

# Pharmacological Inhibition of Transient Receptor Potential Vanilloid 4 (TRPV4) Channel Alleviates Carbon Tetrachloride-Induced Liver Fibrosis in Mice

Jie Fu, Huihui Du, Xiao Zhang and Xundi Xu

Hunan Provincial Key Laboratory of Hepatobiliary Disease Research, 2001 of Jingwei Building,  
The Second Xiangya Hospital, Central South University, Hunan, China

**Background:** Transient receptor potential vanilloid 4 (TRPV4) is a member of the TRP channel family and is involved in diverse physiological and pathological processes. Accumulating evidence from in vitro studies indicates that TRPV4 has a potential role in liver fibrosis, but its precise role in the pathophysiological development of this condition is unclear. Exogenous interventions and endogenous reactions should be considered.

**Methods:** This study used a mouse model of carbon tetrachloride (CCl<sub>4</sub>)-induced liver fibrosis to investigate the effects of intraperitoneal injection of the novel TRPV4 channel selective agonist GSK1016790A (GSK) and antagonist HC-067047 (HC).

**Results:** As compared with the CCl<sub>4</sub> group, collagen fiber deposition and alpha-smooth muscle actin ( $\alpha$ -SMA) levels were markedly higher and hepatic lobule disorganization was worse in the CCl<sub>4</sub>+GSK group, while collagen fiber deposition was significantly lower and hepatic lobule disorganization was less severe in the CCl<sub>4</sub>+HC group.

**Conclusions:** The present findings suggest that activation of TRPV4 channels worsens liver fibrosis and that inhibition of TRPV4 channels may alleviate liver fibrosis in vivo.

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**Key words:** TRPV4, liver fibrosis, in vivo

## Introduction

Fibrosis is often caused by chronic liver injury due to infection, drugs, metabolic disorders, and immune disorders and is a leading cause of cirrhosis<sup>1,2</sup>. In China, an epidemic of hepatitis B infection has increased the prevalence of liver fibrosis<sup>3</sup>. The high risk of end-stage liver disease and hepatic carcinoma, and the fact that such outcomes are always costly and difficult to treat, makes identification of an effective treatment for liver fibrosis highly desirable<sup>4,5</sup>.

The TRPV4 cation channel, a member of the TRPV subfamily, was first described in 2000. It exhibits cation permeability and participates in multiple cellular processes<sup>6,7</sup>. Alteration of TRPV4 channel activity is believed to account for the development and progression of numerous diseases<sup>8–10</sup>, and recent evidence from in vitro

studies suggests that TRPV4 has a role in liver fibrosis<sup>11,12</sup>. However, in vivo studies are required in order to determine the influence of TRPV4.

Using a mouse model of CCl<sub>4</sub>-induced liver fibrosis, and a selective agonist and antagonist of TRPV4<sup>13,14</sup>, we investigated the relation between TRPV4 and liver fibrosis progression in histological and protein studies.

## Materials and Methods

### Animal model

Six-week-old male C57BL/6 mice were purchased from Hunan SJA Laboratory Animal Co. LTD (Changsha, China). All mice were housed in a specific pathogen-free facility and fed on a standard pellet diet and pure water at the animal experiment center of the Second Xiangya Hospital of Central South University. To simulate early

Correspondence to Xundi Xu, Hunan Provincial Key Laboratory of Hepatobiliary Disease Research, 2001 of Jingwei Building, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China

E-mail: xuxundi@csu.edu.cn

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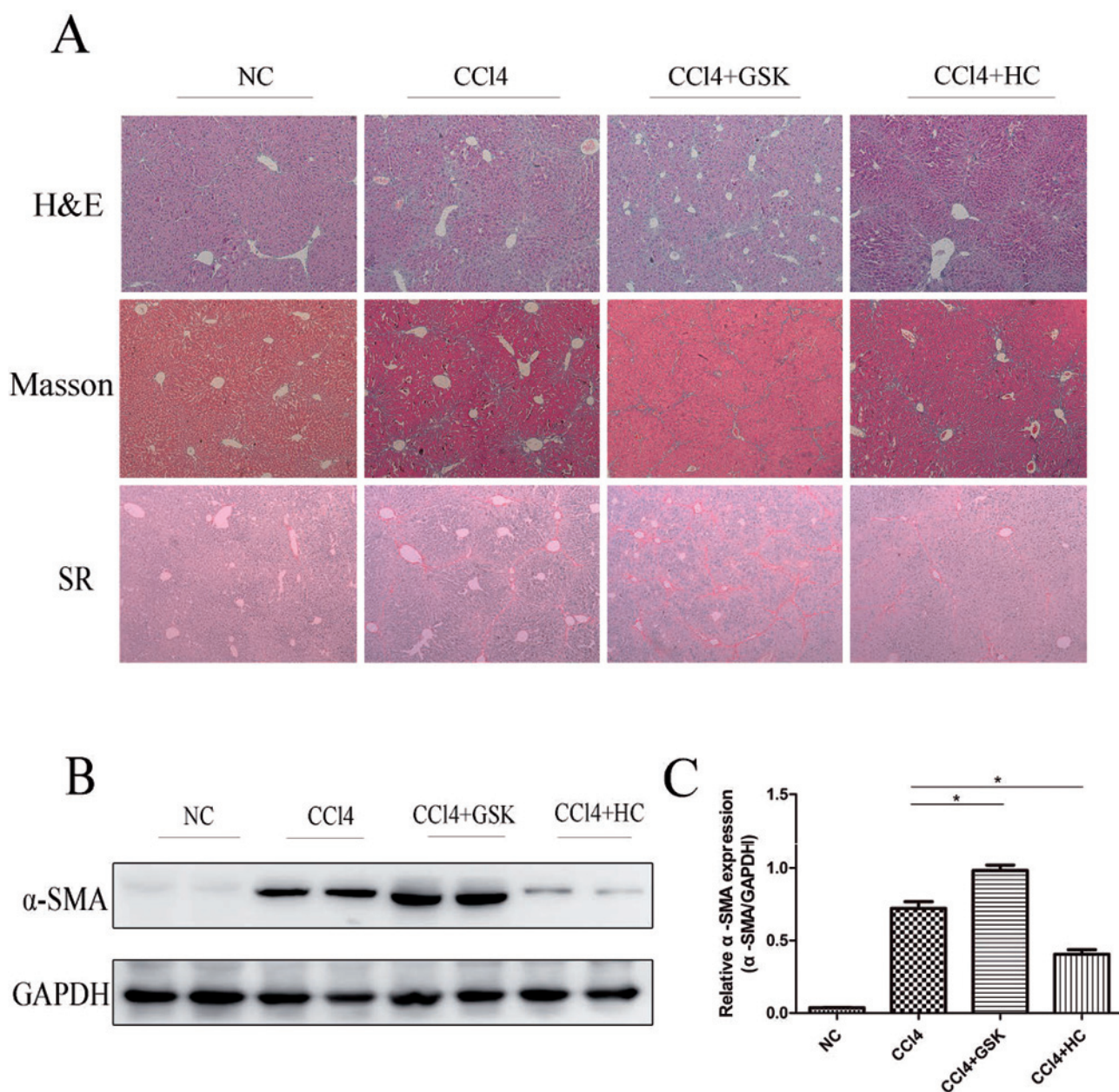


Fig. 1 (A). Histopathological analysis of experimental mice liver sections by HE staining, Masson's trichrome staining, and Sirius red staining in a mouse model of advanced (8-week) liver fibrosis (magnification  $\times 100$ ). (B and C) Western blot analysis of  $\alpha$ -SMA levels in liver tissues. GAPDH was used as an internal reference. \* $P < 0.05$  vs CCl4-treated group. SR, Sirius red.

and advanced hepatic fibrosis, CCl<sub>4</sub> was injected intraperitoneally for 5 and 8 weeks, respectively<sup>1,15</sup>. At both time points, the mice were randomly distributed into four groups: a negative control group (NC group), CCl<sub>4</sub>-induced liver fibrosis group (CCl<sub>4</sub> group), agonist group (CCl<sub>4</sub>+GSK group), and antagonist group (CCl<sub>4</sub>+HC group). Three mice were assigned to each group. The mouse in each group received intraperitoneal injections (5  $\mu$ L/g body weight of CCl<sub>4</sub>/olive oil [1:4 vol/vol]) twice a week, except for the NC group (which received an equal volume of olive oil). The agonist group received

intraperitoneal injections of GSK 10  $\mu$ g/kg. The antagonist group received intraperitoneal injections of HC 10 mg/kg for 2 days during the last 2 weeks of CCl<sub>4</sub> treatment. Drugs were dissolved in 0.5% dimethyl sulfoxide (DMSO) in a total volume of 50  $\mu$ L. Each mouse in the NC and CCl<sub>4</sub> groups received intraperitoneal injections of DMSO 50  $\mu$ L. The mice were killed 24 hours after the final CCl<sub>4</sub> injection, and liver tissues were harvested for further analysis. The experimental protocol was approved by the Central South University Animal Care and Use Committee.

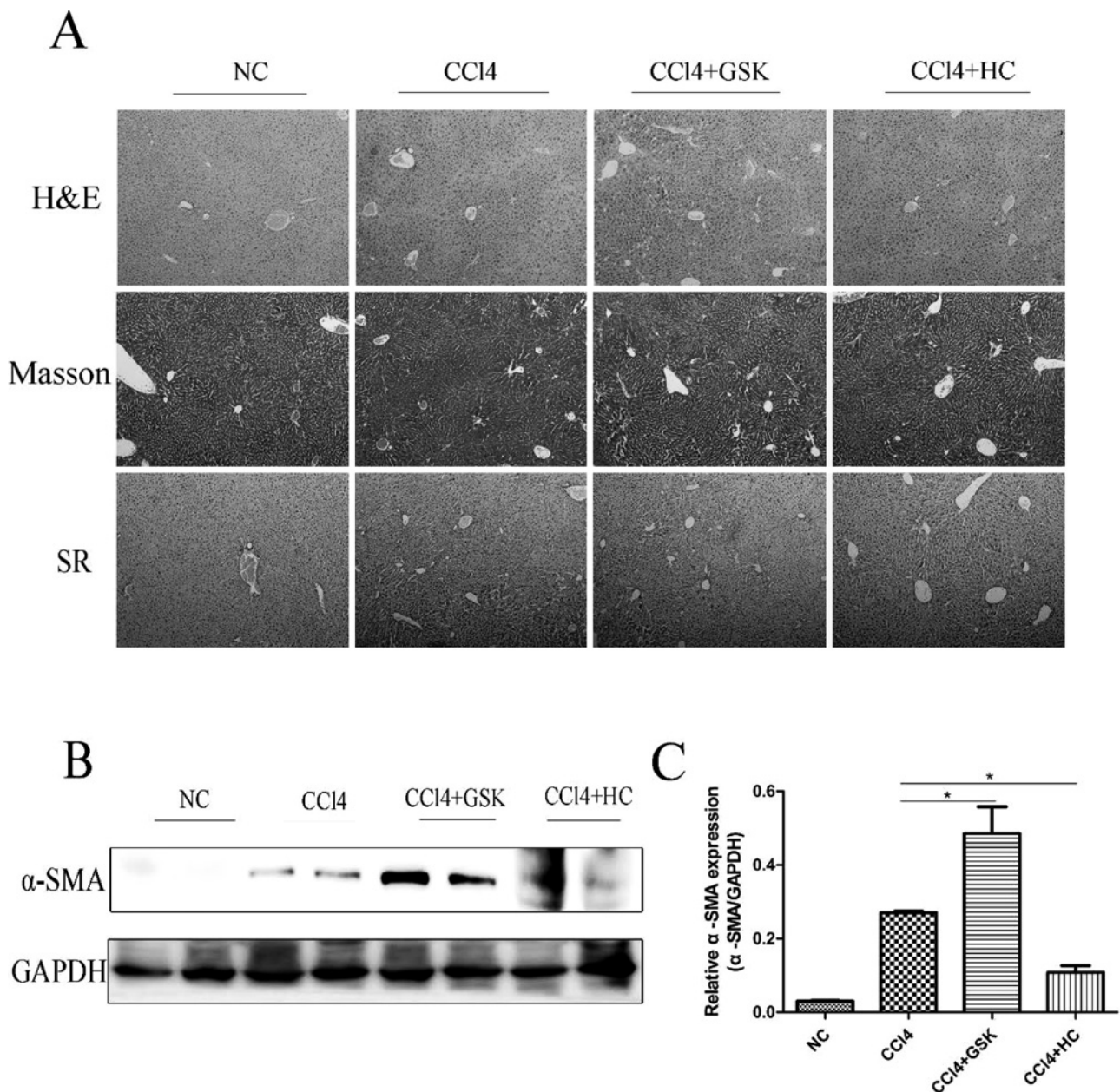


Fig. 2 (A). Histopathological analysis of experimental mice liver sections analyzed by HE staining, Masson’s trichrome staining, and Sirius red staining in a mouse model of early (5-week) liver fibrosis (magnification  $\times 100$ ). (B and C) Western blot analysis of  $\alpha$ -SMA levels in liver tissues. GAPDH was used as an internal reference. \* $P < 0.05$  vs CCl4-treated group. SR, Sirius red.

**Reagents**

GSK1016790A, HC-067047, DMSO, and olive oil were obtained from Sigma Aldrich (USA). Carbon tetrachloride was purchased from Sinopharm (Beijing, China).

**Chemical staining**

The Hematoxylin-Eosin/HE staining kit, Masson’s trichrome staining kit, and Picro Sirius red (SR) solution were obtained from Solarbio Science and Technology (Beijing, China). All staining experiments were conducted according to the manufacturer’s instructions.

**Western blotting**

Whole-liver cell protein lysates were prepared by homogenizing 25 mg of liver tissue in lysis buffer. The procedure for the Western blot assay was reported previously<sup>16</sup>. For Western blotting, the membrane was incubated in the primary antibody  $\alpha$ -SMA (1:400 dilution; Boster Biological Technology, China). GAPDH (1:20,000 dilution; Proteintech) was used as an internal reference.

**Statistical analysis**

All experiments were performed in triplicate, and data are expressed as mean  $\pm$  SD. Data analysis was per-

formed with the SPSS 19.0 software package. Statistical analyses were done by using the Student t-test, and a P value of <0.05 was regarded as statistically significant.

### Results

Pharmacological activation or inhibition of TRPV4 channel activity can affect progression of advanced (8 weeks) and early (5 weeks) liver fibrosis in CCl<sub>4</sub>-treated mice.

To assess the effect of TRPV4 channel activation and suppression on liver fibrosis, we developed a model of advanced liver fibrosis in C57BL/6 mice (8 weeks). Liver fibrosis severity was determined by HE staining, Masson's trichrome staining, and Sirius red staining. All mice treated with CCl<sub>4</sub> (n=3) had liver fibrosis. As compared with the CCl<sub>4</sub> group, deposition of collagen fiber and disorganization of liver lobules were more severe in TRPV4 activator-treated mice and less severe in TRPV4 inhibitor-treated mice (**Fig. 1A**).

We harvested liver tissue and performed Western blotting to analyze  $\alpha$ -SMA, the actin isoform commonly used as a marker of fibroblast formation.  $\alpha$ -SMA expression was upregulated in the activator-treated group and downregulated in the suppressor-treated group (**Fig. 1B, C**). This difference in  $\alpha$ -SMA expression, along with the above histological findings, suggests that pharmacological activation or inhibition of TRPV4 channels regulates progression of advanced liver fibrosis.

The results for the early (5-week) liver fibrosis model were similar to those for the advanced model (**Fig. 2**).

### Discussion

A number of chronic conditions, especially chronic liver disease, can lead to development and progression of liver fibrosis<sup>1,17</sup>. However, although studies of the pathogenesis and treatment of hepatic fibrosis have made immense progress, there is currently no effective treatment<sup>3-5</sup>. Interestingly, during liver injury, activated resident hepatic stellate cells differentiate into fibroblasts and secrete extracellular matrix components such as  $\alpha$ -SMA and collagen I or III. This finding has been a dominant theme in research<sup>1,2</sup>.

The TRPV ion channel family consists of several permeable nonselective members, which are distributed in multiple organs and tissues throughout the body. Activating factors include physical and chemical cues. The TRPV ion channel family is associated with multiple physiological and pathological processes, including calcium influx, intracellular environment stability, and maintenance of vascular function<sup>10,13</sup>. Abnormal expres-

sion of TRPV and alteration of its channel activity are associated with many diseases<sup>6</sup>. We previously reported that TRPV channels are important in the development of hepatocellular carcinoma<sup>8,9</sup>, and recent studies noted that TRPV4 expression was significantly increased in liver fibrotic tissues. Blocking the TRPV4 channels of hepatic stellate cells with selective antagonists significantly inhibits their proliferation<sup>11,12</sup>, perhaps because of autophagy-mediated apoptosis. However, the efficacy of drug-induced changes to the TRPV4 channel on liver fibrosis in vivo is unclear.

In the present study, we developed a mouse model of CCl<sub>4</sub>-induced liver fibrosis and treated the mice with a novel TRPV4 selective agonist, GSK1016790A, and antagonist, HC-067047. Our results suggest that activation of TRPV4 channels aggravates liver fibrosis and that inhibition of the TRPV4 channel alleviates liver fibrosis in vivo. Consistent with the results of previous in vitro experiments, the current findings indicate that TRPV4 is a latent target for treatment of liver fibrosis. Thus, the observed regulatory effects of TRPV4 on liver fibrosis suggest that our findings have broad implications for the study of TRPV4 as a therapeutic target for liver fibrosis in humans.

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**Conflict of Interest:** The authors have no conflicts of interest regarding the publication of this paper.

### References

1. Friedman SL: Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; 134: 1655-1669.
2. Kim KM, Han CY, Kim JY, Cho SS, Kim YS, Koo JH, Lee JM, Lim SC, Kang KW, Kim JS, Hwang SJ, Ki SH, Kim SG: Galpha12 overexpression induced by miR-16 dysregulation contributes to liver fibrosis by promoting autophagy in hepatic stellate cells. *J Hepatol* 2018; 68: 493-504.
3. Dong M, Wu J, Yu X, Li J, Yang S, Qi X, Mao R, Zhang Y, Yu J, Zhu H, Yang F, Qin Y, Zhang J: Validation and comparison of seventeen noninvasive models for evaluating liver fibrosis in Chinese hepatitis B patients. *Liver Int* 2018; 38: 1562-1570.
4. Kaffe E, Katsifa A, Xylourgidis N, Ninou I, Zannikou M, Harokopos V, Foka P, Dimitriadis A, Evangelou K, Moulas AN, Georgopoulou U, Gorgoulis VG, Dalekos GN, Aidinis V: Hepatocyte autotaxin expression promotes liver fibrosis and cancer. *Hepatology* 2017; 65: 1369-1383.
5. Higashi T, Friedman SL, Hoshida Y: Hepatic stellate cells as key target in liver fibrosis. *Adv Drug Deliv Rev* 2017; 121: 27-42.

6. White JP, Cibelli M, Urban L, Nilius B, McGeown JG, Nagy I: TRPV4: Molecular Conductor of a Diverse Orchestra. *Physiol Rev* 2016; 96: 911–973.
7. Szallasi YKaA: Transient receptor potential (TRP) channels: a clinical perspective. *British Journal of Pharmacology* 2014; 171: 2474–2507.
8. Fang Y, Liu G, Xie C, Qian K, Lei X, Liu Q, Liu G, Cao Z, Fu J, Du H, Liu S, Huang S, Hu J, Xu X: Pharmacological inhibition of TRPV4 channel suppresses malignant biological behavior of hepatocellular carcinoma via modulation of ERK signaling pathway. *Biomedicine & Pharmacotherapy* 2018; 101: 910–919.
9. Hu Z, Cao X, Fang Y, Liu G, Xie C, Qian K, Lei X, Cao Z, Du H, Cheng X, Xu X: Transient receptor potential vanilloid-type 2 targeting on stemness in liver cancer. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2018; 105: 697–706.
10. Seth RK, Das S, Dattaroy D, Chandrashekar V, Alhasson F, Michelotti G, Nagarkatti M, Nagarkatti P, Diehl AM, Bell PD, Liedtke W, Chatterjee S: TRPV4 activation of endothelial nitric oxide synthase resists nonalcoholic fatty liver disease by blocking CYP2E1-mediated redox toxicity. *Free Radic Biol Med* 2017; 102: 260–273.
11. Zhan L, Yang Y, Ma TT, Huang C, Meng XM, Zhang L, Li J: Transient receptor potential vanilloid 4 inhibits rat HSC-T6 apoptosis through induction of autophagy. *Mol Cell Biochem* 2015; 402: 9–22.
12. Song Y, Zhan L, Yu M, Huang C, Meng X, Ma T, Zhang L, Li J: TRPV4 channel inhibits TGF-beta1-induced proliferation of hepatic stellate cells. *PLoS One* 2014; 9: e101179.
13. Boehmerle W, Huehnchen P, Lee SLL, Harms C, Endres M: TRPV4 inhibition prevents paclitaxel-induced neurotoxicity in preclinical models. *Exp Neurol* 2018; 306: 64–75.
14. Jie P, Tian Y, Hong Z, Li L, Zhou L, Chen L, Chen L: Blockage of transient receptor potential vanilloid 4 inhibits brain edema in middle cerebral artery occlusion mice. *Front Cell Neurosci* 2015; 9: 141.
15. Mridha AR, Wree A, Robertson AAB, Yeh MM, Johnson CD, Van Rooyen DM, Haczeyni F, Teoh NC, Savard C, Ioannou GN, Masters SL, Schroder K, Cooper MA, Feldstein AE, Farrell GC: NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. *J Hepatol* 2017; 66: 1037–1046.
16. Liu G, Xie C, Fang Y, Qian K, Liu Q, Liu G, Cao Z, Du H, Fu J, Xu X: Splenectomy after partial hepatectomy accelerates liver regeneration in mice by promoting tight junction formation via polarity protein Par 3-aPKC. *Life Sci* 2018; 192: 91–98.
17. Hyun J, Wang S, Kim J, Rao KM, Park SY, Chung I, Ha CS, Kim SW, Yun YH, Jung Y: MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression. *Nat Commun* 2016; 7: 10993.

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