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Research Paper

Hepatic angiokine IFI16/IFI202 couples endothelial senescence and stellate cell activation to drive liver fibrosis

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Abstract

Liver fibrosis, a hallmark of chronic liver injury, is primarily driven by the activation of hepatic stellate cells (HSCs), while liver sinusoidal endothelial cells (LSECs) serve as upstream regulators through secretion of signaling molecules. Here, we investigated the role of interferon-γ–inducible protein 16 (IFI16) and its murine homolog IFI202 as an angiokine secreted by LSECs and a mediator of LSEC–HSC crosstalk during fibrogenesis. We observed progressive upregulation of IFI16 in both hepatic tissues and serum across advancing stages of fibrosis, as revealed by integrated analysis of bulk and single-nucleus RNA sequencing datasets together with human specimens. IFI202 expression and secretion were markedly increased in LSECs in murine models of liver fibrosis induced by CDAA-HFD, DDC diet, and CCl_4, and its release was dependent on gasdermin D–mediated mechanisms. Extracellular IFI202 enhanced HSC activation and promoted LSEC senescence both in monoculture and in multicellular liver spheroids. Genetic and pharmacologic inhibition of IFI202, via AAV-mediated knockdown or neutralizing antibodies, disrupted LSEC–HSC crosstalk and attenuated liver fibrosis. Collectively, IFI16/IFI202 represents a previously unrecognized LSEC-derived angiokine that drives hepatic fibrogenesis and may serve as both a therapeutic target and a circulating biomarker for fibrotic liver diseases.

Keywords: IFI16/IFI202, liver fibrosis, liver sinusoidal endothelial cells, cellular senescence, hepatic stellate cells, MASH

Introduction

Liver fibrosis represents a maladaptive wound-healing response to chronic hepatic injury, characterized by the excessive accumulation of extracellular matrix components [1]. It most commonly arises from metabolic dysfunction, viral hepatitis, and chronic alcohol consumption. Despite these diverse etiologies, a common pathogenic mechanism involves oxidative stress and hepatocellular injury, which stimulates liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), and infiltrating immune cells to release inflammatory and pro-fibrotic cytokines [2]. These signals activate hepatic stellate cells (HSCs), driving their transdifferentiation into myofibroblasts, which

produce excessive collagen and other extracellular matrix components, thereby disrupting liver architecture [3]. Without removal of the injurious stimulus or timely therapeutic intervention, fibrosis may progress to cirrhosis, defined by irreversible architectural distortion and impaired hepatic function. Importantly, cirrhosis is a leading cause of liver-related mortality, accounting for 2.4% of all global deaths, with liver transplantation being the only curative option [4]. Unfortunately, effective diagnostic and therapeutic strategies for liver fibrosis remain elusive due to the complex and incompletely characterized pathogenic mechanisms underlying the disease.

LSECs are highly specialized endothelial cells lining the hepatic sinusoids and represent the most abundant non-parenchymal cell population in the liver [5]. LSECs contribute to the maintenance of hepatic homeostasis through diverse functions, including regulation of vascular tone, antigen presentation, antigen clearance, intrahepatic coagulation control, and the maintenance of HSC quiescence [6]. Unlike conventional capillaries in other organs, LSECs are distinguished by the presence of fenestrae and the absence of a basement membrane, facilitating direct solute exchange between blood and hepatocytes [5]. In response to acute or chronic injury, however, LSECs undergo capillarization, a phenotypic shift characterized by the loss of fenestrae, deposition of basement membrane, acquiring a pro-fibrotic phenotype while losing their ability to maintain HSC quiescence [7]. Furthermore, capillarized LSECs secrete pro-fibrotic mediators, including adipocyte fatty acid–binding protein, endothelin-1, EIIIA-fibronectin, and extracellular vesicles carrying sphingosine kinase 1, to sustain HSC activation [8]. Thus, beyond the traditional HSC-centric paradigm, LSECs are increasingly recognized as active regulators of fibrosis. However, the molecular mechanisms underlying these changes and the regulation of their angiocrine function during liver fibrogenesis are insufficiently understood.

Interferon-γ-inducible protein 16 (IFI16) is an intracellular pattern recognition receptor that detects aberrant or foreign double-stranded DNA, playing a key role in innate immunity [9]. Chronic DNA damage and oxidative stress induce upregulation of IFI16, which promotes cellular senescence in multiple cell types including endothelial cells [10]. The IFI16–cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) signaling cascade activates interferon regulatory factor 3 and NF-κB, inducing type I interferon (IFN) and inflammatory cytokines in keratinocytes and monocytes [11]. We previously showed that IFI16 impairs DNA repair, resulting in cytosolic DNA accumulation which activates the cGAS–STING signaling [12]. Recently, an important role of the cGAS–STING signaling has been recognized in the liver, particularly in pathological conditions. Release of mitochondrial DNA from injured hepatocytes or HSCs activates the cGAS–STING pathway triggering inflammatory and pro-fibrotic responses during progression of metabolic dysfunction–associated steatotic hepatitis (MASH) [13, 14]. Multi-omics analyses showed that elevated hepatic expression of specific IFI16 variants were associated with the progression of metabolic dysfunction–associated steatotic liver diseases (MASLD) [15]. Although these observations suggest that IFI16-associated inflammatory signaling evokes pro-fibrotic responses during the progression of liver fibrosis, the cellular and molecular mechanisms are still poorly understood.

Our previous work demonstrated that IFI202, a murine homolog of IFI16, was secreted by breast cancer cells into circulation in mice, where the IFI200 family member stimulated macrophage cytokine production, creating immunosuppressive tumor microenvironment [16]. Extracellular IFI16 has also been detected in the sera of patients with autoimmune diseases, including Sjögren's syndrome, rheumatoid arthritis, and systemic lupus erythematosus [17-19]. Upon ultraviolet stimulation, IFI16 was released into the extracellular space, functioning as a damage-associated molecular pattern (DAMP) that induced expression of inflammatory cytokines and chemokines via the TLR4 pathway [20]. Together, these observations raise the possibility that IFI16 may exert extracellular functions in the fibrotic liver, prompting us to investigate its pathogenic role in sinusoidal signaling and fibrogenesis.

Materials and Methods

Analysis of public bulk and single-cell transcriptomic datasets

Public human liver RNA-seq datasets were obtained from the NCBI Gene Expression Omnibus under accession numbers GSE256398, GSE206364, GSE244832, GSE151158, GSE193066, GSE159676, and GSE79850. Raw count matrices were directly imported into R (v4.4.3) and analyzed using DESeq2 (v1.46.0). Differential gene expression analysis was performed between healthy and MASH groups with significance thresholds set at $\log_2$fold-change > 0.5 and adjusted p-value < 0.0001. IFI16 expression levels were specifically interrogated across different disease stages. Single-nucleus RNA sequencing (snRNA-seq) data from GSE244832 were analyzed using Seurat (v5.3.0) in R with a pipeline optimized for snRNA-seq

data as described previously [21]. Data were log-normalized, and highly variable features were identified. To correct for inter-sample batch effects, Harmony integration [22] was applied prior to clustering. Low-quality and sample-specific clusters were removed according to the quality control criteria described in the original study, followed by re-clustering using the Leiden algorithm. Cell type annotation was performed using marker gene sets from PanglaoDB [23] combined with hypergeometric enrichment analysis, enabling systematic and liver-specific classification. Dimensional reduction and visualization were conducted using UMAP with a cosine distance metric. IFI16 expression was examined across cell clusters and disease conditions. Plots were generated using ggplot2 (v3.5.2) and patchwork (v1.3.0). Gene Set Variation Analysis using Hallmark gene sets was performed to assess pathway activity, followed by correlation analysis with IFI16 expression.

Human samples

Liver tissue samples from normal, MASL and MASH patients were obtained from the Seoul Metropolitan Government Boramae Medical Center (Seoul, Korea) under institutional review board approval (IRB No. 16-2014-86) [21]. Human serum samples from normal, and patients with MASL and cirrhosis were obtained from the Department of Internal Medicine, School of Medicine, Kyungpook National University (Daegu, Korea) under institutional review board approval (IRB No. KNUH 2014-04-056-015), as previously described [24].

Animal experiments

All animal procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-230602-2-1 and SNU-221214-1-1). Liver fibrosis was induced by feeding mice either a choline-deficient, L-amino acid-defined, high-fat diet (CDAA-HFD, Research Diets Inc) for 7 weeks, or a diet containing 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC, Sigma-Aldrich) for 2 weeks followed by normal chow for a week. The DDC diet was prepared by thoroughly mixing 0.1% (w/w) DDC into a standard chow diet. Also liver fibrosis was induced by intraperitoneal injection of CCl_4 (Sigma-Aldrich; diluted in corn oil, 50 μL/100 g body weight) twice weekly for 4 weeks.

To investigate the role of IFI202, an AAV vector encoding shRNA against IFI202, designated as AAV9-sh*Ifi202* (AAV9-U6-IFI202-sh-CMV-EGFP), and a scrambled control virus, designated as AAV9-shControl (AAV9-U6-SCR-CMV-EGFP), were constructed using standard DNA recombination technique with a pAAV-MCS backbone vector (Cell Biolabs) under the control of a CMV promoter and were packaged using an AAV9 serotype. AAV particles were produced in HEK293T cells at the Virus Facility of the Korea Institute of Science and Technology (Seoul, Korea). The AAV (1×10^{11} genome copies in 100 μL) was delivered to mice by a retro-orbital injection.

Recombinant IFI202 protein tagged with a C-terminal His-tag was produced as previously described [16]. rIFI202 was diluted in PBS, sterilized by filtration, and intraperitoneally injected daily for 2 weeks. At the end of experiments, mice were anesthetized and blood was collected via cardiac puncture. Serum levels of AST, ALT, BUN, and total bilirubin were measured using a FUJI DRI-CHEM 3500s analyzer. Liver lobes were excised and fixed in 10% neutral buffered formalin for paraffin embedding or embedded in optimal cutting temperature compound for cryosection preparation.

Total nitrate/nitrite levels in mouse plasma and conditioned media collected from primary LSECs were measured using a nitric oxide assay kit (Abcam) according to the manufacturer's instructions. Absorbance was measured at 540 nm, and total nitrate/nitrite concentrations were calculated from the standard curve.

Isolation of mouse liver cells and culture

Primary mouse hepatocytes were isolated from 8–10-week-old male C57BL/6N mice as described previously [25]. HSCs, LSECs, and KCs were obtained from the non-parenchymal fraction by centrifugation in a discontinuous Percoll gradient. The top layer enriched in HSCs was collected. LSECs were isolated from the intermediate layer by magnetic-activated cell sorting using CD146 microbeads (Miltenyi Biotech). The unbound fraction remaining after magnetic-activated cell sorting separation was collected as KCs. Isolated cells were suspended in DMEM supplemented with 10% fetal bovine serum. Experiments with primary LSECs were completed within 24 h to preserve their endothelial phenotype. TLR2 signaling in HSCs was inhibited by treatment with C29 (InvivoGen) or OxPAPC (InvivoGen), or by TLR2 siRNA transfection using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions.

To determine senescence-associated β-galactosidase (SA-β-gal) activity, primary LSECs were stained using the senescence cells histochemical staining kit (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, cells were fixed for 6 min, incubated with the staining mixture at 37°C without CO_2, and imaged by light microscopy. SA-β-gal-positive cells were identified by blue staining and

quantified as a percentage of the total cells. Lactate dehydrogenase (LDH) release assays were performed using an LDH cytotoxicity assay kit (Sigma-Aldrich) according to the manufacturer's instructions. The LDH activity in culture media were normalized to Tergitol-induced total lysis, which served as the positive control and was defined as 100% maximal LDH release.

Mouse liver spheroids were generated by combining hepatocytes, non-parenchymal cells including myeloid cells, endothelial cells, T cells, B cells, and NKT cells, and HSCs at proportions adjusted to approximate the physiological cellular composition of human MASH livers [26]. Specifically, hepatocytes, non-parenchymal cells, and HSCs were seeded into 96-well plates and cultured on an orbital shaker. Culture medium was replaced with fetal bovine serum-free fresh medium every 2 days. On day 3 of post-seeding, spheroids were treated with rIFI202 for 7 days. At the end of treatment, spheroids were harvested for immunofluorescence staining and western blot analysis.

Western blotting, Immunoprecipitation, ELISA, immunohistochemistry and qRT-PCR

Western blotting and immunoprecipitation were performed as previously described using specific antibodies (Supplementary Table 1) [16]. For analysis of secreted proteins, serum-free culture supernatants were precipitated with 20% trichloroacetic acid, washed with cold acetone, and subjected to western blotting. Concentrations of IFI16, IFI202, IL-1β, IL-6, and TNF-α in serum or culture supernatants were measured using ELISA kits. For immunofluorescence, specimens were fixed in ice-cold methanol or 4% paraformaldehyde and stained as previously described [12]. Fluorescent images were acquired and analyzed using a confocal microscope and LAS X software. For immunohistochemistry, sections of paraffin-embedded tissue were stained with specific antibodies and examined using an automated multimodal tissue analysis system. The mRNA levels were determined by qRT-PCR using an ABI StepOnePlus™ Real-time PCR system with specific primers (Supplementary Table 2). Relative mRNA expression was calculated relative to controls using the 2^{-DDCT} method [27].

Neutralizing anti-IFI202 antibodies

rIFI202 was used for mouse immunization and monoclonal antibody generation. Hybridomas were generated by electrofusion of spleen cells with SP2/0 myeloma cells, selected in HAT medium, and screened by ELISA for binding to rIFI202. Functional screening involved assessing suppression of IL-6 production in bone marrow–derived macrophages stimulated with 4T1-conditioned medium. Clones with the highest inhibitory activity were subcloned. The isotypes of monoclonal antibodies were determined using the Isostrip Mouse Monoclonal Isotyping kit (Roche) and SBA Clonotyping System-HRP kit (SouthernBiotech). Antibodies were purified from hybridoma supernatants using protein A–coated magnetic beads (GenScript). Purified antibodies were dialyzed against phosphate-buffered saline (pH 7.2–7.4) and stored at 2–8 °C until use. The production of the neutralizing antibody was performed by Hana Pharmaceutical Co., Ltd (Seoul, Korea). For animal experiments, either control IgG or neutralizing anti-IFI202 antibody at a dose of 150 μg in 150 μL PBS was intraperitoneally injected to mice every other day for a total of three weeks.

Statistical analysis

The number of samples is indicated in the figure legends, and three or four independent biological experimental replicates were conducted. All analyses were performed using GraphPad Prism software. Statistical analyses between two groups were conducted using the nonparametric Mann–Whitney *U* test (two-tailed). For experiments with $n = 3$ biological replicates, statistical significance was determined using an unpaired *t*-test, assuming a normal (Gaussian) distribution. When the experiments contain more than two groups, differences between groups were analyzed using one-way ANOVA. Statistical analyses of multiple groups were performed using two-way ANOVA followed by the Bonferroni posttest. Data are presented as the mean ± SD or the mean ± SEM, as indicated in figure legends. Statistical significance was set at $P < 0.05$.

Supplementary Table 1 provides detailed information on the materials.

Results

Hepatic and circulating IFI16 levels are elevated in patients with progressive liver fibrosis

To better understand the underlying pathophysiology associated with the development of liver fibrosis at the molecular level, we performed a differential gene expression analysis using publicly available RNA sequencing datasets of liver tissues from patients with progressive MASH (GSE256398, GSE206364, and GSE244832). By applying thresholds of $\log_2$ fold-change > 0.5 and $p < 0.0001$, we identified 21 genes consistently upregulated across all three datasets (Figure 1A, Supplementary Table 3). Among these genes, we were interested in IFI16, since it has

been predominantly recognized as an intracellular DNA sensor that amplifies cGAS–STING and NF-κB signaling, eventually leading to sustained inflammatory signaling and cellular senescence [11]. Further analysis of chronic liver disease datasets showed a progressive upregulation of IFI16 expression along with advanced disease stages of MASLD. Moreover, elevated levels of hepatic IFI16 transcripts were also observed in patients with cholestatic liver diseases, including primary biliary cholangitis, primary sclerosing cholangitis, and autoimmune hepatitis, suggesting that IFI16 upregulation is a common feature across diverse etiologies of chronic liver injury, potentially with fibrotic changes (Figure 1B).

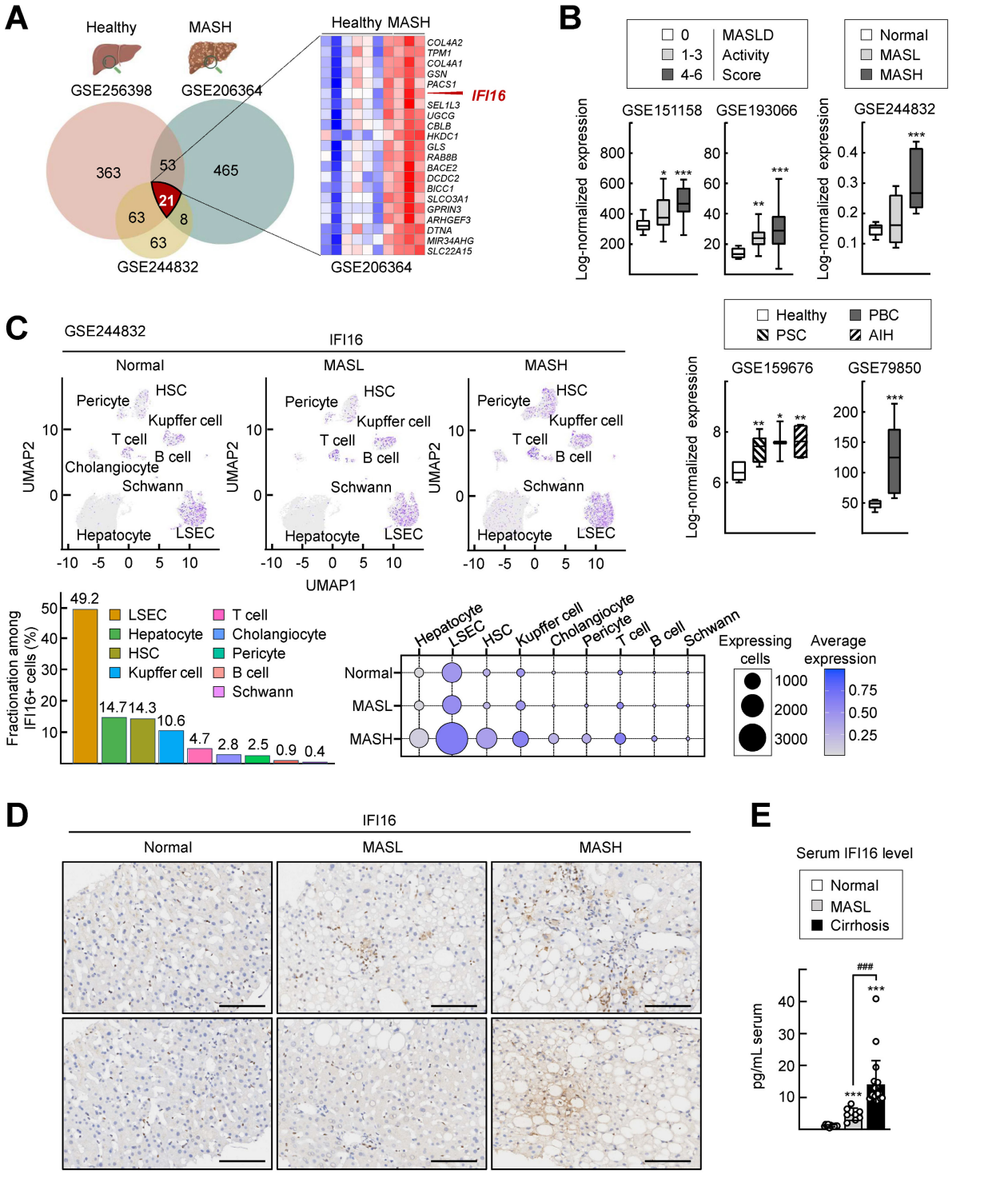


Figure 1. Hepatic and circulating levels of human IFI16 are elevated during progression of MASLD. (A) Differential expression analysis was performed using bulk transcriptomic datasets (GSE256398, GSE206364, and GSE244832). A total of 21 genes were commonly upregulated (log_2fold-change > 0.5, $p < 0.0001$; DESeq2), with a representative heatmap of these genes shown. Sample sizes were as follows: GSE256398, Healthy ($n = 6$) and MASH ($n = 8$); GSE206364, Healthy ($n = 7$) and Cirrhosis ($n =$

3); GSE244832, Normal (*n = 5*) and MASH (*n = 9*). Expression values were normalized by Z-score. **(B)** *IFI16* mRNA levels were analyzed in public datasets (GSE151158, GSE193066, GSE244832, GSE159676, and GSE79850), comparing patients with various liver diseases—including MASLD, PBC, PSC, AIH, and MASH—to healthy individuals or those with a MASLD (previously classified as NAFLD) activity score of 0. GSE151158: 0 (*n = 21*), 1–3 (*n = 23*), 4–6 (*n = 17*); GSE193066: 0 (*n = 4*), 1–3 (*n = 81*), 4–6 (*n = 79*); GSE244832: Normal (*n = 5*), MASL (*n = 4*), MASH (*n = 9*); GSE159676: Healthy (*n = 7*), PSC (*n = 11*), PBC (*n = 3*), AIH (*n = 4*); GSE79850: Healthy (*n = 8*), PBC (*n = 9*). Box-and-whisker plots show the median (line within the box), the interquartile range (bounds of the box), and whiskers extending to values within 1.5 × IQR (Tukey method). Outliers beyond this range are shown as individual dots. Statistical significance was assessed using the two-tailed Mann–Whitney *U* test (n values as indicated above). All data are shown as mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* healthy controls or MASLD activity score 0 (GSE151158 and GSE193066). **(C)** Single-nucleus RNA sequencing data (GSE244832; *n = 18* individuals with varying MASLD stages) were analyzed to visualize IFI16 expression across liver cell populations (top). The UMAP plot shows clustering of hepatic cell populations. IFI16-positive cell population was quantitatively analyzed (bottom left). The dot plot depicts normalized IFI16 expression across these clusters, with a gray-to-blue gradient indicating relative expression (Z-score) (bottom right). **(D)** Representative immunohistochemistry of IFI16 in human liver tissues from normal, MASL, and MASH patients. Scale bar, 100 μm. **(E)** Serum level of human IFI16 quantified using ELISA in normal individuals (*n = 11*), MASL (*n = 8*), and cirrhosis (*n = 21*). Data are shown as mean ± SEM. $^{***}P < 0.001$ *vs* Normal, $^{\#\#\#}P < 0.001$ *vs* MASL. Statistical significance was assessed using the two-tailed Mann–Whitney *U* test. Abbreviations: AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

To delineate the cellular sources of IFI16 within the liver, we analyzed single-nucleus RNA sequencing data from 18 individuals with varying MASLD stages (GSE244832), encompassing nine liver cell types. Across all cell types, IFI16-positive cells were most abundant in LSECs, accounting for approximately 50% of IFI16-expressing cells. Notably, IFI16 expression was predominantly enriched in LSEC and showed a marked increase in MASH, identifying LSECs as the principal cellular source of IFI16 in diseased liver (Figure 1C). To further characterize IFI16-associated transcriptional programs, we performed gene set variation analysis-based correlation assessment. In LSECs, IFI16 expression was associated with inflammatory and endothelial activation pathways, including IFN-γ response, IL2/STAT5 signaling, and apical junction, all of which progressively increased with disease severity (Supplementary Figure 1A). These findings indicate that IFI16 expression in LSECs is closely associated with endothelial dysfunction during MASH progression.

We next examined whether IFI16 was associated with pathogenic programs in other nonparenchymal cell populations. In HSCs, IFI16-correlated transcriptional programs were enriched for fibrogenic and tissue-remodeling pathways, including TGF-β signaling and angiogenesis. In Kupffer cells, IFI16 expression was associated with inflammatory and immune-related pathways, including IL-6/JAK/STAT3, IL-2/STAT5, IFN-γ response, and TGF-β signaling, several of which also became progressively enriched during disease progression. These analyses suggest that, although IFI16 is most prominently enriched in LSECs, its expression is also associated with distinct pathogenic transcriptional programs in multiple hepatic nonparenchymal cell populations during MASH progression (Supplementary Figure 1A).

We performed immunohistochemistry to assess IFI16 expression in liver tissues from patients with metabolic dysfunction–associated steatotic liver (MASL) and MASH. As shown in Figure 1D, IFI16 protein levels in liver increased progressively with MASLD severity. Since elevated circulating IFI16 level has been reported in patients with certain autoimmune diseases such as systemic lupus erythematosus [19], we assessed circulating levels of IFI16 in the serum obtained from normal individuals, patients with MASL and those with cirrhosis [24]. Strikingly, serum IFI16 levels increased in a stage-dependent manner, with the highest concentrations detected in patients with cirrhosis (Figure 1E). Receiver operating characteristic curve analysis suggested that circulating IFI16 has diagnostic potential for distinguishing healthy controls from patients with liver disease and patients with MASL from those with cirrhosis; however, these findings should be interpreted with caution given the limited cohort sizes and require validation in larger and independent cohorts (Supplementary Figure 1C).

IFI202 levels in the liver and the serum are increased after fibrosis-inducing liver injury in mice

To investigate the role of IFI202, a mouse homolog of IFI16, in the progression of chronic liver diseases, we employed diverse mouse models of liver injury [28]. First, mice were fed CDAA-HFD to develop MASH (Figure 2A). Similar to the observation in patients with MASH, IFI202 was markedly upregulated in the liver of MASH mice (Figure 2A). Western blot further confirmed elevated IFI202 expression in the liver of CDAA-HFD-fed mice whereas expression of other IFI200 family members, such as IFI203 and IFI204, was not altered. Increases in the levels of pSTING/STING and IFN-β, which are downstream effectors of IFI16/IFI202 signaling, were observed in the mouse livers (Figure 2A, Supplementary Figure 2). Similarly, hepatic IFI202 protein levels were markedly elevated in mice fed DDC diet, as well as in mice treated with CCl_4, a hepatotoxicant that induces oxidative stress and promotes fibrosis (Figures 2B, 2C and Supplementary Figure 2). Interestingly, serum IFI202 levels were markedly increased in these mouse models of fibrosis-inducing liver injury (Figure 2D). Transcripts of *Ifi202* were largely increased in livers of these mice, suggesting a transcriptional regulation of *Ifi202* under the fibrotic liver microenvironment (Figure 2E).

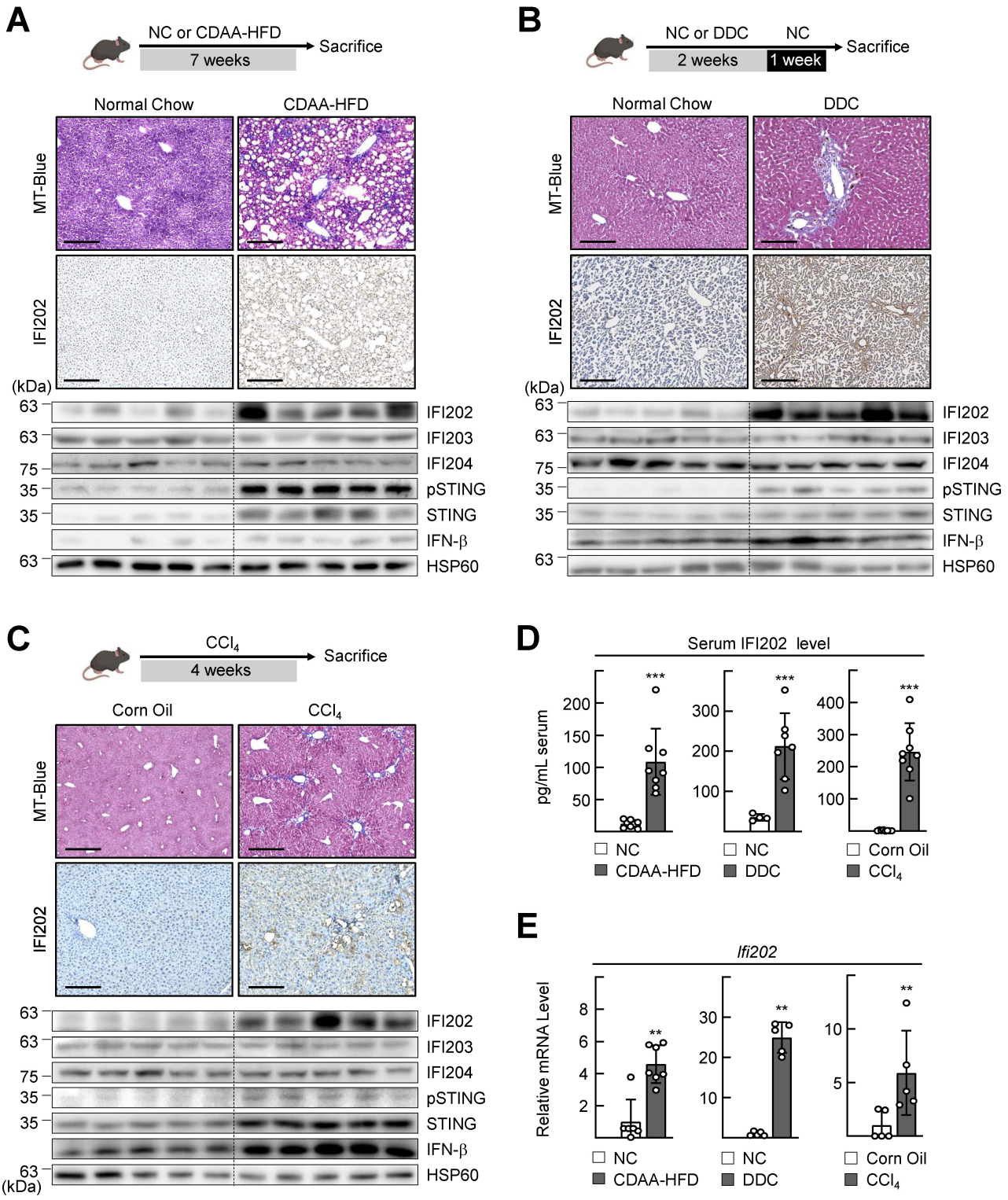


Figure 2. IFI202 levels in the liver and the serum are increased after fibrosis-inducing liver injury in mice. (A) Eight-week-old male mice were fed a CDAA-HFD (*n* = *8*) or normal chow (NC; *n* = *8*) for 7 weeks. Masson's trichrome and IFI202 immunohistochemistry of liver sections are shown (top). Scale bar, 200 μm. Hepatic expression of IFI202, IFI203, IFI204, pSTING, STING, and IFN-β was analyzed by western blot (bottom). **(B)** Seven-week-old male mice were fed a 0.1% DDC diet (*n* = *7*) or NC (*n* = *4*) for 2 weeks, followed by 1 week on NC. Masson's trichrome and IFI202 immunohistochemistry of liver sections are shown (top). Scale bar, 200 μm. Hepatic expression of IFI202, IFI203, IFI204, pSTING, STING, and IFN-β was analyzed by western blot (bottom). **(C)** Seven-week-old male mice received intraperitoneal injections of CCl_4 (*n* = *8*; 50 μL/100 g body weight) or corn oil (*n* = *5*) twice weekly for 4 weeks. Masson's trichrome and IFI202 immunohistochemistry of liver sections are shown (top). Scale bar, 200 μm. Hepatic expression of IFI202, IFI203, IFI204, pSTING, STING, and IFN-β was analyzed by western blot (bottom). **(D)** Serum IFI202 levels were measured by ELISA in NC (*n* = *8*) and CDAA-HFD (*n* = *8*) groups (left). ***$P < 0.001$ *vs* NC. Serum IFI202 levels were measured by ELISA in NC (*n* = *4*) and DDC (*n* = *7*) groups (middle). ***$P < 0.001$ *vs* NC. Serum IFI202 levels were measured by ELISA in corn oil (*n* = *5*) and CCl_4 (*n* = *8*) groups (right). ***$P < 0.001$ *vs* corn oil. (**E**) Liver tissue *Ifi202* mRNA levels were measured by qPCR in NC (*n* = *6*) and CDAA-HFD (*n* = *7*) groups (left), NC (*n* = *5*) and DDC (*n* = *5*) groups (middle), and corn oil (*n* = *5*) and CCl_4 (*n* = *5*) groups (right). **$P < 0.01$ *vs* NC or corn oil. All data are presented as mean ± SD. Statistical significance was determined using the two-tailed Mann–Whitney *U* test. Abbreviations: IFI202, interferon-inducible protein 202; IFI203, interferon-inducible protein 203; IFI204, interferon-inducible protein 204; NC, normal chow; pSTING, phosphorylated stimulator of interferon genes.

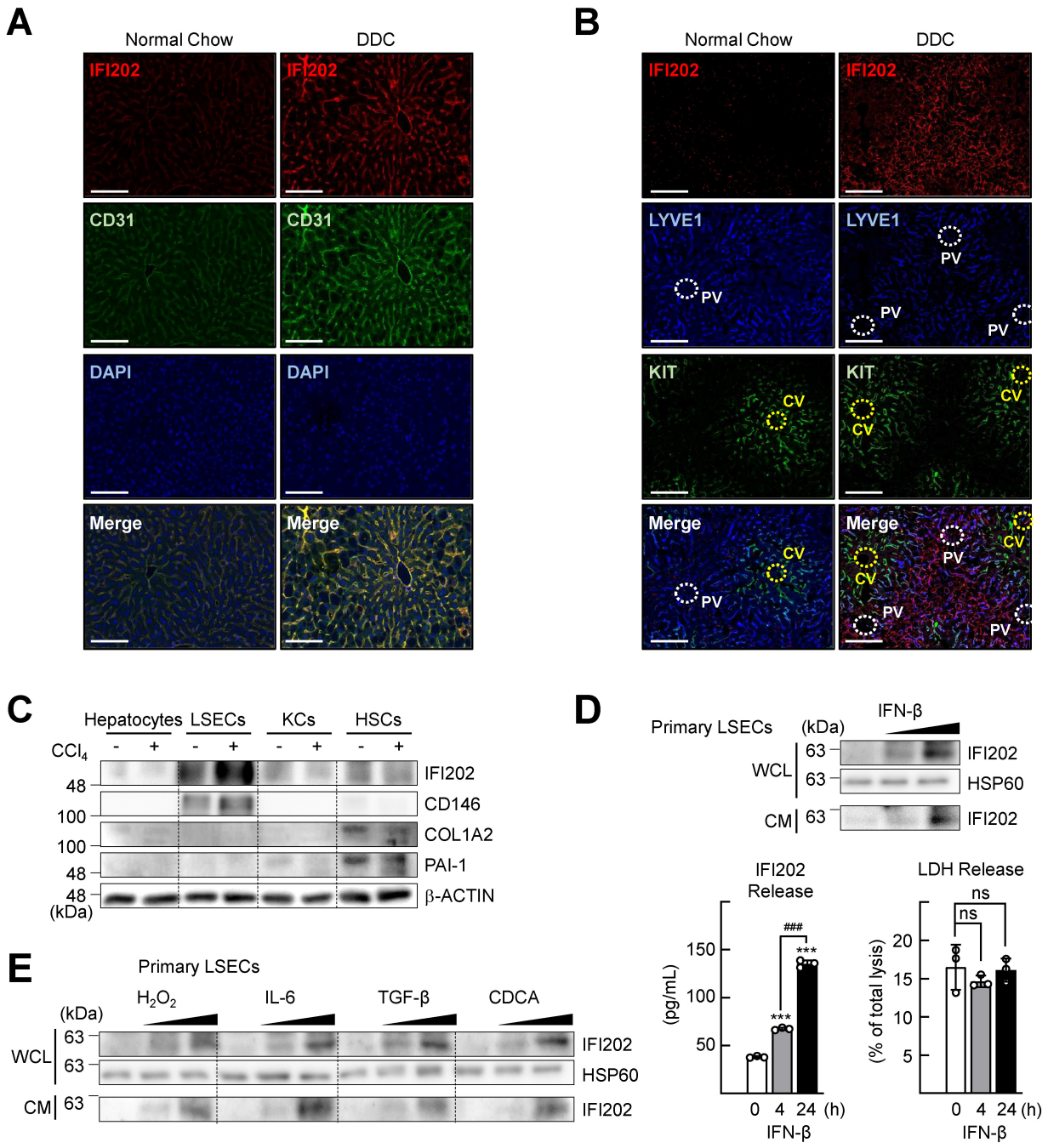


Figure 3. IFI202 is secreted from the LSECs under cellular stress. (A, B) Representative immunofluorescence images of liver sections from mice fed a normal chow or 0.1% DDC diet. IFI202 (red), CD31 (green), and merged images (**A**). IFI202 (red), LYVE1 (blue), KIT (green), and merged images (**B**). Scale bar, 100 μm. (**C**) Western blot analysis of IFI202 expression in primary hepatocytes, LSECs, KCs, and HSCs from mice treated with CCl_4 (50 μL/100 g body weight) or corn oil, collected 48 h after injection. CD146 was used as a marker for LSECs, COL1A2 and PAI-1 for HSCs, and β-ACTIN as the loading control. **(D)** Primary LSECs were seeded at 1 × 10^6 cells/well and treated with IFN-β (0, 0.2, 2 ng/mL). WCL and CM were analyzed by western blot for IFI202 expression and secretion (top). LSECs were treated with IFN-β (10 ng/mL) for 0, 4, or 24 h. CM was collected, and IFI202 concentrations were measured by ELISA (bottom, left). LDH release was quantified in culture media collected under the same conditions and normalized to Tergitol-induced total lysis, which served as the positive control and was defined as 100% maximal LDH release (bottom, right). Data are presented as mean ± SD (*n* = 3). Statistical significance was determined by unpaired *t*-test. $^{***}P < 0.001$ *vs* 0 h, $^{###}P < 0.001$ *vs* 4 h. ns, not significant. **(E)** LSECs were treated for 24 h with H_2O_2 (0, 10, 100 nM), IL-6 (0, 0.2, 2 μg/mL), TGF-β (0, 2, 10 ng/mL) or CDCA (0, 20, 200 μM). WCL and CM were analyzed by western blot for IFI202 expression and secretion. Abbreviations: CM, conditioned medium; IFI202, interferon-inducible protein 202; PAI-1, plasminogen activator inhibitor-1; WCL, whole cell lysate.

IFI202 is secreted from the LSECs under cellular stress *via* pore-forming protein gasdermin D

Immunofluorescence staining of liver tissues from DDC diet–fed mice also demonstrated increased expression of IFI202. Notably, IFI202 co-localized with CD31, a marker of capillarized LSECs in DDC diet- and CDAA-HFD-fed mice (Figure 3A and Supplementary Figure 3A). Moreover, co-staining with LYVE1, which is enriched in LSECs at zones 1–2, and KIT, present in a peri-central region, revealed that

IFI202 was primarily localized to endothelial cells in the peri-portal region (Figure 3B) [29]. Interestingly, IFI202 expression was broadly increased in LSECs across zones 1–3 in CDAA-HFD-fed mice, indicating that the spatial distribution of injury-induced IFI202 appears to vary according to the underlying etiology (Supplementary Figure 3B). To support this observation, IFI202 expression was analyzed in the isolated hepatocytes and non-parenchymal cells from mouse liver. IFI202 was mainly expressed in the LSECs of normal mice and much higher in the LSECs obtained from CCl_4-treated mice (Figure 3C). Treatment with IFN-β increased both the expression and secretion of IFI202 in the LSECs under culture conditions that preserved their endothelial phenotype. IFN-β treatment did not increase LDH release in the same conditions, indicating that IFI202 release is not attributable to secondary leakage caused by cytotoxicity (Figure 3D). In addition, treatment with hydrogen peroxide, pro-fibrogenic cytokines such as IL-6 and TGF-β, and chenodeoxycholic acid led to induction and secretion of IFI202 from the LSECs (Figure 3E, Supplementary Figure 3C). Given that activated KCs and HSCs serve as a major source of IL-6 and TGF-β during fibrogenesis, these findings suggest a potential paracrine signaling could promote IFI202 release from LSECs in the fibrotic liver [30].

Many proteins lacking classical signal peptides, such as high mobility group box 1, have also been reported to be released via gasdermin D (GSDMD) pores [31]. Since IFI202 lacks a signal peptide, we suspected that it may also utilize GSDMD for its secretion. To explore a possible association between IFI202 and GSDMD, we performed co-immunoprecipitation assays, which revealed an association between the two proteins (Figure 4A). An *in vitro* co-immunoprecipitation assay using purified recombinant proteins, further demonstrated that IFI202 physically binds to GSDMD (Figure 4B). When LSECs were treated with necrosulfonamide, an inhibitor of GSDMD, the levels of secreted IFI202 in the culture media were significantly reduced, demonstrating that IFI202 secretion depends on GSDMD-mediated pore formation (Figure 4C). Lastly, treatment of LSECs with hydrogen peroxide increased expression of not only IFI202 but also GSDMD when measured by western blot and immunofluorescence (Figure 4A, 4D). Co-localization of these proteins was evident in the LSECs when cells were treated with hydrogen peroxide (Figure 4D).

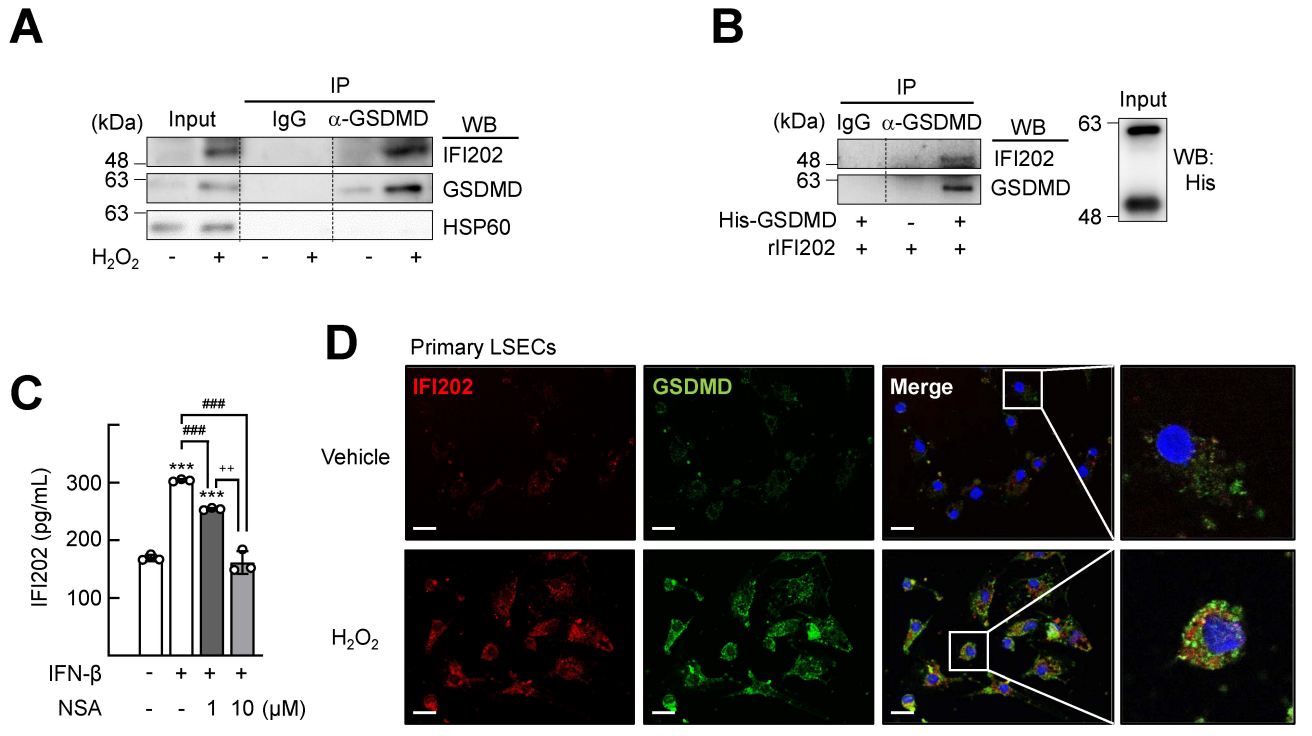


Figure 4. IFI202 is secreted *via* the pore-forming protein gasdermin D in LSECs. (A) EOMA cells treated with H_2O_2 (100 nM, 24 h) were subjected to GSDMD immunoprecipitation followed by western blot. Predicted interaction between GSDMD and IFI202 by AlphaFold2 (mean ipTM score = 0.344). **(B)** Recombinant His-tagged GSDMD (aa 1–325) and recombinant His-tagged IFI202 proteins, alone or in combination, were subjected to immunoprecipitation with an anti-GSDMD antibody or control IgG, followed by western blotting with indicated antibodies. Western blotting with anti-His antibody showed two His-tagged protein bands as input. **(C)** Primary LSECs were treated with IFN-β (0.2 ng/mL) and necrosulfonamide (NSA; 1 or 10 μM) for 24 h. CM was collected, and IFI202 concentrations were measured by ELISA. Data are presented as mean ± SD ($n = 3$). Statistical significance was determined by unpaired *t*-test. $^{***}P < 0.001$ *vs* IFN-β (0 ng/mL) + NSA (0 μM); $^{\#\#\#}P < 0.001$ *vs* IFN-β (0.2 ng/mL) + NSA (0 μM); $^{++}P < 0.01$ *vs* IFN-β (0.2 ng/mL) + NSA (1 μM). **(D)** Primary LSECs treated with H_2O_2 (100 nM, 4 h) were analyzed by immunofluorescence for GSDMD and IFI202 expression and co-localization. Scale bar, 10 μm. Abbreviations: CM, conditioned medium; GSDMD, gasdermin D; IFI202, interferon-inducible protein 202; NSA, necrosulfonamide; WCL, whole cell lysate.

Knockdown of IFI202 alleviates DDC diet-induced fibrosis

As IFI202 was predominantly expressed in liver endothelial cells (Figure 3C), we adopted a liver-targeted knockdown approach using AAV9, which efficiently transduces hepatic tissues. To establish a causal role for IFI202 in liver fibrosis, the DDC diet model was selected as it has been widely used in studies investigating LSEC dysfunction and liver microvascular remodeling during fibrosis [32]. Mice fed a DDC diet were administered either AAV9-sh*Ifi202*, encoding an shRNA targeting *Ifi202*, or a scrambled control virus (AAV9-shControl). Infusion of the AAV9-sh*Ifi202* partially recovered body weight that was decreased upon DDC diet feeding. AST and ALT levels markedly reduced by the AAV9-mediated *Ifi202* knockdown (Figure 5A). Histological analysis showed a decreased Sirius Red–positive area in the livers of AAV9-sh*Ifi202* infused mice relative to control livers. The hepatic expression levels of collagen type I alpha 2 (COL1A2) and α-smooth muscle actin (αSMA) were markedly reduced in the AAV9-sh*Ifi202* infused mice, indicating that the function of IFI202 is associated with liver injury and hepatic fibrogenesis (Figure 5B, Supplementary Figure 4). Since activation of GSDMD has been implicated in cellular senescence, we examined the involvement of IFI202 in cellular senescence [33]. SA-β-galactosidase staining showed that the number of senescent cells was significantly reduced in the AAV9-sh*Ifi202* infused group. Levels of pSTING and p21 were notably decreased, suggesting that IFI202 was involved in the senescence-associated pathways (Figure 5C, Supplementary Figure 4). Expression of LYVE1 was restored after knockdown of IFI202 (Figure 5D). Consistently, mRNA levels of multiple LSEC functional genes, such as *Cd209b* and *Lyve-1*, were restored after knockdown of *Ifi202*, indicating a negative role of IFI202 in the function of LSECs (Figure 5D).

Recombinant IFI202 induces fibrotic changes in the mouse liver

To validate the fibrogenic potential of IFI202 in the liver, recombinant IFI202 protein (rIFI202) was intraperitoneally injected into mice. Body weight decreased, whereas serum AST and ALT levels increased, indicating hepatic injury in the rIFI202-treated mice (Figure 6A). ELISA assay further revealed dose-dependent increases in circulating inflammatory cytokines, including IL-6, TNF-α, and IL-1β (Figure 6B). Levels of COL1A2, αSMA, TGF-β, and pSMAD3 were elevated dose-dependently (Figure 6C, Supplementary Figure 5), Consistently, collagen deposition and αSMA expression level were elevated in the livers of rIFI202-treated mice (Supplementary Figure 5). In addition, SA-β-gal staining revealed increased senescence in the livers of rIFI202-injected mice, accompanied by elevated levels of pSTING, p21, and N-GSDMD, which may underline the mechanism of cellular senescence and extracellular release of IFI202 (Figure 6D, Supplementary Figure 5). To assess the human relevance of these findings, treatment with recombinant IFI16 proteins upregulated expression of COL1A1 and αSMA in LX-2 human HSCs (Supplementary Figure 6). Together, these results indicate that rIFI202 protein alone is sufficient to induce hepatic fibrogenesis, inflammation, and senescence-associated changes in the liver.

Next, we examined the effects of rIFI202 on the primary HSCs and LSECs to understand the mechanism by which IFI202 contributes to liver fibrosis at the cellular level. Considering that IFI202 has been shown to signal through TLR2 and that both HSCs and LSECs express functional TLR2, we speculated that rIFI202 activates downstream signaling *via* this pathway [16, 34]. We observed that the addition of rIFI202 accelerated the activation of primary HSCs in culture, accompanied by increased αSMA expression and activation of canonical fibrogenic signaling pathways (Figure 7A, Supplementary Figure 7A). Interaction between IFI16 and TLR2 was demonstrated by co-immunoprecipitation in LX-2 cells overexpressing tagged IFI16 and TLR2 (Supplementary Figure 7B). rIFI202 treatment markedly increased the phosphorylation of ERK and p65, key downstream effectors of TLR2 signaling (Figure 7B, Supplementary Figure 7C). Notably, pharmacological inhibition of TLR2 using C29 or OxPAPC, or siRNA-mediated knockdown of TLR2 significantly attenuated rIFI202-induced phosphorylation of both ERK and p65, indicating that IFI202-mediated HSC activation occurs via a TLR2-dependent mechanism (Figure 7C, Supplementary Figure 7D, 7E) [35].

Treatment of primary LSECs with rIFI202 decreased the expression levels of the differentiation markers of LSECs, such as *Cd209b* and *Lyve1*, supported a phenotypic shift away from healthy sinusoidal function (Figure 7D). Consistent with our in *vivo* findings, treatment of primary LSECs with rIFI202 induced multiple features of cellular senescence, including reduced nuclear LAMIN B1 expression, a marker of nuclear integrity, and increased p21 expression (Figure 7E). Furthermore, rIFI202 significantly increased SA-β-gal activity compared with vehicle-treated controls. Importantly, the rIFI202-induced senescence phenotype was markedly attenuated by treatment with C-176, a selective covalent inhibitor of murine STING that blocks STING

activation and its downstream signaling (Figure 7F). Collectively, these findings demonstrate that extracellular IFI202 is sufficient to induce senescence-associated changes in primary LSECs and that this process is functionally dependent on STING activation.

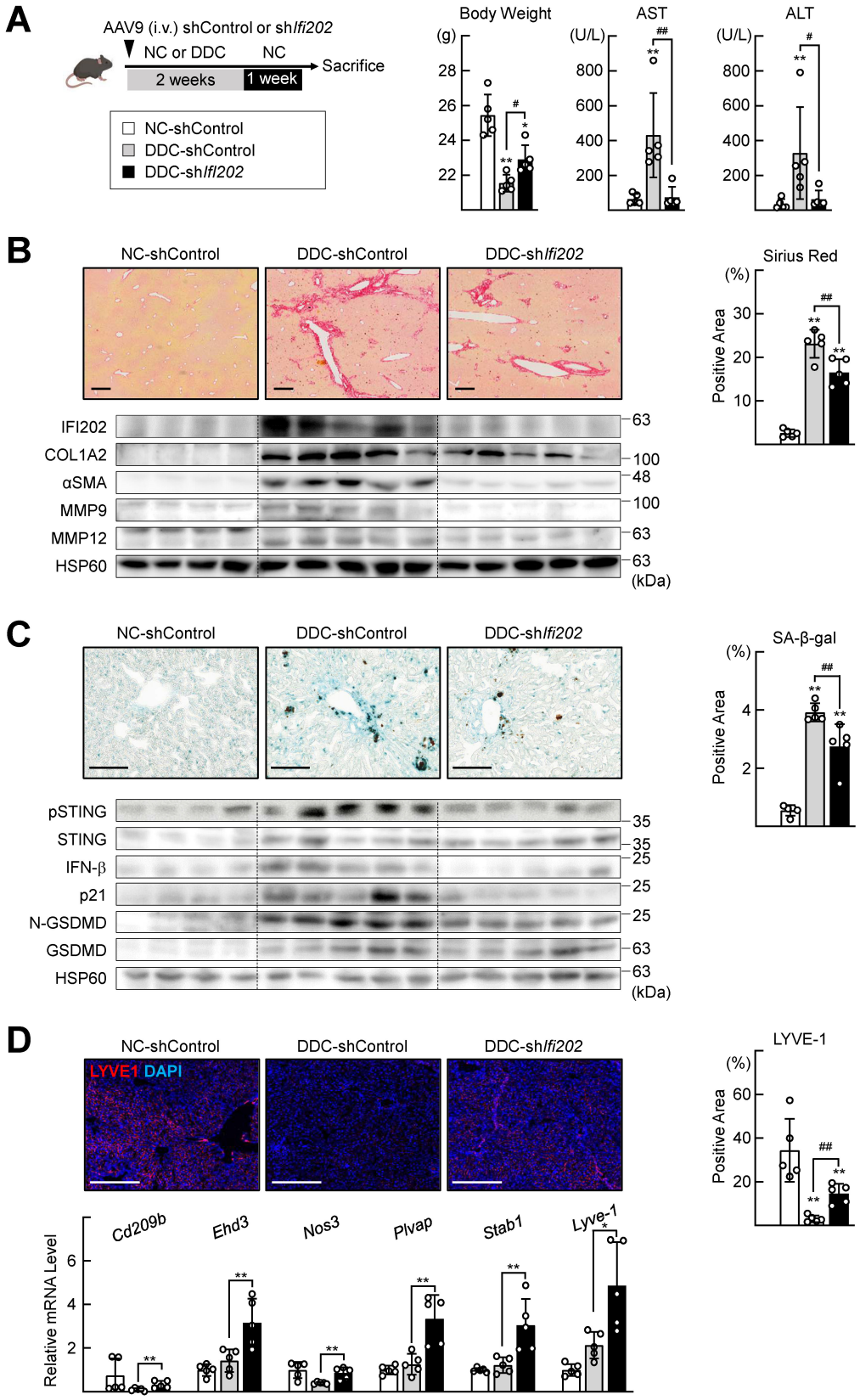


Figure 5. Knockdown of *Ifi202* alleviates DDC diet-induced fibrosis. (A) Experimental design (left). Mice were fed NC, 0.1% DDC diet with shControl, or a 0.1% DDC diet with shRNA-mediated *Ifi202* knockdown (sh*Ifi202*). Body weight and serum AST and ALT levels were measured (right). **(B)** Sirius Red staining of liver sections showing

collagen deposition (left; scale bar, 1 mm) with quantification (right), and western blot analysis of IFI202, COL1A2, αSMA, MMP9, MMP12, and HSP60 as loading control. **(C)** SA-β-gal staining of liver sections (left; scale bar, 100 μm) with quantification (right), and western blot of pSTING, STING, IFN-β, p21, N-GSDMD, and GSDMD in liver tissues. **(D)** Immunofluorescence staining of LYVE1 (red) with DAPI (blue) in liver sections (scale bar, 100 μm) with quantification (right), and qRT-PCR analysis of vascular function-related genes (*Cd209b, Ehd3, Nos3, Plvap, Stab1, Lyve1*). All Data are presented as mean ± SD (*n = 5* per group), except for the western blot analyses, which were performed using *n = 4, 5, and 5* biological samples for the NC-shControl, DDC-shControl, and DDC-sh*Ifi202* groups, respectively. Statistical analyses were performed using the two-tailed Mann–Whitney *U* test. $^{**}P < 0.01$, $^{*}P < 0.05$ *vs* NC-shControl; $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ *vs* DDC-shControl. Abbreviations: GSDMD, gasdermin D; HSP60, heat shock protein 60; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; MMP12, matrix metalloproteinase 12; MMP9, matrix metalloproteinase 9; NC, normal chow; pSTING, phosphorylated stimulator of interferon genes; SA-β-gal, senescence-associated beta-galactosidase.

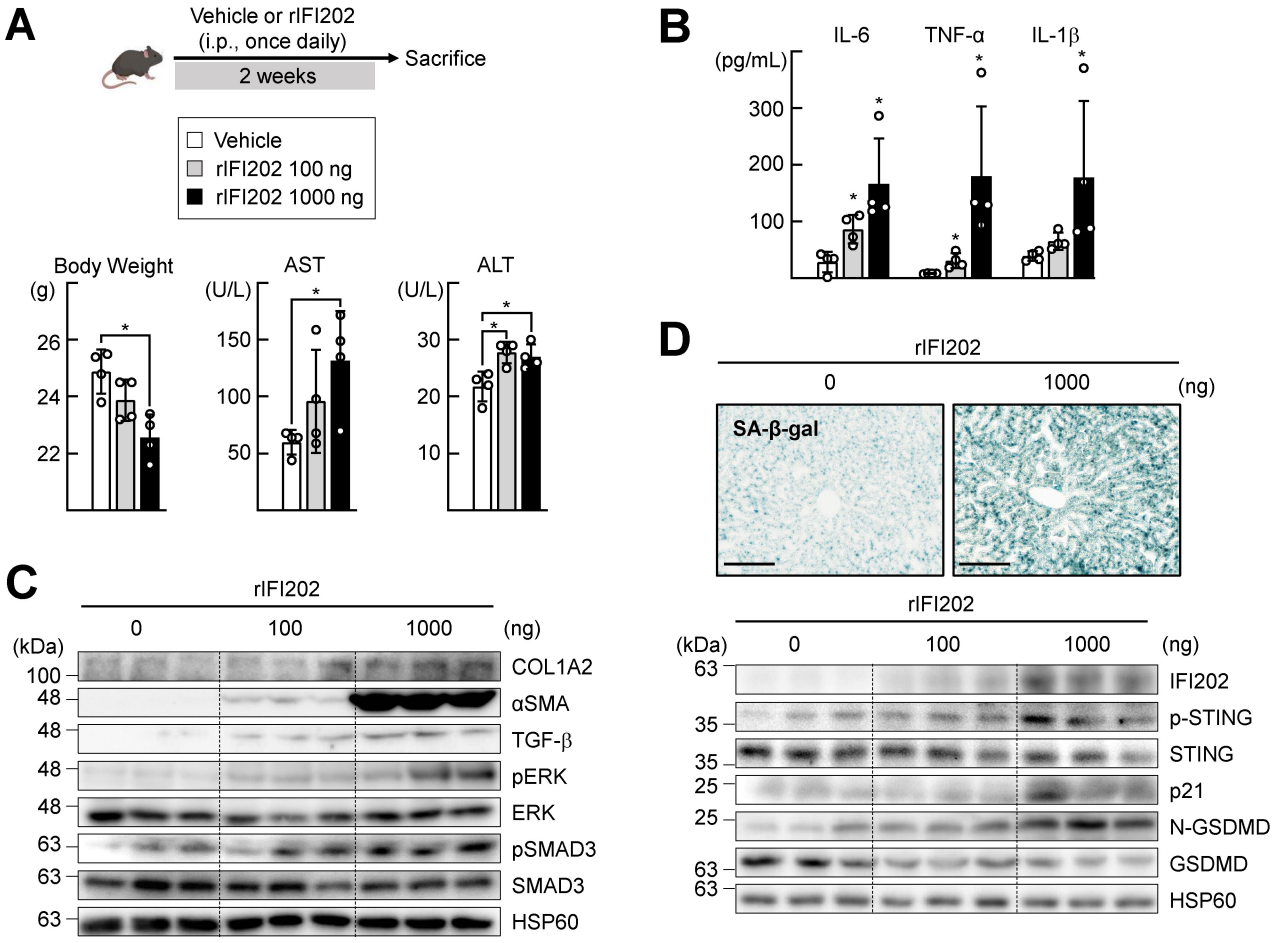


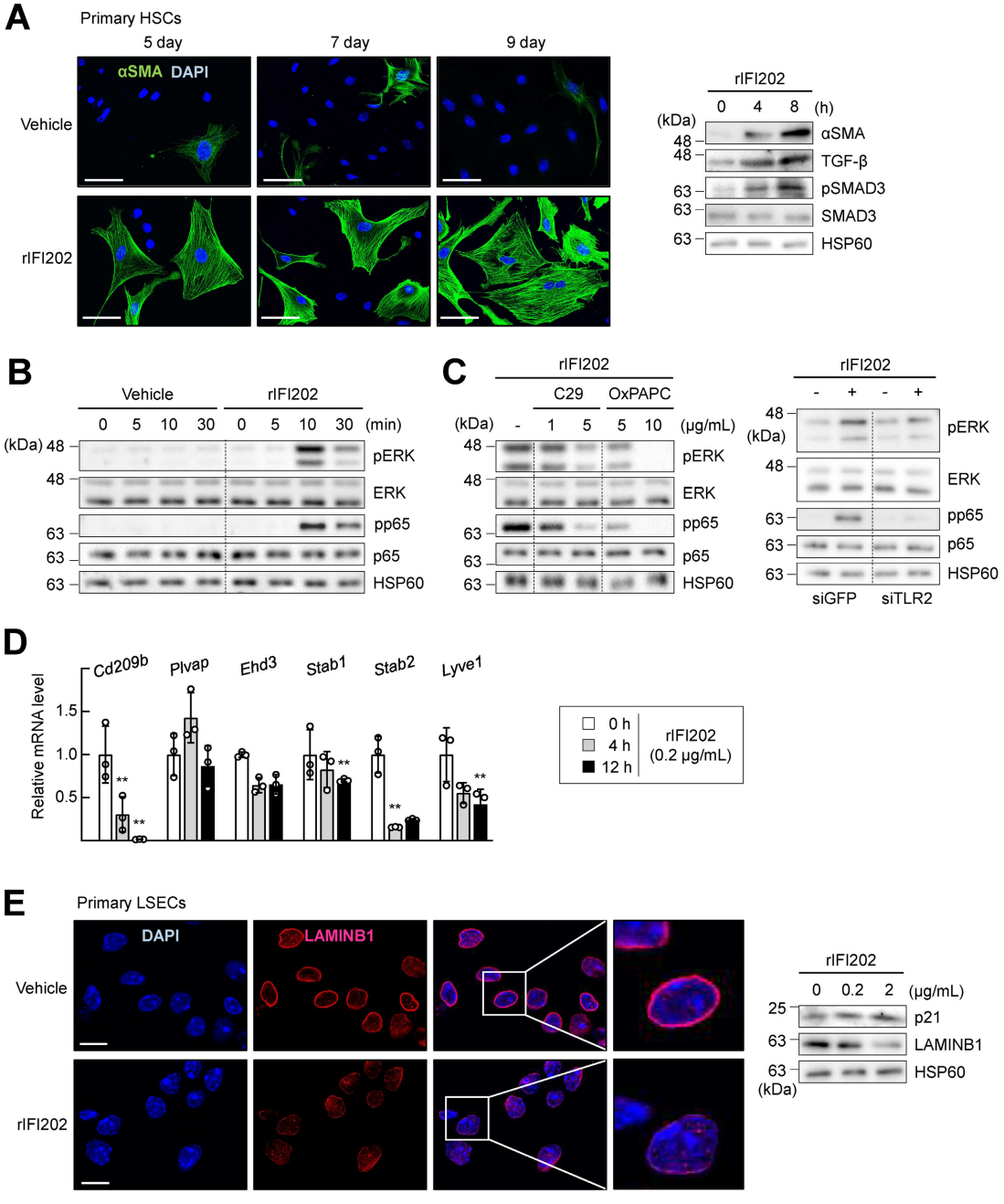
Figure 6. Injection of IFI202 induces fibrotic changes in the liver. (A) Experimental design (top). Eight-week-old male mice were injected intraperitoneally with rIFI202 protein (100 or 1000 ng daily) for 2 weeks. Body weight and serum AST and ALT levels were measured (bottom). **(B)** Serum IL-6, TNF-α, and IL-1β levels measured by ELISA. **(C)** Western blot of COL1A2, αSMA, TGF-β, pERK, and pSMAD3, with ERK, SMAD3, and HSP60 as controls. **(D)** SA-β- galactosidase staining of liver sections (top, scale bar, 100 μm) and western blot of IFI202, pSTING, STING, p21, N-GSDMD and GSDMD in liver tissues (bottom). All data are shown as mean ± SD (*n = 4* per group). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{*}P < 0.05$ *vs* vehicle. Abbreviations: HSP60, heat shock protein 60; pERK, phosphorylated extracellular signal–regulated kinase; pSMAD3, phosphorylated SMAD family member 3; pSTING, phosphorylated stimulator of interferon genes; SA-β-gal, senescence-associated beta-galactosidase.

To support these findings in a more physiologically relevant context, we established a three-dimensional mouse liver spheroid model [36]. Treatment with rIFI202 resulted in increased expression of αSMA, COL1A2, TGF-β, and p21 within the liver spheroids (Figure 7G). Collectively, these data indicate that IFI16/IFI202 mediates both paracrine and autocrine signaling in the fibrotic liver, activating HSCs and inducing LSEC senescence. In conjunction with the findings in Figure 3E, these results collectively delineate a feed-forward regulatory circuit in which IFI202 acts as a key mediator of intercellular crosstalk during liver fibrogenesis.

Neutralizing IFI202 alleviates DDC diet-induced fibrosis

To further validate the role of IFI202 as a key mediator of fibrogenic crosstalk, we generated a neutralizing antibody specific to IFI202 and examined the effects of its blockade. Since cultured LSECs progressively acquire a stress-activated phenotype characterized by activation of inflammatory pathways together with increased expression and secretion of inflammatory mediators, primary HSCs were cultured in the conditioned medium (CM) obtained from LSECs that were pre-treated with either the anti-IFI202 Ab or control IgG [37]. Activation of HSCs was attenuated by

culturing with the CM pretreated with anti-IFI202 Ab, indicating that soluble IFI202 in the CM plays a direct role in promoting HSC activation (Figure 8A). Moreover, administration of anti-IFI202 Ab in the DDC diet-fed mice significantly attenuated liver injury, as shown by restored body weight and reduced serum AST, ALT, BUN and total bilirubin levels (Figure 8B). Histological and immunofluorescence analyses showed a marked decrease in collagen deposition and αSMA expression. Additionally, the levels of LYVE1 as well as multiple LSEC differentiation markers were restored, whereas mRNA levels of capillarized LSEC markers, *Pecam1* and *Edn1*, were reduced, suggesting improved maintenance of LSEC identity. Consistently, the expression of fibrotic and senescence markers was also attenuated (Figure 8C, 8D, and Supplementary Figure 8A). Similarly, administration of anti-IFI202 Ab in the CCl_4-treated mice attenuated liver injury and fibrosis as shown in Figure 8E-8G, Supplementary Figure 8B. Anti-IFI202 Ab administration did not induce detectable toxicity in major extrahepatic organs under the experimental conditions. Also, it did not significantly alter the proportion of neutrophils or any other major immune cell populations compared with the IgG control (Supplementary Figure 8C, 8D). Together, these results support a broader pathogenic role of IFI202 across distinct etiologies of liver fibrosis and suggest potential therapeutic strategies against these diseases.



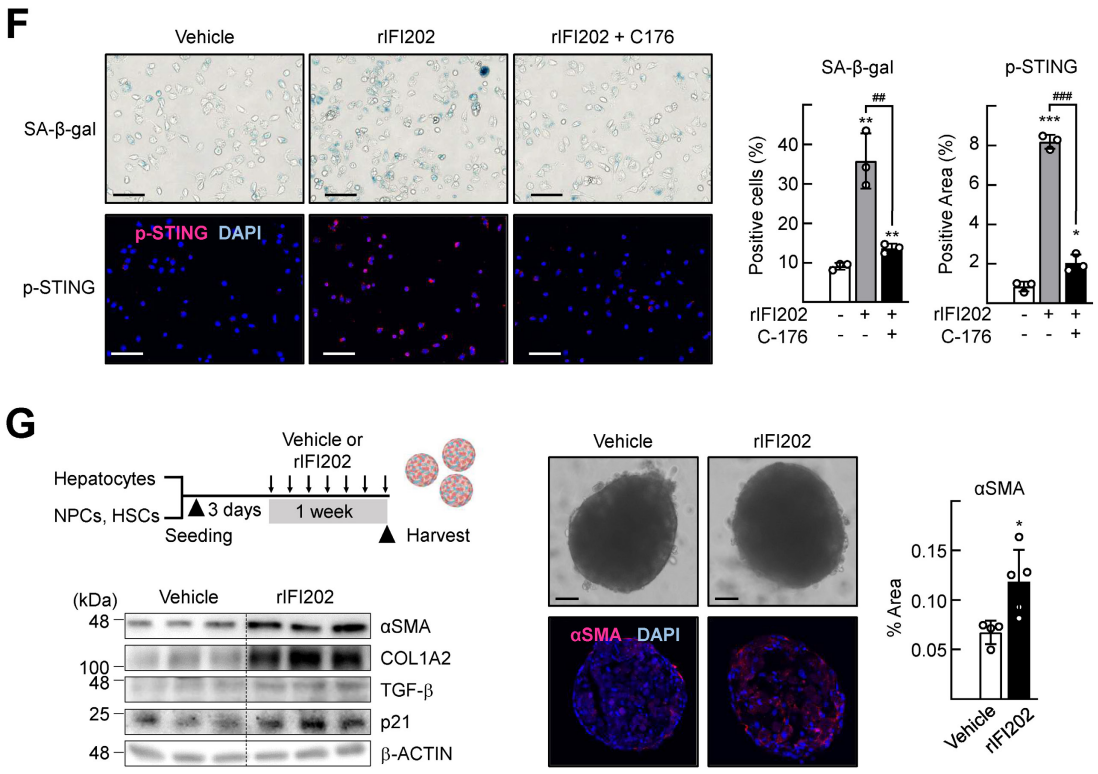


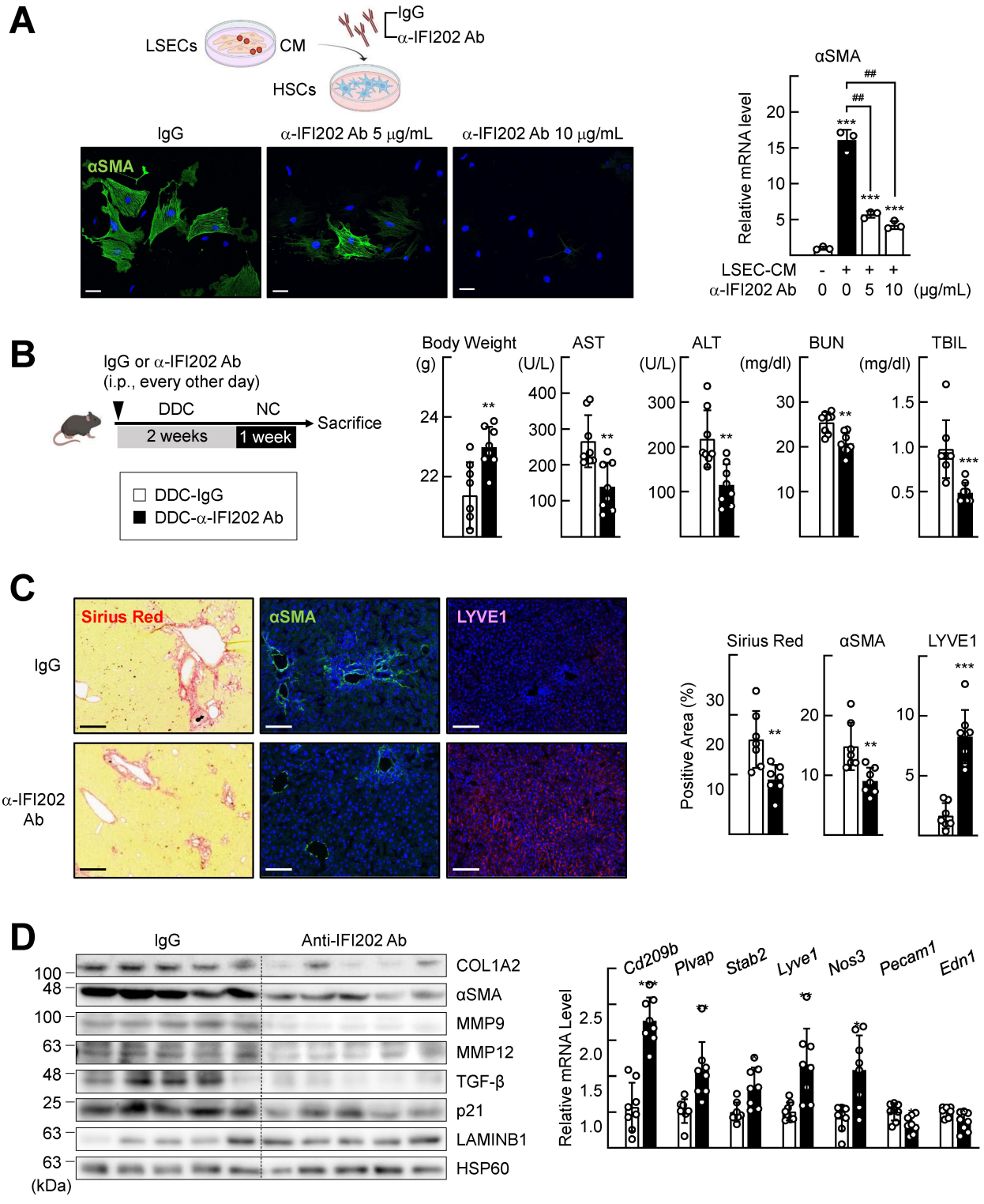
Figure 7. IFI202 induces activation in HSCs and cellular senescence in LSECs. (A) Primary HSCs were seeded at 1 × 10^6 cells/well and treated daily with rIFI202 (0.2 μg/mL) or vehicle beginning 1 day after seeding. Immunofluorescence staining for αSMA (green) with DAPI nuclear counterstain (blue) was performed on days 5, 7, and 9 (left; scale bar, 50 μm). HSCs cultured for 7 days were then treated with rIFI202 (0.2 μg/mL) for 0, 4, or 8 h, and expression of αSMA, TGF-β, pSMAD3, and SMAD3 was analyzed by western blot (right). **(B)** Primary HSCs were seeded at 1 × 10^6 cells/well and, after 2 days of culture, treated with rIFI202 (0.2 μg/mL) or vehicle for 0, 5, 10, and 30 min. The expression levels of pERK, ERK, pp65 and p65 were then analyzed by western blot. **(C)** Primary HSCs were seeded at 1 × 10^6 cells/well and cultured for 2 days, then preincubated for 1 h with TLR2 inhibitors (C29 at 1 and 5 μg/mL or OxPAPC at 5 and 10 μg/mL) followed by treatment with rIFI202 (0.2 μg/mL) for 10 min (left). Primary HSCs were transfected with si-GFP or si-TLR2 for 48 h and subsequently treated with rIFI202 (0.2 μg/mL) for 10 min. The expression levels of p-ERK/ERK, p-p65/p65, and TLR2 were then analyzed by western blotting (right). **(D)** Expression of vascular function-related genes (*Cd209b, Plvap, Ehd3, Nos3, Stab1, Stab2, Lyve1*) was measured by qRT-PCR in LSECs treated with rIFI202 (0.2 μg/mL) or vehicle. Data are shown as mean ± SD ($n = 3$). Statistical significance was determined using unpaired *t*-test. $^{**}P < 0.01$ *vs* 0 h. **(E)** Primary LSECs were seeded at 0.5 × 10^6 cells/well and treated with rIFI202 (0.2 μg/mL, 4 h) or vehicle. Immunofluorescence staining for LAMIN B1 was performed (left; scale bar, 10 μm). Expression of p21 and LAMIN B1 was assessed by western blot (right). **(F)** Primary LSECs were seeded at 0.5 × 10^6 cells/well and treated with rIFI202 (2 μg/mL) or vehicle for 4 h. C-176 (1 μM) was pretreated for 1 h before rIFI202 (2 μg/mL) treatment. SA-β-gal staining and immunofluorescence staining for p-STING were performed (scale bar, 50 μm). SA-β-gal-positive cells were counted and represented as a percentage of the total cells. p-STING-positive area was quantified using image J. Data are presented as mean ± SD ($n = 3$). Statistical significance was determined by unpaired *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* vehicle; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* rIFI202. **(G)** Mouse liver spheroids were treated from day 3 for 7 days with rIFI202 (0.2 μg/mL) or vehicle. On day 10, expression of αSMA, COL1A2, TGF-β, and p21 was analyzed by western blot (left). Spheroids were imaged by light microscopy and stained for αSMA by immunofluorescence, with quantification shown (right, scale bar, 100 μm). Data are shown as mean ± SD ($n = 4$ for vehicle, $n = 5$ for rIFI202). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{*}P < 0.05$ *vs* vehicle. Abbreviations: pERK, phosphorylated extracellular signal–regulated kinase.

To further determine whether IFI202 inhibition functionally restores endothelial function, we assessed eNOS signaling and nitric oxide production. In line with the restored expression of LSEC functional markers, anti-IFI202 Ab-treated liver tissues exhibited increased phosphorylation of eNOS, total eNOS expression, and plasma nitric oxide levels were elevated in both DDC diet-fed and CCl_4-treated mice (Figure 8H, 8I, Supplementary Figure 8E). We further validated these findings in primary LSECs. First, rIFI202 treatment increased the phosphorylation of STING and IRF3, which was attenuated by anti-IFI202 Ab treatment in primary LSECs, confirming the involvement of STING–IRF3 axis in the IFI202-mediated signaling in LSECs. rIFI202 treatment significantly suppressed p-eNOS expression, indicating impaired eNOS activation, and reduced nitric oxide levels in the conditioned medium. Importantly, these effects were fully reversed by neutralization with anti-IFI202 Ab (Figure 8J). Collectively, these results establish that IFI202 impairs endothelial function and that its inhibition effectively restores eNOS activity and nitric oxide production.

Discussion

LSECs are increasingly recognized as central regulators of fibrogenic microenvironments during progression of liver fibrosis. For example, in pathogenesis of MASH, LSECs secreted angiocrine factors, including CXCL chemokines and angiopoietin-2, which promote leukocyte recruitment, endothelial inflammation, and pathological angiogenesis in early disease. As the disease advances, LSECs acquire a pro-fibrogenic phenotype, releasing angiokines such as adipocyte fatty acid–binding

protein that activates HSCs and facilitates extracellular matrix deposition [8, 38, 39]. In this study, we identify IFI16/IFI202 as a novel angiocrine factor that integrates inflammatory and fibrogenic signaling across KCs, LSECs, and HSCs through autocrine and paracrine mechanisms, thereby coordinating microenvironmental remodeling during progression of liver fibrosis.



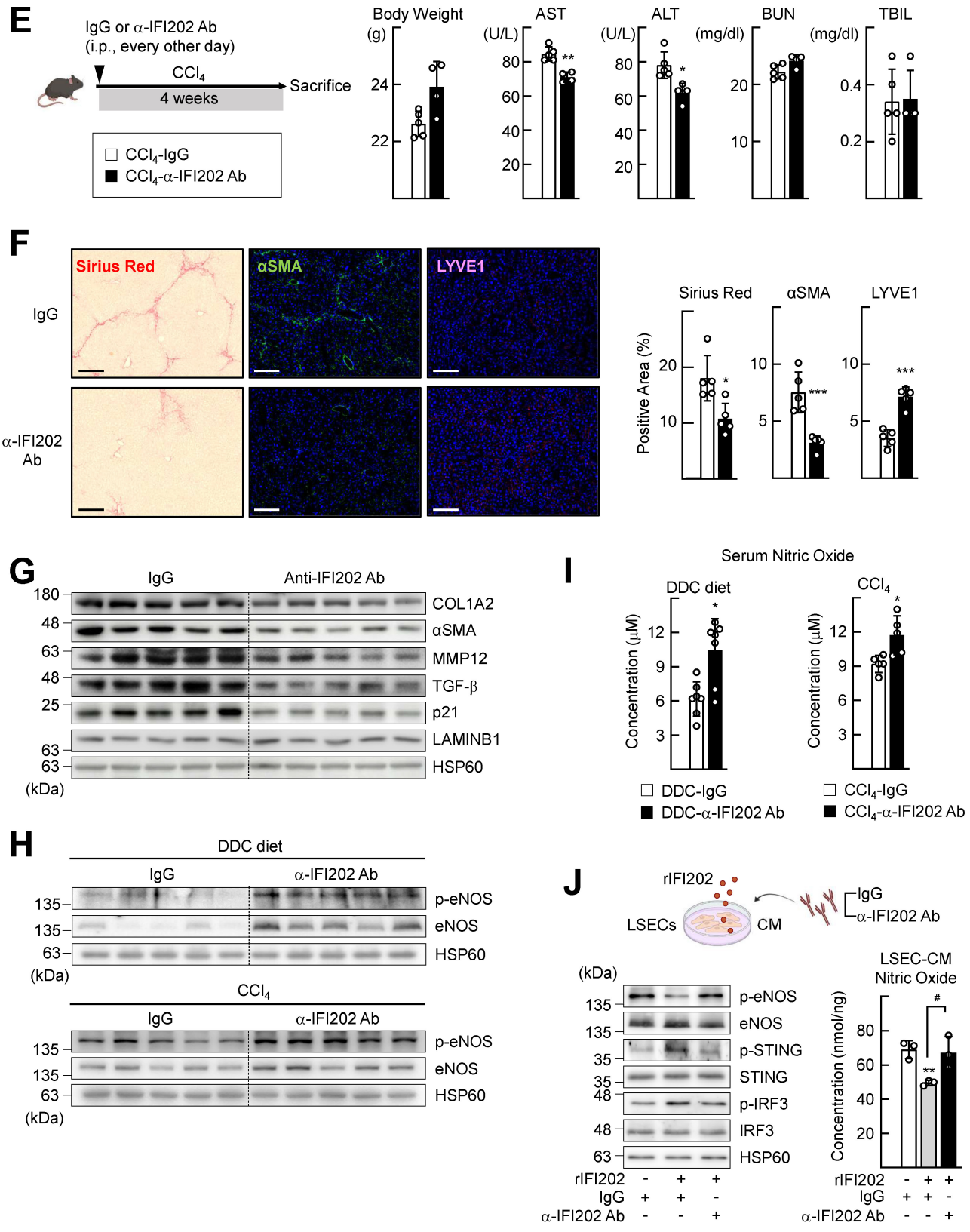


Figure 8. Neutralizing IFI202 antibody alleviates DDC- and CCl_4-induced liver fibrosis. (A) Primary HSCs were seeded at 1 × 10^6 cells/well and cultured for 7 days, then treated with LSEC–CM preincubated for 1 h with either an anti-IFI202 Ab or an IgG. Immunofluorescence staining for αSMA (left; scale bar, 20 μm) and mRNA expression by qRT-PCR (right) were analyzed. Data are shown as mean ± SD ($n = 3$ per group). Statistical significance was determined using an unpaired *t*-test, assuming a normal (Gaussian) distribution. $^{***}P < 0.001$ *vs* IgG–HSC–CM; $^{\#\#}P < 0.01$ *vs* IgG–LSEC–CM. **(B-D)** Mice were fed a 0.1% DDC diet to induce liver fibrosis and were treated with either anti-IFI202 Ab or IgG every other day. **(B)** Schematic overview of the experimental design (left). Body weight and serum AST, ALT, BUN, and total bilirubin levels were measured at the end of the experiment. Data are presented as mean ± SD ($n = 8$ per group). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{***}P < 0.001$, $^{**}P < 0.01$ *vs* DDC–IgG (right). **(C)** Sirius Red staining, immunofluorescence for αSMA and LYVE1 were performed on liver sections to assess collagen deposition and endothelial phenotype (left; scale bar, 1 mm), with quantification shown on the right. Data are presented as mean ± SD ($n = 8$ per group). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{***}P < 0.001$, $^{**}P < 0.01$ *vs* DDC–IgG (right). **(D)** Western blot of liver fibrosis-related proteins (COL1A2, αSMA, MMP9, MMP12, TGF-β, p21,

LAMIN B1) in liver tissues (left). Expression of vascular function-related genes (*Cd209b, Plvap, Stab2, Lyve1, Nos3*) and capillarization-related genes (*Pecam1, Edn1*) were assessed by qRT-PCR (right). Data are presented as mean ± SD (*n = 8* per group). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ *vs* DDC–IgG. **(E-G)** Mice were treated with CCl_4 twice weekly for 4 weeks to induce liver fibrosis and were treated with either anti-IFI202 Ab or IgG every other day. **(E)** Schematic overview of the experimental design (left). Body weight and serum AST, ALT, BUN, and total bilirubin levels were measured at the end of the experiment. Data are presented as mean ± SD (*n = 4-5* per group). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* CCl_4-IgG (right). **(F)** Sirius Red staining, immunofluorescence for αSMA and LYVE1 were performed on liver sections (left; scale bar, 0.15 mm), with quantification shown on the right. Data are presented as mean ± SD (*n = 5* per group). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{*}P < 0.05$, $^{***}P < 0.001$ *vs* CCl_4-IgG (right). **(G)** Western blot of liver fibrosis-related proteins in liver tissues. **(H)** Expression levels of p-eNOS and eNOS in liver tissues were measured by western blotting. HSP60 was shown as loading control. **(I)** Total amount of nitric oxide in plasma was measured. Data are presented as mean ± SD (*n = 7* and *n = 5* for DDC and CCl_4, respectively). Statistical significance was determined using the two-tailed Mann–Whitney *U* test. $^{*}P < 0.05$ *vs* IgG. **(J)** LSECs were treated with rIFI202 (2 μg/mL) and incubated for 16 h with either an anti-IFI202 Ab (10 μg/mL) or an IgG. CM was collected, and total nitric oxide levels were measured (left) and expressions of p-eNOS, eNOS, p-STING, STING, p-IRF3, IRF3 were analyzed by western blot (right). Data are presented as mean ± SD (*n = 3*). Statistical significance was determined by unpaired *t*-test, assuming a normal (Gaussian) distribution. $^{**}P < 0.01$ *vs* IgG; $^{\#}P < 0.05$ *vs* IgG-rIFI202. Abbreviations: CM, conditioned medium; IFI202, interferon-inducible protein 202; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; MMP12, matrix metalloproteinase 12; MMP9, matrix metalloproteinase 9.

IFI16/IFI202 has been well recognized as a cytosolic DNA sensor responding to diverse cellular stresses, including oxidative stress and viral or bacterial infection [9, 10]. Upon binding cytosolic DNA, it activates the cGAS–STING pathway, leading to production of inflammatory mediators, such as type I IFN, IL-6, and the chemokine C–C motif chemokine ligand 20 [11]. Here, we extend this understanding by revealing a DAMP-like function of IFI202. While previous work has suggested extracellular roles for IFI16/IFI202, the mechanistic link between its cytosolic DNA sensing activity and potential as a DAMP remain unclear [16, 19, 20]. Our data suggests that severe metabolic and inflammatory stress induces IFI202 expression, likely through DNA damage and accumulation of cytosolic DNA. Cytosolic DNA in turn triggers inflammasome assembly and caspase-1 activation, leading to cleavage of GSDMD, whose N-terminal fragment forms plasma membrane pores [40]. Consistent with this mechanism, extracellular release of IFI202 was suppressed by the GSDMD inhibitor NSA, implicating that IFI202 secretion occurs via GSDMD-dependent membrane pores (Figure 4). Functionally, LSEC-derived IFI16/IFI202 promoted production of IL-6, TNF-α and IL-1β from KCs, demonstrating its role as a DAMP (Supplementary Figure 9). These findings support a dual function for IFI16/IFI202: intracellularly as a cytosolic DNA sensor of cellular damage and extracellularly as a DAMP that amplifies inflammatory signaling in the hepatic microenvironment. Further studies across different cell types will be critical to determine whether these dual functions are broadly applicable, which would strengthen the rationale for targeting IFI16/IFI202 in inflammation- associated diseases.

Our findings also suggest that IFI16/IFI202 may represent a link between hepatic injury and systemic complications in MASLD. We observed that circulating IFI16/IFI202 was markedly elevated in both MASH patients and murine models, and that administration of rIFI202 induced systemic inflammation with increased IL-1β, IL-6, and TNF-α (Figure 1E, Figure 2D and Figure 6B). These results imply that IFI16/IFI202 functions not only as a marker of hepatic injury but also as an active mediator of extrahepatic pathobiology. This concept is consistent with prior observations that an angiocrine mediator derived from dysfunctional LSECs, leucine-rich alpha-2-glycoprotein 1, was elevated in the serum of patients with MASLD, which contributed to pathological conditions of renal fibrosis and arthritis [24, 41, 42]. Mechanistically, murine IFI202 interacts with TLR2, while human IFI16 binds TLR4, a pathway implicated in renal fibrosis, nephropathy, and muscle atrophy [16, 20, 43]. These findings raise the possibility that IFI16/IFI202–TLR signaling constitutes a shared axis underlying the multi-organ involvement of MASLD. Clinically, elevated serum IFI16 has been reported in patients with systemic lupus erythematosus, many of whom also display abnormal liver function [19, 44]. Given that TLR4 is broadly expressed across hepatic cell types and actively involved in injury responses, increased circulating IFI16 levels from diverse pathological conditions could further amplify hepatic inflammation and fibrosis. Together, these data point to IFI16/IFI202 as a potential driver of liver–multiorgan crosstalk. However, a limitation of this study is the absence of an LSEC-specific conditional knockout model for IFI202. Although our AAV-mediated knockdown supports the functional role of IFI202 in liver fibrosis, this approach does not allow us to specifically define the LSEC-specific contribution of IFI202. Future studies using LSEC-specific conditional knockout mice or AAV systems driven by endothelial-specific promoters will be necessary to more precisely define the role of LSEC-derived IFI202 in liver fibrosis.

Notably, the spatial and transcriptional patterns of IFI16/IFI202 suggest a context-dependent function in LSECs. In the normal liver, IFI16 was broadly distributed in CD31-positive LSECs along the porto-central axis, and IFI16-high LSECs exhibited transcriptional programs associated with DNA repair, p53 signaling, oxidative phosphorylation, and cellular stress responses (Supplementary Figure 1B). These findings suggest that basal IFI16 may contribute to genome surveillance and adaptive stress responses that maintain LSEC homeostasis. During chronic liver injury, however, IFI16/IFI202 was markedly induced

in an etiology-dependent spatial pattern. IFI202 was preferentially increased in periportal LSECs following DDC-induced cholestatic injury, whereas CDAA-HFD induced a broader increase across zones 1–3, suggesting that its spatial induction reflects the regional pattern of LSEC stress rather than an intrinsically periportal-specific function (Figure 3B, Supplementary Figure 3B). Importantly, our functional studies showed that sustained IFI202 elevation promotes LSEC senescence and dysfunction and, following extracellular release, activates HSCs to facilitate fibrogenesis (Figure 7). Together, these findings suggest a transition from a potentially homeostatic role of basal IFI16 in LSECs to a pathological stress response during chronic injury that promotes endothelial dysfunction and fibrogenic remodeling.

The rising prevalence of obesity and metabolic syndrome has markedly increased the global burden of cirrhosis, which progresses stepwise from MASL to MASH [45]. Currently, resmetirom and semaglutide are the FDA-approved therapeutic drugs for MASH with moderate-to-advanced liver fibrosis, primarily targeting metabolic abnormalities [46]. Other investigational agents, such as efruxifermin, an FGF21 analog, are under clinical trials, exerting mainly metabolism-mediated effects with secondary benefits on fibrosis (NCT05039450). However, once HSCs enter the perpetuation phase and acquire a stabilized pro-fibrotic phenotype, the therapeutic efficacy of metabolism-targeting interventions diminishes substantially, underscoring the need for therapies that directly target fibrosis. In this regard, inhibition of TGF-β has received much attention with diverse strategies including neutralizing antibodies [47]. Nonetheless, systemic blockade of TGF-β poses significant safety concerns, given its pleiotropic roles in immune tolerance, tissue homeostasis, and tumor suppression [48]. In this context, we propose IFI16/IFI202 as a novel therapeutic target during the amplified fibrotic response. Notably, IFI16/IFI202 displays an injury-inducible secretion profile, which may reduce the risk of systemic side effects compared with broadly acting cytokine pathways. Furthermore, blockade of IFI16/IFI202 has the potential to interrupt multiple fibrogenic cascades originated from LSECs, KCs, and HSCs. Thus, blockade of IFI16/IFI202 represents a fibrosis-focused strategy that is mechanistically distinct from metabolic modulation. Therapeutic strategies directed at IFI16/IFI202 could therefore be combined with metabolism-regulating agents, thereby expanding the treatment landscape for patients with advanced MASH.

In summary, we identify IFI16/IFI202 as secreted angiokines from liver sinusoidal endothelial cells that contribute to liver fibrosis by promoting endothelial senescence and hepatic stellate cell activation. IFI202 secretion is mediated by gasdermin D, and its inhibition through genetic or antibody-based strategies attenuates fibrosis and restores endothelial function. These findings support IFI16/IFI202 as potential biomarkers and therapeutic targets in chronic liver disease.

Abbreviations

AAV: adeno-associated virus; CDAA-HFD: choline-deficient: L-amino acid-defined: high-fat diet; cGAS: cyclic GMP–AMP synthase; CM: conditioned medium; COL1A2: collagen type I alpha 2; DAMP: damage-associated molecular pattern; DDC: 3:5-diethoxycarbonyl-1:4-dihydrocollidine; GSDMD: gasdermin D; HSCs: hepatic stellate cells; IFI16: Interferon-γ-inducible protein 16; IFN: interferon; KCs: Kupffer cells; LSECs: liver sinusoidal endothelial cells; MASH: metabolic dysfunction–associated steatotic hepatitis; MASL: metabolic dysfunction–associated steatotic liver; MASLD: metabolic dysfunction–associated steatotic liver disease; rIFI202: Recombinant IFI202 protein; STING: stimulator of interferon genes; αSMA: α-smooth muscle actin.

Supplementary Material

Supplementary figures.
https://www.ijbs.com/v23p0528s1.pdf

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Ethical approval and consent to participate

The research protocol was reviewed and approved by the institutional review board of the Seoul Metropolitan Government Boramae Medical Center (IRB No. 16-2014-86) and Kyungpook National University Hospital (IRB No. KNUH 2014-04-056-015). The study was performed in accordance with the Declaration of Helsinki. All animal procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-230602-2-1 and SNU-221214-1-1) and were performed in accordance with its guidelines.

Author contributions

Ga Young Lim, Seung-Su Kim and Mi-Ock Lee designed the study and interpreted the results; Ga

Young Lim, Seung-Su Kim, Ji-Eun Kim, Hae-Min Kwon, Na-Lee Ka, Hyeon Young Kim, Hyeon-Ji Kim, Sang-Heon Lee, Cheolhee Jeong, and Yun-Hee Lee performed the experiments and interpreted data; Won Kim, Jihye You, You Mie Lee and Soo Young Park contributed to analyzing human specimens; Ga Young Lim and Mi-Ock Lee wrote the manuscript; Mi-Ock Lee supervised the research. All authors read and approved the final manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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