Supporting Information

Spatially targeting and regulation of tumor-associated macrophages by raspberry-like micellar system sensitizes pancreatic cancer chemoimmunotherapy

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Supplementary Methods:

1. Preparation of Cy3-PCL-PEG and Cy5-DGL-PEG-PCL

PCL-PEG-COOH and Cy3-NH_2 were linked by an amide bond. Briefly, PCL-PEG-COOH, EDC and NHS were dissolved in dried DMSO in the molecular ratio of 1:1.5:1.5 and reacted at 30°C, and the one ratio of Cy3-NHS was added and reacted for 24 h. The product was obtained by dialysis and lyophilization. PCL-PEG-PEP-DGL and Cy5-NHS were mixed in PBS (pH 8.4) for 4 h, and the production was obtained by dialysis and lyophilization.

2. Preparation of Cy5 labeled D@PP

2.3 mg PCL-PEG-PEP-DGL and 2 mg PCL-PEG-OCH_3 were mixed in DMSO and injected into PBS (pH 8.4) under stirring, then 20 μL Cy5-NHS (5 mg/mL dissolved in DMSO) were added and continued stirring for 4 h, followed by dialysis in PBS (pH 7.4) for 24 h. The solution was concentrated by ultrafiltration. Cy5-DGL was synthesized accordingly.

3. Preparation of DiD encapsulated D@PP

1 mL DiD (100 μg/mL), 2.3 mg PCL-PEG-PEP-DGL and 2 mg PCL-PEG-OCH_3 were dissolved in DMSO and mixed, then the solution was slowly injected into warmed PBS under stirring. DMSO was removed by dialysis and un-encapsulated DiD was removed by filtration with 0.8 μm water filter membrane. The solution was concentrated by ultrafiltration. For the preparation of DiD/PCL-PEG, PCL-PEG-PEP-DGL was replaced by PCL-PEG-COOH. D@PP/DiD was incubated with excess papain for 1 h at 37℃.

4. Preparation of Cy5 and RhoB labeled D@PP or D-PP

Cy5 and RhoB dual labeled Cy5-D@PP/RhoB and Cy5-D-PP/RhoB was prepared by DMSO injection. RhoB was dissolved in DMSO and mixed with D@PP or D-PP (dissolved in DMSO). This solution was injected into warmed PBS (pH 8.4) and Cy5-NHS was added. After stirring for 4 h, the solution was dialysis in PBS for 24 h and filtrated with 0.8 μm water filter. Then, the solution was concentrated by ultrafiltration.

Table 1. Molecular weight of PCL-PEG measured by GPC.

Polymer	Weight average molecular weight (Mw)	Number average molecular Weight (Mn)	Polydispersity index (Mw/Mn)
PCL-PEG-OCH_3	11411	9072	1.258
PCL-PEG-COOH	11070	8882	1.246
PCL-PEG-PEP	13051	10227	1.860

Table 2. Particle size, PDI, potential and loading content of GEM and Wtmn of different nanoparticles (n = 3, mean ± SD).

Drug loaded nanoparticles	Size(nm)	PDI	Zeta potential(mV)	GEM (%)	Wtmn (%)
DGL/GEM	26.85±3.1	0.30±0.01	28.33±3.00	11.3	/
GD@PP	137.60 ±8.27	0.22±0.02	8.56±0.38	3.85	/
D@PP/Wtmn	102.32±10.86	0.291±0.01	16.76±0.51	/	1.51
GD-PP/Wtmn	103.1±2.14	0.245±0.01	4.87±0.64	3.77	1.50
GD@PP/Wtmn	107.93 ±4.25	0.225±0.03	4.91±0.45	3.76	1.50

Supporting Figures

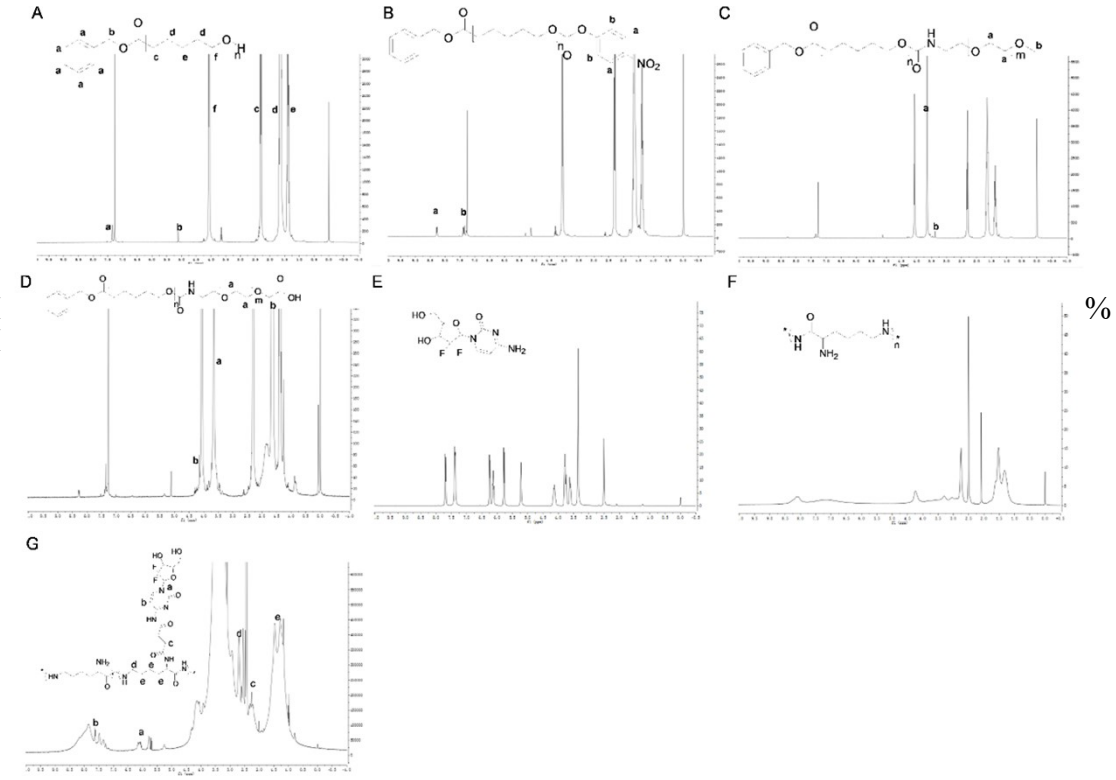

Figure S1 ^{1}H-NMR spectrum of PCL(A), PCL-NPC(B), PCL-PEG-OCH_3(C), PCL-PEG-COOH(D), GEM (E), DGL (F), GEM-DGL (G). PCL, PCL-NPC, PCL-PEG-OCH_3 and PCL-PEG-COOH were dissolved in $CDCL_3$. Other substances were dissolved in DMSO-d_6.

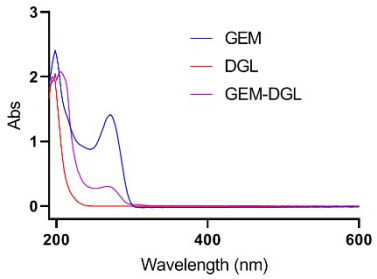

Figure S2 UV spectrum of GEM, DGL and GEM-DGL.

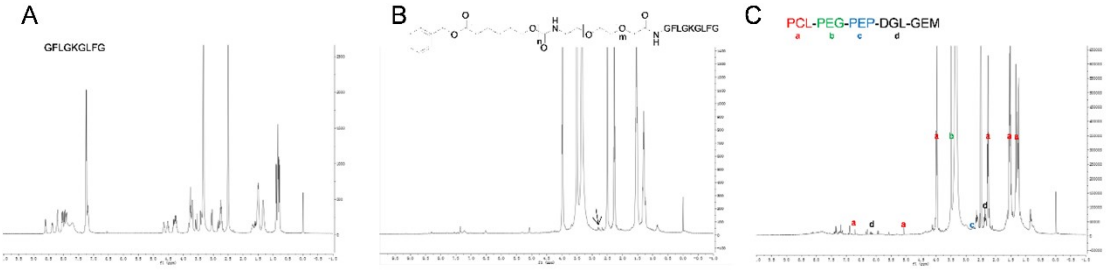

Figure S3 ^{1}H-NMR of substrate peptide GFLGKGLFG (A), PCL-PEG-PEP (B) and PCL-PEG-PEP-DGL-GEM (C) dissolved in DMSO-d_6.

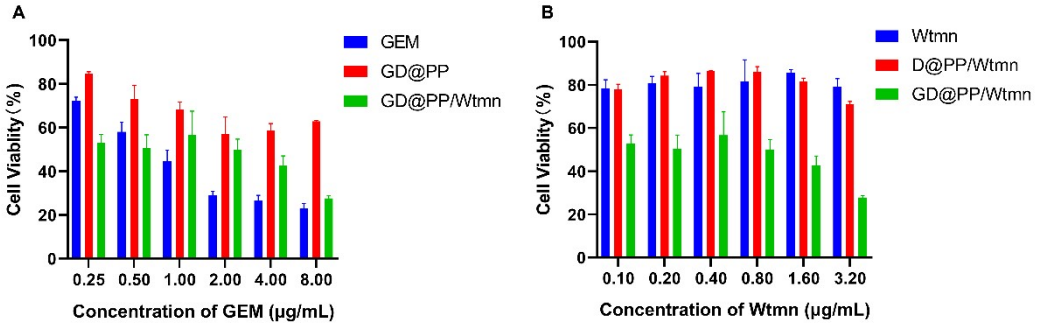

Figure S6 Cell viability of Pan 02 cells after treated with GEM or Wtmn loaded preparations for 48 h (n = 3, mean ± SD).

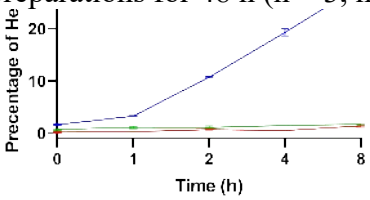

Figure S5 Hemolysis rate of blank carriers (n = 3, mean ± SD).

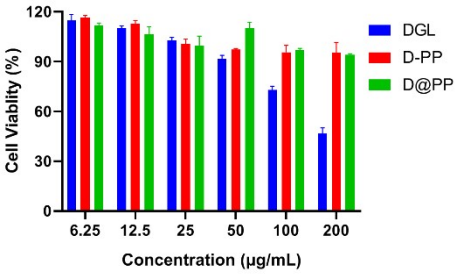

Figure S7 Cytotoxicity of blank carriers on Pan 02 (A) and RAW264.7 cells (B) (n = 3, mean ± SD).

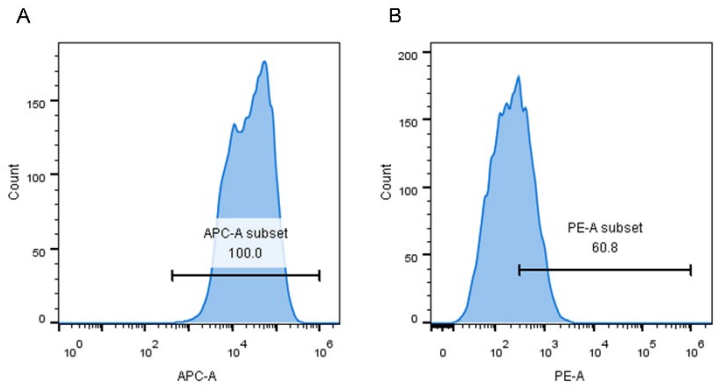

Figure S8 Expression of F4/80 and CD206 in M2 TAMs.

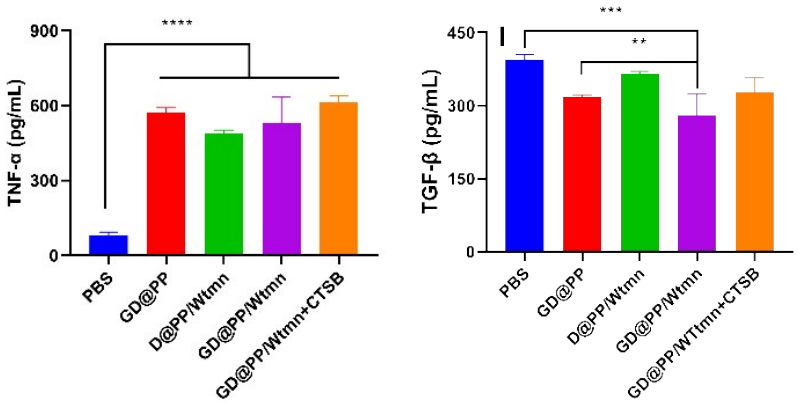

Figure S9 The level of TNF-α and TGF-β in the lower chamber analyzed by ELISA. (n=3, means ± SD).

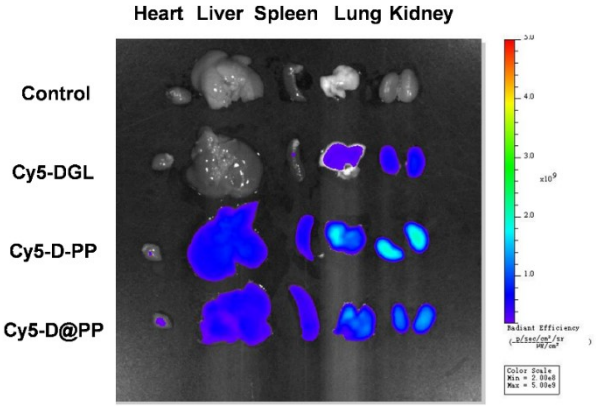

Figure S10 Ex vivo imaging of major organs after intravenous injection of Cy5 labelled nanoparticles.

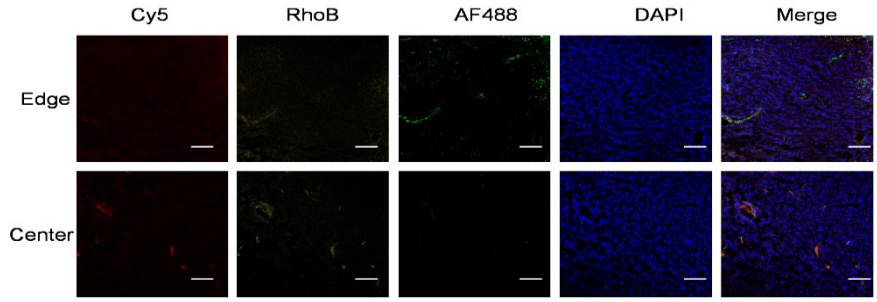

Figure S11 Fluorescence distribution of Cy5-D-PP/RhoB within tumor sections in edge and deep region of tumor. Red: Cy5-DGL, Yellow: PCL-PEG/RhoB, Green: AF488-CD31, Blue: DAPI. Scale bar = 100 μm.

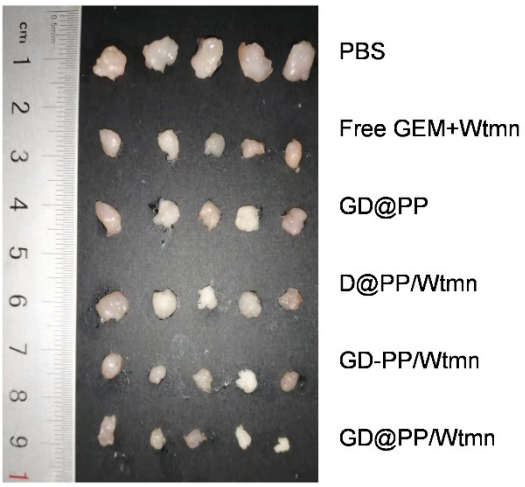

Figure S12 Images of tumors after treatment in each group.

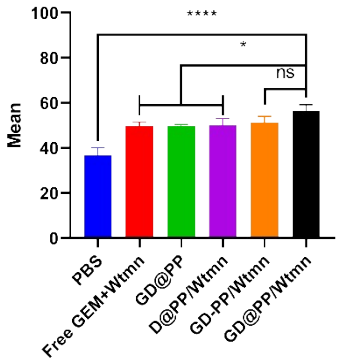

Figure S13 Semi-quantified results of TUNEL staining images of tumors by Image J.

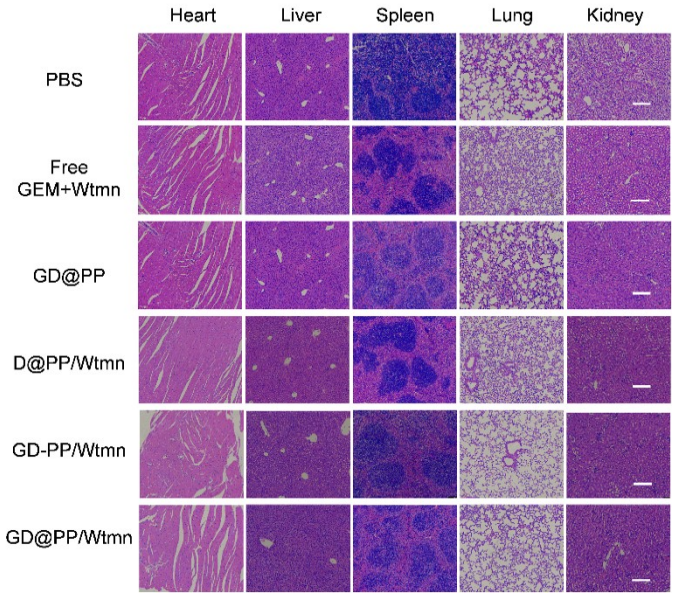

Figure S14 H&E staining images of major organs, scale bar = 200 μm

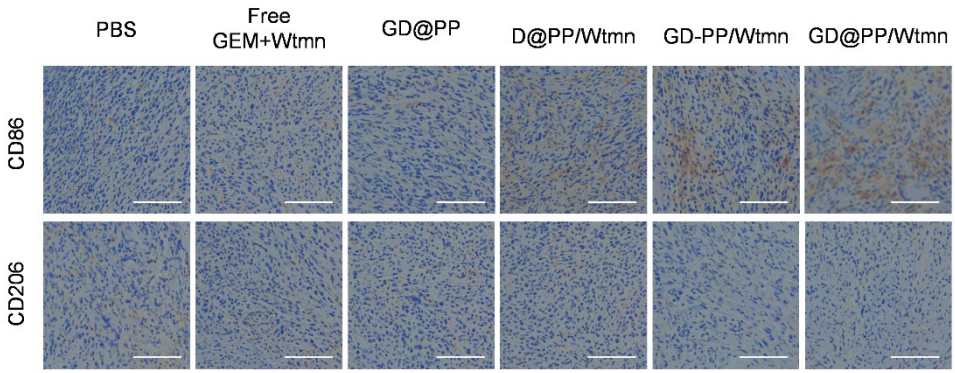

Figure S15 Immunohistochemical staining of CD86 and CD206 of tumor sections after treatment. Scale bar = 100 μm.