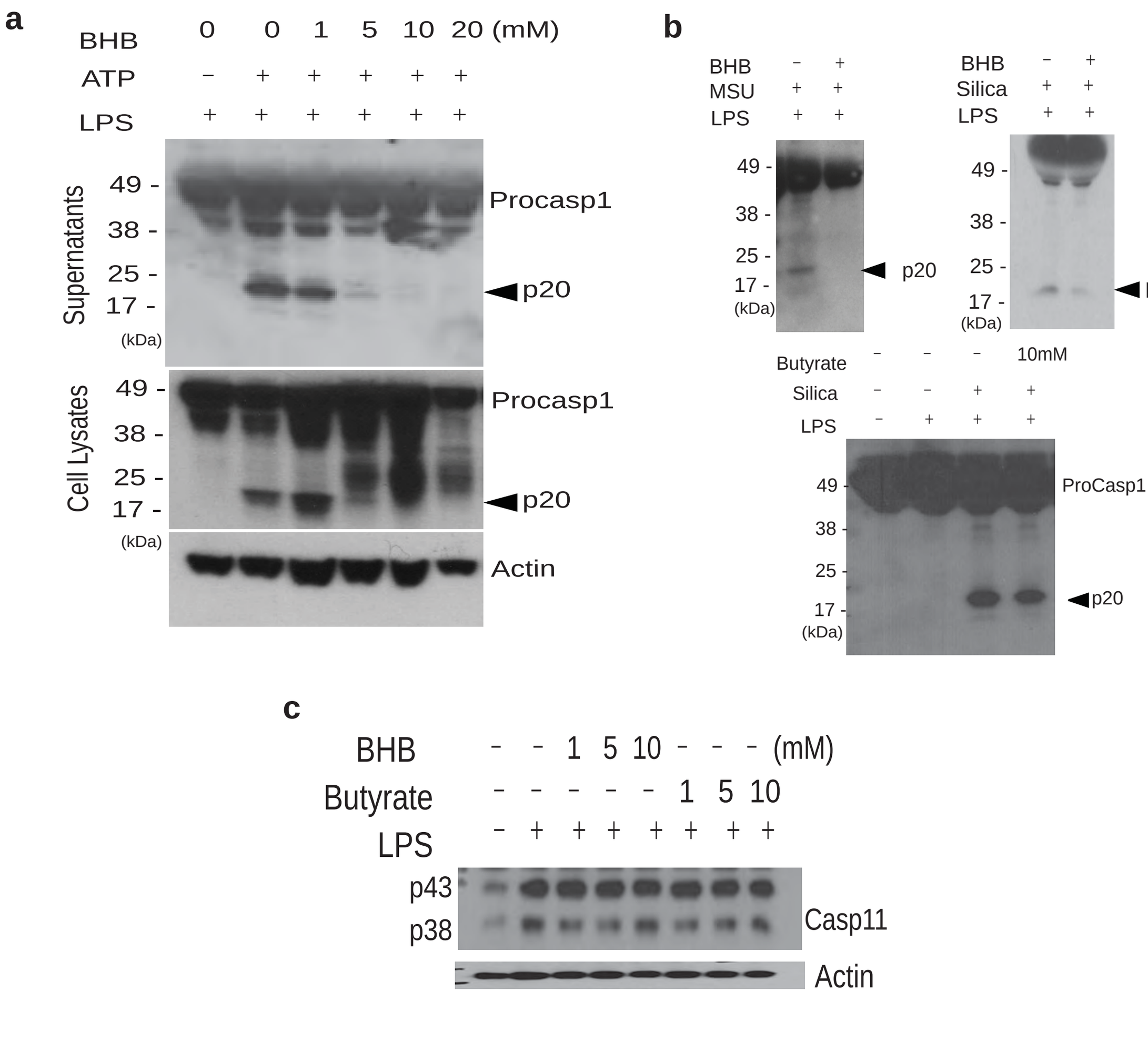
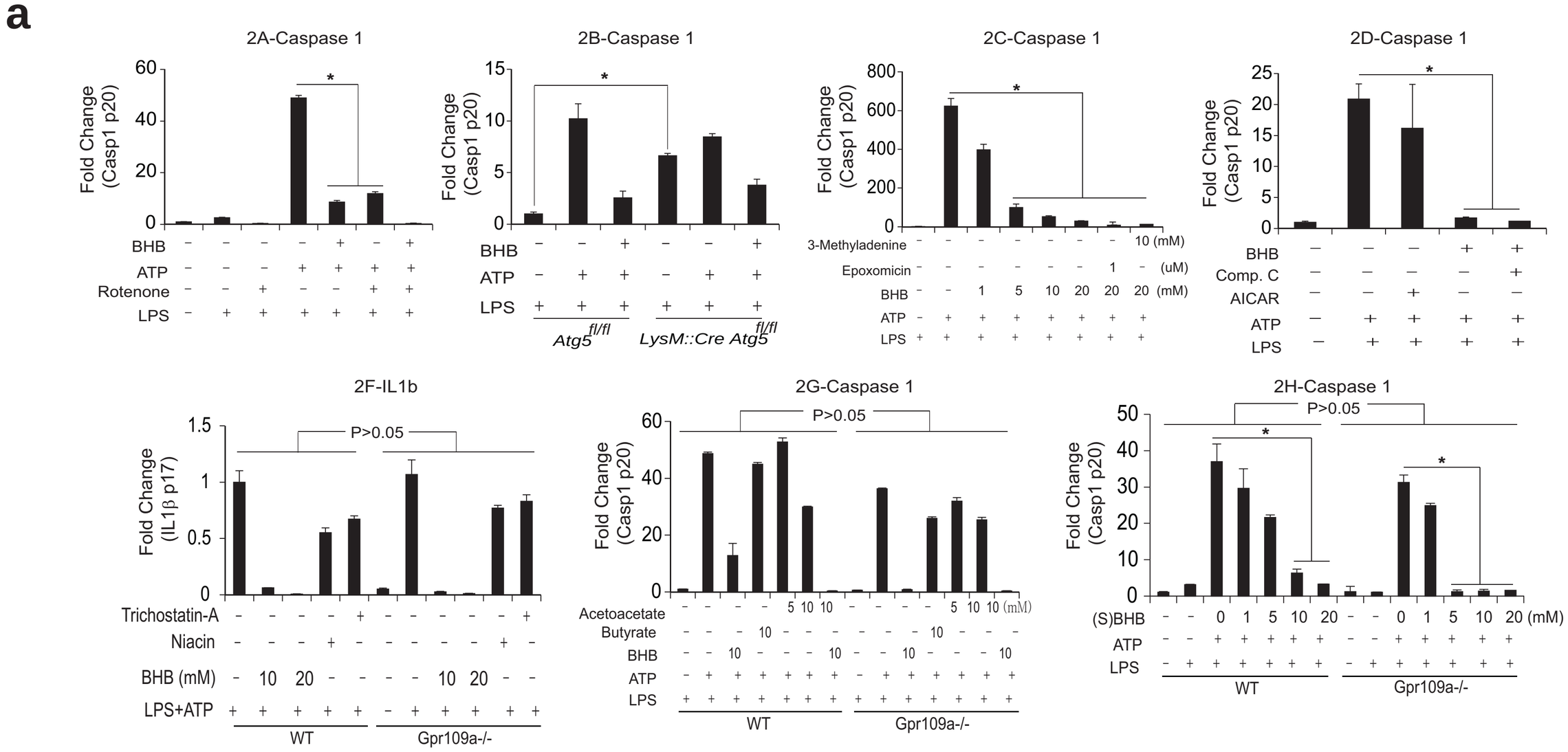


Supplementary Figure 1 Effect of BHB on inflammasome activation

(a) Western blot analysis of caspase1 (active subunit p20) in supernatant and cell lysates of BMDMs primed with LPS for 4 hours and stimulated with ATP for 1 hour in presence of various concentration of D-BHB.
(b) Western blot analysis of caspase1 in BMDMs primed with LPS for 4 hours and stimulated with MSU, Silica for 1 hour in presence of various D-BHB and butyrate (10mM). These experiments were repeated more than 12 times with different combination of treatments.
(c) The immunoblot analysis of caspase-11 in BMDMs treated with LPS for 4h and incubated with BHB and butyrate for 1h.



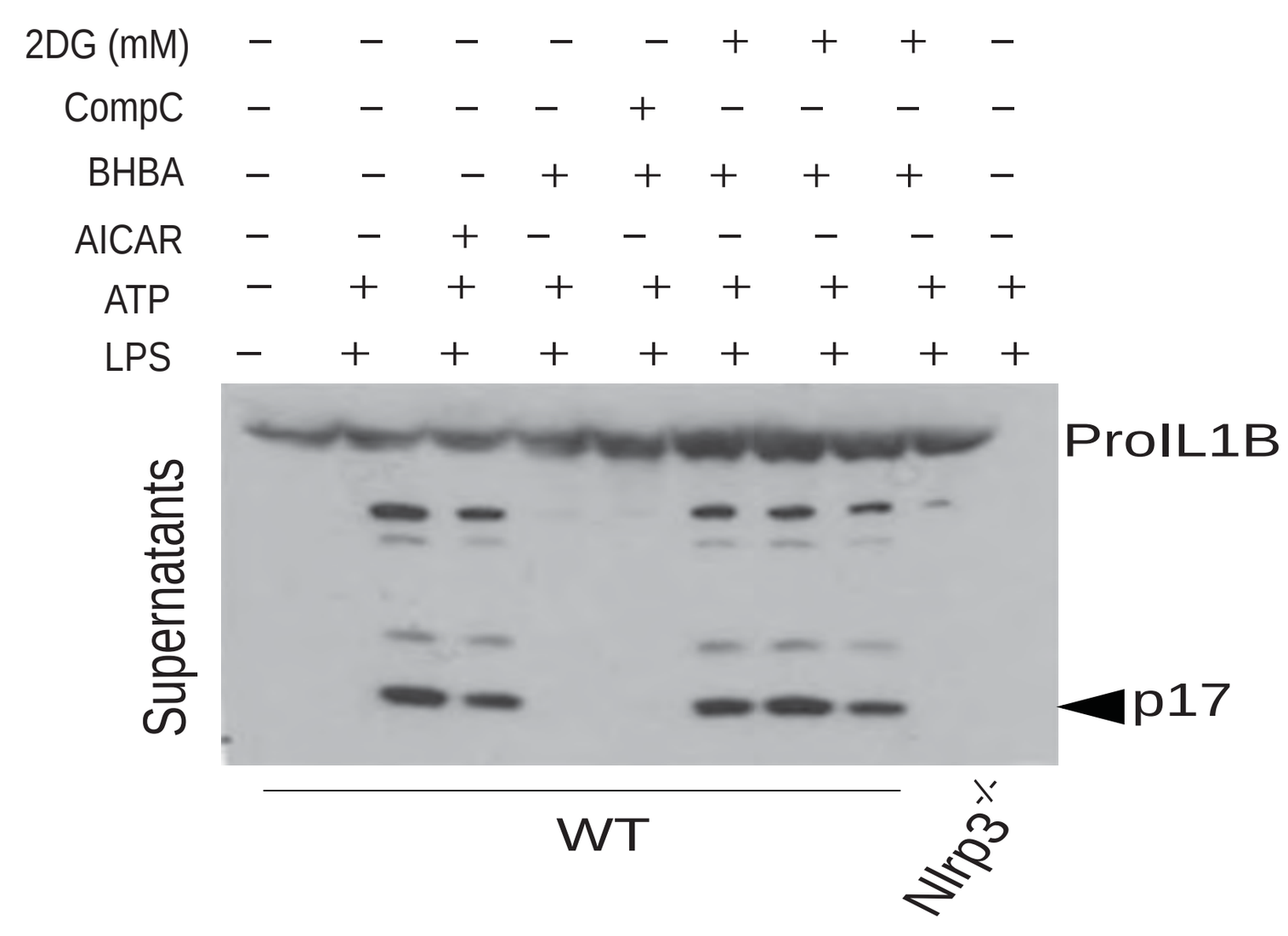
Supplementary Figure 2 BHB does not require ROS and AMPK to block NLRP3.

(a) All bar graphs represent quantitation of p20 caspase1 and p17 band intensity as fold change by normalizing to inactive p48 procaspase-1 and proIL1β. Data are expressed as mean ± S.E.M (*$p<0.05$) from cells derived from six (a) 4 (b) ten (c, d, e) and four (f-h) mice with each independent experiment each carried out in triplicate.

(b) Western blot analysis of caspase1 in BMDMs primed with LPS for 4 hours and stimulated with hydrogen peroxide alone and with ATP for 1 hour.

(c) Caspase-1 western blot in LPS primed BMDM stimulated with ATP and BHB (10mM) in presence of glycolytic inhibitor 2-Deoxyglucose (1mM), AMPK activator (AICAR, 2mM) and AMPK antagonist Compound C (25 μM). The experiments were repeated thrice.

a



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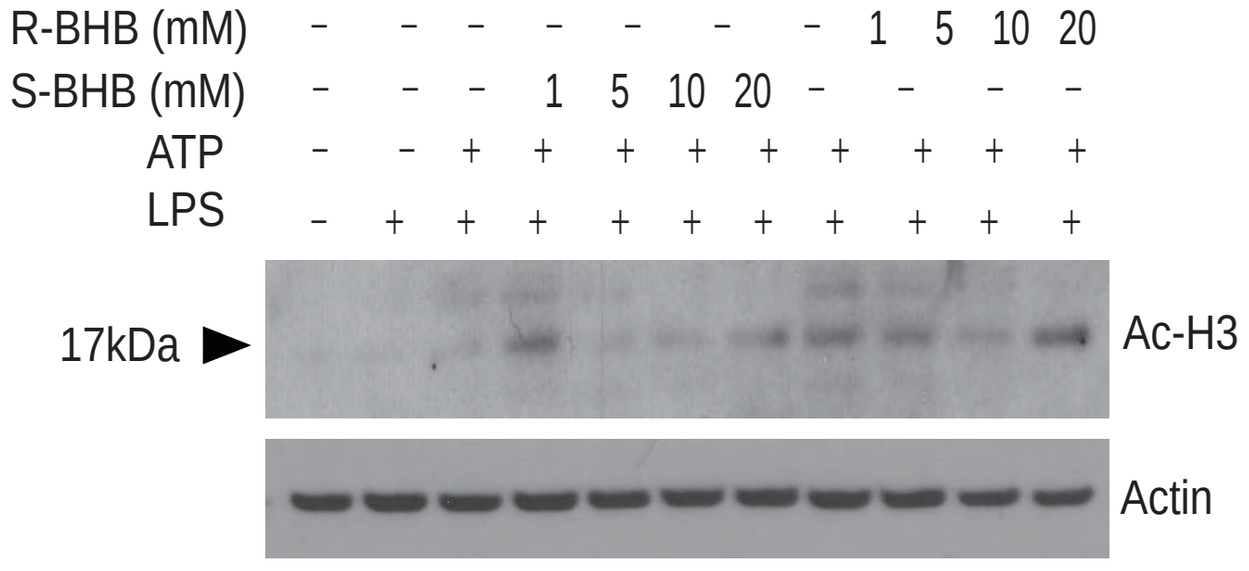


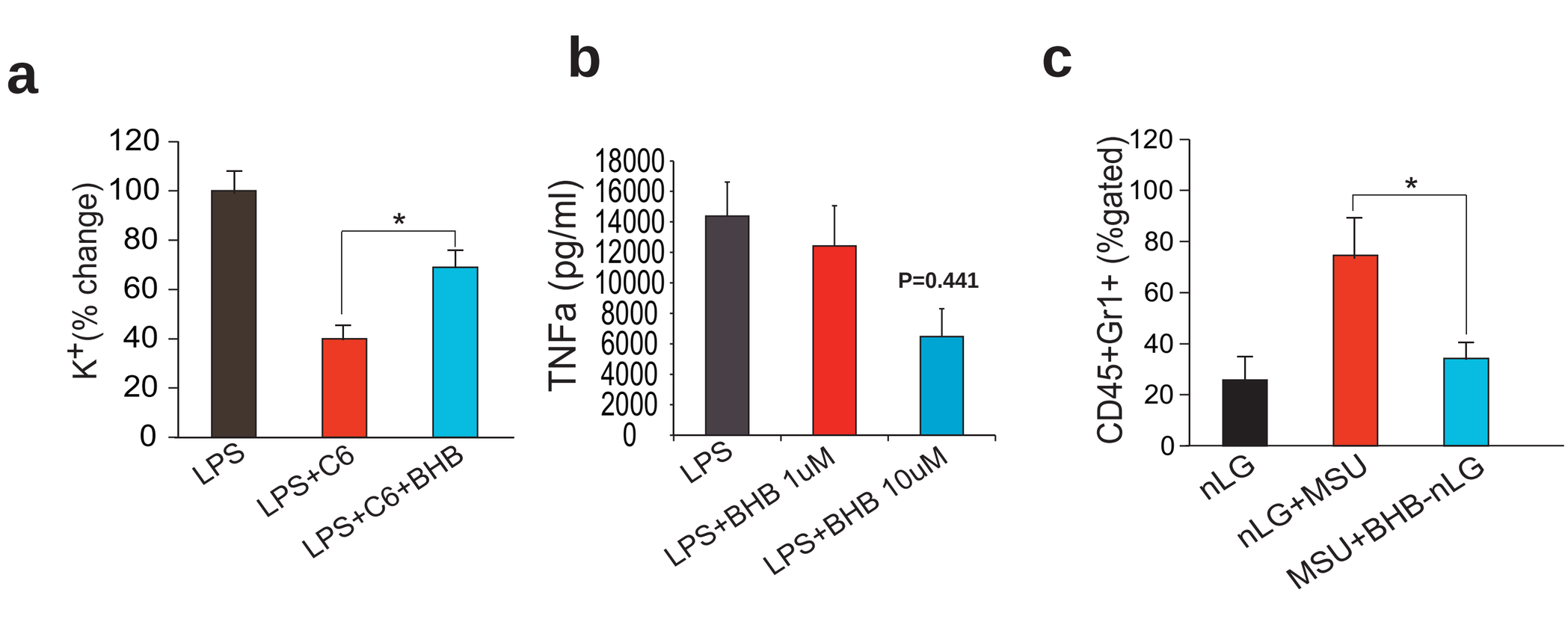
Supplementary Figure 3 BHB induces H3 acetylation and does not require glycolysis to block NLRP3.

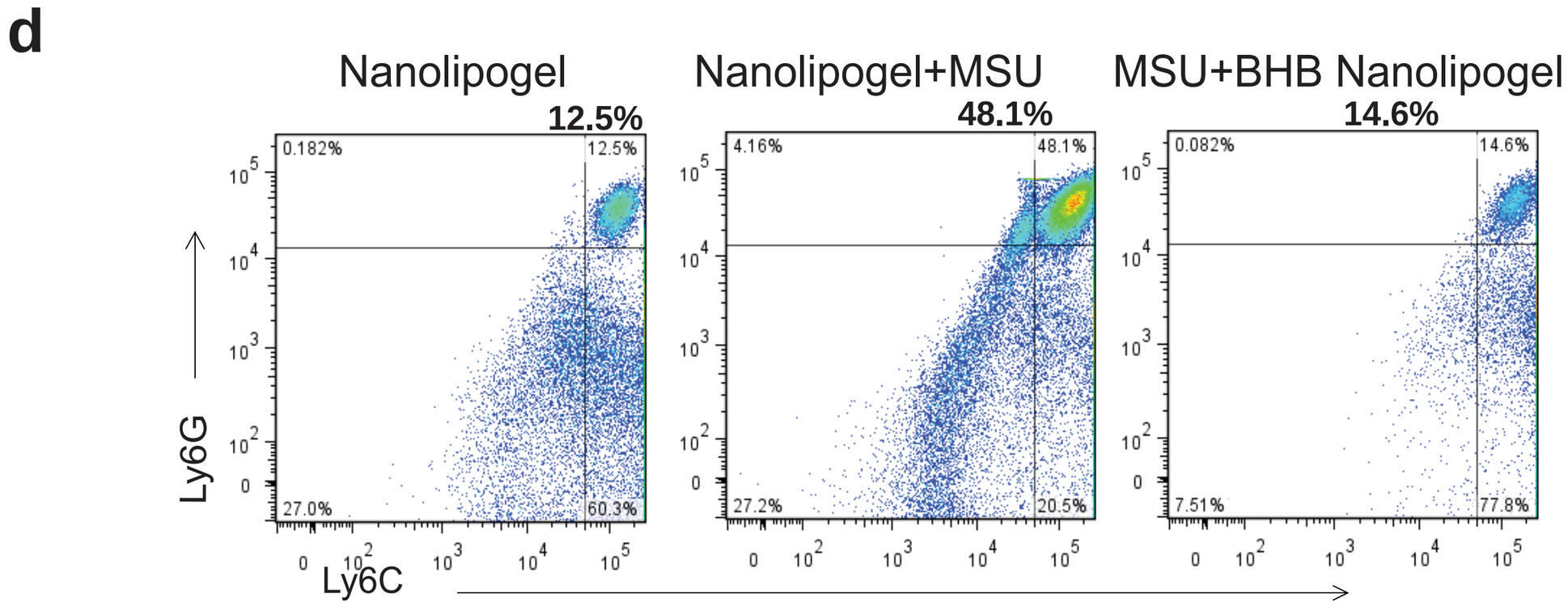
(a,b) Western blot analysis of caspase1 in BMDMs primed with LPS for 4 hours and stimulated with 2DG (0.5, 1, 5 mM), AICAR and Compound C and BHB for 1h on indicated combinations. LPS primed BMDMs were treated with ATP and S or D-BHB for 1h and H3 acytelation was evaluated by western blot. The experiments were repeated at least thrice.

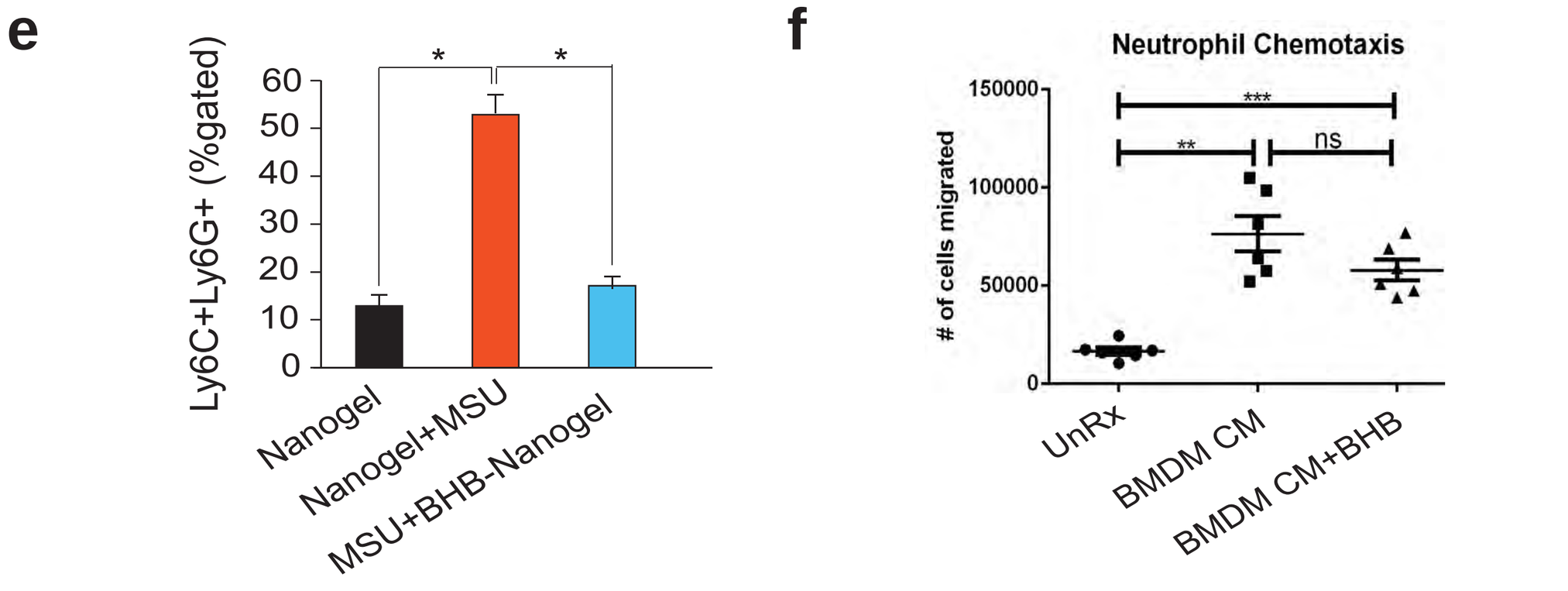
Supplementary Figure 4 Macrophages express ketogenic and ketolytic genes but BHB does not require entry in TCA cycle to block NLRP3.

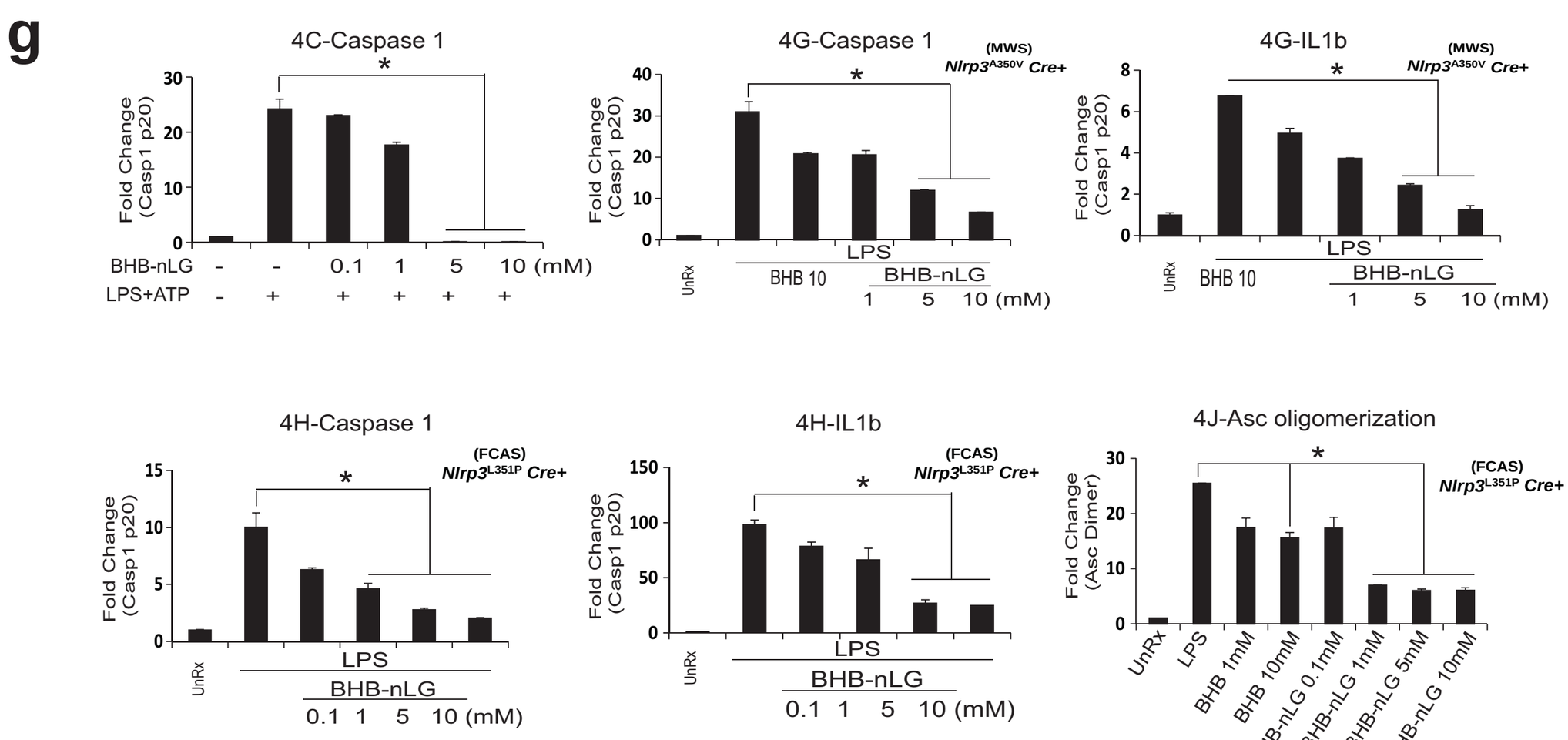
(a) Ketolytic pathway of generation of acetyl coA.
(b) The differential mRNA expression of ketolytic and ketogenic enzymes in M1 and M2 polarized macrophages. Data are expressed as mean ± S.E.M (*p<0.05). The BMDMs from derived from 6 mice were used and experiment was repeated thrice.
(c) Western blot analysis of SCOT and HMGCL expression in BMDMs in response to inflammasome activators and butyrate and BHB.
(d) The caspase-1 activation in LPS primed BMDMs incubated in presence of TCA cycle entry inhibitor AOA (1mM) for 1 hour in presence or absence of ATP and BHB.
(e) The effect of enantiomer, L or (S)- βhydroxybutyrate on Nlrp3 induced caspase-1 activation in BMDMs.
(f) All bar graphs represent quantitation of p20 caspase1, p17 and SCOT band intensity as fold change by normalizing to inactive p48 procaspase-1,proIL1β and actin. Data are expressed as mean ± S.E.M (*p<0.05) from cells derived from five (3a) six (3b,c), mice with each independent experiment carried out in triplicate.

Youm *et al* **Supplementary Fig.4**



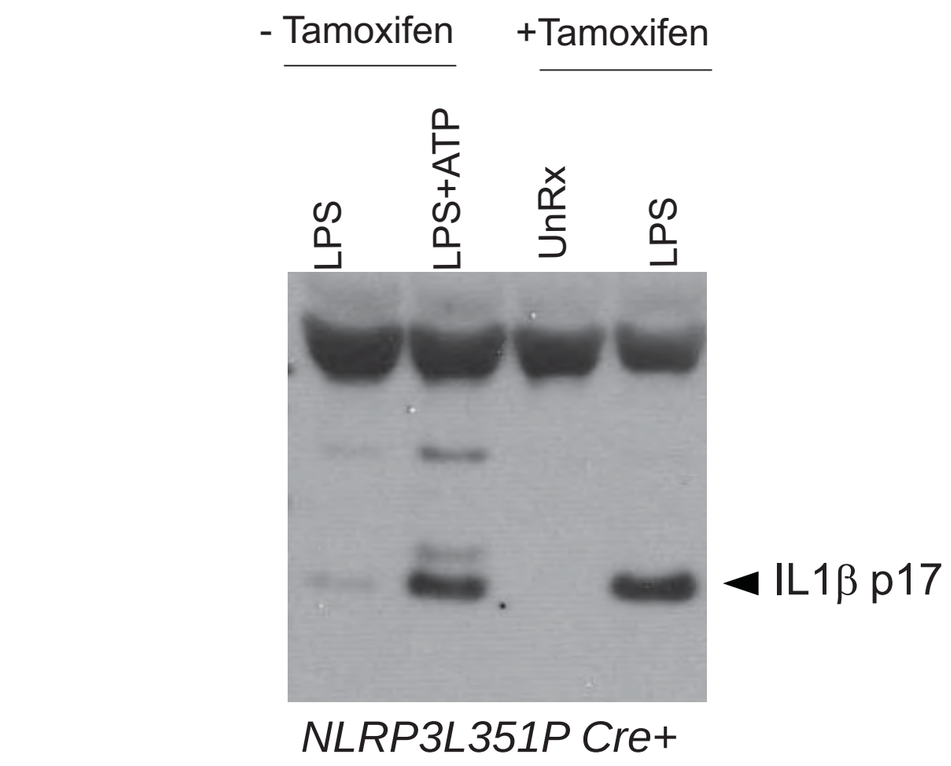


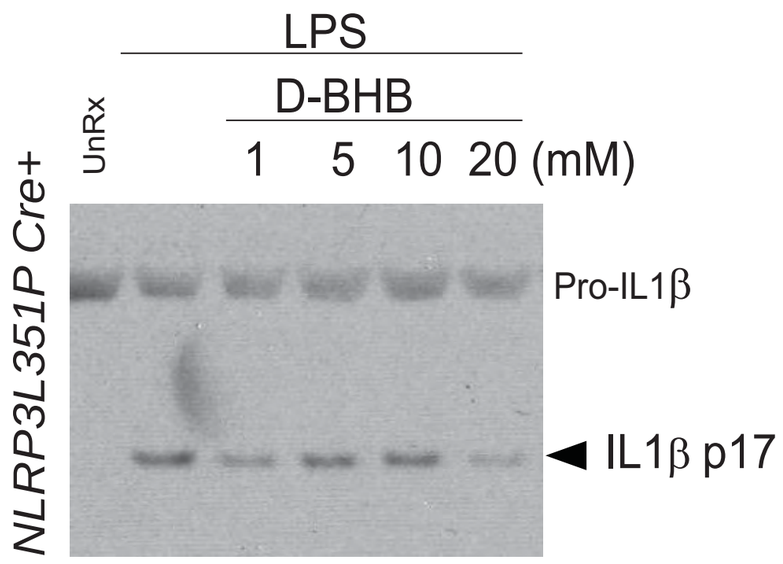


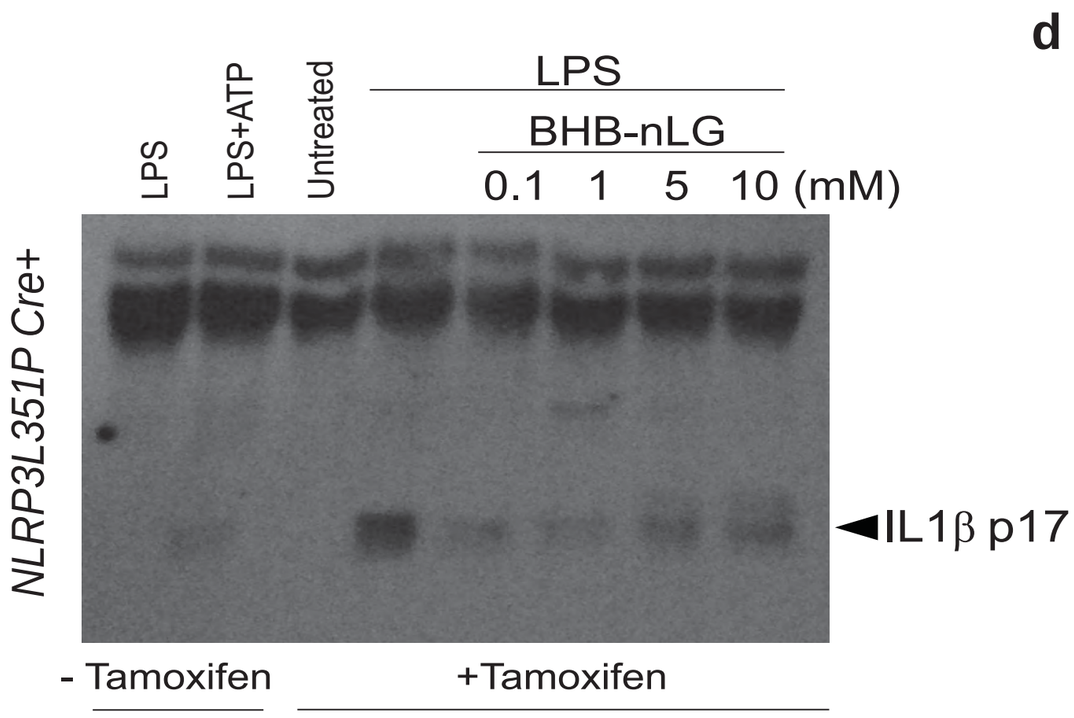


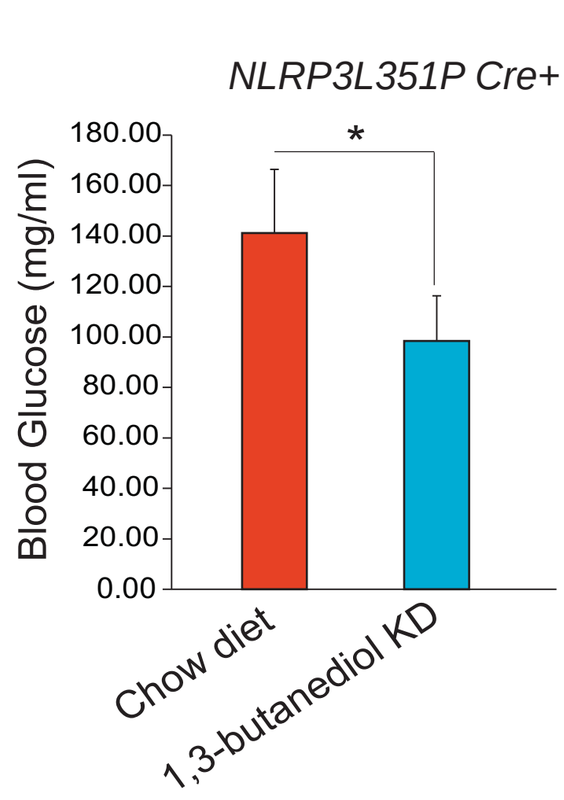
Supplementary Figure 5 BHB nanolipogels block inflammation.

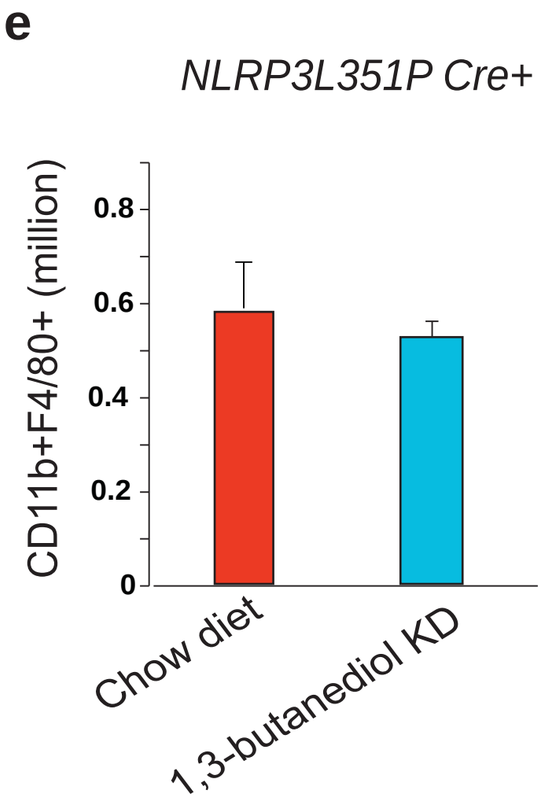
(a) The intracellular potassium levels in LPS primed BMDMs treated with C6 ceramide and BHB for 1h. The BMDMs from total of 5 mice were used with three technical repeats.
(b) Human monocytes were stimulated with vehicle or LPS (1μg/mL) for 4h in presence of increasing concentrations of BHB. The TNFα was measured in supernatants using ELISA.
(c) Quantitation of peritoneal cells stained with CD45 and Gr1 from mice treated intraperitoneally with MSU and BHB-complexed nLGs. (n =6/group).
(d,e,f) Peritoneal cells were stained with Ly6C and Ly6G and double positive neutrophil infiltration in mice 4h after treatment with MSU and BHB-nLGs (n = 4/group).
(g) All bar graphs represent quantitation of p20 caspase1 and p17 band intensity as fold change by normalizing to inactive p48 procaspase-1 and proIL1β. Each experiment was repeated with cells derived from 2 femurs of at least 4-6 mice and repeated thrice. The data are presented as mean ±SEM. *p<0.05.











Supplementary Figure 6 BHB inhibits NLRP3 inflamasome in FCAS mice.

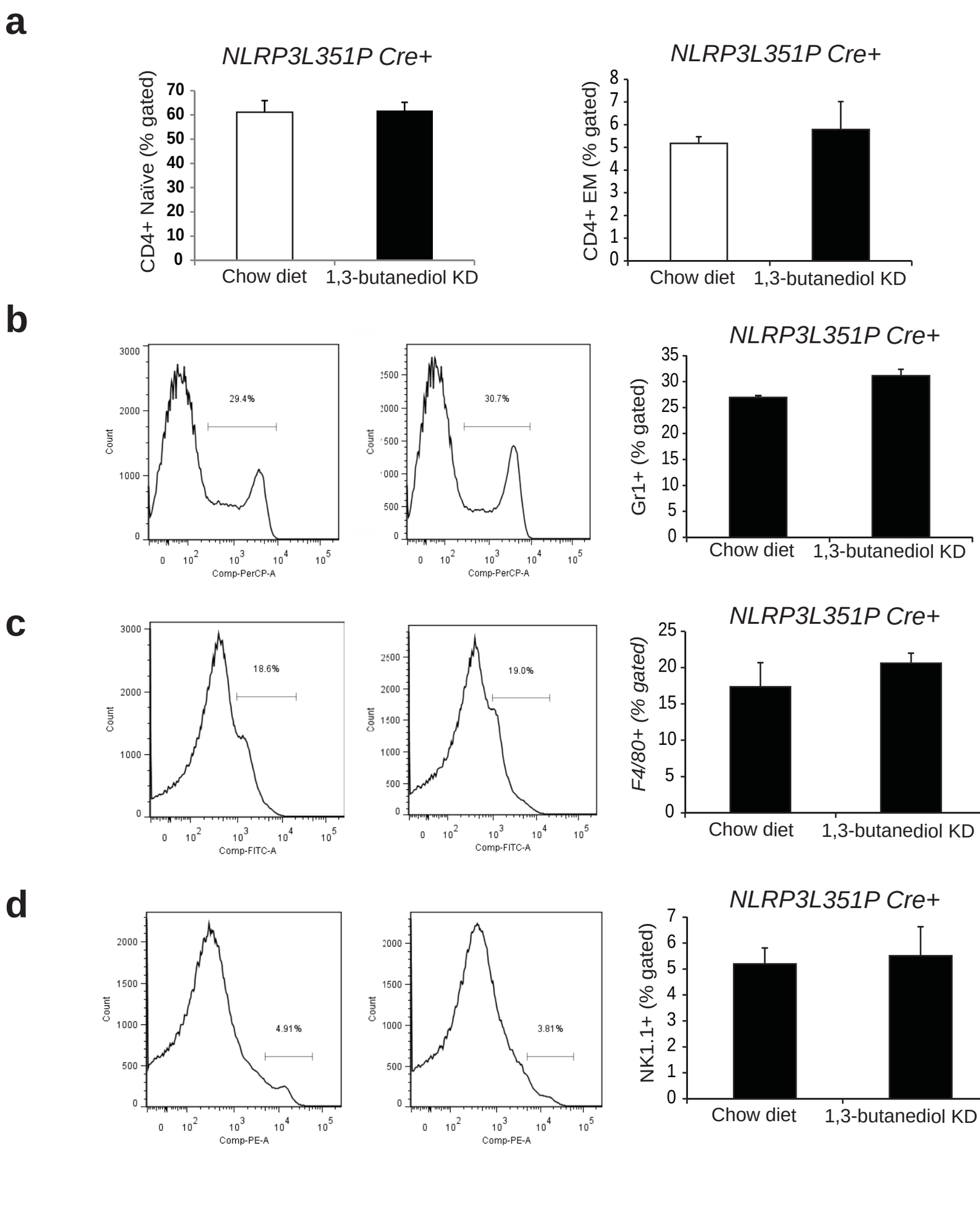
(a) The BM cells from mice (n =6) harbouring the FCAS mutation (*NLRP3*^{L351P}) were treated with tamoxifen on day 6 of macrophage differentiation to induce Cre recombination and activation of NLRP3. The western blot analysis of IL1β in BMDMs primed with LPS alone and treated with or without tamoxifen.

(b) The D-BHB for 1hour and various doses of D-BHB-nLGs. The experiment was repeated twice.

(c) The IL-1β activation (p17) in BMDMs of FCAS mice (*NLRP3*^{L351P}) treated with LPS and D-BHB-nLGs at various concentrations. Cre was induced by tamoxifen injection 24h before LPS treatment. The experiment was repeated twice.

(d) The blood glucose levels were evaluated 3 days post tamoxifen Cre-induced NLRP3 mutation activation (n =6-8/group).

(e) The FCAS mice (*NLRP3*^{L351P}) were fed chow and ketone ester diet (1,3 butanediol, 20% by volume) for one week and peritoneal cells were removed three days after tamoxifen-induced Cre recombination. The peritoneal cells were stained with CD11b and F4/80 and quantified by Flow Cytometry. A total of 6-8 mice/group were used. The data are presented as mean ±SEM. *p<0.05.

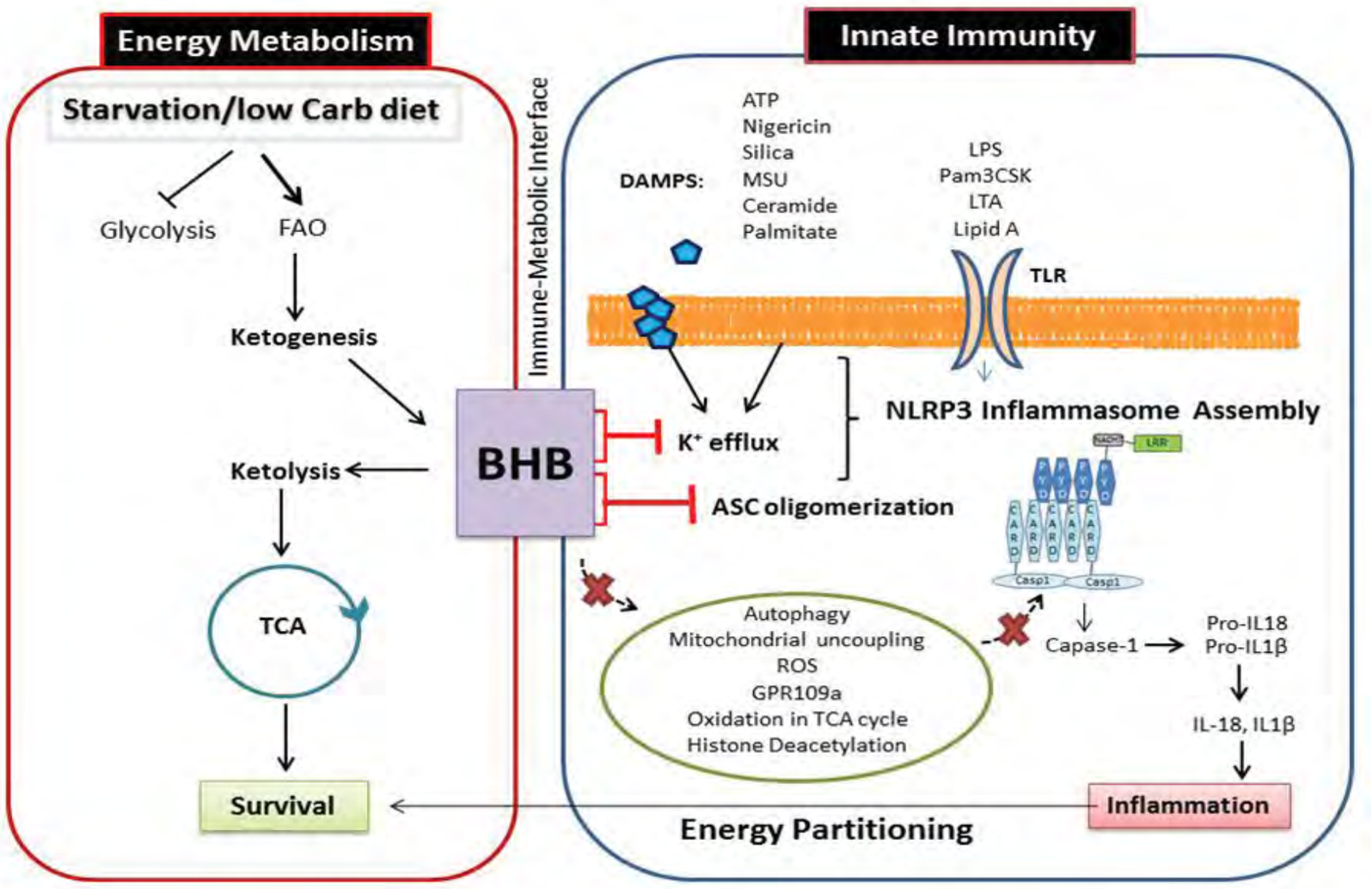


Supplementary Figure 7 Ketone diet does not impact immune cell frequency in spleen.

(a,b,c,d) The BM cells from 8 mice harbouring the FCAS mutation (*NLRP3*^{L351P}) were treated with tamoxifen on day 6 of macrophage differentiation to induce Cre recombination and activation. The data are presented as mean ±SEM. *p<0.05.

Youm *et al* Supplementary Fig.7

a



Supplementary Figure 8 Schematic illustration the proposed model of action of BHB on the NLRP3 inflammasome.

(a) Schematic illustrating the proposed mechanism of action of BHB on the NLRP3 inflammasome in macrophages.

Youm *et al* Supplementary Fig.8