Supporting Information for:

Intrinsic Adjuvanticity of Branched Polyethylenemine *In Vitro and Subcutaneously*

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Supporting Figures:

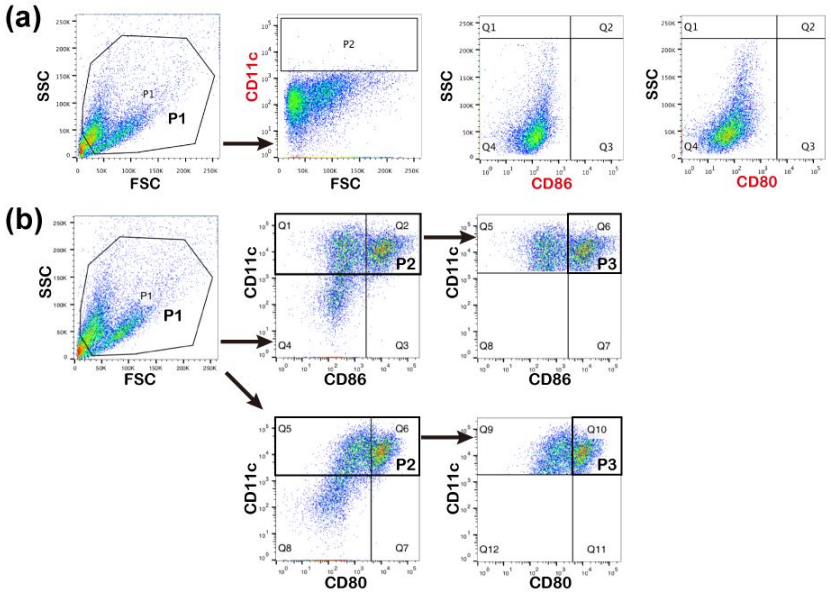

Figure S1. Flow cytometry analysis of BMDCs after stimulation of BPEI or PEG-BPEI. All cells were harvested and (a) stained with corresponding isotype controls of PE-Cy7-CD11c, APC-CD86, PE-Cy5.5-CD80 (marked with red words) to set the gates correctly. (b) P1 was defined as the gate of total cell population. CD11c was identified as the dendritic cell biomarker and all the CD11c positive cells were gated as P2. The percentage of CD86 or CD80 positive cells (P3) and MFI of overall cells in P2 were then analyzed to reflect the maturation state of BMDCs.

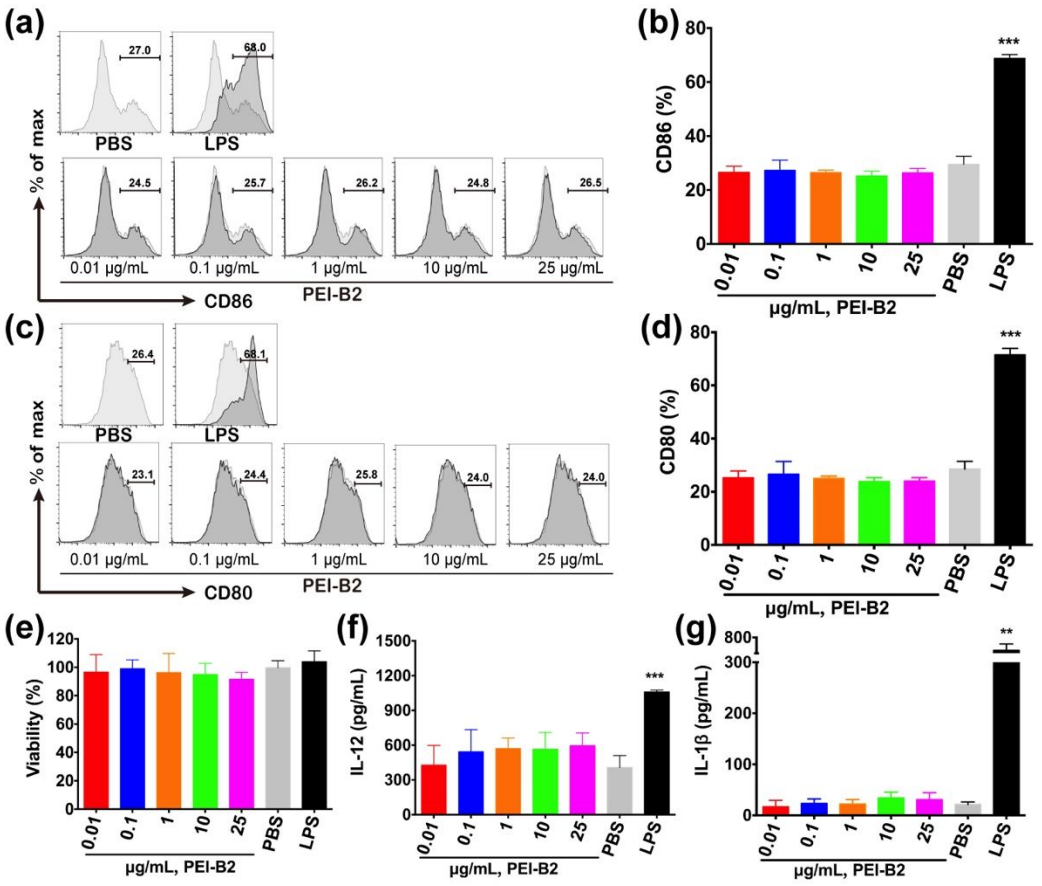

Figure S2. Stimulating ability and cytotoxicity of PEI-B2 to BMDCs after 24 h. (a, b) CD86 and (c, d) CD80 expression on BMDCs and (e) the cell viability. Proinflammatory cytokine secretion of (f) IL-12 and (g) IL-1β by BMDCs. PBS and LPS were applied as negative and positive controls. These experiments were carried out in triplicate and results were shown as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ ($n = 3$).

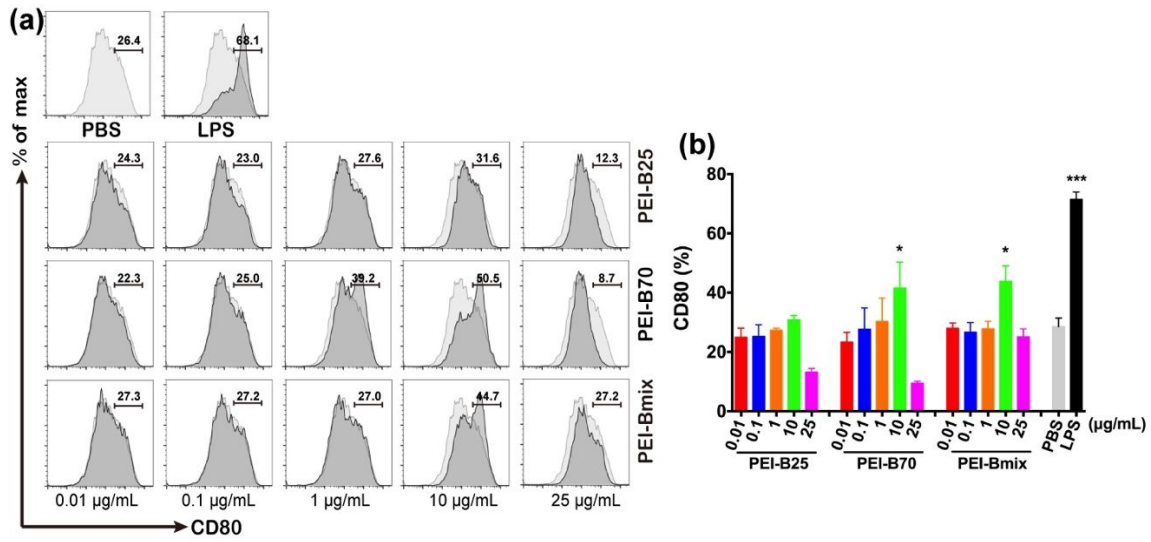

Figure S3. Upregulation of CD80 expression on BMDCs with stimulation of BPEI. (a, b) CD80 expression on BMDCs after 24 h treatment with PEI-B25, PEI-B70, PEI-Bmix of different concentrations or controls. PBS and LPS were applied as negative and positive controls. These experiments were carried out in triplicate and results were shown as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ ($n = 3$).

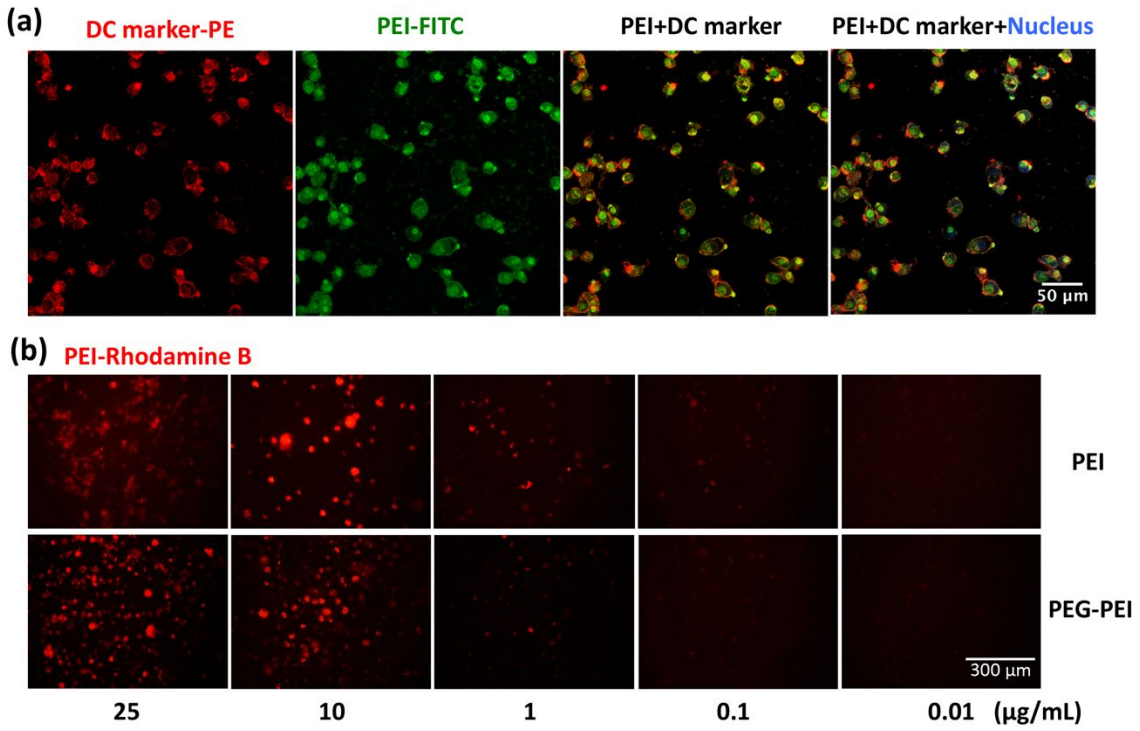

Figure S4. Fluorescence microscopy images of *in vitro* cellular uptake of BPEI or PEG-BPEI by DCs. (a) Confocal microscopic images showing the uptake of 25 kDa BPEI (green) by DC2.4 (red) after 12 h incubation. The cellular nuclei were stained with Hoechst 33258 (blue). (b) BMDCs were incubated with different concentrations of 25 kDa BPEI and PEG-PEI-B25 (red) and observed under fluorescence microscopy after 24 h. The scale bar in the right image can be applied to the others in the same panel.

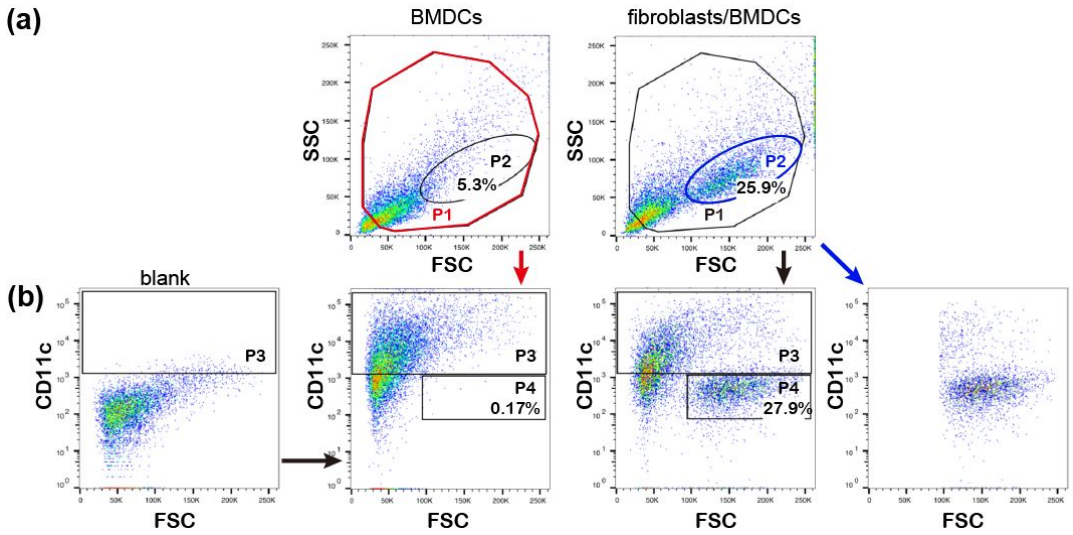

Figure S5. Separation of coculture fibroblast/BMDC population by flow cytometry analysis. (a) All harvested cells were selected and denoted as P1. An extra population of cells was observed in coculture system and selected as P2. (b) The isotype control of PE-Cy7-CD11c was stained to define P3 gate (left). Both BMDCs and coculture cells were stained with PE-Cy7-CD11c antibody. A separate CD11c negative group was observed in fibroblast/BMDC coculture system, and denoted as P4. Cell population in P2 gate matched well with the cell group shown in P4, which indicated the population of fibroblasts.

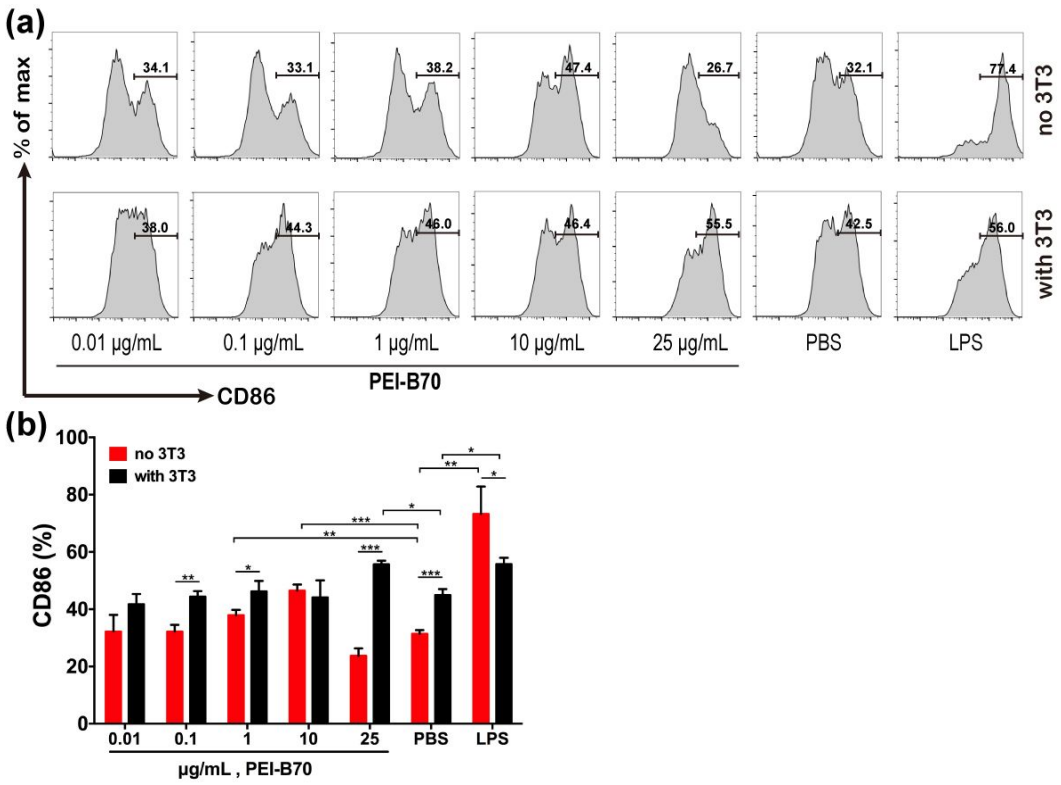

Figure S6. Comparison of BMDC maturation with or without fibroblasts mediation. (a, b) BMDCs were cocultured with PEI-B70, or PEI-B70-treated NIH-3T3 fibroblasts for 24 h. CD86 expression on BMDCs was measured. PBS and LPS were applied as negative and positive controls. These experiments were carried out in triplicate and results were shown as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ ($n = 3$).

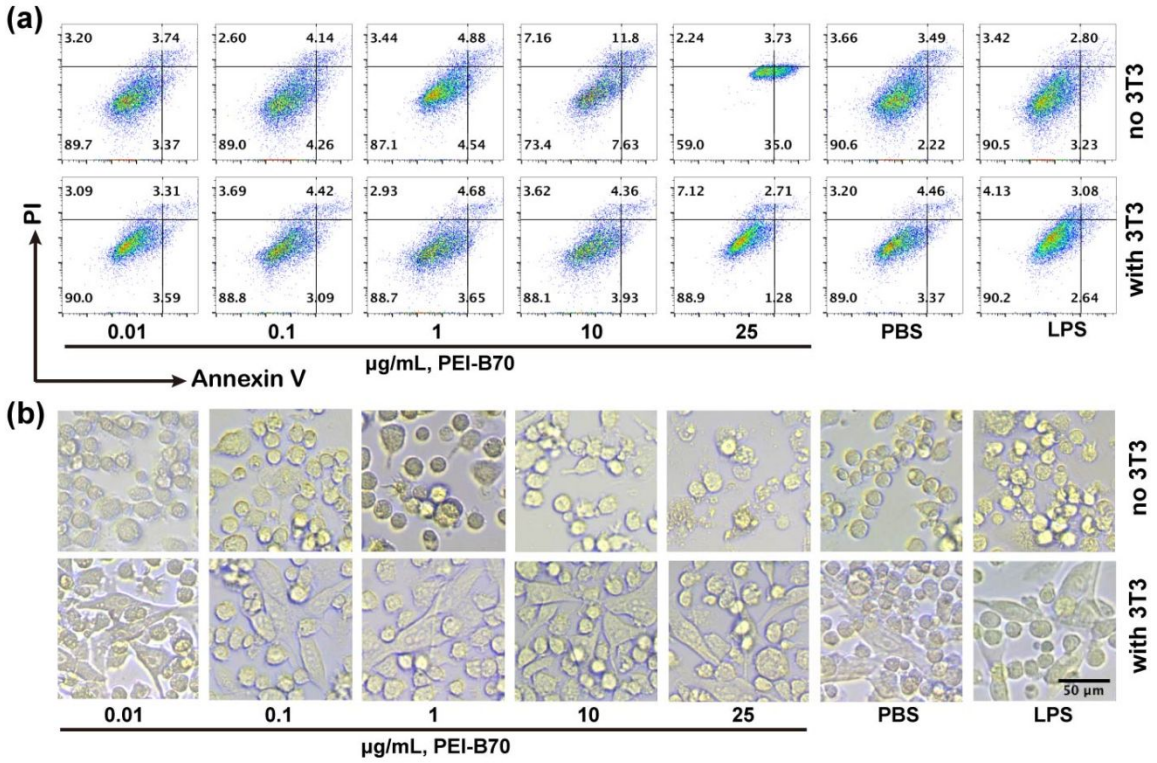

Figure S7. BMDC viability after PEI-B70 treatment with or without fibroblasts mediation. BMDCs were cultured with direct or indirect stimulation of PEI-B70 for 24 h. (a) Flow cytometry analysis of BMDC viability by Annexin V/propidium iodide (PI) assay. (b) Optical microscopy images of BMDC viability with different treatments. The scale bar in the last image is 50 µm and can be applied to the others.

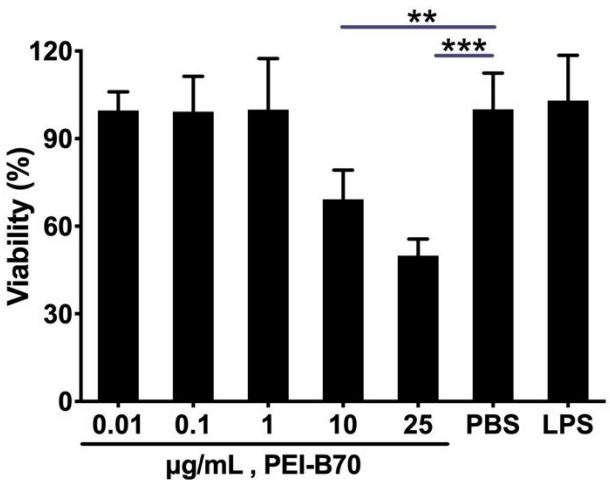

Figure S8. NIH-3T3 murine fibroblasts viability after PEI-B70 treatment for 4 h. Cell viability was assessed by CCK8 kit. The results were shown as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (n = 5).