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KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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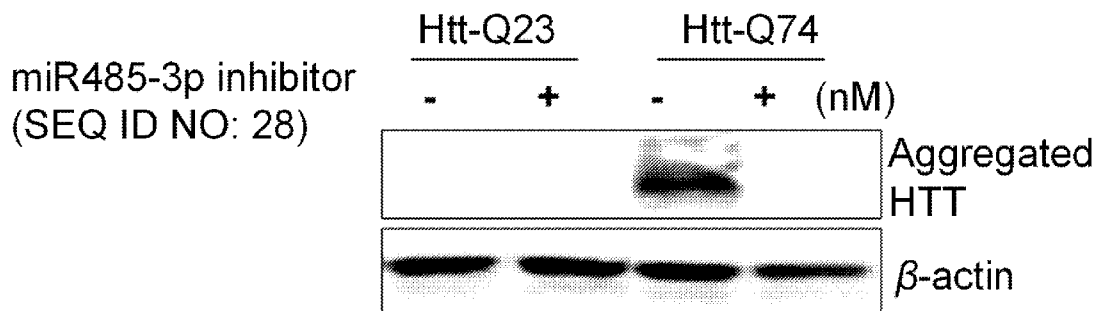
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(54) Title: MIRNA-485 INHIBITOR FOR HUNTINGTON'S DISEASE

Fig. 2B



(57) Abstract: The present disclosure includes the use of a miRNA inhibitor for treating a symptom or condition of Huntington's disease. The miRNA inhibitor useful for the present disclosure can inhibit miR-485 expression and/or activity, which in turn can modulate the level of proteins or gene expression related to Huntington's disease.

MIRNA-485 INHIBITOR FOR HUNTINGTON'S DISEASE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority benefit of U.S. Provisional Application No. 63/047,090, filed July 1, 2020, which is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY
VIA EFS-WEB

[0002] The content of the electronically submitted sequence listing in ASCII text file (Name: 4366_031PC01_Seqlisting_ST25..txt; Size: 77,432 bytes; and Date of Creation: June 29, 2021) filed with the application is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure provides the use of a miR-485 inhibitor (*e.g.*, polynucleotide encoding a nucleotide molecule comprising at least one miR-485 binding site) for the treatment of Huntington's disease.

BACKGROUND OF THE DISCLOSURE

[0004] Huntington's disease is a hereditary, progressive, neurodegenerative disorder. The disorder is caused by an expansion of a repeating CAG triplet series in the huntingtin gene, which results in a huntingtin protein with an abnormally long polyglutamine sequence. Huntington's disease usually manifests in a person's thirties or forties. In some rare cases, Huntington's may manifest in childhood or adolescence. Symptoms include involuntary jerking or twitching of the arms, legs, head, face and upper body (chorea); decline in memory, concentration, judgment, and the ability to plan and organize; and alterations in mood, especially depression, anxiety, and uncharacteristic anger and irritability. Individuals with Huntington's disease live about 15 to 20 years after symptoms begin.

[0005] There is no cure for Huntington's disease and no way to slow or stop the degeneration of brain cells associated with the disease. Treatment is therefore focused on managing

symptoms. Psychotherapy, speech therapy, physical therapy, and occupational therapy may also be used in an attempt to manage symptoms. However, individual responses to different treatment methods are unpredictable and finding an effective treatment regimen is difficult and time consuming. As such, there is a need for effective treatments for Huntington's disease.

BRIEF SUMMARY OF THE DISCLOSURE

[0006] The present disclosure provides a method of treating Huntington's disease in a subject in need thereof comprising administering to the subject a compound that inhibits miR-485 (miRNA inhibitor).

[0007] In some aspects, the subject exhibits one or more characteristics of Huntington's disease comprising irritability, depression, involuntary movements, poor coordination, trouble learning new information or making decisions, uncontrolled movements, emotional problems, and loss of thinking ability (cognition) prior to administration. In some aspects, the subject exhibits, after the administration, an improvement in one or more characteristics of Huntington's disease. In some aspects, the improvement is at least about 1.5 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, or at least about 10 fold compared to the characteristics prior to the administration.

[0008] In some aspects, the Huntington's disease is associated with a decreased level of a SIRT1 protein and/or a SIRT1 gene. In some aspects, the Huntington's disease is associated with a decreased level of a CD36 protein and/or a CD36 gene. In some aspects, the subject has a disease or a condition associated with a decreased level of a PGC-1 α protein and/or a PGC-1 α gene.

[0009] In some aspects, the miRNA inhibitor induces autophagy and/or treats or prevents inflammation.

[0010] In some aspects, the miRNA inhibitor induces neurogenesis. In some aspects, inducing neurogenesis comprises an increased proliferation, differentiation, migration, and/or survival of neural stem cells and/or progenitor cells. In some aspects, inducing neurogenesis comprises an increased number of neural stem cells and/or progenitor cells. In some aspects, inducing neurogenesis comprises an increased axon, dendrite, and/or synapse development.

[0011] In some aspects, the miRNA inhibitor induces phagocytosis.

[0012] In some aspects, the miRNA inhibitor inhibits miR485-3p. In some aspects, the miR485-3p comprises 5'-gucauacacggcucuccucucu-3' (SEQ ID NO: 1).

[0013] In some aspects, the miRNA inhibitor comprises a nucleotide sequence comprising 5'-UGUAUGA-3' (SEQ ID NO: 2) and wherein the miRNA inhibitor comprises about 7 to about 30 nucleotides in length.

[0014] In some aspects, the miRNA inhibitor increases transcription of an SIRT1 gene and/or expression of a SIRT1 protein.

[0015] In some aspects, the miRNA inhibitor comprises at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, or at least 20 nucleotides at the 5' of the nucleotide sequence.

[0016] In some aspects, the miRNA inhibitor comprises at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, or at least 20 nucleotides at the 3' of the nucleotide sequence.

[0017] In some aspects, the miRNA inhibitor has a sequence selected from the group consisting of: 5'-UGUAUGA-3' (SEQ ID NO: 2), 5'-GUGUAUGA-3' (SEQ ID NO: 3), 5'-CGUGUAUGA-3' (SEQ ID NO: 4), 5'-CCGUGUAUGA-3' (SEQ ID NO: 5), 5'-GCCGUGUAUGA-3' (SEQ ID NO: 6), 5'-AGCCGUGUAUGA-3' (SEQ ID NO: 7), 5'-GAGCCGUGUAUGA-3' (SEQ ID NO: 8), 5'-AGAGCCGUGUAUGA-3' (SEQ ID NO: 9), 5'-GAGAGCCGUGUAUGA-3' (SEQ ID NO: 10), 5'-GGAGAGCCGUGUAUGA-3' (SEQ ID NO: 11), 5'-AGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 12), 5'-GAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 13), 5'-AGAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 14), 5'-GAGAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 15); 5'-UGUAUGAC-3' (SEQ ID NO: 16), 5'-GUGUAUGAC-3' (SEQ ID NO: 17), 5'-CGUGUAUGAC-3' (SEQ ID NO: 18), 5'-CCGUGUAUGAC-3' (SEQ ID NO: 19), 5'-GCCGUGUAUGAC-3' (SEQ ID NO: 20), 5'-AGCCGUGUAUGAC-3' (SEQ ID NO: 21), 5'-GAGCCGUGUAUGAC-3' (SEQ ID NO: 22), 5'-AGAGCCGUGUAUGAC-3' (SEQ ID NO: 23), 5'-GAGAGCCGUGUAUGAC-3' (SEQ ID NO: 24), 5'-GGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 25), 5'-AGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 26), 5'-GAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 27), 5'-

AGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 28), and 5'-GAGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 29).

[0018] In some aspects, the miRNA inhibitor has a sequence selected from the group consisting of: 5'-TGTATGA-3' (SEQ ID NO: 62), 5'-GTGTATGA-3' (SEQ ID NO: 63), 5'-CGTGTATGA-3' (SEQ ID NO: 64), 5'-CCGTGTATGA-3' (SEQ ID NO: 65), 5'-GCCGTGTATGA-3' (SEQ ID NO: 66), 5'-AGCCGTGTATGA-3' (SEQ ID NO: 67), 5'-GAGCCGTGTATGA-3' (SEQ ID NO: 68), 5'-AGAGCCGTGTATGA-3' (SEQ ID NO: 69), 5'-GAGAGCCGTGTATGA-3' (SEQ ID NO: 70), 5'-GGAGAGCCGTGTATGA-3' (SEQ ID NO: 71), 5'-AGGAGAGCCGTGTATGA-3' (SEQ ID NO: 72), 5'-GAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 73), 5'-AGAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 74), 5'-GAGAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 75), 5'-TGTATGAC-3' (SEQ ID NO: 76), 5'-GTGTATGAC-3' (SEQ ID NO: 77), 5'-CGTGTATGAC-3' (SEQ ID NO: 78), 5'-CCGTGTATGAC-3' (SEQ ID NO: 79), 5'-GCCGTGTATGAC-3' (SEQ ID NO: 80), 5'-AGCCGTGTATGAC-3' (SEQ ID NO: 81), 5'-GAGCCGTGTATGAC-3' (SEQ ID NO: 82), 5'-AGAGCCGTGTATGAC-3' (SEQ ID NO: 83), 5'-GAGAGCCGTGTATGAC-3' (SEQ ID NO: 84), 5'-GGAGAGCCGTGTATGAC-3' (SEQ ID NO: 85), 5'-AGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 86), 5'-GAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 87), 5'-AGAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 88), and 5'-GAGAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 89).

[0019] In some aspects, the sequence of the miRNA inhibitor is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% sequence identity to 5'-AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'-AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).

[0020] In some aspects, the miRNA inhibitor has a sequence that has at least 90% similarity to 5'-AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'-AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).

[0021] In some aspects, the miRNA inhibitor comprises the nucleotide sequence 5'-AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'-AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88) with one substitution or two substitutions.

[0022] In some aspects, the miRNA inhibitor comprises the nucleotide sequence 5'-AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).

[0023] In some aspects, miRNA inhibitor comprises the nucleotide sequence 5'-AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28).

[0024] In some aspects, the miRNA inhibitor comprises at least one modified nucleotide.

[0025] In some aspects, the at least one modified nucleotide is a locked nucleic acid (LNA), an unlocked nucleic acid (UNA), an arabino nucleic acid (ABA), a bridged nucleic acid (BNA), and/or a peptide nucleic acid (PNA).

[0026] In some aspects, the miRNA inhibitor comprises a backbone modification.

[0027] In some aspects, the backbone modification is a phosphorodiamidate morpholino oligomer (PMO) and/or phosphorothioate (PS) modification.

[0028] In some aspects, the miRNA inhibitor is delivered in a delivery agent.

[0029] In some aspects, the delivery agent is a micelle, an exosome, a lipid nanoparticle, an extracellular vesicle, or a synthetic vesicle.

[0030] In some aspects, the miRNA inhibitor is delivered by a viral vector.

[0031] In some aspects, the viral vector is an AAV, an adenovirus, a retrovirus, or a lentivirus.

[0032] In some aspects, the viral vector is an AAV that has a serotype of AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or any combination thereof.

[0033] In some aspects, the miRNA inhibitor is delivered with a delivery agent.

[0034] In some aspects, the delivery agent comprises a lipidoid, a liposome, a lipoplex, a lipid nanoparticle, a polymeric compound, a peptide, a protein, a cell, a nanoparticle mimic, a nanotube, or a conjugate.

[0035] In some aspects, the delivery agent comprises a cationic carrier unit comprising

[WP]-L1-[CC]-L2-[AM] (formula I)

or

[WP]-L1-[AM]-L2-[CC] (formula II)

wherein

WP is a water-soluble biopolymer moiety;

CC is a positively charged carrier moiety;

AM is an adjuvant moiety; and,

L1 and L2 are independently optional linkers, and

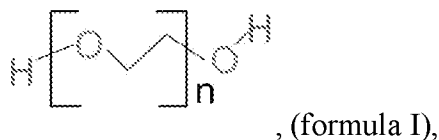
wherein when mixed with a nucleic acid at an ionic ratio of about 1:1, the cationic carrier unit forms a micelle.

[0036] In some aspects, the miRNA inhibitor interacts with the cationic carrier unit via an ionic bond.

[0037] In some aspects, the water-soluble biopolymer comprises poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyglycerol, polyphosphazene, polyoxazolines ("POZ") poly(N-acryloylmorpholine), or any combinations thereof.

[0038] In some aspects, the water-soluble biopolymer comprises polyethylene glycol ("PEG"), polyglycerol, or poly(propylene glycol) ("PPG").

[0039] In some aspects, water-soluble biopolymer comprises:



wherein n is 1-1000.

[0040] In some aspects, n is at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125, at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, or at least about 141.

[0041] In some aspects, the n is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 140 to about 150, or about 150 to about 160.

[0042] In some aspects, the water-soluble biopolymer is linear, branched, or dendritic.

[0043] In some aspects, the cationic carrier moiety comprises one or more basic amino acids.

[0044] In some aspects, the cationic carrier moiety comprises at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50 basic amino acids.

[0045] In some aspects, the cationic carrier moiety comprises about 30 to about 50 basic amino acids.

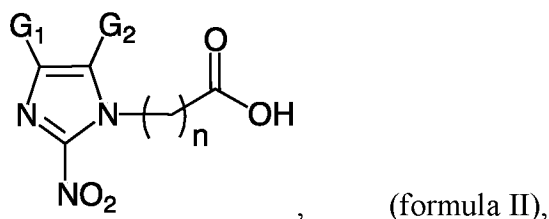
[0046] In some aspects, the basic amino acid comprises arginine, lysine, histidine, or any combination thereof.

[0047] In some aspects, the cationic carrier moiety comprises about 40 lysine monomers.

[0048] In some aspects, the adjuvant moiety is capable of modulating an immune response, an inflammatory response, and/or a tissue microenvironment.

[0049] In some aspects, the adjuvant moiety comprises an imidazole derivative, an amino acid, a vitamin, or any combination thereof.

[0050] In some aspects, the adjuvant moiety comprises:



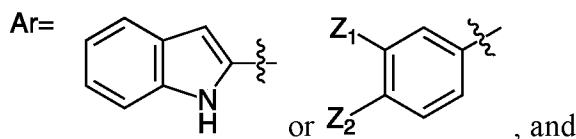
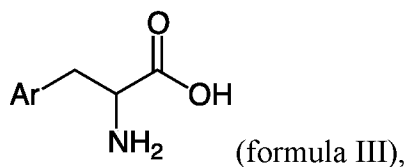
wherein each of G₁ and G₂ is H, an aromatic ring, or 1-10 alkyl, or G₁ and G₂ together form an aromatic ring, and wherein n is 1-10.

[0051] In some aspects, the adjuvant moiety comprises nitroimidazole.

[0052] In some aspects, the adjuvant moiety comprises metronidazole, tinidazole, nimorazole, dimetridazole, pretomanid, ornidazole, megazol, azanidazole, benznidazole, or any combination thereof.

[0053] In some aspects, the adjuvant moiety comprises an amino acid.

[0054] In some aspects, the adjuvant moiety comprises



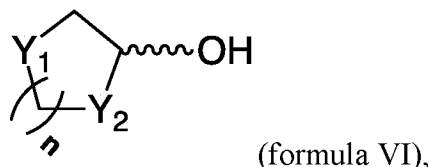
wherein Ar is

wherein each of Z₁ and Z₂ is H or OH.

[0055] In some aspects, the adjuvant moiety comprises a vitamin.

[0056] In some aspects, the vitamin comprises a cyclic ring or cyclic hetero atom ring and a carboxyl group or hydroxyl group.

[0057] In some aspects, the vitamin comprises:



wherein each of Y₁ and Y₂ is C, N, O, or S, and wherein n is 1 or 2.

[0058] In some aspects, the vitamin is selected from the group consisting of vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D2, vitamin D3, vitamin E, vitamin M, vitamin H, and any combination thereof.

[0059] In some aspects, the vitamin is vitamin B3.

[0060] In some aspects, the adjuvant moiety comprises at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, or at least about 20 vitamin B3 units.

[0061] In some aspects, the adjuvant moiety comprises about 10 vitamin B3 units.

[0062] In some aspects, the delivery agent comprises about a water-soluble biopolymer moiety with about 120 to about 130 PEG units, a cationic carrier moiety comprising a poly-lysine with about 30 to about 40 lysines, and an adjuvant moiety with about 5 to about 10 vitamin B3 units.

[0063] In some aspects, the delivery agent is associated with the miRNA inhibitor, thereby forming a micelle.

[0064] In some aspects, the association is a covalent bond, a non-covalent bond, or an ionic bond.

[0065] In some aspects, the cationic carrier unit and the miRNA inhibitor in the micelle is mixed in a solution so that the ionic ratio of the positive charges of the cationic carrier unit and the negative charges of the miRNA inhibitor is about 1:1.

[0066] In some aspects, the cationic carrier unit is capable of protecting the miRNA inhibitor from enzymatic degradation.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0067] FIG. 1 shows an exemplary architecture of a carrier unit of the present disclosure. The example presented includes a cationic carrier moiety, which can interact electrostatically with anionic payloads, *e.g.*, nucleic acids such as antisense oligonucleotides targeting a gene, *e.g.*, miRNA (antimirs). In some aspects, AM can be located between WP and CC. The CC and AM components are portrayed in a linear arrangement for simplicity. However, as described herein, in some aspects, CC and AM can be arranged in a scaffold fashion.

[0068] FIG. 2A shows a schematic overview for aggregated Htt analysis in NSC 34 cells treated with or without miR485-3p inhibitor. FIG. 2B provides western blot analysis showing a decrease in the level of insoluble, aggregated huntingtin (Htt) in Q74-Htt-transfected NSC-34 cells treated with a miR-485-3p inhibitor. NSC-34 cells were transfected with either GFP-tagged wild-type (Q23) or mutant (Q74) Htt.

[0069] FIG. 3A provides western blot analysis showing a decrease in the level of insoluble, aggregated huntingtin (Htt) in Q74-Htt-transfected HEK293T cells treated with a miR-485 inhibitor. FIG. 3B shows a graph quantifying the relative aggregated htt expression level based on the western blot of FIG. 3A.

[0070] FIG. 4A provides western blot analysis showing an increase in the level of the autophagy proteins SIRT1, PGC-1a, p62 and LC3-II in Q74-Htt-transfected HEK293T cells treated with a miR-485 inhibitor compared to Q-23-Htt-transfected HEK293T cells. FIGs. 4B-4E shows a graph quantifying the relative levels of SIRT1, PGC-1a, p62 and LC3-II as determined from the western blot of FIG. 4A, respectively.

[0071] FIG. 5 provides western blot analysis showing a decrease in the level of insoluble, aggregated huntingtin (Htt) in Q74-Htt-transfected PC12 cells treated with a miR-485 inhibitor compared to Q23-Htt-transfected PC12 cells.

[0072] FIG. 6 provides western blot analysis showing an increase in the level of the autophagy proteins SIRT1, PGC-1a, p62 and LC3-II and decrease in cleavage of caspase 3 in Q74-Htt-transfected PC12 cells treated with a miR-485-3p inhibitor compared to Q23-Htt-transfected PC12 cells.

[0073] FIGs. 7A-7E show images visualizing distribution of GFP-tagged-htt in Q74-Htt-transfected PC12 cells (FIGs. 7D and 7E) and GFP-tagged Htt in Q23-Htt-transfected PC12 cells (FIGs. 7B and 7C) treated with a miR-485 inhibitor and control cells (FIG. 7A).

[0074] FIGs. 8A-8D show images visualizing distribution of GFP-tagged-htt in Q74-Htt-transfected (FIGs. 8C and 8D) and Q23-Htt-transfected (FIGs. 8A and 8B) primary cortical neurons treated with a miR-485 inhibitor. The left panels show control treatment and the right panels show miR485-3p inhibitor treatments.

[0075] FIGs. 9A-9R show miR485-3p enhances degradation of Htt aggregates by regulation of autophagy. Immunofluorescence labeling of Htt protein (left panels), LC3B (middle panels), and DAPI (right panels) in the Q23 (FIGs. 9A-9I) or Q74 (FIGs. 9J-9R) transfected PC12 cells, respectively (Scale bars, 20 μ m), with control (top row), 100nM (middle row), or 300nM (bottom row) miR485-3p transfections. White arrowheads show the colocalization of Htt and LC3.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0076] Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to the particular compositions or process steps described, as such can, of course, vary. As will be apparent to those of skill in the art upon reading this disclosure, each of the individual aspects described and illustrated herein has discrete components and features which can be readily separated from or combined with the features of any of the other several aspects without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0077] The headings provided herein are not limitations of the various aspects of the disclosure, which can be defined by reference to the specification as a whole. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

I. Terms

[0078] In order that the present disclosure can be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0079] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. It is further noted that the claims can be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a negative limitation.

[0080] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0081] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0082] Unless defined otherwise, 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 disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0083] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Where a range of values is recited, it is to be understood that each intervening integer value, and each fraction thereof, between the recited upper and lower limits of that range is also specifically disclosed,

along with each subrange between such values. The upper and lower limits of any range can independently be included in or excluded from the range, and each range where either, neither or both limits are included is also encompassed within the disclosure. Thus, ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 10 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

[0084] Where a value is explicitly recited, it is to be understood that values which are about the same quantity or amount as the recited value are also within the scope of the disclosure. Where a combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is within the scope of the disclosure. Conversely, where different elements or groups of elements are individually disclosed, combinations thereof are also disclosed. Where any element of a disclosure is disclosed as having a plurality of alternatives, examples of that disclosure in which each alternative is excluded singly or in any combination with the other alternatives are also hereby disclosed; more than one element of a disclosure can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

[0085] Nucleotides are referred to by their commonly accepted single-letter codes. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Nucleotides are referred to herein by their commonly known one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Accordingly, 'a' represents adenine, 'c' represents cytosine, 'g' represents guanine, 't' represents thymine, and 'u' represents uracil.

[0086] Amino acid sequences are written left to right in amino to carboxy orientation. Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0087] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.*, 10 percent, up or down (higher or lower).

[0088] As used herein, the term "adeno-associated virus" (AAV), includes but is not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, AAVrh.74, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV,

ovine AAV, goat AAV, shrimp AAV, those AAV serotypes and clades disclosed by Gao et al. (*J. Virol.* 78:6381 (2004)) and Moris et al. (*Virology* 33:375 (2004)), and any other AAV now known or later discovered. See, e.g., FIELDS et al. *VIROLOGY*, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). In some aspects, an "AAV" includes a derivative of a known AAV. In some aspects, an "AAV" includes a modified or an artificial AAV.

[0089] The terms "administration," "administering," and grammatical variants thereof refer to introducing a composition, such as a miRNA inhibitor of the present disclosure, into a subject via a pharmaceutically acceptable route. The introduction of a composition, such as a micelle comprising a miRNA inhibitor of the present disclosure, into a subject is by any suitable route, including intratumorally, orally, pulmonarily, intranasally, parenterally (intravenously, intra-arterially, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, intrathecally, periocularly or topically. Administration includes self-administration and the administration by another. A suitable route of administration allows the composition or the agent to perform its intended function. For example, if a suitable route is intravenous, the composition is administered by introducing the composition or agent into a vein of the subject.

[0090] As used herein, the term "approximately," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain aspects, the term "approximately" refers to a range of values that fall within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0091] As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, that are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[0092] In some aspects, two or more sequences are said to be "completely conserved" or "identical" if they are 100% identical to one another. In some aspects, two or more sequences are said to be "highly conserved" if they are at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some aspects, two or more sequences are said to be "highly conserved" if they are about 70% identical, about 80% identical, about 90% identical, about 95%, about 98%, or about 99% identical to one another. In some aspects, two or more

sequences are said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some aspects, two or more sequences are said to be "conserved" if they are about 30% identical, about 40% identical, about 50% identical, about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. Conservation of sequence can apply to the entire length of a polynucleotide or polypeptide or can apply to a portion, region or feature thereof.

[0093] The term "derived from," as used herein, refers to a component that is isolated from or made using a specified molecule or organism, or information (*e.g.*, amino acid or nucleic acid sequence) from the specified molecule or organism. For example, a nucleic acid sequence that is derived from a second nucleic acid sequence can include a nucleotide sequence that is identical or substantially similar to the nucleotide sequence of the second nucleic acid sequence. In the case of nucleotides or polypeptides, the derived species can be obtained by, for example, naturally occurring mutagenesis, artificial directed mutagenesis or artificial random mutagenesis. The mutagenesis used to derive nucleotides or polypeptides can be intentionally directed or intentionally random, or a mixture of each. The mutagenesis of a nucleotide or polypeptide to create a different nucleotide or polypeptide derived from the first can be a random event (*e.g.*, caused by polymerase infidelity) and the identification of the derived nucleotide or polypeptide can be made by appropriate screening methods, *e.g.*, as discussed herein. In some aspects, a nucleotide or amino acid sequence that is derived from a second nucleotide or amino acid sequence has a sequence identity of at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% to the second nucleotide or amino acid sequence,

respectively, wherein the first nucleotide or amino acid sequence retains the biological activity of the second nucleotide or amino acid sequence.

[0094] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide.

[0095] The terms "complementary" and "complementarity" refer to two or more oligomers (*i.e.*, each comprising a nucleobase sequence), or between an oligomer and a target gene, that are related with one another by Watson-Crick base-pairing rules. For example, the nucleobase sequence "T-G-A (5'→3')," is complementary to the nucleobase sequence "A-C-T (3'→ 5')." Complementarity can be "partial," in which less than all of the nucleobases of a given nucleobase sequence are matched to the other nucleobase sequence according to base pairing rules. For example, in some aspects, complementarity between a given nucleobase sequence and the other nucleobase sequence can be about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. Accordingly, in certain aspects, the term "complementary" refers to at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% match or complementarity to a target nucleic acid sequence (*e.g.*, miR-485 nucleic acid sequence). Or, there can be "complete" or "perfect" (100%) complementarity between a given nucleobase sequence and the other nucleobase sequence to continue the example. In some aspects, the degree of complementarity between nucleobase sequences has significant effects on the efficiency and strength of hybridization between the sequences.

[0096] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In certain aspects, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0097] The terms "excipient" and "carrier" are used interchangeably and refer to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound, *e.g.*, a miRNA inhibitor of the present disclosure.

[0098] The term "expression," as used herein, refers to a process by which a polynucleotide produces a gene product, *e.g.*, RNA or a polypeptide. It includes without limitation transcription of the polynucleotide into micro RNA binding site, small hairpin RNA (shRNA), small interfering RNA (siRNA), or any other RNA product. It includes, without limitation, transcription of the polynucleotide into messenger RNA (mRNA), and the translation of mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be, *e.g.*, a nucleic acid, such as an RNA produced by transcription of a gene. As used herein, a gene product can be either a nucleic acid, RNA or miRNA produced by the transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, phosphorylation, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0099] As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules. Generally, the term "homology" implies an evolutionary relationship between two molecules. Thus, two molecules that are homologous will have a common evolutionary ancestor. In the context of the present disclosure, the term homology encompasses both to identity and similarity.

[0100] In some aspects, polymeric molecules are considered to be "homologous" to one another if at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% of the monomers in the molecule are identical (exactly the same monomer) or are similar (conservative substitutions). The term "homologous" necessarily refers to a comparison between at least two sequences (*e.g.*, polynucleotide sequences).

[0101] In the context of the present disclosure, substitutions (even when they are referred to as amino acid substitution) are conducted at the nucleic acid level, *i.e.*, substituting an amino acid residue with an alternative amino acid residue is conducted by substituting the codon encoding the first amino acid with a codon encoding the second amino acid.

[0102] As used herein, the term "identity" refers to the overall monomer conservation between polymeric molecules, *e.g.*, between polynucleotide molecules. The term "identical" without any additional qualifiers, *e.g.*, polynucleotide A is identical to polynucleotide B, implies the polynucleotide sequences are 100% identical (100% sequence identity). Describing two sequences as, *e.g.*, "70% identical," is equivalent to describing them as having, *e.g.*, "70% sequence identity."

[0103] Calculation of the percent identity of two polypeptide or polynucleotide 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 polypeptide or polynucleotide sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain aspects, 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 100% of the length of the reference sequence. The amino acids at corresponding amino acid positions, or bases in the case of polynucleotides, are then compared.

[0104] When a position in the first sequence is occupied by the same amino acid or nucleotide 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.

[0105] Suitable software programs that can be used to align different sequences (*e.g.*, polynucleotide sequences) are available from various sources. One suitable program to determine percent sequence identity is *bl2seq*, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). *Bl2seq* performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.*, Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at www.ebi.ac.uk/Tools/psa.

[0106] Sequence alignments can be conducted using methods known in the art such as MAFFT, Clustal (ClustalW, Clustal X or Clustal Omega), MUSCLE, etc.

[0107] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0108] In certain aspects, the percentage identity (%ID) or of a first amino acid sequence (or nucleic acid sequence) to a second amino acid sequence (or nucleic acid sequence) is calculated as $\%ID = 100 \times (Y/Z)$, where Y is the number of amino acid residues (or nucleobases) scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[0109] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (*e.g.*, crystallographic protein structures), functional data (*e.g.*, location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.*, from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

[0110] As used herein, the terms "isolated," "purified," "extracted," and grammatical variants thereof are used interchangeably and refer to the state of a preparation of desired composition of the present disclosure, *e.g.*, a miRNA inhibitor of the present disclosure, that has undergone one or more processes of purification. In some aspects, isolating or purifying as used herein is the process of removing, partially removing (*e.g.*, a fraction) of a composition of the present disclosure, *e.g.*, a miRNA inhibitor of the present disclosure from a sample containing contaminants.

[0111] In some aspects, an isolated composition has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or

amount. In other aspects, an isolated composition has an amount and/or concentration of desired composition of the present disclosure, at or above an acceptable amount and/or concentration and/or activity. In other aspects, the isolated composition is enriched as compared to the starting material from which the composition is obtained. This enrichment can be by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, at least about 99.99%, at least about 99.999%, at least about 99.9999%, or greater than 99.9999% as compared to the starting material.

[0112] In some aspects, isolated preparations are substantially free of residual biological products. In some aspects, the isolated preparations are 100% free, at least about 99% free, at least about 98% free, at least about 97% free, at least about 96% free, at least about 95% free, at least about 94% free, at least about 93% free, at least about 92% free, at least about 91% free, or at least about 90% free of any contaminating biological matter. Residual biological products can include abiotic materials (including chemicals) or unwanted nucleic acids, proteins, lipids, or metabolites.

[0113] The term "linked" as used herein refers to a first amino acid sequence or polynucleotide sequence covalently or non-covalently joined to a second amino acid sequence or polynucleotide sequence, respectively. The first amino acid or polynucleotide sequence can be directly joined or juxtaposed to the second amino acid or polynucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first polynucleotide sequence to a second polynucleotide sequence at the 5'-end or the 3'-end, but also includes insertion of the whole first polynucleotide sequence (or the second polynucleotide sequence) into any two nucleotides in the second polynucleotide sequence (or the first polynucleotide sequence, respectively). The first polynucleotide sequence can be linked to a second polynucleotide sequence by a phosphodiester bond or a linker. The linker can be, *e.g.*, a polynucleotide.

[0114] A "miRNA inhibitor," as used herein, refers to a compound that can decrease, alter, and/or modulate miRNA expression, function, and/or activity. The miRNA inhibitor can be a polynucleotide sequence that is at least partially complementary to the target miRNA nucleic acid sequence, such that the miRNA inhibitor hybridizes to the target miRNA sequence. For instance, in some aspects, a miR-485 inhibitor of the present disclosure comprises a nucleotide sequence encoding a nucleotide molecule that is at least partially complementary to the target miR-485

nucleic acid sequence, such that the miR-485 inhibitor hybridizes to the miR-485 sequence. In further aspects, the hybridization of the miR-485 to the miR-485 sequence decreases, alters, and/or modulates the expression, function, and/or activity of miR-485 (*e.g.*, hybridization results in an increase in the expression of SIRT1 protein and/or SIRT1 gene).

[0115] The terms "miRNA," "miR," and "microRNA" are used interchangeably and refer to a microRNA molecule found in eukaryotes that is involved in RNA-based gene regulation. The term will be used to refer to the single-stranded RNA molecule processed from a precursor. In some aspects, the term "antisense oligomers" can also be used to describe the microRNA molecules of the present disclosure. Names of miRNAs and their sequences related to the present disclosure are provided herein. MicroRNAs recognize and bind to target mRNAs through imperfect base pairing leading to destabilization or translational inhibition of the target mRNA and thereby downregulate target gene expression. Conversely, targeting miRNAs via molecules comprising a miRNA binding site (generally a molecule comprising a sequence complementary to the seed region of the miRNA) can reduce or inhibit the miRNA-induced translational inhibition leading to an upregulation of the target gene.

[0116] The terms "mismatch" or "mismatches" refer to one or more nucleobases (whether contiguous or separate) in an oligomer nucleobase sequence (*e.g.*, miR-485 inhibitor) that are not matched to a target nucleic acid sequence (*e.g.*, miR-485) according to base pairing rules. While perfect complementarity is often desired, in some aspects, one or more (*e.g.*, 6, 5, 4, 3, 2, or 1 mismatches) can occur with respect to the target nucleic acid sequence. Variations at any location within the oligomer are included. In certain aspects, antisense oligomers of the disclosure (*e.g.*, miR-485 inhibitor) include variations in nucleobase sequence near the termini, variations in the interior, and if present are typically within about 6, 5, 4, 3, 2, or 1 subunits of the 5' and/or 3' terminus. In some aspects, one, two, or three nucleobases can be removed and still provide on-target binding.

[0117] As used herein, the terms "modulate," "modify," and grammatical variants thereof, generally refer when applied to a specific concentration, level, expression, function or behavior, to the ability to alter, by increasing or decreasing, *e.g.*, directly or indirectly promoting/stimulating/up-regulating or interfering with/inhibiting/down-regulating the specific concentration, level, expression, function or behavior, such as, *e.g.*, to act as an antagonist or agonist. In some instances, a modulator can increase and/or decrease a certain concentration, level, activity or function relative to a control, or relative to the average level of activity that would

generally be expected or relative to a control level of activity. In some aspects, a miRNA inhibitor disclosed herein, *e.g.*, a miR-485 inhibitor, can modulate (*e.g.*, decrease, alter, or abolish) miR-485 expression, function, and/or activity, and thereby, modulate SIRT1 protein or gene expression and/or activity.

[0118] "Nucleic acid," "nucleic acid molecule," "nucleotide sequence," "polynucleotide," and grammatical variants thereof are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the disclosure comprises one or more nucleic acids as described herein.

[0119] The terms "pharmaceutically acceptable carrier," "pharmaceutically acceptable excipient," and grammatical variations thereof, encompass any of the agents approved by a regulatory agency of the U.S. Federal government or listed in the U.S. Pharmacopeia for use in animals, including humans, as well as any carrier or diluent that does not cause the production of undesirable physiological effects to a degree that prohibits administration of the composition to a subject and does not abrogate the biological activity and properties of the administered compound. Included are excipients and carriers that are useful in preparing a pharmaceutical composition and are generally safe, non-toxic, and desirable.

[0120] As used herein, the term "pharmaceutical composition" refers to one or more of the compounds described herein, such as, *e.g.*, a miRNA inhibitor of the present disclosure, mixed or intermingled with, or suspended in one or more other chemical components, such as pharmaceutically acceptable carriers and excipients. One purpose of a pharmaceutical composition is to facilitate administration of preparations comprising a miRNA inhibitor of the present disclosure to a subject.

[0121] The term "polynucleotide," as used herein, refers to polymers of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof.

[0122] In some aspects, the term refers to the primary structure of the molecule. Thus, the term includes triple-, double- and single-stranded deoxyribonucleic acid ("DNA"), as well as triple-, double- and single-stranded ribonucleic acid ("RNA"). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the polynucleotide.

[0123] In some aspects, the term "polynucleotide" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, shRNA, siRNA, miRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

[0124] In some aspects of the present disclosure, a polynucleotide can be, *e.g.*, an oligonucleotide, such as an antisense oligonucleotide. In some aspects, the oligonucleotide is an RNA. In some aspects, the RNA is a synthetic RNA. In some aspects, the synthetic RNA comprises at least one unnatural nucleobase. In some aspects, all nucleobases of a certain class have been replaced with unnatural nucleobases (*e.g.*, all uridines in a polynucleotide disclosed herein can be replaced with an unnatural nucleobase, *e.g.*, 5-methoxyuridine).

[0125] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length, *e.g.*, that are encoded by the SIRT1 gene. The polymer can comprise modified amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for

example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids such as homocysteine, ornithine, p-acetylphenylalanine, D-amino acids, and creatine), as well as other modifications known in the art. The term "polypeptide," as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function.

[0126] Polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing.

[0127] A polypeptide can be a single polypeptide or can be a multi-molecular complex such as a dimer, trimer or tetramer. They can also comprise single chain or multichain polypeptides. Most commonly disulfide linkages are found in multichain polypeptides. The term polypeptide can also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid. In some aspects, a "peptide" can be less than or equal to about 50 amino acids long, *e.g.*, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 amino acids long.

[0128] The terms "prevent," "preventing," and variants thereof as used herein, refer partially or completely delaying onset of an disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular disease, disorder, and/or condition; partially or completely delaying progression from a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some aspects, preventing an outcome is achieved through prophylactic treatment.

[0129] As used herein, the terms "promoter" and "promoter sequence" are interchangeable and refer to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters can be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters can direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters." Promoters that cause a gene to be expressed in a specific cell type are commonly referred to as "cell-specific promoters" or "tissue-

specific promoters." Promoters that cause a gene to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters" or "cell differentiation-specific promoters." Promoters that are induced and cause a gene to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as "inducible promoters" or "regulatable promoters." It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths can have identical promoter activity.

[0130] The promoter sequence is typically bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. In some aspects, a promoter that can be used with the present disclosure includes a tissue specific promoter.

[0131] As used herein, "prophylactic" refers to a therapeutic or course of action used to prevent the onset of a disease or condition, or to prevent or delay a symptom associated with a disease or condition.

[0132] As used herein, a "prophylaxis" refers to a measure taken to maintain health and prevent the onset of a disease or condition, or to prevent or delay a symptom associated with a disease or condition.

[0133] As used herein, the term "gene regulatory region" or "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions can include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites, or stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0134] In some aspects, a miR-485 inhibitor disclosed herein (*e.g.*, a polynucleotide encoding a RNA comprising one or more miR-485 binding site) can include a promoter and/or other expression (*e.g.*, transcription) control elements operably associated with one or more coding

regions. In an operable association a coding region for a gene product is associated with one or more regulatory regions in such a way as to place expression of the gene product under the influence or control of the regulatory region(s). For example, a coding region and a promoter are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the gene product encoded by the coding region, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Other expression control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can also be operably associated with a coding region to direct gene product expression.

[0135] As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules (*e.g.* miRNA molecules). Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art. It is understood that percentage of similarity is contingent on the comparison scale used, *i.e.*, whether the nucleic acids are compared, *e.g.*, according to their evolutionary proximity, charge, volume, flexibility, polarity, hydrophobicity, aromaticity, isoelectric point, antigenicity, or combinations thereof.

[0136] The terms "subject," "patient," "individual," and "host," and variants thereof are used interchangeably herein and refer to any mammalian subject, including without limitation, humans, domestic animals (*e.g.*, dogs, cats and the like), farm animals (*e.g.*, cows, sheep, pigs, horses and the like), and laboratory animals (*e.g.*, monkey, rats, mice, rabbits, guinea pigs and the like) for whom diagnosis, treatment, or therapy is desired, particularly humans. The methods described herein are applicable to both human therapy and veterinary applications.

[0137] As used herein, the phrase "subject in need thereof" includes subjects, such as mammalian subjects, that would benefit from administration of a miRNA inhibitor of the disclosure (*e.g.*, miR-485 inhibitor), *e.g.*, to increase the expression level of SIRT1 protein and/or SIRT1 gene.

[0138] As used herein, the term "therapeutically effective amount" is the amount of reagent or pharmaceutical compound comprising a miRNA inhibitor of the present disclosure that is sufficient to produce a desired therapeutic effect, pharmacologic and/or physiologic effect on a

subject in need thereof. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

[0139] The terms "treat," "treatment," or "treating," as used herein refers to, *e.g.*, the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration or elimination of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition. The term also includes prophylaxis or prevention of a disease or condition or its symptoms thereof.

[0140] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence.

[0141] A "vector" refers to any vehicle for the cloning of and/or transfer of a nucleic acid into a host cell. A vector can be a replicon to which another nucleic acid segment can be attached so as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of replication *in vivo*, *i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors are known and used in the art including, for example, plasmids, modified eukaryotic viruses, or modified bacterial viruses. Insertion of a polynucleotide into a suitable vector can be accomplished by ligating the appropriate polynucleotide fragments into a chosen vector that has complementary cohesive termini.

[0142] Vectors can be engineered to encode selectable markers or reporters that provide for the selection or identification of cells that have incorporated the vector. Expression of selectable markers or reporters allows identification and/or selection of host cells that incorporate and express other coding regions contained on the vector. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like. Examples of reporters known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), β -galactosidase (LacZ), β -glucuronidase (Gus), and the like. Selectable markers can also be considered to be reporters.

II. Methods of Use

Treating Huntington's Disease

[0143] In some aspects, the present disclosure provides a method of treating Huntington's disease in a subject in need thereof by administering to the subject a compound that inhibits miR-485 (miRNA inhibitor). In some aspects, the subject exhibits one or more characteristics of irritability, depression, involuntary movements, poor coordination, trouble learning new information or making decisions, uncontrolled movements, emotional problems, and loss of thinking ability (cognition) before administration. In some aspects, the subject exhibits, after the administration, an improvement in one or more characteristics of Huntington's disease. In some aspects, the improvement is at least about 1.5 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, or at least about 10 fold compared to the characteristics prior to the administration.

SIRT1 Regulation

[0144] In some aspects, the Huntington's disease is associated with a decreased level of a SIRT1 protein and/or a SIRT1 gene. In some aspects, the miR-485 inhibitor increases expression of a SIRT1 protein and/or a SIRT1 gene in the subject.

[0145] Sirtuin 1 (SIRT1), also known as NAD-dependent deacetylase sirtuin-1, is a protein that in humans is encoded by the *SIRT1* gene. The *SIRT1* gene is located on chromosome 10 in humans (nucleotides 67,884,656 to 67,918,390 of GenBank Accession Number NC_000010.11, plus strand orientation). Synonyms of the *SIRT1* gene, and the encoded protein thereof, are known and include "regulatory protein SIR2 homolog 1," "silent mating-type information regulation 2 homolog 1," "SIR2," "SIR2-Like Protein 1," "SIR2L1," "SIR2alpha," "Sirtuin Type 1," "hSIRT1," or "hSIR2."

[0146] There are at least two known isoforms of human SIRT1 protein, resulting from alternative splicing. SIRT1 isoform 1 (UniProt identifier: Q96EB6-1) consists of 747 amino acids and has been chosen as the canonical sequence (SEQ ID NO: 31). SIRT1 isoform 2 (also known as "delta-exon8") (UniProt identifier: Q96EB6-2) consists of 561 amino acids and differs from the canonical sequence as follows: 454-639: missing (SEQ ID NO: 32). Table 1 below provides the sequences for the two SIRT1 isoforms.

Table 1. SIRT1 Protein Isoforms

Isoform 1 (UniProt: Q96EB6-1) (SEQ ID NO: 31)	MADEAALALQPGGSPSAAGADREAASSPAGEPLRKRPRRDGPGLERSPGEPGGAAPEREV PAAARGCPGAAAAALWREAEAEAAAAGGEQEAQATAAAGEGDNGPGLQGSPREPPLADNL YDEDDDDGEEEEEEEEAAAAAIGYRDNLLFGDEIITNGFHSCESEDEDRASHASSSDWTPRP RIGPYTFVQQHLMIGTDPRTILKDLLPETIPPELDDMTLWQIVINILSEPPKRRKKRDI NTIEDAVKLLQECKKIIVLTGAGVSVSCGIPDFRSRDGIYARLAVDFPDLDPQAMFDIE YFRKDP RPFFKFAKEIYPGQFQPSLCHKFIALS DKEGKLLRNYTQNIDTLEQVAGIQRII QCHGSFATASCLICKYKVDCEAVRGDIFNQVVP RCP RCPADEPLAIMKPEIVFFGENLPE QFHRAMKYDKDEVDLLIVIGSSLKVRPVALIPSSIPHEVPQILINREPLPHLHFDVELLG DCDVINELCHRLGGEYAKLCCNPVKLSEITEKPPRTQKELAYLSELPTPLHVSSEDSSS PERTSPPDSSVIVTLLDQAAKSNDDLDVSESKGCMEEKPQEVQTSRNVESIAEQMENPDL KNVGSSTGEKNERTSVAGTVRKCPNVRVAKEQISRRLDGNQYLFLPPNRYIFHGAEVYSD SEDDLSSSSCGSNSDSGTCQSPSLEEPMEDESEIEEFYNGLEDEPDVPERAGGAGFGTD GDDQEAINAEISVKQEVTD MNYP SNKS
Isoform 2 (UniProt: Q96EB6-2) (SEQ ID NO: 32)	MADEAALALQPGGSPSAAGADREAASSPAGEPLRKRPRRDGPGLERSPGEPGGAAPEREV PAAARGCPGAAAAALWREAEAEAAAAGGEQEAQATAAAGEGDNGPGLQGSPREPPLADNL YDEDDDDGEEEEEEEEAAAAAIGYRDNLLFGDEIITNGFHSCESEDEDRASHASSSDWTPRP RIGPYTFVQQHLMIGTDPRTILKDLLPETIPPELDDMTLWQIVINILSEPPKRRKKRDI NTIEDAVKLLQECKKIIVLTGAGVSVSCGIPDFRSRDGIYARLAVDFPDLDPQAMFDIE YFRKDP RPFFKFAKEIYPGQFQPSLCHKFIALS DKEGKLLRNYTQNIDTLEQVAGIQRII QCHGSFATASCLICKYKVDCEAVRGDIFNQVVP RCP RCPADEPLAIMKPEIVFFGENLPE QFHRAMKYDKDEVDLLIVIGSSLKVRPVALIPSNQYLFLPPNRYIFHGAEVYSDSEDDVL SSSSCGSNSDSGTCQSPSLEEPMEDESEIEEFYNGLEDEPDVPERAGGAGFGTDGDDQEA INAEISVKQEVTD MNYP SNKS

[0147] As used herein, the term "SIRT1" includes any variants or isoforms of SIRT1 which are naturally expressed by cells. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of SIRT1 isoform 1. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of SIRT1 isoform 2. In further aspects, a miR-485 inhibitor disclosed herein can increase the expression of both SIRT1 isoform 1 and isoform 2. Unless indicated otherwise, both isoform 1 and isoform 2 are collectively referred to herein as "SIRT1."

[0148] In some aspects, a miR-485 inhibitor of the present disclosure increases the expression of SIRT1 protein and/or SIRT1 gene by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, or at least about 300% compared to a reference (*e.g.*, expression of SIRT1 protein and/or SIRT1 gene in a corresponding subject that did not receive an administration of the miR-485 inhibitor).

[0149] Not to be bound by any one theory, in some aspects, a miR-485 inhibitor disclosed herein increases the expression of SIRT1 protein and/or SIRT1 gene by reducing the expression

and/or activity of miR-485, e.g., miR-485-3p. In some aspects, a miR-485 inhibitor of the present disclosure can reduce the expression and/or activity of miR-485-3p.

[0150] In some aspects, a miR-485 inhibitor disclosed herein decreases the expression and/or activity of miR-485-3p by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100% compared to a reference (*e.g.*, miR-485-3p expression in a corresponding subject that did not receive an administration of the miR-485 inhibitor). In certain aspects, a miR-485 inhibitor disclosed herein decreases the expression and/or activity of miR-485-5p by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100% compared to a reference (*e.g.*, miR-485-5p expression in a corresponding subject that did not receive an administration of the miR-485 inhibitor). In further aspects, a miR-485 inhibitor disclosed herein decreases the expression and/or activity of both miR-485-3p and miR-485-5p by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100% compared to a reference (*e.g.*, miR-485-3p and miR-485-5p expression in a corresponding subject that did not receive an administration of the miR-485 inhibitor). In some aspects, the expression of miR-485-3p and/or miR-485-5p is completely inhibited after the administration of the miR-485 inhibitor.

[0151] As described herein, a miR-485 inhibitor of the present disclosure can increase the expression of SIRT1 protein and/or SIRT1 gene when administered to a subject. Accordingly, in some aspects, the present disclosure provides a method of treating Huntingtont's disease associated with an abnormal (*e.g.*, reduced) level of a SIRT1 protein and/or SIRT1 gene in a subject in need thereof.

CD36 Regulation

[0152] In some aspects, the Huntington's disease is associated with a decreased level of a CD36 protein and/or a CD36 gene. In some aspects, the miR-485 inhibitor increases expression of a CD36 protein and/or a CD36 gene in a subject in need thereof.

[0153] Cluster determinant 36 (CD36) is also known as platelet glycoprotein 4, is a protein that in humans is encoded by the *CD36* gene. The *CD36* gene is located on chromosome 7 (nucleotides 80,602,656 to 80,679,277 of GenBank Accession Number NC_000007.14, plus strand orientation). Synonyms of the *CD36* gene, and the encoded protein thereof, are known and include

"platelet glycoprotein IV," "fatty acid translocase," "scavenger receptor class B member 3," "glycoprotein 88," "glycoprotein IIb," "glycoprotein IV," "thrombospondin receptor," "GPIIIB," "PAS IV," "GP3B," "GPIV," "FAT," "GP4," "BDPLT10," "SCARB3," "CHDS7," "PASIV," or "PAS-4."

[0154] There are at least four known isoform of human CD36 protein, resulting from alternative splicing. CD36 isoform 1 (UniProt identifier: P16671-1) consists of 472 amino acids and has been chosen as the canonical sequence (SEQ ID NO: 36). CD36 isoform 2 (also known as "ex8-del") (UniProt identifier: P16671-2) consists of 288 amino acids and differs from the canonical sequence as follows: 274-288: SIYAVFESDVNLKGI → ETCVHFTSSFSVCKS; and 289-472: missing (SEQ ID NO: 37). CD36 Isoform 3 (also known as "ex6-7-del") (UniProt identifier: P16671-3) consists of 433 amino acids and differs from the canonical sequence as follows: 234-272: missing (SEQ ID NO: 38). CD36 isoform 4 (also known as "ex4-del" (UniProt identifier: P16671-4) consists of 412 amino acids and differs from the canonical sequence as follows: 144-203: missing (SEQ ID NO: 39). Table 2 below provides the sequences for the four CD36 isoforms.

Table 2. CD36 Protein Isoforms

Isoform 1 (UniProt:P16671-1) (SEQ ID NO: 36)	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDLLIQKTIKKQVVLEEGTIAFKNWVKTGTE VYRQFWIFDVQNPQEVMNSSNIQVKQRGPYTYRVRFLAKENVTDQEDNTVSFLQPNGA IFEPSLSVGTEADNFTVLNLAVAAAASHIYQNQFVQMILNSLINKSKSSMFQVRTLRELLW GYRDPFLSLVPYPVTTTGVGLFYPYNNADGVYKVFNGKDNISKVAIIDTYKGKRNLSEYWE SHCDMINGTDAASFPPFVEKSQVLQFFSSDICRSIYAVFESDVNLKGI PVYRFVLPSKAF ASPVENPDNYCFCTEKIISKNTSYGVLDISKCKEGRPVYISLPHFLYASPDVSEPIDGL NPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSEKIQVLKLNKRNIVPILWLN ETG TIGDEKANMFRSQVTGKINLLGLIEMILLSVGVMFVAFMISYCACRSKTIK
Isoform 2 (UniProt:P16671-2) (SEQ ID NO: 37)	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDLLIQKTIKKQVVLEEGTIAFKNWVKTGTE VYRQFWIFDVQNPQEVMNSSNIQVKQRGPYTYRVRFLAKENVTDQEDNTVSFLQPNGA IFEPSLSVGTEADNFTVLNLAVAAAASHIYQNQFVQMILNSLINKSKSSMFQVRTLRELLW GYRDPFLSLVPYPVTTTGVGLFYPYNNADGVYKVFNGKDNISKVAIIDTYKGKRNLSEYWE SHCDMINGTDAASFPPFVEKSQVLQFFSSDICRETCVHFTSSFSVCKS
Isoform 3 (UniProt:P16671-3) (SEQ ID NO: 38)	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDLLIQKTIKKQVVLEEGTIAFKNWVKTGTE VYRQFWIFDVQNPQEVMNSSNIQVKQRGPYTYRVRFLAKENVTDQEDNTVSFLQPNGA IFEPSLSVGTEADNFTVLNLAVAAAASHIYQNQFVQMILNSLINKSKSSMFQVRTLRELLW GYRDPFLSLVPYPVTTTGVGLFYPYNNADGVYKVFNGKDNISKVAIIDTYKGKRSIYAVF ESDVNLKGI PVYRFVLPSKAFASPVENPDNYCFCTEKIISKNTSYGVLDISKCKEGRPV YISLPHFLYASPDVSEPIDGLNPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSEKI

	QVLKLNKRNYIVPILWLNETGTIGDEKANMFRSQVTGKINLLGLIEMILLSVGVMFVAF MISYCACRSKTIK
Isoform 4 (UniProt: P16671-4) (SEQ ID NO: 39)	MGCDRNCGLIAGAVIGAVLAVFGGILMPVGDLLIQKTIKKQVWLEEGTIAFKNWVKGTGE VYRQFWIFDVQNPQEVMMNSSNIQVKQRGPYTYRVRF LAKENV TQDAEDNTVSFLQPNGA IFEPSLSVGTEADNFTVLNLAVAYNNTADGVYKVFNGKDNISKVAIIDTYKGKRNL SYWE SHCDMINGTDAASFPPFVEKSQVLQFFSSDICRSIYAVFESDVNLKGIPVYRVFLPSKAF ASPVENPDNYCFCTEKIISKNCTSYGVLDISKCKEGRPVYISLPHFLYASPDVSEPIDGL NPNEEEHRTYLDIEPITGFTLQFAKRLQVNLLVKPSEKIQVLKLNKRNYIVPILWLNETG TIGDEKANMFRSQVTGKINLLGLIEMILLSVGVMFVAFMISYCACRSKTIK

[0155] As used herein, the term "CD36" includes any variants or isoforms of CD36 which are naturally expressed by cells. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of CD36 isoform 1. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of CD36 isoform 2. In some aspect, a miR-485 inhibitor disclosed herein can increase the expression of CD36 isoform 3. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of CD36 isoform 4. In further aspects, a miR-485 inhibitor disclosed herein can increase the expression of both CD36 isoform 1 and isoform 2, and/or isoform 3 and isoform 4, and/or isoform 1 and isoform 4, and/or isoform 2 and isoform 3. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of all CD36 isoforms. Unless indicated otherwise, isoform 1, isoform 2, isoform 3, and isoform 4 are collectively referred to herein as "CD36."

[0156] In some aspects, a miR-485 inhibitor of the present disclosure increases the expression of CD36 protein and/or CD36 gene by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, or at least about 300% compared to a reference (*e.g.*, expression of CD36 protein and/or CD36 gene in a corresponding subject that did not receive an administration of the miR-485 inhibitor).

[0157] Not to be bound by any one theory, in some aspects, a miR-485 inhibitor disclosed herein increases the expression of CD36 protein and/or CD36 gene by reducing the expression and/or activity of miR-485. There are two known mature forms of miR-485: miR-485-3p and miR-485-5p. As disclosed herein, in some aspects, a miR-485 inhibitor of the present disclosure can reduce the expression and/or activity of miR-485-3p. In some aspects, a miR-485 inhibitor can reduce the expression and/or activity of miR-485-5p. In further aspects, a miR-485 inhibitor disclosed herein can reduce the expression and/or activity of both miR-485-3p and miR-485-5p.

PGC1 Regulation

[0158] In some aspects, the Huntington's disease is associated with a decreased level of a PGC-1 α protein and/or a PGC-1 α gene. In some aspects, the miR-485 inhibitor increases expression of a PGC-1 α protein and/or a PGC-1 α gene in a subject in need thereof.

[0159] Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1- α), also known as PPARG Coactivator 1 Alpha or Ligand Effect Modulator-6, is a protein that in humans is encoded by the *PPARGC1A* gene. The *PGC1- α* gene is located on chromosome 4 in humans (nucleotides 23,792,021 to 24,472,905 of GenBank Accession Number NC_000004.12, plus strand orientation). Synonyms of the *PGC1- α* gene, and the encoded protein thereof, are known and include "PPARGC1A," "LEM6," "PGC1," "PGC1A," "PGC-1v," "PPARGC1," "PGC1alpha," or "PGC-1(alpha)."

[0160] There are at least nine known isoforms of human PGC1- α protein, resulting from alternative splicing. PGC1- α isoform 1 (UniProt identifier: Q9UBK2-1) consists of 798 amino acids and has been chosen as the canonical sequence (SEQ ID NO: 40). PGC1- α isoform 2 (also known as "Isoform NT-7a") (UniProt identifier: Q9UBK2-2) consists of 271 amino acids and differs from the canonical sequence as follows: 269-271: DPK $\rightarrow$ LFL; 272-798: Missing (SEQ ID NO: 41). PGC1- α isoform 3 (also known as "Isoform B5") (UniProt identifier: Q9UBK2-3) consists of 803 amino acids and differs from the canonical sequence as follows: 1-18: MAWDMCNQDSESVWSDIE $\rightarrow$ MDETSRLEEDWKKVLQREAGWQ (SEQ ID NO: 42). PGC1- α isoform 4 (also known as "Isoform B4") (UniProt identifier: Q9UBK2-4) consists of 786 amino acids and differs from the canonical sequence as follows: 1-18: MAWDMCNQDSESVWSDIE $\rightarrow$ MDEGYF (SEQ ID NO: 43). PGC1- α isoform 5 (also known as "Isoform B4-8a") (UniProt identifier: Q9UBK2-5) consists of 289 amino acids and differs from the canonical sequence as follows: 1-18: MAWDMCNQDSESVWSDIE $\rightarrow$ MDEGYF; 294-301: LTPPTTPP $\rightarrow$ VKTNLISK; 302-798: Missing (SEQ ID NO: 44). PGC1- α isoform 6 (also known as "Isoform B5-NT") (UniProt identifier: Q9UBK2-6) consists of 276 amino acids and differs from the canonical sequence as follows: 1-18: MAWDMCNQDSESVWSDIE $\rightarrow$ MDETSRLEEDWKKVLQREAGWQ; 269-271: DPK $\rightarrow$ LFL; 272-798: Missing (SEQ ID NO: 45). PGC1- α isoform 7 (also known as "B4-3ext") (UniProt identifier: Q9UBK2-7) consists of 138

amino acids and differs from the canonical sequence as follows: 1-18: MAWDMCNQDSESVWSDIE → MDEGYF; 144-150: LKKLLLA → VRTLPTV; 151-798: Missing (SEQ ID NO: 46). PGC1- α isoform 8 (also known as "Isoform 8a") (UniProt identifier: Q9UBK2-8) consists of 301 amino acids and differs from the canonical sequence as follows: 294-301: LTPPTTPP → VKTNLISK; 302-798: Missing (SEQ ID NO: 47). PGC1- α isoform 9 (also known as "Isoform 9" or "L-PGG-1 α ") (UniProt identifier: Q9UBK2-9) consists of 671 amino acids and differs from the canonical sequence as follows: 1-127: Missing (SEQ ID NO: 48). Table 3 below provides the sequences for the nine PGC1- α isoforms.

Table 3. PGC1- α Protein Isoforms

Isoform 1 (UniProt: Q9UBK2-1) (SEQ ID NO: 40)	MAWDMCNQDSESVWSDIECAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGLKWCS DQSEIISNQYNNEPSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDGDTV TDNEASPSSMPDGTTPPPQEAEEPSLLKKLLLAPANTQLSYNECSGLSTQNHANHNH RIRTNP AIVKTENSWSNKAKSICQQQKPQRRPCSELLKYLTNDPPHTKPTENRNSS RDCKTSKK KSHTSQSQHLQAKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGT AGLTPPTTP PHKANQDNPFRA SPKLSCKTVPPPSKKPRYSESSGTQGNNSTKKGPEQSELYAQLSK SSVLTGGHEERKTKRPSLRLFGDHDYCSINSKTEILINISQELQDSRQLENKDVSSD WQGGICSSDSDQCYLRETLEASKQVSPCSTRKQLQDQEI RAELNKHFGHPSQAVFDDEADK TGELRDSDFSNEQFSKLPMFINSGLAMDGLFDDSEDESDKLSYPWDGTQSYSLF NVSPSCSSFNSPCRDSVSPPKSLFSQRPQRMRSR SRFSRHRSCSRSPYSR SRSRSPGSRSSSRSC YYYESSHYRHRTHRNSPLYVRSR SRSPYSRRPRYDSYEEYQHERL KREEYRREYEKRESE RAKQ RERQ RQKAIEERRVIYVGKIRPD TTRTELDRFEVFG EIEECTVNL RDDGDSYGF ITYRYTCD AFAALENGYTLRRS NETDFELYFCGRKQFFKSNYADL DSNSDDFDPASTKSKY DSLDFDSLKEAQRSLRR
Isoform 2 (UniProt: Q9UBK2-2) (SEQ ID NO: 41)	MAWDMCNQDSESVWSDIECAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGLKWCS DQSEIISNQYNNEPSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDGDTV TDNEASPSSMPDGTTPPPQEAEEPSLLKKLLLAPANTQLSYNECSGLSTQNHANHNH RIRTNP AIVKTENSWSNKAKSICQQQKPQRRPCSELLKYLTNDPPHTKPTENRNSS RDCKTSKK KSHTSQSQHLQAKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGT AGLTPPTTP PHKANQDNPFRA SPKLSCKTVPPPSKKPRYSESSGTQGNNSTKKGPEQSELYAQLSK SSVLTGGHEERKTKRPSLRLFGDHDYCSINSKTEILINISQELQDSRQLENKDVSSD WQGGICSSDSDQCYLRETLEASKQVSPCSTRKQLQDQEI RAELNKHFGHPSQAVFDDEADK TGELRDSDFSNEQFSKLPMFINSGLAMDGLFDDSEDESDKLSYPWDGTQSYSLF NVSPSCSSFNSPCRDSVSPPKSLFSQRPQRMRSR SRFSRHRSCSRSPYSR SRSRSPGSRSSSRSC YYYESSHYRHRTHRNSPLYVRSR SRSPYSRRPRYDSYEEYQHERL KREEYRREYEKRESE RAKQ RERQ RQKAIEERRVIYVGKIRPD TTRTELDRFEVFG EIEECTVNL RDDGDSYGF ITYRYTCD AFAALENGYTLRRS NETDFELYFCGRKQFFKSNYADL DSNSDDFDPASTKSKY DSLDFDSLKEAQRSLRR
Isoform 3 (UniProt: Q9UBK2-3) (SEQ ID NO: 42)	MDETS PRLEEDWKKVLQREAGWQCAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGL KWCS DQSEIISNQYNNEPSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDG DVT TDNEASPSSMPDGTTPPPQEAEEPSLLKKLLLAPANTQLSYNECSGLSTQNHANHNH RIRTNP AIVKTENSWSNKAKSICQQQKPQRRPCSELLKYLTNDPPHTKPTENRNSS RDCKTSKK KSHTSQSQHLQAKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGTAGLT PPTTP PHKANQDNPFRA SPKLSCKTVPPPSKKPRYSESSGTQGNNSTKKGPEQSELYAQLSK SSVLTGGHEERKTKRPSLRLFGDHDYCSINSKTEILINISQELQDSRQLENKDV SSDWQGGICSSDSDQCYLRETLEASKQVSPCSTRKQLQDQEI RAELNKHFGHPSQAVFDDEADK TGELRDSDFSNEQFSKLPMFINSGLAMDGLFDDSEDESDKLSYPWDGTQSYSLF NVSPSCSSFNSPCRDSVSPPKSLFSQRPQRMRSR SRFSRHRSCSRSPYSR SRSRSPGSRSSSRSC YYYESSHYRHRTHRNSPLYVRSR SRSPYSRRPRYDSYEEYQHERL KREEYRREYEKRESE RAKQ RERQ RQKAIEERRVIYVGKIRPD TTRTELDRFEVFG EIEECTVNL RDDGDSYGF ITYRYTCD AFAALENGYTLRRS NETDFELYFCGRKQFFKSNYADL DSNSDDFDPASTKSKY DSLDFDSLKEAQRSLRR

Isoform 4 (UniProt: Q9UBK2-4) (SEQ ID NO: 43)	MDEGYFCAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGLKWCSQSEIISNQYNNE PSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDGDVTTDNEASPSSMPDGT PPPQEAEEPSLLKKLLAPANTQLSYNECSGLSTQNHANHNHRI RTNPAIVKTENSWSNK AKSICQQQKPQRRPCSELLKYLTTNDPPHTKPTENRNSSRDCKTSKKKSHTQSQSQHLQ AKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGTAGLTPPTTPPHKANQDNPFRA SPKLKSSCKTVVPPPSKKPRYSESSGTQGNNSTKKGPEQSELYAQLSKSSVL TGGHEERK TKRPSLRLFGDHDYCQSINSKTEILINISQELQDSRQLENKDVSSDWQGGQICSSTDSQC YLRETL EASKQVSPCSTRKQLQDQEI RAE LNKHFGHPSQAVFDDEADKTGELRDSDFSNE QFSKLP MF INSLAMDGLFDDSEDESDKLSYPWDGTQSYSLFNVSPSCSSFNSPCRDVS PPKSLFSQRPQRMRSRSRFSRHRSCSRSPYSRSRSRSPGSRSSSRSCYYYESSHYRHRT HRNSPLYVRSRSRSPYSRRPRYDSYEEYQHERLKREYRREYEKRESERAKQRRERQKQA IEERRVIYVGKIRPDTTRTEL RDRFEVFGIEECTVNL RDDGDSYGFITYRYTCDFAAL ENGYTLRRSNETDFELYFCGRKQFFKSNYADLDSNSDDFDPASTKSKYDSLDFDLSLLKEA QRS LRR
Isoform 5 (UniProt: Q9UBK2-5) (SEQ ID NO: 44)	MDEGYFCAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGLKWCSQSEIISNQYNNE PSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDGDVTTDNEASPSSMPDGT PPPQEAEEPSLLKKLLAPANTQLSYNECSGLSTQNHANHNHRI RTNPAIVKTENSWSNK AKSICQQQKPQRRPCSELLKYLTTNDPPHTKPTENRNSSRDCKTSKKKSHTQSQSQHLQ AKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGTAGVKTNLISK
Isoform 6 (UniProt: Q9UBK2-6) (SEQ ID NO: 45)	MDETSPRLEEDWKKVLQREAGWQCAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGL KWCSQSEIISNQYNNEPSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDG DVTTDNEASPSSMPDGT PPPQEAEEPSLLKKLLAPANTQLSYNECSGLSTQNHANHNHRI RTNPAIVKTENSWSNKAKSICQQQKPQRRPCSELLKYLTTNDPPHTKPTENRNSSRDCK TSKKKSHTQSQSQHLQAKPTTSLPLTPESP NLF L
Isoform 7 (UniProt: Q9UBK2-7) (SEQ ID NO: 46)	MDEGYFCAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGLKWCSQSEIISNQYNNE PSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDGDVTTDNEASPSSMPDGT PPPQEAEEPSLVRTLPTV
Isoform 8 (UniProt: Q9UBK2-8) (SEQ ID NO: 47)	MAWDMCNQDSES VWS D IECAALVGEDQPLCPDLPELDLSELDVNDLDTDSFLGGLKWCS QSEIISNQYNNEPSNIFEKIDEENEANLLAVLTETLDSLVPDEDGLPSFDALTDGDVTTD NEASPSSMPDGT PPPQEAEEPSLLKKLLAPANTQLSYNECSGLSTQNHANHNHRI RTNP AIVKTENSWSNKAKSICQQQKPQRRPCSELLKYLTTNDPPHTKPTENRNSSRDCKTSKK KSHTQSQSQHLQAKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGTAGVKTNLIS K
Isoform 9 (UniProt: Q9UBK2-9) (SEQ ID NO: 48)	MPDGT PPPQEAEEPSLLKKLLAPANTQLSYNECSGLSTQNHANHNHRI RTNPAIVKTEN SWSNKAKSICQQQKPQRRPCSELLKYLTTNDPPHTKPTENRNSSRDCKTSKKKSHTQSQ S QHLQAKPTTSLPLTPESPNDPKGSPFENKTIERTLSVELSGTAGLTPPTTPPHKANQD NPFRA SPKLKSSCKTVVPPPSKKPRYSESSGTQGNNSTKKGPEQSELYAQLSKSSVL TGG HEERKTKRPSLRLFGDHDYCQSINSKTEILINISQELQDSRQLENKDVSSDWQGGQICSST DSDQCYLRETL EASKQVSPCSTRKQLQDQEI RAE LNKHFGHPSQAVFDDEADKTGELRDS DFSNEQFSKLP MF INSLAMDGLFDDSEDESDKLSYPWDGTQSYSLFNVSPSCSSFNSPC RDSVPPKSLFSQRPQRMRSRSRFSRHRSCSRSPYSRSRSRSPGSRSSSRSCYYYESSH

	YRHRTHRNSPLYVRSRSPYSRRPRYDSYEEYQHERLKREEYRREYEKRESERAKQRE QRQKAIEERRVIYVGKIRPDTRTELDRFEVFGIEECTVNLRDDGDSYGFITYRYTCD AFAALENGYTLRRSNETDFELYFCGRKQFFKSNYADLDSNSDDFDPASTKSKYDSLDFDS LLKEAQRSLRR
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[0161] As used herein, the term "PGC1- α " includes any variants or isoforms of PGC1- α which are naturally expressed by cells. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 1. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 2. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 1. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 2. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 2. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 3. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 4. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 5. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 6. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 7. In some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 8. Accordingly, in some aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 9. In further aspects, a miR-485 inhibitor disclosed herein can increase the expression of PGC1- α isoform 1, isoform 2, isoform 3, isoform 4, isoform 5, isoform 6, isoform 7, isoform 8, and isoform 9. Unless indicated otherwise, both isoform 1 and isoform 2 are collectively referred to herein as "PGC1- α ."

[0162] In some aspects, a miR-485 inhibitor of the present disclosure increases the expression of PGC1- α protein and/or PGC1- α gene by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, or at least about 300% compared to a reference (*e.g.*, expression of PGC1- α protein and/or PGC1- α gene in a corresponding subject that did not receive an administration of the miR-485 inhibitor).

[0163] Not to be bound by any one theory, in some aspects, a miR-485 inhibitor disclosed herein increases the expression of PGC1- α protein and/or PGC1- α gene by reducing the expression and/or activity of miR-485. There are two known mature forms of miR-485: miR-485-3p and miR-485-5p. In some aspects, a miR-485 inhibitor of the present disclosure can reduce the expression

and/or activity of miR-485-3p. In some aspects, a miR-485 inhibitor can reduce the expression and/or activity of miR-485-5p. In further aspects, a miR-485 inhibitor disclosed herein can reduce the expression and/or activity of both miR-485-3p and miR-485-5p.

[0164] In some aspects, administering a miR-485 inhibitor disclosed herein can improve one or more symptoms of Huntington's disease associated with abnormal (*e.g.*, reduced) levels of SIRT1 protein and/or SIRT1 gene. In some aspects, administering a miR-485 inhibitor disclosed herein can improve one or more symptoms of Huntington's disease associated with abnormal (*e.g.*, reduced) levels of CD36 protein and/or CD36 gene. In some aspects, administering a miR-485 inhibitor disclosed herein can improve one or more symptoms of Huntington's disease associated with abnormal (*e.g.*, reduced) levels of PGC1- α protein and/or PGC1- α gene. Non-limiting examples of such symptoms are described below.

[0165] In some aspects, administering a miR-485 inhibitor of the present disclosure reduces the occurrence or risk of occurrence of one or more symptoms of Huntington's disease in a subject by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0166] In some aspects, administering a miR-485 inhibitor of the present disclosure reduces memory loss in a subject suffering from Huntington's disease compared to a reference (*e.g.*, memory loss in the subject prior to the administering). In some aspects, administering a miR-485 inhibitor of the present disclosure reduces memory loss or the risk of occurrence of memory loss in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0167] In some aspects, administering a miR-485 inhibitor of the present disclosure improves memory retention in a subject suffering from Huntington's disease compared to a reference (*e.g.*, memory retention in the subject prior to the administering). In some aspects,

administering a miR-485 inhibitor of the present disclosure improves and/or increases memory retention in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0168] In some aspects, administering a miR-485 inhibitor of the present disclosure improves spatial working memory in a subject suffering from Huntington's disease compared to a reference (*e.g.*, spatial working memory in the subject prior to the administering). As used herein, the term "spatial working memory" refers to the ability to keep spatial information activity in working memory over a short period of time. In some aspects, spatial working memory is improved and/or increased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0169] In some aspects, administering a miR-485 inhibitor of the present disclosure increases the phagocytic activity of scavenger cells (*e.g.*, glial cells) (*e.g.*, by increasing the expression of CD36 protein and/or CD36 gene) in a subject suffering from Huntington's disease compared to a reference (*e.g.*, phagocytic activity in the subject prior to the administering). In some aspects, administering a miR-485 inhibitor of the present disclosure increases dendritic spine density of a neuron in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0170] In some aspects, administering a miR-485 inhibitor of the present disclosure reduces an amyloid beta (A β) plaque load in a subject suffering from Huntington's disease (*e.g.*, by increasing the expression of CD36 protein and/or CD36 gene) compared to a reference (*e.g.*, amyloid beta (A β) plaque load in the subject prior to the administering). As used herein, "amyloid

beta plaque" refers to all forms of aberrant deposition of amyloid beta including large aggregates and small associations of a few amyloid beta peptides and can contain any variation of the amyloid beta peptides. Amyloid beta (A β) plaque is known to cause neuronal changes, *e.g.*, aberrations in synapse composition, synapse shape, synapse density, loss of synaptic conductivity, changes in dendrite diameter, changes in dendrite length, changes in spine density, changes in spine area, changes in spine length, or changes in spine head diameter. In some aspects, administering a miR-485 inhibitor of the present disclosure reduces an amyloid beta plaque load in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0171] In some aspects, administering a miR-485 inhibitor disclosed herein increases neurogenesis in a subject suffering from Huntington's disease (*e.g.*, by increasing the expression of CD36 protein and/or CD36 gene) compared to a reference (*e.g.*, neurogenesis in the subject prior to the administering). As used herein, the term "neurogenesis" refers to the process by which neurons are created. Neurogenesis encompasses proliferation of neural stem and progenitor cells, differentiation of these cells into new neural cell types, as well as migration and survival of the new cells. The term is intended to cover neurogenesis as it occurs during normal development, predominantly during pre-natal and peri-natal development, as well as neural cells regeneration that occurs following disease, damage or therapeutic intervention. Adult neurogenesis is also termed "nerve" or "neural" regeneration. In some aspects, administering a miR-485 inhibitor of the present disclosure increases neurogenesis in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0172] In some aspects, increasing and/or inducing neurogenesis is associated with increased proliferation, differentiation, migration, and/or survival of neural stem cells and/or progenitor cells. Accordingly, in some aspects, administering a miR-485 inhibitor of the present disclosure can increase the proliferation of neural stem cells and/or progenitor cells in the subject.

In certain aspects, the proliferation of neural stem cells and/or progenitor cells is increased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor). In some aspects, the survival of neural stem cells and/or progenitor cells is increased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0173] In some aspects, increasing and/or inducing neurogenesis is associated with an increased number of neural stem cells and/or progenitor cells. In certain aspects, the number of neural stem cells and/or progenitor cells is increased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0174] In some aspects, increasing and/or inducing neurogenesis is associated with increased axon, dendrite, and/or synapse development. In certain aspects, axon, dendrite, and/or synapse development is increased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0175] In some aspects, administering a miR-485 inhibitor of the present disclosure increases dendritic spine density of a neuron in a subject suffering from Huntington's disease compared to a reference (*e.g.*, dendritic spine density of a neuron in the subject prior to the administering). In some aspects, administering a miR-485 inhibitor of the present disclosure increases dendritic spine density of a neuron in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%,

at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0176] In some aspects, administering a miR-485 inhibitor disclosed herein decreases the loss of dendritic spines of a neuron in a subject suffering from Huntington's disease compared to a reference (*e.g.*, loss of dendritic spines of a neuron in the subject prior to the administering). In certain aspects, administering a miR-485 inhibitor decreases the loss of dendritic spines of a neuron in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0177] In some aspects, administering a miR-485 inhibitor of the present disclosure decreases neuroinflammation (*e.g.*, by increasing the expression of SIRT1 protein and/or SIRT1 gene) in a subject suffering from Huntington's disease compared to a reference (*e.g.*, neuroinflammation in the subject prior to the administering). In certain aspects, administering a miR-485 inhibitor decreases neuroinflammation in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor). In some aspects, decreased neuroinflammation comprises glial cells producing decreased amounts of inflammatory mediators. Accordingly, in certain aspects, administering a miR-485 inhibitor disclosed herein to a subject suffering from Huntington's disease decreases the amount of inflammatory mediators produced by glial cells by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%,

at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor). In some aspects, an inflammatory mediator produced by glial cells comprises TNF- α . In some aspects, the inflammatory mediator comprises IL-1 β . In some aspects, an inflammatory mediator produced by glial cells comprises both TNF- α and IL-1 β .

[0178] In some aspects, administering a miR-485 inhibitor disclosed herein increases autophagy (*e.g.*, by increasing the expression of a SIRT1 protein and/or SIRT1 gene) in a subject suffering from Huntington's disease. As used herein, the term "autophagy" refers to cellular stress response and a survival pathway that is responsible for the degradation of long-lived proteins, protein aggregates, as well as damaged organelles in order to maintain cellular homeostasis. Not surprisingly, abnormalities of autophagy have been associated with number of diseases, including many neurodegenerative diseases such as Huntington's disease. In some aspects, administering a miR-485 inhibitor disclosed herein to a subject suffering from Huntington's disease increases autophagy by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 150%, at least about 200%, or at least about 300% or more, compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0179] As is known in the art, subjects suffering from Huntington's disease exhibit certain motor and/or non-motor symptoms. For instance, non-limiting examples of motor symptoms associated with Huntington's disease include resting tremor, reduction of spontaneous movement (bradykinesia), involuntary movements (chorea), rigidity, postural instability, freezing of gait, impaired handwriting (micrographia), decreased facial expression, delayed start of movement (akinesia), motor impersistence, slurred speech, swallowing difficulties, and uncontrolled rapid movements. Non-limiting examples of non-motor symptoms associated with Huntington's disease include autonomic dysfunction, neuropsychiatric problems (mood, cognition, behavior, or thought alterations), sensory alterations (especially altered sense of smell), and sleep difficulties.

[0180] In some aspects, administering a miR-485 inhibitor of the present disclosure improves one or more motor symptoms in a subject suffering from Huntington's disease compared to a reference (*e.g.*, corresponding motor symptoms in the subject prior to the administering). In certain aspects, administering a miR-485 inhibitor of the present disclosure improves one or more

motor symptoms in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0181] In some aspects, administering a miR-485 inhibitor of the present disclosure improves one or more non-motor symptoms in a subject suffering from Huntington's disease compared to a reference (*e.g.*, corresponding non-motor symptom in the subject prior to the administering). In certain aspects, administering a miR-485 inhibitor disclosed herein improves one or more non-motor symptoms in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0182] In some aspects, administering a miR-485 inhibitor disclosed herein improves synaptic function in a subject suffering from Huntington's disease compared to a reference (*e.g.*, synaptic function in the subject prior to the administering). As used herein, the term "synaptic function," refers to the ability of the synapse of a cell (*e.g.*, a neuron) to pass an electrical or chemical signal to another cell (*e.g.*, a neuron). In some aspects, administering a miR-485 inhibitor of the present disclosure improves synaptic function in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300% or more compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0183] In some aspects, administering a miR-485 inhibitor of the present disclosure can prevent, delay, and/or ameliorate the loss of synaptic function in a subject suffering from Huntington's disease compared to a reference (*e.g.*, loss of synaptic function in the subject prior to the administering). In some aspects, administering a miR-485 inhibitor prevents, delays, and/or ameliorates the loss of synaptic function in a subject suffering from Huntington's disease by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% compared to a reference (*e.g.*, subjects that did not receive an administration of the miR-485 inhibitor).

[0184] In some aspects, a miR-485 inhibitor disclosed herein can be administered by any suitable route known in the art. In certain aspects, a miR-485 inhibitor is administered parenterally, intramuscularly, subcutaneously, ophthalmic, intravenously, intraperitoneally, intradermally, intraorbitally, intracerebrally, intracranially, intracerebroventricularly, intraspinally, intraventricular, intrathecally, intracisternally, intracapsularly, intratumorally, or any combination thereof.

[0185] In some aspects, a miR-485 inhibitor of the present disclosure can be used in combination with one or more additional therapeutic agents. In some aspects, the additional therapeutic agent and the miR-485 inhibitor are administered concurrently. In certain aspects, the additional therapeutic agent and the miR-485 inhibitor are administered sequentially.

[0186] In some aspects, the additional therapeutic agent reduces motor or non-motor symptoms. In some aspects, the additional therapeutic agent is a drug to control movement (*e.g.* tetrabenazine), an antipsychotic drug (*e.g.* haloperidol, risperidone, olanzapine and quetiapine), an antidepressant (*e.g.* citalopram, fluoxetine, and sertraline), a mood-stabilizing drug (*e.g.* divalproex, carbamazepine, and lamotrigine), mantadine, levetiracetam, clonazepam, or combinations thereof.

[0187] In some aspects, a miR-485 inhibitor of the present disclosure can be used to treat Huntington's disease in a subject who has failed to respond to other treatments (*e.g.* tetrabenazine, haloperidol, citalopram, divalproex, clonazepam, etc.).

[0188] In some aspects, the administration of a miR-485 inhibitor disclosed herein does not result in any adverse effects. In certain aspects, miR-485 inhibitors of the present disclosure do

not adversely affect body weight when administered to a subject. In some aspects, miR-485 inhibitors disclosed herein do not result in increased mortality or cause pathological abnormalities when administered to a subject.

III. miRNA-485 Inhibitors

[0189] Disclosed herein are compounds that can inhibit miR-485 activity (miR-485 inhibitor). In some aspects, a miR-485 inhibitor of the present disclosure comprises a nucleotide sequence encoding a nucleotide molecule that comprises at least one miR-485 binding site, wherein the nucleotide molecule does not encode a protein. As described herein, in some aspects, the miR-485 binding site is at least partially complementary to the target miRNA nucleic acid sequence (*i.e.*, miR-485), such that the miR-485 inhibitor hybridizes to the miR-485 nucleic acid sequence.

[0190] In some aspects, the miR-485 binding site of a miR inhibitor disclosed herein has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence complementarity to the nucleic acid sequence of a miR-485. In certain aspects, the miR-485 binding site is fully complementary to the nucleic acid sequence of a miR-485.

[0191] The miR-485 hairpin precursor can generate both miR-485-5p and miR-485-3p. In the context of the present disclosure "miR-485" encompasses both miR-485-5p and miR-485-3p unless specified otherwise. The human mature miR-485-3p has the sequence 5'-GUCAUACACGGCUCUCCUCUCU-3' (SEQ ID NO: 1; miRBase Acc. No. MIMAT0002176). A 5' terminal subsequence of miR-485-3p 5'-UCAUACA-3' (SEQ ID NO: 49) is the seed sequence. The human mature miR-485-5p has the sequence 5'-AGAGGCUGGCCGUGAUGAAUUC-3' (SEQ ID NO: 33; miRBase Acc. No. MIMAT0002175). A 5' terminal subsequence of miR-485-5p 5'-GAGGCUG-3' (SEQ ID NO: 50) is the seed sequence.

[0192] As will be apparent to those in the art, the human mature miR-485-3p has significant sequence similarity to that of other species. For instance, the mouse mature miR-485-3p differs from the human mature miR-485-3p by a single amino acid at each of the 5'- and 3'- ends (*i.e.*, has an extra "A" at the 5'-end and missing "C" at the 3'-end). The mouse mature miR-485-3p has the following sequence: 5'-AGUCAUACACGGCUCUCCUCUC-3' (SEQ ID NO: 34; miRBase Acc. No. MIMAT0003129; underlined portion corresponds to overlap to human mature miR-485-3p). The sequence for the mouse mature miR-485-5p is identical to that of the human: 5'-agaggcuggccgugaugaauuc-3' (SEQ ID NO: 33; miRBase Acc. No. MIMAT0003128). In certain

aspects, a miR-485 inhibitor disclosed herein is capable of binding to miR-485-3p and/or miR-485-5p from both human and mouse.

[0193] In some aspects, the miR-485 binding site is a single-stranded polynucleotide sequence that is complementary (*e.g.*, fully complementary) to a sequence of a miR-485-3p (or a subsequence thereof). In some aspects, the miR-485-3p subsequence comprises the seed sequence. Accordingly, in certain aspects, the miR-485 binding site has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence complementarity to the nucleic acid sequence set forth in SEQ ID NO: 49. In certain aspects, the miR-485 binding site is complementary to miR-485-3p except for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mismatches. In further aspects, the the miR-485 binding site is fully complementary to the nucleic acid sequence set forth in SEQ ID NO: 1.

[0194] In some aspects, the miR-485 binding site is a single-stranded polynucleotide sequence that is complementary (*e.g.*, fully complementary) to a sequence of a miR-485-5p (or a subsequence thereof). In some aspects, the miR-485-5p subsequence comprises the seed sequence. In certain aspects, the miR-485 binding site has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence complementarity to the nucleic acid sequence set forth in SEQ ID NO: 50. In certain aspects, the miR-485 binding site is complementary to miR-485-5p except for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mismatches. In further aspects, the miR-485 binding site is fully complementary to the nucleic acid sequence set forth in SEQ ID NO: 35.

[0195] The seed region of a miRNA forms a tight duplex with the target mRNA. Most miRNAs imperfectly base-pair with the 3' untranslated region (UTR) of target mRNAs, and the 5' proximal "seed" region of miRNAs provides most of the pairing specificity. Without being bound to any theory, it is believed that the first nine miRNA nucleotides (encompassing the seed sequence) provide greater specificity whereas the miRNA ribonucleotides 3' of this region allow for lower sequence specificity and thus tolerate a higher degree of mismatched base pairing, with positions 2-7 being the most important. Accordingly, in specific aspects of the present disclosure,

the miR-485 binding site comprises a subsequence that is fully complementary (*i.e.*, 100% complementary) over the entire length of the seed sequence of miR-485.

[0196] miRNA sequences and miRNA binding sequences that can be used in the context of the disclosure include, but are not limited to, all or a portion of those sequences in the sequence listing provided herein, as well as the miRNA precursor sequence, or complement of one or more of these miRNAs. Any aspects of the disclosure involving specific miRNAs or miRNA binding sites by name is contemplated also to cover miRNAs or complementary sequences thereof whose sequences are at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to the mature sequence of the specified miRNA sequence or complementary sequence thereof.

[0197] In some aspects, miRNA binding sequences of the present disclosure can include additional nucleotides at the 5', 3', or both 5' and 3' ends of those sequences in the sequence listing provided herein, as long as the modified sequence is still capable of specifically binding to miR-485. In some aspects, miRNA binding sequences of the present disclosure can differ in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides with respect to those sequences in the sequence listing provided, as long as the modified sequence is still capable of specifically binding to miR-485.

[0198] It is also specifically contemplated that any methods and compositions discussed herein with respect to miRNA binding molecules or miRNA can be implemented with respect to synthetic miRNAs binding molecules. It is also understood that the disclosures related to RNA sequences in the present disclosure are equally applicable to corresponding DNA sequences.

[0199] In some aspects, a miRNA-485 inhibitor of the present disclosure comprises at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, or at least 20 nucleotides at the 5' of

the nucleotide sequence. In some aspects, a miRNA-485 inhibitor comprises at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, or at least 20 nucleotides at the 3' of the nucleotide sequence.

[0200] In some aspects, a miR-485 inhibitor disclosed herein is about 6 to about 30 nucleotides in length. In certain aspects, a miR-485 inhibitor disclosed herein is 7 nucleotides in length. In further aspects, a miR-485 inhibitor disclosed herein is 8 nucleotides in length. In some aspects, a miR-485 inhibitor is 9 nucleotides in length. In some aspects, a miR-485 inhibitor of the present disclosure is 10 nucleotides in length. In certain aspects, a miR-485 inhibitor is 11 nucleotides in length. In further aspects, a miR-485 inhibitor is 12 nucleotides in length. In some aspects, a miR-485 inhibitor disclosed herein is 13 nucleotides in length. In certain aspects, a miR-485 inhibitor disclosed herein is 14 nucleotides in length. In some aspects, a miR-485 inhibitor disclosed herein is 15 nucleotides in length. In further aspects, a miR-485 inhibitor is 16 nucleotides in length. In certain aspects, a miR-485 inhibitor of the present disclosure is 17 nucleotides in length. In some aspects, a miR-485 inhibitor is 18 nucleotides in length. In some aspects, a miR-485 inhibitor is 19 nucleotides in length. In certain aspects, a miR-485 inhibitor is 20 nucleotides in length. In further aspects, a miR-485 inhibitor of the present disclosure is 21 nucleotides in length. In some aspects, a miR-485 inhibitor is 22 nucleotides in length.

[0201] In some aspects, a miR-485 inhibitor disclosed herein comprises a nucleotide sequence that is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% identical to a sequence selected from SEQ ID NOs: 2 to 30. In certain aspects, a miR-485 inhibitor comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 2 to 30, wherein the nucleotide sequence can optionally comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mismatches.

[0202] In some aspects, a miRNA inhibitor comprises 5'-UGUAUGA-3' (SEQ ID NO: 2), 5'-GUGUAUGA-3' (SEQ ID NO: 3), 5'-CGUGUAUGA-3' (SEQ ID NO: 4), 5'-CCGUGUAUGA-3' (SEQ ID NO: 5), 5'-GCCGUGUAUGA-3' (SEQ ID NO: 6), 5'-AGCCGUGUAUGA-3' (SEQ ID NO: 7), 5'-GAGCCGUGUAUGA-3' (SEQ ID NO: 8), 5'-AGAGCCGUGUAUGA-3' (SEQ ID NO:

9), 5'-GAGAGCCGUGUAUGA-3' (SEQ ID NO: 10), 5'-GGAGAGCCGUGUAUGA-3' (SEQ ID NO: 11), 5'-AGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 12), 5'-GAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 13), 5'-AGAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 14), or 5'-GAGAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 15).

[0203] In some aspects, the miRNA inhibitor has 5'-UGUAUGAC-3' (SEQ ID NO: 16), 5'-GUGUAUGAC-3' (SEQ ID NO: 17), 5'-CGUGUAUGAC-3' (SEQ ID NO: 18), 5'-CCGUGUAUGAC-3' (SEQ ID NO: 19), 5'-GCCGUGUAUGAC-3' (SEQ ID NO: 20), 5'-AGCCGUGUAUGAC-3' (SEQ ID NO: 21), 5'-GAGCCGUGUAUGAC-3' (SEQ ID NO: 22), 5'-AGAGCCGUGUAUGAC-3' (SEQ ID NO: 23), 5'-GAGAGCCGUGUAUGAC-3' (SEQ ID NO: 24), 5'-GGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 25), 5'-AGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 26), 5'-GAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 27), 5'-AGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 28), or 5'-GAGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 29).

[0204] In some aspects, the miRNA inhibitor comprises 5'-TGTATGA-3' (SEQ ID NO: 62), 5'-GTGTATGA-3' (SEQ ID NO: 63), 5'-CGTGTATGA-3' (SEQ ID NO: 64), 5'-CCGTGTATGA-3' (SEQ ID NO: 65), 5'-GCCGTGTATGA-3' (SEQ ID NO: 66), 5'-AGCCGTGTATGA-3' (SEQ ID NO: 67), 5'-GAGCCGTGTATGA-3' (SEQ ID NO: 68), 5'-AGAGCCGTGTATGA-3' (SEQ ID NO: 69), 5'-GAGAGCCGTGTATGA-3' (SEQ ID NO: 70), 5'-GGAGAGCCGTGTATGA-3' (SEQ ID NO: 71), 5'-AGGAGAGCCGTGTATGA-3' (SEQ ID NO: 72), 5'-GAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 73), 5'-AGAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 74), 5'-GAGAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 75); 5'-TGTATGAC-3' (SEQ ID NO: 76), 5'-GTGTATGAC-3' (SEQ ID NO: 77), 5'-CGTGTATGAC-3' (SEQ ID NO: 78), 5'-CCGTGTATGAC-3' (SEQ ID NO: 79), 5'-GCCGTGTATGAC-3' (SEQ ID NO: 80), 5'-AGCCGTGTATGAC-3' (SEQ ID NO: 81), 5'-GAGCCGTGTATGAC-3' (SEQ ID NO: 82), 5'-AGAGCCGTGTATGAC-3' (SEQ ID NO: 83), 5'-GAGAGCCGTGTATGAC-3' (SEQ ID NO: 84), 5'-GGAGAGCCGTGTATGAC-3' (SEQ ID NO: 85), 5'-AGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 86), 5'-GAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 87), 5'-AGAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 88), or 5'-GAGAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 89).

[0205] In some aspects, a miRNA inhibitor disclosed herein (*i.e.*, miR-485 inhibitor) comprises a nucleotide sequence that is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%,

at least about 90%, or at least about 95% identical to 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88). In some aspects, the miRNA inhibitor comprises a nucleotide sequence that has at least 90% similarity to 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88). In some aspects, the miRNA inhibitor comprises the nucleotide sequence 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88) with one substitution or two substitutions. In certain aspects, the miRNA inhibitor comprises the nucleotide sequence 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).

[0206] In some aspects, a miR-485 inhibitor of the present disclosure comprises the sequence disclosed herein, e.g., any one of SEQ ID NOs: 2 to 35, or 62 to 89 and at least one, at least two, at least three, at least four or at least five additional nucleic acid at the N terminus, at least one, at least two, at least three, at least four, or at least five additional nucleic acid at the C terminus, or both. In some aspects, a miR-485 inhibitor of the present disclosure comprises the sequence disclosed herein, e.g., any one of SEQ ID NOs: 2 to 35 or 62 to 89, and one additional nucleic acid at the N terminus and/or one additional nucleic acid at the C terminus. In some aspects, a miR-485 inhibitor of the present disclosure comprises the sequence disclosed herein, e.g., any one of SEQ ID NOs: 2 to 35 or 62 to 89, and one or two additional nucleic acids at the N terminus and/or one or two additional nucleic acids at the C terminus. In some aspects, a miR-485 inhibitor of the present disclosure comprises the sequence disclosed herein, e.g., any one of SEQ ID NOs: 2 to 35 or 62 to 89, and one to three additional nucleic acids at the N terminus and/or one to three additional nucleic acids at the C terminus. In some aspects, a miR-485 inhibitor comprises 5'- GAGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 29).

[0207] In some aspects, a miR-485 inhibitor of the present disclosure comprises one miR-485 binding site. In further aspects, a miR-485 inhibitor disclosed herein comprises at least two miR-485 binding sites. In certain aspects, a miR-485 inhibitor comprises three miR-485 binding sites. In some aspects, a miR-485 inhibitor comprises four miR-485 binding sites. In some aspects, a miR-485 inhibitor comprises five miR-485 binding sites. In certain aspects, a miR-485 inhibitor comprises six or more miR-485 binding sites. In some aspects, all the miR-485 binding sites are identical. In some aspects, all the miR-485 binding sites are different. In some aspects, at least one of the miR-485 binding sites is different. In some aspects, all the miR-485 binding sites are miR-485-3p binding sites. In other aspects, all the miR-485 binding sites are miR-485-5p binding sites.

In further aspects, a miR-485 inhibitor comprises at least one miR-485-3p binding site and at least one miR-485-5p binding site.

III.a. Chemically Modified Polynucleotides

[0208] In some aspects, a miR-485 inhibitor disclosed herein comprises a polynucleotide which includes at least one chemically modified nucleoside and/or nucleotide. When the polynucleotides of the present disclosure are chemically modified the polynucleotides can be referred to as "modified polynucleotides."

[0209] A "nucleoside" refers to a compound containing a sugar molecule (*e.g.*, a pentose or ribose) or a derivative thereof in combination with an organic base (*e.g.*, a purine or pyrimidine) or a derivative thereof (also referred to herein as "nucleobase"). A "nucleotide" refers to a nucleoside including a phosphate group. Modified nucleotides can be synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides.

[0210] Polynucleotides can comprise a region or regions of linked nucleosides. Such regions can have variable backbone linkages. The linkages can be standard phosphodiester linkages, in which case the polynucleotides would comprise regions of nucleotides.

[0211] The modified polynucleotides disclosed herein can comprise various distinct modifications. In some aspects, the modified polynucleotides contain one, two, or more (optionally different) nucleoside or nucleotide modifications. In some aspects, a modified polynucleotide can exhibit one or more desirable properties, *e.g.*, improved thermal or chemical stability, reduced immunogenicity, reduced degradation, increased binding to the target microRNA, reduced non-specific binding to other microRNA or other molecules, as compared to an unmodified polynucleotide.

[0212] In some aspects, a polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor) is chemically modified. As used herein, in reference to a polynucleotide, the terms "chemical modification" or, as appropriate, "chemically modified" refer to modification with respect to adenosine (A), guanosine (G), uridine (U), thymidine (T) or cytidine (C) ribo- or deoxyribonucleosides in one or more of their position, pattern, percent or population, including, but not limited to, its nucleobase, sugar, backbone, or any combination thereof.

[0213] In some aspects, a polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor) can have a uniform chemical modification of all or any of the same nucleoside type or a population of modifications produced by downward titration of the same starting modification in

all or any of the same nucleoside type, or a measured percent of a chemical modification of all any of the same nucleoside type but with random incorporation. In further aspects, the polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor) can have a uniform chemical modification of two, three, or four of the same nucleoside type throughout the entire polynucleotide (such as all uridines and/or all cytidines, etc. are modified in the same way).

[0214] Modified nucleotide base pairing encompasses not only the standard adenine-thymine, adenine-uracil, or guanine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures. One example of such non-standard base pairing is the base pairing between the modified nucleobase inosine and adenine, cytosine or uracil. Any combination of base/sugar or linker can be incorporated into polynucleotides of the present disclosure.

[0215] The skilled artisan will appreciate that, except where otherwise noted, polynucleotide sequences set forth in the instant application will recite "T"s in a representative DNA sequence but where the sequence represents RNA, the "T"s would be substituted for "U"s. For example, TD's of the present disclosure can be administered as RNAs, as DNAs, or as hybrid molecules comprising both RNA and DNA units.

[0216] In some aspects, the polynucleotide (*e.g.*, a miR-485 inhibitor) includes a combination of at least two (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, 20 or more) modified nucleobases.

[0217] In some aspects, the nucleobases, sugar, backbone linkages, or any combination thereof in a polynucleotide are modified by at least about 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100%.

(i) Base Modification

[0218] In certain aspects, the chemical modification is at nucleobases in a polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor). In some aspects, the at least one chemically modified nucleoside is a modified uridine (*e.g.*, pseudouridine (ψ), 2-thiouridine (s2U), 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), or 5-methoxy-uridine (mo5U)), a modified

cytosine (*e.g.*, 5-methyl-cytidine (m5C)) a modified adenosine (*e.g.*, 1-methyl-adenosine (m1A), N6-methyl-adenosine (m6A), or 2-methyl-adenine (m2A)), a modified guanosine (*e.g.*, 7-methyl-guanosine (m7G) or 1-methyl-guanosine (m1G)), or a combination thereof.

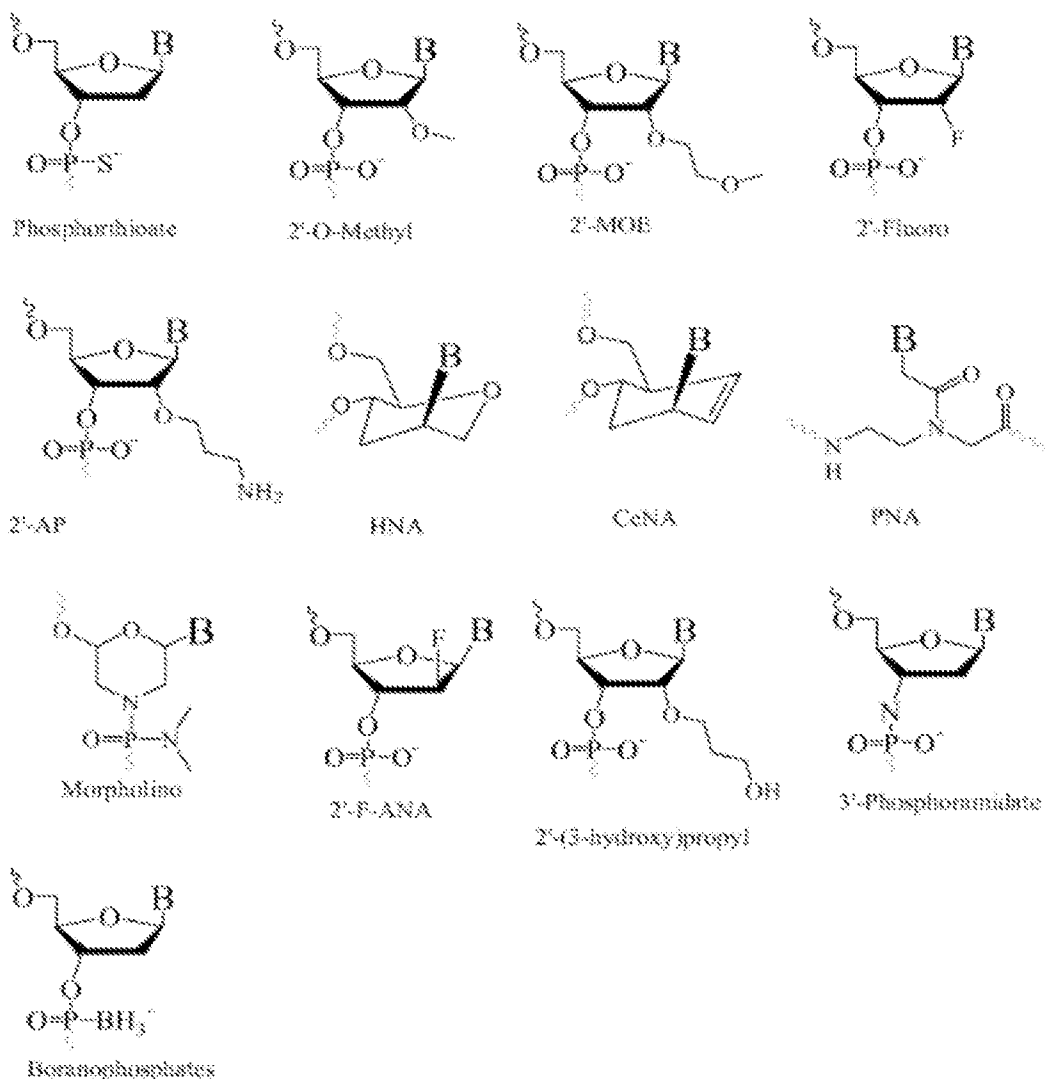
[0219] In some aspects, the polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor) is uniformly modified (*e.g.*, fully modified, modified throughout the entire sequence) for a particular modification. For example, a polynucleotide can be uniformly modified with the same type of base modification, *e.g.*, 5-methyl-cytidine (m5C), meaning that all cytosine residues in the polynucleotide sequence are replaced with 5-methyl-cytidine (m5C). Similarly, a polynucleotide can be uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified nucleoside such as any of those set forth above.

[0220] In some aspects, the polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor) includes a combination of at least two (*e.g.*, 2, 3, 4 or more) of modified nucleobases. In some aspects, at least about 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% of a type of nucleobases in a polynucleotide of the present disclosure (*e.g.*, a miR-485 inhibitor) are modified nucleobases.

(ii) Backbone modifications

[0221] In some aspects, the polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) can include any useful linkage between the nucleosides. Such linkages, including backbone modifications, that are useful in the composition of the present disclosure include, but are not limited to the following: 3'-alkylene phosphonates, 3'-amino phosphoramidate, alkene containing backbones, aminoalkylphosphoramidates, aminoalkylphosphotriesters, boranophosphates, -CH₂-O-N(CH₃)-CH₂-, -CH₂-N(CH₃)-N(CH₃)-CH₂-, -CH₂-NH-CH₂-, chiral phosphonates, chiral phosphorothioates, formacetyl and thioformacetyl backbones, methylene (methylimino), methylene formacetyl and thioformacetyl backbones, methyleneimino and methylenehydrazino backbones, morpholino linkages, -N(CH₃)-CH₂-CH₂-, oligonucleosides with heteroatom internucleoside linkage, phosphinates, phosphoramidates, phosphorodithioates, phosphorothioate internucleoside linkages, phosphorothioates, phosphotriesters, PNA, siloxane backbones, sulfamate backbones, sulfide sulfoxide and sulfone backbones, sulfonate and

sulfonamide backbones, thionoalkylphosphonates, thionoalkylphosphotriesters, and thionophosphoramidates.



[0222] In some aspects, the presence of a backbone linkage disclosed above increase the stability and resistance to degradation of a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor).

[0223] In some aspects, at least about 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% of the backbone linkages in a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) are modified (*e.g.*, all of them are phosphorothioate).

[0224] In some aspects, a backbone modification that can be included in a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) comprises phosphorodiamidate morpholino oligomer (PMO) and/or phosphorothioate (PS) modification.

(iii) Sugar modifications

[0225] The modified nucleosides and nucleotides which can be incorporated into a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) can be modified on the sugar of the nucleic acid. In some aspects, the sugar modification increases the affinity of the binding of a miR-485 inhibitor to miR-485 nucleic acid sequence. Incorporating affinity-enhancing nucleotide analogues in the miR-485 inhibitor, such as LNA or 2'-substituted sugars, can allow the length and/or the size of the miR-485 inhibitor to be reduced.

[0226] In some aspects, at least about 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% of the nucleotides in a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) contain sugar modifications (*e.g.*, LNA).

[0227] In some aspects, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 nucleotide units in a polynucleotide of the present disclosure are sugar modified (*e.g.*, LNA).

[0228] Generally, RNA includes the sugar group ribose, which is a 5-membered ring having an oxygen. Exemplary, non-limiting modified nucleotides include replacement of the oxygen in ribose (*e.g.*, with S, Se, or alkylene, such as methylene or ethylene); addition of a double bond (*e.g.*, to replace ribose with cyclopentenyl or cyclohexenyl); ring contraction of ribose (*e.g.*, to form a 4-membered ring of cyclobutane or oxetane); ring expansion of ribose (*e.g.*, to form a 6- or 7-membered ring having an additional carbon or heteroatom, such as for anhydrohexitol, altritol, mannitol, cyclohexanyl, cyclohexenyl, and morpholino that also has a phosphoramidate backbone); multicyclic forms (*e.g.*, tricyclo; and "unlocked" forms, such as glycol nucleic acid (GNA) (*e.g.*, R-GNA or S-GNA, where ribose is replaced by glycol units attached to phosphodiester bonds), threose nucleic acid (TNA, where ribose is replaced with α -L-threofuranosyl-(3'→2')) , and peptide nucleic acid (PNA, where 2-amino-ethyl-glycine linkages replace the ribose and phosphodiester backbone). The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding

carbon in ribose. Thus, a polynucleotide molecule can include nucleotides containing, *e.g.*, arabinose, as the sugar.

[0229] The 2' hydroxyl group (OH) of ribose can be modified or replaced with a number of different substituents. Exemplary substitutions at the 2'-position include, but are not limited to, H, halo, optionally substituted C₁₋₆ alkyl; optionally substituted C₁₋₆ alkoxy; optionally substituted C₆₋₁₀ aryloxy; optionally substituted C₃₋₈ cycloalkyl; optionally substituted C₃₋₈ cycloalkoxy; optionally substituted C₆₋₁₀ aryloxy; optionally substituted C₆₋₁₀ aryl-C₁₋₆ alkoxy, optionally substituted C₁₋₁₂ (heterocycl)oxy; a sugar (*e.g.*, ribose, pentose, or any described herein); a polyethyleneglycol (PEG), -O(CH₂CH₂O)_nCH₂CH₂OR, where R is H or optionally substituted alkyl, and n is an integer from 0 to 20 (*e.g.*, from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20); "locked" nucleic acids (LNA) in which the 2'-hydroxyl is connected by a C₁₋₆ alkylene or C₁₋₆ heteroalkylene bridge to the 4'-carbon of the same ribose sugar, where exemplary bridges include methylene, propylene, ether, amino bridges, aminoalkyl, aminoalkoxy, amino, and amino acid.

[0230] In some aspects, nucleotide analogues present in a polynucleotide of the present disclosure (*i.e.*, mir-485 inhibitor) comprise, *e.g.*, 2'-O-alkyl-RNA units, 2'-OMe-RNA units, 2'-O-alkyl-SNA, 2'-amino-DNA units, 2'-fluoro-DNA units, LNA units, arabino nucleic acid (ANA) units, 2'-fluoro-ANA units, HNA units, INA (intercalating nucleic acid) units, 2'MOE units, or any combination thereof. In some aspects, the LNA is, *e.g.*, oxy-LNA (such as beta-D-oxy-LNA, or alpha-L-oxy-LNA), amino-LNA (such as beta-D-amino-LNA or alpha-L-amino-LNA), thio-LNA (such as beta-D-thio-LNA or alpha-L-thio-LNA), ENA (such a beta-D-ENA or alpha-L-ENA), or any combination thereof. In further aspects, nucleotide analogues that can be included in a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) comprises a locked nucleic acid (LNA), an unlocked nucleic acid (UNA), an arabino nucleic acid (ABA), a bridged nucleic acid (BNA), and/or a peptide nucleic acid (PNA).

[0231] In some aspects, a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) can comprise both modified RNA nucleotide analogues (*e.g.*, LNA) and DNA units. In some aspects, a miR-485 inhibitor is a gapmer. See, *e.g.*, U.S. Pat. Nos. 8,404,649; 8,580,756; 8,163,708; and 9,034,837; all of which are herein incorporated by reference in their entireties. In some aspects, a miR-485 inhibitor is a micromir. See U.S. Pat. Appl. Publ. No. US20180201928, which is herein incorporated by reference in its entirety.

[0232] In some aspects, a polynucleotide of the present disclosure (*i.e.*, miR-485 inhibitor) can include modifications to prevent rapid degradation by endo- and exo-nucleases. Modifications include, but are not limited to, for example, (a) end modifications, *e.g.*, 5' end modifications (phosphorylation, dephosphorylation, conjugation, inverted linkages, *etc.*), 3' end modifications (conjugation, DNA nucleotides, inverted linkages, *etc.*), (b) base modifications, *e.g.*, replacement with modified bases, stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, or conjugated bases, (c) sugar modifications (*e.g.*, at the 2' position or 4' position) or replacement of the sugar, as well as (d) internucleoside linkage modifications, including modification or replacement of the phosphodiester linkages.

IV. Vectors and Delivery Systems

[0233] In some aspects, the miR-485 inhibitors of the present disclosure can be administered, *e.g.*, to a subject suffering from a disease or condition associated with abnormal (*e.g.*, reduced) level of a SIRT1 protein and/or SIRT1 gene, using any relevant delivery system known in the art. In certain aspects, the delivery system is a vector. Accordingly, in some aspects, the present disclosure provides a vector comprising a miR-485 inhibitor of the present disclosure.

[0234] In some aspects, the vector is viral vector. In some aspects, the viral vector is an adenoviral vector or an adenoassociated viral vector. In certain aspects, the viral vector is an AAV that has a serotype of AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or any combination thereof. In some aspects, the adenoviral vector is a third generation adenoviral vector. ADEASY™ is by far the most popular method for creating adenoviral vector constructs. The system consists of two types of plasmids: shuttle (or transfer) vectors and adenoviral vectors. The transgene of interest is cloned into the shuttle vector, verified, and linearized with the restriction enzyme PmeI. This construct is then transformed into ADEASIER-1 cells, which are BJ5183 *E. coli* cells containing PADEASY™. PADEASY™ is a ~33Kb adenoviral plasmid containing the adenoviral genes necessary for virus production. The shuttle vector and the adenoviral plasmid have matching left and right homology arms which facilitate homologous recombination of the transgene into the adenoviral plasmid. One can also co-transform standard BJ5183 with supercoiled PADEASY™ and the shuttle vector, but this method results in a higher background of non-recombinant adenoviral plasmids. Recombinant adenoviral plasmids are then verified for size and proper restriction digest patterns to determine that the transgene has been inserted into the adenoviral plasmid, and that other patterns of recombination have not occurred. Once verified, the recombinant plasmid is linearized with PacI to create a linear dsDNA construct

flanked by ITRs. 293 or 911 cells are transfected with the linearized construct, and virus can be harvested about 7-10 days later. In addition to this method, other methods for creating adenoviral vector constructs known in the art at the time the present application was filed can be used to practice the methods disclosed herein.

[0235] In some aspects, the viral vector is a retroviral vector, e.g., a lentiviral vector (e.g., a third or fourth generation lentiviral vector). Lentiviral vectors are usually created in a transient transfection system in which a cell line is transfected with three separate plasmid expression systems. These include the transfer vector plasmid (portions of the HIV provirus), the packaging plasmid or construct, and a plasmid with the heterologous envelop gene (*env*) of a different virus. The three plasmid components of the vector are put into a packaging cell which is then inserted into the HIV shell. The virus portions of the vector contain insert sequences so that the virus cannot replicate inside the cell system. Current third generation lentiviral vectors encode only three of the nine HIV-1 proteins (Gag, Pol, Rev), which are expressed from separate plasmids to avoid recombination-mediated generation of a replication-competent virus. In fourth generation lentiviral vectors, the retroviral genome has been further reduced (see, e.g., TAKARA® LENTI-X™ fourth-generation packaging systems).

[0236] Any AAV vector known in the art can be used in the methods disclosed herein. The AAV vector can comprise a known vector or can comprise a variant, fragment, or fusion thereof. In some aspects, the AAV vector is selected from the group consisting of AAV type 1 (AAV1), AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh.74, avian AAV, bovine AAV, canine AAV, equine AAV, goat AAV, primate AAV, non-primate AAV, bovine AAV, shrimp AAV, snake AAV, and any combination thereof.

[0237] In some aspects, the AAV vector is derived from an AAV vector selected from the group consisting of AAV1, AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh.74, avian AAV, bovine AAV, canine AAV, equine AAV, goat AAV, primate AAV, non-primate AAV, ovine AAV, shrimp AAV, snake AAV, and any combination thereof.

[0238] In some aspects, the AAV vector is a chimeric vector derived from at least two AAV vectors selected from the group consisting of AAV1, AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh.74, avian

AAV, bovine AAV, canine AAV, equine AAV, goat AAV, primate AAV, non-primate AAV, ovine AAV, shrimp AAV, snake AAV, and any combination thereof.

[0239] In certain aspects, the AAV vector comprises regions of at least two different AAV vectors known in the art.

[0240] In some aspects, the AAV vector comprises an inverted terminal repeat from a first AAV (*e.g.*, AAV1, AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh.74, avian AAV, bovine AAV, canine AAV, equine AAV, goat AAV, primate AAV, non-primate AAV, ovine AAV, shrimp AAV, snake AAV, or any derivative thereof) and a second inverted terminal repeat from a second AAV (*e.g.*, AAV1, AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh.74, avian AAV, bovine AAV, canine AAV, equine AAV, goat AAV, primate AAV, non-primate AAV, ovine AAV, shrimp AAV, snake AAV, or any derivative thereof).

[0241] In some aspects, the AAV vector comprises a portion of an AAV vector selected from the group consisting of AAV1, AAV2, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh.74, avian AAV, bovine AAV, canine AAV, equine AAV, goat AAV, primate AAV, non-primate AAV, ovine AAV, shrimp AAV, snake AAV, and any combination thereof. In some aspects, the AAV vector comprises AAV2.

[0242] In some aspects, the AAV vector comprises a splice acceptor site. In some aspects, the AAV vector comprises a promoter. Any promoter known in the art can be used in the AAV vector of the present disclosure. In some aspects, the promoter is an RNA Pol III promoter. In some aspects, the RNA Pol III promoter is selected from the group consisting of the U6 promoter, the H1 promoter, the 7SK promoter, the 5S promoter, the adenovirus 2 (Ad2) VAI promoter, and any combination thereof. In some aspects, the promoter is a cytomegalovirus immediate-early gene (CMV) promoter, an EF1a promoter, an SV40 promoter, a PGK1 promoter, a Ubc promoter, a human beta actin promoter, a CAG promoter, a TRE promoter, a UAS promoter, a Ac5 promoter, a polyhedrin promoter, a CaMKIIa promoter, a GAL1 promoter, a GAL10 promoter, a TEF promoter, a GDS promoter, a ADH1 promoter, a CaMV35S promoter, or a Ubi promoter. In a specific aspect, the promoter comprises the U6 promoter.

[0243] In some aspects, the AAV vector comprises a constitutively active promoter (constitutive promoter). In some aspects, the constitutive promoter is selected from the group consisting of hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin promoter, cytomegalovirus (CMV), simian virus (*e.g.*, SV40), papilloma virus,

adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, a retrovirus long terminal repeat (LTR), Murine stem cell virus (MSCV) and the thymidine kinase promoter of herpes simplex virus.

[0244] In some aspects, the promoter is an inducible promoter. In some aspects, the inducible promoter is a tissue specific promoter. In certain aspects, the tissue specific promoter drives transcription of the coding region of the AAV vector in a neuron, a glial cell, or in both a neuron and a glial cell.

[0245] In some aspects, the AAV vector comprises one or more enhancers. In some aspects, the one or more enhancer are present in the AAV alone or together with a promoter disclosed herein. In some aspects, the AAV vector comprises a 3'UTR poly(A) tail sequence. In some aspects, the 3'UTR poly(A) tail sequence is selected from the group consisting of bGH poly(A), actin poly(A), hemoglobin poly(A), and any combination thereof. In some aspects, the 3'UTR poly(A) tail sequence comprises bGH poly(A).

[0246] In some aspects, a miR-485 inhibitor disclosed herein is administered with a delivery agent. Non-limiting examples of delivery agents that can be used include a lipidoid, a liposome, a lipoplex, a lipid nanoparticle, a polymeric compound, a peptide, a protein, a cell, a nanoparticle mimic, a nanotube, a micelle, or a conjugate.

[0247] Thus, in some aspects, the present disclosure also provides a composition comprising a miRNA inhibitor of the present disclosure (*i.e.*, miR-485 inhibitor) and a delivery agent. In some aspects, the delivery agent comprises a cationic carrier unit comprising

[WP]-L1-[CC]-L2-[AM] (formula I)

or

[WP]-L1-[AM]-L2-[CC] (formula II)

wherein

WP is a water-soluble biopolymer moiety;

CC is a positively charged carrier moiety;

AM is an adjuvant moiety; and,

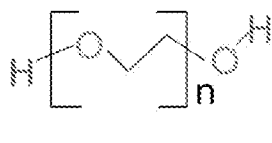
L1 and L2 are independently optional linkers, and

wherein when mixed with a nucleic acid at an ionic ratio of about 1:1, the cationic carrier unit forms a micelle.

[0248] In some aspects, composition comprising a miRNA inhibitor of the present disclosure (*i.e.*, miR-485 inhibitor) interacts with the cationic carrier unit via an ionic bond.

[0249] In some aspects, the cationic carrier units of the present disclosure comprise at least one water-soluble biopolymer. The term "water-soluble biopolymer" as used herein refers to a biocompatible, biologically inert, non-immunogenic, non-toxic, and hydrophilic polymer, *e.g.*, PEG.

[0250] In some aspects, the water-soluble biopolymer comprises poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyglycerol, polyphosphazene, polyoxazolines ("POZ") poly(N-acryloylmorpholine), or any combinations thereof. In some aspects, the water-soluble biopolymer comprises polyethylene glycol ("PEG"), polyglycerol, or poly(propylene glycol) ("PPG"). In some aspects, the water-soluble polymer comprises:



(formula III),

wherein n is 1-1000.

[0251] In some aspects, the n is at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125, at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, or at least about 141. In some aspects, the n is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 140 to about 150, about 150 to about 160.

[0252] In some aspects, the water-soluble polymer is linear, branched, or dendritic. In some aspects, the cationic carrier moiety comprises one or more basic amino acids.

[0253] In some aspects, the cationic carrier moiety comprises at least three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, at least about 32, at least about 33, at least about 34, at least about 35, at least about 36, at least

about 37, at least about 38, at least about 39, at least about 40, at least about 41, at least about 42, at least about 43, at least about 44, at least about 45, at least about 46, at least about 47, at least about 48, at least about 49, at least about 50, at least about 51, at least about 52, at least about 53, at least about 54, at least about 55, at least about 56, at least about 57, at least about 58, at least about 59, at least about 60, at least about 61, at least about 62, at least about 63, at least about 64, at least about 65, at least about 66, at least about 67, at least about 68, at least about 69, at least about 70, at least about 71, at least about 72, at least about 73, at least about 74, at least about 75, at least about 76, at least about 77, at least about 78, at least about 79, at least about 80 basic amino acids, *e.g.*, lysines, arginines, or combinations thereof.

[0254] In some aspects, the cationic carrier unit comprises at least about 40 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 45 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 50 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 55 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 60 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 65 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 70 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 75 basic amino acids, *e.g.*, lysines. In some aspects, the cationic carrier unit comprises at least about 80 basic amino acids, *e.g.*, lysines.

[0255] In some aspects, the number of basic amino acids, *e.g.*, lysines, arginines, histidines, or combinations thereof, can be adjusted based on the length of the anionic payload. As used herein the term "payload" refers to a biologically active molecule, *e.g.*, a therapeutic agent (*e.g.*, miR485-3p inhibitor) that can interact by itself or via an adapter with a cationic carrier unit of the present disclosure, and be included within the core of a micelle of the present disclosure. For example, an anionic payload with a longer sequence can be paired with higher number of basic amino acids, *e.g.*, lysines. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit can be calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, *e.g.*, oligonucleotide, *e.g.*, antimir (N/P ratio) is about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, or about 3. In some aspects, the number of basic amino acids, *e.g.*, lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, *e.g.*,

oligonucleotide, e.g., antimir (N/P ratio) is about 1.3 to about 1.7, e.g., about 1.5. In some aspects, the number of basic amino acids, e.g., lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., oligonucleotide, e.g., antimir (N/P ratio) is about 1.4. In some aspects, the number of basic amino acids, e.g., lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., oligonucleotide, e.g., antimir (N/P ratio) is about 1.6. In some aspects, the number of basic amino acids, e.g., lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., oligonucleotide, e.g., antimir (N/P ratio) is about 1.3. In some aspects, the number of basic amino acids, e.g., lysines, in the cationic carrier unit is calculated so that the molar ratio of protonated amine in polymer to phosphate in an anionic payload, e.g., oligonucleotide, e.g., antimir (N/P ratio) is about 1.7.

[0256] A person of skill in the art would understand that since a role of the cationic carrier moiety is to neutralize negative charges on the payload (e.g., negative charges in the phosphate backbone of an miRNA inhibitor) via electrostatic interaction, in some aspects (e.g., when the payload is a nucleic acid such as an antimir), the length of the cationic carrier, number of positively charged groups on the cationic carrier, and distribution and orientation of charges present on the cationic carrier will depend on the length and charge distribution on the payload molecule.

[0257] In some aspects, the cationic carrier comprises between about 5 and about 10, between about 10 and about 15, between about 15 and about 20, between about 20 and about 25, between about 25 and about 30, between about 30 and about 35, between about 35 and about 40, between about 40 and about 45, between about 45 and about 50, between about 50 and about 55, between about 55 and about 60, between about 60 and about 65, between about and about 70, between about 70 and about 75, or between about 75 and about 80 basic amino acids. In some specific aspects, the positively charged carrier comprises between 30 and about 50 basic amino acids. In some specific aspects, the positively charged carrier comprises between 70 and about 80 basic amino acids.

[0258] In some aspects, the basic amino acid comprises arginine, lysine, histidine, or any combination thereof. In some aspects, the basic amino acid is a D-amino acid. In some aspects, the basic amino acid is an L-amino acid. In some aspects, the positively charged carrier comprises D-amino acids and L-amino acids. In some aspects, the basic amino acid comprises at least one unnatural amino acid or a derivative thereof. In some aspects, the basic amino acid is arginine,

lysine, histidine, L-4-aminomethyl-phenylalanine, L-4-guanidine-phenylalanine, L-4-aminomethyl-N-isopropyl-phenylalanine, L-3-pyridyl-alanine, L-trans-4-aminomethylcyclohexyl-alanine, L-4-piperidiny-alanine, L-4-aminocyclohexyl-alanine, 4-guanidinobutyric acid, L-2-amino-3-guanidinopropionic acid, DL-5-hydroxylysine, pyrrolysine, 5-hydroxy-L-lysine, methyllysine, hypusine, or any combination thereof. In a particular aspect, the positively charged carrier comprises about 40 lysines. In a particular aspect, the positively charged carrier comprises about 50 lysines. In a particular aspect, the positively charged carrier comprises about 60 lysines. In a particular aspect, the positively charged carrier comprises about 70 lysines. In a particular aspect, the positively charged carrier comprises about 80 lysines. In a particular aspect, the positively charged carrier comprises about 30 lysines. In a particular aspect, the positively charged carrier comprises about 40 lysines. In a particular aspect, the positively charged carrier comprises about 38 lysines. In a particular aspect, the positively charged carrier comprises about 32 lysines. In a particular aspect, the positively charged carrier comprises about 35 lysines. In a particular aspect, the positively charged carrier comprises about 64 lysines. In a particular aspect, the positively charged carrier comprises about 63 lysines.

[0259] In some aspects, the cationic carrier moiety binds to a single payload molecule. In other aspects, a cationic carrier moiety can bind to multiple payload molecules, which may be identical or different.

[0260] In some aspects, the positive charges of the cationic carrier moiety and negative charges of a nucleic acid payload are at an ionic ratio of about 5:1, about 4:1, about 3:1, about 2.9:1, about 2.8:1, about 2.7:1, about 2.6:1, about 2.5:1, about 2.4:1, about 2.3:1, about 2.2:1, about 2:1, about 1.9:1, about 1.8:1, about 1.7:1, about 1.6:1, about 1.5:1, about 1.4:1, about 1.3:1, about 1.2:1, about 1.1:1, about 1:1, about 1:1.1, about 1:1.2, about 1:1.3, about 1:1.4, about 1:1.5, about 1:1.6, about 1:1.7, about 1:1.8, about 1:1.9, about 1:2, about 1:2.1, about 1:2.2, about 1:2.3, about 1:2.4, about 1:2.5, about 1:2.6, about 1:2.7, about 1:2.8, about 1:2.9, about 1:3, about 1:4, or about 1:5. In some aspects, the positive charges of the cationic carrier moiety and the negative charged of the nucleic acid payload are at a charge ratio of 1:1. In some aspects, the positive charges of the cationic carrier moiety and the negative charges of the nucleic acid payload are at a charge ratio of 3:2. In some aspects, the positive charges of the cationic carrier moiety and the negative charges of the nucleic acid payload are at a charge ratio of 2:3.

[0261] In some aspects, the cationic carrier units of the present disclosure can further comprise at least one crosslinking moiety (CM). The term "crosslinking moiety" refers to a moiety

or portion of a polymer block comprising a plurality of agents that are capable of forming crosslinks. In some aspects, the number of agents that are capable of forming crosslinks comprises an amino acid with a side chain of a crosslinking agent. In some aspects, the CM comprises a biopolymer, *e.g.*, a peptide (*e.g.*, a polylysine) linked to a crosslinking agent.

[0262] In some aspects, the crosslinking moiety comprises one or more amino acids (*e.g.*, lysine, arginine, histidine, or a combination thereof). In some aspects, the crosslinking moiety comprises at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30 amino acids, or at least about 35 amino acids, *e.g.*, lysines, arginines, or combinations thereof, each of which is linked to a crosslinking agent.

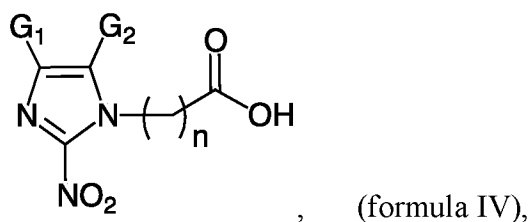
[0263] In some aspects, the lysines of the crosslinking moiety possess a neutral charge (*e.g.*, contain a tertiary amine). In some aspects, lysines of the crosslinking moiety contain a thiol (*e.g.*, lysine-thiol) and a tertiary amine, such that the lysines possess a neutral charge. In some aspects, the crosslinking moiety forms a crosslink through the tertiary amine. In some aspects, the crosslinking moiety forms a crosslink through the thiol. As used herein, “lysine” in the context of the crosslinking moiety, refers to lysines with a neutral charge (*e.g.*, containing a tertiary amine), such that the lysines of the crosslinking moiety do not contribute to the overall charge of the carrier unit. In some aspects, the lysines of the crosslinking moiety are linked to a crosslinking agent through an amide bond.

[0264] In some aspects, the crosslinking moiety comprises at least about 10 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 11 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 12 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 13 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 14 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 15 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 16 amino

acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 17 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 18 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 19 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 20 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 23 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent. In some aspects, the crosslinking moiety comprises at least about 35 amino acids, *e.g.*, lysines, each of which is linked to a crosslinking agent (*e.g.*, lysine-thiol).

[0265] In some aspects, a crosslinking agent is a thiol. In some aspects, a crosslinking agent is a thiol derivative.

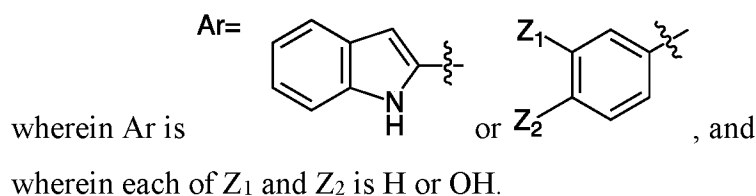
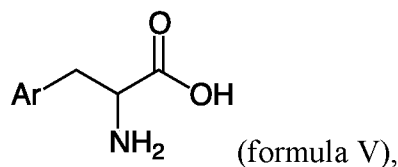
[0266] In some aspects, the adjuvant moiety is capable of modulating an immune response, an inflammatory response, and/or a tissue microenvironment. In some aspects, the adjuvant moiety comprises an imidazole derivative, an amino acid, a vitamin, or any combination thereof. In some aspects, the adjuvant moiety comprises:



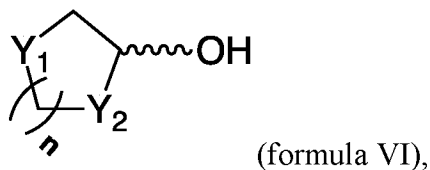
wherein each of G₁ and G₂ is H, an aromatic ring, or 1-10 alkyl, or G₁ and G₂ together form an aromatic ring, and wherein n is 1-10.

[0267] In some aspects, the adjuvant moiety comprises nitroimidazole. In some aspects, the adjuvant moiety comprises metronidazole, tinidazole, nimorazole, dimetridazole, pretomanid, ornidazole, megazol, azanidazole, benznidazole, or any combination thereof. In some aspects, the adjuvant moiety comprises an amino acid.

[0268] In some aspects, the adjuvant moiety comprises



[0269] In some aspects, the adjuvant moiety comprises a vitamin. In some aspects, the vitamin comprises a cyclic ring or cyclic hetero atom ring and a carboxyl group or hydroxyl group. In some aspects, the vitamin comprises:



wherein each of Y₁ and Y₂ is C, N, O, or S, and wherein n is 1 or 2.

[0270] In some aspects, the vitamin is selected from the group consisting of vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D2, vitamin D3, vitamin E, vitamin M, vitamin H, and any combination thereof. In some aspects, the vitamin is vitamin B3.

[0271] In some aspects, the adjuvant moiety comprises at least about two, at least about three, at least about four, at least about five, at least about six, at least about seven, at least about eight, at least about nine, at least about ten, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 26, at least about 27, at least about 28, at least about 29, at least about 30, at least about 31, at least about 32, at least about 33, at least about 34, at least about 35, at least about 36, at least about 37, at least about 38, at least about 39, or at least about 40 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 30 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 31 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 32 amino acids (e.g., lysines),

each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 33 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 34 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 35 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 36 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 37 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 38 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 39 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects, the adjuvant moiety comprises about 40 amino acids (e.g., lysines), each of which is linked to vitamin B3.

[0272] In some aspects the adjuvant moiety comprises from about 20 to about 25 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 25 to about 30 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 30 to about 35 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 35 to about 40 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 40 to about 45 amino acids (e.g., lysines), each of which is linked to vitamin B3, or about 45 to about 50 amino acids (e.g., lysines), each of which is linked to vitamin B3. In some aspects the adjuvant moiety comprises from about 20 to about 30 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 30 to about 40 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 40 to about 50 amino acids (e.g., lysines), each of which is linked to vitamin B3, about 25 to about 35 amino acids (e.g., lysines), each of which is linked to vitamin B3, or about 35 to about 45 amino acids (e.g., lysines), each of which is linked to vitamin B3.

[0273] In some aspects, the adjuvant moiety comprises at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, or at least about 20 vitamin B3 units. In some aspects, the adjuvant moiety comprises about 10 vitamin B3 units.

[0274] In some aspects, the cationic carrier unit comprises a targeting moiety, which is linked to the water-soluble polymer optionally via a linker. As used herein, the term "targeting moiety" refers to a biorecognition molecule that binds to a specific biological substance or site. In some aspects, the targeting moiety is specific for a certain target molecule (e.g., a ligand targeting

a receptor, or an antibody targeting a surface protein), tissue (*e.g.*, a molecule that would preferentially carry the micelle to a specific organ or tissue, *e.g.*, liver, brain, or endothelium), or facilitate transport through a physiological barrier (*e.g.*, a peptide or other molecule that may facilitate transport across the brain blood barrier or plasma membrane).

[0275] For targeting a payload (*e.g.*, a nucleotide molecule, *e.g.*, miR485-3p inhibitor) according to the present disclosure, a targeting moiety can be coupled to a cationic carrier unit, and therefore, to the external surface of a micelle, whereas the micelle has the payload entrapped within its core.

[0276] In some aspects, the targeting moiety is a targeting moiety capable of targeting the micelle of the present disclosure to a tissue. In some aspects, the tissue is liver, brain, kidney, lung, ovary, pancreas, thyroid, breast, stomach, or any combination thereof. In some aspects, the tissue is cancer tissue, *e.g.*, liver cancer, brain cancer, kidney cancer, lung cancer, ovary cancer, pancreas cancer, thyroid cancer, breast cancer, stomach cancer, or any combination thereof.

[0277] In some aspects, the tissue is a tissue in the central nervous system, *e.g.*, neural tissue. In some aspects, the targeting moiety targeting the central nervous system is capable being transported by Large-neutral Amino Acid Transporter 1 (LAT1). LAT1 (SLC7A5) is a transporter for both the uptake of large neutral amino acids and a number of pharmaceutical drugs. LAT1 can transport drugs such as L-dopa or gabapentin. LAT1 is consistently expressed at high levels in brain microvessel endothelial cells. Being a solute carrier located primarily in the BBB, targeting the micelles of the present disclosure to LAT1 allows delivery through the BBB. In some aspects, the targeting moiety targeting a micelle of the present disclosure to the LAT1 transporter is an amino acid, *e.g.*, a branched-chain or aromatic amino acid. In some aspects, the amino acid is valine, leucine, and/or isoleucine. In some aspects, the amino acid is tryptophan and/or tyrosine. In some aspects, the amino acid is tryptophan. In other aspects, the amino acid is tyrosine.

[0278] In some aspects, the targeting moiety is a LAT1 ligand selected from tryptophan, tyrosine, phenylalanine, tryptophan, methionine, thyroxine, melphalan, L-DOPA, gabapentin, 3,5-I-diiodotyrosine, 3-iodo-I-tyrosine, fenclonine, acivicin, leucine, BCH, methionine, histidine, valine, or any combination thereof. In some aspects, a targeting moiety comprises tyrosine, which can bind to LAT1 and cross BBB. In some aspects, a targeting moiety comprises lysine, which can bind to LAT1 and cross BBB. In some aspects, a targeting moiety comprises glutamine, which can bind to LAT1 and cross BBB. In some aspects, a targeting moiety comprises phenylalanine, which can bind to GABA receptors, LAT1, CNS reverse transcriptase inhibitors, and/or dopamine

(DA) receptors and cross BBB. Dopamine receptors are a class of G protein-coupled receptors that are prominent in the vertebrate central nervous system (CNS). Dopamine receptors activate different effectors through not only G-protein coupling, but also signaling through different protein (dopamine receptor-interacting proteins) interactions. The neurotransmitter dopamine is the primary endogenous ligand for dopamine receptors.

[0279] Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, cognition, memory, learning, and fine motor control, as well as modulation of neuroendocrine signaling. Abnormal dopamine receptor signaling and dopaminergic nerve function is implicated in several neuropsychiatric disorders. Thus, dopamine receptors are common neurologic drug targets; antipsychotics are often dopamine receptor antagonists while psychostimulants are typically indirect agonists of dopamine receptors.

[0280] In some aspects, a targeting moiety comprises valine, which can bind to CNS reverse transcriptase inhibitors and cross BBB. In some aspects, a targeting moiety comprises tryptophan, which can bind to GABA receptors and/or CNS reverse transcriptase inhibitors and cross BBB. In some aspects, a targeting moiety comprises leucine, which can bind to GABA receptors and/or CNS reverse transcriptase inhibitors and cross BBB. In some aspects, a targeting moiety comprises methionine, which can bind to GABA receptors and/or CNS reverse transcriptase inhibitors and cross BBB. In some aspects, a targeting moiety comprises histidine, which can bind to GABA receptors and cross BBB. In some aspects, a targeting moiety comprises isoleucine, which can bind to CNS reverse transcriptase inhibitors and cross BBB. In some aspects, a targeting moiety comprises Glutathione, which can bind to GSH transporter and cross BBB. In some aspects, a targeting moiety comprises Glutathione-Met, which can bind to GSH transporter and cross BBB. In some aspects, a targeting moiety comprises Urea/Thiourea, which can bind to Nitric oxide synthase (NOS) and bind to BBB. In some aspects, a targeting moiety comprises NAD⁺/NADH, which is capable of crossing BBB by REDOX mechanism. In some aspects, a targeting moiety comprises purine and can cross BBB. Additional examples of targeting moieties for CNS targeting are shown in Sutura et al. (2016): Small endogenous molecules as moiety to improve targeting of CNS drugs, Expert Opinion on Drug Delivery, DOI: 10.1080/17425247.2016.1208651, which is incorporated herein by reference in its entirety.

[0281] In some aspects, the composition comprises a water-soluble biopolymer moiety with about 120 to about 130 PEG units, a cationic carrier moiety comprising a poly-lysine with about 30 to about 40 lysines, and an adjuvant moiety with about 5 to about 10 vitamin B3 units.

[0282] The present disclosure also provides a micelle comprising a miRNA inhibitor of the present disclosure (*i.e.*, miR-485 inhibitor) wherein the miRNA inhibitor and the delivery agent are associated with each other.

[0283] In some aspects, the association is a covalent bond, a non-covalent bond, or an ionic bond. In some aspects, the positive charge of the cationic carrier moiety of the cationic carrier unit is sufficient to form a micelle when mixed with the miR-485 inhibitor disclosed herein in a solution, wherein the overall ionic ratio of the positive charges of the cationic carrier moiety of the cationic carrier unit and the negative charges of the miR-485 inhibitor (or vector comprising the inhibitor) in the solution is about 1:1.

[0284] In some aspects, the cationic carrier unit is capable of protecting the miRNA inhibitor of the present disclosure (*i.e.*, miR-485 inhibitor) from enzymatic degradation. See WO2020261227A1, which is herein incorporated by reference in its entirety.

[0285] In some aspects, the cationic carrier unit is associated with a miRNA inhibitor of the present disclosure (*e.g.*, miR485-3p inhibitor). In some aspects, the cationic carrier unit associated with a miRNA inhibitor of the present disclosure (*e.g.*, miR485-3p inhibitor) comprises 80 lysine residues, wherein 64 lysine residues are unmodified (*e.g.*, contain a positively charged amine, *e.g.*, -NH₃⁺) and wherein 16 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol). In some aspects, the cationic carrier unit associated with a miRNA inhibitor of the present disclosure (*e.g.*, miR485-3p inhibitor) comprises 80 lysine residues, wherein 40 lysine residues are unmodified (*e.g.*, contain positively charged amine) and wherein 35 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol) and wherein 5 lysine residues are modified to contain an adjuvant moiety (*e.g.*, a vitamin). In some aspects, the cationic carrier unit associated with a miRNA inhibitor of the present disclosure (*e.g.*, miR485-3p inhibitor) comprises 80 lysine residues, wherein 38 lysine residues are unmodified (*e.g.*, contain positively charged quaternary amine) and wherein 23 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol) and wherein 19 lysine residues are modified to contain an adjuvant moiety (*e.g.*, a vitamin). In some aspects, the cationic carrier unit associated with a miRNA inhibitor of the present disclosure (*e.g.*, miR485-3p inhibitor) comprises 80 lysine residues, wherein 32 lysine residues are unmodified (*e.g.*, contain a positively charged amine, *e.g.*, -NH₃⁺) and wherein 16 lysine residues are modified for crosslinking (*e.g.*, linked to a thiol, alkyl thiol, or lysine-thiol) and wherein 32 lysine residues are modified to contain an adjuvant moiety (*e.g.*, a vitamin). In some aspects, the cationic carrier

unit associated with a miRNA inhibitor of the present disclosure (e.g., miR485-3p inhibitor) comprises 80 lysine residues, wherein 63 lysine residues are unmodified (e.g., contain positively charged quaternary amine) and wherein 17 lysine residues are modified to contain an adjuvant moiety (e.g., a vitamin).

VI. Pharmaceutical compositions

[0286] In some aspects, the present disclosure also provides pharmaceutical compositions comprising a miR-485 inhibitor disclosed herein (e.g., a polynucleotide or a vector comprising the miR-485 inhibitor) that are suitable for administration to a subject. The pharmaceutical compositions generally comprise a miR-485 inhibitor described herein (e.g., a polynucleotide or a vector) and a pharmaceutically-acceptable excipient or carrier in a form suitable for administration to a subject. Pharmaceutically acceptable excipients or carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition.

[0287] Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions comprising a miR-485 inhibitor of the present disclosure. (See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. 18th ed. (1990)). The pharmaceutical compositions are generally formulated sterile and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

VII. Kits

[0288] The present disclosure also provides kits or products of manufacture, comprising a miRNA inhibitor of the present disclosure (e.g., a polynucleotide, vector, or pharmaceutical composition disclosed herein) and optionally instructions for use, e.g., instructions for use according to the methods disclosed herein. In some aspects, the kit or product of manufacture comprises a miR-485 inhibitor (e.g., vector, e.g., an AAV vector, a polynucleotide, or a pharmaceutical composition of the present disclosure) in one or more containers. In some aspects, the kit or product of manufacture comprises miR-485 inhibitor (e.g., a vector, e.g., an AAV vector, a polynucleotide, or a pharmaceutical composition of the present disclosure) and a brochure. One skilled in the art will readily recognize that miR-485 inhibitors disclosed herein (e.g., vectors, polynucleotides, and pharmaceutical compositions of the present disclosure, or combinations thereof) can be readily incorporated into one of the established kit formats which are well known in the art.

[0289] The following examples are offered by way of illustration and not by way of limitation.

Examples

Example 1: Preparation of miR-485 Inhibitor

[0290] **(a) Synthesis of alkyne modified tyrosine:** An alkyne modified tyrosine was generated as an intermediate for the synthesis of a tissue specific targeting moiety (TM, see FIG. 1) of a cationic carrier unit to direct micelles of the present disclosure to the LAT1 transporter in the BBB.

[0291] A mixture of N-(tert-butoxycarbonyl)-L-tyrosine methyl ester (Boc-Tyr-OMe) (0.5g, 1.69 mmol) and K₂CO₃ (1.5 equiv., 2.54 mmol) in acetonitrile (4.0 ml) was added drop by drop to propargyl bromide (1.2 equiv., 2.03 mmol). The reaction mixture was heated at 60 °C overnight. After the reaction, the reaction mixture was extracted using water:ethyl acetate (EA). Then, the organic layer was washed using a brine solution. The crude material was purified by flash column (EA in hexane 10%). Next, the resulting product was dissolved in 1,4-dioxane (1.0 ml) and 6.0 M HCl (1.0 ml). The reaction mixture was heated at 100 °C overnight. Next, the dioxane was removed and extracted by EA. Aqueous NaOH (0.5 M) solution was added to the mixture until the pH value become 7. The reactant was concentrated by evaporator and centrifuged at 12,000 rpm at 0°C. The precipitate was washed with deionized water and lyophilized.

[0292] **(b) Synthesis of poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-PLL):** This synthesis step generated the water-soluble biopolymer (WP) and cationic carrier (CC) of a cationic carrier unit of the present disclosure (see FIG. 1).

[0293] Poly(ethylene glycol)-*b*-poly(L-lysine) was synthesized by ring opening polymerization of Lys(TFA)-NCA with monomethoxy PEG (MeO-PEG) as a macroinitiator. In brief, MeO-PEG (600 mg, 0.12 mmol) and Lys(TFA)-NCA (2574 mg, 9.6 mmol) were separately dissolved in DMF containing 1M thiourea and DMF(or NMP). Lys(TFA)-NCA solution was dropped into the MeO-PEG solution by micro syringe and the reaction mixture was stirred at 37 °C for 4 days. The reaction bottles were purged with argon and vacuum. All reactions were conducted in argon atmosphere. After the reaction, the mixture was precipitated into an excess amount of diethyl ether. The precipitate was re-dissolved in methanol and precipitated again into cold diethyl ether. Then it was filtered and white powder was obtained after drying in vacuo. For the deprotection of TFA group in PEG-PLL(TFA), the next step was followed.

[0294] MeO-PEG-PLL(TFA) (500 mg) was dissolved in methanol (60 mL) and 1N NaOH (6 mL) was dropped into the polymer solution with stirring. The mixture was maintained for 1 day with stirring at 37°C. The reaction mixture was dialyzed against 10 mM HEPES for 4 times and distilled water. White powder of PEG-PLL was obtained after lyophilization.

[0295] **(b) Synthesis of azido-poly(ethylene glycol)-*b*-poly(L-lysine) (N₃-PEG-PLL):** This synthesis step generated the water-soluble biopolymer (WP) and cationic carrier (CC) of a cationic carrier unit of the present disclosure (see FIG. 1).

[0296] Azido-poly(ethylene glycol)-*b*-poly(L-lysine) was synthesized by ring opening polymerization of Lys(TFA)-NCA with azido-PEG (N₃-PEG). In brief, N₃-PEG (300 mg, 0.06 mmol) and Lys(TFA)-NCA (1287 mg, 4.8 mmol) were separately dissolved in DMF containing 1M thiourea and DMF(or NMP). Lys(TFA)-NCA solution was dropped into the N₃-PEG solution by micro syringe and the reaction mixture was stirred at 37 °C for 4 days. The reaction bottles were purged with argon and vacuum. All reactions were conducted in argon atmosphere. After the reaction, the mixture was precipitated into an excess amount of diethyl ether. The precipitate was re-dissolved in methanol and precipitated again into cold diethyl ether. Then it was filtered and white powder was obtained after drying in vacuo. For the deprotection of TFA group in PEG-PLL(TFA), the next step was followed.

[0297] N₃-PEG-PLL (500 mg) was dissolved in methanol (60 mL) and 1N NaOH (6 mL) was dropped into the polymer solution with stirring. The mixture was maintained for 1 day with stirring at 37°C. The reaction mixture was dialyzed against 10 mM HEPES for 4 times and distilled water. White powder of N₃-PEG-PLL was obtained after lyophilization.

[0298] **(c) Synthesis of (methoxy or) azido-poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/mercaptopropanamide) (N₃-PEG-PLL(Nic/SH)):** In this step, the tissue-specific adjuvant moieties (AM, see FIG. 1) were attached to the WP-CC component of a cationic carrier unit of the present disclosure. The tissue-specific adjuvant moiety (AM) used in the cationic carrier unit was nicotinamide (vitamin B3). This step would yield the WP-CC-AM components of the cationic carrier unit depicted in FIG. 1.

[0299] Azido-poly(ethylene glycol)-*b*-poly(L-lysine/nicotinamide/mercaptopropanamide) (N₃-PEG-PLL(Nic/SH)) was synthesized by chemical modification of N₃-PEG-PLL and nicotinic acid in the presence of EDC/NHS. N₃-PEG-PLL (372 mg, 25.8 μmol) and nicotinic acid (556.7 mg, 1.02 equiv. to NH₂ of PEG-PLL) were separately dissolved in mixture of deionized water and

methanol (1:1). EDC•HCl (556.7 mg, 1.5 equiv. to NH₂ of N₃-PEG-PLL) was added into nicotinic acid solution and NHS (334.2 mg, 1.5 equiv. to NH₂ of PEG-PLL) stepwise added into the mixture.

[0300] The reaction mixture was added into the N₃-PEG-PLL solution. The reaction mixture was maintained at 37 °C for 16 hours with stirring. After 16 hours, 3,3'-dithiodipropionic acid (36.8 mg, 0.1 equiv.) was dissolved in methanol, EDC•HCl (40.3 mg, 0.15 equiv.), and NHS (24.2 mg, 0.15 equiv.) were dissolved each in deionized water. Then, NHS and EDC•HCl were added sequentially into 3,3'-dithiodipropionic acid solution. The mixture solution was stirred for 4 hours at 37 °C after adding crude N₃-PEG-PLL(Nic) solution.

[0301] For purification, the mixture was dialyzed against methanol for 2 hours, added DL-dithiothreitol (DTT, 40.6 mg, 0.15 equiv.), then activated for 30 min.

[0302] For removing the DTT, the mixture was dialyzed sequentially methanol, 50 % methanol in deionized water, deionized water.

[0303] **d) Synthesis of Phenyl alanine-poly(ethylene glycol)-b-poly(L-lysine/nicotinamide/mercaptopropanamide) (Phe-PEG-PLL(Nic/SH)):** In this step, the tissue-specific targeting moiety (TM) was attached to the WP-CC-AM component synthesized in the previous step. The TM component (phenylalanine) was generated by reaction of the intermediate generated in step (a) with the product of step (c).

[0304] To target brain endothelial tissue in blood vessels, as a LAT1 targeting amino acid, phenyl alanine was introduced by click reaction between N₃-PEG-PLL(Nic/SH) and alkyne modified tyrosine in the presence of copper catalyst. In brief, N₃-PEG-PLL(Nic/SH) (130 mg, 6.5 μmol) and alkyne modified phenyl alanine (5.7 mg, 4.0 equiv.) were dissolved in deionized water (or 50 mM sodium phosphate buffer). Then, CuSO₄•H₂O (0.4 mg, 25 mol%) and Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 3.4 mg, 1.2 equiv.) were dissolved deionized water and added N₃-PEG-PLL(Nic/SH) solution. Then, sodium ascorbate (3.2 mg, 2.5 equiv.) were added into the mixture solution. The reaction mixture was maintained with stirring for 16 hours at room temperature. After the reaction, the mixture was transferred into dialysis membranes (MWCO = 7,000) and dialyzed against deionized water for 1 day. The final product was obtained after lyophilization.

[0305] **(e) Polyion Complex (PIC) micelle preparation** - Once the cationic carrier units of the present disclosure were generated as described above, micelles were produced. The micelles described in the present example comprised cationic carrier units combined with an antisense oligonucleotide payload.

[0306] Nano sized PIC micelles were prepared by mixing MeO- or Phe-PEG-PLL(Nic) and miRNA. PEG-PLL(Nic) was dissolved in HEPES buffer (10 mM) at 0.5 mg/mL concentration. Then a miRNA solution (22.5 μ M) in RNase free water was mixed with the polymer solution at 2:1 (v/v) ratio of miRNA inhibitor (SEQ ID NOs: 2-30) (*e.g.*, 5'-AGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 28) to polymer.

[0307] The mixing ratio of polymer to anti-miRNA was determined by optimizing micelle forming conditions, *i.e.*, ratio between amine in polymer (carrier of the present disclosure) to phosphate in anti-miRNA (payload). The mixture of polymer (carrier) and anti-miRNA (payload) was vigorously mixed for 90 seconds by multi-vortex at 3000 rpm, and kept at room temperature for 30 min to stabilize the micelles.

[0308] Micelles (10 μ M of Anti-miRNA concentration) were stored at 4 °C prior to use. MeO- or Phe- micelles were prepared using the same method, and different amounts of Phe-containing micelles (25% ~75%) were also prepared by mixing both polymers during micelle preparation.

Example 2: Effect of miR-485 inhibitors on Htt degradation in NSC-34 cells

[0309] Cell culture

[0310] Mouse motor neuron-like hybrid cell line (NSC-34) cells were purchased from Cedarlane Labs. NSC-34 cells were maintained in culture medium (DMEM supplemented with 10% (vol/vol) fetal bovine serum (Gibco), 100 units/ml penicillin, 50 μ g/ml streptomycin) and maintained at 37°C in a humidified 5% CO₂ incubator.

[0311] Western blot (insoluble fraction)

[0312] To obtain the insoluble fraction from NSC-34 cells, the cell pellet was lysed in insoluble extraction buffer [50mM Tris-HCl (pH 7.5) + 2% SDS] containing protease/phosphatase inhibitor cocktail on ice for 30 minutes. The lysates were centrifuged at 4°C for 15 minutes at 13,000 rpm. Protein was quantified using a bicinchoninic acid (BCA) assay kit (Bio-Rad Laboratories, Cat#5000116) and adjusted to the same final concentration. After denaturation, the lysates were processed for western blotting to measure insoluble Htt. The samples were separated by SDS–polyacrylamide gel electrophoresis, transferred to PVDF membranes and incubated with mouse anti-Htt primary antibody (Merck, Cat# MAB5374, 1:1000). The results were visualized using an enhanced chemiluminescence system.

[0313] Results

[0314] To determine the effect of miR-485 inhibitors on huntingtin (Htt) degradation, NSC-34 cells (mouse motor neuron like cells) were transfected with GFP-tagged wild-type (Q23) (pEGFP-Q23) or mutant (Q74) (pEGFP-Q74) Htt. After transfection with GFP-tagged wild-type Q23 (pEGFP-Q23) Htt or mutant Q74 (pEGFP-Q74) Htt, miR485-3p inhibitor was co-treated in transfected NSC34. The insoluble fraction was obtained as previously described (after 48 hours) and the level of insoluble, aggregated Htt in Q74-GFP-tagged NSC-34 cells and Q24-GFP-tagged NSC-34 cells was analyzed. Insoluble, aggregated Htt decreased significantly in micelle containing miR485-3p inhibitor (5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28)) treated-NSC-34 cells expressing mutated Htt (FIGs. 2A and 2B).

Example 3: Effect of miR-485 inhibitors on Htt degradation in HEK293T, PC12, and primary cortical neurons

[0315] To determine whether miR485-3p ASO treatment can attenuate Htt aggregation, HEK293T and PC12 cells were plated in 6-well plates overnight and transfected with 2 µg Q23-EGFP, Q74-EGFP using JETOPTIMUS® Transfection Reagent (polyplus) and treated with miR485-3p ASO (i.e., SEQ ID NO: 28) for 48 hours at a final concentration of 50, 100, and 300 nM. As shown in FIGs. 3A and 3B, western blot analysis revealed that Htt-Q74 overexpression increased Htt aggregation. The cells were analyzed by fluorescence microscopy and lysates were harvested for western blot analysis.

[0316] Htt aggregation (puncta) at 48 hours posttransfection was assessed under fluorescence microscopy on the basis of enhanced green fluorescence gene (EGFP) expression.

[0317] After the treatment described above, cells were harvested and lysed with ice-cold RIPA buffer (iNtRON Biotechnology) containing protease/phosphatase inhibitor cocktail (Cell signaling Technology, Cat#5872) on ice for 30 minutes. The lysates were centrifuged at 13,000 rpm for 15 minutes at 4 °C, and supernatants were collected. The samples were separated by SDS–polyacrylamide gel electrophoresis, transferred to PVDF membranes and incubated with the following primary antibodies: mouse anti-HTT clone EM48(1:1000, Sigma-Aldrich, St Louis, MO, USA) mouse anti-SIRT1 (1:1000; Abcam, Cambridge, MA, USA), mouse anti-PGC-1a (1:1000; Abcam, Cambridge, MA, USA), mouse anti-p62 (1:1000; Abcam, Cambridge, MA, USA), rabbit anti-cleaved caspase-3 (1:1000; Cell Signaling Technology, Danvers, MA, USA), mouse anti-GFP (1:1000; anta Cruz Biotechnology), rabbit anti-LC3B (1:1000; Novus Biologicals, Centennial, CO, USA), and mouse anti-β-actin (Santa Cruz Biotechnology). Subsequently, the membranes were incubated with secondary antibodies for 1 hour at room temperature, and the

bands were detected using Western-blot detection reagents (Thermo Fisher Scientific, Rockford, IL, USA). For quantitative analyses, the density of each band was measured using a Computer Imaging Device and accompanying software (Fuji Film, Tokyo, Japan), and the levels were quantitatively expressed as the density normalized to the housekeeping protein band for each sample.

[0318] Western blot analysis revealed that HTT-Q74 overexpression increased HTT aggregation. However, miR485-3p ASO treatment decreased HTT aggregation, indicating that miR485-3p ASO treatment can suppress Htt aggregation in HTT-Q74-overexpressing HEK293T cells (FIGs. 3A and 3B).

[0319] Because autophagy can remove intracellular misfolded proteins, such as Htt aggregates, autophagy related proteins were analyzed by western blot analysis. FIGs. 4A through 4E show that miR485-3p ASO treatment markedly upregulated autophagy stimulators, such as SIRT1, PGC-1a, p62 and LC3-II, demonstrating miR485-3p ASO decreased Htt aggregation through autophagic mechanisms. Reduced Htt aggregation was also observed in PC12 cells treated with miR485-3p ASO by stimulating autophagy through increased expression of SIRT1, PGC-1a, p62 and LC3. (FIGs. 5 and 6). In addition, miR485-3p ASO treatment decreased cleavage of caspase-3, a marker of apoptosis (FIG. 6), demonstrating that Htt induced neuronal apoptosis was blocked by miR485-3p ASO.

[0320] To further validate the effect of miR485-3p ASO treatment on reduction of Htt aggregates, PC12 and primary cortical neurons expressing GFP-tagged wild-type (Q23) or mutant (Q74) Htt were treated with miR485-3p ASO. Under control conditions, cells expressing Q23-Htt showed diffuse GFP distribution with no detectable aggregates in either PC12 cells (FIGs. 7A-7E) or primary cortical neurons (FIGs. 8A-8D). In contrast, both cell types expressing Q74-Htt displayed Htt aggregates and miR-485-3p ASO treatment reduced Htt aggregation compared to control PC12 cells (FIGs. 7A-7E) and primary cortical neurons (FIGs. 8A-8D).

[0321] Primary cortical neurons were cultured from embryonic day 17 mice. In brief, cortices were dissected and incubated in ice-cold HBSS (Welgene, LB003-02) solution and dissociated in accumax (sigma, Cat#A7089) for 15 minutes at 37°C. The cultures were rinsed twice in HBSS. Mouse neurons were resuspended in neurobasal media (Gibco, Cat#21103049) containing 2% B27 (Gibco, Cat#17504), 1% sodium pyruvate, and 1% P/S. Cells were filtered through a 70µm cell strainer (SPL, 93070), plated on culture plates and maintained at 37°C in a

humidified 5% CO₂ incubator. The medium was changed every 3 days and then after 10 days in vitro, cells were used for experiments.

[0322] All data are expressed as the mean±s.d. Post-hoc comparisons (Student–Newman–Keuls test) were performed using Prism 8.

Example 4: miR485-3p ASO enhances degradation of Htt aggregates by regulation of autophagy

[0323] To further validate that miR485-3p ASO treatment can promote degradation of Htt aggregates, PC12 cells were transfected with Htt Q23 or Htt Q74 in combination with miR485-3p (5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28). Compared with Q23 (FIGs. 9A-9I) transfected cells, increased aggregation of Htt protein was detectable in Q74 transfected PC12 cells (PJ-9R), and was drastically decreased after miR485-3p ASO transfection (Figs. 9A-9R). A higher abundance of autophagic vacuoles were also observed (e.g., LC3B with Htt protein colocalized with LC3B in Q74-miR485-3p ASO compared with Q74-Conrol), indicating that miR-485-3p ASO can significantly reduce levels of Htt aggregation in Q74-miR485-3p ASO transfected cells.

[0324] PC12 cells (8×10^4) were plated in 12-well plates overnight. PC12 cells obtained from the American Type Culture Collection (Bethesda, MD, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Welgene) with 10% fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin; and kept at 37 °C in a humidified atmosphere of 5% CO₂. Cells were cultured in 24-well plates and were transfected after 24 hours of culture with 2 µg of pEGFP HTT (Q23)/mHTT (Q74) plasmid with TransIT-X2™ (Mirus). After transfections and media changes, cells were transfected with miR485-3p ASO. After 48 hours of transfection, cells grown on coverslips were fixed with a 4% paraformaldehyde solution, blocked, and then incubated with the primary and corresponding fluorescence-conjugated secondary antibodies. Aggregation of HTT protein and autophagy were assessed under fluorescence microscopy on the basis of enhanced green fluorescence gene (EGFP) expression and LC3B puncta.

[0325] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections can set forth one or more but not all exemplary aspects of the present disclosure as

contemplated by the inventor(s), and thus, are not intended to limit the present disclosure and the appended claims in any way.

[0326] The present disclosure has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[0327] The foregoing description of the specific aspects will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0328] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

[0329] The contents of all cited references (including literature references, patents, patent applications, and websites) that can be cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose, as are the references cited therein.

WHAT IS CLAIMED IS:

1. A method of treating Huntington's disease in a subject in need thereof comprising administering to the subject a compound that inhibits miR-485 (miRNA inhibitor).
2. The method of claim 1, wherein the subject exhibits one or more characteristics of Huntington's disease comprising irritability, depression, involuntary movements, poor coordination, trouble learning new information or making decisions, uncontrolled movements, emotional problems, and loss of thinking ability (cognition) prior to administration.
3. The method of claim 2, wherein the subject exhibits, after the administration, an improvement in one or more characteristics of Huntington's disease.
4. The method of claim 3, wherein the improvement is at least about 1.5 fold, at least about 2 fold, at least about 3 fold, at least about 4 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, or at least about 10 fold compared to the characteristics prior to the administration.
5. The method of any one of claims 1 to 4, wherein the Huntington's disease is associated with a decreased level of a SIRT1 protein and/or a SIRT1 gene.
6. The method of any one of claims 1 to 5, wherein the miRNA inhibitor induces autophagy and/or treats or prevents inflammation.
7. The method of any one of claims 1 to 6, wherein the Huntington's disease is associated with a decreased level of a CD36 protein and/or a CD36 gene.
8. The method of any one of claims 1 to 7, wherein the subject has a disease or a condition associated with a decreased level of a PGC-1 α protein and/or a PGC-1 α gene.
9. The method of any one of claims 1 to 8, wherein the miRNA inhibitor induces neurogenesis.
10. The method of claim 9, wherein inducing neurogenesis comprises an increased proliferation, differentiation, migration, and/or survival of neural stem cells and/or progenitor cells.
11. The method of claim 9 or 10, wherein inducing neurogenesis comprises an increased number of neural stem cells and/or progenitor cells.

12. The method of any one of claims 9 to 11, wherein the inducing neurogenesis comprises an increased axon, dendrite, and/or synapse development.
13. The method of any one of claims 1 to 12, wherein the miRNA inhibitor induces phagocytosis.
14. The method of any one of claims 1 to 13, wherein the miRNA inhibitor inhibits miR485-3p.
15. The method of claim 14, wherein the miR485-3p comprises 5'-gucauacacggcucuccucucu-3' (SEQ ID NO: 1).
16. The method of any one of claims 1 to 15, wherein the miRNA inhibitor comprises a nucleotide sequence comprising 5'-UGUAUGA-3' (SEQ ID NO: 2) and wherein the miRNA inhibitor comprises about 7 to about 30 nucleotides in length.
17. The method of any one of claims 1 to 16, wherein the miRNA inhibitor increases transcription of an SIRT1 gene and/or expression of a SIRT1 protein.
18. The method of any one of claims 1 to 17, wherein the miRNA inhibitor comprises at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, or at least 20 nucleotides at the 5' of the nucleotide sequence.
19. The method of any one of claims 1 to 18, wherein the miRNA inhibitor comprises at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, or at least 20 nucleotides at the 3' of the nucleotide sequence.
20. The method of any one of claims 1 to 19, wherein the miRNA inhibitor has a sequence selected from the group consisting of: 5'-UGUAUGA-3' (SEQ ID NO: 2), 5'-GUGUAUGA-3' (SEQ ID NO: 3), 5'-CGUGUAUGA-3' (SEQ ID NO: 4), 5'-CCGUGUAUGA-3' (SEQ ID NO: 5),

5'-GCCGUGUAUGA-3' (SEQ ID NO: 6), 5'-AGCCGUGUAUGA-3' (SEQ ID NO: 7), 5'-GAGCCGUGUAUGA-3' (SEQ ID NO: 8), 5'-AGAGCCGUGUAUGA-3' (SEQ ID NO: 9), 5'-GAGAGCCGUGUAUGA-3' (SEQ ID NO: 10), 5'-GGAGAGCCGUGUAUGA-3' (SEQ ID NO: 11), 5'-AGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 12), 5'-GAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 13), 5'-AGAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 14), 5'-GAGAGGAGAGCCGUGUAUGA-3' (SEQ ID NO: 15); 5'-UGUAUGAC-3' (SEQ ID NO: 16), 5'-GUGUAUGAC-3' (SEQ ID NO: 17), 5'-CGUGUAUGAC-3' (SEQ ID NO: 18), 5'-CCGUGUAUGAC-3' (SEQ ID NO: 19), 5'-GCCGUGUAUGAC-3' (SEQ ID NO: 20), 5'-AGCCGUGUAUGAC-3' (SEQ ID NO: 21), 5'-GAGCCGUGUAUGAC-3' (SEQ ID NO: 22), 5'-AGAGCCGUGUAUGAC-3' (SEQ ID NO: 23), 5'-GAGAGCCGUGUAUGAC-3' (SEQ ID NO: 24), 5'-GGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 25), 5'-AGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 26), 5'-GAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 27), 5'-AGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 28), and 5'-GAGAGGAGAGCCGUGUAUGAC-3' (SEQ ID NO: 29).

21. The method of any one of claims 1 to 15 or 17-18, wherein the miRNA inhibitor has a sequence selected from the group consisting of: 5'-TGTATGA-3' (SEQ ID NO: 62), 5'-GTGTATGA-3' (SEQ ID NO: 63), 5'-CGTGTATGA-3' (SEQ ID NO: 64), 5'-CCGTGTATGA-3' (SEQ ID NO: 65), 5'-GCCGTGTATGA-3' (SEQ ID NO: 66), 5'-AGCCGTGTATGA-3' (SEQ ID NO: 67), 5'-GAGCCGTGTATGA-3' (SEQ ID NO: 68), 5'-AGAGCCGTGTATGA-3' (SEQ ID NO: 69), 5'-GAGAGCCGTGTATGA-3' (SEQ ID NO: 70), 5'-GGAGAGCCGTGTATGA-3' (SEQ ID NO: 71), 5'-AGGAGAGCCGTGTATGA-3' (SEQ ID NO: 72), 5'-GAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 73), 5'-AGAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 74), 5'-GAGAGGAGAGCCGTGTATGA-3' (SEQ ID NO: 75); 5'-TGTATGAC-3' (SEQ ID NO: 76), 5'-GTGTATGAC-3' (SEQ ID NO: 77), 5'-CGTGTATGAC-3' (SEQ ID NO: 78), 5'-CCGTGTATGAC-3' (SEQ ID NO: 79), 5'-GCCGTGTATGAC-3' (SEQ ID NO: 80), 5'-AGCCGTGTATGAC-3' (SEQ ID NO: 81), 5'-GAGCCGTGTATGAC-3' (SEQ ID NO: 82), 5'-AGAGCCGTGTATGAC-3' (SEQ ID NO: 83), 5'-GAGAGCCGTGTATGAC-3' (SEQ ID NO: 84), 5'-GGAGAGCCGTGTATGAC-3' (SEQ ID NO: 85), 5'-AGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 86), 5'-GAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 87), 5'-AGAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 88), and 5'-GAGAGGAGAGCCGTGTATGAC-3' (SEQ ID NO: 89).

22. The method of any one of claims 1 to 19, wherein the sequence of the miRNA inhibitor is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% sequence identity to 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).
23. The method of claim 22, wherein the miRNA inhibitor has a sequence that has at least 90% similarity to 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).
24. The method of any one of claims 1 to 23, wherein the miRNA inhibitor comprises the nucleotide sequence 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88) with one substitution or two substitutions.
25. The method of any one of claims 1 to 23, wherein the miRNA inhibitor comprises the nucleotide sequence 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28) or 5'- AGAGGAGAGCCGTGTATGAC -3' (SEQ ID NO: 88).
26. The method of any one of claims 1 to 23, wherein the miRNA inhibitor comprises the nucleotide sequence 5'- AGAGGAGAGCCGUGUAUGAC -3' (SEQ ID NO: 28).
27. The method of any one of claims 1 to 26, wherein the miRNA inhibitor comprises at least one modified nucleotide.
28. The method of claim 27, wherein the at least one modified nucleotide is a locked nucleic acid (LNA), an unlocked nucleic acid (UNA), an arabino nucleic acid (ABA), a bridged nucleic acid (BNA), and/or a peptide nucleic acid (PNA).
29. The method of any one of claims 1 to 28, wherein the miRNA inhibitor comprises a backbone modification.
30. The method of claim 29, wherein the backbone modification is a phosphorodiamidate morpholino oligomer (PMO) and/or phosphorothioate (PS) modification.
31. The method of any one of claims 1 to 30, wherein the miRNA inhibitor is delivered in a delivery agent.

32. The method of claim 31, wherein the delivery agent is a micelle, an exosome, a lipid nanoparticle, an extracellular vesicle, or a synthetic vesicle.
33. The method of any one of claims 1 to 32, wherein the miRNA inhibitor is delivered by a viral vector.
34. The method of claim 33, wherein the viral vector is an AAV, an adenovirus, a retrovirus, or a lentivirus.
35. The method of claim 34, wherein the viral vector is an AAV that has a serotype of AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, or any combination thereof.
36. The method of any one claims 1 to 35, wherein the miRNA inhibitor is delivered with a delivery agent.
37. The method of claim 36, wherein the delivery agent comprises a lipidoid, a liposome, a lipoplex, a lipid nanoparticle, a polymeric compound, a peptide, a protein, a cell, a nanoparticle mimic, a nanotube, or a conjugate.
38. The method of claim 36 or 37, wherein the delivery agent comprises a cationic carrier unit comprising

[WP]-L1-[CC]-L2-[AM] (formula I)

or

[WP]-L1-[AM]-L2-[CC] (formula II)

wherein

WP is a water-soluble biopolymer moiety;

CC is a positively charged carrier moiety;

AM is an adjuvant moiety; and,

L1 and L2 are independently optional linkers, and

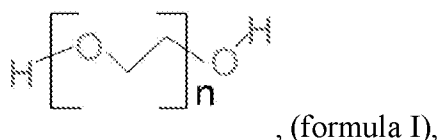
wherein when mixed with a nucleic acid at an ionic ratio of about 1:1, the cationic carrier unit forms a micelle.

39. The method of claim 38, wherein the miRNA inhibitor interacts with the cationic carrier unit via an ionic bond.

40. The method of claims 38 or 39, wherein the water-soluble biopolymer comprises poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α -hydroxy acid), poly(vinyl alcohol), polyglycerol, polyphosphazene, polyoxazolines ("POZ") poly(N-acryloylmorpholine), or any combinations thereof.

41. The method of claims 38 to 40, wherein the water-soluble biopolymer comprises polyethylene glycol ("PEG"), polyglycerol, or poly(propylene glycol) ("PPG").

42. The method of any one of claims 38 to 41, wherein the water-soluble biopolymer comprises:



wherein n is 1-1000.

43. The method of claim 42, wherein the n is at least about 110, at least about 111, at least about 112, at least about 113, at least about 114, at least about 115, at least about 116, at least about 117, at least about 118, at least about 119, at least about 120, at least about 121, at least about 122, at least about 123, at least about 124, at least about 125, at least about 126, at least about 127, at least about 128, at least about 129, at least about 130, at least about 131, at least about 132, at least about 133, at least about 134, at least about 135, at least about 136, at least about 137, at least about 138, at least about 139, at least about 140, or at least about 141.

44. The method of claim 42, wherein the n is about 80 to about 90, about 90 to about 100, about 100 to about 110, about 110 to about 120, about 120 to about 130, about 140 to about 150, or about 150 to about 160.

45. The method of any one of claims 38 to 44, wherein the water-soluble biopolymer is linear, branched, or dendritic.

46. The method of any one of claims 38 to 45, wherein the cationic carrier moiety comprises one or more basic amino acids.

47. The method of claim 46, wherein the cationic carrier moiety comprises at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22,

at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50 basic amino acids.

48. The method of claim 47, wherein the cationic carrier moiety comprises about 30 to about 50 basic amino acids.

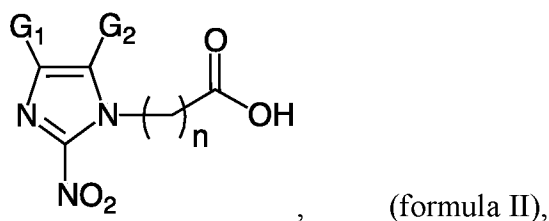
49. The method of claim 47 or claim 48, wherein the basic amino acid comprises arginine, lysine, histidine, or any combination thereof.

50. The method of any one of claims 38 to 49, wherein the cationic carrier moiety comprises about 40 lysine monomers.

51. The method of any one of claims 38 to 50, wherein the adjuvant moiety is capable of modulating an immune response, an inflammatory response, and/or a tissue microenvironment.

52. The method of any one of claims 38 to 51, wherein the adjuvant moiety comprises an imidazole derivative, an amino acid, a vitamin, or any combination thereof.

53. The composition of claim 52, wherein the adjuvant moiety comprises:



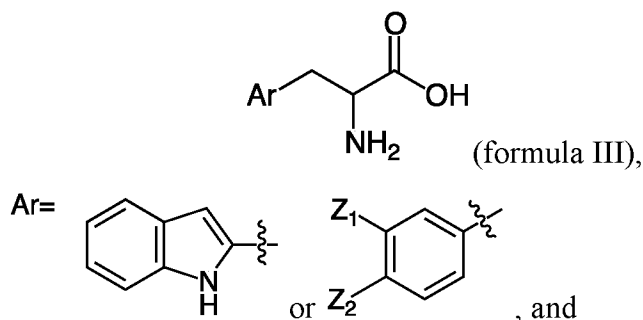
wherein each of G₁ and G₂ is H, an aromatic ring, or 1-10 alkyl, or G₁ and G₂ together form an aromatic ring, and wherein n is 1-10.

54. The method of claim 52, wherein the adjuvant moiety comprises nitroimidazole.

55. The method of claim 52, wherein the adjuvant moiety comprises metronidazole, tinidazole, nimorazole, dimetridazole, pretomanid, ornidazole, megazol, azanidazole, benznidazole, or any combination thereof.

56. The method of any one of claims 38 to 52, wherein the adjuvant moiety comprises an amino acid.

57. The method of claim 56, wherein the adjuvant moiety comprises



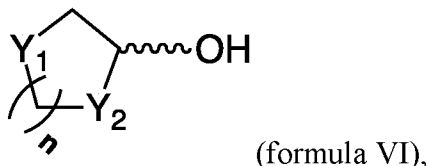
wherein Ar is

wherein each of Z₁ and Z₂ is H or OH.

58. The method of any one of claims 38 to 51, wherein the adjuvant moiety comprises a vitamin.

59. The method of claim 58, wherein the vitamin comprises a cyclic ring or cyclic hetero atom ring and a carboxyl group or hydroxyl group.

60. The method of claim 58 or claim 59, wherein the vitamin comprises:



wherein each of Y₁ and Y₂ is C, N, O, or S, and wherein n is 1 or 2.

61. The method of any one of claims 58 to 60, wherein the vitamin is selected from the group consisting of vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B6, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D2, vitamin D3, vitamin E, vitamin M, vitamin H, and any combination thereof.

62. The method of any one of claims 58 to 61, wherein the vitamin is vitamin B3.

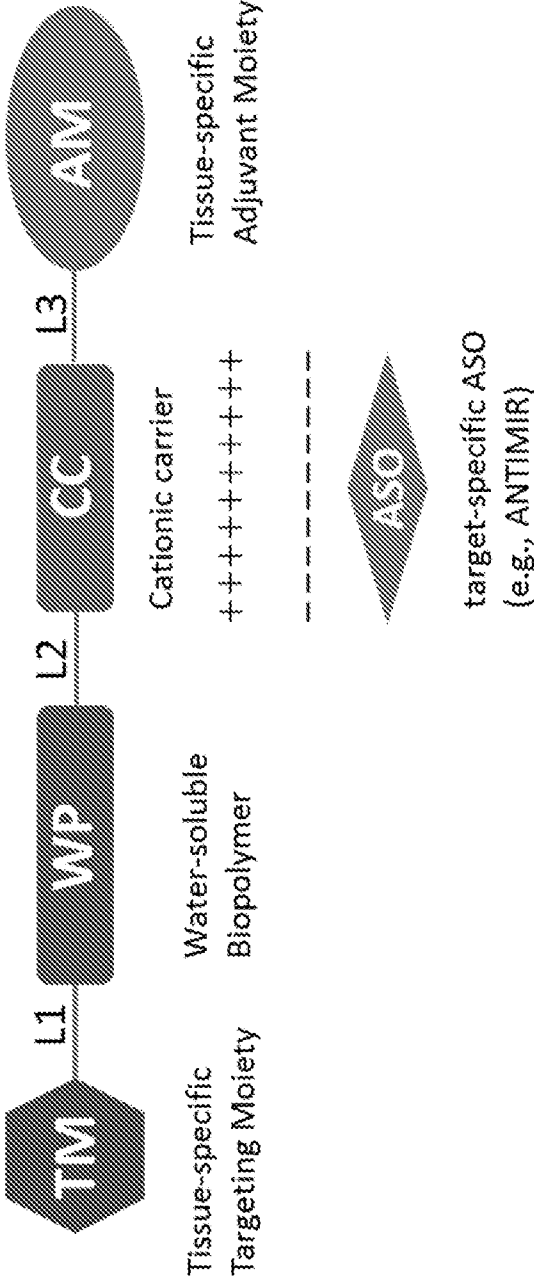
63. The method of any one of claims 58 to 62, wherein the adjuvant moiety comprises at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, or at least about 20 vitamin B3 units.

64. The method of claim 63, wherein the adjuvant moiety comprises about 10 vitamin B3 units.
65. The method of any one of claims 58 to 64, wherein the delivery agent comprises about a water-soluble biopolymer moiety with about 120 to about 130 PEG units, a cationic carrier moiety comprising a poly-lysine with about 30 to about 40 lysines, and an adjuvant moiety with about 5 to about 10 vitamin B3 units.
66. The method of any one of claims 58 to 65, wherein the delivery agent is associated with the miRNA inhibitor, thereby forming a micelle.
67. The method of claim 66, wherein the association is a covalent bond, a non-covalent bond, or an ionic bond.
68. The method of claim 66 or claim 67, wherein the cationic carrier unit and the miRNA inhibitor in the micelle is mixed in a solution so that the ionic ratio of the positive charges of the cationic carrier unit and the negative charges of the miRNA inhibitor is about 1:1.
69. The method of any one of claims 66 to 68, wherein the cationic carrier unit is capable of protecting the miRNA inhibitor from enzymatic degradation.

Fig. 1

Cationic Carrier Unit

L1, L2, L3 – Optional Linker moieties (each moiety can comprise one or more linkers, which can be the same of different)



Anionic Payload

Fig. 2A

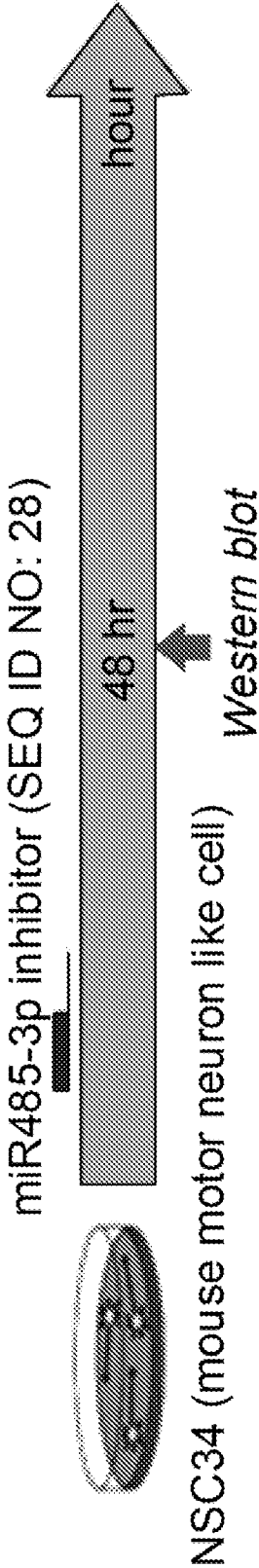
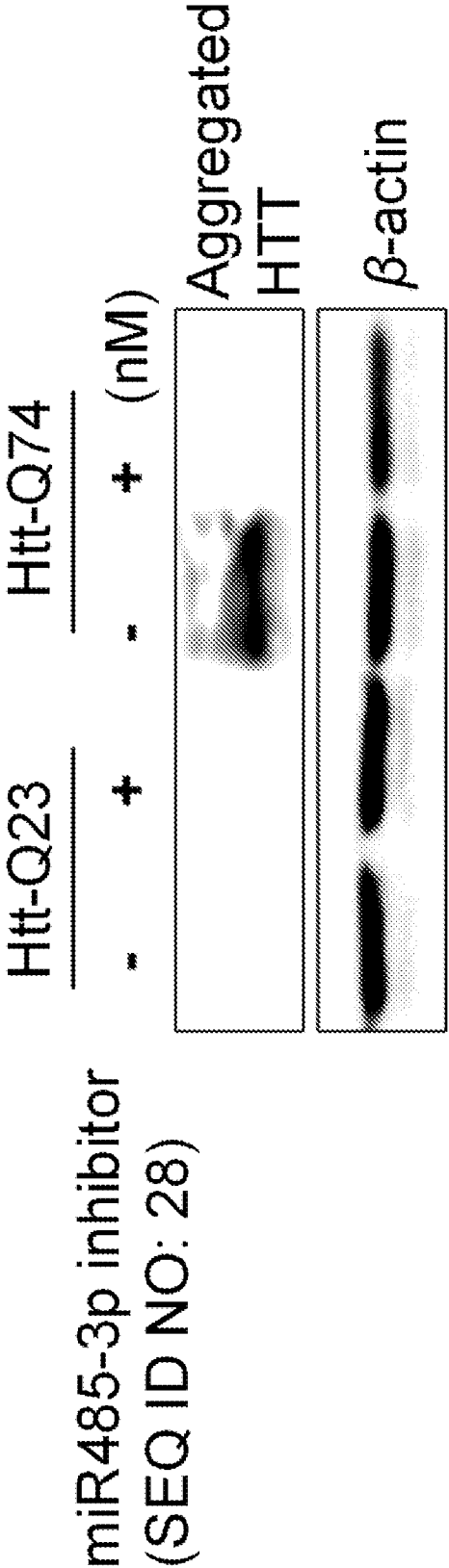


Fig. 2B



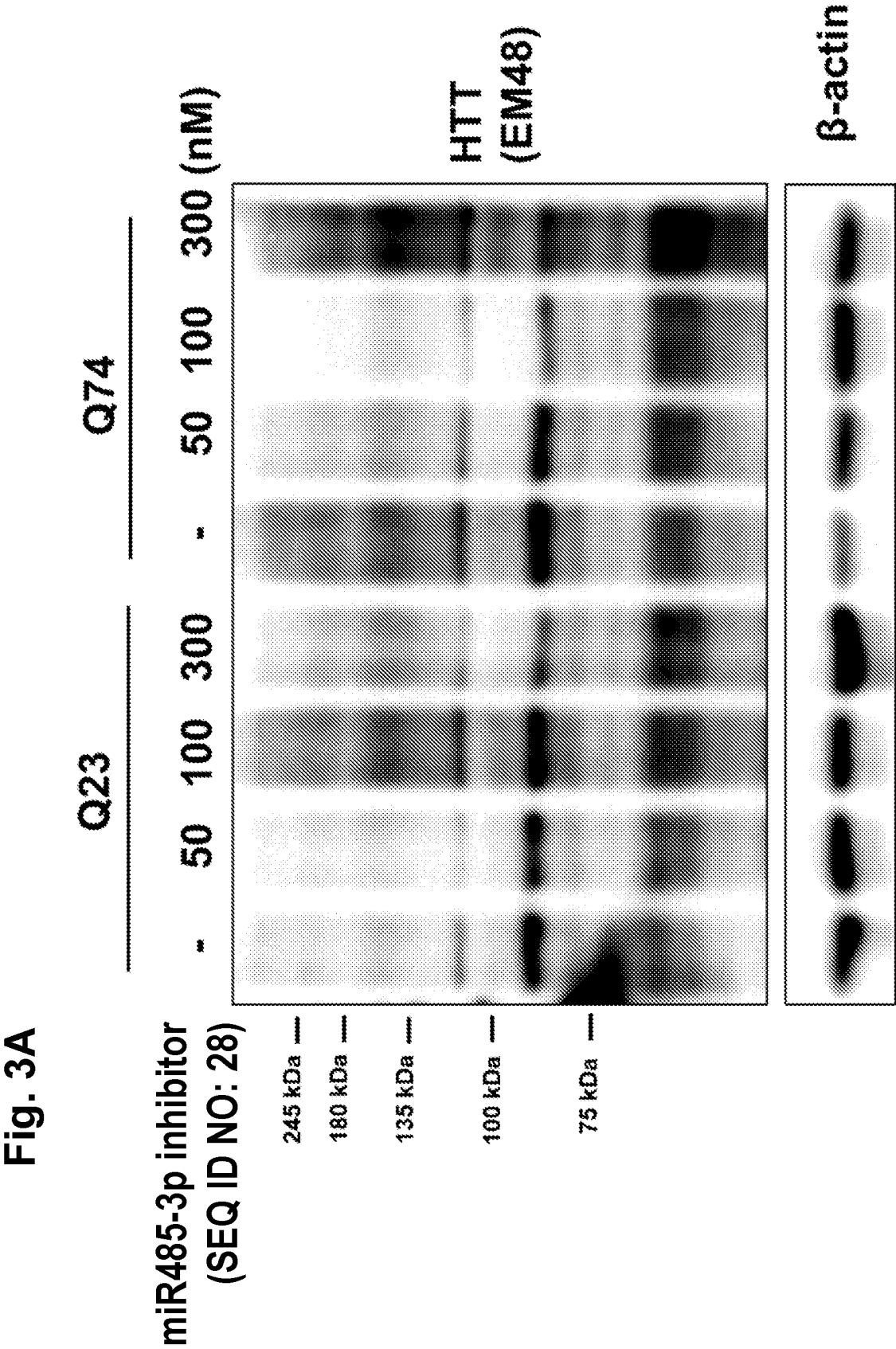


Fig. 3B

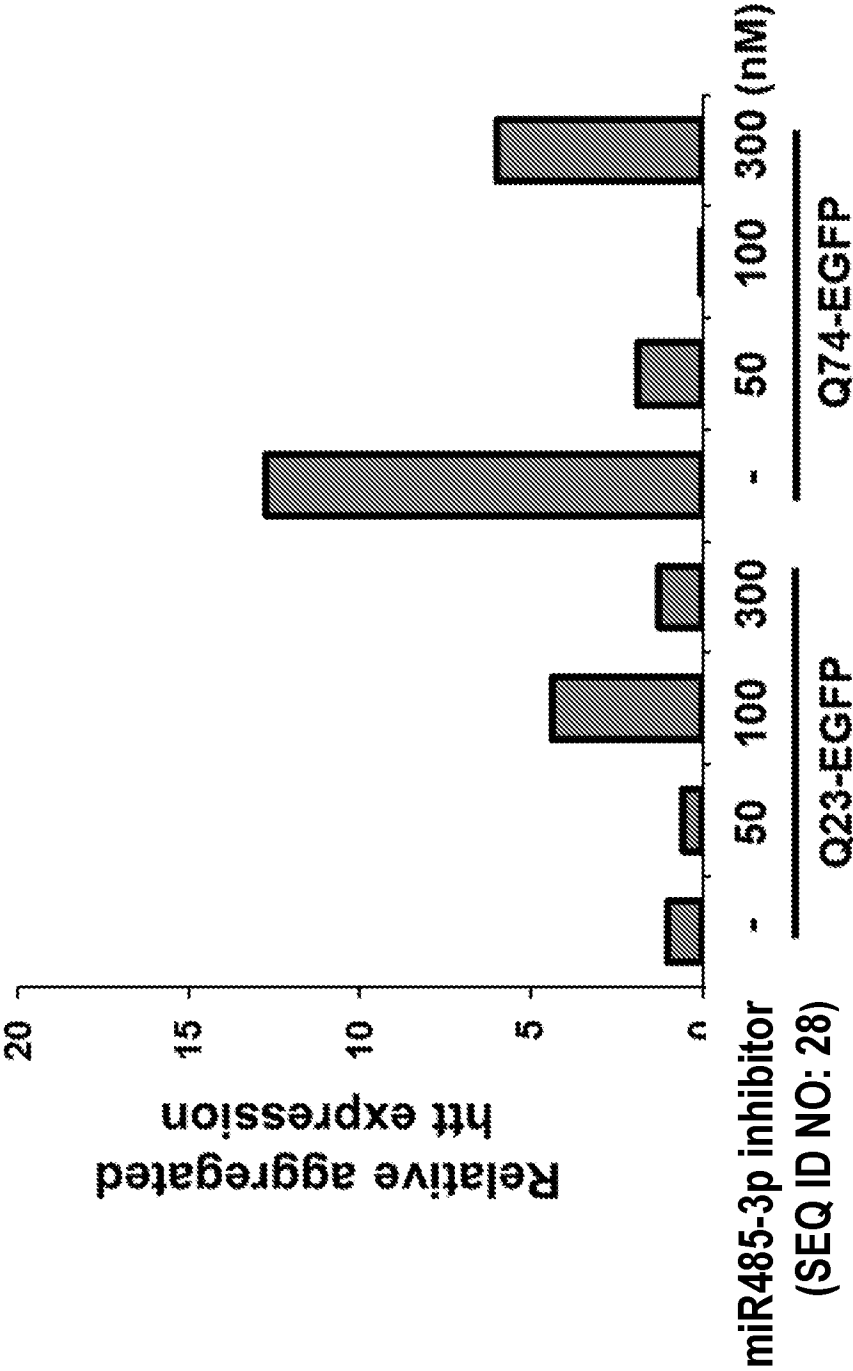


Fig. 4A

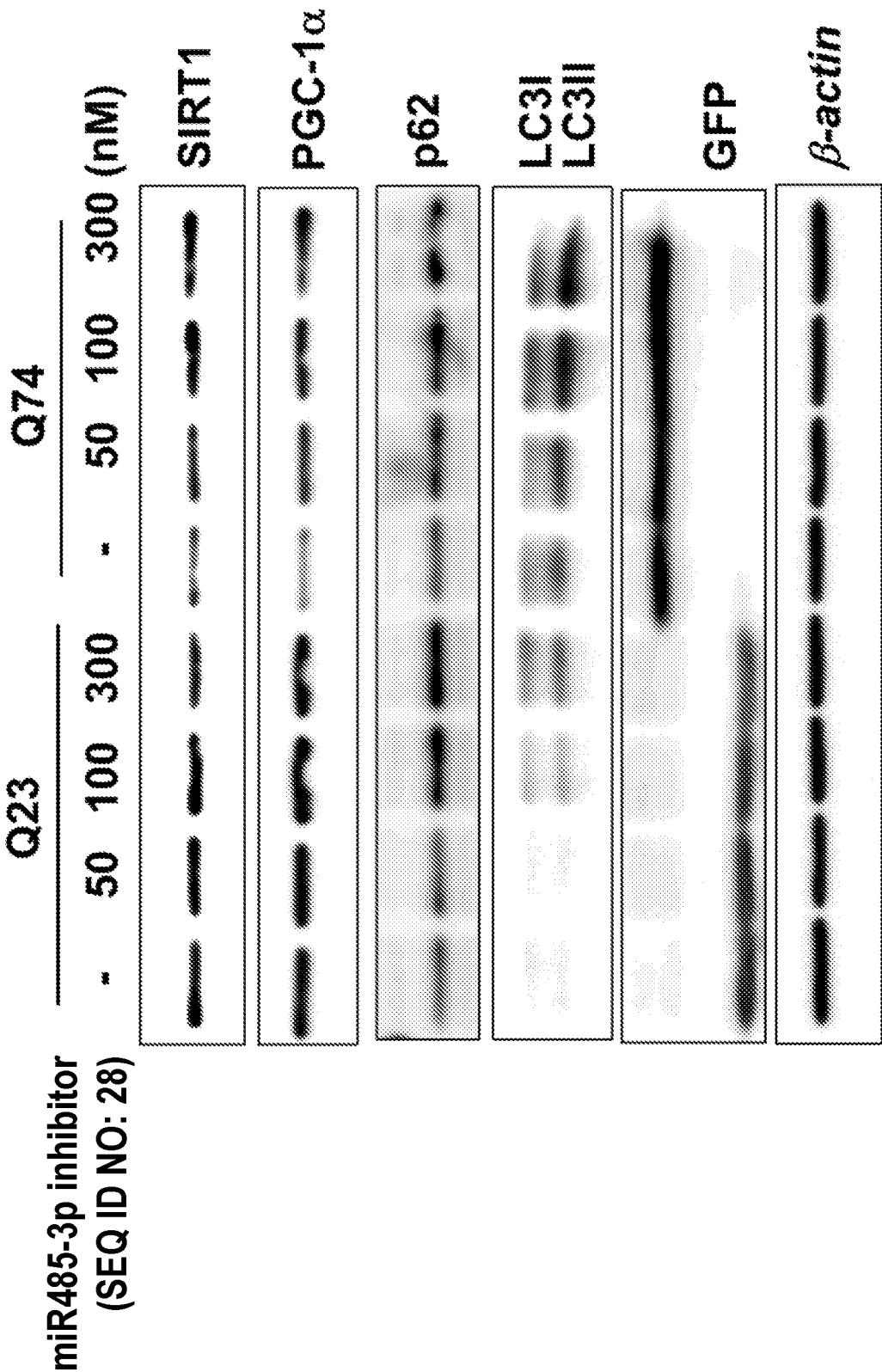


Fig. 4B

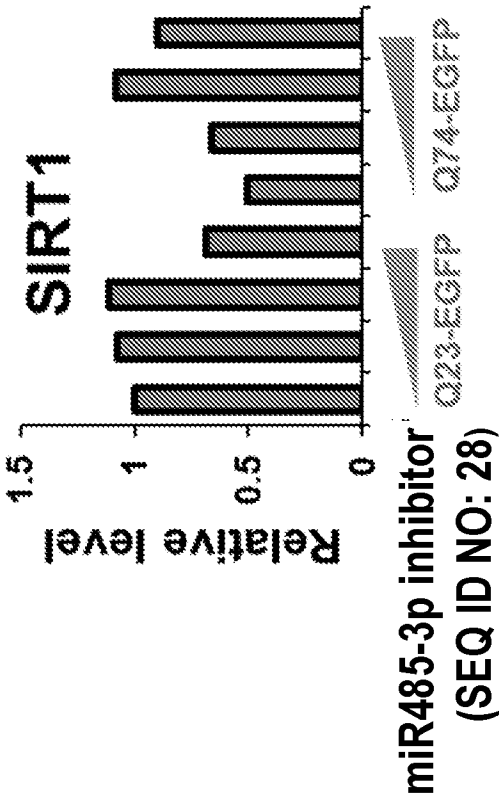


Fig. 4C

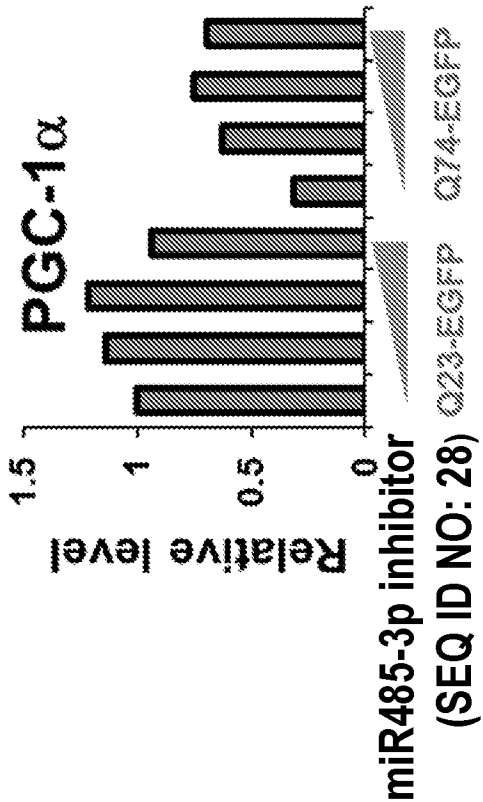


Fig. 4D

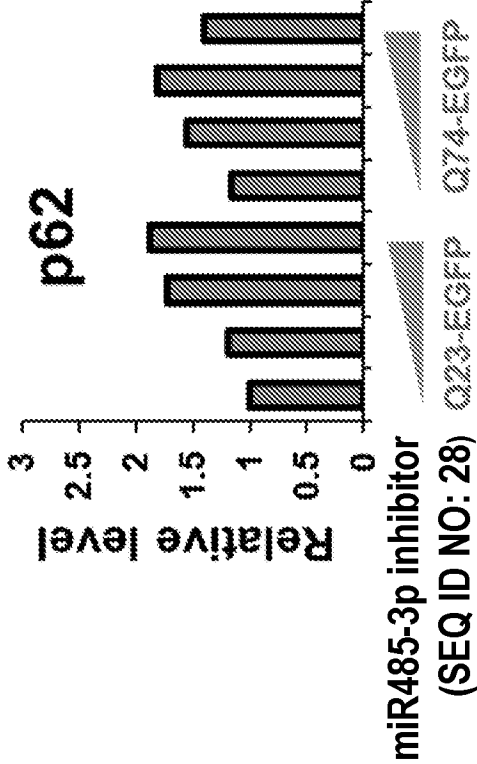


Fig. 4E

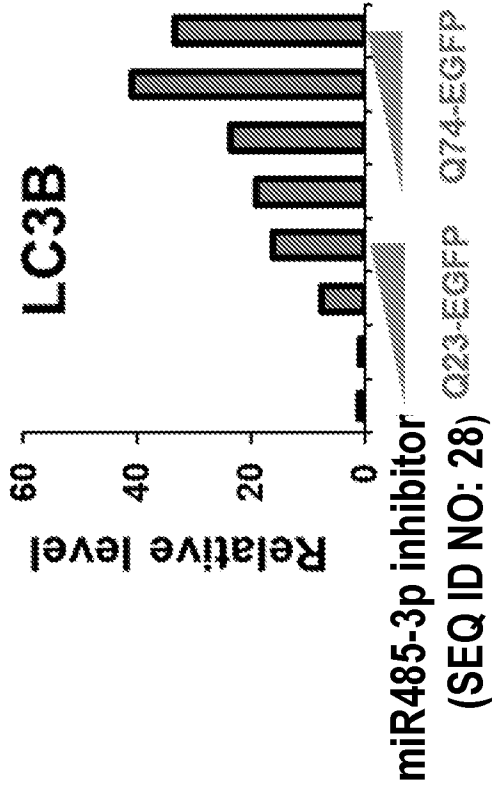


Fig. 5

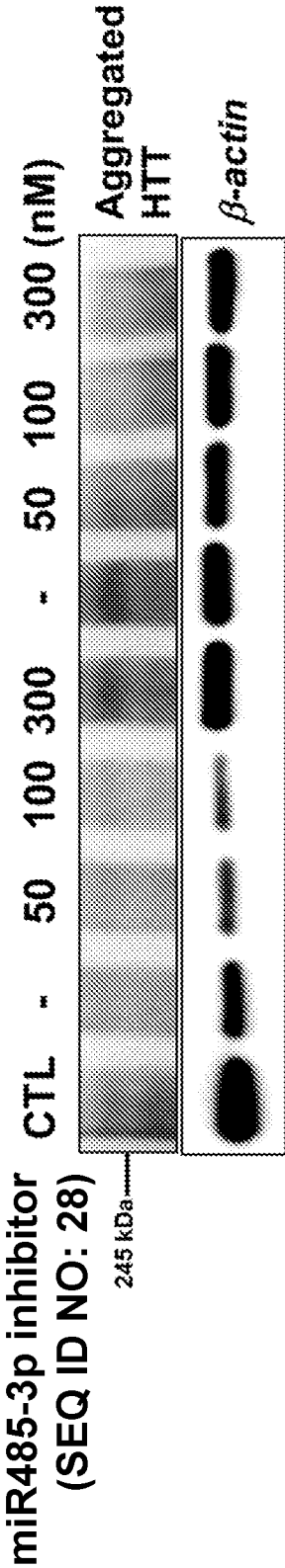


Fig. 6

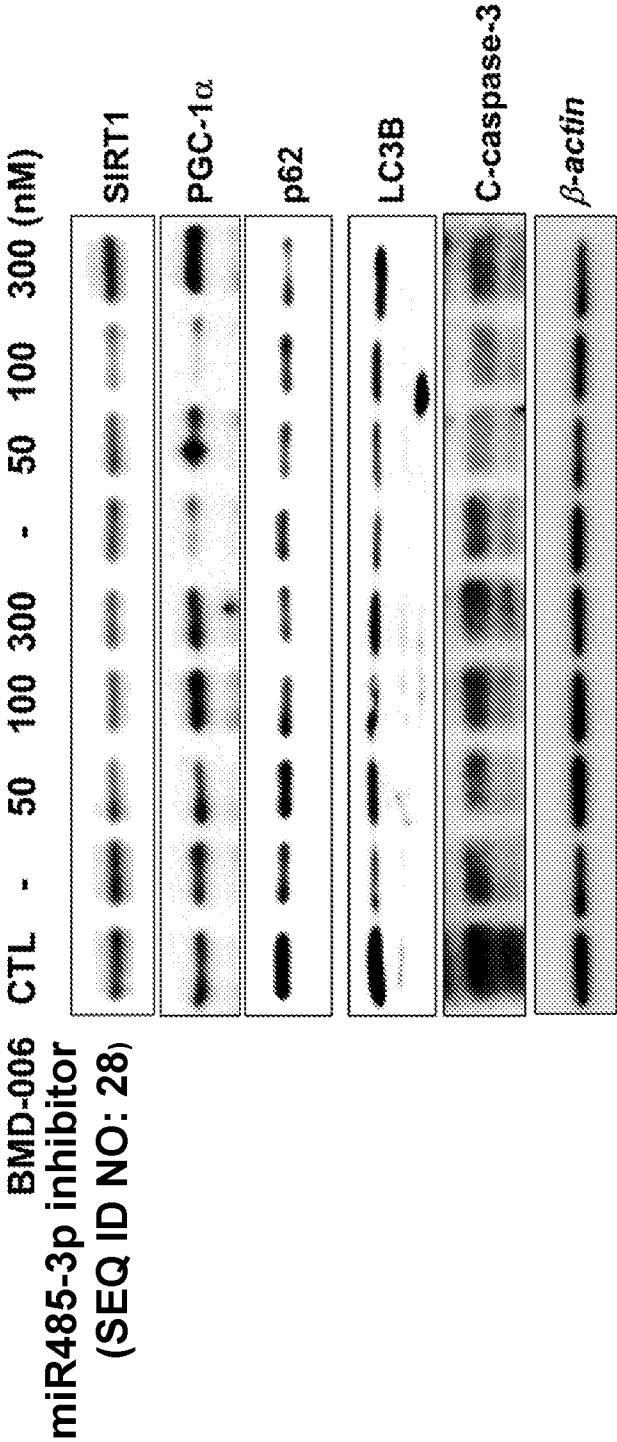


Fig. 7A

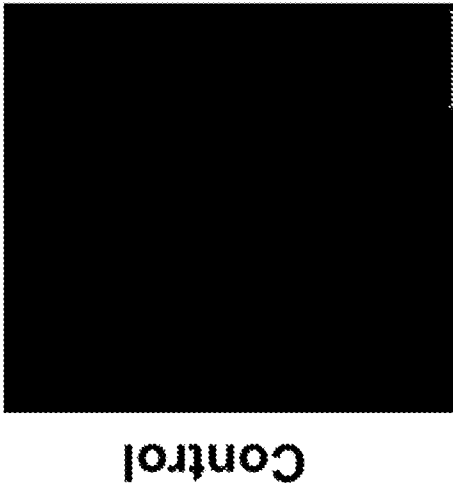


Fig. 7B

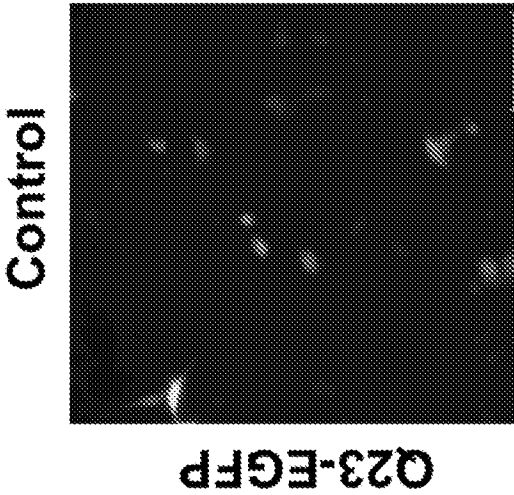


Fig. 7C

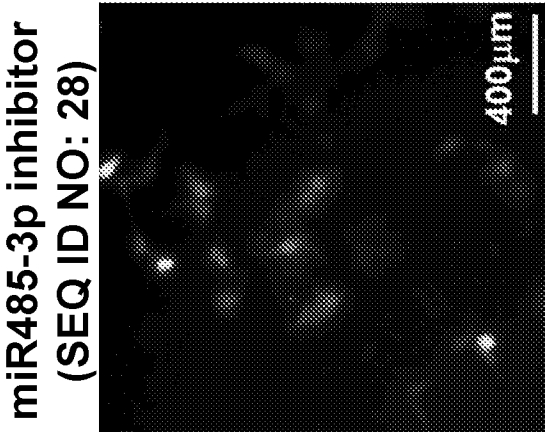


Fig. 7D

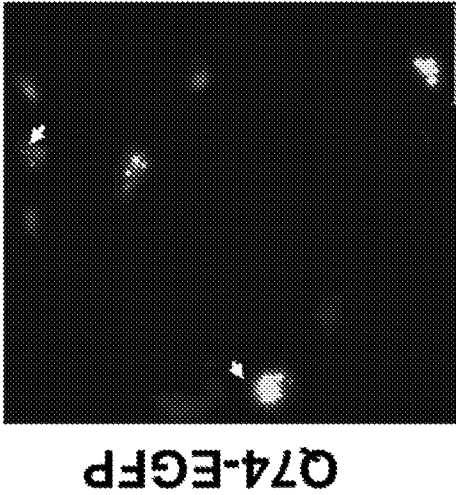


Fig. 7E

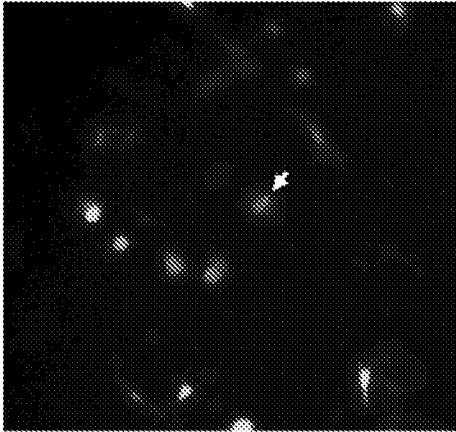


Fig. 8B

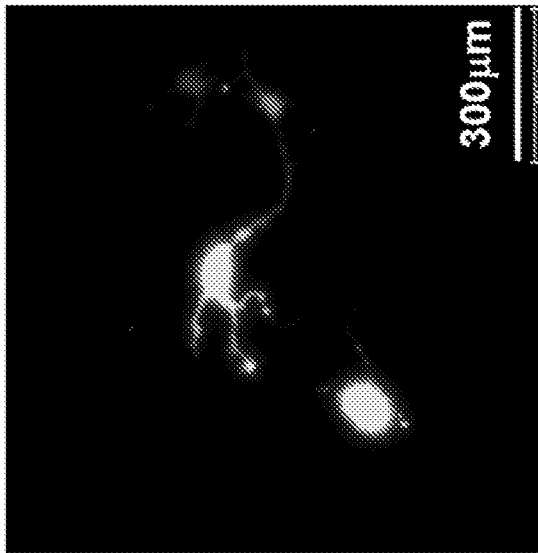
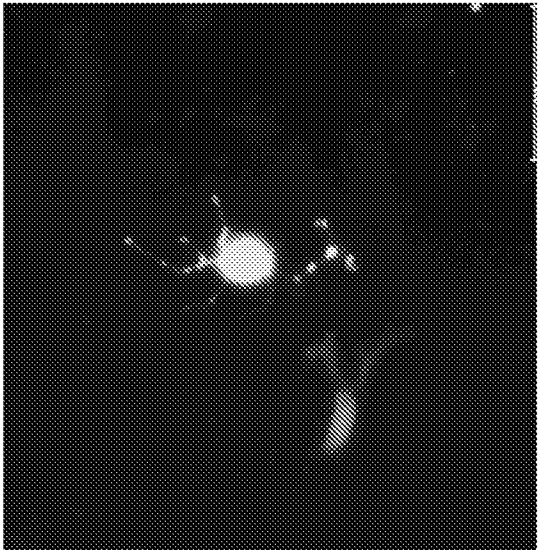


Fig. 8D

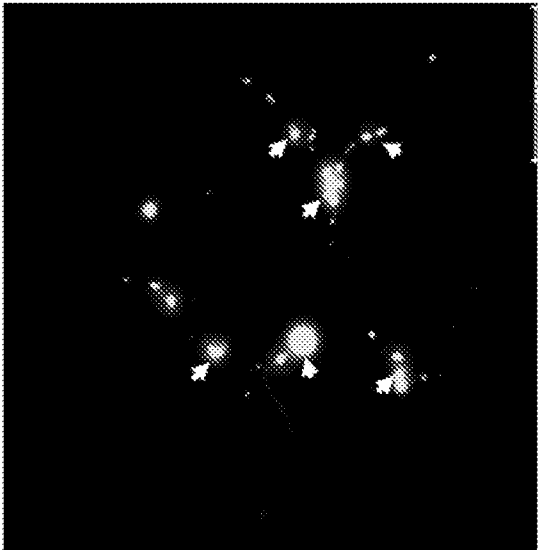


Fig. 8A

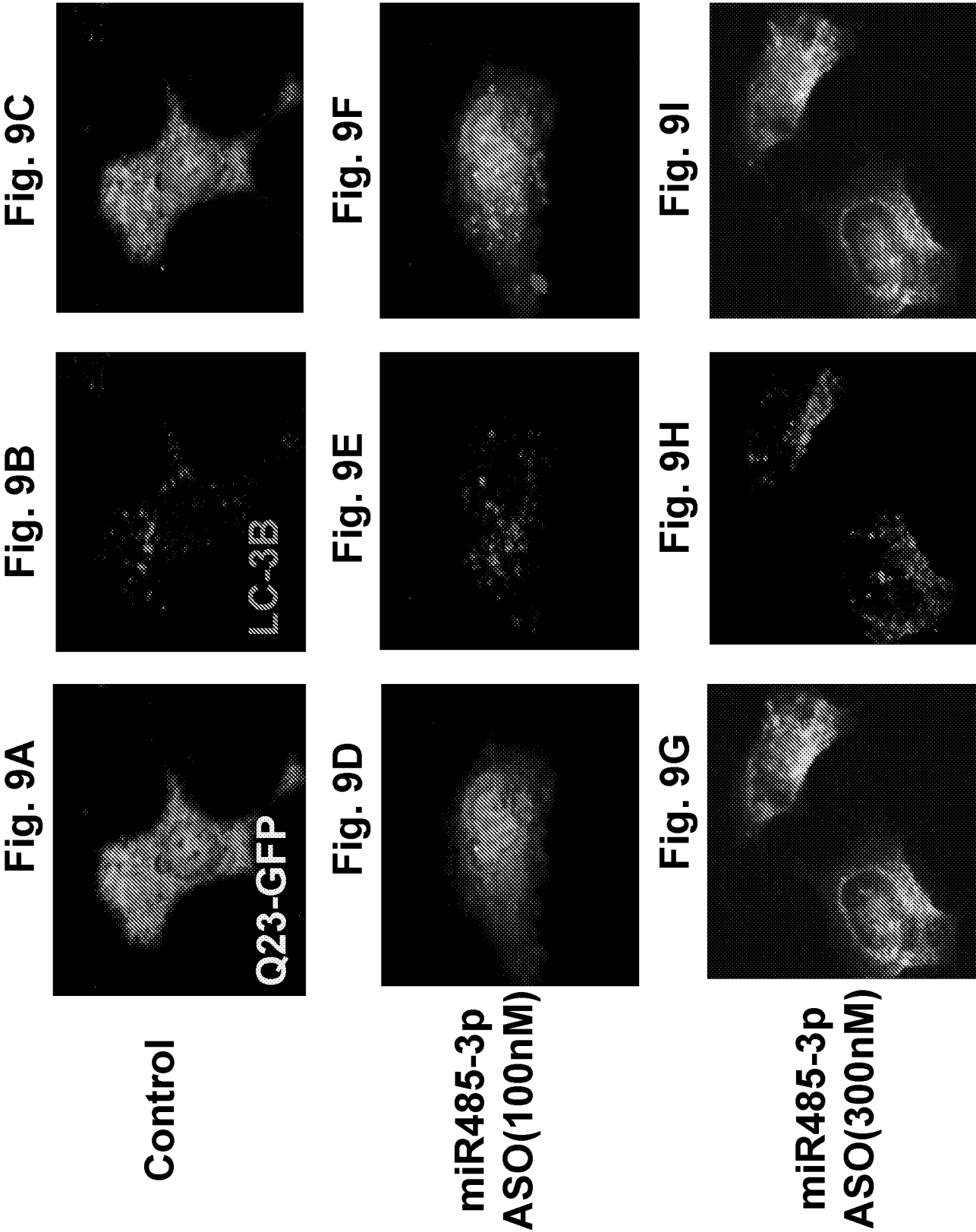


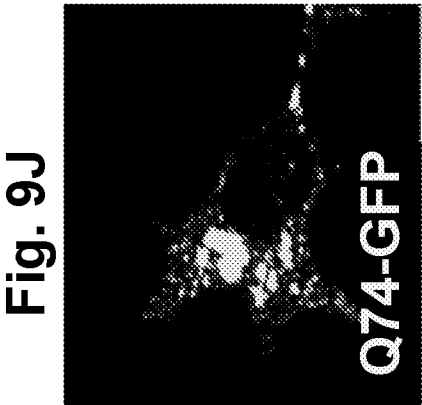
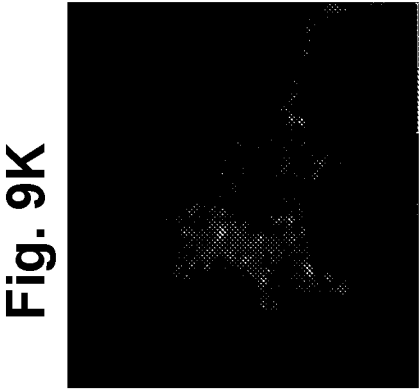
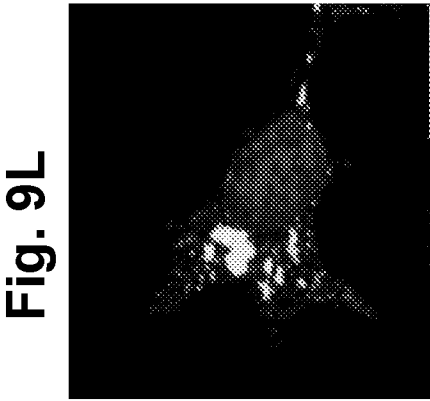
Q23-EGFP

Fig. 8C

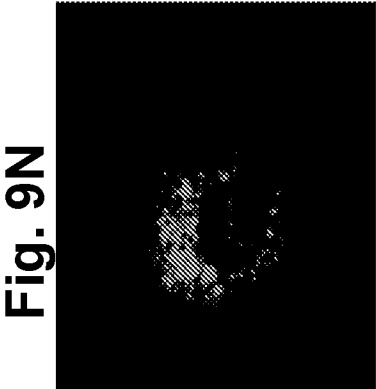
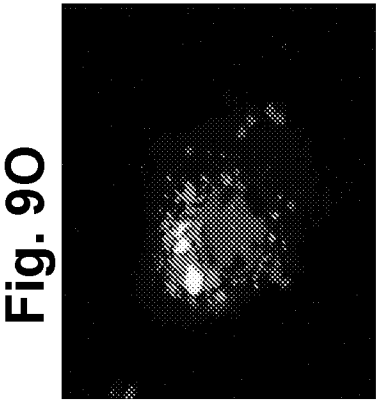


Q74-EGFP

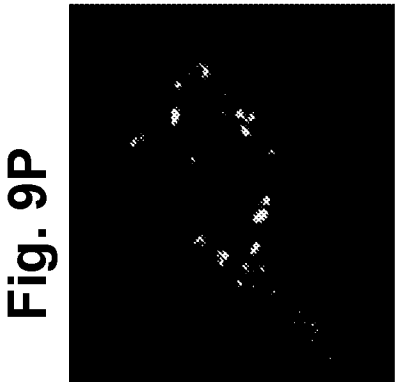
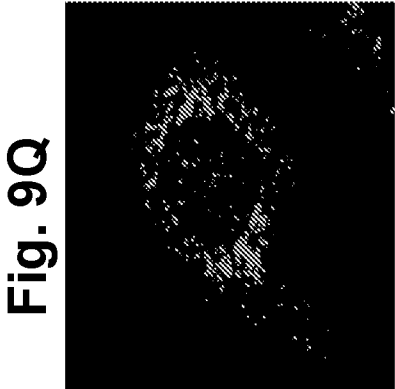
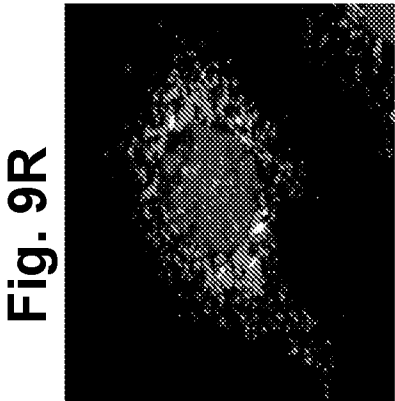




Control



**miR485-3p
ASO(100nM)**



**miR485-3p
ASO(300nM)**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/055890

A. CLASSIFICATION OF SUBJECT MATTER

A61K 48/00(2006.01)i; **A61K 31/7088**(2006.01)i; **A61K 9/107**(2006.01)i; **A61K 47/10**(2006.01)i; **A61K 47/18**(2006.01)i;
A61K 47/22(2006.01)i; **A61P 25/14**(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 48/00(2006.01); A61K 9/00(2006.01)

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

Korean utility models and applications for utility models
 Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: Huntington's disease, miR-485, miRNA inhibitor, SIRT1, neurodegenerative disease, CD36, PGC-1a

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COHEN, JONATHAN E. et al., 'MicroRNA regulation of homeostatic synaptic plasticity', Proceedings of the National Academy of Sciences of the United States of America, 12 July 2011 (Publication date), Vol. 108, No. 28, pages 11650-11655	1-4
Y	abstract; and pages 11652, 11654	5
Y	LLORET, ALEJANDRO et al., 'PGC-1a, Sirtuins and PARPs in Huntington's disease and other neurodegenerative conditions: NAD+ to rule them all', Neurochemical Research, 7 May 2019 (Online publication date), Vol. 44, pages 2423-2434	5
A	MARTI, EULALIA et al., 'A myriad of miRNA variants in control and Huntington's disease brain regions detected by massively parallel sequencing', Nucleic Acids Research, 30 June 2010 (Online publication date), Vol. 38, No. 20, pages 7219-7235	1-5
A	KOH, HAN SEOK et al., 'Reducing miR485-3p ameliorates Alzheimer's disease pathology by regulation of amyloid beta and neuro-inflammation', Research Square, 29 April 2020, pages 1-18	1-5
	abstract; and pages 1-18	

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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

Date of the actual completion of the international search

05 October 2021

Date of mailing of the international search report

05 October 2021

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/055890

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018-139819 A1 (BIORCHESTRA LTD.) 02 August 2018 (2018-08-02) abstract; and claims 1-13	1-5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/055890**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2021/055890**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **10,15,23,28,30,32,34,35,37,39,43,44,47,48,53-55,57,59,64,67**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 10, 15, 23, 28, 30, 32, 34, 35, 37, 39, 43, 44, 47, 48, 53-55, 57, 59, 64 and 67 refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a).

3. ☒ Claims Nos.: **6-9,11-14,16-22,24-27,29,31,33,36,38,40-42,45,46,49-52,56,58,60-63,65,66,68,69**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/IB2021/055890

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)		Publication date (day/month/year)	
WO	2018-139819	A1	02 August 2018	KR	10-2019-0051510	A	15 May 2019
				KR	10-2020-0043322	A	27 April 2020
				KR	10-2074407	B1	06 February 2020
				KR	10-2105016	B1	28 April 2020
				WO	2018-139759	A1	02 August 2018