

Supplementary Materials for

Engineering autologous tumor cell vaccine to locally mobilize antitumor immunity in tumor surgical bed

Lei Fang, Zitong Zhao, Jue Wang, Pengcheng Zhang, Yaping Ding, Yanyan Jiang, Dangge Wang*, Yaping Li*

*Corresponding author. Email: dgwang@simm.ac.cn (D.W.); ypli@simm.ac.cn (Y.L.)

Published 19 June 2020, *Sci. Adv.* **6**, eaba4024 (2020)

DOI: [10.1126/sciadv.aba4024](https://doi.org/10.1126/sciadv.aba4024)

This PDF file includes:

Table S1

Figs. S1 to S9

Table S1. List of antibodies used for flow cytometric examination.

Antibody	Clone	Fluorophore	Detector	Company
CD3e	145-2C11	PerCP-Cy5.5	FL3-H	eBioscience
CD8a	53-6.7	FITC	FL1-H	eBioscience
CD8a	53-6.7	PE	FL2-H	eBioscience
CD86	GL1	PE	FL2-H	eBioscience
CD4	H129.19	FITC	FL1-H	BD Pharmingen
CD11c	N418	FITC	FL1-H	BD Pharmingen
CD80	16-10A1	PE	FL2-H	BD Pharmingen
CD86	GL1	PE-Cy7	FL3-H	BD Pharmingen
CD25	3C7	APC	FL4-H	BD Pharmingen
FoxP3	MF23	PE	FL2-H	BD Pharmingen
IFN- γ	XMG1.2	FITC	FL1-H	BD Pharmingen
CD44	IM7	PerCP-Cy5.5	FL3-H	BioLegend
CD127	A7R34	PE	FL2-H	BioLegend
OVA tetramer-SIINFEKL	-	PE	FL2-H	MBL

Supplemental Figures

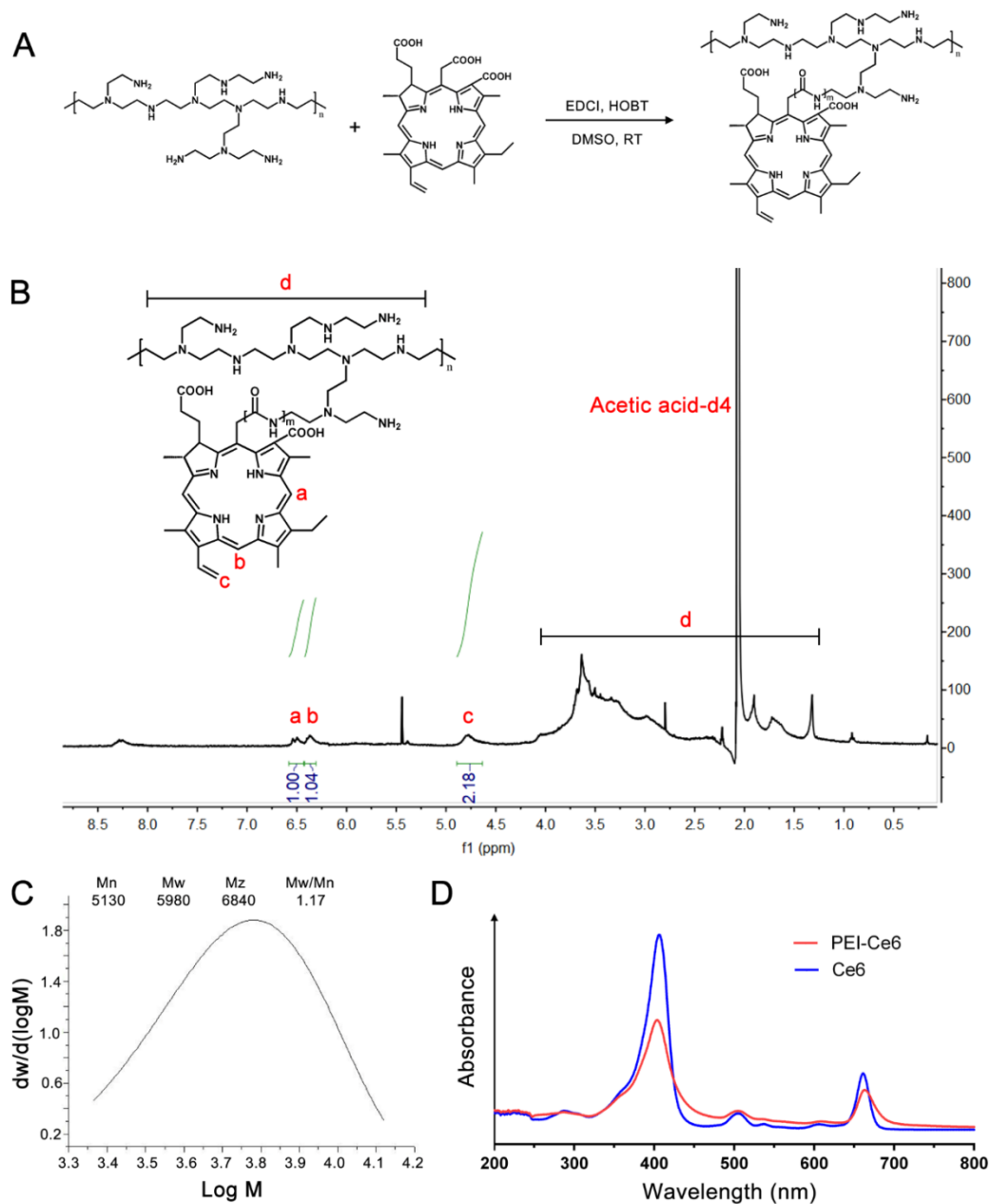


Fig. S1. Synthesis and characterization of PEI-Ce6. (A) Synthetic route of PEI-Ce6.

(B) $^1\text{H-NMR}$ spectra of PEI-Ce6 in acetic acid- d_4 .

(C) Gel permeation chromatography (GPC) examination of molecular weight and polydispersity index

(PDI) of PEI-Ce6. **(D)** UV-vis spectrum of PEI-Ce6 and Ce6.

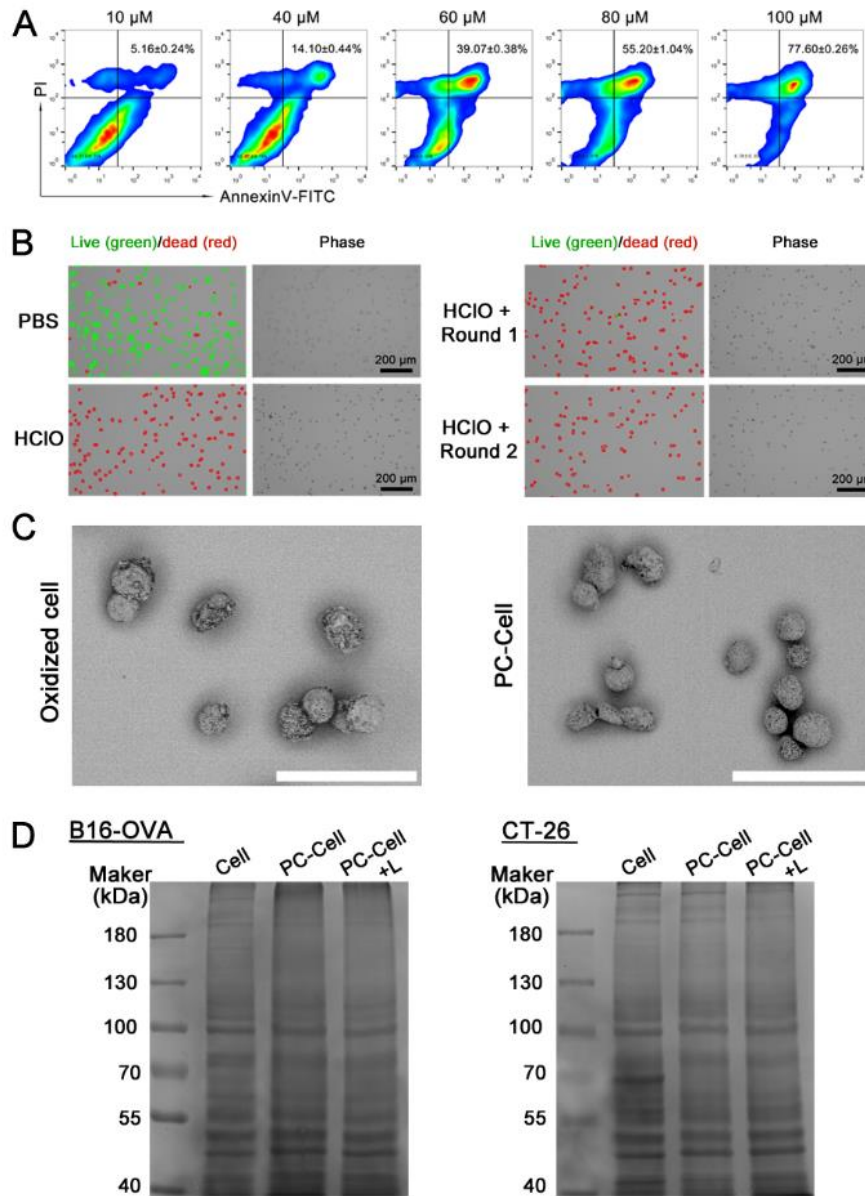


Fig. S2. Apoptosis, SEM images and protein electrophoresis of oxidized tumour cells and PC-Cell. (A) Screening of HClO concentrations to induce apoptosis/necrosis in tumour cells by Annexin V/PI apoptosis detection kits. (B) Live-dead staining assay and phase images of tumour cells treated by HClO and freeze-thaw cycles. (C) SEM images of oxidized tumour cells and PC-Cell. Scale bar, 20 μm. (D) SDS-PAGE electrophoresis of proteins in B16-OVA and CT26-derived PC-Cell complexes with or without laser irradiation (300 mW/cm², 5 min).

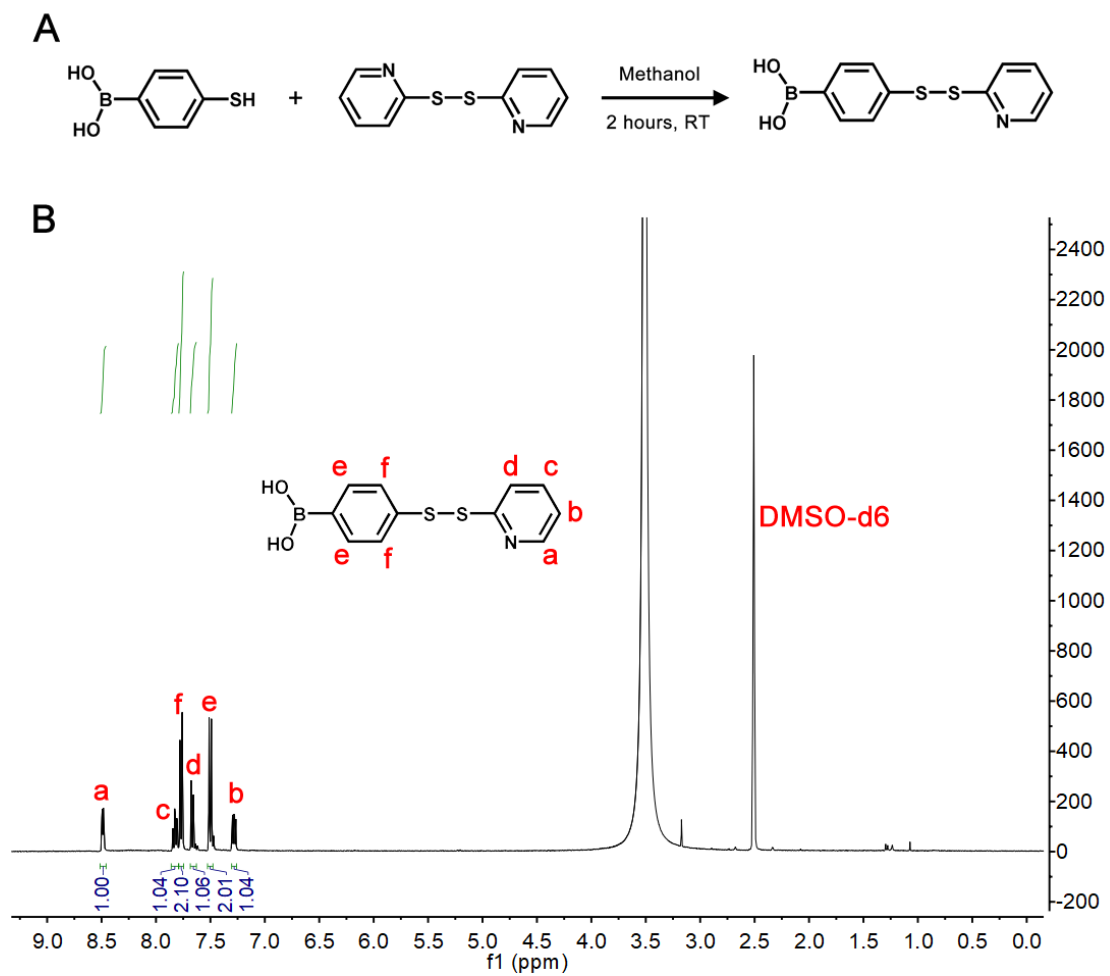


Fig. S3. Synthesis of 4-(dipyridyl disulfide)-phenylboronic acid. (A) Synthetic route. **(B)** ¹H-NMR spectra of the product in DMSO-d₆.

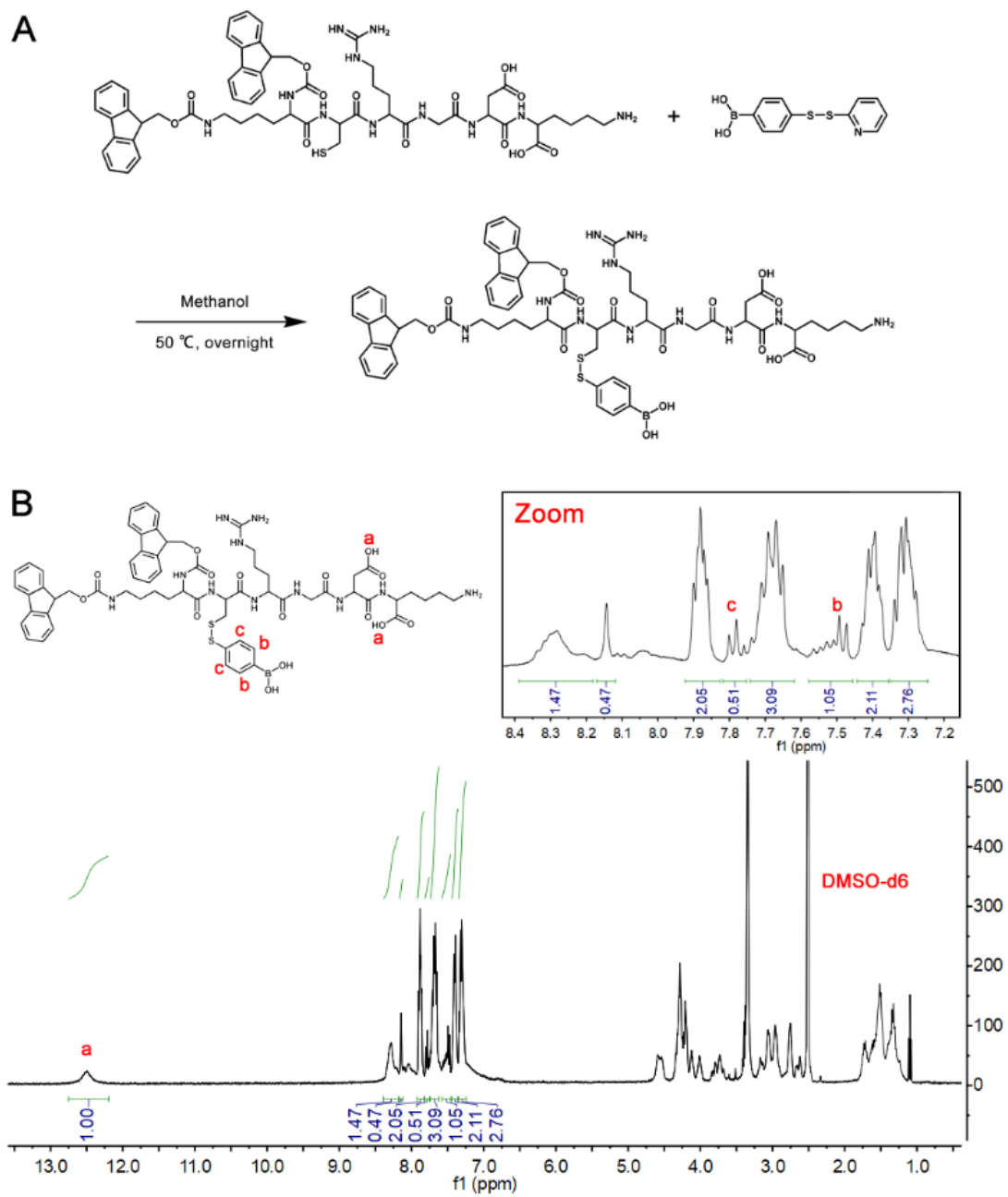


Fig. S4. Synthesis and characterization of FK-PBA. (A) Synthetic route of FK-PBA. (B) $^1\text{H-NMR}$ spectra of FK-PBA in DMSO- d_6 .

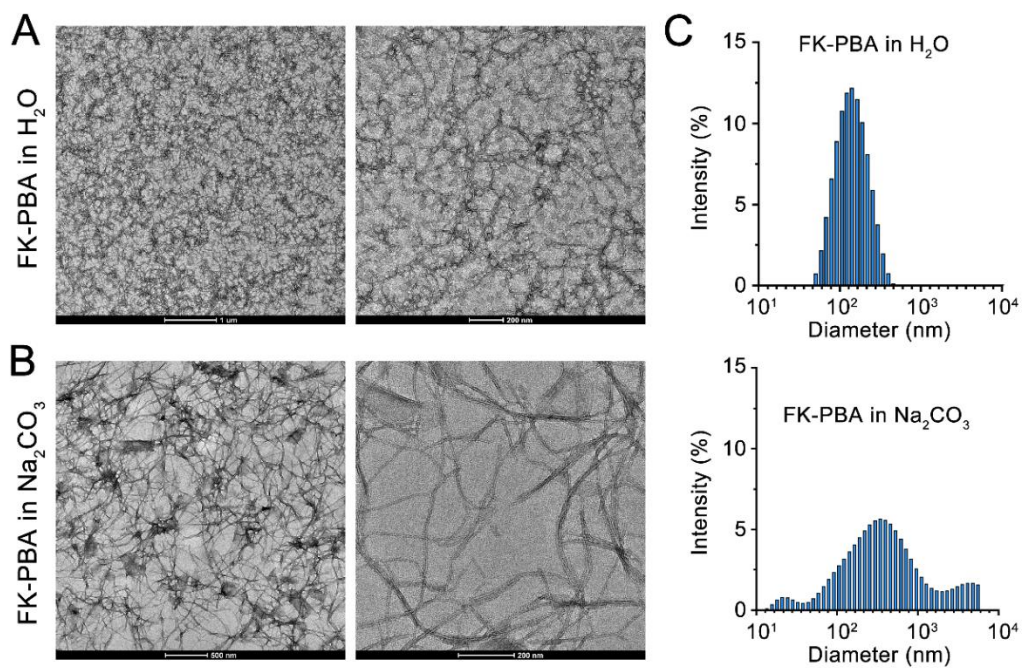


Fig. S5. TEM and DLS examination of FK-PBA. (A) TEM of FK-PBA dispersed in H₂O. (B) TEM of FK-PBA dispersed in 1 mM of Na₂CO₃. (C) DLS examination of FK-PBA in different medium.

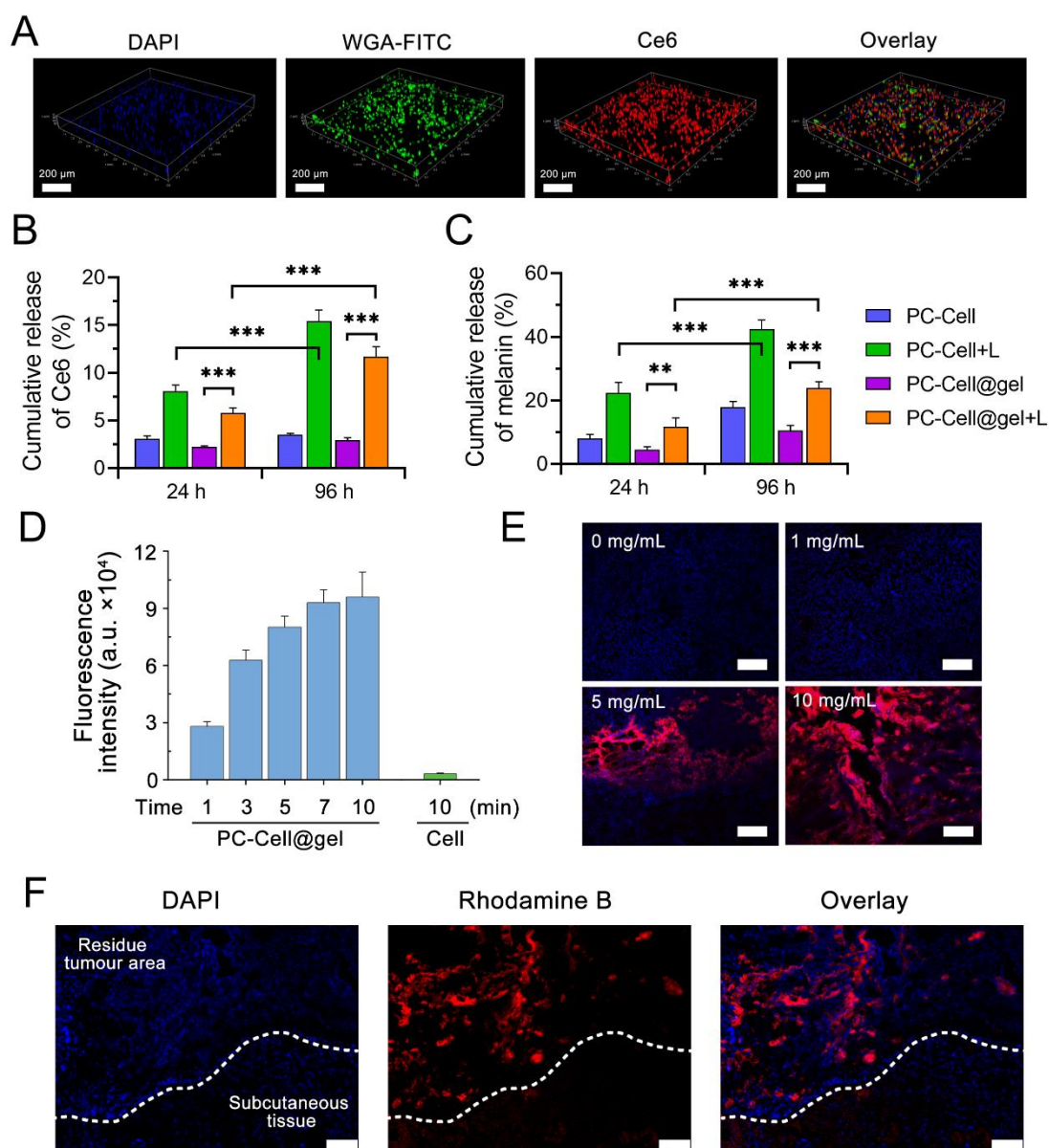


Fig. S6. Release, photodynamic property of PC-Cell@gel and its on-demand gelation in tumour surgical bed. (A) 3D construction image of FK-PBA hydrogel encapsulating PC-Cell. Cell membrane was labeled with WGA-FITC. Scale bar, 200 μ m. (B) Cumulative release of Ce6, and (C) melanin from B16-F10-Luc cell-driven PC-Cell or PC-Cell@gel after laser irradiation (300 mW/cm², 5 min) at 24 h and 96 h, respectively. (D) ROS generation examined by SOSG probe after irradiated by 655 nm laser for various time duration. The Ce6 concentration and laser power density

were fixed at 10 $\mu\text{g}/\text{mL}$ and 300 mW/cm^2 , respectively. (E) Postoperative B16-OVA tumour-bearing mice were first locally injected with FK-PBA at 1, 5 and 10 mg/mL , respectively. Rhodamine B dispersed in 1 mM Na_2CO_3 solution was sequentially injected at 4 h. Tissues in surgical bed were collected and sliced for CLSM imaging at 72 h. Scale bar, 100 μm . (F) Residual tumour was harvested from mice treated by 10 mg/mL of FK-PBA and sliced for CLSM imaging at 72 h. Scale bar, 100 μm . Data are means \pm SD. $**P < 0.01$, $***P < 0.001$.

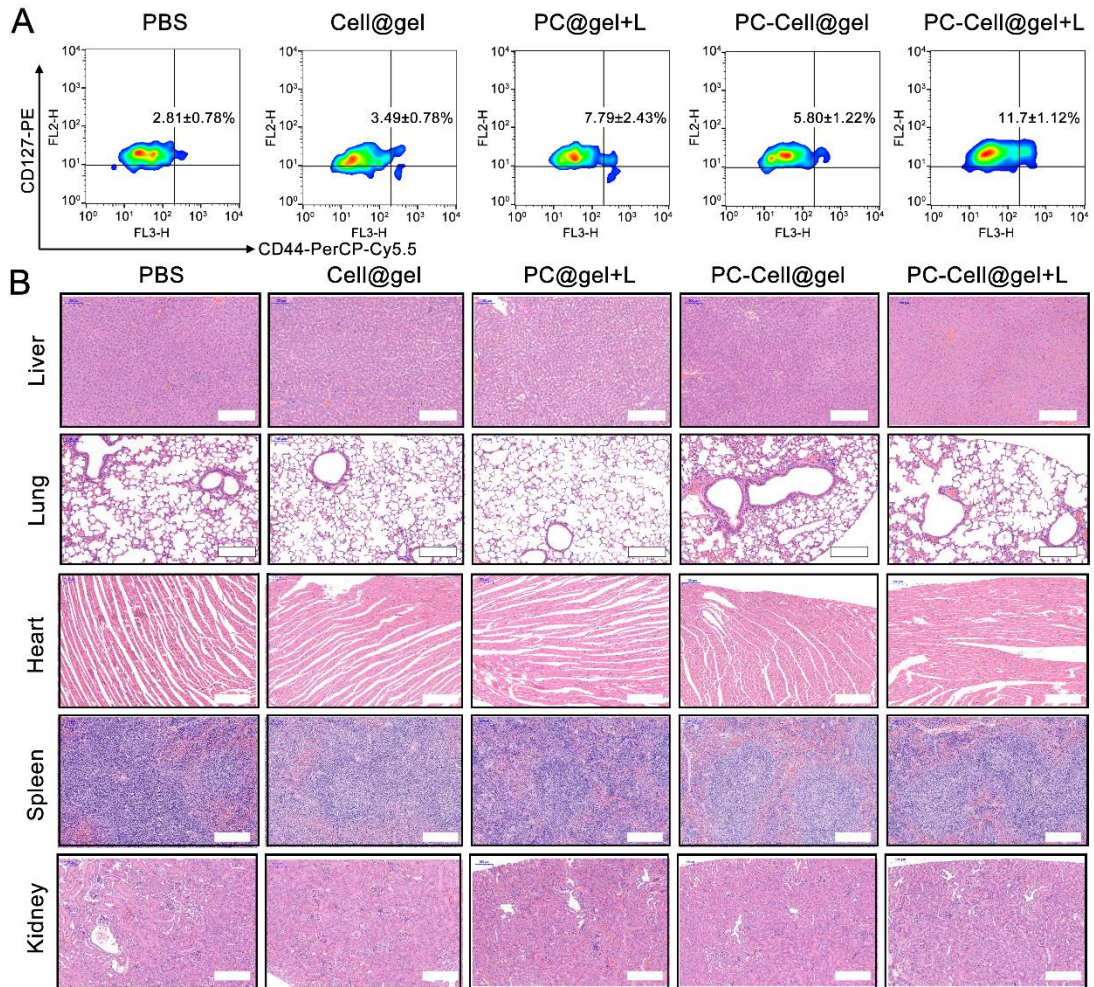


Fig. S7. Immune memory effects and histochemical assay in vivo. (A) Frequency of central memory CD8⁺ T cells in lymph nodes on day 30 of the anti-relapse study. (B) H&E staining of major organs (liver, lung, heart, spleen and kidney). Scale bar, 200 μ m.

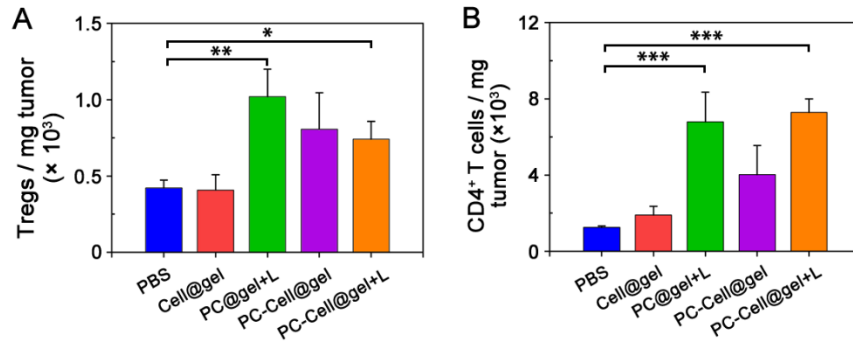


Fig. S8. Weight-normalized number of (A) Tregs, and (B) CD4⁺ T cells in tumour surgical bed in control and treated groups (n = 3). Data are means \pm SD. * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$.**

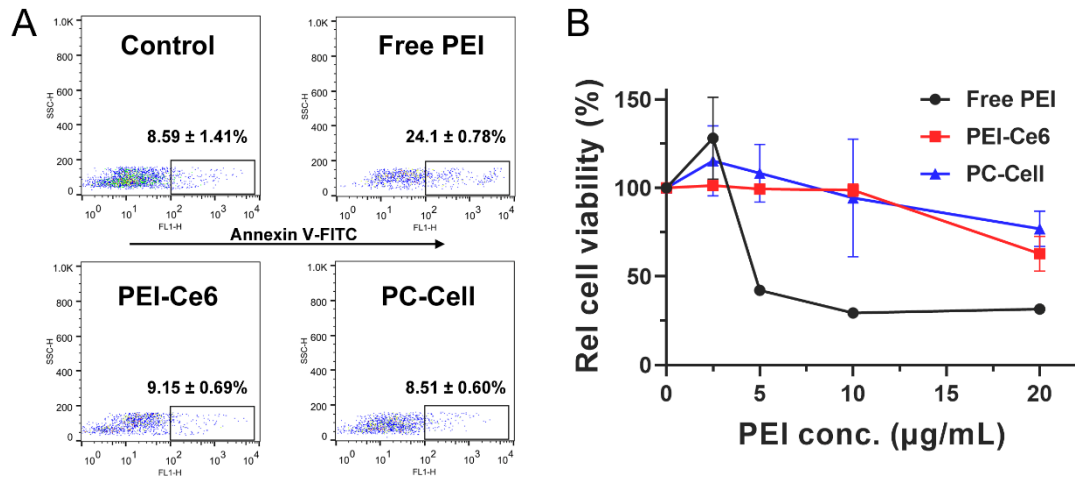


Fig. S9. Cytotoxicity analysis of PEI and PC-Cell on tumour-infiltrated lymphocytes ex vivo. (A) Apoptosis examination of samples with equal 5.0 µg/mL of PEI treated at 4 h. **(B)** Relative cell viability of lymphocytes incubated with various suspensions for 48 h were examined by CCK-8 assay kit. Data are means ± SD.