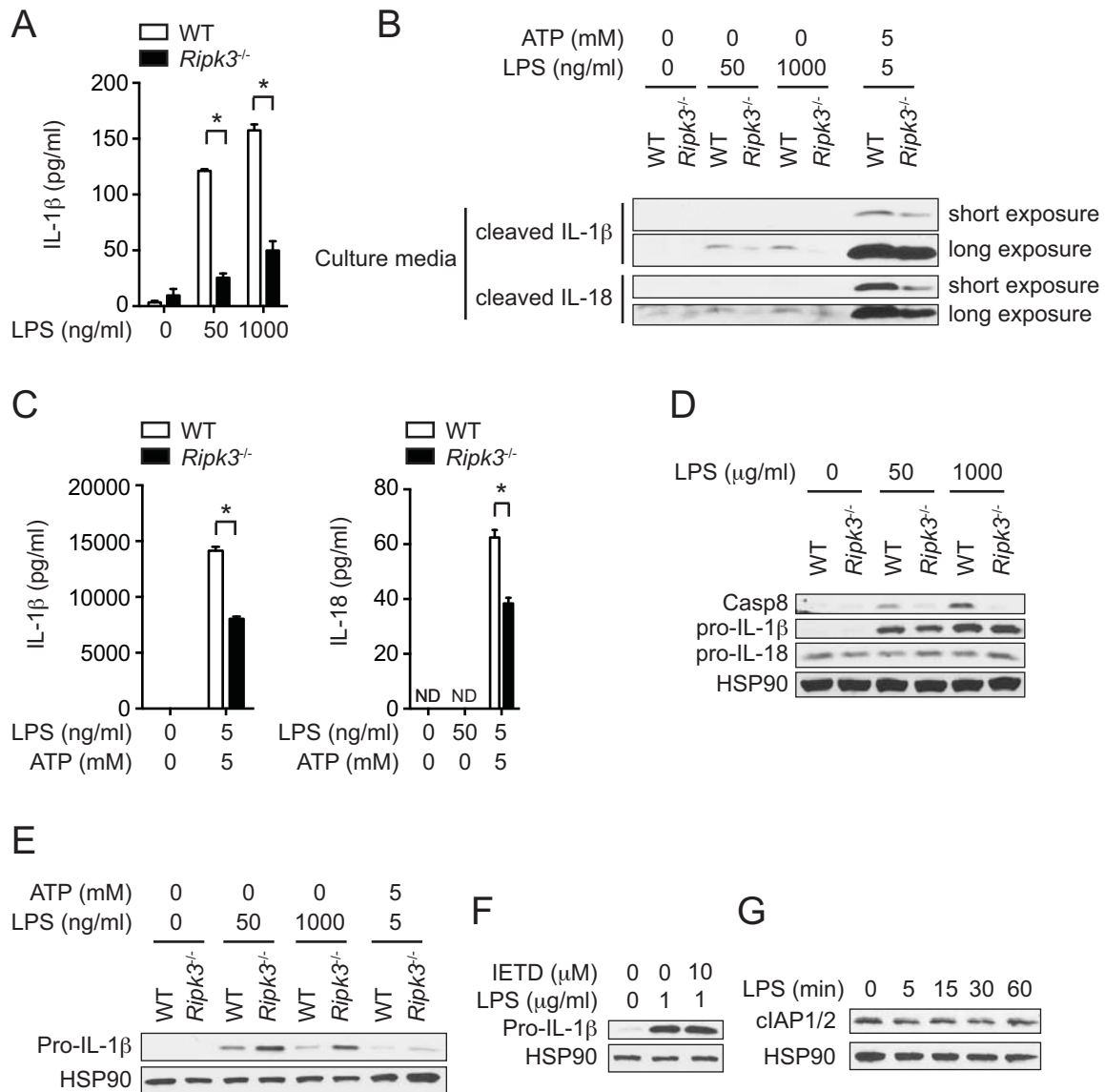


## Supplemental Fig. 1



### Supplemental Fig. 1. LPS-induced IL-1 $\beta$ and IL-18 secretion is impaired in *Ripk3*<sup>-/-</sup> BMDCs.

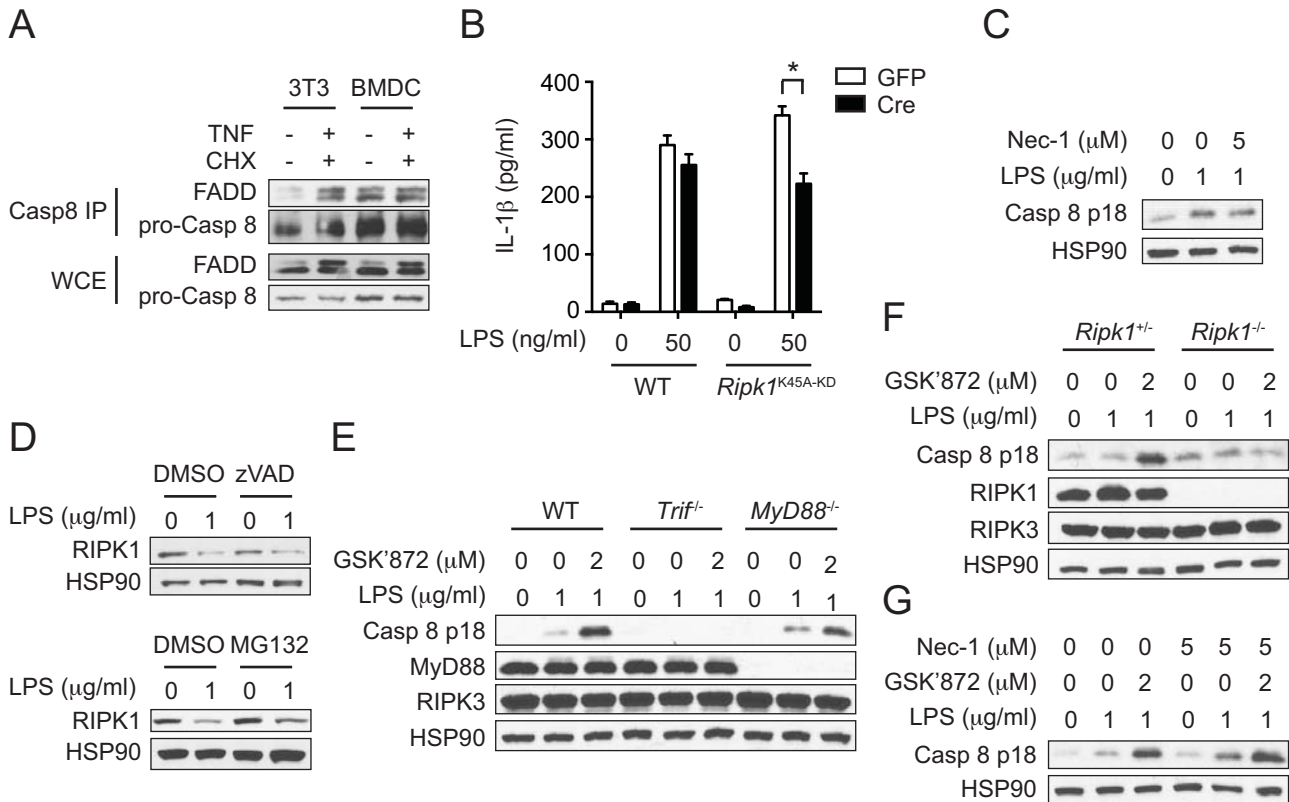
(A-C) WT and *Ripk3*<sup>-/-</sup> BMDCs were treated with LPS for 6 hours. For LPS and ATP stimulation, cells were treated with LPS for 3 hours. ATP was added for the last 30 min. Culture media were tested for IL-1 $\beta$  and IL-18 secretion by (A and C) ELISA or (B) western blotting. Results shown are mean  $\pm$  SEM. Asterisks:  $p < 0.05$ . ND: not detected.

(D and E) WT and *Ripk3*<sup>-/-</sup> BMDCs were treated with LPS for (D) 1 hour or (E) 6 hours. For LPS and ATP stimulation, cells were treated with LPS for 3 hours. ATP was added for the last 30 min. Whole cell extracts (WCEs) were subjected to western blotting.

(F) WT BMDCs pretreated with z-IETD-fmk (IETD) for 1 hour were stimulated with LPS for 1 hour. WCEs were subjected to western blotting.

(G) WCEs from BMDCs treated with 100 ng/ml LPS for the indicated time were subjected to western blotting using pan-cIAP1/2 antibody.

## Supplemental Fig. 2



### Supplemental Fig. 2. LPS-induced caspase 8 activation in BMDCs requires TRIF and RIPK1, but not MyD88 or RIPK1 kinase activity.

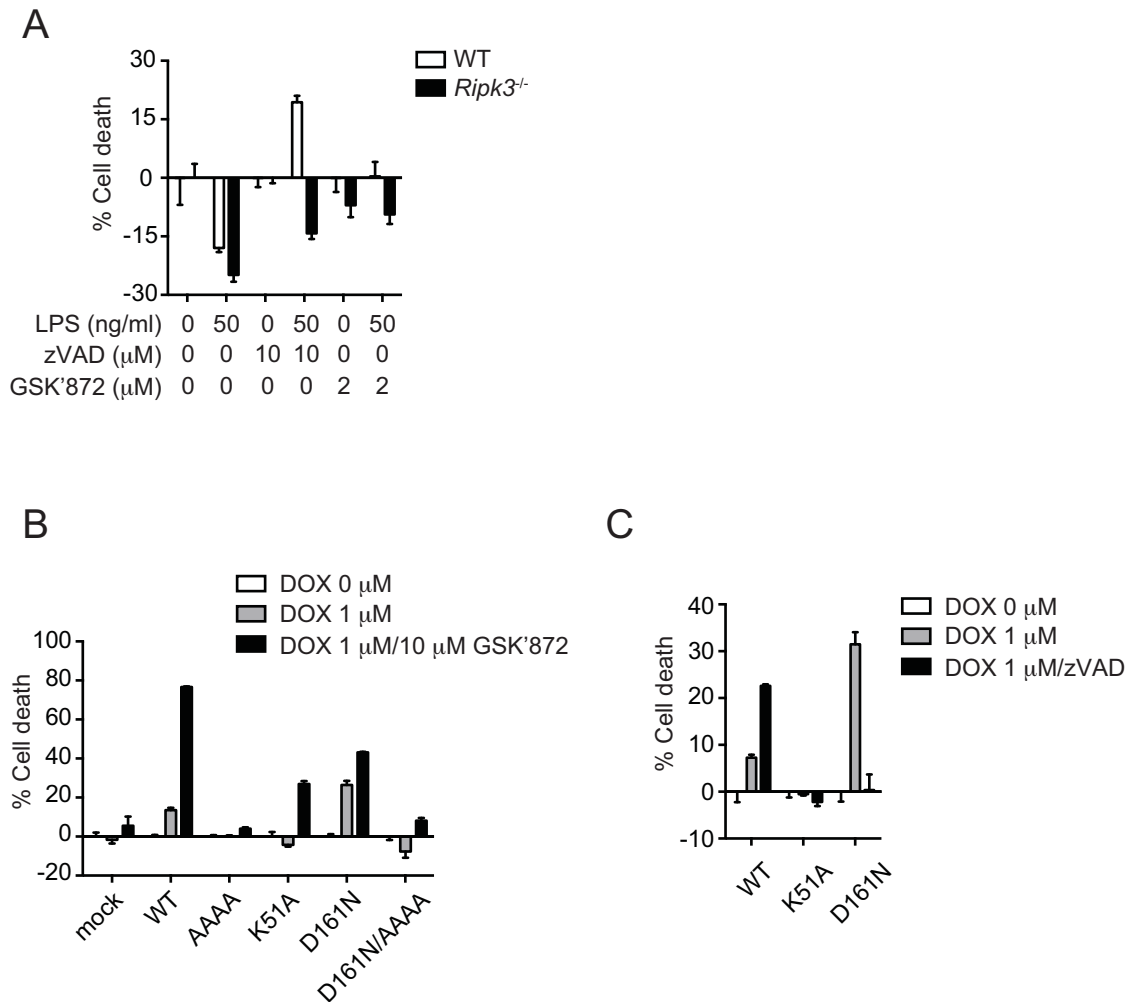
(A) WT 3T3 fibroblasts and BMDCs pretreated with 1 μg/ml cycloheximide (CHX) for 1 hour were stimulated with 100 ng/ml TNF for 2 hours. Whole cell extracts (WCEs) were subjected to immunoprecipitation using anti-caspase 8 antibody, followed by western blotting.

(B) WT or *Ripk1*<sup>K45A-KD</sup> BMDCs transduced with GFP or Cre-expressing vector were stimulated with LPS for 6 hours. The amount of IL-1β in culture media was determined by ELISA (n=4). Results shown are mean ± SEM. Asterisks: p < 0.05.

(C and D) WT BMDCs were pretreated with Nec-1, 10 μM z-VAD-fmk (zVAD), or 2 μM MG132 for 1 hour and then treated with LPS for 1 hour. WCEs were subjected to western blotting.

(E) BMDCs with indicated genotypes, (F) DCs from *Ripk1*<sup>-/-</sup> fetal liver cells, or (G) WT BMDCs were pretreated with RIPK3 inhibitor GSK'872 and/or Nec-1 for 1 hour and then treated with LPS for 1 hour. WCEs were subjected to western blotting.

### Supplemental Fig. 3



#### Supplemental Fig. 3. RIPK3 kinase inhibitor induces caspase 8-dependent apoptosis requires an intact RHIM.

(A) BMDCs preterated with either z-VAD-fmk or GSK'872 for 1 hour were treated with LPS for 16 hours. Cell death was determined by CellTiter-Glo Luminescent Cell Viability Assay.

(B) *Ripk3*<sup>-/-</sup> fibroblasts were transduced with lentivirus expressing the indicated RIPK3 mutants. Cells were treated with doxycycline and GSK'872 as indicated. Cell death was measured 16 hours later.

(C) Cell death was measured as in (B), except that cells were treated with 10  $\mu$ M z-VAD-fmk (zVAD) where indicated.