Supplementary Material

Nanoparticle Delivery Improves the Pharmacokinetic Properties of Cyclic Dinucleotide STING Agonists to Open a Therapeutic Window for Intravenous Administration

Mohamed Wehbe, Lihong Wang-Bishop, Kyle W. Becker, Daniel Shae, Jessalyn J. Baljon, Xinyi He, Plamen Christov, Kelli L. Boyd, Justin M. Balko, and John T. Wilson

Supplementary Figures:

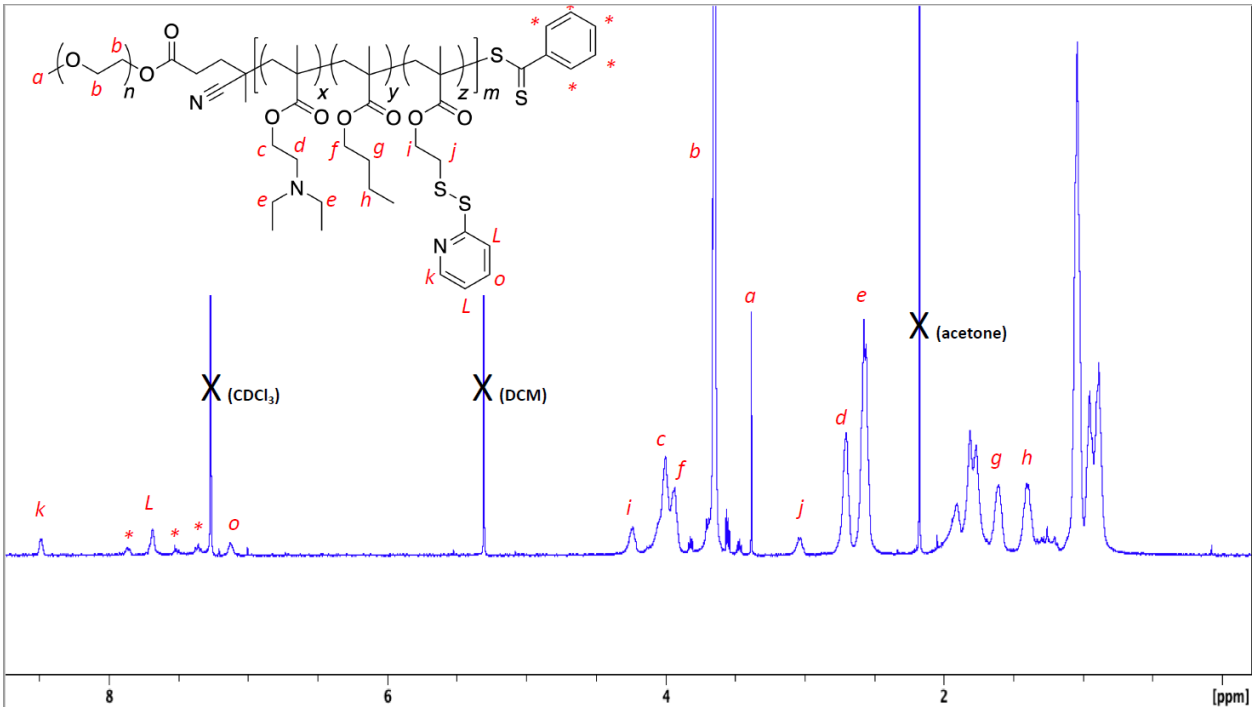

Figure S1: Representative 1 H NMR spectrum of Poly[(ethylene glycol)-*block*-[(2-diethylaminoethyl methacrylate)-*co*-(butyl methacrylate)-*co*-(pyridyl disulfide ethyl methacrylate)].

Table S1: Polymer Characterization

Polymer	DEAEMA(%)	BMA (%)	PDSMA (%)	2nd block DP	Total Molecular Weight (KDa)*
PEG2$_{kDa}$-bDBP$_{4.5kDa}$	50.6	42	7.4	26.5	6.85

* Molecular weight was estimated based on percent monomer conversion as determined by ^{1}H NMR.

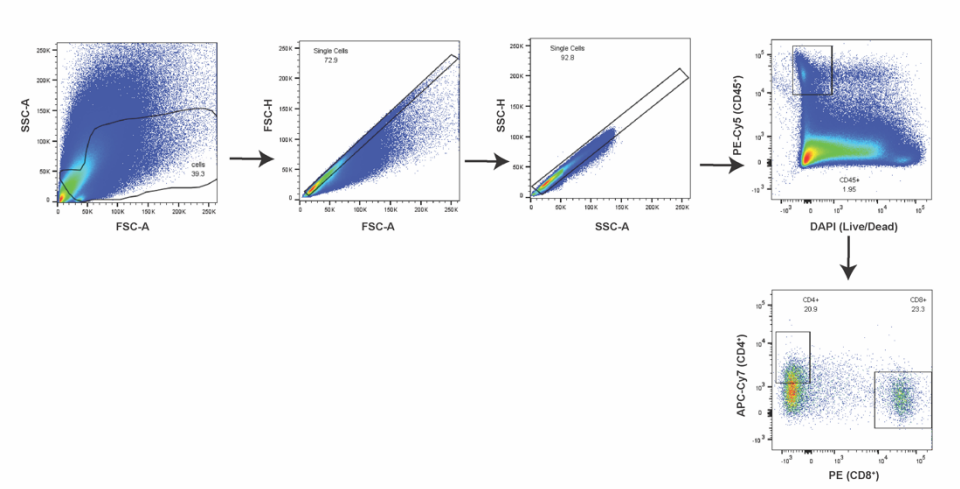

Figure S2: Gating scheme for flow cytometric analysis of T- cell populations in the TME.

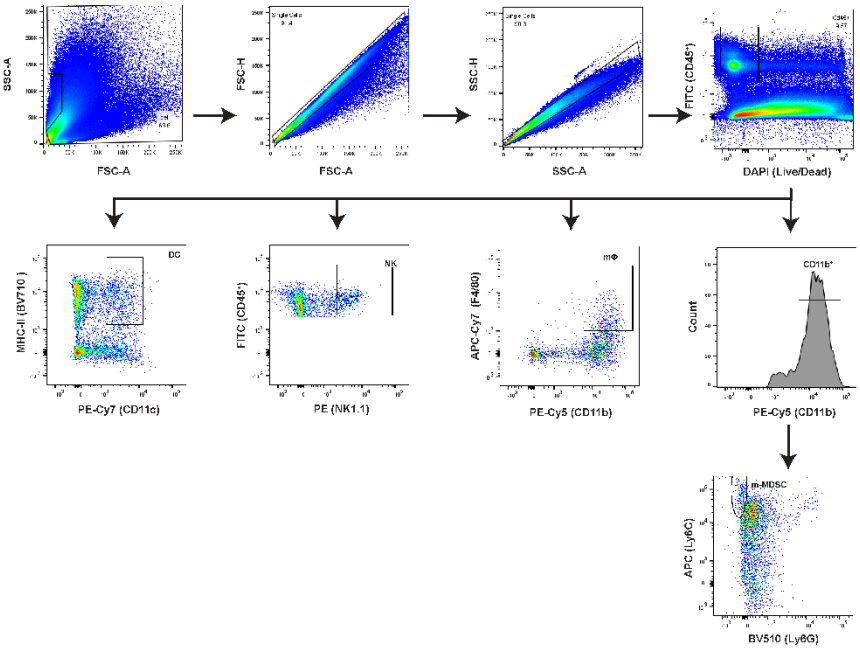

Figure S3: Gating scheme for flow cytometric analysis of immune cell populations in the TME.

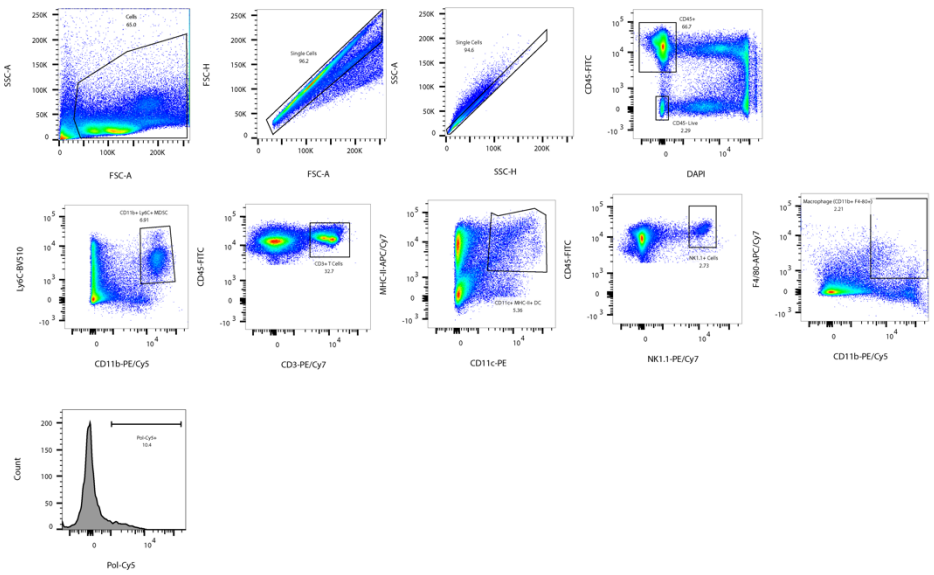

Figure S4: Gating scheme for flow cytometric Cy5 nanoparticle uptake by cell populations in the TME.

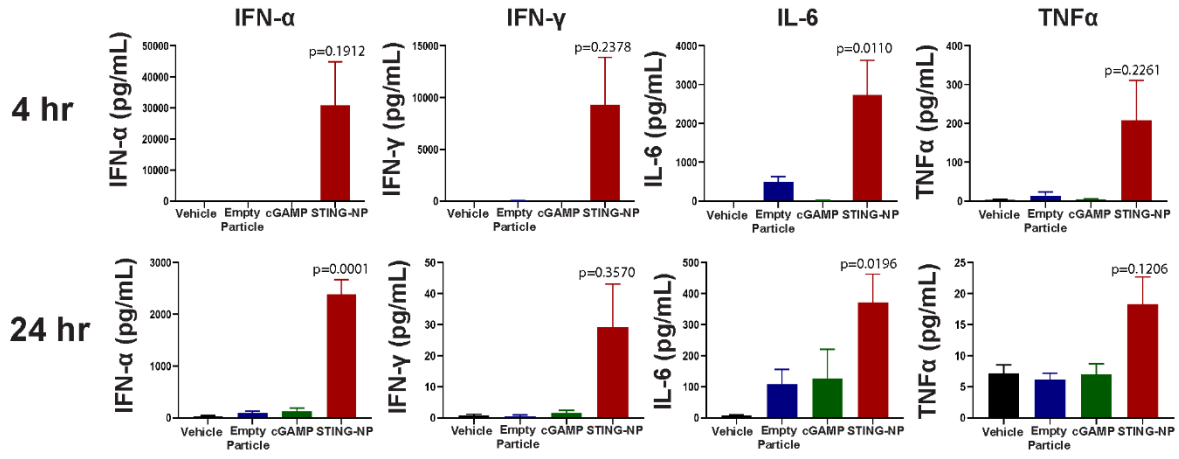

Figure S5: Plasma concentrations of cytokines (of IFN-α, IFN-γ IL-6 and TNF-α) at 4 and 24 hr post-treatment. C57BL/6 mice were treated with Vehicle (PBS), empty particle, cGAMP and STING-NP intravenously, plasma was separated and frozen (-80^{o}C) until analysis by multiplexed bead array.

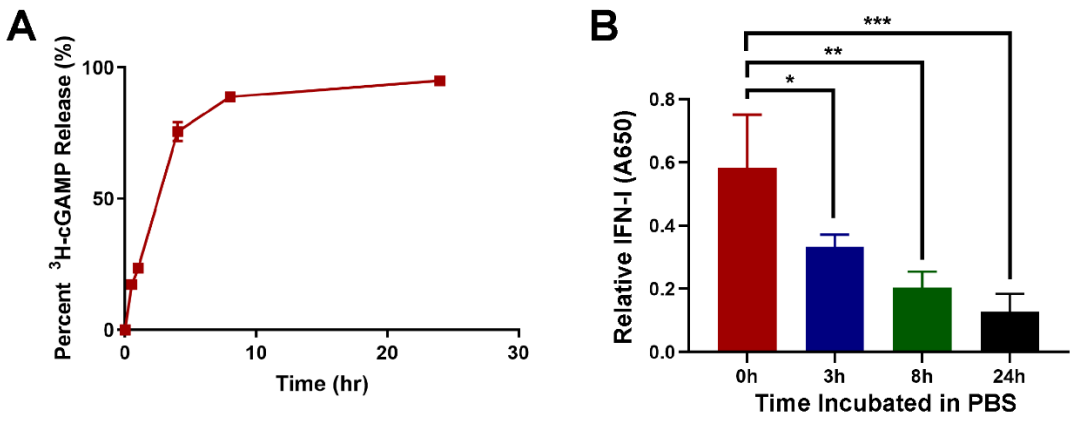

Figure S6: cGAMP release from polymersomes. (A) 3H-cGAMP release was measured using slide-a-lyzer® mini dialysis device (20KDa) over 24 hours. (B) Activity of STING-NPs (125 nM) in RAW-Blue™ reporter cells upon incubation with PBS at 37°C for indicated times. *P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 indicate a statistically significant difference relative to "0 hr" by one-way ANOVA and Tukey's post-hoc test.

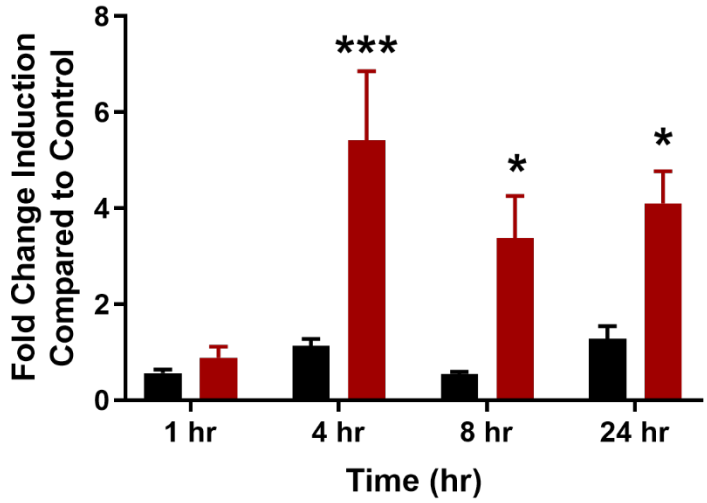

Figure S7: Expression of PD-L1 in tumors treated with cGAMP (black) or STING-NP (red). (**A**) 6-8-week-old C57BL/6 B16-F10 tumor bearing mice were treated with vehicle, cGAMP (20 µg per mouse), or STING-NP (10 µg cGAMP per mouse). qRT-PCR was used to measure *Pdl1* transcript levels in cGAMP or STING-NP treated tumor. (n=4, data is shown as mean ± SD fold change over vehicle treated mice). *P<0.05, ***P<0.001, indicate a statistically significant difference determined by one-way ANOVA followed by Tukey's adjustment to account for multiple comparisons.