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PTP1B-Mediated Dephosphorylation of SRC Controls Fibrogenic Cellular Activation

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Abstract

Liver fibrosis, the precursor to cirrhosis and hepatocellular carcinoma (HCC), represents a global health crisis with millions affected and few effective treatments available. This progressive pathology is characterized by excessive extracellular matrix (ECM) deposition, primarily driven by the activation of hepatic stellate cells (HSCs) into profibrogenic myofibroblasts. Elucidating the molecular control of HSC activation is paramount for therapeutic development. Protein-tyrosine phosphatase 1B (PTP1B), an established target in metabolic diseases, has emerging pro-fibrotic roles, as evidenced by protection against liver fibrosis in PTP1B-deficient mice. However, the regulatory function of PTP1B in human-relevant models remains poorly understood. Here, we define the role and mechanistic significance of PTP1B in primary human HSCs and investigate its clinical relevance in human fibrotic liver. Using loss- and gain-of-function approaches in primary human HSCs, we demonstrate that PTP1B significantly promotes HSC activation, evidenced by enhanced proliferation, migration, and increased expression of key ECM genes and collagen production. Mechanistically, PTP1B acts as a positive regulator of the pro-fibrotic SRC kinase, directly dephosphorylating the inhibitory tyrosine 527 (Y527) residue to induce its activation. Pharmacological inhibition of SRC effectively reversed the PTP1B-driven pro-fibrotic phenotypes. Consistent with our findings, re-analysis of human fibrotic liver tissues revealed that PTP1B and SRC expressions are significantly upregulated and positively correlated with ECM genes. Collectively, these findings establish a novel PTP1B-SRC signaling axis that critically drives human HSC activation and hepatic fibrogenesis, positioning PTP1B as a high-potential therapeutic target for liver fibrosis.

Keywords:

PTP1B;
HSC Activation;
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Liver Fibrosis;
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1- Introduction

Liver fibrosis is a major, progressive global health concern defined by the excessive accumulation of extracellular matrix (ECM) proteins, which profoundly compromises hepatic function and architecture. It results from chronic liver injury due to diverse etiologies, including viral hepatitis, metabolic dysfunction-associated steatotic liver disease (MASLD), alcohol abuse, and autoimmune diseases. Untreated fibrosis progresses to cirrhosis—a state of irreversible structural damage and impaired function—which is the leading risk factor for hepatocellular carcinoma (HCC) [1, 2]. Notably, recent epidemiological trends indicate that MASLD has become the fastest-growing cause of cirrhosis worldwide. While the first FDA-approved therapies for advanced MASLD represent a major milestone, they primarily target upstream metabolic dysregulation. However, a substantial proportion of patients continue to show fibrotic progression despite metabolic improvement, underscoring the urgent need for strategies that directly target the antifibrotic pathways within the liver [3, 4]. The pathogenesis of liver fibrosis is primarily driven by the activation of hepatic stellate cells (HSCs) into profibrogenic myofibroblasts. This activation leads to the excessive production and accumulation of ECM proteins, particularly type I collagen, disrupting parenchymal and vascular architecture [5, 6].

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While rodent models have identified numerous targets for reversing this process, translating these findings into clinical success remains a formidable obstacle. Recent single-cell transcriptomic and functional analyses of human HSCs have revealed substantial species-specific differences in signaling pathways, activation states, and myofibroblast plasticity compared with rodent counterparts [7, 8]. This underscores a critical gap in our understanding: many pathways established in rodents do not faithfully replicate the regulatory landscape of human HSC activation, thereby limiting the clinical translation of novel therapeutics [9]. Importantly, emerging evidence indicates that post-translational regulatory mechanisms (particularly phosphorylation-dependent signaling networks) play a central role in shaping human-specific fibrotic responses [10].

Protein-tyrosine phosphatase 1B (PTP1B), which is encoded by the *PTPN1* gene, is a ubiquitously expressed intracellular non-receptor protein tyrosine phosphatase that has emerged as a key regulatory node [11]. PTP1B is well-established as a critical regulator of metabolic homeostasis, contributing to conditions such as obesity and type 2 diabetes through the promotion of insulin resistance and lipogenesis [12–15]. Beyond these metabolic roles, PTP1B activity is strongly implicated in organ fibrosis. For instance, genetic deletion or pharmacological inhibition of PTP1B reduced cardiac hypertrophy and fibrosis in mouse models of chronic heart failure [16]. In the liver, PTP1B expression is upregulated in various murine models of fibrosis, and its deficiency significantly attenuates HSC activation and overall liver fibrogenesis [17, 18]. Furthermore, *in vitro* studies using rodent HSCs demonstrated that PTP1B overexpression prevents HSC inactivation and its inhibition suppresses TGF-β-induced activation [17]. However, the specific regulatory role and downstream signaling of PTP1B in the context of human HSCs remain largely undefined.

To address the critical gap in our understanding of PTP1B in human HSCs, which is essential for translational relevance; we employed loss- and gain-of-function strategies in primary human HSCs to define how PTP1B regulates activation and profibrogenic responses. We report that PTP1B promotes HSC activation and profibrogenic responses by establishing a novel axis that constitutively activates SRC kinase. Mechanistically, PTP1B achieves SRC kinase activation by directly dephosphorylating the inhibitory tyrosine 527 (Y527) residue on SRC. Furthermore, our re-analysis of human fibrotic liver tissues shows that PTP1B and SRC levels are significantly elevated and correlated with fibrogenic gene expression. These findings establish the PTP1B–SRC signaling axis as a critical driver of human liver fibrogenesis, positioning PTP1B as a viable, actionable therapeutic target for human liver fibrosis.

2- Materials and Methods

An overview of the study design and experimental workflow is shown in Figure 1.

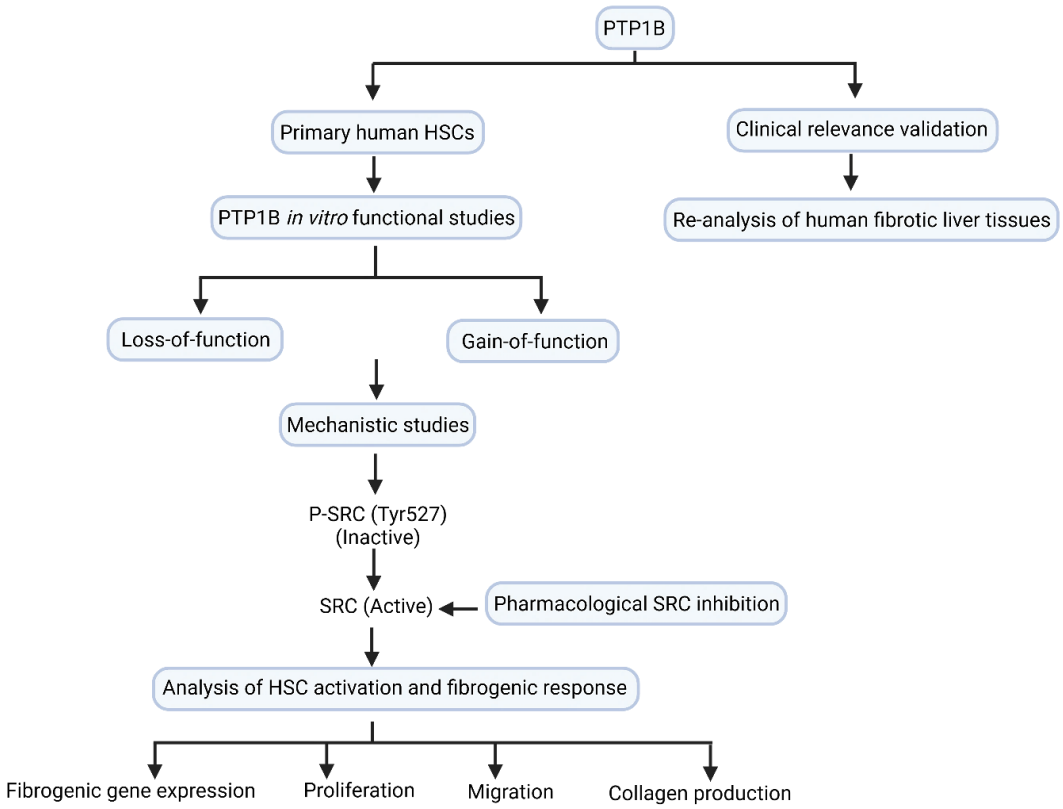


Figure 1. Schematic overview of the experimental workflow

2-1-Cell Culture

Primary human HSCs (lot no. 27742; cat. no. 5300; ScienCell Research Laboratories, Carlsbad, CA, USA) were cultured in the manufacturer's recommended medium (cat. no. 5301; ScienCell Research Laboratories) supplemented with 1X proprietary growth factors (cat. no. 5352; ScienCell Research Laboratories) and 1% penicillin/streptomycin (cat. no. 0503; ScienCell Research Laboratories). HEK 293FT cells (cat. no. R70007; Thermo Fisher Scientific, Waltham, MA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) (cat. no. 11965092; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (cat. no. A5256701; Thermo Fisher Scientific) and 1% antibiotic-antimycotic (cat. no. 15-240-062; Thermo Fisher Scientific). All cells were maintained in a humidified incubator at 37°C with 5% CO_2.

2-2-Loss-of-Function Experiments

To achieve PTP1B knockdown, two independent short hairpin RNA (shRNA) sequences targeting human *PTPN1* transcripts were designed and cloned into the inducible Tet-pLKO-puro lentiviral vector (cat. no. 21915; Addgene, Watertown, MA, USA). For viral particle production, HEK 293FT cells were simultaneously co-transfected with three plasmids: the shRNA-containing lentiviral backbone plasmid, the packaging plasmid psPAX2 (cat. no. 12260; Addgene), and the envelope plasmid pMD2.G (cat. no. 14887; Addgene), using a standard calcium phosphate transfection (Kingston, Chen and Rose, 2003). Viral supernatants were collected 48 and 72 hours after transfection. Concentrated viral particles were transduced into HSCs at a multiplicity of infection of 3 using a spinoculation technique (800 ×g for 40 min at 37°C) [19]. To obtain stable integrants, transduced cells were subjected to antibiotic selection using puromycin (cat. no. P8833; Sigma‐Aldrich, St. Louis, MO, USA) at a working concentration of 0.5 µg/mL. Selection was maintained for 7–10 days until all non-transduced cells were removed. For induction of shRNA expression, cells were treated with 0.2 µg/mL doxycycline (cat. no. D9891; Sigma-Aldrich) for 3 days and used for subsequent experiments. Primers sequences for shRNA cloning are provided in Table A1.

2-3-Gain-of-Function Experiments

For PTP1B overexpression, the full‐length human *PTPN1* open reading frame was amplified from complementary DNA (cDNA) of HSCs and inserted into the pLenti-TetO-PM lentiviral vector (cat. no. 197387; Addgene). Lentivirus production and transduction were performed as described above. PTP1B-inducible HSCs were maintained in a medium containing 0.5 µg/mL blasticidin (cat. no. A1113903; Thermo Fisher Scientific) for selection and induced with 0.2 µg/mL doxycycline for 3 days before subsequent experiments. Cloning primer sequences are provided in Table A1.

2-4-SRC Inhibitor Treatment

Saracatinib (cat. no. HY-101053; MedChemExpress, Monmouth Junction, NJ, USA) was dissolved in dimethyl sulfoxide (DMSO) to generate a 10 mM stock solution. PTP1B-inducible HSCs were pre-treated with 0.2 µg/mL doxycycline for 72 hours at 37°C to induce PTP1B expression. Induced cells were then treated with 10 µM SRC inhibitor or an equivalent volume of vehicle (DMSO) for 24 hours [20]. Following treatment, cells were harvested for subsequent assays.

2-5-Cell Proliferation Assay

Cell proliferation was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (cat. no. M6494; Thermo Fisher Scientific), as previously described [21]. Briefly, cells were plated in 96-well plates and allowed to grow for 24, 48, 72, or 96 hours. At each designated time point, 100 µL of 0.5 mg/mL MTT in basal medium was added to each well, followed by a 60-min incubation at 37°C. Then, the formazan crystals were solubilized in 100 µL DMSO, and absorbance at 570 nm was measured using a BioTek Synergy HTX spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

2-6-Cell Migration Assay

Cell migration was evaluated using a transwell migration assay [22]. Briefly, cells were serum-starved for 24 hours. Then, 1×10^5 cells in FBS-free medium were seeded into the upper chamber of transwell inserts with 8-µm pores (cat. no. 725321; NEST Scientific, Wuxi, China). The lower chamber contained complete growth medium. After 24 hours at 37°C, non-migrated cells were removed from the upper surface using a cotton swab. Migrated cells on the lower surface were fixed and stained with 0.2% crystal violet solution (cat. no. V5265; Sigma-Aldrich) for 10 min at room temperature. Excess stain was removed by rinsing with water. Images were acquired using brightfield microscopy on an EVOS M7000 Imaging System (Thermo Fisher Scientific). Cell migration was quantified by counting the number of stained cells in 10 representative fields per well using ImageJ software (version 1.54g; NIH).

2-7- Analysis of mRNA Expression

Total RNA was extracted using the GenUP Total RNA Kit (cat. no. BR0700903; Biotechrabbit, Berlin, Germany), and cDNA was synthesized using the iScript Reverse Transcription Supermix (cat. no. 1708840; Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed using the CAPITAL qPCR Green Mix HRox (cat. no. BR0501702; Biotechrabbit) on an Applied Biosystems QuantStudio 5 Dx Real-Time PCR System (Thermo Fisher Scientific). The cycling protocol included an initial denaturation at 95°C for 10 minutes, followed by 35 cycles at 95°C for 15 seconds (denaturation) and 60°C for 20 seconds (annealing/extension). Gene expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method and normalized to ribosomal protein L19 (*RPL19*) as the reference gene. Primer sequences are listed in Table A1.

2-8- Immunofluorescence Staining

Cells were prepared for immunofluorescence staining by initial fixation in 4% paraformaldehyde for 15 minutes at room temperature, blocked with 1% bovine serum albumin (BSA) in phosphate saline buffer for 1 hour, and incubated overnight at 4°C with an anti-type I collagen antibody (1:200; cat. no. ab34710; Abcam, Cambridge, UK) diluted in the same buffer. Alexa Fluor 488-conjugated anti-rabbit secondary antibody (1:500; cat. no. A-21206; Thermo Fisher Scientific) was applied for 1 hour at room temperature in the dark. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; cat. no. D9542; Sigma-Aldrich). Fluorescence images were acquired using an EVOS M7000 cell imaging system (Thermo Fisher Scientific). Fluorescence intensity was quantified for collagen staining levels from 30 random cells using ImageJ software (version 1.54g; NIH).

2-9- Western Blot Analysis

Cell lysate preparation using radioimmunoprecipitation assay (RIPA) buffer, protein separation by SDS-PAGE, and protein transfer to a nitrocellulose membrane were performed as previously described [23]. Membranes were incubated overnight with primary antibodies against PTP1B (1:1,000; cat. no. 5311; Cell Signaling Technology, Danvers, MA, USA), p-SRC (Tyr527) (1:1,000; cat. no. 2105; Cell Signaling Technology), SRC (1:1,000; cat. no. 2123; Cell Signaling Technology), and β-actin (1:3,000; cat. no. 3700; Santa Cruz, Dallas, CA, USA). HRP-conjugated goat anti-rabbit or anti-mouse IgG secondary antibodies (1:5,000; cat. no. 7074 and 7076, respectively; Cell Signaling Technology) were applied for 1 hour at room temperature. Signals were developed with ECL substrate (cat. no. RPN3004; Cytiva, Marlborough, MA, USA) and imaged using a UVP ChemStudio system (Analytik Jena, San Jose, CA, USA). Band intensities were quantified using FIJI software, with phosphorylated proteins normalized to total PTP1B and total PTP1B normalized to β-actin. Unprocessed blots are presented in Figure A1.

2-10- Analysis of PTPN1 and SRC Expression in Human Tissues

RNA-seq data of *PTPN1* and *SRC* in normal, fibrotic, and cirrhotic human liver samples were re-analyzed from the GepLiver database (*http://www.gepliver.org/*) [24]. Samples were categorized into three groups based on the corresponding liver fibrosis scores: none (METAVIR F0/Ishak 0), low (METAVIR F1-2/Ishak 1-2), and high (METAVIR F3/Ishak 3-5). Spearman's correlation coefficient (r) was used to analyze associations between *PTPN1* or *SRC* and the expression of ECM genes (e.g., *COL1A1*, *COL1A2*, *COL3A1*, and *COL4A1*).

2-11- Statistical Analysis

Data were expressed as the mean ± SEM. Comparisons between two groups were performed using two-tailed unpaired Student's *t*-tests. Multiple-group comparisons were evaluated by one-way ANOVA followed by Tukey's post hoc test. Statistical analyses were conducted using GraphPad Prism software (version 10.0; GraphPad, La Jolla, CA, USA). Significance thresholds were set as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

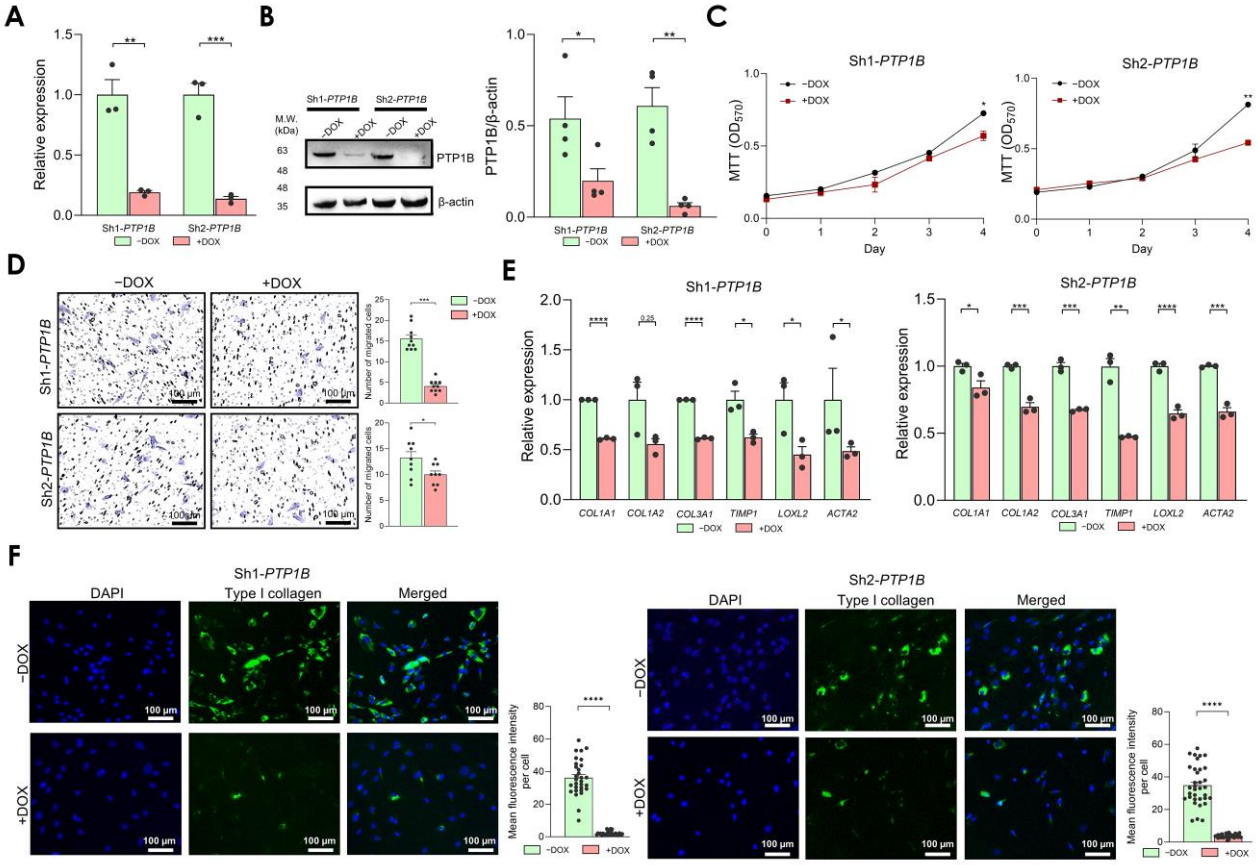
3- Results and Discussion

3-1- Knockdown of PTP1B Suppresses HSC Activation and Fibrogenic Responses

To elucidate the functional role of PTP1B in human HSC activation, we first employed a loss-of-function strategy using two independent PTP1B-specific shRNA constructs. The qRT-PCR analysis confirmed a significant reduction in *PTPN1* expression in both shRNA-induced groups compared with controls (Figure 2-A). This knockdown efficiency was further validated at the protein level by Western blot analysis (Figure 2-B).

Functionally, PTP1B silencing markedly attenuated key features of HSC activation. Both cell proliferation, as measured by the MTT assay, and cell migration, as assessed by the transwell assay, were significantly inhibited in PTP1B-depleted HSCs compared with controls (Figures 2-C and 2-D). Furthermore, knockdown of PTP1B

significantly reduced the expression of several critical fibrosis-associated genes, including *COL1A1*, *COL1A2*, *COL3A1*, and *ACTA2* (Figure 2-E). To further validate the pro-fibrotic role of PTP1B, we assessed markers involved in collagen cross-linking (*LOXL2*) and the inhibition of matrix degradation (*TIMP1*). The consistent downregulation of these markers upon PTP1B knockdown suggests that PTP1B orchestrates a comprehensive fibrogenesis program beyond simple collagen synthesis. Consistently, immunofluorescence staining demonstrated a distinct reduction in type I collagen protein levels in PTP1B-silenced cells (Figure 2-F). These comprehensive results confirm that the endogenous expression of PTP1B is essential for maintaining the activated phenotypes and associated fibrogenic gene expression in human HSCs.



(A) qRT-PCR analysis of *PTPN1* mRNA expression in HSCs upon shRNA-mediated knockdown. (B) Western blot analysis of PTP1B protein levels in HSCs upon shRNA-mediated knockdown. β-actin was used as a loading control. (C) MTT analysis showing the effect of PTP1B knockdown on HSC proliferation over time. (D) Transwell cell migration analysis of HSCs upon shRNA-mediated knockdown. (E) qRT-PCR analysis of fibrotic mRNA expression (*COL1A1*, *COL1A2*, *COL3A1*, *TIMP1*, *LOXL2*, and *ACTA2*) in HSCs upon shRNA-mediated knockdown. (F) Representative immunofluorescence images and quantification of type I collagen protein levels in HSCs upon shRNA-mediated knockdown. Data are presented as means ± SEM, with n ≥ 3 per group from at least two independent experiments. Statistical significance was determined using Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. All fluorescent images were captured with consistent exposure settings across all samples for comparison. Scale bars = 100 μm. Abbreviations: HSC, hepatic stellate cell; qRT-PCR, quantitative real-time PCR; shRNA, short hairpin RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4′,6-diamidino-2-phenylindole.

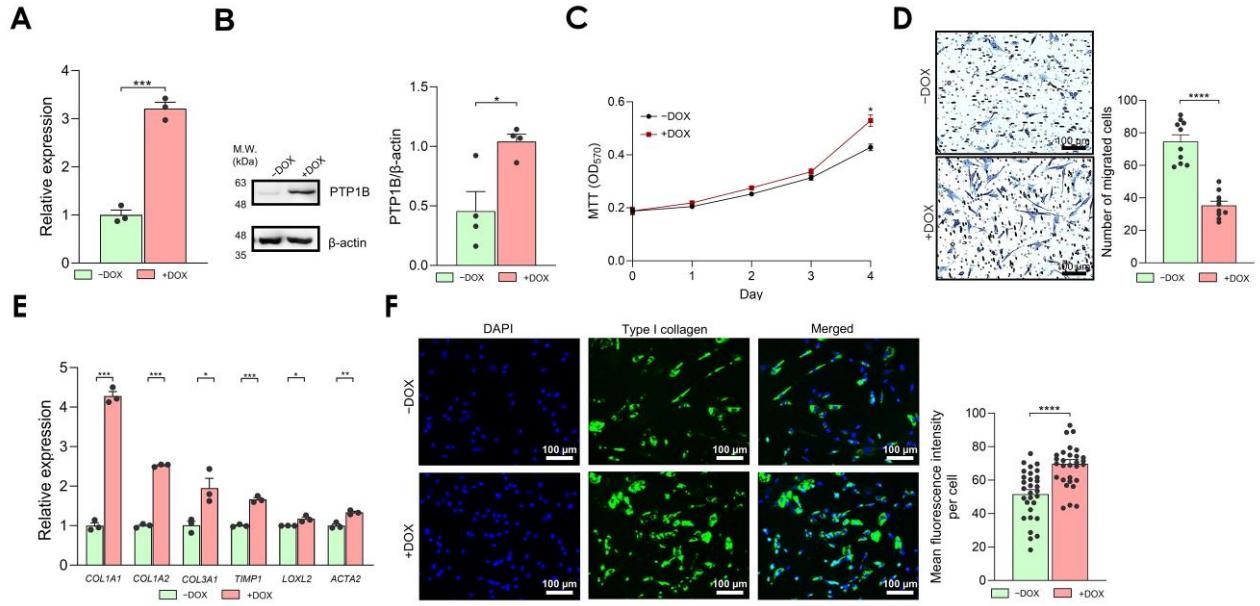
Figure 2. PTP1B depletion reduces human HSC activation and diminishes fibrogenic activity

3-2- Overexpression of PTP1B Promotes HSC Activation and Fibrosis

To further validate the functional role of PTP1B in HSCs, we examined the effects of its stable overexpression. qRT-PCR and Western blot analyses confirmed that PTP1B expression was significantly increased in HSCs transduced with a PTP1B-overexpressing lentivirus compared with control cells (Figures 3-A and 3-B), establishing a successful gain-of-function model.

In direct contrast to the knockdown results, PTP1B overexpression markedly enhanced both HSC proliferation (measured by MTT assay) and migration (assessed by the transwell assay) (Figures 3-C and 3-D), reinforcing the concept that PTP1B acts as a positive regulator of HSC activation. Furthermore, the elevated expression of PTP1B led to significant upregulation of the mRNA levels of key fibrosis-related genes, including *COL1A1*, *COL1A2*, *COL3A1*, *TIMP1*, *LOXL2*, and *ACTA2* (Figure 3-E). Consistently, immunofluorescence staining demonstrated a substantial increase in type I collagen deposition in PTP1B-overexpressing cells (Figure 3-F). Collectively, these data provide robust evidence that PTP1B acts as a potent pro-fibrogenic driver that actively promotes the activation and fibrogenic activity

of human HSCs. Together with the knockdown data, these reciprocal phenotypes provide compelling evidence that PTP1B functions as a driver of fibrogenic activation in human HSCs. This bidirectional modulation mirrors earlier findings in murine systems, but importantly demonstrates that increased PTP1B expression alone is sufficient to amplify fibrotic programs in human HSC cells [17, 18]. Given that HSC activation in chronic liver disease is sustained rather than transient, our findings support a model in which elevated PTP1B contributes to the persistence of the activated myofibroblast phenotypes.



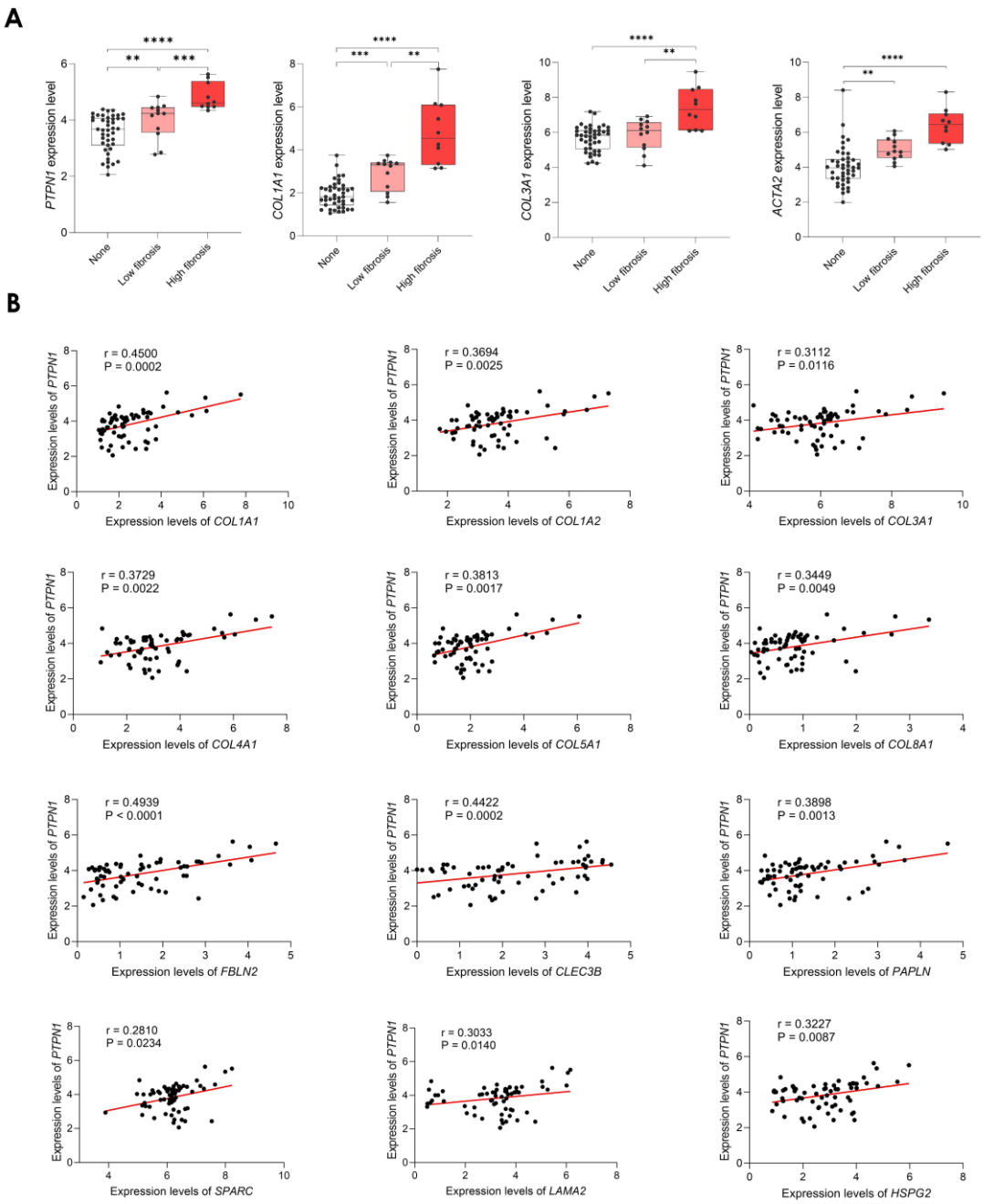
(A) qRT-PCR analysis of *PTPN1* mRNA expression in hepatic stellate cells (HSCs) upon protein-tyrosine phosphatase 1B (PTP1B) overexpression. (B) Western blot analysis of PTP1B protein levels in HSCs upon PTP1B overexpression. β-actin was used as a loading control. (C) MTT assay showing the effect of PTP1B overexpression on HSC proliferation over time. (D) Transwell cell migration analysis of HSCs upon PTP1B overexpression. (E) qRT-PCR analysis of fibrotic mRNA expression (*COL1A1*, *COL1A2*, *COL3A1*, *TIMP1*, *LOXL2*, and *ACTA2*) in HSCs upon PTP1B overexpression. (F) Representative immunofluorescence images and quantification of type I collagen protein levels in HSCs upon PTP1B overexpression. Data are presented as means ± standard error of the mean, with n ≥ 3 per group from at least two independent experiments. Statistical significance was determined using Student's *t*-test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. All fluorescent images were captured with consistent exposure settings across all samples for comparison. Scale bars = 100 μm. Abbreviations: HSC, hepatic stellate cells; qRT-PCR, quantitative real-time PCR; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4′,6-diamidino-2-phenylindole.

Figure 3. PTP1B overexpression enhances activation of hepatic stellate cells and elevates fibrogenic responses

3-3- PTPN1 Expression is Elevated and Positively Correlated with ECM Gene Expression in Human Liver Fibrosis

We next explored the clinical relevance of PTP1B (*PTPN1* gene) expression by re-analyzing RNA-sequencing data from the GepLiver database. Consistently, *PTPN1* expression was significantly higher in human liver samples with both low- and high-grade fibrosis compared with healthy controls (Figure 4-A). This upregulation paralleled the increased expression of established ECM-related genes, such as *COL1A1*, *COL3A1*, and *ACTA2* (Figure 4-A). Demographic stratification revealed that *PTPN1* expression was significantly elevated in fibrotic liver tissues compared with normal controls in individuals aged 50–69 years, but not in those aged 18–49 years or ≥70 years. In addition, *PTPN1* expression was significantly increased in fibrotic liver tissues from male patients but not from female patients (Figures A2(a) and A2(b)). Moreover, etiology-based analysis demonstrated that *PTPN1* expression was significantly upregulated in hepatitis virus-associated fibrotic livers compared with controls (Figure A2(c)).

To quantify the relationship between *PTPN1* expression and fibrogenesis markers, we conducted Spearman's correlation analyses against a panel of 12 ECM-related genes previously shown to be upregulated in fibrotic human liver tissues [25, 26]. This panel included major ECM components, specifically fibrillar collagens (*COL1A1*, *COL1A2*, *COL3A1*, and *COL5A1*), basement membrane-associated proteins (*COL4A1*, *COL8A1*, *LAMA2*, and *HSPG2*), and other matrix proteins (*FBLN2*, *SPARC*, *CLEC3B*, and *PAPLN*). The analysis demonstrated a statistically significant positive correlation ($P < 0.05$) between *PTPN1* expression and the expression of all 12 tested ECM-related genes (Figure 4-B). The correlation coefficients (r) ranged from approximately 0.28 to 0.49, indicating a consistent association. Specifically, *PTPN1* showed the strongest correlation with *FBLN2* (r = 0.49) and highly significant correlations with *COL1A1*, *CLEC3B*, and *PAPLN*. These findings indicate that *PTPN1* upregulation is strongly associated with enhanced ECM gene expression in human liver fibrosis, implicating its involvement in the pathological progression of the disease.

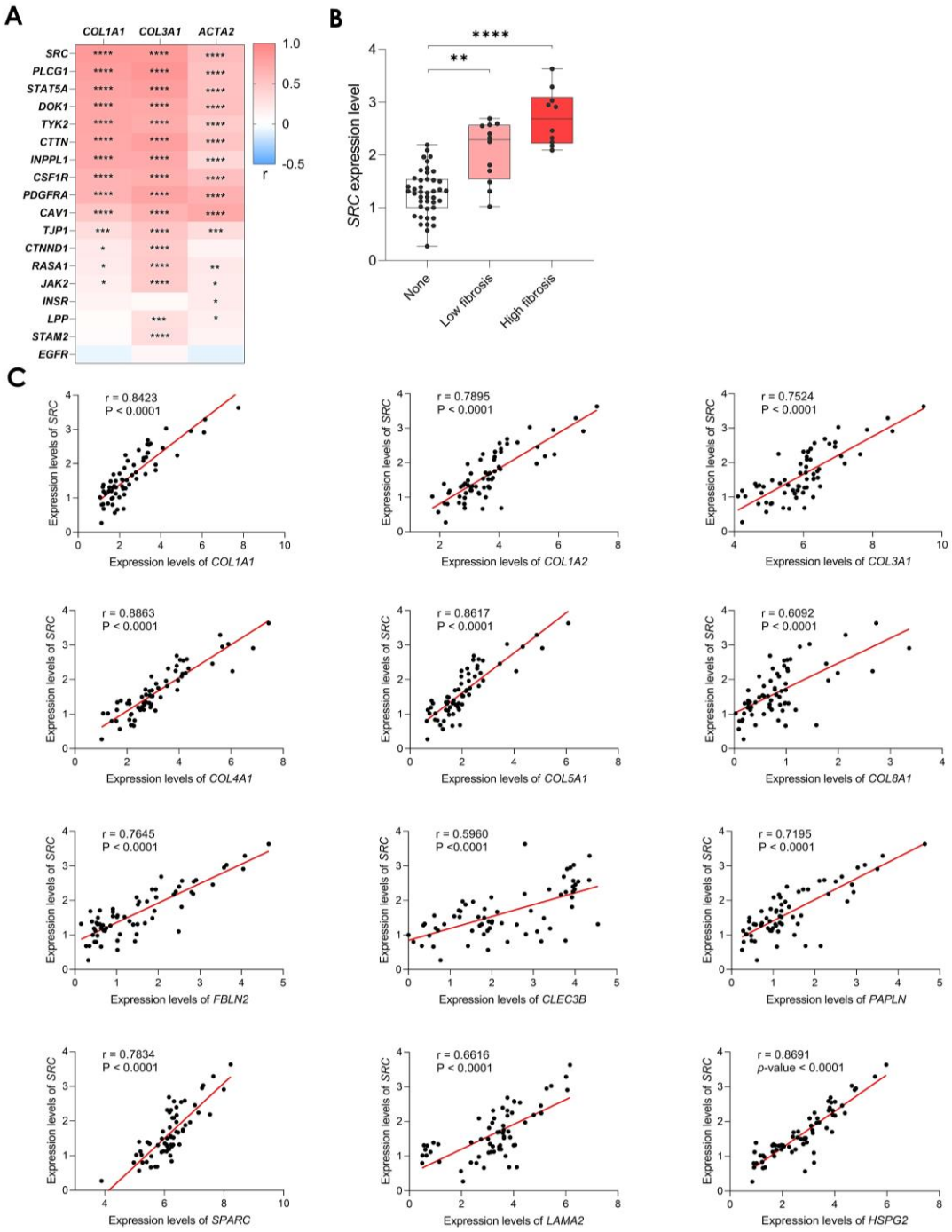


(A) Re-analysis of *PTPN1*, *COL1A1*, *COL3A1*, and *ACTA2* mRNA expression in healthy human livers (N = 43) and livers with low (N = 12) and high (N = 10) grades of fibrosis. Data were obtained from the GepLiver database (http://www.gepliver.org/) and are shown as box-and-whisker plots. Statistical significance was determined using one-way ANOVA with Tukey's multiple comparison test. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. (B) Spearman correlation (r) between *PTPN1* expression (Y-axis) and the panel of ECM gene expression (X-axis) in human liver samples.

Figure 4. *PTPN1* is elevated in fibrotic human livers and shows a positive association with extracellular matrix gene expression

3-4- SRC Expression is Elevated and Correlates with ECM Genes in Fibrotic Human Livers

To explore the molecular mechanisms by which PTP1B drives HSC activation, we investigated the relationship between known PTP1B target genes and key fibrotic markers in the human GepLiver database [27]. Among the tested targets, the non-receptor tyrosine kinase SRC exhibited the strongest positive correlation with the expression of core fibrotic genes, including *COL1A1*, *COL3A1*, and *ACTA2* (Figure 5-A). Notably, *SRC* mRNA levels were significantly elevated in human fibrotic liver tissues compared with healthy controls, with the highest expression observed in patients with high-grade fibrosis (Figure 5-B). Similar to *PTPN1* expression, demographic stratification showed a significant elevation of *SRC* transcript levels in male patients and those aged 50–69 years, corresponding to a high-risk demographic for MASLD-related cirrhosis (Figures A3(a) and A3(b)). Elevated *SRC* expression was particularly pronounced in hepatitis virus-associated fibrotic livers (Figure A3(c)). While statistical significance was not achieved across all demographic subgroups, we observed a consistent trend of upregulation for both *PTPN1* and *SRC* genes across all age groups and both genders compared to their respective healthy controls. Collectively, these findings indicate that *SRC* upregulation accompanies fibrosis progression and aligns with the expression pattern of *PTPN1* in human liver disease.



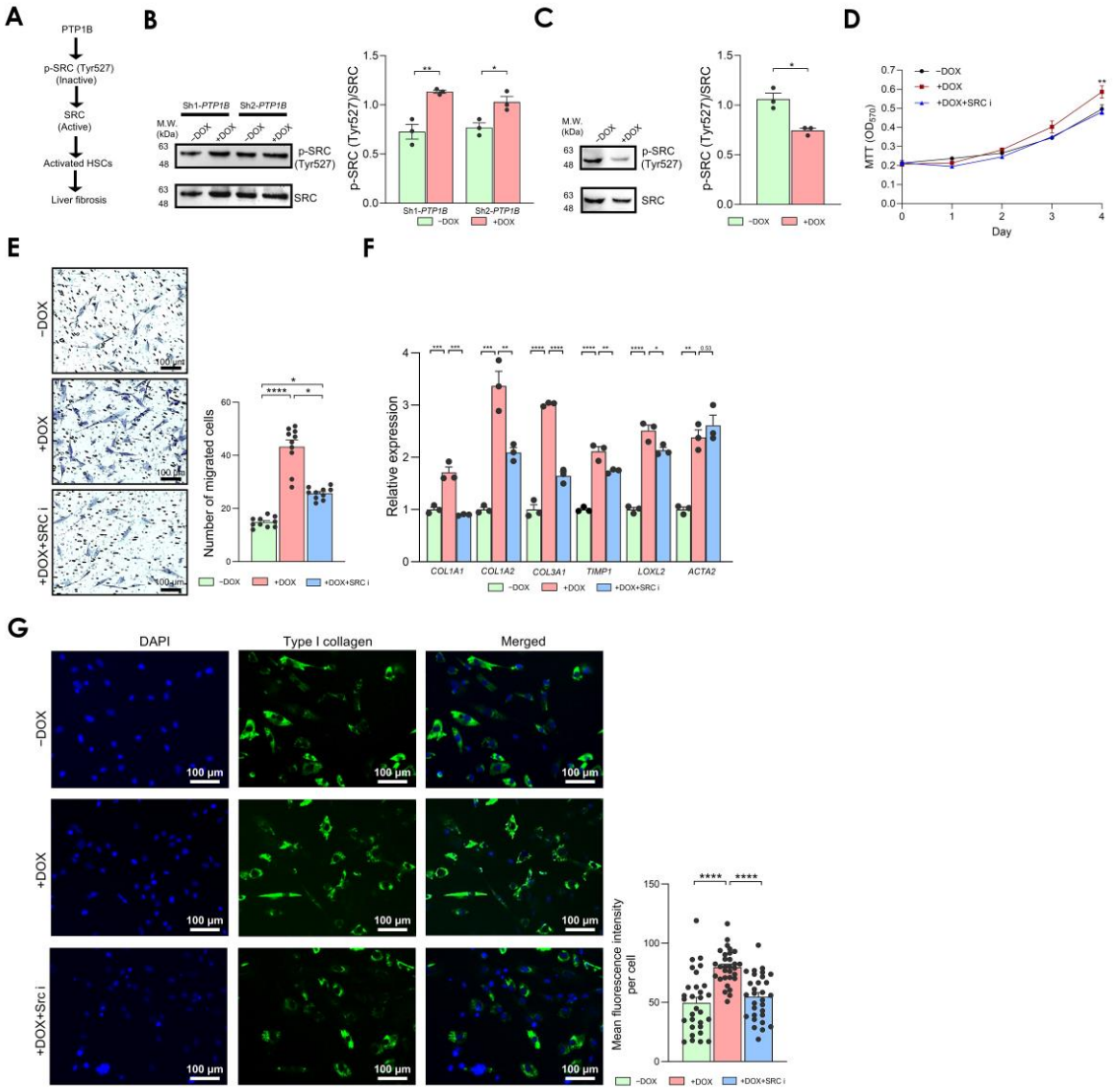
(A) Spearman correlation (r) between SRC target genes and fibrotic genes, *COL1A1*, *COL3A1*, and *ACTA2* in human liver samples. Red indicates strong positive correlation, while blue indicates moderate positive correlation. (B) Re-analysis of *SRC* mRNA expression in healthy human livers (N = 43) and livers with low (N = 12) and high (N = 10) grades of fibrosis. Data were obtained from the GepLiver database and are shown as box-and-whisker plots. Statistical significance was determined using one-way ANOVA with Tukey's multiple comparison test. $^{**}P < 0.01$ and $^{****}P < 0.0001$. (C) Spearman correlation (r) between *SRC* expression (Y-axis) and the panel of ECM gene expression (X-axis) in human liver samples.

Figure 5. *SRC* is increased in fibrotic human livers and positively correlates with extracellular matrix gene expression

We further analyzed the correlation between *SRC* expression and the same panel of 12 ECM-associated genes used in the *PTPN1*-ECM analysis (Section 3-3). The analysis revealed a widespread and highly significant positive correlation between *SRC* expression and all 12 ECM genes (Figure 5-C). Specifically, SRC exhibited strong positive correlations (r ranging from 0.72 to 0.89, $P < 0.0001$) with most ECM genes, and highly significant correlations with *COL1A1*, *COL8A1*, *CLEC3B*, and *PAPLN*. Supporting our hypothesis, SRC activation has previously been reported in models of pulmonary and renal fibrosis, as well as in mouse and human cirrhotic liver tissues [28–30]. Together, these findings strongly position SRC as a critical and clinically relevant downstream effector of PTP1B in mediating HSC activation and fibrogenic responses. Importantly, although SRC has been independently implicated in hepatic fibrosis, its direct regulatory linkage to PTP1B in human HSCs has not been previously established. Thus, these correlative analyses provided a strong rationale for mechanistically interrogating the PTP1B–SRC axis in the context of human HSC activation.

3-5- PTP1B Promotes Fibrogenesis by Activating the SRC Signaling Axis

Previous mechanistic studies have established that PTP1B activates SRC kinase by dephosphorylating the negative regulatory tyrosine residue, Y527, thereby enhancing its kinase activity [31]. Given the elevated expression of SRC observed in human fibrotic liver samples (Section 3-4), we hypothesized that PTP1B drives HSC activation and fibrogenesis through the SRC signaling pathway (Figure 6-A).



(A) Schematic diagram illustrating the hypothesized role of protein-tyrosine phosphatase 1B (PTP1B) in regulating the activation of hepatic stellate cells (HSCs) and fibrosis via SRC. (B) Western blot analysis showing the phosphorylation level of SRC at the inhibitory site Tyr527 in HSCs upon shRNA-mediated PTP1B knockdown and (C) PTP1B overexpression. (D) MTT assay of HSC proliferation in PTP1B overexpression cells treated with an SRC inhibitor (saracatinib). (E) Transwell cell migration analysis in PTP1B-overexpressing HSCs treated with an SRC inhibitor. (F) qRT-PCR analysis of fibrotic gene expression (*COL1A1*, *COL1A2*, *COL3A1*, *TIMP1*, *LOXL2*, and *ACTA2*) in PTP1B-overexpressing HSCs exposed to an SRC inhibitor. (G) Representative immunofluorescence images and quantification of type I collagen in PTP1B-overexpressing HSCs exposed to an SRC inhibitor. Data are presented as means ± standard error of the mean, with n = 3 per group from at least two independent experiments. Statistical significance was determined using Student's *t*-test or one-way ANOVA with Tukey's multiple comparison test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. All fluorescent images were taken with consistent exposure settings across all samples for comparison. Scale bars = 100 μm. Abbreviations: HSCs, hepatic stellate cells; qRT-PCR, quantitative real-time PCR; shRNA, short hairpin RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4′,6-diamidino-2-phenylindole

Figure 6. Protein-tyrosine phosphatase 1B regulates fibrosis by activating SRC signaling

To test this hypothesis, we first examined the direct regulatory effect of PTP1B on SRC dephosphorylation in HSCs. Western blot analysis demonstrated that PTP1B knockdown led to a significant increase in phosphorylated SRC (p-SRC) at tyrosine 527, confirming enhanced inhibitory phosphorylation (Figure 6-B). Conversely, PTP1B overexpression resulted in a marked decrease in p-SRC (Figure 6-C). These results confirm that PTP1B regulates SRC activity by controlling its inhibitory phosphorylation state.

To functionally validate the crucial role of SRC as the downstream effector, we treated PTP1B-overexpressing HSCs with the SRC inhibitor, saracatinib, and found that pharmacological inhibition of SRC completely abolished the pro-fibrotic effects induced by PTP1B overexpression, returning HSC proliferation and migration to levels comparable to those of control cells (Figures 6-D and 6-E). Furthermore, SRC inhibition effectively attenuated the PTP1B-mediated

profibrotic gene expression, evidenced by a significant reduction in the mRNA levels of ECM-related genes and a subsequent decrease in type I collagen deposition (Figures 6-F and 6-G). These rescue experiments definitively establish SRC as the principal downstream effector of PTP1B-mediated HSC activation. Collectively, these findings establish a novel PTP1B–SRC signaling axis in human HSCs and demonstrate that PTP1B promotes HSC activation and fibrogenesis primarily through the activation of SRC signaling via dephosphorylation at the inhibitory tyrosine 527 residue.

The progressive nature of liver fibrosis, which often culminates in severe endpoints such as cirrhosis and HCC, highlights an urgent need for targeted therapeutic strategies, especially given the rising prevalence of MASLD. The sustained activation of HSCs remains the central pathological event driving this progression, yet the specific molecular mechanisms governing this cellular transition, particularly in the human context, have not yet been fully elucidated. In this study, we established that PTP1B is a critical positive regulator of human HSC activation and fibrogenic activity. Our findings define a novel PTP1B-SRC signaling axis that drives these fibrogenic responses, providing mechanistic insights into the progression of human liver fibrosis.

Our findings demonstrate, for the first time in the human context, that PTP1B is essential for maintaining the activated, pro-fibrotic phenotypes of human HSCs. Through complementary loss- and gain-of-function experiments, we observed a reciprocal and powerful modulation of key fibrogenic hallmarks. Specifically, PTP1B silencing significantly attenuated all tested pro-fibrotic features, including reduced cell proliferation, migration, ECM gene expression, and type I collagen deposition. Conversely, PTP1B overexpression markedly enhanced these phenotypes, establishing that endogenous PTP1B is a required driver to maintain the activated state of human HSCs (Figure 7). These results extend and validate prior observations in rodent models, where PTP1B deficiency reduces fibrosis in mice subjected to bile duct ligation (BDL), underscoring a conserved pro-fibrotic role for PTP1B across species [18]. Consistent with our finding, PTP1B mRNA and protein levels have been reported to increase in a time-dependent manner following TGF-β-stimulation in HSCs [17]. Furthermore, *in vivo* studies have shown that PTP1B expression is significantly upregulated during the fibrotic phase and subsequently declines during the resolution of fibrosis [18, 32].

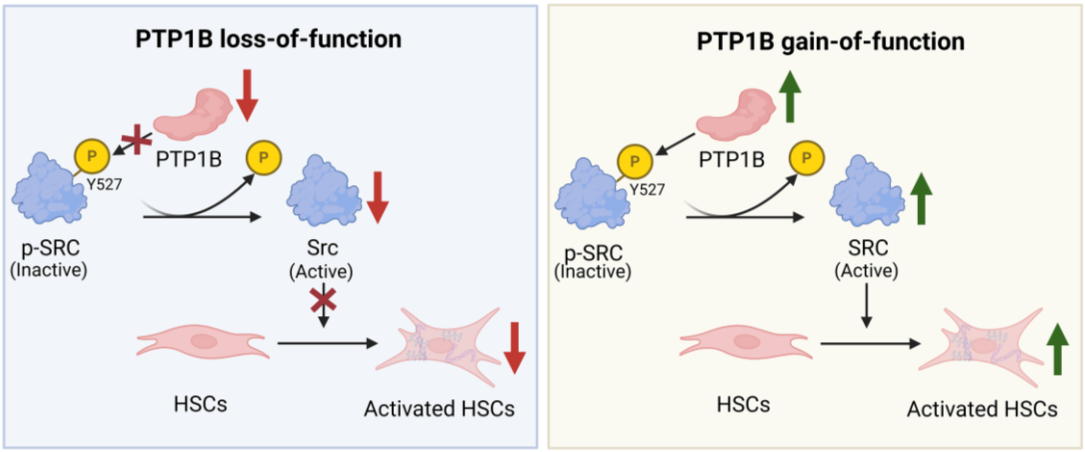


Figure 7. Model illustrating protein-tyrosine phosphatase 1B-mediated regulation of fibrosis via SRC activation

Model illustrating protein-tyrosine phosphatase 1B (PTP1B)-mediated promotion of HSC activation and fibrogenesis by activating SRC signaling via dephosphorylation of the inhibitory tyrosine 527 residue. In loss-of-function conditions, PTP1B silencing diminishes SRC dephosphorylation, leading to the accumulation of inactive p-SRC and reduced active SRC levels, thereby limiting HSC activation. In contrast, gain-of-function experiments show that increased PTP1B enhances SRC dephosphorylation, elevates active SRC levels, and consequently drives HSC activation and pro-fibrotic responses.

To further contextualize these findings within human disease, our re-analysis of the GepLiver database revealed that while the PTP1B-SRC axis is broadly associated with human liver fibrosis, its transcriptomic signature is most pronounced in males and in individuals aged 50–69 years. This demographic alignment is particularly noteworthy as it mirrors the high-risk populations for MASLD-related cirrhosis. The observed trends in other groups—even those without statistical significance—suggest that this axis is a general feature of fibrogenesis, though its magnitude may be modulated by hormonal factors or the duration of chronic injury [33]. Collectively, these findings suggest that PTP1B functions as a dynamic regulator that sustains HSC activation during fibrotic progression. By defining this functional role in primary human cells and reinforcing it with clinical tissue data, our work provides a critical translational link, confirming PTP1B's central importance in human fibrosis biology.

A major contribution of this work is the identification of the PTP1B–SRC axis as a key pathway through which PTP1B promotes fibrosis. We show that PTP1B directly regulates SRC activity by dephosphorylating the inhibitory Tyr527 residue, thereby increasing active SRC. Dephosphorylation of SRC at Tyr527 removes an inhibitory “latch,”

enabling SRC to adopt an open, active conformation that drives fibrogenic signaling. The Tyr527 dephosphorylation event represents the primary, rate-limiting step required for SRC activation, preceding autophosphorylation at Tyr416, which is commonly used as a marker of SRC kinase activity [34, 35]. Activated SRC is known to regulate multiple pro-fibrotic pathways, including TGF-β/Smad signaling and ECM gene transcription, consistent with its established roles in fibrotic tissues [34, 36]. In support of this mechanism, our functional data showed that PTP1B knockdown increased p-SRC levels and impaired downstream fibrogenic signaling, while PTP1B overexpression enhanced SRC activation.

Crucially, pharmacological SRC inhibition abolished the pro-fibrotic effects of PTP1B overexpression, definitively establishing SRC as the principal downstream effector of PTP1B-mediated HSC activation. This regulatory interaction is not unique to the liver; prior studies have reported a similar PTP1B-mediated activation of SRC via Y527/Y530 dephosphorylation in other cell types. For instance, PTP1B regulates SRC activity to enhance oxidative phosphorylation efficiency in rat brain mitochondria [34] and acts as an activator of SRC to increase tumorigenicity in colon cancer [37]. Furthermore, PTP1B-mediated SRC activation and subsequent ERK1/2 activation promote proliferation and metastasis in non-small-cell lung cancer [38]. The consistent utilization of the PTP1B-SRC mechanism in these diverse biological processes reinforces its biological significance as a crucial molecular switch governing cellular activation and proliferation. Our findings integrate these observations into a unified model in which PTP1B sustains human HSC activation by maintaining SRC in an active signaling state.

The functional and mechanistic findings from our human HSC models are strongly supported by transcriptomic evidence derived from human fibrotic liver tissues. Our re-analysis of the GepLiver database revealed that both *PTPN1* (encoding PTP1B) and *SRC* are significantly upregulated and positively correlated with ECM gene expression in fibrotic patient samples, reflecting disease progression. This clinical observation aligns with recent findings showing SRC elevation in primary human HSCs, in livers of thioacetamide (TAA)-treated mice, and in cirrhotic tissues from patients [30]. Furthermore, *in vivo* studies robustly demonstrate that SRC inhibition attenuates TAA-induced increases in α-SMA, type I collagen, and connective tissue growth factor, reinforcing its central and actionable role in hepatic fibrogenesis [30]. Beyond the liver, activated SRC is also implicated in other organ fibrosis models, including pulmonary and renal fibrosis [29]. The consistency between our novel PTP1B-SRC mechanism and the expression patterns observed across patient disease progression and diverse *in vivo* models underscores the immediate clinical and therapeutic importance of the PTP1B–SRC pathway.

PTP1B has also been reported to regulate fibrosis through pathways beyond the SRC axis, underscoring its multifaceted role in pathology. For example, PTP1B deficiency in mouse models protects against bile acid-induced fibrosis, not only by enhancing resistance to cell death but also by controlling the balance of internal stress signals (oxidative stress) in the liver [18]. This protection occurs through mechanisms that are independent of the SRC pathway. Furthermore, in alcoholic liver injury, PTP1B promotes macrophage activation by modulating NF-kB signaling, thereby contributing to inflammatory and fibrotic progression [39]. The therapeutic potential is further highlighted by studies showing that natural PTP1B inhibitors, such as luteolin-7-diglucuronide and ferulic acid, reduce fibrosis in mouse models, in part by inhibiting PTP1B activity and consequently activating AMPK [40, 41]. While our integrative analyses and functional experiments identify SRC as the dominant downstream driver of PTP1B-mediated fibrogenic signaling in human HSCs, we acknowledge that the contribution of other pathways cannot be excluded and may become more prominent in specific disease contexts or stages of injury. Elucidating additional pathways will be an important focus of future studies. Collectively, these findings from diverse models and pathways strongly reinforce the broader, complex pro-fibrotic role of PTP1B in liver pathology.

The identification of the PTP1B–SRC signaling axis presents a high-potential therapeutic target for liver fibrosis. The clinical feasibility of targeting this pathway is strongly supported by the existence of pharmacologic inhibitors for both components. For example, SRC inhibition, using agents such as saracatinib, has already demonstrated significant anti-fibrotic efficacy *in vivo*, reducing fibrosis markers and suppressing Smad3 signaling [30]. Although SRC family kinase inhibitors may exhibit off-target effects, the concordance between our pharmacological rescue experiments and genetic modulation of PTP1B strongly supports the specificity and functional relevance of the PTP1B–SRC axis. Future studies employing isoform-specific SRC inhibition or genetic silencing approaches will help to further refine the contribution of individual SRC family members in HSC biology. Similarly, PTP1B is a well-established therapeutic target for metabolic diseases, leading to the development of several small-molecule inhibitors, including JTT-551, trodusquemine (MSI-1436), and DPM-1001 [42–44]. Recent evidence shows that MSI-1436 reduces *ex vivo* liver inflammation and fibrosis in horses with metabolic syndrome, further supporting the translational viability of PTP1B-targeted therapies [45]. The established safety profiles and clinical availability of these PTP1B inhibitors offer a compelling translational opportunity for treating human liver disease. Given that PTP1B is tightly linked to metabolic dysfunction, which is highly prevalent in MASLD, targeting this phosphatase offers the unique dual therapeutic benefit of inhibiting HSC activation via the SRC pathway while simultaneously managing associated metabolic comorbidities.

Although our findings provide strong mechanistic and translational evidence for the PTP1B–SRC axis in the activation of human HSCs, a key limitation is the lack of *in vivo* validation using cell type-specific PTP1B knockout models. While previous studies have shown that global PTP1B deletion protects mice from BDL-induced fibrosis, the systemic effects inherent to global knockout mice complicate the definitive attribution of the anti-fibrotic effect solely

to HSCs [18]. Therefore, future studies will require the development and use of conditional knockout models with HSC-specific deletion of PTP1B to rigorously define the cell-autonomous contribution of PTP1B to HSC activation and fibrogenesis *in vivo*. Such strategies have been successfully applied in metabolic disease research, where tissue-specific PTP1B deletion revealed distinct physiological roles [46, 47]. Applying similar conditional genetic approaches in fibrosis models will provide definitive *in vivo* evidence for the HSC-specific contribution of PTP1B to liver fibrosis progression. Future studies leveraging these conditional knockout mice will therefore be critical to fully unravel the cell-specific functions of PTP1B and fully validate its therapeutic potential.

4- Conclusion

In this study, we establish PTP1B as a central and indispensable regulator of human HSC activation, providing mechanistic and translational insights into its role in hepatic fibrogenesis. Using complementary loss- and gain-of-function approaches in primary human HSCs, we demonstrate that PTP1B is both necessary and sufficient to maintain the activated myofibroblast phenotypes. Modulation of PTP1B expression led to reciprocal effects on core fibrogenic hallmarks, including HSC proliferation, migratory capacity, ECM gene expression, and type I collagen deposition. These functional data define PTP1B as a key determinant of sustained HSC activation and extend previous observations from rodent models into a clinically relevant human context, thereby strengthening the translational significance of our findings.

Mechanistically, we identify the SRC kinase as the dominant downstream effector of PTP1B in human HSCs. PTP1B directly activates SRC by dephosphorylating its inhibitory tyrosine 527 residue, thereby relieving SRC autoinhibition and sustaining pro-fibrotic signaling. Pharmacological inhibition of SRC using the selective inhibitor saracatinib effectively reversed PTP1B-driven fibrogenic phenotypes, functionally validating SRC as an essential mediator of PTP1B activity in human HSCs. Importantly, transcriptomic re-analyses of human fibrotic liver tissues revealed coordinated upregulation of *PTPN1* (PTP1B), *SRC*, and ECM-associated genes across fibrosis stages, with strong positive correlations between PTP1B/SRC expression and established fibrotic markers. These human tissue data firmly support the clinical relevance of the PTP1B–SRC axis and link its activation to fibrosis severity and pathological matrix remodeling. While PTP1B has been implicated in additional SRC-independent pathways in other liver disease contexts, our findings identify SRC as the dominant driver of PTP1B-mediated fibrogenic signaling in human HSCs. Collectively, this study defines a previously underappreciated phosphatase-driven mechanism of liver fibrosis and provides a strong rationale for repurposing PTP1B-targeted therapies, originally developed for metabolic diseases, as a novel antifibrotic strategy to address a critical unmet clinical need.

5- Declarations

5-1- Author Contributions

Conceptualization, B.B. and C.A.; methodology, B.B. and N.S.; software, B.B.; validation, N.S. and C.A.; formal analysis, B.B.; investigation, B.B. and N.S.; resources, C.A.; data curation, B.B.; writing—original draft preparation, B.B.; writing—review and editing, B.B., N.S., and C.A.; visualization, B.B.; supervision, project administration, and funding acquisition, C.A. All authors have read and approved the final version of the manuscript.

5-2- Data Availability Statement

The data presented in this study are available on request from the corresponding author.

5-3- Funding and Acknowledgements

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5-4- Institutional Review Board Statement

Not applicable.

5-5- Informed Consent Statement

Not applicable.

5-6- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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Appendix I: Information

Table A1. Sequences of primers used in this study

Primer name	Sequence (5′→3′)
Cloning Primer	
T_Sh1_PTP1N (PTP1B)	CCGGTTTGACCATAGTCGGATTAAACTCGAGTTTAATCCGACTATGGTCAAATTTTT
B_Sh1_PTP1N (PTP1B)	AATTAAAAA TTTGACCATAGTCGGATTAAACTCGAGTTTAATCCGACTATGGTCAAA
T_Sh2_PTP1N (PTP1B)	CCGGCTGTGATCGAAGGTGCCAAATCTCGAGATTTGGCACCTTCGATCACAGTTTTT
B_Sh2_PTP1N (PTP1B)	AATTAAAAACTGTGATCGAAGGTGCCAAATCTCGAGATTTGGCACCTTCGATCACAG
F_outer of PTP1N (PTP1B)	CTAGAGCGGCAGACGGC
R_outer of PTP1N (PTP1B)	GACAGTGGGTGGAGGTGGAG
F_inner of PTP1N (PTP1B) EcoRI	CGCTGAATTCGCCGCCACCATGGAGATGGAAAAGGAGTT
R_inner of PTP1N (PTP1B) BamHI	CGCAGGATCCCTATGTGTTGCTGTTGAACAGGAAC
RT-qPCR Primer	
F_PTP1N (PTP1B)	GCATAGGACAGTGGTAATGCG
R_PTP1N (PTP1B)	AACTCACAGGGAAAGCAGAGG
F_COL1A1	CCCAAGGCTTCCAAGGTC
R_COL1A1	GGACGACCAGGTTTTCCAG
F_COL1A2	GAGTCCGAGGACCTAATGGA
R_COL1A2	AGGGGAACCAGGAAGACCT
F_COL3A1	TGGTGGTAAAGGCGAAATG
R_COL3A1	AGTCCAGGAGCACCATTAGC
F_COL4A1	GGGTGAACCAGGAAAAATTG
R_COL4A1	GGAAAGCCTCGGTCTCCTT
F_TIMP1	CTGTTGTTGCTGTGGCTGAT
R_TIMP1	AACTTGGCCCTGATGACG
F_ LOXL2	TTGGAGGACACAGAATGTGAA
R_ LOXL2	AGTCGATGTCATGGCGGTA
F_ACTA2	CTGTTCCAGCCATCCTTCA
R_ ACTA2	TCATGATGCTGTTGTAGGTGGT
F_RPL19	GCTCTTTCCTTTCGCTGCT
R_RPL19	CATTGGTCTCATTGGGGTCT

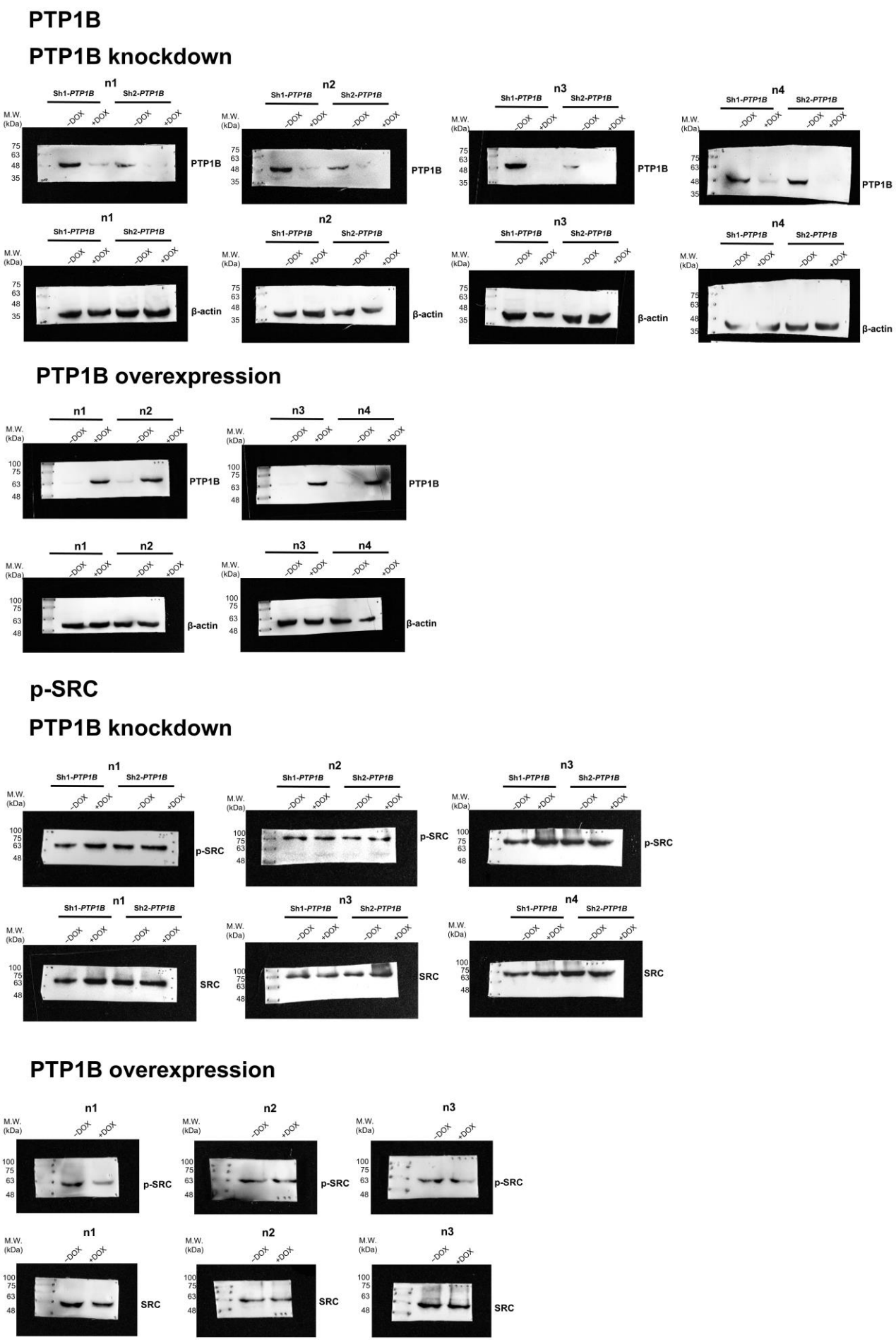

Figure A1. Original Western blot analysis (Relevant to Figures 1b, 2b, 5b, and 5c)

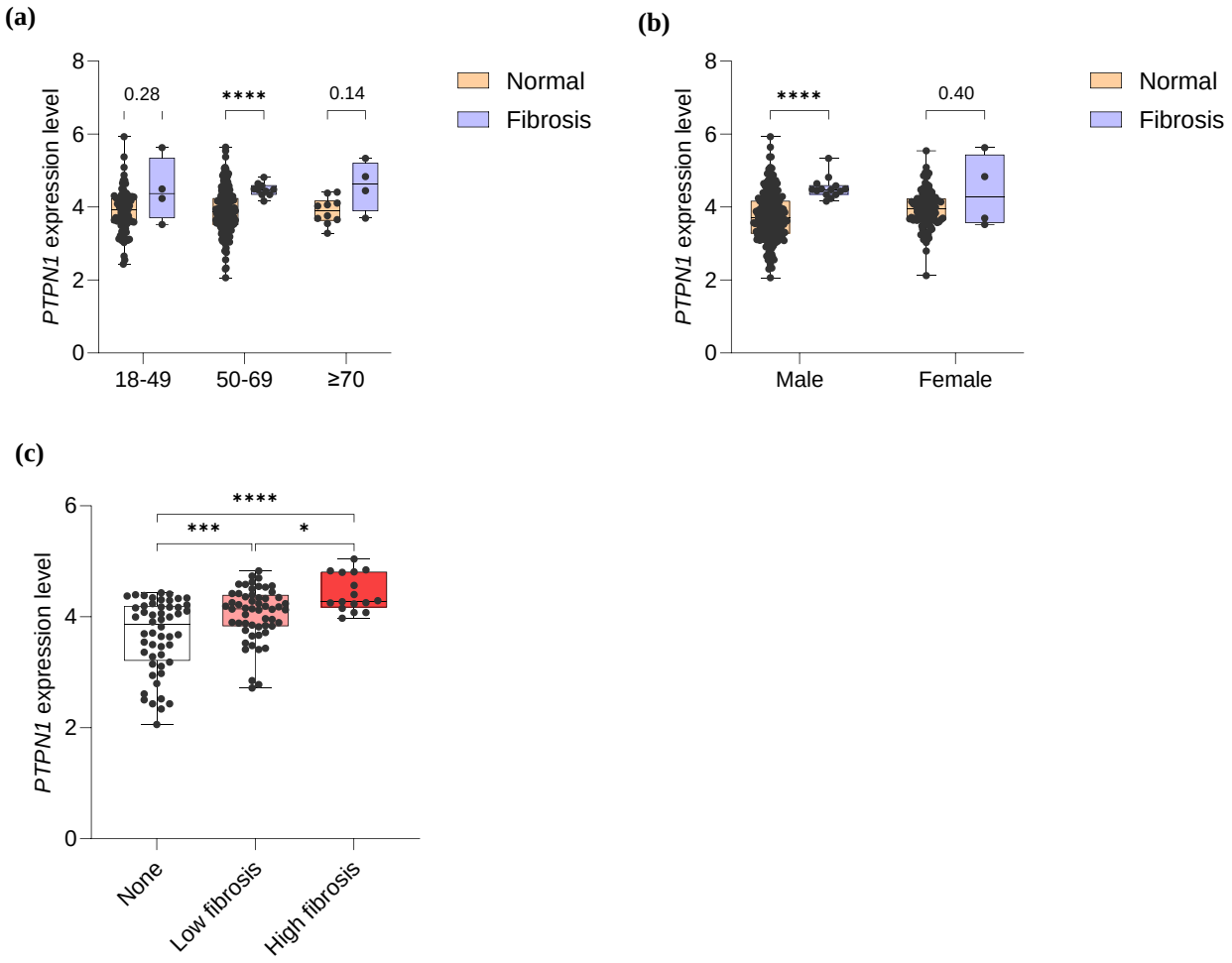

Figure A2. *PTPN1* expression in normal and fibrotic human livers

Re-analysis of *PTPN1* mRNA expression in healthy human livers and fibrotic livers stratified by demographic variables. (A) Age distribution (Normal: male, N = 205; female, N = 107; Fibrosis: male, N = 14; female, N = 4). (B) Gender distribution (Normal: age 18–49 years, N = 86; age 50–69 years, N = 187; age ≥ 70 years, N = 10; Fibrosis: age 18–49 years, N = 4; age 50–69 years, N = 10; age ≥70 years, N = 4). (C) Re-analysis of *PTPN1* mRNA expression in healthy human livers (N = 43) and hepatitis virus-associated fibrotic livers with low (N = 12) and high (N = 10) grades of fibrosis. Data were obtained from the GepLiver database (http://www.gepliver.org/) and are shown as box-and-whisker plots. Statistical significance was determined using Student's *t*-test or one-way ANOVA with Tukey's multiple comparison test. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$.

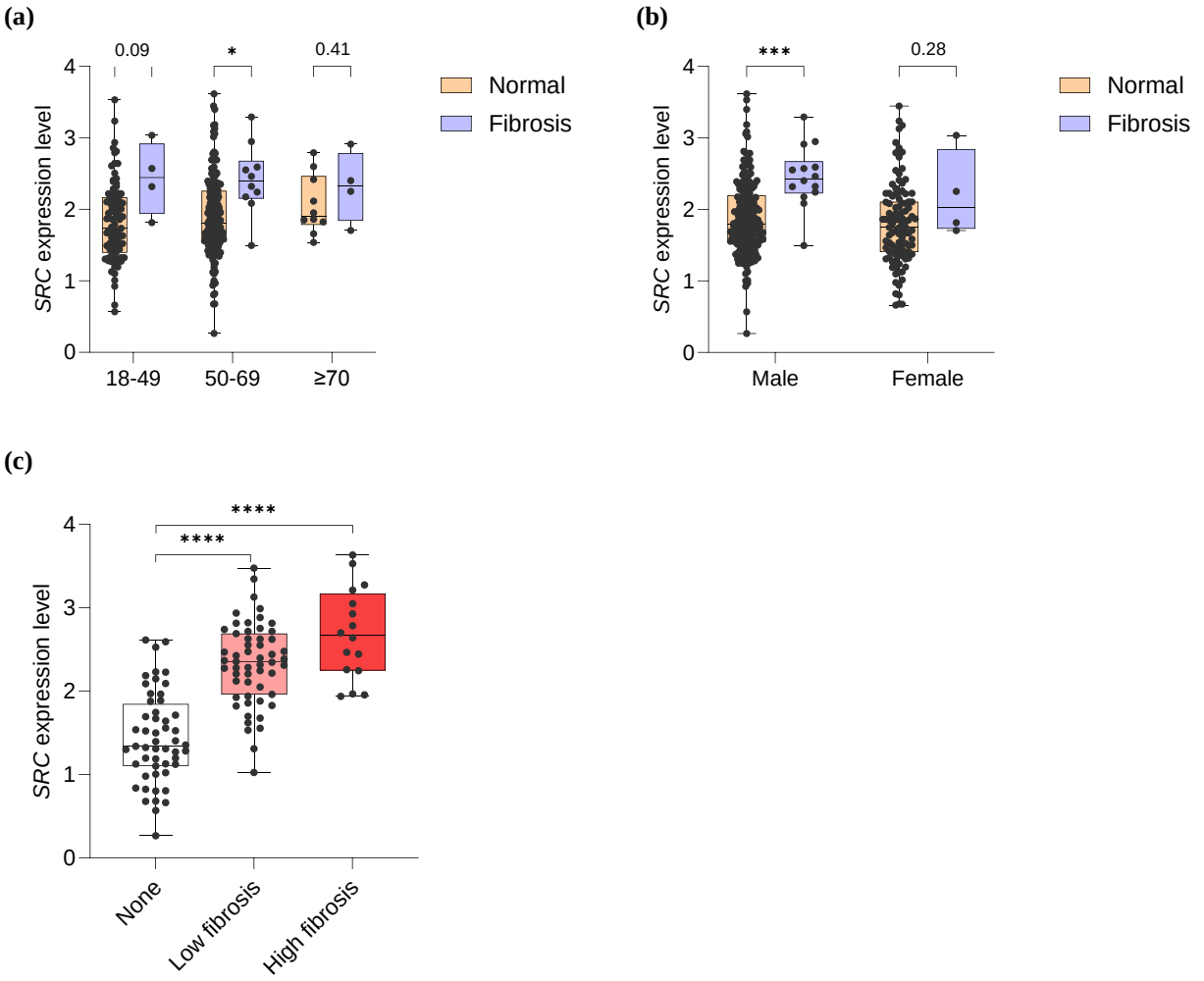

Figure A3. *SRC* expression in normal and fibrotic human livers

Re-analysis of *SRC1* mRNA expression in healthy human livers and fibrotic livers stratified by demographic variables. (A) Age distribution (Normal: male, N = 205; female, N = 107; Fibrosis: male, N = 14; female, N = 4). (B) Gender distribution (Normal: age 18–49 years, N = 86; age 50–69 years, N = 187; age ≥ 70 years, N = 10; Fibrosis: age 18–49 years, N = 4; age 50–69 years, N = 10; age ≥70 years, N = 4). (C) Re-analysis of *SRC* mRNA expression in healthy human livers (N = 43) and hepatitis virus-associated fibrotic livers with low (N = 12) and high (N = 10) grades of fibrosis. Data were obtained from the GepLiver database (http://www.gepliver.org/) and are shown as box-and-whisker plots. Statistical significance was determined using Student's *t*-test or one-way ANOVA with Tukey's multiple comparison test. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$.