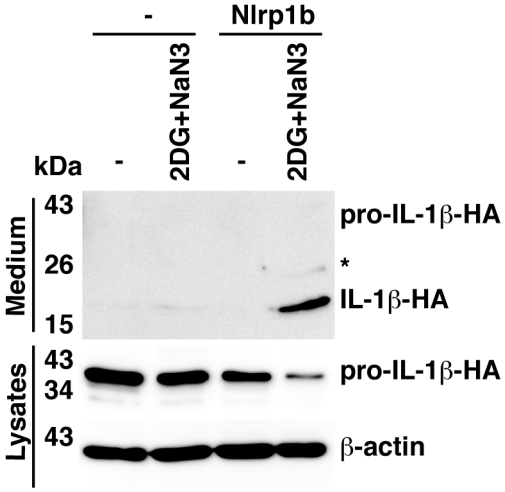

Figure S1. Processing of pro-IL-1β in response to ATP-depletion is dependent on Nlrp1b. Plasmids pcDNA3-pro-caspase-1-T7 and pcDNA3-pro-IL-1β-HA were co-transfected with or without pNTAP-Nlrp1b allele 1 into HT1080 cells. Approximately 24 h after transfection, the cells were treated with 50 mM 2DG and 10 mM NaN_3 for 3 h. The cell lysates were probed for HA-tagged pro-IL-1β and β-actin by immunoblotting. The supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β and pro-IL-1β by immunoblotting. The blot represents three independent experiments.

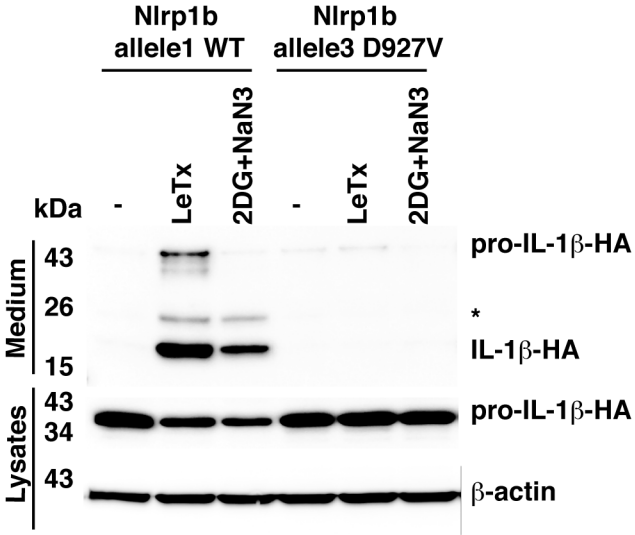

Figure S2. Nlrp1b allele 3 D927V does not support pro-IL-1β processing in response to ATP depletion. Plasmids pcDNA3-pro-caspase-1-T7 and pcDNA3-pro-IL-1β-HA were co-transfected with pNTAP-Nlrp1b allele 1 or pNTAP-Nlrp1b allele 3 D927V into HT1080 cells. Approximately 24 h after transfection, the cells were treated with LeTx or with 50 mM 2DG and 10 mM NaN_3 for 3 h. The cell lysates were probed for HA-tagged pro-IL-1β and β-actin by immunoblotting. The supernatants were immunoprecipitated with anti-HA antibodies and then probed for HA-tagged IL-1β and pro-IL-1β by immunoblotting. The blot represents three independent experiments.

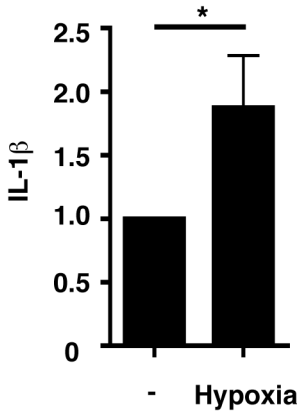

Figure S3. Hypoxia increases pro-IL-1β processing in minimal medium. The experiment shown in Fig. 2D and two replicates were quantified. Asterisk indicates a statistically significant difference ($p<0.05$, Student's t-test).

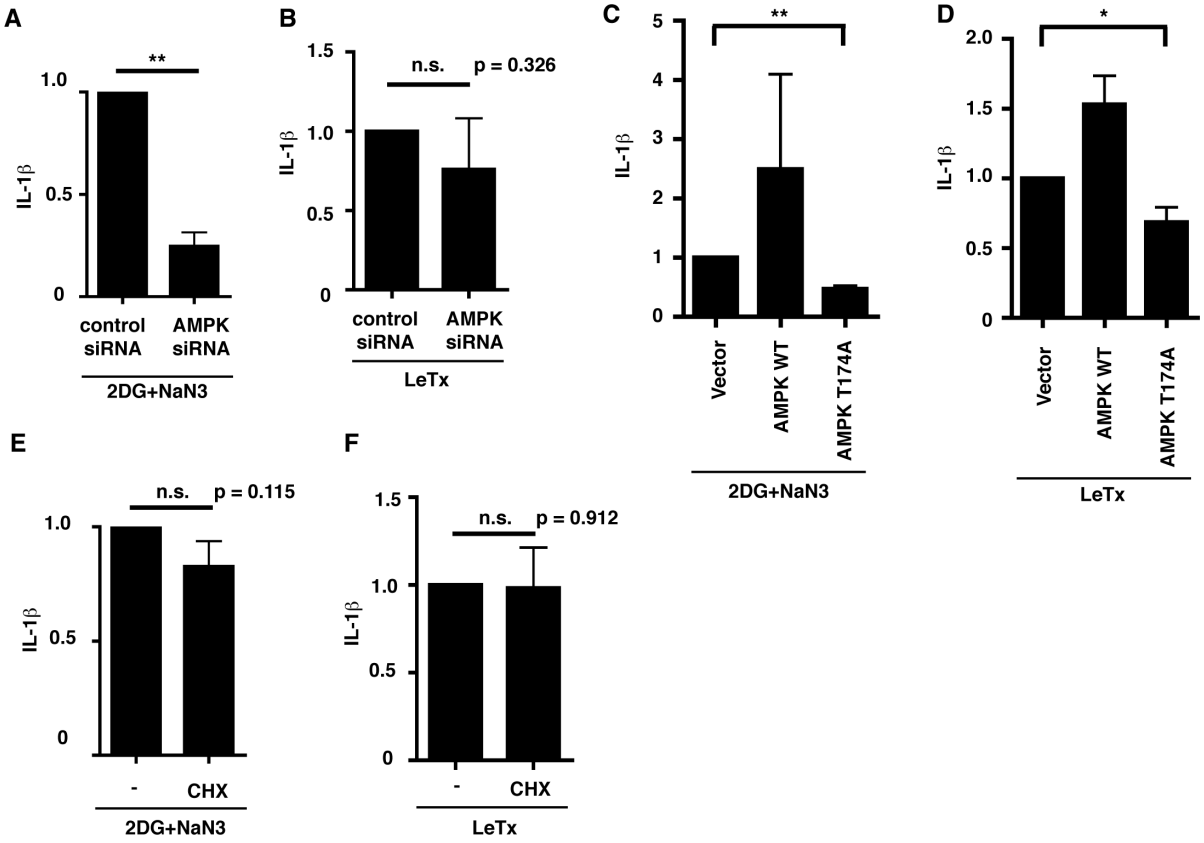

Figure S4. AMPK facilitates Nlrp1b activity. Experiments shown in Figure 3 and two replicates were quantified (* $p<0.05$; ** $p<0.01$, Student's t-test).

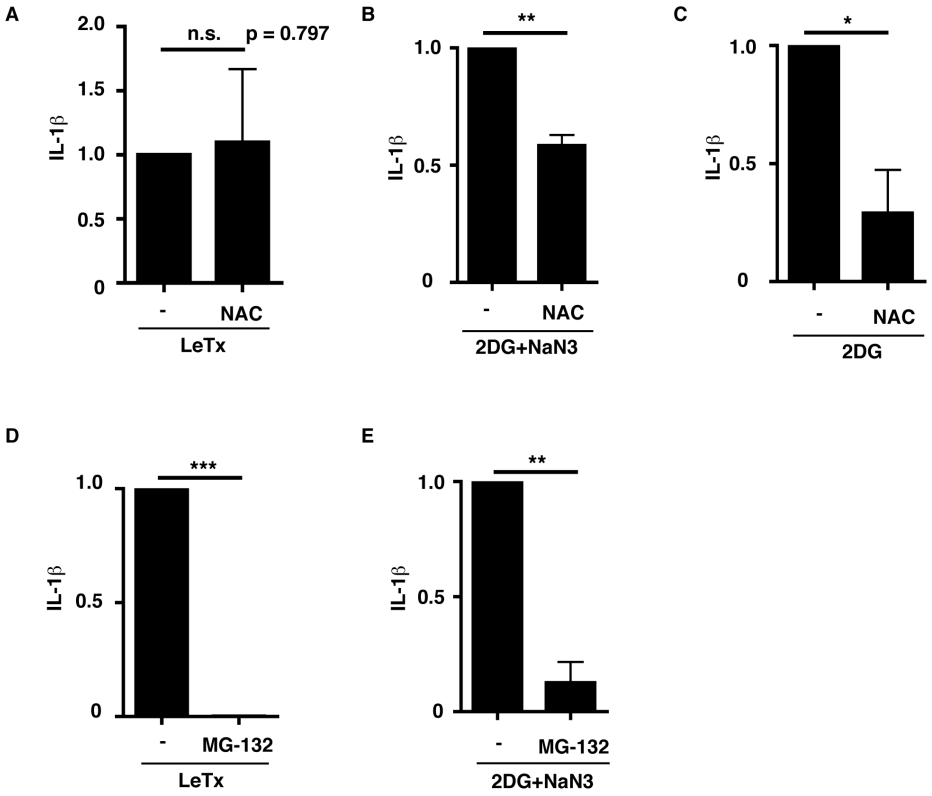

Figure S5. Effects of N-acetylcysteine and MG-132 on inflammasome activity. Experiments shown in Figure 4 and two replicates were quantified (* p<0.05; ** p<0.01; *** p<0.001, Student's t-test).

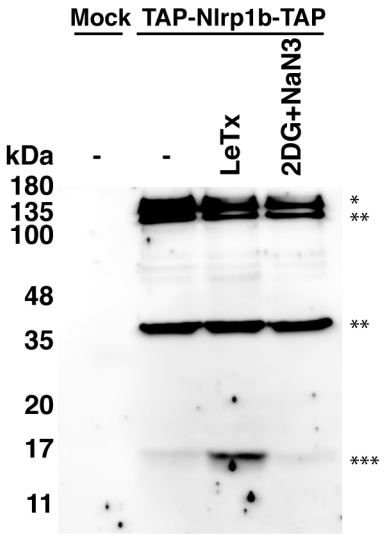

Figure S6. Nlrp1b cleavage is not detected in response to ATP depletion. pNTAP-Nlrp1b-CTAP (encodes Nlrp1b allele 1 fused to amino- and carboxy-terminal TAP tags) was transfected into HT1080 cells. Approximately 24 h after transfection, cells were treated with LeTx or 2DG/NaN_3 for 3 h. The TAP-tagged proteins were isolated using streptavidin-agarose and detected by immunblotting using an anti-calmodulin binding peptide antibody. The single asterisk indicates full-length Nlrp1b; double asterisks indicate the amino- and carboxy-terminal auto-cleavage products of Nlrp1b; the triple asterisk denotes a low molecular weight band that is consistent with a LeTx-dependent cleavage product (~16 kDa: ~8 kDa TAP-tag plus ~8 kDa Nlrp1b fragment).

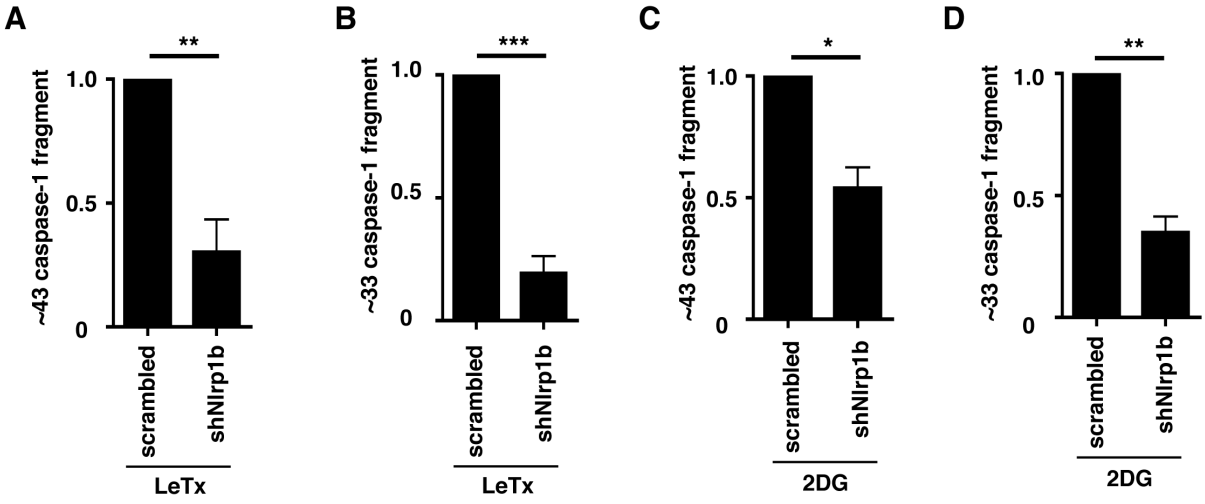

Figure S7. LeTx and 2DG activate the Nlrp1b inflammasome in J774A cells. The ~43 kDa and ~33 kDa caspase-1 fragments shown in Fig. 7 were quantified with those from two other independent experiments (* $p<0.05$; ** $p<0.01$; *** $p<0.001$, Student's t-test).