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(71) Applicant: **THE UNIVERSITY OF BRITISH COLUMBIA** [CA/CA]; #103-6190 Agronomy Road, Vancouver, British Columbia V6T 1Z3 (CA).

(72) Inventors: **HAYDEN, Michael R.**; 4484 West 7th Avenue, Vancouver, British Columbia V6R 1W9 (CA). **PATANKER, Jay**; #219-1741 West 10th Avenue, Vancouver, British Columbia V6J 2A5 (CA).

(74) Agent: **MACINS, Andris I. D.**; C6 Patent Group Incorporated, Operation as Carbon Patent Group, Unit 203A - 116 Geary Avenue, Toronto, Ontario M6H 4H1 (CA).

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(54) **Title**: MODULATION OF CYP8B1 FOR THE PREVENTION AND TREATMENT OF LIVER FIBROSIS AND METABOLIC DISORDERS

(57) **Abstract**: Provided are oligomers, methods, uses and pharmaceutical compositions for reducing the expression of Sterol 12-α hydroxylase (Cyp8b1) in a cell or tissue with an oligomer described herein in a biologically effective amount sufficient for the treatment of liver fibrosis. The oligomer may be between 4 to 49 nucleobases in length which comprises a contiguous nucleotide sequence of a total of between 4 to 49 nucleotides, wherein the contiguous nucleotide sequence is targeted to hybridize to a portion of SEQ ID NO: 104: wherein the oligomer, when bound to the portion of SEQ ID NO: 104 reduces expression of Sterol 12-α hydroxylase (Cyp8b1) in a cell or tissue.

MODULATION OF CYP8B1 FOR THE PREVENTION AND TREATMENT OF LIVER FIBROSIS AND METABOLIC DISORDERS

TECHNICAL FIELD

The present invention relates to compositions that reduce the expression of the Cyp8b1 gene. The compositions are useful for the prevention and/or treatment of liver fibrosis, chronic liver disease, autoimmune heptatitis, chronic viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. The compositions are also useful for the prevention and treatment of metabolic syndrome, obesity and diabetes.

BACKGROUND

Hepatic bile acid (BA) metabolism is closely linked to the regulation of cholesterol homeostasis and the digestion and absorption of fat through the intestine. The conversion of cholesterol to BAs and the biliary secretion of cholesterol are the two significant pathways for elimination of excessive cholesterol from the body. The immediate products of the BA synthetic pathways in humans are the primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA). Sterol 12-α hydroxylase (Cyp8b1) acts in the neutral BA synthesis pathway and catalyzes the conversion of 7α-hydroxy-4-cholesten-3-one into 7α,12α-dihydroxy-4-cholesten-3-one [Lefebvre, P., *et al.*, *Role of bile acids and bile acid receptors in metabolic regulation.* Physiol Rev, 2009. **89**(1): p. 147-91]. The biosynthetic end products of these two steroids are CA and CDCA, the relative levels of, which determine the hydrophobicity and biological properties of the bile pool [Jones, R.D., *et al.*, *Delineation of biochemical, molecular, and physiological changes accompanying bile acid pool size restoration in Cyp7a1(-/-) mice fed low levels of cholic acid.* Am J Physiol Gastrointest Liver Physiol, 2012. **303**(2): p. G263-74; and Staels, B. and V.A. Fonseca, *Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration.* Diabetes Care, 2009. **32 Suppl 2**: p. S237-45].

Cyp8b1 is a liver-specific gene and no significant expression has been detected in other organs [Jones, R.D., *et al.*, *Delineation of biochemical, molecular, and physiological changes accompanying bile acid pool size restoration in Cyp7a1(-/-) mice fed low levels of cholic acid.* Am J Physiol Gastrointest Liver Physiol, 2012. **303**(2): p. G263-74]. Mice lacking Cyp8b1 are characterized by a complete absence of cholic acid (CA) and CA regulates the synthesis, intestinal absorption and hepatic storage of cholesterol. The overall bile acid pool size is increased in Cyp8b1 knockout mice and is predominantly composed of CDCA and bile acids derived from CDCA such as α, β, ω-muricholic acids (MCA) and ursodeoxycholic acid (UDCA). In addition to surfactant properties, bile acids also act as signaling molecules, activating nuclear farnesoid X receptor (FXR) and G-protein coupled receptor (TGR5) [Lefebvre, P., *et al.*, *Role of bile acids and bile acid receptors in metabolic regulation.* Physiol Rev, 2009. **89**(1): p. 147-91]. Note that different bile acids have opposing effects on FXR activation. Cholic acid is an FXR agonist, whereas α, β, ω- MCA and UDCA are FXR antagonists and inhibition of FXR shows beneficial effects in human obesity [Mueller, M., *et al.*, *Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity.* J Hepatol, 2015. **62**(6): p. 1398-404; and Sayin, S.I., *et al.*, *Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist.* Cell Metab, 2013. **17**(2): p. 225-35]. The functional activation of TGR5 in the intestinal L-cells releases the incretin hormone, glucagon-like peptide -1 (GLP-1) and GLP-1 mimetic drugs improve steatosis in mouse models of NAFLD [Liu, J., *et al.*, *GLP-1 receptor agonists: effects on the progression of non-alcoholic fatty liver disease.* Diabetes Metab Res Rev, 2015. **31**(4): p. 329-35].

Non-alcoholic fatty liver disease (NAFLD) is characterized by an excessive fat accumulation in the liver, termed hepatic steatosis. It is diagnosed as fat exceeding 5% of liver mass occurring due to oversupply of lipids that exceeds the rates of lipid oxidation and export by the liver. NAFLD has become the most common liver disorder in the industrialized world affecting an estimated 150

million people worldwide and together with its downstream sequela, hepatic insulin resistance, is a major contributor to morbidity arising from metabolic disorders [Pagano, G., *et al.*, *Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association.* Hepatology, 2002. **35**(2): p. 367-72; and Perry, R.J., *et al.*, *The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes.* Nature, 2014. **510**(7503): p. 84-91]. There is no consensus on the predominant source of hepatic lipids in NAFLD and there are no treatments available to date. In a certain proportion of individuals a simple steatosis can progress to steato-hepatitis or non-alcoholic steatohepatitis (NASH) characterized by increased inflammation and fibrosis of the liver. The build-up of toxic levels of certain lipids, especially cholesterol has been linked to the progression to NASH. In individuals with NAFLD, dyslipidemia may also manifest as an increase in circulating LDL cholesterol and triglycerides and a reduction in HDL cholesterol [Chatrath, H., R. Vuppalanchi, and N. Chalasani, *Dyslipidemia in patients with nonalcoholic fatty liver disease.* Semin Liver Dis, 2012. **32**(1): p. 22-9; and Kumar, R., *et al.*, *Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver disease in Indian patients with normal body mass index: Do they differ from obese or overweight non-alcoholic fatty liver disease?* Indian J Endocrinol Metab, 2013. **17**(4): p. 665-71].

Cholesterol is a key component of cellular membranes and is crucial for normal cellular and tissue function. Cellular cholesterol levels are controlled through a tightly regulated feedback system. Hepatic cholesterol metabolism is dysregulated in NAFLD patients with increased cholesterol biosynthesis caused by a transcriptional induction of genes involved in cholesterol biosynthesis [Chun, Y.S., *et al.*, *Cholesterol regulates HERG K+ channel activation by increasing phospholipase C beta1 expression.* Channels (Austin), 2013. **7**(4): p. 275-87]. Diets containing >0.5% of cholesterol or high cholesterol diet (HCD), are associated with accelerated fat accumulation in the hepatocytes [Almena, M. and I. Merida, *Shaping up the membrane: diacylglycerol coordinates spatial orientation of signaling.* Trends Biochem Sci, 2011. **36**(11): p. 593-603; and Teratani, T., *et al.*, *A high-cholesterol diet exacerbates liver fibrosis in mice via*

accumulation of free cholesterol in hepatic stellate cells. Gastroenterology, 2012. **142**(1): p. 152-164.e10]. Dietary sterol overload, the liver increases the synthesis of free fatty acids via *de novo* lipogenesis, a prerequisite to minimize the free cholesterol toxicity by esterification into cholesteryl esters [Almena, M. and I. Merida, *Shaping up the membrane: diacylglycerol coordinates spatial orientation of signaling.* Trends Biochem Sci, 2011. **36**(11): p. 593-603; Teratani, T., *et al.*, *A high-cholesterol diet exacerbates liver fibrosis in mice via accumulation of free cholesterol in hepatic stellate cells.* Gastroenterology, 2012. **142**(1): p. 152-164.e10; and Kumashiro, N., *et al.*, *Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease.* Proc Natl Acad Sci U S A, 2011. **108**(39): p. 16381-5].

In humans, an increase in hepatic free cholesterol clearly distinguishes simple NAFLD from NASH [Ginsberg, H.N., *Is the slippery slope from steatosis to steatohepatitis paved with triglyceride or cholesterol?* Cell Metab, 2006. **4**(3): p. 179-81; and Tomita, K., *et al.*, *Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice.* Hepatology, 2014. **59**(1): p. 154-69]. Free cholesterol alone is sufficient to cause the three major pathological transitions in the liver, leading to the progression of NAFLD to NASH. Free cholesterol, **i)** potentiates triacylglycerol accumulation in hepatocytes causing steatosis, **ii)** sensitizes hepatic stellate cells to TGF-β leading to fibrosis and **iii)** activates Kupffer cell inflammatory pathways [Teratani, T., *et al.*, *A high-cholesterol diet exacerbates liver fibrosis in mice via accumulation of free cholesterol in hepatic stellate cells.* Gastroenterology, 2012. **142**(1): p. 152-164.e10; Tomita, K., *et al.*, *Free cholesterol accumulation in hepatic stellate cells: mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice.* Hepatology, 2014. **59**(1): p. 154-69; and van der Veen, J.N., *et al.*, *Cholesterol feeding strongly reduces hepatic VLDL-triglyceride production in mice lacking the liver X receptor alpha.* J Lipid Res, 2007. **48**(2): p. 337-47]. In addition, mitochondrial free cholesterol accumulation leads to an induction of apoptosis in hepatocytes, leading to further inflammatory cell infiltration.

Other studies with mice having a targeted disruption of *Cyp8b1* (*Cyp8b1*$^{-/-}$) suggest a role for the enzyme's activity in metabolic syndrome and diabetes. Kaur and colleagues showed that the absence of Cyp8b1 correlated with increased GLP-1 release and improved β-cell function [Kaur, A., *et al.*, *Loss of Cyp8b1 improves glucose homeostasis by increasing GLP-1.* Diabetes, 2015. **64**(4): p. 1168-79]. *Cyp8b1*$^{-/-}$ x *ApoE*$^{-/-}$ mice show reduced atherosclerotic plaques, owing to decreased levels of apolipoprotein B (ApoB)-containing lipoproteins in the plasma, reduced hepatic cholesteryl esters and enhanced bile acid synthesis [Slatis, K., *et al.*, *Abolished synthesis of cholic acid reduces atherosclerotic development in apolipoprotein E knockout mice.* J Lipid Res, 2010. **51**(11): p. 3289-98].

PCT patent application WO2010009534 discloses the identification of genes related to lipid metabolism, more particularly genes which are modulated in the absence of apolipoprotein A1 (ApoA1). Thus, WO2010009534 provides 1) screening methods for the identification of compounds that modulate the activity and/or expression level of more or more high-density lipoprotein cholesterol (HDLc) related genes or encoded proteins identified herein; 2) methods of treating or preventing lipid metabolism associated diseases; 3) diagnostic methods and kits for detecting a predisposition to lipid metabolism associated diseases and disorders; 4) animal models for identifying compounds for treating, preventing or decreasing susceptibility to a lipid metabolism associated disease or a HDL-metabolism disease; and 5) HDLc related genes and encoded proteins.

PCT patent application WO2014018375 describes embodiments that relate to the diagnosis, prevention and/or treatment of diseases related to abnormal lipid metabolism. More particularly, disclosed therein is the discovery in human subjects having elevated HDL of mutated human CYP8B1 encoding genes and their encoded proteins, and exploitation of this discovery for use in compositions and methods for the diagnosis, treatment and prevention of cardiovascular diseases, such as dyslipidemia, atherosclerosis, low HDL diseases and related disorders, including methods for identifying agents that modulate CYP8B1 activity. Accordingly there are provided methods for detecting,

diagnosing, prognosing or determining a predisposition to diseases related to abnormal lipid metabolism, as well as CYP8B1-directed screening assays, kits, antibodies, agents, nucleic acids, polypeptides, cells, vectors, transgenic animals and compositions.

SUMMARY

The present invention provides methods of treating or preventing diabetes, obesity, metabolic syndrome, NAFLD or NASH in a subject, a method comprising administering a therapeutically effective amount of a sterol 12-α hydroxylase (Cyp8b1) inhibitor compound to a subject.

The Cyp8b1 inhibitor compound may be an interference oligonucleotide. The compound may be an RNA interference, antisense compound or a locked-nucleic acid gapmer oligonucleotide. The compound may be an oligonucleotide with substantial sequence similarity to SEQ ID NO: 1-2.

Illustrative embodiments of the present invention provide an oligomer of between 4 to 49 nucleobases in length which comprises a contiguous nucleotide sequence of a total of between 4 to 49 nucleotides, wherein the contiguous nucleotide sequence is targeted to hybridize to a portion of SEQ ID NO: 104:

ATGGTTCTCTGGGGTCCAGTGCTGGGAGCTCTGCTGGTGGTCATTGCTGGA□ACCTGTG
CCTGCCAGGGATGCTCCGACAACGCAGGCCATGGGAGCCCCCTCTGGACAAGGGTACCG
TGCCCTGGCTTGGCCATGCCATGGCTTTCCGGAAGAATATGTTTGAATTTC□GAAGCGC
ATGAGGACCAAGCATGGGGATGTGTTCACAGTGCAGCTAGGGGGCCAGTAC□TCACCTT
CGTCATGGACCCCCTCTCCTTTGGCTCCATCCTCAAGGACACACAGAGAAAACTAGACT
TTGGGCAATATGCAAAAAAACTGGTGCTGAAGGTATTTGGATACCGTTCAG□GCAAGGG
GACCATGAGATGATACACTCAGCCAGCACCAAGCATCTGAGGGGGGATGGC□TGAAGGA
TCTTAATGAGACCATGCTGGACAGCCTGTCCTTTGTAATGCTGACGTCCAAAGGCTGGA
GTCTGGATGCCAGTTGCTGGCATGAGGACAGCCTCTTTCGCTTCTGCTATTACATCTTG
TTCACAGCTGGCTACCTGAGCTTGTTCGGCTACACGAAGGACAAGGAGCAGGACCTGCT
ACAGGCAGGAGAGTTATTCATGGAGTTCCGCAAGTTTGACCTTCTTTTCCCAAGGTTTG
TCTACTCCCTGCTGTGGCCCCGGGAGTGGCTAGAAGTGGGCCGACTCCAGCGTCTCTTT
CACAAGATGCTCTCCGTGAGCCACAGCCAGGAGAAGGAGGGCATCAGCAAC□GGCTGGG

CAACATGCTTCAGTTTCTGAGGGAGCAGGGGGTACCCTCAGCTATGCAGGACAAGTTCA
ACTTCATGATGCTCTGGGCCTCCCAGGGGAACACGGGGCCTACCTCTTTCTGGGCCCTC
TTGTACCTCCTGAAGCACCCAGAAGCTATTCGGGCTGTGAGGGAGGAAGCTACCCAGGT
CCTGGGTGAGGCCAGGCTGGAGACCAAGCAGTCCTTTGCCTTCAAACTCGGTGCCCTGC
AACACACCCCAGTTCTAGACAGCGTGGTGGAGGAGACGCTGCGGCTGAGGGCTGCACCC
ACCCTCCTCAGGTTGGTTCATGAAGACTATACCCTGAAGATGTCCAGTGGGCAGGAGTA
TCTGTTCCGCCATGGAGACATCCTGGCCCTCTTTCCCTACCTCTCAGTGCACATGGACC
CTGACATCCACCCTGAGCCCACCGTCTTCAAGTACGATCGCTTCCTCAACCCTAATGGC
AGCCGGAAAGTGGACTTCTTCAAGACAGGCAAGAAGATCCACCACTACACCATGCCCTG
GGGTTCGGGCGTTTCCATCTGCCCTGGGAGGTTCTTTGCACTCAGTGAGGTGAAGCTCT
TTATCCTGCTTATGGTCACACACTTTGACTTAGAGTTGGTGGACCCTGACACACCACTA
CCCCATGTTGACCCGCAGCGCTGGGGTTTTGGCACCATGCAGCCCAGCCACGATGTGCG
CTTCCGCTACCGCCTGCATCCTACAGAGTGA (SEQ ID NO: 104), wherein the oligomer, when bound to the portion of SEQ ID NO: 104 reduces expression of Sterol 12-α hydroxylase (Cyp8b1) in a cell or tissue.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer is between 14 to 29 nucleotides in length.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer is between 18 to 23 nucleotides in length.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer is between 21 to 27 nucleotides in length.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the cell or tissue is a liver cell or a liver tissue.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer comprises a sequence selected from the group consisting of SEQ ID NOs 57 to 103, 105 and 106:

SEQ ID NO	*Sequence 5'-3'*
57	CGGAAGAATATGTTTGAATTTCT
58	TGGGCAATATGCAAAAAAACTGG
59	AATGAGACCATGCTGGACAGCCT
60	CCGCAAGTTTGACCTTCTTTTCC
61	GGGCAACATGCTTCAGTTTCTGA

SEQ ID NO	Sequence 5'-3'
62	AGGACAAGTTCAACTTCATGATG
63	GCCCTCTTGTACCTCCTGAAGCA
64	CTCCTCAGGTTGGTTCATGAAGA
65	AAGACTATACCCTGAAGATGTCC
66	TCATGAAGACTATACCCTGAAGA
67	CCGGAAAGTGGACTTCTTCAAGA
68	GTGAAGCTCTTTATCCTGCTTAT
69	CACACACTTTGACTTAGAGTTGG
70	AGTGCTGGGAGCTCTGCTGGTGGTCAT
71	ATGCCATGGCTTTCCGGAAGAATATGT
72	CGTCATGGACCCCCTCTCCTTTGGCTC
73	ACTTTGGGCAATATGCAAAAAAACTGG
74	ATGGCTTGAAGGATCTTAATGAGACCA
75	AATGAGACCATGCTGGACAGCCTGTCC
76	TGGAGTTCCGCAAGTTTGACCTTCTTT
77	ACATGCTTCAGTTTCTGAGGGAGCAGG
78	ACCCTCAGCTATGCAGGACAAGTTCAA
79	CTCTTGTACCTCCTGAAGCACCCAGAA
80	CTGCGGCTGAGGGCTGCACCCACCCTC
81	GAAGACTATACCCTGAAGATGTCCAGT
82	ATGGCAGCCGGAAAGTGGACTTCTTCA
83	TCCACTACACCATGCCCTGGGGTTCGG
84	TGAAGCTCTTTATCCTGCTTATGGTCAC
85	GACTTAGAGTTGGTGGACCCTGACACA
86	TCCAGTGCTGGGAG
87	CCGGAAGAATATGT
88	ACAGAGAAAACTAG
89	ATGGCTTGAAGGAT
90	ATGCTGGACAGCCT
91	TGGAGTTCCGCAAG
92	TCTGAGGGAGCAGG
93	CAGCTATGCAGGAC
94	GTTCAACTTCATGA
95	TTGTACCTCCTGAA
96	AGAAGCTATTCGGG
97	GGCTGAGGGCTGCA
98	TCCTCAGGTTGGTT
99	ACTATACCCTGAAG
100	AAAGTGGACTTCTT
101	TACACCATGCCCTG
102	TGAAGCTCTTTATC
103	ACTTAGAGTTGGTG

SEQ ID NO	Sequence 5'-3'
105	GCTCACTTCCACCCACTCCC
106	TTCATCTCGCTGAGGGCAAA

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Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer is modified to include one ore more locked nucleic acid bases and one or more phosphorothioate modification.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the contiguous nucleotide sequence comprises nucleotide analogues.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer has a modified internucleoside linkage.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the modified internucleoside linkage is a peptide-nucleic acid linkage, a morpholino linkage, a N3' to P5' phosphoramidate linkage, a methylphosphonate linkage or a phosphorothioate linkage.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer has one or more modified sugar moiety.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the modified sugar moiety is 2 ' -O-alkyl oligoribonucleotide.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer is a gapmer.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer has a 2'MOE gapmer modification.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer has a modified nucleobase.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the modified nucleobase is a 5-methyl pyrimidine or a 5- propynyl pyrimidine.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the one or more nucleotide analogues comprise locked nucleic acids (LNA) units.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the LNA units consist of beta-D- oxy-LNA monomers.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer is selected from the group consisting of: 5' +G*+C*+T*+C*A*C*T*T*C*C*A*C*C*C*A*C*+T*+C*+C*+C 3' (SEQ ID NO:1); and 5' +T*+T*+C*+A*T*C*T*C*G*C*T*G*A*G*G*G*+C*+A*+A*+A3' (SEQ ID NO: 2), wherein '+' denotes a locked nucleic acid base and '*' indicates a phosphorothioate modification.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer comprises a single stereoisomer.

Illustrative embodiments of the present invention provide an oligomer described herein wherein the oligomer comprises a mixture of stereoisomers.

Illustrative embodiments of the present invention provide a pharmaceutical composition comprising an oligomer described herein and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

Illustrative embodiments of the present invention provide a method of reducing the expression of Sterol 12-α hydroxylase (Cyp8b1) in a cell or tissue, comprising contacting the cell or tissue with an effective amount of an oligomer described herein or a pharmaceutical composition described herein.

Illustrative embodiments of the present invention provide a method described herein wherein the cell is within a tissue of a mammal.

Illustrative embodiments of the present invention provide a method of prophylactic treating or treating a disease selected from the group consisting of: Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes, in a subject, comprising administering to the subject an effective amount of an oligomer described herein or administering a an effective amount of a

pharmaceutical composition described herein, wherein the subject is currently suffering from or at risk for suffering from liver fibrosis.

Illustrative embodiments of the present invention provide a method of described herein wherein the subject is a mammal.

Illustrative embodiments of the present invention provide a method described herein wherein the mammal is a human.

Illustrative embodiments of the present invention provide use of an oligomer described herein for the treatment of a disease selected from the group consisting of: Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

Illustrative embodiments of the present invention provide use of an oligomer described herein in the preparation of a medicament for the treatment of a disease selected from the group consisting of Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

Illustrative embodiments of the present invention provide use of a pharmaceutical composition described herein for the treatment of a disease selected from the group consisting of Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

Illustrative embodiments of the present invention provide a use described hierein wherein the disease is liver fibrosis.

Illustrative embodiments of the present invention provide an oligomer described herein for use in the treatment of a disease selected from the group consisting of Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver

disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

Illustrative embodiments of the present invention provide a commercial package, comprising: a. an oligomer of any one of claims 1 to 21; and b. instructions for the treatment of a disease selected from the group consisting of: Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

Illustrative embodiments of the present invention provide a commercial package described herein wherein the instructions are for the treatment of liver fibrosis.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: (A) Shows a comparison of whole livers and liver sections from mice that were fed normal chow (Chow) or a high-cholesterol diet (HCD). Right panel show the whole liver appearance of chow and HCD; center panel shows Massons' Trichrome staining of 10μM liver sections (200X); and right panel shows the steatosis area of the liver. The histogram shows the quantitation of the steatosis area, as a percentage of total area (B) Oil Red O staining of 25μM cryosections of livers (400X) showing neutral lipid staining. (C) Massons' trichrome staining of 10μM liver sections (200X) and analysis of collagen positive area, histogram: quantitation of collagen positive area, percent of total area. * $p<0.05$, *** $p<0.0001$, mean ± SEM, Mann-Whitney test.

Figure 2: (A) Representative micrographs (200X) from 9 control (+/+) and 9 Cyp8b1 knockout mice (-/-) fed 0.5% high cholesterol diet for 12 weeks. (B) Representative micrographs (200X) from livers stained for Massons' Trichrome and analysis of steatosis area. The histogram shows a quantitation of the

steatosis area (as a percentage of total area). n= 8-9, *** p<0.001, mean ± SEM, Mann-Whitney test.

Figure 3: (A) Total triglyceride levels and (B) total cholesterol levels from the livers of control (+/+) and Cyp8b1 knockout mice (-/-) fed 0.5% high cholesterol diet for 12 weeks. n = 4, *** p<0.001 and ** p<0.01, Student's t-test.

Figure 4: (A) Representative micrographs (200X) from livers stained for Massons' Trichrome and analysis of collagen positive area, histogram: collagen positive area (percent of total area). n= 8-9, * p<0.05, mean ± SEM, Mann-Whitney test. (B) Biochemical estimation of total collagen in the livers of 4 control (+/+) and 5 Cyp8b1 knockout mice (-/-) fed HCD.* p<0.05, mean ± SEM, Students t-test.

Figure 5: (A) Representative micrographs (200X) from livers stained for α-SMA (Red) and nuclei (blue = DAPI) and the analysis of α-SMA positive area from 5 control (+/+) and 5 Cyp8b1 knockout mice (-/-) livers, (B) histogram: α-SMA positive area (percent of total area). n= 5, ** p<0.01, mean ± SEM, Mann-Whitney test. (C) Representative western blot analysis of Calnexin as control and α-SMA proteins in +/+ and -/- liver homogenates, histogram: quantification of relative band densities from 4 control (+/+) and 4 Cyp8b1 knockout (-/-) liver homogenates relative to those of Calnexin. (D) Hepatic mRNA expression of various fibrotic genes relative to Cyclophilin. n = 4, mean ± SEM, *p<0.05, randomization and bootstrapping tests.

Figure 6: (A) Representative micrographs (400X) from livers stained for Massons' Trichrome and analysis of inflammatory infiltrates (groups of dense eosinophilic nuclei marked yellow) in control (+/+) and Cyp8b1 knockout (-/-) livers from mice fed HCD. (B) Inflammation scores based on numbers of infiltrating leucocytes invading the liver parenchyma in control (+/+) and Cyp8b1 knockout (-/-) livers. n = 5-6, mean ± SEM, *** p < 0.0001, Mann-Whitney test. (C) mRNA expression of various inflammatory genes relative to Cyclophilin as house-keeping control, from the livers of control and Cyp8b1 knockout mice fed HCD. n = 4, mean ± SEM, *p<0.05, randomization and bootstrapping tests.

Figure 7: (A) mRNA expression of various FXR target genes relative to Cyclophilin as house-keeping control, from the livers of control (+/+) and Cyp8b1 knockout (-/-) mice fed HCD. n = 4, mean ± SEM, *p<0.05, randomization and bootstrapping tests. (B) mRNA expression of FXR target genes *Bsep* and *Mdr3* and lipogenic genes *Fas* and *Scd1* in isolated primary steatotic hepatocytes treated with 5, 50 and 100 micro molar alpha-muricholic acid. n = 3, mean ± SEM, ns =not significant, *p<0.05, **p<0.01 and ***p<0.001. Students' t-test.

Figure 8: Relative Cyp8b1 transcript levels in primary mouse hepatocytes upon 72h of treatment with two LNA gapmer hybrid oligonucleotides. Sequence Cyp8b1 (2_1) (SEQ ID NO:1) produced a 60% knockdown at the highest concentration tested. No differences in cell death were observed as measured with the LDH release assay at any of the concentrations.

Figure 9 shows Western blot analysis for Cyp8b1 or the Calnexin control following Cyp8b1 silencing in HepG2 cells using siRNAs: A) HDL2 si1; B) HDL2 si 101; C) HDL2 si 103; D) HDL2 si 109; E) HDL2 Dsi 2; and F) HDL2 Dsi 13.

Figure 10 shows Western blot analysis for Cyp8b1 or the Calnexin control following Cyp8b1silencing in HepG2 cells using LNA gapmers: A) HDL2 LNA 1; and B) HDL2 LNA 3.

Figure 11 shows *in vivo* silencing with HDL2 LNA 500125 5'-ATCTCGCTGAGGGCA-3': A) Concentration dependent increase in fecal fat excretion; B) Enhanced fecal fat mass at 3mg/kg/day; C) Increased fecal cholesterol excretion; D) Body weight of mice before and after treatment with either HDL2 LNA or negative control LNA; and E) Hepatic Cyp8b1 protein levels and quantification.

Figure 12 shows lower fat mass and increased metabolic rate in Cyp8b1 knockout mice: A) graph of body weight in grams vs. time for both knockout and control; B) graph of body weight as a percentage vs. time for both knockout and control; C) graph of organ weight in grams of various organs for both knockout and control; D) graph of body composition in weight for fat mass, lean mass and body weight for both knockout and control; E) graph of food intake for day, night and total for both knockout and control; F) graph of oxygen consumption in

milliliters per hour vs. time in hours for both knockout and control; G) graph of carbon dioxide production in milliliters per hour vs time in hours for both knockout and control; H) graph of respiratory exchange ratio vs. time in hours for both knockout and control and a second graph of mean respiratory exchange ratio during the day and during the night for both knockout and control; I) graph of energy expenditure in kilocalories per hour vs. time in hours for both knockout and control; and J) graph of locomotor activity in counts vs. time in hours for both knockout and control and a second graph of locomotor activity in counts during the day and during the night and total locomotor counts for both knockout and control.

Figure 13 shows lower hepatic damage and increased fecal cholesterol and fat excretion in Cyp8b1 knockout mice: A) graph showing AST in milliU/mL for both knockout and control; B) graph showing fecal dry weight in grams/day for both knock out and control; C) graph showing fecal lipid excretion in mg/day for both knockout and control; D) graph showing cholesterol excretion in mg/day for both knockout and control; and E) graph showing free fatty acids (FFA) excretion in µmol/day for both knockout and control.

Figure 14 A) shows two pie charts depicting the phylum level microbiota for cyp8b1 knockout mice and control mice; B) is a dendrogram distance tree of the microbiota; and C) shows bar graphs of the Genus level microbiota.

Figure 15 A) shows Mean free energy structure of the Hs_Cyp8b1 cDNA of minimum free energy of -577.70 kcal/mol; B) shows a plot depicting the interaction free energy (GREY) ΔGi and the energy needed to open existing structures in the longer sequence (BLACK) on the y-axis and nucleotide position on the x-axis; and C) shows the base pair probabilities of targeting sites.

DETAILED DESCRIPTION

Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the art of the invention. As employed throughout the specification, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

As used herein a 'subject' refers to an animal or a mammal. Specific animals include rat, mouse, dog, cat, cow, sheep, horse, pig or primate. A subject may further be a human, alternatively referred to as a patient. A subject may further be a transgenic animal. A subject may further be a rodent, such as a mouse or a rat.

As used herein, an 'inhibitor' refers to a drug, compound or an agent that restrains, retards, or otherwise causes inhibition of a physiological, chemical or enzymatic action or function. An inhibitor may cause at least 5% decrease in enzyme activity. An inhibitor may also refer to a drug, compound or agent that prevents or reduces the expression, transcription or translation of a gene or protein. An inhibitor may reduce or prevent the function of a protein, for instance binding to and/or activating another protein or receptor. An inhibitor may reduce or prevent the interaction of an enzyme or protein with another enzyme or protein. According to certain embodiments of the invention, there are provided compounds which are sterol 12-α hydroxylase (Cyp8b1) inhibitors.

The term Cyp8b1 or sterol 12-alpha hydroxlase refers to the polypeptide expression product of the Cyp8b1 gene, the human orthologue corresponding to EntrezGene # 1582, NCBI Reference Protein NP_004382. Homologous Cyp8b1 enzymes are found in other species which bear sequence similarity to human Cyp8b1 and may thus find utility in the present invention, including but not limited to mouse (EntrezGene # 13124; NCBI Reference Protein NP_034142) and rat (EntrezGene # 81924; NCBI Reference Protein NP_112520.1).

The compounds of the invention may be RNA compounds, for instance RNA interference compounds. The term 'RNA' or 'RNA compound' as used in the present invention includes RNA molecules, RNA segments and RNA fragments. RNA may be single stranded, double stranded, synthetic, isolated, partially isolated, essentially pure or recombinant. RNA interference compounds may be comprised of a single stereoisomer or a combination of selected stereoisomers. RNA compounds may be naturally occurring, or they may be altered such that they differ from naturally occurring RNA compounds. Alterations may include addition, deletion, substitution or modification of existing nucleotides. Such

nucleotides may be either naturally occurring, or non-naturally occurring nucleotides. Alterations may also involve addition of non-nucleotide material, for instance at the end or ends of an existing RNA compound, or at a site that is internal to the RNA compound (i.e. at one or more nucleotides).

The RNA compounds of the invention are capable of target-specific modulation of gene expression and typically exert their effect either by mediating degradation of the mRNA products of the target gene, or by preventing protein translation from the mRNA of the target gene. Such RNA compounds may thus also be referred to as 'RNA interference compounds'. The overall effect of interference with mRNA function is modulation of expression of the product of a target gene. In the context of this invention, 'modulation' means either inhibition or stimulation – i.e. either a decrease or an increase in expression. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay or reverse transcriptase PCR of mRNA expression, Western blot or ELISA assay of protein expression, or by immunoprecipitation assay of protein expression.

In some embodiments of the invention, the RNA compounds are antisense RNA compounds. Antisense RNA compounds are typically single stranded RNA compounds which bind to complementary RNA compounds, such as target mRNA molecules, and block translation from the complementary RNA compounds by sterically interfering with the normal translational machinery. This process is usually passive, in that it does not require or involve additional enzymes to mediate the RNA interference process. Specific targeting of antisense RNA compounds to inhibit the expression of a desired gene may generally involve designing the antisense RNA compound to have a homologous, complementary sequence to the desired gene. Perfect homology is not necessary for the RNA interference effect. In one embodiment of the invention, the antisense RNA compounds include any RNA compound with sufficient complementary homology to bind to the Cyp8b1 mRNA transcript causing a reduction in translation of Cyp8b1 protein.

In other embodiments of the invention, the RNA compounds may be small interfering RNA (siRNA) compounds. Typically, siRNA compounds are short double stranded RNA compounds between 4 and 49 nucleotides in length. More preferably, siRNA compounds are between 16 to 29 nucleotides in length, even more preferably between 18 to 23 nucleotides in length and most preferably between 21-23 nucleotides in length. The siRNA compounds may include short nucleotide 'overhangs' on each end, which are single stranded extensions which are not paired with a complementary base on the opposite strand. The overhangs would preferably be on the 3' end of each strand of the siRNA compound, and are typically 1-3 nucleotides in length. The siRNA compounds of the present invention may be synthesized as individual strands which are subsequently annealed to produce the double stranded siRNA compound. Alternately, the siRNA compounds may derived from a short hairpin RNA (shRNA) molecule, or from a longer RNA compound, which has been processed by the cellular enzyme called dicer, which processes the longer RNA compounds to produce siRNA compounds. Generally, siRNA compounds mediate RNA interference via an enzyme-dependent process in which the target mRNA is degraded, such that it can no longer be translated into its associated protein product. Not being bound by theory, the double stranded siRNA compounds are separated into single stranded molecules and integrated into an activated 'RISC complex'. After integration into the RISC, siRNAs base-pair to their target mRNA and induce cleavage of the mRNA, thereby preventing it from being used as a translation template.

The antisense compounds and siRNA compounds can be modified to enhance the stability of the oligonucleotides, particularly for *in vivo* use. Numerous examples of methods for designing and optimizing antisense RNA compounds are found in the journal literature [Peek, A.S. and M.A. Behlke, *Design of active small interfering RNAs.* Curr Opin Mol Ther, 2007. **9**(2): p. 110-8; Patzel, V., *In silico selection of active siRNA.* Drug Discov Today, 2007. **12**(3-4): p. 139-48; and Pan, W.H. and G.A. Clawson, *Identifying accessible sites in RNA: the first step in designing antisense reagents.* Curr Med Chem, 2006.

13(25): p. 3083-103]. Perfect sequence complementarity is not necessary for the siRNA compound or antisense compound to modulate expression of the target gene. The present invention provides non-limiting examples of locked nucleic acid gapmer oligonucleotides which modulate the expression of Cyp8b1 and are thus Cyp8b1 inhibitors.

"Oligomer" as used herein is meant to encompass any nucleic acid silencing agent (for example, siRNA, miRNA, ASO in all of their modified forms as described herein) and compositions comprising the nucleic acid silencing agent. An oligomer may act by hybridizing to a target sequence.

The term 'medicament' as used herein refers to a composition that may be administered to a patient or test subject and is capable of producing an effect in the patient or test subject. The effect may be chemical, biological or physical, and the patient or test subject may be human, or a non-human animal, such as a rodent or transgenic mouse, or a dog, cat, cow, sheep, horse, hamster, guinea pig, rabbit or pig. The medicament may be comprised of the effective chemical entity alone or in combination with a pharmaceutically acceptable excipient.

The term 'pharmaceutically acceptable excipient' may include any and all solvents, dispersion media, coatings, antibacterial, antimicrobial or antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. An excipient may be suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, intrathecal, topical or oral administration. An excipient may include sterile aqueous solutions or dispersions for extemporaneous preparation of sterile injectable solutions or dispersion. Use of such media for preparation of medicaments is known in the art [Remington, *The Science and Practice of Pharmacy*, Edited by Allen, Loyd V., Jr, 22nd edition, 2012, Pharmaceutical Press].

Compositions or compounds according to some embodiments of the invention may be administered in any of a variety of known routes. Examples of methods that may be suitable for the administration of a compound include orally, intravenous, inhalation, intramuscular, subcutaneous, intraperitoneal, intra-rectal or intra-vaginal suppository, sublingual, and the like. The compounds of the

present invention may be administered as a sterile aqueous solution, or may be administered in a fat-soluble excipient, or in another solution, suspension, patch, tablet or paste format as is appropriate. Other agents may be included in combination with the compounds of the present invention to aid uptake or metabolism, or delay dispersion within the host, such as in a controlled-release formulation. Examples of controlled release formulations will be known to those of skill in the art, and may include microencapsulation, embolism within a carbohydrate or polymer matrix, and the like. Other methods known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences", (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, Pa.; and Remington, *The Science and Practice of Pharmacy*, Edited by Allen, Loyd V., Jr, 22nd edition, 2012, Pharmaceutical Press]

Compounds and compositions as described herein or for use as described herein may be administered by means of a medical device or appliance such as an implant, graft, prosthesis, stent, etc. Also, implants may be devised which are intended to contain and release such compounds or compositions. An example would be an implant made of a polymeric material adapted to release the compound over a period of time.

The dosage of the compositions or compounds of some embodiments of the invention may vary depending on the route of administration (oral, intravenous, or the like) and the form in which the composition or compound is administered (solution, controlled release or the like). Determination of appropriate dosages is within the ability of one of skill in the art. As used herein, an 'effective amount', a 'therapeutically effective amount', or a 'pharmacologically effective amount' of a medicament refers to an amount of a medicament present in such a concentration to result in a therapeutic level of drug delivered over the term that the drug is used. This may be dependent on mode of delivery, time period of the dosage, age, weight, general health, sex and diet of the subject receiving the medicament. Methods of determining effective amounts are known in the art. It is understood that it could be potentially beneficial to restrict delivery of the compounds of the invention to the target tissue or cell in which inhibition of

Cyp8b1 is desired, such as the liver for example. It is also understood that it may be desirable to target the compounds of the invention to a desired tissue or cell type. The compounds of the invention may thus be coupled to a targeting moiety. The compounds of the invention may be coupled to a cell uptake moiety. The targeting moiety may also function as the cell uptake moiety.

"Oligomer" as used herein is meant to encompass any nucleic acid silencing agent (for example, siRNa, miRNA, ASO in all of their modified forms as described herein) and compositions comprising the nucleic acid silencing agent. An oligomer may act by hybridizing to a target sequence.

A phosphorothioate oligonucleotide bond modification alters the phosphate linkage by replacing one of the non-bridging oxygens with sulfur. The introduction of phosphorothioate linkages alters the chemical properties of the oligonucleotide. In particular, the addition of phosphorothioate linkages reduces nuclease degradation of the oligonucleotide, thereby increasing the half-life *in situ*. Accordingly, this modification is particularly useful for antisense oligonucleotides, which when introduced into cells or biological matrices can interact with target nucleic acids to silence the expression of a particular transcript. Oligonucleotides containing phosphorothioate linkages accomplish this feat either through direct blockage of translation or enabling enzymatic degradation of the target transcript (for example, via RNase H).

Although phosphorothioate linkages provide improved half-life, the introduction of these linkages into an oligonucleotide may also introduce limitations to their function as antisense oligonucleotides. Each phosphorothioate linkage creates a chiral center at each bond, which may result in multiple isomers of the oligonucleotide generated during synthesis and the isomers may have differential characteristics and functional properties. However, much of the isomer effects may be mitigated through careful positioning of the modifications or by using additional modifications in conjunction with the phosphorothioate bonds.

One or more of the phosphodiester linkages of the oligonucleotide moiety may be modified by replacing one or both of the two bridging oxygen atoms of

the linkage with analogues such as -NH, -CH2, or -S. Other oxygen analogues known in the art may also be used.

A "modified oligonucleotide" as used herein is meant to include oligonucleotides that are substituted or modified. In addition to the naturally occurring primary bases adenine, guanine, cytosine, and thymine, or other natural bases such as inosine, deoxyinosine, and hypoxanthine, there are numerous other modifications. For example, isosteric purine 2 ' deoxy-furanoside analogues, 2 ' -deoxynebularine or 2 ' deoxyxanthosine, or other purine and pyrimidine analogues such as 5-methyl pyrimidine or a 5-propynyl pyrimidine may also be utilized to improve stability and target hybridization.

A "modified sugar" as used herein when discussing an oligonucleotide moiety, a sugar modified or replaced so as to be ribose, glucose, sucrose, or galactose, or any other sugar. Alternatively, the oligonucleotide may have one or more of its sugars substituted or modified in its 2' position, i.e. 2 ' alkyl or 2'-0-alkyl. An example of a 2 ' -O-alkyl sugar is a 2 ' -O-methylribonucleotide. Furthermore, the oligonucleotide may have one or more of its sugars substituted or modified to form an α-anomeric sugar.

"Second-generation" oligonucleotides as used herein may be defined as oligonucleotides that are resistant to degradation by cellular nucleases and capable of hybridizing specifically to their target precursor mRNA or mRNA with equal or higher affinity than first generation ASOs. An example of a 2nd generation ASO is a 2'-O-(2-Methoxyethyl)-RNA (2'MOE gapmer modification). With a 2'-MOE or a 2'OMe gapmer the 5' and 3' ends may have 2'-MOE modified nucleotides to protect against degradation, but the gap between the 5' and 3' ends may be unmodified phosphodiester or phosphorothioate linkages that are substrates for RNase H. Numerous other chemical modifications have been developed to improve ASOs. For example, morpholino, N3' to P5' phosphoramidate, and methylphosphonate chemical modifications are known in the art (N. Dias, and C. A. Stein 2002). Furthermore, peptide nucleic acids (PNAs) may also be used.

"LNA" as used herein refers to a Locked Nucleic Acid, which is an RNA analog in which the ribose ring is connected by a methylene bridge between the 2'-O and 4'-C atoms thus "locking" the ribose ring in the ideal conformation for Watson-Crick binding. When incorporated into a DNA or RNA oligonucleotide LNAs make the pairing with a complementary nucleotide strand more rapid and increases the stability of the resulting duplex. LNA oligonucleotides have better thermal stability when hybridized to a complementary DNA or RNA strand. Furthermore, LNA oligonucleotides may be made shorter than traditional DNA or RNA oligonucleotides. LNA oligonucleotides are especially useful to detect small or highly similar targets.

A "nucleic acid silencing agent" or an "agent" refers to a composition that acts in a sequence specific manner to effect a reduction in the level of a product (a "gene product") of a given nucleic acid sequence (e.g. a 'gene'). The reduction may be effected by interference with any of the processing of a pre-mRNA following transcription from the DNA of a cell or subject (e.g. splicing, 5' capping, 5' or 3' processing, or export of the processed mRNA to the cytoplasm) or by interference with translation of a mature mRNA, or by specific, directed destruction of the pre-mRNA or mature mRNA. Antisense (ASO) and RNA interference (RNAi - effected by short interfering RNA, or siRNA) are two examples of such methods; microRNA (miRNA) is another.

An antisense oligonucleotide (ASO) is an oligonucleotide that is complementary to a specific RNA sequence, and when hybridized to this specific sequence, interferes with processing or translation of the RNA or triggers degradation of the specific RNA by enzymatic pathways (for example, RNAse H-dependent degradation). The nucleosides comprising an ASO may be purine or pyrimidine nucleosides, or a combination of purine and pyrimidine nucleosides, connected by an internucleoside linkage. ASOs are described generally in, for example, Crooke 2004. Annu. Rev. Med 55:61-95; and in Curr Mol Med 4:465-487. An siRNA is a short (20-30 nucleotide) double-stranded RNA (or modified RNA) molecule that may effect a reduction in the level of a gene product by allowing for specific destruction of mRNA via the RNA interference pathway. The

specific mRNA is degraded in the cytoplasm by the RNA-induced silencing complex (RISC). An miRNA is a short (20-30 nucleotide) single-stranded RNA molecule that may effect a reduction in the level of a gene product. An miRNA is complementary to a part of an mRNA, either a coding region or a non-translated region (e.g. 5' untranslated region (UTR), 3' UTR). The miRNA may anneal to form a double-stranded complex and trigger degradation in a process similar to that of siRNA. Translation may also be disrupted by miRNA. A DNA ASO, commonly referred to simply as an ASO, is a short (12-50 nucleotide) single stranded DNA (or modified DNA) molecule that may effect a reduction in the level of a gene product by inducing specific destruction of pre-mRNA or mRNA via RNase H-mediated cleavage. The specific pre-mRNA or mRNA can be degraded in the nucleus and/or the cytoplasm by induction of RNAseH cleavage of DNA-RNA heteroduplexes. A DNA ASO, commonly referred to simply as an ASO, is a short (12-50 nucleotide) single stranded DNA (or modified DNA) molecule that may effect a reduction in the level of a gene product by inducing specific destruction of pre-mRNA or mRNA via RNase H-mediated cleavage. The specific pre-mRNA or mRNA can be degraded in the nucleus and/or the cytoplasm by induction of RNAseH cleavage of DNA-RNA heteroduplexes.

The term 'nucleoside' refers to a molecule of ribose or deoxyribose sugar bonded through carbon-1 of the sugar ring to a nitrogenous base. Examples of nitrogenous bases include purines such as adenine, guanine, 6-thioguanine, hypoxanthine, xanthine, and pyrimidines such as cytosine, thymine and uracil. Examples of purine nucleosides include adenosine (A), guanosine (G), inosine (I), 2'-O-methyl-inosine, 2'-O-methyl-adenosine, 2'-O-methyl-guanine, 2-chlorodeoxyadenosine, 7-halo-7-deaza-adenosine, 7-halo-7-deaza-guanine, 7-propyne-7-deaza adenosine, 7-propyne-7-deaza-guanine, 2-amino-adenosine, 7-deazainosine, 7- thia-7,9-dideazainosine, formycin B, 8-Azainosine, 9-deazainosine, allopurinol riboside, 8-bromo-inosine, 8-chloroinosine, 7-deaza-2-deoxy-xanthosine, 7-Deaza-8-aza-adenosine, 7-deaza-8-aza-guanosine, 7-deaza-8-aza-deoxyadenosine, 7-deaza-8-aza-deoxyguanosine, 7-deaza-adenosine, 7-deaza-guanosine, 7-deaza-deoxyadenosine, 7-deaza-

deoxyguanosine, 8-amino-adenosine, 8-amino-deoxyadenosine, 8-amino-guanosine, 8-amino-deoxyguanosine,3-deaza-deoxyadenosine, 3-deaza-adenosine, 6-thio-deoxyguanosine, and the like, and other purine nucleosides as described in Freier et al 1997 (Nucleic Acids Res. 25:4429-4443), incorporated herein by reference. Examples of pyrimidine nucleosides include deoxyuridine (dU), uridine (U), cytidine (C), deoxycytidine (dC), thymidine (T), deoxythymidine (dT), 5-fluoro-uracil, 5-bromouracil, 2'-O-methyl-uridine, 2'-O-methyl cytidine, 5-iodouracil, 5-methoxy-ethoxy-methyl-uracil, 5-propynyl deoxyuridine, pseudoisocytidine, 5-azacytidine, 5-(1-propynyl)cytidine, 2'-deoxypseudouridine, 4-thio-deoxythymidine, 4-thio-deoxyuridine, and the like, and other substituted pyrimidines as disclosed in Freier et al,1997 (Nucleic Acids Res. 25:4429-4443). Purine or pyrimidine nucleosides also include phosphoramidite derivatives used in oligonucleotide synthesis using standard methods.

"Nucleoside" also includes nucleosides having substituted ribose sugars (bicyclic or otherwise). Some representative patents and publications that teach the preparation of non-bicyclic modified sugars include, but are not limited to, U.S. Patents: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; 5,700,920; and 6,600,032; and WO 2005/121371. Some representative patents and publications that teach the preparation of bicyclic modified sugars include, but are not limited to, 'locked nucleic acids', such as those described in WO 99/14226, WO 00/56746, WO 00/56748, WO 01/25248, WO 0148190, WO 02/28875, WO 03/006475, WO 03/09547, WO 2004/083430, US 6,268,490, US 6,794499, US 7,034,133. Other examples of substituted ribose sugars are described in, for example, Freier, 1997 (Nucleic Acids Res. 25:4429-4443) and Herdewijn et al., 2000. (Antisense Nucleic Acid Drug Dev 10:297-310) both of which are incorporated by reference herein.

A 'nucleotide' refers to a nucleoside having an internucleoside linkage group bonded through the carbon-5 of the sugar ring, usually a mono-, di- or tri-phosphate, and may be suitable for enzymatic polymerization. In other

examples, the nucleotides may be phosphoramidites, suitable for non-enzymatic polymerization or synthesis of nucleic acid polymers.

An internucleoside linkage group refers to a group capable of coupling two nucleosides, as part of an oligonucleotide backbone. Examples of internucleoside linkage groups are described by Praseuth et al (Biochimica et Biophysica Acta 1489:181-206) and Summerton et al 1997. (Antisense and Nucleic Acid Drug Dev 7:187-195), both of which are incorporated herein by reference. For example, phosphodiester (PO_4-), phosphorothioate ($PO3_S$-), phosphoramidate (N3'-P5') (PO_3NH) and methylphosphonate (PO_3CH_3), peptidic linkages ("PNA"), and the like; see, for example, US Patent 5969118. Inclusion of such modified linkage groups, modified ribose sugars or nitrogenous bases in an oligonucleotide may reduce the rate of hydrolysis of the oligonucleotide *in vitro* or *in vivo*.

"Gapmer" or "gap oligomer", as used herein, refers to a chimeric oligomer having a central portion (a "gap") flanked by 3' and 5' "wings", wherein the gap has a modification that is different as compared to each of the wings. Such modifications may include nucleobase, monomeric linkage, and sugar modifications as well as the absence of a modification (such as unmodified RNA or DNA). Accordingly, a gapmer may be as simple as RNA wings separated by a DNA gap. In some cases, the nucleotide linkages in the wings may be different than the nucleotide linkages in the gap. In certain embodiments, each wing comprises nucleotides with high affinity modifications and the gap comprises nucleotides that do not comprise that modification. Alternatively, the nucleotides in the gap and the nucleotides in the wings may have high affinity modifications, but the high affinity modifications in the gap are different than the high affinity modifications in each of the wings. The modifications in the wings may confer resistance to cleavage by endogenous nucleases, including RNaseH, while the modifications in the gap may be substrates for RNase H. The modifications in the wings may confer resistance to cleavage by endogenous nucleases, including RNaseH, while the modifications in the gap may be substrates for RNase H. The modifications in the wings may be the same or different from one

another. The nucleotides in the gap may be unmodified and nucleotides in the wings may be modified. A gapmer has a wing-gap-wing ratio, which may be represented numerically (wing#-gap#-wing#). The gapmer may be symmetric (for example, 9-13-9, 9-12-9, 9-11-9, 9-10-9, 9-9-9, 9-8-9, 9-7-9, 9-6-9, 9-5-9, 9-4-9, 9-3-9, 9-2-9, 9-1-9, 8-15-8, 8-14-8, 8-13-8, 8-12-8, 8-11-8, 8-10-8, 8-9-8, 8-8-8, 8-7-8, 8-6-8, 8-5-8, 8-4-8, 8-3-8, 8-2-8, 8-1-8, 7-15-7, 7-14-7, 7-13-7, 7-12-7, 7-11-7, 7-10-7, 7-9-7, 7-8-7, 7-7-7, 7-6-7, 7-5-7, 7-4-7, 7-3-7, 7-2-7, 7-1-7, 6-15-6, 6-14-6, 6-13-6, 6-12-6, 6-11-6, 6-10-6, 6-9-6, 6-8-6, 6-7-6, 6-6-6, 6-5-6, 6-4-6, 6-3-6, 6-2-6, 6-1-6, 5-15-5, 5-14-5, 5-13-5, 5-12-5, 5-11-5, 5-10-5, 5-9-5, 5-8-5, 5-7-5, 5-6-5, 5-5-5, 5-4-5, 5-3-5, 5-2-5, 5-1-5, 4-17-4, 4-16-4, 4-15-4, 4-14-4, 4-13-4, 4-12-4, 4-11-4, 4-10-4, 4-9-4, 4-8-4, 4-7-4, 4-6-4, 4-5-4, 4-4-4, 4-3-4, 3-24-3, 3-23-3, 3-22-3, 3-21-3, 3-20-3, 3-19-3, 3-18-3, 3-17-3, 3-16-3, 3-15-3, 3-14-3, 3-13-3, 3-12-3, 3-11-3, 3-10-3, 3-9-3, 3-8-3, 3-7-3, 3-6-3, 3-5-3, 3-4-3, 2-26-2, 2-25-2, 2-24-2, 2-22-2, 2-21-2, 2-20-2, 2-19-2, 2-18-2, 2-17-2, 2-16-2, 2-15-2, 2-14-2, 2-13-2, 2-12-2, 2-11-2, 2-10-2, 2-9-2, 2-8-2, 2-7-2, 2-6-2, 2-5-2, 1-26-1, 1-25-1, 1-24-1, 1-22-1, 1-21-1, 1-20-1, 1-19-1, 1-18-1, 1-17-1, 1-16-1, 1-15-1, 1-14-1, 1-13-1, 1-12-1, 1-11-1, 1-10-1, 1-9-1, 1-8-1 or 1-7-1). The gapmer may be asymmetric (for example, 8-13-9, 8-12-9, 8-11-9, 8-10-9, 8-9-9, 8-8-9, 8-7-9, 8-6-9, 8-5-9, 8-4-9, 8-3-9, 8-2-9, 8-1-9, 7-15-8, 7-14-8, 7-13-8, 7-12-8, 7-11-8, 7-10-8, 7-9-8, 7-8-8, 7-7-8, 7-6-8, 7-5-8, 7-4-8, 7-3-8, 7-2-8, 7-1-8, 6-15-7, 6-14-7, 6-13-7, 6-12-7, 6-11-7, 6-10-7, 6-9-7, 6-8-7, 6-7-7, 6-6-7, 6-5-7, 6-4-7, 6-3-7, 6-2-7, 6-1-7, 5-15-6, 5-14-6, 5-13-6, 5-12-6, 5-11-6, 5-10-6, 5-9-6, 5-8-6, 5-7-6, 5-6-6, 5-5-6, 5-4-6, 5-3-6, 5-2-6, 5-1-6, 4-15-5, 4-14-5, 4-13-5, 4-12-5, 4-11-5, 4-10-5, 4-9-5, 4-8-5, 4-7-5, 4-6-5, 4-5-5, 4-4-5, 4-3-5, 4-2-5, 4-1-5, 3-17-4, 3-16-4, 3-15-4, 3-14-4, 3-13-4, 3-12-4, 3-11-4, 3-10-4, 3-9-4, 3-8-4, 3-7-4, 3-6-4, 3-5-4, 3-4-4, 3-3-4, 2-24-3, 2-23-3, 2-22-3, 2-21-3, 2-20-3, 2-19-3, 2-18-3, 2-17-3, 2-16-3, 2-15-3, 2-14-3, 2-13-3, 2-12-3, 2-11-3, 2-10-3, 2-9-3, 2-8-3, 2-7-3, 2-6-3, 2-5-3, 2-4-3, 1-26-2, 1-25-2, 1-24-2, 1-22-2, 1-21-2, 1-20-2, 1-19-2, 1-18-2, 1-17-2, 1-16-2, 1-15-2, 1-14-2, 1-13-2, 1-12-2, 1-11-2, 1-10-2, 1-9-2, 1-8-2, 1-7-2, 3-26-1, 3-25-1, 3-24-1, 3-22-1, 3-21-1, 3-20-1, 3-19-1, 3-18-1, 3-17-1, 3-16-1, 3-15-1, 3-14-1, 4-13-1, 4-12-1, 4-11-1, 4-10-1, 3-9-1, 3-8-1 or 4-7-1).

A chimeric antisense oligonucleotide with a deoxy gap region which is greater than 10 nucleotides in length may be referred as a "gap-widened antisense oligonucleotide". The wing regions may be one to eight high-affinity modified nucleotides in length. The gap-widened antisense oligonucleotides may be 12 to 30 nucleotides in length capable of having, for example, various wing-gap-wing ratio may be selected from: 2-15-1, 1-15-2, 1-14-3, 3-14-1, 1-13-4, 4-13-1, 2-13-3, 3-13-2, 1-12-5, 5-12-1, 2-12-4, 4-12-2, 3-12-3, 1-11-6, 6-11-1, 2-11-5, 5-11-2, 3-11-4, 4-11-3, 2-16-1, 1-16-2, 1-15-3, 3-15-1, 2-15-2, 1-14-4, 4-14-1, 2-14-3, 3-14-2, 1-13-5, 5-13-1, 2-13-4, 4-13-2, 3-13-3, 1-12-6, 6-12-1, 2-12-5, 5-12-2, 3-12-4, 4-12-3, 1-11-7, 7-11-1, 2-11-6, 6-11-2, 3-11-5, 5-11-3, 4-11-4, 1-18-1, 1-17-2, 2-17-1, 1-16-3, 1-16-3, 2-16-2, 1-15-4, 4-15-1, 2-15-3, 3-15-2, 1-14-5, 5-14-1, 2-14-4, 4-14-2, 3-14-3, 1-13-6, 6-13-1, 2-13-5, 5-13-2, 3-13-4, 4-13-3, 1-12-7, 7-12-1, 2-12-6, 6-12-2, 3-12-5, 5-12-3, 4-12-4, 1-11-8, 8-11-1, 2-11-7, 7-11-2, 3-11-6, 6-11-3, 4-11-5, 5-11-4, 1-18-1, 1-17-2, 2-17-1, 1-16-3, 3-16-1, 2-16-2, 1-15-4, 4-15-1, 2-15-3, 3-15-2, 1-14-5, 2-14-4, 4-14-2, 3-14-3, 1-13-6, 6-13-1, 2-13-5, 5-13-2, 3-13-4, 4-13-3, 1-12-7, 7-12-1, 2-12-6, 6-12-2, 3-12-5, 5-12-3, 4-12-4, 1-11-8, 8-11-1, 2-11-7, 7-11-2, 3-11-6, 6-11-3, 4-11-5, 5-11-4, 1-19-1, 1-18-2, 2-18-1, 1-17-3, 3-17-1, 2-17-2, 1-16-4, 4-16-1, 2-16-3, 3-16-2, 1-15-5, 2-15-4, 4-15-2, 3-15-3, 1-14-6, 6-14-1, 2-14-5, 5-14-2, 3-14-4, 4-14-3, 1-13-7, 7-13-1, 2-13-6, 6-13-2, 3-13-5, 5-13-3, 4-13-4, 1-12-8, 8-12-1, 2-12-7, 7-12-2, 3-12-6, 6-12-3, 4-12-5, 5-12-4, 2-11-8, 8-11-2, 3-11-7, 7-11-3, 4-11-6, 6-11-4, 5-11-5, 1-20-1, 1-19-2, 2-19-1, 1-18-3, 3-18-1, 2-18-2, 1-17-4, 4-17-1, 2-17-3, 3-17-2, 1-16-5, 2-16-4, 4-16-2, 3-16-3, 1-15-6, 6-15-1, 2-15-5, 5-15-2, 3-15-4, 4-15-3, 1-14-7, 7-14-1, 2-14-6, 6-14-2, 3-14-5, 5-14-3, 4-14-4, 1-13-8, 8-13-1, 2-13-7, 7-13-2, 3-13-6, 6-13-3, 4-13-5, 5-13-4, 2-12-8, 8-12-2, 3-12-7, 7-12-3, 4-12-6, 6-12-4, 5-12-5, 3-11-8, 8-11-3, 4-11-7, 7-11-4, 5-11-6, 6-11-5, 1-21-1, 1-20-2, 2-20-1, 1-20-3, 3-19-1, 2-19-2, 1-18-4, 4-18-1, 2-18-3, 3-18-2, 1-17-5, 2-17-4, 4-17-2, 3-17-3, 1-16-6, 6-16-1, 2-16-5, 5-16-2, 3-16-4, 4-16-3, 1-15-7, 7-15-1, 2-15-6, 6-15-2, 3-15-5, 5-15-3, 4-15-4, 1-14-8, 8-14-1, 2-14-7, 7-14-2, 3-14-6, 6-14-3, 4-14-5, 5-14-4, 2-13-8, 8-13-2, 3-13-7, 7-13-3, 4-13-6, 6-13-4, 5-13-5, 1-12-10, 10-12-1, 2-12-9, 9-12-2, 3-12-8, 8-12-3, 4-12-7, 7-12-4, 5-12-6, 6-12-5, 4-11-8, 8-11-4, 5-11-7, 7-11-5, 6-

11-6, 1-22-1, 1-21-2, 2-21-1, 1-21-3, 3-20-1, 2-20-2, 1-19-4, 4-19-1, 2-19-3, 3-19-2, 1-18-5, 2-18-4, 4-18-2, 3-18-3, 1-17-6, 6-17-1, 2-17-5, 5-17-2, 3-17-4, 4-17-3, 1-16-7, 7-16-1, 2-16-6, 6-16-2, 3-16-5, 5-16-3, 4-16-4, 1-15-8, 8-15-1, 2-15-7, 7-15-2, 3-15-6, 6-15-3, 4-15-5, 5-15-4, 2-14-8, 8-14-2, 3-14-7, 7-14-3, 4-14-6, 6-14-4, 5-14-5, 3-13-8, 8-13-3, 4-13-7, 7-13-4, 5-13-6, 6-13-5, 4-12-8, 8-12-4, 5-12-7, 7-12-5, 6-12-6, 5-11-8, 8-11-5, 6-11-7, or 7-11-6. The gap-widened antisense oligonucleotides may have a 2-16-2, 3-14-3, or 4-12-4 wing-gap-wing ratio.

As used herein, the term "high-affinity modification" in relation to a nucleotide refers to a nucleotide having at least one modified nucleobase, internucleoside linkage or sugar moiety, such that the modification increases the affinity of an antisense compound comprising the modified nucleotide to a target nucleic acid. High-affinity modifications include, but are not limited to, bicyclic nucleic acid (BNA)s, LNAs and 2'-MOE. Furthermore, the desirable potency and toxicity characteristics may be obtained by selecting the nucleotide modifications, nucleotide analogues, modified inter-nucleoside linkages, including one or more modified sugar moieties and/or a gapmer wing-gap-wing ratio (for example, see US20100197762).

Examples

The following examples are illustrative of some of the embodiments of the invention described herein. These examples do not limit the spirit or scope of the invention in any way.

Methods

Histochemical staining:

Massons' Trichrome: Mice were sacrificed and livers were immediately fixed in 4% PFA overnight and later paraffin embedded. 10 micron slices of the paraffin embedded livers were made and stained with the Massons' Trichrome staning kit (Thermo Scientific, Canada) as per manufacturer's instructions.

Oil Red O: Mice were sacrificed and livers were immediately snap frozen in liquid nitrogen. 25 micron cryosections were prepared from OCT fixed livers on

a cryotome and stained with 0.5% Oil red O in isopropanol (Sigma Aldrich, MO, USA).

Immunofluorescence staining:

Mice were sacrificed and livers were immediately fixed in 4% PFA overnight and later paraffin embedded. 20 micron sections were prepared and processed for staining of alpha- smooth muscle actin (Abcam, MA, USA) using fluorescently tagged secondary antibody. Images were taken on an Olympus fluroscence microscope.

Western blotting:

Livers from HCD-fed control and Cp8b1 knockout mice were homogenized in radioimmunoprecipitation assay buffer and whole cell lysates were prepared and solubilized in Laemmli sample buffer. Proteins were resolved on self-cast 10% polyacrylamide gels and blotted onto PVDF membranes, blocked with 5% bovine serum albumin and probed with the polyclonal anti-alpha-smooth muscle actin antibody (Abcam, MA, USA) or the anti-Calnexin polyclonal primary antibody (Santa Cruz biotechnologies, CA, USA) overnight. After incubation with horseradish peroxidase-conjugated secondary antibody (Invitrogen, Carlsbad, CA), the immunoreactive proteins were detected using the enhanced chemiluminescence detection reagent (GE Healthcare, Piscataway, NJ) on the BioRad Chemidoc.

Biochemical estimations:

Tissues were weighed and lysed in PBS and homogenized using the bead homogenization method. The lipids were extracted using Folch's solvent extraction method. Triglycerides and total cholesterol was measured enzymatically using commercial kits (Infinity, Thermo Scientific, VA, USA).

Estimation of liver collagen:

Hepatic levels of collagen were measured as previously described [Mueller, M., *et al.*, *Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity.* J Hepatol, 2015. **62**(6): p. 1398-404]. Briefly, liver tissues were homogenized in 2N NaOH and autoclaved for 25 min. The hydrolyzates were cooled to room temperature and filtered through Whatmann filter paper No. 1 to remove particulate matter. Standard dilutions of 4-hydroxyproline (Sigma Aldrich, GmbH, Germany), samples and blanks in acetate-citrate buffer pH 6.5 were mixed in freshly prepared chloramine T and allowed to react at room temperature for 25 min. Color development was achieved by the addition of fresh Ehrlich's aldehyde reagent and incubation at 65°C for 20 min. Absorbances were measured at 550 nm. Results were plotted as mg collagen/g liver using the following formula:

g collagen = (g 4-hydroxyproline / ml)* dilution factor * conversion factor(7:5)

Quantitative Real time PCR:

Total RNA was isolated from tissues using an RNA extraction kit (Qiagen, Toronto, Ontario, Canada) and from islets using the RNeasy kit (Life Technologies, Burlington, Ontario, Canada). One microgram of RNA was used to synthesize cDNA using the Superscript III First-Strand Synthesis kit (Life Technologies). Primers were purchased from IDT Canada and the sequences used for measuring gene expression were as follows:

Name	SEQ ID NO:	Forward	SEQ ID NO:	Reverse
Cd36	3	ATGGGCTGTGATC GGAACTG	4	GTCTTCCCAATAAGCAT GTCTCC
Gpat1	5	ACAGTTGGCACAA TAGACGTTT	6	CCTTCCATTTCAGTGTT GCAGA
Fas	7	GGAGGTGGTGAT AGCCGGTAT	8	TGGGTAATCCATAGAG CCCAG
Scd1	9	TTCTTGCGATACA CTCTGGTGC	10	CGGGATTGAATGTTCTT GTCGT
Col1a1	11	GCTCCTCTTAGGG GCCACT	12	CCACGTCTCACCATTG GGG
Col3a1	13	CTGTAACATGGAA ACTGGGGAAA	14	CCATAGCTGAACTGAA AACCACC
Timp1	15	GCAACTCGGACCT GGTCATAA	16	CGGCCCGTGATGAGAA ACT

Name	SEQ ID NO:	Forward	SEQ ID NO:	Reverse
Timp2	17	TCAGAGCCAAAGC AGTGAGC	18	GCCGTGTAGATAAACT CGATGTC
aSMA	19	GTCCCAGACATCA GGGAGTAA	20	TCGGATACTTCAGCGT CAGGA
TNFa	21	GACGTGGAACTG GCAGAAGAG	22	TTGGTGGTTTGTGAGT GTGAG
Infg	23	ATGAACGCTACAC ACTGCATC	24	CCATCCTTTTGCCAGTT CCTC
IL1b	25	GCAACTGTTCCTG AACTCAACT	26	ATCTTTTGGGGTCCGT CAACT
IL6	27	TAGTCCTTCCTAC CCCAATTTCC	28	TTGGTCCTTAGCCACTC CTTC
Bsep	29	TCTGACTCAGTGA TTCTTCGCA	30	CCCATAAACATCAGCC AGTTGT
Shp	31	TGGGTCCCAAGG AGTATGC	32	GCTCCAAGACTTCACA CAGTG
ApoC2	33	ATGGGGTCTCGGT TCTTCCT	34	GTCTTCTGGTACAGGT CTTTGG
ApoE	35	CTGACAGGATGCC TAGCCG	36	CGCAGGTAATCCCAGA AGC
Mdr3	37	CAGCAGTCAGTGT GCTTACAA	38	ATGGCTCTTTTATCGGC CTCA
Pltp	39	CGCAAAGGGCCA CTTTTACTA	40	GCCCCCATCATATAAG AACCAG
Heplip	41	ATGGGAAATCCCC TCCAAATCT	42	GTGCTGAGGTCTGAGA CGA

Primary mouse hepatocyte isolation and LNA mediated inhibition:

Primary hepatocytes from mice were isolated as previously described. Briefly, forward perfusion of the liver by cannulating the portal vein with a 23 X 3/4th butterfly cannula and sequential perfusion with Hanks balanced salt solution containing EDTA and later with collagenase type IV in Dulbeccos minimum essential medium (DMEM) was performed in anesthetized mice. Digested hepatocytes were triturated, centrifuged, sieved and cultured on collagen coated plates at a density of 60,000 cell per cm2. Two hours after attachment, cells were washed and maintained in DMEM:F12 medium without serum. For the silencing experiments, cells were treated in growth medium with the two LNAs oligonucleotides of the following sequence:

Cyp8b1(2_1) 5' +G*+C*+T*+C*A*C*T*T*C*C*A*C*C*C*A*C*+T*+C*+C*+C 3'

(SEQ ID NO:1)

Cyp8b1(2_3) 5' +T*+T*+C*+A*T*C*T*C*G*C*T*G*A*G*G*G*+C*+A*+A*+A3'

(SEQ ID NO: 2)

for 72h after which cells were harvested and mRNA was isolated and processed for Quantitative Real time PCR as above. '+' in the sequence denotes a locked nucleic acid base and '*' indicates a phosphorothioate modification.

Example 1- High cholesterol diet leads to NAFLD and steatohepatitis

Wild type C57Bl6J mice were fed a high cholesterol diet (HCD) of chow diet supplemented with 0.5% (w/w) cholesterol for 12 weeks. This led to an induction of steatosis and continuation of the diet for up to 16 weeks led to the development of fibrosis. As shown in Figure 1A, HCD fed mice have a 12 fold induction in steatosis area. Liver sections were stained with Oil Red O to reveal neutral lipids and as shown in Figure 1B, HCD feeding led to a significant increase in Oil Red O-positivity. Liver sections were stained using Massons' Trichrome to reveal collagen which revealed a 24 fold induction in collagen positive area (Figure 1C). This model was used as a standard model for the study of NAFLD / NASH in all following studies.

Example 2 - Cyp8b1 knockout mice on HCD have reduced steatosis, hepatic triglycerides and cholesterol.

Cyp8b1 knockout and control mice were fed HCD for 16 weeks and NAFLD and NASH development was analyzed. Representative micrographs from 9 knockout and 9 control mice are shown in Figure 2A, and analysis of steatosis area in Figure 2B indicates that Cyp8b1 knockout mice are protected from steatosis as compared to wild type mice. The amount of hepatic triglyceride (Figure 3A) and total cholesterol accumulation in Cyp8b1 knockout mice is significantly reduced compared with control mice (Figure 3B).

Example 3 - Cyp8b1 knockout mice have reduced collagen deposition and reduced liver fibrosis.

The liver sections were stained with Masons' Trichrome to reveal collagen (blue), which was quantified and found to be reduced in Cyp8b1 knockout mice (Figure 4A). The total amount of 4-hydroxyproline was estimated biochemically from liver homogenates and expressed in terms collagen/g liver tissue. Cyp8b1 knockout mice had significantly lower amount of collagen per g. liver tissue compared with control mice (Figure 4B).

The activation of hepatic stellate cells leads to fibrotic gene expression and α-smooth muscle actin (α-SMA) serves as a marker for stellate cell activation. Therefore, liver sections from control and Cyp8b1 knockout mice were stained with anti- α-SMA antibody and the positive area was quantified. Cyp8b1 knockout mice had significantly reduced α-SMA positive area (Figure 5A and B). Quantification of α-SMA protein from western blot also revealed significantly reduced α-SMA positivity in Cyp8b1 knockout mice compared with controls (Figure 5C). A Q-RT PCR analysis of gene expression of fibrotic markers revealed that Cyp8b1 knockout mice had a significantly reduced expression of Tissue Inhibitor Of Metalloproteinases (*Timp*)-1, *Timp2*, collagen, type I, alpha 1 (*Col1a1*) and *α-SMA* (Figure 5D).

Example 4 - Cyp8b1 knockout mice have reduced inflammation.

Liver sections from Massons' Trichrome were analyzed for inflammatory cell infiltration defined as 3 or more dense eosinophilic nuclei invading the hepatic parenchyma. Analysis revealed a reduced inflammatory score for Cyp8b1 knockout mice compared with controls (Figure 6A and B). A Q-RT PCR analysis of gene expression of inflammatory markers revealed that Cyp8b1 knockout mice had a significant reduction in the expression of interleukin 1β (Il1 β) (Figure 6B)

Example 5 - FXR target genes are downregulated in the livers of Cyp8b1 knockout mice.

To test whether the relative enrichment of FXR antagonistic bile acids such as α, β, ω-MCA and UDCA have functional impact on hepatic FXR

activation, the gene expression of FXR target genes in Cyp8b1 knockout and control livers was quantified. Analysis revealed that most of the well characterized FXR target genes such as bile salt export pump (*Bsep*), ATP-binding cassette, sub-family B (MDR/TAP), member 1 *(Mdr3*), Hepatic lipase (*Lipc*), phospholipid transfer protein (*Pltp*) and apolipoprotein C2 (*ApoC2*) were downregulated in Cyp8b1 knockout mice livers compared with controls (Figure 7A). To directly measure the effects exerted by the altered bile acid composition of Cyp8b1 knockout mice on hepatocyte gene expression, primary mouse hepatocytes isolated from wild type C57bL6J mice fed HCD were treated for 16 weeks and FXR target gene expression was measured. Analysis revealed that α-MCA dose dependently repressed the transcripts of not only *Bsep* and *Mdr3* but also 2 important lipogeneic genes *Fas* and *Scd1*, UDCA and β-MCA were used as positive controls in these experiments (Figure 7B).

Example 6 - Locked nucleic acid (LNA)/DNA hybrid oligonucleotides targeting Cyp8b1 leads to a dose dependent knockdown.

To test whether Cyp8b1 expression yields to antisense oligonucleotide (ASO)- mediated knockdown, two LNA/DNA hybrid gapmer ASOs were designed and tested on mouse hepatocytes. Unassisted delivery of one of the LNA (SEQ ID NO: 1) produces a dose-dependent knockdown of up to 60% after 72h of treatment (Figure 8).

Cyp8b1(2_1) 5' +G*+C*+T*+C*A*C*T*T*C*C*A*C*C*C*A*C*+T*+C*+C*+C 3'

(SEQ ID NO:1)

Cyp8b1(2_3) 5' +T*+T*+C*+A*T*C*T*C*G*C*T*G*A*G*G*G*+C*+A*+A*+A3'

(SEQ ID NO: 2)

Where '+' in the sequence denotes a locked nucleic acid base and '*' indicates a phosphorothioate modification.

Example 7 – *in vivo* knockdown Study

Mice and treatments:

Twelve, 17 week old, C57Bl6J female mice were divided into two groups and were given *ad libitum* access to water and a diet supplemented with 0.5% w/w cholesterol (high cholesterol diet [HCD], TD10401; Harlan Diets, Madison, WI). One group received 3mg/kg of negative control LNA Gapmer of the sequence 5'-AACACGTCTATACGC-3' and the other group received 3mg/kg of HDL2_LNA_500125 LNA Gapmer of the sequence 5'-ATCTCGCTGAGGGCA-3'. The Gapmers were administered intraperitoneally for 5 consecutive days in physiological saline. A set of 3 additional mice were administered 2, 4 and 5 mg/kg of the HDL2_LNA_500125 LNA Gapmer for the same duration of time. The LNA Gapmers were commercially produced such that the three 5' and 3' nucleotides were locked nucleic acids and each nucleotide was phosphorothioate modified. Gapmer oligobnucleotides were commercially produced and purified using anion-exchange HPLC to meet *in vivo* grade puriety by Exiqon (Vedbaek, Denmark).

Measurement of fecal fat mass and cholesterol excretion:

For the estimation of fecal excretion of dietary cholesterol, fecal pellets were collected over 72 h from mice that were fed HCD. Lipids were extracted from lyophilized, pulverized feces using chloroform: methanol: NP40 (7:11:0.1) in a volume 20 times the weight of the sample. Lipid extracts were then air dried and weighed to calculate the fat mass. Extracts were resuspended in 200 µl sterile phosphate-buffered saline containing 1% Triton X 100, followed by enzymatic quantification of total cholesterol using biochemical kits according to manufacturer's instructions (Infinity, Thermo Scientific, Middleton, VA).

siRNA transfections and cell culture:

HepG2 cells (ATCC HB-8065^{TM}) were split and plated at a cell density of $4X10^5$ cell per well of a 12 well plate. Cells were cultured in incubators 37°C with 5% CO_2 in Dulbecco's minimum essential medium (DMEM) (Gibco ThermoFisher Scientific, Burlington, ON), supplemented with 10% fetal calf serum and antibiotics. Once the cells reached 80% confluence, they were transfected using

the designated sequence of siRNA / LNA Gapmers using the Lipfectamine RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA) in the Opti MEM reduced serum medium (Gibco, ThermoFisher Scientific, Burlington, ON) for 48 hours. At the end of 48 hours, the cells were lysed in 50 µL RIPA buffer (Sigma Aldrich, St. Louis, MO) and the protein concentration from the lysates was measured using the BCA protein quantification kit. Proteins were resolved using 10% polyacrylamide gels and western blotting was performed as described above. The amount of Cyp8b1 protein was detected using anti-Cyp8b1 antibody (Abcam, catalogue number ab191910) in conjunction with anti-rabbit secondary antibody.

Sequences used for silencing:

All sequences were purchased from IDT technologies (Coralville, IO). LNA Gapmers were purchased from Exiqon (Vedbaek, Denmark).

HDL2 LNA 1

5'GGGAGTGGCTAGAAGTGGGC-3' (SEQ ID NO: 43)

HDL2 LNA 3

5'TTTGCACTCAGTGAGGTGAA3' (SEQ ID NO: 44)

HDL2_si_1

5'-CTGAAGCACCCAGAAGCTATT-3' (SEQ ID NO: 45)

5'-AATAGCTTCTGGGTGCTTCAG-3' (SEQ ID NO: 46)

HDL2_si101

5'-AATTCAAACATATTCTTCCGG-3' (SEQ ID NO: 47)

5'-GGAAGAATATGTTTGAATTTC-3' (SEQ ID NO: 48)

HDL2_si103

5'-AGTTTTTTTGCATATTGCCCA-3' ((SEQ ID NO: 49)

5'-GGCAATATGCAAAAAAACTGG-3' (SEQ ID NO: 50)

HDL2_si109

5'-TTGAAGAAGTCCACTTTCCGG-3' (SEQ ID NO: 51)

5'-GGAAAGTGGACTTCTTCAAGA -3' (SEQ ID NO: 52)

Dsi2

5'-TGGGAGCCCCCTCTGGACAAGGGT-3' (SEQ ID NO: 53)

5'-ACCCTTGTCCAGAGGGGGCTCCCA-3' (SEQ ID NO: 54)

Dsi13

5'-GGTTGGTTCATGAAGACTATACCCT-3' (SEQ ID NO: 55)

5'- AGGGTATAGTCTTCATGAACCAACC-3' (SEQ ID NO: 56)

Results:

Results are displayed visually in Figures 9, 10 and 11.

Cholesterol lowering drugs such as statins in combination with Ezetimibe to inhibit absorption, were recently shown to reduce NASH related pathology in a mouse model (Rooyen, D. M. Van, Gan, L. T., Yeh, M. M., Haigh, W. G., Larter, C. Z., Ioannou, G., Farrell, G. C. (2013). Pharmacological cholesterol lowering reverses fibrotic NASH in obese, diabetic mice with metabolic syndrome. *Journal of Hepatology*, *59*(1), 144–152. http://doi.org/10.1016/j.jhep.2013.02.024). This has driven the support for a renewed consideration of statin prescription to patients with NAFLD (Egan, M., Prasad, S., Medicine, M. F., & Hickner, J. (2011). Statins for patients with nonalcoholic fatty liver? *The Journal of Family Practice*, *60*(9), 536–538.). Previous studies have shown that the absence of CA and relative enrichment of MCA alters the hydrophobicity of bile in Cyp8b1-/- mice, that leads to reduced intestinal absorption of lipids, especially of cholesterol (Kaur, A., Patankar, JV., de Haan, W., Ruddle, P., Wijesekara, N., Groen, AK., Verchere, CB., Singaraja, RR., Hayden, MR. 2015. "Loss of Cyp8b1 improves glucose homeostasis by increasing GLP-1." *Diabetes* 64 (4): 1168-1179). Therefore it is hypothesized that the Cyp8b1-/- mice would be resistant to the non-alcoholic steatohepatitis.

Example 8 – Lower fat mass and increased metabolic rate in Cyp8b1 knockout mice and Lower hepatic damage and increased fecal cholesterol and fat excretion in Cyp8b1 knockout mice

Animals and diets:

$Cyp8b1^{+/-}$ mice were purchased from the University of California, Davis, Knockout Mouse Project (KOMP) repository (Cyp8b1tm1 (KOMP)Vlcg). $Cyp8b1^{+/+}$ (control) and $Cyp8b1^{-/-}$ (knockout) mice were obtained by breeding $Cyp8b1^{+/-}$ mice. This study used male and female mice, aged 3–6 months. The mice had *ad libitum* access to water and were fed a diet supplemented with 0.5% w/w cholesterol (high cholesterol diet [HCD], TD10401; Harlan Diets, Madison, WI) for 12 - 16 weeks or methionine choline- deficient diet [MCD], A02082002B ; Research Diets, New Brunswick, NJ. For experiments involving fasting, mice were fasted for 4h (7:00 A.M.–11:00 A.M.) before the procedure. Blood for all experiments was drawn from the medial saphenous vein in EDTA coated capillary tubes. All experiments were approved by the University of British Columbia Animal Care Committee.

Analysis of body composition and energy metabolism

Lean and fat mass were quantified by EchoMRI-100 (Echo Medical Systems, Houston, TX, USA) with quantitative magnetic resonance-based method.

For simultaneous measurement of multiple metabolic parameters, mice were housed in PhenoMaster metabolic cages (TSE Systems, Bad Homberg, Germany) for 4 consecutive days. All calculated values for VO2, VCO2, RER, locomotor activity, food intake and water intake are based on measurements obtained over 60 hours post-acclimatization. Mice were allowed to acclimatize for at least 24 hours in the metabolic cages prior to actual data collection. Oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured. Respiratory exchange ratio (RER) was calculated as RER = VCO2/VO2 and energy expenditure (EE) as EE = 3.185 x VO2 + 1.232 x VCO2. Locomotor activity was tracked by the total counts of infra-red beam breaks. Food and water intakes were monitored through weight sensors.

Biochemical parameters:

Levels of plasma triglycerides (TG), cholesterol (C) and cholesterol ester (CE) were performed using enzymatic kits according to manufacturer's protocols (Infinity, Thermo Scientific, Middleton, VA). Levels of lactate dehydrogense (LDH), were measured from the media after incubation of primary hepatocytes with LPS or TNF at indicated concentration and time using the LDH cytotoxicity assay kit according to manufacturer's instructions (Thermo Scientific, Middleton, CA). The levels of serum aspartate aminotransferase (AST) and alanine were determined using commercially available kits from Sigma Aldrich, St. Louis, MO.

Hepatic and cellular TG, C and CE estimation:

TG, C and CE concentrations were determined from lipid extracts of liver lysates or cultured cells using enzymatic kits as mentioned above. Briefly, liver samples from Cyp8b1$^{+/+}$ and Cyp8b1$^{-/-}$ mice were weighed, homogenized, and lipids were extracted in chloroform: methanol: NP40 (7:11:0.1) in a volume 20 times the weight of the sample. Lipid extracts were then air dried and resuspended in 200 µl sterile phosphate-buffered saline followed by enzymatic quantification.

Fecal excretion of dietary cholesterol:

For the estimation of fecal excretion of dietary cholesterol, fecal pellets were collected over 72 h from mice that were fed HCD. Lipids were extracted from lyophilized, pulverized feces using chloroform: methanol: NP40 (7:11:0.1) as described for tissues above. Total cholesterol in the feces was measured using biochemical kits followed by spectrophotometric quantification.

Isolation, culture and treatment of primary hepatocytes and Kupffer cells (KCs):

Hepatocytes were isolated after portal cannulation and collagenase perfusion of the liver as previously published. Briefly, mice were anesthetized using a single terminal dose of Avertin (250 mg/kg), the portal vein was exposed and cannulated using a 23G x ¾" butterfly cannula (BD, Vacutainer #367297) and the inferior vena cava was incised to allow outflow. The liver was perfused

with Hanks balanced salt solution containing HEPES and devoid of Calcium at 9mL/minute after which the solution was changed to Ca and Mg free, low glucose (1g/L) DMEM containing 100-125 CDU/ml of Collagenase type IV (Sigma #C5138, St. Louis, MO) and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin). A digestion time of 8-10 minutes was allowed depending on the size of the liver and the degree of steatohepatitis with steatohepatitic livers requiring longer. The *in situ* digested livers were then carefully excised from the body cavity and cells were dissociated, collagenase activity was inhibited using ice cold DMEM: F12 supplemented with 10% fetal bovine serum (FBS) and filtered through a 480 mm stainless steel wire mesh. Followed by a low speed centrifugation, the cell pellet was resuspended in DMEM: F12 supplemented with 10% FBS and filtered through 70 µm cell strainer, washed, counted and plated on collagen coated (5 µg rat tail collagen/cm^2) plates at a density of 3.75* 10^3 cells/cm^2. Non-adherent cells were washed-off 4 hours after plating and cells were cultured in DMEM: F12 containing 10% fetal calf serum and antibiotics overnight. For the isolation of KCs from the non-parenchymal fraction, perfused and digested livers were centrifuged at 50 x *g* and the supernatant was reserved. The supernatant was centrifuged at 2430 rpm to collect the cells. The cell pellet was washed twice in cold DMEM: F12 and then resuspended in DMEM: F12 with 10% FBS and subjected to differential adhesion on non-tissue culture treated plates. Unattached cells were washed after 1 hour of incubation and adherent cells were detached using Trypsin-EDTA (0.25%), washed, counted and cultured at 5000-10000 cells/ well in DMEM: F12 with 10% FCS on tissue-culture plates overnight. Both, hepatocytes and KCs were used in experiments the following day. Media, antibiotics and FBS were purchased from Gibco, ThermoFisher Scientific, Burlington, ON.

Western blotting:

Livers from HCD-fed Cyp8b1$^{+/+}$ and Cyp8b1$^{-/-}$ mice were homogenized in radioimmunoprecipitation assay buffer and whole cell lysates were prepared and solubilized in Laemmli sample buffer. Proteins were resolved on self-made

agarose gels of appropriate percentage using reagents from commercial providers, blotted onto Polyvinylidene fluoride membranes, blocked with 5% bovine serum albumin / dried milk powder and probed with the appropriate polyclonal primary antibodies overnight. After incubation with anti-host Ig horseradish peroxidase-conjugated secondary antibodies (Invitrogen, Carlsbad, CA), the immunoreactive proteins were detected using the enhanced chemiluminescence detection reagent (GE Healthcare, Piscataway, NJ / Thermo Scientific, Rockford IL). Antibodies against smooth muscle actin alpha 2 (αSMA), fatty acid synthase (FAS), Sterol 12-α hydroxylase (CYP8B1), sterol regulatory element binding protein (SREBP)-1 and 2 were purchased from Abcam, Cambridge, MA. Antibody against Calnexin was purchased from Santa Cruz Biotechnology, Santa Cruz, CA. Relative densities of protein of interest versus housekeeping protein Calnexin were calculated from the percent area under the curve generated using Image J.

Results are displayed visually in Figures 12 and 13. As shown in Figure 13, a 4 fold increase in fecal lipid excretion (Figure 13C) and a 2.8 fold increase in fecal cholesterol excretion (Figure 13D) in Cyp8b1-/- mice was observed compared with Cyp8b1+/+ mice. The reduction in cholesterol absorption and the repression of hepatic DNL clearly contribute to the reduction in the susceptibility to steatohepatitis of Cyp8b1-/- mice.

Example 9 - Favourable alterations in gut Microbiota of Cyp8b1-/- mice.

The pool size and composition of BA greatly influences the composition of gut microbiota (Hofmann, A. F., & Eckmann, L. (2006). How bile acids confer gut mucosal protection against bacteria. *Pnas*, *103*(10), 4333–4334. http://doi.org/10.1073/pnas.0600780103). The obstruction of bile flow causes small intestinal bacterial overgrowth and a compromised epithelial barrier, which can be reversed by oral administration of bile acids (Inagaki, T., Moschetta, A., Lee, Y.-K., Peng, L., Zhao, G., Downes, M., Kliewer, S. A. (2006). Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *PNAS*, *103*(10), 3920–5.). Gut microbiota composition in turn influences hepatic

tolerance and immune-suppression. Moreover, recent data strongly indicates that dietary cholesterol directly induces inflammation and reduces barrier function in the intestine (Progatzky, F., Sangha, N. J., Yoshida, N., McBrien, M., Cheung, J., Shia, A., Dallman, M. J. (2014). Dietary cholesterol directly induces acute inflammasome-dependent intestinal inflammation. *Nature Communications*, *5*, 5864.). Therefore, we hypothesized that the absence of CA and the associated changes in BA composition in Cyp8b1-/- mice and the reduced uptake of cholesterol, would alter the gut microbial community structure and inflammation in the gut.

Analysis of gut microbiota:

The pyrosequencing and bioinformatics analysis of bacterial 16S rRNA was performed by the commercial vendor and service provider, Microbiome Insights Inc, Vancouver, BC, using samples from the jejunum of Cyp8b1+/+ and Cyp8b1-/- mice after 12 weeks of HCD diet.

Sample Preparation

Bacterial DNA was isolated from the intestines using standardized protocols for the PowerSoil Extraction kit (Mo Bio Laboratories, Qiagen, Carlsbad, CA), which has been previously validated for samples in our lab. Purified DNA was amplified using dual-barcoded primers targeting the V4 region of the 16S rRNA gene. Amplicons were subjected to 2 x 250 paired-end sequencing on a MiSeq. Additional wet-lab protocols, including library normalization and PhiX% spike-in, are included in Kozich et al. (2013).

Sequence curation and analysis

We sequenced 16S rRNA gene V4 amplicons generated from 14 mouse intestine samples on a MiSeq. MiSeq-generated Fastq files were quality-filtered and clustered into 97% similarity operational taxonomic units (OTUs) using the mothur software package [http://www.mothur.org]. We obtained 87,116 high-

quality reads and 2,305 OTUs (including those occurring once with a count of 1, or singletons).

Taxonomic composition

High quality reads were classified using Greengenes v. 13_8 as the reference database. We aggregated OTUs into phylum-level and genus-level taxa and plotted the relative abundance of the most abundant taxa. In the figure legends, "Other" represents unclassified and lower-abundance taxa.

Results:

The microbiota composition of the small intestines from HCD fed Cyp8b1+/+ and Cyp8b1-/- mice were analyzed using the 16S rDNA variable region 4 (V4) amplicon sequencing method. Analysis revealed phylum and genus level changes in microbial composition of the jenunum in Cyp8b1-/- mice (Figures 14A-C). We calculated the ecological similarity represented in Figure 14 B, using Pearsons correlation. The analysis showed that Cyp8b1-/- mice have distinct gut microbial communities. At the phylum level (Figure 14A), Cyp8b1-/- mice showed an increase in the fermicute to bacteroidetes ratio. The levels of fermicutes were increased whereas those of bacteriodetes were reduced. These changes are characteristic of those seen in lean mice or mice that have undergone weight loss (Zhang, C., Li, S., Yang, L., Huang, P., Li, W., Wang, S., & Zhao, G. (2013). Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nature Communications*, *4*, 1–10.). The proportions of cholesterol degrading phyla, Acidobacteria and Actinobacteria, increased in Cyp8b1-/- mice from 1% to 2% and from 2% to 6% respectively, compared with those in Cyp8b1+/+ mice (Casabon, I. (2014). Actinobacterial Acyl Coenzyme A Synthetases Involved in Steroid, J. Bacteriol *196*(3), 579–587.; and Casabon, I., Crowe, A. M., Liu, J., & Eltis, L. D. (2013). FadD3 is an acyl-CoA synthetase that initiates catabolism of cholesterol rings C and D in actinobacteria. Mol Microbiol*87*269–283.). At the genus level, we identified a huge increase in the proportion of Allobaculum (18 fold increase). The levels of Allobaculum

negatively correlate with body weight and plasma leptin levels and positively correlate with lifespan (Zhang, C., Li, S., Yang, L., Huang, P., Li, W., Wang, S., & Zhao, G. (2013). Structural modulation of gut microbiota in life-long calorie-restricted mice. *Nature Communications*, *4*, 1–10.). Interestingly, the levels of Bifidobacterium, a cholesterol-owering probiotic microbial phylum, were also increased in Cyp8b1-/- mice (6 fold increase) (Zanotti, I., Turroni, F., Piemontese, A., Mancabelli, L., Milani, C., Viappiani, A., Sinderen, D. Van. (2015). Evidence for cholesterol-lowering activity by Bifidobacterium bifidum PRL2010 through gut microbiota modulation, 6813–6829.; and Bordoni, A., Amaretti, A., Leonardi, A., Boschetti, E., Danesi, F., Matteuzzi, D., ... Rossi, M. (2013). Cholesterol-lowering probiotics : in vitro selection and in vivo testing of bifidobacteria, 8273–8281). These changes are indicative of beneficial alterations in the composition of gut microbiota of Cyp8b1-/- mice.

Example 10 - Sequences for different targeting strategies:

mRNA secondary structure prediction:

A given single stranded nucleic acid molecule has a high propensity to form secondary structures by establishing intra-molecular base pairing. The simple complimentary Watson-Crick rules for base pairing and GU pairing dictate the formation of ordered helices and less ordered loops within the molecule(Mathews, D. (2004) Using an RNA secondary structure partition function to determine confidence in base pairs predicted by free energy minimization. RNA. 10(8): 1178-1190). Dynamic algorithms have been developed that can predict with greater accuracy the secondary structure of a given nucleic acid sequence. Importantly, free energy minimization alone is sufficient to predict 40% of potential base pairing(Mathews, D. (2004) Using an RNA secondary structure partition function to determine confidence in base pairs predicted by free energy minimization. RNA. 10(8): 1178-1190,). This, together with considerations of entropy of stacking co-axial helices separated by single base pair mismatches yields a high degree of confidence in prediction of secondary mRNA structures(Mathews, D (2004) Using an RNA secondary structure partition

function to determine confidence in base pairs predicted by free energy minimization. RNA. 10(8): 1178-1190). Based on these criteria a freely accessible web-based program suite, The ViennaRNA Web Service has been developed and can be accessed here: http://rna.tbi.univie.ac.at/.The website provides number of prediction tools which reveal the thermodynamic properties and emergent base-pairing probabilities leading to the secondary structure of a given RNA molecule. The RNAUp tool is then employed to predict the interaction free energy and the energy needed to open an existing secondary structure in the given sequence(Lorenz, R. et al., 2011 ViennaRNA Package 2.0. Algorithms Mol Biol.6: 26)(Gruber et al., 2008 The Vienna RNA Websuite. Nucleic Acids Res. July 1; 36: W70-W74).

The RNAeval tool was used to get a detailed thermodynamic description (loop free-energy decomposition) of the cDNA sequence of the human CYP8B1 mRNA. This permits the generation of the secondary structure shown in Figure 15A. The probability of base-pairing for each nucleotide is color coded shown in the scale / legend below with blue indicating a base pairing probability of 0 and red indicating maximum probability of 1. RNAup tool was used to determine the required interaction free energy for various regions within the secondary structure shown in Figure 15B. Based on this regions of interest were identified marked as shown Figure 15C (sequences marked with boxes). The regions of interest were then utilized to derive the desired sequences of targeting molecules for each of the different strategies outlined in the tables below after applying constraints against sequence homology using simple BLAST search and high GC content.

siRNA (21 nucleotides + 2 overhang)		
SEQ ID NO	*Base pair position*	*Sequence 5'-3'*
57	148	CGGAAGAATATGTTTGAATTTCT
58	402	TGGGCAATATGCAAAAAAACTGG
59	419	AATGAGACCATGCTGGACAGCCT
60	618	CCGCAAGTTTGACCTTCTTTTCC
61	765	GGGCAACATGCTTCAGTTTCTGA
62	815	AGGACAAGTTCAACTTCATGATG
63	879	GCCCTCTTGTACCTCCTGAAGCA

siRNA (21 nucleotides + 2 overhang)		
SEQ ID NO	*Base pair position*	*Sequence 5'-3'*
64	1065	CTCCTCAGGTTGGTTCATGAAGA
65	1085	AAGACTATACCCTGAAGATGTCC
66	1080	TCATGAAGACTATACCCTGAAGA
67	1242	CCGGAAAGTGGACTTCTTCAAGA
68	1347	GTGAAGCTCTTTATCCTGCTTAT
69	1373	CACACACTTTGACTTAGAGTTGG

Dicer substrate siRNA (3'-2X deoxy nucleotides + 23 nucleotides + 2 overhang)		
SEQ ID NO:	*Base pair position*	*Sequence 5'-3'*
70	18	AGTGCTGGGAGCTCTGCTGGTGGTCAT
71	134	ATGCCATGGCTTTCCGGAAGAATATGT
72	236	CGTCATGGACCCCCTCTCCTTTGGCTC
73	292	ACTTTGGGCAATATGCAAAAAAACTGG
74	401	ATGGCTTGAAGGATCTTAATGAGACCA
75	418	AATGAGACCATGCTGGACAGCCTGTCC
76	610	TGGAGTTCCGCAAGTTTGACCTTCTTT
77	769	ACATGCTTCAGTTTCTGAGGGAGCAGG
78	800	ACCCTCAGCTATGCAGGACAAGTTCAA
79	883	CTCTTGTACCTCCTGAAGCACCCAGAA
80	1042	CTGCGGCTGAGGGCTGCACCCACCCTC
81	1084	GAAGACTATACCCTGAAGATGTCCAGT
82	1234	ATGGCAGCCGGAAAGTGGACTTCTTCA
83	1279	TCCACTACACCATGCCCTGGGGTTCGG
84	1348	TGAAGCTCTTTATCCTGCTTATGGTCAC
85	1383	GACTTAGAGTTGGTGGACCCTGACACA

Gapmer anti-sense oligonucleotides of the configuration (4X) LNA -(6X) PS - (4X)LNA LNA = locked nucleic acid; PS = phosphorothioate modification		
SEQ ID NO:	*Base pair position*	*Sequence 5'-3'*
86	15	TCCAGTGCTGGGAG
87	147	CCGGAAGAATATGT
88	279	ACAGAGAAAACTAG
89	401	ATGGCTTGAAGGAT
90	427	ATGCTGGACAGCCT
91	610	TGGAGTTCCGCAAG
92	782	TCTGAGGGAGCAGG
93	805	CAGCTATGCAGGAC

Gapmer anti-sense oligonucleotides of the configuration (4X) LNA -(6X) PS - (4X)LNA LNA = locked nucleic acid; PS = phosphorothioate modification		
SEQ ID NO:	*Base pair position*	*Sequence 5'-3'*
94	822	GTTCAACTTCATGA
95	885	TTGTACCTCCTGAA
96	906	AGAAGCTATTCGGG
97	1045	GGCTGAGGGCTGCA
98	1066	TCCTCAGGTTGGTT
99	1087	ACTATACCCTGAAG
100	1245	AAAGTGGACTTCTT
101	1284	TACACCATGCCCTG
102	1348	TGAAGCTCTTTATC
103	1384	ACTTAGAGTTGGTG

> Hs_CYP8B1 cDNA sequence (SEQ ID NO: 104)

ATGGTTCTCTGGGGTCCAGTGCTGGGAGCTCTGCTGGTGGTCATTGCTGGATACCTGTG
CCTGCCAGGGATGCTCCGACAACGCAGGCCATGGGAGCCCCCTCTGGACAAGGGTACCG
TGCCCTGGCTTGGCCATGCCATGGCTTTCCGGAAGAATATGTTTGAATTTCTGAAGCGC
ATGAGGACCAAGCATGGGGATGTGTTCACAGTGCAGCTAGGGGGCCAGTACTTCACCTT
CGTCATGGACCCCCTCTCCTTTGGCTCCATCCTCAAGGACACACAGAGAAAACTAGACT
TTGGGCAATATGCAAAAAAACTGGTGCTGAAGGTATTTGGATACCGTTCAGTGCAAGGG
GACCATGAGATGATACACTCAGCCAGCACCAAGCATCTGAGGGGGGATGGCTTGAAGGA
TCTTAATGAGACCATGCTGGACAGCCTGTCCTTTGTAATGCTGACGTCCAAAGGCTGGA
GTCTGGATGCCAGTTGCTGGCATGAGGACAGCCTCTTTCGCTTCTGCTATTACATCTTG
TTCACAGCTGGCTACCTGAGCTTGTTCGGCTACACGAAGGACAAGGAGCAGGACCTGCT
ACAGGCAGGAGAGTTATTCATGGAGTTCCGCAAGTTTGACCTTCTTTTCCCAAGGTTTG
TCTACTCCCTGCTGTGGCCCCGGGAGTGGCTAGAAGTGGGCCGACTCCAGCGTCTCTTT
CACAAGATGCTCTCCGTGAGCCACAGCCAGGAGAAGGAGGGCATCAGCAACTGGCTGGG
CAACATGCTTCAGTTTCTGAGGGAGCAGGGGGTACCCTCAGCTATGCAGGACAAGTTCA
ACTTCATGATGCTCTGGGCCTCCCAGGGGAACACGGGGCCTACCTCTTTCTGGGCCCTC
TTGTACCTCCTGAAGCACCCAGAAGCTATTCGGGCTGTGAGGGAGGAAGCTACCCAGGT
CCTGGGTGAGGCCAGGCTGGAGACCAAGCAGTCCTTTGCCTTCAAACTCGGTGCCCTGC
AACACACCCCAGTTCTAGACAGCGTGGTGGAGGAGACGCTGCGGCTGAGGGCTGCACCC
ACCCTCCTCAGGTTGGTTCATGAAGACTATACCCTGAAGATGTCCAGTGGGCAGGAGTA
TCTGTTCCGCCATGGAGACATCCTGGCCCTCTTTCCCTACCTCTCAGTGCACATGGACC
CTGACATCCACCCTGAGCCCACCGTCTTCAAGTACGATCGCTTCCTCAACCCTAATGGC
AGCCGGAAAGTGGACTTCTTCAAGACAGGCAAGAAGATCCACCACTACACCATGCCCTG
GGGTTCGGGCGTTTCCATCTGCCCTGGGAGGTTCTTTGCACTCAGTGAGGTGAAGCTCT
TTATCCTGCTTATGGTCACACACTTTGACTTAGAGTTGGTGGACCCTGACACACCACTA
CCCCATGTTGACCCGCAGCGCTGGGGTTTTGGCACCATGCAGCCCAGCCACGATGTGCG
CTTCCGCTACCGCCTGCATCCTACAGAGTGA

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. Furthermore, numeric ranges are provided so that the range of values is recited in addition to the individual values within the recited range being specifically recited in the absence of the range. The word "comprising" is used herein as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention. Furthermore, material appearing in the background section of the specification is not an admission that such material is prior art to the invention. Any priority document(s) are incorporated herein by reference as if each individual priority document were specifically and individually indicated to be incorporated by reference herein and as though fully set forth herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

What is claimed is:

1. An oligomer of between 4 to 49 nucleobases in length which comprises a contiguous nucleotide sequence of a total of between 4 to 49 nucleotides, wherein the contiguous nucleotide sequence is targeted to hybridize to a portion of SEQ ID NO: 104:

ATGGTTCTCTGGGGTCCAGTGCTGGGAGCTCTGCTGGTGGTCATTGCTGGATACCTGTG
CCTGCCAGGGATGCTCCGACAACGCAGGCCATGGGAGCCCCCTCTGGACAAGGGTACCG
TGCCCTGGCTTGGCCATGCCATGGCTTTCCGGAAGAATATGTTTGAATTTCTGAAGCGC
ATGAGGACCAAGCATGGGGATGTGTTCACAGTGCAGCTAGGGGGCCAGTACTTCACCTT
CGTCATGGACCCCCTCTCCTTTGGCTCCATCCTCAAGGACACACAGAGAAAACTAGACT
TTGGGCAATATGCAAAAAAACTGGTGCTGAAGGTATTTGGATACCGTTCAGTGCAAGGG
GACCATGAGATGATACACTCAGCCAGCACCAAGCATCTGAGGGGGGATGGCTTGAAGGA
TCTTAATGAGACCATGCTGGACAGCCTGTCCTTTGTAATGCTGACGTCCAAAGGCTGGA
GTCTGGATGCCAGTTGCTGGCATGAGGACAGCCTCTTTCGCTTCTGCTATTACATCTTG
TTCACAGCTGGCTACCTGAGCTTGTTCGGCTACACGAAGGACAAGGAGCAGGACCTGCT
ACAGGCAGGAGAGTTATTCATGGAGTTCCGCAAGTTTGACCTTCTTTTCCCAAGGTTTG
TCTACTCCCTGCTGTGGCCCCGGGAGTGGCTAGAAGTGGGCCGACTCCAGCGTCTCTTT
CACAAGATGCTCTCCGTGAGCCACAGCCAGGAGAAGGAGGGCATCAGCAACTGGCTGGG
CAACATGCTTCAGTTTCTGAGGGAGCAGGGGGTACCCTCAGCTATGCAGGACAAGTTCA
ACTTCATGATGCTCTGGGCCTCCCAGGGGAACACGGGGCCTACCTCTTTCTGGGCCCTC
TTGTACCTCCTGAAGCACCCAGAAGCTATTCGGGCTGTGAGGGAGGAAGCTACCCAGGT
CCTGGGTGAGGCCAGGCTGGAGACCAAGCAGTCCTTTGCCTTCAAACTCGGTGCCCTGC
AACACACCCCAGTTCTAGACAGCGTGGTGGAGGAGACGCTGCGGCTGAGGGCTGCACCC
ACCCTCCTCAGGTTGGTTCATGAAGACTATACCCTGAAGATGTCCAGTGGGCAGGAGTA
TCTGTTCCGCCATGGAGACATCCTGGCCCTCTTTCCCTACCTCTCAGTGCACATGGACC
CTGACATCCACCCTGAGCCCACCGTCTTCAAGTACGATCGCTTCCTCAACCCTAATGGC
AGCCGGAAAGTGGACTTCTTCAAGACAGGCAAGAAGATCCACCACTACACCATGCCCTG
GGGTTCGGGCGTTTCCATCTGCCCTGGGAGGTTCTTTGCACTCAGTGAGGTGAAGCTCT
TTATCCTGCTTATGGTCACACACTTTGACTTAGAGTTGGTGGACCCTGACACACCACTA

CCCCATGTTGACCCGCAGCGCTGGGGTTTTGGCACCATGCAGCCCAGCCACGATGTGCG CTTCCGCTACCGCCTGCATCCTACAGAGTGA (SEQ ID NO: 104)

wherein the oligomer, when bound to the portion of SEQ ID NO: 104 reduces expression of Sterol 12-α hydroxylase (Cyp8b1) in a cell or tissue.

2. The oligomer of claim 1 wherein the oligomer is between 14 to 29 nucleotides in length.

3. The oligomer of claim 1 or 2 wherein the oligomer is between 18 to 23 nucleotides in length.

4. The oligomer of any one of claims 1 to 3 wherein the oligomer is between 21 to 27 nucleotides in length.

5. The oligomer of any one of claims 1 to 4 wherein the cell or tissue is a liver cell or a liver tissue.

6. The oligomer of any one of claims 1 to 5 wherein the oligomer comprises a sequence selected from the group consisting of SEQ ID NOs 57 to 103, 105 and 106:

SEQ ID NO	*Sequence 5'-3'*
57	CGGAAGAATATGTTTGAATTTCT
58	TGGGCAATATGCAAAAAAACTGG
59	AATGAGACCATGCTGGACAGCCT
60	CCGCAAGTTTGACCTTCTTTTCC
61	GGGCAACATGCTTCAGTTTCTGA
62	AGGACAAGTTCAACTTCATGATG
63	GCCCTCTTGTACCTCCTGAAGCA
64	CTCCTCAGGTTGGTTCATGAAGA
65	AAGACTATACCCTGAAGATGTCC
66	TCATGAAGACTATACCCTGAAGA
67	CCGGAAAGTGGACTTCTTCAAGA
68	GTGAAGCTCTTTATCCTGCTTAT
69	CACACACTTTGACTTAGAGTTGG
70	AGTGCTGGGAGCTCTGCTGGTGGTCAT

SEQ ID NO	*Sequence 5'-3'*
71	ATGCCATGGCTTTCCGGAAGAATATGT
72	CGTCATGGACCCCCTCTCCTTTGGCTC
73	ACTTTGGGCAATATGCAAAAAAACTGG
74	ATGGCTTGAAGGATCTTAATGAGACCA
75	AATGAGACCATGCTGGACAGCCTGTCC
76	TGGAGTTCCGCAAGTTTGACCTTCTTT
77	ACATGCTTCAGTTTCTGAGGGAGCAGG
78	ACCCTCAGCTATGCAGGACAAGTTCAA
79	CTCTTGTACCTCCTGAAGCACCCAGAA
80	CTGCGGCTGAGGGCTGCACCCACCCTC
81	GAAGACTATACCCTGAAGATGTCCAGT
82	ATGGCAGCCGGAAAGTGGACTTCTTCA
83	TCCACTACACCATGCCCTGGGGTTCGG
84	TGAAGCTCTTTATCCTGCTTATGGTCAC
85	GACTTAGAGTTGGTGGACCCTGACACA
86	TCCAGTGCTGGGAG
87	CCGGAAGAATATGT
88	ACAGAGAAAACTAG
89	ATGGCTTGAAGGAT
90	ATGCTGGACAGCCT
91	TGGAGTTCCGCAAG
92	TCTGAGGGAGCAGG
93	CAGCTATGCAGGAC
94	GTTCAACTTCATGA
95	TTGTACCTCCTGAA
96	AGAAGCTATTCGGG
97	GGCTGAGGGCTGCA
98	TCCTCAGGTTGGTT
99	ACTATACCCTGAAG
100	AAAGTGGACTTCTT
101	TACACCATGCCCTG
102	TGAAGCTCTTTATC
103	ACTTAGAGTTGGTG
105	GCTCACTTCCACCCACTCCC
106	TTCATCTCGCTGAGGGCAAA

.

7. The oligomer of claim 6 wherein the oligomer is modified to include one ore more locked nucleic acid bases and one or more phosphorothioate modification.

8. The oligomer of any one of claims 1 to 7 wherein the contiguous nucleotide sequence comprises nucleotide analogues.

9. The oligomer of any one of claims 1 to 8 wherein the oligomer has a modified internucleoside linkage.

10. The oligomer of claim 9 wherein the modified internucleoside linkage is a peptide-nucleic acid linkage, a morpholino linkage, a N3' to P5' phosphoramidate linkage, a methylphosphonate linkage or a phosphorothioate linkage.

11. The oligomer of any one of claims 1 to 10 wherein the oligomer has one or more modified sugar moiety.

12. The oligomer of claim 11 wherein the modified sugar moiety is 2 ' -O-alkyl oligoribonucleotide.

13. The oligomer of any one of claims 1 to 12 wherein the oligomer is a gapmer.

14. The oligomer of claim 13 wherein the oligomer has a 2'MOE gapmer modification.

15. The oligomer of any one of claims 1 to 14 wherein the oligomer has a modified nucleobase.

16 The oligomer of claim 15 wherein the modified nucleobase is a 5-methyl pyrimidine or a 5- propynyl pyrimidine.

17. The oligomer of claim 16 wherein the one or more nucleotide analogues comprise locked nucleic acids (LNA) units.

18. The oligomer according to claim 16 or 17 wherein the LNA units consist of beta-D- oxy-LNA monomers.

19. The oligomer of claim 7 wherein the oligomer is selected from the group consisting of:
5' +G*+C*+T*+C*A*C*T*T*C*C*A*C*C*C*A*C*+T*+C*+C*+C 3' (SEQ ID NO:1); and
5' +T*+T*+C*+A*T*C*T*C*G*C*T*G*A*G*G*G*+C*+A*+A*+A3' (SEQ ID NO: 2)
wherein '+' denotes a locked nucleic acid base and '*' indicates a phosphorothioate modification.

20. The oligomer of any one of claims 1 to 19 wherein the oligomer comprises a single stereoisomer.

21. The oligomer of any one of claims 1 to 19 wherein the oligomer comprises a mixture of stereoisomers.

22. A pharmaceutical composition comprising the oligomer of any one of claims 1 to 21 and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

23. A method of reducing the expression of Sterol 12-α hydroxylase (Cyp8b1) in a cell or tissue, comprising contacting the cell or tissue with an effective amount of an oligomer of any one of claims 1 to 21 or a pharmaceutical composition of claim 22.

24. The method of claim 23 wherein the cell is within a tissue of a mammal.

25. A method of prophylactic treating or treating a disease selected from the group consisting of: Liver fibrosis, Chronic liver disease, autoimmune hepatitis,

chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes, in a subject, comprising administering to the subject an effective amount of an oligomer of any one of claims 1 to 21 or administering a an effective amount of a pharmaceutical composition of claim 22, wherein the subject is currently suffering from or at risk for suffering from liver fibrosis.

26. The method of claim 25 wherein the subject is a mammal.

27. The method of claim 24 or 25 wherein the mammal is a human.

28. Use of an oligomer of any one of claims 1 to 21 for the treatment of a disease selected from the group consisting of: Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

29. Use of an oligomer of any one of claims 1 to 21 in the preparation of a medicament for the treatment of a disease selected from the group consisting of Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

30. Use of a pharmaceutical composition of claim 22 for the treatment of a disease selected from the group consisting of Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

31. The use of claim 29 or 30 wherein the disease is liver fibrosis.

32. An oligomer of any one of claims 1 to 21 for use in the treatment of a disease selected from the group consisting of Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

33. A commercial package, comprising:

a. an oligomer of any one of claims 1 to 21; and

b. instructions for the treatment of a disease selected from the group consisting of: Liver fibrosis, Chronic liver disease, autoimmune hepatitis, chronic viral hepatitis, alcoholic liver disease, liver fibrosis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, obesity, and diabetes.

34. The commercial package of claim 33 wherein the instructions are for the treatment of liver fibrosis.

Fig. 1

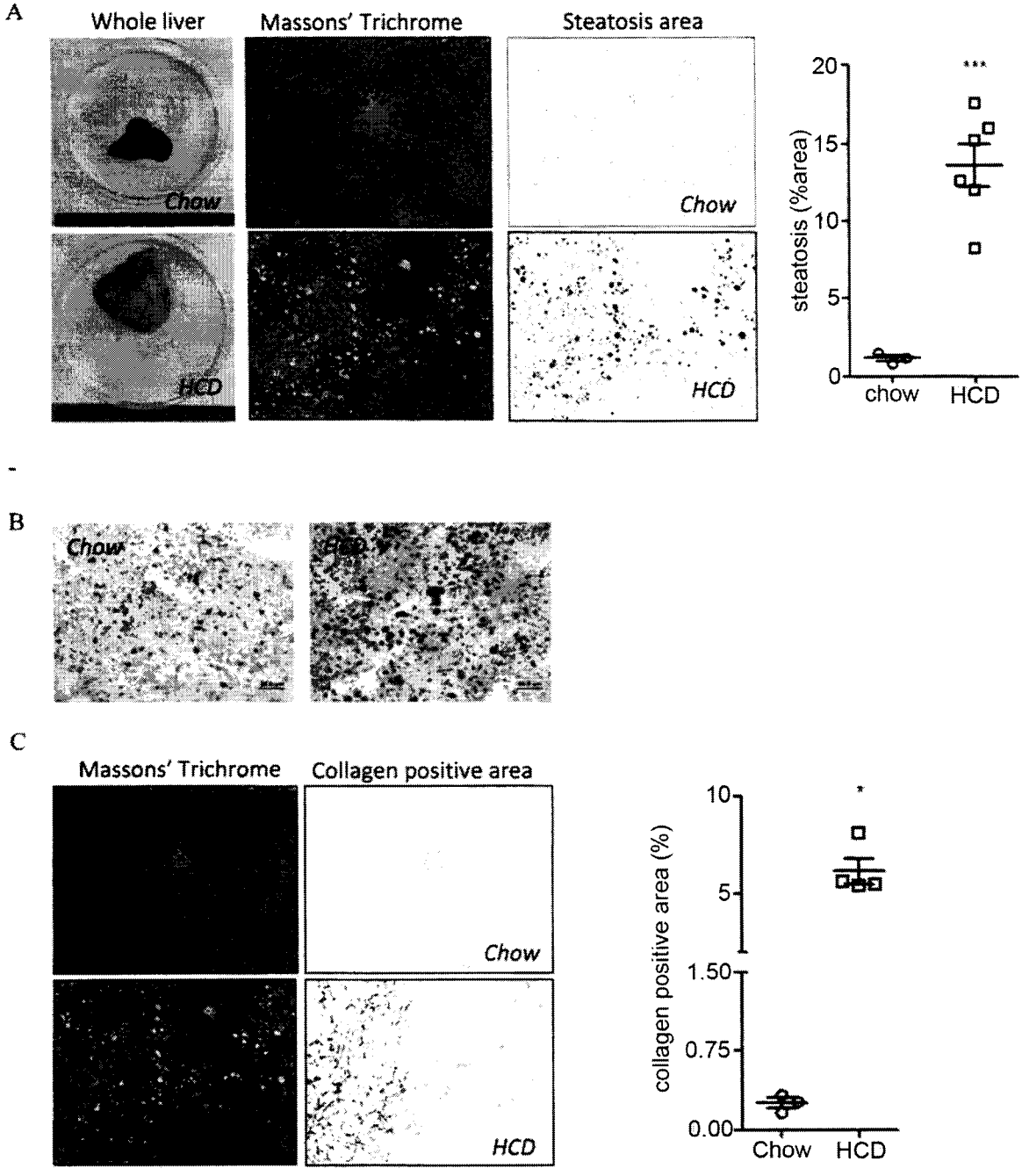
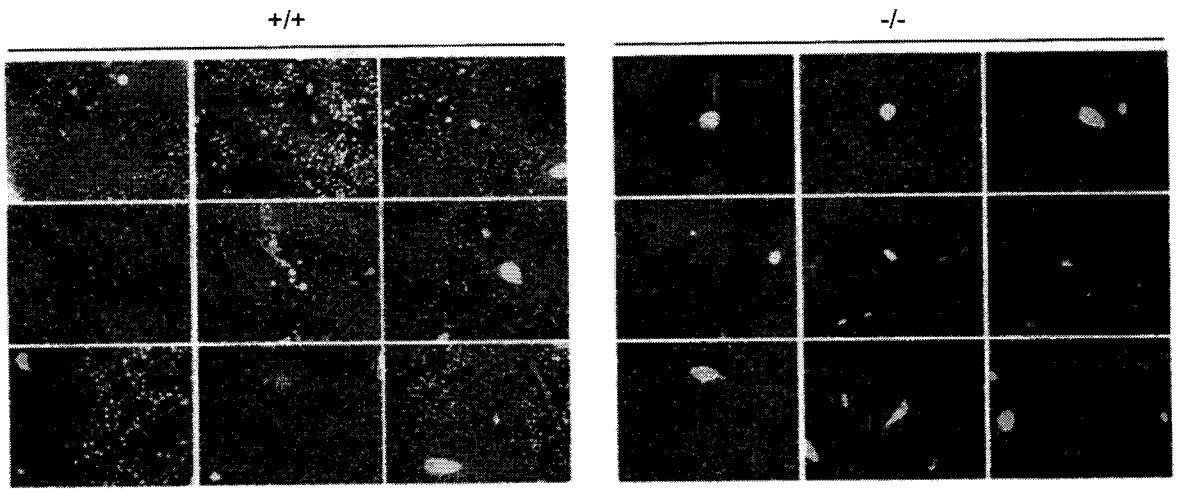

A
Whole liver
Massons' Trichrome
Steatosis area
Chow
HCD
steatosis (%area)
20
15
10
5
0
***
chow
HCD
B
Chow
HCD
C
Massons' Trichrome
Collagen positive area
Chow
HCD
collagen positive area (%)
10
5
1.50
0.75
0.00
*
Chow
HCD

Fig. 2

A



B

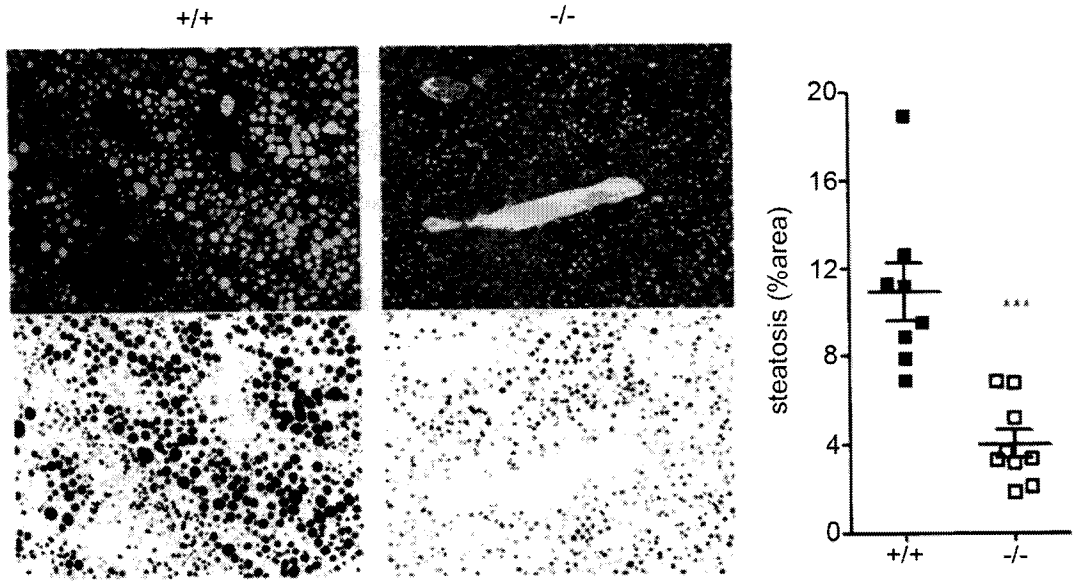
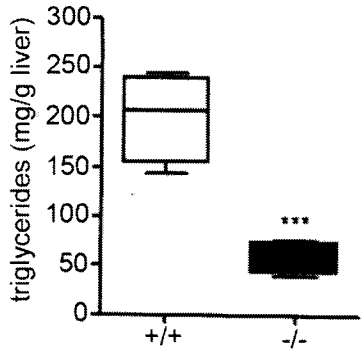


Fig. 3

A


triglycerides (mg/g liver)
300
250
200
150
100
50
0
***
+/+
-/-

B

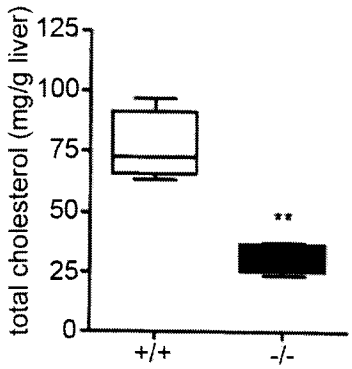

total cholesterol (mg/g liver)
125
100
75
50
25
0
**
+/+
-/-

Fig. 4

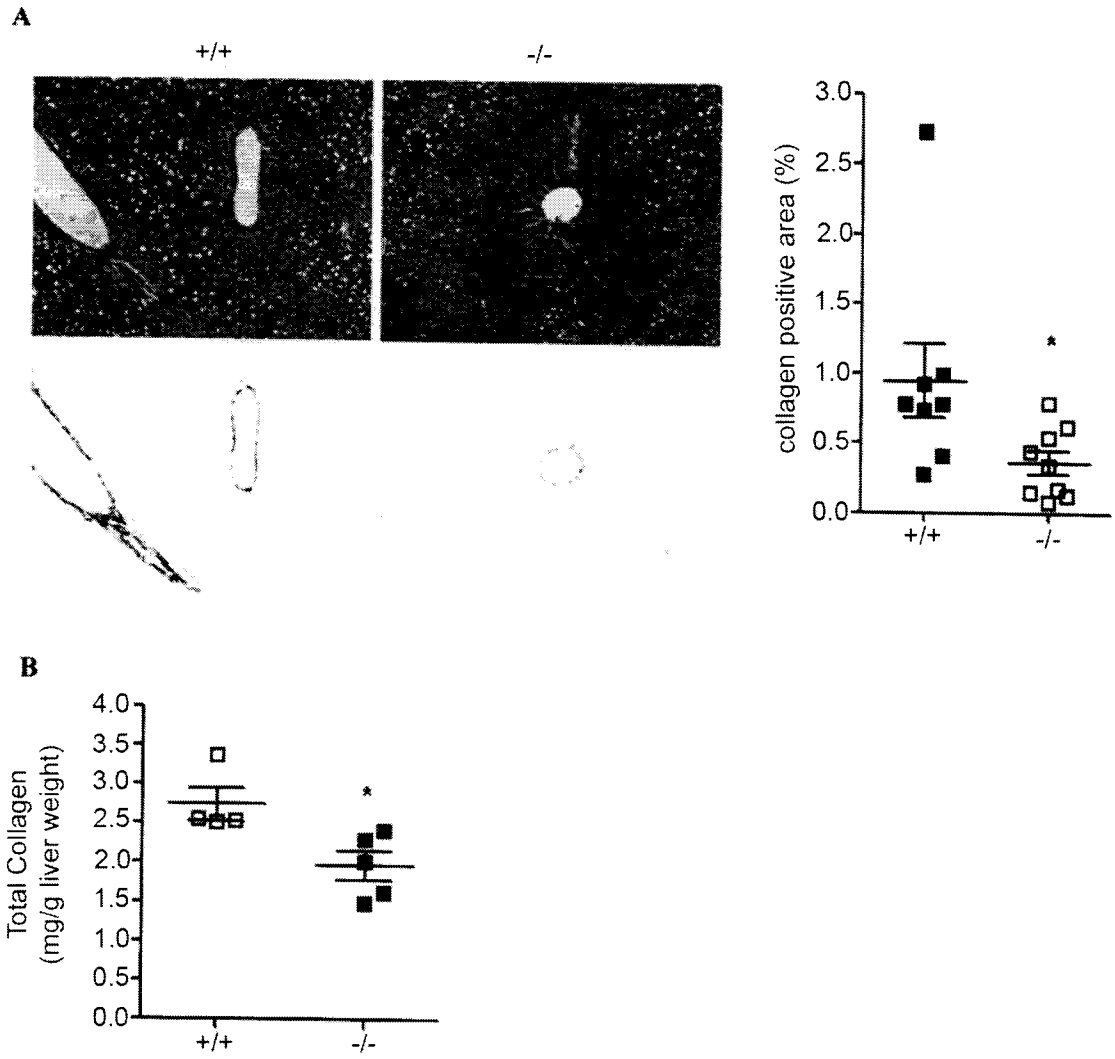

A
+/+
-/-
collagen positive area (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
+/+
-/-
*
B
Total Collagen (mg/g liver weight)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
+/+
-/-
*

Fig. 5

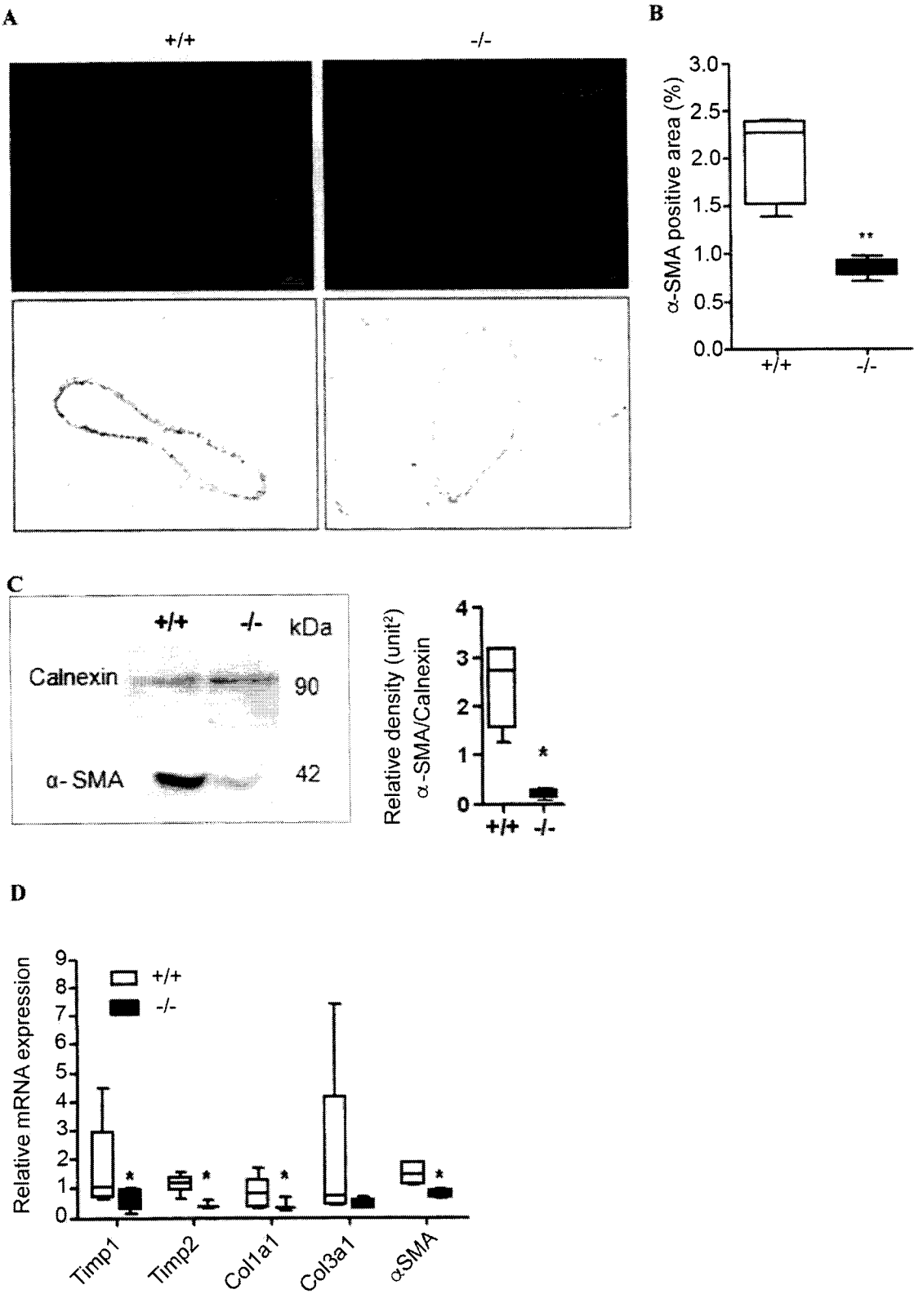

A
+/+
-/-
B
α-SMA positive area (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
+/+
-/-
C
+/+
-/-
kDa
Calnexin
90
α- SMA
42
Relative density (unit²)
α-SMA/Calnexin
4
3
2
1
0
*
+/+
-/-
D
Relative mRNA expression
9
8
7
6
5
4
3
2
1
0
+/+
-/-
*
Timp1
Timp2
Col1a1
Col3a1
αSMA

Fig. 6

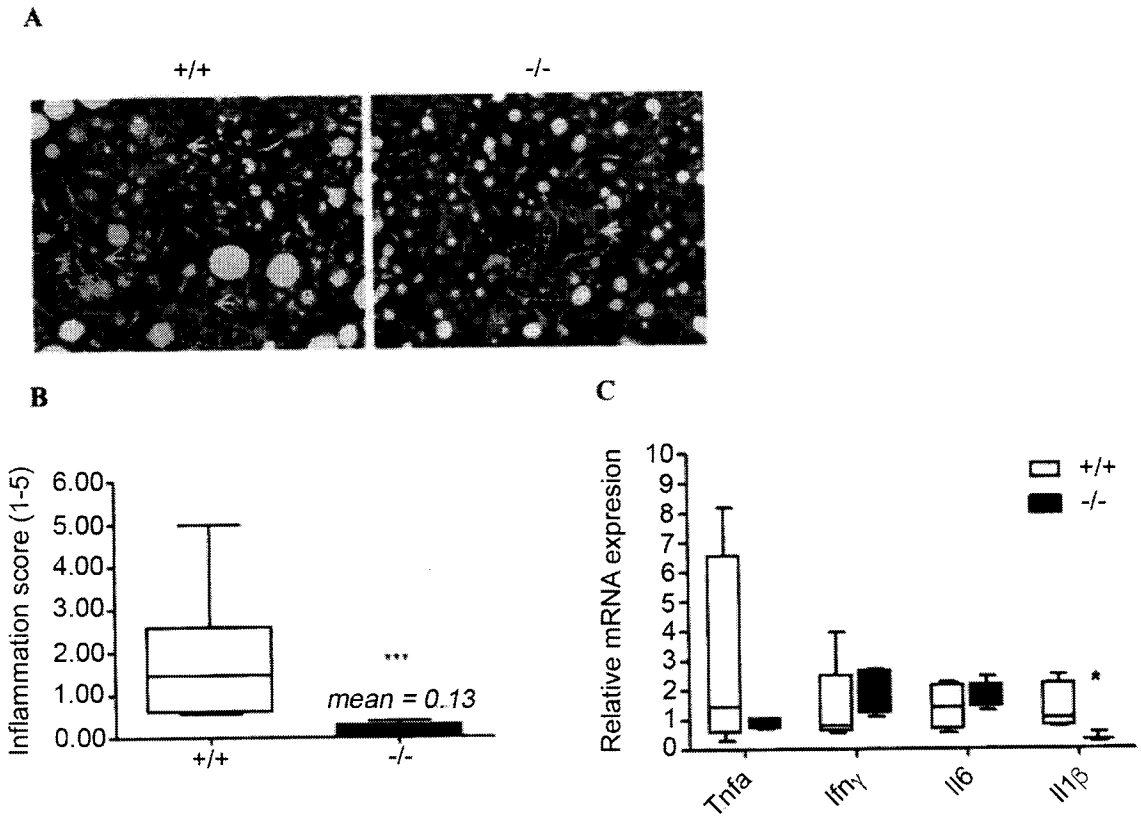
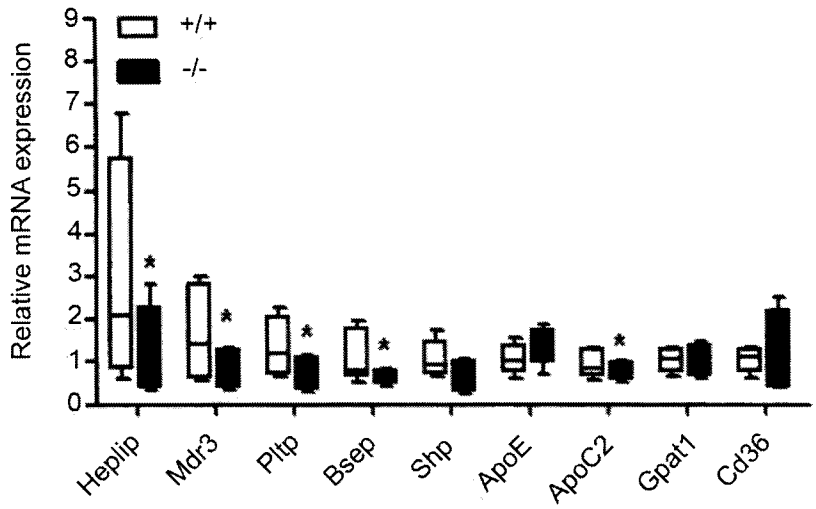

A
+/+
-/-
B
Inflammation score (1-5)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
***
mean = 0.13
+/+
-/-
C
Relative mRNA expresion
10
9
8
7
6
5
4
3
2
1
0
+/+
-/-
*
Tnfa
Ifnγ
Il6
Il1β

Fig. 7

A


+/+
-/-
Relative mRNA expression
0
1
2
3
4
5
6
7
8
9
Heplip
Mdr3
Pltp
Bsep
Shp
ApoE
ApoC2
Gpat1
Cd36

B

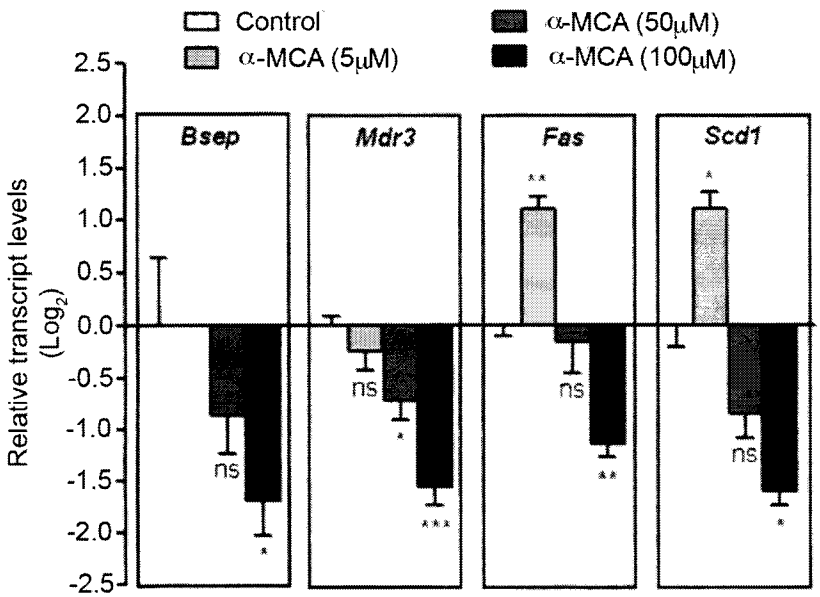
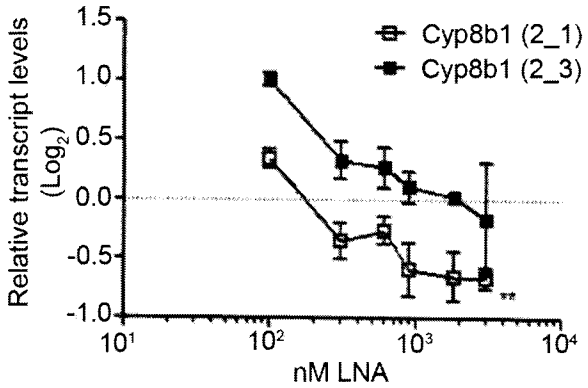

Control
α-MCA (5μM)
α-MCA (50μM)
α-MCA (100μM)
Relative transcript levels (Log2)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Bsep
Mdr3
Fas
Scd1
ns
*
**
***

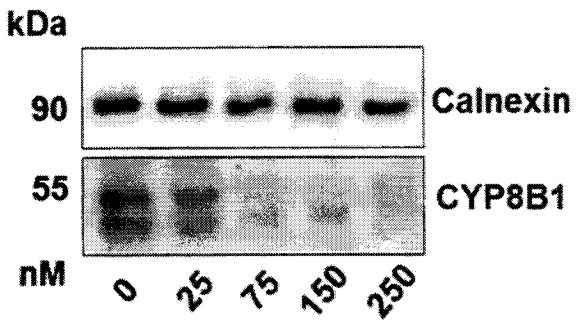
Fig. 8

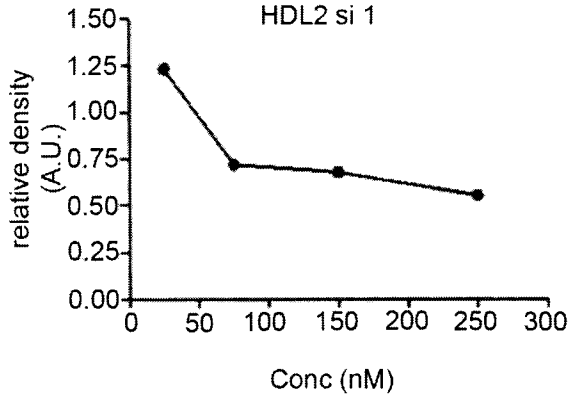


** = Spearman correlation n = 3, p = 0.006

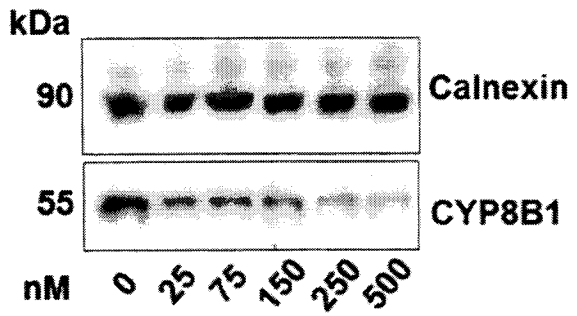
Fig. 9

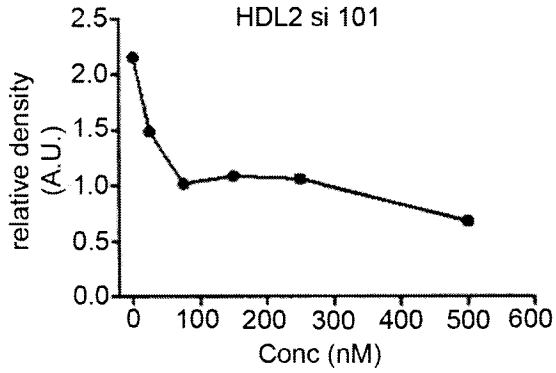
A)


kDa
90
55
nM
0
25
75
150
250
Calnexin
CYP8B1

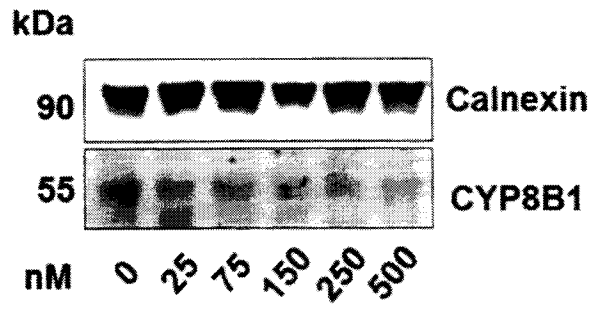

HDL2 si 1
relative density (A.U.)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
250
300
Conc (nM)

B)


kDa
90
55
nM
0
25
75
150
250
500
Calnexin
CYP8B1


HDL2 si 101
relative density (A.U.)
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
Conc (nM)

C)


kDa
90
55
nM
0
25
75
150
250
500
Calnexin
CYP8B1

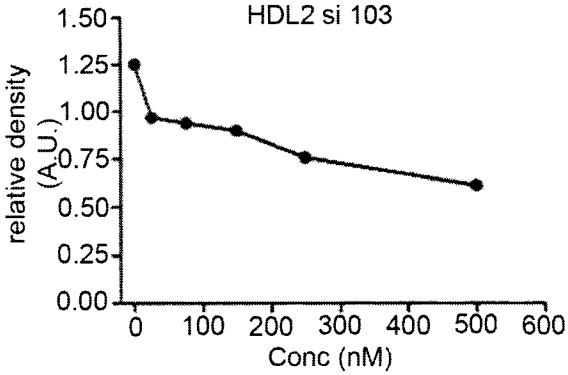
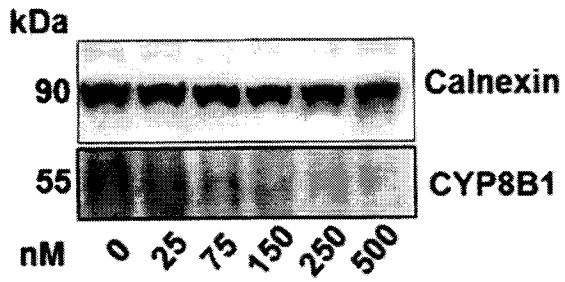
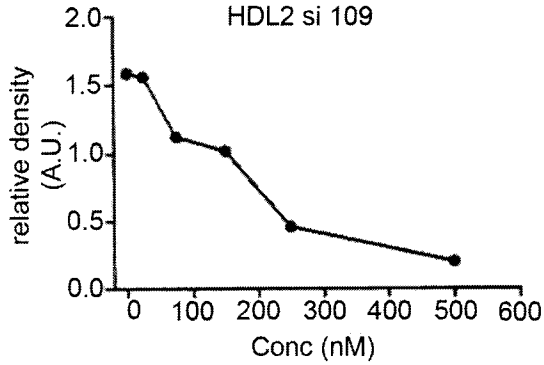

HDL2 si 103
relative density (A.U.)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
100
200
300
400
500
600
Conc (nM)

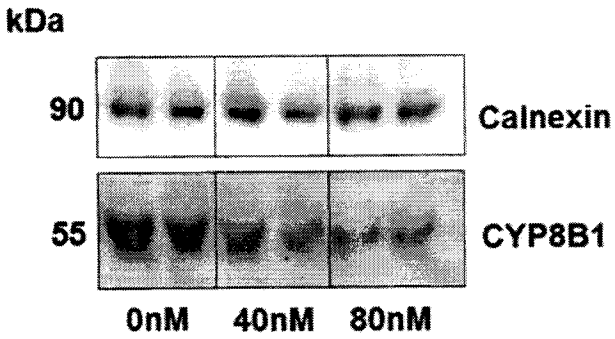
Fig 9 (cont)

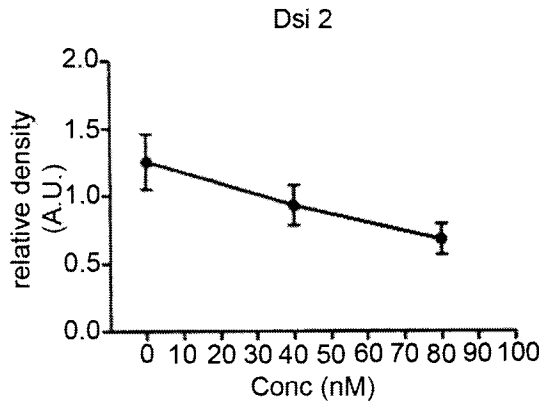
D)



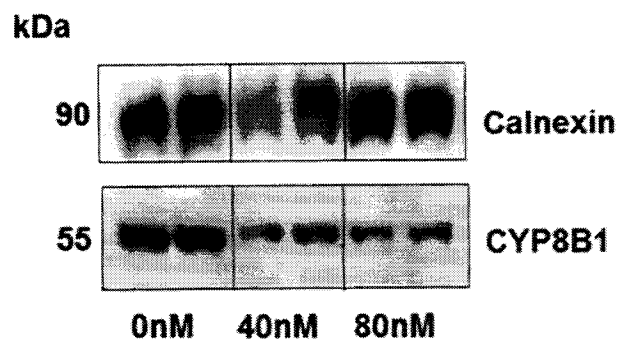


E)





F)



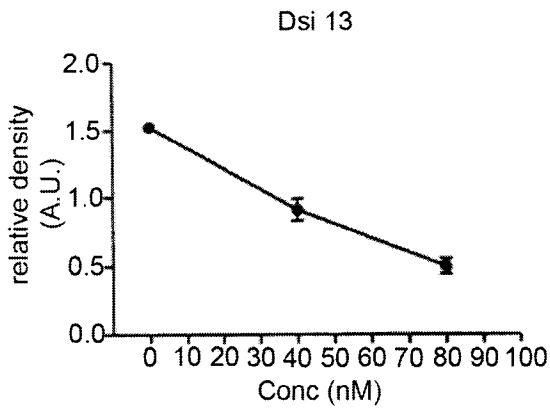
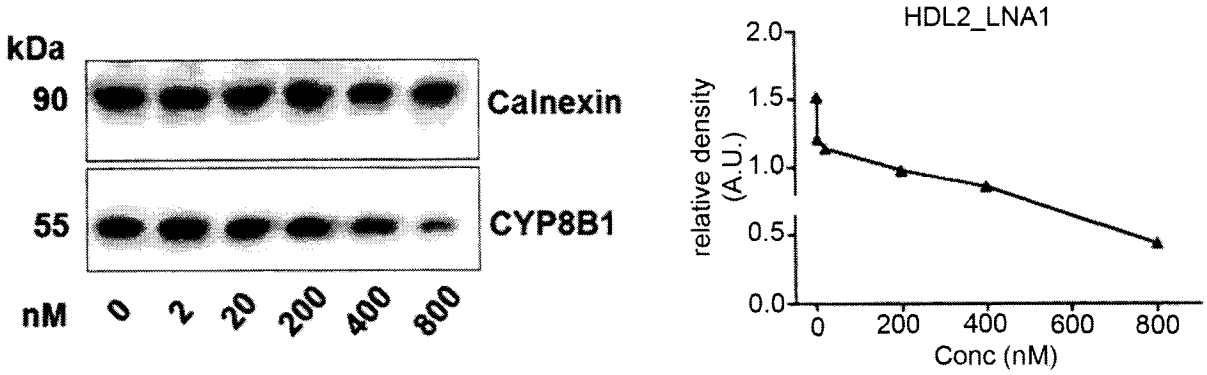


Fig. 10:

A)


kDa
90
Calnexin
55
CYP8B1
nM 0 2 20 200 400 800
HDL2_LNA1
relative density (A.U.)
2.0
1.5
1.0
0.5
0.0
0 200 400 600 800
Conc (nM)

B)

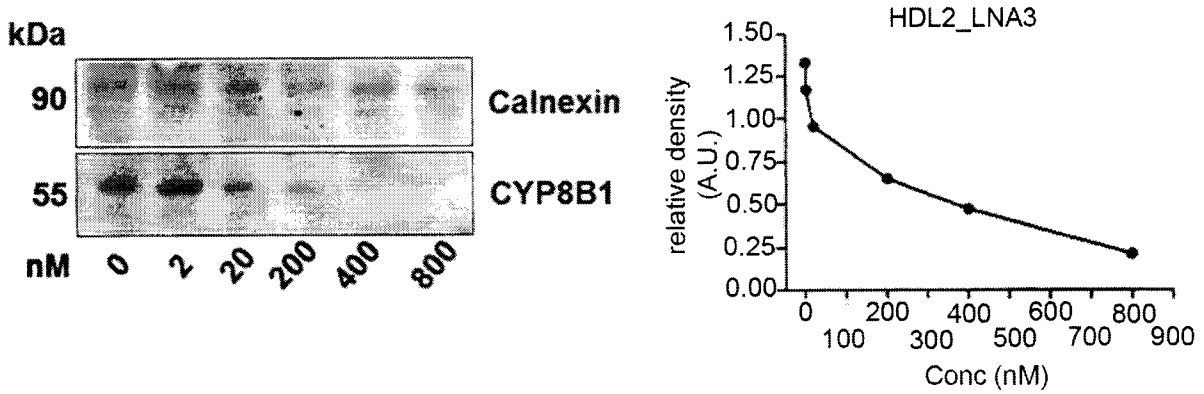
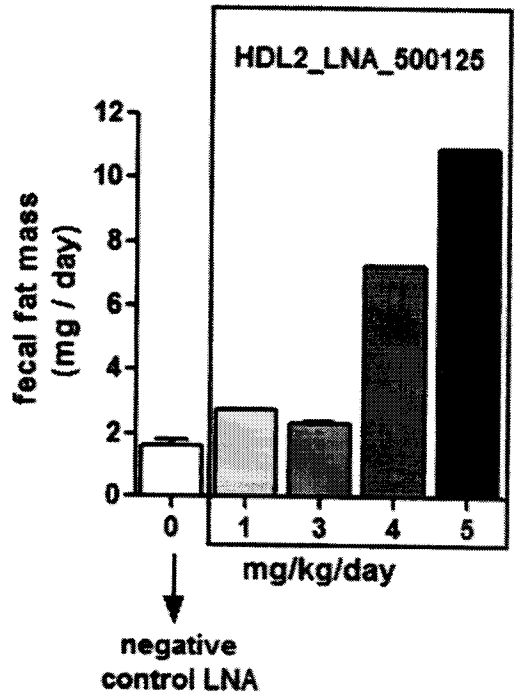

kDa
90
Calnexin
55
CYP8B1
nM 0 2 20 200 400 800
HDL2_LNA3
relative density (A.U.)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0 200 400 600 800
100 300 500 700 900
Conc (nM)

Fig. 11:

A)


HDL2_LNA_500125
fecal fat mass (mg / day)
12
10
8
6
4
2
0
0
1
3
4
5
mg/kg/day
negative control LNA

B)

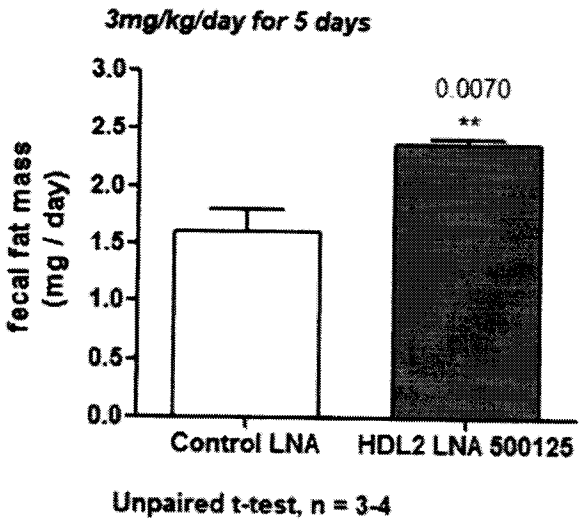
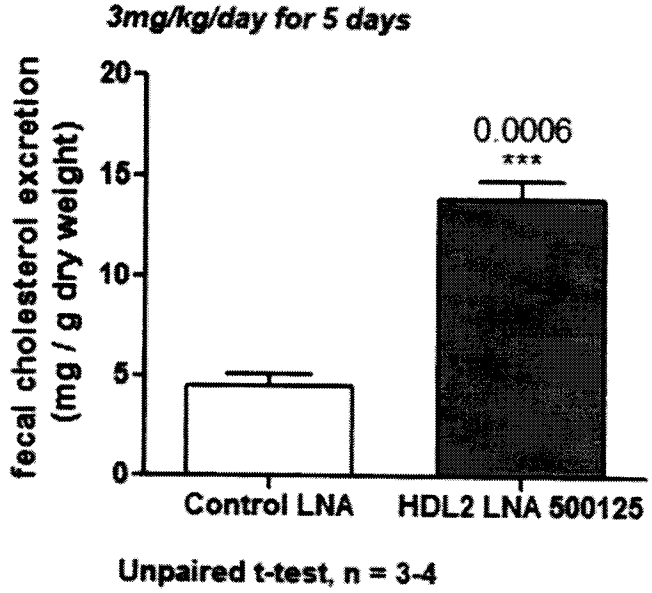

3mg/kg/day for 5 days
fecal fat mass (mg / day)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0070
**
Control LNA
HDL2 LNA 500125
Unpaired t-test, n = 3-4

Fig. 11 (cont)

C)



D)

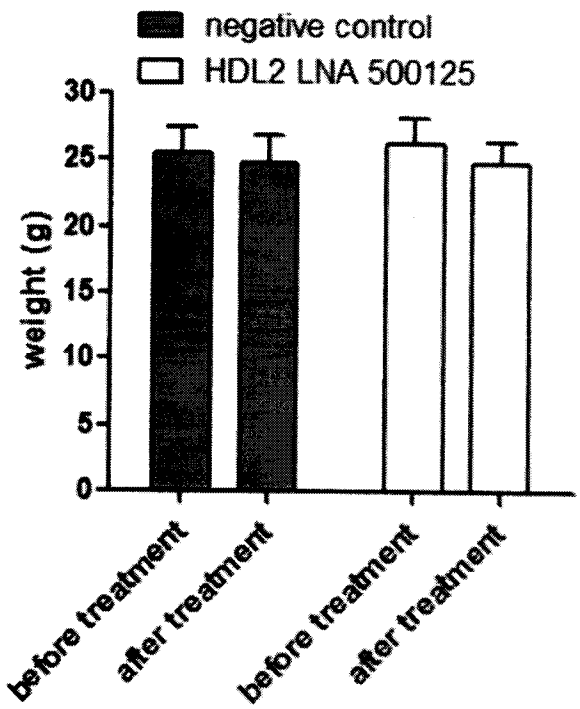


Fig. 11 (cont)

E)

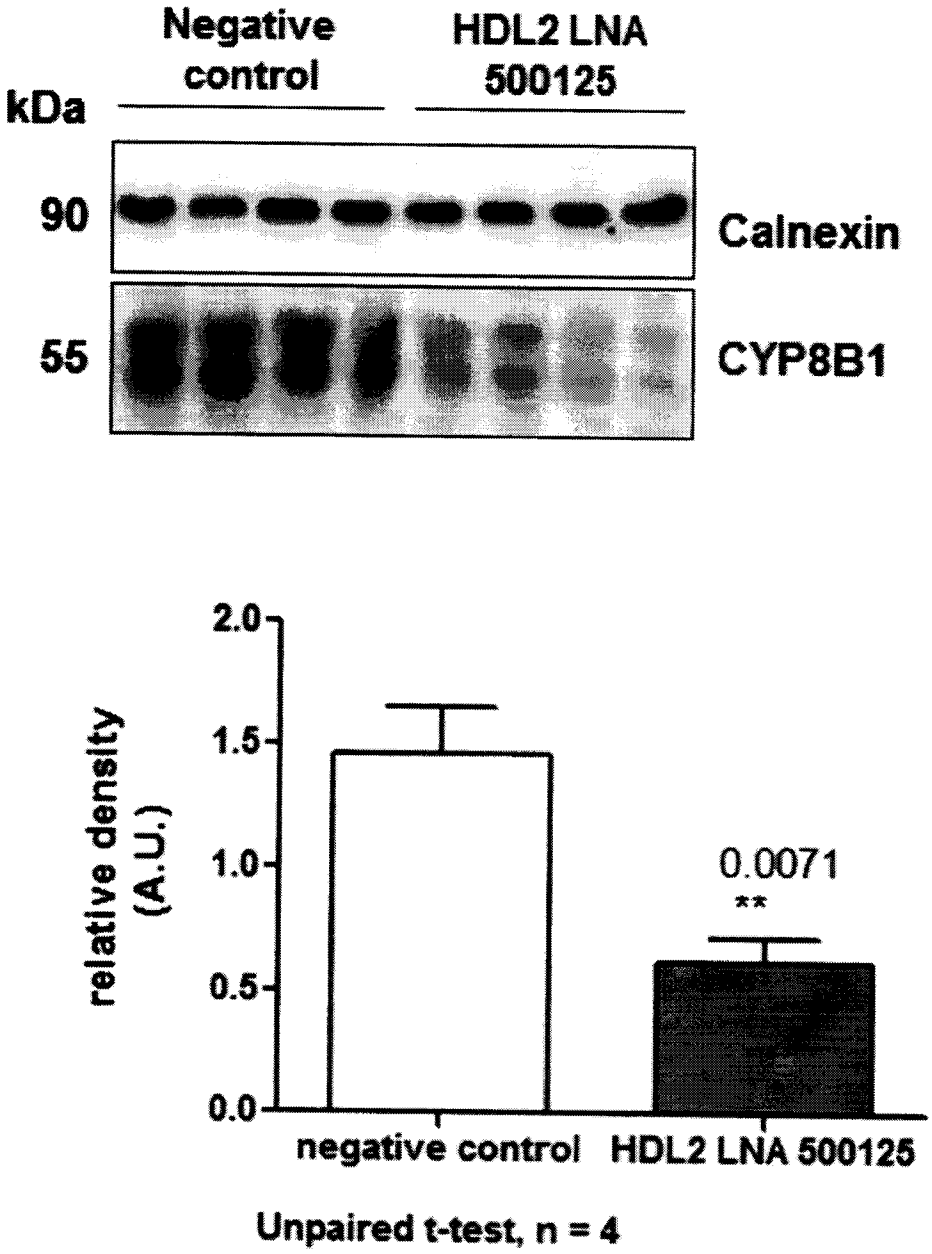

Negative control
HDL2 LNA 500125
kDa
90
Calnexin
55
CYP8B1
relative density (A.U.)
2.0
1.5
1.0
0.5
0.0
0.0071
**
negative control
HDL2 LNA 500125
Unpaired t-test, n = 4

Fig. 12

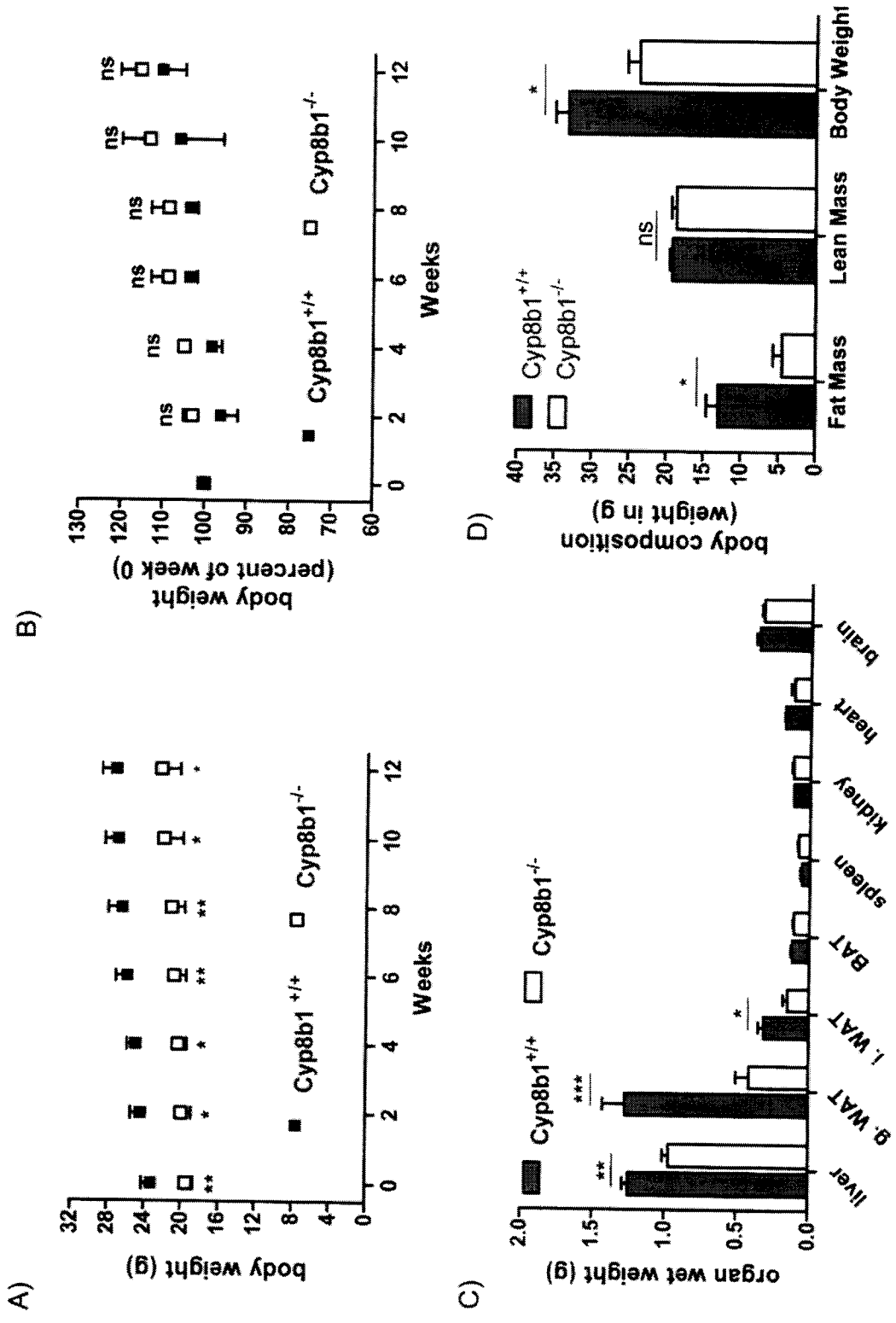

A)
body weight (g)
Weeks
Cyp8b1+/+
Cyp8b1-/-
B)
body weight (percent of week 0)
Weeks
Cyp8b1+/+
Cyp8b1-/-
ns
C)
organ wet weight (g)
Cyp8b1+/+
Cyp8b1-/-
liver
g. WAT
i. WAT
BAT
spleen
kidney
heart
brain
D)
body composition (weight in g)
Cyp8b1+/+
Cyp8b1-/-
Fat Mass
Lean Mass
Body Weight
ns

Fig. 12 (cont)

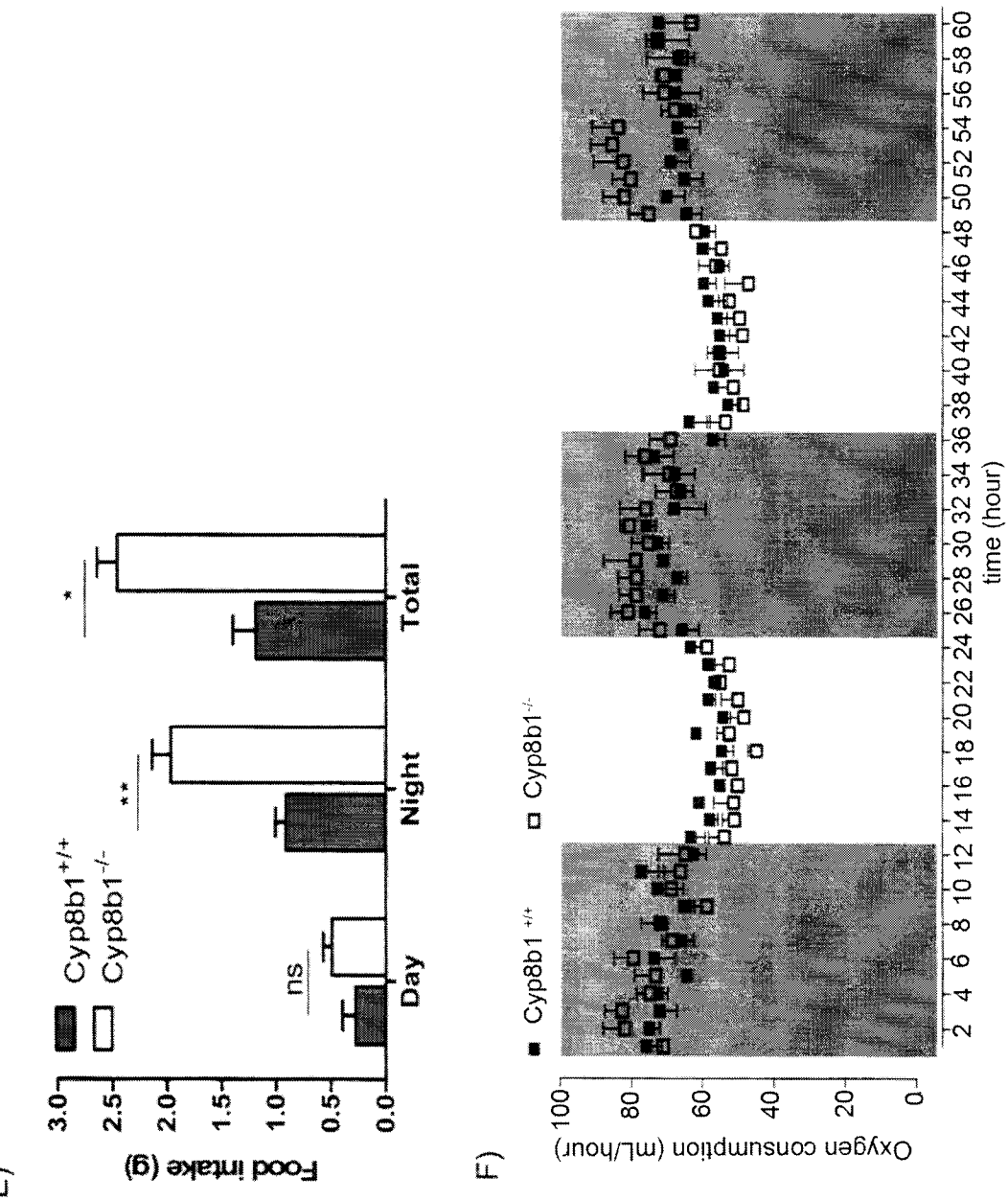

E)
Cyp8b1+/+
Cyp8b1-/-
Food intake (g)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ns
**
*
Day
Night
Total
F)
Cyp8b1 +/+
Cyp8b1-/-
Oxygen consumption (mL/hour)
100
80
60
40
20
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
time (hour)

Fig. 12 (cont)

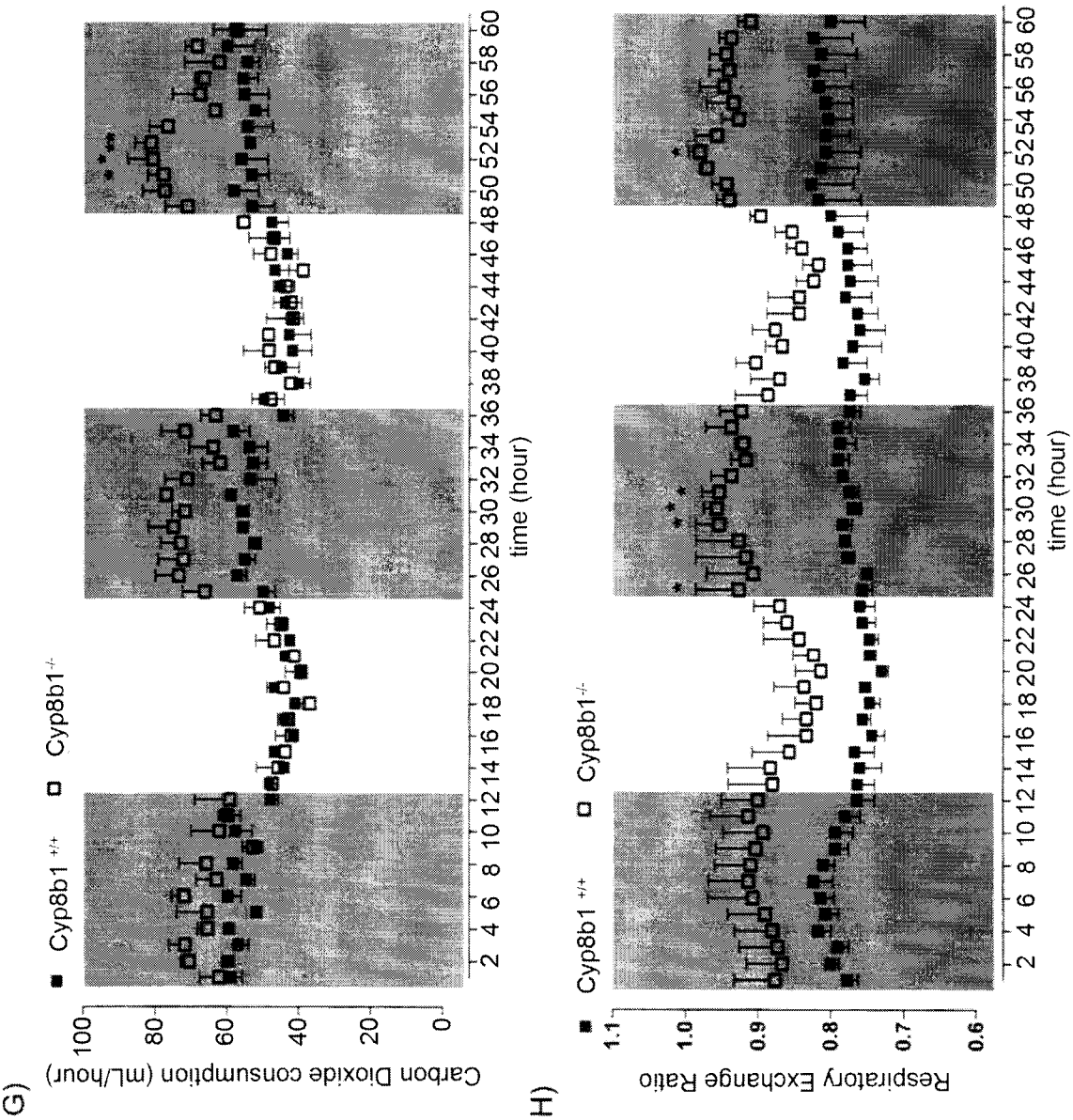

G)
Cyp8b1 +/+
Cyp8b1 -/-
Carbon Dioxide consumption (mL/hour)
time (hour)
H)
Respiratory Exchange Ratio
time (hour)
Respiratory Exchange Ratio (mean RER)
Day
Night

Fig. 12 (cont)

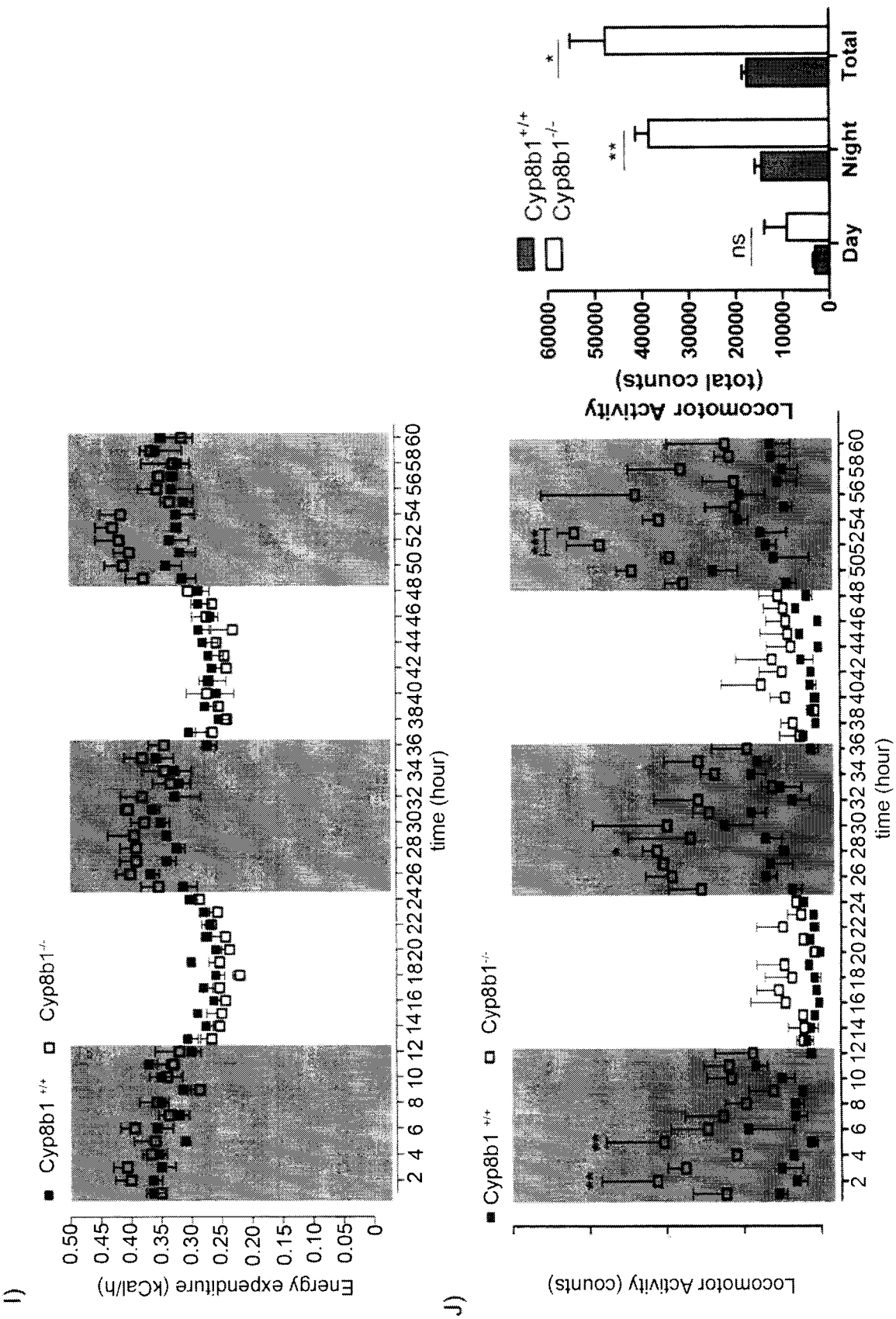

I)
Cyp8b1 +/+
Cyp8b1 -/-
Energy expenditure (kCal/h)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
time (hour)
J)
Cyp8b1 +/+
Cyp8b1 -/-
Locomotor Activity (counts)
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
time (hour)
Locomotor Activity
(total counts)
60000
50000
40000
30000
20000
10000
0
Cyp8b1 +/+
Cyp8b1 -/-
ns
**
*
Day
Night
Total

Fig. 13

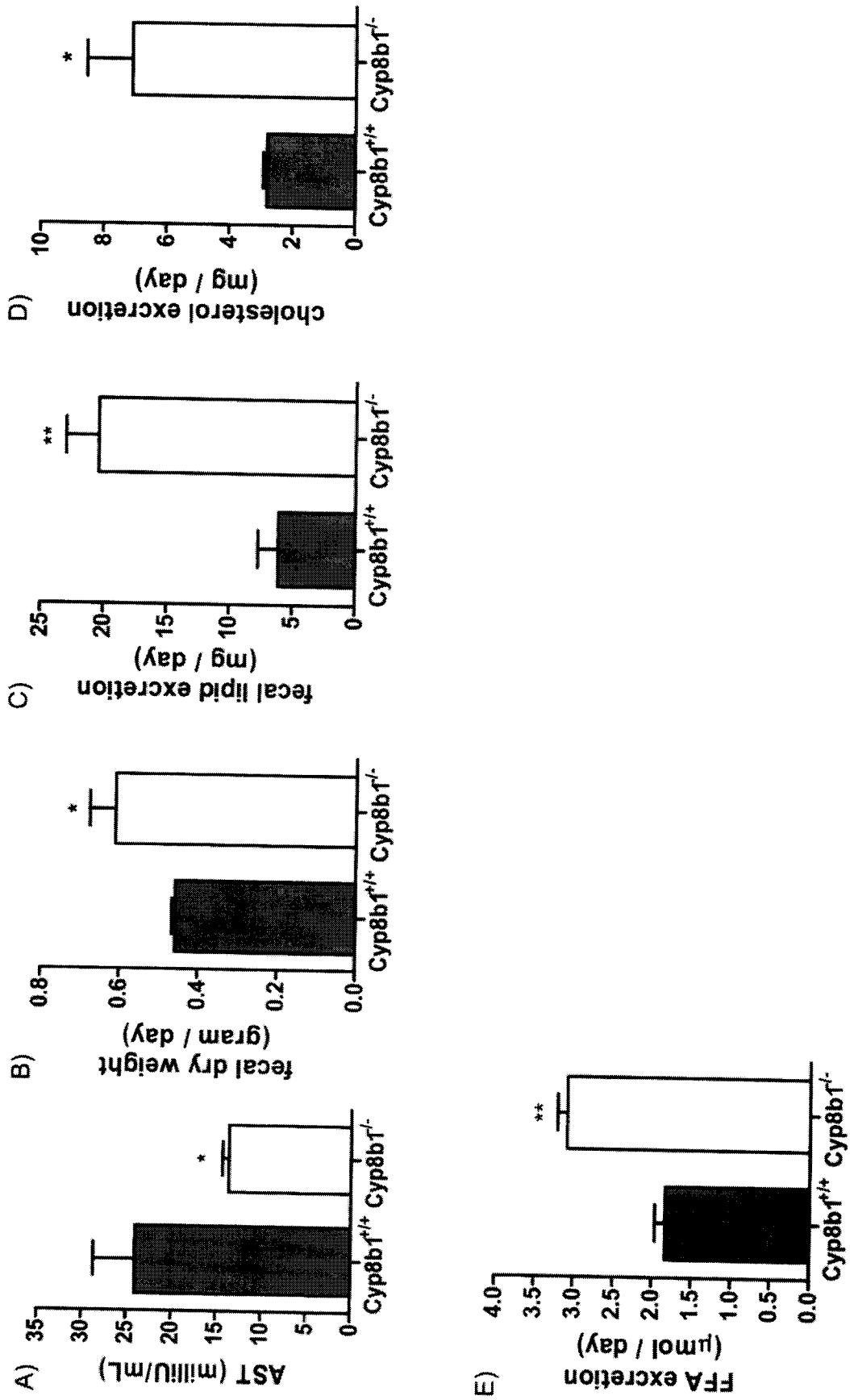

A)
AST (milliU/mL)
B)
fecal dry weight (gram / day)
C)
fecal lipid excretion (mg / day)
D)
cholesterol excretion (mg / day)
E)
FFA excretion (µmol / day)
Cyp8b1+/+
Cyp8b1-/-
*
**

Fig. 14

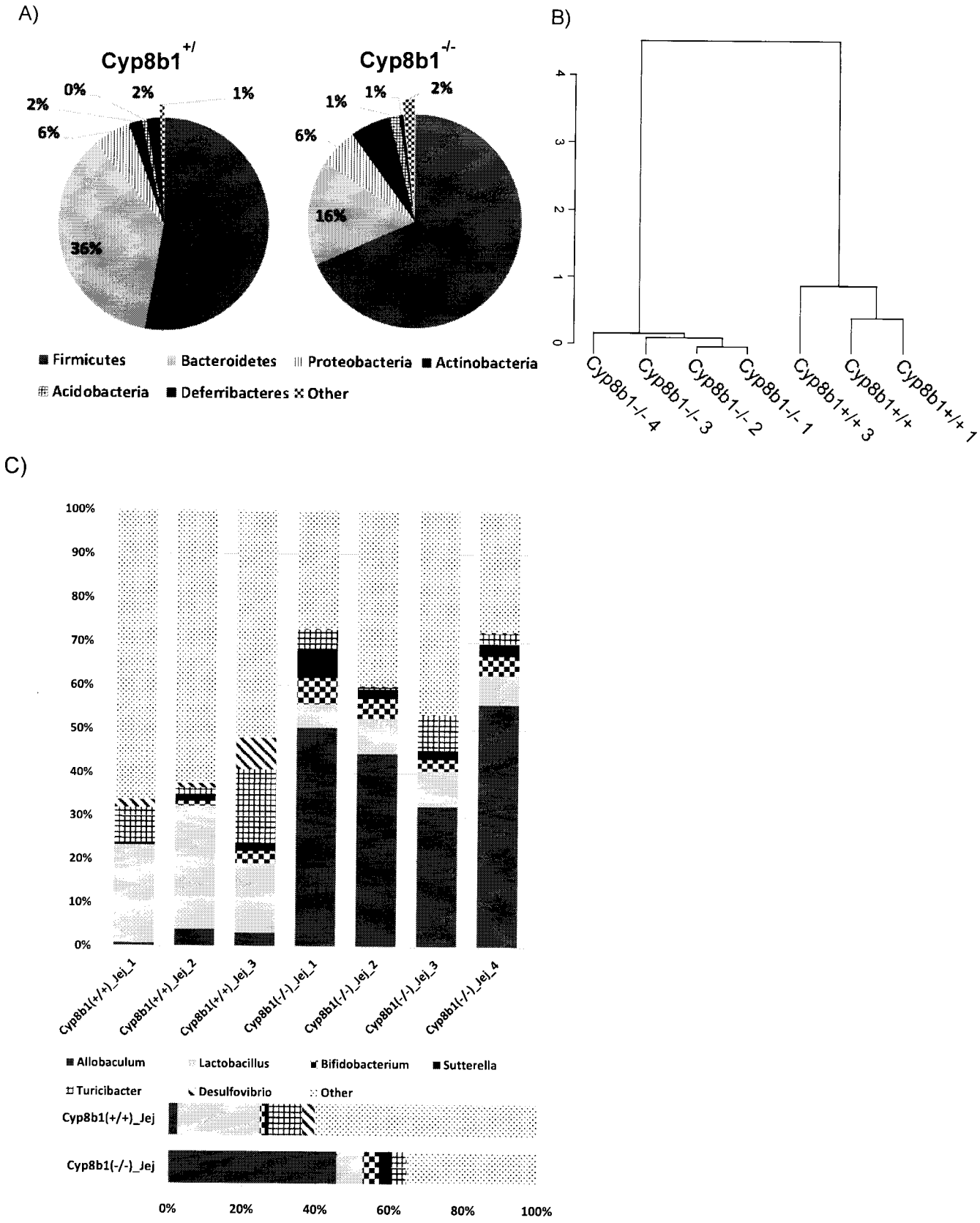
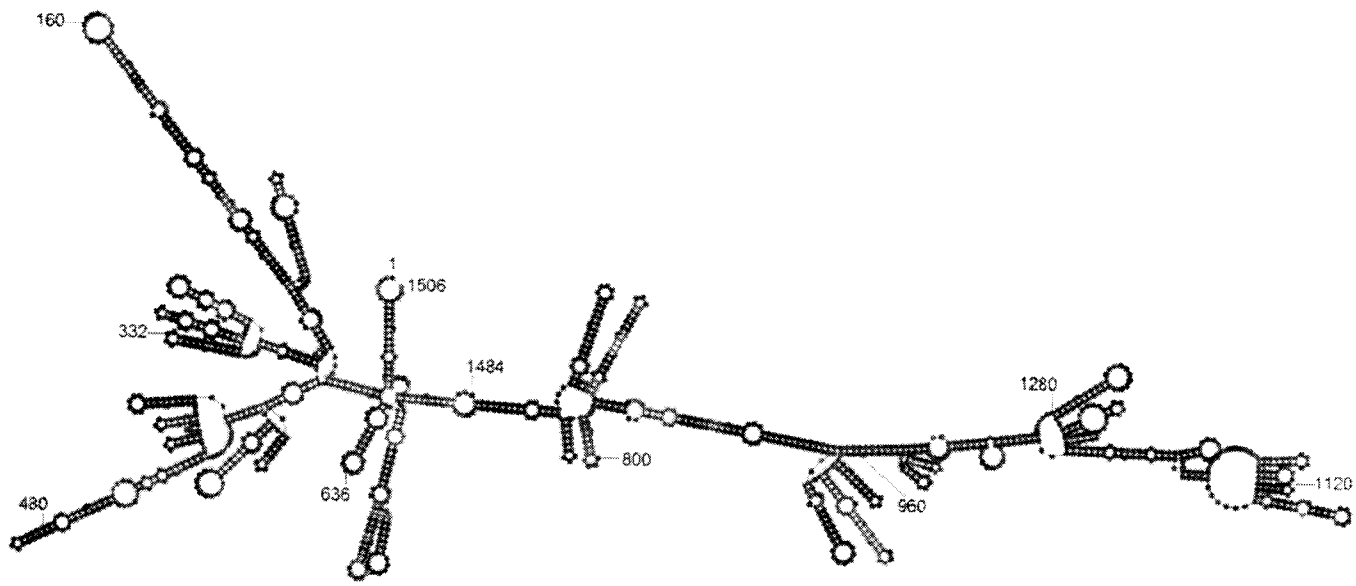

A)
Cyp8b1 +/
0%
2%
1%
2%
6%
36%
Cyp8b1 -/-
1%
1%
2%
6%
16%
Firmicutes
Bacteroidetes
Proteobacteria
Actinobacteria
Acidobacteria
Deferribacteres
Other
B)
4
3
2
1
0
Cyp8b1-/-_4
Cyp8b1-/-_3
Cyp8b1-/-_2
Cyp8b1-/-_1
Cyp8b1+/+_3
Cyp8b1+/+
Cyp8b1+/+_1
C)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Cyp8b1(+/+)_Jej_1
Cyp8b1(+/+)_Jej_2
Cyp8b1(+/+)_Jej_3
Cyp8b1(-/-)_Jej_1
Cyp8b1(-/-)_Jej_2
Cyp8b1(-/-)_Jej_3
Cyp8b1(-/-)_Jej_4
Allobaculum
Lactobacillus
Bifidobacterium
Sutterella
Turicibacter
Desulfovibrio
Other
Cyp8b1(+/+)_Jej
Cyp8b1(-/-)_Jej
0%
20%
40%
60%
80%
100%

Fig. 15

A)


160
1
1506
332
1484
1280
800
480
636
960
1120

B)

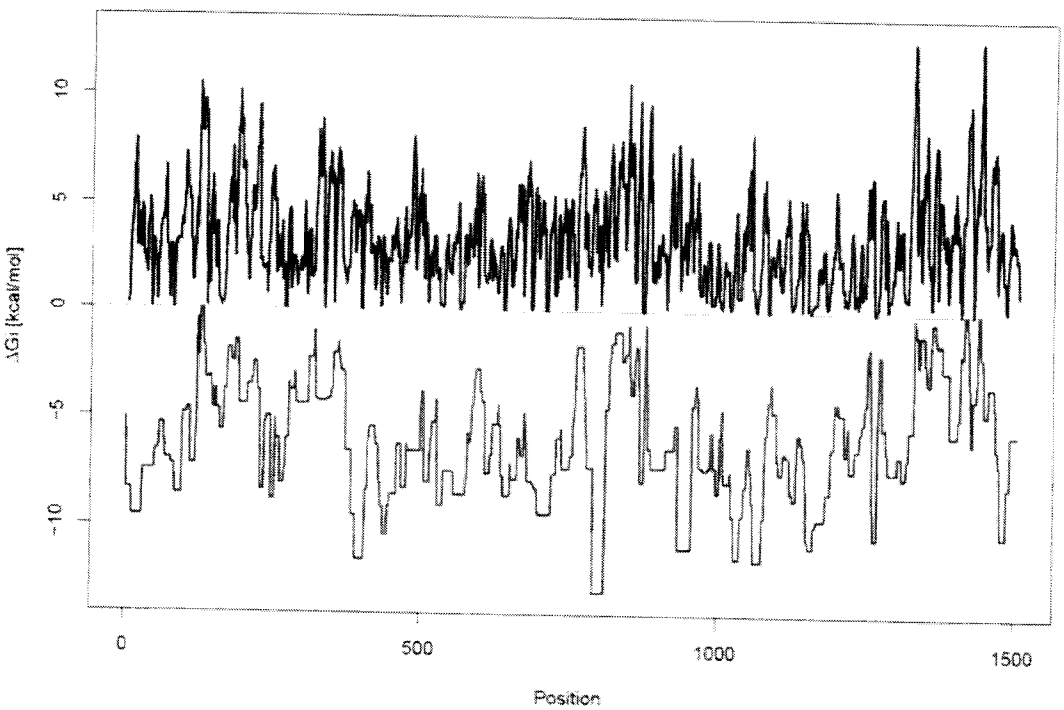
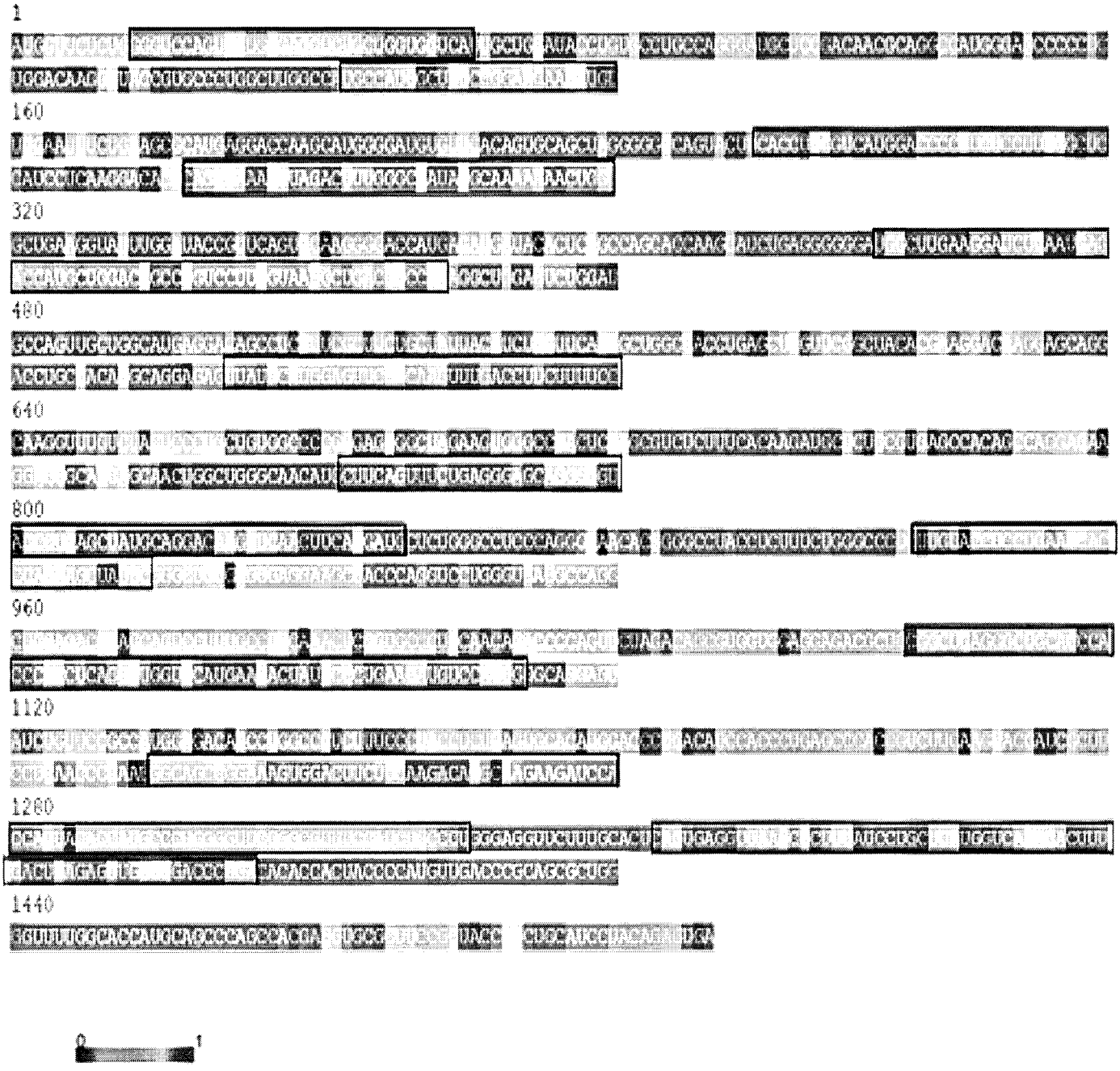

ΔGi [kcal/mol]
10
5
0
-5
-10
0
500
1000
1500
Position

FIG. 15 (cont)

C)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/000181

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 15/113*** (2010.01), ***A61K 31/711*** (2006.01), ***A61K 31/7115*** (2006.01), ***A61K 31/712*** (2006.01), ***A61K 31/7125*** (2006.01), ***A61P 1/16*** (2006.01), ***A61P 3/00*** (2006.01), ***C07H 21/04*** (2006.01), ***C12N 15/53*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
keywords used across the whole IPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Biosis, CAplus, Medline, Canadian Patent Database, FAMPAT, GenomeQuest (GQ Plus, Geneseq; SEQ ID NO:57-106); Keywords: Cyp8b1, sterol 12alpha hydroxylase, antisense, RNAi, siRNA, shRNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US2011054005A1 (NAITO, Y. et al) 03 March 2011 (03-03-2011) - the entire document; SEQ ID NO: 221144; 221149; 221155; 221159	1-6, 8-14, 20, 22, 28-34
Y		7, 15-17, 21
A		18, 19
Y	WO2004083430 A2 (ELMÉN, J. et al) 30 September 2004 (30-09-2004) - the entire document	7, 15-17, 21
A		1-6, 8-14, 18-20, 22, 28-34

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 27 September 2016 (27-09-2016)	Date of mailing of the international search report 06 October 2016 (06-10-2016)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Michael W. De Vouge (819) 639-7794

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAUR, A. et al. Loss of Cyp8b1 improves glucose homeostasis by increasing GLP-1. Diabetes, issue of April 2015 (04-2015), Volume 64, No. 4, pages 1168-1179, ISSN 1939-327X. - cited in the description	1-22, 28-34
A	WO2014018375A1 (FRANCHINI, P.L.A. et al) 30 January 2014 (30-01-2014) - cited in the description	1-22, 28-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/000181

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 23-27
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 23-27 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the oligomer of claim 1.With particular regard to claim 23, the limitation of dependent claim 24 clearly suggests that methods of therapy of the human or animal body are within the scope of claim 23.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/000181

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2011054005A1	03 March 2011 (03-03-2011)	AU2005248147A1	08 December 2005 (08-12-2005)
		CA2566286A1	08 December 2005 (08-12-2005)
		CN101052717A	10 October 2007 (10-10-2007)
		EP1752536A1	14 February 2007 (14-02-2007)
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		KR20070085113A	27 August 2007 (27-08-2007)
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WO2004083430A2	30 September 2004 (30-09-2004)	WO2004083430A3	11 November 2004 (11-11-2004)
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		AU2004221760B2	18 March 2010 (18-03-2010)
		CA2519860A1	30 September 2004 (30-09-2004)
		DE602004023279D1	05 November 2009 (05-11-2009)
		DK1606406T3	14 December 2009 (14-12-2009)
		DK1606406T4	16 December 2013 (16-12-2013)
		DK1713912T3	16 December 2013 (16-12-2013)
		DK2141234T3	20 June 2016 (20-06-2016)
		EP1606406A2	21 December 2005 (21-12-2005)
		EP1606406B1	23 September 2009 (23-09-2009)
		EP1606406B2	27 November 2013 (27-11-2013)
		EP1713912A1	25 October 2006 (25-10-2006)
		EP1713912B1	18 September 2013 (18-09-2013)
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		ES2332178T3	28 January 2010 (28-01-2010)
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		JP2006520760A	14 September 2006 (14-09-2006)
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		US8653252B2	18 February 2014 (18-02-2014)
		US2014235844A1	21 August 2014 (21-08-2014)
		US9297010B2	29 March 2016 (29-03-2016)
		US2008249039A1	09 October 2008 (09-10-2008)
		WO2005073378A1	11 August 2005 (11-08-2005)
WO2014018375A1	30 January 2014 (30-01-2014)	None	