Supporting Information to:

Mechanisms of Concanavalin A-induced cytokine synthesis by hepatic stellate cells: Distinct roles of interferon regulatory factor-1 in liver injury

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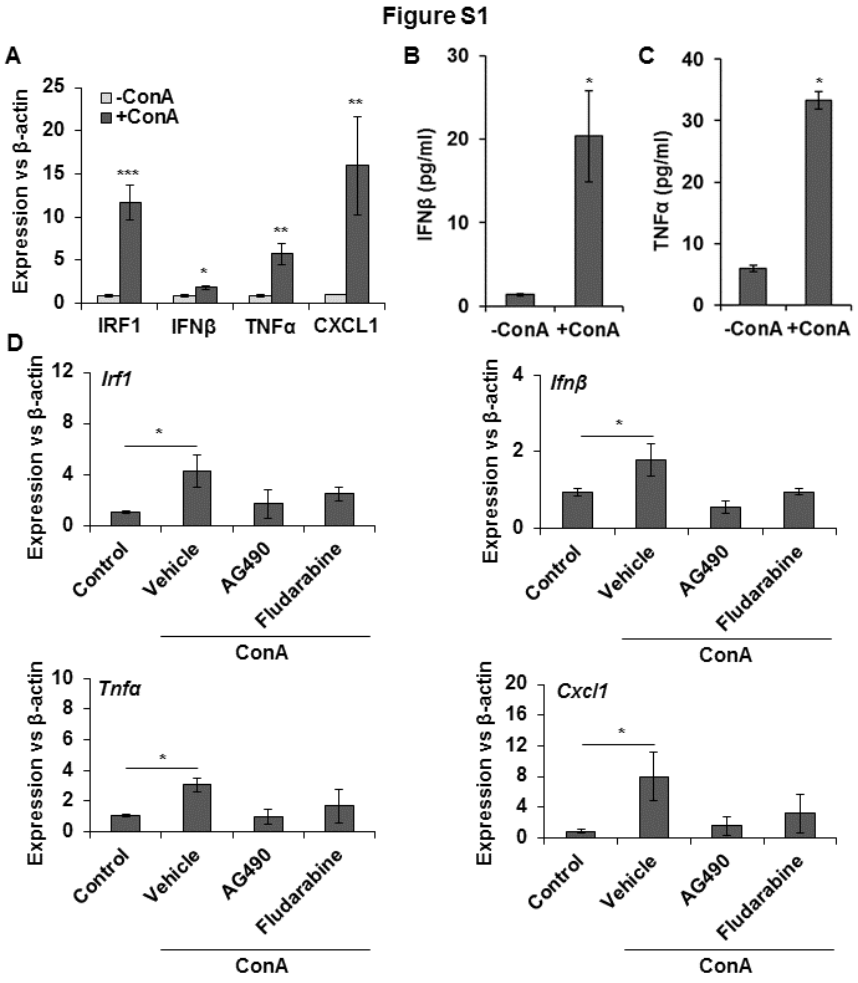

Figure S1: ConA-induced expression of cytokines in rat HSCs. (A) mRNA expression of indicated mediators in rat HSCs stimulated with ConA (50 μg/ml) for 6h. (B-C) Culture supernatants from ConA-stimulated HSCs were aspirated at 8h for determination of TNFα and IFNβ by ELISA. (D) HSCs were preincubated in presence of DMSO-vehicle, JAK2 (AG490; 50 μM) or STAT1 (Fludarabine; 10 μM) inhibitors for 30 min, then stimulated with ConA (50 μg/ml) for 6h. Expression of indicated mRNAs was measured by qRT-PCR. The data are representative of three independent experiments, each performed in duplicate or triplicate. *p<0.05; **p<0.005; ***p<0.0005.

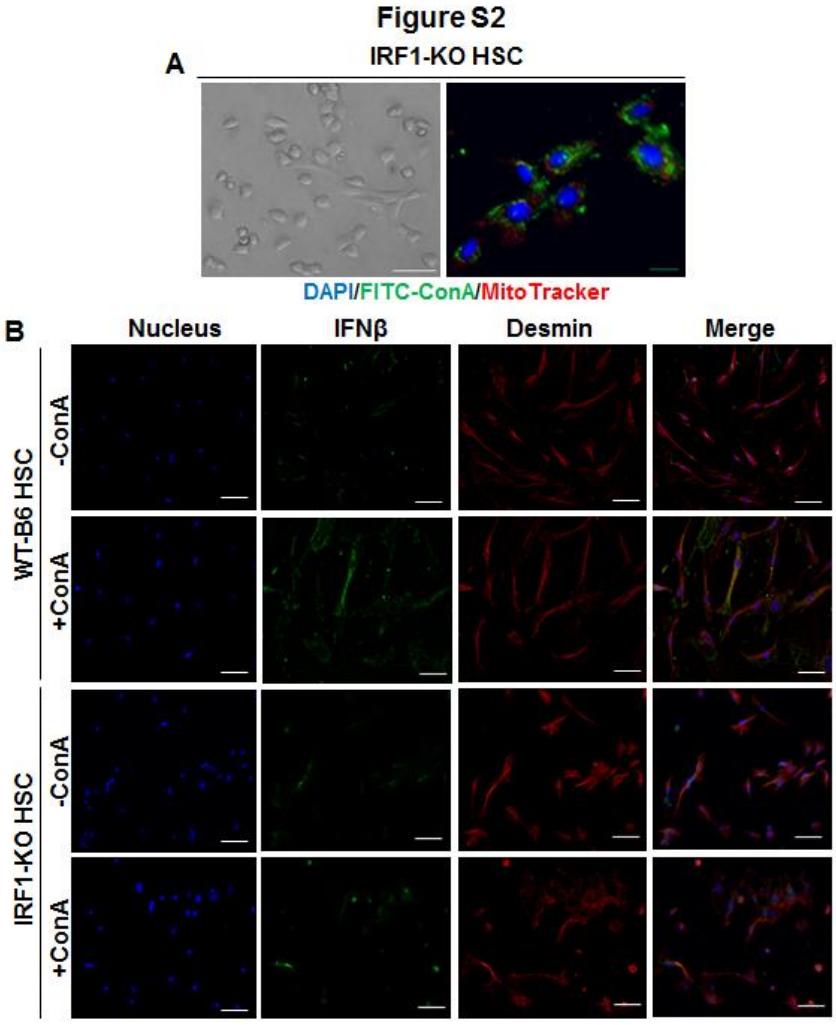

Figure S2: Binding of ConA and ConA-induced expression of IFNβ in IRF1-KO HSCs. (A) Immunofluorescence image showing binding/internalization of FITC-ConA in HSCs isolated from IRF1-KO mice. MitoTracker Red (100 nM) was used to stain mitochondria. Image on the left shows phase-contrast micrograph of IRF1-KO HSCs. Scale bars, 20 µm. (B) WT or IRF1-KO HSCs were incubated in presence of ConA (50 µg/ml) for 6h and intracellular accumulation of IFNβ was determined as described in the Methods section.

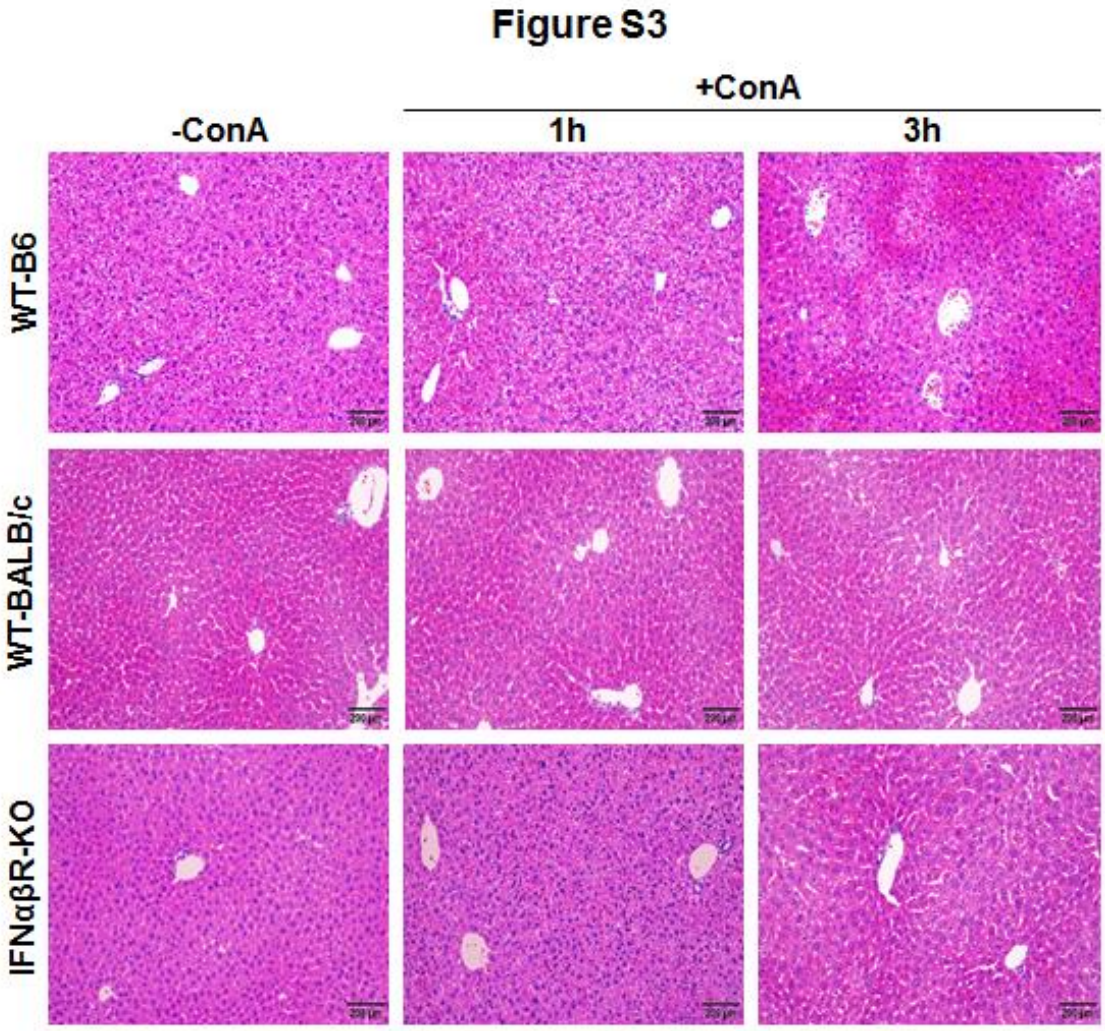

Figure S3: ConA-induced acute hepatitis in mice. Representative images of H/E-stained liver sections from mice treated with ConA (20 mg/kg) for 0, 1 or 3h. Scale bars, 200 μm.

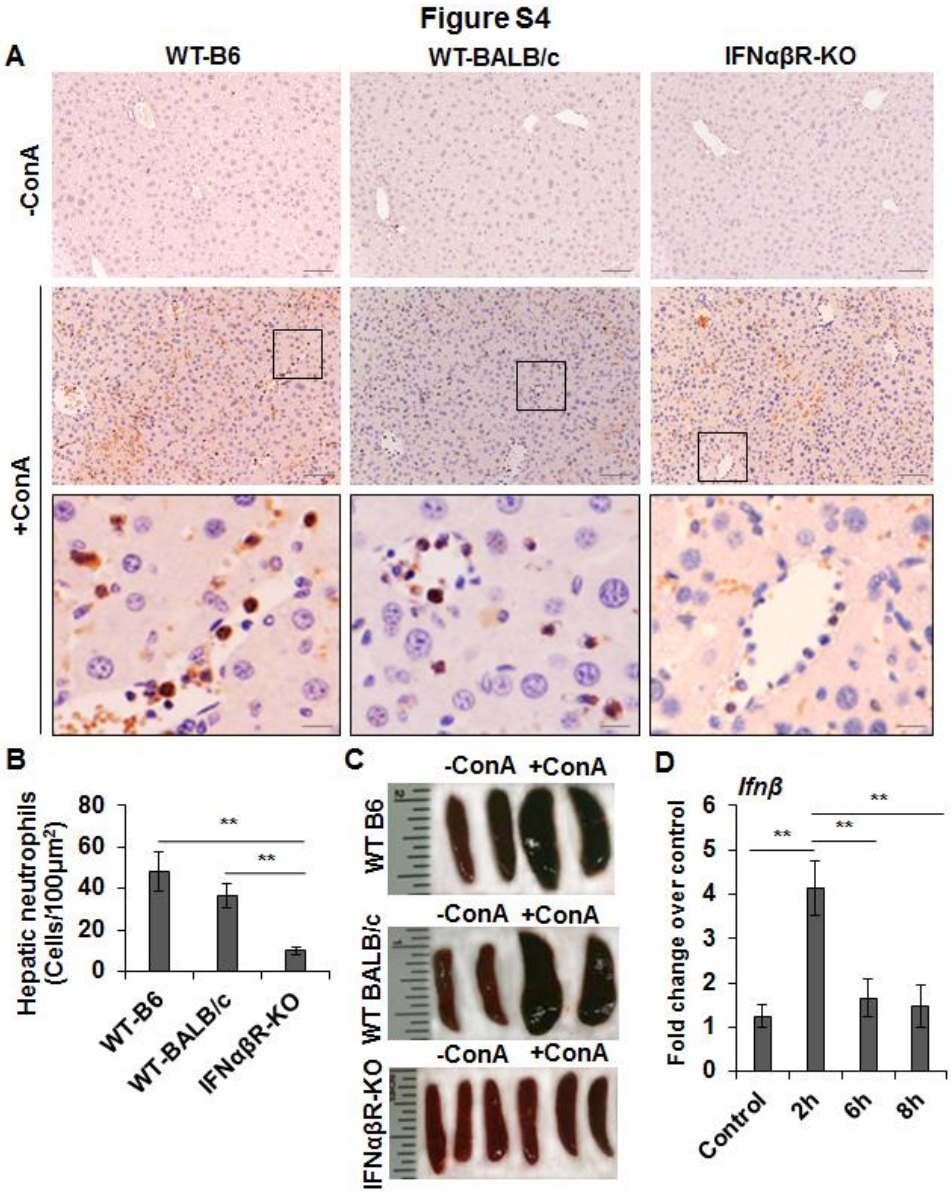

Figure S4: Neutrophil infiltration in ConA-treated mice. Mice were euthanized 6h (WT-B6) or 8h (WT-BALB/c or IFNαβR-KO) after administering 20 mg/kg ConA. (A) Liver sections were immunostained to detect neutrophils. Hardly any neutrophils are observed in the livers of vehicle-treated mice; large number of neutrophil infiltrates are seen in ConA-treated WT mice compared to very few in the IFNαβR-KO mice. Scale bars, 100 μm (upper and middle panels) and 50 μm (lower panels). (B) Bar graph shows quantification of infiltrated hepatic neutrophils. (C) Spleen size of ConA-treated mice show enlargement in WT but not in IFNαβR-KO mice. (D) IFNβ mRNA expression in ConA-challenged IFNαβR-KO mice at indicated time points. (n=4 to 6 mice/group). **$p < 0.005$.

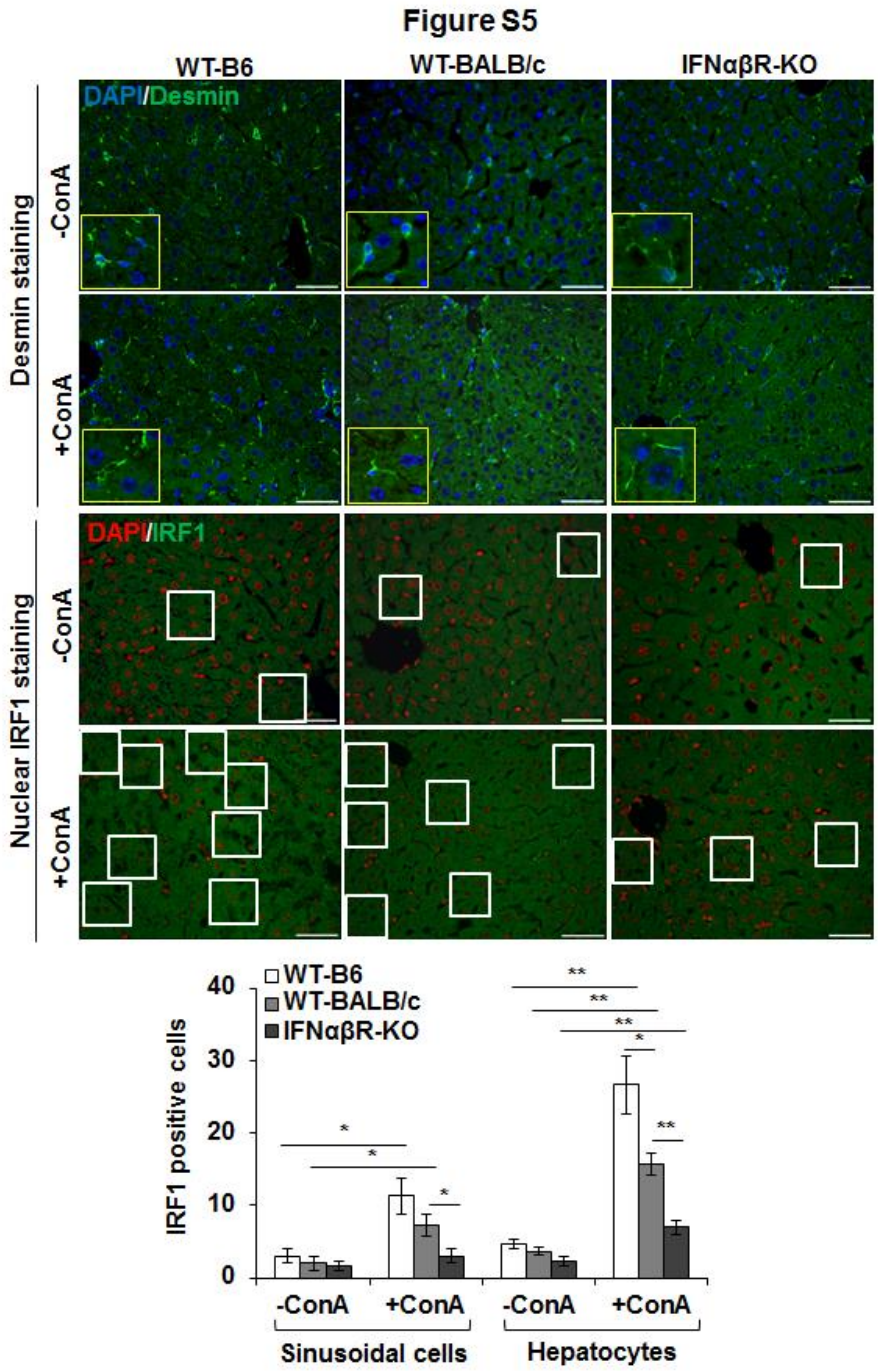

Figure S5: Nuclear IRF1 translocation in ConA-treated WT and IFNαβR-KO mice. Mice were euthanized 6h (WT-B6) or 8h (WT-BALB/c or IFNαβR-KO) after administering 20 mg/kg ConA. Representative sections of the vehicle- or ConA-treated livers were immunostained for the expression of desmin (inset) or nuclear IRF1 (boxed area). Scale bars, 50 μm. Bar graph shows quantification of nuclear IRF1-positive nonparenchymal cells and hepatocytes. *p<0.05; **p<0.005.

Figure S6

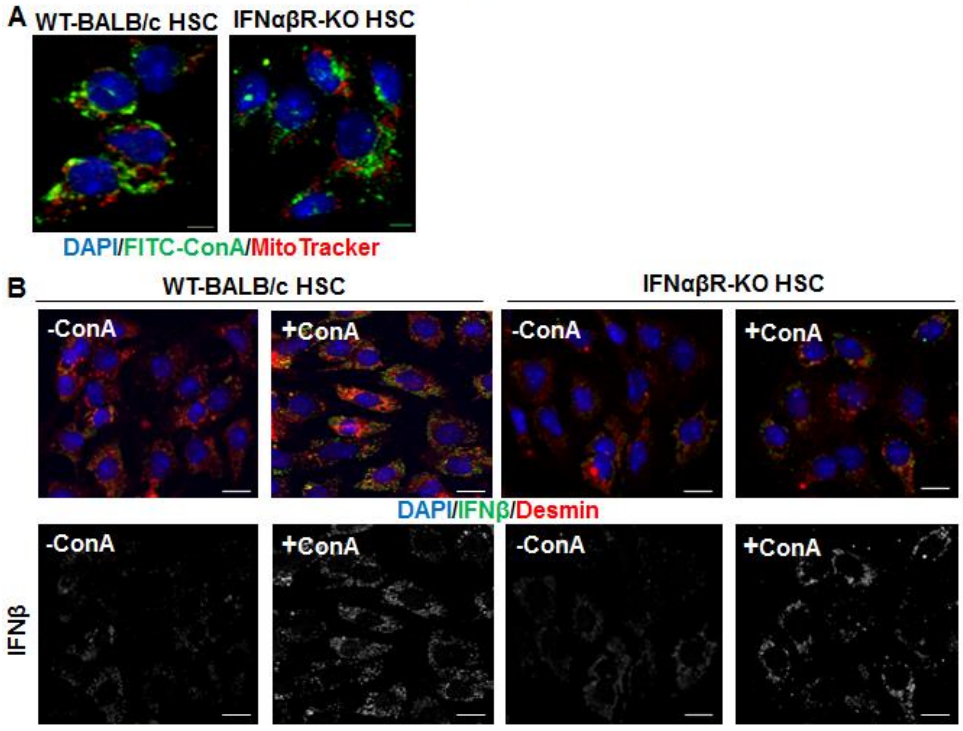

Figure S6: Binding and effects of ConA in HSCs from IFNαβR-KO mice. (A) Immunofluorescence image shows binding/internalization of FITC-ConA in HSCs isolated from WT-BALB/c and IFNαβR-KO mice. DAPI (blue) and MitoTracker Red (100 nM) was used to stain nuclei and mitochondria, respectively. (B) WT-BALB/c or IFNαβR-KO HSCs were incubated in presence of ConA (50 µg/ml) for 6h and intracellular accumulation of IFNβ was determined. Scale bars, 20 µm.

Figure S7

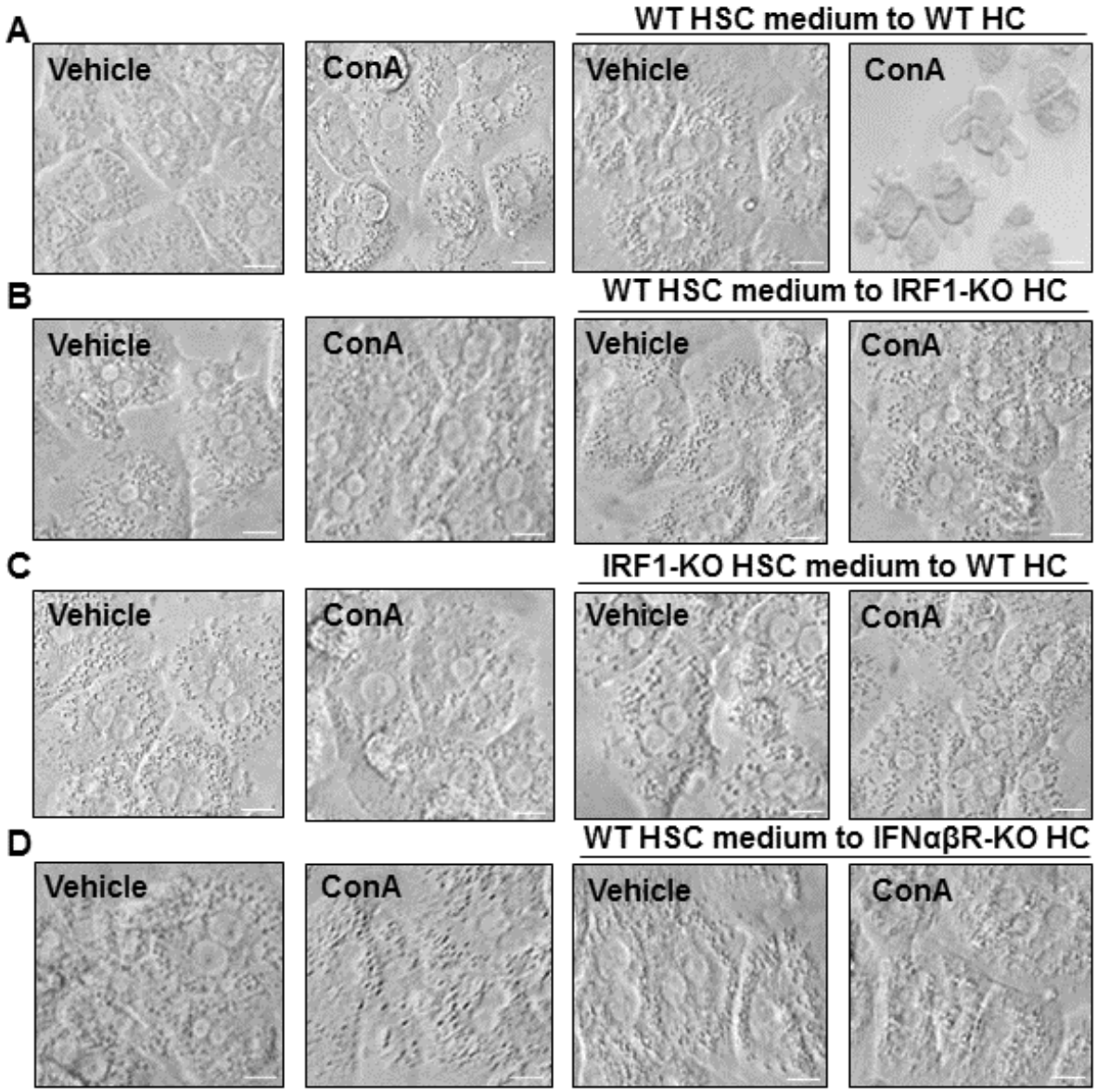

Figure S7: Hepatocytes from IRF1-KO and IFNαβR-KO are resistant to oxidative stress and apoptosis by ConA-stimulated WT HSCs. (A-D) Phase contrast images of hepatocytes (WT or IRF1-KO or IFNαβR-KO) incubated in medium without or with 50 μg/ml ConA or medium conditioned by HSCs (WT or IRF1-KO) for 8h in absence or presence of ConA. Scale bars, 20 μm.

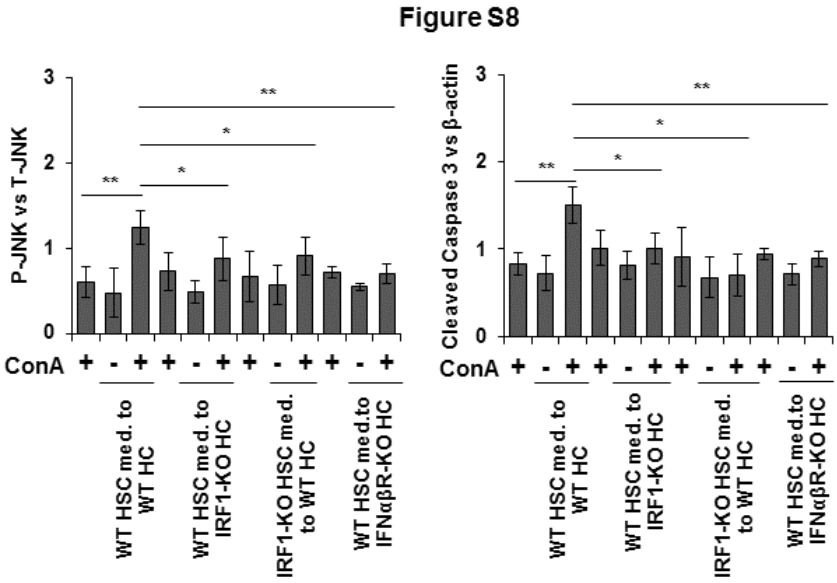

Figure S8: ConA-stimulated WT HSCs do not stimulate JNK or caspase 3 activation in hepatocytes from IRF1-KO and IFNαβR-KO mice. Bar graphs show densitometric analysis of the immunoblots in Figure 6A, for P-JNK and cleaved caspase-3 normalized to T-JNK and β-actin, respectively. *p<0.05; **p<0.005.