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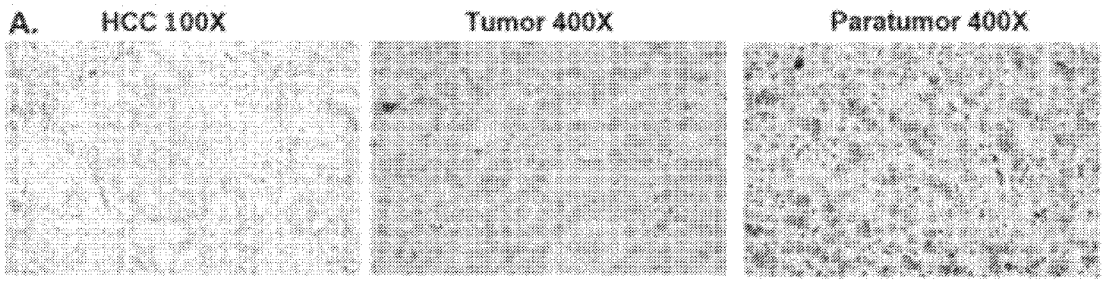
(54) Title: OSR1 GENE THERAPY FOR LIVER CONDITIONS

FIG. 1A



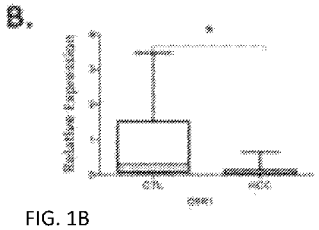


FIG. 1B

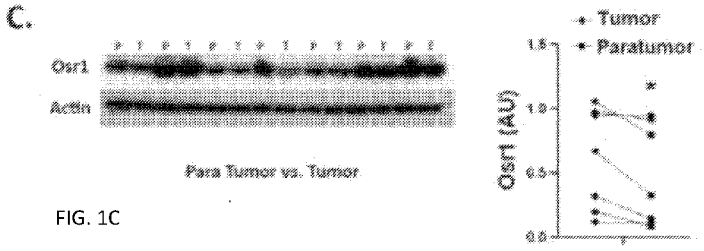


FIG. 1C

FIGS. 1A, 1B and 1C

(57) **Abstract:** Embodiments of the disclosure encompass methods and treatments for a liver medical condition, including at least nonalcoholic steatohepatitis or hepatocellular carcinoma. In specific embodiments, an *Osr1* polynucleotide or the Osr1 gene product encoded therefrom are the therapeutic agent(s) for the liver medical condition. In specific embodiments, the *Osr1* polynucleotide is comprised in an adeno-associated viral vector.

[Continued on next page]

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— *of inventorship (Rule 4.17(iv))*

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OSR1 GENE THERAPY FOR LIVER CONDITIONS

BACKGROUND

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/508,589, filed June 16, 2023, which is incorporated by reference herein in its entirety.

I. Technical Field

[0002] This disclosure relates at least to the fields of hepatology, oncology, cell biology, molecular biology, molecular genetics, and medicine.

II. Background

[0003] Hepatocellular carcinoma (HCC), the fastest-growing lethal cancer in the US, is predicted to rank third among the leading causes of cancer-related death by 2030(1). This alarming trend has been attributed to factors such as increasing obesity, diabetes, and non-alcoholic steatohepatitis (NASH). From 2002 to 2016, the proportion of patients with NASH and HCC in the US increased significantly, with the incidence of HCC with NASH rising 11.8-fold (2). The 45-60 age population was found to have the highest proportional increase in HCC (3). Many preexisting conditions, including liver cirrhosis, non-alcoholic fatty liver disease (NAFLD), viral hepatitis, and alcoholic liver disease, contribute to HCC, with NASH and alcohol being the leading causes in the US (1).

[0004] NAFLD, the most common liver disease, is observed in approximately 30% of the general population, and about one-third of NAFLD cases progress to NASH. Up to 13% of cirrhotic NASH cases eventually manifest into HCC (2). In Texas, HCC is a significant health problem, with higher incidence rates observed in individuals with lower socioeconomic status and among immigrant Hispanics(4, 5). According to the Texas Cancer Registry, the national incidence rate of liver cancer in Texas in 2018 was 58% higher than the national incidence rate. The mean yearly age-adjusted incidence rate of HCC is 2.7 times higher in Hispanic individuals compared to Non-Hispanic White individuals(6). NAFLD has become a pressing issue in Texas, with a 49% occurrence rate reported in South Texas (7, 8), necessitating urgent prevention and treatment strategies for HCC.

[0005] Despite advanced research and the discovery of novel treatment options, HCC generally has a poor prognosis, with a five-year survival rate of 20-40% (1). Traditional less invasive treatments such as radiation therapy and chemotherapy have limited success rates and significant side effects (9), underscoring the need for a better understanding of HCC to identify specific molecular targets for effective treatment.

[0006] Metabolic reprogramming, which allows cancer cells to meet their increasing requirements for survival and proliferation, is one of the key hallmarks of cancer (10). While the Warburg effect, characterized by increased glucose uptake and preferential lactate production, is well-known, most cancer cells still retain mitochondrial respiration to support rapid proliferation (11-13). Mitochondrial chaperones, including Heat Shock Proteins (HSPs), play a crucial role in supporting the excessive metabolism required for tumor growth (14). Mitochondrial HSP60 (mtHSP60), a major chaperone involved in protein homeostasis in mitochondria, is responsible for folding and assembly of key proteins involved in the electron transport chain (ETC) and oxidative phosphorylation (OXPHOS) (15, 16). Maintaining the quality control of mitochondrial proteins by mtHSP60 is essential for the efficiency and integrity of ETC and OXPHOS. Dysfunctional mtHSP60 has been shown to play anti-apoptotic and oncogenic roles in HCC (15). However, the details of dysfunctional mitochondrial chaperones in HCC cell metabolism are not well-described, limiting the development of effective mitochondrial-targeting drugs for HCC treatment.

[0007] The Odd-skipped related 1 (Osr1) gene has been identified as a tumor suppressor in scientific research. Osr1's homolog, Odd-skipped (Odd), was initially discovered as a pair-rule gene essential for segmentation in Drosophila (17, 18). In murine models, Osr1 has been found to regulate the development of critical organs like the heart, lungs, and kidneys (19-22). Recently, a new role was uncovered for Osr1 in the progression of non-alcoholic steatohepatitis (NASH), a liver disease associated with obesity (23-25). NAFLD/NASH was induced in mice with Osr1 heterozygous ($Osr1^{+/-}$) gene status by treating them with a high-fat diet (HFD) or HFD plus diethylnitrosamine (DEN) for 8 weeks. Notably, $Osr1^{+/-}$ mice showed more severe liver injury and higher NASH scores upon both HFD and HFD+DEN treatment. Osr1 has been reported as a novel tumor suppressor gene in various types of cancer (26, 27), and has been proposed as a potential prognostic biomarker in leukemia and gastric cancer (26, 28, 29). Furthermore, Osr1 expression is epigenetically regulated in several cancer cell lines (22, 27, 30).

SUMMARY

[0008] Embodiments of the disclosure encompass methods and compositions related to treatment or prevention of one or more liver medical conditions in mammals. In some embodiments, the liver condition is cancer, whereas in other conditions the liver condition is not cancer. In specific embodiments, the liver condition is hepatocellular carcinoma. In specific embodiments, the liver condition is nonalcoholic fatty liver disease (NAFLD), including nonalcoholic fatty liver (NAFL) and/or non-alcoholic steatohepatitis (NASH). In certain embodiments, the liver condition is Hepatitis A, Hepatitis B, Hepatitis C, fatty liver disease, chronic liver failure, acute liver failure, a genetic disease (such as hemochromatosis, Wilson's disease, and Alpha-1 antitrypsin deficiency), Autoimmune hepatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis, and so forth. Methods and compositions of the disclosure may be provided to an individual to avoid further damage to the liver. Methods and compositions of the disclosure may be provided to an individual to avoid need of a liver transplant. Methods and compositions of the disclosure may be provided to an individual at risk for any liver disease. A person at risk for needing methods and/or compositions of the disclosure include at least heavy alcohol use, obesity, high cholesterol, type 2 diabetes, metabolic syndrome, family history of liver disease, blood-borne viruses, such as Hepatitis B and C, autoimmune hepatitis exposure to toxic chemicals, pesticides, or other people's blood and body fluids, certain prescription or herbal medicines, tattoos or body piercings, shared needles, and/or sex without protection, as examples.

[0009] In particular embodiments, the methods and compositions of the disclosure concern use of gene therapy compositions that provide a specific gene for treatment or prevention of any liver condition. In some embodiments, the gene therapy compositions provide more molecules of the gene product in the liver compared to in the absence of the gene therapy. In specific cases, the gene therapy compositions provide a greater level of gene product in a certain subcellular compartment of cells in the liver. In specific embodiments, the gene is the *Odd-skipped related 1* (*Osr1*) gene that encodes the Osr1 protein, and in particular cases the liver condition is treated or prevented by Osr1 based on a role other than as a tumor suppressor. In specific embodiments, part or all of the *Osr1* gene is utilized in the therapeutic compositions.

[0010] In specific embodiments of the disclosure, gene therapy for liver cancer introduces additional levels of Osr1 to liver cells, and in some cases is delivered by a viral vector, such as an adeno-associated virus (AAV). In particular embodiments, the gene therapy is effective in

blocking liver cancer development in mouse studies. In certain embodiments, the present disclosure concerns gene therapy of nonalcoholic hepatosteatosis (NASH) and hepatocellular carcinoma (HCC), such as *via* AAV8L-Osr1.

[0011] In certain embodiments, a composition, such as any viral particle encompassed herein, is administered to an individual with a liver condition, including NASH or HCC. The administration may be by any suitable route or delivery regimen. In particular embodiments, the viral particle comprises part or all of an *Osr1* polynucleotide that encodes a functional Osr1 protein.

[0012] Embodiments of the disclosure encompass compositions comprising an Osr1 polynucleotide, or a functional fragment thereof, comprised in an adeno-associated viral vector (AAV). In specific embodiments, the AAV is comprised in a pharmaceutically acceptable carrier. In some embodiments, the AAV is an AAV type 1 particle, AAV type 2 particle, AAV type 3 particle, AAV type 5 particle, AAV type 6 particle, AAV type 8 particle, or AAV type 9 particle. In particular embodiments, the *Osr1* polynucleotide encodes an Osr1 polypeptide. The Osr1 polypeptide may comprise an amino acid sequence having at least 90% sequence identity with SEQ ID NO:2. The Osr1 polypeptide may comprise an amino acid sequence having the amino acid sequence of SEQ ID NO:2. In some embodiments, the *Osr1* polynucleotide lacks an intron and/or is operably linked to one or more regulatory sequences. In specific embodiments, the one or more regulatory sequences are active in liver cells. In certain embodiments, the *Osr1* polynucleotide comprises nucleic acid sequence that is at least 90% identical to SEQ ID NO:1. The Osr1 polynucleotide may comprise SEQ ID NO:1.

[0013] In certain embodiments, there is a composition comprising an AAV particle comprising an Osr1 polynucleotide or a functional fragment thereof. In some embodiments, the AAV is an AAV type 1 particle, AAV type 5 particle, AAV type 8 particle, or AAV type 9 particle. In certain embodiments, the Osr1 polynucleotide comprises nucleic acid sequence that is at least 90% identical to SEQ ID NO:1. The Osr1 polynucleotide may comprise nucleic acid sequence that is SEQ ID NO:1. The Osr1 polynucleotide may encode an Osr1 polypeptide comprising amino acid sequence that is at least 90% to SEQ ID NO:2. In some embodiments, the Osr1 polynucleotide encodes an Osr1 polypeptide comprising amino acid sequence that is SEQ ID NO:2.

[0014] In particular embodiments, there is a method of treating or preventing a liver medical condition in an individual, comprising the step of delivering to the individual a therapeutically

effective amount of any composition encompassed herein. The liver medical condition may be nonalcoholic fatty liver disease or hepatocellular carcinoma. In some embodiments, the Osr1 polynucleotide is comprised in a vector, such as an AAV particle, and in some cases it is an AAV type 1 particle, AAV type 5 particle, AAV type 8 particle, or AAV type 9 particle. The individual may be at risk for nonalcoholic fatty liver disease or hepatocellular carcinoma or both. An individual at risk for nonalcoholic fatty liver disease includes an individual having high blood fat levels, such as triglycerides or LDL ("bad") cholesterol, having diabetes or prediabetes, having high blood pressure, having Metabolic syndrome, and/or having an excessive waist circumference (*e.g.*, a waist circumference of more than 35 inches for biological females and 40 inches for biological males).

[0015] In some cases, the individual is an infant, child, adolescent, or adult. The composition may be delivered directly to the liver or delivered systemically.

[0016] In particular embodiments, there is a kit comprising any composition encompassed herein, said composition housed in a suitable container.

[0017] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the disclosure can be used to achieve methods of the disclosure.

[0018] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

[0019] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims herein. It should be appreciated by those skilled in the art that the conception and specific embodiments disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present designs. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope as set forth in the appended claims. The novel features which are believed to be characteristic of

the designs disclosed herein, both as to the organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The embodiments of the disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0021] **FIGS. 1A-1C**. OSR1 expression is significantly decreased in the HCC tumor tissue in both human and mouse models. (**1A**) Immunohistochemical (IHC) staining of OSR1 in liver tissue from patients with HCC (N=5). (**1B**) The mRNA level of OSR1 is significantly reduced in the liver of patients with HCC (n=24/group). *P<0.05. (**1C**) Protein expression of Osr1 is significantly decreased in HCC tumor tissue compared to para-tumor tissue in mice (N=7/group). *P<0.05.

[0022] **FIGS. 2A-2C**. Disrupting *Osr1* expression significantly promoted HCC tumor growth in both chemical-induced and diet-induced HCC mouse models. To assess tumor growth, measurements were taken including the ratio of liver weight to body weight, the size of the largest tumor in each HCC mouse, and the total count of visible tumors (with a diameter >2 mm). (**2A**) HCC was induced by DEN+CCl4 treatment for 20 weeks in both *WT* and *Osr1*$^{+/-}$ mice (N=7-8). (**2B**) HCC induction was performed in STAMTM *WT* and *Osr1*$^{+/-}$ mice (n=6-7). (**2C**) HCC was induced by DEN+CCl4 treatment for 20 weeks in *Osr1F* and *Osr1ΔHep* mice (N=7-8). *P<0.05; **P<0.01.

[0023] **FIG. 3.** Deletion of Osr1 in macrophages did not significantly reduce carcinogen-induced HCC tumor progression in mice. The tumor size and number in Osr1$^{\Delta M\varphi}$ mice was only marginally increased, compared to the control mice. n=7. HCC was induced by DEN+CCl4 treatment for 20 weeks in Osr1F and Osr1ΔM (myeloid-specific osr1 deletion)mice (N=7-8).

[0024] **FIGS. 4A-4E.** In the NAFLD-induced HCC model, lower Osr1 levels promoted tumor growth. **(4A)** According to the STAM model, neonatal Osr1$^{+/-}$ or WT mice were exposed to a low dose streptozotocin (STZ, 200μg) at P2 and are fed 60% HFD at the age of week 4 and continued

this diet until being sacrificed. **FIGS. 4B-4E** show that enhancing *Osr1* expression significantly inhibited tumor growth in HCC mice. **(4B)** Weaned mice were exposed to AAV-GFP (control group) or AAV-Osr1 infection (Osr1 group). Seven days later, the mice were treated with DEN+CCl4 for 20 weeks to induce HCC tumor development. **(4C)** Immunohistochemical (IHC) staining of Osr1 demonstrated enhanced expression of Osr1 in the liver of the Osr1 group compared to the control group. **(4D)** Mice in the Osr1 group exhibited significantly reduced tumor number and size compared to those in the control group (N=7-8). *P<0.05. **(4E)** Mice infected with AAV-Osr1 exhibited repressed cell proliferation in the liver, as indicated by immunofluorescent (IF) staining of p-H3S10. N=7-8. *P<0.05.

[0025] **FIGS. 5A-5C.** Disrupting Osr1 expression promoted cell proliferation. **(5A)** The *Osr1*$^{+/-}$ *vs. WT* mice presented significantly more proliferating cells at the margins of the tumor and para-tumor tissue, detected by the BrdU incorporation. For the BrdU incorporation assay, mice were injected with BrdU 24 and 48 hours before being sacrificed. n=5. **(5B)** Overactivation of JNK signaling was detected in the tumor tissue of *Osr1*+/- *vs. WT* mice. N=6, *P<0.05. **(5C)** Enhancing Osr1 expression in the HepG2 cells resulted in repressed cell proliferation detected by BrdU incorporation. N=3

[0026] **FIG. 6 .** Deleting Osr1 resulted in enhanced mitochondrial OXPHOS in the HCC tumor cells. Osr1KO (*Osr1*$^{\Delta Hep}$) tumor cells exhibited elevated levels of the unbound form of NAD(P)H and the bound form of FAD, indicating heightened OXPHOS in comparison to the WT tumor cells, accompanied by a greater redox ratio. n=150-300 cells/group, *P<0.05; **P<0.01; ***P<0.001

[0027] **FIGS. 7A-7C** Osr1 overexpression prevented carcinogen-induced HCC tumor progression in WT mice. **(7A)** AAV8L-Osr1 or empty AAV8L vectors (1.0×10^{10} gc/mouse) were injected into the wildtype mice and three days later, mice were treated with DEN and CCl_4 for 20 weeks. **(7B)** IHC of Osr1 in the liver of wildtype mice infected with AAV8L-vector or AAV8L-Osr1. **(7C)** Osr1 overexpression established before exposure to carcinogens significantly inhibited tumor growth. n=7, *p<0.05.

[0028] **FIG. 8.** The *Osr1*$^{+/-}$ vs. WT mice, presented significantly more proliferating cells at the margins of the tumor and paratumor tissue, detected by the BrdU incorporation

[0029] (upper panels) and TUNEL assay (lower panels). For the BrdU incorporation assay, mice were injected with BrdU 24 and 48 hours before being sacrificed. n=5.

[0030] **FIGS. 9A-9B.** FLIM results for cellular metabolism of HepG2 with Osr1 overexpression. **(9A)** Under basal (control), glucose, or PA treatment, overexpressing Osr1 resulted in changes of NAD(P)H α1, FAD intensity, and FLIRR that suggested inhibited OXPHOS. ** $p<0.01$, *** $p<0.001$, n=200-600 cells. FLIRR is defined as the fraction of enzyme-bound NAD(P)H divided by the fraction of enzyme-bound FAD. **(9B)** UMAP analysis indicated well-separated populations of control and Osr1-overexpressed HepG2 cells under PA treatment, suggesting that Osr1 overexpression results in distinct metabolic responses to PA treatment. n=200-600.

[0031] **FIGS. 10A-10B.** HepG2 cells overexpressing *OSR1* demonstrated reduced mitochondrial ATP production. **(10A)** The ATP production rate was evaluated using the Seahorse Real-Time ATP Rate Assay. Oxygen consumption rate (OCR) values were recorded sequentially after the injection of oligomycin (OM), FCCP, and rotenone plus antimycin A (Rot + AA). N=5, *$P<0.05$. **(10B)** Analysis using Uniform Manifold Approximation and Projection (UMAP) revealed that while glucose treatment could rescue metabolic alterations induced by *OSR1* overexpression, palmitic acid (PA) treatment did not have the same effect. N=200-300 cells per group.

[0032] **FIGS. 11A-11C**. The expression of Osr1 has been detected within the mitochondria of hepatocytes. (**11A**) IF staining was performed on isolated primary hepatocytes using antibodies against Osr1, MitoTracker, and DAPI. (**11B** and **11C**) A Mitochondria Isolation Kit (Abcam, USA) was utilized to extract mitochondrial proteins from whole cell lysates. The isolation process resulted in a pellet containing mitochondrial proteins (M) and a supernatant containing non-mitochondrial proteins (S). Western Blots were performed on primary hepatocytes and cardiomyocytes isolated from WT mice (B) or HepG2 or Huh7 cells (**11C**).

[0033] **FIG. 12**. Deleting Osr1 did not have an impact on the mitochondria biogenesis. Real-time PCR was performed to quantify mtDNA levels using the mitochondrial D-loop region relative to nuclear RNA. n=5-6 mice/group.

[0034] **FIGS. 13A-13B**. Osr1, Atp5f1β, and Hsp60 showed the possibility of interactions. (**13A**) Immunofluorescent (IF) staining revealed colocalization of Osr1 with Atp5f1β and Hsp60. (**13B**) Coimmunoprecipitation (Co-IP) assays were conducted to investigate the interaction of Osr1 with Atp5f1β and HSP60 in protein extracts from the tumor or normal liver tissue.

[0035] **FIGS. 14A-14D.** Atp5f1b expression was depending on the Osr1 levels. (**14A**) $Osr1^{+/-}$ tumor tissue vs. WT tumor tissues. (**14B**) $Osr1^{\Delta Hep}$ hepatocytes vs. $Osr1^{F}$ hepatocytes. (**14C**) HepG2 cells with Osr1 overexpression vs. HepG2 control cells. N=5, * $P<0.05$. (**14D**) Ubiquitination, detected by immunoprecipitation of the anti-ATP5F1β, followed by anti-ubiquitin immunoblotting was performed in HepG2 cells treated with either scramble siRNA (C: Control) or OSR1 siRNA (O: OSR1 downregulation). The OSR1 siRNA-treated HepG2 cells had a reduced level of ATP5F1β-specific ubiquitination, compared to the control cells.

[0036] **FIG. 15** shows that Osr1 overexpression reduced HCC tumor size.

[0037] **FIG. 16** demonstrates that Osr1 overexpression prevented HCC. MOI (gc/cell)

[0038] **FIG. 17** shows that Osr1 treatment-induced human HCC tumor organoid death *in vitro*.

DETAILED DESCRIPTION

I. Examples of Definitions

[0039] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the measurement or quantitation method. In specific embodiments, the term "about" is used according to its plain and ordinary meaning in the area of cell and molecular biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. "About" when referring to a measurable value, such as an amount of a composition, a dose, a time, a temperature, and the like, is meant to encompass variations of ± 20%, ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

[0040] The use of the word "a" or "an" when used in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0041] The phrase "and/or" means "and" or "or". To illustrate, A, B, and/or C includes: A alone, B alone, C alone, a combination of A and B, a combination of A and C, a combination of B and C, or a combination of A, B, and C. In other words, "and/or" operates as an inclusive or.

[0042] The words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing,

such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0043] The compositions and methods for their use can "comprise," "consist essentially of," or "consist of" any of the ingredients or steps disclosed throughout the specification. Compositions and methods "consisting essentially of" any of the ingredients or steps disclosed limits the scope of the claim to the specified materials or steps which do not materially affect the basic and novel characteristic of the claimed invention.

[0044] The words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0045] The term "adeno-associated virus" (AAV) in the context of the present disclosure includes without limitation AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV and any other AAV now known or later discovered, or engineered from the above-mentioned AAV. The AAV may be an AAV-PHP.eB, AAV-PHP.B, AAV-PHP.S, AAV-PHP.B4, AAV-PHP.B5, AAV-PHP.N, AAV-CAP-B1, AAV-CAP-B10, or AAV-CAP-B22, for example.

[0046] As used herein, the term "therapeutically effective amount" is synonymous with "effective amount", "therapeutically effective dose", and/or "effective dose" and refers to the amount of compound that will elicit the biological, cosmetic or clinical response being sought by the practitioner in an individual in need thereof. The appropriate effective amount to be administered for a particular application of the disclosed methods can be determined by those skilled in the art, using the guidance provided herein. For example, an effective amount can be extrapolated from in vitro and in vivo assays as described in the present specification. One skilled in the art will recognize that the condition of the individual can be monitored throughout the course of therapy and that the effective amount of a compound or composition disclosed herein that is administered can be adjusted accordingly.

[0047] Reference throughout this specification to "one embodiment," "an embodiment," "a particular embodiment," "a related embodiment," "a certain embodiment," "an additional

embodiment," or "a further embodiment" or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[0048] As used herein, the term "individual" or "subject" generally refers to an individual in need of a therapy for a liver condition. The individual can be a mammal, such as a human, dog, cat, horse, pig or rodent. The individual can be one that has or is suspected of having or at risk for having a liver disease or liver medical condition. The individual may have a disease or be suspected of having the disease. The individual may be asymptomatic. The individual may be of any gender or biological sex.

[0049] As used herein, the term "regulatory sequence" refers to any segment of a nucleic acid that is capable of increasing or decreasing the expression of an Osr1 polynucleotide. The regulatory sequence may be proximate to the gene it is regulating, such as, for example, a nucleic acid with less than 100, less than 50, less than 20, less than 10, or less than 5 nucleotides separating the 5' end or 3' end of the gene from the 5' end or 3' end of the regulatory sequence. The regulatory system may be distant to the gene it is regulating, such as, for example, a nucleic acid with 100 or more, 200 or more, 300 or more, 400 or more, 500 or more, 600 or more, 700 or more, 800 or more, 900 or more, 1000 or more nucleotides separating the 5' end or 3' end of the gene from the 5' end or 3' end of the regulatory sequence. A regulatory sequence may comprise a promoter and/or an enhancer. A regulatory sequence may or may not be on the same nucleic acid molecule of the gene the regulatory sequence is regulating. A regulatory sequence, including any regulatory sequence, promoter, and/or enhancer used herein, may be named by the gene in which it regulates. For example, the Osr1 promoter used in compositions of the disclosure may comprise the regulatory sequence that regulates the human Osr1 gene. In some embodiments, a heterologous promoter with respect to the Osr1 polynucleotide may be employed instead.

[0050] As used herein, the terms "treatment," "treat," or "treating" refers to intervention in an attempt to alter the natural course of the individual or cell being treated, and may be performed either for prophylaxis or during the course of pathology of a liver disease or liver medical condition. Treatment may serve to accomplish one or more of various desired outcomes, including,

for example, preventing occurrence or recurrence of disease, alleviation of symptoms, and diminishment of any direct or indirect pathological consequences of the disease, lowering the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

II. Embodiments of the Disclosure

[0051] Embodiments of the disclosure concern gene therapy methods and compositions for treatment of any liver condition, including at least hepatocellular carcinoma and non-alcoholic steatohepatitis, with gene therapy.

[0052] Hepatocellular carcinoma (HCC) is the fastest-growing lethal cancer in the US and is predicted to rank third among the leading causes of cancer-related death by 2030. HCC generally has a poor prognosis with a five-year survival of 20–40%, despite advanced research and the discovery of novel treatment options for HCC patients. Due to the current advanced technologies combined with the multi-disciplinary approaches, treatment modalities for HCC have evolved and given a variety of options, including liver transplant, surgical resection, and transarterial chemoembolization (TACE). However, determining the best treatment option is challenging and traditional treatments, such as surgery, radiation therapy, and chemotherapy, have limited success rates and often cause severe side effects. These facts make it crucial to discover novel treatment options for HCC patients. Gene therapy has the potential to create ever-expanding therapeutic opportunities *via* gene addition and gene editing, through the delivery of non-viral or viral vectors. Vectors based on adeno-associated virus (AAV) has shown the most promise for liver-targeted gene therapy, evidenced by the success of clinical trial using liver-directed AAV gene therapy in treating severe hemophilia A . The liver has less than 1% of dividing hepatocytes and AAV vectors can efficiently infect non-dividing hepatocytes upon intravenous administration. Recombinant AAV is thought to be non-pathogenic in humans and weakly immunogenic. In addition, AAV has slow turnover rates under physiological conditions in adults, making it possible to induce long-term, maybe even life-long treatment effects. The present disclosure concerns evaluation of gene therapy delivered by AAV8L that overexpresses the Oddskipped related 1 (Osr1), reported as a tumor suppressor gene, for treating HCC using mouse models. It is shown herein that this method reduced the tumor size by 600% and the tumor number by 500% in HCC mice.

[0053] The disclosure introduces several innovative aspects that contribute to the understanding and potential treatment of HCC.

[0054] (1) Noncanonical role of Osr1: The disclosure explores the nontraditional role of Osr1 in HCC cell metabolism. The discovery of Osr1's expression in hepatocyte mitochondria reveals a unique characteristic that distinguishes it as a potential target for HCC treatment. This noncanonical function of Osr1 provides a novel perspective on its involvement in HCC progression.

[0055] (2) Mitochondrial localization of Osr1: The disclosure uncovers the surprising finding that Osr1 is exclusively expressed in the mitochondria of hepatocytes, rather than being a traditional transcription factor. This highlights a previously unknown aspect of Osr1's function and suggests a role in mitochondrial metabolism and energy production in HCC.

[0056] (3) Metabolic reprogramming in HCC: The disclosure proposes that Osr1 mediates mitochondrial chaperone activity and controls OXPHOS and mitochondrial ATP production in HCC hepatocytes. This points to the significance of metabolic reprogramming in HCC and the potential for targeting metabolic pathways for therapeutic interventions.

[0057] (4) Therapeutic potential of targeting Osr1 and ATP5f1b chaperone: The disclosure identifies Osr1 as a promising therapeutic target for HCC treatment, given its unique localization in hepatocyte mitochondria. Targeting Osr1 holds the potential for developing effective and less toxic treatments for HCC. Additionally, the study sheds light on the role of mitochondrial chaperones in HCC cell metabolism, providing insights that could aid in the development of mitochondrial-targeting drugs for HCC treatment.

[0058] (5) Application of FLIM technology: The disclosure utilizes fluorescence lifetime imaging microscopy (FLIM) technology to monitor hepatocyte metabolism in real-time in live mice. This innovative approach enables the dynamic visualization and understanding of metabolic changes associated with Osr1, offering valuable insights into HCC tumor size, management, and treatment response prediction.

[0059] (6) Integration of advanced computational methods: The disclosure incorporates advanced deep-learning computational methods, including AlphaFold 2, RosettaDock, and GROMACS, to model protein interactions. These computational tools complement traditional experimental assays like co-immunoprecipitation and yeast two-hybrid assays, enhancing the

understanding of Osr1's interaction with mitochondrial chaperones and its impact on HCC cell metabolism.

[0060] Overall, the disclosure's innovations lie in its characterization of the noncanonical role of Osr1, the use of FLIM technology for real-time metabolic monitoring, the integration of advanced computational methods, the identification of Osr1 and ATP5F1 chaperone as a potential therapeutic target, and the comprehensive approach taken to unravel the complexities of HCC cell metabolism. These innovations advance the understanding of HCC and have implications for the development of new therapeutic strategies.

[0061] The following embodiments are encompassed herein: (1) To characterize the roles of Osr1, Atp5f1β, and Hsp60 in HCC development, one can utilize hepatocyte-specific Cas9-Tg models and perform *in vivo* gene modification using *AAV8* infection. (2) The study may utilize fluorescence lifetime imaging microscopy (FLIM) technology and Vevo 3100 advanced micro-ultrasound imaging system to monitor hepatocyte metabolism and tumor growth in real-time in live mice. These innovative approaches enable dynamic visualization and correlate tumor growth with cell metabolism. (3) One can incorporate advanced deep-learning computational methods, including AlphaFold 2, RosettaDock, and GROMACS, to model protein interactions, in certain embodiments. These computational tools complement traditional experimental assays like co-immunoprecipitation (co-IP) and yeast two-hybrid (Y2H) assays, as well as the cutting-edge TurboID technology, enhancing the understanding of mitochondrial chaperones with Osr1's interaction and its impact on HCC cell metabolism. (4) Embodiments of drug screening may use Cell Painting, a high-content image-based method, to capture various cellular features in a single assay. This can generate rich, multiparametric data to analyze cellular responses to nearly 60,000 drugs and small molecules, including FDA-approved clinical candidates and off-patent drugs with favorable attributes. Additionally, one can use optical metabolic imaging with an automated multi-photon fluorescent lifetime imaging platform. Data analysis may incorporate conventional statistics and advanced machine learning/AI techniques for high-throughput data interpretation, in certain embodiments.

[0062] In particular embodiments of the disclosure, Osr1 orchestrates OXPHOS by facilitating mitochondrial chaperone activity targeting the degradation of ATP synthase. Downregulation of Osr1, as observed in HCC tumors, leads to hyperactivation of OXPHOS, promoting metabolic adaptation required for tumor cell proliferation.

III. Odd-skipped related 1 (Osr1) Gene Therapy and Related Compositions

[0063] Embodiments of the disclosure utilize all, or a functional part, of an *Osr1* polynucleotide as gene therapy for a liver condition in an individual in need thereof. In some embodiments, the gene therapy comprises an *Osr1* polynucleotide that is capable of producing part or all of an Osr1 polypeptide sufficient to improve at least one symptom of a liver condition, including at least HCC or NASH.

[0064] The skilled artisan recognizes that there are a variety of names for the gene, including Odd-Skipped Related Transcription Factor, ODD, Protein Odd-Skipped-Related 1, Odd-Skipped Related Transcription Factor 1, Odd-Skipped Related 1 (Drosophila), Odd-Skipped (Drosophila) Homolog, Odd-Skipped Related 1, and Odd-Skipped Homolog.

[0065] Embodiments of the disclosure concern compositions, including gene therapies, viral particles, and nucleic acids, that are useful for increasing the level of Osr1 in cells of an individual with any liver deficiency, including in liver cells, and the level is increased over the level present in the absence of any therapy encompassed herein in another individual. In some embodiments, the composition comprises a viral particle. The viral particle may comprise one or more nucleic acids. The nucleic acids may encode for an Osr1 gene product. An "Osr1 gene product" describes a polypeptide generated from transcription and translation of a Osr1 polynucleotide, such as an Osr1 gene. The nucleic acid that encodes an Osr1 gene product may be an Osr1 gene from any organism, including for example, a worm, a fruit fly, a mouse, a rat, any non-human primate, and a human.

[0066] One example of an *Osr1* polynucleotide is provided at the National Center for Biotechnology Information GenBank® database at NM_145260 (SEQ ID NO:1)

[0067] 1 gagtgagtcg gagctcggcc tctccccggc acgttctcag ctgctcctgg ttcagaccca
[0068] 61 gcgagggagc cgcgagcgag gctcaccgtc cccggcgtgc aggatccggg gctgctgagc
[0069] 121 gctcgctccc gcgtgtccgg cgcttggagt ccccgcggca ggagaggagt cgggacacta
[0070] 181 gagctccagg ggcgcctgtg ggctccaggg cctccggctt ccccagtccc cttcagctaa
[0071] 241 agccccagag acgtgctcag ccccaggacc tctgcggaac aagatccgga ttgagaagcc
[0072] 301 actgcaacta ccgaaatggg cagcaaaacc ttgccggcgc cggtgcctat ccacccttcc
[0073] 361 ctgcagctca ccaactactc cttccttcag gcagtgaacg gcctgcccac agtgccttcg
[0074] 421 gaccatctgc ccaacctgta tggtttcagc gcgttgcacg ctgtgcacct gcatcagtgg
[0075] 481 acgctgggct acccggccat gcacttgccg cgctcttctt tctccaaagt gccgggcacg
[0076] 541 gtgtccagct tggtggatgc gcgcttccag ctgcccgcct ttccctggtt ccctcatgtc
[0077] 601 attcaaccca agcccgagat caccgctgga ggcagcgttc cagcgctcaa gaccaagccg
[0078] 661 cgctttgatt ttgccaacct ggccttggca gcaacgcaag aagatccggc caagctcggt

[0079] 721 cgcggggagg gcccaggctc ccctgcaggt gggctgggtg ccctcctcga cgtgaccaag

[0080] 781 ctgtctccag aaaagaagcc cacaagggga cgtctgcctt ccaagaccaa gaaggaattc

[0081] 841 gtctgcaagt tctgtggccg ccacttcacc aagtcctaca acctacttat ccatgagcgg

[0082] 901 acgcacaccg acgagcggcc ctacacctgt gacatctgcc acaaagcctt ccggaggcaa

[0083] 961 gaccacctgc gagaccacag atatattcac tccaaagaga agcccttcaa gtgtcaagag

[0084] 1021 tgtgggaaag gattctgcca gtccaggact ctcgctgtcc acaagacgct acactcacag

[0085] 1081 gtgaaggagc tcaaaacctc caagatcaaa tgctaaatag aacctgtggg tcacaaggac

[0086] 1141 cctaggccca gcggccctct cctccatagg gaccagaagc ctgactctgg cgggcagcgg

[0087] 1201 gagaggcgct cgctcgggac cttccacctc tccaacattg tcccctgggt ccctggcgca

[0088] 1261 cgcggcactt cagagccccg cccggggccg cgaccccgac cgtccctgct gctcccctag

[0089] 1321 gacgcggcta aactcttggc caaaaggcgg tactcacgtg gcggaaggga aactgcatta

[0090] 1381 aaaaaagaac gaaagctccg cggagccgca gcggcgcctc tcccagaagt attacttttt

[0091] 1441 ctattgttat tttatacgtt ttctttttta tttttgtctt tgaccatata agcttgtaac

[0092] 1501 tctgactgcg gagagtgagt ggagagagga gaggcaacga agtccttagc actggcagct

[0093] 1561 cccacctcct cctcccgccc ctgcccccac ccccggagcc tgcaaaagtg taatccctgt

[0094] 1621 atctgcttca gcctcctgcc ctagggaccg cggggcagcc ccctcccagc tcttgcagct

[0095] 1681 cagctggcgg ccgtgggctc cgggactagg agagcgggac ggggacctcg ggcctggggt

[0096] 1741 ctgcaggggg cccggcctcc gctgccgcga cactgcgagg gagcagccag ccaagagccg

[0097] 1801 ggcgttatat tgcgattggc actttatgct gaccatcggt aacggacatt tatcactgga

[0098] 1861 gttttttttt tttttaacta ttaaataaac ggttatttta caggca

[0099] In some embodiments, the nucleic acid comprises sequence of an Osr1 polynucleotide comprising at least, or exactly, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% sequence identity with SEQ ID NO:1, or any range or value derivable therein. In some embodiments, the nucleic acid comprises an Osr1 polynucleotide comprising SEQ ID NO:1.

[0100] In certain embodiments, there is a viral particle that comprises a nucleic acid that comprises sequence of an Osr1 polynucleotide comprising at least, or exactly, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% sequence identity with SEQ ID NO:1, or any range or value derivable therein. In some embodiments, there is a viral particle that comprises a nucleic acid comprises an Osr1 polynucleotide comprising SEQ ID NO:1.

[0101] In specific embodiments, all of SEQ ID NO:1 is utilized in a therapeutic composition.

[0102] In some embodiments, the nucleic acid encoding an Osr1 gene product encodes exons of the gene, including only encodes exons of the gene in some cases. The nucleic acid may or may not lack introns. The nucleic acid may comprise at least one regulatory sequence. In some

embodiments, the regulatory sequence, including a promoter, induces constitutive expression, including constitutive expression in neurons. In some embodiments, the viral particle comprises nucleic acids encoding for at least one regulatory sequence and at least one transgene. The regulatory sequence may be capable of inducing constitutive expression of the transgene(s) in any cell (including liver cells) when the cell is transduced by the viral particle. In some embodiments, the viral particle comprises nucleic acids encoding one or more transgenes and comprises one or more regulatory sequences that induce expression of the transgene(s), including specifically in liver cells, in some cases. Any regulatory sequence for expression of the Osr1 polynucleotide may comprise one or more enhancers.

[0103] The Osr1 polynucleotide may encode an example of an Osr1 polypeptide, such as at GenBank® NP_660303.1, such as below:

MGSKTLPAPVPIHPSLQLTNYSFLQAVNGLPTVPSDHLPNLYGF
SALHAVHLHQWTLGYPAMHLPRSSFSKVPGTVSSLVDARFQLPAFPWFPHVIQPKPEI
TAGGSVPALKTKPRFDFANLALAATQEDPAKLGRGEGPGSPAGGLGALLDVTKLSPEK
KPTRGRLPSKTKKEFVCKFCGRHFTKSYNLLIHERTHTDERPYTCDICHKAFRRQDHL
RDHRYIHSKEKPFKCQECGKGFCQSRTLAVHKTLHSQVKELKTSKIKC (SEQ ID NO:2)

[0104] In some embodiments, the nucleic acid encodes an Osr1 gene product comprising an amino acid sequence having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% sequence identity with SEQ ID NO:2, or any range or value derivable therein. In some embodiments, the nucleic acid encodes an Osr1 gene product comprising SEQ ID NO:2.

[0105] In certain embodiments, there is a viral particle that comprises a nucleic acid that encodes for an amino acid sequence having, having at least, or having at most 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with SEQ ID NO:2, or any range or value derivable therein. In certain embodiments, there is a viral particle that comprises a nucleic acid that encodes for an amino acid sequence having 100% identity to SEQ ID NO:2.

[0106] In some embodiments, the viral particle comprises a nucleic acid sequence encoding SEQ ID NO:2, wherein 1, 2 or fewer, 3 or fewer, 4 or fewer, 5 or fewer, 6 or fewer, 7 or fewer, 8

or fewer, 9 or fewer, 10 or fewer, 12 or fewer, 15 or fewer, 20 or fewer, 25 or fewer, 30 or fewer, 40 or fewer, or 50 or fewer of the codons within SEQ ID NO:2 is substituted with another codon, optionally comprising a conservative amino acid substitution or silent mutation, and/or are deleted and/or an insertion (including 5' and/or 3' extensions) of 1, 2 or fewer, 3 or fewer, 4 or fewer, 5 or fewer, 6 or fewer, 7 or fewer, 8 or fewer, 9 or fewer, 10 or fewer, 12 or fewer, 15 or fewer, 20 or fewer, 25 or fewer, 30 or fewer, 40 or fewer, or 50 or fewer codons or any combination of substitutions, deletions and/or insertions, wherein the substitutions, deletions and/or insertions do not unduly impair the structure and/or function of Osr1.

[0107] Certain embodiments of the disclosure concern viral particles, including those useful for treating any encephalopathy. In some embodiments, the viral particle is an adeno-associated virus (AAV). The viral particle may transduce cells of the liver, in specific cases. The viral particle may comprise one or more nucleic acids. The nucleic acids may encode for an entire Osr1 polypeptide or a functional fragment of the Osr1 polypeptide.

[0108] Conservative amino acid substitutions are known in the art. In particular embodiments, a conservative amino acid substitution includes substitutions within one or more of the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and/or phenylalanine, tyrosine.

[0109] In certain embodiments, the viral particle comprises one or more nucleic acid sequences encoding a regulatory sequence. The regulatory sequence may be a promoter and/or enhancer for genes specifically expressed in cells of the liver. It is specifically contemplated that promoters and/or enhancers specific for liver cell types may be excluded from certain embodiments of the disclosure. In some embodiments, the regulatory sequence comprises a promoter selected from the group consisting of CMV (Cytomegalovirus) promoter; SV40 (Simian virus 40) promoter; EF1α (Elongation Factor 1 alpha) promoter; CAG (CMV immediate-early enhancer/chicken β-actin/rabbit β-globin hybrid promoter) promoter; Ubiqutin promoter; RSV (Rous Sarcoma Virus) promoter; PGK (Phosphoglycerate kinase) promoter; MSCV (Murine Stem Cell Virus) promoter; EF1α (Elongation Factor 1 alpha) promoter; hCMV (Human Cytomegalovirus) promoter, etc.

[0110] A variety of promoter/enhancer elements may be used depending on the level and tissue-specific expression desired. The promoter/enhancer may be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer may be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional

initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

[0111] Promoter/enhancer elements can be native to the target cell or subject to be treated and/or native to the heterologous nucleic acid sequence. The promoter/enhancer element is generally chosen so that it will function in the target cell(s) of interest. In representative embodiments, the promoter/enhancer element is a mammalian promoter/enhancer element. The promoter/enhance element may be constitutive or inducible.

In some embodiments, the viral particle comprises an AAV particle, such as any AAV particle encompassed herein. The viral particle may be an AAV-PHP.eB particle, AAV-PHP.B particle, AAV-PHP.S particle, AAV-PHP.B4 particle, AAV-PHP.B5 particle, AAV-PHP.N particle, AAV-CAP-B1 particle, AAV-CAP-B10 particle, AAV-CAP-B22 particle, AAV type 1 particle, AAV type 5 particle, AAV type 8 particle, or AAV type 9 particle. In some embodiments, the viral particle is an AAV-PHP.eB particle.

[0112] In some embodiments, the viral particle is a particle described in one or more of PCT Patent Application Publications WO2020206189, WO2020028751, WO2020168145, WO2020077165, WO2021025995, WO2017197355, WO2018022905, WO201700671, WO2017218842, WO2016154344, WO2017192750; WO2016081811, WO2019222329 and WO2019028306, all of which are incorporated by reference herein in their entirety.

[0113] In some embodiments, the composition comprises at least one nucleic acid molecule that encodes for one or more gene products capable of generating one or more viral particles, including any viral particle encompassed herein. The nucleic acid(s) may comprise one or more plasmids, including any viral plasmids. The plasmid(s) may be an AAV plasmid, including an AAV-PHP.eB, AAV-PHP.B, AAV-PHP.S, AAV-PHP.B4, AAV-PHP.B5, AAV-PHP.N, AAV-CAP-B1, AAV-CAP-B10, AAV-CAP-B22, AAV type 1, AAV type 5, AAV type 8, or AAV type 9 plasmid. The plasmid(s) may be an AAV plasmid as described in one or more of PCT Patent Application Publications WO2020206189, WO2020028751, WO2020168145, WO2020077165, WO2021025995, WO2017197355, WO2018022905, WO201700671, WO2017218842, WO2016154344, WO2017192750; WO2016081811, WO2019222329 and WO2019028306, all of which are incorporated by reference herein in their entirety. The nucleic acid may comprise a sequence that encodes for a transgene. The transgene may encode any gene

product encompassed herein, including any Osr1 gene product encompassed herein. The nucleic acid molecules may comprise any regulatory sequence encompassed herein.

[0114] Certain embodiments of the disclosure concern methods of producing viral particles. In some embodiments, the method comprises providing to a cell in vitro, (a) a template comprising (i) a nucleic acid encoding for an Osr1 gene product, and (ii) packaging signal sequences sufficient for the encapsidation of an AAV template into virus particles (e.g., one or more (e.g., two) terminal repeats, such as AAV terminal repeats), and (b) AAV sequences sufficient for replication and encapsidation of the template into viral particles (e.g., the AAV rep and AAV cap sequences encoding an AAV capsid). The template and AAV replication and capsid sequences are provided under conditions such that recombinant virus particles comprising the template packaged within the capsid are produced in the cell. The method can further comprise the step of collecting the virus particles from the cell. Virus particles may be collected from the medium and/or by lysing the cells.

[0115] The cell is typically a cell that is permissive for AAV viral replication. Any suitable cell known in the art may be employed, such as mammalian cells. Also suitable are trans-complementing packaging cell lines that provide functions deleted from a replication-defective helper virus, e.g., 293 cells or other E1a trans-complementing cells.

[0116] In particular embodiments, the present disclosure provides a pharmaceutical composition comprising a vector, including a virus vector, of the disclosure in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and will preferably be in solid or liquid particulate form.

IV. Administration of Therapeutic Compositions

[0117] Certain embodiments of the disclosure concern the treatment of an individual with a liver condition of any kind. In specific embodiments, the liver condition is the result of genetics, exposure to a pathogen, deleterious behavioral choices, or the onset of disease, such as cancer.

In some embodiments, an individual is treated by a method comprising administering a therapeutically effective amount of one or more compositions encompassed herein, including any viral particle herein, to the individual. The composition may increase the level of a heterologous transgene, including Osp1, in the individual, including in cells of the individual, such as liver cells of the individual. In some embodiments, the composition administered to the individual increases the level of Osr1 to a level greater than in a control individual (i.e. an individual that has not been administered the therapy). In some embodiments, the compositions restore, improve, or cure defective liver function in the individual. In some embodiments, the compositions reduce the number and/or severity of symptoms of liver failure or a liver medical condition in the individual. In some embodiments, the compositions reduce tumor load in the individual. In some embodiments, the compositions reduce the number and/or size of fatty deposits in the liver.

[0118] Effective dosages of the viral particles to be administered to a subject will depend upon the mode of administration, the disease or condition to be treated, the individual subject's condition, the particular virus vector, and the Osr1 nucleic acid to be delivered, and can be determined in a routine manner. Examples of effective doses for achieving therapeutic effects include virus titers of at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^10, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} transducing units or more.

[0119] Example modes of administration include direct delivery to the liver (such as by injection) oral, rectal, transmucosal, topical, intranasal, inhalation (e.g., via an aerosol), buccal (e.g., sublingual), vaginal, intrathecal, intraocular, transdermal, in utero (or in ovo), parenteral (e.g., intravenous, subcutaneous, intradermal, intramuscular (including administration to skeletal, diaphragm and/or cardiac muscle), intradermal, intrapleural, intracerebral, intracerebroventricular, intraparenchymal, and intraarticular), topical (e.g., to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intro-lymphatic, and the like, as well as direct tissue or organ injection (e.g., to brain, liver, muscle, etc.). The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular vector and/or viral particle that is being used. The vector may or may not be delivered *via* cells and by any suitable route.

[0120] In some embodiments, the viral particle is administered directly to the liver. Direct administration can result in high specificity of transduction of liver cells, e.g., wherein at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more of the transduced cells are liver cells. Any method known in the art to administer vectors directly to the liver can be used. In some embodiments, the vectors are administered directly to hepatocytes, hepatic stellate cell and liver-infiltrated macrophages, or a combination thereof.

[0121] In some embodiments, the viral vector is administered in a liquid formulation by direct injection to the liver (any part thereof, including the hepatic vein). In some embodiments, the vector can be delivered via a reservoir and/or pump. In other embodiments, the vector may be provided by intra-nasal administration of an aerosol formulation. As a further alternative, the vector may be administered as a solid, slow-release formulation.

[0122] Embodiments of the disclosure include at least two ways to deliver the AAV particles. In some embodiments, one can inject the AAV particles directly into the liver tissues. In some embodiments, one can deliver the particles systemically, such as by injection into a blood vessel, and then let the AAV particles migrate to the liver. In particular embodiments, one or more AAV particles of the disclosure has the ability to migrate to the liver. In embodiments wherein any AAV particle is considered to have very weak or no ability to migrate to the liver, one can inject the AAV particles directly into the liver tissues.

V. Pharmaceutical Compositions and Delivery

[0123] The disclosure concerns compositions comprising, consisting essentially of, or consisting of an adeno-associated virus (AAV) particle comprising a nucleic acid comprising a sequence encoding part or all of an Osr1 gene product, including wherein the AAV particle comprises one or more elements that imparts activity of the particle to transduce cells of the liver of an individual in need thereof. In cases wherein the nucleic acid encodes part of the Osr1 gene product, the part will be functional in treating a medical condition associated with reduced levels of Osr1, as addressed elsewhere herein.

[0124] In particular embodiments, any composition encompassed herein comprises a pharmaceutically acceptable (*e.g.*, physiologically acceptable) carrier. When the composition

consists essentially of the inventive particle and a pharmaceutically acceptable carrier, additional components can be included that do not materially affect the composition (*e.g.*, adjuvants, buffers, stabilizers, anti-inflammatory agents, solubilizers, preservatives, *etc.*). When the composition consists of the inventive particle and the pharmaceutically acceptable carrier, the composition does not comprise any additional components. Any suitable carrier can be used within the context of the disclosure, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the composition may be administered and the particular method used to administer the composition. The composition optionally can be sterile with the exception of the composition components described herein. The composition can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. The compositions can be generated in accordance with conventional techniques described in, *e.g.*, Remington: The Science and Practice of Pharmacy, 21st Edition, Lippincott Williams & Wilkins, Philadelphia, Pa. (2001).

[0125] Suitable formulations for the composition include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain anti-oxidants, buffers, and bacteriostats, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, immediately prior to use. Extemporaneous solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. In one embodiment, the carrier is a buffered saline solution. In one embodiment, the inventive particle is administered in a composition formulated to protect the composition and its contents from damage prior to administration. For example, the composition can be formulated to reduce damage from devices used to prepare, store, or administer the particle, such as glassware, syringes, or needles. The composition can be formulated to decrease the light sensitivity and/or temperature sensitivity of the nucleic acid. To this end, the composition may comprise a pharmaceutically acceptable liquid carrier, such as, for example, those described above, and a stabilizing agent selected from the group consisting of polysorbate 80, L-arginine, polyvinylpyrrolidone, trehalose, and combinations thereof. Use of such a composition extends the shelf life of the gene transfer vector, facilitate administration, and increase the efficiency of the inventive method. Formulations for gene transfer vector-containing

compositions are further described in, for example, Wright et al., Curr. Opin. Drug Discov. Devel., 6(2): 174-178 (2003) and Wright et al., Molecular Therapy, 12: 171-178 (2005))

[0126] The composition also can be formulated to enhance transduction efficiency. In addition, one of ordinary skill in the art will appreciate that the inventive gene transfer composition can be present in a composition with other therapeutic or biologically-active agents. For example, factors that control inflammation, such as ibuprofen or steroids, can be part of the composition to reduce swelling and inflammation associated with in vivo administration of the gene transfer composition. Immune system stimulators or adjuvants, *e.g.*, interleukins, lipopolysaccharide, and double-stranded RNA, can be administered to enhance or modify the anti-tau immune response. Antibiotics, *i.e.*, microbicides and fungicides, can be present to treat existing infection and/or reduce the risk of future infection, such as infection associated with gene transfer procedures.

[0127] Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

[0128] In certain embodiments, a formulation of the present disclosure comprises a biocompatible polymer selected from the group consisting of polyamides, polycarbonates, polyalkylenes, polymers of acrylic and methacrylic esters, polyvinyl polymers, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses, polypropylene, polyethylenes, polystyrene, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), poly(lactide-co-caprolactone), polysaccharides, proteins, polyhyaluronic acids, polycyanoacrylates, and blends, mixtures, or copolymers thereof.

[0129] The composition can be administered in or on a device (such as a mechanical reservoir) that allows controlled or sustained release, such as a sponge, biocompatible meshwork, mechanical reservoir, or mechanical implant. Implants, devices, such as an implantable device, *e.g.*, a mechanical reservoir or an implant or a device comprised of a polymeric composition, are particularly useful for administration of the inventive gene transfer vector. The composition also can be administered in the form of sustained-release formulations (see, e.g., U.S. Pat. No. 5,378,475) comprising, for example, gel foam, hyaluronic acid, gelatin, chondroitin sulfate, a

polyphosphoester, such as bis-2-hydroxyethyl-terephthalate (BHET), and/or a polylactic-glycolic acid.

[0130] Delivery of the compositions comprising the inventive gene transfer compositions may be intracerebral (including but not limited to intracerebroventricular, intraparenchymal, intraventricular, or intracisternal), intrathecal (including but not limited to lumbar or cisterna magna), or systemic, including but not limited to intravenous, or any combination thereof, using devices known in the art. Delivery may also be via surgical implantation of an implanted device.

[0131] The dose of the gene transfer composition administered to a mammal will depend on a number of factors, including the size (mass) of the mammal, the extent of any side-effects, the particular route of administration, and the like. In one embodiment, the inventive method comprises administering a "therapeutically effective amount" of the composition comprising the inventive gene transfer composition described herein. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount may vary according to factors such as the extent of the disease, age, gender, and/or weight of the individual, and the ability of the gene transfer composition to elicit a desired response in the individual. The dose of gene transfer composition required to achieve a particular therapeutic effect may be determined by standard means. An example of a range of doses includes 10^{11} vg/kg to 10^{14} vg/kg.

[0132] In one embodiment of the disclosure, the composition is administered once to the mammal. However, in certain cases, it may be appropriate to administer the composition multiple times during a therapeutic period to ensure sufficient exposure of cells to the composition. For example, the composition may be administered to the mammal two or more times (e.g., 2, 3, 4, 5, 6, 6, 8, 9, or 10 or more times) during a therapeutic period.

[0133] The present disclosure provides pharmaceutically acceptable compositions that comprise a therapeutically-effective amount of composition comprising a nucleic acid sequence that encodes a functional Osr1.

VI. Polypeptides

[0134] In specific embodiments, instead of, or in addition to, utilizing gene therapy with an Osr1 polynucleotide, one may employ all or a functional part of an Osr1 polypeptide as the

therapeutic agent for any liver condition. One example of an Osr1 polypeptide includes SEQ ID NO:2.

[0135] As used herein, an Osr1 "protein" or "polypeptide" refers to a molecule comprising at least five amino acid residues. As used herein, the term "wild-type" refers to the endogenous version of a molecule that occurs naturally in an organism. In some embodiments, wild-type versions of a protein or polypeptide are employed, however, in many embodiments of the disclosure, a modified protein or polypeptide is employed. The terms described above may be used interchangeably. A "modified protein" or "modified polypeptide" or a "variant" refers to a protein or polypeptide whose chemical structure, particularly its amino acid sequence, is altered with respect to the wild-type protein or polypeptide. In some embodiments, a modified/variant protein or polypeptide has at least one modified activity or function (recognizing that proteins or polypeptides may have multiple activities or functions). It is specifically contemplated that a modified/variant protein or polypeptide may be altered with respect to one activity or function yet retain a wild-type activity or function in other respects.

[0136] Where a protein is specifically mentioned herein, it is in general a reference to a native (wild-type) or recombinant (modified) protein or, optionally, a protein in which any signal sequence has been removed. The protein may be isolated directly from the organism of which it is native, produced by recombinant DNA/exogenous expression methods, or produced by solid-phase peptide synthesis (SPPS) or other in vitro methods. In particular embodiments, there are isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a polypeptide. The term "recombinant" may be used in conjunction with a polypeptide or the name of a specific polypeptide, and this generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated in vitro or that is a replication product of such a molecule.

[0137] In certain embodiments the size of a protein or polypeptide (wild-type or modified) may comprise, but is not limited to, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 255, 260, 265, or 266 amino acid residues or greater, and any range derivable therein, or derivative of a corresponding

amino sequence described or referenced herein. It is contemplated that polypeptides may be mutated by truncation, rendering them shorter than their corresponding wild-type form, also, they might be altered by fusing or conjugating a heterologous protein or polypeptide sequence with a particular function (e.g., for targeting or localization, for purification purposes, etc.). As used herein, the term "domain" refers to any distinct functional or structural unit of a protein or polypeptide, and generally refers to a sequence of amino acids with a structure or function recognizable by one skilled in the art.

[0138] The nucleotide as well as the protein, polypeptide, and peptide sequences for various genes have been previously disclosed, and may be found in the recognized computerized databases. Two commonly used databases are the National Center for Biotechnology Information's Genbank and GenPept databases (on the World Wide Web at ncbi.nlm.nih.gov/) and The Universal Protein Resource (UniProt; on the World Wide Web at uniprot.org). The coding regions for these genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

[0139] It is contemplated that in compositions of the disclosure, there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein).

[0140] The *Osr1* gene therapy agents of the disclosure may be administered by any suitable route of administration. In some embodiments, the *Osr1* gene therapy is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the antibiotic is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. The appropriate dosage may be determined based on the type of liver medical condition to be treated, severity and course of the condition, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

[0141] The *Osr1* gene therapy treatments may include various "unit doses." Unit dose is defined as containing a predetermined-quantity of the therapeutic composition. The quantity to be

administered, and the particular route and formulation, is within the skill of determination of those in the clinical arts. A unit dose need not be administered as a single injection but may comprise continuous infusion over a set period of time. In some embodiments, a unit dose comprises a single administrable dose.

[0142] In some embodiments, *Osr1* gene therapy comprises a nucleic acid encoding for the Osr1 protein, a vector comprising the nucleic acid encoding for the Osr1 protein, or a cell comprising the nucleic acid encoding for the Osr1 protein, or a vector comprising the nucleic acid encoding for the Ors1 protein. In some embodiments, a single dose of the Osr1 gene therapy is administered. In some embodiments, multiple doses of the Osr1 gene therapy are administered. In cases wherein multiple doses of *Osr1* gene therapy are administered to an individual, they may or may not be administered in the same route, and/or they may or may not be administered in the same dosage. In cases wherein multiple doses of *Osr1* gene therapy are administered to an individual, the duration of time between the administrations is within about or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or within 1-24 hours, 1-18 hours, 1-12 hours, 1-6 hours, 6-24 hours, 6-18 hours, 6-12 hours, 12-24 hours, 12-18 hours, or 18-24 hours. In cases wherein multiple doses of *Osr1* gene therapy are administered to an individual, the duration of time between the administrations is within about or exactly 1, 2, 3, 4, 5, 6, or 7 days, or within 1-7 days, 1-6 days, 1-5 days, 1-4 days, 1-3 days, 1-2 days, 2-7 days, 2-6 days, 2-5 days, 2-4 days, 2-3 days, 3-7 days, 3-6 days, 3-5 days, 3-4 days, 4-7 days, 4-6 days, 4-5 days, 5-7 days, 5-6 days, or 6-7 days. In cases wherein multiple doses of *Osr1* gene therapy are administered to an individual, the duration of time between the administrations is within about or exactly 1, 2, 3, or 4 weeks, or within 1-4 weeks, 1-3 weeks, 1-2 weeks, 2-4 weeks, 2-3 weeks, or 3-4 weeks. In cases wherein multiple doses of *Osr1* gene therapy are administered to an individual, the duration of time between the administrations is within about or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or within 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-12, 3-11, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-12, 7-11, 7-10, 7-9, 7-8, 8-12, 8-11, 8-10, 8-9, 9-12, 9-11, 9-10, 10-12, 10-11, or 11-12 months. In cases wherein multiple doses of *Osr1* gene therapy are administered to an individual, the duration of time between the administrations is within about or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more years, or within 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-

4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10 years.

[0143] In some embodiments, the gene therapy is administered at a dose of about or substantially exactly 1.0×10^{10} genomic copies/individual. The dose may be in a range between about 1.0×10^{7} to about 1.0×10^{10} genomic copies/individual and including any range derivable therein.

[0144] In some embodiments, the gene therapy is administered at a dose of between 1 mg/kg and 100 mg/kg. In some embodiments, the therapy is administered at a dose of at least, at most, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 mg/kg.

[0145] The quantity to be administered, both according to number of treatments and unit dose, depends on the treatment effect desired. An effective dose is understood to refer to an amount necessary to achieve a particular effect. In the practice in certain embodiments, it is contemplated that doses in the range from 10 mg/kg to 200 mg/kg can affect the protective capability of these agents. Thus, it is contemplated that doses include doses of about 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, and 200, 300, 400, 500, 1000 μg/kg, mg/kg, μg/day, or mg/day or any range derivable therein. Furthermore, such doses can be administered at multiple times during a day, and/or on multiple days, weeks, or months.

[0146] In certain embodiments, the effective dose of the pharmaceutical composition is about 1 μM to 150 μM. In another embodiment, the effective dose is about 4 μM to 100 μM; or about 1 μM to 100 μM; or about 1 μM to 50 μM; or about 1 μM to 40 μM; or about 1 μM to 30 μM; or about 1 μM to 20 μM; or about 1 μM to 10 μM; or about 10 μM to 150 μM; or about 10 μM to 100 μM; or about 10 μM to 50 μM; or about 25 μM to 150 μM; or about 25 μM to 100 μM; or about 25 μM to 50 μM; or about 50 μM to 150 μM; or about 50 μM to 100 μM (or any range derivable therein). In other embodiments, the dose being administered to a subject: about, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75,

76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 μM or any range derivable therein.

[0147] Precise amounts of the therapeutic composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the patient, the route of administration, the intended goal of treatment (alleviation of symptoms versus cure) and the potency, stability and toxicity of the particular therapeutic substance or other therapies a subject may be undergoing.

[0148] It will be understood by those skilled in the art and made aware that dosage units of μg/kg or mg/kg of body weight can be converted and expressed in comparable concentration units of μg/ml or mM, such as 4 μM to 100 μM. The applicable conversion factors and physiological assumptions to be made concerning uptake and concentration measurement are well-known and would permit those of skill in the art to convert one concentration measurement to another and make reasonable comparisons and conclusions regarding the doses, efficacies and results described herein.

[0149] In certain instances, it will be desirable to have multiple administrations of the composition, e.g., 2, 3, 4, 5, 6 or more administrations.

[0150] The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, including a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in immunogenic and therapeutic compositions is contemplated. Supplementary active ingredients, such as other anti-infective agents and vaccines, can also be incorporated into the compositions.

[0151] The active compounds can be formulated for direct administration to a liver. The active compound can be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, subcutaneous, or intraperitoneal routes. Typically, such compositions can be prepared as either liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[0152] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including, for example, aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0153] In embodiments where the therapy comprises Osr1 proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0154] A pharmaceutical composition can include a solvent or dispersion medium containing, 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 anti-bacterial and anti-fungal 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.

[0155] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization or an equivalent procedure. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of

the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0156] Administration of the compositions will typically be via any common route. This includes, but is not limited to oral, or intravenous administration. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, or intranasal administration. Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. Administration may be systemic or local.

[0157] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of solutions described above.

[0158] In particular embodiments, an individual having one or more symptoms of a liver condition is subject to testing to confirm if a liver condition is present. For example, an individual having one or more of the following symptoms may be suspected of having hepatocellular carcinoma: abdominal discomfort or distention (enlargement); weight loss; jaundice (yellowing of the skin and whites of the eyes); gastrointestinal hemorrhage (bleeding); nausea or vomiting; persistent itching; and/or fever. In another case, an individual having one or more of the following symptoms may be suspected of having NASH: intense itching; abdominal swelling; easy bruising and bleeding; jaundice (yellowing of the skin and eyes); spider-like blood vessels beneath the skin's surface; behavior changes, confusion, and slurred speech.

[0159] In any case where an individual has one or more symptoms of a liver condition, they may be subject to one or more tests to confirm whether a liver condition is present in the individual. Examples of testing include imaging tests, such as CT or MRI; liver biopsy; or blood tests, such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), albumin and total protein, bilirubin, gamma-glutamyltransferase (GGT), L-lactate dehydrogenase (LD), Prothrombin time (PT), or a combination thereof.

[0160] Upon diagnosis of a liver condition, such as hepatocellular carcinoma or NASH, an individual is provided a therapeutically effective amount of Osr1 therapy, including Osr1 gene therapy. In particular embodiments, the Osr1 therapy improves at least one symptom of the liver condition. In particular embodiments, the Osr1 therapy delays the onset of a liver condition. In

particular embodiments, the Osr1 therapy reduces the severity of one or more symptoms of a liver condition. In particular embodiments, the Osr1 therapy provides reduction in the severity, including to an undetectable level, in one or more symptoms of a liver condition. In particular embodiments, the Osr1 therapy results in complete remission of a liver condition, including in some embodiments complete remission faster than other treatments or no treatments.

[0161] The therapy encompassed herein may comprise administration of a Osr1 gene therapy agent (or Osr1 protein therapy) or a combination of therapeutic agents, such as a Osr1 gene therapy and a second therapy. The Osr1 gene therapy may be administered in any suitable manner known in the art. In embodiments where more than one therapy is provided, they may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, the gene therapy and a second treatment are administered in a separate composition. In some embodiments, the gene therapy and second treatment are in the same composition.

A. Other Agents

[0162] It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment. These additional agents include agents that treat liver conditions. The additional agent(s) may be tailored to the liver medical condition being treated. For example, an individual with hepatocellular carcinoma may also have had, is having, and/or will have surgery, radiation (internal or external), ablation with heat or cold, immunotherapy, and so forth.

B. Polypeptide Expression

[0163] In some aspects, there are nucleic acid molecule encoding polypeptides or peptides of the disclosure (*e.g*, the Osr1 gene). These may be generated by methods known in the art, e.g., isolated from B cells of mice that have been immunized and isolated, phage display, expressed in any suitable recombinant expression system and allowed to assemble to form antibody molecules or by recombinant methods.

1. Expression

The Osr1 nucleic acid molecules may be used to express large quantities of Osr1 polypeptides *in vivo* or *ex vivo*. In particular embodiments, expression of the Osr1 nucleic acid may be regulated by any suitable kind of promoter, such as constitutive, conditional, tissue-specific, inducible, *etc*. In specific embodiments, the expression is controlled by a promoter that is active in liver cells, and in some cases the promoter is active only in liver cells or is more active in liver cells than in non-liver cells. In some embodiments, there is utilized one or more enhancers and/or one or more promoter sequences of a hepatic gene. Examples include alpha1-antitrypsin promoter alone or linked to the albumin and/or hepatitis B enhancers.

2. Vectors

[0164] In some aspects, contemplated are expression vectors comprising a nucleic acid molecule encoding a Osr1 polypeptide of the desired sequence or a portion thereof (e.g., a fragment containing one or more domains thereof that are sufficient for improving at least one symptom of a liver medical condition). In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

[0165] To express the Osr1 polypeptides of the disclosure, DNAs encoding the polypeptides or peptides are inserted into expression vectors such that the gene area is operatively linked to transcriptional and translational control sequences. In specific embodiments, the vector comprises any suitable AAV vector. In some aspects, a vector that encodes a functionally complete Osr1 sequence with appropriate restriction sites engineered so that any sequences can be easily inserted and expressed. Typically, expression vectors used in any of the host cells contain sequences for plasmid or virus maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" typically include one or more of the following operatively linked nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Such sequences and methods of using the same are well known in the art.

[0166] In some embodiments, the vector is an Adenovirus (AdV); Lentivirus (LV); Adeno-associated virus (AAV); Retrovirus (RV); Herpes simplex virus (HSV); Vaccinia virus (VV); or Sendai virus (SeV). In specific embodiments, the virus is an AAV, such as AAV8, AAV9, AAV2, AAV3, AAV5, or AAV6.

3. Expression Systems

[0167] Numerous expression systems exist that comprise at least a part or all of the expression vectors discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with an embodiment to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Commercially and widely available systems include in but are not limited to bacterial, mammalian, yeast, and insect cell systems. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Those skilled in the art are able to express a vector to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide using an appropriate expression system.

C. Methods of Gene Transfer

[0168] Suitable methods for Osr1 nucleic acid delivery to effect expression of Osr1 gene product are anticipated to include virtually any method by which a nucleic acid (e.g., DNA, including viral and nonviral vectors) can be introduced into a cell, a tissue or an organism, as described herein or as would be known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of DNA such as by injection (U.S. Patents 5,994,624,5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection (Harland and Weintraub, 1985; U.S. Patent 5,789,215, incorporated herein by reference); by electroporation (U.S. Patent No. 5,384,253, incorporated herein by reference); by calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe et al., 1990); by using DEAE dextran followed by polyethylene glycol (Gopal, 1985); by direct sonic loading (Fechheimer et al., 1987); by liposome mediated transfection (Nicolau and Sene, 1982; Fraley et al., 1979; Nicolau et al., 1987; Wong et al., 1980; Kaneda et al., 1989; Kato et al., 1991); by microprojectile

bombardment (PCT Application Nos. WO 94/09699 and 95/06128; U.S. Patents 5,610,042; 5,322,783, 5,563,055, 5,550,318, 5,538,877 and 5,538,880, and each incorporated herein by reference); by agitation with silicon carbide fibers (Kaeppler et al., 1990; U.S. Patents 5,302,523 and 5,464,765, each incorporated herein by reference); by Agrobacterium mediated transformation (U.S. Patents 5,591,616 and 5,563,055, each incorporated herein by reference); or by PEG mediated transformation of protoplasts (Omirulleh et al., 1993; U.S. Patents 4,684,611 and 4,952,500, each incorporated herein by reference); by desiccation/inhibition mediated DNA uptake (Potrykus et al., 1985). Other methods include viral transduction, such as gene transfer by lentiviral or retroviral transduction.

[0169] In another aspect, contemplated are the use of host cells into which a recombinant Osr1 expression vector has been introduced. An expression construct encoding an Osr1 protein can be transfected into cells according to a variety of methods known in the art. Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. Some vectors may employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. One of skill in the art would understand the conditions under which to incubate host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

[0170] For stable transfection of mammalian cells, it is known, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods known in the arts.

VII. Subjects

[0171] The subject (or individual or patient) that is the recipient of methods and compositions of the disclosure may be any subject with a liver condition, including at least HCC and NASH. The subject (or individual or patient) that is the recipient of methods and compositions of the

disclosure may be any subject with insufficient levels of Osr1 for any reason. The subject may be any animal, including a human and non-human animal. Non-human animals include all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, although mammals are envisioned as subjects, such as non-human primates, sheep, dogs, cats, cows and horses. The subject may also be livestock such as, cattle, swine, sheep, poultry, and horses, or pets, such as dogs and cats.

[0172] Particular subjects include human subjects suffering from or at risk for the medical diseases and conditions described herein. The subject may be generally diagnosed with the condition of the disclosure by skilled artisans, such as a medical practitioner. In some cases, the methods of the disclosure include steps of determining the presence of insufficient Osr1 levels in a suitable sample from the subject, such as a liver sample, including a liver biopsy. In some cases, the methods of the disclosure include steps of determining the presence of a liver condition.

[0173] The methods of the disclosure described herein can be employed for subjects of any species, gender, sex, age, ethnic population, and/or genotype. Accordingly, the term subject includes males and females, and it includes elderly, elderly-to-adult transition age subjects, adults, adult-to-pre-adult transition age subjects, and pre-adults, including adolescents, children, and infants. Examples of human ethnic populations include Caucasians, Asians, Hispanics, Africans, African Americans, Native Americans, Semites, and Pacific Islanders.

[0174] The term subject also includes subjects of any genotype or phenotype as long as they are in need of the disclosed methods and compositions, as described above. In addition, the subject can have the genotype or phenotype for any hair color, eye color, skin color or any combination thereof. The term subject includes a subject of any body height, body weight, or any organ or body part size or shape.

VIII. Kits of the Disclosure

[0175] Any of the Osr1 viral and/or non-viral compositions described herein or similar thereto may be comprised in a kit. In a non-limiting example, one or more reagents for use in methods for preparing viral particles may be comprised in a kit. Such reagents may include cells, vectors, one or more growth factors, one or more costimulatory factors, media, enzymes, buffers, nucleotides, salts, primers, compounds, and so forth. The kit components are provided in suitable container means.

[0176] Some components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present disclosure also will typically include a means for containing the components in close confinement for commercial sale. Such containers may include injection or blow molded plastic containers into which the desired vials are retained.

[0177] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly useful. In some cases, the container means may itself be a syringe, pipette, and/or other such like apparatus, or may be a substrate with multiple compartments for a desired reaction.

[0178] Some components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means. The kits may also comprise a second container means for containing a sterile acceptable buffer and/or other diluent.

[0179] In specific embodiments, reagents and materials include primers for amplifying desired sequences, nucleotides, suitable buffers or buffer reagents, salt, and so forth, and in some cases the reagents include apparatus or reagents for isolation of a particular desired cell(s).

IX. Examples

[0180] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

GENERAL EMBODIMENTS

[0181] The present disclosure concerns the embodiment that Osr1 regulates cell OXPHOS in HCC via mitochondrial chaperone HSP60-mediated ATP5F1 degradation. The present disclosure will determine that Osr1 inhibits HCC tumor growth. The role of Osr1 in HCC cell metabolism will be determined. In specific embodiments, this will establish that rapid HCC tumor growth is caused by lower expression of Osr1 which reprograms the cell metabolism via utilizing OXPHOS as the major energy source. It will be determined how Osr1 regulates cell metabolism, therefore addressing that Osr1 assists mitochondrial chaperon of ATP synthase (ATP5F1α). Such embodiments for a noncanonical role of Osr1 offers novel perspectives on the biology of mitochondria in hepatocytes and contributes to a better understanding of the distinctive characteristics of hepatocytes and the development of HCC.

[0182] The present disclosure provides Osr1 as a therapeutic target for the treatment of hepatocellular carcinoma (HCC). Targeting Osr1 for the treatment of HCC is highly specific, efficient, and low in toxicity because of the unique feature of hepatocyte Osr1 in mitochondria.

EXAMPLE 2

THE ROLE OF OSR1 IN PREVENTING HCC

[0183] Previous studies have identified Osr1 as a tumor suppressor gene in various cancer types (26, 27), but its role in hepatocellular carcinoma (HCC) has not been explored. In HCC patients, there was a significant decrease in Osr1 expression in HCC tumor tissue compared to paratumor tissue in human patients (**FIG. 1A**). This decrease in Osr1 expression was replicated in an experimental mouse model of HCC (**FIG. 1B**). These findings indicate that OSR1 plays a role in HCC development and progression, in specific embodiments.

[0184] To investigate the role of Osr1 in HCC tumor growth, the inventor exposed *WT* or *Osr1*$^{+/-}$ mice to a combination of diethylnitrosamine (DEN) and the hepatotoxin carbon tetrachloride (CCl4) for 20 weeks (**FIG. 2A**). As global Osr1 deletion is fatal during embryonic development (Wang et al. Sci Rep. Aug 23 2019;9(1):12300; Coulter and Wieschaus Genes Dev. Dec 1988;2(12B):1812-23; Nusslein-Volhard and Wieschaus, Nature. Oct 30 1980;287(5785):795-801; Zhou et al. J Mol Cell Cardiol. 2015 2015;(85)), *Osr1*$^{+/-}$ mice were utilized for the study. Remarkably, *Osr1*$^{+/-}$ livers developed significantly more tumors, with larger

average sizes of visible tumors (diameter >2mm). Furthermore, these findings were replicated in an MAFLD/diabetes model, STAM™ (**FIG. 2B**) (Wallrabe et al., Sci Rep. Jan 8 2018;8(1):79). These results indicate that lower levels of Osr1 are associated with increased tumor growth, indicating that Osr1 inhibits HCC tumor growth.

[0185] There were similar phenotypes of increased tumor size and number in mice with hepatocyte-specific knockout of *Osr1* (*Osr1*$^{\Delta Hep}$ or *Osr1*$^{fl/fl}$; *Alb*$^{Cre/}$) compared to their littermate controls (*Osr1*^{F} or *Osr1*$^{fl/fl}$) (**FIG. 2C**). In addition, it was considered whether the deletion of *Osr1* in macrophages (*Osr1*$^{\Delta M\varphi}$ or *Osr1*$^{fl/fl}$; *LysM*$^{Cre/+}$) might promote tumor growth, as these mice presented exacerbated liver inflammation 30. However, *Osr1*$^{\Delta M\varphi}$ mice only developed marginally larger or more HCC tumors than the littermate control mice ($P<0.1$, data not shown). These results indicate that the role of macrophage Osr1 is not as critical as that of hepatocyte Osr1 in tumor growth.

[0186] FIG. 3 HCC was induced by DEN+CCl4 treatment for 20 weeks in Osr1F and Osr1ΔM (myeloid-specific osr1 deletion)mice (N=7-8).

[0187] Furthermore, these findings were replicated in a NAFLD/diabetes model, STAM™ (**FIG. 4A**). Similarly, STAM™ *Osr1*$^{+/-}$ mice developed more and larger HCC tumors Lower levels of Osr1 are associated with increased tumor growth, indicating that Osr1 inhibits HCC tumor growth. The inventor enhanced Osr1 expression in WT mice using AAV8L vectors (*AAV8L-Osr1*) one week before DEN and CCl4 exposure (**FIG. 4B**), as confirmed by IHC staining of Osr1 (**FIG. 4C**). Interestingly, 84% (6 out of 7) of control *WT* mice developed HCC, while only 55% (4 out of 7) of *AAV8L-Osr1* mice did. Moreover, overall tumor size was reduced by 600% and tumor number by 500% in mice with *Osr1* overexpression (**FIG. 4D**). As *Osr1* overexpression was induced before tumorigenesis and some *WT* mice still developed HCC, these findings indicate that additional Osr1 expression may inhibit tumor growth rather than blocking tumorigenesis; therefore, enhanced Osr1 having therapeutic potential in the treatment of HCC.

[0188] *Osr1* expression was negatively associated with cell proliferation. The observations indicated a significant increase in BrdU-labeled proliferating cells around the margins of the tumor and para-tumor tissue in *Osr1*+/- mice (**FIG. 5A**), associated with an overaction of JNK1 signaling (**FIG. 5B**), compared to those in their control mice. These results were consistent with a reduced number of BrdU-labeled HepG2 cells when *OSR1* expression was enhanced (**FIG. 5C**). However, there were no noticeable changes in cell survival, although it appeared that WT mice had only a

few apoptotic cells present at the margins of the tumor and para-tumor tissue (data not shown). These results indicate that the increased tumor growth observed in *Osr1*$^{+/-}$ mice is due to increased cell proliferation rather than changes in cell survival.

[0189] Adequate ATP levels are essential for fueling energy synthesis and nucleotide biosynthesis during the proliferation of cancer cells (Iguchi et al., Sci Rep. Dec 4 2020;10(1):21268; Gerresheim et al., Int J Mol Sci. Mar 15 2019;20(6)). Elegant studies have demonstrated a close relationship between elevated OXPHOS levels and malignant behavior of HCC (Birsoy et al., Cell. Jul 30 2015;162(3):540-51; Sullivan et al., Cell. Jul 30 2015;162(3):552-63; Sezai et al., Liver. Feb 1993;13(1):31-5.; Dubaquie et al., Proc Natl Acad Sci U S A. Aug 19 1997;94(17):9011-6). Using Fluorescence Lifetime Imaging Microscopy (FLIM) technology, the inventor confirmed enhanced OXPHOS in tumor cells in fresh liver tissue from mice with HCC (**FIG. 6**). FLIM operates by detecting NAD(P)H and FAD through their autofluorescence, providing metabolic information from live cells in a label-free, non-contact manner (Alard et al., PLoS One. Feb 7 2011;6(2):e14654). To investigate whether Osr1 plays a role in the metabolism of HCC tumor cells, FLIM was performed on fresh liver tissue collected from the *WT* or *Osr1KO (Osr1ΔHep)* mice with HCC. The FLIM technology detected increased NAD(P)H α1 and FAD α1, indicating elevated levels of the unbound form of NAD(P)H and the bound form of FAD in the *Osr1KO* tumor cells (**FIG. 6**). Importantly, deleting Osr1 also led to a more bound form of FAD in the para tumor cells. In addition, the *Osr1KO* tumor cells presented a higher redox ratio (**FIG. 6**), indicating a shift towards a more reduced cellular environment, consistent with heightened OXPHOS. These findings indicate that Osr1 plays a crucial role in regulating HCC tumor cell metabolism, specifically in OXPHOS, and further supports the potential of targeting Osr1 as a therapeutic strategy for HCC treatment.

[0190] To further characterize this, Osr1 was overexpressed in WT mice using AAV8L vectors (AAV8L-Osr1) one week before DEN and CCl4 exposure, as described 25 (**FIG. 7A**). The overexpression of Osr1 in hepatocytes was confirmed by immunohistochemistry staining of Osr1 (**FIG. 7B**). Interestingly, 84% (6 out of 7) of control WT mice developed HCC, while only 55% (4 out of 7) of WT mice injected with AAV8L-Osr1 did. Moreover, overall tumor size was reduced by 600% and tumor number by 500% in mice with Osr1 overexpression (**FIG. 7C**). As Osr1 overexpression was induced before tumorigenesis and some WT mice still developed HCC, these

findings indicate that additional Osr1 expression may inhibit tumor growth rather than blocking tumorigenesis.

[0191] To explore whether the changes in Osr1 expression influenced tumor growth through cell proliferation or apoptosis, we conducted further investigation. The observations indicated that there was a significant increase in BrdU-labeled proliferating cells around the margins of the tumor and paratumor tissue in $Osr1^{+/-}$ mice compared to those in WT mice (as shown in the upper panels of **FIG. 8**). However, we did not observe any noticeable changes in cell survival, although it appeared that WT mice had only a few apoptotic cells present at the margins of the tumor and paratumor tissue (as shown in the lower panels of **FIG. 8**).

[0192] Based on these results, it was considered that Osr1 inhibits HCC tumor growth through the repression of cell proliferation in HCC.

EXAMPLE 3

HCC CELL PROLIFERATION REQUIRES OSR1

[0193] In further characterize the role of Osr1 in HCC tumor growth, various *in vivo* and *in vitro* assays are employed.

[0194] *In vivo*, mice with different Osr1 genotypes, including *Osr1*$^{+/-}$, WT, $Osr1^{\Delta hep}$, or $Osr1^{F}$, that have developed HCC tumors are injected with BrdU to label cell proliferation prior to sacrifice. Liver tissues are collected and subjected to histopathological assays such as H&E staining, Sirius red staining, F4/80 staining, and TUNEL assay to evaluate tumor biology. Immunohistochemical (IHC) staining for BrdU, p-H3S10, or Ki-67 is performed to assess changes in cell proliferation. Furthermore, RT-PCR is conducted to evaluate the expression of genes involved in inflammation (*Tnfα, Il1β, Il6, Mcp-1, Inf-γ, etc)*, proliferation *(p53, p21, p27, Cyc-D1, Cyc-D2, Cyc-E, Cyc-A, Tgfβ, Pten, etc*), apoptosis (*Casp-3,7, 8,9, Bcl2, Bax, Fas, etc*) and migration (*E-cad, N-cad, Pai, Timp1, MMP2,9,13, etc*). Additionally, the activation of important signaling pathways such as PI3K/AKT/mTOR and RAS/RAF/MEK/ERK is assessed by western blots using extracted total protein from the liver tumor or peritumor tissue.

[0195] *In vitro*, Hepa1-6 cells with disrupted Osr1 expression or with Osr1 overexpression is evaluated using various methods including BrdU incorporation, real-time PCR for cell cycle/proliferation genes, and western blots to assess the activation of important proliferation-related signaling pathways such as MAPK and P53 signaling. These *in vitro* assays will provide further insights into the role of Osr1 in regulating cell proliferation in HCC.

[0196] In specific embodiments, disrupting Osr1 expression results in more cell proliferation both *in vivo* and *in vitro*. On the contrary, overexpressing *Osr1* inhibits cell proliferation.

EXAMPLE 4

OSR1 REPRESSES HCC TUMOR GROWTH

[0197] Tumor growth is assessed in three different models with Osr1 overexpression:

[0198] (1) Carcinogen-induced model: AAV8L-Osr1 or empty AAV8L (1.0×10^{10} gc/mouse) are injected into the WT mice exposed to DEN and CCl_4 treatment for week 16 (HCC phase). Mice are sacrificed at week 20 for tumor growth and tumor biology assessment as described elsewhere herein.

[0199] (2) NAFLD-induced model: AAV8L-Osr1 or empty AAV8L (1.0×10^{10} gc/mouse) are injected into the STAM™ mice at 16 weeks (HCC phase) and sacrificed at the age of 20 weeks for tumor growth and tumor biology assessment as described elsewhere herein.

[0200] (3) Xenografted liver cancer: With this model, two different sets of animal studies may be utilized to test the treatment efficiency of AAV8L-OSR1 on HCC at two different stages.

[0201] (a) H22 human HCC tumor cells will be infected by empty AAV8L (control) or AAV8L-OSR1 (treated) *in vitro*. Once the infection is validated, one million (in a volume of 100 μL) of the Matrigel/H22 cell suspension are injected subcutaneously into the hind leg of each athymic Nude mouse at the age of 10-12 weeks. Specifically, the control H22 cells are injected into the left leg, while treated H22 cells are injected into the right leg. Tumor size is assessed twice every week, by comparing the growth of the tumor on the right side of the leg to that of the left leg using standard statistical analysis. When tumors developed from the control cells reach the volume of $2000mm^3$, the mouse is sacrificed for tumor biology assessment as described elsewhere herein.

[0202] (b) One million (in a volume of 100 μL) of the Matrigel/H22 cell suspension is given to the Nude mice *via* orthotopic injection to establish the tumor. Once established, which usually took 2 weeks, empty AAV8L vectors (control) or AAV8L-Osr1(treated) are administered to the mice *via* intravenous injection. One week later, one can start to monitor tumor growth twice a week by MRI. The efficacy of the AAV8L- OSR1 is assessed by comparing the growth of the tumor in the treated mice to that of the control group using standard statistical analysis. At the end of the study, the tumors are harvested and analyzed histologically to determine the effect of the therapy on tumor morphology and biology as described elsewhere herein.

[0203] In specific embodiments, a decreased expression of Osr1 promotes tumor growth by increasing cell proliferation. Conversely, introducing Osr1 expression leads to the inhibition of cell proliferation, thus inhibiting the growth of HCC tumors in all three models.

EXAMPLE 5

ELUCIDATING THE ROLE OF OSR1 IN HCC CELL METABOLISM

[0204] Osr1 regulates oxidative phosphorylation (OXPHOS) in macrophages (25). To characterize whether Osr1 also plays a role in cell metabolism in liver cancer cells, its expression was examined in HepG2 cells, which have been shown to express low levels of Osr1 (data not shown), like the human and mouse HCC tumor cells (FIG. 1). To assess the effect of Osr1 overexpression on cell metabolism, overexpression of Osr1 was induced in HepG2 cells and compared to control HepG2 cells.

[0205] A working mechanism of Fluorescence lifetime imaging microscopy (FLIM) is to detect NAD(P)H and FAD by their autofluorescence, resolving metabolic information from live cells in a label-free and non-contact manner [36]. FLIM results revealed that Osr1 overexpression in HepG2 cells led to increased NAD(P)H α1, decreased FAD intensity, and decreased FLIRR (=NAD(P)H α2/FAD α1), suggesting inhibition of OXPHOS (**FIG. 9A**). UMAP visualizations of the FLIM data further suggested that Osr1 overexpression significantly reprogrammed the response of HepG2 cells to palmitic acid (PA) that stimulated fatty acid oxidation (FAO) and OXPHOS (**FIG. 9B**). This was confirmed by Seahorse-based assays, which revealed that *OSR1* overexpression decreased the mito-ATP production rate without affecting the glycol-ATP production rate, consequently leading to a reduced OXPHOS% (**FIG. 10A**), implying an inhibitory role of *OSR1* on OXPHOS. Furthermore, FLIM analysis was conducted on HepG2 cells overexpressing *OSR1* under basal conditions, glucose treatment, or palmitic acid (PA) treatment. Uniform Manifold Approximation and Projection (UMAP) visualizations of the FLIM data demonstrated distinct clusters between control and *OSR1*-overexpressed HepG2 cells under basal and PA treatment, whereas the clusters were mixed under glucose treatment (**FIG. 10B**). Given that glucose treatment promotes glycolysis and PA treatment stimulates mitochondrial OXPHOS, these results suggest the glycolysis did not depend on the *OSR1* level, but OXPHOS did.

EXAMPLE 6

DISRUPTING OSR1 EXPRESSION PROMOTES OXPHOS AND ATP PRODUCTION

[0206] *In vivo* studies are useful in cancer metabolism because of the complexity of the tumor microenvironment and the different levels of nutrients and oxygen *in vivo*.

[0207] *In vivo* studies are conducted using a FLIM (fluorescence lifetime imaging microscopy) technology to monitor the real-time metabolism of hepatocytes in live mice. Specifically, one can use *Osr1*$^{+/-}$, WT, *Osr1*$^{\Delta Hep}$, or *Osr1*^{F} mice with HCC induced by DEN plus CCl_4. FLIM images are captured from both tumor tissue and para-tumor tissue using a customized multiphoton fluorescence microscope. The procedure involves anesthetizing the mice with isoflurane, excising the skin above the liver, and implanting an optical window (mm size). FLIM images are acquired at 40X (1.1 NA) from six to ten laterally different locations of tumor and peritumor tissue, with z-depth slices obtained at intervals of 10 μm to 200 μm (20 total z-slices from 0-200 mm). Six mice from each group may be imaged. Autofluorescence metrics, including the redox ratio, short and long NAD(P)H lifetimes, the fraction of free and protein-bound NAD(P)H, short and long FAD lifetimes, and the fraction of free and protein-bound FAD, in both the cytosol and mitochondria, are quantified and compared among the groups. To evaluate the relative OXPHOS activity between *Osr1*$^{+/-}$ vs. WT or *Osr1*$^{\Delta Hep}$ vs. *Osr1*^{F} mice, one can calculate the mitochondrial protein-bound NAD(P)H intensity, mitochondrial protein-bound FAD intensity, and the FLIRR value, which is defined as the fraction of bound NAD(P)H (α2) divided by the fraction of bound FAD (α1).

[0208] Furthermore, ATP production may be measured using Seahorse-based assays on primary hepatocytes isolated from *Osr1*$^{+/-}$, WT, *Osr1*$^{\Delta Hep}$, or *Osr1*^{F} liver under different treatment conditions, including basal (fasting), glucose, or PA treatment, using the Agilent Seahorse XF Real-Time ATP Rate Assay Kit.

[0209] Additionally, to determine if changes in ATP production due to low levels of Osr1 are associated with alterations in protein expression of the ETC, an OXPHOS cocktail assay may be performed using mitochondria-extracted protein from isolated hepatocytes of *Osr1*$^{+/-}$ vs. *WT*, or *Osr1*$^{\Delta Hep}$ *vs. Osr1*^{F} mice. This assay measures the expression levels of Complex I subunit NDUFB8, Complex II subunit 30kDa, Complex III subunit Core 2, Complex IV subunit II, and ATP synthase subunit alpha (ATP5F1α).

[0210] In specific embodiments, FLIM images capture real-time metabolic alterations in hepatocytes with HCC, providing evidence for the role of Osr1 in mediating mitochondrial OXPHOS. The findings from both FLIM and Seahorse assays may further support this consideration, in specific embodiments. Additionally, the results of the OXPHOS cocktail assay sheds light on the relative levels of OXPHOS complexes in mouse mitochondria, identifying specific targets of Osr1 deficiency that impact OXPHOS, in certain embodiments.

EXAMPLE 7

OSR1 OVEREXPRESSION INDUCES METABOLIC REWIRING IN COMBATTING HCC

[0211] In an initial study, it was demonstrated that Osr1 overexpression effectively prevented the progression of carcinogen-induced HCC tumors in WT mice (**FIG. 7**). Briefly, WT mice at the age of 6 weeks are injected with AAV8L-Osr1 or empty AAV8L vectors (1.0×10^{10} gc/mouse). Three days later, mice were treated with DEN and CCl4 for 20 weeks. Building upon the initial data, one can characterize the metabolic alterations associated with Osr1-overexpressing using a well-established mouse model as described in **FIG. 7A**. An additional group of normal WT mice of the same age but without any treatment may be included as a reference (Ref group) to establish the baseline characteristics of metabolism. At week 20 of the experimental period, FLIM technology is employed to record real-time metabolic changes in hepatocytes in living mice, as described elsewhere herein, with data collected and analyzed accordingly.

[0212] Furthermore, one can measure ATP production adaptations resulting from Osr1 overexpression using Seahorse-based assays with primary hepatocytes. Two groups are established: **(1)** the Control group, comprising hepatocytes infected with empty AAV8L, and **(2)** the Osr1 group, composed of hepatocytes infected with AAV8L-Osr1.

[0213] One can compare FLIM and Seahorse assay results between the following groups: **(1)** Ref vs. Control groups to assess ATP/metabolic alterations associated with HCC tumorigenesis, **(2)** Control vs. Osr1 group to examine ATP/metabolic reconfiguration due to Osr1 overexpression, and **(3)** Osr1 vs. Ref group to determine the level of ATP/metabolic correction resulting from Osr1 overexpression.

[0214] In specific embodiments, in comparison to the Ref group, the FLIM, and Seahorse assay results reveal that the Control group exhibits increased glycolysis resulting from heightened glucose uptake, along with elevated OXPHOS and ATP production due to enhanced mitochondrial

biogenesis, as supported by previous research [39]. In certain embodiments, the Osr1 group demonstrate a recovery or partial recovery of OXPHOS and ATP production compared to the Control group, providing evidence that Osr1 overexpression induces metabolic reconfigurations that promote metabolic homeostasis and suppress HCC development.

EXAMPLE 8

THE ROLE OF OSR1 IN THE DEGRADATION OF ATP SYNTHASE BY MITOCHONDRIAL CHAPERONES

[0215] Molecular chaperones are essential for maintaining the balance of enzymatic proteins involved in mitochondrial energy production 40. One such chaperone, HSP60, works in conjunction with its co-chaperone HSP10 to interact with proteins in critical metabolic pathways like glycolysis, TCA cycle, and OXPHOS within the mitochondria 41. In cancer cells, inhibition of HSP60 disrupts the integrity of the mitochondrial respiratory chain and impairs OXPHOS 42-44. Research has also shown that mtHSP60 acts as a chaperone for ATP Synthase 16,45,46, and failure to fold the ATP Synthase β-Subunit can result in neurodegenerative conditions 16. These findings underscore the crucial role of mitochondrial chaperones in cellular metabolism and cancer development, although further investigation is needed for a comprehensive understanding.

[0216] Interestingly, while Osr1 is traditionally thought to be a transcription factor localized in the nucleus, the inventor revealed significant expression of Osr1 in the cytosol of human hepatocytes (**FIG. 1**). An initial study indicated a role for Osr1 in mitochondrial OXPHOS, which was supported by colocalization with MitoTracker, a fluorescent dye specifically targeting mitochondria (**FIG. 11A**). Furthermore, the findings were substantiated by the identification of Osr1 in isolated mitochondrial proteins which was exclusive to hepatocytes but not cardiomyocytes and macrophages (**FIG. 11B**), indicating a distinctive, non-traditional role of Osr1 in hepatocytes. Consistently, a similar pattern of specific mitochondrial localization of OSR1 was discerned in two widely used HCC cell lines, HepG2 and Huh7 (**FIG. 11C**). These findings highlight a unique, oncanonical role of Osr1 in hepatocytes and indicate its involvement in regulating mitochondrial function and metabolism in HCC cells.

[0217] To investigate the involvement of Osr1 in mitochondrial biogenesis, real-time PCR was performed to quantify mtDNA levels using the mitochondrial D-loop region relative to nuclear RNA. The results demonstrate that deleting *Osr1* did not affect mitochondrial biogenesis (**FIG.**

12), consistent with the observation that the mitochondrial mass was not affected in *Osr1ΔHep* hepatocytes, as measured by MitoTracker (data not shown). These findings indicate that Osr1 may not play a direct role in regulating mitochondrial biogenesis in HCC cells.

[0218] To further investigate the potential involvement of Osr1 in mitochondrial function, the inventor conducted a Mass Spec Pull-down Analysis (MSPA) using an anti-Osr1 antibody to capture its interactive proteins from primary hepatocytes and identified a specific set of 44 Osr1-binding proteins that were localized in the mitochondria. Among these proteins, HSP60, HSP70, HSP10, ATP5F1α, ATP5F1β, and malate dehydrogenase (MDH) scored among the top six when considering scores and coverage (**Table 1**), indicating a reliable interaction between Osr1 and these proteins. Notably, ATP5F1β and MDH are known targets of HSP60/10 chaperones based on previous studies 16,45-47.

Table 1. Selected Mitochondrial Osr1-binding proteins

Pathway	Osr1-binding Proteins	Score	Coverage
Chaperone	60 kDa heat shock protein (HSP60)	109.73	52.71
	Stress-70 protein (HSP70)	17.44	17.67
	10 kDa heat shock protein (HSP10)	4.24	31.37
OXPHOS	ATP synthase subunit beta (ATP5F1b)	20.13	16.64
	ATP synthase subunit alpha	30.66	30.74
	Electron transfer flavoprotein subunit	1.72	5.41
TCA cycle	Malate dehydrogenase	5.22	11.24
	Succinate-semialdehyde	1.62	3.55
	Isocitrate dehydrogenase	1.88	7.74
	Succinate dehydrogenase flavoprotein	1.95	1.66

[0219] The confirmed colocalization of Osr1, Atp5f1b, and Hsp60 in primary hepatocytes (**FIG. 13A**) strongly supports the presence of an interaction between Osr1 and the Hsp60/10 and Atp5f1b complex. To further investigate this interaction, co-immunoprecipitation (co-IP) studies were performed using anti-Osr1 or anti-Hsp60 antibodies with protein lysates extracted from normal liver tissue or isolated HCC tumor tissue (**FIG. 13B**). The findings indicated that in both normal and tumor tissues, there was an interaction between Atp5f1b and Hsp60, as well as between Atp5f1b and Osr1. However, precipitation of Osr1 (using anti-Hsp60) or Hsp60 (using anti-Osr1) only occurred in the normal tissue, not in the tumor tissue that expresses lower levels of Osr1 (**FIG. 13B**). This suggests that the interaction between Osr1 and Hsp60 may be indirect and

possibly mediated through their binding with Atp5f1β. Together the findings suggest that Osr1 plays a role in regulating chaperone activity targeting Atp5f1β.

[0220] The findings in **FIG. 14** provide compelling evidence of the intricate relationship between Osr1 and Atp5f1β expression. Western blot analyses demonstrated that Atp5f1β expression negatively depended on the Osr1 levels, in tumor tissue of *Osr1+/- vs. WT* (**FIG. 14A**), in hepatocytes of *Osr1ΔHep* vs. *Osr1F* (**FIG. 14B**), or HepG2 cells with *OSR1* overexpression (**FIG. 14C**). In contrast, HSP60 expression remained unchanged under all conditions examined. Because the mRNA level of Atp5f1β was independent of *Osr1* expression (*Osr1+/- vs. WT:* Fold change: 0.93±0.12, P=0.8701), these findings indicate that Osr1 may play a critical role in regulating Atp5f1β turnover and ATP production in HCC cell metabolism. To determine if OSR1 facilitates ATP5F1β degradation, ATP5F1β ubiquitination was detected in HepG2 cells treated with scramble siRNA or *OSR1 siRNA* following a standard ubiquitination assay protocol 48. The results showed a reduced ubiquitination of ATP5F1β in the HepG2 cells with *OSR1* downregulation (**FIG. 14D**). These findings indicate that OSR1 may play a role in regulating ATP5F1β protein levels by facilitating its degradation, eventually contributing to the cell metabolism.

[0221] Chaperone proteins play two important roles in mitochondria: assisting in the folding of newly synthesized proteins and correcting the misfolding of proteins, as well as directing the elimination of mitochondrial client proteins towards cellular proteolytic machinery for degradation 40. Because Osr1 is a tumor suppressor gene 26,27, it is unlikely that it inhibits the process of correcting protein misfolding. Furthermore, the initial data shows a negative correlation between the Osr1 level and Atp5f1b. Based on this, it was considered that Osr1 interacts with ATP5f1b to facilitate its mitochondrial chaperone-mediated degradation. The data indicate that Osr1 is critical in regulating the chaperone function of Hsp60/10, and its interaction with ATP5f1b may be a crucial mechanism through which Osr1 exerts its non-canonical function in hepatocyte metabolism.

EXAMPLE 9

DETERMINING THE INTERACTIONS BETWEEN OSR1 AND ATP5F1B

[0222] To assess the interactions between Osr1 and Atp5f1b, mitochondrial protein extracts obtained from primary hepatocytes isolated from $Osr1^{\Delta Hep}$ or $Osr1^{F}$ mice are used for Co-IP

experiments. If Osr1 is detected in the Atp5f1b co-immunoprecipitants (IP) in western blot assay, then the results would indicate their interaction *in vivo*. In parallel, one could also perform quantitative proteomics analysis of Osr1 immunoprecipitants in wild-type and mutant backgrounds. Significant enrichment of peptides in the IP would indicate the two proteins are in the same complex. Additionally, this approach aids in the identification of the chaperone complexes by identifying their shared interactive proteins. Established protocols for this purpose may be utilized 48-51.

[0223] The Yeast Two-Hybrid assay may be carried out using a commercially available kit following the manufacturer's instructions (Clontech, CA). Briefly, GAL4 DNA-BD/Osr1 will be used as the bait, and Gal4-AD/Atp5f1b is used as the prey to test the direct interaction between Osr1 and Atp5f1b. Additionally, the GAL4 DNA-binding domain (DNA-BD)/Osr1 may be used as the bait, and the Gal4 activation domain (AD)/Hsp60 may be used as the prey to test the interaction between Osr1 and Hsp60. Furthermore, additional setups may be prepared by interchanging the bait and prey to thoroughly investigate the protein interactions and obtain comprehensive insights into the molecular interplay between Osr1 and Hsp60 or Apt5f1b.

[0224] A next step is to identify the interaction interface between Osr1 and Apt5f1b, and then identify the critical residues in the two proteins that are specifically involved in this interaction. Similar to an approach in dissecting the SWI2/SNF2 ATPase-Serrate interaction 48, one can generate multiple truncated mutants of Osr1 and Apt5f1b and use the Y2H assay to map their interaction interface to a smaller domain or region. One can then perform alanine-scanning mutagenesis on the short regions of the interaction interface between the two proteins and conduct further Y2H assays to precisely determine the residues that are directly involved in their interaction.

[0225] Additionally, AlphaFold 2 multimer may be employed, which is a deep learning model that accurately predicts the three-dimensional structure of proteins from their amino acid sequences. Using this approach, one can predict the 3D structures of the Osr1-Apt5f1b complex and evaluate the docking position using molecular docking software, such as RosettaDock. Using this approach, one can model the interaction between the two proteins by predicting their possible binding orientations and calculating the binding energy for each orientation. The orientation with the lowest binding energy is generally regarded as the most stable and likely to occur in real-life scenarios. A few residues may be selected for Y2H assays to validate their interaction.

[0226] Through Y2H and AlphaFold2 techniques, one can have identified the precise region of Osr1 that interacts with Atp5f1b and pinpoint the specific point mutation (Osr1$^{\Delta}$) that disrupts this interaction. The plasmid embedding Osr1$^{\Delta}$ may be generated using Phusion Site-Directed Mutagenesis Kit (ThermoFisher). One can use these variants to further perform functional analysis to examine whether the interaction between Osr1 and Atp5f1b is critical to regulating the chaperone degradation of Atp5f1b, which further regulates the cell metabolism of the HCC tumor.

[0227] HepG2 cells with a low expression level of Osr1 may undergo transfection with plasmids containing either mock, wildtype Osr1 or Osr1$^{\Delta}$ variants. After 24-hour culture, total protein is extracted for a co-immunoprecipitation (co-IP) study. One purpose of this study is to evaluate whether the interaction between Osr1, Atp5f1b, and Hsp60 persists in the Osr1$^{\Delta}$ group. Furthermore, a seahorse-based experiment, as described elsewhere herein, may be conducted to determine whether disrupting the interaction between Osr1 and Atp5f1b has an impact on cellular oxidative phosphorylation (OXPHOS) and ATP production.

[0228] In specific embodiments, the interaction of Osr1 and Atp5f1b is verified by utilizing co-IP techniques and Y2H assay. Using a truncated Y2H assay, in specific embodiments one can identify the interaction region that contains a maximum of five amino acids. This identification may be further confirmed by utilizing AlphaFold2 to simulate protein-protein interactions between Osr1 and Atp5f1b, molecular docking to predict the interaction, and molecular dynamics simulations to investigate the complex's stability and dynamics. In certain embodiments, the Osr1$^{\Delta}$ group exhibits a loss of interaction between Osr1 and Atp5f1, while the interaction between Atp5f1 and Hsp60 remains intact. In contrast to the Osr1 group, which experiences inhibited OXPHOS and ATP production, the Osr1Δ group in specific embodiments undergoes minimal changes in cell metabolism and ATP production. However, in some embodiments, Osr1$^{\Delta}$ still exerts some repression on OXPHOS or ATP production, although to a lesser extent compared to the Osr1 group. In certain embodiments, this indicates the presence of other targets for Osr1 in cellular metabolism that are unrelated to the Osr1$^{\Delta}$ site.

EXAMPLE 10

OSR1 ASSISTS THE CHAPERONE ACTIVITY TARGETING ATP5F1B DEGRADATION.

[0229] In specific embodiments, the studies described in this Example are conducted under the following examples of conditions:

[0230] (a) Isolated normal and HCC tumor cells are compared to determine if the obtained results correlate with Osr1 expression levels.

[0231] (b) $Osr1^{\Delta Hep}$ and Osr1F hepatocytes are compared to elucidate the causative effects of Osr1 deletion in hepatocytes.

[0232] (c) HepG2 cells are transfected with Osr1, $Osr1^{\Delta}$, or subjected to mock transfection to explore the effect of disrupting the interaction between Osr1 and Atp5f1b.

[0233] (d) $Osr1^{\Delta Hep}$ hepatocytes are infected with AAV8L-Osr1 or empty AAV8L to characterize the normalizing effects.

[0234] (e) $Osr1^{\Delta Hep}$ hepatocytes are infected with AAV8L-Atp5f1b-siRNA or empty AAV8L to determine the rescue effect of Atp5f1b downregulation under Osr1 deletion.

[0235] (f) $Osr1^{\Delta Hep}$ hepatocytes are infected with AAV8L-Hsp60-siRNA or empty AAV8L to characterize the rescue effect of Hsp60 downregulation under Osr1 deletion.

[0236] Study 1: To characterize if Osr1 facilitates Atp5f1b degradation chaperoned by Hsp60/10, Atp5f1b ubiquitination is detected in cultured cells following a standard ubiquitination assay protocol [52]. Ubiquitination, detected by immunoprecipitation of the anti-Atp5f1b, followed by anti-ubiquitin immunoblotting, is quite specific. Briefly, cells are cultured for 24 hours with translation inhibitor, cycloheximide. Cells are collected in a lysis buffer and sheared by sonication. Next, immunoprecipitation is performed using either Protein A- or G- conjugated antibodies against anti- Atp5f1b. The cell lysate-bead mixture is subjected to western blot analysis using anti-ubiquitin, anti-Osr1, and anti-Atp5f1b, to determine the level of degradation.

[0237] Study 2: Atp5f1b is a key component of the mitochondrial ATP synthase, serving as the β subunit of the proton channel. It plays a critical role in connecting the F1 and F0 complexes by forming a peripheral stalk, which acts as a stator to prevent specific subunits from rotating with the central rotary element [53]. This role of Atp5f1b contributes to the structural integrity and functional regulation of the ATP synthase complex, facilitating the efficient synthesis of ATP in

the mitochondria. Hence, in specific embodiments, changes in mitochondrial ATP production and mitochondrial membrane serve as markers of ATP synthase activity.

[0238] (a) ATP synthesis: One can investigate whether the observed overexpression of ATP5F1b is associated with increased ATP production in mitochondria. Elsewhere herein is description of measuring the ATP production rate in cultured liver cancer cells and primary hepatocytes, dependent on the level of Osr1, serving to address this question. Furthermore, one can assess if the upregulation of Atp5f1b, resulting from Osr1 downregulation, leads to the activation of ATP synthase using a commercially available Mitochondrial ATP synthase Activity Assay Kit (BioVision, Waltham, WA). This assay involves the hydrolysis of ATP to ADP by ATP synthase, followed by oxidation of NADH to NAD+ in the presence of ADP, ATP synthase converter, and enzyme mix, which can be monitored by a change in absorbance at 340 nm.

[0239] (b) Mitochondrial membrane potential: ATP synthesis is tightly linked to the mitochondrial membrane potential, the electrical potential difference across the inner membrane. Increased ATP production may cause an imbalance in the mitochondrial membrane potential, which may be assessed using a commercially available JC-1-Mitochondrial Membrane Potential Assay Kit (Abcam, Boston, MA).

[0240] In specific embodiments, restoring Osr1 expression normalizes the reduced degradation of Atp5f1b due to Osr1 deletion. However, in certain embodiments $Osr1^{\Delta}$ should not normalize Atp5f1b degradation due to disrupted interaction between them. A lower level of Osr1 would lead to reduced degradation of Atp5f1b, which can be restored by the downregulation of Atp5f1b. However, in at least some cases downregulation of Hsp60 recovers Atp5f1b degradation in $Osr1^{\Delta Hep}$ hepatocytes, as the chaperone activity of Hsp60/10 targeting Atp5f1b degradation requires assistance from Osr1. Similarly, consistent results in the ATP synthase activity assay and membrane potential assays align with the altered Atp5f1b degradation in each specific condition. These findings supports the embodiment that Osr1 regulates mitochondrial ATP production through its involvement in the chaperone activity targeting Atp5f1b degradation.

EXAMPLE 11

THERAPEUTIC USE TARGETING ATP5F1B CHAPERONE IN HCC TREATMENT

[0241] An objective of this Example is to characterize the role of Atp5f1b chaperone in HCC tumor growth using two different models: the carcinogen-induced and the NAFLD-induced HCC model.

[0242] To achieve this, *AAV8L-Atp5f1b* siRNA, *AAV8L- Hspd1* siRNA or empty AAV8L vectors are injected into WT mice exposed to DEN and CCl_4 treatment for 16 weeks (early HCC phase) or STAM™ mice at 16 weeks (HCC phase) in the respective models. The tumor growth is monitored as described elsewhere herein. (*Hspd1* gene encodes Hsp60 in mice.)

[0243] Furthermore, the study will also characterize if disrupting Atp5f1b chaperone inhibits rapid tumor growth due to Osr1 disruption in Osr1 transgenic mice ($Osr1^{+/-}$ or $Osr1^{\Delta Hep}$). Similar procedures are followed as in the carcinogen or NAFLD-induced models, and tumor growth is monitored as described elsewhere herein. The results provide insight into the therapeutic targets for HCC treatment.

[0244] In specific embodiments, interfering with the Atp5f1b chaperone through *AAV8L-Atp5f1b* or *AAV8L-Hspd1* siRNA injection result in reduced tumor growth compared to control mice injected with empty AAV8L vectors, supporting the embodiment that disrupting the Atp5f1b chaperone is a therapeutic approach for HCC. These findings also demonstrate the functional role of Osr1 in mediating the chaperone activity of Hsp60 to degrade Atp5f1b *in vivo*, in specific embodiments.

EXAMPLE 12

TREATMENT OF LIVER MEDICAL CONDITIONS

[0245] In particular embodiments, liver medical conditions are treated by increasing expression of Osr1. In **FIG. 15,** Osr1 overexpression reduced tumor size of HCC tumors. In **FIG. 16,** Osr1 overexpression treated HCC. Ultrasound images of healthy mouse liver and mouse liver with HCC. Detected on week 19, treatment for 5 weeks. The healthy liver tissue appeared uniform and consistent in texture, with clear vascular structures. In contrast, the liver tissue with HCC, treated with AAV-GFP, showed a heterogeneous echotexture, suggesting the development of cirrhosis. Importantly, multiple focal lesions (arrows) appeared hyperechoic (brighter), indicating tumor formation. The HCC liver treated with AAV-Osr1 showed an improvement with no visible tumor observed and a more uniform texture. **FIG. 17** shows that Osr1 treatment-induced human HCC tumor organoid death *in vitro*.

* * *

[0246] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of

this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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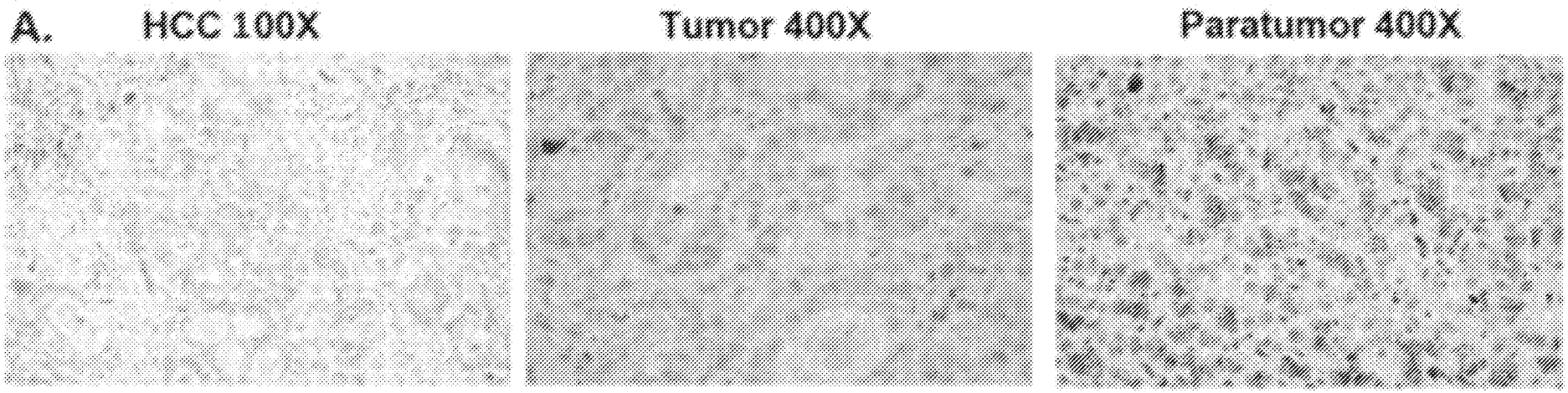
WHAT IS CLAIMED IS:

1. A composition comprising an *Osr1* polynucleotide, or a functional fragment thereof, comprised in an adeno-associated viral vector (AAV).
2. The composition of claim 1, wherein the AAV is comprised in a pharmaceutically acceptable carrier.
3. The composition of claim 1 or 2, wherein the AAV is an AAV type 1 particle, AAV type 2 particle, AAV type 3 particle, AAV type 5 particle, AAV type 6 particle, AAV type 8 particle, or AAV type 9 particle.
4. The composition of any one of claims 1-3, wherein the AAV is AAV8L.
5. The composition of any one of claims 1-4, wherein the *Osr1* polynucleotide encodes an Osr1 polypeptide.
6. The composition of claim 5, wherein the Osr1 polypeptide comprises an amino acid sequence having at least 90% sequence identity with SEQ ID NO:2.
7. The composition of claim 5, wherein the Osr1 polypeptide comprises an amino acid sequence having the amino acid sequence of SEQ ID NO:2.
8. The composition of any one of claims 1-7, wherein the Osr1 polynucleotide lacks an intron.
9. The composition of any one of claims 1-8, wherein the Osr1 polynucleotide is operably linked to one or more regulatory sequences.
10. The composition of claim 9, wherein the one or more regulatory sequences are active in liver cells.
11. The composition of any one of claims 1-10, wherein the *Osr1* polynucleotide comprises nucleic acid sequence that is at least 90% identical to SEQ ID NO:1.
12. The composition of any one of claims 1-10, wherein the *Osr1* polynucleotide comprises SEQ ID NO:1.
13. A composition comprising an AAV particle comprising an *Osr1* polynucleotide or a functional fragment thereof.
14. The composition of claim 13, wherein the AAV is an AAV type 1 particle, AAV type 5 particle, AAV type 8 particle, or AAV type 9 particle.
15. The composition of claim 13 or 14, wherein the Osr1 polynucleotide comprises nucleic acid sequence that is at least 90% identical to SEQ ID NO:1.

16. The composition of claim 13 or 14, wherein the Osr1 polynucleotide comprises nucleic acid sequence that is SEQ ID NO:1.
17. The composition of any one of claims 13-16, wherein the *Osr1* polynucleotide encodes an Osr1 polypeptide comprising amino acid sequence that is at least 90% to SEQ ID NO:2.
18. The composition of any one of claims 13-16, wherein the *Osr1* polynucleotide encodes an Osr1 polypeptide comprising amino acid sequence that is SEQ ID NO:2.
19. A method of treating or preventing a liver medical condition in an individual, comprising the step of delivering to the individual a therapeutically effective amount of the composition of any one of claims 1-18.
20. The method of claim 19, wherein the liver medical condition is nonalcoholic fatty liver disease or hepatocellular carcinoma.
21. The method of claim 19 or 20, wherein the *Osr1* polynucleotide is comprised in a vector.
22. The method of claim 21, wherein the vector is an AAV particle.
23. The method of claim 22, wherein the AAV particle is an AAV type 1 particle, AAV type 5 particle, AAV type 8 particle, or AAV type 9 particle.
24. The method of any one of claims 19-23, wherein the individual is at risk for nonalcoholic fatty liver disease or hepatocellular carcinoma.
25. The method of any one of claims 19-24, wherein the individual is an infant, child, or adolescent.
26. The method of any one of claims 19-24, wherein the individual is an adult.
27. The method of any one of claims 19-26, wherein the composition is delivered directly to the liver.
28. The method of any one of claims 19-26, wherein the composition is delivered systemically.
29. A kit comprising the composition of any one of claims 1-18, said composition housed in a suitable container.

FIG. 1A



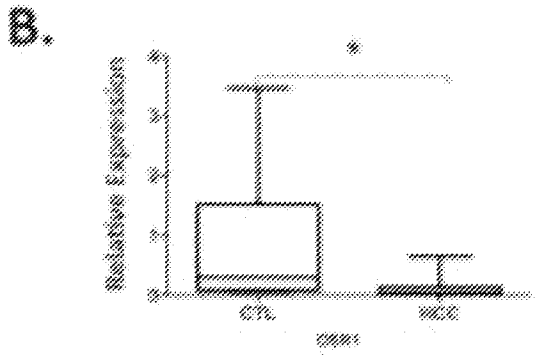


FIG. 1B

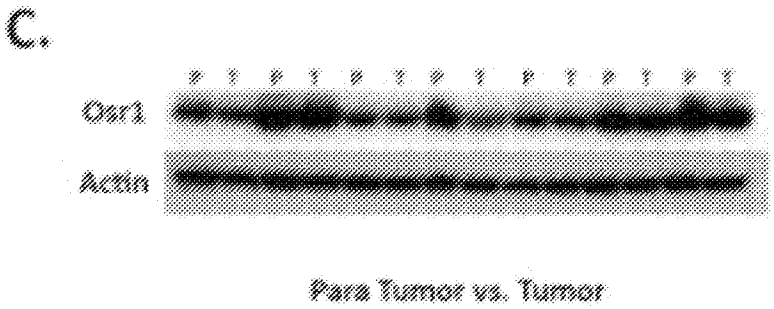
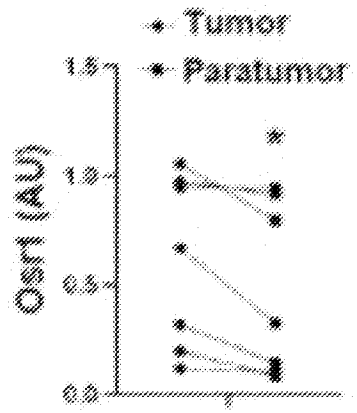


FIG. 1C



FIGS. 1A, 1B and 1C

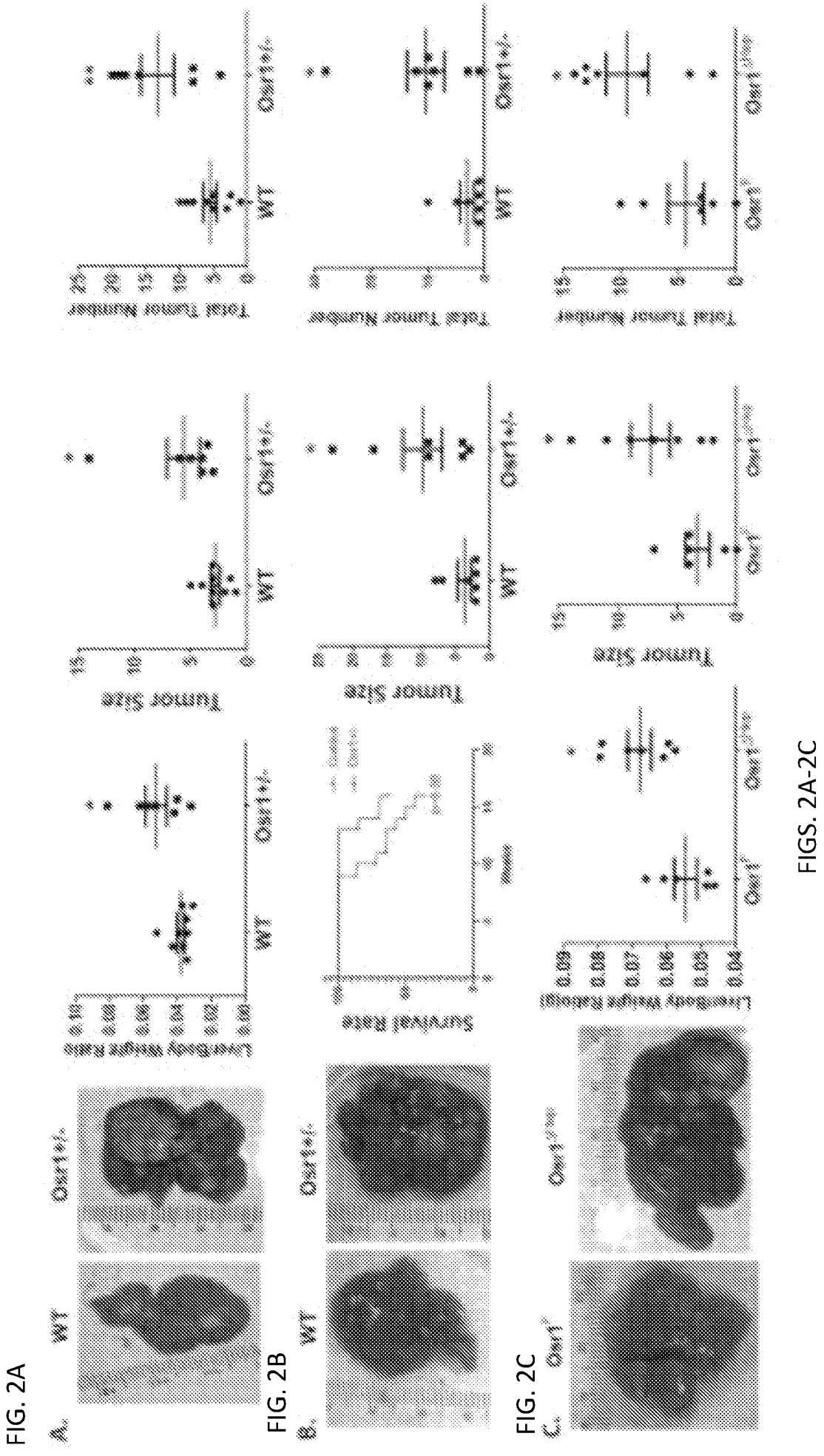


FIG. 2A

FIG. 2B

FIG. 2C

FIGS. 2A-2C

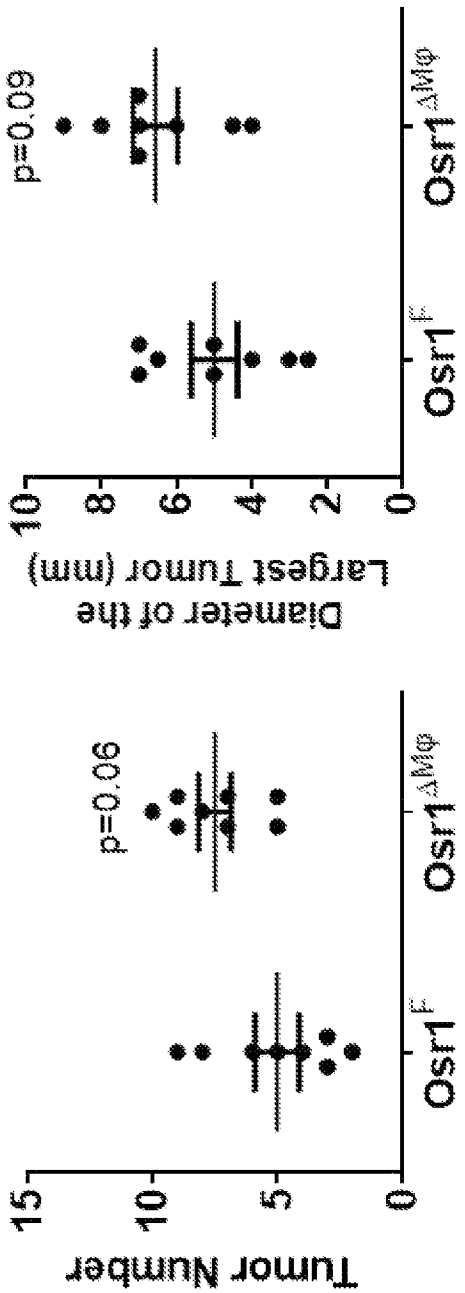

Tumor Number
15
10
5
0
p=0.06
Osr1F
Osr1ΔMφ
Diameter of the Largest Tumor (mm)
10
8
6
4
2
0
p=0.09
Osr1F
Osr1ΔMφ

FIG. 3

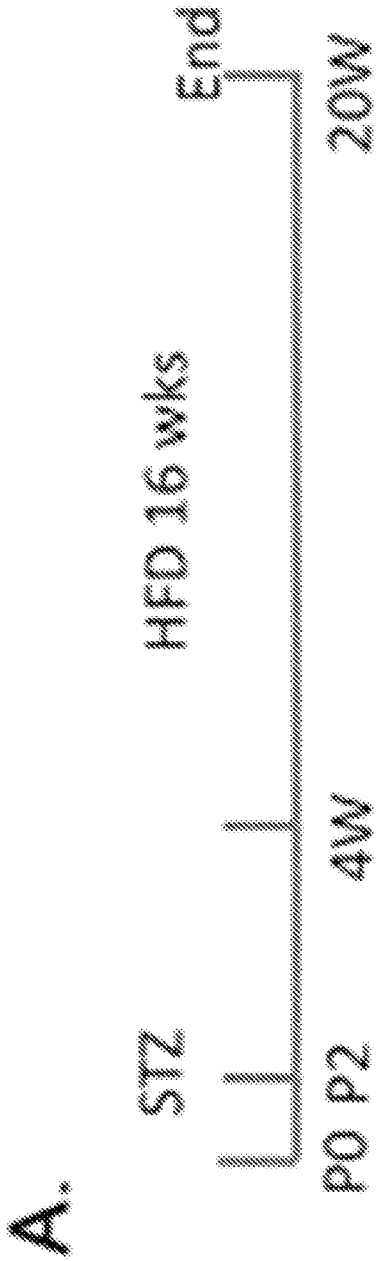


FIG. 4A

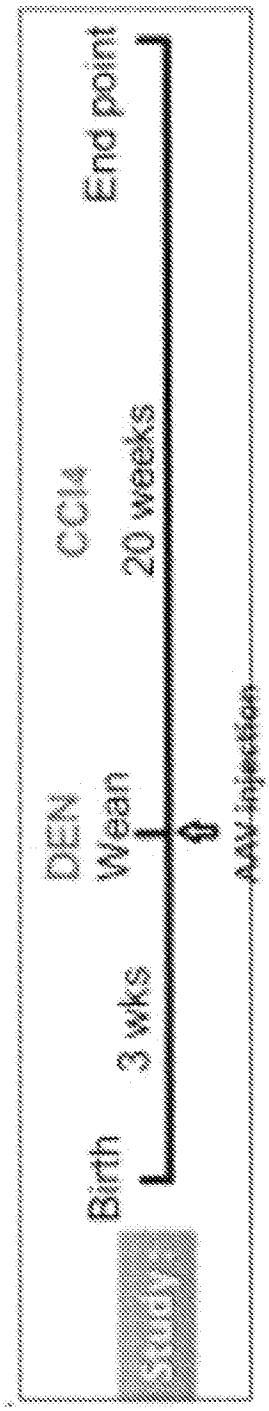


FIG.4B

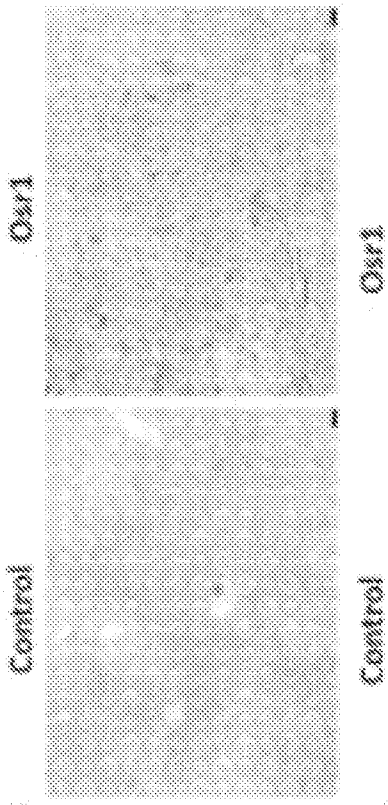


FIG.4C

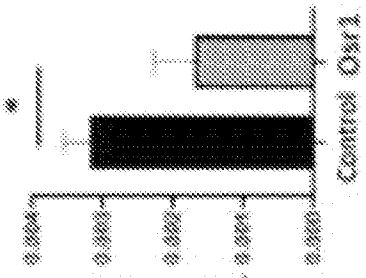


FIG. 4E

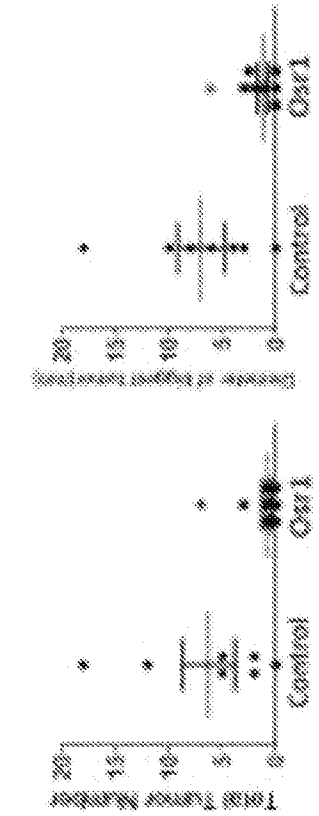


FIG.4D

FIGS. 4B-4E

FIG. 5A.

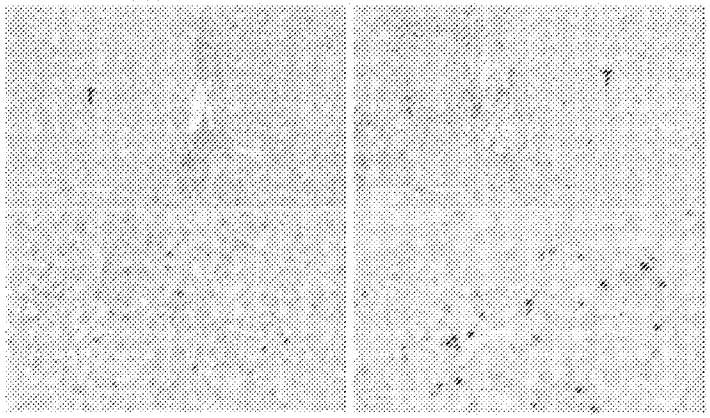


FIG. 5B. Tumor

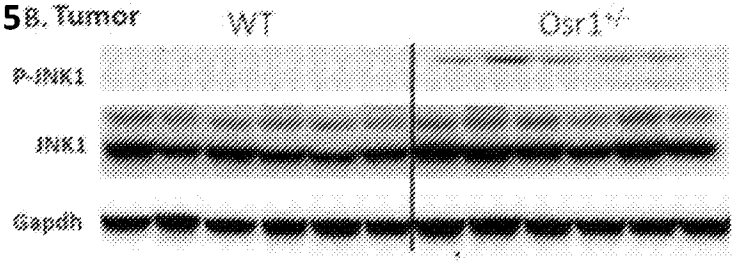
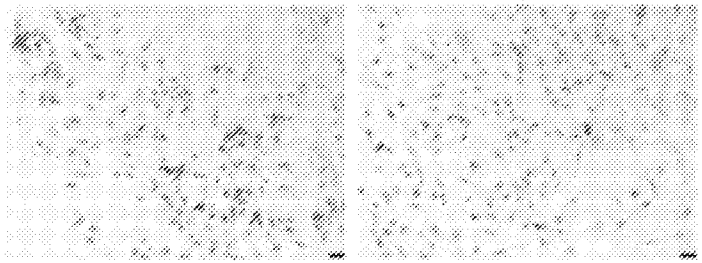


FIG. 5C.



FIGS. 5A-5C

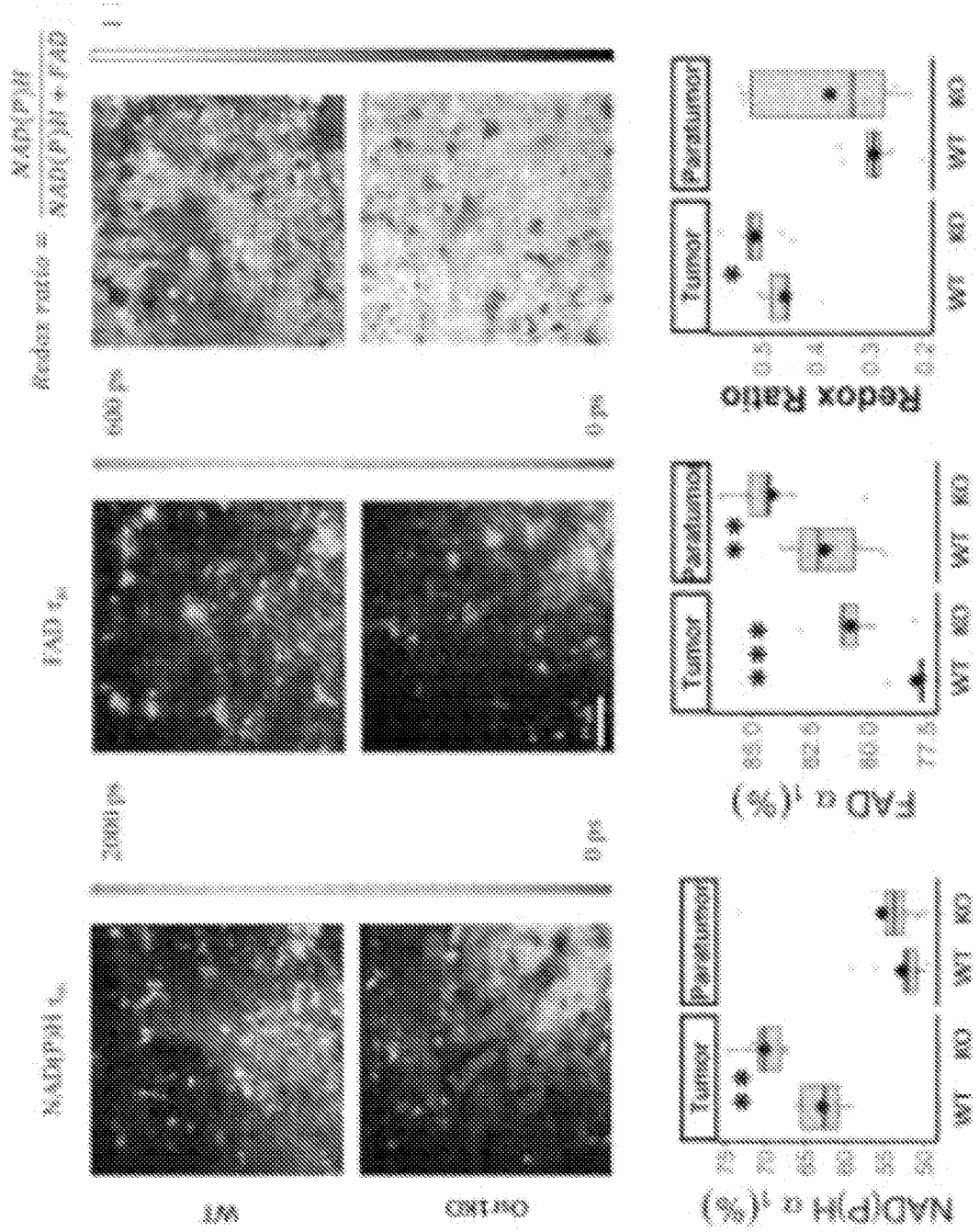

WT
OxTKO
NAD(P)H τ1
FAD τ1
Redox ratio = NAD(P)H / (NAD(P)H + FAD)
200 μm
50 μm
Tumor
Peritumor
WT
KO
NAD(P)H α1 (%)
FAD α1 (%)
Redox Ratio

FIG. 6

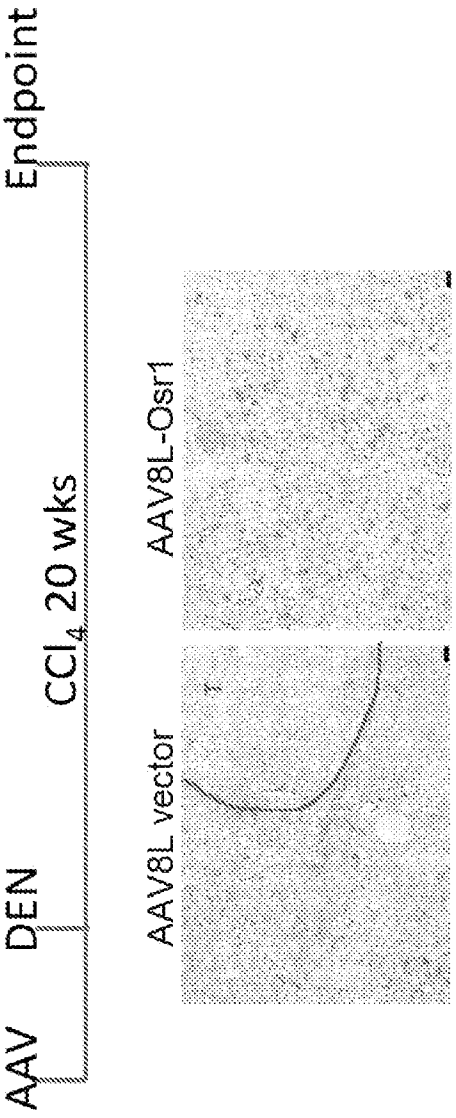


FIG. 7A.

FIG. 7 B.

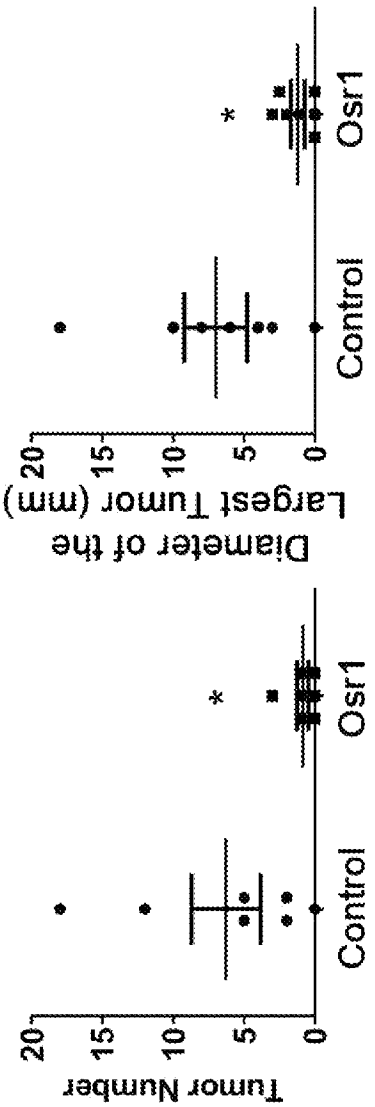


FIG. 7C.

FIGS. 7A-7C

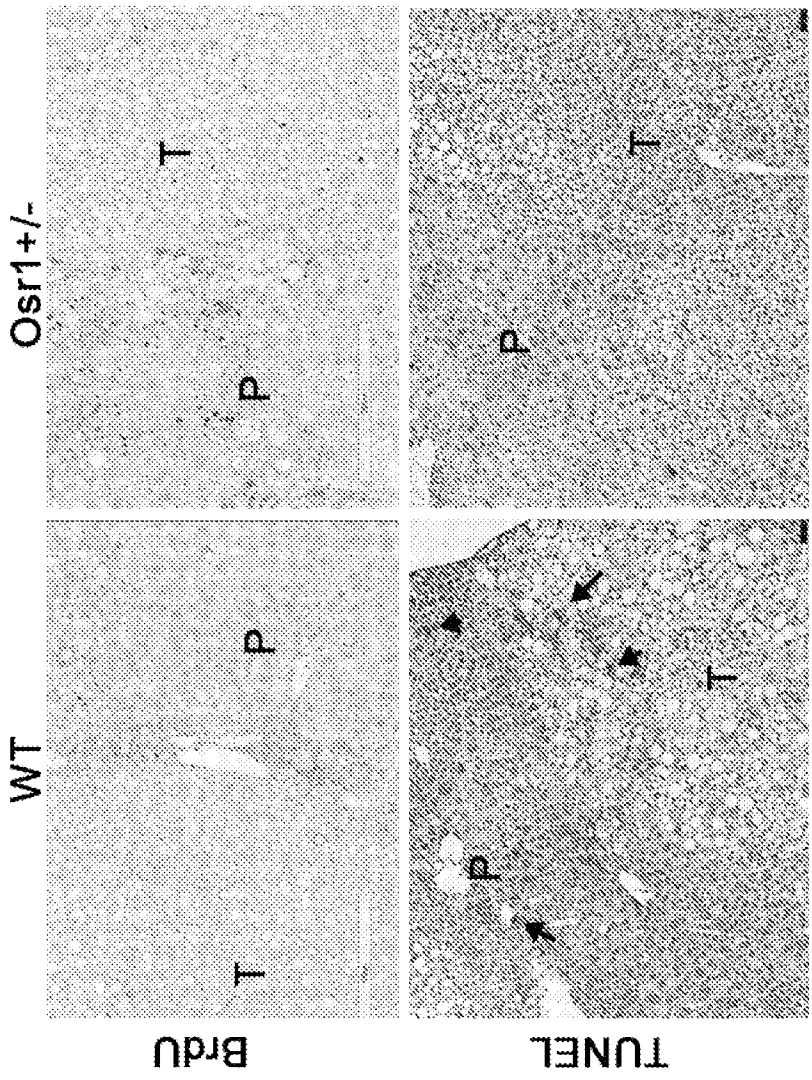


FIG. 8

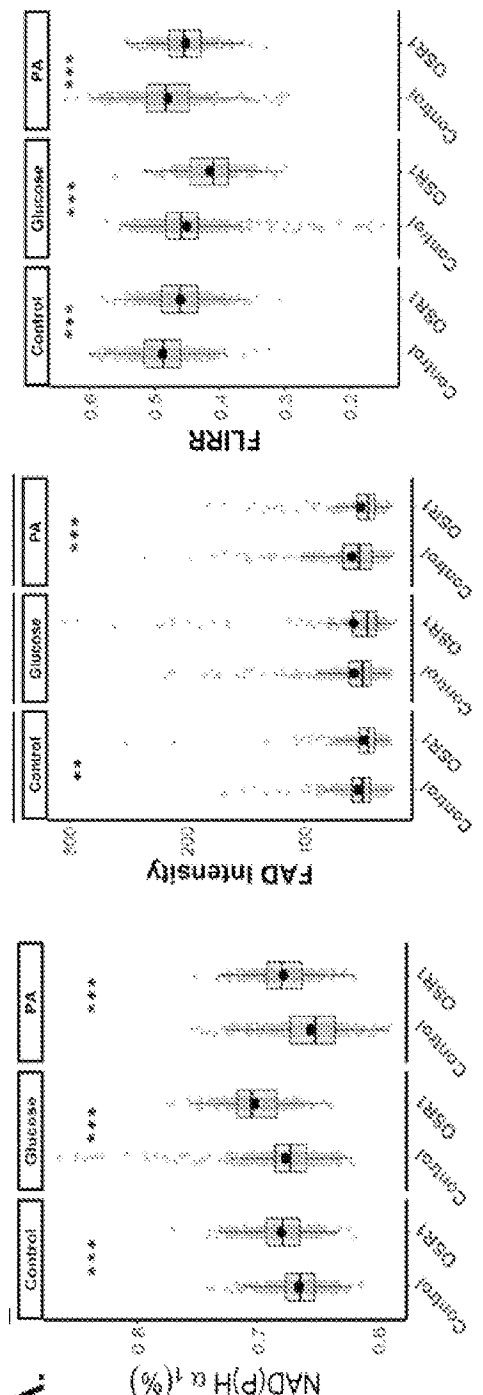

A.
NAD(P)H α1(%)
FAD intensity
FLIRR
Control
Glucose
PA
OSR1
Control
B.
Basal
Glucose
PA
Control
OSR1
UMAP Dimension 1
UMAP Dimension 2

FIG. 9A-9B

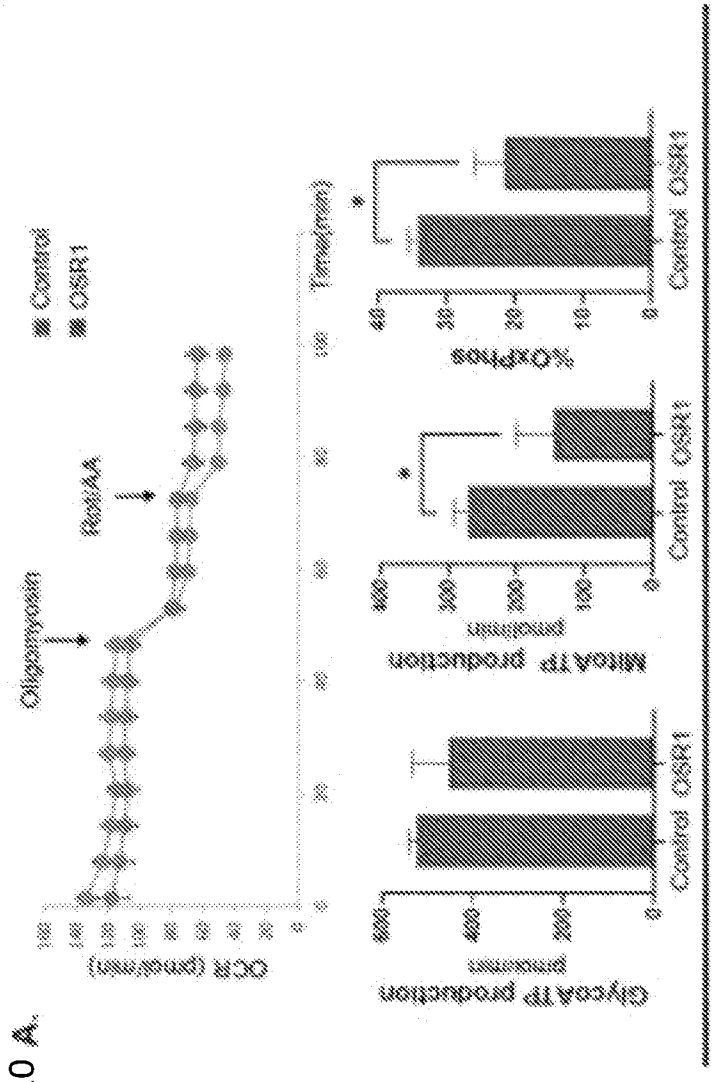


FIG. 10 A.

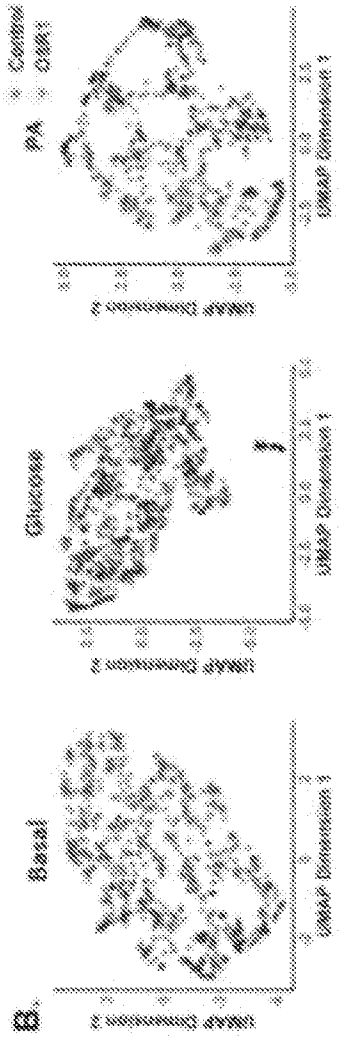


FIG. 10 B.

FIGS. 10A-10B

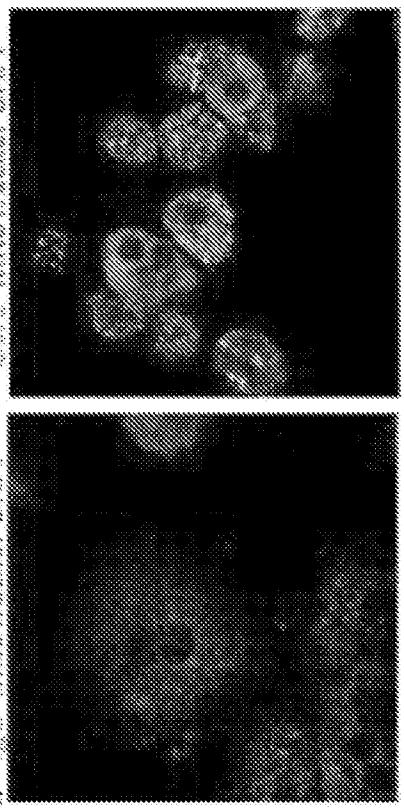


FIG. 11

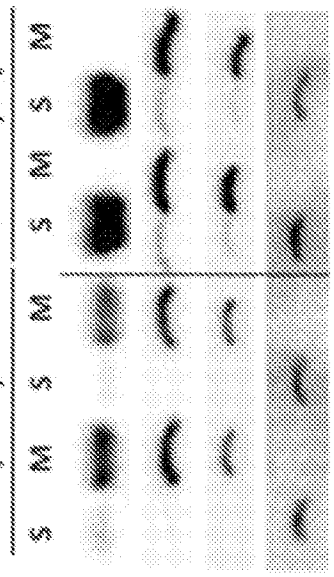


FIG. 11

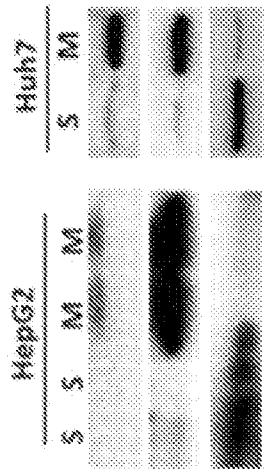


FIG. 11

FIGS. 11A-11C

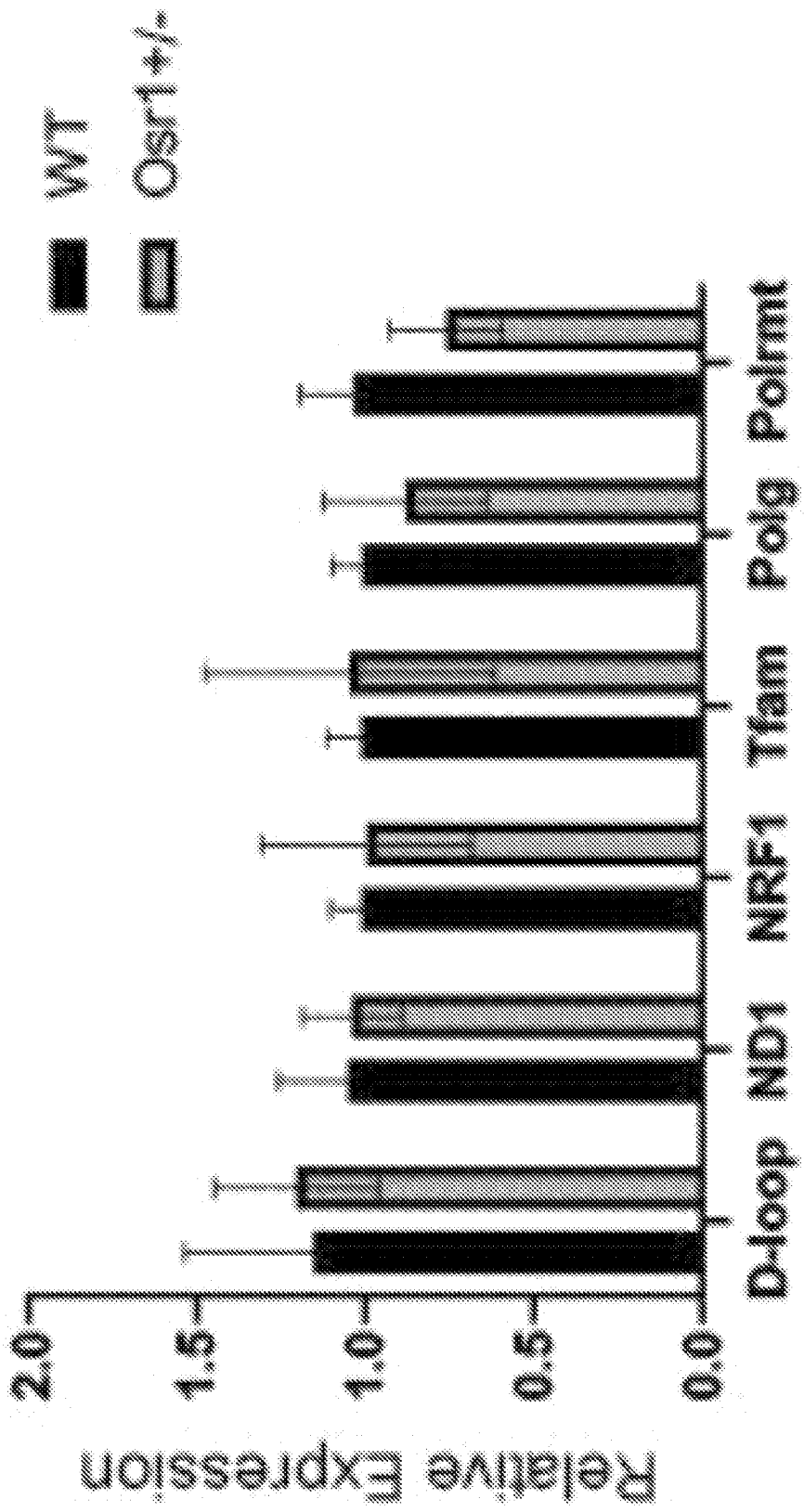

WT
Osr1+/-
Relative Expression
2.0
1.5
1.0
0.5
0.0
D-loop
ND1
NRF1
Tfam
Polg
Polrmt

FIG. 12

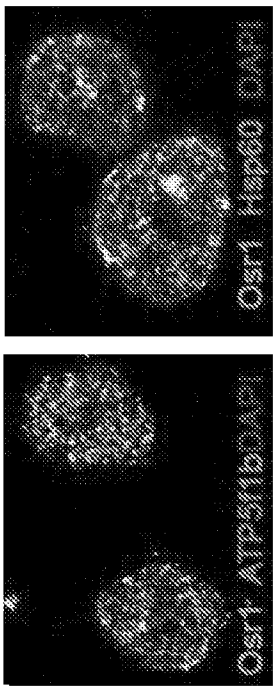


FIG. 13 A.

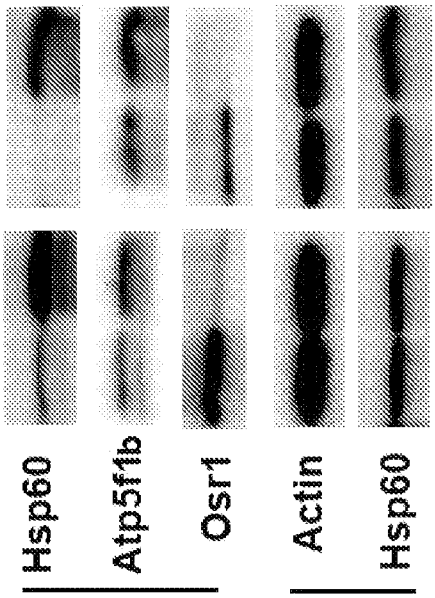


FIG. 13 B.

FIGS. 13A-13B

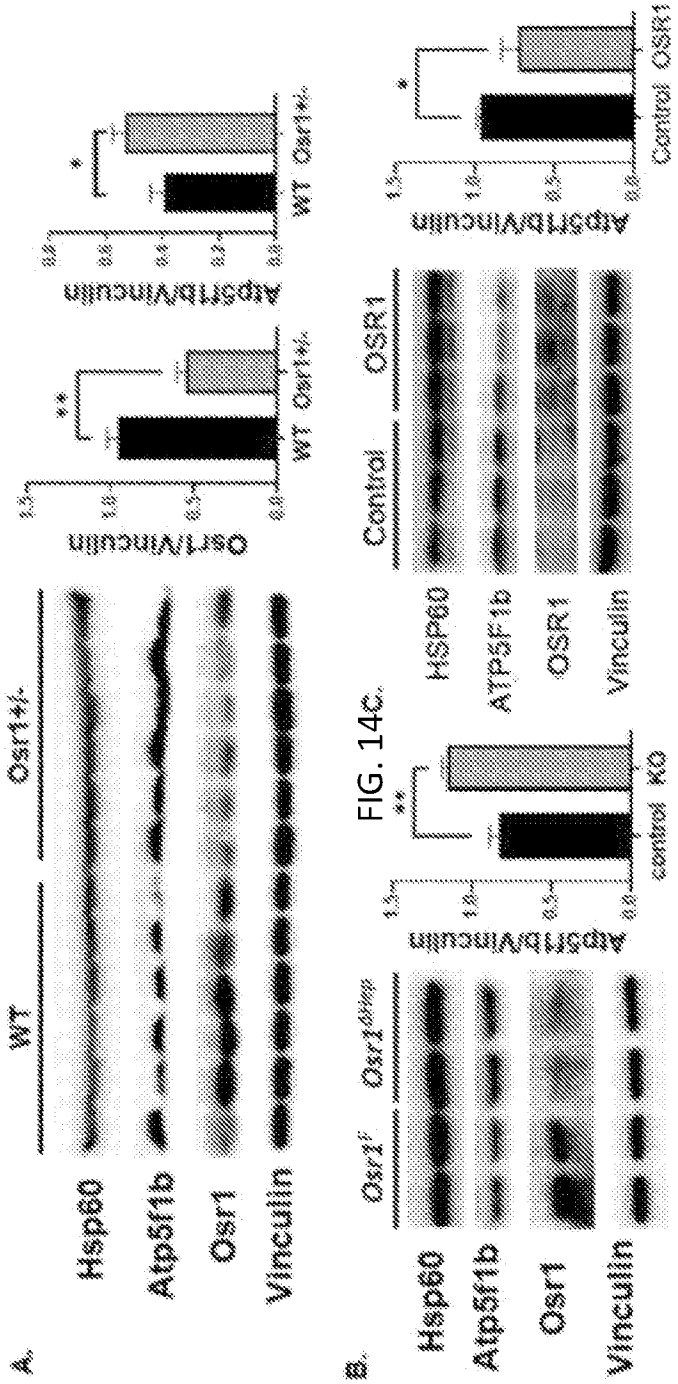


FIG. 14 A.

FIG. 14 B.

FIG. 14C.

FIG. 14 D.

FIGS. 14A-14D

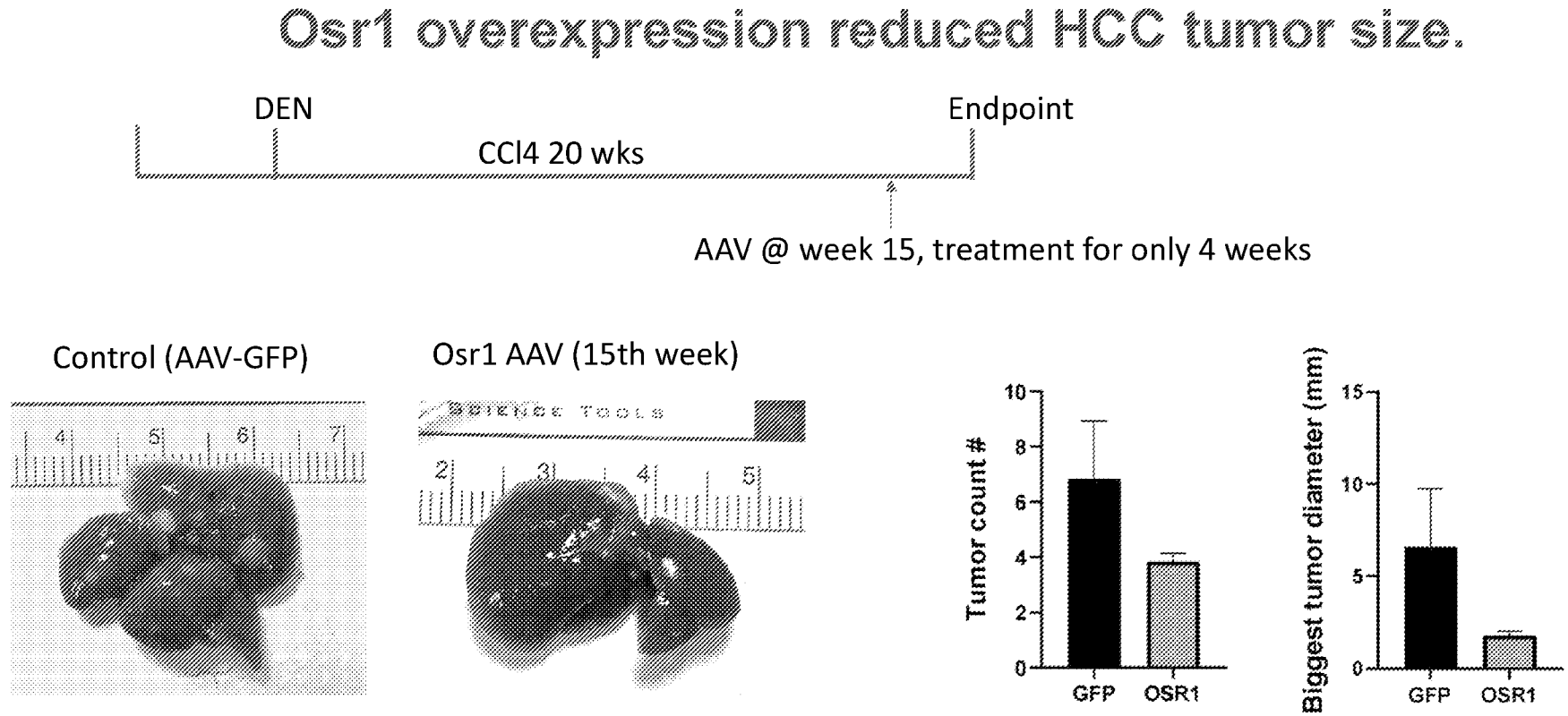

Osr1 overexpression reduced HCC tumor size.
DEN
Endpoint
CCl4 20 wks
AAV @ week 15, treatment for only 4 weeks
Control (AAV-GFP)
Osr1 AAV (15th week)
Tumor count #
GFP
OSR1
Biggest tumor diameter (mm)
GFP
OSR1
N=6 for each group

FIG. 15

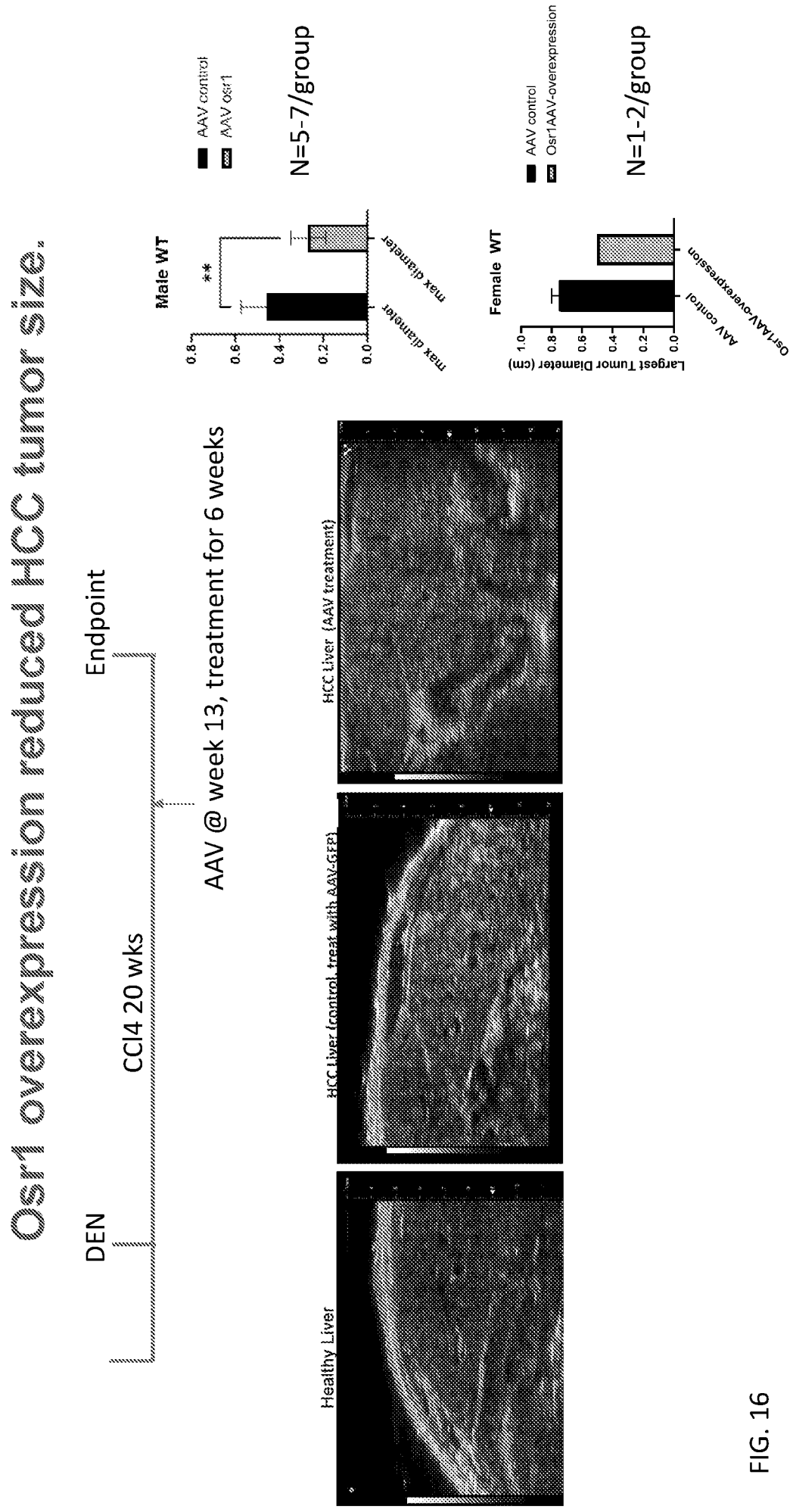

Osr1 overexpression reduced HCC tumor size.
DEN
CCl4 20 wks
AAV @ week 13, treatment for 6 weeks
Endpoint
Healthy Liver
HCC Liver (control, treat with AAV-GFP)
HCC Liver (AAV treatment)
Male WT
AAV control
AAV osr1
N=5-7/group
max diameter
max diameter
**
0.0
0.2
0.4
0.6
0.8
Female WT
AAV control
Osr1AAV-overexpression
N=1-2/group
Largest Tumor Diameter (cm)
AAV control
Osr1AAV-overexpression
0.0
0.2
0.4
0.6
0.8
1.0

FIG. 16

Osr1 treatment-induced human HCC tumor organoid death in vitro

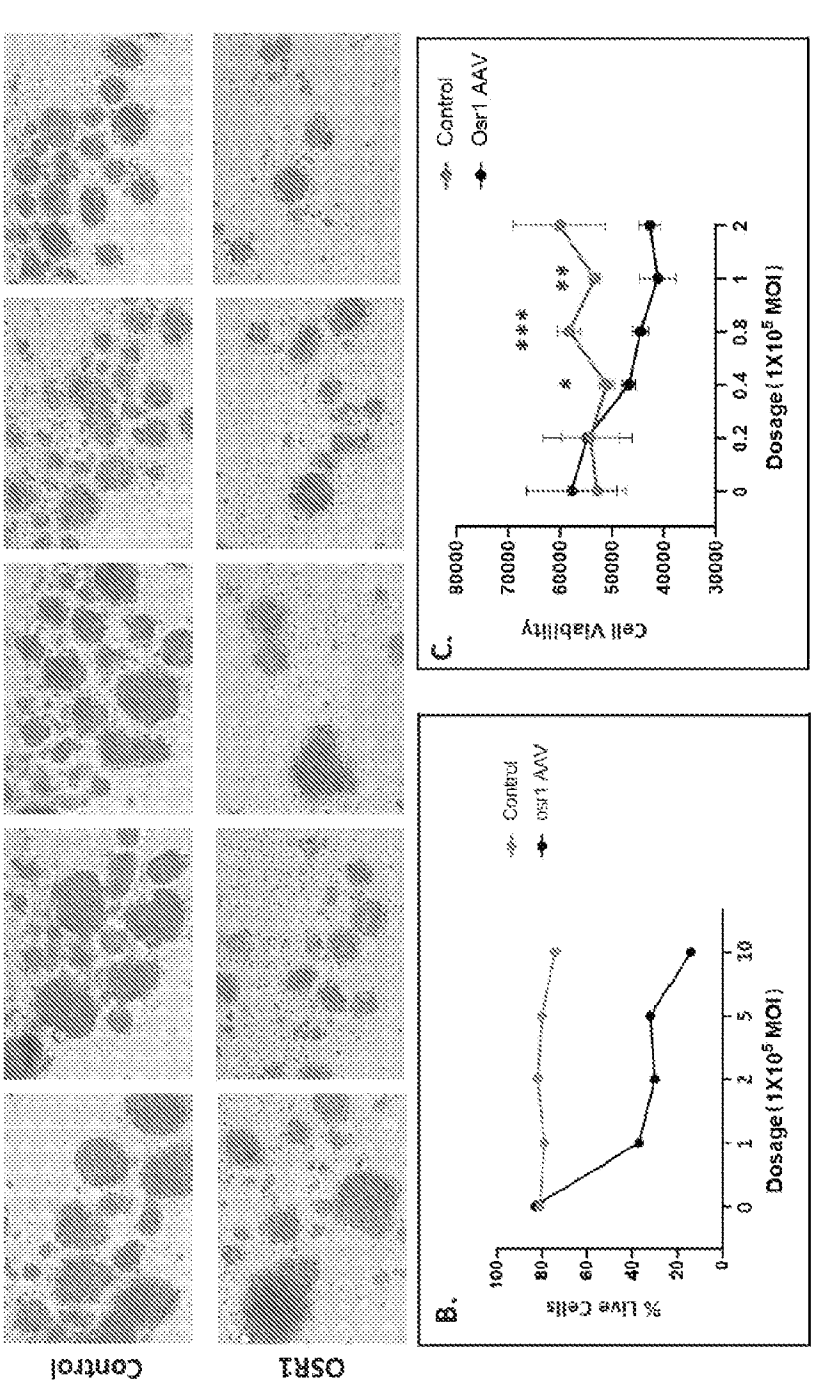


FIG. 17