**Figure S1.** Knockdown of ALDOA reduced accumulation of damaged mitochondria. (A) Immunoblot analysis of ALDOA in J774A.1 cells treated with various glycolytic intermediates (200 μM), pre-incubated with SLO for 5 min. (B) FBP aldolase activity was detected in J774A.1 cells treated with Various glycolytic intermediates (200 μM), pre-incubated with SLO for 5 min. (C) MTT analysis of surviving fraction. Various glycolytic intermediates (200 μM) as indicated were individually added to the 2-h glucose-starved J774A.1 cells pre-incubated with SLO for 5 min. (D) Immunoblot analysis of ALDOA in J774A.1 cells stably expressing *Aldoa* shRNA. (E) Flow cytometry analysis of mitochondrial membrane potential by JC-1 staining in LPS-primed J774A.1 cells stably expressing shRNA against *Aldoa*, stimulated with ATP (5 mM) for 1 h, nigericin (4 μM) for 3 h, MSU (150 μg/ml) for 6 h. (F) Quantitative real-time PCR analysis of mtDNA released from LPS-primed J774A.1 cells treated as above. Data are presented as mean ± SD (n = 3). \*P < 0.05, \*\*P < 0.01.

**Figure S2.** Knockdown of ALDOA induced mitophagy. (A and B) FACS analysis LPS-primed J774A.1 cells stably expressing shRNA against *Aldoa* and mito-Keima, stimulated with or without ATP (5 mM) for 0.5 h and 1 h and nigericin (4 μM) for 1.5 h and 3 h. (C) Lysosomal mito-Keima signals were examined in LPS-primed J774A.1 cells stably expressing shRNA against *Aldoa* by confocal microscopy, stimulated with or without ATP (5 mM) for 1 h and nigericin (4 μM) for 3 h. (D) FACS analysis LPS-primed J774A.1 cells stably expressing shRNA against *Aldoa* and mito-Keima treated with CQ (50 μM), stimulated with nigericin (4 μM) for 3 h. (E and F) Intracellular distribution of PRKN (E) or Poly-Ub (F) and mitochondria (TOMM20) in LPS-primed J774A.1 cells stably expressing *Aldoa* shRNA, stimulated with ATP or nigericin, examined by confocal microscopy. Scale bars: 10 µm. Data are presented as mean ± SD (n = 3).

**Figure S3.** Knockdown of ALDOA suppressed NLRP3 inflammasome activation by triggering PRKN-dependent mitophagy. (A) Immunoblot analysis of ATG5 and ALDOA in J774A.1 cells stably expressing shRNA against *Aldoa* and *Atg5*. (B) Immunoblot analysis of SQSTM1 and ALDOA in J774A.1 cells stably expressing shRNA against *Aldoa* and *Sqstm1*. (C) Immunoblot analysis of PRKN and ALDOA in J774A.1 cells stably expressing shRNA against *Aldoa* and *Prkn*. (D) Flow cytometry analysis (left) and quantification (right) of mitochondrial ROS by MitoSOX staining in LPS-primed J774A.1 cells stably expressing shRNA against *Aldoa* treated with CQ, stimulated with nigericin (4 μM) for 3 h. (E) Flow cytometry analysis (left) and quantification (right) of mitochondrial status in LPS-primed J774A.1 cells stably expressing shRNA against *Aldoa*, treated with CQ, stimulated with nigericin (4 μM) for 3 h. (F) Quantitative real-time PCR analysis of mtDNA released from LPS-primed J774A.1 cells treated with CQ and nigericin (4 μM) for 3 h. (G) Immunoblot analysis of OPTN (left), CALCOCO2 (right) and ALDOA in J774A.1 cells stably expressing shRNA against *Aldoa* and *Optn* or *Calcoco2*. Data are presented as mean ± SD (n = 3). \*\*P < 0.01.

**Figure S4.** Knockdown of ALDOA triggered mitophagy via AMPK. (A) GSEA analyses of regulation of autophagy gene sets in J774A.1 cells stably expressing *Aldoa* shRNA vs non-specific control shRNA. (B) Heatmap of autophagy regulation genes from three independent samples in J774A.1 cells stably expressing *Aldoa* shRNA vs non-specific control shRNA. Gene expression values were colored red for high abundance and blue for low abundance. (C) Immunoblot analysis of PRKAA/AMPKα and ALDOA (top) or quantitative real-time PCR analysis of PRKAA1/AMPKα1 and PRKAA2/AMPKα2 (bottom) in J774A.1 cells stably expressing shRNA against *Aldoa* and *Prkaa*. *\*P* < 0.05, *\*\*P* < 0.01 compared with control group. (D) Immunoblot analysis of p-EIF4EBP1 (Ser65), EIF4EBP1, p-RPS6KB1/S6K1 (Thr389) and RPS6KB1/S6K1 (D) in J774A.1 cells stably expressing shRNA against *Aldoa* and *Prkaa* (top) or shRNA against *Aldoa* treated with compound C (bottom). (E) FACS analysis LPS-primed J774A.1 cells stably expressing mito-Keima and shRNA against *Aldoa* and *Prkaa* (top) or shRNA against *Aldoa* treated with compound C (bottom), stimulated with nigericin (4 μM) for 3 h. (F) Immunoblot analysis of ATP6V1B2, ATP6V0c and ALDOA in ALDOA immunoprecipitates and TCL of J774A.1 cells stably expressing *Aldoa* shRNA and ALDOA WT, D34S, R43A, K147Q, E188Q, or K230A variant cDNA. Data are presented as mean ± SD (n = 3).

**Figure S5.** LYG-202 bound to ALDOA at D34 and R43 residues. (A) CETSA melt curve of β-actin for heat treatment of differentiated THP-1 cells and J774A.1 cells in the absence and in the presence of LYG-202 (4 μM). (B) Drug concentration dependence isometric line of β-actin in the presence of increasing concentrations of LYG-202 in differentiated THP-1 cells and J774A.1 cells. (C) Immunoblot analysis of LAMTOR1 and ALDOA in FLAG immunoprecipitates and TCL of HEK293T cells expressing FLAG-ALDOA WT, D34S, R43A, or D34S/R43A variant cDNA. (D-F) The interaction between LYG-202 and mouse (Mm) ALDOA D34S (D), R43A (E), or D34S/R43A (F) variant was detected by MST. Data are presented as mean ± SD (n = 3).

**Figure S6.** LYG-202 suppressed NLRP3 inflammasome activation. (A) ELISA of TNF in supernatants of LPS-primed differentiated THP-1 cells treated with 4 μM of LYG-202, followed by stimulation with ATP (5 mM) for 1 h, nigericin (4 μM) for 3 h, MSU (150 μg/ml) for 6 h. (B) MTT analysis of surviving fraction in differentiated THP-1 cells, J774A.1 cells and BMDMs treated with 1, 2, 4 μM of LYG-202. (C) ELISA of IL1B in supernatants of LPS-primed WT, *nlrp3-/-* or *casp1-/-* BMDMs that were stimulated with nigericin. (D) ELISA of IL1B in supernatants of LPS-primed differentiated THP-1 cells, J774A.1 cells and BMDMs treated with 1, 2, 4 μM of LYG-202, followed by stimulation with nigericin. (E) CASP1 activity of LPS-primed differentiated THP-1 cells, J774A.1 cells and BMDMs treated with 1, 2, 4 μM of LYG-202, followed by stimulation with nigericin. (F) Immunoblot analysis of IL1B and CASP1 in supernatants (SN) and extracts (Input) of LPS-primed differentiated THP-1 cells, J774A.1 cells and BMDMs treated with 1, 2, 4 μM of LYG-202, followed by stimulation with nigericin. Data are presented as mean ± SD (n = 3). *\*P* < 0.05, *\*\*P* < 0.01 compared with control group; *#P* < 0.05, *##P* < 0.01 compared with nigericin group.

**Figure S7.** LYG-202 reduced accumulation of damaged mitochondria. (A) Flow cytometry analysis (left) and quantification (right) of mitochondrial ROS by MitoSOX staining in LPS-primed differentiated THP-1 cells treated with 4 μM of LYG-202, followed by stimulation with ATP (5 mM) for 1 h, nigericin (4 μM) for 3 h, MSU (150 μg/ml) for 6 h. *\*\*P* < 0.01. (B) Flow cytometry analysis (left) and quantification (right) of mitochondrial status in LPS-primed differentiated THP-1 cells treated as above. Gates represent cells with damaged mitochondria. *\*P* < 0.05, *\*\*P* < 0.01. (C) Flow cytometry analysis of mitochondrial membrane potential by JC-1 staining in LPS-primed differentiated THP-1 cells treated as above. *\*\*P* < 0.01. (D) Quantitative real-time PCR analysis of mtDNA released from LPS-primed differentiated THP-1 cells treated as above. *\*P* < 0.05. (E) Quantification of MitoSOX staining in LPS-primed BMDMs treated with 4 μM of LYG-202, followed by stimulation with ATP, nigericin, or MSU. *\*P* < 0.05, *\*\*P* < 0.01. (F) Quantification of MitoSOX staining in LPS-primedWT, *nlrp3-/-* or *casp1-/-* BMDMs treated with 4 μM of LYG-202, followed by stimulation with nigericin. *\*\*P* < 0.01 compared with control group; *#P* < 0.05, *##P* < 0.01 compared with nigericin group. Data are presented as mean ± SD (n = 3).

**Figure S8.** LYG-202 triggered mitophagy to inhibit NLRP3 inflammasome. (A) FACS analysis LPS-primed J774A.1 cells expressing mito-Keima treated with 4 μM of LYG-202, followed by stimulation with nigericin. (B and C) Intracellular distribution (B) of SQSTM1 and mitochondria (TOMM20) in LPS-primed differentiated THP-1 cells treated with 4 μM of LYG-202, followed by stimulation with nigericin (4 μM) for 3 h, examined by confocal microscopy and (C) quantitated by counting cells with SQSTM1 aggregation on mitochondria. Scale bars: 10 µm. (D) Immunoblot analysis of IL1B and CASP1 in supernatants (SN) and extracts (Input) of LPS-primed differentiated THP-1 cells treated with 4 μM of LYG-202 or 50 μM of CQ, followed by stimulation with nigericin (4 μM) for 3 h. (E) Quantification of MitoSOX staining in LPS-primed differentiated THP-1 cells treated with 4 μM of LYG-202 or 50 μM of CQ, followed by stimulation with nigericin (4 μM) for 3 h. (F) Immunoblot analysis of ATG5 in J774A.1 cells stably expressing shRNA against *Atg5*. (G) Immunoblot analysis of SQSTM1 in J774A.1 cells stably expressing shRNA against *Sqstm1*. (H) Immunoblot analysis of PRKAA/AMPKα in J774A.1 cells stably expressing shRNA against *Prkaa*. Data are presented as mean ± SD (n = 3). *\*P* < 0.05, *\*\*P* < 0.01.