Supplementary information

Nanoparticle-sensitized photoporation enables inflammasome activation studies in targeted single cells

Aranit Harizaj[1], Filip Van Hauwermeiren[2,3], Stephan Stremersch[1], Riet De Rycke[5,6], Herlinde De Keersmaecker[1,4], Toon Brans[1,4], Juan C. Fraire[1], Karolien Grauwen[2], Stefaan C. De Smedt[1,4], Ine Lentacker[1],
Mohamed Lamkanfi[2,3,#,*], Kevin Braeckmans[1,4,#,*]

1. Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Science, Ghent University, Ghent, 9000, Belgium
2. Janssen Immunosciences, Pharmaceutical Companies of Johnson & Johnson, Beerse, 2340, Belgium
3. Department of Internal Medicine and Pediatrics, Ghent University, Ghent, 9000, Belgium
4. Centre for Advanced Light Microscopy, Ghent University, Ghent, 9000, Belgium
5. Department of Biomedical Molecular Biology, Ghent University, Ghent, 9052, Belgium and VIB Center for Inflammation Research, Ghent, 9052, Belgium
6. Expertise Centre for Transmission Electron Microscopy and VIB BioImaging Core, Ghent University, Ghent, Belgium

Shared senior authors

* Corresponding authors: Kevin.Braeckmans@UGent.be, Mohamed.Lamkanfi@UGent.be

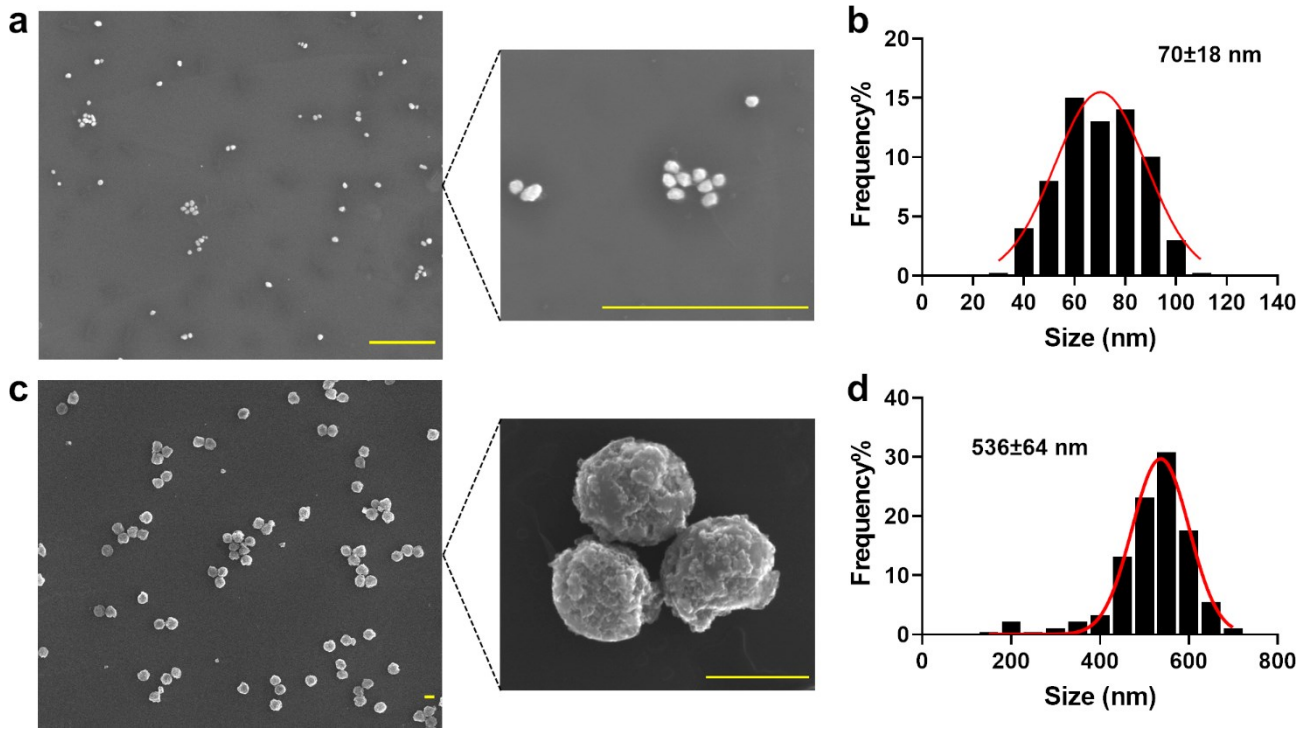

Fig. S1 (a) SEM images of PDDAC coated AuNPs with a magnified view on the right side. Scale bar = 500 nm **(b)** Histogram showing the corresponding size distribution for PDDAC coated AuNPs which confirms the successful synthesis of AuNPs with a mean size of 70 nm. **(c)** SEM images of MagPs with a magnified view on the right side. Scale bar = 500 nm. **(d)** Histogram showing the corresponding size distribution for MagPs with a mean size of 536 nm.

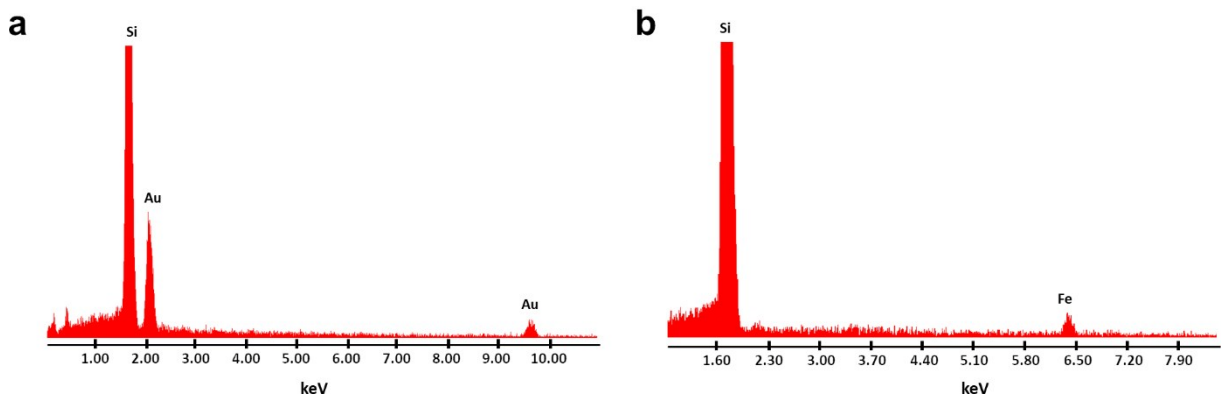

Fig. S2 EDS spectra for PDDAC coated AuNPs **(a)** and MagPs **(b)**. **(a)** The Au peaks confirm the purity of AuNPs. **(b)** An iron peak can be seen for MagPs which results from the iron oxide shell.

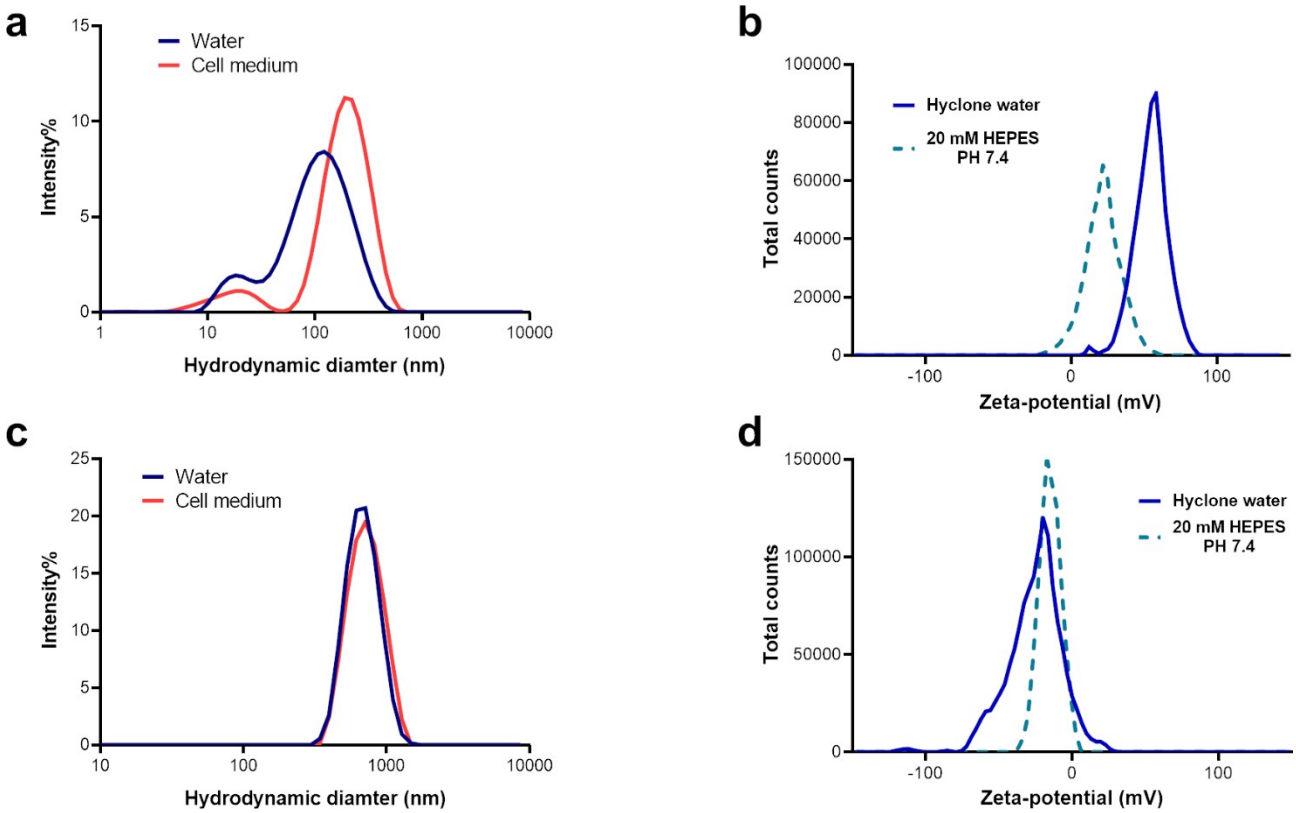

Fig. S3 Physicochemical properties of PDDAC coated AuNPs and MagPs as measured by DLS. **(a)** Hydrodynamic diameter of AuNPs measured in both hyclone water (blue) and full cell culture medium (red). **(b)** Zeta-potential measurement of AuNPs as measured in respectively hyclone water (blue curve) and HEPES buffer (green striped line, 20 mM, PH = 7.4). **(c)** Hydrodynamic diameter of MagPs measured in both hyclone water (blue) and full cell culture medium (red). **(d)** Zeta-potential measurement of MagPs as measured in respectively hyclone water (blue curve) and HEPES buffer (green striped line, 20 mM, PH = 7.4).

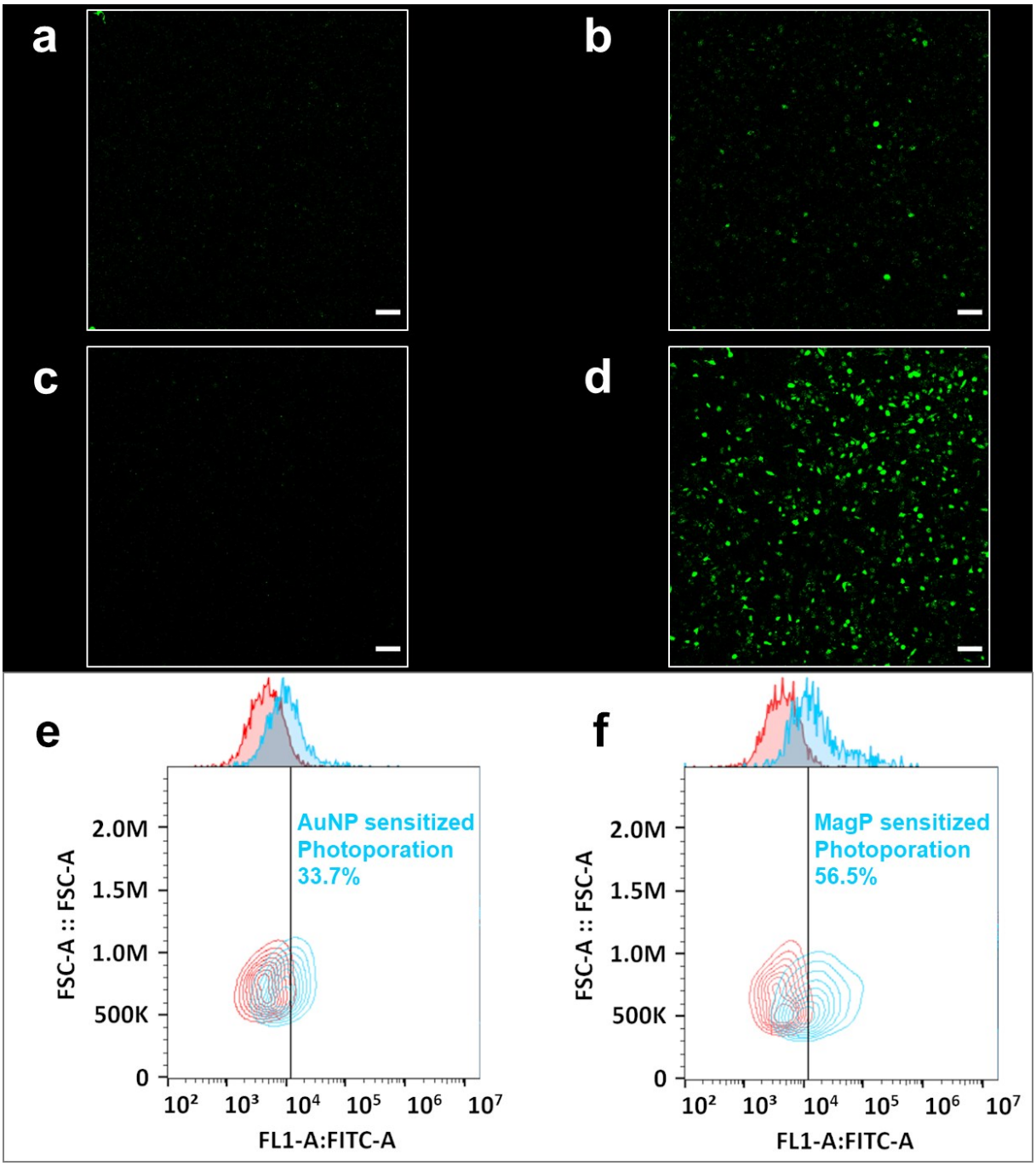

Fig. S4 (a-b) Confocal microscopy images of macrophages incubated with FD150 and AuNPs as control **(a)** and macrophages photoporated with AuNPs and FD150 **(b)** (scale bar = 100 µm). **(c-d)** Confocal microscopy images of macrophages incubated with FD150 and MagPs as a control **(c)** and macrophages photoporated with MagPs and FD150 **(d)** (scale bar = 100 µm). **(e)** AuNP-sensitized photoporation of FD150 as measured with flow cytometry (blue). Control is incubated with FD150 and AuNPs (red). **(f)** MagP-sensitized photoporation of FD150 as measured with flow cytometry (blue). Control is incubated with FD150 and MagPs (red).

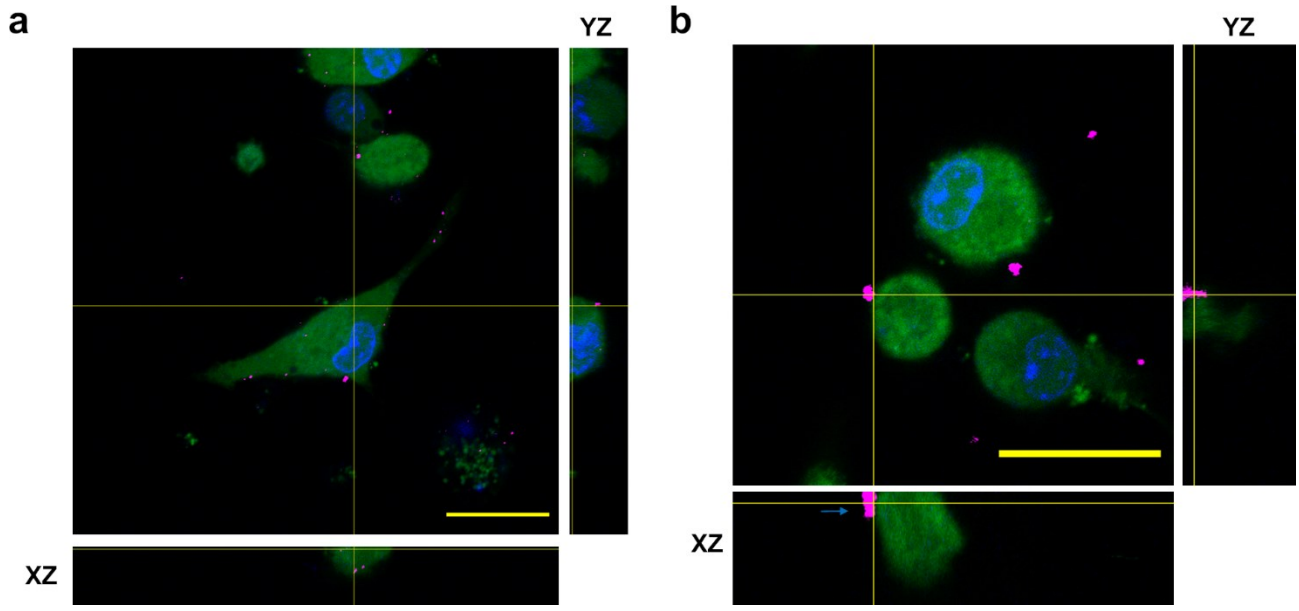

Fig. S5 Confocal reflection microscopy images of macrophages for the visualization of respectively (a) AuNPs and (b) MagPs. The particles are shown in pink (false-colored based on their scattering signal), nucleus is shown in blue (stained with Hoechst 33342) and the cytosol is shown in green (stained with calcein AM). Scale bar = 20 μm.

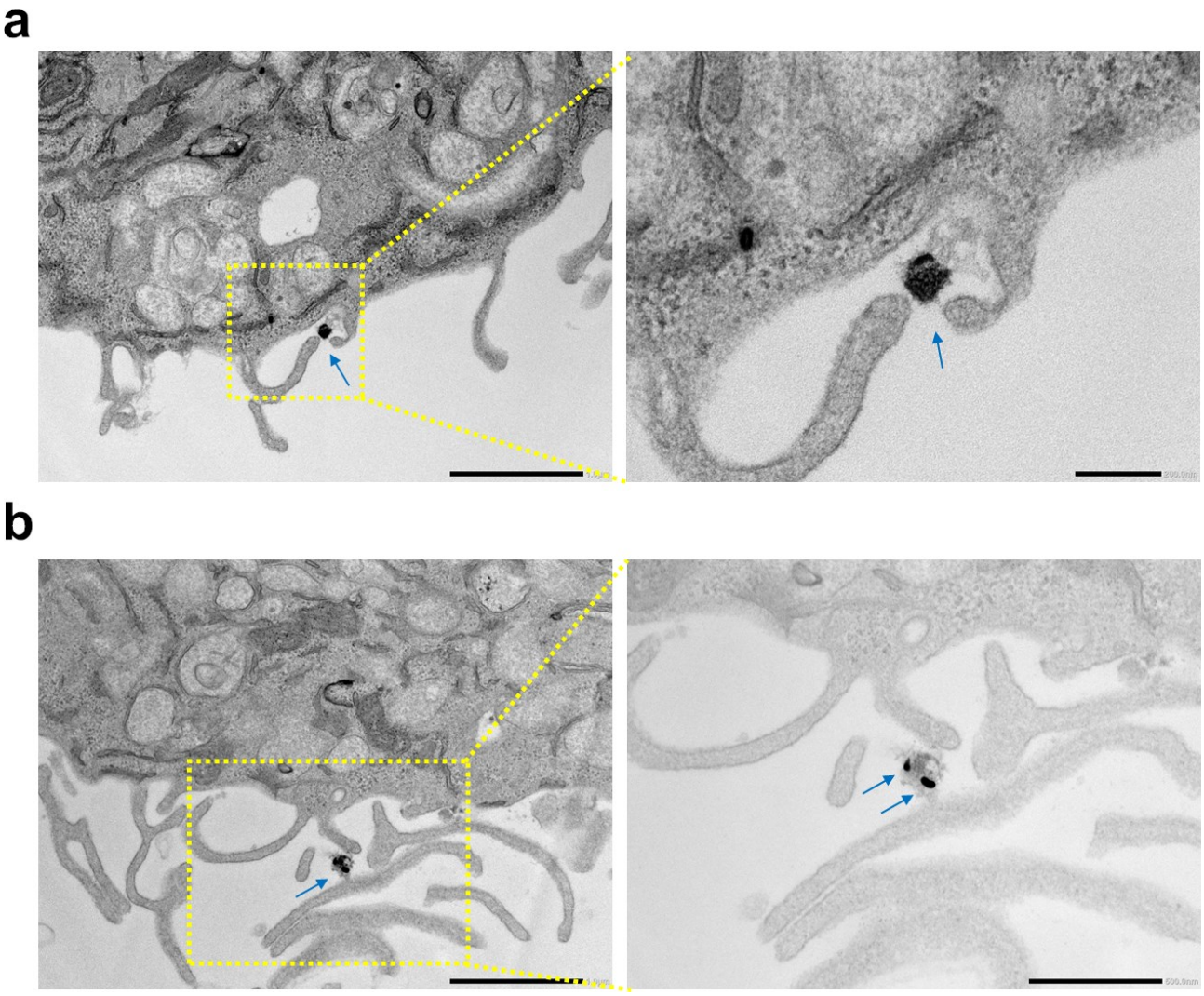

Fig. S6 Transmission electron microscopy images of macrophages incubated with AuNPs. (a-b) TEM images showing AuNPs engulfed by pseudopodia of the macrophage (blue arrows) ((a-b) scale bar = 1 μm) with a magnified view on the right side ((a) scale bar = 200 nm; (b) scale bar = 500 nm).

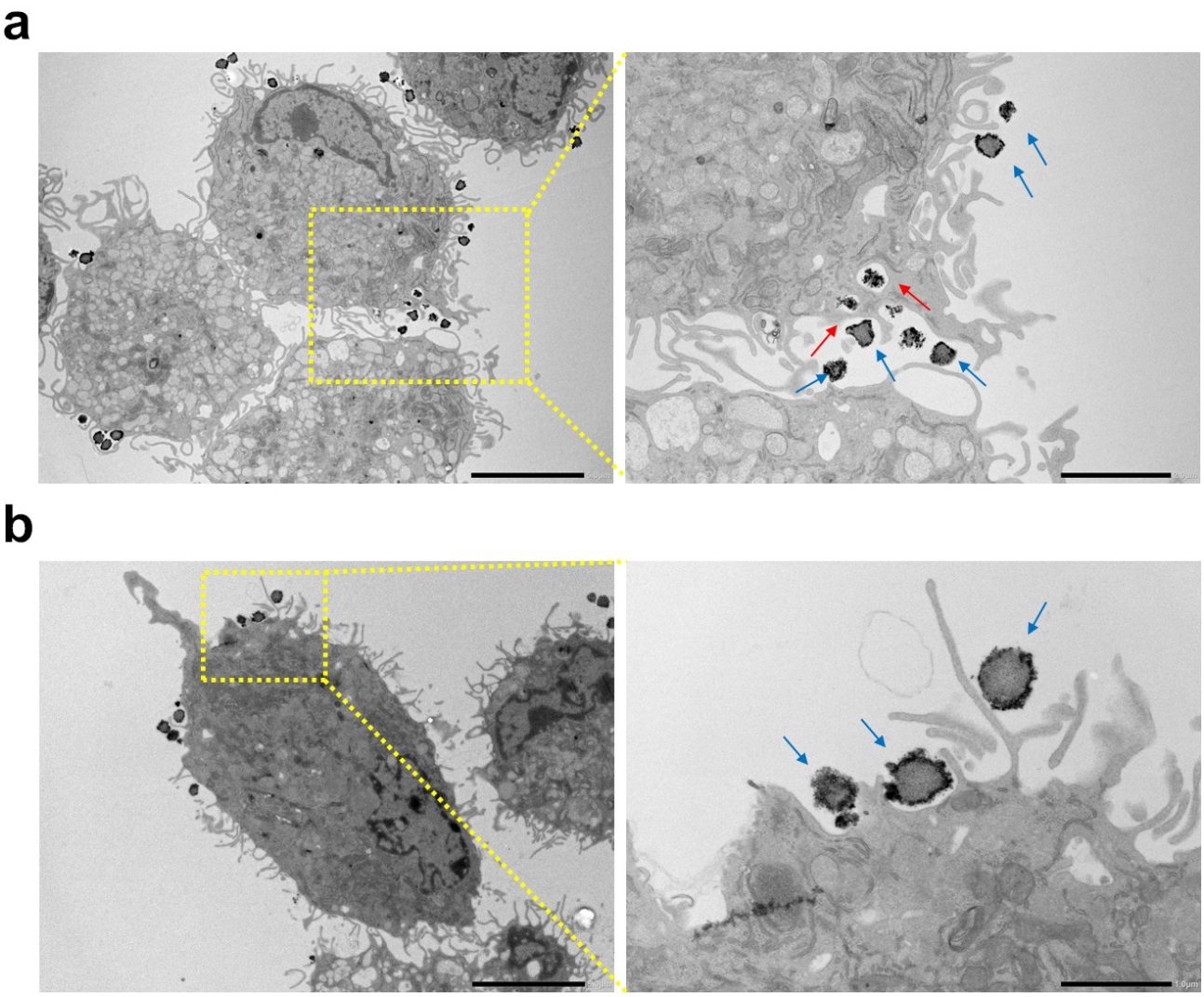

Fig. S7 Transmission electron microscopy images of macrophages incubated with MagPs. (a) and (b) are two examples of TEM images showing MagPs engulfed by the pseudopodia of the macrophages (blue arrows) or internalized just below the plasma membrane (red arrows) (scale bar = 5 µm) with a magnified view on the right side ((a) scale bar = 2 µm, (b) scale bar = 1 µm).

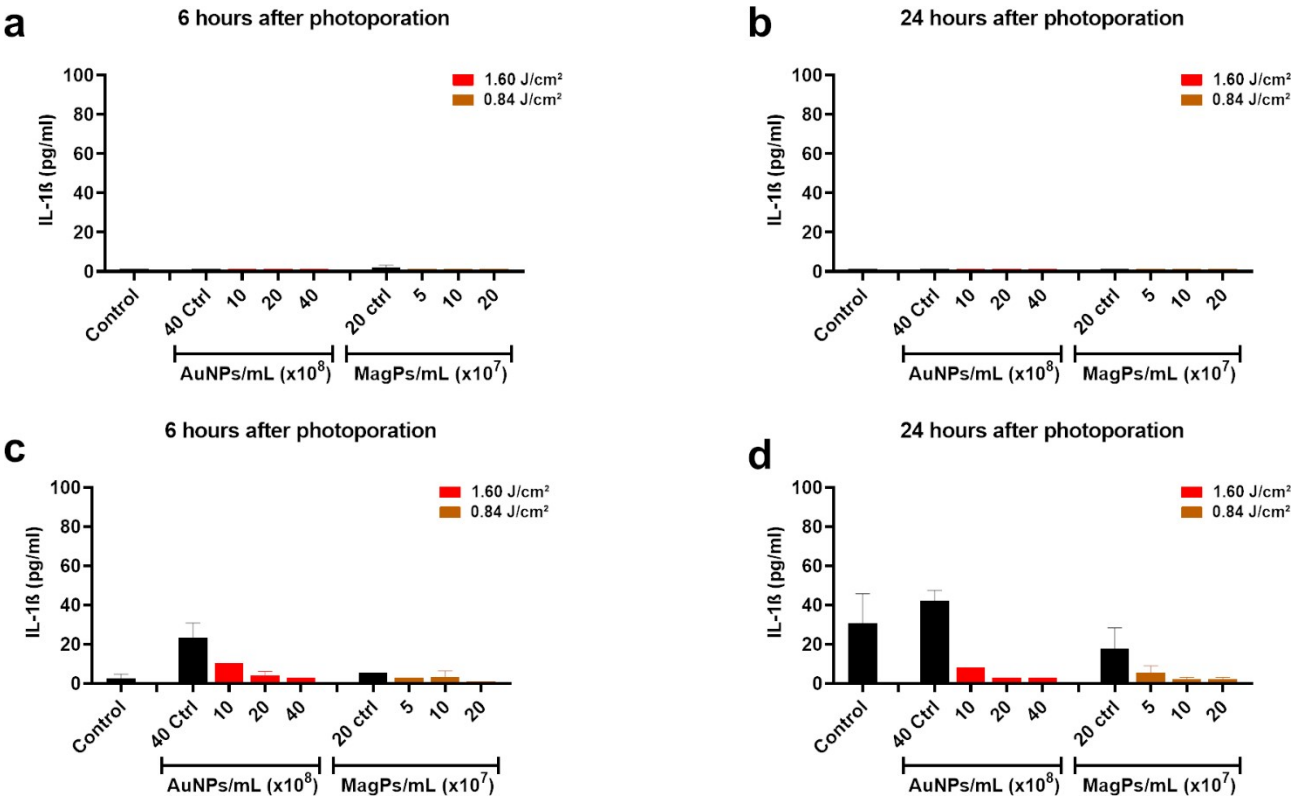

Fig. S8 Secretion of IL-1β after AuNP- or MagP-sensitized photoporation. In case of AuNP-sensitized photoporation, a laser fluence of 1.6 J/cm² was used, for MagP-sensitized photoporation, a laser fluence of 0.84 J/cm² was used. **(a,b)** IL-1β secretion from non-primed macrophages after respectively 6 hours **(a)** and 24 hours **(b)**. **(c,d)** IL-1β secretion from PAM3-CSK4 primed macrophages after respectively 6 hours **(c)** and 24 hours **(d)**. '40 Ctrl' and '20 Ctrl' refers to cells incubated with nanoparticles alone (i.e. without laser irradiation) at the highest tested concentration, being 40×10^8 particles/ml in case of AuNPs and 20×10^7 particles/ml for MagPs.

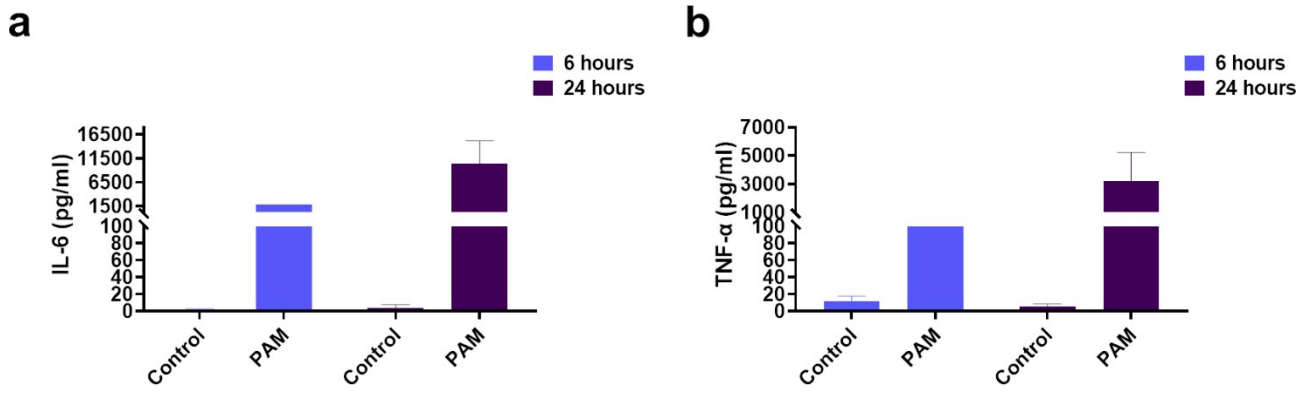

Fig. S9 Secretion of IL-6 and TNF-α before and after priming with PAM3-CSK4. **(a)** IL-6 secretion from PAM3-CSK4 primed macrophages after respectively 6 hours (blue) and 24 hours (dark purple). (**b)** TNF-α secretion from PAM3-CSK4 primed macrophages after respectively 6 hours (blue) and 24 hours (dark purple).

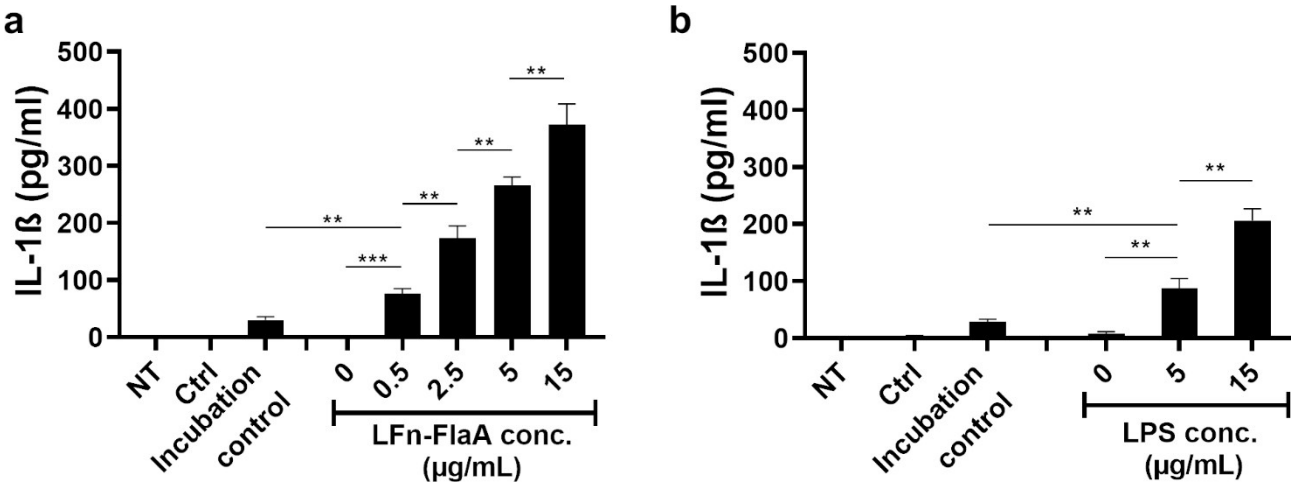

Fig. S10 IL-1β release after MagP-sensitized photoporation (5 x 10^7 MagPs/mL) with increasing concentration of LFn-FlaA **(a)** and LPS **(b)** as measured with ELISA. For the incubation control a concentration of 15 µg/mL was used for both respectively LFn-FlaA and LPS. (NT = non-treated, Ctrl = control). Data are shown as mean ± SD. Statistical significance: Student's t-test, ** $p \leq 0.01$, *** $p \leq 0.001$.