Supporting information for

# Polymer-Coated Gold Nanospheres Do Not Impair the Innate Immune Function of Human B Lymphocytes *in Vitro*

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**Light microscopy images.** In order to assess a potential increase in cell death upon 24 h GNP exposure (20 μg/ml), phase contrast images of the APC cultures were obtained. GNP formulations caused no significant impact upon APC density (Fig. S1). Interaction of citrate-GNS was observed with both MDMs and MDDCs (Fig. S1B and S1C), although not with B cells (Fig. S1A). For the polymer-coated GNP, images were unable to show interactions with the different cell types. Thus, in view of the lack of sensitivity of light microscopy, in order to correctly evaluate GNP-cell association, other methods were used (DF-HSI, ICP-MS, flow cytometry). Addition of R848 immunostimulant did not cause any qualitative increases in cell death or GNP-cell interaction. LPS was used as an additional positive control to highlight morphological changes of activated MDM and MDDC,.

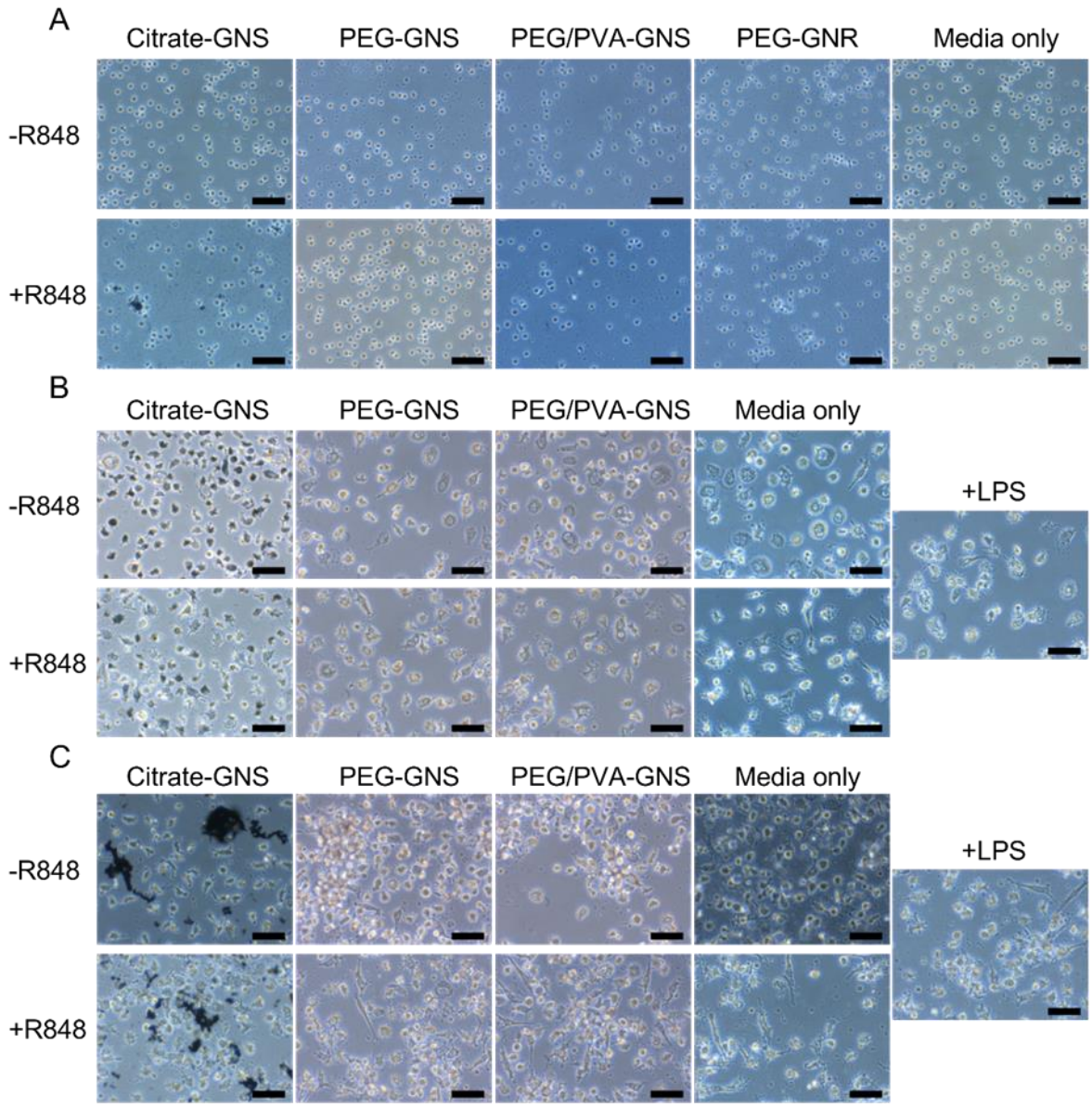


**Figure S1.** Light microscopy phase contrast images of antigen-presenting cells (APCs) exposed to gold nanoparticles (GNPs). B cells **(A),** MDMs **(B)** and MDDCs **(C)** Cells were exposed for 24 h to GNS (20 μg/ml) with different surface functionalizations. B cells were as well exposed to PEG-GNR (20 μg/ml). All APCs were incubated with or without immunostimulant R848 (2 μg/ml). MDM and MDDCs were also incubated with LPS only (100 ng/ml). Scale bars: 50 μm.

**Table S1.** ICP-MS parameters optimized for the three collision cell modes.

| Collision cell modes: | | [No gas] | [He] | [HEHe] |
|---|---|---|---|---|
| Plasma mode | | Low matrix | Low matrix | Low matrix |
| Plasma RF Power(W) | | 1550 | 1550 | 1550 |
| Collision gas (He) flow | (mL/min) | 0 | 4.3 | 10.0 |
| Nebulizer gas (Ar) flow | (L/min) | 1.0 | 1.0 | 1.0 |
| Auxiliary gas (Ar) flow | (L/min) | 0.9 | 0.9 | 0.9 |
| Plasma gas (Ar) flow | (L/min) | 15.0 | 15.0 | 15.0 |
| Extract 2 (V) | | -165 | -165 | -165 |
| Omega bias (V) | | -90 | -90 | -90 |
| Omega lens (V) | | 8.8 | 8.8 | 8.8 |
| Deflect (V) | | 13.6 | 0.2 | -73.2 |
| OctP RF (V) | | 200 | 200 | 190 |
| Energy discrimination | (V) | 5.0 | 3.0 | 7.0 |

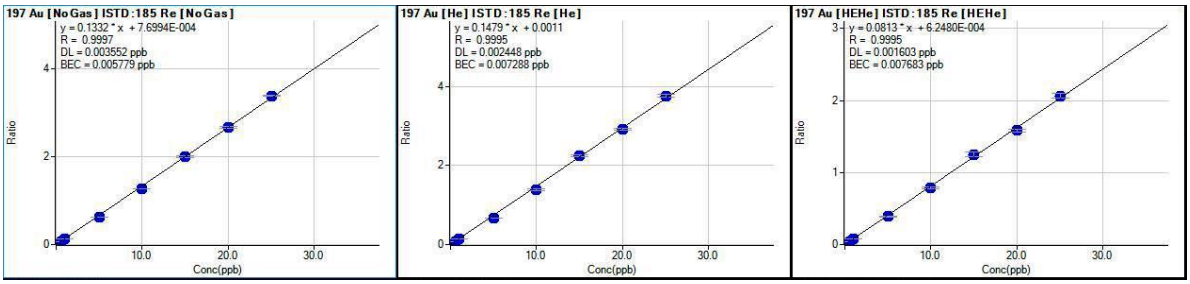


**Figure S2.** Calibration curves for $^{197}$Au in the three collision cell modes.

**PEG-GNR uptake by B lymphocytes.** PEG-GNR were not detected in B lymphocytes by DF-HSI (Fig. S3). A low amount of PEG-GNR associating with B cells was further confirmed and quantified by highly sensitive ICP-MS (as described in the main text manuscript).

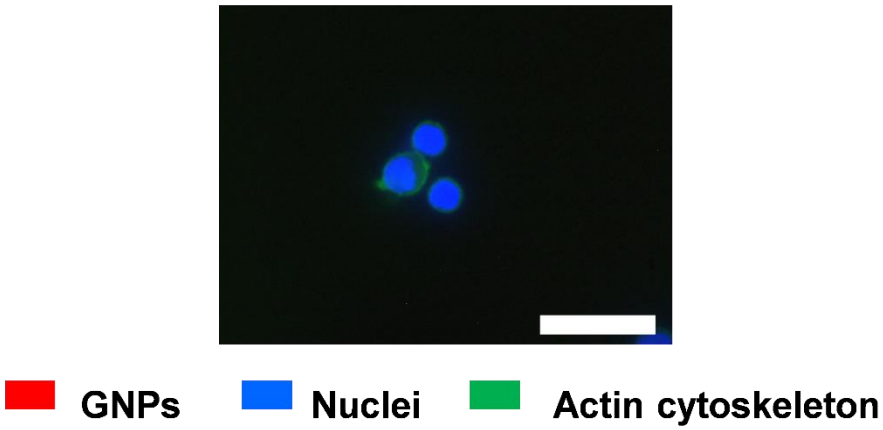


**Figure S3.** Dark field hyperspectral image of B lymphocytes exposed to PEG-GNR (20 μg/ml) for 24 h. Scale bar: 5 μm.

**GNP-cell association *via* Flow Cytometry.** GNPs that are internalized can be assessed by changes in the light scatter detected by flow cytometry. As indicated below, exposure to citrate GNS caused an increase in the side scatter (SSC) for B cells, but not exposure to polymer-protected GNS (Figure S4). In contrast, in MDMs and MDDCs exposed to polymer-protected GNS, an increase in SSC was observed.

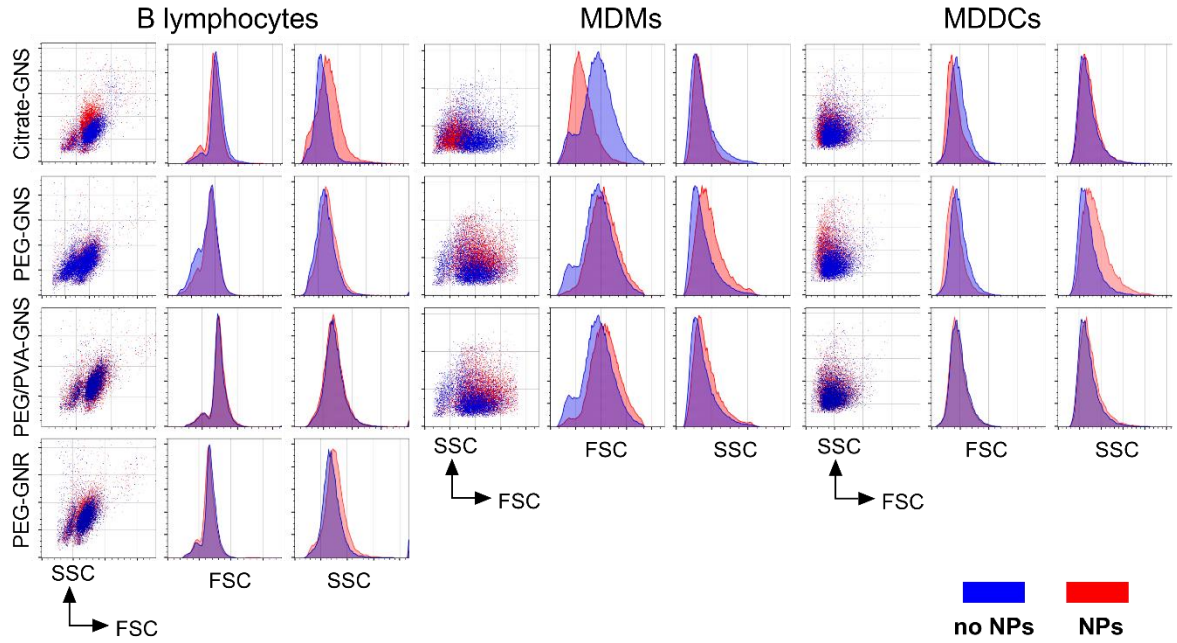


**Figure S4.** FSC/SSC flow cytometry dot plots and histograms of antigen-presenting cells exposed to GNPs. All APCs were exposed for 24 h to GNS (20 μg/ml) with different surface functionalizations. B cells were as well exposed to PEG-GNR (20 μg/ml).

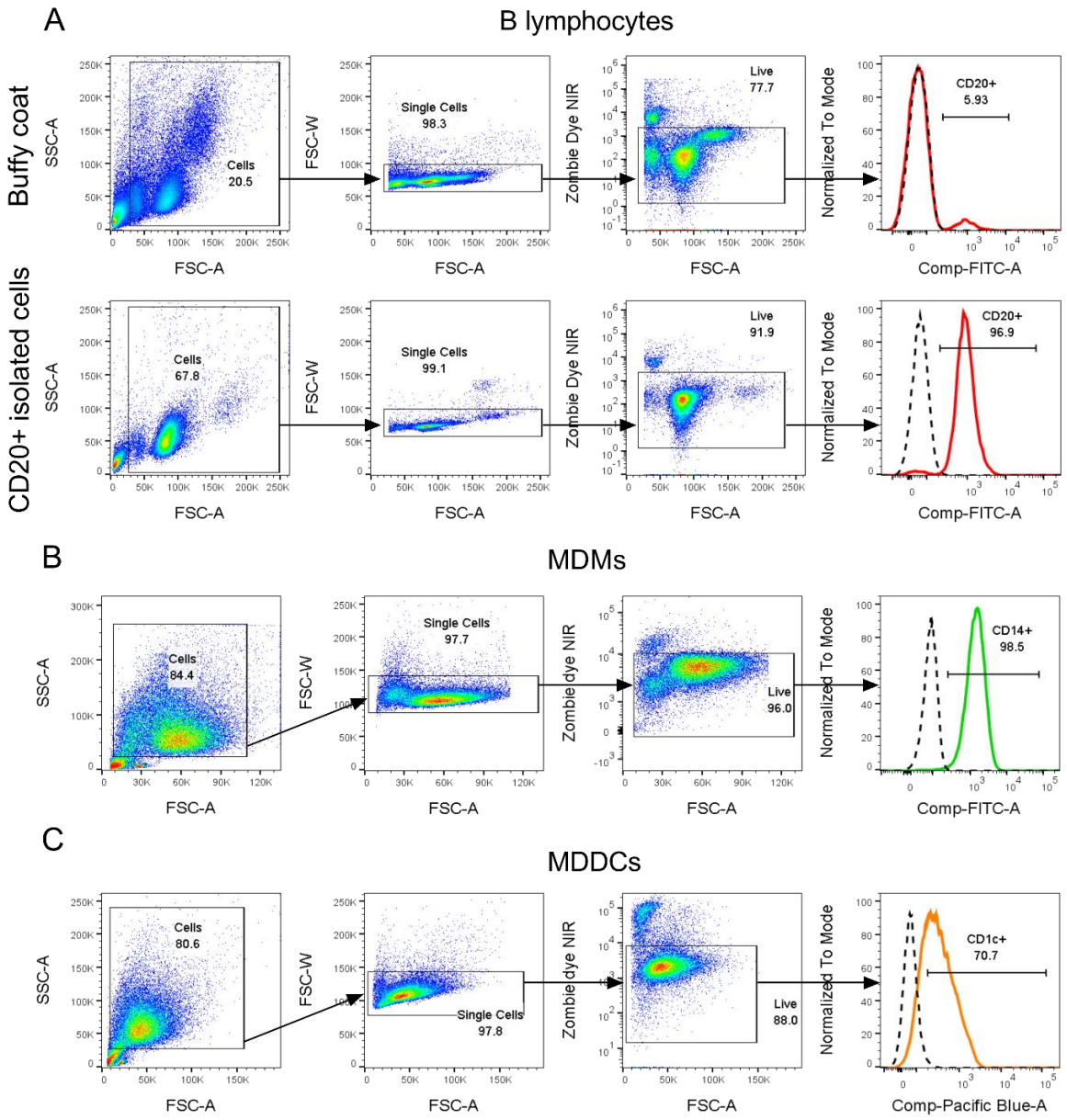


**Figure S5.** Gating strategy and B lymphocyte purity. All cells were first gated to exclude dead cells and cell debris followed by gating of single cells only. Next, live cells were determined by Zombie NIR viability dye. B cells were defined as percentage of CD20-FITC positive cells, before (buffy coat) and after purification. Approximately 96% B cell purity was achieved throughout the experiments **(A)**. MDMs were defined as CD14-FITC positive **(B)** and MDDCs as CD1c-Pacific Blue positive cells **(C)**.

**MDM and MDDC activation.** To compare how GNPs influence the activation potential of different APCs, MDMs and MDDCs were exposed to all the formulations of GNS at 20 μg/ml for 24 h. In general, polymer-coated GNS did not impair the expression of activation markers in naïve as well as in R848-stimulated MDMs and MDDCs (Figure S6). It was observed that high MDM uptake of citrate-GNS interfered with Pacific Blue and PE-Texas Red channel, resulting in false decrease of MFI for CD69 and MHC II markers (Figure S6A). No such interference was detected for B cells and MDDCs. There was an upregulation of CD86 in MDDCs due to PEG-GNS without R848. However, an endotoxin test later confirmed contamination of that specific PEG-GNS batch, which resulted in a false positive increase of CD86 in MDDCs (Figure S6B and Figure S9). LPS served as a positive control for innate immune cell activation.

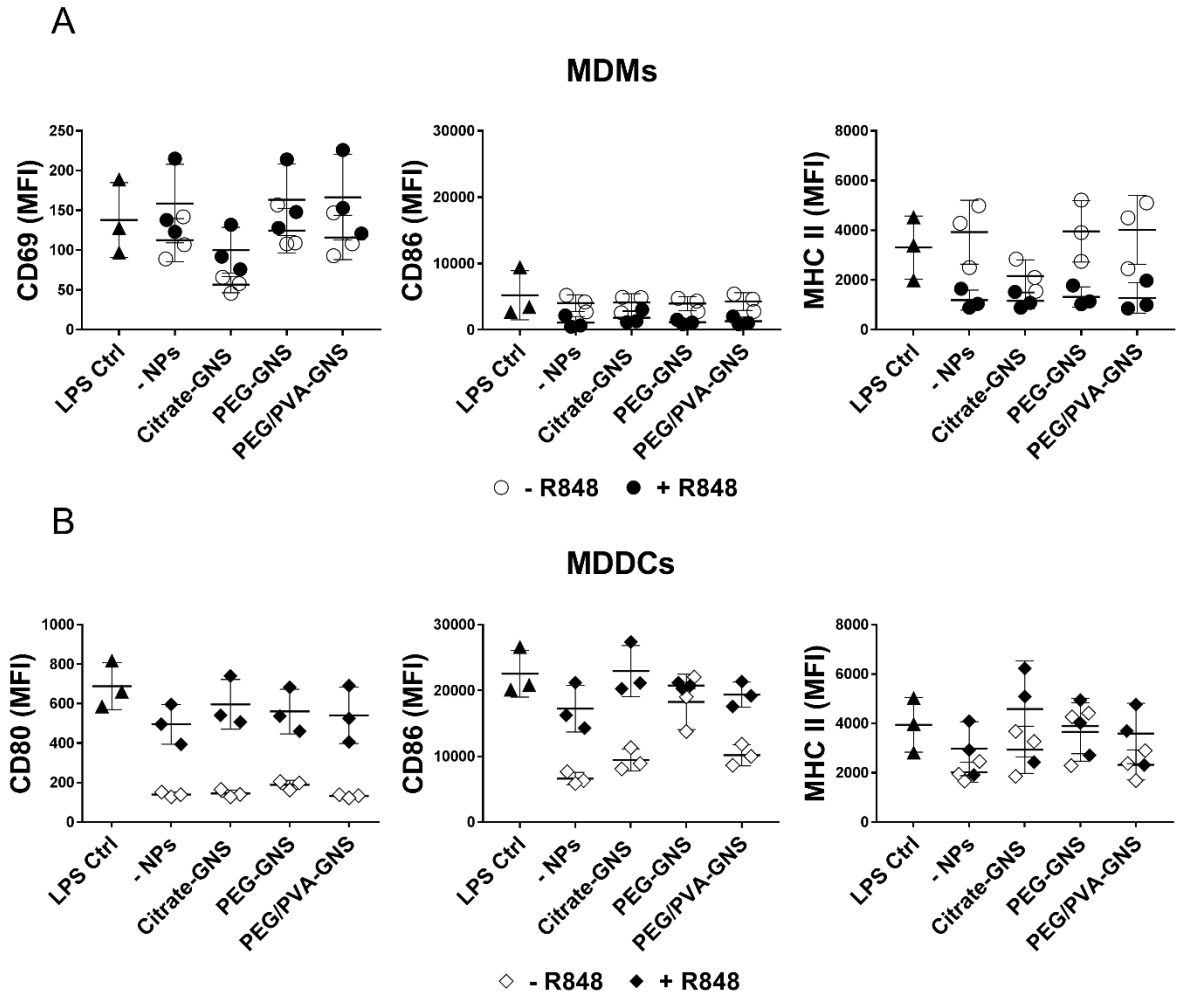


**Figure S6.** MDM and MDDC activation. Surface activation markers on MDMs (CD69, CD86 and MHC-II) **(A)** and MDDCs (CD80, CD86, MHC II) **(B)** measured by flow cytometry after 24 h exposure of cells to GNS (20 µg/ml) with different surface functionalization. Cells were incubated with or without the immunostimulant R848 (2 µg/ml) and with LPS only (100 ng/ml). Each point represents one donor (n=3). Error bars: mean ± SD. Data were evaluated by two-way ANOVA, followed by Tukey's multiple comparison test.

**MDM and MDDC pro-inflammatory response.** MDMs and MDDCs were tested for the changes in their cytokine release, following 24h exposure with different surface functionalized GNS (20 μg/ml). TNF-α and IL-1β release was not affected by GNS in MDM (Figure 7A) and TNF-α and IL-6 were not altered in MDDCs (Figure S7B), whether the cells were stimulated with R848 immunostimulant or not. LPS served as positive control for innate immune cell activation.

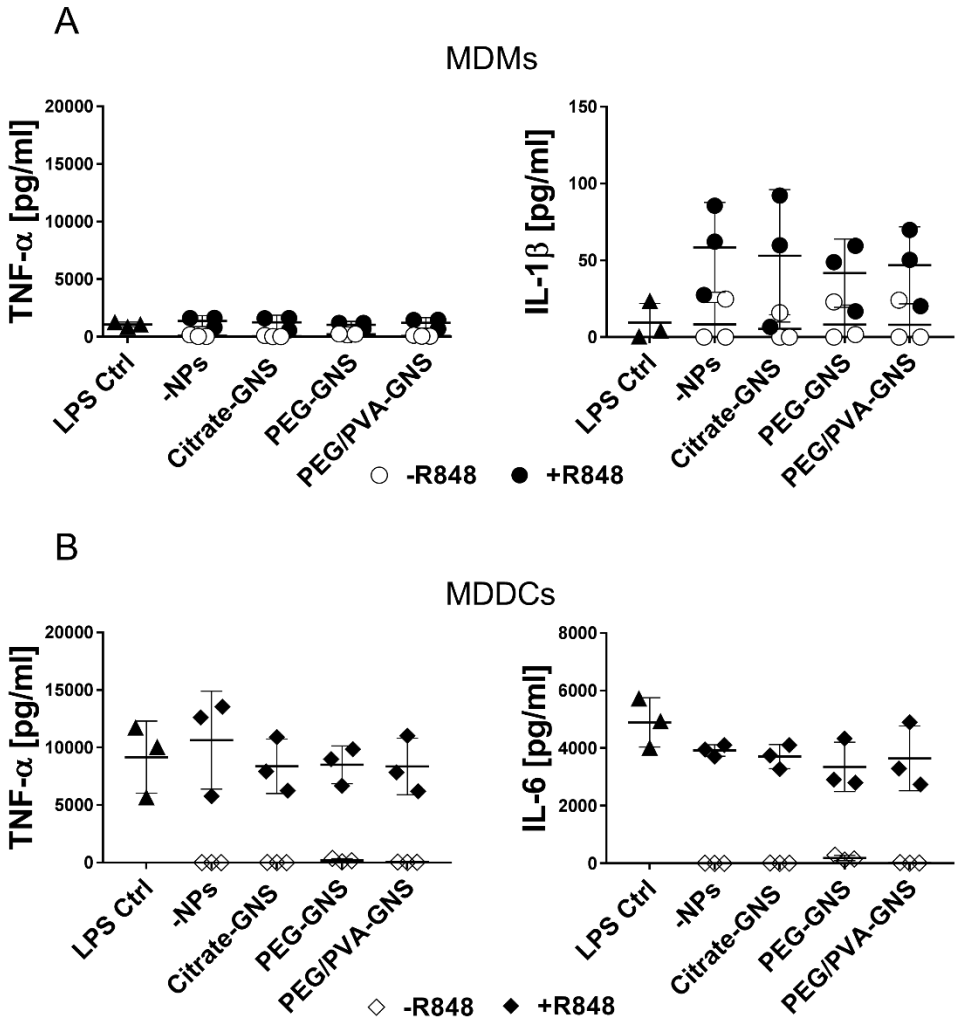


**Figure S7.** MDM and MDDC pro-inflammatory response. Pro-inflammatory cytokines released by MDMs (TNF-α, IL1-β) **(A)** and MDDCs (TNF-α, IL-6) **(B)** after 24 h exposure of B cells to GNS (20 μg/ml) with different surface functionalization. Cells were incubated with or without the immunostimulant R848 (2 μg/ml) and with LPS only (100 ng/ml). Each point represents one donor (n=3). Error bars: mean ± SD. Data were evaluated by two-way ANOVA, followed by Tukey's multiple comparison test.

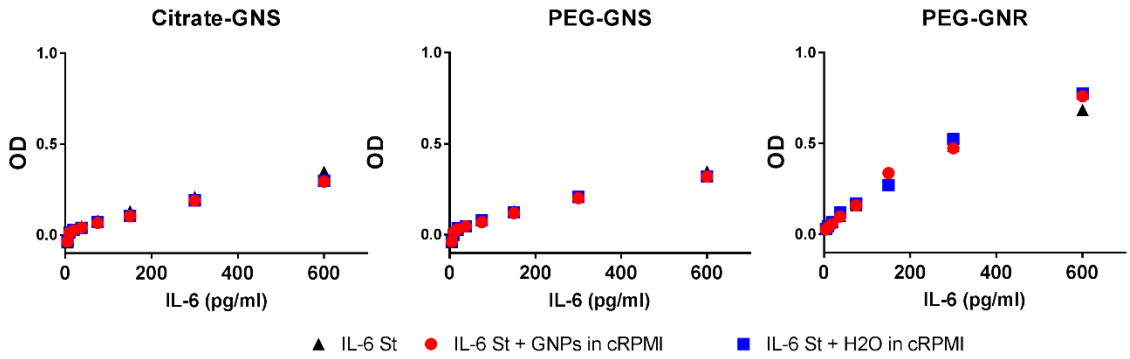


**Figure S8.** Gold nanoparticle interference test. Gold nanoparticle interference with ELISA by cytokine absorption to GNP surface. Citrate-GNS, PEG-GNS and PEG-GNR were incubated in complete culture media with human plasma for 24 h, 37 °C, 5% $CO_2$. $H_2O$ was added to the media as a negative control. Samples were then incubated with IL-6 standards for 1 h at RT, followed by the normal ELISA protocol. The experiment was performed once. Each point on the graphs represents mean of triplicates. RPMI+HP: complete culture medium consisting of RPMI with 1% Pen/Strep, 1% L-Glutamine, 10% human plasma.

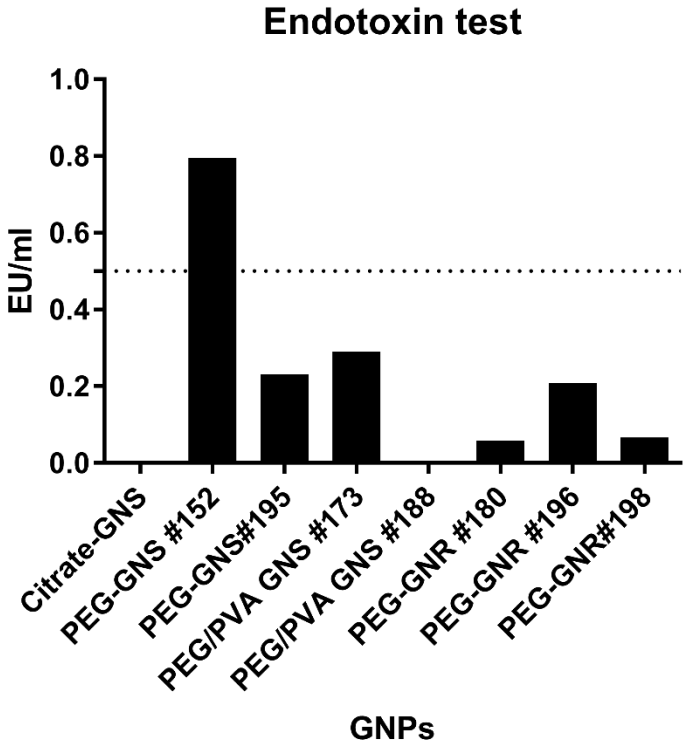


Figure S9: Endotoxin test of different batches of GNPs used in the study. All batches were below the FDA recommended level (> 0.5 EU/ml).[1] except batch #152, which was used for exposure of MDDCs.

(1) Food and Drug Admistration (FDA). Guidance for Industry Pyrogen and Endotoxins Testing https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM310098.pdf (accessed Mar 30, 2019).