Supplemental Data

Prolonged fasting suppresses mitochondrial NLRP3 inflammasome assembly and execution via SIRT3 mediated activation of Superoxide Dismutase 2.

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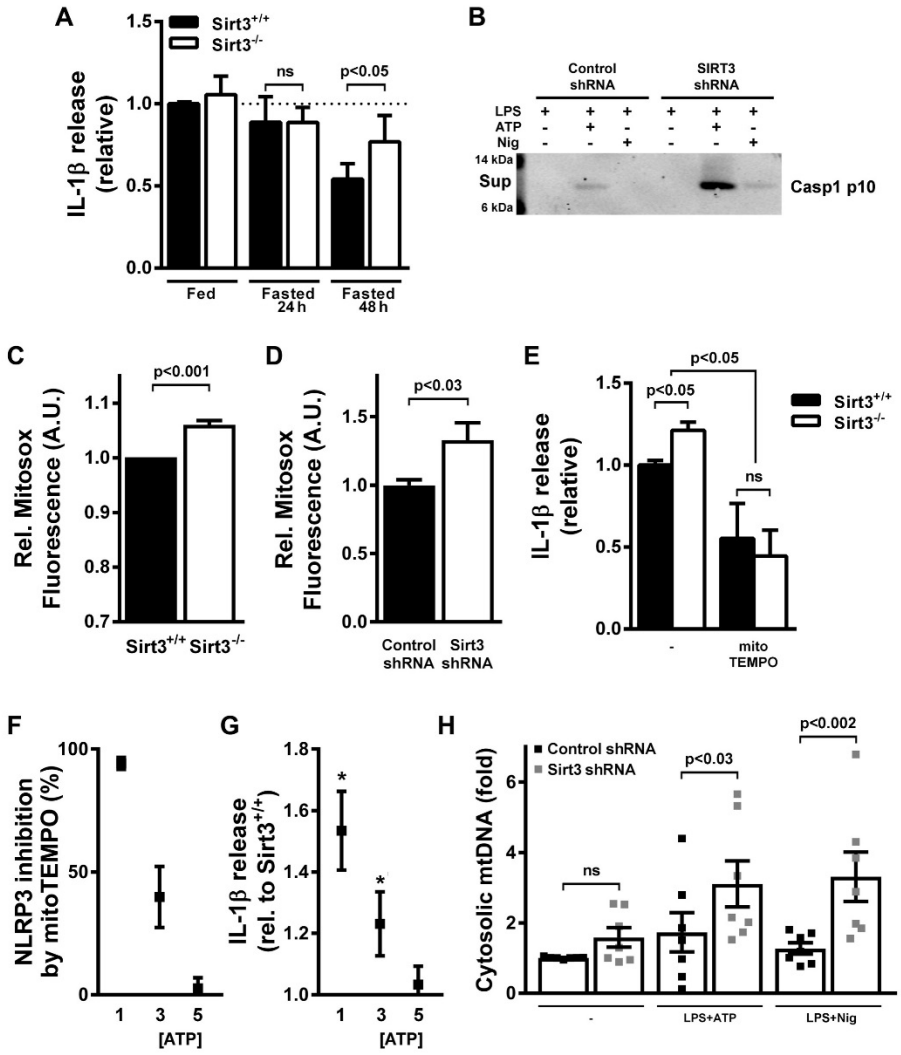

Figure S1

Figure S1. (**A**) IL-1β release to culture supernatants of peritoneal macrophages obtained from fed, 24-hour fasted or 48-hour fasted SIRT3+/+ or SIRT3-/- mice primed with LPS and stimulated with 5 mM ATP. IL-1β was measured by ELISA and is represented relative to the release in cells obtained from fed SIRT3+/+ mice. Bars represent mean ± SEM (n=5-9). *P < 0.05. (**B**) Immunoblot of release of active caspase-1 (p10 subunit) to supernatants of control or SIRT3 KD J774A.1 cells primed with LPS and left untreated or stimulated with 5 mM ATP or nigericin. (**C**) Mitochondrial ROS levels in LPS-treated BMDMs. Bars represent mean ± SEM (n=4). (**D**) Mitochondrial ROS levels in LPS-treated J774A.1 cells. Bars represent mean ± SEM (n=7). (**E**) IL-1β release to culture supernatants of BMDMs primed with LPS and stimulated with 3 mM ATP, left untreated or treated with 500 μM mitoTEMPO for 15 minutes prior to ATP stimulation. IL-1β was measured by ELISA and is represented relative to the release in untreated $SIRT3^{+/+}$. Bars represent mean ± SEM (n=4). (**F**) Inhibition by mitoTEMPO of the NLRP3 inflammasome (measured as release of IL-1β by ELISA) in BMDMs primed with LPS and stimulated with 1, 3 or 5 mM ATP. Bars represent mean ± SEM (n=4). (**G**) Ratio of release of IL-1β comparing $SIRT3^{-/-}$ to $SIRT3^{+/+}$ BMDMs primed with LPS and stimulated with 1, 3 or 5 mM ATP. Cytokines were measured by ELISA. Bars represent mean ± SEM (n=6). (**H**) Measurement of mitochondrial genomic DNA extrusion in control or SIRT3 knockdown J774A.1 macrophages left untreated or primed with LPS and stimulated with ATP or nigericin. Bars represent mean ± SEM (n=7).

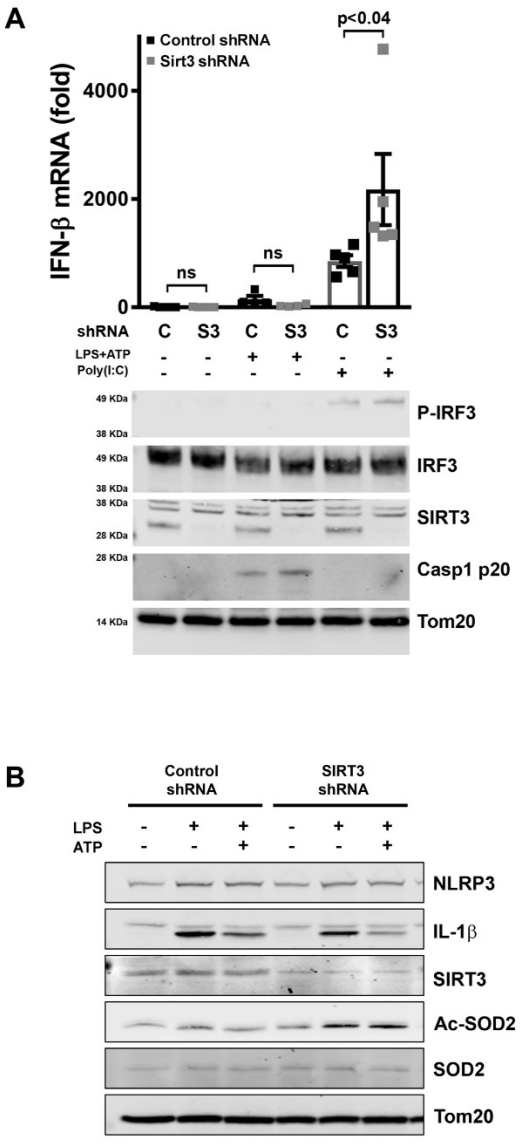

Figure S2

Figure S2. (**A**) Transcript levels of interferon β in response to inflammasome induction and in response to poly (I:C) comparing control and SIRT3 KD J774A.1 macrophages. Immunoblot shows the corresponding phosphorylation of interferon regulatory factor 3 and the cleavage of caspase 1 in response to the same triggers. The respective SIRT3 levels are shown and protein loading is represented by the levels of Tom 20. (**B**) Immunoblot analysis of steady-state protein levels of inflammasome components in control or SIRT3 KD J774A.1 cells left untreated or primed with LPS and left unstimulated or stimulated with 5 mM ATP.

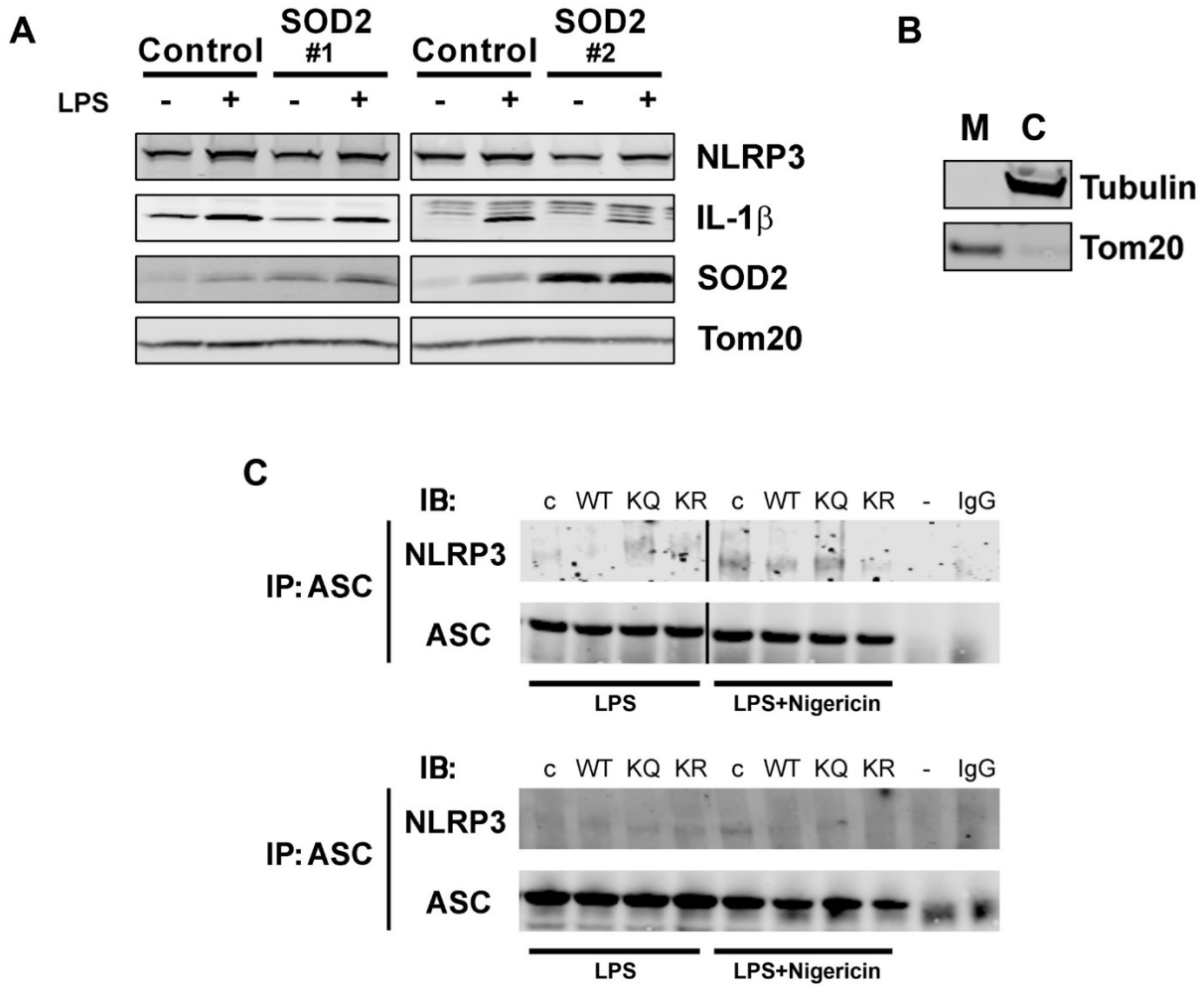

Figure S3

Figure S3. (**A**) The steady-state protein levels of NLRP3 and IL-1β in the presence or absence of LPS in two different SOD2 overexpressing clones of THP-1 macrophages (#1, low SOD2; #2, high SOD2) was analyzed by immunoblot. (**B**) The purity of mitochondrial fractions was tested by probing for the cytosolic protein tubulin, which is present in cytosolic fractions (C) but not mitochondria (M). (**C**) Replicates for the immunoprecipitation study shown in figure 4.

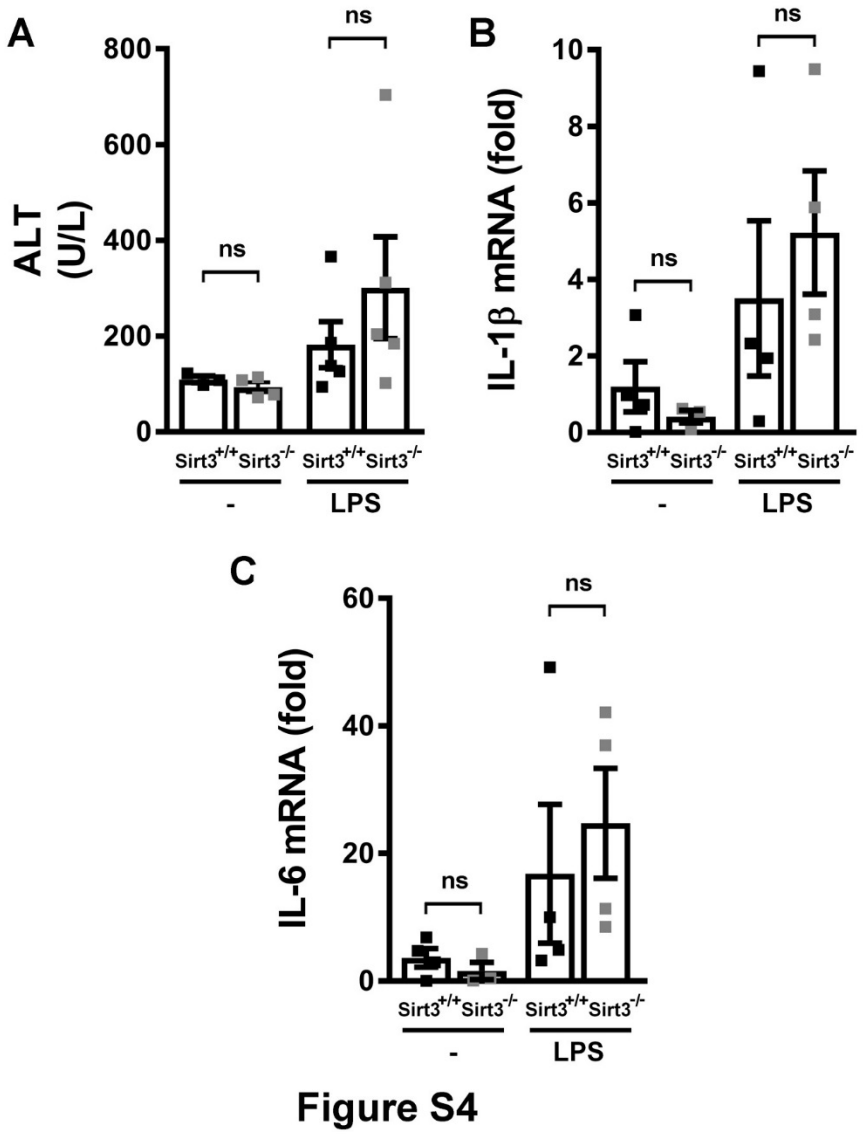

Figure S4. Fed SIRT3$^{+/+}$ or SIRT3$^{-/-}$ mice were injected intraperitoneally with PBS (-) or 1 mg/Kg LPS, and 2 hours later ALT (**A**) in the serum, and the mRNA of IL-1β (**B**) and IL-6 (**C**) in the cells that mobilize to the peritoneal cavity were measured. Bars represent mean ± SEM (A, n=5; B, C: n=4).