Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Nanoparticle vaccines can be designed to induce pDC support of mDCs for increased antigen display

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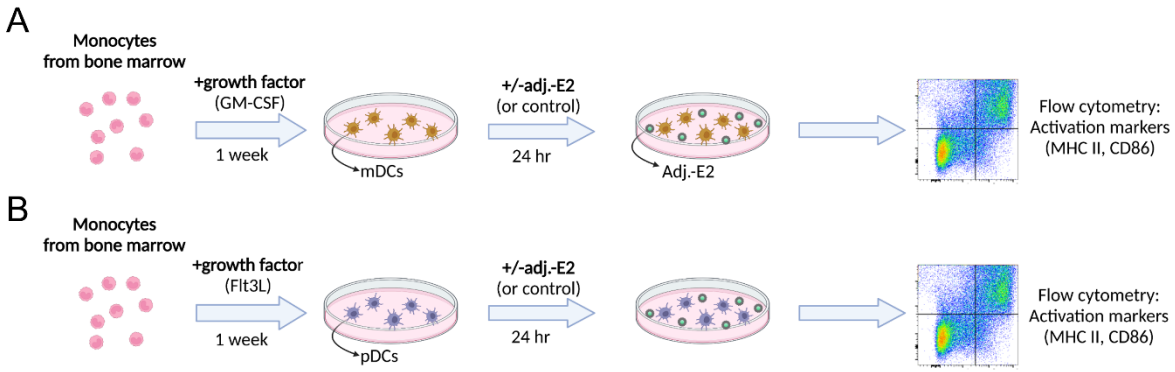

Figure SI-1. Culturing and activation of mDCs and pDCs. A) Myeloid DC culturing and activation. Monocytes were harvested from C57BL/6 bone marrow on Day 0 and differentiated for one week with GM-CSF. Collected mDCs were incubated with PBS (negative control), 100 ng/mL LPS (positive control), E2, free adjuvant, or adjuvant-E2 for 24 hr and analyzed for activation marker expression (MHC II and CD86) using flow cytometry. **B)** Plasmacytoid DC culturing and activation. Plasmacytoid DCs were cultured and characterized in a similar manner to mDCs, but were differentiated with Flt3L growth factor instead of GM-CSF and used 200 ng/mL LPS stimulation as positive control. Additionally, mDCs and pDCs were gated for different populations. *Adj: adjuvant.*

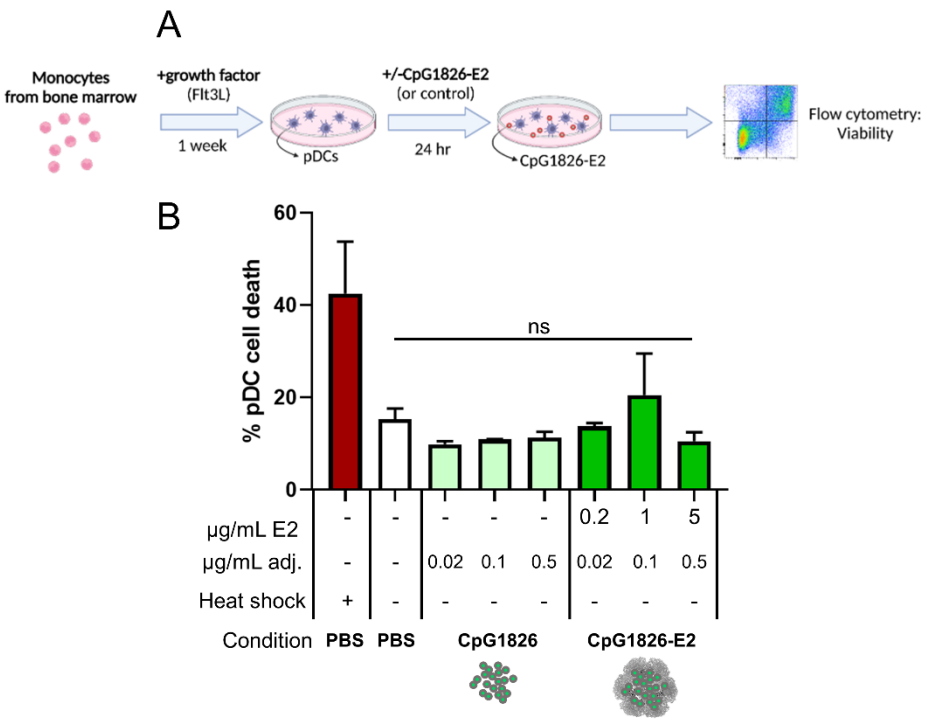

Figure SI-2. Assessment of pDC viability following activation. A) Plasmacytoid DC culturing, activation, and viability assessment. Monocytes were harvested from C57BL/6 bone marrow on Day 0 and differentiated for one week with Flt3L. Plasmacytoid DCs were incubated with PBS, free CpG1826, or CpG1826-E2 for 24 hr. The viability of pDCs ($CD11c^{low}B220^{high}$) was assessed using a live/dead stain with flow cytometry. **B)** Percentage of pDC cell death following 24 hr incubation with PBS, CpG1826, or CpG1826-E2. For each condition or formulation, corresponding concentrations of E2 and adjuvant are displayed, in addition to whether samples were heat shocked following lifting. The white and red bars indicate PBS-treated pDC controls of non-heat shocked (negative control) or heat shocked (positive control) following lifting, respectively. Light and dark green bars correspond to free or E2-encapsulated CpG1826, respectively. *Mean ± SEM. Statistics: 1-way ANOVA, Bonferroni's test. 3 replicates each for 2 independent biological replicates. ns = no significant differences. Adj: adjuvant.*

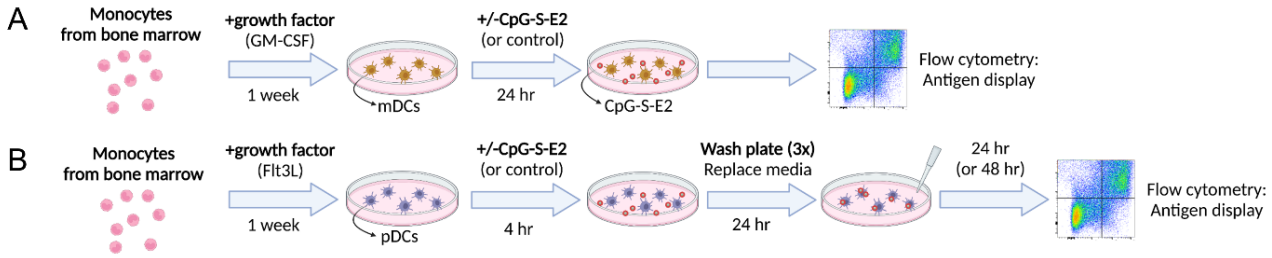

Figure SI-3. Culturing and antigen display studies for mDCs and pDCs. A) Myeloid DC culturing and antigen display. Monocytes were harvested from C57BL/6 bone marrow on Day 0 and differentiated for one week with GM-CSF. Collected mDCs were incubated with 1 µg/mL CpG1826-SIINFEKL-E2 or control for 24 hr then analyzed for SIINFEKL antigen display. **B)** Plasmacytoid DC culturing and antigen display. Plasmacytoid DCs were differentiated over one week with Flt3L and incubated with condition for 4 hr, followed by washing of wells and media replacement. SIINFEKL antigen display by pDCs was assessed after an additional 24 hr or 48 hr incubation period. The wash step is consistent with the procedure for supernatant transfer studies, where a wash step was introduced to minimize residual formulation or CpG + S transfer from pDCs to mDCs. *CpG: CpG1826. S: SIINFEKL.*

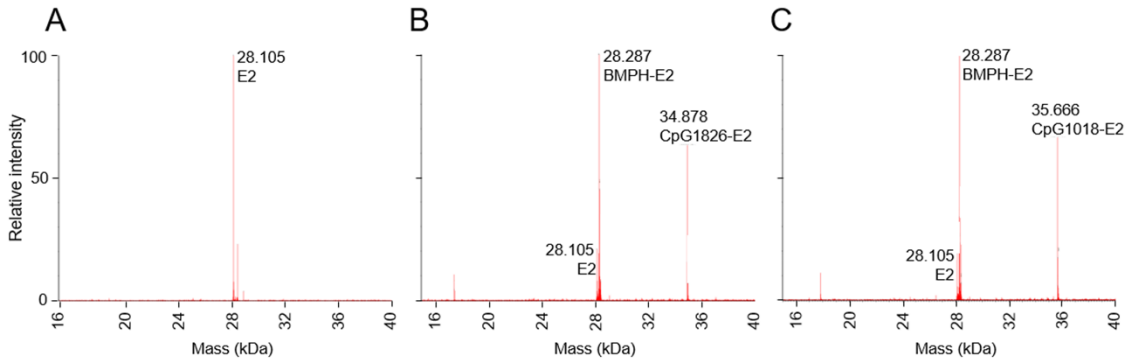

Figure SI-4. Representative mass spectra of nanoparticles. Displayed are mass spectrometry data for **A)** E2, **B)** CpG1826-E2, and **C)** CpG1018-E2. For each, relative intensity for different masses (kDa) are shown. Mass data for free ssRNA and ssRNA-E2 could not be obtained, presumably due to matrix-RNA interactions or incompatible protocol conditions. Major peaks are labeled with observed mass and corresponding species. E2 monomer conjugated to the BMPH linker is abbreviated as BMPH-E2.

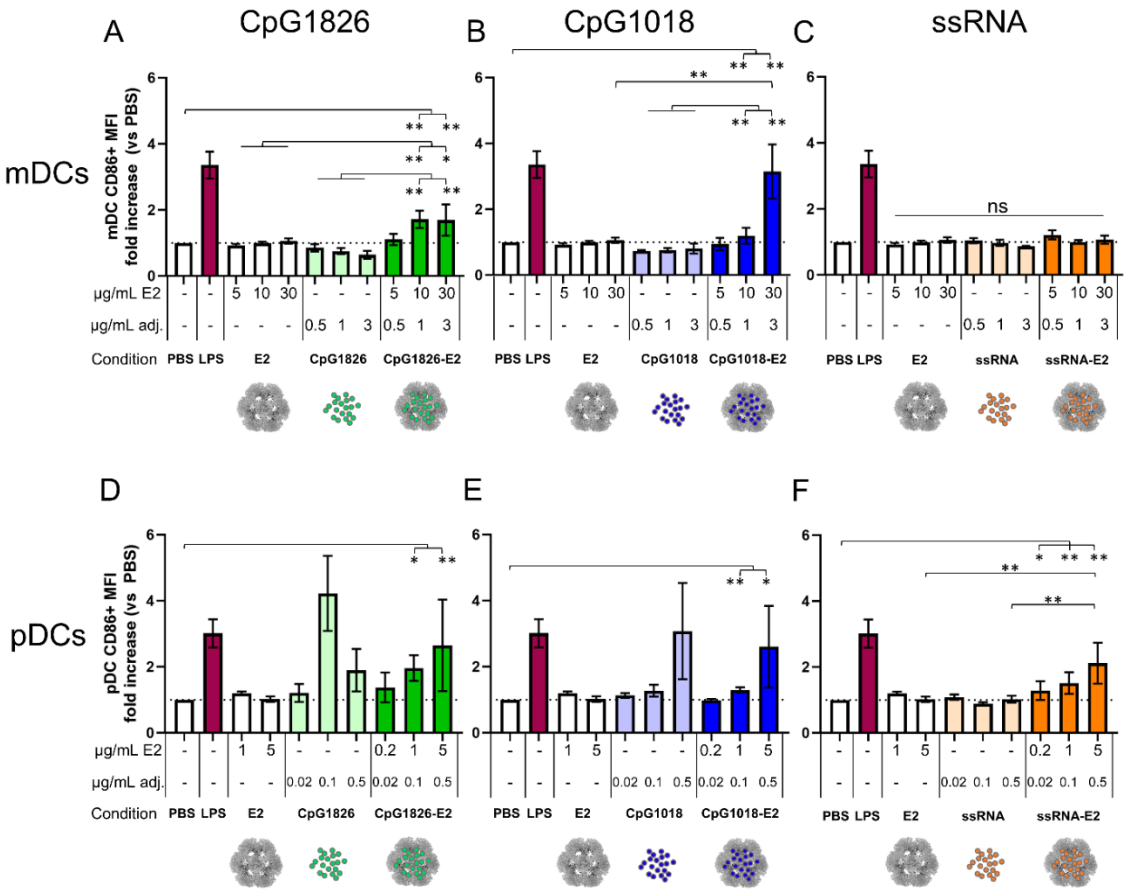

Figure SI-5. CD86 response of mDCs and pDCs to encapsulated TLR agonists. Myeloid DC **(A-C)** or plasmacytoid DC **(D-F)** CD86+ MFI fold increase versus PBS control. For each condition or formulation, corresponding concentrations of E2 and adjuvant are displayed. White bars are PBS- and E2-only controls. Red bars indicate 100 ng/mL LPS (mDC positive control) or 200 ng/mL LPS (pDC positive control). Green, blue, and orange bars correspond to free (light bars) or encapsulated (dark bars) CpG1826, CpG1018, or ssRNA, respectively. **A)** CD86 expression of mDCs incubated with PBS, LPS, E2, and CpG1826 controls or CpG1826-E2. **B)** CD86 expression of mDCs incubated with CpG1018-E2 or control. **C)** CD86 expression of mDCs incubated with ssRNA-E2 or control. **D)** CD86 expression of pDCs incubated with CpG1826-E2 or control. **E)** CD86 expression of pDCs incubated with CpG1018-E2 or control. **F)** CD86 expression of pDCs incubated with ssRNA-E2 or control. *Mean ± SEM. Statistics: 1-way ANOVA, Bonferroni's test. ≥3 independent biological replicates, 2-3 technical replicates per independent biological replicate. Adj: adjuvant. *p ≤ 0.05, **p ≤ 0.01*. Experimental procedure is outlined in **Figure SI-1**.

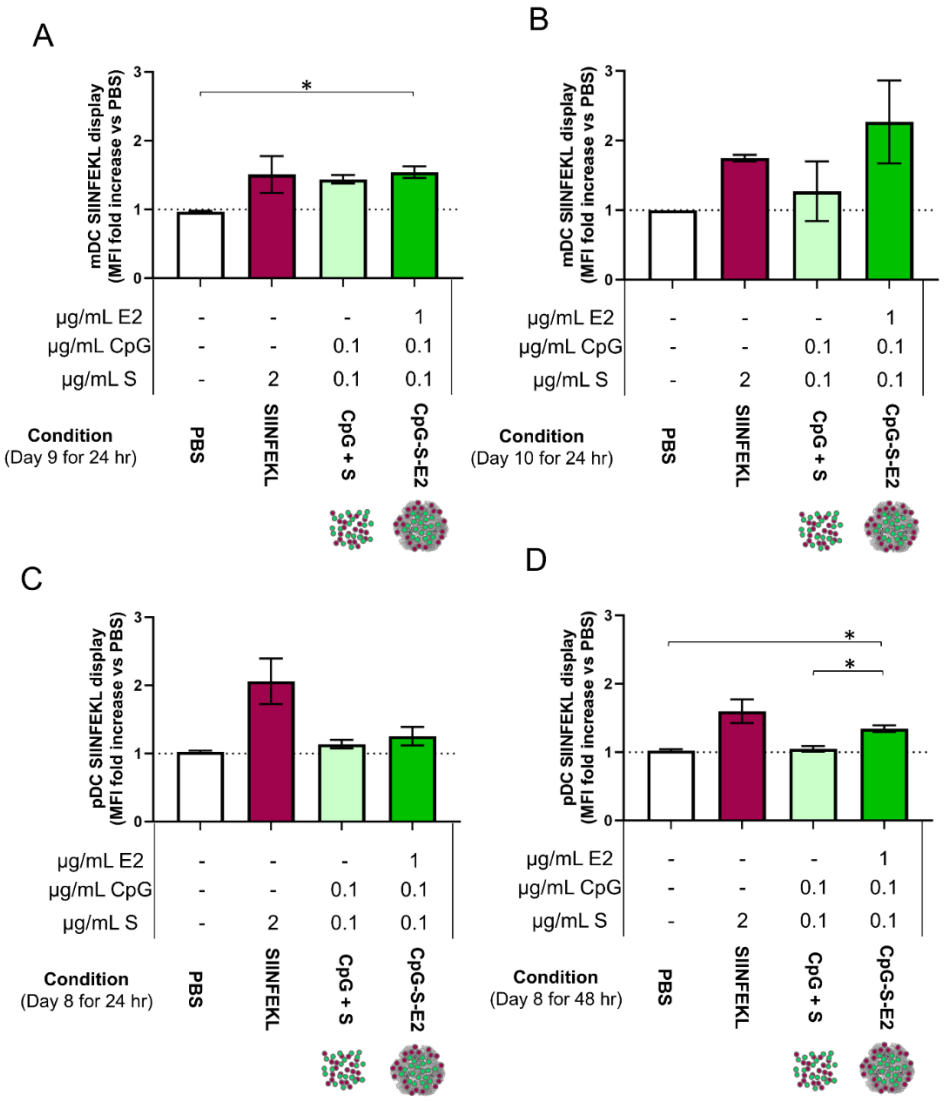

Figure SI-6. Both mDC and pDC subsets can display antigen. SIINFEKL display of mDCs after a 24 hr incubation period starting on **A)** Day 9 or on **B)** Day 10. Also shown is SIINFEKL display of pDCs after an incubation period starting on Day 8 for a duration of **C)** 24 hr or **D)** 48 hr. Plotted is fold increase in SIINFEKL display versus PBS control, with enhanced SIINFEKL display rising above 1. For each condition, corresponding concentrations of E2 and CpG are displayed. Red bars indicate 2 µg/mL free SIINFEKL (positive control). Light green bars correspond to free CpG with SIINFEKL, while dark green bars correspond to CpG-S-E2 containing an equivalent dose of adjuvant and antigen. *Mean ± SEM. Statistics: 1-way ANOVA, Bonferroni's test. 3 independent biological replicates, 2-3 technical replicates per independent biological replicate. CpG: CpG1826. S: SIINFEKL. *p ≤ 0.05.* Experimental procedure is outlined in **Figure SI-3**.

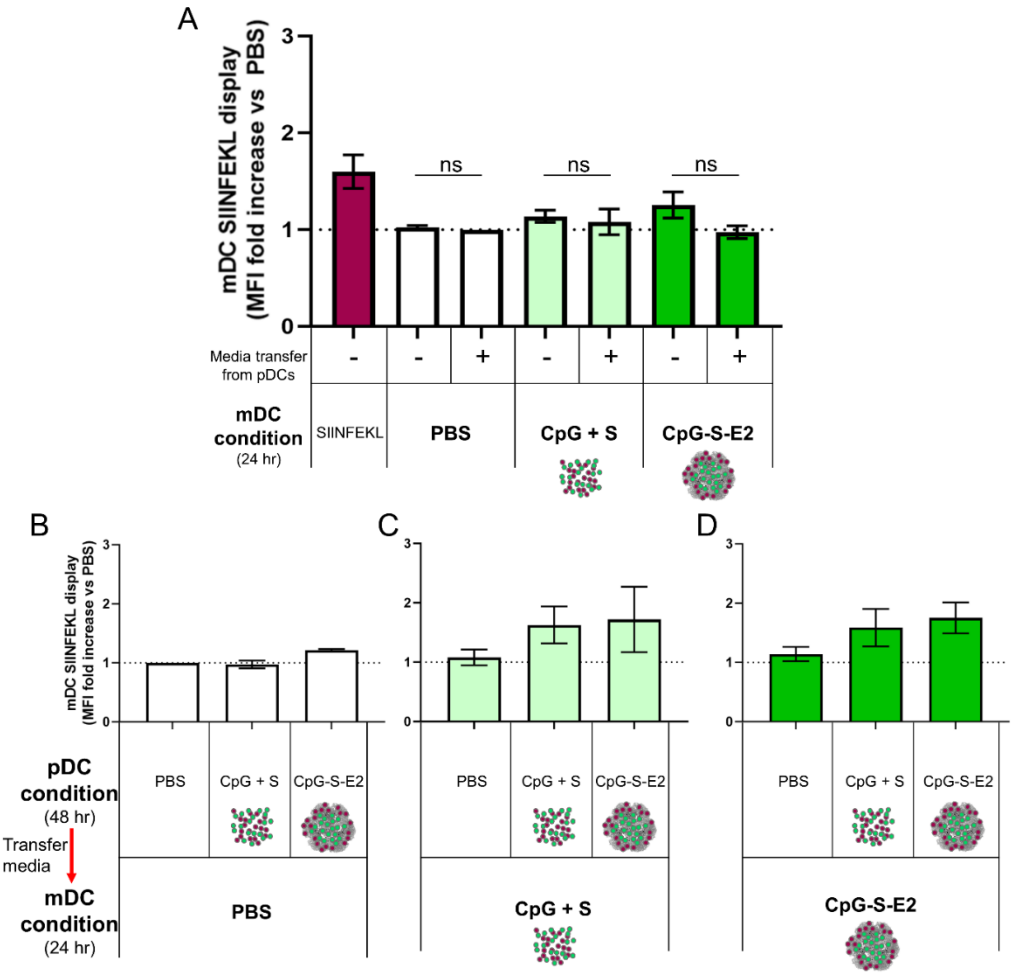

Figure SI-7. Plasmacytoid DCs aid mDCs to display antigen (with pDC incubation period of 48 hr). A) Myeloid DC SIINFEKL display with or without media transfer from pDCs that were incubated with PBS for 48 hr. **(B-D)** Myeloid DC SIINFEKL display following supernatant transfer protocol for mDCs or pDCs incubated with **B)** PBS, **C)** 0.1 μg/mL CpG with 0.1 μg/mL SIINFEKL, and **D)** 1 μg/mL CpG-S-E2 for 24 hr starting on Day 10 (mDCs) or 48 hr starting on Day 8 (pDCs). Shown is display fold increase in antigen display versus that of mDCs incubated with PBS. *Mean ± SEM. Statistics: 1-way ANOVA, Bonferroni's test. 3 independent biological replicates, 2-3 technical replicates per independent biological replicate. Abbreviations: CpG: CpG1826. S: SIINFEKL.* Experimental procedure is outlined in **Figure 6A**.