SUPPLEMENTAL MATERIAL

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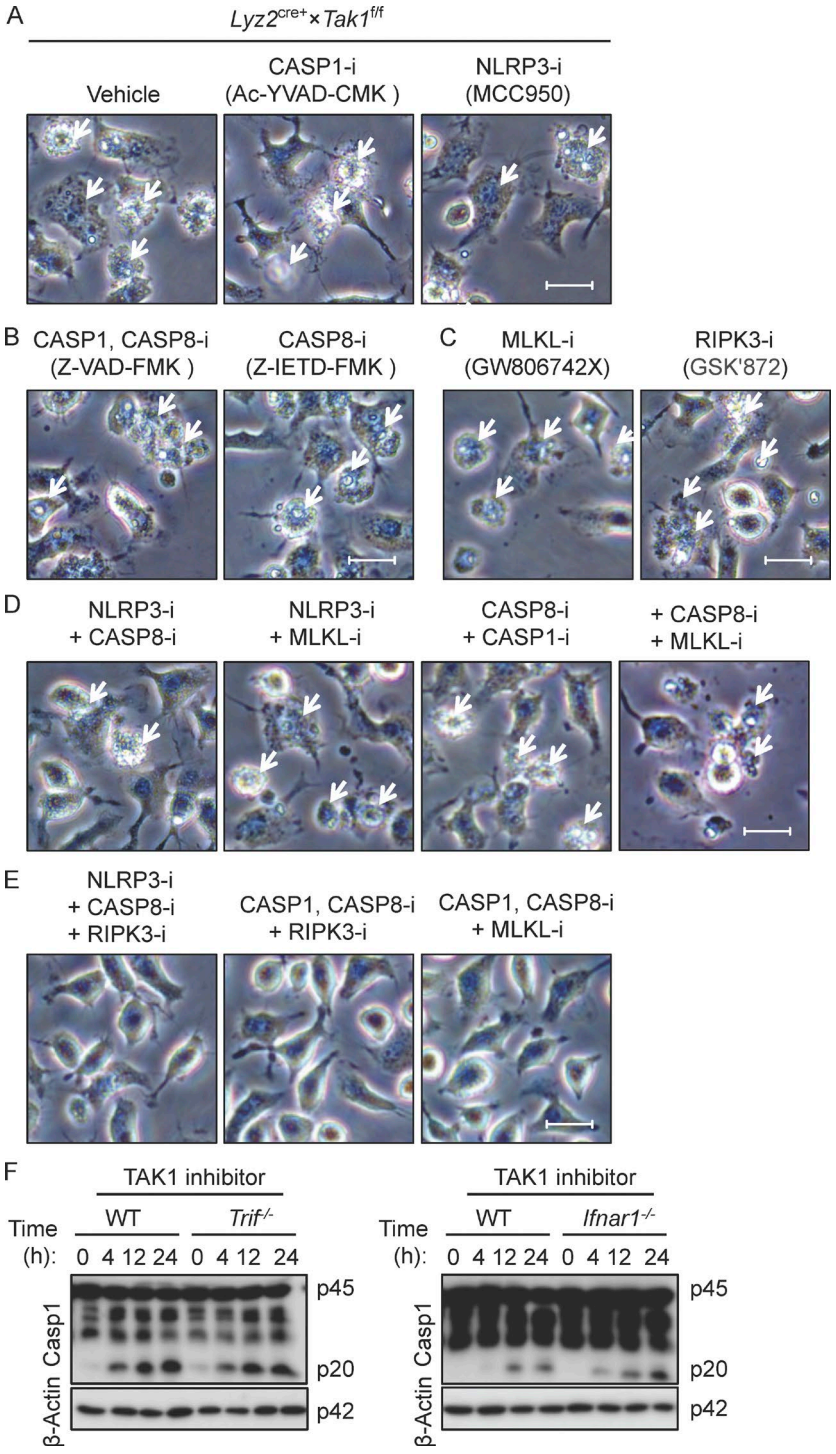

Figure S1. **A combination of inhibitors that specifically block apoptosis, necroptosis, and pyroptosis rescue TAK1-deficient BMDMs from cell death. (A–E)** Microscopic analysis of cell death in *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ BMDMs treated with DMSO control (left: vehicle) or inhibitors at 24 h in culture. Bars, 20 µm. **(A)** Treated with inhibitors of inflammasome components, CASP1-i (middle) or NLRP3-i (right). **(B)** Treated with apoptosis inhibitors Pan-CASP-i (left) or CASP8-i (right). **(C)** Treated with necroptosis inhibitors MLKL-I (left) or RIPK3-i (right). **(D)** Treated with combinations of inhibitors targeting dual pathways of cell death including NLRP3-i + CASP8-i or NLRP3-i + MLKL-i or CASP8-i + CASP1-i or CASP8-i + MLKL-i. **(E)** Treated with combinations of inhibitors targeting three major programmed pathways of cell death including NLRP3-i + CASP8-i + RIPK3-i or Pan CASP-i + RIPK3-i or Pan CASP-i + MLKL-i. Arrows indicate dead cells. **(F)** Immunoblot analysis of pro–caspase-1 (p45) and the active caspase-1 subunit p20 (p20) in TAK1i-treated *Trif*$^{-/-}$ and *Ifnar*$^{-/-}$ compared with WT BMDMs. Data are a representative of three independent experiments (A–F). "p" in Western blots denotes protein molecular weight.

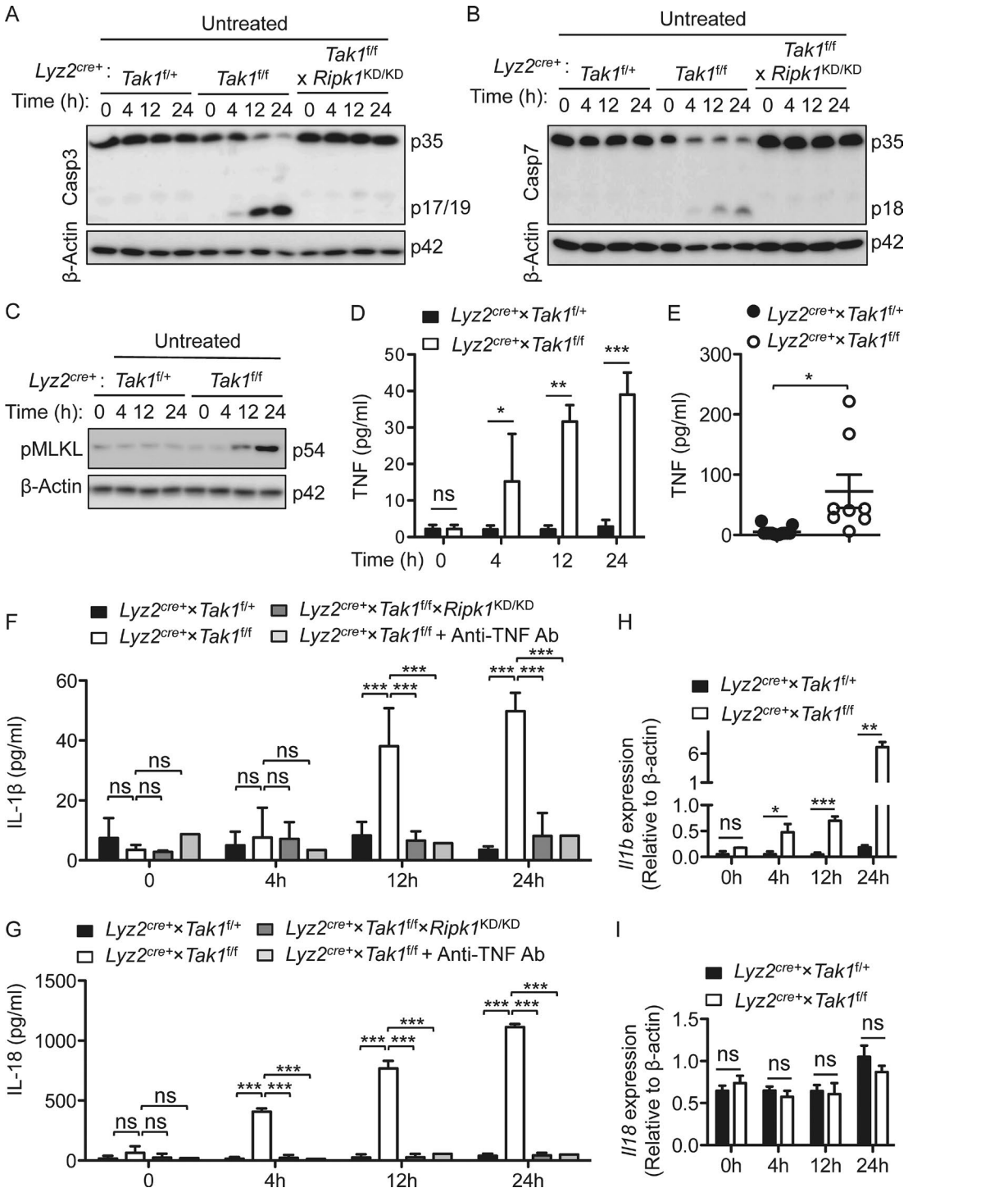

Figure S2. **TAK1 deficiency results in spontaneous cell death and inflammatory cytokine secretion in BMDMs. (A and B)** Analysis of caspase-3 and caspase-7 cleavage in untreated *Lyz2*$^{cre+}$ × *Tak1*$^{f/+}$ (TAK1 HT-control) and *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ (TAK1 KO) and *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ × *Ripk1*^{K45A} (TAK1 KO with RIPK1 kinase dead) BMDMs. **(C–E)** Phosphorylation status of MLKL (C), quantification of secreted TNF from BMDMs (D), and TNF levels in serum from TAK1 KO (*n* = 13) and HT-control (*n* = 8). **(F and G)** Quantification of secreted IL-1β (F) and IL-18 (G) in HT-control, *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ (TAK1 KO), *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ × *Ripk1*^{K45A} (TAK1 KO with RIPK1 kinase–dead), and *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ (TAK1 KO) BMDMs treated with anti-TNF Ab that were left in culture for the indicated times (*n* = 3). **(H and I)** Quantitative PCR analysis of *Il1b* (H) and *Il18 from* untreated TAK1 KO BMDMs compared with TAK1 HT-control (*n* = 3). '"p" in Western blots denotes protein molecular weight. All data are presented as mean ± SEM. P < 0.05 is considered statistically significant. *, P < 0.05; **, P < 0.01; ***, P < 0.001 (two-tailed *t* test). Data are representative of three independent experiments.

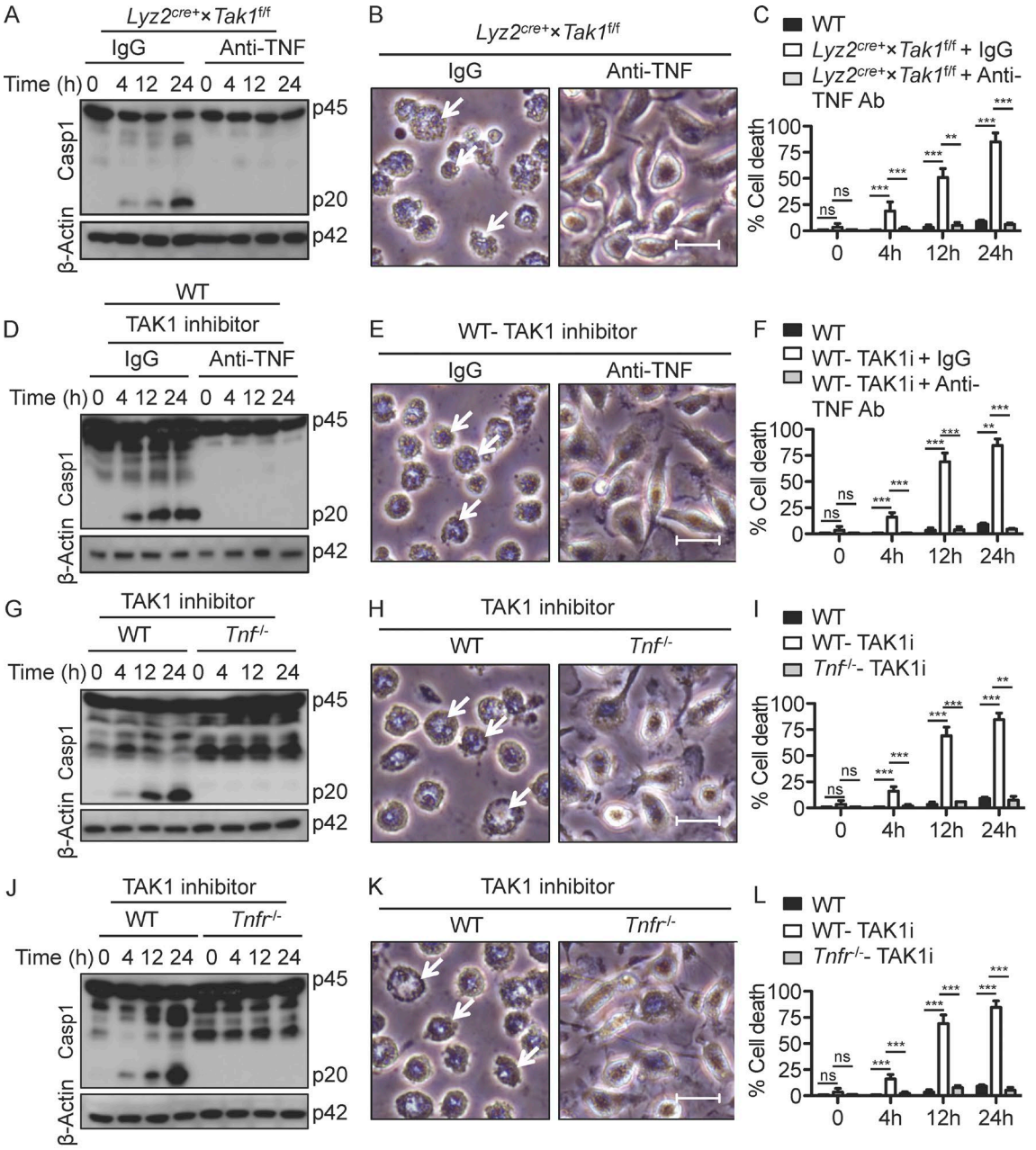

Figure S3. **TNF signaling is critical for spontaneous NLRP3 inflammasome activation in TAK1-deficient mice. (A–C)** Immunoblot analysis of pro–caspase-1 (p45) and the active caspase-1 subunit p20 (p20; A) and cell death analysis by microscopy (B) or by LDH release (C) in *Lyz2*$^{cre+}$ × *Tak1*$^{f/f}$ BMDMs treated with TNF-neutralizing Ab or its corresponding isotype IgG control. **(D–F)** Immunoblot analysis of pro–caspase-1 (p45) and the active caspase-1 subunit p20 (p20; D) and cell death analysis by microscopy (E) or by LDH release (F) in TAK1i-treated WT BMDMs in the presence of TNF-neutralizing Ab or its corresponding isotype IgG control. **(G–I)** Immunoblot analysis of pro–caspase-1 (p45) and the active caspase-1 subunit p20 (p20; G) and cell death analysis by microscopy (H) or by LDH release (I) in TAK1i-treated WT or *Tnf*$^{-/-}$ BMDMs assessed at the indicated times. **(J–L)** Immunoblot analysis of pro–caspase-1 (p45) and the active caspase-1 subunit p20 (p20; J) and cell death analysis by microscopy (K) or by LDH release, $n = 3$ (L) in TAK1i-treated WT or *Tnfr*$^{-/-}$ BMDMs assessed at the indicated times. Bars, 20 µm (B, E, H, and K). Arrows indicate dead cells (B, E, H, and K). "p" in Western blots denotes protein molecular weight (A, D, G, and J). All data are presented as mean ± SEM. $P < 0.05$ is considered statistically significant. **, $P < 0.01$; ***, $P < 0.001$ (two-tailed *t* test). Data are representative of three independent experiments (A–L).