

## SUPPLEMENTARY FIGURES

**Fig. S1.** 3-MA alters LPS-induced cytokine secretion in dendritic cells and human macrophages. BMDC were treated with LPS (10 ng/ml) and indicated concentrations of 3MA for 24 h and supernatants analysed by ELISA for (A) IL-1 $\beta$  and (B) IL-6. (C) Human peripheral blood monocyte-derived macrophages from 2 donors were treated with LPS (100 ng/ml) and indicated concentrations of 3MA for 24 h and supernatants tested for IL-1 $\beta$  by ELISA. Data are means  $\pm$  s.d., \* significant difference from cells treated with LPS alone,  $p < 0.05$ . (one-way ANOVA, followed by Tukey's HSD test).

**Fig. S2.** 3-MA-induced inflammasome activation is independent of NLRC4 and dependent on TRIF. (A) BMDC were treated with PAM3CysK4 (PAM, 1  $\mu$ g/ml), poly(I:C) (p(I:C), 10  $\mu$ g/ml), R837 (10  $\mu$ g/ml), R848 (5  $\mu$ g/ml) and CpG (10  $\mu$ g/ml) with either ATP (5 mM) or 3MA (10 mM) for 24 h. Supernatants were tested for IL-1 $\beta$  by ELISA. (B) BMDC from wild type (WT) C57Bl/6 or *NLRC4*<sup>-/-</sup> mice were treated with LPS (10 ng/ml) plus indicated concentrations of 3MA for 24 h. ELISA analysis of (C) IL-1 $\beta$  and (D) IL-6 from BMDC treated with LPS (10 ng/ml) plus 3MA (10 mM) and different concentrations of KCl or the cathepsin B inhibitor CA-074 Me. Supernatants were analysed by ELISA for IL-1 $\beta$ . Data are means  $\pm$  s.d., \* significant difference from cells treated with LPS alone,  $p < 0.05$ . (one-way ANOVA, followed by Tukey's HSD test).

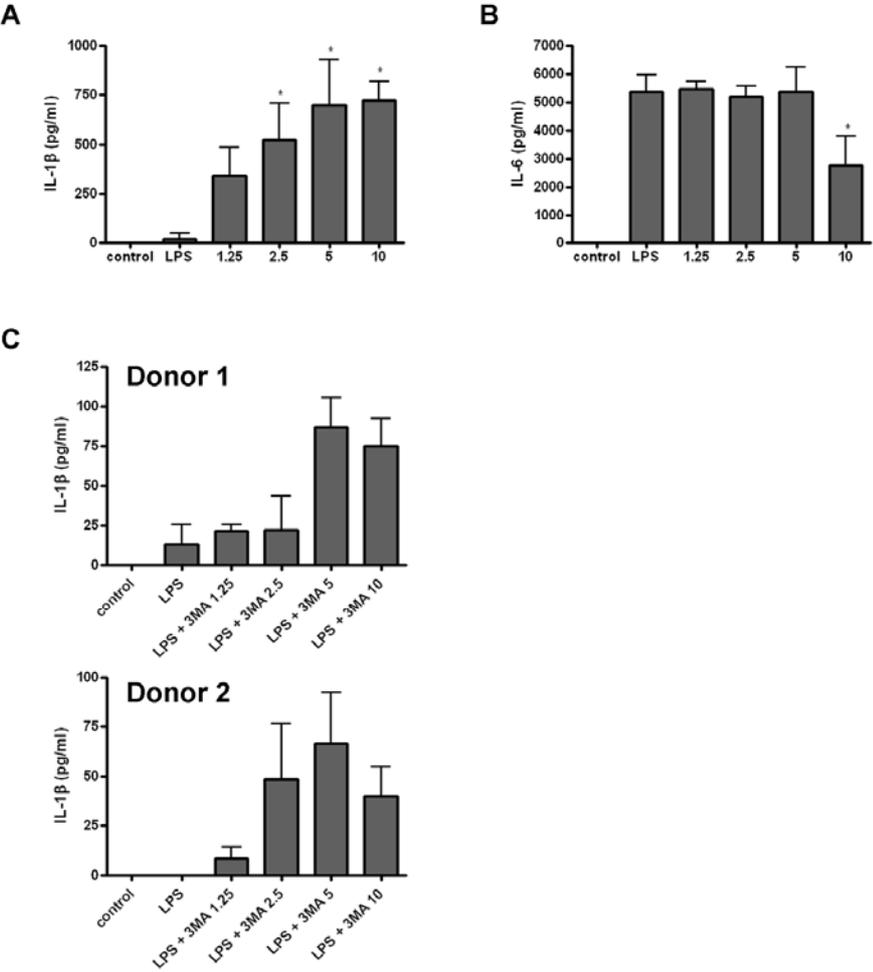
**Fig. S3.** The ROS scavenger N-acetyl-L-cysteine (NAC) inhibits caspase 1 processing in dendritic cells deficient in autophagy. BMDC were treated with LPS (10 ng/ml) and 3-MA (at concentrations indicated) for 24 h in the presence or absence of NAC (25 mM). Cell lysates were analysed by Western blot with anti-caspase 1 antibody.

**Fig. S4.** Rapamycin induces autophagy in LPS-treated macrophages. iBMM stably transfected with GFP-LC3 (green) were treated for 20 h with LPS (100 ng/ml), followed by rapamycin (rapa, 50  $\mu$ g/ml) for 4 h. Cells were fixed and nuclei stained with bisbenzimidazole H 33258 (blue). Cells were analysed by confocal microscopy. Scalebar = 10  $\mu$ m.

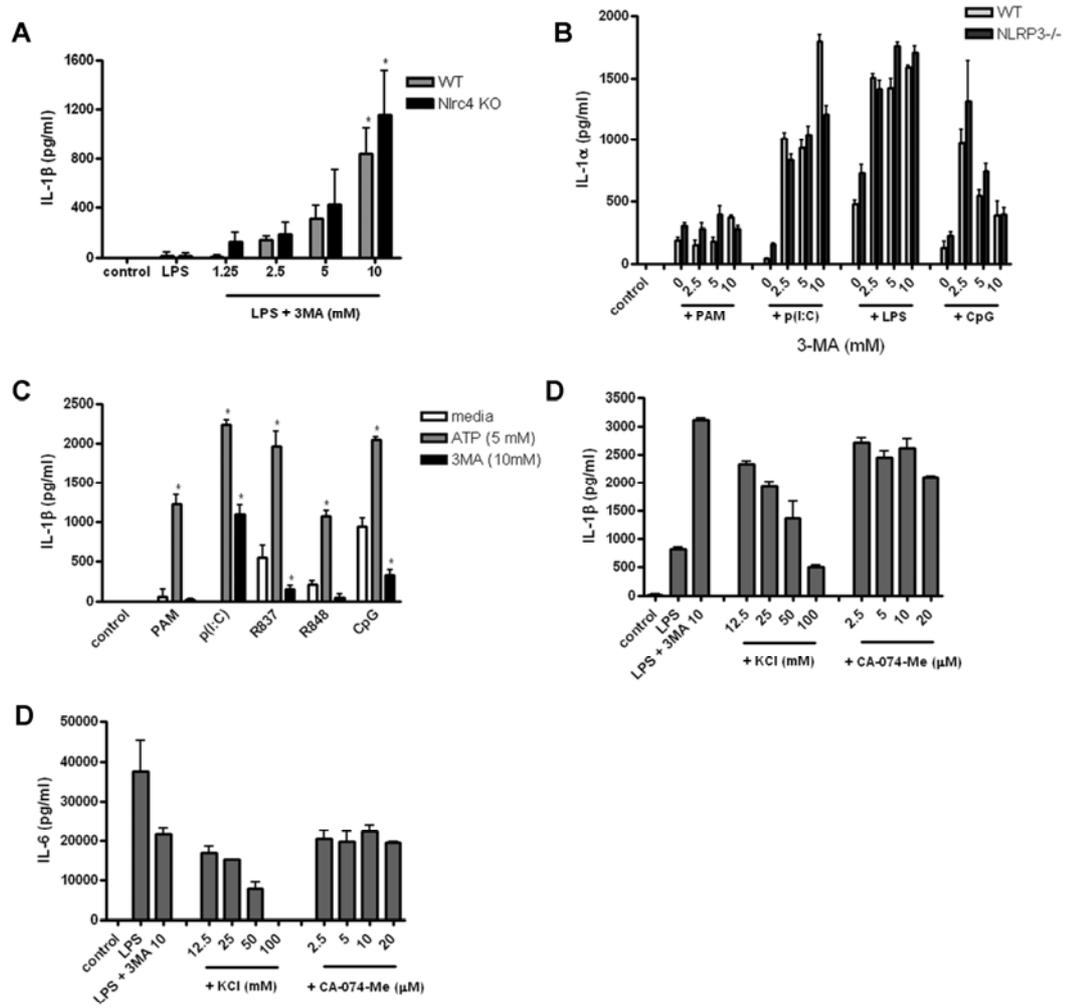
**Fig. S5.** Caspase 1 is not targeted by autophagosomes. BMM stably transfected with GFP-LC3 (green) were treated with or without LPS (100 ng/ml) for 6 h, fixed and stained with antibody against caspase 1 (red). Nuclei were stained with bisbenzimidazole H 33258 (blue). Cells were analysed by confocal microscopy. Scalebar = 10  $\mu$ m.

**Fig. S6.** Cytokine levels in the lavage of mice treated with LPS and rapamycin. Levels of (A) IL-1 $\beta$  and (B) IL-6 were measured in peritoneal lavage fluid from mice injected with LPS or LPS plus rapamycin.

Figure S1



## Supplemental Figure 2



**Figure S3**

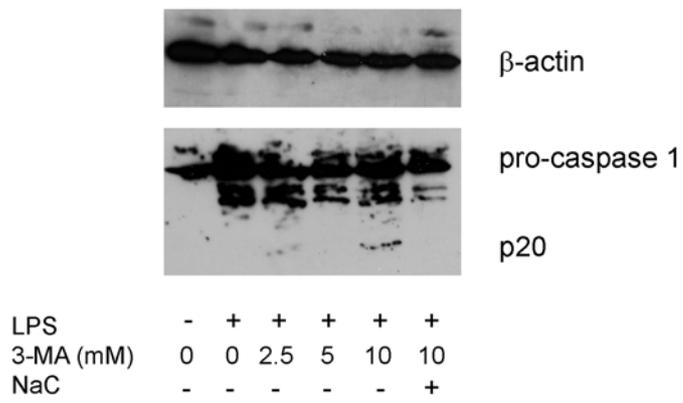
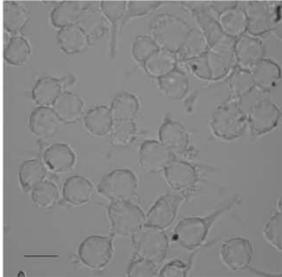
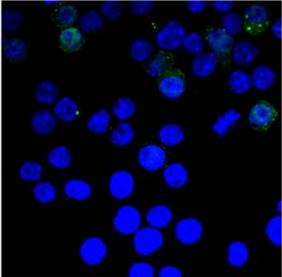
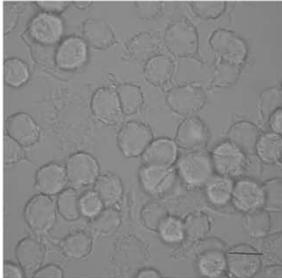
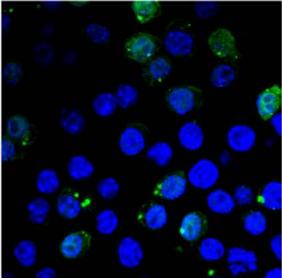


Figure S4

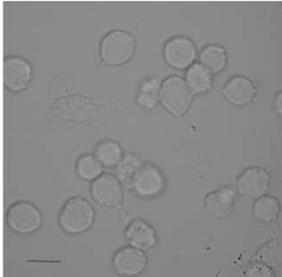
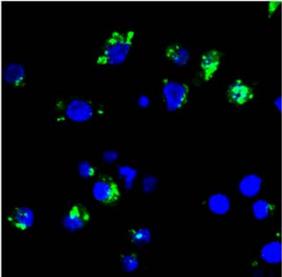
control



LPS



LPS + rapa



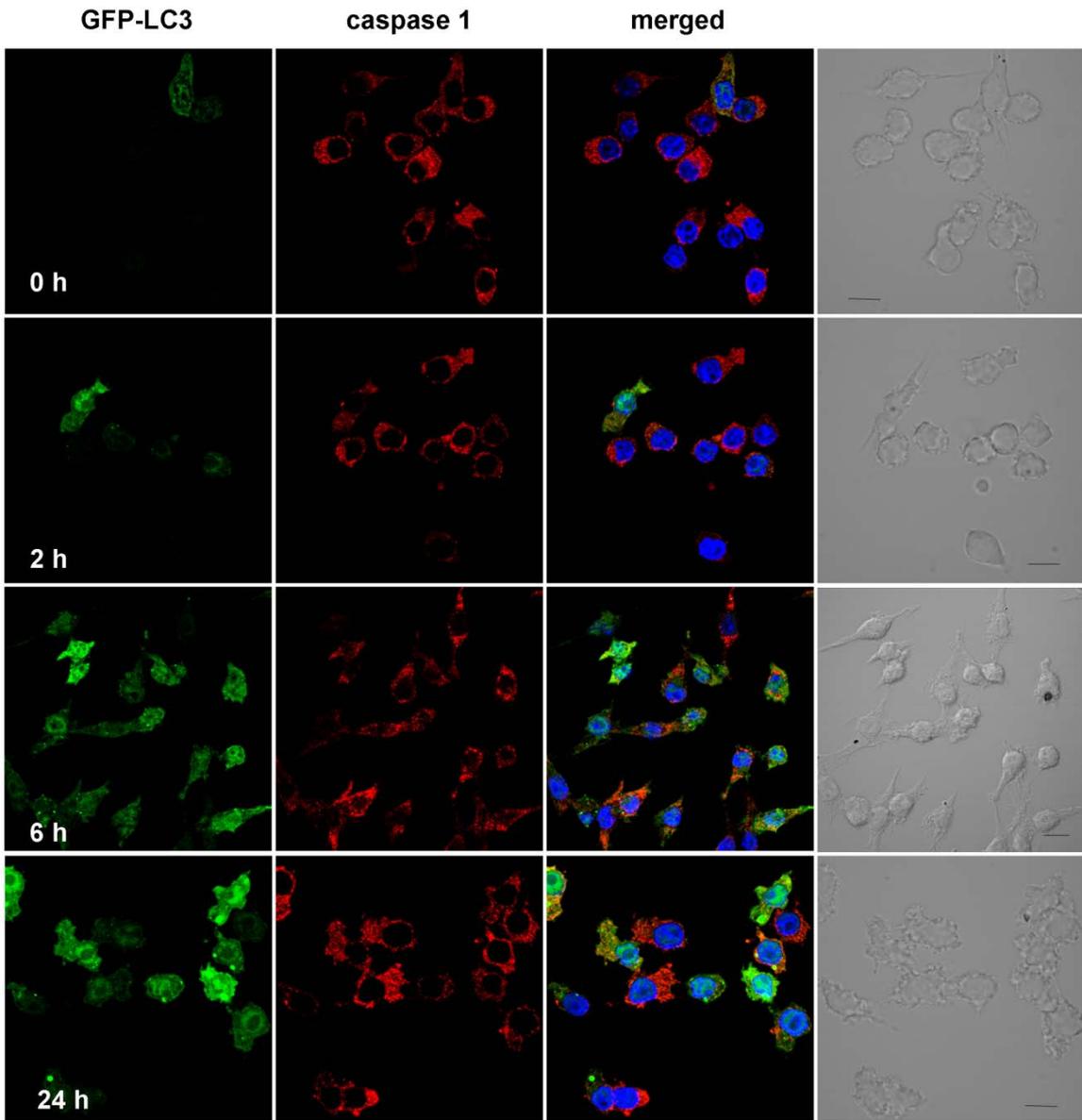
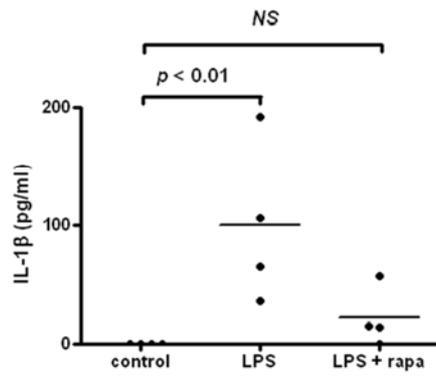


Figure S5

Figure S6

**A**



**B**

