Expanded View Figures

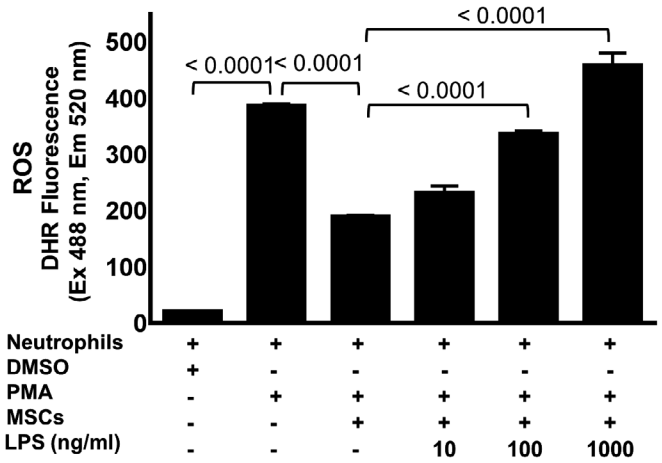
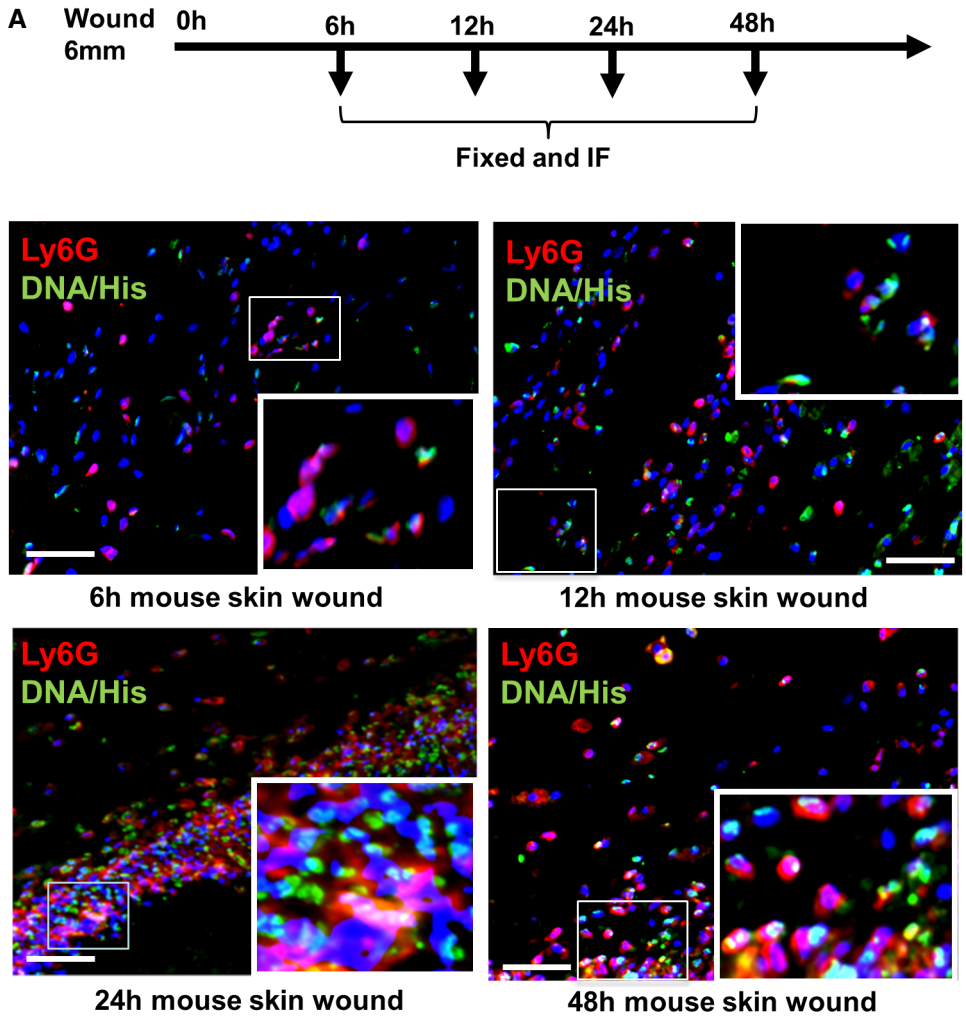


Figure EV1. LPS-treated MSCs augment neutrophil activation and enhanced ROS production.
Graph shows quantification of ROS by measuring fluorescence intensity of DHR123 dye. The MSCs were primed with increasing concentrations of 10 ng/ml, 100 ng/ml, and 1,000 ng/ml LPS followed by co-culture with neutrophils. The MSCs were then incubated with ROS-specific dye DHR123, and the fluorescence was read after 60 min of incubation at 488/520 nm with spectrophotometer. Incubation of neutrophils with PMA alone served as a positive control and DMSO-treated neutrophils as a negative control. Statistical analysis was performed using one-way ANOVA, and values are represented as mean ± SEM, three biological replicates.

Figure EV2. Recruitment of neutrophils increases with time after induction of wounds in mice.

A Immunostaining of Ly6G (green) and DNA-histone (DNA-Hist, red) was performed at 6, 12, 24, and 48 h after induction of full-thickness wounds. Nuclei were stained by DAPI (blue). Staining with Ly6G for neutrophils and DNA-histone for NET formation allowed to study neutrophil recruitment and NET formation. To facilitate comparison, areas inside the rectangles are shown at 5× magnification in the insets. Scale bars: 50 μm

B Quantitative analysis of Ly6G and DNA-histone double-positive cells at different time intervals. Statistical analysis was performed using one-way ANOVA, and values are represented as mean ± SEM, six biological replicates.



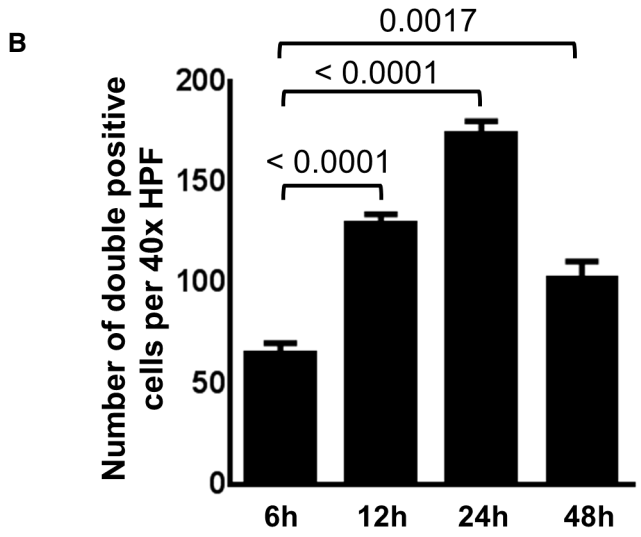


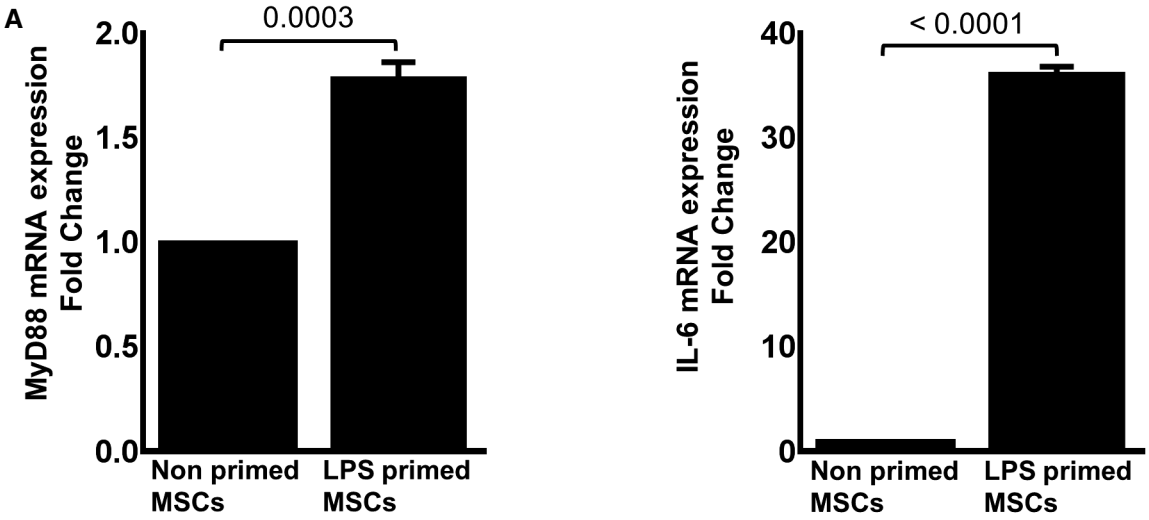
Figure EV2.

Figure EV3. LPS-treated MSCs activate MyD88 and IL-6 expression in the TLR4 signaling pathway.

A Human MSCs were treated with LPS (100 ng/ml) for 24 h. The mRNA expression of MyD88 and IL-6 was assessed by qRT–PCR analysis at 24 h. MyD88 (upper left panel) and IL-6 gene expression (upper right panel) revealed an increase in MyD88 and IL-6 in LPS-primed MSCs. Statistical analysis was performed using unpaired *t*-test, and values are represented as mean ± SEM, three biological replicates.

B The TLR4 signaling pathway is depicted. Upon LPS binding, conformational changes in the TLR4 receptor led to recruitment of intracellular TLR domains harboring adaptor proteins. A bifurcation in MyD88-dependent signaling converges to NF-κB and pro-inflammatory cytokine release, among them IL-6, and the TRIF signaling pathway relaying its signals to downstream effectors like the transcription factor IRF which transactivates type 1 interferons. The right panel depicts representative Western blot analyses with a time-dependent regulation of TLR4 and components of the TLR4 signaling pathway after exposure of MSCs with LPS. p65, a component of the heterodimeric NF-κB transcription factor, and the IRF-1 transcription factor are swiftly induced at 0.5 h, but down-regulated to basal levels at 6 h after LPS challenge of MSCs. The target genes of NF-κB are induced at 0.5 h, and this induction is differentially maintained. The expression of indicated proteins was analyzed in the cell lysates from LPS (100 ng/ml)- or PBS-treated MSCs, three biological replicates.

Source data are available online for this figure.



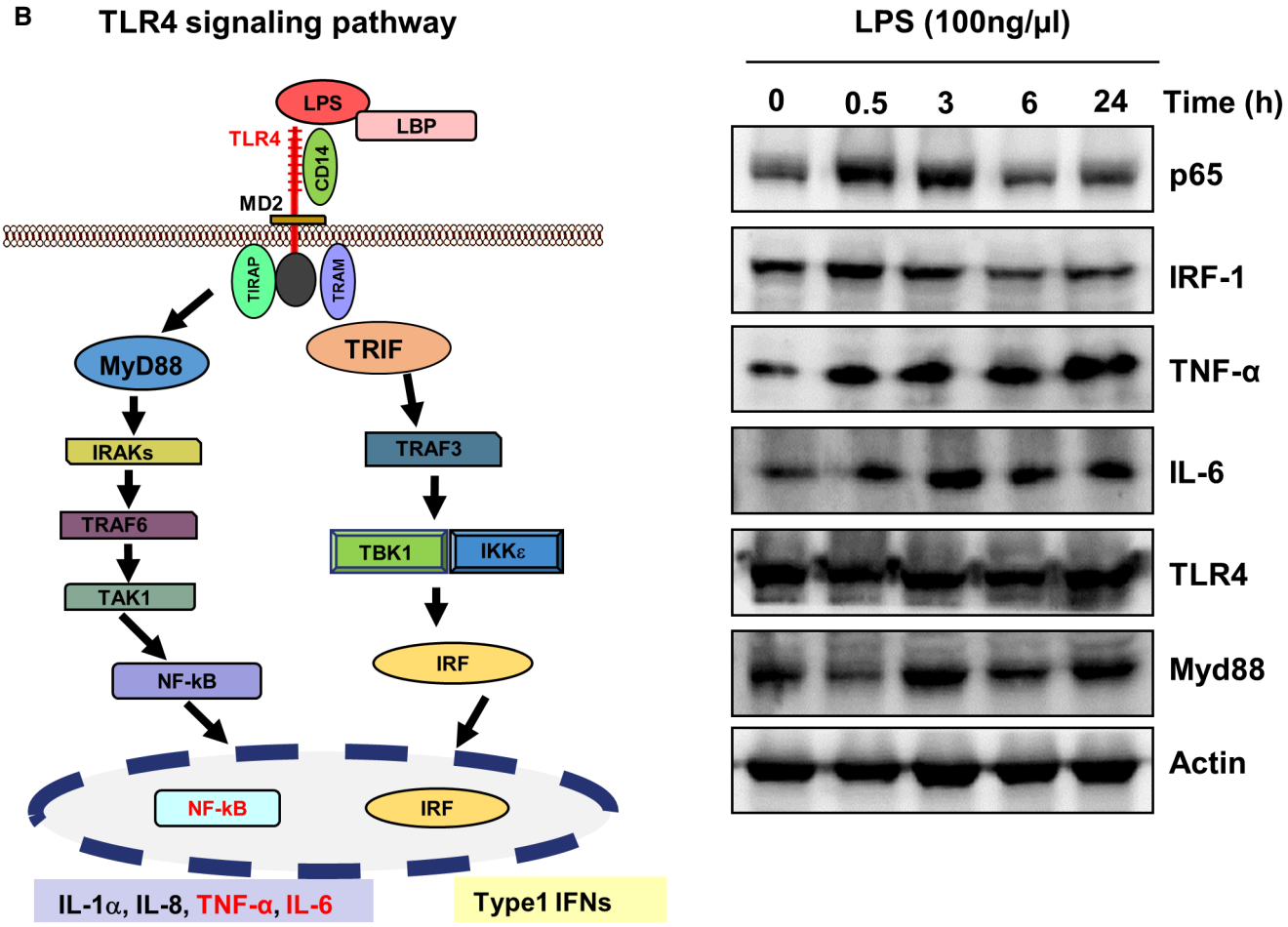


Figure EV3.

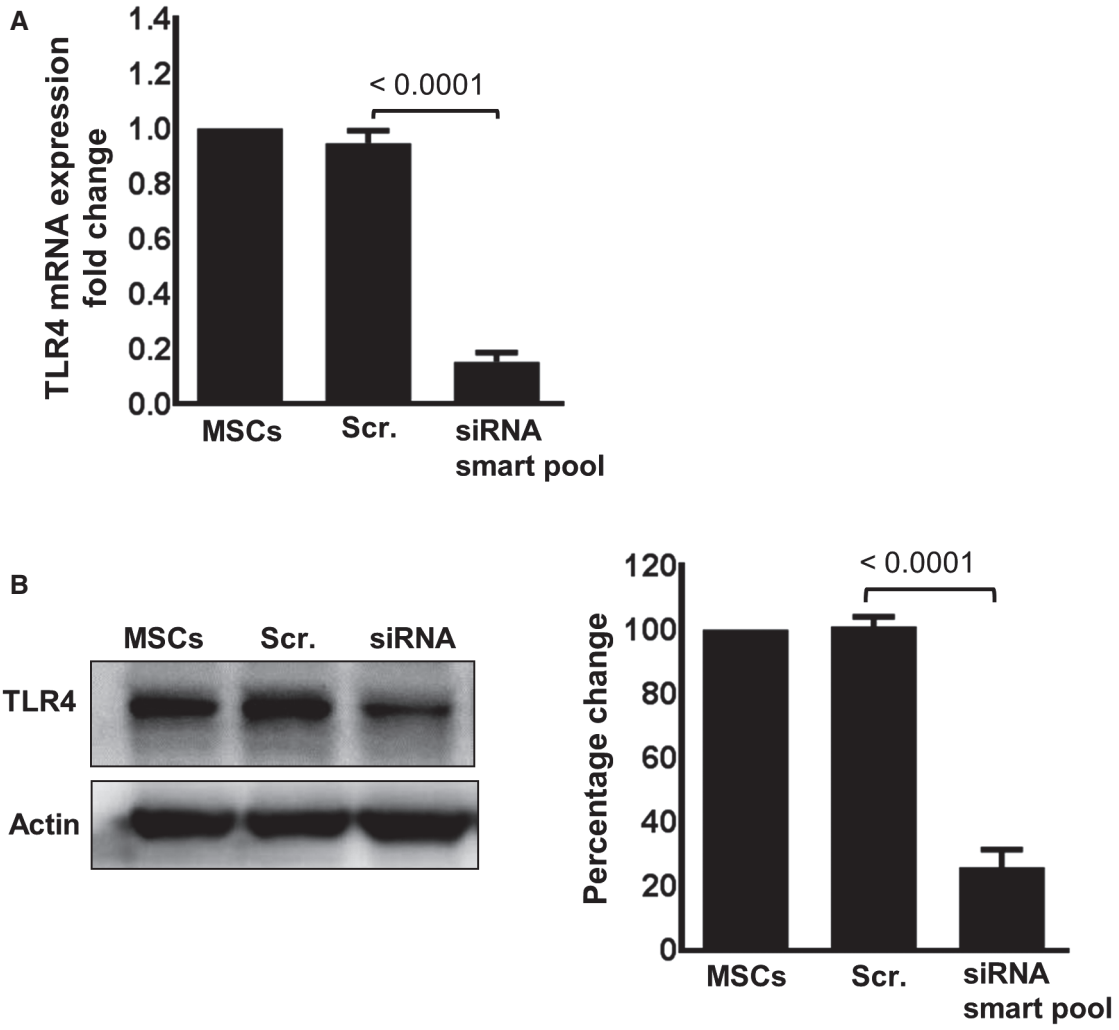


Figure EV4. TLR4-silenced MSCs reveal suppressed expression of TLR4 at transcriptional and protein levels.

A MSCs were either treated with a scrambled control siRNA (Scr. siRNA) or with four different siRNAs targeting the TLR4 receptor (siRNA smart pool). Forty-eight hours after transfection, TLR4-silenced and non-silenced MSCs were analyzed for RNA expression by qPCR. A representative experiment is depicted. Statistical analysis was performed using unpaired *t*-test, and values are represented as mean ± SEM, three biological replicates.

B Seventy-two hours after transfection, TLR4-silenced and non-silenced MSCs were treated with 100 ng/ml LPS for 16 h and subjected to Western blot analysis for protein expression. Densitometric assessment shows the percentage of the change in TLR4 expression in TLR4-silenced and non-silenced MSC. A representative experiment is depicted. Statistical analysis was performed using unpaired *t*-test, and values are represented as mean ± SEM, three biological replicates.

Source data are available online for this figure.

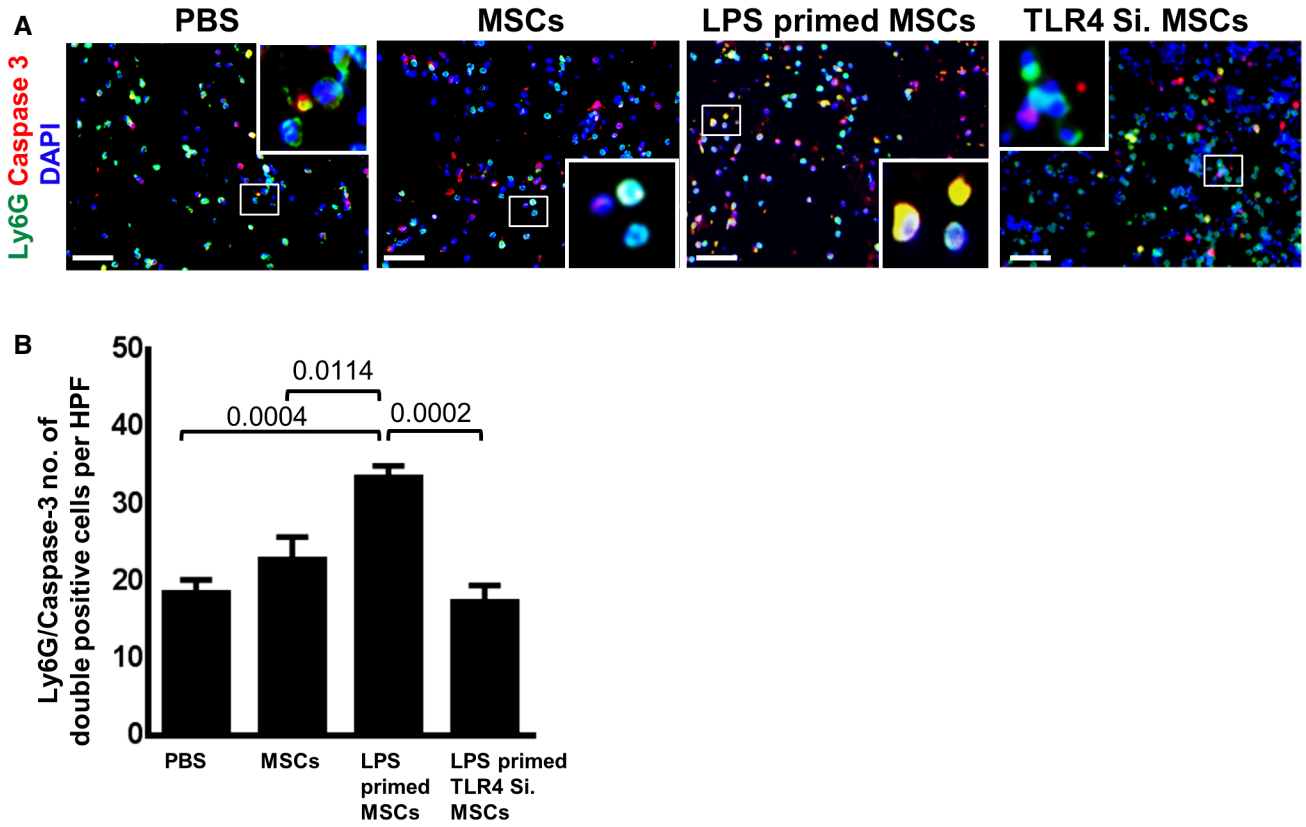


Figure EV5. A significant increase in the caspase 3 activity of neutrophils at an early stage of wound healing in wounds injected with LPS-primed MSCs.

A Representative photomicrographs of sections from differently injected day 3 wounds stained for Ly6G$^+$ neutrophils (green) and caspase 3 (red). Nuclei are stained with DAPI (blue). Double staining was performed for sections of day 3 wounds injected with PBS, non-primed MSCs, LPS-primed MSCs, and LPS-primed TLR4-silenced MSCs. Double-stained cells indicate apoptotic neutrophils. To facilitate comparison, areas inside the rectangles are shown at 5× magnification in the insets. Scale bars: 50 μm.

B Quantification of Ly6G and caspase double-positive cells on sections of differently injected day 3 wounds. A significant increase in double-positive cells (Ly6G and caspase 3) in sections of wounds injected with LPS-primed MSCs as opposed to wounds injected with LPS-primed TLR4-silenced MSCs and PBS control. Double-positive cells were counted; statistical analysis was performed using one-way ANOVA, and values are represented as mean ± SEM, six biological replicates.