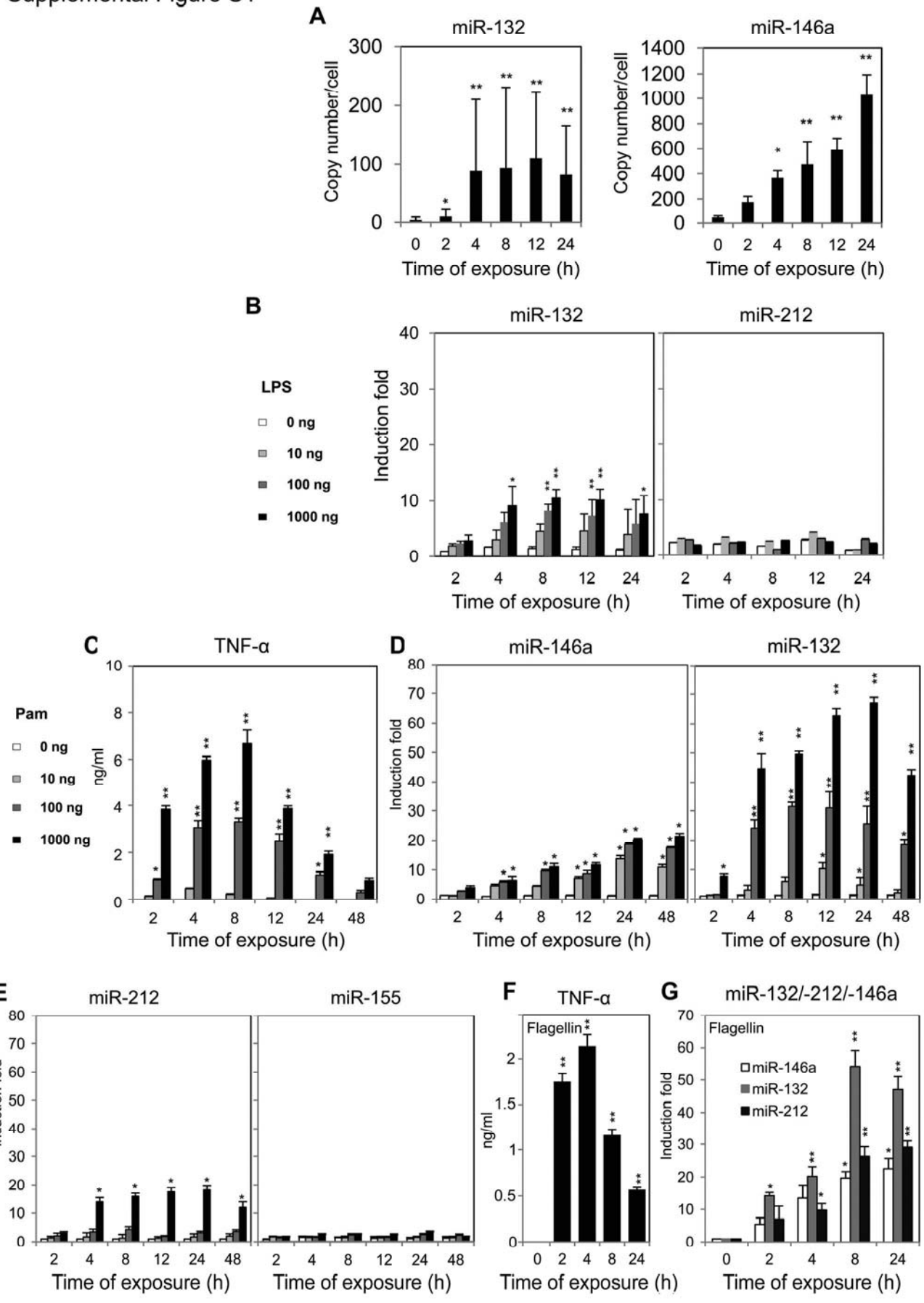
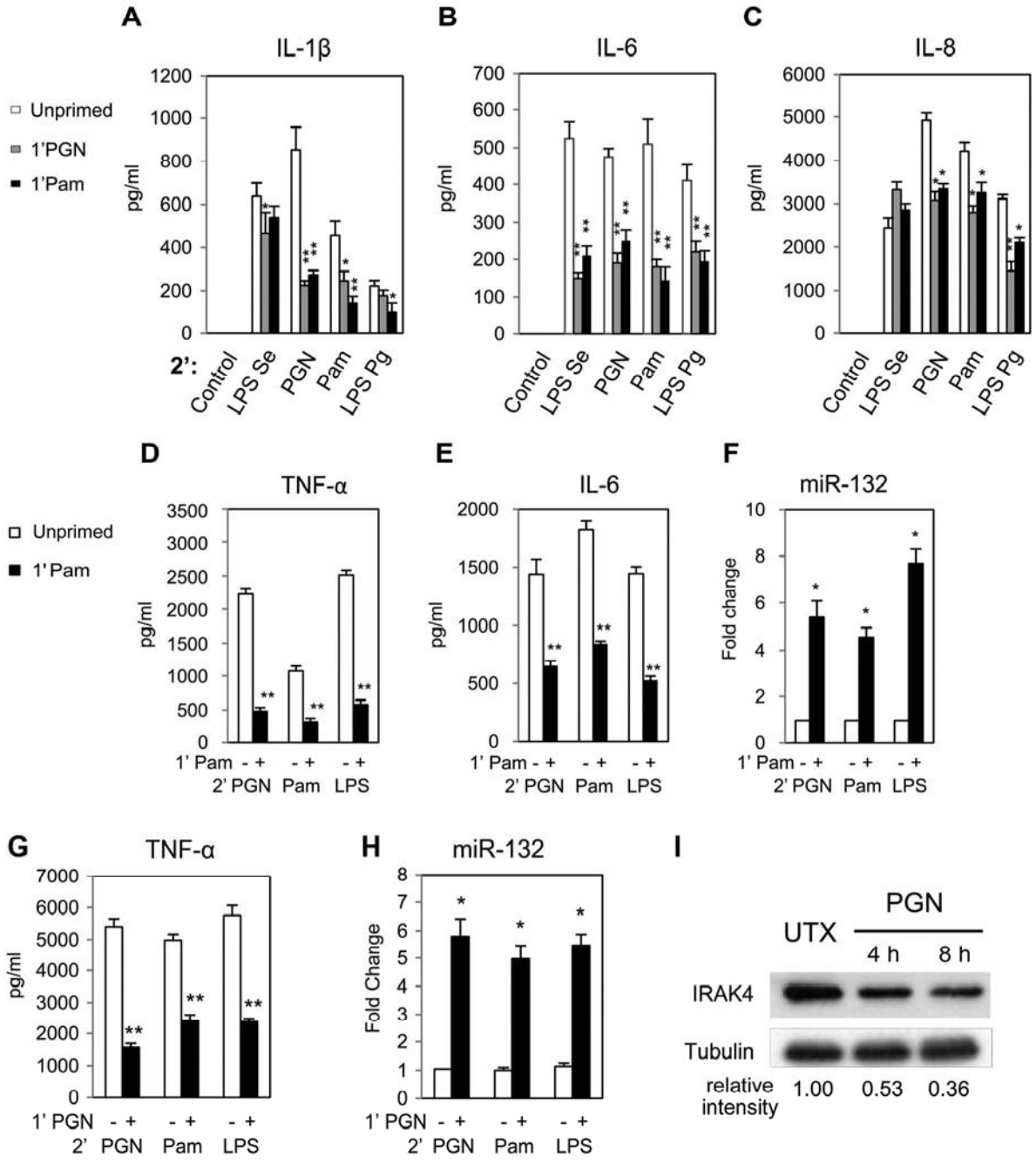
Supplemental Figure S1

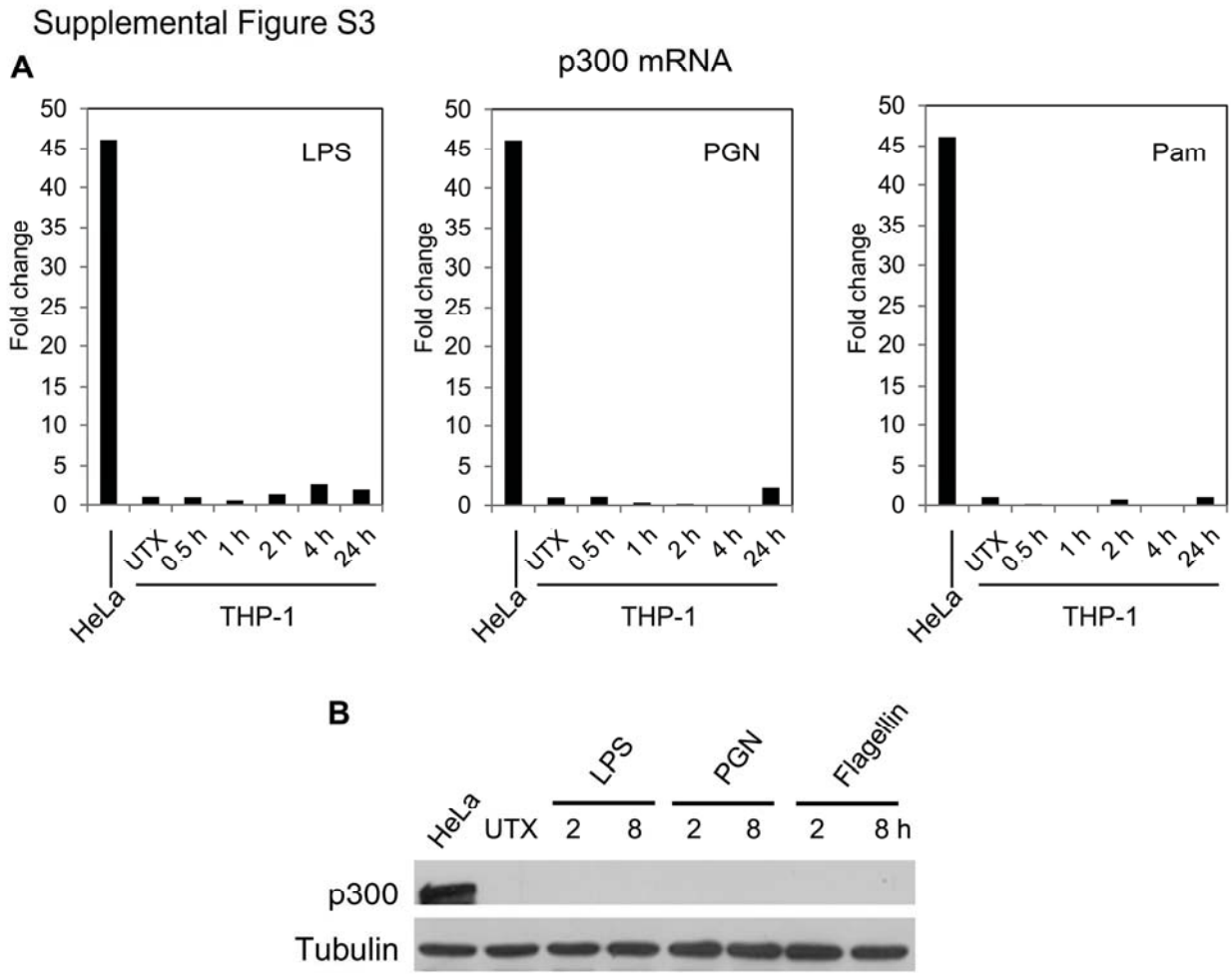


Supplementary Figure S1. **Supplemental data on the characterization of miRNA expression and TNF-α production during TLR-ligand stimulation of THP1 monocytes.** (**A**) Time-course analysis of copy number of miR-132 and miR-146a in PGN-stimulated THP-1 monocytes. Copy number of miR-132 and miR-146a in THP-1 cells stimulated for 0-24 h (horizontal axis) with PGN (2500 ng/ml). (**B**) Induction of miR-132 and miR-212 by LPS treatment in THP-1 monocytes. qRT-PCR analysis of dose- and time-dependent expression of miR-132 and miR-212 in THP-1 monocytes stimulated for 2-24 h with 0-1000 ng/ml LPS. (**C**) TNF-α production by THP-1 cells stimulated with Pam. THP-1 monocytes were incubated for 2-48 h with 0-1000 ng/ml Pam and TNF-α in culture supernatant was measured by ELISA. (**D-E**) qRT-PCR analysis of miR-146a, miR-132, miR-212, and miR-155 expression kinetics in Pam-treated THP-1 cell. (**F**) TNF-α production by THP-1 monocytes stimulated for 0-24 h with 300 ng/ml flagellin. (**G**) qRT-PCR analysis of miR-146a, miR-132, and miR-212 expression in the same THP-1 cells stimulated with 300 ng/ml flagellin. All miRNA expressions were normalized to RNU44. Data are from three independent experiments (mean ± s.d.). $*P < 0.05$; $**P < 0.01$ (two-tailed unpaired *t*-test) compared with time 0 controls (A,F-G) or untreated controls (B-D).

Supplemental Figure S2



Supplementary Figure S2. **Supplemental data on cytokine production in THP-1 cells, human PBMCs, and mouse RAW264.7 cells primed with PGN or Pam and challenged by other TLR-ligands.** (**A-C**) Diminished proinflammatory cytokine secretions by PGN- and Pam-primed THP-1 monocytes. ELISA of IL-1β (**A**), IL-6 (**B**), and IL-8 (**C**) production by THP-1 monocytes primed with or without PGN or Pam (500 ng/ml) for 18 h and then challenged with various ligands (horizontal axis) for 3 h (IL-1β) or 24 h (IL-6 and IL-8). (**D-F**) High levels of miR-132 may account for Pam-induced tolerance in human PBMCs. TNF-α (**D**) and IL-6 (**E**) production by human PBMCs primed with or without Pam (1000 ng/ml) for 18 h and then challenged with various ligands (horizontal axis) for 3 h (TNF-α) or 24 h (IL-6). (**F**) qRT-PCR analysis of miR-132 in the PBMC treated as in **D**. (**G-I**) High levels of miR-132 may promote PGN-induced tolerance in mouse RAW264.7 cells. (**G**) TNF-α production by RAW264.7 cells primed with or without PGN (1000 ng/ml) for 18 h and then challenged with various ligands (horizontal axis) for 3 h. (**H**) qRT-PCR analysis of miR-132 in the purified total RNA obtained from cells described in **G**. (**I**) Immunoblot analysis of IRAK4 in RAW264.7 cells stimulated for 4 and 8 h with PGN (2000 ng/ml). Tubulin serves as a loading control. Data and error bars (mean ± s.d.) are from either from two (**A-C** and **G-H)** or three (**D-F**) independent experiments. **P* < 0.05; ***P* < 0.01 (two-tailed unpaired *t*-test) compared with unprimed control.



Supplementary Figure S3. **p300 expression in THP-1 cells stimulated by LPS, PGN, Pam, and flagellin.** (**A**) Little or no expression of p300 mRNA detected in THP-1 cells untreated (UTX) or treated with 1 μg/ml of LPS, PGN, or Pam for different time points as indicated up to 24 h. Positive control RNA from HeLa cells was included for the qRT-PCR analysis. (**B**) Immunoblot analysis of p300 in THP-1 cells stimulated for 2 or 8 h with 1 μg/ml of LPS, PGN, or flagellin. Cell lysate from HeLa cells was included as a positive control and tubulin analyzed as a loading control.