Pentablock copolymer micelle nanoadjuvants enhance cytosolic delivery of antigen and improve vaccine efficacy while inducing low inflammation

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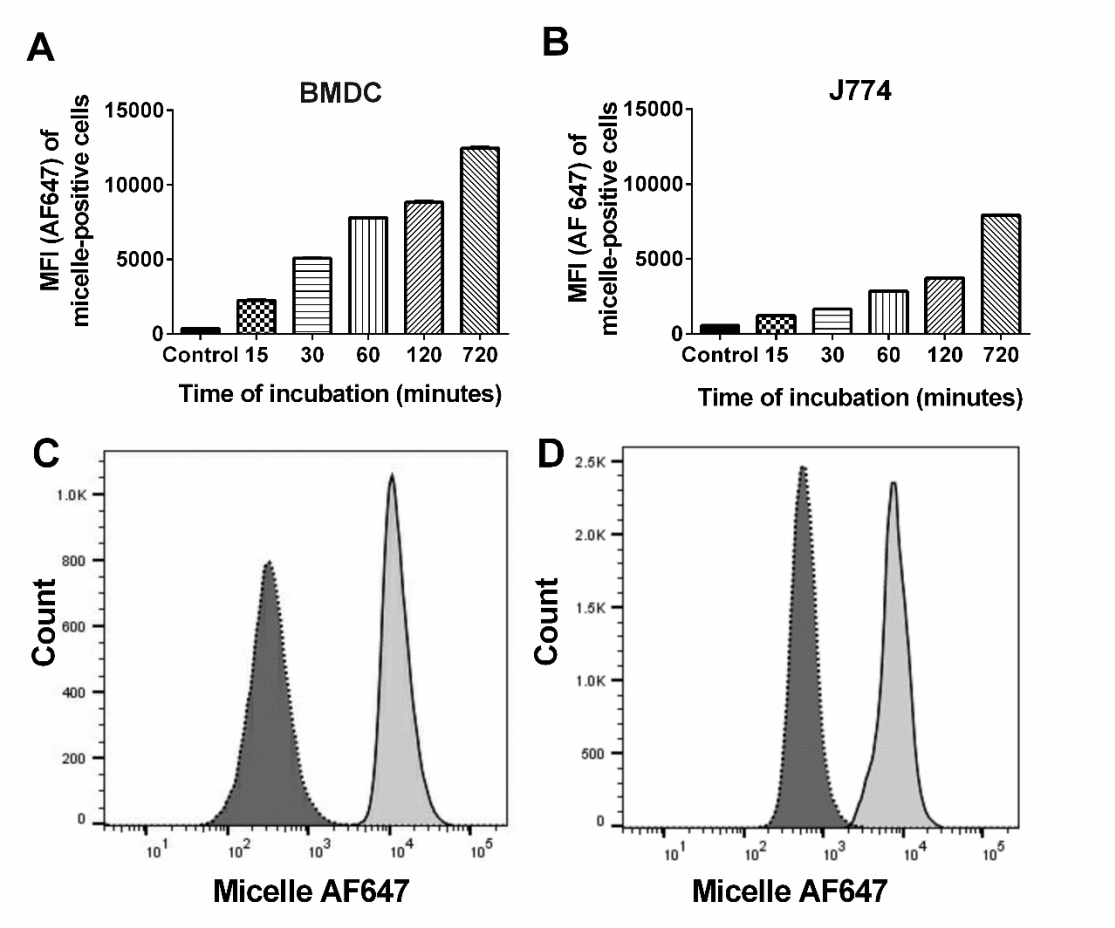

Figure S1. Mean fluorescence intensity (MFI) of micelle-positive A) BALB/c-derived BMDCs and B) J774 cells. Cells were stimulated with 12.5 µg/mL of PBC micelles labeled with AF647 for multiple lengths of time washed and fixed in FACS buffer. Internalization was measured using flow cytometry and the MFI was determined using FlowJo software. A representative histogram demonstrating the shift in the cell population treated with micelles (shown in gray,

solid outline) compared to control (shown in black, dotted outline) is shown for C) BMDCs and D) J774 cells after 12-hour (720 min) incubation. Data representative of three independent experiments and indicated as mean with standard error of mean.

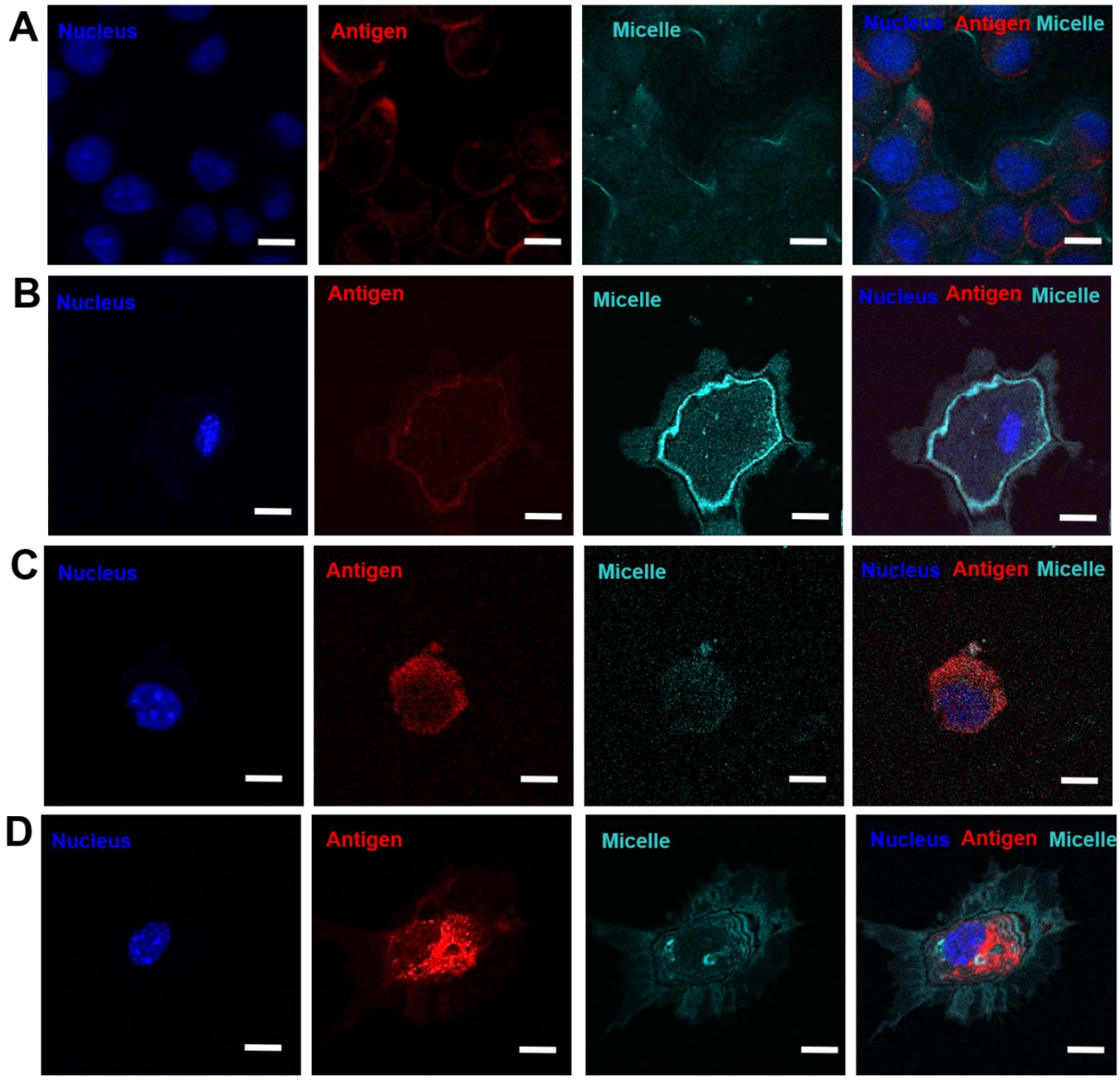

Figure S2. Trafficking of antigen into A) BALB/c-derived BMDCs and B) J774 cells incubated for 15 min with pentablock copolymer (PBC)

micelles (12.5 µg/mL) and antigen (20 µg/mL); C) BMDCs and D) J774 cells incubated for 2 hours with PBC micelles-antigen complex. Cells seeded at a concentration of 2.5 10^6 cells/mL on glass coverslips were incubated with the micelles and ovalbumin for the required amount of time. Scale bars indicate A) 50 µm and B), C) and D) 5 µm. Nuclei were stained with Hoescht (blue). Ovalbumin antigen (AF594) and PBC micelles (AF647) are denoted by red and cyan, respectively. Data are representative of three independent experiments.

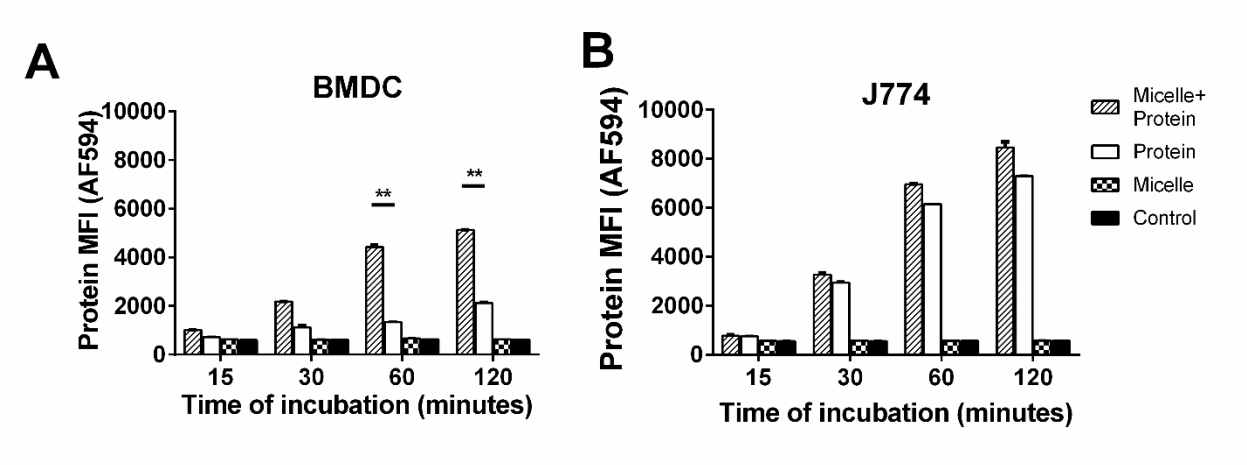

Figure S3. Time-course of the ovalbumin cellular uptake when incubated with pentablock copolymer (PBC) micelles for A) BMDCs and B) J774 cells. The cells were incubated with the micelle-Ova complexes, micelle formulation, Ova (ie. protein) alone and unstimulated control for the indicated times and mean fluorescence intensity (MFI), based on Ova uptake, was calculated using FlowJo software. Data represented as mean of triplicates with standard error of mean. **indicates statistical significance determined using unpaired t-test with $p<0.002$, n=3.

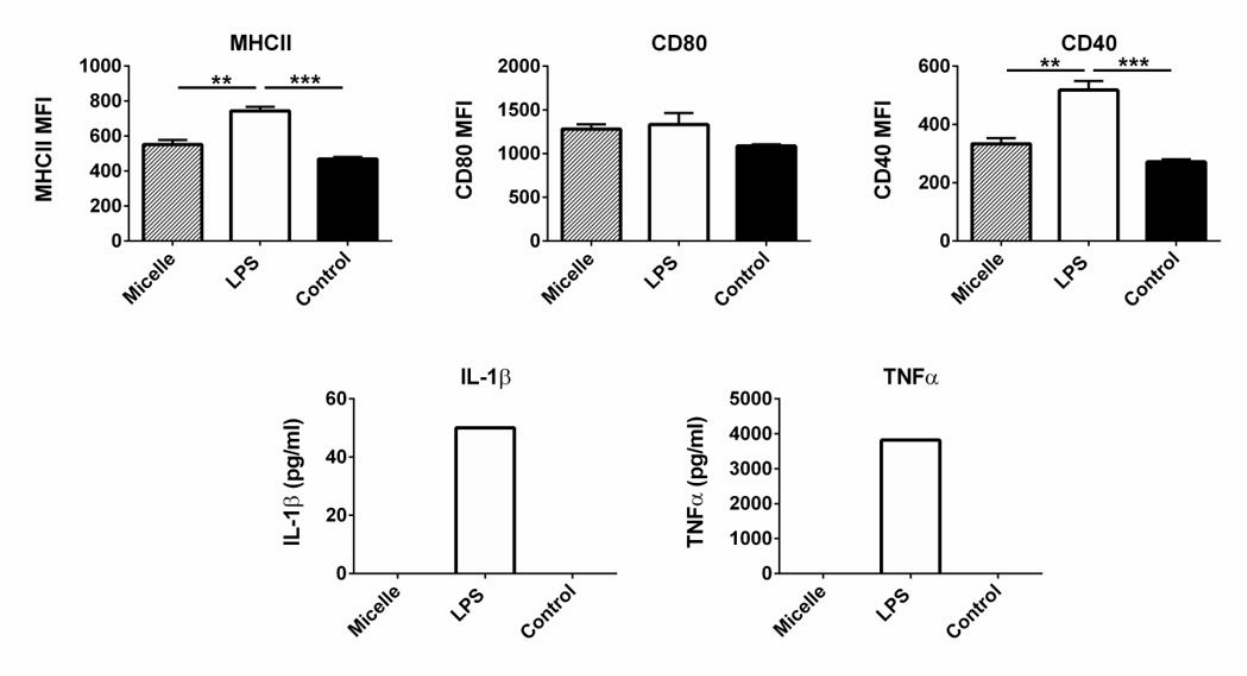

Figure S4. Pentablock copolymer (PBC) micelles did not show significant upregulation of costimulatory molecules in bone marrow macrophages and did not induce pro-inflammatory cytokine secretion. BMMs at a concentration of 2.5 10^6 cells/mL were stimulated for 48 hours with micelle or LPS (PBC micelles at the concentration of 12.5 µg/mL and LPS at 1 µg/mL) . Cell surface markers, MHCII, CD80 and CD40 upregulation were analyzed using flow cytometry and cytokine secretion in supernatants were analyzed using Multiplex bead assay. Data represented as mean with standard error of mean. Statistical significance indicated by $^{**}p<0.005$ and $^{***}p<0.0005$. n=3.

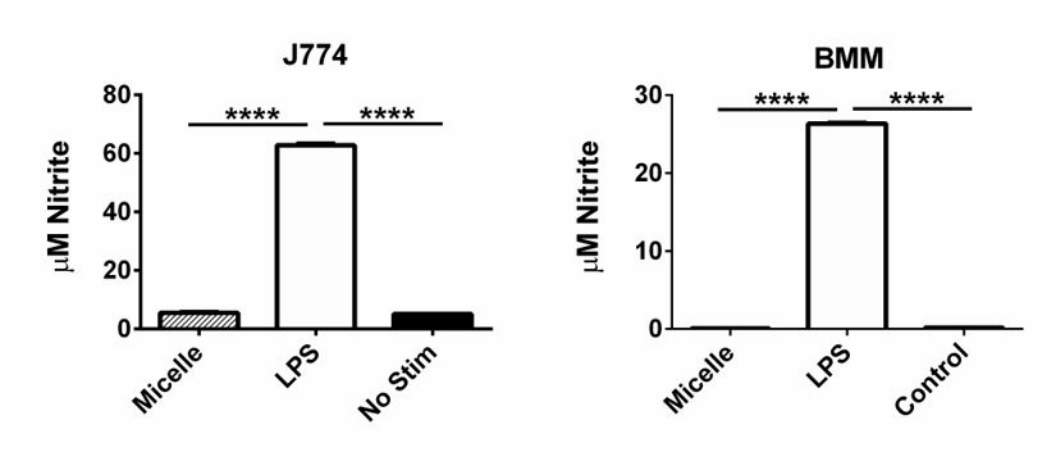

Figure S5. PBC micelle adjuvants do not induce nitric oxide in J774 cells and bone marrow macrophages. Cells were stimulated with micelles and controls for 48 hours. Supernatants were collected to analyze NO production using Griess assay. Data represented as mean nitrile production with standard error of mean. **** indicates statistical significance with $p<0.0001$.