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SUPPLEMENTARY INFORMATION

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Group A streptococcal M protein activates the NLRP3 inflammasome

J. Andrés Valderrama[1,2], Angelica M. Riestra[1], Nina J. Gao[1], Christopher N. LaRock[1], Naveen Gupta[3], Syed Raza Ali[1], Hal M. Hoffman[1,3], Partho Ghosh[2]* and Victor Nizet[1,4]*

[1]Department of Pediatrics, University of California, San Diego, La Jolla, CA 92093, USA. [2]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA. [3]Department of Medicine, University of California, San Diego, La Jolla, CA 92093, USA. [4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA. *e-mail: pghosh@ucsd.edu; vnizet@ucsd.edu

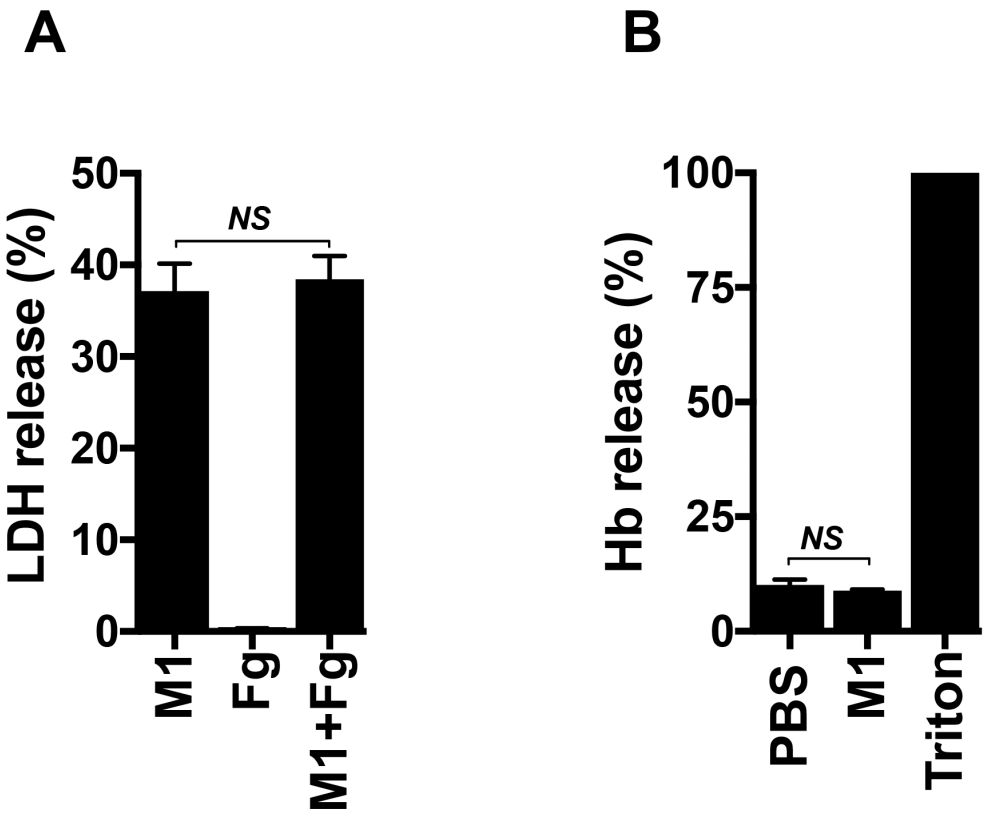
Supplementary Information

Group A Streptococcal M Protein Activates the NLRP3 Inflammasome

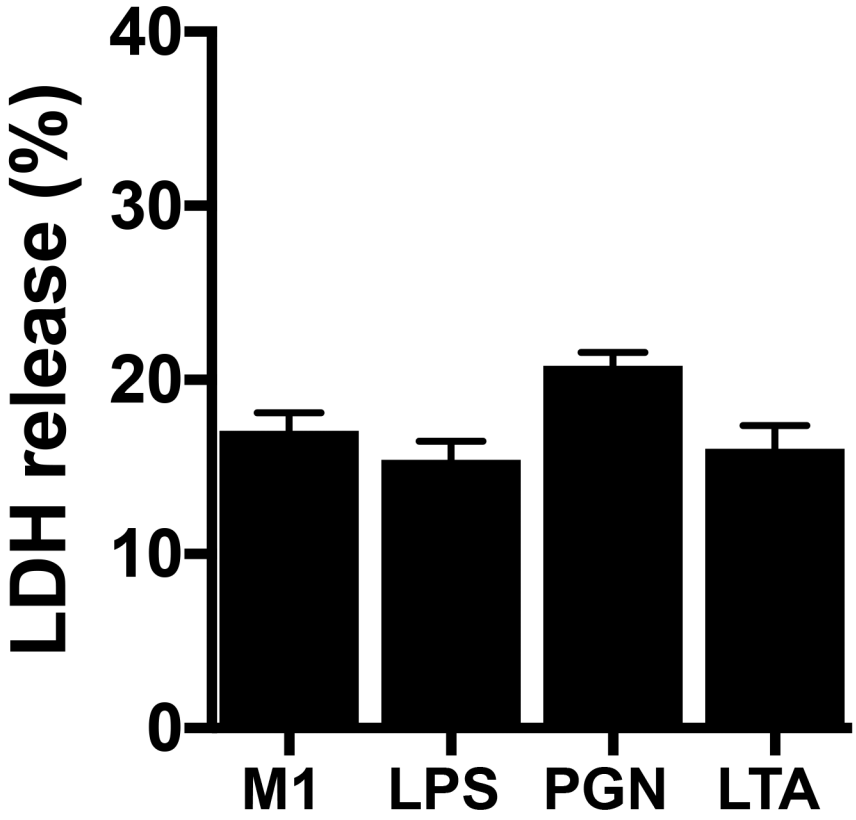
J. Andrés Valderrama[1,2], Angelica M. Riestra[1], Nina J. Gao[1], Christopher N. LaRock[1], Naveen Gupta[3], Syed Raza Ali[1], Hal M. Hoffman[1,3], Partho Ghosh[2]* and Victor Nizet[1,4] *

[1]Department of Pediatrics, University of California, San Diego, La Jolla, CA 92093, USA. [2]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA. [3]Department of Medicine, University of California, San Diego, La Jolla, CA 92093, USA. [4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA.

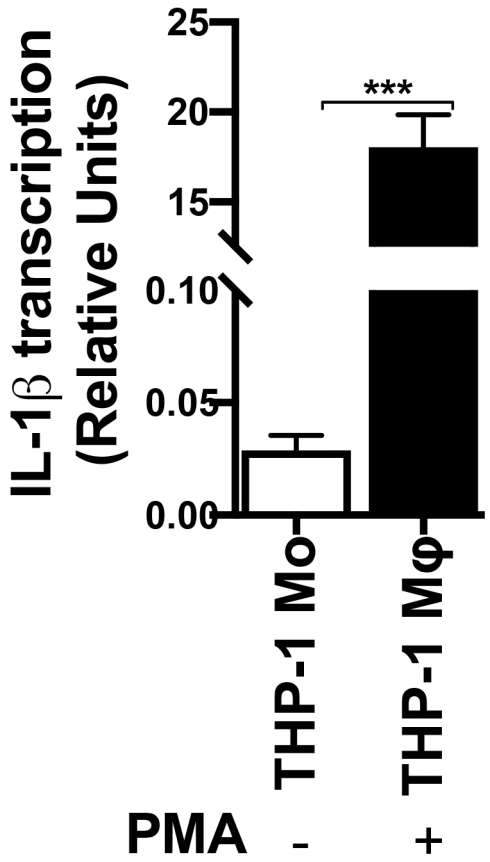
Contents: Supplementary Figures 1-10 and corresponding legends.



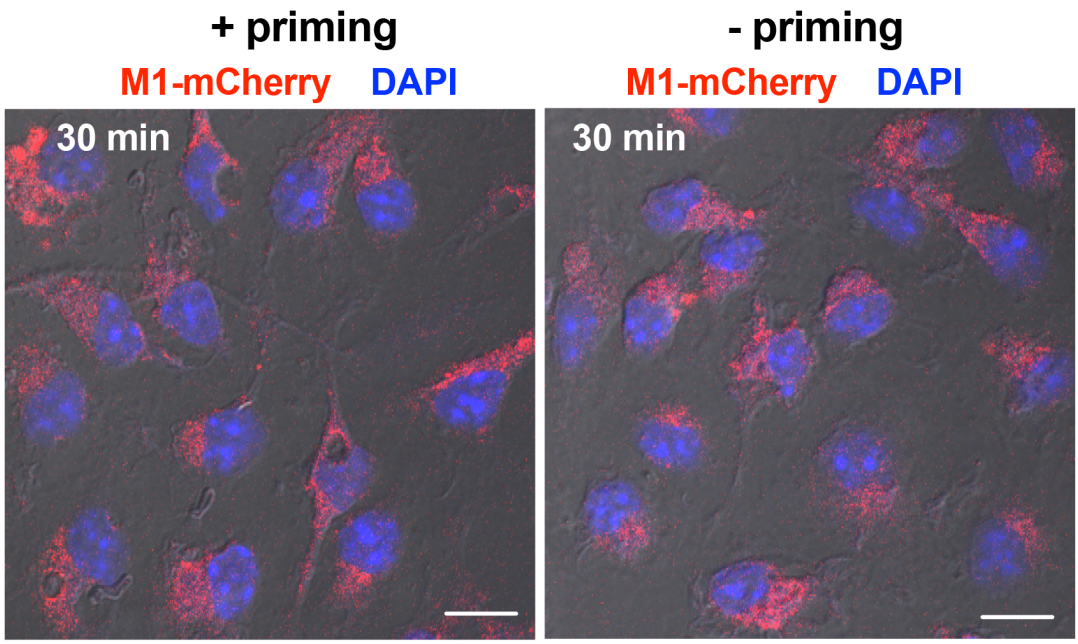
Supplementary Figure 1. M1-induced cell death is fibrinogen-independent in macrophages and does not occur in red blood cells. a, Percentage of LDH released from THP1-Mϕ after 2 h treatment in the presence of M1 (2 μM), human fibrinogen (Fg, 1 mg ml^{-1}) or M1+Fg (M1, 2 μM + Fg, 1 mg ml^{-1}). **b,** Percentage of hemoglobin released (Hb % release) from human erythrocytes after 2 h treatment in the presence of M1 (2 μM). PBS and triton 0.1 % were used as the minimal or maximal inducers of Hb release, respectively. Data are plotted as the mean ± SEM and represent two independent experiments performed in duplicate and analyzed by Student's *t*-test. *NS* = not significant (P>0.05).



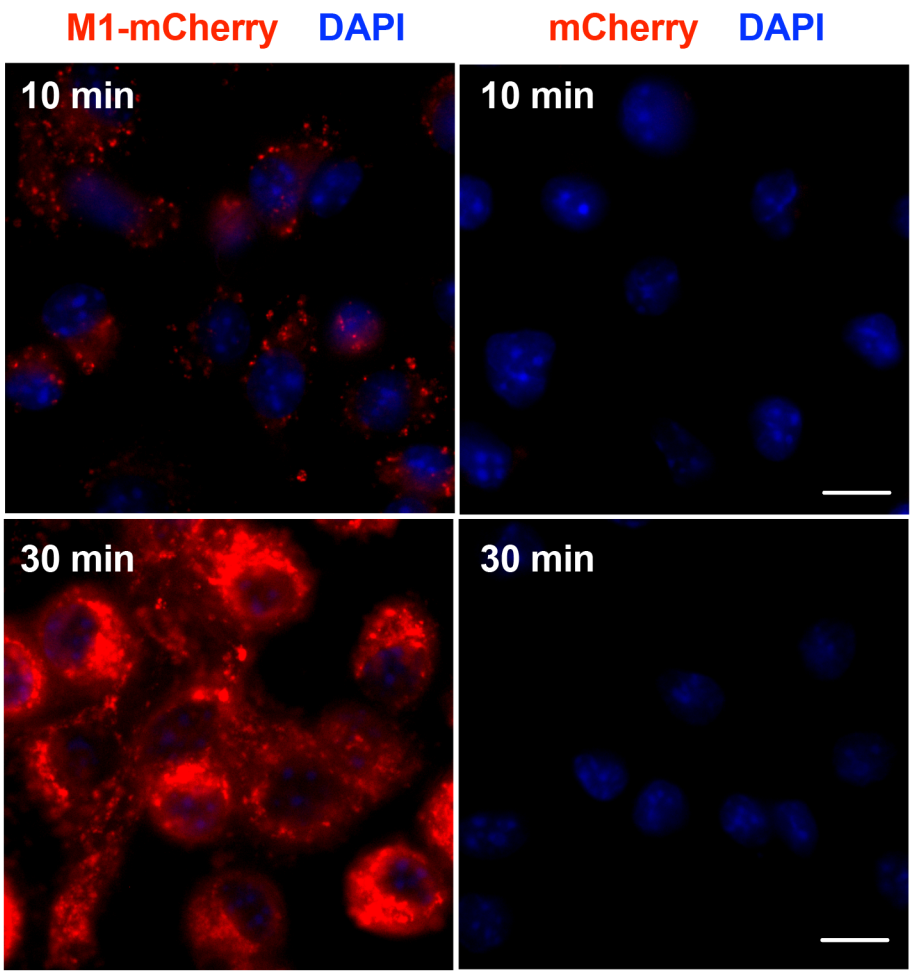
Supplementary Figure 2. Macrophage viability upon treatment with M1 or various TLR agonists. Percentage of LDH released from THP1-Mϕ after 15 h stimulation with M1 (0.02 μM), lipopolysaccharide (LPS, 100 ng ml^{-1}), peptidoglycan (PGN, 1 μg ml^{-1}), or lipoteichoic acid (LTA, 1 μg ml^{-1}). Data are plotted as the mean ± SEM and represent three independent experiments performed in triplicate.



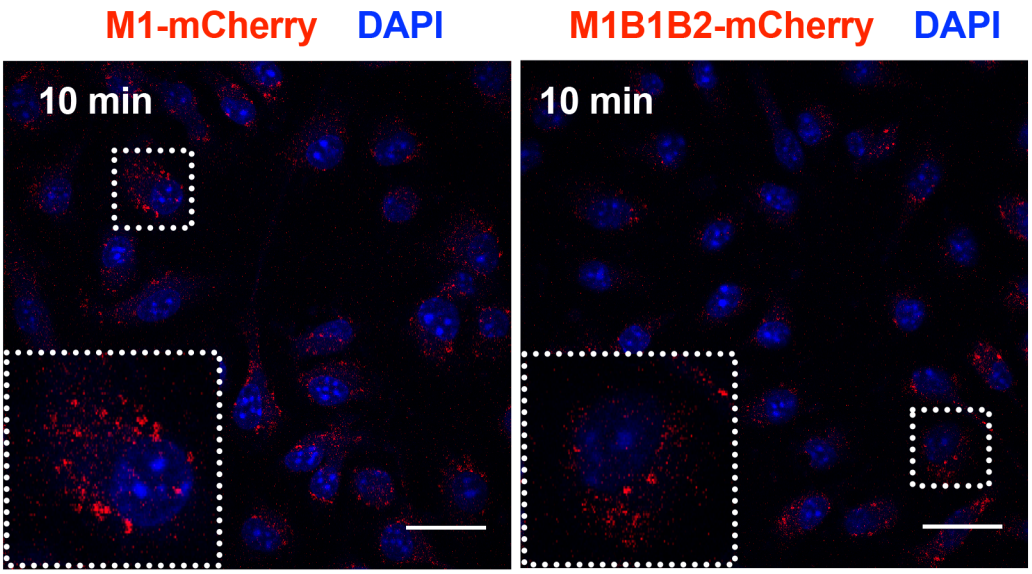
Supplementary Figure 3. PMA triggers signal 1 in THP-1 differentiated macrophages. qPCR analysis of IL-1β gene expression from undifferentiated THP-1 cells (THP1 Mo, - PMA) or THP-1 differentiated to macrophages (THP1-Mϕ, + PMA). The qPCR results were calculated by Delta-Delta CT method with the *gadph* gene as internal control (housekeeping gene). Data are plotted as the mean ± SEM and represent three independent experiments performed in triplicate and analyzed by Student's *t*-test. *NS* = not significant (P>0.05), ***P<0.001).



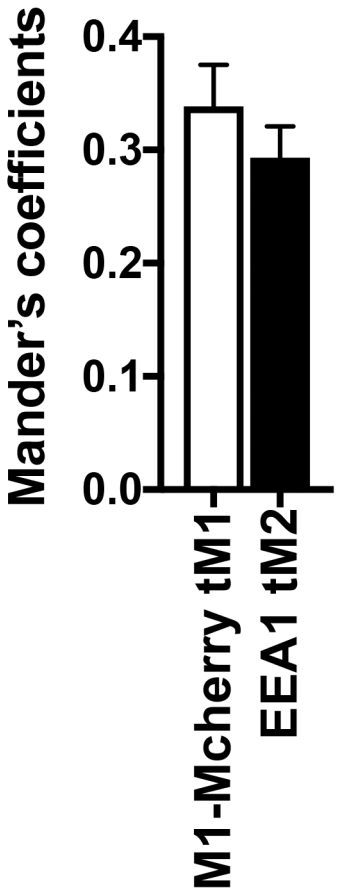
Supplementary Figure 4. M1 uptake occurs independently of inflammasome signal one. Confocal microscopy images of BMDMs primed (left) or unprimed (right) cells with LPS (10 ng ml^{-1}) for 4 h and then incubated with 2 μM of M1-mCherry for 30 minutes. DAPI (blue) and M1-mCherry (red) merge of fluorescence and phase contrast images are shown; scale bar = 10μm. Images are representative of two independent experiments.



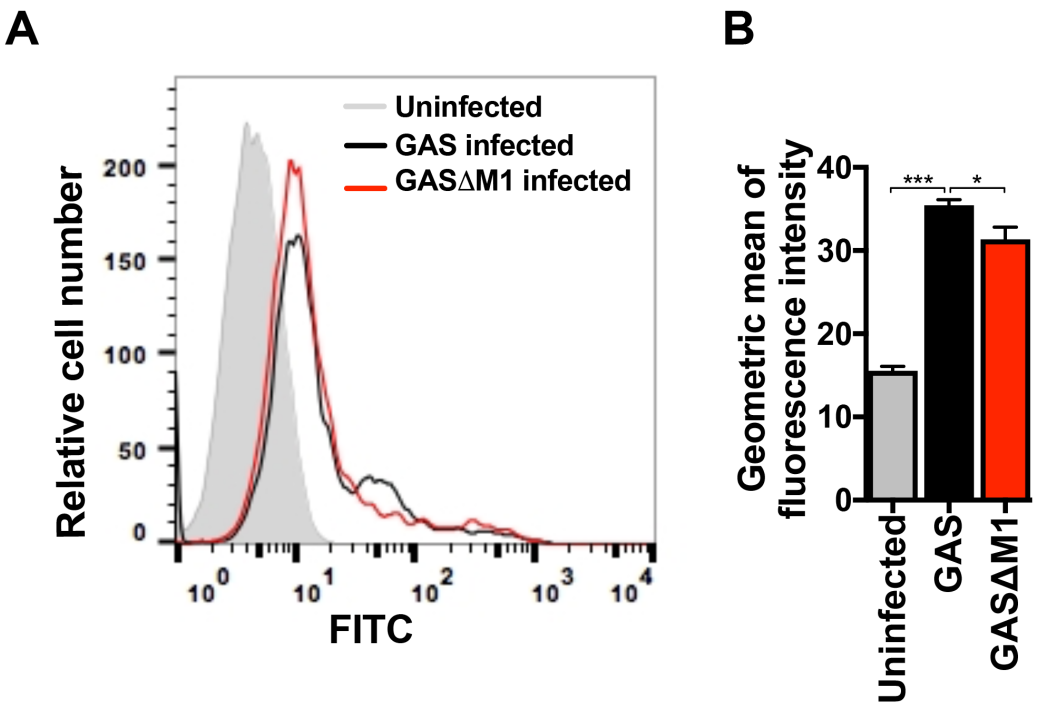
Supplementary Figure 5. mCherry is not taken up by macrophages. Fluorescence microscopy of BMDMs incubated with 2 μM of M1-mCherry (left) or 2 μM of mCherry (right) for 10 (top) or 30 min (bottom). DAPI (blue) and M1-mCherry (red) merged images are shown; scale bar = 30μm. Images are representative of two independent experiments.



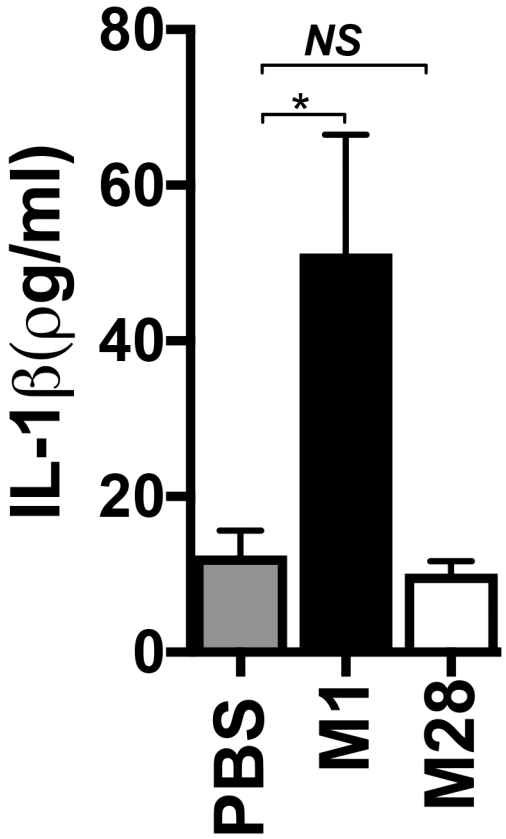
Supplementary Figure 6. M1 B repeats are not required for M1 uptake. Confocal microscopy images of BMDMs incubated with 2 μM of M1-mCherry (left) or 2 μM of M1ΔB1ΔB2-mCherry (right) for 10 min. DAPI (blue) and M1-mCherry (red) merged images are shown. Insets are magnifications of the dashed boxed regions in the merged images; scale bar = 30μm. Images are representative of two independent experiments.



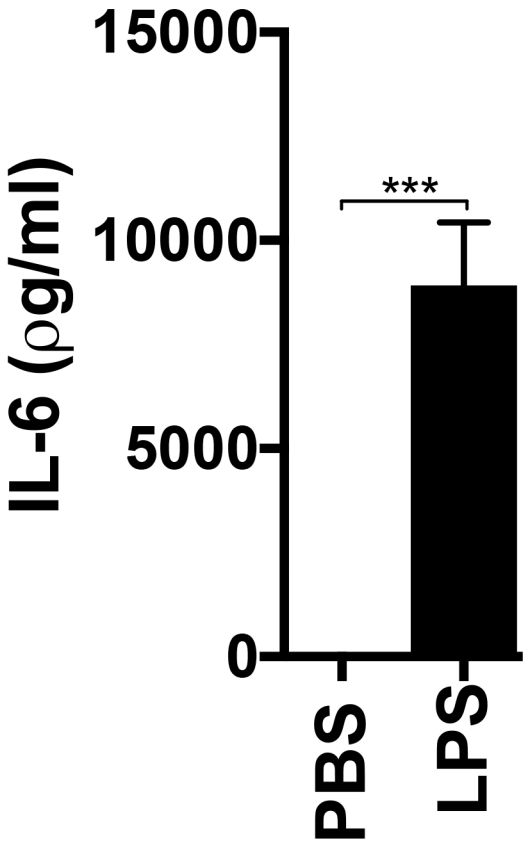
Supplementary Figure 7. Quantification of colocalization between M1-mCherry and the early endosomal marker EEA1. Thresholded Mander's correlation coefficients tM1 and tM2 represent the fraction of overlapped signal of each channel. Data are plotted as the mean ± SEM and show quantification in 15 representative cells shown in **Figure 4d**



Supplementary Figure 8. Binding of GAS to macrophages is not strongly affected by M1. THP1-Mϕ infected with FITC-labeled GAS or GASΔM1 bacteria at MOI 10. Macrophage-bacteria interactions were examined 2 h post-infection by flow cytometry. Events were gated in the population of macrophages. Uninfected THP1-Mϕ served as a negative control. A representative histogram of FITC fluorescence is shown in panel **a** and panel **b** shows the geometric mean of fluorescence intensity ± SEM and represent three independent experiments performed in triplicate and analyzed by Student's *t*-test. *NS* = not significant (P>0.05), *P<0.05, ***P<0.001.



Supplementary Figure 9. Comparison of M1 and M28 proteins induction of IL-1β *in vivo*. Detection of IL-1β from peritoneal lavage fluid of wild type C57BL/6 mice, 4 h after injection with 150 μg purified M1 or M28 protein. Control group animals were injected with PBS. Cytokine quantification was performed by ELISA. Data are plotted as the mean ± SEM, N=5 and were analyzed by Student's *t*-test. *NS* = not significant (P>0.05) and *P<0.05).



Supplementary Figure 10. Detection of IL-6 *in vivo*. Detection of IL-6 from peritoneal lavage fluid of wild type C57BL/6 mice, 4 h after injection with 10 mg/kg LPS. Control group animals were injected with PBS. Cytokine quantification was performed by ELISA. Data are plotted as the mean ± SEM, N=4 and were analyzed by Student's *t*-test (***P<0.001).