Supplementary Materials for

Caspase-1 regulates TBK1 and PPARα to suppress steatosis-associated hepatocarcinogenesis

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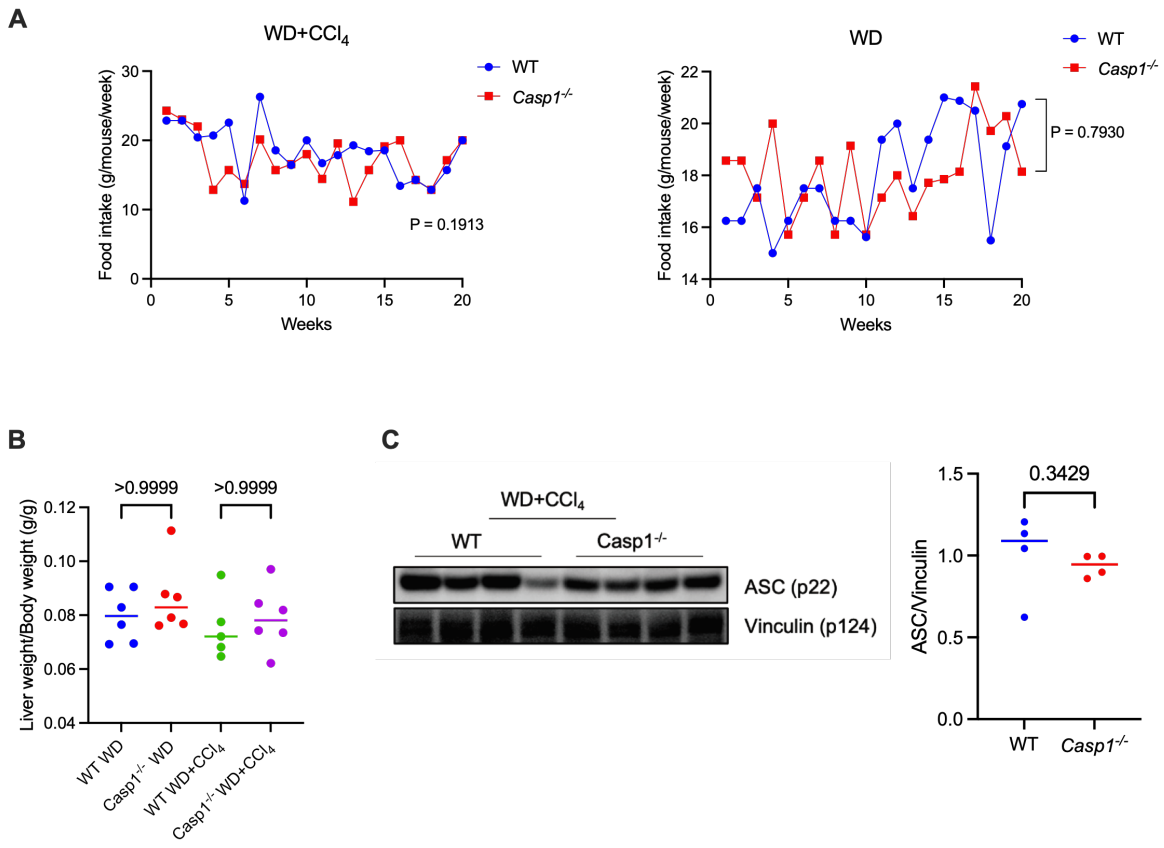
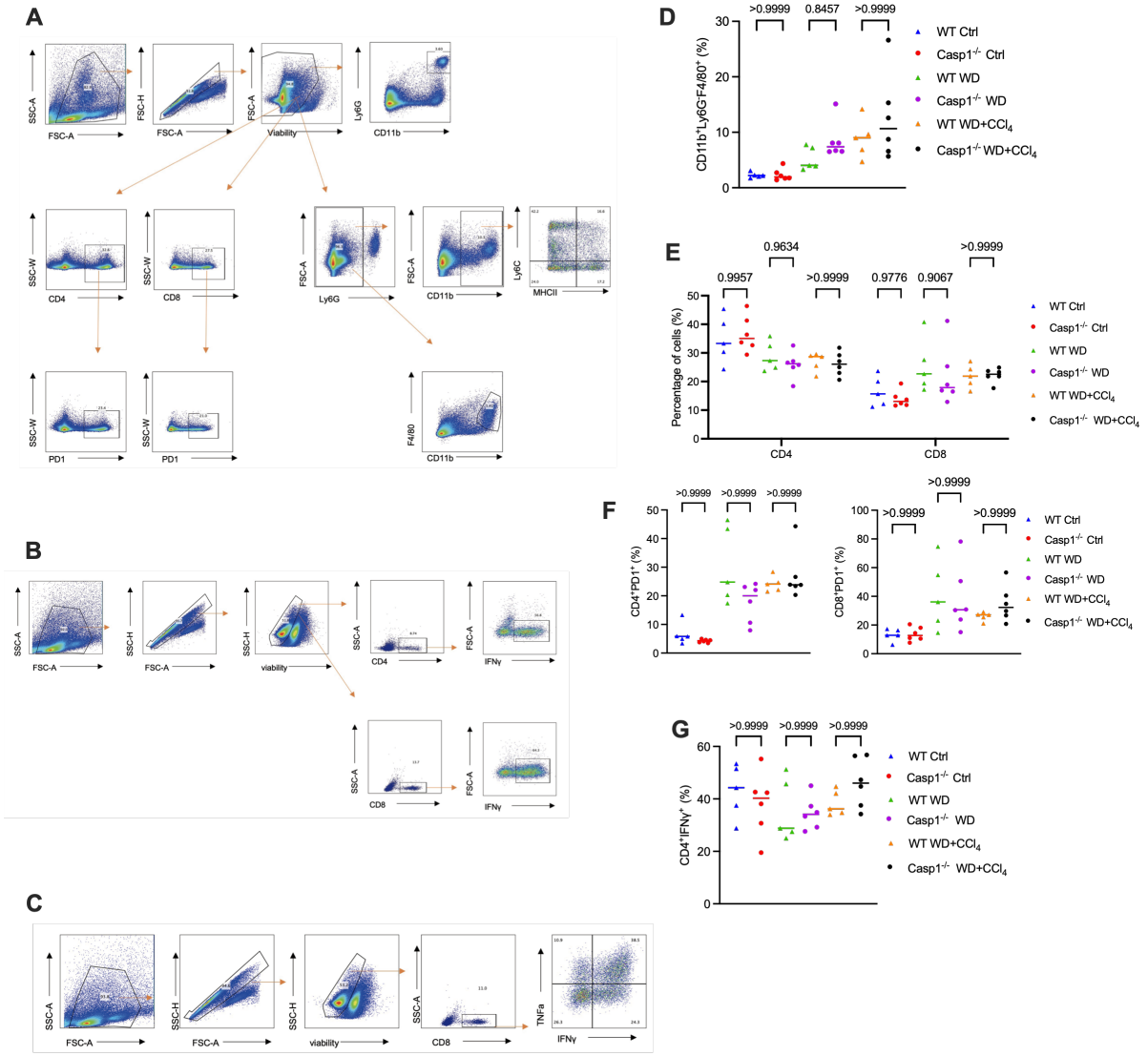
Supplemental Figures 1 – 6



Supplemental Figure 1. Metabolic parameters and inflammasome-related protein expression in WT and *Casp1*$^{-/-}$ mice.

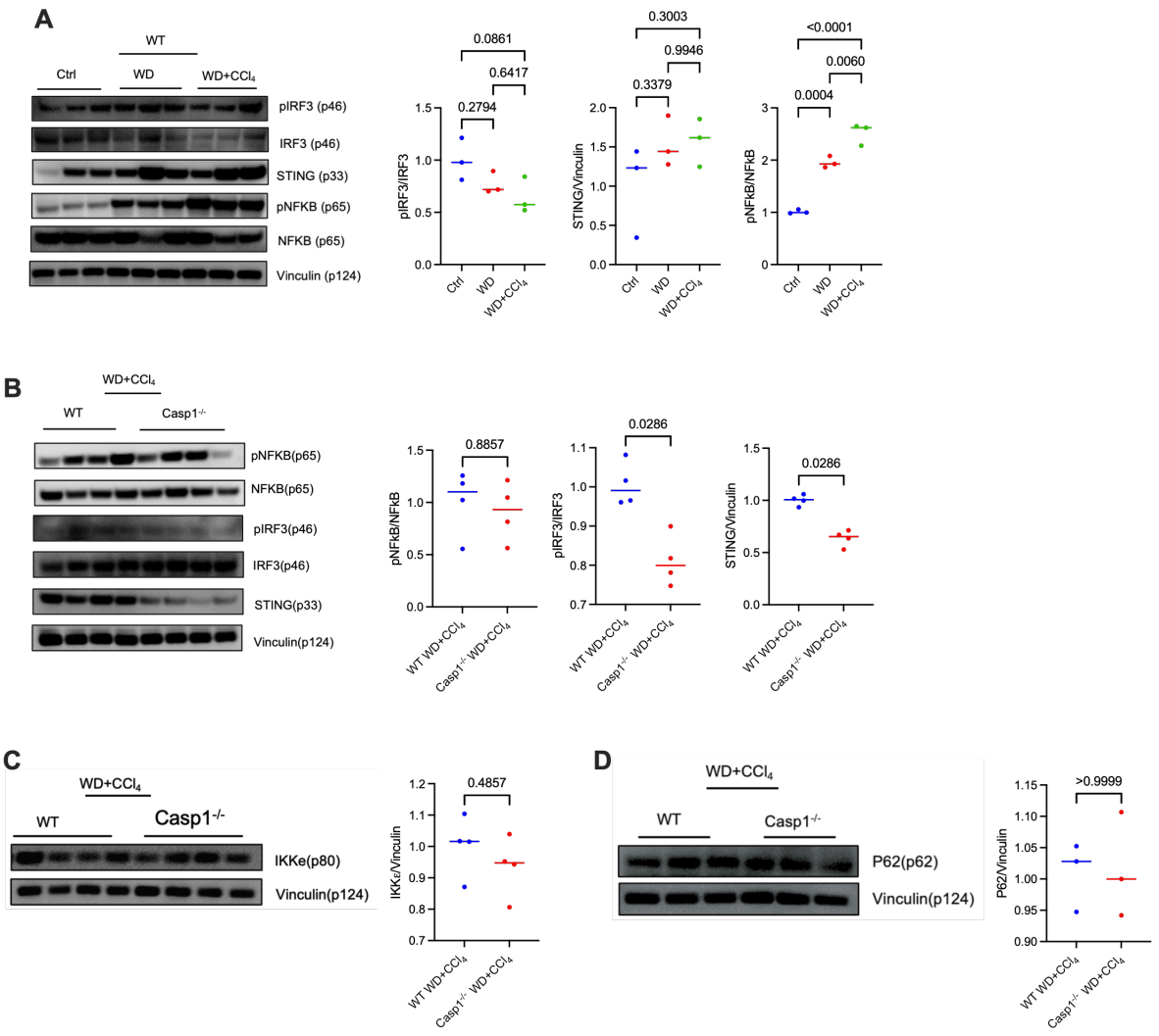
A) Mean weekly food intake over the first 20 weeks in the indicated groups. Data were analysed by two-way ANOVA. **B**) Liver-to-body weight ratio of WT or *Casp1*$^{-/-}$ mice fed a Western diet (WD) or WD combined with CCl_4 treatment. Data were analysed by one-way ANOVA. **C**) Immunoblot analysis of ASC protein levels in liver tissues from WT or *Casp1*$^{-/-}$ mice treated with WD + CCl_4. Data were analysed using two-way ANOVA (A), Kruskal-Wallis test with Dunn´s multiple comparisons test (B) or unpaired Mann-Whitney test (C).



Supplemental Figure 2. Flow cytometric analysis of hepatic T cell populations.

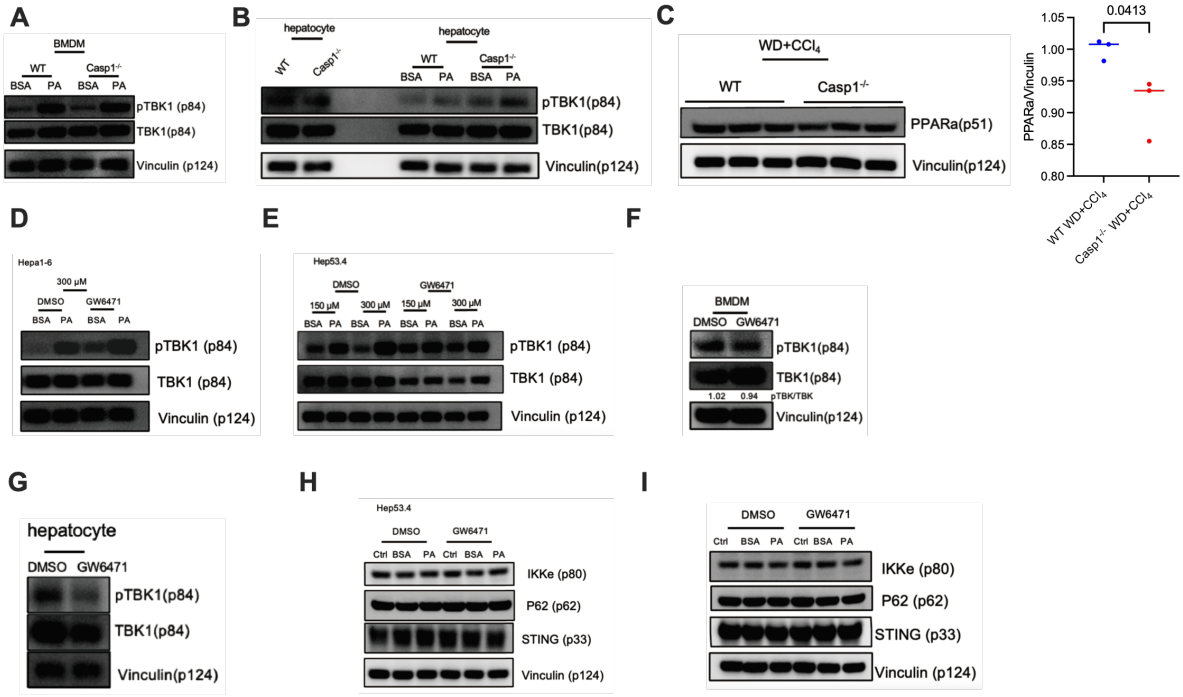
A-C) Gating strategy used for flow cytometry analysis of liver immune cells. **D**) Quantification of hepatic macrophages. **E)** Quantification of hepatic $CD4^+$ and $CD8^+$ T cells in the indicated groups. **F**) Quantification of PD-1 expression on hepatic $CD4^+$ and $CD8^+$ T cells. **G**) Quantification of IFNγ-producing $CD4^+$ T cells.

Data were analysed using ordinary two-way ANOVA with Sidak´s multiple comparisons test (E) or Kruskal-Wallis test with Dunn´s multiple comparisons test (D, F, G).

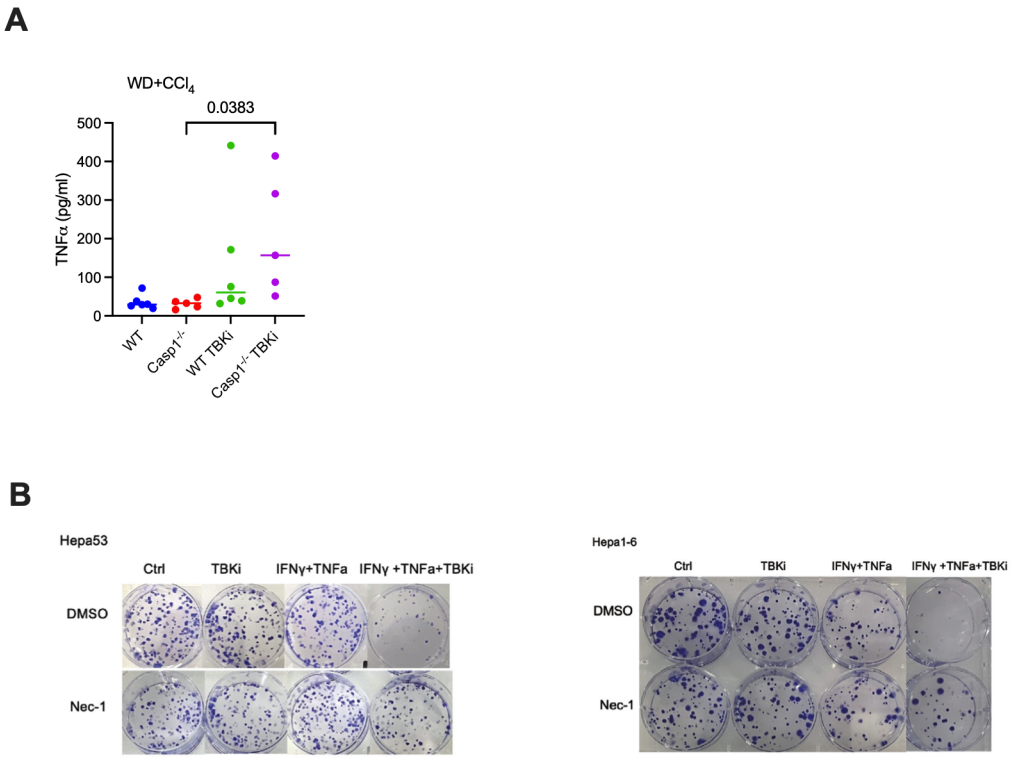


Supplemental Figure 3. Western blot analysis of inflammasome- and signaling-related proteins in liver tissue.

A) Immunoblot analysis of the indicated proteins in liver lysates from WT mice under control, WD, or WD+CCl_4 conditions, with corresponding densitometric quantification. **B-D**) Immunoblot analysis of the indicated proteins in liver lysates from WT and *Casp1*$^{-/-}$ mice treated with WD+CCl_4. Data were analysed using Kruskal-Wallis test with Dunn´s multiple comparisons test (A) or Mann-Whitney test (B-D).

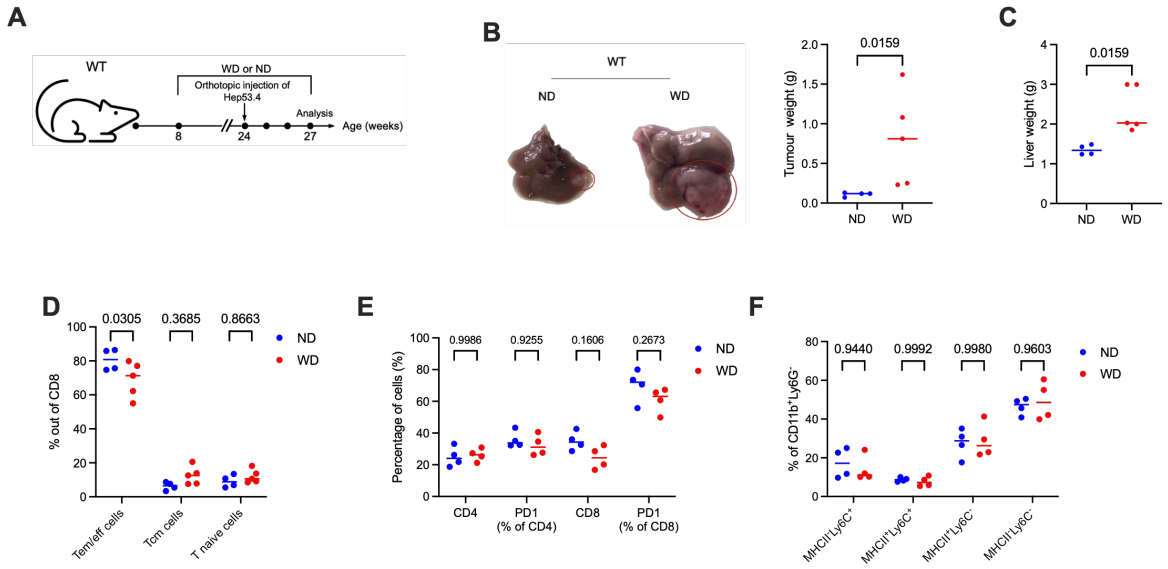


Supplemental Figure 4. TBK1 signaling modulation by fatty acids and PPARα inhibition. **A-B**) Immunoblot analysis of phosphorylated TBK1 (pTBK1) and total TBK1 in bone marrow–derived macrophages (BMDMs) or primary hepatocytes treated with BSA or 300 μM palmitic acid (PA) for 24 h. **C**) Immunoblot analysis of PPARα expression in liver lysates from WT or *Casp1*$^{-/-}$ mice treated with WD+CCl_4. **D**) Immunoblot analysis of pTBK1 and TBK1 in Hepa1-6 cells treated with BSA or 300 μM PA for 24 h. **E**) Immunoblot analysis of pTBK1 and TBK1 in Hep53.4 cells treated with 100 μM or 300 μM BSA or PA for 24 h. **F-G**) Immunoblot analysis of pTBK1 and TBK1 in BMDMs (f) or hepatocytes (g) from WT mice treated with 10 μM GW6471 for 24 h. **H-I**) Immunoblot analysis of IKKε, p62 and STING in Hep53.4 and Hepa1-6 cells treated with 10 μM GW6471 for 24 h and/or 300 μM BSA or PA for 24 h.



Supplemental Figure 5. Effects of TBK1 inhibition on hepatoma cell growth.

A) ELISA of TNFα secretion from liver macrophages treated with TBKi or DMSO. **B)** Colony formation assays visualized by crystal violet staining in Hepa1-6 and Hep53.4 cells cultured under the indicated conditions. Data were analyzed using Kruskal-Wallis test with Dunn´s multiple comparisons test (A)



Supplemental Figure 6. Orthotopic liver tumor model and immune profiling.

A) Schematic illustration of the orthotopic liver tumor model established in WT mice. **B**) Representative images of livers and quantification of tumor weight. Data were analysed using an unpaired two-tailed Student's t-test. **C**) Total liver weight from mice fed a normal diet or Western diet (WD). Data were analysed using an unpaired two-tailed Student's t-test. **D**) Flow cytometric quantification of $CD8^+$ T cell subsets, including effector memory/effector T cells (Tem/Teff; $CD44^+CD62L^-$), central memory T cells (Tcm; $CD44^+CD62L^+$), and naïve T cells ($CD44^-CD62L^+$). **E**) Flow cytometric quantification of $CD4^+$ and $CD8^+$ T cells and PD-1 expression on $CD4^+$ and $CD8^+$ T cells. **F**) Flow cytometric quantification of $MHCII^+Ly6C^+$ cells within the $CD11b^+Ly6G^-$ population across experimental groups. Data were analysed using unpaired Mann-Whitney test (B and C) or two-way ANOVA with Sidak´s multiple comparisons test (D-F).