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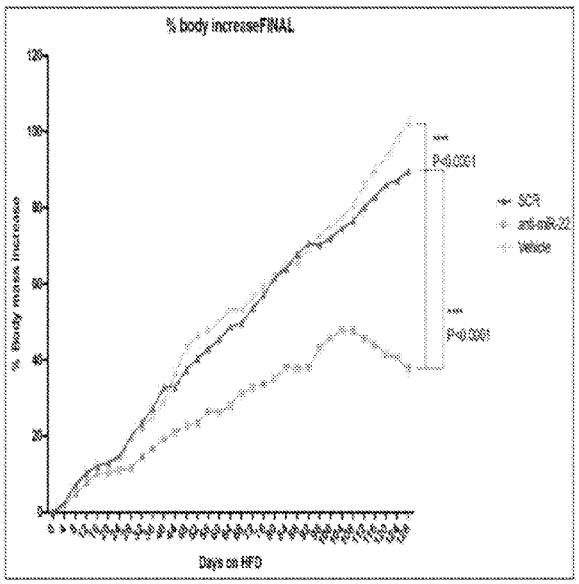
(54) Title: MICRO-RNA AND OBESITY

FIGURE 11

A



(57) Abstract: The present disclosure provides a method of treating or preventing metabolic disorders by administering agents that inhibit the activity of microRNAs that modulate metabolism.

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[Continued on next page]

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MICRO-RNA AND OBESITY

PRIORITY

[0001] This application claims the benefit of, and claims priority to, U.S. Provisional Application No. 62/642,934, filed March 14, 2018, the contents of which is hereby incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0002] The disclosure relates to the treatment and prevention of metabolic disorders by administering agents that modulate the activity or expression of microRNAs.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: BID-005PC1_ST25.txt; date created: March 14, 2019; file size: 323,976 bytes)

BACKGROUND

[0004] MicroRNAs (miRNA or miR) are nucleic acid molecules that regulate the expression of target genes. MiRNAs are typically short (typically 18-24 nucleotides) and act as repressors of target mRNAs by promoting their degradation, when their sequences are perfectly complementary, and/or by inhibiting translation, when their sequences contain mismatches. Functional analyses of miRNAs have revealed that these small non-coding RNAs contribute to different physiological and metabolic processes, including regulating genes associated with metabolic disorders. Metabolic disorders are characterized by one or more abnormalities in metabolic function in the body. Metabolic disorders are also characterized by obesity and weight gain, a deficiency in insulin production, or a deficiency in sensitivity to insulin. Some metabolic disorders are related to defects the body's use of blood glucose, resulting in abnormally high levels of blood glucose. Metabolic disorders affect millions of people worldwide, and can be life-threatening disorders. With the incidence of obesity on the rise in the United States, there is a critical need to develop more effective therapies to reduce the health risks and alleviate the symptoms associated with obesity, overeating, excessive bodyweight gain or excessive accumulation of fat. As such, there is a need

for method and compositions to treat, prevent, or delay the onset of metabolic disorders.

SUMMARY

[0005] The present disclosure provides new methods for the treatment or prevention of metabolic disorders by administering agents that modulate the activity or expression of microRNAs, for example by inhibiting microRNA expression and/or activity. Such inhibition can be mediated by sequence specific chemically modified oligonucleotides, including for example, locked nucleic acid (LNA). Inhibitors based on LNA technology, among others, when directed at the metabolic gene regulating miRNAs disclosed herein provide for an effective method of treating or preventing metabolic disorders.

[0006] In one aspect, the present disclosure provides a method for treating or preventing a metabolic disorder, comprising administering an effective amount of an inhibitor of miR-22 to a subject in need thereof.

[0007] In some embodiments, the expression and/or activity of miR-22 is reduced in the subject following administration of the inhibitor.

[0008] In some embodiments, the inhibitor of miR-22 is an oligonucleotide-based inhibitor. In some embodiments, the oligonucleotide-based inhibitor comprises a sequence that is at least about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% complementary to a mature sequence of miR-22. In some embodiments, the oligonucleotide-based inhibitor comprises deoxynucleotides or ribonucleotides. In some embodiments, the oligonucleotide-based inhibitor is single stranded. In some embodiments, wherein the oligonucleotide-based inhibitor is double stranded.

[0009] In some embodiments, the oligonucleotide-based inhibitor comprises one or more chemically modified nucleotides.

[0010] In some embodiments, the chemically modified nucleotides are locked nucleotides (LNAs).

[0011] In some embodiments, the oligonucleotide-based inhibitor comprises about 25, about 20, about 15, about 10, about 9, about 8, about 7, about 6, or about 5 or fewer nucleotides. In some embodiments, the oligonucleotide-based inhibitor is conjugated to one or more N-

acetylgalactosamine (GalNAc) moieties.

[0012] In some embodiments, the oligonucleotide-based inhibitor is an antisense oligonucleotide inhibitor. In some embodiments, the oligonucleotide-based inhibitor is a small interfering RNA (siRNA). In some embodiments, the oligonucleotide-based inhibitor is an aptamer.

[0013] In some embodiments, the inhibitor of miR-22 is a peptide-based or protein-based inhibitor. In some embodiments, the protein-based inhibitor in an antibody or an antigen-binding portion thereof. In some embodiments, the inhibitor of miR-22 is a small molecule-based inhibitor.

[0014] In some embodiments, the metabolic disorder is obesity.

[0015] In some embodiments, the subject is suffering from Prader-Willi Syndrome.

[0016] In some embodiments, the subject is suffering from hypercholesterolemia.

[0017] In some embodiments, the subject harbors a fat mass and obesity-associated protein (FTO) variant and/or shows an upregulation of FTO expression and/or activity.

[0018] In some embodiments, the subject is obese and has a body mass index of greater than about 30. In some embodiments, the subject is overweight and has a body mass index of about 25-29.9.

[0019] In some embodiments, the method induces weight loss. In some embodiments, the method induces a total weight loss of about 1%, about 5%, about 10%, about 15%, about 20%, or about 25% or more in the subject. In some embodiments, the method prevents weight gain.

[0020] In some embodiments, the method reduces or prevents the growth of adipose tissue. In some embodiments, the method impairs adipocyte differentiation.

[0021] In some embodiments, the metabolic disorder is a fatty liver disease. In some embodiments, the fatty liver disease is selected from non-alcoholic fatty acid liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH). In some embodiments, the method reduces or prevents liver steatosis.

[0022] In some embodiments, the method reduces or prevents liver fibrosis.

[0023] In some embodiments, the method reduces the activity and/or expression of FTO, ALKB Homologous 5 (ALKBH5), CCAAT/enhancer binding protein alpha (CEBPα), peroxisome proliferator activated receptor gamma (PPARγ), and/or ATP citrate lyase (ACLY).

[0024] In some embodiments, the method increases the activity and/or expression of phosphatase and tensin homolog (PTEN) and/or tet methylcytosine dioxygenase 2 (TET2).

[0025] In some embodiments, the method alters the activity and/or expression of PPARγ co-activator-α (PGC1-α), Specific Protein 1 (SP1), Fibroblast Grow Factor 21 (FGF-21), Uncoupled protein 1 (UCP1), DNA Damage Included Transcript 4 (DDIT-4, REDD1), tumor protein p63 (TP63), fibroblast growth factor 1 (FGF1), and/or Methyltransferase like 3 (METTL3). In some embodiments the method alters the level of DNA or RNA methylation, or affects the m6A level at RNA level.

[0026] Any aspect or embodiment described herein can be combined with any other aspect or embodiment as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0028] Figure 1A-C are schematics showing the regulation profile of miR-22. Figure 1A is a schematic showing the position of miR-22 in in 3rd exon in non-coding transcript MGC14376. Figure 1B shows PTEN targeting miRNAs and Figure 1C shows that miR-22 directly targets PTEN and TET to promote tumorigenesis and metastasis.

[0029] Figures 2A-D show that miR-22 overexpression affects weight of mice. Figure 2A is a picture showing the feeding regimen of Wild Type (WT) and Transgenic (Tg) mice. Figure 2B is an immunohistochemical staining of WT liver and miR-22 Tg mice liver. Figure 2C is a graph showing the average weight of WT versus miR-22 Tg mice and Figure 2D is a bar graph showing the average weight of WT and miR-22 T mice colony. These data indicate that miR-22 overexpression affects weight of mice. The data in the right (red) bars in each data pair is for the with miR-2 Tg mice.

[0030] Figure 3A-C are a pair of bar graphs and a line graph showing that miR-22 null mice fail to gain weight on High Fat Diet (HFD). Figure 3A shows the percentage of body mass increase the entire colony after 2 weeks on the HFD and Figure 3B shows the percentage of body mass

increase the entire colony after 4 weeks on the HFD. Figure 3C shows the change in grams of KO vs WT from week 1 to 8 from the start of HFD.

[0031] Figure 4A-F are a series of bar graphs showing that miR-22 null mice fail to gain weight on HFD. Figure 4A shows the percentage of body mass increase in female mice after 2 weeks on the HFD. Figure 4B shows the percentage of body mass increase in female mice after 4 weeks on the HFD. Figure 4C shows the percentage of body mass increase in female mice after 8 weeks on the HFD. Figure 4D shows the change in grams in KO vs WT mice after 2 weeks. Figure 4E shows the change in grams in KO vs WT mice after 4 weeks. Figure 4F shows the change in grams in KO vs WT mice after 8 weeks.

[0032] Figure 5A-D are a series of graphs showing that miR-22 null mice show a different metabolism compared with WT when fed with HFD. Figure 5A shows percentage of fat and lean mass (Figure 5B), (% of total body mass) of miR-22 KO and WT mice pre (day 0) and post 8 weeks on HFD. miR-22 KO cohort has a statistically significant lower fat mass compared to WT after 8 weeks on HFD. Lean mass in miR-22 KO cohort is not affected by HFD, contrary top WT cohort that increase the % of fat mass and decrease the % of lean mass after HFD. Figures 5C and 5D show a series of parameters collected in metabolic cages. At steady state both miR-22KO and WT mice have the same metabolism. Once that mice are challenged with HFD miR-22KO cohort is able to not reduce its energy expenditure, while WT cohort does. Both VCO2 and VO2 are significant higher in miR-22KO cohort compared with WT, even if KO mice are less active than WT. The data in the right (red) bars in each bar graph of Figures 5C and 5D is for the with miR-22KO mice.

[0033] Figure 6 is a bar graph showing no difference between WT and KO cohort in food consumption (HFD).

[0034] Figure 7A-B is a bar graph and Oil-Red-O stain showing that down regulation of miR-22 impairs Mouse Embryonic Fibroblast (MEF) ability to differentiate in adipocytes. Figure 7A is a bar graph showing adipocyte differentiation in MEFs, indicating that miR-22-/- MEFs show 27% less adipocytes than WT. Figure 7B is an Oil-Red-O stain showing MEF from WT, miR-$22^{+/-}$ and miR-$22^{-/-}$, cultured in Adipo-differentiative media for 5 days.

[0035] Figure 8 shows the design of anti-miR-22 LNA.

[0036] Figure 9 is a pictorial showing the in vivo experimental planning and conditions for miR-22-/- and WT mice on HFD following transfection of Vehicle (VCH), Scramble Control RNA (SCR) and Locked Nucleic Acid (LNA).

[0037] Figure 10 is a bar graph showing that there is no difference between treated and non-treated mice in food consumption for (Δ) Vehicle, (◊) SCR, (□) anti-miR-22.

[0038] Figure 11A-B are line graphs showing an in vivo pharmacological inhibition of miR-22 prevents mice from becoming obese. Figure 11A shows the final percentage body increase. Figure 11B shows in vivo silencing of miR-22 in DIO mice. In both figures, at the final time points, the order of data going from top to bottom is Vehicle (in green), SCR (in red), and anti-miR-22 (in blue).

[0039] Figure 12 is a western blot showing that anti-miR-22 therapy in-vivo is able to increase protein level of major miR-22 targets in the liver.

[0040] Figure 13A-B is a series of bar graphs (Figure 13A) and immunohistochemical staining (Figure 13B), showing that anti-miR-22 treatment doesn't affect liver lipid composition but profoundly suppresses liver steatosis.

[0041] Figure 14A-B is a series of bar graphs showing relative mRNA level in the liver of mice treated with VHL, SCR LNA or LNA anti-miR-22. Figure 14A shows mRNA expression of TET2 and PTEN. Figure 14B shows mRNA expression of FTO, FTO and CEBPa.

[0042] Figure 15A-C is a series of bar graphs showing relative mRNA level in Brown adipose tissue (BAT) of mice treated with VHL, SCR LNA or LNA anti-miR-22. Figure 15A shows mRNA expression of TET2 and PTEN. Figure 15B shows mRNA expression of FTO, CEBPa and PPARg and Figure 15C shows mRNA expression of UCP1 and CD36.

[0043] Figure 16A-C is a series of bar graphs showing relative mRNA levels in White adipose tissue (WAT) of mice treated with VHL, SCR LNA or LNA anti-miR-22. Figure 16A are bar graphs showing TET2 and PTEN mRNA expression levels. Figure 16B are bar graphs showing FTO, CEBPa and PPARg mRNA expression levels and Figure 16C are bar graphs showing, UCP1 and CD36 expression levels.

[0044] Figure 17 is a pictorial of a curative approach showing miR-22 -/- and WT mice on a HFD

treated with an anti-miR-22-LNA, SCR and a VHL and placed on a second HFD regimen.

[0045] Figure 18 is a line graph showing the results of the curative approach whereby there is a significant reduction in body weight in mice already obese and fed with a HFD. After 3 ½ months of treatment, a significant reduction in body weight was observed in obese mice (average weight >40 g) and fed with HFD. Mice were sacrificed, tissue collected, RNA from livers used for RNAseq.

[0046] Figure 19A-C are three pictures showing that miR-22 pharmacological inhibition reverts Obese phenotype in mice. Figure 19A shows fat pad from mice treated with VHL, Figure 19B shows Fat pad from mice treated with anti-miR-22 and Figure 19C shows fat pad from mice treated with SCR.

[0047] Figure 20 is an RNA-seq plot showing the hierarchy cluster analysis from mice liver indicating that miR-22 pharmacological inhibition and genetic Knockout (KO) cluster together, indicating that the treatment is on target and that KO phenotype can be mimicked using LNA construct.

[0048] Figure 21 is an RNA-Seq plot showing the gene ontology analysis in mice liver indicating that top down regulated pathway in KO and LNA treated mice are lipid metabolism and biosynthesis related.

[0049] Figure 22 is a western blot showing that anti-miR-22 therapy in-vivo strongly down regulate ATP-citrate lysase (ACL). Anti-miR-22 therapy *in vivo* strongly downregulates ACL.

[0050] Figure 23 is an Oil-Red-O staining showing that Pharmacological inhibition of miR-22 is effective in impairing MEFs adipocytic differentiation.

[0051] Figure 24 is an Oil- Red O staining (Figure 24A) and a bar graph (Figure 24B) showing that anti-miR-22 treatment in Human Primary Mesenchymal cells cultured in Adipo differentiation media for 2 weeks with or without LNA anti-miR-22. Un- assisted uptake 500nM (LNAs added every 2 days). In Figure 24B, the data in the right (red) bars in each bar graph is for the with LNA#10-treated cells.

[0052] Figure 25 is a bar graph showing that pharmacological inhibition of miR-22 is effective in impairing MEFs adipocytic differentiation. The order of data in the bar graphs, when reading from

left to right corresponds with the legend (at the right of the graph) when reading from top to bottom.

[0053] Figure 26 Schematic representation of metabolic network that it is orchestrated by miR-22. miR-22 can target multiple metabolic player (directly or indirectly) at the same time.

[0054] Figure 27A-B show that, Fat Mass and Obesity-Associated protein (FTO) expression is profoundly down-regulated during adipose induced differentiation in miR-22 deficient MEF but not in WT MEF. Figure 27A is a representation of the FTO gene and Figure 27B is a bar graph showing FTO mRNA expression level under steady state and differentiation media conditions. In Figure 27B, the order of data in the bar graphs, reading from left to right, is WT (in black), miR-22+/- (in grey), and miR-22-/- (in blue).

[0055] Figure 28A-D shows that miR-22 down regulation (genetic or pharmacological) increase level of RNA m6A. Figure 28A shows the chemical reaction. Figure 28B, Figure 28C and Figure 28D are bar graphs showing the percentage and relative amount of m6A levels in mice on a HFD.

[0056] Figure 29 are a series of immunohistochemical images showing that miR-22 genetic depletion doesn't affect liver function in 2-month old mice; n=8.

[0057] Figure 30 are a series of immunohistochemical images showing that miR-22 genetic depletion doesn't affect liver function and that miR-22 KO mice do not show any liver related disease or dysfunction at old age.

[0058] Figure 31 are a series of immunohistochemical images showing that anti-miR-22 LNA treatment prevent liver steatosis in diet induced obese mice. Images are at 10x magnification.

[0059] Figure 32 are a series of immunohistochemical images showing the effects of miR-22 Overexpression and Underexpression on Liver Fibrosis.

[0060] Figure 33A-C shows that miR-22 Overexpression Affects Liver Function feed with normal chow: Fatty Liver and Fibrosis in WT-mice (Figure 33A) and miR-22 Tg mice (Figure 33B). Figure 33C shows FSP-1 positive cells in liver from mouse on a normal diet in WT and miR-22 Tg mice.

[0061] Figure 34A-C show that miR-22 Overexpression Affects Liver Function: Fatty Liver and Fibrosis in WT-mice (Figure 34A) and miR-22 +/- mice (Figure 34B). Figure 34C shows FSP-1 positive cells in liver from mouse on a HFD in WT and miR-22 +/- mice.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0062] The present disclosure is based on the discovery that miRNAs, including miR-22, can regulate targets that are linked to a variety of metabolic disorders, including Obesity, Prader-Willi Syndrome, Hypercholesterolemia, Fatty Liver Disease, Non-Fatty Acid Liver Disease (NAFLD) and/or Non-Alcoholic Steatohepatitis (NASH).

[0063] The present disclosure includes targeting miRNAs, including miR-22, with various inhibitors for the treatment and/or prevention of diseases the cause of which can be influenced by modulating the metabolism, for example, metabolic disorders, including Obesity, Prader-Willi Syndrome, Hypercholesterolemia, NAFLD and/or NASH.

[0064] MiR-22 directly targets phosphatase and tensin homolog (PTEN) and tet methylcytosine dioxygenase (TET) to promote tumorigenesis, metastasis and metabolic disorders. More than 60 PTEN-targeting miRNAs and no less than 30 new proto-oncogenic genetic loci are studied in human cancer. PTEN-targeting miRNAs are highly conserved evolutionarily among vertebrates and ubiquitously expressed in various tissues, (Lagos-Quintana *et al.,* 2001, 2002; Neely *et al.,* 2006). By targeting PTEN, miR-22 is remains metabolically relevant, as PTEN lowering or its elevation triggers a Warburg- or an anti-Warburg metabolic state respectively. In some embodiments, miR-22 targeting genes regulate metabolism and fatty acid oxidation or biogenesis.

[0065] In one aspect, the methods of the present disclosure provide a method for treating or preventing a metabolic disorder, comprising administering an effective amount of an inhibitor of miR-22 to a subject in need thereof.

[0066] For example, such inhibition could be mediated by sequence specific chemically modified oligonucleotides. An exemplary modification is a locked nucleic acid (LNA) in which the nucleic acid's ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon, which locks the ribose in the *3'-endo* conformation. The LNA inhibitors, among others, when directed at the metabolism gene-regulating miRNAs disclosed herein, provide for cost effective agents that can be delivered efficiently and possess sufficient bioavailability for the treatment and prevention of various metabolic disorders.

[0067] The present disclosure further provides uses of any methods or combinations described herein in the manufacture of a medicament for treating a disease. Such diseases include, for

example, metabolic disorders or a disease influenced by modulating the metabolism (*e.g.,* Fat related metabolism and synthesis pathway).

[0068] In some embodiments of the methods of the disclosure, miRNAs, including miR-22 regulate fat related metabolism and synthesis pathway targets and/or genes. In some embodiments, the fat related metabolism and synthesis genes include Fat mass and obesity-associated protein (FTO), CCAAT/enhancer binding protein alpha (CEBPa), peroxisome proliferator activated receptor gamma (PPARg), phosphatase and tensin homolog (PTEN), tet methylcytosine dioxygenase 2 (TET2), ATP citrate lyase (ACLY), bone morphogenetic protein 7 (BMP-7) and/or sirtuin 1 (SIRT-1).

[0069] In some embodiments, the fat related metabolism and synthesis genes include FTO. In some embodiments, the method reduces the activity and/or expression of fat mass and obesity-associated protein (FTO), CEBPα, and/or PPARγ.

[0070] In some embodiments, the method reduces the activity and/or expression of ALKB Homologous 5 (ALKBH5). In some embodiments, the method increases the activity and/or expression of PTEN and/or TET2.

[0071] In some embodiments, the method alters the activity and/or expression of PPARγ co-activator-α (PGC1-α), Specific Protein 1 (SP1), Fibroblast Grow Factor 21 (FGF-21), Uncoupled protein 1 (UCP1), DNA Damage Included Transcript 4 (DDIT-4, REDD1), Methyltransferase like 3 (METTL3), tumor protein p63 (TP63) and/or fibroblast growth factor 1 (FGF1).

[0072] In some embodiments the method alters the level of DNA or RNA methylation, or affects the m6A level at RNA level.

[0073] In some embodiments, the present disclosure treats or prevents metabolic disorders (by way of non-limiting example, Obesity, Prader-Willi Syndrome, Hypercholesterolemia, Fatty Liver Disease, NAFLD and/or NASH) in a subject through the inhibition of a miRNA. MiRNAs are short nucleic acid molecules that are able to regulate the expression of target genes. See review by Carrington *et al.,* Science, Vol. 301(5631):336-338, 2003. MiRNAs are often between about 18 to 24 nucleotides in length. MiRNAs act as repressors of target mRNAs by promoting their degradation, when their sequences are perfectly complementary, and/or by inhibiting translation,

when their sequences contain mismatches.

[0074] Without being bound by theory, mature miRNAs are believed to be generated by pol II or pol III and arise from initial transcripts termed pri-miRNAs. These pri-miRNAs are frequently several thousand bases long and are therefore processed to make much shorter mature miRNAs. These pri-miRNAs may be multicistronic and result from the transcription of several clustered sequences that organize what may develop into many miRNAs. The processing to yield miRNAs may be two-steps. First, pri-miRNAs may be processed in the nucleus by the RNase Drosha into about 70- to about 100-nucleotide hairpin-shaped precursors (pre-miRNAs). Second, after transposition to the cytoplasm, the hairpin pre-miRNAs may be further processed by the RNase Dicer to produce a double-stranded miRNA. The mature miRNA strand may then be incorporated into the RNA-induced silencing complex (RISC), where it may associate with its target mRNAs by base-pair complementarity and lead to suppression of protein expression. The other strand of the miRNA duplex that is not preferentially selected for entry into a RISC silencing complex is known as the passenger strand or minor miRNA or star (*) strand. This strand may be degraded. It is understood that, unless specified, as used herein a miRNA may refer to pri- and/or pre- and/or mature and/or minor (star) strand and/or duplex version of miRNA.

[0075] In some embodiments, miRNA genes may be located within introns of protein-coding genes or within introns or exons of noncoding transcriptional units. The expression of intronic miRNAs may coincide with that of the hosting transcriptional units because they are typically oriented in the same direction and are coordinately expressed with the pre-mRNAs in which they reside.

[0076] In some embodiments, miRNAs may bind to sequences within the 3' untranslated region (3'UTR) of target gene transcripts. In some embodiments, miRNAs may bind to sequences outside of the 3'UTR of target gene transcripts. In some embodiments, miRNAs may bind to both within and outside the 3'UTR of target gene transcripts.

[0077] In some embodiments, nucleotide pairing between the second and seventh nucleotides of the miRNA (the miRNA seed sequence) and the corresponding sequence along the target 3'UTR (seed match) may occur for target recognition. Accordingly, the binding between miRNA and target may comprise about a 5 nucleotide base pairing. Additionally, the binding between miRNA

and target may comprise more than a 5 nucleotide base pairing. In some embodiments, the binding between an miRNA and the gene that it regulates may be mediated by the miRNA binding up to 2, up to 4, up to 6, up to 8, or up to 10 sites of the target nucleic acid.

[0078] MiRNAs of the present disclosure may regulate nucleic acids, including but not limited to metabolic-critical genes such as genes of a marker linked to a metabolic disorder etiology, by direct binding. This binding may be perfectly complementary to the target nucleic acid or contain mismatches. The effect of this binding may be to promote degradation and/or to inhibit translation of the target.

[0079] In some embodiments, the present invention treats or prevents metabolic disorder in a subject through the inhibition of miRNAs, such as miR-22. In some embodiments, the nucleic acid encoding mir-22 comprises or consists of AAGCUGCCAGUUGAAGAACUGU (SEQ ID NO: 1).

[0080] The predicted miR-22 hairpin precursor is contained entirely within exon 2 of a noncoding transcript, C17orf91, and the splicing pattern is generally conserved in human and mouse, despite the lack of protein-coding potential. *See* Rodriguez *et al.,* Identification of mammalian microRNA host genes and transcription units. Genome Res. 2004 Oct;14(10A):1902-10. Deletion of exon 2 of C17orf91 encompassing mir-22 in mouse models has revealed that miR-22 may play a role in cardiac hypertrophy and remodeling by targeting SIRT1 (NAD-dependent deacetylase sirtuin-1), HDAC4 (histone deacetylase 4), PURB (purine-rich element binding protein B) and PTEN. *See* Gurha *et al.,* Targeted deletion of microRNA-22 promotes stress-induced cardiac dilation and contractile dysfunction. Circulation. 2012 Jun 5;125(22):2751-61; Huang *et al.,* MicroRNA-22 regulates cardiac hypertrophy and remodeling in response to stress. Circ Res. 2013 Apr 26;112(9):1234-43.

[0081] Additionally, it has been observed that AKT, a downstream target of PTEN, activated mir-22 transcription, suggesting a regulatory loop in the oncogenic PI3K/AKT signaling pathway. *See* Bar *et al.,* miR-22 forms a regulatory loop in PTEN/AKT pathway and modulates signaling kinetics. PLoS One. 2010;5(5): e10859.

[0082] The present invention, in some embodiments, shows that miR-22 may function as an epigenetic modifier and EMT promoter independently of its ability to target Pten. In some

embodiments, the present disclosure includes treatment or prevention of metabolic disorder in a subject through the prevention of an increase and/or causing of a decrease in fat mass and obesity-associated protein (FTO) variant and/or shows a downregulation of FTO expression and/or activity. FTO is an enzyme that in humans is encoded by the FTO gene located on chromosome 16. As one homolog in the AlkB family proteins, it is the first mRNA demethylase that has been identified. Certain variants of the FTO gene are associated with obesity in humans.

[0083] In some embodiments, the sequence of the inhibitor is conserved across species. In some embodiments, the sequence of the inhibitor is taken, in part, from the sequence of a human transcript. In some embodiments, the inhibitor is selected to reduce the expression and/or activity of the target miRNA, by way of non-limiting example, miR-22, in a subject.

[0084] In some embodiments, an inhibitor of miRNA is an antisense oligonucleotide. Antisense oligonucleotides can include ribonucleotides or deoxyribonucleotides or a combination thereof. Antisense oligonucleotides may have at least one chemical modification (non-limiting examples are sugar or backbone modifications).

[0085] In some embodiments, the chemical modification is one or more of a phosphorothioate, 2'-0-Methyl, or 2'-O-Methoxyethyl, 2'-0-alkyl-RNA unit, 2'-OMe-RNA unit, 2'-amino-DNA unit, 2'-fluoro-DNA unit (including, but not limited to, a DNA analogue with a substitution to a fluorine at the 2' position (2' F)), LNA unit, PNA unit, HNA unit, INA unit, and a 2' MOE RNA unit.

[0086] Suitable antisense oligonucleotides can be comprised of one or more conformationally constrained or bicyclic sugar nucleoside modifications (BSN) that confer enhanced thermal stability to complexes formed between the oligonucleotide containing BSN and their complementary miRNA target strand. For example, in one embodiment, the antisense oligonucleotides contain at least one locked nucleic acid. Locked nucleic acids (LNAs) contain a 2'-0, 4'-C-methylene ribonucleoside (structure A) wherein the ribose sugar moiety is in a locked conformation. In another embodiment, the antisense oligonucleotides contain at least one 2', 4'-C-bridged 2' deoxyribonucleoside (CDNA, structure B). *See, e.g.,* U.S. Patent No. 6,403,566 and Wang *et al.,* (1999) Bioorganic and Medicinal Chemistry Letters, Vol. 9: 1147-1150, both of which are herein incorporated by reference in their entireties. In yet another embodiment, the antisense oligonucleotides contain at least one modified nucleoside having the structure shown in structure

C. The antisense oligonucleotides targeting miRNAs that regulate fat related metabolism and synthesis pathway targets can contain combinations of BSN (LNA, CDNA, and the like) or other modified nucleotides, and ribonucleotides or deoxyribonucleotides.

A. B. C.

[0087] Alternatively, the antisense oligonucleotides can comprise peptide nucleic acids (PNAs), which contain a peptide-based backbone rather than a sugar-phosphate backbone. Other modified sugar or phosphodiester modifications to the antisense oligonucleotide are also contemplated. By way of non-limiting examples, other chemical modifications can include 2'-o-alkyl (*e.g.*, 2'-0-methyl, 2'-o-methoxyethyl), 2'-fluoro, and 4'-thio modifications, and backbone modifications, such as one or more phosphorothioate, morpholino, or phosphonocarboxylate linkages (*see, e.g.*, U.S. Patent Nos. 6,693,187 and 7,067,641, which are herein incorporated by reference in their entireties). In one embodiment, antisense oligonucleotides targeting oncogenic miRNAs contain 2'-0-methyl sugar modifications on each base and are linked by phosphorothioate linkages. Antisense oligonucleotides, particularly those of shorter lengths (*e.g.*, less than 16 nucleotides, 7-8 nucleotides) can comprise one or more affinity enhancing modifications, such as, but not limited to, LNAs, bicyclic nucleosides, phosphonoformates, 2' o-alkyl modifications, and the like. In some embodiments, suitable antisense oligonucleotides are 2'-0-methoxyethyl gapmers which contain 2'-O-methoxyethyl-modified ribonucleotides on both 5' and 3' ends with at least ten deoxyribonucleotides in the center. These gapmers are capable of triggering RNase H-dependent degradation mechanisms of RNA targets. Other modifications of antisense oligonucleotides to enhance stability and improve efficacy, such as those described in U.S. Patent No. 6,838,283, which is herein incorporated by reference in its entirety, are known in the art and are suitable for use in the methods of the invention. For instance, and not intending to be limiting, to facilitate in vivo delivery and stability, the antisense oligonucleotide can be linked to a steroid, such as cholesterol moiety, a vitamin, a fatty acid, a carbohydrate or glycoside, a peptide, or other small

molecule ligand at its 3' end.

[0088] In some embodiments, antisense oligonucleotides useful for inhibiting the activity of miRNAs, including, for example, miR-22, are about 5 to about 25 nucleotides in length, about 10 to about 30 nucleotides in length, or about 20 to about 25 nucleotides in length. In certain embodiments, antisense oligonucleotides targeting oncogenic miRNAs are about 8 to about 18 nucleotides in length, in other embodiments about 12 to about 16 nucleotides in length, and in other embodiments about 7 to about 8 nucleotides in length. Any 7-mer or longer complementary to an oncogenic miRNA may be used, i.e., any anti-miR complementary to the 5' end of the miRNA and progressing across the full complementary sequence of the miRNA. By way of non-limiting example, the antisense oligonucleotides targeting oncogenic miRNAs, including, for example, miR-22, are about 5, or about 6, or about 7, or about 8, or about 9, or about 10, or about 11, or about 12, or about 13, or about 14, or about 15, or about 16, or about 17, or about 18, or about 19, or about 20, or about 21, or about 22, or about 23, or about 24, or about 25, or about 26, or about 27, or about 28, or about 29, or about 30 nucleotides in length.

[0089] Antisense oligonucleotides can comprise a sequence that is at least partially complementary to a mature or minor (i.e. star) oncogenic miRNA sequence, *e.g.,* at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% complementary to a mature or minor (*i.e.,* star) oncogenic miRNA sequence. In some embodiments, the antisense oligonucleotide can be substantially complementary to a mature or minor oncogenic miRNA sequence, that is at least about 90%, 95%, 96%, 97%, 98%, or 99% complementary to a target polynucleotide sequence. In one embodiment, the antisense oligonucleotide comprises a sequence that is 100% complementary to a mature or minor oncogenic miRNA sequence.

[0090] As used herein, substantially complementary refers to a sequence that is at least about 95%, 96%, 97%, 98%, 99%, or 100% complementary to a target polynucleotide sequence (non- limiting examples are mature, minor, precursor miRNA, or pri-miRNA sequence of, for example, miR-22).

[0091] In some embodiments, the antisense oligonucleotides are antagomirs. Antagomirs are single-stranded, chemically-modified ribonucleotides that are at least partially complementary to miRNAs and therefore may silence them. *See, e.g.,* Kriitzfeldt, *et al.,* Nature (2005) 438 (7068): 685-9. Antagomirs may comprise one or more modified nucleotides, such as 2'-0-methyl-sugar

modifications. In some embodiments, antagomirs comprise only modified nucleotides. Antagomirs can also comprise one or more phosphorothioate linkages resulting in a partial or full phosphorothioate backbone. To facilitate in vivo delivery and stability, the antagomir can be linked to a cholesterol or other moiety at its 3' end. Antagomirs suitable for inhibiting can be about 15 to about 50 nucleotides in length, about 18 to about 30 nucleotides in length, and about 20 to about 25 nucleotides in length. The antagomirs can be at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% complementary to a mature or minor oncogenic miRNA sequence. In some embodiments, the antagomir may be substantially complementary to a mature or minor oncogenic miRNA sequence, that is at least about 95%, 96%, 97%, 98%, or 99% complementary to a target polynucleotide sequence. In other embodiments, the antagomirs are 100% complementary to a mature or minor oncogenic miRNA sequence.

[0092] Antisense oligonucleotides or antagomirs may comprise a sequence that is substantially complementary to a precursor miRNA sequence (pre-miRNA) or primary miRNA sequence (pri-miRNA) of an oncogenic miRNA. In some embodiments, the antisense oligonucleotide comprises a sequence that is located outside the 3'-untranslated region of a target of that miRNA. In some embodiments, the antisense oligonucleotide comprises a sequence that is located inside the 3'-untranslated region of a target of that miRNA.

[0093] Any of the inhibitors or agonists of the oncogenic miRNAs described herein, including but not limited to miR-22, can be delivered to a target cell by delivering to the cell an expression vector encoding the miRNA inhibitors or agonists. A vector is a composition of matter which can be used to deliver a nucleic acid of interest to the interior of a cell. Numerous vectors are known in the art, including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term vector includes an autonomously replicating plasmid or a virus. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, and the like. An expression construct can be replicated in a living cell, or it can be made synthetically. For purposes of this application, the terms expression construct, expression vector, and vector are used interchangeably to demonstrate the application of the invention in a general, illustrative sense, and are not intended to limit the invention.

[0094] In one embodiment, an expression vector for expressing an inhibitor of an oncogenic

miRNA, *e.g.,* miR-22, comprises a promoter operably linked to a polynucleotide encoding an antisense oligonucleotide. The sequence of the expressed antisense oligonucleotide may be partially or perfectly complementary to a mature or minor sequence of an oncogenic miRNA. The phrase operably linked or under transcriptional control as used herein means that the promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.

[0095] As used herein, a promoter refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. Suitable promoters include, but are not limited to, RNA pol I, pol II, pol III, and viral promoters (*e.g.,* human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter, and the Rous sarcoma virus long terminal repeat).

[0096] In certain embodiments, the promoter operably linked to a polynucleotide encoding a miRNA inhibitor or a polynucleotide encoding a metabolism gene regulating miRNA and/or miRNA targeting genes of markers linked to metabolic etiologies can be an inducible promoter. Inducible promoters are known in the art and include, but are not limited to, the tetracycline promoter, the metallothionein IIA promoter, the heat shock promoter, the steroid/thyroid hormone/retinoic acid response elements, the adenovirus late promoter, and the inducible mouse mammary tumor virus LTR.

[0097] Methods of delivering expression constructs and nucleic acids to cells are known in the art and can include, by way of non-limiting example, calcium phosphate co-precipitation, electroporation, microinjection, DEAE-dextran, lipofection, transfection employing polyamine transfection reagents, cell sonication, gene bombardment using high velocity microprojectiles, and receptor-mediated transfection.

[0098] The present invention also includes scavenging or clearing inhibitors of oncogenic miRNAs following treatment. Scavengers may include isolated nucleic acids that are complementary to miRNA inhibitors or vectors expressing the same. Therefore, they may bind to miRNA inhibitors or vectors expressing the same and, in doing so, prevent the binding between miRNA and target.

[0099] In some embodiments, the present disclosure provides a method of treating or preventing

a metabolic disorder in a subject in need thereof. In some embodiments, the present disclosure provides a method of treating or preventing metabolic disorder, including Obesity, Prader-Willi Syndrome, Hypercholesterolemia, Fatty Liver Disease, Non Alcoholic Fatty Liver Disease (NAFLD) and/or Non-Alcoholic Steatohepatitis (NASH) in a subject.

Metabolic disorders

[00100] The term "metabolic disorder" as used in context of this invention refers to a disease or condition that is impacted by the presence, level or activity of brown adipose tissue, plasma glucose concentration, plasma insulin level and/or body fat content. In some embodiments, the metabolic disorder or condition also includes, but is not limited to, Metabolic Syndrome, impaired glucose tolerance, elevated plasma insulin concentrations and insulin resistance, dyslipidemia, hyperglycemia, hyperlipidemia, hypertension, lipodystrophy, cardiovascular disease, respiratory problems or conditions. Metabolic disorders of particular interest are Obesity, Prader-Willi Syndrome, Hypercholesterolemia, Fatty Liver Disease, including Non-Fatty Acid Liver Disease (NAFLD) and/or Non-Alcoholic Steatohepatitis (NASH).

Obesity

[00101] In some embodiments, the present disclosure relates to obesity. Obesity is a chronic disease that is highly prevalent in modern society and is associated not only with a social stigma, but also with decreased life span and numerous medical problems, including diabetes mellitus, insulin resistance, hypertension, hypercholesterolemia, cholelithiasis, osteoarthritis, orthopedic injury, thromboembolic disease, cancer, and coronary heart disease. Rissanen *et al.,* British Medical Journal, 301: 835-837 (1990). In some embodiments, Obesity can be calculated using the body mass index (BMI: body weight per height in meters squared). In some embodiments, obesity is defined as an otherwise healthy subject that has a BMI greater than or equal to 30 kg/m2, or a condition whereby a subject with at least one co-morbidity has a BMI greater than or equal to 27 kg/m2. In some embodiments of the method of the disclosure, the subject is obese and has a body mass index of greater than about 30. In some embodiments, the subject is overweight and has a body mass index of about 25-29.9. In some embodiments, the method induces weight loss. In some embodiments, the method prevents weight gain. In some embodiments, the method prevents the growth of adipose tissue and impair adipocyte differentiation.

[00102] In some embodiments, the present disclosure provides for methods of treatment comprising administering an inhibitor of miR-22 (and compounds related to miR-22) and/or uses of miR-22 (and compounds related to miR-22) in the treatment of, or manufacture of a medicament for, obesity and overweight, and related conditions. In some embodiments, the present disclosure provides a method for treating or preventing obesity, comprising administering an effective amount of miR-22 (and compounds related to inhibiting miR-22) to a patient in need thereof. In some aspects, the present disclosure provides a method for weight management, comprising administering an effective amount of an inhibitor of miR-22 (and compounds related to inhibiting miR-22) to induce weight loss and/or to prevent weight gain in a patient in need thereof.

[00103] In some embodiments, the present disclosure relates to a method for inducing weight loss or preventing weight gain (or treating or preventing obesity or inducing weight loss or preventing weight gain in a patient that does not substantially change caloric intake), comprising administering an effective amount of an inhibitor of miR-22 (and compounds related to miR-22) to a patient that: has undertaken or will undertake a surgery of the digestive system; is greater than about 80-100 pounds overweight; has a BMI of greater than about 35; or has a health problem related to obesity.

[00104] In some embodiments the surgery of the digestive system is one or more of those classified under ICD-9-CM: Operations on the Digestive System and therefore may include Operations on esophagus; Incision and excision of stomach; Other operations on stomach; Incision, excision, and anastomosis of intestine; Other operations on intestine; Operations on appendix; Operations on rectum, rectosigmoid and perirectal tissue; Operations on anus; Operations on liver; Operations on gallbladder and biliary tract; Operations on pancreas; Repair of hernia; and Other operations on abdominal region.

[00105] In some embodiments, the surgery of the digestive system is one or more of a restrictive surgery and/or malabsorptive procedure, including, for example, vertical banded gastroplasty (VBG, *e.g.,* stomach stapling); gastric banding (*e.g.,* LAP-BAND or REALIZE); sleeve gastrectomy; gastric bypass surgery (*e.g.,* Roux-en-Y gastric bypass), biliopancreatic diversion and a cosmetic surgery (*e.g.,* liposuction, such as, for example, suction-assisted liposuction (SAL); ultrasound-assisted liposuction (UAL); power-assisted liposuction (PAL); twin-cannula (assisted) liposuction (TCAL or TCL); external ultrasound-assisted liposuction (XUAL or EUAL); water-

assisted liposuction (WAL); laser assisted liposuction; tumescent liposuction; and cryolipolysis).

[00106] In some embodiments, the health problem related to obesity is selected from cardiovascular diseases (*e.g.,* high cholesterol, hypercholesterolemia, low HDL, high HDL, hypertension, coronary artery disease, heart failure), sleep apnea (including obstructive sleep apnea), osteoarthritis, thyroid problems, dementia, gout, asthma, gastroesophageal reflux disease, and chronic renal failure. In some embodiments, the health problem related to obesity is heart disease, sleep apnea, or high cholesterol.

[00107] In some aspects, the present disclosure provides for uses and methods for inducing weight loss or preventing weight gain, comprising administering an effective amount of an inhibitor of miR-22 (and compounds related to inhibiting miR-22) to a patient in need thereof; wherein the patient does not substantially change caloric intake. In some embodiments, the caloric intake is high, relative to guidelines, such as the USDA tables. In some embodiments, the patient's caloric intake is 2000-10000 calories/day, or greater than about 2000 calories/day, or about 2200 calories/day, or about 2400 calories/day, or about 2600 calories/day, or about 2800 calories/day, or about 3000 calories/day, or about 3200 calories/day, or about 3400 calories/day, or about 3600 calories/day, or about 3800 calories/day, or about 4000 calories/day, or about 5000 calories/day, or about 6000 calories/day. In some embodiments, the patient has a high caloric intake and does not gain weight or even loses weight. Therefore, the present disclosure provides for an effect without life style changes that often reduce patient adherence (*e.g.,* failed dieting). In some embodiments, the patient's caloric intake is not restricted by more than about 20%, or not by more than about 10%, or not by more than about 5% of the patient's caloric intake at the start of treatment. In some embodiments, a high proportion of the patient's caloric intake is "empty calories," i.e. calories from solid fats and/or added sugars. In some embodiments, greater than about 15%, or 20%, or 25%, or 30%, or 35%, or 50% of the patient's caloric intake is empty calories. Even in these embodiments, a patient may not gain weight or even lose weight.

[00108] In some embodiments, the patient of the present disclosure is overweight or obese. In some embodiments, the patient of the present disclosure suffers from central obesity. In some embodiments, the obesity of one of simple obesity (alimentary obesity; usually resulting from consumption of more calories than the body can utilize), secondary obesity (usually resulting from an underlying medical condition, such as, for example, Cushing's syndrome and polycystic ovary

syndrome), and childhood obesity. In some embodiments, the obesity is classified as: Class I, which includes a BMI between 30 and 34.99; Class II, which includes BMIs of 35 to 39.99; and Class III, which includes a BMI of over 40. Further, the present disclosure provides for obesity of any of classes I, II, or III that is further classified as severe, morbid, and super obesity. In some embodiments, the patient is at risk of further weight gain, as assessed by, for example, daily caloric intake.

[00109] In some embodiments, the weight management/weight loss/anti-obesity effects of an inhibitor of miR-22 (and compounds related to miR-22) can be assessed using various techniques and indices. In some embodiments, assessment before, during, and after treatment is undertaken. In some embodiments, body mass index (BMI), a measure of a person's weight taking into account height, may be used. In some embodiments, a patient described herein has a BMI that provides an "overweight" classification, i.e. 25-29.9, such as, for example, about 25, or about 25.5, or about 26, or about 26.5, or about 27, or about 27.5, or about 28, or about 28.5, or about 29, or about 29.5. In some embodiments, a patient described herein has a BMI that provides an "obese" classification, i.e. greater than 30, such as, for example, about 30, or about 31, or about 32, or about 33, or about 34, or about 35, or about 36, or about 37, or about 38, or about 39, or about 40, or about 50. In some embodiments, body volume index (BVI) is used. BVI uses 3D software to create a 31) image of a person so BVI can differentiate between people with the same BMI rating, but who have a different shape and different weight distribution. BVI measures where a person's weight and the fat are located on the body, rather than total weight or total fat content and places emphasis on the weight carried around the abdomen, commonly known as central obesity. In some embodiments, whole-body air displacement plethysmography (ADP) is used to assess the weight management/weight loss/anti-obesity effects of miR-22 (and compounds related to miR-22). In some embodiments, simple weighing is used in the present invention. In some embodiments, skinfold calipers or "pinch test," bioelectrical impedance analysis, hydrostatic weighing, or dual-energy X-ray absorptiometry (DEXA) may be used.

[00110] In some embodiments, simple circumferential measurement of the body may be used. In some embodiments, a patient of the present disclosure has a waist circumference exceeding about 35 inches, or about 36 inches, or about 37 inches, or about 38 inches, or about 39 inches, or about 40 inches, or about 41 inches, or about 42 inches, or about 43 inches, or about 44 inches, or about

45 inches, or about 46 inches, or about 47 inches, or about 48 inches, or about 50 inches, or about 55 inches, or about 60 inches. In some embodiments, the patient is male human with a waist circumference exceeding 40 inches. In some embodiments, the patient is a female human with a waist circumference exceeding 35 inches.

[00111] The methods of the disclosure may be used to treat humans having a body fat percentage above the recommended body fat percentage, i.e., at least in the "overweight" range, or at least in the "obese" range. The body fat percentage will differ between women and men. Specifically, for women, the methods of the disclosure may be used to treat a female human having a body fat percentage of at least about 25%, above 25%, at least about 32%, or above 32%. For men, the methods of the disclosure may be used to treat a male human having a body fat percentage of at least about 14%, above 14%, at least about 18%, above 18%, at least about 25%, or above 25%. Body fat percentage may be estimated using any method accepted in the art, including, for example, near infrared interactance, dual energy X-ray absorptiometry, body density measurement, bioelectrical impedance analysis, and the like.

[00112] The methods of the disclosure may be used to treat a patient who is a man that is greater than 100 pounds' overweight and/or has waist circumference exceeding 40 inches. The methods of the disclosure may be used to treat a patient who is a woman that is greater than 80 pounds' overweight and/or waist circumference exceeding 35 inches.

[00113] In some embodiments, the disclosure provides for an inhibitor of miR-22 (and compounds related to inhibiting miR-22) being used to treat and/or prevent certain disorders associated with being overweight. For example, miR-22 (and compounds related to miR-22) find use in cardiovascular diseases (*e.g.,* high cholesterol, hypercholesterolemia, low HDL, high HDL, hypertension, coronary artery disease, heart failure), sleep apnea (including obstructive sleep apnea), osteoarthritis, thyroid problems, dementia, gout, asthma, gastroesophageal reflux disease, and chronic renal failure.

[00114] In some embodiments, the inhibitor of miR-22 (and compounds related to miR-22) administration and/or use prevents or reduces the growth of adipose tissue. In some embodiments. miR-22 (and compounds related to miR-22) effects one or more of white adipose tissue (WAT) and brown adipose tissue (BAT), including, for example, visceral adipose tissue (VAT), abdominal

subcutaneous adipose tissue (ASAT), or ectopic fat. Such an effect may be assessed by, for example, using any of the techniques described herein (*e.g.*, BMI, weight for-stature indexes, skinfold measures, electrical bioimpedance analysis, etc.), as well as various imaging techniques, including computed tomography (CT), magnetic-resonance imaging (MRI, including transverse body scans), dual energy X-ray absorptiometry (DXA).

[00115] miR-22 (and compounds related to miR-22) may also be used in combination with dietary therapy, behavioral therapy, physical therapy, exercise, and weight loss surgery, or a combination of two or more such therapies. In some embodiments, the subject is on a calorie restricted diet. In some embodiments, the subject engages in or is to engage in a physical exercise or physical therapy regimen. In some embodiments, the subject has undergone, or will undergo, weight loss surgery. In some embodiments, an inhibitor of miR-22 (and compounds related to inhibiting miR-22) may be in combination with additional agents or may be administered to patient undergoing treatment with various agents.

[00116] For example, including, but not limited to, embodiments pertaining to obesity and/or weight reduction/loss, the additional agents may include one or more of orlistat (*e.g.*, ALLI, XENICAL), loracaserin (*e.g.*, BELVIQ), phentermine-topiramate (*e.g.*, QSYMIA), sibutramine (*e.g.*, REDUCTIL or MERIDIA), rimonabant (ACOMPLIA), exenatide (*e.g.*, BYETTA), pramlintide (*e.g.*, SYMLIN) phentermine, benzphetamine, diethylpropion, phendimetrazine, bupropion, and metformin.

[00117] Agents that interfere with the body's ability to absorb specific nutrients in food are among the additional agents, *e.g.*, orlistat (*e.g.*, ALLI, XENICAL), glucomannan, and guar gum. Agents that suppress apetite are also among the additional agents, e.g. catecholamines and their derivatives (such as phentermine and other amphetamine-based drugs), various antidepressants and mood stabilizers (*e.g.*, bupropion and topiramate), anorectics (*e.g.*, dexedrine, digoxin). Agents that increase the body's metabolism are also among the additional agents.

[00118] In some embodiments, additional agents may be selected from among appetite suppressants, neurotransmitter reuptake inhibitors, dopaminergic agonists, serotonergic agonists, modulators of GABAcrgic signaling, anticonvulsants, antidepressants, monoamine oxidase inhibitors, substance P (NKl) receptor antagonists, melanocortin receptor agonists and antagonists,

lipase inhibitors, inhibitors of fat absorption, regulators of energy intake or metabolism, cannabinoid receptor modulators, agents for treating addiction, agents for treating metabolic syndrome, peroxisome proliferator-activated receptor (PPAR) modulators; dipeptidyl peptidase 4 (DPP-4) antagonists, agents for treating cardiovascular disease, agents for treating elevated triglyceride levels, agents for treating low HDL, agents for treating hypercholesterolemia, and agents for treating hypertension. Some agents for cardiovascular disease include statins (*e.g.,* lovastatin, atorvastatin, fluvastatin, rosuvastatin, simvastatin and pravastatin) and omega-3 agents (*e.g.,* LOVAZA, EPANOVA, VASCEPA, esterified omega-3's in general, fish oils, krill oils, algal oils). In some embodiments, additional agents may be selected from among amphetamines, benzodiazepines, sulfonyl ureas, meglitinides, thiazolidinediones, biguanides, beta-blockers. ACE inhibitors, diuretics, nitrates, calcium channel blockers, phenlermine, sibutramine, lorcaserin, cetilistat, rimonabant, taranabant, topiramate, gabapentin, valproate, vigabatrin, bupropion, tiagabine, sertraline, fluoxetine, trazodone, zonisamide, methylphenidate, varenicline, naltrexone, diethylpropion, phendimetrazine, repaglinide, nateglinide, glimepiride, metformin, pioglitazone, rosiglilazone, and sitagliptin.

Prader-Willi syndrome

[00119] In some embodiments, the method relates to Prader-Willi syndrome. Prader-Willi syndrome is a complex genetic condition that affects many parts of the body. In infancy, the condition is characterized by weak muscle tone (hypotonia), feeding difficulties, poor growth, and delayed development. Beginning in childhood, affected individuals develop an insatiable appetite, which leads to chronic overeating (hyperphagia) and obesity. In some embodiments, the subject is suffering from Prader-Willi syndrome. In some embodiments, Prader-Willi syndrome, particularly those with obesity, also develop type 2 diabetes mellitus.

Fatty Liver Disease

[00120] In some embodiments, the method of the disclosure relates to Fatty Liver Disease. A substantial fraction of the population is affected by fatty liver disease, also known as nonalcoholic steatohepatitis (NASH). NASH is often associated with obesity and diabetes. Hepatic steatosis, the presence of droplets of triglycerides with hepatocytes, predisposes the liver to chronic inflammation (detected in biopsy samples as infiltration of inflammatory leukocytes), which can

lead to fibrosis and cirrhosis. Fatty liver disease is generally detected by observation of elevated serum levels of liver-specific enzymes such as the transaminases ALT and AST, which serve as indices of hepatocyte injury, as well as by presentation of symptoms which include fatigue and pain in the region of the liver, though definitive diagnosis often requires a biopsy. The anticipated benefit is a reduction in liver inflammation and fat content, resulting in attenuation, halting, or reversal of the progression of NASH toward fibrosis and cirrhosis. In some embodiments, the method reduces or prevents liver steatosis. In some embodiments, the method reduces or prevents liver fibrosis.

[00121] In some embodiments, the method is a method of treating NASH by administering the inhibitor of miR-22 described herein. The NASH patient can be a high risk NASH patient. A "high risk NASH patient" refers to characterization by one or more of: NAS≧4; baseline fibrosis stage 2 or 3; or baseline fibrosis stage 1 with a comorbidity (type 2 diabetes, BMI≧30 kg/m2 or ALT≧60 U/L).

[00122] In some embodiments, the inhibitor of miR-22 reduces one or more of steatosis, mixed acinar inflammation, and hepatocellular ballooning and/or pericellular fibrosis.

[00123] In some embodiments, the inhibitor of miR-22 reduces one or more of steatosis.

[00124] In some embodiments, the inhibitor of miR-22 treats mild, grade 1 NASH, or moderate, grade 2 NASH, or severe, grade 3 NASH, as described in Brunt, *et al.* Am. J. Gastroenterology, Vol. 94, No. 9 (1999), the entirety of which is incorporated by reference in its entirety:

Mild, grade 1	Steatosis (predominantly macrovesicular) involving up to 66% of biopsy; may see occasional ballooned zone 3 hepatocytes; scattered rate intra-acinar pmn's ± intra-acinar lymphocytes; no or mild portal chronic inflammation.
Moderate, grade 2	Steatosis of any degree; ballooning of hepatocytes (predominantly zone 3) obvious; intra-acinar pmn's noted, may be associated with zone 3 pericellular fibrosis; portal and intra-acinar chronic inflammation noted, mild to moderate.
Severe, grade 3	Panacinar steatosis; ballooning and disarray obvious, predominantly in zone 3; intra-acinar inflammation noted as scattered pmn's, pms's associated with ballooned hepatocytes ± mild chronic inflammation; portal chronic inflammation mild or moderate, not marked.

[00125] In some embodiments, the inhibitor of miR-22 treats NASH of any of the following stages: Stage 0, No fibrosis; Stage 1, Zone 3 pericellular/sinusoidal fibrosis, focal or extensive; Stage 2, as in stage 1 plus portal fibrosis, focal or extensive; Stage 3, bridging fibrosis, focal or extensive; and Stage 4 cirrhosis (+/- residual pericellular fibrosis).

[00126] In some embodiments, the inhibitor of miR-22 treats NASH of any activity score (NAS), as described in Kleiner, et al., Hepatology, 2005. 41(6): p. 1313-21, the entirety of which is incorporated by reference in its entirety:

Histological feature	Definition	Score
Steatosis	<5%	0
	5-33%	1
	33-66%	2
	>66%	3
Lobular inflammation*	None	0
	<2 foci	1
	2-4 foci	2
	>4 foci	3
Ballooning**	None	0
	Few cells	1
	Prominent	2

*The number of foci was counted per 200x field for lobular inflammation

**Few ballooned cells indicate rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline

[00127] In some embodiments, the inhibitor of miR-22 reduces the NASH to less than 8, or less than 7, or less than 6, or less than 5, or less than 4, or less than 3, or less than 2, or less than 1. In some embodiments, the AP-based reduces the NAS to about 7, or to about 6, or less than 5, or less than 4, or to about 3, to about 2, or to about 1.

[00128] In some embodiments, the inhibitor of miR-22 reduces steatosis by about 5%, or about 10%, or about 15%, or about 20%, or about 25%, or about 30%, or about 35%, or about 40%, or about 45%, or about 50%, or about 55%, or about 60%, or about 65%, or about 70%, or about 75%, or about 80%, or about 85%, or about 90%, or about 95%.

[00129] In some embodiments, the inhibitor of miR-22 reduces lobular inflammation to less than 4 foci, or less than 3 foci, or less than 2 foci, or less than 1 focus.

[00130] In some embodiments, the inhibitor of miR-22 reduces ballooning to a score of 0 or 1 per the scale above.

[00131] In some embodiments, the inhibitor of miR-22 treats a subject at risk for NASH, such as a subject suffering from various acquired metabolic diseases, such as obesity, diabetes (*e.g.,* type 2), hypertriglyceridemia, rapid weight loss, and malnutrition. In some embodiments, the inhibitor of miR-22 treats a subject at risk for NASH, such as a subject suffering from various genetic metabolic diseases, such as Wilson disease, tyrosinemia, and abetalipoproteinemia. In some embodiments, the inhibitor of miR-22 treats a subject at risk for NASH, such as a subject suffering from various other factors such as lipodystrophy and jejunoileal bypass. In some embodiments, the inhibitor of miR-22 treats a subject at risk for NASH, such as a subject undergoing treatment with one or more of amiodarone, chemotherapeutic agents (*e.g.,* irinotecan), tamoxifen, steroids, estrogens, diethylstilbestrol, methotrexate, calcium channel blockers (*e.g.,* nifedipine, verapamil, and diltiazem).

[00132] In some embodiments, the present disclosure provides methods that reduce or prevent fibrosis. Direct markers of fibrosis include procollagen type (I, III, IV), matrix metalloproteinases, cytokines, and chemokines. In some embodiments, the present invention provides methods that reduce or prevent enhancement of extracellular matrix synthesis, *e.g.,* by activated stellate cells. In some embodiments, the present invention provides methods that modulate levels of TIMP-1. In some embodiments, the present disclosure provides methods that reduce or prevent serum levels

of hyaluronic acid.

[00133] In some embodiments, the effect of the inhibitor of miR-22 is monitored using tests that monitor one or more of combines hyaluronic acid, tissue inhibitor of a metalloproteinase-1 (TIMP-1), and alpha-2-macroglobulin (*e.g.,* FIBROSpect II).

[00134] In some embodiments, the present invention provides methods that reduce or prevent cirrhosis.

[00135] In some embodiments, the present disclosure provides methods that modulate one or more of platelet count, prothrombin time, albumin, total bilirubin, and serum aminotransferase. In some embodiments, the present invention provides methods that modulate serum fibrotic markers, such as hyaluronic acid (HA) and alpha-2-macroglobulin.

Hypercholesterolemia

[00136] In some embodiments, the method of the disclosure relates to hypercholesterolemia. Hypercholesterolemia is a condition characterized by elevated serum cholesterol. Elevated serum cholesterol levels affect a substantial fraction of the population and are an important risk factor for atherosclerosis and myocardial infarction. Cholesterol-lowering drugs such as HMG-CoA reductase inhibitors (statins) can be administered to hypercholesterolemia patients in addition to agents of the invention, optionally incorporated into the same pharmaceutical. In some embodiments, the hypocholesterolemia is on-familial hypercholesterolemia which is a condition characterized by elevated serum cholesterol that is not the result of a single gene mutation. In some embodiments, the hypercholesterolemia is polygenic hypercholesterolemia which is a condition characterized by elevated cholesterol that results from the influence of a variety of genetic factors. In certain embodiments, polygenic hypercholesterolemia may be exacerbated by dietary intake of lipids. In some embodiments, the hypercholesterolemia is Familial hypercholesterolemia (FH) which is an autosomal dominant metabolic disorder characterized by a mutation in the LDL-receptor (LDL-R) gene, markedly elevated LDL-C and premature onset of atherosclerosis. A diagnosis of familial hypercholesterolemia is made when an individual meets one or more of the following criteria: genetic testing confirming 2 mutated LDL-receptor genes; genetic testing confirming one mutated LDL-receptor gene; document history of untreated serum LDL-cholesterol greater than 500 mg/dL; tendinous and/or cutaneous xanthoma prior to age 10 years;

or, both parents have documented elevated serum LDL-cholesterol prior to lipid-lowering therapy consistent with heterozygous familial hypercholesterolemia. In some embodiments, the hypercholesterolemia is Homozygous familial hypercholesterolemia or HoFH which is a condition characterized by a mutation in both maternal and paternal LDL-R genes. In some embodiments, the hypercholesterolemia is Heterozygous familial hypercholesterolemia or HeFH which is a condition characterized by a mutation in either the maternal or paternal LDL-R gene.

[00137] In some embodiments of the methods of the disclosure, the wild type human FTO gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001080432.2; SEQ ID NO: 13).

[00138] In some embodiments of the methods of the disclosure, the wild type human FTO gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001073901.1; SEQ ID NO: 14).

[00139] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_004364.4, transcript variant 1; SEQ ID NO: 15).

[00140] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_004355.2, transcript variant 1; SEQ ID NO: 16).

[00141] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001285829.1, transcript variant 2; SEQ ID NO: 17).

[00142] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NM_001285829.1, transcript variant 2; SEQ ID NO: 18).

[00143] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001287424.1, transcript variant 3; SEQ ID NO: 19).

[00144] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number:

NP_001274353.1, transcript variant 3; SEQ ID NO: 20).

[00145] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001287435.1, transcript variant 4; SEQ ID NO: 21).

[00146] In some embodiments of the methods of the disclosure, the wild type human CEBPa gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001274364.1, transcript variant 4; SEQ ID NO: 22).

[00147] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_138712.3, transcript variant 1; SEQ ID NO: 23).

[00148] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_619726.2, transcript variant 1; SEQ ID NO: 24).

[00149] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_015869.4, transcript variant 2; SEQ ID NO: 25).

[00150] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_056953.2, transcript variant 2; SEQ ID NO: 26).

[00151] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_138711.3, transcript variant 3; SEQ ID NO: 27).

[00152] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_619725.2, transcript variant 3; SEQ ID NO: 28).

[00153] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_005037.5, transcript variant 4; SEQ ID NO: 29).

[00154] In some embodiments of the methods of the disclosure, the wild type human PPARg gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number:

NP_005028.4, transcript variant 4; SEQ ID NO: 30).

[00155] In some embodiments of the methods of the disclosure, the wild type human PTEN gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_000314.6, transcript variant 1; SEQ ID NO: 31).

[00156] In some embodiments of the methods of the disclosure, the wild type human PTEN gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_000305.3, transcript variant 1; SEQ ID NO: 32).

[00157] In some embodiments of the methods of the disclosure, the wild type human PTEN gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001304717.2, transcript variant 1; SEQ ID NO: 33).

[00158] In some embodiments of the methods of the disclosure, the wild type human PTEN gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001291646.2, transcript variant 1; SEQ ID NO: 34).

[00159] In some embodiments of the methods of the disclosure, the wild type human PTEN gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001304718.1, transcript variant 2; SEQ ID NO: 35).

[00160] In some embodiments of the methods of the disclosure, the wild type human PTEN gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001291647.1, transcript variant 2; SEQ ID NO: 36).

[00161] In some embodiments of the methods of the disclosure, the wild type human TET2 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001127208.2, transcript variant 1; SEQ ID NO: 37).

[00162] In some embodiments of the methods of the disclosure, the wild type human TET2 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001120680.1, transcript variant 1; SEQ ID NO: 38).

[00163] In some embodiments of the methods of the disclosure, the wild type human TET2 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_017628.4, transcript variant 2; SEQ ID NO: 39).

[00164] In some embodiments of the methods of the disclosure, the wild type human TET2 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number:

NP_060098.3, transcript variant 2; SEQ ID NO: 40).

[00165] In some embodiments of the methods of the disclosure, the wild type human BMP-7 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001719.2, transcript variant 1; SEQ ID NO: 41).

[00166] In some embodiments of the methods of the disclosure, the wild type human BMP-7 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001710.1, transcript variant 1; SEQ ID NO: 42).

[00167] In some embodiments of the methods of the disclosure, the wild type human SIRT-1 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_012238.4, transcript variant 1; SEQ ID NO: 43).

[00168] In some embodiments of the methods of the disclosure, the wild type human SIRT-1 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_036370.2, transcript variant 1; SEQ ID NO: 44).

[00169] In some embodiments of the methods of the disclosure, the wild type human SIRT-1 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001142498.1, transcript variant 2; SEQ ID NO: 45).

[00170] In some embodiments of the methods of the disclosure, the wild type human SIRT-1 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001135970.1, transcript variant 2; SEQ ID NO: 46).

[00171] In some embodiments of the methods of the disclosure, the wild type human SIRT-1 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number: NM_001314049.1, transcript variant 3; SEQ ID NO: 47).

[00172] In some embodiments of the methods of the disclosure, the wild type human SIRT-1 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number: NP_001300978.1, transcript variant 3; SEQ ID NO: 48).

[00173] In some embodiments of the methods of the disclosure, the wild type human PGC1-α gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_001330751, transcript variant 1; SEQ ID NO: 49).

[00174] In some embodiments of the methods of the disclosure, the wild type human PGC1a gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number

NP_001317680: transcript variant 2; SEQ ID NO: 50).

[00175] In some embodiments of the methods of the disclosure, the wild type human PGC1-α gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_013261, transcript variant 2; SEQ ID NO: 51).

[00176] In some embodiments of the methods of the disclosure, the wild type human PGC1a gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_037393: transcript variant 2; SEQ ID NO: 52).

[00177] In some embodiments of the methods of the disclosure, the wild type human PGC1-α gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_001330752.1, transcript variant 3; SEQ ID NO: 53).

[00178] In some embodiments of the methods of the disclosure, the wild type human PGC1a gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_001317681.1, transcript variant 3; SEQ ID NO: 54).

[00179] In some embodiments of the methods of the disclosure, the wild type human PGC1-α gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_001330753.1, transcript variant 4; SEQ ID NO: 55).

[00180] In some embodiments of the methods of the disclosure, the wild type human PGC1a gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_001317682, transcript variant 4; SEQ ID NO: 56).

[00181] In some embodiments of the methods of the disclosure, the wild type human SP-1 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_138473.2; SEQ ID NO: 57).

[00182] In some embodiments of the methods of the disclosure, the wild type human SP-1 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_612482; SEQ ID NO: 58).

[00183] In some embodiments of the methods of the disclosure, the wild type human FGF-21 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_019113.3; SEQ ID NO: 59).

[00184] In some embodiments of the methods of the disclosure, the wild type human FGF-21 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession

number NP_061986.1; SEQ ID NO: 60).

[00185] In some embodiments of the methods of the disclosure, the wild type human UCP1 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_021833.4; SEQ ID NO: 61).

[00186] In some embodiments of the methods of the disclosure, the wild type human UCP1 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_068605.1; SEQ ID NO: 62).

[00187] In some embodiments of the methods of the disclosure, the wild type human DDIT-4 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_019058.3; SEQ ID NO: 63).

[00188] In some embodiments of the methods of the disclosure, the wild type human DDIT-4 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_061931.1; SEQ ID NO: 64).

[00189] In some embodiments of the methods of the disclosure, the wild type human METTL3 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_019852.4; SEQ ID NO: 65).

[00190] In some embodiments of the methods of the disclosure, the wild type human METTL3 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_062826.2; SEQ ID NO: 66).

[00191] In some embodiments of the methods of the disclosure, the wild type human FGF1gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_000800.4; SEQ ID NO: 67).

[00192] In some embodiments of the methods of the disclosure, the wild type human FGF1 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number NP_000791.1; SEQ ID NO: 68).

[00193] In some embodiments of the methods of the disclosure, the wild type human TP63 gene of the disclosure consists of or comprises the nucleic acid sequence (Genbank Accession number NM_001114978.1; SEQ ID NO: 69).

[00194] In some embodiments of the methods of the disclosure, the wild type human TP63 gene of the disclosure consists of or comprises the amino acid sequence (Genbank Accession number

NP_001108450.1; SEQ ID NO: 70).

[00195] As used herein, the term subject or patient refers to any vertebrate including, without limitation, humans and other primates (*e.g.,* chimpanzees and other apes and monkey species), farm animals (*e.g.,* cattle, sheep, pigs, goats, and horses), domestic mammals (*e.g.,* dogs and cats), laboratory animals (*e.g.,* rodents such as mice, rats, and guinea pigs), and birds (*e.g.,* domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like). In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[00196] Another embodiment of the present invention is a pharmaceutical composition, or use of pharmaceutical composition, comprising an inhibitor of a miRNA, such as miR-22, and a pharmaceutically acceptable carrier. Where clinical applications are contemplated, pharmaceutical compositions may be prepared in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[00197] In one embodiment, a pharmaceutical composition comprises an effective dose of a miRNA inhibitor, by way of non-limiting example, an antisense oligonucleotide directed to miR-22, and a pharmaceutically acceptable carrier. An effective dose is an amount sufficient to affect a beneficial or desired clinical result. An effective dose of a miRNA inhibitor of the invention may be from about 1 mg/kg to about 100 mg/kg, about 2.5 mg/kg to about 50 mg/kg, or about 5 mg/kg to about 25 mg/kg. The precise determination of what would be considered an effective dose may be based on factors individual to each patient, including their size, age, type of metabolic disorder, and nature of inhibitor or agonist (non-limiting examples include antagomir, expression construct, antisense oligonucleotide, polynucleotide duplex, etc.). Therefore, dosages can be readily ascertained by those of ordinary skill in the art from this disclosure and the knowledge in the art. For example, doses may be determined with reference Physicians' Desk Reference, 66th Edition, PDR Network; 2012 Edition (December 27, 2011), the contents of which are incorporated by reference in its entirety.

[00198] A beneficial or desired treatment result may include, inter alia, a reduction a body mass index, weight loss or a marker that is associated with the presence of metabolic disorder as compared to what is observed without administration of the inhibitor. A beneficial or desired

treatment result may also include, inter alia, an increased or decreased presence of a marker or gene that is associated with a reduction of metabolic disorder as compared to what is observed without administration of the inhibitor. In some embodiments, the marker or gene is fat mass and obesity-associated protein (FTO), CEBPa, and/or PPARγ, ALKBH5, and ACLY. In some embodiments, there is a perturbation in activity and/or expression of FTO, CEBPa, PPARa, ACLY, SP-1, PGC1a, ALKBH5, SIRT-1, TP63, FGF1, and/or DDIT4. In some embodiments, the marker or gene is fat mass and obesity-associated protein (FTO).

[00199] Colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes, may be used as delivery vehicles for the oligonucleotide inhibitors of oncogenic miRNA function, polynucleotides encoding Fat related metabolism and synthesis pathway targets miRNA agonists, or constructs expressing particular miRNA inhibitors or agonists. Commercially available fat emulsions that are suitable for delivering the nucleic acids of the disclosure to adipose tissues (*e.g.,* adipocytes) include INTRALIPIDO, LIPOSYN®, LIPOSYN® II, LIPOSYN® III, Nutrilipid, and other similar lipid emulsions. A colloidal system for use as a delivery vehicle in vivo is a liposome (i.e., an artificial membrane vesicle). The preparation and use of such systems is well known in the art. Exemplary formulations are also disclosed in US 5,981,505; US6,217,900; US 6,383,512; US 5,783,565; US 7,202,227; US 6,379,965; US 6,127,170; US 5,837,533; US 6,747,014; and W003/093449, which are herein incorporated by reference in their entireties.

[00200] One will generally desire to employ appropriate salts and buffers to render delivery vehicles stable and allow for uptake by target cells. Aqueous compositions of the present invention comprise an effective amount of the delivery vehicle comprising the inhibitor polynucleotides (*e.g.,* liposomes or other complexes or expression vectors) dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The phrases pharmaceutically acceptable or pharmacologically acceptable refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, pharmaceutically acceptable carrier includes solvents, buffers, solutions, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like acceptable for use in formulating pharmaceuticals, such as pharmaceuticals suitable for

administration to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions, provided they do not inactivate the vectors or polynucleotides of the compositions.

[00201] The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention may be via any common route so long as the target tissue is available via that route. This includes oral, nasal, or buccal. Alternatively, administration may be by intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection, or by direct injection into adipose tissue. The agents disclosed herein may also be administered by catheter systems. Such compositions would normally be administered as pharmaceutically acceptable compositions as described herein.

[00202] The active compounds may also be administered parenterally or intraperitoneally. By way of illustration, solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations generally contain a preservative to prevent the growth of microorganisms.

[00203] The pharmaceutical forms suitable for injectable use or catheter delivery include, for example, sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Generally, these preparations are sterile and fluid to the extent that easy injectability exists. Preparations should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Appropriate solvents or dispersion media may contain, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases,

it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00204] Sterile injectable solutions may be prepared by incorporating the active compounds in an appropriate amount into a solvent along with any other ingredients (for example as enumerated above) as desired, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the desired other ingredients, *e.g.,* as enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation include vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient(s) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00205] Upon formulation, solutions may be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations may easily be administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution generally is suitably buffered and the liquid diluent first rendered isotonic with, for example, sufficient saline or glucose. Such aqueous solutions may be used, for example, for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Preferably, sterile aqueous media are employed as is known to those of skill in the art, particularly in light of the present disclosure. By way of illustration, a single dose may be dissolved in 1 ml of isotonic NaC1 solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (*see, e.g.,* Remington Pharmaceutical Sciences, 15th Edition, pages 1035-1038 and 1570-1580, the contents of which are hereby incorporated by reference). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the FDA Office of Biologics standards.

[00206] In some embodiments, of the present disclosure includes a method of treating or preventing metabolic disorder in a subject in need thereof comprising administering to the subject:

a first inhibitor of a first miRNA, wherein the miRNA is miR-22 and a second inhibitor of a second miRNA, wherein the miRNA is a regulator of a metabolism-related gene. In some embodiments, the second miRNA is a known miR inhibitor, including, by way of non-limiting example, those disclosed in International Patent Publication No. WO 2012/142313, the contents of which are hereby incorporated by reference in their entirety. In some embodiments, the first and second inhibitors may be administered in either order (*e.g.,* first then second or second then first) or concurrently.

[00207] In some embodiments, of the present disclosure includes a method of treating or preventing metabolic disorder in a subject in need thereof comprising administering to the subject a first agent that is or comprises an inhibitor of miR-22 and a second agent that is or comprises at least one other metabolic disorder biologic, therapeutic or drug. In some embodiments, the first and second inhibitors may be administered in either order (*e.g.,* first then second or second then first) or concurrently.

[00208] The invention also provides kits that can simplify the administration of any agent described herein, such as an inhibitor of an oncogenic miRNA, including antisense oligonucleotide directed to miR-22. An exemplary kit of the invention comprises any composition described herein in unit dosage form. In one embodiment, the unit dosage form is a container, such as a pre-filled syringe, which can be sterile, containing any agent described herein and a pharmaceutically acceptable carrier, diluent, excipient, or vehicle. The kit can further comprise a label or printed instructions instructing the use of any agent described herein. The kit may also include a lid speculum, topical anesthetic, and a cleaning agent for the administration location. The kit can further comprise one or more additional agent, such as a second inhibitor of an oncogenic miRNA, or a biologic, therapeutic, chemotherapeutic or drug described herein. In one embodiment, the kit comprises a container containing an effective amount of a composition of the invention and an effective amount of another composition, such those described herein.

EXAMPLES

[00209] In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

Example 1: MiR-22 role in obesity

[00210] miR-22 directly targets PTEN and TET to promote tumorigenesis, metastasis and other metabolic disorders. More than 60 PTEN-targeting miRNAs and no less than 30 new proto-oncogenic genetic loci were studied in human cancer. Highly conserved evolutionarily among vertebrates and ubiquitously expressed in various tissues, (Lagos-Quintana *et al.,* 2001, 2002; Neely *et al.,* 2006). By targeting PTEN, miR-22 is remains metabolically relevant, as PTEN lowering or its elevation triggers a Warburg- or an anti-Warburg metabolic state respectively. Figure 2A-D shows that miR-22 overexpression affects weight of mice.

miR-22 Knockout approach

[00211] To assess whether, the effect on a mouse's weight and fat accumulation is due to miR-22 overexpression and to evaluate the differential increase in mice weight during the time and differential consumption of food, 2 month old mice (start day), Wildtype (Wt) and miR-22 Transgenic (Tg) mice were placed on a High Fat (60%) Diet. Mice Weight was monitored 2 times/week and food usage monitored 1 time/week. (*see* Figure 3A-C and Figure 4A-F). Transgenic miR-22 (Tg) mice developed an obese phenotype on a Non-Diet (ND) while miR-22 Knockout mice (KO) mice failed to gain weight on HFD. Mouse Embryonic Fibroblast (MEF) miR-22 deficient cells showed an impaired ability to differentiate in adipocytes. This phenotype is correlated with a differential gene expression for a panel of different genes that are involved in adipocyte differentiation and generally in fat metabolism. Father, results indicate that the effect of miR-22 on weight gain is Leptin (or Leptin like) independent (*see* Figures 5A-D and Figure 6).

Example 2: Inhibition of miR-22 as therapy for Obesity

[00212] All the LNA anti-miR-22 are useful in both human and mouse. Host gene showed a 49% complementarity between human and mouse and LNA anti HG-miR-22 works predominately in human.

Design of anti-miR-22 Locked Nucleic Acid (LNA)

[00213] LNA was designed to cover the seed sequence, contain between 8 nt and 20 nt in length, have a length-specific fraction of LNAs allowed and as high a binding affinity to miR-22 as possible.

[00214] The sequence was validated in an assay by optimization of protocol for LNA assisted uptake (Lipo200 transfection), an adherent cell line, FAM labeled LNA was used and the biological effect validated by Identifying the most potent anti-miR-22 in adherent cell line assisted and un-assisted uptake (analysis of miR-22 level pre and post treatment and TET2 activity and protein level). The aim was to use the anti-miR in a mouse model with the most potent anti-miR for use for *in-vivo* treatment, *see* Figure 33A-C for confirmation results. In the below sequences, capital letters are LNA-modified and lower-case letters are unmodified; the orientations for the miR-22 (SEQ ID NO: 1) and for the anti-miR-22 oligonucleotides (SEQ ID NO: 2 to SEQ ID NO: 10) are orientated 5' to 3'. Figure 8 shows the anti-miR-22 oligonucleotides orientated 3' to 5' as they would be when hybridizing to miR-22. The oligonucleotides of SEQ ID NO: 11 and SEQ ID NO: 12, orientated 5' to 3', are scrambled sequences and do not hybridize to the miR-22 (SEQ ID NO: 1).

hsa-miR-22	AAGCUGCCAGUUGAAGAACUGU	(SEQ ID NO: 1)
CRM0008	TGGCAGCT	(SEQ ID NO: 2)
CRM0009	CtTcaACtgGcAgCT	(SEQ ID NO: 4)
CRM0010	CTTcaACtgGCAgCT	(SEQ ID NO: 5)
CRM0011	TCtTCAaCtgGCAgCT	(SEQ ID NO: 6)
CRM0012	TCtTcaaCtGGCAgCT	(SEQ ID NO: 7)
CRM0013	TCtTCAacTgGCAgCT	(SEQ ID NO: 8)
CRM0014	TTctTCAacTgGCAgCT	(SEQ ID NO: 9)
CRM0015	GTtctTcaaCtgGCaGCT	(SEQ ID NO: 10)
CRM0016	CGaATAgTtaGTAgCG	(SEQ ID NO: 11)
CRM0017	FAM labelled-CGaATAgTtaGTAgCG	(SEQ ID NO: 12)

Anti-miR-22 therapy in-vivo

[00215] In an in vivo experimental planning for prevention, see Figure 9, 2 months old miR-22-/- and WT on HFD were transfected with Vehicle (VCH), Scramble Control RNA (SCR) and Locked Nucleic Acid (LNA) and treated with a Loading dose 20mg/kg (first time) and a Maintenance dose 10mg/kg weekly IP injection un-assisted uptake. There was no difference between treated and non-treated mice in food consumption, *see* Figure 10. In vivo pharmacological inhibition of miR-22 prevented mice from becoming obese and anti-miR-22 therapy *in vivo* was able to increase protein

level of major miR-22 targets in the liver. Anti-miR-22 treatment did not affect liver lipid composition but profoundly suppressed liver steatosis.

[00216] To evaluate potential gene expression of Fat metabolism, synthesis, differentiation in treated and untreated mice, RNAs from Livers, White adipose tissue (WAT) and Brown adipose tissue (BAT) were extracted from the mice treated with VHL, SCR LNA or LNA anti-miR-22 and mRNA expression of TET2, PTEN (Positive Control), FTO, CEBPa, PPARg which are involved in fat related metabolism and synthesis pathway and UCP1 and CD36 (as Brown marker) were a determined, (*see* Figure 12).

[00217] In a curative approach, miR-22-/- and WT mice on a HFD treated with an anti-miR-22-LNA, SCR and a VHL and placed on a second HFD regimen. After 3.5 months of treatment there was a significant reduction in body weight in mice already obese (average weight > 40g) and fed with HFD. Mice were sacrificed, tissues collected, RNA from Livers used for RNAseq (Figures 16A-C, 17). miR-22 pharmacological inhibition was shown to revert Obese phenotype in mice (Figure 18). Figure 19A-C is an RNA-seq plot showing the hierarchy cluster analysis from mice liver indicating that miR-22 pharmacological inhibition and genetic Knockout (KO) cluster together, indicating that the treatment is on target and that KO phenotype can be mimicked using LNA construct and an RNA-Seq plot showing the gene ontology analysis in mice liver indicating that top down regulated pathway in KO and LNA treated mice are lipid metabolism and biosynthesis related is depicted in Figure 20. Anti-miR-22 therapy in vivo strongly downregulated ACL and pharmacological inhibition of miR-22 was shown to be effective in impairing MEFs adipocytic differentiation.

[00218] Results indicate that anti-miR-22 therapy prevents mice from gaining weight when fed with HFD chow, reverses obese phenotype in obese mice fed with HFD chow, does not affect food consumption and efficiently target the liver and WAT in-vivo. Additionally, anti-miR-22 treatment affects the protein level of miR-22 target genes and does not affect any specific lipid class but is able to reduce the overall amount of total lipids in mice.

Example 3: miR-22 control obesity and Fat Mass and Obesity-Associated protein (FTO).

[00219] miR-22 directly targets PTEN and TET to promote tumorigenesis and metastasis. PTEN as many other miR-22 targeting genes are involved in metabolism and fatty acid oxidation or

biogenesis including for example SIRT-1, BMP-7, PPAR-alpha, PPAR-γ, SP-1, PGC1a, FGF-21, UCP1, Methyltransferase like 3 and DDIT4. FTO expression was profoundly down-regulated during adipose induced differentiation in miR-22 deficient MEF but not in WT MEF (Figure 26) and miR-22 down regulation (genetic or pharmacological) increased levels of RNA m6A (Figure 27A-B). Mir-22 downregulation was shown to not affect liver function or show any liver related disease or dysfunction at old age (Figures 28A-B, 29).

[00220] To evaluate whether miR-22 Overexpression affects Liver function, Fatty Liver and Fibrosis, Mice between 8 and 10 months old were fed with normal diet. miR-22 OE was shown to lead to a fatty liver and increase the presence of FSP-1 positive cells FSP-1 identify a sub-population of macrophages in liver, associated with fibrosis.

[00221] Overall, miR-22 in an onco-miR (PTEN targeting, MEF transformation, EMT), miR-22 is 100% conserved in human and mouse, miR-22 OE leads to and obese phenotype in ND, miR-22 KO mice don't gain weight in HFD, miR-22 pharmacologically silencing impaired MEF and human MES to differentiate in adipocyte, LNA anti-miR-22 treatment prevent mice to become obese in a prevention setting, LNA anti-miR-22 treatment reverts obese phenotype in obese mice fed with HFD, miR-22 silencing doesn't show any liver toxicity, miR-22 silencing prevent liver from steatosis and fibrosis, miR-22 can target multiple genes related to metabolism and lipid biogenesis at the same time.

OTHER EMBODIMENTS

[00222] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

INCORPORATION BY REFERENCE

[00223] All patents and publications referenced herein are hereby incorporated by reference in their entireties.

[00224] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[00225] As used herein, all headings are simply for organization and are not intended to limit the disclosure in any way.

CLAIMS

What is claimed is:

1. A method for treating or preventing a metabolic disorder, comprising administering an effective amount of an inhibitor of miR-22 to a subject in need thereof.

2. The method of claim 2, wherein the expression and/or activity of miR-22 is reduced in the subject following administration of the inhibitor.

3. The method of claim 1 or 2, wherein the inhibitor of miR-22 is an oligonucleotide-based inhibitor.

4. The method of claim 3, wherein the oligonucleotide-based inhibitor comprises a sequence that is at least about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% complementary to a mature sequence of miR-22.

5. The method of claim 3 or 4, wherein the oligonucleotide-based inhibitor comprises deoxynucleotides or ribonucleotides.

6. The method of any one of claims 3-5, wherein the oligonucleotide-based inhibitor is single stranded.

7. The method of any one of claims 3-5, wherein the oligonucleotide-based inhibitor is double stranded.

8. The method of any one of claims 3-7, wherein the oligonucleotide-based inhibitor comprises one or more chemically modified nucleotides.

9. The method of claim 8, wherein the chemically modified nucleotides are locked nucleotides (LNAs).

10. The method of any one of claims 3-9, wherein the oligonucleotide-based inhibitor comprises about 25, about 20, about 15, about 10, about 9, about 8, about 7, about 6, or about 5 or fewer nucleotides.

11. The method of any one of claims 3-10, wherein the oligonucleotide-based inhibitor is conjugated to one or more N-acetylgalactosamine (GalNAc) moieties.

12. The method of any one of claims 3-11, wherein the oligonucleotide-based inhibitor is an antisense oligonucleotide inhibitor.

13. The method of any one of claims 3-11, wherein the oligonucleotide-based inhibitor is a small interfering RNA (siRNA).

14. The method of any one of claims 3-11, wherein the oligonucleotide-based inhibitor is an aptamer.

15. The method of claim 1 or 2, wherein the inhibitor of miR-22 is a peptide-based or protein-based inhibitor.

16. The method of claim 15, wherein the protein-based inhibitor in an antibody or an antigen-binding portion thereof.

17. The method of claim 1 or 2, wherein the inhibitor of miR-22 is a small molecule-based inhibitor.

18. The method of any one of the above claims, wherein the metabolic disorder is obesity.

19. The method of claim 18, wherein the subject is suffering from Prader-Willi Syndrome.

20. The method of claim 18, wherein the subject is suffering from hypercholesterolemia.

21. The method of claim 18, wherein the subject harbors a fat mass and obesity-associated protein (FTO) variant and/or shows an upregulation of FTO expression and/or activity.

22. The method of any one of claims 18-21, wherein the subject is obese and has a body mass index of greater than about 30.

23. The method of any one of claims 18-21, wherein the subject is overweight and has a body mass index of about 25-29.9.

24. The method of any one of claims 18-23, wherein the method induces weight loss.

25. The method of claim 24, wherein the method induces a total weight loss of about 1%, about 5%, about 10%, about 15%, about 20%, or about 25% or more in the subject.

26. The method of any one of claims 18-23, wherein the method prevents weight gain.

27. The method of any one of claims 18-26, wherein the method reduces or prevents the growth of adipose tissue.

28. The method of any one of claims 18-26, wherein the method impairs adipocyte differentiation.

29. The method of any one of the above claims, wherein the metabolic disorder is a fatty liver disease.

30. The method of claim 29, wherein the fatty liver disease is selected from non-alcoholic fatty acid liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH).

31. The method of claim 29 or 30, wherein the method reduces or prevents liver steatosis.

32. The method of any one of claims 29-31, wherein the method reduces or prevents liver fibrosis.

33. The method of any one of claims 1-32, wherein the method reduces the activity and/or expression of fat mass and obesity-associated protein (FTO), ALKB Homologous 5 (ALKBH5), CCAAT/enhancer binding protein alpha (CEBPα), peroxisome proliferator activated receptor gamma (PPARγ), peroxisome proliferator activated receptor alpha (PPARa), ATP citrate lyase (ACLY), PPARγ co-activator-α (PGC1-α), Specific Protein 1 (SP1), Fibroblast Grow Factor 21 (FGF-21), Uncoupled protein 1 (UCP1), DNA Damage Included Transcript 4 (DDIT-4, REDD1), tumor protein p63 (TP63), fibroblast growth factor 1 (FGF1), and/or Methyltransferase like 3 (METTL3).

34. The method of any one of claims 1-33, wherein the method increases the activity and/or expression of phosphatase and tensin homolog (PTEN) and/or tet methylcytosine dioxygenase 2 (TET2).

FIGURE 1

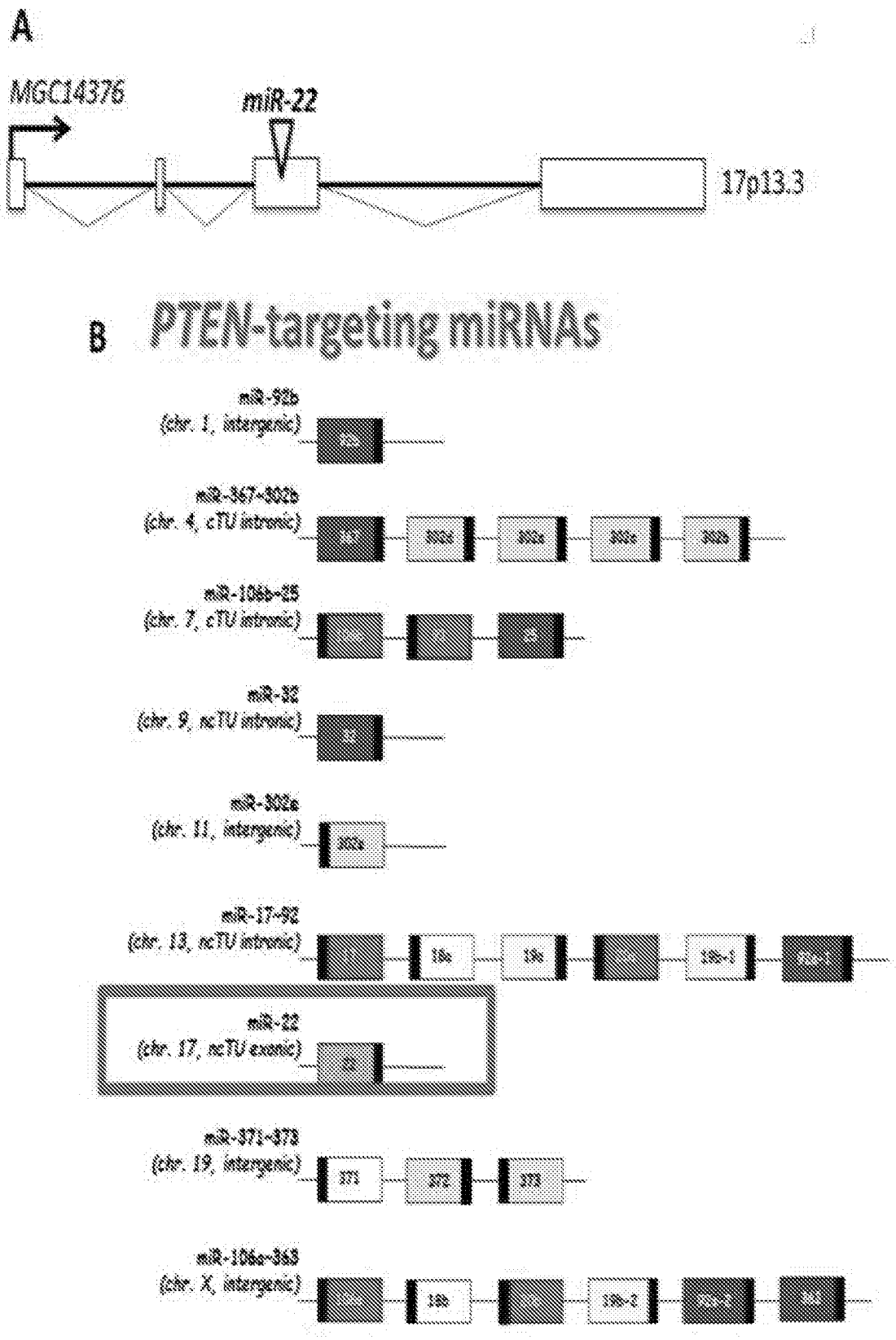

A
MGC14376
miR-22
17p13.3
B
PTEN-targeting miRNAs
miR-92b
(chr. 1, intergenic)
miR-367~302b
(chr. 4, cTU intronic)
miR-106b~25
(chr. 7, cTU intronic)
miR-92
(chr. 9, ncTU intronic)
miR-302a
(chr. 11, intergenic)
miR-17~92
(chr. 13, ncTU intronic)
miR-22
(chr. 17, ncTU exonic)
miR-371~373
(chr. 19, intergenic)
miR-106a~363
(chr. X, intergenic)

FIGURE 1 (Continued)

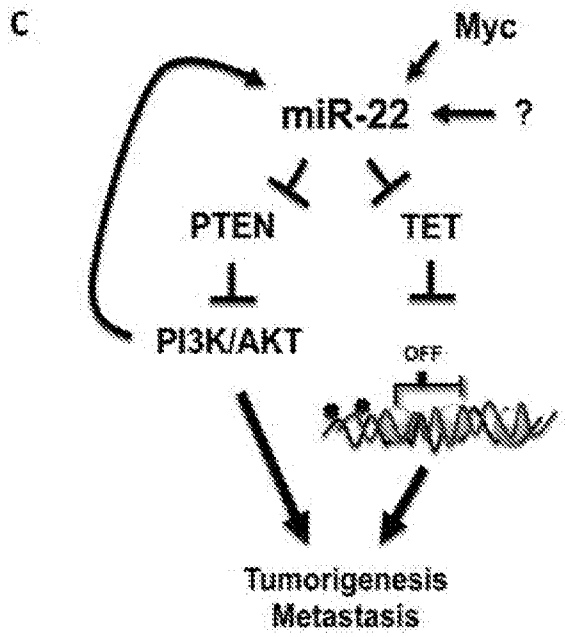


FIGURE 2

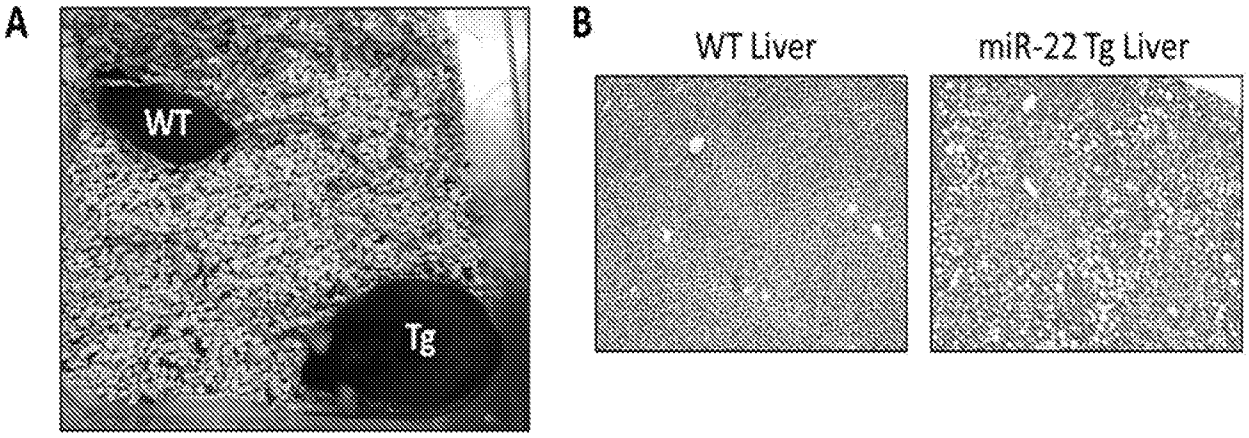
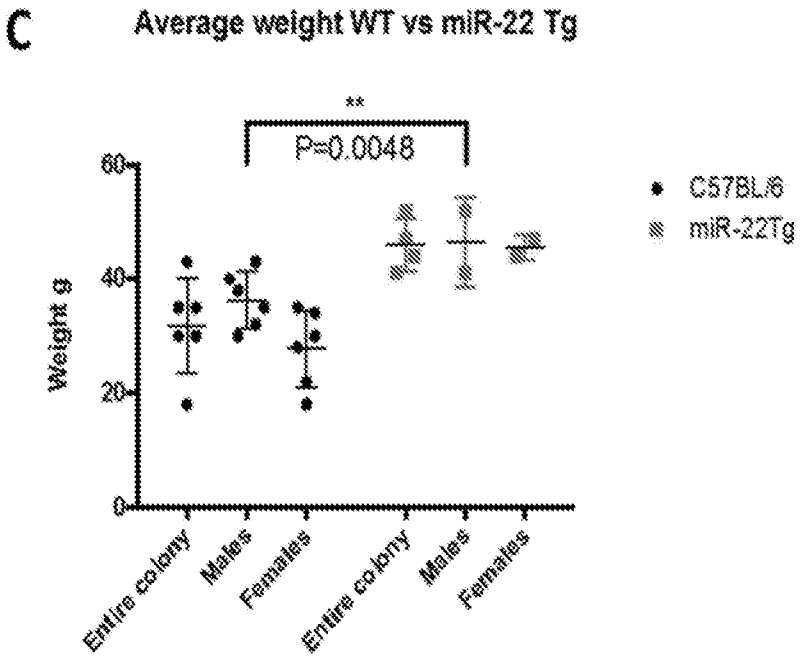


FIGURE 2 (Continued)



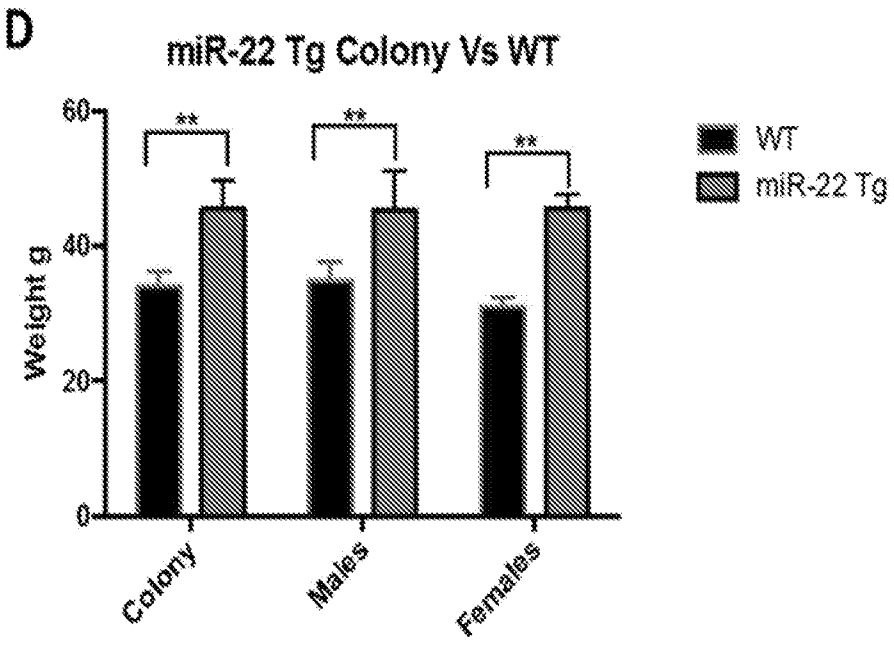


FIGURE 3

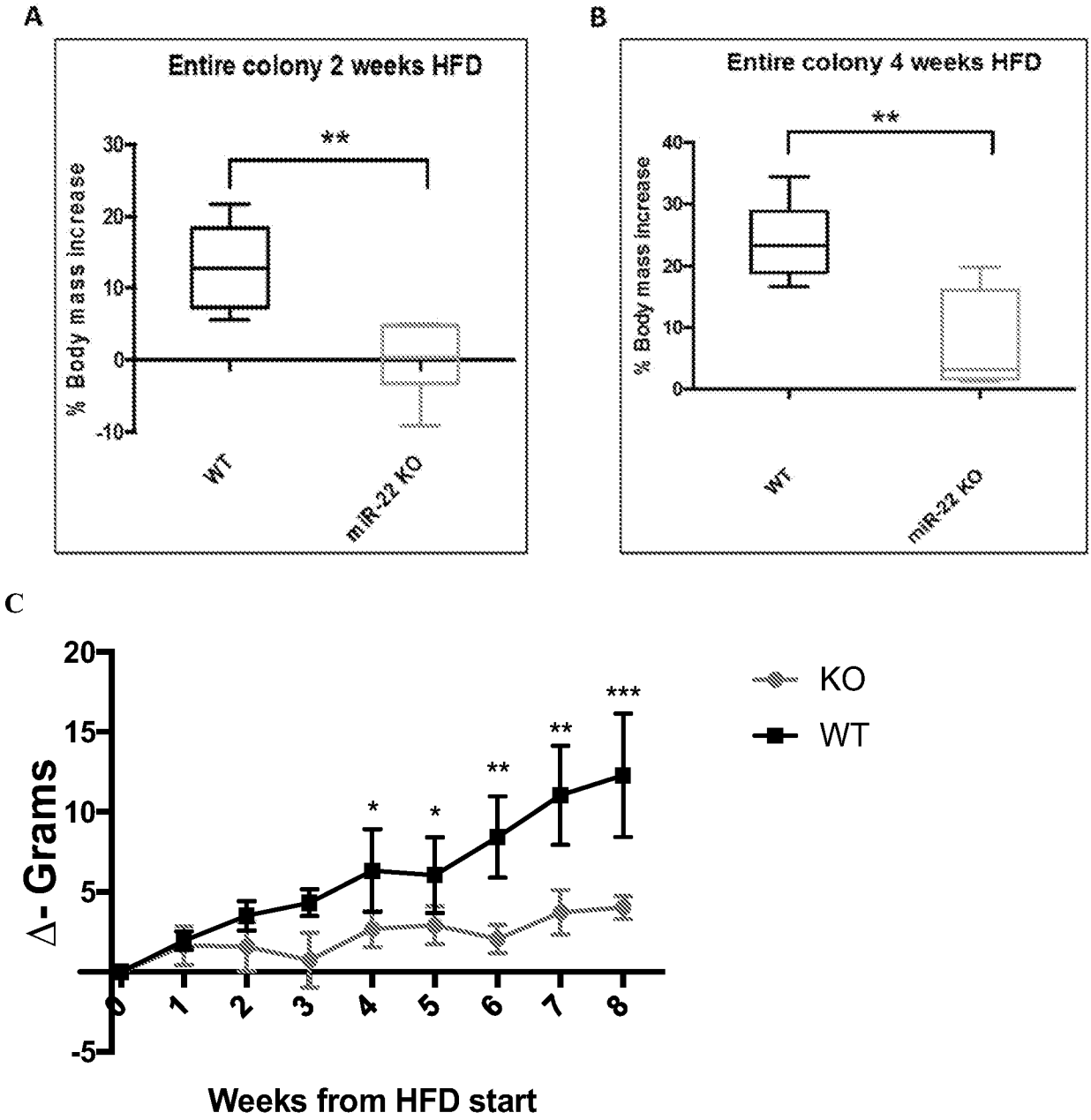
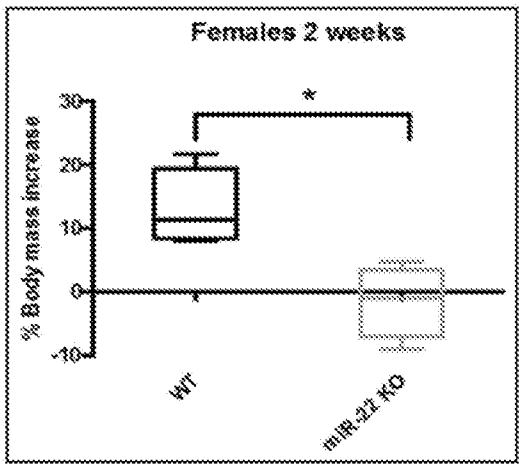
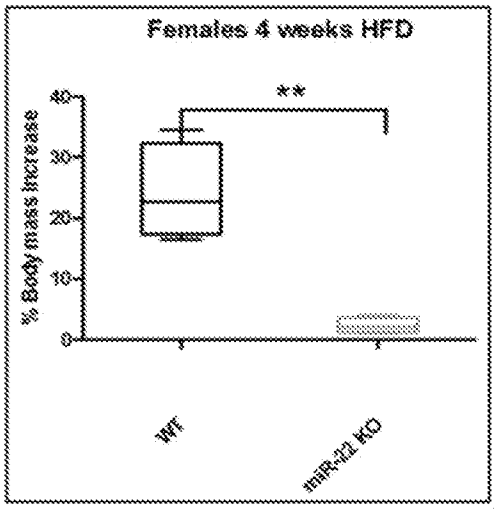

A
Entire colony 2 weeks HFD
% Body mass increase
30
20
10
0
-10
**
WT
miR-22 KO
B
Entire colony 4 weeks HFD
% Body mass increase
40
30
20
10
0
**
WT
miR-22 KO
C
Δ- Grams
20
15
10
5
-5
0
1
2
3
4
5
6
7
8
*
*
**
**
***
KO
WT
Weeks from HFD start

FIGURE 4

A


Females 2 weeks
*
% Body mass increase
30
20
10
0
-10
WT
miR-22 KO

B


Females 4 weeks HFD
**
% Body mass increase
40
30
20
10
0
WT
miR-22 KO

C

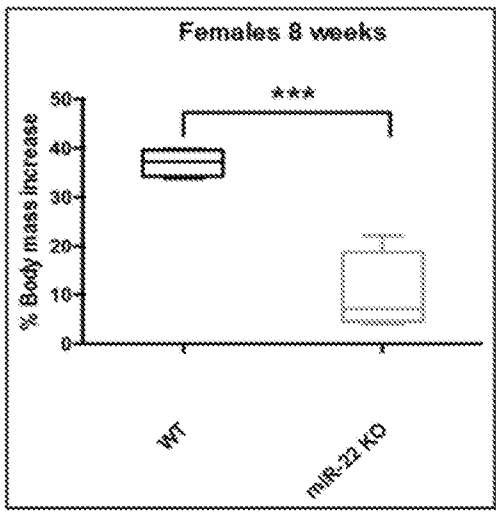

Females 8 weeks
***
% Body mass increase
50
40
30
20
10
0
WT
miR-22 KO

FIGURE 4 (Continued)

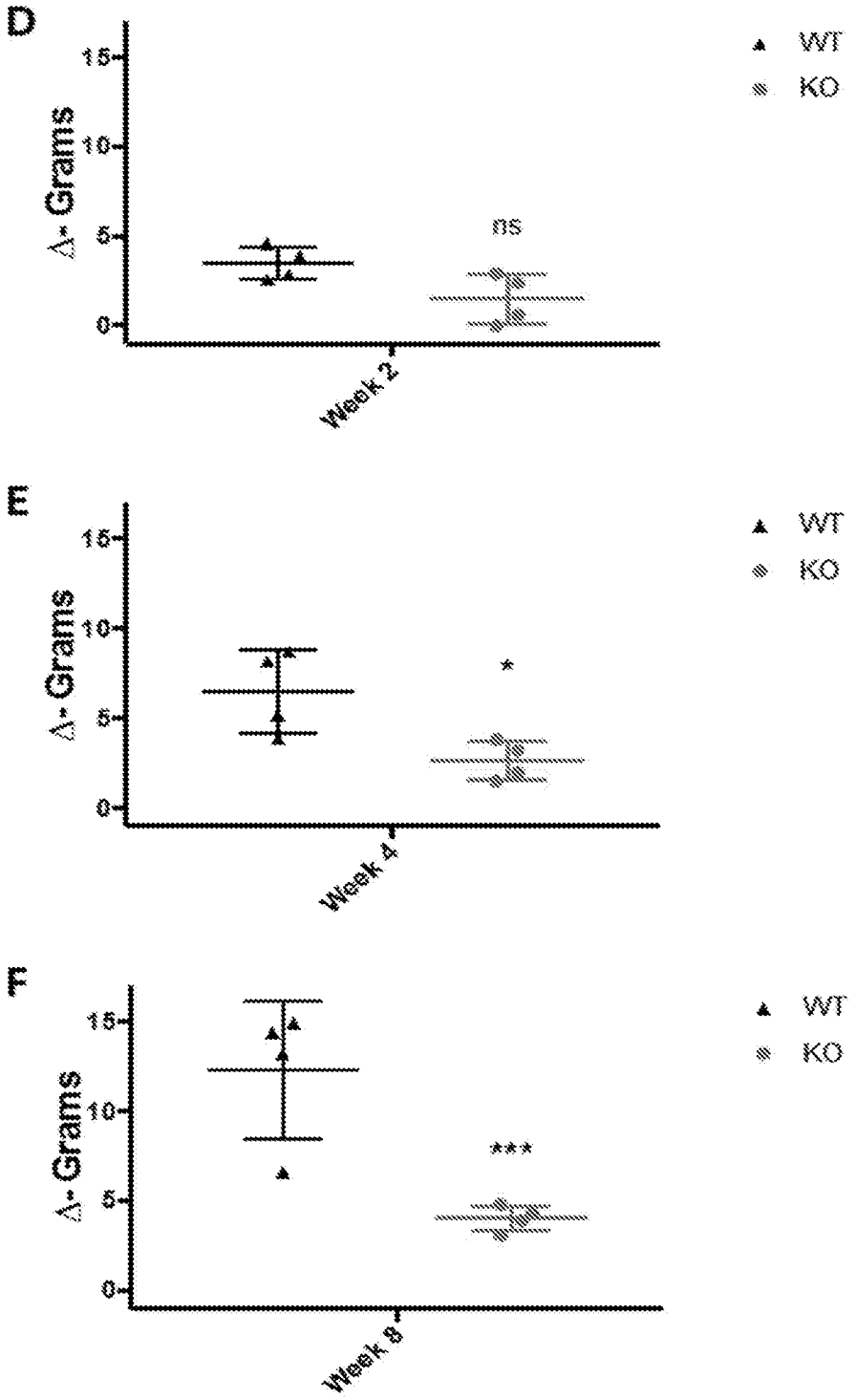


FIGURE 5

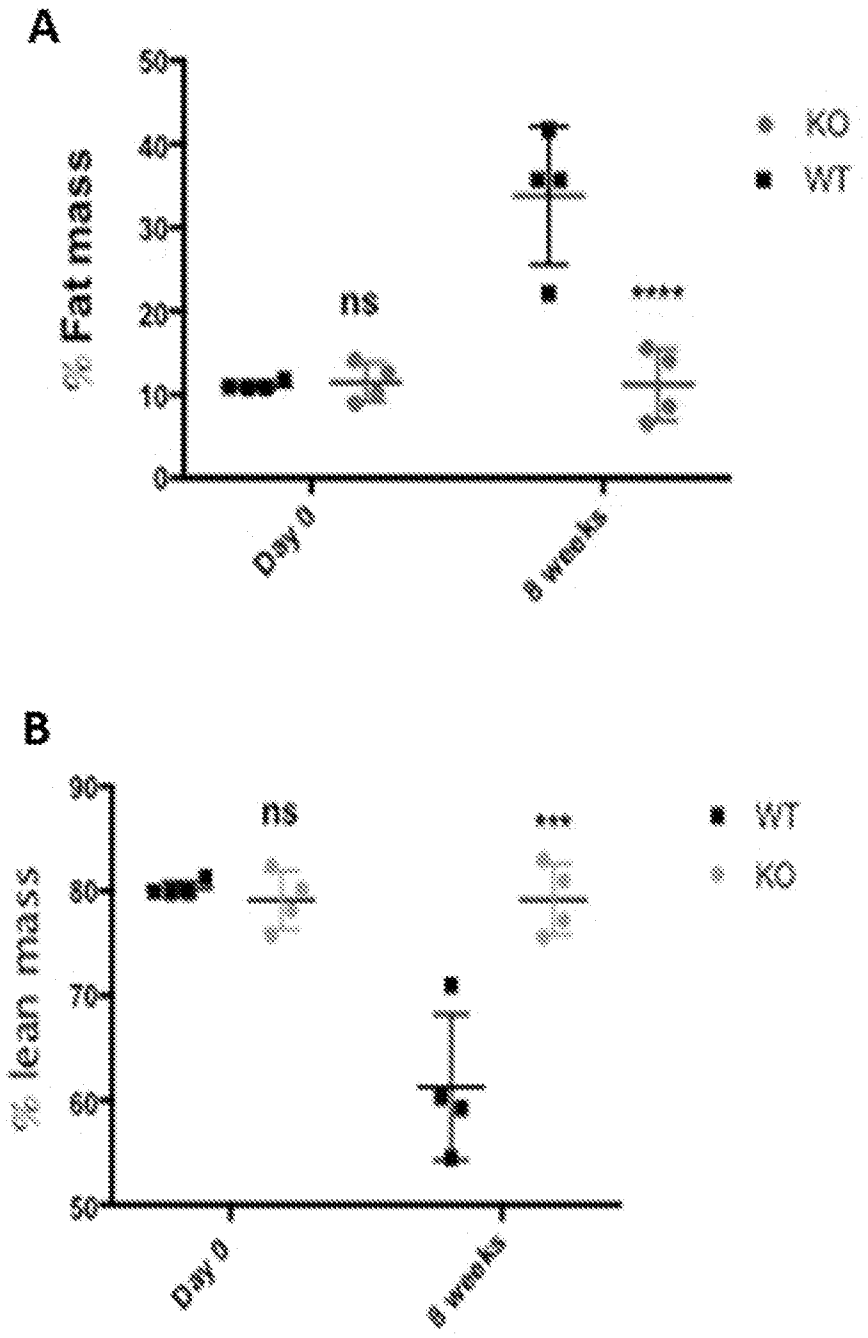

A
% Fat mass
50
40
30
20
10
0
ns
****
KO
WT
Day 0
8 weeks
B
% lean mass
90
80
70
60
50
ns
***
WT
KO
Day 0
8 weeks

FIGURE 5 (Continued)

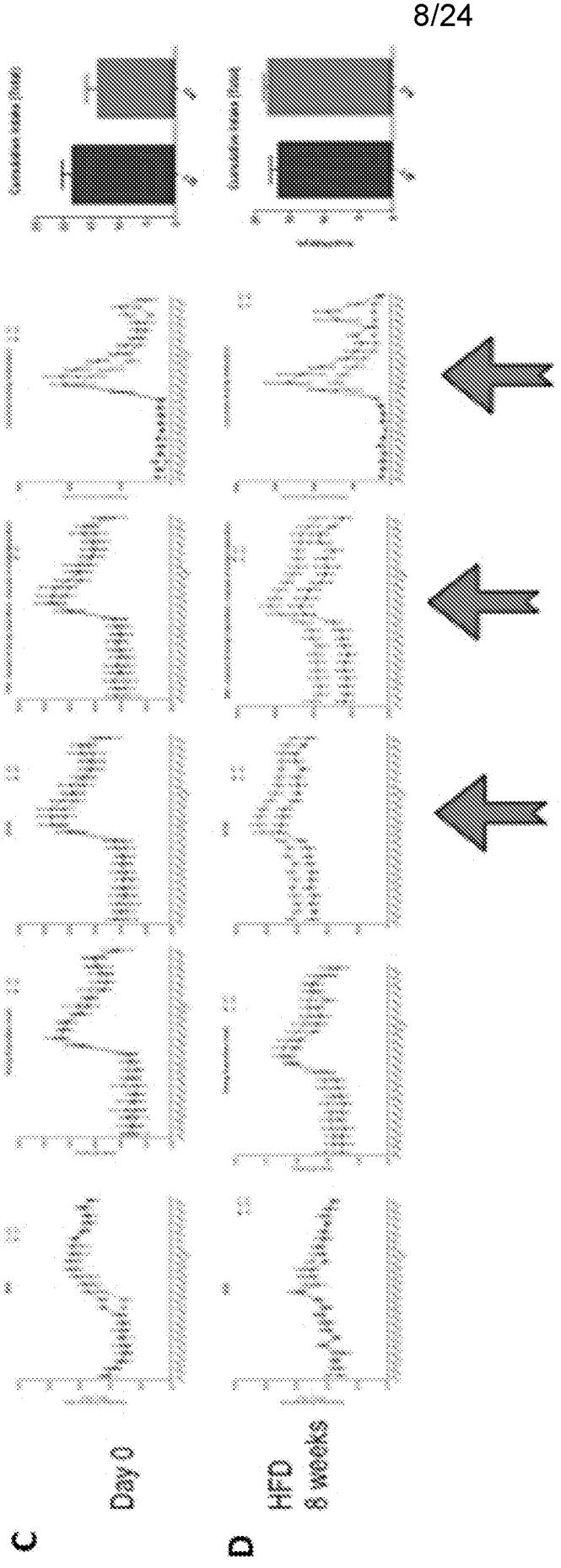


FIGURE 6

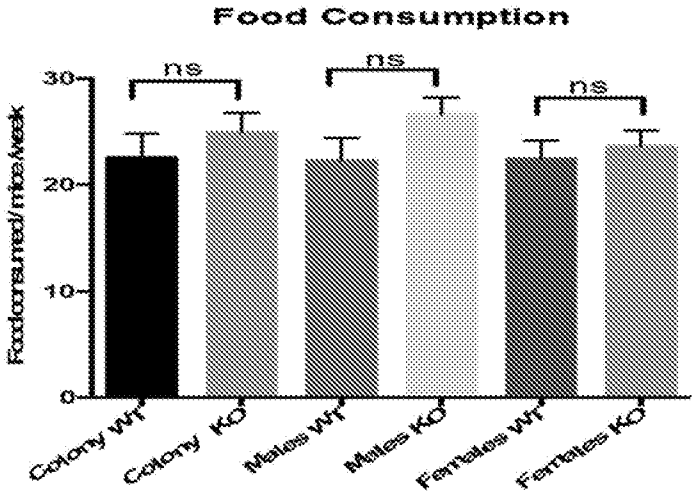


FIGURE 7

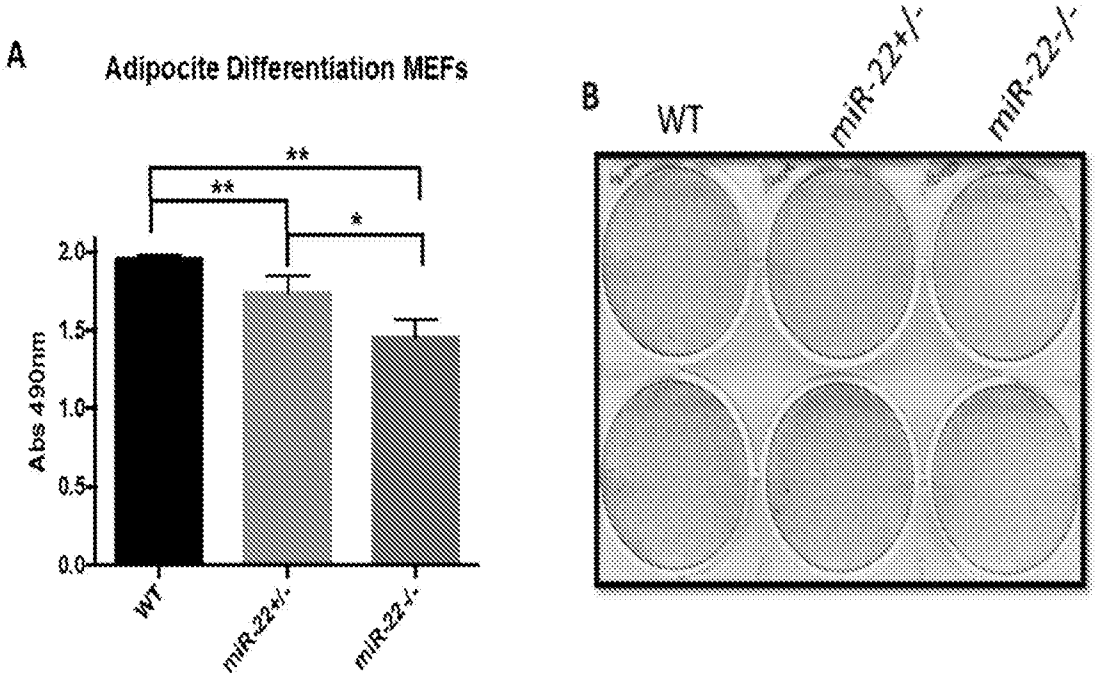


FIGURE 8

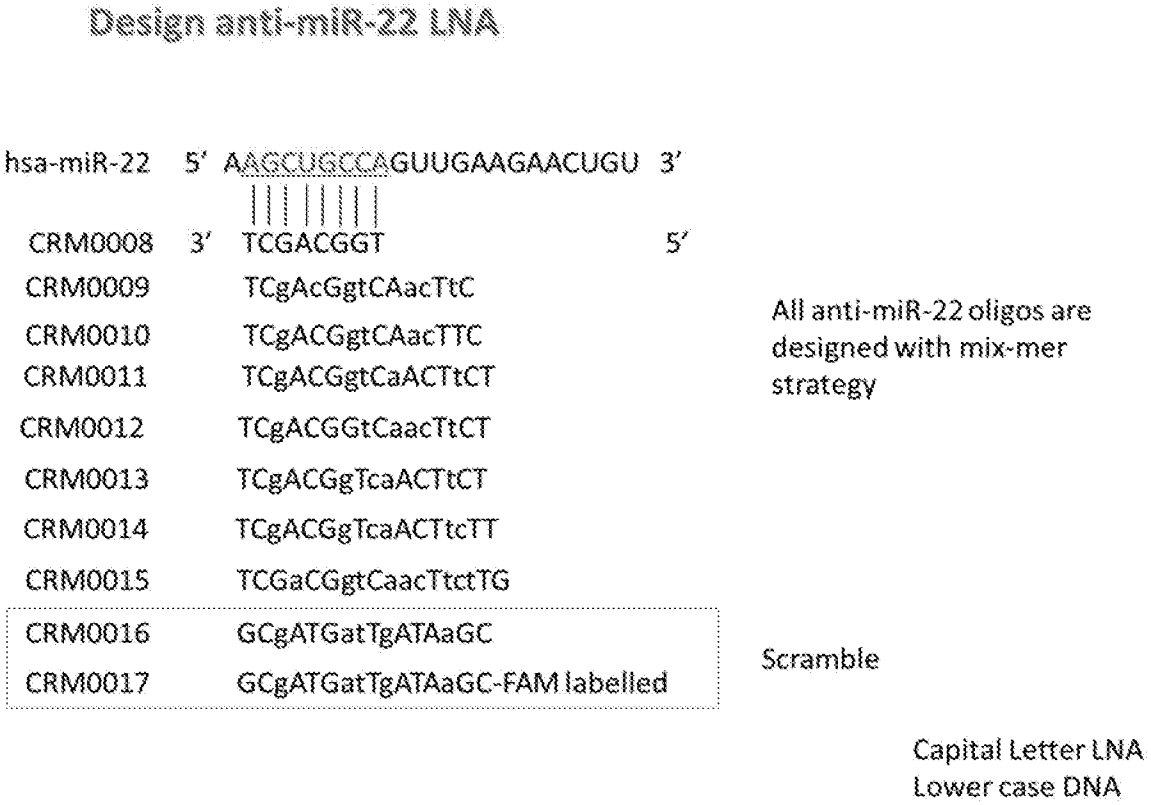
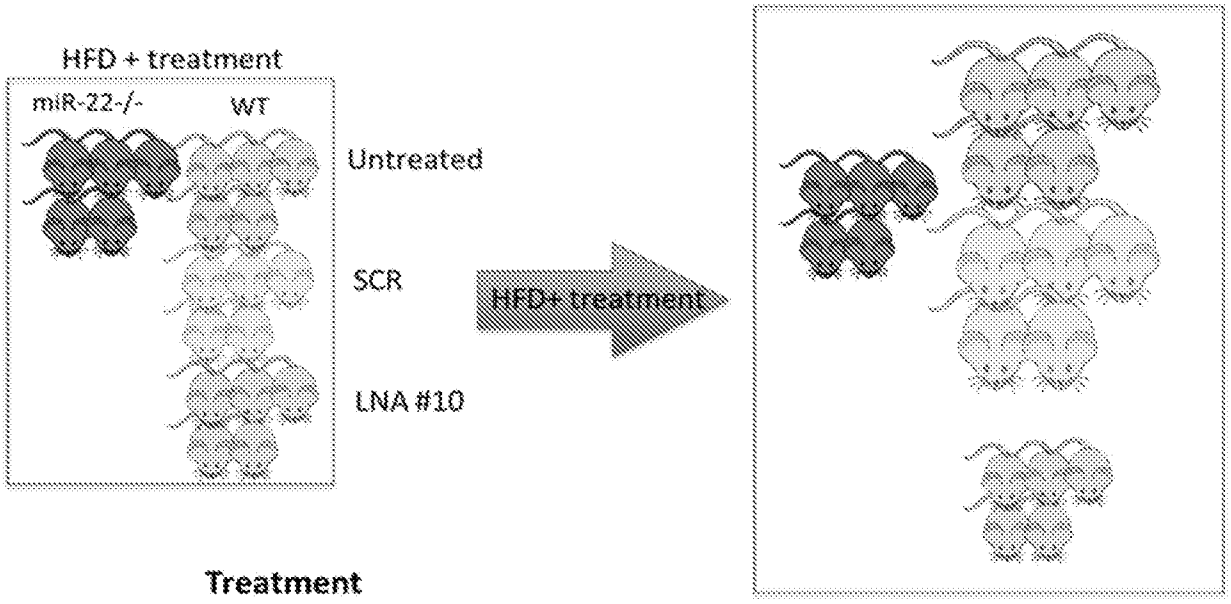

Design anti-miR-22 LNA
hsa-miR-22 5′ AAGCUGCCAGUUGAAGAACUGU 3′
CRM0008 3′ TCGACGGT 5′
CRM0009 TCgAcGgtCAacTtC
CRM0010 TCgACGgtCAacTTC
CRM0011 TCgACGgtCaACTtCT
CRM0012 TCgACGGtCaacTtCT
CRM0013 TCgACGgTcaACTtCT
CRM0014 TCgACGgTcaACTtcTT
CRM0015 TCGaCGgtCaacTtctTG
CRM0016 GCgATGatTgATAaGC
CRM0017 GCgATGatTgATAaGC-FAM labelled
All anti-miR-22 oligos are designed with mix-mer strategy
Scramble
Capital Letter LNA
Lower case DNA

FIGURE 9



Treatment

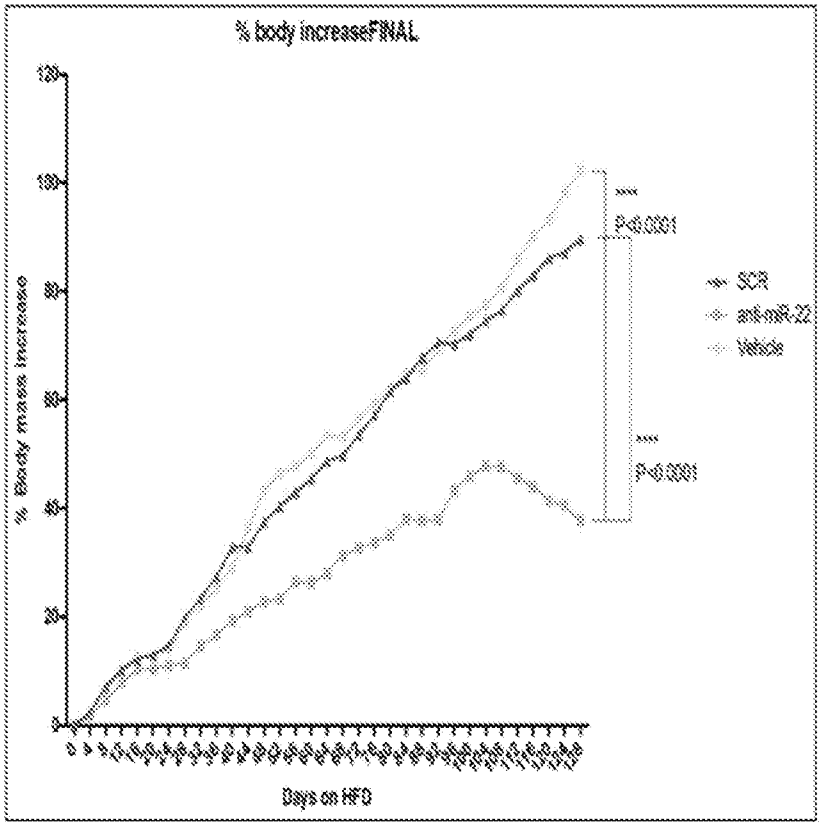
Loading dose 20mg/kg (first time)
Maintenance dose 10mg/kg weekly
IP injection un-assisted uptake

All mice were 2 months old at the beginning of the experiment

FIGURE 10

FIGURE 11

A


% body increaseFINAL
% Body mass increase
Days on HFD
SCR
anti-miR-22
Vehicle
****
P<0.0001
****
P<0.0001

B

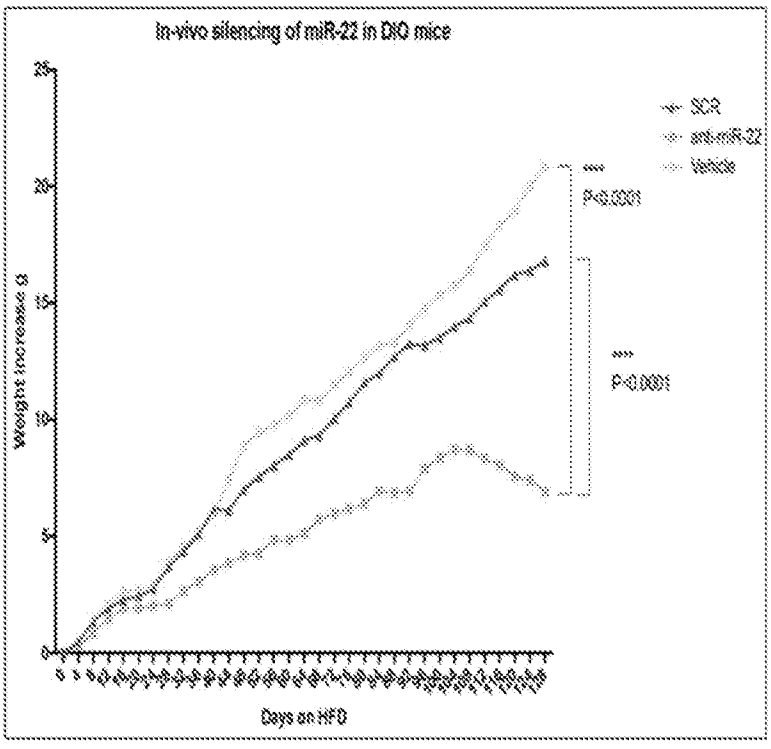
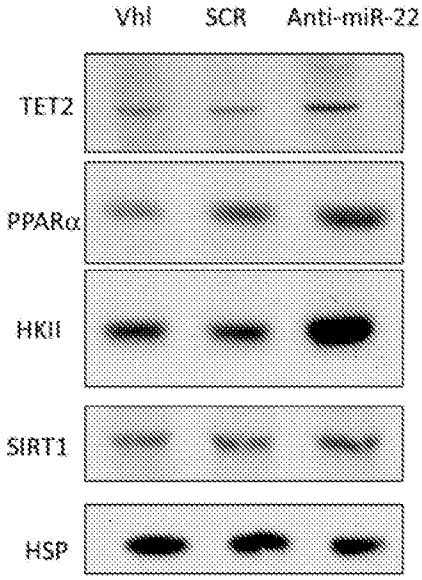

In-vivo silencing of miR-22 in DIO mice
Weight increase g
Days on HFD
SCR
anti-miR-22
Vehicle
****
P<0.0001
****
P<0.0001

FIGURE 12



Liver sample from treated and un-treated mice

FIGURE 13

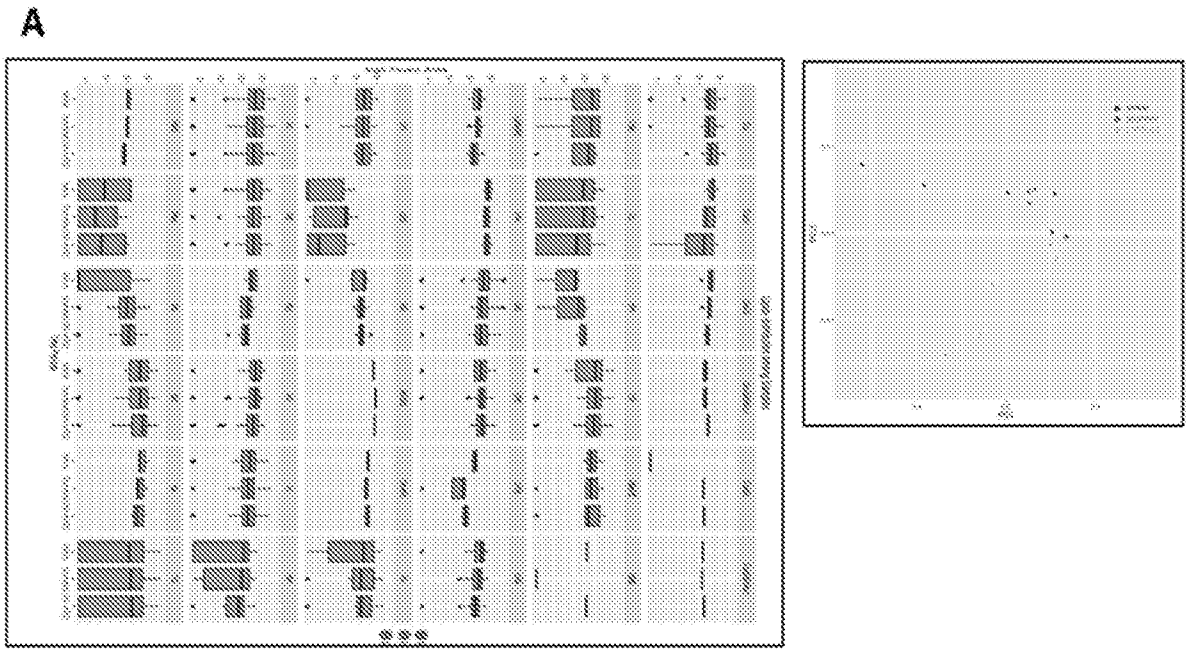


FIGURE 13 (Continued)

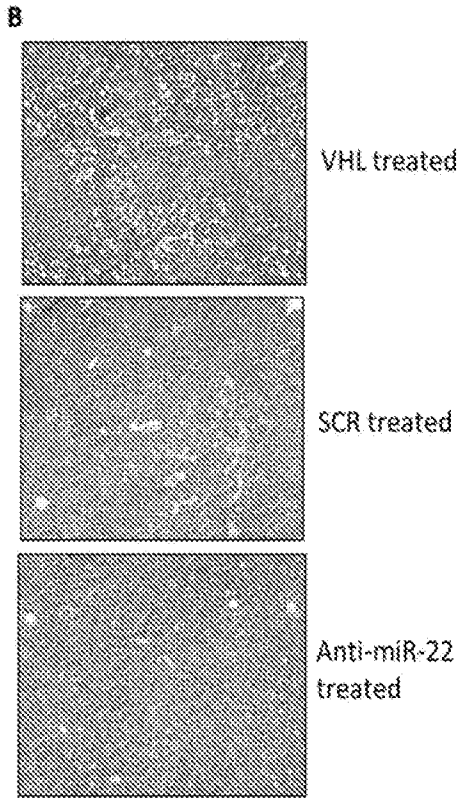


FIGURE 14

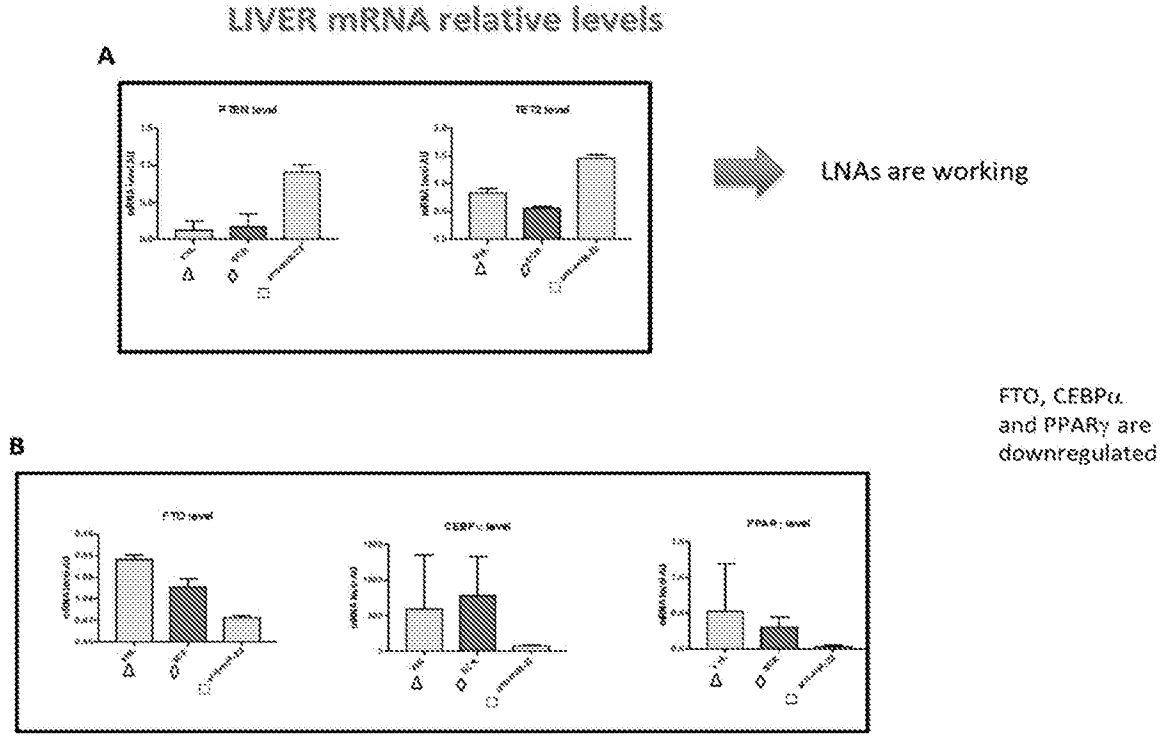


FIGURE 15

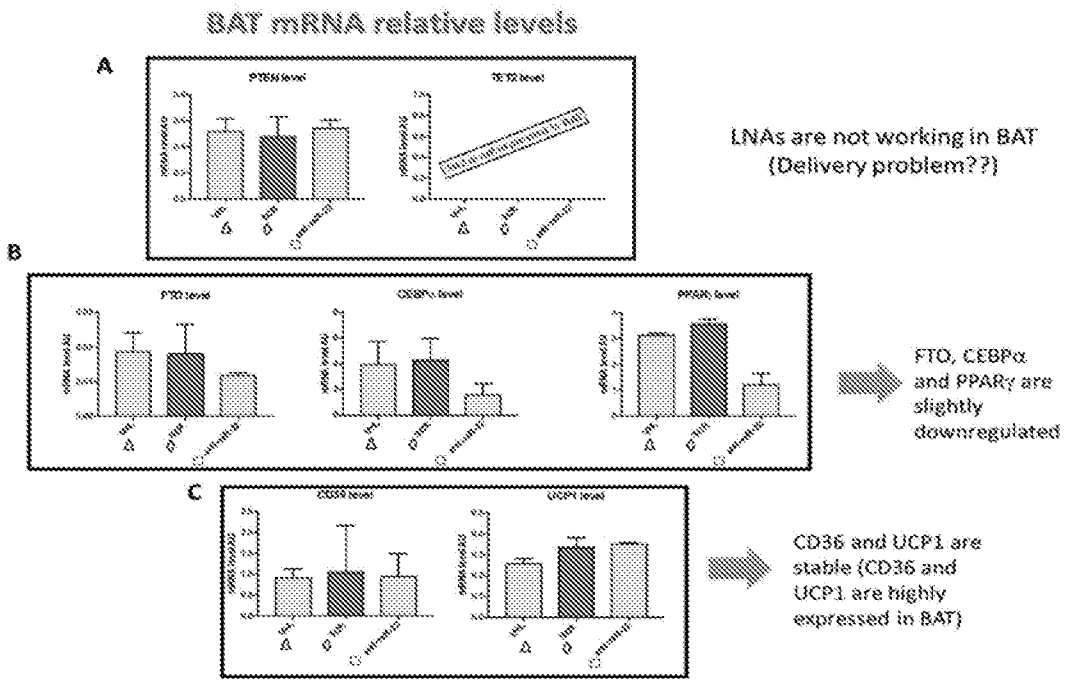


FIGURE 16

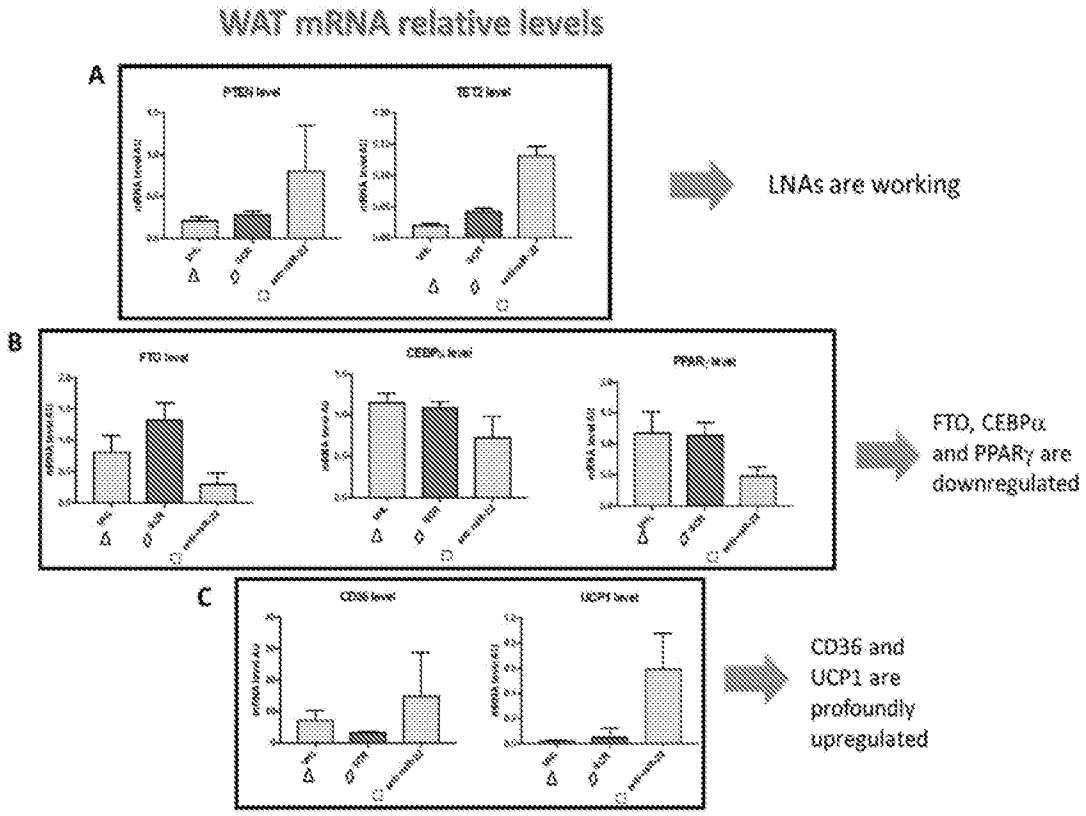


FIGURE 17

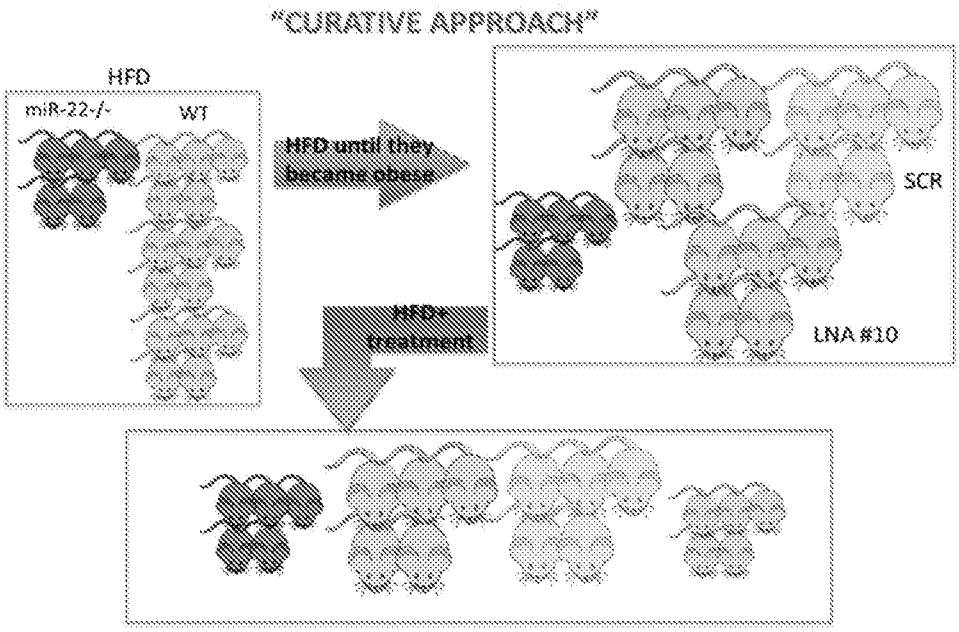


FIGURE 18

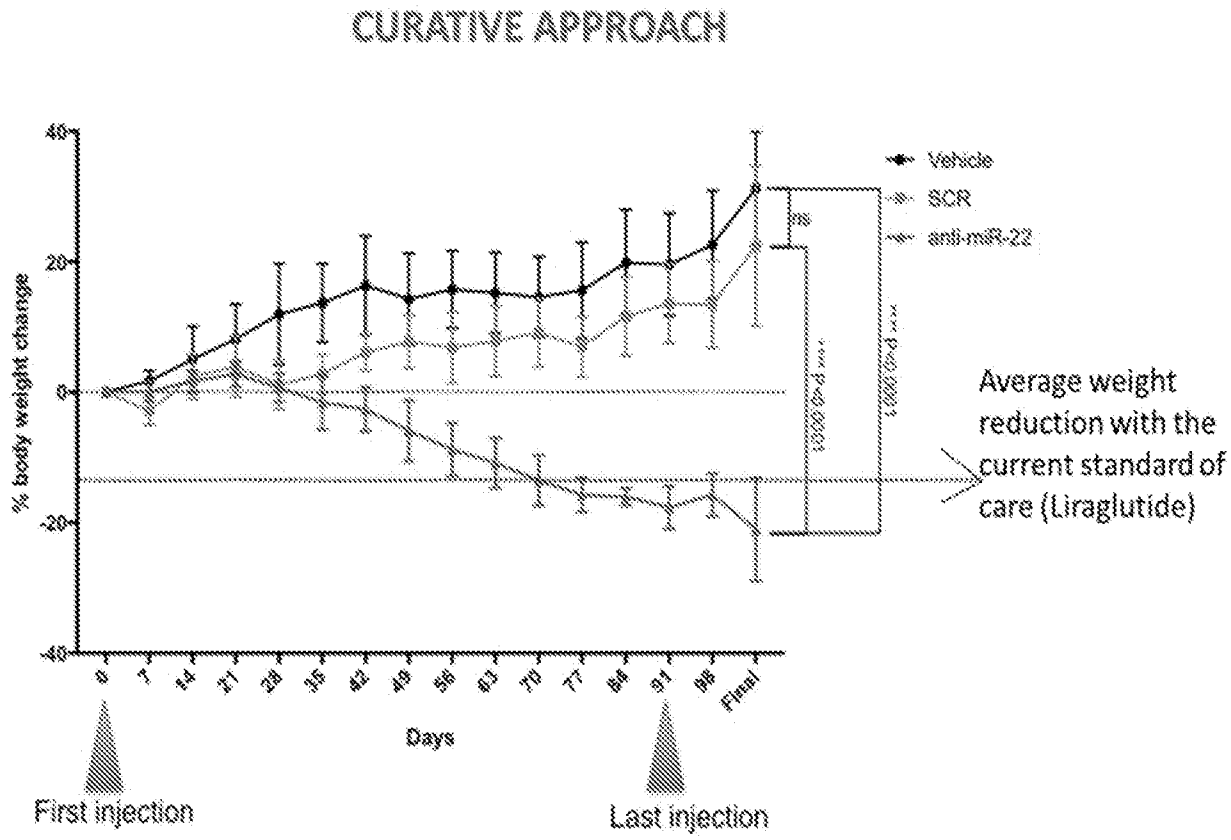


FIGURE 19

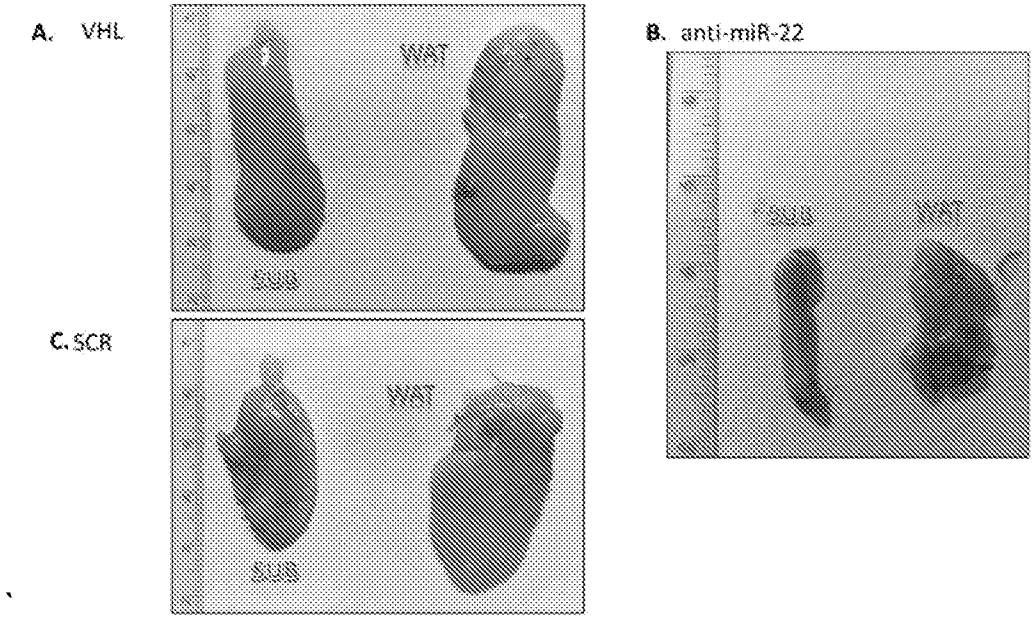


FIGURE 20

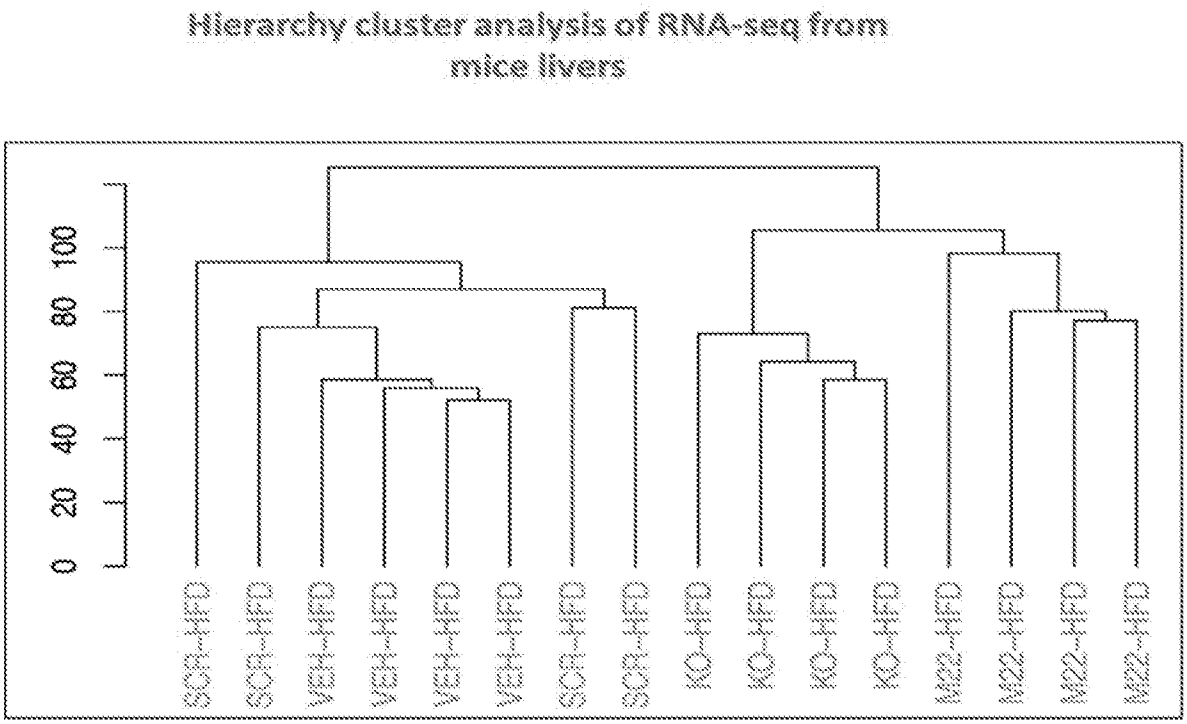


FIGURE 21

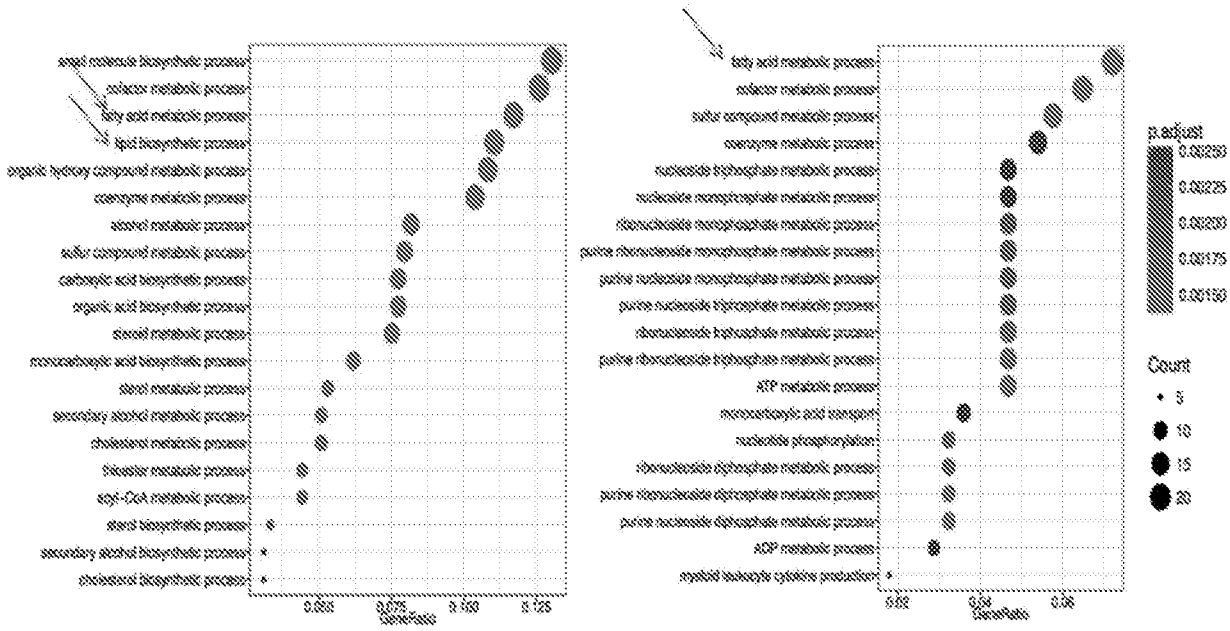


FIGURE 22

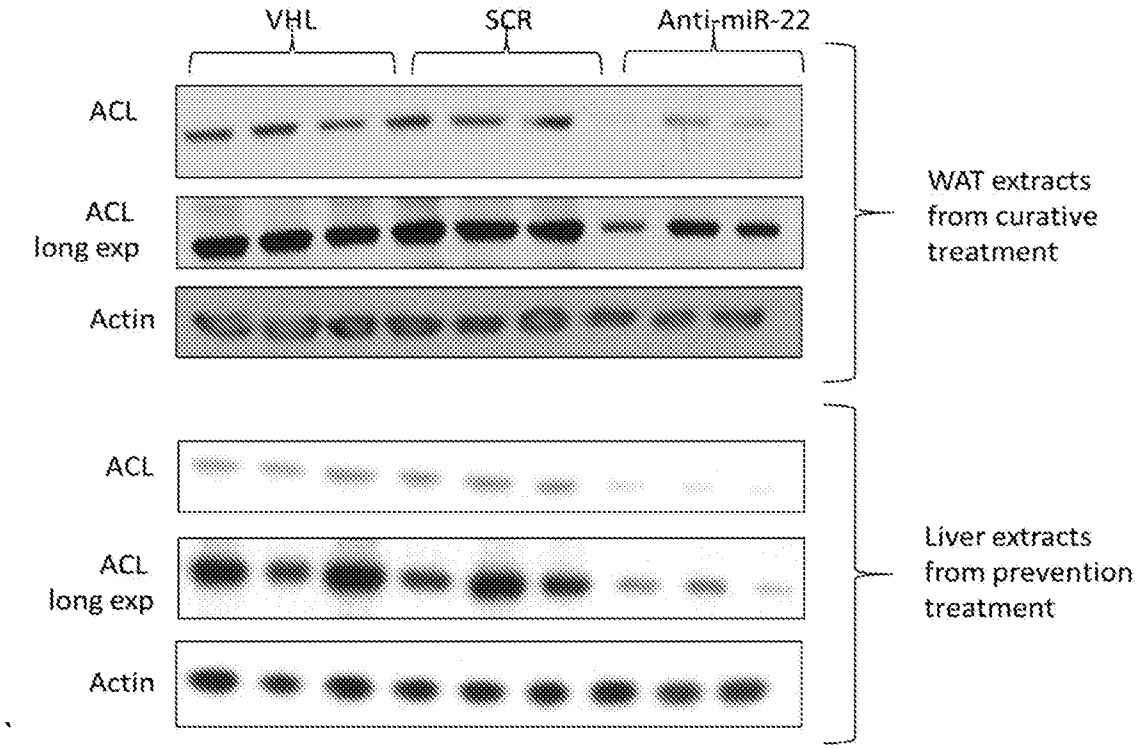


FIGURE 23

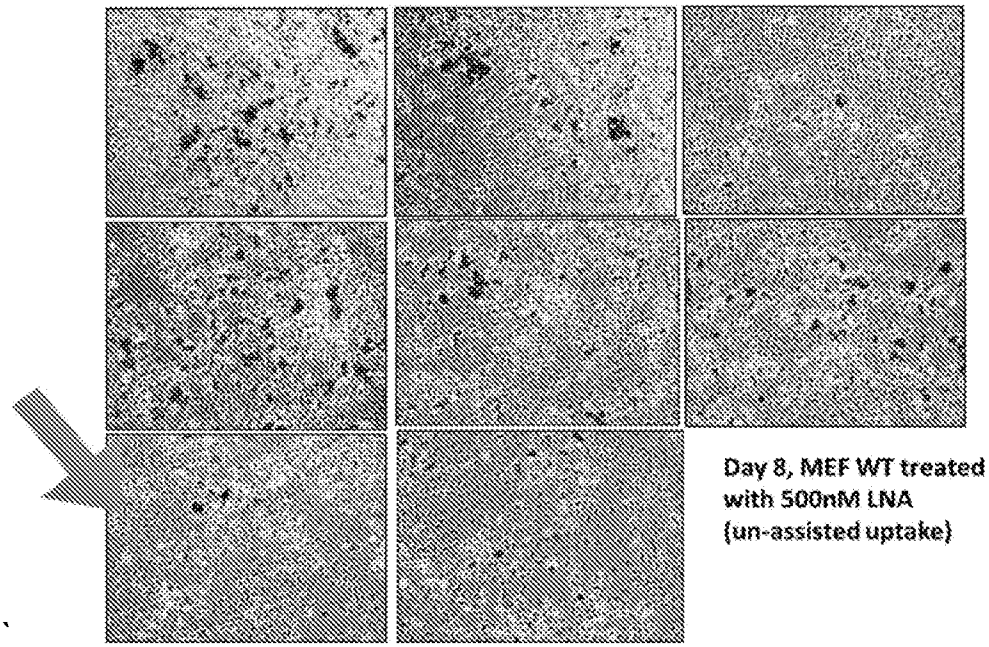


FIGURE 24

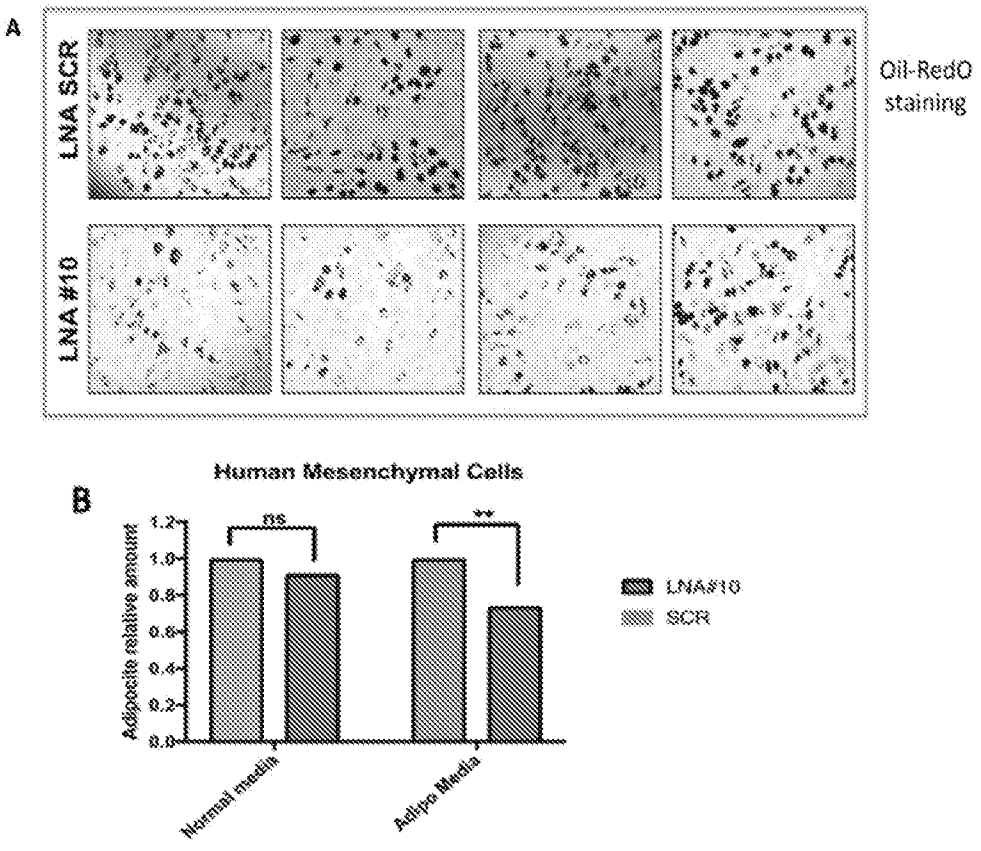


FIGURE 25

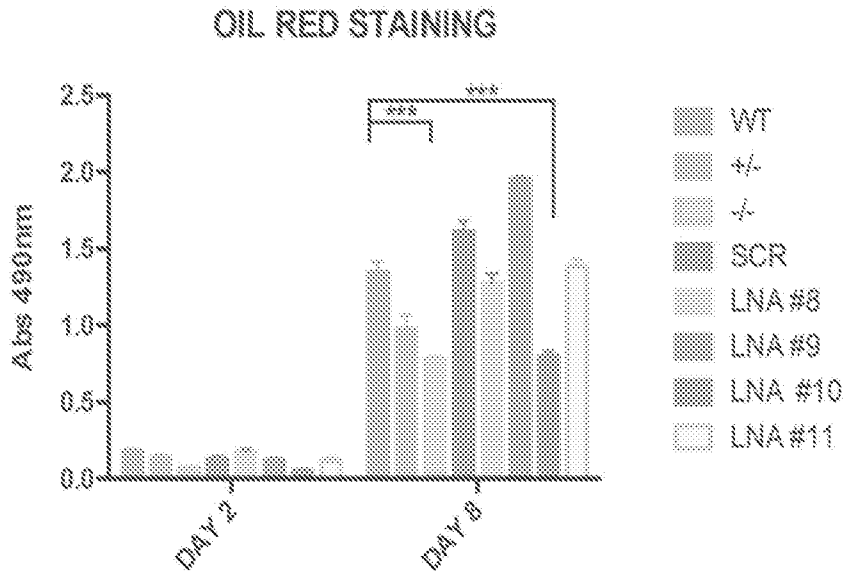


FIGURE 26

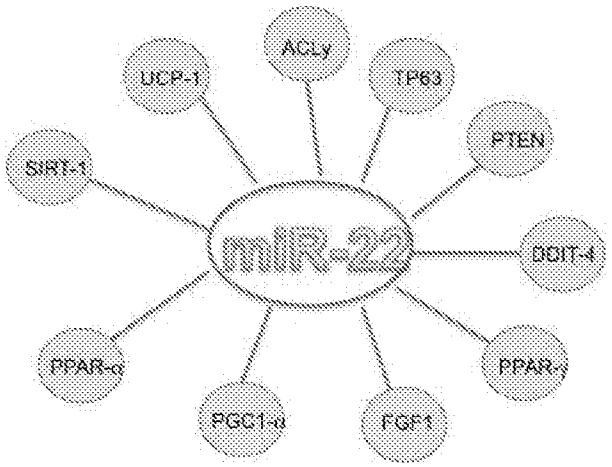


FIGURE 27

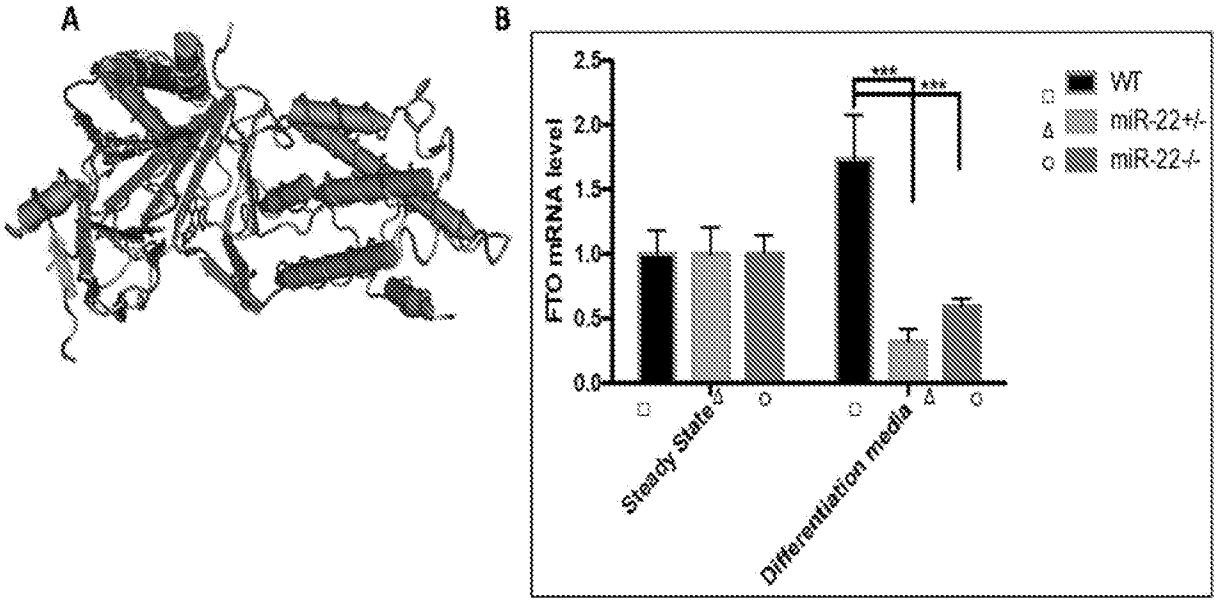
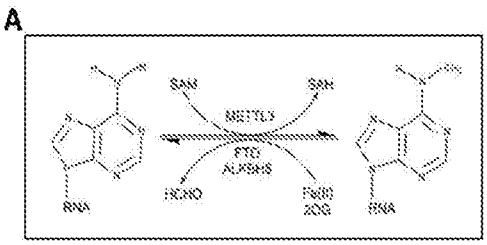
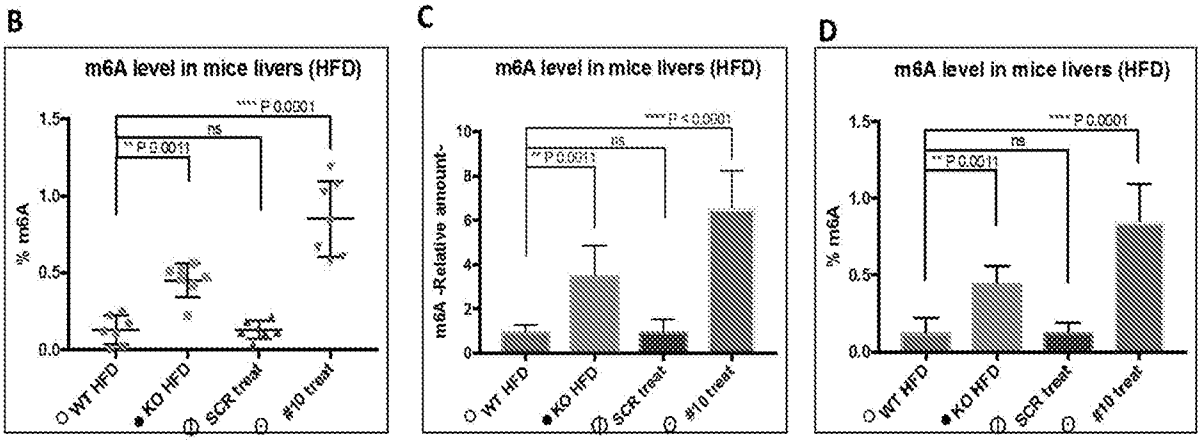


FIGURE 28





RNA from Liver (n=7)
Colorimetric assay

FIGURE 29

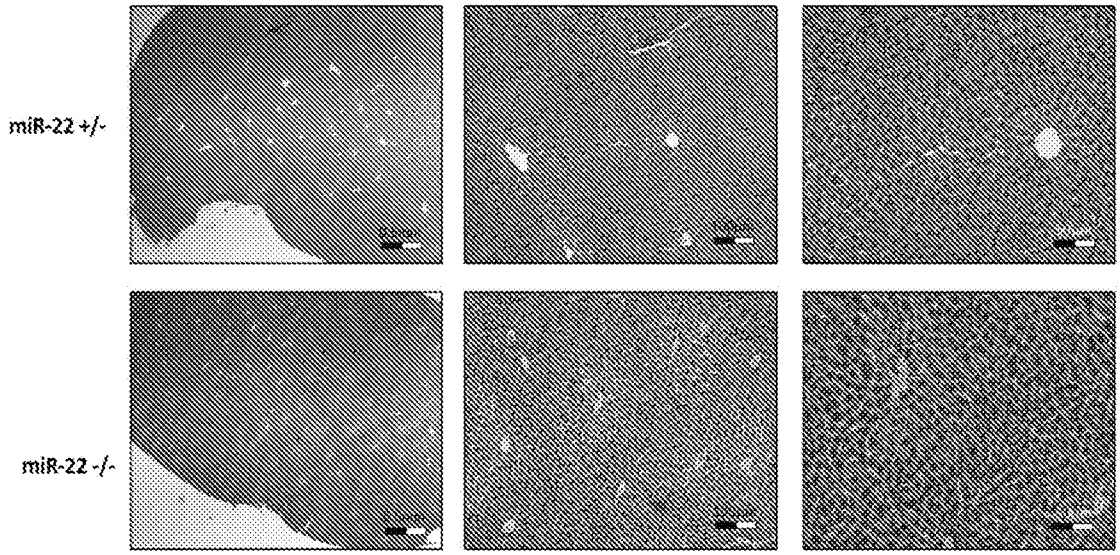


FIGURE 30

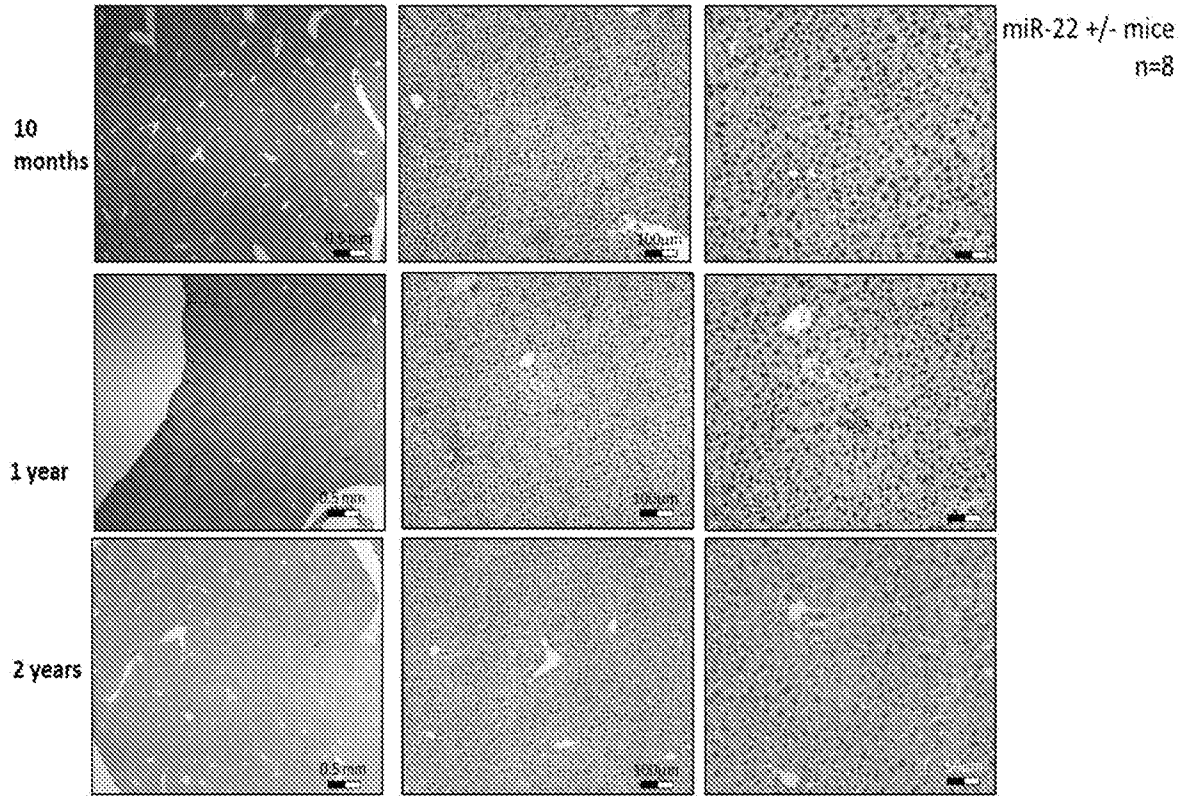


FIGURE 31

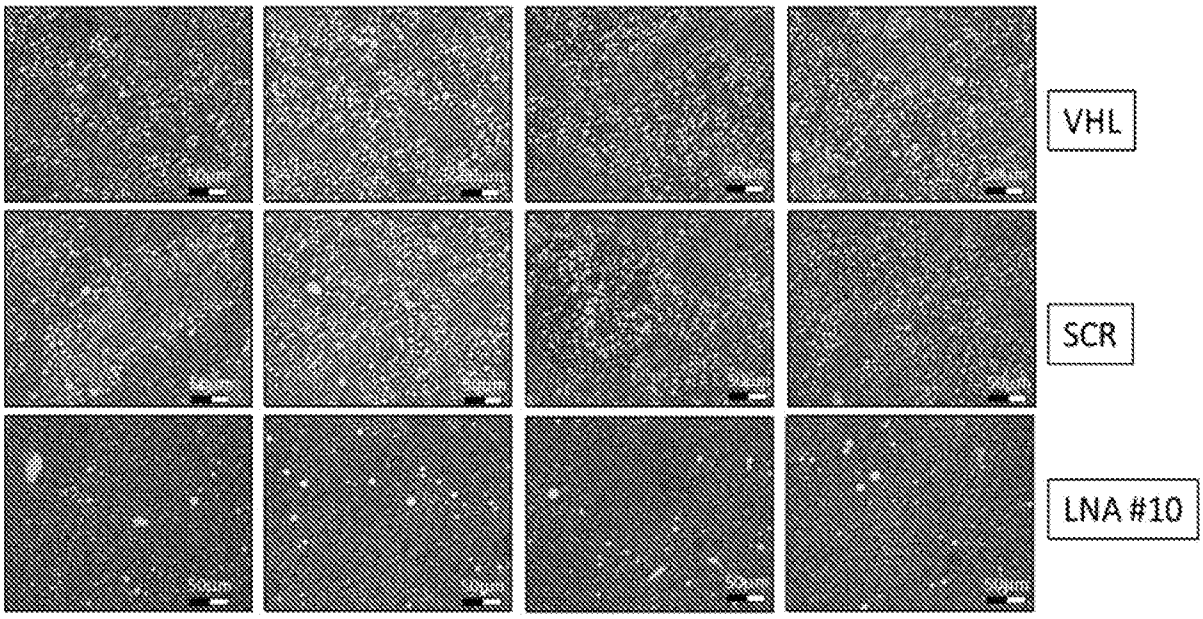


FIGURE 32

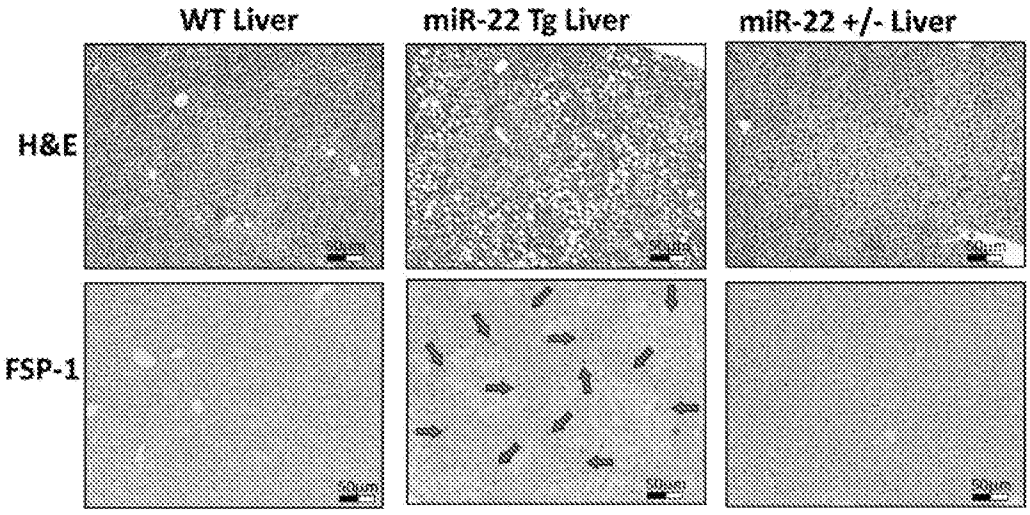


FIGURE 33

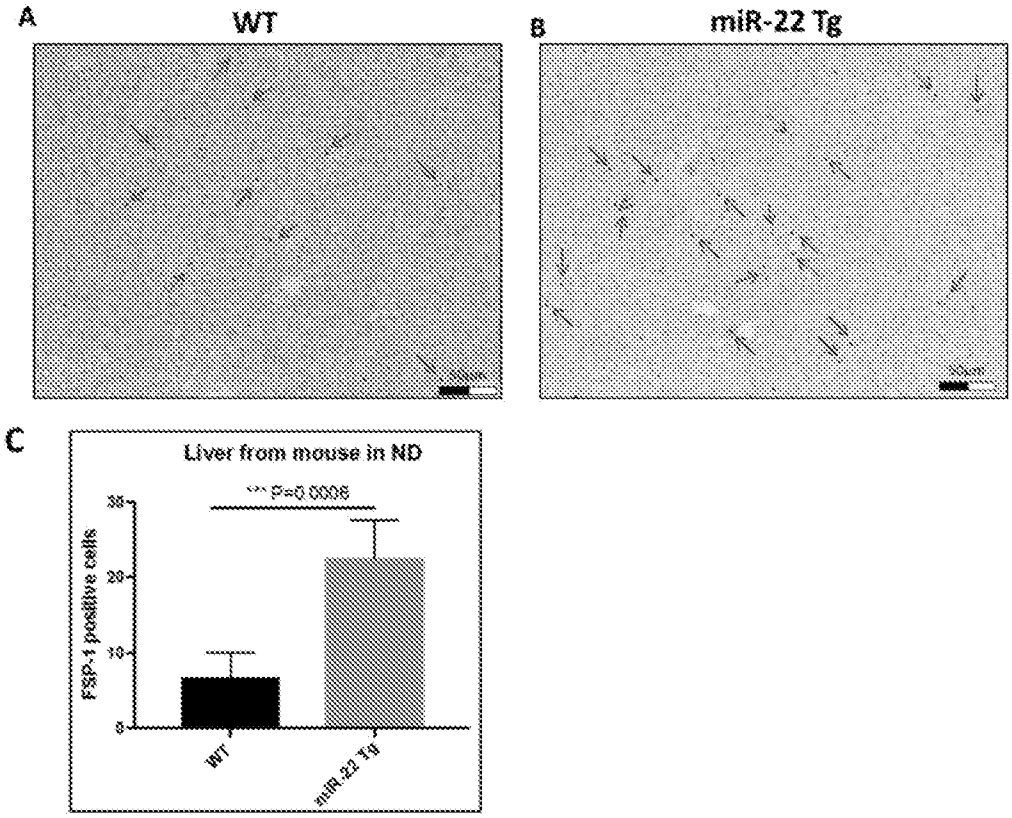
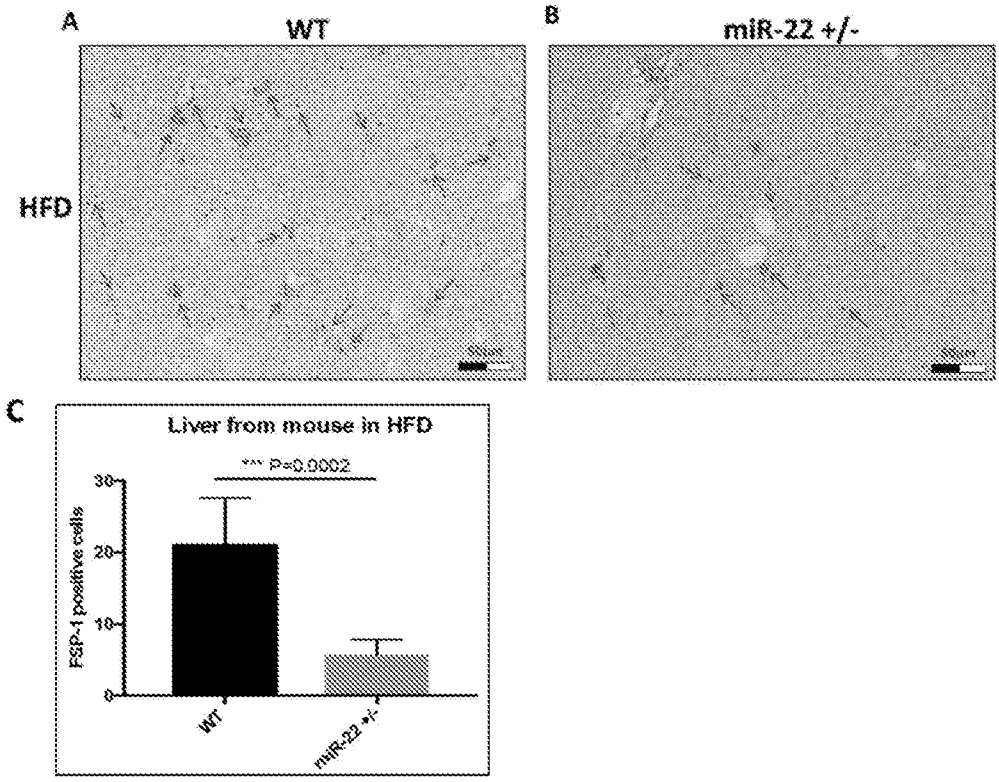


FIGURE 34



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/22350

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/11, C12N 15/113 (2019.01)
CPC - C12N 15/115, C12N 2310/113 , C12N 2310/11, C12N 2310/321, C12Q 2600/178, C12N 2310/141

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2017/187426 A1 (APTAMIR THERAPEUTICS INC) 2 November 2017 (02.11.2017) para [0006], [0008], [0009], [0012], [0087], [0142], [0146], [0219]	1-3, 15, 17 ---------- 4, 16
Y	US 2014/0314833 A1 (MIRNA THERAPEUTICS INC) 23 October 2014 (23.10.2014) para [0023], [0086]	4
Y	WO 2012/080459 A1 (SANOFI) 21 June 2012 (21.06.2012) pg 44, ln 25-32 - pg 45, ln 1	16

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2019	07 JUN 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/22350

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

3. ☒ Claims Nos.: 5-14 and 18-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.