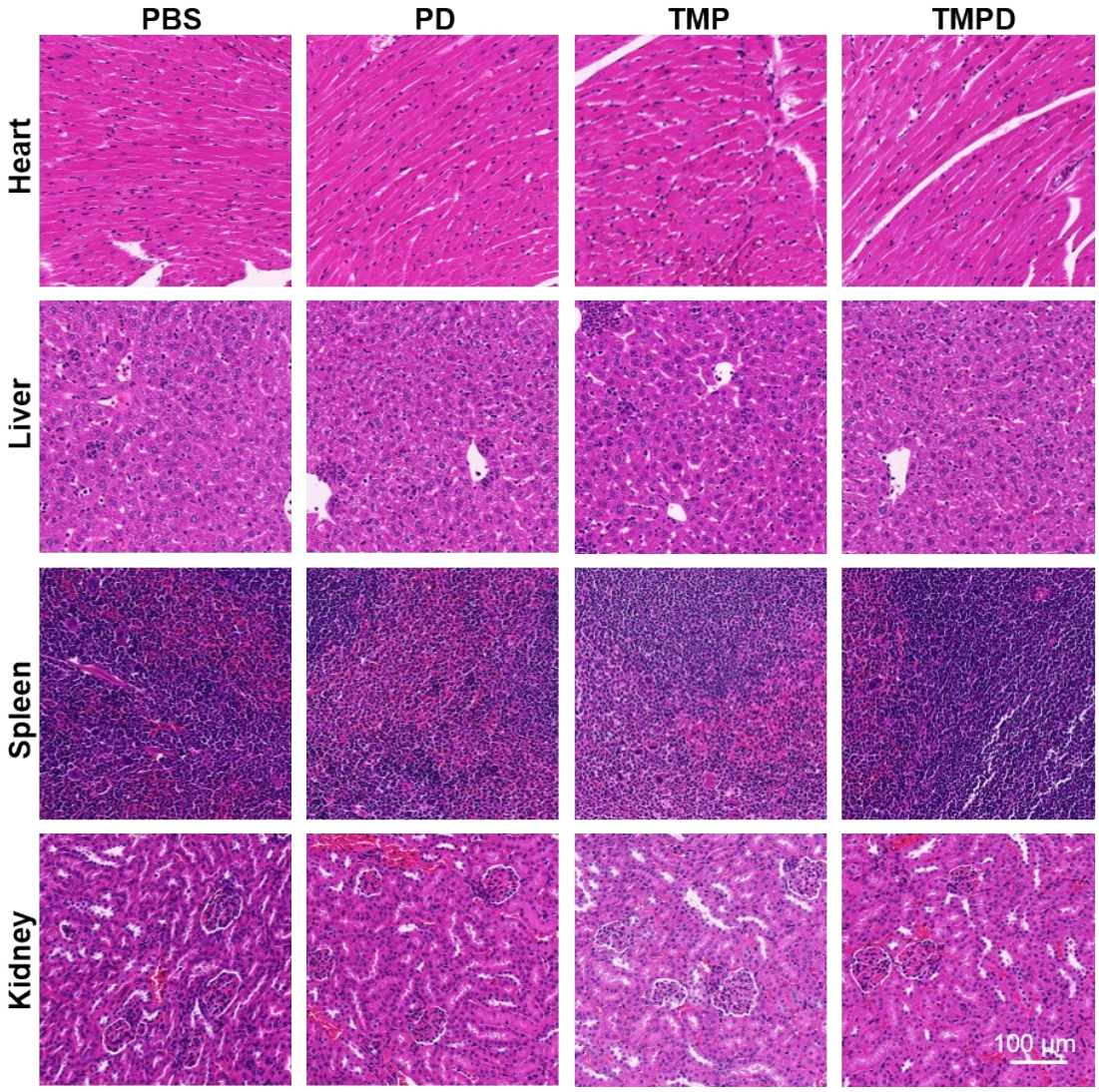

Figure S7. H&E staining of main organs in different groups.

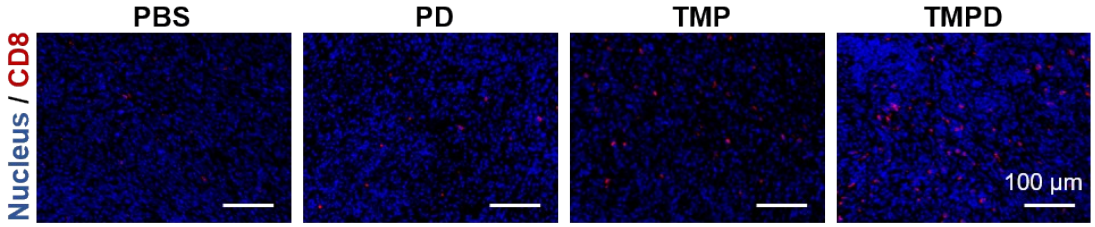

Figure S8. $CD8^+$ fluorescence staining of 4T1 tumor tissues from different treatment groups.

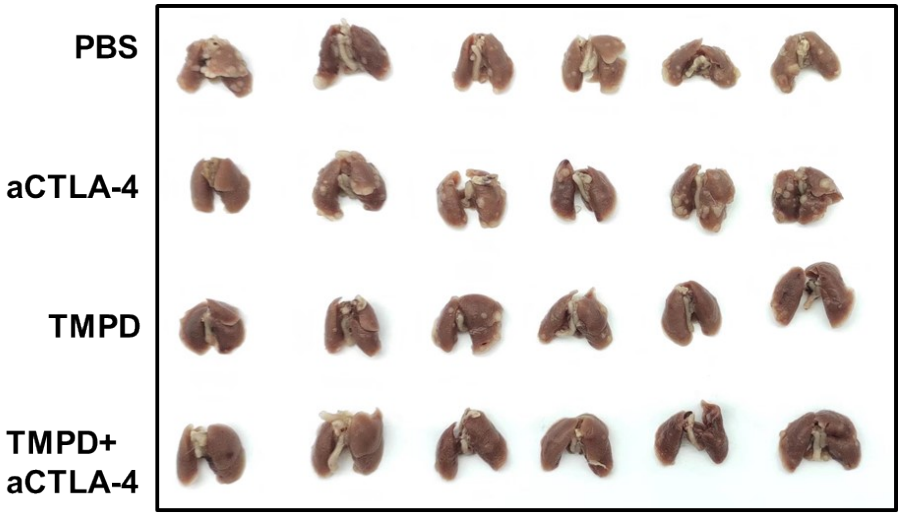

Figure S9. The photograph of lung nodules in different groups (n=6 per group).

	PD	TMP	TMPD
DOX concentration (μg/mL)	17.21	28.40	6.77

Table S1. The half maximal inhibitory concentration (IC50) values of DOX in different groups.

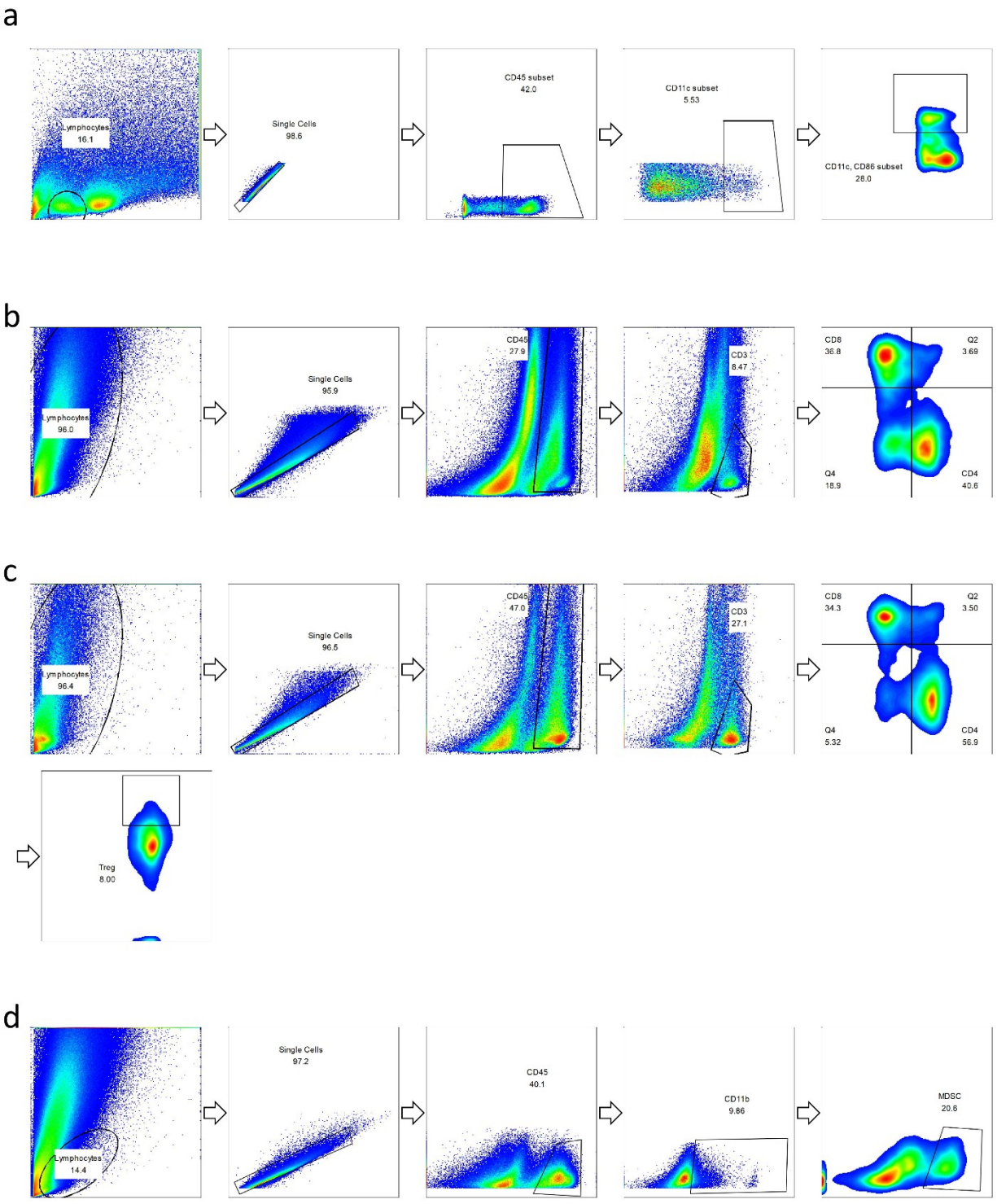

Figure S10 : Gating strategy in Figure 6