

MODULATION OF TJP1 EXPRESSION TO TREAT LIVER DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 10202110245U, filed 16 September 2021, and Singapore patent application No. 10202113389U, filed 01 December 2021, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention generally relates to molecular biology. In particular, the present invention relates to a method of treating liver diseases by targeting Tjp1.

BACKGROUND OF THE INVENTION

[0003] Liver diseases affect many patients worldwide every year. For example, liver cancer is the third most common cause of cancer death globally as well as in the Asia Pacific region. Liver diseases can cause different levels of damage to the liver. Some liver disease causes liver fibrosis where excessive connective tissue builds up in the liver. Others cause cirrhosis, which is a more severe form of liver fibrosis that results in the distortion of the liver architecture. These are often associated with a disruption of bile flow, which leads to liver diseases such as, but not limited to cholestasis. Currently, different therapies such as drug therapy, surgery or trans-arterial therapy are available for liver diseases. Despite advances in these diverse therapeutic options, successfully treating liver diseases remains an unmet clinical challenge. Therapeutic approaches allowing survival of the cells in the liver or replacement of liver would be of tremendous economic and social impact. In view of the above problems, there is a need to provide an alternative method for treating a liver disease in a subject.

SUMMARY

[0003a] In one aspect, the present disclosure relates to use of a Tjp1 inhibitor in the manufacture of a medicament for treating a liver disease in a subject, wherein the Tjp1 inhibitor is a nucleic acid.

[0003b] In another aspect, the present disclosure relates to use of a Tjp1 inhibitor in the manufacture of a medicament for regenerating a biliary system in a subject, wherein the Tjp1 inhibitor is a nucleic acid.

[0004] In one example, there is provided a method for treating a liver disease in a subject, wherein the method comprises administering of a pharmaceutically effective amount of a Tjp1 inhibitor to the subject.

[0005] In another example, there is provided a method of regenerating a biliary system in a subject, wherein the method comprises administering of a pharmaceutically effective amount of a Tjp1 inhibitor to the subject.

[0006] In another example, there is provided a nucleic acid encoding a Tjp1 inhibitor,
 5 wherein the nucleic acid comprises at least 60%, or at least 80% identity to a sequence selected from a group consisting of 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4),
 5'- ATTTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91),
 5'- CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5),
 5'-AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92),
 10 5'-CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6),
 5'-AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93),
 5'-GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104),
 5'-AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105),
 5'-AAGGAAATTCACAAGATAGT-3' (SEQ ID NO: 106),
 15 5'-TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107),
 5'-ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108),
 5'-AAGAACTAGATGAACTCTTA-3' (SEQ ID NO: 109),
 5'-CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110),
 5'-CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111),
 20 5'-AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), and
 5'-TAGATAATACACCACTACATT-3' (SEQ ID NO: 113).

[0007] In another example, there is provided a nucleic acid encoding a Tjp1 inhibitor, wherein the nucleic acid comprises at least 60% identity to a sequence selected from the group consisting of:

- 25 i) a combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides;
- 30 ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 5 with

the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; and

- iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides.

[0008] In another example, there is provided a nucleic acid encoding a Tjp inhibitor, wherein the nucleic acid comprises at least 60% identity to a sequence selected from the group consisting of: 5'-

CCGGCGTGGATTGAACTTACTAAATCTCGAGATTTAGTAAGTT

CAATCCACGTTTTTTG-3' (SEQ ID NO: 1), 5'-

CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG

TTTTTG-3' (SEQ ID NO: 2), and 5'-**CCGGCGGCCATTTGAACGCAAATTTCTCGAG**

AAATTTGCGTTCAAATGGCCGTTTTTG-3' (SEQ ID NO: 3).

[0009] In another example, there is provided a nucleic acid encoding a Tjp inhibitor, wherein the nucleic acid comprises at least 60% identity to a sequence selected from a group consisting of 5'-UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94),

5'-AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95),

5'-ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96),

5'-AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97),

5'-UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98),

5'-UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99),

5'-UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100),

5'-AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101),

5'-UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) and

5'-AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103).

[0010] In yet another example, there is provided a nucleic acid encoding a Tjp inhibitor, wherein the nucleic acid comprises a sequence selected from a group consisting of 5'-

UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'-

AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'-

ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'-

AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'-

UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'-
 UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'-
 UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'-
 AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'-
 5 UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) and 5'-
 AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103).

[0011] In another example, there is provided a kit comprising the Tjp1 inhibitor as defined herein and/or the nucleic acid as defined herein.

10

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0013] **Fig. 1** shows a series of experimental data comparing the effects in liver anatomy and function of Tjp1 conditional knockout (cKO) and control mice. **Fig. 1A** is a series of photos of immunofluorescence microscopy (IF) for Tjp1 (ZO-1) (left) and Tjp2 (ZO-2) (second from left), hematoxylin and eosin (H&E) and Sirius red staining. Tjp1 is absent from hepatocytes and cholangiocytes of the liver of Tjp1 cKO mice. Nuclei are labelled with DAPI (third from left) in the photos of immunofluorescence microscopy, and the merged images (right) represent superimposed images of the Tjp1 (ZO-1), Tjp2 (ZO-2) and DAPI images. H&E and Sirius red staining do not reveal apparent changes in liver histology or fibrosis, respectively. **Fig. 1B** shows 4 graphs representing the liver to body weight ratio, and levels of plasma bile acid (BA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in control and Tjp1 cKO mice. Liver to body weight ratio, and plasma bile acid (BA), ALT and AST levels are comparable in control and Tjp1 cKO mice. **Fig. 1C** shows photos of immunofluorescence staining for Tjp1 and Tjp2. **Fig. 1D** shows an image of a Western blot analysis of Claudin-1 (Cldn1), Claudin-2 (Cldn2), Claudin-3 (Cldn3), Occludin, Cingulin (Cgn), E-cadherin and Vinculin in liver cells from control and Tjp1 cKO mice. The respective graph showing the relative protein expression is also provided. **Fig. 1E** shows photos of immunofluorescence microscopy for Cldn1, Cldn2, Cldn3 and Cingulin (Cgn) and carcinoembryonic antigen-related cell adhesion molecule (Ceacam). Figs. 1C-1E show that conditional deletion of Tjp1 in mouse liver does not alter expression and localization of tight junction components. Western blot

analysis. **Fig. 1F** shows electron microscopy (EM) images of the liver tissue from control and Tjp1 cKO mice. The images showing typical electron dense tight junction plaque (black box) in the vicinity of the bile canaliculi and microvilli. Conditional deletion of Tjp1 has no apparent effect on tight junction morphology as assessed by EM. **Fig. 1G** is a graph showing the levels of 4kDa FITC dextran in control, Tjp cKO and LPS-treated mice. 4kDa FITC dextran was injected into the tail vein and its transfer to the bile assessed. Deletion of Tjp1 does not affect the bile-blood barrier. LPS-treated mice were used as positive control, wherein the bile-blood barrier was compromised by LPS injection, resulting in detectable FITC-dextran leakage. **Fig. 1** shows that conditional deletion of Tjp1 in mouse liver has no apparent effect on liver histology or function.

[0014] **Fig. 2** shows a series of experimental data showing the effects of thioacetamide (TAA) induced injury to the liver in the control and Tjp1 conditional knockout (cKO) mice. **Fig. 2A** shows images of the livers in TAA treated Tjp1 cKO and wild-type (WT) control mice. The gross anatomy of the liver in TAA treated Tjp1 cKO mice remains unchanged, whereas the liver of the WT mice exhibited some signs of damage as shown by the areas circled by white dotted lines. **Fig. 2B** is a line graph showing the survival of Tjp1 cKO and control mice after TAA injection. Tjp1 cKO mice subjected to prolonged TAA treatment do not show an increased mortality as observed in controls. **Fig. 2C** are graphs showing the levels of ALT and AST in Tjp1 cKO and control mice at 6, 24 and 48 hours after TAA treatment. Plasma ALT and AST levels decreased in TAA treated Tjp1 cKO mice as compared to controls, indicating that the hepatic deletion of Tjp1 attenuates liver injury. **Fig. 2D** is a compilation of images of H&E staining of control and Tjp1 cKO livers. The quantification of necrosis is provided in the accompanying graph. TAA-induced liver necrosis is significantly suppressed in Tjp1 cKO livers as compared to controls. The white arrow shows the necrotic area in the liver tissue. **Fig. 2E** shows images of H&E staining and graphs of plasma ALT and AST levels of Tjp1 cKO mice and control mice treated with TAA. Tjp1 deletion protects from chronic liver injury as assessed by biochemistry and H&E staining. Plasma ALT and AST levels, and liver histology were significantly less affected in Tjp1 cKO mice treated with TAA for 18 days as compared to corresponding controls. **Fig. 2F** shows images of Sirius red staining and a graph showing the quantification of the percentage of Sirius red positive area. Liver fibrosis after 18 days of TAA administration was reduced in Tjp1 cKO liver as compared to controls. **Fig. 2G** shows a series of column graphs that presents the expression levels of fibrosis markers monitored by

qRT-PCR in the liver of chronically TAA-treated Tjp1 cKO and control wild type (WT) mice. The fibrosis markers are α SMA, CK19, collagen 1a1, osteopontin, TIMP1, TGF β , CTGF, PDGFR β and PDGF β , and all the markers have lower expression levels in the liver of chronically TAA-treated Tjp1 cKO mice as compared to corresponding controls. **Fig. 2H** shows images of CK19 staining and a graph of the percentage CK19 positive area. Chronic TAA-induced CK19 staining is lower in Tjp1 cKO liver as compared to corresponding controls. **Fig. 2I** shows images of caspase 3 staining and a graph of the percentage caspase 3 positive area. A positive caspase 3 staining represents the presence of apoptosis. Chronic TAA exposure leads to fewer caspase-3 positive cells in the Tjp1 cKO liver as compared to corresponding controls, indicative to less cell death. Thus, **Fig. 2** illustrates that the inactivation of Tjp1 protects mice from TAA induced acute and chronic liver injury.

[0015] **Fig. 3** shows a series of experimental data illustrating the effects of 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC) diet induced injury to the liver in the control and Tjp1 conditional knockout (cKO) mice. **Fig. 3A** shows images of the livers in DDC fed Tjp1 cKO and wild-type (WT) control mice. The Tjp1 cKO liver shows normal size and coloration as compared to the enlarged control liver from mice fed with DDC diet for 7 days. **Fig. 3B** shows line graphs quantifying the weight of the liver and spleen against body weight of Tjp1 cKO and control mice with and without DDC diet induced injury. The DDC diet induced increases in liver and spleen to body weight ratio in control mice. This increase in liver and spleen to body weight ratio is suppressed or abrogated in Tjp1 cKO animals. **Fig. 3C** is a compilation of images of H&E staining of control and Tjp1 cKO livers. After being fed DDC diet for 28 days, massive ductular reaction was observed in the liver of control mice, but not in Tjp1 cKO mice. **Fig. 3D** are graphs showing the levels of BA, plasma alkaline phosphatase (AP), ALT, AST and bilirubin in Tjp1 cKO and control mice with and without DDC diet. The levels of BA, AP, ALT, AST and bilirubin remains unchanged in Tjp1 cKO mice with DDC diet, showing that inactivation of Tjp1 protects the liver from DDC diet induced liver injury. **Fig. 3E** shows images of Sirius red staining and a graph showing the quantification of the percentage of Sirius red positive area. DDC diet induced liver fibrosis as assessed by Sirius red staining was reduced in Tjp1 cKO liver as compared to controls. **Fig. 3F** shows images of CK19 staining and a graph of the percentage CK19 positive area. DDC diet induced ductular reaction, monitored by staining for the cholangiocyte marker CK19, is suppressed in Tjp1 cKO liver. **Fig. 3G** shows a series of column graphs that presents the expression levels of fibrosis

markers monitored by qRT-PCR in the liver of Tjp1 cKO and control wild type (WT) mice with and without DDC diet. The fibrosis markers are α SMA, CK19, epithelial cellular adhesion molecule (EpCam), collagen 1, TIMP1, TGF β 1, CTGF, PDGFR β and PDGF β , and all the markers have lower expression levels in the liver of DDC fed Tjp1 cKO mice as compared to the corresponding control. **Fig. 3H** shows an image of a Western blot analysis of α SMA, Smad2, phosphorylated SMAD2 (pSMAD2) in liver cells from control and Tjp1 cKO mice fed with standard chow, DDC diet for 7 and 28 days. GAPDH serves as a reference control. **Fig. 3I** shows photos of immunofluorescence staining for α SMA (left), DAPI (center) and merged (right) of liver samples obtained from DDC fed Tjp1 cKO and control mice. **Fig. 3J** shows immunohistochemistry images stained for laminin 1-2 and collagen 1 of liver samples obtained from DDC fed Tjp1 cKO and control mice. **Fig. 3K** shows a series of column graphs that presents the expression levels of macrophage markers CD11b and F4/80, and key inflammatory cytokines TNF α , interleukin 6 (IL-6) and osteopontin monitored by qRT-PCR in the liver of Tjp1 cKO and control wild type (WT) mice with and without DDC diet. The expression levels of the macrophage markers and inflammatory cytokines are lower in the liver of DDC fed Tjp1 cKO mice in comparison to the corresponding control. **Fig. 3** illustrates that the inactivation of Tjp1 protects mice from DDC diet induced liver injury.

Fig. 4 shows a series of experimental data showing the effects of bile duct ligation (BDL) induced liver injury in control and Tjp1 conditional knockout (cKO) mice. **Fig. 4A** shows a column graph quantifying the mRNA expression levels of Tjp1 and Tjp2 in the liver of sham or BDL treated groups of control and Tjp1 cKO mice. **Fig. 4B** shows immunohistochemistry images of liver of sham or BDL treated groups of control and Tjp1 cKO mice. Graphs showing the percentage necrosis area, liver-to-body weight ratio, and plasma levels of ALT, AST are also presented. The BDL induced liver injury, as assessed by liver necrosis, liver-to-body weight ratio and plasma ALT and AST, are reduced in Tjp1 cKO animals. **Fig. 4C** shows images of Sirius red staining and a graph showing the quantification of the percentage of Sirius red positive area. BDL induced liver fibrosis as assessed by Sirius red staining was reduced in Tjp1 cKO liver as compared to controls. **Fig. 4D** shows a column graph of mRNA expression levels of inflammatory markers (collagen 1A, EPCAM, α SMA, MMP9, Timp-1, CTGF and TGF β) in the liver of Tjp1 cKO and control wild type (WT) mice with and without BDL. The protein expression of TIMP is also shown in the Western blot, wherein the relative expression is presented in the column graph. The expression levels of the inflammatory markers are lower

in the liver of BDL Tjp1 cKO mice in comparison to the corresponding control. **Fig. 4E** shows images of CK19 staining, a graph of the percentage CK19 positive area, and another graph quantifying the CK19 mRNA expression. BDL-induced ductular reaction, monitored by staining for the cholangiocyte marker CK19, is suppressed in Tjp1 cKO liver. **Fig. 4F** presents a series of staining images and quantification of F4/80 and CD11b positive macrophages and neutrophils to illustrate reduced liver immune cell infiltration in Tjp1 cKO mice after BDL. The other series of column graphs and images of Western blot to illustrate reduced expression levels of inflammatory cytokines (CCL21, C-reactive, endoglin, ICAM-1, IGFBP-1, MMP9, Myeloperoxidase, osteopontin, TNF α , IL-6, and IL-11b) in Tjp1 cKO mice after BDL. **Fig. 4G** shows graphs that present the expression levels of liver and plasma bile acids (BA), plasma AP and bilirubin, which shows reduced cholestasis and improved liver function in Tjp1 cKO mice after BDL. **Fig. 4H** shows graphs of total bile and bile acids in control and Tjp1 cKO mice after BDL. Lower bile acid concentration in the bile of Tjp1 cKO mice after BDL may contribute the reduced liver injury. **Fig. 4I** shows a series of column graphs that presents the expression levels of genes involved in bile acid synthesis (Cyp7a1, Cyp7b1, Cyp8b1, Cyp27a1, FXR and SHP-1) and bile acid transport (NTCP, Oatp1, Oatp2, ABCB11, ABCB4, ABCC2, ABCC3 and ABCC4). **Fig. 4J** shows a column graph of mRNA expression levels of Cyp3a11, Sult2a1, Ugt1a1, CAR and PXR in the liver of Tjp1 cKO and control wild type (WT) mice with and without BDL. **Fig. 4K** shows images of Ki67 and cleaved caspase 3 staining, as well as graphs of the number of Ki67 and cleaved caspase 3 positive cells. Increased hepatocyte proliferation (shown by Ki67 staining and quantification) and reduced apoptosis (shown by Caspase-3 staining and quantification) in the Tjp1 cKO mouse liver after BDL may contribute to reduced liver injury. **Fig. 4** illustrates that the inactivation of Tjp1 protects mice from bile duct ligation (BDL) induced liver injury.

[0016] **Fig. 5** shows a series of experimental data showing the effects of the inactivation of Tjp1 in the liver of Yap cKO mouse conditional knockout (cKO) mice. **Fig. 5A** shows immunohistochemistry images of liver of control and Tjp1 deletion in Yap cKO mice. Graphs showing the percentage necrosis area, liver and spleen to body weight ratio, and plasma bile acid and bilirubin are also presented. Liver specific Tjp1 deletion in the Yap cKO background improves the liver phenotype of the Yap cKO mouse. **Fig. 5B** shows images of Sirius red staining and a graph showing the quantification of the percentage of Sirius red positive area. Plasma ALT and AST expression levels are also assessed. Reduced plasma ALT and AST in

Tjp1 inactivated livers shows improved healing to liver injury and reduced Sirius red levels show suppressed liver fibrosis. **Fig. 5C** shows column graphs of mRNA expression levels of fibrosis marker (α SMA, CK19, collagen 1, TIMP1, TGF β , CTGF, EpCam, PDGFR β and PDGF β) in the liver of Yap cKO and control wild type (WT) mice with and without Tjp1 inactivation. **Fig. 5D** is an image of a Western blot showing protein expression levels of fibrosis marker (α SMA, Laminin 1-2 and osteopontin) in the liver of Yap cKO and control wild type (WT) mice with and without Tjp1 inactivation. GAPDH serves as a reference control. **Fig. 5E** shows images of immunohistochemistry staining of fibrosis marker (α SMA, collagen 1, and Laminin1-2) in the liver of Yap cKO and control wild type (WT) mice with and without Tjp1 inactivation. **Fig. 5F** is a series of column graphs to illustrate reduced expression levels of inflammatory cytokines (F4/80, CD11b, osteopontin, TNF α , IL-6, and IL-1b) in the liver of Yap cKO and control wild type (WT) mice with and without Tjp1 inactivation. **Fig. 5G** shows images of bile duct marker CK19 immunohistochemistry staining in the liver of Yap cKO and control wild type (WT) mice with and without Tjp1 inactivation. The biliary tree, which is absent from the Yap cKO liver, is restored in the Tjp1 Yap cKO liver. **Fig. 5H** shows graphs that present the liver and spleen to body weight ratio, and the levels of serum bile acids (BA), plasma AP ALT, AST and bilirubin. Prolonged Tjp1 inactivation further normalizes liver and spleen to body weight ratio, and further improves liver function as assessed by serum bile acid, levels. **Fig. 5** illustrates that the inactivation of Tjp1 ameliorates the liver phenotype of the Yap cKO mouse.

[0017] **Fig. 6A** shows 3 exemplary short hairpin molecule sequences. The sequences targeting Tjp1 in the short hairpin molecule are in bold, and the remaining sequences are required for the short hairpin formation in the transcribed shRNA. These short hairpin molecules were selected based on the ability of the transcribed shRNAs to silence Tjp1 expression in tissue culture cells. The short hairpin molecules were incorporated into an AAV8 vector downstream of the hepatocyte specific thyroxine binding globulin (TBG) promotor to form different AAV8 TBG-shTjp1 vectors encoding for the Tjp1 shRNAs for selective expression in liver hepatocytes. **Fig. 6B** shows photos of immunofluorescence staining for Tjp1 in liver samples of mice injected with the different AAV8 TBG-shTjp1 vectors encoding for the Tjp1 shRNAs. A DNA encoding scrambled (AAV-Scr) was used as negative control. AAV vectors comprising DNA encoding Tjp1 shRNA#21 (AAV-Tjp1#21), Tjp1 shRNA#22 (AAV-Tjp1#22) and Tjp1 shRNA#13 (AAV-Tjp1#13) showed

reduced expression of Tjp1. **Fig. 6C** shows images of Sirius red staining of liver samples, and graphs of plasma AST and ALT levels. Tamoxifen inducible deletion of Tjp1 in Tjp1^{flf} Alb-Cre^{ERT2} mice (e.g. Tjp1 icKO^{HC}) and injection of AAV-Tjp1#21 can ameliorate liver fibrosis as shown by Sirius red staining. Plasma AST and ALT levels are also reduced in both groups, therefore showing that liver injury can be suppressed. Both the tamoxifen induced deletion and the AAV-Tjp1#21 mediated silencing of Tjp1 specifically occur in hepatocytes. **Fig. 6** illustrates that Tjp1 inactivation or silencing improves liver injury in the Mdr2 mouse model.

[0018] **Fig. 7** shows images of livers and Sirius red staining of liver samples.

Carcinogenesis was assessed based on the liver of mice at 12 months of age (P360). Tumors can be seen on the liver from ABCB4^{-/-} mouse, but none were seen on the liver from ABCB4^{-/-}Tjp1 cKO mouse. Liver fibrosis was assessed by Sirius red staining in liver tissues of mice at 6 months of age (P180), whereby reduced Sirius red staining was observed in the ABCB4^{-/-}Tjp1 cKO mouse liver, showing that Tjp1 inactivation suppressed liver fibrosis. **Fig. 7** illustrates that inactivation of Tjp1 suppresses liver carcinogenesis in the Mdr2 (Abcb4) KO mouse model.

[0019] **Fig. 8A** shows photos of immunofluorescence staining of AAV8 (2x10⁷) expressing Tjp1 short hairpin molecules in the liver. Expression of Tjp1 in the liver of mice injected with AAV8 carrying either scrambled (Scr) control short hairpin molecule (AAV8 Scr) (top row) or Tjp1 short hairpin molecules (AAV8 Tjp1) (bottom row) is monitored using immunofluorescence microscopy. Tjp2 expression is not affected by the short hairpin molecule. The graph shows the relative Tjp1 mRNA expression obtained from and quantitative reverse transcription polymerase chain reaction (qRT-PCR) of the livers from the different groups. Data for DNA encoding shRNA #21 (SEQ ID NO: 2) was shown and similar results were obtained for the other short hairpin molecule. **Fig. 8B** shows graphs of blood biochemistry levels for control or Yap cKO mice injected with AAV8 Scr or AAV8 shTjp1 mice. The graphs showed a reduction of levels in plasma bile acids (BA) plasma, alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin in the AAV8 shTjp1 mice when compared to the AAV8 Scr mice, indicating improvements for the different parameters in AAV8 shTjp1 injected mice. **Fig. 8C** are photos of sirius red and Ck19 stained tissues to monitor fibrosis and the biliary system, respectively. AAV8 short hairpin molecule suppresses fibrosis and induces the reformation of the biliary system in Yap cKO mice. The

graphs illustrate the levels of sirius red and Ck19 expressed. **Fig. 8D** are photos of sirius red staining and graphs showing the levels of AST and ALT plasma levels in Mdr2/Abcb4 KO mice injected with AAV8 Scr or AAV8 shTjp1 vectors. **Fig. 8** illustrates the effect of AAV8 mediated delivery of Tjp1 short hairpin molecule expressed from a liver specific promoter, and shows that AAV8 expressing Tjp1 short hairpin molecules from a liver specific promoter are liver protective in the Yap cKO and the Mdr2/Abcb4 KO mouse models.

DETAILED DESCRIPTION

[0020] The liver is a metabolic hub responsible for physiologic functions including amino acid, carbohydrate, and lipid metabolism; detoxification; and bile secretion. Enterohepatic circulation of metabolites is required for digestion and metabolic homeostasis. Bile acid (BA) synthesis occurs in hepatocytes, and drainage from the liver is performed by cholangiocyte-lined bile ducts. Hepatocytes and cholangiocytes rely on tight junctions (TJs) to establish the blood-bile barrier (BBB) that segregates bile from the blood circulation. TJs are also thought to reinforce cell polarity by maintaining the segregation of distinct proteins to the apical and basolateral membranes. The polarized distribution of specific transporters contributes to the BBB because it is critical for the directional collection of BAs from the blood and their release into the bile by hepatocytes, or the concentration of bile in the bile ducts.

[0021] The dysregulation of any one of the liver functions described above can lead to many different liver diseases. Liver diseases are major causes of illness and mortality worldwide. Liver diseases can ultimately lead to liver failure, which is a life-threatening condition. Despite the availability of different drugs and treatments to treat liver disease, the outcomes remain dismal. For example, ursodeoxycholic acid (UDCA) has been the accepted therapy for a liver disease known as primary biliary cholangitis (PBC) since 2016. However, the efficacy remains debatable as it does not provide any improvements to survival or liver histology. Presently, effective medical therapy for patients suffering from liver diseases and injury remains to be unmet. In view of the above, there is a need to provide an alternative method for treating a liver disease in a subject and to regenerate a biliary system in a subject.

[0022] The inventors of the present disclosure have found an alternative method for treating a liver disease in a subject. Thus, in one aspect, the present invention provides a method for treating a liver disease in a subject comprises administering of (a pharmaceutically effective amount of) a Tjp1 inhibitor to the subject. The administration of a Tjp1 inhibitor inhibits Tjp1

expression. In some examples, the subject has had a liver disease or suffered from a liver injury. Without being bound by theory, the Tjp1 inhibition reduces recruitment of immune cells such as macrophage and neutrophil, and also reduces levels of inflammatory cytokines and chemokines (Figs 2-4). This decreases the inflammatory reaction that takes place after a liver disease or injury and allows the process of regeneration of biliary system to take place in the liver (Fig. 5).

[0023] As used herein the terms Tjp1 (Tight Junction Protein 1) and ZO-1 (Zonula Occludens-1) are used interchangeably. The terms "Tjp1" and "ZO-1" refers to an actin-binding scaffold protein that is associated with tight junctions, which are critical for the biliary-blood barrier and in human patients. Exemplary nucleic acid sequences of Tjp1 include, but are not limited to SEQ ID NO: 7, 8, 10, 11, 114 or 115. Exemplary amino acid sequences of Tjp1 include, but are not limited to SEQ ID NOs: 9 or 12.

[0024] In one example, the Tjp1 inhibitor described herein inhibits Tjp1 expression. The term "inhibit" is used herein generally to mean a decrease of the amount as compared to an untreated subject or a control. However, for avoidance of doubt, "inhibit" means a decrease of Tjp1 levels sufficient to cause any improvement in a subject suffering from liver disease. In one example, inhibition means a decrease by at least 10% as compared to an untreated subject, or at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease of Tjp1 expression as compared to an untreated subject, or any decrease between 10-100% of Tjp1 expression as compared to an untreated subject. As used herein, the term "control" refers to a population that is statistically similar to the set being tested, on which no changes are implemented. A non-limiting example of control as used herein is wild type mice (i.e. mice without Tjp1 knock-out).

[0025] It would be appreciated that any methods known in the art to be capable of reducing or inactivating Tjp1 expression or activity would be suitable for use in the methods as described herein. A person skilled in the art will appreciate that the list of the types of Tjp1 inhibitors included herein is not exhaustive. The examples of the types of Tjp1 inhibitors include, but are not limited to, a small molecule, an antibody, a polypeptide, a nucleic acid such as, but not limited to DNA, complementary DNA, siRNA or shRNA, and any other biological or chemical entity capable of inhibiting Tjp1 expression, function or activity. In one example, Tjp1 inhibitor includes, but is not limited to, a nucleic acid, a small molecule, an antibody, a polypeptide, and

the like. A person skilled in the art will also appreciate that in some examples, the antibody that can be used as Tjp1 inhibitor is a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof. In some examples, the antibody include, but is not limited to, a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fv fragment, a diabody, or a single chain antibody molecule. In some examples, the antibody is a human antibody.

[0026] As would be appreciated by the person skilled in the art, nucleic acid sequences such as short hairpin molecules, vectors, DNA inserts, shRNAs can be designed to target Tjp1. In one example, the nucleic acid that is used as Tjp1 inhibitor includes, but is not limited to, a short hairpin molecule, an shRNA, an siRNA, an antisense oligonucleotide (AON), a gapmer, a short hairpin Antisense Oligonucleotide (shAON), and the like. The term “short hairpin molecule” as used herein refers to an artificial nucleic acid sequence that has a hairpin-like structure with 5'- and 3' tails. The short hairpin molecule can be a DNA sequence, an RNA sequence, or combinations thereof. The short hairpin molecule usually comprises a sequence that is identical or complementary to a sequence or part of a sequence of a target gene. In one example, the short hairpin molecule can be a DNA sequence that is inserted into a vector, such as plasmid vector or a viral vector, from which a short hairpin RNA is produced in the cell. A person skilled in the art would appreciate that the short hairpin molecule will be transcribed and processed by the cellular machinery in the cell into a molecule that can bind to a region in the mRNA of the target (for example, an mRNA that may be translated as Tjp1 protein). This will lead to the degradation of the target mRNA and prevent production of the Tjp1 protein, thereby silencing target gene expression via RNA interference. In another example, the nucleic acid is further transcribed into a short hairpin RNA (shRNA) and processed so that it binds to an mRNA encoding Tjp1 or a homolog thereof. In another example, when a nucleic acid is used as a Tjp1 inhibitor, the nucleic acid may be transcribed into a shRNA that is processed and binds to or interacts with mRNA which encodes Tjp1 and forms a shRNA-mRNA complex with the mRNA encoding Tjp1. In one example, when the nucleic acid that is used as Tjp1 inhibitor is transcribed into a shRNA and processed to bind to or interact with the mRNA that encodes the Tjp1 and forms a nucleic acid-mRNA complex, the mRNA in the shRNA-mRNA complex is cleaved and/or is not translated.

[0027] The term “homolog” as used herein refers to the existence of shared ancestry between genes (or structures) in different taxa. In other words, homology is the relationship between biological structures or sequences that are derived from a common ancestor and that ultimately have the same or similar functions (that is a biological equivalent). In terms of sequence homology, DNA or protein sequences are defined in terms of shared ancestry. The term “sequence homology” is often used in place of the term “sequence similarity”, or *vice versa*. In one example, such sequence similarity can include the ability of the sequence to bind and modulate the function of the target, for example but not limited to binding and modulating the function of a Tjp1 mRNA using a short hairpin molecule, shRNA, siRNA, antisense oligonucleotide (AON), gapmer, or short hairpin antisense oligonucleotide (shAON). Thus, “homolog” refers to a sequence that is highly conserved (a conservative sequence) if it does not change or only shows minimal changes between the species.

[0028] The Tjp1 inhibitor can be a nucleic acid sequence that is identical or complementary to a Tjp1 coding sequence, either in its entirety or in part. The Tjp1 inhibitor can also be a nucleic acid sequence that is capable of inhibiting Tjp1. In one example, the Tjp1 inhibitor is a nucleic acid that has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to a sequence that is capable of inhibiting Tjp1. In one example, the Tjp inhibitor is a nucleic acid sequence comprising part of a Tjp1 sequence that is, but not limited to 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-

ATTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-AAGGAAATTTTACAAAGATAGT-3' (SEQ ID NO: 106), 5'-TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-AAGAACTAGATGAAACTCTTA-3' (SEQ ID NO: 109), 5'-CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-

AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), or 5'-TAGATAATACACCACTACATT-3' (SEQ ID NO: 113). In another example, the Tjp1 inhibitor is a nucleic acid sequence comprising at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to a sequence that is, but

5 not limited to 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-ATTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-

10 AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-AAGGAAATTTCACAAGATAGT-3' (SEQ ID NO: 106), 5'-TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-

15 ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-AAGAACTAGATGAACTCTTA-3' (SEQ ID NO: 109), 5'-CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), or 5'-

20 TAGATAATACACCACTACATT-3' (SEQ ID NO: 113).

[0029] In another example, the Tjp1 inhibitor is a nucleic acid not limited to i) a combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91,

25 and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide

30 sequence comprising 1 to 10 nucleotides; or iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of

SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides. In another example, the Tjp1 inhibitor is a nucleic acid comprising at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to i) a combination of

5 SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5

10 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; or iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 1

15 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides. In another example, SEQ ID NOs 4, 5 or 6 is flanked at the 5' end by a nucleotide sequence comprising 3 to 8 nucleotides, 4 to 6 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides; a nucleotide sequence of 4 to 15

20 nucleotides, 8 to 13 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides connect the 3' end of SEQ ID NO: 4, 5 or 6 with the 5' end of SEQ ID NO: 91, 92 or 93 respectively; and SEQ ID NOs 91, 92 or 93 is flanked at the 3' end by a nucleotide sequence comprising 3 to 8 nucleotides, 4 to 6 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides. In yet another example, the Tjp1 inhibitor is a nucleic acid not limited to i) a

25 combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 4 nucleotides, wherein a nucleotide sequence of 6 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence comprising 6 nucleotides; ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5

30 is flanked at the 5' end by a nucleotide sequence comprising 4 nucleotides, wherein a nucleotide sequence of 6 nucleotides connects the 3' end of SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising

6 nucleotides; or iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 4 nucleotides, wherein a nucleotide sequence of 6 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 6 nucleotides.

[0030] In yet another example, the Tjp1 inhibitor is a nucleic acid, but not limited to 5'-CCGGCGTGGATTGAACTTACTAAATCTCGAGATTAGTAAGTTCAATCCAC

GTTTTTG-3' (SEQ ID NO: 1), 5'-

CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG

TTTTTG-3' (SEQ ID NO: 2), or 5'-

CCGGCGGCCATTTGAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCG

TTTTTG-3' (SEQ ID NO: 3). It can be seen that the residues in bold in SEQ ID NOs: 1-3 are

identical to the sequences in SEQ ID NOs: 4-6 and 91-93. SEQ ID NOs: 4-6 correspond to the

Tjp1 coding sequence, and SEQ ID NOs: 91-93 correspond to the complementary sequences,

wherein the complementary sequences are responsible for binding to the Tjp1 mRNA, thereby

inhibiting Tjp1. The remaining sequences in SEQ ID NOs: 1-3 are required to create the short

hairpin formation in the short hairpin molecule and can be any sequence that is capable of

forming the short hairpin molecule. In yet another example, the Tjp1 inhibitor is a nucleic acid

comprising at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at

least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity

to a sequence that is, but not limited to 5'-

CCGGCGTGGATTGAACTTACTAAATCTCGAGATTAGTAAGTTCAATCCACGT

TTTTTG-3' (SEQ ID NO: 1), 5'-

CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG

TTTTTG-3' (SEQ ID NO: 2), or 5'

CCGGCGGCCATTTGAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCG

TTTTTG-3' (SEQ ID NO: 3). In yet another example, the Tjp1 inhibitor is a short hairpin

molecule comprising the sequence that is, but not limited to 5'-

CCGGCGTGGATTGAACTTACTAAATCTCGAGATTAGTAAGTTCAATCCACGT

TTTTTG-3' (SEQ ID NO: 1), 5'-

CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG

TTTTTG-3' (SEQ ID NO: 2), or 5'-

CCGGCGGCCATTTGAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCG

TTTTTG-3' (SEQ ID NO: 3). In yet another example, the Tjp1 inhibitor is a short hairpin molecule of at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a

sequence that is, but not limited to 5'-

CCGGCGTGGAATTGAACTTACTAAATCTCGAGATTAGTAAGTTCAATCCACGT
TTTTTG-3' (SEQ ID NO: 1), 5'-

CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG
TTTTTG-3' (SEQ ID NO: 2), or 5'-

CCGGCGGCCATTTGAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCG
TTTTTG-3' (SEQ ID NO: 3).

[0031] In yet another example, the Tjp1 inhibitor is an siRNA comprising a sequence of, but not limited to 5'-UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'-

AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'-

ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'-

AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'-

UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'-

UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'-

UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'-

AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'-

UAUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 102) or 5'-

AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103). In yet another example, the Tjp1

inhibitor is a nucleic acid comprising at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at

least 99%, or 100% identity to a sequence that is, but not limited to 5'-

UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'-

AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'-

ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'-

AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'-

UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'-

UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'-

UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'-

AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'-
 UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) or 5'-
 AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103).

[0032] The present invention also provides a nucleic acid encoding a Tjp1 inhibitor,
 5 wherein the nucleic acid has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%,
 at least 85%, at least 90%, or at least 95% identity to a sequence that is capable of inhibiting
 Tjp1. In another example, the nucleic acid comprises part of a Tjp1 sequence that is, but not
 limited to 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-
 ATTTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-
 10 CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-
 AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-
 CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-
 AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-
 GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-
 15 AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-
 AAGGAAATTTACACAAGATAGT-3' (SEQ ID NO: 106), 5'-
 TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-
 ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-
 AAGAACTAGATGAACTCTTA-3' (SEQ ID NO: 109), 5'-
 20 CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-
 CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-
 AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), or 5'-
 TAGATAATACACCACTACATT-3' (SEQ ID NO: 113). In another example, the nucleic acid
 has at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least
 25 90%, or at least 95% identity to a sequence that is, but not limited to 5'-
 CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-
 ATTTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-
 CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-
 AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-
 30 CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-
 AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-
 GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-

AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-
 AAGGAAATTTACACAAGATAGT-3' (SEQ ID NO: 106), 5'-
 TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-
 ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-
 5 AAGAACTAGATGAAACTCTTA-3' (SEQ ID NO: 109), 5'-
 CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-
 CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-
 AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), or 5'-
 TAGATAATACACCACTACATT-3' (SEQ ID NO: 113).

- 10 [0033] In another example, the nucleic acid encoding a Tjp1 inhibitor is, but not limited to
- i) a combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence
- 15 comprising 1 to 10 nucleotides; ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; or iii) a combination of SEQ
- 20 ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides. In another example, the nucleic acid encoding a Tjp1 inhibitor is, but not limited to at least 60%,
- 25 at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to i) a combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide
- 30 sequence comprising 1 to 10 nucleotides; ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of

SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; or iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides. In another example, SEQ ID NOs 4, 5 or 6 is flanked at the 5' end by a nucleotide sequence comprising 3 to 8 nucleotides, 4 to 6 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides; a nucleotide sequence of 4 to 15 nucleotides, 8 to 13 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides connect the 3' end of SEQ ID NO: 4, 5 or 6 with the 5' end of SEQ ID NO: 91, 92 or 93 respectively; and SEQ ID NOs 91, 92 or 93 is flanked at the 3' end by a nucleotide sequence comprising 3 to 8 nucleotides, 4 to 6 nucleotides, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides. In yet another example, the nucleic acid encoding a Tjp1 inhibitor is, but not limited to i) a combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 4 nucleotides, wherein a nucleotide sequence of 6 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence comprising 6 nucleotides; ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5 is flanked at the 5' end by a nucleotide sequence comprising 4 nucleotides, wherein a nucleotide sequence of 6 nucleotides connects the 3' end of SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising 6 nucleotides; or iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 4 nucleotides, wherein a nucleotide sequence of 6 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 6 nucleotides.

[0034] In yet another example, the nucleic acid comprises a sequence that is, but not limited to

5'-CCGGCGTGGATTGAACTTACTAAATCTCGAGATTAGTAAGTTCA
ATCCACGTTTTTG-3' (SEQ ID NO: 1), 5'-
CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG
TTTTTG-3' (SEQ ID NO: 2), or 5'-
CCGGCGGCCATTGAAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCG

TTTTTG-3' (SEQ ID NO: 3). In yet another example, the nucleic acid comprises a sequence of at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a sequence that is, but not limited to 5'-

- 5 CCGGCGTGGAATTGAACTTACTAAATCTCGAGATTTAGTAAGTTCAATCCACGT
TTTTG-3' (SEQ ID NO: 1), 5'-
CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGGTT
TTTG-3' (SEQ ID NO: 2), or 5'-
CCGGCGGCCATTGTAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCGTT
10 TTTG-3' (SEQ ID NO: 3).

[0035] The nucleic acid encoding a Tjp1 inhibitor can also be an siRNA. In one example, the nucleic acid encoding a Tjp1 inhibitor comprises a sequence that is, but not limited to 5'-

- UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'-
AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'-
15 ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'-
AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'-
UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'-
UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'-
UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'-
20 AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'-
UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) and 5'-
AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103). In one example, the nucleic acid
encoding a Tjp1 inhibitor has at least 60%, at least 65%, at least 70%, at least 75%, at least
80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least
25 99%, or 100% identity to a sequence that is, but not limited to 5'-
UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'-
AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'-
ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'-
AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'-
30 UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'-
UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'-
UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'-

AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'-
UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) and 5'-
AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103).

[0036] Some of the nucleic acid sequences as disclosed herein are DNA sequences (such as
5 any one of SEQ ID NOs: 1 to 6, 91 to 93 or 104 to 113, or combinations thereof). In one
example, only one nucleic acid sequences as disclosed in any one or SEQ ID NOs: 1 to 6, 91
to 93 or 104 to 113 can be inserted into a single vector. In another example, more than one
nucleic acid sequences as disclosed in any one or SEQ ID NOs: 1 to 6, 91 to 93 or 104 to 113
can be inserted into a single vector thereby a single vector will express more than one short
10 hairpin molecules. Without wishing to be bound by theory, combination of multiple short
hairpin molecules may increase the inhibitory effects of short hairpin molecules on Tjp1
expression or activity.

[0037] Other nucleic acid sequences as disclosed herein are RNA sequences, for example,
siRNA sequences (such as any one of SEQ ID NOs: 94-103, or combinations thereof). Without
15 wishing to be bound by theory, combination of siRNAs may increase the inhibitory effects of
the siRNAs on Tjp1 expression or activity.

[0038] The Tjp1 inhibitor can be administered using any delivery system known in the art.
The delivery method of the Tjp1 inhibitor includes, but is not limited to delivery method using
virus-mediated delivery system. A person skilled in the art will appreciate that the list of viruses
20 for the administration of the Tjp1 inhibitors via virus-mediated delivery system listed herein is
not exhaustive. The examples of such viruses include, but are not limited to a retrovirus, an
adenovirus, an adeno-associated virus, a herpes simplex virus, and the like. In one example,
wherein when the delivery system of the Tjp1 inhibitor is virus-mediated delivery system, the
virus includes, but is not limited to, a retrovirus, an adenovirus, an adeno-associated virus, a
25 herpes simplex virus, and the like. In one example, wherein when the delivery system of the
Tjp1 inhibitor is virus-mediated delivery system, the virus is an adeno-associated virus (AAV).
The adeno-associated virus that can be used for the delivery of Tjp1 inhibitor has a variety of
serotype. In one example, the adeno-associated virus that is used for the delivery of Tjp1
inhibitor includes, but is not limited to AAV serotype 1, AAV serotype 2, AAV serotype 3,
30 AAV serotype 4, AAV serotype 5, AAV serotype 6, AAV serotype 7, AAV serotype 8, AAV
serotype 9, AAV serotype 10, AAV serotype 11, and the like. In one example, the adeno-
associated virus that is used for the delivery of Tjp1 inhibitor is AAV serotype 8.

[0039] In one example, wherein when the delivery system of the Tjp1 inhibitor is virus-mediated delivery system, the Tjp1 inhibitor comprises about 1×10^7 - 1×10^{16} AAV, or about 1×10^7 - 1×10^9 AAV, about 1×10^9 - 1×10^{11} AAV, about 1×10^{11} - 1×10^{13} AAV, about 1×10^{13} - 1×10^{15} AAV, about 1×10^{14} - 1×10^{16} AAV, or about 1×10^7 AAV, about 2×10^7 AAV, about 3×10^7 AAV, about 4×10^7 AAV, about 5×10^7 AAV, about 6×10^7 AAV, about 7×10^7 AAV, about 8×10^7 AAV, about 9×10^7 AAV, about 1×10^8 AAV, about 2×10^8 AAV, about 3×10^8 AAV, about 4×10^8 AAV, about 5×10^8 AAV, about 6×10^8 AAV, about 7×10^8 AAV, about 8×10^8 AAV, about 9×10^8 AAV, about 1×10^9 AAV, about 2×10^9 AAV, about 3×10^9 AAV, about 4×10^9 AAV, about 5×10^9 AAV, about 6×10^9 AAV, about 7×10^9 AAV, about 8×10^9 AAV, about 9×10^9 AAV, about 1×10^{10} AAV, about 2×10^{10} AAV, about 3×10^{10} AAV, about 4×10^{10} AAV, about 5×10^{10} AAV, about 6×10^{10} AAV, about 7×10^{10} AAV, about 8×10^{10} AAV, about 9×10^{10} AAV, about 1×10^{11} AAV, about 2×10^{11} AAV, about 3×10^{11} AAV, about 4×10^{11} AAV, about 5×10^{11} AAV, about 6×10^{11} AAV, about 7×10^{11} AAV, about 8×10^{11} AAV, about 9×10^{11} AAV, about 1×10^{12} AAV, about 2×10^{12} AAV, about 3×10^{12} AAV, about 4×10^{12} AAV, about 5×10^{12} AAV, about 6×10^{12} AAV, about 7×10^{12} AAV, about 8×10^{12} AAV, about 9×10^{12} AAV, about 1×10^{13} AAV, about 2×10^{13} AAV, about 3×10^{13} AAV, about 4×10^{13} AAV, about 5×10^{13} AAV, about 6×10^{13} AAV, about 7×10^{13} AAV, about 8×10^{13} AAV, about 9×10^{13} AAV, about 1×10^{14} AAV, about 2×10^{14} AAV, about 3×10^{14} AAV, about 4×10^{14} AAV, about 5×10^{14} AAV, about 6×10^{14} AAV, about 7×10^{14} AAV, about 8×10^{14} AAV, about 9×10^{14} AAV, about 1×10^{15} AAV, about 2×10^{15} AAV, about 3×10^{15} AAV, about 4×10^{15} AAV, about 5×10^{15} AAV, about 6×10^{15} AAV, about 7×10^{15} AAV, about 8×10^{15} AAV, about 9×10^{15} AAV, or about 1×10^{16} AAV.

[0040] In another example, the nucleic acid sequences as disclosed herein (such as any one of SEQ ID NOs: 1 to 6 or 91 to 113), in full or fragments thereof, can be delivered as single strand nucleic acids (e.g. as siRNAs, antisense oligonucleotides, etc), either individually or in combinations thereof or as a DNA. These nucleic acids can be DNA (comprising thymidine) or RNA (comprising uracil) or even contain non-natural nucleotides that can bind the target. These nucleotides can also comprise additional modifications to enhance their stability. They can also be coupled to, for example, a N-acetylgalactosamine (GalNAc)-conjugation delivery system for nucleic acid.

[0041] The present invention provides Tjp1 inhibitor for use in therapy. In another example, the present invention provides a Tjp1 inhibitor for use in treating a liver disease. In yet another example, the present invention provides use of a Tjp1 inhibitor in the manufacture of a

medicament for treating a liver disease. The term “liver disease” as used herein refers to abnormalities in liver structure and/or function. The expression disease or disorder can be used interchangeably. The abnormalities can occur to any structure that is found in the liver, including but not limited to cells such as hepatocytes, blood vessels in the liver or bile duct.

5 These abnormalities can occur spontaneously, or be induced by non-liver cells, for example but not limited to immune cell infiltration mediated inflammation, or be induced by toxins, chemicals or drugs. The liver forms part of the biliary system, wherein the liver, bile duct and gall bladder work together to produce, store, secrete, and transport bile. Due to the close relationship of the organs in the biliary system, it would be appreciated that any abnormality
10 in the biliary system can cause a liver disorder. Examples of liver diseases include, but are not limited to cholestasis, liver cancer, alcoholic liver disease, nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cholestatic liver disease, hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, liver fibrosis, liver cirrhosis, cholestasis-related progressive bile duct injury, cystic fibrosis-associated liver disease, thioacetamide (TAA)-
15 related liver disease, 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-related liver disease, bile duct ligation liver injury, Yes-associated Protein (YAP)-related liver disease, Mdr2-related liver disease, a disease related to the exposure to medications that affect cholesterol/bile acid (BA) biosynthesis and/or metabolism, a disease related to genetic mutations that affect cholesterol/bile acid (BA) biosynthesis and/or metabolism, or a disease related to the
20 compromise of the integrity the bile blood barrier.

[0042] The terms “cholestasis” and “cholestatic liver disease” as used herein refer to a decrease in bile flow from the liver to the gall bladder due to impaired secretion by hepatocytes, to obstruction of bile flow through intrahepatic or extrahepatic bile ducts or as a consequence of liver damage (to hepatocytes or bile duct epithelial cells) caused by other diseases or injury
25 in the liver. Many liver diseases have been shown to eventually lead to cholestasis. This results in the retention of bile salts in the liver which, under normal conditions, are excreted into bile. Examples of cholestasis include, but are not limited to intrahepatic cholestasis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), pregnancy-related intrahepatic cholestasis, neonatal cholestasis, progressive familial intrahepatic cholestasis type 3,
30 cholestatic fibrosis or biliary atresia. Causes of intrahepatic cholestasis include immune-mediated conditions such as, but not limited to primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), intrahepatic cholestasis of pregnancy, progressive familial

intrahepatic cholestasis type 3, and cystic fibrosis-associated liver disease, exposure to medications (steroids, nonsteroidal anti-inflammatory drugs, antibiotics, anti-diabetic agents) and genetic mutations (including inactivation of *TJP2* or $\Delta 4$ -3-oxosteroid 5 β -reductase deficiency) that affect cholesterol/bile acid (BA) biosynthesis and/or metabolism or integrity the bile blood barrier. Cholestasis causes progressive bile duct injury, resulting in further retention of toxic hydrophobic bile acids. This leads to persistent and extensive damage to the bile duct, and ultimately causes damage to the liver.

[0043] Some of the liver diseases can result in the activation of inflammatory processes such as, but not limited to, immune cell infiltration, increased expression of inflammatory cytokines and/or chemokines. Long term inflammatory response can cause liver fibrosis, which can lead to liver cirrhosis. The liver diseases can also cause necrosis of cells in the liver. Tjp1 inhibition can reduce the onset and progression of these effects. For example, Tjp1 inhibition can reduce the levels of TAA-induced liver necrosis (Fig. 2D), liver fibrosis (Figs. 2F-2G, 3E, 4C, 5B), and inflammation and immune cell infiltration in the liver (Fig. 3K, 4D)

[0044] Tjp1 inhibition also provides a protective role in liver cancer, as shown by reduced levels of liver fibrosis in Mdr2 KO mice lacking Tjp1 (Fig. 7). Liver cancer can occur in livers damaged by genetic defects, alcohol abuse, or chronic infection with diseases such as hepatitis B and C. Chronic cholestasis, liver fibrosis and inflammation are also known to predispose a subject to liver cancer. In one example, liver cancer can be, but is not limited to hepatocellular carcinoma, cholangiocarcinoma, hepatoblastoma, or metastatic liver cancer.

[0045] This concomitant inactivation of Tjp1 resulted in the regeneration of a biliary system (Fig. 5G). Thus, in another aspect, the present invention provides a method for regenerating a biliary system in a subject, wherein the method comprises administering of a pharmaceutically effective amount of Tjp1 inhibitor to the subject. The terms “biliary system”, “biliary tract” and “biliary tree” as used herein refer to the ducts and organs that function to produce, store, secrete, and transport bile. The organs of the biliary system include, but are not limited to the liver, bile ducts (intra-hepatic and/or extrahepatic) and gall bladder. The biliary system is part of the liver, therefore any biliary disease would also be recognized to be a liver disease. In another example, the present invention provides a Tjp1 inhibitor for use in regenerating a biliary system. In yet another example, the present invention provides use of a Tjp1 inhibitor in the manufacture of a medicament for regenerating a biliary system.

[0046] In another aspect, the present invention provides a kit comprising a Tjp1 inhibitor as described herein and/or the nucleic acid as described herein. In one example, the present invention provides a pharmaceutical composition comprising a Tjp1 inhibitor as described herein. In yet another example, the pharmaceutical composition further comprises one or more
5 pharmaceutically acceptable excipients, vehicles or carriers. Therefore, in one example, the pharmaceutical composition comprising the Tjp1 inhibitor as disclosed herein may further comprise a compound selected from, but not limited to, a pharmaceutically acceptable carrier, a liposomal carrier, an excipient, an adjuvant or combinations thereof.

[0047] As used in this application, the singular form “a,” “an,” and “the” include plural
10 references unless the context clearly dictates otherwise. For example, the term “a genetic marker” includes a plurality of genetic markers, including mixtures and combinations thereof.

[0048] As used herein, the terms “increase” and “decrease” refer to the relative alteration of a chosen trait or characteristic in a subset of a population in comparison to the same trait or characteristic as present in the whole population. An increase thus indicates a change on a
15 positive scale, whereas a decrease indicates a change on a negative scale. The term “change”, as used herein, also refers to the difference between a chosen trait or characteristic of an isolated population subset in comparison to the same trait or characteristic in the population as a whole. However, this term is without valuation of the difference seen.

[0049] As used herein, the term “about” in the context of concentration of a substance, size
20 of a substance, length of time, or other stated values means +/- 5% of the stated value, or +/- 4% of the stated value, or +/- 3% of the stated value, or +/- 2% of the stated value, or +/- 1% of the stated value, or +/- 0.5% of the stated value.

[0050] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and
25 brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4,
30 from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0051] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein
5 have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and
10 variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0052] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part
15 of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0053] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups,
20 those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[0054] **Material and Methods**

25 [0055] ***Mouse disease models***

[0056] To characterize the role of Tjp1/ZO-1 in the liver, Tjp1 was inactivated in a liver specific manner in different mouse models. Albumin (Alb)-Cre driver lines were used to achieve deletion of Tjp1 in early liver development, both in hepatocytes and cholangiocytes, in Tjp1 conditional knockout (cKO) mice. Alb-CreERT2 or Sox9-CreERT2 lines were used to
30 achieve tamoxifen inducible Tjp1 conditional knockout (Tjp1 icKO). This inducible Tjp1 conditional knockout system can be applied specifically to hepatocytes or cholangiocytes to

obtain mice with inducible Tjp1 conditional knockout hepatocytes (Tjp1 icKOHC mice) or cholangiocytes (Tjp1 icKOCC mice).

[0057] **Experimental assays**

[0058] Different assays were established, including plasma and liver biochemistry, to
 5 monitor liver function and histology, as well as immunohistochemical assays to assess tissue changes and fibrosis. Immunostaining for different markers was used to assess changes at the cellular level. EM and tracer permeability assays in vivo were used to assess the tight junction barrier. To assess if deletion of the Tjp1 had an effect on disease susceptibility, several liver disease models were established, including feeding of diets supplemented with thioacetamide
 10 (TAA), 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), bile duct ligation (BDL), or crossing to other liver disease models such as the Mdr2 KO and Yap cKO mice.

[0059] The effect of the absence of Tjp1/ZO-1 in cholangiocytes and/or hepatocytes was assessed using standard biochemical assays for liver injury and function (e.g. blood AST, ALT and bilirubin levels, blood and liver bile acid (BA) levels). Histology, immunohistochemistry,
 15 immunofluorescence microscopy and Western blot analysis were used to identify hepatocytes and cholangiocytes, assess liver fibrosis (Sirius red, collagen, α -smooth muscle actin), monitor the expression levels and localization of hepatocyte and cholangiocyte markers of interest, and assess immune cell infiltration. Quantitative RT-PCR was carried out to determine changes in liver detoxifying enzyme and bile acid transporter expression in response to Tjp1 inactivation.

20 [0060] **Mouse strains, genotyping**

[0061] Animal experimentation was approved by the relevant IACUC (Protocol #171211 and #201558) under SPF conditions. Tjp1 was inactivated by crossing C57BL/6Tac *Tjp1^{F/F}* mice to Alb-Cre mice for constitutive deletion in hepatocytes and cholangiocytes (*Tjp1 cKO*). The Alb-Cre and the Rosa26:LacZ (Rosa26:Lox-STOP-Lox-LacZ) reporter line (B6;129S4-Gt(ROSA)26Sortm1Sor/J)³², used for lineage tracing, were from Jackson Laboratory. Mice
 25 were backcrossed into the *Tjp1^{F/F}* background. For genotyping, genomic DNA isolated from tail clippings was amplified using primer-1 (5'-CTT CTC TGA CCC TAC ACA GCT ACC-3') (SEQ ID NO: 13) and primer-2 (5'-ATC GTG TGG GAA AGA CAA GC-3') (SEQ ID NO: 14), yielding a 279 bp (wild-type allele) or a 471 bp (conditional mutant allele) fragment.
 30 C57BL/6Tac *Tjp1^{F/F}* littermates served as controls. *Tjp1^{F/F}* and Alb-Cre mice were crossed with Yap^{F/F} mice²³ to generate Tjp1 Yap conditional knockout (cKO) mice. Male animals were used, but female mice showed comparable results. For genotyping, genomic DNA isolated

from tail clippings was amplified using Yap specific primer 1 (5'-CCA TTT GTC CTC ATC TCT TAC TAA C-3') (SEQ ID NO: 15) and primer 2 (5'-GAT TGG GCA CTG TCA ATT AAT GGG CTT-3') (SEQ ID NO: 16), yielding a 498 bps (wild type allele) or a 597 bps conditional mutant allele) fragment. *Abcb4/Mdr2* (FVB.129P2-*Abcb4*^{tm1Bor}/J) (Jackson Laboratory) were crossed with *Tjp1*^{F/F} animals to delete *Tjp1*.

[0062] **Thioacetamice (TAA) treatment**

[0063] Thioacetamice (TAA) (Cat #163678, Sigma) was dissolved in PBS and intraperitoneally injected into the mice at 200 mg/kg. For acute liver injury, the mice were injected with TAA and liver samples were collected 6, 24, and 48 hours later. For chronic liver injury, the mice were injected with TAA every two days, 3 times per week for 6 weeks. After the 18th injection, the liver samples were collected for analysis.

[0064] **DDC-diet**

[0065] Mice were fed with chow diet supplemented with 0.1% 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC: Cat #D80002, Sigma) for 28 days. When treated with tamoxifen, mice were provided with the DDC-diet one week after the last tamoxifen dose.

[0066] **Bile duct ligation**

[0067] 8-10 week old mice were anesthetized and their common bile ducts were ligated. The mice with ligated common bile ducts were kept for 7 days and then sacrificed and liver samples were collected for analysis. To make sure the ligation was tight, only those mice with more than 100µl bile in their gallbladder were used.

[0068] **Assessment of hepatic barrier function**

[0069] FITC-Dextran 4kDa (MW 4000, Sigma-Aldrich) was dissolved in PBS (25 mg/ml). Mice received 100 µl of the tracer solution via tail-vein injection and were sacrificed 8 minutes later. The bile was collected from gallbladder and FITC-fluorescence measured in a plate reader (Tecan). To disrupt hepatic tight junctions (TJs), LPS (2mg/kg) was injected into control mice 16 hours before tracer injection. The number of mice per cohort analyzed are given in the figure legend and samples were analyzed in 2-3 different and independent experimental runs.

[0070] **Serum and tissue biochemical analyses**

[0071] Kits were used to determine bilirubin, serum alanine aminotransferase (ALT), alkaline phosphatase (AP) and aspartate aminotransferase (AST) (Teco Diagnostics), or plasma total BA (Diazyme Laboratories) levels. For liver BA levels, 100 mg liver tissue was ground in liquid nitrogen, suspended in 1 mL water, sonicated, centrifuged, and BA levels were

determined in the supernatant. The numbers of mice per cohort analysed are given in the respective figure legends, and samples were analysed in 2 or 3 different and independent experimental runs.

[0072] **Electron microscopy**

5 [0073] Mouse livers were perfused with 2.5% glutaraldehyde fixative via the inferior vena cava, cut into small pieces and fixed for 24 hours. Samples were, rinsed with PBS, post-fixed (1% osmium tetroxide, 1h), rinsed, dehydrated in ethanol, and embedded in resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and viewed with a transmission EM (JEM-1010). For morphometric analysis, the length and width of the tight junction (TJ) plaque
10 were measured. For width, the distance between two bordering cells was measured along the TJ plaque at five different locations and the average used as the width of that particular TJ. Microvilli protruding into the canaliculus were counted and normalized to circumference unit. To account for possible differences due to location in the tissue, blocks from both the edge and center of liver lobes (4 blocks for each animal) were sectioned. Since the measurements are
15 also influenced by the plane of the section, each block was sectioned from different angles to normalize for these differences.

[0074] **Histology**

[0075] Freshly dissected livers were fixed in 4% paraformaldehyde overnight, processed, and embedded in paraffin. 5 μ m sections were stained with H&E or Sirius red and imaged with
20 a Zeisscam camera on a Zeiss Axio microscope. Five mice per cohort and images from at least 2 slides for each mouse were analyzed and used for quantification.

[0076] **Immunohistochemistry**

[0077] Paraffin blocks were sectioned at a thickness of 5 μ m. For immunohistochemistry, antigens were retrieved by steaming the slides for 20 min in a 2100 Retriever (Pick Cell
25 Laboratories). The slides were then stained with primary antibodies against cleaved caspase 3 (rabbit, Cat #9661, Cell Signaling), Laminin1-2 (rabbit; Cat #ab11575, Abcam), Collagen (rabbit; Cat #NB600-408, NOVUS Biologicals), F4/80 (rat; Cat #600-404, NOVUS Biologicals), CD11b (rat; Cat #ab8878, Abcam), Ki67 (rabbit; Cat #9129 Cell Signaling), Ck19 (rat; Troma III, DSHB, 1:20 dilution), and compatible biotin conjugated secondary
30 antibodies (Invitrogen).

[0078] **Immunofluorescence microscopy**

[0079] Paraffin sections were de-waxed and antigens retrieved by steaming for 20 min (2100 Retriever; Pick Cell Laboratories). Frozen tissues were embedded in OCT and 5µm sections stained with primary antibodies against ZO-1 (rat; Cat# R26.4C DSHB), Claudin-1 (rabbit; Cat #71-7800 Invitrogen), Claudin-2 (rabbit; Cat #32-5600 Invitrogen), Claudin-3 (rabbit; Cat #34-1700 Invitrogen), Cingulin (rabbit; Cat #36-4401 Invitrogen), CK19 (rat; Cat #TromaIII DSHB), DPPIV (goat; Cat #AF954 R&D and compatible fluorescently labeled secondary antibodies (Invitrogen, 1:200 dilution). Nuclei were labeled with DAPI. Images were obtained using Zeiss LSM800 confocal microscope. Three mice per cohort and at least 5 sections for each mouse were analyzed for quantification.

10 [0080] **Edu labelling**

[0081] 5-ethynyl-2'-deoxyuridine (Edu) is dissolved in DMSO (1 mg/10 µl) and further diluted in PBS (1 mg/100 µl). This solution was injected intraperitoneally (1 mg/g body weight) 1 hour before sacrificing the mice. Images were taken from at least 3 independent mice using a Zeiss LSM800 confocal microscope and Zen v3.4 (blue edition) software.

15 [0082] **LacZ staining**

[0083] Livers were dissected seven days after the last tamoxifen injection, frozen in optimal cutting temperature compound (OCT), and 10 µm-thick sections cut and mounted on slides. After fixation in formalin for 10 minutes, LacZ staining was carried out (NovaUltra kit) per the manufacturer's protocol. Slides were counterstained with Nuclear Faster Red for 3–5 minutes.

20 Three mice per cohort and at least 5 sections for each mouse were analysed.

[0084] **Western blotting**

[0085] Fresh liver samples were frozen in liquid nitrogen, crushed into powder and lysed for 15 min on ice in lysis buffer (50-mM Tris-HCl, pH7.5, 100 mM NaCl, 1 mM MgCl₂, and 0.5% Triton X-100, supplemented with protease inhibitor cocktail and one PhosSTOP tablet per 10ml [Cat.# 04 906 837 01, Roche]). Lysates were sonicated and centrifuged (13,000 × g for 15 min) at 4°C. Supernatants were collected and equal amounts of protein were fractionated by SDS-polyacrylamide gel electrophoresis and subjected to Western blotting using antibodies against Claudin-1 (rabbit; Cat # ab15098, Abcam), Claudin-2 (rabbit; Cat #32-5600 Invitrogen), Claudin-3 (rabbit; Cat #34-1700, Invitrogen), Occludin (rabbit; Cat #71-1500, Invitrogen), Cingulin (rabbit; Cat #36-4401 Invitrogen), E-cadherin (mouse; Cat #610181, BD Biosciences), Smad2 (rabbit; Cat #5339, Cell Signaling), Phospho-Smad2 (rabbit; Cat #3108, Cell Signaling), Laminin1-2 (rabbit; Cat #ab11575, Abcam), αSMA (rabbit; Cat #ab5694,

Abcam), Timp-1 (rabbit; Cat #ab211926, Abcam), ICAM-1 (mouse; Cat #MA5406, ThermoFisher), Osteopontin (mouse; Cat #MA5-17180), Vinculin (mouse; Cat #V9131, Sigma), or GAPDH (Mouse; Cat # sc-47724, Santa Cruz). Vinculin or GAPDH served as loading control. Primary and secondary antibodies were diluted 1:1000 and 1:3000, respectively. Samples from at least 3 independent mice per cohort were analyzed using ImageJ v15.3.

[0086] **mRNA extraction and Real-Time PCR**

[0087] Total messenger RNA (mRNA) was extracted from whole liver and processed for quantitative reverse-transcription polymerase chain reaction using a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems) and specific primers (SEQ ID NOs: 17-90). mRNA expression levels were normalized to GAPDH. Samples were isolated from at least 3 mice per cohort, pooled and run in triplicates. Each such experiment was independently repeated three times and all the data were combined for analyses.

[0088] **AAV8 vector with DNA encoding Tjp1 shRNA mediating gene silencing**

[0089] Three different DNAs that encode shTjp1 shRNAs (5'-CCGGCGTGGATTGAACTTACTAAATCTCGAGATTTAGTAAGTTCAATCCACGT TTTTG-3' (SEQ ID NO: 1), 5'-CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTTCGCGG TTTTGTG-3' (SEQ ID NO: 2), and 5'-CCGGCGGCCATTGTAACGCAAATTTCTCGAGAAATTTGCGTTCAAATGGCCG TTTTGTG-3' (SEQ ID NO: 3) were generated. The DNAs encoding scrambled shRNA or one of the shTjp1 shRNAs were injected into 2-month old Mdr2/ABCB4 KO mice via orbital injection (5×10^{10} to 2.5×10^{10} virus particles per mouse). These mice were sacrificed 2, 4 or 6 months after injection. Liver samples were collected for analysis. Sequences mediating the interaction with the target, Tjp1, are in bold.

[0090] **Experimental Results**

[0091] *Tjp1 is not required for liver development and dispensable for liver structure and function*

[0092] The albumin promoter is activated during liver development in common precursors that later give rise to both cholangiocytes and hepatocytes, resulting in floxed or deleted genes in both cell types of the Alb-Cre mice. In Tjp1 cKO mice, Tjp1 is inactivated and therefore absent from hepatocytes and cholangiocytes of the adult organ. This did not result

in an overt phenotype with regards to liver function (Fig. 1B), liver histology (Fig. 1A), tight junction marker expression and localization (Figs. 1A, C, and E), or tight junction structure (Fig. 1F) or tight junction function, which is assessed by *in vivo* permeability of 4 kDa FITC-dextran (Fig 1G).

5 [0093] ***Inactivation of Tjp1 is protective in several liver disease models***

[0094] Given the absence of an overt phenotype after liver-specific inactivation of Tjp1, the effect the absence of Tjp1 was assessed in different liver disease models using thioacetamide (TAA), 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC) diet and bile duct

10 DDC diet induced liver injury and bile duct ligation are commonly used to achieve cholestatic disease (choleostasis) models. In addition, Yap cKO and Mdr2 knockout (KO) mice were used. Yap cKO mice have defective bile duct formation during liver development and is a good model for biliary disease. Mdr2 KO mice are considered to be good animal models for bile acid induced liver diseases in humans with defects in Mdr3, which is the

15 human homolog of mouse Mdr2. Examples of such bile acid induced liver diseases include but are not limited to chronic inflammatory biliary liver disease, liver fibrosis and liver cirrhosis or primary sclerosing cholangitis.

[0095] The detrimental effects of TAA (Fig. 2), DDC (Fig. 3) diet feeding, and bile duct ligation (Fig. 4) on the liver can be observed from the plasma and liver biochemistry, liver

20 function, liver histopathology and liver fibrosis observed in the control wild type mice. These effects were abrogated in the Tjp1 cKO mice. Similarly, Yap cKO and Mdr2 KO mice also showed signs of diseased and damaged livers. The extent of damage was reduced after deleting Tjp1 in Yap cKO (Fig. 5) and Mdr2 KO (Fig. 6) mice.

[0096] In Yap cKO mice, the concomitant inactivation of Tjp1 resulted in the regeneration

25 of a biliary system, shown by the positive CK19 staining which represents the proliferation of bile ducts (Fig. 5G). While CK19 can be used to show the presence of bile ducts, increased levels of CK19 staining when compared to a wild type control presents a different interpretation as it represents the proliferation of reactive bile ducts in biliary disorders or when induced by liver injuries, otherwise known as Ductular reaction (Fig. 3F).

30 [0097] In the DDC diet induced liver injury, BDL induced liver injury and Yap KO models, macrophage and neutrophil recruitment and inflammatory cytokine and chemokine levels were reduced in the ligated Tjp1 cKO liver (Figs. 3K, 4F and 5F). Several bile acid synthesis

(e.g. Cyp7a1) and transporter (e.g. Abcb11, Abcb4, Abcc2 and Abcc3) genes were upregulated in ligated Tjp1 cKO livers as assessed by qRT-PCR (Figs. 4I and 4J). On the other hand, the expression of some of these transporter genes was lower in sham operated Tjp1 cKO livers in comparison to control livers, indicating a critical role of injury in their upregulation in the Tjp1 cKO livers. Similar changes in BA transporters and detoxification enzymes were also observed in other liver injury models after inactivation of Tjp1. In the BDL model, the concentrations of total and individual bile acids were reduced in the ligated Tjp1 cKO liver, suggesting lower hepatotoxicity when compared to ligated controls (Table 1 and Fig. 4H). These findings suggest that the deletion of Tjp1 confers protective effects on liver injury induced by hepatotoxic compounds, BDL or gene inactivation.

Bile acid name	Control (nM)	Tjp1 cKO (nM)	P value
α -muricholic acid (α -MCA)	17.35 $\pm$ 9.60	9.40 $\pm$ 1.48	P<0.05
β -muricholic acid (β -MCA)	171.48 $\pm$ 125.16	79.82 $\pm$ 42.40	P<0.05
ω -muricholic acid (ω -MCA)	39.54 $\pm$ 23.44	14.51 $\pm$ 4.59	P<0.005
Cholic acid (CA)	9.76 $\pm$ 7.61	5.23 $\pm$ 0.39	n.s.
Glycochenodeoxycholic acid (GCDCA)	1.57 $\pm$ 0.40	1.31 $\pm$ 0.17	n.s.
Glycohyocholic acid (GHCA)	23.81 $\pm$ 22.44	3.53 $\pm$ 0.72	P<0.05
Glycocholic acid (GCA)	41.98 $\pm$ 35.49	13.35 $\pm$ 4.18	P<0.05
Taurolithocholic acid (TLCA)	3.04 $\pm$ 1.66	1.23 $\pm$ 0.46	P<0.005
Taurodeoxycholic acid (TDCA)	47.31 $\pm$ 79.74	6.49 $\pm$ 4.58	n.s.
Tauroursodeoxycholic acid (TUDCA)	1303.81 $\pm$ 856.82	245.75 $\pm$ 99.60	P<0.005
Tauro-b-muricholic acid (T-b-MCA)	291835 $\pm$ 222119	56785 $\pm$ 13824	P<0.005
Taurocholic acid (TCA)	130790 $\pm$ 117325	35640 $\pm$ 8086	P<0.05

[0098] **Table 1.** Concentration of bile acids (nM) in BDL mice with and without Tjp1 inactivation. n.s. stands for not significant.

[0099] ***Validation of Tjp1 as a therapeutic target for cholestasis***

[00100] In order to determine if the protective effect of knocking-out Tjp1 could be recapitulated by silencing Tjp1 specifically in the liver using AAV8 vectors, several DNAs encoding shTJP1 RNAs were screened in mouse hepatocyte cells. Three DNAs encoding shTjp1 RNAs that showed effective silencing of Tjp1 expression in tissue culture cells (Fig. 6A) were used to generate AAV8 vectors comprising the DNA encoding shTjp1 shRNA, in which expression of the shRNA is driven by a hepatocyte specific promotor (e.g. thyroxine binding globulin (TBG)) to ensure hepatocyte-specific silencing of Tjp1. Mice were then injected with different doses of AAV8 with DNAs encoding shTjp1 or scrambled shRNA at 2-months of age. AAV8 with DNA encoding shTjp1#21 showed significant silencing of Tjp1 expression in the liver (Fig. 6B). When injected into Mdr2 KO mice, AAV8 with DNA encoding shTjp1#21 was able to reduce serum AST and ALT levels, as well as liver fibrosis (Fig. 6C). Improvements to the efficacy can be made by identifying more efficient DNAs encoding Tjp1 shRNAs and/or the dosing of the AAV8 vector to enhance silencing or infection efficiency, respectively. Different chemistries of gene silencing or exon-skipping antisense oligonucleotides with a liver specific targeting module (e.g. GalNAc) can represent an alternative modality.

[00101] The siRNAs of SEQ ID NOs: 94-103 can, when injected into the bile duct ligated or the Yap cKO mouse models, inhibit Tjp1 and establish the beneficial effect on cholestasis (data not shown).

[00102] Inactivation of Tjp1 in the liver did not noticeably affect liver development, nor liver histology of function, as assessed by blood biochemistry and histological assays. At the cellular level, the expression and localization of key markers for tight junctions and cell polarity were normal. The structural and functional integrity of tight junctions, in particular the bile-blood barrier, also remained intact. Based on the results, the livers of the Tjp1 cKO mice did not exhibit any phenotype differences when compared to the livers of the wild type mice. The Tjp1 cKO mice however were conferred protection from adverse effects when subjected to different types of liver injury protocols. Tjp1 cKO mice showed better blood and liver biochemistry, less liver fibrosis and inflammation when compared to the corresponding control mice. This was attributed to the absence of Tjp1 from hepatocytes. In

Yap cKO mice, concomitant inactivation of Tjp1 rescued the formation of a biliary system. In Mdr2 KO animals, hepatic deletion of Tjp1 suppressed liver injury and fibrosis. The beneficial effect of knocking out Tjp1 in the liver could be recapitulated using a therapeutic modality in the form of a DNA encoding Tjp1 shRNA expressed from a liver specific promoter and delivered in an AAV8 vector.

[00103] ***Inactivation of Tjp1 suppresses hepatocellular carcinoma (HCC)***

[00104] As outlined above, inactivating Tjp1 in the liver is protective against different types of liver insults. Chronic exposure to such insults can lead to liver cancer. To assess whether Tjp1 would have a protective role in liver cancer, Tjp1 was inactivated in Mdr2/Abcb4 KO mice. Mdr2/Abcb4 KO is a well-established liver cirrhosis cancer model, where animals spontaneously develop hepatocellular carcinoma (HCC) by 12 months of age (P360). Sirius red staining of liver samples from mice at 6 months of age (P180) showed extensive fibrosis in the liver Mdr2 KO mice (Fig. 7). In contrast, little to no fibrosis was observed in the liver of Tjp1 Mdr2 cKO mice, which were indistinguishable from those of wild-type controls (Fig. 7). The livers from P360 mice and mice of up to 18 months of age were observed, and no tumors were detected in the Tjp1 cKO Mdr2 KO livers. This result contrasts with the livers from the control Mdr2 KO mice, which developed multiple hepatocellular tumors. Thus, in this HCC model, deletion of Tjp1 suppresses liver carcinogenesis, likely by attenuating liver fibrosis and inflammation.

[00105] As shown above, hepatic deletion of Tjp1 in Mdr2 KO mice suppressed the onset of hepatocellular cancer. Tjp1 is known to be considered as a tumor suppressor. It was previously shown that decreased Tjp1 expression was associated with tumor metastasis of liver cancer. However, the Tjp1/ZO-1 expression was analyzed in established tumors and not during the early stages of tumorigenesis. Therefore, the downregulation of Tjp1 expression in an established tumor may be linked to metastasis, rather than to tumor development itself. It was also shown that ZO-1 is downregulated in liver cancer and only upregulated again in metastasis. This could be the result of carcinogenesis and has nothing to do with modulating the process of carcinogenesis. Merely showing the up- or down-regulation in cancer, or correlation with a process does not prove that a gene/protein is an oncogene or tumor suppressor, nor does it show that modulation of its expression affects tumorigenesis. The present application uses mice with healthy livers and the results show that inactivation of Tjp1 has a protective function against liver diseases, including

liver carcinogenesis. It can therefore be appreciated that a therapeutic target against Tjp1 can be used to treat liver diseases and/or liver cancer.

[00106] Table 2 below details the SEQ ID Nos referenced herein and their corresponding sequences. A brief description of the sequences is also provided.

5

SEQ ID NO.	Sequence (Nucleotide sequences are in the 5'→3' direction)	Description
1	CCGGCGTGGATTGAACTTACTAAATCTCGAG <u>ATTT</u> <u>AGTAAGTTCAATCCACGTTTTTG</u>	Short hairpin molecule encoding TJP1 shRNA#18. (Residues in bold correspond to Tjp1 binding site 1; residues in <u>underline</u> correspond to the complementary sequence of the residues in bold)
2	CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGA <u>AACT</u> <u>TGCTCATAACTTCGCGGTTTTTG</u>	Short hairpin molecule encoding TJP1 shRNA#21. (Residues in bold correspond to Tjp1 binding site 2; residues in <u>underline</u> correspond to the complementary sequence of the residues in bold)
3	CCGGCGGCCATTTGAACGCAAATTTCTCGAGA <u>AAAT</u> <u>TTGCGTTCAAATGGCCGTTTTTG</u>	Short hairpin molecule encoding TJP1 shRNA #22. (Residues in bold correspond to Tjp1 binding site 3; residues in <u>underline</u> correspond to the complementary sequence of the residues in bold)

4	CGTGGATTGAACTTACTAAAT	Tjp1 binding site 1 – corresponds to residues of 5678- 5698 of SEQ ID NO: 10
5	CCGCGAAGTTATGAGCAAGTT	Tjp1 binding site 2 – corresponds to residues 3986-4006 of SEQ ID NO: 10
6	CGGCCATTTGAACGCAAATTT	Tjp1 binding site 3 – corresponds to residues 5003-5023 of SEQ ID NO: 10
7	gcccgcgggc caccgcctgc aggggtggaa ggggcggcgg cggcgggagc tgcgggccac gcacggcggc ggcggcggga gcggccgggc acgcacggcg acggcggcgg cgggagcggg agcccgggc acgcacggcc gcagctcgg gtgcggcggg cgcgggagcg gtgggagctg tgggaggcgg ggatggccca gagctgatct <i>atgcggcgcc tggaggggct gtgtctgcgg cggcggcggg</i> <i>ggcggcggg cctgcccgc gacgggagcg gggacaagat gaagtaccag</i> <i>aaatacctga cgggtgtgca gatggccatc ggcgtaccc cctccaaccg</i> <i>cggcagctc ctgccgtca agaggaagct gtgggtaacg ccatcctctg</i> <i>aaaatcctaa tgggtgtact tctagtgtca gccaaaggaaa accctcttta</i> <i>agacgaatta aaggagatt acacagaagc aaaagccttg atagcatgga</i> <i>ttctgtgag ctactagca cagcaatgga ggaacagct atatgggaac</i> <i>aacatacagt gacgttcac agggctcctg gatttggatt tggaaattgca</i> <i>atatctggtg gacgagataa tctcatctt cagagtgggg aaacgtcaat</i> <i>agtatttca gatgtgtga aaggaggacc agctgaagga cagctacagg</i> <i>aaaatgaccg agttgcaatg gtaacggag ttcaatgga taatgttgaa</i> <i>catgtctttg ctgttcagca actaaggaaa agtgggaaaa atgcaaaaa</i> <i>tacaattaga aggaagaaga aagtcaaat accagtaagt cgtcctgac</i> <i>ctgaaccagt atctgataat gaagaagata gttatgatga ggaatacat</i> <i>gatccaagaa gtggccggag tgggtgtggt aacagaagga gtgagaagat</i> <i>ttggccgagg gatagaagt caagtagaga gaggagcttg tccccggt</i> <i>cagacaggcg gtacgtggt tccagccagc ctgctaaacc tactaaagtc</i> <i>acactggtga aatcccggaa aatgaagaa tatggtcttc gattggcaag</i> <i>ccatatattt gtaaggaaa ttccacaaga tagtttggca gcaagagatg</i> <i>gcaatattca agaaggtgat gttgtattga agataaatgg tactgtgaca</i> <i>gaaaatatgt cattgacaga tgcaaagaca ttgatagaaa ggtctaaagg</i> <i>caaattaaaa atggtagttc aaagagatga acgggctacg ctattgaatg</i> <i>tccctgatct ttctgacagc atccactctg ctaatgcctc tgagagagac</i> <i>gacatttcag aaattcagtc actggcatca gatcattctg gtcgatcaca</i> <i>cgataggcct ccccgccgca gccggtcacg atctctgac cagcggtcag</i> <i>agccttctga tcatccagg cactcgccgc agcagccaag caatggcagt</i> <i>ctccggagta gagatgaaga gagaatttct aaacctgggg ctgtctaac</i> <i>tcctgtaaag catgtgatg atcacacacc taaaacagtg gaagaagtta</i> <i>cagttgaaag aatgagaaa caaacacctt ctcttcaga accaaagcct</i> <i>gtgtatgcc aagttgggca accagatgtg gatttacctg tcagtccatc</i> <i>tgatggtgtc ctacctaat caactcatga agatgggatt ctccggcca</i>	Human Tjp 1 DNA sequence. (Residues in <i>italics</i> correspond to the open reading frame.)

gcatgaaatt ggtaaaattc agaaaaggag atagtgtggg ttgcggctg gctgggtggaa atgatgttgg aatatittga gctggcgctc tagaagatag ccctgcagcc aaggaaggct tagaggaagg tgatcaaatt ctgagggtaa acaacgtaga tttaacaaat atcataagag aagaagccgt ccttttctg cttgacctcc ctaaaaggaga agaagtgacc atattggctc agaagaagaa ggatgtttat cgtcgattg tagaatcaga ttagggagat tctttctata ttagaacca tttgaatat gaaaaggaat ctccctatgg acttagtttt aacaaaggag aggtgtccg tgtgtggat acctgtaca atggaaaact gggctcttgg ctgctattc gaattggtaa aatcataag gaggtagaac gaggcacat ccctaataag aacagagctg agcagctagc cagtgtacag tatacacttc caaaaacagc aggcgagac cgtgctgact tctggagatt cagaggctt cgcagctcca agagaaatct tcgaaaaagc agagaggatt tgcgcgtca gcctgttcaa acaaagtffc cagcttatga aagagtgggt ctctgagaag ctggatttct gaggcctgta accattttg gaccaatagc tgatgttgc agagaaaagc tgcaagaga agaaccagat attatacaaa ttgcaaagag tgaaccacga gacgttgaa ctgaccaacg tagctctggc attattcgc tgcatacaat aaagcaaatc atagatcaag acaaacatgc ttatttagat gtaacacca atgcagtga tcgtctaac tatgccagt ggtatccaat tgtgtattt cttaaccctg attctaagca aggagtaaaa acaatgagaa tgaggttatg tccagaatct cggaaaagt ccaggaagt atacgagcga tctataaac ttcgtaaaaa taatcccat ctttttaca ctacaattaa cttaaattca atgaatgatg gttggtatg tgcgtgaaa gaagcaattc aacaacagca aaaccagctg gtatgggtt ccgagggaaa ggcggatggt gctacaagt atgacctga ttgcatgat gatgtctgt cctacctgc agctccagt agtgaatact caatgtatg cacggacagt agacacact ctgactatga agacacagac acagaaggcg gggctacac tgatcaagaa ctatgtgaaa ctctaatga tgaggttggg actccaccgg agtctgcat tacacggctc tctgagcctg taagagagga ctctctgga atgcatcatg aaaaccaaac atatctctct tactcaccac aagegcagcc acaaccaatt catagaatag actccccctg atttaagcca gcctctcaac agaaagcaga agcttcatct ccagtcctt accttcgcc tgaaacaaac ccagcatcat caacctctgc tgttaatcat aatgtaaatt taactaatgt cagactggag gagccacccc cagctcttc caactttac tcaccacaag ctgattcttt aagaacacca agtactgagg cagctcacat aatgctaaga gatcaagaac catcattgtc gtcgcatgta gatccaaca aggtgtatag aaaggatcca tateccgagg aatgatgag gcagaaccat gttttgaac agccagccgt tagtcacca gggcacaggc cagacaaaga gcctaactctg acctatgaac cccaactccc atacgtagag aaacaagcca gcagagacct cgagcagccc acatacagat acgagtcctc aagctatag gaccagtitt ctgaaacta tgaacatcgt ctgcgatac aagatcgct ccccatgtat gaagaacagt ggtcatatta tgatgacaaa cagccctacc catctcggcc acctttgat aatcagcact ctcaagacct tgactccaga cagcatcccg aagagtcctc agaacgaggg tactttccac gtttgaaga gccagccct ctgtcttac acagcagacc acgttacgaa caggcaccta gagcatccgc cctgcggcac gaagagcagc cagctcctgg gtatgacaca catggtagac tcagaccgga ageccagccc caccctcag cagggeccaa gectgcagag tccaagcagt attttagca atattcacgc agttacgagc aagttaccac ccaaggattt acctctagag caggctattt tgagcctc catgggtctg cagctgtccc tccgtgata ccttcatctc agcataagcc	
---	--

agaagctctg ccttcaaaca ccaaaccaact gectccaccc ccaactcaaa ccgaagaaga ggaagatcca gcaatgaagc cacagtctgt actcaccaga gttaagatgt ttgaaaacaa aagatctgca tcttagaga ccaagaagga tgtaaatgac actggcagtt ttaagcctcc agaagtagca tctaaacctt caggtgctcc catcattggt cccaaaccca ctctcagaa tcaattcagt gaacatgaca aaactctgta caggatccca gaacctcaaa aacctcaact gaagccacct gaagatattg ttcggtccaa tcattatgac cctgaagaag atgaagaata ttatcgaaaa cagctgtcat acttgaccg aagaagtttt gagaataagc ctctgcaca cattgccgcc agccatctct cagagcctgc aaagccagcg cattctcaga atcaatcaaa ttttctagt tattcttcaa agggaaagcc tctgaagct gatggtgtgg atagatcatt tggcgagaaa cgctatgaac ccatccaggc cactccccct cctctccat tgcctcgc glatgccag ccatctcagc ctgtcaccag cgcgtctctc cacatacatt ctaagggagc acatggtgaa ggaattcag tgcattgga tttcagaat tcttagtgt ccaaaccca cccacctcca tctcagaata agccagcaac ttcagacca ccaaacccag aagatactgc tcaggcagct ttctatcccc agaaaagtgt tccagataaa gccccagta atggaactga acagactcag aaaacagtca ctccagcata caatcgattc acacaaaaac catatacaag ttctgccga ccatttgaac gcaagtgtga aagtcctaaa ttcaatcaca atcttctgcc aagtgaact gcacataaac ctgactgtc ttcaaaaact cccactctc caaaaactct tctgaaatcg cacagtttg cacagcctcc tgagtgtgac agtggagtgt aaactttctc tatccatgca gagaagccta aatacaaat aaataatata agcacagtgc cttaaagctat tctgtgagt ccttcagctg tggaaagga tgaagatgaa gatggtcata ctgtggtggc cacagcccga ggcataattt acagcaatgg gggcgtgctg agttccatag aaactggtgt tagtataatt atccctcaag gagccattcc cgaaggagt gagcaggaat tctatttcaa ggtctgccgg gacaacagca tcttccacc ttagataaa gagaaggtg aaacactgct gactccttg gtgatgtgtg gtcccatgg cctcaagttc ctgaagcctg tggagctgcg ctaccacac tgtcgtcca tgaactctga cggttggtct ttgtcttaa atcatccga ctctcgtc ggtgactcta aaactggca aaacaagtgt ctcccgag atccaaatta tctcgttga gcaactgtg ttctgtctt tattgaccac tttaactct tgaatatag gaacttaaat aatgtgaaac tggattaaac ttaatctaaa tggaccact ctatcaagta ttatacttt tttagagtg atactacagt ttgttagtat gaggcattg ttgaactga taaagatgag tgagcatgcc cctgaacct ggtcggaata catgctacac actgcatgtt tgtattgac gggactgtg gtattggcta gaggttcaaa gatatttgc tttgtattt tttaatttt ttatctga ctgttaact tcacataatt attccgta aaataccagc cagtaaatgg ggggtcattt gaggtctgtt ctcccaaag tacactgtt caaactttac tatggcctg gctagcata cgtacacatt ttattttatt atgcatgaag taatatgca acattttta aatgcacctg gaatatataa ccagtgtgt ggatttaaca gaaatgtaca gcaaggagat ttacaactgg gggagggtga agtgaagaca atgacttact gtacatgaaa acacatttt cttagggaag gatacaaaag catgtgagac tggttccatg gcctcttcag atctctaaact tcacatatt accacagaca tactaaccag cagaaatgcc ttacctcat gttcttaatt cttagctcat tctcctgtg ttactaagtt tttatggctt ttgtcatta tctagatact gtatcatgac aaagactgag tacgttgtgc atttgggtgt ttcaaaaatg tttatcacc cagaagaaaa tagtgggtgt atttggggat attttttct tttttttct tttttttt tttttttt acaaggggca gtgtgtgtt tctgttctt ctggctatgc atttgaat ttgatgtt taaggatgct tttacataat gcgtgcatac	
---	--

	cacttttgtt cttggtttgt aaattaactt ttataaactt tacctttttt atacataaac aagaccacgt ttctaaaggc taccttttga ttctctctg tacctcttga gccttgaact ttgacctctg cagcaataaa gcagegttgc tatgacacat gcaaggteat ttttttaag aaaaaggatg cacagagttg ttacattttt aagtgtgca ttataaagat acagttactc agaattctct agtttgatta aattcttgca aagtatccct actgtaattt gtgatacaat gctgtgccct aaagtgtatt ttttactaa tagacaattt attatggcac atcagcacga tttctgttga gataatacac cactacatc tgtaatcat taggtgtgac tgaatttctt ttgccgttat taaaaatctc aaatttctaa atctccaaaa taaaactttt taaaataaag tgctggcttg gtctgtttgc ccaactgttt ctagtttcat gcagctttat aatcctgttt taaaatcctg cacacaaatc cctatcaccc agegtcacct accacctcgt cgtctgggtg tgcatgcaga atttctccc ttggccagca tgtacagatg ggtgggcagt gctcatctga agggctcaga ctgaagtggg gcagaaggac ctggagacag agtggggagaa ggcagcaggc cgacttcccc ctgtgggtaa acacacaccc ctgcgtggag aaacacccct gcattggggac acacgtgcgt ttgtgtgtgt gcgtgtaacc atttgtatat ggttttatc cccagataaa tagcacgggc attgtttaat gtcaccaca ttgggggaa gaaaatgggt ttagttagca taaatatcc attgtggaca tctacttac ttaactgcca ccccgttga catttggatt attgctatag tgttcagta aacacctttg tggactgaat ccatctatgt ggattttatt ttttcatct ttgttaaggt ataattaca tagagtaagt tactgtttt aaaatgtata gtcaaattt tgacaaacac atactgttga ttaccacgg ttaatacata caacagtctc ttatcttct ctaatcattt gtaccccttt atactcaacc cttctccag cccctggcag gatgactct tctgtttcta tagttgcctt ttgacagtg tcataataat ggaatcatc aata	
8	atgcggcgcc tggaggggct gtgtctgcgg cggcggcgga ggcggcgggg ccctgcccgc gagcggagcg gggacaagat gaagtaccag aaatacctga cgggtctgca gatggccatc ggcgtaccc cctccaaccg cggcagcctc ctgccgtca agaggaagct gtgggtaacg ccatcctctg aaaatcctaa tgggtctact tctagtgtca gccaaagaaa accctcttta agacgaatta aaggagatt acacagaagc aaaagccttg atagcatgga ttctgtgag ctactagca cagcaatgga ggaacagct atatgggaac aacatacagt gacgcttcac agggctcctg gatttggatt tggaattgca atatctggtg gacgagataa tctcattt cagagtgggg aaacgtcaat agtgatttca gatgtgctga aaggaggacc agctgaagga cagctacagg aaaatgaccg agttgcaatg gttacggag ttcaatgga taatgttga catgcttttg ctgttcagca actaaggaaa agtgggaaaa atgcaaaaat tacaattaga aggaagaaga aagttcaat accagtaagt cgtcctgate ctgaaccagt atctgataat gaagaagata gttatgatga ggaatacat gatccaagaa gtggccggag tgggtgtgtt aacagaagga gtgagaagat ttggccgagg gatagaagtg caagtagaga gaggagcttg tccccggt cagacaggcg gtcagtggct tccagccagc ctgctaaacc tactaaagtc acactggtga aatcccggaa aaatgaagaa tatggcttc gattggcaag ccatatatt gttaaggaaa ttccacaaga tagtttggca gcaagagatg gcaatattca agaaggtgat gttgtattga agataaatgg tactgtgaca gaaaatatgt cattgacaga tgcaagaca ttgatagaaa ggtctaaagg caaattaaaa atggtagtgc aaagagatga acgggctacg ctattgaatg tccctgatct ttctgacagc atccactctg ctaatgcctc tgagagagac gacattcag aaattcagtc actggcatca gatcattctg gtcgacaca cgataggcct ccccgccgca gccggtcacg atctctgac cagcggtcag agccttctga tcattccagg cactcgcgc agcagccaag caatggcagt ctccggagta gagatgaaga gagaatttct aaacctgggg ctgtctcaac	Human Tjp1 DNA sequence – open reading frame (residues 211-5796 of SEQ ID NO: 7)

tctgttaaag catgctgatg atcacacacc taaaacagtg gaagaagtta cagttgaaag aaatgagaaa caaacacett ctctccaga accaaagcct gtgtatgcc aagttgggca accagatgtg gatttacctg tcagtccatc tgatggtgc ctacctaatt caactcatga agatgggatt ctccggccca gcatgaaatt ggtaaaattc agaaaaggag atagtgtggg ttgctggctg gctggtggaa atgatgttg aatatttga gctggcgttc tagaagatag cctgcagcc aaggaaggct tagaggaagg tgatcaaatt ctcagggtaa acaacgtaga tttaacaa atcataagag aagaagccgt cctttctcg ctgacctcc ctaaaggaga agaagtgacc atattggctc agaagaagaa ggatgttat cgtcgattg tagaatcaga ttaggagat tctttctata ttagaaccca tttgaatat gaaaaggaat ctccctatgg acttagtttt aacaaaggag aggtgttccg tgttggtgat acctgtaca atggaaaact gggctcttgg ctgctatc gaattggtaa aaatcataag gaggtagaac gaggcacat ccctaataag aacagagctg agcagctagc cagtgtacag tatacacttc caaaaacagc aggcggagac cgtgctgact tctggagatt cagaggtctt cgcagctcca agagaaatct tcgaaaaagc agagaggatt tgtccgctca gcctgttcaa acaaagtctc cagcttatga aagagtgggt ctccgagaag ctggattct gaggcctgta accattttg gaccaatagc tgatgttgc agagaaaagc tggcaagaga agaaccagat atttatcaa ttgcaaagag tgaaccacga gacgtggaa ctgaccaacg tagctctggc attatcgcc tgcatacaat aaagcaaac atagatcaag acaaacatgc ttattagat gtaacaccaa atgcagtga tctcttaac tatgccaggt ggtatccaat tgtgtattt cttaaccctg attctaagca aggagtaaaa acaatgagaa tgaggttatg tccagaatct cggaaaagtg ccaggaagtt atacgagcga tctcataaac ttcttaaaaa taatcccat cttttacaa ctacaattaa cttaaatca atgaatgatg gttggtatgg tgcgtgaaa gaagcaattc aacaacagca aaaccagctg gtatgggttt ccgagggaaa ggcggatgg gctacaagt atgacctga ttgcatgat gatgctctgt cctacctgtc agctccaggt agtgaatact caatgtatag cacggacagt agacacactt ctgactatga agacacagac acagaaggcg gggcctacac tgatcaagaa ctgatgaaa ctctaatga tgaggttggg actccaccgg agtctgcat tacacggctc tctgagcctg taagagagga ctctctgga atgcatcatg aaaaccaaac atatcctct tactcaccac aagcgcagcc acaaccaatt catagaatag actccctgg atttaagcca gcctctcaac agaaagcaga agcttcatct ccagtcctt accttcgcc tgaacaaaac ccagcatcat caacctctgc tgtaatat aatgtaaatt taactaatgt cagactggag gagccaccc cagctcttc cacctcttac tcaccacaag ctgattctt aagaacacca agtactgagg cagctcacat aatgctaaga gatcaagaac catcattgtc gtcgcatgta gatccaacaa aggtgtatag aaaggatcca tacccgagg aaatgatgag gcagaacct gtttgaac agccagccgt tagtaccaca gggcacaggc cagacaaaga gcctaactg acctatgaac ccaactccc atacgtagag aaacaagcca gcagagacct cgagcagccc acatacagat acgagctctc aagctatacg gaccagtttt ctcgaaacta tgaacatcgt ctgcgatacg aagatcgct cccatgtat gaagaacagt ggtcatatta tgatgacaaa cagccctacc catctcggcc acctttgat aatcagcact ctaagacct tgactccaga cagcatccg aagagtctc agaacgaggg tactttccac gtttgaaga gccagccct ctgtcttac acagcagacc acgttacgaa caggcaccta gagcatccgc cctgcggcac gaagagcagc cagctctgg gtatgacaca catggtagac tcagaccgga agcccagccc caccctcag cagggcccaa gcctgcagag tccaagcagt attttgagca atattcacgc agttacgagc aagtaccacc ccaaggatt acctctagag caggtcattt tgagctctc catggtgctg cagctgtccc tccgtgata cttcatctc	
---	--

	agcataagcc agaagctctg ccttcaaaca ccaaaccact gectccacc ccaactcaaa ccgaagaaga ggaagatecca gcaatgaagc cacagtctgt actcaccaga gttaagatgt ttgaaaacaa aagatctgca tcttagaga ccaagaagga tgtaaatgac actggcagtt ttaagcctcc agaagtagca tctaaacct caggtgtctc catcattggt cccaaaccca cttctcagaa tcaattcagt gaacatgaca aaactctgta caggatecca gaacctcaaa aacctcaact gaagccacct gaagatattg ttcggtccaa tcattatgac cctgaagaag atgaagaata ttatgaaaa cagctgtcat actttgaccg aagaagtttt gagaataagc ctctgcaca cattgccgc agccatctct ccgagcctgc aaagccagcg cattctcaga atcaatcaaa ttttctagt tattttcaa agggaaagcc tctgaagct gatggtgtgg atagatcatt tggcgagaaa cgctatgaac ccatccagcg cactccccct cctctccat tgcctcgea gtatgcccg ccatctcagc ctgtaccag cgcgtctctc cacatacatt ctaagggagc acatggtgaa ggtaattcag tgtcattgga tttcagaat tcttagtgt ccaaaccaga ccacctcca tctcagaata agccagcaac ttcagacca ccaaaccgag aagatactgc tcaggcagct ttctatccc agaaaagttt tccagataaa gcccagttta atggaactga acagactcag aaaacagtca ctccagcata caategatcc acacaaaaac catatacaag ttctgcccga ccatttgaac gcaagttga aagtctaaa ttcaatcaca atcttctgcc aagtgaact gcacataaac ctgactgtc ttcaaaaact ccacttctc caaaaactct tgtgaaatcg cacagtttgg cacagcctcc tgagtttgac agtggagttg aaactttctc tatcatgca gagaagccta aatatcaaat aaataatc agcacagtgc ctaaagctat tctgtgagt cttcagctg tggaagagga tgaagatgaa gatggtcata ctgtggtggc cacagcccga ggcataatta acagcaatgg gggcgtgctg agttccatag aaactggtgt tagtataatt atccctcaag gagccattcc cgaaggagtt gagcaggaaa tctatttcaa ggtctgccgg gacaacagca tcttccacc tttagataaa gagaaagggtg aaacactgct gagtccttg gtgatgtgtg gtcccatgg cctcaagttc ctgaagcctg tggagctgcg ctaccacac tgtgctcca tgactctga cggttggtct tttgctctaa aatcatccga ctctcgtcg ggtgatccta aaacctggca aaacaagtg ctccgggag atccaaatta tctcgttga gaaactgtg tttctgctc tattgaccac ttttaa	
9	MRRLEGLCLRRRRRRRRGPARERSGDKMKYQKYLTVLQM AIGVTPSTAMEETAIWEQHTVTLHRAPGFGFGIAISGGRD NPHFQSGETSIVISDV LKGGPAEGQLQENDRVAMVNGVS MDNVEHAFVQQLRKSGKNAKITIRKKKKVQIPVSRPDPE PVSDNEEDSYDEEIHDP RSGRSGVVNRRSEKIWPRDRSAS RERSLSPRSDRRSVASSQPAKPTK VTLVKS RKN EYGLRL ASHIFVKEISQDSL AARDGNIQEGDVVLKINGTVTENMSL TDAKTLIERSKGKLMVVQ RDERATLLNVPDLSDSIHSAN ASERDDISEIQSLASDHSGRSHDRPPRRSRSPDQRSEPSD HSRHSPQQPSNGSLRSRDEERISKPGAVSTPVKHADDHTP KTVEEVTVRNEKQTPSLPEPKPVYAQVGQPDVDLPVSPS DGVLPNSTHEDGILRPSMKLVKFRKGDSVGLRLAGGNDV GIFVAGVLEDSPAAKEGLEEGDQILRVNNVDFTNIREEAV LFLDLPKGEEVTLAQKKKDVYRRIVESDVGDSFYIRTHF EYEKESPYGLSFNKGEVFRVVD TLYNGKLG SWLAIRIGKN HKEVERGIIPKNRAEQLASVQYTL PKTAGGDRADFWRF RGLRSSKRNLRSREDLSAQPVQTKFPAYERVVLREAGFL RPVTIFGPIADVAREKLAREEPDIYQIAKSEPRDAGTDQRS	Human Tjp1 amino acid sequence

	SGIIRLHTIKQIIDQDKHALLDVTPNAVDRNLNYAQWYPIVV FLNPDSKQGVKTMRLCPESRKSARKLYERSHKLRKNN HHLFTTTINLNSMNDGWYGALKEAIQQQQNQLVWVSEG KADGATSDDLHLHDDRLSYLSAPGSEYSMYSTDSRHTSD YEDTDTEGGAYTDQELDETLNDEVGTPPESAITRSSEPVR EDSSGMHHENQTYPPYSPQAQPQPIHRIDSPGFKPASQQK AEASSVPYLSPETNPASSTSAVNHNVNLTNVRLEETPAP STSYSPQADSLRTPSTEAAHIMLRDQEPSLSSHVDPTKVYR KDPYPEEMMRQNHVLKQPAVSHPGHRPDKEPNLTYEPQL PYVEKQASRDLEQPTYRYESSSYTDQFSRNYEHRLRYEDR VPMYEEQWSYDDKQPYPSRPPFDNQHSQDLDSRQHPEE SSERGYFPRFEPPAPLSYDSRPRYEQAPRASALRHEEQPAP GYDTHGRLRPEAQPHPSAGPKPAESKQYFEQYSRSYEQVP PQGFTSRAGHFEPLHGAAAVPPLIPSSQHKPEALPSNTKPL PPPPTQTEEEEDPAMKPQSVLTRVKMFENKRSASLETKKD VNDTGSFKPPEVASKPSGAPIIGPKPTSQNFSEHDKTLYRI PEPQKPQLKPPEDIVRSNHYDPEEDEEYRKQLSYFDRRSF ENKPPAHIAASHLSEPAKPAHSQNQSNFSSYSSKGKPPAD GVDRSFGKRYEPIQATPPPPPLPSQYAQPSQPVTSASLHH SKGAHGEGNSVSLDFQNSLVSKPDPPPSQNKPATFRPPNR EDTAQAAFYPPQKSFPDKAPVNGTEQTQKTVTPAYNRFTP KPYTSSARPFERKFESPKFNHLLPSETAHKPDLSKKTPTSP KTLVKSHSLAQPFEDSGVETFSIHAEKPKYQINNISTVPK AIPVSPSAVEEDEDEDGHTVVATARGIFNSNGGVLSSIETG VSIIPQGAIEGVEQEIFYKVCRDNSILPLDKEKGETLLSP LVMCGPHGLKFLKPVELRLPHCASMTPDGWSFALKSSDS SSGDPKTWQNKCLPGDPNYLVGANCVSVLIDHF	
10	cgctgagtt gcccgcgacg gctctgccg ccgacggcac gctctcggc ggccgcgct tccggggaag ttacgtcgg gacgagcgt tggaggagac gcccggggt gtaggggaca gccggaggcc cgggtactgc ggagcggcga gccggcgag ggcggcgag gccgagcagg cgcccggtg tgcccgcgg agaagcccg gcggggcgga cgttcccg actttgtcc cactgaatc ccctcccg gggccgggccc ttccggcct ccccgcccc tccccgctc gtccccgga gatgttatg cggacggtg cgtgaggagc gggcgggccc gcggcgga gtttcgggc cgaggagct cgcgcggcg ggagagagac aagatgtccg ccagggcgcc gcccgctaag agcacagcaa tggaggaaac agctatatgg gaacagcaca cagtgcgct tcacagggt cctgggttg gatttgaat tgaatctct ggtggaagag ataactctca tttcagagt ggggaaacct ccatagtat ttctgatgtg taaaaggag ggccagctga aggacagcta caggaaaatg accgagttgc aatggtaac ggagttcaa tggaatacgt tgaacatgct ttgtgttc agcagctaag gaagagtgg aaaaacgcaa aaattactat ccgaaggaag aagaaagttc agatccctgt aagtcacca gactctgagc cgggtgtga taatgaagac gatagttatg acgaagaagt gcatgacca agagctggcc gcggtgctt agcgaacaga aggagcgaga agagctgggc aagggatagg agtgcaagca gggagaggag cctgtccct cgctcggaca ggcgtccgt ggctccagt cagcccgcaa agcccccaa ggtcacactg gtgaagtctc ggaaaaatga agaatatgt cttcgacgg ccagccacat attgtaaaag gaaattcac aagatagtt	Mouse Tjp 1 DNA sequence (GenBank: D14340.1) (Residues in <i>italics</i> correspond to the open reading frame.)

ggcagcaaga gatggtgaca ttcaagaagg ggaatgtgtc ttgaagataa atggtactgt gacagaaaat atgtcattga cagatgcaaa aacactgata gaaaggtcta aaggcaagtt aaaaatggta gtgcaagag atgagcgggc taccttactg aacgtccctg accttcgga tagtatccat tctgctaag cctcggaag agatgacatt tcagaaattc agtcactagc gtcagacat tcaggctgct cgcattgacag gccaccccg cgcagccagt cacgatctcc tgaccaacgt tcagagccct ccgatcattc cagcagctc ccacagcagc ccagcaatgg cagtctccg agcagagagg aagagcgaat gtctaaacct ggggccatct caactcctgt aaaacatgta gacgatcacc cacccaaagc agtgaagaa gttacagttg agaaaaatga gaagcagacg cccactcttc cagaaccgaa acctgtgtat gctcaagttg gacaaccaga tgtggattta cccgcagcc ctctgtatgg tgctctgctt aattcagctc atgaagacgg gatacttagg cccagcatga aactggtaaa attcagaaaa ggagatagtg tggtgttgcg actagctggt ggaaatgatg tcggaatatt ttagctggc gtctagaag atagccctgc agccaaagaa ggcttagagg aaggtgatca aattctcagg gtgaacaatg tagatttcac aaatatcata agggaaagagg ccgtcctttt cctccttgac ctccctaaag gtgaagaagt gaccatactg gtcagaaga agaaggacgt ttatcgccgc attgtagaat cagatgtagg agattcattc tatattagaa cgcattttga atatgaaaaa gaatctcctt acggacttag tttaacaaa ggagaggtgt tccgggtcgt ggatacttta tacaatggaa agctgggctc ttggcttgcc attcgaattg gcaaaaatca taaggaggta gaacgaggca tcatcccaaa taagaacaga gctgaacagt tagccagtgt acaglacaca ctcccaaaaga cagcgggtgg tgatcgggca gacttctgga gggttcgagg tcttcgcagc tccaagagaa atcttcgaaa aagcagagag gactgtcag ctcagccagt tcaactaag tteccagctt atgaaaggtt tgttctcga gaagctggat tctaagacc tgtaaccatc ttggaccaa tagctgatgt tgccagagaa aagttggcaa gagaggagcc agatatctat cagattgcaa aaagtgaact acgagacgct gggactgacc atcgtagctc tggcatcatt cgccttcata caataaagca aatcatagat caggataaac atgctttatt agatgtaacg ccaaatgcgg ttgatcgtct taattatgcg cagtgtgtc caattgtgt gttectaac cctgactcta agcaaggtgt aaaaacaatg aggatgaggt tgtgtccgga gtctcgaaa agcggcagga agctatatga acgctctcat aagcttcgta agaacaatca ccatctcttc acaactacaa ttaacttaaa ctcaatgaat gatggttggg acggtgccct gaaagaagcg attcagcagc aacagaacca gctgggtgtg gtctctgagg ggaaggcgga tgggtctaca agtgatgacc ttgatttgca tgacgatcgt ctgtctacc tgcagcccc aggtagttag tactcaatgt atagcacgga cagtagacac acttctgact atgaagacac agatacagaa ggcggggcct acactgatca agaactagat gaaactctta atgatgaggt ggggactccc ccggagtctg ccattacacg gtcctctgag cctgtaagag aggatctc tggaatgcat catgaaaacc agacataccc tcttactca ccacaagcgc agccacaagc tattcataga atagactccc ctggacttaa gccagcctct caacagaaag cagaagcctc atctccagtc cttaccttt cgcctgaaac aaccacagca tcatcagcct ctgcagttaa tcataatgtc agtgtaacta atgtcagcct ggaggagcct gcccagccc ctccacctc gcacgatca cagcctggtt gtttaggagc accaagtgtc gaggcagctc acgtaggtct cagaggtgaa ggaccacat tgccgccgca tgagaccca gcaaaggtgt acaggaagga gccatattct gaagaaatga tgagacaaaa ccatatttta	
---	--

aaacaaccag ctcttggtca cccagggcag aggccagata aagagccaaa tctagecctat gaaccccaac ttccatatat agaaaaacaa gccagcagag accttgagca gccgtacatac aggtatgagg tctcaagcta cacagaccag ttttctcgga actatgacca tcgcctacgg ttgaagatc gaatccctac ctatgaagac cagtgggtcat attatgatga caaacagccc taccaacctc ggccttttga gaatcagcat ccccgagacc tggactccag acaacatccc gaagaggctt cagaacgagg ttatttccag cgttttgaag agccagcccc tctgtcgtac gacagtagaa cacgctatga gcagctgcct cgaacctcta ctctacgaca tgaagagcag ccagccccctg catatgaggt gcacaacagg tacaggccag aggcacagcc ctattcttca acaggcccta agtcacttga gccaagcag tactttgacc agtaccgcg aagtatgag caagttccac caccaggctt tacctccaaa acaggccatt acgagcctct ccatggtgct gcagttgtcc ctctctgat accttctct caacaaaage cagaagtctt gcgtcggct accaaaccac agcctccacc cccaacctta actgaggagg aggaggatcc agcaatgaaa ccacagtctg tgctaccag agtcaaatg ttgaaaaca aaagatctgc gtctttggag aacaagaaag atgtgaatga cactgccage ttcaagcctc cggaagtagc atctaaacct ccagggtctt ctcttgctgg cctaaacct gtccctcaga gtcagtttag tgagcacgac aaaacgctct acaggtccc agagcctcag aaacctcaag tgaagccacc cgaagatatt gtctgatcaa atcattacga ccctgaagag gatgaagaat attaccggaa acagctctcc tactttgacc gaagaagttt tgagagcaag ccttctgcac atcttctgc tggccatcac tcagagcctg ctaagccagt ccatttctcag agccagccca acttccctag ttactcttca aagggaanaac ccgaaactga tgctgtggat agatcattca gtgagaaacg ttatgatcca gccagggcca cgcctectcc tctccgttg cctcacagt acagccagcc agctccacct ctgtccagct cttctctcca catacattcc aagggcgccc aggggtgaagg caactcagta tcattggatt ttcagaactc atatatgtcc aaaccagacc caccctcctc tcagagttaa ccagcaactt tcagaccacc aactcgagaa gacccccctc agactttcta tccgcagaaa agtttccag acaaagctcc agttaacgga gctgagcaga ctcaaaaaac catcactccg gtgtlacaatc gattcacacc aaagccgtac acaagttctg cccggccatt tgaacgcaaa ttgaaagtc cgaagttcaa ccataatctt ctgccaaagt aaactgtaca taaacctgaa ttgtcttcaa aaactccac ttcccaaaaa actcttatga aagctcatag ttcaacacag cctccagagt ttggcagtgg agttgaaact ttctctgtc acacagataa gcctaaatat caaatgaata atatcagcac catgcctaaa gctgtccctg tgagtcttc agctgtggaa gaagatgaag atgaggatgg tcatactgta gtggctacag ctctgtggcat tttaacagc aatgggtggtg tgttgagttc catagaaacc ggtgttagta taattatccc acaaggagcc attcctgaag gaattgagca agaaatctat ttcaaagtct gcagagacaa tagcattctc ccacctttag ataaagagaa aggtgaaact ctgtgagcc cctagtgtat gtgtggccc catggctca agttctgaa gcccgtggag ctacgttgc cacactgtga ccctaaaacc tggcaaaaca agtgtcttcc tggagatccg aattaccttg ttggagccaa ctgtgtttct gtcctgattg accactttta attctagtgt tataggaact ggactaagca atgtgaacgt ggattgaact tactaaatct aaatggaacc actctaata gtattatatt ttcttagaat ttactgca attggtagta ttaagcattt gttggaactg atgaaggta gcgagcatgc ccctgagcca cggtcagaaa gcatgttaca agctatgtgt tattgagtga agaactgtca ggcattggct agaggttcaa agatattttt gctttgtaat gatttttga ctttttatg gtcactgctt	
--	--

	aacttcacat actgatttcc gttaaaaata ccagccagta aatgggggtg catttgagtt ctgttcttcc caaagtacac tcaaagtta ttatggcctt ggcctagcat acacatttta ttttattata catgaggtaa tgtgcacaca tttttacaa atgcacctgg aatatataac cagtatagtg gatttaacag aaatgtacag cagggggatt tataacttgg ggagggaggg tcaaatgaag acaattactt attgtatatg aaaacacatt tcttttaggg aaggacacca aagcatgtga gcccggttca tggcctctga atctataaat taacatatca ccacagacat gtaaccagca ggaatgcctt accctagtgg gttaattct tccatcatft cgctgtgtat tactaagttt ttatgagttc tgtgcatac tagatactgt accatggaaa agactgagta gatttgggac ttgatggttt cagaaatgtg caaattacct ggaagaaaa aatgggtgta cttaaaatat tttttgaca gggggagtggt cagtctctg tctttgggc tgtgcactg agatgttga tctttcagg acgcatgtac ataatgcgtg cataccactt ttgttctgg tttgtaaatt aactttata aactttacct tttatacat aaacagaacc aagtttctaa ggctacctt gtattctctc ctgtacctct tgagcctga actttgacct ctgcagcaat aaagcagcat ttctacacat acaaggctcat ttttctaaga aaaagaatgc acagagttgt tacattttta agtgcgtcat ttaaaagata cagttactct agtttgatta aattcttga aagtatccct tctgtaaaat ttgtgataca atactgtgcc cttaaagtga ttttttact aatagacagt ttattatggc acatcagcac gatttctgt tagataatac accactacat tctgttaata atcagtaggt gtgaaaggat tcttttgtg gttattaaaa aaaaatctca aatttctaaa tctgcaagaa taaaactttt taaaat	
11	atgtccg ccagggccgc ggccgctaag agcacagcaa tggaggaaac agctatatgg gaacagcaca cagtacgct tcacagggct cctgggtttg gatttggaaat tgcaatctct ggtggaagag ataactctca tttcagagt ggggaaacct ccatagtgtat ttctgatgtg ttaaaaggag ggccagctga aggacagcta caggaaaatg accgagttgc aatggttaac ggagtttcaa tggataacgt tgaacatgct tttctgttc agcagctaag gaagagtggg aaaaacgcaa aaattactat ccgaagggaag aagaaagttc agatccctgt aagtcacca gatcctgagc cgggtgtctga taatgaagac gatagttatg acgaagaagt gcatgacca agagctggcc gcggtgcttt agcgaacaga aggagcgaga agagctgggc aagggatagg agtgcaagca gggagaggag cctgtccct cgtcgggaca ggcggtccgt ggctccagt cagcccgcaa agcccacaa ggtcacactg gtgaagtctc ggaaaaatga agaataaggt cttegaccgg ccagccacat atttgaag gaaatttcac aagatagttt ggcagcaaga gatggtgaca ttcaagaagg ggatgttgc ttgaagataa atggtactgt gacagaaaat atgtcattga cagatgcaa aacactgata gaaaggtcta aaggcaagtt aaaaatggta gtgcaagag atgagcgggc taccttactg aacgtccctg accttctgga tagtatccat tctgtaatg cctcggaag agatgacatt tcagaaatc atgcactagc gtcagacat tcaggtcgt cgcatgacag gccacccgc cgcagccagt cagatctcc tgaccaacgt tcagagccct ccgatcttc cagcagctc ccacagcagc ccagcaatgg cagtctccgg agcagagagg aagagcgaat gtctaaacct ggggccatc caactctgt aaaacatgta gacgatcacc caccaaaagc agtgaagaa gttacagttg agaaaaatga gaagcagacg cccactctc cagaaccgaa acctgtgtat gctcaagttg gacaaccaga tgtggattta cccgtcagcc ctctgatgg tgctctgctt aatcagctc atgaagacgg gatacttagg cccagcatga aactggtaaa attcagaaaa ggagatagtg tgggtttgcg actagctggt ggaaatgatg tcggaatatt ttagctggc gttctagaag atagccctgc agccaaagaa ggcttagagg aaggtgatca aattctcagg gtgaacaatg tagatttcac aaatatcata aggggaagagg ccgtcctttt cctccttgac ctcctaaag	Mouse Tjp1 DNA sequence – open reading frame (residues 404-5641 of SEQ ID NO: 10)

gtgaagaagt gaccatactg gctcagaaga agaaggacgt ttatcgccgc attgtagaat cagatgtagg agattcattc tatattagaa cgcattttga atatgaaaa gaatctcctt acggacttag tttaacaaa ggagagggtgt tccgggtcgt ggatacttta tacaatggaa agctgggctc ttggcttgcc attcgaattg gcaaaaatca taaggaggta gaacgaggca tcatcccaaa taagaacaga gctgaacagt tagccagtgt acagtacaca ctccaaaga cagcgggtgg tgategggca gacttctgga ggttctgagg tcttcgcagc tccaagagaa atcttcgaaa aagcagagag gactgtcag ctacagccagt tcaaactaag ttcccagctt atgaaagggt tgttcttga gaagctggat tctaagacc tgaaccatc ttggacca tagctgatgt tgccagagaa aagtggcaa gagaggagcc agatatctat cagattgcaa aaagtgaact acgagacgt gggactgacc atcgtagctc tgccatcatt cgcctcata caataagca aatcatagat caggataaac atgctttatt agatgtaacg ccaaatgcgg ttgatcgtct taattatgcg cagtgggtatc caattgtgt gttccctaac cctgactcta agcaagggtgt aaaaacaatg aggatgaggt tgtgtccgga gtctcgaaa agcgcagga agctatatga acgtctcat aagcttcgta agaacaatca ccatctcttc acaactacaa ttaacttaaa ctcaatgaat gatggttggt acggtgccct gaaagaagcg attcagcagc aacagaacca gctggtgtgg gtctctgagg ggaaggcgga tgggtctaca agtgatgacc ttgatttga tgacgatcgt ctgtctacc tgcagcccc aggtagttag tactcaatgt atagcacgga cagtagacac acttctgact atgaagacac agatacagaa ggccggggcct aactgatca agaactagat gaaactctta atgatgaggt ggggactccc ccggagtctg ccattacag gtctctgag cctgtaagag aggtatctc tggaatgcat catgaaaacc agacataccc tcttactca ccacaagcgc agccacaagc tattcataga atagactccc ctggacttaa gccagcctct caacagaaag cagaagctc atctcagtc ccttacctt cgcctgaaac aacccagca tcatcagct ctgcagttaa tcaatgtc agtgtaacta atgtcagcct ggaggagcct gccccagccc ctccacctc gacgcata cagcctggtt gtttaggagc accaagtgt gaggcagctc acgtaggtct cagaggtgaa ggaccacat tgccgcgca tgcagaccca gcaagggtgt acaggaagga gccatattct gaagaaatga tgagacaaa ccatatttta aaacaaccag ctcttggtca cccaggcgag aggcagata aagagccaaa tctagcctat gaaccccaac ttccatataf agaaaaacaa gccagcagag accttgagca gccgtcatac aggtatgagg tctcaagcta cacagaccag tttctcgga actatgacca tcgctacgg ttgaagatc gaatccctac ctatgaagac cagtgggtcat attatgatga caacagccc taccaacctc ggcttttga gaatcagcat ccccgagacc tgactccag acaacatccc gaagaggctt cagaacgagg ttatttccag cgttttgaag agccagcccc tctgtctac gacagtagaa cacgctatga gcagctgct cgaacctcta ctctacgaca tgaagagcag ccagccctg catatgaggt gcacaacagg tacaggccag aggcacagcc ctattctca acaggcccta agtcatctga gccaagcag tactttgacc agtaccgcg aagtatgag caagtccac caccaggctt tactccaaa acaggccatt acgagcctt ccatgggtgt cgattgtcc ctctctgat acctctct caacaaaagc cagaagtct gcgtcgggt accaaaccac agcctccacc cccaacctta actgaggagg aggaggatcc agcaatgaaa ccacagtctg tctcaccag agtcaaaatg tttgaacaa aaagatctgc gtctttggag aacaagaaag atgtgaatga cactgccagc ttcaagctc cggaagtgc atctaaacct ccagggtgct ctcttctgg ccctaaacct gtccctcaga gtcagtttag tgagcacgac aaaacgctct acaggctccc agagcctcag aaacctcaag tgaagccacc cgaagatatt	
--	--

	gttcgatcaa atcattacga ccctgaagag gatgaagaat attaccggaa acagctctcc tactttgacc gaagaagttt tgagagcaag ccttctgcac atcttctgc tggccatcac tcagagcctg ctaagccagt ccattctcag agccagecca acttccctag ttactcttca aagggaacac ccgaaactga tgctgtggat agatcattca gtgagaaacg ttatgateca gccaggecca cgcctctccc tctccggtg ccctcacagt acagccagcc agctccacct ctgtccagct cttctctcca catacattcc aagggcgccc aggggtgaagg caactcagta tcattggatt ttcagaactc atatatgtcc aaaccagacc caccceccatc tcagagtaaa ccagcaactt tcagaccacc aactcgagaa gacccccctc agactttcta tccgcagaaa agtttccag acaaagctcc agttaacgga gctgagcaga ctcagaaaac catcactccg gtgtacaatc gattcacacc aaagccgtac acaagttctg cccggccatt tgaacgcaaa ttgaaagtc cgaagttaa ccataatct ctgccaagt aaactgtaca taaacctgaa ttgtcttcaa aaactccac ttccccaaaa actcttatga aagctcatag ttcaacacag cctccagagt ttggcagtgg agttgaaact ttctctgttc acacagataa gcctaaatat caaatgaata atatcagcac catgcctaaa gctgtccctg tgagtccttc agctgtggaa gaagatgaag atgaggatgg tcatactgta gtggctacag ctcgtggcat tttaacagc aatggtggtg tgttgagttc catagaaacc ggtgttagta taattatccc acaaggagcc attctgaag gaattgagca agaaatctat ttcaaagtct gcagagacaa tagcattctc ccacctttag ataaagagaa aggtgaaact ctgtgagcc ccctagtgt gtgtgggccc catggcctca agttctgaa gcccgtggag ctacgttgc cacactgtga cctaaaaacc tggcaaaaca agtgtcttcc tggagatccg aattacctg ttggagccaa ctgtgtttct gtctgattg accactttta a	
12	MSARAAAAKSTAMEETAIWEQHTVTLHRAPGFGFGIAISG GRDNPHFQSGETSIVISDVLKGGPAEGQLQENDRVAMVN GVSMNDNVEHAFVQQLRKSGKNAKITIRRKKKVQIPVSH PDPEPVSDNEDDSYDEEVHDPGRGALANRRSEKSWAR DRSASRERSLSRSDRRSVASSQPAKPTKVTLVKSRKNEE YGLRPASHIFVKEISQDSLARDGDIQEGDVVLKINGTVTE NMSLTDAKTLIERSKGLKLMVVQORDERATLLNVPDLSDSI HSANASERDDISEIQSLASDHSGRSHDRPPRRSQSRSPDQR SEPSDHSTQSPQQPSNGSLRSREEERMSKPGAISTPVKHVD DHPPKAVEEVTVKEKNEKQTPTLPEPKPVYAQVGQPDVDL PVSPSDGALPNSAHEDGILRPSMKLVKFRKGDSVGLRLAG GNDVGIFVAGVLEDSPAAKEGLEEGDQILRVNNVDFTNII REEAVLFLDLDPKGEEVTILAQKKKDVYRRIVESDVGDSF YIRTHFEYEKESPYGLSFNKGEVFRVVDTLYNGKLGSWL AIRIGKNHKEVERGIIPNKNRAEQLASVQYTLPKTAGGDR ADFWRFRLRSSKRNLRSREDLSAQPVQTKFPAYERVV LREAGFLRPVTIFGPIADVAREKLAREEPDIYQIAKSELRD AGTDHRSSGIIRLHTIKQIIDQDKHALLDVTPNAVDRNLNYA QWYPIVVFLNPDSKQGVKTMRLCPESRKSARKLYERS HKLRKNNHHLFTTTINLNSMNDGWYGALKEAIQQQQNQ LVWVSEGKADGATSDDLHDDRSLYSAPGSEYSMYST DSRHTSDYEDTDEGGAYTDQELDETLNDEVGTPPESAIT RSSEPVREDSSGMHHENQTYPPYSPQAQPQAIHRIDSPGLK PASQQKAEASSVPYLSPETTPASSASAVNHNVSVTNVSL EEPAPAPTSHASQPGCLGAPSAEAAHVGLRGEGPPLPPH	Mouse Tjp1 amino acid sequence

	ADPAKVYRKEPYSEEMMRQNHILKQPALGHPGQRPDKEP NLAYEPQLPYIEKQASRDLEQPSYRYEVSSYTDQFSRNYD HRLRFEDRIPTYEDQWSYDDKQPYQPRPFENQHPRDLDS RQHPEEASERGYFQRFEEPAPLSYDSRTRYEQLPRTSTLRH EEQPAPAYEVHNRYRPEAQPYSSSTGPKSSEPKQYFDQYPR SYEQVPPPGFTSKTGHYEPLHGAAVVPPLIPSSQQKPEVLR SATKPQPPPTLTETEEEDPAMKPQSVLTRVKMFENKRSAS LENKKDVNDTASFKPPEVASKPPGASLAGPKVPQSQFSE HDKTLYRLPEPQKPQVKPPEDIVRSNHYDPEEDEEYYRKQ LSYFDRRSFESKPSAHLPAAGHHSEPAKPVHSQSQPNFPSYS SKGKPETDAVDRSFSEKRYDPAQATPPPPPLPSQYSQPAPP LSSSSLHIHSGKAQGEGNSVSLDFQNSYMSKPDPPPSQSKP ATERPPTREDPPQTFYPQKSFPDKAPVNGAEQTQKTITPVY NRFTPKPYTSSARPFERKFESPKFNHNLPSSETVHKPELSS KTPTSPKTLMAHSSTQPPEFGSGVETFSVHTDKPKYQMN NISTMPKAVPVSPSAVEEDEDEDGHTVVATARGIFNSNGG VLSSIETGVSIIPQGAIEGIEQEIYFKVCRDNSILPPLDKEK GETLLSPLVMCGPHGLKFLKPVELRLPHCDPKTWQNKCL PGDPNYLVGANCVSVLIDHF	
13	CTT CTC TGA CCC TAC ACA GCT ACC	Primer 1 for genotyping
14	ATC GTG TGG GAA AGA CAA GC	Primer 2 for genotyping
15	CCA TTT GTC CTC ATC TCT TAC TAA C	Yap specific primer 1
16	GAT TGG GCA CTG TCA ATT AAT GGG CTT	Yap specific primer 2
17	GGCCCAGAGCAAGAGAGGTATCC	Actin forward primer
18	ACGCACGATTTCCCTCTCAGC	Actin reverse primer
19	AGCACAGCAATGGAGGAAACAGCT	Tjp1 forward primer
20	CTGGGTGACTTACAGGGATCTGAA	Tjp1 reverse primer
21	AGCGTGATCAGCGGTTTTG	CK19 forward primer
22	CCTGGTTCTGGCGCTCTATG	CK19 reverse primer
23	GTCATTTGCTCCAAACTGGCG	EPCAM forward primer
24	AGCCCATCGTTGTTCTGGAT	EPCAM reverse primer
25	GACTGGAAGAGCGGAGAGTACTG	Collagen 1A1 forward primer
26	CAGGTCTGACCTGTCTCCATGTT	Collagen 1A1 reverse primer

27	GCAGTGGCTGAACCAAGG	TGF β forward primer
28	AGCAGTGAGCGCTGAATC	TGF β reverse primer
29	GGCATCTGGCATCCTCTTGT	TIMP-1 forward primer
30	TAGCCCTTATGACCAGGTCCG	TIMP-1 reverse primer
31	GCGGTCCTCACCATGAGTCC	MMP9 forward primer
32	TAGCGGTACAAGTATGCCTCTGC	MMP9 reverse primer
33	CCTTCGTGACTACTGCCGAGC	α -SMA forward primer
34	TTCGTGGATGCCCCGCTGACT	α -SMA reverse primer
35	GCAGAAGAAGCCACGCTATG	PdgfRb forward primer
36	CAGGTGGAGTCGTAAGGCAA	PdgfRb reverse primer
37	GAGTCGGCATGAATCGCTG	Pdgfb forward primer
38	TCAAAGGAGCGGATGGAGTG	Pdgfb reverse primer
39	ACATTAAGAAGGGCAAAAAGTGCA	CTGF forward primer
40	AACTTAGCCCTGTATGTCTTCACA	CTGF reverse primer
41	CACGTCGTAGCAAACCACC	TNF α forward primer
42	GGTGAGGAGCACGTAGTC	TNF α reverse primer
43	CCTCTGGTCTTCTGGAGTACC	IL-6 forward primer
44	ACTCCTTCTGTGACTCCAGC	IL-6 reverse primer
45	TGCCACCTTTTGACAGTGATG	IL-1b forward primer
46	ATGTGCTGCTGCGAGATTTG	IL-1b reverse primer
47	AACATGCAACCTGCCACAAC	F4/80 forward primer
48	TTCACAGGATTCGTCCAGGC	F4/80 reverse primer
49	ACACTTTCCTCCAATCGTCC	Osteopontin forward primer
50	TGCCCTTTCCGTTGTTGTCC	Osteopontin reverse primer

51	GCAGCCAGATTGGCTCTTATTTT	CD11b forward primer
52	GCCCTCGAGGCAAGGGA	CD11b reverse primer
53	GGTGGTGAGAGCTTGAAAATGA	Cyp7a1 forward primer
54	GTGGTTCTTGGAGGTGCCTTT	Cyp7a1 reverse primer
55	CTATGGAAGCCCTGCGTGAC	Cyp7b1 forward primer
56	GAGCACAGCCTCAGAACCTC	Cyp7b1 reverse primer
57	TTGCAAATGCTGCCTCAACC	Cyp8b1 forward primer
58	TAACAGTTCGCACACATGGCT	Cyp8b1 reverse primer
59	GAGACAGGAGGGCAAGTACC	Cyp27a1 forward primer
60	TAAGGCATCCGTGTAGAGCG	Cyp27a1 reverse primer
61	GCATTACCAAGAACGCCGTG	FXR forward primer
62	CTGGATTTCAGTTAACAAACCTGT	FXR reverse primer
63	CCAGGCCAGTAGAGTGGTAGC	Shp-1 forward primer
64	GGCAGGCACGTACAAACTC	Shp-1 reverse primer
65	TACGTCCTCAAGGCAGGCATGATC	NTCP forward primer
66	GTAGCCAGTAAGTGTGGTGTCATG	NTCP reverse primer
67	ACGCAAGATCCAACAGAGTGT	OATP-1 forward primer
68	GGGCCAACAATCTTCCCCAT	OATP-1 reverse primer
69	TGAGTTCAGGACACCAAGACTG	OATP-2 forward primer
70	ACCGCCTACCCTATGCCTT	OATP-2 reverse primer
71	CATCTGTAGGGTTGTTGAGTGA	ABCB11 forward primer
72	CAAAGAAGCCAACTCGAGCG	ABCB11 reverse primer
73	ACGGCGACTTTGAACTAGGC	ABCB4 forward primer

74	CCAGTCAGAGTATCGGAACAGT	ABCB4 reverse primer
75	GCAACTCTACTTTTTGGAATCTCT	ABCC2 forward primer
76	CCAAGAGCCAAAGAAAGCCC	ABCC2 reverse primer
77	TGAGGATGCGGTCCTACTGA	ABCC3 forward primer
78	GGCCGTGGGGTCAGAAATAA	ABCC3 reverse primer
79	GACCATGAAGCCTTGCAAAA	ABCC4 forward primer
80	TTGGGCATAGTCCGGTTCTG	ABCC4 reverse primer
81	ACCTGGGTGCTCCTAGCAA	Cyp3a11 forward primer
82	AAATGGCAGAGGTTTGGGC	Cyp3a11 reverse primer
83	CAGACCCGGAGTTACCCAAAG	CAR forward primer
84	AAACCGACTTTGGAGCCGAG	CAR reverse primer
85	GGCCTGACCTTGGCTCATAG	PXR forward primer
86	ACATCTGTGTGTCCTAGACTGT	PXR reverse primer
87	TGTGTGTGTTCCGGTCCCTC	Ugt1a1 forward primer
88	GCTTCAGGTGCTATGACCAC	Ugt1a1 reverse primer
89	CCTCAAAGGAAATGTTCTATTCGGA	Slut2a1 forward primer
90	TCCAGCTCATCTGGCCCTAA	Slut2a1 reverse primer
91	ATTTAGTAAGTTCAATCCACG	Complementary sequence of SEQ ID NO: 4
92	AACTTGCTCATAACTTCGCGG	Complementary sequence of SEQ ID NO: 5
93	AAATTTGCGTTCAAATGGCCG	Complementary sequence of SEQ ID NO: 6
94	UGAAACUCCGUUAACCAUUGC	siRNA Tjp1 sequence 1; targets residues 634-654 of mouse Tjp1 cDNA

		sequence (NM_009386.2) or residues 667-687 of human Tjp1 cDNA sequence (NM_003257.5)
95	AUUGAAACUCCGUUAACCAU	siRNA Tjp1 sequence 2; targets residues 637-657 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 668-688 of human Tjp1 cDNA sequence (NM_003257.5)
96	ACUAUCUUGUGAAAUUUCCU	siRNA Tjp1 sequence 3; targets residues 1033-1053 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 1065-1085 of human Tjp1 cDNA sequence (NM_003257.5)
97	AAAUCAAGGUCAUCACUUGUA	siRNA Tjp1 sequence 4; targets residues 2832-2852 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 2864-2884 of human Tjp1 cDNA sequence (NM_003257.5)
98	UUCAUCUAGUUCUUGAUCAGU	siRNA Tjp1 sequence 5; targets residues 2968-2988 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 3000-3020 of human Tjp1 cDNA sequence (NM_003257.5)
99	UAAGAGUUUCAUCUAGUUCU	siRNA Tjp1 sequence 6; targets

		residues 2976-2996 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 3007-3027 of human Tjp1 cDNA sequence (NM_003257.5)
100	UUCUCUUUAUCUAAAGGUGGG	siRNA Tjp1 sequence 7; targets residues 5445-5465 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 5486-5506 of human Tjp1 cDNA sequence (NM_003257.5)
101	AUCUAAACAGAAAUCGUGCUG	siRNA Tjp1 sequence 8; targets residues 6920-6940 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 7004-7024 of human Tjp1 cDNA sequence (NM_003257.5)
102	UAUCUAAACAGAAAUCGUGCU	siRNA Tjp1 sequence 9; targets residues 6921-6941 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 7005-7025 of human Tjp1 cDNA sequence (NM_003257.5)
103	AAUGUAGUGGUGUAUUAUCUA	siRNA Tjp1 sequence 10; targets residues 6936-6956 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 7020-7040 of human Tjp1

		cDNA sequence (NM_003257.5)
104	GCAATGGTTAACGGAGTTTCA	Tjp1 binding site 4 – corresponds to residues 634-654 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 667-687 of human Tjp1 cDNA sequence (NM_003257.5)
105	AATGGTTAACGGAGTTTCAAT	Tjp1 binding site 5 – corresponds to residues 637-657 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 668-688 of human Tjp1 cDNA sequence (NM_003257.5)
106	AAGGAAATTCACAAGATAGT	Tjp1 binding site 6 – corresponds to residues 1033-1053 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 1065-1085 of human Tjp1 cDNA sequence (NM_003257.5)
107	TACAAGTGATGACCTTGATTT	Tjp1 binding site 7 – corresponds to residues 2832-2852 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 2864-2884 of human Tjp1 cDNA sequence (NM_003257.5)
108	ACTGATCAAGAACTAGATGAA	Tjp1 binding site 8 – corresponds to residues 2968-2988 of mouse Tjp1 cDNA sequence (NM_009386.2) or

		residues 3000-3020 of human Tjp1 cDNA sequence (NM_003257.5)
109	AAGAACTAGATGAAACTCTTA	Tjp1 binding site 9 – corresponds to residues 2976-2996 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 3007-3027 of human Tjp1 cDNA sequence (NM_003257.5)
110	CCCACCTTTAGATAAAGAGAA	Tjp1 binding site 10 – corresponds to residues 5445-5465 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 5486-5506 of human Tjp1 cDNA sequence (NM_003257.5)
111	CAGCACGATTTCTGTTTAGAT	Tjp1 binding site 11 – corresponds to residues 6920-6940 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 7004-7024 of human Tjp1 cDNA sequence (NM_003257.5)
112	AGCACGATTTCTGTTTAGATA	Tjp1 binding site 12 – corresponds to residues 6921-6941 of mouse Tjp1 cDNA sequence (NM_009386.2) or residues 7005-7025 of human Tjp1 cDNA sequence (NM_003257.5)
113	TAGATAATACACCACTACATT	Tjp1 binding site 13 – corresponds to residues 6936-6956 of mouse Tjp1

		cDNA sequence (NM_009386.2) or residues 7020-7040 of human Tjp1 cDNA sequence (NM_003257.5)
114	gtggccgcct gagttgcccg cgacggctct gcccgccgac ggcacgtctc tcggcgcccg cgcgttccgg ggaagtacg tgcgggagca ggctttggag gagacgcccg aggggttagg ggacagccgg agggccgggt actgcggagc ggcgagccgg cggagggcgg cggagggcca gcaggcggcc ggggtgcccc cgcggagaag cccgggcggg gcggacgctt cccggacttt tgccccactt gaateccctc cccgtgggcc gggcctttcc ggctccccc gccctgccc cgctcgtccc cgggagatgt ttatgcggac ggtggcgtga ggagcgggag ggccggcggc gcggagtctt gggcccgagg agcttcgcgc ggcgcggaga gagacaagat gtccgccagg gccgcggccg ctaagagcac agcaatggag gaaacagcta tatgggaaca gcacacagt acgcttcaca gggctcctgg gtttggattt ggaattgcaa tctctggtgg aagagataat cctcatttcc agagtgggga aacctccata gtgattctg atgtgttaaa aggagggcca gctgaaggac agctacagga aaatgaccga gttgcaatgg ttaacggagt ttcaatggat aacgttgaac atgcttttgc tgttcagcag ctaaggaaga gtgggaaaaa cgcaaaaatt actatccgaa ggaagaagaa agttcagatc cctgtaagtc accagatcc tgagccggtg tctgataatg aagacgatag ttatgacgaa gaagtgcattg acccaagagc tggccgcggt gctttagcga acagaaggag cgagaagagc tgggcaaggg ataggagtgc aagcaggag aggagcctgt ccctcgtctc ggacagcggt tccgtggcct ccagtcagcc cgcaaaagcc accaaggtca cactggtgaa gtctcggaaa aatgaagaat atggtcttcg attggccagc catatatttg taaaggaaat ttcacaagat agtttggcag caagagatgg taacattcaa gaaggggatg ttgtctgaa gataaatggt actgtgacag aaaatatgtc attgacagat gcaaaaacac tgatagaaag gtctaaaggc aagttaaaaa tggtagtgca aagagatgag cgggctacct tactgaacgt ccctgacctt tcggatagta tccattctgc taatgcctcg gaaagagatg acatttcaga aattcagta ctagcatcag accattcagg tgcctcgcgt gacaggccac cccgcgcgag ccagtcacga tctcctgacc aacgttcaga gccctccgat cattccacgc agtctccaca gcagcccgag aatggcagtc tccggagcag agaggaagag cgaatgtcta aacctggggc catctcaact cctgtaaaaac atgtagacga tcatccacc aaagcagtg gagaagttac agttgagaaa aatgagaagc agacgcccac tcttcagaa ccgaaacctg tctatgctca agttggacaa ccagatgtgg atttaccgt cagcccttct gatggtgctc tgcctaattc agtcatgaa gacgggatac ttaggcccag catgaaactg gtaaaattca gaaaaggaga tagtgtgggt ttgcgactag ctggtggaaa tgatgtcgga atattttag ctggcgttct agaagatagc cctgcagcca aagaaggctt agaggaaggat gatcaaatc tcagggtgaa caatgtagat ttcacaata tcataaggga agagccgctc ctttctctc ttgacctccc taaagtgaa gaagtgacca tactggctca gaagaagaag gacgtttatc gccgcattgt agaatcagat gtaggagatt cattctatat tagaacgcat ttgaaatag aaaaagaatc tccttacgga cttagtttta acaaaggaga ggtgttccgg gtcgtggata ctttatacaa tggaagctg ggctcttggc ttgccaatcg aattggcaaa aatcataagg aggtagaacg aggcattcat cctaataaga acagagctga acagttagcc agtgtacagt acacacttcc aaagacagcg ggtggtgac gggcagactt	Mouse Tjp1 cDNA sequence (NM_009386.2)

ctggagggtt cgaggcttc gcagctcaa gagaaatctt cgaaaaagca gagaggactt gtcagctcag ccagttcaaa ctaagttccc agcttatgaa aggggtgttc ttcgagaagc tggattccta agacctgtaa ccatcttgg accaatagct gatgttgcca gagaaaaagt ggcaagagag gagccagata tctatcagat tgcaaaaagt gaaccacgag atgctgggac tgaccatcgt agctctggca tcaatgcct tcatacaata aagcaaatca tagatcagga taaacatgct ttattagatg taacgccaaa tgcggttgat cgtcttaatt atgcgcagtg gtatccaatt gttgtgttc ttaacctga ctctaagcaa ggtgtaaaaa caatgaggat gaggtgtgt cggagctc ggaaaagcgc caggaaagcta tatgaacgct ctcataagct tegtgaagac aatcaccatc tcttcaaac tacaattaac ttaaactcaa tgaatgatgg ttggtacggt gccctgaaag aagcgattca gcagcaacag aaccagctgg tgtgggtctc tgaggggaag gcggatggtg ctacaagtga tgacctgat ttgatgacg atcgtctgtc ctacctgtca gccccaggta gtgagtactc aatgtatagc acggacagta gacacacttc tgactatgaa gacacagata cagaaggcgg gccctacact gatcaagaac tagatgaac tcttaatatg gagtgggga cteccccgga gtctgccatt acacggctct ctgagcctgt aagagaggat tctctggaa tgcatcatga aaaccagaca taccctctt actaccaca agcgcagcca caagctatc atagaataga ctccccgga cttaagccag cctctcaaca gaaagcagaa gccctatc cagtccctta ctttgcct gaaacaaccc cagcatcacc agcctctgca gttaatcata atgtcagtgt aactaatgc agcctggagg agcctgcccc agccccccc acctgcacg catcacagcc tggttgttta ggagaccaa gtgctgaggc agctcacgta gtgctcagag gtgaaggacc accattgccg ccgatgcag acccagcaaa ggtgtacagg aaggagccgt attctgaaga aatgatgaga caaaaccata tttaaaaca accagctctt ggcacccag ggcagaggcc agataaagag ccaaatctag cctatgaacc ccaacttcca tatatagaaa aacaagccag cagagacct gagcagccgt catacaggtg tgaggctc agctacacag accagtttct tggaaactat gaccatgcgc tacggtttga agatcgaatc cctacctatg aagaccagtg gtcatattat gatgacaaac agccctacca acctggcct ttgagaatc agcatcccc agacctggac tccagacaac atcccgaaga ggttcagaa cgagggtatt tccagcgtt tgaagageca gccccctgt cgtacgacag tagaacacgc tatgagcagc tgctcgaac ctctactc cgacatgaag agcagccagc cctgcata gaggtgcaca acaggtacag gccagaggca cagccctatt cttaacagg ccctaagtca tctgagccca agcagtactt tgaccagtac ccgcaagt atgagcaagt tcaccacca ggctttacct ccaaacagg ccattacgag cctctccatg gtgctgcagt gtccctct ctgataacct cctctcaaca aaagccagaa gtctgcct cggtacca accacagcct ccaccccaa ccctaactga ggaggaggag gatccagcaa tgaaaccaca gtctgtgctc accagagtca aaatgttga aaacaaaaga tctgcgtctt tggagaacaa gaaagatgtg aatgacactg ccagctttaa gccctcggaa gtgacatcta aacctccagg tgcctctt gctggcccta aacctgtccc tcagagtcag tttagtgagc acgacaaaac gctctacagg ctcccagagc ctcaaaacc tcaagtgaag ccacccgaag atattgttc atcaaatcat tacgacctg aagaggatga agaataattac cggaaacagc tctctactt tgaccgaaga agttttgaga gcaagccttc tgcacatct cctgtggcc atcactcaga gccgtctaag ccagtcatt ctgagagcca gccaacttc tctagtact cttcaaagg aaaacccgaa actgatgctg tggatagatc attcagtga aaacgttatg atccagccca ggccacgct cctctctc cgttgcctc acagtacagc cagccagctc cactctgtc cagctctct ctccacatac attcaaggg cgcccagggt gaaggcaact cagtatcatt ggatttcag aactcatata tgcctaaacc	
--	--

	agaccacccc ccatctcaga gtaaaccage aactttcaga ccaccaactc gagaagacccc cctcagact ttctatccgc agaaaagttt cccagacaaa gctccagtta acggagetga gcagactcag aaaaccatca ctccgggtga caatcgattc acaccaaagc cgtacacaag ttctgcccgg ccatttgaac gcaaatttga aagtcgaag ttcaaccata atcttctgcc aagtgaact gtacataaac ctgaattgtc ttcaaaaact cccacttccc caaaaactct tatgaaagct catagttaa cacagcctcc agagtttgac agtggagttg aaactttctc tgttcacaca gataagccta aatatcaat gaataatc agcaccatgc cttaaagctgt cctgtgagt ccttcagctg tggagaaga tgaagatgag gatggtcata ctgtagtggc tacagctcgt ggcattttta acagcaatgg tgggtgttg agttccatag aaaccgggtg tagtataatt atcccacaag gagccattcc tgaaggaatt gagcaagaaa tctatttcaa agtctgcaga gacaatagca ttctcccacc tttagataaa gagaaagggtg aaactctgct gagcccccta gtgatgtgtg ggccccatgg cctcaagttc ctgaagcccg tggagctacg ctggccacac tgtgacccta aaacctggca aaacaagtg ctctctggag atccgaatta cctgttggga gccaactgtg ttctgtctct gattgaccac tttaattct tagtgtatag gaactggact aagcaatgtg aacgtggatt gaacttacta aatctaaatg gaaccactct aatgagtatt atatttctt agaatttata ctgcaattgg tagtattaag catttgttg aactgatga ggtagcgag catgcccctg agccacggtc agaaaacatg ctacaagcta tgtgttattg agtgaagaac tgcaggcat tggctagagg ttcaaagata ttttgcctt gtaatgatt ttgtacttt ttatggtcac tgcttaactt cacatactga ttccgftaa aaataaccage cagtaaatgg ggggtgcattt gagtctgtt ctttccaaag tacactcaaa gtttattatg gccttggcct agcatacaca tttttttta ttatgcatga ggtaatgtgc acacattttt tacaaatgca cctggaatat ataaccagta tagtggattt aacagaaatg tacagcaggg ggatttataa ctggggagg gaggggtcaaa tgaagacaat tacttattgt atatgaaaac acatttctt tagggaagga caccaaagca tgtgagcccg gtcatggcc tctgaatca taaftaaca tatcaccaca gacatgtaac cagcaggaat gccttacct agtgggttta attctccat catttctgt tgtattacta agttttatg agttctgtgc atatctagat actgtaccat ggaaaagact gagtagattg tggacttgat ggttcagaa atgtgcaat tacttggag aaaataatgg tgtgacttaa aatattttt tgacaggggg agtggcagtc ttctgttct tgggtgtgc atctgagatg ttgatgctt tcaggacgca tgtacataat gcgtgcatac cacttttgtt ctgggttgt aaattaactt ttataaactt tacttttta tacataaaca gaaccaagtt tctaaggcta ccttgtatt ctctctgtga cctcttgagc ctgaaactt gacctctgca gcaataaagc agcatttcta cacatacaag gtcattttc taagaaaaag aatgcacaga gttgttacat tttaagtgc tgcattttaa agatacagtt actctagttt gattaaatc ttgcaaagta tcccttctgt aaaatttgtg atacaatact gtgccctaaa gtgtattttt ttactaatag acagtttatt atggcacatc agcacgattt ctgtttagat aatacaccac tacattctgt taataatcag taggtgtgaa aggatttctt ttgtggttat taaaaaaaaa tctcaattt cttaatctgc aagaataaaa ctttttaaaa taca	
115	gtggccgctg agttgccggc gccggtgag ccagcggacg ccgcgttctt tggcggccgc cggttcccgg gaagttacgt ggcaagccg gcttccgagg agacgccggg aggccacggg tctgtctgac gggcgggga cggggcgagg ccgacgtggc cgggctgca aagctgcggg aggccgagtg ggtggccgcg ctggaggga ggtgccggtc gggcgcgcc cgtggagaag acccgggcgg ggccggcgct tcccggactt ttgtccgagt tgaattccct cccctgggc cgggccctt cggccgccc cggcgtgcc ccgtctgctc tgggagatg	Human Tjp1 cDNA sequence (NM_003257.5)

tttatttggg ctgtggcgtg aggagcgggc gggccagcgc cgcggagttt cgggtccgag gagcctcgcg cggcgtgga gagagacaag atgtccgcca gagctcggc cgccaagagc acagcaatgg 481 aggaaacagc tatatggga caacatacag tgacgttca cagggtcct ggatttggat ttggaattgc aatatctgt ggacgagata atcctcattt tcagagtggg gaaacgtcaa tagtgatttc agatgtgctg aaaggaggac cagctgaagg acagctacag gaaaatgacc gagttgcaat ggtaacgga gttcaatgg ataatttga acatgctttt gctgttcage aactaaggaa aagtgggaaa aatgcaaaaa ttacaattag aaggaagaag aaagtcaaa taccagtaag tcgtctgat cctgaaccag tatctgataa tgaagaagat agttatgatg aggaaataca tgatccaaga agtggccgga gtggtgtggt taacagaagg agtgagaaga ttggccgag ggatagaagt gcaagtagag agaggagctt gtccccgagg tcagacaggc ggtcagtggc ttccagccag cctgctaaac ctactaaagt cacactgggtg aaatcccga aaaatgaaga atatggtctt cgattggcaa gccatatatt tgtaaggaa atttcacaag atagtttggc agcaagagat ggcaatattc aagaaggtga tgtgtattg aagataaatg gtactgtgac agaaaatatg tcattgacag atgcaaagac attgatagaa aggtctaaag gcaaattaaa aatggtagtt caaagagatg aacgggctac gctattgaat gtccctgac tttctgacag catccactct gctaatgcct ctgagagaga cgacattca gaaattcagt cactggcacc agatcattct ggtcgatcac acgataggcc tccccggcg agccgggtcac gatctctga ccagcgggtca gagcctctg atcattccag gcactcgccg cagcagccaa gcaatggcag tctccggagt agagatgaag agagaatttc taaacctggg gctgtctcaa ctctgtaaa gcatgctgat gatcacacac ctaaaacagt ggaagaagtt acagttgaaa gaaatgagaa acaaacacct tctctccag aaccaaagcc tgtgtatgcc caagtgggc aaccagatgt ggatttacct gtcagtccat ctgatggtgt cctacctaat tcaactcatg aagatgggat tctcggccc agcatgaaat tggtaaaatt cagaaaagga gatagtgtgg gtttcggct ggctgggtgga aatgatgttg gaatatttgt agctggcggt ctagaagata gccctgcagc caaggaaggc ttgaggaag gtgatcaat tctcagggtg aacaacgtag attttacaa tatcataaga gaagaagccg tcttttct gcttgacct cctaaaggag aagaagtac catattggct cagaagaaga aggatgttta tcgtcgcat gtagaatcag atgtaggaga tttttctat attagaacct attttgaata tgaaggaa tctccctatg gacttagttt taacaaagga gaggtgttcc gtgtgtgga tacctgtac aatggaaaac tgggtcttg gcttgctatt cgaattggtg aaaatcataa ggaggtagaa cgaggcatca tccctaataa gaacagagct gagcagctag ccagtgtaca gtatacact caaaaacag caggcggaga ccgtgctgac ttctggagat tcagaggtct tcgagctcc aagagaaatc ttgaaaaag cagagaggat ttgtccgtc agcctgttca acaaagttt ccagcttatg aaagagtggg tcttcagaa gctggatttc tgaggcctgt aaccattttt ggaccaatag ctgatgttc cagagaaaag ctggcaagag aagaaccaga tatttatcaa attgcaaaga gtgaaccag agacgtgga actgaccaac gtactctgg cattattgc ctgcatacaa taaagcaat catagatcaa gacaacatg cttattaga tgaacacca aatgcagttg atcgtcttaa ctatgccag tggatccaa ttgtgtatt tcttaacct gattctaagc aaggagtaaa aacaatgaga atgaggttat gtccagaatc tcggaaaagt gccaggaagt tatcagagcg atctcataaa ctctgtaaaa ataatacca tcttttaca actacaatta actaaatc aatgaatgat ggttggtatg gtgcgtgaa agaagcaatt caacaacagc aaaaccagct ggtatgggtt tccgagggaa aggcggatgg tgctacaagt gatgacctg atttgcata tgatcgtctg tctacctgt cagctccagg tagtgaatac tcaatgtata gcacggacag tagacacact tctgactatg aagacacaga cacagaaggc ggggcctaca	
---	--

ctgatcaaga actagatgaa actcttaatg atgagggtgg gactccaccg gagtctgcca ttacacggtc ctctgagcct gtaagagagg actcctctgg aatgcatcat gaaaacaaaa catacctcc ttactacca caagcgcagc cacaaccaat tcatagaata gactccccctg gatttaagcc agcctctcaa cagaaagcag aagcttcac tccagtcctt tacctttgc ctgaaacaaa cccagcatca tcaacctctg ctgttaatca taatgtaaat ttaactaatg tcagactgga ggagcccacc ccagtcctt ccacctctta ctaccacaa gctgattctt taagaacacc aagtactgag gcagtcaca taatgctaag agatcaagaa ccatactgt cgtcgcattg agatccaaca aagggtgata gaaaggatcc atatcccag gaaatgatga ggcagaacca tgttttga cagccagccg ttatgccccc agggcacagg ccagacaaag agcctaatt gacctatgaa ccccaactcc catacgtaga gaaacaagcc agcagagacc tcgagcagcc cacatacaga tacgagtcct caagctatac ggaccagt tctcgaaact atgaacatcg tctcgcatac gaagatcgcg tccccatgta tgaagaacag tggatcatatt atgatgacaa acagccctac ccactctggc cacttttga taatcagcac tctcaagacc ttgactccag acagcatccc gaagagtcct cagaacgagg gtactttcca cgttttgaag agccagcccc tctgtcttac gacagcagac caggttacga acaggcacct agagcatccg cctgcggca cgaagagcag ccagctctg ggtatgacac acatggtaga ctcagaccgg aagccagcc ccaccttca gcagggccca agcctgcaga gtccaagcag tattttgagc aataatcac cagttacgag caagtaccac ccaaggatt tacctctaga gcaggtcatt ttgagcctct ccattggtct gcagctgtcc ctccgtgat acctcatct cagcataagc cagaagctct gccttcaaac accaaaccac tgcctccacc cccaactcaa accgaagaag aggaagatcc agcaatgaag ccacagtctg tactcaccag agttaagatg ttgaaaaca aaagatctgc atccttagag accaagaagg atgtaatga cactggcagt ttaagcctc cagaagtagc atctaaacct tcaggtgctc ccatactgg tcccaaccc acttctcaga atcaattcag tgaacatgac aaaactctgt acaggatccc agaactcaa aaactcaac tgaagccacc tgaagatatt gtccgtcca atcattatga cctgaagaa gatgaagaat attatcgaaa acagctgta tactttgacc gaagaagttt tgagaataag cctctgcac acattgccgc cagcatctc tccagcctg caaagccagc gcattctcag aatcaatcaa atttttctag ttattctca aagggaagc ctctgaagc tgatggtgtg gatagatcat ttggcgagaa acgtatgaa cccatccagg ccactcccc tctctctca ttgccctgc agtatgccc gccatctcag cctgtacca gcgcgtctct ccacatacat tctaaggag cacatggtga aggtaatca gtgtcattgg atttcagaa ttccttagtg tcaaacag acccactcc atctcagaat aagccagca ctttcagacc accaaacga gaagatactg ctcaggcagc ttctatccc cagaaaagtt ttccagataa agccccagtt aatggaactg aacagactca gaaaacagtc actccagcat acaatcgatt cacacaaaa ccatatacaa gttctgccc accatttgaa cgcaagttg aaagtctaa attcaatcac aatcttctgc caagtgaac tgcacataaa cctgactgt cttcaaaaac tcccacttct ccaaaaact ttgtgaaac gcacagttg gcacagcctc ctgagttga cagtggagt gaaacttct ctatccatgc agagaagcct aaatatcaa taaataatat cagcacagt cctaaagcta ttctgtgag tcttcagct gtggaagagg atgaagatga agatggtcat actgtggtgg ccacagcccc aggcataatt aacagcaatg ggggcgtgct gattccata gaaactggtg ttagtataat tatccctcaa ggagccattc ccgaaggagt tgagcaggaa atctattca aggtctgccg ggacaacagc atcttccac ctttagataa agagaaaggt gaaacactgc tgagtcctt ggtgatgtgt ggtcccatg gcctcaagtt cctgaagcct gtggagctgc gcttaccaca ctgtgacct aaaactggc aaaacaagt tcttcccga gatccaaatt	
--	--

atctcgttgg agcaaactgt gtttctgtcc ttattgacca cttttaactc ttgaaatata ggaacttaaa taatgtgaaa ctggattaaa cttaatctaa atggaaccac tctatcaagt attataccct ttttagagtt gatactacag ttgttagta tgaggcattt gtttgaactg ataaagatga gtgagcatgc ccctgaacca tggctcggaaa acatgctaca cactgcatgt ttgtgattga cgggactgtt ggtattggct agaggttcaa agatattttg ctttgtgatt ttgttaattt tttatcgtc actgcttaac ttcacatatt gatttccgtt aaaaatccag ccagtaaatg ggggtgcatt tgaggctctg tcttccaaa gtacactgtt tcaaacttta ctatggccct ggcctagcat acgtacacat tttattttat tatgcatgaa gtaatatgca cacattttt aaatgcacct ggaatatata accagtgttg tggatttaac agaaatgtac agcaaggaga ttacaactg ggggagggtg aagtgaagac aatgacttac tgtacatgaa aacacatttt tcttagggaa ggatacaaaa gcatgtgaga ctggttccat ggctcttca gatcttaac ttcaccatat taccacagac atactaacca gcagaaatgc cttacctca tgttcttaat tcttagctca ttctcttgt gttactaagt tttatggct ttgtgcatt atctagatac tgtatcatga caaagactga gtacgttgtg catttgggtg ttccagaaat gtgttatcac ccagaagaaa atagtgggtg gatttgggga tattttttc tttctttc tttctttt tttttttt gacaaggggc agtgggtggt ttctgttct tctggctatg catttgaaaa ttgtatgtt ttaaggatgc ttgtacataa tgcgtgcata ccactttgt tcttggttg taaattaact ttataaaact ttacctttt tatacataa caagaccag ttctaaagg ctaccttgt attctctct gtacctctg agcctgaac tttgacctct gcagcaataa agcagcgtt ctatgacaca tgcaaggta tttttttaa gaaaaaggat gcacagagtt gttacattt taagtgtgc atttaaaaga tacagtact cagaattctc tagtttgatt aaattctgc aaagtatccc tactgtaatt tgtgatacaa tgctgtgccc taaagtgtat tttttacta atagacaatt tattatggca catcagcacg attctgttt agataataca ccactacatt ctgttaatca ttagggtgta ctgaattct tttggcgtta ttaaaaatct caaatttcta aatctccaaa ataaaacttt taaaataaa gtgtgtgctt ggtctgttg ccactgtt tctagtcca tgcagctta taactctgt ttaaaatct gcacacaaat ccctatcac cagcgtcac taccacctg tctctgggt ttgcatgcag aattctccc ctggccagc atgtacagat ggttgggcag tgcctatctg aagggtcag actgaagtgg ggcagaagga cctggagaca gagtgggaga aggcagcagg ccgacttccc cctgtgggt aacacacacc cctgcgtgga gaaacacccc tgcattggga cacacgtgcg ttgtgtgtg tgcgtgtaac cattgtata tggttttatt cccagataa atagcacggg cattgtttta tgcaccac atttggggga agaaaatggg tttagtgagc ataaatatt cattgtggac atctactta cttactgcc accctgttg acatttgat tattgtata gtgttcagt aacacctt gtggactgaa tccatctatg tggattttat ttttctc tttgtaagg tataattac atagagtaag ttactgttt taaaatgtat agttcaaatt ttgacaaaca catactgtt atttaccag gttaatacat acaacagtt cttatctt tctaatcatt tgtacctt tatactaac ccttctcca gccctggca ggatgatct tctgtttct atagtgcct tttgacagt gtcatataaa tggaatcata caata	
--	--

Claims

1. Use of a Tjp1 inhibitor in the manufacture of a medicament for treating a liver disease in a subject, wherein the Tjp1 inhibitor is a nucleic acid.
2. Use of a Tjp1 inhibitor in the manufacture of a medicament for regenerating a biliary system in a subject, wherein the Tjp1 inhibitor is a nucleic acid.
3. The use of claim 1 or 2, wherein the nucleic acid is selected from the group consisting of a short hairpin molecule, an shRNA, an siRNA, an antisense oligonucleotide (AON), a gapmer, and a short hairpin Antisense Oligonucleotide (shAON).
4. The use of any one of claims 1 to 3, wherein the nucleic acid comprises at least 90% identity to a sequence selected from a group consisting of 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-ATTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-AAGGAAATTTCAACAAGATAGT-3' (SEQ ID NO: 106), 5'-TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-AAGAACTAGATGAACTCTTA-3' (SEQ ID NO: 109), 5'-CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), and 5'-TAGATAATACACCACTACATT-3' (SEQ ID NO: 113).

5. The use of any one of claims 1 to 4, wherein the nucleic acid comprises at least 95% identity to a sequence selected from a group consisting of 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-ATTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-AAGGAAATTTCAACAAGATAGT-3' (SEQ ID NO: 106), 5'-TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-AAGAACTAGATGAACTCTTA-3' (SEQ ID NO: 109), 5'-CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), and 5'-TAGATAATACACCACTACATT-3' (SEQ ID NO: 113).
6. The use of any one of the preceding claims, wherein the nucleic acid comprises a sequence selected from a group consisting of 5'-CGTGGATTGAACTTACTAAAT-3' (SEQ ID NO: 4), 5'-ATTAGTAAGTTCAATCCACG-3' (SEQ ID NO: 91), 5'-CCGCGAAGTTATGAGCAAGTT-3' (SEQ ID NO: 5), 5'-AACTTGCTCATAACTTCGCGG-3' (SEQ ID NO: 92), 5'-CGGCCATTTGAACGCAAATTT-3' (SEQ ID NO: 6), 5'-AAATTTGCGTTCAAATGGCCG-3' (SEQ ID NO: 93), 5'-GCAATGGTTAACGGAGTTTCA-3' (SEQ ID NO: 104), 5'-AATGGTTAACGGAGTTTCAAT-3' (SEQ ID NO: 105), 5'-AAGGAAATTTCAACAAGATAGT-3' (SEQ ID NO: 106), 5'-TACAAGTGATGACCTTGATTT-3' (SEQ ID NO: 107), 5'-ACTGATCAAGAACTAGATGAA-3' (SEQ ID NO: 108), 5'-AAGAACTAGATGAACTCTTA-3' (SEQ ID NO: 109), 5'-

CCCACCTTTAGATAAAGAGAA-3' (SEQ ID NO: 110), 5'-
 CAGCACGATTTCTGTTTAGAT-3' (SEQ ID NO: 111), 5'-
 AGCACGATTTCTGTTTAGATA-3' (SEQ ID NO: 112), and 5'-
 TAGATAATACACCACTACATT-3' (SEQ ID NO: 113).

5

7. The use of any one of any one of the preceding claims, wherein the nucleic acid comprises at least 90% identity to a sequence selected from the group consisting of:

- i) a combination of SEQ ID NO: 4 and SEQ ID NO: 91, wherein SEQ ID NO: 4 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 4 with the 5' end of SEQ ID NO: 91, and wherein SEQ ID NO: 91 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides;
- ii) a combination of SEQ ID NO: 5 and SEQ ID NO: 92, wherein SEQ ID NO: 5 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 5 with the 5' end of SEQ ID NO: 92, and wherein SEQ ID NO: 92 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides; and
- iii) a combination of SEQ ID NO: 6 and SEQ ID NO: 93, wherein SEQ ID NO: 6 is flanked at the 5' end by a nucleotide sequence comprising 1 to 10 nucleotides, wherein a nucleotide sequence of 1 to 20 nucleotides connects the 3' end of SEQ ID NO: 6 with the 5' end of SEQ ID NO: 93, and wherein SEQ ID NO: 93 is flanked at the 3' end by a nucleotide sequence comprising 1 to 10 nucleotides.

10

15

20

25 8. The use of any one of the preceding claims, wherein the nucleic acid comprises at least 90% identity to a sequence selected from the group consisting of:

**5'-CCGGCGTGGAATTGAACTTACTAAATCTCGAGATTTAGTAAGTTCAAT
 CCACGTTTTTG-3' (SEQ ID NO: 1),**

**5'-CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTT
 CGCGGTTTTTG-3' (SEQ ID NO: 2), and**

**5'-CCGGCGGCCATTTGAACGCAAATTTCTCGAGAAATTTGCGTTCAAAT
 GGCCGTTTTTG-3' (SEQ ID NO: 3).**

30

9. The use of any one of the preceding claims, wherein the nucleic acid comprises at least 95% identity to a sequence selected from the group consisting of:

5'-CCGGCGTGGATTGAACTTACTAAATCTCGAGATTTAGTAAGTTCAAT
5 CCACGTTTTTTG-3' (SEQ ID NO: 1),

5'-CCGGCCGCGAAGTTATGAGCAAGTTCTCGAGAACTTGCTCATAACTT
CGCGGTTTTTTG-3' (SEQ ID NO: 2), and

5'-CCGGCGGGCCATTTGAACGCAAATTTCTCGAGAAATTTGCGTTCAAAT
GGCCGTTTTTTG-3' (SEQ ID NO: 3).

10. The use of any one of claims 1 to 3, wherein the nucleic acid is an siRNA.

11. The use of claim 11, wherein the siRNA comprises at least 90% identity to a sequence selected from a group consisting of 5'-UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'- AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'- ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'- AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'- UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'- UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'- UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'- AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'- UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) and 5'- AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103).

12. The use of claim 10 or 11, wherein the siRNA comprises a sequence selected from a group consisting of 5'-UGAAACUCCGUUAACCAUUGC-3' (SEQ ID NO: 94), 5'- AUUGAAACUCCGUUAACCAUU-3' (SEQ ID NO: 95), 5'- ACUAUCUUGUGAAAUUUCCUU-3' (SEQ ID NO: 96), 5'- AAAUCAAGGUCAUCACUUGUA-3' (SEQ ID NO: 97), 5'- UUCAUCUAGUUCUUGAUCAGU-3' (SEQ ID NO: 98), 5'- UAAGAGUUUCAUCUAGUUCUU-3' (SEQ ID NO: 99), 5'- UUCUCUUUAUCUAAAGGUGGG-3' (SEQ ID NO: 100), 5'-

AUCUAAACAGAAAUCGUGCUG-3' (SEQ ID NO: 101), 5'-
 UAUCUAAACAGAAAUCGUGCU-3' (SEQ ID NO: 102) and 5'-
 AAUGUAGUGGUGUAUUAUCUA-3' (SEQ ID NO: 103).

- 5 13. The use of any one of the preceding claims, wherein the liver disease is selected from
 the group consisting of cholestasis, liver cancer, alcoholic liver disease, nonalcoholic
 steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), cholestatic liver
 10 disease, hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E, liver fibrosis, liver
 cirrhosis, cholestasis-related progressive bile duct injury, cystic fibrosis-associated
 liver disease, thioacetamide (TAA)-related liver disease, 3,5-Diethoxycarbonyl-1,4-
 Dihydrocollidine (DDC)-related liver disease, bile duct ligation liver injury, Yes-
 associated Protein (YAP)-related liver disease, Mdr2-related liver disease, a disease
 15 related to the exposure to medications that affect cholesterol/bile acid (BA) biosynthesis
 and/or metabolism, a disease related to genetic mutations that affect cholesterol/bile
 acid (BA) biosynthesis and/or metabolism, and a disease related to the compromise of
 the integrity the bile blood barrier.

14. The use of claim 13, wherein cholestasis is selected from a group consisting of
 intrahepatic cholestasis, primary biliary cholangitis (PBC), primary sclerosing
 20 cholangitis (PSC), pregnancy-related intrahepatic cholestasis, neonatal cholestasis,
 progressive familial intrahepatic cholestasis type 3, cholestatic fibrosis, and biliary
 atresia.

15. The use of claim 13, wherein the liver cancer is selected from a group consisting of
 25 hepatocellular carcinoma, cholangiocarcinoma, hepatoblastoma, and metastatic liver
 cancer.