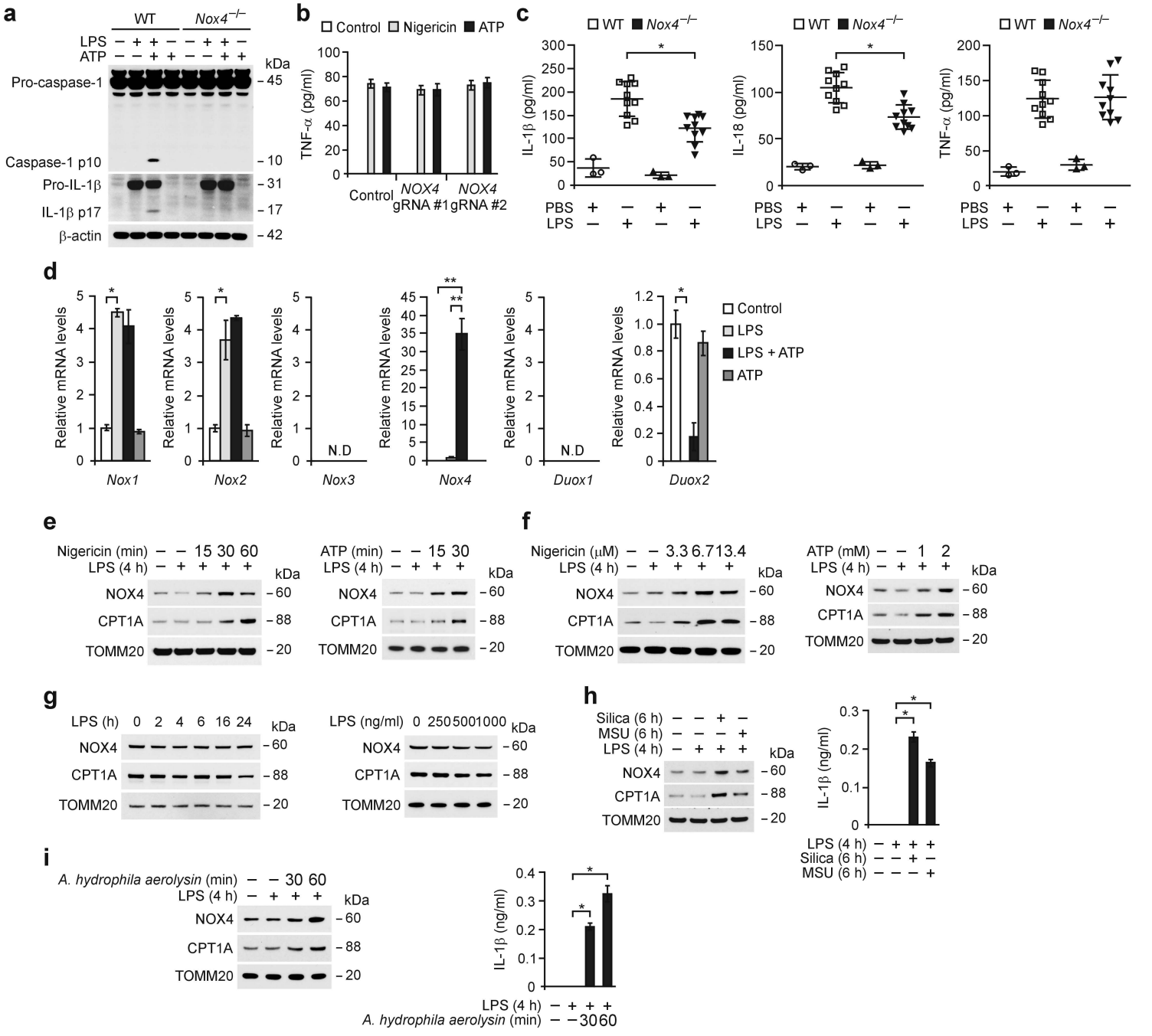
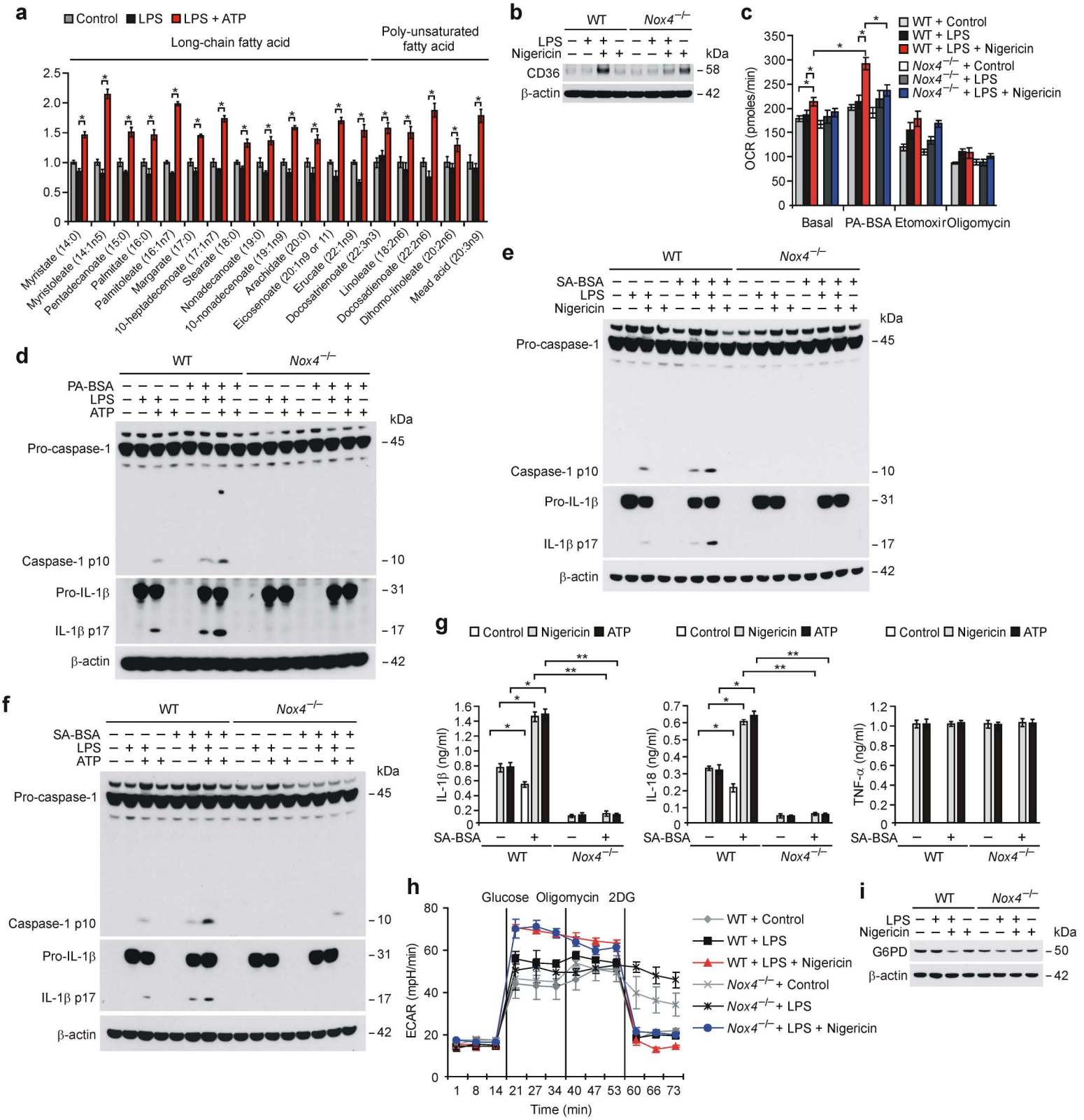
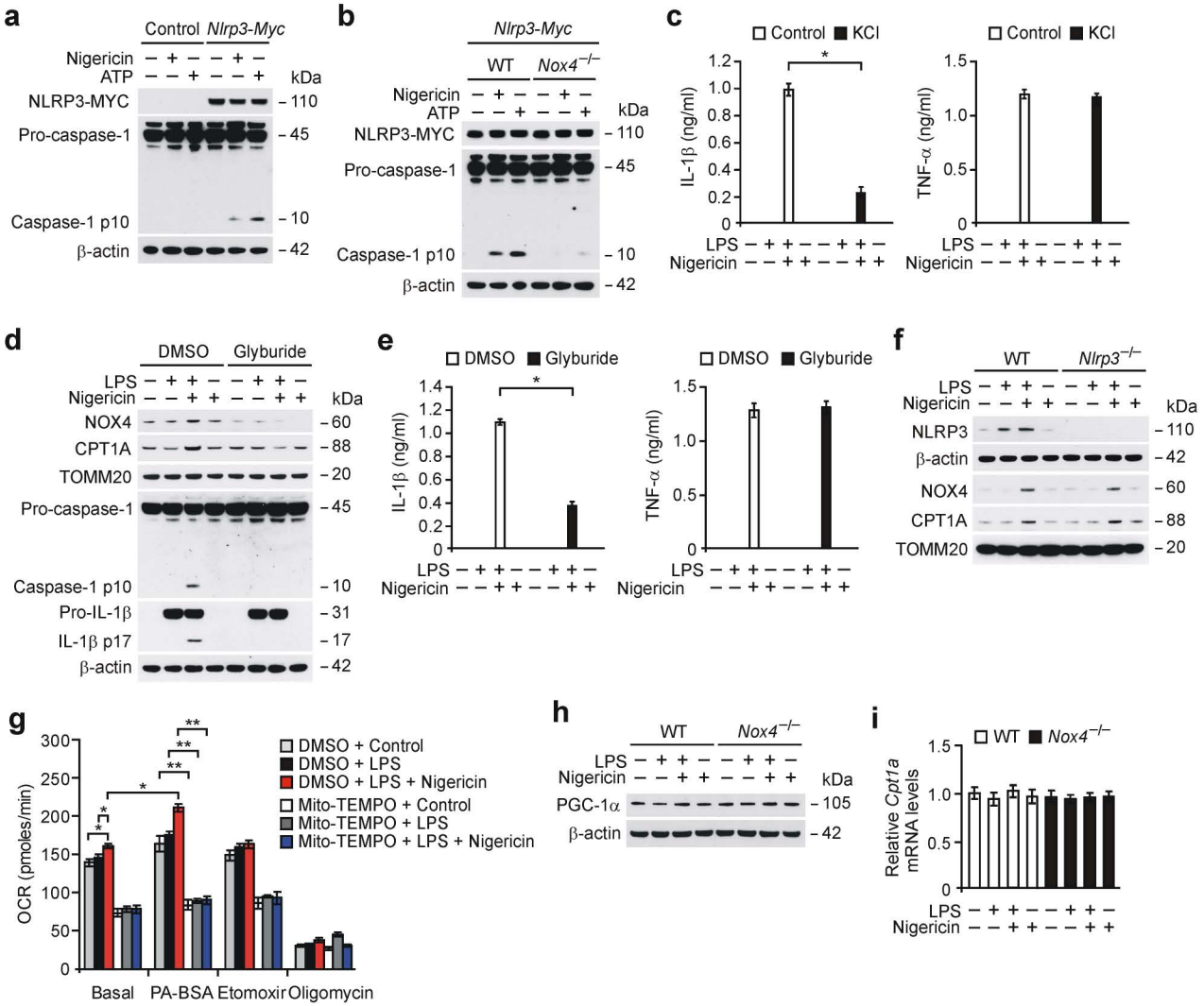


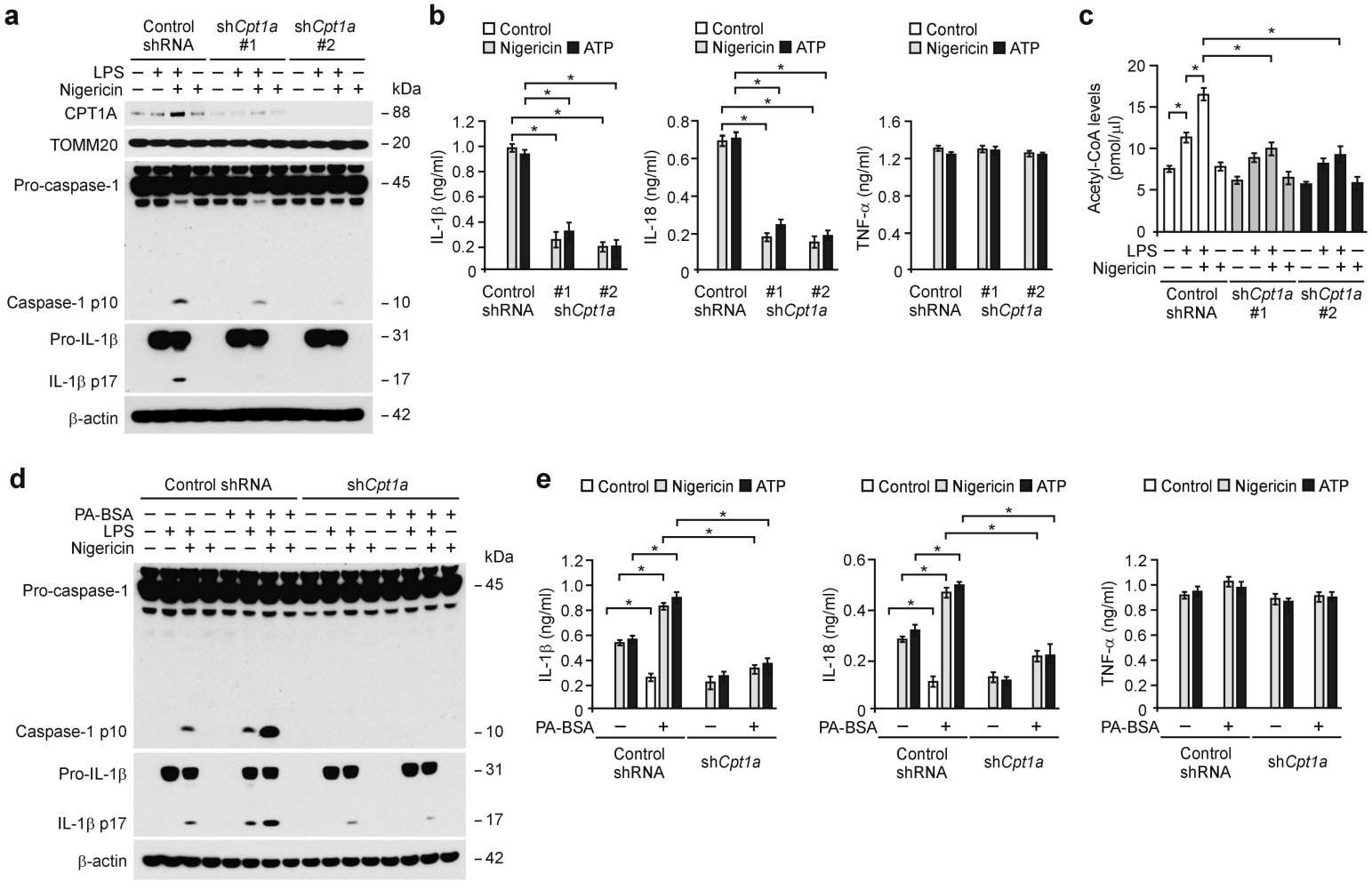
Supplementary figure 1 NOX4 positively regulates NLRP3 inflammasome activation. (**a**) Immunoblot for caspase-1 and IL-1β from LPS-primed WT and *Nox4*$^{-/-}$ BMDMs stimulated with ATP. (**b**) ELISA for TNF-α from primary human macrophages transduced with lentivirus expressing two independent *NOX4*-target gRNAs (*NOX4* gRNA #1 and *NOX4* gRNA #2) or control plasmid (Control), and stimulated with LPS and nigericin or ATP. (**c**) ELISA for IL-1β, IL-18 and TNF-α in plasma of WT and *Nox4*$^{-/-}$ mice after intraperitoneal injection (i.p.) of LPS (10 mg/kg) or PBS for 24 h (PBS, $n = 3$ and LPS, $n = 10$). (**d**) Quantitative PCR for *Nox1*, *Nox2*, *Nox3*, *Nox4*, *Duox1* and *Duox2* gene from WT BMDMs treated with LPS and ATP. Not detected (N.D). (**e**,**f**) Immunoblot for NOX4 and CPT1A in mitochondrial fraction from LPS-primed WT BMDMs stimulated with nigericin or ATP. (**g**) Immunoblot for NOX4 and CPT1A in mitochondrial fraction from WT BMDMs incubated with LPS. (**h**,**i**) Immunoblot for NOX4 and CPT1A in mitochondrial fraction and ELISA for IL-1β from LPS-primed WT BMDMs stimulated with silica (200 μM, 6 h), MSU (200 μM, 6 h) or *A. hydrophila aerolysin* (10 ng/ml, 0, 30, 60 min). β-actin and TOMM20 served as the standard. Data are derived from $n = 6$ (**a**); $n = 5$ (**d**); $n = 8$ (**e**,**f**); $n = 6$ (**g**,**h**); $n = 5$ (**i**); mice and $n = 3$ (**b**) human subjects. All data are mean ± s.d., $^{**}P < 0.01$, $^{*}P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



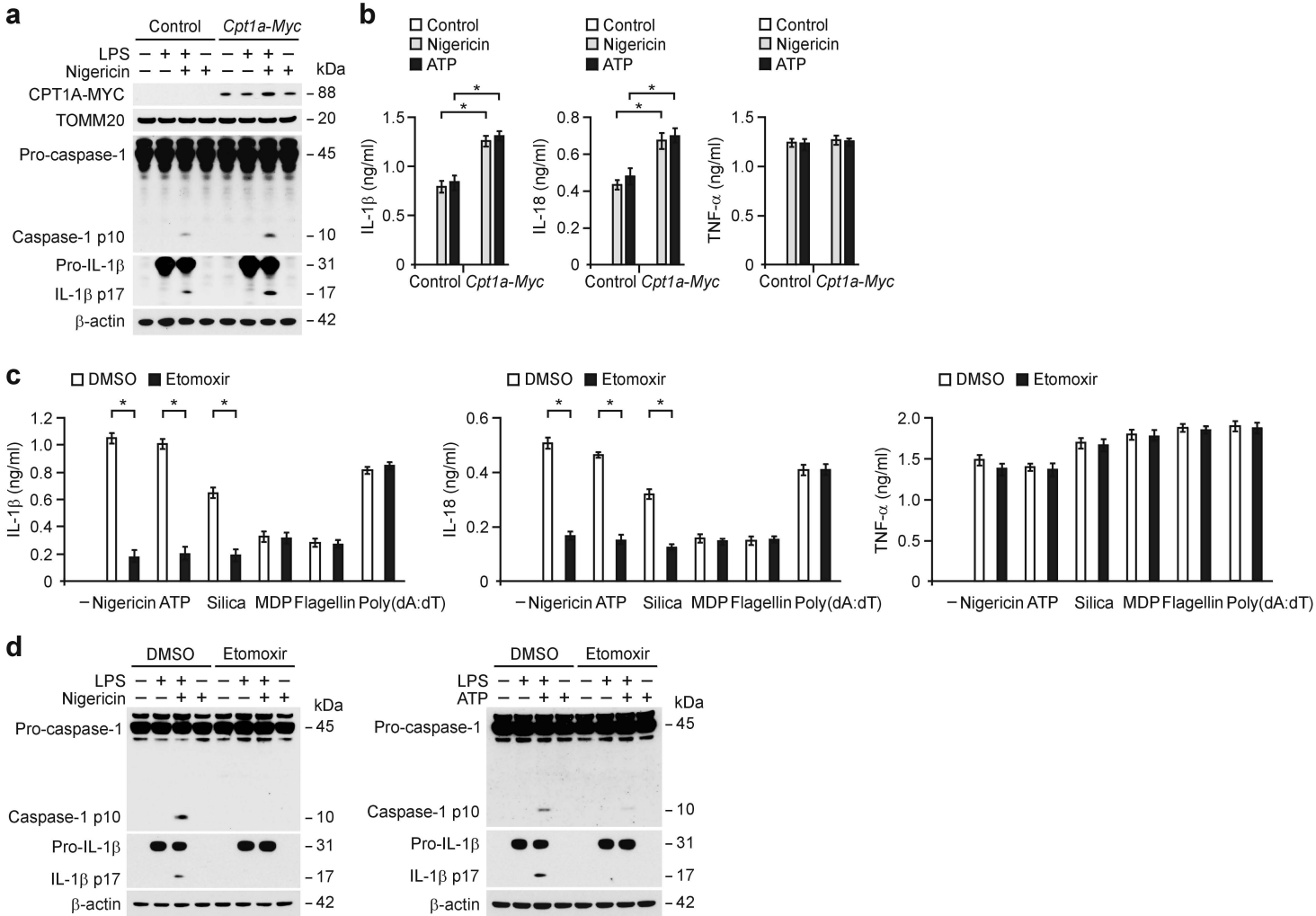
Supplemetary figure 2 NOX4 induces FAO in NLRP3 inflammasome activation. (**a**) Metabolon profiles in WT BMDMs stimulated with LPS and ATP. (**b**) Immunoblot analysis for CD36 in WT and *Nox4*$^{-/-}$ BMDMs stimulated with LPS and nigericin. (**c**) OCR in WT and *Nox4*$^{-/-}$ BMDMs stimulated with LPS and nigericin. (**d**) Immunoblot analysis for caspase-1 and IL-1β in WT and *Nox4*$^{-/-}$ BMDMs incubated with palmitate-BSA (PA-BSA) and ATP after LPS. (**e**,**f**) Immunoblot analysis for caspase-1 and IL-1β and (**g**) ELISA for IL-1β, IL-18 and TNF-α in WT and *Nox4*$^{-/-}$ BMDMs incubated with stearic acid-BSA (SA-BSA) and nigericin or ATP after LPS. (**h**) ECAR in WT and *Nox4*$^{-/-}$ BMDMs stimulated with LPS and nigericin. (**i**) Immunoblot analysis for G6PD in WT and *Nox4*$^{-/-}$ BMDMs stimulated with LPS and nigericin. β-actin served as the standard. Data are derived from $n = 12$ (**a**); $n = 3$ (**b**); $n = 6$ (**c**); $n = 5$ (**d**); $n = 8$ (**e**,**f**); $n = 6$ (**g**); $n = 3$ (**h**,**i**) mice. All data are mean ± s.d., $^{**}P < 0.01$, $^{*}P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



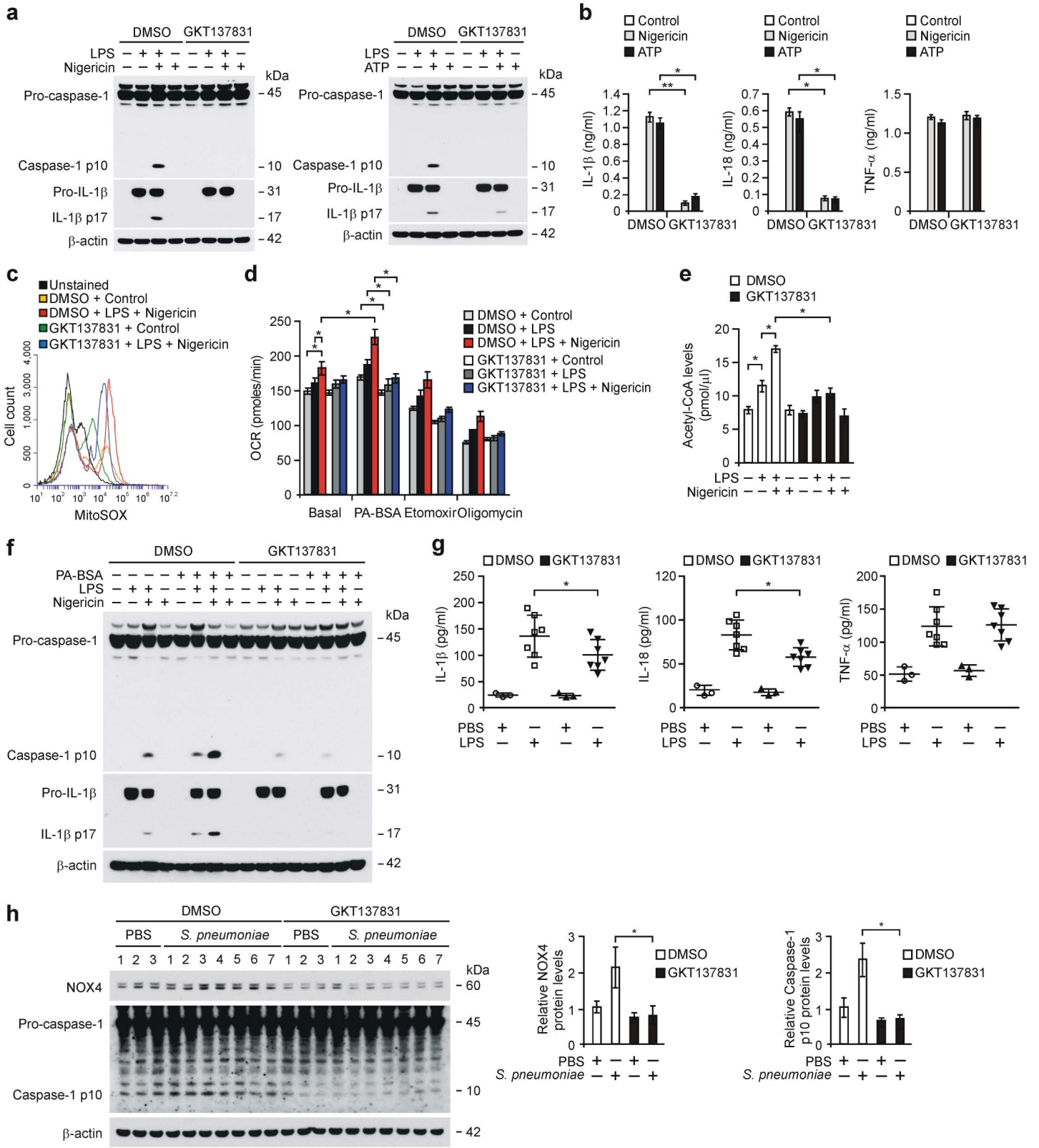
Supplementary figure 3 NOX4-dependent FAO positively regulates NLRP3 inflammasome activation. (**a**) Immunoblot for NLRP3 and caspase-1 from control and *Nlrp3* over-expressed BMDMs stimulated with nigericin or ATP. (**b**) Immunoblot for NLRP3 and caspase-1 from control and *Nlrp3* over-expressed WT and *Nox4*$^{-/-}$ BMDMs stimulated with nigericin or ATP. (**c**) ELISA for IL-1β and TNF-α from WT BMDMs pre-treated with KCl (100 mM, 1 h) before nigericin stimulation after LPS. (**d**) Immunoblot for NOX4 and CPT1A in mitochondrial faction, caspase-1 and IL-1β in cytosolic fraction and (**e**) ELISA for IL-1β and TNF-α from WT BMDMs pre-treated with Glyburide (200 μM, 1 h) before nigericin stimulation after LPS. (**f**) Immunoblot for NLRP3 in cytosolic fraction, NOX4 and CPT1A in mitochondrial fraction from LPS-primed WT and *Nlrp3*$^{-/-}$ BMDMs stimulated with nigericin. (**g**) OCR in WT BMDMs pre-treated with Mito-TEMPO (100 μM, 1 h) before nigericin stimulation after LPS. (**h**) Immunoblot analysis for PGC-1α and (**i**) Quantitative PCR for *Cpt1a* gene expression from LPS-primed WT and *Nox4*$^{-/-}$ BMDMs stimulated with nigericin. β-actin and TOMM20 served as the standard. Data are derived from $n = 3$ (**a**); $n = 5$ (**b**); $n = 6$ (**c**); $n = 3$ (**d,e**); $n = 3$ (**f**); $n = 5$ (**g**); $n = 3$ (**h**,**i**) mice. All data are mean ± s.d., $^{**}P < 0.01$, $^{*}P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



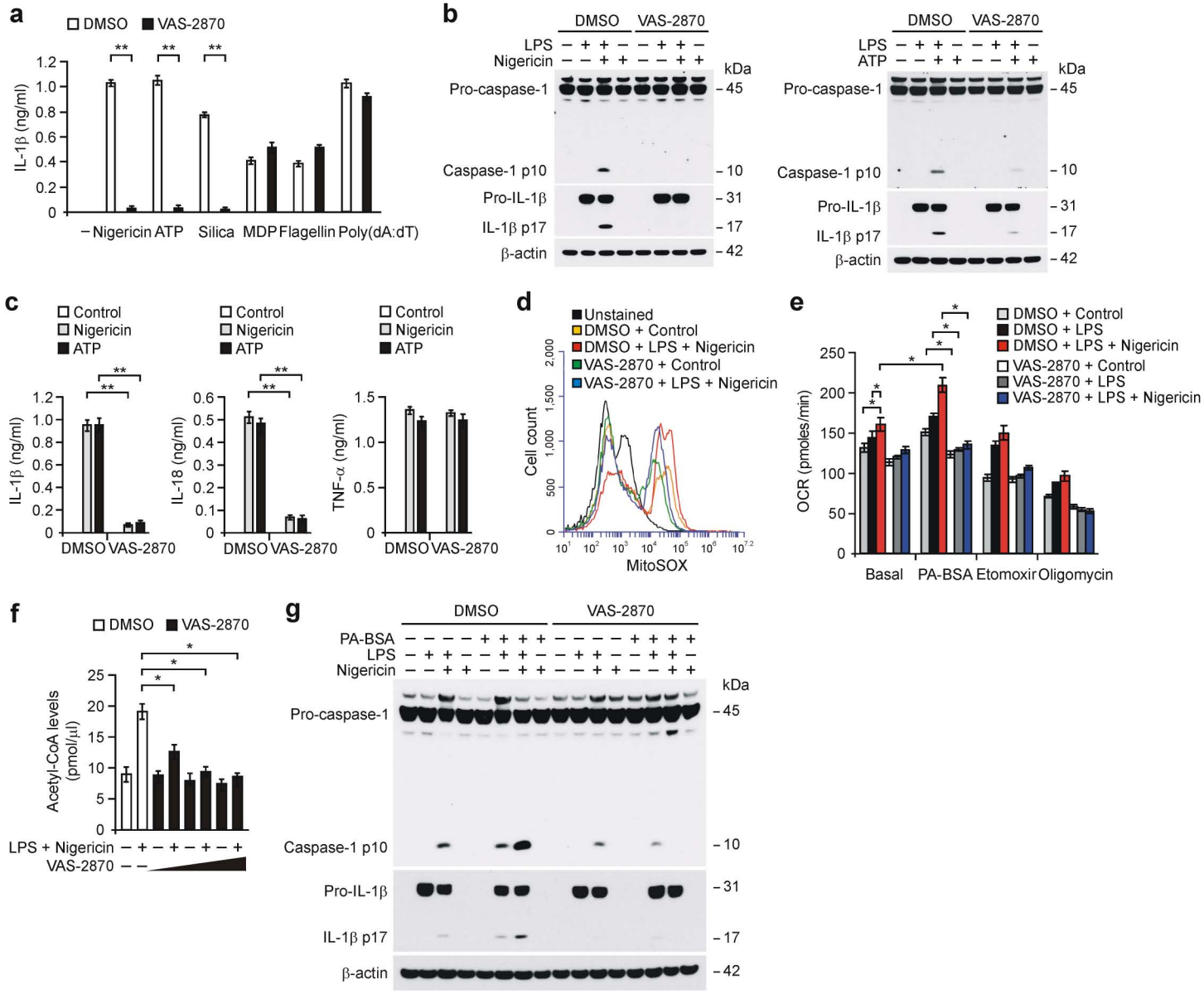
Supplementary figure 4 CPT1A positively regulates NLRP3 inflammasome activation. (**a**) Immunoblot analysis for CPT1A in mitochondrial fraction, caspase-1 and IL-1β cytosolic fraction and (**b**) ELISA for IL-1β, IL-18 and TNF-α and (**c**) Intracellular acetyl-CoA levels from WT peritoneal macrophages transduced with lentivirus expressing non-target shRNA (Control shRNA) or two independent shRNAs for *Cpt1a* (sh*Cpt1a*#1 and sh*Cpt1a*#2), and stimulated with LPS and nigericin or ATP. (**d**) Immunoblot analysis for caspase-1 and IL-1β and (**e**) ELISA for IL-1β, IL-18 and TNF-α from WT peritoneal macrophages transduced with lentivirus expressing non-target shRNA (Control shRNA) or shRNAs for *Cpt1a* (sh*Cpt1a*), and incubated with palmitate-BSA (PA-BSA) and nigericin after LPS. β-actin and TOMM20 served as the standard. Data are derived from $n = 10$ (**a**); $n = 6$ (**b**,**c**); $n = 10$ (**d**); $n = 6$ (**e**) mice. All data are mean ± s.d., $^*P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



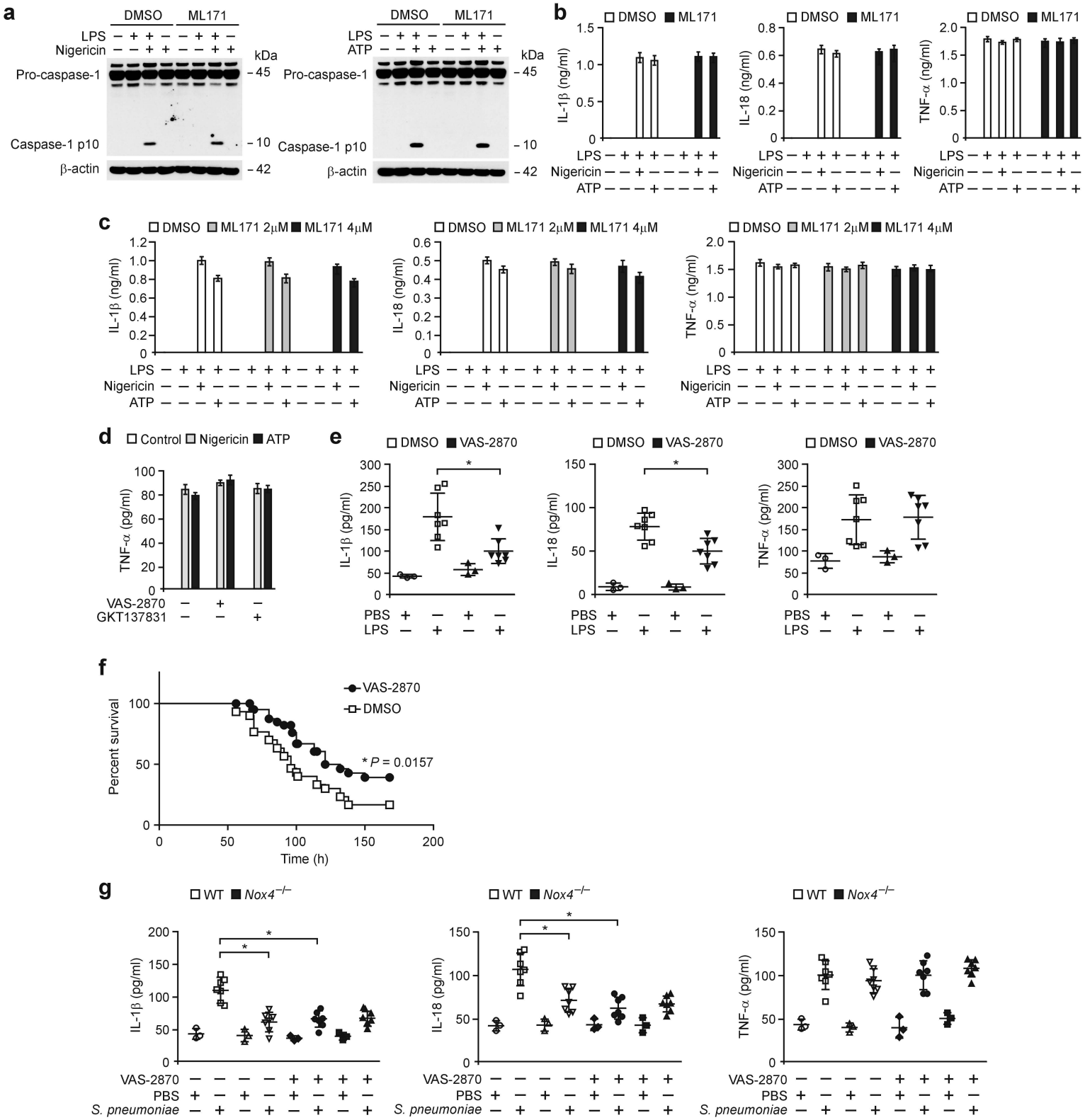
Supplementary figure 5 Inhibition of CPT1A suppresses NLRP3 inflammasome activation. (**a**) Immunoblot analysis for CPT1A in mitochondrial fraction, caspase-1 and IL-1β in cytosolic fraction and (**b**) ELISA for IL-1β, IL-18 and TNF-α from control and *Cpt1a* over-expressed WT BMDMs stimulated with LPS and nigericin. (**c**) ELISA for IL-1β, IL-18 and TNF-α in WT BMDMs pre-treated with etomoxir (200 μM, 2 h) or DMSO before nigericin, ATP, silica, MDP, flagellin or poly(dA:dT) stimulation after LPS. (**d**) Immunoblot analysis for caspase-1 and IL-1β from WT BMDMs pre-treated with etomoxir or DMSO before nigericin or ATP stimulation after LPS. β-actin and TOMM20 served as the standard. Data are derived from $n = 6$ (**a**,**b**); $n = 5$ (**c**); $n = 6$ (**d**) mice. All data are mean ± s.d., $^*P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



Supplementary figure 6 GKT137831 suppresses NLRP3 inflammasome activation. (**a**) Immunoblot for caspase-1 and IL-1β and (**b**) ELISA for IL-1β, IL-18 and TNF-α from WT BMDMs pre-treated with GKT137831 (20 μM, 2 h) or DMSO before nigericin or ATP stimulation after LPS. (**c**) Flow cytometry analysis of WT peritoneal macrophages stained with MitoSOX and treated with GKT137831 (20 μM, 1h) before LPS and nigericin stimulation. (**d**) OCR in WT BMDMs pre-treated with GKT137831 (20 μM, 2 h) before nigericin stimulation after LPS. (**e**) Intracellular acetyl-CoA levels in WT BMDMs pre-treated with GKT137831 (20 μM, 1 h) before LPS and nigericin stimulation. (**f**) Immunoblot for caspase-1 and IL-1β from WT BMDMs pre-treated with GKT137831 (20 μM, 2 h) before palmitate-BSA (PA-BSA) and nigericin incubation after LPS. (**g**) ELISA for IL-1β, IL-18 and TNF-α in plasma of WT mice after i.p. of GKT137831 (20 mg/kg, 3 h) then i.p. of LPS (10 mg/kg) or PBS for 24 h (PBS, $n = 3$ and LPS, $n = 7$). (**h**) Immunoblot for NOX4 and caspase-1 in lung tissues from WT mice after i.p. of GKT137831, then infection of *S. pneumoniae* or PBS for 24 h. β-actin served as the standard. Data are derived from $n = 6$ (**a**,**b**); $n = 3$ (**c**); $n = 6$ (**d**); $n = 3$ (**e**); $n = 4$ (**f**) mice. All data are mean ± s.d., $^{**}P < 0.01$, $^{*}P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



Supplementary figure 7 VAS-2870 suppresses NLRP3 inflammasome activation. (**a**) ELISA for IL-1β in WT peritoneal macrophages pre-treated with VAS-2870 (1 μM, 2 h) before nigericin, ATP, silica, MDP, flagellin or poly(dA:dT) stimulation after LPS. (**b**) Immunoblot analysis for caspase-1 and IL-1β from WT BMDMs pre-treated with VAS-2870 before nigericin or ATP stimulation after LPS. (**c**) ELISA for IL-1β, IL-18 and TNF-α from WT BMDMs pre-treated with VAS-2870 before nigericin or ATP stimulation after LPS. (**d**) Flow cytometry analysis of WT peritoneal macrophages stained with MitoSOX and then treated with VAS-2870 before LPS and nigericin stimulation. (**e**) OCR in WT BMDMs pre-treated with VAS-2870 (0.5 μM, 2 h) before nigericin stimulation after LPS. (**f**) Intracellular acetyl-CoA levels in WT BMDMs pre-treated with VAS-2870 (0, 0.5, 1, 2 μM, 2 h) before nigericin stimulation after LPS. (**g**) Immunoblot analysis for caspase-1 and IL-1β from WT BMDMs pre-treated with VAS-2870 (1 μM, 2 h) before palmitate-BSA (PA-BSA) and nigericin incubation after LPS. β-actin served as the standard. Data are derived from $n = 10$ (**a**); $n = 5$ (**b**,**c**); $n = 3$ (**d**); $n = 3$ (**e**); $n = 3$ (**f**,**g**) mice. All data are mean ± s.d., $^{**}P < 0.01$, $^{*}P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.



Supplementary figure 8 Inhibition of NOX4 suppresses NLRP3 inflammasome activation. (**a**) Immunoblot for caspase-1 and (**b**) ELISA for IL-1β, IL-18 and TNF-α from WT peritoneal macrophages pre-treated with ML171 (1 μM, 2 h) or DMSO before nigericin or ATP stimulation after LPS. (**c**) ELISA for IL-1β, IL-18 and TNF-α from WT peritoneal macrophages pre-treated with ML171 (2 or 4 μM, 2 h) for before nigericin or ATP stimulation after LPS. (**d**) ELISA for TNF-α from primary human macrophages pre-treated with GKT137831 (20 μM, 1 h), VAS-2870 (20 μM, 1 h) or DMSO before nigericin or ATP stimulation after LPS. (**e**) ELISA for IL-1β, IL-18 and TNF-α in plasma from WT mice after intraperitoneal injection (i.p.) of VAS-2870 (10 mg/kg, 6 h) or DMSO, then i.p. of LPS (10 mg/kg, 24 h) or PBS (PBS, $n = 3$ and LPS, $n = 7$). (**f**) Survival curve was determined from WT mice treated with VAS-2870 (10 mg/kg) or DMSO by i.p. at 12 h after *S. pneumoniae* infection (Control, $n = 30$ and VAS-2870, $n = 30$, $P = 0.0157$ by log-rank test). (**g**) ELISA for IL-1β, IL-18 and TNF-α in lung tissues from WT and *Nox4*$^{-/-}$ mice after i.p. of VAS-2870 (10 mg/kg, 3 h) or DMSO, then infection of *S. pneumoniae* or PBS for 24 h (PBS, $n = 3$ and *S. pneumoniae*, $n = 7$). β-actin served as the standard. Data are derived from $n = 3$ (**a**,**b**); $n = 5$ (**c**) mice; $n = 3$ (**d**) human subjects. All data are mean ± s.d., $^*P < 0.05$ by ANOVA. Data are representative of three independent experiemnts and each carried out in triplicate.