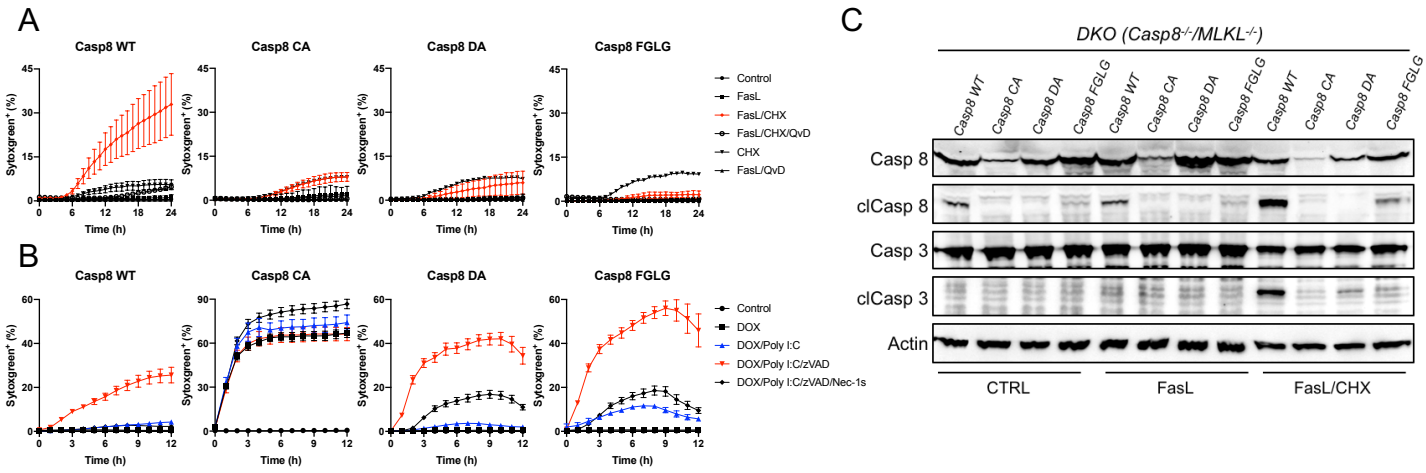

D

Casp8$^{WT/FGLG}$ x *Casp8*$^{WT/FGLG}$

Casp8	Expected %	Expected #	Observed #
WT/WT	25	43.5	44
WT/FGLG	50	87	87
FGLG/FGLG	25	43.5	**43**
	100	174	174

E

Casp8$^{WT/DA}$ x *Casp8*$^{WT/DA}$

Casp8	Expected %	Expected #	Observed #
WT/WT	25	38.25	48
WT/DA	50	76.5	70
DA/DA	25	38.25	**35**
	100	153	153

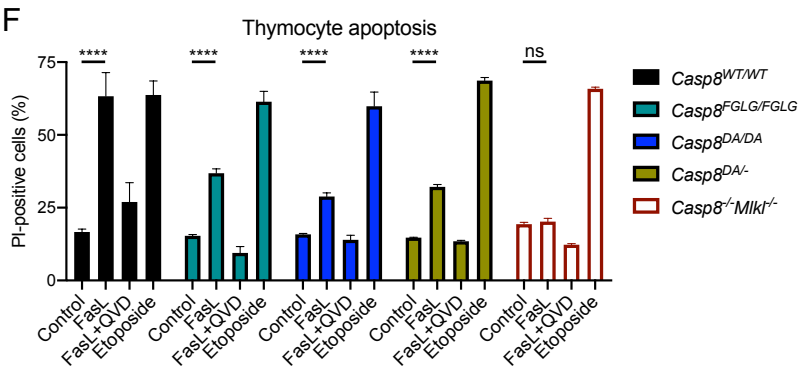

Figure S1: *Casp8*$^{FGLG/FGLG}$ and *Casp8*$^{DA/DA}$ MEFs are resistant to apoptosis and retain the ability to block necroptosis, Related to Figure 1.

A: *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ MEFs expressing caspase-8 WT, C362A, D387A or F122GL123G were stimulated as indicated. Cell death was monitored by SYTOX Green uptake using an Incucyte Kinetic Live Cell Imager. B: *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ MEFs expressing doxycycline (DOX)-inducible MLKL and Caspase-8 WT, C362A, D387A or F122GL123G were stimulated as indicated. Cell death was monitored by SYTOX Green uptake using an Incucyte Kinetic Live Cell Imager. C: Western blots for Caspase-8, cleaved Caspase-8, Caspase-3, cleaved Caspase-3 and β-Actin in cell lysates of *Casp8*$^{-/-}$*Mlkl*$^{-/-}$ MEFs expressing Caspase-8 WT, CA, DA or FGLG that were stimulated as indicated for 20 hours. D, E: Expected and observed frequency of genotypes in offspring from intercrosses of *Casp8*$^{WT/FGLG}$ (D) and *Casp8*$^{WT/DA}$ (E) mice. F: Cell death of thymocytes isolated from mice of the indicated genotypes by propidium iodide (PI) uptake as determined by flow cytometry 24 hours after indicated treatment.

**** p<0.0001, ns not significant

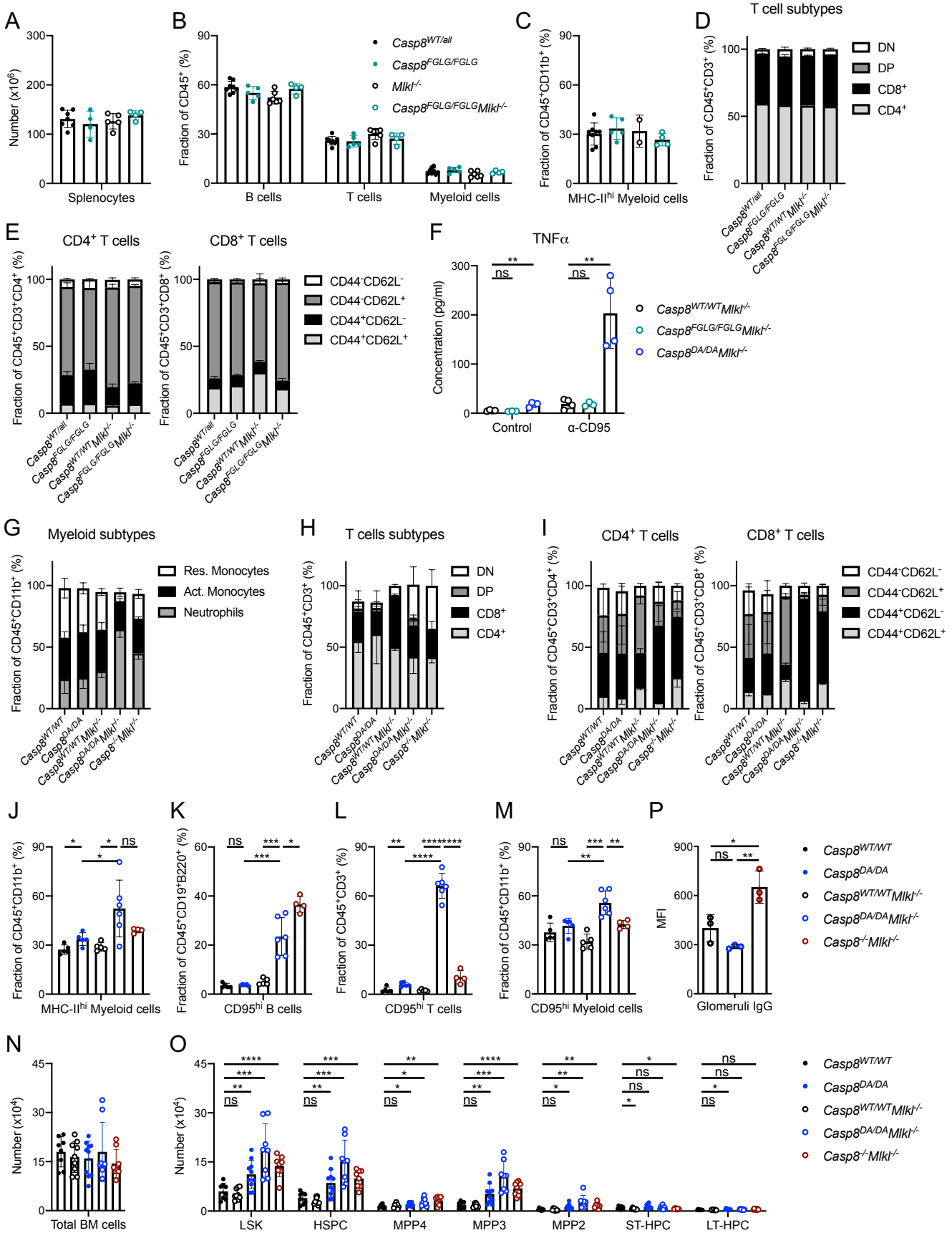

Figure S2: Ablation of *Mlkl* controls inflammation in *Casp8*$^{DA/DA}$, but not *Casp8*$^{FGLG/FGLG}$ mice, Related to Figure 2.
A: Total splenic cell numbers. B, C: Percentages of splenic B cells, T cells and Myeloid cells (B), and MHC-II^{hi} Myeloid cells (C) as determined by flow cytometry. D, E: T cell subtypes (D) and CD44 and CD62L status of CD4$^+$ (left) and CD8$^+$ (right) T cells (E) as determined by flow cytometry. F: TNFα concentration in sera of mice 4 hours post-IP injection with 1.2 mg/kg a-CD95 or PBS as measured by ELISA. G, H: Myeloid cell subtypes (G) and T cell subtypes (H) as determined by flow cytometry. I: CD44 and CD62L status of CD4$^+$ (left) and CD8$^+$ (right) T cells as determined by flow cytometry. J-M: MHC-II^{hi} Myeloid cells (J), and CD95^{hi} B cells (K), T cells (L) and Myeloid cells (M) as determined by flow cytometry. N: Total bone-marrow (BM) cell numbers. O: Bone-marrow stem cell subsets as determined by flow cytometry. P: IgG deposition in glomeruli as determined by IF on kidney sections.
**** p<0.0001, *** p<0.0005, ** p<0.005, * p<0.05, ns not significant

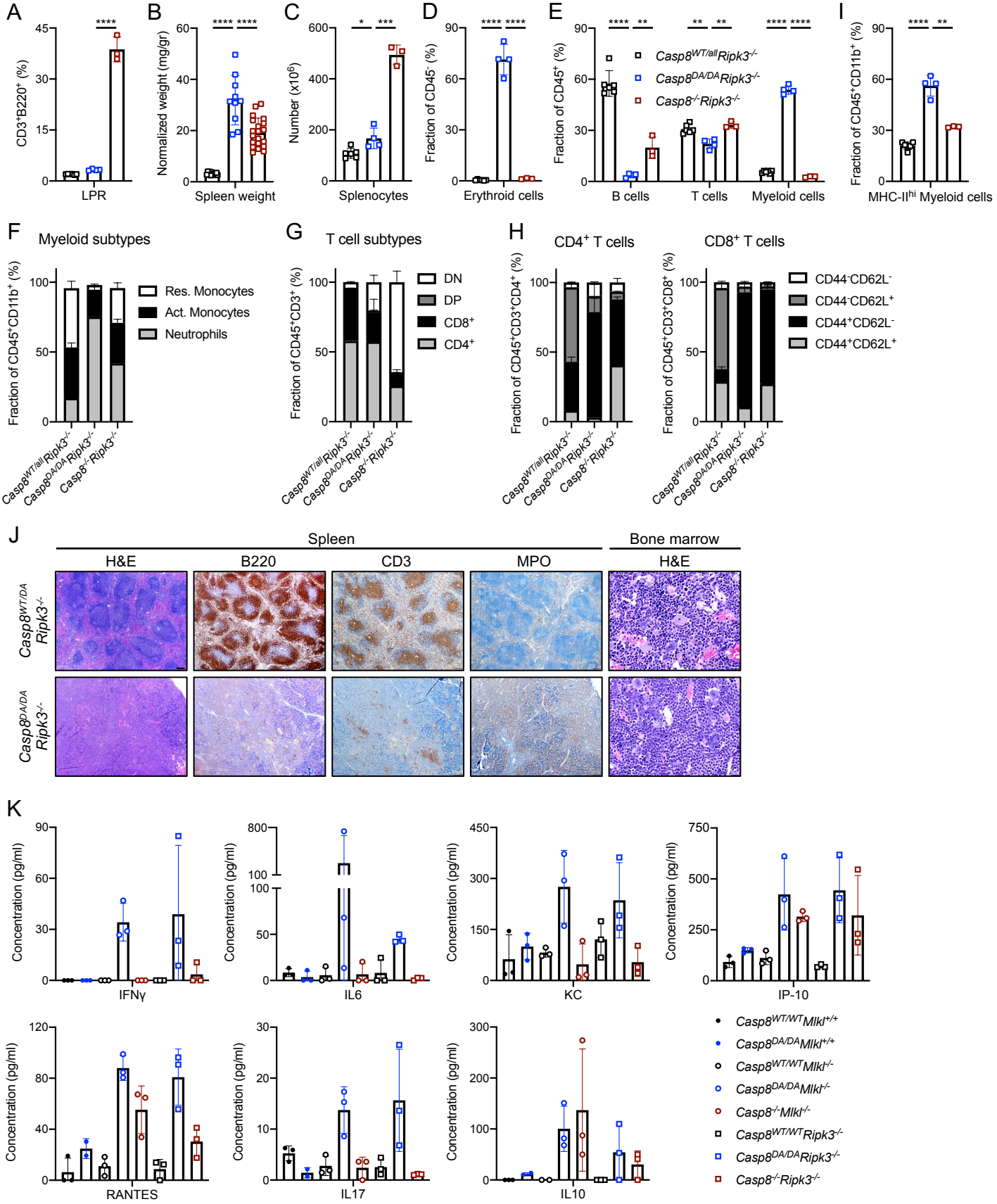

Figure S3: Ablation of *Ripk3* controls lethal inflammation in *Casp8*$^{DA/DA}$ mice, Related to Figure 2.

A: Percentages of splenic CD3$^+$B220$^+$ cells as determined by flow cytometry. B: Normalized spleen weight. C: Total splenic cell numbers. D, E: Percentages of splenic Erythroid cells (D), B cells, T cells and Myeloid cells (E) as determined by flow cytometry. F, G: Myeloid cell subtypes (F) and T cell subtypes (G) as determined by flow cytometry. H: CD44 and CD62L status of CD4$^+$ (left) and CD8$^+$ (right) T cells as determined by flow cytometry. I: MHC-II^{hi} Myeloid cells as determined by flow cytometry. J: Representative images of H&E of spleen (left) and bone marrow (right) and immunohistochemistry (IHC) for B220, CD3 and MPO on lung sections (middle). K: Serum levels of the indicated cytokines as measured by Multiplex ELISA.

**** p<0.0001, *** p<0.0005, ** p<0.005, * p<0.05

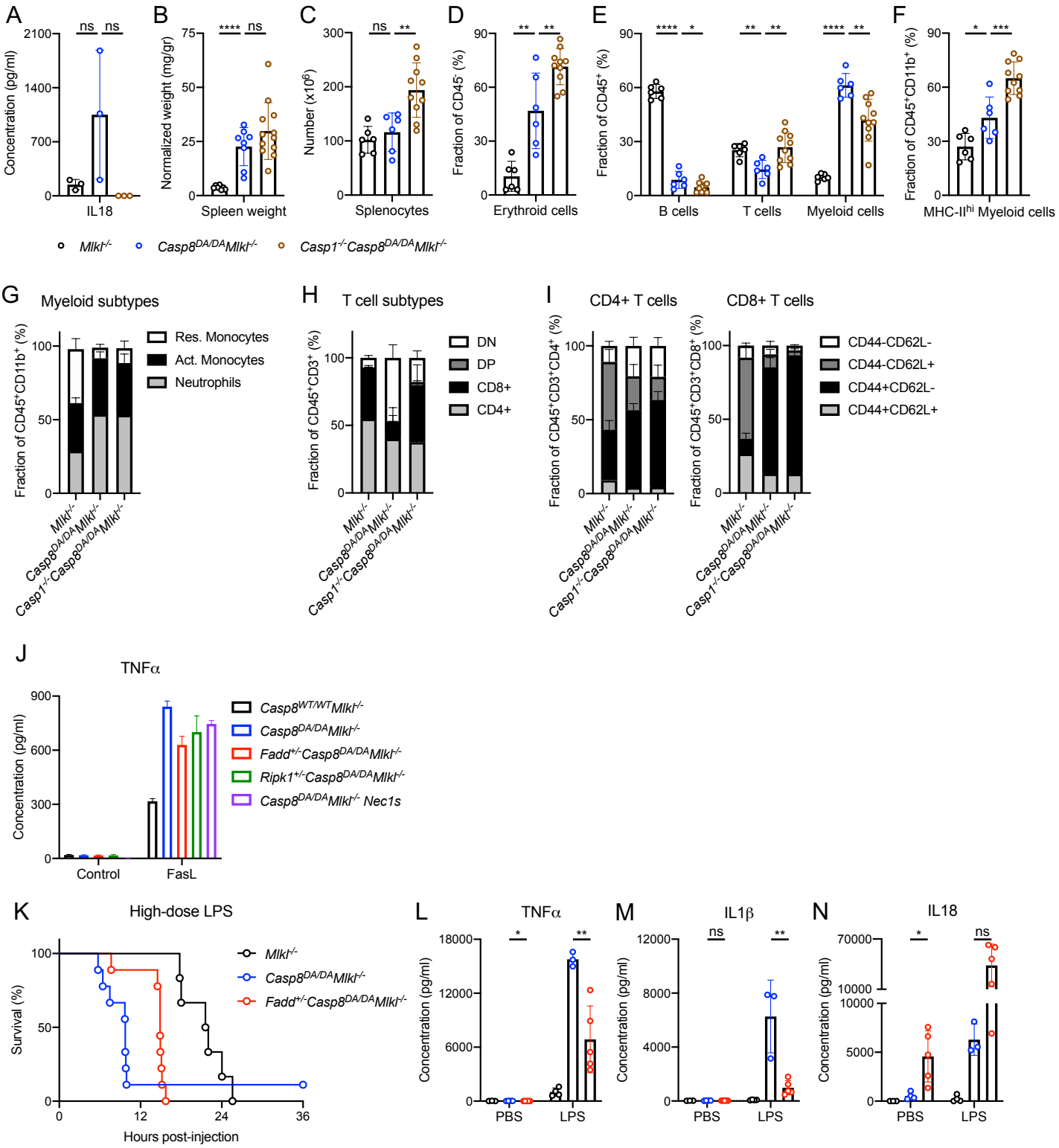

Figure S4: The inflammatory phenotype of *Casp8$^{DA/DA}$Mlkl$^{-/-}$* mice is mediated by FADD and RIPK1, but not by Caspase-1 and/or -11, Related to Figure 4.

A: Serum cytokine concentration of IL18 as measured by ELISA. B: Normalized spleen weight. C: Total splenic cell numbers. D-F: Percentages of splenic Erythroid cells (D), B cells, T cells and Myeloid cells (E) and MHC-II^{hi} Myeloid cells (F) as determined by flow cytometry. G, H: Myeloid cell subtypes (G) and T cell subtypes (H) as determined by flow cytometry. I: CD44 and CD62L status of CD4^{+} (left) and CD8^{+} (right) T cells as determined by flow cytometry. J: TNFα concentration in supernatant 24 hours post-CD95L stimulation. K: Survival of mice of the indicated genotypes after 56 mg/kg IP-injected LPS. *Mlkl$^{-/-}$* n=6, *Casp8$^{DA/DA}$Mlkl$^{-/-}$* n=9, *Fadd$^{+/-}$Casp8$^{DA/DA}$Mlkl$^{-/-}$* n=9. L-N: Serum concentration of TNFα (L), IL1β (M), and IL18 (N) in mice 2 hours post-IP injection with 56 mg/kg LPS or PBS as measured by ELISA. BMDMs generated from mice of the indicated genotypes were pre-stimulated with LPS (20 ng/ml, overnight) and treated with CD95L.

**** p<0.0001, *** p<0.0005, ** p<0.005, * p<0.05, ns not significant

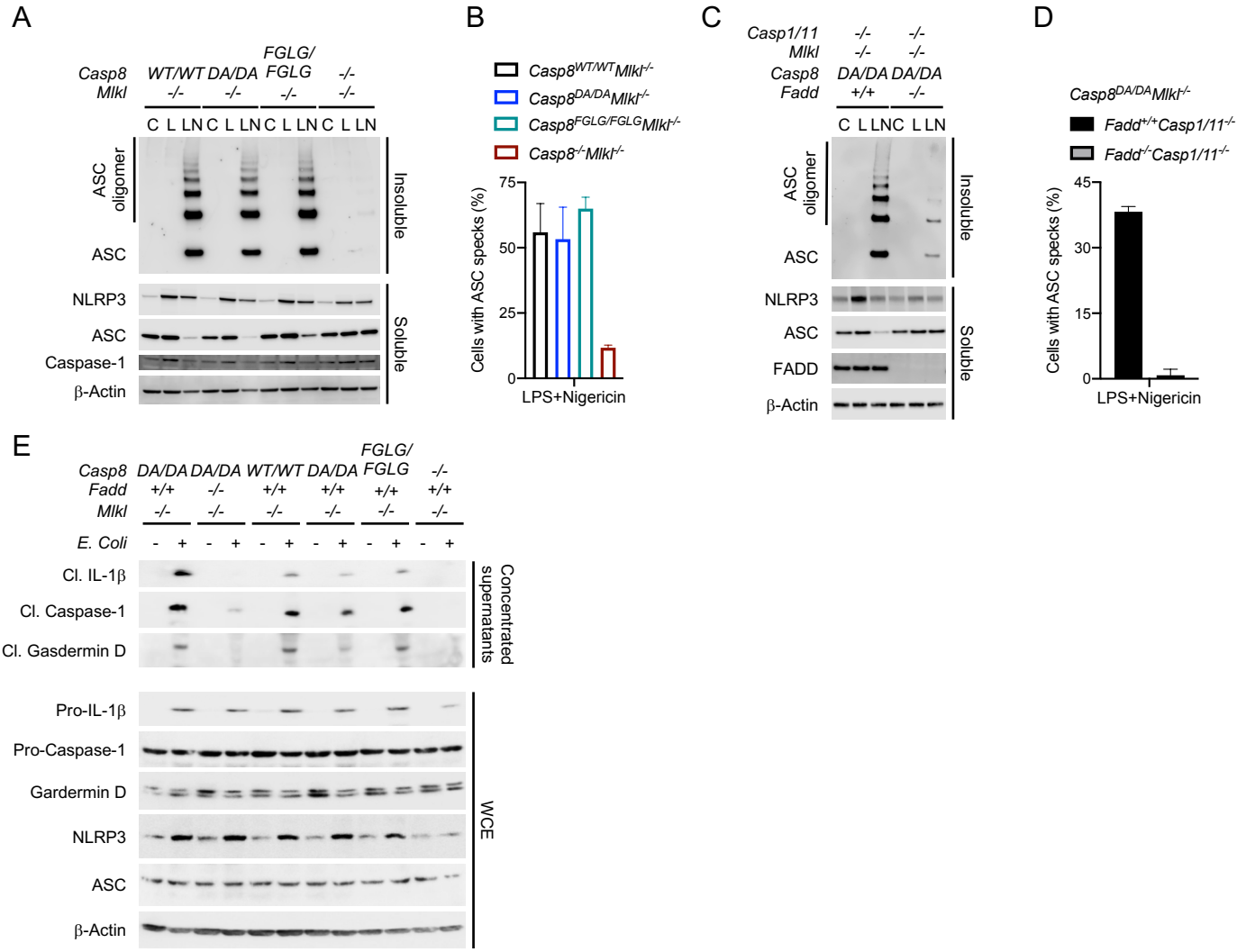

Figure S5: Caspase-8 and FADD are required for optimal inflammasome activation in macrophages, Related to Figure 6.

A, C: Western blots for ASC in the cross-linked insoluble fraction and ASC, NLRP3, Caspase-1 and β-Actin in the soluble fraction of lysates from LPS and nigericin-treated BMDMs generated from mice of the indicated genotypes. C: Control, L: LPS, LN: LPS+nigericin. B, D: ASC speck formation in LPS and nigericin-treated BMDMs generated from mice of the indicated genotypes as determined by IF. E: Western blots for cleaved (Cl.) IL1β, cleaved Caspase-1 and cleaved Gasdermin D in concentrated supernatants, and pro-IL1β, pro-Caspase-1, Gasdermin D, NLRP3, ASC and β-Actin in whole cell extracts (WCE) of BMDMs generated from mice of the indicated genotypes upon infection with *E. coli*.

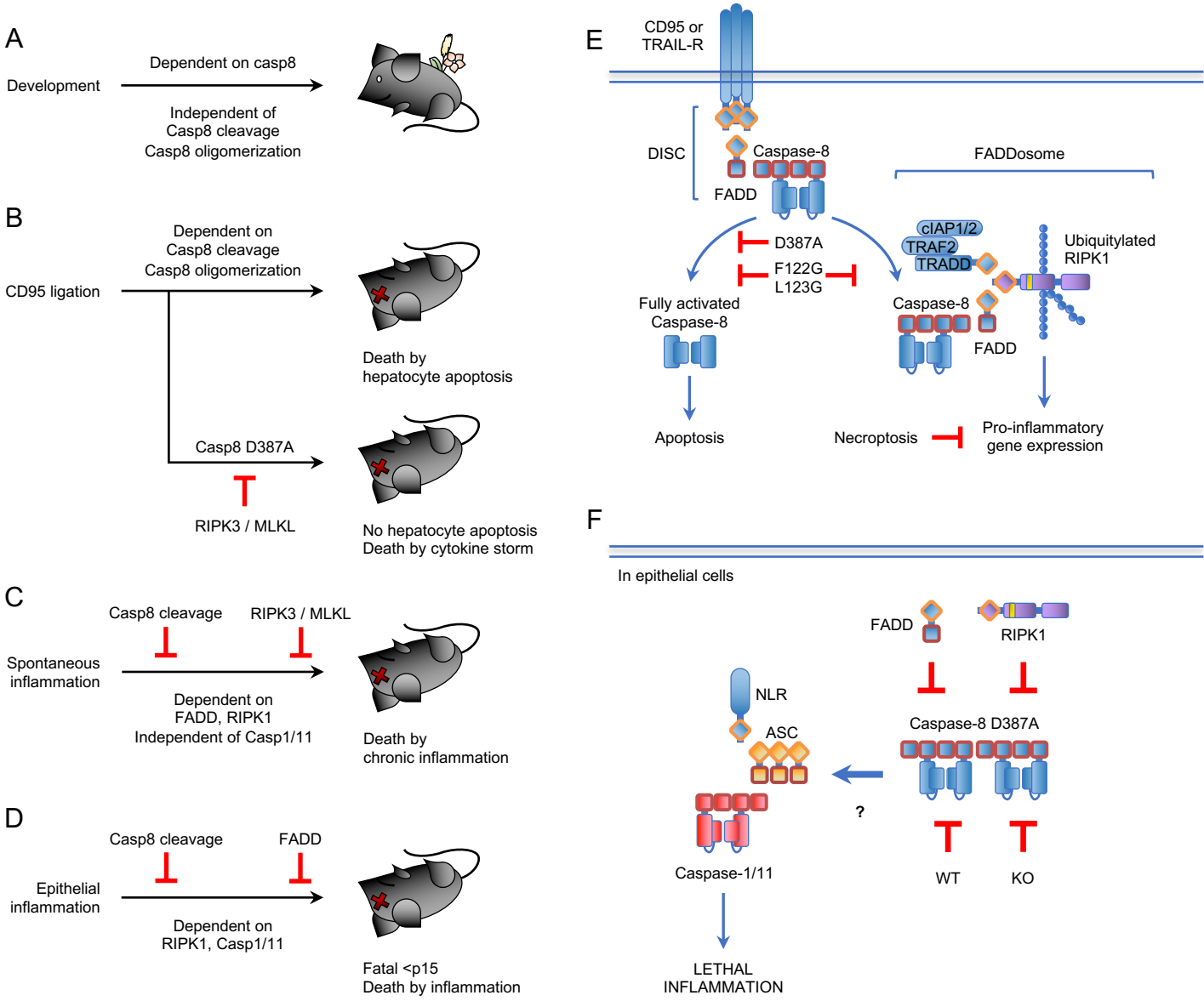

Figure S6: Regulation of Caspase-8-mediated inflammation, Related to Figures 1-6.
Schematic representations of the findings of this study.
A: The influence of Caspase-8 on mouse development.
B: The influence of Caspase-8 on the lethal effects of *in vivo* CD95 ligation.
C: The influence of Caspase-8 and necroptosis on spontaneous inflammation *in vivo*.
D: The influence of Caspase-8 and FADD on epithelial inflammation *in vivo*.
E: The effects of Caspase-8s D387A and F122GL123G mutations on CD95-mediated signaling.
F: The effects of Caspase-8s D387A mutation on ASC oligomerization.