In vivo studies on the effect of co-encapsulation of CpG DNA and antigen in acid-degradable microparticle vaccines

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—Supporting Information—

Analysis of 3'-CpG Macromonomer by HPLC. The 3'-methacrylamide-modified oligonucleotide **8** was analyzed for purity by reversed phase high-performance liquid chromatography (HPLC) using a 0.1 M triethylammonium acetate buffer (pH 7.0) and a gradient of 0 to 40% acetonitrile over 15 min followed by a gradient to 100% acetonitrile over the next 5 min. All HPLC runs were performed at a flow rate of 0.2 mL/min using a Symmetry C-18 column (3.5 μm, 2.1 x 150 mm, Waters) and an inline

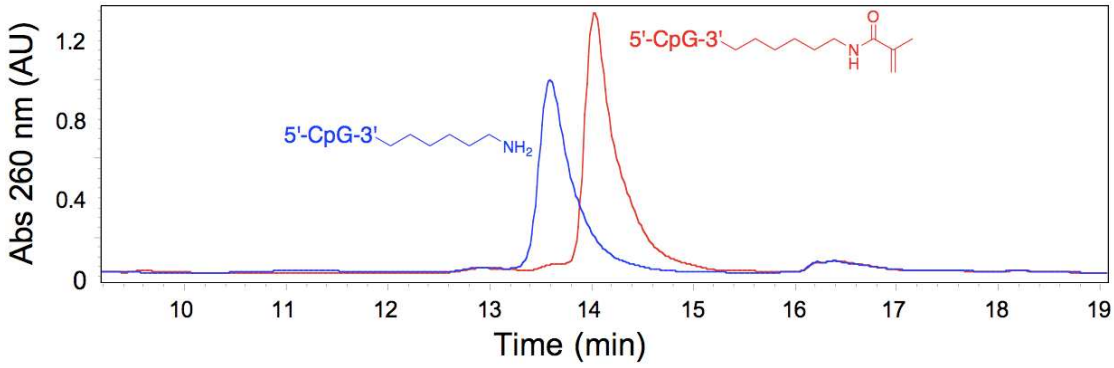

Figure S1. Analysis of macromonomer **8** by HPLC showed a single signal with a slightly longer retention time than the amine-modified oligonucleotide **6**.

996 photodiode array detector (Waters). Representative chromatograms are presented in Figure S1.

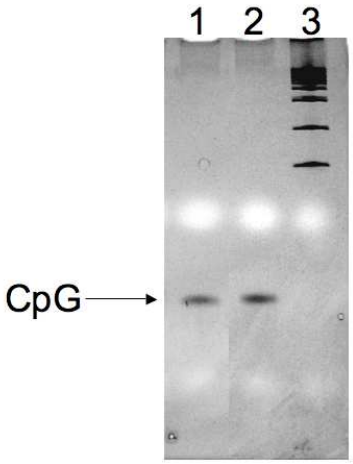

Figure S2. Analysis of macromonomer **8** by PAGE. Lane 1: unmodified CpG DNA; lane 2: macromonomer **8**; lane 3: 50-2000 bp ladder.

PAGE Analysis of Macromonomer 8. The 3'-CpG macromonomer **8** was further analyzed for purity using polyacrylamide gel electrophoresis. Oligonucleotides (150 ng/well) were run on a 15% TBE-urea gel (BioRad) for 1 h at 150 V in TBE buffer (pH 8.3, 1 mM EDTA, 45 mM boric acid, 45 mM Tris). The gel, which is presented in Figure S2, was stained with ethidium bromide and imaged under UV irradiation.

Analysis of CpG-polymer Conjugates using Gel Permeation Chromatography. Particles containing either 3'- or 5'-linked CpG were hydrolyzed overnight in acidic buffer (300 mM acetate, pH 5). The resulting solutions were dialyzed against deionized water at 4 ºC for 2 d using dialysis tubing with a 10,000 molecular weight cutoff. The purified polymers were then lyophilized and analyzed using gel permeation chromatography with 0.1 M $NaNO_3$ as a mobile phase, injected polymer concentrations of 5 mg/mL, two Suprema columns (1000 then 3000) connected in series (both 10 μm, 8 x 300 mm, Polymer Standards Service), a flow rate of 1 mL/min, and a 2414 differential refractometer (Waters) and a 996 photodiode array detector (Waters). Particles containing no CpG were also degraded and analyzed in the same fashion to serve as a negative control. As illustrated in Figure S3, the 3'- and 5'-linked CpG-polymer conjugates gave similar chromatograms with the refractive index (arising from the polymer backbone) and UV (arising from absorption of DNA at 260 nm) traces overlapping almost exactly. These data provide evidence for the successful copolymerization of both CpG macromonomers into the polyacrylamide backbone with an even distribution of the CpG throughout the polymer samples.

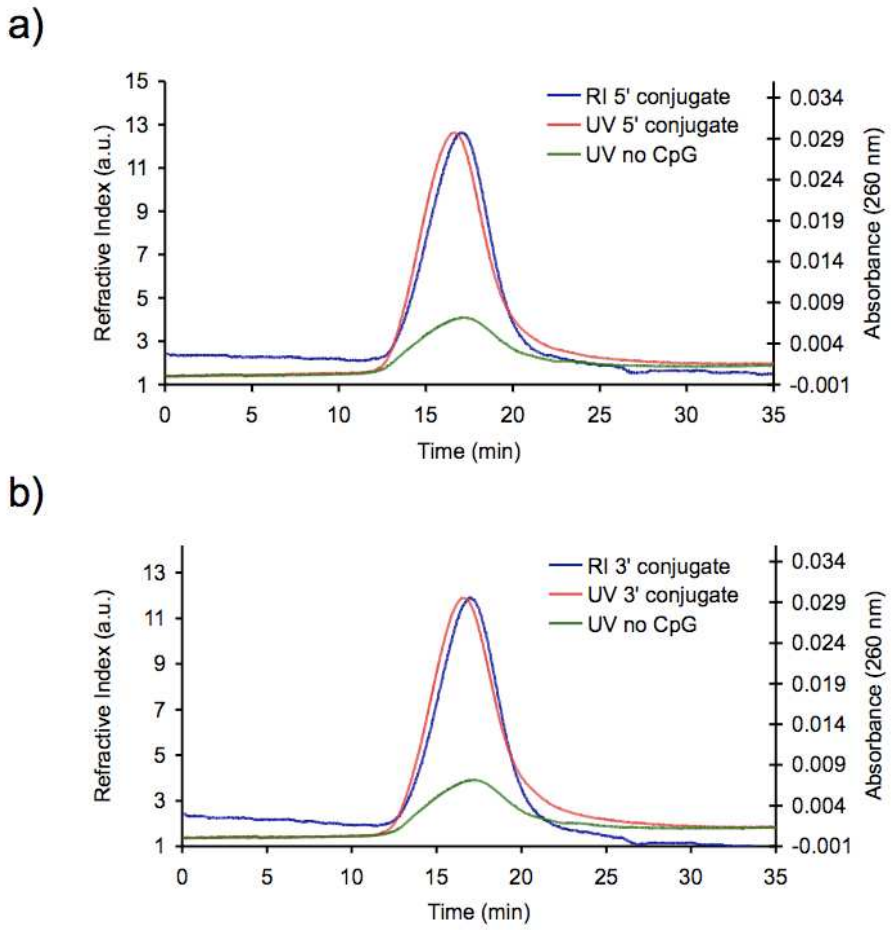

Figure S3. Analysis of CpG-polymer conjugates using gel permeation chromatography. Polymers containing (a) 5'-linked and (b) 3'-linked CpG gave overlapping refractive index and UV traces, thus suggesting successful copolymerization of the CpG macromonomers into the polyacrylamide backbone with an even distribution of the CpG throughout the polymer sample. The UV trace of particles made without a CpG macromonomer is included as a negative control for DNA absorption at 260 nm.

CD86 Expression by BMDCs treated with CpG-loaded particles was quantified as described in the main text. Representative histograms are presented in Figure S4.

Representative Flow Cytometry Histograms from the *in vivo* cytotoxicity experiment described in the main text are presented in Figure S5.

Plots of Tumor Volume vs. Time from the tumor experiment described in the text are shown in Figure S6. Tumor volume was calculated using the equation: volume = 0.5 x (longest diameter) x (shortest diameter)^2.

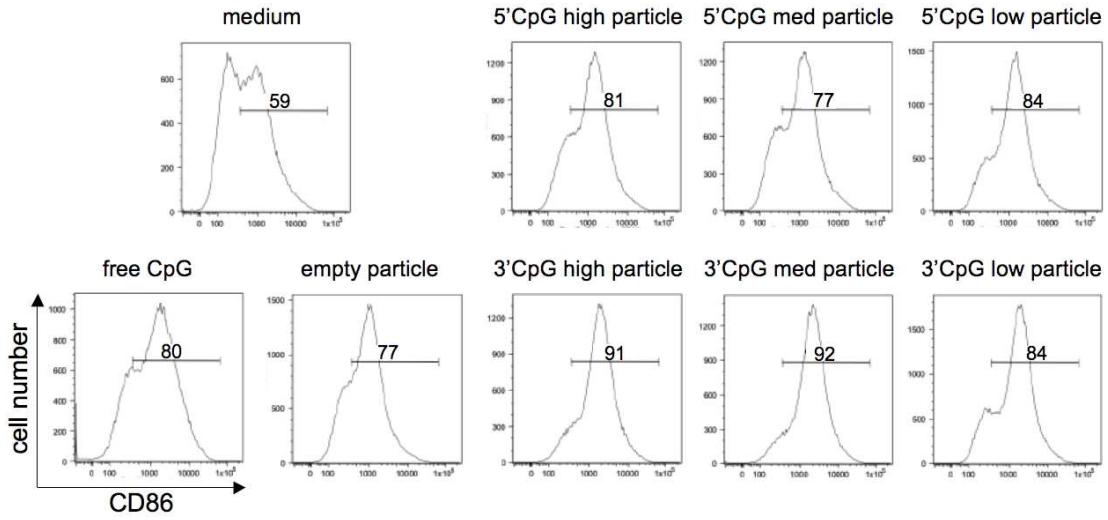

Figure S4. Expression of CD86 by BMDCs pulsed with 3'- or 5'-linked CpG particles or control samples. Numbers in histograms represent percent of CD86 postive DCs after overnight culture with samples normalized to 300 ng CpG. Data are representative of three experiments with similar results.

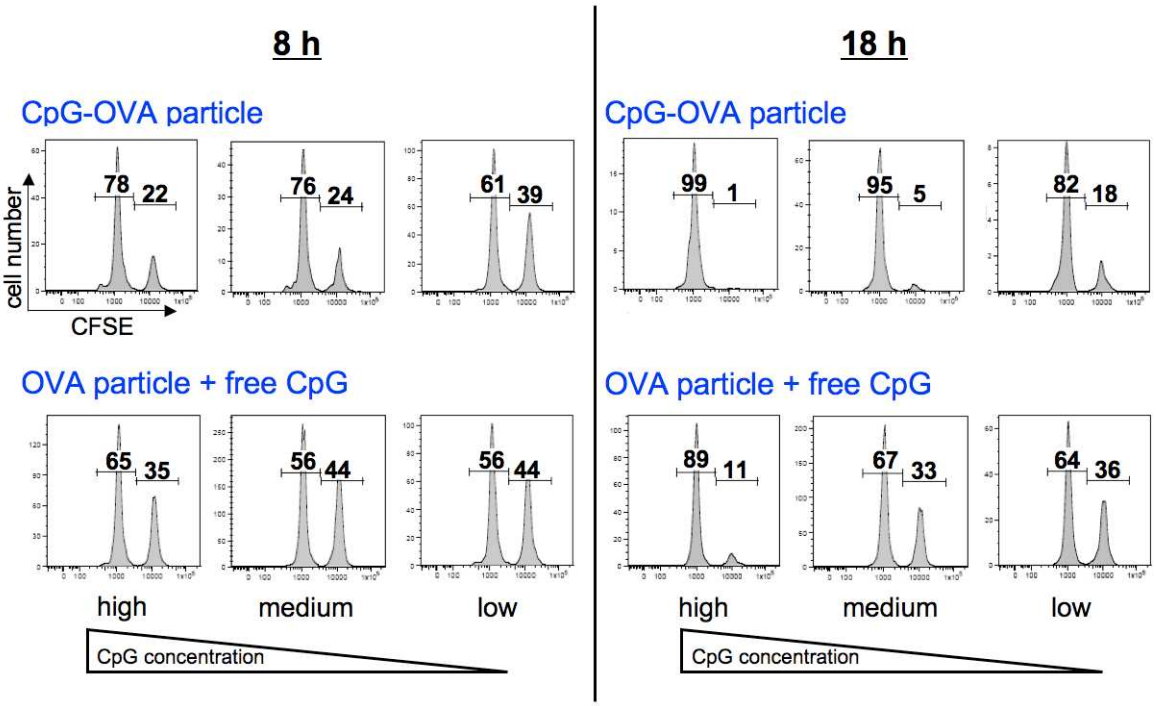

Figure S5. Specific lysis of SIINFEKL-pulsed cells in mice immunized with 3'-CpG-OVA particles or OVA particles plus free CpG after 8 (left side) and 18 hours (right side). Histograms show percentage of remaining CFSE stained target cells (SIINFEKL-pulsed, population on right) or unpulsed control cells (population on left) and are representative of five experiments with similar results. High, medium and low CpG loadings correspond to 9, 3, and 1 µg CpG per mg particles respectively.

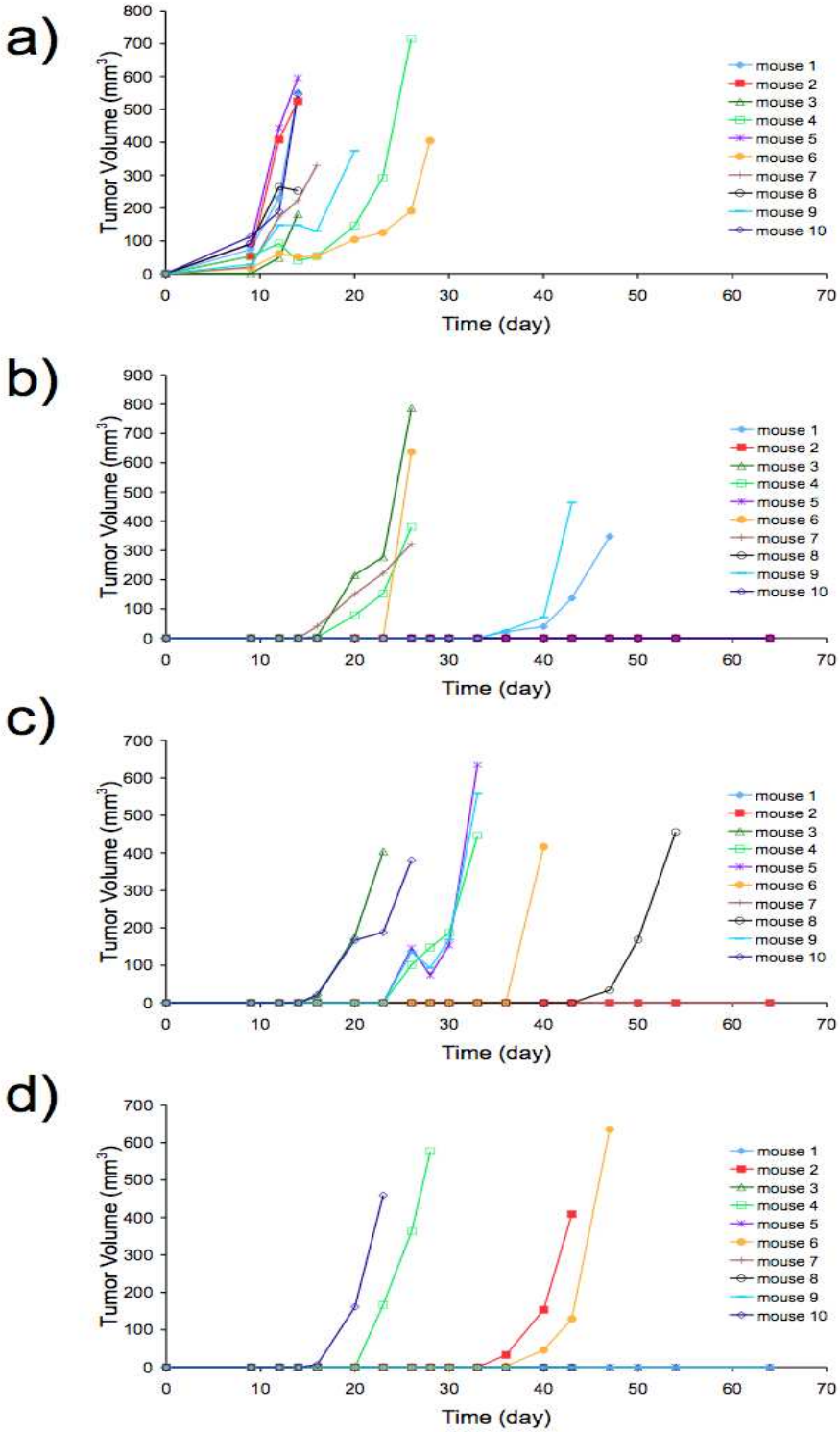

Figure S6. Plots of tumor volume vs. time for mice treated with (a) PBS, (b) free OVA plus free CpG, (c) OVA particles plus free CpG, or (d) 3'-CpG/OVA particles.