Supporting Information for

Delivery of 5'-Triphosphate RNA with Endosome Releasing Nanoparticles Potently Activates RIG-I to Improve Cancer Immunotherapy

Max Jacobson[1], Lihong Wang-Bishop[1], Kyle W. Becker[1], and John T. Wilson[1-5,*]

[1]Department of Chemical and Biomolecular Engineering; Vanderbilt University

Nashville, TN 37235, USA

[2]Department of Biomedical Engineering; Vanderbilt University

[3]Vanderbilt Center for Immunobiology; Vanderbilt University

[4]Vanderbilt Institute for Infection, Immunology and Inflammation; Vanderbilt University

[5]Vanderbilt-Ingram Cancer Center; Vanderbilt University

*CORRESPONDING AUTHOR. John T. Wilson; Tel: +1-615-322-6406; e-mail:

john.t.wilson@vanderbilt.edu

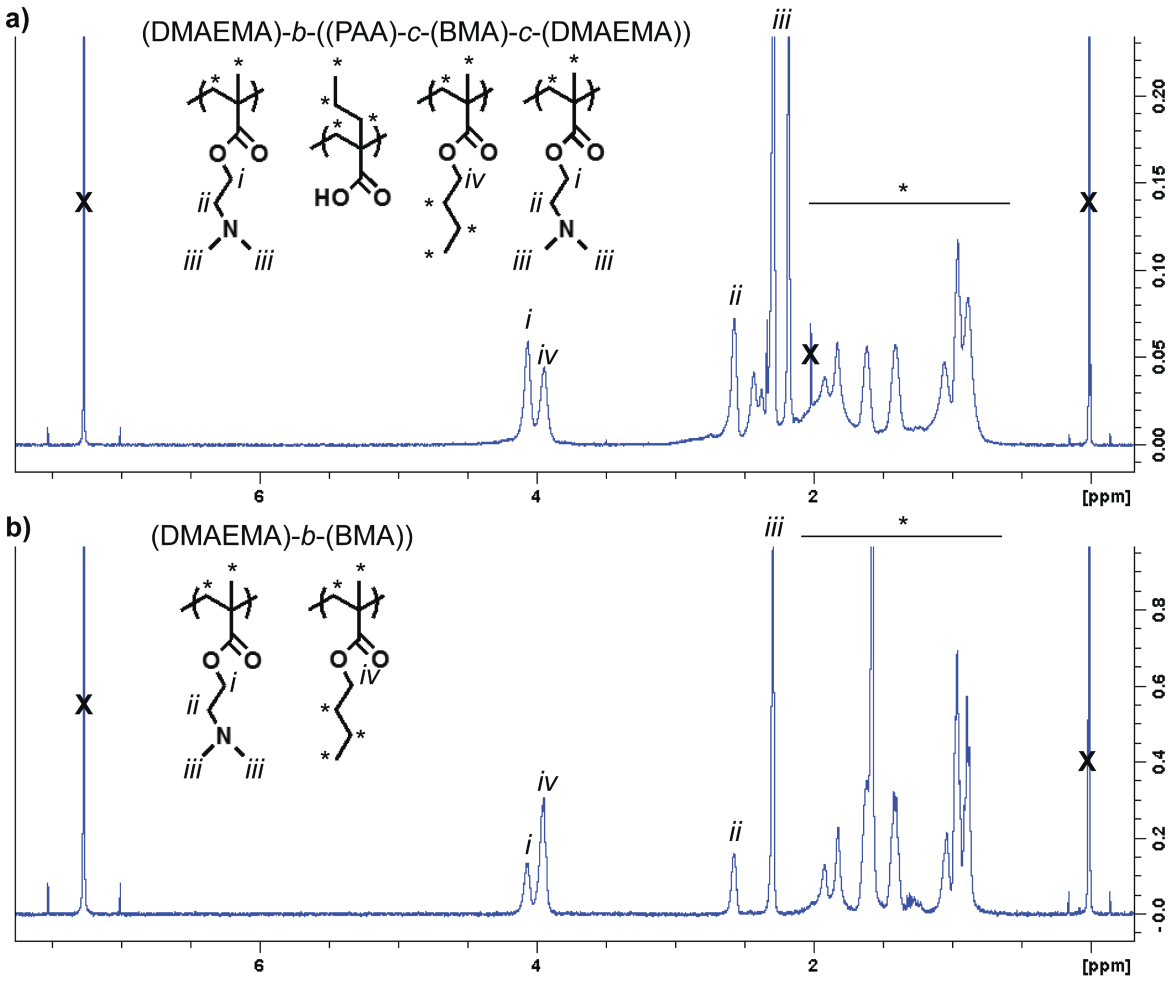

Figure S1. ^{1}H NMR Characterization of diblock copolymers. (a) DMAEMA-*b*-(PAA-*c*-BMA-*c*-DMAEMA) and (b) DMAEMA-*b*-BMA were characterized using NMR. Peaks denoted with an X originate from solvent or TMS.

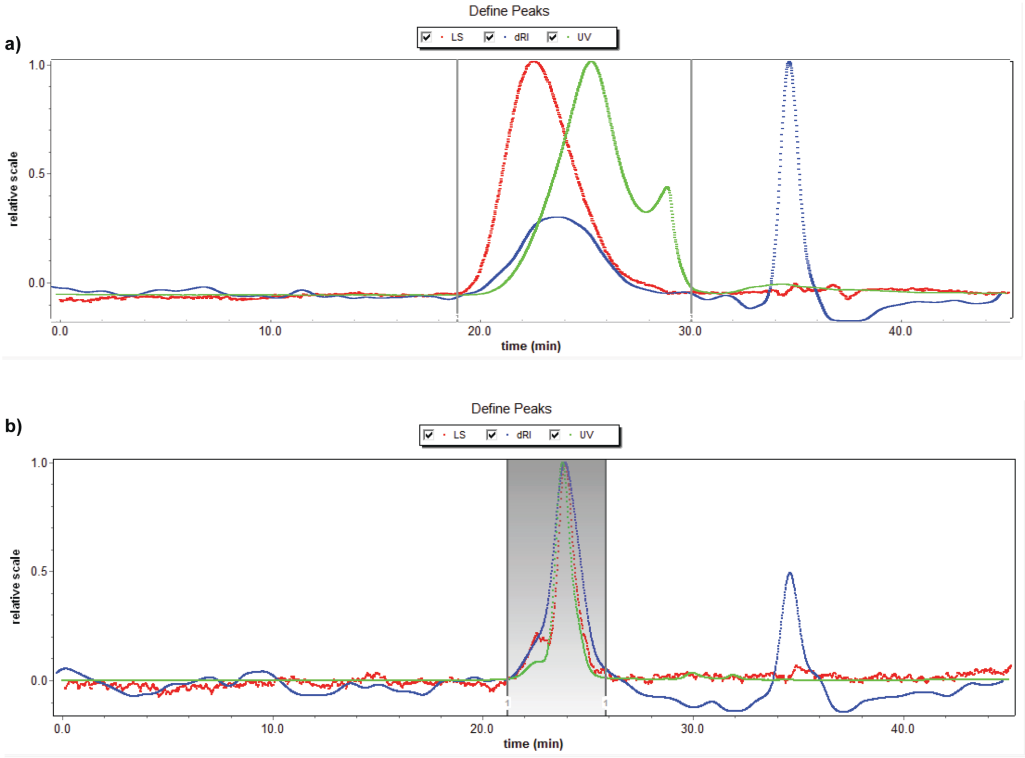

Figure S2. GPC spectra of diblock copolymers (a) DMAEMA-*b*-(PAA-*c*-BMA-*c*-DMAEMA) and (b) DMAEMA-*b*-BMA were characterized using GPC.

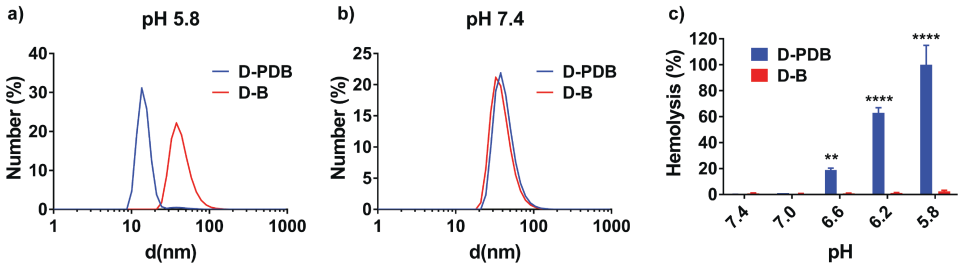

Figure S3. DMAEMA-*b*-BMA (D-B) is not pH-responsive or membrane disruptive. D-PDB and D-B were suspended in (a) pH 5.8 or (b) pH 7.4 PBS and particle size distribution was measured using DLS. (c) Erythrocytes were incubated with 10 μg/mL D-PDB or D-B in PBS at pH 5.8, pH 6.2, pH 6.6, pH 7.0, or pH 7.4 and membrane disruption was quantified using spectrophotometric determination of hemoglobin leakage as an indicator of hemolysis. Significance is between D-PDB and D-B treatment at the same pH.

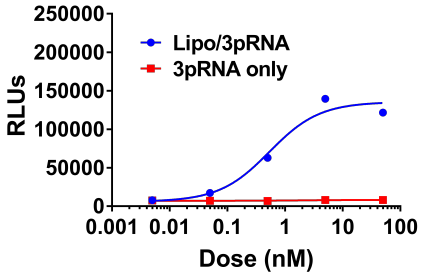

Figure S4. 3pRNA without a transfection agent or other carrier does not activate RIG-I. A549-dual reporter cells were treated with Lipofectamine complexed with 3pRNA or 3pRNA only at doses ranging from 0.05 nM to 50nM 3pRNA.

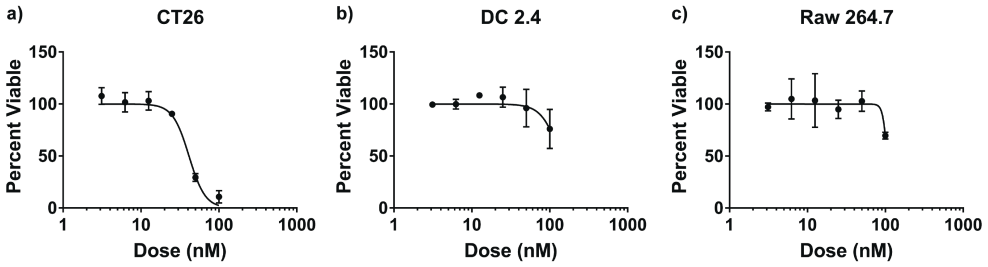

Figure S5. D-PDB is not cytotoxic at relevant concentrations *in vitro*. (a) CT26 cells, (b) DC 2.4 cells, and (c) RAW 264.7 cells were treated with NP/OH-RNA at doses between 1.5 nM-100 nM RNA.

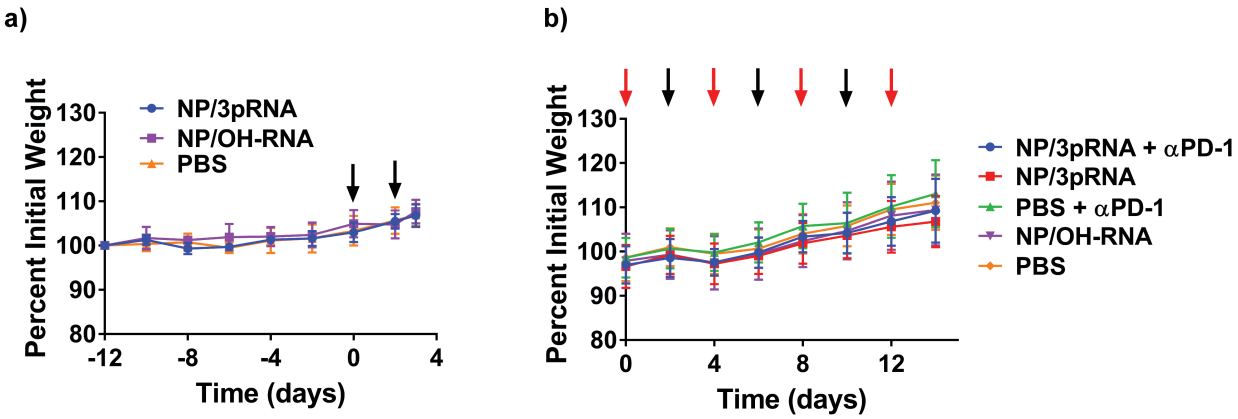

Figure S6. *In vivo* treatment regimens do not result in toxicity-related weight loss. Mice were weighed every two days following CT26 cell injection. (a) Mice from studies detailed in Figure 4 were weighed after tumor cell injection. The mice exhibited no weight loss from any of the treatment regimens. Mice were treated with NPs only at days indicated by the black arrow. (b) Mice from the study detailed in Figure 5 were weighed after tumor cell injection (d-12). The mice exhibited no weight loss from any of the treatment regimens. Mice were injected with anti-PD1 and NPs at days indicated by the red arrow. Mice were treated with NPs only at days indicated by the black arrow.

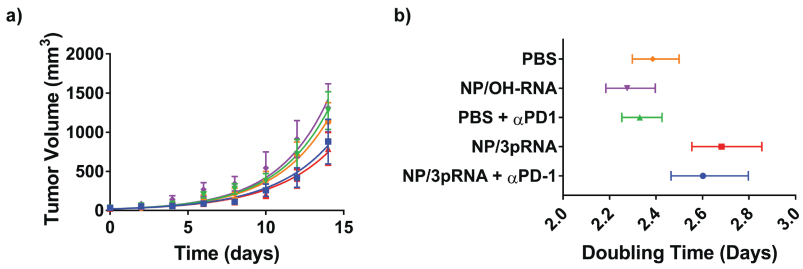

Figure S7. Intratumoral administration of NP/3pRNA + αPD1 and NP/3pRNA results in an increased average tumor volume doubling time in CT26 colon cancer model. (a) Tumor

growth plots were fit using an exponential curve. Initial tumor volume was assumed to be the minimum measured in the study for each group. (b) Tumor doubling time derived from exponential fits for each group plotted with 95% confidence intervals.

Polymer	Mn 1st block	Mn 2nd block	PAA (%)	BMA (%)	DMAEMA (%)	PDI
D-PDB	10300	31000	28	39	33	1.24
D-B	9900	34122	0	100	0	1.09

Table S1. Summary of polymer properties. 1st block Mn, 2nd block Mn, 2nd block composition, and PDI of both polymers were determined from ^{1}H NMR and GPC analysis.

Doubling time (Days)	NP/3pRNA + αPD1	NP/3pRNA	PBS + αPD1	NP/OH-RNA	PBS
Mean	2.602	2.682	2.330	2.276	2.386
Upper 95% CL	2.797	2.855	2.426	2.397	2.499
Lower 95% CL	2.465	2.554	2.253	2.184	2.298

Table S2. Summary of doubling time analysis. Mean doubling time derived from each exponential fit as well as the 95% upper and lower confidence limits (CL).