Hos et al., https://doi.org/10.1083/jcb.201701107

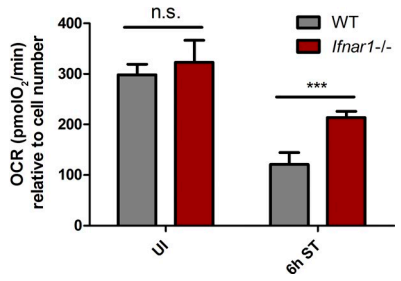

Figure S1. **IFN-I signaling exacerbates *S.* Typhimurium–induced mitochondrial damage (related to Fig. 1).** Analysis of OCR by Seahorse assay. OCRs of *S.* Typhimurium–infected WT and *Ifnar1*$^{-/-}$ BMDMs were analyzed by Seahorse assay 6 h after infection. OCR was normalized to cell number. The assay was performed with five replicates each and results are presented as means ± SD. pmolO_2/min, picomoles of oxygen per minute. UI, uninfected. ST, *S.* Typhimurium. ***, P < 0.001. n.s., not significant.

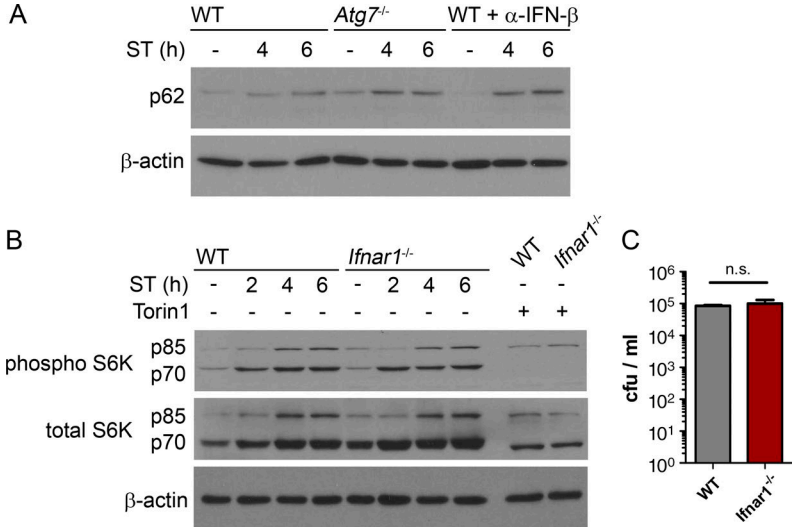

Figure S2. **Mitochondrial damage results in the transient induction of autophagy (related to Fig. 2).** (A) Immunoblot analysis of p62 expression. WT BMDMs were pretreated with anti-IgG or neutralizing antibodies against IFN-β (α-IFN-β) for 2 h. WT BMDMs were then infected with *S.* Typhimurium for the indicated time in the presence of anti-IgG or anti–IFN-β and then immunoblotted for p62. Autophagy-deficient *Atg7*$^{-/-}$ BMDMs infected with *S.* Typhimurium were used as a control for p62 accumulation. (B) Immunoblot analysis of mTOR activation. WT and *Ifnar1*$^{-/-}$ BMDMs were infected with *S.* Typhimurium for the indicated time or treated with Torin1 (10 µM for 4 h) and then immunostained for phospho-S6K and total S6K. p70 S6 kinase is a downstream target of mTOR and is therefore used as a marker for mTOR activation. Torin1 inhibits mTOR and was used as a control. (C) Assessment of bacterial burden. WT and *Ifnar1*$^{-/-}$ BMDMs were infected with *S.* Typhimurium for 24 h, and bacterial colony-forming units (cfu) were counted to assess bacterial burden. Results are representative of two independent experiments performed with three replicates each and are presented as means ± SD. ST, *S.* Typhimurium. n.s., not significant.

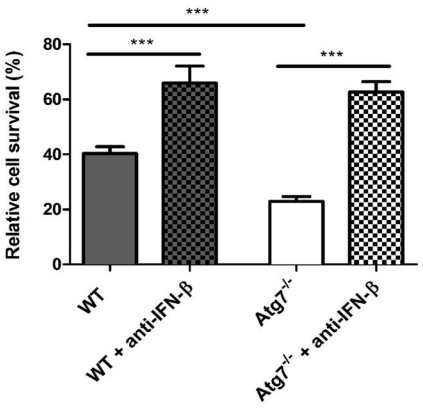

Figure S3. **p62 levels contribute to IFN-I–mediated cell death upon *S.* Typhimurium infection (related to Fig. 3).** Luminescence analysis of autophagy- and IFN-β–dependent cell death. WT and autophagy-deficient *Atg7*$^{-/-}$ BMDMs were treated with anti-IgG or neutralizing antibodies against IFN-β as described in Fig. S2 A. WT and *Atg7*$^{-/-}$ BMDMs were then infected with *S.* Typhimurium for 6 h, and luminescence was determined to analyze cell viability. Values were normalized to the luminescence signal of uninfected WT BMDMs treated with anti-IgG antibodies. Results are representative of three independent experiments performed in quintuplicates and are presented as means ± SD. ***, $P < 0.001$.

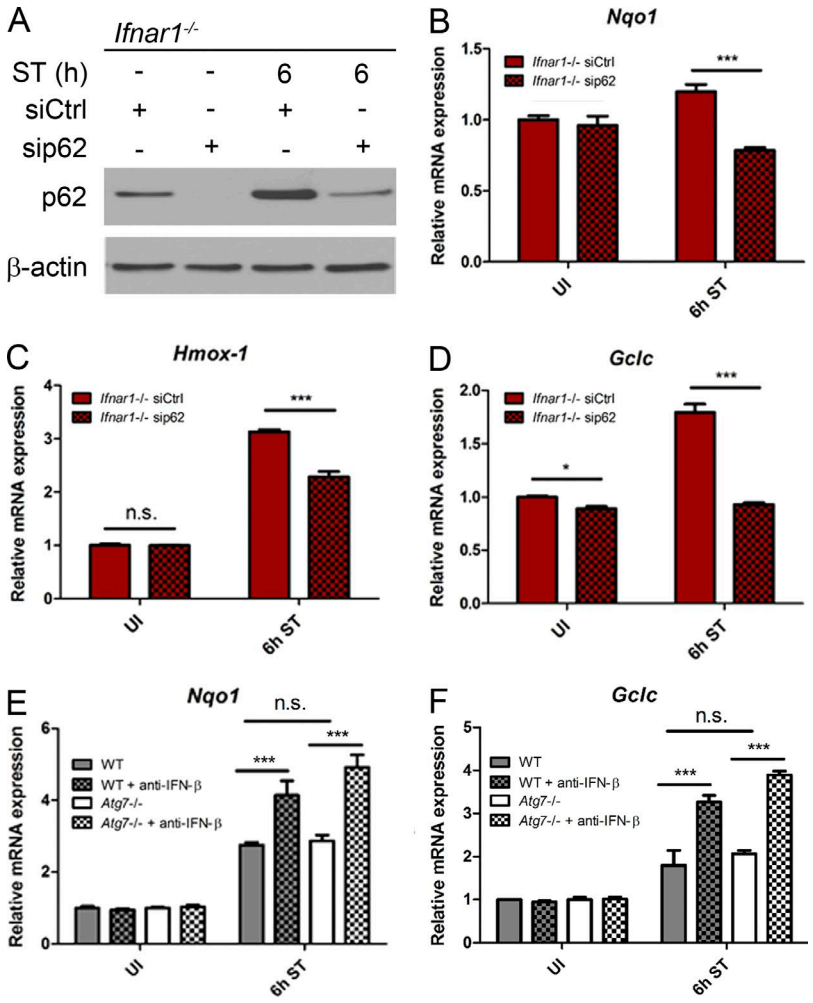

Figure S4. **IFN-I signaling attenuates Nrf2 activation and antioxidative stress responses during *S.* Typhimurium infection (related to Fig. 4).** (A) Immunoblot analysis of p62 knockdown efficiency. *Ifnar1*$^{-/-}$ BMDMs were transfected with nontargeting (siCtrl) or *Sqstm1*/p62-specific siRNA (sip62), and p62 knockdown efficiency was determined in total cell lysates by immunoblot. (B–D) p62-dependent expression of Nrf2 target genes. *Ifnar1*$^{-/-}$ BMDMs were transfected as described in A. Transfected *Ifnar1*$^{-/-}$ BMDMs were infected with *S.* Typhimurium for 6 h, and relative mRNA expression levels of *Nqo1* (B), *Hmox-1* (C), and *Gclc* (D) were determined by real-time PCR. Values were normalized to the amounts of mRNA in uninfected siCtrl BMDMs. (E and F) Autophagy- and IFN-β–dependent expression of Nrf2 target genes. WT and autophagy-deficient *Atg7*$^{-/-}$ BMDMs were treated with anti-IgG or neutralizing antibodies against IFN-β as described in Fig. S2 A. WT and *Atg7*$^{-/-}$ BMDMs were infected with *S.* Typhimurium for 6 h, and relative mRNA expression levels of *Nqo1* (E) and *Gclc* (F) were determined by real-time PCR. Values were normalized to the amounts of mRNA in uninfected BMDMs treated with anti-IgG. (B–F) Data are means ± SD from three independent experiments performed in triplicates. ST, *S.* Typhimurium. UI, uninfected. *, $P < 0.05$; ***, $P < 0.001$. n.s., not significant.

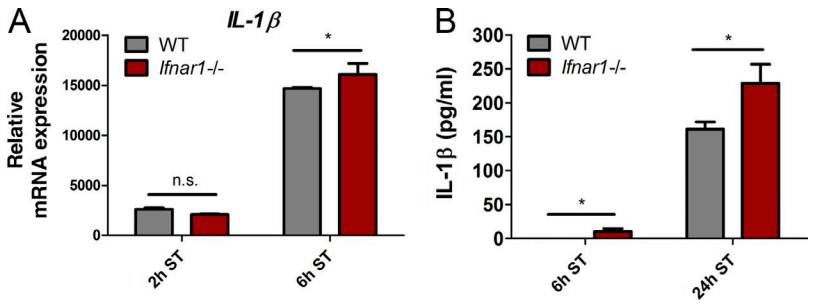

Figure S5. **Nrf2 activation upon *S.* Typhimurium infection is regulated by RIP3 (related to Fig. 6).** (A) Relative *IL-1β* mRNA expression. WT and *Ifnar1*$^{-/-}$ BMDMs were infected with *S.* Typhimurium for the indicated time, and *IL-1β* mRNA expression was determined by real-time PCR. Values were normalized to the amounts of mRNA in uninfected BMDMs. Data are means ± SD from two independent experiments with three replicates each. (B) Secretion of IL-1β. WT and *Ifnar1*$^{-/-}$ BMDMs were infected with *S.* Typhimurium for the indicated time, and IL-1β was measured in the supernatants by ELISA. Data represent three independent experiments performed in triplicates and are means ± SD. ST, *S.* Typhimurium. *, $P < 0.05$. n.s., not significant.