

Supporting Information

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Immunogenic Nanovesicle-Tandem-Augmented Chemoimmunotherapy via Efficient Cancer-Homing Delivery and Optimized Ordinal-Interval Regime

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Supporting Information

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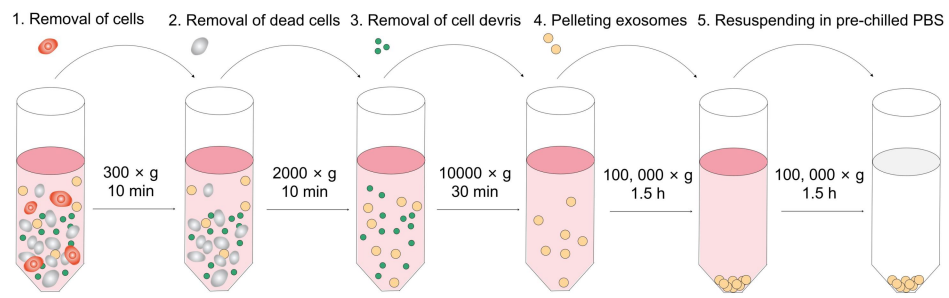


Figure S1 Schematic representation of TEDV isolation using the differential centrifugation method.

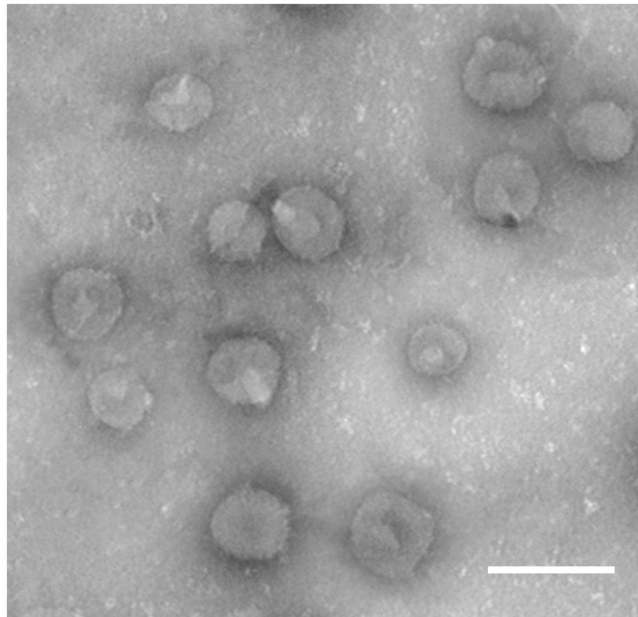


Figure S2 TEM image of TEDV. Scale bar = 200 nm.

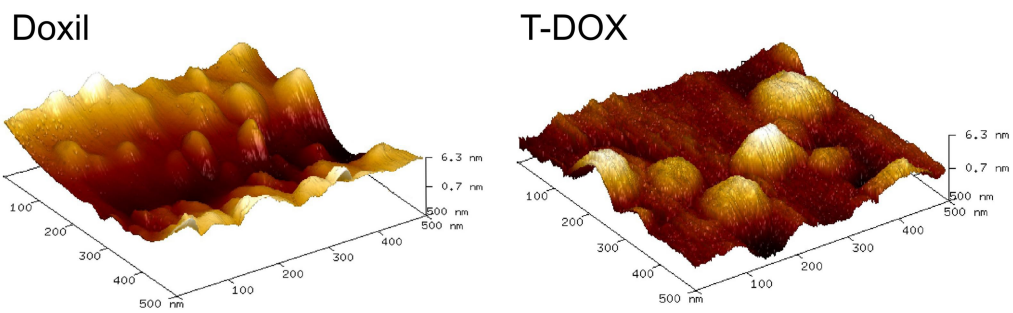


Figure S3 AFM images of Doxil and T-DOX.

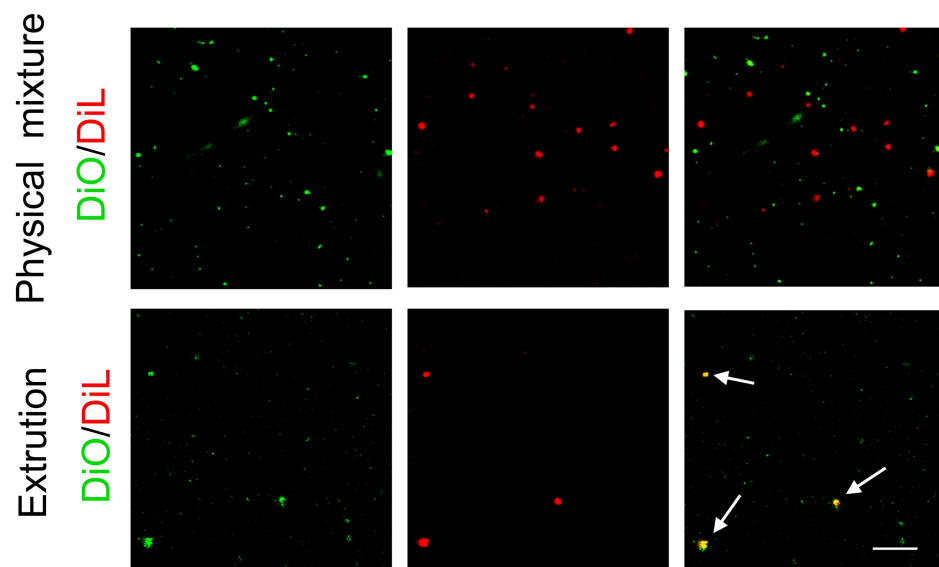


Figure S4 CLSM images of physical mixture and extrusion of TEDVs and liposomes. (red = liposome, green = TEDV; Scale bar = 2 μ m).

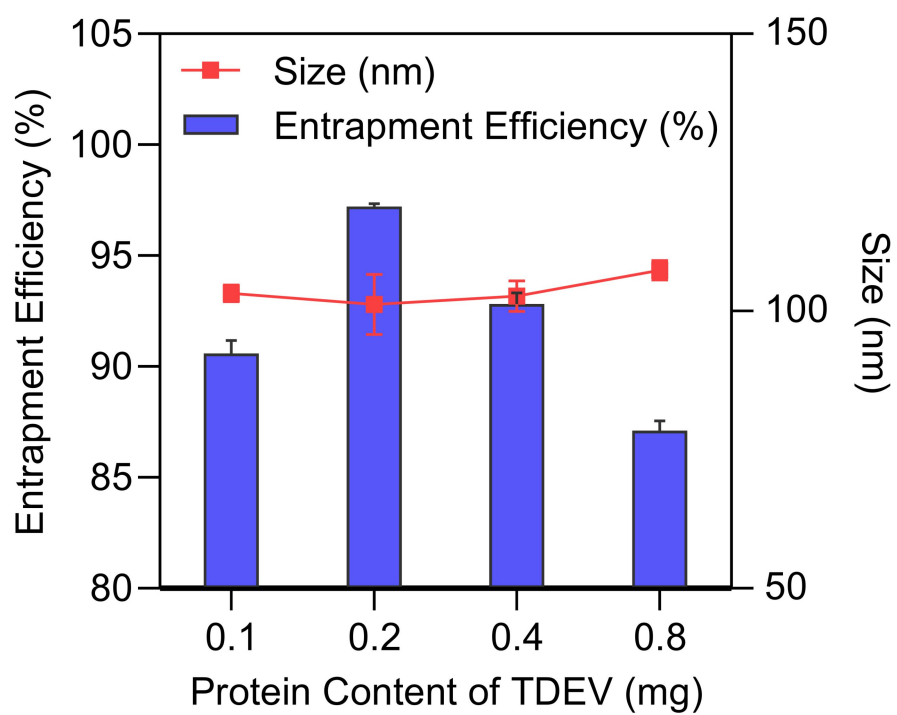


Figure S5 Hydrodynamic diameter and entrapment efficiency of T-DOX with different formulations (n = 3).

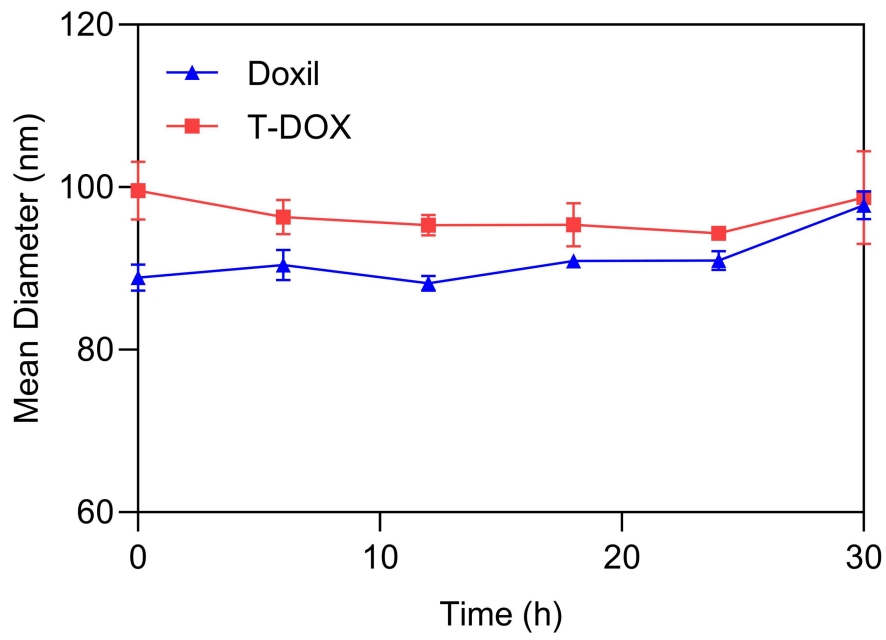


Figure S6 *In vitro* stability of Doxil and T-DOX in PBS (pH 7.4) containing 10% fetal bovine serum (FBS) for 30 h (n = 3).

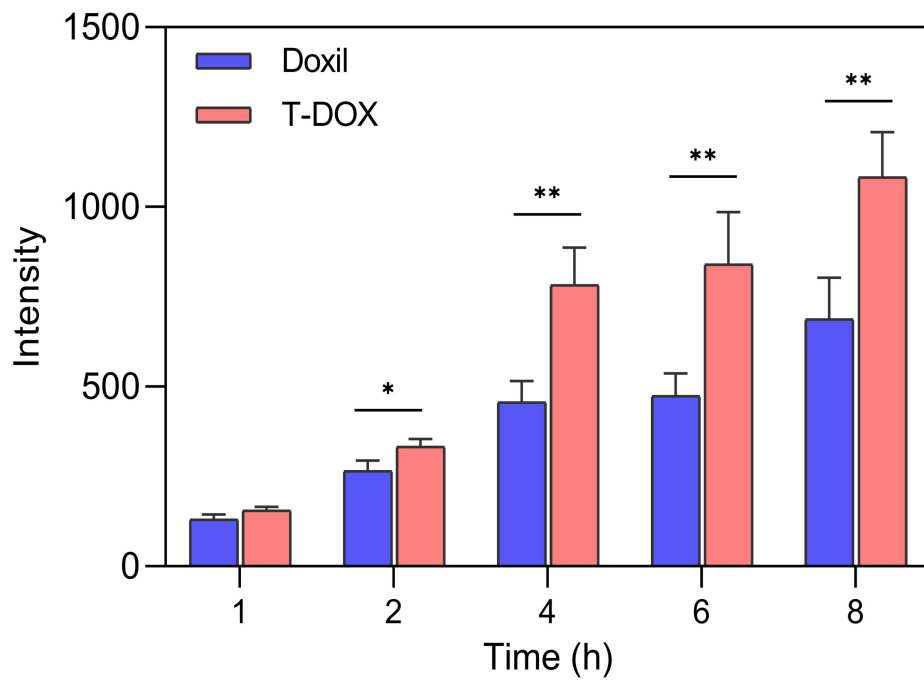


Figure S7 Flow cytometry analysis of 4T1 cells incubated with Doxil or T-DOX at different time points (n = 3). Significant values are presented as mean \pm SD; *P < 0.05; **P < 0.01; ***P < 0.001.

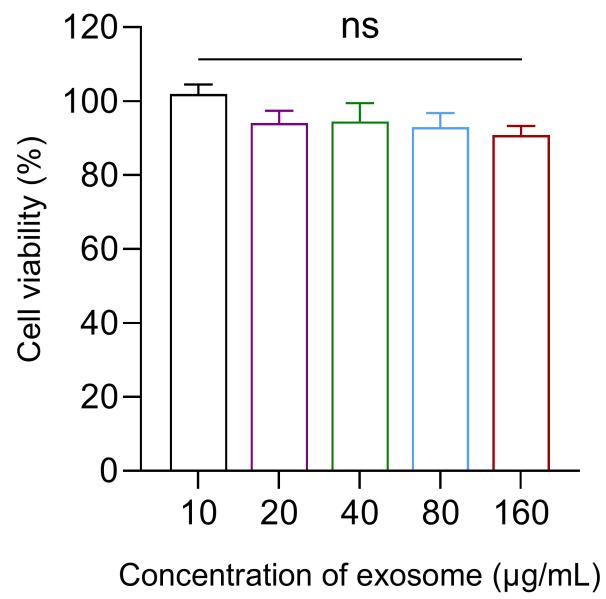


Figure S8 *In vitro* cytotoxicity of blank biohybrid exosome against 4T1 cells for 48 h (n = 3).

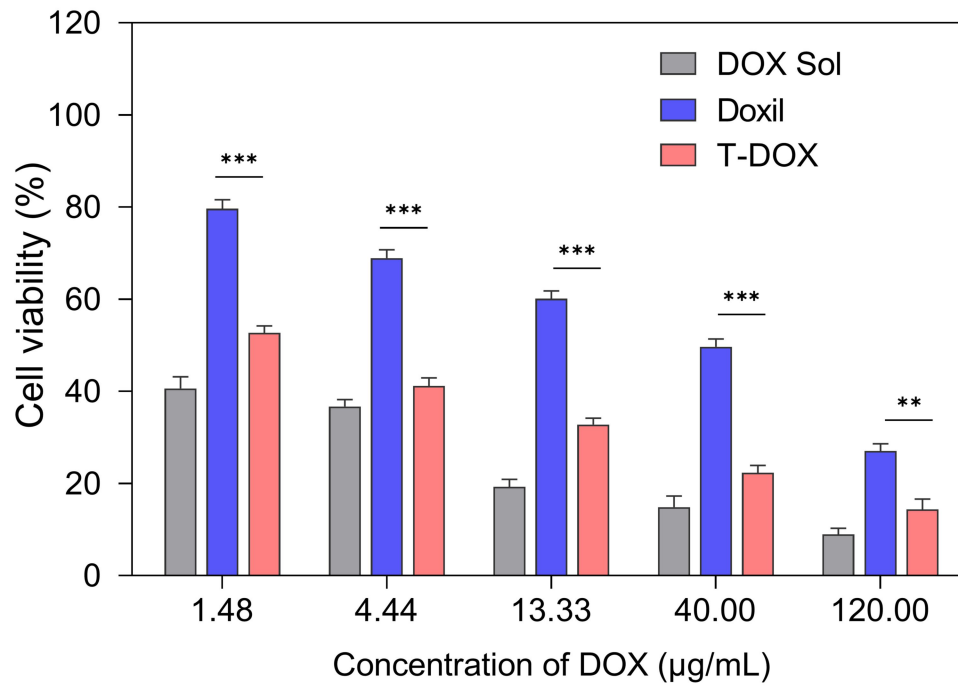


Figure S9 *In vitro* cytotoxicity of DOX Sol, Doxil, or T-DOX against 4T1 cells for 48 h (n = 3).

Significant values are presented as mean \pm SD; *P<0.05; **P<0.01; ***P<0.001.

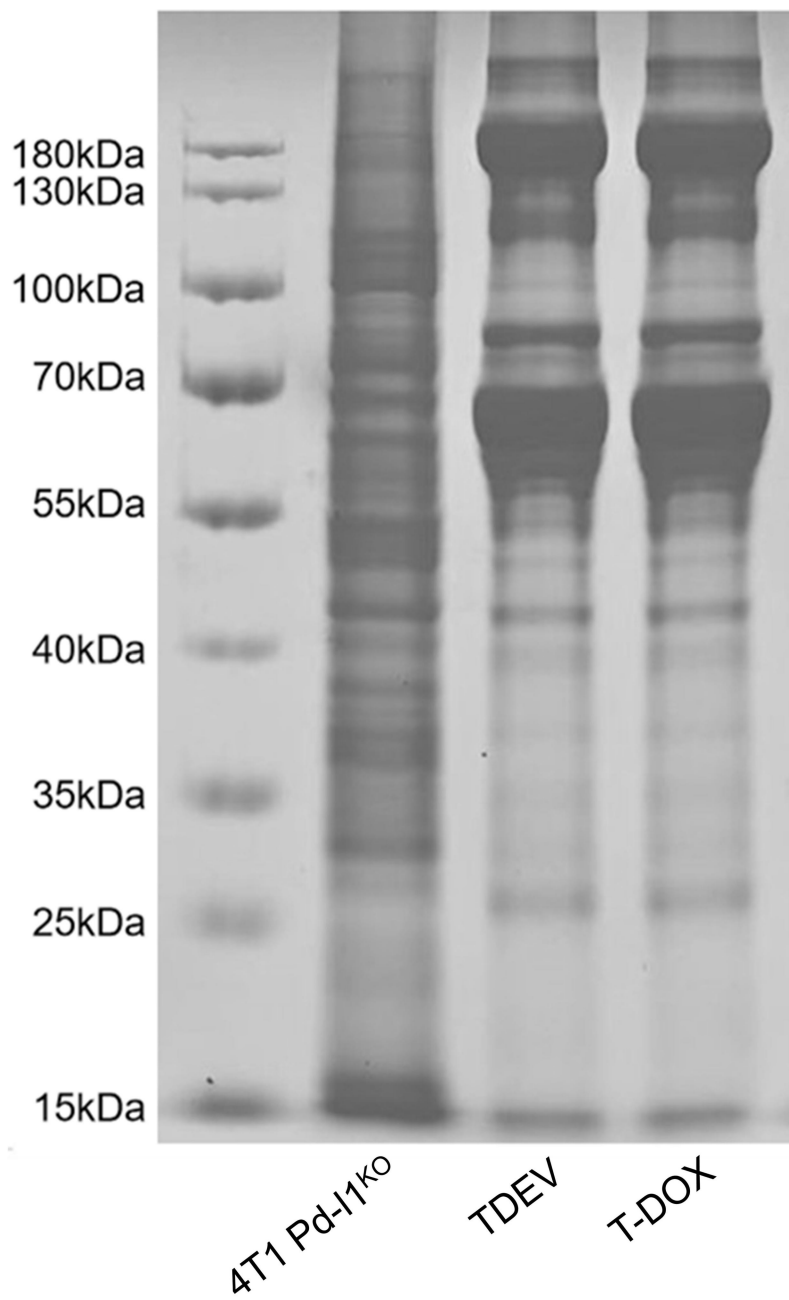


Figure S10 SDS-PAGE protein analysis of 4T1 Pd-I1^{KO}, TEDV, and T-DOX.

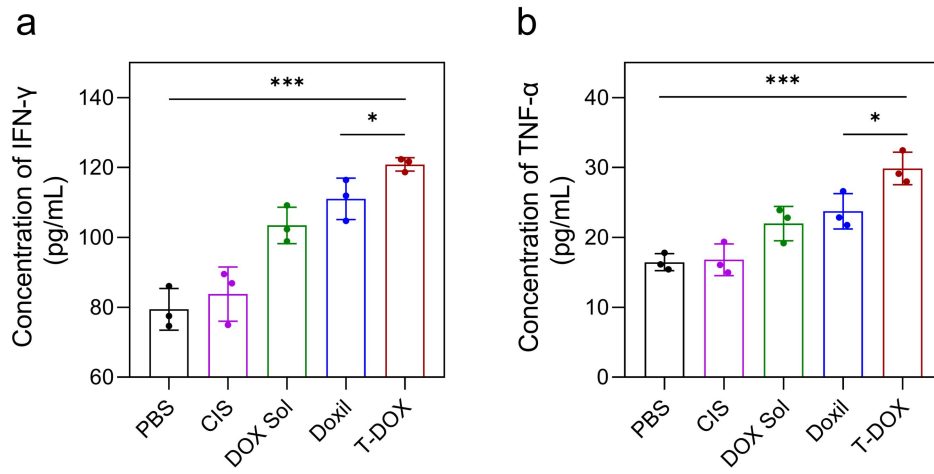


Figure S11 (a) IFN- γ and (b) TNF- α in tumors following various treatments (n = 3). Significant values are presented as mean \pm SD; *P<0.05; **P<0.01; ***P<0.001.

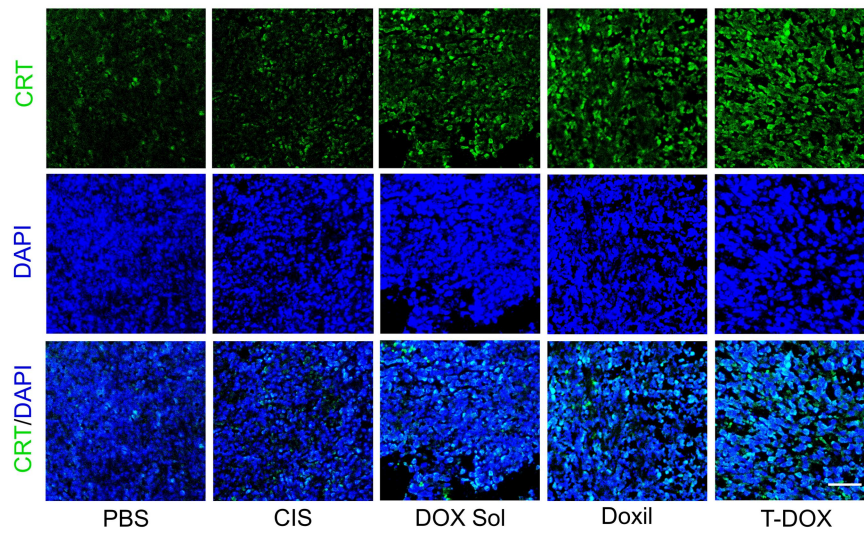


Figure S12 CRT staining of tumor slices after various treatments. Scale bar = 50 μ m.

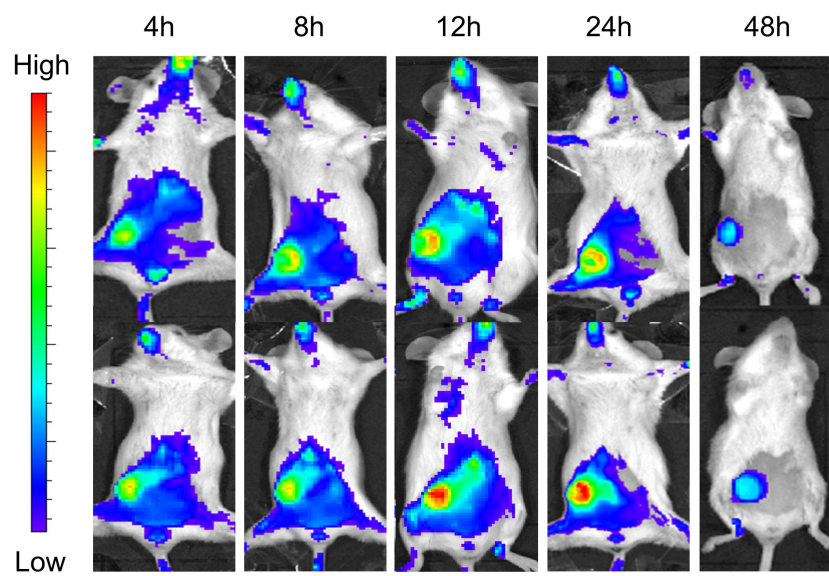


Figure S13 *In vivo* fluorescence imaging in orthotopic 4T1 tumor-bearing mice at 4 h, 8 h, 12 h, 24 h, and 48 h following injection with Doxil^{DiR} and T-DOX^{DiR}.

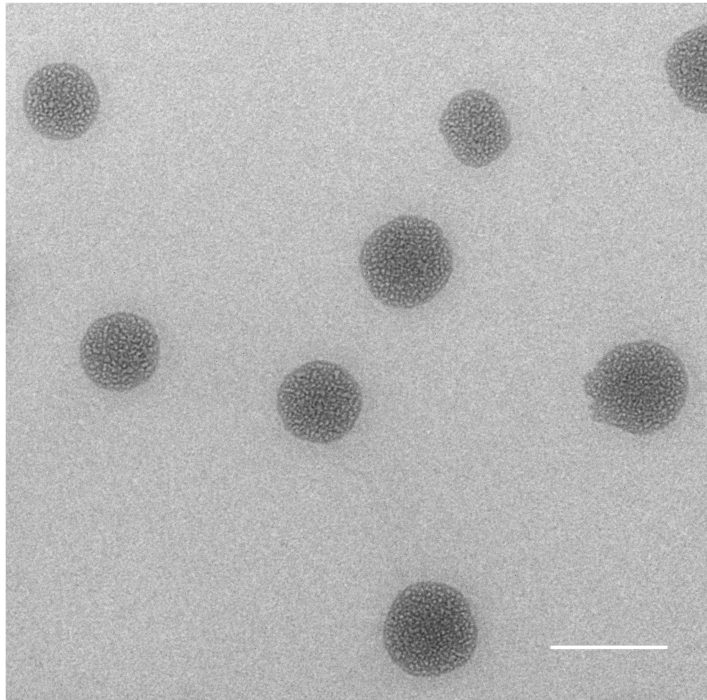


Figure S14 TEM image of S-PD. Scale bar = 200 nm.

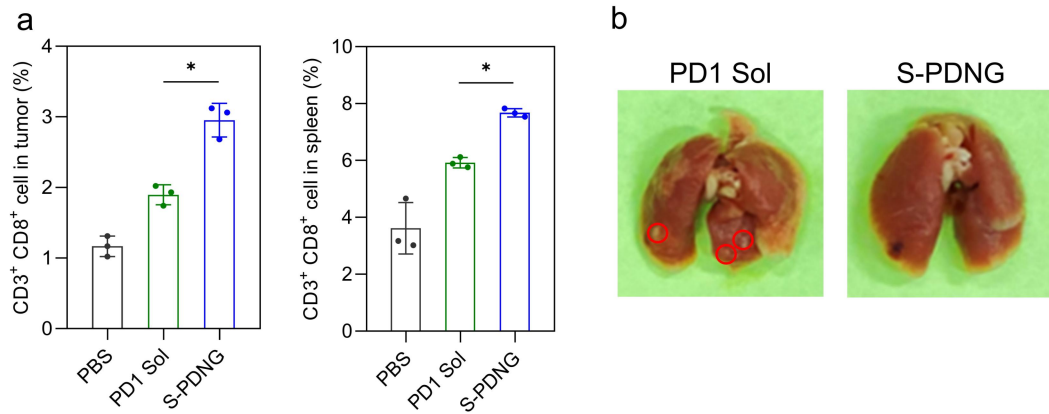


Figure S15 (a) CD8⁺ T cell ratios in tumors and splenocytes of the 4T1 tumor-bearing mice after various treatments (n = 3). Significant values are presented as mean ± SD; *P < 0.05; **P < 0.01; ***P < 0.001. (b) Lung tissues collected from various treatments. Red circles demonstrate the visible metastatic site.

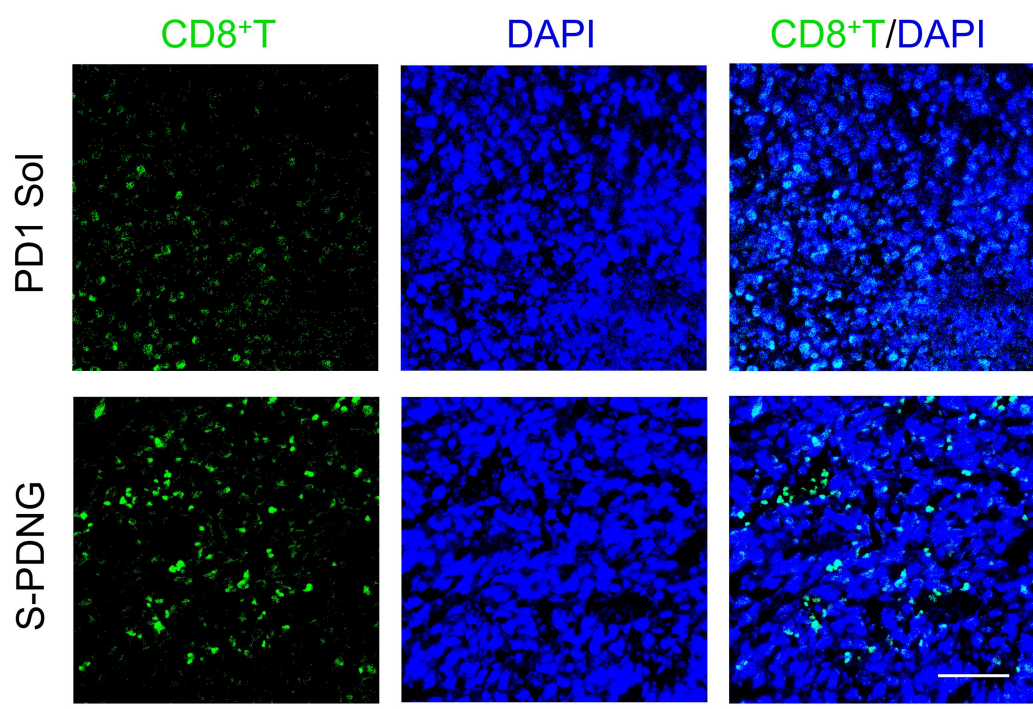


Figure S16 CD8 staining of tumors slices following PD1 Sol and S-PDNG treatment. Scale bar = 50 μ m.

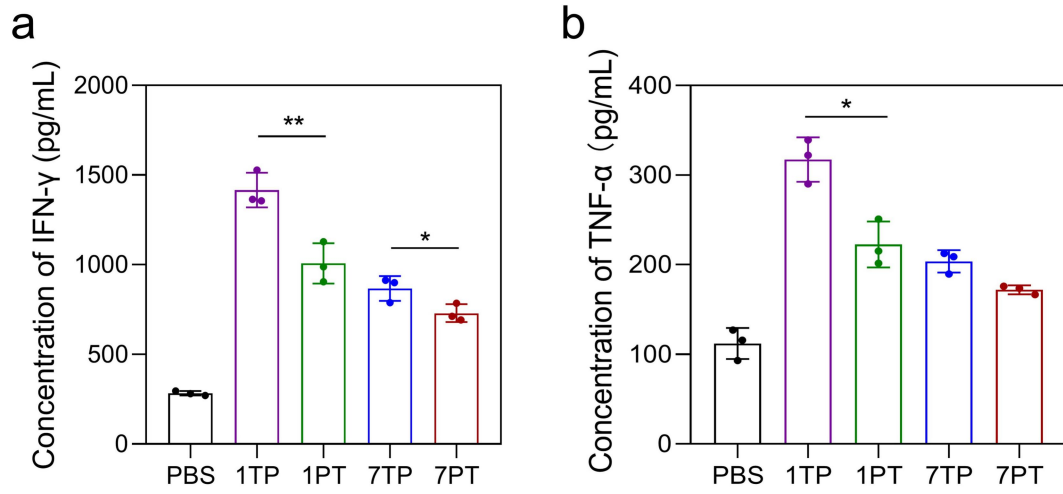


Figure S17 (a) IFN- γ and (b) TNF- α in tumors following sequential dosing of T-DOX and S-PDNGs. (n = 3). Significant values are presented as mean \pm SD; *P < 0.05; **P < 0.01; ***P < 0.001.

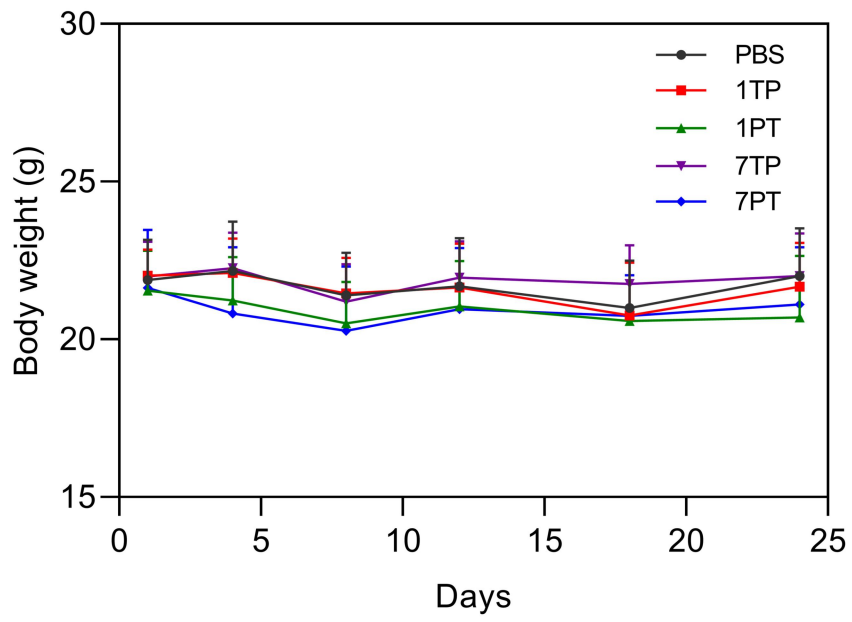


Figure S18 Body weight of mice following sequential dosing of T-DOX and S-PDNG (n = 3).

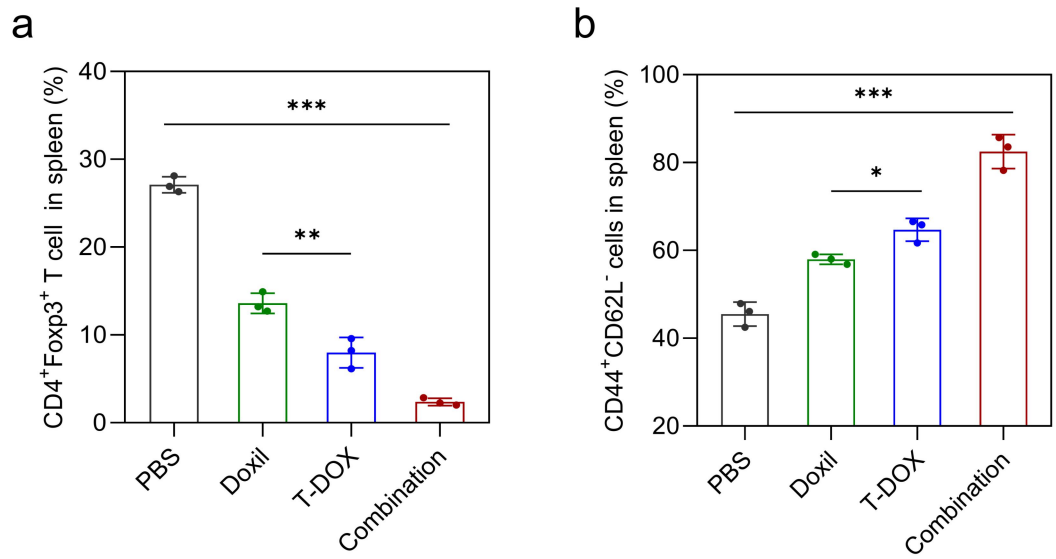


Figure S19 (a) Treg and (b) Memory T cell ratios in splenocytes of 4T1 tumor-bearing mice after various treatments (n = 3). Significant values are presented as mean \pm SD; *P < 0.05; **P < 0.01; ***P < 0.001.

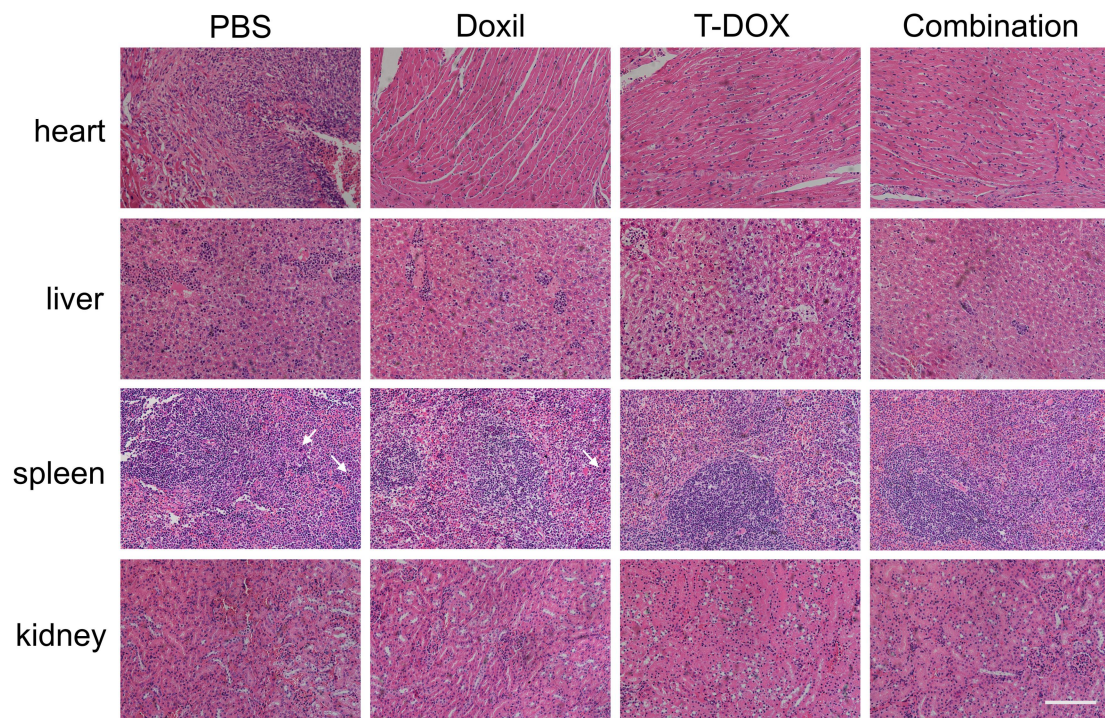


Figure S20 Pathological analyses of heart, liver, spleen, and kidney of the orthotopic 4T1 tumor-bearing mice in the PBS, Doxil, T-DOX, and combination-treated groups on day 11. White arrows demonstrate the lung metastases. Scale bar = 100 μ m.

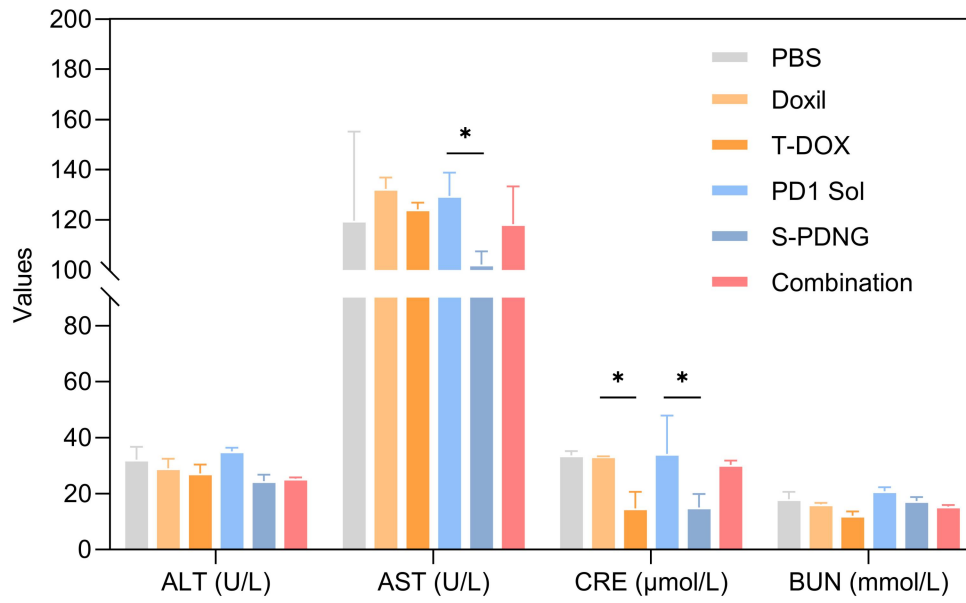


Figure S21 Blood chemistry analysis of the orthotopic 4T1 tumor-bearing mice after various treatments (n = 3). Significant values are presented as mean \pm SD; *P<0.05; **P<0.01; ***P<0.001.

Table S1 Antibodies used in the study.

Antibodies	Company	Catalog No.	Application	Dilution fold
Anti-CD4	ABclonal	A0362	WB, IF	500, 50
Anti-CD8a	Bioss	bs-10699R	WB, IF	1000, 200
Anti-CD81	ProteinTech	66866-1-Ig	WB	1000
Anti-TSG101	ProteinTech	67381-1-Ig	WB	5000
Anti-CRT	ProteinTech	27298-1-AP	WB, IF	2000, 250
APC Anti-PD-L1	Abcam	ab205921	FC	200
Anti-PD-L1	ProteinTech	66248-1-Ig	WB,IF	2000,50
FITC anti-CD3	Biolegend	100204	FC	200
PercP anti-CD4	Biolegend	100539	FC	200
APC anti-CD8A	Biolegend	300926	FC	200
PercP anti-CD3	Biolegend	103132	FC	200
PE anti-CD44	Biolegend	100422	FC	200
FITC anti-CD8	Biolegend	100706	FC	200
APC/Cy7 anti-CD62L	Biolegend	104428	FC	200
PE anti-CD86	Biolegend	105007	FC	200
FITC anti-CD80	Biolegend	104705	FC	200
PE anti-FoxP3	Biolegend	126403	FC	200
APC anti-IL-17a	Biolegend	506915	FC	200