The effects of CD14 and IL-27 on induction of endotoxin tolerance in human monocytes and macrophages

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Running title: *IL-27 enhances LPS-TLR4-CD14 signaling*

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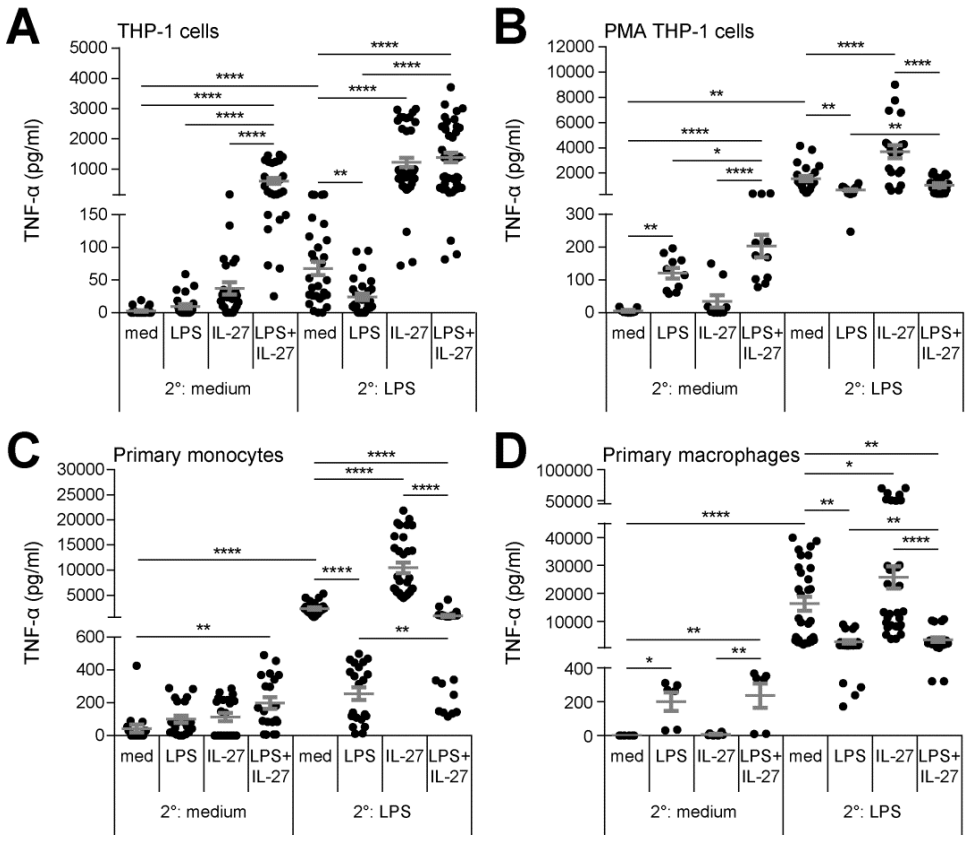
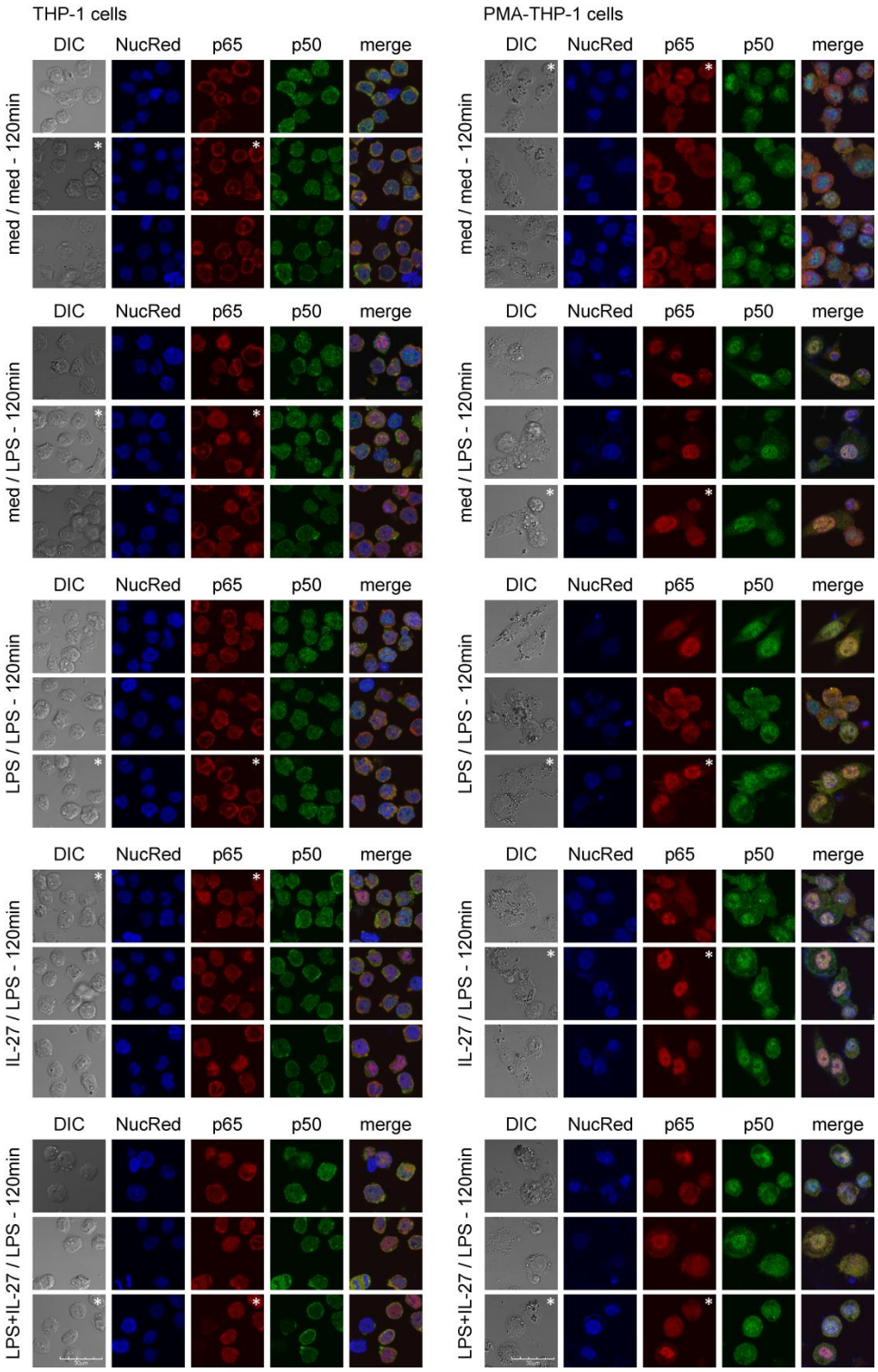
Supplementary Material Included:

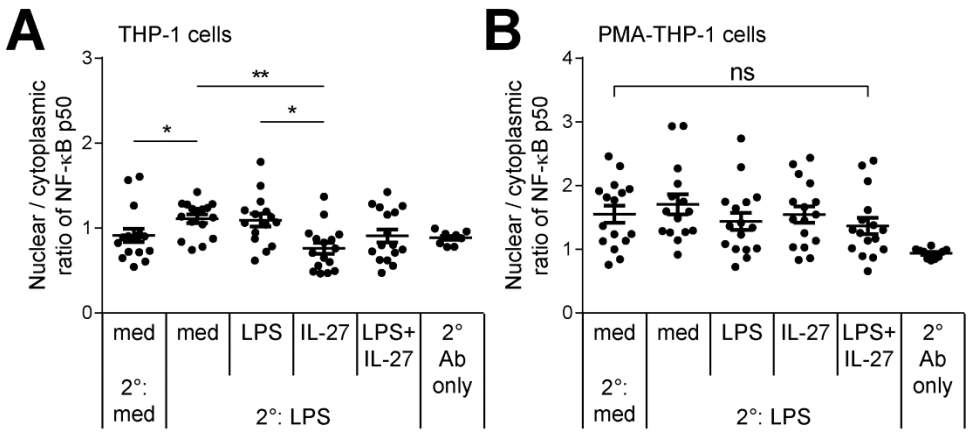
Supplementary Figures 1-5



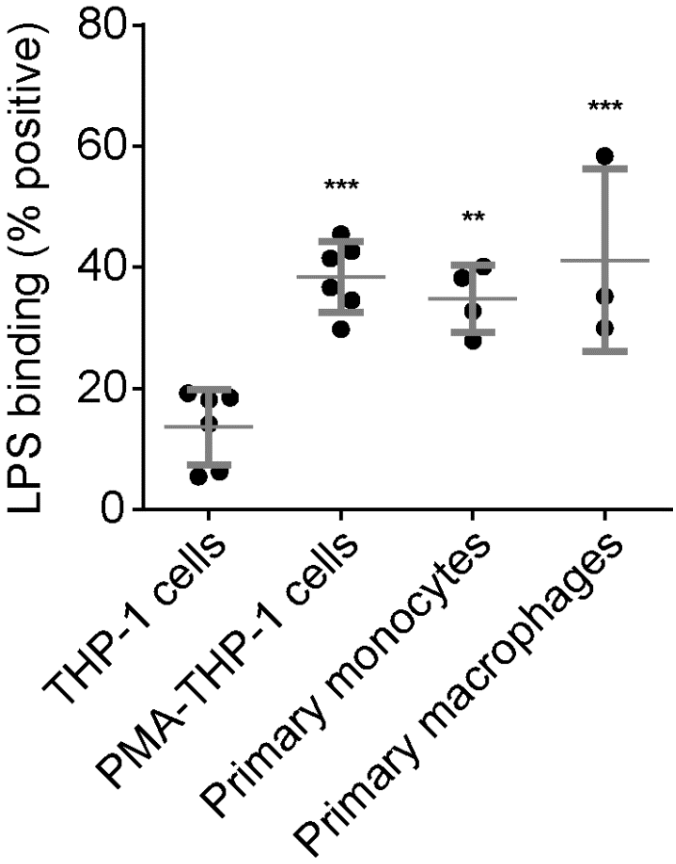
Supplementary Figure 1. Co-treatment of IL-27 with a tolerizing dose of LPS inhibits induction of endotoxin tolerance in human monocytes and macrophages. (A) THP-1 cells, (B) PMA-THP-1 cells, (C) primary human monocytes, and (D) primary human macrophages were stimulated with LPS (10ng/ml), IL-27 (100ng/ml), or LPS + IL-27 simultaneously for 24 hours (1°). Cells were washed and challenged with LPS (100ng/ml) for 4 hours (2°). TNF-α production was measured in cell-free supernatants by ELISA. Graphs present mean ± SEM of at least six independent experiments or at least six different blood donors.


THP-1 cells
PMA-THP-1 cells
DIC
NucRed
p65
p50
merge
med / med - 120min
med / LPS - 120min
LPS / LPS - 120min
IL-27 / LPS - 120min
LPS+IL-27 / LPS - 120min

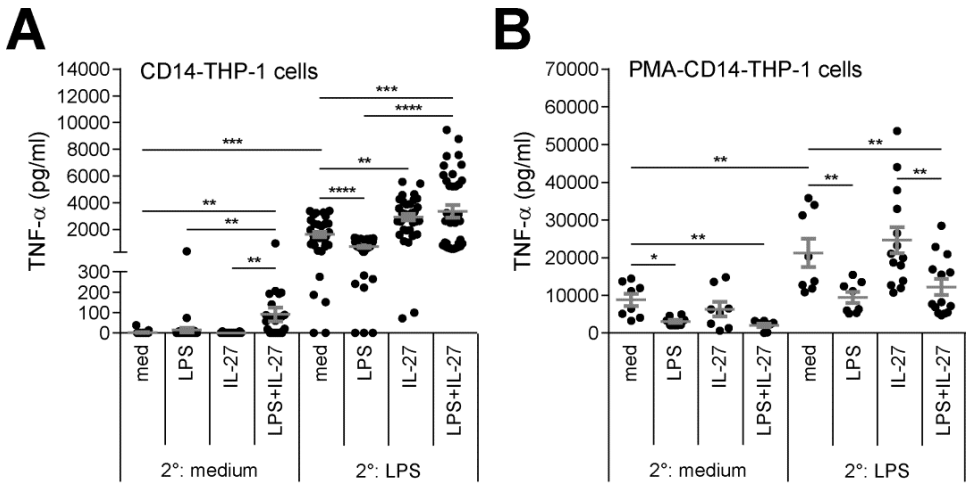
Supplementary Figure 2. Representative images of NF-κB p65 and p50 in endotoxin tolerized THP-1 and PMA-THP-1 cells in the presence or absence of IL-27. THP-1 cells and PMA-THP-1 cells were stimulated with LPS (10ng/ml), IL-27 (100ng/ml), or LPS + IL-27 simultaneously for 24 hours. Cells were washed and challenged with LPS (100ng/ml) for 2 hours. Cells were stained with anti-human NF-κB p65 (*red*), anti-human NF-κB p50 (*green*), and NucRed DNA stain (*blue*). Images are representative of three independent experiments with three images captured for each condition. Differential interference contrast (DIC) images are shown on the left of the panels. Merged images display overlays of NucRed, p65, and p50. Scale bar of 30μm is displayed at the bottom left for each cell type. Images with asterisks (*) are presented in Figure 3A and B.



Supplementary Figure 3. IL-27 differentially affects NF-κB p50 activity in tolerized THP-1 cells relative to PMA-THP-1 cells. (A) THP-1 cells and (B) PMA-THP-1 cells were stimulated with LPS (10ng/ml), IL-27 (100ng/ml), or LPS + IL-27 simultaneously for 24 hours. Cells were washed and challenged with LPS (100ng/ml) for 2 hours. Cells were stained with anti-NF-κB p50 and NucRed DNA stain. Relative brightness of NF-κB p50 was measured in identically sized regions of the cell nucleus and cytoplasm. Nuclear / cytoplasmic ratios were calculated for each condition. Graphs present mean ± SEM of 18 cells per condition.



Supplementary Figure 4. THP-1 cells bind less LPS-Alexa compared to PMA-THP-1 cells, primary monocytes, or primary macrophages. (A) THP-1 cells, (B) PMA-THP-1 cells, (C) primary monocytes, and (D) primary macrophages were incubated with or without LPS-AlexaFluor488 (LPS-Alexa; 100ng/ml) for 1 hour at 37°C. LPS-Alexa bound was measured by flow cytometry. Graph presents mean ± SD of percent positive cells with LPS-Alexa.



Supplementary Figure 5. Overexpression of CD14 in THP-1 cells does not alter IL-27-mediated inhibition of endotoxin tolerance. (A) CD14-THP-1 cells and (B) PMA-CD14-THP-1 cells were stimulated with LPS (10ng/ml), IL-27 (100ng/ml), or LPS + IL-27 simultaneously for 24 hours (1°). Cells were washed and challenged with LPS (100ng/ml) for 4 hours (2°). TNF-α production was measured in cell-free supernatants by ELISA. Graphs present mean ± SEM of at least six independent experiments.