Supplemental data

UCP2 promotes inflammation in sepsis through FASN-dependent NLRP3-inflammasome activation

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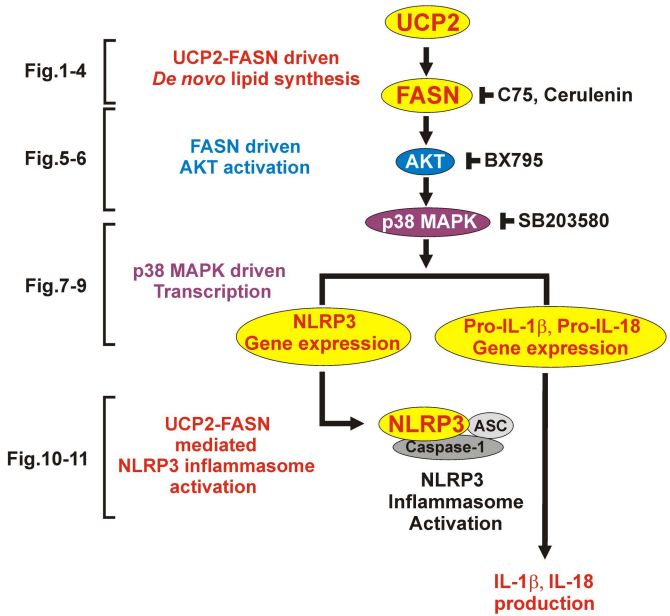
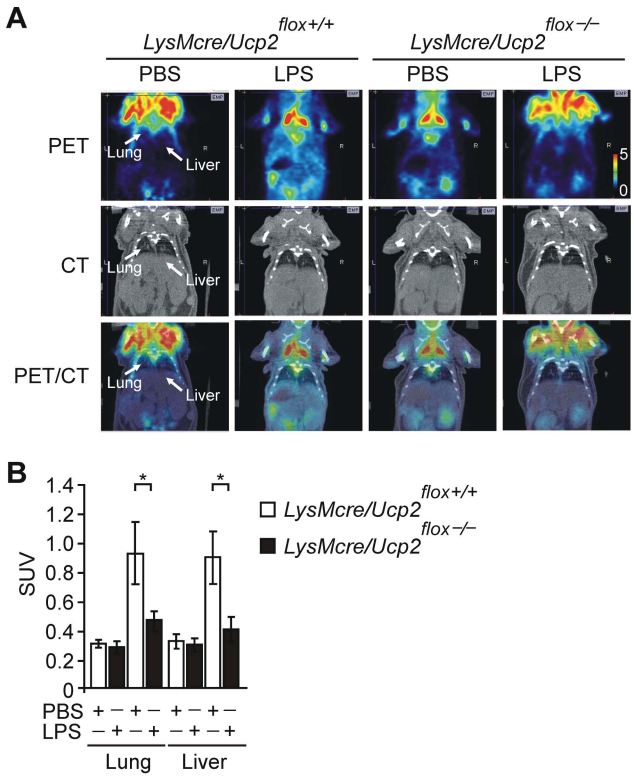
Supplemental Figure 1-14 and Supplemental Table 1



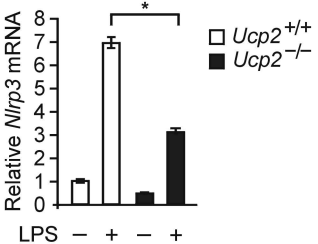
Supplemental Figure 1.

Summary of localization and relevance for individual data in our whole mechanism.



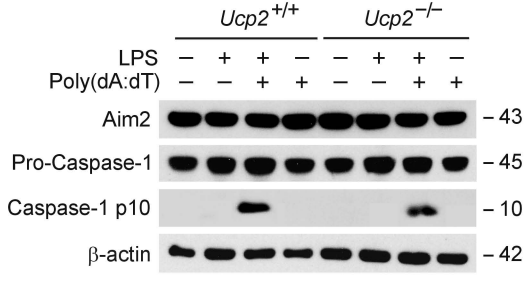
Supplemental Figure 2.

Deficiency of UCP2 suppresses glucose utilization in vivo. (**A**) Coronal SUV of PET/CT imaging using ^{18}F-FDG of lung and liver from LysMcre/*Ucp2*$^{flox+/+}$ or LysMcre/*Ucp2*$^{flox-/-}$ mice after injection of LPS for 4 h (10 mg/kg, i.p.). Image is representative of three independent experiments. (**B**) Quantification of coronal SUV in PET/CT imaging from A. n = 3 per group, **P*<0.05 by ANOVA.



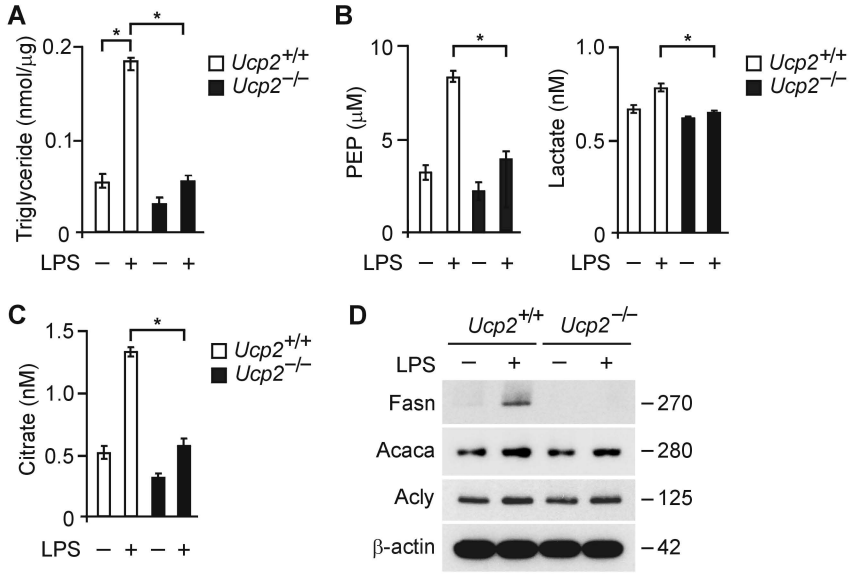
Supplemental Figure 3.

Quantitative PCR analysis for *Nlrp3* gene expression in *Ucp2*$^{+/+}$ or *Ucp2*$^{-/-}$ BMDMs treated with LPS (500 ng/ml) for 4 h. *P<0.05 by ANOVA.



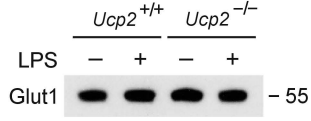
Supplemental Figure 4.

Deficiency of UCP2 does not suppress AIM2-mediated caspase-1 activation in macrophages. Immunoblot analysis for AIM2 and caspase-1 in cell lysates from *Ucp2*$^{+/+}$ or *Ucp2*$^{-/-}$ BMDMs treated with LPS (500 ng/ml) for 4 h and followed by incubation with poly(dA:dT) (1 μg/ml) for 6 h. β-actin served as the standard.



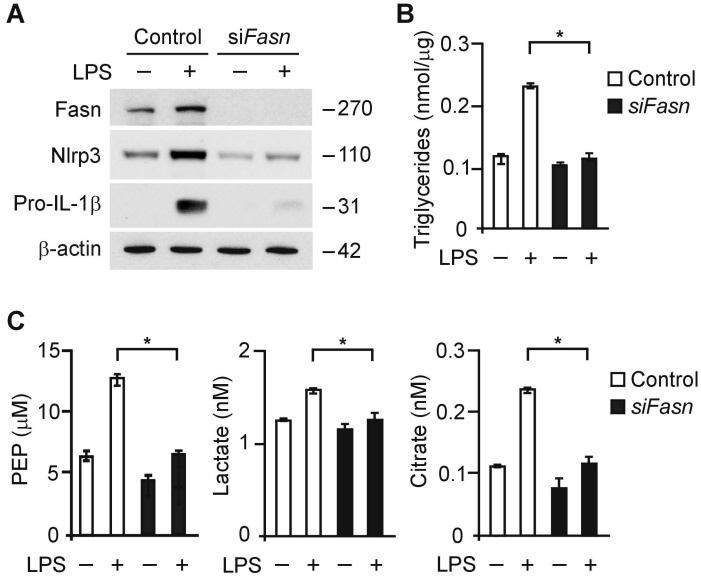
Supplemental Figure 5.

UCP2 regulates lipid synthesis via FASN in macrophages. (**A**) The measurement of triglycerides (TG) in *Ucp2*$^{+/+}$ or *Ucp2*$^{-/-}$ peritoneal macrophages treated with LPS (500 ng/ml) for 4 h. **P*<0.05 by ANOVA. (**B**) The measurement of PEP and lactate production in *Ucp2*$^{-/-}$ or *Ucp2*$^{+/+}$ peritoneal macrophages treated with LPS (500 ng/ml) for 4 h. **P*<0.05 by ANOVA. (**C**) The measurement of citrate production in *Ucp2*$^{+/+}$ or *Ucp2*$^{-/-}$ peritoneal macrophages treated with LPS (500 ng/ml) for 4 h. **P*<0.05 by ANOVA. (**D**) Immunoblot analysis for FASN, ACACA and ACLY in cell lysates from *Ucp2*$^{+/+}$ or *Ucp2*$^{-/-}$ peritoneal macrophages treated with LPS (500 ng/ml) for 4 h. β-actin served as the standard.



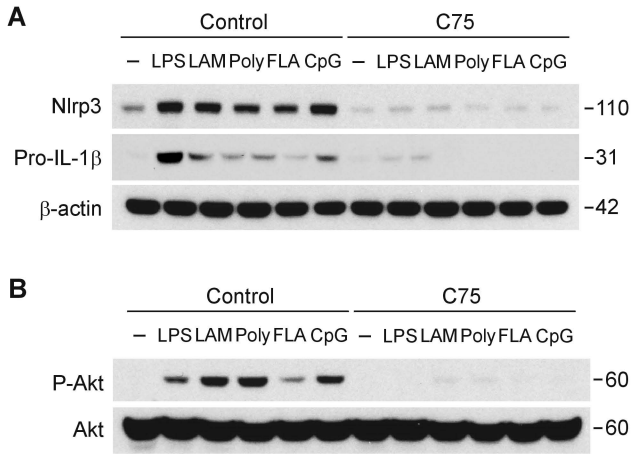
Supplemental Figure 6.

Deficiency of UCP2 does not affect GLUT1 expression in macrophages. Immunoblot analysis for GLUT1 in cell lysates from *Ucp2*$^{+/+}$ or *Ucp2*$^{-/-}$ BMDMs treated with LPS (500 ng/ml) for 4 h.



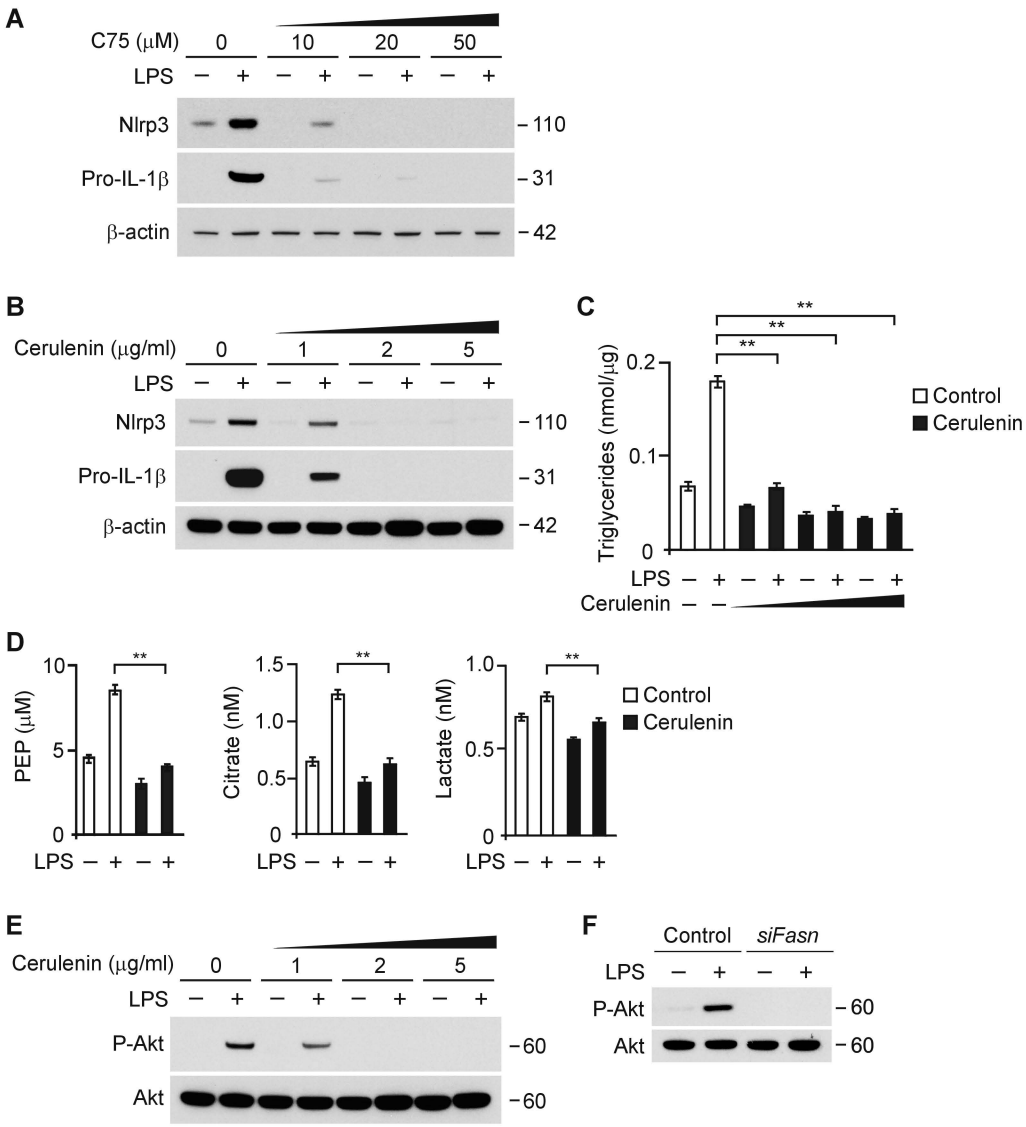
Supplemental Figure 7.

FASN regulates NLRP3 and IL-1β expression in macrophages. (**A**) Immunoblot analysis for FASN, NLRP3 and IL-1β in cell lysates from mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), and treated with LPS (500 ng/ml) for 4 h. β-actin served as the standard. (**B**) The measurement of triglycerides (TG) production in mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), treated with LPS (500 ng/ml) for 4 h. **P*<0.05 by ANOVA. (**C**) The measurement of PEP, citrate and lactate production in mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), and treated with LPS (500 ng/ml) for 4 h. **P*<0.05 by ANOVA.



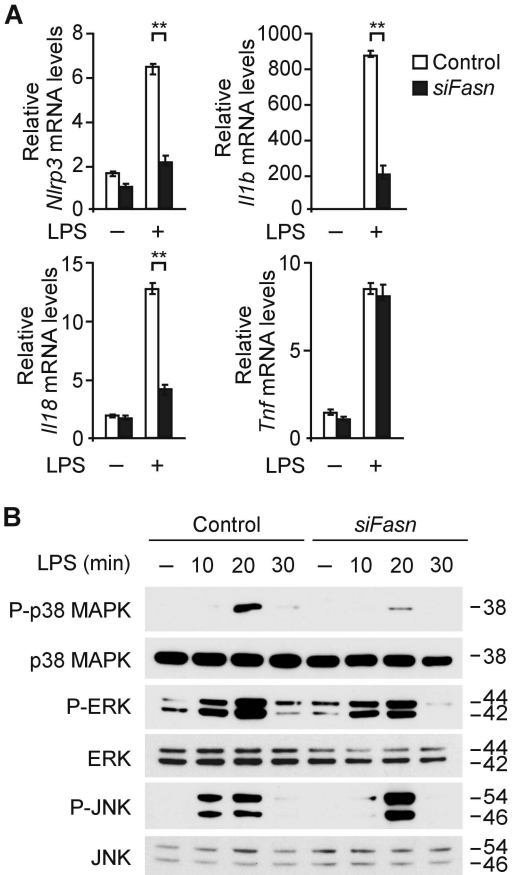
Supplemental Figure 8.

Inhibition of FASN by C75 suppresses NLRP3 and IL-1β expression in response to TLR agonists in macrophages. (**A**) Immunoblot analysis for NLRP3 and IL-1β in cell lysates from wild type BMDMs pre-treated with C75 (20 μM) for 2 h before stimulation with TLR agonists (LPS; 500 ng/ml, LAM; 20 μg/ml, Poly(I:C); 10 μg/ml, Flagellin; 40 ng/ml, CpG oligo; 5 μM) for 6 h. β-actin served as the standard. (**B**) Immunoblot analysis of the phosphorylation of AKT on Ser-473 in cell lysates from wild type BMDMs pre-treated with C75 (20 μM) for 2 h before stimulation with TLR agonists (LPS; 500 ng/ml, LAM; 20 μg/ml, Poly(I:C); 10 μg/ml, Flagellin; 40 ng/ml, CpG oligo; 5 μM) for 6 h. Total AKT served as the standard.



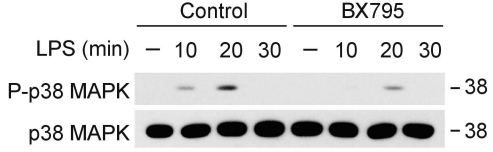
Supplemental Figure 9.

FASN regulates NLRP3 and IL-1β expression through AKT activation in macrophages. (**A**) Immunoblot analysis for NLRP3 and IL-1β in cell lysates from wild type peritoneal macrophages pre-treated with C75 (0, 10, 20 and 50 μM) for 2 h before stimulation with LPS (500 ng/ml, 4 h). β-actin served as the standard. (**B**) Immunoblot analysis for NLRP3 and IL-1β, and (**C**) The measurement of triglycerides (TG) production in cell lysates from wild type peritoneal macrophages pre-treated with cerulenin (0, 1, 2 and 5 μg/ml) for 2 h before stimulation with LPS (500 ng/ml, 4 h). β-actin served as the standard. ***P*<0.01 by ANOVA. (**D**) The measurement of PEP, citrate and lactate production from wild type peritoneal macrophages pre-treated with cerulenin (2 μg/ml) for 2 h before stimulation with LPS (500 ng/ml, 4 h). ***P*<0.01 by ANOVA. (**E**) Immunoblot analysis of the phosphorylation of AKT on Ser-473 in cell lysates from wild type peritoneal macrophages pre-treated with cerulenin (0, 1, 2 and 5 μg/ml) for 2 h before stimulation with LPS (500 ng/ml, 4 h). Total AKT served as the standard. (**F**) Immunoblot analysis of the phosphorylation of AKT on Ser-473 in cell lysates from mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), treated with LPS (500 ng/ml) for 4 h. Total AKT served as the standard.



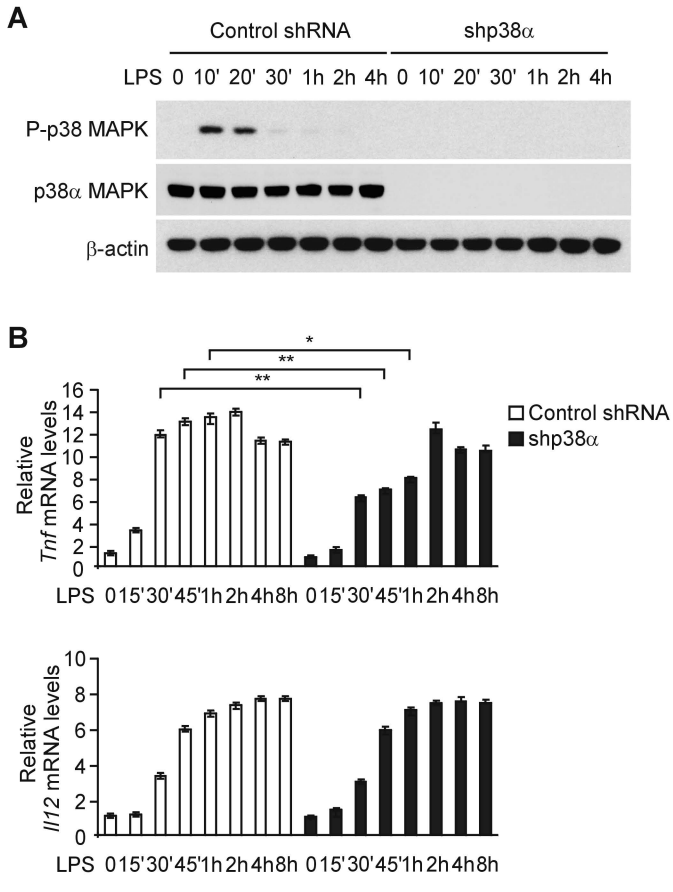
Supplemental Figure 10.

Deficiency of FASN suppresses the transcription of NLRP3 and IL-1β gene through p38 MAPK in macrophages**.** (**A**) Quantitative PCR analysis for *Nlrp3*, *Il1b*, *Il18* and *Tnf* gene expression from mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), and stimulated with LPS (500 ng/ml) for 4 h. ***P*<0.01 by ANOVA. (**B**) Immunoblot analysis for activation of p38 MAPK, ERK and JNK in cell lysates from mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), and stimulated with LPS (500 ng/ml) for 0, 10, 20 and 30 min.



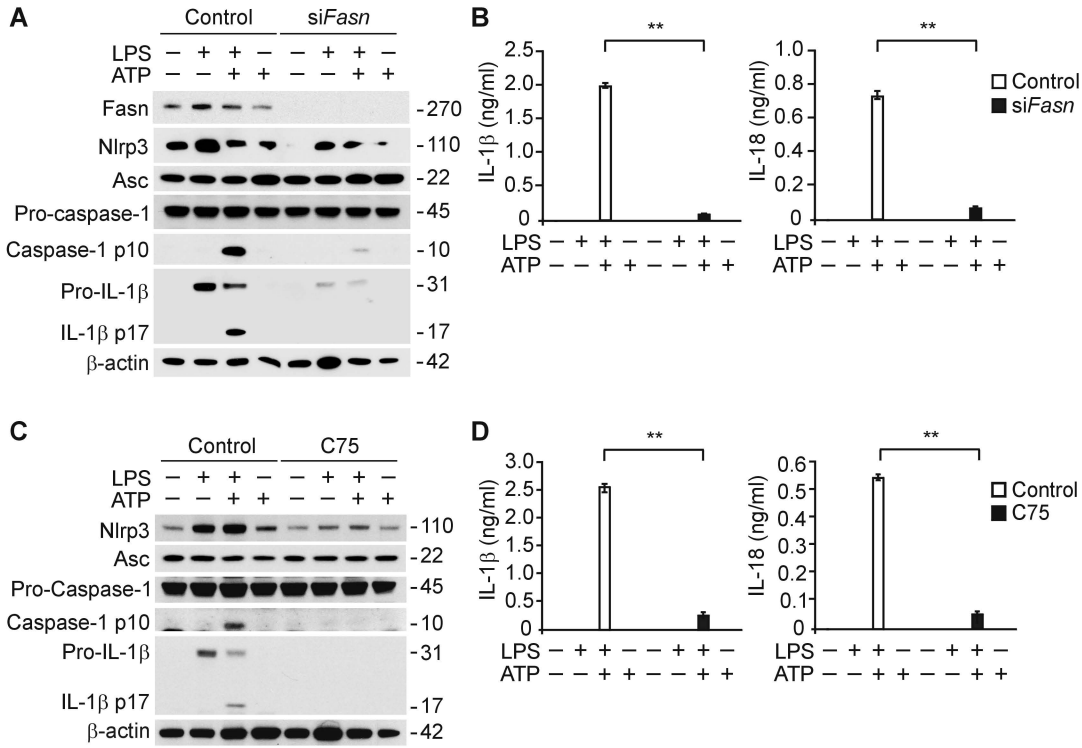
Supplemental Figure 11.

Inhibition of AKT activation suppresses the activation of p38 MAPK in macrophages**.** Immunoblot analysis for activation of p38 MAPK in cell lysates from wild type BMDMs pre-treated with BX795 (10 μM) for 1 h before stimulation with LPS (500 ng/ml) for 0, 10, 20 and 30 min. p38 MAPK served as the standard.



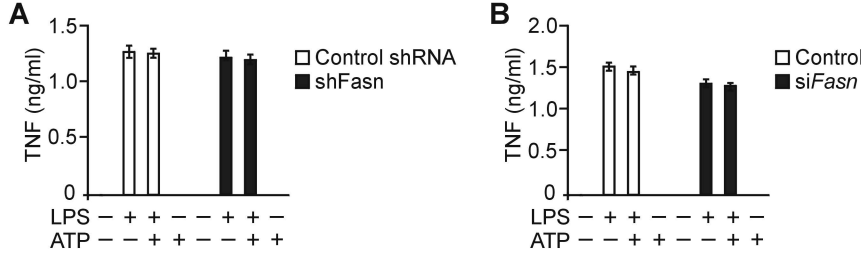
Supplemental Figure 12.

Inhibition of p38 MAPK activation suppresses the transcription of NLRP3 and IL-1β gene in macrophages**.** (**A**) Immunoblot analysis for activation of p38 MAPK in cell lysates from wild type mice peritoneal macrophages transduced with lentiviruses expressing control shRNA or shRNA for p38α (shp38α), and stimulated with LPS (500 ng/ml) for 0, 10 min, 20 min, 30 min, 1 h, 2 h and 4 h. (**B**) Quantitative PCR analysis for *Tnf* and *Il12* gene expression from wild type mice peritoneal macrophages transduced with lentiviruses expressing control shRNA or shRNA for p38α (shp38α), and stimulated with LPS (500 ng/ml) for 0, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h and 8 h. $^{**}P<0.01$, $^{*}P<0.05$ by ANOVA.



Supplemental Figure 13.

FASN regulates NLRP3 mediated caspase-1 activation in macrophages. (**A**) Immunoblot analysis for caspase-1 and IL-1β of cell lysates from mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), treated with LPS (500 ng/ml) for 4 h, followed by incubation with ATP (5 mM) for 30 min. β-actin served as the standard. (**B**) Luminex assay and ELISA assay for IL-1β and IL-18 in the media from A. ***P*<0.01 by ANOVA. (**C**) Immunoblot analysis for IL-1β and NLRP3 of cell lysates from wild-type BMDMs pre-treated with C75 (20 μM) for 2 h before stimulation with LPS (500 ng/ml) for 4 h, followed by incubation with ATP (5 mM) for 30 min. β-actin served as the standard. (**D**) Luminex assay and ELISA assay for IL-1β and IL-18 in the media from C. ***P*<0.01 by ANOVA.



Supplemental Figure 14.

Deficiency of FASN does not affect TNF secretion in macrophages. (**A**) ELISA assay for TNF in the media from wild type mice peritoneal macrophages transduced with lentiviruses expressing non-target shRNA (Control shRNA) or shRNA for Fasn (shFasn), and stimulation with LPS (500 ng/ml) for 4 h, followed by incubation with ATP (5 mM) for 30 min. (**B**) Luminex assay and ELISA assay for TNF in the media from mouse J774A.1 macrophages transfected with control siRNA or siRNA for *Fasn* (*siFasn*), and stimulation with LPS (500 ng/ml) for 4 h, followed by incubation with ATP (5 mM) for 30 min.

Supplemental Table 1. Demographics of Brigham and Women's Hospital Registry of Critical Illness Patients.

	Control (N = 21)	Sepsis (N = 29)	P-value
Age, mean (SD)	51.5 (14.5)	61.3 (14.4)	0.02*
Gender N (%) Male Female	 8 (38.1) 13 (61.9)	 15 (51.7) 14 (48.3)	0.40
Race N (%) White Black Hispanic Asian/Pacific Islander	 15 (23.8) 5 (4.8) 1 (71.4) 0 (0)	 24 (82.8) 3 (10.3) 2 (6.9) 0 (0)	0.44
Length of stay, d, median (range)	17 (3, 45)	13 (4, 43)	0.74
In-hospital mortality, N (%) Yes No	 7 (33.3) 14 (66.6)	 2 (6.9) 27 (93.1)	0.03*

*Represents significant differences Control vs other patient groups.
(*P value < 0.05, Wilcoxon Rank Sum test for continuous data and Fisher's Exact test for categorical data).