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(54) **Title:** RNAI CONSTRUCTS FOR INHIBITING SCAP EXPRESSION AND METHODS OF USE THEREOF

(57) **Abstract:** The disclosure relates to RNAi constructs, such as siRNA, for reducing expression of the SCAP gene. Methods of using such RNAi constructs to treat or prevent liver disease, such as nonalcoholic fatty liver disease (NAFLD), are also described.

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RNAI CONSTRUCTS FOR INHIBITING SCAP EXPRESSION AND METHODS OF USE THEREOF

FIELD

[0001] The present disclosure relates to compositions and methods for modulating liver expression of SREBP Cleavage Activating Protein (SCAP). In particular, the present disclosure relates to nucleic acid-based therapeutics for reducing SCAP expression via RNA interference and methods of using such nucleic acid-based therapeutics to treat or prevent liver disease, such as nonalcoholic fatty liver disease (NAFLD).

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 550 kilobyte XML document named "A-2911-WO01-SEC.xml," created on May 19, 2023.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0003] This application claims the benefit of U.S. Provisional Application No. 63/345,513, filed on May 25, 2022, which is hereby incorporated by reference in its entirety.

BACKGROUND

[0004] Comprising a spectrum of hepatic pathologies, nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world, the prevalence of which doubled in the last 20 years and now is estimated to affect approximately 20% of the world's population (Sattar et al. (2014) BMJ 349: g4596; Loomba and Sanyal (2013) Nature Reviews Gastroenterology & hepatology 10(11):686-690; Kim and Kim (2017) Clin Gastroenterol Hepatol 15(4):474-485; Petta et al. (2016) Dig Liver Dis 48(3):333-342). NAFLD begins with the accumulation of triglyceride in the liver and is defined by the presence of cytoplasmic lipid droplets in more than 5% of hepatocytes in an individual 1) without a history of significant alcohol consumption and 2) in which the diagnosis of other types of liver disease have been excluded (Zhu et al (2016) World J Gastroenterol 22(36):8226-33; Rinella (2015) JAMA 313(22):2263-73; Yki-Jarvinen (2016) Diabetologia 59(6):1104-11). In some individuals the accumulation of ectopic fat in the liver, called steatosis, triggers inflammation and hepatocellular injury leading to a more advanced stage of disease called nonalcoholic steatohepatitis (NASH) (Rinella, *supra*). As of 2015, 75-100 million Americans are predicted to have NAFLD, with

NASH accounting for approximately 10-30% of NAFLD diagnoses (Rinella, *supra*; Younossi et al (2016) Hepatology 64(5):1577-1586).

[0005] One of the key triggers for the onset of NAFLD is loss of triglyceride (TG) homeostasis in the liver, resulting in increased TG and fatty acid accumulation. Over time the lipotoxicity exhausts the adaptive and regenerative responses of the hepatocytes, enabling inflammation, activation of the innate immune system, and steatohepatitis. Progressive NASH triggers collagen deposition or fibrosis within the liver. As the grade of fibrosis worsens, the liver in 10%-29% of individuals with NASH becomes cirrhotic and finally culminates in hepatocellular cancer.

[0006] The Sterol Response Element Binding Protein (SREBP) family plays an important role in regulating *de novo* lipogenesis and TG accumulation within the liver. SREBPs are synthesized as inactive precursors in the endoplasmic reticulum (ER). SREBP Cleavage Activating Protein (SCAP) is the only known regulator of the transcription factors of the SREBP family. Immediately after synthesis, SCAP forms a complex with SREBPs and escorts the SREBPs to the Golgi vesicles. Here, SREBPs are further processed to release the active amino terminal of the transcription factor. Active SREBP translocates to the nucleus and binds to SREBP response elements to drive transcriptional activation of target genes. Thus, inhibiting SCAP function may prevent processing of active SREBP and reduce lipogenesis and TG accumulation in the liver.

[0007] Currently, NAFLD symptoms are managed via weight loss and treatment of any secondary conditions, as no pharmacologic treatments have been approved. Thus, there is a need for compositions and methods that treat NAFLD in affected individuals.

BRIEF SUMMARY

[0008] The present disclosure provides an RNAi construct comprising a sense strand and an antisense strand, wherein the RNAi construct inhibits the expression of a SREBP Cleavage Activating Protein (SCAP) mRNA. In certain embodiments, the RNAi construct comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence listed in Table 1.

[0009] In some embodiments, the sense strand of the RNAi constructs described herein comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length. In these and other embodiments, the sense and antisense strands each are about 15 to about 30 nucleotides in length. In some embodiments, the RNAi constructs comprise at least one blunt end. In other

embodiments, the RNAi constructs comprise at least one nucleotide overhang. Such nucleotide overhangs may comprise at least 1 to 6 unpaired nucleotides and can be located at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense and antisense strand. In certain embodiments, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the sense strand and the 3' end of the antisense strand. In other embodiments, the RNAi constructs comprise an overhang of two unpaired nucleotides at the 3' end of the antisense strand and a blunt end of the 3' end of the sense strand/5' end of the antisense strand.

[0010] The RNAi constructs of the disclosure may comprise one or more modified nucleotides, including nucleotides having modifications to the ribose ring, nucleobase, or phosphodiester backbone. In some embodiments, modifications to the ribose ring of the RNAi constructs include one or more 2'-modifications. Such 2'-modifications can include 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, or bicyclic nucleic acids (BNA). Modifications to the ribose ring may also include incorporation of glycol nucleic acids (GNAs), in which the ribose ring is replaced with propylene glycol. In one particular embodiment, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof. In some embodiments, all of the nucleotides in the sense and antisense strand of the RNAi construct are modified nucleotides. Abasic nucleotides may be incorporated into the RNAi constructs of the disclosure, for example, as the terminal nucleotide at the 3' end, the 5' end, or both the 3' end and the 5' end of the sense strand. In such embodiments, the abasic nucleotide may be inverted, e.g., linked to the adjacent nucleotide through a 3'-3' internucleotide linkage or a 5'-5' internucleotide linkage.

[0011] In some embodiments, the RNAi constructs comprise at least one backbone modification, such as a modified internucleotide or internucleoside linkage. In certain embodiments, the RNAi constructs described herein comprise at least one phosphorothioate internucleotide linkage. In particular embodiments, the phosphorothioate internucleotide linkages may be positioned at the 3' or 5' ends of the sense and/or antisense strands.

[0012] The disclosure also provides a composition comprising the aforementioned RNAi construct and a pharmaceutically acceptable carrier, excipient, or diluent, as well as methods of reducing the expression of SCAP in a patient in need thereof comprising administering to the patient the aforementioned RNAi construct or composition.

[0013] The disclosure also includes RNAi constructs for use in reducing expression of SCAP, such as for the treatment of NAFLD or NASH. Also provided is the use an RNAi construct that inhibits SCAP expression for the preparation of a medicament for treating NAFLD.

DETAILED DESCRIPTION

[0014] The present disclosure is based, in part, on the design and generation of RNAi constructs that target the SREBP Cleavage Activating Protein (SCAP) gene and reduce expression of SCAP in liver cells. The inhibition of SCAP expression is useful for treating or preventing conditions associated with SCAP expression, including liver-related diseases, such as, for example, simple fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis (irreversible, advanced scarring of the liver), or SCAP-mediated hyperlipidemia or hypertriglyceridemia.

[0015] The disclosure provides compositions and methods for regulating the expression of the gene encoding SCAP. In some embodiments, the gene may be within a cell or subject, such as a mammal (e.g., a human). In some embodiments, compositions of the disclosure comprise RNAi constructs that target a SCAP mRNA and reduce SCAP expression in a cell or mammal. Such RNAi constructs are useful for treating or preventing various forms of liver-related diseases, such as, for example, simple fatty liver (steatosis), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), cirrhosis (irreversible, advanced scarring of the liver), or SCAP-mediated hyperlipidemia or hypertriglyceridemia (see, e.g., Lee et al., *Experimental & Molecular Medicine*, 52: 724-729 (2020)).

[0016] As discussed above, SCAP (SREBP cleavage-activating protein) is a sterol-regulated escort protein that transports SREBPs from their site of synthesis in the endoplasmic reticulum to their site of cleavage in the Golgi, in which they are cleaved sequentially by two proteases that release the cytosolic NH_2-terminal transcription factor domains. Cell culture experiments have demonstrated that mutant cells lacking SCAP have low levels of SREBP precursors, apparently because these proteins are unstable in the absence of SCAP. In addition, SCAP-deficient CHO cells cannot synthesize cholesterol, and they require external sources of cholesterol for growth (Rawson et al., *J Biol Chem.*, *274*: 28549-28556 (1999)). SCAP also functions as a cholesterol sensor, which is mediated by the polytopic membrane domain of SCAP. Point mutations in this sterol-sensing motif prevent sterol repression of SREBP cleavage and lead to unregulated overproduction of cholesterol.

[0017] Control of lipid synthesis is especially important in the liver, which synthesizes lipids not only for its own use but also for export into the plasma as lipoproteins. Levels of plasma lipoprotein cholesterol are lowered by treatment with statins. In addition, hepatic fatty acid synthesis is elevated when plasma insulin rises, such as may occur in obesity and noninsulin-dependent diabetes mellitus. In the liver, overexpression of the soluble transcription-activating domain of SREBP-1a in transgenic mice leads to a massive fatty liver. Transgenic mice overexpressing a sterol-resistant mutant SCAP developed livers which overproduced cholesterol and failed to show normal feedback inhibition when fed cholesterol, suggesting that SCAP plays a regulatory role in the liver. Mice with a conditional SCAP deficiency in the liver exhibited reduced basal rates of cholesterol and fatty acid synthesis in liver, primarily due to decreases in mRNAs encoding multiple biosynthetic enzymes (Matsuda et al., Genes & Development 15: 1206-121 (2001). In addition, NASH/NAFLD patients exhibit increased expression and transcriptional activity of SREBP1c and its target genes. Taken together, these data provide *in vivo* evidence that SCAP and the SREBPs are required for hepatic lipid synthesis.

[0018] RNA interference (RNAi) is the process of introducing exogeneous RNA into a cell leading to specific degradation of the mRNA encoding the targeted protein with a resultant decrease in protein expression. Advances in both the RNAi technology and hepatic delivery, as well as growing positive outcomes with other RNAi-based therapies, suggest RNAi as a compelling means to therapeutically treat NAFLD by directly targeting genes that regulate lipid synthesis in liver, such as SCAP.

[0019] As used herein, the term "RNAi construct" refers to an agent comprising an RNA molecule that is capable of downregulating expression of a target gene (e.g., SCAP) via an RNA interference mechanism when introduced into a cell. "RNA interference" is the process by which a nucleic acid molecule induces the cleavage and degradation of a target RNA molecule (e.g. messenger RNA or mRNA molecule) in a sequence-specific manner, e.g. through an RNA induced silencing complex (RISC) pathway. In some embodiments, the RNAi construct comprises a double-stranded RNA (dsRNA) molecule comprising two antiparallel strands of contiguous nucleotides that are sufficiently complementary to each other to hybridize to form a duplex region. A double-stranded RNAi construct also may be referred to as an RNAi "trigger." The terms "hybridize" or "hybridization" refer to the pairing of complementary polynucleotides, typically via hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary bases in the two polynucleotides. The strand comprising a region having a sequence that is substantially complementary to a target sequence (e.g., target mRNA) is referred to as the "antisense strand." The "sense strand" refers to the strand that

includes a region that is substantially complementary to a region of the antisense strand. In some embodiments, the sense strand may comprise a region that has a sequence that is substantially identical to the target sequence.

[0020] In certain embodiments, the sense strand and antisense strand of the double-stranded RNA may be two separate molecules that hybridize to form a duplex region but are otherwise unconnected. Such double-stranded RNA molecules formed from two separate strands are referred to as "small interfering RNAs" or "short interfering RNAs" (siRNAs). siRNAs are a class of non-coding, double-stranded RNA molecules that are typically about 20-27 base pairs and are central to RNAi. Thus, in some embodiments, the RNAi constructs of the disclosure comprise an siRNA. In other embodiments, the RNAi construct may be a microRNA (also known as "miRNA" or "mature miRNA"). miRNAs are small (approximately 18-24 nucleotides in length), non-coding RNA molecules present in plants, animals, and some viruses. miRNAs resemble siRNA, but miRNAs originate from endogenous precursor hairpin RNA structures. miRNAs regulate gene expression by base-pairing to complementary regions of target mRNAs and directing the cleavage of the target RNA via the RISC pathway.

[0021] In some embodiments, the disclosure provides an RNAi construct directed to SCAP. In some embodiments, the RNAi construct is an siRNA that comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region that is complementary to SCAP mRNA sequence. The region of the RNAi antisense strand may be complementary to any suitable region of a SCAP mRNA sequence. For example, the antisense strand may comprise a region that is complementary to the coding region or the 3' untranslated region (UTR) of a SCAP mRNA sequence.

[0022] A double-stranded RNAi molecule may include chemical modifications to ribonucleotides, including modifications to the ribose sugar, base, or backbone components of the ribonucleotides, such as those described herein or known in the art. Any such modifications, as used in a double-stranded RNA molecule (e.g. siRNA, shRNA, or the like), are encompassed by the term "double-stranded RNA" for the purposes of this disclosure.

[0023] As used herein, a first sequence is "complementary" to a second sequence if a polynucleotide comprising the first sequence can hybridize to a polynucleotide comprising the second sequence to form a duplex region under certain conditions, such as physiological conditions. Other such conditions can include moderate or stringent hybridization conditions, which are known to those of skill in the art. A first sequence is considered to be fully complementary (100% complementary) to a second sequence if a polynucleotide comprising the first sequence base pairs with a polynucleotide comprising the second sequence over the entire

length of one or both nucleotide sequences without any mismatches. A sequence is "substantially complementary" to a target sequence if the sequence is at least about 80%, 85%, 90%, 95%,96%, 97%, 98%, 99% or 100% complementary to a target sequence. Percent complementarity can be calculated by dividing the number of bases in a first sequence that are complementary to bases at corresponding positions in a second or target sequence by the total length of the first sequence. A sequence may also be said to be substantially complementary to another sequence if there are no more than 5, 4, 3, 2, or 1 mismatch over a 30 base pair duplex region when the two sequences are hybridized. Generally, if any nucleotide overhangs, as defined herein, are present, the sequence of such overhangs is not considered in determining the degree of complementarity between two sequences. By way of example, a sense strand of 21 nucleotides in length and an antisense strand of 21 nucleotides in length that hybridize to form a 19 base pair duplex region with a 2-nucleotide overhang at the 3' end of each strand would be considered to be fully complementary as the term is used herein.

[0024] In some embodiments, a region of the antisense strand comprises a sequence that is fully complementary to a region of the target RNA sequence (e.g., SCAP mRNA). In such embodiments, the sense strand may comprise a sequence that is fully complementary to the sequence of the antisense strand. In other such embodiments, the sense strand may comprise a sequence that is substantially complementary to the sequence of the antisense strand, e.g., having 1, 2, 3, 4, or 5 mismatches in the duplex region formed by the sense and antisense strands. In certain embodiments, it is preferred that any mismatches occur within the terminal regions (e.g. within 6, 5, 4, 3, 2, or 1 nucleotides of the 5' and/or 3' ends of the strands). In one embodiment, any mismatches in the duplex region formed from the sense and antisense strands desirably occur within 6, 5, 4, 3, 2, or 1 nucleotides of the 5' end of the antisense strand.

[0025] Where the two substantially complementary strands of a dsRNA are comprised of separate RNA molecules, those molecules need not, but can be, covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3 '-end of one strand and the 5' -end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs in the duplex is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

[0026] In other embodiments, the sense strand and the antisense strand that hybridize to form a duplex region may be part of a single RNA molecule, i.e., the sense and antisense strands are

part of a self-complementary region of a single RNA molecule. In such cases, a single RNA molecule comprises a duplex region (also referred to as a stem region) and a loop region. The 3' end of the sense strand is connected to the 5' end of the antisense strand by a contiguous sequence of unpaired nucleotides, which will form the loop region. The loop region is typically of a sufficient length to allow the RNA molecule to fold back on itself such that the antisense strand can base pair with the sense strand to form the duplex or stem region. The loop region can comprise from about 3 to about 25, from about 5 to about 15, or from about 8 to about 12 unpaired nucleotides. As noted herein, such RNA molecules with at least partially self-complementary regions are referred to as "short hairpin RNAs" (shRNAs). In some embodiments, the loop region can comprise at least 1, 2, 3, 4, 5, 10, 20, or 25 unpaired nucleotides. In other embodiments, the loop region can have 10, 9, 8, 7, 6, 5, 4, 3, 2, or fewer unpaired nucleotides. In certain embodiments, the RNAi constructs disclosed herein comprise an shRNA. The length of a single, at least partially self-complementary RNA molecule can be from about 35 nucleotides to about 100 nucleotides, from about 45 nucleotides to about 85 nucleotides, or from about 50 to about 60 nucleotides and comprise a duplex region and loop region each having the lengths recited herein.

[0027] In some embodiments, the RNAi constructs disclosed herein comprise a sense strand and an antisense strand, wherein the antisense strand comprises a region having a sequence that is substantially or fully complementary to a SCAP messenger RNA (mRNA) sequence. As used herein, a "SCAP mRNA sequence" refers to any messenger RNA sequence, including splice variants, encoding a SCAP protein, including SCAP protein variants or isoforms from any species (e.g. mouse, rat, non-human primate, human). SCAP also is known in the art as SREBF chaperone.

[0028] A SCAP mRNA sequence also includes the transcript sequence expressed as its complementary DNA (cDNA) sequence. A cDNA sequence refers to the sequence of an mRNA transcript expressed as DNA bases (e.g. guanine, adenine, thymine, and cytosine) rather than RNA bases (e.g. guanine, adenine, uracil, and cytosine). Thus, the antisense strand of the RNAi constructs disclosed herein may comprise a region having a sequence that is substantially or fully complementary to a target SCAP mRNA sequence or SCAP cDNA sequence. A SCAP mRNA or cDNA sequence can include, but is not limited to, any SCAP mRNA or cDNA sequence such as can be derived from the NCBI Reference sequence NM_001320044.2 or NM_012235.4.

[0029] A region of the antisense strand can be substantially complementary or fully complementary to at least 15 consecutive nucleotides of the SCAP mRNA sequence. In some embodiments, the target region of the SCAP mRNA sequence to which the antisense strand

comprises a region of complementarity can range from about 15 to about 30 consecutive nucleotides, from about 16 to about 28 consecutive nucleotides, from about 18 to about 26 consecutive nucleotides, from about 17 to about 24 consecutive nucleotides, from about 19 to about 25 consecutive nucleotides, from about 19 to about 23 (e.g., 19, 20, 21, 22, or 23) consecutive nucleotides, or from about 19 to about 21 consecutive nucleotides. In certain embodiments, the region of the antisense strand comprising a sequence that is substantially or fully complementary to a SCAP mRNA sequence may, in some embodiments, comprise at least 19 contiguous nucleotides from an antisense sequence listed in Table 1. In some embodiments, the sense and/or antisense sequence comprises at least 15 consecutive nucleotides (e.g., at least 16, 17, or 18 consecutive nucleotides) from a sequence listed in Table 1 with no more than 1, 2, or 3 nucleotide mismatches.

[0030] The sense strand of the RNAi construct typically comprises a sequence that is sufficiently complementary to the sequence of the antisense strand such that the two strands hybridize under physiological conditions to form a duplex region. A "duplex region" refers to the region in two complementary or substantially complementary polynucleotides that form base pairs with one another, either by Watson-Crick base pairing or other hydrogen bonding interaction, to create a duplex between the two polynucleotides. The duplex region of the RNAi construct should be of sufficient length to allow the RNAi construct to enter the RNA interference pathway, e.g. by engaging the Dicer enzyme and/or the RISC complex (described below). For instance, in some embodiments, the duplex region is about 15 to about 30 base pairs in length. Other lengths for the duplex region within this range are also suitable, such as about 15 to about 28 base pairs, about 15 to about 26 base pairs, about 15 to about 24 base pairs, about 15 to about 22 base pairs, about 17 to about 28 base pairs, about 17 to about 26 base pairs, about 17 to about 24 base pairs, about 17 to about 23 base pairs, about 17 to about 21 base pairs, about 19 to about 25 base pairs, about 19 to about 23 base pairs, or about 19 to about 21 base pairs. In one embodiment, the duplex region is about 17 to about 24 base pairs in length. In another embodiment, the duplex region is about 19 to about 21 base pairs in length. For example, the duplex region may be about 19 base pairs in length.

[0031] In some embodiments, an RNAi construct disclosed herein contains a duplex region of about 17 to about 24 nucleotides that interacts with a target RNA sequence, e.g., a SCAP target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double-stranded RNA introduced into cells can be broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) Genes Dev. 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs

with characteristic two base 3' overhangs (Bernstein, et al., (2001) Nature 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) Cell, 107: 309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) Genes Dev. 15: 188).

[0032] For embodiments in which the sense strand and antisense strand are two separate molecules (e.g., an siRNA RNAi construct), the sense strand and antisense strand need not be the same length as the length of the duplex region. For instance, one or both strands maybe longer than the duplex region and have one or more unpaired nucleotides or mismatches flanking the duplex region. Thus, in some embodiments, the RNAi construct comprises at least one nucleotide overhang. As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that extend beyond the duplex region at the terminal ends of the strands. Nucleotide overhangs are typically created when the 3' end of one strand extends beyond the 5' end of the other strand or when the 5' end of one strand extends beyond the 3' end of the other strand. The length of a nucleotide overhang generally is between 1 and 6 nucleotides, 1 and 5 nucleotides, 1 and 4 nucleotides, 1 and 3 nucleotides, 2 and 6 nucleotides, 2 and 5 nucleotides, or 2 and 4 nucleotides. In some embodiments, the nucleotide overhang comprises 1, 2, 3, 4, 5, or 6 nucleotides. In one particular embodiment, the nucleotide overhang comprises 1 to 4 nucleotides. In certain embodiments, the nucleotide overhang comprises 2 nucleotides. The nucleotides in the overhang can be ribonucleotides, deoxyribonucleotides, or modified nucleotides as described herein. In some embodiments, the overhang comprises a 5'-uridine-uridine-3' (5'-UU-3') dinucleotide. In such embodiments, the UU dinucleotide may comprise ribonucleotides or modified nucleotides, e.g., 2'-modified nucleotides. In other embodiments, the overhang comprises a 5'-deoxythymidine-deoxythymidine-3' (5'-dTdT-3') dinucleotide.

[0033] The nucleotide overhang can be at the 5' end or 3' end of one or both strands. For example, in one embodiment, the RNAi construct comprises a nucleotide overhang at the 5' end and the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises a nucleotide overhang at the 5' end and the 3' end of the sense strand. In some embodiments, the RNAi construct comprises a nucleotide overhang at the 5' end of the sense strand and the 5' end of the antisense strand. In other embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand and the 3' end of the antisense strand.

[0034] The RNAi constructs may comprise a single nucleotide overhang at one end of the double-stranded RNA molecule and a blunt end at the other. A "blunt end" means that the sense

strand and antisense strand are fully base-paired at the end of the molecule and there are no unpaired nucleotides that extend beyond the duplex region. In some embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand and a blunt end at the 5' end of the sense strand and 3' end of the antisense strand. In other embodiments, the RNAi construct comprises a nucleotide overhang at the 3' end of the antisense strand and a blunt end at the 5' end of the antisense strand and the 3' end of the sense strand. In certain embodiments, the RNAi construct comprises a blunt end at both ends of the double-stranded RNA molecule. In such embodiments, the sense strand and antisense strand have the same length and the duplex region is the same length as the sense and antisense strands (i.e., the molecule is double-stranded over its entire length).

[0035] The sense strand and antisense strand can each independently be any suitable length, such as about 15 to about 30 nucleotides in length, about 18 to about 28 nucleotides in length, about 19 to about 27 nucleotides in length, about 19 to about 25 nucleotides in length, about 19 to about 23 nucleotides in length, about 21 to about 25 nucleotides in length, or about 21 to about 23 nucleotides in length. In certain embodiments, the sense strand and antisense strand are each about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25 nucleotides in length. In some embodiments, the sense strand and antisense strand are of the same length but form a duplex region that is shorter than the strands such that the RNAi construct has two nucleotide overhangs. For instance, in one embodiment, the RNAi construct comprises (i) a sense strand and an antisense strand that are each 21 nucleotides in length, (ii) a duplex region that is 19 base pairs in length, and (iii) nucleotide overhangs of 2 unpaired nucleotides at both the 3' end of the sense strand and the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises (i) a sense strand and an antisense strand that are each 23 nucleotides in length, (ii) a duplex region that is 21 base pairs in length, and (iii) nucleotide overhangs of 2 unpaired nucleotides at both the 3' end of the sense strand and the 3' end of the antisense strand. In other embodiments, the sense strand and antisense strand have the same length and form a duplex region over their entire length such that there are no nucleotide overhangs on either end of the double-stranded molecule. In one such embodiment, the RNAi construct is blunt ended and comprises (i) a sense strand and an antisense strand, each of which is 21 nucleotides in length, and (ii) a duplex region that is 21 base pairs in length. In another embodiment, the RNAi construct is blunt ended and comprises (i) a sense strand and an antisense strand, each of which is 23 nucleotides in length, and (ii) a duplex region that is 23 base pairs in length.

[0036] In other embodiments, the sense strand or the antisense strand is longer than the other strand and the two strands form a duplex region having a length equal to that of the shorter

strand such that the RNAi construct comprises at least one nucleotide overhang. For example, in one embodiment, the RNAi construct comprises (i) a sense strand that is 19 nucleotides in length, (ii) an antisense strand that is 21 nucleotides in length, (iii) a duplex region of 19 base pairs in length, and (iv) a single nucleotide overhang of 2 unpaired nucleotides at the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises (i) a sense strand that is 21 nucleotides in length, (ii) an antisense strand that is 23 nucleotides in length, (iii) a duplex region of 21 base pairs in length, and (iv) a single nucleotide overhang of 2 unpaired nucleotides at the 3' end of the antisense strand.

[0037] The antisense strand of the RNAi constructs disclosed herein can comprise the sequence of any one of the antisense sequences listed in Table 1 or the sequence of nucleotides 1-18, 2-18, 1-19, 2-19, 1-21, or 2-21 of any of these antisense sequences. Each of the antisense sequences listed in Table 1 comprises a sequence of 16-19 consecutive nucleotides that is complementary to a SCAP mRNA sequence plus a two-nucleotide overhang sequence. Thus, in some embodiments, the antisense strand comprises a sequence of nucleotides 1-18, 2-18, 1-19, 2-19, 1-21, or 2-21 of any one of SEQ ID NOs: 148-294 or SEQ ID NOs: 442-588. Likewise, the sense strand of the RNAi constructs disclosed herein can comprise the sequence of any one of the sense sequences listed in Table 1 or the sequence of nucleotides 1-18, 2-18, 1-19, 2-19, 1-21, or 2-21 of any of these sense sequences. Thus, in some embodiments, the sense strand comprises a sequence of nucleotides 1-18, 2-18, 1-19, 2-19, 1-21, or 2-21 of any one of SEQ ID NOs: 1-147 or SEQ ID NOs: 295-441.

Modified Nucleotides

[0038] The RNAi constructs disclosed herein, such as those listed in Table 1, may comprise one or more modified nucleotides. A "modified nucleotide" refers to a nucleotide that has one or more chemical modifications to the nucleoside, nucleobase, pentose ring, or phosphate group. As used herein, modified nucleotides do not encompass ribonucleotides containing adenosine monophosphate, guanosine monophosphate, uridine monophosphate, and cytidine monophosphate, and deoxyribonucleotides containing deoxyadenosine monophosphate, deoxyguanosine monophosphate, deoxythymidine monophosphate, and deoxycytidine monophosphate. However, the RNAi constructs may comprise combinations of modified nucleotides, ribonucleotides, and deoxyribonucleotides. Incorporation of modified nucleotides into one or both strands of double-stranded RNA molecules can improve the *in vivo* stability of the RNA molecules, e.g., by reducing the molecules' susceptibility to nucleases and other

degradation processes. The potency of RNAi constructs for reducing expression of the target gene can also be enhanced by incorporation of modified nucleotides.

[0039] In certain embodiments, the modified nucleotides have a modification of the ribose sugar. These sugar modifications can include modifications at the 2' and/or 5' position of the pentose ring as well as bicyclic sugar modifications. A 2'-modified nucleotide refers to a nucleotide having a pentose ring with a substituent at the 2' position other than H or OH. Such 2' modifications include, but are not limited to, 2'-O-alkyl (e.g. O-C1-C10 or O-C1-C10 substituted alkyl), 2'-O-allyl (O-$CH_2CH{=}CH_2$), 2'-C-allyl, 2'-fluoro, 2'-O-methyl (OCH_3), 2'-O-methoxyethyl (O-$(CH_2)_2OCH_3$), 2'-OCF_3, 2'-O$(CH_2)_2SCH_3$, 2'-O-aminoalkyl, 2'-amino (e.g., NH_2), 2'-O-ethylamine, and 2'-azido. Modifications at the 5' position of the pentose ring include, but are not limited to, 5'-methyl (R or S); 5'-vinyl, and 5'-methoxy.

[0040] A "bicyclic sugar modification" refers to a modification of the pentose ring where a bridge connects two atoms of the ring to form a second ring resulting in a bicyclic sugar structure. In some embodiments, the bicyclic sugar modification comprises a bridge between the 4' and 2' carbons of the pentose ring. Nucleotides comprising a sugar moiety with a bicyclic sugar modification are referred to herein as "bicyclic nucleic acids," "bridged nucleic acids," or "BNAs." A "locked nucleic acid" (LNA) is a 2',4'-bicyclic nucleic acid (2',4'-BNA) in which the ribose ring is locked by a methylene bridge that connects 2'-oxygen and 4'-carbon. Exemplary bicyclic sugar modifications include, but are not limited to, α-L-Methyleneoxy (4'-CH_2-O-2') bicyclicnucleic acid (BNA); β-D-Methyleneoxy (4'-CH_2-O-2') BNA (LNA); Ethyleneoxy (4'-(CH_2)2-O-2') BNA; Aminooxy (4'- CH_2-O-N(R)- 2')BNA; Oxyamino (4'-CH_2-N(R)-O-2') BNA; Methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA (also referred to as constrained ethyl or cEt); methylene-thio (4'- CH_2-S-2') BNA; methylene-amino (4'- CH_2-N(R)-2') BNA; methyl carbocyclic (4'- CH_2-CH(CH3)-2') BNA; propylene carbocyclic (4'-(CH_2)3-2') BNA; and Methoxy(ethyleneoxy) (4'-$CH(CH_2OMe)$-O-2')BNA (also referred to as constrained MOE or cMOE). These and other sugar-modified nucleotides that can be incorporated into the RNAi constructs disclosed herein are described in, e.g., U.S. Patent 9,181,551, U.S. Patent Publication No. 2016/0122761, and Deleavey and Damha, *Chemistry and Biology*, *19*: 937-954 (2012).

[0041] In some embodiments, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, 2'-O-allyl modified nucleotides, bicyclic nucleic acids (BNAs), or combinations thereof. In certain embodiments, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, 2'-O-methoxyethyl modified nucleotides, or combinations thereof.

In one particular embodiment, the RNAi constructs comprise one or more 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof.

[0042] Both the sense and antisense strands of the RNAi constructs can comprise one or multiple modified nucleotides. For instance, in some embodiments, the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modified nucleotides. In certain embodiments, all nucleotides in the sense strand are modified nucleotides. In some embodiments, the antisense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modified nucleotides. In other embodiments, all nucleotides in the antisense strand are modified nucleotides. In certain other embodiments, all nucleotides in the sense strand and all nucleotides in the antisense strand are modified nucleotides. In these and other embodiments, the modified nucleotides can be 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, or combinations thereof.

[0043] In certain embodiments, the modified nucleotides incorporated into one or both of the strands of the RNAi constructs of the invention have a modification of the nucleobase (also referred to herein as "base"). A "modified nucleobase" or "modified base" refers to a base other than the naturally occurring purine bases adenine (A) and guanine (G) and pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases can be synthetic or naturally occurring modifications and include, but are not limited to, universal bases, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine (X), hypoxanthine (I), 2-aminoadenine, 6-methyladenine, 6-methylguanine, and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[0044] In some embodiments, the modified base is a universal base. A "universal base" refers to a base analog that indiscriminately forms base pairs with all of the natural bases in RNA and DNA without altering the double helical structure of the resulting duplex region. Universal bases are known to those of skill in the art and include, but are not limited to, inosine, C-phenyl, C-naphthyl and other aromatic derivatives, azole carboxamides, and nitroazole derivatives, such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole.

[0045] Other suitable modified bases that can be incorporated into the RNAi constructs of the invention include those described in Herdewijn, Antisense Nucleic Acid Drug Dev., Vol. 10:

297–310, 2000 and Peacock *et al.*, J. Org. Chem., Vol. 76: 7295–7300, 2011, both of which are hereby incorporated by reference in their entireties. The skilled person is well aware that guanine, cytosine, adenine, thymine, and uracil may be replaced by other nucleobases, such as the modified nucleobases described above, without substantially altering the base pairing properties of a polynucleotide comprising a nucleotide bearing such replacement nucleobase.

[0046] In some embodiments, the sense and antisense strands of the RNAi constructs may comprise one or more abasic nucleotides. An "abasic nucleotide" or "abasic nucleoside" is a nucleotide or nucleoside that lacks a nucleobase at the 1' position of the ribose sugar. In certain embodiments, the abasic nucleotides are incorporated into the terminal ends of the sense and/or antisense strands of the RNAi constructs. In one embodiment, the sense strand comprises an abasic nucleotide as the terminal nucleotide at its 3' end, its 5' end, or both its 3' and 5' ends. In another embodiment, the antisense strand comprises an abasic nucleotide as the terminal nucleotide at its 3' end, its 5' end, or both its 3' and 5' ends. In such embodiments in which the abasic nucleotide is a terminal nucleotide, it may be an inverted nucleotide – that is, linked to the adjacent nucleotide through a 3'-3' internucleotide linkage (when on the 3' end of a strand) or through a 5'-5' internucleotide linkage (when on the 5' end of a strand) rather than the natural 3'-5' internucleotide linkage. Abasic nucleotides may also comprise a sugar modification, such as any of the sugar modifications described above. In certain embodiments, abasic nucleotides comprise a 2'-modification, such as a 2'-fluoro modification, 2'-O-methyl modification, or a 2'-H (deoxy) modification. In one embodiment, the abasic nucleotide comprises a 2'-O-methyl modification. In another embodiment, the abasic nucleotide comprises a 2'-H modification (i.e. a deoxy abasic nucleotide).

[0047] In some embodiments, all pyrimidine nucleotides preceding an adenosine nucleotide in the sense strand and/or in the antisense strand are modified nucleotides. For example, where the sequence 5'-CA-3' or 5'-UA-3' appears in either strand, the cytidine and uridine nucleotides are modified nucleotides, preferably 2'-O-methyl modified nucleotides. In certain embodiments, all pyrimidine nucleotides in the sense strand are modified nucleotides (e.g. 2'-O-methyl modified nucleotides), and the 5' nucleotide in all occurrences of the sequence 5'-CA-3' or 5'-UA-3' in the antisense strand are modified nucleotides (e.g. 2'-O-methyl modified nucleotides). In other embodiments, all nucleotides in the duplex region are modified nucleotides. In such embodiments, the modified nucleotides are preferably 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, or combinations thereof.

[0048] In embodiments in which the RNAi construct comprises a nucleotide overhang, the nucleotides in the overhang can be ribonucleotides, deoxyribonucleotides, or modified

nucleotides. In one embodiment, the nucleotides in the overhang are deoxyribonucleotides, e.g., deoxythymidine. In another embodiment, the nucleotides in the overhang are modified nucleotides. For instance, in some embodiments, the nucleotides in the overhang are 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, 2'-methoxyethyl modified nucleotides, or combinations thereof.

[0049] The RNAi constructs of the disclosure may also comprise one or more modified internucleotide linkages. As used herein, the term "modified internucleotide linkage" refers to an internucleotide linkage other than the natural 3' to 5' phosphodiester linkage. In some embodiments, the modified internucleotide linkage is a phosphorous-containing internucleotide linkage, such as a phosphotriester, an aminoalkyl phosphotriester, an alkylphosphonate (e.g., methylphosphonate, 3'-alkylene phosphonate), a phosphinate, a phosphoramidate (e.g., 3'-aminophosphoramidate and aminoalkylphosphoramidate), a phosphorothioate (P=S), a chiralphosphorothioate, a phosphorodithioate, a thionophosphoramidate, a thionoalkylphosphonate, athionoalkylphosphotriester, and a boranophosphate. In one embodiment, a modified internucleotide linkage is a 2' to 5' phosphodiester linkage. In other embodiments, the modified internucleotide linkage is a non-phosphorous-containing internucleotide linkage and thus can be referred to as a modified internucleoside linkage. Such non-phosphorous-containing linkages include, but are not limited to, morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane linkages (-O-Si(H)$_2$-O-); sulfide, sulfoxide and sulfone linkages; formacetyl and thioformacetyl linkages; alkene containing backbones; sulfamate backbones; methylenemethylimino (-CH$_2$-N(CH$_3$)-O-CH$_2$-) and methylenehydrazino linkages; sulfonate and sulfonamide linkages; amide linkages; and others having mixed N, O, S and CH$_2$ component parts. In one embodiment, the modified internucleoside linkage is a peptide-based linkage (e.g., aminoethylglycine) to create a peptide nucleic acid or PNA, such as those described in U.S. Patents 5,539,082; 5,714,331; and 5,719,262. Other suitable modified internucleotide and internucleoside linkages that may be employed in the disclosed RNAi constructs are described in U.S. Patents 6,693,187 and 9,181,551, U.S. Patent Publication No. 2016/0122761, and Deleavey and Damha, *supra*.

[0050] In certain embodiments, the RNAi constructs comprise one or more phosphorothioate internucleotide linkages. The phosphorothioate internucleotide linkages may be present in the sense strand, antisense strand, or both strands of the RNAi constructs. For instance, in some embodiments, the sense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. In other embodiments, the antisense strand comprises 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. In still other

embodiments, both strands comprise 1, 2, 3, 4, 5, 6, 7, 8, or more phosphorothioate internucleotide linkages. The RNAi constructs can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both the 3'- and 5'- ends of the sense strand, the antisense strand, or both strands. For instance, in certain embodiments, the RNAi construct comprises about 1 to about 6 or more (e.g., about 1, 2, 3, 4, 5, 6 or more) consecutive phosphorothioate internucleotide linkages at the 3'-end of the sense strand, the antisense strand, or both strands. In other embodiments, the RNAi construct comprises about 1 to about 6 or more (e.g., about 1, 2, 3, 4, 5, 6 or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the sense strand, the antisense strand, or both strands. In one embodiment, the RNAi construct comprises a single phosphorothioate internucleotide linkage at the 3' end of the sense strand and a single phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at the 3' end of the antisense strand (i.e., a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at the 3' end of the antisense strand). In another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand. In yet another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at the 5' end of the sense strand. In still another embodiment, the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the sense strand (i.e. a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at both the 5' and 3' ends of the antisense strand and a phosphorothioate internucleotide linkage at the first and second internucleotide linkages at both the 5' and 3' ends of the sense strand). In any of the embodiments in which one or both strands comprise one or more phosphorothioate internucleotide linkages, the remaining internucleotide linkages within the strands can be the natural 3' to 5' phosphodiester linkages. For instance, in some embodiments, each internucleotide linkage of the sense and antisense strands is selected from phosphodiester and phosphorothioate, wherein at least one internucleotide linkage is a phosphorothioate.

[0051] In embodiments in which the RNAi construct comprises a nucleotide overhang, two or more of the unpaired nucleotides in the overhang can be connected by a phosphorothioate internucleotide linkage. In certain embodiments, all the unpaired nucleotides in a nucleotide overhang at the 3' end of the antisense strand and/or the sense strand are connected by

phosphorothioate internucleotide linkages. In other embodiments, all the unpaired nucleotides in a nucleotide overhang at the 5' end of the antisense strand and/or the sense strand are connected by phosphorothioate internucleotide linkages. In still other embodiments, all the unpaired nucleotides in any nucleotide overhang are connected by phosphorothioate internucleotide linkages.

[0052] In some embodiments, the 5' end of the sense strand, antisense strand, or both the antisense and sense strands of the disclosed RNAi constructs comprises a phosphate moiety. As used herein, the term "phosphate moiety" refers to a terminal phosphate group that includes unmodified phosphates (-O-P=O)(OH)OH) as well as modified phosphates. Modified phosphates include phosphates in which one or more of the O and OH groups are replaced with H, O, S, N(R) or alkyl where R is H, an amino protecting group or unsubstituted or substituted alkyl. Exemplary phosphate moieties include, but are not limited to, 5'-monophosphate; 5'diphosphate; 5'-triphosphate; 5'-guanosine cap (7-methylated or non-methylated); 5'-adenosinecap or any other modified or unmodified nucleotide cap structure; 5'-monothiophosphate (phosphorothioate); 5'-monodithiophosphate (phosphorodithioate); 5'-alpha-thiotriphosphate; 5'-gamma-thiotriphosphate, 5'-phosphoramidates; 5'-vinylphosphates; 5'-alkylphosphonates (wherein "alkyl" can be methyl, ethyl, isopropyl, propyl, etc.); and 5'-alkyletherphosphonates (wherein "alkylether" can be methoxymethyl, ethoxymethyl, etc.).

[0053] The modified nucleotides that can be incorporated into the RNAi constructs disclosed herein may have more than one chemical modification described herein. For instance, the modified nucleotide may have a modification to the ribose sugar as well as a modification to the nucleobase. By way of example, a modified nucleotide may comprise a 2' sugar modification (e.g., 2'-fluoro or 2'-O-methyl) and comprise a modified base (e.g., 5-methyl cytosine or pseudouracil). In other embodiments, the modified nucleotide may comprise a sugar modification in combination with a modification to the 5' phosphate that would create a modified internucleotide or internucleoside linkage when the modified nucleotide was incorporated into a polynucleotide. For instance, in some embodiments, the modified nucleotide may comprise a sugar modification, such as a 2'-fluoro modification, a 2'-O-methyl modification, or a bicyclic sugar modification, as well as a 5' phosphorothioate group. Accordingly, in some embodiments, one or both strands of the RNAi constructs comprise a combination of 2' modified nucleotides or BNAs and phosphorothioate internucleotide linkages. In certain embodiments, both the sense and antisense strands of the RNAi constructs comprise a combination of 2'-fluoro modified nucleotides, 2'-O-methyl modified nucleotides, and

phosphorothioate internucleotide linkages. Exemplary RNAi constructs comprising modified nucleotides and internucleotide linkages are shown in Table 1 and Table 2.

Function of RNAi Constructs

[0054] The RNAi constructs disclosed herein desirably reduce or inhibit the expression of SCAP in cells, particularly liver cells. Accordingly, in one embodiment, the present disclosure provides a method of reducing SCAP expression in a cell by contacting the cell with any RNAi construct described herein. The cell may be *in vitro* or *in vivo*. SCAP expression can be assessed by measuring the amount or level of SCAP mRNA, SCAP protein, or another biomarker linked to SCAP expression. The reduction of SCAP expression in cells or animals treated with an RNAi construct disclosed herein can be determined relative to the SCAP expression in cells or animals not treated with the RNAi construct or treated with a control RNAi construct. For instance, in some embodiments, reduction of SCAP expression is assessed by (a) measuring the amount or level of SCAP mRNA in liver cells treated with a RNAi construct disclosed herein, (b) measuring the amount or level of SCAP mRNA in liver cells treated with a control RNAi construct (e.g., RNAi construct directed to a RNA molecule not expressed in liver cells or a RNAi construct having a nonsense or scrambled sequence) or no construct, and (c) comparing the measured SCAP mRNA levels from treated cells in (a) to the measured SCAP mRNA levels from control cells in (b). The SCAP mRNA levels in the treated cells and controls cells can be normalized to RNA levels for a control gene (e.g., 18S ribosomal RNA) prior to comparison. SCAP mRNA levels can be measured by a variety of methods, including Northern blot analysis, nuclease protection assays, fluorescence *in situ* hybridization (FISH), reverse-transcriptase (RT)-PCR, real-time RT-PCR, quantitative PCR, and the like.

[0055] In other embodiments, reduction of SCAP expression is assessed by (a) measuring the amount or level of SCAP protein in liver cells treated with a RNAi construct disclosed herein, (b) measuring the amount or level of SCAP protein in liver cells treated with a control RNAi construct (e.g., RNAi construct directed to a RNA molecule not expressed in liver cells or a RNAi construct having a nonsense or scrambled sequence) or no construct, and (c) comparing the measured SCAP protein levels from treated cells in (a) to the measured SCAP protein levels from control cells in (b). SCAP protein levels can be measured using any suitable method known to those of skill in the art, including but not limited to, western blots, immunoassays (e.g., ELISA), and flow cytometry. Any suitable method of measuring SCAP mRNA or protein can be used to assess the efficacy of the RNAi constructs disclosed herein.

[0056] In some embodiments, the methods to assess SCAP expression levels are performed *in vitro* in cells that natively express SCAP (e.g., liver cells) or cells that have been engineered to express SCAP. In certain embodiments, the methods are performed *in vitro* in liver cells. Suitable liver cells include, but are not limited to, primary hepatocytes (e.g. human, non-human primate, or rodent hepatocytes), HepAD38 cells, HuH-6 cells, HuH-7 cells, HuH-5-2 cells, BNLCL2 cells, Hep3B cells, or HepG2 cells. In one embodiment, the liver cells are Hep3B cells. In another embodiment, the liver cells are HepG2 cells.

[0057] In other embodiments, the methods to assess SCAP expression levels are performed *in vivo*. For example, the RNAi constructs and any control RNAi constructs can be administered to an animal (e.g., rodent or non-human primate), and SCAP mRNA or protein levels may be assessed in liver tissue harvested from the animal following treatment. Alternatively or additionally, a biomarker or functional phenotype associated with SCAP expression can be assessed in the treated animals.

[0058] In certain embodiments, expression of SCAP is reduced in liver cells by at least 40%, at least 45%, or at least 50% by an RNAi construct disclosed herein. In some embodiments, expression of SCAP is reduced in liver cells by at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% by an RNAi construct disclosed herein. In other embodiments, the expression of SCAP is reduced in liver cells by about 90% or more, e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more by an RNAi construct disclosed herein. The percent reduction of SCAP expression can be measured by any of the methods described herein or otherwise known in the art. For instance, in certain embodiments, the RNAi constructs inhibit at least 40% of SCAP expression at 5 nM in Hep3B cells (contains wild type SCAP) *in vitro*. In related embodiments, the RNAi constructs inhibit at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of SCAP expression at 5 nM in Hep3B cells *in vitro*. In other embodiments, the RNAi constructs inhibit at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of SCAP expression at 5 nM in Hep3B cells *in vitro*. In certain embodiments, the RNAi constructs disclosed herein inhibit at least 40% of SCAP expression at 5 nM in HepG2 cells *in vitro*. In related embodiments, the RNAi constructs disclosed herein inhibit at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of SCAP expression at 5 nM in HepG2 cells *in vitro*. In other embodiments, the RNAi constructs inhibit at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of SCAP expression at 5 nM in HepG2 cells *in vitro*. In certain embodiments, the RNAi constructs inhibit at least 40% of SCAP expression at 5 nM in CHO transfected cells expressing human SCAP *in vitro*. In

related embodiments, the RNAi constructs inhibit at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75% of SCAP expression at 5 nM in CHO transfected cells expressing human SCAP *in vitro*. In other embodiments, the RNAi constructs inhibit at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98% of SCAP expression at 5 nM in CHO transfected cells expressing human SCAP *in vitro*. Reduction of SCAP can be measured using a variety of techniques including, for example, RNA FISH or droplet digital PCR (see, e.g., Kamitaki et al., *Digital PCR. Methods in Molecular Biology*, *1768*: 401-422 (2018). doi:10.1007/978-1-4939-7778-9_23).

[0059] In some embodiments, an IC_{50} value is calculated to assess the potency of an RNAi construct disclosed herein for inhibiting SCAP expression in liver cells. An "IC_{50} value" is the dose/concentration required to achieve 50% inhibition of a biological or biochemical function. In other embodiments, the potency of an RNAi construct may be assessed by calculating an "AC_{50}" value, which is the dose/concentration required to achieve 50% activation of a biological or biochemical function. The IC_{50} value or AC_{50} value of any substance or antagonist can be determined by constructing a dose-response curve and examining the effect of different concentrations of the substance or antagonist on expression levels or functional activity in any assay. IC_{50} values can be calculated for a given antagonist or substance by determining the concentration needed to inhibit half of the maximum biological response or native expression levels. Thus, the IC_{50} value for any RNAi construct can be calculated by determining the concentration of the RNAi construct needed to inhibit half of the native SCAP expression level in liver cells (e.g., SCAP expression level in control liver cells) in any assay, such as an immunoassay, RNA FISH assay, or a droplet digital PCR assay. Similarly, AC_{50} values can be calculated for a given substance by determining the concentration needed to activate half of the maximum biological response or native expression levels. The RNAi constructs disclosed herein may inhibit SCAP expression in liver cells (e.g. Hep3B cells) with an IC_{50} of less than about 20 nM (e.g., less than about 15 nM, 10 nM, 5 nM, or 1 nM). For example, the disclosed RNAi constructs may inhibit SCAP expression in liver cells with an IC_{50} of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits SCAP expression in liver cells (e.g., Hep3B cells) with an IC_{50} of about 1 nM to about 10 nM (e.g., about 5 nM). The RNAi constructs disclosed herein may inhibit SCAP expression in liver cells (e.g., HepG2 cells) with an IC_{50} of less than about 20 nM. For example, the RNAi constructs may inhibit SCAP expression in liver cells with an IC_{50} of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about

0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits SCAP expression in liver cells (e.g., HepG2 cells) with an IC_{50} of about 1 nM to about 10 nM (e.g., about 5 nM). The RNAi constructs disclosed herein may inhibit SCAP expression in cells (e.g., CHO-transfected cells) expressing human SCAP with an IC_{50} of less than about 20 nM. For example, the RNAi constructs inhibit SCAP expression in SCAP -expressing cells with an IC_{50} of about 0.001 nM to about 20 nM, about 0.001 nM to about 10 nM, about 0.001 nM to about 5 nM, about 0.001 nM to about 1 nM, about 0.1 nM to about 10 nM, about 0.1 nM to about 5 nM, or about 0.1 nM to about 1 nM. In certain embodiments, the RNAi construct inhibits SCAP expression in SCAP -expressing cells with an IC_{50} of about 1 nM to about 10 nM (e.g., about 5 nM).

[0060] The RNAi constructs disclosed herein can readily be made using techniques known in the art, such as, for example, conventional nucleic acid solid phase synthesis. The polynucleotides of the RNAi constructs can be assembled on a suitable nucleic acid synthesizer utilizing standard nucleotide or nucleoside precursors (e.g., phosphoramidites). Automated nucleic acid synthesizers are sold commercially by several vendors, including DNA/RNA synthesizers from Applied Biosystems (Foster City, CA), MerMade synthesizers from BioAutomation (Irving, TX), and OligoPilot synthesizers from GE Healthcare Life Sciences (Pittsburgh, PA).

[0061] The 2' silyl protecting group can be used in conjunction with acid labile dimethoxytrityl (DMT) at the 5' position of ribonucleosides to synthesize oligonucleotides via phosphoramidite chemistry. Final deprotection conditions are known not to significantly degrade RNA products. All syntheses can be conducted in any automated or manual synthesizer on large, medium, or small scale. The syntheses may also be carried out in multiple well plates, columns, or glass slides.

[0062] The 2'-O-silyl group can be removed via exposure to fluoride ions, which can include any source of fluoride ion, e.g., those salts containing fluoride ion paired with inorganic counterions, e.g., cesium fluoride and potassium fluoride or those salts containing fluoride ion paired with an organic counterion, e.g., a tetraalkylammonium fluoride. A crown ether catalyst can be utilized in combination with the inorganic fluoride in the deprotection reaction. Exemplary fluoride ion sources include, but are not limited to, tetrabutylammonium fluoride or aminohydrofluorides (e.g., combining aqueous HF with triethylamine in a dipolar aprotic solvent, e.g., dimethylformamide).

[0063] The choice of protecting groups for use on the phosphite triesters and phosphotriesters can alter the stability of the triesters towards fluoride. Methyl protection of the phosphotriester or phosphite triester can stabilize the linkage against fluoride ions and improve process yields.

[0064] Since ribonucleosides have a reactive 2' hydroxyl substituent, it may be desirable to protect the reactive 2' position in RNA with a protecting group that is orthogonal to a 5'-O-dimethoxytrityl protecting group, e.g., one stable to treatment with acid. Silyl protecting groups meet this criterion and can be readily removed in a final fluoride deprotection step that can result in minimal RNA degradation.

[0065] Tetrazole catalysts can be used in the standard phosphoramidite coupling reaction. Exemplary catalysts include, e.g., tetrazole, S-ethyl-tetrazole, benzylthiotetrazole, and pnitrophenyltetrazole.

[0066] Additional methods of synthesizing the RNAi constructs described herein will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. Other synthetic chemistry transformations, protecting groups (e.g., for hydroxyl, amino, etc., present on the bases) and protecting group methodologies (protection and deprotection) useful in synthesizing the RNAi constructs described herein are known in the art and include, for example, those described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); T. W. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof. Custom synthesis of RNAi constructs is also available from several commercial vendors, including Dharmacon, Inc. (Lafayette, CO), AxoLabs GmbH (Kulmbach, Germany), and Ambion, Inc. (Foster City, CA).

[0067] The RNAi constructs disclosed herein may comprise a ligand. As used herein, a "ligand" refers to any compound or molecule that can interact with another compound or molecule, either directly or indirectly. The interaction of a ligand with another compound or molecule may elicit a biological response (e.g., initiate a signal transduction cascade, induce receptor mediated endocytosis) or may just be a physical association. The ligand can modify one or more properties of the double-stranded RNA molecule to which is attached, such as the pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties of the RNA molecule.

[0068] The ligand may comprise a serum protein (e.g., human serum albumin, low-density lipoprotein, globulin), a cholesterol moiety, a vitamin (e.g., biotin, vitamin E, vitamin B12), a folate moiety, a steroid, a bile acid (e.g., cholic acid), a fatty acid (e.g., palmitic acid, myristic acid), a carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid), a glycoside, a phospholipid, or an antibody or binding fragment thereof (e.g., a whole antibody or binding fragment that targets the RNAi construct to a specific cell type, such as liver cells). Other examples of ligands include dyes, intercalating agents (e.g., acridines), cross-linkers (e.g., psoralene, mitomycin C), porphyrins (e.g., TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g., EDTA), lipophilic molecules (e.g., adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-BisO(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, 03-(oleoyl)lithocholic acid, 03-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine), peptides (e.g., antennapedia peptide, Tat peptide, RGD peptides), alkylating agents, polymers (e.g., polyethylene glycol (PEG), PEG-40K), poly amino acids, and polyamines (e.g., spermine, spermidine).

[0069] In certain embodiments, the ligands have endosomolytic properties. The endosomolytic ligands promote the lysis of the endosome and/or transport of the RNAi construct, or its components, from the endosome to the cytoplasm of the cell. The endosomolytic ligand may be a polycationic peptide or peptidomimetic which shows pH-dependent membrane activity and fusogenicity. In one embodiment, the endosomolytic ligand assumes its active conformation at endosomal pH. The "active" conformation is that conformation in which the endosomolytic ligand promotes lysis of the endosome and/or transport of the RNAi construct, or its components, from the endosome to the cytoplasm of the cell. Exemplary endosomolytic ligands include the GALA peptide (Subbarao et al., Biochemistry, Vol. 26: 2964-2972, 1987), the EALA peptide (Vogel et al., J. Am. Chem. Soc., Vol. 118: 1581-1586, 1996), and their derivatives (Turk et al., Biochem. Biophys. Acta, Vol. 1559: 56-68, 2002). In one embodiment, the endosomolytic component may contain a chemical group (e.g., an amino acid) which will undergo a change in charge or protonation in response to a change in pH. The endosomolytic component may be linear or branched.

[0070] In some embodiments, the ligand comprises a lipid or other hydrophobic molecule. In one embodiment, the ligand comprises a cholesterol moiety or other steroid. Cholesterol conjugated oligonucleotides have been reported to be more active than their unconjugated counterparts (Manoharan, Antisense Nucleic Acid Drug Development, Vol. 12: 103-228, 2002). Ligands comprising cholesterol moieties and other lipids for conjugation to nucleic acid

molecules have also been described in U.S. Patents 7,851,615; 7,745,608; and 7,833,992. In another embodiment, the ligand may comprise a folate moiety. Polynucleotides conjugated to folate moieties can be taken up by cells via a receptor-mediated endocytosis pathway. Such folate-polynucleotide conjugates are described in, e.g., U.S. Patent 8,188,247.

[0071] Given that SCAP is expressed in liver cells (e.g., hepatocytes), in certain embodiments, it is desirable to specifically deliver the RNAi construct to liver cells. In some embodiments, RNAi constructs can be specifically targeted to the liver by employing ligands that bind to or interact with proteins expressed on the surface of liver cells. For example, in certain embodiments, a ligand may comprise one or more antigen binding proteins (e.g. antibodies or binding fragments thereof (e.g. Fab, scFv)) that specifically bind to a receptor expressed on hepatocytes.

[0072] In certain embodiments, the ligand comprises a carbohydrate. A "carbohydrate" refers to a compound made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched, or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Carbohydrates include, but are not limited to, sugars (e.g., monosaccharides, disaccharides, trisaccharides, tetrasaccharides, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides, such as starches, glycogen, cellulose, and polysaccharide gums. In some embodiments, the carbohydrate incorporated into the ligand is a monosaccharide selected from a pentose, hexose, or heptose and di- and tri-saccharides including such monosaccharide units. In other embodiments, the carbohydrate incorporated into the ligand is an amino sugar, such as galactosamine, glucosamine, N-acetylgalactosamine, and N-acetylglucosamine.

[0073] In some embodiments, the ligand comprises a hexose or hexosamine. The hexose may be selected from glucose, galactose, mannose, fucose, or fructose. The hexosamine may be selected from fructosamine, galactosamine, glucosamine, or mannosamine. In certain embodiments, the ligand comprises glucose, galactose, galactosamine, or glucosamine. In one embodiment, the ligand comprises glucose, glucosamine, or N-acetylglucosamine. In another embodiment, the ligand comprises galactose, galactosamine, or N-acetyl-galactosamine. In particular embodiments, the ligand comprises N-acetyl-galactosamine. Ligands comprising glucose, galactose, and N-acetyl-galactosamine (GalNAc) are particularly effective in targeting compounds to liver cells (see, e.g., D'Souza and Devarajan, J. Control Release, Vol. 203: 126-139, 2015). Examples of GalNAc- or galactose-containing ligands that can be incorporated into the RNAi constructs disclosed herein are described in U.S. Patents 7,491,805; 8,106,022; and

8,877,917; U.S. Patent Publication No. 2003/0130186; and WIPO Publication No. WO 2013/166155.

[0074] In certain embodiments, the ligand comprises a multivalent carbohydrate moiety. As used herein, a "multivalent carbohydrate moiety" refers to a moiety comprising two or more carbohydrate units capable of independently binding or interacting with other molecules. For example, a multivalent carbohydrate moiety comprises two or more binding domains comprised of carbohydrates that can bind to two or more different molecules or two or more different sites on the same molecule. The valency of the carbohydrate moiety denotes the number of individual binding domains within the carbohydrate moiety. For instance, the terms "monovalent," "bivalent," "trivalent," and "tetravalent" with reference to the carbohydrate moiety refer to carbohydrate moieties with one, two, three, and four binding domains, respectively. The multivalent carbohydrate moiety may comprise a multivalent lactose moiety, a multivalent galactose moiety, a multivalent glucose moiety, a multivalent N-acetyl-galactosamine moiety, a multivalent N-acetyl-glucosamine moiety, a multivalent mannose moiety, or a multivalent fucose moiety. In some embodiments, the ligand comprises a multivalent galactose moiety. In other embodiments, the ligand comprises a multivalent N-acetyl-galactosamine moiety. In these and other embodiments, the multivalent carbohydrate moiety is bivalent, trivalent, or tetravalent. In such embodiments, the multivalent carbohydrate moiety can be bi-antennary or tri-antennary. In one particular embodiment, the multivalent N-acetyl-galactosamine moiety is trivalent or tetravalent. In another particular embodiment, the multivalent galactose moiety is trivalent or tetravalent. An exemplary GalNAc-containing ligand for incorporation into the RNAi constructs disclosed herein includes a tri-antennary GalNAc-containing ligand (also referred to as "GalNAc3").

[0075] The ligand can be attached or conjugated to the RNA molecule of the RNAi construct directly or indirectly. For instance, in some embodiments, the ligand is covalently attached directly to the sense or antisense strand of the RNAi construct. In other embodiments, the ligand is covalently attached via a linker to the sense or antisense strand of the RNAi construct. The ligand can be attached to nucleobases, sugar moieties, or internucleotide linkages of polynucleotides (e.g., sense strand or antisense strand) of the RNAi constructs disclosed herein. Conjugation or attachment to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In certain embodiments, the 2-, 6-, 7-, or 8-positions of a purine nucleobase are attached to a ligand. Conjugation or attachment to pyrimidine nucleobases or derivatives thereof can also occur at any position. In some embodiments, the 2, 5-, and 6-positions of a pyrimidine nucleobase can be attached to a ligand. Conjugation or

attachment to sugar moieties of nucleotides can occur at any carbon atom. Exemplary carbon atoms of a sugar moiety that can be attached to a ligand include the 2', 3', and 5' carbon atoms. The 1' position also can be attached to a ligand, such as in abasic nucleotides. Internucleotide linkages can also support ligand attachments. For phosphorus-containing linkages (e.g., phosphodiester, phosphorothioate, phosphorodithiotate, phosphoroamidate, and the like), the ligand can be attached directly to the phosphorus atom or to an O, N, or S atom bound to the phosphorus atom. For amine- or amide-containing internucleoside linkages (e.g., PNA), the ligand can be attached to the nitrogen atom of the amine or amide or to an adjacent carbon atom.

[0076] In certain embodiments, the ligand may be attached to the 3' or 5' end of either the sense or antisense strand. In certain embodiments, the ligand is covalently attached to the 5' end of the sense strand. For example, in some embodiments, the ligand is attached to the 5'-terminal nucleotide of the sense strand. In certain such embodiments, the ligand is attached at the 5'-position of the 5'-terminal nucleotide of the sense strand. In other embodiments, the ligand is covalently attached to the 3' end of the sense strand. For example, in some embodiments, the ligand is attached to the 3'-terminal nucleotide of the sense strand. In certain such embodiments, the ligand is attached at the 3'-position of the 3'-terminal nucleotide of the sense strand. In alternative embodiments, the ligand is attached near the 3' end of the sense strand, but before one or more terminal nucleotides (i.e. before 1, 2, 3, or 4 terminal nucleotides). In some embodiments, the ligand is attached at the 2'-position of the sugar of the 3'-terminal nucleotide of the sense strand.

[0077] In certain embodiments, the ligand is attached to the sense or antisense strand via a linker. A "linker" is an atom or group of atoms that covalently joins a ligand to a polynucleotide component of the RNAi construct. The linker may be from about 1 to about 30 atoms in length, from about 2 to about 28 atoms in length, from about 3 to about 26 atoms in length, from about 4 to about 24 atoms in length, from about 6 to about 20 atoms in length, from about 7 to about 20 atoms in length, from about 8 to about 20 atoms in length, from about 8 to about 18 atoms in length, from about 10 to about 18 atoms in length, and from about 12 to about 18 atoms in length. In some embodiments, the linker may comprise a bifunctional linking moiety, which generally comprises an alkyl moiety with two functional groups. One of the functional groups is selected to bind to the compound of interest (e.g., sense or antisense strand of the RNAi construct) and the other is selected to bind essentially any selected group, such as a ligand as described herein. In certain embodiments, the linker comprises a chain structure or an oligomer of repeating units, such as ethylene glycol or amino acid units. Examples of functional groups that are typically employed in a bifunctional linking moiety include, but are not limited to,

electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In some embodiments, bifunctional linking moieties include amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), and the like.

[0078] Linkers that may be used to attach a ligand to the sense or antisense strand in the RNAi constructs include, but are not limited to, pyrrolidine, 8-amino-3,6-di oxaoctanoic acid, succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, 6-aminohexanoic acid, substituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} alkynyl. Preferred substituent groups for such linkers include, but are not limited to, hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl, and alkynyl.

[0079] In certain embodiments, the linkers are cleavable. A cleavable linker is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In some embodiments, the cleavable linker is cleaved at least 10 times, 20 times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least 100 times faster in the target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum).

[0080] Cleavable linkers are susceptible to cleavage agents, e.g., pH, redox potential, or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linker by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linker by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

[0081] A cleavable linker may comprise a moiety that is susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable group that is cleaved at a preferred pH, thereby releasing the RNA molecule from the ligand inside the cell, or into the desired compartment of the cell.

[0082] A linker can include a cleavable group that is cleavable by a particular enzyme. The type of cleavable group incorporated into a linker can depend on the cell to be targeted. For example, liver-targeting ligands can be linked to RNA molecules through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other types of cells rich in esterases include cells of the lung, renal cortex, and testis. Linkers that contain peptide bonds can be used when targeting cells rich in peptidases, such as liver cells and synoviocytes.

[0083] In general, the suitability of a candidate cleavable linker can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linker. It will also be desirable to also test the candidate cleavable linker for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It may be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In some embodiments, useful candidate linkers are cleaved at least 2, 4, 10, 20, 50, 70, or 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood or serum (or under *in vitro* conditions selected to mimic extracellular conditions).

[0084] In other embodiments, redox cleavable linkers are utilized. Redox cleavable linkers are cleaved upon reduction or oxidation. An example of reductively cleavable group is a disulfide linking group (-S-S-). To determine if a candidate cleavable linker is a suitable "reductively cleavable linker," or, for example, is suitable for use with a particular RNAi construct and particular ligand, one or more methods described herein can be used. For example, a candidate linker can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent known in the art, which mimics the rate of cleavage that would be observed in a cell, e.g., a target cell. The candidate linkers can also be evaluated under conditions which are selected to mimic blood or serum conditions. In a specific embodiment, candidate linkers are cleaved by at most 10% in the blood. In other embodiments, useful candidate linkers are degraded at least 2, 4, 10, 20, 50,70, or 100 times faster in the cell (or under *in vitro* conditions selected to mimic intracellular conditions) as compared to blood (or under *in vitro* conditions selected to mimic extracellular conditions).

[0085] In yet other embodiments, phosphate-based cleavable linkers are cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that hydrolyzes phosphate groups in cells are enzymes, such as phosphatases in cells. Examples of phosphate-based cleavable groups are -O-P(O)(ORk)-O-, -O-P(S)(ORk)-O-, -O-P(S)(SRk)-O-, -S-P(O)(ORk)-O-, -O-P(O)(ORk)-S-, -S-P(O)(ORk)-S-, -O-P(S)(ORk)-S-, -S-P(S)(ORk)-O-, -O-P(O)(Rk)-O-, -O-P(S)(Rk)-O-, -S-P(O)(Rk)-O-, -S-P(S)(Rk)-O-, -S-P(O)(Rk)-S-, -O-P(S)(Rk)-S-, where Rk can be hydrogen or C_1-C_{10} alkyl. Specific embodiments include -O-P(O)(OH)-O-, -O-P(S)(OH)-O-, -O-P(S)(SH)-O-, -S-P(O)(OH)-O-, -O-P(O)(OH)-S-, -S-P(O)(OH)-S-, -O-P(S)(OH)-S-, -SP(S)(OH)-O-, -O-P(O)(H)-O-, -O-P(S)(H)-O-, -S-P(O)(H)-O-, -S-P(S)(H)-O-, -S-P(O)(H)-S-, -O-P(S)(H)-S-. Another specific embodiment is -O-P(O)(OH)-O-. These candidate linkers can be evaluated using methods analogous to those described above.

[0086] In other embodiments, the linkers may comprise acid cleavable groups, which are groups that are cleaved under acidic conditions. In some embodiments, acid cleavable groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.5, 5.0, or lower), or by agents, such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes, can provide a cleaving environment for acid cleavable groups. Examples of acid cleavable linking groups include, but are not limited to, hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). A specific embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl, pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

[0087] In other embodiments, the linkers may comprise ester-based cleavable groups, which are cleaved by enzymes, such as esterases and amidases in cells. Examples of ester-based cleavable groups include, but are not limited to, esters of alkylene, alkenylene and alkynylene groups. Ester cleavable groups have the general formula -C(O)O-, or -OC(O) -. These candidate linkers can be evaluated using methods analogous to those described above.

[0088] In further embodiments, the linkers may comprise peptide-based cleavable groups, which are cleaved by enzymes, such as peptidases and proteases in cells. Peptide-based cleavable groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (-C(O)NH-). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide-based cleavage group is generally limited to the peptide bond

(i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula -NHCHR^{A}C(O)NHCHR^{B}C(O)-, where R^A and R^B are the side chains of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

[0089] Other types of linkers suitable for attaching ligands to the sense or antisense strands in the RNAi constructs described herein are known in the art and can include the linkers described in, e.g., U.S. Patents 7,723,509; 8,017,762; 8,828,956; 8,877,917; and 9,181,551.

[0090] In certain embodiments, the ligand covalently attached to the sense or antisense strand of the RNAi constructs comprises a GalNAc moiety, e.g., a multivalent GalNAc moiety. In some embodiments, the multivalent GalNAc moiety is a trivalent GalNAc moiety and is attached to the 3' end of the sense strand. In other embodiments, the multivalent GalNAc moiety is a trivalent GalNAc moiety and is attached to the 5' end of the sense strand. In yet other embodiments, the multivalent GalNAc moiety is a tetravalent GalNAc moiety and is attached to the 3' end of the sense strand. In still other embodiments, the multivalent GalNAc moiety is a tetravalent GalNAc moiety and is attached to the 5' end of the sense strand.

[0091] In some embodiments, the RNAi constructs disclosed herein may be delivered to a cell or tissue of interest by administering a vector that encodes and controls the intracellular expression of the RNAi construct. A "vector" (also referred to herein as an "expression vector") is a composition of matter which can be used to deliver a nucleic acid of interest to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated viral vectors, retroviral vectors, and the like. A vector can be replicated in a living cell, or it can be made synthetically.

[0092] Generally, a vector for expressing an RNAi construct will comprise one or more promoters operably linked to sequences encoding the RNAi construct. The phrases "operably linked," "operatively linked," or "under transcriptional control" may be used interchangeably herein to indicate when a promoter is in the correct location and orientation in relation to a polynucleotide sequence to control the initiation of transcription by RNA polymerase and expression of the polynucleotide sequence. A "promoter" refers to a sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene sequence. Suitable promoters include, but are not limited to,

RNA pol I, pol II, HI or U6 RNA pol III, and viral promoters (e.g., human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, and the Rous sarcoma virus long terminal repeat). In some embodiments, an HI or U6RNA pol III promoter is employed. The promoter can be a tissue-specific or inducible promoter. Of particular interest are liver-specific promoters, such as promoter sequences from the human alpha-1 antitrypsin gene, albumin gene, hemopexin gene, and hepatic lipase gene. Inducible promoters include, for example, promoters regulated by ecdysone, estrogen, progesterone, tetracycline, and isopropyl-PD1-thiogalactopyranoside (IPTG).

[0093] When the RNAi construct comprises an siRNA, the two separate strands (sense and antisense strand) can be expressed from a single vector or two separate vectors. For example, in some embodiments, the sequence encoding the sense strand is operably linked to a promoter on a first vector and the sequence encoding the antisense strand is operably linked to a promoter on a second vector. In such an embodiment, the first and second vectors are co-introduced, e.g., by infection or transfection, into a target cell, such that the sense and antisense strands, once transcribed, will hybridize intracellularly to form the siRNA molecule. In another embodiment, the sense and antisense strands are transcribed from two separate promoters located in a single vector. In such embodiments, the sequence encoding the sense strand may be operably linked to a first promoter and the sequence encoding the antisense strand may be operably linked to a second promoter, wherein the first and second promoters are located in a single vector. In one embodiment, the vector comprises a first promoter operably linked to a sequence encoding the siRNA molecule, and a second promoter operably linked to the same sequence in the opposite direction, such that transcription of the sequence from the first promoter results in the synthesis of the sense strand of the siRNA molecule and transcription of the sequence from the second promoter results in synthesis of the antisense strand of the siRNA molecule.

[0094] When the RNAi construct comprises a shRNA, a sequence encoding the single, at least partially self-complementary RNA molecule is operably linked to a promoter to produce a single transcript. In some embodiments, the sequence encoding the shRNA comprises an inverted repeat joined by a linker polynucleotide sequence to produce the stem and loop structure of the shRNA following transcription.

[0095] In some embodiments, the vector encoding an RNAi construct is a viral vector. Various viral vector systems that are suitable to express the RNAi constructs described herein include, but are not limited to, adenoviral vectors, retroviral vectors (e.g., lentiviral vectors, maloney murine leukemia virus), adeno-associated viral vectors; herpes simplex viral vectors; SV40 vectors; polyoma viral vectors; papilloma viral vectors; picornaviral vectors; and pox viral

vectors (e.g., vaccinia virus). In certain embodiments, the viral vector is a retroviral vector (e.g., lentiviral vector).

[0096] Various vectors suitable for use in the disclosure, methods for inserting nucleic acid sequences encoding siRNA or shRNA molecules into vectors, and methods of delivering the vectors to the cells of interest are known in the art (see, e.g., Dornburg , Gene Therap., Vol. 2: 301-310, 1995; Eglitis, Biotechniques, Vol. 6: 608-614, 1988; Miller, HumGene Therap., Vol. 1: 5-14, 1990; Anderson, Nature, Vol. 392: 25-30, 1998; Rubinson D A et al., Nat. Genet., Vol. 33: 401-406, 2003; Brummelkamp et al., Science, Vol. 296: 550-553, 2002; Brummelkamp et al., Cancer Cell, Vol. 2: 243-247, 2002; Lee et al., Nat Biotechnol, Vol. 20: 500-505, 2002; Miyagishi et al., Nat Biotechnol, Vol. 20: 497-500, 2002; Paddison et al., GenesDev, Vol. 16: 948-958, 2002; Paul et al., Nat Biotechnol, Vol. 20: 505-508, 2002; Sui et al., Proc Natl Acad Sci USA, Vol. 99: 5515-5520, 2002; and Yu et al., Proc Natl Acad Sci USA, Vol. 99: 6047-6052, 2002).

Compositions

[0097] The disclosure also provides compositions and formulations comprising the RNAi constructs described herein and pharmaceutically acceptable carriers, excipients, or diluents. Such compositions and formulations are useful for reducing expression of SCAP in a subject in need thereof. Where clinical applications are contemplated, pharmaceutical compositions and formulations will be prepared in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[0098] The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier, excipient, or diluent" includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, etc., acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the RNAi constructs of the present disclosure, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or RNAi constructs of the compositions.

[0099] Compositions and methods for the formulation of pharmaceutical compositions depend on several criteria, including, but not limited to, route of administration, type and extent of disease or disorder to be treated, and dose to be administered. In some embodiments, the pharmaceutical compositions are formulated based on the intended route of delivery. For instance, in certain embodiments, the pharmaceutical compositions are formulated for parenteral delivery. Parenteral forms of delivery include intravenous, intraarterial, subcutaneous, intrathecal, intraperitoneal, and intramuscular injection or infusion. In one embodiment, the pharmaceutical composition is formulated for intravenous delivery. In such an embodiment, the pharmaceutical composition may include a lipid-based delivery vehicle. In another embodiment, the pharmaceutical composition is formulated for subcutaneous delivery. In such an embodiment, the pharmaceutical composition may include a targeting ligand (e.g., GalNAc-containing ligands described herein).

[0100] In some embodiments, the pharmaceutical compositions comprise an effective amount of an RNAi construct described herein. An "effective amount" is an amount sufficient to produce a beneficial or desired clinical result. In some embodiments, an effective amount is an amount sufficient to reduce SCAP expression in hepatocytes of a subject. In some embodiments, an effective amount may be an amount sufficient to only partially reduce SCAP expression, for example, to a level comparable to expression of the wild-type SCAP allele in human heterozygotes.

[0101] An effective amount of an RNAi construct disclosed herein may be from about 0.01 mg/kg body weight to about 100 mg/kg body weight, about 0.05 mg/kg body weight to about 75mg/kg body weight, about 0.1 mg/kg body weight to about 50 mg/kg body weight, about 1 mg/kg to about 30 mg/kg body weight, about 2.5 mg/kg of body weight to about 20 mg/kg bodyweight, or about 5 mg/kg body weight to about 15 mg/kg body weight. The pharmaceutical composition comprising an effective amount of RNAi construct can be administered weekly, biweekly, monthly, quarterly, or biannually. The precise determination of what would be considered an effective amount and frequency of administration may be based on several factors, including a patient's size, age, gender, type of disorder to be treated (e.g., steatosis, NAFLD, NASH, or cirrhosis), particular RNAi construct employed, and route of administration. Estimates of effective dosages and *in vivo* half-lives for any particular RNAi construct disclosed herein can be ascertained using conventional methods and/or testing in appropriate animal models.

[0102] Colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems, including oil-in-water emulsions, micelles, mixed

micelles, and liposomes, may be used as delivery vehicles for the RNAi constructs disclosed herein or vectors encoding such constructs. Commercially available fat emulsions that are suitable for delivering the nucleic acids include INTRALIPID®, LIPOSYN®, LIPOSYN®II, LIPOSYN®III, NUTRILIPID, and other similar lipid emulsions. A preferred colloidal system for use as a delivery vehicle *in vivo* is a liposome (i.e., an artificial membrane vesicle). The RNAi constructs may be encapsulated within liposomes, such as cationic liposomes. Alternatively, RNAi constructs disclosed herein may be complexed to lipids, such as cationic lipids. Suitable lipids and liposomes include neutral (e.g., dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), and dipalmitoyl phosphatidylcholine (DPPC)), distearolyphosphatidyl choline), negative (e.g., dimyristoylphosphatidyl glycerol (DMPG)), and cationic (e.g., dioleoyltetramethylaminopropyl (DOTAP) and dioleoylphosphatidyl ethanolamine (DOTMA)). The preparation and use of such colloidal dispersion systems is well known in the art. Exemplary formulations also are disclosed in, e.g., U.S. Patents 5,783,565; 5,837,533; 5,981,505; 6,127,170; 6,217,900; 6,379,965; 6,383,512; 6,747,014; 7,202,227; and WO 03/093449.

[0103] In some embodiments, the RNAi constructs disclosed herein are fully encapsulated in a lipid formulation, e.g., to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle. As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a noncationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). SNALPs and SPLPs are exceptionally useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous injection and accumulate at distal sites (e.g., sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The nucleic acid-lipid particles typically have a mean diameter of about 50 nm to about 150 nm, about 60 nm to about 130 nm, about 70 nm to about 110 nm, or about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids present in the nucleic acid-lipid particles desirably are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Patents 5,976,567; 5,981,501; 6,534,484; 6,586,410; and 6,815,432; and PCT Publication No. WO 96/40964.

[0104] Pharmaceutical compositions suitable for injections include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of

sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by using a coating (such as lecithin), by maintaining the required particle size (in the case of dispersion), and/or by using surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, such as, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, isotonic agents (e.g., sugars or sodium chloride) may be included in the composition. Prolonged absorption of the injectable compositions can be brought about by including absorption-delaying agents, such as, for example, aluminum monostearate and gelatin.

[0105] Sterile injectable solutions may be prepared by incorporating an appropriate amount of the RNAi construct (alone or complexed with a ligand) into a solvent along with any other ingredients (such as described above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suitable methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0106] The compositions provided herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include, for example, acid addition salts (formed with free amino groups) derived from inorganic acids (e.g., hydrochloric or phosphoric acids), or from organic acids (e.g., acetic, oxalic, tartaric, mandelic, and the like). Salts formed with free carboxyl groups can also be derived from inorganic bases (e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides) or from organic bases (e.g., isopropylamine, trimethylamine, histidine, procaine, and the like).

[0107] For parenteral administration in an aqueous solution, for example, a solution generally is suitably buffered, and a liquid diluent is first rendered isotonic with, e.g., sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. Sterile aqueous media desirably are employed as is known to those of skill in the art. By way of illustration, a single dose may be

dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA standards. In certain embodiments of the disclosure, a pharmaceutical composition comprises or consists of a sterile saline solution and an RNAi construct described herein. In other embodiments, a pharmaceutical composition comprises or consists of an RNAi construct described herein and sterile water (e.g., water for injection, WFI). In still other embodiments, a pharmaceutical composition comprises or consists of an RNAi construct described herein and phosphate-buffered saline (PBS).

[0108] In some embodiments, the pharmaceutical compositions are packaged with or stored within a device for administration. Devices for injectable formulations include, but are not limited to, injection ports, pre-filled syringes, auto injectors, injection pumps, on-body injectors, and injection pens. Devices for aerosolized or powder formulations include, but are not limited to, inhalers, insufflators, aspirators, and the like. Thus, the present disclosure includes administration devices comprising a pharmaceutical composition for treating or preventing one or more of the disorders described herein.

Methods for Inhibiting SCAP Expression

[0109] The present disclosure also provides methods of inhibiting expression of a SCAP gene in a cell. The methods include contacting a cell with an RNAi construct, e.g., double-stranded RNAi construct, in an amount effective to inhibit expression of SCAP in the cell. Contacting a cell with an RNAi construct, e.g., a double-stranded RNAi construct, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the RNAi construct includes contacting a cell or group of cells within a subject, e.g., a human subject, with the RNAi construct. Combinations of *in vitro* and *in vivo* methods of contacting a cell also are within the scope of the present disclosure.

[0110] The present disclosure provides methods for reducing or inhibiting expression of SCAP in a subject in need thereof as well as methods of treating or preventing conditions, diseases, or disorders associated with SCAP expression or activity. A "condition, disease, or disorder associated with SCAP expression" refers to conditions, diseases, or disorders in which SCAP expression levels are altered or where elevated expression levels of SCAP are associated with an increased risk of developing the condition, disease, or disorder.

[0111] Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, e.g., a tri-antennary GalNAc ligand (GalNAc3), or any other ligand that directs the RNAi construct to a site of interest.

[0112] In one embodiment, contacting a cell with an RNAi includes "introducing" or "delivering the RNAi into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an RNAi can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. For *in vivo* introduction, for example, RNAi can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell may be accomplished using methods known in the art, such as electroporation and lipofection. Additional approaches are described herein below and/or are known in the art.

[0113] The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

[0114] The phrase "inhibiting expression of a SCAP" is intended to refer to inhibition of expression of any SCAP gene (such as, e.g., a mouse SCAP gene, a rat SCAP gene, a monkey SCAP gene, or a human SCAP gene) as well as variants or mutants of a SCAP gene. Thus, the SCAP gene may be a wild-type SCAP gene, a mutant SCAP gene, or a transgenic SCAP gene in the context of a genetically manipulated cell, group of cells, or organism.

[0115] "Inhibiting expression of a SCAP gene" includes any level of inhibition of a SCAP gene, e.g., at least partial suppression of the expression of a SCAP gene. The expression of the SCAP gene may be assessed based on the level, or the change in the level, of any variable associated with SCAP gene expression, e.g., SCAP mRNA level or SCAP protein level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

[0116] Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with SCAP expression compared with a control level. The control level may be any type of control level that is utilized in the art, e.g., a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, e.g., buffer only control or inactive agent control). In some embodiments, expression of a SCAP gene is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%,at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about

60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

[0117] Inhibition of the expression of a SCAP gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a SCAP gene is transcribed and which has or have been treated (e.g., by contacting the cell or cells with an RNAi construct disclosed herein, or by administering an RNAi construct disclosed herein to a subject in which the cells are or were present), such that the expression of a SCAP gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). Inhibition may be assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(mRNA\ in\ control\ cells) - (mRNA\ in\ treated\ cells)}{mRNA\ in\ control\ cells} \times 100\%$$

[0118] Alternatively, inhibition of the expression of a SCAP gene may be assessed in terms of a reduction of a parameter that is functionally linked to SCAP gene expression, e.g., SCAP protein expression or SREBP protein family activities. SCAP gene silencing may be determined in any cell expressing SCAP, either endogenously or recombinantly, by any assay known in the art.

[0119] Inhibition of the expression of a SCAP protein may be manifested by a reduction in the level of the SCAP protein that is expressed by a cell or group of cells (e.g., the level of protein expressed in a sample obtained from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

[0120] A control cell or group of cells that may be used to assess the inhibition of the expression of a SCAP gene includes a cell or group of cells that has not yet been contacted with an RNAi construct disclosed herein. For example, the control cell or group of cells may be derived from an individual subject (e.g., a human or animal subject) prior to treatment of the subject with an RNAi construct.

[0121] The level of SCAP mRNA that is expressed by a cell or group of cells, or the level of circulating SCAP mRNA, may be determined using any method known in the art for assessing mRNA expression, such as those mentioned above. In some embodiments, the level of expression of SCAP in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., mRNA of the SCAP gene. In this regard, for example, RNA may be extracted from cells using RNA extraction techniques including, for example, acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen), or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton et al., Nuc. Acids Res., 12:7035), northern blotting, *in situ* hybridization, and microarray analysis. Circulating SCAP mRNA may be detected using methods described in WO 2012/177906.

[0122] In one embodiment, the level of expression of SCAP is determined using a nucleic acid probe. The term "probe," as used herein, refers to any molecule that is capable of selectively binding to a specific SCAP sequence. Probes can be synthesized by one of skill in the art or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

[0123] Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses, and probe arrays. One method for the determination of mRNA levels involves contacting isolated mRNA with a nucleic acid molecule (probe) that can hybridize to SCAP mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of SCAP mRNA.

[0124] An alternative method for determining the level of expression of SCAP in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, e.g., by RT-PCR (see, e.g., U.S. Patent 4,683,202), ligase chain reaction (Barany (1991) Proc. Natl. Acad. Sci. USA 88: 189-193), self-sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87: 1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86: 1173-1177), Q-Beta

Replicase (Lizardi et al. (1988) Bio/Technology 6: 1197), rolling circle replication (Lizardi et al., *supra*; and U.S Patent 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In some aspects of the disclosure, the level of expression of SCAP may be determined by quantitative fluorogenic RT-PCR {i.e., the TAQMAN™ System). The expression levels of SCAP mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids) (see, e.g., U.S. Patents 5,445,934; 5,677,195; 5,770,722; 5,744,305; and 5,874,219). The determination of SCAP expression level may also comprise using nucleic acid probes in solution. In certain embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR).

[0125] The level of SCAP protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, Western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, etc.

[0126] In some embodiments, the efficacy of the methods disclosed herein can be monitored by detecting or monitoring a reduction in a symptom of a SCAP-associated disease, such as gastrointestinal pain, difficulty breathing, high blood pressure, or swelling of the extremities, face, larynx, upper respiratory tract, abdomen, trunk, and genitals. These symptoms may be assessed *in vitro* or *in vivo* using any method known in the art.

[0127] In some embodiments, the RNAi construct or a composition comprising the RNAi construct is administered to a subject such that the RNAi construct is delivered to a specific site within the subject. The inhibition of expression of SCAP may be assessed using measurements of the level or change in the level of SCAP mRNA or SCAP protein in a sample derived from fluid or tissue from the specific site within the subject. In some embodiments, the RNAi construct may be delivered to a site such as the liver, choroid plexus, retina, and pancreas. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites. The site may also include cells that express a particular type of receptor.

Methods of Treating or Preventing SCAP-Associated Diseases

[0128] The present disclosure provides therapeutic and prophylactic methods which include administering to a subject with a SCAP-associated disease, disorder, and/or condition, or prone to developing a SCAP-associated disease, disorder, and/or condition, an RNAi construct, compositions (e.g., pharmaceutical compositions) comprising an RNAi construct, or vectors comprising an RNAi construct as described herein. Non-limiting examples of SCAP-associated diseases include, for example, fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), cirrhosis of the liver, accumulation of fat in the liver, inflammation of the liver, hepatocellular necrosis, liver fibrosis, obesity, nonalcoholic fatty liver disease (NAFLD), hypertriglyceridemia, and hyperlipidemia. In one embodiment, the SCAP-associated disease is NAFLD. In another embodiment, the SCAP-associated disease is NASH. In another embodiment, the SCAP-associated disease is fatty liver (steatosis). In another embodiment, the SCAP-associated disease is insulin resistance.

[0129] In certain embodiments, the present disclosure provides a method for reducing the expression of SCAP in a patient in need thereof comprising administering to the patient any of the RNAi constructs described herein. The term "patient," as used herein, refers to a mammal, including humans, and can be used interchangeably with the term "subject." The expression level of SCAP in hepatocytes in the patient desirably is reduced following administration of the RNAi construct as compared to the SCAP expression level in a patient not receiving the RNAi construct.

[0130] The methods disclosed herein are useful for treating a subject having a SCAP-associated disease, e.g., a subject that would benefit from reduction in SCAP gene expression and/or SCAP protein production. In one aspect, the present disclosure provides methods of reducing the level of SCAP gene expression in a subject having nonalcoholic fatty liver disease (NAFLD). In another aspect, the present disclosure provides methods of reducing the level of SCAP protein in a subject with NAFLD.

[0131] The treatment methods (and uses) disclosed herein include administering to the subject, e.g., a human, a therapeutically effective amount of the disclosed RNAi construct targeting a SCAP gene, a pharmaceutical composition comprising the RNAi construct, or a vector comprising the RNAi construct.

[0132] In one aspect, the disclosure provides methods of preventing at least one symptom in a subject having NAFLD, e.g., the presence of elevated hedgehog signaling pathways, fatigue, weakness, weight loss, loss of appetite, nausea, abdominal pain, spider-like blood vessels,

yellowing of the skin and eyes (jaundice), itching, fluid buildup and swelling of the legs (edema), abdomen swelling (ascites), and mental confusion. The methods include administering to the subject a prophylactically effective amount of the RNAi construct, e.g., siRNA, pharmaceutical compositions comprising the RNAi construct, or vectors encoding the RNAi construct, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in SCAP gene expression. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (e.g., prevention of disease onset).

[0133] In another aspect, the present disclosure provides uses of a therapeutically effective amount of an RNAi construct disclosed herein for treating a subject, e.g., a subject that would benefit from a reduction and/or inhibition of SCAP gene expression. In a further aspect, the present disclosure provides uses of an RNAi construct, e.g., a siRNA, targeting an SCAP gene or pharmaceutical composition comprising an RNAi construct targeting an SCAP gene in the manufacture of a medicament for treating a subject, e.g., a subject that would benefit from a reduction and/or inhibition of SCAP gene expression and/or SCAP protein production, such as a subject having a disorder that would benefit from reduction in SCAP gene expression, e.g., a SCAP-associated disease.

[0134] The disclosure provides uses of an RNAi construct, e.g., a siRNA, disclosed herein for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of SCAP gene expression and/or SCAP protein production. For example, the disclosure provides uses of the RNAi construct described herein, compositions comprising same, and vectors comprising same, in the treatment of NAFLD.

[0135] In a further aspect, the present disclosure provides uses of the disclosed RNAi construct, compositions comprising same, or a vector comprising same, in the manufacture of a medicament for preventing at least one symptom in a subject suffering from a disorder that would benefit from a reduction and/or inhibition of SCAP gene expression and/or SCAP protein production, such as a SCAP-associated disease.

[0136] In one embodiment, an RNAi construct targeting SCAP is administered to a subject having a SCAP-associated disease, e.g., nonalcoholic fatty liver disease (NAFLD), such that the expression of a SCAP gene, e.g., in a cell, tissue, blood or other tissue or fluid of the subject is reduced by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%,

70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more when the RNAi construct is administered to the subject.

[0137] The methods and uses disclosed herein include administering a composition described herein such that expression of the target SCAP gene is decreased for any suitable amount of time, such as for about 1, 2, 3, 4 5, 6, 7, 8, 12, 16, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, or about 80 hours. In one embodiment, expression of the target SCAP gene is decreased for an extended duration, e.g., at least about two, three, four, five, six, seven days or more, e.g., about one week, two weeks, three weeks, or about four weeks or longer.

[0138] Administration of the RNAi construct according to the methods and uses disclosed herein may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with a SCAP-associated disease, e.g., NAFLD. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%. Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of NAFLD may be assessed, for example, by periodic monitoring of NAFLD symptoms, liver fat levels, or expression of downstream genes. Comparison of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. In connection with the administration of an RNAi targeting SCAP or pharmaceutical composition thereof, "effective against" an SCAP -associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating NAFLD and/or an SCAP -associated disease and the related causes.

[0139] A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or

more can be indicative of effective treatment. Efficacy for a given RNAi construct or formulation of that construct can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

[0140] Subjects can be administered any therapeutically effective amount of the RNAi construct. Exemplary ranges of therapeutically effective amounts of the RNAi construct include, but are not limited to, from about 0.01 mg/kg body weight to about 100 mg/kg body weight, about 0.05 mg/kg body weight to about 75mg/kg body weight, about 0.1 mg/kg body weight to about 50 mg/kg body weight, about 1 mg/kg to about 30 mg/kg body weight, or about 2.5 mg/kg of body weight to about 20 mg/kg bodyweight. Values and ranges intermediate to the recited ranges also are encompassed by the present disclosure.

[0141] Administration of the RNAi construct, or a composition comprising same, can reduce the presence of SCAP protein levels, e.g., in a cell, tissue, blood, urine or other compartment of the patient by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31 %, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more.

[0142] Before administration of a full dose of the RNAi, patients can be administered a smaller dose, such as a 5% infusion, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (e.g., TNF-alpha or INF-alpha) levels.

[0143] Owing to the inhibitory effects on SCAP expression, a composition according to the disclosure or a pharmaceutical composition prepared therefrom can enhance the quality of life of a patient suffering from a SCAP-associated disease (e.g., NAFLD).

[0144] An RNAi disclosed herein may be administered in "naked" form, where the modified or unmodified RNAi construct is directly suspended in aqueous or suitable buffer solvent, as a "free RNAi." A free RNAi is administered in the absence of a pharmaceutical composition. The free RNAi may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the

buffer solution is phosphate buffered saline (PBS). The pH and osmolality of the buffer solution containing the RNAi can be adjusted such that it is suitable for administering to a subject.

[0145] Alternatively, an RNAi construct disclosed herein may be administered as a pharmaceutical composition, such as a liposomal formulation.

[0146] Subjects that would benefit from a reduction and/or inhibition of SCAP gene expression are those having nonalcoholic fatty liver disease (NAFLD) and/or another SCAP-associated disease or disorder as described herein or otherwise known in the art.

[0147] The disclosure further provides methods and uses of an RNAi construct or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of SCAP gene expression, e.g., a subject having a SCAP-associated disease, in combination with other pharmaceuticals and/or other therapeutic methods, e.g., with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

[0148] For example, in certain embodiments, an RNAi construct targeting a SCAP gene is administered in combination with, e.g., an agent useful in treating a SCAP-associated disease. For example, additional therapeutics and therapeutic methods suitable for treating a subject that would benefit from reduction in SCAP expression, e.g., a subject having a SCAP-associated disease, include an RNAi construct targeting a different portion of the SCAP gene, a therapeutic agent, and/or procedures for treating a SCAP-associated disease or a combination of any of the foregoing.

[0149] In certain embodiments, a first RNAi construct targeting a portion of a SCAP gene is administered in combination with a second RNAi construct targeting a different portion of the SCAP gene. For example, the first RNAi construct may comprise a first sense strand and a first antisense strand forming a double stranded region, wherein substantially all of the nucleotides of said first sense strand and substantially all of the nucleotides of the first antisense strand are modified nucleotides, wherein said first sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker; and the second RNAi construct may comprise a second sense strand and a second antisense strand forming a double stranded region, wherein substantially all of the nucleotides of the second sense strand and substantially all of the nucleotides of the second antisense strand are modified nucleotides, wherein the second sense strand is conjugated to a ligand attached at the 3'-terminus, and wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker. In one embodiment, all of the nucleotides of the first and second sense strand and/or all of the nucleotides of the first and

second antisense strand comprise a modification. The modified nucleotides may be any one or combination of the modified nucleotides described herein.

[0150] In other embodiments, a first RNAi construct targeting a SCAP gene is administered in combination with a second RNAi construct targeting a gene that is different from the SCAP gene. For example, the RNAi construct targeting the SCAP gene may be administered in combination with an RNAi construct targeting the patatin like phospholipase domain containing 3 (PNPLA3) gene. The I148M mutant PNPLA3 protein is a therapeutic target with strong human genetic validation for the treatment of non-alcoholic steatohepatitis (NASH). PNPLA3 I148M expression leads to accumulation of excess liver fat and drives non-alcoholic fatty liver disease (NAFLD)-associated phenotypes with increased incidence, progression, and severity. The first RNAi construct targeting a SCAP gene and the second RNAi construct targeting a different gene, e.g., the PNPLA3 gene, may be administered as parts of the same pharmaceutical composition. Alternatively, the first RNAi construct targeting a SCAP gene and the second RNAi construct targeting a different gene, e.g., the PNPLA3 gene, may be administered as parts of different pharmaceutical compositions.

[0151] The RNAi construct and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, e.g., parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

[0152] The present disclosure also provides methods of using an RNAi construct disclosed herein and/or a composition containing an RNAi construct to reduce and/or inhibit SCAP expression (gene or protein expression) in a cell. In yet other aspects, use of an RNAi construct and/or a composition comprising an RNAi construct disclosed herein for the manufacture of a medicament for reducing and/or inhibiting SCAP gene expression in a cell are provided. In still other aspects, the present disclosure provides an RNAi and/or a composition comprising an RNAi construct described herein for use in reducing and/or inhibiting SCAP protein production in a cell. In yet other aspects, use of an RNAi construct and/or a composition comprising an RNAi construct described herein for the manufacture of a medicament for reducing and/or inhibiting SCAP protein production in a cell are provided. The methods and uses include contacting the cell with an RNAi construct, e.g., a siRNA, disclosed herein and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcript of a SCAP gene, thereby inhibiting expression of the SCAP gene or inhibiting SCAP protein production in the cell. Reduction in gene expression can be assessed by any methods known in the art or described herein for determining mRNA or protein levels.

[0153] In the methods and uses disclosed herein the cell may be contacted *in vitro* or *in vivo*, i.e., the cell may be outside (e.g., in cell culture) or within a subject. A cell suitable for treatment using the methods disclosed herein may be any cell that expresses an SCAP gene, e.g., a cell from a subject having NAFLD or a cell comprising an expression vector comprising a SCAP gene or portion of a SCAP gene. A suitable cell for use in the disclosed methods includes, for example, a mammalian cell, e.g., a primate cell (such as a human cell or a non-human primate cell, e.g., a monkey cell or a chimpanzee cell), a non-primate cell (such as a cow cell, a pig cell, a camel cell, a llama cell, a horse cell, a goat cell, a rabbit cell, a sheep cell, a hamster, a guinea pig cell, a cat cell, a dog cell, a rat cell, a mouse cell, a lion cell, a tiger cell, a bear cell, or a buffalo cell), a bird cell (e.g., a duck cell or a goose cell), or a whale cell. In one embodiment, the cell is a human cell.

[0154] SCAP gene expression may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

[0155] SCAP protein production may be inhibited in the cell by at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or about 100%.

[0156] The *in vivo* methods and uses disclosed herein may include administering to a subject a composition containing an RNAi construct, where the RNAi construct includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the SCAP gene of the subject. When the organism to be treated is a human, the composition can be administered by any means known in the art including, but not limited to subcutaneous, intravenous, oral, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal, and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the

compositions are administered by subcutaneous or intravenous infusion or injection. In one embodiment, the compositions are administered by subcutaneous injection.

[0157] In some embodiments, the administration is via a depot injection. A depot injection may release the RNAi construct in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, e.g., a desired inhibition of SCAP, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In some embodiments, the depot injection is a subcutaneous injection.

[0158] In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the RNAi construct to the subject.

[0159] The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

[0160] The methods and uses include administering to the mammal, e.g., a human, a composition comprising an RNAi construct, e.g., an siRNA, that targets a SCAP gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the SCAP gene, thereby inhibiting expression of the SCAP gene in the mammal. Reduction in gene expression and/or protein expression can be assessed in a sample obtained from the RNAi construct-administered subject by any method known in the art or described herein. In one embodiment, a tissue sample serves as the tissue material for monitoring the reduction in SCAP gene and/or protein expression. In another embodiment, a blood sample serves as the tissue material for monitoring the reduction in SCAP gene and/or protein expression.

[0161] In some embodiments, verification of RISC-mediated cleavage of a target mRNA (e.g., SCAP mRNA) *in vivo* following administration of an RNAi construct may be assessed by performing 5 '-RACE or modifications of the protocol as known in the art (Lasham A et al., (2010) Nucleic Acid Res., 38 (3) p-el9; and Zimmermann et al. (2006) Nature 441: 111-4).

[0162] It is understood that all ribonucleic acid sequences disclosed herein can be converted to deoxyribonucleic acid sequences by substituting a thymine base for a uracil base in the

sequence. Likewise, all deoxyribonucleic acid sequences disclosed herein can be converted to ribonucleic acid sequences by substituting a uracil base for a thymine base in the sequence. Deoxyribonucleic acid sequences, ribonucleic acid sequences, and sequences containing mixtures of deoxyribonucleotides and ribonucleotides of all sequences disclosed herein are encompassed by the present disclosure.

[0163] Additionally, any nucleic acid sequences disclosed herein may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as "RNA" or "DNA" to describe modified polynucleotides is, in certain instances, arbitrary. For example, a polynucleotide comprising a nucleotide having a 2'-OH substituent on the ribose sugar and a thymine base could be described as a DNA molecule having a modified sugar (2'-OH for the natural 2'-H of DNA) or as an RNA molecule having a modified base (thymine (methylated uracil) for natural uracil of RNA).

[0164] Accordingly, nucleic acid sequences provided herein, including but not limited to those set forth in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to, such nucleic acids having modified nucleobases. By way of a further example and without limitation, a polynucleotide having the sequence "ATCGATCG" encompasses any polynucleotides having such a sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence "AUCGAUCG" and those having some DNA bases and some RNA bases such as "AUCGATCG," and polynucleotides having other modified bases, such as "ATmeCGAUCG," wherein meC indicates a cytosine base comprising a methyl group at the 5-position.

[0165] The following examples, including the experiments conducted and the results achieved, are provided for illustrative purposes only and are not to be construed as limiting the scope of the appended claims.

EXAMPLES

[0166] All animal experiments described herein were approved by the Institutional Animal Care and Use Committee (IACUC) of Amgen and cared for in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council (U.S.)). Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (U.S.), and National Academies Press (U.S.) (2011) *Guide for the care and use of laboratory animals. 8th Ed.*, National Academies Press, Washington, D.C. Mice were single-housed in an air-conditioned room at 22±2°C with a twelve-hour light; twelve-hour

darkness cycle (0600-1800 hours). Animals had *ad libitum* access to a regular chow diet (Envigo, 2920X, or a diet as stated otherwise) and to water (reverse osmosis-purified) via automatic watering system, unless otherwise indicated. At termination, blood was collected by cardiac puncture under deep anesthesia, and then, following Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines, euthanized by a secondary physical method.

EXAMPLE 1: Selection, Design and Synthesis of Modified SCAP siRNA molecules

[0167] The identification and selection of optimal sequences for therapeutic siRNA molecules targeting the SREBP Cleavage Activating Protein (SCAP) gene were identified using bioinformatics analysis of a human SCAP transcript. The siRNA triggers were prepared as chemically modified siRNA duplexes consisting of a sense (passenger) and anti-sense (guide) strand. Both strands were 18-23 nucleotides in length with a two base pair 3' overhang. The siRNAs were modified such that the natural 2'-OH in the ribose of each nucleotide was substituted with either a 2'-OMe or 2'-F group, and two phosphodiester internucleotide linkages at each end of both strands were replaced with phosphorothioates to reduce degradation by exonucleases. The unmodified and modified antisense and sense siRNA sequences generated are shown in Table 1.

[0168] The nucleotide sequences in Table 1 and other parts of the application are listed according to the following notations: A, U, G, and C =corresponding ribonucleotide; dT = deoxythymidine; dA = deoxyadenosine; dC = deoxycytidine; dG = deoxyguanosine; invAb = inverted Abasic; invDT = inverted deoxythymidine; invDA = inverted deoxyadenosine; invDC = inverted deoxycytidine; invDG = inverted deoxyguanosine; a, u, g, and c = corresponding 2'-O-methyl ribonucleotide; Af, Uf, Gf, and Cf = corresponding 2'-deoxy-2'-fluoro ("2'-fluoro") ribonucleotide; Ab = Abasic; MeO-I = 2' methoxy inosine; GNA = glycol nucleic acid; sGNA = glycol nucleic acid with 3' phosphorothioate; LNA = locked nucleic acid. Insertion of an "s" in the sequence indicates that the two adjacent nucleotides are connected by a phosphorothiodiester group (e.g. a phosphorothioate internucleotide linkage). Unless indicated otherwise, all other nucleotides are connected by 3'-5' phosphodiester groups.

Table 1. Unmodified and Modified siRNA Sequences Directed to SCAP

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
1	ggaccuGfuGfGfAfAfuucacscs[invAb]	1	ggaccuguggaauucacc	295	usGfsgugaAfuuccAfcAfgguccsusu	148	uggugaauucccacagguccuu	442
2	ggaccuGfuGfGfAfAfuucacscs[invAb]	2	ggaccuguggaauucacc	296	usGfsgUfgAfAfuuccAfcAfgguccsusu	149	uggugaauucccacagguccuu	443
3	ggacCfuGfUfGfGfaauucaccs[invAb]	3	ggaccuguggaauucacc	297	usGfsgugaAfuuccacAfgGfuccsusu	150	uggugaauucccacagguccuu	444
4	ggacCfuGfuGfgaauucaccs[invAb]	4	ggaccuguggaauucacc	298	usGfsgugaauuccAfcAfgGfuccsusu	151	uggugaauucccacagguccuu	445
5	ggaccuGfuGfGfAfAfuucaccaus[invAb]	5	ggaccuguggaauucaccau	299	usGfsgugaAfuuccAfcAfgguccsusu	152	uggugaauucccacagguccuu	446
6	ggaccuGfuGfGfAfAfuucaccs[invAb]	6	ggaccuguggaauucacc	300	usGfsgugaAfuuccAfcAfggusesc	153	uggugaauucccacaggucc	447
7	gcgagaUfuUfUfCfCfccuacscs[invAb]	7	gcgagauuuuccccuacc	301	asGfsguagGfggaaAfaUfcucgcsusu	154	agguaggggaaaaucucgcuu	448
8	gcgagaUfuUfUfCfCfccuacscs[invAb]	8	gcgagauuuuccccuacc	302	asGfsgUfaGfGfggaaAfaUfcucgcsusu	155	agguaggggaaaaucucgcuu	449
9	gcgaGfaUfUfUfUfccccuaccs[invAb]	9	gcgagauuuuccccuacc	303	asGfsguagGfggaaaaUfcUfcgcsusu	156	agguaggggaaaaucucgcuu	450
10	gcgaGfaUfuUfucccctuaccs[invAb]	10	gcgagauuuuccccuacc	304	asGfsguaggggaaAfaUfcUfcgcsusu	157	agguaggggaaaaucucgcuu	451
11	gcgagaUfuUfUfCfCfccuaccs[invAb]	11	gcgagauuuuccccuacc	305	asGfsguagGfggaaAfaUfcucsgsc	158	agguaggggaaaaucucgc	452
12	gcgagaUfuUfUfCfCfccuaccuus[invAb]	12	gcgagauuuuccccuaccuu	306	asGfsguagGfggaaAfaUfcucgcsusu	159	agguaggggaaaaucucgcuu	453

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
13	ccugucCfaUfUfUfGfAfcau ucsgs[invAb]	13	ccuguccauugaca uucg	307	asCfsgaauGfucaaUfgG facaggsusu	160	acgaaugucaa uggacagguu	454
14	ccugucCfaUfUfUfGfAfcau ucsgs[invAb]	14	ccuguccauugaca uucg	308	asCfsgAfaUfGfucaaUf gGfacaggsusu	161	acgaaugucaa uggacagguu	455
15	ccugUfcCfAfUfUfgacau ucgs[invAb]	15	ccuguccauugaca uucg	309	asCfsgaauGfucaaugGfa Cfaggsusu	162	acgaaugucaa uggacagguu	456
16	ccugUfcCfaUfugacauuc gs[invAb]	16	ccuguccauugaca uucg	310	asCfsgaaugucaaUfgGfa Cfaggsusu	163	acgaaugucaa uggacagguu	457
17	ccugucCfaUfUfGfAfcau ucguus[invAb]	17	ccuguccauugaca uucguu	311	asCfsgaauGfucaaUfgG facaggsusu	164	acgaaugucaa uggacagguu	458
18	ccugucCfaUfUfGfAfcau ucgs[invAb]	18	ccuguccauugaca uucg	312	asCfsgaauGfucaaUfgG facasgsg	165	acgaaugucaa uggacagg	459
19	guccauUfgAfCfAfUfucg ccsgs[invAb]	19	guccauugacauuc gccg	313	asCfsggcgAfauguCfaA fuggacsusu	166	acggcgaaugu caauggacuu	460
20	cuguccauUfgAfCfAfUfu cgccgs[invAb]	20	cuguccauugacau ucgccg	314	asCfsggcgAfauguCfaA fuggacagsusu	167	acggcgaaugu caauggacagu u	461
21	cuguccAfuUfGfAfCfauu cgccgs[invAb]	21	cuguccauugacau ucgccg	315	asCfsggcgAfaugucaAf uGfgacagsusu	168	acggcgaaugu caauggacagu u	462
22	cuguccauUfgAfCfAfUfu cgccgs[invAb]	22	cuguccauugacau ucgccg	316	asCfsggcgAfauguCfaA fuggacsasg	169	acggcgaaugu caauggacag	463
23	cuguccAfuUfgAfcauucg ccgs[invAb]	23	cuguccauugacau ucgccg	317	asCfsggcgaauguCfaAf uGfgacagsusu	170	acggcgaaugu caauggacagu u	464
24	guccauUfgAfCfAfUfucg ccguus[invAb]	24	guccauugacauuc gccguu	318	asCfsggcgAfauguCfaA fuggacsasg	171	acggcgaaugu caauggacag	465
25	ggaaagAfgCfCfGfAfgua ucsus[invAb]	25	ggaaagagccgagu aucu	319	asAfsgauaCfucggCfuC fuuuccsusu	172	aagauacucgg cucuuuccuu	466

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
26	ggaaagAfgCfCfGfAfgua ucsus[invAb]	26	ggaaagagccgagu aucu	320	asAfsgAfuAfCfucggCf uCfuuuccsusu	173	aagauacucgg cucuuuccuu	467
27	ggaaAfgAfgCfcgaguauc us[invAb]	27	ggaaagagccgagu aucu	321	asAfsgauacucggCfuCf uUfuccsusu	174	aagauacucgg cucuuuccuu	468
28	ggaaagAfgCfCfGfAfgua ucuuus[invAb]	28	ggaaagagccgagu aucuuu	322	asAfsgauaCfucggCfuC fuuuccsusu	175	aagauacucgg cucuuuccuu	469
29	ggaaagAfgCfCfGfAfgua ucus[invAb]	29	ggaaagagccgagu aucu	323	asAfsgauaCfucggCfuC fuuuscsc	176	aagauacucgg cucuuucc	470
30	ggaaAfgAfGfCfCfgagua ucus[invAb]	30	ggaaagagccgagu aucu	324	asAfsgauaCfucggcuCf uUfuccsusu	177	aagauacucgg cucuuuccuu	471
31	aaagagCfcGfAfGfUfauc uuscs[invAb]	31	aaagagccgaguau cuuc	325	asGfsaagaUfacucGfgC fucuuususu	178	agaagauacuc ggcucuuuuu	472
32	ggaaagagCfcGfAfGfUfa ucuucs[invAb]	32	ggaaagagccgagu aucuuc	326	asGfsaagaUfacucGfgC fucuuuccsusu	179	agaagauacuc ggcucuuuccu u	473
33	ggaaagAfgCfCfGfAfgua ucuucs[invAb]	33	ggaaagagccgagu aucuuc	327	asGfsaagaUfacucggCfu Cfuuuccsusu	180	agaagauacuc ggcucuuuccu u	474
34	ggaaagagCfcGfAfGfUfa ucuucs[invAb]	34	ggaaagagccgagu aucuuc	328	asGfsaagaUfacucGfgC fucuuuscsc	181	agaagauacuc ggcucuuucc	475
35	ggaaagAfgCfcGfaguauc uucs[invAb]	35	ggaaagagccgagu aucuuc	329	asGfsaagauacucGfgCfu Cfuuuccsusu	182	agaagauacuc ggcucuuuccu u	476
36	aaagagCfcGfAfGfUfauc uucuus[invAb]	36	aaagagccgaguau cuucuu	330	asGfsaagaUfacucGfgC fucuuuscsc	183	agaagauacuc ggcucuuucc	477
37	gcggcgAfgAfUfUfUfucc ccsus[invAb]	37	gcggcgagauuuuc cccu	331	usAfsggggAfaaauCfuC fgccgcsusu	184	uaggggaaaau cucgccgcuu	478
38	ggcgagAfuUfUfUfCfccc uascs[invAb]	38	ggcgagauuuuccc cuac	332	asGfsuaggGfgaaaAfuC fucgccsusu	185	aguaggggaaa aucucgccuu	479

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
39	aagauaUfcUfCfGfGfgcc uuscs[invAb]	39	aagauaucucgggc cuuc	333	asGfsaaggCfccgaGfaU faucuususu	186	agaaggcccga gauaucuuuu	480
40	cgggccUfuCfUfAfCfaac casus[invAb]	40	cgggccuucuacaa ccau	334	asAfsugguUfguagAfaG fgcccgsusu	187	aaugguuguag aaggcccguu	481
41	cugcagUfuUfAfGfAfggg acscs[invAb]	41	cugcaguuuagagg gacc	335	asGfsguccCfucuaAfaC fugcagsusu	188	aggucccucua aacugcaguu	482
42	acggggAfcCfUfGfUfuac agsas[invAb]	42	acggggaccuguua caga	336	asUfscuguAfacagGfuC fcccgususu	189	aucuguaacag guccccguuu	483
43	cggcucAfaCfGfGfUfucc cusus[invAb]	43	cggcucaacgguuc ccuu	337	asAfsagggAfaccgUfuG fagccgsusu	190	aaagggaaccg uugagccguu	484
44	uguacuGfaCfUfGfGfcag ccsgs[invAb]	44	uguacugacuggca gccg	338	asCfsggcuGfccagUfcA fguacasusu	191	acggcugccag ucaguacauu	485
45	ccccugCfaGfUfUfUfaga ggsgs[invAb]	45	ccccugcaguuuag aggg	339	usCfsccucUfaaacUfgCf aggggsusu	192	ucccucuaaacu gcagggguu	486
46	uguccaUfuGfAfCfAfuuc gcscs[invAb]	46	uguccauugacauu cgcc	340	asGfsgcgaAfugucAfaU fggacasusu	193	aggcgaauguc aauggacauu	487
47	ggcuggCfaCfCfGfUfugu cusgs[invAb]	47	ggcuggcaccguug ucug	341	asCfsagacAfacggUfgC fcagccsusu	194	acagacaacggu gccagccuu	488
48	ggcucaAfcGfGfUfUfccc uusgs[invAb]	48	ggcucaacgguucc cuug	342	usCfsaaggGfaaccGfuU fgagccsusu	195	ucaagggaacc guugagccuu	489
49	gcucaaCfgGfUfUfCfccu ugsas[invAb]	49	gcucaacgguuccc uuga	343	asUfscaagGfgaacCfgU fugagcsusu	196	aucaagggaacc guugagcuu	490
50	caacggUfuCfCfCfUfuga uusus[invAb]	50	caacgguucccuug auuu	344	asAfsaaucAfagggAfaC fcguugsusu	197	aaaaucaaggga accguuguu	491
51	acgguuCfcCfUfUfGfauu ucsus[invAb]	51	acgguucccuugau uucu	345	asAfsgaaaUfcaagGfgA faccgususu	198	aagaaaucaagg gaaccguuu	492
52	gugggaUfgUfAfCfUfgac ugsgs[invAb]	52	gugggauguacuga cugg	346	asCfscaguCfaguaCfaUf cccacsusu	199	accagucaguac aucccacuu	493

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
53	cugaaaCfuCfCfCfCfuug ccsas[invAb]	53	cugaaacucccuu gcca	347	asUfsggcaAfggggAfgU fuucagsusu	200	auggcaagggg aguuucaguu	494
54	gauccuGfaCfAfUfCfauu ggsgs[invAb]	54	gauccugacaucau uggg	348	usCfsccaaUfgaugUfcA fggaucsusu	201	ucccaaugaug ucaggaucuu	495
55	uggucc AfcGfUfGfCfacu ucsas[invAb]	55	uggucc acgugcac uuca	349	usUfsgaagUfgcacGfuG fgaccasusu	202	uugaagugcac guggaccauu	496
56	guggacUfcUfGfAfCfcgc aasas[invAb]	56	guggacucugaccg caaa	350	asUfsuugcGfgucaGfaG fuccacsusu	203	auuugcgguca gaguccacuu	497
57	ggaggaGfaUfUfGfGfugu cgscs[invAb]	57	ggaggagauuggu gucgc	351	asGfscgacAfccaaUfcUf ccuccsusu	204	agcgacaccaau cuccuccuu	498
58	aguguuUfcCfCfUfGfgca casas[invAb]	58	aguguuucccuggc acaa	352	asUfsugugCfcaggGfaA facacususu	205	auugugccagg gaaacacuuu	499
59	ggagauUfgGfUfGfUfcgc ugsas[invAb]	59	ggagauugguguc gcuga	353	asUfscagcGfacacCfaAf ucuccsusu	206	aucagcgacacc aaucuccuu	500
60	ucgcugAfgCfUfCfAfucc ccscs[invAb]	60	ucgcugagcucauc cccc	354	asGfsggggAfugagCfuC fagcgasusu	207	agggggaugag cucagcgauu	501
61	uguccc GfgGfCfAfUfucc aascs[invAb]	61	uguccc gggcauuc caac	355	asGfsuuggAfaugcCfcG fggacasusu	208	aguuggaaugc ccgggacauu	502
62	ggaguuCfuGfUfCfUfcuu ugscs[invAb]	62	ggaguucugucucu uugc	356	asGfscaaaGfagacAfgA facuccsusu	209	agcaaagagaca gaacuccuu	503
63	gagguaAfcCfUfGfGfggg ccsus[invAb]	63	gagguaaccuggg gccu	357	asAfsggccCfccagGfuU faccucsusu	210	aaggcccccagg uuaccucuu	504
64	acaccaUfcAfCfGfUfugc agscs[invAb]	64	acaccaucacguug cagc	358	asGfscugcAfacguGfaU fggugususu	211	agcugcaacgu gaugguguuu	505
65	cggcuaCfuUfCfAfCfccu agsus[invAb]	65	cggcuacuucaccc uagu	359	asAfscuagGfgugaAfgU fagccgsusu	212	aacuaggguga aguagccguu	506
66	cauugaCfcAfGfAfCfcau ggsus[invAb]	66	cauugaccagacca uggu	360	asAfsccauGfgucuGfgU fcaaugsusu	213	aaccauggucu ggucaauguu	507

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
67	aggagaUfuGfGfUfGfucgcusgs[invAb]	67	aggagauuggugucgcug	361	usCfsagcgAfcaccAfaUfcuccususu	214	ucagcgacaccaaucuccuuu	508
68	ccgccuUfcCfCfUfCfuucggsgs[invAb]	68	ccgccuucccucuucggg	362	asCfsccgaAfgaggGfaAfggcggsusu	215	acccgaagagggaaggcgguu	509
69	auugaaGfgGfGfUfGfcugugscs[invAb]	69	auugaaggggugcugugc	363	asGfscacaGfcaccCfcUfucaaususu	216	agcacagcaccccuucaauuu	510
70	ccugguccAfcGfUfGfCfacuucas[invAb]	70	ccugguccacgugcacuuca	364	usUfsgaagUfgcacGfuGfgaccaggsusu	217	uugaagugcacguggaccaggu	511
71	ccugguCfcAfCfGfUfgcacuucas[invAb]	71	ccugguccacgugcacuuca	365	usUfsgaagUfgcacguGfgAfccaggsusu	218	uugaagugcacguggaccaggu	512
72	ugguccAfcGfUfGfCfacuucaaus[invAb]	72	ugguccacgugcacuucaau	366	usUfsgaagUfgcacGfuGfgaccasgsg	219	uugaagugcacguggaccagg	513
73	ccugguccAfcGfUfGfCfacuucas[invAb]	73	ccugguccacgugcacuuca	367	usUfsgaagUfgcacGfuGfgaccasgsg	220	uugaagugcacguggaccagg	514
74	ccugguCfcAfcGfugcacuucas[invAb]	74	ccugguccacgugcacuuca	368	usUfsgaagugcacGfuGfgAfccaggsusu	221	uugaagugcacguggaccaggu	515
75	ggagauUfgGfUfGfUfcgcugsas[invAb]	75	ggagauuggugucgcuga	369	asUfscAfgCfGfacacCfaAfucuccsusu	222	aucagcgacaccaaucuccuu	516
76	acaccaUfcAfCfGfUfugcagscs[invAb]	76	acaccaucacguugcagc	370	asGfscUfgCfAfacguGfaUfggugususu	223	agcugcaacgugaugguguuu	517
77	ggagAfuUfGfGfUfgucgcugas[invAb]	77	ggagauuggugucgcuga	371	asUfscagcGfacaccaAfuCfuccsusu	224	aucagcgacaccaaucuccuu	518
78	acacCfaUfCfAfCfguugcagcs[invAb]	78	acaccaucacguugcagc	372	asGfscugcAfacgugaUfgGfugususu	225	agcugcaacgugaugguguuu	519
79	ggagAfuUfgGfugucgcugas[invAb]	79	ggagauuggugucgcuga	373	asUfscagcgacacCfaAfuCfuccsusu	226	aucagcgacaccaaucuccuu	520

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
80	acacCfaUfcAfcguugcagcs[invAb]	80	acaccaucacguugcagc	374	asGfscugcaacguGfaUfgGfugususu	227	agcugcaacgugaugguguuu	521
81	ggagauUfgGfUfGfUfcgcugas[invAb]	81	ggagauuggugucgcuga	375	asUfscagcGfacacCfaAfucuscsc	228	aucagcgacaccaaucucc	522
82	acaccaUfcAfCfGfUfugcagcs[invAb]	82	acaccaucacguugcagc	376	asGfscugcAfacguGfaUfggusgsu	229	agcugcaacgugauggugu	523
83	ggagauUfgGfUfGfUfcgcugauus[invAb]	83	ggagauuggugucgcugauu	377	asUfscagcGfacacCfaAfucuccsusu	230	aucagcgacaccaaucuccuu	524
84	acaccaUfcAfCfGfUfugcagcuus[invAb]	84	acaccaucacguugcagcuu	378	asGfscugcAfacguGfaUfggugususu	231	agcugcaacgugaugguguuu	525
85	ucgcugAfgCfUfCfAfuccccscs[invAb]	85	ucgcugagcucauccccc	379	asGfsgGfgGfAfugagCfuCfagcgasusu	232	agggggaugagcucagcgauu	526
86	ucgcUfgAfGfCfUfcaucccccs[invAb]	86	ucgcugagcucauccccc	380	asGfsggggAfugagcuCfaGfcgasusu	233	agggggaugagcucagcgauu	527
87	ucgcUfgAfgCfucaucccccs[invAb]	87	ucgcugagcucauccccc	381	asGfsggggaugagCfuCfaGfcgasusu	234	agggggaugagcucagcgauu	528
88	ucgcugAfgCfUfCfAfuccccuus[invAb]	88	ucgcugagcucauccccuu	382	asGfsggggAfugagCfuCfagcgasusu	235	agggggaugagcucagcgauu	529
89	ucgcugAfgCfUfCfAfuccccscs[invAb]	89	ucgcugagcucauccccc	383	asGfsggggAfugagCfuCfagcsgsa	236	agggggaugagcucagcga	530
90	ggcucaAfcGfGfUfUfcccuusgs[invAb]	90	ggcucaacgguucccuug	384	usCfsaAfgGfGfaaccGfuUfgagccsusu	237	ucaagggaaccguugagccuu	531
91	ggcuCfaAfCfGfGfuucccuugs[invAb]	91	ggcucaacgguucccuug	385	usCfsaaggGfaaccguUfgAfgccsusu	238	ucaagggaaccguugagccuu	532
92	ggcuCfaAfcGfguucccuugs[invAb]	92	ggcucaacgguucccuug	386	usCfsaagggaaccGfuUfgAfgccsusu	239	ucaagggaaccguugagccuu	533
93	ggcucaAfcGfGfUfUfcccuugaus[invAb]	93	ggcucaacgguucccuugau	387	usCfsaaggGfaaccGfuUfgagccsusu	240	ucaagggaaccguugagccuu	534

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
94	ggcucaAfcGfGfUfUfcccuugs[invAb]	94	ggcucaacgguucccuug	388	usCfsaaggGfaaccGfuUfgagscsc	241	ucaagggaaccguugagcc	535
95	gcggcgagAfuUfUfUfUfCfccuacs[invAb]	95	gcggcgagauuuuuccccuac	389	asGfsuaggGfgaaaAfuCfucgccgcsusu	242	aguagggaaaaucucgccgcu	536
96	cggcucaaCfgGfUfUfUfCfccuugas[invAb]	96	cggcucaacgguuccccuuga	390	asUfscaagGfgaacCfgUfugagccgsusu	243	aucaagggaaccguugagccguu	537
97	gcggcgAfgAfUfUfUfUfuccccuacs[invAb]	97	gcggcgagauuuuuccccuac	391	asGfsuaggGfgaaaauCfuCfgccgcsusu	244	aguagggaaaaucucgccgcu	538
98	cggcucAfaCfGfGfUfUfuccccuugas[invAb]	98	cggcucaacgguuccccuuga	392	asUfscaagGfgaaccgUfuGfagccgsusu	245	aucaagggaaccguugagccguu	539
99	gcggcgAfgAfuUfuuccccuacs[invAb]	99	gcggcgagauuuuuccccuac	393	asGfsuagggaaaAfuCfuCfgccgcsusu	246	aguagggaaaaucucgccgcu	540
100	cggcucAfaCfgGfuuccccuugas[invAb]	100	cggcucaacgguuccccuuga	394	asUfscaagggaacCfgUfuGfagccgsusu	247	aucaagggaaccguugagccguu	541
101	ggcgagAfuUfUfUfUfCfcccuacuus[invAb]	101	ggcgagauuuuuccccuacuu	395	asGfsuaggGfgaaaAfuCfucgccsgsc	248	aguagggaaaaucucgccgc	542
102	gcucaaCfgGfUfUfUfCfccuugauus[invAb]	102	gcucaacgguuccccuugauu	396	asUfscaagGfgaacCfgUfugagccsgsg	249	aucaagggaaccguugagccg	543
103	gcggcgagAfuUfUfUfUfCfccuacs[invAb]	103	gcggcgagauuuuuccccuac	397	asGfsuaggGfgaaaAfuCfucgccsgsc	250	aguagggaaaaucucgccgc	544
104	cggcucaaCfgGfUfUfUfCfccuugas[invAb]	104	cggcucaacgguuccccuuga	398	asUfscaagGfgaacCfgUfugagccsgsg	251	aucaagggaaccguugagccg	545
105	agaagauaUfcUfCfGfGfgfccuucs[invAb]	105	agaagauaucucgggccuuc	399	asGfsaaggCfccgaGfaUfaucuuscsu	252	agaaggcccgagauaucuucu	546

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
106	agaagauaUfcUfCfGfGfg ccuucs[invAb]	106	agaagauaucucgg gccuuc	400	asGfsaaggCfccgaGfaU faucuucususu	253	agaaggcccga gauaucuucuu u	547
107	agaagaUfaUfCfUfCfggg ccuucs[invAb]	107	agaagauaucucgg gccuuc	401	asGfsaaggCfccgagaUfa Ufcuucususu	254	agaaggcccga gauaucuucuu u	548
108	agaagaUfaUfcUfcgggcc uucs[invAb]	108	agaagauaucucgg gccuuc	402	asGfsaaggcccgaGfaUfa Ufcuucususu	255	agaaggcccga gauaucuucuu u	549
109	aagauaUfcUfCfGfGfgcc uucus[invAb]	109	aagauaucucgggc cuucuu	403	asGfsaaggCfccgaGfaU faucuuscsu	256	agaaggcccga gauaucuucu	550
110	ccugguccAfcGfUfGfCfa cuucas[invAb]	110	ccugguccacgugc acuuca	404	usUfsgaagUfgcacguGf gAfccaggsusu	257	uugaagugcac guggaccaggu u	551
111	gcggcgagAfuUfUfUfCfc ccuacs[invAb]	111	gcggcgagauuuuuc cccuac	405	asGfsuaggGfgaaaauCf uCfgccgcsusu	258	aguaggggaaa aucucgccgcu u	552
112	cggcucaaCfgGfUfUfCfc cuugas[invAb]	112	cggcucaacgguuc ccuuga	406	asUfscaagGfgaaccgUfu Gfagccgsusu	259	aucaaggaacc guugagccguu	553
113	agaagauaUfcUfCfGfGfg ccuucs[invAb]	113	agaagauaucucgg gccuuc	407	asGfsaaggCfccgagaUfa Ufcuucususu	260	agaaggcccga gauaucuucuu u	554
114	ccugguccAfcGfUfGfCfa cuucas[invAb]	114	ccugguccacgugc acuuca	408	usUfsgaagUfgcacGfuG fgAfccaggsusu	261	uugaagugcac guggaccaggu u	555
115	gcggcgagAfuUfUfUfCfc ccuacs[invAb]	115	gcggcgagauuuuuc cccuac	409	asGfsuaggGfgaaaAfuC fuCfgccgcsusu	262	aguaggggaaa aucucgccgcu u	556

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
116	cggcucaaCfgGfUfUfCfc cuugas[invAb]	116	cggcucaacgguuc ccuuga	410	asUfscaagGfgaacCfgU fuGfagccgsusu	263	aucaagggaacc guugagccguu	557
117	agaagauaUfcUfCfGfGfg ccuucs[invAb]	117	agaagauaucucgg gccuuc	411	asGfsaaggCfccgaGfaU faUfcuucususu	264	agaaggcccga gauaucuucuu u	558
118	ccugguCfcAfcGfugcacu ucas[invAb]	118	ccugguccacgugc acuuca	412	usUfsgaaGfUfgcacguG fgAfccaggsusu	265	uugaagugcac guggaccaggu u	559
119	gcggcgAfgAfuUfuucccc uacs[invAb]	119	gcggcgagauuuuc cccuac	413	asGfsuagGfGfgaaaauC fuCfgccgcsusu	266	aguaggggaaa aucucgccgcu u	560
120	cggcucAfaCfgGfuucccu ugas[invAb]	120	cggcucaacgguuc ccuuga	414	asUfscaaGfGfgaaccgU fuGfagccgsusu	267	aucaagggaacc guugagccguu	561
121	agaagaUfaUfcUfcgggcc uucs[invAb]	121	agaagauaucucgg gccuuc	415	asGfsaagGfCfccgagaU faUfcuucususu	268	agaaggcccga gauaucuucuu u	562
122	ggagauUfgGfUfGfUfcgc ugas[invAb]	122	ggagauugguguc gcuga	416	asUfscagcGfacaccaAfu Cfuccsusu	269	aucagcgacacc aaucuccuu	563
123	ucgcugAfgCfUfCfAfucc cccs[invAb]	123	ucgcugagcucauc cccc	417	asGfsggggAfugagcuCf aGfcgasusu	270	agggggaugag cucagcgauu	564
124	acaccaUfcAfCfGfUfugc agcs[invAb]	124	acaccaucacguug cagc	418	asGfscugcAfacgugaUf gGfugususu	271	agcugcaacgu gaugguguuu	565
125	ggcucaAfcGfGfUfUfccc uugs[invAb]	125	ggcucaacgguucc cuug	419	usCfsaaggGfaaccguUf gAfgccsusu	272	ucaagggaacc guugagccuu	566
126	ggagauUfgGfUfGfUfcgc ugas[invAb]	126	ggagauuggguguc gcuga	420	asUfscagcGfacacCfaAf uCfuccsusu	273	aucagcgacacc aaucuccuu	567
127	ggagAfuUfgGfugucgcug as[invAb]	127	ggagauuggguguc gcuga	421	asUfscagCfGfacaccaAf uCfuccsusu	274	aucagcgacacc aaucuccuu	568

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
128	ucgcugAfgCfUfCfAfucc cccs[invAb]	128	ucgcugagcucauc cccc	422	asGfsggggAfugagCfuC faGfcgasusu	275	aggggaugag cucagcgauu	569
129	ucgcUfgAfgCfucaucccccc s[invAb]	129	ucgcugagcucauc cccc	423	asGfsggGfAfugagcuC faGfcgasusu	276	aggggaugag cucagcgauu	570
130	acaccaUfcAfCfGfUfugc agcs[invAb]	130	acaccaucacguug cagc	424	asGfscugcAfacguGfaU fgGfugususu	277	agcugcaacgu gaugguguuu	571
131	acacCfaUfcAfcguugcagc s[invAb]	131	acaccaucacguug cagc	425	asGfscugCfAfacgugaU fgGfugususu	278	agcugcaacgu gaugguguuu	572
132	ggcucaAfcGfGfUfUfccc uugs[invAb]	132	ggcucaacgguucc cuug	426	usCfsaaggGfaaccGfuU fgAfgccsusu	279	ucaagggaacc guugagccuu	573
133	ggcuCfaAfcGfguuccuu gs[invAb]	133	ggcucaacgguucc cuug	427	usCfsaagGfGfaaccguU fgAfgccsusu	280	ucaagggaacc guugagccuu	574
134	ucgcugAfgCfUfCfAfucc cccs[invAb]	134	ucgcugagcucauc cccc	428	asGfsgGfgGfAfugagCf uCfagcsgsa	281	aggggaugag cucagcga	575
135	ucgcugAfgCfUfCfAfucc ccscs[invAb]	135	ucgcugagcucauc cccc	429	asGfsgGfgGfAfugagcu CfaGfcgasusu	282	aggggaugag cucagcgauu	576
136	ugguCfcAfCfGfUfgcacu ucaaus[invAb]	136	ugguccacgugcac uucaau	430	usUfsgaagUfgcacguGf gAfccasgsg	283	uugaagugcac guggaccagg	577
137	ugguccAfcGfUfGfCfacu ucaaus[invAb]	137	ugguccacgugcac uucaau	431	usUfsgaagUfgcacguGf gAfccasgsg	284	uugaagugcac guggaccagg	578
138	ugguccAfcGfUfGfCfacu ucaaus[invAb]	138	ugguccacgugcac uucaau	432	usUfsgaagUfgcacGfuG fgAfccasgsg	285	uugaagugcac guggaccagg	579
139	ggagauUfgGfUfGfUfcgc ugauus[invAb]	139	ggagauugguguc gcugauu	433	asUfscagcGfacacCfaAfu Cfuccsusu	286	aucagcgcacc aaucuccuu	580
140	ggagauUfgGfUfGfUfcgc ugauus[invAb]	140	ggagauugguguc gcugauu	434	asUfscagcGfacacCfaAf uCfuccsusu	287	aucagcgcacc aaucuccuu	581
141	ggagAfuUfGfGfUfgucgc ugauus[invAb]	141	ggagauugguguc gcugauu	435	asUfscagcGfacaccAfu Cfuccsusu	288	aucagcgcacc aaucuccuu	582

Duplex No.	Sense (5'-3') modified	SEQ ID NO (Sense-Mod)	Sense (5'-3') unmodified	SEQ ID NO (Sense-Unmod)	Antisense (5'-3') modified	SEQ ID NO (Antisense-Mod)	Antisense (5'-3') unmodified	SEQ ID NO (Antisense-Unmod)
142	acaccaUfcAfCfGfUfugc agcuus[invAb]	142	acaccaucacguug cagcuu	436	asGfscugcAfacguGfaU fgGfugususu	289	agcugcaacgu gauggugguuu	583
143	acaccaUfcAfCfGfUfugc agcuus[invAb]	143	acaccaucacguug cagcuu	437	asGfscugcAfacgugaUf gGfugususu	290	agcugcaacgu gauggugguuu	584
144	ggcuCfaAfCfGfGfuuccc uugaus[invAb]	144	ggcucaacgguucc cuugau	438	usCfsaaggGfaaccguUf gAfgccsusu	291	ucaagggaacc guugagccuu	585
145	ggcuCfaAfCfGfGfuuccc uugs[invAb]	145	ggcucaacgguucc cuug	439	usCfsaaggGfaaccguUf gAfgscsc	292	ucaagggaacc guugagcc	586
146	cggcucAfaCfGfGfUfucc cuugas[invAb]	146	cggcucaacgguuc ccuuga	440	asUfscaagGfgaaccgUfu Gfagcscsg	293	aucaagggaacc guugagccg	587
147	cggcucAfaCfGfGfUfucc cuugas[invAb]	147	cggcucaacgguuc ccuuga	441	asUfscaagGfgaacCfgU fuGfagcscsg	294	aucaagggaacc guugagccg	588

EXAMPLE 2: Efficacy of select SCAP siRNA molecules in RNA FISH assay

[0169] siRNA molecules synthesized in Example 1 were screened in a fluorescent *in situ* hybridization assay targeting ribonucleic acid molecules (RNA FISH) to determine IC_{50} and maximum activity values.

[0170] The RNA FISH assay was carried out to measure SCAP mRNA knockdown by test siRNAs. Hep3B cells (purchased from ATCC) were cultured in minimal essential medium (MEM, Corning) supplemented with 10% Fetal Bovine Serum (FBS, Sigma) and 1% penicillin-streptomycin (P-S, Corning). The siRNA transfection was performed as follows: 1 μL of test siRNAs and 4 μL of plain MEM were added to PDL-coated CellCarrier-384 Ultra assay plates (PerkinElmer) by BioMek FX (Beckman Coulter). 5 μL of Lipofectamine RNAiMAX (Thermo Fisher Scientific), pre-diluted in plain MEM (0.035 μL of RNAiMAX in 5 μL MEM), was then dispensed into the assay plates by Multidrop Combi Reagent Dispenser (Thermo Fisher Scientific). After a 20 minute incubation of the siRNA/RNAiMAX mixture at room temperature (RT), 30 μL of Hep3B cells (2000 cells per well) in MEM supplemented with 10% FBS and 1% P-S were added to the transfection complex using Multidrop Combi Reagent Dispenser, and the assay plates were kept at RT for 20 minutes prior to moving them to an incubator. Cells were incubated for 72 hours at 37 °C and 5% CO_2.

[0171] The RNA FISH assay was performed using the Affymetrix QuantiGene® View RNA HC Screening Assay kit (QVP0011), the Affymetrix View HC Signal Amplification Kit 3-plex (QVP0213), and Affymetrix gene specific probes (SCAP Human 0.33mL View RNA Type 6 (650 label) VA6-20279-01 and PPIB Human 0.44mL View RNA Type 1 (488 label) VA1-10148-01) following the manufacturer's protocol.

[0172] Plates were first rehydrated with sequential 100%, 70%, and 50% ethanol washes. Cells were then washed with PBS, and then permeabilized and protease-digested according to the kit instructions. The target Working Probe Sets were prepared according to the manufacturer's protocol, added to the wells, and incubated for 3 hours at 40°C. The manufacturer's protocol was followed for the sequential hybridizations with the Working Probe Sets, the Working PreAmps, the Working Amps, and the Working LPs. Last, nuclei counterstains were applied (Hoechst 33342 and Cell Mask Blue; Molecular Probes). Plates were incubated for 30 minutes at room temperature, washed with PBS, overlaid with 80μl of PBS, and then the plates were sealed for imaging.

[0173] All plates were imaged on an Opera Phenix High Content Screening System (PerkinElmer), using the UV Channel for Hoechst 33342 and Cell Mask Blue, the 488 Channel for Type1 probes, and the 647 Channel for Type6 probes.

[0174] RNA FISH data was analyzed using Columbus software and images were generated using Genedata Screener. The images were analyzed to obtain mean spot counts per cell. The spot counts were normalized using the high (containing phosphate-buffered saline, Corning) and low (without target probe pairs) control wells. The normalized values against the total siRNA concentrations were plotted and the data were fit to a four-parameter sigmoidal model in Genedata Screener (Genedata) to obtain IC_{50} and maximum activity.

[0175] The results of the assay are shown in Table 2. SCAP activity is expressed as a percentage of knockdown compared to control. Negative values indicate a decrease in SCAP levels. Additional testing of several siRNA triggers was performed, the results of which are shown in the last column of Table 2.

Table 2. RNA FISH Assay with modified SCAP siRNA

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
1	ggaccuGfuGfGfAfAfuucacscs[invAb]	1	usGfsgugaAfuuccAfcAfgguccsusu	148	3.24	-73.7	
2	ggaccuGfuGfGfAfAfuucacscs[invAb]	2	usGfsgUfgAfAfuuccAfcAfgguccsusu	149	1.04	-82.7	
3	ggacCfuGfUfGfGfaauucaccs[invAb]	3	usGfsgugaAfuuccacAfgGfuccsusu	150	1.23	-80.6	
4	ggacCfuGfuGfgaauucaccs[invAb]	4	usGfsgugaauuccAfcAfgGfuccsusu	151	1.18	-78.5	
5	ggaccuGfuGfGfAfAfuucaccaus[invAb]	5	usGfsgugaAfuuccAfcAfgguccsusu	152	--	-34.6	-64.0
6	ggaccuGfuGfGfAfAfuucaccs[invAb]	6	usGfsgugaAfuuccAfcAfggucscsc	153	6.63	-65.7	-54.9
7	gcgagaUfuUfUfCfCfccuacscs[invAb]	7	asGfsguagGfggaaAfaUfcucgcsusu	154	0.916	-68.8	-70.9
8	gcgagaUfuUfUfCfCfccuacscs[invAb]	8	asGfsgUfaGfGfggaaAfaUfcucgcsusu	155	1.13	-81.7	
9	gcgaGfaUfUfUfUfccccuaccs[invAb]	9	asGfsguagGfggaaaaUfcUfcgcsusu	156	1.82	-85.0	
10	gcgaGfaUfuUfucccccuaccs[invAb]	10	asGfsguaggggaaAfaUfcUfcgcsusu	157	1.89	-73.0	
11	gcgagaUfuUfUfCfCfccuaccs[invAb]	11	asGfsguagGfggaaAfaUfcucsgsc	158	3.52	-63.9	-57.6
12	gcgagaUfuUfUfCfCfccuaccuus[invAb]	12	asGfsguagGfggaaAfaUfcucgcsusu	159	7.67	-69.7	-64.8
13	ccugucCfaUfUfGfAfcauucsgs[invAb]	13	asCfsgaauGfucaaUfgGfacaggsusu	160	3.76	-69.2	-63.3
14	ccugucCfaUfUfGfAfcauucsgs[invAb]	14	asCfsgAfaUfGfucaaUfgGfacaggsusu	161	1.44	-82.4	
15	ccugUfcCfAfUfUfgacauucgs[invAb]	15	asCfsgaauGfucaaugGfaCfaggsusu	162	0.62	-88.5	
16	ccugUfcCfaUfugacauucgs[invAb]	16	asCfsgaaugucaaUfgGfaCfaggsusu	163	6.04	-55.4	-40.3
17	ccugucCfaUfUfGfAfcauucguus[invAb]	17	asCfsgaauGfucaaUfgGfacaggsusu	164	1.65	-77.8	
18	ccugucCfaUfUfGfAfcauucgs[invAb]	18	asCfsgaauGfucaaUfgGfacasgsg	165	16.10	-68.3	-61.0
19	guccauUfgAfCfAfUfucgccsgs[invAb]	19	asCfsggcgAfauguCfaAfuggacsusu	166	1.61	-78.5	

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
20	cuguccauUfgAfCfAfUfucgccgs[invAb]	20	asCfsggcgAfauguCfaAfuggacagsusu	167	1.17	-86.61	
21	cuguccAfuUfGfAfCfauucgccgs[invAb]	21	asCfsggcgAfaugucaAfuGfgacagsusu	168	1.83	-88.59	
22	cuguccauUfgAfCfAfUfucgccgs[invAb]	22	asCfsggcgAfauguCfaAfuggacsasg	169	1.67	-82.83	
23	cuguccAfuUfgAfcauucgccgs[invAb]	23	asCfsggcgaauguCfaAfuGfgacagsusu	170	3.35	-81.06	
24	guccauUfgAfCfAfUfucgccguus[invAb]	24	asCfsggcgAfauguCfaAfuggacsasg	171	2.75	-81.36	
25	ggaaagAfgCfCfGfAfguaucsus[invAb]	25	asAfsgauaCfucggCfuCfuuuccsusu	172	1.44	-84.3	
26	ggaaagAfgCfCfGfAfguaucsus[invAb]	26	asAfsgAfuAfCfucggCfuCfuuuccsusu	173	1.4	-73.61	
27	ggaaAfgAfgCfcgaguaucus[invAb]	27	asAfsgauacucggCfuCfuUfuccsusu	174	2.7	-83.17	
28	ggaaagAfgCfCfGfAfguaucuuus[invAb]	28	asAfsgauaCfucggCfuCfuuuccsusu	175	1.05	-76.92	
29	ggaaagAfgCfCfGfAfguaucus[invAb]	29	asAfsgauaCfucggCfuCfuuuscsc	176	3.02	-79.38	
30	ggaaAfgAfGfCfCfgaguaucus[invAb]	30	asAfsgauaCfucggcuCfuUfuccsusu	177	1.82	-88.68	
31	aaagagCfcGfAfGfUfaucuuscs[invAb]	31	asGfsaagaUfacucGfgCfucuuususu	178	1.37	-90	
32	ggaaagagCfcGfAfGfUfaucuucs[invAb]	32	asGfsaagaUfacucGfgCfucuuuccsusu	179	2.18	-79.50	
33	ggaaagAfgCfCfGfAfguaucuucs[invAb]	33	asGfsaagaUfacucggCfuCfuuuccsusu	180	0.486	-80.75	
34	ggaaagagCfcGfAfGfUfaucuucs[invAb]	34	asGfsaagaUfacucGfgCfucuuuscsc	181	3.03	-83.15	
35	ggaaagAfgCfcGfaguaucuucs[invAb]	35	asGfsaagauacucGfgCfuCfuuuccsusu	182	3.01	-91.24	
36	aaagagCfcGfAfGfUfaucuucuus[invAb]	36	asGfsaagaUfacucGfgCfucuuuscsc	183	2.91	-82.11	
37	gcggcgAfgAfUfUfUfucccccsus[invAb]	37	usAfsggggAfaaauCfuCfgccgcsusu	184	2.69	-61.2	-63.6
38	ggcgagAfuUfUfUfCfcccuascs[invAb]	38	asGfsuaggGfgaaaAfuCfucgccsusu	185	1.50	-79.1	
39	aagauaUfcUfCfGfGfgccuuscs[invAb]	39	asGfsaaggCfccgaGfaUfaucuususu	186	2.50	-73.0	
40	cgggccUfuCfUfAfCfaaccasus[invAb]	40	asAfsugguUfguagAfaGfgcccgsusu	187	3.28	-73.1	
41	cugcagUfuUfAfGfAfgggacscs[invAb]	41	asGfsguccCfucuaAfaCfugcagsusu	188	1.76	-78.3	

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
42	acggggAfcCfUfGfUfuacagsas[invAb]	42	asUfscuguAfacagGfuCfcccgususu	189	1.45	-84.3	
43	cggcucAfaCfGfGfUfucccusus[invAb]	43	asAfsagggAfaccgUfuGfagccgsusu	190	14.90	-63.7	-32.2
44	uguacuGfaCfUfGfGfcagccsgs[invAb]	44	asCfsggcuGfccagUfcAfguacasusu	191	3.59	-53.2	-41.7
45	ccccugCfaGfUfUfUfagaggsgs[invAb]	45	usCfsccucUfaaacUfgCfaggggsusu	192	12.20	-50.0	-49.1
46	uguccaUfuGfAfCfAfuucgcscs[invAb]	46	asGfsgcgaAfugucAfaUfggacasusu	193	0.55	-87.4	
47	ggcuggCfaCfCfGfUfugucusgs[invAb]	47	asCfsagacAfacggUfgCfcagccsusu	194	3.20	-48.1	-8.4
48	ggcucaAfcGfGfUfUfcccuusgs[invAb]	48	usCfsaaggGfaaccGfuUfgagccsusu	195	3.10	-79.8	
49	gcucaaCfgGfUfUfCfccuugsas[invAb]	49	asUfscaagGfgaacCfgUfugagcsusu	196	2.20	-92.1	
50	caacggUfuCfCfCfUfugauusus[invAb]	50	asAfsaaucAfagggAfaCfcguugsusu	197	0.97	-79.4	
51	acgguuCfcCfUfUfGfauuucsus[invAb]	51	asAfsgaaaUfcaagGfgAfaccgususu	198	0.67	-89.9	
52	gugggaUfgUfAfCfUfgacugsgs[invAb]	52	asCfscaguCfaguaCfaUfcccacsusu	199	5.38	-59.6	-39.1
53	cugaaaCfuCfCfCfCfuugccsas[invAb]	53	asUfsggcaAfggggAfgUfuucagsusu	200	--	-20.0	-7.6
54	gauccuGfaCfAfUfCfauuggsgs[invAb]	54	usCfsccaaUfgaugUfcAfggaucsusu	201	2.77	-66.2	-32.8
55	ugguccAfcGfUfGfCfacuucsas[invAb]	55	usUfsgaagUfgcacGfuGfgaccasusu	202	1.70	-78.7	
56	guggacUfcUfGfAfCfcgcaasas[invAb]	56	asUfsuugcGfgucaGfaGfuccacsusu	203	5.67	-47.1	-23.4
57	ggaggaGfaUfUfGfGfugucgscs[invAb]	57	asGfscgacAfccaaUfcUfccuccsusu	204	0.91	-80.6	
58	aguguuUfcCfCfUfGfgcacasas[invAb]	58	asUfsugugCfcaggGfaAfacacususu	205	2.22	-57.4	-45.6
59	ggagauUfgGfUfGfUfcgcugsas[invAb]	59	asUfscagcGfacacCfaAfucuccsusu	206	3.50	-84.1	
60	ucgcugAfgCfUfCfAfucccscs[invAb]	60	asGfsggggAfugagCfuCfagcgasusu	207	3.30	-75.0	
61	ugucccGfgGfCfAfUfuccaascs[invAb]	61	asGfsuuggAfaugcCfcGfggacasusu	208	5.09	-51.0	-32.3
62	ggaguuCfuGfUfCfUfcuuugscs[invAb]	62	asGfscaaaGfagacAfgAfacuccsusu	209	0.31	-91.4	
63	gagguaAfcCfUfGfGfgggccsus[invAb]	63	asAfsggccCfccagGfuUfaccucsusu	210	2.24	-62.1	-48.2

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
64	acaccaUfcAfCfGfUfugcagscs[invAb]	64	asGfscugcAfacguGfaUfggugususu	211	3.50	-86.7	
65	cggcuaCfuUfCfAfCfccuagsus[invAb]	65	asAfscuagGfgugaAfgUfagccgsusu	212	17.80	-55.9	-37.4
66	cauugaCfcAfGfAfCfcauggsus[invAb]	66	asAfsccauGfgucuGfgUfcaaugsusu	213	3.28	-54.7	-42.2
67	aggagaUfuGfGfUfGfucgcusgs[invAb]	67	usCfsagcgAfcaccAfaUfcuccususu	214	0.79	-71.5	
68	ccgccuUfcCfCfUfCfuucggsgs[invAb]	68	asCfsccgaAfgaggGfaAfggcggsusu	215	--	-40.5	-31.4
69	auugaaGfgGfGfUfGfcugugscs[invAb]	69	asGfscacaGfcaccCfcUfucaaususu	216	2.73	-71.0	
70	ccugguccAfcGfUfGfCfacuucas[invAb]	70	usUfsgaagUfgcacGfuGfgaccaggsusu	217	3.51	-77.78	
71	ccugguCfcAfCfGfUfgcacuucas[invAb]	71	usUfsgaagUfgcacguGfgAfccaggsusu	218	1.83	-76.44	
72	ugguccAfcGfUfGfCfacuucaaus[invAb]	72	usUfsgaagUfgcacGfuGfgaccasgsg	219	7.48	-88.25	
73	ccugguccAfcGfUfGfCfacuucas[invAb]	73	usUfsgaagUfgcacGfuGfgaccasgsg	220	5.25	-80.05	
74	ccugguCfcAfcGfugcacuucas[invAb]	74	usUfsgaagugcacGfuGfgAfccaggsusu	221	5.77	-81.11	
75	ggagauUfgGfUfGfUfcgcugsas[invAb]	75	asUfscAfgCfGfacacCfaAfucuccsusu	222	2.9	-81.52	
76	acaccaUfcAfCfGfUfugcagscs[invAb]	76	asGfscUfgCfAfacguGfaUfggugususu	223	3.93	-90.02	
77	ggagAfuUfGfGfUfgucgcugas[invAb]	77	asUfscagcGfacaccaAfuCfuccsusu	224	500	-8.18	-64.7
78	acacCfaUfCfAfCfguugcagcs[invAb]	78	asGfscugcAfacgugaUfgGfugususu	225	4.2	-86.99	
79	ggagAfuUfgGfugucgcugas[invAb]	79	asUfscagcgacacCfaAfuCfuccsusu	226	6.53	-88.09	
80	acacCfaUfcAfcguugcagcs[invAb]	80	asGfscugcaacguGfaUfgGfugususu	227	7	-87.92	
81	ggagauUfgGfUfGfUfcgcugas[invAb]	81	asUfscagcGfacacCfaAfucuscsc	228	12.1	-71.94	
82	acaccaUfcAfCfGfUfugcagcs[invAb]	82	asGfscugcAfacguGfaUfggusgsu	229	16.6	-76.00	
83	ggagauUfgGfUfGfUfcgcugauus[invAb]	83	asUfscagcGfacacCfaAfucuccsusu	230	6.5	-85.33	
84	acaccaUfcAfCfGfUfugcagcuus[invAb]	84	asGfscugcAfacguGfaUfggugususu	231	7.52	-78.50	
85	ucgcugAfgCfUfCfAfucccscs[invAb]	85	asGfsgGfgGfAfugagCfuCfagcgasusu	232	3.65	-76.02	

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
86	ucgcUfgAfGfCfUfcaucccccs[invAb]	86	asGfsggggAfugagcuCfaGfcgasusu	233	4.87	-78.97	
87	ucgcUfgAfgCfucaucccccs[invAb]	87	asGfsggggaugagCfuCfaGfcgasusu	234	17.4	-64.39	-44.7
88	ucgcugAfgCfUfCfAfucccccuus[invAb]	88	asGfsggggAfugagCfuCfagcgasusu	235	14.8	-63.94	-55.5
89	ucgcugAfgCfUfCfAfucccccs[invAb]	89	asGfsggggAfugagCfuCfagcsgsa	236	12.5	-66.64	-57.4
90	ggcucaAfcGfGfUfUfcccuusgs[invAb]	90	usCfsaAfgGfGfaaccGfuUfgagccsusu	237	4.7	-85.50	
91	ggcuCfaAfCfGfGfuucccuugs[invAb]	91	usCfsaaggGfaaccguUfgAfgccsusu	238	7.08	-86.03	
92	ggcuCfaAfcGfguucccuugs[invAb]	92	usCfsaagggaaccGfuUfgAfgccsusu	239	9.84	-79.22	
93	ggcucaAfcGfGfUfUfcccuugaus[invAb]	93	usCfsaaggGfaaccGfuUfgagccsusu	240	22.6	-80.97	
94	ggcucaAfcGfGfUfUfcccuugs[invAb]	94	usCfsaaggGfaaccGfuUfgagscsc	241	20.5	-63.66	-55.4
95	gcggcgagAfuUfUfUfCfcccuacs[invAb]	95	asGfsuaggGfgaaaAfuCfucgccgcsusu	242	3.88	-82.42	
96	cggcucaaCfgGfUfUfCfccuugas[invAb]	96	asUfscaagGfgaacCfgUfugagccgsusu	243	4.55	-85.51	
97	gcggcgAfgAfUfUfUfucccccuacs[invAb]	97	asGfsuaggGfgaaaauCfuCfgccgcsusu	244	3.09	-80.60	
98	cggcucAfaCfGfGfUfucccuugas[invAb]	98	asUfscaagGfgaaccgUfuGfagccgsusu	245	3.29	-92.30	
99	gcggcgAfgAfuUfuucccccuacs[invAb]	99	asGfsuaggggaaaAfuCfuCfgccgcsusu	246	9.43	-79.94	
100	cggcucAfaCfgGfuucccuugas[invAb]	100	asUfscaagggaacCfgUfuGfagccgsusu	247	4.23	-89.56	
101	ggcgagAfuUfUfUfCfcccuacuus[invAb]	101	asGfsuaggGfgaaaAfuCfucgccsgsc	248	17.9	-78.53	
102	gcucaaCfgGfUfUfCfccuugauus[invAb]	102	asUfscaagGfgaacCfgUfugagcscsg	249	5.36	-88.46	
103	gcggcgagAfuUfUfUfCfcccuacs[invAb]	103	asGfsuaggGfgaaaAfuCfucgccsgsc	250	6.13	-78.57	
104	cggcucaaCfgGfUfUfCfccuugas[invAb]	104	asUfscaagGfgaacCfgUfugagcscsg	251	3.3	-90.52	
105	agaagauaUfcUfCfGfGfgccuucs[invAb]	105	asGfsaaggCfccgaGfaUfaucuuscsu	252	7.32	-71.83	
106	agaagauaUfcUfCfGfGfgccuucs[invAb]	106	asGfsaaggCfccgaGfaUfaucuucususu	253	5.56	-70.77	
107	agaagaUfaUfCfUfCfgggccuucs[invAb]	107	asGfsaaggCfccgagaUfaUfcuucususu	254	35.6	-45.72	-25.7

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
108	agaagaUfaUfcUfcgggccuucs[invAb]	108	asGfsaaggcccgaGfaUfaUfcuucususu	255	500	-21.94	-11.6
109	aagauaUfcUfCfGfGfgccuucuus[invAb]	109	asGfsaaggCfccgaGfaUfaucuuscsu	256	8.22	-72.74	
110	ccugguccAfcGfUfGfCfacuucas[invAb]	110	usUfsgaagUfgcacguGfgAfccaggsusu	257	6.21	-74.11	
111	gcggcgagAfuUfUfUfCfcccuacs[invAb]	111	asGfsuaggGfgaaaauCfuCfgccgcsusu	258	5.7	-77.61	
112	cggcucaaCfgGfUfUfCfccuugas[invAb]	112	asUfscaagGfgaaccgUfuGfagccgsusu	259	3.81	-92.67	
113	agaagauaUfcUfCfGfGfgccuucs[invAb]	113	asGfsaaggCfccgagaUfaUfcuucususu	260	38.8	-55.21	-29.1
114	ccuggucc AfcGfUfGfCfacuucas[invAb]	114	usUfsgaagUfgcacGfuGfgAfccaggsusu	261	5.26	-74.45	
115	gcggcgagAfuUfUfUfCfcccuacs[invAb]	115	asGfsuaggGfgaaaAfuCfuCfgccgcsusu	262	5.58	-82.96	
116	cggcucaaCfgGfUfUfCfccuugas[invAb]	116	asUfscaagGfgaacCfgUfuGfagccgsusu	263	3.43	-84.24	
117	agaagauaUfcUfCfGfGfgccuucs[invAb]	117	asGfsaaggCfccgaGfaUfaUfcuucususu	264	28.7	-56.99	-21.7
118	ccuggu CfcAfcGfugcacuucas[invAb]	118	usUfsgaaGfUfgcacguGfgAfccaggsusu	265	3.3	-75.82	
119	gcggcgAfgAfuUfuucccccuacs[invAb]	119	asGfsuagGfGfgaaaauCfuCfgccgcsusu	266	4.89	-82.00	
120	cggcucAfaCfgGfuucccuugas[invAb]	120	asUfscaaGfGfgaaccgUfuGfagccgsusu	267	2.6	-94.24	
121	agaagaUfaUfcUfcgggccuucs[invAb]	121	asGfsaagGfCfccgagaUfaUfcuucususu	268	24.3	-61.77	-42.7
122	ggagauUfgGfUfGfUfcgcugas[invAb]	122	asUfscagcGfacaccaAfuCfuccsusu	269	10.9	-82.25	
123	ucgcugAfgCfUfCfAfucccccs[invAb]	123	asGfsggggAfugagcuCfaGfcgasusu	270	8.51	-69.15	-69.4
124	acaccaUfcAfCfGfUfugcagcs[invAb]	124	asGfscugcAfacgugaUfgGfugususu	271	5.34	-80.19	
125	ggcucaAfcGfGfUfUfcccuugs[invAb]	125	usCfsaaggGfaaccguUfgAfgccsusu	272	6.52	-81.92	
126	ggagauUfgGfUfGfUfcgcugas[invAb]	126	asUfscagcGfacacCfaAfuCfuccsusu	273	9.62	-86.67	
127	ggagAfuUfgGfugucgcugas[invAb]	127	asUfscagCfGfacaccaAfuCfuccsusu	274	8.3	-83.21	
128	ucgcugAfgCfUfCfAfucccccs[invAb]	128	asGfsggggAfugagCfuCfaGfcgasusu	275	7.23	-71.83	
129	ucgcUfgAfgCfucaucccccs[invAb]	129	asGfsgggGfAfugagcuCfaGfcgasusu	276	5.58	-72.60	

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	RNA FISH IC50 IP [nM]	RNA FISH max activity (%)	RNA FISH Additional Test Avg Activity (%)
130	acaccaUfcAfCfGfUfugcagcs[invAb]	130	asGfscugcAfacguGfaUfgGfugususu	277	500	-83.34	
131	acacCfaUfcAfcguugcagcs[invAb]	131	asGfscugCfAfacgugaUfgGfugususu	278	9.05	-85.16	
132	ggcucaAfcGfGfUfUfcccuugs[invAb]	132	usCfsaaggGfaaccGfuUfgAfgccsusu	279	4.92	-86.26	
133	ggcuCfaAfcGfguucccuugs[invAb]	133	usCfsaagGfGfaaccguUfgAfgccsusu	280	4.45	-87.64	
134	ucgcugAfgCfUfCfAfucccccs[invAb]	134	asGfsgGfgGfAfugagCfuCfagcsgsa	281	24.6	-56.17	-64.7
135	ucgcugAfgCfUfCfAfucccccscs[invAb]	135	asGfsgGfgGfAfugagcuCfaGfcgasusu	282	7.15	-74.41	
136	ugguCfcAfCfGfUfgcacuucaaus[invAb]	136	usUfsgaagUfgcacguGfgAfccasgsg	283	3.71	-79.80	
137	ugguccAfcGfUfGfCfacuucaaus[invAb]	137	usUfsgaagUfgcacguGfgAfccasgsg	284	5.24	-78.85	
138	ugguccAfcGfUfGfCfacuucaaus[invAb]	138	usUfsgaagUfgcacGfuGfgAfccasgsg	285	7.83	-81.55	
139	ggagauUfgGfUfGfUfcgcugauus[invAb]	139	asUfscagcGfacaccaAfuCfuccsusu	286	8.21	-77.02	
140	ggagauUfgGfUfGfUfcgcugauus[invAb]	140	asUfscagcGfacacCfaAfuCfuccsusu	287	8.24	-83.79	
141	ggagAfuUfGfGfUfgucgcugauus[invAb]	141	asUfscagcGfacaccaAfuCfuccsusu	288	10.6	-83.56	
142	acaccaUfcAfCfGfUfugcagcuus[invAb]	142	asGfscugcAfacguGfaUfgGfugususu	289	18	-76.24	
143	acaccaUfcAfCfGfUfugcagcuus[invAb]	143	asGfscugcAfacgugaUfgGfugususu	290	18.4	-76.34	
144	ggcuCfaAfCfGfGfuucccuugaus[invAb]	144	usCfsaaggGfaaccguUfgAfgccsusu	291	11.2	-80.59	
145	ggcuCfaAfCfGfGfuucccuugs[invAb]	145	usCfsaaggGfaaccguUfgAfgscsc	292	17.8	-64.03	-68.1
146	cggcucAfaCfGfGfUfucccuugas[invAb]	146	asUfscaagGfgaaccgUfuGfagcscsg	293	5.28	-91.97	
147	cggcucAfaCfGfGfUfucccuugas[invAb]	147	asUfscaagGfgaacCfgUfuGfagcscsg	294	500	-28.59	-90.2

Example 3: Efficacy Screening of Select SCAP siRNA Molecules in a Mouse Model

[0176] Male Balbc mice at 7-8 weeks of age and above 20 grams body weight were obtained from Charles River Laboratories (Charles River Laboratories, Inc, MA). Mice were dosed with an AAV8 viral vector for robust hepatic expression. Each animal was dosed with $1x10^{12}$ viral particles reconstituted in 200 µl of cell culture grade PBS. The AAV vector encoded a luciferase gene-based reporter construct. Following intraperitoneal administration of Xenolight Rediject D-Luciferin substrate (Perkim Elmer), for 10 minutes, the luciferase activity was analyzed by measuring light signals generated from the luciferin/luciferase reaction. These light signals were indicated as flux. Bioluminescence imaging (BLI) was performed using the IVIS Lumina S5 Spectrum pre-clinical *in vivo* imaging instrument from Perkin Elmer. The BLI image analysis was performed by placing a small and identical region of interest (ROI) on the liver and analyzed using the Living Image Software 4.7.2.

[0177] To monitor SCAP siRNA activity, the 3'UTR region of the luciferase reporter gene included specific SCAP mRNA sequences to which the siRNA can bind and initiate degradation of the luciferase reporter gene. Two weeks following AAV administration, mice were imaged for baseline luciferase activity. Animals were then randomized according to the baseline image intensity and grouped into 5 animals per siRNA. On the following day, mice were subcutaneously dosed at 3 mg per kg of body weight (mpk) with SCAP siRNA conjugated to tri-antennary GalNAc (GalNAc3) at the 5' end of the sense strand. Four weeks after the siRNA dose, BLI imaging was performed to monitor luciferase activity. siRNA potency at week 4 shown in Table 3 as % silencing data, calculated by normalizing the ratio of week 4 (Wk4) flux: baseline flux for each siRNA group to the PBS group. A total of 158 triggers were tested, several of which included different chemical modifications.

Table 3. *In vivo* Potency of SCAP siRNA

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
1	ggaccuGfuGfGfAfAfuucacscs[invAb]	1	usGfsgugaAfuuccAfcAfgguccsusu	148	3mpk	-80.34
2	ggaccuGfuGfGfAfAfuucacscs[invAb]	2	usGfsgUfgAfAfuuccAfcAfgguccsusu	149	3mpk	-15.88
3	ggacCfuGfUfGfGfaauucaccs[invAb]	3	usGfsgugaAfuuccacAfgGfuccsusu	150	3mpk	-53.3
4	ggacCfuGfuGfgaauucaccs[invAb]	4	usGfsgugaauuccAfcAfgGfuccsusu	151	3mpk	-42.49
5	ggaccuGfuGfGfAfAfuucaccaus[invAb]	5	usGfsgugaAfuuccAfcAfgguccsusu	152	3mpk	-63.11
6	ggaccuGfuGfGfAfAfuucaccs[invAb]	6	usGfsgugaAfuuccAfcAfgguscsc	153	3mpk	-3.65
7	gcgagaUfuUfUfCfCfccuacscs[invAb]	7	asGfsguagGfggaaAfaUfcucgcsusu	154	3mpk	-86.33
8	gcgagaUfuUfUfCfCfccuacscs[invAb]	8	asGfsgUfaGfGfggaaAfaUfcucgcsusu	155	3mpk	-79.59
9	gcgaGfaUfUfUfUfccccuaccs[invAb]	9	asGfsguagGfggaaaaUfcUfcgcsusu	156	3mpk	-72.82
10	gcgaGfaUfuUfuccccuaccs[invAb]	10	asGfsguaggggaaAfaUfcUfcgcsusu	157	3mpk	-84.66
11	gcgagaUfuUfUfCfCfccuaccs[invAb]	11	asGfsguagGfggaaAfaUfcucsgsc	158	3mpk	-47.38
12	gcgagaUfuUfUfCfCfccuaccuus[invAb]	12	asGfsguagGfggaaAfaUfcucgcsusu	159	3mpk	-81.46
13	ccugucCfaUfUfGfAfcauucsgs[invAb]	13	asCfsgaauGfucaaUfgGfacaggsusu	160	3mpk	-65.41
14	ccugucCfaUfUfGfAfcauucsgs[invAb]	14	asCfsgAfaUfGfucaaUfgGfacaggsusu	161	3mpk	-77.07
15	ccugUfcCfAfUfUfgacauucgs[invAb]	15	asCfsgaauGfucaaugGfaCfaggsusu	162	3mpk	-79.08
16	ccugUfcCfaUfugacauucgs[invAb]	16	asCfsgaaugucaaUfgGfaCfaggsusu	163	3mpk	-73.33
17	ccugucCfaUfUfGfAfcauucguus[invAb]	17	asCfsgaauGfucaaUfgGfacaggsusu	164	3mpk	-83.3
18	ccugucCfaUfUfGfAfcauucgs[invAb]	18	asCfsgaauGfucaaUfgGfacasgsg	165	3mpk	-27.8
19	guccauUfgAfCfAfUfucgccsgs[invAb]	19	asCfsggcgAfauguCfaAfuggacsusu	166	3mpk	-71.1
20	cuguccauUfgAfCfAfUfucgccgs[invAb]	20	asCfsggcgAfauguCfaAfuggacagsusu	167	3mpk	-57.77
21	cuguccAfuUfGfAfCfauucgccgs[invAb]	21	asCfsggcgAfaugucaAfuGfgacagsusu	168	3mpk	-64.67

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
22	cuguccauUfgAfCfAfUfucgccgs[invAb]	22	asCfsggcgAfauguCfaAfuggacsasg	169	3mpk	-73.67
23	cuguccAfuUfgAfcauucgccgs[invAb]	23	asCfsggcgaauguCfaAfuGfgacagsusu	170	3mpk	-56.27
24	guccauUfgAfCfAfUfucgccguus[invAb]	24	asCfsggcgAfauguCfaAfuggacsasg	171	3mpk	-79.22
25	ggaaagAfgCfCfGfAfguaucsus[invAb]	25	asAfsgauaCfucggCfuCfuuuccsusu	172	3mpk	-76.7
26	ggaaagAfgCfCfGfAfguaucsus[invAb]	26	asAfsgAfuAfCfucggCfuCfuuuccsusu	173	3mpk	-86.88
27	ggaaAfgAfgCfcgaguaucus[invAb]	27	asAfsgauacucggCfuCfuUfuccsusu	174	3mpk	-64.51
28	ggaaagAfgCfCfGfAfguaucuuus[invAb]	28	asAfsgauaCfucggCfuCfuuuccsusu	175	3mpk	-88.89
29	ggaaagAfgCfCfGfAfguaucus[invAb]	29	asAfsgauaCfucggCfuCfuuuscsc	176	3mpk	-44.87
30	ggaaAfgAfGfCfCfgaguaucus[invAb]	30	asAfsgauaCfucggcuCfuUfuccsusu	177	3mpk	-69.31
31	aaagagCfcGfAfGfUfaucuuscs[invAb]	31	asGfsaagaUfacucGfgCfucuuususu	178	3mpk	-78
32	ggaaagagCfcGfAfGfUfaucuucs[invAb]	32	asGfsaagaUfacucGfgCfucuuuccsusu	179	3mpk	-67.98
33	ggaaagAfgCfCfGfAfguaucuucs[invAb]	33	asGfsaagaUfacucggCfuCfuuuccsusu	180	3mpk	-86.86
34	ggaaagagCfcGfAfGfUfaucuucs[invAb]	34	asGfsaagaUfacucGfgCfucuuuscsc	181	3mpk	-66.29
35	ggaaagAfgCfcGfaguaucuucs[invAb]	35	asGfsaagauacucGfgCfuCfuuuccsusu	182	3mpk	-66.65
36	aaagagCfcGfAfGfUfaucuucuus[invAb]	36	asGfsaagaUfacucGfgCfucuuuscsc	183	3mpk	-74.06
37	gcggcgAfgAfUfUfUfucccсsus[invAb]	37	usAfsggggAfaaauCfuCfgccgcsusu	184	3mpk	-14.96
38	ggcgagAfuUfUfUfCfcccuascs[invAb]	38	asGfsuaggGfgaaaAfuCfucgccsusu	185	3mpk	-88.43
39	aagauaUfcUfCfGfGfgccuuscs[invAb]	39	asGfsaaggCfccgaGfaUfaucuususu	186	3mpk	-77.32
40	cgggccUfuCfUfAfCfaaccasus[invAb]	40	asAfsugguUfguagAfaGfgcccgsusu	187	3mpk	0
41	cugcagUfuUfAfGfAfgggacscs[invAb]	41	asGfsguccCfucuaAfaCfugcagsusu	188	3mpk	-54.07
42	acggggAfcCfUfGfUfuacagsas[invAb]	42	asUfscuguAfacagGfuCfcccgususu	189	3mpk	0
43	cggcucAfaCfGfGfUfucccusus[invAb]	43	asAfsagggAfaccgUfuGfagccgsusu	190	3mpk	-66.71
44	uguacuGfaCfUfGfGfcagccsgs[invAb]	44	asCfsggcuGfccagUfcAfguacasusu	191	3mpk	-45.12

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
45	ccccugCfaGfUfUfUfagaggsgs[invAb]	45	usCfsccucUfaaacUfgCfaggggsusu	192	3mpk	0
46	uguccaUfuGfAfCfAfuucgcscs[invAb]	46	asGfsgcgaAfugucAfaUfggacasusu	193	3mpk	-69.36
47	ggcuggCfaCfCfGfUfugucusgs[invAb]	47	asCfsagacAfacggUfgCfcagccsusu	194	--	--
48	ggcucaAfcGfGfUfUfcccuusgs[invAb]	48	usCfsaaggGfaaccGfuUfgagccsusu	195	3mpk	-87.62
49	gcucaaCfgGfUfUfCfccuugsas[invAb]	49	asUfscaagGfgaacCfgUfugagcsusu	196	3mpk	-86.43
50	caacggUfuCfCfCfUfugauusus[invAb]	50	asAfsaaucAfagggAfaCfcguugsusu	197	3mpk	-60.78
51	acgguuCfcCfUfUfGfauuucsus[invAb]	51	asAfsgaaaUfcaagGfgAfaccgususu	198	3mpk	-74.51
52	gugggaUfgUfAfCfUfgacugsgs[invAb]	52	asCfscaguCfaguaCfaUfcccacsusu	199	3mpk	-20.77
53	cugaaaCfuCfCfCfCfuugccsas[invAb]	53	asUfsggcaAfggggAfgUfuucagsusu	200	--	--
54	gauccuGfaCfAfUfCfauuggsgs[invAb]	54	usCfsccaaUfgaugUfcAfggaucsusu	201	--	--
55	ugguccAfcGfUfGfCfacuucsas[invAb]	55	usUfsgaagUfgcacGfuGfgaccasusu	202	3mpk	-73.63
56	guggacUfcUfGfAfCfcgcaasas[invAb]	56	asUfsuugcGfgucaGfaGfuccacsusu	203	--	--
57	ggaggaGfaUfUfGfGfugucgscs[invAb]	57	asGfscgacAfccaaUfcUfccuccsusu	204	3mpk	-34.27
58	aguguuUfcCfCfUfGfgcacasas[invAb]	58	asUfsugugCfcaggGfaAfacacususu	205	--	--
59	ggagauUfgGfUfGfUfcgcugsas[invAb]	59	asUfscagcGfacacCfaAfucuccsusu	206	3mpk	-76.45
60	ucgcugAfgCfUfCfAfucccscs[invAb]	60	asGfsggggAfugagCfuCfagcgasusu	207	3mpk	-45.22
61	ugucccGfgGfCfAfUfuccaascs[invAb]	61	asGfsuuggAfaugcCfcGfggacasusu	208	--	--
62	ggaguuCfuGfUfCfUfcuuugscs[invAb]	62	asGfscaaaGfagacAfgAfacuccsusu	209	3mpk	-48.69
63	gagguaAfcCfUfGfGfgggccsus[invAb]	63	asAfsggccCfccagGfuUfaccucsusu	210	--	--
64	acaccaUfcAfCfGfUfugcagscs[invAb]	64	asGfscugcAfacguGfaUfggugususu	211	--	--
65	cggcuaCfuUfCfAfCfccuagsus[invAb]	65	asAfscuagGfgugaAfgUfagccgsusu	212	--	--
66	cauugaCfcAfGfAfCfcauggsus[invAb]	66	asAfsccauGfgucuGfgUfcaaugsusu	213	--	--
67	aggagaUfuGfGfUfGfucgcusgs[invAb]	67	usCfsagcgAfcaccAfaUfcuccususu	214	3mpk	-73.73

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
68	ccgccuUfcCfCfUfCfuucgggsgs[invAb]	68	asCfsccgaAfgaggGfaAfggcggsusu	215	--	--
69	auugaaGfgGfGfUfGfcugugscs[invAb]	69	asGfscacaGfcaccCfcUfucaaususu	216	--	--
70	ccugguccAfcGfUfGfCfacuucas[invAb]	70	usUfsgaagUfgcacGfuGfgaccaggsusu	217	3mpk	-59.29
71	ccugguCfcAfCfGfUfgcacuucas[invAb]	71	usUfsgaagUfgcacguGfgAfccaggsusu	218	3mpk	-83.99
72	ugguccAfcGfUfGfCfacuucaaus[invAb]	72	usUfsgaagUfgcacGfuGfgaccasgsg	219	3mpk	-70.67
73	ccugguccAfcGfUfGfCfacuucas[invAb]	73	usUfsgaagUfgcacGfuGfgaccasgsg	220	3mpk	-63.01
74	ccugguCfcAfcGfugcacuucas[invAb]	74	usUfsgaagugcacGfuGfgAfccaggsusu	221	3mpk	-49.48
75	ggagauUfgGfUfGfUfcgcugsas[invAb]	75	asUfscAfgCfGfacacCfaAfucuccsusu	222	3mpk	-67.13
76	acaccaUfcAfCfGfUfugcagscs[invAb]	76	asGfscUfgCfAfacguGfaUfggugususu	223	--	--
77	ggagAfuUfGfGfUfgucgcugas[invAb]	77	asUfscagcGfacaccaAfuCfuccsusu	224	3mpk	-64.32
78	acacCfaUfCfAfCfguugcagcs[invAb]	78	asGfscugcAfacgugaUfgGfugususu	225	--	--
79	ggagAfuUfgGfugucgcugas[invAb]	79	asUfscagcgacacCfaAfuCfuccsusu	226	3mpk	-69.66
80	acacCfaUfcAfcguugcagcs[invAb]	80	asGfscugcaacguGfaUfgGfugususu	227	--	--
81	ggagauUfgGfUfGfUfcgcugas[invAb]	81	asUfscagcGfacacCfaAfucuscsc	228	3mpk	-70.56
82	acaccaUfcAfCfGfUfugcagcs[invAb]	82	asGfscugcAfacguGfaUfggusgsu	229	--	--
83	ggagauUfgGfUfGfUfcgcugauus[invAb]	83	asUfscagcGfacacCfaAfucuccsusu	230	3mpk	-85.28
84	acaccaUfcAfCfGfUfugcagcuus[invAb]	84	asGfscugcAfacguGfaUfggugususu	231	--	--
85	ucgcugAfgCfUfCfAfucccccscs[invAb]	85	asGfsgGfgGfgAfugagCfuCfagcgasusu	232	3mpk	-81.14
86	ucgcUfgAfGfCfUfcaucccccs[invAb]	86	asGfsggggAfugagcuCfaGfcgasusu	233	3mpk	-74.05
87	ucgcUfgAfgCfucaucccccs[invAb]	87	asGfsggggaugagCfuCfaGfcgasusu	234	3mpk	-66.76
88	ucgcugAfgCfUfCfAfucccccuus[invAb]	88	asGfsggggAfugagCfuCfagcgasusu	235	3mpk	-73.73
89	ucgcugAfgCfUfCfAfucccccs[invAb]	89	asGfsggggAfugagCfuCfagcsgsa	236	3mpk	-74.58
90	ggcucaAfcGfGfUfUfcccuusgs[invAb]	90	usCfsaAfgGfGfaaccGfuUfgagccsusu	237	3mpk	-68.23

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
91	ggcuCfaAfCfGfGfGfuucccuugs[invAb]	91	usCfsaaggGfaaccguUfgAfgccsusu	238	3mpk	-94.43
92	ggcuCfaAfcGfguucccuugs[invAb]	92	usCfsaagggaaccGfuUfgAfgccsusu	239	3mpk	-89.89
93	ggcucaAfcGfGfGfUfUfcccuugaus[invAb]	93	usCfsaaggGfaaccGfuUfgagccsusu	240	3mpk	-94.81
94	ggcucaAfcGfGfGfUfUfcccuugs[invAb]	94	usCfsaaggGfaaccGfuUfgagscsc	241	3mpk	-91.36
95	gcggcgagAfuUfUfUfUfCfcccuacs[invAb]	95	asGfsuaggGfgaaaAfuCfucgccgcsusu	242	--	--
96	cggcucaaCfgGfUfUfUfCfccuugas[invAb]	96	asUfscaagGfgaacCfgUfugagccgsusu	243	3mpk	-87.69
97	gcggcgAfgAfUfUfUfUfucccccuacs[invAb]	97	asGfsuaggGfgaaaauCfuCfgccgcsusu	244	--	--
98	cggcucAfaCfGfGfUfUfcccuugas[invAb]	98	asUfscaagGfgaaccgUfuGfagccgsusu	245	3mpk	-94.03
99	gcggcgAfgAfuUfuuccccuacs[invAb]	99	asGfsuagggaaaAfuCfuCfgccgcsusu	246	--	--
100	cggcucAfaCfgGfuuccccuugas[invAb]	100	asUfscaagggaacCfgUfuGfagccgsusu	247	3mpk	-67.91
101	ggcgagAfuUfUfUfUfCfcccuacuus[invAb]	101	asGfsuaggGfgaaaAfuCfucgccsgsc	248	--	--
102	gcucaaCfgGfUfUfUfCfccuugauus[invAb]	102	asUfscaagGfgaacCfgUfugagcscsg	249	3mpk	-70.86
103	gcggcgagAfuUfUfUfUfCfcccuacs[invAb]	103	asGfsuaggGfgaaaAfuCfucgccsgsc	250	--	--
104	cggcucaaCfgGfUfUfUfCfccuugas[invAb]	104	asUfscaagGfgaacCfgUfugagcscsg	251	3mpk	-92.8
105	agaagauaUfcUfCfGfGfgccuucs[invAb]	105	asGfsaaggCfccgaGfaUfaucuuscsu	252	--	--
106	agaagauaUfcUfCfGfGfgccuucs[invAb]	106	asGfsaaggCfccgaGfaUfaucuucusu	253	--	--
107	agaagaUfaUfCfUfUfCfgggccuucs[invAb]	107	asGfsaaggCfccgagaUfaUfcuucusu	254	--	--
108	agaagaUfaUfcUfcgggccuucs[invAb]	108	asGfsaaggcccgaGfaUfaUfcuucusu	255	--	--
109	aagauaUfcUfCfGfGfgccuucuus[invAb]	109	asGfsaaggCfccgaGfaUfaucuuscsu	256	--	--
110	ccuggucc AfcGfUfUfGfCfacuucas[invAb]	110	usUfsgaagUfgcacguGfgAfccaggsusu	257	--	--
111	gcggcgagAfuUfUfUfUfCfcccuacs[invAb]	111	asGfsuaggGfgaaaauCfuCfgccgcsusu	258	--	--
112	cggcucaaCfgGfUfUfUfCfccuugas[invAb]	112	asUfscaagGfgaaccgUfuGfagccgsusu	259	--	--
113	agaagauaUfcUfCfGfGfgccuucs[invAb]	113	asGfsaaggCfccgagaUfaUfcuucususu	260	--	--

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
114	ccugguccAfcGfUfGfCfacuucas[invAb]	114	usUfsgaagUfgcacGfuGfgAfccaggsus u	261	--	--
115	gcggcgagAfuUfUfUfCfcccuacs[invAb]	115	asGfsuaggGfgaaaAfuCfuCfgccgcsusu	262	--	--
116	cggcucaaCfgGfUfUfCfccuugas[invAb]	116	asUfscaagGfgaacCfgUfuGfagccgsusu	263	--	--
117	agaagauaUfcUfCfGfGfgccuucs[invAb]	117	asGfsaaggCfccgaGfaUfaUfcuucususu	264	--	--
118	ccugguCfcAfcGfugcacuucas[invAb]	118	usUfsgaaGfUfgcacguGfgAfccaggsus u	265	--	--
119	gcggcgAfgAfuUfuucccuacs[invAb]	119	asGfsuagGfGfgaaaauCfuCfgccgcsusu	266	--	--
120	cggcucAfaCfgGfuucccuugas[invAb]	120	asUfscaaGfGfgaaccgUfuGfagccgsusu	267	--	--
121	agaagaUfaUfcUfcgggccuucs[invAb]	121	asGfsaagGfCfccgagaUfaUfcuucususu	268	--	--
122	ggagauUfgGfUfGfUfcgcugas[invAb]	122	asUfscagcGfacaccaAfuCfuccsusu	269	--	--
123	ucgcugAfgCfUfCfAfucccccs[invAb]	123	asGfsggggAfugagcuCfaGfcgasusu	270	--	--
124	acaccaUfcAfCfGfUfugcagcs[invAb]	124	asGfscugcAfacgugaUfgGfugususu	271	--	--
125	ggcucaAfcGfGfGfUfUfcccuugs[invAb]	125	usCfsaaggGfaaccguUfgAfgccsusu	272	--	--
126	ggagauUfgGfUfGfUfcgcugas[invAb]	126	asUfscagcGfacacCfaAfuCfuccsusu	273	--	--
127	ggagAfuUfgGfugucgcugas[invAb]	127	asUfscagCfGfacaccaAfuCfuccsusu	274	--	--
128	ucgcugAfgCfUfCfAfucccccs[invAb]	128	asGfsggggAfugagCfuCfaGfcgasusu	275	--	--
129	ucgcUfgAfgCfucauccccccs[invAb]	129	asGfsgggGfAfugagcuCfaGfcgasusu	276	--	--
130	acaccaUfcAfCfGfUfugcagcs[invAb]	130	asGfscugcAfacguGfaUfgGfugususu	277	--	--
131	acacCfaUfcAfcguugcagcs[invAb]	131	asGfscugCfAfacgugaUfgGfugususu	278	--	--
132	ggcucaAfcGfGfGfUfUfcccuugs[invAb]	132	usCfsaaggGfaaccGfuUfgAfgccsusu	279	--	--
133	ggcuCfaAfcGfguucccuugs[invAb]	133	usCfsaagGfGfaaccguUfgAfgccsusu	280	--	--
134	ucgcugAfgCfUfCfAfucccccs[invAb]	134	asGfsgGfgGfgAfugagCfuCfagcsgsa	281	--	--
135	ucgcugAfgCfUfCfAfucccccscs[invAb]	135	asGfsgGfgGfgAfugagcuCfaGfcgasusu	282	--	--

Duplex No.	Sense (5'-3')	SEQ ID NO: (sense)	Antisense (5'-3')	SEQ ID NO: (antisense)	BLI Dose	BLI wk4 % silencing
136	ugguCfcAfCfGfUfgcacuucaaus[invAb]	136	usUfsgaagUfgcacguGfgAfccasgsg	283	--	--
137	ugguccAfcGfUfGfCfacuucaaus[invAb]	137	usUfsgaagUfgcacguGfgAfccasgsg	284	--	--
138	ugguccAfcGfUfGfCfacuucaaus[invAb]	138	usUfsgaagUfgcacGfuGfgAfccasgsg	285	--	--
139	ggagauUfgGfUfGfUfcgcugauus[invAb]	139	asUfscagcGfacaccaAfuCfuccsusu	286	--	--
140	ggagauUfgGfUfGfUfcgcugauus[invAb]	140	asUfscagcGfacacCfaAfuCfuccsusu	287	--	--
141	ggagAfuUfGfGfUfgucgcugauus[invAb]	141	asUfscagcGfacaccaAfuCfuccsusu	288	--	--
142	acaccaUfcAfCfGfUfugcagcuus[invAb]	142	asGfscugcAfacguGfaUfgGfugususu	289	--	--
143	acaccaUfcAfCfGfUfugcagcuus[invAb]	143	asGfscugcAfacgugaUfgGfugususu	290	--	--
144	ggcuCfaAfCfGfGfuucccuugaus[invAb]	144	usCfsaaggGfaaccguUfgAfgccsusu	291	--	--
145	ggcuCfaAfCfGfGfuucccuugs[invAb]	145	usCfsaaggGfaaccguUfgAfgscsc	292	--	--
146	cggcucAfaCfGfGfUfucccuugas[invAb]	146	asUfscaagGfgaaccgUfuGfagcscsg	293	--	--
147	cggcucAfaCfGfGfUfucccuugas[invAb]	147	asUfscaagGfgaacCfgUfuGfagcscsg	294	--	--

[0178] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention. To the extent that any of the definitions or terms provided in the references incorporated by reference differ from the terms and discussion provided herein, the present terms and definitions control.

[0179] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and examples detail certain preferred embodiments of the invention and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

CLAIMS:

1. An RNAi construct comprising a sense strand and an antisense strand, wherein the antisense strand comprises a region having at least 15 contiguous nucleotides differing by no more than 3 nucleotides from an antisense sequence listed in Table 1, and wherein the RNAi construct inhibits the expression of a SREBP Cleavage Activating Protein (SCAP) mRNA.

2. The RNAi construct of claim 1, wherein the sense strand comprises a sequence that is sufficiently complementary to the sequence of the antisense strand to form a duplex region of about 15 to about 30 base pairs in length.

3. The RNAi construct of claim 1 or claim 2, wherein the duplex region is about 17 to about 24 base pairs in length.

4. The RNAi construct of any one of claims 1-3, wherein the duplex region is about 19 to about 21 base pairs in length.

5. The RNAi construct of claim 4, wherein the duplex region is 19 base pairs in length.

6. The RNAi construct of any one of claims 1-5, wherein the sense strand and the antisense strand are each about 15 to about 30 nucleotides in length.

7. The RNAi construct of claim 6, wherein the sense strand and the antisense strand are each about 19 to about 27 nucleotides in length.

8. The RNAi construct of claim 7, wherein the sense strand and the antisense strand are each about 21 to about 25 nucleotides in length.

9. The RNAi construct of claim 8, wherein the sense strand and the antisense strand are each about 21 to about 23 nucleotides in length.

10. The RNAi construct of any one of claims 1-9, which comprises at least one blunt end.

11. The RNAi construct of any one of claims 1-10, which comprises at least one nucleotide overhang of 1 to 4 unpaired nucleotides.

12. The RNAi construct of claim 11, wherein the nucleotide overhang has two unpaired nucleotides.

13. The RNAi construct of claim 11 or 12, wherein the RNAi construct comprises a nucleotide overhang at the 3' end of the sense strand, the 3' end of the antisense strand, or the 3' end of both the sense strand and the antisense strand.

14. The RNAi construct of any one of claims 11-13, wherein the nucleotide overhang comprises a 5'-UU-3' dinucleotide or a 5'-dTdT-3' dinucleotide.

15. The RNAi construct of any one of claims 1-14, wherein the RNAi construct comprises at least one modified nucleotide.

16. The RNAi construct of claim 15, wherein the modified nucleotide is a 2'-modified nucleotide.

17. The RNAi construct of claim 15, wherein the modified nucleotide is a 2'-fluoro modified nucleotide, a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-O-allyl modified nucleotide, a bicyclic nucleic acid (BNA), a glycol nucleic acid, an inverted nucleotide, or combinations thereof.

18. The RNAi construct of claim 17, wherein the modified nucleotide is a 2'-O-methyl modified nucleotide, a 2'-O-methoxyethyl modified nucleotide, a 2'-fluoro modified nucleotide, or combinations thereof.

19. The RNAi construct of claim any one of claims 15-18, wherein all of the nucleotides in the sense and antisense strands are modified nucleotides.

20. The RNAi construct of claim 19, wherein the modified nucleotides are 2'-O-methyl modified nucleotides, 2'-fluoro modified nucleotides, or combinations thereof.

21. The RNAi construct of any one of claims 1-20, which comprises at least one phosphorothioate internucleotide linkage.

22. The RNAi construct of claim 21, wherein the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at the 3' end of the antisense strand.

23. The RNAi construct of claim 21, wherein the RNAi construct comprises two consecutive phosphorothioate internucleotide linkages at both the 3' and 5' ends of the antisense strand and two consecutive phosphorothioate internucleotide linkages at the 5' end of the sense strand.

24. The RNAi construct of any one of claims 1-23, wherein the antisense strand comprises a sequence selected from the antisense sequences listed in Table 1.

25. The RNAi construct of any one of claims 1-24, wherein the sense strand comprises a sequence selected from the sense sequences listed in Table 1.

26. The RNAi construct of any one of claims 1-25, wherein the RNAi construct is any one of the duplex compounds listed in Table 1.

27. The RNAi construct of any one of claims 1-26, which is a small interfering RNA (siRNA).

28. The RNAi construct of any one of claims 1-27, wherein the RNAi construct reduces the expression level of SCAP in liver cells following incubation with the RNAi construct as compared to the SCAP expression level in liver cells that have been incubated with a control RNAi construct.

29. The RNAi construct of claim 28, wherein the liver cells are Hep3B or HepG2 cells.

30. The RNAi construct of any one of claims 1-29, wherein the RNAi construct inhibits at least 40% of SCAP expression at 5 nM in Hep3B cells *in vitro*.

31. The RNAi construct of any one of claims 1-29, wherein the RNAi construct inhibits at least 40% of SCAP expression at 5 nM in HepG2 cells *in vitro*.

32. The RNAi construct of any one of claims 1-31, wherein the RNAi construct inhibits SCAP expression in Hep3B cells with an IC50 of less than about 5 nM.

33. The RNAi construct of any one of claims 1-31, wherein the RNAi construct inhibits SCAP expression in HepG2 cells with an IC50 of less than about 5 nM.

34. The RNAi construct of any one of claims 1-33, further comprising a ligand that binds to one or more proteins expressed on the surface of liver cells.

35. A composition comprising the RNAi construct of any one of claims 1-34 and a pharmaceutically acceptable carrier, excipient, or diluent.

36. A method for reducing the expression of SCAP in a patient in need thereof comprising administering to the patient the RNAi construct of any one of claims 1-34.

37. A method for reducing the expression of SCAP in a patient in need thereof comprising administering to the patient the composition of claim 35.

38. The method of claim 36 or claim 37, wherein the expression level of SCAP in hepatocytes is reduced in the patient following administration of the RNAi construct as compared to the SCAP expression level in a patient not receiving the RNAi construct.

39. The method of any one of claims 36-38, wherein the patient suffers from nonalcoholic fatty liver disease (NAFLD).

40. The method of claim 39, wherein the patient suffers from non-alcoholic steatohepatitis (NASH).

41. An RNAi construct of any one of claims 1-34 or a composition of claim 35 for use in the treatment of NAFLD.

42. Use of an RNAi construct of any one of claims 1-34 or a composition of claim 35 for the preparation of a medicament for treating NAFLD.