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Suppressor of Cytokine Signaling Expression with Increasing Severity of Murine Hepatic Ischemia-Reperfusion Injury

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Abstract

Background/Aims—Preservation of function requires that the cellular events initiated when hepatic ischemia is followed by reperfusion (IR) be tightly regulated. One important mechanism modulating this cytokine-directed response to injury is the Suppressors of Cytokine Signaling, SOCS1 and SOCS3, which ensure appropriate intensity and duration of cytokine signaling through negative feedback on the JAK-STAT pathway. The potential contribution of SOCS1 and SOCS3-mediated regulation to the evolution of hepatic IR and injury control is unknown.

Methods—In a murine model of hepatic IR, we demonstrate that expression of *Tnf*, *Il-1β*, and *Il-6* parallels increasing histologic injury severity. Despite early intense phosphorylation of both STAT1 and STAT3 after severe IR injury, negligible nuclear translocation of activated STAT1 was detected, suggesting that induction of nuclear target genes through JAK-STAT is predominantly via STAT3.

Results—*Socs3* was expressed across the IR injury spectrum (mild to severe) while *Socs1* was induced only in the face of severe IR injury.

Severe IR in *Il-6* deficient mice confirmed that *Il-6*, acting via STAT3, serves as a primary inducer of both protective mechanisms.

Conclusion—Under the influence of IL-6-mediated STAT3 signaling, *Socs1* serves as a complimentary regulatory mechanism when *Socs3* is insufficient to limit cytokine-mediated inflammation after hepatic IR.

Keywords

liver; SOCS1; SOCS3; interleukin 6; cytokine signaling

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Introduction

The evolution of a survivable hepatic ischemia-reperfusion (IR) injury requires the acute inflammatory phase to be tightly regulated. While the inflammatory responses that characterize IR are fairly well defined, relatively little is known about which regulatory pathways modulate injury severity and thus may be exploitable to improve outcome.

TNF, IL-1 β , IL-6 and IFN γ are among the cytokines identified as important contributors to IR injury (1-6). IL-6 and IFN γ in particular mediate their effects through Janus kinase (JAK) cell signaling pathways (7-9). A targeted gene response begins when a cytokine, bound to its membrane receptor, initiates the association of JAK with signal transducers and activators of transcription (STATs). Transcription of secondary inflammatory mediators follows translocation of phosphorylated STATs to the nucleus. The parallel initiation of negative regulatory mechanisms ensures that the duration and intensity of this response to injury is controlled. One important mechanism of control is the Suppressor of Cytokine Signaling (SOCS) family of proteins, which interfere with JAK-STAT coupling to inhibit propagation of pro-inflammatory cytokine signaling through a negative feedback loop (9). Deletion of the *Socs1* gene appears to result in unchecked cytokine signaling duration while *Socs3* primarily regulates the magnitude of cytokine-mediated responses (10).

We and others have shown that exogenous pre-treatment with IFN γ or IL-6 results in a significant reduction in hepatic IR injury (1,3). Since these pro-inflammatory mediators are potent inducers of SOCS1 and SOCS3 (7), induction of these regulatory mechanisms may play a significant role in determining whether cytokine-mediated IR resolves or progresses to irreversible necrosis. The goal of this study was to determine the effect of increasingly severe liver IR injury on the induction of SOCS regulatory mechanisms. Our data show that *Socs3* expression spans the range of IR injury from mild to severe, suggesting that it acts as a general regulatory mechanism in acute inflammation. Under the influence of IL-6 and STAT3, *Socs1* is induced with increasingly severe ischemic injury, suggesting that it serves as a complementary means of defense when SOCS3 is insufficient to limit cytokine signaling.

Experimental Procedures

Animals

Animal protocols were approved by the Veterans Affairs and the University of Washington IACUC Committees. C57/BL6 or *Il-6* $-/-$ male mice on a C57/BL6 background (6-8 weeks, Jackson Labs, Maine) weighing 20-25 gm, were fed standard rodent chow and subjected to 12h day/night light cycles.

Surgical preparation and sampling

After 1 week acclimatization, mice underwent *in situ* partial liver IR (6 per group) under isoflurane anesthesia. An atraumatic vascular clip was applied to the porta hepatis, such that the cephalad lobes were ischemic while the posterior and caudate lobes remained perfused as previously described (3,11). Animals were maintained under anesthesia on a heating pad at 39°C with the abdomen temporarily closed for the period of ischemia. After 20 or 90 min of ischemia, perfusion was re-established. After abdominal closure and recovery, animals were allowed *ad libitum* access to food and water. Cohorts of animals were euthanized 1, 2, 4, 8, or 24h after reperfusion. Parallel animals undergoing laparotomy without vascular occlusion served as sham-operated controls. At the time of CO₂ inhalation euthanasia, serum was collected by cardiac puncture and stored at -80° for glutamate pyruvate transaminase (GPT) analysis (Sigma). Liver from sham, ischemic and perfused lobes was preserved in 10% formalin

for histology or snap frozen in liquid nitrogen and stored at -80°C for RNA, nuclear extract and protein analysis.

Preparation and analysis of RNA

Total RNA was prepared from frozen liver tissues using Trizol/chloroform extraction (Invitrogen). Total RNA purity and concentration were determined using a spectrophotometer (BioRad). Wavelength ratios (260:280) between 1.7 and 2.0 were indicative of acceptable RNA quality. RNA integrity of 1µg samples was confirmed by electrophoresis on 1% agarose/ethidium bromide gels.

Real Time Polymerase Chain Reactions (RT-PCR)

DNase purified total RNA from ischemic, perfused and sham treated liver was reverse transcribed into cDNA and subjected to semi-quantitative RT-PCR utilizing FAM-labeled primers for the genes of interest (Applied Biosystems) on ABI Prism® 7000 instrumentation and software. Delta delta Ct values were calculated by subtracting Ct values for the gene of interest from values for β-actin (housekeeping gene) and then subtracting the delta Ct value for each gene from that of non-operated control mice. Fold induction was calculated by normalizing values to non-operated wild-type mice.

Western Blot

Ischemic and perfused liver tissue samples were homogenized in lysis buffer containing protease inhibitors and protein concentrations determined as previously described (8). Samples (50µg) diluted in sample buffer were subjected to SDS-PAGE and transferred to PVDF membranes (Amersham Biosciences). Membranes were blocked with 5% milk in TBST and incubated overnight at 4°C with primary antibodies to phospho-STAT1 and total STAT1, phospho-STAT3 and total STAT3 (Cell Signaling) in 5% BSA/TBST, followed by 5 washes in 1X TBST. After 1h incubation at room temperature in anti-rabbit horse radish peroxidase secondary antibody in 0.5% milk/TBST, membranes were washed and signals detected using ECL (Amersham). Membranes were exposed to Xray film (Kodak) and densitometry on scanned film was performed using NIH Image-J software.

Electrophoretic Mobility Shift Assays (EMSA)

Nuclear extracts were prepared as described (8) from snap frozen liver from ischemic and perfused liver and protein concentrations determined. Nuclear protein (5µg) was incubated with 0.2ng of ³²P-labeled SIE oligonucleotide probe for 30 minutes at room temperature. For supershift experiments, 1µg of specific antibodies to STAT1 (C-136X) or STAT3 (C-20X) (Santa Cruz) were added 30min prior to the addition of labeled probe. Samples were then subjected to electrophoresis through 5% polyacrylamide Tris-glycine-EDTA gels. Dried gels were exposed to film at -80°C with intensifying screens. IL-6 treated Hep-G2 cells served as a positive control (8).

Statistical analysis

Data are expressed as means ± SEM. Two-way ANOVA, verified by Bonferroni-Dunn post hoc testing, was performed on ischemic, perfused and sham samples for each condition using GraphPad Prism 4.00 software for GPT and expression of inflammatory cytokines, STATs, *Socs1*, and *Socs3*. Compared values were accepted as showing significant differences for $P \leq 0.05$.

Results

Kinetics of increasing injury severity (C57Blk6)

Hepatocellular damage was initially assessed using GPT levels and histology for the spectrum of IR injury. GPT paralleled ischemic severity. As shown in Figure 1A, 20min of ischemia resulted in transaminase release comparable to sham operation throughout reperfusion. With 90 min of ischemia (B), GPT was elevated within 1 hour, reached significantly different peak levels 4h after reperfusion. Levels for both IR groups returned to shams levels by 24h post-reperfusion.

Histologic examination of ischemic liver after mild injury (20min IR) showed scattered hepatocellular degeneration (cellular swelling) with minimal parenchymal alteration over time. With prolonged ischemia (90min IR), injury evolved throughout reperfusion. Initial mild focal hepatocellular degeneration after 1h of reperfusion was followed by sinusoidal neutrophil accumulation and parenchymal infiltration within 2-4h reperfusion (histology not shown). Figure 1(C, D) compares representative tissue sections 24h after reperfusion. Liver lobes subjected to 20min of ischemia (C) showed residual cellular edema but no neutrophil infiltration. With 90min ischemia (D), extensive areas of coagulative necrosis with hemorrhage and neutrophil infiltration were observed. In addition to the presented mild and severe injury extremes, we also examined intermediate levels of injury, observing gradations of transaminase release and histologic change after 45 or 60min ischemia and comparable reperfusion (unpublished data). Liver from perfused and sham treated lobes showed normal histology for the spectrum of injury severity (data not shown).

Cytokine expression profile

RT-PCR analysis of total RNA isolated from ischemic, perfused, and sham-treated liver profiled the kinetics of *Tnf*, *Il-1 β* and *Il-6* expression for mild vs. severe injury (Figure 2). Mild IR did not stimulate appreciable cytokine expression in perfused or ischemic tissue after IR (A, C, E). After severe IR, *Tnf* expression (B) was significantly elevated in ischemic tissue when compared with parallel perfused lobes ($p < 0.001$ at 4h and $p < 0.01$ at 8h or compared with 20min IR tissues at 1h, 4, and 8h ($p < 0.05$ at 1, 8h, $p < 0.001$ at 4h). *Il-1 β* expression (D) was similarly significantly enhanced in ischemic liver within 4h and 8h after 90min IR compared with the associated perfused lobes as well as ischemic or perfused tissues after 20min IR ($p < 0.001$). *Il-6* expression (F) closely mimicked that of *Tnf*, showing significant differences between mild and severe injury in ischemic tissues at 1, 4 and 8h of reperfusion ($p < 0.01$, 0.001, 0.05 respectively). Perfused liver in the 90min IR group showed significantly less *Il-6* expression compared with parallel ischemic tissue. *Il-10* and *Il-12* expression in ischemic liver was not statistically different from sham after 90 min IR. *Ifn γ* was not detected in ischemic or perfused liver after either mild or severe injury (data not shown). All cytokine RNA levels returned to baseline within 24h reperfusion.

STAT activation parallels injury severity

Given the cytokine profiles of mild and severe IR injury, we examined the phosphorylation of STAT1 and STAT3 as an indication of JAK-STAT transcription factor activation for the injury spectrum. Figure 3 shows representative immunoblots of phospho- and total STAT1 (A, ischemic; B perfused) and phospho- and total STAT3 (D, ischemic; E, perfused) with their respective densitometry analyses (C - STAT1, F - STAT3) after mild or severe IR. Despite low levels of cytokine gene expression, phosphorylation of STAT1 and STAT3 in ischemic and perfused liver peaked 2h after reperfusion and remained detectable up to 8h after mild IR. With increasing injury severity, phosphorylation of STAT1 was initiated earlier in both ischemic and perfused lobes ($p < 0.05$, 20min ischemia vs. 90min ischemia at 1h reperfusion).

Phosphorylation of STAT3 showed a similar pattern to STAT1, peaking 2h after mild IR injury but showing earlier activation with increasing injury severity.

Induction of target genes, including *Socs1* and *Socs3*, requires nuclear translocation of the phosphorylated activated transcription factor(s). To corroborate activation of phosphorylated STAT1 and STAT3 signaling and confirm nuclear translocation with DNA binding, nuclear extracts prepared from ischemic and perfused liver lobes harvested 2h after reperfusion were subjected to EMSA techniques. As shown in Figure 4, STAT3 homodimers and STAT3/STAT1 heterodimers but no STAT1 homodimers were seen in ischemic or perfused tissues after mild or severe IR. Supershift with antibodies specific for STAT3 and STAT1 identified STAT3 as the predominant signaling mechanism during IR.

SOCS induction and injury severity

Given the regulation of JAK-STAT mediated cytokine signaling duration and intensity by SOCS proteins (9,10,12) and our evidence for strong IR activation of STAT3 signaling, we investigated the relationship between *Socs3* and *Socs1* expression and injury severity. As shown in Figure 5, short duration ischemia (A) induced a ten-fold increase in *Socs3* expression in both ischemic and perfused lobes within 2h reperfusion, with gradual return toward baseline within 24h. Paralleling the pattern of STAT3 expression, *Socs3* was induced earlier in reperfusion (within 1h) and peaked later (4h) after prolonged ischemia (90min), with expression dominating in the ischemic liver (B). *Socs1* mRNA expression more closely paralleled injury severity. Mild ischemia led to minimal *Socs1* expression throughout reperfusion (C). With 90min IR (D), significant *Socs1* expression was induced within 2h of reperfusion in both directly injured lobes and perfused liver, was sustained through 4h of reperfusion and remained detectable 24h after reperfusion. These data show that *Socs3* is up-regulated across the spectrum of mild to severe IR and thus suggest a potential role for SOCS3 in the initial response to an injury. The correlation of *Socs1* expression only with severe injury suggests a second tier, complementary role, induced when cytokine signaling exceeds the regulatory capacity of SOCS3.

IL-6 mediates induction of SOCS1 and SOCS

Although IL-6 is a potent regulator of SOCS3 and SOCS1 is primarily induced by IFN γ (9, 12,13), each of these cytokines is capable of inducing both genes (12). The early and robust expression of *Il-6* after IR would be expected to induce *Socs3*. Minimal detection of *Ifn γ* coupled with the distinct lack of STAT1 nuclear translocation suggests that IL-6 maybe responsible for *Socs1* induction as well.

To test this hypothesis, 90min IR was performed in mice with deletion of the *Il-6* gene (*Il-6*^{-/-} on C57Bk6 background; *Il-6* KO) and tissues examined 2h after reperfusion. Ischemic liver histology showed extensive bridging areas of hepatocellular degeneration, characterized by cell swelling accompanied by individual cell necrosis and neutrophil margination (Figure 6 A, B). By comparison, ischemic tissues from wild-type control animals showed only focal and mild degenerative changes. GPT levels in the *Il-6* KO mice were double that of wild-type (mean 4617 units/L $\pm$ 954 vs. 2476 units/L $\pm$ 520), corroborating the histologic impression of increased injury severity in the absence of *Il-6*.

IR induced significantly less *Socs1* and *Socs3* expression in ischemic and perfused lobes from *Il-6* KO mice compared with perfused liver from wild-type controls (Figure 6 C, D). *Socs1* and *Socs3* expression was significantly decreased in ischemic and perfused lobes post IR compared with control perfused liver. EMSA affirmed nuclear translocation of phosphorylated STAT3 homodimers, STAT3/1 heterodimers, or STAT1 homodimers (data not shown). The lack of

nuclear STAT3 or STAT1 likely accounts for the observed sharp decline in *Socs3* and *Socs1* expression and may underlie the increased injury severity.

Discussion

The complex cell signaling events initiated after IR requires that the pro-inflammatory response to injury be countered by effective regulatory mechanisms if injury is to be controlled. By investigating both injury evolution and regulation in parallel, this study documents for the first time the relationships between hepatic IR injury progression, induction of pro-inflammatory mediators and the activation of negative regulatory cytokine signaling mechanisms.

The spectrum of liver IR injury evolves from mild hepatocyte swelling, through neutrophil infiltration, to patchy centrilobular necrosis as ischemia is prolonged. Significant elevations in serum transaminases occur only in the face of active cellular destruction. While a harbinger of injury progression toward tissue necrosis, circulatory clearance of GPT cannot, however be construed as indicating recovery but merely a lack of ongoing hepatocyte death.

Although previous reports have documented cytokine induction in ischemic liver prior to and immediately after reperfusion (14,15), we have extended these observations. Cytokine mRNA levels remain low in perfused lobes after mild or severe IR injury, emphasizing the impact of prolonged local hypoxia on cellular inflammatory mediator induction. Inflammatory cytokine expression varies with ischemic injury severity as evidenced by significant differences in *Tnf*, *Il-6*, and *Il-1 β* expression after mild and severe injuries throughout reperfusion. Early cytokine gene expression in severely ischemic tissue ebbs only to rebound and peak within 4h of reperfusion, and then gradually returns to normal levels within 24h of reperfusion. This phasic pattern of cytokine expression after severe hepatic IR supports the presence of a dynamic regulatory control mechanism.

Their ability to regulate cytokine signaling duration and magnitude through distinct though complementary mechanisms places SOCS1 and SOCS3 at a central point in the response to IR injury. As for many biological systems, there appears to be partial functional redundancy between SOCS1 and SOCS3 (12), and their roles in cytokine regulation extend beyond direct JAK-STAT negative feedback. Although inflammatory mediators such as TNF are primarily regulated through non-JAK-STAT signaling pathways, cross-regulation through SOCS has been observed (16). The association of *Socs1* and *Socs3* expression with *Tnf*, *Il-1 β* , and *Il-6* in ischemic tissue after IR emphasizes the potential importance of these negative regulators to the local control of injury. Induction in perfused lobes, despite minimal local tissue cytokine gene expression, however, suggests the influence by circulating inflammatory mediators.

SOCS3 is believed to primarily affect the intensity of cytokine signaling (12). We report that *Socs3* is induced in both ischemic and perfused liver lobes across the full range of IR injury, despite minimal associated cytokine expression (20min IR). This suggests that SOCS3 serves as an initial regulatory mechanism, even when local injury is mild and exposure to systemic cytokines is low. As the cytokine response escalates, *Socs3* expression is initiated earlier and peaks later, mirroring the magnitude of *Tnf*, *Il-1 β* and *Il-6* expression, particularly in ischemic liver. SOCS1, reported to modulate the duration of cytokine signaling, appears to be reserved for severe ischemic injury, initiated as a second tier mechanism of control. Although like *Socs3*, the pattern of expression for *Socs1* mirrors the ebb and flow of inflammatory cytokines, SOCS1 and SOCS3 proteins inhibit JAK-initiated phosphorylation of STATs through distinct mechanisms. Thus SOCS1 and SOCS3 likely serve complimentary and potentially additive roles in the negative regulation of cytokine signaling, with SOCS1 serving as a back-up negative-feedback mechanism when SOCS3-mediated cytokine suppression is insufficient to control IR injury.

IL-6 acts as a primary inducer of both *Socs3* and *Socs1* after IR. Their patterns of induction, coupled with the extensive degenerative injury and distinct lack *Socs1* and *Socs3* after IR in *Il-6*KO mice support this hypothesis. Given the negligible nuclear translocation of STAT1 after hepatic IR in the presence or absence of *Il-6*, *Socs1* induction appears to be via STAT3. Although IL-6 signals efficiently through STAT3 (17), STAT3 induction of *Socs1* is comparatively inefficient (18,19). Intense activation and translocation of STAT3 via high levels of *Il-6* may therefore be necessary to initiate *Socs1*-mediated feedback inhibition, accounting for its expression only after severe IR.

The relationship between the inflammatory response to injury and clinical outcome is the subject of active study. IL-6 in particular has been cited as a potent marker of injury severity and mortality in trauma and multiple organ failure (20,21). Our data raises the possibility that high levels of IL-6 associated with poor clinical outcome after major injury may, in fact, be a marker of inadequate negative regulatory mechanisms. Conceptually, this may be important to our understanding of multiple organ dysfunction and other clinical outcomes. If regulatory mechanisms, induced in response to local or remote injury are insufficient, the return to homeostasis is impeded and the stage is set for progression of an unchecked inflammatory response to multiple organ failure.

In conclusion, SOCS3 participates in the acute regulatory response to hepatic IR and may be sufficient to control cytokine expression when injury is mild. With increasing injury severity, SOCS1 induction provides a second tier, complementary mechanism of potential injury control. *Il-6* acts as a primary inducer of both *Socs3* and *Socs1* expression and cytokine regulation, signaling through STAT3.

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Abbreviations

IR	ischemia-reperfusion
SOCS1	suppressor of cytokine signaling1
SOCS3	suppressor of cytokine signaling3
STAT1	signal transducer and activators of transcription1
STAT3	signal transducer and activators of transcription3
GPT	glutamate pyruvate transaminase
EMSA	electro-mobility shift assays
RT-PCR	Real time polymerase chain reaction

Figure 1. Kinetics of Increasing Injury Severity after IR

Serum glutamate pyruvate transaminase (GPT) levels correlated with injury progression. While 20min of ischemia (mild injury, **A**) resulted in transaminase release comparable to sham. After 90min of ischemia (severe injury, **B**), GPT was elevated within 1 hour and reached significantly elevated levels compared with mild injury 4h after reperfusion (** $p < 0.001$). Histology confirmed a spectrum of IR injury 24hr after reperfusion, ranging from hepatocyte edema after 20min ischemia (**C** mild injury) through extensive coagulative necrosis after 90min ischemia (**D** severe injury). Panels show tissues at 4X magnification.

Figure 2. Cytokine Kinetics of Increasing Injury Severity

Inductions of *Tnf*, *Il-1 β* , and *Il-6* after 20min or 90min IR are shown (A, B, C respectively). Values were determined by RT-PCR. Expression for the gene of interest was normalized to β -actin loading and is expressed as fold induction over non-operated controls. Minimal cytokine expression was induced in ischemic and perfused lobes compared to controls after short duration ischemia (20min). Prolonged ischemia (90min) induced significant cytokine expression of all three inflammatory mediators, beginning within 1h reperfusion. Direct ischemic injury induced significantly greater gene expression compared to perfused liver throughout reperfusion, with maximal expression at 4h and 8h reperfusion (*p<0.05, **p<0.01, ***p<0.001).

*** $p < 0.001$). Significant differences between ischemic and perfused lobes after 20 or 90min ischemic were also confirmed ($\ddagger$ $p < 0.05$, $\ddagger\ddagger\ddagger$ $p < 0.001$).

Figure 3. Activation of STAT1 and STAT3 after Hepatic IR

Representative immunoblots of phospho- and total STAT1 (**A**, ischemic lobe; **B** perfused lobe) and phospho- and total STAT3 (**D**, ischemic lobe; **E** perfused lobe) are shown with their respective densitometry analyses (**C** - STAT1, **F** - STAT3) for mild or severe IR. Both STAT1 and STAT3 showed early phosphorylation (within 1h reperfusion) after severe IR when compared with a lesser injury, in particular in the ischemic lobes ($p < 0.05$) 90min ischemic liver compared with 20min ischemic or perfused liver for STAT1) Densitometry analysis was performed as described in Material and Methods.

Figure 4. Nuclear STAT signaling after Hepatic IR

A representative SIE EMSA of nuclear extracts from ischemic and perfused liver lobes after 90min ischemia followed by 2h reperfusion is shown. STAT1 and STAT3 homodimers and STAT3/STAT1 heterodimers are identified by the black arrows. HepG2 cells stimulated with IL-6 and liver after partial hepatectomy served as positive controls. After 2h reperfusion, only STAT3 homodimers and STAT3/STAT1 heterodimers are identified in nuclear extracts. Supershift analysis for STAT1 and for STAT3 (lanes labeled supershift) confirms STAT3 as the dominant nuclear signaling mechanism after IR (shown by an asterisks over an arrow).

Figure 5. *Socs3* and *Socs1* after Hepatic IR

RT-PCR quantitation of *Socs3* (A, B) and *Socs1* (C, D) are shown. Although *Socs3* was induced across the spectrum of hepatic IR injury, significantly greater expression was noted within 2h reperfusion after mild ischemia in both ischemic and perfused lobes (** $p < 0.001$ compared with 90min IR). With more severe IR (90min), *Socs3* induction was initiated earlier and peaked later ($p < 0.01$ at 4h reperfusion) in both ischemic and perfused liver compared with liver lobes after mild injury. These data suggest that *Socs3* likely serves as an early regulatory control mechanism in the response to an acute inflammatory injury. *Socs1* showed minimal expression after mild IR but significant induction in both ischemic and perfused liver after 90min IR at 2 and 4h reperfusion (** $p < 0.01$, *** $p < 0.001$). The correlation of *Socs1* with

increased injury severity suggests that this negative regulator may serve as a reserve and complementary mechanism of injury control when Socs3-mediated control is insufficient.

Figure 6. *Il-6* Depletion Increases Injury Severity after 90min IR

Histology comparing ischemic liver from mice with *Il-6* gene deletion (IL-6KO) versus wild-type controls after 90min ischemia followed by 2h reperfusion is shown. Panels show H&E stained ischemic tissues at 4X magnification with 20X insets (A wild-type, B IL-6KO).

Deletion of *Il-6* results in extensive bridging areas of hepatocellular degeneration while wild-type controls exhibits only focal degenerative changes. Induction of *Socs1* (C) and *Socs3* (D) after hepatic IR as measured by RT-PCT and normalized to β -actin is significantly reduced in the absence of *Il-6*, particularly in perfused liver from *Il-6* KOs compared with wild-type mice (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).