

SUPPLEMENTARY FIGURE LEGENDS

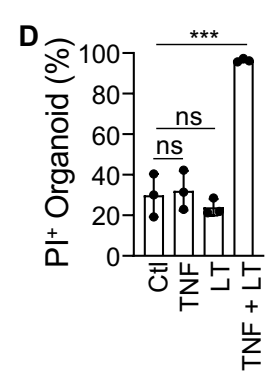
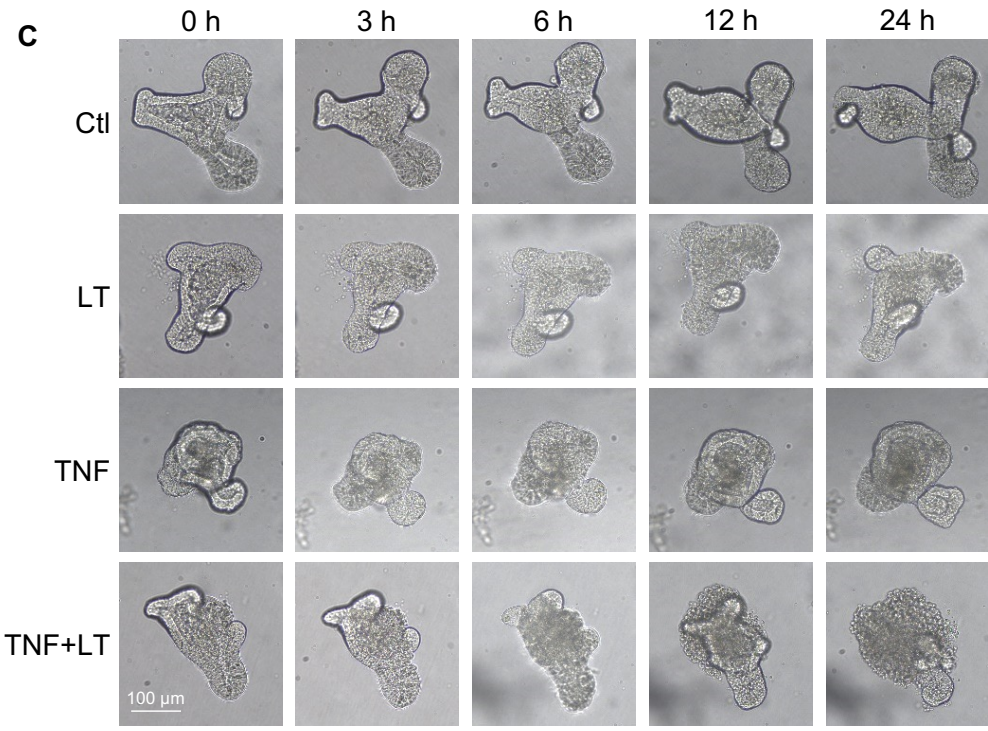
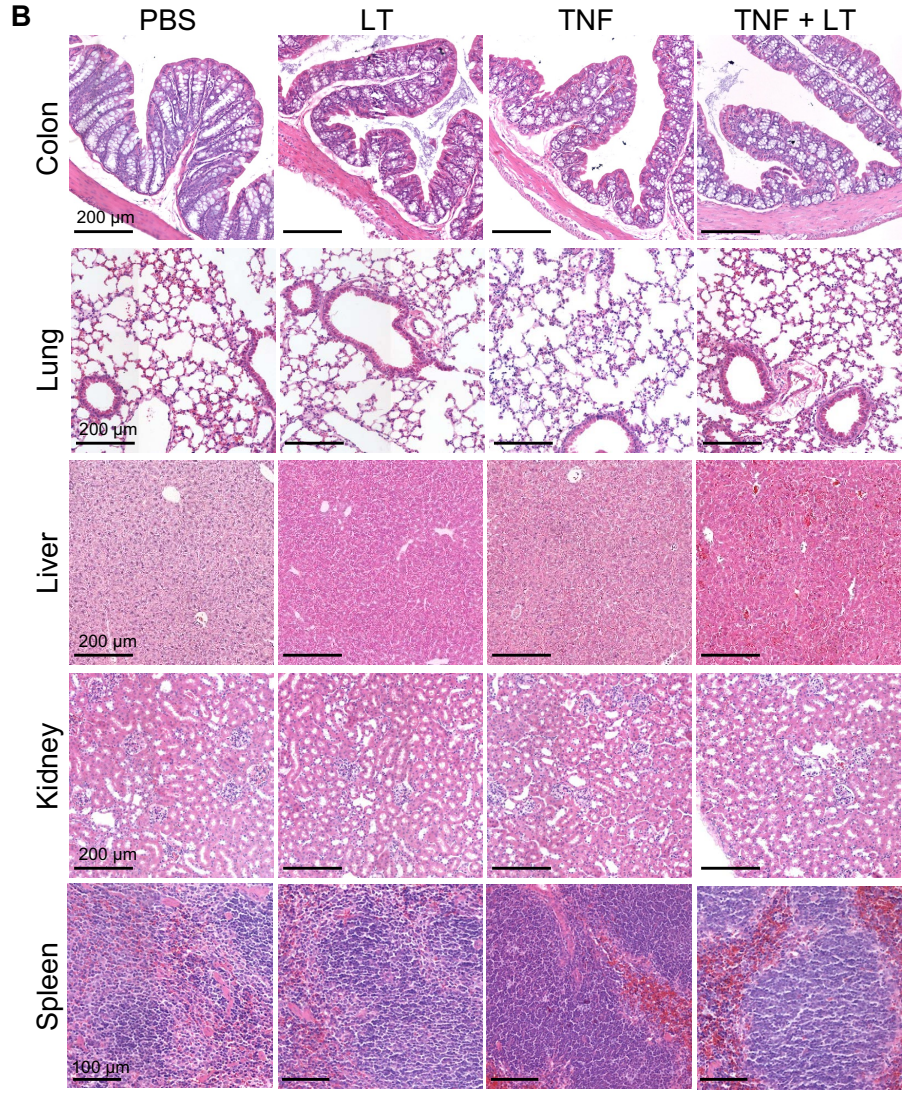
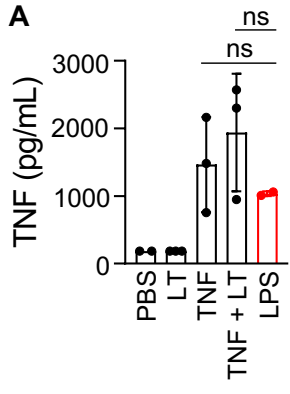
Supplementary Fig. 1. Co-treatment of sub-lethal dose of TNF+LT induced intestinal tissue damages.

(A) WT mice were i.v. injected with PBS, TNF, LT, or TNF+LT, or intraperitoneally (i.p.) injected with lipopolysaccharide (LPS) (50 mg/kg). Mice were euthanized and sera were collected for measurement of TNF 4 hours after challenge. LPS treatment was included as a positive control. Error bars represented the standard deviations of the means of technical triplicates pooled from two independent experiments. *P* values were calculated using an unpaired Student *t* test. ns, not significant.

(B) WT mice were i.v. injected with TNF, LT, TNF+LT, or PBS. Mice were euthanized and tissues were collected 4 hours after challenge. H&E staining of indicated tissues was shown. Scale bars, 200 μm or 100 μm as indicated. Images represent 4 mice per treatment pooled from two independent experiments.

(C and D) Small intestinal organoids from WT mice were treated with TNF (10 ng/mL) with or without LT (2 $\mu\text{g/mL}$ of LF and 2 $\mu\text{g/mL}$ of PA). Representative organoid images were taken at indicated time points after treatment (scale bar, 100 μm) (C) and percentages of PI-positive organoids were calculated

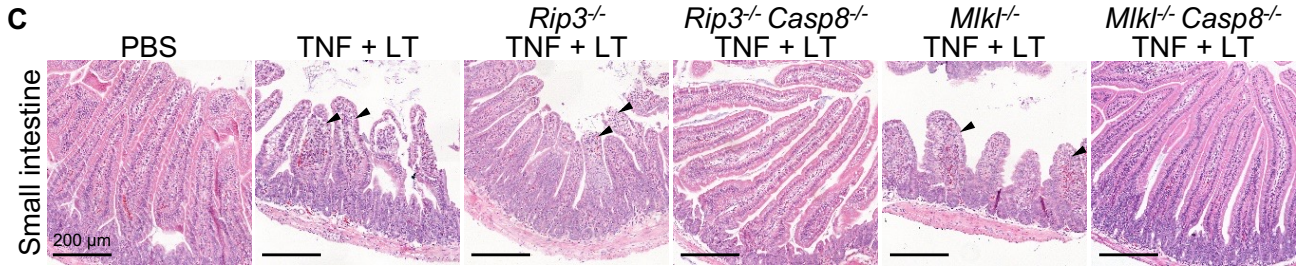
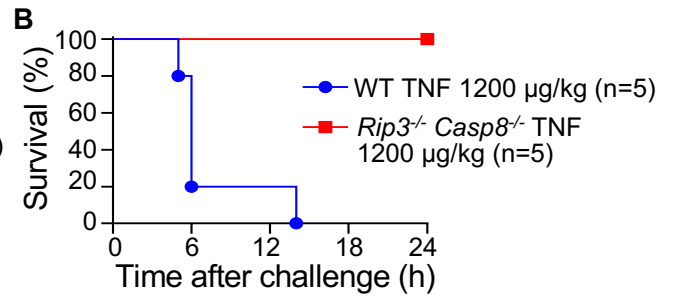
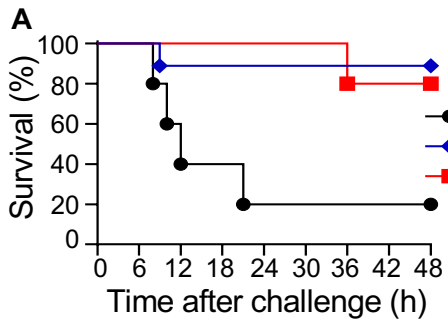
(D). *P* values were calculated using an unpaired Student *t* test. ***, $P < 0.001$; ns, not significant. Error bars represented the standard deviations of the means of technical triplicates pooled from two independent experiments.



Supplementary Fig. 2. Apoptosis and necroptosis pathways complement each other in mediating death of B6 mice co-stimulated with TNF and LT.

(A and B) Mice of indicated genotypes were i.v. injected with TNF (240 µg/kg in (A) and 1200 µg/kg in (B)) and monitored for survival rate.

(C) Mice of indicated genotypes were i.v. injected with TNF plus LT. Mice were euthanized and tissues were collected 4 hours after challenge. H&E staining was applied in small intestines. Scale bars, 200 µm. Images represent 3 mice per treatment pooled from two independent experiments.



Supplementary Fig. 3. RIP3- and Caspase-8-dependent signaling in intestinal epithelial cells determines LT+TNF-induced animal death.

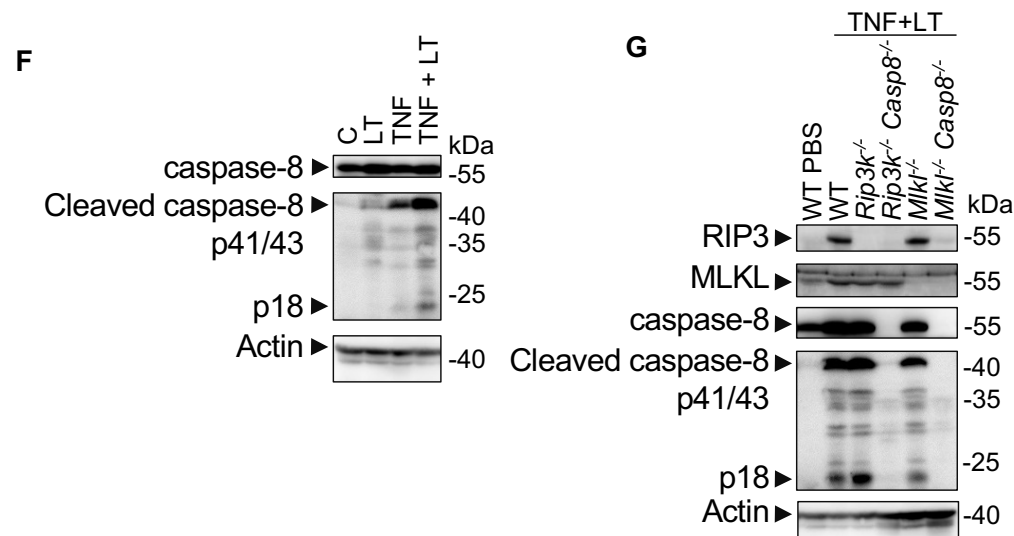
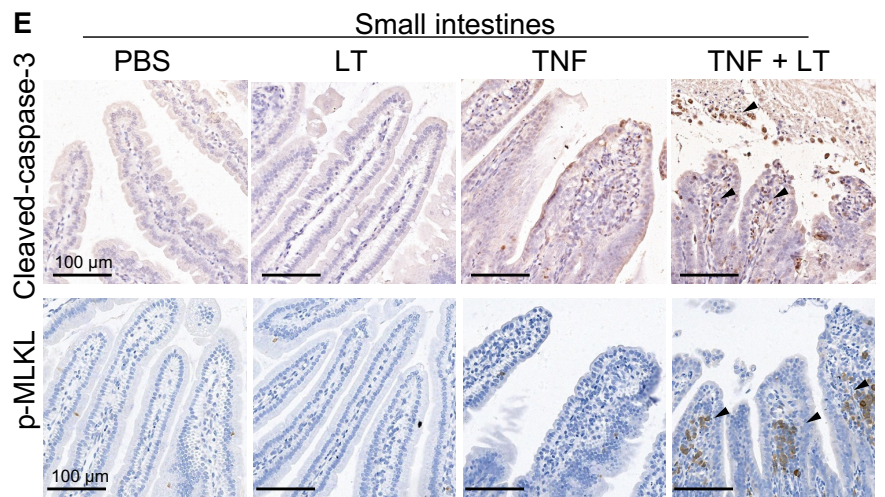
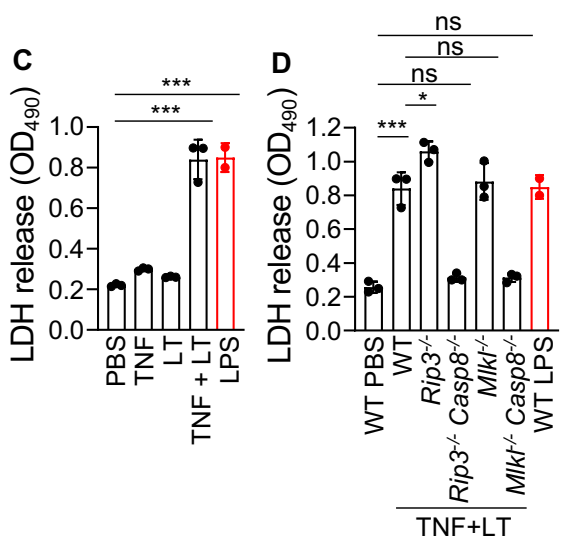
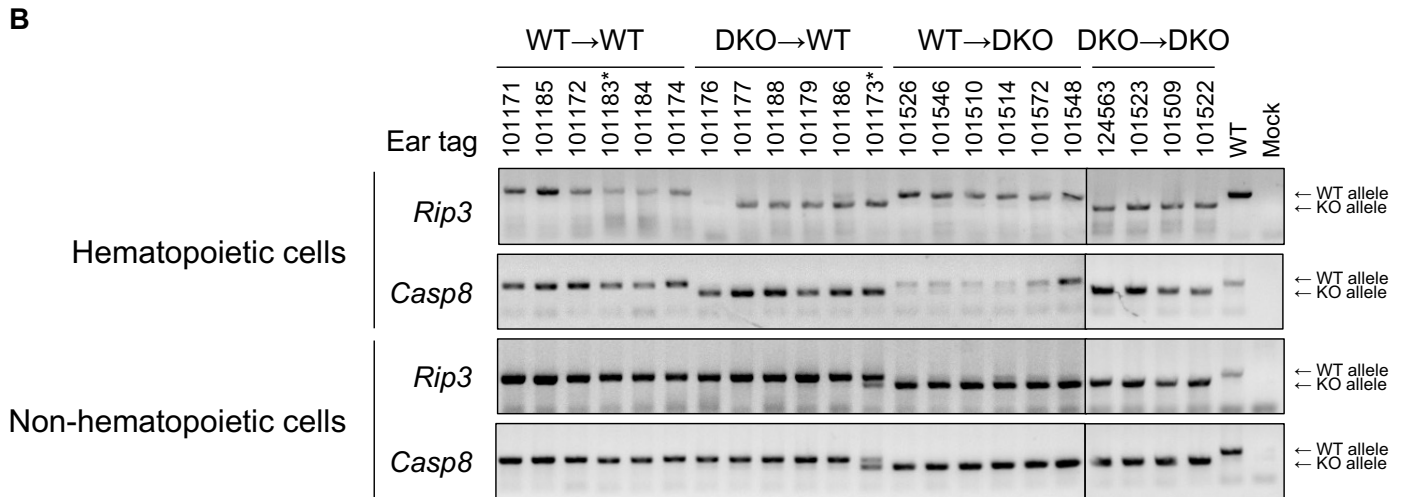
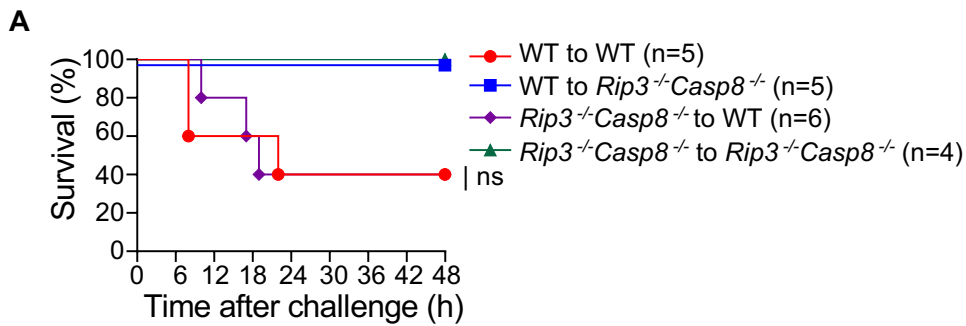
(A) WT or *Rip3*^{-/-}*Casp8*^{-/-} recipient mice were irradiated and transplanted with bone marrow cells of indicated genotypes. TNF plus LT was subsequently i.v. injected into recipient mice and survival rates were monitored.

(B) Genotyping of chimeric mice after bone marrow transplantation. KO, *Rip3* or *Casp8* knockout. DKO, *Rip3* and *Casp8* double knockout. * stands for the mouse which was not irradiated successfully and thus was excluded from the TNF+LT-challenge.

(C and D) WT mice (C) or mice of indicated genotypes (D) were i.v. injected with TNF, LT, TNF+ LT, or PBS. Mice were euthanized and tissues were collected 4 hours after challenge and lactate dehydrogenase (LDH) release in mouse serum was measured. Sera from mice i.p. injected with 50 mg/kg of LPS for 4 hours were included as positive controls. Error bars represented the standard deviations of the means of technical triplicates pooled from two independent experiments. *P* values were calculated using an unpaired Student *t* test. *, *P* < 0.05; ***, *P* < 0.001; ns, not significant.

(E) WT mice were i.v. injected with TNF, LT, TNF+LT, or PBS. Mice were euthanized and tissues were collected 4 hours after challenge. Immunohistochemistry (IHC) staining of small intestines for cleaved-caspase-3 and phospho-MLKL was performed. Scale bars, 100 μm. Images represent 4 mice per treatment pooled from two independent experiments.

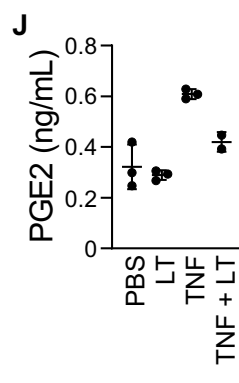
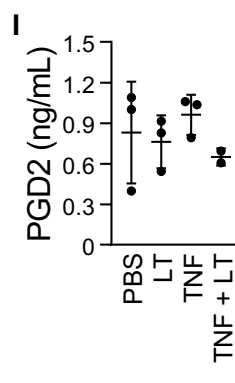
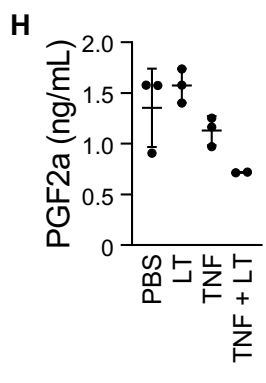
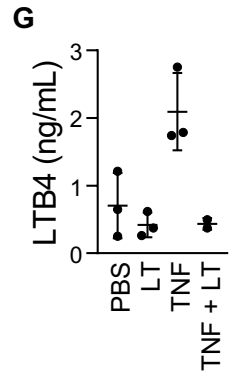
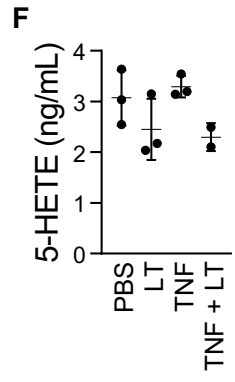
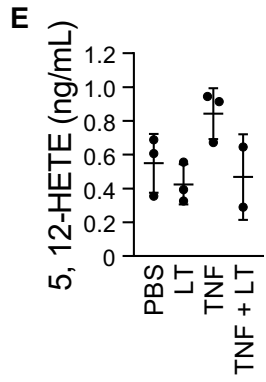
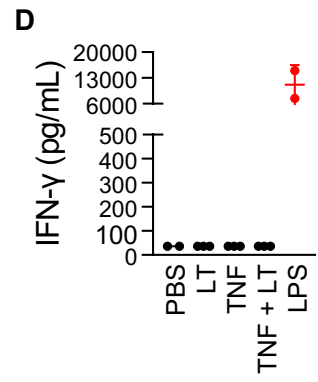
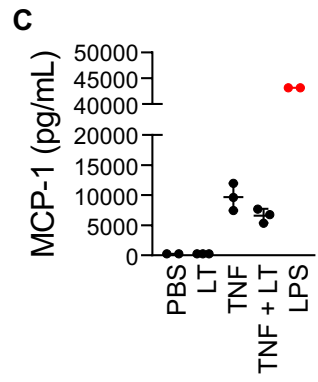
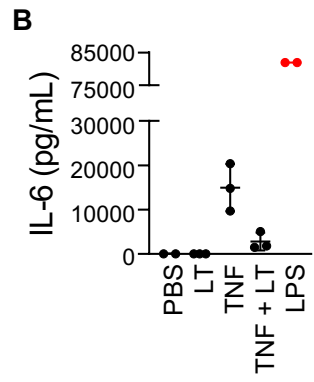
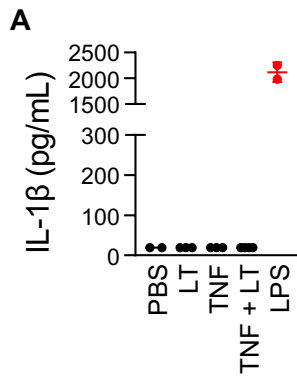
(F and G) WT mice (F) or mice of indicated genotypes (G) were i.v. injected with TNF, LT, TNF+ LT, or PBS. Small intestinal epithelia were isolated 4 hours post-challenge and subjected to western blot. Data represent 3 mice per treatment pooled from two independent experiments.



Supplementary Fig. 4. Changes in cytokines or lipid mediators do not correlate with

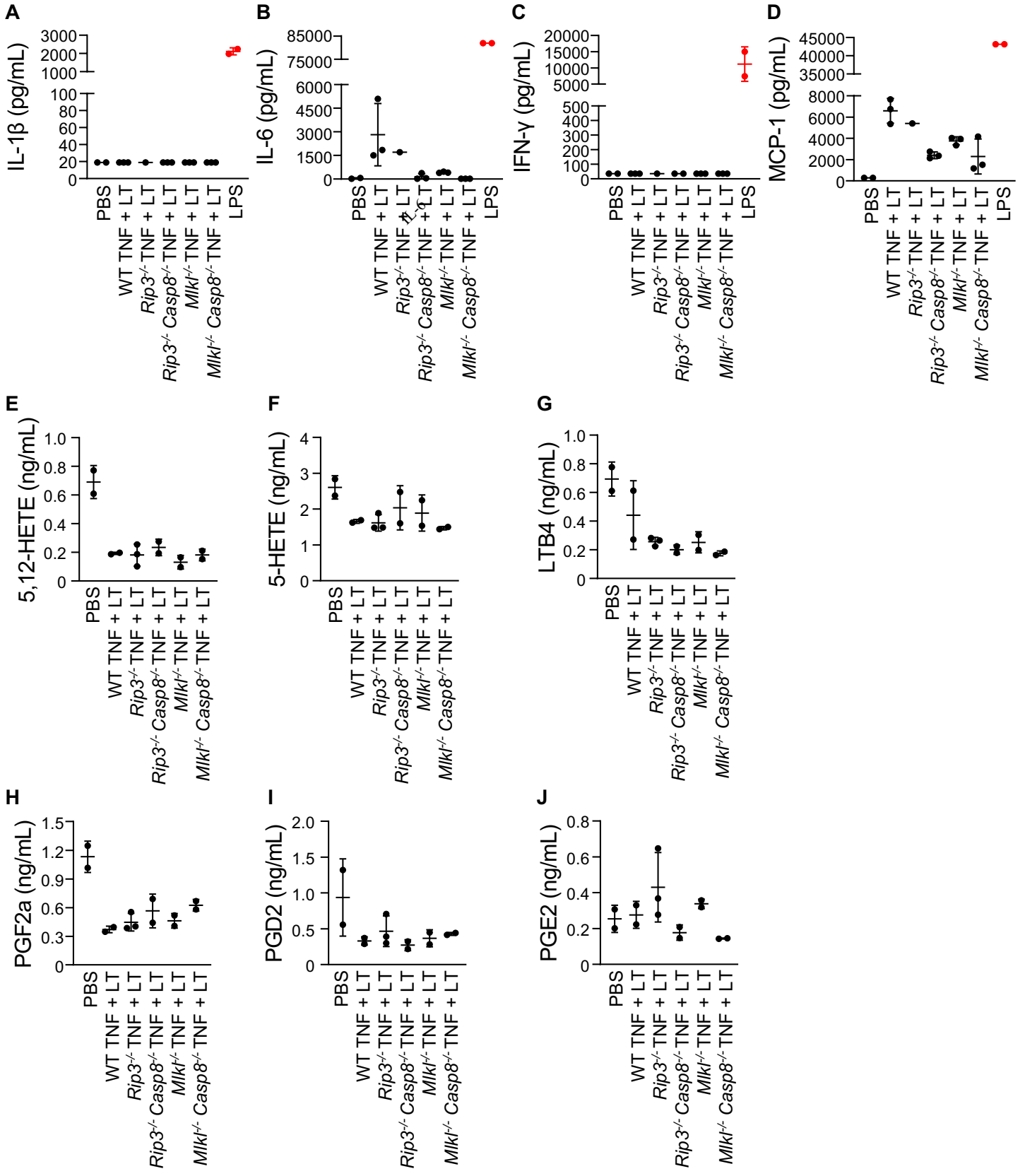
TNF+LT-induced mouse death.

WT mice were i.v. injected with TNF, LT, TNF+LT, or PBS. Mice were euthanized and sera were collected for measurement of cytokines and lipid mediators 4 hours after challenge. Sera from mice i.p. injected with 50 mg/kg of LPS for 4 hours were included as positive controls (A to D). Error bars represented the standard deviations of the means of technical triplicates pooled from two independent experiments.



Supplementary Fig. 5. Changes in cytokines or lipid mediators do not correlate with resistance of *Rip3^{-/-} Casp8^{-/-}* or *Mlkl^{-/-} Casp8^{-/-}* mice to TNF+LT-induced death.

Mice of indicated genotypes were i.v. injected with TNF plus LT. Mice were euthanized and sera were collected for measurement of cytokines and lipid mediators 4 hours after challenge. Sera from mice i.p. injected with 50 mg/kg of LPS for 4 hours were used as positive controls (A to D). Error bars represented the standard deviations of the means of technical triplicates pooled from two independent experiments.



Supplementary Fig. 6. LT induces pyroptosis in peritoneal macrophages of BALB/c but not B6 mice.

(A to C) Peritoneal macrophages from B6 or BALB/c mice were treated with LT (2 $\mu\text{g}/\text{mL}$ of LF and 2 $\mu\text{g}/\text{mL}$ of PA) for 3 hours. ATP loss (A) and LDH release (B) were measured. Error bars represented the standard deviations of the means of technical triplicates pooled from two independent experiments. Cell lysates were subjected to western blot (C).

(D) Cell lysates of HT-29 cells of indicated genotypes were subjected to western blot. Western blot results represent technical triplicates pooled from two independent experiments.

