Supporting Information

GPX4 inhibition synergistically boosts mitochondrial targeting nanoartemisinin-induced apoptosis/ferroptosis combination cancer therapy

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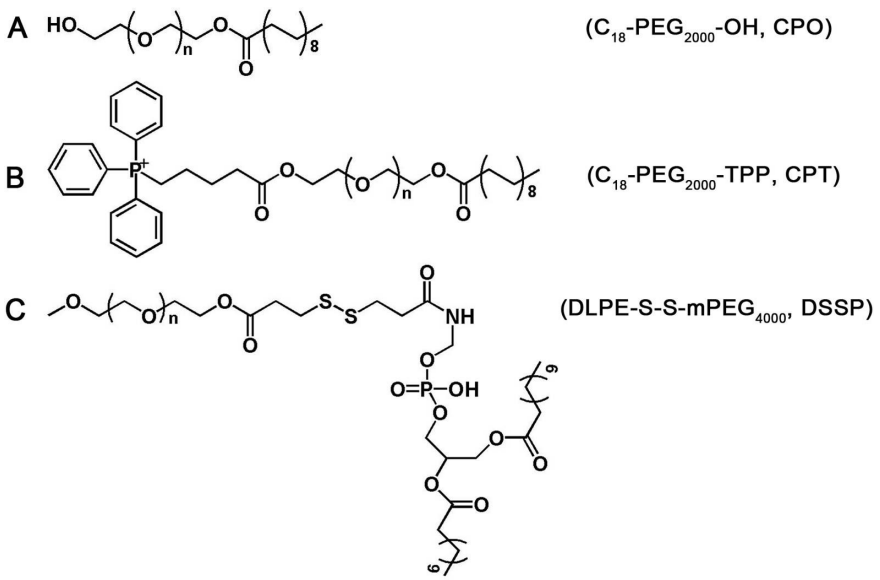

Fig. S1. Chemical Structures of C_{18}-PEG_{2000}-OH (CPO, A), C_{18}-PEG_{2000}-TPP (CPT, B) and DLPE-S-S-$mPEG_{4000}$ (DSSP, C).

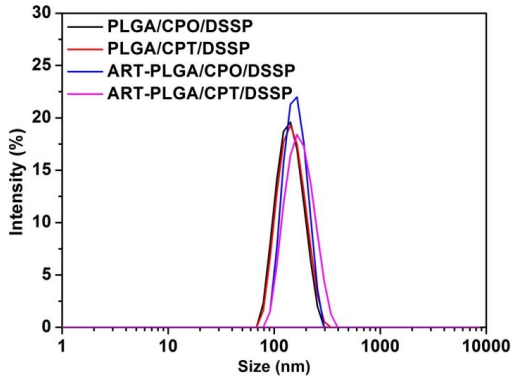

Fig. S2. Average hydrodynamic sizes measured with DLS.

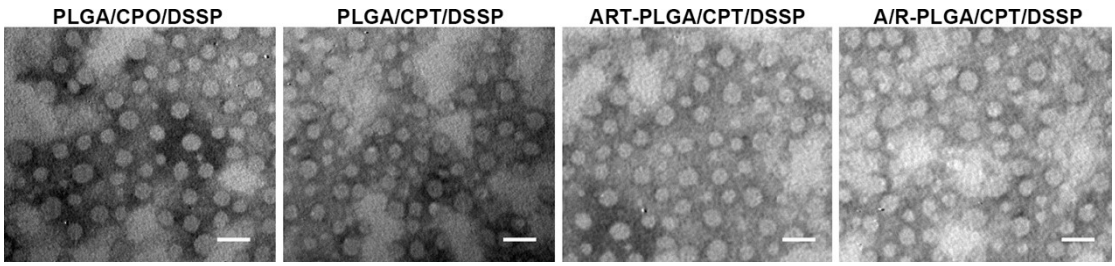

Fig. S3. TEM images of PLGA/CPO/DSSP, PLGA/CPT/DSSP, ART-PLGA/CPT/DSSP, and A/R-PLGA/CPT/DSSP (A/R = 11.1/1). The scale bars are 200 nm.

Tab. S1. Physicochemical characteristics of nanoparticles.

Sample	Size (nm)	PDI	Zeta potential (mV)	DLC (%)
PLGA/CPO/DSSP	139.3	0.136	-3.31	/
PLGA/CPT/DSSP	140.4	0.125	0.16	/
ART-PLGA/CPO/DSSP	150.8	0.068	-1.13	8.39
ART-PLGA/CPT/DSSP	165.3	0.092	0.49	6.62

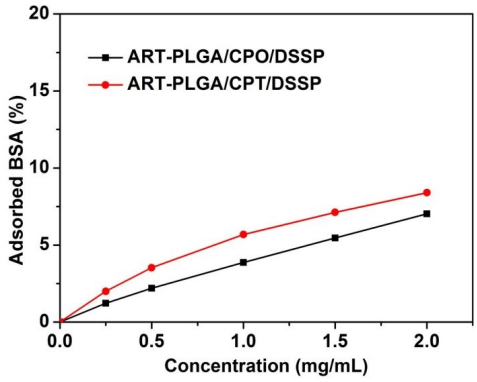

Fig. S4. BSA adsorption of ART-PLGA/CPO/DSSP and ART-PLGA/CPT/DSSP in PBS.

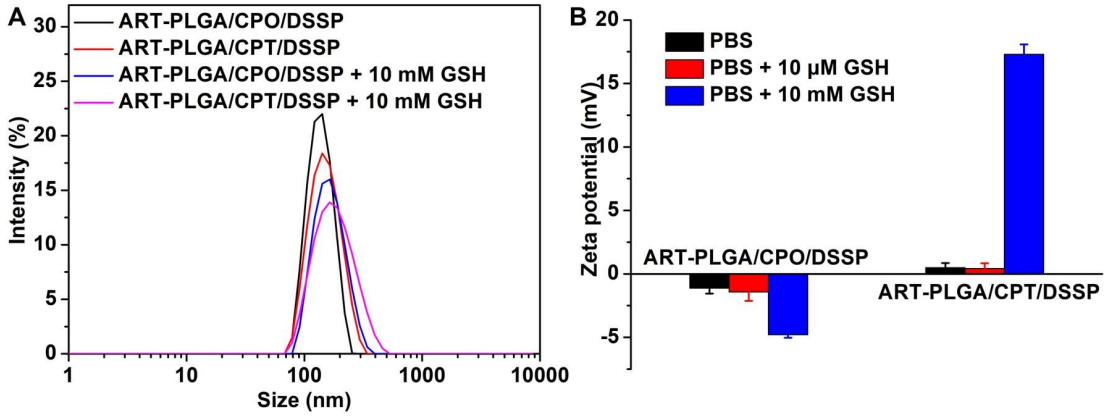

Fig. S5. Sizes and zeta potentials of ART-PLGA/CPO/DSSP and ART-PLGA/CPT/DSSP in PBS containing different concentrations of GSH.

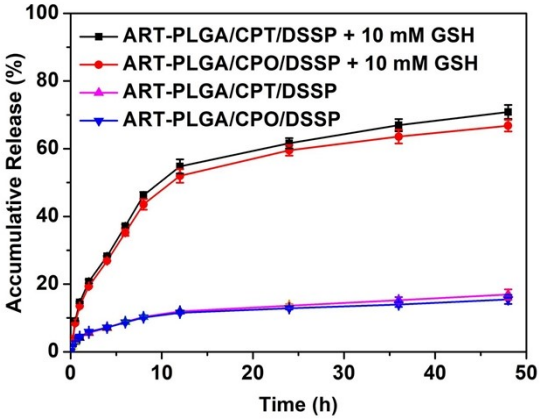

Fig. S6. *In vitro* release of ART from ART-PLGA/CPT/DSSP and ART-PLGA/CPO/DSSP in PBS with or without 10 mM GSH.

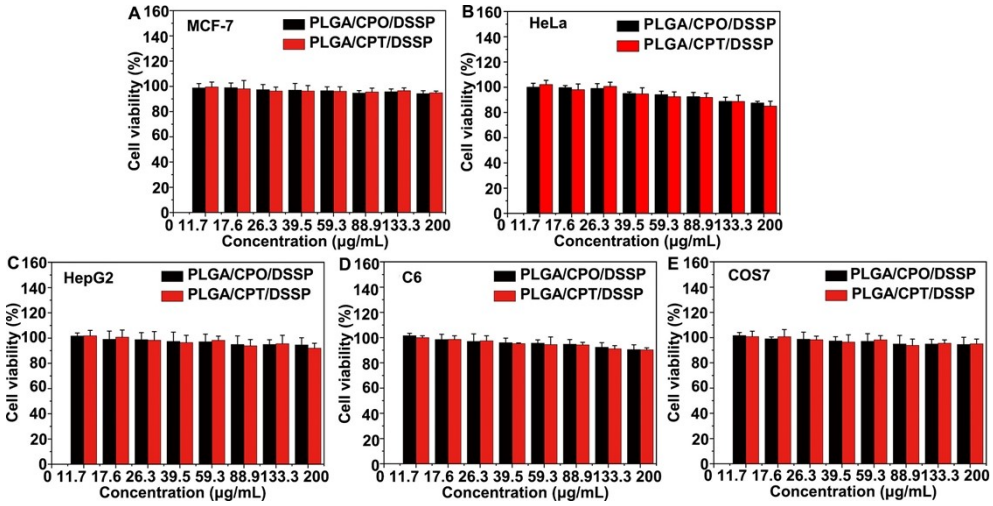

Fig. S7. Cytotoxicity of ART-free nanoparticles against MCF-7, HeLa, HepG2, C6 and COS 7 cells.

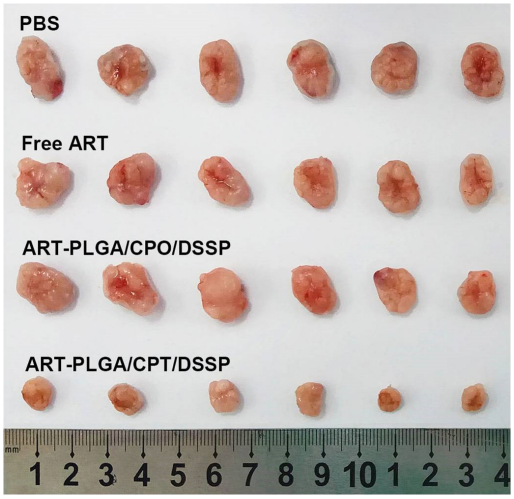

Fig. S8. Tumor images on the 12th day after treatment with PBS, free ART, ART-PLGA/CPO/DSSP, and ART-PLGA/CPT/DSSP, respectively.

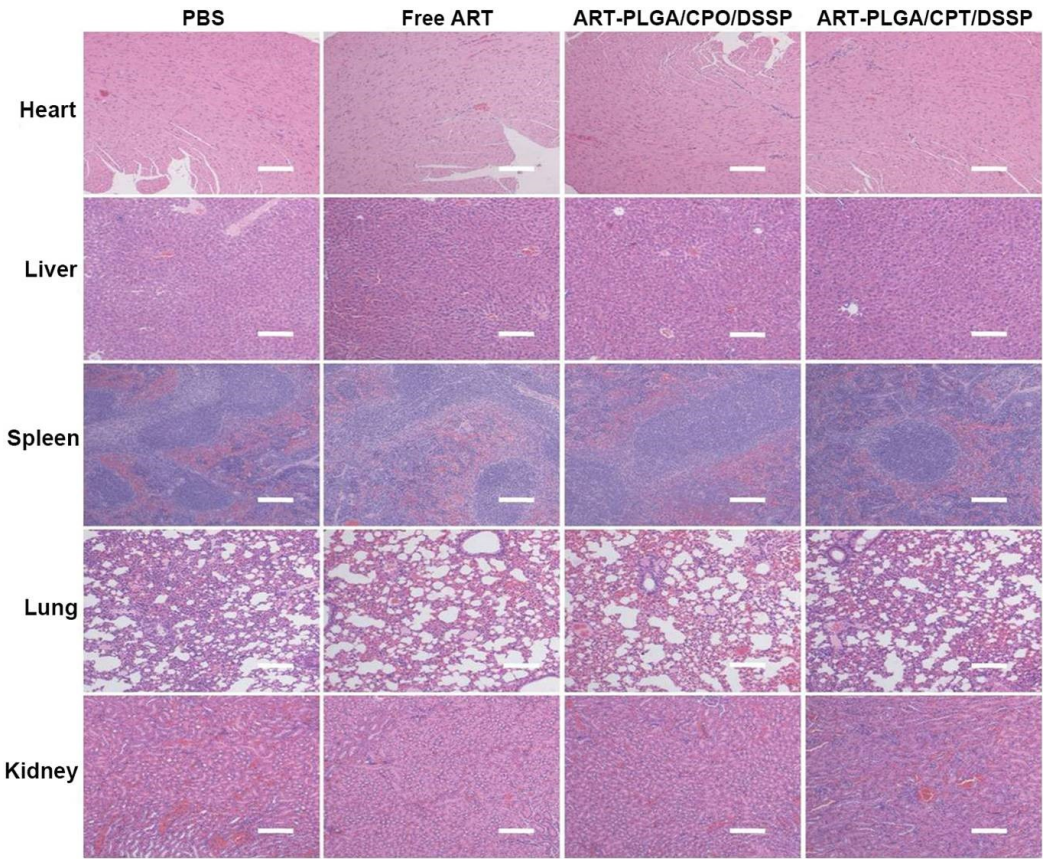

Fig. S9. H&E staining of vital organs after treatment with PBS, free ART, ART-PLGA/CPO/DSSP, and ART-PLGA/CPT/DSSP, respectively. The scale bars are 200 μm.

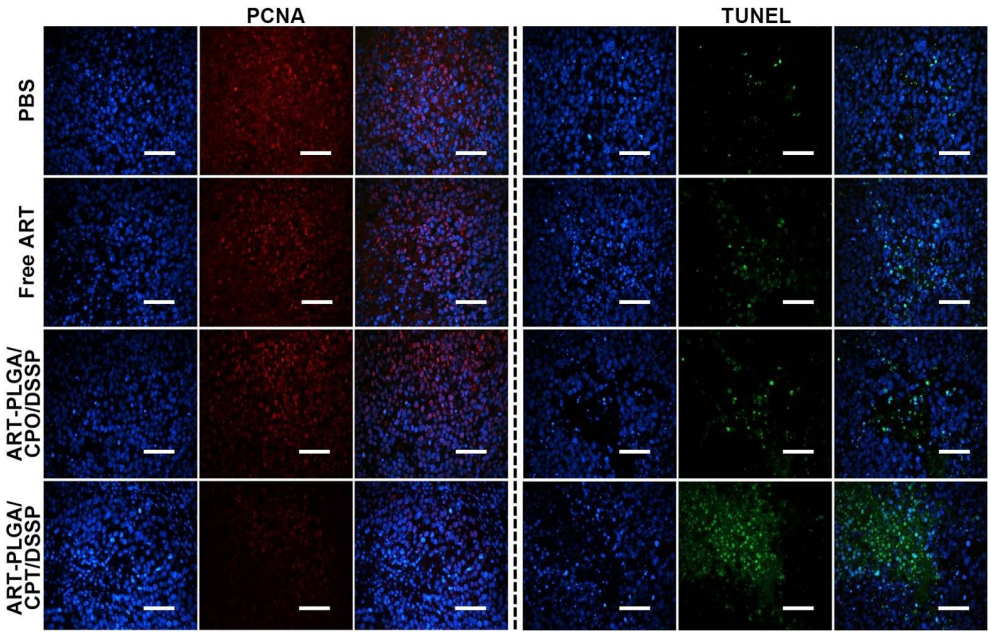

Fig. S10. PCNA and TUNEL analyses of tumor tissues after treatment with PBS, free ART, ART-PLGA/CPO/DSSP, and ART-PLGA/CPT/DSSP, respectively. The scale bars are 100 μm.

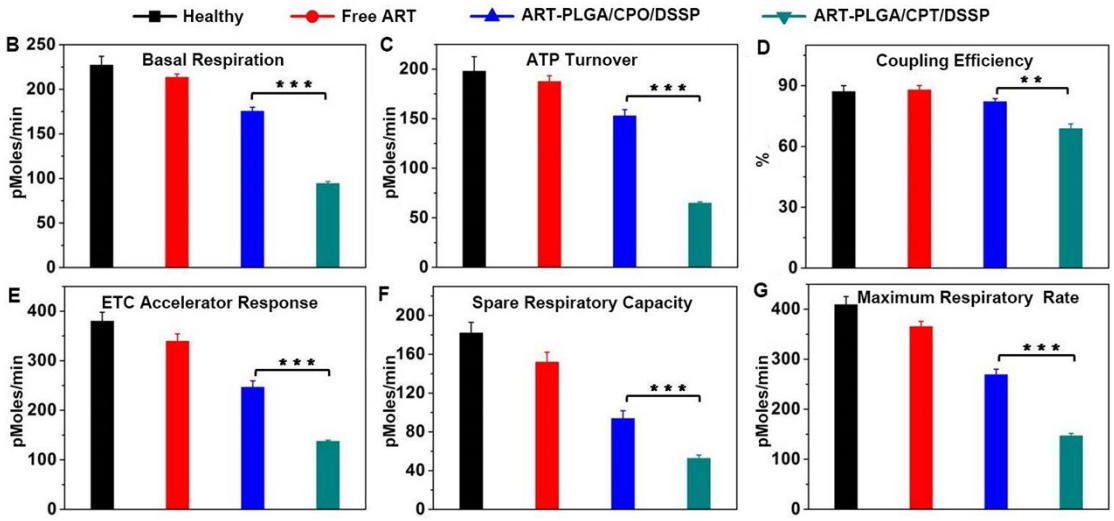

Fig. S11. Mitochondrial bioenergetics analysis of MCF-7 cells after treatment with ART formulations for 24 h, mean ± S.D, n=3, **P < 0.01 and ***P < 0.001.

Tab. S2. Physicochemical characteristics drug loaded PLGA/CPT/DSSP.

Samples	Size (nm)	PDI	Zeta potential (mV)	DLC (%)	
				ART	RSL3
RSL3-PLGA/CPT/DSSP	159.8	0.139	-0.262	/	0.71
ART-PLGA/CPT/DSSP	167.9	0.124	-0.233	6.32	/
A/R = 7.4/1	169.7	0.156	-0.158	6.21	0.84
A/R = 11.1/1	165.4	0.107	0.041	6.35	0.57
A/R = 20.9/1	169.7	0.085	-0.111	6.49	0.31
A/R = 42.5/1	166.1	0.139	0.118	6.37	0.15
A/R = 81.5/1	169.5	0.132	-0.330	6.52	0.08

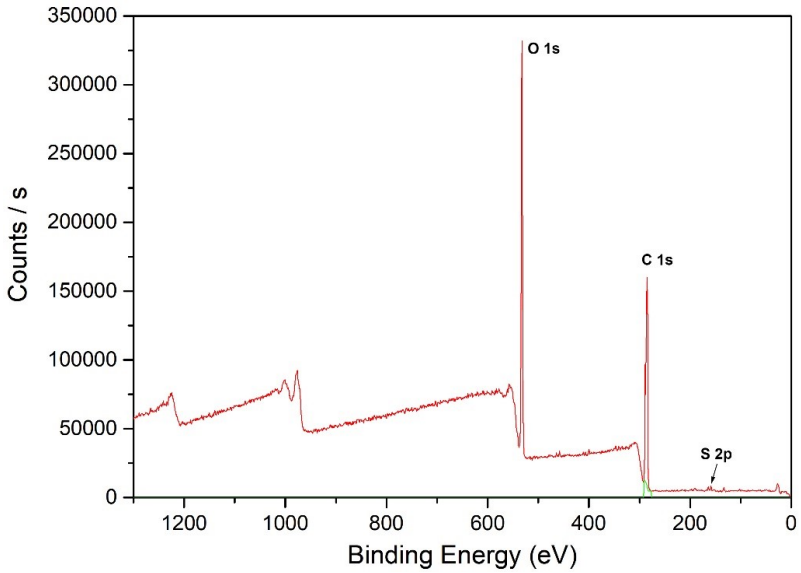

Fig. S12. XPS spectra of A/R-PLGA/CPT/DSSP.

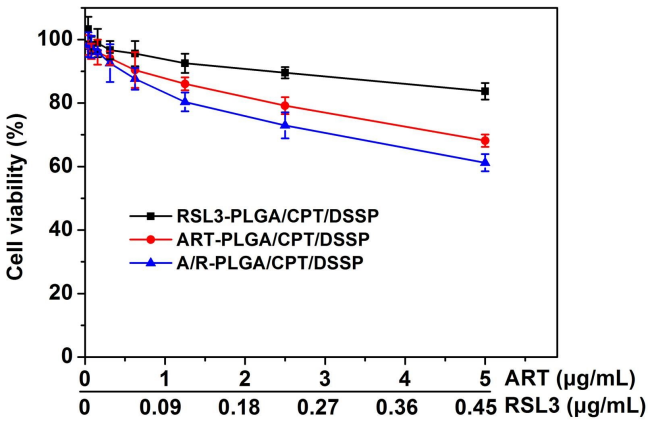

Fig. S13. Cytotoxicity of ART-PLGA/CPT/DSSP, RSL3-PLGA/CPT/DSSP and A/R-PLGA/CPT/DSSP (A/R = 11.1/1) against COS 7 cells.

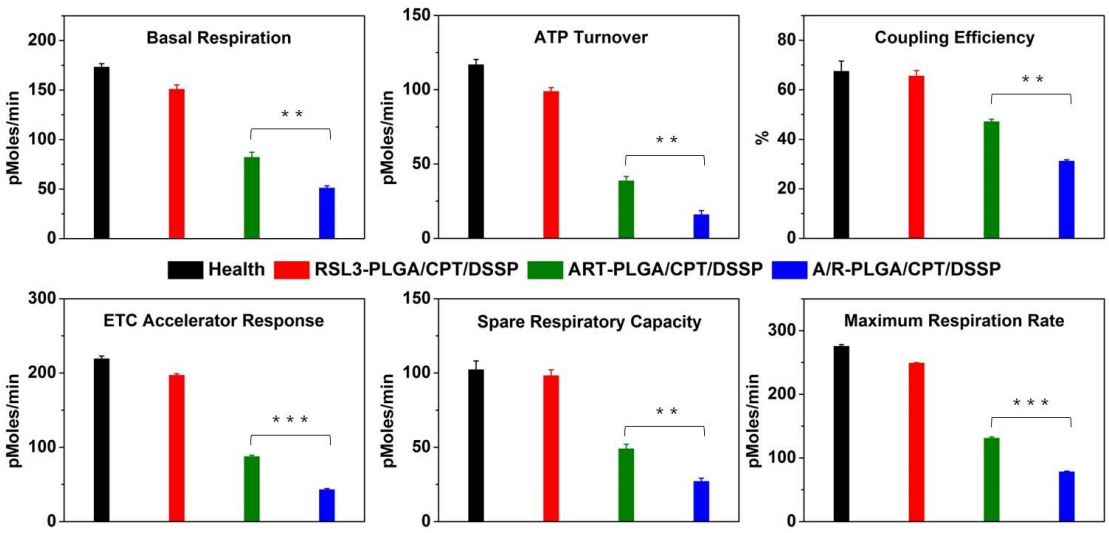

Fig. S14. Basal respiration, ATP turnover, coupling efficiency, electron transport chain (ETC) accelerator response, spare respiratory capacity, and maximum respiration rate of MCF-7 cells after various treatments for 24 h (mean ± S.D, n=3), **P < 0.01, ***P < 0.001.

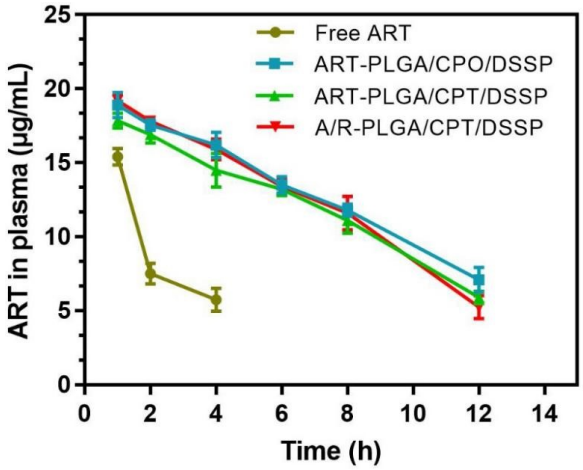

Fig. S15. Blood circulation of free ART and drug-loaded nanopaticles after intravenous injection.

Tab. S3. Pharmacokinetic parameters of free ART and drug-loaded nanopaticles after

intravenous injection at a dose of 5 mg ART per kg body weight (n = 3).

Parameters	Free ART	ART-PLGA/ CPT/DSSP	ART-PLGA/ CPO/DSSP	A/R-PLGA/ CPT/DSSP
$t_{1/2z}$ (h)	0.77	9.96	10.41	9.72
$AUC_{0-\infty}$ (mg/L*h)	55.18	153.24	164.63	160.12
$MRT_{0-\infty}$ (h)	1.58	10.91	12.30	9.42
CL_Z (L/h/kg)	0.090	0.018	0.017	0.018

Parameters and units: half-life ($t_{1/2Z}$, h); area under the curve ($AUC_{0-\infty}$, mg/L×h); mean residence time ($MRT_{0-\infty}$, h); total body clearance (CL_Z, L/h/kg).

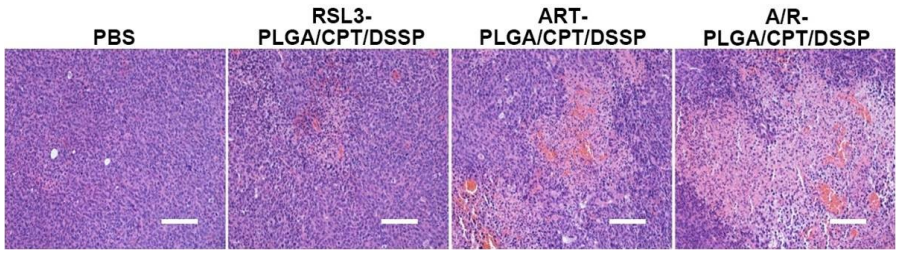

Fig. S16. H&E staining of tumor tissues after various treatments for 18 days. The scale bars are 100 μm.

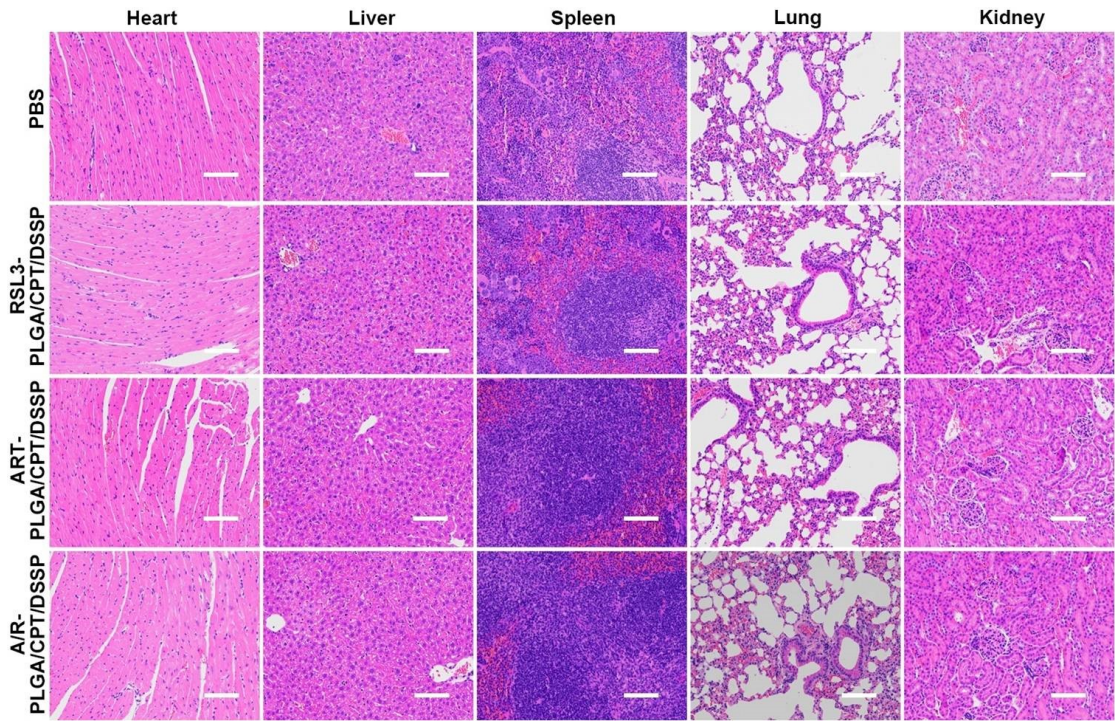

Fig. S17. H&E staining of major organs after treatment with various formulations. The scale bars are 100 μm.

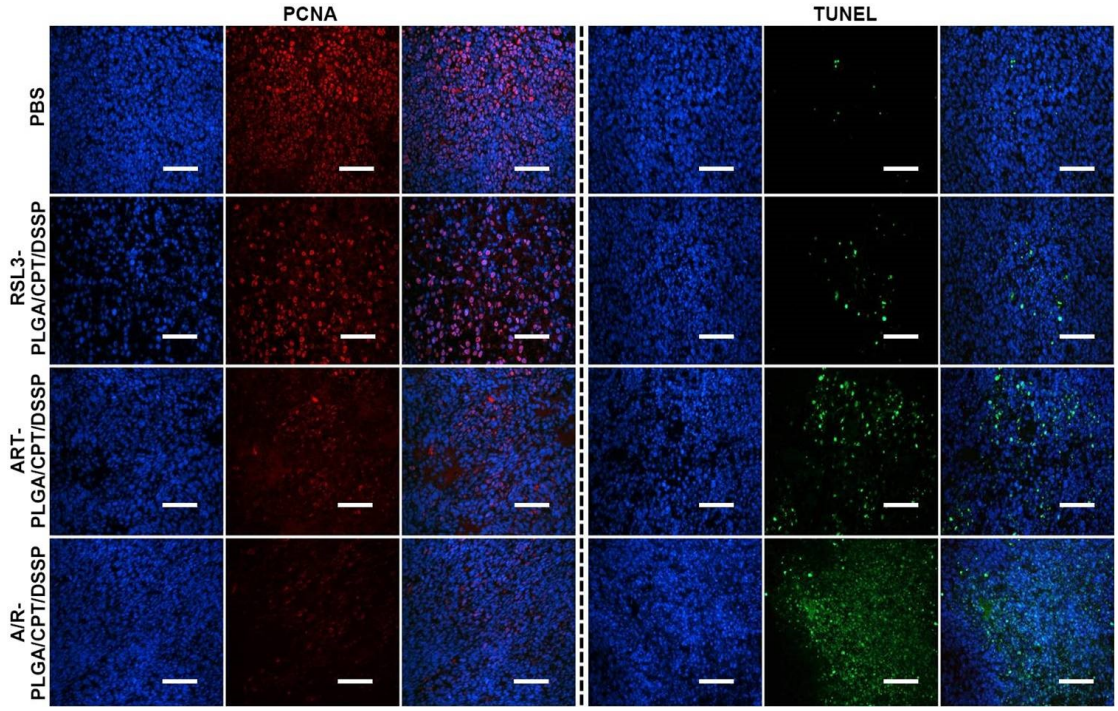

Fig. S18. PCNA and TUNEL analyses of tumor tissues after various treatments. The scale bars are 100 μm.