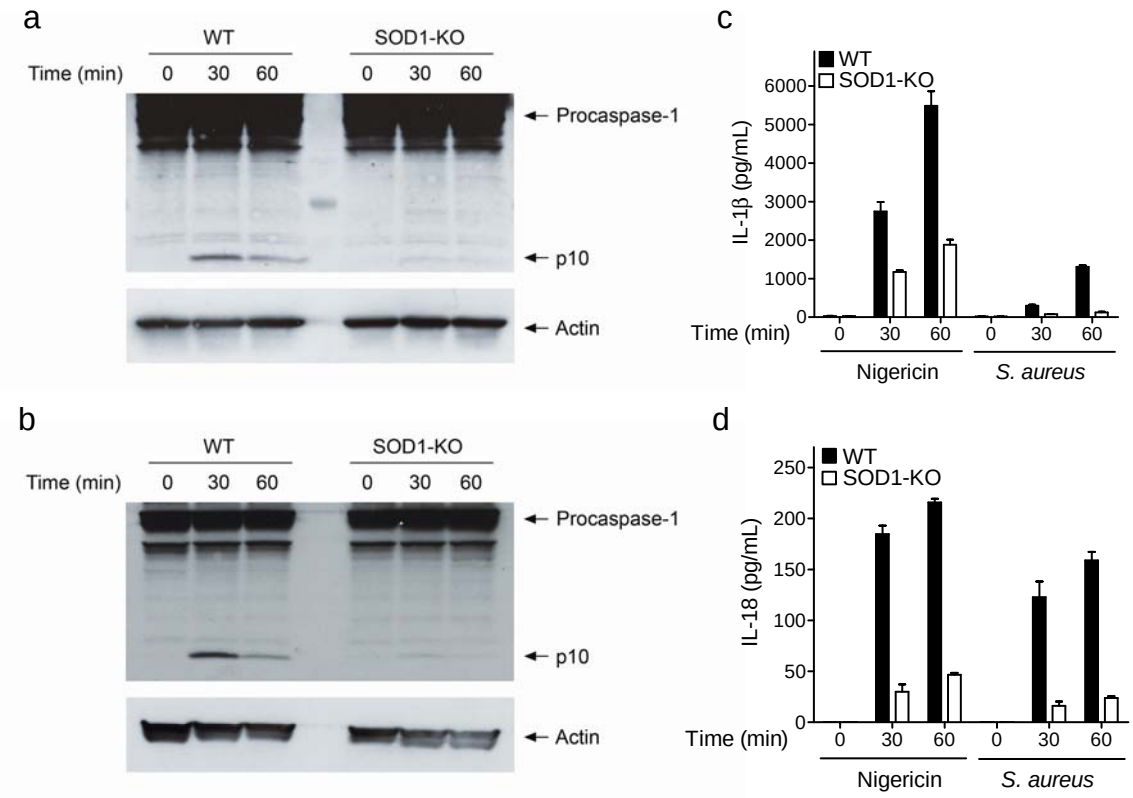
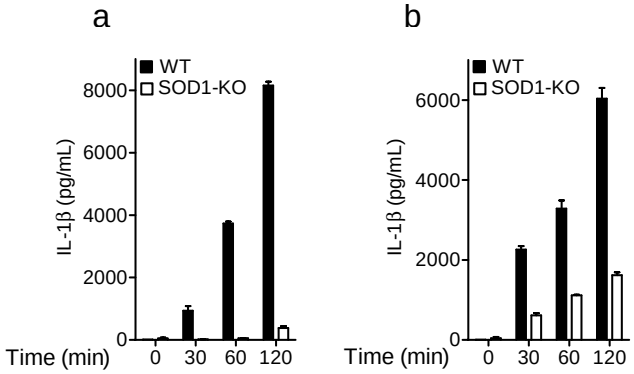
Supplementary Figure 1



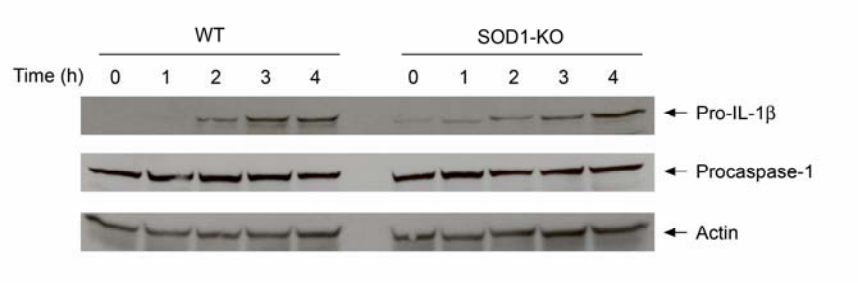
Supplementary Figure 1 SOD1 deficiency blocks caspase-1 activation and maturation of IL-1β and IL-18 upon stimulation with nigericin and *S. aureus* supernatant. (a-c) Peritoneal wild-type and SOD1-deficient macrophages were primed with 500 ng/mL LPS for 3 h or **(d)** left untreated and then pulsed with **(a)** 2.5 μM nigericin or **(b)** 5% *S. aureus* supernatant. **(a,b)** Cell lysates were immunoblotted with an antibody against the p10 subunit of caspase-1. Secretion of mature IL-1β **(c)** and mature IL-18 **(d)** into the cell supernatant was determined by ELISA. Bars represent the mean ± s.e.m of triplicate wells. Results are representative of at least five independent experiments.

Supplementary Figure 2



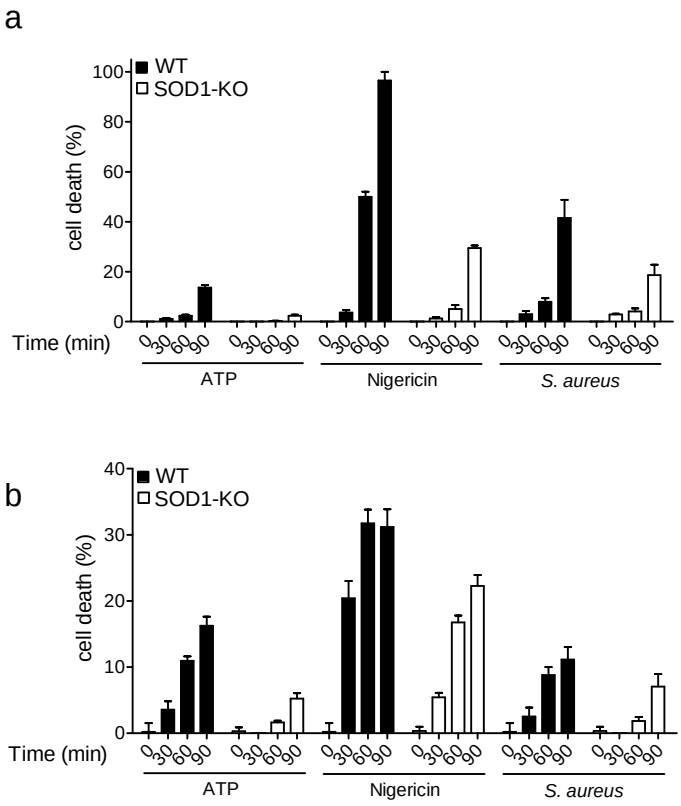
Supplementary Figure 2 SOD1 deficiency blocks maturation of IL-1β upon infection with the gram positive bacteria *L. monocytogenes* and *S. aureus*. (**a,b**) Peritoneal wild-type and SOD1-deficient macrophages primed with 500 ng/mL LPS for 3 h and then infected with *L. monocytogenes* (MOI of 10:1) **(a)** or *S. aureus* (MOI of 100:1) **(b)**. Secretion of mature IL-1β into the cell supernatant was determined by ELISA. Bars represent the mean ± s.e.m of triplicate wells. Results are representative of three independent experiments.

Supplementary Figure 3



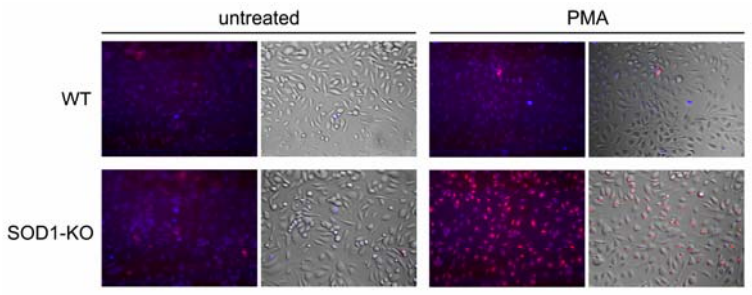
Supplementary Figure 3 Expression levels of caspase-1 and pro-IL-1β are not altered in SOD1-deficient cells. Peritoneal wild-type and SOD1-deficient macrophages stimulated with 500 ng/mL LPS for the indicated time periods and then cell lysates were analysed by immunoblot for caspase-1 and pro-IL-1β expression. Results are representative of at least three independent experiments.

Supplementary Figure 4



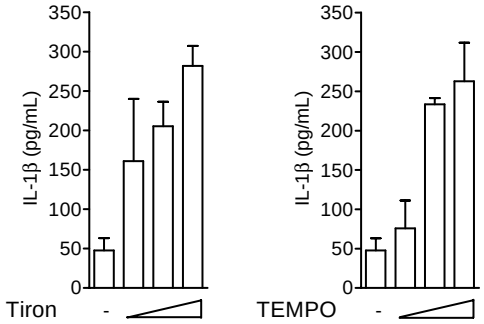
Supplementary Figure 4 SOD1 deficiency impairs cell death. (**a,b**) Peritoneal wild-type and SOD1-deficient macrophages **(a)** primed with 500 ng/mL LPS for 3 h (**a**) or left untreated (**b**) and then pulsed with 2 mM ATP, 2.5 μM nigericin or 5% *S. aureus* supernatant. Cell death was determined at the indicated time points by LDH release into the cell supernatant. Bars represent the mean ± s.e.m of triplicate wells. Results are representative of at least three independent experiments.

Supplementary Figure 5



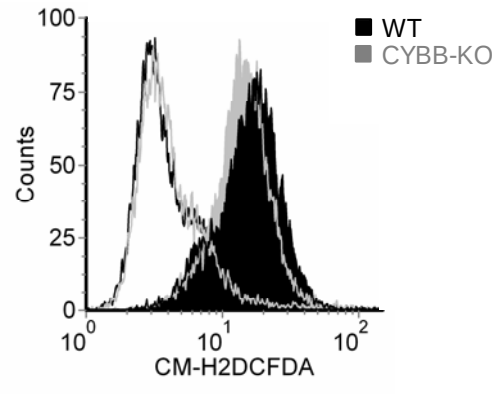
Supplementary Figure 5 Superoxide production is increased in SOD1-deficient macrophages. Peritoneal macrophages were loaded with 5 μg/mL dihydroethidium for 15 min in the dark. Cells were stimulated with 30 nM PMA for 30 min and superoxide production was detected by the conversion of the weakly blue-fluorescent dihydroethidium (350 nm) to the red-fluorescent ethidium (450 nm), which accumulates in the nucleus. Results are representative of at least three independent experiments.

Supplementary Figure 6



Supplementary Figure 6 SOD mimetics increase IL-1β release in SOD1-deficient cells. Peritoneal macrophages primed with LPS for 3h; 30 min before stimulation with 2mM ATP the cells were incubated with various concentrations of the SOD mimetics Tiron or TEMPO. IL-1β release to the supernatant within 1h was determined by ELISA. Results are representative of at least three independent experiments.

Supplementary Figure 7



Supplementary Figure 7 ROS production in response to ATP does not differ in cytochrome b-deficient and wild-type peritoneal macrophages. CM-H_2DCFDA was used as an indicator of ROS levels in wild-type and cytochrome b-deficient macrophages. Cells were either left untreated (lines) or treated with 5mM ATP (filled).

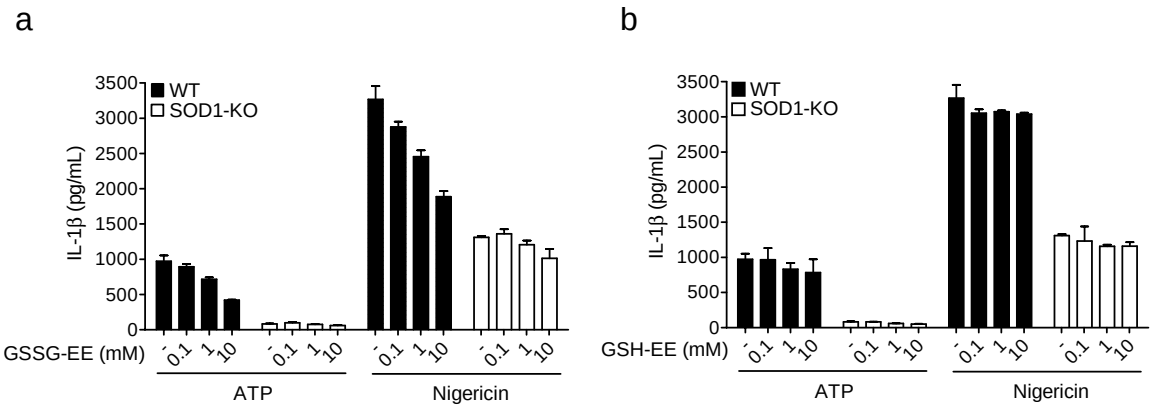
Supplementary Figure 8

$E_h = E_0 + 2.303 * RT / nF * \log([GSSG]/[GSH]^2)$
$E_0 = -0.24$ V (for Glutathione)
R = 8.31 J / deg * mol
F = 96.406 J / V

$\Delta E_{h\_untreated} = E_{h\_SOD1\ null\_(untreated)} - E_{h\_wild\text{-}type\_(untreated)} = 46.2 \pm 10.9$ mV
$\Delta E_{h\_LPS + ATP} = E_{h\_SOD1\ null\_(LPS + ATP)} - E_{h\_wild\text{-}type\_(LPS + ATP)} = 84.6 \pm 18.8$ mV

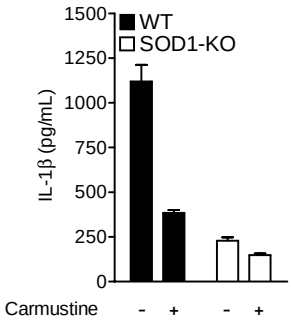
Supplementary Figure 8 The cellular redox state is decreased in SOD1-deficient mice. Wild-type (n = 3) and *SOD1* null (n = 3) mice were either left untreated or challenged with 15 mg/kg of body weight of *E. coli* LPS for 3h plus a successive injection of 250 nmol/kg body weight ATP 30 min prior to being bled. The resulting changes in the redox potential were calculated using the Nernst equation. Results are representative of three independent experiments.

Supplementary Figure 9



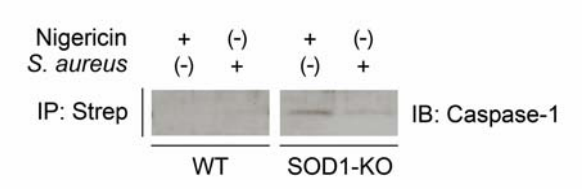
Supplementary Figure 9 Loading of wild-type macrophages with oxidized glutathione reduces IL-1β maturation. (**a,b**) Oxidized glutathione ethylester was prepared by incubation of equimolar ratios of reduced glutathione ethylester with diamide. Mouse peritoneal macrophages were loaded with indicated concentrations of oxidized **(a)** or reduced glutathione ethylester **(b)** for 1h before stimulation with 500 ng/mL LPS for 3h and 2 mM ATP or 1 μM nigericin for 1h. IL-1β maturation was analysed by ELISA from the cell supernatants. Results are representative of at least three independent experiments.

Supplementary Figure 10



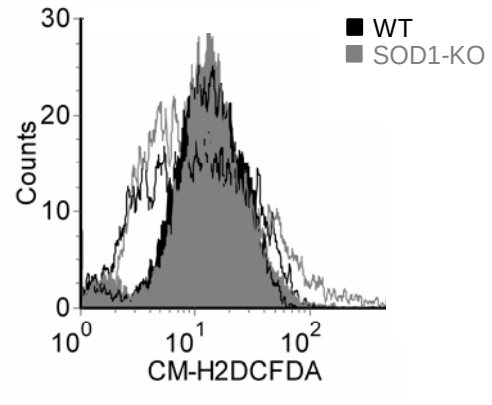
Supplementary Figure 10 Inhibition of glutathione reductase with carmustine reduces IL-1β maturation in wild-type macrophages. Peritoneal macrophages were incubated with 100 μM carmustine for 30 min before stimulation with 500 ng/mL LPS for 3h and 2 mM ATP for 1h. IL-1β maturation was analysed by ELISA from the cell supernatants. Bars represent the mean ± s.e.m of triplicate wells. Results are representative of at least three independent experiments.

Supplementary Figure 11



Supplementary Figure 11 Caspase-1 is glutathionylated in SOD1-deficient cells upon stimulation with nigericin and *S. aureus* supernatant. Glutathionylation of cellular proteins in response to 2 µM nigericin and 5% *S. aureus* supernatant was analysed by immunoblot. Glutathionylated proteins were precipitated with streptavidin and probed with a caspase-1 antibody. Results are representative of at least three independent experiments.

Supplementary Figure 12



Supplementary Figure 12 Hypoxia limits ROS production. Cells were seeded, loaded with CM-H_2DCFDA and either left untreated (lines) or stimulated with ATP (filled) under hypoxic conditions. For determination of ROS production by DCF fluorescence, cells were discharged from the hypoxic chamber and analysed immediately by FACS. Results are representative of three independent experiments.