Supporting information

Poly(I:C)-encapsulating nanoparticles enhance innate immune responses to the tuberculosis vaccine Bacille-Calmette-Guérin (BCG) via synergistic activation of innate immune receptors

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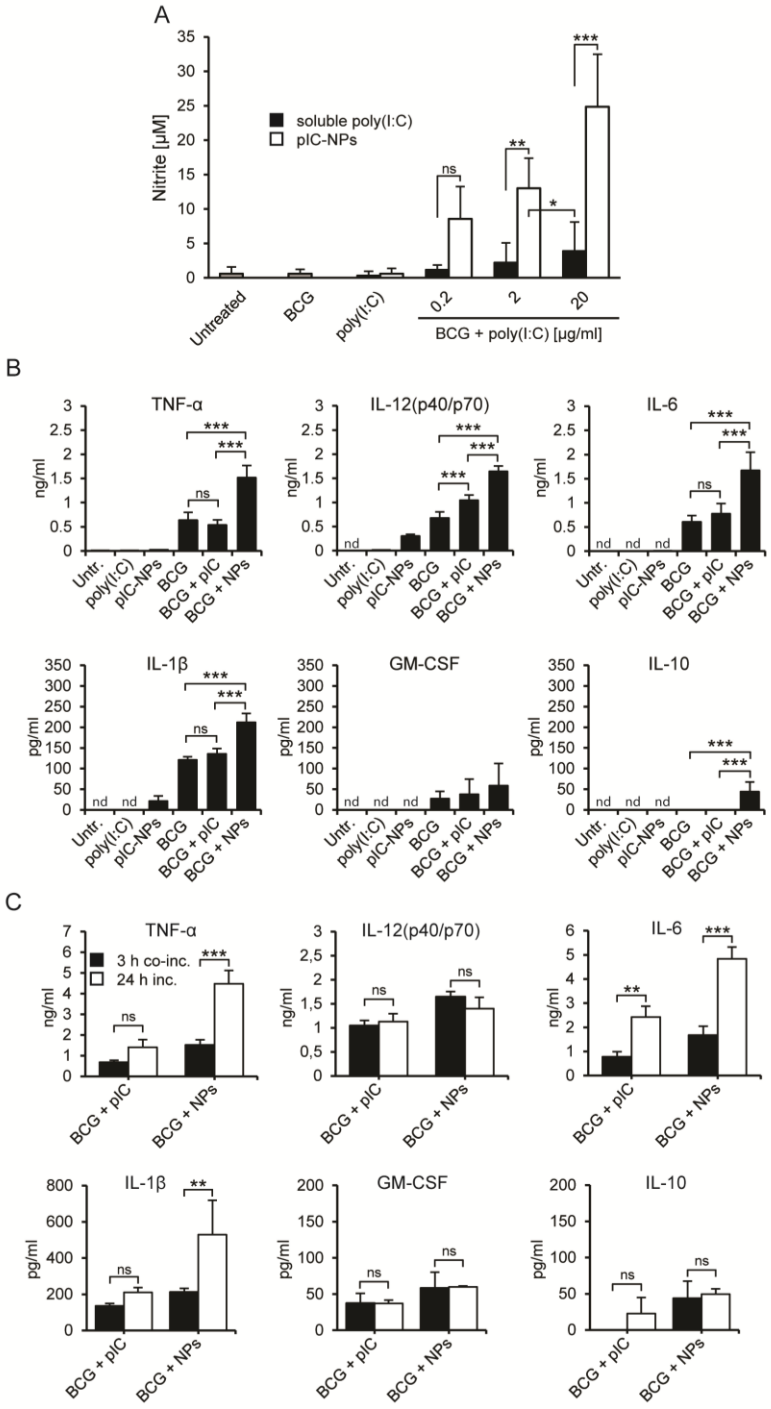
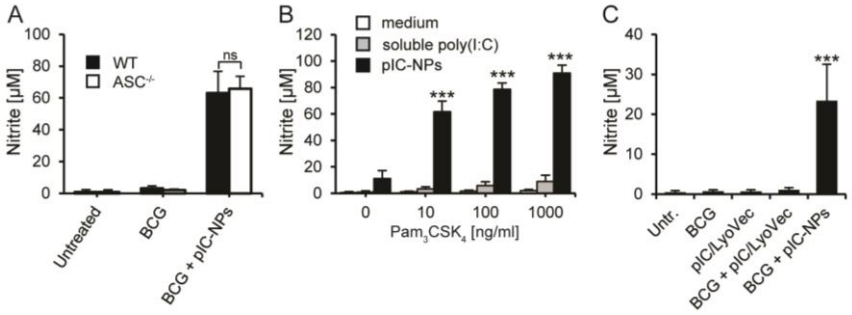
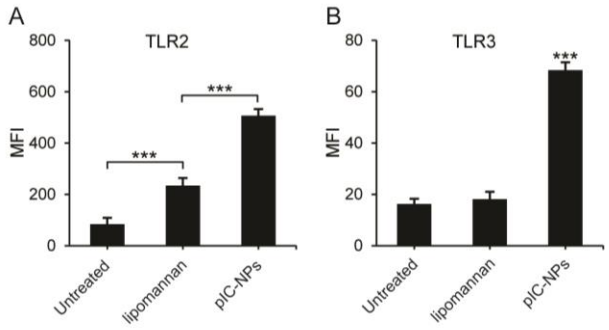


Supplementary Figure S1. Production of nitric oxide (NO) and cytokines in BMDM after short-term coincubation with BCG and soluble poly(I:C) or pIC-NPs. (A) NO production by BMDM coincubated with BCG and soluble poly(I:C) or pIC-NPs at different concentrations for 3 h, followed by wash-out and an additional incubation period of 24 h, after which culture supernatants were harvested and analyzed. As controls, BMDM were left untreated, infected with BCG only or treated with 20 µg/ml soluble poly(I:C) or pIC-NP for 3 h. (B) Production of pro- and antiinflammatory cytokines by BMDM incubated with 20 µg/ml poly(I:C) in soluble or NP-form alone or together with BCG for 3 h. Untreated BMDM or cells

infected with BCG only were used as controls. At 24 h after BCG-infection, cell culture supernatants were collected and analyzed for cytokine levels. (C) Comparison of cytokine production by BCG-infected BMDM after a short-term coincubation with BCG and soluble poly(I:C) or pIC-NPs (both 20 µg/ml) for 3 h, and after a prolonged incubation with soluble poly(I:C) or pIC-NPs for 24 h following BCG infection. Cytokine levels in both groups were analyzed 24 h after infection with BCG. Data are presented as means ± SD of three (B, C) or four (A) independent experiments; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



Supplementary Figure S2. Role of TLR2 and different non-TLR signaling pathways in synergistic induction of NO production in BMDM by pIC-NPs and BCG. (A) NO production in BCG-infected WT and KO-BMDM, deficient in the central inflammasome adaptor protein ASC ($ASC^{-/-}$ BMDM), in response to stimulation with 2 µg/ml pIC-NPs for 24 h. (B) Nitric oxide production in WT-BMDM after co-stimulation with the TLR2 agonist Pam_3CSK_4 at different concentrations and soluble poly(I:C) or pIC-NPs (both at 2 µg/ml) for 24 h. (C) NO production in BCG-infected WT-BMDM after stimulation with 2 µg/ml of the Rig-I/Mda5 agonist poly(I:C)/LyoVec™ or pIC-NPs for 24 h. Data represent means ± SD of three independent experiments; ns, not significant; *** $p < 0.001$.



Supplementary Figure S3. Expression of TLR2 and TLR3 in BMDM stimulated with lipomannan or pIC-NPs. Flow cytometry analysis of the expression of TLR2 (A) and TLR3 (B) in BMDM treated with 500 ng/ml lipomannan for 3 h or with 2 µg/ml pIC-NPs for 24 h in comparison with untreated BMDM. Data are presented as means ± SD of three independent experiments; ns, not significant; *** $p < 0.001$.