Supplementary methods

1. Reagents, kits, and antibodies

PIP was bought from Yuan-Ye Bio-Technology (Shanghai, China). Recombinant human and mouse TGF-β were bought from Huamei Biological (Wuhan, Hubei, China). DAPI was bought from Beyotime Biotechnology (Shanghai, China). Crystal violet was from Servicebio Technology (Wuhan, Hubei, China).

The sense and antisense sequences of siRNAs specific to mouse Nrf2 (siNrf2-m) are 5′-GAG AUG AGC UUA GGG CAA AUU-3′ and 5′-AAU UUG CCC UAA GCU CAU CUC-3′. Sequences of their control siRNAs (siNC-m) are 5′-UUC UCC GAA CGU GUC ACG UTT-3′ and 5′- ACG UGA CAC GUU CGG AGA ATT-3′. The sense and antisense sequences of siRNAs targeting human Nrf2 (siNrf2-h) are 5′-GUG AGA UAC CAU GUU TT-3′ and 5′-AAC GUG GUA GUC UCA CAC CTT-3′. Sequences of their corresponding control siRNAs (siNC-h) are 5′-UUC UCC GAA CGU GUC ACG UTT-3′ and 5′- ACG UGA CAC GUU CGG AGA ATT-3′. SiRNAs were transfected by methods as described previously.[1]

Biochemical assay kits for glutathione (GSH), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), hydroxyproline (HYP), and total antioxidant capacity (T-AOC) were from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The DCFH-DA ROS quantification kit, nuclear protein extraction kit, hematoxylin-eosin (HE) staining kit, hypersensitive chemiluminescence kit, and EdU cell proliferation assay kit were bought from Beyotime Biotechnology (Shanghai, China). ELISA kits for hyaluronic acid (HA) and procollagen III (PC III) were purchased

from ZCIBIO Technology (Shanghai, China).

Primary antibodies against E-cadherin, smad7, Nrf2, Lamin B, HO-1, NQO1, GCLC, and β-actin were bought from Beyotime Biotechnology (Shanghai, China). Primary antibodies against vimentin, α-SMA, smad2, and smad3 were bought from Proteintech Group (Wuhan, Hubei, China). Primary antibodies against phosphorylated smad2 at Ser465/467 (p-smad2), and phosphorylated smad3 at Ser423/425 (p-smad3), and collagen I were purchased from Affinity Biosciences (Changzhou, Jiangsu, China). Secondary antibodies were bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2. Quantification of ROS

Levels of ROS in AML-12 and LX-2 cells were determined by using the DCFH-DA ROS quantification kit following the manufacturer's instructions.

3. Determination of cell proliferation and migration

Proliferation of LX-2 cells was detected by the EdU cell proliferation assay kit following the manufacturer's instructions. Migration of LX-2 cells was analyzed by transwell assay followed by crystal violet staining which was performed as described previously.[2]

4. Immunoblotting and real-time PCR

Total lysates of cultured cells and mouse livers were prepared as described previously.[3] Nuclear extract of cultured cells and mouse livers were prepared by using the nuclear protein extraction kit following the manufacturer's instructions. Then,

immunoblotting was performed and analyzed by methods described previously.[4]

To quantify gene transcription, real-time PCR was performed and analyzed as described previously.[5] The primers sets used in our PCR reaction were provided in Supplementary Table 1 and 2.

5. Animals

Male specific pathogen free Kunming mice weighing 20 to 24 g were bought from the Hubei Experimental Animal Research Center (Wuhan, Hubei, China) and acclimatized for 1 week prior to the experiment.

6. Immunohistochemistry and immunofluorescence staining assays

Immunohistochemical staining of tissue sections and immunofluorescence staining of cultured cells were performed as described previously.[6, 7] DAPI was used in immunofluorescence to stain the nucleus. Immunofluorescence staining images were quantified as described previously.[8]

7. Calculation of liver index and determination of liver biochemical parameters

Liver index was calculated by the formula: Liver index = liver weight (g) / body weight (g) × 100%. Hepatic levels of MDA, GSH, SOD, T-AOC, HYP, GSH-PX, and CAT were measured by using the corresponding assay kits following the manufacturer's instructions. Serum HA and PC III were quantified by using the corresponding ELISA kits according to the manufacturer's instructions.

8. Statistical analysis

The data shown in this manuscript are representatives or statistics (mean ± standard deviation) of results from at least 3 independent experiments or all mice in a group. One-way analysis of variance (ANOVA) was performed to assess difference among groups. If the number of groups was more than two, Tukey's post-test was also used for two-way comparisons.

References to Supplementary methods

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Supplementary tables

Supplementary Table 1 Primer sequences to probe human genes in real-time PCR

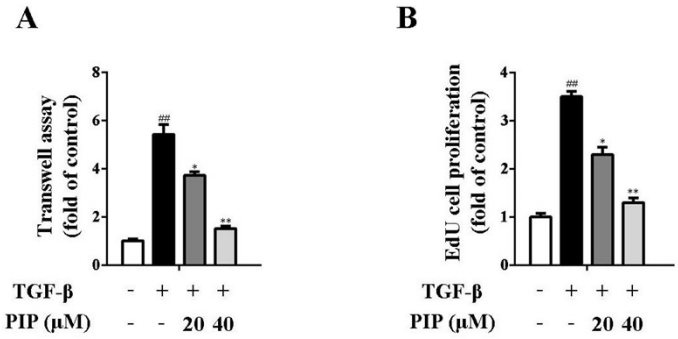
Genes	Forward (5′→3′)	Reverse (5′→3′)
GAPDH	ACCAGTCCATGCCATCAC	TCCACCCCCTGTTGCTGTA
Snail	CCAGACCCACTCAGATGTCAA	GGACTCTTGGTGCTTGTGGA
Twist	GGCTCAGCTACGCCTTCTC	TCCTTCTCTGGAAACAATGACA
Zeb1	TGTGGTAGAACAAATTCAGATTC	GCCCTTCCTTTCCTGTGTCA
Zeb2	GCCTCTGTAGATGGTCCAGTGA	TCACTGCGCTGAAGGTACTC
HO-1	GCCCTGCCCTTCAGCAT	AGCTGCCACATTAGGGTGTCTT
NQO1	GTGGCAGTGGCTCCATGTACTC	CTTGGAAGCCACAGAAATGCAG
GCLC	TTCCTGGACTGATCCCAATTCTG	CTCATCCATCTGGCAACTGTCATTA

Supplementary table 2 Primer sequences to probe mouse genes in real-time PCR

Genes	Forward (5′–3′)	Reverse (5′–3′)
Actin	ACCGTGAAAAGATGACCCAG	TCTCAGCTGTGGTGGTGAAG
Slug	GCACTGTGATGCCCAGTCTA	CAGTGAGGGCAAGAGAAAGG
Snail	CTTGTGTCTGCACGACCTGT	CTTCACATCCGAGTGGGTTT
Twist	GGCTCAGCTACGCCTTCTC	TCCTTCTCTGGAAACAATGACA
Zeb1	TGGCAAGACAACGTGAAAGA	AACTGGGAAAATGCATCTGG
Zeb2	TAGCCGGTCCAGAAGAAATG	GGCCATCTCTTTCCTCCAGT
HO-1	TCTGTCCAATGTGGCCTTCTC	GCTAGGGACCCCAAAAGCTT
NQO1	AGCCAATCAGCGTTCGGTAT	GCCTCCTTCATGGCGTAGTT
GCLC	TGCACATCTACCACGCAGTC	GTCTCAGAGATCGCCTCCA
E-cadherin	GGGTTGTCTCAGCCAATGTT	CACCAACACACCCAGCATAG
Vimentin	AGATCGATGTGGACGTTTCC	CACCTGTCTCCGGTATTCGT
α-SMA	TGACAGAGGCACCACTGAACC	TCCAGAGTCCAGCACAATACCAGT
Collage I	TGTGTGCGATGACGTGCAAT	GGGTCCCTCGACTCCTAC

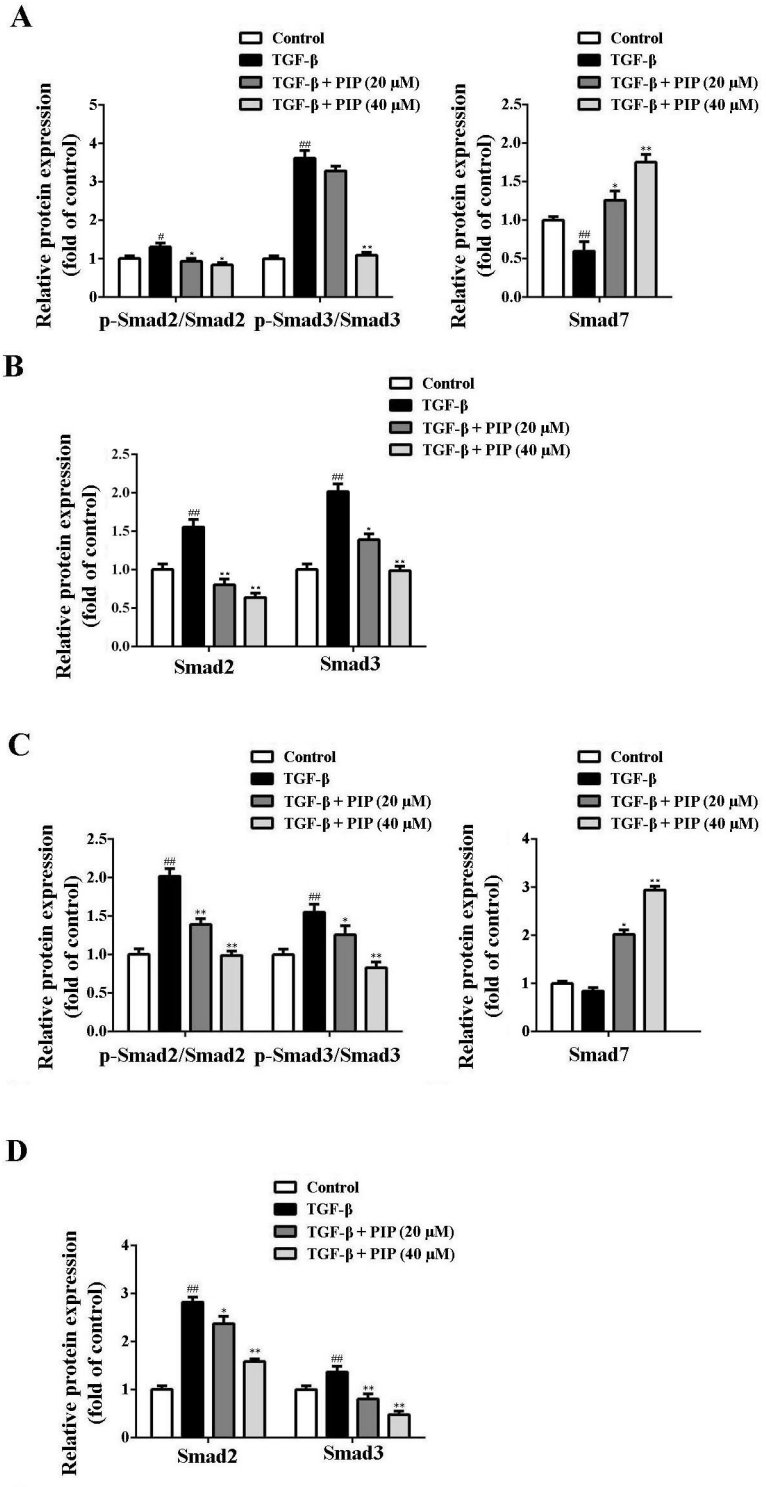
Supplementary figures

Supplementary Figure 1



Supplementary Figure 1 PIP inhibited LX-2 HSC migration and proliferation induced by TGF-β. (A and B) LX-2 HSCs were subjected to the indicated treatment. Then, cells were subjected to transwell assay (A) and EdU incorporation assay (B). ## $P < 0.01$, compared to cells treated with vehicle. * $P < 0.05$, ** $P < 0.01$, compared to cells treated with TGF-β alone.

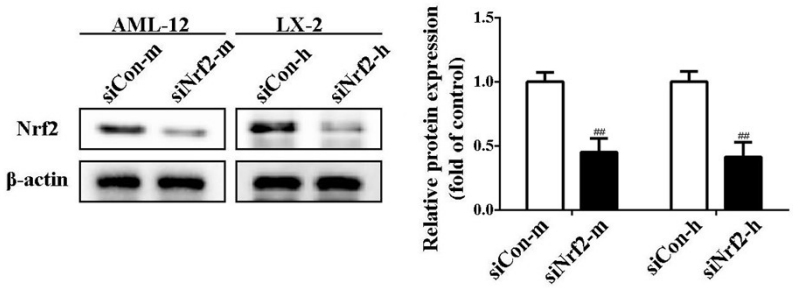
Supplementary Figure 2



Supplementary Figure 2 Effects of PIP on smad2, smad3, and smad7 in AML-

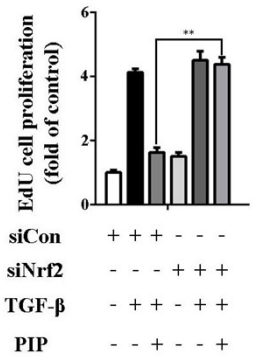
12 and LX-2 cells. (A and B) AML-12 hepatocytes were treated as indicated. Then, levels of p-smad2, p-smad3 in total cell lysates were determined (A, left panel). Levels of smad7 were in nucleus were also detected (A, right panel). (C and D) LX-2 HSCs were subjected to the indicated treatment and the same assays as described in A and B. ## $P < 0.01$, compared to cells treated with vehicle. * $P < 0.05$, ** $P < 0.01$, compared to cells treated with TGF-β alone.

Supplementary Figure 3



Supplementary Figure 3 Levels of Nrf2 were effectively knocked down by the siRNA. The indicated siRNA were transfected into AML-12 and LX-2 cells. Then, intracellular Nrf2 were detected by immunoblotting. ## $P < 0.01$, compared to cells transfected with control siRNAs.

Supplementary Figure 4



Supplementary Figure 4 Knockdown of Nrf2 abrogated the protective effects of PIP on TGF-β-induced proliferation of LX-2 cells. LX-2 cells were subjected to the indicated treatment. Then, cells were subjected to EdU incorporation assay. ** $P < 0.01$, compared to the indicated control.