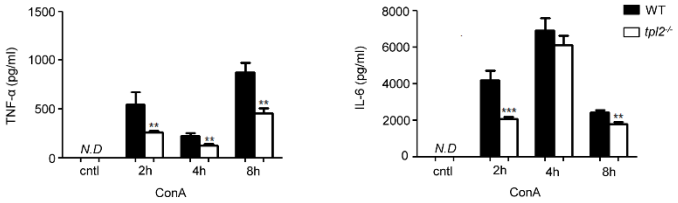
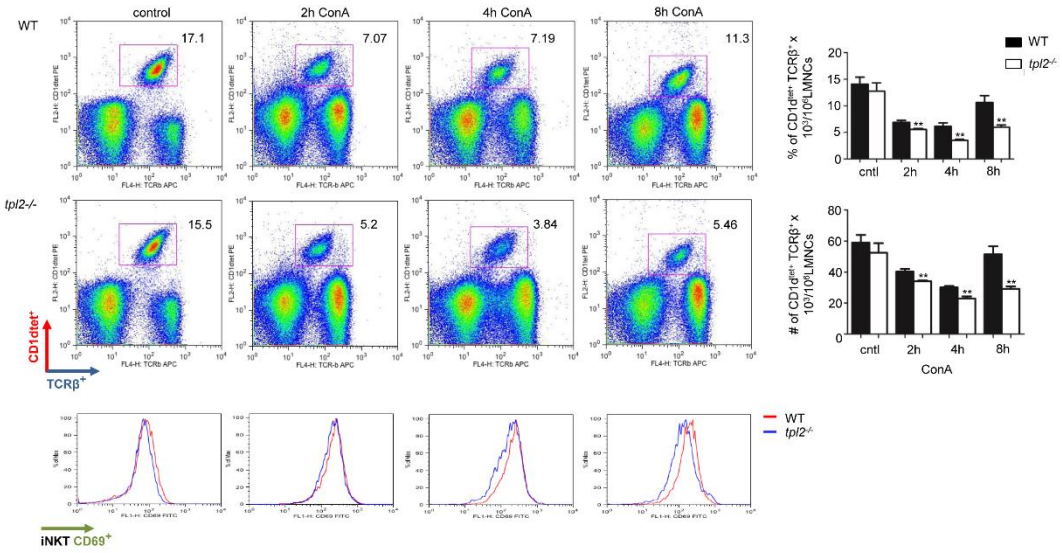
Supplemental Fig. 1



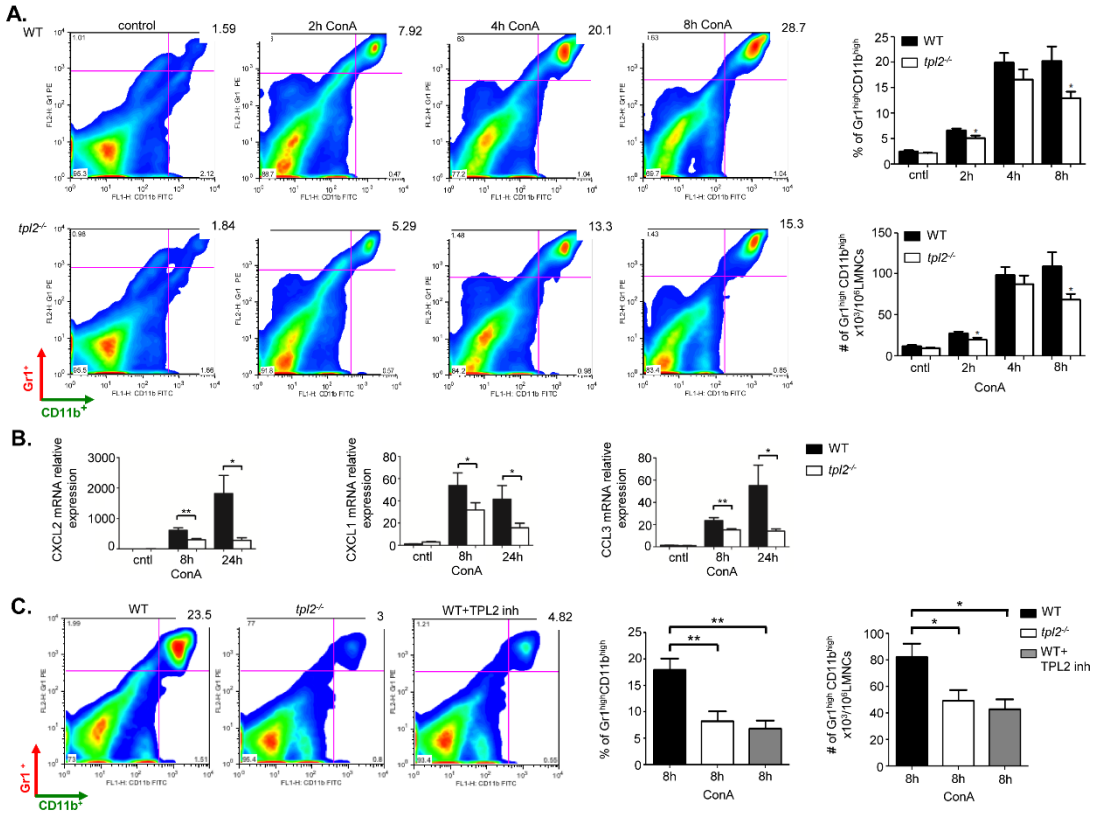
Supplemental Fig. 1: *tpl2*$^{-/-}$ mice display decreased pro-inflammatory cytokine levels following ConA administration. Graphs show serum cytokine levels as determined by ELISA in WT and *tpl2*$^{-/-}$ mice at the indicated time points after ConA treatment. (Data are expressed as the mean ± SEM of n=8-10 mice/per time point, ** $p<0.01$, ***$p<0.001$).

Supplemental Fig. 2



Supplemental Fig. 2: TPL2 kinase affects surface receptor down regulation and expansion of iNKT cells during immune-mediated liver damage. Representative flow cytometric analysis of kinetics and frequency of CD1d^{tet} TCRβ^{+} iNKT cell accumulation and activation (CD69 expression of CD1d^{tet} TCRβ^{+} gated cells) in the liver of WT and *tpl2*$^{-/-}$ mice treated with ConA at various time intervals (n=6-7 mice per group). Percentages and relative numbers of CD1d^{tet} TCRβ^{+} cells/10^{6} liver mononuclear cells (LMNCs) during the different phases of ConA-induced liver injury in WT and *tpl2*$^{-/-}$ mice are shown in the upper and lower right panels, respectively. Data are expressed as the mean ± SEM of 6-7 mice per time point, ** p<0.01.

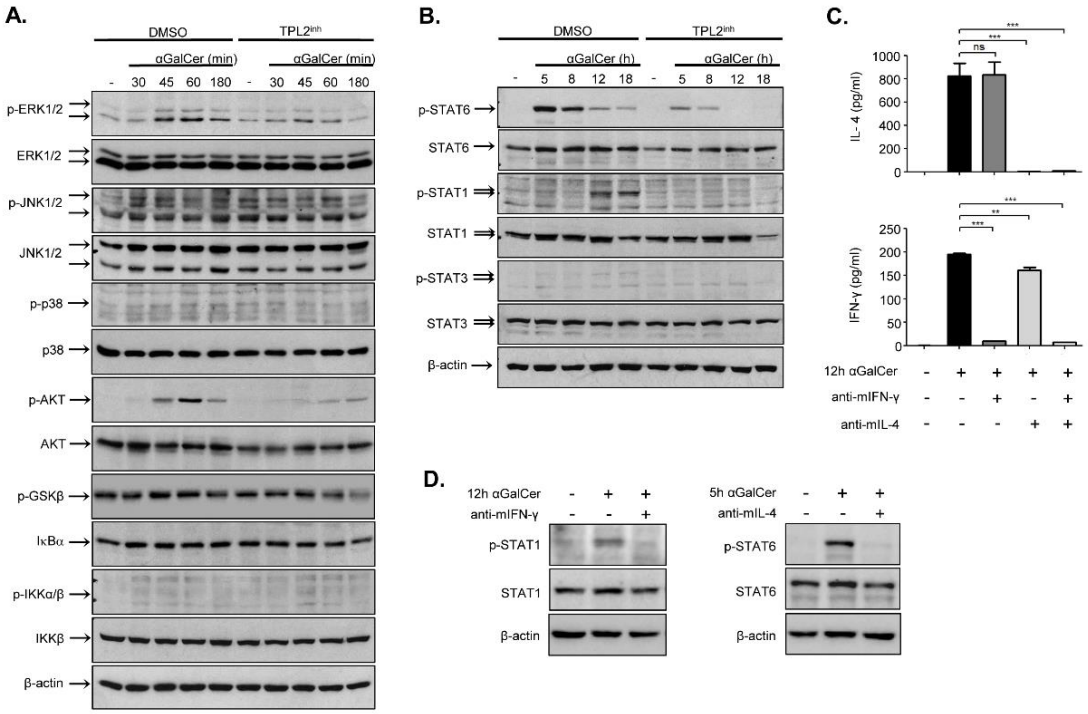
Supplemental Fig. 3



Supplemental Fig. 3: TPL2 ablation of kinase activity results in decreased granulocyte infiltration during immune-mediated liver damage. (A) Representative flow cytometric analysis of kinetics and frequency of neutrophil ($Gr1^{high}$ $CD11b^{high}$) accumulation in the liver of WT and *tpl2*$^{-/-}$ mice treated with ConA at various time intervals (n=8-10 mice per group). Percentages (upper panel) of infiltrating neutrophils and relative numbers (lower panel) of $Gr1^{high}CD11b^{high}$ cells/10^6 liver mononuclear cells (LMNCs) during the different phases of ConA-induced liver injury in WT and *tpl2*$^{-/-}$ mice. Data are expressed as the mean ± SEM of 8-10 mice/per time point, * $p<0.05$, ** $p<0.01$. **(B)** Quantitative Real-Time PCR for CXCL2, CXCL1 and CCL3 mRNA expression in the liver of WT mice treated with saline or ConA at the indicated time points. (Data are expressed as the mean ± SEM of n=8 mice/per time point, * $p<0.05$, **$p<0.001$)**. (C)** Administration of TPL2 kinase inhibitor prior to ConA treatment leads to reduced neutrophil accumulation in the livers of WT mice. Flow cytometric analysis of

$Gr1^{high}CD11b^{high}$ cells in the liver of WT mice treated with ConA and TPL2 inhibitor or vehicle control (n=4-5 mice per group). Percentages (upper panel) of infiltrating neutrophils and relative numbers (lower panel) of $Gr1^{high}CD11b^{high}$ cells/10^6 liver mononuclear cells (LMNCs) during the different phases of ConA-induced liver injury in WT mice treated with ConA and TPL2 inhibitor or vehicle control. Data are expressed as the mean ± SEM of 8-10 mice/per time point, * $p<0.05$, ** $p<0.01$.

Supplemental Fig. 4



Supplemental Fig. 4: Inhibition of TPL2 kinase activity affects ERK1/2 and Akt signaling pathways and decreases STAT1 and STAT6 activation in iNKT cells. (A) DN32.D3 cells were treated with TPL2 inhibitor or vehicle control and further stimulated with αGalCer at various time points. Protein lysates were collected and analyzed for the indicated signalling molecules. β-actin is used as a control for equal loading. Data are representative of three independent experiments. **(B)** DN32.D3 cells were activated with αGalCer in the presence of TPL2 inhibitor or vehicle control. Western blot for phosphorylated and total STAT1, STAT6 and STAT3 was performed. β-actin is used as a control for equal protein loading. Data are representative of three independent experiments. **(C) & (D)** DN32.D3 cells were stimulated with αGalCer in the presence of neutralizing antibodies against IL-4 or IFN-γ as indicated. Culture supernatants and protein lysates were collected and analyzed with ELISA for IFN-γ and IL-4 secretion (C) and Western blot for phosphorylated and total STAT1 and STAT6 respectively (D). β-actin is used as a control for equal loading. Data are representative of three independent experiments.