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Supporting Information

Amplification of Oxidative Stress via Intracellular ROS Production and Antioxidant Consumption by Two Natural Drugs Encapsulated Nanoagents for Efficient Anticancer Therapy

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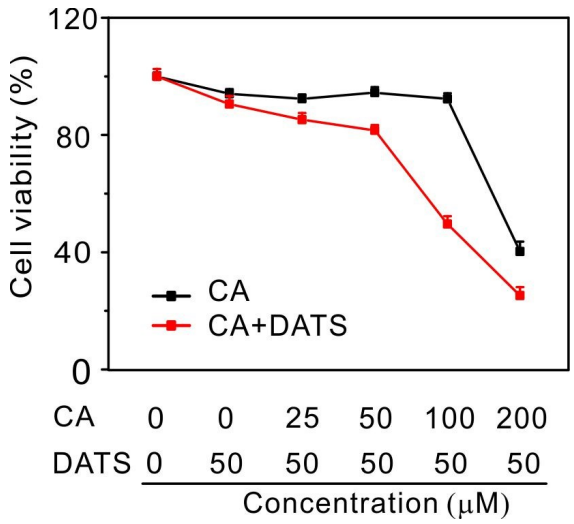

Figure S1. The cytotoxicity of CA, DATS, and CA+DATS on MCF-7 cells after 48 h incubation. The concentration of DATS was fixed at 50 μM, and that of CA was varied in a range of 0-200 μM. The combination of CA at 100 μM and DATS at 50 μM showed the best effect over killing cancer cells (n = 6).

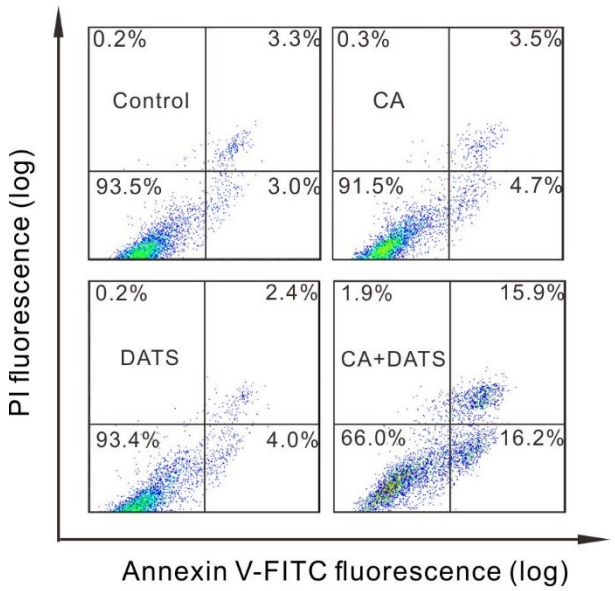

Figure S2. Apoptosis of MCF-7 cells treated with CA (100 μM), DATS (50 μM), and their combination after 24 h incubation. The cells were stained by Annexin V-FITC and PI and analyzed by flow cytometry.

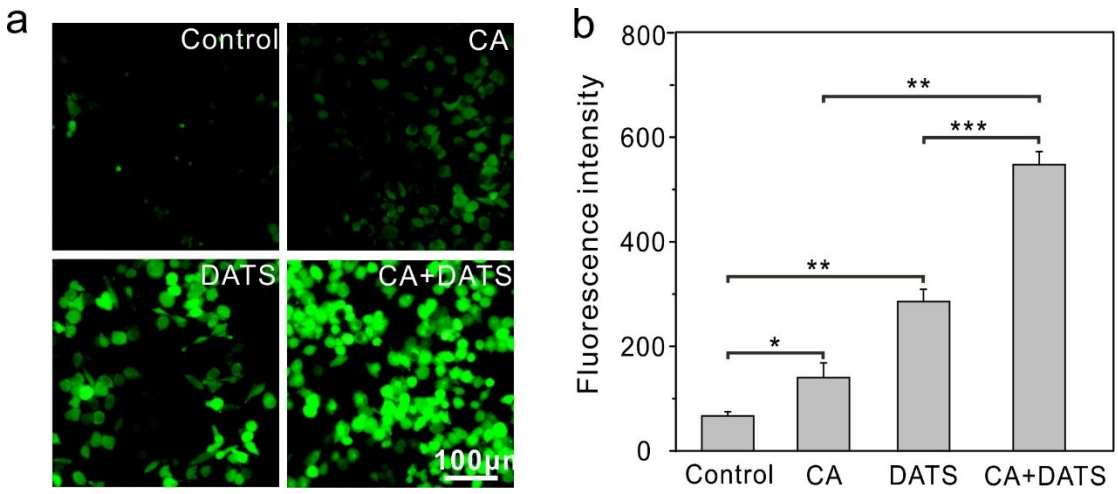

Figure S3. ROS detection on MCF-7 cells. Fluorescence images (a) and the average fluorescence intensities (b) of MCF-7 cells treated with CA (100 μM), DATS (50 μM), and their combination of CA+DATS after 12 h incubation (n = 3). The cells were stained with DCFH-DA. *P< 0.05, **P< 0.01 and ***P< 0.001 are analyzed by student's t-test.

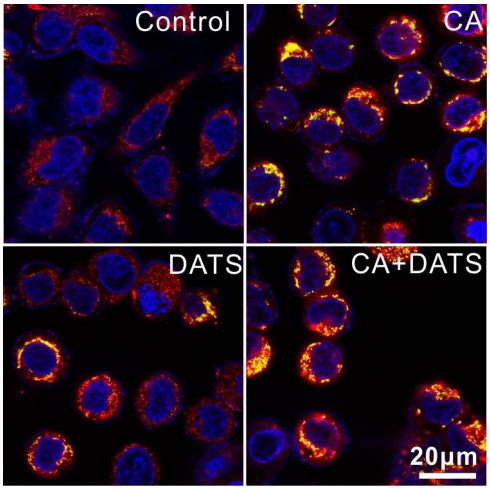

Figure S4. The changes of mitochondrial membrane potential of MCF-7 cells after 2 h incubation with CA (100 μM), DATS (50 μM) and their combination.

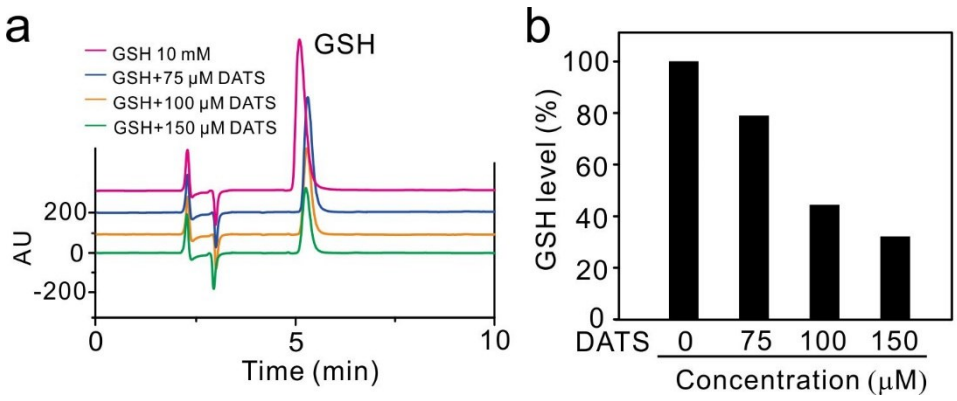

Figure S5. (a) HPLC chromatograms of GSH (10 mM) after incubating with different concentrations of DATS. The typical absorption peak of GSH at 210 nm was detected. (b) The remaining GSH was quantified after incubating with different concentrations of DATS.

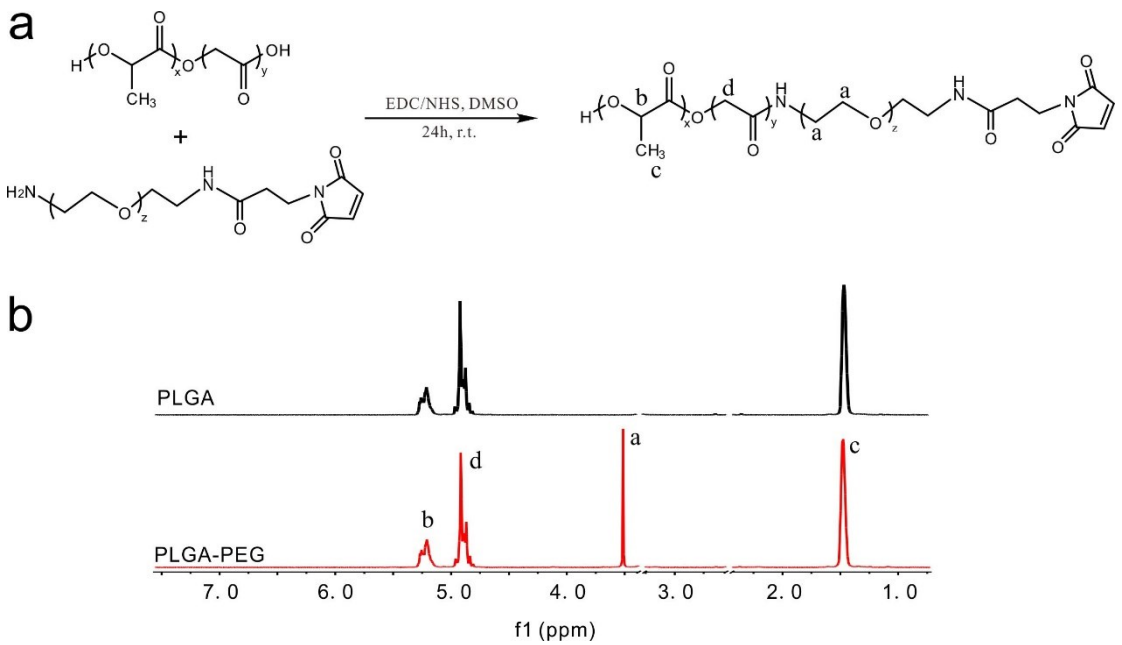

Figure S6. (a) Synthesis of PLGA-PEG-Mal (termed as PLGA-PEG). (b) The ^{1}H NMR spectra of PLGA and PLGA-PEG.

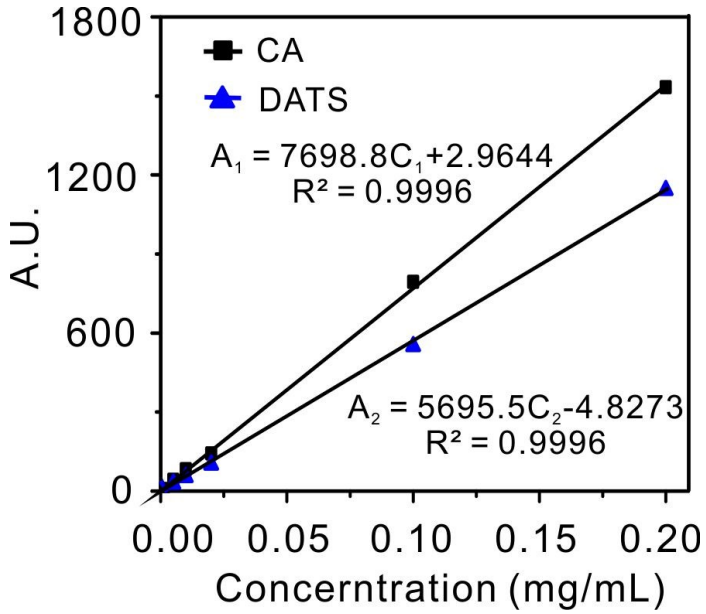

Figure S7. The standard curves for DATS and CA were determined by HPLC in a concentration range of 0-0.2 mg/mL. CA: $A_1 = 7698.8C_1 + 2.9644$ ($R_1 = 0.9996$). DATS: $A_2 = 5695.5C_2 - 4.8273$ ($R_2 = 0.9996$). C_1 and C_2 are the concentrations of CA and DATS (mg/mL), respectively. A_1 and A_2 are the peak areas of CA and DATS, respectively.

Table S1. The formulation of PP-CD.

Formulation Method	Drug Load ratio (%)		Drug encapsulation efficiency (%)	
Nanoprecipitation	CA	1.0 %	CA	1.0 %
	DATS	1.5 %	DATS	20.0 %

PP-CD were fabricated via nanoprecipitation. The amounts of DATS and CA in PP-CD were determined by HPLC. The drug loading ratio and the drug encapsulation efficiency were determined according to the formulas described in the experiment section.

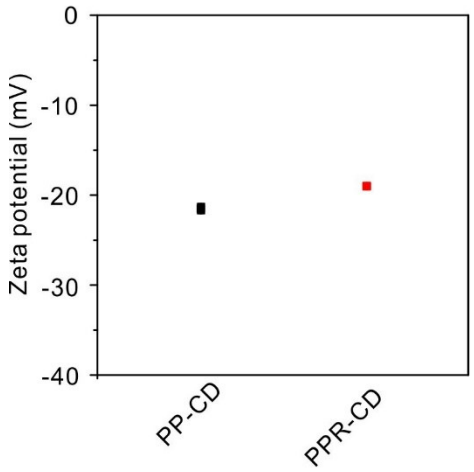

Figure S8. The zeta potentials of PP-CD (-21.5 mV) and PPR-CD (-19.0 mV) in DI water.

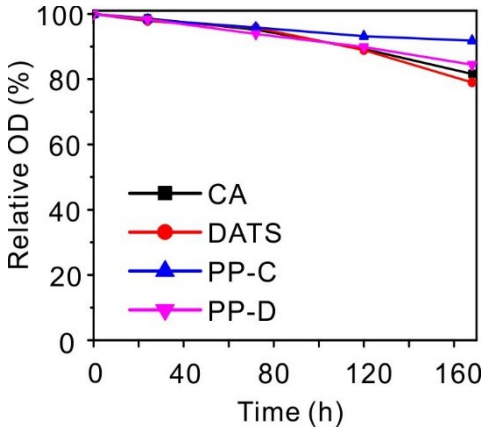

Figure S9. The stability of free CA and DATS and their stability in the nanoparticles of PP-C and PP-D.

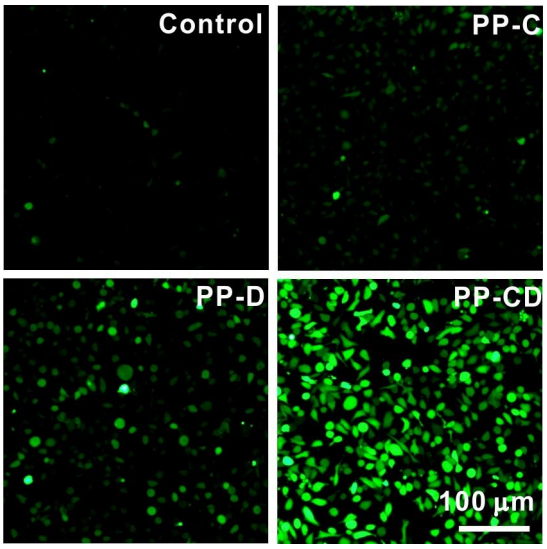

Figure S10. ROS generation in MDA-MB-231 cells treated with PBS, PP-C (CA, 37.5 μM), PP-D (DATS, 40.0 μM) or PP-CD (CA, 37.5 μM; DATS, 40.0 μM) for 12 h. The cells were stained with DCFH-DA.

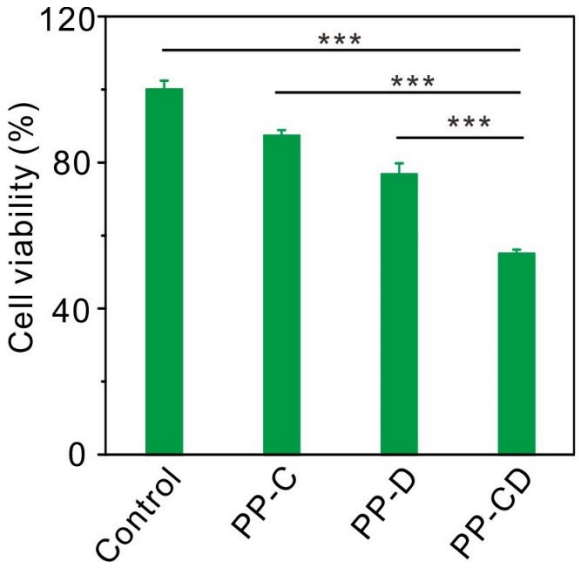

Figure S11. The cytotoxicity of PP-C (CA, 37.5 μM), PP-D (DATS, 40.0 μM) and PP-CD (CA, 37.5 μM; DATS, 40.0 μM) on MDA-MB-231 cells after 24 h incubation. ***P< 0.001 are analyzed by student's t-test.

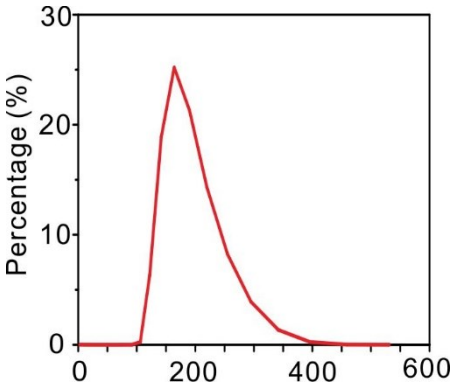

Figure S12. The hydrodynamic diameter of PPR-CD in DI water.

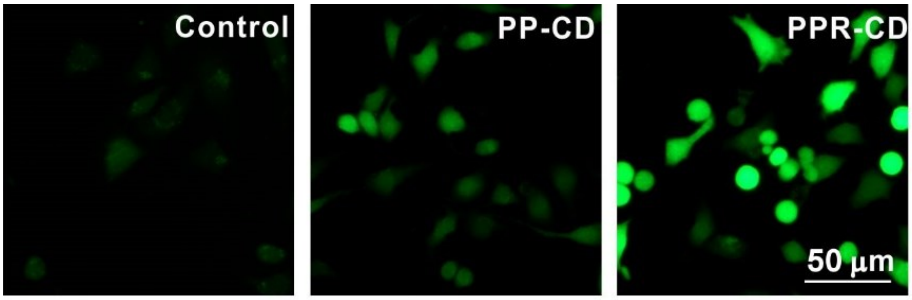

Figure 13. ROS detection of MDA-MB-231 cells treated with PP-CD and PPR-CD for 12 h. The concentration of CA was 37.5 μM, and that of DATS was 40.0 μM. The cells were stained by DCFH-DA.

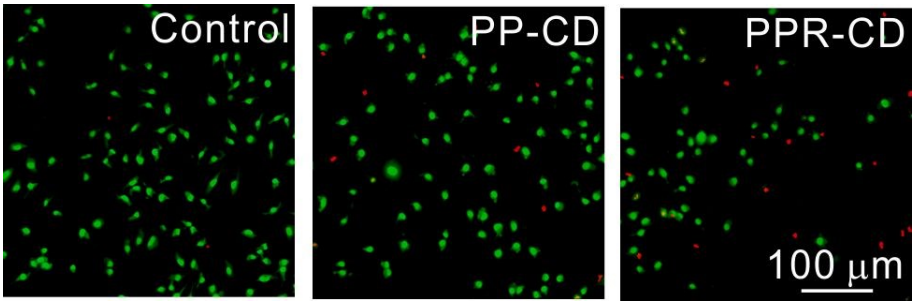

Figure 14. AO/EB-stained MDA-MB-231 cells after incubation with PP-CD and PPR-CD for 24 h. The concentration of CA was 37.5 μM, and that of DATS was 40.0 μM.

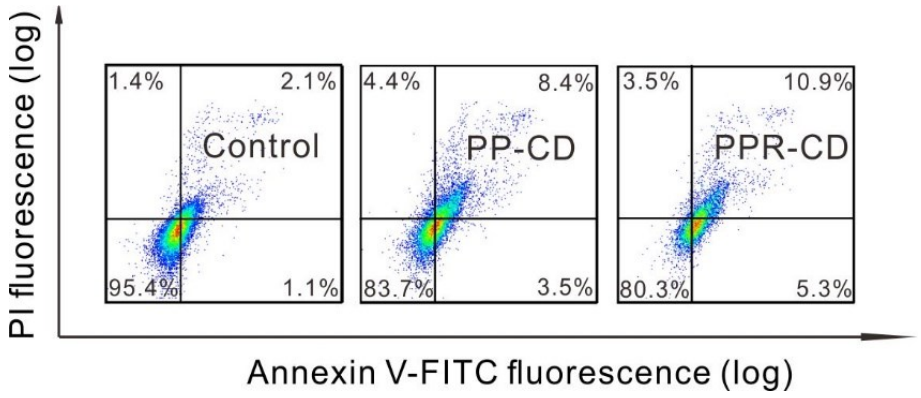

Figure 15. Apoptosis of MDA-MB-231 cells treated with PP-CD and PPR-CD for 24 h. The cells were stained by Annexin V-FITC and PI and analyzed by flow cytometry. The concentration of CA was 25.0 μM, and that of DATS was 26.7 μM.

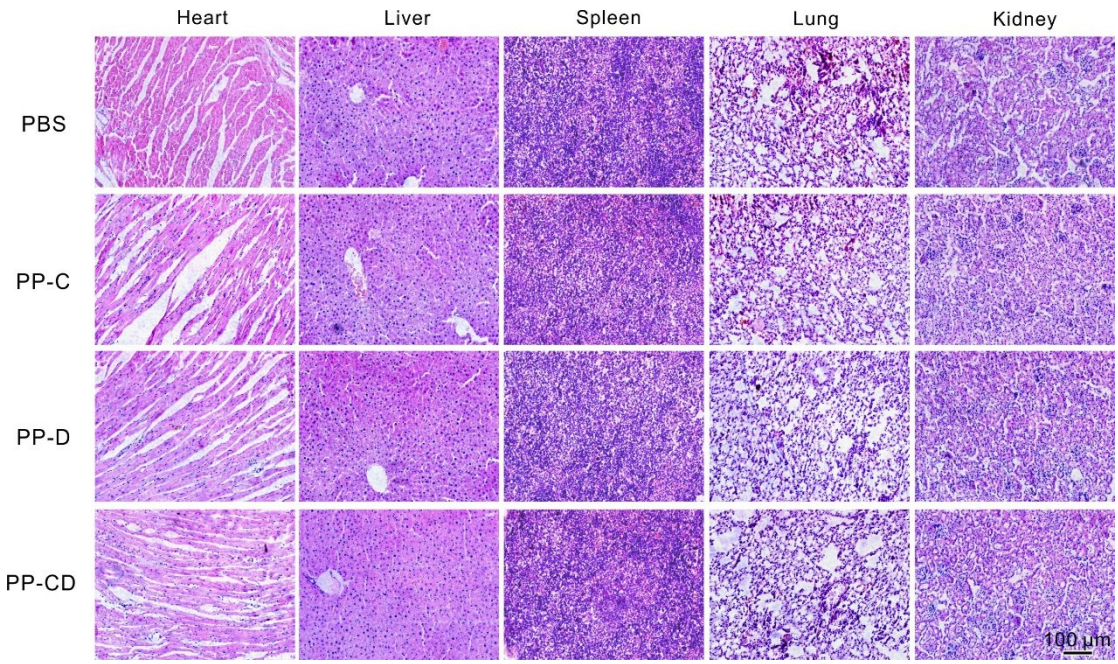

Figure S16. Histological examination of the main organs including heart, liver, spleen, lungs and kidneys harvested from four groups of mice (PBS, PP-C, PP-D, and PP-CD group) after treatment.

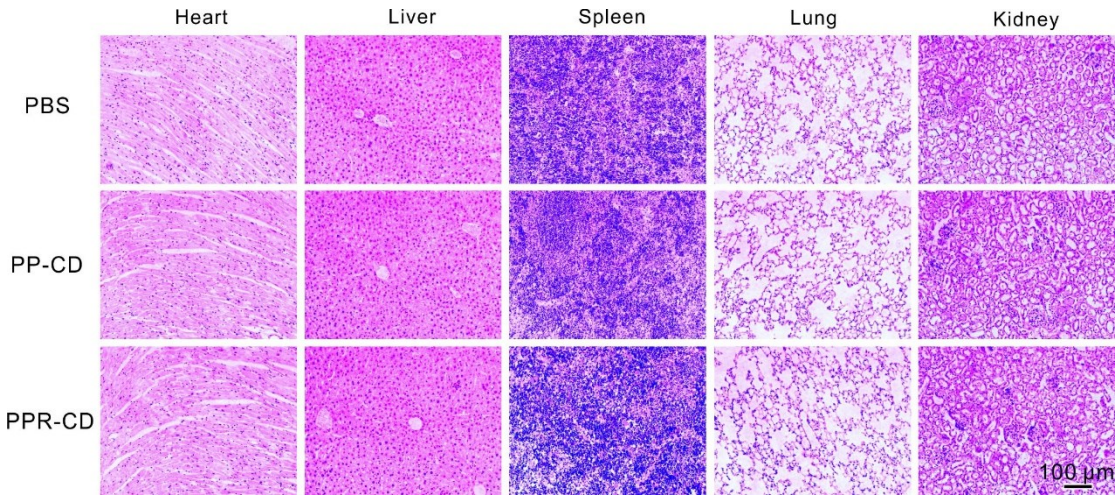

Figure S17. Histological examination of the main organs including heart, liver, spleen, lungs and kidneys harvested from three groups of mice (PBS, PP-CD, and PPR-CD group).