SUPPLEMENTARY METHODS

Microarray analyses for targets of TNFR2. Genome-wide extrahepatic bile duct expression datasets have been previously generated and reported by us (deposited in GEO: GSE46995) (8). Statistical significance was determined by unpaired t-test with a significance of <0.05. To identify molecules linked to TNFR2 function, the ToppGenet algorithm within the Toppgene Suite (http://toppgene.cchmc.org/) was utilized to generate a network of TNFR2-interacting proteins, which identified 29 individual molecules. GeneSpring GX13.0 platform was then used to select genes whose expression differed by ≥1.5-fold between saline and RRV groups with a significance of <0.05 by Welch one-way analysis of variance (ANOVA) and Benjamini-Hochberg multiple testing correction (false discovery rate [FDR] 0.05).

Supplementary Table 1

Oligonucleotide primer sequences and PCR product sizes for mouse TNFα-related genes

Gene	Mouse Primer Sequences	Annealing Temp. (°C)	Product Size (bp)
Tnfa	For: 5'-AAGGGAGAGTGGTCAGTTGCC-3' Rev: 5'-CCTCAGGGAAGAGTCTGGAAAGG-3'	54	95
Tnfr1	For: 5'-ATCCATCAGGGGTCACTGGA-3' Rev: 5'-ACTTGGTGCAGCAGATGGAA-3'	60	117
Tnfr2	For: 5'-ACAAACCGGAACCTGGGTAC-3' Rev: 5'-GTCCGAGGTCTTGTTGCAGA-3'	60	125
Bmx	For: 5'-GGAAAGTTCCTGTGTTGCCA-3' Rev: 5'-TGGGAGCTCTGTGTTTCTCA-3'	60	117
Ask1	For: 5'-CAAATCAGACAGTCCGACGG-3' Rev: 5'-GTGCATTCTGGGAGTCATGG-3'	60	117

Supplementary Table 2

Statistical analysis of growth profiles, symptoms of cholestasis and Kaplan-Meier survival charts.

A) Statistical analysis of growth curves (weights) of experimental groups (*two-tailed unpaired t test with Welch's correction*):

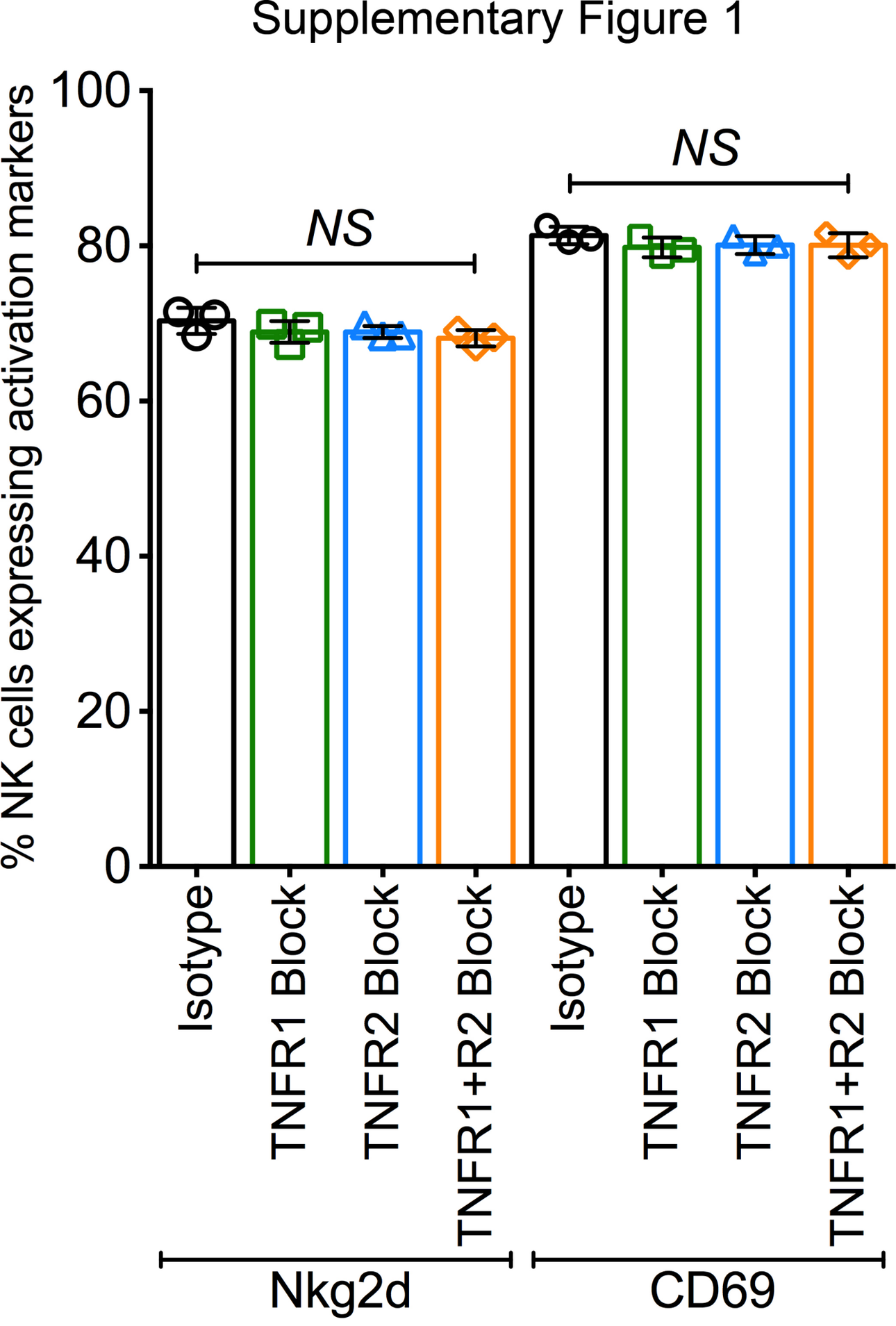
	WT/IgG RRV	TNFa-Ab RRV	TNFR1-Ab RRV	TNFR2-Ab RRV	TNFR1-KO	TNFR2-KO
WT/IgG RRV	XXX	**0.002**	**<0.0001**	**<0.0001**	**0.11**	**<0.0001**
TNFa-Ab RRV	**0.002**	XXX	XXX	XXX	XXX	XXX
TNFR1-Ab RRV	**<0.0001**	XXX	XXX	**0.48**	XXX	XXX
TNFR2-Ab RRV	**<0.0001**	XXX	**0.48**	XXX	XXX	XXX
TNFR1-KO	**0.11**	XXX	XXX	XXX	XXX	**0.0011**
TNFR2-KO	**<0.0001**	XXX	XXX	XXX	**0.0011**	XXX

B) Kaplan-Meier survival plots of experimental groups (*Log-rank [Mantel-Cox] test*):

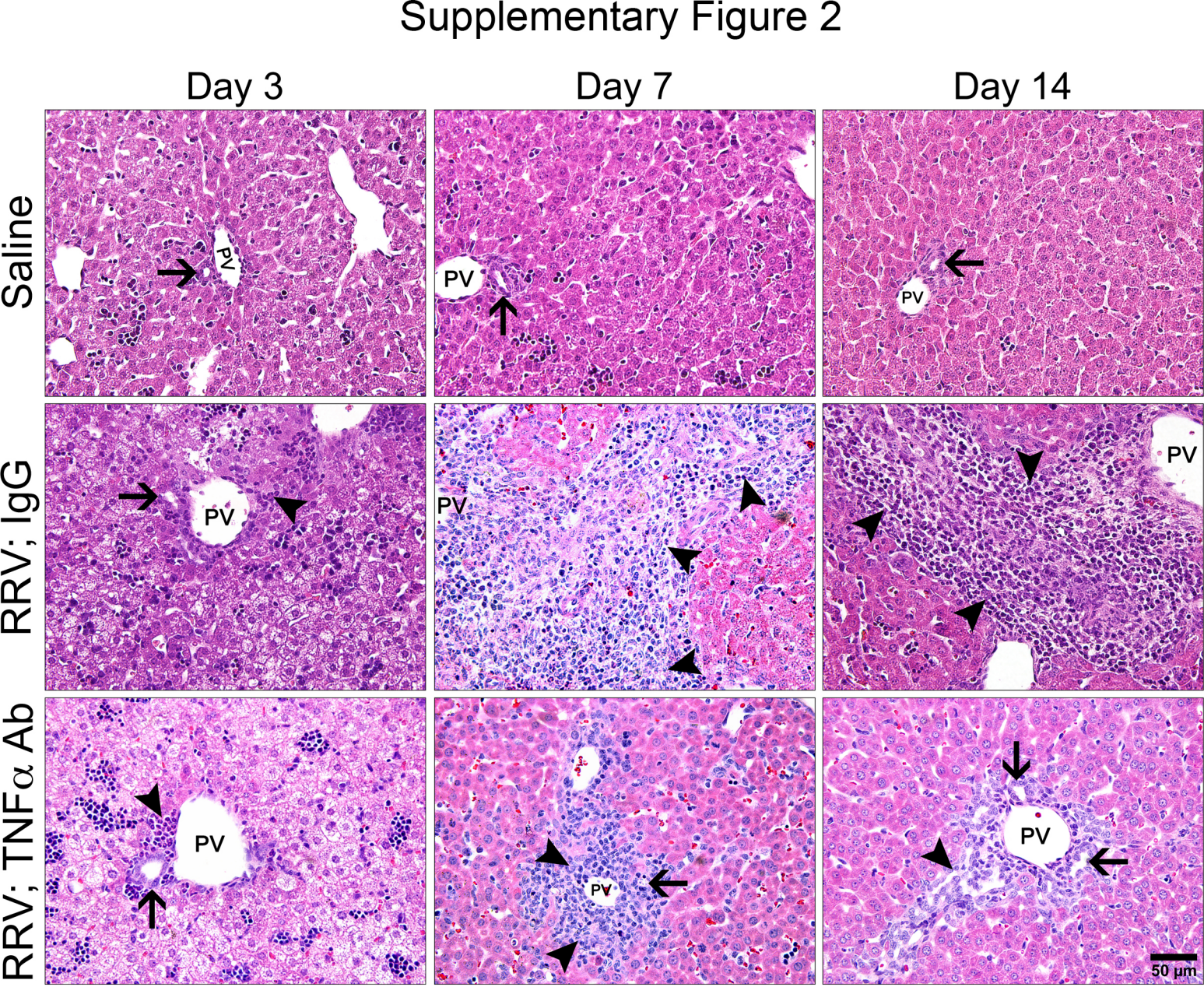
	WT/IgG RRV	TNFa-Ab RRV	TNFR1-Ab RRV	TNFR2-Ab RRV	TNFR1-KO	TNFR2-KO
WT/IgG RRV	XXX	**0.0003**	**<0.0001**	**<0.0001**	**0.94**	**0.001**
TNFa-Ab RRV	**0.0003**	XXX	XXX	XXX	XXX	XXX
TNFR1-Ab RRV	**<0.0001**	XXX	XXX	**0.04**	XXX	XXX
TNFR2-Ab RRV	**<0.0001**	XXX	**0.04**	XXX	XXX	XXX
TNFR1-KO	**0.94**	XXX	XXX	XXX	XXX	**0.01**
TNFR2-KO	**0.001**	XXX	XXX	XXX	**0.01**	XXX

C) Comparative analysis of percent mice jaundiced from experimental cohorts of mice (*Wilcoxon matched-pairs signed rank test*):

	WT/IgG RRV	TNFa-Ab RRV	TNFR1-Ab RRV	TNFR2-Ab RRV	TNFR1-KO	TNFR2-KO
WT/IgG RRV	XXX	**<0.0001**	**0.0005**	**0.0012**	**0.50**	**0.001**
TNFa-Ab RRV	**<0.0001**	XXX	XXX	XXX	XXX	XXX
TNFR1-Ab RRV	**0.0005**	XXX	XXX	**0.21**	XXX	XXX
TNFR2-Ab RRV	**0.0012**	XXX	**0.21**	XXX	XXX	XXX
TNFR1-KO	**0.50**	XXX	XXX	XXX	XXX	**0.004**
TNFR2-KO	**0.001**	XXX	XXX	XXX	**0.004**	XXX

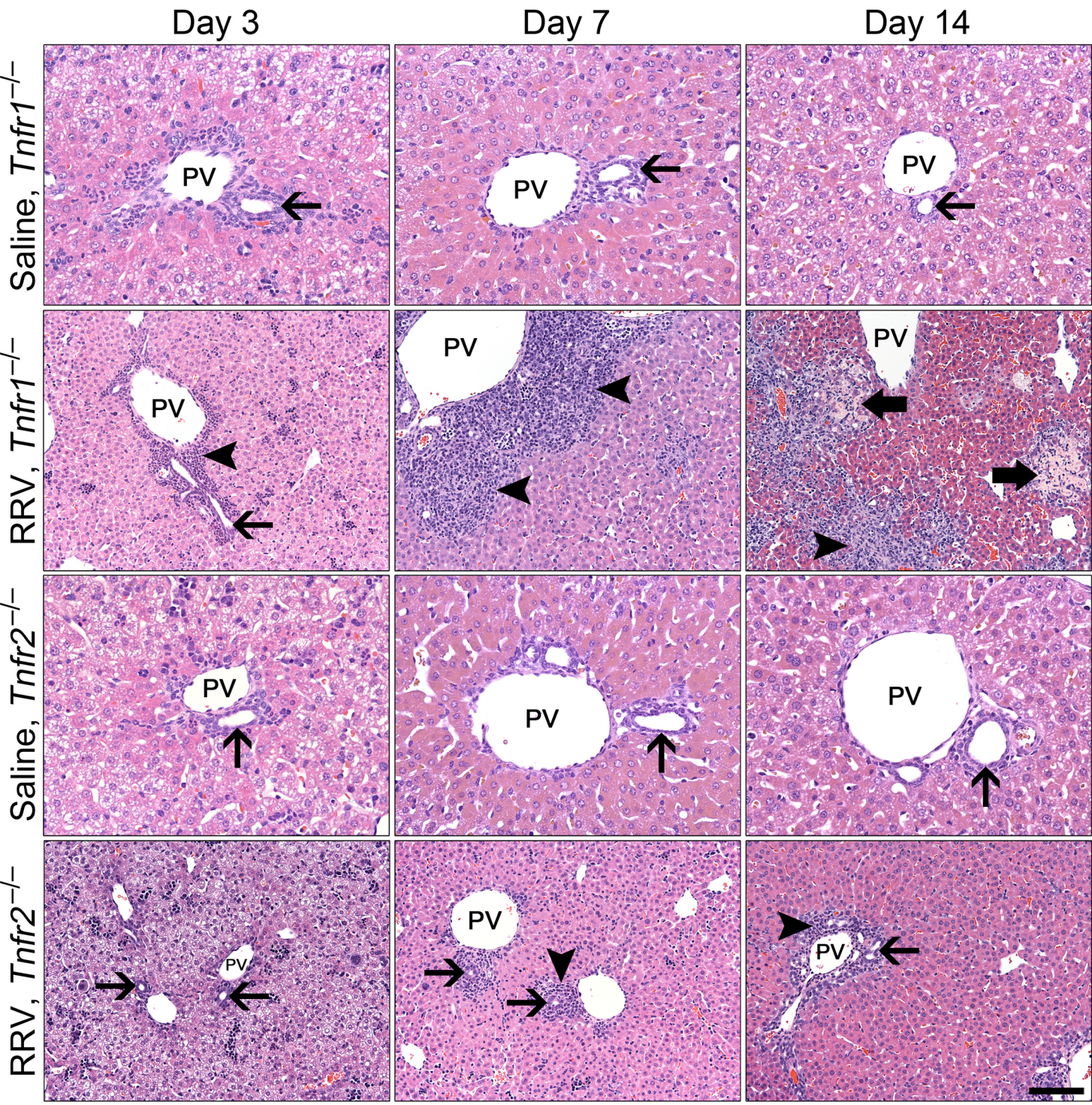


Supplementary Figure 1. Antibody blocking of TNFR1 and/or TNFR2 does not interfere with NK-cell activation status. Quantification of activation markers (Mean ± SD) on NK cells from in vitro cytotoxicity co-culture assays of mCL and NK cells from RRV-primed mice shows similar levels of Nkg2d and CD69 in the presence of antibodies when compared to isotype control. N=4-6 wells/ratio/specimen. Results are representative of 2 independent experiments with NK cells obtained from a pool of 3-4 livers/specimen. NS=no significant difference as determined by 2-tailed unpaired *t* test.



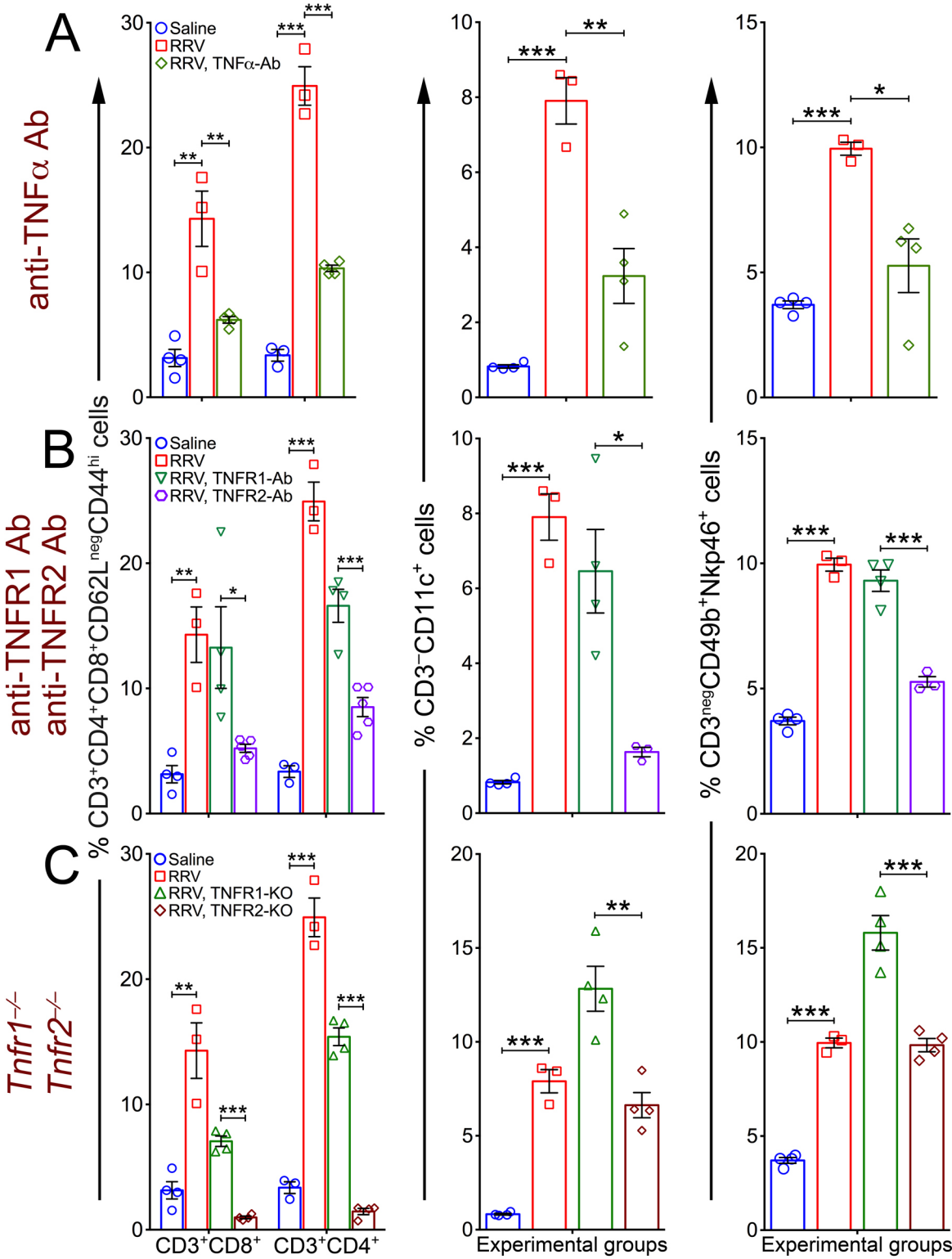
Supplementary Figure 2. Antibodies to TNFα decrease portal inflammation in mice with experimental atresia. Representative hematoxylin/eosin-staining of livers from saline and RRV-injected neonatal mice treated with anti-TNFα or isotype antibodies show decreased portal inflammation in the anti-TNFα group. N=4-6 mice/treatment group/day. Magnification of 400X; scale bar: 50 μm.

Supplementary Figure 3



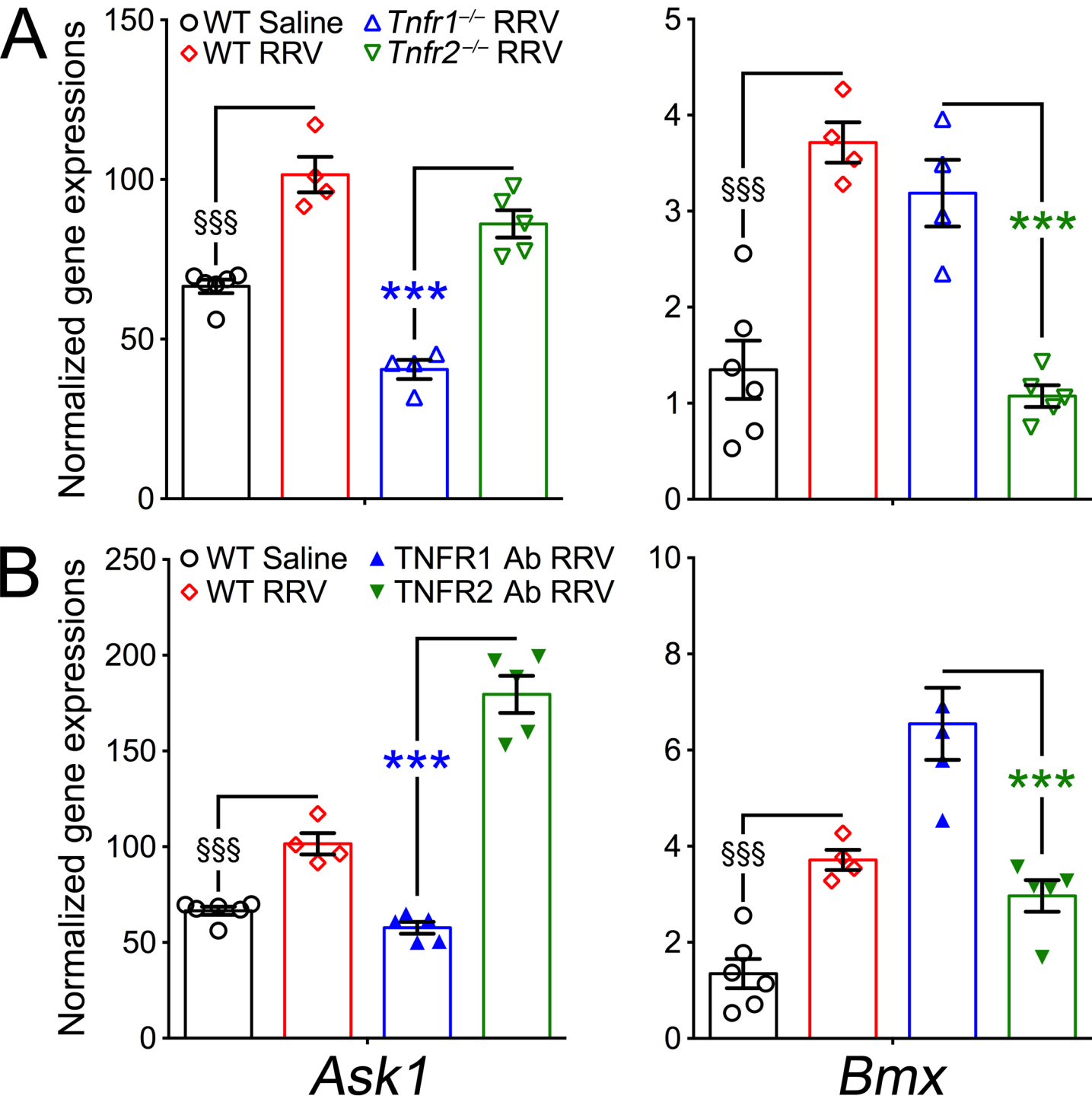
Supplementary Figure 3. Genetic deficiency of TNFR2 but not TNFR1 improves hepatic inflammation. Representative hematoxylin/eosin-staining of livers from *Tnfr1*$^{-/-}$ and *Tnfr2*$^{-/-}$ mice injected with saline show normal hepatic architecture while livers from RRV-challenged *Tnfr2*$^{-/-}$ mice show decreased portal inflammation when compared to *Tnfr1*$^{-/-}$ mice. Thin arrows denote intrahepatic bile ducts, arrowheads depict inflammatory cells and block arrows denote areas of parenchymal necrosis and loss of hepatocytes, PV = portal vein. N=4-6 mice/treatment group/day. Magnification: 400X.

Supplementary Figure 4



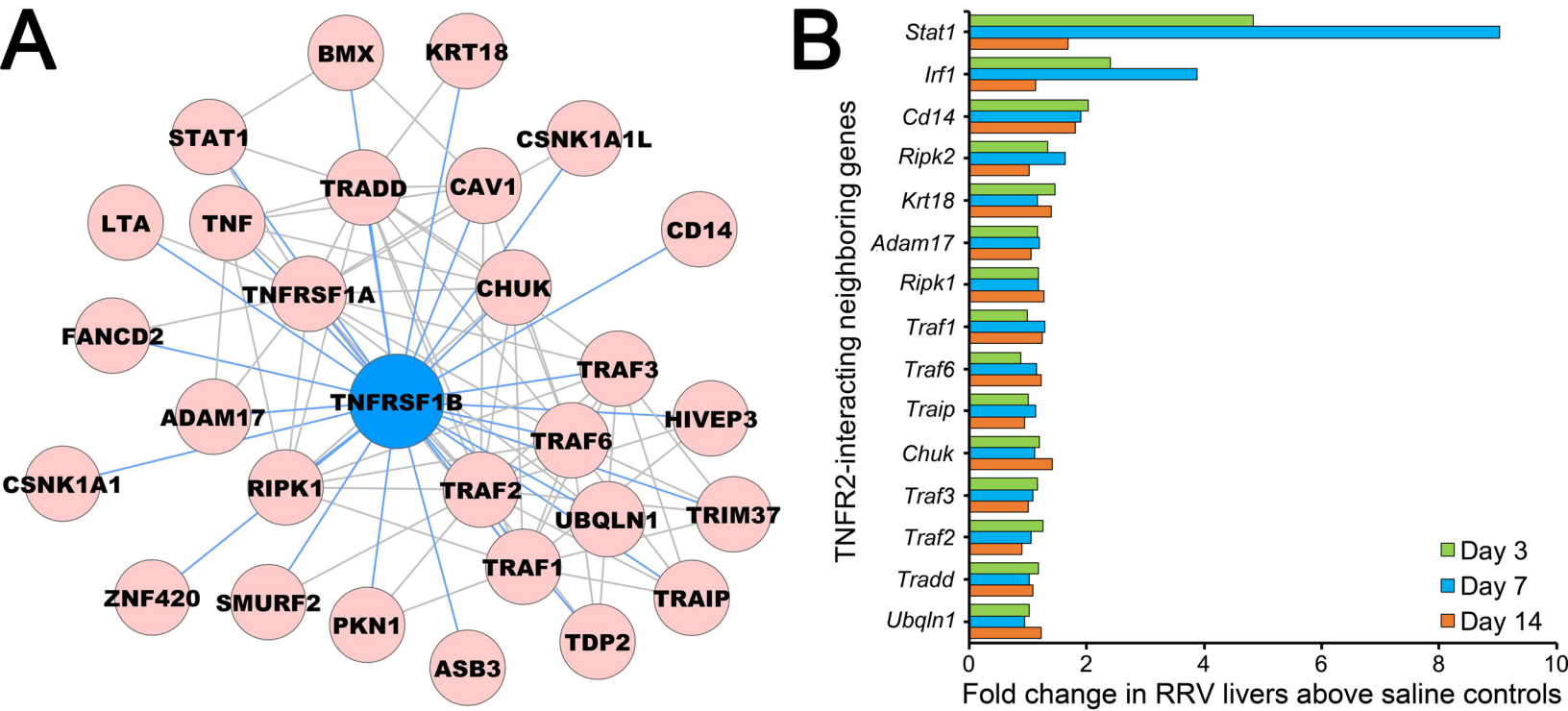
Supplementary Figure 4. TNFα/TNFR signals regulate intrahepatic immune cell populations. In Panel **A**, blocking of TNFα suppresses the expansion of hepatic effector T cells ($CD3^+CD4^+CD8^+$ cells co-stained with $CD62L^{neg}CD44^{hi}$), dendritic cells (DC; $CD3^{neg}CD11c^+$) and NK cells ($CD3^{neg}CD49b^+Nkp46^+$). In panel **B**, the expansion of these cells is suppressed more consistently by antibodies to TNFR2. The same pattern is seen in panel **C**, where the hepatic population of T, DC and NK cells is suppressed consistently in *Tnfr2*$^{-/-}$ mice. Saline and RRV-injected WT mice are depicted for comparison. N=3-4 mice/group. Values are expressed as Mean ± SD. *=P<0.05, **=P<0.01 and ***=P<0.001 as determined by 2-tailed parametric unpaired t test using Welch's correction.

Supplementary Figure 5



Supplementary Figure 5. Expression of TNFR-specific molecules in *Tnfr1*$^{-/-}$ and *Tnfr2*$^{-/-}$ and antibody blocked mice. Real-time PCR based hepatic mRNA expressions at day 7 after RRV challenge of TNFR1-specific *Ask1* and TNFR2-specific *Bmx* tyrosine kinases in *Tnfr1*$^{-/-}$ and *Tnfr2*$^{-/-}$ mice (**A**) and antibody blocked (**B**) mice show biological specificities. WT saline and RRV-challenged groups are depicted for comparative purposes. N=4-6 mice/group. Values are expressed as Mean ± SD. §§§=P<0.001 (WT saline and RRV) and ***=P<0.001 (*Tnfr1*$^{-/-}$, *Tnfr2*$^{-/-}$, TNFR1 Ab or TNFR2 Ab treated) as determined by 2-tailed parametric unpaired *t* test using Welch's correction.

Supplementary Figure 6



Supplementary Figure 6. Bioinformatics analysis of TNFR2-genes in extrahepatic bile ducts links to STAT1-IRF1 pathway. (**A**) Prioritization of TNFR2-neighboring genes by ToppGenet protein-protein interaction network (http://toppgene.cchmc.org) identified 29 genes. (**B**) 15 of these genes were differentially upregulated at day 3, 7 or 14 in extrahepatic bile ducts with greater increases in Stat1 and Irf1 mRNA.