Online Data Supplement

SESN2/sestrin2 suppresses sepsis by inducing mitophagy and inhibiting NLRP3 activation in macrophages

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Figure S1-17

Figure S1

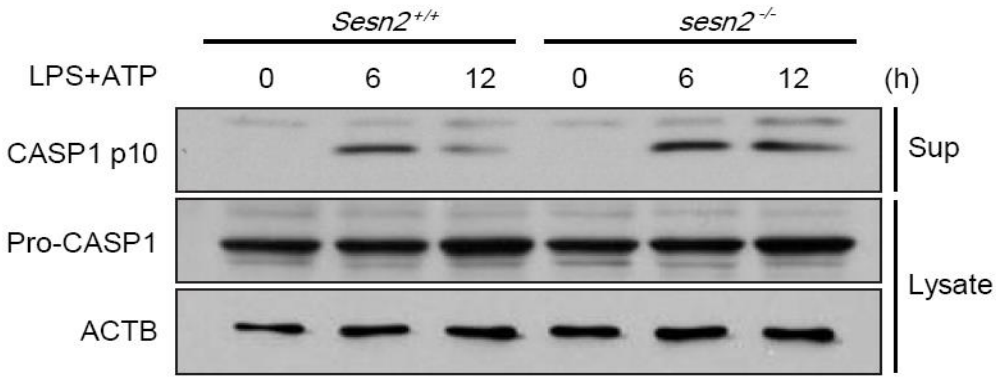

Figure S1. SESN2 suppresses NLRP3-dependent CASP1 activation in response to LPS and ATP. Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDM extracts and supernatants primed with LPS (0, 6, and 12 h) followed by ATP treatment. Data shown are representative of 3 independent experiments.

Figure S2

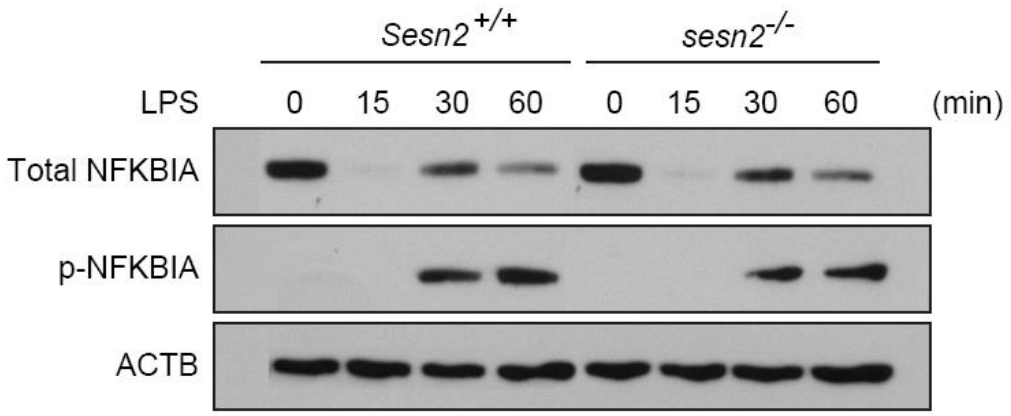

Figure S2. NFKB signaling by LPS is not affected in *sesn2*$^{-/-}$ BMDMs. Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDMs treated with LPS (0, 15, 30, and 60 min). Data shown are representative of 3 independent experiments.

Figure S3

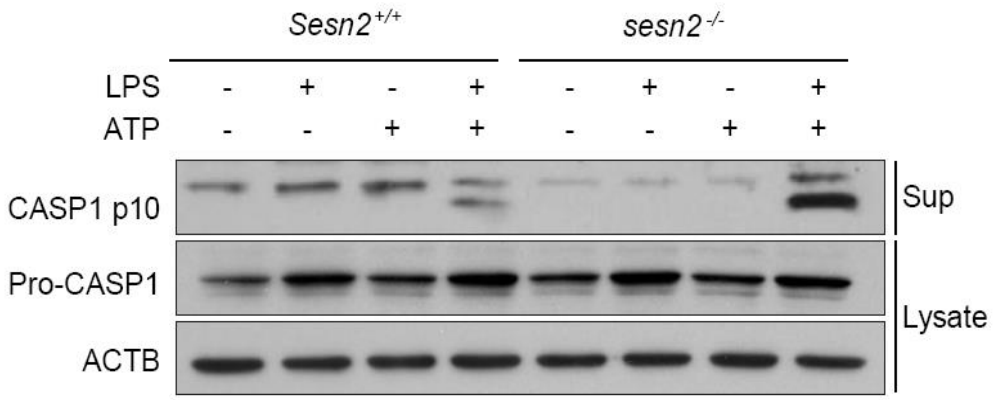

Figure S3. LPS or ATP treatment alone does not induce CASP1 activation. Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDM extracts and supernatants treated with LPS and/or ATP. Data shown are representative of 3 independent experiments.

Figure S4

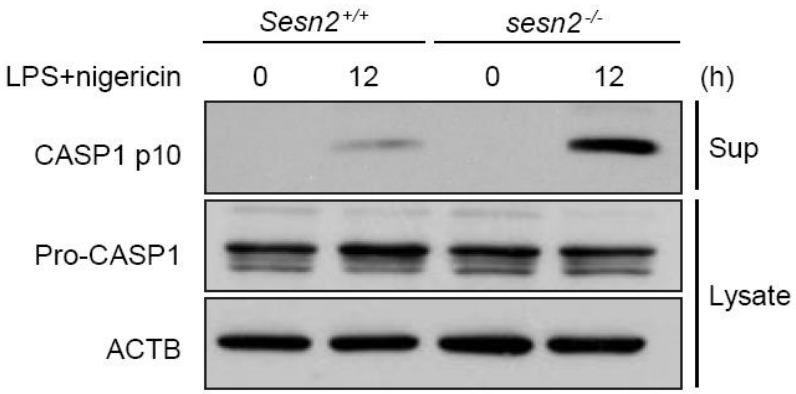

Figure S4. Nigericin induces CASP1 activation in LPS-primed BMDMs. Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDM extracts and supernatants primed with LPS followed by nigericin treatment. Data shown are representative of 3 independent experiments.

Figure S5

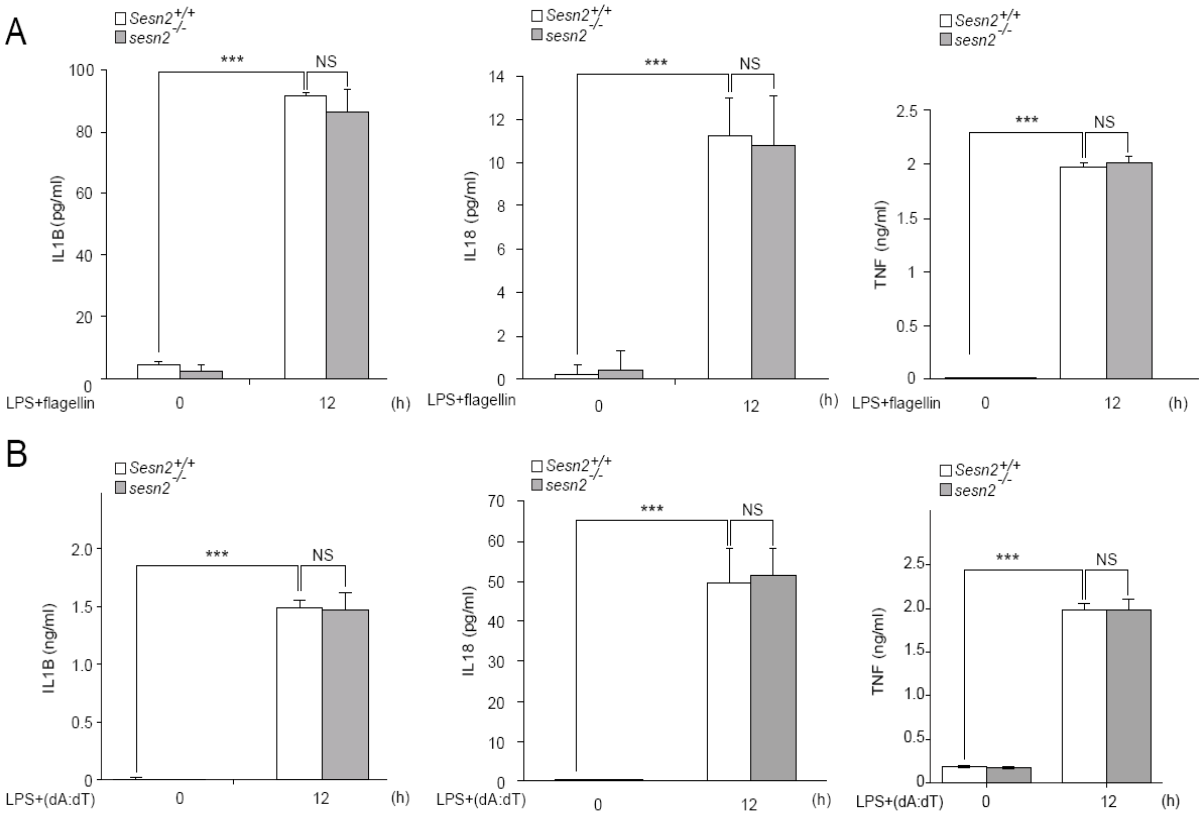

Figure S5. SESN2 does not suppress CASP1 activation in response to extended (12 h) LPS priming followed by flagellin or (dA:dT) treatment. (**A**) Production of IL1B, IL18 and TNF as measured by ELISA from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDMs treated with LPS and flagellin. (**B**) Production of IL1B, IL18 and TNF as measured by ELISA from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDMs treated with LPS and (dA:dT). Data shown are the mean±s.e.m. ***, p<0.005 from an ANOVA followed by Tukey's post hoc test. NS, not significant.

Figure S6

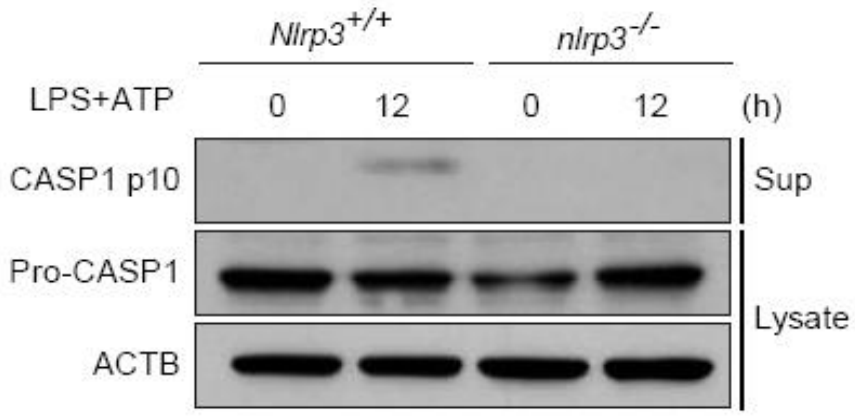

Figure S6. ATP induces NLRP3-dependent CASP1 activation in LPS-primed BMDMs. Immunoblot analysis of the indicated proteins from *Nlrp3*$^{+/+}$ and *nlrp3*$^{-/-}$ BMDM extracts and supernatants primed with LPS followed by ATP treatment. Data shown are representative of 2 independent experiments.

Figure S7

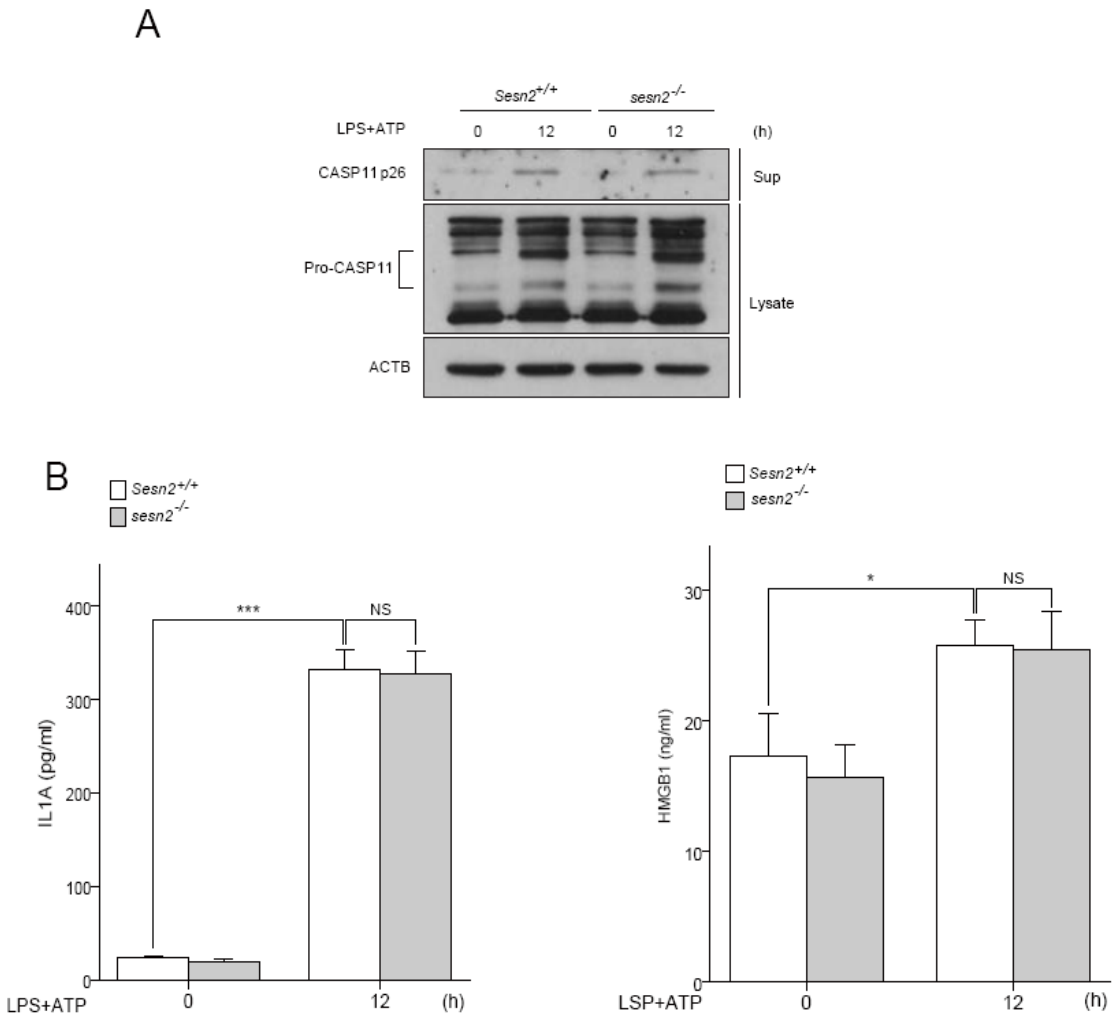

Figure S7. SESN2 is not involved in CASP11 activation in response to extended (12 h) LPS priming followed by ATP treatment. (**A**) Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDM extracts and supernatants primed with LPS followed by ATP treatment. (**B**) Production of IL1A and HMGB1 as measured by ELISA from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDMs treated with LPS and ATP. Data shown are representative of 3 independent experiments and are the mean±s.e.m. *, p<0.05; ***, p<0.005 from an ANOVA followed by Tukey's post hoc test. NS, not significant.

Figure S8

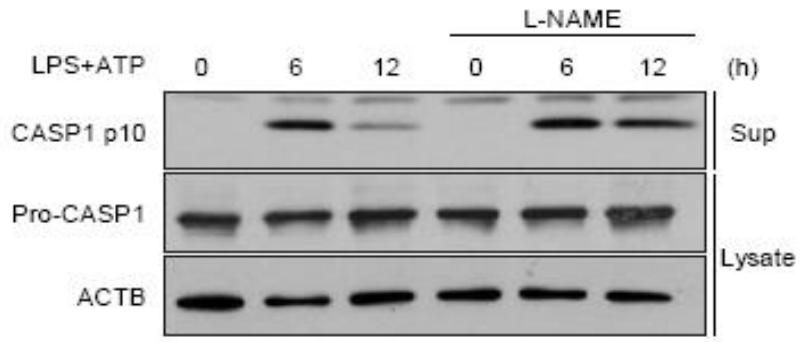

Figure S8. Inhibition of NO production increases CASP1 activation. Immunoblot analysis of the indicated proteins from BMDM extracts and supernatants primed with LPS (0, 6, 12 h) followed by ATP treatment in the presence or absence of L-NAME. Data shown are representative of 3 independent experiments.

Figure S9

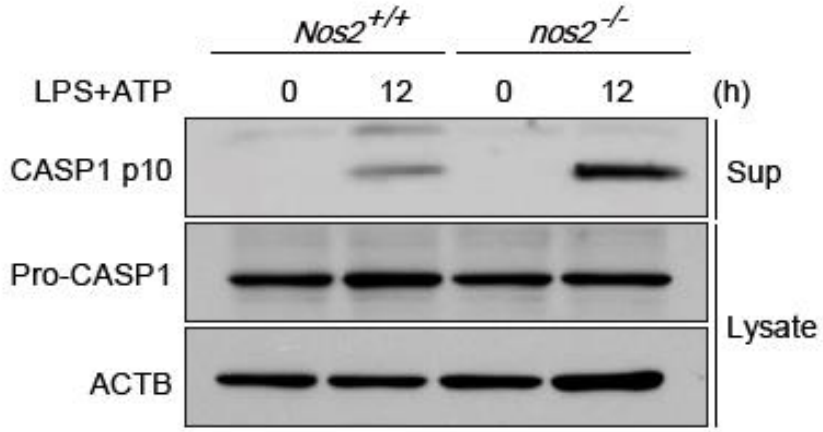

Figure S9. NOS2 suppresses CASP1 activation in response to extended (12 h) LPS priming followed by ATP treatment. Immunoblot analysis of the indicated proteins from *Nos2*$^{+/+}$ and *nos2*$^{-/-}$ BMDM extracts and supernatants primed with LPS followed by ATP treatment. Data shown are representative of 3 independent experiments.

Figure S10

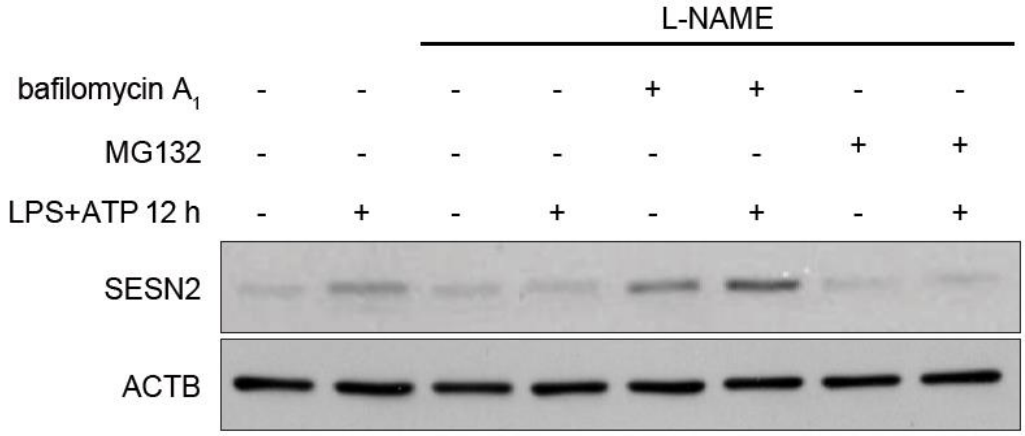

Figure S10. SESN2 degradation in the presence of L-NAME is due to autophagic degradation. Immunoblot analysis of the indicated proteins from BMDM extracts primed with LPS followed by ATP treatment in the presence of L-NAME when pretreated with bafilomycin A_1 or MG132 for 1h prior to LPS and ATP. Data shown are representative of 3 independent experiments. .

Figure S11

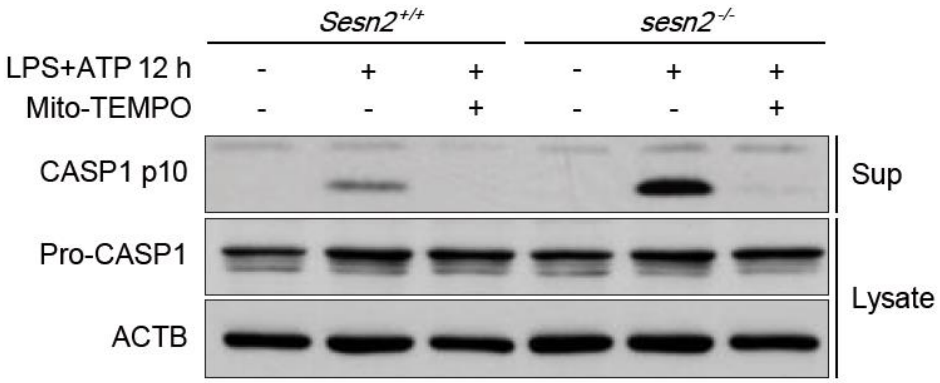

Figure S11. Mitochondrial ROS induce CASP1 activation. Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDM extracts and supernatants incubated with Mito-TEMPO (500 μM) for 1 h followed by LPS and ATP treatment. Data shown are representative of 3 independent experiments.

Figure S12

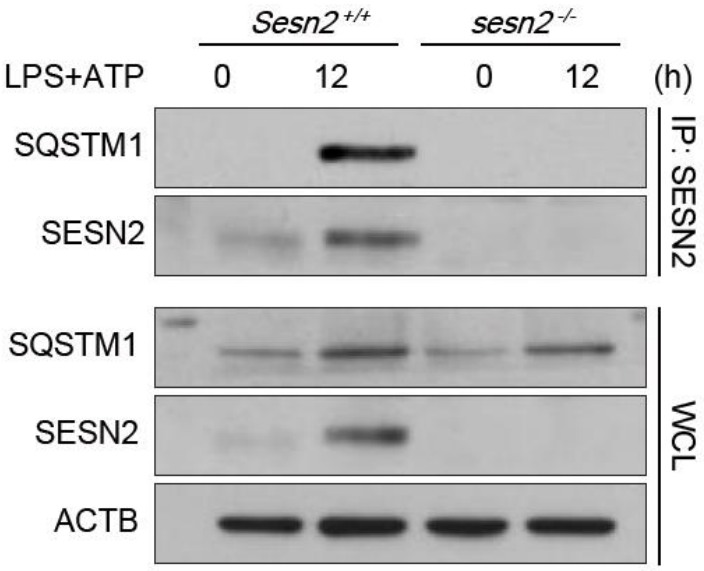

Figure S12. Endogenous SESN2 interacts with SQSTM1 in response to LPS and ATP. Immunoblot analysis of SQSTM1 or SESN2 in SESN2 immunoprecipitates in the lysates of *Sesn2*$^{+/+}$ or *sesn2*$^{-/-}$ BMDMs. (IP: SESN2, upper) and BMDM extracts (whole cell lysates [WCL], lower) primed with LPS followed by ATP treatment. Data shown are representative of 3 independent experiments.

Figure S13

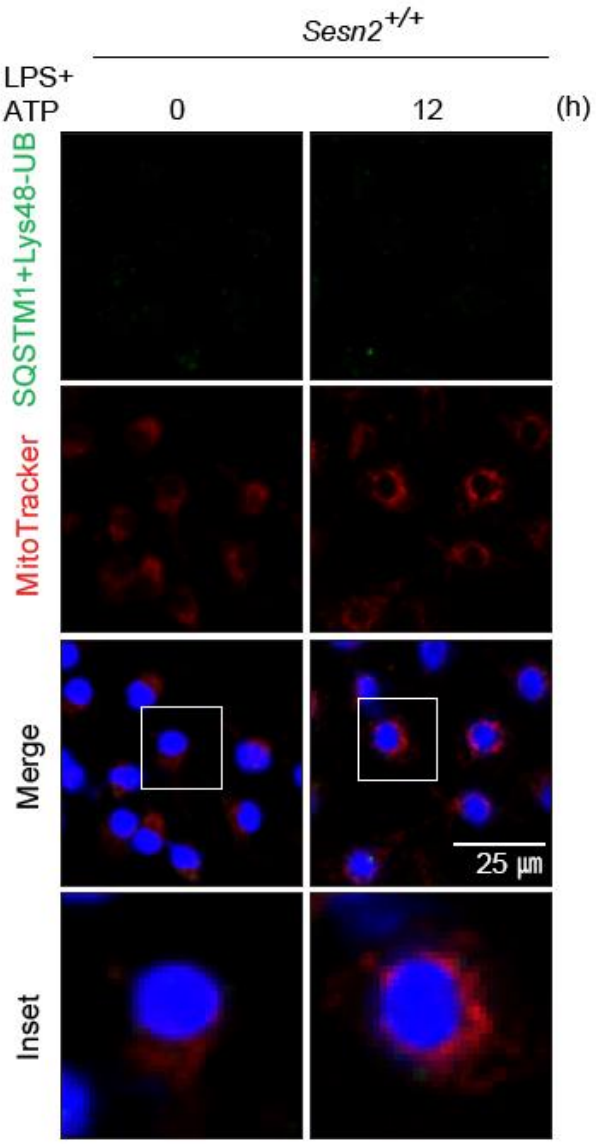

Figure S13. SQSTM1 does not associate with Lys48-ubiquitin (UB) on mitochondria in response to LPS and ATP. PLA of SQSTM1 and Lys48-ubiquitin and their colocalization with MitoTracker Deep Red. Scale bar: 25 μm. Data shown are representative of 3 independent experiments.

Figure S14

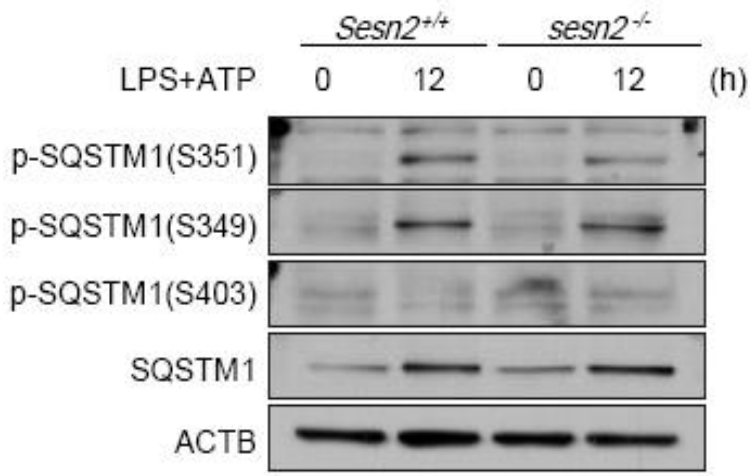

Figure S14. SESN2 does not induce phosphorylation of SQSTM1 in BMDMs. Immunoblot analysis of phosphorylated SQSTM1 from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDM extracts primed with LPS followed by ATP treatment. Data shown are representative of 3 independent experiments.

Figure S15

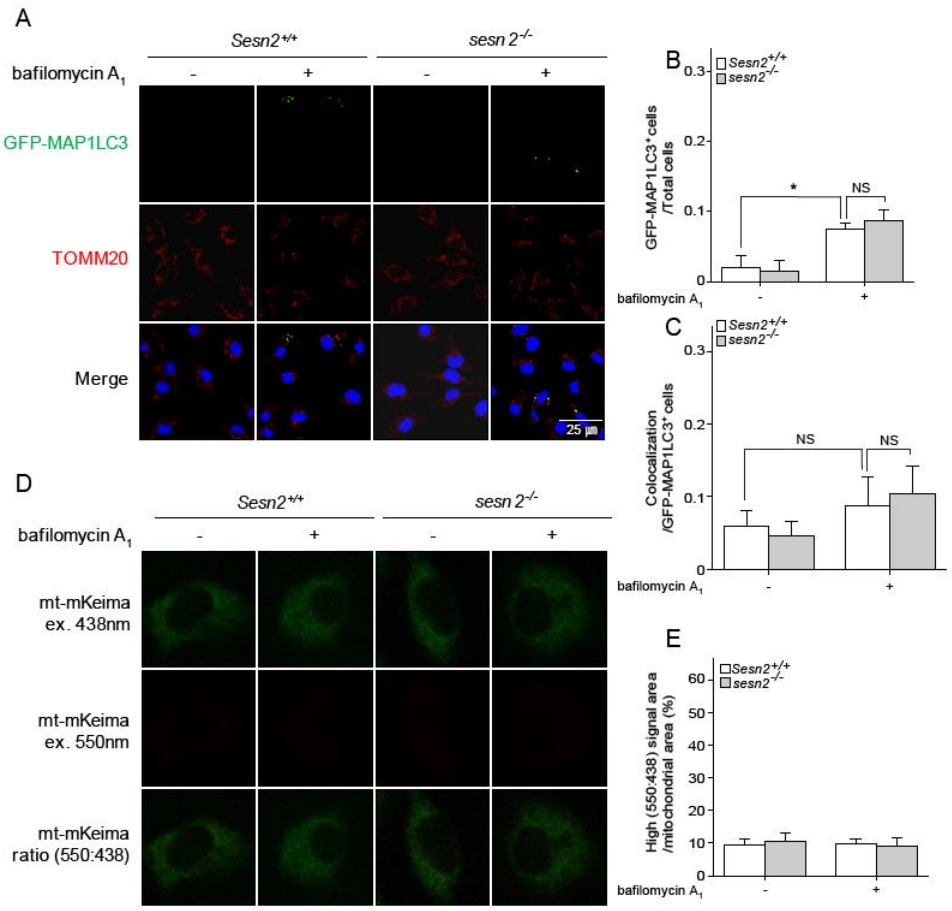

Figure S15. SESN2 is not involved in basal rate of autophagosome formation and mitophagic activity. (**A**-**E**) *Sesn2*$^{+/+}$ GFP-MAP1LC3 and *Sesn2*$^{-/-}$ GFP-MAP1LC3 BMDMs were incubated with bafilomycin A_1. (**A**) Representative confocal immunofluorescence images of GFP-MAP1LC3 and TOMM20. Scale bar: 25 μm. (**B**) Quantification of cells with more than 3 weak GFP-MAP1LC3 puncta from among total cells and (**C**) quantification of cells that have GFP-MAP1LC3 puncta colocalized with mitochondria among cells with GFP-MAP1LC3 puncta (>150 cells per group in 3 independent experiments for **B**, **C**). (**D**) Representative confocal immunofluorescence images of mt-mKeima. Scale bar: 25 μm. (**E**) Quantification of the ratio of high (550_{ex}:438_{ex}) signal area (red) to total mitochondrial area (>30 cells per group in 3 independent experiments). *, $p<0.05$ from an ANOVA followed by Tukey's post hoc test. NS, not significant.

Figure S16

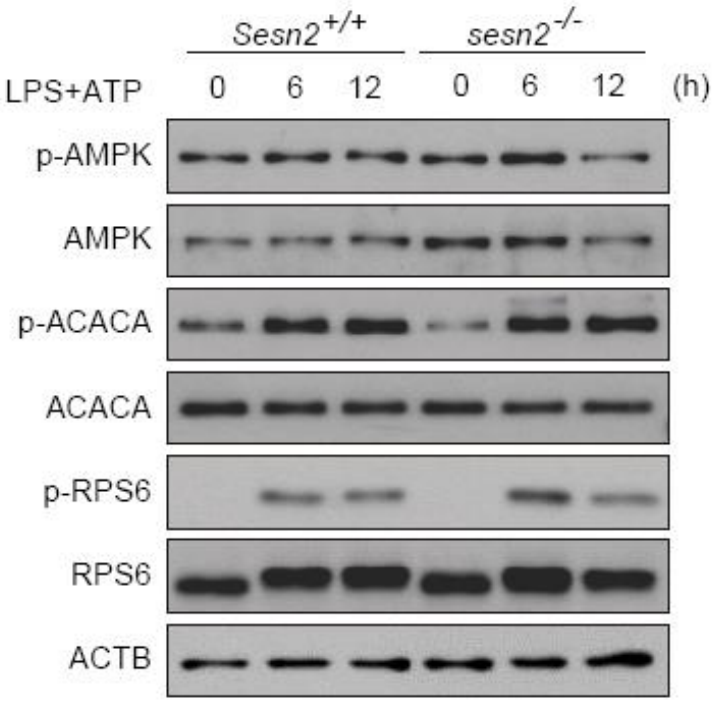

Figure S16. Phosphorylation of AMPK, RPS6, and ACACA are not affected in *sesn2*$^{-/-}$ BMDMs upon stimulation. Immunoblot analysis of the indicated proteins from *Sesn2*$^{+/+}$ and *sesn2*$^{-/-}$ BMDMs primed with LPS (0, 6, and 12 h) followed by ATP treatment. Data shown are representative of 3 independent experiments.

Figure S17

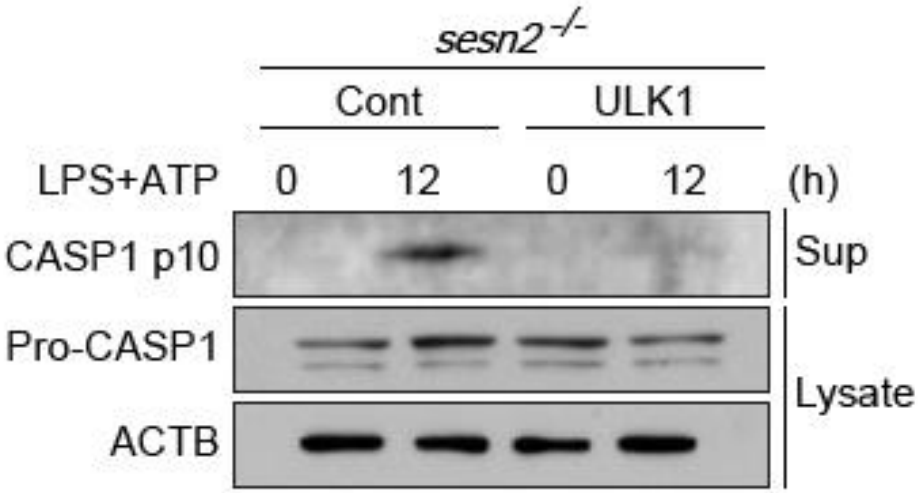

Figure S17. Increased CASP1 activation in *sesn2*$^{-/-}$ BMDMs is decreased by addition of ULK1. Immunoblot analysis of the indicated proteins from *sesn2*$^{-/-}$ BMDM extracts and supernatants when transfected with retrovirus containing control vector, or human ULK1 expression vector, (*sesn2*$^{-/-}$ + Cont, *sesn2*$^{-/-}$ + ULK1, respectively) and primed with LPS followed by ATP treatment. Data shown are representative of 3 independent experiments.