

Figure S1. Inhibition of TAK1 with 5z-7 promotes TNF α -induced cell death. **A.** Cell death assessed by propidium iodide (PI) staining of TAK1+/+ and TAK1-/- MEFs treated with vehicle control, TNF α , or 5z-7 plus TNF α , with or without Nec-1. * $P < 0.05$ vs Con; # $P < 0.05$ vs TAK1+/+ 5z-7+TNF; § $P < 0.05$ vs TAK1-/- 5z-7+TNF. n.s., non-significant. **B.** Western blots for the indicated proteins from TAK1+/+ and TAK1-/- MEFs.

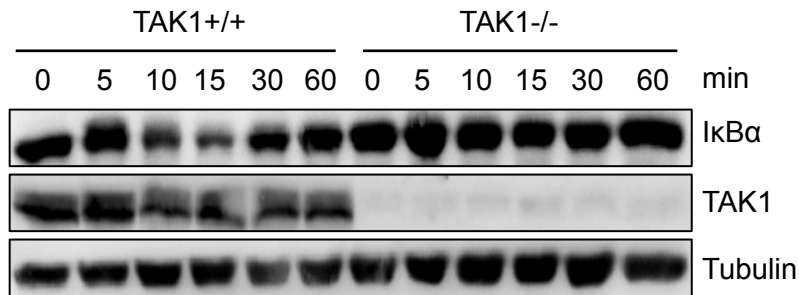
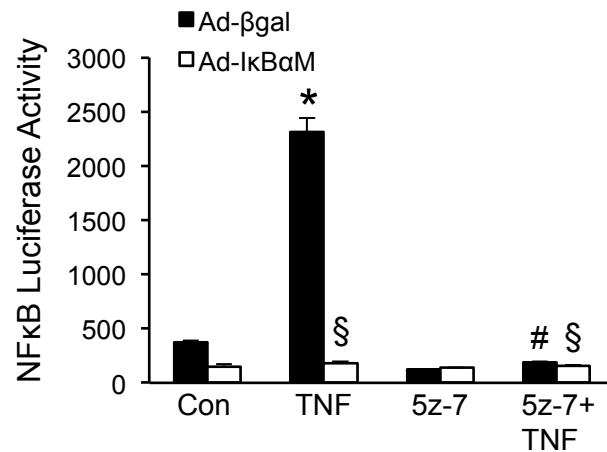
A**B**

Figure S2. TAK1 is essential for TNF α -induced NFκB activation. **A.** Western blotting with the indicated antibodies from cellular extracts of TAK1^{+/+} and TAK1^{-/-} MEFs stimulated with 10 ng/ml TNF α for the indicated time periods. **B.** Relative NFκB-luciferase activity from H9c2 myocytes infected with Ad-βgal or Ad-IκBαM (IκBα S32/36A mutant) along with an adenovirus encoding the NFκB-luciferase reporter for 24 h, followed by treatment with vehicle control, TNF α , 5z-7, or 5z-7 plus TNF α for 12 h. * P < 0.01 vs Con; # P < 0.05 vs TNF. § P < 0.05 vs Ad-βgal TNF.

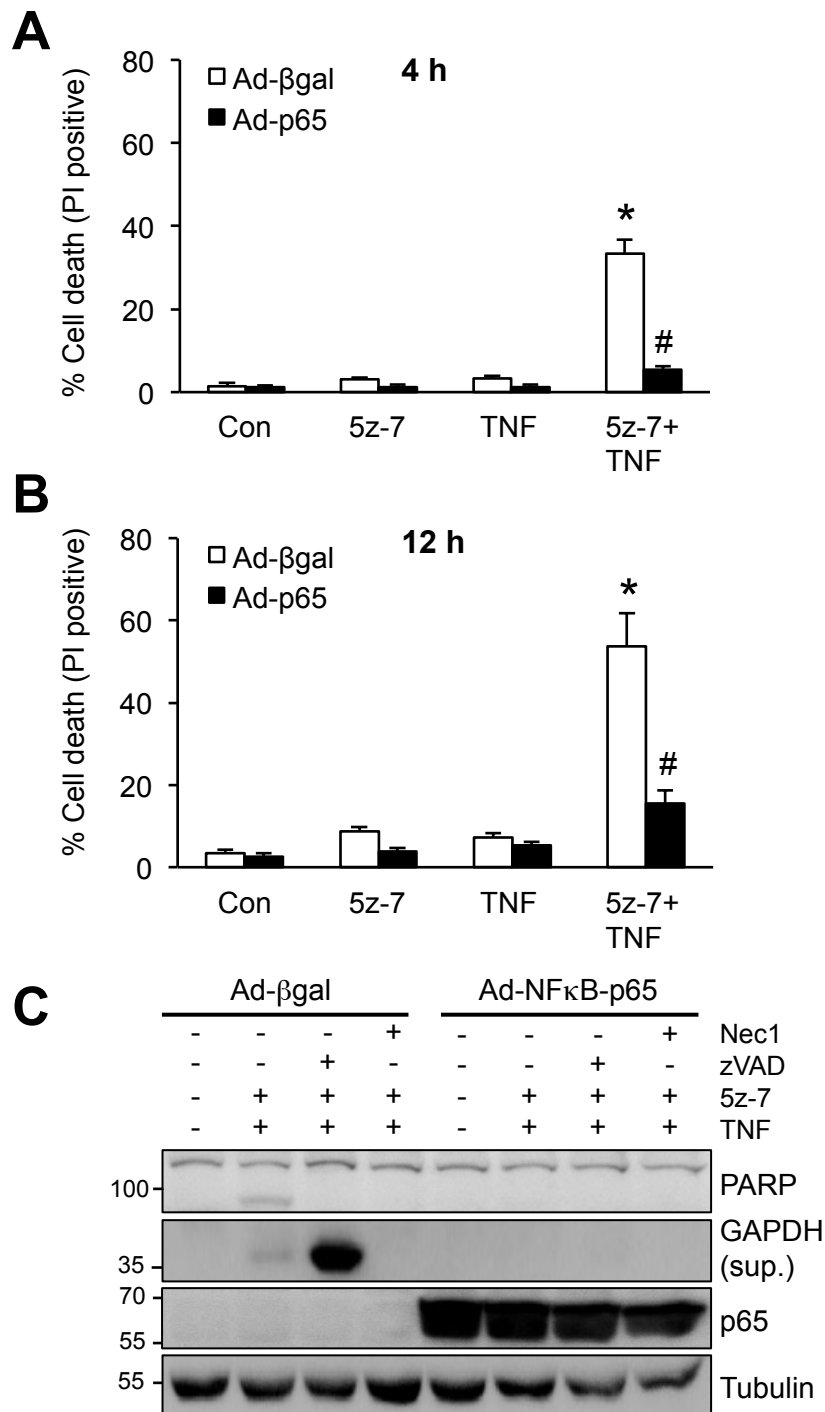


Fig S3. NFκB-p65 inhibits necroptotic cell death triggered by TAK1 inhibition. **A.** Quantification of cell death in H9c2 myocytes infected with β-gal or NFκB-p65 adenoviruses for 24 h, followed by stimulation with vehicle control, TNFα, 5z-7 for 4 h. **P* < 0.05 vs Con. #*P* < 0.05 vs Ad-βgal 5z-7+TNF. **B.** Quantification of cell death in cells treated as in A for 12 h. **P* < 0.05 vs Con; #*P* < 0.05 vs Ad-βgal 5z-7+TNF. **C.** Western blots for the indicated proteins from H9c2 myocytes infected with β-gal or NFκB-p65 adenoviruses for 24 h, followed by treatment with vehicle control or TNFα for 4 h, in the presence or absence of 5z-7 or Nec-1.

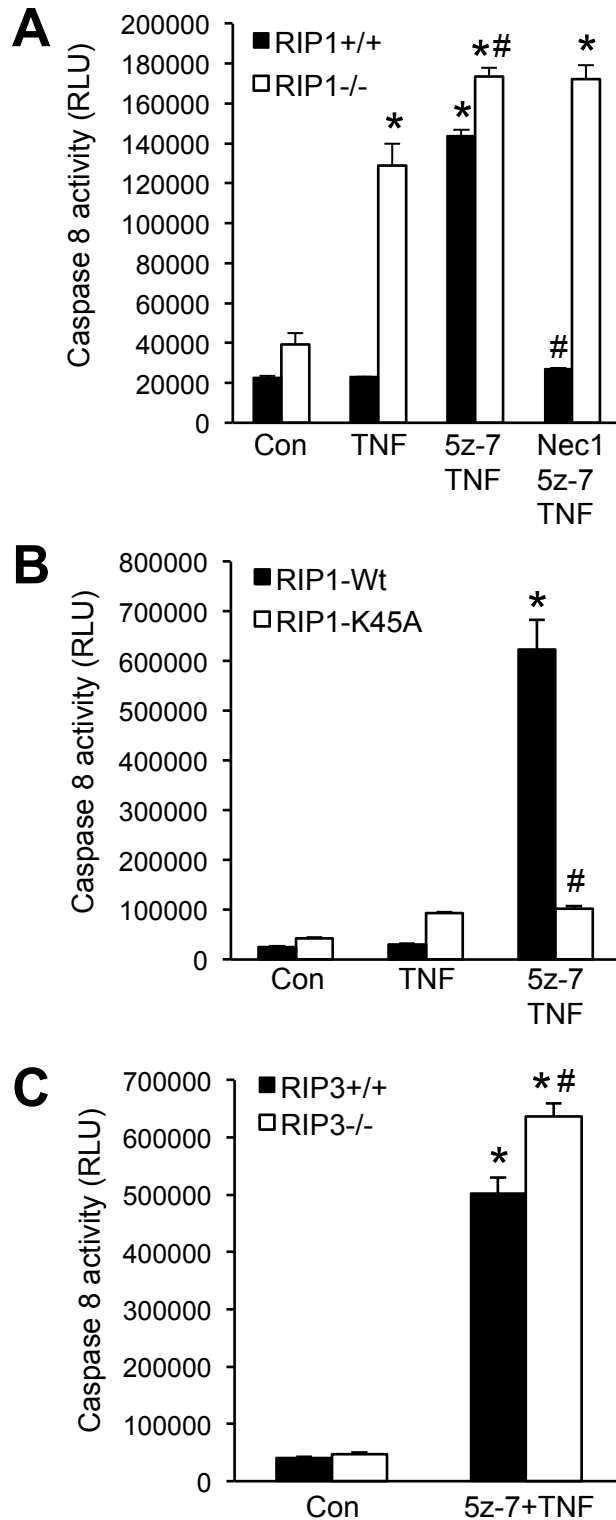


Figure S4. Role of RIP1 and RIP3 in TNF α -induced caspase 8 activity in the setting of TAK inhibition. **A.** Caspase 8 activity in RIP1^{+/+} and RIP1^{-/-} MEFs treated as indicated for 4 h. * $P < 0.01$ vs Con; # $P < 0.05$ vs RIP1^{+/+} 5z-7 plus TNF. **B.** Caspase 8 activity in RIP1^{-/-} MEFs reconstituted with wild-type (Wt) or K45A mutant of RIP, treated as indicated for 4 h. * $P < 0.05$ vs Con; # $P < 0.05$ vs RIP1-Wt. **C.** Caspase 8 activity in RIP3^{+/+} and RIP3^{-/-} MEFs treated with vehicle control or 5z-7 plus TNF α for 4 h. * $P < 0.05$ vs Con; # $P < 0.05$ vs RIP3^{+/+}.