COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER AND METABOLIC DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos. 63/495,479, filed April 11, 2023, and 63/427,685, filed November 23, 2022, the contents of each of which are herein incorporated by reference in their entirety.

BACKGROUND

[0002] Human mitochondrial RNA polymerase, POLRMT (also referred to as hmtRNAP), is a nuclear-encoded single-subunit DNA-dependent RNA polymerase. A primary biological role of POLRMT is to transcribe the mitochondrial genome to produce the RNAs needed for expression of mitochondrial DNA (mtDNA). The mitochondrial genome encodes the various subunits of the electron transport chain (see, *e.g.,* Shokolenko, I.N., et al., Annu. Rev. Biochem., 85, 133-160, 2016). Specifically, transcription of the mitochondrial genome is necessary for the expression of 13 subunits of the oxidative phosphorylation (OXPHOS) system, as well as two rRNAs and 22 tRNAs (see, *e.g.,* Shokolenko, I.N., et al., Frontiers in Bioscience, Landmark, 22, 835-853, 2017). Thus, POLRMT is essential for biogenesis of the OXPHOS system, resulting in ATP production. This, in turn, is vital for energy homeostasis in the cell.

[0003] Dysregulation of POLRMT and the OXPHOS system have been implicated in various disease states including cancer and metabolic disease. High rates of OXPHOS have been shown to support growth in cancer cell lines, including in a subset of diffuse large B cell lymphoma cells (see, *e.g.,* DeBeradinis, R.J., Cancer Cell, 22, 423-24, 2012).

[0004] Cancer is now the second leading cause of death in the United States, with projections indicating that almost two million new cases will be diagnosed in 2022 and over 600,000 deaths will be the result of cancer (see Siegel, R.L. et al., CA Cancer J. Clin. (72) 7-33, 2022). Accordingly, there exists a need to develop new therapeutic strategies for treatment and prevention.

SUMMARY

[0005] The present disclosure is based, at least in part, on the insight that, dysregulation of POLRMT and the OXPHOS system have been implicated in various disease states including cancer and metabolic disease. The present disclosure provides, among other things, the recognition that oligonucleotides that inhibit POLRMT are particularly beneficial as a treatment for cancer and metabolic diseases associated with mitochondrial dysfunction.

[0006] In one aspect, the present disclosure provides an oligonucleotide comprising a sequence that is substantially complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript. In some embodiments, the oligonucleotide comprises a sequence that is at least 85%, at least 90%, or at least 95% complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript. In some embodiments, the oligonucleotide comprises a sequence that is perfectly complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript. In some embodiments, the 8 to 30 contiguous nucleotides is 15 to 25 contiguous nucleotides. In some embodiments, the oligonucleotide is 8 to 30 nucleotides in length. In some embodiments, the oligonucleotide is 18 to 22 nucleotides in length. In some embodiments, the oligonucleotide is 20 nucleotides in length.

[0007] In some embodiments, the POLRMT RNA transcript is a human PORLMT RNA transcript. In some embodiments, the human POLRMT RNA transcript comprises SEQ ID NO: 205. In some embodiments, the 8 to 30 contiguous nucleotides is within or includes an exon region of the POLRMT RNA transcript. In some embodiments, the exon comprises an exon identified in any one of Ensemble ID Nos: ENSE00000655271, ENSE00000655279, and ENSE00000655283. In some embodiments, the oligonucleotide is complementary to 16-20 contiguous nucleotides of a sequence that corresponds to nucleotides 817-845, 2415-2446, or 2978-3008 of SEQ ID NO: 205 (*i.e.*, the nucleotide sequences represented in SEQ ID NOs: 725, 726, or 727).

[0008] In some embodiments, the 8 to 30 contiguous nucleotides comprises a sequence that corresponds to nucleotides 2420-2439, 2422-2441, 2983-3002, 2984-3003, 822-839, 823-840, 2421-2438, 2422-2439, 2423-2440, 2424-2441, 2984-3001, 2985-3002, or 2986-3003 of SEQ ID NO: 205.

[0009] In another aspect, the present disclosure provides, an oligonucleotide comprising a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, the oligonucleotide comprises SEQ ID NO: 594. In some embodiments, the oligonucleotide comprises SEQ ID NO: 612. In some embodiments, the oligonucleotide comprises SEQ ID NO: 632.

[0010] In another aspect, the present disclosure provides, an oligonucleotide comprising a sequence that is substantially complementary to a sequence selected from a group consisting of SEQ ID NOs: 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, the oligonucleotide is at least 85%, at least 90%, or at least 95% complementary to a sequence selected from a group consisting of SEQ ID NOs: 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, the oligonucleotide is perfectly complementary to a sequence selected from a group consisting of SEQ ID NOs: 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 663. In some embodiments, the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 681. In some embodiments, the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 701.

[0011] In some embodiments, the oligonucleotide is a chirally pure oligonucleotide.

[0012] In some embodiments, the oligonucleotide comprises at least one modified nucleotide. In some embodiments, the modified nucleotide comprises a base modification, a sugar modification, a sugar phosphate modification, an internucleotidic linkage modification, or a combination thereof.

[0013] In some embodiments, the internucleotidic linkage modification comprises a phosphorothioate or phosphodithioate linkage modification.

[0014] In some embodiments, the sugar modification comprises a 2'-O-methoxyethyl (2'-MOE) modification, a 2'-Fluoro (2'-F) modification, a 2'-O-methyl (2'-O-Me) modification, an unlocked nucleic acid (UNA), or a locked nucleic acid (LNA).

[0015] In some embodiments, the sugar phosphate modification comprises a phosphorodiamidate morpholino (PMO) modification and/or a peptide nucleic acid (PNA) modification.

[0016] In some embodiments, the base modification comprises a 5'-methylcytosine modification or a G-clamp modification.

[0017] In some embodiments, each nucleotide comprises a phosphorothioate (PS) internucleotide linkage.

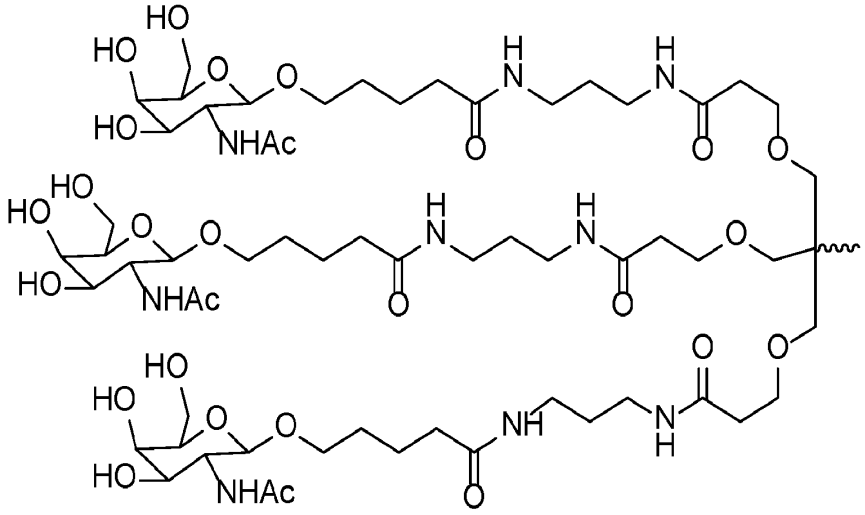
[0018] In some embodiments, the oligonucleotide comprises five nucleotides at the 5'-end and five nucleotides at the 3'-end of the oligonucleotide sequence which contain a 2'-MOE modification. In some embodiments, the oligonucleotide comprises any one of SEQ ID NOs: 728-740.

[0019] In some embodiments, each nucleotide contains a 2'-MOE modification.

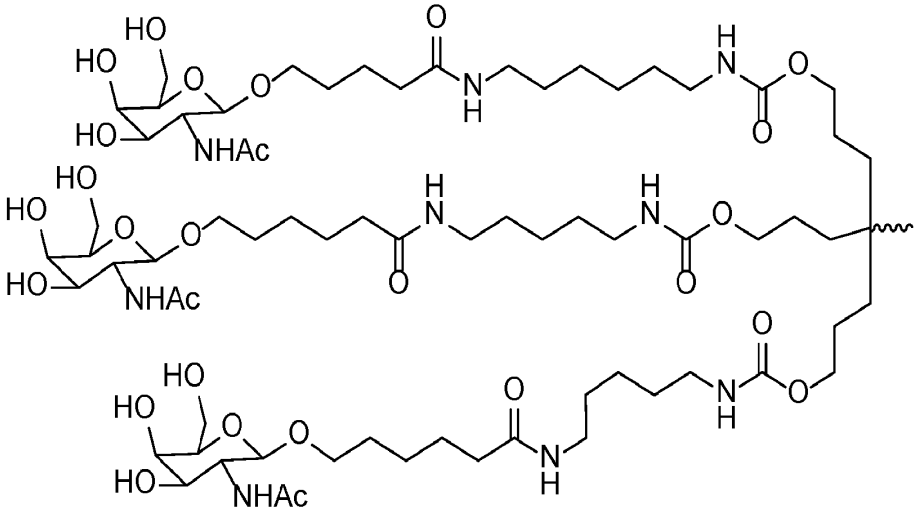
[0020] In some embodiments, the oligonucleotide further comprises at least at least one ligand attached to the 5' end and/or the 3' end. In some embodiments, the ligand comprises at least one lipid, peptide, and/or sugar. In some embodiments, the sugar comprises one or more N-acetylgalactosamine (GalNAc) moieties.

[0021] In some embodiments, the GalNAc moiety comprises a structural formula comprising:

(i) Formula I:

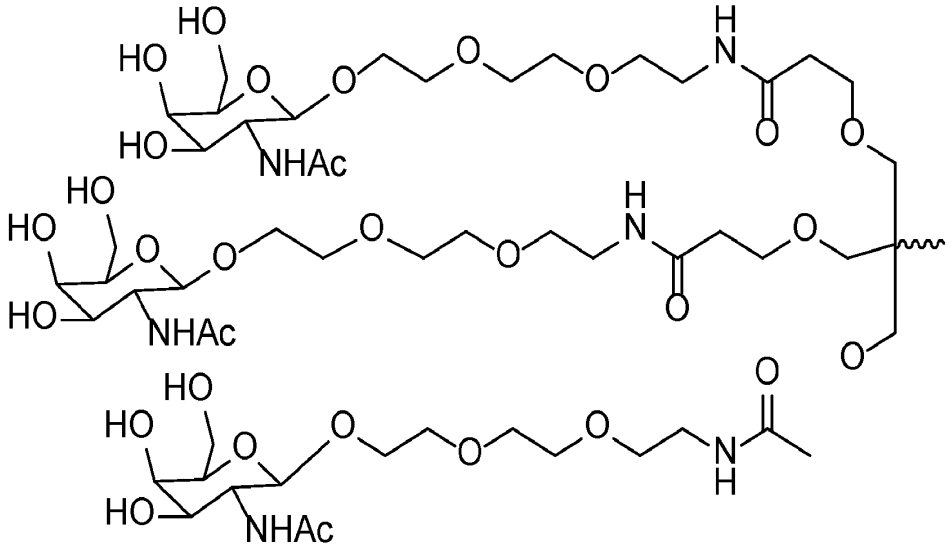


(ii) Formula II:

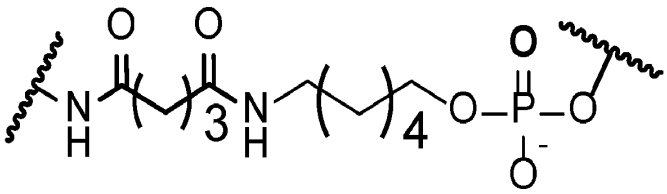


or

(iii) Formula III:



[0022] In some embodiments, the GalNAc moiety is conjugated to the oligonucleotide via a linker. In some embodiments, the linker comprises Formula A as follows:



Formula A.

[0023] In some embodiments, the GalNAc moiety is conjugated to the oligonucleotide via a linker. In some embodiments, a 2' deoxyadenosine phosphodiester is inserted between the oligonucleotide and the one or more GalNAc moieties.

[0024] In some embodiments, the oligonucleotide, when administered to a cell, is capable of reducing the level of POLRMT mRNA expression, POLRMT protein, and/or PORLMT activity in a cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration. In some embodiments, the cell is a human cell.

[0025] In another aspect, the disclosure features an oligonucleotide sequence comprising a sequence that is complementary to a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from a target region that spans between 8 to 30 contiguous nucleotides of SEQ ID NO: 1. In some embodiments, the oligonucleotide comprises a sequence that is complementary to a target region that spans between 8 to 30 contiguous nucleotides of SEQ ID NO: 1. In some embodiments, the target region spans between 15 to 25 contiguous nucleotides of SEQ ID NO: 1. In some embodiments, the target region spans 20 contiguous nucleotides of SEQ ID NO: 1. In some embodiments, the target region comprises an exon region of POLRMT. In some embodiments, the target region comprises a region that corresponds to nucleotides 5696-5715, 8808-8827, 8809-8828, 8811-8830, 16221-16240, 17159-17178, 17314-17333, 17315-17334, 18082-18101, 18083-18102, 18084-18103, 18130-18149, 5680-5699, 8491-8510, 8529-8548, 8569-8588, 8570-8589, 8571-8590, 8572-8591, 8573-8592, 8574-8593, 13322-13341, 13719-13738, 14999-15018, 15092-15111, 15093-15112, 17304-17323, 19309-19328, 20041-20060, 20042-20061, or 21102-21121 of SEQ ID NO: 1.

[0026] In some embodiments, the oligonucleotide comprises a sequence that is substantially complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript.

[0027] In another aspect, the disclosure provides an oligonucleotide comprising a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14. In some embodiments, the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14. In some embodiments, the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 3-14. In some embodiments, the oligonucleotide comprises SEQ ID NO: 11. In some embodiments, the oligonucleotide comprises SEQ ID NO: 12.

[0028] In another aspect, the disclosure provides an oligonucleotide comprising a sequence that is complementary to a sequence that is at least 80% identical to a sequence selected from a group consisting of SEQ ID NOs: 15-26. In some embodiments, the oligonucleotide comprises a sequence that is complementary to a sequence that is at least 90% identical to any one of SEQ ID NOs: 15-26. In some embodiments, the oligonucleotide comprises a sequence that is complementary to a sequence selected from a group consisting of

SEQ ID NOs: 15-26. In some embodiments, the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 23. In some embodiments, the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 24.

[0029] In another aspect, the disclosure provides an oligonucleotide comprising a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NOs: 3-14 and/or is complementary to a nucleotide sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NO: 15-26.

[0030] In another aspect, the disclosure provides an oligonucleotide sequence comprising a sequence that is complementary to a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from a target region that spans between 8 to 30 contiguous nucleotides of SEQ ID NO: 581. In some embodiments, an oligonucleotide comprises a sequence that is complementary to a target region that spans between 8 to 30 contiguous nucleotides of SEQ ID NO: 581. In some embodiments, a target region spans between 15 to 25 contiguous nucleotides of SEQ ID NO: 581. In some embodiments, a target region spans 20 contiguous nucleotides of SEQ ID NO: 581. In some embodiments, a target region comprises an exon region of POLRMT. In some embodiments, a target region comprises a region that corresponds to nucleotides 3348-3367 or 3198-3217 of SEQ ID NO: 581.

[0031] In another aspect, the disclosure provides an oligonucleotide comprising a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 393-486. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 393-486. In some embodiments, an oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 393-486. In some embodiments, an oligonucleotide comprises SEQ ID NO: 434. In some embodiments, an oligonucleotide comprises SEQ ID NO: 442.

[0032] In another aspect, the disclosure provides an oligonucleotide comprising a sequence that is complementary to a sequence that is at least 80% identical to a sequence selected from a group consisting of SEQ ID NOs: 487-580. In some embodiments, an oligonucleotide comprises a sequence that is complementary to a sequence that is at least 90% identical to any one of SEQ ID NOs: 487-580. In some embodiments, an oligonucleotide

comprises a sequence that is complementary to a sequence selected from a group consisting of SEQ ID NOs: 487-580. In some embodiments, an oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 528. In some embodiments, an oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 536.

[0033] In another aspect, the disclosure provides an oligonucleotide comprising a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NOs: 393-486 and/or is complementary to a nucleotide sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NO: 487-580.

[0034] In some embodiments, oligonucleotides according to various aspects of the disclosure are chirally pure oligonucleotides.

[0035] In some embodiments, an oligonucleotide comprises at least one modified nucleotide. In some embodiments, the modified nucleotide comprises a base modification, a sugar or sugar phosphate modification, an internucleotidic linkage modification, or a combination thereof. In some embodiments, the internucleotidic linkage modification comprises a phosphorothioate or phosphodithioate linkage modification. In some embodiments, the sugar or sugar phosphate modification comprises a 2'-O-methoxyethyl (2'-MOE) modification, a 2'-fluoro (2'-F) modification, a 2'-O-methyl (2'-O-Me) modification, a phosphorodiamidate morpholino (PMO) modification, a peptide nucleic acid (PNA) modification, an unlocked nucleic acid (UNA), or a locked nucleic acid (LNA). In some embodiments, the base modification comprises a 5'-methylcytosine modification or a G-clamp modification. In some embodiments, each nucleotide comprises a phosphorothioate (PS) internucleotide linkage. In some embodiments, the oligonucleotide comprises five nucleotides at the 5'-end and five nucleotides at the 3'-end of the oligonucleotide sequence which contain a 2'-MOE modification. In some embodiments, each nucleotide contains a 2'-MOE modification.

[0036] In some embodiments, an oligonucleotide further comprises at least at least one ligand attached to the 5' end and/or the 3' end. In some embodiments, the ligand comprises at least one lipid, peptide, and/or sugar. In some embodiments, the sugar comprises N-acetylgalactosamine (GalNAc) moiety.

[0037] In another aspect, the disclosure provides a composition comprising an oligonucleotide described herein and a carrier and/or excipient.

[0038] In another aspect, the disclosure provides an expression vector comprising one or more sequences encoding one of more oligonucleotides described herein.

[0039] In another aspect, the disclosure provides a method of treating a subject having or at risk of cancer or metabolic disease, the method comprising administering to the subject a composition comprising an effective amount of an oligonucleotide described herein.

[0040] In some embodiments, a level of mitochondrial RNA polymerase (POLRMT) mRNA expression or POLRMT protein in the subject or in a biological sample from the subject after the administration of the composition is reduced relative to a level before the administration of the composition. In some embodiments, the level of POLRMT mRNA expression or POLRMT protein is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration. In some embodiments, the composition is administered intravenously, intrathecally, intramuscularly, orally, intranasaly, or subcutaneously to the subject. In some embodiments, the subject is a human.

[0041] In another aspect, the disclosure provides a method of treating and/or preventing a cancer or a metabolic disease in a subject comprising: administering to the subject an oligonucleotide that is complementary to a target region of a nucleic acid sequence encoding POLRMT.

[0042] In another aspect, the disclosure provides a method of decreasing mitochondrial transcription in a subject that is susceptible to or suffering from cancer or metabolic disease, the method comprising: administering to the subject an oligonucleotide that is complementary to a target region of a nucleic acid sequence encoding POLRMT.

[0043] In some embodiments, the nucleic acid sequence encoding POLRMT comprises SEQ ID NO: 1. In some embodiments, the target region comprises a region that spans between 8 to 30 contiguous nucleotides within SEQ ID NO: 1. In some embodiments, the target region

comprises a region that corresponds to nucleotides 5696-5715, 8808-8827, 8809-8828, 8811-8830, 16221-16240, 17159-17178, 17314-17333, 17315-17334, 18082-18101, 18083-18102, 18084-18103, 18130-18149, 5680-5699, 8491-8510, 8529-8548, 8569-8588, 8570-8589, 8571-8590, 8572-8591, 8573-8592, 8574-8593, 13322-13341, 13719-13738, 14999-15018, 15092-15111, 15093-15112, 17304-17323, 19309-19328, 20041-20060, 20042-20061, or 21102-21121 of SEQ ID NO: 1. In some embodiments, the oligonucleotide comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14. In some embodiments, the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14. In some embodiments, the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 3-14.

[0044] In some embodiments, upon administration of the oligonucleotide to the subject, the level of POLRMT mRNA expression in the subject is decreased. In some embodiments, upon administration of the oligonucleotide to the subject, the level of POLRMT protein or activity in the subject is decreased. In some embodiments, the level of POLRMT mRNA expression, POLRMT protein, or POLRMT activity is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration.

[0045] In some embodiments, the subject is a human. In some embodiments, the metabolic disease comprises include obesity, diabetes, non-alcoholic steatohepatitis (NASH), a disorder of amino acid metabolism (amino acidemias), a disorder of organic acid metabolism (organic acidurias, organic acidemias), a disorder of lipid metabolism (lipid storage disorders), a lysosomal storage disorder, a peroxisomal disorder, phenylketonuria (PKU), a glycogen storage disease, or a urea cycle disorder.

[0046] In some embodiments, the composition is delivered to the liver. In some embodiments, the composition is delivered to the muscle. In some embodiments, the composition is delivered to the CNS. In some embodiments, the composition is delivered to the cerebrospinal fluid.

[0047] In another aspect, the disclosure provides a pharmaceutical composition comprising an oligonucleotide described herein. In some embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable carrier. In some embodiments, the oligonucleotide is formulated in a nanocarrier. In some embodiments, the oligonucleotide is formulated in a lipid nanoparticle (LNP). In some embodiments, the oligonucleotide is conjugated to at least one GalNAc moiety.

[0048] In some embodiments, the composition is formulated for systemic or localized administration. In some embodiments, the composition is formulated for delivery route selected from intrathecal, intramuscular, or intravenous administration.

[0049] In another aspect, the disclosure provides a method of reducing or inhibiting POLRMT expression in a cell, the method comprising contacting the cell with the oligonucleotide described herein. In some embodiments, the level of POLRMT mRNA expression, POLRMT protein, or POLRMT activity is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% relative to the level before the cell is contacted with the oligonucleotide.

[0050] In some embodiments, the cell is in a subject. In some embodiments, the subject is a human. In some embodiments, the human is suffering from or susceptible to cancer or a metabolic disorder.

DEFINITIONS

[0051] Compounds of this invention include those described generally above, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0052] ***Acyl:*** As used herein, the term "acyl" means –C(O)R, wherein R is C_{1-20} aliphatic.

[0053] ***Aliphatic:*** The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle" "cycloaliphatic" or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-6 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-4 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-3 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1-2 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C_3-C_6 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0054] ***Alkyl:*** As used herein, the term "alkyl" means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated and that has a single point of attachment to the rest of the molecule.

[0055] ***Alkenyl:*** As used herein, the term "alkenyl" means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that comprises at least one carbon-carbon double bond and that has a single point of attachment to the rest of the molecule.

[0056] ***Alkynyl:*** As used herein, the term "alkynyl" means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that comprises at least one carbon-carbon triple bond and that has a single point of attachment to the rest of the molecule.

[0057] ***Alkylene:*** As used herein, the term "alkylene" refers to a bivalent alkyl group. An "alkylene chain" is a polymethylene group, i.e., $–(CH_2)_n–$, wherein n is a positive integer,

preferably from 1 to 6, from 1 to 4, from 1 to 3, from 1 to 2, or from 2 to 3. A substituted alkylene chain is a polymethylene group in which one or more methylene hydrogen atoms in the chain are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0058] ***Alkenylene:*** As used herein, the term "alkenylene" refers to a bivalent alkenyl group. A substituted alkenylene chain is a polymethylene group containing at least one carbon-carbon double bond in which one or more hydrogen atoms in the chain are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0059] ***Alkynylene:*** As used herein, the term "alkynylene" refers to a bivalent alkynyl group. A substituted alkynylene chain is a polymethylene group containing at least one carbon-carbon triple bond in which one or more hydrogen atoms in the chain are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0060] ***Approximately:*** As used herein, the terms "approximately" or "about" in reference to a number are generally taken to include numbers that fall within a range of 5%, 10%, 15%, or 20% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

[0061] ***Cancer***: As used herein, the term "cancer" refers to a disease, disorder, or condition in which cells exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they display an abnormally elevated proliferation rate and/or aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In some embodiments, a cancer may be characterized by one or more tumors. Those skilled in the art are aware of a variety of types of cancer including, for example, adrenocortical carcinoma, astrocytoma, basal cell carcinoma, carcinoid, cardiac, cholangiocarcinoma, chordoma, chronic myeloproliferative neoplasms, craniopharyngioma, ductal carcinoma in situ, ependymoma, intraocular melanoma, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), gestational trophoblastic disease, glioma, histiocytosis, leukemia (*e.g.,* acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, myelogenous leukemia, myeloid leukemia), lymphoma (*e.g.,* Burkitt lymphoma [non-Hodgkin lymphoma], cutaneous T cell lymphoma, Hodgkin lymphoma, mycosis fungoides, Sezary syndrome, AIDS-related lymphoma, follicular lymphoma, diffuse large B-cell

lymphoma), melanoma, merkel cell carcinoma, mesothelioma, myeloma (*e.g.*, multiple myeloma), myelodysplastic syndrome, papillomatosis, paraganglioma, pheochromacytoma, pleuropulmonary blastoma, retinoblastoma, sarcoma (*e.g.*, Ewing sarcoma, Kaposi sarcoma, osteosarcoma, rhabdomyosarcoma, uterine sarcoma, vascular sarcoma), Wilms' tumor, and/or cancer of the adrenal cortex, anus, appendix, bile duct, bladder, bone, brain, breast, bronchus, central nervous system, cervix, colon, endometrium, esophagus, eye, fallopian tube, gall bladder, gastrointestinal tract, germ cell, head and neck, heart, intestine, kidney (*e.g.*, Wilms' tumor), larynx, liver, lung (*e.g.*, non-small cell lung cancer, small cell lung cancer), mouth, nasal cavity, oral cavity, ovary, pancreas, rectum, skin, stomach, testes, throat, thyroid, penis, pharynx, peritoneum, pituitary, prostate, rectum, salivary gland, ureter, urethra, uterus, vagina, or vulva.

[0062] ***Aryl:*** As used herein, the term "aryl" used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl", refers to monocyclic and bicyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term "aryl" may be used interchangeably with the term "aryl ring". In certain embodiments of the present invention, "aryl" refers to an aromatic ring system which includes, but not limited to, phenyl, biphenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl", as it is used herein, is a group in which an aromatic ring is fused to one or more non–aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like. In certain preferred embodiments, the term aryl refers to phenyl.

[0063] ***Carbocyclic:*** As used herein, the terms "cycloaliphatic", "carbocycle" or "cycloalkyl" refer to a monocyclic C_3-C_6 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule.

[0064] ***Complementary***: As used herein, in accordance with its art-accepted meaning, "complementary" refers to the capacity for pairing between particular bases, nucleosides, nucleotides or nucleic acids. For example, adenine (A) and uracil (U) are complementary; adenine (A) and thymine (T) are complementary; and guanine (G) and cytosine (C) are complementary and are referred to in the art as Watson-Crick base pairings. If an oligonucleotide, *i.e.*, a molecule comprising nucleotides, at a certain position (at a certain

nucleotide of the oligonucleotide) within its sequence is complementary to a nucleotide in a second oligonucleotide when the oligonucleotides are aligned in anti-parallel orientation, the nucleotides of each oligonucleotide form a complementary base pairing and the oligonucleotides are said to complementary at that certain position. Thus, two oligonucleotides can be characterized by their percent of complimentary base pairing of their nucleotides. For example, the percent complementarity of a first oligonucleotide having a first nucleic acid sequence to a second oligonucleotide having a longer nucleic acid sequence may be evaluated by aligning them in antiparallel orientation and maximizing their complimentary base pairing. When an oligonucleotide is engineered to a target gene, the oligonucleotide may be evaluated for its complementarity to the pre-RNA or mRNA sequence of the target gene, and the alignment is said to be done over a window of evaluation along the RNA sequence. In this example, the percent complementarity of the base pairs in the oligonucleotide to the RNA sequence window is determined by the total number of nucleotides in the oligonucleotide and RNA sequence window that form base pairings, divided by the total number of nucleotides within the RNA sequence window, and multiplying by 100. For example, if the RNA sequence is AATTTGTTATAA, the window of evaluation ("RNA sequence window") may be from nucleic acid at position #3, *i.e.,* T (counting from left to right) to the nucleotide in position number #10, which is also a T. The RNA sequence window of this exemplary RNA sequence is 8 contiguous nucleotides in length. Aligning an oligonucleotide of AAAAAAAA along the aforementioned RNA sequence window would have an optimized alignment resulting in a maximum 75% complementary base parings since there are 6 nucleotides in Watson-Crick base pairings out of a total of 8 nucleotides in the RNA sequence window. A position occupied by two, non-complementary nucleotides constitutes a mismatch, *i.e.,* the position is occupied by a non-complementary base pair. In the above example, 2 of the 8 nucleotides within the RNA sequence window are mismatched. When each nucleotide of an oligonucleotide is base pairing with each nucleotide of a second sequence of equal length (be it another oligonucleotide or RNA sequence window), such sequences can be referred to as "perfectly complementary" (100% complementarity) with respect to each other. Two nucleic acid sequences that are at least 80% complementary over a window of evaluation are considered "substantially complementary" over that window. In certain embodiments, two nucleic acid sequences are at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% complementary over a window of evaluation. Where a first nucleic acid sequence is referred to

as "substantially complementary" with respect to a second nucleic acid sequence herein, they may comprise one or more unmatched bases upon hybridization, *e.g.*, up to about 5%, 10%, 15%, or 20% unmatched bases upon hybridization, *e.g.*, 1, 2, 3, 4, 5, or 6 mismatched base pairs upon hybridization for a duplex up to 30 base pairs. It should be understood that where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs are not regarded as mismatches or unpaired nucleotides with regard to the determination of percent complementarity. "Complementary" sequences, as used herein may include one or more non-Watson-Crick base pairs and/or base pairs formed from non-natural nucleobases, in so far as the 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. Those of ordinary skill in the art are aware that guanine, cytosine, adenine, thymine, and uracil can be replaced by other bases without substantially altering the base pairing properties of a polynucleotide comprising a nucleotide bearing such bases, according to the so-called "wobble" rules (see, e.g., Murphy, FV IV & V Ramakrishnan, V., Nature Structural and Molecular Biology 11: 1251 - 1252 (2004)). For example, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, thymine, or uracil. Thus, nucleotides containing uracil, guanine, thymine, or adenine can be replaced in the nucleic acid sequence of an oligonucleotide described herein by a nucleotide containing, for example, inosine, without decreasing the % complementarity. If a pair of bases is able to base pair (*e.g.*, through Watson-Crick or Wobble base pairing), then such base pairs are considered to be complementary for purposes of determining % complementarity.

[0065] ***Corresponding to***: As used herein, the term "corresponding to" may be used to designate the position/identity of a structural element in a compound or composition through comparison with an appropriate reference compound or composition. For example, in some embodiments, a monomeric residue in a polymer (*e.g.*, an amino acid residue in a polypeptide or a nucleotide residue in an oligonucleotide) may be identified as "corresponding to" a residue in an appropriate reference polymer. For example, those of ordinary skill will appreciate that, for purposes of simplicity, residues in a polypeptide are often designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid "*corresponding to*" a residue at position 190, for example, need not actually be the 190th amino acid in a particular amino acid chain but rather corresponds to the residue found at 190 in the

reference polypeptide; those of ordinary skill in the art readily appreciate how to identify "*corresponding*" amino acids and *"corresponding"* nucleotides. For example, those skilled in the art will be aware of various sequence alignment strategies, including software programs such as, for example, BLAST, CS-BLAST, CUSASW++, DIAMOND, FASTA, GGSEARCH/GLSEARCH, Genoogle, HMMER, HHpred/Hhsearch, IDF, Infernal, KLAST, USEARCH, parasail, PSI-BLAST, PSI-Search, ScalaBLAST, Sequilab, SAM, SSEARCH, SWAPHI, SWAPHI-LS, SWIMM, or SWIPE that can be utilized, for example, to identify "corresponding" residues in polypeptides, oligonucleotides and/or nucleic acid sequences in accordance with the present disclosure.

[0066] ***Halogen:*** As used herein, the term "halogen" means F, Cl, Br, or I.

[0067] ***Heteroaryl:*** As used herein, the terms "heteroaryl" and "heteroar–", used alone or as part of a larger moiety, e.g., "heteroaralkyl", or "heteroaralkoxy", refer to groups having 5 to 10 ring atoms, preferably 5, 6, or 9 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. The term "heteroatom" refers to nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. The terms "heteroaryl" and "heteroar–", as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Nonlimiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4*H*–quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3–b]–1,4–oxazin–3(4H)–one. A heteroaryl group may be mono– or bicyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring", "heteroaryl group", or "heteroaromatic", any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[0068] ***Heteroatom:*** As used herein, the term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

[0069] ***Heterocycle:*** As used herein, the terms "heterocycle", "heterocyclyl", "heterocyclic radical", and "heterocyclic ring" are used interchangeably and refer to a stable 5– to 7–membered monocyclic or 7–10–membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4–dihydro–2*H*–pyrrolyl), NH (as in pyrrolidinyl), or $^+$NR (as in *N*–substituted pyrrolidinyl).

[0070] A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothiophenyl pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms "heterocycle", "heterocyclyl", "heterocyclyl ring", "heterocyclic group", "heterocyclic moiety", and "heterocyclic radical", are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3*H*–indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl, where the radical or point of attachment is on the heterocyclyl ring. A heterocyclyl group may be mono– or bicyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[0071] ***Host cell:*** As used herein, the term "host cell" refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding

generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "*host cell*" as used herein. In some embodiments, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life that are suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence). Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of *E. coli, Bacillus spp., Streptomyces spp.*, etc.), mycobacteria cells, fungal cells, yeast cells (e.g., *S. cerevisiae, S. pombe, P. pastoris, P. methanolica*, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, *Trichoplusia ni*, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO Kl, DXB-1 1 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3 A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes.

[0072] ***Identity***: As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules, such as oligonucleotides) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "substantially identical" to one another if their sequences are at least 80%, 85%, 90%, 95%, or 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (*e.g.*, nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0). In some exemplary embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can alternatively be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0073] ***Linked:*** As used herein, the term "linked", when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another to form a molecular structure that is sufficiently stable so that the moieties remain associated under the conditions in which the linkage is formed and, preferably, under the conditions in which the new molecular structure is used, *e.g.,* physiological conditions. In certain preferred embodiments of the invention the linkage is a covalent linkage. In other embodiments the linkage is noncovalent. Moieties may be linked either directly or indirectly. When two moieties are directly linked, they are either covalently bonded to one another or are in sufficiently close proximity such that intermolecular forces between the two moieties maintain their association. When two moieties are indirectly linked, they are each linked either covalently or noncovalently to a third moiety, which maintains the association between the two moieties. In general, when two moieties are referred to as being linked by a "linker" or "linking moiety" or "linking portion", the linkage between the two linked moieties is indirect, and typically each of the linked moieties is covalently bonded to the linker. The linker can be any suitable moiety that reacts with the two moieties to be linked within a reasonable period of time, under conditions consistent with stability of the moieties (which may be protected as appropriate, depending upon the conditions), and in sufficient amount, to produce a reasonable yield.

[0074] ***Operably linked*:** As used herein, the term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control element "operably linked" to a functional

element is associated in such a way that expression and/or activity of the functional element is achieved under conditions compatible with the control element. In some embodiments, "operably linked" control elements are contiguous (*e.g.*, covalently linked) with the coding elements of interest; in some embodiments, control elements act in trans to or otherwise at a from the functional element of interest.

[0075] ***Optionally substituted or substituted:*** As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[0076] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$, $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; –CH=CHPh, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; $-NO_2$; –CN; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ$, SC(S)SR°; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; –SC(S)SR°, $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)NR^\circ_2$; –

$P(O)_2R°$; -$P(O)R°_2$; -$OP(O)R°_2$; $–OP(O)(OR°)_2$; $SiR°_3$; –($C_{1–4}$ straight or branched alkylene)O–$N(R°)_2$; or –($C_{1–4}$ straight or branched alkylene)C(O)O–$N(R°)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, $C_{1–6}$ aliphatic, $–CH_2Ph$, $–O(CH_2)_{0–1}Ph$, -CH_2-(5-6 membered heteroaryl ring), or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[0077] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $–(CH_2)_{0–2}R^•$, –(haloR•), $–(CH_2)_{0–2}OH$, $–(CH_2)_{0–2}OR^•$, $–(CH_2)_{0–2}CH(OR^•)_2$; -O(haloR•), –CN, $–N_3$, $–(CH_2)_{0–2}C(O)R^•$, $–(CH_2)_{0–2}C(O)OH$, $–(CH_2)_{0–2}C(O)OR^•$, $–(CH_2)_{0–2}SR^•$, $–(CH_2)_{0–2}SH$, $–(CH_2)_{0–2}NH_2$, $–(CH_2)_{0–2}NHR^•$, $–(CH_2)_{0–2}NR^•_2$, $–NO_2$, $–SiR^•_3$, $–OSiR^•_3$, -C(O)SR•, –($C_{1–4}$ straight or branched alkylene)C(O)OR•, or –SSR• wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from $C_{1–4}$ aliphatic, $–CH_2Ph$, $–O(CH_2)_{0–1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[0078] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^*_2$, =NNHC(O)R*, =NNHC(O)OR*, $=NNHS(O)_2R^*$, =NR*, =NOR*, $–O(C(R^*_2))_{2–3}O–$, or $–S(C(R^*_2))_{2–3}S–$, wherein each independent occurrence of R* is selected from hydrogen, $C_{1–6}$ aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $–O(CR^*_2)_{2–3}O–$, wherein each independent occurrence of R* is selected from hydrogen, $C_{1–6}$ aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0079] Suitable substituents on the aliphatic group of R^* include halogen, –$R^\bullet$, -(halo$R^\bullet$), -OH, –$OR^\bullet$, –O(halo$R^\bullet$), –CN, –C(O)OH, –C(O)$OR^\bullet$, –NH_2, –$NHR^\bullet$, –$NR^\bullet_2$, or –NO_2, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, –CH_2Ph, –$O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0080] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include –$R^\dagger$, –$NR^\dagger_2$, –C(O)$R^\dagger$, –C(O)$OR^\dagger$, –C(O)C(O)$R^\dagger$, –$C(O)CH_2C(O)R^\dagger$, –$S(O)_2R^\dagger$, -$S(O)_2NR^\dagger_2$, –C(S)$NR^\dagger_2$, –C(NH)$NR^\dagger_2$, or –$N(R^\dagger)S(O)_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, taken together with their intervening atom(s) form an unsubstituted 3–12–membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0081] Suitable substituents on the aliphatic group of $R^\dagger$ are independently halogen, –$R^\bullet$, (halo$R^\bullet$), –OH, –$OR^\bullet$, –O(halo$R^\bullet$), –CN, –C(O)OH, –C(O)$OR^\bullet$, –NH_2, –$NHR^\bullet$, –$NR^\bullet_2$, or NO_2, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, –CH_2Ph, –$O(CH_2)_{0-1}Ph$, or a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0082] ***Partially unsaturated:*** As used herein, the term "partially unsaturated" refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aryl or heteroaryl moieties, as herein defined.

[0083] ***Recombinant***: As used herein, the term "recombinant" is intended to refer to polypeptides, polynucleotides, or oligonucleotides that are designed, engineered, prepared, expressed, created, manufactured, and/or or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell; polypeptides isolated from a recombinant, combinatorial human polypeptide library; polypeptides isolated from an animal (e.g., a mouse, rabbit, sheep, fish, etc.) that is transgenic for or otherwise has

been manipulated to express a gene or genes, or gene components that encode and/or direct expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof; and/or polypeptides prepared, expressed, created or isolated by any other means that involves splicing or ligating selected nucleic acid sequence elements to one another, chemically synthesizing selected sequence elements, and/or otherwise generating a nucleic acid that encodes and/or directs expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements results from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a natural or synthetic source such as, for example, in the germline of a source organism of interest (e.g., of a human, a mouse, etc.).

[0084] ***Subject:*** As used herein, the term "subject" or "test subject" refers to any organism to which a provided compound or composition is administered in accordance with the present invention *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; *etc.*) and plants. In some embodiments, a subject may be suffering from, and/or susceptible to a disease, disorder, and/or condition.

[0085] ***Substantially:*** As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and/or chemical phenomena.

[0086] ***Suffering from*:** An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[0087] ***Target gene:*** A "target gene", as used herein, refers to a gene whose expression is to be modulated, *e.g.,* inhibited.

[0088] ***Target Region:*** As used herein, the term "target region" refers to a region within the RNA transcript of the target gene where the RNA is to be degraded or translationally

repressed or otherwise inhibited using one or more oligonucleotides. In some embodiments, an oligonucleotide described herein is complementary to a target region (*e.g.*, substantially or perfectly complementary), such that the oligonucleotide is capable of hybridizing to the target region. A target region, as described herein, may be described by its position (*i.e.*, the coordinates of the nucleotides of the target region) within a target RNA sequence or the corresponding region within the target gene sequence. The RNA may be a primary RNA transcript transcribed from the target gene (*e.g.*, a pre-mRNA) or a processed transcript, *e.g.*, mRNA encoding a polypeptide. In some embodiments, a target region of an mRNA is at least long enough to serve as a substrate for RNAase-mediated degradation within that portion in the presence of a suitable oligonucleotides. A target region may be from about 8-36 nucleotides in length, *e.g.*, about 8-30, 10-20, or about 15-30 nucleotides in length. A target region length may have specific value or subrange within the afore-mentioned ranges.

[0089] ***Therapeutic agent:*** As used herein, the phrase "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. In some embodiments, a therapeutic agent is an oligonucleotide designed to target a certain region of target gene.

[0090] ***Therapeutically effective amount:*** As used herein, the term "therapeutically effective amount" means an amount of a substance (*e.g.*, a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, the target cell or tissue, *etc.* For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or signs of the disease, disorder, and/or condition.

In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[0091] ***Treating:*** As used herein, the term "treating" refers to providing treatment, *i.e.*, providing any type of medical or surgical management of a subject. The treatment can be provided in order to reverse, alleviate, inhibit the progression of, prevent or reduce the likelihood of a disease, disorder, or condition, or in order to reverse, alleviate, inhibit or prevent the progression of, prevent or reduce the likelihood of one or more symptoms or manifestations of a disease, disorder or condition. "Prevent" refers to causing a disease, disorder, condition, or symptom or manifestation of such not to occur for at least a period of time in at least some individuals. Treating can include administering an agent to the subject following the development of one or more symptoms or manifestations indicative of a cancer or metabolic-related condition, *e.g.*, in order to reverse, alleviate, reduce the severity of, and/or inhibit or prevent the progression of the condition and/or to reverse, alleviate, reduce the severity of, and/or inhibit or one or more symptoms or manifestations of the condition. A composition of the disclosure can be administered to a subject who has developed cancer or a metabolic-related disorder or is at increased risk of developing such a disorder relative to a member of the general population. A composition of the disclosure can be administered prophylactically, i.e., before development of any symptom or manifestation of the condition. Typically, in this case the subject will be at risk of developing the condition.

[0092] ***Nucleic acid:*** The term "nucleic acid" includes any nucleotides, analogs thereof, and polymers thereof. The term "polynucleotide" or "oligonucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecules and, thus, include double- and single-stranded DNA, and double- and single-stranded RNA. These terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated, protected and/or capped nucleotides or polynucleotides. The terms encompass poly- or oligo-ribonucleotides (RNA) and poly- or oligo-deoxyribonucleotides (DNA); RNA or DNA derived from N-glycosides or C-glycosides of nucleobases and/or modified nucleobases; nucleic acids derived from sugars and/or modified sugars; and nucleic acids derived from phosphate bridges and/or modified phosphorus-

atom bridges (also referred to herein as "internucleotide linkages"). The terms further encompass nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified phosphorus atom bridges. Examples include, and are not limited to, nucleic acids containing ribose moieties, the nucleic acids containing deoxy-ribose moieties and nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. In some embodiments, the prefix poly- refers to a nucleic acid containing 2 to about 10,000, 2 to about 50,000, or 2 to about 100,000 nucleotide monomer units. In some embodiments, the prefix oligo- refers to a nucleic acid containing 2 to about 200 nucleotide monomer units.

[0093] ***Unsaturated:*** The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation.

[0094] ***Vector:*** As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "*plasmid*", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "*expression vectors*."

[0095] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

BRIEF DESCRIPTION OF THE DRAWING

[0096] **FIG. 1** shows a schematic of mitochondria in a cell, including the oxidative phosphorylation system (OXPHOS) and the mitochondrial DNA (mtDNA), which is an exemplary target of the oligonucleotides described herein.

[0097] **FIG. 2** shows a schematic of the various POLRMT RNA transcripts targeted by exemplary oligonucleotides described herein.

[0098] **FIG. 3** shows two exemplary oligonucleotides described herein, including their nucleotide sequence and particular modification pattern. In this figure, a blue shaded circle indicates a 2'-O-MOE group and a red line between nucleotides represents a phosphorothioate (PS) bond.

[0099] **FIG. 4** shows relative POLRMT mRNA expression in HeLa cells transfected with exemplary oligonucleotides.

[0100] **FIG. 5** shows relative CytB mRNA expression in HeLa cells transfected with exemplary oligonucleotides.

[0101] **FIG. 6** shows relative POLRMT mRNA expression in 3T3 cells transfected with exemplary oligonucleotides at 100nM.

[0102] **FIG. 7** shows relative POLRMT mRNA expression in 3T3 cells transfected with exemplary oligonucleotides at 100nM.

[0103] **FIG. 8** shows relative POLRMT mRNA expression in 3T3 cells transfected with exemplary oligonucleotides at 100nM.

[0104] **FIG. 9** shows relative POLRMT mRNA expression in 3T3 cells transfected with exemplary oligonucleotides at 100nM.

[0105] **FIG. 10** shows relative POLRMT mRNA expression in 3T3 cells transfected with exemplary oligonucleotides at 30nM.

[0106] **FIG. 11** shows relative POLRMT mRNA expression in 3T3 cells transfected with exemplary oligonucleotides at 30nM.

[0107] **FIG. 12** shows a schematic of the human POLRMT transcript and exemplary human-mouse matched oligonucleotides arranged based on their target region on the POLRMT transcript. This schematic also identifies three "hotspot" regions identified and described herein.

[0108] **FIG. 13** shows POLRMT expression and cell viability of human 143B and mouse 3T3 cells transfected with exemplary oligonucleotides (corresponding to nucleotide sequences shown in SEQ ID NOs: 612, 613, 623, 624, 632, 633, and 634) at various concentrations. **Panel (A)** shows POLRMT expression in human 143B cells transfected with exemplary oligonucleotides at various concentrations. **Panel (B)** shows viability of human 143B cells transfected with exemplary oligonucleotides at various concentrations. **Panel (C)** shows POLRMT expression in mouse 3T3 cells transfected with exemplary oligonucleotides at various concentrations. **Panel (D)** shows viability of mouse 3T3 cells transfected with exemplary oligonucleotides at various concentrations.

[0109] **FIG. 14** shows POLRMT expression and cell viability of human 143B and mouse 3T3 cells transfected with exemplary oligonucleotides (corresponding to nucleotide sequences shown in SEQ ID NOs: 592, 594, 597, 598, 625, and 626) at various concentrations. **Panel (A)** shows POLRMT expression in human 143B cells transfected with exemplary oligonucleotides at various concentrations. **Panel (B)** shows viability of human 143B cells transfected with exemplary oligonucleotides at various concentrations. **Panel (C)** shows POLRMT expression in mouse 3T3 cells transfected with exemplary oligonucleotides at various concentrations. **Panel (D)** shows viability of mouse 3T3 cells transfected with exemplary oligonucleotides at various concentrations.

[0110] **FIG. 15** shows in vitro toxicity of HepG2 cells and 3T3 cells transfected with various exemplary oligonucleotides described herein at 100nM, expressed as a ratio relative to a vehicle control.

[0111] **FIG. 16** shows expression of POLRMT in HepG2 cells transfected with exemplary oligonucleotides described herein at a concentration of 100nM relative to a vehicle

control (**Panel A**) and viability of HepG2 cells transfected with exemplary oligonucleotides expressed as a percentage (%) relative to a vehicle control (**Panel B**).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Mitochondrial RNA Polymerase (POLRMT), Cancer and Metabolic Disease

[0112] The present disclosure provides, among other things, compositions and methods for treating cancer and metabolic diseases through inhibition of POLRMT.

[0113] Human mitochondrial RNA polymerase, POLRMT (also referred to as hmtRNAP), is a nuclear-encoded single-subunit DNA-dependent RNA polymerase. POLRMT is 1230 amino acids in length and consists of three distinct regions: (1) a C-terminal polymerase domain (CTD) (residues 648–1230); (2) an N-terminal domain (NTD) (residues 369–647); and (3) an N-terminal extension (NTE) (residues 1–368) (see, *e.g.,* Arnold, J.J., et al., Biochim. Biophys. Acta, 1819, 948-960, 2012). It is structurally related to the single-subunit RNA polymerase encoded by bacteriophage T7. The CTD is also known as the catalytic domain due to its function of catalyzing nucleotide incorporation into a growing RNA molecule during transcription. This domain is highly conserved across species, whereas by contrast the NTE demonstrates significant sequence variability, suggesting organism-specific roles for this domain of POLRMT. Structurally, the NTD of POLRMT resembles the N-terminal domain (also called the promoter-binding domain) of T7 RNA polymerase. However, for promoter-specific transcription initiation, POLRMT requires assistance from additional transcription factors, whereas T7 RNA polymerase does not.

[0114] The protein sequence of wildtype human POLRMT is as follows (1230 amino acids):

MSALCWGRGAAGLKRALRPCGRPGLPGKEGTAGGVCGPRRSSSASPQEQDQDRRKDW
GHVELLEVLQARVRQLQAESVSEVVVNRVDVARLPECGSGDGSLQPPRKVQMGAKDA
TPVPCGRWAKILEKDKRTQQMRMQRLKAKLQMPFQSGEFKALTRRLQVEPRLLSKQM
AGCLEDCTRQAPESPWEEQLARLLQEAPGKLSLDVEQAPSGQHSQAQLSGQQQRLLAFF
KCCLLTDQLPLAHHLLVVHHGQRQKRKLLTLDMYNAVMLGWARQGAFKELVYVLFM

VKDAGLTPDLLSYAAALQCMGRQDQDAGTIERCLEQMSQEGLKLQALFTAVLLSEEDR
ATVLKAVHKVKPTFSLPPQLPPPVNTSKLLRDVYAKDGRVSYPKLHLPLKTLQCLFEKQ
LHMELASRVCVVSVEKPTLPSKEVKHARKTLKTLRDQWEKALCRALRETKNRLEREVY
EGRFSLYPFLCLLDEREVVRMLLQVLQALPAQGESFTTLARELSARTFSRHVVQRQRVS
GQVQALQNHYRKYLCLLASDAEVPEPCLPRQYWEELGAPEALREQPWPLPVQMELGKL
LAEMLVQATQMPCSLDKPHRSSRLVPVLYHVYSFRNVQQIGILKPHPAYVQLLEKAAEP
TLTFEAVDVPMLCPPLPWTSPHSGAFLLSPTKLMRTVEGATQHQELLETCPPTALHGAL
DALTQLGNCAWRVNGRVLDLVLQLFQAKGCPQLGVPAPPSEAPQPPEAHLPHSAAPAR
KAELRRELAHCQKVAREMHSLRAEALYRLSLAQHLRDRVFWLPHNMDFRGRTYPCPP
HFNHLGSDVARALLEFAQGRPLGPHGLDWLKIHLVNLTGLKKREPLRKRLAFAEEVMD
DILDSADQPLTGRKWWMGAEEPWQTLACCMEVANAVRASDPAAYVSHLPVHQDGSC
NGLQHYAALGRDSVGAASVNLEPSDVPQDVYSGVAAQVEVFRRQDAQRGMRVAQVL
EGFITRKVVKQTVMTVVYGVTRYGGRLQIEKRLRELSDFPQEFVWEASHYLVRQVFKS
LQEMFSGTRAIQHWLTESARLISHMGSVVEWVTPLGVPVIQPYRLDSKVKQIGGGIQSIT
YTHNGDISRKPNTRKQKNGFPPNFIHSLDSSHMMLTALHCYRKGLTFVSVHDCYWTHA
ADVSVMNQVCREQFVRLHSEPILQDLSRFLVKRFCSEPQKILEASQLKETLQAVPKPGAF
DLEQVKRSTYFFS (**SEQ ID NO: 2**, transit peptide)

[0115] There are 8 known mRNA splice variants of the POLRMT gene, which are publicly available, and identified in ENSEMBL IDs: ENST00000588649.7, ENST00000590573.4, ENST00000590336.2, ENST00000592863.2, ENST00000587057.5, ENST00000590709.3, ENST00000589961.2, and ENST00000592633.5. The POLRMT mRNA transcript that encodes the full-length POLRMT protein (identified above in SEQ ID NO: 2) is identified in ENSEMBL ID: ENST00000588649.7 (corresponding to SEQ ID NO: 205).

[0116] A primary biological role of POLRMT is to transcribe the mitochondrial genome to produce the RNAs needed for expression of mitochondrial DNA (mtDNA). Initiation, elongation, and termination are the three steps of mitochondrial transcription. Each of a light-strand promoter (LSP) and two heavy-strand promoters (HSP-1 and HSP-2) on the mtDNA contains a transcription initiation site (see, e.g., Basu, U. et al., J. Biol. Chem., 295(52), 18406-425, 2020). For promoter-specific transcription initiation, POLRMT requires two transcription factors, TFAM (transcription factor A mitochondrial) and TFB2M (transcription factor B

mitochondrial). *See id.* Various models suggest different mechanisms by which the initiation complex structure with POLRMT, TFAM, and TFB2M comes together to cover the promoter DNA for initiation of transcription. In one current model TFAM recruits POLRMT to the promoter site to form a protein-protein pre-initiation complex, to which TFB2M binds to form the initiation complex, which covers the promoter DNA. *See id.* During initiation, the RNA is elongated to about 8-10 nucleotides in length. Conformational changes occur at that point, including promoter release and displacement of the initiation factors, converting the initiation complex into an elongation complex at which time transcription occurs. *See id.*

[0117] The mitochondrial genome encodes the various subunits of the electron transport chain (see, *e.g.,* Shokolenko, I.N., et al., Annu. Rev. Biochem., 85, 133-160, 2016). Specifically, transcription of the mitochondrial genome is necessary for the expression of 13 subunits of the oxidative phosphorylation (OXPHOS) system, as well as two rRNAs and 22 tRNAs (see, *e.g.,* Shokolenko, I.N., et al., Frontiers in Bioscience, Landmark, 22, 835-853, 2017). Thus, POLRMT is essential for biogenesis of the OXPHOS system, resulting in ATP production. This, in turn, is vital for energy homeostasis in the cell. **FIG. 1** shows a schematic of the mitochondria, including the OXPHOS system and the mtDNA genome.

[0118] Dysregulation of POLRMT and the OXPHOS system have been implicated in various disease states, in particular cancer. Cancer is now the second leading cause of death in the United States, with projections indicating that almost two million new cases will be diagnosed in 2022 and over 600,000 deaths will be the result of cancer (see Siegel, R.L. et al., CA Cancer J. Clin. (72) 7-33, 2022). High rates of OXPHOS have been shown to support growth in cancer cell lines, including in a subset of diffuse large B cell lymphoma cells (see, *e.g.,* DeBeradinis, R.J., Cancer Cell, 22, 423-24, 2012). Noteworthy is the observation that metabolic heterogeneity exists not only between different types of cancer, but also among tumors of the same type. Similarly, in a study using melanoma cell lines representative of various stages of tumor progression and that collectively mimic the mixture of cells found in a tumor, it was found that metastatic cells demonstrated a high OXPHOS capacity (Rodrigues, M.F., et al., Biochem. J. 473: 703-715, 2016). These data suggest mitochondria play a role as cells progress toward metastasis, possibly to provide the energy needed for tumor cell migration and invasion.

[0119] Relatedly, overexpression of POLRMT has been linked to multiple types of cancers, suggesting that it plays a role in tumor growth. Supporting this hypothesis is, for example, a study involving acute myeloid leukemia (AML) cells, which are known to have high oxidative phosphorylation and mitochondrial mass, as well as low respiratory chain spare reserve capacity. POLRMT knockdown in AML cells demonstrated a reduction in POLRMT levels, decreased oxidative phosphorylation, and increased cell death as compared to control AML cells (see Bralha, F.N., et al., Oncotarget, 6(35), 37216-228, 2015). In other work, injection into nude mice of a human breast cancer cell line that overexpresses POLRMT resulted in increased tumor growth, independent of tumor angiogenesis, suggesting that POLRMT should be considered a tumor promoter or metabolic oncogene (Salem, A.F., et al. Cell Cycle, 11(22), 4174-80, 2012). Recently, the expression of POLRMT in non-small cell lung cancer (NSCLC) has been examined (see Zhou, T. et al., Cell Death and Disease, 12, 751, 2021).

[0120] The development of multidrug resistance (MDR) to numerous cancers is associated with poor prognosis and presents significant challenges in the treatment of this disease. Because such resistance encompasses drugs having different structures and mechanisms of action, identifying and targeting a single biochemical pathway that could re-sensitize MDR cancer cells to established chemotherapy would provide a promising treatment strategy (see Yu, H.-J., Front. Chem., 9:775226, 2021). A main reason for the development of MDR is enhanced drug efflux from and decreased drug accumulation in MDR cells due to ATP-dependent protein transporters that pump drugs out of cells. Inhibiting POLRMT and consequently the production of the proteins essential for the OXPHOS system could compromise ATP production and, in turn, the ATP-dependent efflux of chemotherapeutic agents from cancer cells.

[0121] Consistent with the findings that the OXPHOS system and POLRMT may be involved in the etiology of and in some cases overexpressed in some cancers, small molecule inhibitors of POLRMT have been developed (see, e.g., EP 3 598 972 A1; WO 2019/057821 A1; and WO 2020/188049 A1, which are herein incorporated by reference in their entirety). Some of these inhibitors have been shown to be useful in inhibiting cancer cell proliferation without affecting control cells (see Bonekamp, N.A., et al., Nature, 588, 712-716, 2020). The cancer cell toxicity was correlated to a considerable increase in the levels of mono- and diphosphate nucleotides with a concomitant decrease in nucleotide triphosphate levels, all the result of a

debilitated OXPHOS system. Similarly, treatment with POLRMT inhibitors caused a decrease in citric-acid cycle intermediates and ultimately cellular amino acid levels, the result of which is a state of severe energy and nutrient depletion. *See id.* Such inhibitors also produced a decrease in tumor volume in mice with no significant toxicity in control animals. Specifically, mtDNA transcript levels in tumor cells were decreased as compared to transcript levels in differentiated tissue. These data highlight the importance of mtDNA expression in rapidly dividing cells as opposed to post-mitotic tissue, a distinction that may be capitalized on using POLRMT inhibitors that are capable of modulating mtDNA transcription and ultimately the OXPHOS system.

[0122] While mitochondria are an emerging target for cancer treatment, the resistance mechanisms induced by chronic inhibition of mitochondrial function are poorly understood. In view of the challenges presented by drug resistance in cancer chemotherapy, the development of such resistance to small molecule inhibitors of POLRMT has been investigated (see Mennuni, M. et al., EMBO reports, 23: e53054 1-18, 2022). Using a CRISPR-Cas9 whole-genome screen, loss of genes belonging to von Hippel–Lindau (VHL) and mammalian target of rapamycin complex 1 (mTORC1) were the pathways that caused resistance to acute treatment with a POLRMT inhibitor. *See id.* at pp. 1-2. Moreover, dose-escalated chronic treatment of cells with this molecule resulted in drug-resistant cells that had increased levels of mtDNA, thereby giving rise to increased levels of mitochondrial transcripts and proteins. *See id.* at p. 5. The drug-resistant cells maintained higher levels of nucleotide levels, tricarboxylic acid cycle intermediates, and amino acids. *See id.* at p. 7. Notably, the drug-resistant cells did not have mutations in POLRMT that compromise inhibitor binding to the polymerase. *See id.* The development of resistance to POLRMT inhibitors underscores the importance and need for the development of other POLRMT inhibitors to understand and treat cancers of varying types.

[0123] Alterations in the OXPHOS system also have been implicated in the development of metabolic diseases such as insulin resistance and ultimately Type-2 diabetes. In studies involving apoptosis inducing factor (AIF) knockout mice, a primary OXPHOS defect that produced OXPHOS deficiency revealed an increase in insulin sensitivity and resistance to diabetes and obesity (see Pospisilik, J.A., et al., Cell, 131, 476-91, 2007). Correlated with these phenotypic changes were the metabolic alterations of increased glucose uptake and enhanced fuel utilization. Manipulation of the OXPHOS system with POLRMT modulators affords the

potential for further understanding the physiological mechanisms involved in diseases such as diabetes and for the development of novel treatments for intervention of such metabolic disorders.

[0124] In addition to its critical role in transcription, POLRMT acts as the primase for mtDNA replication, thus playing a part in the regulation of mtDNA levels. Human mtDNA is a circular double-stranded DNA that is packaged in DNA-protein structures called mitochondrial nucleoids, for which TFAM is the most abundant structural component (see, e.g., Filograna, R., et al., FEBS Letters, 595, 976-1002, 2021). TFAM facilitates mtDNA compaction, which results in regulating the accessibility of the DNA to cellular replication and transcription components. With respect to mtDNA replication, POLRMT is part of the mtDNA replisome along with the hexameric helicase TWINKLE, the heterotrimeric DNA polymerase gamma (POLγ) and the tetrameric mitochondrial single-stranded DNA-binding protein (mtSSB). *See id.* Its function in this replisome is to synthesize the RNA primers required for the initiation of the synthesis of both strands of mtDNA. While there may be many mechanisms by which mtDNA levels may be regulated, including modulation of POLRMT, what is known to date is that mtDNA copy number can be manipulated through modulation of TFAM expression.

[0125] While the correlation is not completely straightforward, changed levels of mtDNA have been implicated in neurodegenerative disorders, cancer, and aging (see *e.g.*, Filograna, R., et al., FEBS Letters, 595, 976-1002, 2021). Particularly challenging is the attempt to understand the relationship between mtDNA copy number and cancer. It appears that such copy number can correlate with both increased and decreased disease burden. As such, tumor type and stage of disease may be important factors in determining the role of mtDNA copy number in the diagnosis and/or prognosis of cancer. With respect to aging, most data show a reduction in mtDNA levels in the older population. That being said, other study data are inconsistent as to the relationship between mtDNA copy number and longevity. By contrast, there appears to be a clearer correlation between neurodegeneration in Alzheimer's disease and reduction in mtDNA levels. Complicating the understanding of the relationship between mtDNA levels and disease is the role that mtDNA mutations have on various disorders. While accumulation of mtDNA mutations appears to occur in almost all types of cancer, it is unclear whether such mutations are causative of the cancer or merely a by-product of rapid replication in

fast-dividing cells. Nonetheless, since POLRMT plays a key role in mtDNA replication, POLMRT modulation may provide an effective mechanism by which to understand various disease states and how to slow or alter the progression of disease.

[0126] Mutations affecting POLRMT may also cause human disease (see Oláhová, M., et al., Nat. Commun., 12, 1135, 2021). POLRMT variants have been identified in a number of unrelated families. Patients present with multiple phenotypes, including global developmental delay, hypotonia, short stature, and speech/intellectual disability in childhood. POLRMT modulation may provide a mechanism to slow or alter the progression of disease.

[0127] POLRMT is of fundamental importance for both expression and replication of the human mitochondrial genome. While aspects of POLRMT biochemistry are known, its full physiological role in mitochondrial gene expression and homeostasis, as well as its underlying impact in the etiology of various disease states, remains unclear. Its dysfunction and/or deregulation impacts mitochondrial metabolism, sometimes through the OXPHOS system, which ultimately contributes to many metabolic, degenerative and age-related diseases such as cancer, diabetes, obesity, and Alzheimer's disease. Inhibition of POLRMT is one means by which to gain a further understanding of the role of this polymerase in cell physiology and the development of disease. Regulation of metabolic mechanisms, including oxidative phosphorylation, with POLRMT modulators affords an opportunity for intervention in complex disorders. In view of the numerous and varied roles of POLRMT, the need exists for potent and specific modulators of POLRMT.

POLRMT Oligonucleotides

[0128] In some embodiments, the present disclosure provides oligonucleotides that bind to and inhibit expression of messenger RNA (mRNA) produced by a target gene (*e.g.*, POLRMT). As used herein, the terms "oligonucleotides" and "antisense oligonucleotides" are used interchangeably.

[0129] In some embodiments, administration of an oligonucleotide can decrease or inhibit mRNA expression of POLRMT in a subject or in a biological sample compared to a level before administration. In some embodiments, administration of an oligonucleotide can decrease level of POLRMT protein in a subject or in a biological sample compared to a level before

administration. In some embodiments, administration of an oligonucleotide can decrease POLRMT activity (thereby decreasing mitochondrial transcription) in a subject or in a biological sample compared to a level before administration.

[0130] Indications of decreased POLRMT activity include decreased level of mitochondrial transcription. In some embodiments, decreased level of mitochondrial transcription can be measured by total mtDNA. Additionally, decreased mitochondrial transcription can also be indicated by a decrease in mRNA expression of various mitochondrial proteins, for example, decreased mRNA expression of Cytochrome B. Other mitochondrial proteins include various subunits of the electron transport chain (see, *e.g.,* Shokolenko, I.N., et al., Annu. Rev. Biochem., 85, 133-160, 2016), and more specifically, the 13 subunits of the oxidative phosphorylation (OXPHOS) system (see, *e.g.,* Shokolenko, I.N., et al., Frontiers in Bioscience, Landmark, 22, 835-853, 2017). Indications of decreased POLRMT activity also include decreased level ATP production.

[0131] In some embodiments, when oligonucleotides described herein are administered to a cell, level of POLRMT mRNA expression, POLRMT protein, and/or POLRMT activity is reduced in the cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration. In some embodiments, administration of an oligonucleotide can lead to complete or substantially complete inhibition of POLRMT mRNA expression.

[0132] In some embodiments, an oligonucleotide described herein is an RNase H-dependent oligonucleotide, wherein the oligonucleotide induces the degradation of mRNA by RNase H. In some embodiments, an oligonucleotide inhibits expression of a target gene through steric-blocking, wherein the oligonucleotide physically prevents or inhibits the progression of splicing or translational machinery. Oligonucleotides, as described herein, are capable of hybridizing to a target region of a target nucleic acid, resulting in at least one antisense activity. In some embodiments, antisense activity comprises degradation of a target nucleic acid by RNase H. In some embodiments, antisense activity comprises an oligonucleotide physically preventing or inhibiting the progression of splicing or translational machinery.

[0133] In some embodiments, oligonucleotides described herein specifically hybridize to one or more target regions on an RNA transcript of a target gene. In some embodiments, a target region comprises a region of an mRNA (*e.g.,* a region within SEQ ID NO: 205). In some embodiments, a target region comprises a region of a pre-mRNA. In some embodiments, a target region comprises a region of pre-mRNA that spans an exon/intron junction. In some embodiments, a target region comprises a region of pre-mRNA spanning or including an intron region. In some embodiments, a target region corresponds to a region of a DNA sequence, *i.e.,* a target gene sequence. In some embodiments, a target region comprises a region near to, that includes or is within a 5'-UTR region. In some embodiments, a target region comprises a region near to, that includes, or is within a 3'-UTR region. In some embodiments, a target region comprises a region near to, that includes, or is within an exon region (*e.g.,* as shown in the transcripts of FIG. 2).

[0134] Exemplary target regions as described herein are shown in several POLRMT transcripts as shown in FIG. 2. The amino acid and nucleotide sequences of human POLRMT are known in the art and can be found in publicly available databases. For example, POLRMT transcript sequences are identified in Accession Numbers NM_005035.4, XM_005259580.5, XM_047438952.1, and XM_047438951.1 and ENSEMBL IDs of the 8 known mRNA transcripts are identified in: ENST00000588649.7, ENST00000590573.4, ENST00000590336.2, ENST00000592863.2, ENST00000587057.5, ENST00000590709.3, ENST00000589961.2, and ENST00000592633.5. The POLRMT mRNA transcript that encodes the full-length POLRMT protein (SEQ ID NO: 2) is identified in ENSEMBL ID: ENST00000588649.7 (corresponding to SEQ ID NO: 205). Additionally, the full human POLRMT gene sequence is represented in Reference No. NG_023049.1 (SEQ ID NO: 1).

[0135] A POLRMT mRNA transcript sequence is presented herein in SEQ ID NO: 205 (ENSEMBL ID: ENST00000588649.7), where U residues are represented by T residues in the provided sequence. One of ordinary skill in the art will appreciate that where one refers to a sequence as "RNA" or "mRNA" or "pre-mRNA" or "transcript" the actual sequence contains U rather than T, but may be presented either way in the present disclosure.

[0136] Strategies for targeting particular regions of the POLRMT transcript, corresponding to regions with the gene sequence (*e.g.,* SEQ ID NO: 1, NCBI Reference No. NG_023049.1), may be utilized in targeting a region within one or more POLRMT transcripts. **FIG. 2** provides several exemplary POLRMT transcript sequences that may be targeted by oligonucleotides described herein (*e.g.,* as identified in Accession Numbers NM_005035.4 (ENST00000588649.7 corresponding to SEQ ID NO: 205), XM_005259580.5, XM_047438952.1, and XM_047438951.1). One of skill in the art understands that an oligonucleotide targeting a region within SEQ ID NO: 205 may also target the corresponding region in other POLRMT RNA transcripts, although they may vary slightly in the exact coordinates within the nucleic acid sequence. Additionally, one of skill in the art will understand that a target region within a POLRMT transcript (*e.g.,* SEQ ID NO: 205) may be characterized by the corresponding coordinates within the full POLRMT gene sequence (SEQ ID NO: 1).

[0137] In some embodiments, an oligonucleotide is capable of targeting a POLRMT sequence of one or more non-human species, *e.g.,* a non-human primate POLRMT, *e.g., Macaca fascicularis* POLRMT, or *e.g., Chlorocebus sabaeus* in addition to human POLRMT. Such sequences are known in the art and publicly available. In some embodiments, an oligonucleotide is complementary to a target region that is identical in the human and *Macaca fascicularis* POLRMT transcripts. In some embodiments, an oligonucleotide is complementary to a target region of a human POLRMT transcript that differs by 1, 2, or 3 nucleotides from a sequence in a *Macaca fascicularis* POLRMT transcript. It will be appreciated that an oligonucleotide that targets human POLRMT and inhibits or decreases POLRMT expression level may also have such an effect on non-primate POLRMT *e.g.,* rat or mouse POLRMT, particularly if conserved regions of POLRMT transcript are targeted. One of skill in the art understands that a target region of an oligonucleotide within a mouse POLRMT RNA transcript (*e.g.,* SEQ ID NO: 582) may target a corresponding region within a human POLRMT RNA transcript (*e.g.,* SEQ ID NO: 205), particularly if the target region is within a conserved region of POLRMT RNA transcript.

[0138] In some embodiments, an oligonucleotide has a nucleotide sequence comprising a region having sufficient complementarity to a target nucleic acid sequence to allow hybridization and insufficient complementarity to any non-target nucleic acid sequences so as to avoid non-specific hybridization to any non-target nucleic acid sequences under conditions in which

specific hybridization is desired (*e.g.*, under physiological conditions for *in vivo* or therapeutic uses, and under conditions in which assays are performed in the case of *in vitro* assays).

[0139] In some embodiments, the present disclosure provides oligonucleotides that are perfectly complementary to a target nucleotide sequence over the entire length of the oligonucleotide. In some embodiments, an oligonucleotide is at least 95% complementary to a PORLMT nucleotide sequence over the entire length of the oligonucleotide. In some embodiments, an oligonucleotide is at least 90% complementary to a PORLMT nucleotide sequence over the entire length of the oligonucleotide. In some embodiments, an oligonucleotide is at least 85% complementary to a POLRMT nucleotide sequence over the entire length of the oligonucleotide. In some embodiments, an oligonucleotide is at least 80% complementary to a POLRMT nucleotide sequence over the entire length of the oligonucleotide. In some embodiments, an oligonucleotide is between 80% and 100% complementary to a POLRMT nucleotide sequence over the entire length of the oligonucleotide (*i.e.*, substantially complementary). In some embodiments, an oligonucleotide comprises a region that is perfectly complementary to a POLRMT nucleotide sequence and is at least 80% complementary to the POLRMT nucleotide sequence over the entire length of the oligonucleotide. In some embodiments, the region of perfect complementarity is from 6 to 30 nucleotides in length.

[0140] In some embodiments, an oligonucleotide comprises DNA. In some embodiments, an oligonucleotide comprises RNA. In some embodiments, an oligonucleotide comprises both RNA and DNA. In some embodiments, an oligonucleotide is between 5 and 100 nucleotides in length. In some embodiments, an oligonucleotide is between 5 and 90 nucleotides in length. In some embodiments, an oligonucleotide is between 5 and 80 nucleotides in length. In some embodiments, an oligonucleotide is between 5 and 70 nucleotides in length. In some embodiments, an oligonucleotide is between 5 and 60 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 50 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 40 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 30 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 25 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 20 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 15 nucleotides in length. In some embodiments, an oligonucleotide is 5 to 10 nucleotides in length. In some embodiments, an oligonucleotide is 10

to 100 nucleotides in length. In some embodiments, an oligonucleotide is 15 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 20 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 25 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 30 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 40 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 50 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 60 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 70 to 100 nucleotides in length. In some embodiments, an oligonucleotide is 90 to 100 nucleotides in length. In some embodiments, an oligonucleotide is between 8 and 30 nucleotides in length. In some embodiments, an oligonucleotide is 15 to 25 nucleotides in length. In some embodiments, an oligonucleotide is 16 to 22 nucleotides in length. In some embodiments, an oligonucleotide is 18 to 20 nucleotides in length. In some embodiments, an oligonucleotide is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. In some embodiments, an oligonucleotide is 18 nucleotides in length. In some embodiments, an oligonucleotide is 20 nucleotides in length. In some embodiments, an oligonucleotide is 19 nucleotides in length.

[0141] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14, 27-45, 71-147, 205-298, and 587-655. In some embodiments, an oligonucleotide comprises a sequence having at least 85% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14, 27-45, 71-147, 205-298, and 587-655. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14, 27-45, 71-147, 205-298, and 587-655. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14, 27-45, 71-147, 205-298, and 587-655. In some embodiments, an oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 3-14, 27-45, 71-147, 205-298, and 587-655.

[0142] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, and 728-740. In some embodiments, an oligonucleotide comprises a sequence having at least 85% identity to a sequence selected from a

group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, and 728-740. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, and 728-740. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, and 728-740. In some embodiments, an oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, and 728-740.

[0143] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 11. In some embodiments, an oligonucleotide comprises a sequence having at least 85% to SEQ ID NO: 11. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 11. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 11. In some embodiments, an oligonucleotide comprises SEQ ID NO: 11.

[0144] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 12. In some embodiments, an oligonucleotide comprises a sequence having at least 85% to SEQ ID NO: 12. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 12. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 12. In some embodiments, an oligonucleotide comprises SEQ ID NO: 12.

[0145] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 594. In some embodiments, an oligonucleotide comprises a sequence having at least 85% to SEQ ID NO: 594. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 594. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 594. In some embodiments, an oligonucleotide comprises SEQ ID NO: 594.

[0146] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 612. In some embodiments, an oligonucleotide comprises a

sequence having at least 85% to SEQ ID NO: 612. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 612. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 612. In some embodiments, an oligonucleotide comprises SEQ ID NO: 612.

[0147] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 632. In some embodiments, an oligonucleotide comprises a sequence having at least 85% to SEQ ID NO: 632. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 632. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 632. In some embodiments, an oligonucleotide comprises SEQ ID NO: 632.

[0148] In some embodiments, an oligonucleotide comprises a nucleotide sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOs: 3-14, 27-45, 71-147, 205-298, and 587-655 in the following Table 1.

[0149] In some embodiments, an oligonucleotide comprises a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of the sequences listed below in Table 1. Table 1 includes exemplary POLRMT oligonucleotide sequences, the target region of complementarity in the POLRMT mRNA transcript, and the corresponding coordinates of the target region within the POLRMT gene sequence as shown in SEQ ID NO: 1.

Table 1: Oligonucleotide Sequences, Target Region on PORLMT transcript, and Corresponding Coordinates of the Target Region on POLRMT gene sequence (SEQ ID NO: 1)

SEQ ID NO:	Oligonucleotide Sequence (5' to 3')	Target Region	Corresponding Coordinates within SEQ ID NO: 1
3	CCCCAGTCCTTCCTGCGGTC	Exon	5696-5715
4	TCCACATCGAGGCTCAGCTT	Exon	8808-8827
5	CTCCACATCGAGGCTCAGCT	Exon	8809-8828
6	TGCTCCACATCGAGGCTCAG	Exon	8811-8830
7	CCCCAGCTCCTCCCAGTACT	Exon	16221-16240
8	GGCCACCTTCTGGCAGTGCG	Exon	17159-17178
9	GCCACGTCGCTGCCCAGGTG	Exon	17314-17333
10	CGCCACGTCGCTGCCCAGGT	Exon	17315-17334
11	CCCATCCACCACTTTCGGCC	Exon	18082-18101

12	GCCCATCCACCACTTTCGGC	Exon	18083-18102
13	CGCCCATCCACCACTTTCGG	Exon	18084-18103
14	TTCGCCACCTCCATACAGCA	Exon	18130-18149
27	GGTCTTGGTCTTGCTCCTGG	Exon	5680-5699
28	GAGGCTACCATCTCCACTGC	Exon	8491-8510
29	GCATCCTTGGCCCCCATCTG	Exon	8529-8548
30	CTCCAGTATCTTTGCCCAGC	Exon	8569-8588
31	TCTCCAGTATCTTTGCCCAG	Exon	8570-8589
32	TTCTCCAGTATCTTTGCCCA	Exon	8571-8590
33	CTTCTCCAGTATCTTTGCCC	Exon	8572-8591
34	CCTTCTCCAGTATCTTTGCC	Exon	8573-8592
35	TCCTTCTCCAGTATCTTTGC	Exon	8574-8593
36	CACATATACCAGCTCCTTGA	Exon	13322-13341
37	TCCTCCTCAGACAGCAGAAC	Exon	13719-13738
38	CACTGCAGGGTCTTCAAGGG	Exon	14999-15018
39	TGCTTGACCTCCTTGCTTGG	Exon	15092-15111
40	GTGCTTGACCTCCTTGCTTG	Exon	15093-15112
41	TGCCCAGGTGGTTGAAGTGC	Exon	17304-17323
42	TGTAGACTCTTGAAGACCTG	Exon	19309-19328
43	AGCATCATGTGGGAGGAGTC	Exon	20041-20060
44	GAGCATCATGTGGGAGGAGT	Exon	20042-20061
45	TTCAGCTGGCTGGCCTCCAA	Exon	21102-21121
71	GAGATAGTGAGAGGCCTCC	Exon	19284-19302
72	AGGAATACACGTGGTAGAG	Exon	16385-16403
73	CCAGTACTGCCGCGGCAGG	Exon	16210-16228
74	AGTCCAGGATGTCATCCAT	Exon	17473-17491
75	TCTTCTGCTTACGTGTGTT	Exon	19990-20008
76	ATCATGTGGGAGGAGTCCA	Exon	20039-20057
77	GCCTGCAGGACCTGCAGGA	Exon	15742-15748 and 15817-15827
78	CAGCTGGTCAGTGAGCAGG	Exon	8096-8924
79	CTATTTGCTTGACCTTGGA	Exon	19561-19572 and 19809-19815
80	CAAAGCATGGGTACATCCA	Exon	16802-16820
81	AAGGGGTAAAGTGAGAACC	Exon	15684-15702
82	AGAGGTACTTCCTGTAGTG	Exon	15946-15964
83	AACACATATACCAGCTCCT	Exon	13325-13343
84	TTCATGACGGAGACATCAG	Exon	20770-20788
85	GTAGGGCTCGTTTGAGCCC	Exon	5090-5108
86	CCCATGTGGGAGATGAGGC	Exon	19481-19499
87	GAGTCAAGCCGGCATCCTT	Exon	13354-13372
88	CCAAGATCTTCTGGGGCTC	Exon	20998-20999 and 21089-21105
89	CACTGCAGGGTCTTCAAGG	Exon	15000-15018
90	CACACCCTGCTGGCCAGCT	Exon	15045-15063
91	ACGCCGCTCCAGGCCACCCC	5'UTR	5032-5051

92	GCACGCCGCTCCAGGCCACC	5'UTR	5034-5053
93	ACGCACGCCGCTCCAGGCCA	5'UTR	5036-5055
94	GGCCCCAGCAAAGTGCCGAC	exon	5059-5078
95	GTCCTTCCTGCGGTCTTGGT	exon	5691-5710
96	AGTCCTTCCTGCGGTCTTGG	exon	5692-5711
97	CCCCAGTCCTTCCTGCGGTC	exon	5696-5715
98	CACGTGGCCCCAGTCCTTCC	exon	5703-5722
99	CATCCACCCTGTTCACCACC	exon	8450-8469
100	CACATCCACCCTGTTCACCA	exon	8452-8471
101	GCGCCACATCCACCCTGTTC	exon	8456-8475
102	GCCCCCATCTGGACCTTCCT	exon	8520-8539
103	GAACTCCCCGCTCTGGAATG	exon	8647-8666
104	CTTGAACTCCCCGCTCTGGA	exon	8650-8669
105	CGGCCATCTGCTTGCTCAGG	exon	8705-8724
106	CCGGCCATCTGCTTGCTCAG	exon	8706-8725
107	ACCCGGCCATCTGCTTGCTC	exon	8708-8727
108	CTCCAGGCACCCGGCCATCT	exon	8716-8735
109	CCTCCAGGCACCCGGCCATC	exon	8717-8736
110	ATCGAGGCTCAGCTTCCCAG	exon	8803-8822
111	CCTGCTCCACATCGAGGCTC	exon	8813-8832
112	GGCGTTGTACATGTCCAGCG	exon	8986-9005
113	GCATCACGGCGTTGTACATG	exon	8993-9012
114	AGCATCACGGCGTTGTACAT	exon	8995-9014
115	AAGCATCACGGCGTTGTACA	exon	8994-9013
116	TCCAGACACCTTTCGATGGT	exon	13435-13447 and 13666-13672
117	CAGAACGGCGGTGAAGAGTG	exon	13705-13724
118	GCAGAACGGCGGTGAAGAGT	exon	13706-13725
119	GACAGCAGAACGGCGGTGAA	exon	13710-13729
120	ACGGCCTTCAGAACAGTGGC	exon	13743-13762
121	CACCTTGTGCACGGCCTTCA	exon	13753-13772
122	ACACGCCCATCCTTGGCATA	exon	13842-13851 and 14967-14976
123	GGACACACGCCCATCCTTGG	exon	14959-14980
124	AGGACACACGCCCATCCTTG	exon	14963-14981
125	GTAGGACACACGCCCATCCT	exon	14964-14983
126	GGTAGGACACACGCCCATCC	exon	14965-14984
127	GACCTCCTTGCTTGGCAACG	exon	15087-15106
128	TTTCTCCCATTGGTCCCGCA	exon	15603-15622
129	GTGCTTTCTCCCATTGGTCC	exon	15607-15626
130	CCGGCACAGTGCTTTCTCCC	exon	15615-15634
131	CCACTGACCCGCTGCCTCTG	exon	15904-15923
132	CTCCTCCCAGTACTGCCGCG	exon	16215-16234
133	TTGTCCAGGCTGCATGGCAT	exon	16334-16353
134	TCCACCGCCTCGAAGGTCAG	exon	16786-16805

135	ATCCACCGCCTCGAAGGTCA	exon	16787-16806
136	CATCCACCGCCTCGAAGGTC	exon	16788-16807
137	ACATCCACCGCCTCGAAGGT	exon	16789-16808
138	GGTACATCCACCGCCTCGAA	exon	16792-16811
139	GTGCGGCGATGTCCAGGGCA	exon	16829-16848
140	AGAGTGCGGCGATGTCCAGG	exon	16832-16851
141	CAGAGTGCGGCGATGTCCAG	exon	16833-16852
142	CCAGAGTGCGGCGATGTCCA	exon	16834-16853
143	CACCAGAGTGCGGCGATGTC	exon	16836-16855
144	AGCACCAGAGTGCGGCGATG	exon	16838-16857
145	GGTCCAGCACGCGCCCGTTG	exon	16998-17017
146	AGGTCCAGCACGCGCCCGTT	exon	16999-17018
147	GCCAGCTCACGGCGCAGCTC	exon	17140-17159
205	CCGCCGCTCCAGGCCACCCC	exon	5032-5051
206	GCACGCCGCTCCAGGCCACC	exon	5034-5053
207	ACGCACGCCGCTCCAGGCCA	exon	5036-5055
208	GGCCCCAGCAAAGTGCCGAC	exon	5059-5078
209	GTCCTTCCTGCGGTCTTGGT	exon	5691-5710
210	AGTCCTTCCTGCGGTCTTGG	exon	5692-5711
211	CCCCAGTCCTTCCTGCGGTC	exon	5696-5715
212	CACGTGGCCCCAGTCCTTCC	exon	5703-5722
213	CATCCACCCTGTTCACCACC	exon	8450-8469
214	CACATCCACCCTGTTCACCA	exon	8452-8471
215	GCGCCACATCCACCCTGTTC	exon	8456-8475
216	GCCCCCATCTGGACCTTCCT	exon	8520-8539
217	GAACTCCCCGCTCTGGAATG	exon	8647-8666
218	CTTGAACTCCCCGCTCTGGA	exon	8650-8669
219	CGGCCATCTGCTTGCTCAGG	exon	8705-8724
220	CCGGCCATCTGCTTGCTCAG	exon	8706-8725
221	ACCCGGCCATCTGCTTGCTC	exon	8708-8727
222	CTCCAGGCACCCGGCCATCT	exon	8716-8735
223	CCTCCAGGCACCCGGCCATC	exon	8717-8736
224	ATCGAGGCTCAGCTTCCCAG	exon	8803-8822
225	CCTGCTCCACATCGAGGCTC	exon	8813-8832
226	GGCGTTGTACATGTCCAGCG	exon	8986-9005
227	GCATCACGGCGTTGTACATG	exon	8993-9012
228	AGCATCACGGCGTTGTACAT	exon	8994-9013
229	AAGCATCACGGCGTTGTACA	exon	8995-9014
230	TCCAGACACCTTTCGATGGT	exon	13435-13447 and 13666-13672
231	CAGAACGGCGGTGAAGAGTG	exon	13705-13724
232	GCAGAACGGCGGTGAAGAGT	exon	13706-13725
233	GACAGCAGAACGGCGGTGAA	exon	13710-13729
234	ACGGCCTTCAGAACAGTGGC	exon	13743-13762

235	CACCTTGTGCACGGCCTTCA	exon	13753-13772
236	ACACGCCCATCCTTGGCATA	exon	13842-13850 and 14966-14976
237	GGACACACGCCCATCCTTGG	exon	13846-13850 and 14966-14980
238	AGGACACACGCCCATCCTTG	exon	13847-13850 and 14966-14981
239	GTAGGACACACGCCCATCCT	exon	13849-13850 and 14966-14983
240	GGTAGGACACACGCCCATCC	exon	13850-13850 and 14966-14984
241	GACCTCCTTGCTTGGCAACG	exon	15087-15106
242	TTTCTCCCATTGGTCCCGCA	exon	15603-15622
243	GTGCTTTCTCCCATTGGTCC	exon	15607-15626
244	CCGGCACAGTGCTTTCTCCC	exon	15615-15634
245	CCACTGACCCGCTGCCTCTG	exon	15904-15923
246	CTCCTCCCAGTACTGCCGCG	exon	16215-16234
247	TTGTCCAGGCTGCATGGCAT	exon	16334-16353
248	TCCACCGCCTCGAAGGTCAG	exon	16786-16805
249	ATCCACCGCCTCGAAGGTCA	exon	16787-16806
250	CATCCACCGCCTCGAAGGTC	exon	16788-16807
251	ACATCCACCGCCTCGAAGGT	exon	16789-16808
252	GGTACATCCACCGCCTCGAA	exon	16792-16811
253	GTGCGGCGATGTCCAGGGCA	exon	16829-16848
254	AGAGTGCGGCGATGTCCAGG	exon	16832-16851
255	CAGAGTGCGGCGATGTCCAG	exon	16833-16852
256	CCAGAGTGCGGCGATGTCCA	exon	16834-16853
257	CACCAGAGTGCGGCGATGTC	exon	16836-16855
258	AGCACCAGAGTGCGGCGATG	exon	16838-16857
259	GGTCCAGCACGCGCCCGTTG	exon	16998-17017
260	AGGTCCAGCACGCGCCCGTT	exon	16999-17018
261	GCCAGCTCACGGCGCAGCTC	exon	17140-17159
262	CGCCAGCTCACGGCGCAGCT	exon	17141-17160
263	GCAGTGCGCCAGCTCACGGC	exon	17147-17166
264	CTTCTGGCAGTGCGCCAGCT	exon	17153-17172
265	ACCTTCTGGCAGTGCGCCAG	exon	17155-17174
266	CCACCTTCTGGCAGTGCGCC	exon	17157-17176
267	TCCCGGGCCACCTTCTGGCA	exon	17164-17183
268	GACGCGGTCCCGCAGGTGCT	exon	17231-17250
269	GGAAGTCCATGTTGTGCGGC	exon	17259-17278
270	GCGGAAGTCCATGTTGTGCG	exon	17261-17280
271	GTCGCTGCCCAGGTGGTTGA	exon	17309-17328
272	ACGTCGCTGCCCAGGTGGTT	exon	17311-17330
273	CCACGTCGCTGCCCAGGTGG	exon	17313-17332
274	CCCGCGCCACGTCGCTGCCC	exon	17319-17338
275	GGCCCGCGCCACGTCGCTGC	exon	17321-17340
276	GCGGCCCTGGGCGAACTCCA	exon	17345-17364
277	CACCACTTTCGGCCCGTCAA	exon	17506-17512 and 18083-18095

278	CCTCCGCGCCCATCCACCAC	exon	18090-18109
279	TCCTCCGCGCCCATCCACCA	exon	18091-18110
280	GTTCCTCCGCGCCCATCCAC	exon	18093-18112
281	GGGTTCCTCCGCGCCCATCC	exon	18095-18114
282	AGGCCAGCGTCTGCCAGGGT	exon	18111-18130
283	CGGCACATCCGAGGGCTCCA	exon	18562-18581
284	CACGCCGCTGTACACGTCCT	exon	18583-18602
285	CCACGCCGCTGTACACGTCC	exon	18584-18603
286	GCCACGCCGCTGTACACGTC	exon	18585-18604
287	GGCCACGCCGCTGTACACGT	exon	18586-18605
288	TGCGCGGCCACGCCGCTGTA	exon	18591-18610
289	CTGCCTACGGAACACCTCCA	exon	18805-18824
290	TTCCAGCACCTGTGCCACCC	exon	18844-18863
291	GAGGCCTCCCACACGAACTC	exon	19273-19292
292	AGAGGCCTCCCACACGAACT	exon	19274-19293
293	TGAATTCCACCTCCTATTTG	exon	19809-19828
294	CGAAGGTCAGGCCCTTCCTG	exon	20717-20736
295	AGACGAAGGTCAGGCCCTTC	exon	20720-20739
296	GAGACGAAGGTCAGGCCCTT	exon	20721-20740
297	AGAGACGAAGGTCAGGCCCT	exon	20722-20741
298	AGTAACAGTCGTGCACAGAG	exon	20738-20757
587	GAAGTCCATGTTGTGCGGCA	Exon	17258-17277
588	CGGAAGTCCATGTTGTGCGG	Exon	17260-17279
589	CGCGGAAGTCCATGTTGTGC	Exon	17262-17281
590	GCCGCGGAAGTCCATGTTGT	Exon	17264-17283
591	GGCCGCGGAAGTCCATGTTG	Exon	17265-17285
592	CGGCCGCGGAAGTCCATGTT	Exon	17266-17285
593	GCGGCCGCGGAAGTCCATGT	Exon	17267-17286
594	TGCGGCCGCGGAAGTCCATG	Exon	17268-17287
595	GTGCGGCCGCGGAAGTCCAT	Exon	17269-17288
596	CTGCCCAGGTGGTTGAAGTG	Exon	17305-17324
597	TCTGCTTCACCACCTTGCGG	exon	18875-18894
598	GTCTGCTTCACCACCTTGCG	exon	18876-18895
599	ATGAAGTTGGGCGGGAAGCC	Exon	20011-20030
600	GATGAAGTTGGGCGGGAAGC	Exon	20012-20031
601	GGATGAAGTTGGGCGGGAAG	Exon	20013-20032
602	TGGATGAAGTTGGGCGGGAA	Exon	20014-20033
603	GTGGATGAAGTTGGGCGGGA	Exon	20015-20034
604	GAGTGGATGAAGTTGGGCGG	Exon	20017-20036
605	GAGTGGATGAAGTTGGGCGG	Exon	20017-20036
606	ATCATGTGGGAGGAGTCCAG	Exon	20038-20057
607	CATCATGTGGGAGGAGTCCA	Exon	20039-20058
608	GCATCATGTGGGAGGAGTCC	Exon	20040-20059

609	AGGCCCTTCCTGTAGCAGTG	Exon	20071-20081 and 20720-20728
610	CAGGCCCTTCCTGTAGCAGT	Exon	20072-20081 and 20720-20729
611	TCAGGCCCTTCCTGTAGCAG	Exon	20073-20081 and 20720-20730
612	CCCAGCCAAGCATCACGG	Exon	9004-9021
613	GCCCAGCCAAGCATCACG	Exon	9005-9022
614	AGTCCATGTTGTGCGGCA	Exon	17258-17275
615	AAGTCCATGTTGTGCGGC	Exon	17259-17276
616	GAAGTCCATGTTGTGCGG	Exon	17260-17277
617	GGAAGTCCATGTTGTGCG	Exon	17261-17278
618	CGGAAGTCCATGTTGTGC	Exon	17262-17279
619	GCGGAAGTCCATGTTGTG	Exon	17263-17280
620	CGCGGAAGTCCATGTTGT	Exon	17264-17281
621	CCGCGGAAGTCCATGTTG	Exon	17265-17282
622	GCCGCGGAAGTCCATGTT	Exon	17266-17283
623	GGCCGCGGAAGTCCATGT	Exon	17267-17284
624	CGGCCGCGGAAGTCCATG	Exon	17268-17285
625	GCGGCCGCGGAAGTCCAT	Exon	17269-17286
626	TGCGGCCGCGGAAGTCCA	Exon	17270-17287
627	GTGCGGCCGCGGAAGTCC	Exon	17271-17288
628	GCCCAGGTGGTTGAAGTG	Exon	17305-17322
629	TGCCCAGGTGGTTGAAGT	Exon	17306-17323
630	CTGCCCAGGTGGTTGAAG	Exon	17307-17324
631	TGCTTCACCACCTTGCGG	Exon	18875-18892
632	CTGCTTCACCACCTTGCG	Exon	18876-18893
633	TCTGCTTCACCACCTTGC	Exon	18877-18894
634	GTCTGCTTCACCACCTTG	Exon	18878-18895
635	GAACATCTCCTGTAGACT	Exon	19321-19338
636	GAAGTTGGGCGGGAAGCC	Exon	20011-20028
637	TGAAGTTGGGCGGGAAGC	Exon	20012-20029
638	ATGAAGTTGGGCGGGAAG	Exon	20013-20030
639	GATGAAGTTGGGCGGGAA	Exon	20014-20031
640	GGATGAAGTTGGGCGGGA	Exon	20015-20032
641	TGGATGAAGTTGGGCGGG	Exon	20016-20033
642	GTGGATGAAGTTGGGCGG	Exon	20017-20034
643	AGTGGATGAAGTTGGGCG	Exon	20018-20035
644	GAGTGGATGAAGTTGGGC	Exon	20019-20036
645	CATGTGGGAGGAGTCCAG	Exon	20038-20055
646	TCATGTGGGAGGAGTCCA	Exon	20039-20056
647	ATCATGTGGGAGGAGTCC	Exon	20040-20057
648	CATCATGTGGGAGGAGTC	Exon	20041-20058
649	GCATCATGTGGGAGGAGT	Exon	20042-20059
650	AGCATCATGTGGGAGGAG	Exon	20043-20060
651	GCCCTTCCTGTAGCAGTG	Exon	20071-20081 and 20720-20726

652	GGCCCTTCCTGTAGCAGT	Exon	20072-20081 and 20720-20727
653	AGGCCCTTCCTGTAGCAG	Exon	20073-20081 and 20720-20728
654	CAGGCCCTTCCTGTAGCA	Exon	20074-20081 and 20720-20729
655	TCAGGCCCTTCCTGTAGC	Exon	20075-20081 and 20720-20730

[0150] In some embodiments, an oligonucleotide targets a region of a murine POLRMT transcript. In some embodiments, an oligonucleotide targets a region of a mouse POLRMT transcript. The amino acid and nucleotide sequences encoding the mouse POLRMT gene are known in the art and can be found in publicly available databases. For example, mouse POLRMT gene sequences are identified in the sequence span on mouse chromosome 10 corresponding to coordinates GRCm39 10_79571957_79582415 and the POLRMT transcript is represented in, *e.g.,* Reference No. ENSMUST00000161765. In some embodiments, an oligonucleotide targets an exon region within a mouse POLRMT transcript (*e.g.,* the longest exon in a mouse POLRMT transcript represented in Reference No. ENSMUST00000161765, SEQ ID NO: 582).

[0151] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID Nos: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, an oligonucleotide comprises a sequence having at least 85% identity to a sequence selected from a group consisting of SEQ ID Nos: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID Nos: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, an oligonucleotide comprises a sequence selected from a group consisting of SEQ ID Nos: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634. In some embodiments, an oligonucleotide comprises a sequence selected from a group consisting of SEQ ID Nos: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0152] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 434. In some embodiments, an oligonucleotide comprises a sequence having at least 85% to SEQ ID NO: 434. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 434. In some embodiments,

an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 434. In some embodiments, an oligonucleotide comprises SEQ ID NO: 434.

[0153] In some embodiments, an oligonucleotide comprises a sequence having at least 80% identity to SEQ ID NO: 442. In some embodiments, an oligonucleotide comprises a sequence having at least 85% to SEQ ID NO: 442. In some embodiments, an oligonucleotide comprises a sequence having at least 90% identity to SEQ ID NO: 442. In some embodiments, an oligonucleotide comprises a sequence having at least 95% identity to SEQ ID NO: 442. In some embodiments, an oligonucleotide comprises SEQ ID NO: 442.

[0154] In some embodiments, an oligonucleotide comprises a nucleotide sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID Nos: 393-486 in the following Table 2.

[0155] In some embodiments, an oligonucleotide comprises a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of the sequences listed below in Table 2. Table 2 includes exemplary mouse POLRMT oligonucleotide sequences, the target region of complementarity in the mouse POLRMT mRNA transcript, and the corresponding coordinates of the target region within the mouse POLRMT gene sequence as shown in SEQ ID NO: 581.

Table 2: Oligonucleotide Sequences, Target Region, and Corresponding Coordinates of the Target Region on mouse POLRMT gene sequence (SEQ ID NO: 581)

SEQ ID NO:	Oligonucleotide Sequence (5' to 3')	Target Region	Corresponding Coordinates within SEQ ID NO: 581
393	CACGGTGTTGTACATGTGCA	exon	7077-7096
394	ATCACGGTGTTGTACATGTG	exon	7075-7094
395	CATCACGGTGTTGTACATGT	exon	7074-7093
396	GTGCATCTCTCGAGCCACCT	exon	3342-3361
397	TGTGCATCTCTCGAGCCACC	exon	3341-3360
398	CTGTGCATCTCTCGAGCCAC	exon	3340-3359
399	GTGCTGTGCCAGCGACAGGC	exon	3297-3316
400	GTCCATGTTGTGCGGCAACC	exon	3258-3277
401	AGGTCACTGCCCAGGTGGTT	exon	3202-3221
402	TCCATCCACCACTTCCGGCC	exon	2663-2682
403	ACCTCCATGCAGCAGGCCAG	exon	2621-2640
404	CACCTCCATGCAGCAGGCCA	exon	2620-2639

405	CCACCTCCATGCAGCAGGCC	exon	2619-2638
406	GCCACCTCCATGCAGCAGGC	exon	2618-2637
407	TGCCACCTCCATGCAGCAGG	exon	2617-2636
408	CACCACCTTGCGGCTGATGA	exon	2005-2024
409	TTCACCACCTTGCGGCTGAT	exon	2003-2022
410	CTGCTGTCTGTCTCCGTTGT	exon	7107-7126
411	ACCTGCTGTCTGTCTCCGTT	exon	7105-7124
412	GCACCTGCTGTCTGTCTCCG	exon	7103-7122
413	TGTTGTACATGTGCAGTGTG	exon	7082-7101
414	CGGTGTTGTACATGTGCAGT	exon	7079-7098
415	GACCAGGTCAGTGAAGAGCA	exon	5712-5731
416	TCCAGGACCAGGTCAGTGAA	exon	5707-5726
417	CTTCCAGGACCAGGTCAGTG	exon	5705-5724
418	CTCCCCTTCGCTCAGCAGGC	exon	4732-4751
419	ACTCCCCTTCGCTCAGCAGG	exon	4731-4750
420	AACTCCCCTTCGCTCAGCAG	exon	4730-4749
421	GCCCAGGTTATGGGCCAACT	exon	4568-4587
422	CTGTTGTGCAGGGGCCTCCA	exon	4178-4197
423	CAATTGCTTGCCCAGCTGTA	exon	4136-4155
424	CCAATTGCTTGCCCAGCTGT	exon	4135-4154
425	CGCCAATTGCTTGCCCAGCT	exon	4133-4152
426	CCGCCAATTGCTTGCCCAGC	exon	4132-4151
427	TCCGCCAATTGCTTGCCCAG	exon	4131-4150
428	CTCCGCCAATTGCTTGCCCA	exon	4130-4149
429	GCTCCGCCAATTGCTTGCCC	exon	4129-4148
430	AGCTCCGCCAATTGCTTGCC	exon	4128-4147
431	AGGGCATTGCTCCAGCAGAC	exon	3597-3616
432	CGCAGTTCCCCAACTGTGTG	exon	3545-3564
433	GCGCAGTTCCCCAACTGTGT	exon	3544-3563
434	CTCTCGAGCCACCTTGAGGC	exon	3348-3367
435	ACTGTGCATCTCTCGAGCCA	exon	3339-3358
436	GACTGTGCATCTCTCGAGCC	exon	3338-3357
437	AGACTGTGCATCTCTCGAGC	exon	3337-3356
438	CAGACTGTGCATCTCTCGAG	exon	3336-3355
439	GCAGACTGTGCATCTCTCGA	exon	3335-3354
440	GCGGAAGTCCATGTTGTGCG	exon	3252-3271
441	CCGCGGAAGTCCATGTTGTG	exon	3250-3269
442	CGCTAGGTCACTGCCCAGGT	exon	3198-3217
443	GCGCTAGGTCACTGCCCAGG	exon	3197-3216
444	CGCGCTAGGTCACTGCCCAG	exon	3196-3215
445	CCGGCCCTCAGCAAACTCCA	exon	3168-3187
446	TTCCATCCACCACTTCCGGC	exon	2662-2681
447	GCTTCCATCCACCACTTCCG	exon	2660-2679

448	AGCTTCCATCCACCACTTCC	exon	2659-2678
449	CAGCTTCCATCCACCACTTC	exon	2658-2677
450	TCAGCTTCCATCCACCACTT	exon	2657-2676
451	ATCAGCTTCCATCCACCACT	exon	2656-2675
452	CATCAGCTTCCATCCACCAC	exon	2655-2674
453	TCATCAGCTTCCATCCACCA	exon	2654-2673
454	GCTCATCAGCTTCCATCCAC	exon	2652-2671
455	GGCTCATCAGCTTCCATCCA	exon	2651-2670
456	GTGCCACCTCCATGCAGCAG	exon	2616-2635
457	TGTGCCACCTCCATGCAGCA	exon	2615-2634
458	GTGTGCCACCTCCATGCAGC	exon	2614-2633
459	CGTGTGCCACCTCCATGCAG	exon	2613-2632
460	GCGTGTGCCACCTCCATGCA	exon	2612-2631
461	TGCGTGTGCCACCTCCATGC	exon	2611-2630
462	CAGTCCACTTGCGTGCCCAC	exon	2540-2559
463	GGGCAGTCCACTTGCGTGCC	exon	2537-2556
464	GCCATGGGCAGTCCACTTGC	exon	2532-2551
465	CACAGGTCACCCCAGAAACT	exon	2380-2399
466	CACCCACACTGTCTCGGCCT	exon	2271-2290
467	GCACCCACACTGTCTCGGCC	exon	2270-2289
468	TGTTGCCACCTCCCTGTACA	exon	2209-2228
469	TGTGTTGCCACCTCCCTGTA	exon	2207-2226
470	CTGTGTTGCCACCTCCCTGT	exon	2206-2225
471	CTGGCACCCACCTGTGTTGC	exon	2195-2214
472	CACTCCACAGGCCATCCGGC	exon	1536-1555
473	CCCACTCCACAGGCCATCCG	exon	1534-1553
474	GACCCACTCCACAGGCCATC	exon	1532-1551
475	CCACCTTTTACCTGCAGACC	exon	1377-1396
476	GCCACCTTTTACCTGCAGAC	exon	1376-1395
477	GGCCACCTTTTACCTGCAGA	exon	1375-1394
478	CTCCCCGCAGTACAGGACTC	exon	1313-1332
479	CCTCCCCGCAGTACAGGACT	exon	1312-1331
480	CCCTCCCCGCAGTACAGGAC	exon	1311-1330
481	CTCCCCTCCCCGCAGTACAG	exon	1308-1327
482	TCCTGCCCATCCCTCCATCC	exon	1287-1306
483	TTCCTGCCCATCCCTCCATC	exon	1286-1305
484	CCTTTCCTGCCCATCCCTCC	exon	1283-1302
485	GCATGTGTCCAGAAGCAGTC	exon	637-656
486	TTGCGCACCGCCAGAAAGTG	exon	19-38

[0156] In some embodiments, an oligonucleotide of the present disclosure is complementary (*e.g.*, substantially complementary or perfectly complementary) to a region of a POLRMT RNA transcript (*e.g.*, SEQ ID NO: 205). In some embodiments, an oligonucleotide is complementary to a region of a 5' untranslated region (UTR) of a POLRMT RNA transcript. In some embodiments, an oligonucleotide is complementary to a region that is within or includes an exon region of a POLRMT RNA transcript (*e.g.*, within SEQ ID NO: 205). In some embodiments, an oligonucleotide is complementary to a region that is within or includes an intron region of a POLRMT pre-mRNA transcript. In some embodiments, an oligonucleotide is complementary to a region of a POLRMT pre-mRNA transcript that spans an exon/intron junction. In some embodiments, an oligonucleotide is complementary to a region that includes or is within a 3' UTR region of a POLRMT RNA transcript. In some embodiments, an oligonucleotide is complementary to a region of a POLRMT RNA transcript as illustrated in FIG. 2, FIG. 12, and Table 3. In some embodiments, an oligonucleotide sequence is complementary to a region within the POLRMT gene sequence (SEQ ID NO: 1).

[0157] In some embodiments, an oligonucleotide is substantially complementary to 8 to 30 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655271. In some embodiments, an oligonucleotide is perfectly complementary to 8 to 30 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655271. In some embodiments, an oligonucleotide is complementary to 8 to 22 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655271. In some embodiments, an oligonucleotide is complementary to 8 to 19 contiguous nucleotides of SEQ ID NO: 725 (CAACGCCGTGATGCTTGGCTGGGCGCGGC), which corresponds to nucleotides 817-845 of the POLRMT transcript (SEQ ID NO: 205) and to nucleotides 8999-9027 on the POLRMT gene sequence (SEQ ID NO: 1). In some embodiments, an oligonucleotide is complementary to a sequence comprising SEQ ID NO: 681 or 682. In some embodiments an oligonucleotide targets a region within the POLRMT exon identified in Ensemble ID ENSE00000655271 and comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95% or 100% identity to SEQ ID NO: 612 or 613.

[0158] In some embodiments, an oligonucleotide is substantially complementary to 8 to 30 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID

ENSE00000655279. In some embodiments, an oligonucleotide is perfectly complementary to 8 to 30 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655279. In some embodiments, an oligonucleotide is complementary to 8 to 22 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655279. In some embodiments, an oligonucleotide is complementary 8 to 22 contiguous nucleotides of SEQ ID NO: 726 (CGCACAACATGGACTTCCGCGGCCGCACCTAC), which corresponds to nucleotides 2415-2446 of the POLRMT transcript (SEQ ID NO: 205) and nucleotides 117261-17292 of the POLRMT gene sequence (SEQ ID NO: 1). In some embodiments, an oligonucleotide is complementary to a sequence comprising SEQ ID NO: 661, 663, 692, 693, 694, or 695. In some embodiments an oligonucleotide targets a region within the POLRMT exon identified in Ensemble ID ENSE00000655279 and comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95% or 100% identity to SEQ ID NO: 592, 594, 623, 624, 625, or 626.

[0159] In some embodiments, an oligonucleotide is substantially complementary to 8 to 30 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655283. In some embodiments, an oligonucleotide is complementary to 8 to 30 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655283. In some embodiments, an oligonucleotide is complementary to 8 to 22 contiguous nucleotides of the POLRMT exon sequence identified in Ensemble ID ENSE00000655283. In some embodiments, an oligonucleotide is complementary to a sequence that spans 8 to 22 contiguous nucleotides of SEQ ID NO: 727 (ATCACCCGCAAGGTGGTGAAGCAGACGGTGA), which corresponds to nucleotides 2978-3008 of the POLRMT transcript (SEQ ID NO: 205) and nucleotides 18870-18900 of the POLRMT gene sequence (SEQ ID NO: 1). In some embodiments, an oligonucleotide is complementary to a sequence comprising SEQ ID NO: 661, 663, 692, 693, 694, or 695. In some embodiments an oligonucleotide targets a region within the POLRMT exon identified in Ensemble ID ENSE00000655283 and comprises a sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 592, 594, 623, 624, 625, or 626.

[0160] In some embodiments, an oligonucleotide comprises a sequence that is complementary (*e.g.,* substantially complementary or perfectly complementary) to a region within a POLRMT transcript, *e.g.,* POLRMT mRNA or pre-mRNA transcript (*e.g.,* complementary to a nucleotide sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to a target region of SEQ ID NO: 205). In some embodiments, an oligonucleotide is complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript (*i.e.,* the target region) *e.g.,* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides long, although shorter and longer target region are also contemplated.

[0161] In some embodiments, the 8 to 30 contiguous nucleotides on the POLRMT RNA transcript (*i.e.,* the target region) comprises a sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to any one of the sequences listed below in Table 3.

[0162] In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 15-26, 46-64, 299-392, and 656-724. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 85% identity to a sequence selected from a group consisting of SEQ ID NOs: 15-26, 46-64, 299-392, and 656-724. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 15-26, 46-64, 299-392, and 656-724. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 95% identity to a sequence selected from a group consisting of SEQ ID NOs: 15-26, 46-64, 299-392, and 656-724. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence selected from a group consisting of SEQ ID NOs: 15-26, 46-64, 299-392, and 656-724.

[0163] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 23. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 23. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 23. In some embodiments, a target region

comprises a sequence having at least 95% identity to SEQ ID NO: 23. In some embodiments, target region comprises SEQ ID NO: 23.

[0164] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 24. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 24. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 24. In some embodiments, a target region comprises a sequence having at least 95% identity to SEQ ID NO: 24. In some embodiments, target region comprises SEQ ID NO: 24.

[0165] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 663. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 663. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 663. In some embodiments, a target region comprises a sequence having at least 95% identity to SEQ ID NO: 663. In some embodiments, target region comprises SEQ ID NO: 663.

[0166] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 681. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 681. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 681. In some embodiments, a target region comprises a sequence having at least 95% identity to SEQ ID NO: 681. In some embodiments, target region comprises SEQ ID NO: 681.

[0167] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 701. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 701. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 701. In some embodiments, a target region comprises a sequence having at least 95% identity to SEQ ID NO: 701. In some embodiments, target region comprises SEQ ID NO: 701.

[0168] In some embodiments, an oligonucleotide comprises a sequence that is complementary (*e.g.*, substantially complementary or perfectly complementary, and/or that includes no more than 1, 2, 3, or 4 nucleotide mismatches) to 8 to 30 contiguous nucleotides of a

POLRMT transcript (*e.g.,* SEQ ID NO: 205, and correspond to a region within gene sequence SEQ ID NO: 1). In some embodiments, an oligonucleotide comprises a sequence that is complementary to any one of the sequences listed below in Table 3.

[0169] In some embodiments, a target region on the POLRMT RNA transcript comprises a region that corresponds to nucleotides 5696-5715, 8808-8827, 8809-8828, 8811-8830, 16221-16240, 17159-17178, 17314-17333, 17315-17334, 18082-18101, 18083-18102, 18084-18103, 18130-18149, 5680-5699, 8491-8510, 8529-8548, 8569-8588, 8570-8589, 8571-8590, 8572-8591, 8573-8592, 8574-8593, 13322-13341, 13719-13738, 14999-15018, 15092-15111, 15093-15112, 17304-17323, 19309-19328, 20041-20060, 20042-20061, 21102-21121, 5032-5051, 5034-5053, 5036-5055, 5059-5078, 5691-5710, 5692-5711, 5696-5715, 5703-5722, 8450-8469, 8452-8471, 8456-8475, 8520-8539, 8647-8666, 8650-8669, 8705-8724, 8706-8725, 8708-8727, 8716-8735, 8717-8736, 8803-8822, 8813-8832, 8986-9005, 8993-9012, 8994-9013, 8995-9014, 13435-13447 and 13666-13672, 13705-13724, 13706-13725, 13710-13729, 13743-13762, 13753-13772, 13842-13850 and 14966-14976, 13846-13850 and 14966-14980, 13847-13850 and 14966-14981, 13849-13850 and 14966-14983, 13850-13850 and 14966-14984, 15087-15106, 15603-15622, 15607-15626, 15615-15634, 15904-15923, 16215-16234, 16334-16353, 16786-16805, 16787-16806, 16788-16807, 16789-16808, 16792-16811, 16829-16848, 16832-16851, 16833-16852, 16834-16853, 16836-16855, 16838-16857, 16998-17017, 16999-17018, 17140-17159, 17141-17160, 17147-17166, 17153-17172, 17155-17174, 17157-17176, 17164-17183, 17231-17250, 17259-17278, 17261-17280, 17309-17328, 17311-17330, 17313-17332, 17319-17338, 17321-17340, 17345-17364, 17506-17512 and 18083-18095, 18090-18109, 18091-18110, 18093-18112, 18095-18114, 18111-18130, 18562-18581, 18583-18602, 18584-18603, 18585-18604, 18586-18605, 18591-18610, 18805-18824, 18844-18863, 19273-19292, 19274-19293, 19809-19828, 20717-20736, 20720-20739, 20721-20740, 20722-20741, 20738-20757, 17266-17285, 17268-17287, 18875-18894, 18876-18895, 9004-9021, 9005-9022, 17267-17284, 17268-17285, 17269-17286, 17270-17287, 18876-18893, 18877-18894, or 18878-18895 of SEQ ID NO: 1. In some embodiments, a target region on the POLRMT RNA transcript comprises a region that corresponds to nucleotides 2420-2439, 2422-2441, 2983-3002, 2984-3003, 822-839, 823-840, 2421-2438, 2422-2439, 2423-2440, 2424-2441, 2984-3001, 2985-3002, or 2986-3003 of the POLRMT transcript sequence (SEQ ID NO: 205).

Table 3: Target Region Sequence of Human POLRMT

SEQ ID NO:	POLRMT RNA Sequence (5' to 3')
15	GACCGCAGGAAGGACTGGGG
16	AAGCTGAGCCTCGATGTGGA
17	AGCTGAGCCTCGATGTGGAG
18	CTGAGCCTCGATGTGGAGCA
19	AGTACTGGGAGGAGCTGGGG
20	CGCACTGCCAGAAGGTGGCC
21	CACCTGGGCAGCGACGTGGC
22	ACCTGGGCAGCGACGTGGCG
23	GGCCGAAAGTGGTGGATGGG
24	GCCGAAAGTGGTGGATGGGC
25	CCGAAAGTGGTGGATGGGCG
26	TGCTGTATGGAGGTGGCGAA
46	CCAGGAGCAAGACCAAGACC
47	GCAGTGGAGATGGTAGCCTC
48	CAGATGGGGGCCAAGGATGC
49	GCTGGGCAAAGATACTGGAG
50	CTGGGCAAAGATACTGGAGA
51	TGGGCAAAGATACTGGAGAA
52	GGGCAAAGATACTGGAGAAG
53	GGCAAAGATACTGGAGAAGG
54	GCAAAGATACTGGAGAAGGA
55	TCAAGGAGCTGGTATATGTG
56	GTTCTGCTGTCTGAGGAGGA
57	CCCTTGAAGACCCTGCAGTG
58	CCAAGCAAGGAGGTCAAGCA
59	CAAGCAAGGAGGTCAAGCAC
60	GCACTTCAACCACCTGGGCA
61	CAGGTCTTCAAGAGTCTACA
62	GACTCCTCCCACATGATGCT
63	ACTCCTCCCACATGATGCTC
64	TTGGAGGCCAGCCAGCTGAA
148	GGGGTGGCCTGGAGCGGCGT
149	GGTGGCCTGGAGCGGCGTGC
150	TGGCCTGGAGCGGCGTGCGT
151	GTCGGCACTTTGCTGGGGCC
152	ACCAAGACCGCAGGAAGGAC
153	CCAAGACCGCAGGAAGGACT
154	GACCGCAGGAAGGACTGGGG
155	GGAAGGACTGGGGCCACGTG

156	GGTGGTGAACAGGGTGGATG
157	TGGTGAACAGGGTGGATGTG
158	GAACAGGGTGGATGTGGCGC
159	AGGAAGGTCCAGATGGGGGC
160	CATTCCAGAGCGGGGAGTTC
161	TCCAGAGCGGGGAGTTCAAG
162	CCTGAGCAAGCAGATGGCCG
163	CTGAGCAAGCAGATGGCCGG
164	GAGCAAGCAGATGGCCGGGT
165	AGATGGCCGGGTGCCTGGAG
166	GATGGCCGGGTGCCTGGAGG
167	CTGGGAAGCTGAGCCTCGAT
168	GAGCCTCGATGTGGAGCAGG
169	CGCTGGACATGTACAACGCC
170	CATGTACAACGCCGTGATGC
171	ATGTACAACGCCGTGATGCT
172	TGTACAACGCCGTGATGCTT
173	ACCATCGAAAGGTGTCTGGA
174	CACTCTTCACCGCCGTTCTG
175	ACTCTTCACCGCCGTTCTGC
176	TTCACCGCCGTTCTGCTGTC
177	GCCACTGTTCTGAAGGCCGT
178	TGAAGGCCGTGCACAAGGTG
179	TATGCCAAGGATGGGCGTGT
180	CCTGCA**A**GGATGGGCGTGTGTCC
181	CA**A**GGATGGGCGTGTGTCCT
182	AGGATGGGCGTGTGTCCTAC
183	GGATGGGCGTGTGTCCTACC
184	CGTTGCCAAGCAAGGAGGTC
185	TGCGGGACCAATGGGAGAAA
186	GGACCAATGGGAGAAAGCAC
187	GGGAGAAAGCACTGTGCCGG
188	CAGAGGCAGCGGGTCAGTGG
189	CGCGGCAGTACTGGGAGGAG
190	ATGCCATGCAGCCTGGACAA
191	CTGACCTTCGAGGCGGTGGA
192	TGACCTTCGAGGCGGTGGAT
193	GACCTTCGAGGCGGTGGATG
194	ACCTTCGAGGCGGTGGATGT
195	TTCGAGGCGGTGGATGTACC
196	TGCCCTGGACATCGCCGCAC
197	CCTGGACATCGCCGCACTCT
198	CTGGACATCGCCGCACTCTG

199	TGGACATCGCCGCACTCTGG
200	GACATCGCCGCACTCTGGTG
201	CATCGCCGCACTCTGGTGCT
202	CAACGGGCGCGTGCTGGACC
203	AACGGGCGCGTGCTGGACCT
204	GAGCTGCGCCGTGAGCTGGC
299	GGGGTGGCCTGGAGCGGCGG
300	GGTGGCCTGGAGCGGCGTGC
301	TGGCCTGGAGCGGCGTGCGT
302	GTCGGCACTTTGCTGGGGCC
303	ACCAAGACCGCAGGAAGGAC
304	CCAAGACCGCAGGAAGGACT
305	GACCGCAGGAAGGACTGGGG
306	GGAAGGACTGGGGCCACGTG
307	GGTGGTGAACAGGGTGGATG
308	TGGTGAACAGGGTGGATGTG
309	GAACAGGGTGGATGTGGCGC
310	AGGAAGGTCCAGATGGGGGC
311	CATTCCAGAGCGGGGAGTTC
312	TCCAGAGCGGGGAGTTCAAG
313	CCTGAGCAAGCAGATGGCCG
314	CTGAGCAAGCAGATGGCCGG
315	GAGCAAGCAGATGGCCGGGT
316	AGATGGCCGGGTGCCTGGAG
317	GATGGCCGGGTGCCTGGAGG
318	CTGGGAAGCTGAGCCTCGAT
319	GAGCCTCGATGTGGAGCAGG
320	CGCTGGACATGTACAACGCC
321	CATGTACAACGCCGTGATGC
322	ATGTACAACGCCGTGATGCT
323	TGTACAACGCCGTGATGCTT
324	ACCATCGAAAGGTGTCTGGA
325	CACTCTTCACCGCCGTTCTG
326	ACTCTTCACCGCCGTTCTGC
327	TTCACCGCCGTTCTGCTGTC
328	GCCACTGTTCTGAAGGCCGT
329	TGAAGGCCGTGCACAAGGTG
330	TATGCCAAGGATGGGCGTGT
331	CCAAGGATGGGCGTGTGTCC
332	CAAGGATGGGCGTGTGTCCT
333	AGGATGGGCGTGTGTCCTAC
334	GGATGGGCGTGTGTCCTACC
335	CGTTGCCAAGCAAGGAGGTC

336	TGCGGGACCAATGGGAGAAA
337	GGACCAATGGGAGAAAGCAC
338	GGGAGAAAGCACTGTGCCGG
339	CAGAGGCAGCGGGTCAGTGG
340	CGCGGCAGTACTGGGAGGAG
341	ATGCCATGCAGCCTGGACAA
342	CTGACCTTCGAGGCGGTGGA
343	TGACCTTCGAGGCGGTGGAT
344	GACCTTCGAGGCGGTGGATG
345	ACCTTCGAGGCGGTGGATGT
346	TTCGAGGCGGTGGATGTACC
347	TGCCCTGGACATCGCCGCAC
348	CCTGGACATCGCCGCACTCT
349	CTGGACATCGCCGCACTCTG
350	TGGACATCGCCGCACTCTGG
351	GACATCGCCGCACTCTGGTG
352	CATCGCCGCACTCTGGTGCT
353	CAACGGGCGCGTGCTGGACC
354	AACGGGCGCGTGCTGGACCT
355	GAGCTGCGCCGTGAGCTGGC
356	AGCTGCGCCGTGAGCTGGCG
357	GCCGTGAGCTGGCGCACTGC
358	AGCTGGCGCACTGCCAGAAG
359	CTGGCGCACTGCCAGAAGGT
360	GGCGCACTGCCAGAAGGTGG
361	TGCCAGAAGGTGGCCCGGGA
362	AGCACCTGCGGGACCGCGTC
363	GCCGCACAACATGGACTTCC
364	CGCACAACATGGACTTCCGC
365	TCAACCACCTGGGCAGCGAC
366	AACCACCTGGGCAGCGACGT
367	CCACCTGGGCAGCGACGTGG
368	GGGCAGCGACGTGGCGCGGG
369	GCAGCGACGTGGCGCGGGCC
370	TGGAGTTCGCCCAGGGCCGC
371	TTGACGGGCCGAAAGTGGTG
372	GTGGTGGATGGGCGCGGAGG
373	TGGTGGATGGGCGCGGAGGA
374	GTGGATGGGCGCGGAGGAAC
375	GGATGGGCGCGGAGGAACCC
376	ACCCTGGCAGACGCTGGCCT
377	TGGAGCCCTCGGATGTGCCG
378	AGGACGTGTACAGCGGCGTG

379	GGACGTGTACAGCGGCGTGG
380	GACGTGTACAGCGGCGTGGC
381	ACGTGTACAGCGGCGTGGCC
382	TACAGCGGCGTGGCCGCGCA
383	TGGAGGTGTTCCGTAGGCAG
384	GGGTGGCACAGGTGCTGGAA
385	GAGTTCGTGTGGGAGGCCTC
386	AGTTCGTGTGGGAGGCCTCT
387	CAAATAGGAGGTGGAATTCA
388	CAGGAAGGGCCTGACCTTCG
389	GAAGGGCCTGACCTTCGTCT
390	AAGGGCCTGACCTTCGTCTC
391	AGGGCCTGACCTTCGTCTCT
392	CTCTGTGCACGACTGTTACT
656	TGCCGCACAACATGGACTTC
657	CCGCACAACATGGACTTCCG
658	GCACAACATGGACTTCCGCG
659	ACAACATGGACTTCCGCGGC
660	CAACATGGACTTCCGCGGCC
661	AACATGGACTTCCGCGGCCG
662	ACATGGACTTCCGCGGCCGC
663	CATGGACTTCCGCGGCCGCA
664	ATGGACTTCCGCGGCCGCAC
665	CACTTCAACCACCTGGGCAG
666	CCGCAAGGTGGTGAAGCAGA
667	CGCAAGGTGGTGAAGCAGAC
668	GGCTTCCCGCCCAACTTCAT
669	GCTTCCCGCCCAACTTCATC
670	CTTCCCGCCCAACTTCATCC
671	TTCCCGCCCAACTTCATCCA
672	TCCCGCCCAACTTCATCCAC
673	CCGCCCAACTTCATCCACTC
674	CCGCCCAACTTCATCCACTC
675	CTGGACTCCTCCCACATGAT
676	TGGACTCCTCCCACATGATG
677	GGACTCCTCCCACATGATGC
678	CACTGCTACAGGAAGGGCCT
679	ACTGCTACAGGAAGGGCCTG
680	CTGCTACAGGAAGGGCCTGA
681	CCGTGATGCTTGGCTGGG
682	CGTGATGCTTGGCTGGGC
683	TGCCGCACAACATGGACT
684	GCCGCACAACATGGACTT

685	CCGCACAACATGGACTTC
686	CGCACAACATGGACTTCC
687	GCACAACATGGACTTCCG
688	CACAACATGGACTTCCGC
689	ACAACATGGACTTCCGCG
690	CAACATGGACTTCCGCGG
691	AACATGGACTTCCGCGGC
692	ACATGGACTTCCGCGGCC
693	CATGGACTTCCGCGGCCG
694	ATGGACTTCCGCGGCCGC
695	TGGACTTCCGCGGCCGCA
696	GGACTTCCGCGGCCGCAC
697	CACTTCAACCACCTGGGC
698	ACTTCAACCACCTGGGCA
699	CTTCAACCACCTGGGCAG
700	CCGCAAGGTGGTGAAGCA
701	CGCAAGGTGGTGAAGCAG
702	GCAAGGTGGTGAAGCAGA
703	CAAGGTGGTGAAGCAGAC
704	AGTCTACAGGAGATGTTC
705	GGCTTCCCGCCCAACTTC
706	GCTTCCCGCCCAACTTCA
707	CTTCCCGCCCAACTTCAT
708	TTCCCGCCCAACTTCATC
709	TCCCGCCCAACTTCATCC
710	CCCGCCCAACTTCATCCA
711	CCGCCCAACTTCATCCAC
712	CGCCCAACTTCATCCACT
713	GCCCAACTTCATCCACTC
714	CTGGACTCCTCCCACATG
715	TGGACTCCTCCCACATGA
716	GGACTCCTCCCACATGAT
717	GACTCCTCCCACATGATG
718	ACTCCTCCCACATGATGC
719	CTCCTCCCACATGATGCT
720	CACTGCTACAGGAAGGGC
721	ACTGCTACAGGAAGGGCC
722	CTGCTACAGGAAGGGCCT
723	TGCTACAGGAAGGGCCTG
724	GCTACAGGAAGGGCCTGA

[0170] In some embodiments, an oligonucleotide comprises a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NOs: 3-14, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740 and/or is complementary to a nucleotide sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NO: 15-26, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, or 703.

[0171] In some embodiments, an oligonucleotide comprises one or more mismatch(es) (*e.g.,* 1, 2, 3, 4, or 5) with the target region (*i.e.,* a nucleotide that is not complementary with the corresponding nucleotide in the target region sequence).

[0172] In some embodiments, an oligonucleotide is complementary to a target region within the mouse POLRMT RNA transcript (*e.g.,* as shown in SEQ ID NO: 582). In some embodiments, an oligonucleotide is complementary to a target region within the mouse POLRMT transcript (*e.g.,* as shown in SEQ ID NO: 582) and is also complementary to a corresponding target region within the human POLRMT transcript (*e.g.,* as shown in SEQ ID NO: 205), particularly if the target region corresponds to a conserved region between the mouse and human POLRMT sequences. In some embodiments, an oligonucleotide is complementary to a particular target region within the mouse POLRMT RNA transcript that corresponds to a region within the mouse POLRMT gene sequence (*e.g.,* as shown in SEQ ID NO: 581).

[0173] In some embodiments, an oligonucleotide of the present disclosure is complementary to 8 to 30 contiguous nucleotides (*i.e.,* a target region) of a mouse POLRMT RNA transcript. In some embodiments, an oligonucleotide is complementary to a target region of a mouse POLRMT RNA transcript and comprises any one of the sequences as shown in Table 4.

[0174] In some embodiments, an oligonucleotide comprises a sequence that is complementary (*e.g.,* substantially complementary or perfectly complementary) to a region of a mouse POLRMT transcript, *e.g.,* mouse POLRMT mRNA or pre-mRNA RNA transcript (*e.g.,* complementary to a nucleotide sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to a target region of SEQ ID NO: 582). In some embodiments, an oligonucleotide is complementary to 8 to 30 contiguous nucleotides of a mouse PORLMT transcript (*i.e.,* the target region) *e.g.,* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or

30 nucleotides long, although shorter and longer target region are also contemplated. In some embodiments, such an oligonucleotide sequence also targets a corresponding region within a human POLRMT transcript.

[0175] In some embodiments, the 8 to 30 contiguous nucleotides on the POLRMT RNA transcript (*i.e.*, the target region) comprises a sequence that is at least 80%, 85%, 90%, 95%, or 100% identical to any one of the sequences listed below in Table 4.

[0176] In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 85% identity to a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence having at least 95% identity to a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703. In some embodiments, an oligonucleotide is complementary to a target region on the POLRMT RNA transcript that comprises a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0177] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 528. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 528. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 528. In some embodiments, a target region comprises a sequence having at least 95% identity to SEQ ID NO: 528. In some embodiments, target region comprises SEQ ID NO: 528.

[0178] In some embodiments, a target region comprises a sequence having at least 80% identity to SEQ ID NO: 536. In some embodiments, a target region comprises a sequence having at least 85% to SEQ ID NO: 536. In some embodiments, a target region comprises a sequence having at least 90% identity to SEQ ID NO: 536. In some embodiments, a target region comprises a sequence having at least 95% identity to SEQ ID NO: 536. In some embodiments, target region comprises SEQ ID NO: 536.

[0179] In some embodiments, an oligonucleotide comprises a sequence that is complementary (*e.g.*, substantially complementary or perfectly complementary, and/or that includes no more than 1, 2, 3, or 4 nucleotide mismatches) to 8 to 30 contiguous nucleotides of a mouse POLRMT transcript (*e.g.*, SEQ ID NO: 582) and corresponds to a region within the mouse gene sequence (*e.g.*, SEQ ID NO: 581). In some embodiments, an oligonucleotide comprises a sequence that is complementary any one of the sequences listed below in Table 4. In some embodiments, an oligonucleotide may be complementary to any one of the sequences listed below in Table 4 but differs in one or more nucleotides in order to be complementary to the corresponding human POLRMT target region (*e.g.*, within SEQ ID NO: 205, corresponding to a region within the human gene sequence SEQ ID NO: 1).

[0180] SEQ ID NO: 581 In some embodiments, a target region on the mouse POLRMT RNA transcript comprises a region that corresponds to nucleotides 7077-7096, 7075-7094, 7074-7093, 3342-3361, 3341-3360, 3340-3359, 3297-3316, 3258-3277, 3202-3221, 2663-2682, 2621-2640, 2620-2639, 2619-2638, 2618-2637, 2617-2636, 2005-2024, 2003-2022, 7107-7126, 7105-7124, 7103-7122, 7082-7101, 7079-7098, 5712-5731, 5707-5726, 5705-5724, 4732-4751, 4731-4750, 4730-4749, 4568-4587, 4178-4197, 4136-4155, 4135-4154, 4133-4152, 4132-4151, 4131-4150, 4130-4149, 4129-4148, 4128-4147, 3597-3616, 3545-3564, 3544-3563, 3348-3367, 3339-3358, 3338-3357, 3337-3356, 3336-3355, 3335-3354, 3252-3271, 3250-3269, 3198-3217, 3197-3216, 3196-3215, 3168-3187, 2662-2681, 2660-2679, 2659-2678, 2658-2677, 2657-2676, 2656-2675, 2655-2674, 2654-2673, 2652-2671, 2651-2670, 2616-2635, 2615-2634, 2614-2633, 2613-2632, 2612-2631, 2611-2630, 2540-2559, 2537-2556, 2532-2551, 2380-2399, 2271-2290, 2270-2289, 2209-2228, 2207-2226, 2206-2225, 2195-2214, 1536-1555, 1534-1553, 1532-1551, 1377-1396, 1376-1395, 1375-1394, 1313-1332, 1312-1331, 1311-1330, 1308-1327, 1287-1306, 1286-1305, 1283-1302, 637-656, or 19-38 of SEQ ID NO: 581, or a corresponding target region within human POLRMT sequence (as shown in SEQ ID NO: 1).

[0181] In some embodiments, a target region on the POLRMT RNA transcript comprises a region that corresponds to nucleotides 2329-2348, 2331-2350, 3240-3259, 3241-3260, 734-751, 735-752, 2330-2347, 2331-2348, 2332-2349, 2333-2350, 3241-3258, 3242-3259, or 3243-3260 of the mouse PORLMT transcript (SEQ ID NO: 582), or a corresponding region within a human POLRMT transcript sequence (*e.g.*, as shown in SEQ ID NO: 205).

Table 4: Target Region Sequence of mouse POLRMT

SEQ ID NO:	POLRMT RNA sequence (5' to 3')
487	TGCACATGTACAACACCGTG
488	CACATGTACAACACCGTGAT
489	ACATGTACAACACCGTGATG
490	AGGTGGCTCGAGAGATGCAC
491	GGTGGCTCGAGAGATGCACA
492	GTGGCTCGAGAGATGCACAG
493	GCCTGTCGCTGGCACAGCAC
494	GGTTGCCGCACAACATGGAC
495	AACCACCTGGGCAGTGACCT
496	GGCCGGAAGTGGTGGATGGA
497	CTGGCCTGCTGCATGGAGGT
498	TGGCCTGCTGCATGGAGGTG
499	GGCCTGCTGCATGGAGGTGG
500	GCCTGCTGCATGGAGGTGGC
501	CCTGCTGCATGGAGGTGGCA
502	TCATCAGCCGCAAGGTGGTG
503	ATCAGCCGCAAGGTGGTGAA
504	ACAACGGAGACAGACAGCAG
505	AACGGAGACAGACAGCAGGT
506	CGGAGACAGACAGCAGGTGC
507	CACACTGCACATGTACAACA
508	ACTGCACATGTACAACACCG
509	TGCTCTTCACTGACCTGGTC
510	TTCACTGACCTGGTCCTGGA
511	CACTGACCTGGTCCTGGAAG
512	GCCTGCTGAGCGAAGGGGAG
513	CCTGCTGAGCGAAGGGGAGT
514	CTGCTGAGCGAAGGGGAGTT
515	AGTTGGCCCATAACCTGGGC
516	TGGAGGCCCCTGCACAACAG
517	TACAGCTGGGCAAGCAATTG

518	ACAGCTGGGCAAGCAATTGG
519	AGCTGGGCAAGCAATTGGCG
520	GCTGGGCAAGCAATTGGCGG
521	CTGGGCAAGCAATTGGCGGA
522	TGGGCAAGCAATTGGCGGAG
523	GGGCAAGCAATTGGCGGAGC
524	GGCAAGCAATTGGCGGAGCT
525	GTCTGCTGGAGCAATGCCCT
526	CACACAGTTGGGGAACTGCG
527	ACACAGTTGGGGAACTGCGC
528	GCCTCAAGGTGGCTCGAGAG
529	TGGCTCGAGAGATGCACAGT
530	GGCTCGAGAGATGCACAGTC
531	GCTCGAGAGATGCACAGTCT
532	CTCGAGAGATGCACAGTCTG
533	TCGAGAGATGCACAGTCTGC
534	CGCACAACATGGACTTCCGC
535	CACAACATGGACTTCCGCGG
536	ACCTGGGCAGTGACCTAGCG
537	CCTGGGCAGTGACCTAGCGC
538	CTGGGCAGTGACCTAGCGCG
539	TGGAGTTTGCTGAGGGCCGG
540	GCCGGAAGTGGTGGATGGAA
541	CGGAAGTGGTGGATGGAAGC
542	GGAAGTGGTGGATGGAAGCT
543	GAAGTGGTGGATGGAAGCTG
544	AAGTGGTGGATGGAAGCTGA
545	AGTGGTGGATGGAAGCTGAT
546	GTGGTGGATGGAAGCTGATG
547	TGGTGGATGGAAGCTGATGA
548	GTGGATGGAAGCTGATGAGC
549	TGGATGGAAGCTGATGAGCC
550	CTGCTGCATGGAGGTGGCAC
551	TGCTGCATGGAGGTGGCACA
552	GCTGCATGGAGGTGGCACAC
553	CTGCATGGAGGTGGCACACG
554	TGCATGGAGGTGGCACACGC
555	GCATGGAGGTGGCACACGCA
556	GTGGGCACGCAAGTGGACTG
557	GGCACGCAAGTGGACTGCCC
558	GCAAGTGGACTGCCCATGGC
559	AGTTTCTGGGGTGACCTGTG
560	AGGCCGAGACAGTGTGGGTG

561	GGCCGAGACAGTGTGGGTGC
562	TGTACAGGGAGGTGGCAACA
563	TACAGGGAGGTGGCAACACA
564	ACAGGGAGGTGGCAACACAG
565	GCAACACAGGTGGGTGCCAG
566	GCCGGATGGCCTGTGGAGTG
567	CGGATGGCCTGTGGAGTGGG
568	GATGGCCTGTGGAGTGGGTC
569	GGTCTGCAGGTAAAAGGTGG
570	GTCTGCAGGTAAAAGGTGGC
571	TCTGCAGGTAAAAGGTGGCC
572	GAGTCCTGTACTGCGGGGAG
573	AGTCCTGTACTGCGGGGAGG
574	GTCCTGTACTGCGGGGAGGG
575	CTGTACTGCGGGGAGGGGAG
576	GGATGGAGGGATGGGCAGGA
577	GATGGAGGGATGGGCAGGAA
578	GGAGGGATGGGCAGGAAAGG
579	GACTGCTTCTGGACACATGC
580	CACTTTCTGGCGGTGCGCAA
661	AACATGGACTTCCGCGGCCG
663	CATGGACTTCCGCGGCCGCA
666	CCGCAAGGTGGTGAAGCAGA
667	CGCAAGGTGGTGAAGCAGAC
681	CCGTGATGCTTGGCTGGG
682	CGTGATGCTTGGCTGGGC
692	ACATGGACTTCCGCGGCC
693	CATGGACTTCCGCGGCCG
694	ATGGACTTCCGCGGCCGC
695	TGGACTTCCGCGGCCGCA
701	CGCAAGGTGGTGAAGCAG
702	GCAAGGTGGTGAAGCAGA
703	CAAGGTGGTGAAGCAGAC

Modifications

[0182] In some embodiments, an oligonucleotide of the disclosure comprises a sequence based on a phosphodiester backbone (*i.e.*, an unmodified oligonucleotide sequence). In some embodiments, an oligonucleotide of the disclosure includes one or more modified nucleotides.

[0183] The use of naturally occurring nucleic acids (*e.g.*, unmodified DNA or RNA) is limited, for example, by their susceptibility to endo- and exo-nucleases. As such, various

synthetic counterparts have been developed to circumvent these shortcomings. These include synthetic oligonucleotides that contain chemical modification, e.g., base modifications, sugar modifications, backbone modifications, etc., which, among other things, render these molecules less susceptible to degradation and improve other properties of oligonucleotides. Chemical modifications may also lead to certain undesired effects, such as increased toxicities, etc.

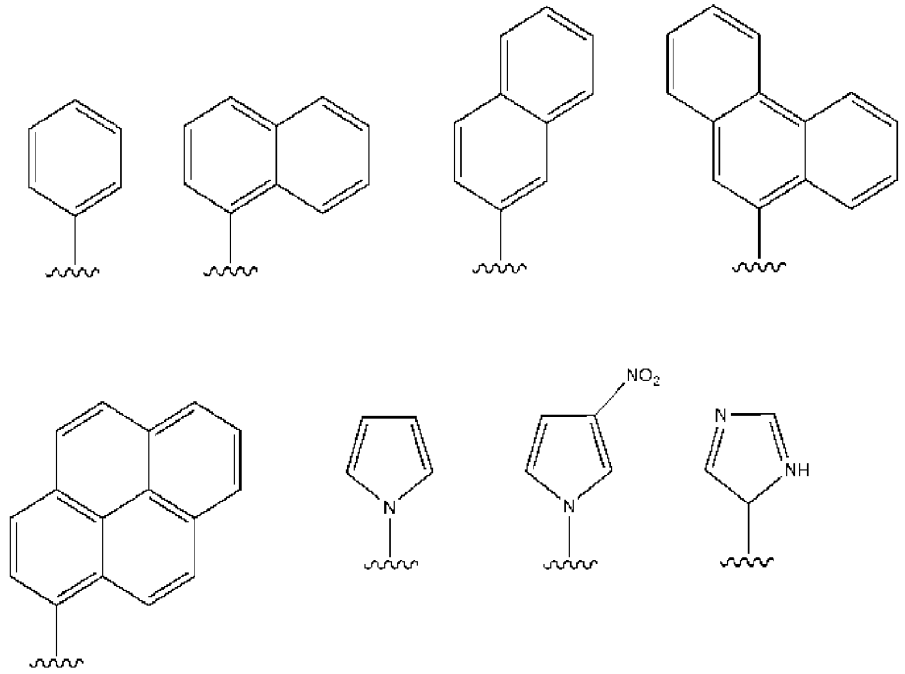
[0184] Such as base sequence, chemical modifications (*e.g.*, modifications of sugar, base, and/or internucleotidic linkages, and patterns thereof), and/or stereochemistry (*e.g.*, stereochemistry of backbone chiral centers (chiral internucleotidic linkages), and/or patterns thereof) can have significant impact on properties, *e.g.*, stability, splicing-altering capabilities, etc. In some embodiments, oligonucleotide properties can be adjusted by optimizing chemical modifications (modifications of base, sugar, and/or internucleotidic linkage) and/or stereochemistry (pattern of backbone chiral centers).

[0185] In some embodiments, a modified nucleotide comprises a base modification, a sugar or sugar phosphate modification, an internucleotidic linkage modification, or a combination thereof.

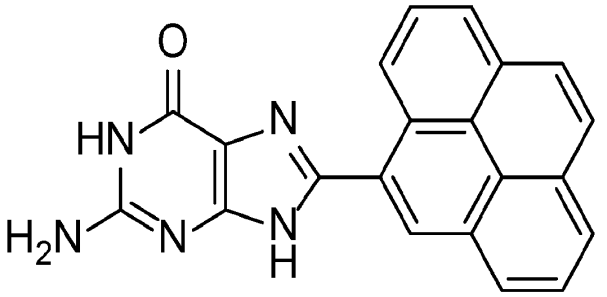
[0186] In some embodiments, an oligonucleotide of the disclosure includes one or more natural nucleobase and/or one or more modified nucleobases derived from a natural nucleobase. Examples include, but are not limited to, uracil, thymine, adenine, cytosine, and guanine having their respective amino groups protected by acyl protecting groups, 2-fluorouracil, 2-fluorocytosine, 5-bromouracil, 5-iodouracil, 2,6-diaminopurine, azacytosine, pyrimidine analogs such as pseudoisocytosine and pseudouracil and other modified nucleobases such as 8-substituted purines, xanthine, or hypoxanthine (the latter two being the natural degradation products).

[0187] Modified nucleobases also include expanded-size nucleobases in which one or more aryl rings, such as phenyl rings, have been added.

[0188] In some embodiments, modified nucleobases comprise any one of the following substituents, each of which is optionally substituted:



[0189] For example, a pyrene-modified guanine base can have the structure



[0190] A person skilled in the art would understand where and how a nucleobase can be modified with any of the foregoing groups.

[0191] In some embodiments, a modified nucleobase is unsubstituted. In some embodiments, a modified nucleobase is substituted. In some embodiments, a modified nucleobase is substituted such that it contains, e.g., heteroatoms, alkyl groups, or linking moieties connected to fluorescent moieties, biotin or avidin moieties, or other protein or peptides. In some embodiments, a modified nucleobase is a "universal base" that is not a

nucleobase in the most classical sense, but that functions similarly to a nucleobase. One representative example of such a universal base is 3-nitropyrrole.

[0192] In some embodiments, an oligonucleotide described herein includes nucleosides that incorporate modified nucleobases and/or nucleobases covalently bound to modified sugars (i.e., a "base modification"). Some examples of nucleosides that incorporate modified nucleobases include 4-acetylcytidine; 5-(carboxyhydroxylmethyl)uridine; 2′-*O*-methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2′-*O*-methylpseudouridine; beta,D-galactosylqueosine; 2′-*O*-methylguanosine; N^6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; 1-methylinosine; 2,2-dimethylguanosine; 2-methyladenosine; 2-methylguanosine; N^7-methylguanosine; 3-methylcytidine; 5-methylcytidine; 5-hydroxymethylcytidine; 5-methylcytosine, 5-formylcytosine; 5-carboxylcytosine; N^6-methyladenosine; 7-methylguanosine; 5-methylaminoethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N^6-isopentenyladenosine; *N*-((9-beta,D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine; *N*-((9-beta,D-ribofuranosylpurine-6-yl)-*N*-methylcarbamoyl)threonine; uridine-5-oxyacetic acid methylester; uridine-5-oxyacetic acid; pseudouridine; queosine; 2-thiocytidine; 5-methyl-2-thiouridine; 2-thiouridine; 4-thiouridine; 5-methyluridine; 2′-*O*-methyl-5-methyluridine; and 2′-*O*-methyluridine. In some embodiments, an oligonucleotide described herein comprises at least one G-clamp modification.

[0193] In some embodiments, nucleosides include 6′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 6′-position and include the analogs described in US Patent No. 7,399,845. In other embodiments, nucleosides include 5′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 5′-position and include the analogs described in U.S. Publ. No. 20070287831. In some embodiments, a nucleobase or modified nucleobase is 5-bromouracil, 5-iodouracil, or 2,6-diaminopurine. In some embodiments, a nucleobase or modified nucleobase is modified by substitution with a fluorescent moiety.

[0194] In some embodiments, an oligonucleotide described herein includes one or more modified nucleotides wherein a phosphate group or linkage phosphorus in the nucleotides are

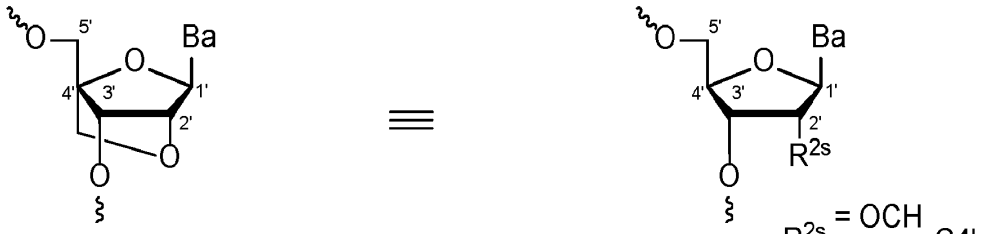
linked to various positions of a sugar or modified sugar. As non-limiting examples, the phosphate group or linkage phosphorus can be linked to the 2′, 3′, 4′ or 5′ hydroxyl moiety of a sugar or modified sugar. Nucleotides that incorporate modified nucleobases as described herein are also contemplated in this context. In some embodiments, the sugar or sugar phosphate modification comprises a 2'-O-methoxyethyl (2'-MOE) modification, a 2'-fluoro (2'-F) modification, a 2'-O-methyl (2'-O-Me) modification, a phosphorodiamidate morpholino (PMO) modification, a peptide nucleic acid (PNA) modification, a glycol nucleic acid (GNA), an unlocked nucleic acid (UNA), or a locked nucleic acid (LNA).

[0195] Other modified sugars can also be incorporated within an oligonucleotide molecule. In some embodiments, a modified sugar contains one or more groups at the 2′ position selected from –F, $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently hydrogen or optionally substituted C_1-C_{10} aliphatic. In some embodiments, a modified sugar contains one or more groups at the 2′ position selected from –F, $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl),$–N(C_1–C_{10}$ alkyl$)_2$, $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl),$–N(C_2–C_{10}$ alkenyl$)_2$, $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$ alkynyl), $–NH–(C_2–C_{10}$ alkynyl),$–N(C_2–C_{10}$ alkynyl$)_2$,$–O–(C_1–C_{10}$ alkylene$)–O–(C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene$)–NH–(C_1–C_{10}$ alkyl),$–O–(C_1–C_{10}$ alkylene$)–N(C_1–C_{10}$ alkyl$)_2$, $–NH–(C_1–C_{10}$ alkylene$)–O–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl$)–(C_1–C_{10}$ alkylene$)–O–(C_1–C_{10}$ alkyl), wherein each alkyl, alkylene, alkenyl and alkynyl may be substituted or unsubstituted. In some embodiments, an alkyl, alkenyl, or alkynyl is substituted by a group selected from $–O(CH_2)_nOCH_3$ or $–O(CH_2)_nNH_2$, wherein n is from 1 to about 10, MOE, DMAOE, and DMAEOE.

[0196] In some embodiments, the 2'-OH of a ribose is replaced with a group selected from –H, –F, $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently hydrogen or optionally substituted C_1-C_{10} aliphatic. In some embodiments, a modified sugar contains one or more groups at the 2′ position selected from –F, $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl),$–N(C_1–C_{10}$ alkyl$)_2$, $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl),$–N(C_2–C_{10}$ alkenyl$)_2$, $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$ alkynyl), $–NH–(C_2–C_{10}$ alkynyl),$–N(C_2–C_{10}$ alkynyl$)_2$,$–O–(C_1–C_{10}$ alkylene$)–O–(C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene$)–NH–(C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene$)–$

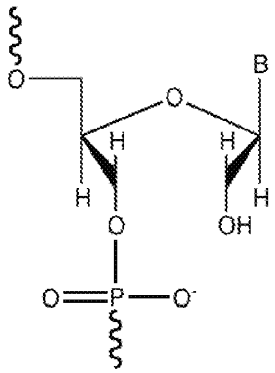
$N(C_1–C_{10}$ alkyl$)_2$, –NH–($C_1–C_{10}$ alkylene)–O–($C_1–C_{10}$ alkyl), or –N($C_1–C_{10}$ alkyl)–($C_1–C_{10}$ alkylene)–O–($C_1–C_{10}$ alkyl), wherein each alkyl, alkylene, alkenyl and alkynyl may be substituted or unsubstituted. In some embodiments, the 2'–OH is replaced with –H (i.e., deoxyribose). In some embodiments, the 2'–OH is replaced with –F. In some embodiments, the 2'–OH is replaced with –OR'. In some embodiments, the 2'–OH is replaced with –OMe. In some embodiments, the 2'–OH is replaced with $–OCH_2CH_2OMe$ (i.e., MOE).

[0197] Modified sugars also include locked nucleic acids (LNAs). In some embodiments, the locked nucleic acid has the structure indicated below. In some embodiments, a locked nucleic acid comprises the structure below, wherein Ba represents a nucleobase or modified nucleobase as described herein, and wherein R^{2s} is $–OCH_2C4'–$



$C2'OCH_2C4'$ = LNA (Locked Nucleic Acid)

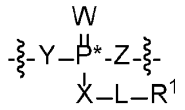
[0198] Modified sugars also include unlocked nucleic acids (UNAs). In some embodiments, an unlocked nucleic acid has the structure indicated below (see *e.g.,* Fluiter, Kees, et al., *Molecular BioSystems* 5.8 (2009): 838-843, which is herein incorporated by reference in its entirety). In some embodiments, a locked nucleic acid comprises the structure below.



Unlocked Nucleic Acid (UNA)

[0199] In some embodiments, an oligonucleotide described herein comprises at least one modified internucleotidic linkage. In some embodiments, an internucleotidic linkage modification comprises a phosphorothioate or phosphodithioate linkage modification.

[0200] In some embodiments, the present invention provides an oligonucleotide comprising one or more modified internucleotidic linkages independently having the structure of formula **I**:



[0201] (**I**)

[0202] wherein:

[0203] P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

[0204] W is O, S or Se;

[0205] each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L;

[0206] L is a covalent bond or an optionally substituted, linear or branched , saturated or unsaturated C_1–C_{10} aliphatic, wherein one or more methylene units of L are optionally and independently replaced by –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′), –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

[0207] R^1 is halogen, R, or an optionally substituted, linear or branched, saturated or unsaturated C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′), –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

[0208] each R′ is independently –R, C(O)R, CO_2R, or –SO_2R, or:

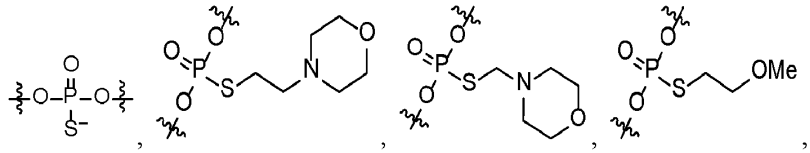
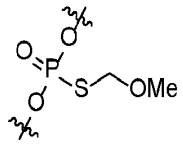
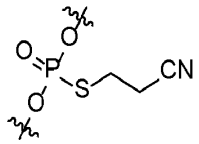
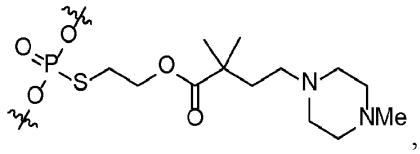
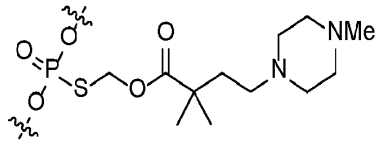
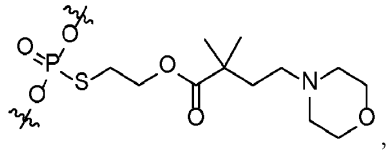
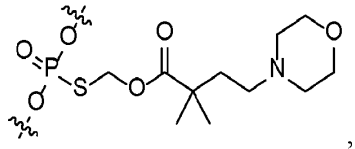
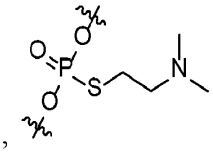
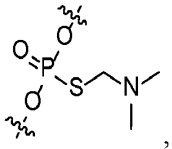
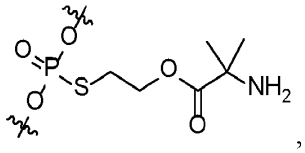
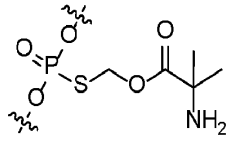
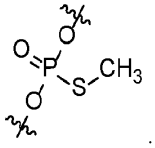
[0209] two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

[0210] two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

[0211] –Cy– is an optionally substituted bivalent ring selected from carbocyclylene, arylene, heteroarylene, or heterocyclylene;

[0212] each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

[0213] each $^{\wedge}$ independently represents a connection to a nucleoside.

[0214] In some embodiments, the internucleotidic linkage having the structure of formula **I** is  , , , ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  .

[0215] Among other things, the present disclosure provides oligonucleotides of various designs, which may comprise various nucleobases and patterns thereof, sugars and patterns thereof, internucleotidic linkages and patterns thereof, and/or additional chemical moieties and patterns thereof as described in the present disclosure. In some embodiments, provided oligonucleotides can decrease the level of POLRMT protein, POLRMT mRNA expression and/or POLRMT activity in a cell of a subject. In some embodiments, such an oligonucleotide has a base sequence which consists of, comprises, or comprises a portion (*e.g.,* a span of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more contiguous bases) of the base sequence of an oligonucleotide disclosed herein, wherein each T can be independently substituted with U and vice versa, and the oligonucleotide comprises at least one non-naturally-occurring modification of a base, sugar and/or internucleotidic linkage.

[0216] According to certain embodiments, various nucleotide modifications or nucleotide modification patterns may be in any of oligonucleotides described herein.

[0217] In some embodiments, an oligonucleotide comprises two or more chemically distinct regions, wherein the regions confer distinct properties on the compound. In some embodiments, at least one region is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid and at least one additional region of the oligonucleotide can serve as a substrate for enzymes (*e.g.,* RNase H) capable of cleaving RNA:DNA or RNA:RNA hybrids. In some embodiments, at least one region of the oligonucleotide can serve as a substrate for enzymes (*e.g.,* RNase H) capable of cleaving RNA:DNA or RNA:RNA hybrids and at least one region can inhibit translation by steric blocking.

[0218] In some embodiments, an oligonucleotide comprises at least one (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) phosphorothioate (PS) internucleotide bond. In some embodiments, an oligonucleotide comprises a sequence where each internucleotidic linkage comprises a phosphorothioate (PS) internucleotide bond. In some embodiments, an oligonucleotide comprises at least one (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) phosphodithioate bond. In some embodiments, an oligonucleotide comprises a sequence where each internucleotidic linkage comprises a phosphodithioate bond.

[0219] In some embodiments, an oligonucleotide comprises at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) 2'-MOE modification.

[0220] In some embodiments, an oligonucleotide comprises five nucleotides at the 5'-end and five nucleotides at the 3'-end which contain a 2'-MOE modification.

[0221] In some embodiments, an oligonucleotide is modified so that each nucleotide comprises a 2'-MOE modification.

[0222] In some embodiments, an oligonucleotide comprises one of the following modification patterns or a portion thereof:

$X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}$ ("4-8-4" 16-mer)

$X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}$ ("3-10-3" 16-mer)

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$ ("5-8-5" 18-mer)

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}$ ("5-9-4" 18-mer)

$X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$ ("4-9-5" 18-mer)

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$ ("5-10-5" 20-mer)

X_{MS}

[0223] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond.

[0224] When DNA-based antisense oligonucleotides (ASO) bind to their cognate mRNA transcripts, the endogenous RNase H enzyme RNASEH1 recognizes RNA–DNA heteroduplex substrates that are formed and further cleaves at the site of ASO binding and results in degradation of the target RNA, thereby silencing target gene expression. Gapmer antisense oligonucleotides (ASOs), consisting of a DNA-based internal 'gap' and RNA-like flanking regions (often consisting of 2′-O-methyl (2′-OMe) or 2′-O-methoxyethyl (2′-MOE) modified bases) bind to target transcripts with high affinity. In some embodiments, oligonucleotides comprise a Gapmer modification pattern.

[0225] In some embodiments, an oligonucleotide comprises any one of the sequences listed in Table 1 or Table 2, or a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to any one of the sequences listed in Table 1 or Table 2 and comprises the following modification pattern:

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

[0226] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond.

[0227] In some embodiments, an oligonucleotide comprises any one of the sequences listed in Table 1 or Table 2, or a sequence that is at least 80%, at least 85%, at least 90%, at least 95% identical to any one of the sequences listed in Table 1 or Table 2 and comprises the following modification pattern:

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$,

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

or

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

[0228] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond.

[0229] In some embodiments, oligonucleotides are provided and/or utilized in salt forms. In some embodiments, oligonucleotides are provided as salts comprising negatively-charged internucleotidic linkages (e.g., phosphorothioate internucleotidic linkages, natural phosphate linkages, etc.) existing as their salt forms. In some embodiments, oligonucleotides are provided as pharmaceutically acceptable salts. In some embodiments, oligonucleotides are provided as metal salts. In some embodiments, metal salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium, and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or

alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using the appropriate hydroxide or amine base. In some embodiments, oligonucleotides are provided as sodium salts. In some embodiments, oligonucleotides are provided as metal salts, e.g., sodium salts, wherein each negatively-charged internucleotidic linkage is independently in a salt form (e.g., for sodium salts, −O−P(O)(SNa)−O− for a phosphorothioate internucleotidic linkage, −O−P(O)(ONa)−O− for a natural phosphate linkage, etc.). In some embodiments, oligonucleotides are provided as ammonium salts.

[0230] In some embodiments, an oligonucleotide can be modified according to any one of the modifications and modification patterns described herein and can also be conjugated to a ligand, *e.g.*, as described herein. In some such embodiments, a ligand can be attached to any of the 3' or 5' terminus of the oligonucleotide sequence.

[0231] In some embodiments, the ligand targets the nucleic acid molecule to hepatocytes. For example, in some embodiments the ligand binds to hepatocyte-specific asialoglycoprotein receptor (ASGPR). In some embodiments, the ligand comprises a galactose derivative, *e.g.*, GalNAc.

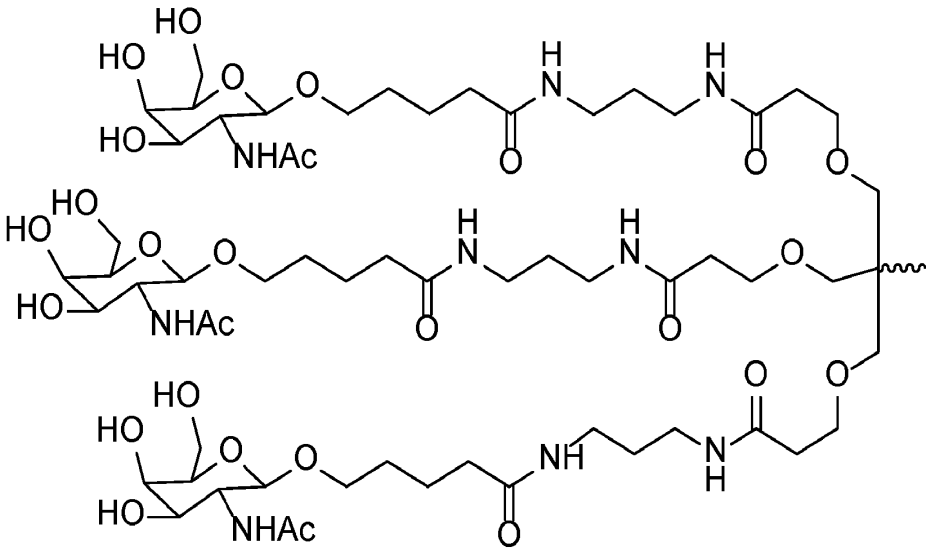
[0232] In some embodiments, an oligonucleotide is conjugated to or otherwise physically associated with one or more moieties that modulate, *e.g.*, enhance, the activity, stability, cellular distribution, and/or cellular uptake of the oligonucleotide and/or alter one or more physical properties of the oligonucleotide, such as charge or solubility. In some embodiments, a moiety may comprise an antibody or ligand. A ligand may be a carbohydrate, lectin, protein, glycoprotein, lipid, cholesterol, steroid, bile acid, nucleic acid hormone, growth factor, or receptor. In some embodiments a biologically inactive variant of a naturally occurring hormone, growth factor, or other ligand may be used. In some embodiments, the moiety comprises a targeting moiety that targets the oligonucleotide to a specified cell type, *e.g.*, a hepatocyte. In some embodiments a targeting moiety binds to hepatocyte-specific asialoglycoprotein receptor (ASGPR).

[0233] In some embodiments, a moiety is attached to an oligonucleotide via a reversible linkage. A "reversible linkage" is a linkage that comprises a reversible bond. A "reversible bond" (also referred to as a labile bond or cleavable bond) is a covalent bond other than a covalent bond to a hydrogen atom that is capable of being selectively broken or cleaved more rapidly than other bonds in a molecule under selected conditions, the bond is capable of being selectively broken or cleaved under conditions that substantially will not break or cleave other covalent bonds in the same molecule. Cleavage or lability of a bond may be described in terms of the half-life ($t_{1/2}$) of bond cleavage (the time required for half of the bonds to cleave).

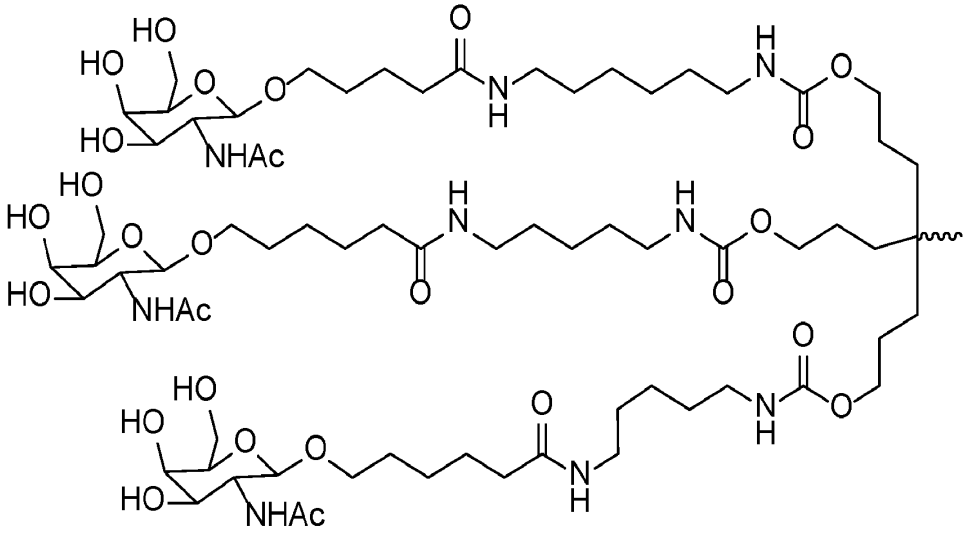
[0234] In some embodiments a moiety attached to an oligonucleotide comprises a carbohydrate. Representative carbohydrates include mono-, di-, tri- and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units. In certain embodiments the carbohydrate comprises galactose or a galactose derivative such as galactosamine, N-formyl-galactosamine, N-acetylgalactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine, and N-iso-butanoylgalactos-amine. In certain embodiments of particular interest, a galactose derivative comprises N-acetylgalactosamine (GalNAc). In certain embodiments, a moiety comprises multiple instances of the galactose or galactose derivative, *e.g.*, multiple N-acetylgalactosamine moieties, *e.g.*, 3 GalNAc moieties (*i.e.*, a triantennary GalNAc). A terminal galactose derivative may be attached to another moiety through the C-1 carbon of the galactose derivative. In some embodiments two or more, *e.g.*, three, galactose derivatives are attached to a moiety that serves as a branch point and that can be attached to an oligonucleotide. In some embodiments, a galactose derivative is linked to the moiety that serves as a branch point via a linker or spacer. In some embodiments, the moiety that serves as a branch point may be attached to an oligonucleotide via a linker or spacer. For example, in some embodiments, a galactose derivative is attached to a branch point via a linker or spacer that comprises an amide, carbonyl, alkyl, oligoethylene glycol moiety, or combination thereof. In some embodiments, at least 3 nucleoside–GalNAc monomers or at least 3 non-nucleoside–GalNAc monomers are incorporated site-specifically into an oligonucleotide. In some embodiments, such incorporation may occur during solid-phase synthesis using phosphoramidite chemistry or via postsynthetic conjugation. In some embodiments, the galactose derivative-containing monomeric units are joined via phosphodiester bonds to each other and/or to nucleosides of the oligonucleotide that

do not have a galactose derivative attached. One of ordinary skill in the art appreciates that the structure of the linking moieties that connect each GalNAc to a branch point may vary.

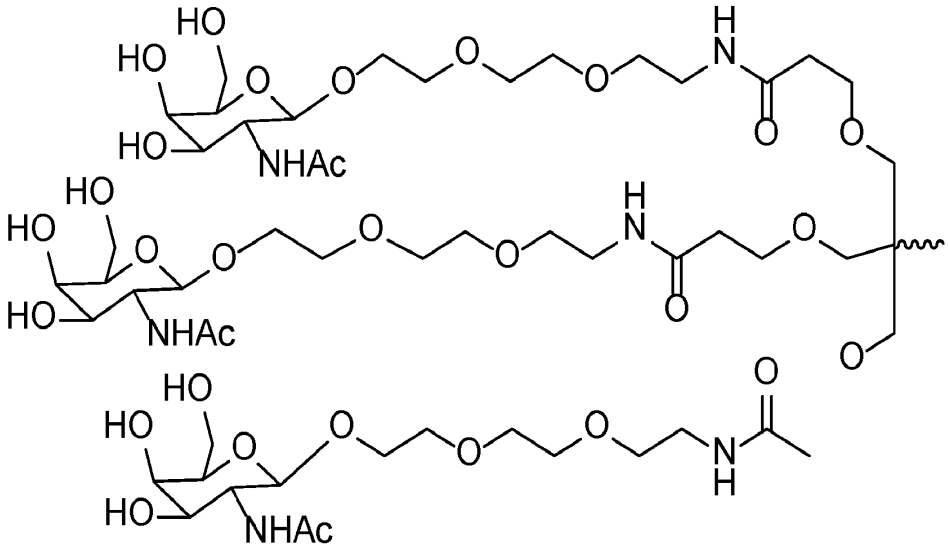
[0235] Exemplary galactose clusters are depicted below.



Formula I



Formula II



Formula III

[0236] In some embodiments, a GalNAc moiety (*e.g.*, a GalNAc moiety as represented in Formulas I-III) is conjugated to the 5' end of an oligonucleotide described herein (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740).

[0237] In some embodiments, a GalNAc moiety (*e.g.*, a GalNAc moiety as represented in Formulas I-III) is conjugated to the 3' end of an oligonucleotide described herein (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740).

[0238] Methods of conjugating oligonucleotides to a GalNAc moiety are known in the art and exemplary methods are disclosed in Østergaard, Michael E., et al., "Efficient synthesis and biological evaluation of 5′-GalNAc conjugated antisense oligonucleotides." *Bioconjugate chemistry* 26.8 (2015): 1451-1455, which is herein incorporated by reference in its entirety.

[0239] In some embodiments, a 2' deoxyadenosine phosphodiester is inserted between the oligonucleotide and the GalNAc conjugate to facilitate metabolic cleavage. Accordingly, in

some embodiments, an oligonucleotide sequence contains an additional adenine (A) nucleotide residue at the 5' or 3' end where a GalNAc moiety is conjugated (see e.g., Østergaard, Michael E., et al., 2015) and the additional A contains a phosphate bond between the A and the 5' or 3' nucleotide of the oligonucleotide. Exemplary modification patterns are shown below:

$A_OX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}A_O$

$A_OX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}A_O$

$A_OX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}A_O$

[0240] where "A" represents an adenine; an "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; an "S" represents a phosphorothioate bond; and an "O" represents a phosphate linkage.

[0241] In some embodiments, an oligonucleotide comprises any one of the sequences listed in Table 1 or Table 2, or a sequence that is at least 80%, at least 85%, at least 90%, at least 95% identical to any one of the sequences listed in Table 1 or Table 2 and comprises the following modification pattern:

$A_OX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}A_O$

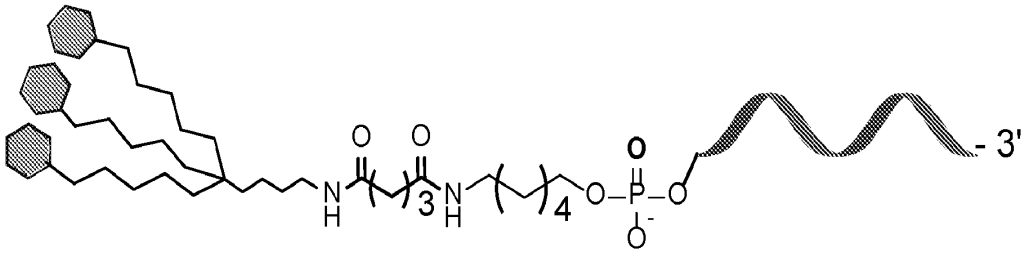
$A_OX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}A_O$

$A_OX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}A_O$

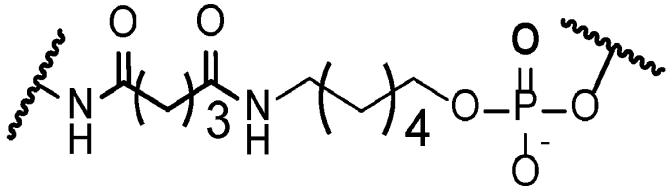
[0242] where "A" represents an adenine; an "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; an "S" represents a phosphorothioate bond; and an "O" represents a phosphate linkage.

[0243] In some embodiments, a linking moiety connects an oligonucleotide described herein (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) to a GalNAc moiety (*e.g.*, as shown in Formulas I-III). In some embodiments, an oligonucleotide described herein is conjugated to GalNAc as depicted below:



5'-triantennary GalNAc-ASO conjugate

[0244] In some embodiments, a linking moiety comprises a structure as depicted below:



Formula A

[0245] In some embodiments, an oligonucleotide (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) is conjugated to a GalNAc moiety as shown in Formula I at its 5' end via a linker as shown in Formula A. In some embodiments, an oligonucleotide (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) is conjugated to a GalNAc moiety as shown in Formula I at its 3' end via a linker as shown in Formula A. In some embodiments, an oligonucleotide (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) additionally comprises a 2' deoxyadenosine phosphodiester inserted between the oligonucleotide and the GalNAc/Linker moiety.

[0246] In some embodiments, an oligonucleotide (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or

728-740) is conjugated to a GalNAc moiety as shown in Formula II at its 5' end via a linker as shown in Formula A. In some embodiments, an oligonucleotide (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) is conjugated to a GalNAc moiety as shown in Formula II at its 3' end via a linker as shown in Formula A. In some embodiments, an oligonucleotide (*e.g.*, an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) additionally comprises a 2' deoxyadenosine phosphodiester inserted between the oligonucleotide and the GalNAc/Linker moiety.

[0247] In some embodiments, an oligonucleotide (e.g., an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) is conjugated to a GalNAc moiety as shown in Formula III at its 5' end via a linker as shown in Formula A. In some embodiments, an oligonucleotide (e.g., an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) is conjugated to a GalNAc moiety as shown in Formula III at its 3' end via a linker as shown in Formula A. In some embodiments, an oligonucleotide (e.g., an oligonucleotide represented in any one of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, 634, or 728-740) additionally comprises a 2' deoxyadenosine phosphodiester inserted between the oligonucleotide and the GalNAc/Linker moiety.

[0248] In certain embodiments, the moiety comprises a lipophilic moiety. In some embodiments, the lipophilic moiety comprises a tocopherol, *e.g.*, alpha-tocopherol. In some embodiments, the lipophilic moiety comprises cholesterol. In some embodiments, the lipophilic compound comprises an alkyl or heteroalkyl group. In some embodiments the lipophilic compound comprises palmitoyl, hexadec-8-enoyl, oleyl, (9E,12E)-octadeca-9,12-dienoyl, dioctanoyl, or C_{16}-C_{20} acyl. In some embodiments, the lipophilic moiety comprises at least 16 carbon atoms. In some embodiments the lipophilic moiety comprises $-(CH_y)_n$-NH-(C=O)-$(CH_x)_m$-CH_3, wherein each of m and n is independently 0-20; and each of x and y is independently 0-2. In some embodiments, n and m are each independently an integer from 1 to 20. In some embodiments n + m is at least 10, 12, 14, or 16.

[0249] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the

structure; for example, the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures including the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention.

Compositions and Administration

[0250] In some embodiments, one or more oligonucleotides as described herein may be formulated in an oligonucleotide composition. In some embodiments, an oligonucleotide composition may comprise oligonucleotides comprising the same nucleotide sequence (*e.g.*, any one of the sequences provided in Table 1 or Table 2). In some embodiments, an oligonucleotide composition may comprise oligonucleotides comprising more than one nucleotide sequence (*e.g.*, more than one of the sequences provided in Table 1 or Table 2).

[0251] In some embodiments, provided oligonucleotide compositions may be or include pure preparations of individual stereochemically isomeric forms of a compound (*e.g.*, comprising a chirally pure oligonucleotide). In some embodiments, provided oligonucleotide compositions may be or include mixtures of two or more stereochemically isomeric forms of the compound. In some embodiments, such mixtures contain equal amounts of different stereochemically isomeric forms. In some embodiments, such mixtures contain different amounts of at least two different stereochemically isomeric forms. In some embodiments, an oligonucleotide composition may contain all diastereomers and/or enantiomers of the compound. In some embodiments, an oligonucleotide composition may contain fewer than all diastereomers and/or enantiomers of a compound. In some embodiments, if a particular enantiomer of an oligonucleotide is desired, it may be prepared, for example, by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the

auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, diastereomeric salts are formed with an appropriate optically-active acid, and resolved, for example, by fractional crystallization.

Pharmaceutical Compositions

[0252] In some embodiments, the present disclosure provides pharmaceutical compositions comprising one or more oligonucleotides. In some embodiments, a pharmaceutical composition comprises a pharmaceutically acceptable carrier.

[0253] In some embodiments, a pharmaceutical composition is formulated for systemic or localized administration. In some embodiments, a pharmaceutical composition is administered via a delivery route selected from intrathecal, oral, intramuscular, or intravenous administration.

[0254] Pharmaceutically acceptable compositions of this disclosure may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents. In some embodiments, an intranasal composition is an intranasal drop or spray (fine mist) in a liquid form such as, for example, a solution, emulsion or suspension.

[0255] Pharmaceutically acceptable compositions of this disclosure may also be adapted for pulmonary administration, such as an inhalation composition to be inhaled by the patient. The inhalation composition can be in the form of a dry powder inhalation composition, a pressurized aerosol inhalation composition or a nebulized inhalation composition (e.g., an aqueous suspension or solution).

[0256] In some embodiments an oligonucleotide is associated with a delivery agent. "Delivery agent" refers to a substance or entity that is non-covalently or covalently associated with an oligonucleotide or is co-administered with an oligonucleotide and serves one or more functions that increase the stability and/or efficacy of the biologically active agent beyond that which would result if the biologically active agent was delivered (*e.g.*, administered to a subject)

in the absence of the delivery agent. For example, a delivery agent may protect an oligonucleotide from degradation, may facilitate entry of an oligonucleotide into cells or into a cellular compartment of interest (*e.g.,* the cytoplasm or mitochondria), and/or may enhance associations with particular cells containing the molecular target to be modulated. Those of ordinary skill in the art are aware of numerous delivery agents that may be used to deliver oligonucleotides. See Dhuri, Karishma, et al., "Antisense oligonucleotides: an emerging area in drug discovery and development." *Journal of clinical medicine* 9.6 (2020), for review of some of these technologies. In some embodiments, *e.g.,* for administering an oligonucleotide systemically, the oligonucleotide may be associated with a delivery agent such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Lipids (*e.g.,* cationic lipids, or neutral lipids), dendrimers, or polymers may be bound to an oligonucleotide or may form a vesicle or micelle that encapsulates an oligonucleotide.

[0257] In some embodiments an oligonucleotide is administered in association with a lipid or lipid-containing particle (e.g., a lipid nanoparticle (LNP)). In some embodiments an oligonucleotide is administered in association with a cationic polymer (which may be a polypeptide or a non-polypeptide polymer), a lipid, a peptide, PEG, cyclodextrin, or combination thereof, which may be in the form of a nanoparticle or microparticle. The lipid or peptide may be cationic. "Nanoparticle" refers to particles with lengths in two or three dimensions greater than 1 nanometer (nm) and smaller than about 150 nm e.g., 20 nm – 50 nm or 50 nm -100 nm. "Microparticle" refers to particles with lengths in two or three dimensions greater than 150 nm and smaller than about 1000 nm. A nanoparticle may have a targeting moiety and/or cell-penetrating moiety or membrane active moiety covalently or noncovalently attached thereto. Nanoparticles, such as lipid nanoparticles, are described in, e.g., Tatiparti et al., Nanomaterials 7:77 (2017).

[0258] In some embodiments, a delivery agent comprises one or more amino acid lipids. Amino acid lipids are molecules containing an amino acid residue (e.g., arginine, homoarginine, norarginine, nor-norarginine, ornithine, lysine, homolysine, histidine, 1-methylhistidine, pyridylalanine, asparagine, N-ethylasparagine, glutamine, 4-aminophenylalanine, the N-methylated versions thereof, and side chain modified derivatives thereof) and one or more lipophilic tails. In some embodiments, a delivery agent comprises a lipopeptide compound

comprising a central peptide and having lipophilic groups attached at each terminus. In some embodiments lipophilic groups can be derived from a naturally occurring lipid. In some embodiments a lipophilic group may comprise a C(1-22)alkyl, C(6-12)cycloalkyl, C(6-12)cycloalkyl-alkyl, C(3-18)alkenyl, C(3-18)alkynyl, C(1-5)alkoxy-C(1-5)alkyl, or a sphinganine, or (2R,3R)-2-amino-1,3-octadecanediol, icosasphinganine, sphingosine, phytosphingosine, or cis-4-sphingenine. The central peptide may comprise a cationic or amphipathic amino acid sequence. Examples of such lipopeptides and their use to deliver nucleic acids are described in, e.g., U.S. Pat. No. 9,220,785.

[0259] In some embodiments an oligonucleotide is conjugated to a delivery agent that is a polymer. Useful delivery polymers include, e.g., poly(acrylate) polymers (see., e.g., US Pat. Pub. No. 20150104408), poly(vinyl ester) polymers (see., e.g., US Pat. Pub. No. 20150110732) and certain polypeptides.

[0260] In some embodiments an oligonucleotide may be administered in "naked" form, *i.e.*, administered in the absence of a delivery agent. The naked oligonucleotide may be in a suitable buffer solution. The buffer solution may, for example, comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In some embodiments, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution can be adjusted such that it is suitable for administering to a subject. In some embodiments, an oligonucleotide is administered not in physical association with a lipid or lipid-containing particle. In some embodiments, an oligonucleotide is administered not in physical association with a nanoparticle or microparticle. In some embodiments, an oligonucleotide is administered not in physical association with a cationic polymer.

[0261] Oligonucleotides described herein, can be incorporated into pharmaceutical compositions. Such pharmaceutical compositions are useful for, among other things, administration and delivery to a subject in vivo or ex vivo. In some embodiments, pharmaceutical compositions also contain a pharmaceutically acceptable carrier or excipient. Such excipients include any pharmaceutical agent, *e.g.*, a pharmaceutical agent that does not itself induce an immune response harmful to the individual receiving the composition, and which may be administered without undue toxicity. As used herein the terms "pharmaceutically

acceptable" and "physiologically acceptable" mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, in vivo delivery or contact. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, glycerol, sugars and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

[0262] Pharmaceutical compositions may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding, free base forms. In some embodiments, a pharmaceutical composition may be a lyophilized powder.

[0263] Pharmaceutical compositions can include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (e.g., oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or in vivo contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules and crystals. Supplementary active compounds (e.g., preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions.

[0264] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration or delivery, as set forth herein or known to one of skill in the art. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes.

[0265] Compositions suitable for parenteral administration can comprise aqueous and non-aqueous solutions, suspensions or emulsions of the active compound, which preparations are

typically sterile and can be isotonic with the blood of the intended recipient. Non-limiting illustrative examples include water, buffered saline, Hanks' solution, Ringer's solution, dextrose, fructose, ethanol, animal, vegetable or synthetic oils. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oil injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility to allow for the preparation of highly concentrated solutions.

[0266] Cosolvents and adjuvants may be added to the formulation. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters. Adjuvants include, for example, surfactants such as, soy lecithin and oleic acid; sorbitan esters such as sorbitan trioleate; and polyvinylpyrrolidone.

[0267] After pharmaceutical compositions have been prepared, they may be placed in an appropriate container and labeled for treatment. Such labeling can include amount, frequency, and method of administration.

[0268] Pharmaceutical compositions and delivery systems appropriate for the compositions, methods and uses of the disclosure are known in the art (see, e.g., Remington: The Science and Practice of Pharmacy. 21st Edition. Philadelphia, PA. Lippincott Williams & Wilkins, 2005).

Dosing and Administration

[0269] Oligonucleotides described herein, or a vector comprising a nucleotide sequence encoding an oligonucleotide described herein, can be used to treat cancer or a metabolic disease or disorder, e.g., subjects suffering from or susceptible to cancer or a metabolic disease or disorder described herein. The mode of administration of pharmaceutical compositions described herein can vary depending upon the desired results. One with skill in the art, *i.e.*, a

physician, is aware that dosage regimens can be adjusted to provide the desired response, *e.g.*, a therapeutic response.

[0270] Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intracerebral, intrathecal (*e.g.*, intracisternal or via a lumbar puncture), intravaginal, transdermal, rectal, by inhalation, or topical, particularly to the ears, nose, eyes, or skin. In some embodiments, compositions of oligonucleotides are delivered to the central nervous system (CNS), *e.g.*, delivered via intracerebroventricular administration.

[0271] In some embodiments, a pharmaceutical composition described herein is delivered to the liver. In some embodiments, a pharmaceutical composition described herein is delivered to the muscle. In some embodiments, a pharmaceutical composition described herein is delivered to the CNS (*e.g.*, via intrathecal administration). In some embodiments, a pharmaceutical composition described herein is delivered to the cerebrospinal fluid.

[0272] Delivery of an oligonucleotide to a cell may be achieved in a number of different ways. *In vivo* delivery may be performed by administering a composition comprising an oligonucleotide to a subject, *e.g.*, by parenteral administration route, *e.g.*, subcutaneous or intravenous or intramuscular administration.

[0273] The disclosure also provides methods for administering an oligonucleotide, or a vector comprising a nucleotide sequence encoding an oligonucleotide described herein, into a cell or an animal. In some embodiments, such methods include contacting a subject (*e.g.*, a cell or tissue of a subject) with, or administering to a subject (*e.g.*, a subject such as a mammal), an oligonucleotide described herein (or a vector comprising a nucleotide sequence encoding an oligonucleotide described herein), such that the oligonucleotide is expressed in the subject (*e.g.*, in a cell or tissue of a subject).

[0274] Compositions of oligonucleotides described herein (or a vector comprising a nucleotide sequence encoding an oligonucleotide described herein) can be administered in a sufficient or effective amount to a subject in need thereof. Doses can vary and depend upon the type, onset, progression, severity, frequency, duration, or probability of the disease to which

treatment is directed, the clinical endpoint desired, previous or simultaneous treatments, the general health, age, gender, race or immunological competency of the subject and other factors that will be appreciated by the skilled artisan. The dose amount, number, frequency or duration may be proportionally increased or reduced, as indicated by any adverse side effects, complications or other risk factors of the treatment or therapy and the status of the subject. The skilled artisan will appreciate the factors that may influence the dosage and timing required to provide an amount sufficient for providing a therapeutic or prophylactic benefit.

[0275] In some embodiments, oligonucleotide compositions are administered to a subject in an amount that is between 0.01 mg/kg and 50 mg/kg. In some embodiments, the oligonucleotide composition is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 15 mg/kg. In some embodiments, the oligonucleotide composition is administered at a dose of about 10 mg/kg to about 30 mg/kg. In some embodiments, the oligonucleotide composition is administered at a dose of about 0.5 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2.0 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, or about 50 mg/kg. In some embodiments, the oligonucleotide composition is administered at a dose of between 0.01 mg/kg and 0.1 mg/kg, between 0.01 mg/kg and 0.1 mg/kg, between 0.1 mg/kg and 1.0 mg/kg, between 1.0 mg/kg and 2.5 mg/kg, between 2.5 mg/kg and 5.0 mg/kg, between 5.0 mg/kg and 10 mg/kg, between 10 mg/kg and 20 mg/kg, between 20 mg/kg and 30 mg/kg, between 30 mg/kg and 40 mg/kg or between 40 mg/kg and 50 mg/kg. In some embodiments, a fixed dose is administered. In some embodiments, the oligonucleotide composition is administered at a dose of between 5 mg and 1.0 g, e.g., between 5 mg and 10 mg, between 10 mg and 20 mg, between 20 mg and 40 mg, between 40 mg and 80 mg, between 80 mg and 160 mg, between 160 mg and 320 mg, between 320 mg and 640 mg, between 640 mg and 1g. In some embodiments, the dose is about 1 mg, 5 mg, 10 mg, 25 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, or 1000 mg.

[0276] In some embodiments, the dose is a daily dose. In some embodiments, the dose is administered according to a dosing regimen with a dosing interval of at least 2 days, *e.g.,* at least 7 days, *e.g.,* about 2, 3, 4, 6, or 8 weeks. For example, in some embodiments, an oligonucleotide composition is administered according to a dosing regimen with a dosing interval of at least 7

days. In some embodiments, an oligonucleotide composition is administered daily, weekly, monthly, or every 2, 3, 4, 5, or 6 months or longer. In some embodiments, any of the doses and/or dosing regimens described herein are administered subcutaneously. In some embodiments, an oligonucleotide composition is administered once and levels of inhibition are subsequently measured, and once the level of inhibition decreases to a certain level, a subsequent dose of the inhibitory composition is administered.

[0277] In some embodiments, a subject exhibits a sustained inhibition of POLRMT, *e.g.*, measured by POLRMT mRNA expression (*e.g.*, in a biological sample) for a period of time that is at least 2 days, *e.g.*, at least 7 days, *e.g.*, about 2, 3, 4, 6, 8, 10, 12, 16, or 20 weeks post-administration.

[0278] An effective amount or a sufficient amount can (but need not) be provided in a single administration, may require multiple administrations, and can (but need not) be, administered alone or in combination with another composition. For example, the amount may be proportionally increased as indicated by the need of the subject, type, status and severity of the disease treated or side effects (if any) of treatment. Amounts considered effective also include amounts that result in a reduction of the use of another treatment, therapeutic regimen or protocol, such as administration of another therapeutic described herein.

[0279] Accordingly, pharmaceutical compositions of the disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve the intended therapeutic purpose. Determining a therapeutically effective dose is well within the capability of a skilled medical practitioner using the techniques and guidance provided in the disclosure. Therapeutic doses can depend on, among other factors, the age and general condition of the subject, the severity of the cancer or metabolic disease or disorder, and the strength of the control sequences regulating the expression levels of the oligonucleotide. Thus, a therapeutically effective amount in humans will fall in a relatively broad range that may be determined by a medical practitioner based on the response of an individual patient to vector-based treatment. Pharmaceutical compositions may be delivered to a subject, so as to allow production of an oligonucleotide described herein in vivo by gene- and or cell-based therapies or by ex-vivo modification of the patient's or donor's cells.

[0280] Methods and uses of the disclosure include delivery and administration systemically, regionally or locally, or by any route, for example, by injection or infusion. Delivery of a pharmaceutical composition in vivo may generally be accomplished via injection using a conventional syringe, although other delivery methods such as convection-enhanced delivery can also be used (see, *e.g.,* U.S. Pat. No. 5,720,720). For example, compositions may be delivered subcutaneously, epidermally, intradermally, intrathecally, intraorbitally, intramucosally, intraperitoneally, intravenously, intra-pleurally, intraarterially, orally, intrahepatically, intracerebroventricularly (*e.g.,* via intracerebroventricular injection), via the portal vein, or intramuscularly. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications. A clinician specializing in the treatment of patients with cancer, or a metabolic disease or disorder may determine the optimal route for administration of an oligonucleotide composition or a vector comprising a nucleotide sequence encoding an oligonucleotide described herein.

[0281] In some embodiments, an oligonucleotide composition may be administered to a subject once daily, weekly, every 2, 3, or 4 weeks, or even at longer intervals. In some embodiments, an oligonucleotide composition described herein may be administered according to a dosing regimen that includes (i) an initial administration that is once daily, weekly, every 2, 3, or 4 weeks, or even at longer intervals; followed by (ii) a period of no administration of, *e.g.,* 1, 2, 3, 4, 5, 6, 8, or 10 months, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 years. In some embodiments, a subject is monitored before and/or following treatment for level of POLRMT mRNA expression and/or activity or POLRMT protein level. In some embodiments, a subject is monitored before and/or following treatment for level of mtDNA, mRNA expression of other mitochondrial genes, or level of other mitochondrial proteins (*e.g.,* indicating decrease in mitochondrial transcription). In some embodiments, a subject is treated, or is retreated, if a measured level of POLRMT mRNA expression and/or POLRMT activity or level of POLRMT protein is more than 10%, 20%, 30%, 40%, 50%, 100%, 200%, or more, relative to measured level in a control subject.

Diseases, Disorders, and Conditions

[0282] The present disclosure provides, among other things, oligonucleotides and compositions comprising the same. In some embodiments, such compositions are used for treating cancer and metabolic diseases through inhibition of POLRMT.

Cancer

[0283] In some embodiments, oligonucleotides described here may be used to treat cancer. Those skilled in the art are aware of a variety of types of cancer including, for example, adrenal gland cancer, anal cancer, adenocarcinoma, adrenocortical carcinoma, astrocytoma, angiosarcoma, basal cell carcinoma, bile duct cancer, bladder cancer, blastic plasmacytoid dendritic cell neoplasm, bone cancer, brain cancer, breast cancer, bronchogenic carcinoma, central nervous system (CNS) cancer, cervical cancer, carcinoid, cardiac, cholangiocarcinoma, chordoma, chronic myeloproliferative neoplasms, craniopharyngioma, cholangiocarcinoma, chondrosarcoma, colon cancer, choriocarcinoma, colorectal cancer, cancer of connective tissue, esophageal cancer, ductal carcinoma in situ, ependymoma,embryonal carcinoma, fibrosarcoma, gall bladder cancer, gastric cancer, glioblastomas, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), gestational trophoblastic disease, glioma, head and neck cancer, hematological cancer, histiocytosis, kidney cancer, intraocular melanoma, leukemias (*e.g.,* acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, myelogenous leukemia, myeloid leukemia, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), liposarcoma, liver cancer, lung cancer, lymphoma (*e.g.,* Burkitt lymphoma [non-Hodgkin lymphoma], cutaneous T cell lymphoma, Hodgkin lymphoma, mycosis fungoides, Sezary syndrome, AIDS-related lymphoma, follicular lymphoma, diffuse large B-cell lymphoma), melanoma, Merkel cell carcinoma, mesothelioma, myeloma (*e.g.,* multiple myeloma), muscular cancer, myxosarcoma, myelodysplastic syndrome, papillomatosis, paraganglioma, pheochromacytoma, pleuropulmonary blastoma, retinoblastoma, sarcoma (*e.g.,* Ewing sarcoma, Kaposi sarcoma, osteosarcoma, rhabdomyosarcoma, uterine sarcoma, vascular sarcoma), neuroblastomas, Wilms' tumor, and/or cancer of the adrenal cortex, anus, appendix, bile duct, bladder, bone, brain, breast, bronchus, central nervous system, cervix, colon, endometrium, esophagus, eye, fallopian tube,

gall bladder, gastrointestinal tract, germ cell, head and neck, heart, intestine, kidney (*e.g.*, Wilms' tumor), larynx, liver, lung (*e.g.*, non-small cell lung cancer, small cell lung cancer), mouth, nasal cavity, oral cavity, ovary, pancreas, rectum, skin, stomach, testes, throat, thyroid, penis, pharynx, peritoneum, pituitary, prostate, rectum, salivary gland, ureter, urethra, uterus, vagina, or vulva.

[0284] In some embodiments, compositions of oligonucleotides described herein may be used to treat a tumor in a subject. In some embodiments, a tumor is or comprises a hematologic malignancy, including but not limited to, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, AIDS-related lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma, Langerhans cell histiocytosis, multiple myeloma, or myeloproliferative neoplasms.

[0285] In some embodiments, a tumor is or comprises a solid tumor, including but not limited to breast carcinoma, a squamous cell carcinoma, a colon cancer, a head and neck cancer, ovarian cancer, a lung cancer, mesothelioma, a genitourinary cancer, a bladder cancer, a rectal cancer, a gastric cancer, or an esophageal cancer.

[0286] In some particular embodiments, a tumor is or comprises an advanced tumor, and/or a refractory tumor. In some embodiments, a tumor is characterized as advanced when certain pathologies are observed in a tumor (*e.g.*, in a tissue sample, such as a biopsy sample, obtained from a tumor) and/or when cancer patients with such tumors are typically considered not to be candidates for conventional chemotherapy. In some embodiments, pathologies characterizing tumors as advanced can include tumor size, altered expression of genetic markers, invasion of adjacent organs and/ or lymph nodes by tumor cells. In some embodiments, a tumor is characterized as refractory when patients having such a tumor are resistant to one or more known therapeutic modalities (*e.g.*, one or more conventional chemotherapy regimens) and/or when a particular patient has demonstrated resistance (*e.g.*, lack of responsiveness) to one or more such known therapeutic modalities.

[0287] In some embodiments, compositions comprising one or more oligonucleotides described herein can be administered in combination with a cancer therapy. The present disclosure is not limited to any specific cancer therapy, and any known or developed cancer

therapy is encompassed by the present disclosure. Known cancer therapies include, *e.g.*, administration of therapeutic cancer vaccines, chemotherapeutic agents, radiation therapy, surgical excision, chemotherapy following surgical excision of tumor, adjuvant therapy, localized hypothermia or hyperthermia, anti-tumor antibodies, and anti-angiogenic agents. In some embodiments, cancer and/or adjuvant therapy includes a TLR agonist (*e.g.*, CpG, Poly I:C, etc., see, *e.g.*, Wittig *et al.*, Crit. Rev. Oncol. Hematol. 94:31-44 (2015); Huen *et al.*, Curr. Opin. Oncol. 26:237-44 (2014); Kaczanowska *et al.*, J. Leukoc. Biol. 93:847-863 (2013)), a STING agonist (see, *e.g.*, US20160362441; US20140329889; Fu *et al.*, Sci. Transl. Med. 7:283ra52 (2015); and WO2014189805), a non-specific stimulus of innate immunity, and/or dendritic cells, or administration of GM-CSF, Interleukin-12, Interleukin-7, Flt-3, or other cytokines. In some embodiments, the cancer therapy is or comprises oncolytic virus therapy, *e.g.*, talimogene leherparepvec. (See, *e.g.*, Fukuhara *et al.*, Cancer Sci. 107:1373-1379 (2016)). In some embodiments, the cancer therapy is or comprises bi-specific antibody therapy (*e.g.*, Choi *et al.*, 2011 *Expert Opin Biol Ther*; Huehls *et al.*, 2015, *Immunol and Cell Biol*). In some embodiments, the cancer therapy is or comprises cellular therapy such as chimeric antigen receptor T (CAR-T) cells, TCR-transduced T cells, dendritic cells, tumor infiltrating lymphocytes (TIL), or natural killer (NK) cells (*e.g.*, as reviewed in Sharpe and Mount, 2015, *Dis Model Mech* 8:337-50).

[0288] In some embodiments, a cancer therapy may include a chemotherapeutic agent. A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, regardless of mechanism of action. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, spindle poison plant alkaloids, cytotoxic/anti-tumor antibiotics, topoisomerase inhibitors, antibodies, photosensitizers, and kinase inhibitors. Non-limiting examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), docetaxel (TAXOTER®, Sanofi-Aventis), 5-FU (fluorouracil, 5-fluorouracil, CAS No. 51-21-8), gemcitabine (GEMZAR®, Lilly), PD-0325901 (CAS No. 391210-10-9, Pfizer), cisplatin (cis-diamine,dichloroplatinum(II), CAS No. 15663-27-1), carboplatin (CAS No. 41575-94-4), paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.), temozolomide (4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo [4.3.0] nona-2,7,9-triene-9-carboxamide, CAS No. 85622-93-1, TEMODAR®, TEMODAL®, Schering Plough), tamoxifen ((Z)-2-[4-(1,2-

diphenylbut-1-enyl)phenoxy]-N,N-dimethyl-ethanamine, NOLVADEX®, ISTUBAL®, VALODEX®), and doxorubicin (ADRIAMYCIN®), Akti-1/2, HPPD, and rapamycin.

[0289] Additional examples of chemotherapeutic agents include: oxaliplatin (ELOXATIN®, Sanofi), bortezomib (VELCADE®, Millennium Pharm.), sutent (SUNITINIB®, SU11248, Pfizer), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), XL-518 (MEK inhibitor, Exelixis, WO 2007/044515), ARRY-886 (Mek inhibitor, AZD6244, Array BioPharma, Astra Zeneca), SF-1126 (PI3K inhibitor, Semafore Pharmaceuticals), BEZ-235 (PI3K inhibitor, Novartis), XL-147 (PI3K inhibitor, Exelixis), PTK787/ZK 222584 (Novartis), fulvestrant (FASLODEX®, AstraZeneca), leucovorin (folinic acid), rapamycin (sirolimus, RAPAMUNE®, Wyeth), lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), lonafarnib (SARASAR™, SCH 66336, Schering Plough), sorafenib (NEXAVAR®, BAY43-9006, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), irinotecan (CAMPTOSAR®, CPT-11, Pfizer), tipifarnib (ZARNESTRA™, Johnson & Johnson), ABRAXANE™ (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), vandetanib (rINN, ZD6474, ZACTIMA®, AstraZeneca), chloranmbucil, AG1478, AG1571 (SU 5271; Sugen), temsirolimus (TORISEL®, Wyeth), pazopanib (GlaxoSmithKline), canfosfamide (TELCYTA®, Telik), thiotepa and cyclosphosphamide (CYTOXAN®, NEOSAR®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, calicheamicin gamma1I, calicheamicin omegaI1 (Angew Chem. Intl. Ed. Engl. (1994) 33:183-186); dynemicin, dynemicin A; bisphosphonates,

such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (Ara-C); cyclophosphamide; thiotepa; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (NAVELBINE®); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®, Roche); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

Metabolic Disorders

[0290] In some embodiments, oligonucleotides described herein may be used to treat a metabolic disease associated with mitochondrial dysfunction. Types of metabolic diseases include obesity, diabetes, non-alcoholic steatohepatitis (NASH), and related metabolic syndromes such as non-alcoholic fatty liver disease (NAFLD), Familial hypercholesterolemia, Hunter syndrome, Metachromatic leukodystrophy, Mitochondrial encephalopathy, lactic acidosis, and Porphyria.

[0291] In some embodiments, a metabolic disorder includes syndromic obesity such as Prader-Willi (PWS) and Bardet-Biedl (BBS) syndromes. In some embodiments, a metabolic disorder includes oligogenic obesity, such as melanocortin 4 receptor (MC4R)-linked obesity (see Rodríguez-López, Raquel, et al., Current Genomics 23.3 (2022): 147, which is herein incorporated by reference).

[0292] In some embodiments, a metabolic disorder includes disorders of amino acid metabolism (amino acidemias) such as Maple Syrup Urine Disease (MSUD), Tyrosinemia, and Homocystinuria.

[0293] In some embodiments, a metabolic disorder includes disorders of organic acid metabolism (organic acidurias, organic acidemias) such as Methylmalonic Aciduria, 3-Methylglutaconic Aciduria -- Barth Syndrome, Glutaric Aciduria, 2-Hydroxyglutaric aciduria – D and L forms, and propionic acidemia.

[0294] In some embodiments, a metabolic disorder includes disorders of Fatty Acid Beta-Oxidation such as MCAD Deficiency, LCHAD, and VLCAD deficiency.

[0295] In some embodiments, a metabolic disorder includes disorders of lipid metabolism (lipid storage disorders) such as Gangliosidoses (*e.g.*, GM1 Gangliosidosis, Tay-Sachs Disease, Sandhoff Disease), Sphingolipidoses (*e.g.*, Fabry Disease, Gaucher Disease, Niemann-Pick Disease, and Krabbe Disease), Mucolipidoses, and Mucopolysaccharidoses.

[0296] In some embodiments, a metabolic disease includes mitochondrial disorders, leading in some cases to muscle damage or muscle wasting. Examples of mitochondrial disorders include mitochondrial cardiomyopathies, Leigh disease, stroke-like episodes (MELAS), MERRF, NARP, and Barth syndrome.

[0297] In some embodiments, a metabolic disorder includes a lysosomal storage disorder, where enzymes in lysosomes that break down waste products of metabolism may be deficient or dysfunctional and cause buildup of toxic substances resulting in various diseases. Examples of lysosomal storage disorders include, *e.g.*, Hurler syndrome (abnormal bone structure and developmental delay).

[0298] In some embodiments, a metabolic disease includes peroxisomal disorders. Similar to lysosomes, peroxisomes are tiny, enzyme-filled spaces within cells. Functional deficiencies of enzymes within peroxisomes can lead to buildup of toxic products of metabolism. Exemplary peroxisomal disorders include Zellweger syndrome (which manifests as abnormal facial features, enlarged liver, and nerve damage in infants), Adrenoleukodystrophy (which is characterized by symptoms of nerve damage that can develop in childhood or early adulthood depending on the form), and Refsum Disease.

[0299] In some embodiments, a metabolic disease includes galactosemia, resulting from impaired breakdown of the sugar galactose, which leads to jaundice, vomiting, and liver enlargement after breast or formula feeding by a newborn.

[0300] In some embodiments, a metabolic disease includes phenylketonuria (PKU) resulting from a deficiency of the enzyme PAH which results in high levels of phenylalanine in the blood. Intellectual disability may result if this condition is not recognized and treated.

[0301] In some embodiments, a metabolic disease includes glycogen storage diseases resulting from problems with sugar storage, which leads to low blood sugar levels, muscle pain, and weakness.

[0302] In some embodiments, a metabolic disease includes Friedreich ataxia, resulting from problems related to a protein called frataxin causing nerve damage and often heart problems. Usually, such a disease results in the inability to walk by young adulthood.

[0303] In some embodiments, a metabolic disease includes metal metabolism disorders. In the blood, levels of trace metals are controlled by special proteins. Inherited metabolic disorders can result in protein malfunction and toxic accumulation of metal in the body. Example metal metabolism disorders include Wilson's disease, where toxic copper levels

accumulate in the liver, brain, and other organs, and Hemochromatosis, where the intestines absorb excessive iron, which builds up in the liver, pancreas, joints, and heart, causing damage.

[0304] In some embodiments, a metabolic disease includes urea cycle disorders such as ornithine transcarbamylase deficiency and citrullinemia.

[0305] All publications, patent applications, patents, and other references mentioned herein, including GenBank Accession Numbers, are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[0306] The disclosure is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the disclosure in any way.

NUMBERED EMBODIMENTS

[0307] Embodiment 1. An oligonucleotide comprising a sequence that is complementary to a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from a target region that spans between 8 to 30 contiguous nucleotides of a POLRMT nucleotide sequence.

[0308] Embodiment 2. The oligonucleotide of embodiment 1, wherein the oligonucleotide comprises a sequence that is complementary to a region that spans between 8 to 30 contiguous nucleotides of a POLRMT nucleotide sequence.

[0309] Embodiment 3. The oligonucleotide of embodiment 1 or embodiment 2, wherein the target region spans between 15 to 25 contiguous nucleotides of a POLRMT nucleotide sequence.

[0310] Embodiment 4. The oligonucleotide sequence of any one of embodiments 1-3, wherein the target region spans 20 contiguous nucleotides of a POLRMT nucleotide sequence.

[0311] Embodiment 5. The oligonucleotide sequence of any one of embodiments 1-4, wherein the target region comprises an exon region of POLRMT nucleotide sequence.

[0312] Embodiment 6. The oligonucleotide of any one of embodiments 1-5, wherein the target region comprises a sequence that corresponds to nucleotides 5696-5715, 8808-8827, 8809-8828, 8811-8830, 16221-16240, 17159-17178, 17314-17333, 17315-17334, 18082-18101, 18083-18102, 18084-18103, 18130-18149, 5680-5699, 8491-8510, 8529-8548, 8569-8588, 8570-8589, 8571-8590, 8572-8591, 8573-8592, 8574-8593, 13322-13341, 13719-13738, 14999-15018, 15092-15111, 15093-15112, 17304-17323, 19309-19328, 20041-20060, 20042-20061, or 21102-21121 of SEQ ID NO: 1.

[0313] Embodiment 7. An oligonucleotide comprising a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0314] Embodiment 8. The oligonucleotide of embodiment 7, wherein the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0315] Embodiment 9. The oligonucleotide of embodiment 7 or embodiment 8, wherein the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 3-14, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0316] Embodiment 10. The oligonucleotide of any one of embodiments 7-9, wherein the oligonucleotide comprises SEQ ID NO: 11.

[0317] Embodiment 11. The oligonucleotide of any one of embodiments 7-9, wherein the oligonucleotide comprises SEQ ID NO: 12.

[0318] Embodiment 12. The oligonucleotide of any one of embodiments 7-9, wherein the oligonucleotide comprises SEQ ID NO: 594.

[0319] Embodiment 13. The oligonucleotide of any one of embodiments 7-9, wherein the oligonucleotide comprises SEQ ID NO: 612.

[0320] Embodiment 14. The oligonucleotide of any one of embodiments 7-9, wherein the oligonucleotide comprises SEQ ID NO: 632.

[0321] Embodiment 15. An oligonucleotide comprising a sequence that is complementary to a sequence that is at least 80% identical to a sequence selected from a group consisting of SEQ ID NOs: 15-26, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0322] Embodiment 16. The oligonucleotide of embodiment 15, wherein the oligonucleotide comprises a sequence that is complementary to a sequence that is at least 90% identical to any one of SEQ ID NOs: 15-26, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0323] Embodiment 17. The oligonucleotide of embodiment 15 or embodiment 16, wherein the oligonucleotide comprises a sequence that is complementary to a sequence selected from a group consisting of SEQ ID NOs: 15-26, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0324] Embodiment 18. The oligonucleotide of any one of embodiments 15-17, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 23.

[0325] Embodiment 19. The oligonucleotide of any one of embodiments 15-17, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 24.

[0326] Embodiment 20. The oligonucleotide of any one of embodiments 15-17, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 663.

[0327] Embodiment 21. The oligonucleotide of any one of embodiments 15-17, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 681.

[0328] Embodiment 22. The oligonucleotide of any one of embodiments 15-17, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 701.

[0329] Embodiment 23. An oligonucleotide comprising a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NOs: 3-14, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634, and/or is complementary to a nucleotide sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NO: 15-26, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0330] Embodiment 24. An oligonucleotide comprising a sequence that is complementary to a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from a target region that spans between 8 to 30 contiguous nucleotides of a mouse POLRMT nucleotide sequence.

[0331] Embodiment 25. The oligonucleotide of embodiment 24, wherein the oligonucleotide comprises a sequence that is complementary to a target region that spans between 8 to 30 contiguous nucleotides of a mouse POLRMT nucleotide sequence.

[0332] Embodiment 26. The oligonucleotide of embodiment 24 or embodiment 25, wherein the target region spans between 15 to 25 contiguous nucleotides of a mouse POLRMT nucleotide sequence.

[0333] Embodiment 27. The oligonucleotide sequence of any one of embodiments 24-26, wherein the target region spans 20 contiguous nucleotides of a mouse POLRMT nucleotide sequence.

[0334] Embodiment 28. The oligonucleotide sequence of any one of embodiments 24-27, wherein the target region comprises an exon region of POLRMT nucleotide sequence.

[0335] Embodiment 29. The oligonucleotide of any one of embodiments 24-28, wherein the target region comprises a sequence that corresponds to nucleotides 3348-3367 or 3198-3217 of SEQ ID NO: 581.

[0336] Embodiment 30. An oligonucleotide comprising a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0337] Embodiment 31. The oligonucleotide of embodiment 30, wherein the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0338] Embodiment 32. The oligonucleotide of embodiment 30 or embodiment 31, wherein the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

[0339] Embodiment 33. The oligonucleotide of any one of embodiments 30-32, wherein the oligonucleotide comprises SEQ ID NO: 434.

[0340] Embodiment 34. The oligonucleotide of any one of embodiments 30-32, wherein the oligonucleotide comprises SEQ ID NO: 442.

[0341] Embodiment 35. The oligonucleotide of any one of embodiments 30-32, wherein the oligonucleotide comprises SEQ ID NO: 594.

[0342] Embodiment 36. The oligonucleotide of any one of embodiments 30-32, wherein the oligonucleotide comprises SEQ ID NO: 612.

[0343] Embodiment 37. The oligonucleotide of any one of embodiments 30-32, wherein the oligonucleotide comprises SEQ ID NO: 632.

[0344] Embodiment 38. An oligonucleotide comprising a sequence that is complementary to a sequence that is at least 80% identical to a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0345] Embodiment 39. The oligonucleotide of embodiment 38, wherein the oligonucleotide comprises a sequence that is complementary to a sequence that is at least 90% identical to any one of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0346] Embodiment 40. The oligonucleotide of embodiment 38 or embodiment 39, wherein the oligonucleotide comprises a sequence that is complementary to a sequence selected from a group consisting of SEQ ID NOs: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0347] Embodiment 41. The oligonucleotide of any one of embodiments 38-40, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 528.

[0348] Embodiment 42. The oligonucleotide of any one of embodiments 38-40, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 536.

[0349] Embodiment 43. The oligonucleotide of any one of embodiments 38-40, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 663.

[0350] Embodiment 44. The oligonucleotide of any one of embodiments 38-40, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 681.

[0351] Embodiment 45. The oligonucleotide of any one of embodiments 38-40, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 701.

[0352] Embodiment 46.An oligonucleotide comprising a sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NOs: 393-486, 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634 and/or is complementary to a nucleotide sequence that differs by no more than 1, 2, 3, or 4 nucleotides from any one of SEQ ID NO: 487-580, 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

[0353] Embodiment 47. The oligonucleotide of any one of embodiments 1-46, wherein the oligonucleotide is a chirally pure oligonucleotide.

[0354] Embodiment 48. The oligonucleotide of any one of embodiments 1-47, wherein the oligonucleotide comprises at least one modified nucleotide.

[0355] Embodiment 49. The oligonucleotide of embodiment 48, wherein the modified nucleotide comprises a base modification, a sugar or sugar phosphate modification, an internucleotidic linkage modification, or a combination thereof.

[0356] Embodiment 50. The oligonucleotide of embodiment 49, wherein the internucleotidic linkage modification comprises a phosphorothioate or phosphodithioate linkage modification.

[0357] Embodiment 51. The oligonucleotide of embodiment 49, wherein the sugar or sugar phosphate modification comprises a 2'-O-methoxyethyl (2'-MOE) modification, a 2'-Fluoro (2'-F) modification, a 2'-O-methyl (2'-O-Me) modification, a phosphorodiamidate morpholino (PMO) modification, a peptide nucleic acid (PNA) modification, an unlocked nucleic acid (UNA), or a locked nucleic acid (LNA).

[0358] Embodiment 52. The oligonucleotide of embodiment 49, wherein the base modification comprises a 5'-methylcytosine modification or a G-clamp modification.

[0359] Embodiment 53. The oligonucleotide of any one of embodiments 48-52, wherein each nucleotide comprises a phosphorothioate (PS) internucleotide linkage.

[0360] Embodiment 54. The oligonucleotide of any one of embodiments 48-53, wherein the oligonucleotide comprises five nucleotides at the 5'-end and five nucleotides at the 3'-end of the oligonucleotide sequence which contain a 2'-MOE modification.

[0361] Embodiment 55. The oligonucleotide of any one of embodiments 48-53, wherein each nucleotide contains a 2'-MOE modification.

[0362] Embodiment 56. The oligonucleotide of any one of embodiments 1-55, further comprising at least at least one ligand attached to the 5' end and/or the 3' end.

[0363] Embodiment 57. The oligonucleotide of embodiment 56, wherein the ligand comprises at least one lipid, peptide, and/or sugar.

[0364] Embodiment 58. The oligonucleotide of embodiment 57, wherein the sugar comprises N-acetylgalactosamine (GalNAc)moiety.

[0365] Embodiment 59. A composition comprising the oligonucleotide of any one of embodiments 1-58 and a carrier and/or excipient.

[0366] Embodiment 60. An expression vector comprising one or more sequences encoding one of more oligonucleotides of any one of embodiments 1-58.

[0367] Embodiment 61. A method of treating a subject having or at risk of cancer or metabolic disease, the method comprising administering to the subject a composition comprising an effective amount of the oligonucleotide of any one of embodiments 1-58.

[0368] Embodiment 62. The method of embodiment 61, wherein, a level of mitochondrial RNA polymerase (POLRMT) mRNA expression or POLRMT protein in the subject or in a biological sample from the subject after the administration of the composition is reduced relative to a level before the administration of the composition.

[0369] Embodiment 63. The method of embodiment 62, wherein the level of POLRMT mRNA expression or POLRMT protein is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration.

[0370] Embodiment 64. The method of any one of embodiments 61-63, wherein the composition is administered intravenously, intrathecally, intramuscularly, orally, intranasaly, or subcutaneously to the subject.

[0371] Embodiment 65. The method of any one of embodiments 61-64, wherein the subject is a human.

[0372] Embodiment 66. A method of treating and/or preventing a cancer or a metabolic disease in a subject, comprising administering to the subject an oligonucleotide that is complementary to a target region of a nucleic acid sequence encoding POLRMT.

[0373] Embodiment 67. A method of decreasing mitochondrial transcription in a subject that is susceptible to or suffering from cancer or metabolic disease, the method comprising: administering to the subject an oligonucleotide that is complementary to a target region of a nucleic acid sequence encoding POLRMT.

[0374] Embodiment 68. The method of embodiment 66 or embodiment 67, wherein the nucleic acid sequence encoding POLRMT comprises SEQ ID NO: 205.

[0375] Embodiment 69. The method of any one of embodiments 66-68, wherein the target region comprises a region that spans between 8 to 30 contiguous nucleotides within SEQ ID NO: 205.

[0376] Embodiment 70. The method of any one of embodiments 66-69, wherein the target region comprises a sequence that corresponds to nucleotides 5696-5715, 8808-8827, 8809-

8828, 8811-8830, 16221-16240, 17159-17178, 17314-17333, 17315-17334, 18082-18101, 18083-18102, 18084-18103, 18130-18149, 5680-5699, 8491-8510, 8529-8548, 8569-8588, 8570-8589, 8571-8590, 8572-8591, 8573-8592, 8574-8593, 13322-13341, 13719-13738, 14999-15018, 15092-15111, 15093-15112, 17304-17323, 19309-19328, 20041-20060, 20042-20061, or 21102-21121 of SEQ ID NO: 1.

[0377] Embodiment 71. The method of any one of embodiments 66-70, wherein the oligonucleotide comprises a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14.

[0378] Embodiment 72. The method of any one of embodiments 66-71, wherein the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 3-14.

[0379] Embodiment 73. The method of any one of embodiments 66-72, wherein the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 3-14.

[0380] Embodiment 74. The method of any one of embodiments 66-73, wherein upon administration of the oligonucleotide to the subject, the level of POLRMT mRNA expression in the subject is decreased.

[0381] Embodiment 75. The method of any one of embodiments 66-74, wherein upon administration of the oligonucleotide to the subject, the level of POLRMT protein or activity in the subject is decreased.

[0382] Embodiment 76. The method of embodiment 74 or embodiment 75, wherein the level of POLRMT mRNA expression, POLRMT protein, or POLRMT activity is reduced by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration.

[0383] Embodiment 77. The method of any one of embodiments 66-76, wherein the subject is a human.

[0384] Embodiment 78. The method of any one of embodiments 66-77, wherein the metabolic disease comprises include obesity, diabetes, non-alcoholic steatohepatitis (NASH), a disorder of amino acid metabolism (amino acidemias), a disorder of organic acid metabolism (organic acidurias, organic acidemias), a disorder of lipid metabolism (lipid storage disorders), a

lysosomal storage disorder, a peroxisomal disorder, phenylketonuria (PKU), a glycogen storage disease, or a urea cycle disorder.

[0385] Embodiment 79. The method of any one of embodiments 66-78, wherein the composition is delivered to the liver.

[0386] Embodiment 80. The method of any one of embodiments 66-78, wherein the composition is delivered to the muscle.

[0387] Embodiment 81. The method of any one of embodiments 66-78, wherein the composition is delivered to the CNS.

[0388] Embodiment 82. The method of any one of embodiments 66-78, wherein the composition is delivered to the cerebrospinal fluid.

[0389] Embodiment 83. A pharmaceutical composition comprising an oligonucleotide of any one of embodiments 1-58.

[0390] Embodiment 84. The pharmaceutical composition of embodiment 83, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.

[0391] Embodiment 85. The pharmaceutical composition of embodiment 83 or embodiment 84, wherein the oligonucleotide is formulated in a nanocarrier.

[0392] Embodiment 86. The pharmaceutical composition of any one of embodiments 83-85, wherein the oligonucleotide is formulated in a lipid nanoparticle (LNP).

[0393] Embodiment 87. The pharmaceutical composition of any one of embodiments 83-86, wherein the oligonucleotide is conjugated to at least one GalNAc moiety.

[0394] Embodiment 88. The pharmaceutical composition of any one of embodiments 83-87, wherein the composition is formulated for systemic or localized administration.

[0395] Embodiment 89. The pharmaceutical composition of embodiment 83-88, wherein the composition is formulated for delivery route selected from intrathecal, intramuscular, or intravenous administration.

[0396] Embodiment 90. A method of reducing or inhibiting POLRMT expression in a cell, the method comprising contacting the cell with the oligonucleotide of any one of embodiments 1-58.

[0397] Embodiment 91. The method of embodiment 90, wherein the level of POLRMT mRNA expression, POLRMT protein, or POLRMT activity is reduced by at least 10%, at least

20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% relative to the level before the cell is contacted with the oligonucleotide.

[0398] Embodiment 92. The method of embodiment 90 or embodiment 91, wherein the cell is in a subject.

[0399] Embodiment 93. The method of embodiment 92, wherein the subject is a human.

[0400] Embodiment 94. The method of embodiment 93, wherein the human is suffering from or susceptible to cancer or a metabolic disorder.

EXAMPLES

Example 1: Knockdown of POLRMT and CytB Expression in HeLa cells

[0401] This example illustrates the design and production of exemplary antisense oligonucleotides (ASOs) that target the POLRMT RNA transcript and are capable of altering mRNA expression of POLRMT in HeLa cells.

[0402] POLRMT is a mitochondrial DNA-directed RNA polymerase that is essential in transcription of circular mammalian mitochondrial DNA (mtDNA).

[0403] To determine whether the ASOs described in this example were able to impair transcription of mtDNA (through knockdown POLRMT expression), expression of Cytochrome B (CytB), a mitochondrial protein, was also measured.

ASOs

[0404] ASOs were designed and synthesized to target different regions of the POLRMT RNA transcript and are characterized by the corresponding region of the POLRMT gene sequence (represented in Reference No. NG_023049.1 and in SEQ ID NO: 1). ASOs were designed using two different strategies.

[0405] The first strategy designed ASOs using software program LNCASO (https://iomics.ugent.be/pjdev/design) and analyzing the longest POLRMT transcript (represented in the sequence Reference No. ENST00000588649.7 and SEQ ID NO: 205). Oligonucleotide length was set to 19 nucleotides. The second strategy designed additional ASOs using the software program PFRED (https://github.com/pfred/pfred-gui/releases/tag/v1.0). For the PFRED program, oligonucleotide length was set to 20 nucleotides and 1 mismatch. The gene base on ENSG ID was searched and the longest POLRMT transcript was chosen as a primary target (represented in the sequence Reference No. ENST00000588649.7 and SEQ ID NO: 205). Oligos with more than 1 mismatch in both cDNA and unspliced mRNA were filtered out. The SVMpred was set as >0.5, PLSpred_optimized was set at >0.8.

[0406] The exemplary ASO sequences, the POLRMT sequence targeted (target region), a description of the target region, and the coordinates of the target region within the POLRMT gene sequence (SEQ ID NO: 1) are shown in Table 5 below.

Table 5

ASO SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Corresponding coordinates within SEQ ID NO: 1
3	CCCCAGTCCTTCCTGCGGTC	15	GACCGCAGGAAGGACTGGGG	Exon	5696-5715
4	TCCACATCGAGGCTCAGCTT	16	AAGCTGAGCCTCGATGTGGA	Exon	8808-8827
5	CTCCACATCGAGGCTCAGCT	17	AGCTGAGCCTCGATGTGGAG	Exon	8809-8828
6	TGCTCCACATCGAGGCTCAG	18	CTGAGCCTCGATGTGGAGCA	Exon	8811-8830
7	CCCCAGCTCCTCCCAGTACT	19	AGTACTGGGAGGAGCTGGGG	Exon	16221-16240
8	GGCCACCTTCTGGCAGTGCG	20	CGCACTGCCAGAAGGTGGCC	Exon	17159-17178
9	GCCACGTCGCTGCCCAGGTG	21	CACCTGGGCAGCGACGTGGC	Exon	17314-17333
10	CGCCACGTCGCTGCCCAGGT	22	ACCTGGGCAGCGACGTGGCG	Exon	17315-17334
11	CCCATCCACCACTTTCGGCC	23	GGCCGAAAGTGGTGGATGGG	Exon	18082-18101
12	GCCCATCCACCACTTTCGGC	24	GCCGAAAGTGGTGGATGGGC	Exon	18083-18102
13	CGCCCATCCACCACTTTCGG	25	CCGAAAGTGGTGGATGGGCG	Exon	18084-18103
14	TTCGCCACCTCCATACAGCA	26	TGCTGTATGGAGGTGGCGAA	Exon	18130-18149
27	GGTCTTGGTCTTGCTCCTGG	46	CCAGGAGCAAGACCAAGACC	Exon	5680-5699
28	GAGGCTACCATCTCCACTGC	47	GCAGTGGAGATGGTAGCCTC	Exon	8491-8510
29	GCATCCTTGGCCCCCATCTG	48	CAGATGGGGGCCAAGGATGC	Exon	8529-8548
30	CTCCAGTATCTTTGCCCAGC	49	GCTGGGCAAAGATACTGGAG	Exon	8569-8588
31	TCTCCAGTATCTTTGCCCAG	50	CTGGGCAAAGATACTGGAGA	Exon	8570-8589
32	TTCTCCAGTATCTTTGCCCA	51	TGGGCAAAGATACTGGAGAA	Exon	8571-8590
33	CTTCTCCAGTATCTTTGCCC	52	GGGCAAAGATACTGGAGAAG	Exon	8572-8591
34	CCTTCTCCAGTATCTTTGCC	53	GGCAAAGATACTGGAGAAGG	Exon	8573-8592
35	TCCTTCTCCAGTATCTTTGC	54	GCAAAGATACTGGAGAAGGA	Exon	8574-8593
36	CACATATACCAGCTCCTTGA	55	TCAAGGAGCTGGTATATGTG	Exon	13322-13341
37	TCCTCCTCAGACAGCAGAAC	56	GTTCTGCTGTCTGAGGAGGA	Exon	13719-13738
38	CACTGCAGGGTCTTCAAGGG	57	CCCTTGAAGACCCTGCAGTG	Exon	14999-15018
39	TGCTTGACCTCCTTGCTTGG	58	CCAAGCAAGGAGGTCAAGCA	Exon	15092-15111
40	GTGCTTGACCTCCTTGCTTG	59	CAAGCAAGGAGGTCAAGCAC	Exon	15093-15112
41	TGCCCAGGTGGTTGAAGTGC	60	GCACTTCAACCACCTGGGCA	Exon	17304-17323
42	TGTAGACTCTTGAAGACCTG	61	CAGGTCTTCAAGAGTCTACA	Exon	19309-19328
43	AGCATCATGTGGGAGGAGTC	62	GACTCCTCCCACATGATGCT	Exon	20041-20060
44	GAGCATCATGTGGGAGGAGT	63	ACTCCTCCCACATGATGCTC	Exon	20042-20061
45	TTCAGCTGGCTGGCCTCCAA	64	TTGGAGGCCAGCCAGCTGAA	Exon	21102-21121

ASO Synthesis

[0407] Exemplary ASOs were synthesized using phosphoramidite synthesis methods that begin with the 3'-most nucleotide and proceed through multiple cycles of the following steps:

deprotection (trityl group is removed from the 5' carbon by trichloroacetic acid (TCA) resulting in a reactive hydroxyl group for the next base to be added), coupling (using tetrazole activation to produce an intermediate that reacts with the hydroxyl group), capping (acetylating reagent is added to react with free hydroxyl groups of oligonucleotides where coupling failed), and stabilization (iodine and water are added to cause oxidation of the phosphite into phosphate leaving a stabilized phosphotriester bond), until the 5'-most nucleotide is attached. Where phosphorothioate bonds are produced in exemplary ASOs, a sulfurizing agent is used in place of iodine/water in the stabilize step, for example, dibenzyl tetrasulfide, Beaucage Reagent (3*H*-1,2-benzodithiol-3-one 1,1-dioxide), 3-ethoxy-1,2,4-dithiazolidin-5-one (EDITH), 1,2,4-dithiazolidine-3,5-dione (DtsNH), 3-amino-1,2,4-dithiazole-5-thione.

[0408] The remaining trityl groups were removed from completed synthesis and from the CPG resulting in a hydroxyl group on both the 3' and 5' ends. The oligo was deprotected using ammonium hydroxide to promote base hydrolysis. The remaining contaminants were removed through desalting. The oligonucleotides were purified via PAGE or HPLC and quality is confirmed using Mass Spectrometry (using either Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) or Electrospray Ionization (ESI)) (see https://eu.idtdna.com/pages/products/functional-genomics/antisense-oligos).

[0409] In this experiment, ASOs contained modifications that included the following modification pattern:

[0410] $X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

[0411] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond. Exemplary ASOs with such a modification pattern are shown in **FIG. 3**.

ASO Transfection

[0412] Human HeLa cells (ATCC CCL-2) were grown at 37 ºC with 5% (v/v) CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

[0413] 6 or 12 µl of each ASO (100µM) was added to 8 µL of DharmaFECT 1 (horizondiscovery, T-2001-02), 1 ml Opti-MEM media (Thermo Fisher Scientific, Cat#11058021), and mixed well. ASOs were tested at two final concentrations 100nM and 200 nM. The mixture was incubated for 15 min at room temperature. 5 mL of 5×10^4/mL Hela cells were added to the mixture and mixed well. The cells and ASO mixture were seeded (2 mL per well) in a 6-well plate and grown for three days.

RT-PCR Protocol

[0414] The following RT-PCR protocol was performed:

1. Transfer cells to eppendorf tubes, pellet cells by centrifuging 500g 5 min, and remove remaining media. Wash the cell pellet with 1 x Phosphate Buffered Saline (PBS). Add 1 mL of trizol solution (Thermo Fisher Scientific, Cat# 15596026) to the HeLa cell pellet. Resuspend the cells completely by vortexing.
2. Incubate tubes at room temperature for 5 min.
3. Add 200 µl chloroform. Vortex for 10 seconds.
4. Incubate for 5 min at room temperature to permit nucleoprotein dissociation.
5. Centrifuge the tubes at 12,000 x g for 15 min at 4 °C.
6. Transfer the upper phase to a new microcentrifuge tube without disturbing the interphase.
7. Add 500 µL of isopropanol to the sample, invert 5 times to mix and incubate 30 min at -20 °C.
8. Centrifuge at a minimum of 12,000 x g for 15 min at 4 °C and remove the supernatant. The RNA will appear as a white pellet in the tube.
9. Wash the pellet with 500 µL of ice-cold 75% ethanol (made with deionized, diethylpyrocarbonate (*DEPC*) treated and 0.22 µm membrane-filtered H_2O).
10. Remove the ethanol, air dry for 5 minutes at room temperature (do not completely dry the RNA) and resuspend the RNA in 100 µL of DEPC-H_2O.
11. Incubate 5 µg RNA with 1 unit of turbo DNase (TURBO DNA-free Kit, Thermo Fisher Scientific, Cat# AM1907) at room temperature for 15 minutes and stop reaction following the manufacturer's instructions.
12. Purify RNA using a Quick-RNA miniprep kit (ZYMO, Cat# R1055).

13. Perform reverse transcription using 1 µg RNA and the iScript cDNA Synthesis Kit (Biorad, 170-8891).
14. Dilute 20 µl of cDNA to a final volume of 200 µl using H_2O. The cDNA is now ready for PCR quantification.

[0415] The iTaq Universal SYBR Green Supermix (Bio-Rad, Cat# #1725121) was used together with primers ordered from Eurofins genomics (shown below in **Table 6**) to detect expression of POLRMT, CytB and 18S rRNA genes. Quantification was performed using the Biorad CFX96 PCR system. The reaction mixtures in each well contained 1 µL forward primer (conc. 5 µM) and 1 µL reverse primer (conc. 5 µM), 2 µL cDNA, 9.5 µL H_2O and 12.5 µL SYBR supermix.

Table 6:

Primer	**Supplier**	**Sequence**	**SEQ ID NO:**	**Storage**
Human 18S_qPCR_F	Eurofins	AGAAACGGCTACCACATCCA	65	-20 °C
Human 18S_qPCR_R	Eurofins	CCCTCCAATGGATCCTCGTT	66	-20 °C
POLRMT_RT_F2	Eurofins	ACT GTT CTG AAG GCC GTG	67	-20 °C
POLRMT_RT_R2	Eurofins	GGT CTT CAA GGG CAG GTG	68	-20 °C
Human CytB_qPCR_F	Eurofins	ACAATTCTCCGATCCGTCCC	69	-20 °C
Human CytB_qPCR_R	Eurofins	GTGATTGGCTTAGTGGGCGA	70	-20 °C

Results – POLRMT Expression

[0416] POLRMT expression in HeLa cells transfected with the exemplary ASOs are shown in FIG. 4. A scrambled ASO (not a perfect match to any human transcripts) was used as a control. A POLRMT siRNA (Horizon Discovery Catalog ID:L-012004-01-0005, smartpool format) was used as a positive control. All results were normalized by 18S expression.

[0417] Of the ASOs tested at 100nM concentration (*i.e.,* ASOs represented in SEQ ID NOs: 3-14), all ASO sequences except SEQ ID NO: 5 showed a reduction of relative POLRMT expression at 100nM (data not shown). SEQ ID NOs: 9 and 10 showed cytotoxic activity and therefore no data was obtained.

[0418] The results in FIG. 4 show that ASOs represented in SEQ ID NOs: 3-8, 13-14, 28-30, 32-37, and 39-43 showed some inhibition of POLRMT expression (decreased expression relative to control). ASOs represented in SEQ ID NOs: 11 and 12 showed excellent inhibition of POLRMT expression at 200 nM. ASOs represented in SEQ ID NOs: 9, 10, and 27 showed cytotoxic activity and therefore no data was obtained.

[0419] These results show that the inhibition activity of the ASO depends on the region of the POLRMT transcript targeted.

Results – CytB Expression

[0420] In addition to knockdown of POLRMT expression, expression of CytB was also measured in the transfected HeLa cells.

[0421] POLRMT is a mitochondrial DNA-directed RNA polymerase that is essential in transcription of circular mammalian mitochondrial DNA (mtDNA). To determine whether the ASOs described in this example were able to impair transcription of mtDNA (through knockdown POLRMT expression), expression of Cytochrome B (CytB), a mitochondrial protein, was measured in the HeLa cells transfected with ASOs.

[0422] CytB expression in HeLa cells transfected with the exemplary ASOs are shown in **FIG. 5**. The same controls were used in this screen. All results were normalized by 18S expression.

[0423] The results in FIG. 5 show that the ASOs targeting POLRMT that were able to knockdown expression of POLRMT mRNA also resulted in decreased expression of CytB in all of the ASOs except ASO represented by SEQ ID NO: 14, where there was a slight increase. These results show that the knockdown in POLRMT expression leads to decreased activity of POLRMT (i.e., transcription of CytB mtDNA). No data was obtained from the ASOs represented in SEQ ID NOs: 9 and 10 due to cytotoxicity.

Example 2: Design and Modification of Additional POLRMT ASO Sequences

[0424] This example illustrates the design of additional exemplary antisense oligonucleotides (ASOs) that target POLRMT RNA.

[0425] ASOs were designed using two different strategies. The first strategy designed ASOs using software program LNCASO (https://iomics.ugent.be/pjdev/design) and analyzing

the longest POLRMT transcript (represented in Reference No. ENST00000588649 and SEQ ID NO: 205). Oligo length was set to 19 nucleotides. The ASOs designed using this strategy are shown in **Table 7** below.

[0426] The second strategy designed additional ASOs using the software program PFRED (https://github.com/pfred/pfred-gui/releases/tag/v1.0). For the PFRED program, oligo length was set to 20 nucleotides and 1 mismatch. The gene base on ENSG ID was searched and the longest POLRMT transcript (represented in Reference No. ENST00000588649 and SEQ ID NO: 205). Oligos with more than 1 mismatch in both cDNA and unspliced mRNA were filtered out. The SVMpred was set as >0.5, PLSpred_optimized was set at >0.7 or > 0.8. The ASOs designed using this strategy are shown in **Table 8** below.

Table 7

ASO SEQ ID NO:	**ASO sequence (5' to 3')**
71	GAGATAGTGAGAGGCCTCC
72	AGGAATACACGTGGTAGAG
73	CCAGTACTGCCGCGGCAGG
74	AGTCCAGGATGTCATCCAT
75	TCTTCTGCTTACGTGTGTT
76	ATCATGTGGGAGGAGTCCA
77	GCCTGCAGGACCTGCAGGA
78	CAGCTGGTCAGTGAGCAGG
79	CTATTTGCTTGACCTTGGA
80	CAAAGCATGGGTACATCCA
81	AAGGGGTAAAGTGAGAACC
82	AGAGGTACTTCCTGTAGTG
83	AACACATATACCAGCTCCT
84	TTCATGACGGAGACATCAG
85	GTAGGGCTCGTTTGAGCCC
86	CCCATGTGGGAGATGAGGC
87	GAGTCAAGCCGGCATCCTT
88	CCAAGATCTTCTGGGGCTC
89	CACTGCAGGGTCTTCAAGG
90	CACACCCTGCTGGCCAGCT

Table 8

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Corresponding coordinates within SEQ ID NO: 1
91	ACGCCGCTCCAGGCCACCCC	148	GGGGTGGCCTGGAGCGGCGT	5'UTR	5032-5051
92	GCACGCCGCTCCAGGCCACC	149	GGTGGCCTGGAGCGGCGTGC	5'UTR	5034-5053
93	ACGCACGCCGCTCCAGGCCA	150	TGGCCTGGAGCGGCGTGCGT	5'UTR	5036-5055
94	GGCCCCAGCAAAGTGCCGAC	151	GTCGGCACTTTGCTGGGGCC	exon	5059-5078
95	GTCCTTCCTGCGGTCTTGGT	152	ACCAAGACCGCAGGAAGGAC	exon	5691-5710
96	AGTCCTTCCTGCGGTCTTGG	153	CCAAGACCGCAGGAAGGACT	exon	5692-5711
97	CCCCAGTCCTTCCTGCGGTC	154	GACCGCAGGAAGGACTGGGG	exon	5696-5715
98	CACGTGGCCCCAGTCCTTCC	155	GGAAGGACTGGGGCCACGTG	exon	5703-5722
99	CATCCACCCTGTTCACCACC	156	GGTGGTGAACAGGGTGGATG	exon	8450-8469
100	CACATCCACCCTGTTCACCA	157	TGGTGAACAGGGTGGATGTG	exon	8452-8471
101	GCGCCACATCCACCCTGTTC	158	GAACAGGGTGGATGTGGCGC	exon	8456-8475
102	GCCCCCATCTGGACCTTCCT	159	AGGAAGGTCCAGATGGGGGC	exon	8520-8539
103	GAACTCCCCGCTCTGGAATG	160	CATTCCAGAGCGGGGAGTTC	exon	8647-8666
104	CTTGAACTCCCCGCTCTGGA	161	TCCAGAGCGGGGAGTTCAAG	exon	8650-8669
105	CGGCCATCTGCTTGCTCAGG	162	CCTGAGCAAGCAGATGGCCG	exon	8705-8724
106	CCGGCCATCTGCTTGCTCAG	163	CTGAGCAAGCAGATGGCCGG	exon	8706-8725
107	ACCCGGCCATCTGCTTGCTC	164	GAGCAAGCAGATGGCCGGGT	exon	8708-8727
108	CTCCAGGCACCCGGCCATCT	165	AGATGGCCGGGTGCCTGGAG	exon	8716-8735
109	CCTCCAGGCACCCGGCCATC	166	GATGGCCGGGTGCCTGGAGG	exon	8717-8736
110	ATCGAGGCTCAGCTTCCCAG	167	CTGGGAAGCTGAGCCTCGAT	exon	8803-8822
111	CCTGCTCCACATCGAGGCTC	168	GAGCCTCGATGTGGAGCAGG	exon	8813-8832
112	GGCGTTGTACATGTCCAGCG	169	CGCTGGACATGTACAACGCC	exon	8986-9005
113	GCATCACGGCGTTGTACATG	170	CATGTACAACGCCGTGATGC	exon	8993-9012
114	AGCATCACGGCGTTGTACAT	171	ATGTACAACGCCGTGATGCT	exon	8995-9014
115	AAGCATCACGGCGTTGTACA	172	TGTACAACGCCGTGATGCTT	exon	8994-9013
116	TCCAGACACCTTTCGATGGT	173	ACCATCGAAAGGTGTCTGGA	exon	13435-13447, 13666-13672
117	CAGAACGGCGGTGAAGAGTG	174	CACTCTTCACCGCCGTTCTG	exon	13705-13724
118	GCAGAACGGCGGTGAAGAGT	175	ACTCTTCACCGCCGTTCTGC	exon	13706-13725
119	GACAGCAGAACGGCGGTGAA	176	TTCACCGCCGTTCTGCTGTC	exon	13710-13729
120	ACGGCCTTCAGAACAGTGGC	177	GCCACTGTTCTGAAGGCCGT	exon	13743-13762
121	CACCTTGTGCACGGCCTTCA	178	TGAAGGCCGTGCACAAGGTG	exon	13753-13772
122	ACACGCCCATCCTTGGCATA	179	TATGCCAAGGATGGGCGTGT	exon	13842-13851,14967-14976
123	GGACACACGCCCATCCTTGG	180	CCTGCA**A**GGATGGGCGTGTGTCC	exon	14959-14980
124	AGGACACACGCCCATCCTTG	181	CA**A**GGATGGGCGTGTGTCCT	exon	14963-14981

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Corresponding coordinates within SEQ ID NO: 1
125	GTAGGACACACGCCCATCCT	182	AGGATGGGCGTGTGTCCTAC	exon	14964-14983
126	GGTAGGACACACGCCCATCC	183	GGATGGGCGTGTGTCCTACC	exon	14965-14984
127	GACCTCCTTGCTTGGCAACG	184	CGTTGCCAAGCAAGGAGGTC	exon	15087-15106
128	TTTCTCCCATTGGTCCCGCA	185	TGCGGGACCAATGGGAGAAA	exon	15603-15622
129	GTGCTTTCTCCCATTGGTCC	186	GGACCAATGGGAGAAAGCAC	exon	15607-15626
130	CCGGCACAGTGCTTTCTCCC	187	GGGAGAAAGCACTGTGCCGG	exon	15615-15634
131	CCACTGACCCGCTGCCTCTG	188	CAGAGGCAGCGGGTCAGTGG	exon	15904-15923
132	CTCCTCCCAGTACTGCCGCG	189	CGCGGCAGTACTGGGAGGAG	exon	16215-16234
133	TTGTCCAGGCTGCATGGCAT	190	ATGCCATGCAGCCTGGACAA	exon	16334-16353
134	TCCACCGCCTCGAAGGTCAG	191	CTGACCTTCGAGGCGGTGGA	exon	16786-16805
135	ATCCACCGCCTCGAAGGTCA	192	TGACCTTCGAGGCGGTGGAT	exon	16787-16806
136	CATCCACCGCCTCGAAGGTC	193	GACCTTCGAGGCGGTGGATG	exon	16788-16807
137	ACATCCACCGCCTCGAAGGT	194	ACCTTCGAGGCGGTGGATGT	exon	16789-16808
138	GGTACATCCACCGCCTCGAA	195	TTCGAGGCGGTGGATGTACC	exon	16792-16811
139	GTGCGGCGATGTCCAGGGCA	196	TGCCCTGGACATCGCCGCAC	exon	16829-16848
140	AGAGTGCGGCGATGTCCAGG	197	CCTGGACATCGCCGCACTCT	exon	16832-16851
141	CAGAGTGCGGCGATGTCCAG	198	CTGGACATCGCCGCACTCTG	exon	16833-16852
142	CCAGAGTGCGGCGATGTCCA	199	TGGACATCGCCGCACTCTGG	exon	16834-16853
143	CACCAGAGTGCGGCGATGTC	200	GACATCGCCGCACTCTGGTG	exon	16836-16855
144	AGCACCAGAGTGCGGCGATG	201	CATCGCCGCACTCTGGTGCT	exon	16838-16857
145	GGTCCAGCACGCGCCCGTTG	202	CAACGGGCGCGTGCTGGACC	exon	16998-17017
146	AGGTCCAGCACGCGCCCGTT	203	AACGGGCGCGTGCTGGACCT	exon	16999-17018
147	GCCAGCTCACGGCGCAGCTC	204	GAGCTGCGCCGTGAGCTGGC	exon	17140-17159
205	CCGCCGCTCCAGGCCACCCC	299	GGGGTGGCCTGGAGCGGCGG	exon	5032-5051
206	GCACGCCGCTCCAGGCCACC	300	GGTGGCCTGGAGCGGCGTGC	exon	5034-5053
207	ACGCACGCCGCTCCAGGCCA	301	TGGCCTGGAGCGGCGTGCGT	exon	5036-5055
208	GGCCCCAGCAAAGTGCCGAC	302	GTCGGCACTTTGCTGGGGCC	exon	5059-5078
209	GTCCTTCCTGCGGTCTTGGT	303	ACCAAGACCGCAGGAAGGAC	exon	5691-5710
210	AGTCCTTCCTGCGGTCTTGG	304	CCAAGACCGCAGGAAGGACT	exon	5692-5711
211	CCCCAGTCCTTCCTGCGGTC	305	GACCGCAGGAAGGACTGGGG	exon	5696-5715
212	CACGTGGCCCCAGTCCTTCC	306	GGAAGGACTGGGGCCACGTG	exon	5703-5722
213	CATCCACCCTGTTCACCACC	307	GGTGGTGAACAGGGTGGATG	exon	8450-8469
214	CACATCCACCCTGTTCACCA	308	TGGTGAACAGGGTGGATGTG	exon	8452-8471
215	GCGCCACATCCACCCTGTTC	309	GAACAGGGTGGATGTGGCGC	exon	8456-8475
216	GCCCCCATCTGGACCTTCCT	310	AGGAAGGTCCAGATGGGGGC	exon	8520-8539
217	GAACTCCCCGCTCTGGAATG	311	CATTCCAGAGCGGGGAGTTC	exon	8647-8666
218	CTTGAACTCCCCGCTCTGGA	312	TCCAGAGCGGGGAGTTCAAG	exon	8650-8669
219	CGGCCATCTGCTTGCTCAGG	313	CCTGAGCAAGCAGATGGCCG	exon	8705-8724

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Corresponding coordinates within SEQ ID NO: 1
220	CCGGCCATCTGCTTGCTCAG	314	CTGAGCAAGCAGATGGCCGG	exon	8706-8725
221	ACCCGGCCATCTGCTTGCTC	315	GAGCAAGCAGATGGCCGGGT	exon	8708-8727
222	CTCCAGGCACCCGGCCATCT	316	AGATGGCCGGGTGCCTGGAG	exon	8716-8735
223	CCTCCAGGCACCCGGCCATC	317	GATGGCCGGGTGCCTGGAGG	exon	8717-8736
224	ATCGAGGCTCAGCTTCCCAG	318	CTGGGAAGCTGAGCCTCGAT	exon	8803-8822
225	CCTGCTCCACATCGAGGCTC	319	GAGCCTCGATGTGGAGCAGG	exon	8813-8832
226	GGCGTTGTACATGTCCAGCG	320	CGCTGGACATGTACAACGCC	exon	8986-9005
227	GCATCACGGCGTTGTACATG	321	CATGTACAACGCCGTGATGC	exon	8993-9012
228	AGCATCACGGCGTTGTACAT	322	ATGTACAACGCCGTGATGCT	exon	8994-9013
229	AAGCATCACGGCGTTGTACA	323	TGTACAACGCCGTGATGCTT	exon	8995-9014
230	TCCAGACACCTTTCGATGGT	324	ACCATCGAAAGGTGTCTGGA	exon	13435-13447 and 13666-13672
231	CAGAACGGCGGTGAAGAGTG	325	CACTCTTCACCGCCGTTCTG	exon	13705-13724
232	GCAGAACGGCGGTGAAGAGT	326	ACTCTTCACCGCCGTTCTGC	exon	13706-13725
233	GACAGCAGAACGGCGGTGAA	327	TTCACCGCCGTTCTGCTGTC	exon	13710-13729
234	ACGGCCTTCAGAACAGTGGC	328	GCCACTGTTCTGAAGGCCGT	exon	13743-13762
235	CACCTTGTGCACGGCCTTCA	329	TGAAGGCCGTGCACAAGGTG	exon	13753-13772
236	ACACGCCCATCCTTGGCATA	330	TATGCCAAGGATGGGCGTGT	exon	13842-13850 and 14966-14976
237	GGACACACGCCCATCCTTGG	331	CCAAGGATGGGCGTGTGTCC	exon	13846-13850 and 14966-14980
238	AGGACACACGCCCATCCTTG	332	CAAGGATGGGCGTGTGTCCT	exon	13847-13850 and 14966-14981
239	GTAGGACACACGCCCATCCT	333	AGGATGGGCGTGTGTCCTAC	exon	13849-13850 and 14966-14983
240	GGTAGGACACACGCCCATCC	334	GGATGGGCGTGTGTCCTACC	exon	13850-13850 and 14966-14984
241	GACCTCCTTGCTTGGCAACG	335	CGTTGCCAAGCAAGGAGGTC	exon	15087-15106
242	TTTCTCCCATTGGTCCCGCA	336	TGCGGGACCAATGGGAGAAA	exon	15603-15622
243	GTGCTTTCTCCCATTGGTCC	337	GGACCAATGGGAGAAAGCAC	exon	15607-15626
244	CCGGCACAGTGCTTTCTCCC	338	GGGAGAAAGCACTGTGCCGG	exon	15615-15634
245	CCACTGACCCGCTGCCTCTG	339	CAGAGGCAGCGGGTCAGTGG	exon	15904-15923
246	CTCCTCCCAGTACTGCCGCG	340	CGCGGCAGTACTGGGAGGAG	exon	16215-16234
247	TTGTCCAGGCTGCATGGCAT	341	ATGCCATGCAGCCTGGACAA	exon	16334-16353
248	TCCACCGCCTCGAAGGTCAG	342	CTGACCTTCGAGGCGGTGGA	exon	16786-16805

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Corresponding coordinates within SEQ ID NO: 1
249	ATCCACCGCCTCGAAGGTCA	343	TGACCTTCGAGGCGGTGGAT	exon	16787-16806
250	CATCCACCGCCTCGAAGGTC	344	GACCTTCGAGGCGGTGGATG	exon	16788-16807
251	ACATCCACCGCCTCGAAGGT	345	ACCTTCGAGGCGGTGGATGT	exon	16789-16808
252	GGTACATCCACCGCCTCGAA	346	TTCGAGGCGGTGGATGTACC	exon	16792-16811
253	GTGCGGCGATGTCCAGGGCA	347	TGCCCTGGACATCGCCGCAC	exon	16829-16848
254	AGAGTGCGGCGATGTCCAGG	348	CCTGGACATCGCCGCACTCT	exon	16832-16851
255	CAGAGTGCGGCGATGTCCAG	349	CTGGACATCGCCGCACTCTG	exon	16833-16852
256	CCAGAGTGCGGCGATGTCCA	350	TGGACATCGCCGCACTCTGG	exon	16834-16853
257	CACCAGAGTGCGGCGATGTC	351	GACATCGCCGCACTCTGGTG	exon	16836-16855
258	AGCACCAGAGTGCGGCGATG	352	CATCGCCGCACTCTGGTGCT	exon	16838-16857
259	GGTCCAGCACGCGCCCGTTG	353	CAACGGGCGCGTGCTGGACC	exon	16998-17017
260	AGGTCCAGCACGCGCCCGTT	354	AACGGGCGCGTGCTGGACCT	exon	16999-17018
261	GCCAGCTCACGGCGCAGCTC	355	GAGCTGCGCCGTGAGCTGGC	exon	17140-17159
262	CGCCAGCTCACGGCGCAGCT	356	AGCTGCGCCGTGAGCTGGCG	exon	17141-17160
263	GCAGTGCGCCAGCTCACGGC	357	GCCGTGAGCTGGCGCACTGC	exon	17147-17166
264	CTTCTGGCAGTGCGCCAGCT	358	AGCTGGCGCACTGCCAGAAG	exon	17153-17172
265	ACCTTCTGGCAGTGCGCCAG	359	CTGGCGCACTGCCAGAAGGT	exon	17155-17174
266	CCACCTTCTGGCAGTGCGCC	360	GGCGCACTGCCAGAAGGTGG	exon	17157-17176
267	TCCCGGGCCACCTTCTGGCA	361	TGCCAGAAGGTGGCCCGGGA	exon	17164-17183
268	GACGCGGTCCCGCAGGTGCT	362	AGCACCTGCGGGACCGCGTC	exon	17231-17250
269	GGAAGTCCATGTTGTGCGGC	363	GCCGCACAACATGGACTTCC	exon	17259-17278
270	GCGGAAGTCCATGTTGTGCG	364	CGCACAACATGGACTTCCGC	exon	17261-17280
271	GTCGCTGCCCAGGTGGTTGA	365	TCAACCACCTGGGCAGCGAC	exon	17309-17328
272	ACGTCGCTGCCCAGGTGGTT	366	AACCACCTGGGCAGCGACGT	exon	17311-17330
273	CCACGTCGCTGCCCAGGTGG	367	CCACCTGGGCAGCGACGTGG	exon	17313-17332
274	CCCGCGCCACGTCGCTGCCC	368	GGGCAGCGACGTGGCGCGGG	exon	17319-17338
275	GGCCCGCGCCACGTCGCTGC	369	GCAGCGACGTGGCGCGGGCC	exon	17321-17340
276	GCGGCCCTGGGCGAACTCCA	370	TGGAGTTCGCCCAGGGCCGC	exon	17345-17364
277	CACCACTTTCGGCCCGTCAA	371	TTGACGGGCCGAAAGTGGTG	exon	17506-17512 and 18083-18095
278	CCTCCGCGCCCATCCACCAC	372	GTGGTGGATGGGCGCGGAGG	exon	18090-18109
279	TCCTCCGCGCCCATCCACCA	373	TGGTGGATGGGCGCGGAGGA	exon	18091-18110
280	GTTCCTCCGCGCCCATCCAC	374	GTGGATGGGCGCGGAGGAAC	exon	18093-18112
281	GGGTTCCTCCGCGCCCATCC	375	GGATGGGCGCGGAGGAACCC	exon	18095-18114
282	AGGCCAGCGTCTGCCAGGGT	376	ACCCTGGCAGACGCTGGCCT	exon	18111-18130
283	CGGCACATCCGAGGGCTCCA	377	TGGAGCCCTCGGATGTGCCG	exon	18562-18581
284	CACGCCGCTGTACACGTCCT	378	AGGACGTGTACAGCGGCGTG	exon	18583-18602

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Corresponding coordinates within SEQ ID NO: 1
285	CCACGCCGCTGTACACGTCC	379	GGACGTGTACAGCGGCGTGG	exon	18584-18603
286	GCCACGCCGCTGTACACGTC	380	GACGTGTACAGCGGCGTGGC	exon	18585-18604
287	GGCCACGCCGCTGTACACGT	381	ACGTGTACAGCGGCGTGGCC	exon	18586-18605
288	TGCGCGGCCACGCCGCTGTA	382	TACAGCGGCGTGGCCGCGCA	exon	18591-18610
289	CTGCCTACGGAACACCTCCA	383	TGGAGGTGTTCCGTAGGCAG	exon	18805-18824
290	TTCCAGCACCTGTGCCACCC	384	GGGTGGCACAGGTGCTGGAA	exon	18844-18863
291	GAGGCCTCCCACACGAACTC	385	GAGTTCGTGTGGGAGGCCTC	exon	19273-19292
292	AGAGGCCTCCCACACGAACT	386	AGTTCGTGTGGGAGGCCTCT	exon	19274-19293
293	TGAATTCCACCTCCTATTTG	387	CAAATAGGAGGTGGAATTCA	exon	19809-19828
294	CGAAGGTCAGGCCCTTCCTG	388	CAGGAAGGGCCTGACCTTCG	exon	20717-20736
295	AGACGAAGGTCAGGCCCTTC	389	GAAGGGCCTGACCTTCGTCT	exon	20720-20739
296	GAGACGAAGGTCAGGCCCTT	390	AAGGGCCTGACCTTCGTCTC	exon	20721-20740
297	AGAGACGAAGGTCAGGCCCT	391	AGGGCCTGACCTTCGTCTCT	exon	20722-20741
298	AGTAACAGTCGTGCACAGAG	392	CTCTGTGCACGACTGTTACT	exon	20738-20757

ASO Synthesis

[0427] Exemplary ASOs are synthesized according to methods described in Example 1 (see https://eu.idtdna.com/pages/products/functional-genomics/antisense-oligos).

[0428] ASOs contain the following modification pattern:

[0429] $X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

[0430] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond.

ASO Transfection

[0431] Human HeLa cells (ATCC CCL-2) are grown at 37 ℃ with 5% (v/v) CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

[0432] 6 or 12 µl of each ASO (100 µM) is added to 8 µL of DharmaFECT 1 (horizondiscovery, T-2001-02), 1 ml Opti-MEM media (Thermo Fisher Scientific,

Cat#11058021), and mixed well (final concentration of ASO is 100nM and 200nM). The mixture is incubated for 15 min at room temperature. 5 mL of 5×10^4/mL Hela cells are added to the mixture and mixed well. The cells and ASO mixture are seeded (2 mL per well) in a 6-well plate and grown for three days.

RT-PCR Protocol

[0433] The RT-PCR protocol is performed according to Example 1 in order to measure relative POLRMT mRNA expression levels.

Example 3: Design and Modification of Additional POLRMT ASO Sequences

[0434] This example illustrates the design of additional exemplary mouse antisense oligonucleotides (ASOs) that target mouse POLRMT RNA.

[0435] ASOs were designed using the software program PFRED (https://github.com/pfred/pfred-gui/releases/tag/v1.0). For the PFRED program, oligo length was set to 20 nucleotides and 1 mismatch. The gene base on ENSG ID was searched and the longest mouse POLRMT transcript (represented in Reference No. ENSMUST00000161765; SEQ ID NO: 582). Oligos with more than 1 mismatch in both cDNA and unspliced mRNA were filtered out. The SVMpred was set as >0.6, PLSpred_optimized was set at >0.8. 0.8.

[0436] Exemplary mouse ASO sequences, the POLRMT sequence targeted (target region), a description of the target region, and the coordinates of the target region within the mouse POLRMT gene sequence (SEQ ID NO: 581) are shown in **Table 9** below.

Table 9

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Start	End
393	CACGGTGTTGTACATGTGCA	487	TGCACATGTACAACACCGTG	Exon	7077	7096
394	ATCACGGTGTTGTACATGTG	488	CACATGTACAACACCGTGAT	Exon	7075	7094
395	CATCACGGTGTTGTACATGT	489	ACATGTACAACACCGTGATG	Exon	7074	7093
396	GTGCATCTCTCGAGCCACCT	490	AGGTGGCTCGAGAGATGCAC	Exon	3342	3361
397	TGTGCATCTCTCGAGCCACC	491	GGTGGCTCGAGAGATGCACA	Exon	3341	3360
398	CTGTGCATCTCTCGAGCCAC	492	GTGGCTCGAGAGATGCACAG	Exon	3340	3359

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Start	End
399	GTGCTGTGCCAGCGACAGGC	493	GCCTGTCGCTGGCACAGCAC	Exon	3297	3316
400	GTCCATGTTGTGCGGCAACC	494	GGTTGCCGCACAACATGGAC	Exon	3258	3277
401	AGGTCACTGCCCAGGTGGTT	495	AACCACCTGGGCAGTGACCT	Exon	3202	3221
402	TCCATCCACCACTTCCGGCC	496	GGCCGGAAGTGGTGGATGGA	Exon	2663	2682
403	ACCTCCATGCAGCAGGCCAG	497	CTGGCCTGCTGCATGGAGGT	Exon	2621	2640
404	CACCTCCATGCAGCAGGCCA	498	TGGCCTGCTGCATGGAGGTG	Exon	2620	2639
405	CCACCTCCATGCAGCAGGCC	499	GGCCTGCTGCATGGAGGTGG	Exon	2619	2638
406	GCCACCTCCATGCAGCAGGC	500	GCCTGCTGCATGGAGGTGGC	Exon	2618	2637
407	TGCCACCTCCATGCAGCAGG	501	CCTGCTGCATGGAGGTGGCA	Exon	2617	2636
408	CACCACCTTGCGGCTGATGA	502	TCATCAGCCGCAAGGTGGTG	Exon	2005	2024
409	TTCACCACCTTGCGGCTGAT	503	ATCAGCCGCAAGGTGGTGAA	Exon	2003	2022
410	CTGCTGTCTGTCTCCGTTGT	504	ACAACGGAGACAGACAGCAG	Exon	7107	7126
411	ACCTGCTGTCTGTCTCCGTT	505	AACGGAGACAGACAGCAGGT	Exon	7105	7124
412	GCACCTGCTGTCTGTCTCCG	506	CGGAGACAGACAGCAGGTGC	Exon	7103	7122
413	TGTTGTACATGTGCAGTGTG	507	CACACTGCACATGTACAACA	Exon	7082	7101
414	CGGTGTTGTACATGTGCAGT	508	ACTGCACATGTACAACACCG	Exon	7079	7098
415	GACCAGGTCAGTGAAGAGCA	509	TGCTCTTCACTGACCTGGTC	Exon	5712	5731
416	TCCAGGACCAGGTCAGTGAA	510	TTCACTGACCTGGTCCTGGA	Exon	5707	5726
417	CTTCCAGGACCAGGTCAGTG	511	CACTGACCTGGTCCTGGAAG	Exon	5705	5724
418	CTCCCCTTCGCTCAGCAGGC	512	GCCTGCTGAGCGAAGGGGAG	Exon	4732	4751
419	ACTCCCCTTCGCTCAGCAGG	513	CCTGCTGAGCGAAGGGGAGT	Exon	4731	4750
420	AACTCCCCTTCGCTCAGCAG	514	CTGCTGAGCGAAGGGGAGTT	Exon	4730	4749
421	GCCCAGGTTATGGGCCAACT	515	AGTTGGCCCATAACCTGGGC	Exon	4568	4587
422	CTGTTGTGCAGGGGCCTCCA	516	TGGAGGCCCCTGCACAACAG	Exon	4178	4197
423	CAATTGCTTGCCCAGCTGTA	517	TACAGCTGGGCAAGCAATTG	Exon	4136	4155
424	CCAATTGCTTGCCCAGCTGT	518	ACAGCTGGGCAAGCAATTGG	Exon	4135	4154
425	CGCCAATTGCTTGCCCAGCT	519	AGCTGGGCAAGCAATTGGCG	Exon	4133	4152
426	CCGCCAATTGCTTGCCCAGC	520	GCTGGGCAAGCAATTGGCGG	Exon	4132	4151
427	TCCGCCAATTGCTTGCCCAG	521	CTGGGCAAGCAATTGGCGGA	Exon	4131	4150
428	CTCCGCCAATTGCTTGCCCA	522	TGGGCAAGCAATTGGCGGAG	Exon	4130	4149
429	GCTCCGCCAATTGCTTGCCC	523	GGGCAAGCAATTGGCGGAGC	Exon	4129	4148
430	AGCTCCGCCAATTGCTTGCC	524	GGCAAGCAATTGGCGGAGCT	Exon	4128	4147
431	AGGGCATTGCTCCAGCAGAC	525	GTCTGCTGGAGCAATGCCCT	Exon	3597	3616
432	CGCAGTTCCCCAACTGTGTG	526	CACACAGTTGGGGAACTGCG	Exon	3545	3564
433	GCGCAGTTCCCCAACTGTGT	527	ACACAGTTGGGGAACTGCGC	Exon	3544	3563
434	CTCTCGAGCCACCTTGAGGC	528	GCCTCAAGGTGGCTCGAGAG	Exon	3348	3367
435	ACTGTGCATCTCTCGAGCCA	529	TGGCTCGAGAGATGCACAGT	Exon	3339	3358
436	GACTGTGCATCTCTCGAGCC	530	GGCTCGAGAGATGCACAGTC	Exon	3338	3357
437	AGACTGTGCATCTCTCGAGC	531	GCTCGAGAGATGCACAGTCT	Exon	3337	3356

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Start	End
438	CAGACTGTGCATCTCTCGAG	532	CTCGAGAGATGCACAGTCTG	Exon	3336	3355
439	GCAGACTGTGCATCTCTCGA	533	TCGAGAGATGCACAGTCTGC	Exon	3335	3354
440	GCGGAAGTCCATGTTGTGCG	534	CGCACAACATGGACTTCCGC	Exon	3252	3271
441	CCGCGGAAGTCCATGTTGTG	535	CACAACATGGACTTCCGCGG	Exon	3250	3269
442	CGCTAGGTCACTGCCCAGGT	536	ACCTGGGCAGTGACCTAGCG	Exon	3198	3217
443	GCGCTAGGTCACTGCCCAGG	537	CCTGGGCAGTGACCTAGCGC	Exon	3197	3216
444	CGCGCTAGGTCACTGCCCAG	538	CTGGGCAGTGACCTAGCGCG	Exon	3196	3215
445	CCGGCCCTCAGCAAACTCCA	539	TGGAGTTTGCTGAGGGCCGG	Exon	3168	3187
446	TTCCATCCACCACTTCCGGC	540	GCCGGAAGTGGTGGATGGAA	Exon	2662	2681
447	GCTTCCATCCACCACTTCCG	541	CGGAAGTGGTGGATGGAAGC	Exon	2660	2679
448	AGCTTCCATCCACCACTTCC	542	GGAAGTGGTGGATGGAAGCT	Exon	2659	2678
449	CAGCTTCCATCCACCACTTC	543	GAAGTGGTGGATGGAAGCTG	Exon	2658	2677
450	TCAGCTTCCATCCACCACTT	544	AAGTGGTGGATGGAAGCTGA	Exon	2657	2676
451	ATCAGCTTCCATCCACCACT	545	AGTGGTGGATGGAAGCTGAT	Exon	2656	2675
452	CATCAGCTTCCATCCACCAC	546	GTGGTGGATGGAAGCTGATG	Exon	2655	2674
453	TCATCAGCTTCCATCCACCA	547	TGGTGGATGGAAGCTGATGA	Exon	2654	2673
454	GCTCATCAGCTTCCATCCAC	548	GTGGATGGAAGCTGATGAGC	Exon	2652	2671
455	GGCTCATCAGCTTCCATCCA	549	TGGATGGAAGCTGATGAGCC	Exon	2651	2670
456	GTGCCACCTCCATGCAGCAG	550	CTGCTGCATGGAGGTGGCAC	Exon	2616	2635
457	TGTGCCACCTCCATGCAGCA	551	TGCTGCATGGAGGTGGCACA	Exon	2615	2634
458	GTGTGCCACCTCCATGCAGC	552	GCTGCATGGAGGTGGCACAC	Exon	2614	2633
459	CGTGTGCCACCTCCATGCAG	553	CTGCATGGAGGTGGCACACG	Exon	2613	2632
460	GCGTGTGCCACCTCCATGCA	554	TGCATGGAGGTGGCACACGC	Exon	2612	2631
461	TGCGTGTGCCACCTCCATGC	555	GCATGGAGGTGGCACACGCA	Exon	2611	2630
462	CAGTCCACTTGCGTGCCCAC	556	GTGGGCACGCAAGTGGACTG	Exon	2540	2559
463	GGGCAGTCCACTTGCGTGCC	557	GGCACGCAAGTGGACTGCCC	Exon	2537	2556
464	GCCATGGGCAGTCCACTTGC	558	GCAAGTGGACTGCCCATGGC	Exon	2532	2551
465	CACAGGTCACCCCAGAAACT	559	AGTTTCTGGGGTGACCTGTG	Exon	2380	2399
466	CACCCACACTGTCTCGGCCT	560	AGGCCGAGACAGTGTGGGTG	Exon	2271	2290
467	GCACCCACACTGTCTCGGCC	561	GGCCGAGACAGTGTGGGTGC	Exon	2270	2289
468	TGTTGCCACCTCCCTGTACA	562	TGTACAGGGAGGTGGCAACA	Exon	2209	2228
469	TGTGTTGCCACCTCCCTGTA	563	TACAGGGAGGTGGCAACACA	Exon	2207	2226
470	CTGTGTTGCCACCTCCCTGT	564	ACAGGGAGGTGGCAACACAG	Exon	2206	2225
471	CTGGCACCCACCTGTGTTGC	565	GCAACACAGGTGGGTGCCAG	Exon	2195	2214
472	CACTCCACAGGCCATCCGGC	566	GCCGGATGGCCTGTGGAGTG	Exon	1536	1555
473	CCCACTCCACAGGCCATCCG	567	CGGATGGCCTGTGGAGTGGG	Exon	1534	1553
474	GACCCACTCCACAGGCCATC	568	GATGGCCTGTGGAGTGGGTC	Exon	1532	1551
475	CCACCTTTTACCTGCAGACC	569	GGTCTGCAGGTAAAAGGTGG	Exon	1377	1396
476	GCCACCTTTTACCTGCAGAC	570	GTCTGCAGGTAAAAGGTGGC	Exon	1376	1395

SEQ ID NO:	ASO sequence (5' to 3')	SEQ ID NO:	POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)	Target region	Start	End
477	GGCCACCTTTTACCTGCAGA	571	TCTGCAGGTAAAAGGTGGCC	Exon	1375	1394
478	CTCCCCGCAGTACAGGACTC	572	GAGTCCTGTACTGCGGGGAG	Exon	1313	1332
479	CCTCCCCGCAGTACAGGACT	573	AGTCCTGTACTGCGGGGAGG	Exon	1312	1331
480	CCCTCCCCGCAGTACAGGAC	574	GTCCTGTACTGCGGGGAGGG	Exon	1311	1330
481	CTCCCCTCCCCGCAGTACAG	575	CTGTACTGCGGGGAGGGGAG	Exon	1308	1327
482	TCCTGCCCATCCCTCCATCC	576	GGATGGAGGGATGGGCAGGA	Exon	1287	1306
483	TTCCTGCCCATCCCTCCATC	577	GATGGAGGGATGGGCAGGAA	Exon	1286	1305
484	CCTTTCCTGCCCATCCCTCC	578	GGAGGGATGGGCAGGAAAGG	Exon	1283	1302
485	GCATGTGTCCAGAAGCAGTC	579	GACTGCTTCTGGACACATGC	Exon	637	656
486	TTGCGCACCGCCAGAAAGTG	580	CACTTTCTGGCGGTGCGCAA	Exon	19	38

ASO Synthesis

[0437] Exemplary ASOs were synthesized according to methods described in Example 1 (see https://eu.idtdna.com/pages/products/functional-genomics/antisense-oligos).

[0438] ASOs contained the following modification pattern:

[0439] $X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$

[0440] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond.

ASO Transfection

[0441] Mouse 3T3 cells (ATCC CRL-1658) were grown at 37 °C with 5% (v/v) CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

[0442] 1.8 or 6 µl of each ASO (100µM) was added to 8 µL of DharmaFECT 1 (horizondiscovery, T-2001-02), 1 ml Opti-MEM media (Thermo Fisher Scientific, Cat#11058021), and mixed well. ASOs were tested at two final concentrations 100nM and 30 nM. The mixture was incubated for 15 min at room temperature. 5 mL of 5×10^4/mL 3T3 cells were added to the mixture and mixed well. The cells and ASO mixture were seeded (2 mL per well) in a 6-well plate and grown for one day.

RT-PCR Protocol

[0443] The following RT-PCR protocol was performed:

1. Transfer 3T3 cells to eppendorf tubes, pellet cells by centrifuging 500g 5 min, and remove remaining media. Wash the cell pellet with 1 x Phosphate Buffered Saline (PBS). Add 1 mL of trizol solution (Thermo Fisher Scientific, Cat# 15596026) to the 3T3 cell pellet. Resuspend the cells completely by vortexing.
2. Incubate tubes at room temperature for 5 min.
3. Add 200 µl chloroform. Vortex for 10 seconds.
4. Incubate for 5 min at room temperature to permit nucleoprotein dissociation.
5. Centrifuge the tubes at 12,000 x g for 15 min at 4 °C.
6. Transfer the upper phase to a new microcentrifuge tube without disturbing the interphase.
7. Add 500 µL of isopropanol to the sample, invert 5 times to mix and incubate 30 min at -20 °C.
8. Centrifuge at a minimum of 12,000 x g for 15 min at 4 °C and remove the supernatant. The RNA will appear as a white pellet in the tube.
9. Wash the pellet with 500 µL of ice-cold 75% ethanol (made with deionized, diethylpyrocarbonate (*DEPC*) treated and 0.22 µm membrane-filtered H_2O).
10. Remove the ethanol, air dry for 5 minutes at room temperature (do not completely dry the RNA) and resuspend the RNA in 100 µL of DEPC-H_2O.
11. Incubate 5 µg RNA with 1 unit of turbo DNase (TURBO DNA-free Kit, Thermo Fisher Scientific, Cat# AM1907) at room temperature for 15 minutes and stop reaction following the manufacturer's instructions.
12. Purify RNA using a Quick-RNA miniprep kit (ZYMO, Cat# R1055).
13. Perform reverse transcription using 1 µg RNA and the iScript cDNA Synthesis Kit (Biorad, 170-8891).
14. Dilute 20 µl of cDNA to a final volume of 200 µl using H_2O. The cDNA is now ready for PCR quantification.

[0444] The iTaq Universal SYBR Green Supermix (Bio-Rad, Cat# #1725121) was used together with primers ordered from Eurofins genomics (shown below in **Table 10**) to detect expression of mouse POLRMT and mouse 18S rRNA genes. Quantification was performed

using the Biorad CFX96 PCR system. The reaction mixtures in each well contained 1 µL forward primer (conc. 5 µM) and 1 µL reverse primer (conc. 5 µM), 2 µL cDNA, 9.5 µL H_2O and 12.5 µL SYBR supermix.

Table 10:

Primer	**Supplier**	**Sequence**	**SEQ ID NO:**	**Storage**
mPOLRMT_F2	Eurofins	CCCACGATGAACGAGGTATG	583	-20 °C
mPOLRMT_R2	Eurofins	CGAGGACTTTAGGGACTTGATG	584	-20 °C
Ms_18S_F1	Eurofins	GCCGCTAGAGGTGAAATTCT	585	-20 °C
Ms_18S_R1	Eurofins	TCGGAACTACGACGGTATCT	586	-20 °C

Results – POLRMT Expression

[0445] POLRMT expression in 3T3 cells transfected with the exemplary ASOs at 100nM and 30nM are shown in FIGs. 6-9 and FIGs. 10-11, respectively. A scrambled ASO (not a perfect match to any mouse transcripts) was used as a control in each PCR plate. All results were normalized by 18S expression.

[0446] Of the ASOs tested at 100nM concentration (*i.e.,* ASOs represented in SEQ ID NOs: 393-486), all of the ASOs showed some inhibition of POLRMT expression (*i.e.,* decreased expression relative to the control). ASOs represented in SEQ ID NOs: 396, 398, 400, 402, 404-407, 418, 419, 424, 430, 434, 442, 449, 462, 472, 480, and 481, showed strong inhibition of POLRMT expression at 100nM.

[0447] Of the ASOs tested at 30nM concentration (*i.e.,* ASOs represented in SEQ ID NOs: 422-479), all ASOs showed some inhibition of POLRMT expression (*i.e.,* decreased expression relative to the control). ASOs represented in SEQ ID NOs: 422, 425, 432-434, 442-444, 446, 458-461, 464, 466, 468, 470, 471, and 477 showed strong inhibition of POLRMT expression at 30nM. ASOs represented in SEQ ID NOs: 434 and 442 showed strong inhibition of POLRMT expression at both 100nM and 30nM concentration.

[0448] These results show that the targeting certain regions of mouse POLRMT with ASO leads to different inhibition activity.

Example 4: Design and Testing of Exemplary Oligonucleotides Cross-reactive in Human and Mouse

[0449] This Example demonstrates exemplary oligonucleotides capable of inhibiting POLRMT expression in mouse and human cells and identifies regions within the POLRMT transcript that, when targeted by oligonucleotides described herein, are effective in inhibiting POLRMT expression.

Oligonucleotides

[0450] Oligonucleotides were designed and synthesized to target different regions of the POLRMT RNA transcript (SEQ ID NO: 205) and target regions on the POLRMNT mRNA transcript are characterized by corresponding region within the POLRMT gene sequence (represented in Reference No. NG_023049.1 and in SEQ ID NO: 1). Oligonucleotides were designed by selecting 16, 18, and 20-mers that target various regions of human POLRMT.

[0451] Exemplary oligonucleotide sequences, the POLRMT target region sequence within the POLRMT RNA transcript are shown in **Table 11** below.

Table 11

ASO SEQ ID NO:	**ASO sequence (5' to 3')**	**SEQ ID NO:**	**POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)**
587	GAAGTCCATGTTGTGCGGCA	656	TGCCGCACAACATGGACTTC
588	CGGAAGTCCATGTTGTGCGG	657	CCGCACAACATGGACTTCCG
589	CGCGGAAGTCCATGTTGTGC	658	GCACAACATGGACTTCCGCG
590	GCCGCGGAAGTCCATGTTGT	659	ACAACATGGACTTCCGCGGC
591	GGCCGCGGAAGTCCATGTTG	660	CAACATGGACTTCCGCGGCC
592	CGGCCGCGGAAGTCCATGTT	661	AACATGGACTTCCGCGGCCG
593	GCGGCCGCGGAAGTCCATGT	662	ACATGGACTTCCGCGGCCGC
594	TGCGGCCGCGGAAGTCCATG	663	CATGGACTTCCGCGGCCGCA
595	GTGCGGCCGCGGAAGTCCAT	664	ATGGACTTCCGCGGCCGCAC
596	CTGCCCAGGTGGTTGAAGTG	665	CACTTCAACCACCTGGGCAG
597	TCTGCTTCACCACCTTGCGG	666	CCGCAAGGTGGTGAAGCAGA
598	GTCTGCTTCACCACCTTGCG	667	CGCAAGGTGGTGAAGCAGAC
599	ATGAAGTTGGGCGGGAAGCC	668	GGCTTCCCGCCCAACTTCAT
600	GATGAAGTTGGGCGGGAAGC	669	GCTTCCCGCCCAACTTCATC
601	GGATGAAGTTGGGCGGGAAG	670	CTTCCCGCCCAACTTCATCC

602	TGGATGAAGTTGGGCGGGAA	671	TTCCCGCCCAACTTCATCCA
603	GTGGATGAAGTTGGGCGGGA	672	TCCCGCCCAACTTCATCCAC
604	GAGTGGATGAAGTTGGGCGG	673	CCGCCCAACTTCATCCACTC
605	GAGTGGATGAAGTTGGGCGG	674	CCGCCCAACTTCATCCACTC
606	ATCATGTGGGAGGAGTCCAG	675	CTGGACTCCTCCCACATGAT
607	CATCATGTGGGAGGAGTCCA	676	TGGACTCCTCCCACATGATG
608	GCATCATGTGGGAGGAGTCC	677	GGACTCCTCCCACATGATGC
609	AGGCCCTTCCTGTAGCAGTG	678	CACTGCTACAGGAAGGGCCT
610	CAGGCCCTTCCTGTAGCAGT	679	ACTGCTACAGGAAGGGCCTG
611	TCAGGCCCTTCCTGTAGCAG	680	CTGCTACAGGAAGGGCCTGA
612	CCCAGCCAAGCATCACGG	681	CCGTGATGCTTGGCTGGG
613	GCCCAGCCAAGCATCACG	682	CGTGATGCTTGGCTGGGC
614	AGTCCATGTTGTGCGGCA	683	TGCCGCACAACATGGACT
615	AAGTCCATGTTGTGCGGC	684	GCCGCACAACATGGACTT
616	GAAGTCCATGTTGTGCGG	685	CCGCACAACATGGACTTC
617	GGAAGTCCATGTTGTGCG	686	CGCACAACATGGACTTCC
618	CGGAAGTCCATGTTGTGC	687	GCACAACATGGACTTCCG
619	GCGGAAGTCCATGTTGTG	688	CACAACATGGACTTCCGC
620	CGCGGAAGTCCATGTTGT	689	ACAACATGGACTTCCGCG
621	CCGCGGAAGTCCATGTTG	690	CAACATGGACTTCCGCGG
622	GCCGCGGAAGTCCATGTT	691	AACATGGACTTCCGCGGC
623	GGCCGCGGAAGTCCATGT	692	ACATGGACTTCCGCGGCC
624	CGGCCGCGGAAGTCCATG	693	CATGGACTTCCGCGGCCG
625	GCGGCCGCGGAAGTCCAT	694	ATGGACTTCCGCGGCCGC
626	TGCGGCCGCGGAAGTCCA	695	TGGACTTCCGCGGCCGCA
627	GTGCGGCCGCGGAAGTCC	696	GGACTTCCGCGGCCGCAC
628	GCCCAGGTGGTTGAAGTG	697	CACTTCAACCACCTGGGC
629	TGCCCAGGTGGTTGAAGT	698	ACTTCAACCACCTGGGCA
630	CTGCCCAGGTGGTTGAAG	699	CTTCAACCACCTGGGCAG
631	TGCTTCACCACCTTGCGG	700	CCGCAAGGTGGTGAAGCA
632	CTGCTTCACCACCTTGCG	701	CGCAAGGTGGTGAAGCAG
633	TCTGCTTCACCACCTTGC	702	GCAAGGTGGTGAAGCAGA
634	GTCTGCTTCACCACCTTG	703	CAAGGTGGTGAAGCAGAC
635	GAACATCTCCTGTAGACT	704	AGTCTACAGGAGATGTTC
636	GAAGTTGGGCGGGAAGCC	705	GGCTTCCCGCCCAACTTC
637	TGAAGTTGGGCGGGAAGC	706	GCTTCCCGCCCAACTTCA
638	ATGAAGTTGGGCGGGAAG	707	CTTCCCGCCCAACTTCAT
639	GATGAAGTTGGGCGGGAA	708	TTCCCGCCCAACTTCATC
640	GGATGAAGTTGGGCGGGA	709	TCCCGCCCAACTTCATCC
641	TGGATGAAGTTGGGCGGG	710	CCCGCCCAACTTCATCCA
642	GTGGATGAAGTTGGGCGG	711	CCGCCCAACTTCATCCAC

643	AGTGGATGAAGTTGGGCG	712	CGCCCAACTTCATCCACT
644	GAGTGGATGAAGTTGGGC	713	GCCCAACTTCATCCACTC
645	CATGTGGGAGGAGTCCAG	714	CTGGACTCCTCCCACATG
646	TCATGTGGGAGGAGTCCA	715	TGGACTCCTCCCACATGA
647	ATCATGTGGGAGGAGTCC	716	GGACTCCTCCCACATGAT
648	CATCATGTGGGAGGAGTC	717	GACTCCTCCCACATGATG
649	GCATCATGTGGGAGGAGT	718	ACTCCTCCCACATGATGC
650	AGCATCATGTGGGAGGAG	719	CTCCTCCCACATGATGCT
651	GCCCTTCCTGTAGCAGTG	720	CACTGCTACAGGAAGGGC
652	GGCCCTTCCTGTAGCAGT	721	ACTGCTACAGGAAGGGCC
653	AGGCCCTTCCTGTAGCAG	722	CTGCTACAGGAAGGGCCT
654	CAGGCCCTTCCTGTAGCA	723	TGCTACAGGAAGGGCCTG
655	TCAGGCCCTTCCTGTAGC	724	GCTACAGGAAGGGCCTGA

[0452] A subset of the oligonucleotides that have a target region sequence identical to the corresponding region on the mouse POLRMT transcript were selected for testing in human 143B cells and mouse 3T3 cells. A schematic of the 13 selected oligonucleotide sequences and their respective target regions on the POLRMT transcript is shown in **FIG. 12** and **Table 12** below.

Table 12

SEQ ID NO:	**ASO Sequence (5' to 3')**	**SEQ ID NO:**	**POLRMT RNA Sequence (5' to 3') (represented as a DNA sequence)**	**Location (human) POLRMT mRNA**	**Location (mouse) POLRMT mRNA**
592	CGGCCGCGGAAGTCCATGTT	661	AACATGGACTTCCGCGGCCG	2420-2439	2329-2348
594	TGCGGCCGCGGAAGTCCATG	663	CATGGACTTCCGCGGCCGCA	2422-2441	2331-2350
597	TCTGCTTCACCACCTTGCGG	666	CCGCAAGGTGGTGAAGCAGA	2983-3002	3240-3259
598	GTCTGCTTCACCACCTTGCG	667	CGCAAGGTGGTGAAGCAGAC	2984-3003	3241-3260
612	CCCAGCCAAGCATCACGG	681	CCGTGATGCTTGGCTGGG	822-839	734-751
613	GCCCAGCCAAGCATCACG	682	CGTGATGCTTGGCTGGGC	823-840	735-752
623	GGCCGCGGAAGTCCATGT	692	ACATGGACTTCCGCGGCC	2421-2438	2330-2347
624	CGGCCGCGGAAGTCCATG	693	CATGGACTTCCGCGGCCG	2422-2439	2331-2348
625	GCGGCCGCGGAAGTCCAT	694	ATGGACTTCCGCGGCCGC	2423-2440	2332-2349
626	TGCGGCCGCGGAAGTCCA	695	TGGACTTCCGCGGCCGCA	2424-2441	2333-2350
632	CTGCTTCACCACCTTGCG	701	CGCAAGGTGGTGAAGCAG	2984-3001	3241-3258
633	TCTGCTTCACCACCTTGC	702	GCAAGGTGGTGAAGCAGA	2985-3002	3242-3259

634	GTCTGCTTCACCACCTTG	703	CAAGGTGGTGAAGCAGAC	2986-3003	3243-3260

Oligonucleotide Synthesis

[0453] Exemplary oligonucleotides were synthesized according to methods described in Example 1 (see https://eu.idtdna.com/pages/products/functional-genomics/antisense-oligos).

[0454] Oligonucleotides contain the following modification pattern:

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$ (for 20-mers) and

$X_{MS}X_{MS}X_{MS}X_{MS}X_{MS}X_SX_SX_SX_SX_SX_SX_SX_SX_{MS}X_{MS}X_{MS}X_{MS}X_{MS}$ (for 18-mers)

[0455] where "X" represents any nucleotide; a "M" represents a 2'-O-MOE group; and an "S" represents a phosphorothioate bond.

Table 13: Exemplary Modified Oligonucleotides (where "s" represents a phosphorothioate bond and "M" represents a 2'-O-MOE group)

Oligonucleotide Base Sequence SEQ ID NO:	**Modified Oligonucleotide SEQ ID NO:**	**Oligonucleotide Sequence (5' to 3')**
592	728	$C_{MS}G_{MS}G_{MS}C_{MS}C_{MS}G_SC_SG_SG_SA_SA_SG_ST_SC_SC_SA_{MS}T_{MS}G_{MS}T_{MS}T_{MS}$
594	729	$T_{MS}G_{MS}C_{MS}G_{MS}G_{MS}C_SC_SG_SC_SG_SG_SA_SA_SG_ST_SC_{MS}C_{MS}A_{MS}T_{MS}G_{MS}$
597	730	$T_{MS}C_{MS}T_{MS}G_{MS}C_{MS}T_ST_SC_SA_SC_SC_SA_SC_SC_ST_ST_{MS}G_{MS}C_{MS}G_{MS}G_{MS}$
598	731	$G_{MS}T_{MS}C_{MS}T_{MS}G_{MS}C_ST_ST_SC_SA_SC_SC_SA_SC_SC_ST_{MS}T_{MS}G_{MS}C_{MS}G_{MS}$
612	732	$C_{MS}C_{MS}C_{MS}A_{MS}G_{MS}C_SC_SA_SA_SG_SC_SA_ST_SC_{MS}A_{MS}C_{MS}G_{MS}G_{MS}$
613	733	$G_{MS}C_{MS}C_{MS}C_{MS}A_{MS}G_SC_SC_SA_SA_SG_SC_SA_ST_{MS}C_{MS}A_{MS}C_{MS}G_{MS}$
623	734	$G_{MS}G_{MS}C_{MS}C_{MS}G_{MS}C_SG_SG_SA_SA_SG_ST_SC_SC_{MS}A_{MS}T_{MS}G_{MS}T_{MS}$
624	735	$C_{MS}G_{MS}G_{MS}C_{MS}C_{MS}G_SC_SG_SG_SA_SA_SG_ST_SC_{MS}C_{MS}A_{MS}T_{MS}G_{MS}$
625	736	$G_{MS}C_{MS}G_{MS}G_{MS}C_{MS}C_SG_SC_SG_SG_SA_SA_SG_ST_{MS}C_{MS}C_{MS}A_{MS}T_{MS}$
626	737	$T_{MS}G_{MS}C_{MS}G_{MS}G_{MS}C_SC_SG_SC_SG_SG_SA_SA_SG_{MS}T_{MS}C_{MS}C_{MS}A_{MS}$
632	738	$C_{MS}T_{MS}G_{MS}C_{MS}T_{MS}T_SC_SA_SC_SC_SA_SC_SC_ST_{MS}T_{MS}G_{MS}C_{MS}G_{MS}$
633	739	$T_{MS}C_{MS}T_{MS}G_{MS}C_{MS}T_ST_SC_SA_SC_SC_SA_SC_SC_{MS}T_{MS}T_{MS}G_{MS}C_{MS}$
634	740	$G_{MS}T_{MS}C_{MS}T_{MS}G_{MS}C_ST_ST_SC_SA_SC_SC_SA_SC_{MS}C_{MS}T_{MS}T_{MS}G_{MS}$

(i) Dose response in Human 143B and mouse 3T3 cells

Oligonucleotide Transfection

[0456] Human 143B cells (ATCC # 8303) and mouse 3T3 cells (ATCC #1658) were grown at 37 ℃ with 5% (v/v) CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

[0457] Each modified oligonucleotide was added to DharmaFECT 1 (horizondiscovery, T-2001-02) and Opti-MEM media (Thermo Fisher Scientific, Cat#11058021), and mixed well to a final concentration of 100nM ASO. Serial dilutions (1:2) were performed for each oligonucleotide resulting in a total of 7 concentrations (100nM, 50nM, 25nM, 12.5nM, 6.25nM, 3.125nM, and 1.5625nM) of each oligonucleotide to be tested in each cell type. The mixture was incubated for 15 min at room temperature. 5 × 104/mL 143B cells or 4 × 104/mL 3T3 cells were added to the oligo/DharmaFECT/Media mixture and mixed well.

[0458] The cells were then seeded in a 6-well plate and grown for one day. Cells were harvested for RT-PCR to assess POLRMT expression and cell viability using Celltiter fluor (Promega Cat #G6080).

RT-PCR Protocol

[0459] The RT-PCR protocol is performed according to Example 1 in order to measure relative POLRMT mRNA expression levels. Expression is shown relative to vehicle control.

Results

[0460] POLRMT expression in both mouse 3T3 and human 143B cells transfected with each modified oligonucleotide is shown in FIG. 13 (for modified oligonucleotides with unmodified base sequence represented in SEQ ID NOs: 612, 613, 623, 624, 632, 633, and 634) and FIG. 14 (for modified oligonucleotides with unmodified base sequence represented in SEQ ID NOs: 592, 594, 597, 598, 625, and 626). Results show that POLRMT expression was inhibited in a dose-dependent manner for each oligonucleotide tested, confirming that the selected oligonucleotides are indeed cross-reactive in human and mouse cells (*i.e.,* can target the

mouse and human POLRMT transcript and are capable of POLRMT knockdown in mouse and human cells).

[0461] Additionally, **FIG. 14**, panel (B) and (D) and **FIG. 15**, panel (B) and (D) shows that the cells transfected with oligonucleotides remained viable.

(ii) Toxicity in HepG2 and 3T3 cells

Oligonucleotide Transfection

[0462] HepG2 cells (ATCC # HB-8065) and mouse 3T3 cells (ATCC #1658) were grown at 37 °C with 5% (v/v) CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

[0463] Modified oligonucleotides were added to DharmaFECT 1 (horizondiscovery, T-2001-02) and Opti-MEM media (Thermo Fisher Scientific, Cat#11058021), and mixed well, the mixture was incubated for 15 min at room temperature. After incubation the mixture was added to 1×10^5/mL HepG2 cells or 4×10^4/mL 3T3 cells to a final concentration of oligonucleotide at 100nM. Two scrambled ASOs and a vehicle treatment were used as controls.

[0464] The cells were seeded to 96-well plate on day 1, transfected with oligonucleotide on day 2, and cell viability and RT-qPCR were carried out on day 3.

Caspase-Glo 3/7 Assay

[0465] In order to assess in vitro toxicity of the exemplary oligonucleotides on HepG2 and 3T3 cells, a Caspase-Glo 3/7 assay was performed (Promega Cat. #G8090).

(iii) Activity in HepG2 cells

Oligonucleotide Transfection in HepG2

[0466] HepG2 cells (ATCC # HB-8065) were grown at 37 °C with 5% (v/v) CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

[0467] Modified oligonucleotides were added to DharmaFECT 1 (horizondiscovery, T-2001-02) and Opti-MEM media (Thermo Fisher Scientific, Cat#11058021), and mixed well, the mixture was incubated for 15 min at room temperature. After incubation the mixture was added to 1×10^5/mL HepG2 cells to a final concentration of oligonucleotide at 100nM.

[0468] The cells and oligonucleotide mixture were seeded in a 6-well plate and grown for one day. Cells were then harvested for either RT-PCR to measure PORLMT expression or to assess cell viability measured using Celltiter fluor (Promega Cat #G6080).

RT-PCR Protocol

[0469] The RT-PCR protocol is performed according to Example 1 in order to measure relative POLRMT mRNA expression levels.

Results

[0470] Results from the expression and toxicity assays are shown in Table 14 below. Exemplary modified oligonucleotides having nucleotide sequences represented in SEQ ID NOs: 592, 594, 612, 623, 625, 626, and 632 were well tolerated in HepG2 and mouse 3T3 cells and showed low toxicity (see FIG. 15 and FIG. 16, Panel B). Exemplary modified oligonucleotides having nucleotide sequences represented in SEQ ID NOs: 594, 612 and 632 showed the best inhibition of POLRMT expression in HepG2 cells (see Table 12 and FIG. 16, Panel A) and also had low toxicity HepG2 cells and mouse 3T3 cells (see FIG. 15 and FIG. 16, Panel B).

[0471] This data suggested that there are three potential "hotspot" regions along the POLRMT transcript that can be targeted to inhibit POLRMT expression. These regions were identified within exons represented by Ensemble IDs ENSE00000655271, ENSE00000655279, and ENSE00000655283. The hotspot regions include spans of nucleotides within the PORLMT human and mouse transcript that comprise SEQ ID NO: 725 [CAACGCCGTGATGCTTGGCTGGGCGCGGC] ("hotspot 1"), SEQ ID NO: 726 [CGCACAACATGGACTTCCGCGGCCGCACCTAC] ("hotspot 2") and SEQ ID NO: 727 [ATCACCCGCAAGGTGGTGAAGCAGACGGTGA] ("hotspot 3"). The first, second and third hotspot regions correspond to positions 817-845, 2415-2446, and 2978-3008 of the human POLRMT mRNA transcript (SEQ ID NO: 205) and positions 729-756, 2324-2355, and 3235-

3265 of the mouse PORLMT transcript (SEQ ID NO: 581), respectively. Additionally, the most effective oligonucleotides targeting these regions consisted of various lengths (18 and 20 nucleotides).

Table 14

SEQ ID NO:	**Length**		**Hotspot**	**Toxicity**	**Efficiency on HepG2 (single dose – 100nM)**
	18nt	**20nt**			
592		X	2	Low	0.229
594		X	2	Low	0.089
597		X	3	High	0.301
598		X	3	High	0.107
612	X		1	Low	0.242
613	X		1	High	0.146
623	X		2	Low	0.265
624	X		2	High*	0.243
625	X		2	Low	0.152
626	X		2	Low	0.176
632	X		3	Low**	0.330
633	X		3	High	0.220
634	X		3	High	0.016

*Showed a slightly higher toxicity in HepG2, but not in 3T3 cells
**Showed high in toxicity in 3T3, but not in HepG2.

EXEMPLARY SEQUENCES

Wild-type human POLRMT gene sequence (corresponding to the whole POLRMT gene)

(NCBI Accession Number: NG_023049.1) **(SEQ ID NO: 1)**

ACCCTCCACGGAGCACGCTGGGCAGAGGGCGCGGCACCAGACACTGAAGAAATGTCCACCGCCTTCTCGC
CAGCAGGAGCCCAGGTCTTCCCTTCTTAGAGTGTCCGCCCCCCACCGCGGAAGGGTGATCTGAGGGCCTT
GTGAGAAATGATGGGGATGGGACCCACAGCGGTGAGACTGGCCCCACCCCAGGCGTCTGAGTTTCTTTAA
TGACACCTCGGAAACATCACTCATATACCACACAATTCACCCACTTACACTGTACAGTTCAGTGATTTTA
TTTTATTATTATTTCTTTGAGACGGAGTCTGGCTCTGTCACCCAGACTGGAGTGCAGTGGTGCGATCTCG
GCTCACTGCAAACTCTGCCTCCCTGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGATTA
CAGGCACCTGCCACCACACCCAGCTAATTTTTGTATTTTTAGTAGAGACAGAGTTTTACCATGTTGGCCA
GGCTGGTCTCAAACTCCTGACCTCGTGATCTACCTGCTTCTGCCTCCCAAAGTGCTGGGATTACAGGCAT
GAGCCACTGCACCCAGCCAGTTCAGTGATTTTAGTACCTCGTAGAGTTGTGCAACCACCACGACTGTCTA
GTTCCAGAAGATTCCATCACCGCAAGAAGAAGCCCCATCAGCTGTCACTCATCCCCTCTGCCAGCCCCCG
GCACCCACACATCCCCTTCCTGCCTCTGTGGATGGGCCTGTTGTGGACATTTCATATAAAGGGGATCACA
CACTGTGTGTCCTTCTGTGTCTGGCGTCTTTCACTGAGCATGACATCCTCAAGGTGCATCCGCGCTGTGG
CTGGGGCAGAGCCTTGCTCCTTTTCACGCCTGTAATCCCAGCTACTCAGGAGGCTGAGGCAGGAGAATCT
ATACAACCCAGGAGGCGGAGGTTGCAATGAGCTGAGATCATGCCACTGCCCTCCAGCCTGGGCGACAGAG
CGAGACTCTGTCTCAAACAAAAACAACAACAACAAACACCGATCAGGGCTGGGCTGGCGCACGCTTTGTA
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GACTTCAGCCCCGCAGTCCTGATTTTGGACTTTTCATTTCCAGAAATGTAAGAAAATGAACGTTGCTTTA
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ACTACCACGTCCGGCTAATTTTTGAATTTTTAGTGGAGACAGGGTTTCACCACGTTGGCCAGGCCAGTCT
CGAACTCCTGACCTCAGGGGATCTGCCTGCCTCAGCCTCCCAAAGTGCTGGAATTACAGGCATGAGCCAC
CATGACCAGCCAATATTGCATTTACTTAATACAGGAGTGGTGGAGACAGATGTAGTTAAAATAAATGGTG
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ACTCCTGACCTCACGTGATCCGCCCGCCTCGGCCTCCCAAAGTGCTGGGATCTCATCAAATAAAAGAAAA
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CCCCAGTAGTGGGTGAGGTCTGAGGTCACAGCCCGCTGGGGCACCCTGAGGTGGACTGAGGCAGGTGAGG
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CCAGCAGGGTGTGCGTGGTGTCCGTGGAGAAGCCCACGTTGCCAAGCAAGGAGGTCAAGCACGCGGTAGG
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CAATGGGAGAAAGCACTGTGCCGGGCGCTGCGGGAGACCAAGAACCGCCTAGAGCGCGAGGTGTACGAGG
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GGGACTACAGGCGCCCGCCACCACACCCGGCTAATTTTTTGTATTTTTTTTTTAGTAGAGACGGGGTTTC
ACCATGTTAGCCAGGATGGTCTCAATCTCCTGACCTCGTGATCCACCCACCTTGGCCTCCGAAAGTGCTG
GGACTACAGGCGTGAGCCACCGCGCCTGGCAGTAAA

The protein sequence of wildtype human POLRMT is as follows (1230 amino acids):

MSALCWGRGAAGLKRALRPCGRPGLPGKEGTAGGVCGPRRSSSASPQEQDQDRRKDWGHVELLEVLQARV
RQLQAESVSEVVVNRVDVARLPECGSGDGSLQPPRKVQMGAKDATPVPCGRWAKILEKDKRTQQMRMQRL
KAKLQMPFQSGEFKALTRRLQVEPRLLSKQMAGCLEDCTRQAPESPWEEQLARLLQEAPGKLSLDVEQAP
SGQHSQAQLSGQQQRLLAFFKCCLLTDQLPLAHHLLVVHHGQRQKRKLLTLDMYNAVMLGWARQGAFKEL
VYVLFMVKDAGLTPDLLSYAAALQCMGRQDQDAGTIERCLEQMSQEGLKLQALFTAVLLSEEDRATVLKA
VHKVKPTFSLPPQLPPPVNTSKLLRDVYAKDGRVSYPKLHLPLKTLQCLFEKQLHMELASRVCVVSVEKP
TLPSKEVKHARKTLKTLRDQWEKALCRALRETKNRLEREVYEGRFSLYPFLCLLDEREVVRMLLQVLQAL
PAQGESFTTLARELSARTFSRHVVQRQRVSGQVQALQNHYRKYLCLLASDAEVPEPCLPRQYWEELGAPE
ALREQPWPLPVQMELGKLLAEMLVQATQMPCSLDKPHRSSRLVPVLYHVYSFRNVQQIGILKPHPAYVQL
LEKAAEPTLTFEAVDVPMLCPPLPWTSPHSGAFLLSPTKLMRTVEGATQHQELLETCPPTALHGALDALT
QLGNCAWRVNGRVLDLVLQLFQAKGCPQLGVPAPPSEAPQPPEAHLPHSAAPARKAELRRELAHCQKVAR
EMHSLRAEALYRLSLAQHLRDRVFWLPHNMDFRGRTYPCPPHFNHLGSDVARALLEFAQGRPLGPHGLDW
LKIHLVNLTGLKKREPLRKRLAFAEEVMDDILDSADQPLTGRKWWMGAEEPWQTLACCMEVANAVRASDP
AAYVSHLPVHQDGSCNGLQHYAALGRDSVGAASVNLEPSDVPQDVYSGVAAQVEVFRRQDAQRGMRVAQV
LEGFITRKVVKQTVMTVVYGVTRYGGRLQIEKRLRELSDFPQEFVWEASHYLVRQVFKSLQEMFSGTRAI
QHWLTESARLISHMGSVVEWVTPLGVPVIQPYRLDSKVKQIGGGIQSITYTHNGDISRKPNTRKQKNGFP
PNFIHSLDSSHMMLTALHCYRKGLTFVSVHDCYWTHAADVSVMNQVCREQFVRLHSEPILQDLSRFLVKR
FCSEPQKILEASQLKETLQAVPKPGAFDLEQVKRSTYFFS (**SEQ ID NO: 2**, transit peptide)

Human POLRMT, mRNA; nuclear gene for mitochondrial product. (RefSeq NM_005035) Reference No. ENST00000588649.7 (**SEQ ID NO: 205**)

GGGGTGGCCTGGAGCGGCGTGCGTAATGTCGGCACTTTGCTGGGGCCGCGGAGCGGCGGGGCTCAAACGA
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GAGACACTGCAGGCGGTGCCCAAGCCAGGGGCCTTCGACCTGGAGCAGGTGAAGCGTTCCACCTACTTCT
TCAGCTGACACCCCGTGAGCCTTGTCAGTGTGTAAATAAAGCTCTTTTGCCACCCCCAGGA

Wild-type mouse POLRMT gene sequence (corresponding Mouse10 dna_chromosome chromosome_GRCm39_10_79571957_79582415) **(SEQ ID NO: 581)**

CTCATTACTTTGGTGGGTTTGCGCACCGCCAGAAAGTGGCGGCTGGCATAGGTAGACACAAACTTTCATT
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TAAATCCAGACCCAGAAAAAGACCAACTTCAAGGGCAGAACAGGCCAAAGCTATAAGGATCGGGGTGCAG

GATCCAGCAAGAAGTACTCCTAACTAAAAGTAGCCAAAGGTCAATGCCAATATCCGTAAGTCCCCAACAG
TGGGTCAGAGACATGGAAAAGCGAGCAACAATCAAAAGTCAAAGTGCCCTGCGGACCTAGAGAGACGTTG
CCAACTAGTGCCCAGCTAAATGGGTCCCCAGAAACTCCTCCATAGGTCAAAGAACAAAGGTTGAAGGTGA
CCGGGATCCTAAGGAAGGGAAGATTATAGTCCTGGATCCGACACTCCTTTACCTTCCTCCGAAGGCGGGC
GGTGGGGCCCAGGGGAGCGCAGAACCCGGCCAAGCCCGGCTGCGCTTCGGGTCCACCGGAGCGCCGACAT
GCCGCCTGCTGCCCGCAGTGCGCACGCGC

***Mouse POLRMT transcript* ENSMUST00000161765.8 *Polrmt-210 cdna* (SEQ ID NO: 582)**

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GCGCCAAAAGGAAGTTGACCAGCAGAAGCAAGCCCTCACACAGGAGTTCTGGACCCTTCACAAGGAGCCC
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CTGCCCTGGACGTCGCTGCATTCTGGTGCCTACTTGCTGAGCTCCACCAAACTAATGCGTGCCACGGAGG
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CACACAGTTGGGGAACTGCGCCTGGCGTGTAAACGGGCATCTGCTGGACTTGGTGCTGCAGATCTTTAGA
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TGCCACCCGGCTCCACACCAGTGCACAAGTCTGAGCTGCGGAAGGAGCTGGCGCGTTGCCTCAAGGTGGC
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TTCGCAGATGAGGTCATGGAGGAGATCTTGGACTCTGCAGACAACCCCTTGACGGGCCGGAAGTGGTGGA
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CCCTGCTGCCTACATTTCCCACCTGCCAGTTCACCAGGTGGGCACGCAAGTGGACTGCCCATGGCTGAGC
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CCGACCTGCCCCAAGATGTGTACAGGGAGGTGGCAACACAGGTGGGTGCCAGGAAACCCCTCCTGTCCCC
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ACCCCTAATCCCATCCAGGTGGAGGAGTTCCGCCAGCAGGACGCCAAGGAGGGTCTACGGGTGGCTCAGG
TGCTGGAGGGCTTCATCAGCCGCAAGGTGGTGAAGCAGACAGTGATGACGGTGGTGTATGGGGTCACACG
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GAAGCCTCACACTACCTCGTGCGCCAGGTCTTCAAAAGTCTACAGGAGATGTTCACCAGCACGCGGGCCA
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CCAGCCCATCCTGGAAGACCTGGCCAAGTTCCTGAAGAAGCGTTTCTGCTCCGTGTCCAGCATCAAGTCC
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CCTTCGATCTAGGACAGGTGATAAGATCCACCTACTTTTTCAGCTGACCCACGCCATGCGGTCCCTAAAC
CTGTGCATAATGTAAATAAATGAAAGTTTGTGTCTACCTATGCCAGCCGCCACTTTCTGGCGGTGCGCAA
ACCCACCAAAGTAATGAG

Table 15: POLRMT Oligonucleotide Sequences

SEQ ID NO:	ASO Sequence (5' to 3')
3	CCCCAGTCCTTCCTGCGGTC
4	TCCACATCGAGGCTCAGCTT
5	CTCCACATCGAGGCTCAGCT
6	TGCTCCACATCGAGGCTCAG
7	CCCCAGCTCCTCCCAGTACT
8	GGCCACCTTCTGGCAGTGCG
9	GCCACGTCGCTGCCCAGGTG
10	CGCCACGTCGCTGCCCAGGT
11	CCCATCCACCACTTTCGGCC
12	GCCCATCCACCACTTTCGGC
13	CGCCCATCCACCACTTTCGG
14	TTCGCCACCTCCATACAGCA
27	GGTCTTGGTCTTGCTCCTGG
28	GAGGCTACCATCTCCACTGC
29	GCATCCTTGGCCCCCATCTG
30	CTCCAGTATCTTTGCCCAGC
31	TCTCCAGTATCTTTGCCCAG
32	TTCTCCAGTATCTTTGCCCA
33	CTTCTCCAGTATCTTTGCCC

34	CCTTCTCCAGTATCTTTGCC
35	TCCTTCTCCAGTATCTTTGC
36	CACATATACCAGCTCCTTGA
37	TCCTCCTCAGACAGCAGAAC
38	CACTGCAGGGTCTTCAAGGG
39	TGCTTGACCTCCTTGCTTGG
40	GTGCTTGACCTCCTTGCTTG
41	TGCCCAGGTGGTTGAAGTGC
42	TGTAGACTCTTGAAGACCTG
43	AGCATCATGTGGGAGGAGTC
44	GAGCATCATGTGGGAGGAGT
45	TTCAGCTGGCTGGCCTCCAA
71	GAGATAGTGAGAGGCCTCC
72	AGGAATACACGTGGTAGAG
73	CCAGTACTGCCGCGGCAGG
74	AGTCCAGGATGTCATCCAT
75	TCTTCTGCTTACGTGTGTT
76	ATCATGTGGGAGGAGTCCA
77	GCCTGCAGGACCTGCAGGA
78	CAGCTGGTCAGTGAGCAGG
79	CTATTTGCTTGACCTTGGA
80	CAAAGCATGGGTACATCCA
81	AAGGGGTAAAGTGAGAACC
82	AGAGGTACTTCCTGTAGTG
83	AACACATATACCAGCTCCT
84	TTCATGACGGAGACATCAG
85	GTAGGGCTCGTTTGAGCCC
86	CCCATGTGGGAGATGAGGC
87	GAGTCAAGCCGGCATCCTT
88	CCAAGATCTTCTGGGGCTC
89	CACTGCAGGGTCTTCAAGG
90	CACACCCTGCTGGCCAGCT
91	ACGCCGCTCCAGGCCACCCC
92	GCACGCCGCTCCAGGCCACC
93	ACGCACGCCGCTCCAGGCCA
94	GGCCCCAGCAAAGTGCCGAC
95	GTCCTTCCTGCGGTCTTGGT
96	AGTCCTTCCTGCGGTCTTGG
97	CCCCAGTCCTTCCTGCGGTC
98	CACGTGGCCCCAGTCCTTCC
99	CATCCACCCTGTTCACCACC
100	CACATCCACCCTGTTCACCA
101	GCGCCACATCCACCCTGTTC

102	GCCCCCATCTGGACCTTCCT
103	GAACTCCCCGCTCTGGAATG
104	CTTGAACTCCCCGCTCTGGA
105	CGGCCATCTGCTTGCTCAGG
106	CCGGCCATCTGCTTGCTCAG
107	ACCCGGCCATCTGCTTGCTC
108	CTCCAGGCACCCGGCCATCT
109	CCTCCAGGCACCCGGCCATC
110	ATCGAGGCTCAGCTTCCCAG
111	CCTGCTCCACATCGAGGCTC
112	GGCGTTGTACATGTCCAGCG
113	GCATCACGGCGTTGTACATG
114	AGCATCACGGCGTTGTACAT
115	AAGCATCACGGCGTTGTACA
116	TCCAGACACCTTTCGATGGT
117	CAGAACGGCGGTGAAGAGTG
118	GCAGAACGGCGGTGAAGAGT
119	GACAGCAGAACGGCGGTGAA
120	ACGGCCTTCAGAACAGTGGC
121	CACCTTGTGCACGGCCTTCA
122	ACACGCCCATCCTTGGCATA
123	GGACACACGCCCATCCTTGG
124	AGGACACACGCCCATCCTTG
125	GTAGGACACACGCCCATCCT
126	GGTAGGACACACGCCCATCC
127	GACCTCCTTGCTTGGCAACG
128	TTTCTCCCATTGGTCCCGCA
129	GTGCTTTCTCCCATTGGTCC
130	CCGGCACAGTGCTTTCTCCC
131	CCACTGACCCGCTGCCTCTG
132	CTCCTCCCAGTACTGCCGCG
133	TTGTCCAGGCTGCATGGCAT
134	TCCACCGCCTCGAAGGTCAG
135	ATCCACCGCCTCGAAGGTCA
136	CATCCACCGCCTCGAAGGTC
137	ACATCCACCGCCTCGAAGGT
138	GGTACATCCACCGCCTCGAA
139	GTGCGGCGATGTCCAGGGCA
140	AGAGTGCGGCGATGTCCAGG
141	CAGAGTGCGGCGATGTCCAG
142	CCAGAGTGCGGCGATGTCCA
143	CACCAGAGTGCGGCGATGTC
144	AGCACCAGAGTGCGGCGATG

145	GGTCCAGCACGCGCCCGTTG
146	AGGTCCAGCACGCGCCCGTT
147	GCCAGCTCACGGCGCAGCTC
205	CCGCCGCTCCAGGCCACCCC
206	GCACGCCGCTCCAGGCCACC
207	ACGCACGCCGCTCCAGGCCA
208	GGCCCCAGCAAAGTGCCGAC
209	GTCCTTCCTGCGGTCTTGGT
210	AGTCCTTCCTGCGGTCTTGG
211	CCCCAGTCCTTCCTGCGGTC
212	CACGTGGCCCCAGTCCTTCC
213	CATCCACCCTGTTCACCACC
214	CACATCCACCCTGTTCACCA
215	GCGCCACATCCACCCTGTTC
216	GCCCCCATCTGGACCTTCCT
217	GAACTCCCCGCTCTGGAATG
218	CTTGAACTCCCCGCTCTGGA
219	CGGCCATCTGCTTGCTCAGG
220	CCGGCCATCTGCTTGCTCAG
221	ACCCGGCCATCTGCTTGCTC
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225	CCTGCTCCACATCGAGGCTC
226	GGCGTTGTACATGTCCAGCG
227	GCATCACGGCGTTGTACATG
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229	AAGCATCACGGCGTTGTACA
230	TCCAGACACCTTTCGATGGT
231	CAGAACGGCGGTGAAGAGTG
232	GCAGAACGGCGGTGAAGAGT
233	GACAGCAGAACGGCGGTGAA
234	ACGGCCTTCAGAACAGTGGC
235	CACCTTGTGCACGGCCTTCA
236	ACACGCCCATCCTTGGCATA
237	GGACACACGCCCATCCTTGG
238	AGGACACACGCCCATCCTTG
239	GTAGGACACACGCCCATCCT
240	GGTAGGACACACGCCCATCC
241	GACCTCCTTGCTTGGCAACG
242	TTTCTCCCATTGGTCCCGCA
243	GTGCTTTCTCCCATTGGTCC
244	CCGGCACAGTGCTTTCTCCC

245	CCACTGACCCGCTGCCTCTG
246	CTCCTCCCAGTACTGCCGCG
247	TTGTCCAGGCTGCATGGCAT
248	TCCACCGCCTCGAAGGTCAG
249	ATCCACCGCCTCGAAGGTCA
250	CATCCACCGCCTCGAAGGTC
251	ACATCCACCGCCTCGAAGGT
252	GGTACATCCACCGCCTCGAA
253	GTGCGGCGATGTCCAGGGCA
254	AGAGTGCGGCGATGTCCAGG
255	CAGAGTGCGGCGATGTCCAG
256	CCAGAGTGCGGCGATGTCCA
257	CACCAGAGTGCGGCGATGTC
258	AGCACCAGAGTGCGGCGATG
259	GGTCCAGCACGCGCCCGTTG
260	AGGTCCAGCACGCGCCCGTT
261	GCCAGCTCACGGCGCAGCTC
262	CGCCAGCTCACGGCGCAGCT
263	GCAGTGCGCCAGCTCACGGC
264	CTTCTGGCAGTGCGCCAGCT
265	ACCTTCTGGCAGTGCGCCAG
266	CCACCTTCTGGCAGTGCGCC
267	TCCCGGGCCACCTTCTGGCA
268	GACGCGGTCCCGCAGGTGCT
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270	GCGGAAGTCCATGTTGTGCG
271	GTCGCTGCCCAGGTGGTTGA
272	ACGTCGCTGCCCAGGTGGTT
273	CCACGTCGCTGCCCAGGTGG
274	CCCGCGCCACGTCGCTGCCC
275	GGCCCGCGCCACGTCGCTGC
276	GCGGCCCTGGGCGAACTCCA
277	CACCACTTTCGGCCCGTCAA
278	CCTCCGCGCCCATCCACCAC
279	TCCTCCGCGCCCATCCACCA
280	GTTCCTCCGCGCCCATCCAC
281	GGGTTCCTCCGCGCCCATCC
282	AGGCCAGCGTCTGCCAGGGT
283	CGGCACATCCGAGGGCTCCA
284	CACGCCGCTGTACACGTCCT
285	CCACGCCGCTGTACACGTCC
286	GCCACGCCGCTGTACACGTC
287	GGCCACGCCGCTGTACACGT

288	TGCGCGGCCACGCCGCTGTA
289	CTGCCTACGGAACACCTCCA
290	TTCCAGCACCTGTGCCACCC
291	GAGGCCTCCCACACGAACTC
292	AGAGGCCTCCCACACGAACT
293	TGAATTCCACCTCCTATTTG
294	CGAAGGTCAGGCCCTTCCTG
295	AGACGAAGGTCAGGCCCTTC
296	GAGACGAAGGTCAGGCCCTT
297	AGAGACGAAGGTCAGGCCCT
298	AGTAACAGTCGTGCACAGAG
393	CACGGTGTTGTACATGTGCA
394	ATCACGGTGTTGTACATGTG
395	CATCACGGTGTTGTACATGT
396	GTGCATCTCTCGAGCCACCT
397	TGTGCATCTCTCGAGCCACC
398	CTGTGCATCTCTCGAGCCAC
399	GTGCTGTGCCAGCGACAGGC
400	GTCCATGTTGTGCGGCAACC
401	AGGTCACTGCCCAGGTGGTT
402	TCCATCCACCACTTCCGGCC
403	ACCTCCATGCAGCAGGCCAG
404	CACCTCCATGCAGCAGGCCA
405	CCACCTCCATGCAGCAGGCC
406	GCCACCTCCATGCAGCAGGC
407	TGCCACCTCCATGCAGCAGG
408	CACCACCTTGCGGCTGATGA
409	TTCACCACCTTGCGGCTGAT
410	CTGCTGTCTGTCTCCGTTGT
411	ACCTGCTGTCTGTCTCCGTT
412	GCACCTGCTGTCTGTCTCCG
413	TGTTGTACATGTGCAGTGTG
414	CGGTGTTGTACATGTGCAGT
415	GACCAGGTCAGTGAAGAGCA
416	TCCAGGACCAGGTCAGTGAA
417	CTTCCAGGACCAGGTCAGTG
418	CTCCCCTTCGCTCAGCAGGC
419	ACTCCCCTTCGCTCAGCAGG
420	AACTCCCCTTCGCTCAGCAG
421	GCCCAGGTTATGGGCCAACT
422	CTGTTGTGCAGGGGCCTCCA
423	CAATTGCTTGCCCAGCTGTA
424	CCAATTGCTTGCCCAGCTGT

425	CGCCAATTGCTTGCCCAGCT
426	CCGCCAATTGCTTGCCCAGC
427	TCCGCCAATTGCTTGCCCAG
428	CTCCGCCAATTGCTTGCCCA
429	GCTCCGCCAATTGCTTGCCC
430	AGCTCCGCCAATTGCTTGCC
431	AGGGCATTGCTCCAGCAGAC
432	CGCAGTTCCCCAACTGTGTG
433	GCGCAGTTCCCCAACTGTGT
434	CTCTCGAGCCACCTTGAGGC
435	ACTGTGCATCTCTCGAGCCA
436	GACTGTGCATCTCTCGAGCC
437	AGACTGTGCATCTCTCGAGC
438	CAGACTGTGCATCTCTCGAG
439	GCAGACTGTGCATCTCTCGA
440	GCGGAAGTCCATGTTGTGCG
441	CCGCGGAAGTCCATGTTGTG
442	CGCTAGGTCACTGCCCAGGT
443	GCGCTAGGTCACTGCCCAGG
444	CGCGCTAGGTCACTGCCCAG
445	CCGGCCCTCAGCAAACTCCA
446	TTCCATCCACCACTTCCGGC
447	GCTTCCATCCACCACTTCCG
448	AGCTTCCATCCACCACTTCC
449	CAGCTTCCATCCACCACTTC
450	TCAGCTTCCATCCACCACTT
451	ATCAGCTTCCATCCACCACT
452	CATCAGCTTCCATCCACCAC
453	TCATCAGCTTCCATCCACCA
454	GCTCATCAGCTTCCATCCAC
455	GGCTCATCAGCTTCCATCCA
456	GTGCCACCTCCATGCAGCAG
457	TGTGCCACCTCCATGCAGCA
458	GTGTGCCACCTCCATGCAGC
459	CGTGTGCCACCTCCATGCAG
460	GCGTGTGCCACCTCCATGCA
461	TGCGTGTGCCACCTCCATGC
462	CAGTCCACTTGCGTGCCCAC
463	GGGCAGTCCACTTGCGTGCC
464	GCCATGGGCAGTCCACTTGC
465	CACAGGTCACCCCAGAAACT
466	CACCCACACTGTCTCGGCCT
467	GCACCCACACTGTCTCGGCC

468	TGTTGCCACCTCCCTGTACA
469	TGTGTTGCCACCTCCCTGTA
470	CTGTGTTGCCACCTCCCTGT
471	CTGGCACCCACCTGTGTTGC
472	CACTCCACAGGCCATCCGGC
473	CCCACTCCACAGGCCATCCG
474	GACCCACTCCACAGGCCATC
475	CCACCTTTTACCTGCAGACC
476	GCCACCTTTTACCTGCAGAC
477	GGCCACCTTTTACCTGCAGA
478	CTCCCCGCAGTACAGGACTC
479	CCTCCCCGCAGTACAGGACT
480	CCCTCCCCGCAGTACAGGAC
481	CTCCCCTCCCCGCAGTACAG
482	TCCTGCCCATCCCTCCATCC
483	TTCCTGCCCATCCCTCCATC
484	CCTTTCCTGCCCATCCCTCC
485	GCATGTGTCCAGAAGCAGTC
486	TTGCGCACCGCCAGAAAGTG
587	GAAGTCCATGTTGTGCGGCA
588	CGGAAGTCCATGTTGTGCGG
589	CGCGGAAGTCCATGTTGTGC
590	GCCGCGGAAGTCCATGTTGT
591	GGCCGCGGAAGTCCATGTTG
592	CGGCCGCGGAAGTCCATGTT
593	GCGGCCGCGGAAGTCCATGT
594	TGCGGCCGCGGAAGTCCATG
595	GTGCGGCCGCGGAAGTCCAT
596	CTGCCCAGGTGGTTGAAGTG
597	TCTGCTTCACCACCTTGCGG
598	GTCTGCTTCACCACCTTGCG
599	ATGAAGTTGGGCGGGAAGCC
600	GATGAAGTTGGGCGGGAAGC
601	GGATGAAGTTGGGCGGGAAG
602	TGGATGAAGTTGGGCGGGAA
603	GTGGATGAAGTTGGGCGGGA
604	GAGTGGATGAAGTTGGGCGG
605	GAGTGGATGAAGTTGGGCGG
606	ATCATGTGGGAGGAGTCCAG
607	CATCATGTGGGAGGAGTCCA
608	GCATCATGTGGGAGGAGTCC
609	AGGCCCTTCCTGTAGCAGTG
610	CAGGCCCTTCCTGTAGCAGT

611	TCAGGCCCTTCCTGTAGCAG
612	CCCAGCCAAGCATCACGG
613	GCCCAGCCAAGCATCACG
614	AGTCCATGTTGTGCGGCA
615	AAGTCCATGTTGTGCGGC
616	GAAGTCCATGTTGTGCGG
617	GGAAGTCCATGTTGTGCG
618	CGGAAGTCCATGTTGTGC
619	GCGGAAGTCCATGTTGTG
620	CGCGGAAGTCCATGTTGT
621	CCGCGGAAGTCCATGTTG
622	GCCGCGGAAGTCCATGTT
623	GGCCGCGGAAGTCCATGT
624	CGGCCGCGGAAGTCCATG
625	GCGGCCGCGGAAGTCCAT
626	TGCGGCCGCGGAAGTCCA
627	GTGCGGCCGCGGAAGTCC
628	GCCCAGGTGGTTGAAGTG
629	TGCCCAGGTGGTTGAAGT
630	CTGCCCAGGTGGTTGAAG
631	TGCTTCACCACCTTGCGG
632	CTGCTTCACCACCTTGCG
633	TCTGCTTCACCACCTTGC
634	GTCTGCTTCACCACCTTG
635	GAACATCTCCTGTAGACT
636	GAAGTTGGGCGGGAAGCC
637	TGAAGTTGGGCGGGAAGC
638	ATGAAGTTGGGCGGGAAG
639	GATGAAGTTGGGCGGGAA
640	GGATGAAGTTGGGCGGGA
641	TGGATGAAGTTGGGCGGG
642	GTGGATGAAGTTGGGCGG
643	AGTGGATGAAGTTGGGCG
644	GAGTGGATGAAGTTGGGC
645	CATGTGGGAGGAGTCCAG
646	TCATGTGGGAGGAGTCCA
647	ATCATGTGGGAGGAGTCC
648	CATCATGTGGGAGGAGTC
649	GCATCATGTGGGAGGAGT
650	AGCATCATGTGGGAGGAG
651	GCCCTTCCTGTAGCAGTG
652	GGCCCTTCCTGTAGCAGT
653	AGGCCCTTCCTGTAGCAG

654	CAGGCCCTTCCTGTAGCA
655	TCAGGCCCTTCCTGTAGC

Table 16: POLRMT Target Region Sequences

SEQ ID NO:	Target Sequence of POLRMT (5' to 3')
15	GACCGCAGGAAGGACTGGGG
16	AAGCTGAGCCTCGATGTGGA
17	AGCTGAGCCTCGATGTGGAG
18	CTGAGCCTCGATGTGGAGCA
19	AGTACTGGGAGGAGCTGGGG
20	CGCACTGCCAGAAGGTGGCC
21	CACCTGGGCAGCGACGTGGC
22	ACCTGGGCAGCGACGTGGCG
23	GGCCGAAAGTGGTGGATGGG
24	GCCGAAAGTGGTGGATGGGC
25	CCGAAAGTGGTGGATGGGCG
26	TGCTGTATGGAGGTGGCGAA
46	CCAGGAGCAAGACCAAGACC
47	GCAGTGGAGATGGTAGCCTC
48	CAGATGGGGGCCAAGGATGC
49	GCTGGGCAAAGATACTGGAG
50	CTGGGCAAAGATACTGGAGA
51	TGGGCAAAGATACTGGAGAA
52	GGGCAAAGATACTGGAGAAG
53	GGCAAAGATACTGGAGAAGG
54	GCAAAGATACTGGAGAAGGA
55	TCAAGGAGCTGGTATATGTG
56	GTTCTGCTGTCTGAGGAGGA
57	CCCTTGAAGACCCTGCAGTG
58	CCAAGCAAGGAGGTCAAGCA
59	CAAGCAAGGAGGTCAAGCAC
60	GCACTTCAACCACCTGGGCA
61	CAGGTCTTCAAGAGTCTACA
62	GACTCCTCCCACATGATGCT
63	ACTCCTCCCACATGATGCTC
64	TTGGAGGCCAGCCAGCTGAA
148	GGGGTGGCCTGGAGCGGCGT
149	GGTGGCCTGGAGCGGCGTGC
150	TGGCCTGGAGCGGCGTGCGT
151	GTCGGCACTTTGCTGGGGCC
152	ACCAAGACCGCAGGAAGGAC

153	CCAAGACCGCAGGAAGGACT
154	GACCGCAGGAAGGACTGGGG
155	GGAAGGACTGGGGCCACGTG
156	GGTGGTGAACAGGGTGGATG
157	TGGTGAACAGGGTGGATGTG
158	GAACAGGGTGGATGTGGCGC
159	AGGAAGGTCCAGATGGGGGC
160	CATTCCAGAGCGGGGAGTTC
161	TCCAGAGCGGGGAGTTCAAG
162	CCTGAGCAAGCAGATGGCCG
163	CTGAGCAAGCAGATGGCCGG
164	GAGCAAGCAGATGGCCGGGT
165	AGATGGCCGGGTGCCTGGAG
166	GATGGCCGGGTGCCTGGAGG
167	CTGGGAAGCTGAGCCTCGAT
168	GAGCCTCGATGTGGAGCAGG
169	CGCTGGACATGTACAACGCC
170	CATGTACAACGCCGTGATGC
171	ATGTACAACGCCGTGATGCT
172	TGTACAACGCCGTGATGCTT
173	ACCATCGAAAGGTGTCTGGA
174	CACTCTTCACCGCCGTTCTG
175	ACTCTTCACCGCCGTTCTGC
176	TTCACCGCCGTTCTGCTGTC
177	GCCACTGTTCTGAAGGCCGT
178	TGAAGGCCGTGCACAAGGTG
179	TATGCCAAGGATGGGCGTGT
180	CCTGCA**A**GGATGGGCGTGTGTCC
181	CA**A**GGATGGGCGTGTGTCCT
182	AGGATGGGCGTGTGTCCTAC
183	GGATGGGCGTGTGTCCTACC
184	CGTTGCCAAGCAAGGAGGTC
185	TGCGGGACCAATGGGAGAAA
186	GGACCAATGGGAGAAAGCAC
187	GGGAGAAAGCACTGTGCCGG
188	CAGAGGCAGCGGGTCAGTGG
189	CGCGGCAGTACTGGGAGGAG
190	ATGCCATGCAGCCTGGACAA
191	CTGACCTTCGAGGCGGTGGA
192	TGACCTTCGAGGCGGTGGAT
193	GACCTTCGAGGCGGTGGATG
194	ACCTTCGAGGCGGTGGATGT
195	TTCGAGGCGGTGGATGTACC

196	TGCCCTGGACATCGCCGCAC
197	CCTGGACATCGCCGCACTCT
198	CTGGACATCGCCGCACTCTG
199	TGGACATCGCCGCACTCTGG
200	GACATCGCCGCACTCTGGTG
201	CATCGCCGCACTCTGGTGCT
202	CAACGGGCGCGTGCTGGACC
203	AACGGGCGCGTGCTGGACCT
204	GAGCTGCGCCGTGAGCTGGC
299	GGGGTGGCCTGGAGCGGCGG
300	GGTGGCCTGGAGCGGCGTGC
301	TGGCCTGGAGCGGCGTGCGT
302	GTCGGCACTTTGCTGGGGCC
303	ACCAAGACCGCAGGAAGGAC
304	CCAAGACCGCAGGAAGGACT
305	GACCGCAGGAAGGACTGGGG
306	GGAAGGACTGGGGCCACGTG
307	GGTGGTGAACAGGGTGGATG
308	TGGTGAACAGGGTGGATGTG
309	GAACAGGGTGGATGTGGCGC
310	AGGAAGGTCCAGATGGGGGC
311	CATTCCAGAGCGGGGAGTTC
312	TCCAGAGCGGGGAGTTCAAG
313	CCTGAGCAAGCAGATGGCCG
314	CTGAGCAAGCAGATGGCCGG
315	GAGCAAGCAGATGGCCGGGT
316	AGATGGCCGGGTGCCTGGAG
317	GATGGCCGGGTGCCTGGAGG
318	CTGGGAAGCTGAGCCTCGAT
319	GAGCCTCGATGTGGAGCAGG
320	CGCTGGACATGTACAACGCC
321	CATGTACAACGCCGTGATGC
322	ATGTACAACGCCGTGATGCT
323	TGTACAACGCCGTGATGCTT
324	ACCATCGAAAGGTGTCTGGA
325	CACTCTTCACCGCCGTTCTG
326	ACTCTTCACCGCCGTTCTGC
327	TTCACCGCCGTTCTGCTGTC
328	GCCACTGTTCTGAAGGCCGT
329	TGAAGGCCGTGCACAAGGTG
330	TATGCCAAGGATGGGCGTGT
331	CCAAGGATGGGCGTGTGTCC
332	CAAGGATGGGCGTGTGTCCT

333	AGGATGGGCGTGTGTCCTAC
334	GGATGGGCGTGTGTCCTACC
335	CGTTGCCAAGCAAGGAGGTC
336	TGCGGGACCAATGGGAGAAA
337	GGACCAATGGGAGAAAGCAC
338	GGGAGAAAGCACTGTGCCGG
339	CAGAGGCAGCGGGTCAGTGG
340	CGCGGCAGTACTGGGAGGAG
341	ATGCCATGCAGCCTGGACAA
342	CTGACCTTCGAGGCGGTGGA
343	TGACCTTCGAGGCGGTGGAT
344	GACCTTCGAGGCGGTGGATG
345	ACCTTCGAGGCGGTGGATGT
346	TTCGAGGCGGTGGATGTACC
347	TGCCCTGGACATCGCCGCAC
348	CCTGGACATCGCCGCACTCT
349	CTGGACATCGCCGCACTCTG
350	TGGACATCGCCGCACTCTGG
351	GACATCGCCGCACTCTGGTG
352	CATCGCCGCACTCTGGTGCT
353	CAACGGGCGCGTGCTGGACC
354	AACGGGCGCGTGCTGGACCT
355	GAGCTGCGCCGTGAGCTGGC
356	AGCTGCGCCGTGAGCTGGCG
357	GCCGTGAGCTGGCGCACTGC
358	AGCTGGCGCACTGCCAGAAG
359	CTGGCGCACTGCCAGAAGGT
360	GGCGCACTGCCAGAAGGTGG
361	TGCCAGAAGGTGGCCCGGGA
362	AGCACCTGCGGGACCGCGTC
363	GCCGCACAACATGGACTTCC
364	CGCACAACATGGACTTCCGC
365	TCAACCACCTGGGCAGCGAC
366	AACCACCTGGGCAGCGACGT
367	CCACCTGGGCAGCGACGTGG
368	GGGCAGCGACGTGGCGCGGG
369	GCAGCGACGTGGCGCGGGCC
370	TGGAGTTCGCCCAGGGCCGC
371	TTGACGGGCCGAAAGTGGTG
372	GTGGTGGATGGGCGCGGAGG
373	TGGTGGATGGGCGCGGAGGA
374	GTGGATGGGCGCGGAGGAAC
375	GGATGGGCGCGGAGGAACCC

376	ACCCTGGCAGACGCTGGCCT
377	TGGAGCCCTCGGATGTGCCG
378	AGGACGTGTACAGCGGCGTG
379	GGACGTGTACAGCGGCGTGG
380	GACGTGTACAGCGGCGTGGC
381	ACGTGTACAGCGGCGTGGCC
382	TACAGCGGCGTGGCCGCGCA
383	TGGAGGTGTTCCGTAGGCAG
384	GGGTGGCACAGGTGCTGGAA
385	GAGTTCGTGTGGGAGGCCTC
386	AGTTCGTGTGGGAGGCCTCT
387	CAAATAGGAGGTGGAATTCA
388	CAGGAAGGGCCTGACCTTCG
389	GAAGGGCCTGACCTTCGTCT
390	AAGGGCCTGACCTTCGTCTC
391	AGGGCCTGACCTTCGTCTCT
392	CTCTGTGCACGACTGTTACT
487	TGCACATGTACAACACCGTG
488	CACATGTACAACACCGTGAT
489	ACATGTACAACACCGTGATG
490	AGGTGGCTCGAGAGATGCAC
491	GGTGGCTCGAGAGATGCACA
492	GTGGCTCGAGAGATGCACAG
493	GCCTGTCGCTGGCACAGCAC
494	GGTTGCCGCACAACATGGAC
495	AACCACCTGGGCAGTGACCT
496	GGCCGGAAGTGGTGGATGGA
497	CTGGCCTGCTGCATGGAGGT
498	TGGCCTGCTGCATGGAGGTG
499	GGCCTGCTGCATGGAGGTGG
500	GCCTGCTGCATGGAGGTGGC
501	CCTGCTGCATGGAGGTGGCA
502	TCATCAGCCGCAAGGTGGTG
503	ATCAGCCGCAAGGTGGTGAA
504	ACAACGGAGACAGACAGCAG
505	AACGGAGACAGACAGCAGGT
506	CGGAGACAGACAGCAGGTGC
507	CACACTGCACATGTACAACA
508	ACTGCACATGTACAACACCG
509	TGCTCTTCACTGACCTGGTC
510	TTCACTGACCTGGTCCTGGA
511	CACTGACCTGGTCCTGGAAG
512	GCCTGCTGAGCGAAGGGGAG

513	CCTGCTGAGCGAAGGGGAGT
514	CTGCTGAGCGAAGGGGAGTT
515	AGTTGGCCCATAACCTGGGC
516	TGGAGGCCCCTGCACAACAG
517	TACAGCTGGGCAAGCAATTG
518	ACAGCTGGGCAAGCAATTGG
519	AGCTGGGCAAGCAATTGGCG
520	GCTGGGCAAGCAATTGGCGG
521	CTGGGCAAGCAATTGGCGGA
522	TGGGCAAGCAATTGGCGGAG
523	GGGCAAGCAATTGGCGGAGC
524	GGCAAGCAATTGGCGGAGCT
525	GTCTGCTGGAGCAATGCCCT
526	CACACAGTTGGGGAACTGCG
527	ACACAGTTGGGGAACTGCGC
528	GCCTCAAGGTGGCTCGAGAG
529	TGGCTCGAGAGATGCACAGT
530	GGCTCGAGAGATGCACAGTC
531	GCTCGAGAGATGCACAGTCT
532	CTCGAGAGATGCACAGTCTG
533	TCGAGAGATGCACAGTCTGC
534	CGCACAACATGGACTTCCGC
535	CACAACATGGACTTCCGCGG
536	ACCTGGGCAGTGACCTAGCG
537	CCTGGGCAGTGACCTAGCGC
538	CTGGGCAGTGACCTAGCGCG
539	TGGAGTTTGCTGAGGGCCGG
540	GCCGGAAGTGGTGGATGGAA
541	CGGAAGTGGTGGATGGAAGC
542	GGAAGTGGTGGATGGAAGCT
543	GAAGTGGTGGATGGAAGCTG
544	AAGTGGTGGATGGAAGCTGA
545	AGTGGTGGATGGAAGCTGAT
546	GTGGTGGATGGAAGCTGATG
547	TGGTGGATGGAAGCTGATGA
548	GTGGATGGAAGCTGATGAGC
549	TGGATGGAAGCTGATGAGCC
550	CTGCTGCATGGAGGTGGCAC
551	TGCTGCATGGAGGTGGCACA
552	GCTGCATGGAGGTGGCACAC
553	CTGCATGGAGGTGGCACACG
554	TGCATGGAGGTGGCACACGC
555	GCATGGAGGTGGCACACGCA

556	GTGGGCACGCAAGTGGACTG
557	GGCACGCAAGTGGACTGCCC
558	GCAAGTGGACTGCCCATGGC
559	AGTTTCTGGGGTGACCTGTG
560	AGGCCGAGACAGTGTGGGTG
561	GGCCGAGACAGTGTGGGTGC
562	TGTACAGGGAGGTGGCAACA
563	TACAGGGAGGTGGCAACACA
564	ACAGGGAGGTGGCAACACAG
565	GCAACACAGGTGGGTGCCAG
566	GCCGGATGGCCTGTGGAGTG
567	CGGATGGCCTGTGGAGTGGG
568	GATGGCCTGTGGAGTGGGTC
569	GGTCTGCAGGTAAAAGGTGG
570	GTCTGCAGGTAAAAGGTGGC
571	TCTGCAGGTAAAAGGTGGCC
572	GAGTCCTGTACTGCGGGGAG
573	AGTCCTGTACTGCGGGGAGG
574	GTCCTGTACTGCGGGGAGGG
575	CTGTACTGCGGGGAGGGGAG
576	GGATGGAGGGATGGGCAGGA
577	GATGGAGGGATGGGCAGGAA
578	GGAGGGATGGGCAGGAAAGG
579	GACTGCTTCTGGACACATGC
580	CACTTTCTGGCGGTGCGCAA
656	TGCCGCACAACATGGACTTC
657	CCGCACAACATGGACTTCCG
658	GCACAACATGGACTTCCGCG
659	ACAACATGGACTTCCGCGGC
660	CAACATGGACTTCCGCGGCC
661	AACATGGACTTCCGCGGCCG
662	ACATGGACTTCCGCGGCCGC
663	CATGGACTTCCGCGGCCGCA
664	ATGGACTTCCGCGGCCGCAC
665	CACTTCAACCACCTGGGCAG
666	CCGCAAGGTGGTGAAGCAGA
667	CGCAAGGTGGTGAAGCAGAC
668	GGCTTCCCGCCCAACTTCAT
669	GCTTCCCGCCCAACTTCATC
670	CTTCCCGCCCAACTTCATCC
671	TTCCCGCCCAACTTCATCCA
672	TCCCGCCCAACTTCATCCAC
673	CCGCCCAACTTCATCCACTC

674	CCGCCCAACTTCATCCACTC
675	CTGGACTCCTCCCACATGAT
676	TGGACTCCTCCCACATGATG
677	GGACTCCTCCCACATGATGC
678	CACTGCTACAGGAAGGGCCT
679	ACTGCTACAGGAAGGGCCTG
680	CTGCTACAGGAAGGGCCTGA
681	CCGTGATGCTTGGCTGGG
682	CGTGATGCTTGGCTGGGC
683	TGCCGCACAACATGGACT
684	GCCGCACAACATGGACTT
685	CCGCACAACATGGACTTC
686	CGCACAACATGGACTTCC
687	GCACAACATGGACTTCCG
688	CACAACATGGACTTCCGC
689	ACAACATGGACTTCCGCG
690	CAACATGGACTTCCGCGG
691	AACATGGACTTCCGCGGC
692	ACATGGACTTCCGCGGCC
693	CATGGACTTCCGCGGCCG
694	ATGGACTTCCGCGGCCGC
695	TGGACTTCCGCGGCCGCA
696	GGACTTCCGCGGCCGCAC
697	CACTTCAACCACCTGGGC
698	ACTTCAACCACCTGGGCA
699	CTTCAACCACCTGGGCAG
700	CCGCAAGGTGGTGAAGCA
701	CGCAAGGTGGTGAAGCAG
702	GCAAGGTGGTGAAGCAGA
703	CAAGGTGGTGAAGCAGAC
704	AGTCTACAGGAGATGTTC
705	GGCTTCCCGCCCAACTTC
706	GCTTCCCGCCCAACTTCA
707	CTTCCCGCCCAACTTCAT
708	TTCCCGCCCAACTTCATC
709	TCCCGCCCAACTTCATCC
710	CCCGCCCAACTTCATCCA
711	CCGCCCAACTTCATCCAC
712	CGCCCAACTTCATCCACT
713	GCCCAACTTCATCCACTC
714	CTGGACTCCTCCCACATG
715	TGGACTCCTCCCACATGA
716	GGACTCCTCCCACATGAT

717	GACTCCTCCCACATGATG
718	ACTCCTCCCACATGATGC
719	CTCCTCCCACATGATGCT
720	CACTGCTACAGGAAGGGC
721	ACTGCTACAGGAAGGGCC
722	CTGCTACAGGAAGGGCCT
723	TGCTACAGGAAGGGCCTG
724	GCTACAGGAAGGGCCTGA

EQUIVALENTS

[0472] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

CLAIMS

We claim:

1. An oligonucleotide comprising a sequence that is substantially complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript.

2. The oligonucleotide of claim 1, wherein the oligonucleotide comprises a sequence that is at least 85%, at least 90%, or at least 95% complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript.

3. The oligonucleotide of claim 1, wherein the oligonucleotide comprises a sequence that is perfectly complementary to 8 to 30 contiguous nucleotides of a POLRMT RNA transcript.

4. The oligonucleotide of claim 1, wherein the 8 to 30 contiguous nucleotides is 15 to 25 contiguous nucleotides.

5. The oligonucleotide of claim 1, wherein the oligonucleotide is 8 to 30 nucleotides in length.

6. This oligonucleotide of claim 1, wherein the oligonucleotide is 18 to 22 nucleotides in length.

7. The oligonucleotide of claim 1, wherein the oligonucleotide is 20 nucleotides in length.

8. The oligonucleotide of claim 1, wherein the POLRMT RNA transcript is a human PORLMT RNA transcript.

9. The oligonucleotide of claim 8, wherein the human POLRMT RNA transcript comprises SEQ ID NO: 205.

10. The oligonucleotide of claim 1, wherein the 8 to 30 contiguous nucleotides is within or includes an exon region of the POLRMT RNA transcript.

11. The oligonucleotide of claim 10, wherein the exon comprises an exon identified in any one of Ensemble ID Nos: ENSE00000655271, ENSE00000655279, and ENSE00000655283.

12. The oligonucleotide of claim 11, wherein the oligonucleotide is complementary to 16-20 contiguous nucleotides of a sequence that corresponds to nucleotides 817-845, 2415-2446, or 2978-3008 of SEQ ID NO: 205 (*i.e.*, the nucleotide sequences represented in SEQ ID NOs: 725, 726, or 727).

13. The oligonucleotide of claim 1, wherein the 8 to 30 contiguous nucleotides comprises a sequence that corresponds to nucleotides 2420-2439, 2422-2441, 2983-3002, 2984-3003, 822-839, 823-840, 2421-2438, 2422-2439, 2423-2440, 2424-2441, 2984-3001, 2985-3002, or 2986-3003 of SEQ ID NO: 205.

14. An oligonucleotide comprising a sequence having at least 80% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

15. The oligonucleotide of claim 14, wherein the oligonucleotide comprises a sequence having at least 90% identity to a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

16. The oligonucleotide of claim 14, wherein the oligonucleotide comprises a sequence selected from a group consisting of SEQ ID NOs: 592, 594, 597, 598, 612, 613, 623, 624, 625, 626, 632, 633, and 634.

17. The oligonucleotide of claim 14, wherein the oligonucleotide comprises SEQ ID NO: 594.

18. The oligonucleotide of claim 14, wherein the oligonucleotide comprises SEQ ID NO: 612.

19. The oligonucleotide of claim 14, wherein the oligonucleotide comprises SEQ ID NO: 632.

20. An oligonucleotide comprising a sequence that is substantially complementary to a sequence selected from a group consisting of SEQ ID NOs: 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

21. The oligonucleotide of claim 20, wherein the oligonucleotide is at least 85%, at least 90%, or at least 95% complementary to a sequence selected from a group consisting of SEQ ID NOs: 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

22. The oligonucleotide of claim 20, wherein the oligonucleotide is perfectly complementary to a sequence selected from a group consisting of SEQ ID NOs: 661, 663, 666, 667, 681, 682, 692, 693, 694, 695, 701, 702, and 703.

23. The oligonucleotide of claim 22, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 663.

24. The oligonucleotide of claim 22, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 681.

25. The oligonucleotide of claim 22, wherein the oligonucleotide comprises a sequence that is complementary to SEQ ID NO: 701.

26. The oligonucleotide of claim 1, wherein the oligonucleotide is a chirally pure oligonucleotide.

27. The oligonucleotide of claim 1, wherein the oligonucleotide comprises at least one modified nucleotide.

28. The oligonucleotide of claim 27, wherein the modified nucleotide comprises a base modification, a sugar modification, a sugar phosphate modification, an internucleotidic linkage modification, or a combination thereof.

29. The oligonucleotide of claim 28, wherein the internucleotidic linkage modification comprises a phosphorothioate or phosphodithioate linkage modification.

30. The oligonucleotide of claim 28, wherein the sugar modification comprises a 2'-O-methoxyethyl (2'-MOE) modification, a 2'-Fluoro (2'-F) modification, a 2'-O-methyl (2'-O-Me) modification, an unlocked nucleic acid (UNA), or a locked nucleic acid (LNA).

31. The oligonucleotide of claim 28, wherein the sugar phosphate modification comprises a phosphorodiamidate morpholino (PMO) modification and/or a peptide nucleic acid (PNA) modification.

32. The oligonucleotide of claim 28, wherein the base modification comprises a 5'-methylcytosine modification or a G-clamp modification.

33. The oligonucleotide of claim 27, wherein each nucleotide comprises a phosphorothioate (PS) internucleotide linkage.

34. The oligonucleotide of claim 27, wherein the oligonucleotide comprises five nucleotides at the 5'-end and five nucleotides at the 3'-end of the oligonucleotide sequence which contain a 2'-MOE modification.

35. The oligonucleotide of claim 34, wherein the oligonucleotide comprises any one of SEQ ID NOs: 728-740.

36. The oligonucleotide of claim 27, wherein each nucleotide contains a 2'-MOE modification.

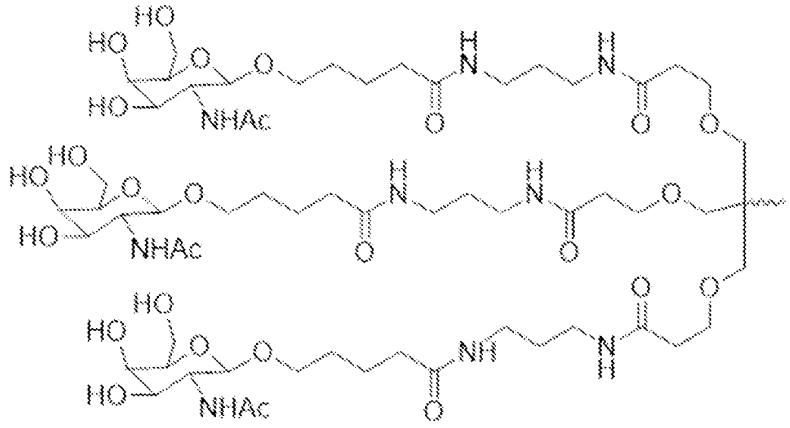
37. The oligonucleotide of claim 1, further comprising at least at least one ligand attached to the 5' end and/or the 3' end.

38. The oligonucleotide of claim 37, wherein the ligand comprises at least one lipid, peptide, and/or sugar.

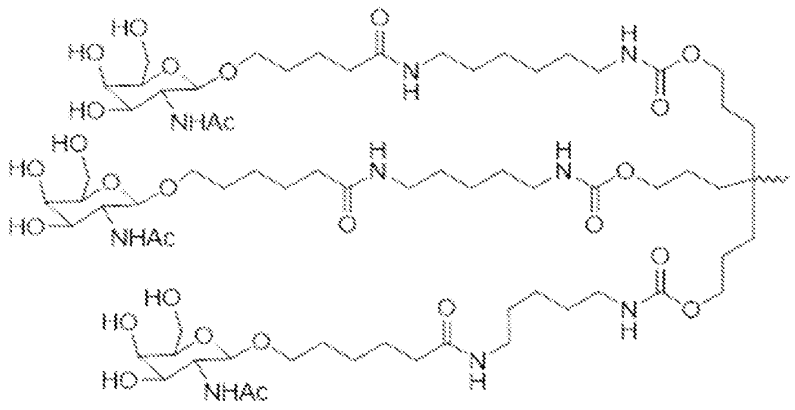
39. The oligonucleotide of claim 38, wherein the sugar comprises one or more N-acetylgalactosamine (GalNAc) moieties.

40. The oligonucleotide of claim 39, wherein the GalNAc moiety comprises a structural formula comprising:

(i) Formula I

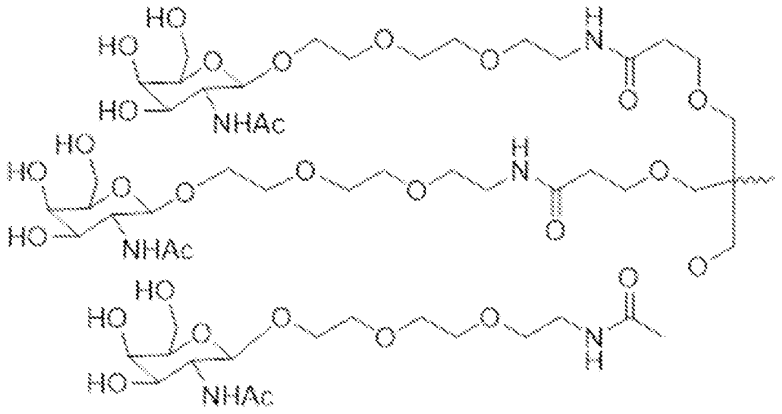


(ii) Formula II:



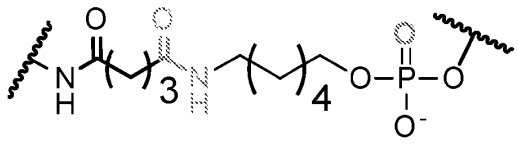
or

(iii) Formula III:



41. The oligonucleotide of claim 40, wherein the GalNAc moiety is conjugated to the oligonucleotide via a linker.

42. The oligonucleotide of claim 41, wherein the linker comprises Formula A as follows:



Formula A.

43. The oligonucleotide of claim 42, wherein a 2' deoxyadenosine phosphodiester is inserted between the oligonucleotide and the one or more GalNAc moieties.

44. The oligonucleotide of claim 1, wherein the oligonucleotide, when administered to a cell, is capable of reducing the level of POLRMT mRNA expression, POLRMT protein, and/or PORLMT activity in a cell by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% relative to a level before the administration.

45. The oligonucleotide of claim 44, wherein the cell is a human cell.