KETOHEXOKINASE (KHK) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 63/154,005, filed on February 26, 2021, U.S. Provisional Application No. 63/223,581, filed on July 20, 2021, and U.S. Provisional Application No. 63/280,668, filed on November 18, 2021. The entire contents of each of the foregoing applications are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 16, 2022, is named 121301_14720_SL.txt and is 572,214 bytes in size.

BACKGROUND OF THE INVENTION

Epidemiological studies have shown that a western diet is one of the leading causes of the modern obesity pandemic. Increase in fructose uptake, associated with the use of enriched soft drinks and processed food, is proposed to be a major contributing factor to the epidemic. High fructose corn sweeteners started gaining widespread use in the food industry by 1967. Although glucose and fructose have the same caloric value per molecule, the two sugars are metabolized differently and utilize different GLUT transporters. Fructose is almost exclusively metabolized in the liver, and unlike the glucose metabolism pathway, the fructose metabolism pathway is not regulated by feedback inhibition by the product (Khaitan Z *et al* ., (2013) *J. Nutr. Metab.* 2013, Article ID 682673, 1-12). While hexokinase and phosphofructokinase (PFK) regulate the production of glyceraldehyde-3-P from glucose, fructokinase or ketohexokinase (KHK), which is responsible for phosphorylation of fructose to fructose-1-phosphate in the liver, it is not down regulated by increasing concentrations of fructose-1-phosphate. As a result, all fructose entering the cell is rapidly phosphorylated. (Cirillo P. *et al* ., (2009) *J. Am. Soc. Nephrol.* 20: 545-553). Continued utilization of ATP to phosphorylate the fructose to fructose-1-phosphate results in intracellular phosphate depletion, ATP depletion, activation of AMP deaminase and formation of uric acid (Khaitan Z. *et al* ., (2013) *J. Nutr. Metab.* Article ID 682673, 1-12). Increased uric acid further stimulates the up-regulation of KHK (Lanaspa M.A. *et al* ., (2012) *PLOS ONE* 7(10): 1-11) and causes endothelial cell and adipocyte dysfunction. Fructose-1-phosphate is subsequently converted to glyceraldehyde by the action of aldolase B and is phosphorylated to glyceraldehyde-3-phosphate. The latter proceeds downstream to the glycolysis pathway to form pyruvate, which enters the citric acid cycle, wherefrom, under well-fed conditions, citrate is exported to the cytosol from the mitochondria, providing Acetyl Coenzyme A for lipogenesis (Figure 1).

The phosphorylation of fructose by KHK, and subsequent activation of lipogenesis leads to, for example, fatty liver, hypertriglyceridemia, dyslipidemia, and insulin resistance. Proinflammatory changes in renal proximal tubular cells have also been shown to be induced by

KHK activity (Cirillo P. *et al* ., (2009) *J. Am. Soc. Nephrol.* 20: 545-553). The phosphorylation of fructose by KHK is associated with diseases, disorders or conditions such as liver disease (*e.g.*, fatty liver, steatohepatitis), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.,* hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving. Accordingly, there is a need in the art for compositions and methods for treating diseases, disorders, and conditions associated with KHK activity.

SUMMARY OF THE INVENTION

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding ketohexokinase (KHK). The KHK gene may be within a cell, *e.g.*, a cell within a subject, such as a human subject.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:2. In one embodiment, the dsRNA agent comprises at least one thermally destabilizing nucleotide modification, *e.g.*, an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), or a glycerol nucleic acid (GNA), *e.g.*, the antisense strand comprises at least one thermally destabilizing nucleotide modification.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding KHK, and wherein the region of complementarity comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23,contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 5, 6, and 8-13.

In one embodiment, the dsRNA agent comprises a sense strand comprising a contiguous nucleotide sequence which has at least 85%, e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6 and 8-13 and an antisense strand

comprising a contiguous nucleotide sequence which has at least 85%, e.g., 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6 and 8-13.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6 and 8-13 and an antisense strand comprising at least 15, e.g., 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6 and 8-13.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6 and 8-13 and an antisense strand comprising at least 15, e.g., 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6 and 8-13.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6 and 8-13 and an antisense strand comprising at least 15, e.g., 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6 and 8-13.

In one embodiment, the dsRNA agent comprises a sense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6 and 8-13 and an antisense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6 and 8-13.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 120-162; 164-188; 181-207; 193-217; 209-231; 283-306; 508-546; 568-603; 596-632; 640-674; 746-806; 806-835; 917-942; 936-084; 1016-1041; 1100-1123; 1149-1175; 1160-1193; 1205-1229; 1252-1283; 1334-1356; 1407-1429; 1472-1497; 1506-1533; 1539-1561; 1704-1727; 1747-1787; 1850-1873; 1936-1964; 1960-1990; 2015-2048; 2060-2095; 2090-2118; 2124-2160; 2181-2200; 2221-2262 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides

differing by no more than three, *e.g.,* 3, 2, 1, or 0, from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 517-539; 521-543; 524-546; 517-546; 581-603; 610-632; 747-769; 749-771; 752-774; 755-777; 757-779; 758-780; 764-786; 776-798; 781-803; 747-803; 920-942; 941-963; 944-966; 950-972; 962-984; 920-984; 1149-1171; 1161-1183; 1165-1187; 1171-1193; 1149-1193; 1205-1227; 1206-1228; 1205-1228; 1334-1356; 1472-1494; 1475-1497; 1472-1497 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0,from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the nucleotide sequence of nucleotides 517-539; 524-546; 517-546; 753-775; 757-779; 753-779; 764-786; 767-789; 768-790; 769-791; 764-791; 773-795; 781-803; 773-803; 753-803; 808-830; 937-959; 941-963; 944-966; 941-966; 948-970; 950-972; 948-972; 1160-1182; 1161-1183; 1160-1183; and 1207-1229 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by nor more than 0, 1, 2, or 3 nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1613400; AD-1613243; AD-1290757.3; AD-1290878.3; AD-1290969.3; AD-1423317.2; AD-1423327.2; AD-1423336.2; AD-1290599.3; AD-1523172.1; AD-1290837.3; AD-1523173.1; AD-1290884.3; AD-1523174.1; AD-1290959.3; AD-1523175.1; AD-1423311.2; AD-1423324.2; AD-1523176.1; AD-1423329.2; AD-1423333.2; AD-1423330.2; AD-1523177.1; AD-1290885.3; AD-1523178.1; AD-1423334.2; AD-1523179.1; AD-1523180.1; AD-1290539.3; and AD-1523181.1.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the nucleotide sequence of nucleotides 495-517, 492-517, 500-529, 514-551, 517-539, 524-546, 517-548, 614-643, 625-647, 625-660, 642-664, 642-672, 753-811, 754-780, 762-791, 764-786, 772-800, 781-803, 805-827, 808-830, 809-831, 792-

838, 931-982, 944-966, 947-969, 948-970, 948-982, 1011-1035, 1021-1043, 1019-1050, 1063-1091, 1150-1192, 1152-1176, 1160-1192, 1160-1182, 1162-1184, 1198-1230, 1198-1221, and 1202-1230 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the nucleotide sequence of nucleotides 513-556, 753-813, 936-981, 1155-1193, 1200-1229, 1704-1727, 1747-1787, 1850-1873, 1936-1964, 1960-1990, 1936-1990, 2015-2048, 2060-2095, 2090-2118, 2060-2118, 2124-2160, 2181-2220, 2221-2249, 2181-2249, 2240-2262, 2221-2262, and 2181-2262 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22 or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence of nucleotides 1207-1229 or 937-959 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence of nucleotides 1207-1229 of SEQ ID NO: 1, and the antisense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the dsRNA agent comprises at least one modified nucleotide.

In one embodiment, substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

In one embodiment, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), *e.g.,* Ggn, Cgn, Tgn, or Agn, a nucleotide comprising a 2' phosphate, *e.g.,* G2p, C2p, A2p, U2p, a vinyl-phosphonate nucleotide; and combinations thereof.

In another embodiment, at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

In one embodiment, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

In some embodiments, the modified nucleotide comprises a short sequence of 3'-terminal deoxythimidine nucleotides (dT).

In some embodiments, the modifications on the nucleotides are 2'-O-methyl, GNA and 2'fluoro modifications.

In some embodiments, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some embodiments, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

The double stranded region may be 19-30 nucleotide pairs in length; 19-25 nucleotide pairs in length;19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

In one embodiment, each strand is independently no more than 30 nucleotides in length.

In one embodiment, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

In one embodiment, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides.

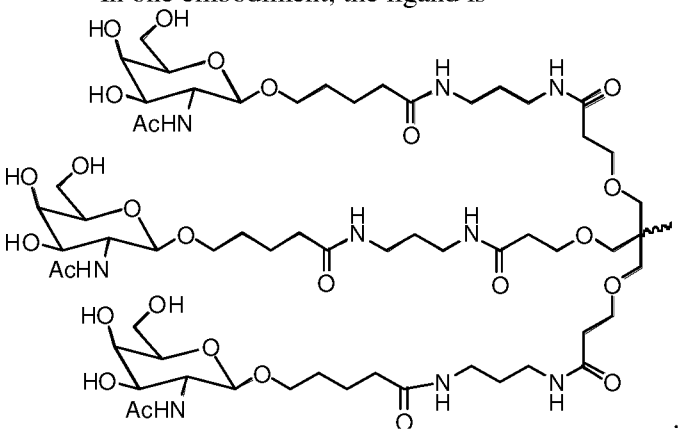
In one embodiment, the dsRNA agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

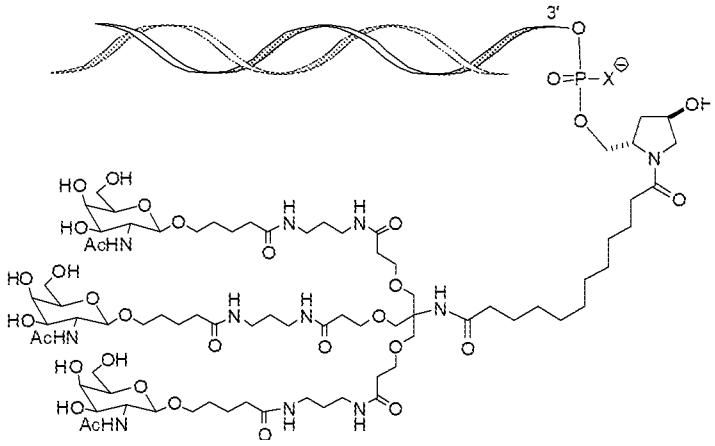
In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is



.

In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, the X is O.

In one embodiment, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.,* the antisense strand or the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

In one embodiment, the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

In one aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, or a pharmaceutically acceptable salt thereof, comprising a sense strand and an antisense strand forming a double stranded region, wherein

a) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-asdCsgadAudCucagdTgCfuuccugcsasc-3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-asdCsaadAcdAccacdGuCfuccguagscsc -3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C,

and U; Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

In one embodiment,

a) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-gscsaggaagCfAfCfugagauucgu-3’ and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-asdCsgadAudCucagdTgCfuuccugcsasc-3’, wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5’-csusacggagAfCfGfugguguuugu-3’ and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5’-asdCsaadAcdAccacdGuCfuccguagscsc -3’, wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

In one embodiment,

a) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5’-gscsaggaagCfAfCfugagauucgu-3’ and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5’-asdCsgadAudCucagdTgCfuuccugcsasc-3’, wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5’-csusacggagAfCfGfugguguuugu-3’ and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5’-asdCsaadAcdAccacdGuCfuccguagscsc -3’, wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

In one embodiment,

a) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5’-gscsaggaagCfAfCfugagauucgu-3’ and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5’-asdCsgadAudCucagdTgCfuuccugcsasc-3’, wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2’-fluoro (2’-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5’-csusacggagAfCfGfugguguuugu-3’ and the nucleotide sequence of the

antisense strand differs by no more than 1 base from the nucleotide sequence 5'-asdCsaadAcdAccacdGuCfuccguagscsc -3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

In one embodiment,

a) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'- asdCsgadAudCucagdTgCfuuccugcsasc-3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'- asdCsaadAcdAccacdGuCfuccguagscsc -3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

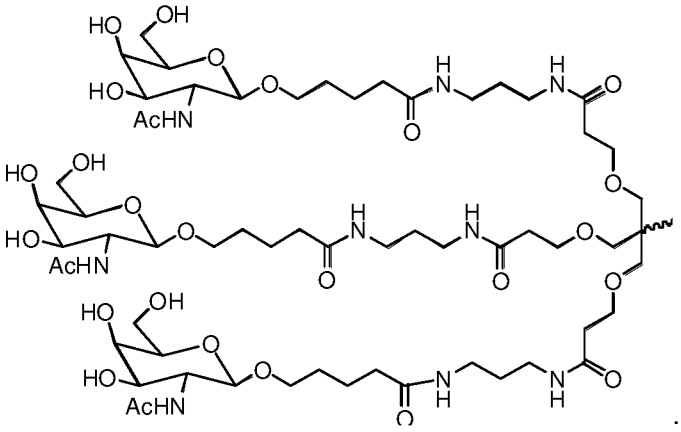
In one embodiment, the dsRNA agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

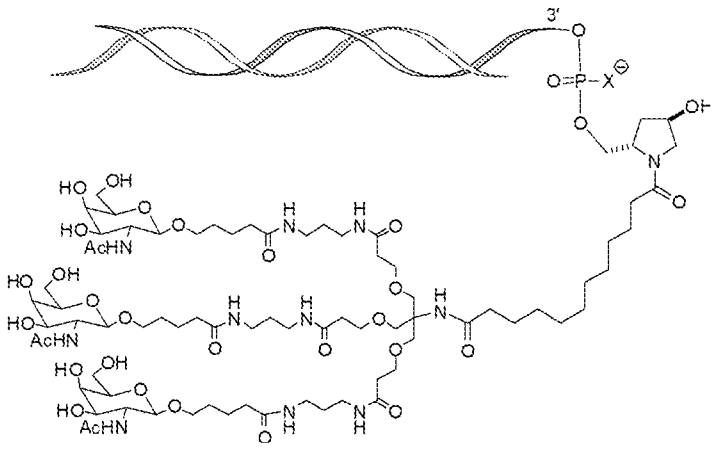
In one embodiment, the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

In one embodiment, the ligand is



.

In one embodiment, the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

In one embodiment, X is O.

The present invention also provides cells containing any of the dsRNA agents of the invention and pharmaceutical compositions comprising any of the dsRNA agents of the invention.

The pharmaceutical composition of the invention may include dsRNA agent in an unbuffered solution, *e.g.,* saline or water, or the pharmaceutical composition of the invention may include the dsRNA agent is in a buffer solution, *e.g.,* a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one aspect, the present invention provides a method of inhibiting expression of a ketohexokinase (KHK) gene in a cell. The method includes contacting the cell with any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby inhibiting expression of the KHK gene in the cell.

In one embodiment, the cell is within a subject, *e.g.,* a human subject, *e.g.,* a subject having a ketohexokinase (KHK)-associated disorder, such as a KHK-associate disorder selected from the group consisting of liver disease (*e.g.,* fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.,* hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

In one embodiment, contacting the cell with the dsRNA agent inhibits the expression of KHK by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one embodiment, inhibiting expression of KHK decreases KHK protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

In one aspect, the present invention provides a method of treating a subject having a disorder that would benefit from reduction in ketohexokinase (KHK) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby treating the subject having the disorder that would benefit from reduction in KHK expression.

In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in ketohexokinase (KHK) expression. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in KHK expression.

In certain embodiments, the KHK-associated disorder is a liver disease, *e.g.*, fatty liver disease such as NAFLD or NASH. In certain embodiments, the KHK-associated disorder is dyslipidemia, *e.g.*, elevated serum triglycerides, elevated serum LDL, elevated serum cholesterol, lowered serum HDL, postprandial hypertriglyceridemia. In another embodiment, the KHK-associated disorder is a disorder of glycemic control, *e.g.*, insulin resistance not resulting from an immune response against insulin, glucose resistance, type 2 diabetes. In certain embodiments, the KHK-associated disorder is a cardiovascular disease, *e.g.*, hypertension, endothelial cell dysfunction. In certain embodiments, the KHK-associated disorder is a kidney disease, *e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease. In certain embodiments, the disease is metabolic syndrome. In certain embodiments, the KHK-associated disorder is a disease of lipid deposition or dysfunction, *e.g.*, visceral adipose deposition, fatty liver, obesity. In certain embodiments, the KHK-associated disorder is a disease of elevated uric acid, *e.g.*, gout, hyperuricemia. In certain embodiments the KHK-associated disorder is an eating disorder such as excessive sugar craving.

In certain embodiments, the administration of the dsRNA to the subject causes a decrease in fructose metabolism. In certain embodiments, the administration of the dsRNA causes a decrease in the level of KHK in the subject, especially hepatic KHK, especially KHK-C in a subject with elevated KHK. In certain embodiments, the administration of the dsRNA causes a decrease in fructose metabolism in the subject. In certain embodiments, the administration of the dsRNA causes a decrease in the level of uric acid, *e.g.*, serum uric acid, in a subject with elevated serum uric acid, *e.g.*, elevated serum uric acid associated with gout. In certain embodiments, the administration of the dsRNA causes a normalization of serum lipids, *e.g.*, triglycerides including postprandial triglycerides, LDL, HDL, or cholesterol, in a subject with at least one abnormal serum lipid level. In certain embodiments, the administration of the dsRNA causes a normalization of lipid deposition, *e.g.*, a decrease of lipid deposition in the liver (*e.g.*, decrease of NAFLD or NASH), a decrease of visceral fat deposition, a decrease in body weight. In certain embodiments, the administration of the dsRNA causes a normalization of insulin or glucose response in a subject with abnormal insulin response not

related to an immune response to insulin, or abnormal glucose response. In certain embodiments, the administration of the dsRNA results in an improvement of kidney function, or a stoppage or reduction of the rate of loss of kidney function. In certain embodiments, the dsRNA causes a reduction of hypertension, *i.e.*, elevated blood pressure.

In certain embodiments, the invention further comprises administering an additional agent to a subject with a KHK-associated disease. In certain embodiments, treatments known in the art for the various KHK-associated diseases are used in combination with the RNAi agents of the invention. Such treatments are discussed below.

In one embodiment, the subject is human.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

In one embodiment, the dsRNA agent is administered to the subject subcutaneously.

In one embodiment, the methods of the invention include further determining the level of KHK in a sample(s) from the subject.

In one embodiment, the level of KHK in the subject sample(s) is a KHK protein level in a blood or serum sample(s).

In one embodiment, the methods of the invention further comprise measuring the uric acid level, especially serum uric acid level, in the subject. In one embodiment, the methods of the invention further comprise measuring the urine fructose level in the subject. In one embodiment, the methods of the invention further comprise measuring a serum lipid level in a subject. In certain embodiments, the methods of the invention further include measuring insulin or glucose sensitivity in a subject. In certain embodiments, a decrease in the levels of expression or activity of fructose metabolism indicates that the KHK-associated disease is being treated or prevented.

The present invention also provides kits comprising any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, and optionally, instructions for use.

The present invention further provides an RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of the invention.

In another embodiment, the RNAi agent is a pharmaceutically acceptable salt thereof. "Pharmaceutically acceptable salts" of each of RNAi agents herein include, but are not limited to, a sodium salt, a calcium salt, a lithium salt, a potassium salt, an ammonium salt, a magnesium salt, an mixtures thereof. One skilled in the art will appreciate that the RNAi agent, when provided as a polycationic salt having one cation per free acid group of the optionally modified phosophodiester backbone and/or any other acidic modifications (e.g., 5'-terminal phosphonate groups). For example, an oligonucleotide of "n" nucleotides in length contains n-1 optionally modified phosophodiesters, so that an oligonucleotide of 21 nt in length may be provided as a salt having up to 20 cations (e.g, 20 sodium cations). Similarly, an RNAi agentshaving a sense strand of 21 nt in length and an antisense strand of 23 nt in length may be provided as a salt having up to 42 cations (e.g, 42 sodium cations). In the preceding example, where the RNAi agent also includes a 5'-terminal phosphate or a 5'-

terminal vinylphosphonate group, the RNAi agent may be provided as a salt having up to 44 cations (e.g, 44 sodium cations).

The present invention is further illustrated by the following detailed description and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the classic and alternative lipogenic pathways of fructose. In the classical pathway, triglycerides (TG) are a direct product of fructose metabolism by the action of multiple enzymes including aldolase B (Aldo B) and fatty acid synthase (FAS). In an alternative pathway, uric acid produced from the nucleotide turnover that occurs during the phosphorylation of fructose to fructose-1-phosphate (F-1-P) results in the generation of mitochondrial oxidative stress (mtROS), which causes a decrease in the activity of aconitase (ACO2) in the Krebs cycle. As a consequence, the ACO2 substrate, citrate, accumulates and is released to the cytosol where it acts as substrate for TG synthesis through the activation of ATP citrate lyase (ACL) and fatty acid synthase. AMPD2, AMP deaminase 2; IMP, inosine monophosphate; PO_4, phosphate (from Johnson *et al* . (2013) *Diabetes*. 62:3307-3315).

Figure 2 is a graph depicting the effect of administration of a single 3 mg/kg dose of the indicated duplexes on the level of KHK mRNA and KHK protein at Day 29 post-dose. The level of mRNA and protein are depicted as % remining normalized to the pre-dose level of mRNA and protein.

Figure 3 is a graph depicting the effect of administration of a single 3 mg/kg dose of the indicated duplexes on the level of KHK mRNA at Day 50 post-dose. The level of mRNA is depicted as % remining normalized to the pre-dose level of mRNA.

Figure 4 is a graph depicting the effect of administration of a single 3 mg/kg dose of the indicated duplexes on the level of KHK mRNA at Day 78 post-dose. The level of mRNA is depicted as % remining normalized to the pre-dose level of mRNA.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a ketohexokinase (KHK) gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (KHK gene) in mammals.

The iRNAs of the invention have been designed to target the human ketohexokinase (KHK) gene, including portions of the gene that are conserved in the ketohexokinase (KHK) orthologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved efficacy, stability, potency, durability, and safety.

Accordingly, the present invention provides methods for treating and preventing a ketohexokinase (KHK)-associated disorder, *e.g.*, liver disease (*e.g.,* fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.,* hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a KHK gene.

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, *e.g.*, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of a KHK gene.

In certain embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of a KHK gene. In some embodiments, such iRNA agents having longer length antisense strands can, for example, include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of iRNAs of the invention enables the targeted degradation of mRNAs of the corresponding gene (KHK gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting a KHK gene can potently mediate RNAi, resulting in significant inhibition of expression of a KHK gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having a KHK-associated disorder, *e.g.*, liver disease (*e.g.,* fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.,* hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

Accordingly, the present invention provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of a KHK gene, *e.g.*, a KHK-associated disorder, such as liver disease (*e.g.,* fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low

HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a KHK gene.

The present invention also provides methods for preventing at least one symptom in a subject having a disorder that would benefit from inhibiting or reducing the expression of a KHK gene, *e.g.*, liver disease (*e.g.*, fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a KHK gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of a KHK gene, *e.g.*, subjects susceptible to or diagnosed with a KHK-associated disorder.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. For example, “sense strand or antisense strand” is understood as “sense strand or antisense strand or sense strand and antisense strand.”

The term “about” is used herein to mean within the typical ranges of tolerances in the art. For example, “about” can be understood as about 2 standard deviations from the mean. In certain embodiments, about means $\pm$10%. In certain embodiments, about means $\pm$5%. When about is

present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least" , "no less than", or "or more" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 19 nucleotides of a 21 nucleotide nucleic acid molecule" means that 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "or less" is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range. As used herein, ranges include both the upper and lower limit.

As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

As used herein, "ketohexokinase," used interchangeably with the term "KHK," refers to the naturally occurring gene that encodes an enzyme that catalyzes conversion of fructose to fructose-1-phosphate. The product of this gene is the first enzyme in the pathway that catabolizes dietary fructose. Alternatively spliced transcript variants encoding different isoforms have been identified. The gene is also known as fructokinase.

Exemplary nucleotide and amino acid sequences of KHK can be found, for example, at GenBank Accession No. XM_017004061.1 (Homo sapiens KHK; SEQ ID NO:1; reverse complement, SEQ ID NO:2); NM_006488.3 (Homo sapiens KHK; SEQ ID NO:3; reverse complement, SEQ ID NO:4); NM_000221.3 (Homo sapiens KHK; SEQ ID NO:5; reverse complement, SEQ ID NO:6); GenBank Accession No. NM_001310524.1 (Mus musculus KHK; SEQ ID NO:7; reverse complement, SEQ ID NO:8); GenBank Accession No. NM_031855.3 (Rattus norvegicus KHK; SEQ ID NO:9; reverse complement, SEQ ID NO:10); GenBank Accession No. XM_005576322.2 (Macaca fascicularis KHK, SEQ ID NO:11; reverse complement, SEQ ID NO:12).

The KHK (Ketohexokinase) gene is located on chromosome 2p23 and encodes ketohexokinase, also known as fructokinase. KHK is a phosphotransferase enzyme with an alcohol as the phosphate acceptor. KHK belongs to the ribokinase family of carbohydrate kinases (Trinh *et al* ., *ACTA Cryst.*, D65: 201-211). Two isoforms of ketohexokinase have been identified, KHK-A and

KHK-C, that result from alternative splicing of the full length mRNA. These isoforms differ by inclusion of either exon 3a or 3c, and differ by 32 amino acids between positions 72 and 115. KHK-C mRNA is expressed at high levels, predominantly in the liver, kidney and small intestine. KHK-C has a much lower K_m for fructose binding than KHK-A, and as a result, is highly effective in phosphorylating dietary fructose. The sequence of a human KHK-C mRNA transcript may be found at, for example, GenBank Accession No. NM_006488.3 (Homo sapiens KHK; SEQ ID NO:3). The sequence of a human KHK-A mRNA transcript may be found at, for example GenBank Accession No. NM_000221.3; SEQ ID NO:5). The sequence of full-length human KHK mRNA is provided in GenBank Accession No. GI: XM_017004061.1 (SEQ ID NO:1).

The iRNA agents provided herein can be capable of silencing one or both KHK isoforms.

Additional examples of KHK mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the Macaca genome project web site.

Further information on KHK can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=khk.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term KHK, as used herein, also refers to variations of the KHK gene including variants provided in the SNP database. Numerous seuqnce variations within the KHK gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=KHK, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a KHK gene, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a KHK gene. In one embodiment, the target sequence is within the protein coding region of KHK.

The target sequence may be from about 19-36 nucleotides in length, *e.g.*, about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In certain embodiments, the target sequence is 19-23 nucleotides in length, optionally 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.,* Table 1). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms "iRNA", "RNAi agent," "iRNA agent,", "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.,* inhibits, the expression of a KHK gene in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.,* a KHK target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is 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). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.,* a KHK gene. Accordingly, the term "siRNA" is also used herein to refer to an iRNA as described above.

In certain embodiments, the RNAi agent may be a single-stranded siRNA (ssRNAi) that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded siRNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al* ., (2012) *Cell* 150:883-

894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al* ., (2012) *Cell* 150:883-894.

In certain embodiments, an "iRNA" for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a "double stranded RNA agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA", refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a KHK gene. In some embodiments of the invention, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide or a modified nucleotide. In addition, as used in this specification, an "iRNA" may include ribonucleotides with chemical modifications; an iRNA may include substantial modifications at multiple nucleotides. As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "iRNA" or "RNAi agent" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 19 to 36 base pairs in length, *e.g.*, about 19-30 base pairs in length, for example, about 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, or 36 base pairs in length, such as about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex region is 19-21 base pairs in length, *e.g.*, 21 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by 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

RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not be, 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 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. In one embodiment of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, a KHK gene, to direct cleavage of the target RNA.

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, a KHK target mRNA sequence, to direct the cleavage of the target RNA.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a double stranded iRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or *vice versa*, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense

strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.*, a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotides, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.*, 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, 10-25 nucleotides, 10-20 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the extended overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNA agent, *i.e.*, no nucleotide overhang. A "blunt ended" double stranded RNA agent is double stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with no nucleotide overhang at one end (*i.e.*, agents with one overhang and one blunt end) or with no nucleotide overhangs at either end. Most often such a molecule will be double-stranded over its entire length.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.*, a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a KHK mRNA.

As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* a KHK nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.*, within 5, 4, or 3 nucleotides of the 5'- or 3'-end of the iRNA. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4

mismatches with the target mRNA, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, *e.g.*, the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the sense strand. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the sense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the antisense strand, *e.g.*, the sense strand includes 4, 3, 2, 1, or 0 mismatches with the antisense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA agent. In some embodiments, the mismatch(s) is not in the seed region.

Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a KHK gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a KHK gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a KHK gene is important, especially if the particular region of complementarity in a KHK gene is known to have polymorphic sequence variation within the population.

The term "sense strand" or "passenger strand" as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing (see, *e.g.*, "Molecular Cloning: A Laboratory Manual, Sambrook, *et al* . (1989) Cold Spring Harbor Laboratory Press). Such conditions can be, for example, "stringent conditions", where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 oC or 70 oC for 12-16 hours followed by washing (see, *e.g.*, "Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, *e.g.*, within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression, in vitro or in vivo. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between two oligonucleotides or polynucleotides, such as the antisense strand of a double stranded RNA agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding a KHK gene). For example, a polynucleotide is complementary to at least a part of a KHK mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a KHK gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target KHK sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target KHK sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1, 3, 5, 7, 9, or 11, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, 9, or 11, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target KHK sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 120-162; 164-188; 181-207; 193-217; 209-231; 283-306; 508-546; 568-603; 596-632; 640-674; 746-806; 806-835; 917-942; 936-084; 1016-1041; 1100-1123; 1149-1175; 1160-1193; 1205-1229; 1252-1283; 1334-1356; 1407-1429; 1472-1497; 1506-1533; 1539-1561; 1704-1727; 1747-1787; 1850-1873; 1936-1964; 1960-1990; 2015-2048; 2060-2095; 2090-2118; 2124-2160; 2181-2200; 2221-2262 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target KHK sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 517-539; 521-543; 524-546; 517-546; 581-603; 610-632; 747-769; 749-771; 752-774; 755-777; 757-779; 758-780; 764-786; 776-798; 781-803; 747-803; 920-942; 941-963; 944-966; 950-972; 962-984; 920-984; 1149-1171; 1161-1183; 1165-1187; 1171-1193; 1149-1193; 1205-1227; 1206-1228; 1205-1228; 1334-1356; 1472-1494; 1475-1497; 1472-1497 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target KHK sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 517-539; 524-546; 517-546; 753-775; 757-779; 753-779; 764-786; 767-789; 768-790; 769-791; 764-791; 773-795; 781-803; 773-803; 753-803; 808-830; 937-959; 941-963; 944-966; 941-966; 948-970; 950-972; 948-972; 1160-1182; 1161-1183; 1160-1183; and

1207-1229 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target KHK sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 495-517, 492-517, 500-529, 514-551, 517-539, 524-546, 517-548, 614-643, 625-647, 625-660, 642-664, 642-672, 753-811, 754-780, 762-791, 764-786, 772-800, 781-803, 805-827, 808-830, 809-831, 792-838, 931-982, 944-966, 947-969, 948-970, 948-982, 1011-1035, 1021-1043, 1019-1050, 1063-1091, 1150-1192, 1152-1176, 1160-1192, 1160-1182, 1162-1184, 1198-1230, 1198-1221, and 1202-1230 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to a fragment of a target KHK sequence and comprise a contiguous nucleotide sequence which is at least 80% complementary over its entire length to a fragment of SEQ ID NO: 1 selected from the group of nucleotides 513-556, 753-813, 936-981, 1155-1193, 1200-1229, 1704-1727, 1747-1787, 1850-1873, 1936-1964, 1960-1990, 1936-1990, 2015-2048, 2060-2095, 2090-2118, 2060-2118, 2124-2160, 2181-2220, 2221-2249, 2181-2249, 2240-2262, 2221-2262, and 2181-2262 of SEQ ID NO: 1, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target KHK sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of any one of Tables 2, 3, 5, 6, and 8-13, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 5, 6, and 8-13, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target KHK sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 2, 4, 6, 8, 10, or 12, or a fragment of any one of SEQ ID NOs:2, 4, 6, 8, 10, or 12, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target KHK sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand

nucleotide sequences in any one of any one of Tables 2, 3, 5, 6, and 8-13, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2, 3, 5, 6, and 8-13, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In certain embodiments, the sense and antisense strands are selected from any one of duplexes AD-1613400; AD-1613243; AD-1290757.3; AD-1290878.3; AD-1290969.3; AD-1423317.2; AD-1423327.2; AD-1423336.2; AD-1290599.3; AD-1523172.1; AD-1290837.3; AD-1523173.1; AD-1290884.3; AD-1523174.1; AD-1290959.3; AD-1523175.1; AD-1423311.2; AD-1423324.2; AD-1523176.1; AD-1423329.2; AD-1423333.2; AD-1423330.2; AD-1523177.1; AD-1290885.3; AD-1523178.1; AD-1423334.2; AD-1523179.1; AD-1523180.1; AD-1290539.3; and AD-1523181.1.

In general, an "iRNA" includes ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a dsRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

In an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al* ., (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

The phrase "contacting a cell with an iRNA," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo* with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the iRNA may be put into physical contact with the cell by the individual performing the method, or alternatively, the iRNA may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the iRNA. Contacting a cell *in vivo* may be done, for example, by injecting the iRNA into or near the tissue where the cell is located, or by injecting the iRNA into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be

contacted is located. For example, the iRNA may contain or be coupled to a ligand, *e.g.*, GalNAc, that directs the iRNA to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an iRNA and subsequently transplanted into a subject.

In certain embodiments, contacting a cell with an iRNA includes "introducing" or "delivering the iRNA into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, iRNA can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

The term "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.*, an iRNA or a plasmid from which an iRNA is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a bird that expresses the target gene, either endogenously or heterologously. In an embodiment, the subject is a human, such as a human being treated or assessed for a disease or disorder that would benefit from reduction in KHK expression; a human at risk for a disease or disorder that would benefit from reduction in KHK expression; a human having a disease or disorder that would benefit from reduction in KHK expression; or human being treated for a disease or disorder that would benefit from reduction in KHK expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result, such as reducing at least one sign or symptom of a KHK-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted KHK expression; diminishing the extent of unwanted KHK activation or stabilization; amelioration or palliation of unwanted KHK activation or stabilization. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.The term "lower" in the context of the level of KHK in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.*, protein or gene expression level. "Lower" in the context of the level of KHK in a subject is a

decrease to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, "lower" is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual, *e.g.*, the level of decrease in bodyweight between an obese individual and an individual having a weight accepted within the range of normal.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, may be treated or ameliorated by a reduction in expression of a KHK gene, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.*, a symptom of unwanted or excessive KHK expression and/or activity, *e.g.,* increased fructose metabolism, elevated uric acid and lipid levels. Without being bound by mechanism, it is known that fructose phosphorylation catalyzed by KHK to form fructose-1-phosphate is not regulated by feedback inhibition which can result in depletion of ATP and intracellular phosphate, and increased AMP levels, which results in the production of uric acid. Further, the fructose-1-phosphate is metabolized to glyceraldehyde which feeds into the citric acid cycle increasing the production of acetyl Co-A stimulating fatty acid synthesis. Diseases and conditions associated with elevated uric acid and fatty acid synthesis include, *e.g.*, liver disease (*e.g.*, fatty liver, steatohepatitis including non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance not related to immune response to insulin, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, disease of lipid deposition or dysfunction (*e.g.*, adipocyte dysfunction, visceral adipose deposition, obesity), disease of elevated uric acid (*e.g.*, hyperuricemia, gout), and eating disorders such as excessive sugar craving. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom or comorbidity associated with such a disease, disorder or condition (*e.g.*, by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed signs or symptoms or disease progression by days, weeks, months or years is considered effective prevention.

As used herein, the term " ketohexokinase (KHK)-associated disease" or "KHK-associated disorder," is a disease or disorder that is caused by, or associated with KHK gene expression or KHK protein production. The term "KHK-associated disease" includes a disease, disorder or condition that would benefit from a decrease in KHK gene expression, replication, or protein activity. Non-limiting examples of KHK-associated diseases include, for example, liver disease (*e.g.*, fatty liver, steatohepatitis including non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance not related to immune response to insulin, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory

changes to the proximal tubules, chronic kidney disease), metabolic syndrome, disease of lipid deposition or dysfunction (*e.g.*, adipocyte dysfunction, visceral adipose deposition, obesity), disease of elevated uric acid (*e.g.*, hyperuricemia, gout), and eating disorders such as excessive sugar craving.

In certain embodiments, a KHK-associated disease is associated with elevated uric acid (*e.g.* hyperuricemia, gout).

In certain embodiments, a KHK-associated disease is associated with elevated lipid levels (*e.g.*, fatty liver, steatohepatitis including non-alcoholic steatohepatitis (NASH), dyslipidemia).

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a KHK-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a KHK-associated disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired effect at a reasonable benefit/risk ratio applicable to any treatment. The iRNA employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds (including salts), materials, compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the

subject being treated. Such carriers are known in the art. Pharmaceutically acceptable carriers include carriers for administration by injection.

The term "lower" in the context of the level of KHK gene expression or KHK protein production in a subject, or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection for the detection method. In certain embodiments, the expression of the target is normalized, *i.e.*, decreased towards or to a level accepted as within the range of normal for an individual without such disorder, *e.g.*, normalization of body weight, blood pressure, or a serum lipid level. As used here, "lower" in a subject can refer to lowering of gene expression or protein production in a cell in a subject does not require lowering of expression in all cells or tissues of a subject. For example, as used herein, lowering in a subject can include lowering of gene expression or protein production in the liver of a subject.

The term "lower" can also be used in association with normalizing a symptom of a disease or condition, *i.e.* decreasing the difference between a level in a subject suffering from a KHK-associated disease towards or to a level in a normal subject not suffering from a KHK-associated disease. For example, if a subject with a normal weight of 70 kg weighs 90 kg prior to treatment (20 kg overweight) and 80 kg after treatment (10 kg overweight), the subject's weight is lowered towards a normal weight by 50% (10/20 x 100%). Similarly, if the HDL level of a woman is increased from 50 mg/dL (poor) to 57 mg/dL, with a normal level being 60 mg/dL, the difference between the prior level of the subject and the normal level is decreased by 70% (difference of 10 mg/dL between subject level and normal is decreased by 7 mg/dL, 7/10 x 100%). As used herein, if a disease is associated with an elevated value for a symptom, "normal" is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, "normal" is considered to be the lower limit of normal.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In some embodiments, a "sample derived from a subject" refers to urine obtained from the subject. A "sample derived from a subject" can refer to blood (which can be readily converted to plasma or serum) drawn from the subject.

II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a KHK gene. In certain embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a KHK gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human having or susceptible to developing a KHK-associated disease. The dsRNAi agent includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a KHK gene. The region of complementarity is about 30 nucleotides or less in length (*e.g.,* about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the KHK gene, the iRNA inhibits the expression of the KHK gene (*e.g.*, a human, a primate, a non-primate, or a rat KHK gene) by at least about 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In certain embodiments, inhibition of expression is determined by the qPCR method provided in the examples herein with the siRNA at, *e.g.*, a 10 nM concentration, in an appropriate organism cell line provided therein. In certain embodiments, inhibition of expression in vivo is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, a mouse or an AAV-infected mouse expressing the human target gene, *e.g.*, when administered as single dose, *e.g.*, at 3 mg/kg at the nadir of RNA expression.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of a KHK gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, *e.g.*, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex structure is 18 to 25 base pairs in length, *e.g.*, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-25, 20-24,20-23, 20-22, 20-21, 21-25, 21-24, 21-23, 21-22, 22-25, 22-24, 22-23, 23-25, 23-24 or 24-25 base pairs in length, for example, 19-21 basepairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

Similarly, the region of complementarity to the target sequence is 15 to 30 nucleotides in length, *e.g.*, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, for example 19-23 nucleotides in length or 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure. In some embodiments, the duplex structure is 19 to 30 base pairs in length. Similarly, the region of complementarity to the target sequence is 19 to 30 nucleotides in length.

In some embodiments, the dsRNA is about 19 to about 23 nucleotides in length, or about 25 to about 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.*, cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.*, a duplex region of about 19 to about 30 base pairs, *e.g.*, about 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.*, 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target KHK gene expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.*, 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art. Double stranded RNAi compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or

solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Similarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 2, 3, 5, 6, and 8-13, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2, 3, 5, 6, and 8-13. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a KHK gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Tables 2, 3, 5, 6, and 8-13, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2, 3, 5, 6, and 8-13.

In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

In certain embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-517197.2; AD-517258.2; AD-516748.2; AD-516851.2; AD-519351.2; AD-519754.2; AD-519828.2; AD-520018.2; AD-520035.2; AD-520062.2; AD-520064.2; AD-520065.2; AD-520067.2; AD-75289.2; AD-520069.2; AD-520099.2; AD-67575.7; AD-520101.2; AD-1193323.1; AD-1193344.1; AD-1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; AD-1193481.1 or AD-67605.7.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of any one of duplexes AD-519345.1, AD-519346.1, AD-519347.1, AD-67554.7, AD-519752.3, AD-1010731.1, AD-1010732.1, AD-519343.1, AD-519344.1, AD-519349.1, AD-519350.1, AD-519753.2, AD-519932.1, AD-519935.2, AD-520018.6, AD-517837.2, AD-805635.2, AD-519329.2, AD-520063.2, AD-519757.2, AD-805631.2, AD-516917.2, AD-516828.2, AD-518983.2, AD-805636.2, AD-519754.7, AD-520062.2, AD-67575.9, AD-518923.3, AD-520053.4, AD-519667.2, AD-519773.2, AD-519354.2, AD-520060.4, AD-520061.4, AD-1010733.2, AD-1010735.2, AD-1193323.1; AD-1193344.1; AD-1193350.1; AD-1193365.1; AD-1193379.1; AD-1193407.1; AD-1193421.1; AD-1193422.1; AD-1193429.1; AD-1193437.1; AD-1193443.1; AD-1193471.1; or AD-1193481.1.

In some embodiments, the sense or antisense strand is selected from the sense or antisense strand of duplex AD-519351.

It will be understood that, although the sequences in, for example, Tables 2, 5, 8 and 10 are not described as modified or conjugated sequences, the RNA of the iRNA of the invention *e.g.*, a

dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 2, 3, 5, 6, and 8-13 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. In other words, the invention encompasses dsRNA of Tables 2, 3, 5, 6, and 8-13 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.*, 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al* ., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) RNA 14:1714-1719; Kim *et al* . (2005) Nat Biotech 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 2, 3, 5, 6, and 8-13. dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having any one of the sequences in any one of Tables 2, 3, 5, 6, and 8-13 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 19, 20, 21, 22, 23 or more contiguous nucleotides derived from any one of the sequences of any one of Tables 2, 3, 5, 6, and 8-13, and differing in their ability to inhibit the expression of a KHK gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 2, 3, 5, 6, and 8-13 identify a site(s) in a KHK transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 19 contiguous nucleotides from any one of the sequences provided in any one of Tables 2, 3, 5, 6, and 8-13 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a KHK gene.

III. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-modified, and does not comprise, *e.g.,* chemical modifications or conjugations known in the art and described herein. In other embodiments, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA or substantially all of the nucleotides of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1 unmodified nucleotides are present in a strand of the iRNA.

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. *et al* . (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby

incorporated herein by reference. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothiotate groups present in the agent. Agents in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothiotate groups present in the agent.

Representative U.S. Patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6, 239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715;

6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH_2 component parts.

Representative U.S. Patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

Suitable RNA mimetics are contemplated for use in iRNAs provided herein, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound in which an RNA mimetic that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative US patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al* ., *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--CH_2-, --CH_2--$N(CH_3)$--O--CH_2--[known as a methylene (methylimino) or MMI backbone], --CH_2--O--$N(CH_3)$--CH_2--, --CH_2--$N(CH_3)$--$N(CH_3)$--CH_2-- and --$N(CH_3)$--CH_2--CH_2-- of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506. The native phosphodiester backbone can be represented as O-P(O)(OH)-OCH2-.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al*., *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, *i.e.*, a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH_2--O--CH_2--$N(CH_3)_2$. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative US patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patent Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxythimidine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl 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

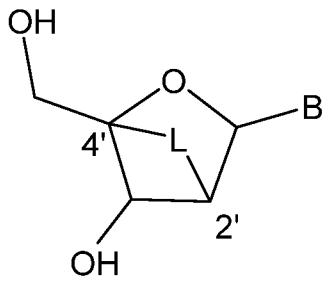
and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al* ., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. Patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

An iRNA agent of the disclosure can also be modified to include one or more bicyclic sugar moieties. A “bicyclic sugar” is a furanosyl ring modified by a ring formed by the bridging of two carbons, whether adjacent or non-adjacent. A “bicyclic nucleoside” (“BNA”) is a nucleoside having a sugar moiety comprising a ring formed by bridging two carbons, whether adjacent or non-adjacent, of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4′-carbon and the 2′-carbon of the sugar ring, optionally, via the 2’-acyclic oxygen atom. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH_2-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al* ., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al* ., (2007) *Mol Canc Ther*

6(3):833-843; Grunweller, A. *et al* ., (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4′ and the 2′ ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4′ to 2′ bridge.

A locked nucleoside can be represented by the structure (omitting stereochemistry),



wherein B is a nucleobase or modified nucleobase and L is the linking group that joins the 2'-carbon to the 4'-carbon of the ribose ring. Examples of such 4′ to 2′ bridged bicyclic nucleosides, include but are not limited to 4′-(CH_2)—O-2′ (LNA); 4′-(CH_2)—S-2′; 4′-(CH_2)$_2$—O-2′ (ENA); 4′-$CH(CH_3)$—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-$CH(CH_2OCH_3)$—O-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4′-$C(CH_3)(CH_3)$—O-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4′-CH_2—$N(OCH_3)$-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4′-CH_2—O—$N(CH_3)$-2′ (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4′-CH_2—N(R)—O-2′, wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4′-CH_2—$C(H)(CH_3)$-2′ (see, *e.g.*, Chattopadhyaya *et al* ., *J. Org. Chem.*, 2009, 74, 118-134); and 4′-CH_2—$C(=CH_2)$-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and U.S. Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133;7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-$CH(CH_3)$-O-2' bridge (*i.e.*, L in the preceding structure). In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2'and C4' carbons of ribose or the C3 and -C5′ carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, U.S. Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, an iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'-C4' have been removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series,* 52, 133-134 (2008) and Fluiter *et al* ., *Mol. Biosyst.,* 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, U.S. Patent No. 8,314,227; and U.S. Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3'- phosphate, inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), and inverted abasic 2'-deoxyribonucleotide (iAb) and others. Disclosure of this modification can be found in WO 2011/005861.

In one example, the 3' or 5' terminal end of a oligonucleotide is linked to an inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), or a inverted abasic 2'-deoxyribonucleotide (iAb). In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted abasic ribonucleotide (iAb). In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted dA (idA).

In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-terminal nucleotides of a sense strand is an inverted dA (idA) and is linked to the preceding nucleotide via a 3'-3'- linkage (e.g., 3'-3'-phosphorothioate linkage).

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, *e.g.,* a 5'-terminal phosphate or phosphate mimic on the antisense strand of an iRNA. Suitable phosphate mimics are disclosed in, for example U.S. Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNA agents of the invention include agents with chemical modifications as disclosed, for example, in WO2013/075035, the entire contents of each of which are incorporated herein by reference. As shown herein and in WO2013/075035, one or more motifs of three identical modifications on three consecutive nucleotides may be introduced into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNA agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNA agents capable of inhibiting the expression of a target gene (*i.e.*, KHK gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, for example, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as "dsRNAi agent." The duplex region of a dsRNAi agent may be, for example, the duplex region can be 27-30 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In certain embodiments, the

overhang regions can include extended overhang regions as provided above. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2`-O-methoxyethyl-5-methyluridine (Teo), 2`-O-methoxyethyladenosine (Aeo), 2`-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof.

For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand, or both strands of the dsRNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In some embodiments, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In some embodiments, this 3'-overhang is present in the antisense strand. In some embodiments, this 3'-overhang is present in the sense strand.

The dsRNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'- end of the sense strand or, alternatively, at the 3'-end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the dsRNAi agent has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In certain embodiments, the dsRNAi agent is a double blune-ended of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, and 9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, and 13 from the 5'end.

In other embodiments, the dsRNAi agent is a double blune-ended of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, and 10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, and 13 from the 5'end.

In yet other embodiments, the dsRNAi agent is a double blune-ended of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, and 11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, and 13 from the 5'end.

In certain embodiments, the dsRNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, and 11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, and 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a two nucleotide overhang. In some embodiments, the two nucleotide overhang is at the 3'-end of the antisense strand.

When the two nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 2'-fluoro, *e.g.,* in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (such as, $GalNAc_3$).

In certain embodiments, the dsRNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the

motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In certain embodiments, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein Dicer cleavage of the dsRNAi agent results in an siRNA comprising the 3'-end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the dsRNAi agent further comprises a ligand.

In certain embodiments, the sense strand of the dsRNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In certain embodiments, the antisense strand of the dsRNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

For a dsRNAi agent having a duplex region of 19-23 nucleotides in length, the cleavage site of the antisense strand is typically around the 10, 11, and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, and 11 positions; the 10, 11, and 12 positions; the 11, 12, and 13 positions; the 12, 13, and 14 positions; or the 13, 14, and 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the dsRNAi agent from the 5'-end.

The sense strand of the dsRNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In some embodiments, the sense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near

the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistries of the motifs are distinct from each other, and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In some embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end, or both ends of the strand.

In other embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end, or both ends of the strand.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two, or three nucleotides.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two, or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2′-hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3'- or 5' terminal position, may only occur in a terminal region, *e.g.,* at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of an RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.,* at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5'-end or ends can be phosphorylated.

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5'- or 3'-overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3'- or 5'-overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'- O-methyl or 2'-fluoro modifications, or others.

In certain embodiments, the N_a or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB…," "AABBAABBAABB…," "AABAABAABAAB…," "AAABAAABAAAB…," "AAABBBAAABBB…," or "ABCABCABCABC…," *etc.*

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or

antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB…", "ACACAC…" "BDBDBD…" or "CDCDCD…," *etc.*

In some embodiments, the dsRNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5' to 3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand, where each A is an unmodified ribonucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand, where each A is an 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In another particular example, the alternating motif in the antisense strand is "BABABA" from 3'-5'of the strand, where each A is a 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand and the alternating motif in the antisense strand is "BABABA" from 3'-5'of the strand, where each A is an unmodified ribonucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand and the alternating motif in the antisense strand is "BABABA" from 3'-5'of the strand, where each A is a 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In some embodiments, the dsRNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, *i.e.*, the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the 2'-F modification, and the 1 position of the antisense strand may start with the 2'- O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand or antisense strand interrupts the initial modification pattern present in

the sense strand or antisense strand. This interruption of the modification pattern of the sense or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing activity against the target gene.

In some embodiments, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "...N_aYYYN_b...," where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, N_a or N_b may be present or absent when there is a wing modification present.

The iRNA may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand, antisense strand, or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, a double-stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In some embodiments, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-end and two phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

In some embodiments, the dsRNAi agent comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within the duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next

to the overhang nucleotide. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, or the 5'end of the antisense strand.

In some embodiments, the 2-nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the dsRNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the dsRNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is sred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In certain embodiments, the dsRNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In certain embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2, or 3 base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair.

In other embodiments, the nucleotide at the 3'-end of the sense strand is deoxythimidine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxythimidine (dT). For example, there is a short sequence of deoxythimidine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

In certain embodiments, the sense strand sequence may be represented by formula (I):

$$5' \; n_p\text{-}N_a\text{-}(X\,X\,X)_i\text{-}N_b\text{-}Y\,Y\,Y\text{-}N_b\text{-}(Z\,Z\,Z)_j\text{-}N_a\text{-}n_q \; 3' \qquad (I)$$

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY, and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. In some embodiments, YYY is all 2'-F modified nucleotides.

In some embodiments, the N_a or N_b comprises modifications of alternating pattern.

In some embodiments, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8; 7, 8, 9; 8, 9, 10; 9, 10, 11; 10, 11,12; or 11, 12, 13) of the sense strand, the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' n_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ib);

5' n_p-N_a-XXX-N_b-YYY-N_a-n_q 3' (Ic); or

5' n_p-N_a-XXX-N_b-YYY-N_b-ZZZ-N_a-n_q 3' (Id).

When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. In some embodiments, N_b is 0, 1, 2, 3, 4, 5, or 6 Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

5' n_p-N_a-YYY- N_a-n_q 3' (Ia).

When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

5' $n_{q'}$-N_a'-(Z'Z'Z')$_k$-N_b'-Y'Y'Y'-N_b'-(X'X'X')$_l$-N'_a-n_p' 3' (II)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification; and

X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the Y'Y'Y' motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14; or 13, 14, 15 of the antisense strand, with the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end. In some embodiments, the Y'Y'Y' motif occurs at positions 11, 12, 13.

In certain embodiments, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

In certain embodiments, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:

5' $n_{q'}$-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'-$n_{p'}$ 3' (IIb);

5' $n_{q'}$-N_a'-Y'Y'Y'-N_b'-X'X'X'-$n_{p'}$ 3' (IIc); or

5' $n_{q'}$-N_a'- Z'Z'Z'-N_b'-Y'Y'Y'-N_b'- X'X'X'-N_a'-$n_{p'}$ 3' (IId).

When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides.

Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. In some embodiments, N_b is 0, 1, 2, 3, 4, 5, or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

5' $n_{p'}$-$N_{a'}$-Y'Y'Y'- $N_{a'}$-$n_{q'}$ 3' (Ia).

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X′, Y′ and Z′ may be the same or different from each other.

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X′, Y′, and Z′, in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In some embodiments, the sense strand of the dsRNAi agent may contain YYY motif occurring at 9, 10, and 11 positions of the strand when the duplex region is 21 nt, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'- end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In some embodiments the antisense strand may contain Y′Y′Y′ motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'- end; and Y′ represents 2'-O-methyl modification. The antisense strand may additionally contain X′X′X′ motif or Z′Z′Z′ motifs as wing modifications at the opposite end of the duplex region; and X′X′X′ and Z′Z′Z′ each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with an antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the dsRNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the iRNA duplex represented by formula (III):

sense: 5' n_p -N_a-(X X X)$_i$ -N_b- Y Y Y -N_b -(Z Z Z)$_j$-N_a-n_q 3'

antisense: 3' n_p'-N_a'-(X'X'X')$_k$-N_b'-Y′Y′Y′-N_b'-(Z′Z′Z′)$_l$-N_a'-n_q' 5'

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p′, q, and q′ are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

Exemplary combinations of the sense strand and antisense strand forming an iRNA duplex include the formulas below:

5' n_p - N_a -Y Y Y -N_a-n_q 3'

3' n_p'-N_a'-Y′Y′Y′ -$N_a'$$n_q'$ 5'

(IIIa)

5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-Y′Y′Y′-N_b'-Z′Z′Z′-$N_a'$$n_q'$ 5'

(IIIb)

5' n_p-N_a- X X X -N_b -Y Y Y - N_a-n_q 3'

3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_a'-n_q' 5'

(IIIc)

5' n_p -N_a -X X X -N_b-Y Y Y -N_b- Z Z Z -N_a-n_q 3'

3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_b'-Z′Z′Z′-N_a-n_q' 5'

(IIId)

When the dsRNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5, or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-

15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b, and N_b' independently comprises modifications of alternating pattern.

Each of X, Y, and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the dsRNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), at least one of the Y nucleotides may form a base pair with one of the Y′ nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y′ nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y′ nucleotides.

When the dsRNAi agent is represented by formula (IIIb) or (IIId), at least one of the Z nucleotides may form a base pair with one of the Z′ nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z′ nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z′ nucleotides.

When the dsRNAi agent is represented as formula (IIIc) or (IIId), at least one of the X nucleotides may form a base pair with one of the X′ nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X′ nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X′ nucleotides.

In certain embodiments, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In certain embodiments, when the dsRNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications and n_p' >0 and at least one n_p' is linked to a neighboring nucleotide a *via* phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , n_p' >0 and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , n_p' >0 and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, when the dsRNAi agent is represented by formula (IIIa), the N_a modifications are 2′-O-methyl or 2′-fluoro modifications , n_p' >0 and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, the dsRNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In some embodiments, the dsRNAi agent is a multimer containing three, four, five, six, or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

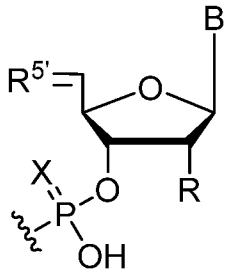
In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

In certain embodiments, an RNAi agent of the invention may contain a low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 10 or fewer nucleotides with 2'-fluoro modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent of the invention contains 10 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 6 nucleotides with a 2'-fluoro modification in the antisense strand. In another specific embodiment, the RNAi agent of the invention contains 6 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

In other embodiments, an RNAi agent of the invention may contain an ultra low number of nucleotides containing a 2'-fluoro modification, *e.g.*, 2 or fewer nucleotides containing a 2'-fluoro modification. For example, the RNAi agent may contain 2, 1 of 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, *e.g.*, 0 nucleotides with a 2-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include WO2007/091269, U.S. Patent No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a 5'- vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or C_{1-20}alkoxy (*e.g.*, methoxy or n-hexadecyloxy);

$R^{5'}$ is =C(H)-P(O)$(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the *E* or *Z* orientation (*e.g.*, *E* orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphonate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphonate structure includes the preceding structure, where R5' is =C(H)-OP(O)(OH)2 and the double bond between the C5' carbon and R5' is in the E or Z orientation (*e.g.*, E orientation).

As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA can be replaced with another moiety, *e.g.*, a non-carbohydrate (such as, cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide *via* a carrier. The carriers include (i) at least one "backbone attachment point," such as, two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom

which provides a backbone attachment point), that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

The iRNA may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group. In some embodiments, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin. In some embodiments, the acyclic group is a serinol backbone or diethanolamine backbone.

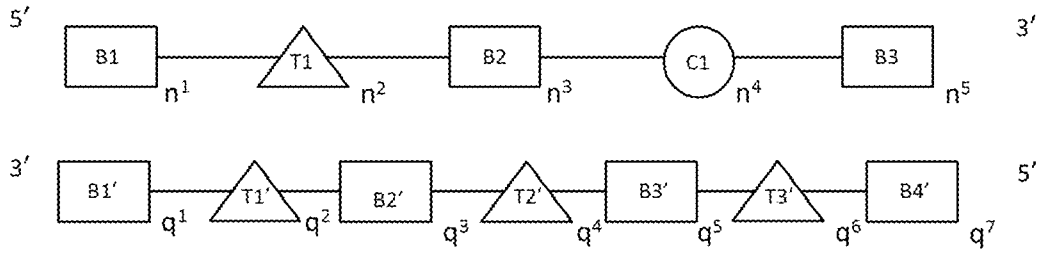
i. Thermally Destabilizing Modifications

In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand. As used herein "seed region" means at positions 2-9 of the 5'-end of the referenced strand or at positions 2-8 of the 5'-end of the refrenced strand. For example, thermally destabilizing modifications can be incorporated in the seed region of the antisense strand to reduce or inhibit off-target gene silencing.

The term "thermally destabilizing modification(s)" includes modification(s) that would result with a dsRNA with a lower overall melting temperature (Tm) than the Tm of the dsRNA without having such modification(s). For example, the thermally destabilizing modification(s) can decrease the Tm of the dsRNA by 1 – 4 °C, such as one, two, three or four degrees Celcius. And, the term "thermally destabilizing nucleotide" refers to a nucleotide containing one or more thermally destabilizing modifications.

It has been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (*e.g.*, one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, such as, positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

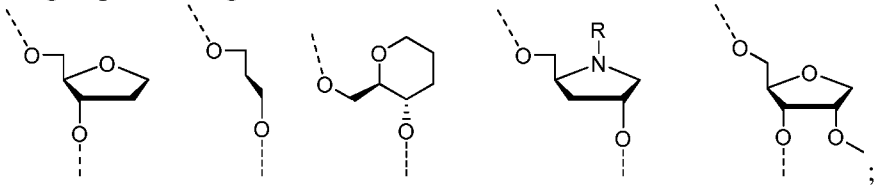
An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



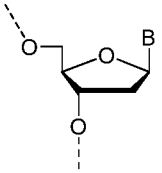
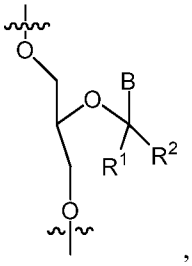
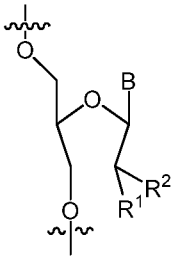
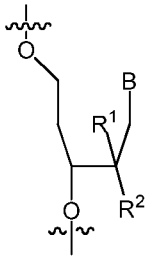
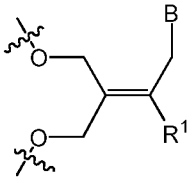
(L),

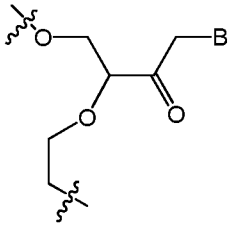
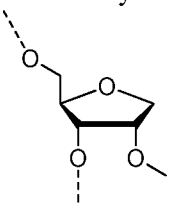
In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In one embodiment, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA, , 2'O-CH2C(O)N(Me)H) modification.

C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand or at positions 2-9 of the 5'-end of the refrenced strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide *e.g.*, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA); and 2'-5'-linked ribonucleotides ("3'-RNA"). In one embodiment, C1 has thermally destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:



; and iii) sugar modification selected from the group consisting of:






2'-deoxy , , , , , and


, wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H, halogen, OR_3, or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar. In one embodiment, the thermally destabilizing modification in C1 is a mismatch selected from the group consisting of G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, and U:T; and optionally, at least one nucleobase in the mismatch pair is a 2'-deoxy nucleobase. In one example, the thermally destabilizing modification in C1 is GNA or  .

T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In one embodiment, T1 is DNA. In one embodiment, T1' is DNA, RNA or LNA. In one embodiment, T2' is DNA or RNA. In one embodiment, T3' is DNA or RNA.

n^1, n^3, and q^1 are independently 4 to 15 nucleotides in length.

n^5, q^3, and q^7 are independently 1-6 nucleotide(s) in length.

n^4, q^2, and q^6 are independently 1-3 nucleotide(s) in length; alternatively, n^4 is 0.

q^5 is independently 0-10 nucleotide(s) in length.

n^2 and q^4 are independently 0-3 nucleotide(s) in length.

Alternatively, n^4 is 0-3 nucleotide(s) in length.

In one embodiment, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, with two phosphorothioate internucleotide linkage modifications

within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, n^4, q^2, and q^6 are each 1.

In one embodiment, n^2, n^4, q^2, q^4, and q^6 are each 1.

In one embodiment, C1 is at position 14-17 of the 5'-end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^4 is 1. In one embodiment, C1 is at position 15 of the 5'-end of the sense strand

In one embodiment, T3' starts at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1.

In one embodiment, T1' starts at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In an exemplary embodiment, T3' starts from position 2 from the 5' end of the antisense strand and T1' starts from position 14 from the 5' end of the antisense strand. In one example, T3' starts from position 2 from the 5' end of the antisense strand and q^6 is equal to 1 and T1' starts from position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In one embodiment, T1' and T3' are separated by 11 nucleotides in length (*i.e.* not counting the T1' and T3' nucleotides).

In one embodiment, T1' is at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose.

In one embodiment, T3' is at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T1 is at the cleavage site of the sense strand. In one example, T1 is at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1. In an exemplary embodiment, T1 is at the cleavage site of the sense strand at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1,

In one embodiment, T2' starts at position 6 from the 5' end of the antisense strand. In one example, T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1.

In an exemplary embodiment, T1 is at the cleavage site of the sense strand, for instance, at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1; T1' is at position 14 from the 5' end of the antisense strand, and q^2 is equal to 1, and the modification to T1' is at the 2' position of a ribose sugar or at positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose; T2' is at positions 6-10 from the 5' end

of the antisense strand, and q^4 is 1; and T3' is at position 2 from the 5' end of the antisense strand, and q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T2' starts at position 8 from the 5' end of the antisense strand. In one example, T2' starts at position 8 from the 5' end of the antisense strand, and q^4 is 2.

In one embodiment, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is 1.

In one embodiment, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 6, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 7, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4,

T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 5, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

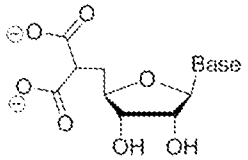
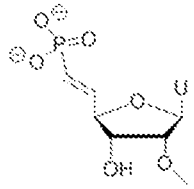
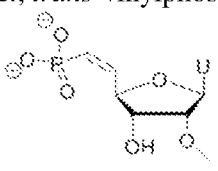
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at

positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

The RNAi agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS_2), 5'-end vinylphosphonate (5'-VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-*C*-malonyl (). When the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either 5'-*E*-VP isomer (*i.e.*, *trans*-vinylphosphonate, ), 5'-*Z*-VP isomer (*i.e.*, *cis*-vinylphosphonate, ), or mixtures thereof.

In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the sense strand. In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-P. In one embodiment, the RNAi agent comprises a 5'-P in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS. In one embodiment, the RNAi agent comprises a 5'-PS in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-VP. In one embodiment, the RNAi agent comprises a 5'-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*E*-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*Z*-VP in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS_2. In one embodiment, the RNAi agent comprises a 5'-PS_2 in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS_2. In one embodiment, the RNAi agent comprises a 5'-deoxy-5'-*C*-malonyl in the antisense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F,

q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense

strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The dsRNA agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof. In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two

phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The dsRNAi RNA agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- P.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F,

q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense

strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof), and a targeting ligand.

In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one embodiment, the 5'- PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense

strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-PS_2 and a

targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof)

and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-PS_2 and a targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The

RNAi agent also comprises a 5'- PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- VP (*e.g.*, a 5'-*E*-VP, 5'-*Z*-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'- PS_2 and a targeting ligand. In one embodiment, the 5'-PS_2 is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand). The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-*C*-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In a particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the dsRNA agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, an RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a deoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, and 13, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, and 14 to 21; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5 to 7, 9, 11 to 13, 15, 17 to 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 8, 10, 14, 16, and 20 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, and 19 to 21, and 2'-F modifications at positions 3, 5, 7, 9 to 11, 13, 16, and 18; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 25 nucleotides;

(ii) 2'-OMe modifications at positions 1, 4, 6, 7, 9, 11 to 13, 15, 17, and 19 to 23, 2'-F modifications at positions 2, 3, 5, 8, 10, 14, 16, and 18, and desoxy-nucleotides (*e.g.* dT) at positions 24 and 25 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a four nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular embodiment, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 19 nucleotides;

(ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 19 and 20, and between nucleotide positions 20 and 21 (counting from the 5' end);

wherein the RNAi agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in any one of Tables 2, 3, 5, 6, and 8-13. These agents may further comprise a ligand.

IV. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA *e.g.*, into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al .*, *Proc. Natl. Acid. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al .*, *Biorg. Med. Chem. Let.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al .*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al .*, *Biorg. Med. Chem. Let.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al .*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al .*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al .*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al .*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al .*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al .*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al .*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al .*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al .*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke *et al .*, J. *Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In certain embodiments, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In some embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. In some embodiments, ligands do not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride

copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, the ligand is a multivalent galactose, *e.g.*, an N-acetyl-galactosamine.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxol,

vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins, *etc*. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems® (Foster City, Calif.). Any other methods for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule may bind a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In certain embodiments, the lipid based ligand binds HSA. In some embodiments, it binds HSA with a sufficient affinity such that the conjugate will be distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In other embodiments, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be distributed to the kidney. Other moieties that target to kidney cells can also be used in place of, or in addition to, the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, such as, a helical cell-permeation agent. In some embodiments, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. In some embodiments, the helical agent is an alpha-helical agent, which has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and

absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: 14). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:15) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:16) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:17) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al* ., Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent *via* an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.,* glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand, *e.g.,* PECAM-1 or VEGF.

A "cell permeation peptide" is capable of permeating a cell, *e.g.,* a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al* ., Nucl. Acids Res. 31:2717-2724, 2003).

C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is advantageous for the *in vivo*

delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* 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; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri-, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (*e.g.,* C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.,* C5, C6, C7, or C8).

In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, *e.g.*, by serving as a ligand for the asialoglycoprotein receptor of liver cells (*e.g.*, hepatocytes).

In some embodiments, the carbohydrate conjugate comprises one or more GalNAc derivatives. The GalNAc derivatives may be attached *via* a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 3' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein. In some embodiments the GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein.

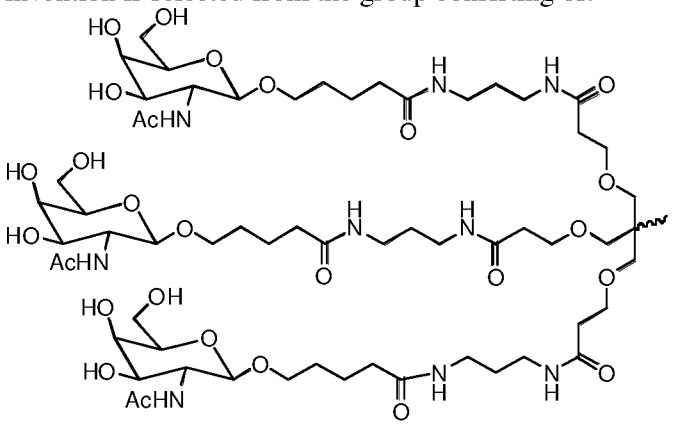
In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a tetravalent linker.

In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

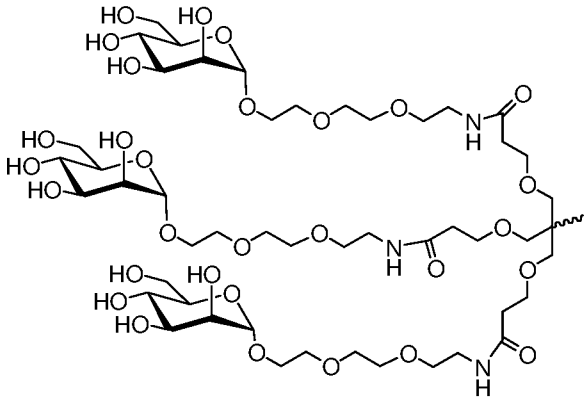
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

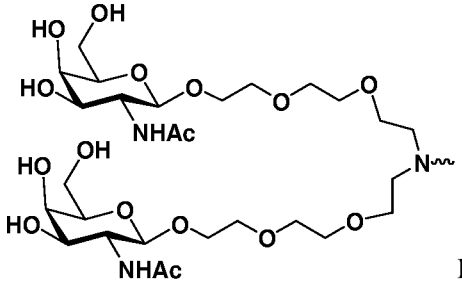
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



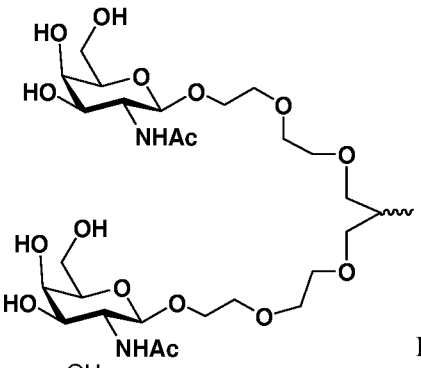
Formula II,



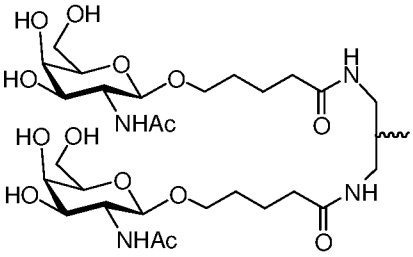
Formula III,



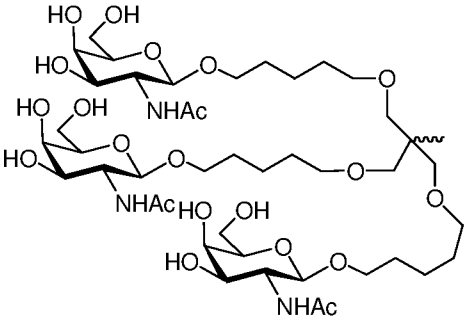
Formula IV,



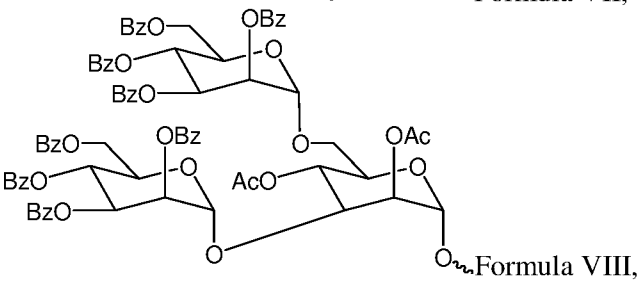
Formula V,



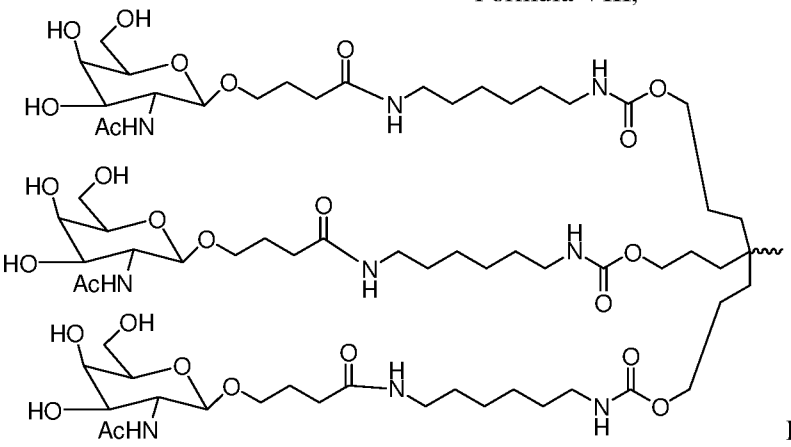
Formula VI,



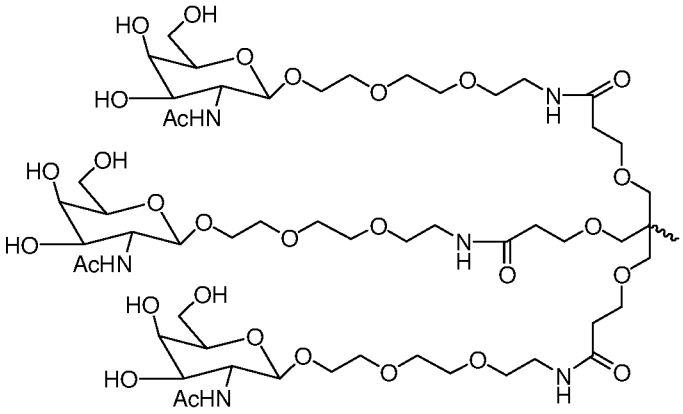
Formula VII,



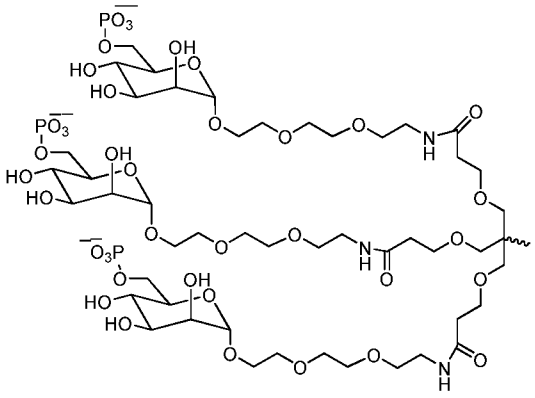
Formula VIII,



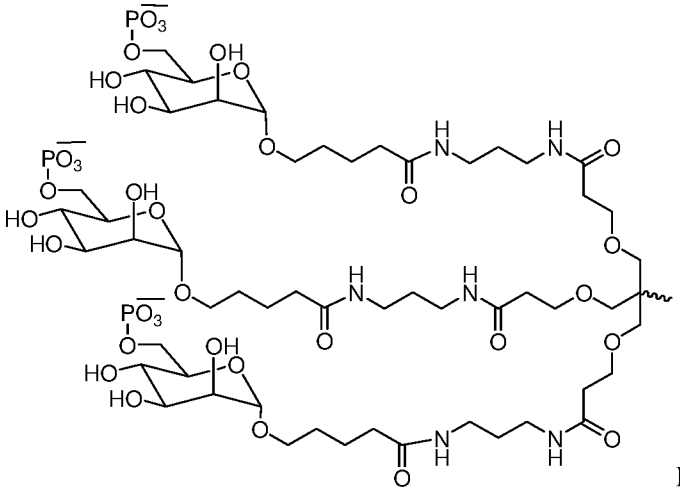
Formula IX,



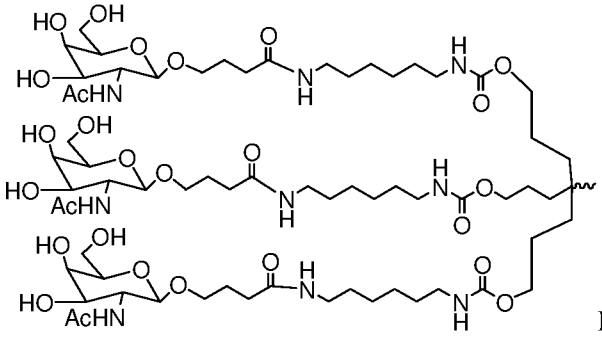
Formula X,



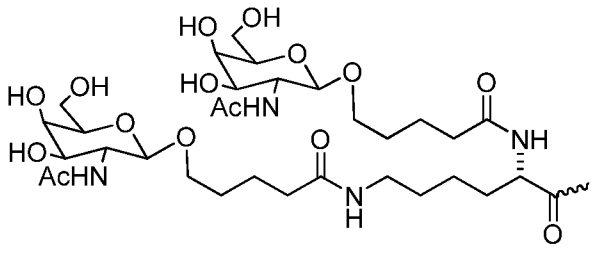
Formula XI,



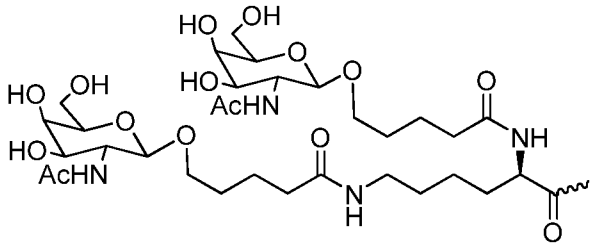
Formula XII,



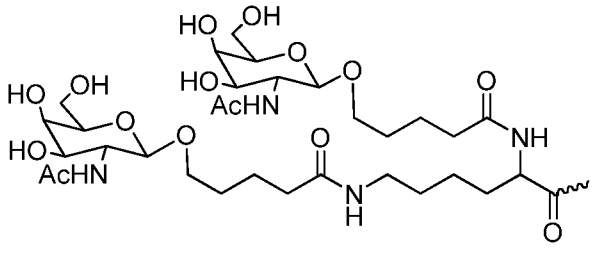
Formula XIII,



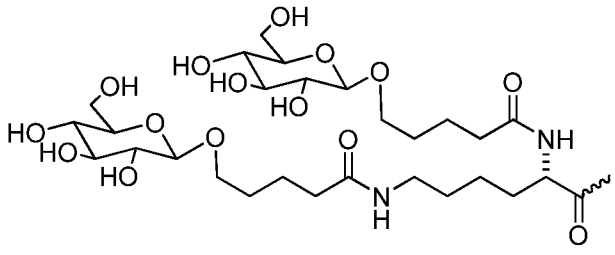
Formula XIV,



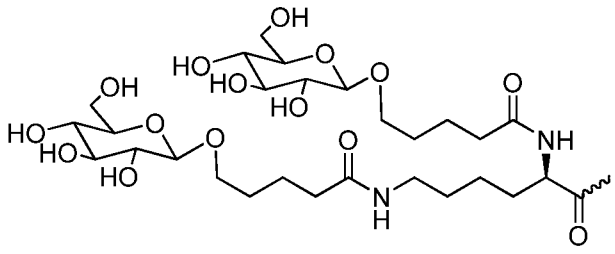
Formula XV,



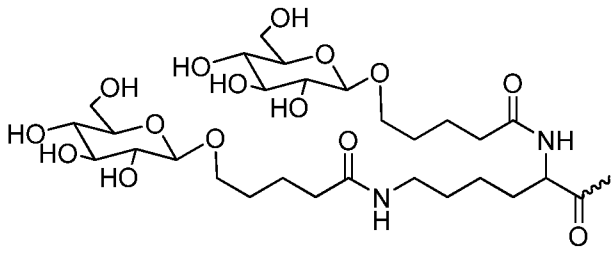
Formula XVI,



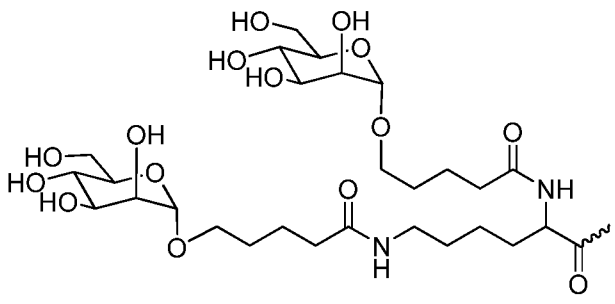
Formula XVII,



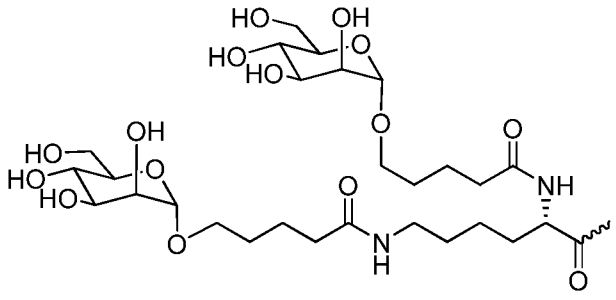
Formula XVIII,



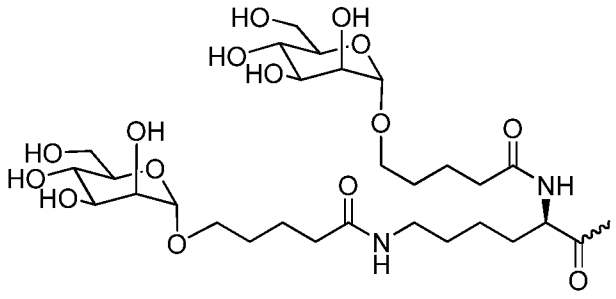
Formula XIX,



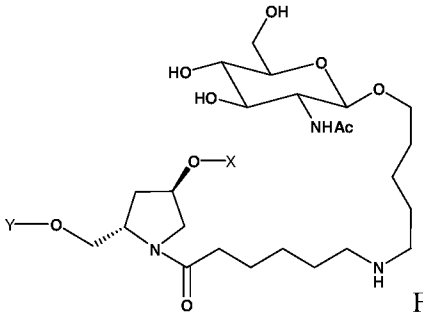
Formula XX,



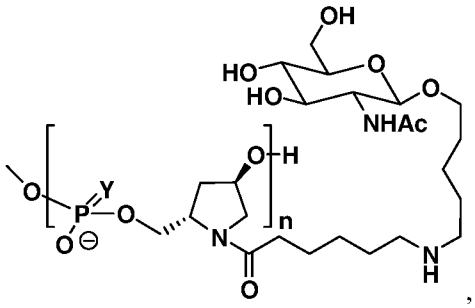
Formula XXI,



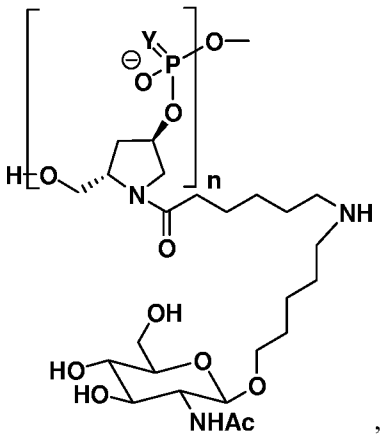
Formula XXII,



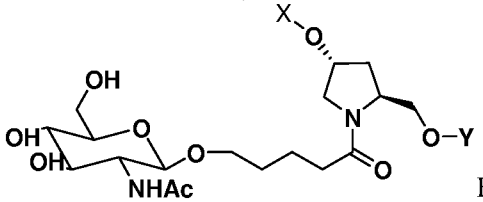
Formula XXIII;



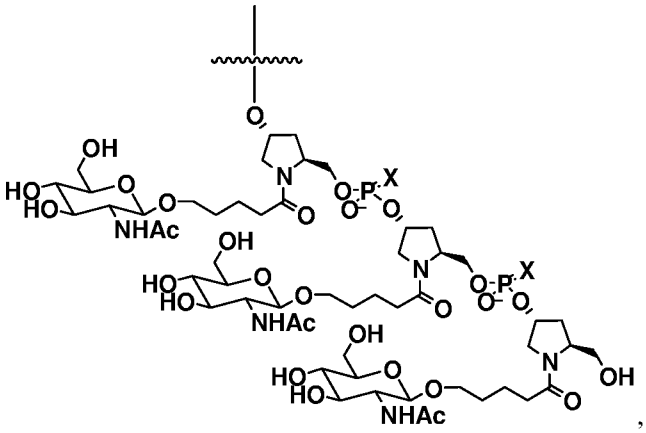
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



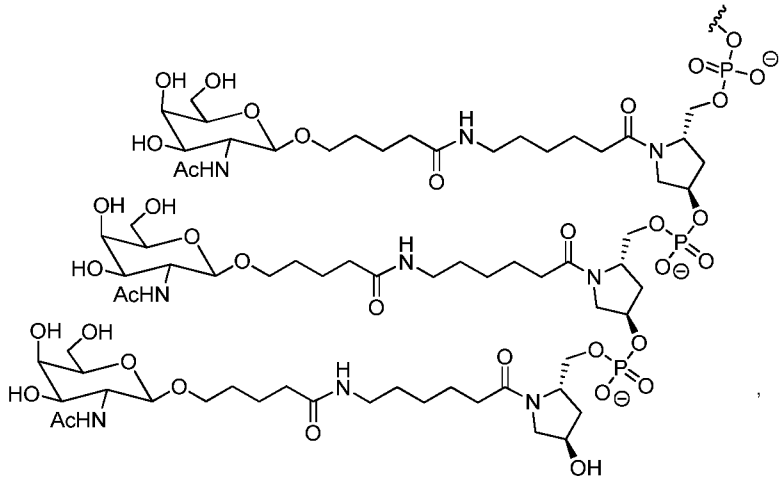
, wherein Y is O or S and n is 3-6 (Formula XXV);



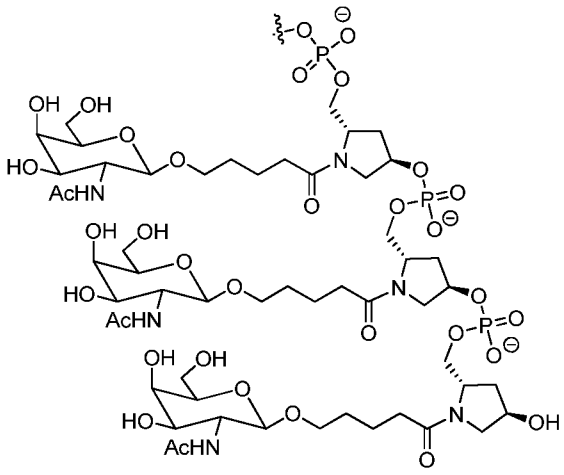
Formula XXVI;



, wherein X is O or S (Formula XXVII);

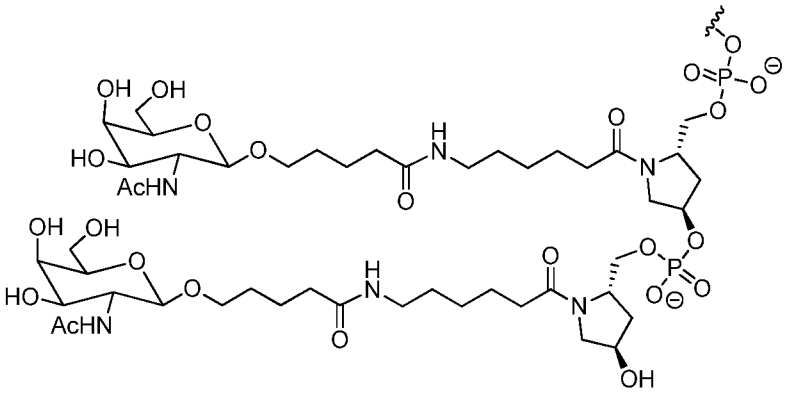


,

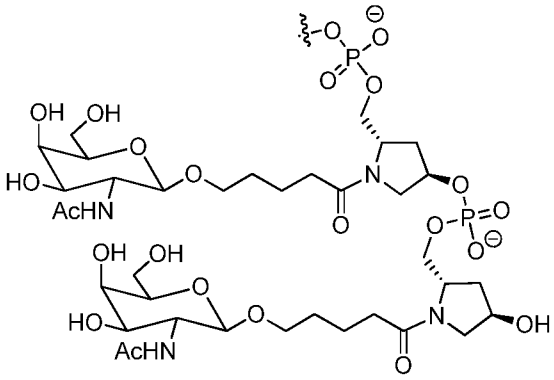


,

Formula XXVII; Formula XXIX;



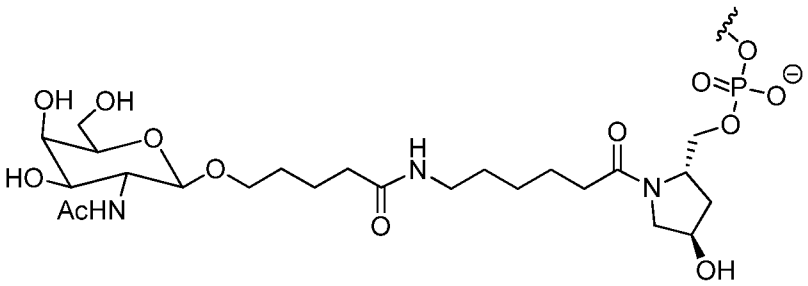
,



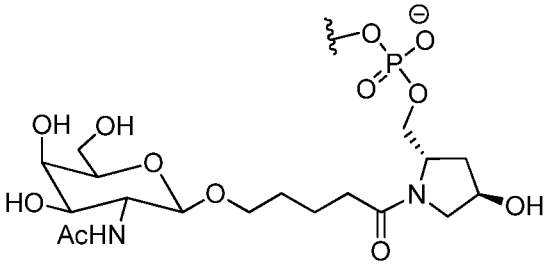
,

Formula XXX;

Formula XXXI;



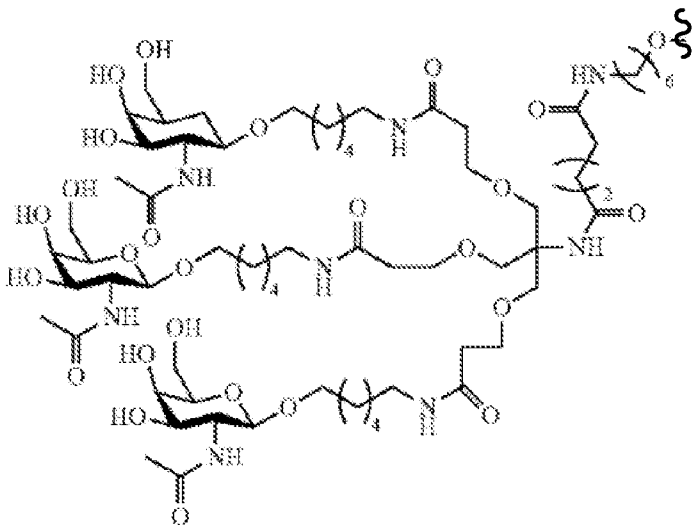
, and



.

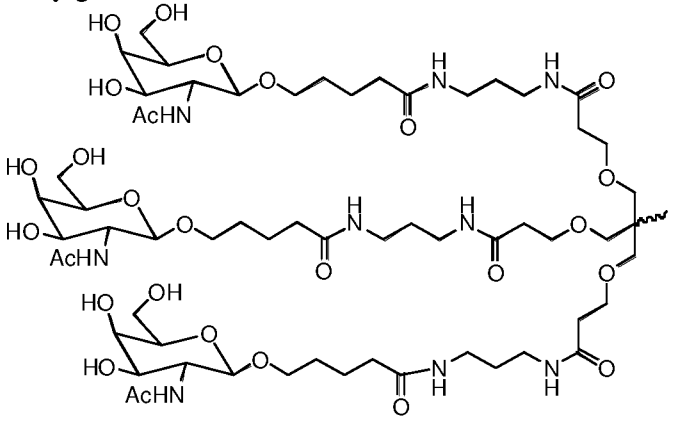
Formula XXXII;

Formula XXXIII.



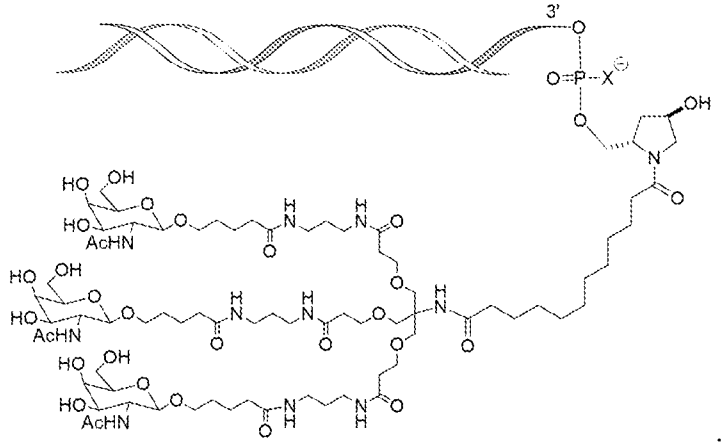
Formula XXXIV.

In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as

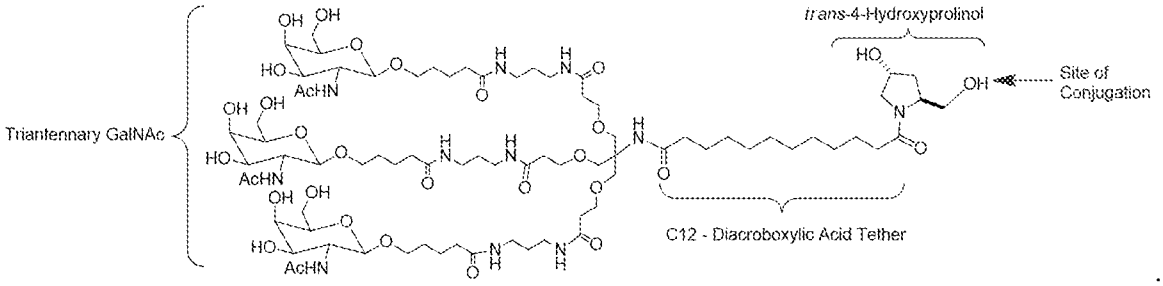


Formula II.

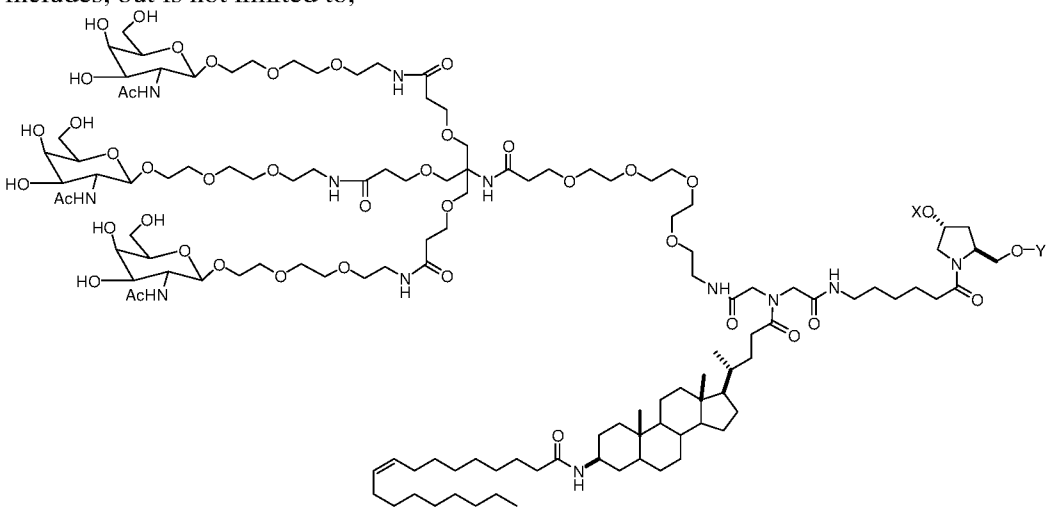
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S



In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:

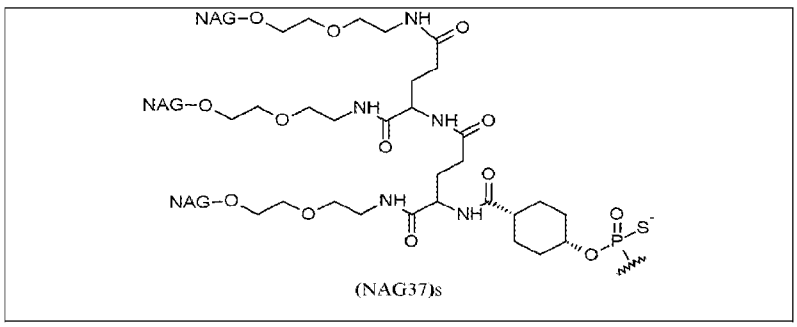


Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXXVI), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises the structure below:



In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc

derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one or more GalNAc or GalNAc derivative attached to the iRNA agent. The GalNAc may be attached to any nucleotide *via* a linker on the sense strand or antsisense strand. The GalNac may be attached to the 5'-end of the sense strand, the 3' end of the sense strand, the 5'-end of the antisense strand, or the 3' – end of the antisense strand. In one embodiment, the GalNAc is attached to the 3' end of the sense strand, *e.g., via* a trivalent linker.

In other embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.,* 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of linkers, *e.g.,* monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention is part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

Additional carbohydrate conjugates and linkers suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.,* covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl,

alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic, or substituted aliphatic. In one embodiment, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

A cleavable linking group 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 one embodiment, the cleavable linking group is cleaved at least about 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 a 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).

Cleavable linking groups 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 linking group 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 linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be 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 linking group that is cleaved at a selected pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid 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 cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group 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 can 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 compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, 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).

i. Redox cleavable linking groups

In certain embodiments, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (-S-S-). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 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). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-based cleavable linking groups

In other embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking 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-, wherein Rk at each occurrence can be, independently, C1-C20 alkyl, C1-C20 haloalkyl, C6-C10 aryl, or C7-C12 aralkyl. Exemplary 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-, -S-P(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-, and -O-P(S)(H)-S-. In certain embodiments, a phosphate-based linking group is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In other embodiments, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In some embodiments acid cleavable linking 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 linking 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). An exemplary 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.

iv. Ester-based linking groups

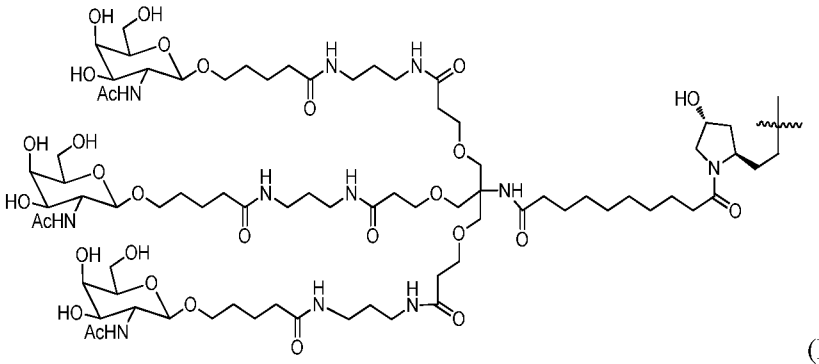
In other embodiments, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include, but are not limited to, esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula -C(O)O-, or -OC(O)-. These candidates can be evaluated using methods analogous to those described above.

v. Peptide-based cleaving groups

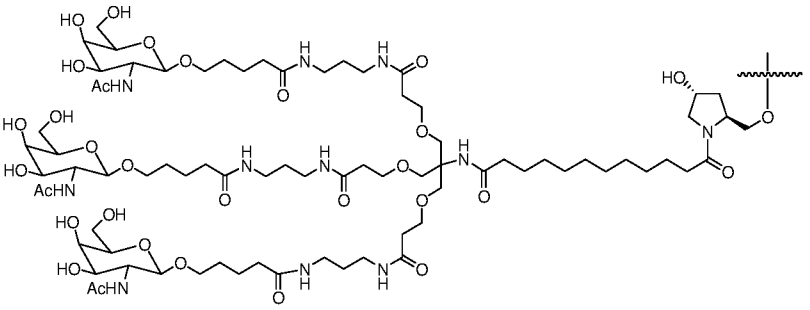
In yet other embodiments, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*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 – NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the

R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

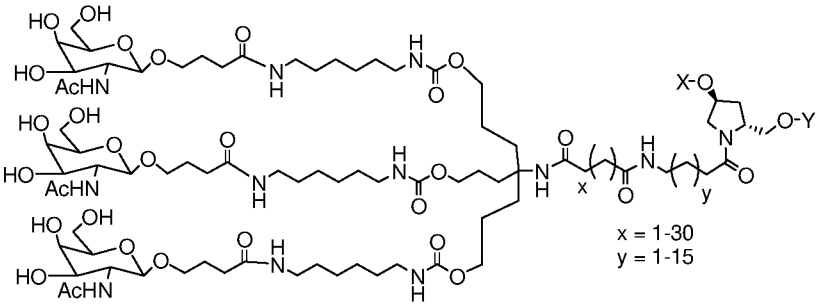
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



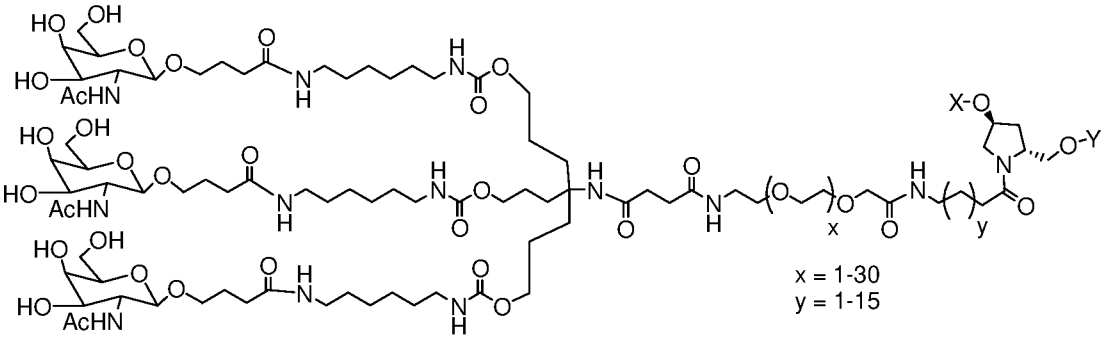
(Formula XXXVII),



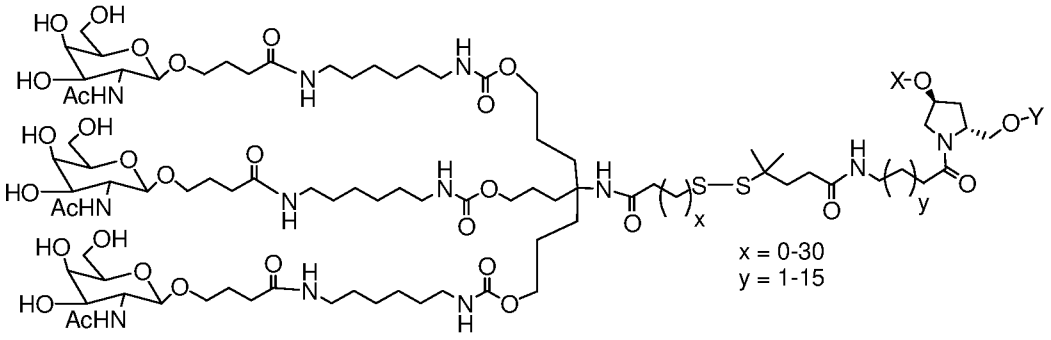
(Formula XXXVIII),



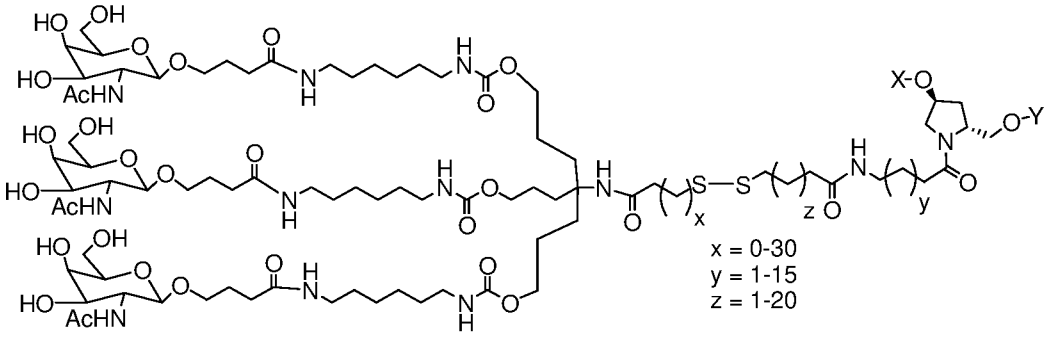
(Formula XXXIX),



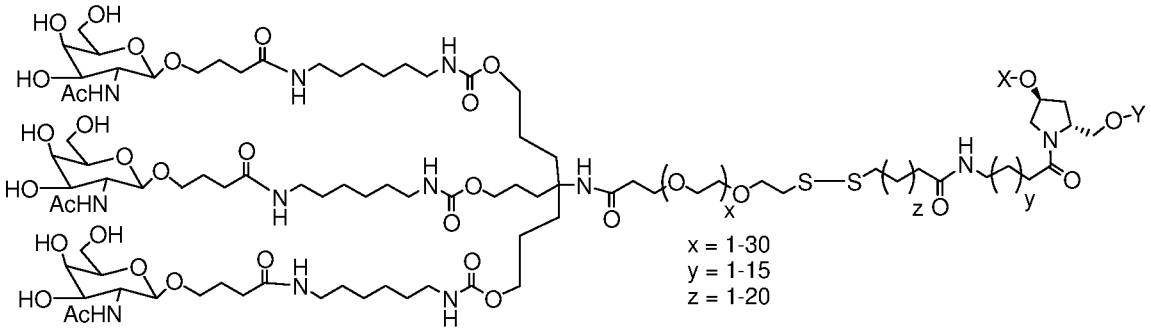
(Formula XL),



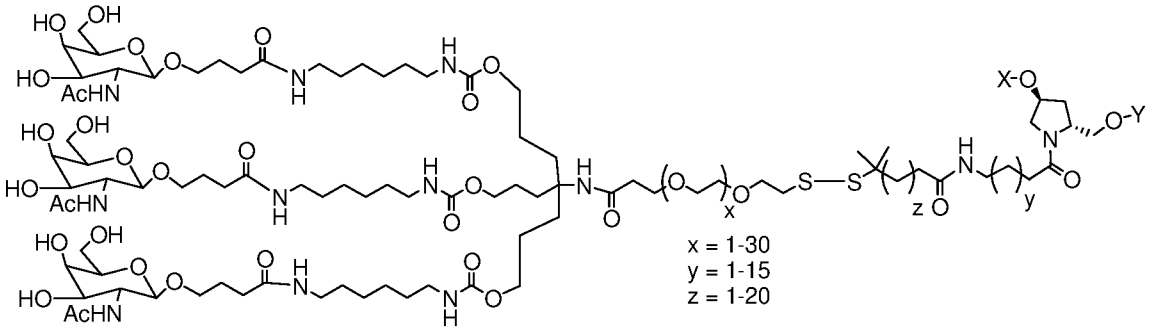
(Formula XLI),



(Formula XLII),



(Formula XLIII), and

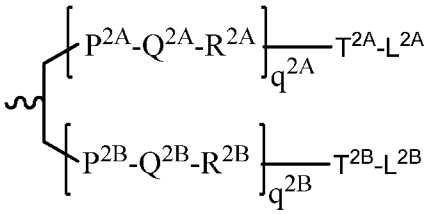


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

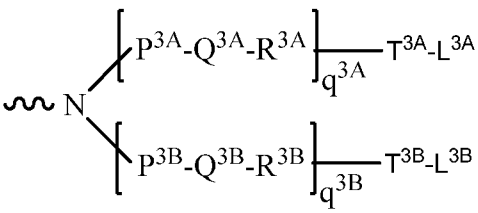
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

Formula XXXXV

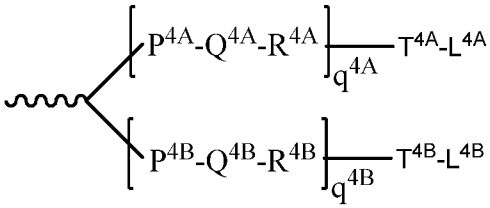


,

Formula XLVI

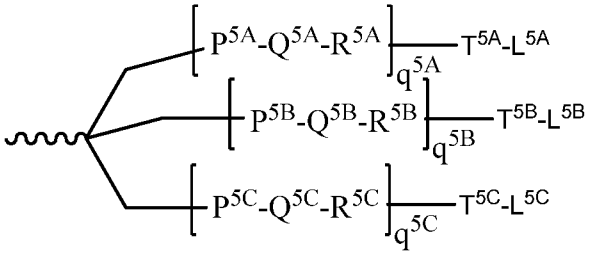


,



Formula (VI)

,



Formula (VII)

;

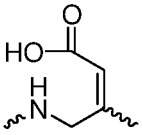
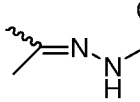
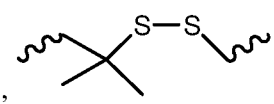
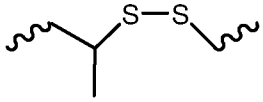
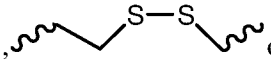
Formula XLVII Formula XLVIII

wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

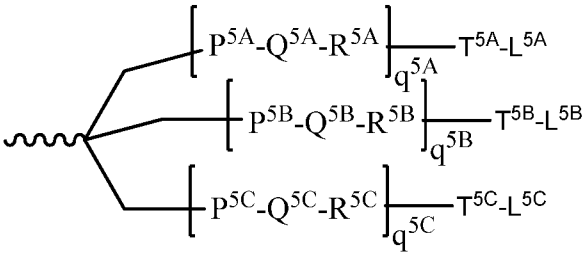
P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-, CO, CH=N-O,  ,  ,  ,  ,  or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XLIX):

Formula XLIX



Formula (VII ,

wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Patent Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928;5,688,941; 6,294,664; 6,320,017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds or "chimeras," in the context of this invention, are iRNA compounds, such as, dsRNAi agents, that contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al*., *Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; Letsinger *et al*., *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan *et al*., *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan *et al*., *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan *et al*., *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et al*., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al*., *EMBO J.*, 1991, 10:111; Kabanov *et al*., *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al*., *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al*., *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al*., *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al*., *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan *et al*., *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra *et al*., *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke *et al*., *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

V. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject susceptible to or diagnosed with a KHK-associated disorder, as described herein) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with an iRNA of the invention (see *e.g.*, Akhtar S. and Julian RL. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of

the delivered molecule in the target tissue. RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al* . (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al* (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., *et al* (2005) *J. Neurophysiol.* 93:594-602). Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al* (2004) *Nature* 432:173-178).

In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see *e.g.*, Kim SH, *et al* (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR, *et al* (2003) *J. Mol. Biol* 327:761-766; Verma, UN, *et al* (2003) Clin. *Cancer Res.* 9:1291-1300; Arnold, AS *et al* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN, *et al* (2003), *supra*), "solid nucleic acid lipid particles" (Zimmermann, TS, *et al* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY, *et al* (2005) *Cancer Gene Ther.* 12:321-328; Pal, A, *et al* (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME, *et al* (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA, *et al* (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., *et al* (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Patent No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector encoded iRNAs of the Invention

iRNA targeting the KHK gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al* ., *TIG.* (1996), 12:5-10; Skillern, A, *et al* ., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and

Conrad, U.S. Patent No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al .*, *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are known in the art.

VI. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of a KHK gene. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, *e.g.*, by subcutaneous (SC) or intravenous (IV) delivery. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a KHK gene.

In some embodiments, the pharmaceutical compositions of the invention are sterile. In another embodiment, the pharmaceutical compositions of the invention are pyrogen free.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of a KHK gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every month, once every 3-6 months, or once

a year. In certain embodiments, the iRNA is administered about once per month to about once per six months.

After an initial treatment regimen, the treatments can be administered on a less frequent basis. Duration of treatment can be determined based on the severity of disease.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that doses are administered at not more than 1, 2, 3, or 4 month intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered about once per month. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered quarterly (*i.e.*, about every three months). In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered twice per year (*i.e.*, about once every six months).

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to mutations present in the subject, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a prophylactically or therapeutically effective amount, as appropriate, of a composition can include a single treatment or a series of treatments.

The iRNA can be delivered in a manner to target a particular tissue (*e.g.*, hepatocytes).

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids, and self-emulsifying semisolids. Formulations include those that target the liver.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers.

A. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and

Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al* ., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution either in the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic,

cationic, and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives, and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

The application of emulsion formulations *via* dermatological, oral, and parenteral routes, and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215).

iii. Microparticles

An iRNA of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al* ., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers and their use in manufacture of pharmaceutical compositions and delivery of pharmaceutical agents are well known in the art.

v. Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Such agent are well known in the art.

vi. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings, or aromatic substances, and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating a KHK-associated disorder, *e.g.*, liver disease (*e.g.,* fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.,* hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

Toxicity and prophylactic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose prophylactically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50, such as, an ED80 or ED90, with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the prophylactically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) or higher levels of inhibition as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents used for the prevention or treatment of a KHK-associated disorder, *e.g.*, liver disease (*e.g.,* fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.,* hypertension, endothelial cell

dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VII. Methods For Inhibiting KHK Expression

The present invention also provides methods of inhibiting expression of a KHK gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNAi agent, in an amount effective to inhibit expression of KHK in the cell, thereby inhibiting expression of KHK in the cell.

Contacting of a cell with an iRNA, *e.g.*, a double stranded RNA agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the iRNA includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the iRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. 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 some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

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

The phrase "inhibiting expression of a KHK" is intended to refer to inhibition of expression of any KHK gene (such as, *e.g.*, a mouse KHK 3 gene, a rat KHK gene, a monkey KHK gene, or a human KHK gene) as well as variants or mutants of a KHK gene. Thus, the KHK gene may be a wild-type KHK gene, a mutant KHK gene, or a transgenic KHK gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a KHK gene" includes any level of inhibition of a KHK gene, *e.g.*, at least partial suppression of the expression of a KHK gene. The expression of the KHK gene may be assessed based on the level, or the change in the level, of any variable associated with KHK gene expression, *e.g.*, KHK mRNA level or KHK 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.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with KHK 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).

It is understood that the degree and duration of elevation of a sign of a KHK-associated disease will vary depending upon the sign. For example, lipid signs, *e.g.*, fasting lipid levels,

NAFLD, NASH, obesity; signs of liver and kidney function, and glucose or insulin response, are durable signs that will not vary in a clinically significant manner within a day or even within a week. Other markers, *e.g.*, serum uric acid and glucose levels, and urine fructose levels, will vary within and likely between days. Blood pressure can be elevated transiently and durably in response to fructose. As fructose likely results in weight gain at least in part by reducing saiety, fructose consumption in conjunction with caloric limitation may not result in weight gain.

Further, depending on the disease state in the subject, as many as one third of adults and two thirds of children malabsorb fructose (Johnson *et al* . (2013) Diabetes. 62:3307-3315), *e.g.*, due to variations in expression of the GLUT5 transporter in the gut. However, repeated exposure to fructose can increase fructose absorption. Fructose metabolism has demonstrated to be different depending on the source of fructose, *e.g.*, in high fructose corn syrup vs. in natural fruit, and at high concentrations, such as those provided by soft drinks, glucose can be converted to fructose by the polyol pathway. However, fructose will have more metabolic effects than glucose. Body composition, *e.g.*, lean body mass, has also been demonstrated to affect fructose metabolism. Therefore, both the timing of testing and controls must be carefully selected.

In some embodiments of the methods of the invention, expression of a KHK gene is inhibited by at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In some embodiments, expression of a KHK gene is inhibited by at least 70%. In some embodiments, expression level is determined using the assay method provided in Example 2 with a 10 nM siRNA concentration in the appropriate species matched cell line.

In certain embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, an AAV-infected mouse expressing the human target gene (*i.e.*, KHK), *e.g.*, when administered as a single dose, *e.g.*, at 3 mg/kg at the nadir of RNA expression. Knockdown of expression of an endogenous gene in a model animal system can also be determined, *e.g.*, after administration of a single dose at, *e.g.*, 3 mg/kg at the nadir of RNA expression. Such systems are useful when the nucleic acid sequence of the human gene and the model animal gene are sufficiently close such that the human iRNA provides effective knockdown of the model animal gene. RNA expression in liver is determined using the PCR methods provided in Example 2.

Inhibition of the expression of a KHK 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 KHK gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of a KHK 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) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In some embodiments, the inhibition is assessed by the method provided in Example 2 using a 10nM siRNA concentration in

the species matched cell line and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

In other embodiments, inhibition of the expression of a KHK gene may be assessed in terms of a reduction of a parameter that is functionally linked to KHK gene expression, *e.g.*, KHK protein level in blood or serum from a subject. KHK gene silencing may be determined in any cell expressing KHK, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a KHK protein may be manifested by a reduction in the level of the KHK protein that is expressed by a cell or group of cells or in a subject sample (*e.g.*, the level of protein in a blood sample derived 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, or the change in the level of protein in a subject sample, *e.g.*, blood or serum derived therefrom.

A control cell, a group of cells, or subject sample that may be used to assess the inhibition of the expression of a KHK gene includes a cell, group of cells, or subject sample that has not yet been contacted with an RNAi agent of the invention. For example, the control cell, group of cells, or subject sample may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent or an appropriately matched population control.

The level of KHK mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of KHK in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the KHK gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using 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, northern blotting, *in situ* hybridization, and microarray analysis.

In some embodiments, the level of expression of KHK 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 KHK. 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.

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 the isolated mRNA with a

nucleic acid molecule (probe) that can hybridize to KHK 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 KHK mRNA.

An alternative method for determining the level of expression of KHK in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Patent No. 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*., U.S. Patent No. 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 particular aspects of the invention, the level of expression of KHK is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System). In some embodiments, expression level is determined by the method provided in Example 2 using, *e.g.,* a 10nM siRNA concentration, in the species matched cell line.

The expression levels of KHK 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 U.S. Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of KHK expression level may also comprise using nucleic acid probes in solution.

In some embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein. In some embodiments, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

The level of KHK 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, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention are assessed by a decrease in KHK mRNA or protein level (*e.g.*, in a liver biopsy).

In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of KHK may be assessed using measurements of the level or change in the level of KHK mRNA or KHK protein in a sample derived from fluid or tissue from the specific site within the subject (*e.g.*, liver or blood).

As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.*, protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

VIII. Prophylactic and Treatment Methods of the Invention

The present invention also provides methods of using an iRNA of the invention or a composition containing an iRNA of the invention to inhibit expression of KHK, thereby preventing or treating an KHK-associated disorder, *e.g.*, liver disease (*e.g.*, fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving. In the methods of the invention the cell may be contacted with the siRNA *in vitro* or *in vivo, i.e.,* the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses a KHK gene, *e.g.*, a liver cell. A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.,* a primate cell (such as a human cell, including human cell in a chimeric non-human animal, or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), or a non-primate cell. In certain embodiments, the cell is a human cell, *e.g.,* a human liver cell. In the methods of the invention, KHK expression is inhibited in the cell by at least 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay.

The *in vivo* methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the KHK gene of the mammal to which the RNAi agent is to be administered. The composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain

embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered by intramuscular injection.

In one aspect, the present invention also provides methods for inhibiting the expression of an KHK gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a KHK gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the KHK gene, thereby inhibiting expression of the KHK gene in the cell. Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein, *e.g.*, in Example 2. Reduction in protein production can be assessed by any methods known it the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the KHK gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in the KHK protein expression. A reduction in the expression of KHK may also be assessed indirectly by measuring a decrease in fructose metabolism by detecting one or more indicators of fructose metabolism, *e.g.*, the presence of fructose in the urine indicating lack of fructose metabolism.

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with a KHK-associated disorder, such as, liver disease (*e.g.*, fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

The present invention further provides methods of prophylaxis in a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction of KHK expression, in a prophylactically effective amount of an iRNA targeting a KHK gene or a pharmaceutical composition comprising an iRNA targeting a KHK gene.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in KHK expression, *e.g.,* a KHK-associated disease, such as liver disease (*e.g.*, fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic

syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

In certain embodiments, the KHK-associated disorder is a liver disease, *e.g.*, fatty liver disease such as NAFLD or NASH. In certain embodiments, the KHK-associated disorder is dyslipidemia, *e.g.*, elevated serum triglycerides, elevated serum LDL, elevated serum cholesterol, lowered serum HDL, postprandial hypertriglyceridemia. In another embodiment, the KHK-associated disorder is a disorder of glycemic control, *e.g.*, insulin resistance not resulting from an immune response against insulin, glucose resistance, type 2 diabetes. In certain embodiments, the KHK-associated disorder is a cardiovascular disease, *e.g.*, hypertension, endothelial cell dysfunction. In certain embodiments, the KHK-associated disorder is a kidney disease, *e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease. In certain embodiments, the disease is metabolic syndrome. In certain embodiments, the KHK-associated disorder is a disease of lipid deposition or dysfunction, *e.g.*, visceral adipose deposition, fatty liver, obesity. In certain embodiments, the KHK-associated disorder is a disease of elevated uric acid, *e.g.*, gout, hyperuricemia. In certain embodiments the KHK-associated disorder is an eating disorder such as excessive sugar craving.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA 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 osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from an inhibition of KHK gene expression are subjects susceptible to or diagnosed with an KHK-associated disorder, such as liver disease (*e.g.*, fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

In an embodiment, the method includes administering a composition featured herein such that expression of the target a KHK gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 1-6, 1-3, or 3-6 months per dose. In certain embodiments, the composition is administered once every 3-6 months.

In some embodiments, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target a KHK gene. Compositions and

methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result prevention or treatment of a KHK-associated disorder, *e.g.*, liver disease (*e.g.*, fatty liver, steatohepatitis, non-alcoholic steatohepatitis (NASH)), dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), cardiovascular disease (*e.g.*, hypertension, endothelial cell dysfunction), kidney disease (*e.g.*, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, chronic kidney disease), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, hyperuricemia, gout, eating disorders, and excessive sugar craving.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

In some embodiments, the iRNA is administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired dose of iRNA to a subject. The injections may be repeated over a period of time.

The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as once per month to once a year. In certain embodiments, the iRNA is administered about once per month to about once every three months, or about once every three months to about once every six months.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of KHK gene expression, *e.g.*, a subject having an KHK-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.

Accordingly, in some aspects of the invention, the methods which include either a single iRNA agent of the invention, further include administering to the subject one or more additional therapeutic agents.

The iRNA agent 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.

IX. Diagnostic Criteria and Treatment for KHK-Associated diseases

Diagnostic criteria, therapeutic agents, and considerations for treatment for various KHK-associated diseases are provided below.

A. Hyperuricemia

Serum uric acid levels are not routinely obtained as clinical lab values. However, hyperuricemia (elevated uric acid) is associated with a number of diseases and conditions including gout, NAFLD, NASH, metabolic disorder, insulin resistance (not resulting from an immune response to insulin), cardiovascular disease, hypertension, and type 2 diabetes. It is expected that decreasing KHK expression can be useful in the prevention or treatment of one or more conditions associated with elevated serum uric acid levels. Further, it is expected that a subject would derive clinical benefit from normalization of serum uric acid levels towards or to a normal serum uric acid level, *e.g.*, no more than 6.8 mg/dl, such as, no more than 6 mg/dl, even in the absence of overt signs or symptoms of one or more conditions associated with elevated uric acid.

Animal models of hyperuricemia include, for example, high fructose diet, *e.g.*, in rats and mice, which can induce one or more of fat accumulation including fatty liver, insulin resistance, type 2 diabetes, obesity including visceral obesity, metabolic syndrome, decreased adiponectin secretion, reduced renal function, and inflammation (see, *e.g.*, Johnson *et al* . (2013) Diabetes. 62:3307-3315). Administration of oxonic acid, a uricase inhibitor, can also be used to induce hyperuricemia (see, *e.g.*,Mazalli *et al* . (2001) Hypertens. 38:1101-1106). Genetic models of hyperuricemia include the B6;129S7-Uox^{tm1Bay}/J mouse available from Jackson Laboratory (/jaxmice.jax.org/strain/002223.html) which develops hyperuricemia, with 10-fold higher levels of serum uric acid levels.

Various treatments for hyperuricemia are known in the art. However, some of the agents can only be used in limited populations. For example, allopurinol is a xanthine oxidase inhibitor that is used to reduce serum uric acid levels for the treatment of a number of conditions, *e.g.*, gout, cardiovascular disease including ischemia-reperfusion injury, hypertension, atherosclerosis, and stroke, and inflammatory diseases (Pacher *et al* ., (2006) Pharma. Rev. 58:87-114). However, the use of allopurinol is contraindicated in subjects with impaired renal function, *e.g.*, chronic kidney disease, hypothyroidism, hyperinsulinemia, or insulin resistance; or in subjects predisposed to kidney disease or impaired renal function, *e.g.*, subjects with hypertension, metabolic disorder, diabetes, and the elderly. Further, allopurinol should not be taken by subjects taking oral coagulants or probencid as well as subjects taking diuretics, especially thiazide diuretics or other drugs that can reduce kidney function or have potential kidney toxicity.

In certain embodiments, the compositions and methods of the invention are used in combination with other compositions and methods to treat hyperuricemia, *e.g.*, allopurinol, oxypurinol, febuxostat. In certain embodiments, the compositions and methods of the invention are used for treatment of subjects with reduced kidney function or susceptible to reduced kidney function, *e.g.*, due to age, comorbidities, or drug interactions.

B. Gout

Gout affects approximately 1 in 40 adults, most commonly men between 30-60 years of age. Gout less commonly affects women. Gout is one of a few types of arthritis where future damage to

joints can be avoided by treatment. Gout is characterized by recurrent attacks of acute inflammatory arthritis caused by an inflammatory reaction to uric acid crystals in the joint due to hyperuricemia resulting from insufficient renal clearance of uric acid or excessive uric acid production. Fructose associated gout is sometimes associated with variants of transporters expressed in the kidney, intestine, and liver. Gout is characterized by the formation and deposition of tophi, monosodium urate (MSU) crystals, in the joints and subcutaneously. Pain associated with gout is not related to the size of the tophi, but is a result of an immune response against the MSU crystals. There is a linear inverse relation between serum uric acid and the rate of decrease in tophus size. For example, in one study of 18 patients with non-tophaceous gout, serum uric acid declined to 2.7–5.4 mg/dL (0.16–0.32 mM) in all subjects within 3 months of starting urate lowering therapy (Pascual and Sivera (2007) Ann. Rheum. Dis. 66:1056-1058). However, it took 12 months with normalized serum uric acid for MSU crystals to disappear from asymptomatic knee or first MTP joints in patients who had gout for less than 10 years, vs. 18 months in those with gout for more than 10 years. Therefore, effective treatment of gout does not require complete clearance of tophi or resolution of all symptoms, *e.g.*, joint pain and swelling, inflammation, but simply a reduction in at least one sign or symptom of gout, *e.g.*, reduction in severity or frequency of gout attacks, in conjunction with a reduction in serum urate levels.

Animal models of gout include oxonic acid- induced hyperuricemia (see, *e.g.*, Jang *et al* . (2014) Mycobiology. 42:296-300).

Currently available treatments for gout are contraindicated or ineffective in a number of subjects. Allopurinol, a common first line treatment to reduce uric acid levels in subjects with gout, is contraindicated in a number of populations, especially those with compromised renal function, as discussed above. Further, a number of subjects fail treatment with allopurinol, *e.g.*, subjects who suffer gout flares despite treatment, or subjects who suffer from rashes or hypersensitivity reactions associated with allopurinol.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of gout, *e.g.*, analgesic or anti-inflammatory agents, *e.g.*, NSAIDS. In certain embodiments, the compositions and methods of the invention are used for treatment of subjects with reduced kidney function or susceptible to reduced kidney function, *e.g.*, due to age, comorbidities, or drug interactions.

C. Liver disease

NAFLD is associated with hyperuricemia (Xu *et al* . (2015) J. Hepatol. 62:1412-1419) which, in turn, is associated with elevated fructose metabolism. The definition of nonalcoholic fatty liver disease (NAFLD) requires that (a) there is evidence of hepatic steatosis, either by imaging or by histology and (b) there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders. In the majority of

patients, NAFLD is associated with metabolic risk factors such as obesity, diabetes mellitus, and dyslipidemia. NAFLD is histologically further categorized into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis (Chalasani *et al* .(2012) Hepatol. 55:2005-2023). It is generally agreed that patients with simple steatosis have very slow, if any, histological progression, while patients with NASH can exhibit histological progression to cirrhotic-stage disease. The long term outcomes of patients with NAFLD and NASH have been reported in several studies. Their findings can be summarized as follows; (a) patients with NAFLD have increased overall mortality compared to matched control populations, (b) the most common cause of death in patients with NAFLD, NAFL, and NASH is cardiovascular disease, and (c) patients with NASH (but not NAFL) have an increased liver-related mortality rate.

Animal models of NAFLD include various high fat- or high fructose-fed animal models. Genetic models of NAFLD include the B6.129S7-Ldlr^{tm1Her}/J and the B6.129S4-Pten^{tm1Hwu}/J mice available from The Jackson Laboratory.

Treatment of NAFLD is typically to manage the conditions that resulted in development of NAFLD. For example, patients with dyslipidemia are treated with agents to normalize cholesterol or triglycerides, as needed, to treat or prevent further progression of NAFLD. Patients with type 2 diabetes are treated with agents to normalize glucose or insulin sensitivity. Lifestyle changes, *e.g.*, changes in diet and exercise, are also used to treat NAFLD. In a mouse model of NAFLD, treatment with allopurinol both prevented the development of hepatic steatosis, but also significantly ameliorated established hepatic steatosis in mice (Xu *et al* ., J. Hepatol. 62:1412-1419, 2015).

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of NAFLD. In certain embodiments, the compositions and methods of the invention are used for treatment of subjects with reduced kidney function or susceptible to reduced kidney function, *e.g.*, due to age, comorbidities, or drug interactions.

D. Dyslipidemia, disorders of glycemic control, metabolic syndrome, and obesity

Dyslipidemia (*e.g.*, hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia), disorders of glycemic control (*e.g.*, insulin resistance, type 2 diabetes), metabolic syndrome, adipocyte dysfunction, visceral adipose deposition, obesity, and excessive sugar craving are associated with elevated fructose metabolism. Characteristics or diagnostic criteria for the conditions are provided below. Animal models of metabolic disorder and the component features include various high fat- or high fructose-fed animal

models. Genetic models include leptin deficient B6.Cg-*Lep*^{ob}/J, commonly known as *ob* or *ob/ob* mice, which are available from The Jackson Laboratory.

Normal and abnormal fasting levels of the lipids are provided in the table below.

Lipid	**Value**	**Interpretation**
Total cholesterol	Below 200 mg/dL	Desirable
	200-239 mg/dL	Borderline high
	240 mg/dL and above	High
LDL cholesterol	Below 70 mg/dL	Best for people who have heart disease or diabetes.
	Below 100 mg/dL	Optimal for people at risk of heart disease.
	100-129 mg/dL	Near optimal if there is no heart disease. High if there is heart disease.
	130-159 mg/dL	Borderline high if there is no heart disease. High if there is heart disease.
	160-189 mg/dL	High if there is no heart disease. Very high if there is heart disease.
	190 mg/dL and above	Very high
HDL cholesterol	Below 40 mg/dL (men) Below 50 mg/dL (women)	Poor
	50-59 mg/dL	Moderate
	60 mg/dL and above	Normal
Triglycerides	Below 150 mg/dL	Desirable
	150-199 mg/dL	Borderline high
	200-499 mg/dL	High
	500 mg/dL and above	Very High

Postprandial hypertriglyceridemia is principally initiated by overproduction or decreased catabolism of triglyceride-rich lipoproteins (TRLs) and is a consequence of predisposing genetic variations and medical conditions such as obesity and insulin resistance.

Insulin resistance is characterized by the presence of at least one of:

1. A fasting blood glucose level of 100-125 mg/dL taken at two different times; or
2. An oral glucose tolerance test with a result of a glucose level of 140-199 mg/dL at 2 hours after glucose consumption.

As used herein, insulin resistance does not include a lack of response to insulin as a result of an immune response to administered insulin as often occurs in late stages of insulin dependent diabetes, especially type 1 diabetes.

Type 2 diabetes is characterized by at least one of:

1. A fasting blood glucose level $\geq$ 126 mg/dL taken at two different times;

2. A hemoglobin A1c (A1C) test with a result of ≥ 6.5% or higher; or

3. An oral glucose tolerance test with a result of a glucose level ≥ 200 mg/dL at 2 hours after glucose consumption.

Pharmacological treatments for type 2 diabetes and insulin resistance include treatment with agents to normalize blood sugar such as metformin (*e.g.*, glucophage, glumetza), sulfonylureas (*e.g.*, glyburide, glipizide, glimepiride), meglitinides (*e.g.*, repaglinide, nateglinide), thiazolidinediones (rosiglitazone, pioglitazone), DPP-4 inhibitors (sitagliptin, saxagliptin, linagliptin), GLP-1 receptor antagonists (exenatide, liraglutide), and SGLT2 inhibitors (*e.g.*, canagliflozin, dapagliflozin).

Obesity is characterized as disease of excess body fat. Body mass index (BMI), which is calculated by dividing body weight in kilograms (kg) by height in meters (m) squared, provides a reasonable estimate of body fat for most, but not all, people. Generally, a BMI below 18.5 is characterized as underweight, 18-.5 to24.9 is normal, 25.0-29.9 is overweight, 30.0-34.9 is obese (class I), 35-39.9 is obese (class II), and 40.0 and higher is extremely obese (class III).

Methods for assessment of subcutaneous vs. visceral fat are provided, for example, in Wajchenberg (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome, Endocr Rev. 21:697-738, which is incorporated herein by reference.

Metabolic syndrome is characterized by a cluster of conditions defined as at least three of the five following metabolic risk factors:

1. Large waistline (≥ 35 inches for women or ≥ 40 inches for men);
2. High triglyceride level (≥ 150 mg/dl);
3. Low HDL cholesterol (≤ 50 mg/dl for women or ≤ 40 mg/dl for men);
4. Elevated blood pressure (≥ 130/85) or on medicine to treat high blood pressure; and
5. High fasting blood sugar (≥ 100 mg/dl) or being in medicine to treat high blood sugar.

As with NAFLD, the agents for treatment of metabolic syndrome depend on the specific risk factors present, *e.g.*, normalize lipids when lipids are abnormal, normalize glucose or insulin sensitivity when they are abnormal.

Metabolic syndrome, insulin resistance, and type 2 diabetes are often associated with decreased renal function or the potential for decreased renal function.

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with dyslipidemia, disorders of glycemic control, metabolic syndrome, and obesity. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are suffering from one or more of cardiovascular disease, hypothyroidism, or inflammatory disease; or elderly subjects (*e.g.*, over 65). In certain embodiments, the compositions and methods are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods

of the invention are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are being treated with diuretics, especially thiazide diuretics.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of metabolic syndrome, insulin resistance, or type 2 diabetes. In certain embodiments, subjects are treated with *e.g.*, agents to decrease blood pressure, *e.g.*, diuretics, beta-blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor antagonists, combined alpha- and beta-blockers, central agonists, peripheral adrenergic inhibitors, and blood vessel dialators; agents to decrease cholesterol, *e.g.*, statins, selective cholesterol absorption inhibitors, resins, or lipid lowering therapies; or agents to normalize blood sugar, *e.g.*, metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor antagonists, and SGLT2 inhibitors.

In certain embodiments, the compositions and methods of the invention are used for treatment of subjects with reduced kidney function or susceptible to reduced kidney function, *e.g.*, due to age, comorbidities, or drug interactions.

The iRNA and additional therapeutic agents may be administered at the same time 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 or by another method known in the art or described herein.

E. Cardiovascular disease

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with cardiovascular disease. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with cardiovascular disease and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with cardiovascular disease who are suffering from one or more of metabolic disorder, insulin resistance, hyperinsulinemia, diabetes, hypothyroidism, or inflammatory disease. In certain embodiments, the compositions and methods are for use in subjects with cardiovascular disease who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with cardiovascular disease who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with cardiovascular disease who are being treated with diuretics, especially thiazide diuretics. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with cardiovascular disease who have failed treatment with allopurinol.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of cardiovascular disease, *e.g.*, agents to decrease blood pressure, *e.g.*, diuretics, beta-blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor antagonists, combined alpha- and beta-blockers, central agonists, peripheral adrenergic inhibitors, and blood vessel dialators; or agents to decrease cholesterol, *e.g.*, statins, selective cholesterol absorption inhibitors, resins, or lipid lowering therapies.

F. Kidney disease

Kidney disease includes, for example, acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, and chronic kidney disease.

Acute kidney (renal) failure occurs when the kidneys suddenly become unable to filter waste products from the blood resulting in accumulation of dangerous levels of wastes in serum and systemic chemical imbalance. Acute kidney failure can develop rapidly over a few hours or a few days, and is most common in individuals who are already hospitalized, particularly in critically ill individuals who need intensive care. Acute kidney failure can be fatal and requires intensive treatment. However, acute kidney failure may be reversible. If you're otherwise in good health, you may recover normal or nearly normal kidney function.

Chronic kidney disease, also called chronic kidney failure, describes the gradual loss of kidney function. When chronic kidney disease reaches an advanced stage, dangerous levels of fluid, electrolytes and wastes can accumulate in the body. Signs and symptoms of kidney disease may include nausea, vomiting, loss of appetite, fatigue and weakness, sleep problems, changes in urine output, decreased mental sharpness, muscle twitches and cramps, hiccups, swelling of feet and ankles, persistent itching, chest pain, if fluid builds up around the lining of the heart, shortness of breath, if fluid builds up in the lungs, high blood pressure (hypertension) that's difficult to control. Signs and symptoms of chronic kidney disease are often nonspecific and can develop slowly, and may not appear until irreversible damage has occurred.

Kidney disease is treated by removing the damaging agent or condition that is causing kidney damage, *e.g.* normalize blood pressure to improve kidney function, end treatment with agents that can induce kidney damage, reduce inflammation that is causing kidney damage, or by providing renal support (*e.g.*, renal dialysis) to assist kidney function.

Renal function is typically determined using one or more routine laboratory tests, BUN (blood urea nitrogen), creatinine (blood), creatinine (urine), or creatinine clearance (see, *e.g.*, www.nlm.nih.gov/medlineplus/ency/article/003435.htm). The tests may also be diagnostic of conditions in other organs.

Generally, a BUN level of 6 to 20 mg/dL is considered normal, although normal values may vary among different laboratories. Elevated BUN level can be indicative of kidney disease, including glomerulonephritis, pyelonephritis, and acute tubular necrosis, or kidney failure.

A normal result for blood creatinine is 0.7 to 1.3 mg/dL for men and 0.6 to 1.1 mg/dL for women. Elevated blood creatinine can be indicative of compromised kidney function due to kidney damage or failure, infection, or reduced blood flow.

Urine creatinine (24-hour sample) values can range from 500 to 2000 mg/day. Results depend on age and amount of lean body mass. Normal results are 14 to 26 mg per kg of body mass per day for men and 11 to 20 mg per kg of body mass per day for women. Abnormal results can be indicative of kidney damage, such as damage to the tubule cells, kidney failure, decreased blood flow to the kidneys, or kidney infection (pyelonephritis).

The creatinine clearance test helps provide information regarding kidney function by comparing the creatinine level in urine with the creatinine level in blood. Clearance is often measured as milliliters per minute (ml/min). Normal values are 97 to 137 ml/min. for men and 88 to 128 ml/min. for women. Lower than normal creatinine clearance can be indicative of kidney damage, such as damage to the tubule cells, kidney failure, decreased blood flow to the kidneys, or reduced glomerular filtration in the kidneys.

In certain embodiements, the compositions and methods of the invention can be used for the treatment of kidney disease. It is expected that such agents would not cause damage to the kidney.

X. Kits

In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a siRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof).

Such kits include one or more dsRNA agent(s) and instructions for use, *e.g.*, instructions for administering a prophylactically or therapeutically effective amount of a dsRNA agent(s). The dsRNA agent may be in a vial or a pre-filled syringe. The kits may optionally further comprise means for administering the dsRNA agent (*e.g.*, an injection device, such as a pre-filled syringe), or means for measuring the inhibition of KHK (*e.g.,* means for measuring the inhibition of KHK mRNA, KHK protein, and/or KHK activity). Such means for measuring the inhibition of KHK may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for determining the therapeutically effective or prophylactically effective amount.

In certain embodiments the individual components of the pharmaceutical formulation may be provided in one container, *e.g.,* a vial or a pre-filled syringe. Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*,

one container for a siRNA compound preparation, and at least another for a carrier compound. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the informal Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA Design

siRNAs targeting the human KHK (human NCBI refseqID: XM_017004061.1; NCBI GeneID: 3795) were designed using custom R and Python scripts. The human KHK REFSEQ mRNA has a length of 2283 bases.

Detailed lists of the unmodified KHK sense and antisense strand nucleotide sequences are shown in Tables 2, 5 and 8. Detailed lists of the modified KHK sense and antisense strand nucleotide sequences are shown in Tables 3, 6 and 9.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

siRNA Synthesis

siRNAs were designed, synthesized, and prepared using methods known in the art.

Briefly, siRNA sequences were synthesized on a 1 μmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or

Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 µL of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

Example 2. In vitro screening methods

HepG2 Cell culture and 96-well transfections

HepG2 cells were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 18.5 µl of Opti-MEM plus 0.25 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of each siRNA duplex to an individual well in a 96-well plate. The mixture was then incubated at room temperature for 15 minutes. Eighty µl of complete growth media without antibiotic containing ~2

x10^4 HepG2 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Dose experiments were performed at 10 nM, 1 nM and 0.1 nM final duplex concentration.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR

Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human KHK, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC50s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 18) and antisense UCGAAGuACUcAGCGuAAGdTsdT (SEQ ID NO: 19).

The results of the transfection assays of the dsRNA agents listed in Tables 2 and 3 in HepG2 cells are shown in Table 4.

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds; and it is understood that when the nucleotide contains a 2'-fluoro modification,

then the fluoro replaces the hydroxy at that position in the parent nucleotide (*i.e.*, it is a 2'-deoxy-2'-fluoronucleotide).

Abbre*via*tion	**Nucleotide(s)**
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Abs	beta-L-adenosine-3`-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3`-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3`-phosphate
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol)

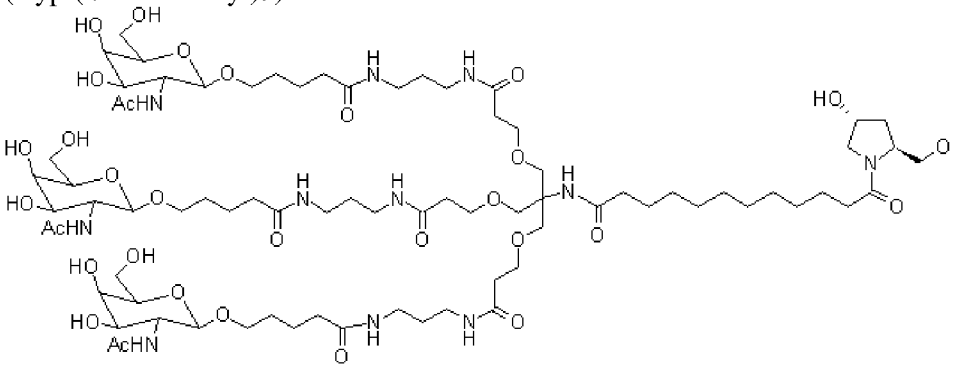
Abbre*via*tion	Nucleotide(s)
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3) 
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose)
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate)
(Agn)	Adenosine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA) S-Isomer
(Ggn)	Guanosine-glycol nucleic acid (GNA) S-Isomer
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
dA	2`-deoxyadenosine-3`-phosphate
dAs	2`-deoxyadenosine-3`-phosphorothioate
dC	2`-deoxycytidine-3`-phosphate
dCs	2`-deoxycytidine-3`-phosphorothioate
dG	2`-deoxyguanosine-3`-phosphate
dGs	2`-deoxyguanosine-3`-phosphorothioate
dT	2`-deoxythimidine-3`-phosphate
dTs	2`-deoxythimidine-3`-phosphorothioate
dU	2`-deoxyuridine
dUs	2`-deoxyuridine-3`-phosphorothioate
(C2p)	cytidine-2`-phosphate
(G2p)	guanosine-2`-phosphate
(U2p)	uridine-2`-phosphate
(A2p)	adenosine-2`-phosphate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
s	phosphorothioate

Table 2. Unmodified Sense and Antisense Strand Sequences of KHK dsRNAs

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290652	GUGGUGUUUGUCAGCAAAGAU	20	810-830	AUCUUUGCUGACAAACACCACGU	244	808-830
AD-1290731	ACGUGGUGUUUGUCAGCAAAU	21	808-828	AUUUGCUGACAAACACCACGUCU	245	806-828
AD-1290560	UCAGAGCAAAUAAAUCUUCCU	22	1336-1356	AGGAAGAUUUAUUUGCUCUGAGG	246	1334-1356
AD-1290854	CAGUUCAAGUGGAUCCACAUU	23	642-662	AAUGUGGAUCCACUUGAACUGGG	247	640-662
AD-1290629	AGUAGCGCAUUUUCUCUUUGU	24	122-142	ACAAAGAGAAAAUGCGCUACUUG	248	120-142
AD-1290517	GCGCAUUUUCUCUUUGCAUUU	25	126-146	AAAUGCAAAGAGAAAAUGCGCUA	249	124-146
AD-1290526	CGCAUUUUCUCUUUGCAUUCU	26	127-147	AGAAUGCAAAGAGAAAAUGCGCU	250	125-147
AD-1290559	GCAUUUUCUCUUUGCAUUCUU	27	128-148	AAGAAUGCAAAGAGAAAAUGCGC	251	126-148
AD-1290548	CAUUUUCUCUUUGCAUUCUCU	28	129-149	AGAGAAUGCAAAGAGAAAAUGCG	252	127-149
AD-1290569	UUUUCUCUUUGCAUUCUCGAU	29	131-151	AUCGAGAAUGCAAAGAGAAAAUG	253	129-151
AD-1290547	UUCUCUUUGCAUUCUCGAGAU	30	133-153	AUCUCGAGAAUGCAAAGAGAAAA	254	131-153
AD-1290544	UCUCUUUGCAUUCUCGAGAUU	31	134-154	AAUCUCGAGAAUGCAAAGAGAAA	255	132-154
AD-1290667	CUCUUUGCAUUCUCGAGAUCU	32	135-155	AGAUCUCGAGAAUGCAAAGAGAA	256	133-155
AD-1290685	UCUUUGCAUUCUCGAGAUCGU	33	136-156	ACGAUCUCGAGAAUGCAAAGAGA	257	134-156
AD-1290695	UUUGCAUUCUCGAGAUCGCUU	34	138-158	AAGCGAUCUCGAGAAUGCAAAGA	258	136-158
AD-1290653	UGCAUUCUCGAGAUCGCUUAU	35	140-160	AUAAGCGAUCUCGAGAAUGCAAA	259	138-160
AD-1290853	GCAUUCUCGAGAUCGCUUAGU	36	141-161	ACUAAGCGAUCUCGAGAAUGCAA	260	139-161
AD-1290990	CAUUCUCGAGAUCGCUUAGCU	37	142-162	AGCUAAGCGAUCUCGAGAAUGCA	261	140-162
AD-1290540	CUUUAAAAAGGUUUGCAUCAU	38	166-186	AUGAUGCAAACCUUUUUAAAGCG	262	164-186
AD-1290580	UUUAAAAAGGUUUGCAUCAGU	39	167-187	ACUGAUGCAAACCUUUUUAAAGC	263	165-187
AD-1290664	UUAAAAAGGUUUGCAUCAGCU	40	168-188	AGCUGAUGCAAACCUUUUUAAAG	264	166-188
AD-1290916	UCAGCUGUGAGUCCAUCUGAU	41	183-203	AUCAGAUGGACUCACAGCUGAUG	265	181-203
AD-1290938	GCUGUGAGUCCAUCUGACAAU	42	186-206	AUUGUCAGAUGGACUCACAGCUG	266	184-206
AD-1290896	CUGUGAGUCCAUCUGACAAGU	43	187-207	ACUUGUCAGAUGGACUCACAGCU	267	185-207
AD-1290914	CCAUCUGACAAGCGAGGAAAU	44	195-215	AUUUCCUCGCUUGUCAGAUGGAC	268	193-215
AD-1290982	CAUCUGACAAGCGAGGAAACU	45	196-216	AGUUUCCUCGCUUGUCAGAUGGA	269	194-216

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290708	GGAAACUAAGGCUGAGAAGUU	46	210-230	AACUUCUCAGCCUUAGUUUCCUC	270	208-230
AD-1290693	GAAACUAAGGCUGAGAAGUGU	47	211-231	ACACUUCUCAGCCUUAGUUUCCU	271	209-231
AD-1290942	AGACCUCUGGGUUGGCUUUCU	48	286-306	AGAAAGCCAACCCAGAGGUCUUG	272	284-306
AD-1290807	AGUAGCCUCAUGGAAGAGAAU	49	514-534	AUUCUCUUCCAUGAGGCUACUCC	273	512-534
AD-1290881	CCUCAUGGAAGAGAAGCAGAU	50	519-539	AUCUGCUUCUCUUCCAUGAGGCU	274	517-539
AD-1290745	CUCAUGGAAGAGAAGCAGAUU	51	520-540	AAUCUGCUUCUCUUCCAUGAGGC	275	518-540
AD-1290814	AUGGAAGAGAAGCAGAUCCUU	52	523-543	AAGGAUCUGCUUCUCUUCCAUGA	276	521-543
AD-1290900	GGAAGAGAAGCAGAUCCUGUU	53	525-545	AACAGGAUCUGCUUCUCUUCCAU	277	523-545
AD-1290964	GAAGAGAAGCAGAUCCUGUGU	54	526-546	ACACAGGAUCUGCUUCUCUUCCA	278	524-546
AD-1290802	AUCAGCCUGGUGGACAAGUAU	55	571-591	AUACUUGUCCACCAGGCUGAUGA	279	569-591
AD-1290816	GUGGACAAGUACCCUAAGGAU	56	580-600	AUCCUUAGGGUACUUGUCCACCA	280	578-600
AD-1290821	GACAAGUACCCUAAGGAGGAU	57	583-603	AUCCUCCUUAGGGUACUUGUCCA	281	581-603
AD-1290870	GAGGACUCGGAGAUAAGGUGU	58	598-618	ACACCUUAUCUCCGAGUCCUCCU	282	596-618
AD-1290984	UAAGGUGUUUGUCCCAGAGAU	59	611-631	AUCUCUGGGACAAACACCUUAUC	283	609-631
AD-1290682	AAGGUGUUUGUCCCAGAGAUU	60	612-632	AAUCUCUGGGACAAACACCUUAU	284	610-632
AD-1290872	CAACUCCUGCACCGUUCUCUU	61	654-674	AAGAGAACGGUGCAGGAGUUGGA	285	652-674
AD-1290663	UCUGCUACAGACUUUGAGAAU	62	748-768	AUUCUCAAAGUCUGUAGCAGACA	286	746-768
AD-1290627	CUGCUACAGACUUUGAGAAGU	63	749-769	ACUUCUCAAAGUCUGUAGCAGAC	287	747-769
AD-1290730	UGCUACAGACUUUGAGAAGGU	64	750-770	ACCUUCUCAAAGUCUGUAGCAGA	288	748-770
AD-1290692	GCUACAGACUUUGAGAAGGUU	65	751-771	AACCUUCUCAAAGUCUGUAGCAG	289	749-771
AD-1290579	CUACAGACUUUGAGAAGGUUU	66	752-772	AAACCUUCUCAAAGUCUGUAGCA	290	750-772
AD-1290591	ACAGACUUUGAGAAGGUUGAU	67	754-774	AUCAACCUUCUCAAAGUCUGUAG	291	752-774
AD-1290539	CAGACUUUGAGAAGGUUGAUU	68	755-775	AAUCAACCUUCUCAAAGUCUGUA	292	753-775
AD-1290611	AGACUUUGAGAAGGUUGAUCU	69	756-776	AGAUCAACCUUCUCAAAGUCUGU	293	754-776
AD-1290530	GACUUUGAGAAGGUUGAUCUU	70	757-777	AAGAUCAACCUUCUCAAAGUCUG	294	755-777
AD-1290576	CUUUGAGAAGGUUGAUCUGAU	71	759-779	AUCAGAUCAACCUUCUCAAAGUC	295	757-779
AD-1290546	UUUGAGAAGGUUGAUCUGACU	72	760-780	AGUCAGAUCAACCUUCUCAAAGU	296	758-780

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290823	AAGGUUGAUCUGACCCAGUUU	73	766-786	AAACUGGGUCAGAUCAACCUUCU	297	764-786
AD-1290757	GUUGAUCUGACCCAGUUCAAU	74	769-789	AUUGAACUGGGUCAGAUCAACCU	298	767-789
AD-1290959	UUGAUCUGACCCAGUUCAAGU	75	770-790	ACUUGAACUGGGUCAGAUCAACC	299	768-790
AD-1290837	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUUGAACUGGGUCAGAUCAAC	300	769-791
AD-1290861	GAUCUGACCCAGUUCAAGUGU	77	772-792	ACACUUGAACUGGGUCAGAUCAA	301	770-792
AD-1290885	CUGACCCAGUUCAAGUGGAUU	78	775-795	AAUCCACUUGAACUGGGUCAGAU	302	773-795
AD-1290970	ACCCAGUUCAAGUGGAUCCAU	79	778-798	AUGGAUCCACUUGAACUGGGUCA	303	776-798
AD-1290962	CCAGUUCAAGUGGAUCCACAU	80	780-800	AUGUGGAUCCACUUGAACUGGGU	304	778-800
AD-1290759	AGUUCAAGUGGAUCCACAUUU	81	782-802	AAAUGUGGAUCCACUUGAACUGG	305	780-802
AD-1290736	UUCAAGUGGAUCCACAUUGAU	82	784-804	AUCAAUGUGGAUCCACUUGAACU	306	782-804
AD-1290739	UCAAGUGGAUCCACAUUGAGU	83	785-805	ACUCAAUGUGGAUCCACUUGAAC	307	783-805
AD-1290828	CAAGUGGAUCCACAUUGAGGU	84	786-806	ACCUCAAUGUGGAUCCACUUGAA	308	784-806
AD-1291001	GCAUCGGAGCAGGUGAAGAUU	85	814-834	AAUCUUCACCUGCUCCGAUGCGU	309	812-834
AD-1290933	CAUCGGAGCAGGUGAAGAUGU	86	815-835	ACAUCUUCACCUGCUCCGAUGCG	310	813-835
AD-1290988	GAGCUCUUCCAGCUGUUUGGU	87	919-939	ACCAAACAGCUGGAAGAGCUCCU	311	917-939
AD-1290955	CUCUUCCAGCUGUUUGGCUAU	88	922-942	AUAGCCAAACAGCUGGAAGAGCU	312	920-942
AD-1290810	CUACGGAGACGUGGUGUUUGU	89	939-959	ACAAACACCACGUCUCCGUAGCC	313	937-959
AD-1290740	UACGGAGACGUGGUGUUUGUU	90	940-960	AACAAACACCACGUCUCCGUAGC	314	938-960
AD-1290752	CGGAGACGUGGUGUUUGUCAU	91	942-962	AUGACAAACACCACGUCUCCGUA	315	940-962
AD-1290878	GGAGACGUGGUGUUUGUCAGU	92	943-963	ACUGACAAACACCACGUCUCCGU	316	941-963
AD-1290599	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCUUUGCUGACAAACACCACG	317	948-970
AD-1290632	GGUGUUUGUCAGCAAAGAUGU	94	951-971	ACAUCUUUGCUGACAAACACCAC	318	949-971
AD-1290584	GUGUUUGUCAGCAAAGAUGUU	95	952-972	AACAUCUUUGCUGACAAACACCA	319	950-972
AD-1290734	UGUUUGUCAGCAAAGAUGUGU	96	953-973	ACACAUCUUUGCUGACAAACACC	320	951-973
AD-1290882	GUUUGUCAGCAAAGAUGUGGU	97	954-974	ACCACAUCUUUGCUGACAAACAC	321	952-974
AD-1290987	UUUGUCAGCAAAGAUGUGGCU	98	955-975	AGCCACAUCUUUGCUGACAAACA	322	953-975
AD-1290963	AAAGAUGUGGCCAAGCACUUU	99	964-984	AAAGUGCUUGGCCACAUCUUUGC	323	962-984

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290710	UGUAUGGUCGUGUGAGGAAAU	100	1019-1039	AUUUCCUCACACGACCAUACAAG	324	1017-1039
AD-1290656	GUAUGGUCGUGUGAGGAAAGU	101	1020-1040	ACUUUCCUCACACGACCAUACAA	325	1018-1040
AD-1290890	UAUGGUCGUGUGAGGAAAGGU	102	1021-1041	ACCUUUCCUCACACGACCAUACA	326	1019-1041
AD-1290887	UGCUCCACUCGGAUGCUUUCU	103	1103-1123	AGAAAGCAUCCGAGUGGAGCAAU	327	1101-1123
AD-1290923	GAGCUGGAGACACCUUCAAUU	104	1151-1171	AAUUGAAGGUGUCUCCAGCUCCC	328	1149-1171
AD-1290904	AGCUGGAGACACCUUCAAUGU	105	1152-1172	ACAUUGAAGGUGUCUCCAGCUCC	329	1150-1172
AD-1290980	CUGGAGACACCUUCAAUGCCU	106	1154-1174	AGGCAUUGAAGGUGUCUCCAGCU	330	1152-1174
AD-1290860	UGGAGACACCUUCAAUGCCUU	107	1155-1175	AAGGCAUUGAAGGUGUCUCCAGC	331	1153-1175
AD-1290969	ACCUUCAAUGCCUCCGUCAUU	108	1162-1182	AAUGACGGAGGCAUUGAAGGUGU	332	1160-1182
AD-1290811	CCUUCAAUGCCUCCGUCAUCU	109	1163-1183	AGAUGACGGAGGCAUUGAAGGUG	333	1161-1183
AD-1290886	UUCAAUGCCUCCGUCAUCUUU	110	1165-1185	AAAGAUGACGGAGGCAUUGAAGG	334	1163-1185
AD-1290668	CAAUGCCUCCGUCAUCUUCAU	111	1167-1187	AUGAAGAUGACGGAGGCAUUGAA	335	1165-1187
AD-1290852	AAUGCCUCCGUCAUCUUCAGU	112	1168-1188	ACUGAAGAUGACGGAGGCAUUGA	336	1166-1188
AD-1290915	CUCCGUCAUCUUCAGCCUCUU	113	1173-1193	AAGAGGCUGAAGAUGACGGAGGC	337	1171-1193
AD-1290874	GUGCAGGAAGCACUGAGAUUU	114	1207-1227	AAAUCUCAGUGCUUCCUGCACGC	338	1205-1227
AD-1290818	UGCAGGAAGCACUGAGAUUCU	115	1208-1228	AGAAUCUCAGUGCUUCCUGCACG	339	1206-1228
AD-1290884	GCAGGAAGCACUGAGAUUCGU	116	1209-1229	ACGAAUCUCAGUGCUUCCUGCAC	340	1207-1229
AD-1290977	UGGCCUGCAGGGCUUUGAUGU	117	1254-1274	ACAUCAAAGCCCUGCAGGCCACA	341	1252-1274
AD-1290961	CUGCAGGGCUUUGAUGGCAUU	118	1258-1278	AAUGCCAUCAAAGCCCUGCAGGC	342	1256-1278
AD-1290834	CAGGGCUUUGAUGGCAUCGUU	119	1261-1281	AACGAUGCCAUCAAAGCCCUGCA	343	1259-1281
AD-1290867	GGGCUUUGAUGGCAUCGUGUU	120	1263-1283	AACACGAUGCCAUCAAAGCCCUG	344	1261-1283
AD-1290879	CUCUGCCUGUGUCCUGUGUUU	121	1409-1429	AAACACAGGACACAGGCAGAGUC	345	1407-1429
AD-1290549	CUCAGAGCAAAUAAAUCUUCU	122	1474-1494	AGAAGAUUUAUUUGCUCUGAGGC	346	1472-1494
AD-1290525	CAGAGCAAAUAAAUCUUCCUU	123	1476-1496	AAGGAAGAUUUAUUUGCUCUGAG	347	1474-1496
AD-1290622	CUCCUCUCAAUGUCUGAACUU	124	1508-1528	AAGUUCAGACAUUGAGAGGAGAA	348	1506-1528
AD-1290638	UCCUCUCAAUGUCUGAACUGU	125	1509-1529	ACAGUUCAGACAUUGAGAGGAGA	349	1507-1529
AD-1290921	CCUCUCAAUGUCUGAACUGCU	126	1510-1530	AGCAGUUCAGACAUUGAGAGGAG	350	1508-1530

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290621	CUCUCAAUGUCUGAACUGCUU	127	1511-1531	AAGCAGUUCAGACAUUGAGAGGA	351	1509-1531
AD-1290775	CUCAAUGUCUGAACUGCUCUU	128	1513-1533	AAGAGCAGUUCAGACAUUGAGAG	352	1511-1533
AD-1290748	AUUCCUGAGGCUCUGACUCUU	129	1541-1561	AAGAGUCAGAGCCUCAGGAAUGC	353	1539-1561
AD-1290865	CUGCGUUGUGCAGACUCUAUU	130	1749-1769	AAUAGAGUCUGCACAACGCAGGG	354	1747-1769
AD-1290897	UGCGUUGUGCAGACUCUAUUU	131	1750-1770	AAAUAGAGUCUGCACAACGCAGG	355	1748-1770
AD-1290989	GCGUUGUGCAGACUCUAUUCU	132	1751-1771	AGAAUAGAGUCUGCACAACGCAG	356	1749-1771
AD-1290983	CGUUGUGCAGACUCUAUUCCU	133	1752-1772	AGGAAUAGAGUCUGCACAACGCA	357	1750-1772
AD-1290909	UUGUGCAGACUCUAUUCCCAU	134	1754-1774	AUGGGAAUAGAGUCUGCACAACG	358	1752-1774
AD-1290993	UAUUCCCACAGCUCAGAAGCU	135	1766-1786	AGCUUCUGAGCUGUGGGAAUAGA	359	1764-1786
AD-1290841	AUUCCCACAGCUCAGAAGCUU	136	1767-1787	AAGCUUCUGAGCUGUGGGAAUAG	360	1765-1787
AD-1290880	CUUGGAGCCCACCUUGGAAUU	137	1938-1958	AAUUCCAAGGUGGGCUCCAAGGG	361	1936-1958
AD-1290747	GGAGCCCACCUUGGAAUUAAU	138	1941-1961	AUUAAUUCCAAGGUGGGCUCCAA	362	1939-1961
AD-1290842	GAGCCCACCUUGGAAUUAAGU	139	1942-1962	ACUUAAUUCCAAGGUGGGCUCCA	363	1940-1962
AD-1290911	AGCCCACCUUGGAAUUAAGGU	140	1943-1963	ACCUUAAUUCCAAGGUGGGCUCC	364	1941-1963
AD-1290926	GCCCACCUUGGAAUUAAGGGU	141	1944-1964	ACCCUUAAUUCCAAGGUGGGCUC	365	1942-1964
AD-1291003	GGCGUGCCUCAGCCACAAAUU	142	1962-1982	AAUUUGUGGCUGAGGCACGCCCU	366	1960-1982
AD-1290931	UCAGCCACAAAUGUGACCCAU	143	1970-1990	AUGGGUCACAUUUGUGGCUGAGG	367	1968-1990
AD-1290764	GGUCCGAUCUGGAACACAUAU	144	2018-2038	AUAUGUGUUCCAGAUCGGACCUC	368	2016-2038
AD-1290763	GUCCGAUCUGGAACACAUAUU	145	2019-2039	AAUAUGUGUUCCAGAUCGGACCU	369	2017-2039
AD-1290670	UCCGAUCUGGAACACAUAUUU	146	2020-2040	AAAUAUGUGUUCCAGAUCGGACC	370	2018-2040
AD-1290712	CCGAUCUGGAACACAUAUUGU	147	2021-2041	ACAAUAUGUGUUCCAGAUCGGAC	371	2019-2041
AD-1290612	AUCUGGAACACAUAUUGGAAU	148	2024-2044	AUUCCAAUAUGUGUUCCAGAUCG	372	2022-2044
AD-1290522	UCUGGAACACAUAUUGGAAUU	149	2025-2045	AAUUCCAAUAUGUGUUCCAGAUC	373	2023-2045
AD-1290528	CUGGAACACAUAUUGGAAUUU	150	2026-2046	AAAUUCCAAUAUGUGUUCCAGAU	374	2024-2046
AD-1290543	UGGAACACAUAUUGGAAUUGU	151	2027-2047	ACAAUUCCAAUAUGUGUUCCAGA	375	2025-2047
AD-1290589	GGAACACAUAUUGGAAUUGGU	152	2028-2048	ACCAAUUCCAAUAUGUGUUCCAG	376	2026-2048
AD-1290800	GGGUGGGUAAGGCCUUAUAAU	153	2064-2084	AUUAUAAGGCCUUACCCACCCUA	377	2062-2084

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290755	GGUGGGUAAGGCCUUAUAAUU	154	2065-2085	AAUUAUAAGGCCUUACCCACCCU	378	2063-2085
AD-1290742	GUGGGUAAGGCCUUAUAAUGU	155	2066-2086	ACAUUAUAAGGCCUUACCCACCC	379	2064-2086
AD-1290563	GUAAGGCCUUAUAAUGUAAAU	156	2070-2090	AUUUACAUUAUAAGGCCUUACCC	380	2068-2090
AD-1290570	AAGGCCUUAUAAUGUAAAGAU	157	2072-2092	AUCUUUACAUUAUAAGGCCUUAC	381	2070-2092
AD-1290515	AGGCCUUAUAAUGUAAAGAGU	158	2073-2093	ACUCUUUACAUUAUAAGGCCUUA	382	2071-2093
AD-1290556	GCCUUAUAAUGUAAAGAGCAU	159	2075-2095	AUGCUCUUUACAUUAUAAGGCCU	383	2073-2095
AD-1290661	GCAUAUAAUGUAAAGGGCUUU	160	2092-2112	AAAGCCCUUUACAUUAUAUGCUC	384	2090-2112
AD-1290555	AUAUAAUGUAAAGGGCUUUAU	161	2094-2114	AUAAAGCCCUUUACAUUAUAUGC	385	2092-2114
AD-1290554	AUAAUGUAAAGGGCUUUAGAU	162	2096-2116	AUCUAAAGCCCUUUACAUUAUAU	386	2094-2116
AD-1290639	UAAUGUAAAGGGCUUUAGAGU	163	2097-2117	ACUCUAAAGCCCUUUACAUUAUA	387	2095-2117
AD-1290618	AAUGUAAAGGGCUUUAGAGUU	164	2098-2118	AACUCUAAAGCCCUUUACAUUAU	388	2096-2118
AD-1290660	CCUGGAUUAAAAUCUGCCAUU	165	2126-2146	AAUGGCAGAUUUUAAUCCAGGUC	389	2124-2146
AD-1290551	CUGGAUUAAAAUCUGCCAUUU	166	2127-2147	AAAUGGCAGAUUUUAAUCCAGGU	390	2125-2147
AD-1290509	GAUUAAAAUCUGCCAUUUAAU	167	2130-2150	AUUAAAUGGCAGAUUUUAAUCCA	391	2128-2150
AD-1290597	AUUAAAAUCUGCCAUUUAAUU	168	2131-2151	AAUUAAAUGGCAGAUUUUAAUCC	392	2129-2151
AD-1290533	AAAUCUGCCAUUUAAUUAGCU	169	2135-2155	AGCUAAUUAAAUGGCAGAUUUUA	393	2133-2155
AD-1290535	AAUCUGCCAUUUAAUUAGCUU	170	2136-2156	AAGCUAAUUAAAUGGCAGAUUUU	394	2134-2156
AD-1290604	AUCUGCCAUUUAAUUAGCUGU	171	2137-2157	ACAGCUAAUUAAAUGGCAGAUUU	395	2135-2157
AD-1290633	CUGCCAUUUAAUUAGCUGCAU	172	2139-2159	AUGCAGCUAAUUAAAUGGCAGAU	396	2137-2159
AD-1290741	ACGCAAUCUGCCUCAAUUUCU	173	2183-2203	AGAAAUUGAGGCAGAUUGCGUUA	397	2181-2203
AD-1290650	CGCAAUCUGCCUCAAUUUCUU	174	2184-2204	AAGAAAUUGAGGCAGAUUGCGUU	398	2182-2204
AD-1290672	GCAAUCUGCCUCAAUUUCUUU	175	2185-2205	AAAGAAAUUGAGGCAGAUUGCGU	399	2183-2205
AD-1290605	AAUCUGCCUCAAUUUCUUCAU	176	2187-2207	AUGAAGAAAUUGAGGCAGAUUGC	400	2185-2207
AD-1290573	AUCUGCCUCAAUUUCUUCAUU	177	2188-2208	AAUGAAGAAAUUGAGGCAGAUUG	401	2186-2208
AD-1290615	UCUGCCUCAAUUUCUUCAUCU	178	2189-2209	AGAUGAAGAAAUUGAGGCAGAUU	402	2187-2209
AD-1290531	CUGCCUCAAUUUCUUCAUCUU	179	2190-2210	AAGAUGAAGAAAUUGAGGCAGAU	403	2188-2210
AD-1290602	UGCCUCAAUUUCUUCAUCUGU	180	2191-2211	ACAGAUGAAGAAAUUGAGGCAGA	404	2189-2211

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290523	GCCUCAAUUUCUUCAUCUGUU	181	2192-2212	AACAGAUGAAGAAAUUGAGGCAG	405	2190-2212
AD-1290514	CAAUUUCUUCAUCUGUCAAAU	182	2196-2216	AUUUGACAGAUGAAGAAAUUGAG	406	2194-2216
AD-1290510	AAUUUCUUCAUCUGUCAAAUU	183	2197-2217	AAUUUGACAGAUGAAGAAAUUGA	407	2195-2217
AD-1290524	AUUUCUUCAUCUGUCAAAUGU	184	2198-2218	ACAUUUGACAGAUGAAGAAAUUG	408	2196-2218
AD-1290836	AAUUCUGCUUGGCUACAGAAU	185	2223-2243	AUUCUGUAGCCAAGCAGAAUUGG	409	2221-2243
AD-1290719	AUUCUGCUUGGCUACAGAAUU	186	2224-2244	AAUUCUGUAGCCAAGCAGAAUUG	410	2222-2244
AD-1290722	UCUGCUUGGCUACAGAAUUAU	187	2226-2246	AUAAUUCUGUAGCCAAGCAGAAU	411	2224-2246
AD-1290687	CUGCUUGGCUACAGAAUUAUU	188	2227-2247	AAUAAUUCUGUAGCCAAGCAGAA	412	2225-2247
AD-1290643	UGCUUGGCUACAGAAUUAUUU	189	2228-2248	AAAUAAUUCUGUAGCCAAGCAGA	413	2226-2248
AD-1290600	GCUUGGCUACAGAAUUAUUGU	190	2229-2249	ACAAUAAUUCUGUAGCCAAGCAG	414	2227-2249
AD-1290507	UUCUUCAUCUGUCAAAUGGAU	191	2200-2220	AUCCAUUUGACAGAUGAAGAAAU	415	2198-2220
AD-1290516	GGAUUAAAAAUCUGCCAUUUAU	192	2129-2149	AUAAAUGGCAGAUUUUAAUCCAG	416	2127-2149
AD-1290527	UGGAUUAAAAAUCUGCCAUUUU	193	2128-2148	AAAAUGGCAGAUUUUAAUCCAGG	417	2126-2148
AD-1290542	UCAAUUUCUUCAUCUGUCAAU	194	2195-2215	AUUGACAGAUGAAGAAAUUGAGG	418	2193-2215
AD-1290552	UUUCUUCAUCUGUCAAAUGGU	195	2199-2219	ACCAUUUGACAGAUGAAGAAAUU	419	2197-2219
AD-1290557	GGCCUUAUAAUGUAAAGAGCU	196	2074-2094	AGCUCUUUACAUUAUAAGGCCUU	420	2072-2094
AD-1290558	UAUAAUGUAAAGGGCUUUAGU	197	2095-2115	ACUAAAGCCCUUUACAUUAUAUG	421	2093-2115
AD-1290561	AUUUUCUCUUUGCAUUCUCGU	198	130-150	ACGAGAAUGCAAAGAGAAAAUGC	422	128-150
AD-1290564	CCUCAAUUUCUUCAUCUGUCU	199	2193-2213	AGACAGAUGAAGAAAUUGAGGCA	423	2191-2213
AD-1290565	CUCAAUUUCUUCAUCUGUCAU	200	2194-2214	AUGACAGAUGAAGAAAUUGAGGC	424	2192-2214
AD-1290574	UAAGGCCUUAUAAUGUAAAGU	201	2071-2091	ACUUUACAUUAUAAGGCCUUACC	425	2069-2091
AD-1290592	CAUAUAAUGUAAAGGGCUUUU	202	2093-2113	AAAAGCCCUUUACAUUAUAUGCU	426	2091-2113
AD-1290609	UGCCAUUUAAUUAGCUGCAUU	203	2140-2160	AAUGCAGCUAAUUAAAUGGCAGA	427	2138-2160
AD-1290624	AUUAUUGUGAGGAUAAAAUCU	204	2242-2262	AGAUUUUAUCCUCACAAUAAUUC	428	2240-2262
AD-1290626	GGUAAGGCCUUAUAAUGUAAU	205	2069-2089	AUUACAUUAUAAGGCCUUACCCA	429	2067-2089
AD-1290635	UUCUGCUUGGCUACAGAAUUU	206	2225-2245	AAAUUCUGUAGCCAAGCAGAAUU	430	2223-2245
AD-1290651	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAAUGUGGAUCCACUUGAACUG	431	781-803

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290654	GGGUAAGGCCUUAUAAUGUAU	208	2068-2088	AUACAUUAUAAGGCCUUACCCAC	432	2066-2088
AD-1290655	GAUCUGGAACACAUAUUGGAU	209	2023-2043	AUCCAAUAUGUGUUCCAGAUCGG	433	2021-2043
AD-1290657	UUUCUCUUUGCAUUCUCGAGU	210	132-152	ACUCGAGAAUGCAAAGAGAAAAU	434	130-152
AD-1290659	CAAUCUGCCUCAAUUUCUUCU	211	2186-2206	AGAAGAAAUUGAGGCAGAUUGCG	435	2184-2206
AD-1290665	AGAGCAAAUAAAUCUUCCUCU	212	1477-1497	AGAGGAAGAUUUAUUUGCUCUGA	436	1475-1497
AD-1290666	ACUUUGAGAAGGUUGAUCUGU	213	758-778	ACAGAUCAACCUUCUCAAAGUCU	437	756-778
AD-1290680	UACAGACUUUGAGAAGGUUGU	214	753-773	ACAACCUUCUCAAAGUCUGUAGC	438	751-773
AD-1290681	UGGGUAAGGCCUUAUAAUGUU	215	2067-2087	AACAUUAUAAGGCCUUACCCACC	439	2065-2087
AD-1290683	UUGCAUUCUCGAGAUCGCUUU	216	139-159	AAAGCGAUCUCGAGAAUGCAAAG	440	137-159
AD-1290684	CGAUCUGGAACACAUAUUGGU	217	2022-2042	ACCAAUAUGUGUUCCAGAUCGGA	441	2020-2042
AD-1290702	UCUGCCAUUUAAUUAGCUGCU	218	2138-2158	AGCAGCUAAUUAAAUGGCAGAUU	442	2136-2158
AD-1290718	CGUGGUGUUUGUCAGCAAAGU	219	948-968	ACUUUGCUGACAAACACCACGUC	443	946-968
AD-1290746	UUGUAUGGUCGUGUGAGGAAU	220	1018-1038	AUUCCUCACACGACCAUACAAGC	444	1016-1038
AD-1290750	CCCAGUGAACCUGCCAAAGAU	221	1706-1726	AUCUUUGGCAGGUUCACUGGGUG	445	1704-1726
AD-1290765	GACGUGGUGUUUGUCAGCAAU	222	946-966	AUUGCUGACAAACACCACGUCUC	446	944-966
AD-1290778	UCAAUGCCUCCGUCAUCUUCU	223	1166-1186	AGAAGAUGACGGAGGCAUUGAAG	447	1164-1186
AD-1290795	UUGCUCCACUCGGAUGCUUUU	224	1102-1122	AAAAGCAUCCGAGUGGAGCAAUU	448	1100-1122
AD-1290796	UUGGAGCCCACCUUGGAAUUU	225	1939-1959	AAAUUCCAAGGUGGGCUCCAAGG	449	1937-1959
AD-1290803	AUCUGACAAGCGAGGAAACUU	226	197-217	AAGUUUCCUCGCUUGUCAGAUGG	450	195-217
AD-1290805	UGGAGCCCACCUUGGAAUUAU	227	1940-1960	AUAAUUCCAAGGUGGGCUCCAAG	451	1938-1960
AD-1290835	GCUCUUCCAGCUGUUUGGCUU	228	921-941	AAGCCAAACAGCUGGAAGAGCUC	452	919-941
AD-1290857	UGCCCACCAGCCUGUGAUUUU	229	1852-1872	AAAAUCACAGGCUGGUGGGCAGG	453	1850-1872
AD-1290863	AAGACCUCUGGGUUGGCUUUU	230	285-305	AAAAGCCAACCCAGAGGUCUUGG	454	283-305
AD-1290875	GCCCACCAGCCUGUGAUUUGU	231	1853-1873	ACAAAUCACAGGCUGGUGGGCAG	455	1851-1873
AD-1290891	ACGGAGACGUGGUGUUUGUCU	232	941-961	AGACAAACACCACGUCUCCGUAG	456	939-961
AD-1290894	AGGUCCGAUCUGGAACACAUU	233	2017-2037	AAUGUGUUCCAGAUCGGACCUCC	457	2015-2037
AD-1290903	GAGUAGCCUCAUGGAAGAGAU	234	513-533	AUCUCUUCCAUGAGGCUACUCCC	458	511-533

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290908	CCAGUGAACCUGCCAAAGAAU	235	1707-1727	AUUCUUUGGCAGGUUCACUGGGU	459	1705-1727
AD-1290910	UAGGGUGGGUAAGGCCUUAUU	236	2062-2082	AAUAAGGCCUUACCCACCCUAUA	460	2060-2082
AD-1290924	UGGGAGUAGCCUCAUGGAAGU	237	510-530	ACUUCCAUGAGGCUACUCCCAGA	461	508-530
AD-1290939	AGGGUGGGUAAGGCCUUAUAU	238	2063-2083	AUAUAAGGCCUUACCCACCCUAU	462	2061-2083
AD-1290946	GGUUGAUCUGACCCAGUUCAU	239	768-788	AUGAACUGGGUCAGAUCAACCUU	463	766-788
AD-1290950	CAUCAGCCUGGUGGACAAGUU	240	570-590	AACUUGUCCACCAGGCUGAUGAC	464	568-590
AD-1290956	AGCUGUGAGUCCAUCUGACAU	241	185-205	AUGUCAGAUGGACUCACAGCUGA	465	183-205
AD-1290971	GCUACGGAGACGUGGUGUUUU	242	938-958	AAAACACCACGUCUCCGUAGCCA	466	936-958
AD-1290973	GUUGUGCAGACUCUAUUCCCU	243	1753-1773	AGGGAAUAGAGUCUGCACAACGC	467	1751-1773

Table 3. Modified Sense and Antisense Strand Sequences of KHK dsRNAs

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290652	gsusggugUfuUfGfUfcagcaaagauL96	1187	asUfscuuUfgcugacaAfaCfaccacsgsu	692	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1290731	ascsguggUfgUfUfUfgucagcaaauL96	469	asUfsuugCfugacaaaCfaCfcacguscsu	693	AGACGUGGUGUUUGUCAGCAAAG	917
AD-1290560	uscsagagCfaAfAfUfaaaucuuccuL96	470	asGfsgaaGfauuuauuUfgCfucugasgsg	694	CCUCAGAGCAAAUAAAUCUUCCU	918
AD-1290854	csasguucAfaGfUfGfgauccacauuL96	471	asAfsuguGfgauccacUfuGfaacugsgsg	695	CCCAGUUCAAGUGGAUCCACAUU	919
AD-1290629	asgsuagcGfcAfUfUfuucucuuuguL96	472	asCfsaaaGfagaaaauGfcGfcuacususg	696	CAAGUAGCGCAUUUUCUCUUUGC	920
AD-1290517	gscsgcauUfuUfCfUfcuuugcauuuL96	473	asAfsaugCfaaagagaAfaAfugcgcsusa	697	UAGCGCAUUUUCUCUUUGCAUUC	921
AD-1290526	csgscauuUfuCfUfCfuuugcauucuL96	474	asGfsaauGfcaaagagAfaAfaugcgscsu	698	AGCGCAUUUUCUCUUUGCAUUCU	922
AD-1290559	gscsauuuUfcUfCfUfuugcauucuuL96	475	asAfsgaaUfgcaaagaGfaAfaaugcsgsc	699	GCGCAUUUUCUCUUUGCAUUCUC	923
AD-1290548	csasuuuuCfuCfUfUfugcauucucuL96	476	asGfsagaAfugcaaagAfgAfaaaugscsg	700	CGCAUUUUCUCUUUGCAUUCUCG	924
AD-1290569	ususuucuCfuUfUfGfcauucucgauL96	477	asUfscgaGfaaugcaaAfgAfgaaaasusg	701	CAUUUUCUCUUUGCAUUCUCGAG	925
AD-1290547	ususcucuUfuGfCfAfuucucgagauL96	478	asUfscucGfagaaugcAfaAfgagaasasa	702	UUUUCUCUUUGCAUUCUCGAGAU	926
AD-1290544	uscsucuuUfgCfAfUfucucgagauuL96	479	asAfsucuCfgagaaugCfaAfagagasasa	703	UUUCUCUUUGCAUUCUCGAGAUC	927
AD-1290667	csuscuuuGfcAfUfUfcucgagaucuL96	480	asGfsaucUfcgagaauGfcAfaagagsasa	704	UUCUCUUUGCAUUCUCGAGAUCG	928
AD-1290685	uscsuuugCfaUfUfCfucgagaucguL96	481	asCfsgauCfucgagaaUfgCfaaagasgsa	705	UCUCUUUGCAUUCUCGAGAUCGC	929
AD-1290695	ususugcaUfuCfUfCfgagaucgcuuL96	482	asAfsgcgAfucucgagAfaUfgcaaasgsa	706	UCUUUGCAUUCUCGAGAUCGCUU	930
AD-1290653	usgscauuCfuCfGfAfgaucgcuuauL96	483	asUfsaagCfgaucucgAfgAfaugcasasa	707	UUUGCAUUCUCGAGAUCGCUUAG	931
AD-1290853	gscsauucUfcGfAfGfaucgcuuaguL96	484	asCfsuaaGfcgaucucGfaGfaaugcsasa	708	UUGCAUUCUCGAGAUCGCUUAGC	932
AD-1290990	csasuucuCfgAfGfAfucgcuuagcuL96	485	asGfscuaAfgcgaucuCfgAfgaaugscsa	709	UGCAUUCUCGAGAUCGCUUAGCC	933
AD-1290540	csusuuaaAfaAfGfGfuuugcaucauL96	486	asUfsgauGfcaaaccuUfuUfuaaagscsg	710	CGCUUUAAAAAGGUUUGCAUCAG	934
AD-1290580	ususuaaaAfaGfGfUfuugcaucaguL96	487	asCfsugaUfgcaaaccUfuUfuuaaasgsc	711	GCUUUAAAAAGGUUUGCAUCAGC	935
AD-1290664	ususaaaaAfgGfUfUfugcaucagcuL96	488	asGfscugAfugcaaacCfuUfuuuaasasg	712	CUUUAAAAAGGUUUGCAUCAGCU	936

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290916	uscsagcuGfuGfAfGfuccaucugauL96	489	asUfscagAfuggacucAfcAfgcugasusg	713	CAUCAGCUGUGAGUCCAUCUGAC	937
AD-1290938	gscsugugAfgUfCfCfaucugacaauL96	490	asUfsuguCfagauggaCfuCfacagcsusg	714	CAGCUGUGAGUCCAUCUGACAAG	938
AD-1290896	csusgugaGfuCfCfAfucugacaaguL96	491	asCfsuugUfcagauggAfcUfcacagscsu	715	AGCUGUGAGUCCAUCUGACAAGC	939
AD-1290914	cscsaucuGfaCfAfAfgcgaggaaauL96	492	asUfsuucCfucgcuugUfcAfgauggsasc	716	GUCCAUCUGACAAGCGAGGAAAC	940
AD-1290982	csasucugAfcAfAfGfcgaggaaacuL96	493	asGfsuuuCfcucgcuuGfuCfagaugsgsa	717	UCCAUCUGACAAGCGAGGAAACU	941
AD-1290708	gsgsaaacUfaAfGfGfcugagaaguuL96	494	asAfscuuCfucagccuUfaGfuuuccsusc	718	GAGGAAACUAAGGCUGAGAAGUG	942
AD-1290693	gsasaacuAfaGfGfCfugagaaguguL96	495	asCfsacuUfcucagccUfuAfguuucscsu	719	AGGAAACUAAGGCUGAGAAGUGG	943
AD-1290942	asgsaccuCfuGfGfGfuuggcuuucuL96	496	asGfsaaaGfccaacccAfgAfggucususg	720	CAAGACCUCUGGGUUGGCUUUCC	944
AD-1290807	asgsuagcCfuCfAfUfggaagagaauL96	497	asUfsucuCfuuccaugAfgGfcuacuscsc	721	GGAGUAGCCUCAUGGAAGAGAAG	945
AD-1290881	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscugCfuucucuuCfcAfugaggscsu	722	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1290745	csuscaugGfaAfGfAfgaagcagauuL96	499	asAfsucuGfcuucucuUfcCfaugagsgsc	723	GCCUCAUGGAAGAGAAGCAGAUC	947
AD-1290814	asusggaaGfaGfAfAfgcagauccuuL96	500	asAfsggaUfcugcuucUfcUfuccausgsa	724	UCAUGGAAGAGAAGCAGAUCCUG	948
AD-1290900	gsgsaagaGfaAfGfCfagauccuguuL96	501	asAfscagGfaucugcuUfcUfcuuccsasu	725	AUGGAAGAGAAGCAGAUCCUGUG	949
AD-1290964	gsasagagAfaGfCfAfgauccuguguL96	502	asCfsacaGfgaucugcUfuCfucuucscsa	726	UGGAAGAGAAGCAGAUCCUGUGC	950
AD-1290802	asuscagcCfuGfGfUfggacaaguauL96	503	asUfsacuUfguccaccAfgGfcugausgsa	727	UCAUCAGCCUGGUGGACAAGUAC	951
AD-1290816	gsusggacAfaGfUfAfcccuaaggauL96	504	asUfsccuUfaggguacUfuGfuccacscsa	728	UGGUGGACAAGUACCCUAAGGAG	952
AD-1290821	gsascaagUfaCfCfCfuaaggaggauL96	505	asUfsccuCfcuuagggUfaCfuugucscsa	729	UGGACAAGUACCCUAAGGAGGAC	953
AD-1290870	gsasggacUfcGfGfAfgauaagguguL96	506	asCfsaccUfuaucuccGfaGfuccucscsu	730	AGGAGGACUCGGAGAUAAGGUGU	954
AD-1290984	usasagguGfuUfUfGfucccagagauL96	507	asUfscucUfgggacaaAfcAfccuuasusc	731	GAUAAGGUGUUUGUCCCAGAGAU	955
AD-1290682	asasggugUfuUfGfUfcccagagauuL96	508	asAfsucuCfugggacaAfaCfaccuusasu	732	AUAAGGUGUUUGUCCCAGAGAUG	956
AD-1290872	csasacucCfuGfCfAfccguucucuuL96	509	asAfsgagAfacggugcAfgGfaguugsgsa	733	UCCAACUCCUGCACCGUUCUCUC	957
AD-1290663	uscsugcuAfcAfGfAfcuuugagaauL96	510	asUfsucuCfaaagucuGfuAfgcagascsa	734	UGUCUGCUACAGACUUUGAGAAG	958
AD-1290627	csusgcuaCfaGfAfCfuuugagaaguL96	511	asCfsuucUfcaaagucUfgUfagcagsasc	735	GUCUGCUACAGACUUUGAGAAGG	959
AD-1290730	usgscuacAfgAfCfUfuugagaagguL96	512	asCfscuuCfucaaaguCfuGfuagcasgsa	736	UCUGCUACAGACUUUGAGAAGGU	960
AD-1290692	gscsuacaGfaCfUfUfugagaagguuL96	513	asAfsccuUfcucaaagUfcUfguagcsasg	737	CUGCUACAGACUUUGAGAAGGUU	961
AD-1290579	csusacagAfcUfUfUfgagaagguuuL96	514	asAfsaccUfucucaaaGfuCfuguagscsa	738	UGCUACAGACUUUGAGAAGGUUG	962
AD-1290591	ascsagacUfuUfGfAfgaagguugauL96	515	asUfscaaCfcuucucaAfaGfucugusasg	739	CUACAGACUUUGAGAAGGUUGAU	963
AD-1290539	csasgacuUfuGfAfGfaagguugauuL96	516	asAfsucaAfccuucucAfaAfgucugsusa	740	UACAGACUUUGAGAAGGUUGAUC	964
AD-1290611	asgsacuuUfgAfGfAfagguugaucuL96	517	asGfsaucAfaccuucuCfaAfagucusgsu	741	ACAGACUUUGAGAAGGUUGAUCU	965
AD-1290530	gsascuuuGfaGfAfAfgguugaucuuL96	518	asAfsgauCfaaccuucUfcAfaagucsusg	742	CAGACUUUGAGAAGGUUGAUCUG	966
AD-1290576	csusuugaGfaAfGfGfuugaucugauL96	519	asUfscagAfucaaccuUfcUfcaaagsusc	743	GACUUUGAGAAGGUUGAUCUGAC	967
AD-1290546	ususugagAfaGfGfUfugaucugacuL96	520	asGfsucaGfaucaaccUfuCfucaaasgsu	744	ACUUUGAGAAGGUUGAUCUGACC	968
AD-1290823	asasgguuGfaUfCfUfgacccaguuuL96	521	asAfsacuGfggucagaUfcAfaccuuscsu	745	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1290757	gsusugauCfuGfAfCfccaguucaauL96	522	asUfsugaAfcugggucAfgAfucaacscsu	746	AGGUUGAUCUGACCCAGUUCAAG	970
AD-1290959	ususgaucUfgAfCfCfcaguucaaguL96	523	asCfsuugAfacuggguCfaGfaucaascsc	747	GGUUGAUCUGACCCAGUUCAAGU	971
AD-1290837	usgsaucuGfaCfCfCfaguucaaguuL96	524	asAfscuuGfaacugggUfcAfgaucasasc	748	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1290861	gsasucugAfcCfCfAfguucaaguguL96	525	asCfsacuUfgaacuggGfuCfagaucsasa	749	UUGAUCUGACCCAGUUCAAGUGG	973
AD-1290885	csusgaccCfaGfUfUfcaaguggauuL96	526	asAfsuccAfcuugaacUfgGfgucagsasu	750	AUCUGACCCAGUUCAAGUGGAUC	974
AD-1290970	ascsccagUfuCfAfAfguggauccauL96	527	asUfsggaUfccacuugAfaCfuggguscsa	751	UGACCCAGUUCAAGUGGAUCCAC	975

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290962	cscsaguuCfaAfGfUfggauccacauL96	528	asUfsgugGfauccacuUfgAfacuggsgsu	752	ACCCAGUUCAAGUGGAUCCACAU	976
AD-1290759	asgsuucaAfgUfGfGfauccacauuuL96	529	asAfsaugUfggauccaCfuUfgaacusgsg	753	CCAGUUCAAGUGGAUCCACAUUG	977
AD-1290736	ususcaagUfgGfAfUfccacauugauL96	530	asUfscaaUfguggaucCfaCfuugaascsu	754	AGUUCAAGUGGAUCCACAUUGAG	978
AD-1290739	uscsaaguGfgAfUfCfcacauugaguL96	531	asCfsucaAfuguggauCfcAfcuugasasc	755	GUUCAAGUGGAUCCACAUUGAGG	979
AD-1290828	csasagugGfaUfCfCfacauugagguL96	532	asCfscucAfauguggaUfcCfacuugsasa	756	UUCAAGUGGAUCCACAUUGAGGG	980
AD-1291001	gscsaucgGfaGfCfAfggugaagauuL96	533	asAfsucuUfcaccugcUfcCfgaugcsgsu	757	ACGCAUCGGAGCAGGUGAAGAUG	981
AD-1290933	csasucggAfgCfAfGfgugaagauguL96	534	asCfsaucUfucaccugCfuCfcgaugscsg	758	CGCAUCGGAGCAGGUGAAGAUGC	982
AD-1290988	gsasgcucUfuCfCfAfgcuguuuggUL96	535	asCfscaaAfcagcuggAfaGfagcucscsu	759	AGGAGCUCUUCCAGCUGUUUGGC	983
AD-1290955	csuscuucCfaGfCfUfguuuggcuaUL96	536	asUfsagcCfaaacagcUfgGfaagagscsu	760	AGCUCUUCCAGCUGUUUGGCUAC	984
AD-1290810	csusacggAfgAfCfGfugguguuuguL96	537	asCfsaaaCfaccacguCfuCfcguagscsc	761	GGCUACGGAGACGUGGUGUUUGU	985
AD-1290740	usascggaGfaCfGfUfgguguuuguuL96	538	asAfscaaAfcaccacgUfcUfccguasgsc	762	GCUACGGAGACGUGGUGUUUGUC	986
AD-1290752	csgsgagaCfgUfGfGfuguuugucauL96	539	asUfsgacAfaacaccaCfgUfcuccgsusa	763	UACGGAGACGUGGUGUUUGUCAG	987
AD-1290878	gsgsagacGfuGfGfUfguuugucaguL96	540	asCfsugaCfaaacaccAfcGfucuccsgsu	764	ACGGAGACGUGGUGUUUGUCAGC	988
AD-1290599	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucuUfugcugacAfaAfcaccascsg	765	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1290632	gsgsuguuUfgUfCfAfgcaaagauguL96	542	asCfsaucUfuugcugaCfaAfacaccsasc	766	GUGGUGUUUGUCAGCAAAGAUGU	990
AD-1290584	gsusguuuGfuCfAfGfcaaagauguuL96	543	asAfscauCfuuugcugAfcAfaacacscsa	767	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1290734	usgsuuugUfcAfGfCfaaagauguguL96	544	asCfsacaUfcuuugcuGfaCfaaacascsc	768	GGUGUUUGUCAGCAAAGAUGUGG	992
AD-1290882	gsusuuguCfaGfCfAfaagaugugguL96	545	asCfscacAfucuuugcUfgAfcaaacsasc	769	GUGUUUGUCAGCAAAGAUGUGGC	993
AD-1290987	ususugucAfgCfAfAfagauguggcuL96	546	asGfsccaCfaucuuugCfuGfacaaascsa	770	UGUUUGUCAGCAAAGAUGUGGCC	994
AD-1290963	asasagauGfuGfGfCfcaagcacuuuL96	547	asAfsaguGfcuuggccAfcAfucuuusgsc	771	GCAAAGAUGUGGCCAAGCACUUG	995
AD-1290710	usgsuaugGfuCfGfUfgugaggaaauL96	548	asUfsuucCfucacacgAfcCfauacasasg	772	CUUGUAUGGUCGUGUGAGGAAAG	996
AD-1290656	gsusauggUfcGfUfGfugaggaaaguL96	549	asCfsuuuCfcucacacGfaCfcauacsasa	773	UUGUAUGGUCGUGUGAGGAAAGG	997
AD-1290890	usasugguCfgUfGfUfgaggaaaggUL96	550	asCfscuuUfccucacaCfgAfccauascsa	774	UGUAUGGUCGUGUGAGGAAAGGG	998
AD-1290887	usgscuccAfcUfCfGfgaugcuuucuL96	551	asGfsaaaGfcauccgaGfuGfgagcasasu	775	AUUGCUCCACUCGGAUGCUUUCC	999
AD-1290923	gsasgcugGfaGfAfCfaccuucaauuL96	552	asAfsuugAfaggugucUfcCfagcucscsc	776	GGGAGCUGGAGACACCUUCAAUG	1000
AD-1290904	asgscuggAfgAfCfAfccuucaauguL96	553	asCfsauuGfaagguguCfuCfcagcuscsc	777	GGAGCUGGAGACACCUUCAAUGC	1001
AD-1290980	csusggagAfcAfCfCfuucaaugccuL96	554	asGfsgcaUfugaagguGfuCfuccagscsu	778	AGCUGGAGACACCUUCAAUGCCU	1002
AD-1290860	usgsgagaCfaCfCfUfucaaugccuuL96	555	asAfsggcAfuugaaggUfgUfcuccasgsc	779	GCUGGAGACACCUUCAAUGCCUC	1003
AD-1290969	ascscuucAfaUfGfCfcuccgucauuL96	556	asAfsugaCfggaggcaUfuGfaaggusgsu	780	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1290811	cscsuucaAfuGfCfCfuccgucaucuL96	557	asGfsaugAfcggaggcAfuUfgaaggsusg	781	CACCUUCAAUGCCUCCGUCAUCU	1005
AD-1290886	ususcaauGfcCfUfCfcgucaucuuuL96	558	asAfsagaUfgacggagGfcAfuugaasgsg	782	CCUUCAAUGCCUCCGUCAUCUUC	1006
AD-1290668	csasaugcCfuCfCfGfucaucuucauL96	559	asUfsgaaGfaugacggAfgGfcauugsasa	783	UUCAAUGCCUCCGUCAUCUUCAG	1007
AD-1290852	asasugccUfcCfGfUfcaucuucaguL96	560	asCfsugaAfgaugacgGfaGfgcauusgsa	784	UCAAUGCCUCCGUCAUCUUCAGC	1008
AD-1290915	csusccguCfaUfCfUfucagccucuuL96	561	asAfsgagGfcugaagaUfgAfcggagsgsc	785	GCCUCCGUCAUCUUCAGCCUCUC	1009
AD-1290874	gsusgcagGfaAfGfCfacugagauuuL96	562	asAfsaucUfcagugcuUfcCfugcacsgsc	786	GCGUGCAGGAAGCACUGAGAUUC	1010
AD-1290818	usgscaggAfaGfCfAfcugagauucuL96	563	asGfsaauCfucagugcUfuCfcugcascsg	787	CGUGCAGGAAGCACUGAGAUUCG	1011
AD-1290884	gscsaggaAfgCfAfCfugagauucguL96	564	asCfsgaaUfcucagugCfuUfccugcsasc	788	GUGCAGGAAGCACUGAGAUUCGG	1012
AD-1290977	usgsgccuGfcAfGfGfgcuuugauguL96	565	asCfsaucAfaagcccuGfcAfggccascsa	789	UGUGGCCUGCAGGGCUUUGAUGG	1013
AD-1290961	csusgcagGfgCfUfUfugauggcauuL96	566	asAfsugcCfaucaaagCfcCfugcagsgsc	790	GCCUGCAGGGCUUUGAUGGCAUC	1014

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290834	csasgggcUfuUfGfAfuggcaucguuL96	567	asAfscgaUfgccaucaAfaGfcccugscsa	791	UGCAGGGCUUUGAUGGCAUCGUG	1015
AD-1290867	gsgsgcuuUfgAfUfGfgcaucguguuL96	568	asAfscacGfaugccauCfaAfagcccsusg	792	CAGGGCUUUGAUGGCAUCGUGUG	1016
AD-1290879	csuscugcCfuGfUfGfuccuguguuuL96	569	asAfsacaCfaggacacAfgGfcagagsusc	793	GACUCUGCCUGUGUCCUGUGUUC	1017
AD-1290549	csuscagaGfcAfAfAfuaaaucuucuL96	570	asGfsaagAfuuuauuuGfcUfcugagsgsc	794	GCCUCAGAGCAAAUAAAUCUUCC	1018
AD-1290525	csasgagcAfaAfUfAfaaucuuccuuL96	571	asAfsggaAfgauuuauUfuGfcucugsasg	795	CUCAGAGCAAAUAAAUCUUCCUC	1019
AD-1290622	csusccucUfcAfAfUfgucugaacuuL96	572	asAfsguuCfagacauuGfaGfaggagsasa	796	UUCUCCUCUCAAUGUCUGAACUG	1020
AD-1290638	uscscucuCfaAfUfGfucugaacuguL96	573	asCfsaguUfcagacauUfgAfgaggasgsa	797	UCUCCUCUCAAUGUCUGAACUGC	1021
AD-1290921	cscsucucAfaUfGfUfcugaacugcuL96	574	asGfscagUfucagacaUfuGfagaggsasg	798	CUCCUCUCAAUGUCUGAACUGCU	1022
AD-1290621	csuscucaAfuGfUfCfugaacugcuuL96	575	asAfsgcaGfuucagacAfuUfgagagsgsa	799	UCCUCUCAAUGUCUGAACUGCUC	1023
AD-1290775	csuscaauGfuCfUfGfaacugcucuuL96	576	asAfsgagCfaguucagAfcAfuugagsasg	800	CUCUCAAUGUCUGAACUGCUCUG	1024
AD-1290748	asusuccuGfaGfGfCfucugacucuuL96	577	asAfsgagUfcagagccUfcAfggaausgsc	801	GCAUUCCUGAGGCUCUGACUCUU	1025
AD-1290865	csusgcguUfgUfGfCfagacucuauuL96	578	asAfsuagAfgucugcaCfaAfcgcagsgsg	802	CCCUGCGUUGUGCAGACUCUAUU	1026
AD-1290897	usgscguuGfuGfCfAfgacucuauuuL96	579	asAfsauaGfagucugcAfcAfacgcasgsg	803	CCUGCGUUGUGCAGACUCUAUUC	1027
AD-1290989	gscsguugUfgCfAfGfacucuauucuL96	580	asGfsaauAfgagucugCfaCfaacgcsasg	804	CUGCGUUGUGCAGACUCUAUUCC	1028
AD-1290983	csgsuuguGfcAfGfAfcucuauuccuL96	581	asGfsgaaUfagagucuGfcAfcaacgscsa	805	UGCGUUGUGCAGACUCUAUUCCC	1029
AD-1290909	ususgugcAfgAfCfUfcuauucccauL96	582	asUfsgggAfauagaguCfuGfcacaascsg	806	CGUUGUGCAGACUCUAUUCCCAC	1030
AD-1290993	usasuuccCfaCfAfGfcucagaagcuL96	583	asGfscuuCfugagcugUfgGfgaauasgsa	807	UCUAUUCCCACAGCUCAGAAGCU	1031
AD-1290841	asusucccAfcAfGfCfucagaagcuuL96	584	asAfsgcuUfcugagcuGfuGfggaausasg	808	CUAUUCCCACAGCUCAGAAGCUG	1032
AD-1290880	csusuggaGfcCfCfAfccuuggaauuL96	585	asAfsuucCfaagguggGfcUfccaagsgsg	809	CCCUUGGAGCCCACCUUGGAAUU	1033
AD-1290747	gsgsagccCfaCfCfUfuggaauuaauL96	586	asUfsuaaUfuccaaggUfgGfgcuccsasa	810	UUGGAGCCCACCUUGGAAUUAAG	1034
AD-1290842	gsasgcccAfcCfUfUfggaauuaaguL96	587	asCfsuuaAfuuccaagGfuGfggcucscsa	811	UGGAGCCCACCUUGGAAUUAAGG	1035
AD-1290911	asgscccaCfcUfUfGfgaauuaagguL96	588	asCfscuuAfauuccaaGfgUfgggcuscsc	812	GGAGCCCACCUUGGAAUUAAGGG	1036
AD-1290926	gscsccacCfuUfGfGfaauuaaggguL96	589	asCfsccuUfaauuccaAfgGfugggcsusc	813	GAGCCCACCUUGGAAUUAAGGGC	1037
AD-1291003	gsgscgugCfcUfCfAfgccacaaauuL96	590	asAfsuuuGfuggcugaGfgCfacgccscsu	814	AGGGCGUGCCUCAGCCACAAAUG	1038
AD-1290931	uscsagccAfcAfAfAfugugacccauL96	591	asUfsgggUfcacauuuGfuGfgcugasgsg	815	CCUCAGCCACAAAUGUGACCCAG	1039
AD-1290764	gsgsuccgAfuCfUfGfgaacacauauL96	592	asUfsaugUfguuccagAfuCfggaccsusc	816	GAGGUCCGAUCUGGAACACAUAU	1040
AD-1290763	gsusccgaUfcUfGfGfaacacauauuL96	593	asAfsuauGfuguuccaGfaUfcggacscsu	817	AGGUCCGAUCUGGAACACAUAUU	1041
AD-1290670	uscscgauCfuGfGfAfacacauauuuL96	594	asAfsauaUfguguuccAfgAfucggascsc	818	GGUCCGAUCUGGAACACAUAUUG	1042
AD-1290712	cscsgaucUfgGfAfAfcacauauuguL96	595	asCfsaauAfuguguucCfaGfaucggsasc	819	GUCCGAUCUGGAACACAUAUUGG	1043
AD-1290612	asuscuggAfaCfAfCfauauuggaauL96	596	asUfsuccAfauaugugUfuCfcagauscsg	820	CGAUCUGGAACACAUAUUGGAAU	1044
AD-1290522	uscsuggaAfcAfCfAfuauuggaauuL96	597	asAfsuucCfaauauguGfuUfccagasusc	821	GAUCUGGAACACAUAUUGGAAUU	1045
AD-1290528	csusggaaCfaCfAfUfauuggaauuuL96	598	asAfsauuCfcaauaugUfgUfuccagsasu	822	AUCUGGAACACAUAUUGGAAUUG	1046
AD-1290543	usgsgaacAfcAfUfAfuuggaauuguL96	599	asCfsaauUfccaauauGfuGfuuccasgsa	823	UCUGGAACACAUAUUGGAAUUGG	1047
AD-1290589	gsgsaacaCfaUfAfUfuggaauugguL96	600	asCfscaaUfuccaauaUfgUfguuccsasg	824	CUGGAACACAUAUUGGAAUUGGG	1048
AD-1290800	gsgsguggGfuAfAfGfgccuuauaaauL96	601	asUfsuauAfaggccuuAfcCfcacccsusa	825	UAGGGUGGGUAAGGCCUUAUAAU	1049
AD-1290755	gsgsugggUfaAfGfGfccuuauaauuL96	602	asAfsuuaUfaaggccuUfaCfccaccscsu	826	AGGGUGGGUAAGGCCUUAUAAUG	1050
AD-1290742	gsusggguAfaGfGfCfcuuauaauguL96	603	asCfsauuAfuaaggccUfuAfcccacscsc	827	GGGUGGGUAAGGCCUUAUAAUGU	1051
AD-1290563	gsusaaggCfcUfUfAfuaauguaaauL96	604	asUfsuuaCfauuauaaGfgCfcuuacscsc	828	GGGUAAGGCCUUAUAAUGUAAAG	1052
AD-1290570	asasggccUfuAfUfAfauguaaagauL96	605	asUfscuuUfacauuauAfaGfgccuusasc	829	GUAAGGCCUUAUAAUGUAAAGAG	1053

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290515	asgsgccuUfaUfAfAfuguaaagaguL96	606	asCfsucuUfuacauuaUfaAfggccususa	830	UAAGGCCUUAUAAUGUAAAGAGC	1054
AD-1290556	gscscuuaUfaAfUfGfuaaagagcauL96	607	asUfsgcuCfuuuacauUfaUfaaggcscsu	831	AGGCCUUAUAAUGUAAAGAGCAU	1055
AD-1290661	gscsauauAfaUfGfUfaaagggcuuuL96	608	asAfsagcCfcuuuacaUfuAfuaugcsusc	832	GAGCAUAUAAUGUAAAGGGCUUU	1056
AD-1290555	asusauaaUfgUfAfAfagggcuuuauL96	609	asUfsaaaGfcccuuuaCfaUfuauausgsc	833	GCAUAUAAUGUAAAGGGCUUUAG	1057
AD-1290554	asusaaugUfaAfAfGfggcuuuagauL96	610	asUfscuaAfagcccuuUfaCfauuausasu	834	AUAUAAUGUAAAGGGCUUUAGAG	1058
AD-1290639	usasauguAfaAfGfGfgcuuuagaguL96	611	asCfsucuAfaagcccuUfuAfcauuasusa	835	UAUAAUGUAAAGGGCUUUAGAGU	1059
AD-1290618	asasuguaAfaGfGfGfcuuuagaguuL96	612	asAfscucUfaaagcccUfuUfacauusasu	836	AUAAUGUAAAGGGCUUUAGAGUG	1060
AD-1290660	cscsuggaUfuAfAfAfaucugccauuL96	613	asAfsuggCfagauuuuAfaUfccaggsusc	837	GACCUGGAUUAAAAUCUGCCAUU	1061
AD-1290551	csusggauUfaAfAfAfucugccauuuL96	614	asAfsaugGfcagauuuUfaAfuccagsgsu	838	ACCUGGAUUAAAAUCUGCCAUUU	1062
AD-1290509	gsasuuaaAfaUfCfUfgccauuuaauL96	615	asUfsuaaAfuggcagaUfuUfuaaucscsa	839	UGGAUUAAAAUCUGCCAUUUAAU	1063
AD-1290597	asusuaaaAfuCfUfGfccauuuaauuL96	616	asAfsuuaAfauggcagAfuUfuuaauscsc	840	GGAUUAAAAUCUGCCAUUUAAUU	1064
AD-1290533	asasaucuGfcCfAfUfuuaauuagcuL96	617	asGfscuaAfuuaaaugGfcAfgauuususa	841	UAAAAUCUGCCAUUUAAUUAGCU	1065
AD-1290535	asasucugCfcAfUfUfuaauuagcuuL96	618	asAfsgcuAfauuaaauGfgCfagauususu	842	AAAAUCUGCCAUUUAAUUAGCUG	1066
AD-1290604	asuscugcCfaUfUfUfaauuagcuguL96	619	asCfsagcUfaauuaaaUfgGfcagaususu	843	AAAUCUGCCAUUUAAUUAGCUGC	1067
AD-1290633	csusgccaUfuUfAfAfuuagcugcauL96	620	asUfsgcaGfcuaauuaAfaUfggcagsasu	844	AUCUGCCAUUUAAUUAGCUGCAU	1068
AD-1290741	ascsgcaaUfcUfGfCfcucaauuucuL96	621	asGfsaaaUfugaggcaGfaUfugcgususa	845	UAACGCAAUCUGCCUCAAUUUCU	1069
AD-1290650	csgscaauCfuGfCfCfucaauuucuuL96	622	asAfsgaaAfuugaggcAfgAfuugcgsusu	846	AACGCAAUCUGCCUCAAUUUCUU	1070
AD-1290672	gscsaaucUfgCfCfUfcaauuucuuuL96	623	asAfsagaAfauugaggCfaGfauugcsgsu	847	ACGCAAUCUGCCUCAAUUUCUUC	1071
AD-1290605	asasucugCfcUfCfAfauuucuucauL96	624	asUfsgaaGfaaauugaGfgCfagauusgsc	848	GCAAUCUGCCUCAAUUUCUUCAU	1072
AD-1290573	asuscugcCfuCfAfAfuuucuucauuL96	625	asAfsugaAfgaaauugAfgGfcagaususg	849	CAAUCUGCCUCAAUUUCUUCAUC	1073
AD-1290615	uscsugccUfcAfAfUfuucuucaucuL96	626	asGfsaugAfagaaauuGfaGfgcagasusu	850	AAUCUGCCUCAAUUUCUUCAUCU	1074
AD-1290531	csusgccuCfaAfUfUfucuucaucuuL96	627	asAfsgauGfaagaaauUfgAfggcagsasu	851	AUCUGCCUCAAUUUCUUCAUCUG	1075
AD-1290602	usgsccucAfaUfUfUfcuucaucuguL96	628	asCfsagaUfgaagaaaUfuGfaggcasgsa	852	UCUGCCUCAAUUUCUUCAUCUGU	1076
AD-1290523	gscscucaAfuUfUfCfuucaucuguuL96	629	asAfscagAfugaagaaAfuUfgaggcsasg	853	CUGCCUCAAUUUCUUCAUCUGUC	1077
AD-1290514	csasauuuCfuUfCfAfucugucaaauL96	630	asUfsuugAfcagaugaAfgAfaauugsasg	854	CUCAAUUUCUUCAUCUGUCAAAU	1078
AD-1290510	asasuuucUfuCfAfUfcugucaaauuL96	631	asAfsuuuGfacagaugAfaGfaaauusgsa	855	UCAAUUUCUUCAUCUGUCAAAUG	1079
AD-1290524	asusuucuUfcAfUfCfugucaaauguL96	632	asCfsauuUfgacagauGfaAfgaaaususg	856	CAAUUUCUUCAUCUGUCAAAUGG	1080
AD-1290836	asasuucuGfcUfUfGfgcuacagaauL96	633	asUfsucuGfuagccaaGfcAfgaauusgsg	857	CCAAUUCUGCUUGGCUACAGAAU	1081
AD-1290719	asusucugCfuUfGfGfcuacagaauuL96	634	asAfsuucUfguagccaAfgCfagaaususg	858	CAAUUCUGCUUGGCUACAGAAUU	1082
AD-1290722	uscsugcuUfgGfCfUfacagaauuauL96	635	asUfsaauUfcuguagcCfaAfgcagasasu	859	AUUCUGCUUGGCUACAGAAUUAU	1083
AD-1290687	csusgcuuGfgCfUfAfcagaauuauuL96	636	asAfsuaaUfucuguagCfcAfagcagsasa	860	UUCUGCUUGGCUACAGAAUUAUU	1084
AD-1290643	usgscuugGfcUfAfCfagaauuauuuL96	637	asAfsauaAfuucuguaGfcCfaagcasgsa	861	UCUGCUUGGCUACAGAAUUAUUG	1085
AD-1290600	gscsuuggCfuAfCfAfgaauuauuguL96	638	asCfsaauAfauucuguAfgCfcaagcsasg	862	CUGCUUGGCUACAGAAUUAUUGU	1086
AD-1290507	ususcuucAfuCfUfGfucaaauggauL96	639	asUfsccaUfuugacagAfuGfaagaasasu	863	AUUUCUUCAUCUGUCAAAUGGAA	1087
AD-1290516	gsgsauuaAfaAfUfCfugccauuuauL96	640	asUfsaaaUfggcagauUfuUfaauccsasg	864	CUGGAUUAAAAUCUGCCAUUUAA	1088
AD-1290527	usgsgauuAfaAfAfUfcugccauuuuL96	641	asAfsaauGfgcagauuUfuAfauccasgsg	865	CCUGGAUUAAAAUCUGCCAUUUA	1089
AD-1290542	uscsaauuUfcUfUfCfaucugucaauL96	642	asUfsugaCfagaugaaGfaAfauugasgsg	866	CCUCAAUUUCUUCAUCUGUCAAA	1090
AD-1290552	ususucuuCfaUfCfUfgucaaaugguL96	643	asCfscauUfugacagaUfgAfagaaasusu	867	AAUUUCUUCAUCUGUCAAAUGGA	1091
AD-1290557	gsgsccuuAfuAfAfUfguaaagagcuL96	644	asGfscucUfuuacauuAfuAfaggccsusu	868	AAGGCCUUAUAAUGUAAAGAGCA	1092

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290558	usasuaauGfuAfAfAfgggcuuuaguL96	645	asCfsuaaAfgcccuuuAfcAfuuauasusg	869	CAUAUAAUGUAAAGGGCUUUAGA	1093
AD-1290561	asusuuucUfcUfUfUfgcauucucguL96	646	asCfsgagAfaugcaaaGfaGfaaaausgsc	870	GCAUUUUCUCUUUGCAUUCUCGA	1094
AD-1290564	cscsucaaUfuUfCfUfucaucugucuL96	647	asGfsacaGfaugaagaAfaUfugaggscsa	871	UGCCUCAAUUUCUUCAUCUGUCA	1095
AD-1290565	csuscaauUfuCfUfUfcaucugucauL96	648	asUfsgacAfgaugaagAfaAfuugagsgsc	872	GCCUCAAUUUCUUCAUCUGUCAA	1096
AD-1290574	usasaggcCfuUfAfUfaauguaaaguL96	649	asCfsuuuAfcauuauaAfgGfccuuascsc	873	GGUAAGGCCUUAUAAUGUAAAGA	1097
AD-1290592	csasuauaAfuGfUfAfaagggcuuuuL96	650	asAfsaagCfccuuuacAfuUfauaugscsu	874	AGCAUAUAAUGUAAAGGGCUUUA	1098
AD-1290609	usgsccauUfuAfAfUfuagcugcauuL96	651	asAfsugcAfgcuaauuAfaAfuggcasgsa	875	UCUGCCAUUUAAUUAGCUGCAUA	1099
AD-1290624	asusuauuGfuGfAfGfgauaaaaucuL96	652	asGfsauuUfuauccucAfcAfauaaususc	876	GAAUUAUUGUGAGGAUAAAAUCA	1100
AD-1290626	gsgsuaagGfcCfUfUfauaauguaauL96	653	asUfsuacAfuuauaagGfcCfuuaccscsa	877	UGGGUAAGGCCUUAUAAUGUAAA	1101
AD-1290635	ususcugcUfuGfGfCfuacagaauuuL96	654	asAfsauuCfuguagccAfaGfcagaasusu	878	AAUUCUGCUUGGCUACAGAAUUA	1102
AD-1290651	gsusucaaGfuGfGfAfuccacauuguL96	655	asCfsaauGfuggauccAfcUfugaacsusg	879	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1290654	gsgsguaaGfgCfCfUfuauaauguauL96	656	asUfsacaUfuauaaggCfcUfuacccsasc	880	GUGGGUAAGGCCUUAUAAUGUAA	1104
AD-1290655	gsasucugGfaAfCfAfcauauuggauL96	657	asUfsccaAfuauguguUfcCfagaucsgsg	881	CCGAUCUGGAACACAUAUUGGAA	1105
AD-1290657	ususucucUfuUfGfCfauucucgaguL96	658	asCfsucgAfgaaugcaAfaGfagaaasasu	882	AUUUUCUCUUUGCAUUCUCGAGA	1106
AD-1290659	csasaucuGfcCfUfCfaauuucuucuL96	659	asGfsaagAfaauugagGfcAfgauugscsg	883	CGCAAUCUGCCUCAAUUUCUUCA	1107
AD-1290665	asgsagcaAfaUfAfAfaucuuccucuL96	660	asGfsaggAfagauuuaUfuUfgcucusgsa	884	UCAGAGCAAAUAAAUCUUCCUCA	1108
AD-1290666	ascsuuugAfgAfAfGfguugaucuguL96	661	asCfsagaUfcaaccuuCfuCfaaaguscsu	885	AGACUUUGAGAAGGUUGAUCUGA	1109
AD-1290680	usascagaCfuUfUfGfagaagguuguL96	662	asCfsaacCfuucucaaAfgUfcuguasgsc	886	GCUACAGACUUUGAGAAGGUUGA	1110
AD-1290681	usgsgguaAfgGfCfCfuuauaauguuL96	663	asAfscauUfauaaggcCfuUfacccascsc	887	GGUGGGUAAGGCCUUAUAAUGUA	1111
AD-1290683	ususgcauUfcUfCfGfagaucgcuuuL96	664	asAfsagcGfaucucgaGfaAfugcaasasg	888	CUUUGCAUUCUCGAGAUCGCUUA	1112
AD-1290684	csgsaucuGfgAfAfCfacauauugguL96	665	asCfscaaUfauguguuCfcAfgaucgsgsa	889	UCCGAUCUGGAACACAUAUUGGA	1113
AD-1290702	uscsugccAfuUfUfAfauuagcugcuL96	666	asGfscagCfuaauuaaAfuGfgcagasusu	890	AAUCUGCCAUUUAAUUAGCUGCA	1114
AD-1290718	csgsugguGfuUfUfGfucagcaaaguL96	667	asCfsuuuGfcugacaaAfcAfccacgsusc	891	GACGUGGUGUUUGUCAGCAAAGA	1115
AD-1290746	ususguauGfgUfCfGfugugaggaauL96	668	asUfsuccUfcacacgaCfcAfuacaasgsc	892	GCUUGUAUGGUCGUGUGAGGAAA	1116
AD-1290750	cscscaguGfaAfCfCfugccaaagauL96	669	asUfscuuUfggcagguUfcAfcugggsusg	893	CACCCAGUGAACCUGCCAAAGAA	1117
AD-1290765	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsugcUfgacaaacAfcCfacgucsusc	894	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1290778	uscsaaugCfcUfCfCfgucaucuucuL96	671	asGfsaagAfugacggaGfgCfauugasasg	895	CUUCAAUGCCUCCGUCAUCUUCA	1119
AD-1290795	ususgcucCfaCfUfCfggaugcuuuuL96	672	asAfsaagCfauccgagUfgGfagcaasusu	896	AAUUGCUCCACUCGGAUGCUUUC	1120
AD-1290796	ususggagCfcCfAfCfcuuggaauuuL96	673	asAfsauuCfcaaggugGfgCfuccaasgsg	897	CCUUGGAGCCCACCUUGGAAUUA	1121
AD-1290803	asuscugaCfaAfGfCfgaggaaacuuL96	674	asAfsguuUfccucgcuUfgUfcagausgsg	898	CCAUCUGACAAGCGAGGAAACUA	1122
AD-1290805	usgsgagcCfcAfCfCfuuggaauuauL96	675	asUfsaauUfccaagguGfgGfcuccasasg	899	CUUGGAGCCCACCUUGGAAUUAA	1123
AD-1290835	gscsucuuCfcAfGfCfuguuuggcuuL96	676	asAfsgccAfaacagcuGfgAfagagcsusc	900	GAGCUCUUCCAGCUGUUUGGCUA	1124
AD-1290857	usgscccaCfcAfGfCfcugugauuuuL96	677	asAfsaauCfacaggcuGfgUfgggcasgsg	901	CCUGCCCACCAGCCUGUGAUUUG	1125
AD-1290863	asasgaccUfcUfGfGfguuggcuuuuL96	678	asAfsaagCfcaacccaGfaGfgucuusgsg	902	CCAAGACCUCUGGGUUGGCUUUC	1126
AD-1290875	gscsccacCfaGfCfCfugugauuuguL96	679	asCfsaaaUfcacaggcUfgGfugggcsasg	903	CUGCCCACCAGCCUGUGAUUUGA	1127
AD-1290891	ascsggagAfcGfUfGfguguuugucuL96	680	asGfsacaAfacaccacGfuCfuccgusasg	904	CUACGGAGACGUGGUGUUUGUCA	1128
AD-1290894	asgsguccGfaUfCfUfggaacacauuL96	681	asAfsuguGfuuccagaUfcGfgaccuscsc	905	GGAGGUCCGAUCUGGAACACAUA	1129
AD-1290903	gsasguagCfcUfCfAfuggaagagauL96	682	asUfscucUfuccaugaGfgCfuacucscsc	906	GGGAGUAGCCUCAUGGAAGAGAA	1130
AD-1290908	cscsagugAfaCfCfUfgccaaagaauL96	683	asUfsucuUfuggcaggUfuCfacuggsgsu	907	ACCCAGUGAACCUGCCAAAGAAA	1131

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1290910	usasggguGfgGfUfAfaggccuuauuL96	684	asAfsuaaGfgccuuacCfcAfcccuasusa	908	UAUAGGGUGGGUAAGGCCUUAUA	1132
AD-1290924	usgsggagUfaGfCfCfucauggaaguL96	685	asCfsuucCfaugaggcUfaCfucccasgsa	909	UCUGGGAGUAGCCUCAUGGAAGA	1133
AD-1290939	asgsggugGfgUfAfAfggccuuauauL96	686	asUfsauaAfggccuuaCfcCfacccusasu	910	AUAGGGUGGGUAAGGCCUUAUAA	1134
AD-1290946	gsgsuugaUfcUfGfAfcccaguucauL96	687	asUfsgaaCfugggucaGfaUfcaaccsusu	911	AAGGUUGAUCUGACCCAGUUCAA	1135
AD-1290950	csasucagCfcUfGfGfuggacaaguuL96	688	asAfscuuGfuccaccaGfgCfugaugsasc	912	GUCAUCAGCCUGGUGGACAAGUA	1136
AD-1290956	asgscuguGfaGfUfCfcaucugacauL96	689	asUfsgucAfgauggacUfcAfcagcusgsa	913	UCAGCUGUGAGUCCAUCUGACAA	1137
AD-1290971	gscsuacgGfaGfAfCfgugguguuuuL96	690	asAfsaacAfccacgucUfcCfguagcscsa	914	UGGCUACGGAGACGUGGUGUUUG	1138
AD-1290973	gsusugugCfaGfAfCfucuauucccuL96	691	asGfsggaAfuagagucUfgCfacaacsgsc	915	GCGUUGUGCAGACUCUAUUCCCA	1139

Table 4. KHK Dose Screen in HepG2 cells

Duplex Name	KHK/gapdh					
	10 nM		1 nM		0.1 nM	
	mean	SD	mean	SD	mean	SD
AD-1290887.1	0.404	0.066	0.946	0.145	1.155	0.187
AD-1290629.1	0.613	0.010	1.098	0.229	1.020	0.166
AD-1290807.1	0.247	0.007	0.663	0.138	1.226	0.074
AD-1290681.1	0.990	0.171	1.179	0.170	1.183	0.178
AD-1290639.1	0.927	0.044	1.302	0.254	1.104	0.178
AD-1290597.1	0.904	0.040	1.425	0.206	1.156	0.123
AD-1290775.1	0.870	0.026	1.498	0.159	1.210	0.162
AD-1290795.1	0.182	0.023	0.526	0.119	1.005	0.106
AD-1291003.1	0.835	0.113	1.353	0.222	1.296	0.081
AD-1290903.1	0.419	0.021	1.151	0.233	0.887	0.055
AD-1290896.1	0.807	0.065	1.389	0.334	0.970	0.054
AD-1290605.1	0.675	0.077	1.474	0.345	0.989	0.083
AD-1290742.1	0.991	0.143	1.355	0.379	1.054	0.145
AD-1290554.1	0.924	0.069	1.578	0.256	1.118	0.082
AD-1290509.1	0.865	0.048	1.256	0.129	1.118	0.165
AD-1290938.1	0.482	0.044	0.772	0.164	0.990	0.327
AD-1290750.1	0.990	0.051	1.077	0.059	1.058	0.116
AD-1290659.1	0.690	0.031	0.937	0.194	0.971	0.100
AD-1290755.1	0.916	0.058	1.122	0.098	1.100	0.119
AD-1290558.1	0.888	0.034	1.064	0.214	0.850	0.035
AD-1290516.1	0.777	0.044	1.046	0.236	0.864	0.132
AD-1290621.1	0.893	0.039	0.990	0.210	0.910	0.148
AD-1290956.1	0.498	0.040	0.582	0.063	0.762	0.089
AD-1290672.1	0.814	0.034	0.828	0.073	0.826	0.210
AD-1290800.1	0.859	0.116	0.898	0.082	0.764	0.096
AD-1290555.1	0.781	0.061	0.843	0.036	0.805	0.143
AD-1290527.1	0.912	0.114	0.908	0.056	0.664	0.037
AD-1290921.1	0.920	0.045	0.896	0.102	0.741	0.082
AD-1290664.1	0.693	0.071	0.956	0.095	0.806	0.035
AD-1290924.1	0.282	0.025	0.576	0.045	0.630	0.011
AD-1290650.1	0.756	0.035	0.914	0.130	0.830	0.081
AD-1290939.1	0.829	0.027	0.873	0.030	0.804	0.128
AD-1290592.1	0.784	0.055	0.894	0.136	0.775	0.069
AD-1290551.1	0.724	0.024	0.891	0.189	0.687	0.009
AD-1290638.1	0.921	0.052	0.976	0.230	0.758	0.071
AD-1290580.1	0.410	0.017	0.702	0.116	0.741	0.112
AD-1290916.1	0.552	0.026	0.694	0.024	0.723	0.105
AD-1290741.1	0.799	0.057	0.864	0.034	0.731	0.075
AD-1290910.1	0.945	0.078	0.948	0.020	0.665	0.030
AD-1290661.1	0.985	0.065	0.937	0.064	0.665	0.011

Duplex Name	KHK/gapdh					
	10 nM		1 nM		0.1 nM	
	mean	SD	mean	SD	mean	SD
AD-1290660.1	0.901	0.159	0.936	0.022	0.912	0.032
AD-1290622.1	1.020	0.207	0.940	0.040	0.932	0.174
AD-1290540.1	0.474	0.033	0.698	0.062	0.978	0.109
AD-1290618.1	1.075	0.102	0.930	0.116	0.786	0.032
AD-1290600.1	1.072	0.213	0.901	0.055	0.953	0.133
AD-1290908.1	0.812	0.052	0.810	0.019	0.802	0.172
AD-1290665.1	0.191	0.030	0.216	0.116	0.656	0.117
AD-1290870.1	0.831	0.092	0.916	0.071	0.731	0.047
AD-1290990.1	0.698	0.020	0.687	0.036	0.785	0.075
AD-1290989.1	0.829	0.167	0.862	0.101	0.607	0.241
AD-1290987.1	0.376	0.042	0.581	0.024	0.985	0.177
AD-1290983.1	0.987	0.249	0.993	0.176	0.883	0.125
AD-1290982.1	0.949	0.138	0.846	0.054	0.866	0.052
AD-1290973.1	0.884	0.229	0.954	0.070	0.813	0.127
AD-1290971.1	0.135	0.043	0.289	0.029	0.699	0.071
AD-1290970.1	0.153	0.039	0.207	0.011	0.660	0.127
AD-1290969.1	0.140	0.034	0.221	0.017	0.608	0.113
AD-1290964.1	0.265	0.038	0.360	0.026	0.709	0.100
AD-1290963.1	0.158	0.045	0.311	0.063	0.739	0.176
AD-1290962.1	0.317	0.114	0.436	0.042	0.730	0.095
AD-1290959.1	0.157	0.044	0.295	0.014	0.835	0.044
AD-1290950.1	0.282	0.044	0.624	0.049	0.896	0.080
AD-1290946.1	0.656	0.156	0.866	0.031	0.883	0.042
AD-1290915.1	0.117	0.040	0.396	0.024	0.882	0.121
AD-1290914.1	0.676	0.046	0.689	0.038	0.862	0.102
AD-1290911.1	1.052	0.136	0.914	0.028	0.930	0.143
AD-1290900.1	0.230	0.034	0.512	0.013	0.805	0.026
AD-1290897.1	1.054	0.014	0.957	0.027	0.867	0.065
AD-1290891.1	0.534	0.134	0.675	0.016	0.853	0.068
AD-1290890.1	1.094	0.160	0.905	0.025	0.945	0.138
AD-1290886.1	0.626	0.146	0.764	0.044	0.953	0.112
AD-1290885.1	0.115	0.017	0.217	0.066	0.770	0.030
AD-1290884.1	0.133	0.025	0.264	0.014	0.783	0.120
AD-1290882.1	0.265	0.032	0.659	0.013	0.897	0.032
AD-1290881.1	0.156	0.052	0.393	0.024	0.952	0.137
AD-1290878.1	0.156	0.026	0.381	0.011	0.933	0.201
AD-1290874.1	0.135	0.006	0.371	0.010	0.827	0.127
AD-1290872.1	0.269	0.055	0.256	0.013	0.606	0.098
AD-1290865.1	0.680	0.264	0.838	0.020	0.895	0.202
AD-1290861.1	0.814	0.323	0.885	0.022	0.961	0.088
AD-1290860.1	0.169	0.022	0.641	0.031	0.957	0.032

Duplex Name	KHK/gapdh					
	10 nM		1 nM		0.1 nM	
	mean	SD	mean	SD	mean	SD
AD-1290857.1	0.656	0.179	0.921	0.068	0.990	0.068
AD-1290854.1	0.532	0.083	0.644	0.032	0.994	0.041
AD-1290853.1	0.550	0.135	0.732	0.018	0.966	0.051
AD-1290852.1	0.750	0.108	0.762	0.161	1.074	0.156
AD-1290842.1	0.887	0.182	0.748	0.049	0.982	0.039
AD-1290837.1	0.409	0.365	0.260	0.016	0.919	0.135
AD-1290828.1	0.370	0.070	0.565	0.059	0.904	0.028
AD-1290823.1	0.213	0.072	0.329	0.034	0.916	0.051
AD-1290821.1	0.198	0.043	0.363	0.012	1.036	0.111
AD-1290818.1	0.053	0.009	0.162	0.011	0.699	0.039
AD-1290816.1	0.208	0.040	0.472	0.026	0.916	0.037
AD-1290814.1	0.116	0.037	0.221	0.012	0.625	0.025
AD-1290811.1	0.120	0.031	0.291	0.023	0.830	0.034
AD-1290810.1	0.194	0.025	0.572	0.033	0.949	0.018
AD-1290802.1	0.332	0.110	0.511	0.004	0.983	0.032
AD-1290778.1	0.146	0.019	0.440	0.037	0.961	0.045
AD-1290765.1	0.086	0.017	0.141	0.010	0.647	0.020
AD-1290759.1	0.262	0.082	0.432	0.008	0.948	0.065
AD-1290757.1	0.136	0.041	0.242	0.011	0.838	0.035
AD-1290752.1	0.155	0.018	0.423	0.074	0.893	0.042
AD-1290746.1	0.123	0.013	0.598	0.099	0.970	0.125
AD-1290745.1	0.199	0.018	0.614	0.068	0.964	0.081
AD-1290740.1	0.138	0.016	0.620	0.120	0.985	0.031
AD-1290739.1	0.379	0.035	1.002	0.121	0.992	0.049
AD-1290736.1	0.525	0.046	1.239	0.176	1.017	0.173
AD-1290734.1	0.512	0.029	1.320	0.191	1.066	0.083
AD-1290731.1	0.154	0.004	0.536	0.100	1.089	0.077
AD-1290718.1	0.128	0.051	0.699	0.158	1.227	0.081
AD-1290710.1	0.237	0.082	0.841	0.138	1.133	0.125
AD-1290702.1	0.771	0.024	1.217	0.214	1.069	0.019
AD-1290695.1	0.379	0.050	0.758	0.127	0.997	0.026
AD-1290685.1	0.373	0.009	0.887	0.154	1.003	0.045
AD-1290683.1	0.363	0.013	0.741	0.104	1.030	0.056
AD-1290668.1	0.096	0.008	0.285	0.042	0.874	0.040
AD-1290667.1	0.549	0.036	0.987	0.122	1.200	0.067
AD-1290657.1	0.459	0.045	1.116	0.220	1.181	0.044
AD-1290656.1	0.591	0.104	1.197	0.190	1.231	0.021
AD-1290653.1	0.325	0.008	0.636	0.092	1.087	0.022
AD-1290652.1	0.106	0.008	0.382	0.063	1.143	0.023
AD-1290651.1	0.232	0.008	0.415	0.053	0.983	0.091
AD-1290633.1	0.943	0.033	0.882	0.084	0.898	0.126

Duplex Name	KHK/gapdh					
	10 nM		1 nM		0.1 nM	
	mean	SD	mean	SD	mean	SD
AD-1290632.1	0.406	0.154	0.773	0.099	0.866	0.099
AD-1290604.1	1.000	0.035	0.737	0.043	0.934	0.078
AD-1290599.1	0.128	0.003	0.200	0.016	0.749	0.027
AD-1290589.1	0.992	0.050	0.731	0.038	0.839	0.047
AD-1290584.1	0.222	0.012	0.362	0.031	0.830	0.037
AD-1290576.1	0.140	0.009	0.218	0.020	0.846	0.118
AD-1290569.1	0.531	0.139	0.575	0.056	0.814	0.192
AD-1290565.1	1.031	0.070	0.703	0.049	0.911	0.086
AD-1290561.1	0.400	0.039	0.640	0.106	1.109	0.180
AD-1290559.1	0.429	0.037	0.573	0.086	1.094	0.053
AD-1290557.1	0.861	0.121	0.917	0.251	1.148	0.214
AD-1290552.1	0.866	0.110	0.759	0.128	0.739	0.317
AD-1290548.1	0.427	0.040	0.436	0.041	0.854	0.037
AD-1290547.1	0.534	0.102	0.569	0.065	1.079	0.218
AD-1290546.1	0.081	0.017	0.127	0.070	0.689	0.115
AD-1290544.1	0.420	0.176	0.533	0.047	0.964	0.045
AD-1290543.1	0.798	0.127	0.855	0.085	0.973	0.049
AD-1290542.1	0.807	0.059	0.730	0.024	0.901	0.187
AD-1290528.1	0.638	0.045	0.777	0.049	1.080	0.063
AD-1290526.1	0.496	0.040	0.680	0.045	1.027	0.164
AD-1290524.1	0.886	0.029	1.005	0.038	0.998	0.132
AD-1290522.1	0.825	0.036	0.937	0.042	0.863	0.034
AD-1290515.1	0.925	0.061	0.886	0.060	0.802	0.049
AD-1290514.1	1.056	0.046	1.130	0.080	0.899	0.030
AD-1290510.1	0.794	0.058	0.921	0.052	0.869	0.073
AD-1290980.1	0.309	0.016	0.756	0.022	0.804	0.042
AD-1290933.1	0.444	0.029	0.747	0.039	0.862	0.125
AD-1290834.1	0.296	0.017	0.611	0.064	0.904	0.119
AD-1290747.1	0.839	0.068	0.831	0.135	1.002	0.093
AD-1290666.1	0.265	0.003	0.457	0.036	0.988	0.117
AD-1290612.1	0.776	0.084	0.811	0.050	0.828	0.027
AD-1290570.1	0.811	0.035	0.909	0.096	0.844	0.016
AD-1290564.1	0.956	0.026	0.910	0.053	1.112	0.182
AD-1290535.1	0.916	0.048	0.820	0.086	0.772	0.175
AD-1290517.1	0.443	0.038	0.508	0.035	0.908	0.157
AD-1291001.1	0.214	0.024	0.423	0.052	0.874	0.134
AD-1290805.1	0.927	0.033	0.863	0.094	0.948	0.152
AD-1290655.1	0.926	0.058	0.999	0.085	0.902	0.068
AD-1290574.1	0.978	0.105	0.874	0.046	0.826	0.069
AD-1290533.1	0.937	0.105	0.788	0.035	0.885	0.090
AD-1290530.1	0.127	0.007	0.230	0.022	0.731	0.057

Duplex Name	KHK/gapdh					
	10 nM		1 nM		0.1 nM	
	mean	SD	mean	SD	mean	SD
AD-1290523.1	0.997	0.162	0.713	0.015	0.937	0.168
AD-1290904.1	0.465	0.035	0.660	0.132	0.973	0.114
AD-1290796.1	0.944	0.075	0.734	0.131	0.936	0.147
AD-1290684.1	1.012	0.060	0.678	0.064	0.957	0.197
AD-1290611.1	0.201	0.009	0.224	0.023	0.781	0.084
AD-1290602.1	0.927	0.141	0.648	0.089	1.032	0.068
AD-1290563.1	0.839	0.111	0.650	0.045	1.135	0.189
AD-1290961.1	0.289	0.029	0.473	0.028	0.923	0.133
AD-1290923.1	0.137	0.005	0.280	0.038	0.771	0.071
AD-1290880.1	0.913	0.055	0.859	0.098	0.855	0.084
AD-1290712.1	0.942	0.014	0.729	0.038	0.935	0.078
AD-1290626.1	0.730	0.044	0.705	0.068	0.991	0.097
AD-1290539.1	0.121	0.012	0.166	0.041	0.609	0.026
AD-1290531.1	0.909	0.182	0.781	0.132	1.007	0.135
AD-1290670.1	0.973	0.038	0.790	0.179	1.075	0.159
AD-1290654.1	0.921	0.046	0.798	0.151	1.175	0.185
AD-1290615.1	0.918	0.026	0.772	0.081	1.243	0.213
AD-1290591.1	0.096	0.006	0.163	0.012	0.767	0.159
AD-1290879.1	0.209	0.012	0.478	0.021	0.920	0.147
AD-1290763.1	0.665	0.047	0.752	0.030	0.810	0.057
AD-1290680.1	0.092	0.013	0.198	0.018	0.759	0.050
AD-1290643.1	0.708	0.049	0.770	0.033	0.873	0.029
AD-1290573.1	0.585	0.040	0.744	0.034	0.909	0.065
AD-1290764.1	0.607	0.049	0.788	0.031	1.020	0.087
AD-1290687.1	0.646	0.032	0.894	0.092	1.038	0.155
AD-1290579.1	0.118	0.007	0.456	0.015	1.022	0.022
AD-1290977.1	0.688	0.059	0.950	0.061	1.176	0.120
AD-1290955.1	0.172	0.011	0.269	0.025	0.963	0.125
AD-1290894.1	0.864	0.038	0.804	0.051	0.997	0.068
AD-1290722.1	0.822	0.068	0.839	0.044	0.907	0.040
AD-1290692.1	0.106	0.011	0.192	0.022	0.840	0.025
AD-1290835.1	0.384	0.030	0.692	0.019	0.975	0.135
AD-1290730.1	0.246	0.020	0.491	0.022	0.990	0.131
AD-1290693.1	0.553	0.051	0.740	0.120	1.105	0.191
AD-1290682.1	0.250	0.020	0.348	0.036	0.884	0.105
AD-1290635.1	0.765	0.068	0.800	0.075	1.070	0.110
AD-1290525.1	0.128	0.019	0.203	0.019	0.958	0.069
AD-1290984.1	0.411	0.044	0.685	0.045	0.754	0.043
AD-1290942.1	0.474	0.024	0.586	0.037	0.673	0.103
AD-1290841.1	0.995	0.059	0.960	0.049	0.767	0.029
AD-1290719.1	0.887	0.047	0.789	0.043	0.913	0.113

Duplex Name	KHK/gapdh					
	10 nM		1 nM		0.1 nM	
	mean	SD	mean	SD	mean	SD
AD-1290708.1	0.751	0.032	0.794	0.046	0.980	0.136
AD-1290627.1	0.222	0.018	0.309	0.132	0.986	0.113
AD-1290624.1	0.828	0.027	0.737	0.053	0.899	0.140
AD-1290560.1	0.145	0.005	0.213	0.021	0.854	0.151
AD-1290993.1	1.013	0.140	0.825	0.053	1.298	0.137
AD-1290988.1	0.433	0.023	0.650	0.031	1.274	0.288
AD-1290863.1	0.856	0.088	0.914	0.050	0.916	0.041
AD-1290836.1	0.818	0.080	0.906	0.072	0.922	0.124
AD-1290663.1	0.247	0.019	0.496	0.025	1.019	0.180
AD-1290549.1	0.126	0.009	0.263	0.019	0.885	0.056
AD-1290931.1	1.106	0.034	0.850	0.043	1.057	0.104
AD-1290926.1	1.096	0.033	0.849	0.016	0.934	0.044
AD-1290909.1	1.023	0.042	0.776	0.065	0.935	0.089
AD-1290875.1	0.982	0.074	0.704	0.047	0.943	0.096
AD-1290867.1	1.168	0.044	0.798	0.033	1.048	0.189
AD-1290803.1	0.646	0.007	0.569	0.021	0.852	0.263
AD-1290748.1	0.830	0.098	0.901	0.126	1.229	0.126
AD-1290609.1	0.822	0.087	0.808	0.259	1.227	0.116
AD-1290556.1	0.915	0.043	0.826	0.240	1.251	0.124
AD-1290507.1	0.927	0.080	0.691	0.130	1.342	0.077
Neg ctrl	1.130	0.343	0.981	0.222	0.863	0.237
Neg ctrl	0.897	0.088	1.089	0.141	1.102	0.113
Pos ctrl	0.091	0.013	0.101	0.008	0.196	0.037

Example 3. Design, Synthesis and *In Vitro* Screening of Additional dsRNA Duplexes

Additional siRNAs were designed, synthesized, and prepared using methods known in the art and described above in Example 1.

Detailed lists of the additional unmodified KHK sense and antisense strand nucleotide sequences are shown in Table 5. Detailed lists of the modified KHK sense and antisense strand nucleotide sequences are shown in Table 6.

For transfections, Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 7.5 µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 2.5 µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty µl of complete growth media without antibiotic containing ~1.5 x10^4 Hep3b cells were then added to the siRNA

mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM final duplex concentration.

Total RNA isolation was performed using DYNABEADS. Briefly, cells were lysed in 10µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 3µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 12µL RT mixture was added to each well, as described below.

For cDNA synthesis, a master mix of 1.5µl 10X Buffer, 0.6µl 10X dNTPs, 1.5µl Random primers, 0.75µl Reverse Transcriptase, 0.75µl RNase inhibitor and 9.9µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

RT-qPCR was performed as described above and relative fold change was calculated as described above.

The results of the transfection assays of the dsRNA agents listed in Tables 5 and 6 in Hep3b cells are shown in Table 7.

Table 8 provides a list of the unmodified KHK sense and antisense strand nucleotide sequences of selected dsRNA agentes. Table 9 provides a list of the modified KHK sense and antisense strand nucleotide sequences of selected dsRNA agents.

Table 5. Unmodified Sense and Antisense Strand Sequences of KHK dsRNAs

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in XM_017004061.1
AD-1423310.1	UUUGAGAAGGUUGAUCUGACU	1140	760-780	AGUCAGAUCAACCUUCUCAAAGU	296	758-780
AD-1423311.1	GACGUGGUGUUUGUCAGCAAU	222	946-966	AUUGCUGACAAACACCACGUCUC	446	944-966
AD-1423312.1	UGCAGGAAGCACUGAGAUUCU	115	1208-1228	AGAATCTCAGUGCUUCCUGCACG	1141	1206-1228
AD-1423313.1	ACAGACUUUGAGAAGGUUGAU	67	754-774	AUCAACCUUCUCAAAGUCUGUAG	291	752-774
AD-1423314.1	GCUACAGACUUUGAGAAGGUU	65	751-771	AACCTUCUCAAAGUCUGUAGCAG	1142	749-771
AD-1423315.1	ACCCAGUUCAAGUGGAUCCAU	79	778-798	AUGGAUCCACUUGAACUGGGUCA	303	776-798
AD-1423316.1	UCAGAGCAAAUAAAUCUUCCU	22	1336-1356	AGGAAGAUUUAUUUGCUCUGAGG	246	1334-1356
AD-1290665.2	AGAGCAAAUAAAUCUUCCUCU	212	1477-1497	AGAGGAAGAUUUAUUUGCUCUGA	436	1475-1497
AD-1423317.1	CUUUGAGAAGGUUGAUCUGAU	71	759-779	AUCAGATCAACCUUCUCAAAGUC	1143	757-779
AD-1423318.1	AUGGAAGAGAAGCAGAUCCUU	52	523-543	AAGGAUCUGCUUCUCUUCCAUGA	276	521-543
AD-1423319.1	GACUUUGAGAAGGUUGAUCUU	70	757-777	AAGATCAACCUUCUCAAAGUCUG	1144	755-777
AD-1423320.1	CUCAGAGCAAAUAAAUCUUCU	122	1474-1494	AGAAGATUUAUUUGCUCUGAGGC	1145	1472-1494
AD-1423321.1	CUCUUCCAGCUGUUUGGCUAU	88	922-942	AUAGCCAAACAGCUGGAAGAGCU	312	920-942
AD-1423322.1	GAGCUGGAGACACCUUCAAUU	104	1151-1171	AAUUGAAGGUGUCUCCAGCUCCC	328	1149-1171
AD-1423323.1	CAAUGCCUCCGUCAUCUUCAU	111	1167-1187	AUGAAGAUGACGGAGGCAUUGAA	335	1165-1187
AD-1423324.1	CCUUCAAUGCCUCCGUCAUCU	109	1163-1183	AGAUGACGGAGGCAUUGAAGGUG	333	1161-1183
AD-1423325.1	CUGCUACAGACUUUGAGAAGU	63	749-769	ACUUCUCAAAGUCUGUAGCAGAC	287	747-769
AD-1423326.1	AAAGAUGUGGCCAAGCACUUU	99	964-984	AAAGTGCUUGGCCACAUCUUUGC	1146	962-984
AD-1423327.1	AAGGUUGAUCUGACCCAGUUU	73	766-786	AAACTGGGUCAGAUCAACCUUCU	1147	764-786
AD-1423328.1	AAGGUGUUUGUCCCAGAGAUU	60	612-632	AAUCTCTGGGACAAACACCUUAU	1148	610-632
AD-1423329.1	GAAGAGAAGCAGAUCCUGUGU	54	526-546	ACACAGGAUCUGCUUCUCUUCCA	278	524-546
AD-1423330.1	GUGUUUGUCAGCAAAGAUGUU	95	952-972	AACATCTUUGCUGACAAACACCA	1149	950-972
AD-1423331.1	GACAAGUACCCUAAGGAGGAU	57	583-603	AUCCTCCUUAGGGUACUUGUCCA	1150	581-603
AD-1423332.1	GUGCAGGAAGCACUGAGAUUU	114	1207-1227	AAAUCUCAGUGCUUCCUGCACGC	338	1205-1227
AD-1290878.2	GGAGACGUGGUGUUUGUCAGU	92	943-963	ACUGACAAACACCACGUCUCCGU	316	941-963
AD-1423334.1	CCUCAUGGAAGAGAAGCAGAU	50	519-539	AUCUGCTUCUCUUCCAUGAGGCU	1151	517-539

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO	Range in XM_017004061.1
AD-1423335.1	CUCCGUCAUCUUCAGCCUCUU	113	1173-1193	AAGAGGCUGAAGAUGACGGAGGC	337	1171-1193
AD-1423336.1	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803

Table 6. Modified Sense and Antisense Strand Sequences of KHK dsRNAs

Duplex Name	Sense sequence 5' to 3'	SEQ ID NO:	Antisense sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
AD-1423310.1	ususugagAfaGfGfUfugaucugacuL96	1153	asGfsucdAg(Agn)ucaaccUfuCfucaaasgsu	1154	ACUUUGAGAAGGUUGAUCUGACC	968
AD-1423311.1	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsugdCu(G2p)acaaacAfcCfacgucsusc	1155	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1423312.1	usgscaggAfaGfCfAfcugagauucuL96	563	asGfsaadTc(Tgn)cagugcUfuCfcugcascsg	1156	CGUGCAGGAAGCACUGAGAUUCG	1011
AD-1423313.1	ascsagacUfuUfGfAfgaagguugauL96	515	asUfscadAc(C2p)uucucaAfaGfucugusasg	1157	CUACAGACUUUGAGAAGGUUGAU	963
AD-1423314.1	gscsuacaGfaCfUfUfugagaagguuL96	513	asAfsccdTu(C2p)ucaaagUfcUfguagcsasg	1158	CUGCUACAGACUUUGAGAAGGUU	961
AD-1423315.1	ascsccagUfuCfAfAfguggauccauL96	527	asUfsggdAu(C2p)cacuugAfaCfuggguscsa	1159	UGACCCAGUUCAAGUGGAUCCAC	975
AD-1423316.1	uscsagagCfaAfAfUfaaaucuuccuL96	470	asGfsgadAg(Agn)uuuauuUfgCfucugasgsg	1160	CCUCAGAGCAAAUAAAUCUUCCU	918
AD-1290665.2	asgsagcaAfaUfAfAfaucuuccucuL96	660	asGfsaggAfagauuuaUfuUfgcucusgsa	884	UCAGAGCAAAUAAAUCUUCCUCA	1108
AD-1423317.1	csusuugaGfaAfGfGfuugaucugauL96	519	asUfscadGa(Tgn)caaccuUfcUfcaaagsusc	1161	GACUUUGAGAAGGUUGAUCUGAC	967
AD-1423318.1	asusggaaGfaGfAfAfgcagauccuuL96	500	asAfsggdAu(C2p)ugcuucUfcUfuccausgsa	1162	UCAUGGAAGAGAAGCAGAUCCUG	948
AD-1423319.1	gsascuuuGfaGfAfAfgguugaucuuL96	518	asAfsgadTc(Agn)accuucUfcAfaagucsusg	1163	CAGACUUUGAGAAGGUUGAUCUG	966
AD-1423320.1	csuscagaGfcAfAfAfuaaaucuucuL96	570	asGfsaadGa(Tgn)uuauuuGfcUfcugagsgsc	1164	GCCUCAGAGCAAAUAAAUCUUCC	1018
AD-1423321.1	csuscuucCfaGfCfUfguuuggcuauL96	536	asUfsagdCc(Agn)aacagcUfgGfaagagscsu	1165	AGCUCUUCCAGCUGUUUGGCUAC	984
AD-1423322.1	gsasgcugGfaGfAfCfaccuucaauuL9	552	asAfsuudGa(Agn)ggugucUfcCfagcucscsc	1166	GGGAGCUGGAGACACCUUCAAU	1000

Duplex Name	Sense sequence 5' to 3'	SEQ ID NO:	Antisense sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
	6				G	
AD-1423323.1	csasaugcCfuCfCfGfucaucuucauL96	559	asUfsgadAg(Agn)ugacggAfgGfcauugsasa	1167	UUCAAUGCCUCCGUCAUCUUCAG	1007
AD-1423324.1	cscsuucaAfuGfCfCfuccgucaucuL96	557	asGfsaudGa(C2p)ggaggcAfuUfgaaggsusg	1168	CACCUUCAAUGCCUCCGUCAUCU	1005
AD-1423325.1	csusgcuaCfaGfAfCfuuugagaaguL96	511	asCfsuudCu(C2p)aaagucUfgUfagcagsasc	1169	GUCUGCUACAGACUUUGAGAAGG	959
AD-1423326.1	asasagauGfuGfGfCfcaagcacuuuL96	547	asAfsagdTg(C2p)uuggccAfcAfucuuusgsc	1170	GCAAAGAUGUGGCCAAGCACUUG	995
AD-1423327.1	asasgguuGfaUfCfUfgacccaguuuL96	521	asAfsacdTg(G2p)gucagaUfcAfaccuuscsu	1171	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1423328.1	asasggugUfuUfGfUfcccagagauuL96	508	asAfsucdTc(Tgn)gggacaAfaCfaccuusasu	1172	AUAAGGUGUUUGUCCCAGAGAUG	956
AD-1423329.1	gsasagagAfaGfCfAfgauccuguguL96	502	asCfsacdAg(G2p)aucugcUfuCfucuucscsa	1173	UGGAAGAGAAGCAGAUCCUGUGC	950
AD-1423330.1	gsusguuuGfuCfAfGfcaaagauguuL96	543	asAfscadTc(Tgn)uugcugAfcAfaacacscsa	1174	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1423331.1	gsascaagUfaCfCfCfuaaggaggauL96	505	asUfsccdTc(C2p)uuagggUfaCfuugucscsa	1175	UGGACAAGUACCCUAAGGAGGAC	953
AD-1423332.1	gsusgcagGfaAfGfCfacugagauuuL96	562	asAfsaudCu(C2p)agugcuUfcCfugcacsgsc	1176	GCGUGCAGGAAGCACUGAGAUUC	1010
AD-1290878.2	gsgsagacGfuGfGfUfguuugucaguL96	540	asCfsugaCfaaacaccAfcGfucuccsgsu	764	ACGGAGACGUGGUGUUUGUCAGC	988
AD-1423334.1	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscudGc(Tgn)ucucuuCfcAfugaggscsu	1177	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1423335.1	csusccguCfaUfCfUfucagccucuuL96	561	asAfsgadGg(C2p)ugaagaUfgAfcggagsgsc	1178	GCCUCCGUCAUCUUCAGCCUCUC	1009
AD-1423336.1	gsusucaaGfuGfGfAfuccacauuguL96	655	asCfsaadTg(Tgn)ggauccAfcUfugaacsusg	1179	CAGUUCAAGUGGAUCCACAUUGA	1103

Table 7. KHK Single Dose Screen in Hep3b cells

Duplex Name	% Message Remaining (qPCR)
AD-1423317	9.38
AD-1423327	7.75
AD-1423336	7.70
AD-1423311	6.06
AD-1423320	21.59
AD-1423324	10.44
AD-1423329	12.28
AD-1423333	9.09
AD-1423330	8.78
AD-1423310	6.66
AD-1423314	8.66
AD-1423316	10.59
AD-1423322	18.97
AD-1423325	16.15
AD-1423334	10.71
AD-1423312	12.77
AD-1423313	9.33
AD-1423315	7.66
AD-1423318	13.43
AD-1423319	8.34
AD-1423321	11.82
AD-1423323	9.65
AD-1423326	5.15
AD-1423328	22.84
AD-1423331	13.21
AD-1423332	10.54
AD-1423335	8.60

Table 8. Unmodified Sense and Antisense Strand Sequences of KHK dsRNAs

Duplex Name	Sense sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Source Name	Antisense sequence 5' to 3'	SEQ ID NO:	Range in GenBank Source Name
AD-1290757.3	GUUGAUCUGACCCAGUUCAAU	1180	XM_017004061.1_769-789_G21U_s	769-789	AUUGAACUGGGUCAGAUCAACCU	298	767-789
AD-1290878.3	GGAGACGUGGUGUUUGUCAGU	92	XM_017004061.1_943-963_C21U_s	943-963	ACUGACAAACACCACGUCUCCGU	316	941-963
AD-1290969.3	ACCUUCAAUGCCUCCGUCAUU	108	XM_017004061.1_1162-1182_C21U_s	1162-1182	AAUGACGGAGGCAUUGAAGGUGU	332	1160-1182
AD-1423317.2	CUUUGAGAAGGUUGAUCUGAU	71	XM_017004061.1_759-779_C21U_s	759-779	AUCAGATCAACCUUCUCAAAGUC	1143	757-779
AD-1423327.2	AAGGUUGAUCUGACCCAGUUU	73	XM_017004061.1_766-786_C21U_s	766-786	AAACTGGGUCAGAUCAACCUUCU	1147	764-786
AD-1423336.2	GUUCAAGUGGAUCCACAUUGU	207		783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803
AD-1290599.3	UGGUGUUUGUCAGCAAAGAUU	93	XM_017004061.1_950-970_G21U_s	950-970	AAUCUUUGCUGACAAACACCACG	317	948-970
AD-1523172.1	UGGUGUUUGUCAGCAAAGAUU	93	XM_017004061.1_950-970_G21U_s	950-970	AAUCTUUGCUGACAAACACCACG	1182	948-970
AD-1290837.3	UGAUCUGACCCAGUUCAAGUU	76	XM_017004061.1_771-791_G21U_s	771-791	AACUUGAACUGGGUCAGAUCAAC	300	769-791
AD-1523173.1	UGAUCUGACCCAGUUCAAGUU	76	XM_017004061.1_771-791_G21U_s	771-791	AACUTGAACUGGGUCAGAUCAAC	1183	769-791
AD-1290884.3	GCAGGAAGCACUGAGAUUCGU	116	XM_017004061.1_1209-1229_G21U_s	1209-1229	ACGAAUCUCAGUGCUUCCUGCAC	340	1207-1229
AD-1523174.1	GCAGGAAGCACUGAGAUUCGU	116	XM_017004061.1_1209-1229_G21U_s	1209-1229	ACGAAUCUCAGUGCUUCCUGCAC	340	1207-1229
AD-1290959.3	UUGAUCUGACCCAGUUCAAGU	75	XM_017004061.1_770-790_s	770-790	ACUUGAACUGGGUCAGAUCAACC	299	768-790
AD-1523175.1	UUGAUCUGACCCAGUUCAAGU	75	XM_017004061.1_770-790_s	770-790	ACUUGAACUGGGUCAGAUCAACC	299	768-790
AD-1423311.2	GACGUGGUGUUUGUCAGCAAU	222		946-966	AUUGCUGACAAACACCACGUCUC	446	944-966
AD-1423324.2	CCUUCAAUGCCUCCGUCAUCU	109	XM_017004061.1_1163-1183_s	1163-1183	AGAUGACGGAGGCAUUGAAGGUG	333	1161-1183
AD-1523176.1	CCUUCAAUGCCUCCGUCAUCU	109	XM_017004061.1_1163-1183_s	1163-1183	AGAUGACGGAGGCAUUGAAGGUG	333	1161-1183

Duplex Name	Sense sequence 5' to 3'	SEQ ID NO:	Source Name	Range in GenBank Source Name	Antisense sequence 5' to 3'	SEQ ID NO:	Range in GenBank Source Name
AD-1423329.2	GAAGAGAAGCAGAUCCUGUGU	54	XM_017004061.1_526-546_C21U_s	526-546	ACACAGGAUCUGCUUCUCUUCCA	278	524-546
AD-1423333.2	GUGGUGUUUGUCAGCAAAGAU	1181	XM_005264298.1_812-830_s	810-830	AUCUTUGCUGACAAACACCACGU	1184	808-830
AD-1423330.2	GUGUUUGUCAGCAAAGAUGUU	95	XM_017004061.1_952-972_G21U_s	952-972	AACATCTUUGCUGACAAACACCA	1149	950-972
AD-1523177.1	GUGUUUGUCAGCAAAGAUGUU	95	XM_017004061.1_952-972_G21U_s	952-972	AACATCUUUGCUGACAAACACCA	1185	950-972
AD-1290885.3	CUGACCCAGUUCAAGUGGAUU	78	XM_017004061.1_775-795_C21U_s	775-795	AAUCCACUUGAACUGGGUCAGAU	302	773-795
AD-1523178.1	CUGACCCAGUUCAAGUGGAUU	78	XM_017004061.1_775-795_C21U_s	775-795	AAUCCACUUGAACUGGGUCAGAU	302	773-795
AD-1423334.2	CCUCAUGGAAGAGAAGCAGAU	50	XM_017004061.1_519-539_s	519-539	AUCUGCTUCUCUUCCAUGAGGCU	1151	517-539
AD-1523179.1	CCUCAUGGAAGAGAAGCAGAU	50	XM_017004061.1_519-539_s	519-539	AUCUGCUUCUCUUCCAUGAGGCU	274	517-539
AD-1523180.1	CCUCAUGGAAGAGAAGCAGAU	50	XM_017004061.1_519-539_s	519-539	AUCUGCTUCUCUUCCAUGAGGCU	1151	517-539
AD-1290539.3	CAGACUUUGAGAAGGUUGAUU	68	XM_017004061.1_755-775_C21U_s	755-775	AAUCAACCUUCUCAAAGUCUGUA	292	753-775
AD-1523181.1	CAGACUUUGAGAAGGUUGAUU	68	XM_017004061.1_755-775_C21U_s	755-775	AAUCAACCUUCUCAAAGUCUGUA	292	753-775

Table 9. Modified Sense and Antisense Strand Sequences of KHK dsRNAs

Duplex Name	Sense Sequence 5’ to 3’	SEQ ID NO:	Antisense Sequence 5’ to 3’	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1290757.3	gsusugauCfuGfAfCfccaguucaauL96	1186	asUfsugaAfcuggucAfgAfucaacscsu	746	AGGUUGAUCUGACCCAGUUCAAG	970
AD-1290878.3	gsgsagacGfuGfGfUfguuugucaguL96	540	asCfsugaCfaaacaccAfcGfucuccsgsu	764	ACGGAGACGUGGUGUUUGUCAGC	988
AD-1290969.3	ascscuucAfaUfGfCfcuccgucauuL96	556	asAfsugaCfggaggcaUfuGfaaggusgsu	780	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1423317.2	csusuugaGfaAfGfGfuugaucugauL96	519	asUfscadGa(Tgn)caaccuUfcUfcaaagsusc	1161	GACUUUGAGAAGGUUGAUCUGAC	967
AD-1423327.2	asasgguuGfaUfCfUfgacccaguuuL96	521	asAfsacdTg(G2p)gucagaUfcAfaccuuscsu	1171	AGAAGGUUGAUCUGACCCAGUUC	969

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1423336.2	gsusucaaGfuGfGfAfuccacauuguL96	655	asCfsaadTg(Tgn)ggauccAfcUfugaacsusg	1179	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1290599.3	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucuUfugcugacAfaAfcaccascsg	765	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1523172.1	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucdTu(U2p)gcugacAfaAfcaccascsg	1188	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1290837.3	usgsaucuGfaCfCfCfaguucaaguuL96	524	asAfscuuGfaacugggUfcAfgaucasasc	748	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1523173.1	usgsaucuGfaCfCfCfaguucaaguuL96	524	asAfscudTg(A2p)acugggUfcAfgaucasasc	1189	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1290884.3	gscsaggaAfgCfAfCfugagauucguL96	564	asCfsgaaUfcucagugCfuUfccugcsasc	788	GUGCAGGAAGCACUGAGAUUCGG	1012
AD-1523174.1	gscsaggaAfgCfAfCfugagauucguL96	564	asCfsgadAu(C2p)ucagugCfuUfccugcsasc	1190	GUGCAGGAAGCACUGAGAUUCGG	1012
AD-1290959.3	ususgaucUfgAfCfCfcaguucaaguL96	523	asCfsuugAfacuggguCfaGfaucaascsc	747	GGUUGAUCUGACCCAGUUCAAGU	971
AD-1523175.1	ususgaucUfgAfCfCfcaguucaaguL96	523	asCfsuudGa(A2p)cuggguCfaGfaucaascsc	1191	GGUUGAUCUGACCCAGUUCAAGU	971
AD-1423311.2	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsugdCu(G2p)acaaacAfcCfacgucsusc	1155	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1423324.2	cscsuucaAfuGfCfCfuccgucaucuL96	557	asGfsaudGa(C2p)ggaggcAfuUfgaaggsusg	1168	CACCUUCAAUGCCUCCGUCAUCU	1005
AD-1523176.1	cscsuucaAfuGfCfCfuccgucaucuL96	557	asGfsaudGa(Cgn)ggaggcAfuUfgaaggsusg	1192	CACCUUCAAUGCCUCCGUCAUCU	1005
AD-1423329.2	gsasagagAfaGfCfAfgauccuguguL96	502	asCfsacdAg(G2p)aucugcUfuCfucuucscsa	1173	UGGAAGAGAAGCAGAUCCUGUGC	950
AD-1423333.2	gsusggugUfuUfGfUfcagcaaagauL96	468	asUfscudTu(G2p)cugacaAfaCfaccacsgsu	1193	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1423330.2	gsusguuuGfuCfAfGfcaaagauguuL96	543	asAfscadTc(Tgn)uugcugAfcAfaacacscsa	1174	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1523177.1	gsusguuuGfuCfAfGfcaaagauguuL96	543	asAfscadTc(U2p)uugcugAfcAfaacacscsa	1194	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1290885.3	csusgaccCfaGfUfUfcaaguggauuL96	526	asAfsuccAfcuugaacUfgGfgucagsasu	750	AUCUGACCCAGUUCAAGUGGAUC	974
AD-1523178.1	csusgaccCfaGfUfUfcaaguggauuL9	526	asAfsucdCa(C2p)uugaacUfgGfgucag	1195	AUCUGACCCAGUUCAAGUGGAUC	974

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
	6		sasu			
AD-1423334.2	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscudGc(Tgn)ucucuuCfcAfugaggscsu	1177	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1523179.1	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscudGc(U2p)ucucuuCfcAfugaggscsu	1196	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1523180.1	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscudGcdTucucuuCfcAfugaggscsu	1197	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1290539.3	csasgacuUfuGfAfGfaagguugauuL96	516	asAfsucaAfccuucucAfaAfgucugsusa	740	UACAGACUUUGAGAAGGUUGAUC	964
AD-1523181.1	csasgacuUfuGfAfGfaagguugauuL96	516	asAfsucdAa(C2p)cuucucAfaAfgucugsusa	1198	UACAGACUUUGAGAAGGUUGAUC	964

Example 4. Design, Synthesis and In Vitro Screening of Additional dsRNA Duplexes

Additional siRNAs were designed, synthesized, and prepared using the methods described above in Example 1.

Detailed lists of the additional unmodified KHK sense and antisense strand nucleotide sequences are shown in Table 10. Detailed lists of the modified KHK sense and antisense strand nucleotide sequences are shown in Table 11.

Table 10. Unmodified Sense and Antisense Strand Sequences of KHK dsRNA

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
AD-1290969	ACCUUCAAUGCCUCCGUCAUU	1199	1162-1182	AAUGACGGAGGCAUUGAAGGUGU	332	1160-1182
AD-1423310	UUUGAGAAGGUUGAUCUGACU	72	760-780	AGUCAGAUCAACCUUCUCAAAGU	296	758-780
AD-1423311	GACGUGGUGUUUGUCAGCAAU	222	946-966	AUUGCUGACAAACACCACGUCUC	446	944-966
AD-1423312	UGCAGGAAGCACUGAGAUUCU	115	1208-1228	AGAATCTCAGUGCUUCCUGCACG	1141	1206-1228
AD-1423317	CUUUGAGAAGGUUGAUCUGAU	71	759-779	AUCAGATCAACCUUCUCAAAGUC	1143	757-779
AD-1423319	GACUUUGAGAAGGUUGAUCUU	70	757-777	AAGATCAACCUUCUCAAAGUCUG	1144	755-777
AD-1423323	CAAUGCCUCCGUCAUCUUCAU	111	1167-1187	AUGAAGAUGACGGAGGCAUUGAA	335	1165-1187
AD-1423327	AAGGUUGAUCUGACCCAGUUU	73	766-786	AAACTGGGUCAGAUCAACCUUCU	1147	764-786
AD-1423329	GAAGAGAAGCAGAUCCUGUGU	54	526-546	ACACAGGAUCUGCUUCUCUUCCA	278	524-546
AD-1423330	GUGUUUGUCAGCAAAGAUGUU	95	952-972	AACATCTUUGCUGACAAACACCA	1149	950-972
AD-1423333	GUGGUGUUUGUCAGCAAAGAU	20	949-969	AUCUTUGCUGACAAACACCACGU	1184	947-969
AD-1423334	CCUCAUGGAAGAGAAGCAGAU	50	519-539	AUCUGCTUCUCUUCCAUGAGGCU	1151	517-539
AD-1423336	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803
AD-1523180	CCUCAUGGAAGAGAAGCAGAU	50	519-539	AUCUGCTUCUCUUCCAUGAGGCU	1151	517-539
AD-1548743	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCTUTGCUGACAAACACCACG	1269	948-970
AD-	GCCUCAUGGAAGAGAAGCAGU	1200	518-538	ACUGCUTCUCUTCCAUGAGGCUA	1270	516-538

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1612957						
AD-1612958	CCUCAUGGAAGAGAAGCAGAU	50	519-539	ATCUGCTUCUCTUCCAUGAGGCU	1271	517-539
AD-1612963	UGGAAGAGAAGCAGAUCCUGU	1201	524-544	ACAGGATCUGCTUCUCUUCCAUG	1272	522-544
AD-1612967	AGAGAAGCAGAUCCUGUGCGU	1202	528-548	ACGCACAGGAUCUGCUUCUCUUC	1273	526-548
AD-1612969	AGAAGCAGAUCCUGUGCGUGU	1203	530-550	ACACGCACAGGAUCUGCUUCUCU	1274	528-550
AD-1612970	GAAGCAGAUCCUGUGCGUGGU	1204	531-551	ACCACGCACAGGAUCUGCUUCUC	1275	529-551
AD-1613059	CAGACUUUGAGAAGGUUGAUU	68	755-775	AAUCAACCUUCTCAAAGUCUGUA	1276	753-775
AD-1613060	AGACUUUGAGAAGGUUGAUCU	69	756-776	AGAUCAACCUUCUCAAAGUCUGU	293	754-776
AD-1613062	ACUUUGAGAAGGUUGAUCUGU	213	758-778	ACAGAUCAACCTUCUCAAAGUCU	1277	756-778
AD-1613070	AAGGUUGAUCUGACCCAGUUU	73	766-786	AAACTGGGUCAGAUCAACCUUCU	1147	764-786
AD-1613073	GUUGAUCUGACCCAGUUCAAU	74	769-789	ATUGAACUGGGTCAGAUCAACCU	1278	767-789
AD-1613074	UUGAUCUGACCCAGUUCAAGU	75	770-790	ACUUGAACUGGGUCAGAUCAACC	299	768-790
AD-1613075	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUTGAACUGGGUCAGAUCAAC	1183	769-791
AD-1613076	GAUCUGACCCAGUUCAAGUGU	77	772-792	ACACTUGAACUGGGUCAGAUCAA	1279	770-792
AD-1613077	AUCUGACCCAGUUCAAGUGGU	1205	773-793	ACCACUTGAACTGGGUCAGAUCA	1280	771-793
AD-1613079	CUGACCCAGUUCAAGUGGAUU	78	775-795	AAUCCACUUGAACUGGGUCAGAU	302	773-795
AD-1613088	UUCAAGUGGAUCCACAUUGAU	82	784-804	ATCAAUGUGGATCCACUUGAACU	1281	782-804
AD-	UCAAGUGGAUCCACAUUGAGU	83	785-805	ACUCAATGUGGAUCCACUUGAAC	1282	783-805

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1613089						
AD-1613090	CAAGUGGAUCCACAUUGAGGU	84	786-806	ACCUCAAUGUGGAUCCACUUGAA	308	784-806
AD-1613091	AAGUGGAUCCACAUUGAGGGU	1206	787-807	ACCCTCAAUGUGGAUCCACUUGA	1283	785-807
AD-1613094	UGGAUCCACAUUGAGGGCCGU	1207	790-810	ACGGCCCUCAATGUGGAUCCACU	1284	788-810
AD-1613095	GGAUCCACAUUGAGGGCCGGU	1208	791-811	ACCGGCCCUCAAUGUGGAUCCAC	1285	789-811
AD-1613237	GUUUGGCUACGGAGACGUGGU	1209	933-953	ACCACGTCUCCGUAGCCAAACAG	1286	931-953
AD-1613238	UUUGGCUACGGAGACGUGGUU	1210	934-954	AACCACGUCUCCGUAGCCAAACA	1287	932-954
AD-1613239	UUGGCUACGGAGACGUGGUGU	1211	935-955	ACACCACGUCUCCGUAGCCAAAC	1288	933-955
AD-1613240	UGGCUACGGAGACGUGGUGUU	1212	936-956	AACACCACGUCTCCGUAGCCAAA	1289	934-956
AD-1613241	GGCUACGGAGACGUGGUGUUU	1213	937-957	AAACACCACGUCUCCGUAGCCAA	1290	935-957
AD-1613242	GCUACGGAGACGUGGUGUUUU	242	938-958	AAAACACCACGTCUCCGUAGCCA	1291	936-958
AD-1613243	CUACGGAGACGUGGUGUUUGU	89	939-959	ACAAACACCACGUCUCCGUAGCC	313	937-959
AD-1613244	UACGGAGACGUGGUGUUUGUU	90	940-960	AACAAACACCACGUCUCCGUAGC	314	938-960
AD-1613245	ACGGAGACGUGGUGUUUGUCU	232	941-961	AGACAAACACCACGUCUCCGUAG	456	939-961
AD-1613246	CGGAGACGUGGUGUUUGUCAU	91	942-962	ATGACAAACACCACGUCUCCGUA	1292	940-962
AD-1613247	GGAGACGUGGUGUUUGUCAGU	92	943-963	ACUGACAAACACCACGUCUCCGU	316	941-963
AD-1613254	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCTUTGCUGACAAACACCACG	1269	948-970
AD-	GGUGUUUGUCAGCAAAGAUGU	94	951-971	ACAUCUTUGCUGACAAACACCAC	1293	949-971

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1613255						
AD-1613256	GUGUUUGUCAGCAAAGAUGUU	95	952-972	AACATCTUUGCTGACAAACACCA	1294	950-972
AD-1613257	UGUUUGUCAGCAAAGAUGUGU	96	953-973	ACACAUCUUUGCUGACAAACACC	320	951-973
AD-1613258	GUUUGUCAGCAAAGAUGUGGU	97	954-974	ACCACATCUUUGCUGACAAACAC	1295	952-974
AD-1613259	UUUGUCAGCAAAGAUGUGGCU	98	955-975	AGCCACAUCUUTGCUGACAAACA	1296	953-975
AD-1613361	AGCUGGAGACACCUUCAAUGU	105	1152-1172	ACAUTGAAGGUGUCUCCAGCUCC	1297	1150-1172
AD-1613362	GCUGGAGACACCUUCAAUGCU	1214	1153-1173	AGCATUGAAGGTGUCUCCAGCUC	1298	1151-1173
AD-1613363	CUGGAGACACCUUCAAUGCCU	106	1154-1174	AGGCAUTGAAGGUGUCUCCAGCU	1299	1152-1174
AD-1613369	ACACCUUCAAUGCCUCCGUCU	1215	1160-1180	AGACGGAGGCATUGAAGGUGUCU	1300	1158-1180
AD-1613370	CACCUUCAAUGCCUCCGUCAU	1216	1161-1181	ATGACGGAGGCAUUGAAGGUGUC	1301	1159-1181
AD-1613371	ACCUUCAAUGCCUCCGUCAUU	108	1162-1182	AAUGACGGAGGCAUUGAAGGUGU	332	1160-1182
AD-1613374	UUCAAUGCCUCCGUCAUCUUU	110	1165-1185	AAAGAUGACGGAGGCAUUGAAGG	334	1163-1185
AD-1613377	AAUGCCUCCGUCAUCUUCAGU	112	1168-1188	ACUGAAGAUGACGGAGGCAUUGA	336	1166-1188
AD-1613378	AUGCCUCCGUCAUCUUCAGCU	1217	1169-1189	AGCUGAAGAUGACGGAGGCAUUG	1302	1167-1189
AD-1613395	AGCGUGCAGGAAGCACUGAGU	1218	1204-1224	ACUCAGTGCUUCCUGCACGCUCC	1303	1202-1224
AD-1613400	GCAGGAAGCACUGAGAUUCGU	116	1209-1229	ACGAAUCUCAGTGCUUCCUGCAC	1304	1207-1229
AD-1613401	CAGGAAGCACUGAGAUUCGGU	1219	1210-1230	ACCGAATCUCAGUGCUUCCUGCA	1305	1208-1230
AD-	UAGCCUCAUGGAAGAGAAGCU	1220	516-536	AGCUTCTCUUCCAUGAGGCUACU	1306	514-536

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1634353						
AD-1634354	GCCUCAUGGAAGAGAAGCAGU	1200	518-538	ACUGCUTCUCUUCCAUGAGGCUA	1307	516-538
AD-1634355	CAUGGAAGAGAAGCAGAUCCU	1221	522-542	AGGATCTGCUUCUCUUCCAUGAG	1308	520-542
AD-1634356	GGAAGAGAAGCAGAUCCUGUU	53	525-545	AACAGGAUCUGCUUCUCUUCCAU	277	523-545
AD-1634357	AGAGAAGCAGAUCCUGUGCGU	1202	528-548	ACGCACAGGAUCUGCUUCUCUUC	1273	526-548
AD-1634358	AGACUUUGAGAAGGUUGAUCU	69	756-776	AGAUCAACCUUCUCAAAGUCUGU	293	754-776
AD-1634359	UGAGAAGGUUGAUCUGACCCU	1222	762-782	AGGGTCAGAUCAACCUUCUCAAA	1309	760-782
AD-1634360	AGAAGGUUGAUCUGACCCAGU	1223	764-784	ACUGGGTCAGAUCAACCUUCUCA	1310	762-784
AD-1634361	GGUUGAUCUGACCCAGUUCAU	239	768-788	AUGAACTGGGUCAGAUCAACCUU	1311	766-788
AD-1634362	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUTGAACUGGGUCAGAUCAAC	1183	769-791
AD-1634363	UCUGACCCAGUUCAAGUGGAU	1224	774-794	AUCCACTUGAACUGGGUCAGAUC	1312	772-794
AD-1634364	UGACCCAGUUCAAGUGGAUCU	1225	776-796	AGAUCCACUUGAACUGGGUCAGA	1313	774-796
AD-1634365	CCCAGUUCAAGUGGAUCCACU	1226	779-799	AGUGGATCCACUUGAACUGGGUC	1314	777-799
AD-1634366	CCAGUUCAAGUGGAUCCACAU	80	780-800	AUGUGGAUCCACUUGAACUGGGU	304	778-800
AD-1634367	GUGGAUCCACAUUGAGGGCCU	1227	789-809	AGGCCCTCAAUGUGGAUCCACUU	1315	787-809
AD-1634368	AGACGUGGUGUUUGUCAGCAU	1228	945-965	AUGCTGACAAACACCACGUCUCC	1316	943-965
AD-1634369	ACGUGGUGUUUGUCAGCAAAU	21	947-967	AUUUGCTGACAAACACCACGUCU	1317	945-967
AD-	UUUGUCAGCAAAGAUGUGGCU	98	955-975	AGCCACAUCUUUGCUGACAAACA	322	953-975

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1634370						
AD-1634371	UGUCAGCAAAGAUGUGGCCAU	1229	957-977	AUGGCCACAUCUUUGCUGACAAA	1318	955-977
AD-1634372	GCAAAGAUGUGGCCAAGCACU	1230	962-982	AGUGCUTGGCCACAUCUUUGCUG	1319	960-982
AD-1634373	CUGGAGACACCUUCAAUGCCU	106	1154-1174	AGGCAUTGAAGGUGUCUCCAGCU	1299	1152-1174
AD-1634374	UGGAGACACCUUCAAUGCCUU	107	1155-1175	AAGGCATUGAAGGUGUCUCCAGC	1320	1153-1175
AD-1634375	GGAGACACCUUCAAUGCCUCU	1231	1156-1176	AGAGGCAUUGAAGGUGUCUCCAG	1321	1154-1176
AD-1634376	ACACCUUCAAUGCCUCCGUCU	1215	1160-1180	AGACGGAGGCAUUGAAGGUGUCU	1322	1158-1180
AD-1634377	CUUCAAUGCCUCCGUCAUCUU	1232	1164-1184	AAGATGACGGAGGCAUUGAAGGU	1323	1162-1184
AD-1634378	UCAAUGCCUCCGUCAUCUUCU	223	1166-1186	AGAAGATGACGGAGGCAUUGAAG	1324	1164-1186
AD-1634379	AUGCCUCCGUCAUCUUCAGCU	1217	1169-1189	AGCUGAAGAUGACGGAGGCAUUG	1302	1167-1189
AD-1634380	UGCCUCCGUCAUCUUCAGCCU	1233	1170-1190	AGGCTGAAGAUGACGGAGGCAUU	1325	1168-1190
AD-1634381	CCUCCGUCAUCUUCAGCCUCU	1234	1172-1192	AGAGGCTGAAGAUGACGGAGGCA	1326	1170-1192
AD-1634382	GAGGAGCGUGCAGGAAGCACU	1235	1200-1220	AGUGCUTCCUGCACGCUCCUCCC	1327	1198-1220
AD-1634383	AGGAGCGUGCAGGAAGCACUU	1236	1201-1221	AAGUGCTUCCUGCACGCUCCUCC	1328	1199-1221
AD-1634384	AGCGUGCAGGAAGCACUGAGU	1218	1204-1224	ACUCAGTGCUUCCUGCACGCUCC	1303	1202-1224
AD-1634385	CGUGCAGGAAGCACUGAGAUU	1237	1206-1226	AAUCTCAGUGCUUCCUGCACGCU	1329	1204-1226
AD-1634386	CUUCAAUGCCUCCGUCAUU	1238	1164-1182	AAUGACGGAGGCAUUGAAGGU	1330	1162-1182
AD-	ACCUACAAUGCCUCCGUCAUU	1239	1162-1182	AAUGACGGAGGCAUUGUAGGUGU	1331	1160-1182

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1634387						
AD-1634388	ACCAUCAAUGCCUCCGUCAUU	1240	1162-1182	AAUGACGGAGGCAUUGAUGGUGU	1332	1160-1182
AD-1634389	ACGUUCAAUGCCUCCGUCAUU	1241	1162-1182	AAUGACGGAGGCAUUGAACGUGU	1333	1160-1182
AD-1634390	ACAUUCAAUGCCUCCGUCAUU	1242	1162-1182	AAUGACGGAGGCAUUGAAUGUGU	1334	1160-1182
AD-1634391	CUUCAAUGCCUCCGUCAUU	1238	1164-1182	AAUGACGGAGGCAUUGAAGGU	1330	1162-1182
AD-1634392	ACCUACAAUGCCUCCGUCAUU	1239	1162-1182	AAUGACGGAGGCAUUGUAGGUGU	1331	1160-1182
AD-1634393	ACCAUCAAUGCCUCCGUCAUU	1240	1162-1182	AAUGACGGAGGCAUUGAUGGUGU	1332	1160-1182
AD-1634394	ACGUUCAAUGCCUCCGUCAUU	1241	1162-1182	AAUGACGGAGGCAUUGAACGUGU	1333	1160-1182
AD-1634395	ACAUUCAAUGCCUCCGUCAUU	1242	1162-1182	AAUGACGGAGGCAUUGAAUGUGU	1334	1160-1182
AD-1634396	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803
AD-1634397	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803
AD-1634398	GUUCUAGUGGAUCCACAUUGU	1243	783-803	ACAATGTGGAUCCACUAGAACUG	1335	781-803
AD-1634399	GUUGAAGUGGAUCCACAUUGU	1244	783-803	ACAATGTGGAUCCACUUCAACUG	1336	781-803
AD-1634400	GUUAAAGUGGAUCCACAUUGU	1245	783-803	ACAATGTGGAUCCACUUUAACUG	1337	781-803
AD-1634401	GUACAAGUGGAUCCACAUUGU	1246	783-803	ACAATGTGGAUCCACUUGUACUG	1338	781-803
AD-1634402	AAGGUUGAUCUGACCUAGUUU	1247	766-786	AAACTAGGUCAGAUCAACCUUCU	1339	764-786
AD-1634403	AAGGUUGAUCUGACUCAGUUU	1248	766-786	AAACTGAGUCAGAUCAACCUUCU	1340	764-786
AD-	AAGUUUGAUCUGACCUAGUUU	1249	766-786	AAACTAGGUCAGAUCAAACUUCU	1341	764-786

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1634404						
AD-1634405	AAUGUUGAUCUGACCUAGUUU	1250	766-786	AAACTAGGUCAGAUCAACAUUCU	1342	764-786
AD-1634406	AAGUUUGAUCUGACUCAGUUU	1251	766-786	AAACTGAGUCAGAUCAAACUUCU	1343	764-786
AD-1634407	AAUGUUGAUCUGACUCAGUUU	1252	766-786	AAACTGAGUCAGAUCAACAUUCU	1344	764-786
AD-1634408	UGGUUUUUGUCAGCAAAGAUU	1253	950-970	AAUCTUTGCUGACAAAAACCACG	1345	948-970
AD-1634409	UGGUCUUUGUCAGCAAAGAUU	1254	950-970	AAUCTUTGCUGACAAAGACCACG	1346	948-970
AD-1634410	UGGAGUUUGUCAGCAAAGAUU	1255	950-970	AAUCTUTGCUGACAAACUCCACG	1347	948-970
AD-1634411	UGUUGUUUGUCAGCAAAGAUU	1256	950-970	AAUCTUTGCUGACAAACAACACG	1348	948-970
AD-1634412	UGCUGUUUGUCAGCAAAGAUU	1257	950-970	AAUCTUTGCUGACAAACAGCACG	1349	948-970
AD-1634413	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCTUTGCUGACAAACACCACU	1350	948-970
AD-1634414	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCTUTGCUGACAAACACCACG	1269	948-970
AD-1634415	CCUCAUGGAAGAGAAUCAGAU	1258	519-539	ATCUGATUCUCTUCCAUGAGGCU	1351	517-539
AD-1634416	CCUCUUGGAAGAGAAUCAGAU	1259	519-539	ATCUGATUCUCTUCCAAGAGGCU	1352	517-539
AD-1634417	CCUGAUGGAAGAGAAUCAGAU	1260	519-539	ATCUGATUCUCTUCCAUCAGGCU	1353	517-539
AD-1634418	CCUAAUGGAAGAGAAUCAGAU	1261	519-539	ATCUGATUCUCTUCCAUUAGGCU	1354	517-539
AD-1634419	CCACAUGGAAGAGAAUCAGAU	1262	519-539	ATCUGATUCUCTUCCAUGUGGCU	1355	517-539
AD-1634420	GACGUGGUGUUUGUCAGCAAU	222	946-966	AUUGCUGACAAACACCACGUCUC	446	944-966
AD-	GACGUGGUGUUUGUCAGCAAU	222	946-966	AUUGCTGACAAACACCACGUCUC	1356	944-966

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in XM_017004061.1
1634421						
AD-1634422	GACGUGGUGUUUGUCAGCAAU	222	946-966	AUUGCTGACAAACACCACGUCUC	1356	944-966
AD-1634423	GUGGUGUUUGUCAGCAAAGAU	20	949-969	AUCUTUGCUGACAAACACCACGU	1184	947-969
AD-1634424	GUGGUGUUUGUCAGUAAAGAU	1263	949-969	AUCUTUACUGACAAACACCACGU	1357	947-969
AD-1634425	GUGGUGUUUGUCAGCAAAGAU	20	949-969	AUCTUTGCUGACAAACACCACGU	1358	947-969
AD-1634426	GUGGAGUUUGUCAGUAAAGAU	1264	949-969	AUCUTUACUGACAAACUCCACGU	1359	947-969
AD-1634427	GUGCUGUUUGUCAGUAAAGAU	1265	949-969	AUCUTUACUGACAAACAGCACGU	1360	947-969
AD-1634428	GUGUUGUUUGUCAGUAAAGAU	1266	949-969	AUCUTUACUGACAAACAACACGU	1361	947-969
AD-1634429	GUCGUGUUUGUCAGUAAAGAU	1267	949-969	AUCUTUACUGACAAACACGACGU	1362	947-969
AD-1634430	GUUGUGUUUGUCAGUAAAGAU	1268	949-969	AUCUTUACUGACAAACACAACGU	1363	947-969
AD-1634431	GUGGAGUUUGUCAGUAAAGAU	1264	949-969	AUCTUTGCUGACAAACUCCACGU	1364	947-969
AD-1634432	GUGCUGUUUGUCAGUAAAGAU	1265	949-969	AUCTUTGCUGACAAACAGCACGU	1365	947-969
AD-1634433	GUGUUGUUUGUCAGUAAAGAU	1266	949-969	AUCTUTGCUGACAAACAACACGU	1366	947-969
AD-1634434	GUCGUGUUUGUCAGUAAAGAU	1267	949-969	AUCTUTGCUGACAAACACGACGU	1367	947-969
AD-1634435	GUUGUGUUUGUCAGUAAAGAU	1268	949-969	AUCTUTGCUGACAAACACAACGU	1368	947-969
AD-1634436	GAAGAGAAGCAGAUCCUGUGU	54	526-546	ACACAGGAUCUGCUUCUCUUCCU	1369	524-546
AD-1634437	GAAGAGAAGCAGAUCCUGUGU	54	526-546	ACACAGGAUCUGCUUCUCUUCCU	1369	524-546

Table 11. Modified Sense and Antisense Strand Sequences of KHK dsRNA

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1290969	ascscuucAfaUfGfCfcuccgucauuL96	1370	asAfsugaCfggaggcaUfuGfaaggusgsu	780	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1423310	ususugagAfaGfGfUfugaucugacuL96	1153	asGfsucdAg(Agn)ucaaccUfuCfucaaasgsu	1154	ACUUUGAGAAGGUUGAUCUGACC	968
AD-1423311	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsugdCu(G2p)acaaacAfcCfacgucsusc	1155	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1423312	usgscaggAfaGfCfAfcugagauucuL96	563	asGfsaadTc(Tgn)cagugcUfuCfcugcascsg	1156	CGUGCAGGAAGCACUGAGAUUCG	1011
AD-1423317	csusuugaGfaAfGfGfuugaucugauL96	519	asUfscadGa(Tgn)caaccuUfcUfcaaagsusc	1161	GACUUUGAGAAGGUUGAUCUGAC	967
AD-1423319	gsascuuuGfaGfAfAfgguugaucuuL96	518	asAfsgadTc(Agn)accuucUfcAfaagucsusg	1163	CAGACUUUGAGAAGGUUGAUCUG	966
AD-1423323	csasaugcCfuCfCfGfucaucuucauL96	559	asUfsgadAg(Agn)ugacggAfgGfcauugsasa	1167	UUCAAUGCCUCCGUCAUCUUCAG	1007
AD-1423327	asasgguuGfaUfCfUfgacccaguuuL96	521	asAfsacdTg(G2p)gucagaUfcAfaccuuscsu	1171	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1423329	gsasagagAfaGfCfAfgauccuguguL96	502	asCfsacdAg(G2p)aucugcUfuCfucuucscsa	1173	UGGAAGAGAAGCAGAUCCUGUGC	950
AD-1423330	gsusguuuGfuCfAfGfcaaagauguuL96	543	asAfscadTc(Tgn)uugcugAfcAfaacacscsa	1174	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1423333	gsusggugUfuUfGfUfcagcaaagauL96	468	asUfscudTu(G2p)cugacaAfaCfaccacsgsu	1193	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1423334	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscudGc(Tgn)ucucuuCfcAfugaggscsu	1177	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1423336	gsusucaaGfuGfGfAfuccacauuguL96	655	asCfsaadTg(Tgn)ggauccAfcUfugaacsusg	1179	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1523180	cscsucauGfgAfAfGfagaagcagauL96	498	asUfscudGcdTucucuuCfcAfugaggscsu	1197	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1548743	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucdTu(Tgn)gcugacAfaAfcaccascsg	1482	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1612957	gscscucaugGfAfAfgagaagcaguL96	1371	asdCsugdCudTcucudTcCfaugaggcsusa	1483	UAGCCUCAUGGAAGAGAAGCAGA	1619

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1612958	cscsucauggAfAfGfagaagcagauL96	1372	asdTscudGcdTucucdTuCfcaugaggscsu	1484	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1612963	usgsgaagagAfAfGfcagauccuguL96	1373	asdCsagdGadTcugcdTuCfucuuccasusg	1485	CAUGGAAGAGAAGCAGAUCCUGU	1620
AD-1612967	asgsagaagcAfGfAfuccugugcguL96	1374	asdCsgcdAcdAggaudCuGfcuucucususc	1486	GAAGAGAAGCAGAUCCUGUGCGU	1621
AD-1612969	asgsaagcagAfUfCfcugugcguguL96	1375	asdCsacdGcdAcaggdAuCfugcuucuscsu	1487	AGAGAAGCAGAUCCUGUGCGUGG	1622
AD-1612970	gsasagcagaUfCfCfugugcgugguL96	1376	asdCscadCgdCacagdGaUfcugcuucsusc	1488	GAGAAGCAGAUCCUGUGCGUGGG	1623
AD-1613059	csasgacuuuGfAfGfaagguugauuL96	1377	asdAsucdAadCcuucdTcAfaagucugsusa	1489	UACAGACUUUGAGAAGGUUGAUC	964
AD-1613060	asgsacuuugAfGfAfagguugaucuL96	1378	asdGsaudCadAccuudCuCfaaagucusgsu	1490	ACAGACUUUGAGAAGGUUGAUCU	965
AD-1613062	ascsuuugagAfAfGfguugaucuguL96	1379	asdCsagdAudCaaccdTuCfucaaaguscsu	1491	AGACUUUGAGAAGGUUGAUCUGA	1109
AD-1613070	asasgguugaUfCfUfgacccaguuuL96	1380	asdAsacdTgdGgucadGaUfcaaccuuscsu	1492	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1613073	gsusugaucuGfAfCfccaguucaauL96	1381	asdTsugdAadCugggdTcAfgaucaacscsu	1493	AGGUUGAUCUGACCCAGUUCAAG	970
AD-1613074	ususgaucugAfCfCfcaguucaaguL96	1382	asdCsuudGadAcuggdGuCfagaucaascsc	1494	GGUUGAUCUGACCCAGUUCAAGU	971
AD-1613075	usgsaucugaCfCfCfaguucaaguuL96	1383	asdAscudTgdAacugdGgUfcagaucasasc	1495	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1613076	gsasucugacCfCfAfguucaaguguL96	1384	asdCsacdTudGaacudGgGfucagaucsasa	1496	UUGAUCUGACCCAGUUCAAGUGG	973
AD-1613077	asuscugaccCfAfGfuucaagugguL96	1385	asdCscadCudTgaacdTgGfgucagauscsa	1497	UGAUCUGACCCAGUUCAAGUGGA	1624
AD-1613079	csusgacccaGfUfUfcaaguggauuL96	1386	asdAsucdCadCuugadAcUfgggucagsasu	1498	AUCUGACCCAGUUCAAGUGGAUC	974
AD-1613088	ususcaagugGfAfUfccacauugauL96	1387	asdTscadAudGuggadTcCfacuugaascsu	1499	AGUUCAAGUGGAUCCACAUUGAG	978
AD-1613089	uscsaaguggAfUfCfcacauugaguL96	1388	asdCsucdAadTguggdAuCfcacuugasasc	1500	GUUCAAGUGGAUCCACAUUGAGG	979
AD-	csasaguggaUfCfCfacauugagguL96	1389	asdCscudCadAugugdGaUfccacuugsasa	1501	UUCAAGUGGAUCCACAUUGAGG	980

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1613090					G	
AD-1613091	asasguggauCfCfAfcauugaggguL96	1390	asdCsccdTcdAaugudGgAfuccacuusgsa	1502	UCAAGUGGAUCCACAUUGAGGGC	1625
AD-1613094	usgsgauccaCfAfUfugagggccguL96	1391	asdCsggdCcdCucaadTgUfggauccascsu	1503	AGUGGAUCCACAUUGAGGGCCGG	1626
AD-1613095	gsgsauccacAfUfUfgagggccgguL96	1392	asdCscgdGcdCcucadAuGfuggauccsasc	1504	GUGGAUCCACAUUGAGGGCCGGA	1627
AD-1613237	gsusuuggcuAfCfGfgagacgugguL96	1393	asdCscadCgdTcuccdGuAfgccaaacsasg	1505	CUGUUUGGCUACGGAGACGUGGU	1628
AD-1613238	ususuggcuaCfGfGfagacgugguuL96	1394	asdAsccdAcdGucucdCgUfagccaaascsa	1506	UGUUUGGCUACGGAGACGUGGUG	1629
AD-1613239	ususggcuacGfGfAfgacgugguguL96	1395	asdCsacdCadCgucudCcGfuagccaasasc	1507	GUUUGGCUACGGAGACGUGGUGU	1630
AD-1613240	usgsgcuacgGfAfGfacgugguguuL96	1396	asdAscadCcdAcgucdTcCfguagccasasa	1508	UUUGGCUACGGAGACGUGGUGUU	1631
AD-1613241	gsgscuacggAfGfAfcgugguguuuL96	1397	asdAsacdAcdCacgudCuCfcguagccsasa	1509	UUGGCUACGGAGACGUGGUGUUU	1632
AD-1613242	gscsuacggaGfAfCfgugguguuuuL96	1398	asdAsaadCadCcacgdTcUfccguagcscsa	1510	UGGCUACGGAGACGUGGUGUUUG	1138
AD-1613243	csusacggagAfCfGfugguguuuguL96	1399	asdCsaadAcdAccacdGuCfuccguagscsc	1511	GGCUACGGAGACGUGGUGUUUGU	985
AD-1613244	usascggagaCfGfUfgguguuuguuL96	1400	asdAscadAadCaccadCgUfcuccguasgsc	1512	GCUACGGAGACGUGGUGUUUGUC	986
AD-1613245	ascsggagacGfUfGfguguuugucuL96	1401	asdGsacdAadAcaccdAcGfucuccgusasg	1513	CUACGGAGACGUGGUGUUUGUCA	1128
AD-1613246	csgsgagacgUfGfGfuguuugucauL96	1402	asdTsgadCadAacacdCaCfgucuccgsusa	1514	UACGGAGACGUGGUGUUUGUCAG	987
AD-1613247	gsgsagacguGfGfUfguuugucaguL96	1403	asdCsugdAcdAaacadCcAfcgucuccsgsu	1515	ACGGAGACGUGGUGUUUGUCAGC	988
AD-1613254	usgsguguuuGfUfCfagcaaagauuL96	1404	asdAsucdTudTgcugdAcAfaacaccascsg	1516	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1613255	gsgsuguuugUfCfAfgcaaagauguL96	1405	asdCsaudCudTugcudGaCfaaacaccsasc	1517	GUGGUGUUUGUCAGCAAAGAUGU	990
AD-1613256	gsusguuuguCfAfGfcaaagauguuL96	1406	asdAscadTcdTuugcdTgAfcaaacacscsa	1518	UGGUGUUUGUCAGCAAAGAUGUG	991

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1613257	usgsuuugucAfGfCfaaagauguguL96	1407	asdCsacdAudCuuugdCuGfacaaacascsc	1519	GGUGUUUGUCAGCAAAGAUGUGG	992
AD-1613258	gsusuugucaGfCfAfaagaugugguL96	1408	asdCscadCadTcuuudGcUfgacaaacsasc	1520	GUGUUUGUCAGCAAAGAUGUGGC	993
AD-1613259	ususugucagCfAfAfagauguggcuL96	1409	asdGsccdAcdAucuudTgCfugacaaascsa	1521	UGUUUGUCAGCAAAGAUGUGGCC	994
AD-1613361	asgscuggagAfCfAfccuucaauguL96	1410	asdCsaudTgdAaggudGuCfuccagcuscsc	1522	GGAGCUGGAGACACCUUCAAUGC	1001
AD-1613362	gscsuggagaCfAfCfcuucaaugcuL96	1411	asdGscadTudGaaggdTgUfcuccagcsusc	1523	GAGCUGGAGACACCUUCAAUGCC	1633
AD-1613363	csusggagacAfCfCfuucaaugccuL96	1412	asdGsgcdAudTgaagdGuGfucuccagscsu	1524	AGCUGGAGACACCUUCAAUGCCU	1002
AD-1613369	ascsaccuucAfAfUfgccuccgucuL96	1413	asdGsacdGgdAggcadTuGfaaggugucscsu	1525	AGACACCUUCAAUGCCUCCGUCA	1634
AD-1613370	csasccuucaAfUfGfccuccgucauL96	1414	asdTsgadCgdGaggcdAuUfgaaggugsusc	1526	GACACCUUCAAUGCCUCCGUCAU	1635
AD-1613371	ascscuucaaUfGfCfcuccgucauuL96	1415	asdAsugdAcdGgaggdCaUfugaaggusgsu	1527	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1613374	ususcaaugcCfUfCfcgucaucuuuL96	1416	asdAsagdAudGacggdAgGfcauugaasgsg	1528	CCUUCAAUGCCUCCGUCAUCUUC	1006
AD-1613377	asasugccucCfGfUfcaucuucaguL96	1417	asdCsugdAadGaugadCgGfaggcauusgsa	1529	UCAAUGCCUCCGUCAUCUUCAGC	1008
AD-1613378	asusgccuccGfUfCfaucuucagcuL96	1418	asdGscudGadAgaugdAcGfgaggcaususg	1530	CAAUGCCUCCGUCAUCUUCAGCC	1636
AD-1613395	asgscgugcaGfGfAfagcacugaguL96	1419	asdCsucdAgdTgcuudCcUfgcacgcuscsc	1531	GGAGCGUGCAGGAAGCACUGAGA	1637
AD-1613400	gscsaggaagCfAfCfugagauucguL96	1420	asdCsgadAudCucagdTgCfuuccugcsasc	1532	GUGCAGGAAGCACUGAGAUUCGG	1012
AD-1613401	csasggaagcAfCfUfgagauucgguL96	1421	asdCscgdAadTcucadGuGfcuuccugscsa	1533	UGCAGGAAGCACUGAGAUUCGGG	1638
AD-1634353	usasgccuCfaUfGfGfaagagaagcuL96	1422	asGfscudTc(Tgn)cuuccaUfgAfggcuascsu	1534	AGUAGCCUCAUGGAAGAGAAGCA	1639
AD-1634354	gscscucaUfgGfAfAfgagaagcaguL96	1423	asCfsugdCu(Tgn)cucuucCfaUfgaggcsusa	1535	UAGCCUCAUGGAAGAGAAGCAGA	1619
AD-	csasuggaAfgAfGfAfagcagauccuL96	1424	asGfsgadTc(Tgn)gcuucuCfuUfccaugsasg	1536	CUCAUGGAAGAGAAGCAGAUCC	1640

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1634355					U	
AD-1634356	gsgsaagaGfaAfGfCfagauccuguuL96	501	asAfscadGg(Agn)ucugcuUfcUfcuuccsasu	1537	AUGGAAGAGAAGCAGAUCCUGUG	949
AD-1634357	asgsagaaGfcAfGfAfuccugugcguL96	1425	asCfsgcdAc(Agn)ggaucuGfcUfucucususc	1538	GAAGAGAAGCAGAUCCUGUGCGU	1621
AD-1634358	asgsacuuUfgAfGfAfagguugaucuL96	517	asGfsaudCa(Agn)ccuucuCfaAfagucusgsu	1539	ACAGACUUUGAGAAGGUUGAUCU	965
AD-1634359	usgsagaaGfgUfUfGfaucugacccuL96	1426	asGfsggdTc(Agn)gaucaaCfcUfucucasasa	1540	UUUGAGAAGGUUGAUCUGACCCA	1641
AD-1634360	asgsaaggUfuGfAfUfcugacccaguL96	1427	asCfsugdGg(Tgn)cagaucAfaCfcuucuscsa	1541	UGAGAAGGUUGAUCUGACCCAGU	1642
AD-1634361	gsgsuugaUfcUfGfAfcccaguucauL96	687	asUfsgadAc(Tgn)ggggucaGfaUfcaaccsusu	1542	AAGGUUGAUCUGACCCAGUUCAA	1135
AD-1634362	usgsaucuGfaCfCfCfaguucaaguuL96	524	asAfscudTg(Agn)acugggUfcAfgaucasasc	1543	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1634363	uscsugacCfcAfGfUfucaaguggauL96	1428	asUfsccdAc(Tgn)ugaacuGfgGfucagasusc	1544	GAUCUGACCCAGUUCAAGUGGAU	1643
AD-1634364	usgsacccAfgUfUfCfaaguggaucuL96	1429	asGfsaudCc(Agn)cuugaaCfuGfggucasgsa	1545	UCUGACCCAGUUCAAGUGGAUCC	1644
AD-1634365	cscscaguUfcAfAfGfuggauccacuL96	1430	asGfsugdGa(Tgn)ccacuuGfaAfcugggsusc	1546	GACCCAGUUCAAGUGGAUCCACA	1645
AD-1634366	cscsaguuCfaAfGfUfggauccacauL96	528	asUfsgudGg(Agn)uccacuUfgAfacuggsgsu	1547	ACCCAGUUCAAGUGGAUCCACAU	976
AD-1634367	gsusggauCfcAfCfAfuugagggccuL96	1431	asGfsgcdCc(Tgn)caauguGfgAfuccacsusu	1548	AAGUGGAUCCACAUUGAGGGCCG	1646
AD-1634368	asgsacguGfgUfGfUfuugucagcauL96	1432	asUfsgcdTg(Agn)caaacaCfcAfcgucuscsc	1549	GGAGACGUGGUGUUUGUCAGCAA	1647
AD-1634369	ascsguggUfgUfUfUfgucagcaaauL96	469	asUfsuudGc(Tgn)gacaaaCfaCfcacguscsu	1550	AGACGUGGUGUUUGUCAGCAAAG	917
AD-1634370	ususugucAfgCfAfAfagauguggcuL96	546	asGfsccdAc(Agn)ucuuugCfuGfacaaascsa	1551	UGUUUGUCAGCAAAGAUGUGGCC	994
AD-1634371	usgsucagCfaAfAfGfauguggccauL96	1433	asUfsggdCc(Agn)caucuuUfgCfugacasasa	1552	UUUGUCAGCAAAGAUGUGGCCAA	1648
AD-1634372	gscsaaagAfuGfUfGfgccaagcacuL96	1434	asGfsugdCu(Tgn)ggccacAfuCfuuugcsusg	1553	CAGCAAAGAUGUGGCCAAGCACU	1649

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1634373	csusggagAfcAfCfCfuucaaugccuL96	554	asGfsgcdAu(Tgn)gaagguGfuCfuccagscsu	1554	AGCUGGAGACACCUUCAAUGCCU	1002
AD-1634374	usgsgagaCfaCfCfUfucaaugccuuL96	555	asAfsggdCa(Tgn)ugaaggUfgUfcuccasgsc	1555	GCUGGAGACACCUUCAAUGCCUC	1003
AD-1634375	gsgsagacAfcCfUfUfcaaugccucuL96	1435	asGfsagdGc(Agn)uugaagGfuGfucuccsasg	1556	CUGGAGACACCUUCAAUGCCUCC	1650
AD-1634376	ascsaccuUfcAfAfUfgccuccgucuL96	1436	asGfsacdGg(Agn)ggcauuGfaAfgguguscsu	1557	AGACACCUUCAAUGCCUCCGUCA	1634
AD-1634377	csusucaaUfgCfCfUfccgucaucuuL96	1437	asAfsgadTg(Agn)cggaggCfaUfugaagsgsu	1558	ACCUUCAAUGCCUCCGUCAUCU	1651
AD-1634378	uscsaaugCfcUfCfCfgucaucuucuL96	671	asGfsaadGa(Tgn)gacggaGfgCfauugasasg	1559	CUUCAAUGCCUCCGUCAUCUUC	1119
AD-1634379	asusgccuCfcGfUfCfaucuucagcuL96	1438	asGfscudGa(Agn)gaugacGfgAfggcaususg	1560	CAAUGCCUCCGUCAUCUUCAGCC	1636
AD-1634380	usgsccucCfgUfCfAfucuucagccuL96	1439	asGfsgcdTg(Agn)agaugaCfgGfaggcasusu	1561	AAUGCCUCCGUCAUCUUCAGCCU	1652
AD-1634381	cscsuccgUfcAfUfCfuucagccucuL96	1440	asGfsagdGc(Tgn)gaagauGfaCfggaggscsa	1562	UGCCUCCGUCAUCUUCAGCCUC	1653
AD-1634382	gsasggagCfgUfGfCfaggaagcacuL96	1441	asGfsugdCu(Tgn)ccugcaCfgCfuccucscsc	1563	GGGAGGAGCGUGCAGGAAGCAC	1654
AD-1634383	asgsgagcGfuGfCfAfggaagcacuuL96	1442	asAfsgudGc(Tgn)uccugcAfcGfcuccuscsc	1564	GGAGGAGCGUGCAGGAAGCACU	1655
AD-1634384	asgscgugCfaGfGfAfagcacugaguL96	1443	asCfsucdAg(Tgn)gcuuccUfgCfacgcuscsc	1565	GGAGCGUGCAGGAAGCACUGAG	1637
AD-1634385	csgsugcaGfgAfAfGfcacugagauuL96	1444	asAfsucdTc(Agn)gugcuuCfcUfgcacgscsu	1566	AGCGUGCAGGAAGCACUGAGAU	1656
AD-1634386	csusucaaUfGfCfcuccgucauuL96	1445	asdAsugdAcdGgaggdCaUfugaagsgsu	1567	ACCUUCAAUGCCUCCGUCAUC	1657
AD-1634387	ascscuacaaUfGfCfcuccgucauuL96	1446	asdAsugdAcdGgaggdCaUfuguaggusgsu	1568	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1634388	ascscaucaaUfGfCfcuccgucauuL96	1447	asdAsugdAcdGgaggdCaUfugauggusgsu	1569	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1634389	ascsguucaaUfGfCfcuccgucauuL96	1448	asdAsugdAcdGgaggdCaUfugaacgusgsu	1570	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-	ascsauucaaUfGfCfcuccgucauuL96	1449	asdAsugdAcdGgaggdCaUfugaaugusgsu	1571	ACACCUUCAAUGCCUCCGUCAU	1004

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1634390					C	
AD-1634391	csusucAfaUfGfCfcuccgucauuL96	1450	asAfsugaCfggaggcaUfuGfaagsgsu	1572	ACCUUCAAUGCCUCCGUCAUC	1657
AD-1634392	ascscuacAfaUfGfCfcuccgucauuL96	1451	asAfsugaCfggaggcaUfuGfuaggusgsu	1573	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1634393	ascscaucAfaUfGfCfcuccgucauuL96	1452	asAfsugaCfggaggcaUfuGfauggusgsu	1574	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1634394	ascsguucAfaUfGfCfcuccgucauuL96	1453	asAfsugaCfggaggcaUfuGfaacgusgsu	1575	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1634395	ascsauucAfaUfGfCfcuccgucauuL96	1454	asAfsugaCfggaggcaUfuGfaaugusgsu	1576	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1634396	gsusucaaguGfGfAfuccacauuguL96	1455	asdCsaadTg(Tgn)ggaudCcAfcuugaacsusg	1577	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1634397	gsusucaaguGfGfAfuccacauuguL96	1455	asdCsaadTg(Tgn)ggaudCcAfcUfugaacsusg	1578	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1634398	gsusucuaGfuGfGfAfuccacauuguL96	1456	asCfsaadTg(Tgn)ggauccAfcUfagaacsusg	1579	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1634399	gsusugaaGfuGfGfAfuccacauuguL96	1457	asCfsaadTg(Tgn)ggauccAfcUfucaacsusg	1580	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1634400	gsusuaaaGfuGfGfAfuccacauuguL96	1458	asCfsaadTg(Tgn)ggauccAfcUfuuaacsusg	1581	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1634401	gsusacaaGfuGfGfAfuccacauuguL96	1459	asCfsaadTg(Tgn)ggauccAfcUfuguacsusg	1582	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1634402	asasgguugaUfCfUfgaccuaguuuL96	1460	asdAsacdTadGgucadGaUfcaaccuuscsu	1583	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1634403	asasgguugaUfCfUfgacucaguuuL96	1461	asdAsacdTgdAgucadGaUfcaaccuuscsu	1584	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1634404	asasguuugaUfCfUfgaccuaguuuL96	1462	asdAsacdTadGgucadGaUfcaaacuuscsu	1585	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1634405	asasuguugaUfCfUfgaccuaguuuL96	1463	asdAsacdTadGgucadGaUfcaacauuscsu	1586	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1634406	asasguuugaUfCfUfgacucaguuuL96	1464	asdAsacdTgdAgucadGaUfcaaacuuscsu	1587	AGAAGGUUGAUCUGACCCAGUUC	969
AD-1634407	asasuguugaUfCfUfgacucaguuuL96	1465	asdAsacdTgdAgucadGaUfcaacauuscsu	1588	AGAAGGUUGAUCUGACCCAGUUC	969

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
AD-1634408	usgsguuuUfuGfUfCfagcaaagauuL96	1466	asAfsucdTu(Tgn)gcugacAfaAfaaccascsg	1589	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634409	usgsgucuUfuGfUfCfagcaaagauuL96	1467	asAfsucdTu(Tgn)gcugacAfaAfgaccascsg	1590	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634410	usgsgaguUfuGfUfCfagcaaagauuL96	1468	asAfsucdTu(Tgn)gcugacAfaAfcuccascsg	1591	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634411	usgsuuguUfuGfUfCfagcaaagauuL96	1469	asAfsucdTu(Tgn)gcugacAfaAfcaacascsg	1592	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634412	usgscuguUfuGfUfCfagcaaagauuL96	1470	asAfsucdTu(Tgn)gcugacAfaAfcagcascsg	1593	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634413	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucdTu(Tgn)gcugacAfaAfcaccascsu	1594	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634414	usgsguguuuGfUfCfagcaaagauuL96	1404	asdAsucdTu(Tgn)gcugdAcAfaAfcaccascsg	1595	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1634415	cscsucauggAfAfGfagaaucagauL96	1471	asdTscudGadTucucdTuCfcaugaggscsu	1596	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1634416	cscsucuuggAfAfGfagaaucagauL96	1472	asdTscudGadTucucdTuCfcaagaggscsu	1597	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1634417	cscsugauggAfAfGfagaaucagauL96	1473	asdTscudGadTucucdTuCfcaucaggscsu	1598	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1634418	cscsuaauggAfAfGfagaaucagauL96	1474	asdTscudGadTucucdTuCfcauuaggscsu	1599	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1634419	cscsacauggAfAfGfagaaucagauL96	1475	asdTscudGadTucucdTuCfcauguggscsu	1600	AGCCUCAUGGAAGAGAAGCAGAU	946
AD-1634420	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsugdCudGacaaacAfcCfacgucsusc	1601	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1634421	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsudGc(Tgn)gacaaacAfcCfacgucsusc	1602	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1634422	gsascgugGfuGfUfUfugucagcaauL96	670	asUfsugcdTg(Agn)caaacAfcCfacgucsusc	1603	GAGACGUGGUGUUUGUCAGCAAA	1118
AD-1634423	gsusggugUfuUfGfUfcagcaaagauL96	468	asUfscudTudGcugacaAfaCfaccacsgsu	1604	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634424	gsusggugUfuUfGfUfcaguaaagauL96	1476	asUfscudTudAcugacaAfaCfaccacsgsu	1605	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-	gsusggugUfuUfGfUfcagcaaagauL96	468	asUfscdTu(Tgn)gcugacaAfaCfaccacsgsu	1606	ACGUGGUGUUUGUCAGCAAAGA	916

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence	SEQ ID NO:
1634425					U	
AD-1634426	gsusggagUfuUfGfUfcaguaaagauL96	1477	asUfscudTudAcugacaAfaCfuccacsgsu	1607	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634427	gsusgcugUfuUfGfUfcaguaaagauL96	1478	asUfscudTudAcugacaAfaCfagcacsgsu	1608	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634428	gsusguugUfuUfGfUfcaguaaagauL96	1479	asUfscudTudAcugacaAfaCfaacacsgsu	1609	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634429	gsuscgugUfuUfGfUfcaguaaagauL96	1480	asUfscudTudAcugacaAfaCfacgacsgsu	1610	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634430	gsusugugUfuUfGfUfcaguaaagauL96	1481	asUfscudTudAcugacaAfaCfacaacsgsu	1611	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634431	gsusggagUfuUfGfUfcaguaaagauL96	1477	asUfscdTu(Tgn)gcugacaAfaCfuccacsgsu	1612	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634432	gsusgcugUfuUfGfUfcaguaaagauL96	1478	asUfscdTu(Tgn)gcugacaAfaCfagcacsgsu	1613	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634433	gsusguugUfuUfGfUfcaguaaagauL96	1479	asUfscdTu(Tgn)gcugacaAfaCfaacacsgsu	1614	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634434	gsuscgugUfuUfGfUfcaguaaagauL96	1480	asUfscdTu(Tgn)gcugacaAfaCfacgacsgsu	1615	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634435	gsusugugUfuUfGfUfcaguaaagauL96	1481	asUfscdTu(Tgn)gcugacaAfaCfacaacsgsu	1616	ACGUGGUGUUUGUCAGCAAAGAU	916
AD-1634436	gsasagagAfaGfCfAfgauccuguguL96	502	asdCsacdAgdGaucudGcUfucucuucscsu	1617	UGGAAGAGAAGCAGAUCCUGUGC	950
AD-1634437	gsasagagAfaGfCfAfgauccuguguL96	502	asCfsacadGg(Agn)ucugcUfuCfucuucscsu	1618	UGGAAGAGAAGCAGAUCCUGUGC	950

Example 5. Design, Synthesis and In Vitro Screening of Additional dsRNA Duplexes

Based on the *in vitro* analyses, structure-active relationship (SAR) analyses were performed. In particular, additional duplexes were designed, synthesized, and assayed.

siRNAs were designed, synthesized, and prepared using the methods described above. *In vitro* screening assays in Hep3B and PCH cells with these siRNAs were performed as described above.

Detailed lists of the unmodified KHK sense and antisense strand nucleotide sequences are shown in Table 12. Detailed lists of the modified KHK sense and antisense strand nucleotide sequences are shown in Table 13.

For transfections, cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 7.5 µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 2.5 µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty µl of complete growth media without antibiotic containing ~1.5 x10^4 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10, 1 and 0.1 nM final duplex concentration.

Total RNA isolation was performed using DYNABEADS. Briefly, cells were lysed in 10µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 3µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 12µL RT mixture was added to each well, as described below.

For cDNA synthesis, a master mix of 1.5µl 10X Buffer, 0.6µl 10X dNTPs, 1.5µl Random primers, 0.75µl Reverse Transcriptase, 0.75µl RNase inhibitor and 9.9µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

RT-qPCR was performed as described above and relative fold change was calculated as described above.The results of the transfection assays of the dsRNA agents listed in Tables 12 and 13 in Hep3B cells are shown in Table 14. The results of the transfection assays of the dsRNA agents listed in Tables 12 and 13 in primary cynomolgus hepatocytes (PCH) are shown in Table 15.

For Dual-Glo® Luciferase assay, cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Dual-Glo® Luciferase constructs were generated in the psiCHECK2 plasmid containing the human KHK genomic sequence. Each dual-luciferase plasmid was co-transfected with siRNA (Tables 12 and 13)

into approximately $2x10^4$ cells using Lipofectamine 2000 (Invitrogen, Carlsbad CA. cat # 11668-019). For each well of a 96 well plate, 0.5 µl of Lipofectamine was added to 100 ng of plasmid vector and a single siRNA (Tables 12 and 13) in 14.8 µl of Opti-MEM and allowed to complex at room temperature for 15 minutes. The mixture was then added to the cells which were resuspended in 80 µl of fresh complete media. Cells were incubated for 24 hours before luciferase was measured. Single dose experiments were performed at 10, 1 and 0.1 nM final duplex concentration.

Forty-eight hours after the siRNAs were transfected, Firefly (transfection control) and Rinella (fused to KHK target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 75 µl of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before lunimescense (500 nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 75 µl of room temperature Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent, quenches the firefly luciferase signal and sustaines luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (KHK) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done in quadruplicates.

Table 16 shows the Dual-Glo® Luciferase assay results of a single dose screen in cells transfected with the indicated agents in Tables 12 and 13.

Table 12. Unmodified Sense and Antisense Strand Sequences of KHK dsRNA

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1290507.2	UUCUUCAUCUGUCAAAUGGAU	1658	2200-2220	AUCCAUUUGACAGAUGAAGAAAU	415	2198-2220
AD-1290509.2	GAUUAAAAUCUGCCAUUUAAU	167	2130-2150	AUUAAAUGGCAGAUUUUAAUCCA	391	2128-2150
AD-1290510.2	AAUUUCUUCAUCUGUCAAAUU	183	2197-2217	AAUUUGACAGAUGAAGAAAUUGA	407	2195-2217
AD-1290514.2	CAAUUUCUUCAUCUGUCAAAU	182	2196-2216	AUUUGACAGAUGAAGAAAUUGAG	406	2194-2216
AD-1290515.2	AGGCCUUAUAAUGUAAAGAGU	158	2073-2093	ACUCUUUACAUUAUAAGGCCUUA	382	2071-2093
AD-1290516.2	GGAUUAAAAUCUGCCAUUUAU	192	2129-2149	AUAAAUGGCAGAUUUUAAUCCAG	416	2127-2149
AD-1290522.2	UCUGGAACACAUAUUGGAAUU	149	2025-2045	AAUUCCAAUAUGUGUUCCAGAUC	373	2023-2045
AD-1290523.2	GCCUCAAUUUCUUCAUCUGUU	181	2192-2212	AACAGAUGAAGAAAUUGAGGCAG	405	2190-2212
AD-1290524.2	AUUUCUUCAUCUGUCAAAUGU	184	2198-2218	ACAUUUGACAGAUGAAGAAAUUG	408	2196-2218
AD-1290527.2	UGGAUUAAAAUCUGCCAUUUU	193	2128-2148	AAAAUGGCAGAUUUUAAUCCAGG	417	2126-2148
AD-1290528.2	CUGGAACACAUAUUGGAAUUU	150	2026-2046	AAAUUCCAAUAUGUGUUCCAGAU	374	2024-2046
AD-1290531.2	CUGCCUCAAUUUCUUCAUCUU	179	2190-2210	AAGAUGAAGAAAUUGAGGCAGAU	403	2188-2210
AD-1290533.2	AAAUCUGCCAUUUAAUUAGCU	169	2135-2155	AGCUAAUUAAAUGGCAGAUUUUA	393	2133-2155
AD-1290535.2	AAUCUGCCAUUUAAUUAGCUU	170	2136-2156	AAGCUAAUUAAAUGGCAGAUUUU	394	2134-2156
AD-1290539.5	CAGACUUUGAGAAGGUUGAUU	68	755-775	AAUCAACCUUCUCAAAGUCUGUA	292	753-775
AD-1290542.2	UCAAUUUCUUCAUCUGUCAAU	194	2195-2215	AUUGACAGAUGAAGAAAUUGAGG	418	2193-2215
AD-1290543.2	UGGAACACAUAUUGGAAUUGU	151	2027-2047	ACAAUUCCAAUAUGUGUUCCAGA	375	2025-2047
AD-1290551.2	CUGGAUUAAAAUCUGCCAUUU	166	2127-2147	AAAUGGCAGAUUUUAAUCCAGGU	390	2125-2147
AD-1290552.2	UUUCUUCAUCUGUCAAAUGGU	195	2199-2219	ACCAUUUGACAGAUGAAGAAAUU	419	2197-2219
AD-1290554.2	AUAAUGUAAAGGGCUUUAGAU	162	2096-2116	AUCUAAAGCCCUUUACAUUAUAU	386	2094-2116
AD-1290555.2	AUAUAAUGUAAAGGGCUUUAU	161	2094-2114	AUAAAGCCCUUUACAUUAUAUGC	385	2092-2114
AD-1290556.2	GCCUUAUAAUGUAAAGAGCAU	159	2075-2095	AUGCUCUUUACAUUAUAAGGCCU	383	2073-2095
AD-1290557.2	GGCCUUAUAAUGUAAAGAGCU	196	2074-2094	AGCUCUUUACAUUAUAAGGCCUU	420	2072-2094
AD-1290558.2	UAUAAUGUAAAGGGCUUUAGU	197	2095-2115	ACUAAAGCCCUUUACAUUAUAUG	421	2093-2115
AD-1290563.2	GUAAGGCCUUAUAAUGUAAAU	156	2070-2090	AUUUACAUUAUAAGGCCUUACCC	380	2068-2090
AD-1290564.2	CCUCAAUUUCUUCAUCUGUCU	199	2193-2213	AGACAGAUGAAGAAAUUGAGGCA	423	2191-2213

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1290565.2	CUCAAUUUCUUCAUCUGUCAU	200	2194-2214	AUGACAGAUGAAGAAAUUGAGGC	424	2192-2214
AD-1290570.2	AAGGCCUUAUAAUGUAAAGAU	157	2072-2092	AUCUUUACAUUAUAAGGCCUUAC	381	2070-2092
AD-1290573.2	AUCUGCCUCAAUUUCUUCAUU	177	2188-2208	AAUGAAGAAAUUGAGGCAGAUUG	401	2186-2208
AD-1290574.2	UAAGGCCUUAUAAUGUAAAGU	201	2071-2091	ACUUUACAUUAUAAGGCCUUACC	425	2069-2091
AD-1290584.2	GUGUUUGUCAGCAAAGAUGUU	95	952-972	AACAUCUUUGCUGACAAACACCA	319	950-972
AD-1290589.2	GGAACACAUAUUGGAAUUGGU	152	2028-2048	ACCAAUUCCAAUAUGUGUUCCAG	376	2026-2048
AD-1290592.2	CAUAUAAUGUAAAGGGCUUUU	202	2093-2113	AAAAGCCCUUUACAUUAUAUGCU	426	2091-2113
AD-1290597.2	AUUAAAAUCUGCCAUUUAAUU	168	2131-2151	AAUUAAAUGGCAGAUUUUAAUCC	392	2129-2151
AD-1290599.7	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCUUUGCUGACAAACACCACG	317	948-970
AD-1290600.2	GCUUGGCUACAGAAUUAUUGU	190	2229-2249	ACAAUAAUUCUGUAGCCAAGCAG	414	2227-2249
AD-1290602.2	UGCCUCAAUUUCUUCAUCUGU	180	2191-2211	ACAGAUGAAGAAAUUGAGGCAGA	404	2189-2211
AD-1290604.2	AUCUGCCAUUUAAUUAGCUGU	171	2137-2157	ACAGCUAAUUAAAUGGCAGAUUU	395	2135-2157
AD-1290605.2	AAUCUGCCUCAAUUUCUUCAU	176	2187-2207	AUGAAGAAAUUGAGGCAGAUUGC	400	2185-2207
AD-1290609.2	UGCCAUUUAAUUAGCUGCAUU	203	2140-2160	AAUGCAGCUAAUUAAAUGGCAGA	427	2138-2160
AD-1290611.3	AGACUUUGAGAAGGUUGAUCU	69	756-776	AGAUCAACCUUCUCAAAGUCUGU	293	754-776
AD-1290612.2	AUCUGGAACACAUAUUGGAAU	148	2024-2044	AUUCCAAUAUGUGUUCCAGAUCG	372	2022-2044
AD-1290615.2	UCUGCCUCAAUUUCUUCAUCU	178	2189-2209	AGAUGAAGAAAUUGAGGCAGAUU	402	2187-2209
AD-1290618.2	AAUGUAAAGGGCUUUAGAGUU	164	2098-2118	AACUCUAAAGCCCUUUACAUUAU	388	2096-2118
AD-1290624.2	AUUAUUGUGAGGAUAAAAUCU	204	2242-2262	AGAUUUUAUCCUCACAAUAAUUC	428	2240-2262
AD-1290626.2	GGUAAGGCCUUAUAAUGUAAU	205	2069-2089	AUUACAUUAUAAGGCCUUACCCA	429	2067-2089
AD-1290633.2	CUGCCAUUUAAUUAGCUGCAU	172	2139-2159	AUGCAGCUAAUUAAAUGGCAGAU	396	2137-2159
AD-1290635.2	UUCUGCUUGGCUACAGAAUUU	206	2225-2245	AAAUUCUGUAGCCAAGCAGAAUU	430	2223-2245
AD-1290639.2	UAAUGUAAAGGGCUUUAGAGU	163	2097-2117	ACUCUAAAGCCCUUUACAUUAUA	387	2095-2117
AD-1290643.2	UGCUUGGCUACAGAAUUAUUU	189	2228-2248	AAAUAAUUCUGUAGCCAAGCAGA	413	2226-2248
AD-1290650.2	CGCAAUCUGCCUCAAUUUCUU	174	2184-2204	AAGAAAUUGAGGCAGAUUGCGUU	398	2182-2204
AD-1290651.2	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAAUGUGGAUCCACUUGAACUG	431	781-803
AD-1290654.2	GGGUAAGGCCUUAUAAUGUAU	208	2068-2088	AUACAUUAUAAGGCCUUACCCAC	432	2066-2088

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1290655.2	GAUCUGGAACACAUAUUGGAU	209	2023-2043	AUCCAAUAUGUGUUCCAGAUCGG	433	2021-2043
AD-1290659.2	CAAUCUGCCUCAAUUUCUUCU	211	2186-2206	AGAAGAAAUUGAGGCAGAUUGCG	435	2184-2206
AD-1290660.2	CCUGGAUUAAAAUCUGCCAUU	165	2126-2146	AAUGGCAGAUUUUAAUCCAGGUC	389	2124-2146
AD-1290661.2	GCAUAUAAUGUAAAGGGCUUU	160	2092-2112	AAAGCCCUUUACAUUAUAUGCUC	384	2090-2112
AD-1290666.2	ACUUUGAGAAGGUUGAUCUGU	213	758-778	ACAGAUCAACCUUCUCAAAGUCU	437	756-778
AD-1290670.2	UCCGAUCUGGAACACAUAUUU	146	2020-2040	AAAUAUGUGUUCCAGAUCGGACC	370	2018-2040
AD-1290672.2	GCAAUCUGCCUCAAUUUCUUU	175	2185-2205	AAAGAAAUUGAGGCAGAUUGCGU	399	2183-2205
AD-1290681.2	UGGGUAAGGCCUUAUAAUGUU	215	2067-2087	AACAUUAUAAGGCCUUACCCACC	439	2065-2087
AD-1290684.2	CGAUCUGGAACACAUAUUGGU	217	2022-2042	ACCAAUAUGUGUUCCAGAUCGGA	441	2020-2042
AD-1290687.2	CUGCUUGGCUACAGAAUUAUU	188	2227-2247	AAUAAUUCUGUAGCCAAGCAGAA	412	2225-2247
AD-1290702.2	UCUGCCAUUUAAUUAGCUGCU	218	2138-2158	AGCAGCUAAUUAAAUGGCAGAUU	442	2136-2158
AD-1290712.2	CCGAUCUGGAACACAUAUUGU	147	2021-2041	ACAAUAUGUGUUCCAGAUCGGAC	371	2019-2041
AD-1290719.2	AUUCUGCUUGGCUACAGAAUU	186	2224-2244	AAUUCUGUAGCCAAGCAGAAUUG	410	2222-2244
AD-1290722.2	UCUGCUUGGCUACAGAAUUAU	187	2226-2246	AUAAUUCUGUAGCCAAGCAGAAU	411	2224-2246
AD-1290741.2	ACGCAAUCUGCCUCAAUUUCU	173	2183-2203	AGAAAUUGAGGCAGAUUGCGUUA	397	2181-2203
AD-1290742.2	GUGGGUAAGGCCUUAUAAUGU	155	2066-2086	ACAUUAUAAGGCCUUACCCACCC	379	2064-2086
AD-1290747.2	GGAGCCCACCUUGGAAUUAAU	138	1941-1961	AUUAAUUCCAAGGUGGGCUCCAA	362	1939-1961
AD-1290750.2	CCCAGUGAACCUGCCAAAGAU	221	1706-1726	AUCUUUGGCAGGUUCACUGGGUG	445	1704-1726
AD-1290755.2	GGUGGGUAAGGCCUUAUAAUU	154	2065-2085	AAUUAUAAGGCCUUACCCACCCU	378	2063-2085
AD-1290763.2	GUCCGAUCUGGAACACAUAUU	145	2019-2039	AAUAUGUGUUCCAGAUCGGACCU	369	2017-2039
AD-1290764.2	GGUCCGAUCUGGAACACAUAU	144	2018-2038	AUAUGUGUUCCAGAUCGGACCUC	368	2016-2038
AD-1290796.2	UUGGAGCCCACCUUGGAAUUU	225	1939-1959	AAAUUCCAAGGUGGGCUCCAAGG	449	1937-1959
AD-1290800.2	GGGUGGGUAAGGCCUUAUAAU	153	2064-2084	AUUAUAAGGCCUUACCCACCCUA	377	2062-2084
AD-1290805.2	UGGAGCCCACCUUGGAAUUAU	227	1940-1960	AUAAUUCCAAGGUGGGCUCCAAG	451	1938-1960
AD-1290836.2	AAUUCUGCUUGGCUACAGAAU	185	2223-2243	AUUCUGUAGCCAAGCAGAAUUGG	409	2221-2243
AD-1290837.5	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUUGAACUGGGUCAGAUCAAC	300	769-791
AD-1290841.2	AUUCCCACAGCUCAGAAGCUU	136	1767-1787	AAGCUUCUGAGCUGUGGGAAUAG	360	1765-1787

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1290842.2	GAGCCCACCUUGGAAUUAAGU	139	1942-1962	ACUUAAUUCCAAGGUGGGCUCCA	363	1940-1962
AD-1290857.2	UGCCCACCAGCCUGUGAUUUU	229	1852-1872	AAAAUCACAGGCUGGUGGGCAGG	453	1850-1872
AD-1290865.2	CUGCGUUGUGCAGACUCUAUU	130	1749-1769	AAUAGAGUCUGCACAACGCAGGG	354	1747-1769
AD-1290875.2	GCCCACCAGCCUGUGAUUUGU	231	1853-1873	ACAAAUCACAGGCUGGUGGGCAG	455	1851-1873
AD-1290880.2	CUUGGAGCCCACCUUGGAAUU	137	1938-1958	AAUUCCAAGGUGGGCUCCAAGGG	361	1936-1958
AD-1290884.5	GCAGGAAGCACUGAGAUUCGU	116	1209-1229	ACGAAUCUCAGUGCUUCCUGCAC	340	1207-1229
AD-1290885.5	CUGACCCAGUUCAAGUGGAUU	78	775-795	AAUCCACUUGAACUGGGUCAGAU	302	773-795
AD-1290894.2	AGGUCCGAUCUGGAACACAUU	233	2017-2037	AAUGUGUUCCAGAUCGGACCUCC	457	2015-2037
AD-1290897.2	UGCGUUGUGCAGACUCUAUUU	131	1750-1770	AAAUAGAGUCUGCACAACGCAGG	355	1748-1770
AD-1290908.2	CCAGUGAACCUGCCAAAGAAU	235	1707-1727	AUUCUUUGGCAGGUUCACUGGGU	459	1705-1727
AD-1290909.2	UUGUGCAGACUCUAUUCCCAU	134	1754-1774	AUGGGAAUAGAGUCUGCACAACG	358	1752-1774
AD-1290910.2	UAGGGUGGGUAAGGCCUUAUU	236	2062-2082	AAUAAGGCCUUACCCACCCUAUA	460	2060-2082
AD-1290911.2	AGCCCACCUUGGAAUUAAGGU	140	1943-1963	ACCUUAAUUCCAAGGUGGGCUCC	364	1941-1963
AD-1290926.2	GCCCACCUUGGAAUUAAGGGU	141	1944-1964	ACCCUUAAUUCCAAGGUGGGCUC	365	1942-1964
AD-1290931.2	UCAGCCACAAAUGUGACCCAU	143	1970-1990	AUGGGUCACAUUUGUGGCUGAGG	367	1968-1990
AD-1290939.2	AGGGUGGGUAAGGCCUUAUAU	238	2063-2083	AUAUAAGGCCUUACCCACCCUAU	462	2061-2083
AD-1290969.7	ACCUUCAAUGCCUCCGUCAUU	108	1162-1182	AAUGACGGAGGCAUUGAAGGUGU	332	1160-1182
AD-1290971.3	GCUACGGAGACGUGGUGUUUU	242	938-958	AAAACACCACGUCUCCGUAGCCA	466	936-958
AD-1290973.2	GUUGUGCAGACUCUAUUCCCU	243	1753-1773	AGGGAAUAGAGUCUGCACAACGC	467	1751-1773
AD-1290983.2	CGUUGUGCAGACUCUAUUCCU	133	1752-1772	AGGAAUAGAGUCUGCACAACGCA	357	1750-1772
AD-1290989.2	GCGUUGUGCAGACUCUAUUCU	132	1751-1771	AGAAUAGAGUCUGCACAACGCAG	356	1749-1771
AD-1290993.2	UAUUCCCACAGCUCAGAAGCU	135	1766-1786	AGCUUCUGAGCUGUGGGAAUAGA	359	1764-1786
AD-1291003.2	GGCGUGCCUCAGCCACAAAUU	142	1962-1982	AAUUUGUGGCUGAGGCACGCCCU	366	1960-1982
AD-1423312.3	UGCAGGAAGCACUGAGAUUCU	115	1208-1228	AGAATCTCAGUGCUUCCUGCACG	1141	1206-1228
AD-1423319.3	GACUUUGAGAAGGUUGAUCUU	70	757-777	AAGATCAACCUUCUCAAAGUCUG	1144	755-777
AD-1423336.7	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803
AD-1548743.7	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCTUTGCUGACAAACACCACG	1269	948-970

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1612957.2	GCCUCAUGGAAGAGAAGCAGU	1200	518-538	ACUGCUTCUCUTCCAUGAGGCUA	1270	516-538
AD-1612963.2	UGGAAGAGAAGCAGAUCCUGU	1201	524-544	ACAGGATCUGCTUCUCUUCCAUG	1272	522-544
AD-1612969.2	AGAAGCAGAUCCUGUGCGUGU	1203	530-550	ACACGCACAGGAUCUGCUUCUCU	1274	528-550
AD-1613059.2	CAGACUUUGAGAAGGUUGAUU	68	755-775	AAUCAACCUUCTCAAAGUCUGUA	1276	753-775
AD-1613060.2	AGACUUUGAGAAGGUUGAUCU	69	756-776	AGAUCAACCUUCUCAAAGUCUGU	293	754-776
AD-1613061.1	GACUUUGAGAAGGUUGAUCUU	70	757-777	AAGATCAACCUTCUCAAAGUCUG	1696	755-777
AD-1613062.2	ACUUUGAGAAGGUUGAUCUGU	213	758-778	ACAGAUCAACCTUCUCAAAGUCU	1277	756-778
AD-1613072.1	GGUUGAUCUGACCCAGUUCAU	239	768-788	ATGAACTGGGUCAGAUCAACCUU	1697	766-788
AD-1613075.2	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUTGAACUGGGUCAGAUCAAC	1183	769-791
AD-1613079.2	CUGACCCAGUUCAAGUGGAUU	78	775-795	AAUCCACUUGAACUGGGUCAGAU	302	773-795
AD-1613087.1	GUUCAAGUGGAUCCACAUUGU	207	783-803	ACAATGTGGAUCCACUUGAACUG	1152	781-803
AD-1613094.2	UGGAUCCACAUUGAGGGCCGU	1207	790-810	ACGGCCCUCAATGUGGAUCCACU	1284	788-810
AD-1613242.2	GCUACGGAGACGUGGUGUUUU	242	938-958	AAAACACCACGTCUCCGUAGCCA	1291	936-958
AD-1613254.2	UGGUGUUUGUCAGCAAAGAUU	93	950-970	AAUCTUTGCUGACAAACACCACG	1269	948-970
AD-1613256.2	GUGUUUGUCAGCAAAGAUGUU	95	952-972	AACATCTUUGCTGACAAACACCA	1294	950-972
AD-1613371.3	ACCUUCAAUGCCUCCGUCAUU	108	1162-1182	AAUGACGGAGGCAUUGAAGGUGU	332	1160-1182
AD-1613400.2	GCAGGAAGCACUGAGAUUCGU	116	1209-1229	ACGAAUCUCAGTGCUUCCUGCAC	1304	1207-1229
AD-1684592.1	GUAGCCUCAUGGAAGAGAAGU	1659	515-535	ACUUCUCUUCCAUGAGGCUACUC	1698	513-535
AD-1684593.1	AGCCUCAUGGAAGAGAAGCAU	1660	517-537	AUGCTUCUCUUCCAUGAGGCUAC	1699	515-537
AD-1684594.1	CUCAUGGAAGAGAAGCAGAUU	51	520-540	AAUCTGCUUCUCUUCCAUGAGGC	1700	518-540
AD-1684595.1	UCAUGGAAGAGAAGCAGAUCU	1661	521-541	AGAUCUGCUUCUCUUCCAUGAGG	1701	519-541
AD-1684596.1	AUGGAAGAGAAGCAGAUCCUU	52	523-543	AAGGAUCUGCUUCUCUUCCAUGA	276	521-543
AD-1684597.1	AAGAGAAGCAGAUCCUGUGCU	1662	527-547	AGCACAGGAUCUGCUUCUCUUCC	1702	525-547
AD-1684598.1	GAGAAGCAGAUCCUGUGCGUU	1663	529-549	AACGCACAGGAUCUGCUUCUCUU	1703	527-549
AD-1684599.1	AAGCAGAUCCUGUGCGUGGGU	1664	532-552	ACCCACGCACAGGAUCUGCUUCU	1704	530-552
AD-1684600.1	AAGCAGAUCCUGUGCGUGGGU	1664	532-552	ACCCACGCACAGGAUCUGCUUCU	1704	530-552
AD-1684601.1	AGCAGAUCCUGUGCGUGGGGU	1665	533-553	ACCCCACGCACAGGAUCUGCUUC	1705	531-553

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1684602.1	AGCAGAUCCUGUGCGUGGGGU	1665	533-553	ACCCCACGCACAGGAUCUGCUUC	1705	531-553
AD-1684603.1	GCAGAUCCUGUGCGUGGGGCU	1666	534-554	AGCCCCACGCACAGGAUCUGCUU	1706	532-554
AD-1684604.1	CAGAUCCUGUGCGUGGGGCUU	1667	535-555	AAGCCCCACGCACAGGAUCUGCU	1707	533-555
AD-1684605.1	AGAUCCUGUGCGUGGGGCUAU	1668	536-556	AUAGCCCCACGCACAGGAUCUGC	1708	534-556
AD-1684606.1	CAGACUUUGAGAAGGUUGAUU	68	755-775	AAUCAACCUUCTCAAAGUCUGUG	1709	753-775
AD-1684607.1	CAGACUUUGAGAAGGUUGAUU	68	755-775	AAUCAACCUUCTCAAAGUCUGCU	1710	753-775
AD-1684608.1	CAGACUUUGAGAAGGUUGAUA	1669	755-775	UAUCAACCUUCTCAAAGUCUGCU	1711	753-775
AD-1684609.1	GACUUUGAGAAGGUUGAUU	1670	757-775	AAUCAACCUUCTCAAAGUCUG	1712	755-775
AD-1684610.1	GACUUUGAGAAGGUUGAUCUU	70	757-777	AAGATCAACCUTCUCAAAGUCUG	1696	755-777
AD-1684611.1	UUUGAGAAGGUUGAUCUGU	1671	760-778	ACAGAUCAACCTUCUCAAAGU	1713	758-778
AD-1684612.1	ACUUUAAGAAGGUUGAUCUGU	1672	758-778	ACAGAUCAACCTUCUUAAAGUCU	1714	756-778
AD-1684613.1	UUGAGAAGGUUGAUCUGACCU	1673	761-781	AGGUCAGAUCAACCUUCUCAAAG	1715	759-781
AD-1684614.1	GAGAAGGUUGAUCUGACCCAU	1674	763-783	AUGGGUCAGAUCAACCUUCUCAA	1716	761-783
AD-1684615.1	GAAGGUUGAUCUGACCCAGUU	1675	765-785	AACUGGGUCAGAUCAACCUUCUC	1717	763-785
AD-1684616.1	AGGUUGAUCUGACCCAGUUCU	1676	767-787	AGAACUGGGUCAGAUCAACCUUC	1718	765-787
AD-1684617.1	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUTGAACUGGGUCAGAUCAGC	1719	769-791
AD-1684618.1	UGAUCUGACCCAGUUCAAGUU	76	771-791	AACUTGAACUGGGUCAGAUCACU	1720	769-791
AD-1684619.1	UGAUCUGACCCAGUUCAAGUA	1677	771-791	UACUTGAACUGGGUCAGAUCACU	1721	769-791
AD-1684620.1	AUCUGACCCAGUUCAAGUU	1678	773-791	AACUTGAACUGGGUCAGAUCG	1722	771-791
AD-1684621.1	GACCCAGUUCAAGUGGAUCCU	1679	777-797	AGGATCCACUUGAACUGGGUCAG	1723	775-797
AD-1684622.1	ACCCAGUUCAAGUGGAUCCAU	79	778-798	AUGGAUCCACUUGAACUGGGUCA	303	776-798
AD-1684623.1	CAGUUCAAGUGGAUCCACAUU	23	781-801	AAUGTGGAUCCACUUGAACUGGG	1724	779-801
AD-1684624.1	AGUUCAAGUGGAUCCACAUUU	81	782-802	AAAUGUGGAUCCACUUGAACUGG	305	780-802
AD-1684625.1	AGUGGAUCCACAUUGAGGGCU	1680	788-808	AGCCCUCAAUGUGGAUCCACUUG	1725	786-808
AD-1684626.1	UGGAUCCACAUUGAGGGCCGU	1207	790-810	ACGGCCCUCAAUGUGGAUCCACU	1726	788-810
AD-1684627.1	GAUCCACAUUGAGGGCCGGAU	1681	792-812	AUCCGGCCCUCAAUGUGGAUCCA	1727	790-812
AD-1684628.1	AUCCACAUUGAGGGCCGGAAU	1682	793-813	AUUCCGGCCCUCAAUGUGGAUCC	1728	791-813

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1684629.1	GAGACGUGGUGUUUGUCAGCU	1683	944-964	AGCUGACAAACACCACGUCUCCG	1729	942-964
AD-1684630.1	CGUGGUGUUUGUCAGCAAAGU	219	948-968	ACUUTGCUGACAAACACCACGUC	1730	946-968
AD-1684631.1	UUGUCAGCAAAGAUGUGGCCU	1684	956-976	AGGCCACAUCUUUGCUGACAAAC	1731	954-976
AD-1684632.1	GUCAGCAAAGAUGUGGCCAAU	1685	958-978	AUUGGCCACAUCUUUGCUGACAA	1732	956-978
AD-1684633.1	UCAGCAAAGAUGUGGCCAAGU	1686	959-979	ACUUGGCCACAUCUUUGCUGACA	1733	957-979
AD-1684634.1	CAGCAAAGAUGUGGCCAAGCU	1687	960-980	AGCUTGGCCACAUCUUUGCUGAC	1734	958-980
AD-1684635.1	AGCAAAGAUGUGGCCAAGCAU	1688	961-981	AUGCTUGGCCACAUCUUUGCUGA	1735	959-981
AD-1684636.1	GAGACACCUUCAAUGCCUCCU	1689	1157-1177	AGGAGGCAUUGAAGGUGUCUCCA	1736	1155-1177
AD-1684637.1	AGACACCUUCAAUGCCUCCGU	1690	1158-1178	ACGGAGGCAUUGAAGGUGUCUCC	1737	1156-1178
AD-1684638.1	GACACCUUCAAUGCCUCCGUU	1691	1159-1179	AACGGAGGCAUUGAAGGUGUCUC	1738	1157-1179
AD-1684639.1	CCUUCAAUGCCUCCGUCAUCU	109	1163-1183	AGAUGACGGAGGCAUUGAAGGUG	333	1161-1183
AD-1684640.1	GCCUCCGUCAUCUUCAGCCUU	1692	1171-1191	AAGGCUGAAGAUGACGGAGGCAU	1739	1169-1191
AD-1684641.1	CUCCGUCAUCUUCAGCCUCUU	113	1173-1193	AAGAGGCUGAAGAUGACGGAGGC	337	1171-1193
AD-1684642.1	GGAGCGUGCAGGAAGCACUGU	1693	1202-1222	ACAGTGCUUCCUGCACGCUCCUC	1740	1200-1222
AD-1684643.1	GAGCGUGCAGGAAGCACUGAU	1694	1203-1223	AUCAGUGCUUCCUGCACGCUCCU	1741	1201-1223
AD-1684644.1	GCGUGCAGGAAGCACUGAGAU	1695	1205-1225	AUCUCAGUGCUUCCUGCACGCUC	1742	1203-1225
AD-1684645.1	GUGCAGGAAGCACUGAGAUUU	114	1207-1227	AAAUCUCAGUGCUUCCUGCACGC	338	1205-1227
AD-1684646.1	CCCAGUGAACCUGCCAAAGAU	221	1706-1726	ATCUTUGGCAGGUUCACUGGGUG	1743	1704-1726
AD-1684647.1	CCAGUGAACCUGCCAAAGAAU	235	1707-1727	ATUCTUTGGCAGGUUCACUGGGU	1744	1705-1727
AD-1684648.1	CUGCGUUGUGCAGACUCUAUU	130	1749-1769	AAUAGAGUCUGCACAACGCAGGG	354	1747-1769
AD-1684649.1	UGCGUUGUGCAGACUCUAUUU	131	1750-1770	AAAUAGAGUCUGCACAACGCAGG	355	1748-1770
AD-1684650.1	GCGUUGUGCAGACUCUAUUCU	132	1751-1771	AGAATAGAGUCTGCACAACGCAG	1745	1749-1771
AD-1684651.1	CGUUGUGCAGACUCUAUUCCU	133	1752-1772	AGGAAUAGAGUCUGCACAACGCA	357	1750-1772
AD-1684652.1	GUUGUGCAGACUCUAUUCCCU	243	1753-1773	AGGGAATAGAGTCUGCACAACGC	1746	1751-1773
AD-1684653.1	UUGUGCAGACUCUAUUCCCAU	134	1754-1774	ATGGGAAUAGAGUCUGCACAACG	1747	1752-1774
AD-1684654.1	UAUUCCCACAGCUCAGAAGCU	135	1766-1786	AGCUTCTGAGCTGUGGGAAUAGA	1748	1764-1786
AD-1684655.1	AUUCCCACAGCUCAGAAGCUU	136	1767-1787	AAGCTUCUGAGCUGUGGGAAUAG	1749	1765-1787

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1684656.1	UGCCCACCAGCCUGUGAUUUU	229	1852-1872	AAAATCACAGGCUGGUGGGCAGG	1750	1850-1872
AD-1684657.1	GCCCACCAGCCUGUGAUUUGU	231	1853-1873	ACAAAUCACAGGCUGGUGGGCAG	455	1851-1873
AD-1684658.1	CUUGGAGCCCACCUUGGAAUU	137	1938-1958	AAUUCCAAGGUGGGCUCCAAGGG	361	1936-1958
AD-1684659.1	UUGGAGCCCACCUUGGAAUUU	225	1939-1959	AAAUTCCAAGGTGGGCUCCAAGG	1751	1937-1959
AD-1684660.1	UGGAGCCCACCUUGGAAUUAU	227	1940-1960	ATAATUCCAAGGUGGGCUCCAAG	1752	1938-1960
AD-1684661.1	GGAGCCCACCUUGGAAUUAAU	138	1941-1961	ATUAAUTCCAAGGUGGGCUCCAA	1753	1939-1961
AD-1684662.1	GAGCCCACCUUGGAAUUAAGU	139	1942-1962	ACUUAATUCCAAGGUGGGCUCCA	1754	1940-1962
AD-1684663.1	AGCCCACCUUGGAAUUAAGGU	140	1943-1963	ACCUTAAUUCCAAGGUGGGCUCC	1755	1941-1963
AD-1684664.1	GCCCACCUUGGAAUUAAGGGU	141	1944-1964	ACCCTUAAUUCCAAGGUGGGCUC	1756	1942-1964
AD-1684665.1	GGCGUGCCUCAGCCACAAAUU	142	1962-1982	AAUUTGTGGCUGAGGCACGCCCU	1757	1960-1982
AD-1684666.1	UCAGCCACAAAUGUGACCCAU	143	1970-1990	ATGGGUCACAUTUGUGGCUGAGG	1758	1968-1990
AD-1684667.1	AGGUCCGAUCUGGAACACAUU	233	2017-2037	AAUGTGTUCCAGAUCGGACCUCC	1759	2015-2037
AD-1684668.1	GGUCCGAUCUGGAACACAUAU	144	2018-2038	ATAUGUGUUCCAGAUCGGACCUC	1760	2016-2038
AD-1684669.1	GUCCGAUCUGGAACACAUAUU	145	2019-2039	AAUATGTGUUCCAGAUCGGACCU	1761	2017-2039
AD-1684670.1	UCCGAUCUGGAACACAUAUUU	146	2020-2040	AAAUAUGUGUUCCAGAUCGGACC	370	2018-2040
AD-1684671.1	CCGAUCUGGAACACAUAUUGU	147	2021-2041	ACAATATGUGUTCCAGAUCGGAC	1762	2019-2041
AD-1684672.1	CGAUCUGGAACACAUAUUGGU	217	2022-2042	ACCAAUAUGUGTUCCAGAUCGGA	1763	2020-2042
AD-1684673.1	GAUCUGGAACACAUAUUGGAU	209	2023-2043	ATCCAATAUGUGUUCCAGAUCGG	1764	2021-2043
AD-1684674.1	AUCUGGAACACAUAUUGGAAU	148	2024-2044	ATUCCAAUAUGTGUUCCAGAUCG	1765	2022-2044
AD-1684675.1	UCUGGAACACAUAUUGGAAUU	149	2025-2045	AAUUCCAAUAUGUGUUCCAGAUC	373	2023-2045
AD-1684676.1	CUGGAACACAUAUUGGAAUUU	150	2026-2046	AAAUTCCAAUATGUGUUCCAGAU	1766	2024-2046
AD-1684677.1	UGGAACACAUAUUGGAAUUGU	151	2027-2047	ACAATUCCAAUAUGUGUUCCAGA	1767	2025-2047
AD-1684678.1	GGAACACAUAUUGGAAUUGGU	152	2028-2048	ACCAAUTCCAATAUGUGUUCCAG	1768	2026-2048
AD-1684679.1	UAGGGUGGGUAAGGCCUUAUU	236	2062-2082	AAUAAGGCCUUACCCACCCUAUA	460	2060-2082
AD-1684680.1	AGGGUGGGUAAGGCCUUAUAU	238	2063-2083	ATAUAAGGCCUTACCCACCCUAU	1769	2061-2083
AD-1684681.1	GGGUGGGUAAGGCCUUAUAAU	153	2064-2084	ATUATAAGGCCTUACCCACCCUA	1770	2062-2084
AD-1684682.1	GGUGGGUAAGGCCUUAUAAUU	154	2065-2085	AAUUAUAAGGCCUUACCCACCCU	378	2063-2085

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1684683.1	GUGGGUAAGGCCUUAUAAUGU	155	2066-2086	ACAUTATAAGGCCUUACCCACCC	1771	2064-2086
AD-1684684.1	UGGGUAAGGCCUUAUAAUGUU	215	2067-2087	AACATUAUAAGGCCUUACCCACC	1772	2065-2087
AD-1684685.1	GGGUAAGGCCUUAUAAUGUAU	208	2068-2088	ATACAUTAUAAGGCCUUACCCAC	1773	2066-2088
AD-1684686.1	GGUAAGGCCUUAUAAUGUAAU	205	2069-2089	ATUACATUAUAAGGCCUUACCCA	1774	2067-2089
AD-1684687.1	GUAAGGCCUUAUAAUGUAAAU	156	2070-2090	ATUUACAUUAUAAGGCCUUACCC	1775	2068-2090
AD-1684688.1	UAAGGCCUUAUAAUGUAAAGU	201	2071-2091	ACUUTACAUUATAAGGCCUUACC	1776	2069-2091
AD-1684689.1	AAGGCCUUAUAAUGUAAAGAU	157	2072-2092	ATCUTUACAUUAUAAGGCCUUAC	1777	2070-2092
AD-1684690.1	AGGCCUUAUAAUGUAAAGAGU	158	2073-2093	ACUCTUTACAUTAUAAGGCCUUA	1778	2071-2093
AD-1684691.1	GGCCUUAUAAUGUAAAGAGCU	196	2074-2094	AGCUCUTUACATUAUAAGGCCUU	1779	2072-2094
AD-1684692.1	GCCUUAUAAUGUAAAGAGCAU	159	2075-2095	ATGCTCTUUACAUUAUAAGGCCU	1780	2073-2095
AD-1684693.1	GCAUAUAAUGUAAAGGGCUUU	160	2092-2112	AAAGCCCUUUACAUUAUAUGCUC	384	2090-2112
AD-1684694.1	CAUAUAAUGUAAAGGGCUUUU	202	2093-2113	AAAAGCCCUUUACAUUAUAUGCU	426	2091-2113
AD-1684695.1	AUAUAAUGUAAAGGGCUUUAU	161	2094-2114	ATAAAGCCCUUTACAUUAUAUGC	1781	2092-2114
AD-1684696.1	UAUAAUGUAAAGGGCUUUAGU	197	2095-2115	ACUAAAGCCCUTUACAUUAUAUG	1782	2093-2115
AD-1684697.1	AUAAUGUAAAGGGCUUUAGAU	162	2096-2116	ATCUAAAGCCCTUUACAUUAUAU	1783	2094-2116
AD-1684698.1	UAAUGUAAAGGGCUUUAGAGU	163	2097-2117	ACUCTAAAGCCCUUUACAUUAUA	1784	2095-2117
AD-1684699.1	AAUGUAAAGGGCUUUAGAGUU	164	2098-2118	AACUCUAAAGCCCUUUACAUUAU	388	2096-2118
AD-1684700.1	CCUGGAUUAAAAUCUGCCAUU	165	2126-2146	AAUGGCAGAUUTUAAUCCAGGUC	1785	2124-2146
AD-1684701.1	CUGGAUUAAAAUCUGCCAUUU	166	2127-2147	AAAUGGCAGAUTUUAAUCCAGGU	1786	2125-2147
AD-1684702.1	UGGAUUAAAAUCUGCCAUUUU	193	2128-2148	AAAATGGCAGATUUUAAUCCAGG	1787	2126-2148
AD-1684703.1	GGAUUAAAAUCUGCCAUUUAU	192	2129-2149	ATAAAUGGCAGAUUUUAAUCCAG	1788	2127-2149
AD-1684704.1	GAUUAAAAUCUGCCAUUUAAU	167	2130-2150	ATUAAATGGCAGAUUUUAAUCCA	1789	2128-2150
AD-1684705.1	AUUAAAAUCUGCCAUUUAAUU	168	2131-2151	AAUUAAAUGGCAGAUUUUAAUCC	392	2129-2151
AD-1684706.1	AAAUCUGCCAUUUAAUUAGCU	169	2135-2155	AGCUAATUAAATGGCAGAUUUUA	1790	2133-2155
AD-1684707.1	AAUCUGCCAUUUAAUUAGCUU	170	2136-2156	AAGCTAAUUAAAUGGCAGAUUUU	1791	2134-2156
AD-1684708.1	AUCUGCCAUUUAAUUAGCUGU	171	2137-2157	ACAGCUAAUUAAAUGGCAGAUUU	395	2135-2157
AD-1684709.1	UCUGCCAUUUAAUUAGCUGCU	218	2138-2158	AGCAGCTAAUUAAAUGGCAGAUU	1792	2136-2158

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1684710.1	CUGCCAUUUAAUUAGCUGCAU	172	2139-2159	ATGCAGCUAAUTAAAUGGCAGAU	1793	2137-2159
AD-1684711.1	UGCCAUUUAAUUAGCUGCAUU	203	2140-2160	AAUGCAGCUAATUAAAUGGCAGA	1794	2138-2160
AD-1684712.1	ACGCAAUCUGCCUCAAUUUCU	173	2183-2203	AGAAAUTGAGGCAGAUUGCGUUA	1795	2181-2203
AD-1684713.1	CGCAAUCUGCCUCAAUUUCUU	174	2184-2204	AAGAAATUGAGGCAGAUUGCGUU	1796	2182-2204
AD-1684714.1	GCAAUCUGCCUCAAUUUCUUU	175	2185-2205	AAAGAAAUUGAGGCAGAUUGCGU	399	2183-2205
AD-1684715.1	CAAUCUGCCUCAAUUUCUUCU	211	2186-2206	AGAAGAAAUUGAGGCAGAUUGCG	435	2184-2206
AD-1684716.1	AAUCUGCCUCAAUUUCUUCAU	176	2187-2207	ATGAAGAAAUUGAGGCAGAUUGC	1797	2185-2207
AD-1684717.1	AUCUGCCUCAAUUUCUUCAUU	177	2188-2208	AAUGAAGAAAUTGAGGCAGAUUG	1798	2186-2208
AD-1684718.1	UCUGCCUCAAUUUCUUCAUCU	178	2189-2209	AGAUGAAGAAATUGAGGCAGAUU	1799	2187-2209
AD-1684719.1	CUGCCUCAAUUUCUUCAUCUU	179	2190-2210	AAGATGAAGAAAUUGAGGCAGAU	1800	2188-2210
AD-1684720.1	UGCCUCAAUUUCUUCAUCUGU	180	2191-2211	ACAGAUGAAGAAAUUGAGGCAGA	404	2189-2211
AD-1684721.1	GCCUCAAUUUCUUCAUCUGUU	181	2192-2212	AACAGATGAAGAAAUUGAGGCAG	1801	2190-2212
AD-1684722.1	CCUCAAUUUCUUCAUCUGUCU	199	2193-2213	AGACAGAUGAAGAAAUUGAGGCA	423	2191-2213
AD-1684723.1	CUCAAUUUCUUCAUCUGUCAU	200	2194-2214	ATGACAGAUGAAGAAAUUGAGGC	1802	2192-2214
AD-1684724.1	UCAAUUUCUUCAUCUGUCAAU	194	2195-2215	ATUGACAGAUGAAGAAAUUGAGG	1803	2193-2215
AD-1684725.1	CAAUUUCUUCAUCUGUCAAAU	182	2196-2216	ATUUGACAGAUGAAGAAAUUGAG	1804	2194-2216
AD-1684726.1	AAUUUCUUCAUCUGUCAAAUU	183	2197-2217	AAUUTGACAGATGAAGAAAUUGA	1805	2195-2217
AD-1684727.1	AUUUCUUCAUCUGUCAAAUGU	184	2198-2218	ACAUTUGACAGAUGAAGAAAUUG	1806	2196-2218
AD-1684728.1	UUUCUUCAUCUGUCAAAUGGU	195	2199-2219	ACCATUTGACAGAUGAAGAAAUU	1807	2197-2219
AD-1684729.1	UUCUUCAUCUGUCAAAUGGAU	191	2200-2220	ATCCAUTUGACAGAUGAAGAAAU	1808	2198-2220
AD-1684730.1	AAUUCUGCUUGGCUACAGAAU	185	2223-2243	ATUCTGTAGCCAAGCAGAAUUGG	1809	2221-2243
AD-1684731.1	AUUCUGCUUGGCUACAGAAUU	186	2224-2244	AAUUCUGUAGCCAAGCAGAAUUG	410	2222-2244
AD-1684732.1	UUCUGCUUGGCUACAGAAUUU	206	2225-2245	AAAUTCTGUAGCCAAGCAGAAUU	1810	2223-2245
AD-1684733.1	UCUGCUUGGCUACAGAAUUAU	187	2226-2246	ATAATUCUGUAGCCAAGCAGAAU	1811	2224-2246
AD-1684734.1	CUGCUUGGCUACAGAAUUAUU	188	2227-2247	AAUAAUTCUGUAGCCAAGCAGAA	1812	2225-2247
AD-1684735.1	UGCUUGGCUACAGAAUUAUUU	189	2228-2248	AAAUAATUCUGTAGCCAAGCAGA	1813	2226-2248
AD-1684736.1	GCUUGGCUACAGAAUUAUUGU	190	2229-2249	ACAATAAUUCUGUAGCCAAGCAG	1814	2227-2249

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in XM_017004061.1
AD-1684737.1	AUUAUUGUGAGGAUAAAAUCU	204	2242-2262	AGAUTUTAUCCTCACAAUAAUUC	1815	2240-2262

Table 13. Modified Sense and Antisense Strand Sequences of KHK dsRNA

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1290507.2	ususcuucAfuCfUfGfucaaauggauL96	1816	asUfsccaUfuugacagAfuGfaagaasasu	863	AUUUCUUCAUCUGUCAAAUGGAA	1087
AD-1290509.2	gsasuuaaAfaUfCfUfgccauuuaauL96	615	asUfsuaaAfuggcagaUfuUfuaaucscsa	839	UGGAUUAAAAUCUGCCAUUUAAU	1063
AD-1290510.2	asasuuucUfuCfAfUfcugucaaauuL96	631	asAfsuuuGfacagaugAfaGfaaauusgsa	855	UCAAUUUCUUCAUCUGUCAAAUG	1079
AD-1290514.2	csasauuuCfuUfCfAfucugucaaauL96	630	asUfsuugAfcagaugaAfgAfaauugsasg	854	CUCAAUUUCUUCAUCUGUCAAAU	1078
AD-1290515.2	asgsgccuUfaUfAfAfuguaaagaguL96	606	asCfsucuUfuacauuaUfaAfggccususa	830	UAAGGCCUUAUAAUGUAAAGAGC	1054
AD-1290516.2	gsgsauuaAfaAfUfCfugccauuuauL96	640	asUfsaaaUfggcagauUfuUfaauccsasg	864	CUGGAUUAAAAUCUGCCAUUUAA	1088
AD-1290522.2	uscsuggaAfcAfCfAfuauuggaauuL96	597	asAfsuucCfaauauguGfuUfccagasusc	821	GAUCUGGAACACAUAUUGGAAUU	1045
AD-1290523.2	gscscucaAfuUfUfCfuucaucuguuL96	629	asAfscagAfugaagaaAfuUfgaggcsasg	853	CUGCCUCAAUUUCUUCAUCUGUC	1077
AD-1290524.2	asusuucuUfcAfUfCfugucaaauguL96	632	asCfsauuUfgacagauGfaAfgaaaususg	856	CAAUUUCUUCAUCUGUCAAAUGG	1080
AD-1290527.2	usgsgauuAfaAfAfUfcugccauuuuL96	641	asAfsaauGfgcagauuUfuAfauccasgsg	865	CCUGGAUUAAAAUCUGCCAUUUA	1089
AD-1290528.2	csusggaaCfaCfAfUfauuggaauuuL96	598	asAfsauuCfcaauaugUfgUfuccagsasu	822	AUCUGGAACACAUAUUGGAAUUG	1046
AD-1290531.2	csusgccuCfaAfUfUfucuucaucuuL96	627	asAfsgauGfaagaaauUfgAfggcagsasu	851	AUCUGCCUCAAUUUCUUCAUCUG	1075
AD-1290533.2	asasaucuGfcCfAfUfuuaauuagcuL96	617	asGfscuaAfuuaaaugGfcAfgauuususa	841	UAAAAUCUGCCAUUUAAUUAGCU	1065
AD-1290535.2	asasucugCfcAfUfUfuaauuagcuuL96	618	asAfsgcuAfauuaaauGfgCfagauususu	842	AAAAUCUGCCAUUUAAUUAGCUG	1066
AD-1290539.5	csasgacuUfuGfAfGfaagguugauuL96	516	asAfsucaAfccuucucAfaAfgucugsusa	740	UACAGACUUUGAGAAGGUUGAUC	964
AD-1290542.2	uscsaauuUfcUfUfCfaucugucaauL96	642	asUfsugaCfagaugaaGfaAfauugasgsg	866	CCUCAAUUUCUUCAUCUGUCAAA	1090
AD-1290543.2	usgsgaacAfcAfUfAfuuggaauuguL96	599	asCfsaauUfccaauauGfuGfuuccasgsa	823	UCUGGAACACAUAUUGGAAUUGG	1047
AD-1290551.2	csusggauUfaAfAfAfucugccauuuL96	614	asAfsaugGfcagauuuUfaAfuccagsgsu	838	ACCUGGAUUAAAAUCUGCCAUUU	1062
AD-1290552.2	ususucuuCfaUfCfUfgucaaaugguL96	643	asCfscauUfugacagaUfgAfagaaasusu	867	AAUUUCUUCAUCUGUCAAAUGGA	1091
AD-1290554.2	asusaaugUfaAfAfGfggcuuuagauL96	610	asUfscuaAfagcccuuUfaCfauuausasu	834	AUAUAAUGUAAAGGGCUUUAGAG	1058

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1290555.2	asusauaaUfgUfAfAfagggcuuuauL96	609	asUfsaaaGfcccuuuaCfaUfuauausgsc	833	GCAUAUAAUGUAAAGGGCUUUAG	1057
AD-1290556.2	gscscuuaUfaAfUfGfuaaagagcauL96	607	asUfsgcuCfuuuacauUfaUfaaggcscsu	831	AGGCCUUAUAAUGUAAAGAGCAU	1055
AD-1290557.2	gsgsccuuAfuAfAfUfguaaagagcuL96	644	asGfscucUfuuacauuAfuAfaggccsusu	868	AAGGCCUUAUAAUGUAAAGAGCA	1092
AD-1290558.2	usasuaauGfuAfAfAfgggcuuuaguL96	645	asCfsuaaAfgcccuuuAfcAfuuauasusg	869	CAUAUAAUGUAAAGGGCUUUAGA	1093
AD-1290563.2	gsusaaggCfcUfUfAfuaauguaaauL96	604	asUfsuuaCfauuauaaGfgCfcuuacscsc	828	GGGUAAGGCCUUAUAAUGUAAAG	1052
AD-1290564.2	cscsucaaUfuUfCfUfucaucugucuL96	647	asGfsacaGfaugaagaAfaUfugaggscsa	871	UGCCUCAAUUUCUUCAUCUGUCA	1095
AD-1290565.2	csuscaauUfuCfUfUfcaucugucauL96	648	asUfsgacAfgaugaagAfaAfuugagsgsc	872	GCCUCAAUUUCUUCAUCUGUCAA	1096
AD-1290570.2	asasggccUfuAfUfAfauguaaagauL96	605	asUfscuuUfacauuauAfaGfgccuusasc	829	GUAAGGCCUUAUAAUGUAAAGAG	1053
AD-1290573.2	asuscugcCfuCfAfAfuuucuucauuL96	625	asAfsugaAfgaaauugAfgGfcagaususg	849	CAAUCUGCCUCAAUUUCUUCAUC	1073
AD-1290574.2	usasaggcCfuUfAfUfaauguaaaguL96	649	asCfsuuuAfcauuauaAfgGfccuuascsc	873	GGUAAGGCCUUAUAAUGUAAAGA	1097
AD-1290584.2	gsusguuuGfuCfAfGfcaaagauguuL96	543	asAfscauCfuuugcugAfcAfaacacscsa	767	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1290589.2	gsgsaacaCfaUfAfUfuggaauugguL96	600	asCfscaaUfuccaauaUfgUfguuccsasg	824	CUGGAACACAUAUUGGAAUUGGG	1048
AD-1290592.2	csasuauaAfuGfUfAfaagggcuuuuL96	650	asAfsaagCfccuuuacAfuUfauaugscsu	874	AGCAUAUAAUGUAAAGGGCUUUA	1098
AD-1290597.2	asusuaaaAfuCfUfGfccauuuaauuL96	616	asAfsuuaAfauggcagAfuUfuuaauscsc	840	GGAUUAAAAUCUGCCAUUUAAUU	1064
AD-1290599.7	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucuUfugcugacAfaAfcaccascsg	765	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1290600.2	gscsuuggCfuAfCfAfgaauuauuguL96	638	asCfsaauAfauucuguAfgCfcaagcsasg	862	CUGCUUGGCUACAGAAUUAUUGU	1086
AD-1290602.2	usgsccucAfaUfUfUfcuucaucuguL96	628	asCfsagaUfgaagaaaUfuGfaggcasgsa	852	UCUGCCUCAAUUUCUUCAUCUGU	1076
AD-1290604.2	asuscugcCfaUfUfUfaauuagcuguL96	619	asCfsagcUfaauuaaaUfgGfcagaususu	843	AAAUCUGCCAUUUAAUUAGCUGC	1067
AD-1290605.2	asasucugCfcUfCfAfauuucuucauL96	624	asUfsgaaGfaaauugaGfgCfagauusgsc	848	GCAAUCUGCCUCAAUUUCUUCAU	1072
AD-1290609.2	usgsccauUfuAfAfUfuagcugcauuL96	651	asAfsugcAfgcuaauuAfaAfuggcasgsa	875	UCUGCCAUUUAAUUAGCUGCAUA	1099
AD-1290611.3	asgsacuuUfgAfGfAfagguugaucuL96	517	asGfsaucAfaccuucuCfaAfagucusgsu	741	ACAGACUUUGAGAAGGUUGAUCU	965

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1290612.2	asuscuggAfaCfAfCfauauuggaauL96	596	asUfsuccAfauaugugUfuCfcagauscsg	820	CGAUCUGGAACACAUAUUGGAAU	1044
AD-1290615.2	uscsugccUfcAfAfUfuucuucaucuL96	626	asGfsaugAfagaaauuGfaGfgcagasusu	850	AAUCUGCCUCAAUUUCUUCAUCU	1074
AD-1290618.2	asasuguaAfaGfGfGfcuuuagaguuL96	612	asAfscucUfaaagcccUfuUfacauusasu	836	AUAAUGUAAAGGGCUUUAGAGUG	1060
AD-1290624.2	asusuauuGfuGfAfGfgauaaaaucuL96	652	asGfsauuUfuauccucAfcAfauaaususc	876	GAAUUAUUGUGAGGAUAAAAUCA	1100
AD-1290626.2	gsgsuaagGfcCfUfUfauaauguaauL96	653	asUfsuacAfuuauaagGfcCfuuaccscsa	877	UGGGUAAGGCCUUAUAAUGUAAA	1101
AD-1290633.2	csusgccaUfuUfAfAfuuagcugcauL96	620	asUfsgcaGfcuaauuaAfaUfggcagsasu	844	AUCUGCCAUUUAAUUAGCUGCAU	1068
AD-1290635.2	ususcugcUfuGfGfCfuacagaauuuL96	654	asAfsauuCfuguagccAfaGfcagaasusu	878	AAUUCUGCUUGGCUACAGAAUUA	1102
AD-1290639.2	usasauguAfaAfGfGfgcuuuagaguL96	611	asCfsucuAfaagcccuUfuAfcauuasusa	835	UAUAAUGUAAAGGGCUUUAGAGU	1059
AD-1290643.2	usgscuugGfcUfAfCfagaauuauuuL96	637	asAfsauaAfuucuguaGfcCfaagcasgsa	861	UCUGCUUGGCUACAGAAUUAUUG	1085
AD-1290650.2	csgscaauCfuGfCfCfucaauuucuuL96	622	asAfsgaaAfuugaggcAfgAfuugcgsusu	846	AACGCAAUCUGCCUCAAUUUCUU	1070
AD-1290651.2	gsusucaaGfuGfGfAfuccacauuguL96	655	asCfsaauGfuggauccAfcUfugaacsusg	879	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1290654.2	gsgsguaaGfgCfCfUfuauaauguauL96	656	asUfsacaUfuauaaggCfcUfuacccsasc	880	GUGGGUAAGGCCUUAUAAUGUAA	1104
AD-1290655.2	gsasucugGfaAfCfAfcauauuggauL96	657	asUfsccaAfuauguguUfcCfagaucsgsg	881	CCGAUCUGGAACACAUAUUGGAA	1105
AD-1290659.2	csasaucuGfcCfUfCfaauuucuucuL96	659	asGfsaagAfaauugagGfcAfgauugscsg	883	CGCAAUCUGCCUCAAUUUCUUCA	1107
AD-1290660.2	cscsuggaUfuAfAfAfaucugccauuL96	613	asAfsuggCfagauuuuAfaUfccaggsusc	837	GACCUGGAUUAAAAUCUGCCAUU	1061
AD-1290661.2	gscsauauAfaUfGfUfaaagggcuuuL96	608	asAfsagcCfcuuuacaUfuAfuaugcsusc	832	GAGCAUAUAAUGUAAAGGGCUUU	1056
AD-1290666.2	ascsuuugAfgAfAfGfguugaucuguL96	661	asCfsagaUfcaaccuuCfuCfaaaguscsu	885	AGACUUUGAGAAGGUUGAUCUGA	1109
AD-1290670.2	uscscgauCfuGfGfAfacacauauuuL96	594	asAfsauaUfguguuccAfgAfucggascsc	818	GGUCCGAUCUGGAACACAUAUUG	1042
AD-1290672.2	gscsaaucUfgCfCfUfcaauuucuuuL96	623	asAfsagaAfauugaggCfaGfauugcsgsu	847	ACGCAAUCUGCCUCAAUUUCUUC	1071
AD-1290681.2	usgsgguaAfgGfCfCfuuauaauguuL96	663	asAfscauUfauaaggcCfuUfacccascsc	887	GGUGGGUAAGGCCUUAUAAUGUA	1111
AD-1290684.2	csgsaucuGfgAfAfCfacauauugguL96	665	asCfscaaUfauguguuCfcAfgaucgsgsa	889	UCCGAUCUGGAACACAUAUUGGA	1113
AD-1290687.2	csusgcuuGfgCfUfAfcagaauuauuL96	636	asAfsuaaUfucuguagCfcAfagcagsasa	860	UUCUGCUUGGCUACAGAAUUAUU	1084
AD-1290702.2	uscsugccAfuUfUfAfauuagcugcuL96	666	asGfscagCfuaauuaaAfuGfgcagasusu	890	AAUCUGCCAUUUAAUUAGCUGCA	1114
AD-1290712.2	cscsgaucUfgGfAfAfcacauauuguL96	595	asCfsaauAfuguguucCfaGfaucggsasc	819	GUCCGAUCUGGAACACAUAUUGG	1043
AD-1290719.2	asusucugCfuUfGfGfcuacagaauuL96	634	asAfsuucUfguagccaAfgCfagaaususg	858	CAAUUCUGCUUGGCUACAGAAUU	1082
AD-1290722.2	uscsugcuUfgGfCfUfacagaauuauL96	635	asUfsaauUfcuguagcCfaAfgcagasasu	859	AUUCUGCUUGGCUACAGAAUUAU	1083

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1290741.2	ascsgcaaUfcUfGfCfcucaauuucuL96	621	asGfsaaaUfugaggcaGfaUfugcgususa	845	UAACGCAAUCUGCCUCAAUUUCU	1069
AD-1290742.2	gsusggguAfaGfGfCfcuuauaauguL96	603	asCfsauuAfuaaggccUfuAfcccacscsc	827	GGGUGGGUAAGGCCUUAUAAUGU	1051
AD-1290747.2	gsgsagccCfaCfCfUfuggaauuaauL96	586	asUfsuaaUfuccaaggUfgGfgcuccsasa	810	UUGGAGCCCACCUUGGAAUUAAG	1034
AD-1290750.2	cscscaguGfaAfCfCfugccaaagauL96	669	asUfscuuUfggcagguUfcAfcugggsusg	893	CACCCAGUGAACCUGCCAAAGAA	1117
AD-1290755.2	gsgsugggUfaAfGfGfccuuauaauuL96	602	asAfsuuaUfaaggccuUfaCfccaccscsu	826	AGGGUGGGUAAGGCCUUAUAAUG	1050
AD-1290763.2	gsusccgaUfcUfGfGfaacacauauuL96	593	asAfsuauGfuguuccaGfaUfcggacscsu	817	AGGUCCGAUCUGGAACACAUAUU	1041
AD-1290764.2	gsgsuccgAfuCfUfGfgaacacauauL96	592	asUfsaugUfguuccagAfuCfggaccsusc	816	GAGGUCCGAUCUGGAACACAUAU	1040
AD-1290796.2	ususggagCfcCfAfCfcuuggaauuuL96	673	asAfsauuCfcaaggugGfgCfuccaasgsg	897	CCUUGGAGCCCACCUUGGAAUUA	1121
AD-1290800.2	gsgsguggGfuAfAfGfgccuuauaauL96	601	asUfsuauAfaggccuuAfcCfcacccsusa	825	UAGGGUGGGUAAGGCCUUAUAAU	1049
AD-1290805.2	usgsgagcCfcAfCfCfuuggaauuauL96	675	asUfsaauUfccaagguGfgGfcuccasasg	899	CUUGGAGCCCACCUUGGAAUUAA	1123
AD-1290836.2	asasuucuGfcUfUfGfgcuacagaauL96	633	asUfsucuGfuagccaaGfcAfgaauusgsg	857	CCAAUUCUGCUUGGCUACAGAAU	1081
AD-1290837.5	usgsaucuGfaCfCfCfaguucaaguuL96	524	asAfscuuGfaacugggUfcAfgaucasasc	748	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1290841.2	asususcccAfcAfGfCfucagaagcuuL96	584	asAfsgcuUfcugagcuGfuGfggaausasg	808	CUAUUCCCACAGCUCAGAAGCUG	1032
AD-1290842.2	gsasgcccAfcCfUfUfggaauuaaguL96	587	asCfsuuaAfuuccaagGfuGfggcucscsa	811	UGGAGCCCACCUUGGAAUUAAGG	1035
AD-1290857.2	usgscccaCfcAfGfCfcugugauuuuL96	677	asAfsaauCfacaggcuGfgUfgggcasgsg	901	CCUGCCCACCAGCCUGUGAUUUG	1125
AD-1290865.2	csusgcguUfgUfGfCfagacucuauuL96	578	asAfsuagAfgucugcaCfaAfcgcagsgsg	802	CCCUGCGUUGUGCAGACUCUAUU	1026
AD-1290875.2	gscsccacCfaGfCfCfcugugauuuguL96	679	asCfsaaaUfcacaggcUfgGfugggcsasg	903	CUGCCCACCAGCCUGUGAUUUGA	1127
AD-1290880.2	csusuggaGfcCfCfAfccuuggaauuL96	585	asAfsuucCfaagguggGfcUfccaagsgsg	809	CCCUUGGAGCCCACCUUGGAAUU	1033
AD-1290884.5	gscsaggaAfgCfAfCfugagauucguL96	564	asCfsgaaUfcucagugCfuUfccugcsasc	788	GUGCAGGAAGCACUGAGAUUCGG	1012
AD-1290885.5	csusgaccCfaGfUfUfcaaguggauuL96	526	asAfsuccAfcuugaacUfgGfgucagsasu	750	AUCUGACCCAGUUCAAGUGGAUC	974
AD-1290894.2	asgsguccGfaUfCfUfggaacacauuL96	681	asAfsuguGfuuccagaUfcGfgaccuscsc	905	GGAGGUCCGAUCUGGAACACAUA	1129
AD-1290897.2	usgscguuGfuGfCfAfgacucuauuuL96	579	asAfsauaGfagucugcAfcAfacgcasgsg	803	CCUGCGUUGUGCAGACUCUAUUC	1027
AD-1290908.2	cscsagugAfaCfCfUfgccaaagaauL96	683	asUfsucuUfuggcaggUfuCfacuggsgsu	907	ACCCAGUGAACCUGCCAAAGAAA	1131
AD-1290909.2	ususgugcAfgAfCfUfcuauucccauL96	582	asUfsgggAfauagaguCfuGfcacaascsg	806	CGUUGUGCAGACUCUAUUCCCAC	1030
AD-1290910.2	usasggguGfgGfUfAfaggccuuauuL96	684	asAfsuaaGfgccuuacCfcAfcccuasusa	908	UAUAGGGUGGGUAAGGCCUUAUA	1132
AD-1290911.2	asgscccaCfcUfUfGfgaauuaagguL96	588	asCfscuuAfauuccaaGfgUfgggcuscsc	812	GGAGCCCACCUUGGAAUUAAGGG	1036
AD-1290926.2	gscsccacCfuUfGfGfaauuaaggguL96	589	asCfsccuUfaauuccaAfgGfugggcsusc	813	GAGCCCACCUUGGAAUUAAGGGC	1037

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1290931.2	uscsagccAfcAfAfAfugugacccauL96	591	asUfsgggUfcacauuuGfuGfgcugasgsg	815	CCUCAGCCACAAAUGUGACCCAG	1039
AD-1290939.2	asgsggugGfgUfAfAfggccuuauauL96	686	asUfsauaAfggccuuaCfcCfacccusasu	910	AUAGGGUGGGUAAGGCCUUAUAA	1134
AD-1290969.7	ascscuucAfaUfGfCfcuccgucauuL96	556	asAfsugaCfggaggcaUfuGfaaggusgsu	780	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1290971.3	gscsuacgGfaGfAfCfgugguguuuuL96	690	asAfsaacAfccacgucUfcCfguagcscsa	914	UGGCUACGGAGACGUGGUGUUUG	1138
AD-1290973.2	gsusugugCfaGfAfCfucuauucccuL96	691	asGfsggaAfuagagucUfgCfacaacsgsc	915	GCGUUGUGCAGACUCUAUUCCCA	1139
AD-1290983.2	csgsuuguGfcAfGfAfcucuauuccuL96	581	asGfsgaaUfagagucuGfcAfcaacgscsa	805	UGCGUUGUGCAGACUCUAUUCCC	1029
AD-1290989.2	gscsguugUfgCfAfGfacucuauuucuL96	580	asGfsaauAfgagucugCfaCfaacgcsasg	804	CUGCGUUGUGCAGACUCUAUUCC	1028
AD-1290993.2	usasuuccCfaCfAfGfcucagaagcuL96	583	asGfscuuCfugagcugUfgGfgaauasgsa	807	UCUAUUCCCACAGCUCAGAAGCU	1031
AD-1291003.2	gsgscgugCfcUfCfAfgccacaaauuL96	590	asAfsuuuGfuggcugaGfgCfacgccscsu	814	AGGGCGUGCCUCAGCCACAAAUG	1038
AD-1423312.3	usgscaggAfaGfCfAfcugagauucuL96	563	asGfsaadTc(Tgn)cagugcUfuCfcugcascsg	1156	CGUGCAGGAAGCACUGAGAUUCG	1011
AD-1423319.3	gsascuuuGfaGfAfAfgguugaucuuL96	518	asAfsgadTc(Agn)accuucUfcAfaagucsusg	1163	CAGACUUUGAGAAGGUUGAUCUG	966
AD-1423336.7	gsusucaaGfuGfGfAfuccacauuguL96	655	asCfsaadTg(Tgn)ggauccAfcUfugaacsusg	1179	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1548743.7	usgsguguUfuGfUfCfagcaaagauuL96	541	asAfsucdTu(Tgn)gcugacAfaAfcaccascsg	1482	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1612957.2	gscscucaugGfAfAfgagaagcaguL96	1371	asdCsugdCudTcucudTcCfaugaggcsusa	1483	UAGCCUCAUGGAAGAGAAGCAGA	1619
AD-1612963.2	usgsgaagagAfAfGfcagauccuguL96	1373	asdCsagdGadTcugcdTuCfucuuccasusg	1485	CAUGGAAGAGAAGCAGAUCCUGU	1620
AD-1612969.2	asgsaagcagAfUfCfcugugcguguL96	1375	asdCsacdGcdAcaggdAuCfugcuucuscsu	1487	AGAGAAGCAGAUCCUGUGCGUGG	1622
AD-1613059.2	csasgacuuuGfAfGfaagguugauuL96	1377	asdAsucdAadCcuucdTcAfaagucugsusa	1489	UACAGACUUUGAGAAGGUUGAUC	964
AD-1613060.2	asgsacuuugAfGfAfagguugaucuL96	1378	asdGsaudCadAccuudCuCfaaagucusgsu	1490	ACAGACUUUGAGAAGGUUGAUCU	965
AD-1613061.1	gsascuuugaGfAfAfgguugaucuuL96	1817	asdAsgadTcdAaccudTcUfcaaagucsusg	1859	CAGACUUUGAGAAGGUUGAUCUG	966
AD-1613062.2	ascsuuugagAfAfGfguugaucuguL96	1379	asdCsagdAudCaaccdTuCfucaaagcscsu	1491	AGACUUUGAGAAGGUUGAUCUGA	1109

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1613072.1	gsgsuugaucUfGfAfcccaguucauL96	1818	asdTsgadAcdTgggudCaGfaucaaccsusu	1860	AAGGUUGAUCUGACCCAGUUCAA	1135
AD-1613075.2	usgsaucugaCfCfCfaguucaaguuL96	1383	asdAscudTgdAacugdGgUfcagaucasasc	1495	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1613079.2	csusgacccaGfUfUfcaaguggauuL96	1386	asdAsucdCadCuugadAcUfgggucagsasu	1498	AUCUGACCCAGUUCAAGUGGAUC	974
AD-1613087.1	gsusucaaguGfGfAfuccacauuguL96	1455	asdCsaadTgdTggaudCcAfcuugaacsusg	1861	CAGUUCAAGUGGAUCCACAUUGA	1103
AD-1613094.2	usgsgauccaCfAfUfugagggccguL96	1391	asdCsggdCcdCucaadTgUfggauccascsu	1503	AGUGGAUCCACAUUGAGGGCCGG	1626
AD-1613242.2	gscsuacggaGfAfCfgugguguuuuL96	1398	asdAsaadCadCcacgdTcUfccguagcscsa	1510	UGGCUACGGAGACGUGGUGUUUG	1138
AD-1613254.2	usgsguguuuGfUfCfagcaaagauuL96	1404	asdAsucdTudTgcugdAcAfaacaccascsg	1516	CGUGGUGUUUGUCAGCAAAGAUG	989
AD-1613256.2	gsusguuuguCfAfGfcaaagauguuL96	1406	asdAscadTcdTuugcdTgAfcaaacacscsa	1518	UGGUGUUUGUCAGCAAAGAUGUG	991
AD-1613371.3	ascscuucaaUfGfCfcuccgucauuL96	1415	asdAsugdAcdGgaggdCaUfugaaggusgsu	1527	ACACCUUCAAUGCCUCCGUCAUC	1004
AD-1613400.2	gscsaggaagCfAfCfugagauucguL96	1420	asdCsgadAudCucagdTgCfuuccugcsasc	1532	GUGCAGGAAGCACUGAGAUUCGG	1012
AD-1684592.1	gsusagccUfcAfUfGfgaagagaaguL96	1819	asCfsuudCudCuuccauGfaGfgcuacsusc	1862	GAGUAGCCUCAUGGAAGAGAAGC	2008
AD-1684593.1	asgsccucAfuGfGfAfagagaagcauL96	1820	asUfsgcdTudCucuuccAfuGfaggcusasc	1863	GUAGCCUCAUGGAAGAGAAGCAG	2009
AD-1684594.1	csuscaugGfaAfGfAfgaagcagauuL96	499	asAfsucdTgdCuucucuUfcCfaugagsgsc	1864	GCCUCAUGGAAGAGAAGCAGAUC	947
AD-1684595.1	uscsauggAfaGfAfGfaagcagaucuL96	1821	asGfsaudCudGcuucucUfuCfcaugasgsg	1865	CCUCAUGGAAGAGAAGCAGAUCC	2010
AD-1684596.1	asusggaaGfaGfAfAfgcagauccuuL96	500	asAfsggdAudCugcuucUfcUfuccausgsa	1866	UCAUGGAAGAGAAGCAGAUCCUG	948
AD-1684597.1	asasgagaAfgCfAfGfauccugugcuL96	1822	asGfscadCadGgaucugCfuUfcucuuscsc	1867	GGAAGAGAAGCAGAUCCUGUGCG	2011
AD-1684598.1	gsasgaagCfaGfAfUfccugugcguuL96	1823	asAfscgdCadCaggaucUfgCfuucucsusu	1868	AAGAGAAGCAGAUCCUGUGCGUG	2012
AD-1684599.1	asasgcagauCfCfUfgugcgugggguL96	1824	asdCsccdAcdGcacadGgAfucugcuus	1869	AGAAGCAGAUCCUGUGCGUGGGG	2013

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
			csu			
AD-1684600.1	asasgcagAfuCfCfUfgugcguggguL96	1825	asCfsccaCfgcacaggAfuCfugcuuscsu	1870	AGAAGCAGAUCCUGUGCGUGGGG	2013
AD-1684601.1	asgscagaucCfUfGfugcgugggguL96	1826	asdCsccdCadCgcacdAgGfaucugcsusc	1871	GAAGCAGAUCCUGUGCGUGGGGC	2014
AD-1684602.1	asgscagaUfcCfUfGfugcgugggguL96	1827	asCfscccAfcgcacagGfaUfcugcususc	1872	GAAGCAGAUCCUGUGCGUGGGGC	2014
AD-1684603.1	gscsagauCfcUfGfUfgcgugggcuL96	1828	asGfsccdCc(Agn)cgcacaGfgAfucugcsusu	1873	AAGCAGAUCCUGUGCGUGGGGCU	2015
AD-1684604.1	csasgaucCfuGfUfGfcgugggcuuL96	1829	asAfsgcdCcdCacgcacAfgGfaucugscsu	1874	AGCAGAUCCUGUGCGUGGGGCUA	2016
AD-1684605.1	asgsauccUfgUfGfCfgugggcuauL96	1830	asUfsagdCcdCcacgcaCfaGfgaucusgsc	1875	GCAGAUCCUGUGCGUGGGGCUAG	2017
AD-1684606.1	csasgacuuuGfAfGfaagguugauuL96	1377	asdAsucdAadCcuucdTcAfaagucugsusg	1876	UACAGACUUUGAGAAGGUUGAUC	964
AD-1684607.1	csasgacuuuGfAfGfaagguugauuL96	1377	asdAsucdAadCcuucdTcAfaagucugscsu	1877	UACAGACUUUGAGAAGGUUGAUC	964
AD-1684608.1	csasgacuuuGfAfGfaagguugauaL96	1831	usdAsucdAadCcuucdTcAfaagucugscsu	1878	UACAGACUUUGAGAAGGUUGAUC	964
AD-1684609.1	gsascuuuGfAfGfaagguugauuL96	1832	asdAsucdAadCcuucdTcAfaagucsusg	1879	CAGACUUUGAGAAGGUUGAUC	2018
AD-1684610.1	gsascuuugaGfAfAfgguugaucuuL96	1817	asdAsgadTc(Agn)accudTcUfcAfaagucsusg	1880	CAGACUUUGAGAAGGUUGAUCUG	966
AD-1684611.1	ususugagAfAfGfguugaucuguL96	1833	asdCsagdAudCaaccdTuCfucaaasgsu	1881	ACUUUGAGAAGGUUGAUCUGA	2019
AD-1684612.1	ascsuuuaagAfAfGfguugaucuguL96	1834	asdCsagdAudCaaccdTuCfuuaaagcscsu	1882	AGACUUUGAGAAGGUUGAUCUGA	1109
AD-1684613.1	ususgagaAfgGfUfUfgaucugaccuL96	1835	asGfsgudCadGaucaacCfuUfcucaasasg	1883	CUUUGAGAAGGUUGAUCUGACCC	2020
AD-1684614.1	gsasgaagGfuUfGfAfucugacccauL96	1836	asUfsggdGudCagaucaAfcCfuucucsasa	1884	UUGAGAAGGUUGAUCUGACCCAG	2021
AD-1684615.1	gsasagguUfgAfUfCfugacccaguuL96	1837	asAfscudGgdGucagauCfaAfccuucsusc	1885	GAGAAGGUUGAUCUGACCCAGUU	2022
AD-1684616.1	asgsguugAfuCfUfGfacccaguucuL96	1838	asGfsaadCudGggucagAfuCfaaccususc	1886	GAAGGUUGAUCUGACCCAGUUCA	2023
AD-1684617.1	usgsaucugaCfCfCfaguucaaguuL96	1383	asdAscudTgdAacugdGgUfcagaucasgsc	1887	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1684618.1	usgsaucugaCfCfCfaguucaaguuL96	1383	asdAscudTgdAacugdGgUfcagaucas	1888	GUUGAUCUGACCCAGUUCAAGUG	972

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
			csu			
AD-1684619.1	usgsaucugaCfCfCfaguucaaguaL96	1839	usdAscudTgdAacugdGgUfcagaucascsu	1889	GUUGAUCUGACCCAGUUCAAGUG	972
AD-1684620.1	asuscugaCfCfCfaguucaaguuL96	1840	asdAscudTgdAacugdGgUfcagauscsg	1890	UGAUCUGACCCAGUUCAAGUG	2024
AD-1684621.1	gsascccaGfuUfCfAfaguggauccuL96	1841	asGfsgadTcdCacuugaAfcUfgggucsasg	1891	CUGACCCAGUUCAAGUGGAUCCA	2025
AD-1684622.1	ascsccagUfuCfAfAfguggauccauL96	527	asUfsggdAudCcacuugAfaCfugggucsa	1892	UGACCCAGUUCAAGUGGAUCCAC	975
AD-1684623.1	csasguucAfaGfUfGfgauccacauuL96	471	asAfsugdTgdGauccacUfuGfaacugsgsg	1893	CCCAGUUCAAGUGGAUCCACAUU	919
AD-1684624.1	asgsuucaAfgUfGfGfauccacauuuL96	529	asAfsaudGudGgauccaCfuUfgaacusgsg	1894	CCAGUUCAAGUGGAUCCACAUUG	977
AD-1684625.1	asgsuggaUfcCfAfCfauugagggcuL96	1842	asGfsccdCudCaaugugGfaUfccacususg	1895	CAAGUGGAUCCACAUUGAGGGCC	2026
AD-1684626.1	usgsgaucCfaCfAfUfugagggccguL96	1843	asCfsggcCfcucaaugUfgGfauccascsu	1896	AGUGGAUCCACAUUGAGGGCCGG	1626
AD-1684627.1	gsasuccaCfaUfUfGfagggccggauL96	1844	asUfsccdGgdCccucaaUfgUfggaucscsa	1897	UGGAUCCACAUUGAGGGCCGGAA	2027
AD-1684628.1	asusccacAfuUfGfAfgggccggaauL96	1845	asUfsucdCgdGcccucaAfuGfuggauscsc	1898	GGAUCCACAUUGAGGGCCGGAAC	2028
AD-1684629.1	gsasgacgUfgGfUfGfuuugucagcuL96	1846	asGfscudGadCaaacacCfaCfgucucscsg	1899	CGGAGACGUGGUGUUUGUCAGCA	2029
AD-1684630.1	csgsugguGfuUfUfGfucagcaaaguL96	667	asCfsuudTgdCugacaaAfcAfccacgsusc	1900	GACGUGGUGUUUGUCAGCAAAGA	1115
AD-1684631.1	ususgucaGfcAfAfAfgauguggccuL96	1847	asGfsgcdCadCaucuuuGfcUfgacaasasc	1901	GUUUGUCAGCAAAGAUGUGGCCA	2030
AD-1684632.1	gsuscagcAfaAfGfAfuguggccaauL96	1848	asUfsugdGcdCacaucUfuGfcugacsasa	1902	UUGUCAGCAAAGAUGUGGCCAAG	2031
AD-1684633.1	uscsagcaAfaGfAfUfguggccaaguL96	1849	asCfsuudGgdCcacaucUfuUfgcugascsa	1903	UGUCAGCAAAGAUGUGGCCAAGC	2032
AD-1684634.1	csasgcaaAfgAfUfGfuggccaagcuL96	1850	asGfscudTgdGccacauCfuUfugcugsasc	1904	GUCAGCAAAGAUGUGGCCAAGCA	2033
AD-1684635.1	asgscaaaGfaUfGfUfggccaagcauL96	1851	asUfsgcdTudGgccacaUfcUfuugcusgsa	1905	UCAGCAAAGAUGUGGCCAAGCAC	2034
AD-1684636.1	gsasgacaCfcUfUfCfaaugccuccuL96	1852	asGfsgadGgdCauugaaGfgUfgucucsc	1906	UGGAGACACCUUCAAUGCCUCCG	2035

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
			sa			
AD-1684637.1	asgsacacCfuUfCfAfaugccuccguL96	1853	asCfsggdAgdGcauugaAfgGfugucuscsc	1907	GGAGACACCUUCAAUGCCUCCGU	2036
AD-1684638.1	gsascaccUfuCfAfAfugccuccguuL96	1854	asAfscgdGadGgcauugAfaGfgugucsusc	1908	GAGACACCUUCAAUGCCUCCGUC	2037
AD-1684639.1	cscsuucaAfuGfCfCfuccgucaucuL96	557	asGfsaudGadCggaggcAfuUfgaaggsusg	1909	CACCUUCAAUGCCUCCGUCAUCU	1005
AD-1684640.1	gscscuccGfuCfAfUfcuucagccuuL96	1855	asAfsggdCudGaagaugAfcGfgaggcsasu	1910	AUGCCUCCGUCAUCUUCAGCCUC	2038
AD-1684641.1	csusccguCfaUfCfUfucagccucuuL96	561	asAfsgadGgdCugaagaUfgAfcggagsgsc	1911	GCCUCCGUCAUCUUCAGCCUCUC	1009
AD-1684642.1	gsgsagcgUfgCfAfGfgaagcacuguL96	1856	asCfsagdTgdCuuccugCfaCfgcuccsusc	1912	GAGGAGCGUGCAGGAAGCACUGA	2039
AD-1684643.1	gsasgcguGfcAfGfGfaagcacugauL96	1857	asUfscadGudGcuuccuGfcAfcgcucscsu	1913	AGGAGCGUGCAGGAAGCACUGAG	2040
AD-1684644.1	gscsgugcAfgGfAfAfgcacugagauL96	1858	asUfscudCadGugcuucCfuGfcacgcsusc	1914	GAGCGUGCAGGAAGCACUGAGAU	2041
AD-1684645.1	gsusgcagGfaAfGfCfacugagauuuL96	562	asAfsaudCudCagugcuUfcCfugcacsgsc	1915	GCGUGCAGGAAGCACUGAGAUUC	1010
AD-1684646.1	cscscaguGfaAfCfCfugccaaagauL96	669	asdTscudTudGgcagdGuUfcacugggsusg	1916	CACCCAGUGAACCUGCCAAAGAA	1117
AD-1684647.1	cscsagugAfaCfCfUfgccaaagaauL96	683	asdTsucdTudTggcadGgUfucacuggsgsu	1917	ACCCAGUGAACCUGCCAAAGAAA	1131
AD-1684648.1	csusgcguUfgUfGfCfagacucuauuL96	578	asdAsuadGadGucugdCaCfaacgcagsgsg	1918	CCCUGCGUUGUGCAGACUCUAUU	1026
AD-1684649.1	usgscguuGfuGfCfAfgacucuauuuL96	579	asdAsaudAgdAgucudGcAfcaacgcasgsg	1919	CCUGCGUUGUGCAGACUCUAUUC	1027
AD-1684650.1	gscsguugUfgCfAfGfacucuauucuL96	580	asdGsaadTadGagucdTgCfacaacgcsasg	1920	CUGCGUUGUGCAGACUCUAUUCC	1028
AD-1684651.1	csgsuuguGfcAfGfAfcucuauuccuL96	581	asdGsgadAudAgagudCuGfcacaacgscsa	1921	UGCGUUGUGCAGACUCUAUUCCC	1029
AD-1684652.1	gsusugugCfaGfAfCfucuauucccuL96	691	asdGsggdAadTagagdTcUfgcacaacgsgsc	1922	GCGUUGUGCAGACUCUAUUCCCA	1139
AD-1684653.1	ususgugcAfgAfCfUfcuauucccauL96	582	asdTsggdGadAuagadGuCfugcacaascsg	1923	CGUUGUGCAGACUCUAUUCCCAC	1030

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1684654.1	usasuuccCfaCfAfGfcucagaagcuL96	583	asdGscudTcdTgagcdTgUfgggaauasgsa	1924	UCUAUUCCCACAGCUCAGAAGCU	1031
AD-1684655.1	asusucccAfcAfGfCfucagaagcuuL96	584	asdAsgcdTudCugagdCuGfugggaausasg	1925	CUAUUCCCACAGCUCAGAAGCUG	1032
AD-1684656.1	usgscccaCfcAfGfCfcugugauuuuL96	677	asdAsaadTcdAcaggdCuGfgugggcasgsg	1926	CCUGCCCACCAGCCUGUGAUUUG	1125
AD-1684657.1	gscsccacCfaGfCfCfugugauuuguL96	679	asdCsaadAudCacagdGcUfggugggcsasg	1927	CUGCCCACCAGCCUGUGAUUUGA	1127
AD-1684658.1	csusuggaGfcCfCfAfccuuggaauuL96	585	asdAsuudCcdAaggudGgGfcuccaagsgsg	1928	CCCUUGGAGCCCACCUUGGAAUU	1033
AD-1684659.1	ususggagCfcCfAfCfcuuggaauuuL96	673	asdAsaudTcdCaaggdTgGfgcuccaasgsg	1929	CCUUGGAGCCCACCUUGGAAUUA	1121
AD-1684660.1	usgsgagcCfcAfCfCfuuggaauuauL96	675	asdTsaadTudCcaagdGuGfggcuccasasg	1930	CUUGGAGCCCACCUUGGAAUUAA	1123
AD-1684661.1	gsgsagccCfaCfCfUfuggaauuaauL96	586	asdTsuadAudTccaadGgUfgggcuccsasa	1931	UUGGAGCCCACCUUGGAAUUAAG	1034
AD-1684662.1	gsasgcccAfcCfUfUfggaauuaaguL96	587	asdCsuudAadTuccadAgGfugggcucscsa	1932	UGGAGCCCACCUUGGAAUUAAGG	1035
AD-1684663.1	asgscccaCfcUfUfGfgaauuaagguL96	588	asdCscudTadAuuccdAaGfgugggcuscsc	1933	GGAGCCCACCUUGGAAUUAAGGG	1036
AD-1684664.1	gscsccacCfuUfGfGfaauuaaggguL96	589	asdCsccdTudAauucdCaAfggugggcsusc	1934	GAGCCCACCUUGGAAUUAAGGGC	1037
AD-1684665.1	gsgscgugCfcUfCfAfgccacaaauuL96	590	asdAsuudTgdTggcudGaGfgcacgccscsu	1935	AGGGCGUGCCUCAGCCACAAAUG	1038
AD-1684666.1	uscsagccAfcAfAfAfugugacccauL96	591	asdTsggdGudCacaudTuGfuggcugasgsg	1936	CCUCAGCCACAAAUGUGACCCAG	1039
AD-1684667.1	asgsguccGfaUfCfUfggaacacauuL96	681	asdAsugdTgdTuccadGaUfcggaccuscsc	1937	GGAGGUCCGAUCUGGAACACAUA	1129
AD-1684668.1	gsgsuccgAfuCfUfGfgaacacauauL96	592	asdTsaudGudGuuccdAgAfucggaccsusc	1938	GAGGUCCGAUCUGGAACACAUAU	1040
AD-1684669.1	gsusccgaUfcUfGfGfaacacauauuL96	593	asdAsuadTgdTguucdCaGfaucggacscsu	1939	AGGUCCGAUCUGGAACACAUAUU	1041
AD-1684670.1	uscscgauCfuGfGfAfacacauauuuL96	594	asdAsaudAudGuguudCcAfgaucggascsc	1940	GGUCCGAUCUGGAACACAUAUUG	1042
AD-1684671.1	cscsgaucUfgGfAfAfcacauauuguL96	595	asdCsaadTadTgugudTcCfagaucggsa	1941	GUCCGAUCUGGAACACAUAUUGG	1043

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
			sc			
AD-1684672.1	csgsaucuGfgAfAfCfacauauugguL96	665	asdCscadAudAugugdTuCfcagaucgsgsa	1942	UCCGAUCUGGAACACAUAUUGGA	1113
AD-1684673.1	gsasucugGfaAfCfAfcauauuggauL96	657	asdTsccdAadTaugudGuUfccagaucsgsg	1943	CCGAUCUGGAACACAUAUUGGAA	1105
AD-1684674.1	asuscuggAfaCfAfCfauauuggaauL96	596	asdTsucdCadAuaugdTgUfuccagauscsg	1944	CGAUCUGGAACACAUAUUGGAAU	1044
AD-1684675.1	uscsuggaAfcAfCfAfuauuggaauuL96	597	asdAsuudCcdAauaudGuGfuuccagasusc	1945	GAUCUGGAACACAUAUUGGAAUU	1045
AD-1684676.1	csusggaaCfaCfAfUfauuggaauuuL96	598	asdAsaudTcdCaauadTgUfguuccagsasu	1946	AUCUGGAACACAUAUUGGAAUUG	1046
AD-1684677.1	usgsgaacAfcAfUfAfuuggaauuguL96	599	asdCsaadTudCcaaudAuGfuguuccasgsa	1947	UCUGGAACACAUAUUGGAAUUGG	1047
AD-1684678.1	gsgsaacaCfaUfAfUfuggaauugguL96	600	asdCscadAudTccaadTaUfguguuccsasg	1948	CUGGAACACAUAUUGGAAUUGGG	1048
AD-1684679.1	usasggguGfgGfUfAfaggccuuauuL96	684	asdAsuadAgdGccuudAcCfcacccuasusa	1949	UAUAGGGUGGGUAAGGCCUUAUA	1132
AD-1684680.1	asgsggugGfgUfAfAfggccuuauauL96	686	asdTsaudAadGgccudTaCfccacccusasu	1950	AUAGGGUGGGUAAGGCCUUAUAA	1134
AD-1684681.1	gsgsguggGfuAfAfGfgccuuauaauL96	601	asdTsuadTadAggccdTuAfcccacccsusa	1951	UAGGGUGGGUAAGGCCUUAUAAU	1049
AD-1684682.1	gsgsugggUfaAfGfGfccuuauaauuL96	602	asdAsuudAudAaggcdCuUfacccaccscsu	1952	AGGGUGGGUAAGGCCUUAUAAUG	1050
AD-1684683.1	gsusggguAfaGfGfCfcuuauaauguL96	603	asdCsaudTadTaaggdCcUfuacccacscsc	1953	GGGUGGGUAAGGCCUUAUAAUGU	1051
AD-1684684.1	usgsgguaAfgGfCfCfuuauaauguuL96	663	asdAscadTudAuaagdGcCfuuacccascsc	1954	GGUGGGUAAGGCCUUAUAAUGUA	1111
AD-1684685.1	gsgsguaaGfgCfCfUfuauaauguauL96	656	asdTsacdAudTauaadGgCfcuuacccsasc	1955	GUGGGUAAGGCCUUAUAAUGUAA	1104
AD-1684686.1	gsgsuaagGfcCfUfUfauaauguaauL96	653	asdTsuadCadTuauadAgGfccuuaccscsa	1956	UGGGUAAGGCCUUAUAAUGUAAA	1101
AD-1684687.1	gsusaaggCfcUfUfAfuaauguaaauL96	604	asdTsuudAcdAuuaudAaGfgccuuacscsc	1957	GGGUAAGGCCUUAUAAUGUAAAG	1052
AD-1684688.1	usasaggcCfuUfAfUfaauguaaaguL96	649	asdCsuudTadCauuadTaAfggccuuascsc	1958	GGUAAGGCCUUAUAAUGUAAAGA	1097

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1684689.1	asasggccUfuAfUfAfauguaaagauL96	605	asdTscudTudAcauudAuAfaggccuusasc	1959	GUAAGGCCUUAUAAUGUAAAGAG	1053
AD-1684690.1	asgsgccuUfaUfAfAfuguaaagaguL96	606	asdCsucdTudTacaudTaUfaaggccususa	1960	UAAGGCCUUAUAAUGUAAAGAGC	1054
AD-1684691.1	gsgsccuuAfuAfAfUfguaaagagcuL96	644	asdGscudCudTuacadTuAfuaaggccsusu	1961	AAGGCCUUAUAAUGUAAAGAGCA	1092
AD-1684692.1	gscscuuaUfaAfUfGfuaaagagcauL96	607	asdTsgcdTcdTuuacdAuUfauaaggcscsu	1962	AGGCCUUAUAAUGUAAAGAGCAU	1055
AD-1684693.1	gscsauauAfaUfGfUfaaagggcuuuL96	608	asdAsagdCcdCuuuadCaUfuauaugcsusc	1963	GAGCAUAUAAUGUAAAGGGCUUU	1056
AD-1684694.1	csasuauaAfuGfUfAfaagggcuuuuL96	650	asdAsaadGcdCcuuudAcAfuuauaugscsu	1964	AGCAUAUAAUGUAAAGGGCUUUA	1098
AD-1684695.1	asusauaaUfgUfAfAfagggcuuuauL96	609	asdTsaadAgdCccuudTaCfauuauausgsc	1965	GCAUAUAAUGUAAAGGGCUUUAG	1057
AD-1684696.1	usasuaauGfuAfAfAfgggcuuuaguL96	645	asdCsuadAadGcccudTuAfcauuauasusg	1966	CAUAUAAUGUAAAGGGCUUUAGA	1093
AD-1684697.1	asusaaugUfaAfAfGfggcuuuagauL96	610	asdTscudAadAgcccdTuUfacauuausasu	1967	AUAUAAUGUAAAGGGCUUUAGAG	1058
AD-1684698.1	usasauguAfaAfGfGfgcuuuagaguL96	611	asdCsucdTadAagccdCuUfuacauuasusa	1968	UAUAAUGUAAAGGGCUUUAGAGU	1059
AD-1684699.1	asasuguaAfaGfGfGfcuuuagaguuL96	612	asdAscudCudAaagcdCcUfuuacauusasu	1969	AUAAUGUAAAGGGCUUUAGAGUG	1060
AD-1684700.1	cscsuggaUfuAfAfAfaucugccauuL96	613	asdAsugdGcdAgauudTuAfauccaggsusc	1970	GACCUGGAUUAAAAUCUGCCAUU	1061
AD-1684701.1	csusggauUfaAfAfAfucugccauuuL96	614	asdAsaudGgdCagaudTuUfaauccagsgsu	1971	ACCUGGAUUAAAAUCUGCCAUUU	1062
AD-1684702.1	usgsgauuAfaAfAfUfcugccauuuuL96	641	asdAsaadTgdGcagadTuUfuaauccasgsg	1972	CCUGGAUUAAAAUCUGCCAUUUA	1089
AD-1684703.1	gsgsauuaAfaAfUfCfugccauuuauL96	640	asdTsaadAudGgcagdAuUfuuaauccsasg	1973	CUGGAUUAAAAUCUGCCAUUUAA	1088
AD-1684704.1	gsasuuaaAfaUfCfUfgccauuuaauL96	615	asdTsuadAadTggcadGaUfuuuaaucscsa	1974	UGGAUUAAAAUCUGCCAUUUAAU	1063
AD-1684705.1	asusuaaaAfuCfUfGfccauuuaauuL96	616	asdAsuudAadAuggcdAgAfuuuuaauscsc	1975	GGAUUAAAAUCUGCCAUUUAAUU	1064
AD-1684706.1	asasaucuGfcCfAfUfuuaauuagcuL96	617	asdGscudAadTuaaadTgGfcagauuus	1976	UAAAAUCUGCCAUUUAAUUAGCU	1065

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
			usa			
AD-1684707.1	asasucugCfcAfUfUfuaauuagcuuL96	618	asdAsgcdTadAuuaadAuGfgcagauus usu	1977	AAAAUCUGCCAUUUAAUUAGCUG	1066
AD-1684708.1	asuscugcCfaUfUfUfaauuagcuguL96	619	asdCsagdCudAauuadAaUfggcagaus usu	1978	AAAUCUGCCAUUUAAUUAGCUGC	1067
AD-1684709.1	uscsugccAfuUfUfAfauuagcugcuL96	666	asdGscadGcdTaauudAaAfuggcagas usu	1979	AAUCUGCCAUUUAAUUAGCUGCA	1114
AD-1684710.1	csusgccaUfuUfAfAfuuagcugcauL96	620	asdTsgcdAgdCuaaudTaAfauggcags asu	1980	AUCUGCCAUUUAAUUAGCUGCAU	1068
AD-1684711.1	usgsccauUfuAfAfUfuagcugcauuL96	651	asdAsugdCadGcuaadTuAfaauggcas gsa	1981	UCUGCCAUUUAAUUAGCUGCAUA	1099
AD-1684712.1	ascsgcaaUfcUfGfCfcucaauuucuL96	621	asdGsaadAudTgaggdCaGfauugcgus usa	1982	UAACGCAAUCUGCCUCAAUUUCU	1069
AD-1684713.1	csgscaauCfuGfCfCfucaauuucuuL96	622	asdAsgadAadTugagdGcAfgauugcgs usu	1983	AACGCAAUCUGCCUCAAUUUCUU	1070
AD-1684714.1	gscsaaucUfgCfCfUfcaauuucuuuL96	623	asdAsagdAadAuugadGgCfagauugcs gsu	1984	ACGCAAUCUGCCUCAAUUUCUUC	1071
AD-1684715.1	csasaucuGfcCfUfCfaauuucuucuL96	659	asdGsaadGadAauugdAgGfcagauugs csg	1985	CGCAAUCUGCCUCAAUUUCUUCA	1107
AD-1684716.1	asasucugCfcUfCfAfauuucuucauL96	624	asdTsgadAgdAaauudGaGfgcagauus gsc	1986	GCAAUCUGCCUCAAUUUCUUCAU	1072
AD-1684717.1	asuscugcCfuCfAfAfuuucuucauuL96	625	asdAsugdAadGaaaudTgAfggcagaus usg	1987	CAAUCUGCCUCAAUUUCUUCAUC	1073
AD-1684718.1	uscsugccUfcAfAfUfuucuucaucuL96	626	asdGsaudGadAgaaadTuGfaggcagas usu	1988	AAUCUGCCUCAAUUUCUUCAUCU	1074
AD-1684719.1	csusgccuCfaAfUfUfucuucaucuuL96	627	asdAsgadTgdAagaadAuUfgaggcags asu	1989	AUCUGCCUCAAUUUCUUCAUCUG	1075
AD-1684720.1	usgsccucAfaUfUfUfcuucaucuguL96	628	asdCsagdAudGaagadAaUfugaggcas gsa	1990	UCUGCCUCAAUUUCUUCAUCUGU	1076
AD-1684721.1	gscscucaAfuUfUfCfuucaucuguuL96	629	asdAscadGadTgaagdAaAfuugaggcs asg	1991	CUGCCUCAAUUUCUUCAUCUGUC	1077
AD-1684722.1	cscsucaaUfuUfCfUfucaucugucuL96	647	asdGsacdAgdAugaadGaAfauugaggs csa	1992	UGCCUCAAUUUCUUCAUCUGUCA	1095
AD-1684723.1	csuscaauUfuCfUfUfcaucugucauL96	648	asdTsgadCadGaugadAgAfaauugags gsc	1993	GCCUCAAUUUCUUCAUCUGUCAA	1096

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-1684724.1	uscsaauuUfcUfUfCfaucugucaauL96	642	asdTsugdAcdAgaugdAaGfaaauugasgsg	1994	CCUCAAUUUCUUCAUCUGUCAAA	1090
AD-1684725.1	csasauuuCfuUfCfAfucugucaaauL96	630	asdTsuudGadCagaudGaAfgaaauugsasg	1995	CUCAAUUUCUUCAUCUGUCAAAU	1078
AD-1684726.1	asasuuucUfuCfAfUfcugucaaauuL96	631	asdAsuudTgdAcagadTgAfagaaauusgsa	1996	UCAAUUUCUUCAUCUGUCAAAUG	1079
AD-1684727.1	asusuucuUfcAfUfCfugucaaauguL96	632	asdCsaudTudGacagdAuGfaagaaaususg	1997	CAAUUUCUUCAUCUGUCAAAUGG	1080
AD-1684728.1	ususucuuCfaUfCfUfgucaaaugguL96	643	asdCscadTudTgacadGaUfgaagaaasusu	1998	AAUUUCUUCAUCUGUCAAAUGGA	1091
AD-1684729.1	ususcuucAfuCfUfGfucaaauggauL96	639	asdTsccdAudTugacdAgAfugaagaasasu	1999	AUUUCUUCAUCUGUCAAAUGGAA	1087
AD-1684730.1	asasuucuGfcUfUfGfgcuacagaauL96	633	asdTsucdTgdTagccdAaGfcagaauusgsg	2000	CCAAUUCUGCUUGGCUACAGAAU	1081
AD-1684731.1	asusucugCfuUfGfGfcuacagaauuL96	634	asdAsuudCudGuagcdCaAfgcagaaususg	2001	CAAUUCUGCUUGGCUACAGAAUU	1082
AD-1684732.1	ususcugcUfuGfGfCfuacagaauuuL96	654	asdAsaudTcdTguagdCcAfagcagaasusu	2002	AAUUCUGCUUGGCUACAGAAUUA	1102
AD-1684733.1	uscsugcuUfgGfCfUfacagaauuauL96	635	asdTsaadTudCuguadGcCfaagcagasasu	2003	AUUCUGCUUGGCUACAGAAUUAU	1083
AD-1684734.1	csusgcuuGfgCfUfAfcagaauuauuL96	636	asdAsuadAudTcugudAgCfcaagcagsasa	2004	UUCUGCUUGGCUACAGAAUUAUU	1084
AD-1684735.1	usgscuugGfcUfAfCfagaauuauuuL96	637	asdAsaudAadTucugdTaGfccaagcasgsa	2005	UCUGCUUGGCUACAGAAUUAUUG	1085
AD-1684736.1	gscsuuggCfuAfCfAfgaauuauuguL96	638	asdCsaadTadAuucudGuAfgccaagcsasg	2006	CUGCUUGGCUACAGAAUUAUUGU	1086
AD-1684737.1	asusuauuGfuGfAfGfgauaaaaucuL96	652	asdGsaudTudTauccdTcAfcaauaaususc	2007	GAAUUAUUGUGAGGAUAAAAUCA	1100

Table 14. KHK Single Dose Screen in Hep3b Cells

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1290635.2	98.30	12.80	109.90	0.00	133.00	0.00
AD-1684641.1	25.30	0.00	51.50	14.40	18.60	10.60
AD-1684620.1	7.20	2.70	11.30	0.70	10.10	2.10
AD-1684619.1	6.60	1.50	13.70	3.60	17.90	2.90
AD-1684610.1	7.60	1.60	15.40	3.70	25.00	7.40
AD-1684617.1	7.60	1.40	13.30	5.40	12.50	4.00
AD-1613079.2	9.70	2.20	36.70	8.80	33.60	5.70
AD-1684618.1	5.60	0.70	13.00	4.60	9.50	1.60
AD-1684622.1	8.30	2.50	14.00	2.30	17.60	4.50
AD-1684607.1	8.30	0.90	14.40	2.70	12.10	2.10
AD-1684614.1	7.80	1.50	22.00	7.60	23.30	7.70
AD-1613059.2	8.50	0.50	11.70	1.40	11.80	1.50
AD-1613075.2	6.90	0.70	16.00	4.90	19.60	6.80
AD-1613061.1	5.90	2.20	8.00	2.50	10.50	6.10
AD-1684608.1	10.20	2.80	19.20	3.00	18.80	6.80
AD-1684624.1	16.50	3.50	31.80	5.70	50.70	12.80
AD-1423319.3	9.20	0.70	14.40	2.10	14.90	1.60
AD-1684606.1	8.10	2.00	12.40	2.20	14.10	4.40
AD-1684609.1	8.40	1.20	17.00	2.90	14.00	4.00
AD-1684616.1	15.20	2.90	29.70	8.40	31.50	3.00
AD-1613060.2	13.60	1.70	27.00	1.90	29.80	9.50
AD-1613087.1	16.80	2.90	35.50	6.30	39.80	10.40
AD-1290539.5	12.30	2.80	18.30	2.90	21.70	2.50
AD-1290885.5	17.50	5.90	41.70	7.50	63.60	1.40
AD-1613062.2	12.10	2.10	24.10	4.90	28.60	9.00
AD-1684611.1	14.70	3.70	22.30	2.40	26.50	6.10
AD-1684623.1	18.60	3.60	31.90	3.00	59.60	13.80
AD-1612969.2	15.40	4.00	30.40	10.90	52.70	6.80
AD-1613256.2	8.30	1.20	23.60	6.70	33.40	3.80
AD-1423312.3	5.70	2.30	20.50	5.50	16.00	5.80
AD-1548743.7	14.60	2.40	24.20	2.30	34.60	8.10
AD-1684596.1	21.40	8.00	20.40	0.70	24.30	6.00
AD-1423336.7	10.90	1.50	32.80	3.40	36.60	5.80
AD-1612963.2	12.30	4.10	26.50	5.90	28.70	7.10
AD-1290611.3	15.60	3.80	32.40	7.40	54.20	10.00
AD-1613254.2	11.60	2.20	20.70	4.50	24.00	2.80
AD-1684612.1	11.70	1.00	19.50	2.70	31.70	9.10
AD-1684645.1	14.90	2.80	28.80	6.70	35.70	4.70
AD-1684621.1	21.20	6.50	59.50	12.40	42.00	9.10

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1290837.5	17.70	6.20	49.70	7.60	69.90	15.10
AD-1684598.1	37.30	9.50	67.00	19.00	66.40	10.90
AD-1684638.1	23.00	6.60	47.50	13.90	40.40	7.20
AD-1290599.7	11.00	1.20	18.60	3.30	40.00	5.00
AD-1613400.2	7.80	1.70	19.10	7.80	23.00	2.20
AD-1684615.1	17.50	3.20	41.00	2.90	52.70	6.40
AD-1684630.1	14.20	1.30	45.40	10.30	51.20	10.00
AD-1613072.1	43.70	14.40	72.70	6.50	86.70	11.40
AD-1684632.1	17.50	5.60	36.80	7.70	53.40	3.40
AD-1613242.2	11.60	2.60	28.40	9.80	35.20	8.90
AD-1684605.1	53.80	6.00	72.00	8.60	102.80	17.40
AD-1684594.1	16.70	3.90	27.10	4.20	35.80	9.10
AD-1612957.2	14.90	5.10	29.60	7.40	25.90	6.30
AD-1290971.3	13.60	2.40	37.60	9.50	36.90	12.60
AD-1684595.1	29.60	9.90	49.80	12.20	52.40	9.80
AD-1613371.3	14.20	4.40	27.70	5.80	32.10	2.80
AD-1684593.1	20.10	5.10	32.70	10.70	39.60	13.90
AD-1684639.1	13.50	4.50	35.10	6.50	39.90	2.30
AD-1613094.2	16.80	5.20	38.70	4.90	58.50	17.50
AD-1684597.1	30.30	3.00	61.00	14.40	48.90	4.70
AD-1290563.2	50.40	7.90	92.60	14.00	95.40	26.70
AD-1684599.1	41.60	13.60	66.40	17.20	66.80	14.50
AD-1684640.1	17.80	3.60	50.90	10.60	62.70	11.10
AD-1290584.2	32.10	9.80	70.00	11.80	76.10	20.40
AD-1684627.1	20.10	10.50	54.00	12.10	71.60	11.20
AD-1290969.7	18.50	5.40	31.80	7.00	34.30	5.10
AD-1290651.2	51.80	11.50	100.80	20.90	117.60	24.10
AD-1684628.1	17.40	7.00	26.80	5.30	34.10	6.40
AD-1290884.5	20.80	7.20	37.60	7.60	39.00	5.00
AD-1684633.1	25.60	5.90	52.30	3.50	77.80	17.00
AD-1684637.1	31.90	10.90	55.00	9.20	68.60	17.60
AD-1684629.1	38.00	5.90	86.00	13.60	91.70	16.40
AD-1684635.1	32.60	4.30	87.20	14.10	45.90	4.10
AD-1684613.1	43.20	9.20	84.20	23.20	75.90	26.30
AD-1684604.1	47.30	8.80	78.10	15.50	83.00	11.40
AD-1290666.2	28.30	8.10	62.20	16.50	74.10	11.60
AD-1684634.1	39.90	11.00	76.80	9.60	86.20	17.40
AD-1684600.1	70.30	11.40	101.80	5.30	63.80	22.40
AD-1684644.1	68.00	19.10	159.30	17.60	131.10	15.50
AD-1684643.1	96.80	16.70	130.10	23.00	140.40	19.50

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1684601.1	92.10	22.20	136.00	33.00	115.30	33.70
AD-1684626.1	35.10	7.20	65.30	10.50	66.90	5.70
AD-1684642.1	72.30	18.80	116.80	22.20	124.30	8.60
AD-1684592.1	68.50	13.90	120.10	23.20	85.70	6.80
AD-1684636.1	52.00	18.10	88.40	34.10	87.30	10.60
AD-1684603.1	133.70	13.10	143.80	21.30	128.00	22.60
AD-1684631.1	114.50	35.20	163.20	10.70	162.80	24.90
AD-1684625.1	74.60	12.90	121.80	25.30	105.40	20.30
AD-1290523.2	38.30	4.50	44.10	3.20	49.20	15.10
AD-1684602.1	91.40	21.30	151.40	24.60	110.60	24.70
AD-1290570.2	113.50	16.00	137.90	22.90	94.90	11.30
AD-1684666.1	116.20	5.80	109.50	14.40	143.90	23.60
AD-1290865.2	58.40	13.50	64.50	10.30	77.90	7.80
AD-1290589.2	92.60	17.80	108.80	5.90	113.00	7.50
AD-1684681.1	111.40	18.20	129.40	13.10	131.70	21.70
AD-1684689.1	148.40	36.10	91.30	3.00	122.40	27.70
AD-1684708.1	51.20	10.40	83.10	17.60	93.00	21.90
AD-1290557.2	118.30	12.80	76.90	5.70	125.20	33.40
AD-1290515.2	106.60	14.40	116.50	20.20	98.10	9.30
AD-1290741.2	109.20	9.20	119.20	28.90	144.10	20.80
AD-1684713.1	91.70	14.60	84.70	7.90	116.30	17.60
AD-1290650.2	89.60	6.80	75.90	10.30	123.80	18.30
AD-1290897.2	54.80	10.60	81.70	22.90	75.20	24.00
AD-1290556.2	123.30	14.80	92.50	13.30	115.10	12.00
AD-1290750.2	93.70	13.30	95.10	14.20	100.10	12.50
AD-1684714.1	98.00	6.20	136.70	24.30	118.10	12.60
AD-1290654.2	102.80	15.70	119.50	14.80	134.00	32.20
AD-1290909.2	91.20	10.30	108.90	34.40	81.90	22.00
AD-1684674.1	96.60	21.20	118.40	19.30	112.90	27.70
AD-1684712.1	46.90	8.60	88.20	13.40	75.30	13.50
AD-1290796.2	111.90	12.00	119.70	14.30	171.60	18.40
AD-1290612.2	65.20	11.20	94.10	16.20	87.40	11.00
AD-1290633.2	106.60	11.10	94.20	9.50	97.00	23.00
AD-1684691.1	142.10	25.10	112.20	13.20	129.20	9.80
AD-1290659.2	79.40	14.80	103.40	10.30	120.70	13.50
AD-1684686.1	107.60	17.70	129.20	33.80	108.30	23.50
AD-1290604.2	60.90	12.20	64.30	15.40	76.30	16.10
AD-1290574.2	121.10	5.80	122.10	15.40	128.60	12.90
AD-1290609.2	87.60	13.30	111.90	17.40	91.70	17.20
AD-1290911.2	134.00	15.60	65.80	14.70	82.30	16.50

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1290615.2	110.30	11.00	127.90	11.60	158.20	13.00
AD-1684710.1	95.20	12.40	103.60	33.60	117.10	10.50
AD-1684709.1	89.50	19.50	81.20	18.80	92.00	6.10
AD-1290533.2	91.20	19.70	104.90	13.90	99.00	13.30
AD-1684671.1	78.00	7.50	102.80	18.70	98.50	11.20
AD-1684650.1	108.50	3.10	127.80	12.40	118.90	14.50
AD-1684711.1	109.10	20.40	116.90	21.40	106.80	24.30
AD-1684670.1	73.90	14.70	104.70	17.20	127.80	8.90
AD-1684692.1	96.10	16.30	113.50	17.10	103.70	8.30
AD-1290939.2	112.00	10.60	108.90	18.70	101.00	11.20
AD-1684662.1	113.70	21.40	115.40	20.80	117.00	19.60
AD-1684700.1	97.00	21.20	99.20	20.90	65.60	13.10
AD-1290742.2	120.00	18.60	140.80	26.00	97.10	12.40
AD-1684715.1	85.70	18.20	72.70	18.50	120.60	8.70
AD-1290894.2	102.60	11.70	125.50	28.90	149.60	44.30
AD-1684718.1	112.50	13.00	122.40	22.90	136.80	18.20
AD-1290702.2	112.80	29.70	73.50	8.90	80.50	18.40
AD-1684688.1	106.50	25.50	124.40	36.40	123.10	16.60
AD-1684690.1	110.50	5.50	94.50	13.40	106.60	11.50
AD-1684672.1	94.00	12.20	91.00	4.70	98.10	9.30
AD-1684684.1	124.60	21.80	134.40	17.30	121.20	11.40
AD-1684723.1	122.90	4.90	137.70	20.30	142.70	35.90
AD-1684651.1	121.50	44.40	94.60	19.10	107.00	15.50
AD-1684683.1	86.10	5.00	122.80	16.60	91.60	12.10
AD-1290973.2	135.00	19.60	121.30	17.90	129.00	27.10
AD-1290857.2	106.00	12.00	105.00	22.10	89.90	13.10
AD-1684649.1	61.80	9.50	90.40	9.30	67.00	31.50
AD-1290516.2	106.50	19.40	84.40	15.50	70.60	6.80
AD-1290554.2	71.60	21.20	72.80	12.90	71.10	20.50
AD-1290509.2	115.50	16.80	112.90	19.60	92.90	15.10
AD-1290660.2	132.60	30.90	135.00	21.70	98.20	28.10
AD-1684698.1	116.30	8.30	111.20	6.20	90.70	25.00
AD-1290670.2	122.20	6.90	135.80	24.50	150.90	20.40
AD-1684673.1	101.80	11.20	103.80	5.00	105.40	30.20
AD-1684646.1	148.30	19.10	159.50	17.50	128.30	21.40
AD-1290597.2	109.30	23.60	97.80	15.50	109.10	34.10
AD-1290573.2	71.20	7.50	88.60	4.80	93.50	6.40
AD-1684707.1	88.20	7.50	94.50	13.40	91.10	19.00
AD-1684722.1	102.60	11.40	147.30	34.10	162.60	1.40
AD-1290639.2	88.00	11.40	74.20	8.40	83.20	11.10

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1290551.2	100.60	27.30	74.50	19.50	105.80	31.00
AD-1684655.1	146.00	14.60	127.60	21.40	109.60	18.60
AD-1684678.1	148.20	38.60	144.00	24.60	160.70	11.20
AD-1290800.2	129.20	17.20	141.00	10.40	119.30	16.10
AD-1684726.1	83.90	6.80	95.10	21.20	153.80	21.30
AD-1290764.2	85.70	9.50	100.30	16.60	89.50	14.40
AD-1290672.2	90.20	14.50	108.20	6.50	122.60	29.80
AD-1684685.1	158.00	23.20	147.70	27.30	131.50	17.80
AD-1290528.2	17.30	2.30	26.80	4.40	35.90	7.40
AD-1684696.1	83.80	15.70	87.80	20.90	56.60	13.20
AD-1290836.2	120.80	24.10	138.00	20.80	153.80	22.50
AD-1684682.1	99.50	7.40	121.50	22.20	102.10	19.30
AD-1684659.1	61.20	13.60	89.50	3.40	102.00	18.30
AD-1684669.1	101.90	15.80	114.60	19.50	112.10	24.30
AD-1684704.1	115.60	9.50	143.60	36.20	111.10	34.60
AD-1684716.1	83.10	11.60	87.30	7.90	106.30	22.40
AD-1684699.1	106.50	14.40	111.70	29.80	64.20	17.80
AD-1290510.2	112.30	18.30	137.60	27.30	155.40	21.70
AD-1290531.2	87.20	9.20	113.60	33.80	125.10	21.60
AD-1684703.1	101.00	14.00	104.40	15.90	81.80	17.40
AD-1290910.2	96.60	6.80	96.30	35.70	123.50	13.10
AD-1684705.1	98.30	15.30	108.60	32.30	96.40	21.30
AD-1290618.2	109.80	9.20	117.20	13.60	72.30	17.00
AD-1684668.1	95.50	5.80	105.80	16.30	103.00	8.00
AD-1684701.1	61.40	12.80	59.40	11.60	81.70	15.50
AD-1290542.2	110.40	18.30	120.10	23.60	110.00	3.90
AD-1290626.2	92.50	9.90	95.20	10.10	105.00	8.60
AD-1684724.1	98.40	8.20	106.50	12.10	144.80	26.30
AD-1290535.2	131.60	18.00	137.80	13.30	127.70	33.60
AD-1290558.2	129.70	41.60	107.30	30.00	61.90	12.50
AD-1684647.1	118.10	43.70	124.50	25.80	94.60	17.50
AD-1290763.2	91.60	6.90	121.20	18.40	105.70	16.10
AD-1684677.1	94.50	18.30	97.10	22.20	103.50	4.00
AD-1684658.1	115.10	25.50	108.90	16.00	126.20	12.60
AD-1684687.1	92.20	17.60	107.40	15.70	104.80	11.50
AD-1684719.1	97.00	5.10	121.20	5.20	106.00	10.70
AD-1290681.2	130.40	12.30	120.30	17.30	113.50	18.10
AD-1684653.1	133.20	34.50	112.90	7.20	94.80	25.20
AD-1290841.2	166.20	35.70	140.10	1.50	147.10	15.70
AD-1290687.2	83.90	6.80	140.00	15.70	138.80	12.90

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1290592.2	75.50	4.00	63.30	22.80	53.40	4.00
AD-1290522.2	86.40	5.50	102.50	18.10	95.70	11.40
AD-1290880.2	125.50	24.70	94.30	11.10	126.00	12.60
AD-1684693.1	56.90	19.40	53.80	7.10	46.50	6.60
AD-1684679.1	96.50	11.30	111.50	11.40	115.70	21.40
AD-1290555.2	102.60	12.20	59.80	6.50	74.00	33.30
AD-1684648.1	64.70	25.60	86.20	8.10	69.90	28.10
AD-1684725.1	97.40	10.30	109.20	10.50	143.70	21.00
AD-1684706.1	37.50	4.70	46.80	16.60	55.10	14.90
AD-1684663.1	111.70	15.50	118.30	30.30	86.10	35.10
AD-1684702.1	100.20	11.00	79.80	12.00	81.80	10.10
AD-1291003.2	110.40	24.90	108.30	14.50	122.30	20.40
AD-1684664.1	110.20	5.00	117.70	22.00	98.80	7.20
AD-1684697.1	58.70	13.20	66.60	14.80	62.10	15.80
AD-1290514.2	89.80	6.40	108.60	7.20	151.30	18.10
AD-1290989.2	93.40	7.70	101.00	18.70	110.30	13.70
AD-1684657.1	90.30	11.70	98.80	16.00	99.70	29.30
AD-1684654.1	128.30	24.50	109.50	22.10	105.60	33.40
AD-1684695.1	106.60	19.70	91.90	14.50	58.70	7.60
AD-1290712.2	78.60	8.50	126.00	31.80	96.20	13.60
AD-1290931.2	144.80	16.70	127.50	23.50	120.60	18.50
AD-1684729.1	117.20	9.80	134.20	14.70	138.70	25.30
AD-1290805.2	87.90	18.00	94.50	33.50	102.90	20.50
AD-1290755.2	117.00	13.30	124.90	28.50	125.00	29.10
AD-1290527.2	124.10	2.80	76.20	21.60	92.30	31.20
AD-1684652.1	102.00	14.00	96.20	28.30	72.30	18.50
AD-1290507.2	107.20	18.20	118.90	22.10	145.70	41.10
AD-1290747.2	119.20	36.80	103.60	5.30	102.40	14.10
AD-1290926.2	104.90	20.70	105.00	24.10	85.60	16.00
AD-1290983.2	93.70	26.10	136.80	26.50	117.40	15.60
AD-1684680.1	119.70	9.90	115.70	20.40	112.70	18.00
AD-1290605.2	82.10	14.20	96.30	22.40	90.80	9.80
AD-1290842.2	147.60	23.00	145.10	24.30	151.50	27.20
AD-1290565.2	90.90	12.90	107.40	14.80	162.30	28.00
AD-1290661.2	81.70	18.70	82.70	7.80	73.00	24.20
AD-1684667.1	41.60	3.10	54.00	5.90	61.80	5.20
AD-1290684.2	85.00	8.00	92.90	11.80	101.60	7.90
AD-1290524.2	114.60	17.20	143.40	15.60	139.30	26.90
AD-1290655.2	142.20	24.00	128.40	16.50	136.10	21.10
AD-1684660.1	110.20	18.10	134.30	16.20	122.60	20.30

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1684656.1	87.70	37.40	75.00	26.10	76.20	25.20
AD-1684676.1	37.60	2.80	58.60	12.60	72.40	5.00
AD-1684727.1	104.30	31.00	110.10	20.50	117.50	12.10
AD-1684665.1	115.00	28.50	115.70	16.40	105.90	4.90
AD-1290993.2	126.50	14.30	107.50	12.10	98.50	26.30
AD-1684721.1	84.80	4.40	115.10	9.00	144.20	10.10
AD-1290543.2	41.90	8.40	52.50	9.70	54.00	10.30
AD-1290719.2	102.80	11.70	107.60	10.40	136.50	13.40
AD-1290602.2	97.50	7.70	111.60	23.90	116.30	21.10
AD-1290564.2	51.40	1.80	55.70	6.00	75.50	15.00
AD-1684737.1	96.80	16.70	111.80	9.20	105.40	18.20
AD-1684694.1	63.70	21.10	65.10	20.50	72.20	15.80
AD-1290875.2	116.90	11.10	123.80	27.30	72.10	27.80
AD-1684732.1	99.40	14.40	91.50	17.70	138.80	7.00
AD-1684717.1	49.00	11.30	101.40	15.10	104.80	3.30
AD-1290908.2	118.70	7.20	117.70	15.60	97.80	14.70
AD-1684720.1	86.00	2.70	90.20	15.90	112.10	6.60
AD-1290552.2	98.50	16.80	121.20	17.10	137.80	29.70
AD-1684728.1	101.60	25.20	113.60	20.10	136.80	29.60
AD-1684731.1	137.20	10.40	126.20	11.10	142.60	2.40
AD-1290722.2	137.00	19.70	166.40	13.80	170.50	18.70
AD-1684730.1	130.20	30.50	117.60	15.60	117.80	9.00
AD-1684736.1	128.90	12.90	137.40	13.70	135.60	30.10
AD-1684733.1	169.30	14.60	170.60	26.10	178.70	11.10
AD-1684661.1	31.60	6.40	41.70	8.40	48.80	15.50
AD-1684734.1	80.20	13.20	65.20	20.10	85.00	20.90
AD-1684735.1	114.70	26.20	94.40	18.00	119.80	18.20
AD-1290600.2	120.90	18.80	101.00	13.30	118.60	9.70
AD-1290624.2	130.50	13.50	141.60	27.20	115.50	17.40
AD-1290643.2	111.40	14.00	62.10	21.50	114.00	21.40
AD-1684675.1	94.00	21.20	89.60	10.90	84.00	7.70

Table 15. KHK Single Dose Screen in PCH Cells

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1290635.2	23.50	10.50	0.00	0.00	21.60	5.20
AD-1684641.1	0.00	0.00	0.00	0.00	6.00	0.20
AD-1684620.1	1.00	0.10	1.70	0.50	3.20	0.30

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1684619.1	1.30	0.10	2.00	0.10	2.80	0.30
AD-1684610.1	2.00	0.60	2.10	0.20	4.20	0.50
AD-1684617.1	1.70	0.10	2.10	0.40	3.00	0.50
AD-1613079.2	1.40	0.40	2.30	0.20	3.90	1.00
AD-1684618.1	1.00	0.30	2.30	0.30	3.40	0.60
AD-1684622.1	1.80	0.20	2.40	0.20	4.20	0.80
AD-1684607.1	1.60	0.10	2.50	0.40	5.60	0.80
AD-1684614.1	2.10	0.70	2.60	0.30	4.30	0.30
AD-1613059.2	1.90	0.40	2.70	0.40	5.10	0.20
AD-1613075.2	1.10	0.30	2.70	0.30	3.50	0.20
AD-1613061.1	1.90	0.70	2.80	0.30	5.00	0.80
AD-1684608.1	2.10	0.30	2.80	0.20	6.10	1.10
AD-1684624.1	1.70	0.20	2.80	1.00	7.00	1.30
AD-1423319.3	1.90	0.40	2.90	0.80	4.90	0.70
AD-1684606.1	2.00	0.30	3.10	0.20	5.80	0.80
AD-1684609.1	2.00	0.30	3.10	0.30	5.50	0.60
AD-1684616.1	2.20	0.50	3.10	0.80	5.20	1.40
AD-1613060.2	2.00	0.70	3.30	0.20	6.30	1.40
AD-1613087.1	1.50	0.20	3.30	0.10	5.30	0.70
AD-1290539.5	2.50	0.10	3.80	0.10	6.20	0.70
AD-1290885.5	2.00	0.40	3.90	0.60	6.30	0.90
AD-1613062.2	2.80	0.40	3.90	0.10	6.90	0.80
AD-1684611.1	2.00	0.20	4.00	0.80	7.70	0.80
AD-1684623.1	3.10	0.30	4.00	0.80	7.90	1.80
AD-1612969.2	3.90	0.40	4.70	0.60	11.30	1.20
AD-1613256.2	3.60	0.40	4.70	1.10	7.20	1.00
AD-1423312.3	3.60	1.70	4.80	0.90	7.60	2.20
AD-1548743.7	4.40	1.30	4.90	0.30	9.30	1.90
AD-1684596.1	4.40	0.60	4.90	0.70	13.40	3.90
AD-1423336.7	2.40	0.70	5.00	1.30	8.00	2.30
AD-1612963.2	4.40	0.70	5.00	0.30	12.50	1.60
AD-1290611.3	2.60	0.70	5.20	1.10	12.60	3.50
AD-1613254.2	3.50	1.00	5.20	1.40	9.80	2.60
AD-1684612.1	3.40	1.00	5.30	0.90	9.60	0.40
AD-1684645.1	3.00	0.60	5.50	1.20	6.20	0.40
AD-1684621.1	3.10	0.40	5.80	0.10	12.10	3.10
AD-1290837.5	2.50	0.60	5.90	0.80	13.90	1.50
AD-1684598.1	4.60	1.10	6.00	0.70	14.80	2.90
AD-1684638.1	4.00	1.30	6.30	1.10	8.60	1.80
AD-1290599.7	3.70	1.00	6.50	1.10	11.80	1.80
AD-1613400.2	4.50	1.30	6.60	1.40	10.30	2.40

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1684615.1	2.70	0.80	6.60	1.10	13.00	1.90
AD-1684630.1	4.00	1.70	6.70	1.30	9.60	1.70
AD-1613072.1	3.30	1.00	6.90	0.70	12.30	1.80
AD-1684632.1	4.10	1.50	7.00	1.30	8.00	1.10
AD-1613242.2	5.10	2.20	7.10	1.20	9.20	2.30
AD-1684605.1	7.30	1.30	7.60	0.90	17.10	1.90
AD-1684594.1	6.70	1.00	7.70	1.20	15.60	2.10
AD-1612957.2	5.00	0.80	7.80	1.80	14.00	1.10
AD-1290971.3	6.20	1.90	8.10	1.50	12.80	2.00
AD-1684595.1	5.90	0.70	8.20	2.20	18.90	1.30
AD-1613371.3	4.40	1.10	8.80	1.00	9.60	1.10
AD-1684593.1	6.30	0.80	8.80	1.10	12.00	2.50
AD-1684639.1	5.70	1.90	8.80	1.70	19.10	5.30
AD-1613094.2	6.90	1.20	9.70	0.90	19.90	4.60
AD-1684597.1	5.70	1.00	9.70	1.60	22.40	2.80
AD-1290563.2	17.60	5.00	9.80	0.00	27.40	8.90
AD-1684599.1	4.90	1.80	9.90	1.80	19.80	2.70
AD-1684640.1	6.40	2.70	10.10	1.50	18.20	4.00
AD-1290584.2	5.50	2.50	10.40	1.30	15.80	3.80
AD-1684627.1	5.90	1.70	12.30	1.00	21.60	3.70
AD-1290969.7	6.70	2.40	12.60	4.00	16.80	5.60
AD-1290651.2	3.90	1.10	13.30	1.50	24.70	4.10
AD-1684628.1	9.10	2.40	13.30	0.20	18.50	2.20
AD-1290884.5	5.40	1.60	13.60	1.70	22.20	5.90
AD-1684633.1	8.20	3.80	14.20	0.90	27.10	5.90
AD-1684637.1	7.80	3.50	15.10	2.00	23.00	2.60
AD-1684629.1	5.80	1.90	16.90	3.20	24.00	1.40
AD-1684635.1	12.10	2.70	17.10	1.20	27.50	7.70
AD-1684613.1	7.10	2.10	18.20	3.80	35.50	5.20
AD-1684604.1	11.80	0.40	19.90	0.60	41.60	2.50
AD-1290666.2	10.00	3.00	20.80	4.80	37.00	2.30
AD-1684634.1	10.00	4.00	22.70	4.00	37.30	6.30
AD-1684600.1	11.60	1.90	27.90	0.80	53.10	5.40
AD-1684644.1	15.60	4.80	28.80	3.10	55.40	19.80
AD-1684643.1	12.20	4.20	30.20	2.80	41.90	4.30
AD-1684601.1	16.00	2.10	31.20	3.80	60.10	6.00
AD-1684626.1	21.50	6.40	31.60	5.10	60.50	16.20
AD-1684642.1	13.50	4.60	35.50	5.20	56.50	8.40
AD-1684592.1	21.80	1.90	35.70	5.00	59.70	6.20
AD-1684636.1	11.30	4.50	38.10	6.60	59.60	14.00
AD-1684603.1	22.70	4.00	51.50	2.10	77.80	11.40

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1684631.1	21.40	7.60	53.80	9.50	68.00	7.30
AD-1684625.1	12.80	4.50	54.20	7.70	67.50	14.20
AD-1290523.2	68.70	17.50	67.80	25.20	105.40	19.60
AD-1684602.1	37.60	3.30	69.00	5.10	99.90	5.60
AD-1290570.2	113.90	17.40	74.40	15.30	118.80	10.50
AD-1684666.1	114.80	4.60	75.40	12.40	99.60	7.20
AD-1290865.2	90.60	6.20	76.50	35.10	107.90	14.00
AD-1290589.2	133.30	30.50	77.30	8.40	120.40	23.60
AD-1684681.1	118.30	35.00	80.70	18.10	115.30	14.10
AD-1684689.1	145.00	30.40	82.20	13.40	100.00	21.80
AD-1684708.1	85.20	12.90	83.50	7.60	89.10	5.30
AD-1290557.2	143.10	27.50	85.40	13.60	142.40	27.30
AD-1290515.2	146.90	30.00	85.90	15.30	152.80	28.10
AD-1290741.2	77.20	17.70	86.30	19.50	88.50	16.50
AD-1684713.1	81.30	21.10	86.80	14.00	89.80	15.00
AD-1290650.2	70.10	15.20	87.00	13.50	93.80	15.50
AD-1290897.2	84.50	12.20	88.50	35.40	98.20	8.90
AD-1290556.2	130.10	24.90	88.60	21.40	149.90	29.90
AD-1290750.2	41.60	15.10	89.20	24.30	131.10	34.80
AD-1684714.1	84.20	10.00	89.20	7.70	80.10	8.10
AD-1290654.2	117.30	18.70	89.50	16.40	107.40	13.50
AD-1290909.2	79.50	5.30	89.50	12.00	87.40	12.00
AD-1684674.1	109.80	18.20	89.60	26.20	94.20	6.20
AD-1684712.1	75.20	12.40	89.70	6.10	87.70	10.50
AD-1290796.2	94.60	12.30	90.70	8.80	104.30	13.60
AD-1290612.2	141.60	32.40	90.80	5.60	116.90	22.40
AD-1290633.2	92.10	16.50	90.80	4.20	91.90	11.20
AD-1684691.1	130.90	21.50	90.80	10.80	147.30	22.50
AD-1290659.2	77.40	11.60	91.50	10.90	83.40	8.80
AD-1684686.1	115.30	23.00	91.80	23.80	132.80	20.30
AD-1290604.2	102.20	21.70	92.60	11.90	91.50	11.60
AD-1290574.2	118.10	16.60	93.80	4.40	138.30	11.10
AD-1290609.2	76.10	7.50	93.90	12.10	79.40	4.90
AD-1290911.2	83.20	10.90	94.10	22.60	85.00	20.70
AD-1290615.2	83.40	11.20	94.90	16.30	87.60	9.90
AD-1684710.1	83.80	5.80	95.20	11.00	89.50	7.60
AD-1684709.1	74.10	5.00	95.40	15.30	91.70	6.20
AD-1290533.2	60.80	2.90	96.00	21.40	98.10	5.80
AD-1684671.1	109.90	24.30	96.10	27.80	114.60	15.80
AD-1684650.1	88.00	6.10	96.50	24.20	100.70	11.50
AD-1684711.1	74.10	6.90	97.00	3.80	91.30	7.20

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1684670.1	87.20	10.00	97.30	28.90	90.90	5.20
AD-1684692.1	141.10	33.00	97.50	14.20	136.30	32.50
AD-1290939.2	122.30	10.60	98.30	11.70	128.50	6.70
AD-1684662.1	99.20	17.30	98.60	8.40	100.00	20.40
AD-1684700.1	99.30	9.10	98.60	9.60	106.60	10.90
AD-1290742.2	129.20	16.80	98.90	12.20	128.20	17.60
AD-1684715.1	93.70	10.90	98.90	10.10	100.70	9.00
AD-1290894.2	68.90	12.30	99.60	29.10	80.80	16.30
AD-1684718.1	83.00	3.60	99.60	5.80	96.10	14.40
AD-1290702.2	82.00	17.20	100.60	20.70	90.80	15.10
AD-1684688.1	126.60	29.80	100.60	9.30	144.00	31.80
AD-1684690.1	156.10	29.90	101.30	11.40	156.50	25.20
AD-1684672.1	119.50	27.80	101.40	23.10	125.10	34.20
AD-1684684.1	109.90	17.40	102.00	9.20	116.50	8.10
AD-1684723.1	89.40	12.20	102.40	7.90	107.30	6.20
AD-1684651.1	77.60	7.90	102.50	16.10	86.80	14.20
AD-1684683.1	122.10	20.10	102.50	12.50	135.40	19.70
AD-1290973.2	66.80	15.60	103.80	13.80	93.70	11.40
AD-1290857.2	92.30	5.20	103.90	6.10	105.60	6.90
AD-1684649.1	78.90	6.80	103.90	27.90	103.80	11.00
AD-1290516.2	89.30	12.20	105.20	7.00	107.80	12.00
AD-1290554.2	101.40	2.80	105.20	10.30	102.20	6.50
AD-1290509.2	93.00	10.80	105.40	4.20	113.40	8.80
AD-1290660.2	101.60	7.50	105.40	4.70	98.80	6.00
AD-1684698.1	105.40	4.50	105.60	11.70	110.20	7.20
AD-1290670.2	88.70	6.50	106.10	15.30	94.50	10.60
AD-1684673.1	88.30	5.20	106.10	2.50	94.50	11.50
AD-1684646.1	68.80	21.90	106.60	19.00	127.30	25.00
AD-1290597.2	105.80	10.00	107.40	3.40	110.00	12.20
AD-1290573.2	97.40	12.60	107.90	15.20	92.20	10.80
AD-1684707.1	86.40	2.60	108.00	4.80	98.50	11.00
AD-1684722.1	96.60	11.70	108.00	12.10	96.90	8.80
AD-1290639.2	106.10	7.10	108.40	8.50	103.50	6.20
AD-1290551.2	111.70	7.50	109.50	7.80	104.80	7.70
AD-1684655.1	91.50	9.00	109.50	7.40	94.60	5.10
AD-1684678.1	91.10	9.20	109.50	5.60	88.50	3.60
AD-1290800.2	107.10	7.30	109.60	10.90	93.70	8.50
AD-1684726.1	104.90	16.30	109.60	14.60	114.50	8.20
AD-1290764.2	77.50	7.80	110.10	17.00	93.00	12.00
AD-1290672.2	99.10	10.80	110.70	9.90	104.30	13.40
AD-1684685.1	133.70	29.90	110.70	29.30	128.50	32.50

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1290528.2	45.40	4.40	110.80	15.30	84.60	13.70
AD-1684696.1	114.00	11.70	111.00	5.70	120.50	6.60
AD-1290836.2	118.10	8.10	111.70	12.30	123.80	4.70
AD-1684682.1	111.90	18.40	111.80	9.50	124.20	10.20
AD-1684659.1	97.90	4.90	111.90	8.60	99.50	10.20
AD-1684669.1	64.50	9.70	111.90	15.10	81.40	7.10
AD-1684704.1	108.80	8.60	112.00	6.30	97.30	6.70
AD-1684716.1	102.80	16.80	112.00	22.40	98.40	12.60
AD-1684699.1	103.80	5.50	112.40	6.40	105.30	13.70
AD-1290510.2	99.80	10.70	112.70	7.50	115.90	10.10
AD-1290531.2	113.00	19.70	113.00	27.70	118.50	20.40
AD-1684703.1	85.30	9.20	113.40	14.70	107.50	6.00
AD-1290910.2	109.50	15.50	113.50	20.80	133.70	28.50
AD-1684705.1	94.20	4.80	113.50	8.20	113.00	5.90
AD-1290618.2	113.70	12.60	114.20	7.30	103.40	5.70
AD-1684668.1	74.50	13.50	114.20	6.00	84.80	9.70
AD-1684701.1	92.50	14.90	114.30	10.30	105.10	6.80
AD-1290542.2	104.10	14.20	114.40	10.00	123.10	10.40
AD-1290626.2	119.70	13.70	114.40	15.00	144.90	14.70
AD-1684724.1	107.40	11.70	114.40	19.80	121.90	13.70
AD-1290535.2	90.80	4.90	114.60	14.70	101.60	7.00
AD-1290558.2	109.40	6.40	114.90	15.30	115.40	12.00
AD-1684647.1	99.40	27.50	114.90	19.80	136.90	32.80
AD-1290763.2	75.90	7.30	115.50	16.90	111.20	14.60
AD-1684677.1	91.30	8.80	115.50	15.40	95.80	18.60
AD-1684658.1	92.50	14.90	116.50	15.90	109.20	12.60
AD-1684687.1	114.50	18.10	116.90	13.90	132.30	10.30
AD-1684719.1	102.40	6.30	117.20	11.70	96.70	8.20
AD-1290681.2	107.00	9.00	117.60	11.00	117.40	4.40
AD-1684653.1	80.70	3.20	117.70	17.10	81.80	9.30
AD-1290841.2	75.10	4.00	118.00	11.00	85.70	4.70
AD-1290687.2	107.70	12.90	118.20	6.40	124.20	4.50
AD-1290592.2	119.00	9.20	118.30	4.30	120.60	11.50
AD-1290522.2	118.50	37.70	118.50	20.40	136.80	37.60
AD-1290880.2	96.70	19.90	118.70	21.80	110.10	20.90
AD-1684693.1	114.30	7.70	118.70	10.70	116.50	13.10
AD-1684679.1	117.20	20.30	118.80	6.80	126.50	13.80
AD-1290555.2	125.00	8.30	118.90	4.70	113.50	16.10
AD-1684648.1	81.50	12.40	119.10	21.00	95.30	6.60
AD-1684725.1	114.50	12.10	119.50	17.20	127.60	7.20
AD-1684706.1	89.90	9.20	119.60	27.40	124.10	22.90

	10 nM		1 nM		0.1 nM	
Duplex ID	Average % message remaining	STDEV	Average % message remaining	STDEV	Average % message remaining	STDEV
AD-1684663.1	82.10	7.70	120.20	13.70	84.40	10.90
AD-1684702.1	98.10	2.80	120.40	5.00	107.50	3.20
AD-1291003.2	85.70	8.50	120.50	8.30	95.90	9.20
AD-1684664.1	87.30	9.50	120.70	17.50	98.60	17.10
AD-1684697.1	86.40	5.20	121.00	8.60	109.80	7.60
AD-1290514.2	110.00	18.00	121.20	8.80	125.50	8.40
AD-1290989.2	88.80	3.60	121.60	11.60	110.00	12.50
AD-1684657.1	82.50	2.60	121.60	14.20	104.60	5.40
AD-1684654.1	81.10	4.50	121.80	15.10	101.60	2.30
AD-1684695.1	103.90	12.30	122.10	6.90	105.10	10.90
AD-1290712.2	122.10	28.40	122.40	10.60	114.50	28.30
AD-1290931.2	82.00	3.10	122.40	14.10	89.10	11.20
AD-1684729.1	104.60	9.20	123.10	7.70	123.50	12.60
AD-1290805.2	101.60	3.30	123.20	9.40	100.50	5.90
AD-1290755.2	120.80	13.20	123.60	3.30	110.50	19.00
AD-1290527.2	105.70	3.50	123.80	6.20	116.20	6.30
AD-1684652.1	81.80	9.60	123.80	14.40	91.50	12.50
AD-1290507.2	119.00	5.80	124.40	10.90	119.80	5.30
AD-1290747.2	91.90	6.10	124.50	3.30	102.50	17.60
AD-1290926.2	74.50	8.60	125.30	8.90	92.90	15.90
AD-1290983.2	88.30	10.80	125.70	4.10	96.00	6.30
AD-1684680.1	105.10	11.90	125.90	2.90	126.30	18.10
AD-1290605.2	99.20	7.80	126.50	19.90	107.40	14.50
AD-1290842.2	100.80	20.90	127.20	11.10	105.20	14.70
AD-1290565.2	104.90	15.40	127.30	19.20	120.90	17.40
AD-1290661.2	115.40	2.60	127.70	5.70	116.60	9.50
AD-1684667.1	79.60	10.90	129.10	19.10	93.80	5.00
AD-1290684.2	100.20	19.10	129.20	10.90	111.00	9.40
AD-1290524.2	114.10	8.60	129.40	21.20	134.10	19.90
AD-1290655.2	84.20	4.60	129.90	7.20	91.70	2.40
AD-1684660.1	106.80	17.30	130.10	13.70	119.80	14.80
AD-1684656.1	94.10	9.30	130.40	15.20	90.80	14.70
AD-1684676.1	125.80	31.60	130.70	10.10	141.50	42.50
AD-1684727.1	112.30	11.80	130.80	15.60	134.10	10.20
AD-1684665.1	82.50	19.00	131.30	9.90	103.40	6.40
AD-1290993.2	81.50	10.60	132.90	17.10	96.40	5.80
AD-1684721.1	118.30	29.70	133.40	22.80	129.60	16.70
AD-1290543.2	117.30	33.60	133.80	5.80	134.30	21.30
AD-1290719.2	125.50	4.70	134.50	18.60	135.30	13.50
AD-1290602.2	122.90	13.50	135.30	23.70	117.30	12.20
AD-1290564.2	105.80	15.20	136.20	13.00	109.20	11.40

	10 nM		1 nM		0.1 nM	
Duplex ID	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**	**Average % message remaining**	**STDEV**
AD-1684737.1	139.10	33.20	136.40	12.50	142.70	28.20
AD-1684694.1	113.60	7.10	136.60	11.80	118.50	7.50
AD-1290875.2	100.10	5.10	138.20	5.60	104.20	4.30
AD-1684732.1	115.40	13.50	139.80	6.40	131.10	6.80
AD-1684717.1	119.30	16.20	140.00	30.80	120.10	24.90
AD-1290908.2	96.80	28.00	140.10	27.80	147.20	37.70
AD-1684720.1	124.30	14.00	140.50	23.90	120.80	14.90
AD-1290552.2	123.10	4.70	142.50	18.90	137.50	12.20
AD-1684728.1	128.40	15.80	143.40	23.80	139.10	18.30
AD-1684731.1	131.10	12.90	147.20	5.60	148.60	29.20
AD-1290722.2	121.90	13.20	147.80	22.40	124.10	19.20
AD-1684730.1	144.30	31.10	150.10	41.90	121.70	5.50
AD-1684736.1	132.70	21.70	151.10	19.90	144.20	15.80
AD-1684733.1	130.50	19.30	151.20	22.00	141.30	22.00
AD-1684661.1	117.60	34.20	151.80	0.00	134.50	29.30
AD-1684734.1	140.80	29.20	153.70	37.90	150.30	28.10
AD-1684735.1	147.10	29.80	154.10	1.60	159.80	22.20
AD-1290600.2	130.70	35.00	160.60	27.90	150.40	19.70
AD-1290624.2	150.80	32.00	161.90	26.00	151.90	33.80
AD-1290643.2	139.30	26.20	163.80	27.20	160.60	26.00
AD-1684675.1	116.00	28.80	NA	0.00	130.20	26.80

Table 16. KHK Single Dose Screen (Dual-Luciferase Assay)

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1290635.2	27.55	4.72	75.07	12.04	82.87	2.65
AD-1684641.1	110.35	9.02	108.94	4.27	110.57	4.47
AD-1684620.1	49.87	8.22	58.56	6.05	70.53	11.50
AD-1684619.1	52.62	8.75	75.71	10.40	80.49	2.91
AD-1684610.1	37.56	5.38	67.47	3.80	80.31	4.72
AD-1684617.1	55.49	13.45	70.80	1.77	75.55	1.72
AD-1613079.2	53.10	14.44	59.41	4.50	81.54	6.24
AD-1684618.1	55.17	9.77	69.69	5.23	72.30	5.02
AD-1684622.1	63.23	13.55	78.66	4.27	89.31	3.28
AD-1684607.1	58.97	7.60	72.73	7.51	76.63	5.82
AD-1684614.1	64.70	10.39	80.88	8.02	79.18	5.68
AD-1613059.2	51.21	4.83	77.16	7.06	74.45	6.20
AD-1613075.2	60.08	8.05	77.04	7.40	83.17	6.93
AD-1613061.1	33.86	3.11	66.45	5.22	75.37	1.85
AD-1684608.1	57.25	9.23	78.62	6.26	78.71	3.21
AD-1684624.1	79.54	24.34	87.68	11.58	90.36	3.70
AD-1423319.3	36.18	4.92	71.09	4.29	81.57	7.77
AD-1684606.1	54.06	6.46	69.09	8.66	81.86	3.98
AD-1684609.1	50.63	11.26	69.42	5.35	75.07	4.46
AD-1684616.1	68.70	8.45	83.79	6.71	90.02	5.65
AD-1613060.2	66.82	13.96	88.93	7.79	85.14	3.13
AD-1613087.1	74.02	4.74	88.43	10.42	86.90	7.15
AD-1290539.5	54.13	8.10	76.99	5.02	77.45	2.84
AD-1290885.5	61.23	8.10	86.40	22.40	86.33	2.17
AD-1613062.2	41.02	9.84	61.06	10.45	78.05	7.96
AD-1684611.1	28.66	7.54	68.17	4.16	73.93	11.62
AD-1684623.1	70.49	21.68	78.20	8.68	93.68	4.29
AD-1612969.2	82.38	14.04	98.61	3.54	88.83	5.84
AD-1613256.2	63.57	23.77	80.73	3.90	85.86	7.48
AD-1423312.3	78.78	18.49	89.56	6.87	93.64	11.24
AD-1548743.7	60.42	7.72	80.40	4.82	87.16	5.21
AD-1684596.1	66.99	3.42	89.91	18.17	93.12	9.16
AD-1423336.7	55.41	10.18	75.06	10.07	81.43	5.09
AD-1612963.2	68.21	8.77	90.00	8.17	89.11	10.05
AD-1290611.3	72.79	17.88	87.65	5.54	89.02	6.88
AD-1613254.2	60.32	9.01	93.11	8.72	91.19	7.21
AD-1684612.1	57.05	5.54	68.77	6.05	77.64	4.46
AD-1684645.1	94.74	17.21	100.78	3.85	97.29	4.48
AD-1684621.1	70.57	13.92	96.48	4.17	102.25	8.16
AD-1290837.5	75.64	11.34	87.27	7.07	89.95	5.79

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1684598.1	82.26	9.02	100.74	8.99	95.70	14.97
AD-1684638.1	90.83	19.35	99.80	1.21	88.22	5.61
AD-1290599.7	81.02	16.64	88.84	3.66	96.80	4.47
AD-1613400.2	78.98	6.10	101.81	4.90	97.90	11.65
AD-1684615.1	67.00	20.89	97.66	10.46	88.82	5.13
AD-1684630.1	92.58	21.02	102.41	5.76	95.70	6.29
AD-1613072.1	74.57	8.15	92.03	6.87	101.02	3.25
AD-1684632.1	123.67	22.63	120.79	8.89	111.58	5.69
AD-1613242.2	78.42	23.84	97.73	5.29	91.80	1.46
AD-1684605.1	100.37	8.25	97.95	10.50	93.62	9.84
AD-1684594.1	72.68	15.57	112.86	9.89	96.08	5.44
AD-1612957.2	84.90	22.12	94.33	10.29	95.29	8.77
AD-1290971.3	90.45	5.32	92.69	5.86	100.99	5.05
AD-1684595.1	67.25	5.15	83.63	8.61	97.72	8.62
AD-1613371.3	84.73	8.63	93.73	5.20	92.88	3.87
AD-1684593.1	95.59	10.06	105.09	8.84	99.26	9.30
AD-1684639.1	111.42	26.90	100.56	13.89	100.10	10.09
AD-1613094.2	109.88	20.02	106.26	7.18	91.52	4.75
AD-1684597.1	87.17	18.25	98.70	10.75	97.29	11.97
AD-1290563.2	57.60	6.91	70.25	6.46	93.86	10.89
AD-1684599.1	95.44	9.63	99.22	5.79	96.16	3.23
AD-1684640.1	68.26	5.61	95.79	2.54	102.60	4.75
AD-1290584.2	61.38	5.41	94.01	8.50	94.05	2.24
AD-1684627.1	90.44	15.14	92.95	9.50	93.69	9.49
AD-1290969.7	81.86	22.36	100.31	15.03	104.51	10.34
AD-1290651.2	97.29	8.97	93.94	5.58	90.64	2.47
AD-1684628.1	99.71	21.99	94.37	8.89	95.59	1.56
AD-1290884.5	89.60	2.17	98.52	11.66	105.58	5.25
AD-1684633.1	124.09	6.72	124.21	8.35	126.12	7.08
AD-1684637.1	87.99	25.10	99.86	3.94	98.08	2.65
AD-1684629.1	96.24	14.82	108.74	6.76	98.94	1.93
AD-1684635.1	115.43	19.21	110.59	14.86	111.51	5.28
AD-1684613.1	77.44	17.90	86.13	7.31	96.31	11.48
AD-1684604.1	126.73	12.80	104.44	9.66	88.10	9.76
AD-1290666.2	77.77	7.66	85.66	5.31	91.09	7.78
AD-1684634.1	101.54	9.42	112.46	7.92	102.84	8.33
AD-1684600.1	92.36	15.27	102.10	14.83	98.62	7.39
AD-1684644.1	94.78	12.14	105.73	11.26	91.40	7.74
AD-1684643.1	134.90	25.23	106.06	3.63	114.45	5.37
AD-1684601.1	109.99	17.91	106.64	6.25	93.03	6.35
AD-1684626.1	105.90	5.90	97.95	4.84	97.84	3.22

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1684642.1	121.11	34.83	118.91	13.95	106.61	2.62
AD-1684592.1	93.35	12.76	103.28	10.86	103.22	9.57
AD-1684636.1	101.15	23.41	102.25	10.70	108.30	10.50
AD-1684603.1	105.78	12.89	103.83	10.25	94.04	10.93
AD-1684631.1	88.92	2.51	98.87	14.74	103.10	3.82
AD-1684625.1	98.13	10.61	116.53	26.66	103.80	4.63
AD-1290523.2	36.07	3.49	83.69	18.88	78.13	21.59
AD-1684602.1	108.47	14.10	109.97	12.29	100.25	5.84
AD-1290570.2	69.96	7.85	86.80	14.21	101.61	7.01
AD-1684666.1	77.63	4.44	85.12	7.88	89.66	10.91
AD-1290865.2	67.01	0.58	88.22	11.34	96.40	4.28
AD-1290589.2	67.56	4.89	85.24	5.97	97.93	8.15
AD-1684681.1	86.44	3.20	88.04	8.65	103.81	12.28
AD-1684689.1	49.57	6.76	86.48	5.18	90.62	11.35
AD-1684708.1	23.03	1.62	33.48	7.99	57.59	11.14
AD-1290557.2	94.58	3.42	98.87	10.22	103.18	8.22
AD-1290515.2	48.39	6.21	70.61	2.16	86.52	1.87
AD-1290741.2	62.89	8.45	108.61	21.31	83.31	16.19
AD-1684713.1	25.62	2.85	56.78	8.85	73.15	15.46
AD-1290650.2	45.96	4.20	75.32	14.73	88.04	15.00
AD-1290897.2	91.50	12.78	99.78	4.12	95.69	6.37
AD-1290556.2	46.60	6.23	72.83	12.88	95.77	2.43
AD-1290750.2	100.69	11.17	112.50	12.75	98.54	9.04
AD-1684714.1	28.09	1.61	63.87	15.58	58.74	14.71
AD-1290654.2	59.38	5.24	82.45	5.68	92.90	11.63
AD-1290909.2	79.81	7.84	85.50	1.84	99.95	6.60
AD-1684674.1	38.19	5.91	68.84	4.29	86.91	7.62
AD-1684712.1	27.74	1.92	51.61	7.17	81.25	10.84
AD-1290796.2	90.91	8.26	93.92	11.82	98.49	8.90
AD-1290612.2	42.68	2.55	66.55	14.42	68.94	7.63
AD-1290633.2	50.34	3.85	73.42	11.49	83.99	10.78
AD-1684691.1	66.75	8.42	91.12	4.34	97.18	11.63
AD-1290659.2	29.24	3.16	58.78	1.46	83.51	13.24
AD-1684686.1	55.60	5.55	81.33	10.32	90.49	12.00
AD-1290604.2	30.50	1.61	58.09	12.94	75.45	12.12
AD-1290574.2	62.06	4.41	79.92	5.27	89.83	8.75
AD-1290609.2	27.15	4.45	50.65	5.55	73.67	12.54
AD-1290911.2	96.37	7.95	93.75	11.00	96.22	5.42
AD-1290615.2	51.72	2.67	70.39	15.92	77.60	16.60
AD-1684710.1	42.53	5.32	73.27	11.74	68.13	11.94
AD-1684709.1	58.70	4.19	79.18	25.68	86.01	19.30

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1290533.2	47.59	4.90	68.99	11.94	77.30	6.48
AD-1684671.1	47.59	1.95	61.26	3.20	82.05	10.96
AD-1684650.1	46.11	8.10	76.50	10.07	82.83	4.08
AD-1684711.1	25.37	2.16	39.63	9.66	66.61	6.31
AD-1684670.1	61.98	4.96	79.82	3.32	86.14	2.50
AD-1684692.1	51.68	5.44	73.68	5.82	94.81	6.71
AD-1290939.2	79.47	12.39	91.84	15.17	102.79	10.83
AD-1684662.1	92.36	5.74	89.87	6.57	98.46	7.31
AD-1684700.1	49.06	2.35	66.17	5.75	80.59	11.96
AD-1290742.2	97.05	12.34	108.91	9.16	98.27	8.80
AD-1684715.1	39.93	3.07	71.43	4.49	66.16	5.22
AD-1290894.2	69.53	5.96	86.15	6.51	89.41	8.71
AD-1684718.1	38.19	2.50	56.43	6.04	89.12	4.16
AD-1290702.2	63.45	4.81	94.75	15.47	93.98	25.50
AD-1684688.1	31.21	4.38	62.76	5.56	71.36	5.67
AD-1684690.1	37.78	7.44	65.65	6.32	81.53	5.19
AD-1684672.1	50.47	5.26	77.82	8.07	89.96	7.10
AD-1684684.1	34.84	3.57	66.09	6.84	81.50	13.30
AD-1684723.1	35.25	2.97	68.48	5.60	65.99	9.83
AD-1684651.1	70.03	6.39	85.13	4.79	94.44	5.75
AD-1684683.1	54.95	5.39	77.78	7.33	95.72	7.28
AD-1290973.2	98.50	6.54	92.81	14.03	98.60	4.58
AD-1290857.2	87.52	2.70	96.73	5.63	99.17	2.18
AD-1684649.1	72.47	0.92	84.56	8.23	89.82	2.21
AD-1290516.2	26.76	0.49	42.76	4.24	59.84	6.99
AD-1290554.2	61.58	4.05	88.87	14.21	74.37	3.21
AD-1290509.2	73.11	5.62	99.31	14.02	101.98	16.69
AD-1290660.2	54.56	9.39	83.38	0.31	86.16	6.94
AD-1684698.1	49.13	6.23	101.74	19.35	58.63	21.67
AD-1290670.2	70.45	5.20	85.30	12.39	83.73	9.68
AD-1684673.1	54.65	6.25	78.09	7.36	92.77	10.76
AD-1684646.1	88.86	5.66	109.84	2.99	100.07	6.89
AD-1290597.2	64.33	3.82	104.86	17.05	78.64	14.59
AD-1290573.2	21.93	2.25	51.90	11.20	61.08	10.20
AD-1684707.1	25.65	1.98	47.58	11.42	58.67	13.41
AD-1684722.1	42.02	2.86	72.20	13.41	91.03	17.74
AD-1290639.2	65.65	8.85	106.60	6.21	80.86	3.84
AD-1290551.2	37.93	4.95	63.38	4.48	69.08	10.49
AD-1684655.1	82.73	4.01	88.85	9.58	98.35	13.91
AD-1684678.1	71.26	6.57	96.82	5.79	98.92	5.96
AD-1290800.2	81.68	8.76	95.82	8.76	99.82	11.38

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1684726.1	28.47	1.78	45.62	14.88	47.10	11.51
AD-1290764.2	67.56	8.24	67.75	7.72	79.61	6.78
AD-1290672.2	42.65	3.57	64.81	14.49	73.51	15.34
AD-1684685.1	52.36	4.45	80.46	8.33	90.09	6.80
AD-1290528.2	40.22	5.18	55.62	6.12	74.10	6.88
AD-1684696.1	36.27	4.80	43.87	3.17	104.26	16.60
AD-1290836.2	78.37	9.59	84.56	11.22	101.22	23.26
AD-1684682.1	80.66	18.04	114.19	18.74	92.36	3.20
AD-1684659.1	80.80	5.53	92.64	9.37	100.32	4.86
AD-1684669.1	61.93	5.52	72.55	4.18	83.30	4.78
AD-1684704.1	73.43	3.74	102.98	4.47	82.11	11.96
AD-1684716.1	49.39	6.90	78.93	10.67	88.70	8.71
AD-1684699.1	32.13	1.49	49.17	12.29	88.94	7.66
AD-1290510.2	29.78	2.09	61.76	7.12	57.41	3.23
AD-1290531.2	30.64	2.31	61.41	11.62	88.80	10.45
AD-1684703.1	29.94	4.56	48.74	5.70	64.27	5.80
AD-1290910.2	78.87	4.68	83.35	7.19	102.58	9.62
AD-1684705.1	84.81	5.73	78.46	14.78	108.49	29.28
AD-1290618.2	38.80	2.21	91.85	24.03	70.60	14.74
AD-1684668.1	82.61	3.09	85.84	5.55	102.22	13.23
AD-1684701.1	33.37	3.20	59.65	10.73	79.48	7.48
AD-1290542.2	25.33	4.70	75.56	5.50	43.19	10.83
AD-1290626.2	42.99	7.92	66.24	9.90	78.99	6.15
AD-1684724.1	25.49	3.64	41.90	10.84	75.17	17.57
AD-1290535.2	39.61	4.59	86.53	3.83	75.49	9.54
AD-1290558.2	52.87	4.30	77.41	14.99	88.24	18.29
AD-1684647.1	63.31	20.44	88.14	11.46	99.17	9.42
AD-1290763.2	76.79	3.45	91.24	4.92	100.24	8.61
AD-1684677.1	49.89	7.84	62.12	3.31	80.47	5.14
AD-1684658.1	61.99	9.91	82.39	6.38	93.13	3.41
AD-1684687.1	56.84	10.02	73.94	6.08	90.30	8.39
AD-1684719.1	21.60	1.78	54.66	6.44	49.60	12.61
AD-1290681.2	70.01	13.50	102.05	13.79	97.11	8.26
AD-1684653.1	77.63	3.92	90.78	7.67	107.04	10.77
AD-1290841.2	102.02	12.88	92.72	10.43	92.89	8.78
AD-1290687.2	24.79	5.58	47.49	9.56	54.96	7.96
AD-1290592.2	42.32	3.00	63.61	7.19	81.99	13.27
AD-1290522.2	63.08	8.25	80.77	2.61	89.07	3.72
AD-1290880.2	86.62	8.68	84.96	10.84	112.23	7.89
AD-1684693.1	33.57	4.76	72.88	8.53	56.56	8.52
AD-1684679.1	63.98	10.81	81.92	8.14	99.47	7.56

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1290555.2	29.21	2.95	57.32	7.73	76.70	14.67
AD-1684648.1	57.07	4.43	72.65	5.51	90.63	6.01
AD-1684725.1	37.50	5.23	60.12	13.34	75.61	7.15
AD-1684706.1	34.25	5.45	66.03	7.18	61.51	7.34
AD-1684663.1	100.81	7.18	100.10	13.78	98.87	4.95
AD-1684702.1	27.84	1.80	55.04	5.17	62.99	6.59
AD-1291003.2	99.82	7.41	90.75	6.78	96.81	11.67
AD-1684664.1	98.11	1.83	95.21	12.95	100.40	8.82
AD-1684697.1	63.74	8.80	90.40	16.93	79.86	15.55
AD-1290514.2	25.53	1.05	39.72	6.89	59.21	5.46
AD-1290989.2	80.25	6.00	95.06	4.87	94.19	9.09
AD-1684657.1	85.45	4.39	92.61	11.75	107.59	8.01
AD-1684654.1	93.92	6.75	95.83	4.68	99.57	13.80
AD-1684695.1	40.77	3.71	87.34	16.28	65.10	12.55
AD-1290712.2	76.80	6.49	89.36	6.93	97.09	5.67
AD-1290931.2	74.20	8.48	91.78	7.74	93.55	7.40
AD-1684729.1	32.72	5.04	71.92	14.70	63.69	5.82
AD-1290805.2	85.12	3.08	83.71	7.55	90.13	4.27
AD-1290755.2	82.43	5.17	101.69	9.25	96.50	4.82
AD-1290527.2	35.84	2.68	72.84	6.30	61.60	13.91
AD-1684652.1	85.57	2.57	95.34	9.86	91.15	4.65
AD-1290507.2	29.99	3.00	86.33	6.23	57.42	9.02
AD-1290747.2	93.73	11.80	93.12	7.86	93.91	7.92
AD-1290926.2	105.81	5.23	93.36	5.15	101.13	11.67
AD-1290983.2	83.97	2.62	84.46	5.64	102.69	8.80
AD-1684680.1	75.84	12.82	88.75	9.33	92.50	2.09
AD-1290605.2	71.92	10.68	111.52	16.71	79.13	8.92
AD-1290842.2	104.49	5.65	91.21	5.53	95.02	7.94
AD-1290565.2	34.69	2.42	75.14	21.84	83.67	22.69
AD-1290661.2	47.11	5.63	82.93	15.48	77.74	26.12
AD-1684667.1	50.51	6.46	74.42	2.87	89.86	7.71
AD-1290684.2	61.88	5.00	86.54	7.60	91.09	8.89
AD-1290524.2	55.34	5.89	100.08	30.59	97.04	35.30
AD-1290655.2	55.60	8.94	79.31	8.29	86.56	4.09
AD-1684660.1	90.30	7.72	90.68	6.87	89.53	4.84
AD-1684656.1	99.30	6.66	87.28	5.54	93.70	10.20
AD-1684676.1	42.60	3.34	64.98	4.82	81.15	8.48
AD-1684727.1	34.77	9.34	62.64	16.13	56.10	7.77
AD-1684665.1	88.07	7.37	85.96	2.62	96.42	4.35
AD-1290993.2	126.19	4.37	103.72	5.04	100.36	8.22
AD-1684721.1	30.93	4.69	73.02	5.08	74.51	8.53

Duplex Name	RLuc/FLuc		RLuc/FLuc		RLuc/FLuc	
	10 nM		1 nM		0.1 nM	
	Avg (%)	SD (%)	Avg (%)	SD (%)	Avg (%)	SD (%)
AD-1290543.2	60.43	2.14	78.76	1.16	86.30	3.38
AD-1290719.2	72.55	5.47	106.96	11.38	89.56	13.94
AD-1290602.2	36.81	5.78	75.60	6.07	63.11	4.79
AD-1290564.2	46.18	1.47	88.84	15.84	89.88	12.30
AD-1684737.1	19.91	2.75	65.84	2.75	50.34	9.86
AD-1684694.1	37.30	6.64	65.54	8.09	68.75	11.54
AD-1290875.2	88.36	6.67	87.65	4.57	95.91	11.39
AD-1684732.1	21.92	1.86	70.21	17.18	48.70	2.36
AD-1684717.1	20.14	3.23	53.41	2.38	49.49	5.05
AD-1290908.2	69.14	8.90	91.09	8.96	105.33	6.07
AD-1684720.1	25.19	1.57	65.20	9.13	55.67	9.89
AD-1290552.2	60.07	5.86	110.60	5.95	68.03	4.85
AD-1684728.1	32.65	4.26	60.26	10.61	81.39	13.63
AD-1684731.1	71.00	4.39	97.76	10.63	103.47	10.13
AD-1290722.2	56.44	2.77	96.22	25.60	84.59	1.63
AD-1684730.1	55.45	5.72	103.19	33.31	94.48	30.63
AD-1684736.1	22.63	1.19	55.13	6.21	63.03	19.64
AD-1684733.1	31.22	2.64	74.74	15.23	80.11	13.22
AD-1684661.1	88.94	5.85	86.32	8.94	96.70	2.89
AD-1684734.1	24.44	1.34	55.52	4.03	41.46	7.30
AD-1684735.1	27.36	1.31	62.50	9.06	50.38	6.57
AD-1290600.2	63.33	8.27	96.26	30.33	114.38	17.93
AD-1290624.2	34.72	1.33	92.38	34.13	71.36	13.25
AD-1290643.2	31.00	3.54	63.18	15.27	67.58	13.33
AD-1684675.1	43.08	8.00	63.69	6.05	73.37	3.64

Example 6: Effects of siRNA –GalNAC Conjugates in Non-Human Primates

The effect of candidates identified from the *in vitro* studies described above, duplexes AD-1613062, AD-1613073, AD-1613242, AD-1613243, AD-1613246, AD-1613247, AD-1613400, AD-1634397, AD-1634424, and AD-1634425, were further investigated for their effectiveness in non-human primates. Specifically, a single dose of 3 mg/kg of AD-1613062, AD-1613073, AD-1613242, AD-1613243, AD-1613246, AD-1613247, AD-1613400, AD-1634397, AD-1634424, or AD-1634425 was subcutaneously administered to cynomolgous monkeys. Sera and tissue samples were collected at Days 29, 50, and 78 post-dose.

mRNA was extracted from liver tissue by a magnetic bead-based extraction method using the NucleoMag RNA kit by Macherey-Nagel. cDNA was generated using Applied Biosystems SuperScript IV VILO master mix. Taqman probe-based qPCR was used to quantify KHK mRNA which was normalized to the geometric mean of two housekeeping genes, ARL6IP4 and PPIB.

Targeted quantitation of KHK protein in cynomolgus monkey liver was performed via liquid chromatography coupled to mass spectrometer (LC-MS) using parallel reaction monitoring (PRM) in positive ion mode. The signature peptide sequence selected for quantitation was HLGFQSAGEALR (UniProtKB - A0A2K5V1R8-1, amino acid positions 198-209).

Figure 2 shows the effect of administration of a single 3 mg/kg dose of the selected duplexes on the level of KHK mRNA and protein at Day 29 post-dose.

Figure 3 shows the effect of administration of a single 3 mg/kg dose of the selected duplexes on the level of KHK mRNA at Day 50 post-dose.

Figure 4 shows the effect of administration of a single 3 mg/kg dose of the selected duplexes on the level of KHK mRNA at Day 78 post-dose.

The data demonstrate that the indicated agents, *e.g.*, AD-1613400 and AD-1613243, are effective in durably and potently inhibiting KHK mRNA and protein expression.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding KHK, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 5, 6, and 8-13.

2. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6, and 8-13 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6, and 8-13.

3. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6, and 8-13 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6, and 8-13.

4. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6, and 8-13 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6, and 8-13.

5. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, 6, and 8-13 and an antisense strand comprising a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2, 3, 5, 6, and 8-13.

6. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 120-162; 164-188; 181-207; 193-217; 209-231; 283-306; 508-546; 568-603; 596-632;

640-674; 746-806; 806-835; 917-942; 936-084; 1016-1041; 1100-1123; 1149-1175; 1160-1193; 1205-1229; 1252-1283; 1334-1356; 1407-1429; 1472-1497; 1506-1533; 1539-1561; 1704-1727; 1747-1787; 1850-1873; 1936-1964; 1960-1990; 2015-2048; 2060-2095; 2090-2118; 2124-2160; 2181-2200; 2221-2262 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

7. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 517-539; 521-543; 524-546; 517-546; 581-603; 610-632; 747-769; 749-771; 752-774; 755-777; 757-779; 758-780; 764-786; 776-798; 781-803; 747-803; 920-942; 941-963; 944-966; 950-972; 962-984; 920-984; 1149-1171; 1161-1183; 1165-1187; 1171-1193; 1149-1193; 1205-1227; 1206-1228; 1205-1228; 1334-1356; 1472-1494; 1475-1497; 1472-1497 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

8. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 517-539; 524-546; 517-546; 753-775; 757-779; 753-779; 764-786; 767-789; 768-790; 769-791; 764-791; 773-795; 781-803; 773-803; 753-803; 808-830; 937-959; 941-963; 944-966; 941-966; 948-970; 950-972; 948-972; 1160-1182; 1161-1183; 1160-1183; and 1207-1229 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

9. The dsRNA agent of any one of claims 1-8, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1613400; AD-1613243; AD-1290757.3; AD-1290878.3; AD-1290969.3; AD-1423317.2; AD-1423327.2; AD-1423336.2; AD-1290599.3; AD-1523172.1; AD-1290837.3; AD-1523173.1; AD-1290884.3; AD-1523174.1; AD-1290959.3; AD-1523175.1; AD-1423311.2; AD-1423324.2; AD-1523176.1; AD-1423329.2; AD-1423333.2; AD-1423330.2; AD-1523177.1; AD-1290885.3; AD-1523178.1; AD-1423334.2; AD-1523179.1; AD-1523180.1; AD-1290539.3; and AD-1523181.1.

10. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense

strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 495-517, 492-517, 500-529, 514-551, 517-539, 524-546, 517-548, 614-643, 625-647, 625-660, 642-664, 642-672, 753-811, 754-780, 762-791, 764-786, 772-800, 781-803, 805-827, 808-830, 809-831, 792-838, 931-982, 944-966, 947-969, 948-970, 948-982, 1011-1035, 1021-1043, 1019-1050, 1063-1091, 1150-1192, 1152-1176, 1160-1192, 1160-1182, 1162-1184, 1198-1230, 1198-1221, and 1202-1230 of SEQ ID NO: 1,and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

11. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 513-556, 753-813, 936-981, 1155-1193, 1200-1229, 1704-1727, 1747-1787, 1850-1873, 1936-1964, 1960-1990, 1936-1990, 2015-2048, 2060-2095, 2090-2118, 2060-2118, 2124-2160, 2181-2220, 2221-2249, 2181-2249, 2240-2262, 2221-2262, and 2181-2262 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

12. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the nucleotide sequence of nucleotides 1207-1229 or 937-959 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

13. The dsRNA agent of claim 12, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the nucleotide sequence of nucleotides 1207-1229 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from the corresponding nucleotide sequence of SEQ ID NO:2.

14. The dsRNA agent of any one of claims 1-13, wherein the dsRNA agent comprises at least one modified nucleotide.

15. The dsRNA agent of any one of claims 1-14, wherein substantially all of the nucleotides of the sense strand; substantially all of the nucleotides of the antisense strand comprise a modification; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification.

16. The dsRNA agent of any one of claims 1-15, wherein all of the nucleotides of the sense strand comprise a modification; all of the nucleotides of the antisense strand comprise a modification; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a modification.

17. The dsRNA agent of any one of claims 14-16, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythimidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a nucleotide comprising a 2' phosphate, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

18. The dsRNA agent of any one of claims 14-16, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

19. The dsRNA agent of any one of claims 14-16, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, a vinyl-phosphonate nucleotide; and combinations thereof.

20. The dsRNA agent of any one of claims 14-16, wherein at least one of the modifications on the nucleotides is a thermally destabilizing nucleotide modification.

21. The dsRNA agent of claim 20, wherein the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; and destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acids (UNA), and a glycerol nucleic acid (GNA).

22. The dsRNA agent of any one of claims 1-21, wherein the double stranded region is 19-30 nucleotide pairs in length.

23. The dsRNA agent of claim 22, wherein the double stranded region is 19-25 nucleotide pairs in length.

24. The dsRNA agent of claim 22, wherein the double stranded region is 19-23 nucleotide pairs in length.

25. The dsRNA agent of claim 22, wherein the double stranded region is 23-27 nucleotide pairs in length.

26. The dsRNA agent of claim 22, wherein the double stranded region is 21-23 nucleotide pairs in length.

27. The dsRNA agent of any one of claims 1-26, wherein each strand is independently no more than 30 nucleotides in length.

28. The dsRNA agent of any one of claims 1-27, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

29. The dsRNA agent of any one of claims 1-28, wherein the region of complementarity is at least 17 nucleotides in length.

30. The dsRNA agent of any one of claims 1-29, wherein the region of complementarity is between 19 and 23 nucleotides in length.

31. The dsRNA agent of any one of claims 1-30, wherein the region of complementarity is 19 nucleotides in length.

32. The dsRNA agent of any one of claims 1-31, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

33. The dsRNA agent of any one of claims 1-31, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

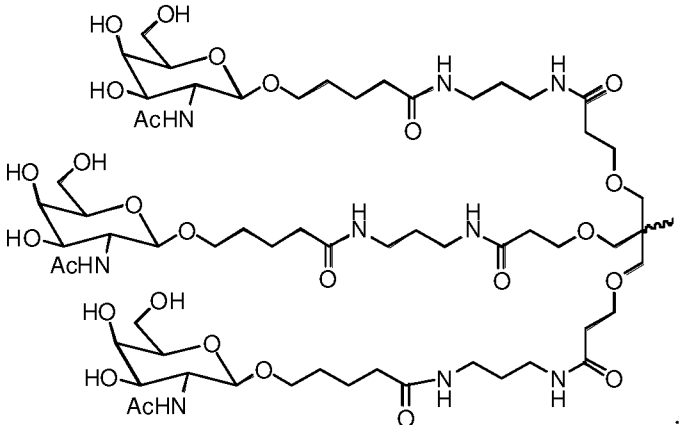
34. The dsRNA agent of any one of claims 1-33, further comprising a ligand.

35. The dsRNA agent of claim 34, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

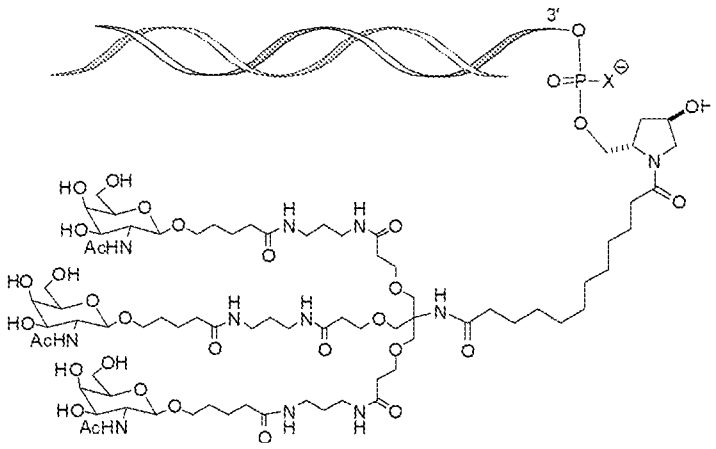
36. The dsRNA agent of claim 34 or 35, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

37. The dsRNA agent of any one of claims 34-36, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

38. The dsRNA agent of claim 36 or 37, wherein the ligand is



39. The dsRNA agent of claim 38, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

40. The dsRNA agent of claim 39, wherein the X is O.

41. The dsRNA agent of any one of claims 1-40, wherein the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

42. The dsRNA agent of claim 41, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

43. The dsRNA agent of claim 42, wherein the strand is the antisense strand.

44. The dsRNA agent of claim 42, wherein the strand is the sense strand.

45. The dsRNA agent of claim 41, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

46. The dsRNA agent of claim 45, wherein the strand is the antisense strand.

47. The dsRNA agent of claim 45, wherein the strand is the sense strand.

48. The dsRNA agent of claim 41, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the both the 5'- and 3'-terminus of one strand.

49. The dsRNA agent of claim 48, wherein the strand is the antisense strand.

50. The dsRNA agent of any one of claims 1-49, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

51. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of ketohexokinase (KHK) in a cell, or a pharmaceutically acceptable salt thereof, comprising a sense strand and an antisense strand forming a double stranded region, wherein

a) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-asdCsgadAudCucagdTgCfuuccugcsasc-3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand differs by no more than 4 bases from the nucleotide sequence 5'-asdCsaadAcdAccacdGuCfuccguagscsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

52. The dsRNA agent of claim 51, wherein

a) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-asdCsgadAudCucagdTgCfuuccugcsasc-3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 3 bases from the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand differs by no more than 3 bases from the nucleotide sequence 5'-asdCsaadAcdAccacdGuCfuccguagscsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

53. The dsRNA agent of claim 51, wherein

a) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-asdCsgadAudCucagdTgCfuuccugcsasc-3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 2 bases from the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand differs by no more than 2 bases from the nucleotide sequence 5'-asdCsaadAcdAccacdGuCfuccguagscsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

54. The dsRNA agent of claim 51, wherein

a) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-asdCsgadAudCucagdTgCfuuccugcsasc-3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand differs by no more than 1 base from the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand differs by no more than 1 base from the nucleotide sequence 5'-asdCsaadAcdAccacdGuCfuccguagscsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

55. The dsRNA agent of claim 51, wherein

a) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-gscsaggaagCfAfCfugagauucgu-3' and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'- asdCsgadAudCucagdTgCfuuccugcsasc-3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dT are 2′-deoxy C, A, and T; and s is a phosphorothioate linkage; or

b) the nucleotide sequence of the sense strand comprises the nucleotide sequence 5'-csusacggagAfCfGfugguguuugu-3' and the nucleotide sequence of the antisense strand comprises the nucleotide sequence 5'- asdCsaadAcdAccacdGuCfuccguagscsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U; Cf and Uf are 2′-deoxy-2'-fluoro (2'-F) C and U; dC, dA, and dG are 2′-deoxy C, A, and G; and s is a phosphorothioate linkage.

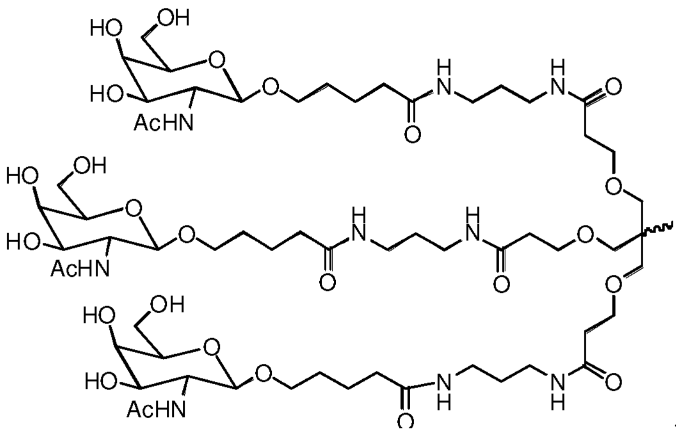
56. The dsRNA agent of any one of claims 50-55, further comprising a ligand.

57. The dsRNA agent of claim 56, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

58. The dsRNA agent of claim 56 or 57, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

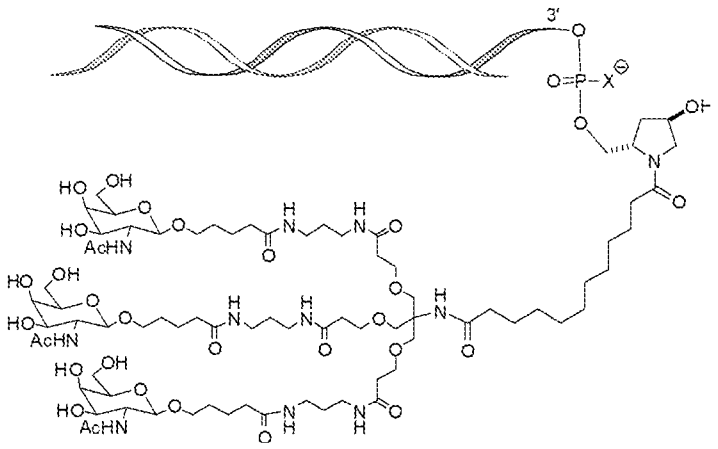
59. The dsRNA agent of any one of claims 56-58, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

60. The dsRNA agent of claim 58 or 59, wherein the ligand is



.

61. The dsRNA agent of claim 60, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

62. The dsRNA agent of claim 61, wherein the X is O.

63. A cell containing the dsRNA agent of any one of claims 1-62.

64. A pharmaceutical composition for inhibiting expression of a gene encoding ketohexokinase (KHK) comprising the dsRNA agent of any one of claims 1-62.

65. The pharmaceutical composition of claim 64, wherein dsRNA agent is in an unbuffered solution.

66. The pharmaceutical composition of claim 65, wherein the unbuffered solution is saline or water.

67. The pharmaceutical composition of claim 64, wherein said dsRNA agent is in a buffer solution.

68. The pharmaceutical composition of claim 67, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

69. The pharmaceutical composition of claim 68, wherein the buffer solution is phosphate buffered saline (PBS).

70. A method of inhibiting expression of a ketohexokinase (KHK) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-62, or the pharmaceutical composition of any one of claims 64-69, thereby inhibiting expression of the KHK gene in the cell.

71. The method of claim 70, wherein the cell is within a subject.

72. The method of claim 71, wherein the subject is a human.

73. The method of claim 71 or 72, wherein the subject has a KHK-associated disorder.

74. The method of claim 73, wherein the KHK-associated disorder is a liver disease selected from the group consisting of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

75. The method of claim 73, wherein the KHK-associated disorder is dyslipidemia selected from the group consisting of hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia, adipocyte dysfunction, visceral adipose deposition, obesity, and metabolic syndrome.

76. The method of claim 73, wherein the KHK-associated disorder is a disorder of glycemic control selected from the group consisting of insulin resistance, type 2 diabetes, and glucose intolerance.

77. The method of claim 73, wherein the KHK-associated disorder is a cardiovascular disease selected from the group consisting of hypertension and endothelial cell dysfunction.

78. The method of claim 73, wherein the KHK-associated disorder is a kidney disease selected from the group consisting of acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, and chronic kidney disease.

79. The method of claim 73, wherein the KHK-associated disorder is hyperuricemia.

80. The method of claim 73, wherein the KHK-associated disorder is gout.

81. The method of any one of claims 56-66, wherein contacting the cell with the dsRNA agent inhibits the expression of KHK by at least 50%, 60%, 70%, 80%, 90%, or 95%.

82. The method of any one of claims 70-81, wherein inhibiting expression of KHK decreases KHK protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

83. A method of treating a subject having a disease or disorder that would benefit from reduction in ketohexokinase (KHK) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-62 or the pharmaceutical composition of any one of claims 64-69, thereby treating the subject having the disorder that would benefit from reduction in KHK expression.

84. A method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in ketohexokinase (KHK) expression, comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-62 or the pharmaceutical composition of any one of claims 64-69, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in KHK expression.

85. The method of claim 83 or 84, wherein the disorder is a KHK-associated disorder.

86. The method of claim 85, wherein the KHK-associated disorder is a liver disease selected from the group consisting of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).

87. The method of claim 85, wherein the KHK-associated disorder is dyslipidemia selected from the group consisting of hyperlipidemia, high LDL cholesterol, low HDL cholesterol, hypertriglyceridemia, postprandial hypertriglyceridemia, adipocyte dysfunction, visceral adipose deposition, obesity, and metabolic syndrome.

88. The method of claim 85, wherein the KHK-associated disorder is a disorder of glycemic control selected from the group consisting of insulin resistance, type 2 diabetes, and glucose intolerance.

89. The method of claim 85, wherein the KHK-associated disorder is a cardiovascular disease selected from the group consisting of hypertension and endothelial cell dysfunction.

90. The method of claim 85, wherein the KHK-associated disorder is a kidney disease selected from the group consisting of acute kidney disorder, tubular dysfunction, proinflammatory changes to the proximal tubules, and chronic kidney disease.

91. The method of claim 85, wherein the KHK-associated disorder is hyperuricemia.

92. The method of claim 85, wherein the KHK-associated disorder is gout.

93. The method of any one of claims 83-92, wherein the subject is human.

94. The method of claim 93, wherein the subject has or is prone to compromised renal function.

95. The method of any one of claims 83-94, wherein the administration of the dsRNA agent to the subject causes a decrease in one or more serum lipid and/or a decrease in KHK protein accumulation.

96. The method of any one of claims 83-95, wherein the administration of the dsRNA agent to the subject causes a decrease in fructose metabolism.

97. The method of any one of claims 83-96, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

98. The method of any one of claims 83-97, wherein the dsRNA agent is administered to the subject subcutaneously.

99. The method of any one of claims 83-98, further comprising determining the level of KHK in a sample(s) from the subject.

100. The method of claim 99, wherein the level of KHK in the subject sample(s) is a KHK protein level in a blood or serum sample(s).

101. The method of claim 83-100, further comprising determining a level of fructose metabolism in a sample(s) from the subject.

102. The method of claim 83-101, further comprising determining a level of uric acid in a sample(s) from the subject.

103. The method of claim 83-102, further comprising determining a level of serum lipid in a sample(s) from the subject.

104. The method of any one of claims 83-103, further comprising administering to the subject an additional therapeutic agent for treatment of a KHK-associated disorder.

105. A kit comprising the dsRNA agent of any one of claims 1-62 or the pharmaceutical composition of any one of claims 64-69.

106. A vial comprising the dsRNA agent of any one of claims 1-62 or the pharmaceutical composition of any one of claims 64-69.

107. A syringe comprising the dsRNA agent of any one of claims 1-62 or the pharmaceutical composition of any one of claims 64-69.

108. An RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of claims 1-62.