

Supplementary information

Platelet backpacking nanoparticles based on bacterial outer membrane vesicles enhanced photothermal- immune anti-tumor therapy

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Supplementary tables and figures:

	Size (nm)	Zeta Potential (mV)	Particles concentration ($\times 10^8 / \mu\text{g protein}$)
OMVs	150.2 ± 0.97	-27.38 ± 0.83	3.614 ± 0.2
IR780-SLN	61.60 ± 1.2	6.96 ± 1.8	—
IR780-SLN@O-P	82.46 ± 0.5	-28.57 ± 0.76	—

Table S1 Particle size and zeta potential of OMVs and prepared nanoparticles. (n = 3, mean \pm SD)

	EE (%)
IR780-SLN	$87.59\% \pm 3.0\%$
IR780-SLN@O-P	$89.52\% \pm 0.19\%$

Table S2 Encapsulation efficiency (EE) of IR780. (n = 3, mean \pm SD)

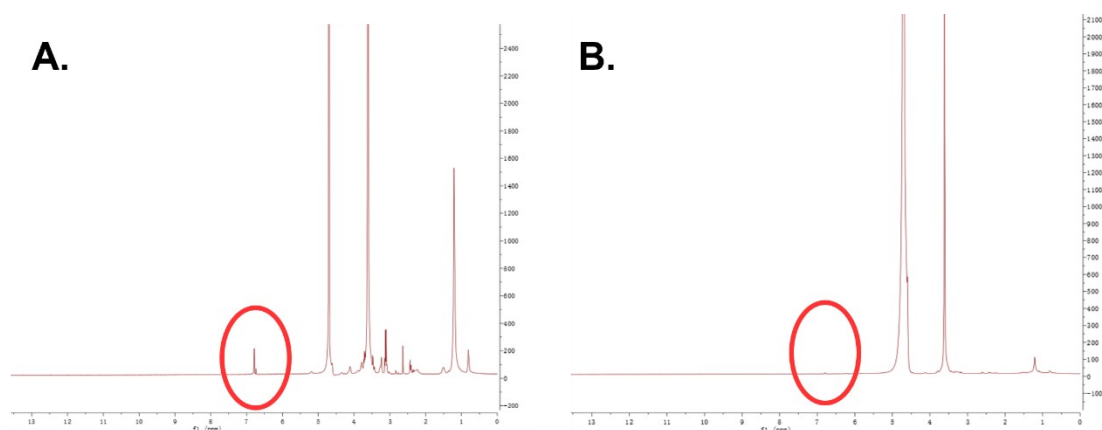


Fig S1 ^1H NMR spectrum (**A.**) the reactant DSPE-PEG₂₀₀₀-Mal, (**B.**) the product PSN-DP. (Red circles indicate characteristic peak locations)

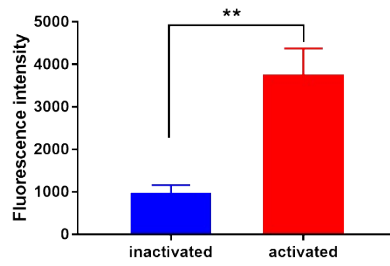


Fig S2 Changes of CD62P expression in platelets before and after activation. (n = 3, mean \pm SD, **p < 0.01)

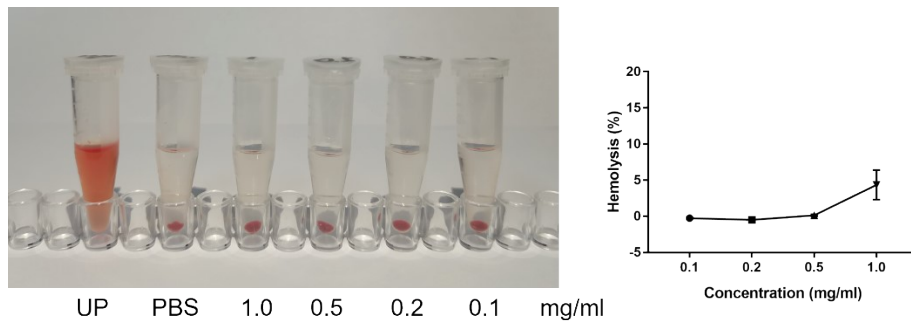


Fig S3 Blood compatibility test of SLN@O-P nanoparticles. (incubated with 2% red blood cell suspension, n = 3, mean \pm SD)

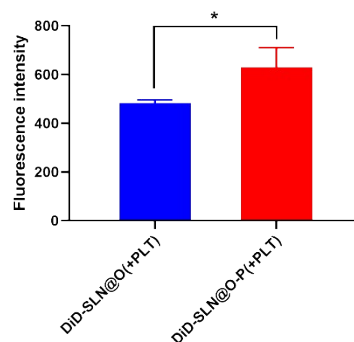


Fig S4 Flow cytometry data of SLN@O and SLN@O-P nanoparticles uptaken by 4T1 cells after pre-incubation with activated platelets. (Nanoparticles labeled with fluorescent dye DiD) (n = 3, mean \pm SD, *p < 0.05)

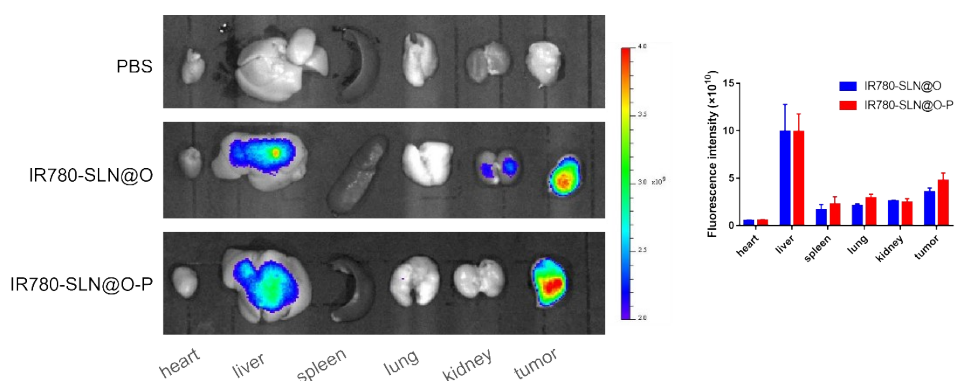


Fig S5 Images of major organs and tumors *ex vivo* and semi-quantitative statistics of fluorescence intensity after 24 hours. (n = 3, mean \pm SD)

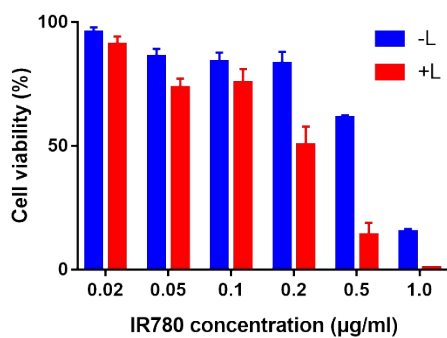


Fig S6 Cytotoxicity of free IR780 to 4T1 cells. (+L means laser irradiation, -L means without. n = 3, mean \pm SD)

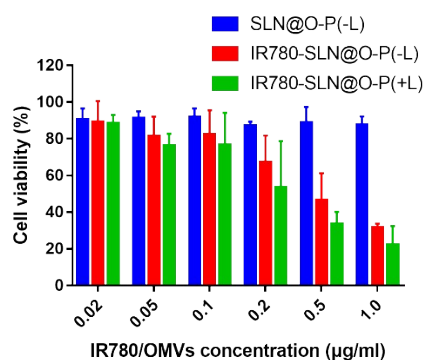


Fig S7 Cytotoxicity of nanoparticles (IR780-SLN@O-P and SLN@O-P) to 4T1 cells. (+L means laser irradiation, -L means without. n = 3, mean \pm SD)

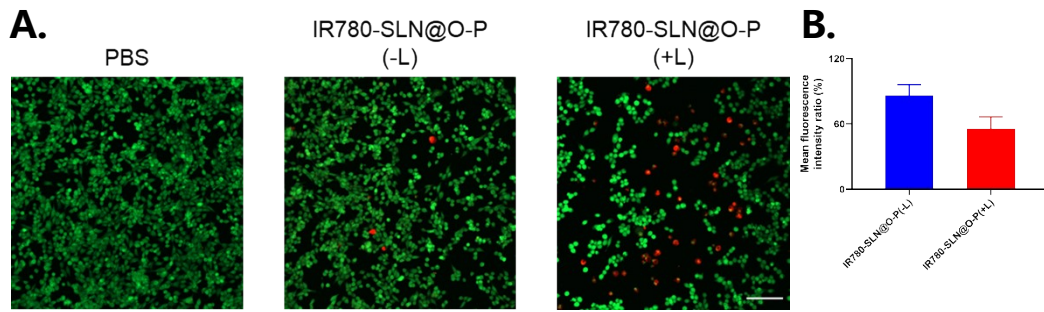


Fig S8 (A.) CLSM images of 4T1 cell activity affected by IR780-SLN@O-P NPs with or without laser irradiation. (Green fluorescence of calcein-AM labeled living cells and red fluorescence of Propidium Iodide labeled dead cells. Scale bar: 100 μ m) **(B.)** Semi-quantitative statistics of fluorescence intensity of live cells (green) compared with PBS group.

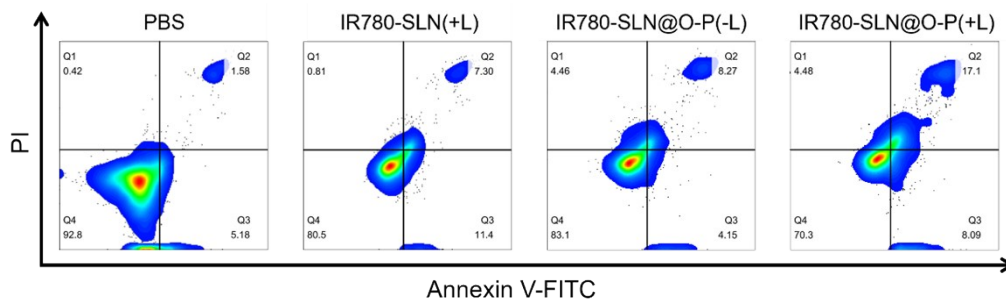


Fig S9 Flow cytometry images of apoptosis after different nanoparticle treatment and laser irradiation.

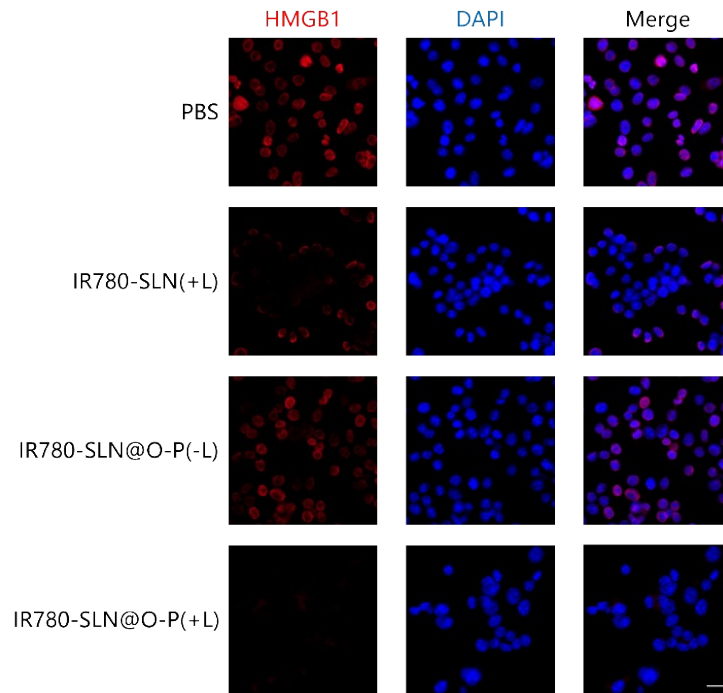


Fig S10 CLSM images of HMGB1 released from 4T1 cells after different nanoparticle treatments and laser irradiation. (Red fluorescence AF647 labeled HMGB1. Scale bar: 20 μm)

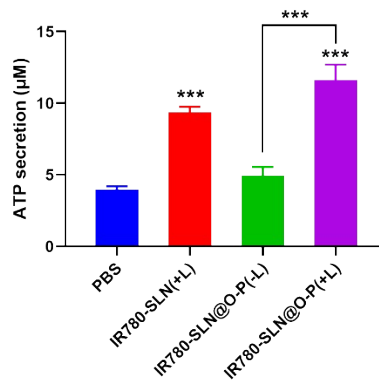


Fig S11 ATP secretion in 4T1 cells after different nanoparticle treatments and laser irradiation. (n = 3, mean \pm SD, ***p < 0.001)

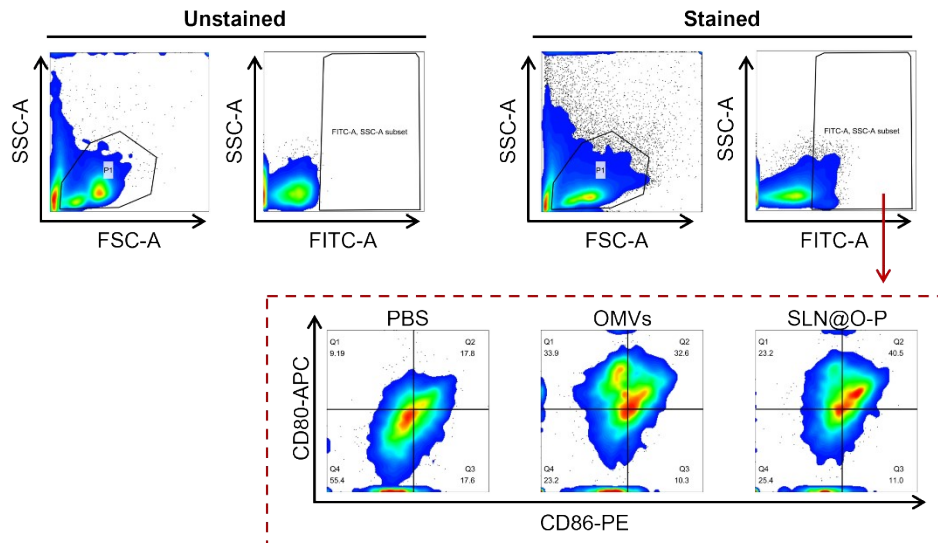


Fig S12 Flow cytometry procedure and gating strategy for the detection of mature DCs in draining lymph nodes.

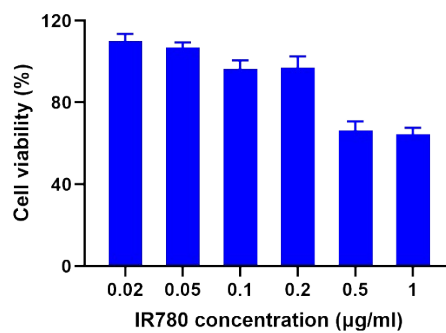


Fig S13 Cytotoxicity of IR780-SLN@O-P nanoparticles to platelets. (n = 3, mean ± SD)

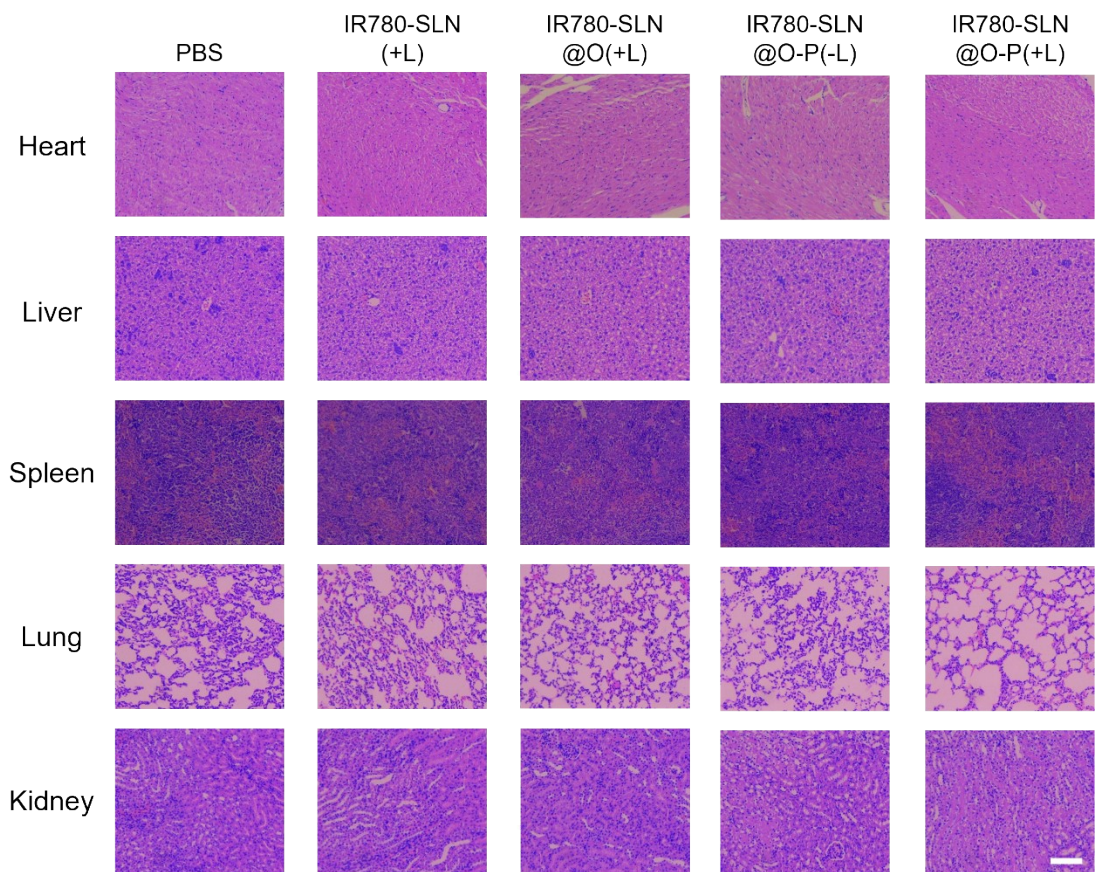


Fig S14 HE stained section images of major organs from 4T1-bearing orthotopic tumor mice after treatment. (scale bar: 200 μm)

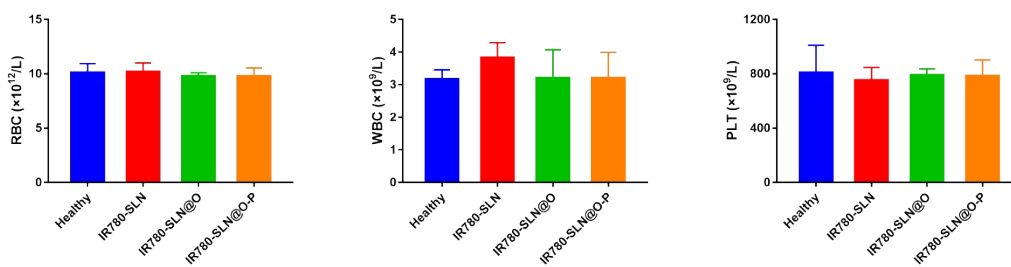


Fig S15 Routine blood test of healthy mice after injection. (n = 3, mean ± SD)

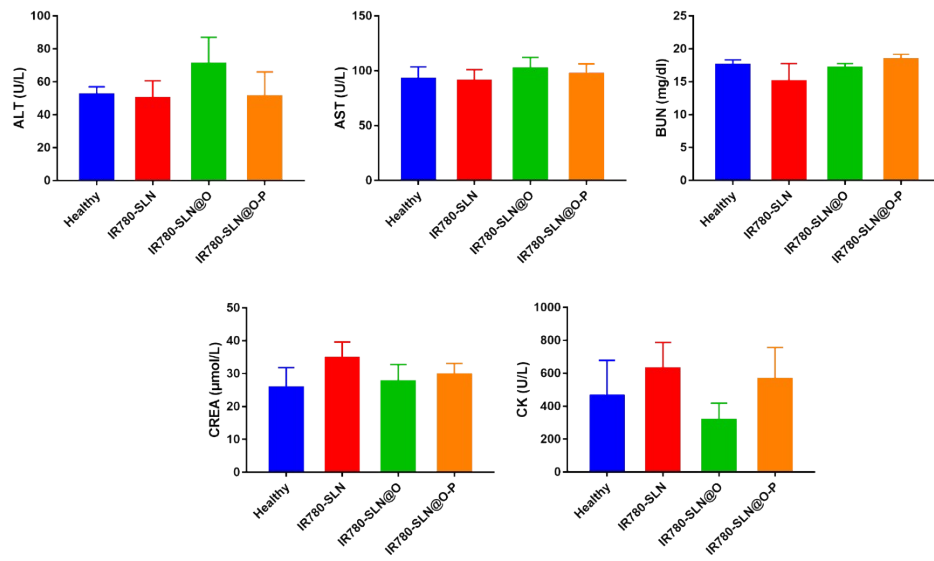


Fig S16 Serum biochemical indices of healthy mice after injection. (n = 3, mean \pm SD)