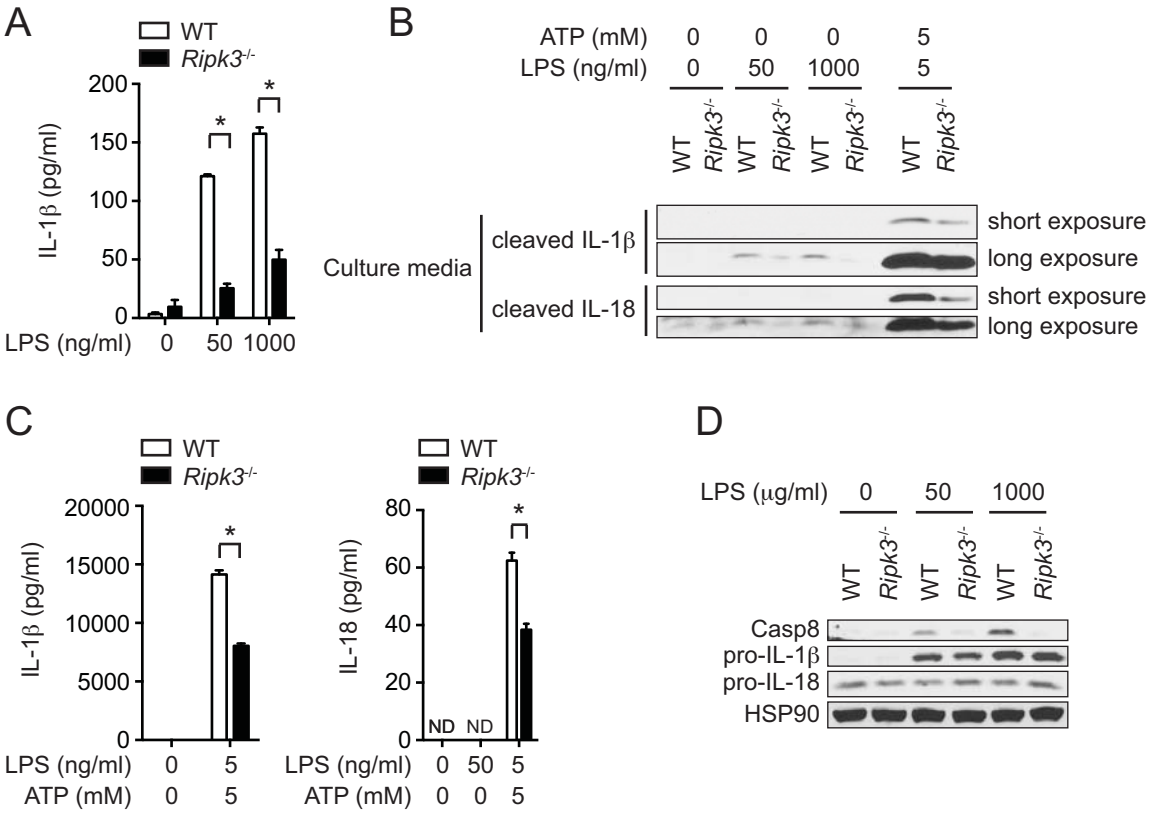
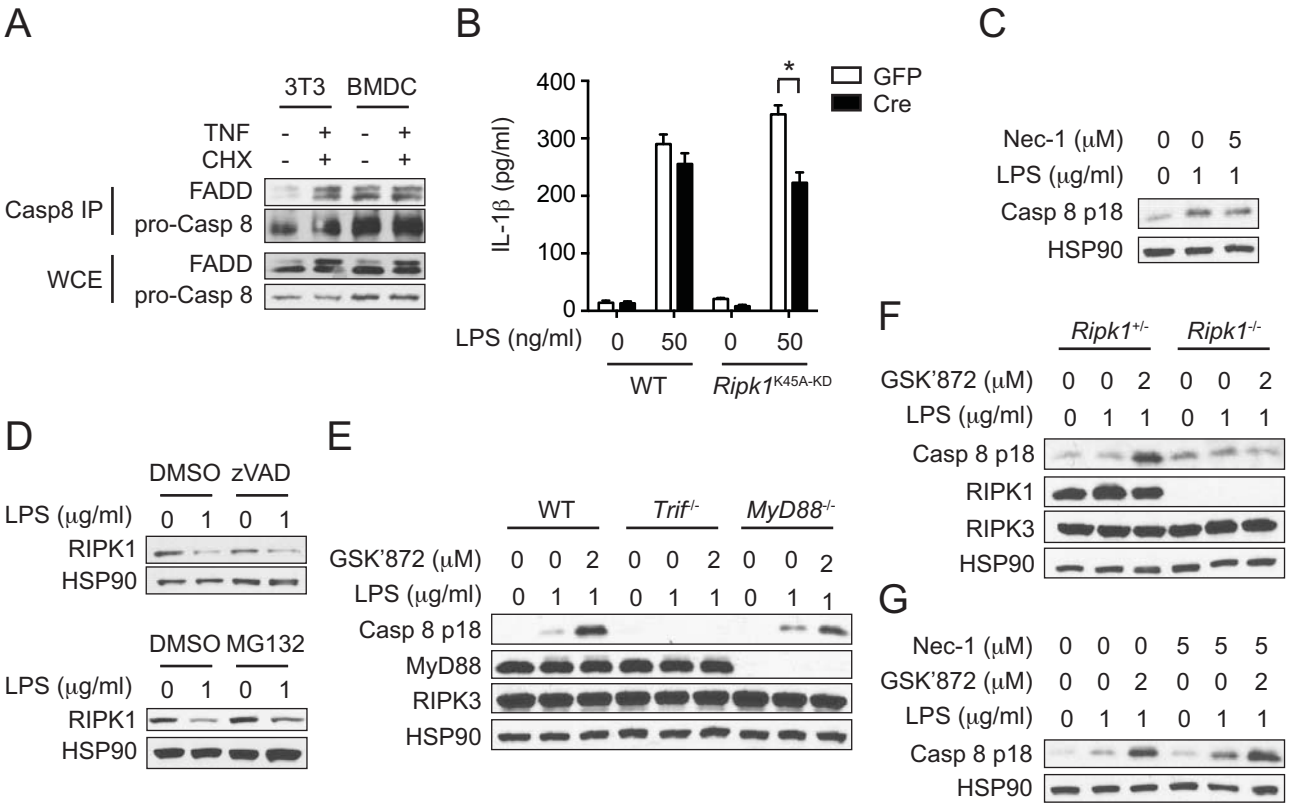
Supplemental Fig. 1



Supplemental Fig. 1. LPS-induced IL-1β and IL-18 secretion is impaired in *Ripk3*$^{-/-}$ BMDCs.
(A-C) WT and *Ripk3*$^{-/-}$ 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-1β and IL-18 secretion by **(A and C)** ELISA or **(B)** western blotting. Results shown are mean ± SEM. Asterisks: $p < 0.05$. ND: not detected.
(D and E) WT and *Ripk3*$^{-/-}$ 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.

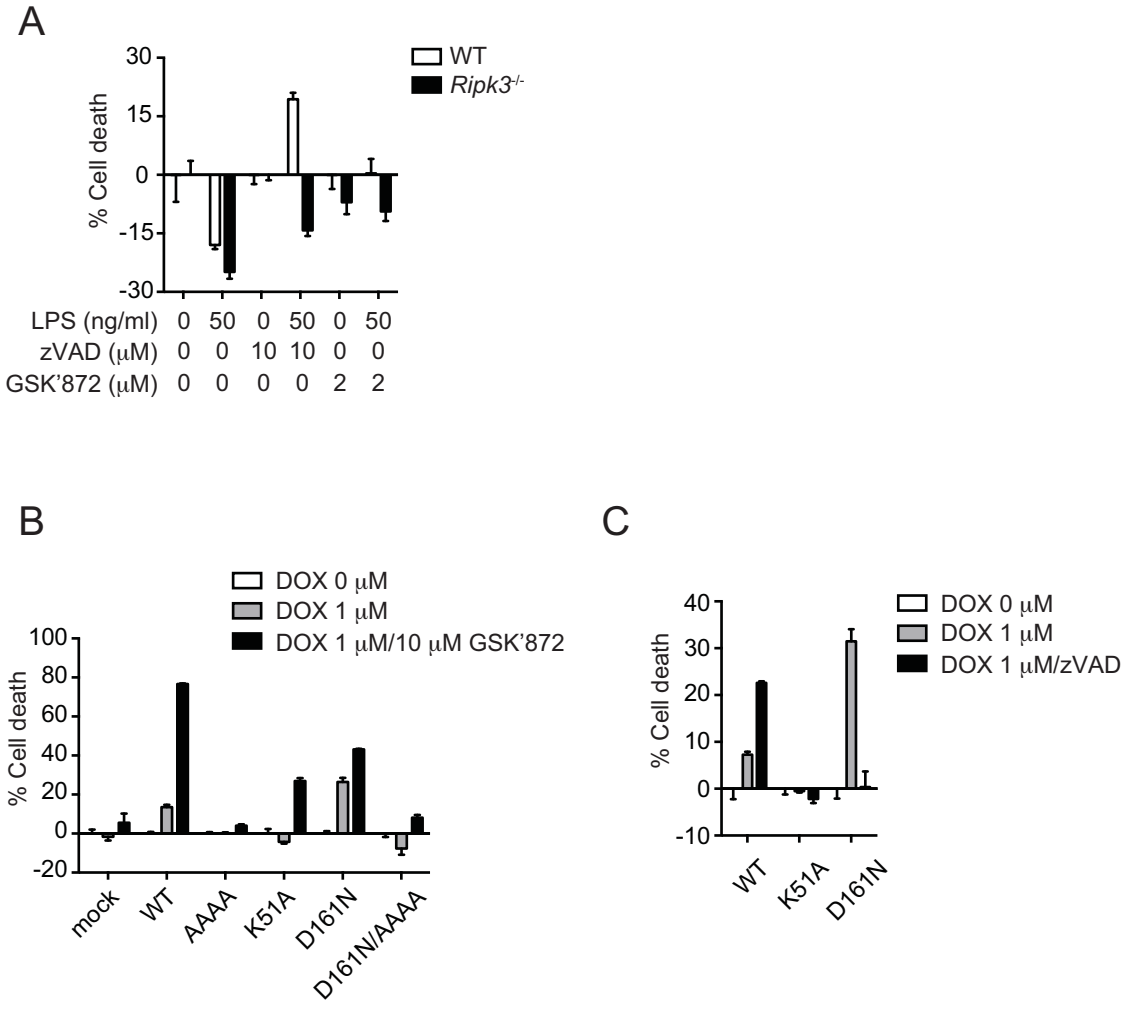
(A) WT 3T3 fibroblasts and BMDCs pretreated with 1 μg/ml cycloheximide (CHX) for 1 hour were stimulated with 100 ng/ml TNF for 2 hours. Whole cell extracts (WCEs) were subjected to immunoprecipitation using anti-caspase 8 antibody, followed by western blotting.

(B) WT or *Ripk1*$^{K45A-KD}$ 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 wtih 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*$^{-/-}$ 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*$^{-/-}$ 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 μM z-VAD-fmk (zVAD) where indicated.