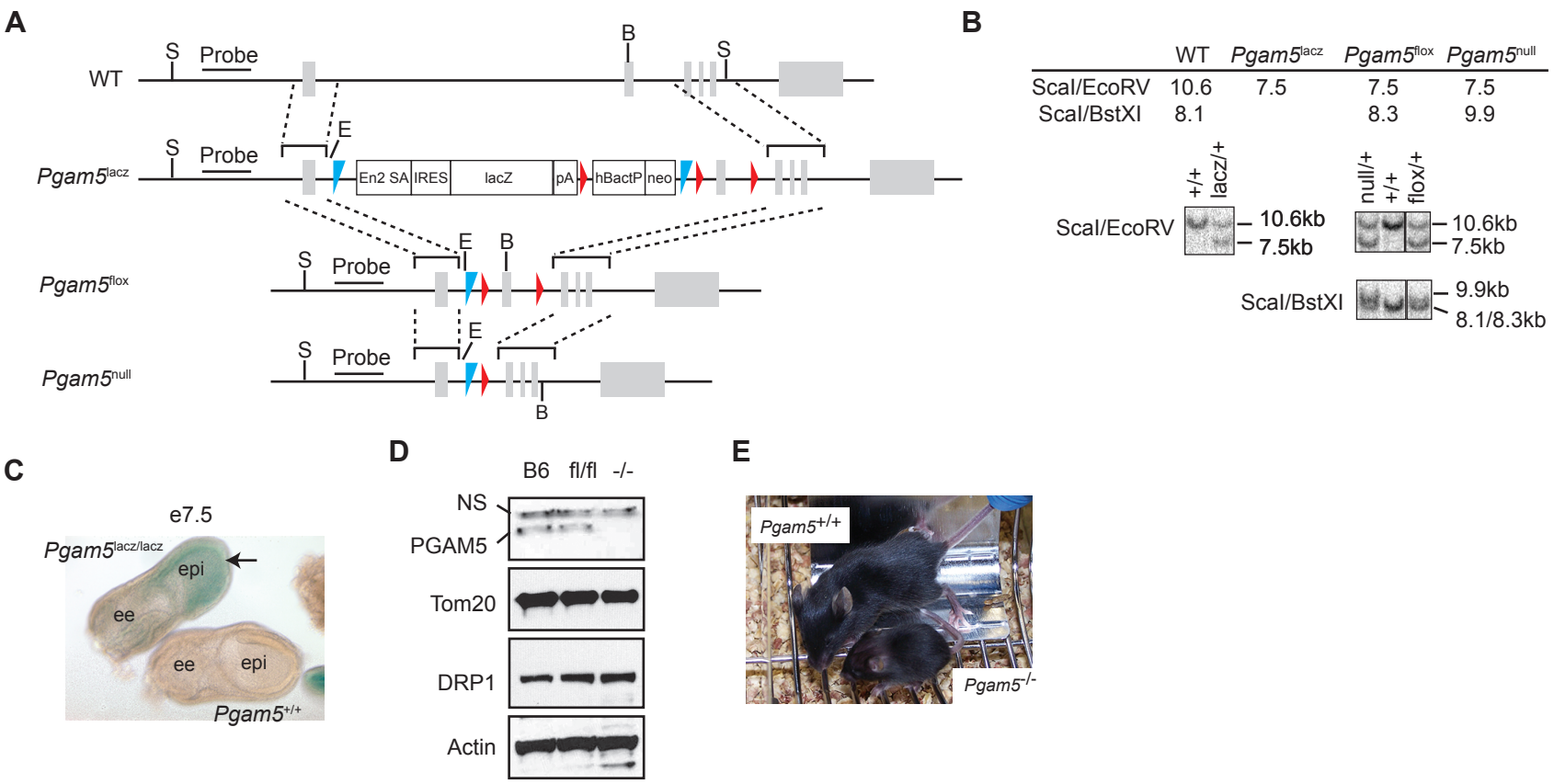
Supplemental Figure 1



Supplemental figure 1. Generation of *Pgam5*$^{-/-}$ mice.

(**A**) *Pgam5* gene targeting strategy. Exons are shown as gray boxes. En2SA, engrailed 2 splice acceptor. IRES, internal ribosome entry site. lacZ, β-galactosidase. pA, polyadenylation sequences. hBactP, human β-actin promoter. Neo, neomycin resistance gene. Loxp and FRT sequences are indicated, as red and blue triangles, respectively. Restriction enzyme sites: S, ScaI; E, EcoRI; B, BstXI. Solid black lines represent Southern blot probe used for genotyping.

(**B**) Genomic DNA from mice of the indicated genotypes was digested by restriction enzymes and subsequently subjected to Southern blotting analysis using 5' probe shown in **A**. The predicted sizes of the bands in kb are shown in the table above.

(**C**) β-galactosidase staining of E7.5 embryos from *Pgam5*$^{lacZ/lacZ}$ mice. The arrow indicates positive staining in the epiblast (epi). ee: extra-embryonic maternal tissues. A wild type embryo (below) was shown for comparison.

(**D**) Western blot of whole cell lysates from C57BL/6, *Pgam5*$^{fl/fl}$ and *Pgam5*$^{-/-}$ BMDMs. NS: non-specific signal.

(**E**) Picture of 3 weeks old wild type and *Pgam5*$^{-/-}$ littermates shows that *Pgam5*$^{-/-}$ mice were smaller in size.

Figure S2

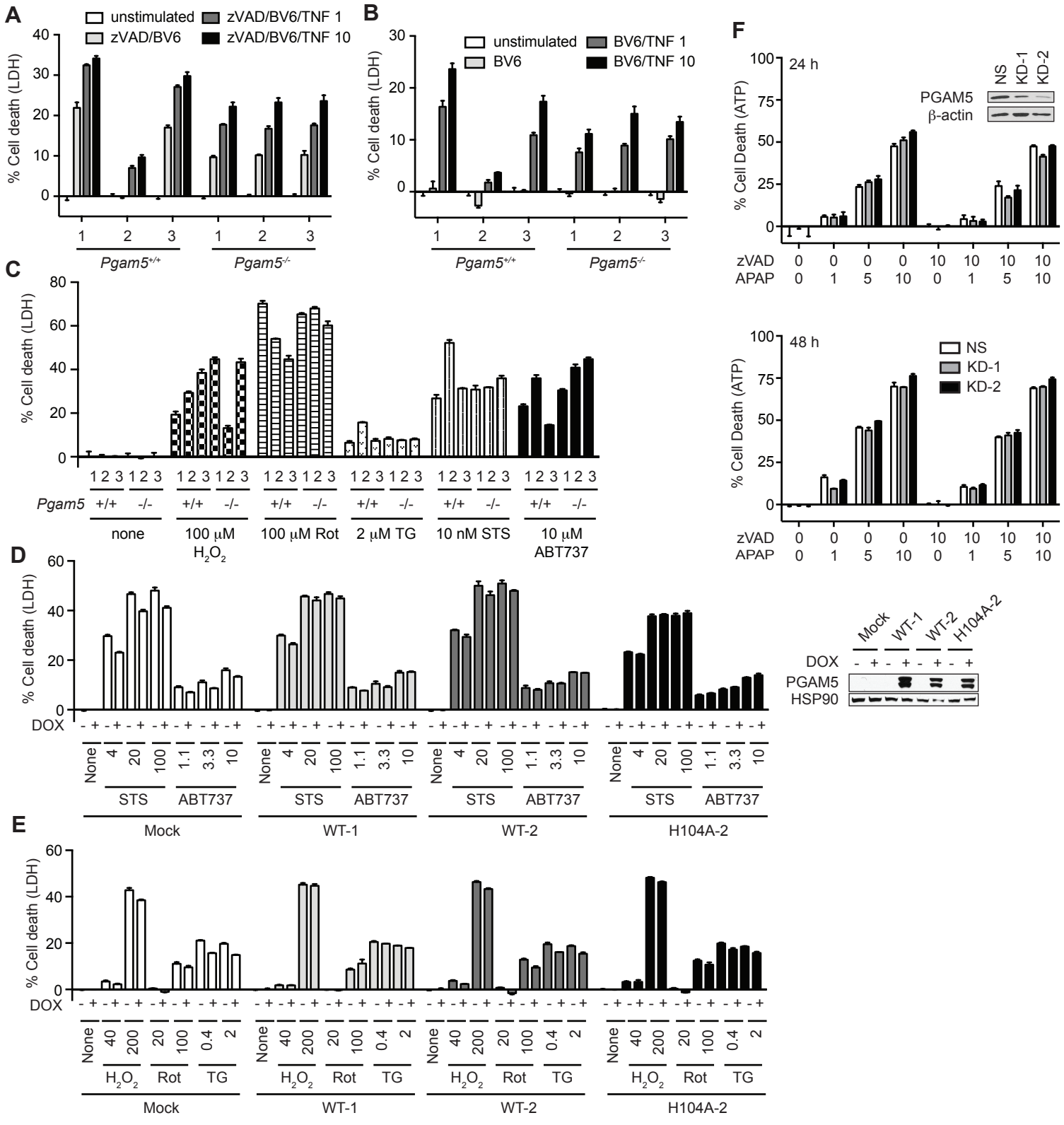


Figure S2. PGAM5 is dispensable for apoptosis and necroptosis induced by multiple stimuli.
(**A-C**) Three different primary MEF lines from *Pgam5*$^{+/+}$ and *Pgam5*$^{-/-}$ mice were treated for 24 hours as indicated. TNF was used at 1 or 10 ng/ml as indicated. Rot: Rottlerin, TG: thapsigargin, STS: staurosporine.
(**D, E)** SV40-immortalized *Pgam5*$^{-/-}$ MEFs reconstituted with PGAM5 wild type isoform 1 (WT-1), isoform-2 (WT-2) or phospatase-dead isoform 2 (H104A-2) were treated with H_2O_2 (μM), Rot (μM), TG (μM), STS (nM), or ABT737 (μM) for 24 hours. PGAM5 expression was induced by 1 μg/ml doxycycline (DOX) for 24 hours before stimulation with the indicated agents. PGAM5 expression by Western blot after induction with 1 μg/ml doxycycline (DOX) for 24 hours was shown on the right.
(**F**) HepG2 cells transduced with *Pgam5* shRNA (KD) were treated by acetaminophen (APAP, mM) with/without z-VAD-fmk (zVAD, μM). NS, non-specific shRNA. Knockdown of PGAM5 expression was confirmed by western blotting.

Figure S3

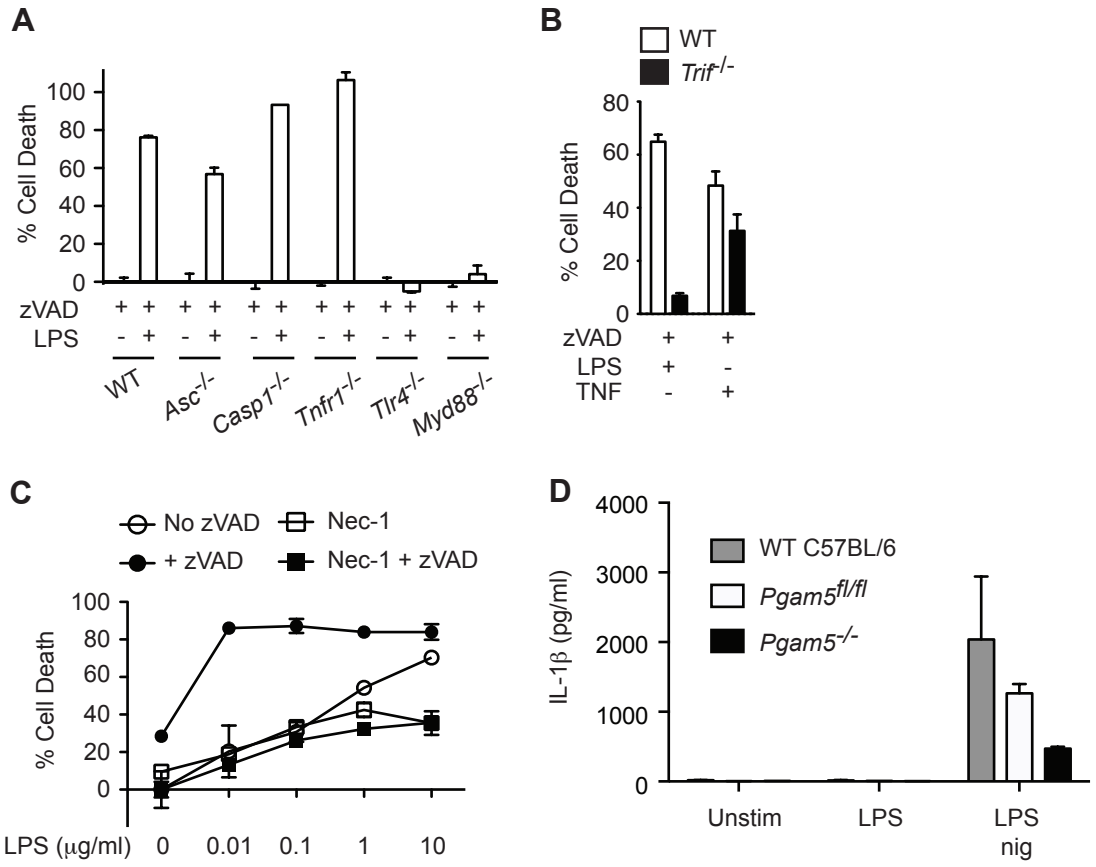


Fig. S3. LPS-induced necroptosis in macrophages requires TRIF, MyD88, and RIPK1 kinase acitivity.
(**A-B**) J2-transformed macrophages of the indicated genotypes or (**C**) wild type (WT) J2 macrophages were pretreated with z-VAD-fmk (zVAD) and/or Nec-1 for 1 hour, followed by stimulation with LPS. Cell death was measured 6 hours later.
(**D**) IL-1β secretion by LPS+nigericin treated BMDMs from mice of the indicated genotypes showed that PGAM5 is required for optimal IL-1β secretion.