Deletion of tumour necrosis factor α receptor 1 elicits an increased TH17 immune response in the chronically inflamed liver

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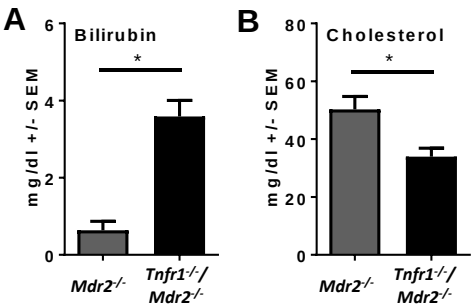
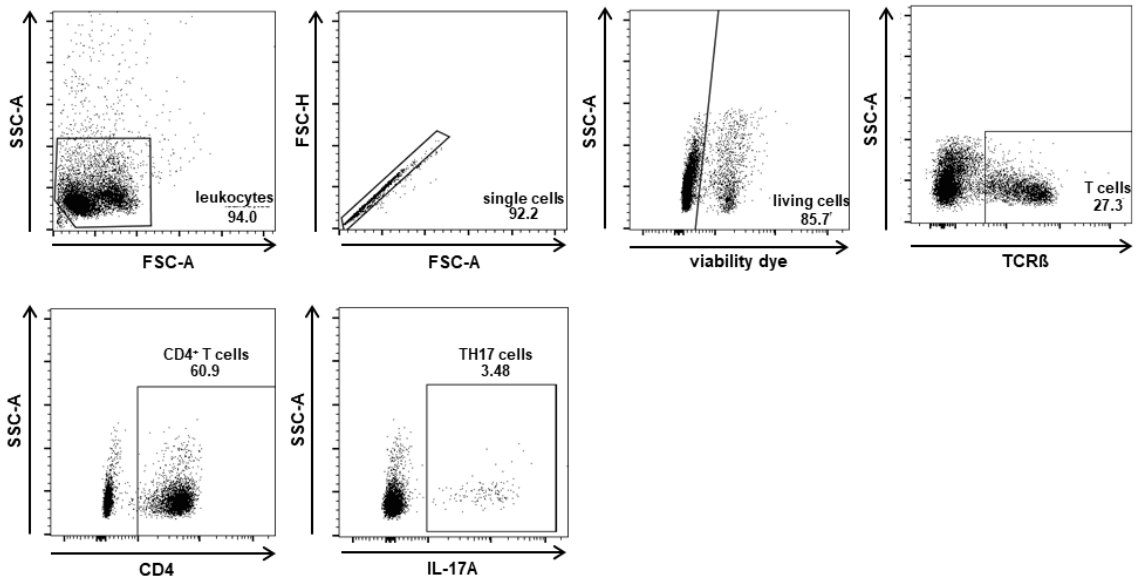
Supplementary Figures

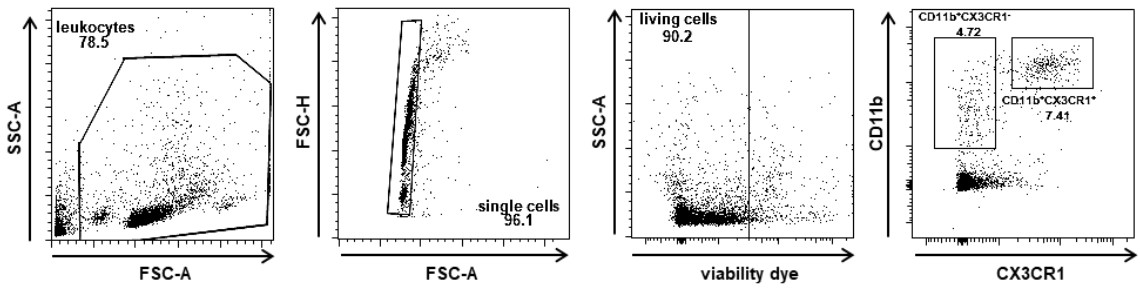


Supplementary Figure 1: Marker of cholestasis in *Tnfr1*$^{-/-}$*/Mdr2*$^{-/-}$ mice.
(A) Plasma concentrations of bilirubin and
(B) cholesterol in *Mdr2*$^{-/-}$ (n = 4) and *Tnfr1*$^{-/-}$*/Mdr2*$^{-/-}$ (n = 5) mice (10-12 weeks of age). *P ≤ 0.05

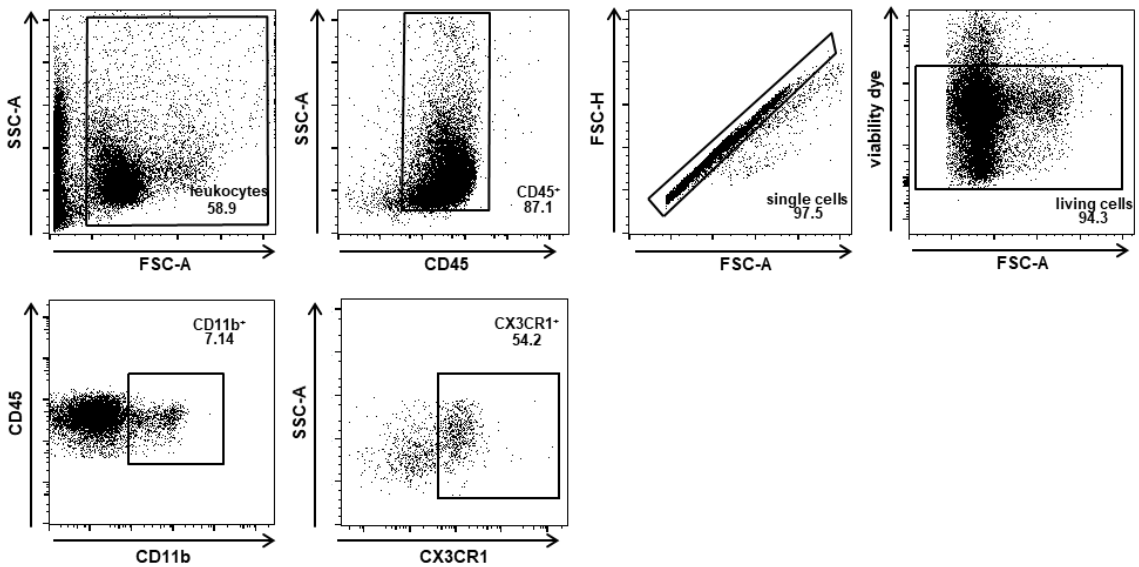
A Gating strategy: flow cytometric anaylsis of TH17 cells (Figure 3)



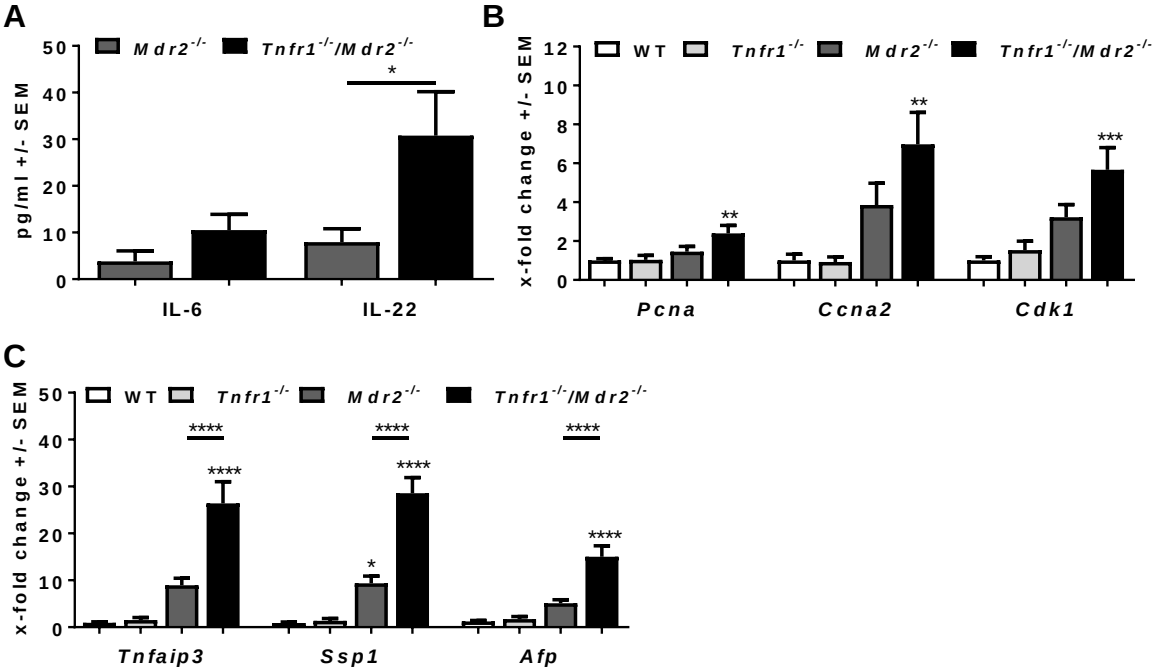
B Gating strategy: fluorescence-activated cell sort of CD11b^{+}CX3CR1$^{-/+}$ cells (12w; Figure 4)



C Gating strategy: flow cytometric analysis of CD11b^{+}CX3CR1^{+} cells (24w; Figure 5)

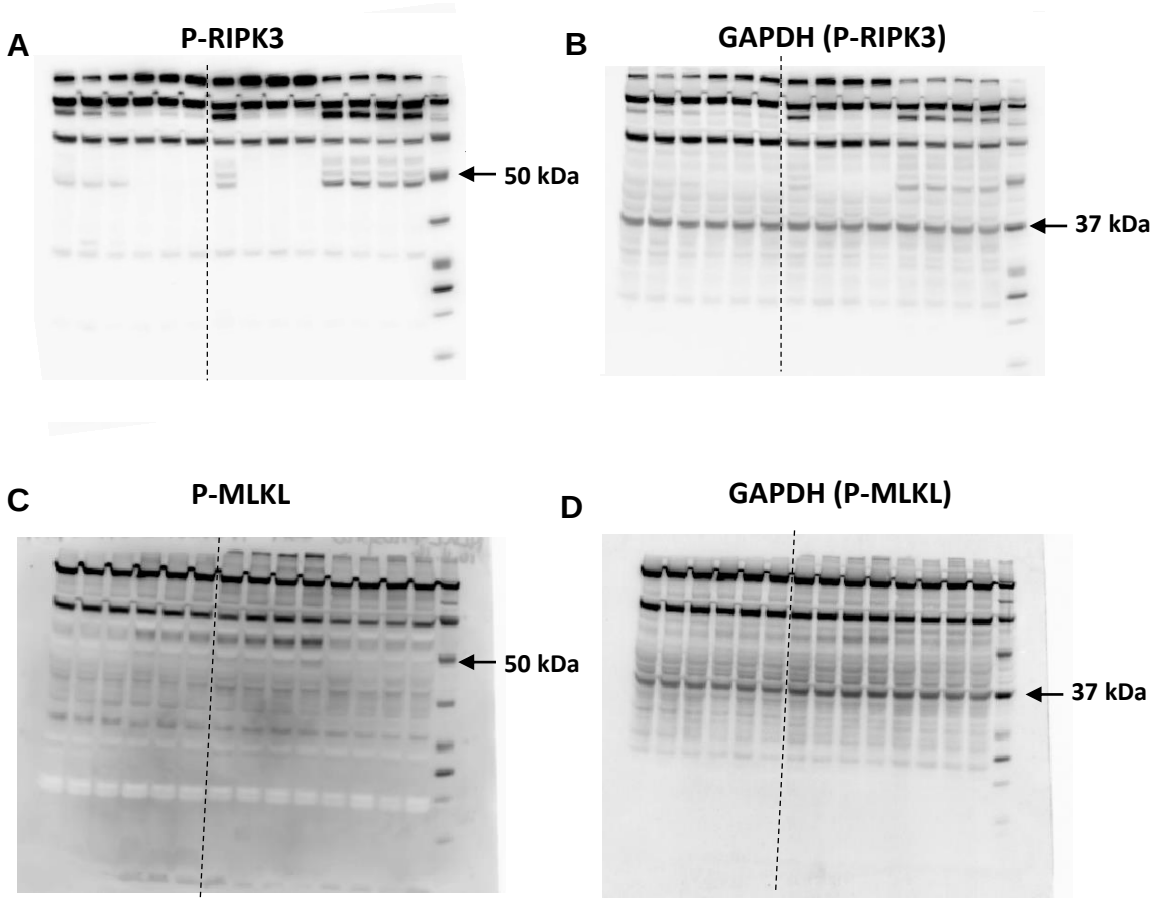


Supplementary Figure 2: Gating strategies.
For all analysis living total leukocytes were determined by forward / sideward scatter, and exclusion of dead cells. (A) TH17 cells were defined as TCRß$^{+}$ CD4^{+} IL-17^{+} cells. (B) Cells were sorted for CD11b^{+} and CX3CR1$^{+/-}$. (C) Cells were defined as CD45^{+} CD11b^{+} and CX3CR1^{+}.



Supplementary Figure 3: Hepatic proliferation and differentiation in *Tnfr1*$^{-/-}$*/Mdr2*$^{-/-}$ mice. (A) Plasma levels of STAT3 inducing cytokines IL-6 and IL-22 in *Mdr2*$^{-/-}$ (n = 8) and *Tnfr1*$^{-/-}$*/Mdr2*$^{-/-}$ (n = 8) mice measured via Legendplex. (B) Hepatic gene expression analysis of proliferation marker *Pcna*, *CcnA2* and *Cdk1* and (C) tumour marker *Tnfaip3*, *Ssp1*, and *Afp* in WT (n ≥ 10), *Tnfr1*$^{-/-}$ (n = 9), *Mdr2*$^{-/-}$ (n ≥ 9) and *Tnfr1*$^{-/-}$*/Mdr2*$^{-/-}$ (n ≥ 10) determined by RT-qPCR. *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.

Full length western blots pf phosphorylated RIP3, MLKL and GAPDH as loading control



Supplementary Figure 4: Full length western blots of data presented in Figure 4B.
The liver tissue samples lysates for the western blot analysis of P-RIPK3 and P-MLKL were derived from the same experiment and gels/blots were processed in parallel. Gels were loaded as follows, left lane 1-3 WT, Lane 4-6: *Tnfr1*$^{-/-}$, lane 7-10: *Mdr2*$^{-/-}$, lane 11-14: *Tnfr1*$^{-/-}$*/Mdr2*$^{-/-}$, Lane 15: molecular marker (Precision Plus Protein WesternC Standards). Each lane depicts one animal. Only lanes 7-15 are displayed in the main article, dashed lines indicate were images were cropped.