Supporting Information

Biomimetic Protein Nanoparticles Facilitate Enhanced Dendritic Cell Activation and Cross-Presentation

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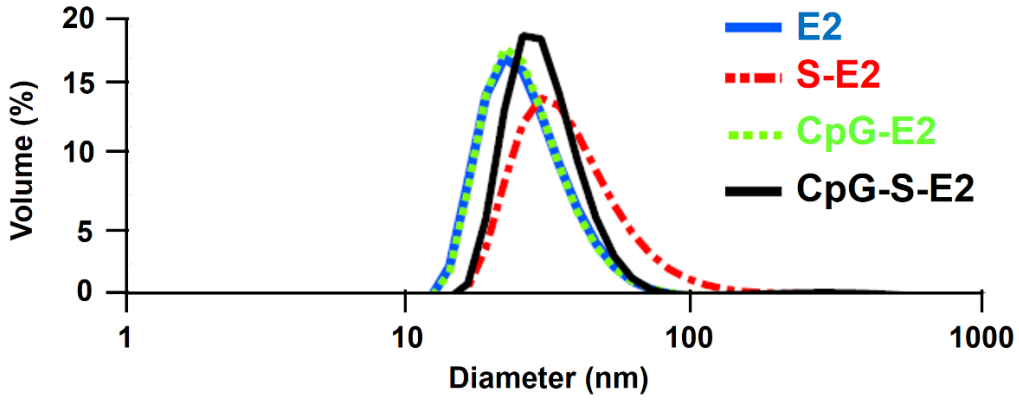

Figure S1. Representative dynamic light scattering graph of the functionalized nanoparticles. A total of three independent experiments were carried out on each nanoparticle, with n=3 size measurements for each experiment.

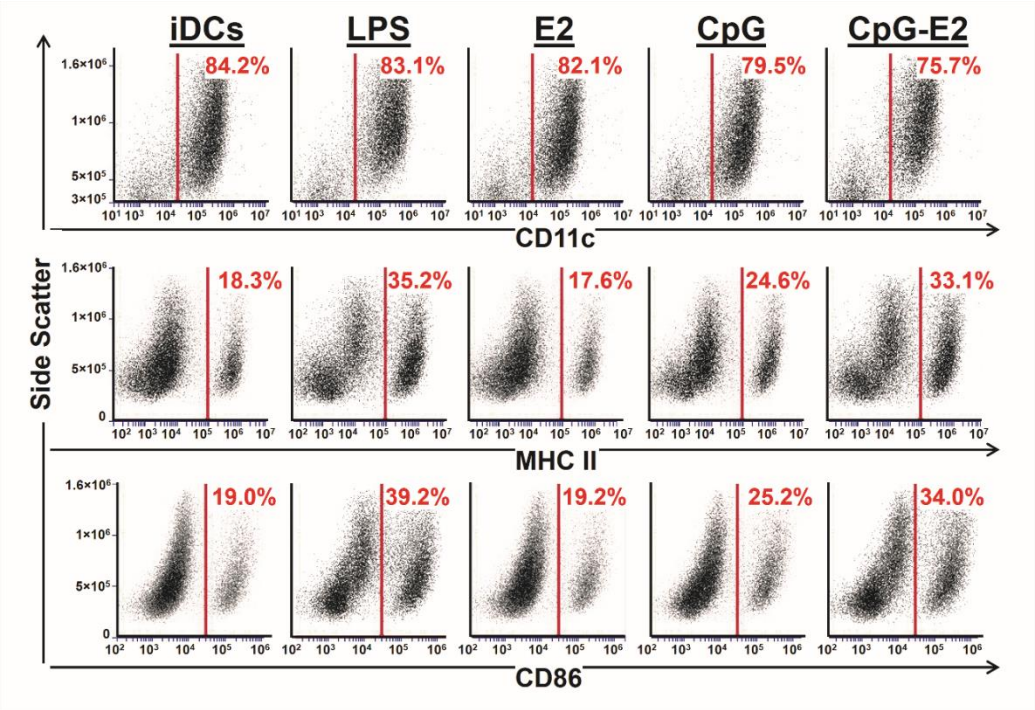

Figure S2. A representative BMDC activation experiment analyzed by flow cytometry following 24 hours incubation with E2, CpG-E2, or free CpG (0.5 µg/ml equivalent CpG concentration). Rows display different surface markers, and columns correspond to the different compounds added (iDCs = immature DCs). Events to the right of the red bar are considered positive for the specific marker.

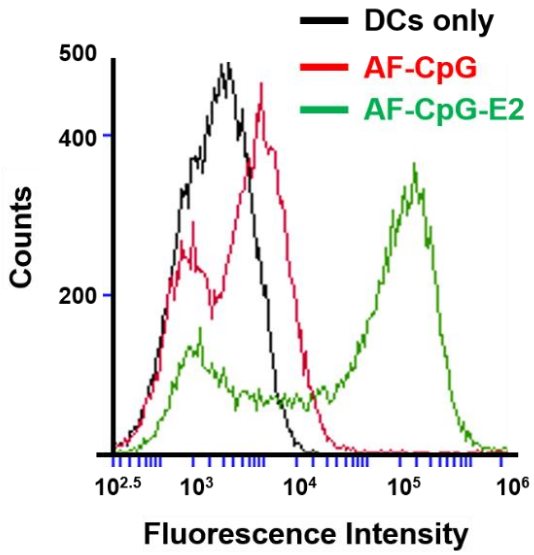

Figure S3. BMDCs show increased uptake of E2-encapsulated CpG, relative to unbound CpG. A representative BMDC uptake experiment analyzed by flow cytometry following 2 hours incubation with 0.5 µg/ml Alexa Fluor 488-conjugated CpG (AF-CpG) or Alexa Fluor 488-conjugated CpG-E2 (AF-CpG-E2, 0.5 µg/ml CpG). A shift in the MFI of > 25-fold was observed when incubated with AF-CpG-E2, relative to AF-CpG, indicative of increased uptake.

Methods for Figure S3: CpG-E2 (at ~25 CpG molecules per E2 nanoparticle) were reacted with AlexaFluor 488 carboxylic acid succinimidyl ester (Invitrogen) for 1.5 hours at room temperature to yield ~25 dye molecules per E2 nanoparticle (AF-CpG-E2), giving a dye-to-CpG ratio of 1:1. Unbound CpG with a 5' AlexaFluor 488 (AF-CpG) was synthesized by Integrated DNA Technologies at a dye-to-CpG ratio of 1:1. Immature BMDCs harvested on day 8 of culture were incubated with unbound AF-CpG or AF-CpG-E2 particle at equivalent CpG concentrations for 2 hours at 37°C, harvested, and analyzed by flow cytometry. (n=3)

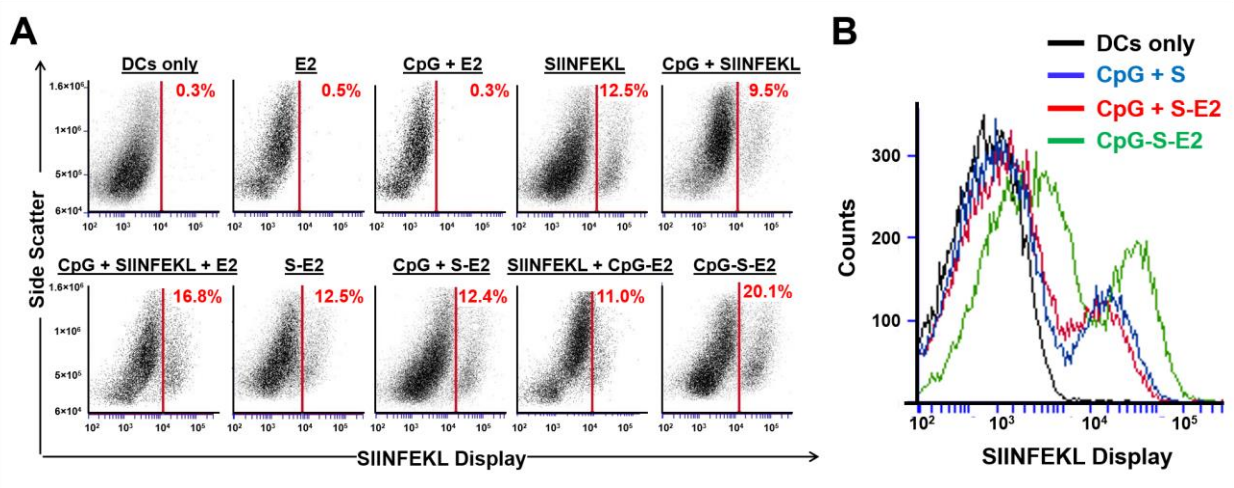

Figure S4. Depicted are representative BMDC cross-presentation experiment analyzed by flow cytometry following incubation with antigen (0.5 µg/ml equivalent SIINFEKL concentration) and staining with monocloncal antibody 25-D1.16. A) Representative dot plot with the events to the right of the red bars are considered positive for SIINFEKL display in the context of H-2K^{b}.
B) Overlay of representative histograms of DCs only, unbound CpG + unbound SIINFEKL (S), unbound CpG + S-E2, and CpG-S-E2.

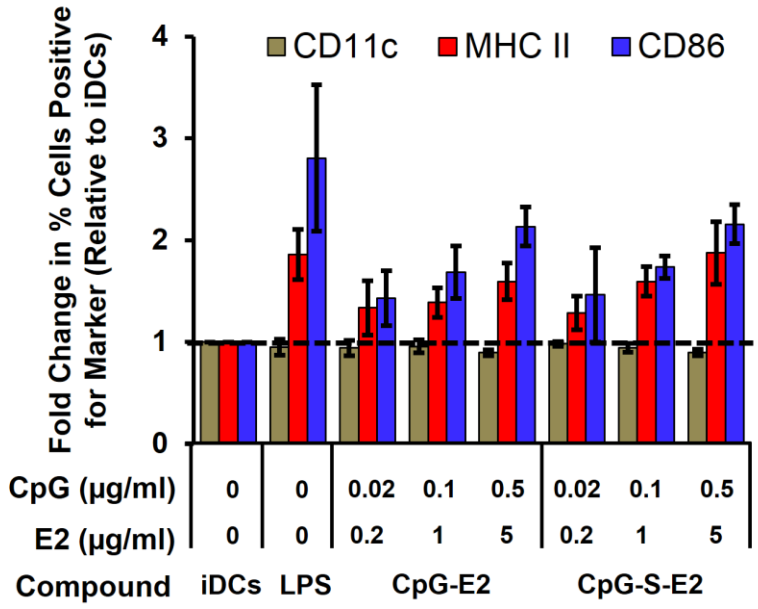

Figure S5. No significant difference was observed between the CpG-E2 particle and CpG-S-E2 particle with respect to BMDC activation at a given CpG concentration. Activation extent was determined by measuring MFI for the expression of MHC II and CD86 (n ≥ 4 independent experiments).

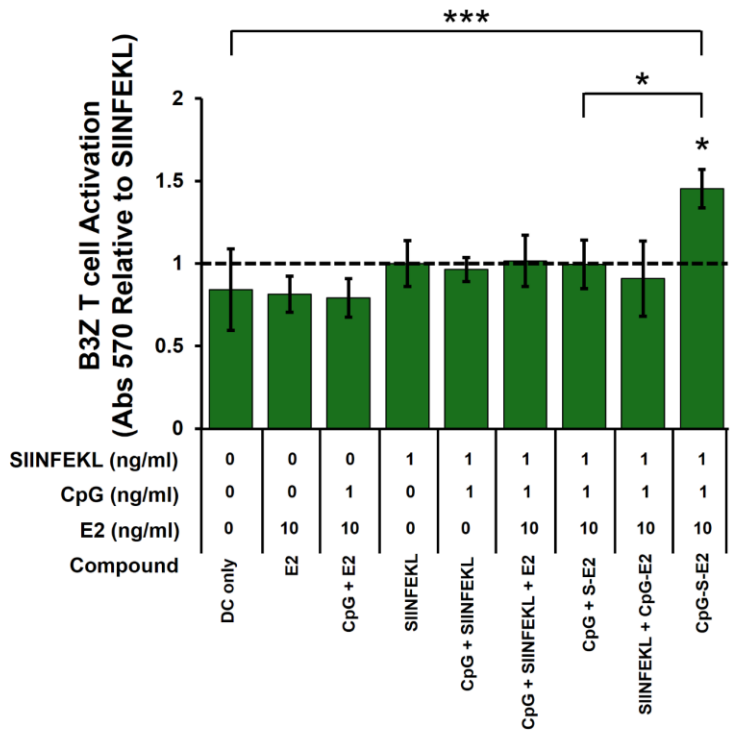

Figure S6. BMDC incubated with CpG-S-E2 facilitate greater CD8 T cell activation than any other peptide delivery strategy after 48 hour incubation. Significantly greater B3Z activation was achieved for BMDCs incubated with CpG-S-E2, relative to all unbound SIINFEKL formulations (* $p < 0.05$). Comparisons with CpG + S-E2 (* $p < 0.05$) and with DC-only background (*** $p < 0.001$) also yielded statistically significant differences. Data is presented as mean ± SD ($n \geq 3$ independent experiments), and significance was determined by a one-way ANOVA followed by a Bonferroni post-test comparing the set of data including DC-only control and all formulations containing peptide.