Electronic Supplementary Material

Peptide nanotube loaded with a STING agonist, c-di-GMP, enhance cancer immunotherapy against melanoma

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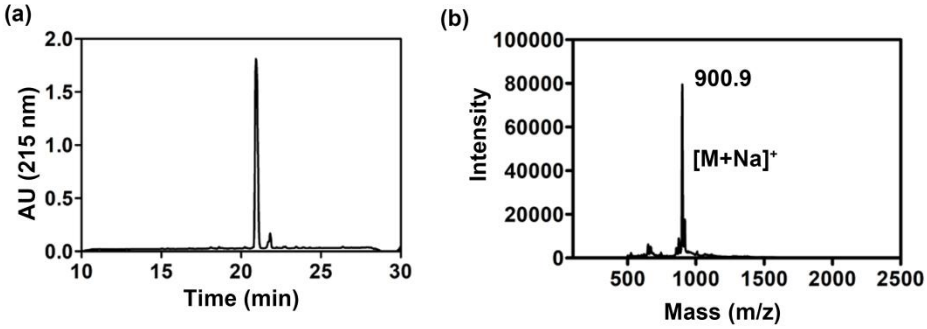

Figure S1 Synthesis and characterization of KL-7. (a) KL-7 peptide was purified by RP-HPLC (Column, Welch, C18, 300 Å, 10 × 250 mm). Linear acetonitrile gradient elution (20%–60% acetonitrile/0.1% TFA) was performed at the flow rate of 3 mL·min^{-1} and the detection wavelength was 215 nm. (b) The molecular weight of the KL-7 peptide was determined by MALDI-TOF MS.

Table S1 The encapsulation rate and cumulative release rate of c-di-GMP-PNT

Sample	Encapsulation efficiency (%)	Cumulative release (%)
c-di-GMP-PNT	51.64	98.8

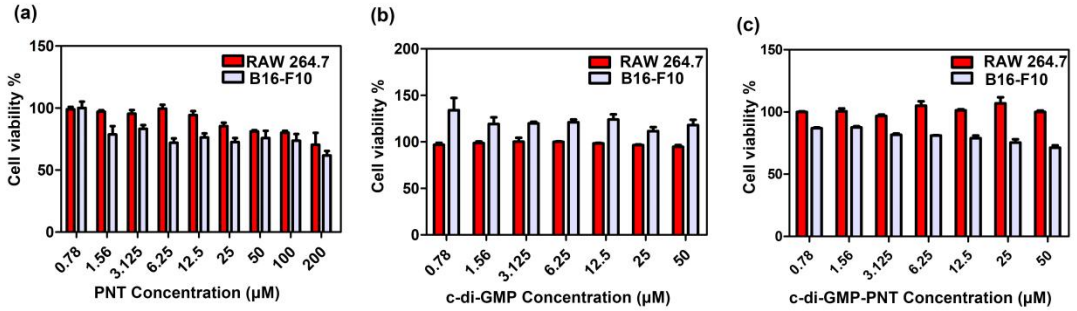

Figure S2 Cytotoxicity was determined by the MTT method. (a)–(c) RAW 264.7 cells and B16-F10 cells were treated with PNT, c-di-GMP, and c-di-GMP-PNT for 24 h (Bottom). Data are shown as mean ± standard deviation (n = 3).

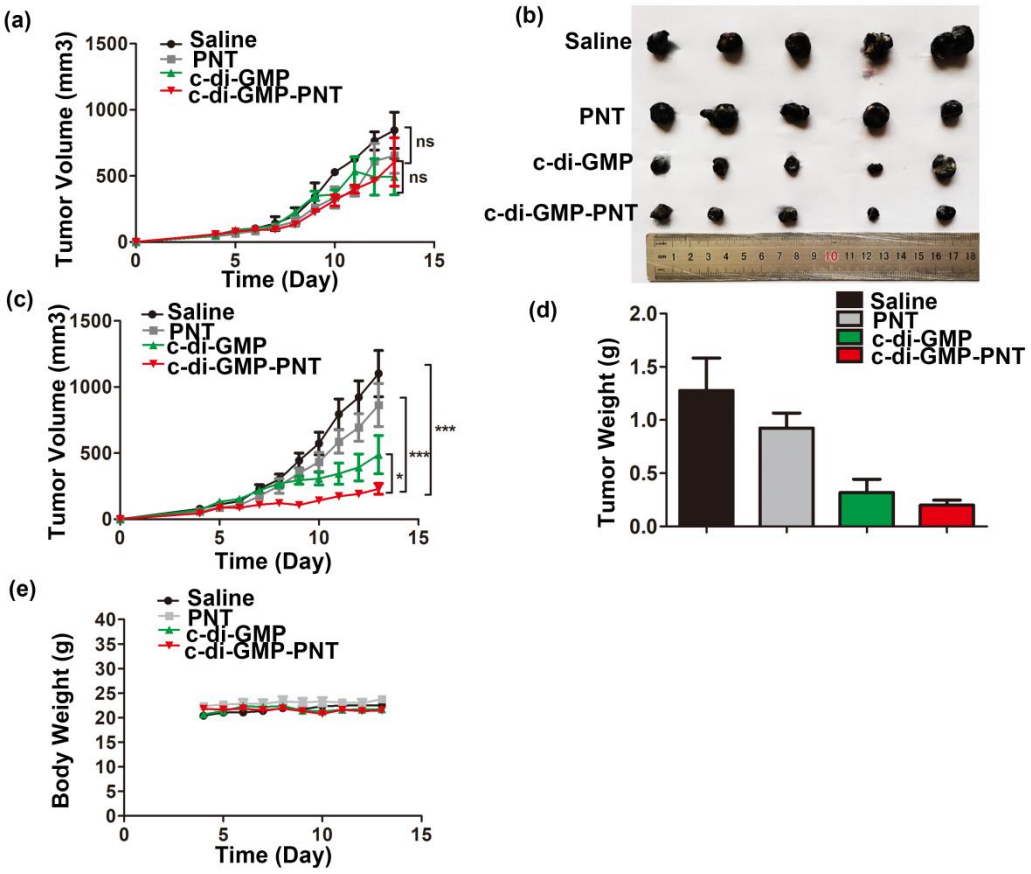

Figure S3 PNT enhances the antitumor effect of c-di-GMP *in vivo*. Schematic diagram of the therapeutic unilateral tumor-bearing mouse model. Balb/c mice were subcutaneously injected with 2×10^6 B16-F10 cells in the flank at day 0. Intratumoral injections of c-di-GMP (1 μg per mouse) and c-di-GMP-PNT (equivalent to 1 μg c-di-GMP per mouse), were started on the 4th, 6th, and 8th. The tumor volume (a) was monitored. Intratumoral injections of c-di-GMP (5 μg per mouse) and c-di-GMP-PNT (equivalent to 5 μg c-di-GMP per mouse), were started on the 4th, 6th, and 8th. All mice were sacrificed on day 13, representative tumors in each group (b) and the average tumor weight (d) were examined, respectively. The tumor volume (c) and body weight (e) were monitored. Data are shown as mean ± SD (*$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$). ns: no significant differences ($p > 0.05$).

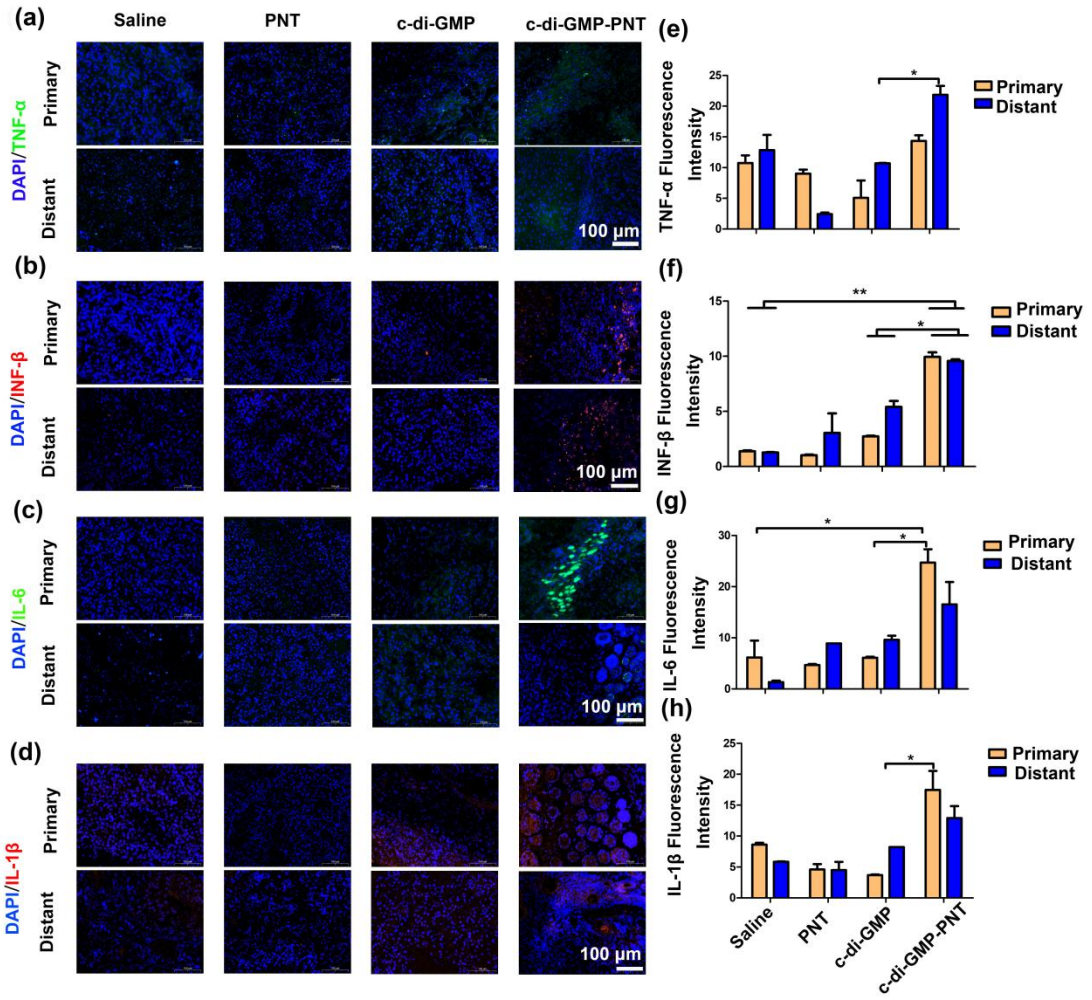

Figure S4 Systemic anti-tumor immune response to treat bilateral tumors. The bilateral B16-F10 tumor model, with saline, PNT, free c-di-GMP (10 μg per mouse) and c-di-GMP-PNT (equivalent to 10 μg c-di-GMP per mouse) was injected into the primary tumor *in situ*. And without treatment of distant tumors. (a)–(d) Tumor slices were stained with INF-β, TNF-α, IL-6, and IL-1β antibodies. (e)–(h) By Image J quantitative analysis of the INF-β, TNF-α, IL-6, and IL-1β positive fluorescence intensity acquired from (a)–(d), respectively. All scale bars: 100 μm. Data are shown as mean ± SD (*$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$).

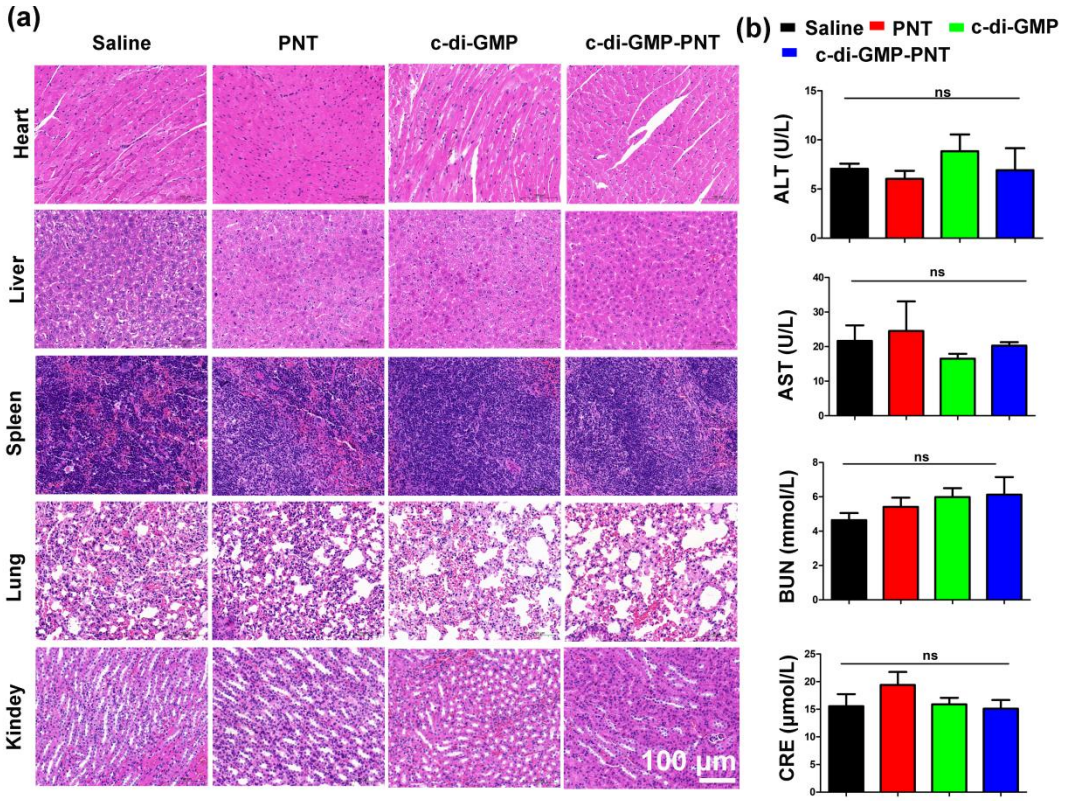

Figure S5 Safety evaluation was conducted for different treatment groups. (a) H&E morphological evaluation of the heart, liver, spleen, lung, and kidney of tumor-bearing mice, scale bars: 100 μm. (b) Blood biochemical marker analysis of tumor-bearing mice. ns: no significant differences ($p > 0.05$).