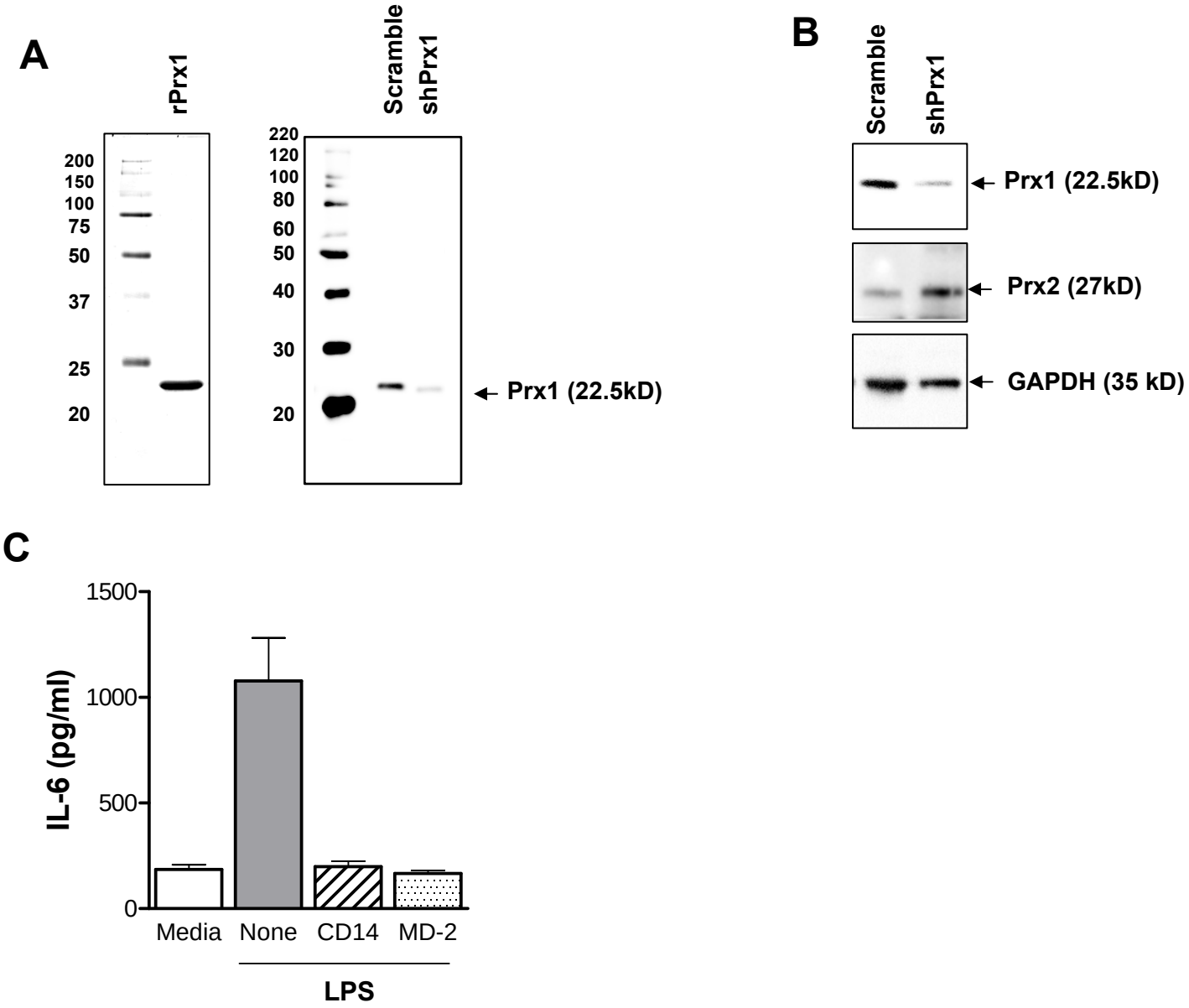
(A) Stable transfectants of the RAW264.7 macrophage cell line containing control (open bar) or MyD88 DN (filled bars) expressing plasmids were stimulated with 100 nM LPS or 1000 nM Prx1 for 24 h and the resulting supernatants were assayed for IL-6 expression by ELISA. ELISA analysis was performed in three independent experiments; error bars represent standard deviation. Asterisks indicate a P value $\leq$ 0.001. (B) TG-elicited macrophages isolated from C3H/HeNCr ($TLR4^{+/+}$) and C3H/HeNJ ($TLR4^{-/-}$) mice were stimulated with 200 nM Prx1 in complete media. At the indicated time points cells were stained with FITC conjugated antibodies to NFκB p65 and DRAQ5 (nuclear stain) for 10 min and analyzed using Amnis technology. The furthest right column shows a pixel by pixel statistical analysis of the similarity of NFκB and nuclear staining. (C) The average numerical value of the overall similarity coefficients for each time point in both C3H/HeNCr (filled circles) and C3H/HeNJ (open circles) macrophages is; error bars represent standard deviation. (D) TG-elicited macrophages were incubated with the indicated concentrations of Prx1 for 1 hour. EMSA analysis was performed as described in Materials and Methods.

Supplementary Figure 1. (A) Cell lysate isolated from PC-3M cells (right panel) engineered to express control (Scramble) shRNA or Prx1 specific shRNA (shPrx1) was separated by gel electrophoresis, blotted and probed with antibodies specific for Prx1. (B) Expression of shRNA specific for Prx1 leads to decreased Prx1 levels. PC3-M cell lines engineered to express either control shRNA (Scramble) or shRNA specific for Prx1` were harvested and analyzed for expression of Prx1 or Prx2 by Western analysis. (C) TG-elicited macrophages were isolated from C57BL/6 mice and stimulated with LPS in the presence or absence of control or blocking antibodies to CD14 or MD2 for 24h. Supernatants were collected and analyzed by IL-6 ELISA kits. Results are presented as pg/ml; error bars represent SEM.



Supplementary Figure 1