

Supplemental Figures and Legends

Figure S1

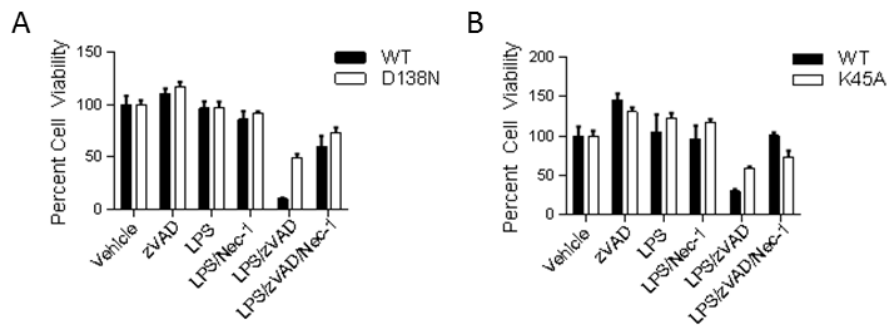


Figure S1: LPS with zVAD induces RIPK1 kinase-dependent cell death.

(A) Cell viability of wild type (WT) and D138N RIPK1 BMDMs (D138N) treated for 24 hours and evaluated by ATP assay. (B) Cell viability of WT and K45A RIPK1 BMDMs (K45A) treated for 24 hours and evaluated by ATP assay. Data are representative. Error bars reflect SD from the mean. BMDMs were treated with LPS=10 ng/mL, zVAD=50 μ M, and/or Nec-1s=30 μ M where indicated.

Figure S2

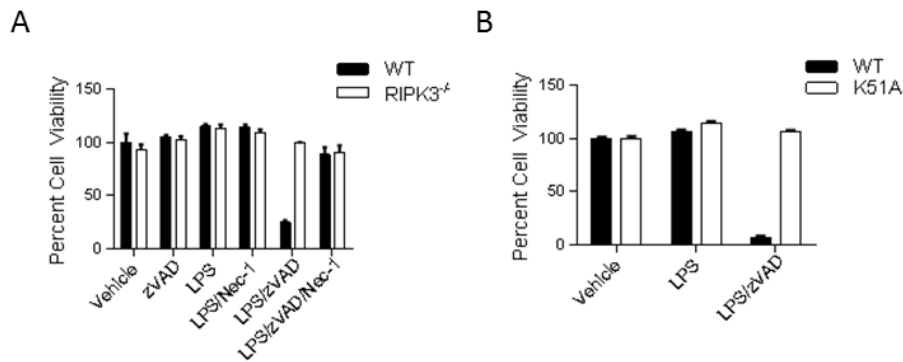


Figure S2: RIPK3 is required for cell death induced by LPS with zVAD.

(A) Cell viability of wild type (WT) and RIPK3 knockout (*Ripk3*^{-/-}) BMDMs treated for 24 hours and evaluated by ATP assay. (B) Cell viability of WT and K51A RIPK3 BMDMs (K51A) treated for 24 hours and evaluated by ATP assay. Data are representative. Error bars reflect SD from the mean. BMDMs were treated with LPS=10 ng/mL, zVAD=50 μ M, and/or Nec-1s=30 μ M where indicated.

Figure S3

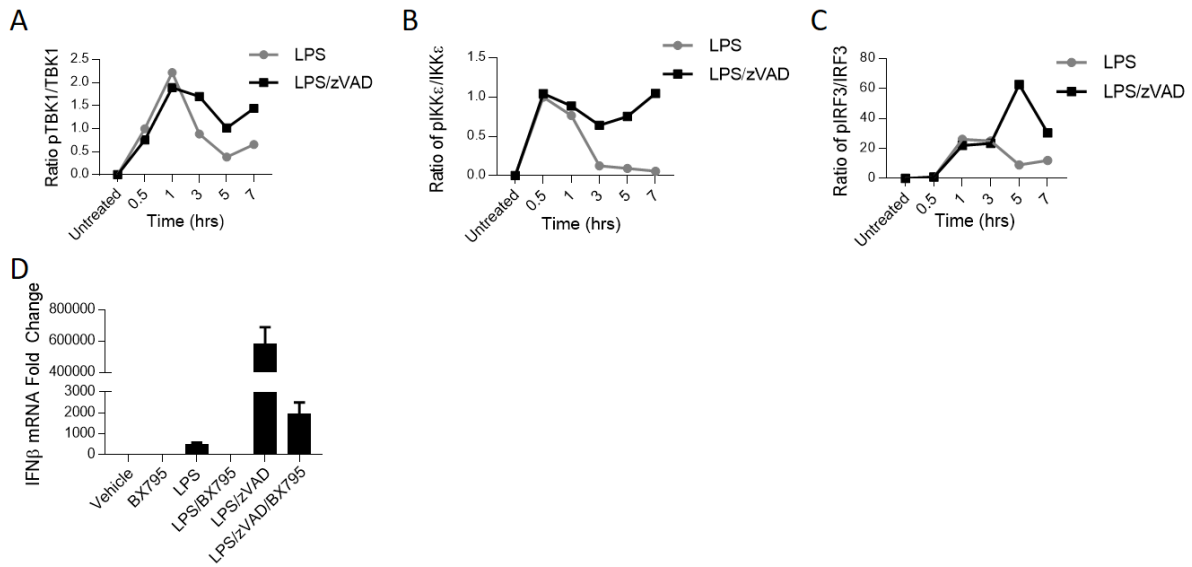


Figure S3: TRIF, TBK1/IKKε, and IRF3 are important for LPS/zVAD induced IFNβ synthesis.

(A-C) Densitometry analysis Western blot timecourse phosphorylation of TBK1 (A), IKKε (B), and IRF3 (C) in wild type BMDMs. (D) qRT-PCR of *Ifnb* mRNA expression in wild type BMDMs treated with TBK1/IKKε inhibitor (BX795) for 5-7 hrs. Data are representative. Error bars reflect SD from the mean. BMDMs were treated with LPS=10 ng/mL, zVAD=50 μM, Nec-1s=30μM and/or BX795=1μM where indicated.

Figure S4

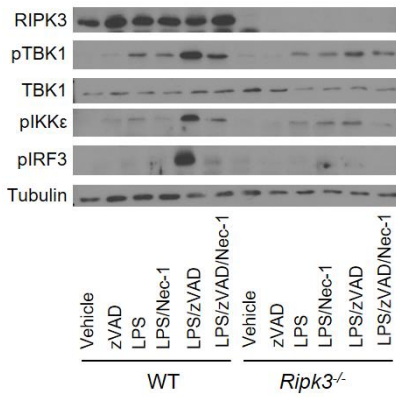


Figure S4: IFN-I pathway activation by LPS with zVAD is dependent on RIPK3.

Western analysis of TBK1, IKKε, and IRF3 phosphorylation in wild type (WT) and *Ripk3*^{-/-} BMDMs treated for 3-4 hours. Data is representative. BMDMs were treated with LPS=10 ng/mL, zVAD=50 μM, and/or Nec-1s=30μM where indicated.