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## Authors

Yue, Shi
Zhu, Jianjun
Zhang, Ming
et al.

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# The Myeloid Heat Shock Transcription Factor 1/β-Catenin Axis Regulates NLR Family, Pyrin Domain-Containing 3 Inflammasome Activation in Mouse Liver Ischemia/Reperfusion Injury

Shi Yue,[1*] Jianjun Zhu,[2*] Ming Zhang,[2] Changyong Li,[1] Xingliang Zhou,[3] Min Zhou,[1] Michael Ke,[1] Ronald W. Busuttil,[1] Qi-Long Ying,[3] Jerzy W. Kupiec-Weglinski,[1] Qiang Xia,[2] and Bibo Ke[1]

Heat shock transcription factor 1 (HSF1) has been implicated in the differential regulation of cell stress and disease states. β-catenin activation is essential for immune homeostasis. However, little is known about the role of macrophage HSF1-β-catenin signaling in the regulation of NLRP3 inflammasome activation during ischemia/reperfusion (I/R) injury (IRI) in the liver. This study investigated the functions and molecular mechanisms by which HSF1-β-catenin signaling influenced NLRP3-mediated innate immune response *in vivo* and *in vitro*. Using a mouse model of IR-induced liver inflammatory injury, we found that mice with a myeloid-specific HSF1 knockout ($HSF1^{M\text{-}KO}$) displayed exacerbated liver damage based on their increased serum alanine aminotransferase levels, intrahepatic macrophage/neutrophil trafficking, and proinflammatory interleukin (IL)-1β levels compared to the HSF1-proficient ($HSF1^{FL/FL}$) controls. Disruption of myeloid HSF1 markedly increased transcription factor X-box-binding protein (XBP1), NLR family, pyrin domain-containing 3 (NLRP3), and cleaved caspase-1 expression, which was accompanied by reduced β-catenin activity. Knockdown of XBP1 in HSF1-deficient livers using a XBP1 small interfering RNA ameliorated hepatocellular functions and reduced NLRP3/cleaved caspase-1 and IL-1β protein levels. In parallel *in vitro* studies, HSF1 overexpression increased β-catenin (Ser552) phosphorylation and decreased reactive oxygen species (ROS) production in bone-marrow-derived macrophages. However, myeloid HSF1 ablation inhibited β-catenin, but promoted XBP1. Furthermore, myeloid β-catenin deletion increased XBP1 messenger RNA splicing, whereas a CRISPR/CRISPR-associated protein 9-mediated XBP1 knockout diminished NLRP3/caspase-1. *Conclusion:* The myeloid HSF1-β-catenin axis controlled NLRP3 activation by modulating the XBP1 signaling pathway. HSF1 activation promoted β-catenin, which, in turn, inhibited XBP1, leading to NLRP3 inactivation and reduced I/R-induced liver injury. These findings demonstrated that HSF1/β-catenin signaling is a novel regulator of innate immunity in liver inflammatory injury and implied the therapeutic potential for management of sterile liver inflammation in transplant recipients. (HEPATOLOGY 2016;64:1683-1698).

Hepatic dysfunction or failure caused by ischemia-reperfusion (I/R) injury (IRI) is a major problem following liver transplantation.[1] Oxidative stress has been recognized as an important factor in the pathogenesis of hepatic IRI.[2] Hepatic I/R (HIR) activates liver macrophages (Kupffer cells; KCs) to generate reactive oxygen species (ROS), leading to sterile inflammation in the liver. Indeed,

*Abbreviations: ANOVA, analysis of variance; ASC, apoptosis-associated speck-like protein containing CARD; ATP, adenosine triphosphate; β-catenin$^{FL/FL}$, floxed β-catenin; β-catenin$^{M\text{-}KO}$, myeloid-specific β-catenin knockout; BMMs, bone-derived macrophages; Cas9, CRISPR-associated protein 9; CMV, cytomegalovirus; CRISPR, clustered regularly interspaced short palindromic repeats; CXCL-10, chemokine (C-X-C motif) ligand 10; DAMPs, danger-associated molecular patterns; ER, endoplasmic reticulum; GFP, green fluorescent protein; HIF, hypoxia-inducible transcription factor; HIR, hepatic I/R; HSF1, heat shock transcription factor 1; $HSF1^{FL/FL}$, floxed HSF1; $HSF1^{M\text{-}KO}$, myeloid-specific HSF1 knockout; HSPs, heat shock proteins; IL, interleukin; I/R, ischemia/reperfusion; IRE1α, inositol-requiring enzyme 1 alpha; IRI, ischemia/reperfusion injury; KCs, Kupffer cells; LPS, lipopolysaccharide; LysM, lysozyme M; mAbs, monoclonal antibodies; MPO, myeloperoxidase; mRNA, messenger RNA; MyD88, myeloid differentiation factor 88; NLRP3, NLR family, pyrin domain-containing 3; NS, nonspecific; PAMPs, pathogen-associated molecular patterns; qRT-PCR, quantitative real-time polymerase chain reaction; ROS, reactive oxygen species; sALT, serum alanine aminotransferase; siRNA, small interfering RNA; STAT3, signal transducer and activator of transcription 3; XBP1s, soluble XBP1; TLR4, Toll-like receptor 4; TNF, tumor necrosis factor; TRAF6,*

ROS functions as an alarm signal that triggers efficient defense responses by modulating specific signal transduction pathways. One signaling pathway that leads to ROS production triggers NLR family, pyrin domain-containing 3 (NLRP3) inflammasome activity.[3,4]

NLRP3 is a member of the NLR family of cytosolic pattern recognition receptors. NLRP3 mediates caspase-1 activation with the adaptor protein, apoptosis-associated speck-like protein containing CARD (ASC), to induce maturation and secretion of interleukin (IL)-1$\beta$ in response to various pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) released from stressed or injured cells.[5] The active NLRP3 inflammasome drives the innate immune response toward invading pathogens and cellular damage and regulates the adaptive immune response.[6,7] A number of human heritable and acquired diseases with dysregulated inflammasome activity have strongly highlighted the importance of the NLRP3 inflammasome in regulation of immune responses.[8] As a danger signal sensor, NLRP3 is essential for initiation of profound sterile inflammatory injury.[9,10]

Recently, heat shock transcription factor 1 (HSF1), which is a transcription factor for heat shock proteins (HSPs), was shown to facilitate cell survival and proliferation against severe stress insults.[11] Indeed, HSF1 is induced by various stressors, including oxidative stress. In unstressed cells, HSF1 is bound in the cytoplasm and forms multimeric protein complexes containing either HSP40/70 or HSP90. When stress causes protein unfolding, HSF1 is released from the complex and translocated to the nucleus, where it binds a heat shock response element to induce transcriptional activity.[12] HSF1 overexpression protected cardiomyocytes and improved cell survival against stress-mediated cell apoptosis.[13] Increased HSF1 expression promoted anti-inflammatory IL-10 activity and diminished proinflammatory cytokine expression in macrophages.[14] Additionally, HSF1 conferred protection against inflammatory injury and bacterial infection.[15,16] Induction of HSF1 prevented lipopolysaccharide (LPS)-induced liver damage by inhibiting HSP90-mediated inflammatory response.[17] We previously found that signal transducer and activator of transcription 3 (STAT3)-induced $\beta$-catenin inhibited Toll-like receptor 4 (TLR4)-driven inflammatory response in liver IRI.[18] Induction of the TLR4-mediated innate immune response was dependent on activation of the transcription factor, X-box-binding protein 1 (XBP1), in macrophages.[19] Indeed, XBP1

*TNF receptor-associated factor 6; TRIF, Toll/IL-1 receptor domain-containing adapter inducing interferon-β; WT, wild type; XBP1, X-box-binding protein.*

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## ARTICLE INFORMATION:

**From the [1]The Dumont-UCLA Transplant Center, Division of Liver and Pancreas Transplantation, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, CA; [2]Department of Liver Surgery, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; and [3]Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at USC, Department of Stem Cell Biology & Regenerative Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA.**

## ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

**Bibo Ke, M.D., Ph.D.**
**The Dumont-UCLA Transplant Center, Department of Surgery, David Geffen School of Medicine at UCLA**
**77-120 CHS**
**10833 Le Conte Avenue**
**Los Angeles, CA 90095**
**E-mail: bke@mednet.ucla.edu**
**Tel: +1-310-794-7557; or**

**Qiang Xia, M.D., Ph.D.**
**Department of Liver Surgery, Renji Hospital, Shanghai Jiaotong University School of Medicine**
**160 Pujian Road**
**Shanghai 200127, China**
**E-mail: xiaqiang@shsmu.edu.cn**
**Tel: +86-21-68383775**

is a key component of the endoplasmic reticulum (ER) stress response that is required for optimal and sustained production of proinflammatory cytokines during the inflammatory response.[19] XBP1 deficiency in mice increased their susceptibility to bacterial infections and impaired host defenses.[19] Furthermore, engagement of TLR4 and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) is crucial for mitochondrial/cellular ROS generation,[20] which is required for NLRP3 activation.[3] Disruption of NLRP3 or its adaptor protein, ASC, depressed caspase-1 activity and IL-1$\beta$ production, resulting in reduced I/R-triggered liver inflammation.[10,21] Although the roles of HSF1 in the protection of cells and organisms against severe stress insults have been established,[16,22] whether and how HSF1-$\beta$-catenin signaling regulates NLRP3 inflammasome activation in liver IRI are unknown.

Here, we identified a novel functional role and regulatory mechanism of HSF1-$\beta$-catenin signaling on the NLRP3-mediated innate immune response in liver sterile inflammatory injury. We demonstrated that activation of myeloid HSF1 modulated liver inflammatory response by promoting $\beta$-catenin signaling and depressing XBP1, which, in turn, inhibited NLRP3 activation and reduced I/R-triggered liver inflammatory injury.

# Materials and Methods

## ANIMALS

Floxed HSF1 ($HSF1^{FL/FL}$) and $\beta$-catenin ($\beta$-$catenin^{FL/FL}$) mice and mice expressing the Cre recombinase under the control of the lysozyme M (LysM) promoter (LysM-Cre) were used to generate myeloid-specific HSF1 ($HSF1^{M\text{-}KO}$) and $\beta$-catenin knockout ($\beta$-$catenin^{M\text{-}KO}$) mice. This study was performed in strict accord with the recommendations in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Bethesda, MD). The study protocols were approved by the Institutional Animal Care and Use Committee of the University of California at Los Angeles (Los Angeles, CA) and Shanghai Jiaotong University (Shanghai, China). See the Supporting Materials.

## MOUSE LIVER IRI MODEL AND TREATMENT

We used an established mouse model of warm hepatic ischemia followed by reperfusion, as previously described.[23] Some animals were injected by their tail veins 4 hours preceding ischemia with an Alexa Fluor 488-labeled nonspecific (control) small interfering RNA (siRNA), XBP1 siRNAs, or NLRP3 siRNA (2 mg/kg) mixed with mannose-conjugated polymers at a ratio determined according to the manufacturer's instructions as described.(24) See the Supporting Materials.

## HEPATOCELLULAR FUNCTION ASSAY

Serum alanine aminotransferase (sALT) levels, which are an indicator of hepatocellular injury, were measured by IDEXX Laboratories (Westbrook, ME).

## HISTOLOGY, IMMUNOHISTOCHEMISTRY, AND IMMUNOFLUORESCENCE STAINING

Liver sections were stained with hematoxylin and eosin. IRI severity was graded using Suzuki's criteria.[25] Liver macrophages and neutrophils were detected using primary rat antimouse $CD11b^{+}$ and Ly6G monoclonal antibodies (mAbs; BD Biosciences, San Jose, CA). Immunofluorescence staining was used to identify *in vivo* mannose-mediated delivery of siRNA into macrophages. See the Supporting Materials.

## QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION ANALYSIS

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed as described.[26] The primer sequences used for the amplification are shown in Supporting Table S1. See the Supporting Materials.

## WESTERN BLOTTING ANALYSIS

Protein was extracted from liver tissue or cell cultures as described.[26] The polyclonal rabbit anti-mouse phos-IRE1$\alpha$, monoclonal rabbit anti-mouse HSF1, phos-$\beta$-catenin, $\beta$-catenin, phos-Stat3, TLR4, TRAF6, XBP1s, NLRP3, cleaved caspase-1, and $\beta$-actin antibodies were used. See the Supporting Materials.

## LENTIVIRAL VECTOR PRODUCTION

The pSIN-$\beta$-catenin vector expresses $\beta$-catenin and contains an EF2 promoter and a puromycin gene.[27] The pLJM1-GFP (green fluorescent protein) vector

contains cytomegalovirus (CMV)-driven GFP. pSPAX2 and pCMV-VSV-G are lentiviral packaging plasmids. 293T cells were cotransfected with pSin-β-catenin (or pLJM1-GFP), pSPAX2, and pCMV-VSV-G using the Lipofectamine LTX Plus reagent to package lentiviruses according to the manufacturer's instructions (Life Technologies, NY). See the Supporting Materials.

The lentiviral clustered regularly interspaced short palindromic repeats receptor (CRISPR) XBP1 knockout vector was constructed by first cloning the XBP1 single-guide RNA sequences into the site of *BsmB*I of the LentiCRISPRv2 vector as described.[28] Lentiviral vector production was performed as described above. LentiCRISPRv2-XBP1 KO (LV-CRISPR-XBP1 KO), pSPAX2, and pCMV-VSV-G were used to package the viruses. The pLJM1-GFP virus was used as a control. See the Supporting Materials.

## ISOLATION OF HEPATOCYTES AND LIVER KCs

Primary hepatocytes and liver KCs were isolated from the $HSF1^{FL/FL}$ and $HSF1^{M\text{-}KO}$ mice as described.[26,29] Purity of KCs in ischemic liver was 80% based on immunofluorescence staining for $CD11b^{+}$. See the Supporting Materials.

## BONE MARROW-DERIVED MACROPHAGE ISOLATION AND *IN VITRO* TRANSFECTION

Murine bone marrow-derived macrophages (BMMs) were generated as described.[30] Cells ($1 \times 10^6$/well) cultured for 7 days were transfected with pBabe-HSF1 or the control vector. In some experiments, cells were transduced with a lentivirus expressing β-catenin, the CRISPR/Cas9 (CRISPR-associated protein 9) XBP1 knockout, or the pLJM1-GFP vector. See the Supporting Materials.

## ENZYME-LINKED IMMUNOSORBENT ASSAY

Murine BMMs culture supernatants were harvested for cytokine analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure IL-1β, TNF-α, and chemokine (C-X-C motif) ligand 10 (CXCL-10) levels.

## ROS ASSAY

ROS production in BMMs was measured using the Carboxy-H2DFFDA kit. ROS production by BMMs was analyzed and quantified by fluorescence microscopy according to the manufacturer's instructions. See the Supporting Materials.

## CASPASE-1 ENZYME ACTIVITY ASSAY

Caspase-1 enzymatic activity in BMMs from β-$catenin^{FL/FL}$, β-$catenin^{M\text{-}KO}$, and $HSF1^{M\text{-}KO}$ mice was determined using a colorimetric assay kit as described.[21] See the Supporting Materials.

## STATISTICAL ANALYSIS

Data are expressed as the mean ± SD and analyzed using the Permutation *t* test and Pearson correlation. Per comparison, two-sided *P* values less than 0.05 were considered statistically significant. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. We applied Welch's ANOVA to perform multiple group comparisons when the groups showed unequal variances. All analyses were performed using SAS/STAT software (version 9.4).

# Results

## MYELOID-SPECIFIC HSF1 DEFICIENCY INCREASES HEPATOCELLULAR DAMAGE IN LIVER IRI

Mouse livers from the myeloid-specific HSF1-deficient ($HSF1^{M\text{-}KO}$) and HSF1-proficient ($HSF1^{FL/FL}$) mice were subjected to 90 minutes of warm ischemia followed by 6 hours of reperfusion. We isolated both hepatocytes and KCs from these ischemic livers and found that, $HSF1^{M\text{-}KO}$ did not change hepatocyte HSF1 expression. However, HSF1 expression was undetectable in KCs from the $HSF1^{M\text{-}KO}$, but not in $HSF1^{FL/FL}$ mice (Fig. 1A). Hepatocellular functions were evaluated by measuring serum sALT levels (IU/L). sALT levels were increased in $HSF1^{M\text{-}KO}$ mice compared to $HSF1^{FL/FL}$ controls (Fig. 1B; 8,825 ± 714.2 vs. 4,765 ± 629.3; $P < 0.01$). These data correlated with Suzuki's histological grading of liver IRI. $HSF1^{FL/FL}$ mouse livers showed mild-to-moderate edema, sinusoidal congestion,

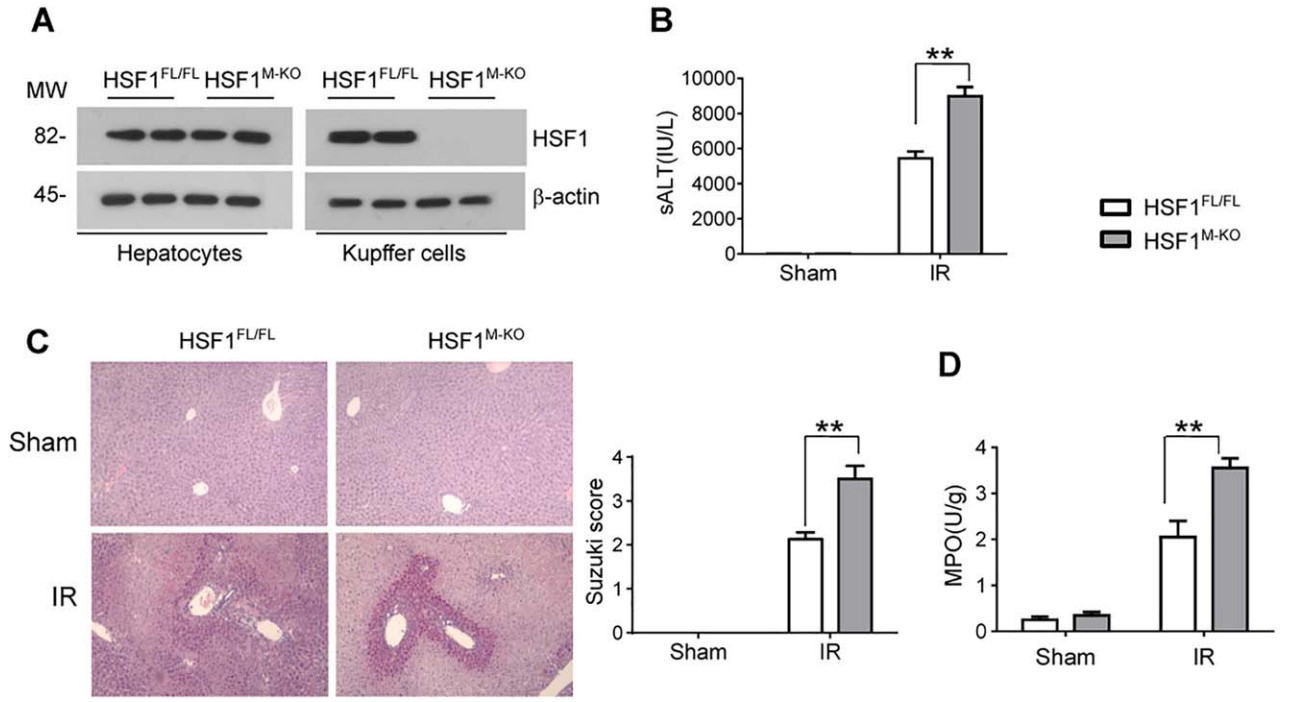


**FIG. 1.** Myeloid-specific HSF1 deficiency increases hepatocellular damage in liver IRI. Mice were subjected to 90 minutes of partial liver warm ischemia, followed by 6 hours of reperfusion. (A) Western blottings for detection of HSF1 in hepatocytes and liver KCs. Representative of three experiments. (B) Hepatocellular function in serum samples was evaluated by sALT levels (IU/L). Results expressed as mean ± SD (n = 4-6 samples/group). $^{**}P < 0.01$. (C) Representative histological staining (hematoxylin and eosin) of ischemic liver tissue. Results representative of 4-6 mice/group; original magnification, ×100. Liver damage, evaluated by Suzuki's histological score. $^{**}P < 0.01$. (D) Liver neutrophil accumulation, analyzed by MPO activity (U/g). Mean ± SD (n = 4-6 samples/group). $^{**}P < 0.01$. Abbreviation: MW, molecular weight.

and mild necrosis (Fig. 1C; score = 2.13 ± 0.08). In contrast, $HSF1^{M\text{-}KO}$ mouse livers displayed severe edema, sinusoidal congestion, and extensive hepatocellular necrosis (Fig. 1C; score = 3.65 ± 0.15; $P < 0.01$). Consistent with the histopathological and hepatocellular function data, myeloperoxidase (MPO) levels, which reflect liver neutrophil activity (U/g), were significantly elevated in the $HSF1^{M\text{-}KO}$ group, but not in the $HSF1^{FL/FL}$ group (Fig. 1D; 3.55 ± 0.15 vs. 2.05 ± 0.25; $P < 0.01$).

## MYELOID-SPECIFIC HSF1 DEFICIENCY INCREASES MACROPHAGE/NEUTROPHIL TRAFFICKING AND PROINFLAMMATORY MEDIATORS IN I/R-STRESSED LIVER

We analyzed macrophage and neutrophil accumulation in IR-stressed livers by immunohistochemistry staining. $HSF1^{M\text{-}KO}$ ischemic livers exhibited increased $CD11b^{+}$ macrophages infiltration (39.5 ± 1.5) compared to $HSF1^{FL/FL}$ controls (Fig. 2A; 18.3 ± 0.8; $P < 0.01$). These results were supported by the RT-PCR analysis in which $HSF1^{M\text{-}KO}$ increased IL-1$\beta$, TNF-$\alpha$, and CXCL-10 messenger RNA (mRNA) levels compared to $HSF1^{FL/FL}$ controls (Fig. 2B). Further immunostaining analysis revealed increased neutrophil trafficking in $HSF1^{M\text{-}KO}$ livers compared to $HSF1^{FL/FL}$ controls (Fig. 2C; 95.6 ± 3.38 vs. 43.6 ± 1.1; $P < 0.01$), which was companied by augmented CXCL-1 mRNA levels (Fig. 2D).

## MYELOID-SPECIFIC HSF1 DEFICIENCY DEPRESSES $\beta$-CATENIN SIGNALING AND ENHANCES XBP1/NLRP3 ACTIVATION IN I/R-STRESSED LIVER

Next, we analyzed whether HSF1 regulated innate immune response in IR-triggered liver injury.

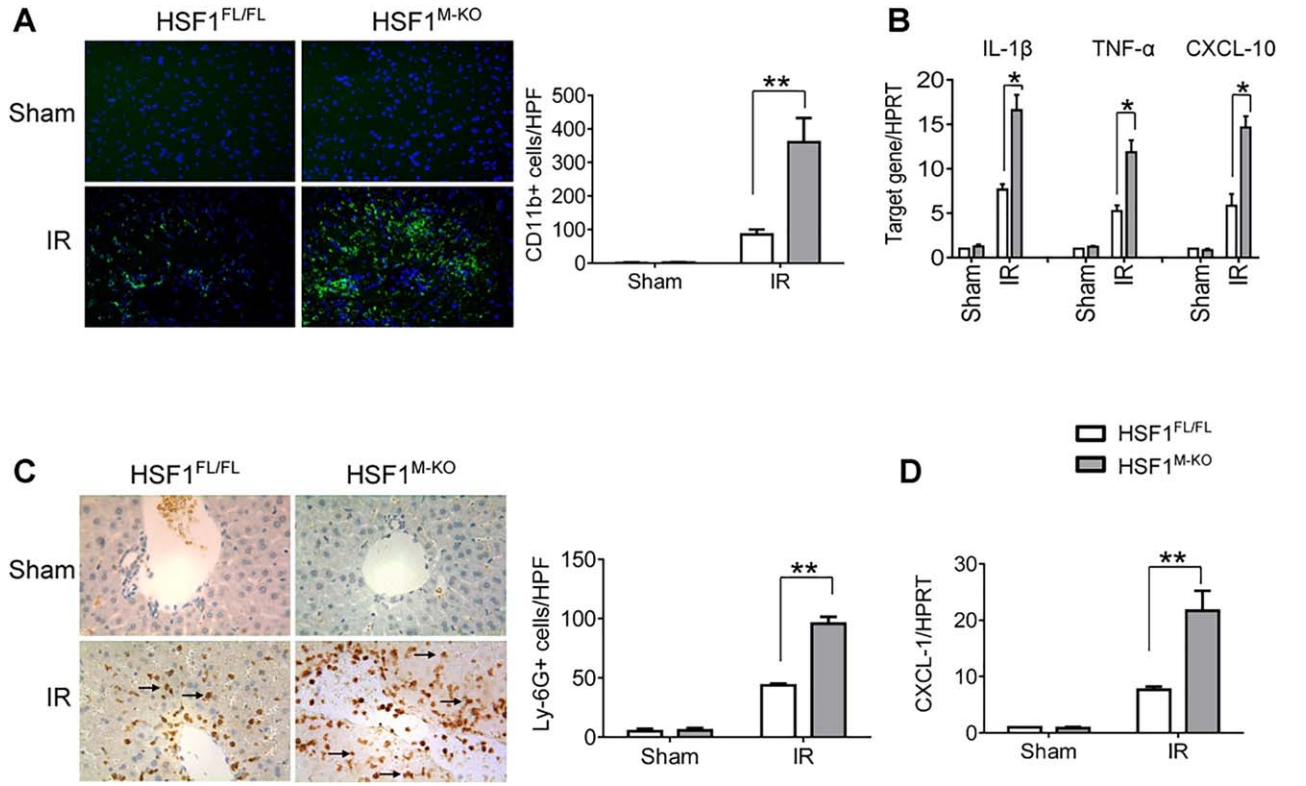


**FIG. 2.** Myeloid-specific HSF1 deficiency increases macrophage/neutrophil trafficking and proinflammatory mediators in I/R-stressed liver. Liver macrophages and neutrophils were detected by immunohistochemical staining using mAbs against mouse $CD11b^+$ and Ly6G in $HSF1^{FL/FL}$ (▭) and $HSF1^{M\text{-}KO}$ (▬) mice. (A) Immunohistochemical staining of $CD11b^+$ macrophages in ischemic livers. Quantification of $CD11b^+$ macrophages per high-power field. Results scored semiquantitatively by averaging number of positively stained cells (mean ± SD)/field at 200× magnification. Representative of 4-6 mice/group. $^{**}P < 0.01$. (B) qRT-PCR-assisted detection of IL-1$\beta$, TNF-$\alpha$, and CXCL-10 in mouse livers. Each column represents the mean ± SD (n = 3-4 samples/group). $^{*}P < 0.05$. (C) Immunohistochemical staining of $Ly6G^+$ neutrophils in ischemic livers. Quantification of $Ly6G^+$ neutrophils per high-power field (HPF; original magnification, ×200). Representative of 4-6 mice/group. $^{**}P < 0.01$. (D) qRT-PCR-assisted detection of CXCL-1 in mouse livers. Each column represents the mean ± SD (n = 3-4 samples/group). $^{**}P < 0.01$.

Following 6 hours of reperfusion after 90 minutes of ischemia, $HSF1^{M\text{-}KO}$ augmented spliced XBP1 (XBP1s), NLRP3, and IL-1$\beta$ mRNA expression in ischemic livers, as compared to $HSF1^{FL/FL}$ controls (Fig. 3A). Western blotting analysis revealed elevated XBP1s, NLRP3, and cleaved caspase-1 protein levels (Fig. 3B) in $HSF1^{M\text{-}KO}$ livers, which resulted in increased IL-1$\beta$, TNF-$\alpha$, and CXCL-10 production (Fig. 3C). Moreover, decreased STAT3 and $\beta$-catenin phosphorylation at Ser552 was found in $HSF1^{M\text{-}KO}$, but not the $HSF1^{FL/FL}$, livers post-HIR (Fig. 3D). To investigate whether HSF1 specifically influenced $\beta$-catenin activation in liver KCs, we isolated both hepatocytes and KCs from ischemic livers from $HSF1^{FL/FL}$ and $HSF1^{M\text{-}KO}$ mice. $HSF1^{M\text{-}KO}$ did not change hepatocyte $\beta$-catenin activation. However, reduced KC $\beta$-catenin phosphorylation was observed in $HSF1^{M\text{-}KO}$, but not in $HSF1^{FL/FL}$, mice (Fig. 3E).

## NLRP3 ACTIVATION IN MYELOID HSF1-DEFICIENT LIVER CONTRIBUTES TO I/R-TRIGGERED LIVER INFLAMMATION

To evaluate whether NLRP3 activation in $HSF1^{M\text{-}KO}$ livers contributed to IR-induced liver inflammation, we disrupted NLRP3 in $HSF1^{M\text{-}KO}$ livers using an NLRP3 siRNA with an *in vivo* mannose-mediated delivery system that enhances delivery to cells expressing a mannose-specific membrane receptor to macrophages, as

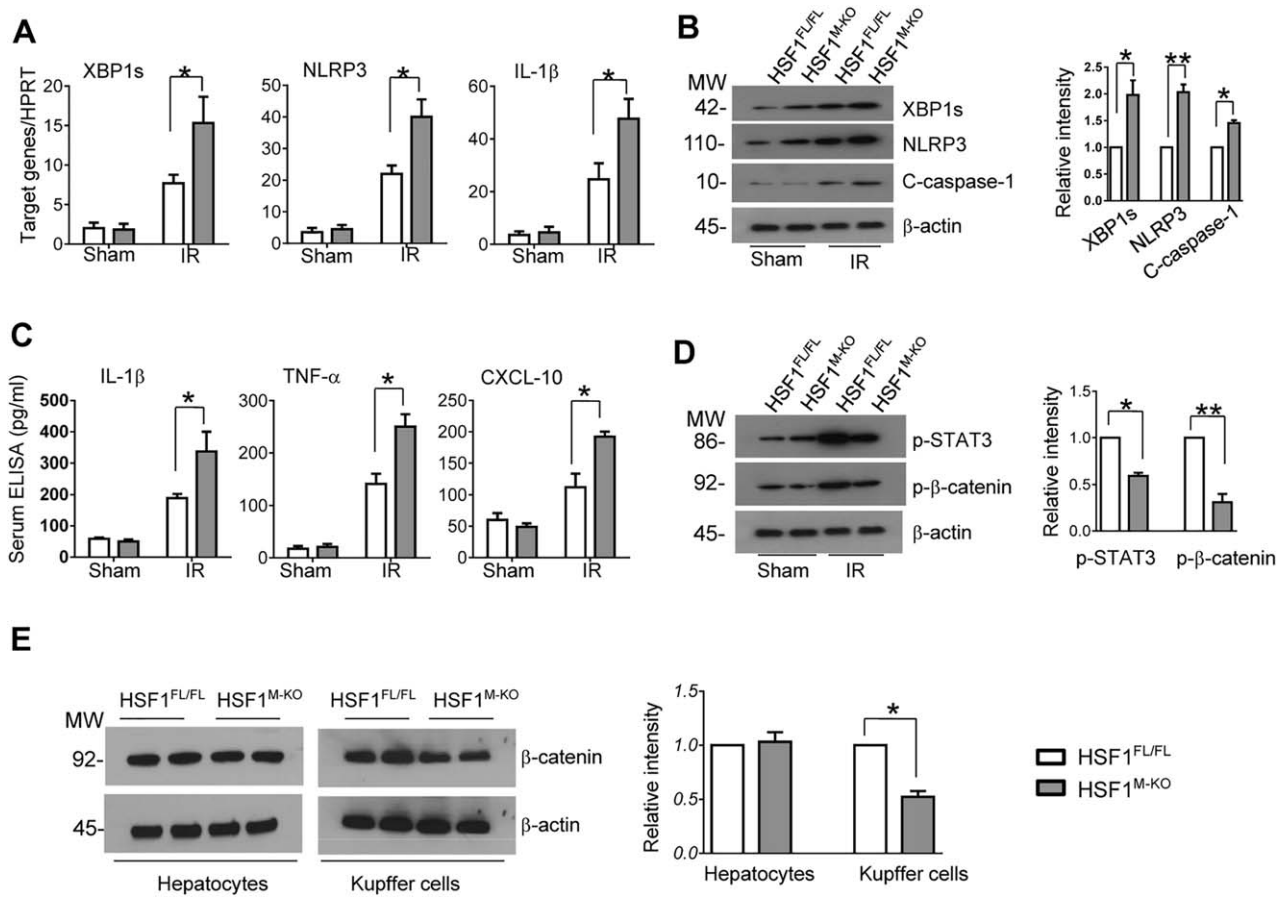


**FIG. 3.** Myeloid-specific HSF1 deficiency depresses $\beta$-catenin signaling and enhances XBP1/NLRP3 activation in I/R-stressed liver. (A) qRT-PCR-assisted detection of mRNA coding for XBP1s, NLRP3, and IL-1$\beta$ in mouse livers. Each column represents the mean ± SD (n = 3-4 samples/group). $^{*}P < 0.05$. (B) Western-assisted analysis and relative density ratio of XBP1s, NLRP3, and cleaved caspase-1. Representative of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. (C) ELISA analysis of IL-$\beta$, TNF-$\alpha$, and CXCL-10 levels in animal serum. Mean ± SD (n = 3-4 samples/group). $^{*}P < 0.05$. (D) Western blotting analysis and relative density ratio of p-Stat3 and $\beta$-catenin. Representative of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. (E) Western-assisted analysis and relative density ratio of $\beta$-catenin in hepatocytes and liver KCs. Representative of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. Abbreviations: HPRT, hypoxanthine-guanine phosphoribosyltransferase; MW, molecular weight; p, phosphorylated.

described.[24,31] Disruption of NLRP3 with siRNA treatment reduced IR-induced liver damage as evidenced by the decreased Suzuki's histological score (Fig. 4A; score = 0.95 ± 0.21 vs. 3.75 ± 0.35; $P < 0.01$) and sALT levels (Fig. 4B; 3,931 ± 799 vs. 9,014 ± 814; $P < 0.01$) compared to nonspecific (NS) siRNA-treated controls. Moreover, NLRP3 siRNA administration in HSF1$^{M\text{-}KO}$ ischemic livers decreased CD11b$^{+}$ macrophages (Fig. 4C; 58.7 ± 11.8 vs. 337.5 ± 23.9; $P < 0.01$) and neutrophil (Fig. 4D; 32.75 ± 6.26 vs. 117 ± 12.1; $P < 0.01$) accumulation compared to NS siRNA-treated controls. These results were consistent with the ELISA analysis, in which the NLRP3 blockade in HSF1$^{M\text{-}KO}$ reduced serum IL-1$\beta$ levels (Fig. 4E; 88.25 ± 24.7 vs. 380 ± 133.6; $P < 0.05$) and liver TNF-$\alpha$ and CXCL-10 mRNA levels compared to NS siRNA-treated controls (Fig. 4F).

## XBP1 IS REQUIRED FOR NLRP3 ACTIVATION IN MYELOID HSF1-DEFICIENT LIVER IN RESPONSE TO I/R

Because myeloid-specific HSF1 deletion induced XBP1 activation, we examined whether XBP1 affected NLRP3 function in I/R-triggered liver inflammation. We disrupted XBP1 in HSF1$^{M\text{-}KO}$ livers using a

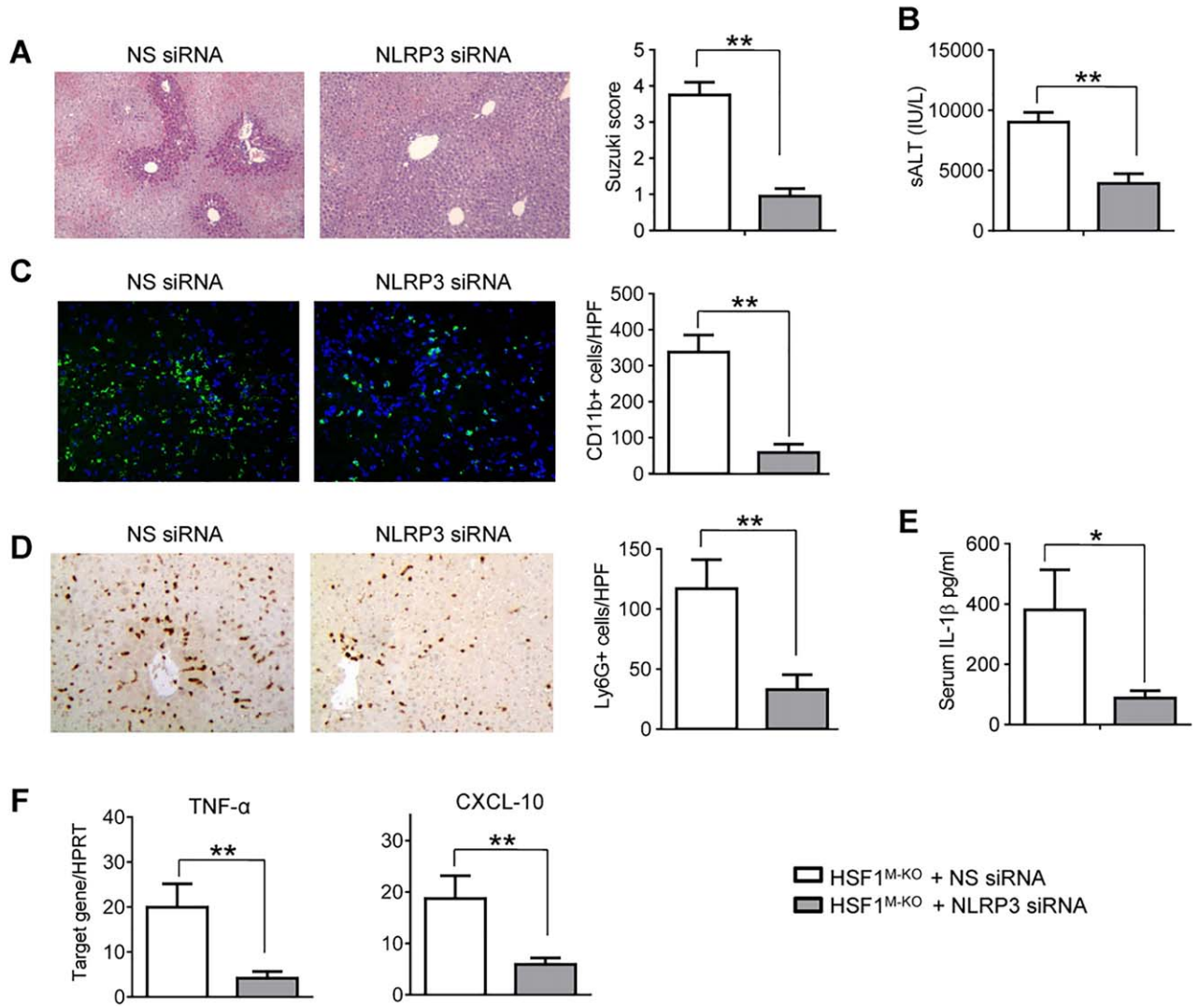


**FIG. 4.** NLRP3 activation in myeloid HSF1-deficient liver contributes to I/R-triggered liver inflammation. $HSF1^{M\text{-}KO}$ mice were injected by tail vein with Alexa Fluor 488-labeled NS control siRNAs (▭) or NLRP3 siRNA(■) (2 mg/kg) mixed with mannose-conjugated polymers at 4 hours before ischemia. (A) Representative histological staining (hematoxylin and eosin) of ischemic liver tissue. Results representative of 4-6 mice/group; original magnification, ×100. Severity of liver IRI was evaluated by Suzuki's histological grading. $^{**}P < 0.01$. (B) Hepatocellular function was evaluated by sALT levels (IU/L). Results expressed as mean ± SD (n = 4-6 samples/group). $^{**}P < 0.01$. (C) Immunohistochemical staining of $CD11b^+$ macrophages in ischemic livers. Quantification of $CD11b^+$ macrophages per high power field (HPF). Results scored semiquantitatively by averaging number of positively stained cells (mean ± SD)/field at 200× magnification. Representative of 4-6 mice/group. $^{**}P < 0.01$. (D) Immunohistochemical staining of $Ly6G^+$ neutrophils in ischemic livers. Quantification of $Ly6G^+$ neutrophils per HPF (original magnification, ×200). Representative of 4-6 mice/group. $^{**}P < 0.01$. (E) ELISA analysis of IL-$\beta$ levels in animal serum. Mean ± SD (n = 3-4 samples/group). $^{*}P < 0.05$. (F) qRT-PCR-assisted detection of mRNA coding for TNF-α and CXCL-10. Each column represents the mean ± SD (n = 3-4 samples/group). $^{**}P < 0.01$. Abbreviation: HPRT, hypoxanthine-guanine phosphoribosyltransferase.

mannose-mediated XBP1 siRNA *in vivo*. The mannose-mediated Alexa Fluor 488-labeled siRNA (green) delivery efficiently transduced into macrophages (red) in I/R-stressed livers (Fig. 5A). Livers in $HSF1^{M\text{-}KO}$ mice with NS siRNA treatment revealed significant edema, severe sinusoidal congestion/cytoplasmic vacuolization, and extensive (30%-50%) necrosis (Fig. 4B; score = 3.9 ± 0.1). In contrast, livers in mice treated with mannose-mediated XBP1 siRNA showed mild-to-moderate edema without necrosis (Fig. 5B; score = 2.0 ± 0.1; $P < 0.01$). Consistent with these data, sALT levels were significantly

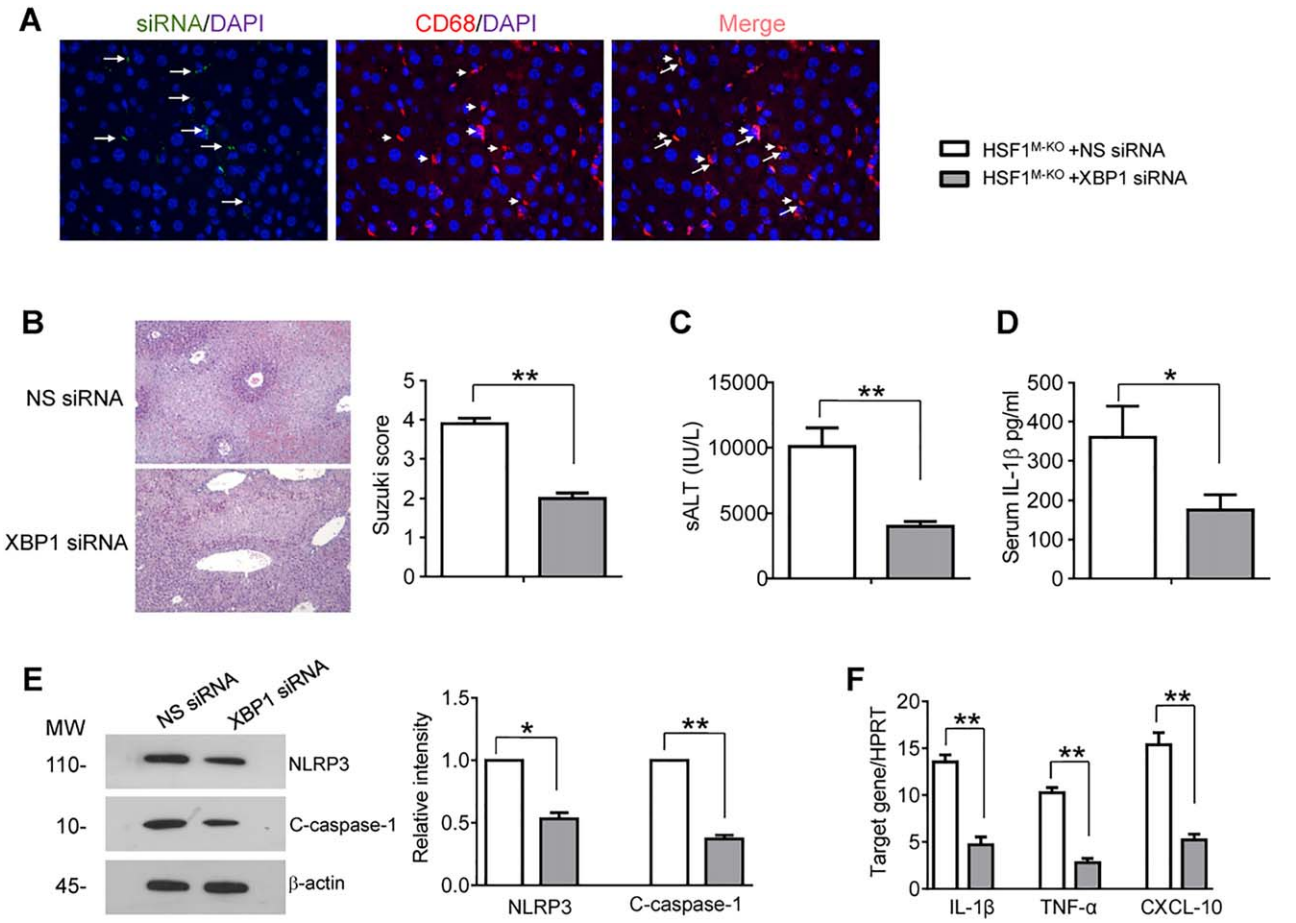


**FIG. 5.** XBP1 is required for NLRP3 activation in myeloid HSF1-deficient liver in response to I/R. $HSF1^{M\text{-}KO}$ mice were injected by tail vein with Alexa Fluor 488-labeled NS control siRNAs (▭) or XBP1 siRNA(▬) (2 mg/kg) mixed with mannose-conjugated polymers at 4 hours before ischemia. (A) Immunofluorescence staining of Alexa Fluor 488-labeled control siRNA (long arrow) and CD68-positive macrophages (short arrow) in ischemic liver lobes. Note: Green: Alexa Fluor 488-labeled siRNA; red: macrophage marker detected with CD68 mAb; blue: DAPI nuclear stain. Original magnification, ×200; Representative of 3-4 mice/group (B) Representative histological staining (hematoxylin and eosin) of ischemic liver tissue. Results representative of 4-6 mice/group; original magnification, ×100. Severity of liver IRI was evaluated by Suzuki's histological grading. $^{**}P < 0.01$. (C) Hepatocellular function was evaluated by sALT levels (IU/L). Results expressed as mean ± SD (n = 4-6 samples/group). $^{**}P < 0.01$. (D) ELISA analysis of IL-$\beta$ levels in animal serum. Mean ± SD (n = 3-4 samples/group). $^{*}P < 0.05$. (E) Western blottings analysis and relative density ratio of NLRP3 and cleaved caspase-1. Representative of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. (F) qRT-PCR-assisted detection of mRNA coding for IL-1$\beta$, TNF-$\alpha$, and CXCL-10. Each column represents the mean ± SD (n = 3-4 samples/group). $^{**}P < 0.01$. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; HPRT, hypoxanthine-guanine phosphoribosyltransferase; MW, molecular weight.

decreased in XBP1 siRNA knock-down mice compared to NS siRNA-treated controls (Fig. 5C; 3,992 ± 190 vs. 10,080 ± 722; $P < 0.001$). Moreover, XBP1 siRNA treatment in $HSF1^{M\text{-}KO}$ livers reduced serum IL-1$\beta$ release (Fig. 5D; 175.75 ± 19.3 vs. 360 ± 40.2; $P < 0.05$) and NLRP3 and cleaved caspase-1 protein expression (Fig. 5E), which were accompanied by decreased IL-1$\beta$, TNF-$\alpha$, and CXCL-10 production compared to the NS siRNA-treated group (Fig. 5F).

## DISRUPTION OF MYELOID HSF1 INHIBITS $\beta$-CATENIN ACTIVITY BUT ENHANCES NLRP3 INFLAMMASOME ACTIVATION IN MACROPHAGES

Having demonstrated that myeloid HSF1 activates $\beta$-catenin signaling to regulate IR-triggered liver inflammation, next we tested the regulatory role of

HSF1 during inflammatory response in macrophages. BMMs from wild-type (WT) mice were transfected with the HSF1-expressing vector, pBabe-HSF1, or the control vector followed by LPS stimulation. Transfection of pBabe-HSF1 increased HSF1 mRNA (Supporting Fig. S1A) and protein expression levels compared to controls (Fig. 6A). Moreover, overexpression of HSF1 by pBabe-HSF1 transfection increased STAT3 and β-catenin phosphorylation at Ser552 (Fig. 6A), which is a site associated with enhanced β-catenin transcriptional activity,[32] and decreased IL-1β, TNF-α, and CXCL-10 expression (Fig. 6B). Interestingly, pBabe-HSF1-mediated transfection markedly decreased ROS production in LPS-stimulated macrophages compared to controls (Fig. 6C; 18.0 ± 1.73 vs. 46.67 ± 2.96; $P < 0.01$).

To determine the importance of HSF1 for NLRP3 functions in macrophages, BMMs were isolated from $HSF1^{FL/FL}$ and $HSF1^{M\text{-}KO}$ mice and then cultured with LPS. HSF1 deficiency increased NLRP3 mRNA expression levels in LPS-stimulated BMMs (Supporting Fig. S1B). NLRP3 and cleaved caspase-1 protein expression was consistently increased in $HSF1^{M\text{-}KO}$ BMMs, but not $HSF1^{FL/FL}$ cells, after LPS stimulation (Fig. 6D). Moreover, $HSF1^{M\text{-}KO}$ cells exhibited significantly decreased β-catenin phosphorylation at Ser552 (Fig. 6D) accompanied by increased IL-1β, TNF-α, and CXCL-10 production (Fig. 6E). Strikingly, $HSF1^{M\text{-}KO}$ cells displayed significantly increased ROS production compared to $HSF1^{FL/FL}$ controls (Fig. 6F; 78.33 ± 3.28 vs. 46.33 ± 1.85; $P < 0.01$) in response to LPS stimulation.

## MYELOID β-CATENIN SIGNALING IS ESSENTIAL FOR HSF1-MEDIATED IMMUNE REGULATION OF NLRP3 FUNCTIONS IN MACROPHAGES

To elucidate the mechanisms of β-catenin in HSF1-mediated immune regulation of NLRP3 function, we cultured BMMs from $\beta\text{-catenin}^{FL/FL}$ and $\beta\text{-catenin}^{M\text{-}KO}$ mice and then transfected them with the HSF1 expression vector, pBabe-HSF1, or the control vector followed by LPS stimulation. Clearly, pBabe-HSF1 transfection in $\beta\text{-catenin}^{FL/FL}$ cells markedly increased HSF1 and β-catenin expression, but inhibited TLR4 expression (Fig. 7A), compared to the control vector-transfected cells. However, $\beta\text{-catenin}^{M\text{-}KO}$ cells exhibited augmented TLR4 expression even though these cells were transfected with pBabe-HSF1 (Fig. 7A). Moreover, $\beta\text{-catenin}^{M\text{-}KO}$ enhanced NLRP3 activation in BMMs based on the increased NLRP3 mRNA levels (Supporting Fig. S1C) and NLRP3 and cleaved caspase-1 protein expression levels (Fig. 7A) and activity (Supporting Fig. S1D). Because transcription factor XBP1 activation is induced by TLR4 signaling and β-catenin is crucial for regulation of TLR4-driven inflammatory response in macrophages,[18,19] we investigated spliced XBP1 expression in $\beta\text{-catenin}^{M\text{-}KO}$ cells. Interestingly, $\beta\text{-catenin}^{M\text{-}KO}$ increased TRAF6 and inositol-requiring enzyme 1 alpha (IRE1α) phosphorylation (Fig. 7B), resulting in increased splicing XBP1 mRNA (Supporting Figure S1E) and protein expression in response to LPS stimulation compared to $\beta\text{-catenin}^{FL/FL}$ cells (Fig. 7B). Furthermore, increased IL-1β production was found in the $\beta\text{-catenin}^{M\text{-}KO}$ cells, but not in the $\beta\text{-catenin}^{FL/FL}$ cells (Fig. 7C; 1,101.0 ± 60.1 vs. 515.7 ± 60.82; $P < 0.01$).

## HSF1-β-CATENIN AXIS INHIBITS XBP1-DEPENDENT NLRP3 ACTIVATION IN MACROPHAGES

To dissect the regulatory mechanisms underlying the effect of the HSF1-β-catenin axis on NLRP3 functions in macrophages, BMMs from $HSF1^{M\text{-}KO}$ mice were transfected with a lentivirus expressing β-catenin. In contrast to the lentiviral-mediated GFP (LV-pLJM1-GFP) control, transfection of $HSF1^{M\text{-}KO}$ cells with the lentivirus expressing β-catenin (pSIN-β-catenin) increased β-catenin expression, but inhibited IRE1α, XBP1, NLRP3, and cleaved caspase-1 expression (Fig. 7D) in response to LPS stimulation. To determine the role of macrophage XBP1 in NLRP3 activation, we disrupted XBP1 by using a CRISPR/Cas9 XBP1 knockout vector (LV-CRISPR-XBP1 KO) in BMMs from $HSF1^{M\text{-}KO}$ mice. Strikingly, knockdown of XBP1 in LV-CRISPR-XBP1 KO-treated cells led to decreased NLRP3 and cleaved caspase-1 expression (Fig. 7E), which was confirmed by examining the caspase-1 activity (Supporting Fig. S1F) in response to LPS stimulation compared to the LV-pLJM1-GFP-treated control. Moreover, IL-1β production was decreased in the XBP1 knockdown group, but not in the control group (Fig. 7F; 1,063.0 ± 96.4 vs. 2,638 ± 337; $P < 0.01$). Similarly, XBP1 knockdown reduced TNF-α and CXCL-10 expression in $HSF1^{M\text{-}KO}$ BMMs after LPS stimulation (Supporting Fig. S1G).

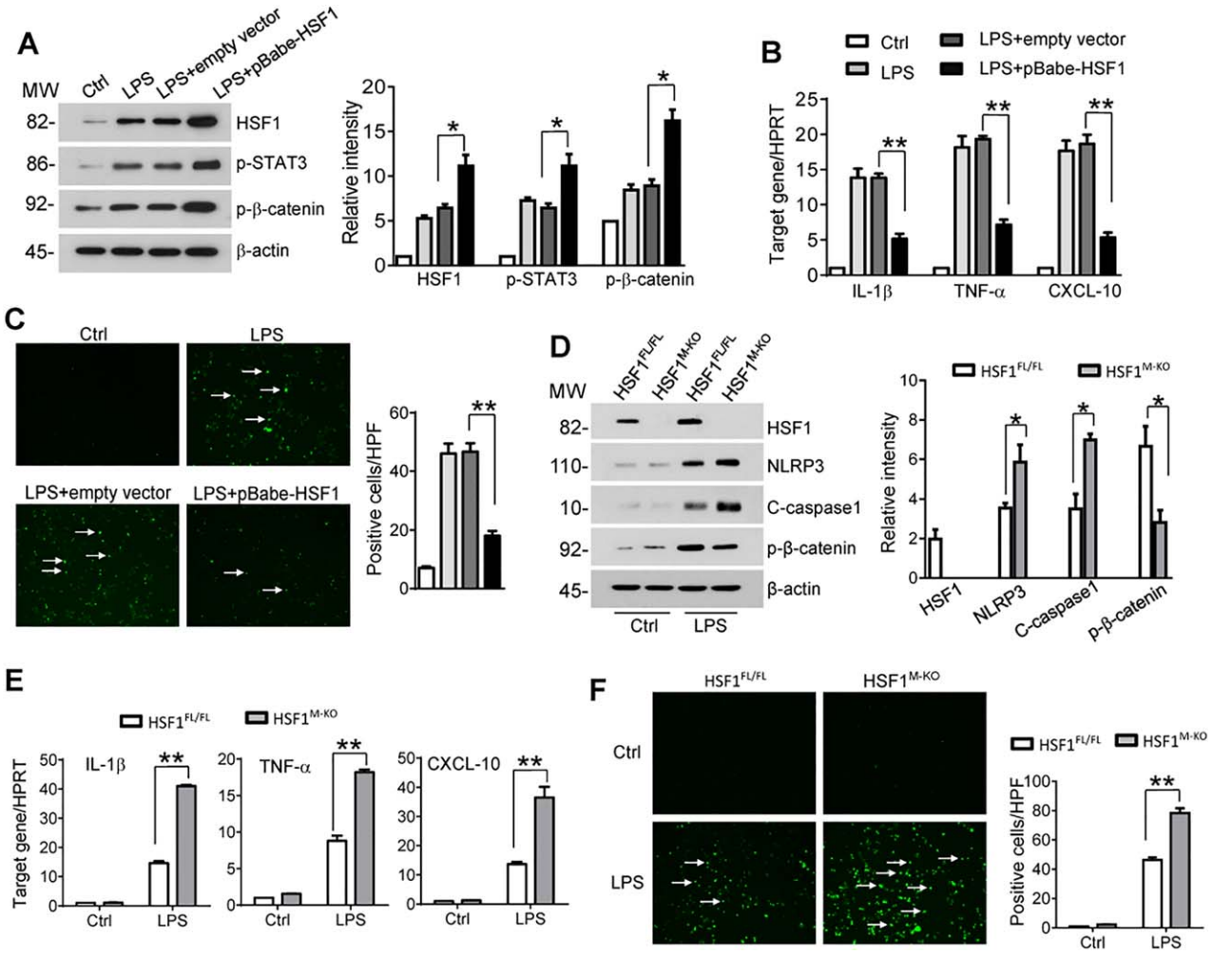


**FIG. 6.** Disruption of myeloid HSF1 inhibits β-catenin activity, but enhances NLRP3 inflammasome activation in macrophages. Murine BMMs from WT mice were transfected with control vector (▭) or pBabe-HSF1(▬) followed by LPS (100 ng/mL) stimulation. (A) Western blotting analysis and relative density ratio of HSF1, p-Stat3, and p-β-catenin. Representative of three experiments. $^{*}P < 0.05$. (B) qRT-PCR-assisted detection of mRNA coding for IL-1β, TNF-α, and CXCL-10. Each column represents mean ± SD (n = 3-4 samples/group). $^{**}P < 0.01$. (C) ROS production was detected by carboxy-H2DFFDA in LPS-stimulated BMMs from WT mice. Positive green-fluorescent-labeled cells were counted blindly in 10 high power fields (HPF)/section (200×). Quantification of ROS-producing BMMs (green) per HPF 200×). $^{**}P < 0.01$. (D) BMMs from $HSF1^{FL/FL}$ (▭) and $HSF1^{M\text{-}KO}$ (▬) mice were incubated with LPS (100 ng/mL). Western-assisted analysis and relative density ratio of HSF1, NLRP3, cleaved caspase-1, and p-β-catenin in LPS-stimulated cells. Representative of three experiments. $^{*}P < 0.05$. (E) qRT-PCR-assisted detection of mRNA coding for IL-1β, TNF-α, and CXCL-10. Each column represents mean ± SD (n = 3-4 samples/group). $^{**}P < 0.01$. (F) ROS production was detected by carboxy-H2DFFDA in LPS-stimulated BMMs from $HSF1^{FL/FL}$ and $HSF1^{M\text{-}KO}$ mice. Positive green-fluorescent-labeled cells were counted blindly in 10 HPF/section (200×). Quantification of ROS-producing BMMs (green) per HPF (200×). $^{**}P < 0.01$. Abbreviations: Ctrl, control;; HPRT, hypoxanthine-guanine phosphoribosyltransferase; MW, molecular weight; p, phosphorylated.

# Discussion

To the best of our knowledge, this is the first study to document the key role of the myeloid HSF1-β-catenin axis in regulation of NLRP3 inflammasome-mediated innate immune responses in liver sterile inflammatory injury. In this study, we demonstrate the following: (1) Myeloid HSF1 is essential for the control of liver inflammation through the activation of β-catenin signaling; (2) β-catenin activation inhibits NLRP3/caspase-1 activation and reduces IL-1β level; and (3) the myeloid HSF1-β-catenin axis regulates NLRP3 functions in an XBP1-dependent manner. Our results highlight the importance of myeloid HSF1

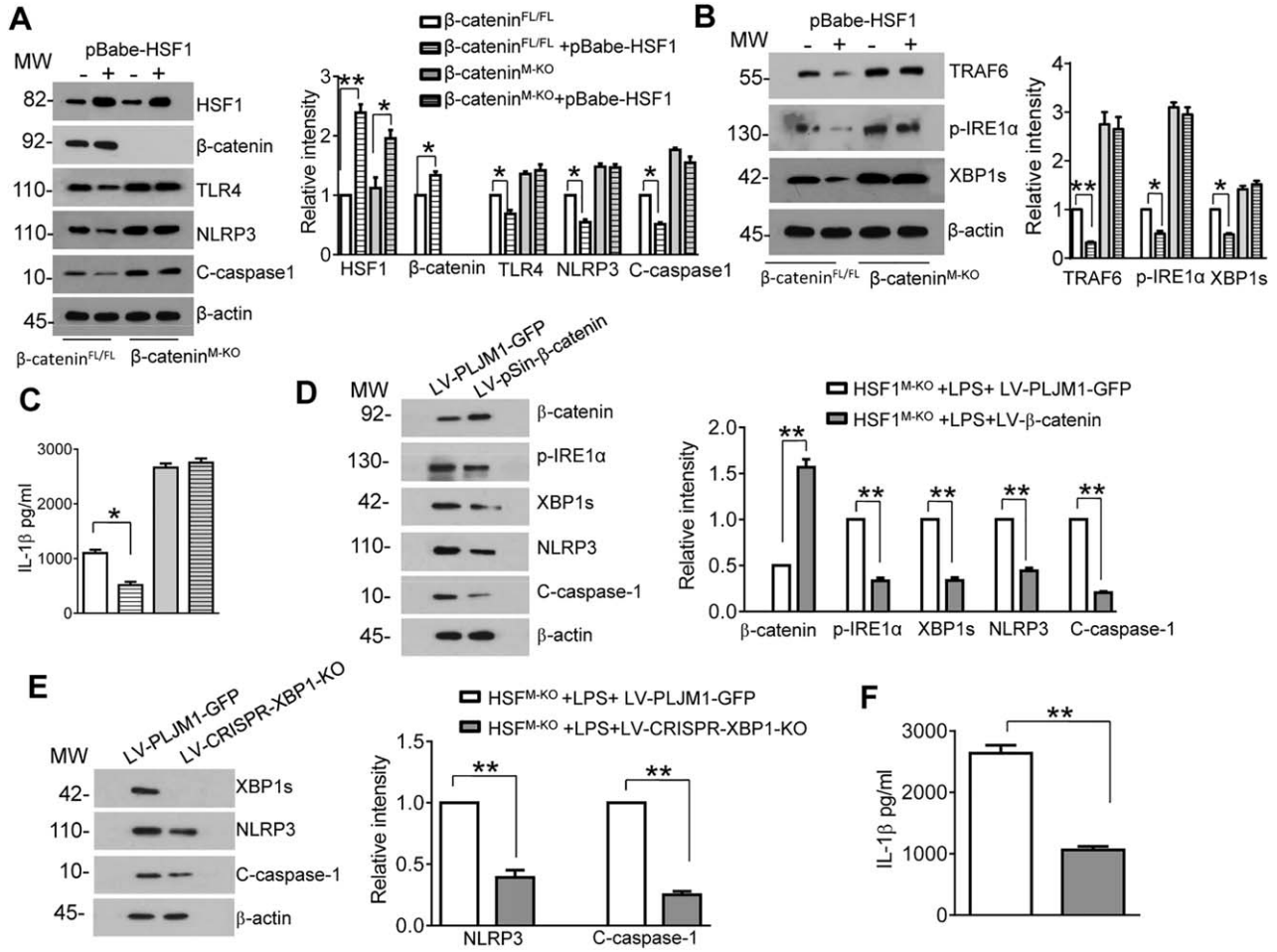


**FIG. 7.** Myeloid *β*-catenin signaling is essential for HSF1-mediated immune regulation of XBP1-dependent NLRP3 activation in macrophages. BMMs from *β*-catenin$^{FL/FL}$ (▭) and *β*-catenin$^{M\text{-}KO}$ (▬) mice were transfected with pBabe-HSF1 or control vector followed by LPS (100 ng/mL) stimulation. (A) Western-assisted analysis and relative density ratio of HSF1, *β*-catenin, and TLR4, NLRP3, and cleaved caspase-1. Representative of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. (B) Western blotting analysis and their relative density ratio of TRAF6, p-IRE1α, and XBP1s. Representative of three experiments. $^{*}P < 0.05$; $^{**}P < 0.01$. (C) ELISA-assisted production of IL-1*β* in cell-culture supernatants. Mean ± SD (n = 3-4 samples/group). $^{*}P < 0.05$. (D) BMMs from HSF1$^{M\text{-}KO}$ mice were transduced with lentivirus-expressing *β*-catenin (LV-pSin-*β*-catenin (▬), LV-CRISPR/Cas9 XBP1 knockout (KO) (■), or LV-pLJM1-GFP controls (▭). After 24-48 hours, cells were supplemented with 100 ng/mL of LPS for additional 6 hours. Western blotting analysis and their relative density ratio of *β*-catenin, p-IRE1α, XBP1s, NLRP3, and cleaved caspase-1 in LV-pSin-*β*-catenin- or LV-pLJM1-GFP-transduced cells. Representative of three experiments. $^{**}P < 0.01$. (E) Western blotting analysis and their relative density ratio of XBP1s, NLRP3, and cleaved caspase-1 in LV-CRISPR/Cas9-XBP1 KO- or LV-pLJM1-GFP-transduced cells. Representative of three experiments. $^{**}P < 0.01$. (F) ELISA-assisted production of IL-*β* in cell-culture supernatants. Mean ± SD (n = 3-4 samples/group). $^{**}P < 0.01$. Abbreviations: MW, molecular weight; p, phosphorylated.

in orchestrating *β*-catenin signaling, XBP1/NLRP3 activation, and the local sterile inflammation cascade in the I/R-stressed liver.

The NLRP3 inflammasome is activated by a wide range of danger signals.[5] Generation of ROS by oxidative stress is a crucial element for NLRP3 activation.[33] Stress-induced ROS mediates the host-defense response by modulating several signaling pathways to induce NLRP3 activation.[3] Previous studies have shown that HSF1 inhibits ROS-mediated cell death by regulating c-Jun N-terminal kinase activity.[13] We found that HSF1 overexpression increased STAT3 and *β*-catenin phosphorylation and decreased ROS production in macrophages. Consistent with our previous report that *β*-catenin regulated TLR4-mediated inflammatory response by controlling

dendritic cell maturation and functions to program innate and adaptive immunity,[18] our present results suggest that β-catenin plays a pivotal role in HSF1-mediated immune regulation.

With the dramatic expression of β-catenin and its potent ability to regulate ROS production in macrophages, HSF1 could act as a native regulator for ROS-induced NLRP3 activation during inflammatory response. Using BMMs from HSF1$^{M\text{-}KO}$ mice, we found that HSF1 deletion activated NLRP3 and caspase-1, which is a key mediator in the processing of the proinflammatory cytokine, IL-1β, from an inactive precursor to an active, secreted molecule to trigger innate immune response.[34] Notably, NLRP3 and caspase-1 activation was accompanied by decreased β-catenin phosphorylation in HSF1-deficient cells after LPS stimulation, suggesting the importance of β-catenin in mediating HSF1-mediated immune regulation of NLRP3 functions. Further evidence for a role for β-catenin in the control of NLRP3 functions was obtained from β-catenin$^{M\text{-}KO}$ mice. We found that deletion of β-catenin in cells from β-catenin$^{M\text{-}KO}$ mice reversed HSF1-mediated inhibition of TLR4, NLRP3, and caspase-1 activation compared to BMMs from β-catenin$^{FL/FL}$ mice. β-catenin$^{M\text{-}KO}$ increased I/R-induced liver damage (Supporting Fig. S3). Indeed, β-catenin signaling has been shown to possess multiple regulatory functions during inflammatory response. Increasing β-catenin activation may inhibit nuclear factor kappa B activation or promote phosphoinositide-3 kinase/protein kinase B, leading to inhibition of the innate TLR4-mediated inflammatory response in a negative feedback regulatory mechanism.[18] Moreover, TLR4 signaling is relayed by the adapter molecule, myeloid differentiation factor 88 (MyD88), and Toll/IL-1 receptor domain-containing adapter inducing interferon-β (TRIF)-mediated pathways.[35] TRIF has been linked to NLRP3 activation by promoting caspase-11 activity during Gram-negative bacterial infection.[36] TLR4 stimulation may increase ER stress sensor kinase IRE1α activation,[37] indicating that the unfolded protein response may be required for the innate immune response. Interestingly, our data revealed that increasing macrophage β-catenin expression inhibited IRE1α phosphorylation. However, deletion of β-catenin in cells from β-catenin$^{M\text{-}KO}$ mice increased the activation of IRE1α and its downstream transcription factor, XBP1, resulting in augmented NLRP3/caspase-1 activity. Because inflammasome NLRP3/caspase-1 is responsible for activation of innate immunity, our findings suggest that β-catenin may interact with XBP1 to regulate the innate inflammatory process in macrophages.

The cross-talk between cellular stress response and innate immune signaling is less clear. Previous studies have demonstrated that the cellular stress caused by oxygen deficiency (hypoxia) induces the inflammatory response associated with ischemic and inflammatory diseases through a hypoxia-inducible transcription factor (HIF) signaling pathway.[38] Induction of HIF-1α contributes to cytokine activation and development of LPS-induced sepsis in a TLR4-dependent manner.[39] Moreover, adenosine triphosphate (ATP), released from intracellular storage under ischemic or hypoxic conditions, plays a role as the predominant signaling molecule in triggering inflammatory response through the activation of purinergic P2 receptors.[40] Indeed, the purinergic receptor, P2X7R, has been shown to mediate ATP-driven NLRP3 activation,[41] suggesting the convergence of multiple signaling pathways on NLRP3 activation. Cell stress can trigger an innate immune response, such as the ER-stress sensor kinase IRE1α inducing XBP1 activation by TLR-mediated signaling pathways.[19] β-catenin activation inhibited TLR4 signaling, which implies a functional link between β-catenin and XBP1 in the stress-mediated immune response. In our current study, increased IRE1α phosphorylation and mature XBP1 production were observed in BMMs from β-catenin$^{M\text{-}KO}$ mice in response to TLR4 agonist (LPS) stimulation. However, β-catenin overexpression by lentivirus-mediated transduction suppressed IRE1α-mediated XBP1 activation. IRE1α is a Ser/Thr protein kinase and endoribonuclease that controls the processing of the splicing mRNA encoding XBP1, which forms the potent transcription factor XBPs.[42,43] Using lentivirus CRISPR/Cas9-mediated genome editing, we found that knockout of XBP1 in macrophages inhibited NLRP3/caspase-1 activation and reduced proinflammatory IL-1β. This finding suggests that macrophage XBP1 expression is essential for the NLRP3-mediated innate immune response.

The mechanisms underlying myeloid HSF1-mediated immune regulation appear to involve multiple signaling pathways. Several key factors may contribute to HSF1-mediated immune regulation in I/R-triggered liver inflammation. First, upon liver exposure to I/R stress, macrophages are critical for the initial uptake of pathogens, such as PAMPs and DAMPs, to trigger inflammation by activating innate TLR4, which activates the stress sensor, IRE1α, and the spliced form of XBP1 (XBP1s), resulting in increased expression of

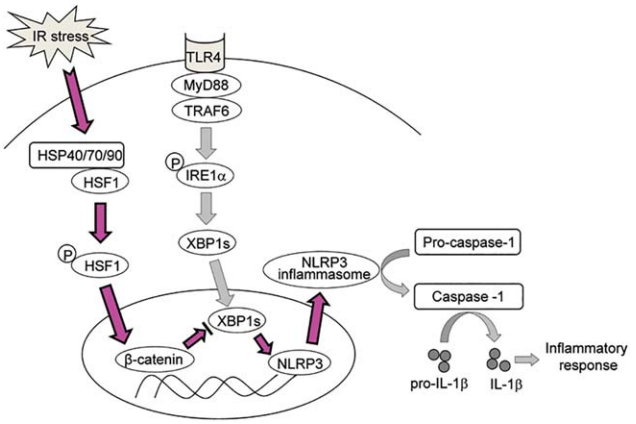


**FIG. 8.** Schematic illustration of HSF1-$\beta$-catenin axis in the regulation of innate immune response in I/R-stressed liver inflammation. This novel signaling pathway is indicated by the solid pink arrow. HSF1 can be induced in I/R-stressed livers. HSF1 induction increases $\beta$-catenin translocation from the cytoplasm to the nucleus, resulting in enhanced $\beta$-catenin transcriptional activity, which inhibits XBP1 activation in response to TLR/TRAF6 stimulation in macrophages. Moreover, suppression of XBP1 activity diminishes NLRP3 functions, leading to reduced caspase-1 activation, and the maturation and secretion of IL-1$\beta$ in liver IRI.

proinflammatory cytokines. Second, myeloid-specific, HSF1-deficient livers exhibited reduced $\beta$-catenin activity, leading to augmented IRE1$\alpha$-mediated XBP1 activation that, in turn, enhanced NLRP3-mediated innate immunity. Third, neutrophils are also activated during liver IRI by IL-1/TNF-$\alpha$ and contribute to local inflammation. Our results showed that myeloid-specific, HSF1-deficient livers exhibited increased neutrophil accumulation based on the MPO assay, $Ly6G^{+}$ staining, and CXCL-1 expression. Furthermore, $HSF1^{M\text{-}KO}$ resulted in severe liver damage compared to $HSF1^{FL/FL}$ controls. Hence, myeloid HSF1 ablation leads to neutrophil activation, which further enhances the hepatic innate immune cascade.

The question arises as to what other mechanisms may confer $\beta$-catenin with the ability to selectively affect TLR4-induced XBP1 activation in HSF1-mediated immune regulation. We showed that activation of $\beta$-catenin signaling inhibited XBP1 activity in macrophages. Moreover, LPS-induced splicing of XBP1 was dependent on the adaptors, MyD88 or TRIF. Both of these adaptors can engage the signaling molecule, TRAF6,[44] suggesting that TRAF6 is required for XBP1 activation in response to TLR stimulation. Our results showed that $\beta$-catenin deficiency in $\beta$-$catenin^{M\text{-}KO}$ macrophages augmented TRAF6 expression and IRE1$\alpha$-induced XBP1 activity compared to $\beta$-$catenin^{FL/FL}$ controls. These data were consistent with other reports that TRAF6 activated the NADPH oxidase, NOX2, which induced ROS production and shifted the redox balance to trigger IRE1$\alpha$ activation.[37,45] Thus, $\beta$-catenin controls IRE1$\alpha$-induced XBP1 activation through TRAF6 regulation. Consistent with this finding, knockdown of XBP1 with a CRISPR/Cas9 knockout system resulted in reduced NLRP3 and caspase-1 activity, suggesting that IRE1$\alpha$-induced XBP1 could regulate innate immune response through a novel mechanism independently of protein misfolding in macrophages. Indeed, increasing evidence demonstrates that IRE1$\alpha$ activates the innate NLRP3 signaling pathways through promoting thioredoxin-interacting protein in response to ER stress.[46] Engagement of XBP1 by a signaling peptide targeting the ER receptor triggers NLRP3-mediated immune response.[47] Our *in vivo* results revealed an unexpected role of XBP1 in controlling the dynamic cross-talk with NLRP3 in HSF1-$\beta$-catenin axis-mediated immune regulation.

Based on the emerging function of HSF1 in protection against oxidative stress-induced injury, it is becoming clear that HSF1-$\beta$-catenin signaling is a key player in the regulation of immunity during liver IRI. A recent study with small-molecule human HSF1 activators, which increased protein translation and HSF1 activation in mammalian cells, demonstrated their therapeutic benefits against inflammatory injury in neurodegenerative disease.[48] Thus, the development of HSF1 activators could be a potential intervention for treatment of I/R-induced liver inflammation. Next, patient samples from liver transplantation could be used to provide proof-of-principle studies for this pathway in transplant patients. By analyzing the levels of HSF1 in these samples, we can use a small-molecule human HSF1 activator to test the regulatory role of the HSF1-$\beta$-catenin axis in liver transplant patients.

Figure 8 depicts the putative molecular mechanisms by which the myeloid HSF1/$\beta$-catenin axis may regulate innate immunity in I/R-triggered liver sterile inflammation. Previous studies have suggested that TLR signaling induces IRE1$\alpha$-mediated XBP1 activation.[19] Activation of NLRP3 mediates caspase-1 activation and IL-1$\beta$ secretion.[5] This study reveals the novel signaling pathway by which the HSF1-$\beta$-catenin axis regulates NLRP3 activation through control of XBP1 activation in liver inflammatory injury. HSF1

can be induced in I/R-stressed livers. HSF1 induction increases $\beta$-catenin translocation and activity, which inhibits XBP1 activation in response to TLR/TRAF6 stimulation in macrophages. Moreover, inhibition of XBP1 activity diminishes NLRP3 functions, leading to reduced caspase-1 activation, and the maturation and secretion of IL-1$\beta$ in liver IRI.

In conclusion, we demonstrate that myeloid HSF1 regulates innate immune responses in liver sterile inflammatory injury. HSF1 controls NLRP3 functions by promoting $\beta$-catenin signaling and inhibiting XBP1 activation in the I/R-stressed liver. By identifying the molecular pathways by which HSF1 regulates NLRP3-mediated innate immunity, our findings provide the rationale for novel therapeutic approaches to ameliorate I/R-triggered liver inflammation and injury.

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Author names in bold designate shared co-first authorship.

# Supporting Information

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