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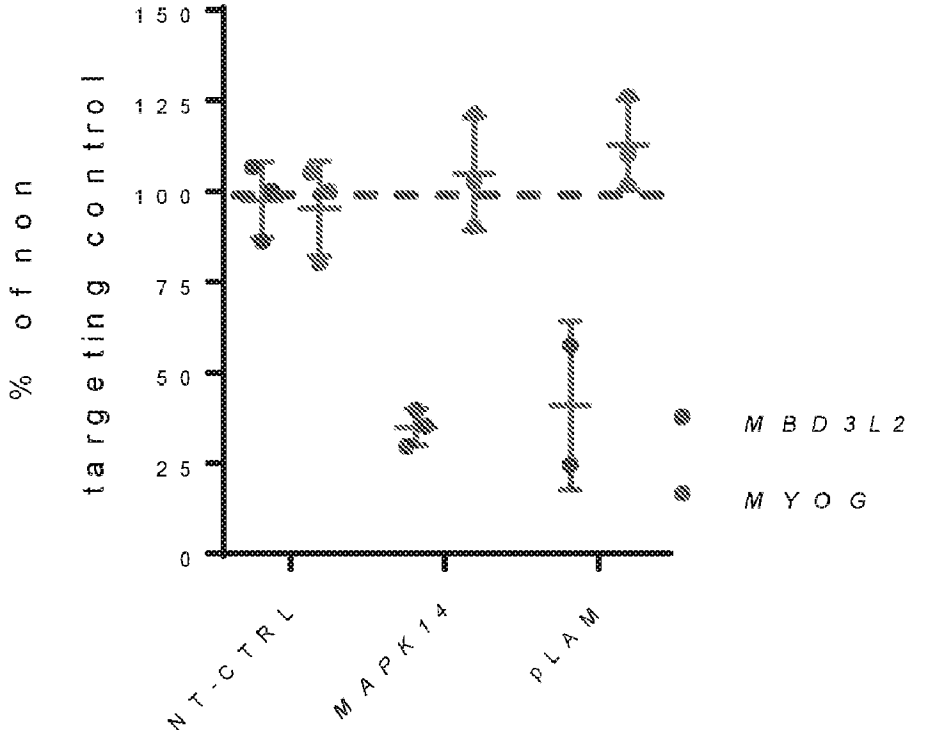
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(54) Titre : UTILISATION D'INHIBITEURS DE P38 POUR REDUIRE L'EXPRESSION DE DUX4
(54) Title: USE OF P38 INHIBITORS TO REDUCE EXPRESSION OF DUX4

FIG. 6C



(57) Abrégé/Abstract:
The present invention relates to compositions and methods of inhibiting p38 kinase to reduce gene and protein expression of DUX4 and downstream genes regulated by DUX4. The present invention further relates to methods for treating patients suffering from diseases associated with increased expression of DUX4 or expression of an aberrant form of DUX4, such as Facioscapulohumeral muscular dystrophy (FSHD).

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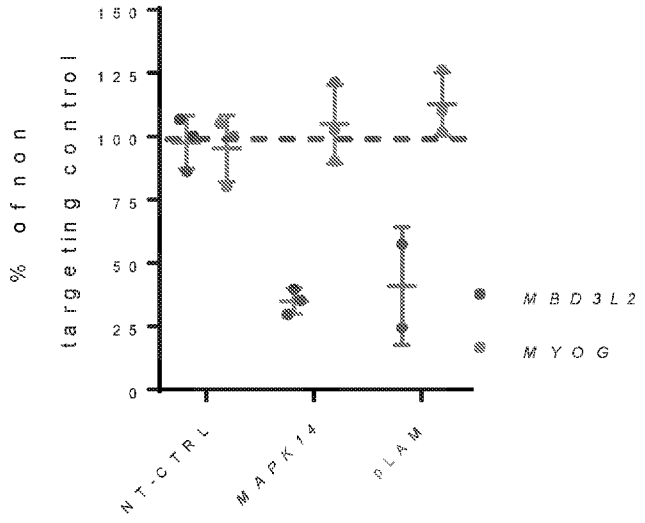
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(54) Title: USE OF P38 INHIBITORS TO REDUCE EXPRESSION OF DUX4

FIG. 6C



(57) Abstract: The present invention relates to compositions and methods of inhibiting p38 kinase to reduce gene and protein expression of DUX4 and downstream genes regulated by DUX4. The present invention further relates to methods for treating patients suffering from diseases associated with increased expression of DUX4 or expression of an aberrant form of DUX4, such as Facioscapulohumeral muscular dystrophy (FSHD).

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USE OF P38 INHIBITORS TO REDUCE EXPRESSION OF DUX4

Cross Reference to Related Applications

[0001] This application claims priority to U.S. Provisional Application No. 62/568,673, filed on October 5, 2017; U.S. Provisional Application No. 62/568,754, filed on October 5, 2017; U.S. Provisional Application No. 62/682,563, filed on June 8, 2018; and U.S. Provisional Application No. 62/682,565, filed on June 8, 2018; all of which are incorporated by reference herein in their entireties.

Sequence Listing

[0002] The instant application contains a Sequence Listing that has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 4, 2018, is named FULC_027_02WO_ST25.txt and is 27 KB in size.

Field of the Disclosure

[0003] The present invention relates to compositions and methods of inhibiting p38 kinase to reduce gene and protein expression of DUX4 and downstream genes regulated by DUX4. The present invention further relates to methods for treating subjects with disease and disorders associated with increased expression of DUX4 or expression of an aberrant form of DUX4, such as Facioscapulohumeral muscular dystrophy (FSHD).

Background

[0004] The muscular dystrophies (MD) are a group of more than 30 different genetic diseases characterized by progressive weakness and degeneration of the skeletal muscles that control movement. Some forms of MD occur in infancy or childhood, while others may not appear until middle age or older. The various MD diseases differ in terms of the distribution and extent of muscle weakness (some forms of MD also affect cardiac muscle), age of onset, rate of progression, and pattern of inheritance.

[0005] Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular dystrophy and affects approximately 1 in 15,000 people worldwide. FSHD is caused by genetic mutations resulting in the epigenetic derepression of the DUX4 gene, which makes this disease unique among muscular dystrophies. FSHD's primary

manifestations are weakness and wasting of muscles of the face, shoulder girdle, upper arms, and trunk, and impacts lower extremities in more severe cases.

[0006] Genetic mutations associated with FSHD lead to a partial decompaction of the D4Z4 chromatin structure and a resulting failure to repress DUX4, a transcription factor encoded by the D4Z4 unit, in skeletal muscle. FSHD1, representing about 95% of FSHD cases reported, is associated with deletions of macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, leaving 1–10 D4Z4 repeats (reviewed in Tawil et. al., 2014). FSHD2 is caused by mutations in Structural Maintenance of Chromosomes Flexible Hinge Domain Containing 1 gene (*SMCHD1*) on chromosome 18 (reviewed in van der Maarel et. al., 2007). Both FSHD1 and FSHD2 mutations lead to loss of repression at the 4q35 D4Z4 repeat array, allowing aberrant transcription in muscle of a full-length form of Double homeobox 4, DUX4, mRNA (*DUX4-fl*), which encodes the double homeobox 4 (DUX4) transcription factor (Tawil et. al., 2014). *DUX4-fl* RNA isoforms found associated with FSHD vary only in the 3′ untranslated region and have no identified functional distinction.

[0007] There is currently no approved treatment that can halt or reverse the effects of FSHD, although nonsteroidal anti-inflammatory drug are often prescribed to improve comfort and mobility. Clearly, therefore, there is a need in the art for new methods for reducing the expression levels of DUX4, e.g., *DUX4-fl* mRNA and/or DUX4 protein, e.g., to treat FSHD and other diseases. The present invention meets this need.

Brief Description of the Drawings

[0008] FIGS. 1A and 1B show expression of DUX4 protein and RNA in FSHD myotubes. FIG. 1A includes micrographs of FSHD myotubes stained using an antibody that binds DUX4 protein and/or 4′,6-diamidino-2-phenylindole (DAPI; to detect nuclei). Mature FSHD myotubes showed actin striations in culture (not shown) and expressed DUX4 protein in discrete sets of nuclei contained within a differentiated myotube (FIG. 1A). FIG. 1B is a graph showing relative expression of DUX4 mRNA in FSHD myotubes and myotubes from an isogenic wild type (healthy) control.

[0009] FIG. 2 is a graph showing mRNA expression of the indicated DUX4 regulated genes in wild type myotubes treated with DMSO, or FSHD myotubes treated with FTX-2 or DMSO. For each indicated gene, the bars from left to right correlate to wild type myotubes treated with DMSO, FSHD myotubes treated with DMSO, and FSHD myotubes treated with FTX-2 (*DUX4*-targeted ASO).

[0010] FIGS. 3A- 3C show reduction of *MBD3L2* mRNA in FSHD myotubes treated with *DUX4*-targeted ASOs. *MBD3L2* was normalized to *POLR2A* mRNA as measured by qPCR. FIG. 3A is a graph showing grouped plate quality control data comparing *MBD3L2* expression in FSHD myotubes treated with DMSO control or 1 μM *DUX4*-targeted ASOs, and healthy normal isogenic wild-type myotubes (WT). FIG. 3B is a graph showing dose-dependent reduction of *MBD3L2* mRNA expression in FSHD myotubes treated with different dilutions of the *DUX4*-targeted ASO (FTX-2). FIG. 3C shows plate-based assay statistics comparing *MBD3L2* signal in FSHD myotubes treated with DMSO to *DUX4*-targeted ASOs or wild-type myotubes treated with DMSO.

[0011] FIGS. 4A-4D are graphs showing expression levels of *MBD3L2* mRNA and *MYOG* mRNA in FSHD myotubes treated with the indicated p38α/β inhibitors relative to treatment with DMSO control. The p38α/β inhibitors included SB 239063 (FIG. 4A), VX-702 (FIG. 4B), Pamapimod (FIG. 4C), and TAK-715 (FIG. 4D). The structures of the inhibitors are also provided.

[0012] FIGS. 5A and 5B show data from FSHD myotubes treated with Pamapimod. FIG. 5A is a graph showing that dose-dependent reduction in *DUX4-fl* mRNA (filled circles) and *MBD3L2* mRNA (open circles). FIG. 5B shows micrographs of FSHD myotubes treated with either DMSO or Pamapimod.

[0013] FIGS. 6A-6C are graphs showing mRNA levels of *MAPK14* (FIG. 6A) and *MBD3L2* (FIG. 6B and FIG. 6C) in FSHD myotubes treated with siRNAs targeting p38a MAPK14 (siMAPK14 85 and siMAPK14 86; FIG. 6A and FIG. 6B) or treated with p38a kinase (MAPK14 and DUX4 pLAM) Cas9/sgRNA RNPs (FIG. 6C), as compared to non-targeting control (NT CTRL). In FIG. 6C, for each treatment, the results shown left to right correspond to *MBD3L2* and *MYOG*, respectively.

[0014] FIG. 7 is a graph showing expression levels of DUX4 protein, *MBD3L2* mRNA, and p-HSP27 protein in FSHD myotubes following treatment with increasing dosages of FTX-1821 (structure shown), as a percentage of DMSO control treatment levels. Bars represent standard deviation.

[0015] FIGS. 8A and 8B show the effect of FTX-1821 on myotube formation. FIG. 8A provides representative images of morphology of immortalized FSHD myotubes obtained after treatment with vehicle (DMSO) or the indicated concentrations of FTX-1821, and staining with antibodies against MHC and DAPI (nuclear stain). FIG. 8B is a graph showing

quantification of nuclei in myotubes, as defined by MHC staining, after treatment with FTX-1821 at concentrations tested. Bars represent standard deviation of three replicates.

[0016] FIGS. 9A and 9B show the results of apoptosis assays in FSHD myotubes in vitro. FIG. 9A provides micrographs of FSHD myotubes stained for active caspase-3 (as a marker of apoptosis) or DAPI. Apoptosis was detected in a sporadic manner in a subset of myotubes in culture as shown by white circles in the left panel and in the magnified region to the right. FIG. 9B is a graph showing quantification of active caspase-3 signal in FSHD myotubes treated with the indicated concentrations of FTX 1821.

[0017] FIGS. 10A and 10B illustrate the identification of genes downregulated in FSHD myotubes by FTX-1821. FIG. 10A is a heatmap, which illustrates differentially expressed genes identified by RNA-seq profiling. Three replicates for each condition were analyzed by RNA-seq and genes were clustered by the direction and intensity of change as indicated. The color bar indicates the normalized changes observed, e.g., genes that were downregulated by FTX-1821 are enriched in samples treated with only DMSO. Down-regulated genes are listed in FIG. 10A. FIG. 10B is a graph showing the normalized expression level reads of the DUX4 target genes that were downregulated upon treatment with FTX-1821 in wild type cells treated with vehicle control DMSO, FSHD cells treated with DMSO, or FSHD cells treated with FTX-1821.

[0018] FIG. 11 is a graph showing mRNA expression levels by qRT-PCR of the DUX4 target gene, *MBD3L2* (normalized to *POLR2A*), in myotubes derived from four distinct FSHD patient myoblast lines, FTCE-016, -020, -197, -196 and two wild type (WT) control lines, following the indicated treatment with DMSO vehicle control, FTX-1821 or FTX-839.

[0019] FIGS. 12A and 12B provide information on various p38 inhibitors. FIG. 12A is a table of data summarizing pharmacology for the indicated p38α and β inhibitors, including IC50 for reducing *MBD3L2* expression in FSHD cells. Comparable *MBD3L2* IC_{50} values are shown, indicating inhibition of DUX4 downstream gene expression in FSHD myotubes across a broad structural panel of p38α and β inhibitors reported to have similar enzyme potencies. These data indicate that p38 inhibition result in DUX4 target gene, *MBD3L2*, reduction IC_{50} values in the range of ~6-68 nM. FIG. 12B provides the compound structures of the p38 inhibitors listed in FIG. 12A.

[0020] FIG. 13 is a table of various cell lines utilized in "clinical trial in a dish," which shows diversity of genotypes, and includes both primary and immortalized lines, as well as FSHD1 and FSHD2 patient lines.

[0021] FIGS. 14A and 14B are graphs showing *MBD3L2* mRNA expression normalized to POLR2A (by qRT-PCR) (FIG. 14A) and apoptosis as measured by cleaved caspase-3 (FIG. 14B) determined in nine FSHD1 and three FSHD2 patient myotubes (listed in Table 2, FIG. 14B contains only two FSHD2 cell lines) following treatment with FTX-1821, FTX-839, or DMSO vehicle control.

[0022] FIG. 15 is a graph showing the time course of plasma exposure, trapezius muscle exposure and p38 target engagement (Phosphorylated p38α:Total p38α Ratio) in the rat following oral administration of 0.3 mg/kg FTX-1821.

[0023] FIG. 16 is a graph showing MBD3L2 mRNA levels in A4 and C6 xenografted TA muscles.

[0024] FIG. 17 is a graph showing phosphor/total MC2 ratio in mouse trapezius muscles following treatment with vehicle control or p38 inhibitor, FTX-2865.

[0025] FIG. 18 is a graph showing MBD3L2 mRNA levels in C6 xenografted TA muscles following treatment with vehicle control or p38 inhibitor, FTX-2865.

Summary of the Invention

[0026] The present disclosure provides methods of reducing the expression a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in cells, comprising contacting the cells with an agent that results in a reduction of active p38 protein in the cell, thereby reducing expression the DUX4 polypeptide or the polypeptide encoded by the downstream target gene of DUX4. These methods may be practiced using a variety of different types of agents, and for modulating a variety of different biological processes in the cell, as well as for treating subjects for diseases associated with aberrant DUX4 expression, such as FSHD.

[0027] In certain embodiments of any of the methods disclosed herein, the cell is a muscle cell, optionally a terminally differentiated muscle cell. In some embodiments, the cell has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell, e.g., a cell obtained from a healthy subject. In some embodiments, the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell. In certain embodiments, the cell is associated with facioscapulohumeral muscular

dystrophy (FSHD), e.g., it was obtained from a subject diagnosed with FSHD or is present within a subject diagnosed with FSHD. In some embodiments, the cell comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises ≤7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35. In some embodiments, the cell comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene. In some embodiments, the cell comprises at least one non-deleted 4qA allele.

[0028] In certain embodiments of the methods disclosed herein, the agent inhibits the expression or activity, or reduces the amount, of the p38 protein, wherein the activity is optionally kinase activity.

[0029] In some embodiments, the agent inhibits the expression of the p38 protein. In particular embodiments, the agent binds a polynucleotide encoding the p38 protein, or binds an antisense polynucleotide thereof. In particular embodiments, the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide.

[0030] In some embodiments, the agent inhibits the activity of the p38 protein. In particular embodiments, the agent binds the p38 protein. In particular embodiments, the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some embodiments, the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule.

[0031] In certain embodiments of any of the methods disclosed herein, the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

[0032] In particular embodiments of any of the methods disclosed herein, the expression or the activity of the p38 protein, or the amount of the p38 protein, is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

[0033] In a related embodiment, the present disclosure provides a method of treating or preventing a disease or disorder associated with increased expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in a subject in need thereof, comprising providing to the subject a pharmaceutical composition comprising an agent that results in a reduction in the amount of active p38 protein in one or

more tissue of the subject, thereby reducing expression of the DUX4-fl mRNA, the DUX4 protein, or the polypeptide encoding the downstream target gene in one or more tissue of the subject. In some embodiments, the disease or disorder is a facioscapulohumeral muscular dystrophy (FSHD), optionally FSHD1 or FSHD2. In certain embodiments, the subject comprises reduced repression at a D4Z4 locus. In some embodiments, the subject comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises $\leq$7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35. In some embodiments, the subject comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene. In some embodiments, the subject comprises at least one non-deleted 4qA allele. In certain embodiments, the expression or the activity of, or the amount of, the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% in a muscle tissue of the subject. In some embodiments, the method decreases muscle degeneration in the subject. In some embodiments, the method reduces apoptosis of muscle cells in the subject. In some embodiments, the muscle tissue is terminally differentiated. In particular embodiments, the pharmaceutical composition is provided to the subject parenterally or orally. In certain embodiments, the pharmaceutical composition is provided to a muscle tissue of the subject, optionally parenterally or intramuscularly. In particular embodiments, the method further comprises providing to the subject a second agent or therapy for treating the disease or disorder associated with increased expression of a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4.

[0034] The present disclosure also provides a unit dosage form of a pharmaceutical composition comprising an agent that results in a reduction in the amount of active p38 protein in a cell, and a pharmaceutically acceptable carrier, diluent or excipient, wherein the unit dosage form is effective to reduce expression or activity of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in one or more cell or tissue in a subject to whom the unit dosage form is administered. In particular embodiments, the agent binds the DUX4 polypeptide or binds a polynucleotide encoding the DUX4 polypeptide. In some embodiments, the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide. In some embodiments, the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some embodiments, the agent comprises a small molecule, optionally an organic molecule

or an inorganic molecule. In certain embodiments, the tissue is muscle tissue, optionally wherein the tissue comprises cells comprising a mutation associated with facioscapulohumeral muscular dystrophy (FSHD).

[0035] In a further related embodiment, the present disclosure provides a method of reducing apoptosis of a cell, e.g., a muscle cell, comprising contacting the cell with an agent that results in a reduction in the amount of active p38 protein in the cell, optionally wherein the muscle cell is terminally differentiated, thereby reducing expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in the cell. In some embodiments, the cell has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell. In some embodiments, the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell. In particular embodiments, the cell comprises one or more mutations associated with FSHD. In certain embodiments, the agent inhibits the expression of the p38 protein, optionally wherein the agent binds a polynucleotide encoding the p38 protein, or an antisense polynucleotide thereof. For instance, in some embodiments, the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, or antisense oligonucleotide, e.g., which targets p38. In some embodiments, the agent inhibits the activity of the p38 protein, optionally wherein the agent binds the p38 protein. In some embodiments, the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some embodiments, the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule. In particular embodiments, the expression or the activity of the p38 protein, the DUX4 protein, or the polypeptide encoded by the DUX4 downstream gene is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%. In particular embodiments, the method reduces apoptosis of muscle cells in a muscle tissue at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% as compared to a control, e.g., an untreated cell.

[0036] In certain embodiments of any of the methods disclosed herein, the agent reduces expression of DUX4 or the downstream target gene. In certain embodiments, the agent binds

a p38 protein, e.g., p38-α or p38-β, or binds a polynucleotide encoding the p38 protein, e.g., p38-α or p38-β, or an antisense polynucleotide thereof. In particular embodiments, the agent comprises or consists of: a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, CRISPR gRNA, or antisense oligonucleotide. In particular embodiments, the agent comprises or consists of: a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In particular embodiments, the agent comprises: a small molecule, optionally an organic molecule or an inorganic molecule. In some embodiments, the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A. In particular embodiments, the downstream target gene is MBD3L2, ZSCAN4, LEUTX, PRAMEF2, TRIM43, or KHDC1L.

[0037] In certain embodiments of any of the methods disclosed herein, the agent binds a p38 protein, e.g., p38-α or p38-β, or binds a polynucleotide encoding a p38 protein e.g., p38-α or p38-β. In some embodiments, the agent comprises or consists of: a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, mRNA, CRISPR gRNA, modified mRNA, morpholino, or antisense oligonucleotide. In some embodiments, the mRNA or modified mRNA encodes an antibody or a functional fragment thereof. In some embodiments, the agent comprises or consists of: a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In some embodiments, the agent comprises or consists of a gene therapy vector, e.g., a viral vector comprising a nucleic acid sequence encoding a polynucleotide or polypeptide inhibitor of p38, e.g., p38-α or p38-β, or other target. In some embodiments, the agent comprises or consists of a small molecule, optionally an organic molecule or an inorganic molecule. In some embodiments, the downstream target is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A. In some embodiments, the downstream target gene is MBD3L2, ZSCAN4, LEUTX, PRAMEF2, TRIM43, or KHDC1L. In some embodiments, the downstream target gene is CCNA1.

Detailed Description of the Invention

[0038] The present invention is based, in part, on the discovery that inhibition of p38 kinase, e.g., p38-α, results in reduced expression of DUX4 and downstream genes regulated by DUX4. Accordingly, the invention includes methods and compositions related to using an inhibitor of p38, e.g., p38-α, (alone or in combination with another agent) to reduce the

expression and/or activity levels of DUX4 and/or any of its downstream target genes, e.g., in the treatment or prevention of diseases associated with aberrant DUX4 expression, such as FSHD, a type of muscular dystrophy. This may be accomplished in a variety of ways, e.g., reducing expression of the DUX4-fl mRNA, reducing expression of the DUX4 protein, inhibiting DUX4 protein activity; CRISPR genome editing, and/or inducing degradation of the DUX4 protein.

[0039] The muscular dystrophies are a diverse group of genetic diseases that cause progressive weakness of the body's muscles. Some types of muscular dystrophy will present symptoms in early childhood, while other types will appear in adulthood. Different muscle groups also may be affected depending on the type of muscular dystrophy. See, e.g., Isin Dalkilic and Louis M Kunkel. Nearly 30 genes are known to give rise to various forms of muscular dystrophy, which differ in age of onset, severity, and muscle groups affected. The number of genes identified increases each year, adding to our understanding as well as revealing the overall complexity of the pathogenesis of these diseases.

[0040] For example, two common muscular dystrophies – Duchenne Muscular Dystrophy (DMD) and Facioscapulohumeral dystrophy (FSHD) – are considered to be unique diseases with some shared characteristics. Similarities between DMD and FSHD include that both are genetic diseases and symptoms include muscle loss with muscle weakness leading to disability (therefore both DMD and FSHD are grouped in the large category of muscular dystrophies, which means muscle degeneration). However, DMD and FSHD have very different etiology and disease diagnosis (dystrophin loss in DMD vs expression of DUX4-myotoxin in FSHD). For example, in DMD, mutations in the DMD gene (>2000 known) result in dysfunctional or missing dystrophin. In FSHD, the disease is due to overexpression of the *DUX4* gene in muscle tissue; it is not due to point mutations in the gene (DUX4 protein is expressed when the number of *D4Z4* repeats in the *DUX4* gene is between 1 and 8, or when repression is lost at the D4Z4 by mutations in other silencing machinery). Other differences include that only skeletal muscle is involved in FSHD, whereas both skeletal and cardiac muscle are affected in DMD; the diaphragm is involved in DMD but not FSHD; generally there is childhood onset in DMD but adult/adolescent onset in FSHD; and onset with ambulatory involvement in DMD but onset with face and proximal arm/shoulders in FSHD. Another important distinction is that there is response to steroids in DMD but not in FSHD. In addition, the approved treatment for DMD (Exondys-51 in the US; Ataluren in the EU) will not have any effect in FSHD. Finally, only males are affected in DMD while there is equal involvement of both sexes in FSHD.

[0041] FSHD also has an unusual pathology, and it is unique among muscular dystrophies in that its development requires both genetic and epigenetic conditions. The genetic condition is the presence of a complete *DUX4* gene. The *DUX4* gene is a retrogene normally expressed in germ line and early embryonic cells, but it is repressed by *D4Z4* repeat-induced silencing in adult tissues (Ehrlich and Lacey, 2012). Each *D4Z4* element contains a promoter and the DUX4 ORF, but lacks a polyadenylation signal (PAS), resulting in rapid *DUX4* mRNA degradation. In contrast, transcripts initiated in the distal *D4Z4* unit on a 4qA permissive allele extend outside of the repeat array and reach a PAS in the flanking pLAM sequence (reviewed in Tawil et al., 2014; Himeda et al., 2015). The resulting poly-A tail stabilizes the DUX4 mRNAs and allows for their translation into a protein that is not normally expressed in healthy muscle and is toxic to skeletal muscle function. Two enhancers, DUX4 myogenic enhancer 1 (DME1) and DME2, which activate DUX4-fl expression in skeletal myocytes, have been described to regulate DUX4-fl expression in FSHD (Himeda et al., 2014).

[0042] FSHD1, FSHD2 stages in early development as well as germline formation stages appear to confer a transcriptionally permissive conformation to *D4Z4* chromatin. This is evidenced by changes in histone modification, partial but variable hypomethylation of *D4Z4* in FSHD1, and more extensive hypomethylation in FSHD2 (Himeda et al., 2015). However, D4Z4 hypomethylation does not suffice for the disease, since there is an absence of muscular dystrophy symptoms in patients with ICF (immunodeficiency, centromeric region instability and facial anomalies), a rare, unrelated DNA hypomethylation-associated disease in which *D4Z4* is strongly hypomethylated (OMIM Entry - # 614069).

[0043] DUX4 is a homeobox transcription factor protein, and expression of DUX4 in muscle induces a transcriptional program leading to expression of downstream genes and protein products that are not normally expressed in skeletal muscle. For example, DUX4 expression results in the induction of several germline genes in FSHD skeletal muscles and in transfected cells (Yao et al, 2014; Ehrlich and Lacey, 2012). Many of these novel transcripts are expressed in FSHD muscle cells but not in control muscle cells (Yao et al., 2014; Homma et al., 2015; Shadle et al., 2017; Bosnakovski et al., 2014). Since some of the downstream target genes of DUX4 encode transcription factors, DUX4 pathological activation leads to a large gene expression deregulation cascade in muscle, which causes the disease (Yao et al., 2014; Homma et al., 2015; Shadle et al., 2017; Bosnakovski et al., 2014).

[0044] Endogenous (in the FSHD myofiber) and forced DUX4 expression in muscle cells is toxic, leads to apoptosis and oxidative stress, and interferes with myogenesis and sarcomere function (Rickard et al., 2015; Homma et al., 2015; Bosnokovski et al., 2014; Tawil et al.,

2014; Himeda et al., 2015). Clinical heterogeneity in both disease progression and age of onset can be accounted for, in part, by epigenetic instability leading to progressive changes in DUX4 transcription. The role of DNA hypomethylation and permissive DUX4 transcription is exemplified by the high clinical severity observed in patients who inherited combined FSHD1 and 2 defects (reviewed in Tawil et al., 2014; van der Maarel et al., 2007). Clinical heterogeneity is also explained by differences in the severity of D4Z4 repeat shortening, with more severe phenotype and younger age at onset in patients with shorter repeats (1-3) compared to patients with less severely contracted repeats (4-7).

[0045] DUX4 is now recognized as the cause of the pathology of FSHD, since activation of its target genes is the main molecular signature in FSHD muscle (Reviewed in Tawil et al., 2014; Himeda et al., 2015). Major downstream target genes are members of highly homologous gene families that are clustered spatially on chromosomes, including PRAMEF (preferentially expressed in melanoma), TRIM (tripartite motif-containing), MBDL (methyl-CpG binding protein-like), ZSCAN (zinc finger and SCAN domain containing) and RFPL (ret-finger protein-like) families (Geng et al., 2012; Yao et al., 2014; Shadle et al., 2017; Ehrlich and Lacey, 2012; Tawil et al., 2014; van der Maarel et al., 2007). Discrimination between FSHD and control skeletal muscle can be made using ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, ZNF280A etc. (described in but not limited to Yao et al., 2014; Shadle et al., 2017; Ehrlich and Lacey, 2012).

[0046] Annotated chemical probes were screened to identify disease-modifying small molecule drug targets that reduce DUX4 expression in FSHD myotubes. These screens identified multiple chemical scaffolds that inhibit the activity of p38 mitogen-activated protein kinase alpha (MAPK14 or p38α). As described in the accompanying Examples, it has been shown that knockdown of the MAPK14 gene using small interfering RNA (siRNA) technology or CRISPR-mediated genome editing with specific guide RNA's (gRNAs) that selectively target the alpha isoform of p38 kinase also reduces DUX4 and DUX4-related downstream gene expression in FSHD myotubes. It was also found that selective p38α and β kinase inhibitors specifically reduced DUX4 and its downstream genes in FSHD myotubes, thereby impacting the core pathophysiology of the FSHD disease process (data exemplified herein). The same experiments revealed that p38α and β kinase inhibitors do not impact myogenin or the expression of other myogenic factors, nor do they impact proliferation of myoblasts or differentiation of myoblasts exhibited by myogenic fusion in FSHD myotubes,

thereby demonstrating that the effect is not due to overall toxicity to muscle. These p38 kinase inhibitor small molecules reduce the expression of DUX4 and related downstream genes, thereby impacting pathophysiology of the FSHD disease process, including reducing apoptotic cell death. p38-mediated DUX4 reduction would be expected to impact downstream inflammatory, fatty infiltration, and fibrotic processes in FSHD.

[0047] Members of the p38 MAPK family, composed of α, β, γ and δ, isoforms are encoded by separate genes that play a critical role in cellular responses needed for adaptation to stress and survival (reviewed in Whitmarsh 2010; Martin et al., 2014; Krementsov et al., 2013). In many inflammatory diseases, including cardiovascular and other chronic diseases, these same p38 MAPK stress-induced signals can trigger maladaptive responses that aggravate, rather than alleviate, the disease (reviewed in Whitmarsh 2010; Martin et al., 2014). Indeed, in skeletal muscle, a variety of cellular stresses including chronic exercise, insulin exposure and altered endocrine states, myoblast differentiation into myocytes, reactive oxygen species, as well as apoptosis, have all been shown to induce the p38 kinase pathway (Keren, et.al., 2006; Zarubin et al., 2006). In fact, the p38 kinase pathway can be activated by a number of external stimuli, including pro-inflammatory cytokines and cellular stress, leading to activation of the dual-specificity MAPK kinases MKK3 and MKK6. Activation of MKK3 and MKK6, which in turn phosphorylate p38 in its activation loop, trigger downstream phosphorylation events. These include phosphorylation of HSP27, MAPKAPK2 (MK2) and a variety of transcription factors, culminating in transcriptional changes in the nucleus. A modest number of p38-regulated transcripts and a large number of downstream effectors of p38 kinase have been identified (described in Cuenda et al., 2007 and Kyriakis et.al., 2001, Viemann et al. 2004).

[0048] Several compounds from different chemical scaffolds that inhibit the p38α MAPK signaling pathway have entered clinical trials in diverse (non-neuromuscular) indications, including rheumatoid arthritis, chronic obstructive pulmonary disease, pain, cardiovascular diseases, and cancer. Inhibition of p38α and β in clinical trials has proven to be safe. In vitro and in vivo pharmacology suggest that p38α target engagement in these clinical studies was robust, as demonstrated by measuring reduction in phosphorylation of HSP27 (an indirect target) and pMK2 (a direct target).

[0049] p38α MAPK is known to play critical roles in skeletal muscle biology, specifically in abrogating proliferating myoblasts to differentiation and subsequently fusion to form multi-nucleated myotubes. Treatment of muscular dystrophy patients that are constitutively undergoing processes of degeneration and regeneration with p38α inhibitors would not be

obvious. Complete knockout (KO) of p38α is embryonically lethal. Embryonic rescue allows for survival of pups to a few days postnatal and isolation of satellite cells to study myogenic precursors lacking p38α. Myoblasts completely lacking p38α express significantly less critical differentiation genes and show severe deficits in fusion. Histology of P2 pups show significantly increased cycling satellite cells and a left-shifted fiber distribution. (Perdiguero et. al, 2007). Importantly, KO of p38α in mature muscle (cre driven by Myl1 promoter) shows no deficiencies in early time points, but mice deficient in p38α at 6 months of age show significantly greater regeneration and type I fibers, as well as a smaller fiber distribution compared to controls (Wissing et. al, 2014). These data suggest that inhibition of p38α would trigger skeletal muscle regeneration in diseases deficient in regeneration in addition to FSHD by a mechanism independent of regulation of DUX4 expression.

[0050] In skeletal muscle, p38 has been shown to regulate gene expression during myogenesis. p38γ has been shown to be required for myogenesis using both specific gene knock out and conditional knock out approaches (Cuenda et.al., 2007; Kerin et.al., 2006; Aouadi et.al., 2006). In the adult, selective inhibitors of p38α and β avoid p38γ-related impact to myogenesis.

[0051] The present disclosure finds that p38 is activated during myogenesis, and that inhibition of p38α and β by molecules exemplified herein, including FTX-839, FTX-1821, etc., profoundly reduces DUX4 expression and its downstream gene program in FSHD myotubes (data exemplified herein). Without wishing to be bound by theory, p38α appears to directly regulate DUX4 expression by impacting the activity of critical myogenic enhancers required for pathologic DUX4 expression at the level of the mutated D4Z4 locus with shorter repeats (FSHD1) or SMCHD1 mutations (FSHD2) or when repression is lost by other mechanisms in the muscle of FSHD patients. This is a differentiated mechanism from the previous clinical studies, which targeted functions of p38 in the cytoplasm and failed to show efficacy in numerous diseases, including rheumatoid arthritis, pain, depression, chronic obstructive pulmonary disease, and cardiovascular disease. Inhibitors of p38 have never been explored clinically for FSHD.

[0052] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

[0053] As used in this specification, the term "and/or" is used in this disclosure to either "and" or "or" unless indicated otherwise.

[0054] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply

the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0055] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0056] "Administration" refers herein to introducing an agent or composition into a subject or contacting an agent or composition with a cell and/or tissue.

[0057] In certain aspects, the disclosure includes a method for reducing the expression or activity of a DUX4 gene, mRNA, or polypeptide, or for reducing the expression or activity of a DUX4 downstream gene or polypeptide, including but not limited to any of those disclosed herein, in a cell, tissue, organ, or subject. In particular embodiments, the DUX4 mRNA is DUX4-fl. As used herein, the term "DUX4 downstream gene" refers to a gene that is transcriptionally activated (i.e., its expression is increased) by DUX4, and the term "DUX4 downstream polypeptide" refers to the encoded polypeptide. Illustrative examples if DUX4 downstream genes are provided herein. In certain embodiments, the DUX4 downstream gene is selected from those shown in FIG. 10A.

[0058] The methods disclosed herein may be practiced in vitro or in vivo, and in certain embodiments, the methods comprise contacting a cell, tissue, organ or subject with a p38 inhibitor, resulting in a reduced amount of active p38 protein in the cell, tissue, organ or subject. The term "p38 inhibitor" may refer to any agents that results in a reduced amount of active p38 protein in the cell, tissue, organ, or subject. The amount of active p38 protein in a cell may be reduced via a variety of means, including but not limited to reducing the total amount of p38 protein or inhibiting one or more activity of the p38 protein. In various embodiments, a p38 inhibitor may inhibit the expression of a p38 gene, a p38 mRNA, or a p38 protein, and/or a p38 inhibitor may inhibit a biological activity of a p38 protein. In certain embodiments, the biological activity is kinase activity. For example, a p38 inhibitor may competitively bind to the ATP-binding site of p38 MAPK and inhibit its kinase activity, or it may allosterically block the kinase activity of the p38 MAPK. In certain embodiments, a p38 inhibitor causes increased degradation of a p38 protein. In particular embodiments, the

p38 gene or p38 protein is a mammalian p38 gene or mammalian p38 protein, e.g., a human p38 gene or human p38 protein, e.g., a human p38-α (MAPK14) or p38-β (MAPK11) gene or protein.

[0059] p38 MAP Kinase (MAPK), also called RK or CSBP (Cytokinin Specific Binding Protein), is the mammalian orthologue of the yeast Hog1p MAP kinase, which participates in a signaling cascade controlling cellular responses to cytokines and stress. Four p38 MAP kinases, p38-α (MAPK14), -β (MAPK11), -γ (MAPK12 / ERK6), and –δ (MAPK13 / SAPK4), have been identified. These include various isoforms. In particular embodiments, any of these may be targeted by the methods disclosed herein. In certain embodiments, the p38 inhibitor inhibits p38-α (MAPK14) or p38-β (MAPK11), e.g., human versions of these genes or proteins.

[0060] In certain embodiment, the targeted p38 protein comprises the amino acid sequence set forth below or disclosed in GenBank accession NP_001306.1 for p38 kinase (mitogen-activated protein kinase 14 isoform 1, homo sapiens):

MSQERPTFYRQELNKTIWEVPERYQNLSPVGSGAYGSVCAAFDTKTGLRVAVKKLS
RPFQSIIHAKRTYRELRLLKHMKHENVIGLLDVFTPARSLEEFNDVYLVTHLMGADL
NNIVKCQKLTDDHVQFLIYQILRGLKYIHSADIIHRDLKPSNLAVNEDCELKILDFGLA
RHTDDEMTGYVATRWYRAPEIMLNWMHYNQTVDIWSVGCIMAELLTGRTLFPGTD
HIDQLKLILRLVGTPGAELLKKISSESARNYIQSLTQMPKMNFANVFIGANPLAVDLL
EKMLVLDSDKRITAAQALAHAYFAQYHDPDDEPVADPYDQSFESRDLLIDEWKSLT
YDEVISFVPPPLDQEEMES (SEQ ID NO:1).

[0061] In certain embodiments, the targeted p38 gene, cDNA, mRNA or coding sequence comprises the p38-α nucleic acid sequence set forth below or disclosed in GenBank accession NM_001315.2, or a complement thereof:

TTCTCTCACGAAGCCCCGCCCGCGGAGAGGTTCCATATTGGGTAAAATCTCGGCT
CTCGGAGAGTCCCGGGAGCTGTTCTCGCGAGAGTACTGCGGGAGGCTCCCGTTTG
CTGGCTCTTGGAACCGCGACCACTGGAGCCTTAGCGGGCGCAGCAGCTGGAACG
GGAGTACTGCGACGCAGCCCGGAGTCGGCCTTGTAGGGGCGAAGGTGCAGGGAG
ATCGCGGCGGGCGCAGTCTTGAGCGCCGGAGCGCGTCCCTGCCCTTAGCGGGGC
TTGCCCCAGTCGCAGGGGCACATCCAGCCGCTGCGGCTGACAGCAGCCGCGCGC
GCGGGAGTCTGCGGGGTCGCGGCAGCCGCACCTGCGCGGGCGACCAGCGCAAGG
TCCCCGCCCGGCTGGGCGGGCAGCAAGGGCCGGGGAGAGGGTGCGGGTGCAGG
CGGGGGCCCCACAGGGCCACCTTCTTGCCCGGCGGCTGCCGCTGGAAAATGTCTC

AGGAGAGGCCCACGTTCTACCGGCAGGAGCTGAACAAGACAATCTGGGAGGTGC
CCGAGCGTTACCAGAACCTGTCTCCAGTGGGCTCTGGCGCCTATGGCTCTGTGTG
TGCTGCTTTTGACACAAAAACGGGGTTACGTGTGGCAGTGAAGAAGCTCTCCAG
ACCATTTCAGTCCATCATTCATGCGAAAAGAACCTACAGAGAACTGCGGTTACTT
AAACATATGAAACATGAAAATGTGATTGGTCTGTTGGACGTTTTTACACCTGCAA
GGTCTCTGGAGGAATTCAATGATGTGTATCTGGTGACCCATCTCATGGGGGCAGA
TCTGAACAACATTGTGAAATGTCAGAAGCTTACAGATGACCATGTTCAGTTCCTT
ATCTACCAAATTCTCCGAGGTCTAAAGTATATACATTCAGCTGACATAATTCACA
GGGACCTAAAACCTAGTAATCTAGCTGTGAATGAAGACTGTGAGCTGAAGATTC
TGGATTTTGGACTGGCTCGGCACACAGATGATGAAATGACAGGCTACGTGGCCA
CTAGGTGGTACAGGGCTCCTGAGATCATGCTGAACTGGATGCATTACAACCAGA
CAGTTGATATTTGGTCAGTGGGATGCATAATGGCCGAGCTGTTGACTGGAAGAAC
ATTGTTTCCTGGTACAGACCATATTAACCAGCTTCAGCAGATTATGCGTCTGACA
GGAACACCCCCCGCTTATCTCATTAACAGGATGCCAAGCCATGAGGCAAGAAAC
TATATTCAGTCTTTGACTCAGATGCCGAAGATGAACTTTGCGAATGTATTTATTG
GTGCCAATCCCCTGGCTGTCGACTTGCTGGAGAAGATGCTTGTATTGGACTCAGA
TAAGAGAATTACAGCGGCCCAAGCCCTTGCACATGCCTACTTTGCTCAGTACCAC
GATCCTGATGATGAACCAGTGGCCGATCCTTATGATCAGTCCTTTGAAAGCAGGG
ACCTCCTTATAGATGAGTGGAAAAGCCTGACCTATGATGAAGTCATCAGCTTTGT
GCCACCACCCCTTGACCAAGAAGAGATGGAGTCCTGAGCACCTGGTTTCTGTTCT
GTTGATCCCACTTCACTGTGAGGGGAAGGCCTTTTCACGGGAACTCTCCAAATAT
TATTCAAGTGCCTCTTGTTGCAGAGATTTCCTCCATGGTGGAAGGGGGTGTGCGT
GCGTGTGCGTGCGTGTTAGTGTGTGTGCATGTGTGTGTCTGTCTTTGTGGGAGGG
TAAGACAATATGAACAAACTATGATCACAGTGACTTTACAGGAGGTTGTGGATG
CTCCAGGGCAGCCTCCACCTTGCTCTTCTTTCTGAGAGTTGGCTCAGGCAGACAA
GAGCTGCTGTCCTTTTAGGAATATGTTCAATGCAAAGTAAAAAAATATGAATTGT
CCCCAATCCCGGTCATGCTTTTGCCACTTTGGCTTCTCCTGTGACCCCACCTTGAC
GGTGGGGCGTAGACTTGACAACATCCCACAGTGGCACGGAGAGAAGGCCCATAC
CTTCTGGTTGCTTCAGACCTGACACCGTCCCTCAGTGATACGTACAGCCAAAAAG
GACCAACTGGCTTCTGTGCACTAGCCTGTGATTAACTTGCTTAGTATGGTTCTCA
GATCTTGACAGTATATTTGAAACTGTAAATATGTTTGTGCCTTAAAAGGAGAGAA
GAAAGTGTAGATAGTTAAAAGACTGCAGCTGCTGAAGTTCTGAGCCGGGCAAGT
CGAGAGGGCTGTTGGACAGCTGCTTGTGGGCCCGGAGTAATCAGGCAGCCTTCA
TAGGCGGTCATGTGTGCATGTGAGCACATGCGTATATGTGCGTCTCTCTTTCTCCC

TCACCCCCAGGTGTTGCCATTTCTCTGCTTACCCTTCACCTTTGGTGCAGAGGTTT
CTTGAATATCTGCCCCAGTAGTCAGAAGCAGGTTCTTGATGTCATGTACTTCCTG
TGTACTCTTTATTTCTAGCAGAGTGAGGATGTGTTTTGCACGTCTTGCTATTTGAG
CATGCACAGCTGCTTGTCCTGCTCTCTTCAGGAGGCCCTGGTGTCAGGCAGGTTT
GCCAGTGAAGACTTCTTGGGTAGTTTAGATCCCATGTCACCTCAGCTGATATTAT
GGCAAGTGATATCACCTCTCTTCAGCCCCTAGTGCTATTCTGTGTTGAACACAAT
TGATACTTCAGGTGCTTTTGATGTGAAAATCATGAAAAGAGGAACAGGTGGATG
TATAGCATTTTTATTCATGCCATCTGTTTTCAACCAACTATTTTTGAGGAATTATC
ATGGGAAAAGACCAGGGCTTTTCCCAGGAATATCCCAAACTTCGGAAACAAGTT
ATTCTCTTCACTCCCAATAACTAATGCTAAGAAATGCTGAAAATCAAAGTAAAAA
ATTAAAGCCCATAAGGCCAGAAACTCCTTTTGCTGTCTTTCTCTAAATATGATTA
CTTTAAAATAAAAAAGTAACAAGGTGTCTTTTCCACTCCTATGGAAAAGGGTCT
TCTTGGCAGCTTAACATTGACTTCTTGGTTTGGGGAGAAATAAATTTTGTTTCAGA
ATTTTGTATATTGTAGGAATCCTTTGAGAATGTGATTCCTTTTGATGGGGAGAAA
GGGCAAATTATTTTAATATTTTGTATTTTCAACTTTATAAAGATAAAATATCCTCA
GGGGTGGAGAAGTGTCGTTTTCATAACTTGCTGAATTTCAGGCATTTTGTTCTAC
ATGAGGACTCATATATTTAAGCCTTTTGTGTAATAAGAAAGTATAAAGTCACTTC
CAGTGTTGGCTGTGTGACAGAATCTTGTATTTGGGCCAAGGTGTTTCCATTTCTCA
ATCAGTGCAGTGATACATGTACTCCAGAGGGACAGGGTGGACCCCCTGAGTCAA
CTGGAGCAAGAAGGAAGGAGGCAGACTGATGGCGATTCCCTCTCACCCGGGACT
CTCCCCCTTTCAAGGAAAGTGAACCTTTAAAGTAAAGGCCTCATCTCCTTTATTG
CAGTTCAAATCCTCACCATCCACAGCAAGATGAATTTTATCAGCCATGTTTGGTT
GTAAATGCTCGTGTGATTTCCTACAGAAATACTGCTCTGAATATTTTGTAATAAA
GGTCTTTGCACATGTGACCACATACGTGTTAGGAGGCTGCATGCTCTGGAAGCCT
GGACTCTAAGCTGGAGCTCTTGGAAGAGCTCTTCGGTTTCTGAGCATAATGCTCC
CATCTCCTGATTTCTCTGAACAGAAAACAAAAGAGAGAATGAGGGAAATTGCTA
TTTTATTTGTATTCATGAACTTGGCTGTAATCAGTTATGCCGTATAGGATGTCAGA
CAATACCACTGGTTAAAATAAAGCCTATTTTTCAAATTTAGTGAGTTTCTCAAGTT
TATTATATTTTTCTCTTGTTTTTATTTAATGCACAATATGGCATTATATCAATATCC
TTTAAACTGTGACCTGGCATACTTGTCTGACAGATCTTAATACTACTCCTAACATT
TAGAAAATGTTGATAAAGCTTCTTAGTTGTACATTTTTTGGTGAAGAGTATCCAG
GTCTTTGCTGTGGATGGGTAAAGCAAAGAGCAAATGAACGAAGTATTAAGCATT
GGGGCCTGTCTTATCTACACTCGAGTGTAAGAGTGGCCGAAATGACAGGGCTCA
GCAGACTGTGGCCTGAGGGCCAAATCTGGCCCACCACCTGTTTGGTGTAGCCTGC

TAAGAATGGCTTTTACATTTTTAAATGGTTGGGAAAGAAAAAAAAAGAAGTAGT
AGATTTTGTAGCATGTGATGTAAGTAATGTAAAACTTAAATTCCAGTATCCATAA
ATAAAGTTTTATGAGAACAGA (SEQ ID NO:2).

[0062] In certain embodiment, the targeted p38 protein comprises the amino acid sequence set forth below or disclosed in GenBank accession NP_002742.3 for p38 kinase (mitogen-activated protein kinase 11 isoform 1, homo sapiens):

MSGPRAGFYRQELNKTVWEVPQRLQGLRPVGSGAYGSVCSAYDARLRQKVAVKKL
SRPFQSLIHARRTYRELRLLKHLKHENVIGLLDVFTPATSIEDFSEVYLVTTLMGADL
NNIVKCQALSDEHVQFLVYQLLRGLKYIHSAGIIHRDLKPSNVAVNEDCELRILDFGL
ARQADEEMTGYVATRWYRAPEIMLNWMHYNQTVDIWSVGCIMAELLQGKALFPG
SDYIDQLKRIMEVVGTPSPEVLAKISSEHARTYIQSLPPMPQKDLSSIFRGANPLAIDLL
GRMLVLDSDQRVSAAEALAHAYFSQYHDPEDEPEAEPYDESVEAKERTLEEWKELT
YQEVLSFKPPEPPKPPGSLEIEQ (SEQ ID NO:3).

[0063] In certain embodiments, the targeted p38 gene, cDNA, mRNA or coding sequence comprises the p38-β nucleic acid sequence set forth below or disclosed in GenBank accession NM_002751.6, or a complement thereof:

CGCCGCCTCCGCCGCCCTCCGCTCCGCTCGGCTCGGGCTCGGCTCGGGCGCGGGC
GCGGGGCGCGGGGCTGGGCCCGGGCGGAGCGGCGGCTGCTCCGGACATGTCGGG
CCCTCGCGCCGGCTTCTACCGGCAGGAGCTGAACAAGACCGTGTGGGAGGTGCC
GCAGCGGCTGCAGGGGCTGCGCCCGGTGGGCTCCGGCGCCTACGGCTCCGTCTG
TTCGGCCTACGACGCCCGGCTGCGCCAGAAGGTGGCGGTGAAGAAGCTGTCGCG
CCCCTTCCAGTCGCTGATCCACGCGCGCAGAACGTACCGGGAGCTGCGGCTGCTC
AAGCACCTGAAGCACGAGAACGTCATCGGGCTTCTGGACGTCTTCACGCCGGCC
ACGTCCATCGAGGACTTCAGCGAAGTGTACTTGGTGACCACCCTGATGGGCGCC
GACCTGAACAACATCGTCAAGTGCCAGGCGCTGAGCGACGAGCACGTTCAATTC
CTGGTTTACCAGCTGCTGCGCGGGCTGAAGTACATCCACTCGGCCGGGATCATCC
ACCGGGACCTGAAGCCCAGCAACGTGGCTGTGAACGAGGACTGTGAGCTCAGGA
TCCTGGATTTCGGGCTGGCGCGCCAGGCGGACGAGGAGATGACCGGCTATGTGG
CCACGCGCTGGTACCGGGCACCTGAGATCATGCTCAACTGGATGCATTACAACC
AAACAGTGGATATCTGGTCCGTGGGCTGCATCATGGCTGAGCTGCTCCAGGGCA
AGGCCCTCTTCCCGGGAAGCGACTACATTGACCAGCTGAAGCGCATCATGGAAG
TGGTGGGCACACCCAGCCCTGAGGTTCTGGCAAAAATCTCCTCAGAACACGCCC
GGACATATATCCAGTCCCTGCCCCCCATGCCCCAGAAGGACCTGAGCAGCATCTT

CCGTGGAGCCAACCCCCTGGCCATAGACCTCCTTGGAAGGATGCTGGTGCTGGA
CAGTGACCAGAGGGTCAGTGCAGCTGAGGCACTGGCCCACGCCTACTTCAGCCA
GTACCACGACCCCGAGGATGAGCCAGAGGCCGAGCCATATGATGAGAGCGTTGA
GGCCAAGGAGCGCACGCTGGAGGAGTGGAAGGAGCTCACTTACCAGGAAGTCCT
CAGCTTCAAGCCCCCAGAGCCACCGAAGCCACCTGGCAGCCTGGAGATTGAGCA
GTGAGGTGCTGCCCAGCAGCCCCTGAGAGCCTGTGGAGGGGCTTGGGCCTGCAC
CCTTCCACAGCTGGCCTGGTTTCCTCGAGAGGCACCTCCCACACTCCTATGGTCA
CAGACTTCTGGCCTAGGACCCCTCGCCTTCAGGAGAATCTACACGCATGTATGCA
TGCACAAACATGTGTGTACATGTGCTTGCCATGTGTAGGAGTCTGGGCACAAGTG
TCCCTGGGCCTACCTTGGTCCTCCTGTCCTCTTCTGGCTACTGCACTCTCCACTGG
GACCTGACTGTGGGGTCCTAGATGCCAAAGGGGTTCCCCTGCGGAGTTCCCCTGT
CTGTCCCAGGCCGACCCAAGGGAGTGTCAGCCTTGGGCTCTCTTCTGTCCCAGGG
CTTTCTGGAGGACGCGCTGGGGCCGGGACCCCGGGAGACTCAAAGGGAGAGGTC
TCAGTGGTTAGAGCTGCTCAGCCTGGAGGTAGGGGGCTGTCTTGGTCACTGCTGA
GACCCACAGGTCTAAGAGGAGAGGCAGAGCCAGTGTGCCACCAGGCTGGGCAG
GGACAACCACCAGGTGTCAAATGAGAAAAGCTGCCTGGAGTCTTGTGTTCACCC
GTGGGTGTGTGTGGGCACGTGTGGATGAGCGTGCACTCCCCGTGTTCATATGTCA
GGGCACATGTGATGTGGTGCGTGTGAATCTGTGGGCGCCCAAGGCCAGCAGCCA
TATCTGGCAAGAAGCTGGAGCCGGGGTGGGTGTGCTGTTGCCTTCCCTCTCCTCG
GTTCCTGATGCCTTGAGGGGTGTTTCAGACTGGCGGCTCCAGTGGGCCAAAGGGC
AACCACATGAGCATGGGCAGGGGCTTTCTCCTTGGATGTGGGACCCACAGCAGC
TTCCTGAGGCTGGGGGTGGGTGGGTGGGTGGTTTGGCCTTGAGGACGCTAGGGC
AGGCAGCACACCTGGATGTGGACTTGGACTCGGACACTTCTGCCCTGCACCCTGG
CCCGCTCTCTACCTCTGCCCACCGTTGTGGCCCTGCAGCCGGAGATCTGAGGTGC
TCTGGTCTGTGGGTCAGTCCTCTTTCCTTGTCCCAGGATGGAGCTGATCCAGTAA
CCTCGGAGACGGGACCCTGCCCAGAGCTGAGTTGGGGGTGTGGCTCTGCCCTGG
AAAGGGGGTGACCTCTTGCCTCGAGGGGCCCAGGGAAGCCTGGGTGTCAAGTGC
CTGCACCAGGGGTGCACAATAAAGGGGGTTCTCTCTCAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAA (SEQ ID NO:4)

[0064] In certain embodiments, methods disclosed herein comprising contacting a cell, tissue, organ, or subject with a p38 inhibitor, are practiced to inhibit or decrease the expression or activity of DUX4 or one or more DUX4 downstream genes. In certain embodiments, the DUX4 or DUX4 downstream gene is a human gene. For example, the

DUX4 double homeobox 4 (Homo sapiens) gene may comprise the nucleotide sequence set forth below or disclosed in GenBank accession NG_034189.2, or a complement thereof:

ATGGCCCTCCCGACACCCTCGGACAGCACCCTCCCCGCGGAAGCCCGGGGACGA
GGACGGCGACGGAGACTCGTTTGGACCCCGAGCCAAAGCGAGGCCCTGCGAGCC
TGCTTTGAGCGGAACCCGTACCCGGGCATCGCCACCAGAGAACGGCTGGCCCAG
GCCATCGGCATTCCGGAGCCCAGGGTCCAGATTTGGTTTCAGAATGAGAGGTCA
CGCCAGCTGAGGCAGCACCGGCGGGAATCTCGGCCCTGGCCCGGGAGACGCGGC
CCGCCAGAAGGCCGGCGAAAGCGGACCGCCGTCACCGGATCCCAGACCGCCCTG
CTCCTCCGAGCCTTTGAGAAGGATCGCTTTCCAGGCATCGCCGCCCGGGAGGAGC
TGGCCAGAGAGACGGGCCTCCCGGAGTCCAGGATTCAGATCTGGTTTCAGAATC
GAAGGGCCAGGCACCCGGGACAGGGTGGCAGGGCGCCCGCGCAGGCAGGCGGC
CTGTGCAGCGCGGCCCCCGGCGGGGGTCACCCTGCTCCCTCGTGGGTCGCCTTCG
CCCACACCGGCGCGTGGGGAACGGGGCTTCCCGCACCCCACGTGCCCTGCGCGC
CTGGGGCTCTCCCACAGGGGGCTTTCGTGAGCCAGGCAGCGAGGGCCGCCCCCG
CGCTGCAGCCCAGCCAGGCCGCGCCGGCAGAGGGGATCTCCCAACCTGCCCCGG
CGCGCGGGGATTTCGCCTACGCCGCCCCGGCTCCTCCGGACGGGGCGCTCTCCCA
CCCTCAGGCTCCTCGCTGGCCTCCGCACCCGGGCAAAAGCCGGGAGGACCGGGA
CCCGCAGCGCGACGGCCTGCCGGGCCCCTGCGCGGTGGCACAGCCTGGGCCCGC
TCAAGCGGGGCCGCAGGGCCAAGGGGTGCTTGCGCCACCCACGTCCCAGGGGAG
TCCGTGGTGGGGCTGGGGCCGGGGTCCCCAGGTCGCCGGGGCGGCGTGGGAACC
CCAAGCCGGGGCAGCTCCACCTCCCCAGCCCGCGCCCCCGGACGCCTCCGCCTCC
GCGCGGCAGGGGCAGATGCAAGGCATCCCGGCGCCCTCCCAGGCGCTCCAGGAG
CCGGCGCCCTGGTCTGCACTCCCCTGCGGCCTGCTGCTGGATGAGCTCCTGGCGA
GCCCGGAGTTTCTGCAGCAGGCGCAACCTCTCCTAGAAACGGAGGCCCCGGGGG
AGCTGGAGGCCTCGGAAGAGGCCGCCTCGCTGGAAGCACCCCTCAGCGAGGAAG
AATACCGGGCTCTGCTGGAGGAGCTTTAGGACGCGGGGTTGGGACGGGGTCGGG
TGGTTCGGGGCAGGGCGGTGGCCTCTCTTTCGCGGGGAACACCTGGCTGGCTACG
GAGGGGCGTGTCTCCGCCCCGCCCCCTCCACCGGGCTGACCGGCCTGGGATTCCT
GCCTTCTAGGTCTAGGCCCGGTGAGAGACTCCACACCGCGGAGAACTGCCATTCT
TTCCTGGGCATCCCGGGGATCCCAGAGCCGGCCCAGGTACCAGCAGGTGGGCCG
CCTACTGCGCACGCGCGGGTTTGCGGGCAGCCGCCTGGGCTGTGGGAGCAGCCC
GGGCAGAGCTCTCCTGCCTCTCCACCAGCCCACCCCGCCGCCTGACCGCCCCCTC
CCCACCCCCACCCCCCACCCCCGGAAAACGCGTCGTCCCCTGGGCTGGGTGGAG
ACCCCCGTCCCGCGAAACACCGGGCCCCGCGCAGCGTCCGGGCCTGACACCGCT

CCGGCGGCTCGCCTCCTCTGCGCCCCCGCGCCACCGTCGCCCGCCCGCCCGGGCC
CCTGCAGCCTCCCAGCTGCCAGCACGGAGCGCCTGGCGGTCAAAAGCATACCTC
TGTCTGTCTTTGCCCGCTTCCTGGCTAGACCTGCGCGCAGTGCGCACCCCGGCTG
ACGTGCAAGGGAGCTCGCTGGCCTCTCTGTGCCCTTGTTCTTCCGTGAAATTCTG
GCTGAATGTCTCCCCCCACCTTCCGACGCTGTCTAGGCAAACCTGGATTAGAGTT
ACATCTCCTGGATGATTAGTTCAGAGATATATTAAAATGCCCCCTCCCTGTGGAT
CCTATAG (SEQ ID NO:5).

[0065] For example, the DUX4 double homeobox 4 [Homo sapiens] mRNA gene may comprise the nucleotide sequence set forth below or disclosed in GenBank accession NM_001293798.2, or a complement thereof:

ATGGCCCTCCCGACACCCTCGGACAGCACCCTCCCCGCGGAAGCCCGGGGACGA
GGACGGCGACGGAGACTCGTTTGGACCCCGAGCCAAAGCGAGGCCCTGCGAGCC
TGCTTTGAGCGGAACCCGTACCCGGGCATCGCCACCAGAGAACGGCTGGCCCAG
GCCATCGGCATTCCGGAGCCCAGGGTCCAGATTTGGTTTCAGAATGAGAGGTCA
CGCCAGCTGAGGCAGCACCGGCGGGAATCTCGGCCCTGGCCCGGGAGACGCGGC
CCGCCAGAAGGCCGGCGAAAGCGGACCGCCGTCACCGGATCCCAGACCGCCCTG
CTCCTCCGAGCCTTTGAGAAGGATCGCTTTCCAGGCATCGCCGCCCGGGAGGAGC
TGGCCAGAGAGACGGGCCTCCCGGAGTCCAGGATTCAGATCTGGTTTCAGAATC
GAAGGGCCAGGCACCCGGGACAGGGTGGCAGGGCGCCCGCGCAGGCAGGCGGC
CTGTGCAGCGCGGCCCCCGGCGGGGGTCACCCTGCTCCCTCGTGGGTCGCCTTCG
CCCACACCGGCGCGTGGGGAACGGGGCTTCCCGCACCCCACGTGCCCTGCGCGC
CTGGGGCTCTCCCACAGGGGGCTTTCGTGAGCCAGGCAGCGAGGGCCGCCCCCG
CGCTGCAGCCCAGCCAGGCCGCGCCGGCAGAGGGGATCTCCCAACCTGCCCCGG
CGCGCGGGGATTTCGCCTACGCCGCCCCGGCTCCTCCGGACGGGGCGCTCTCCCA
CCCTCAGGCTCCTCGCTGGCCTCCGCACCCGGGCAAAAGCCGGGAGGACCGGGA
CCCGCAGCGCGACGGCCTGCCGGGCCCCTGCGCGGTGGCACAGCCTGGGCCCGC
TCAAGCGGGGCCGCAGGGCCAAGGGGTGCTTGCGCCACCCACGTCCCAGGGGAG
TCCGTGGTGGGGCTGGGGCCGGGGTCCCCAGGTCGCCGGGGCGGCGTGGGAACC
CCAAGCCGGGGCAGCTCCACCTCCCCAGCCCGCGCCCCCGGACGCCTCCGCCTCC
GCGCGGCAGGGGCAGATGCAAGGCATCCCGGCGCCCTCCCAGGCGCTCCAGGAG
CCGGCGCCCTGGTCTGCACTCCCCTGCGGCCTGCTGCTGGATGAGCTCCTGGCGA
GCCCGGAGTTTCTGCAGCAGGCGCAACCTCTCCTAGAAACGGAGGCCCCGGGGG
AGCTGGAGGCCTCGGAAGAGGCCGCCTCGCTGGAAGCACCCCTCAGCGAGGAAG
AATACCGGGCTCTGCTGGAGGAGCTTTAGGACGCGGGGTCTAGGCCCGGTGAGA

GACTCCACACCGCGGAGAACTGCCATTCTTTCCTGGGCATCCCGGGGATCCCAGA
GCCGGCCCAGGTACCAGCAGACCTGCGCGCAGTGCGCACCCCGGCTGACGTGCA
AGGGAGCTCGCTGGCCTCTCTGTGCCCTTGTTCTTCCGTGAAATTCTGGCTGAAT
GTCTCCCCCCACCTTCCGACGCTGTCTAGGCAAACCTGGATTAGAGTTACATCTC
CTGGATGATTAGTTCAGAGATATATTAAAATGCCCCCTCCCTGTGGATCCTATAG
(SEQ ID NO:6).

[0066] In particular embodiment, the DUX4 polypeptide sequence is as set forth below or disclosed in GenBank accession NP_001280727.1:

MALPTPSDSTLPAEARGRGRRRRLVWTPSQSEALRACFERNPYPGIATRERLAQAIGI
PEPRVQIWFQNERSRQLRQHRRESRPWPGRRGPPEGRRKRTAVTGSQTALLLRAFEK
DRFPGIAAREELARETGLPESRIQIWFQNRRARHPGQGGRAPAQAGGLCSAAPGGGH
PAPSWVAFAHTGAWGTGLPAPHVPCAPGALPQGAFVSQAARAAPALQPSQAAPAE
GISQPAPARGDFAYAAPAPPDGALSHPQAPRWPPHPGKSREDRDPQRDGLPGPCAVA
QPGPAQAGPQGQGVLAPPTSQGSPWWGWGRGPQVAGAAWEPQAGAAPPPQPAPPD
ASASARQGQMQGIPAPSQALQEPAPWSALPCGLLLDELLASPEFLQQAQPLLETEAP
GELEASEEAASLEAPLSEEEYRALLEEL (SEQ ID NO:7).

[0067] Sequences of DUX4 downstream genes or targets are known in the art and illustrative DUX4 downstream genes are provided by the accession numbers shown below:

MBD3L2:

Genomic nucleotide accession NC_000019.10 (7049340..7051735)

mRNA nucleotide accession NM_144614.3

protein polypeptide accession NP_653215.2

ZSCAN4:

NC_000019.10 (57651497..57679152)

NM_152677.2

NP_689890.1

LEUTX:

NC_000019.10 (39776594..39786135)

NM_001143832.1

NP_001137304.1

PRAMEF2:

NC_000001.11 (12857086..12861909)

NM_023014.1

NP_075390.1

TRIM43:

NC_000002.12 (95592018..95599723)

NM_138800.2

NP_620155.1

KHDC1L:

NC_000006.12 (73223544..73225452, complement)

NM_001126063.2

NP_001119535.1

[0068] Methods of determining the expression level of p38, DUX4, or a DUX4 downstream gene or polypeptide in a biological sample, e.g., tissue, are known in the art and include, e.g., RT-PCR and FACS.

[0069] In one embodiment, a method of reducing the expression of a DUX4 mRNA (e.g., DUX4-fl), a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in a cell, tissue, organ, or subject, comprises contacting the cell, tissue, organ, or subject with an agent that results in a reduced amount of active p38 protein (also referred to herein as a p38 inhibitor), e.g., an inhibitor of p38-α and/or p38-β. In certain embodiments, the agent inhibits the expression or activity of a p38 protein. In certain embodiments, the agent causes increased degradation of a p38 protein, e.g., p38-α and/or p38-β. In particular embodiments, the cell or tissue is contacted with an amount of the agent effective to reduce the expression or activity of a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in the cell or tissue. In certain embodiments, the cell or tissue is contacted with an amount of the agent effective to reduce the amount of active p38 protein in the cell or tissue. In particular embodiments, the cells are muscle cells. In certain embodiments, the cells are terminally differentiated, e.g., terminally differentiated muscle cells. In some embodiments, the cells have an increased expression level of the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level in a control cell. In certain embodiments, the cells are associated with facioscapulohumeral muscular dystrophy (FSHD), e.g., FSHD1 or FSHD2. For example, the cells may be derived from or obtained from cells or tissue from a subject diagnosed with FSHD. Methods disclosed herein may be practiced in vitro or in vivo.

[0070] In an embodiment, the disclosure provides a method of reducing apoptosis of a cell or tissue, comprising contacting the cell or tissue with an agent that inhibits the expression or activity of a p38 protein (also referred to herein as a p38 inhibitor), e.g., an inhibitor of p38-α

and/or p38-β. In particular embodiments, the cell or tissue is contacted with an amount of the agent effective to reduce the expression or activity of a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in the cell or tissue. In certain embodiments, the cell or tissue is contacted with an amount of the agent effective to reduce the amount of active p38 protein in the cell or tissue. In particular embodiments, the cells are muscle cells. In certain embodiments, the cells are terminally differentiated, e.g., terminally differentiated muscle cells. In some embodiments, the cells have an increased expression level of the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level in a control cell (i.e., before treatment). In certain embodiments, the cells are associated with facioscapulohumeral muscular dystrophy (FSHD), e.g., FSHD1 or FSHD2. For example, the cells may be derived from or obtained from cells or tissue from a subject diagnosed with FSHD. Methods disclosed herein may be practiced in vitro or in vivo.

[0071] In a related aspect, the disclosure includes a method of treating or preventing a disease or disorder associated with increased activity or expression of a DUX4 protein or a downstream target gene of DUX4 in a subject in need thereof, comprising providing to a subject a pharmaceutical composition comprising an effective amount of an agent that reduces the amount of active p38 protein (e.g., p38-α and/or p38-β) in the subject, or in certain cells or tissue of the subject. In some embodiments, the agent inhibits the expression or activity of a p38 protein, e.g., p38-α and/or p38-β. In certain embodiments, the agent induces degradation of the p38 protein. In certain embodiments, the agent inhibits activity of a p38 protein, e.g., inhibits kinase activity of the p38 protein. In particular embodiments of any of the methods, the p38 inhibitor reduces expression of DUX4 and/or one or more DUX4 downstream genes in cells or tissue of the subject.

[0072] In particular embodiments of methods of treatment disclosed herein, the disease or disorder is selected from FSHD 1, FSHD2, Immunodeficiency, Centromere instability and Facial anomalies syndrome (ICF), amyotrophic lateral sclerosis (ALS), inclusion body myositis (IBM), Ewing's Sarcoma, soft tissue sarcoma, rhabdomyosarcoma, and adult and pediatric B-cell acute lymphoblastic leukemia.

[0073] In particular embodiments of any of the methods disclosed herein, the subject is diagnosed with FSHD1 or FSHD2, and in certain embodiments, the subject comprises one or more genetic mutation associated with FSHD1 and/or FSHD2. In certain embodiments, the subject comprises reduced repression at a D4Z4 locus.

[0074] In certain embodiments of any of the methods disclosed herein, the subject is identified as having FSHD based upon the presence of a transcriptionally active DUX4. In another embodiment, the subject is identified as having FSHD based upon the presence of increased expression levels of one or more downstream genes, e.g., ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, or ZNF280A relative to a healthy control. In another embodiment, the subject is identified as having FSHD based upon the presence of a transcriptionally active DUX4 and increased expression levels of one or more DUX4 downstream genes, e.g., ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, or ZNF280A.

[0075] In another embodiment, the method may include measuring the expression level of one or more of DUX4 and DUX4 downstream genes, e.g., DUX4, ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, or ZNF280A, in the subject prior to the administration of the p38 kinase inhibitor. The method may further include determining that the subject is in need of treatment if the expression level of one or more of DUX4 and DUX4 downstream genes, e.g., DUX4, ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, and ZNF280A KHDC1L is/are elevated relative to a healthy control.

[0076] In another embodiment, the method may include measuring the expression level of one or more of DUX 4 and DUX4 downstream genes, e.g., DUX4, ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, or ZNF280A, in the cells of the subject before and after the administration of the p38 kinase inhibitor. The method may include comparing the expression level of one or more of DUX4 and DUX4 downstream genes, e.g., DUX4, ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, or ZNF280A in the subject before and after the administration of the p38 kinase inhibitor. The method may include determining the effectiveness of treatment by the comparing of the expression level of one or more of DUX4 and DUX4 downstream genes, e.g., DUX4, ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, and ZNF280A before

and after the administration of the p38 kinase inhibitor, wherein a decrease in the expression level(s) is indicative of effective treatment.

[0077] In some embodiments, the p38 kinase inhibitor reduces one or more downstream genes selected from ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, and ZNF280A.

[0078] In one embodiment, a transcriptional modulator of DUX4 and downstream genes ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15, and ZNF280A are inhibited by p38 kinase.

[0079] In particular embodiments, the subject comprises contraction of 4q35A D4Z4 array, such that the subject comprises $\leq$ 10 or $\leq$ 7 repeats (FSHD1). In certain embodiments, the subject, or one or more cells or tissues of the subject, comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises $\leq$7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35. In certain embodiments, the subject comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene. In certain embodiments, the subject comprises at least one non-deleted 4qA allele. In certain embodiments, the subject comprises at least one non-deleted 4qA allele and an SMCHD1 mutation (FSHD2). In some embodiments, the subject is wheelchair bound (e.g., CSS 4.5 and 5). In certain embodiments, during or following treatment, the subject displays a reduced or decreased amount or rate of muscle degeneration e.g., a subject diagnosed with FSHD1 or FSHD2. In certain embodiments, during or following treatment, the subject displays a reduction of skeletal muscle replacement by fat, e.g., as determined via quantitative MRI, e.g., a reduction of at least 5%, at least 10%, at least 20%, at least 30%, at least 50%, or at least 70%. In certain embodiments, during or following treatment, the subject displays evidence of benefit on one or more of the following Clinical Outcome Assessments:

- Shoulder/arm function as measured by Reachable Work Space (RWS) w/wo weights;
- Mobility as measured by Time Up and Go (TUG) or a similar assay;
- Patient reports of Activities of Daily Life (ADLs) and Quality of Life (QOL); and

- Quantitative skeletal muscle strength as measured by dynamometry.

[0080] In some embodiments, the subject displays any of these improvements for at least some time, e.g., for at least one week, one month, two months, six months, or one year following either commencement or cessation of treatment.

[0081] In another aspect, the disclosure provides the disclosed methods of using p38 inhibitors, e.g., an inhibitor of p38-α or p38-β, for treatment for FSHD1, FSHD2, ICF, and diseases where similar pathological changes are found, such ALS and IBM (Tawil et al., 2014).

[0082] In particular embodiments of any of the methods described herein, the pharmaceutical composition is provided to the subject parenterally.

[0083] In particular embodiments of any of the methods described herein, the pharmaceutical composition is provided to a muscle tissue of the subject.

[0084] In some certain embodiments, any of the methods described herein that comprise providing to the subject a p38 inhibitor may further comprise providing to the subject an additional therapy.

[0085] In particular embodiments, the additional therapy comprises clinical management. In one embodiment, the present invention provides a method for treating or preventing of FSHD 1, FSHD2, ICF, ALS, IBM, Ewing's Sarcoma, soft tissue sarcoma, rhabdomyosarcoma, and adult and pediatric B-cell acute lymphoblastic leukemia, where p38 inhibitors are used to decrease DUX4 and/or downstream gene and/or protein expression and/or activity and may be combined with clinical management involving physical therapy, aerobic exercise, respiratory function therapy, and/or orthopedic interventions.

[0086] In particular embodiments, the additional therapy comprises providing to the subject one or more myostatin inhibitors, anti-inflammatory agents, or gene therapy vectors, e.g., to reduce pathogenic DUX4 protein production in FSHD by controlling D4Z4 methylation, suppressing DUX4 mRNA, and/or inhibiting DUX4 signaling pathways. In one embodiment, the present disclosure provides a method of treatment of FSHD 1, FSHD2, ICF, ALS or IBM, in a subject in need thereof, where p38 inhibitors, e.g., inhibitors of p38-α or p38-β, are used to reduce DUX4 and downstream gene and/or protein expression and may be combined with myostatin inhibitors, anti-inflammatory agents, and/or gene therapy, e.g., to reduce pathogenic DUX4 protein production in FSHD by controlling D4Z4 methylation, suppressing DUX4 mRNA, and inhibiting DUX4 pathways. In certain embodiments, the methods are practiced using an inhibitor of p38 and a myostatin inhibitor. Particular

myostatin pathway inhibitors that act extracellularly by either binding myostatin directly (Fstl3, Follistatin, myostatin antibody, GASP1, myostatin propeptide, decorin peptides, ActRIIB-Fc) or by binding its receptor complex (ActRIIB antibody) in order to block myostatin engaging its receptor complex and activating downstream signaling may be used in certain embodiments. Some of the myostatin inhibitors are naturally occurring (myostatin propeptide, Gasp1, follistatin, Fstl3) whereas others are engineered (myostatin antibody, ActRIIB antibody, ActRIIB-Fc).

[0087] In particular embodiments, the additional therapy comprises providing to the subject an inhibitor of DUX4 or a DUX4 downstream target or gene, e.g., an inhibitor that inhibits expression of DUX-4fl mRNA and/or DUX4 protein (or expression of mRNA or protein of a DUX4 downstream target) or an inhibitor that inhibits DUX4 activity, e.g., its activity as a transcriptional activator, or activity of a DUX4 downstream target. In particular embodiments, the inhibitor induces degradation of DUX4 polypeptide or DUX4 downstream target polypeptide. In particular embodiments, the inhibitor is an siRNA, miRNA, gRNA, shRNA, or antisense oligonucleotide that specifically binds a nucleic acid sequence or antisense thereof of a DUX4 or a DUX4 downstream target gene. In one embodiment, the present invention provides a method of treatment of FSHD 1, FSHD2, ICF, ALS or IBM, where p38 inhibitors are used to reduce DUX4 and downstream gene and protein expression and may be combined with an inhibitor of DUX4 or a DUX4 downstream target, e.g., small interfering RNA (siRNA), small hairpin RNA (shRNA), guide RNA (gRNA), microRNA (miRNA) and antisense oligonucleotides directed at DUX4 and/or one or more DUX4 downstream target transcripts (e.g., DNA or mRNA).

[0088] In certain embodiments, the present invention provides a method to use small molecule inhibitors of p38 kinase, e.g., an inhibitor of p38-α or p38-β, to reduce DUX4 and downstream gene expression in FSHD skeletal muscle myotubes to treat FSHD or any other disease or disorder disclosed herein and/or related to aberrant DUX4 expression or activity.

[0089] In some embodiments, p38, e.g., p38-α and/or p38-β, is inhibited by any of the small molecules or other agents disclosed herein.

[0090] p38 inhibitors and/or other agents and compositions (e.g., inhibitors) described herein can be formulated in any manner suitable for a desired administration route (e.g., parenteral or oral administration). In some embodiments, contacting an agent or composition with a cell and/or tissue is a result of administration of or providing an agent or composition to a subject. In some embodiments, an agent or composition (e.g., a p38 inhibitor) is administered at least

1, 2, 3, 4, 5, 10, 15, 20, or more times. In some embodiments of combination therapies, administration of a first agent or composition is followed by or occurs overlapping with or concurrently with the administration of a second agent or composition. The first and second agent or composition may be the same or they may be different. In some embodiments, the first and second agents or compositions are administered by the same actor and/or in the same geographic location. In some embodiments, the first and second agents or compositions are administered by different actors and/or in different geographical locations. In some embodiments, multiple agents described herein are administered as a single composition.

[0091] A wide variety of administration methods may be used in conjunction with the p38 inhibitors according to the methods disclosed herein. For example, p38 inhibitors may be administered or coadministered topically, orally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraoccularly, via local delivery (for example by catheter or stent), subcutaneously, intraadiposally, intraarticularly, intrathecally, transmucosally, pulmonary, or parenterally, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot or reservoir, for example, subcutaneously or intramuscularly.

[0092] "Subjects" includes animals (e.g., mammals, swine, fish, birds, insects etc.). In some embodiments, subjects are mammals, particularly primates, especially humans. In some embodiments, subjects are livestock such as cattle, sheep, goats, cows, swine, and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals such as dogs and cats. In some embodiments (e.g., particularly in research contexts) subjects are rodents (e.g., mice, rats, hamsters), rabbits, primates, or swine such as inbred pigs and the like. The terms "subject" and "patient" are used interchangeably herein.

[0093] "Tissue" is an ensemble of similar cells from the same origin that together carry out a specific function. In certain embodiments, tissue is muscle tissue.

[0094] Methods disclosed herein may be practiced with any agent capable of inhibiting expression or activity of a p38 gene or protein, e.g., an inhibitor of a p38-α or p38-β gene or protein, including but not limited to any of those disclosed herein.

[0095] In particular embodiments, methods disclosed herein result in a decrease in an expression level or activity of DUX4 and/or one or more DUX4 downstream genes in cells or

tissues (e.g., within a subject), e.g., as compared to the expression level or activity in control cells or tissue not contacted with a p38 inhibitor, or a reference level. "Decrease" refers to a decrease of at least 5%, for example, at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or 100%, for example, as compared to the reference level. Decrease also means decreases by at least 1-fold, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 1000-fold or more, for example, as compared to the level of a reference or control cells or tissue.

[0096] Methods described herein may be practiced using any type of inhibitor that results in a reduced amount or level of an active p38 protein, e.g., in a cell or tissue, e.g., a cell or tissue in a subject. In particular embodiments, the p38 inhibitor causes a reduction in active p38 protein (e.g., active p38-α and/or p38-β), a reduction in total p38 protein (e.g., total p38-α and/or p38-β protein), a reduction in p38 mRNA (e.g., p38-α and/or p38-β mRNA), and/or a reduction in p38 protein activity (e.g., p38-α and/or p38-β kinase activity) in a cell or tissue contacted with the p38 inhibitor. In particular embodiments, the p38 inhibitor causes a reduction in p38-α and/or p38-β signaling pathway activity or expression. In certain embodiments, the reduction is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, as compared to the level in the same type of cell or tissue not contacted with the p38 inhibitor. Methods of measuring total p38 protein or mRNA levels, or p38 kinase activity, in a cell are known in the art. In certain embodiments, the inhibitor inhibits or reduces p38 activity or expression, e.g., mRNA and/or protein expression. In certain embodiments, the inhibitor causes increased degradation of the p38 protein, resulting in lower amounts of p38 protein in a cell or tissue. Particular methods may also employ any type of inhibitor of expression or activity of DUX4 or a DUX4 downstream gene. In certain instances, the inhibitor inhibits both p38-α and p38-β proteins, while in other instances, the inhibitor selectively or preferentially inhibits either p38-α or p38-β. In certain embodiments, the inhibitor does not inhibit p38-γ.

[0097] Inhibitors that may be used to practice the disclosed methods include but are not limited to agents that inhibit or reduce or decrease the expression or activity of a biomolecule, (e.g., a protein or nucleic acid), such as but not limited to a p38-α or p38-β gene, mRNA or protein. In certain embodiments, an inhibitor can cause increased degradation of the biomolecule. In particular embodiments, an inhibitor can inhibit a biomolecule by competitive, uncompetitive, or non-competitive means. Exemplary inhibitors include, but are not limited to, nucleic acids, DNA, RNA, gRNA, shRNA, siRNA, modified mRNA (mRNA),

microRNA (miRNA), proteins, protein mimetics, peptides, peptidomimetics, antibodies, small molecules, small organic molecules, inorganic molecules, chemicals, analogs that mimic the binding site of an enzyme, receptor, or other protein, e.g., that is involved in signal transduction, therapeutic agents, pharmaceutical compositions, drugs, and combinations of these. In some embodiments, the inhibitor can be a nucleic acid molecule including, but not limited to, siRNA that reduce the amount of functional protein in a cell. Accordingly, compounds or agents said to be "capable of inhibiting" a particular protein, e.g., p38, comprise any type of inhibitor. In certain embodiments, a p38 inhibitor or an inhibitor of DUX4 or a DUX4 downstream target gene is any of the different classes of inhibitors disclosed herein or any other.

[0098] In particular embodiments, a p38 inhibitor (or other inhibitor) comprises a nucleic acid that binds to a p38 gene (e.g., *MAPK14* or *MAPK11* gene) or mRNA (or other target gene or mRNA). Accordingly, a nucleic acid inhibitor may comprise a sequence complementary to a target polynucleotide sequence, e.g., the p38-α sequence disclosed herein, or a region thereof, or an antisense thereof. In particular embodiments, a nucleic acid inhibitor comprises at least 8, at least 10, at least 12, at least 14, at least 16, at least 20, at least 24, or at least 30 nucleotide sequence corresponding to or complementary to a target polynucleotide sequence or antisense thereof.

[0099] In certain embodiments, a nucleic acid inhibitor is an RNA interference or anti-sense RNA agent or a portion or mimetic thereof, or a morpholino, that decreases the expression of a target gene when administered to a cell. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. In some embodiments, expression of a target gene is reduced by 10%, 25%, 50%, 75%, or even 90-100%.

[0100] A "complementary" nucleic acid sequence is a nucleic acid sequence capable of hybridizing with another nucleic acid sequence comprised of complementary nucleotide base pairs. By "hybridize" is meant pair to form a double-stranded molecule between complementary nucleotide bases (*e.g.*, adenine (A) forms a base pair with thymine (T), as does guanine (G) with cytosine (C) in DNA) under suitable conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

[0101] "Antisense" refers to a nucleic acid sequence, regardless of length, that is complementary to a nucleic acid sequence. In certain embodiments, antisense RNA refers to single stranded RNA molecules that can be introduced to an individual cell, tissue, or subject and results in decreased expression of a target gene through mechanisms that do not rely on endogenous gene silencing pathways. An antisense nucleic acid can contain a modified backbone, for example, phosphorothioate, phosphorodithioate, or others known in the art, or may contain non-natural internucleoside linkages. Antisense nucleic acid can comprise, e.g., locked nucleic acids (LNA).

[0102] "RNA interference" as used herein refers to the use of agents that decrease the expression of a target gene by degradation of a target mRNA through endogenous gene silencing pathways (e.g., Dicer and RNA-induced silencing complex (RISC)). RNA interference may be accomplished using various agents, including shRNA and siRNA. "Short hair-pin RNA" or "shRNA" refers to a double stranded, artificial RNA molecule with a hairpin turn that can be used to silence target gene expression via RNA interference (RNAi). Expression of shRNA in cells is typically accomplished by delivery of plasmids or through viral or bacterial vectors. shRNA is an advantageous mediator of RNAi in that it has a relatively low rate of degradation and turnover. Small interfering RNA (siRNA) is a class of double-stranded RNA molecules, usually 20-25 base pairs in length, similar to miRNA, and operating within the RNA interference (RNAi) pathway. It interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, preventing translation. In certain embodiments, an siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. siRNAs can be introduced to an individual cell and/or culture system and result in the degradation of target mRNA sequences. "Morpholino" as used herein refers to a modified nucleic acid oligomer wherein standard nucleic acid bases are bound to morpholine rings and are linked through phosphorodiamidate linkages. Similar to siRNA and shRNA, morpholinos bind to complementary mRNA sequences. However, morpholinos function through steric-inhibition of mRNA translation and alteration of mRNA splicing rather than targeting complementary mRNA sequences for degradation.

[0103] In certain embodiments, a nucleic acid inhibitor is a messenger RNA that may be introduced into a cell, wherein it encodes a polypeptide inhibitor of p38 or other target disclosed herein. In particular embodiments, the mRNA is modified, e.g., to increase its stability or reduce its immunogenicity, e.g., by the incorporation of one or more modified nucleosides. Suitable modifications are known in the art.

[0104] In certain embodiments, an inhibitor comprises an expression cassette that encodes a polynucleotide or polypeptide inhibitor of p38 or other target disclosed herein. In particular embodiments, the expression cassette is present in a gene therapy vector, for example a viral gene therapy vector. A variety of gene therapy vectors, including viral gene therapy vectors are known in the art, including, for example, AAV-based gene therapy vectors.

[0105] In some embodiments, an inhibitor is a polypeptide inhibitor. In particular embodiments, a polypeptide inhibitor binds to a target polypeptide such as p38, thus inhibiting its activity, e.g., kinase activity. Examples of polypeptide inhibitors include any types of polypeptides (e.g., peptides and proteins), such as antibodies and fragments thereof.

[0106] An "antibody" is an immunoglobulin (Ig) molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, or polypeptide, through at least one epitope recognition site, located in the variable region of the Ig molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof, such as dAb, Fab, Fab', $F(ab')_2$, Fv, single chain (scFv), synthetic variants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen-binding fragment of the required specificity, chimeric antibodies, nanobodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen-binding site or fragment of the required specificity.

[0107] "Fragment" refers to a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids. A "functional fragment" of an antibody is a fragment that maintains one or more activities of the antibody, e.g., it binds the same epitope and or possesses a biological activity of the antibody. In particular embodiments, a functional fragment comprises the six CDRs present in the antibody.

[0108] In certain embodiments, the inhibitor induces degradation of a target polypeptide, e.g., p38 protein. For example, inhibitors include proteolysis targeting chimeras (PROTAC), which induce selective intracellular proteolysis of target proteins. PROTACs include functional domains, which may be covalently linked protein-binding molecules: one is capable of engaging an E3 ubiquitin ligase, and the other binds to the target protein meant for degradation. Recruitment of the E3 ligase to the target protein results in ubiquitination and subsequent degradation of the target protein by the proteasome. In particular embodiments, an inhibitor is a PROTAC that targets a p38 protein (e.g., p38-α and/or p38-β).

[0109] In certain embodiments, an inhibitor is a small molecule inhibitor, or a stereoisomer, enantiomer, diastereomer, isotopically-enriched, pro-drug, or pharmaceutically acceptable salt thereof. In particular embodiments, the p38 inhibitor inhibits p38-α and/or p38-β. In particular embodiments, it does not significantly inhibit p38-γ. In particular embodiments, a small molecule inhibitor of p38 includes but is not limited to any of the small molecule compounds disclosed herein, including but not limited to those shown in FIG. 12B. A variety of p38 inhibitors are known and available, and some are in clinical development. Any of these may be used. These include, but are not limited to, ARRY-797, VX-745, VX-702, RO-4402257, SCIO- 469, BIRB-796, SD-0006, PH-797804, AMG-548, LY2228820, SB-681323 and GW-856553. Illustrative inhibitor compounds also include, but are not limited to:
N-(4-(2-ethyl-4-(m-tolyl)thiazol-5-yl)pyridin-2-yl)benzamide;
2-(2,4-difluorophenyl)-6-(1-(2,6-difluorophenyl)ureido)nicotinamide;
6-(2,4-difluorophenoxy)-8-methyl-2-((tetrahydro-2H-pyran-4-yl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one;
6-(2,4-difluorophenoxy)-2-((1,5-dihydroxypentan-3-yl)amino)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;
(R)-6-(2-(4-fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidin-3-yl)-2-(o-tolyl)pyridazin-3(2H)-one;
6-(5-(cyclopropylcarbamoyl)-3-fluoro-2-methylphenyl)-N-neopentylnicotinamide;
5-(2-(tert-butyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl)-3-neopentyl-3H-imidazo[4,5-b]pyridin-2-amine;
2-(6-chloro-5-((2R,5S)-4-(4-fluorobenzyl)-2,5-dimethylpiperazine-1-carbonyl)-1-methyl-1H-indol-3-yl)-N,N-dimethyl-2-oxoacetamide;
1-(3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)-3-(4-(2-morpholinoethoxy)naphthalen-1-yl)urea;
4-((5-(cyclopropylcarbamoyl)-2-methylphenyl)amino)-5-methyl-N-propylpyrrolo[2,1-f][1,2,4]triazine-6-carboxamide;
3-(3-bromo-4-((2,4-difluorobenzyl)oxy)-6-methyl-2-oxopyridin-1(2H)-yl)-N,4-dimethylbenzamide;
1-(3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)-3-(5-fluoro-2-((1-(2-hydroxyethyl)-1H-indazol-5-yl)oxy)benzyl)urea;
8-(2,6-difluorophenyl)-2-((1,3-dihydroxypropan-2-yl)amino)-4-(4-fluoro-2-methylphenyl)pyrido[2,3-d]pyrimidin-7(8H)-one;

5-(2,6-dichlorophenyl)-2-((2,4-difluorophenyl)thio)-6H-pyrimido[1,6-b]pyridazin-6-one; (5-(2,4-difluorophenoxy)-1-isobutyl-1H-indazol-6-yl)((2-(dimethylamino)ethyl)-l2-azaneyl)methanone; and
(R)-2-((2,4-difluorophenyl)amino)-7-(2,3-dihydroxypropoxy)-10,11-dihydro-5H-dibenzo[a,d][7]annulen-5-one.

[0110] Certain inhibitor compounds of the present invention may exist in stereoisomeric forms (e.g., they may contain one or more asymmetric carbon atoms or may exhibit cis-trans isomerism). Some compounds may include more than one asymmetric carbon atoms. "Stereoisomer" refers to a compound that differ in orientation (R/S) about one or more asymmetric carbon atom(s), or differs in orientation (cis:trans) about a double bond. The term stereoisomer may also encompass atropisomers, which arise from hindered rotation about a single bond, e.g., in compounds having a substituted biphenyl moiety. An "enantiomer" is a compound that is a mirror image of another compound, i.e., all asymmetric carbon atoms of an enantiomer exist in opposite orientation (R/S) with respect to the other compound. A "diastereomer" is a compound that is not a mirror image of another compound, but includes one or more asymmetric carbon atoms existing in opposite orientation (R/S) with respect to the other compound. The embodiments of the present invention may include mixtures of stereoisomers, or may include a single stereoisomer. Single enantiomers or diastereomers may be prepared beginning with chiral reagents or by stereoselective or stereospecific synthetic techniques. Alternatively, the single enantiomers or diastereomers may be isolated from mixtures by standard chiral chromatographic or crystallization techniques. "Isotopically-enriched" refers to a compound wherein one or more atoms is enriched with an isotope beyond its natural abundance. For example, the natural abundance of deuterium is 0.015%. One of ordinary skill in the art recognizes that in all chemical compounds with a H atom, the H atom actually represents a mixture of H and D, with about 0.015% being D. An isoptically-enriched compound may have one or more specific chemical sites wherein the H/D ratio is greater than 0.015%. An isotopically-enriched compound may be refered to as isotopically-labeled.

[0111] In certain embodiments, the inhibitor comprises one or more components of a gene editing system. As used herein, the term "gene editing system" refers to a protein, nucleic acid, or combination thereof that is capable of modifying a target locus of an endogenous DNA sequence when introduced into a cell. Numerous gene editing systems suitable for use in the methods of the present invention are known in the art including, but not limited to, zinc-finger nuclease systems, TALEN systems, and CRISPR/Cas systems.

[0112] In some embodiments, the gene editing system used in the methods described herein is a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR Associated) nuclease system, which is an engineered nuclease system based on a bacterial system that can be used for mammalian genome engineering. Generally, the system comprises a CRISPR-associated endonuclease (for example, a Cas endonuclease) and a guide RNA (gRNA). The gRNA is comprised of two parts; a crispr-RNA (crRNA) that is specific for a target genomic DNA sequence, and a trans-activating RNA (tracrRNA) that facilitates endonuclease binding to the DNA at the targeted insertion site. In some embodiments, the crRNA and tracrRNA may be present in the same RNA oligonucleotide, referred to as a single guide-RNA (sgRNA). In some embodiments, the crRNA and tracrRNA may be present as separate RNA oligonucleotides. In such embodiments, the gRNA is comprised of a crRNA oligonucleotide and a tracrRNA oligonucleotide that associate to form a crRNA:tracrRNA duplex. As used herein, the term "guide RNA" or "gRNA" refers to the combination of a tracrRNA and a crRNA, present as either an sgRNA or a crRNA:tracrRNA duplex.

[0113] In some embodiments, the CRISPR/Cas systems comprise a Cas protein, a crRNA, and a tracrRNA. In some embodiments, the crRNA and tracrRNA are combined as a duplex RNA molecule to form a gRNA. In some embodiments, the crRNA:tracrRNA duplex is formed *in vitro* prior to introduction to a cell. In some embodiments, the crRNA and tracrRNA are introduced into a cell as separate RNA molecules and crRNA:tracrRNA duplex is then formed intracellularly. In some embodiments, polynucleotides encoding the crRNA and tracrRNA are provided. In such embodiments, the polynucleotides encoding the crRNA and tracrRNA are introduced into a cell and the crRNA and tracrRNA molecules are then transcribed intracellularly. In some embodiments, the crRNA and tracrRNA are encoded by a single polynucleotides. In some embodiments, the crRNA and tracrRNA are encoded by separate polynucleotides.

[0114] In some embodiments, a Cas endonuclease is directed to the target insertion site by the sequence specificity of the crRNA portion of the gRNA, which may include a protospacer motif (PAM) sequence near the target insertion site. A variety of PAM sequences suitable for use with a particular endonuclease (*e.g.*, a Cas9 endonuclease) are known in the art (*See e.g.*, Nat Methods. 2013 Nov; 10(11): 1116–1121 and Sci Rep. 2014; 4: 5405).

[0115] The specificity of a gRNA for a target locus is mediated by the crRNA sequence, which comprises a sequence of about 20 nucleotides that are complementary to the DNA sequence at a target locus, e.g., complementary to a p38-α or p-38-β DNA sequence. In some

embodiments, the crRNA sequences used in the methods of the present invention are at least 90% complementary to a DNA sequence of a target locus. In some embodiments, the crRNA sequences used in the methods of the present invention are at least 95%, 96%, 97%, 98%, or 99% complementary to a DNA sequence of a target locus. In some embodiments, the crRNA sequences used in the methods of the present invention are 100% complementary to a DNA sequence of a target locus, e.g., a *MAPK14* or *MAPK11* gene. In some embodiments, the crRNA sequences described herein are designed to minimize off-target binding using algorithms known in the art (*e.g.*, Cas-OFF finder) to identify target sequences that are unique to a particular target locus or target gene.

[0116] In some embodiments, the endonuclease is a Cas protein or ortholog. In some embodiments, the endonuclease is a Cas9 protein. In some embodiments, the Cas9 protein is derived from *Streptococcus pyogenes* (*e.g.*, SpCas9), *Staphylococcus aureus* (*e.g.*, SaCas9), or *Neisseria meningitides* (NmeCas9). In some embodiments, the Cas endonuclease is a Cas9 protein or a Cas9 ortholog and is selected from the group consisting of SpCas9, SpCas9-HF1, SpCas9-HF2, SpCas9-HF3, SpCas9-HF4, SaCas9, FnCpf, FnCas9, eSpCas9, and NmeCas9. In some embodiments, the endonuclease is selected from the group consisting of C2C1, C2C3, Cpf1 (also referred to as Cas12a), Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csnl and Csx12), Cas10, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, Csx10, Csx16, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, and Csf4. In some embodiments, the Cas9 is a Cas9 nickase mutant. Cas9 nickase mutants comprise only one catalytically active domain (either the HNH domain or the RuvC domain).

[0117] In particular aspects, the disclosure includes compositions, e.g., pharmaceutical compositions comprising an inhibitor of p38, including any of the various classes of inhibitors described herein. The invention encompasses pharmaceutical compositions comprising a p38 inhibitor and a pharmaceutically acceptable carrier, diluent or excipient. Any inert excipient that is commonly used as a carrier or diluent may be used in compositions of the present invention, such as sugars, polyalcohols, soluble polymers, salts and lipids. Sugars and polyalcohols which may be employed include, without limitation, lactose, sucrose, mannitol, and sorbitol. Illustrative of the soluble polymers which may be employed are polyoxyethylene, poloxamers, polyvinylpyrrolidone, and dextran. Useful salts include, without limitation, sodium chloride, magnesium chloride, and calcium chloride. Lipids which may be employed include, without limitation, fatty acids, glycerol fatty acid esters, glycolipids, and phospholipids.

[0118] In addition, the pharmaceutical compositions may further comprise binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate, Primogel), buffers (e.g., tris-HCL, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g., sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol, cyclodextrins), a glidant (e.g., colloidal silicon dioxide), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g., carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g., sucrose, aspartame, citric acid), flavoring agents (e.g., peppermint, methyl salicylate, or orange flavoring), preservatives (e.g., thimerosal, benzyl alcohol, parabens), lubricants (e.g., stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g., colloidal silicon dioxide), plasticizers (e.g., diethyl phthalate, triethyl citrate), emulsifiers (e.g., carbomer, hydroxypropyl cellulose, sodium lauryl sulfate, methyl cellulose, hydroxyethyl cellulose, carboxymethylcellulose sodium), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g., ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

[0119] In one embodiment, the pharmaceutical compositions are prepared with carriers that will protect the p38 inhibitor against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0120] Additionally, the invention encompasses pharmaceutical compositions comprising any solid or liquid physical form of a p38 inhibitor. For example, the p38 inhibitor can be in a crystalline form, in amorphous form, and have any particle size. The particles may be

micronized, or may be agglomerated, particulate granules, powders, oils, oily suspensions or any other form of solid or liquid physical form.

[0121] When p38 inhibitors exhibit insufficient solubility, methods for solubilizing the compounds may be used. Such methods are known to those of skill in this art, and include, but are not limited to, pH adjustment and salt formation, using co-solvents, such as ethanol, propylene glycol, polyethylene glycol (PEG) 300, PEG 400, DMA (10-30%), DMSO (10-20%), NMP (10-20%), using surfactants, such as polysorbate 80, polysorbate 20 (1-10%), cremophor EL, Cremophor RH40, Cremophor RH60 (5-10%), Pluronic F68/Poloxamer 188 (20-50%), Solutol HS15 (20-50%), Vitamin E TPGS, and d-a-tocopheryl PEG 1000 succinate (20-50%), using complexation such as HP β–CD and SBE β–CD (10-40%), and using advanced approaches such as micelles, addition of a polymer, nanoparticle suspensions, and liposome formation.

[0122] p38 inhibitors may also be administered or coadministered in slow release dosage forms. p38 inhibitors may be in gaseous, liquid, semi-liquid or solid form, formulated in a manner suitable for the route of administration to be used. For oral administration, suitable solid oral formulations include tablets, capsules, pills, granules, pellets, sachets and effervescent, powders, and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, syrups, emulsions, oils and the like. For parenteral administration, reconstitution of a lyophilized powder is typically used.

[0123] Suitable doses of the p38 inhibitors for use in treating the diseases or disorders described herein can be determined by those skilled in the relevant art. Therapeutic doses are generally identified through a dose ranging study in humans based on preliminary evidence derived from the animal studies. Doses should be sufficient to result in a desired therapeutic benefit without causing unwanted side effects. Mode of administration, dosage forms and suitable pharmaceutical excipients can also be well used and adjusted by those skilled in the art. All changes and modifications are envisioned within the scope of the present patent application.

[0124] In certain embodiments, the disclosure includes unit dosage forms of a pharmaceutical composition comprising an agent that inhibits expression or activity of a p38 polypeptide (or results in reduced levels of an active p38 protein) and a pharmaceutically acceptable carrier, diluent or excipient, wherein the unit dosage form is effective to reduce expression of a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in one or more tissue in a subject to whom the unit dosage form is administered. In certain

embodiments, the downstream target gene is MBD3L2, ZSCAN4, LEUTX, PRAMEF2, TRIM43, or KHDC1L. In certain embodiments, the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A. In certain embodiments, the tissue is muscle tissue. In certain embodiments, the tissue is terminally differentiated, e.g., terminally differentiated muscle tissue. In certain embodiments, the tissue comprises cells comprising a mutation associated with facioscapulohumeral muscular dystrophy (FSHD). In particular embodiments, the agent binds a p38 polypeptide (e.g., p38-α or p38-β) or binds a polynucleotide encoding the p38 polypeptide. In certain embodiments, the agent comprises or consists of: a nucleic acid, optionally a DNA, RNA, modified mRNA (mmRNA), shRNA, siRNA, guide RNA (gRNA), microRNA (miRNA) or antisense oligonucleotide. In other embodiments, the agent comprises or consists of: a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof. In other embodiments, the agent comprises: a small molecule, optionally an organic molecule or an inorganic molecule. In other embodiments, the agent comprises a gene expression cassette, optionally a gene therapy vector, that expression a polynucleotide or polypeptide agent that inhibits expression or activity of a p38 polypeptide.

[0125] In particular embodiments, the unit dosage forms comprise an effective amount, an effective concentration, and/or an inhibitory concentration, of a p38 inhibitor to treat a disease or disorder associated with increased activity or expression of DUX4 and/or a downstream DUX4 target gene, including any of the disease or disorders disclosed herein, e.g., FSHD.

[0126] "Pharmaceutical compositions" include compositions of one or more agents capable of is capable of being administered or delivered to a subject and/or cell for the prevention and/or treatment of a particular disease or disorder.

[0127] "Pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0128] "Pharmaceutically acceptable carrier" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic

agent, solvent, surfactant, and/or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans and/or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen- free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations. Except insofar as any conventional media and/or agent is incompatible with the agents of the present disclosure, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[0129] "Effective amount" as used herein refers to an amount of an agent effective in achieving a particular effect, e.g., reducing DUX4-fl mRNA or DUX4 protein, or mRNA or protein of one or more DUX4 downstream targets in a cell, tissue, organ or subject. In the context of therapeutic treatment of a subject, an effective amount may be, e.g., an amount effective or sufficient to reduce one or more disease symptoms in the subject, e.g., a subject with FSHD. In certain embodiments, the reduction is at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, or at least 70%, as compared to the amount prior to or without treatment.

[0130] "Effective Concentration" as used herein refers to the minimum concentration (mass/volume) of an agent and/or composition required to result in a particular physiological effect. As used herein, effective concentration typically refers to the concentration of an agent required to increase, activate, and/or enhance a particular physiological effect.

[0131] "Inhibitory Concentration" "Inhibitory Concentration" is the minimum concentration (mass/volume) of an agent required to inhibit a particular physiological effect. As used herein, inhibitory concentration typically refers to the concentration of an agent required to decrease, inhibit, and/or repress a particular physiological effect.

[0132] In some embodiments, an agent or compound described herein may be administered at a dosage from about 1 mg/kg to about 300 mg/kg. In another embodiment, an agent or

compound described herein may be administered at a dosage from about 1 mg/kg to about 20 mg/kg. For example, the agent or compound may be administered to a subject at a dosage of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 mg/kg, or within a range between any of the proceeding values, for example, between about 10 mg/kg and about 15 mg/kg, between about 6 mg/kg and about 12 mg/kg, and the like. In another embodiment, an agent or compound described herein is administered at a dosage of ≤15 mg/kg. For example, an agent or compound may be administered at 15 mg/kg per day for 7 days for a total of 105 mg/kg per week. For example, a compound may be administered at 10 mg/kg twice per day for 7 days for a total of 140 mg/kg per week.

[0133] In many embodiments, the dosages described herein may refer to a single dosage, a daily dosage, or a weekly dosage. In one embodiment, an agent or compound may be administered once per day. In another embodiment, a compound may be administered twice per day. In some embodiments, an agent or compound may be administered three times per day. In some embodiments, a compound may be four times per day. In some embodiments, an agent or compound described herein may be administered 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 times per week. In other embodiments, the compound is administered once biweekly.

[0134] In some embodiments, an agent or compound described herein may be administered orally. In some embodiments, an agent or compound described herein may be administered orally at a dosage of ≤15 mg/kg once per day.

[0135] The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage regimen for a particular situation is within the skill of the art. For convenience, the total daily dosage may be divided and administered in portions during the day as required.

[0136] The dosage regimen utilizing the disclosed compound is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal or hepatic function of the patient; and the particular disclosed compound employed. A physician or veterinarian of ordinary skill in the art can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[0137] The amount and frequency of administration of the compounds of the invention and/or the pharmaceutically acceptable salts thereof will be regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the symptoms being treated.

[0138] In some aspects, the present invention relates to methods used for screening to identify drug targets using small molecules and/or genomic tools (e.g., small interfering RNA (siRNA), small hairpin RNA (shRNA), microRNA (miRNA), antisense oligonucleotides and gene therapeutic viruses) that reduce the expression and/or activity of DUX4 and downstream transcripts, MBD3L2, in FSHD myotubes. In particular embodiments, the methods comprise contacting a myotube comprising FSHD cells, for example, cells comprising an FSHD1 and/or an FSHD2 defect (e.g., mutation) with one or more candidate agent, and then determining whether myotubes contacted with the candidate agent have reduced DUX4 activity, reduced levels of DUX4 mRNA or protein, reduced activity of one or more downstream genes regulated by DUX4; or reduced levels of one or more downstream genes regulated by DUX4, as compared to the levels in myotubes contacted with a negative control, e.g., vehicle only. Candidate agents associated with reduced activity or expression levels of DUX4 and/or a DUX4 downstream gene are then identified, and the targets that they modulate may be identified. For example, in the case of siRNA, the gene target of the siRNA associated with reduced expression of DUX4 and/or a DUX4 downstream target gene is identified as a drug target for treating diseases or disorders associated with aberrant expression of DUX4 and/or a downstream DUX4 target gene, including any of those described herein, e.g., FSHD. Similarly, in the case of small molecules, the drug target of the small molecule associated with reduced activity or expression of DUX4 and/or a DUX4 downstream target gene is identified as a drug target for treating diseases or disorders associated with aberrant expression of DUX4 and/or a downstream DUX4 target gene, including any of those described herein, e.g., FSHD. In certain embodiments, the methods may be practiced by assessing a physical or qualitative property of the myotubes, in order to identify a candidate agent and its target, which may be used to improve the physical or qualitative property of the myotubes, thus identifying the target as a therapeutic target for treating diseases or disorders associated with aberrant expression of DUX4 and/or a downstream DUX4 target gene, including any of those described herein, e.g., FSHD.

[0139] In certain embodiments, the disclosure includes a method of identifying an agent that inhibits expression of a DUX4 protein, or inhibits expression of a protein encoded by a downstream gene target of DUX4, the method comprising: contacting a myotube prepared from cells associated with facioscapulohumeral muscular dystrophy (FSHD) with a candidate agent; and determining an expression level of the DUX4 protein, a polynucleotide encoding the DUX4 protein, the downstream gene target of DUX4, or a polynucleotide encoding the downstream gene target of DUX4 in the myotube, wherein the candidate agent is identified as

an agent that inhibits expression of the DUX4 protein, or the protein encoded by the downstream gene target, if the expression level determined after the contacting is reduced as compared to the expression level of the DUX4 protein or the protein encoded by the downstream gene target in a myotube prepared from cells associated with FSHD not contacted with the candidate agent or contacted with a negative control agent.

[0140] In certain embodiments of any of the methods described herein, the candidate agent may be any of the classes of inhibitors disclosed herein, including small molecules, polypeptides, and nucleic acids, such as, e.g., wherein the candidate agent comprises or consists of: a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, miRNA, siRNA, or antisense oligonucleotide; a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, gene therapy vector, or an antibody or functional fragment thereof; or a small molecule, optionally an organic molecule or an inorganic molecule.

[0141] In certain embodiments of any of the methods, the downstream target gene is, for example, MBD3L2, ZSCAN4, LEUTX, PRAMEF2, TRIM43, or KHDC1L. In certain embodiments, the downstream target gene is, for example, ZSCAN4, LEUTX, PRAMEF2, TRIM43, MBD3L2, KHDC1L, RFPL2, CCNA1, SLC34A2, TPRX1, PRAMEF20, TRIM49, PRAMEF4, PRAME6, or PRAMEF15.

[0142] In certain embodiments, any of the methods described herein are performed by screening a library of potential candidate agents. In certain embodiments, the methods are performed using high throughput assays.

[0143] In certain embodiments, the methods are performed using mature patient derived FSHD myotubes.

[0144] In one embodiment, a library of small molecules are used to screen for target modifiers of DUX4 or downstream target genes expression or activity in FSHD myotubes. Three days prior to treatment, cells are plated at 15,000 cells per well in a gelatinized 96 well plate with skeletal muscle growth medium (PromoCell, C-23060) with 20% FBS and Pen/Strep (Gibco, 15140148). On the day of treatment, the media is changed to Skeletal Muscle Cell Differentiation Medium (PromoCell, C-23061) supplemented with 20% KnockOut Serum replacement (Gibco, 10828010) or NbActiv4 medium (BrainBits Nb4-500) and Pen/Strep. p38 modulating agents are added at desired concentration into the culture media containing differentiated FSHD myotubes and cultured for 3-4 days in incubator. Myotubes are removed from incubator and RNA is extracted using RNeasy Micro Plus Kit (Qiagen Cat No./ID:74034). cDNA is prepared from the extracted RNA for Taqman Gene

Expression assay to measure DUX4 or downstream target genes expression. *POL2RA* transcript is used as endogenous control.

EXAMPLES

[0145] The studies described in the following Examples were performed using the Materials and Methods described below.

Abbreviations

ASO	antisense oligonucleotides
DAPI	4',6-diamidino-2-phenylindole (dihydrochloride)
DMSO	dimethyl sulfoxide
DUX4	double homeobox 4
DUX4-fl	double homeobox 4 full length
FSHD	facioscapulohumeral muscular dystrophy
gRNA	guide RNA
MBD3L2	methyl CpG binding domain protein 3 like 2
MHC	myosin heavy chain
MPAK14	mitogen-activated protein kinase 14
mRNA	messenger RNA
MYOG	myogenin (myogenic factor 4)
p HSP27	phosphorylated heat shock protein 27
PCR	polymerase chain reaction
pLAM	polyadenylation signal sequence
POLR2A	RNA Polymerase II Subunit A
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
sgRNA	single guide RNA
siRNA	small interfering RNA

General Materials and Methods

Human skeletal muscle myoblasts:

[0146] FTCE-00016-01 (immortalized FSDH myoblast line, 6.3 repeats) and isogenic lines, A4 control healthy normal, and C12 FSHD myoblasts were used for all studies (as described in Mamchaoui et al., 2011; Thorley et al., 2016). Four distinct primary patient myoblast

lines, FTCE-016, -020, -197, -196 were provided by R. Tawil. The FSHD myoblasts were shown to express aberrant DUX4 via demethylation of the D4Z4 on chromosome 4q35.

Media components and tissue culture materials included:

[0147] Skeletal Muscle Growth Medium (PromoCell, C-23160) supplemented with 15% FBS (Hyclone, SH30071) and Pen/Strep (Gibco, 15140148). NbActiv4 (BrainBits Nb4-500) and Pen/Strep (Differentiation media). EmbryoMax 0.1% Gelatin Solution (EMDmillipore ES-006-B). PBS (Gibco, 10010023), Tissue culture treated 96-well microplate (Corning, CLS3595),TC-Treated Multiwell Cell Culture Plat (Falcon, 353046).

Real Time PCR reagents and kits:

[0148] Lysis buffer-Roche Realtime Ready lysis buffer 19.5 μL.(for 20 μL) (Roche, 07248431001), DNAse I (Ambion, AM2222) 0.25 μL, Protector RNase Inhibitor (Roche, 3335402001) 0.25 μL, RNeasy Micro Kit (Qiagen, 74004), Taqman Preamp Master Mix (ThermoFisher Scientific, 4391128), Taqman Multiplex Master Mix (ThermoFisher Scientific, 4484262), ZSCAN4 Taqman Assay (ThermoFisher Scientific, Hs00537549_m1, FAM-MGB), MYOG Taqman Assay (ThermoFisher Scientific, Hs01072232_m1, JUN-QSY), RPLP0 Taqman Assay (ThermoFisher Scientific, Hs99999902_m1), and LEUTX Taqman Assay (ThermoFisher Scientific, Hs00418470_m1).

Antisense oligonucleotides (ASOs):

[0149] ASOs were purchased from Exiqon: FTSE-000001 (DUX4 ASO from Exiqon, CAGCGTCGGAAGGTGG (SEQ ID NO:18), 300610)), and non-targeting ASO (Exiqon, AACACGTCTATACGC (SEQ ID NO:19, 300610).

Gelatin Coating of Tissue Culture Dishes:

[0150] Performed three days prior to treatment, 0.1% gelatin solution was made by combining 1 g gelatin (e.g. Sigma G9391) and 1 L tissue culture grade water; autoclaved for 30 minutes to dissolve, and sterilized. Sufficient 0.1% gelatin to coat dish was applied using a sterile pipette, then the solution was aspirated, and the dishes were air-dried and stored at room temperature.

Cell plating:

[0151] Performed three days prior to treatment, 10,000 cells were plated per well on gelatinized 96-well plates, or 100,000 cells on gelatinized 6-well plates.

Antisense oligonucleotide and compound treatment:

[0152] For ASO or compound treatments, cells were plated into 100 μL of Promocell growth medium containing ASO or compounds at the described concentrations.

Skeletal muscle myotube differentiation:

[0153] On day 0, the media was changed to differentiation media. Plates were removed from the incubator and the growth medium was aspirated, plates were washed once with PBS, 100 µL for 96-wells and 1 mL for a 6-well plate, 100 µL or 2 mL of differentiation medium was added per well, 96- or 6-well respectively. Antisense oligonucleotides or drug were added at the desired concentration, and the plates were put back in the incubator and incubated for 3-4 days.

RNA preparation:

[0154] Cells were removed from the incubator and media aspirated. Cells were quickly lysed following one of the following protocols: for lysis in 96-well plates, direct lysis and one-step RT-Preamp qPCR was performed according to the protocol described below. For each 96-well, a mix containing: 19.5 µL Roche Realtime Ready lysis buffer, 0.25 µL RNAse inhibitor, 0.25 µL DNAseI (from Thermo not the included one in the kit) was prepared. 20 µL of the mix was added to each well, mixed 5 times, and incubated 5 minutes at RT or alternatively shaken vigorously for 15 minutes. Lysis was observed under the microscope. Samples were frozen -80 °C at least for 15 minutes.

qPCR One Step:

[0155] For qPCR, cell lysate was diluted 1:10, and 2 µL was used for a 10 µL 1-step RT-qPCR reaction for detection of GAPDH, RPLP0, TBP, MYOG, FRG1, MYH3, ACTN2, etc.. Per 10 µL reaction, the reaction mix included: 2 µL of RNA (1:10 dilution lysate), 5 µL of Fast Advanced Taqman Master Mix (2X), 0.25 µL of RT enzyme mix (40X), 0.5 µL of Taqman probe set (20X), and 2.25 µL of H_20. The following reaction protocol was run on the QuantStudio 7: 48 °C for 15 min, 50 °C for 2 min, 95 °C for 30 sec, 40x, 95 °C for 5 sec, 60 °C for 30 sec, after which the plates were read as specified by the manufacturer (Thermo).

[0156] 1-step RT-Preamplification was used for detection of DUX4 downstream genes, i.e., ,MBD3L2, ZSCAN4, LEUTX, TRIM43, KHDC1L, and POL2RA-VIC was used as endogenous control. Per 10 µL reaction, the reaction mix included: 2.25 µL of RNA (1:10 dilution lysate), 5 µL of Taqman Pre-Amp Master Mix (2X), 0.25 µL of RT enzyme mix (40X), 2.5 µL of Taqman probe set (0.2X)*. * For pooling the TaqMan Assays, equal volumes of each 20× TaqMan® Gene Expression Assay were used, and up to 100 assays were combined. For example, to pool 50 TaqMan assays, 10 µL of each assay were combined in a microcentrifuge tube. The pooled TaqMan assays were diluted using 1× TE buffer so that each assay was at a final concentration of 0.2×. For the above example, 500 µL of 1× TE buffer was added to the pooled TaqMan assays for a total final volume of 1 mL.

The QuantStudio7 protocol was used at 48 °C 15 min, 95 °C 10 min, 10 cycles: 95 °C 15 sec, 60 °C 4 min, and 4 °C infinite. Samples were then diluted to 50 µL and continued with the qPCR step. Per 10 µL reaction, the reaction mix included: 2 µL of Preamp dilution, 5 µL of Fast Advanced Taqman Master Mix (2X), 0.5 µL of Taqman probe set (20X), and 2.5 µL of H_20. When multiplexing, the volume was adjusted to 10 µL total. The following program was run on the QuantStudio7: 50 °C for 2 min, 95 °C for 30 sec, 40x, 95 °C for 5 sec, 60 °C for 30 sec, and plates were read as per the manufacturers specifications (Thermo).

Methods for total RNA extraction from myotubes using RNeasy Micro Plus Kit:

[0157] In a 6 well plate, 450 µL Buffer RLT Plus was added. Lysate was homogenized by transfer of the lysate to a gDNA Eliminator spin column placed in a 2 mL collection tube (supplied), the column was centrifuged for 30 s at ≥8000 x g (≥10,000 rpm), and then the column was discarded while saving the flow-through. 250 µL of ethanol (35% final) was added to the flow-through, and mixed well by pipetting (not centrifuged). The samples were then transferred, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 mL collection tube (supplied). The columns were centrifuged for 15 s at ≥8000 x g. Flow-through was discarded or collected for protein precipitation. 700 µL of Buffer RW1 was added to the RNeasy MinElute spin column, which was then centrifuged for 15 s at ≥8000 x g, after which the flow-through was discarded. DNAse treatment was performed by gently mixing 10 µL DNAseI with 70 µL of Buffer RDD, and the resulting solution was added directly to the column, which was incubated at room temperature for 20 min. Then, 700 µL of Buffer RW1 (per manufactures specification) was added to the RNeasy MinElute spin column, the column was centrifuged for 15 s at ≥8000 x g. and the flow-through discarded. 500 µL Buffer RPE was added to the RNeasy MinElute spin column, which was then centrifuged for 15 s at ≥8000 x g, after which the flow-through was discarded. 500 µL of 80% ethanol was added to the RNeasy MinElute spin column, the column was centrifuged for 2 min at ≥8000 x g to wash the spin column membrane, and the collection tube was discarded with the flow-through. The RNeasy MinElute spin column was placed in a new 2 mL collection tube (supplied), centrifuged at full speed for 5 min to dry the membrane, and the collection tube was discarded with the flow through. The RNeasy MinElute spin column was placed in a new 1.5 mL collection tube (supplied). 14 µL of RNase-free water was added directly to the center of the spin column membrane, which was then centrifuged for 1 min at full speed to elute the RNA. Approximately 12 µL of RNA was eluted.

Detection of DUX4-fl using method described by Himeda et al. 2015:

[0158] cDNA preparation. 10 µL reactions included 1 µL of RNA (1 µg), 0.5 µL of Oligo dT, 0.5 µL of 10 mM dNTPs, and 4.5 µL of H_20. Reaction samples were incubated at 65 °C for 2 min and quickly moved to ice and held at least 1 min before adding the enzyme mix, which included 2 µL of 5x First strand Buffer, 0.5 µL of 0.1M DTT, 0.5 µL of RNAse inhibitor, 0.5 µL of SSIV RT. The samples were incubated at 55 °C for 20 min and 80 °C for 10 min, followed by cool down to 4 °C. DUX4 pre-amplification was performed in a 10 µL reaction mixture containing 1 µL of RT reaction, 2 µL of 5X GC buffer, 0.8 µL of DMSO, 0.2 µL of 10 mM dNTPs, 0.2 µL of 10 µM TJ38F, 0.2 µL of 10 µM TJ40R, 0.1 µL of Phusion II DNA pol, and 5.5 µL of H_20. The following protocol was run on the QuantStudio 7: 98 °C 2 min, 10 cycles of 98 °C 15 seconds, 64 °C 20 seconds, 72 °C 15 seconds, and 4 °C infinite.

[0159] DUX4 qPCR with nested primers was performed in a 10 µL reaction containing 1 µL of DUX4 pre amplification DNA, 5 µL of 2X IQ SYBR Mix, 0.4 µL of 10 µM TJ38F, 0.4 µL of 10 µM TJ41R, and 3.2 µL H_20. The following protocol was run on the QuantStudio7: 95 °C 3 min, 40 cycles of 95 °C 10 seconds, 64 °C 15 seconds, 72 °C 20 seconds, 86 °C 10 seconds, then the plates were read on QuantStudio7 as per manufactures instruction (Thermo). Ct values were extracted from the QuantStudio Realtime PCR software, and Genedata was used to calculate relative levels of expression using POLR2A as a housekeeping gene.

RNAseq methods:

[0160] The 40 bp single-end reads from Illumina had good quality by checking with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were mapped to hg19 using TopHat v2.1.1 (Kim et al., 2013) with options as ''solexa1.3-quals'' mode and ''no-novel-juncs.'' The gene model for TopHat was created by merging known gene in gtf format with kgXref table. Both known gene and kgXref were downloaded from UCSC table browser in hg19 assembly. The read counts were obtained using featureCounts function from Subread package with strandness option as –r 2. Reads were normalized with DESeq2 (Love et al., 2014).

FSHD myotube immunocytochemistry:

[0161] Briefly, cells were fixed in 4% paraformaldehyde and permeabilized in 4% paraformaldehyde (PFA) for 10 min at room temperature. Cells were permeabilized with PBST (1 x PBS solution with 0.1% Triton X-100) before blocking with 10% Normal Donkey Serum or 3% BSA (NDS) in PBST. Cells were then incubated with appropriately diluted primary antibodies in PBST with 5% NDS for 1 hours at room temperature or 12 hours at 4

°C, washed with PBST for 3 times at room temperature, and then incubated with desired secondary antibodies in TBST with 5%NDS and DAPI to counterstain the nuclei. DUX4 was detected by immunocytochemistry using the E5-5 antibody in differentiated FSHD myotubes. Activated Caspase-3 was detected using a commercially available antivbody (https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-antibody/9661).

RNAseq methods:

[0162] The 40 bp single-end reads from Illumina had good quality by checking with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were mapped to hg19 using TopHat v2.1.1. The gene model for TopHat was created by merging knownGene in gtf format with kgXref table. Both known gene and kgXref were downloaded from UCSC table browser in hg19 assembly. The read counts were obtained using feature Counts function from Subread package with strandness option as –r 2. Reads were normalized with DESeq2. The biological replicates in the neuron samples, processed at different time periods, had batch effect as suggested by principle component analysis. Consequently, Combat was used for reducing this batch effect. Calculated standard RPKM expression values. Total gene signature was very small and defined at standard statistical cutoffs: 86/19,799 mRNA genes. DUX4-regulated gene signature was a majority of total signature: 77/86 mRNA genes = 90%. Non-DUX4 regulated genes was a minority of total signature with moderate fold changes: 9/86 mRNA genes = 10%; 2-2.7X logFC.

Methods for siRNA and Cas9/sgRNA RNP transduction of FSHD myotubes:

[0163] Synthetic crRNAs were purchased from Thermo Fisher Scientific and annealing to tracrRNAs was performed according to specifications. In short, crRNAs and tracrRNA were resuspended in TE buffer at 100 μM, mixed, and diluted 5-fold in annealing buffer. Annealing was performed in a ProFlex PCR system following manufacturer's recommendation. 100 ng of assembled crRNA:tracrRNA were incubated with 500 ng of TrueCut Cas9 (ThermoFisher, #A36497) in the resuspension buffer provided with the Neon transfection system kit (ThermoFisher, #MPK10096). After 15 minute incubation, the reaction was used to transfect 50,000 myoblasts according to the methods described. Sequences used for the targeting of MAPK14 (3 sgRNAs) and pLAM region (polyadenylation sequence of DUX4, 4 gRNAs) were: NT-CTRL, GTATTACTGATATTGGTGGG (SEQ ID NO:8); MAPK14, GCTGAACAAGACAATCTGGG (SEQ ID NO:9), CTGCTTTTGACACAAAAACG (SEQ ID NO:10), CTTATCTACCAAATTCTCCG (SEQ ID NO:11); pLAM,

AGAATTTCACGGAAGAACAA (SEQ ID NO:12), CAGGTTTGCCTAGACAGCGT (SEQ ID NO:13), ATTAAAATGCCCCCTCCCTG (SEQ ID NO:14), AATCTTCTATAGGATCCACA (SEQ ID NO:15), and siRNA MAPK14, Antisense: UAGAUUACUAGGUUUUAGGTC (SEQ ID NO:16), Sense: CCUAAAACCUAGUAAUCUATT (SEQ ID NO:17).

In Vivo Materials and Methods

Rats for PK/PD Studies:

[0164] Male Sprague Dawley rats (6-8 weeks of age) were supplied by Hilltop Lab Animals, Inc. (USA). Following arrival at Wuxi AppTec, animals were assessed for their general health by a member of veterinary staff or other designated personnel. Animals were acclimated for at least 2 days (upon arrival at WuXi AppTec) before commencement of the study. Animals were individually housed during acclimation and throughout the study. The animal room environment was controlled according to facility operation (target conditions: temperature 20 to 26°C, relative humidity 30 to 70%, 12-hour cycle of lights on and off). Lights, temperature and relative humidity are constantly monitored by AmegaView Environmental Monitoring System. The diet (Certified Rodent Diet #5002, PMI Feeds, Inc., Brentwood, MO) was irradiated pellets with double package; diet lot number and specifications will be recorded in study notebook and archived at WuXi AppTec. Water (reverse osmosis) was provided to the animals ad libitum. Periodic analyses of water quality were performed, and the results were archived at WuXi AppTec. There are no known contaminants in the diet or water that, at the levels of detection, is expected to interfere with the outcome of the study. In study # FULTH- 20171120 rats were fasted overnight prior to drug administration: rats were allowed free access to water at all times and were fed 4 hours post dosing. For study # FULTH-20171228 rats were allowed ad-libitum access to food and water throughout the study.

Mice for Xenograft Studies:

[0165] Male NOD.Cg-Rag1tm1Mom Il2rgtm1Wjl/SzJ (Nod-Rag) mice (6-8 weeks of age) were supplied by the University of Maryland VR breeding colony (USA). Animals were housed in the UMB central animal facility at Howard Hall at the University of Maryland. Animals were group housed (4/cage) during acclimation (4-5 days), throughout the engraftment procedure and throughout the drug treatment study. The animal room environment was controlled according to facility operation (target conditions: temperature 20 to 26°C, relative humidity 30 to 70%, 12-hour cycle of lights on and off). Lights, temperature and relative humidity are constantly monitored by AmegaView Environmental Monitoring

System. The diet (LabDiets 5P76 22.5% protein rodent chow) was provided ad libitum during the study. Sterilized water was provided ad libitum. There are no known contaminants in the diet or water that were expected to interfere with the outcome of the study.

Creation of FSHD and Control Xenograft Mice:

[0166] FSHD and control mice were generated by xenografting C6 and A4 IPSC-derived human immortalized isogeneic myoblast cell lines into the bilateral tibialis anterior (TA) muscles of approximately 8-week old male Nod-Rag mice. To create the human muscle xenografts, a niche to seed the A4 or C6 cells within the TA of the mouse hindlimb was created by X-irradiating the hindlimbs of the 8-week-old immunodeficient NRG mice, to prevent mouse muscle regeneration. One day later, 50 ul of a 2% BaCl2 solution was injected along the length of each TA to eliminate the mouse muscle tissue. Following mouse tissue ablation, 2x106 C6 cells were injected into each, bilateral, TA region and allowed to develop for four weeks. Following engraftment of the A4 or C6 cells, the animals were exposed to 4 weeks of intermittent neuromuscular electrical stimulation (iNMES) in order to improve the engraftment of the human cells as described by Sakellariou et al., 2016.

Test Article Formulation and Preparation

[0167] Appropriate amount of FTX-1821 were accurately weighed and mixed with appropriate volume of excipients (0.5% (1% DMSO:99% methyl cellulose) in water to get a uniform suspension with a final concentration of 0.03 mg/mL. Formulation was prepared on the day of the study and was dosed within 1 hour of preparation. Dose volume given to animals was 10mL/kg. Two 20-50 µL aliquots of dose solution were taken from each formulation and set aside for the determination of dose accuracy by LC-MS/MS.

[0168] Appropriate amount of FTX-2865 were accurately weighed and mixed with appropriate volume of sterile 0.9% saline for injection to achieve a clear solution with a final concentration 1 mg/mL. Formulation was prepared on the day of the study and was given to animals using a dose volume of 10mL/kg.

FTX-1821 Test Article Administration and PK/PD Study Design

[0169] The dosing solution of FTX-1821 (0.03mg/mL) was administered via oral gavage at a dose volume of 10 mL/kg in order to yield a final dose of 0.3 mg/kg. following Wuxi facility SOPs. Dose volume was determined by measured body weight prior to dosing. The dosing solution concentrations (mg/ML), dose volumes (mL/kg) and final dose (mg/kg) for the respective treatment groups were recorded in the included excel study sheet. Feeding condition: overnight fast, food return 4 hours after dosing.

[0170] FTX-2865 Test Article Administration and Xenograft Study Design

[0171] The dosing solution of FTX-2865 (1mg/mL) was administered via IP injection at a dose volume of 10 mL/kg in order to yield a final dose of 10 mg/kg (for each dose). 0.9% sterile saline was administered via IP injection at a dose volume of 10 mL/kg as a vehicle control (for each dose). Dose volume was determined by measured body weight prior to the morning dosing. The dosing solution concentrations (mg/ML), dose volumes (mL/kg) and final dose (mg/kg) for the respective treatment groups were recorded in the included excel study sheet. BID injections were spaced approximately 12-hours apart to maximize target coverage. Study animals received a total of 14 injections of vehicle or FTX-2865 over 8 days and were sacrificed approximately 1-hour after the final morning injection on day 8.

Sample Collection:

[0172] Blood samples for PK: Approximately 100 µl of blood sample was obtained via jugular vein or tail vein at each pre-defined time point. Blood samples were placed in pre-chilled collecting tubes treated with EDTA-K2 as anti-coagulant and placed on ice until centrifugation.

[0173] Plasma collection for PK assessment: PK blood samples were centrifuged at 4°C, 3000g for 15 min within half an hour for plasma collection. Plasma samples were stored in polypropylene tubes or 96-well plates, quickly frozen on dry ice and stored at -70°C until LC/MS/MS analysis.

[0174] Muscle collection for PK and PD assessment: Bilateral tibialis anterior and trapezius muscles were collected following blood collection by cardiac puncture. Each muscle from left and right sides was quickly weighed separately and placed in separate tubes, then snap frozen on dry ice. Muscles from one side were used for the measurement of compound concentration, those from the other side were sent to the sponsor for PD analysis. Dosing was staggered so that sample collection was carried out at approximately the same time at the end of the day.

Sample Processing for PK Analysis:

[0175] Plasma Sample Preparation for LC/MS Analysis: An aliquot of 10 µL plasma sample was protein precipitated with 150 µL IS solution (100 ng/mL Labetalol & 100 ng/mL Tolbutamide & 100 ng/mL Diclofenac in ACN), the mixture was vortex-mixed well and centrifuged at 4000 rpm for 15 min, 4 °C. An aliquot of 80 µL supernatant was transferred to sample plate and mixed with 80 µL water, then the plate was shaken at 800 rpm for 10 min. 1 µL supernatant was then injected for LC-MS/MS analysis. Muscle sample preparation for LC/MS Analysis: The muscle samples were homogenized in water in a 1:4 ratio (w/v) using

Omni bead ruptor. The homogenates were then used for the measurement of drug concentration. An aliquot of 20 μL muscle tissue homogenate was protein precipitated with 200 μL IS solution (100 ng/mL Labetalol & 100 ng/mL Tolbutamide & 100 ng/mL Diclofenac in ACN), the mixture was vortex-mixed well and centrifuged at 4000 rpm for 15 min, 4 °C. An aliquot of 80 μL supernatant was transferred to sample plate and mixed with 80 μL water, then the plate was shaken at 800 rpm for 10 min. 0.3 μL supernatant was then injected for LC-MS/MS analysis.

Analytical Method (LC/MS, non-GLP):

[0176] Technical details used to execute analytical methods include: Instrument: LCMS Triple Quad QTRAP 6500+ (SCIEX, MA, USA), Matrix:Male SD rat plasma (EDTA-K2), Internal standard(s):100 ng/mL Labetalol & 100 ng/mL Tolbutamide & 100 ng/mL and Diclofenac in CAN, MS conditions ESI: positive , SRM detection of FTX-1821: [Mouse + Human]+ m/z 383.838>299.231; Labetalol (IS): [M+H]+ m/z 329.2/162.1;Tolbutamide (IS): [M+H]+ m/z 271.1/155; Calibration curve: 1.00-3000 ng/mL for FTX001821-02 in male SD rat plasma (EDTA-K2) and muscle homogenate

Quantitation method: The peak area of the test article in samples and in the standard solution sample was determined by LC/UV or LC-MS/MS method. Method acceptance criteria: Linearity: ≥75% STDs was back calculated to within ±20% of their nominal values (±25% for LLOQ) in biofluid and within 25% of their nominal values (30% for LLOQ) in tissue homogenate and feces sample. QC: ≥ 67% all QCs was back calculated to within ±20% of their nominal values for biofluid and within 25% of their nominal values for tissue and feces samples. Specificity: The mean calculated concentration of analyte in the single blank matrix was less than 0.5 times of the LLOQ. Sensitivity: The LLOQ in biofluid and tissue homogenate was 1-3 ng/mL. Carryover: The mean calculated carryover concentration in the single blank matrix immediately after the highest standard injection was less than LLOQ.

Protocol for Cryofracture, Lysis and Preparation of Muscle Tissue for Immunoassay Assessment of Target Engagement:

[0177] Approximately 50mg of muscle tissue was placed on dry ice. Muscle samples were cut, as needed to obtain the 50mg weight using a clean razor blade per specimen. 50mg of muscle tissue was placed into a pre-labeled TT1 Covaris bag (Covaris, MA, USA) and kept on dry ice. The TT1 Covaris bag was submerged in liquid nitrogen and the sample was cryofractured in the Covaris cryoPREP (Covaris, MA, USA) on setting “5”. The TT1 bag was rotated 180° and steps 2-a were repeated. The sample was transferred into a pre-

weighed/labeled tube and maintained on dry ice until all samples were prepared. Sample weights were recorded. RIPA lysis buffer was prepared (R0278-500ML, Sigma, MO, USA). For 10ml, two Roche PhosSTOP phosphatase inhibitor tablets and one Roche EDTA-free protease inhibitor tablet were added. To the cryofractured material, 8µl per mg of RIPA buffer were added to each tube and each tube was vortexed until the lysate was homogeneous. Lysates were maintained on ice until all samples were processed. The lysate was cleared by centrifugation at 13,000g for 5 minutes at 4°C. The supernatant was transferred to a new tube and snap frozen in liquid nitrogen (setting aside 10µl for protein assay). To measure the protein content of each sample, a Bradford DC protein assay (5000112, Bio-Rad, CA, USA) was conducted. Samples were diluted 1:20 in PBS for protein assessment.

Phospho MK2 and Total MK2 Immunoassay:

[0178] The homogenized trapezius muscle lysate was assessed using an internally developed Meso Scale Discovery (MSD) multiplexed phospho MK2 / total MK2 immunoassay (Meso Scale Diagnostics, MD, USA). For each sample, 50 µL of muscle lysate, equal to 50 µg of protein, was loaded onto the MSD assay. Protein concentrations in muscle lysates were determined by a Bradford DC protein assay as described above. Samples were assessed in duplicate. Muscle samples were incubated on a pre-coated MSD plate overnight at 4°C while on an orbital shaker (300 rpm) and assessed the following morning.

Protocol for Cryofracture, RNA Extraction and RNA Purification of Muscle Tissue and Quantitative PCR Assay Assessment of MBD3L2 and CDKN1B:

[0179] Approximately 3-5mg of TA muscle tissue was placed on dry ice. Muscle tissue was placed into a pre-labeled TT1 Covaris bag (Covaris, MA, USA) and kept on dry ice. The TT1 Covaris bag was submerged in liquid nitrogen and the sample was cryofractured in the Covaris cryoPREP (Covaris, MA, USA) on setting "5". The TT1 bag was rotated 180° and steps 2-a were repeated. The sample was transferred into a pre-weighed/labeled tube and maintained on dry ice until all samples were prepared. RNA was purification using Zymo Direct-zol microprep RNA kit (CA, USA) from 3-5 mg of cryofractured muscle tissue. cDNA was synthesized from the RNA template via reverse transcription. The targeted transcripts were then pre-amplified in a 14-cycle PCR assay using diluted, human-specific TaqMan probes. Gene expression was analyzed in a qPCR assay using the human specific TaqMan probes. The relative expression level was normalized to CDKN1B expression using $2^{-\Delta Ct}$ method.

Data Analysis:

[0180] Plasma and muscle concentration versus time were analyzed by non-compartmental approaches using the Phoenix WinNonlin 6.3 software (Cetera, NJ, USA). C0, CLp, Vdss, Cmax, Tmax, T½, AUC(0-t), AUC(0-inf), MRT(0-t) , MRT(0-inf), %F and graphs of plasma and muscle concentration versus time profile and PD endpoints are reported using GraphPad Prizm software version 7 (CA, USA). Muscle PD was assessed via one-way ANOVA using GraphPad Prizm software version 7 (CA, USA). The effect of C6 vs. A4 cell engraftment on MBD3L2 mRNA in xenografted muscles was assessed via two-tailed T-test using GraphPad Prizm software version 7 (CA, USA). The effect of FTX-2865 vs. vehicle treatment on MBD3L2 mRNA in FSHD xenografted muscles was assessed via two-tailed T-test using GraphPad Prizm software version 7 (CA, USA).

EXAMPLE 1

REPRESSION OF DUX4 USING SEQUENCE DIRECTED ANTISENSE OLIGONUCLEOTIDE REDUCES DOWNSTREAM TARGET GENES

[0181] Wild type myotubes were treated with DMSO control vehicle, and mature patient-derived FSHD myotubes that express DUX4 protein were treated with DMSO vehicle control or 1 μM of a DUX4 sequence-directed antisense oligonucleotide (ASO; FTX-2) purchased from Exiqon. After treatment, the myotubes were lysed in 19.5 μL of Roche Real Time Ready Lysis Buffer, 0.25 μL of DNAse1 (Ambion, AM2222), 0.25 μL of Protector RNase Inhibitor (Roche, 3335402001), and the RNA was collected in an RNeasy Micro Kit Master Mix. Expression levels of DUX4-regulated downstream genes (ZSCAN4, TRIM43, MBD3L2, LEUTX, and KHDC1L) was determined by real time PCR (ThermoFisher Scientific, 4484262), ZSCAN4 Taqman Assay (ThermoFisher Scientific, Hs00537549_m1, FAM-MGB), MYOG Taqman Assay (ThermoFisher Scientific, Hs01072232_m1, JUN-QSY), RPLP0 Taqman Assay (ThermoFisher Scientific, Hs99999902_m1), and/or LEUTX Taqman Assay (ThermoFisher Scientific, Hs00418470_m1). Ct values were extracted from QuantStudio Realtime PCR software, and Genedata was used to calculate relative levels of expression using POLR2A as a housekeeping gene.

[0182] The results showed that FSHD myotubes treated with DUX4 sequence directed ASO express reduced amounts of DUX4 and theDUX4 downstream transcription factor target genes, ZSCAN4, TRIM43, MBD3L2, LEUTX, and KHDC1L, as compared to FSHD myotubes treated with DMSO vehicle control (FIG. 2).

[0183] The data in FIG. 3A are grouped plate quality control data comparing expression of MBD3L2 mRNA in FSHD myotubes treated with DMSO control or 1 μM DUX4 ASO, and healthy normal isogenic control myotubes. FIG. 3B shows pharmacologic quality control data and dose dependent reduction of DUX4 and the downstream gene, *MBD3L2*, using different dilutions of the DUX4 ASO. FIG. 3C shows plate-based assay statistics comparing FSHD myotubes treated with DMSO to WT: Z' is 0.512 and Signal to Noise (S/N) is 5.1, and FSHD myotubes treated with DMSO or DUX4 ASO: Z' is 0.319 and Signal to Noise (S/N) is 4.6.

EXAMPLE 2

p38 Small Molecule Inhibitors Reduce *MBD3L2* mRNA Expression

[0184] Wild type myotubes and mature patient-derived FSHD myotubes that express DUX4 protein were treated with DMSO vehicle control or multiple concentrations of various p38α/β inhibitors with different ranges of isoform and kinome selectivity, including SB239063 (FIG. 4A), VX-702 (FIG. 4B), Pamapimod (FIG. 4C), and TAK-715 (FIG. 4D). After treatment, the control and treated cells were processed for realtime PCR quantification of MBD3L2 mRNA (DUX4 downstream gene) and myogenin (MYOG) mRNA (control) expression. These p38α/β inhibitors showed potent (IC_{50} approximately <10 nM, FIGS. 4A-D) reduction of MBD3L2 mRNA expression with no impact to MYOG mRNA expression in FSHD myotubes.

[0185] In FSHD myotubes, p38 inhibitors (e.g., Pamapimod) dose-dependently reduced DUX4 mRNA and DUX4 downstream gene MBD3L2 mRNA expression without impacting myotube formation. When compared to DMSO treatment, 10, 100, and 1000 nM FTX000839 (Pamapimod) dose-dependently reduced both DUX4-fl and MBD3L2 downstream gene mRNA levels normalized to POLR2A mRNA, as measured by qPCR and Taqman in FSHD myotubes (FIG. 5A) without impacting differentiation into myotubes (FIG. 5B). The data show that p38 inhibitors dose-dependently reduce MBD3L2 mRNA expression without impacting myogenin mRNA expression.

EXAMPLE 3

p38 MAPK14 mRNA and MBD3L2 mRNA Reduction via siRNA Knockdown

[0186] p38α MAPK14 85 and p38α MAPK14 86 siRNAs were transfected into patient FSHD myotubes as described in Materials and Methods. Each of p38α MAPK14 85 siRNA and p38α MAPK14 86 siRNA (to a lesser extent) reduced p38 MAPK14 expression, as shown in FIG. 6A, and MBD3L2 mRNA (DUX4 target gene) expression, as shown in FIG. 6B, as compared to non-target control siRNAs (NT CTRL 1 and NT CTRL 2). The data shows that

genomic reduction of p38α MAPK14 >50% specifically reduced DUX4 and downstream target genes, as exemplified by MBD3L2.

EXAMPLE 4

MBD3L2 mRNA Reduction via p38α Kinase Cas9/sgRNA RNPs

[0187] CRISPR gRNA targeting of MAPK14 or pLAM (polyadenylation signal sequence for DUX4) was conducted as described in Materials and Methods. CRISPR gRNA targeted to MAPK14 or pLAM (polyadenylation signal sequence for DUX4) resulted in a reduction in expression of MBD3L2 but no MYOG. The data indicates that genomic reduction of p38α MAPK14 specifically reduced DUX4 and downstream target genes, as exemplified by MBD3L2.

EXAMPLE 5

FTX-1821 downregulates DUX4 protein and MBD3L2 mRNA

[0188] Patient-derived FSHD myotubes (with 6 repeats of D4Z4 arrays) were treated with DMSO vehicle control and different FTX-1821 concentrations, and DUX4 protein and MBD3L2 mRNA levels were determined as described in Methods and Materials. For DUX4 and MBD3L2, four biological replicates were analyzed. In addition, pHSP27 levels were determined. For pHSP27 quantification, three replicates were obtained in two independent experiments.

[0189] Treatment of the FSHD patient derived myotubes with FTX 1821 resulted in a concentration-dependent reduction of DUX4 protein (IC_{50} = 25nM) and MBD3L2 mRNA (IC_{50} = 25nM) that correlated with the changes observed in phospho HSP27 levels (IC_{50} = 10nM) as evidence of target engagement (FIG. 7). The results were indicative of a concentration-dependent reduction of DUX4 protein (IC_{50} = 25 nM) and MBD3L2 mRNA (IC_{50} = 10 nM). The reductions in DUX4 protein and MBD3L2 mRNA correlated with the observed changes in p-HSP27 levels (IC_{50} = 10 nM) as evidence of target engagement. These results indicate that p38α pathway inhibition by FTX-1821 results in potent DUX4 protein and MBD3L2 mRNA downregulation.

EXAMPLE 6

FTX-1821 Does Not Affect Myotube Formation

[0190] Immortalized FHSD myotubes were differentiated and treated with DMSO vehicle control or FTX-1821 at concentrations of 1 μM, 0.33 μM, 0.11 μM, or 0.037 μM. After 4 days, the cells were fixed and stained with antibodies directed against MHC or DAPI. See FIG. 8A. The nuclei in myotubes were quantified according to MHC staining (FIG. 8B).

The results showed no changes in myotube formation or fusion after treatment with FTX-1821 at concentrations tested.

EXAMPLE 7

FTX-1821 REDUCES APOPTOSIS IN FSHD MYOTUBES

[0191] Apoptosis was measured by active Caspase-3 levels in FSHD myotubes *in vitro* as described in Materials and Methods. Apoptosis was detected in a sporadic manner in a subset of myotubes in culture as shown by the white circles and magnified region in FIG. 9A. Active Caspase-3 signal was quantified in FSHD myotubes that had been treated with FTX-1821 at different concentrations (FIG. 9B). The results showed a dose-dependent reduction of apoptotic signal, as indicated by the reduction in detection of active Caspase 3 (IC_{50} = 45 nM), and this effect was specific to FSHD myotubes compared to control myotubes. No change in active Caspase-3 signal was observed following DMSO treatment.

EXAMPLE 8

FTX-1821 REDUCES PATHOLOGIC DUX4 TRANSCRIPTIONAL PROGRAM EXPRESSION

[0192] Studies were conducted as described in Methods and Materials to identify genes in the DUX4 pathway whose expression in down-regulated in FSHD myotubes treated with FTX-1821 as compared to FSHD myotubes treated with DMSO vehicle control. In addition, gene expression was also determined in wild type myotubes treated with DMSO. Three replicates for each condition were analyzed by RNA-seq and genes were clustered by the direction and intensity of change.

[0193] As shown in the heatmap of FIG. 10A, a number of differentially expressed genes were identified by RNA-seq profiling. The bar indicates the normalized changes observed, e.g., genes that were downregulated by FTX-1821 are enriched in samples treated with only DMSO. The expression of these genes was normalized upon treatment with FTX-1821 (1 μM) and closer resembled the observations in wild type cells. Calculated using standard RPKM expression values, the total gene signature was very small and defined at standard statistical cutoffs: 86/19,799 mRNA genes. DUX4-regulated gene signature was a majority of the total signature, and these genes are listed in FIG. 10A. Non-DUX4-regulated genes were minority of the total signature with moderate fold changes: 9/86 mRNA genes = 10%; 2-2.7X logFC. FIG. 10B shows the normalized reads, as described in Materials and Methods, of the DUX4 target genes that were downregulated upon treatment with FTX-1821. Three independent replicates per group were analyzed.

EXAMPLE 9

REDUCTION OF MBD3L2 MRNA IN VARIOUS FSHD1 GENOTYPES AND PHENOTYPES

[0194] The ability of p38 inhibitors to reduce expression of DUX4 target genes in cells obtained from patients having various different FSHD1 genotypes was conducted as described in Methods and Materials. Four distinct FSHD patient myoblast lines, i.e., FTCE-016, -020, -197, and -196 (kindly provided Rabi Tawil) were treated with FTX-1821 (1 μM) or FTX-839 (1 μM), and mRNA levels of the DUX4 target gene, MBD3L2, were determined following treatment.

[0195] MBD3L2 expression levels were reduced in all of the FSHD lines, resulting in levels similar to those measured in healthy controls, FTCE-396 and FTCE-014 (FIG. 11). This is evidence of DUX4 target gene reduction by p38 inhibitors across myotubes derived from diverse FSHD1 genotypes and phenotypes (similar results were observed for FSHD2, data not shown).

EXAMPLE 10

REDUCTION OF MBD3L2 MRNA FROM FSHD1 AND FSHD2 GENOTYPES AND PHENOTYPES

[0196] To assess the treatment effect of p38 selective inhibition using FTX-1821 in FSHD1 and FSHD2 cells, primary myoblast lines were kindly provided by Rabi Tawil at the University of Rochester. FIG. 13 summarizes the genotypes and phenotypes of 13 FSHD1 and 3 FSHD2 patient myoblasts used in the study. The various FSHD1 and FSHD2 myoblasts were treated with DMSO, FTX-1821 or FTX-839 (1 μM), and following treatment, mRNA expression levels of the DUX4 target gene, MBD3L2, were determined. In addition, apoptosis was determined by measuring active caspase-3 in the FSHD1 and FSHD2 lines.

[0197] Each of the various FSHD1 and FSHD2 myoblasts showed a reduction of MBD3L2 (FIG. 14A, top 11 lines). The reduction resulted in expression levels similar to those in healthy control lines (CTRL- FTCE-014) (FIG. 14A, bottom 2 lines). In addition, treatment with FTX-839 showed a reduction in apoptosis across both FSHD1 and FSHD2 lines, to a level that was similar to the amount determined in a healthy control line (CTRL- FTCE-014) (FIG. 14B). These results indicate that clinical FSHD biopsy myoblasts, when differentiated into myotubes, show a reduction in both pathologic DUX4 downstream gene expression and resulting cell death across both FSHD1 and FSHD2 genotypes and phenotypes.

EXAMPLE 11

TARGET ENGAGEMENT IN MUSCLE OF WILD TYPE RATS FOLLOWING TREATMENT WITH A POTENT AND SELECTIVE P38 INHIBITOR

[0198] The pharmacokinetic properties of FTX-1821 were studied in an animal model. FTX-1821 was orally dosed to fasted or unfasted male Sprague-Dawley rats (N=6 animals per time

point and treatment group), and phospho p38α:total p38α levels were determined. Pharmacodynamic analysis of p38 system target engagement in muscle tissue was performed by measuring the change in phospho MAP kinase-activated protein kinase 2 (MK2) to total MK2 ratio before and after drug treatment. All methods used are described in the Materials and Methods section.

[0199] FTX-1821 exhibited plasma pharmacokinetic properties similar to those described previously (Aston et al., 2009; data not shown). These studies additionally demonstrated rapid distribution of FTX-1821 to multiple muscles and plasma. Muscle to plasma exposure ratios were equal to or greater than 1 in the rat when clinically relevant plasma exposures were achieved.

[0200] Pharmacodynamic analysis demonstrated that a single, oral dose of FTX-1821 (0.3mg/kg) resulted in clinically relevant plasma concentrations (Barbour et al., 2012) and significantly decreased the phospho MK2 to total MK2 ratio in rat trapezius muscle within 1-hour of drug treatment (FIG. 15). P38 system target engagement persisted for at least 12 hours following the single dose of FTX-1821 (FIG. 15). P38 system target engagement in trapezius muscle was maximal when plasma and muscle concentrations of FTX-1821 were greater than 20 ng.mL or ng.g and declined at timepoints when exposures decreased. The muscle concentrations of FTX-1821 achieved in the rat study are predicted to result in >70% reduction at Cmax in DUX4 dependent target genes in FSHD patient muscle biopsies based upon *in vitro* data in FSHD myotubes (above).

[0201] This pharmacokinetic and pharmacodynamic analysis indicated that maximal inhibition of the p38 system in muscle was achieved when plasma FTX-1821 concentrations were greater than 20 ng/mL and that significant p38 pathway inhibition would be expected in human muscle, with clinical doses of 7.5 or 15 mg BID (Barbour et al., 2012).

EXAMPLE 12

INHIBITION OF THE DUX4 GENOMIC PROGRAM IN FSHD XENOGRAFTED MICE FOLLOWING TREATMENT WITH A POTENT AND SELECTIVE P38 INHIBITOR

[0202] FSHD and control muscle xenograft mice were generated by xenografting C6 (FSHD) and A4 (control) IPSC-derived human immortalized isogeneic myoblast cell lines into the bilateral tibialis anterior (TA) muscles of approximately 8-week old male Nod-Rag mice as described by Sakellariou et al., 2016. Following the 4-week long engraftment and INMES procedure, the FSHD xenografted animals were treated with BID injections of either vehicle or FTX-2865 (10 mg/kg) for 8 days (a total of 14 injections) and were sacrificed at approximately the time of maximal plasma concentrations (Tmax) 1-hour after the final

morning injection on Day 8. At sacrifice, plasma, trapezius muscle and bilateral tibialis anterior muscles were collected and flash frozen for analysis of pharmacokinetic endpoints, target engagement and DUX4 dependent mRNAs. MBD3L2 was assessed by qPCR using a human specific probe and was normalized to the housekeeping gene CDKN1B. pMK2 and MK2 protein concentrations were assessed by a quantitative MSD assay.

[0203] Analysis of TA tissue by qPCR from animals engrafted for 4-6 weeks with A4 or C6 myoblast tissues demonstrated a significant ($p<0.05$) and >10-fold increase in MBD3L2 and other Dux4 dependent genes (not shown) in the FSHD (C6) vs. control (A4) xenografted TA muscles (FIG. 16). N=8 TA samples per group.

[0204] Treatment of FSHD xenografted animals with the potent and selective p38 inhibitor, FTX-2865, produced p38 system target engagement, as measured by a change in phospho MAP kinase-activated protein kinase 2 (MK2) to total MK2 ratio of >50% in the TA and trapezius muscles of wild-type mice following repeated BID administration of a 10mg/kg dose given via intraperitoneal (IP) injection (data not shown). FTX-2865 treatment significantly ($p<0.05$) decreased the ratio of phospho to total MK2 in mouse trapezius muscle, indicating significant p38 system engagement, and also indicating sufficient drug concentrations in the skeletal muscles of the animals to inhibit the p38 system by >80% (FIG. 17; N=8 trapezius samples per group). In addition, FTX-2865 treatment significantly ($p<0.05$) decreased the expression of MBD3L2 in the FSHD xenografted TA muscles compared to vehicle treated animals, indicating suppression of the pathologic DUX4 gene program by p38 inhibition (FIG. 18; N=5-7 TA samples per group).

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All publications and patent applications described herein are hereby incorporated by reference in their entireties.

While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and other variations thereof

will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

Claims

1. A method of reducing the expression of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in a cell, comprising contacting the cell with an agent that results in a reduction of active p38 protein in the cell, thereby reducing expression the DUX4 polypeptide or the polypeptide encoded by the downstream target gene of DUX4.

2. The method of claim 1, wherein the agent inhibits the expression or activity, or reduces the amount, of the p38 protein, wherein the activity is optionally kinase activity.

3. The method of claim 1 or claim 2, wherein the cell is a muscle cell, optionally a terminally differentiated muscle cell.

4. The method of claim 1, wherein the cell has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell.

5. The method of claim 4, wherein the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell.

6. The method of any of claims 1-5, wherein the cell is associated with facioscapulohumeral muscular dystrophy (FSHD).

7. The method of claim 6, wherein the cell comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises $\leq$7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35.

8. The method of any of claims 1-7, wherein the cell comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene.

9. The method of claim 8, wherein the cell comprises at least one non-deleted 4qA allele.

10. The method of any of claims 1-9, wherein the agent inhibits the expression of the p38 protein.

11. The method of claim 10, wherein the agent binds a polynucleotide encoding the p38 protein, or an antisense polynucleotide thereof.

12. The method of any of claims 1-11, wherein the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide.

13. The method of any of claims 1-9, wherein the agent inhibits the activity of the p38 protein.

14. The method of any of claims 1-9 and 13, wherein the agent binds the p38 protein.

15. The method of any of claims 1-9, 13 and 14, wherein the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof.

16. The method of any of claims 1-9, 13, and 14, wherein the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule.

17. The method of any of claims 1-16, wherein the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

18. The method of any of claims 1-17, wherein the expression or the activity of the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

19. A method of treating or preventing a disease or disorder associated with increased expression of a DUX4-fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in a subject in need thereof, comprising providing to the subject a pharmaceutical composition comprising an agent that results in a reduced amount of active p38 protein in one or more tissue of the subject, thereby reducing expression of the DUX4-fl mRNA, the DUX4 protein, or the polypeptide encoding the downstream target gene in one or more tissue of the subject.

20. The method of claim 19, wherein the disease or disorder is a facioscapulohumeral muscular dystrophy (FSHD), optionally FSHD1 or FSHD2.

21. The method of claim 19 or claim 20, wherein the subject comprises reduced repression at a D4Z4 locus.

22. The method of any of claims 19-21, wherein the subject comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises $\leq$7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35.

23. The method of claim 19 or claim 20, wherein the subject comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene.

24. The method of claim 23, wherein the subject comprises at least one non-deleted 4qA allele.

25. The method of any of claims 19-24, wherein the agent inhibits the expression of the p38 protein.

26. The method of any of claims 19-25, wherein the agent binds a polynucleotide encoding the p38 protein, or an antisense polynucleotide thereof.

27. The method of any of claims 19-26, wherein the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide.

28. The method of any of claims 19-24, wherein the agent inhibits the activity of the p38 protein.

29. The method of any of claims 19-24 and 28, wherein the agent binds the p38 protein.

30. The method of any of claims 19-24, 28 and 29, wherein the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof.

31. The method of any of claims 19-24, 28 and 29, wherein the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule.

32. The method of any of claims 19-31, wherein the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

33. The method of any of claims 19-32, wherein the expression or the activity of the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% in a muscle tissue of the subject.

34. The method of any of claims 19-33, wherein the method inhibits the expression or activity of the p38 protein in a muscle tissue of the subject.

35. The method of any of claims 19-34, wherein the method decreases muscle degeneration in the subject.

36. The method of any of claims 19-35, wherein the method reduces apoptosis of muscle cells in the subject.

37. The method of claim 36, wherein the muscle tissue is terminally differentiated.

38. The method of claim 11, wherein the pharmaceutical composition is provided to the subject parenterally.

39. The method of claim 11, wherein the pharmaceutical composition is provided to a muscle tissue of the subject.

40. The method of any of claims 19-39, wherein the method further comprises providing to the subject a second agent for treating the disease or disorder associated with increased expression of a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4.

41. A unit dosage form of a pharmaceutical composition comprising an agent that results in a reduced amount of active p38 protein in a cell, and a pharmaceutically acceptable carrier, diluent or excipient, wherein the unit dosage form is effective to reduce expression or activity of a DUX4-fl mRNA, a DUX4 polypeptide, or a polypeptide encoded by a downstream target gene of DUX4, in one or more cell or tissue in a subject to whom the unit dosage form is administered.

42. The unit dosage form of claim 41, wherein the agent binds the DUX4 polypeptide or binds a polynucleotide encoding the DUX4 polypeptide.

43. The unit dosage form of claim 41 or claim 42, wherein the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, gRNA, shRNA, siRNA, or antisense oligonucleotide.

44. The unit dosage form of claim 41 or claim 42, wherein the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof.

45. The unit dosage form of 41 or claim 42, wherein the agent comprises a small molecule, optionally an organic molecule or an inorganic molecule.

46. The unit dosage form of any of claims 41-45, wherein the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

47. The unit dosage form of any of claims 41-46, wherein the tissue is muscle tissue, optionally wherein the tissue comprises cells comprising a mutation associated with facioscapulohumeral muscular dystrophy (FSHD).

48. A method of reducing apoptosis of a muscle cell, comprising contacting the cell with an agent that results in a reduced amount of active p38 protein in the cell, optionally wherein the muscle cell is terminally differentiated, thereby reducing expression of a DUX4-

fl mRNA, a DUX4 protein, or a polypeptide encoded by a downstream target gene of DUX4, in the cell.

49. The method of claim 48, wherein the cell has an increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, as compared to the expression level of the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, in a control cell.

50. The method of claim 49, wherein the increased expression level of the DUX4-fl mRNA, the DUX4 polypeptide, or the polypeptide encoded by the downstream target gene, is due to reduced repression at a D4Z4 locus in the cell.

51. The method of any of claims 48-50, wherein the cell is associated with facioscapulohumeral muscular dystrophy (FSHD).

52. The method of any of claims 48-51, wherein the cell comprises a deletion of one or more macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35, optionally wherein the cell comprises $\leq$7 macrosatellite D4Z4 repeats in the subtelomeric region of chromosome 4q35.

53. The method of any of claims 48-52, wherein the cell comprises one or more mutations in a Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1 (SMCHD1) gene.

54. The method of claim 53, wherein the cell comprises at least one non-deleted 4qA allele.

55. The method of any of claims 53-59, wherein the agent inhibits the expression of the p38 protein.

56. The method of any of claims 48-55, wherein the agent binds a polynucleotide encoding the p38 protein, or an antisense polynucleotide thereof.

57. The method of any of claims 48-56, wherein the agent comprises or consists of a nucleic acid, optionally a DNA, RNA, shRNA, siRNA, or antisense oligonucleotide.

58. The method of any of claims 48-54, wherein the agent inhibits the activity of the p38 protein.

59. The method of any of claims 48-54 and 58, wherein the agent binds the p38 protein.

60. The method of any of claims 48-54, 583 and 59, wherein the agent comprises or consists of a polypeptide, optionally a protein, a peptide, a protein mimetic, a peptidomimetic, or an antibody or functional fragment thereof.

61. The method of any of claims 48-54, 58, and 59, wherein the agent comprises a small molecule, optionally a small organic molecule or a small inorganic molecule.

62. The method of any of claims 48-61, wherein the downstream target gene is RFPL2, CCNA1, SLC34A2, TPRX1, KHDC1L, ZSCAN4, PRAMEF20, TRIM49, PRAMEF4, PRAME6, PRAMEF15 or ZNF280A.

63. The method of any of claims 48-62, wherein the expression or the activity of the p38 protein is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

64. The method of any of claims 48-63, wherein the method reduces apoptosis of muscle cells in a muscle tissue at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%.

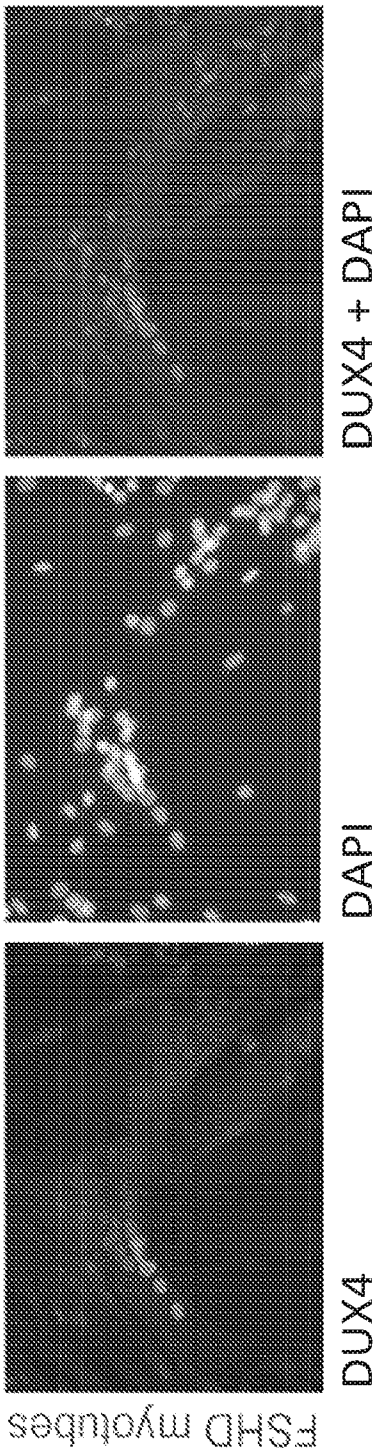


FIG. 1A

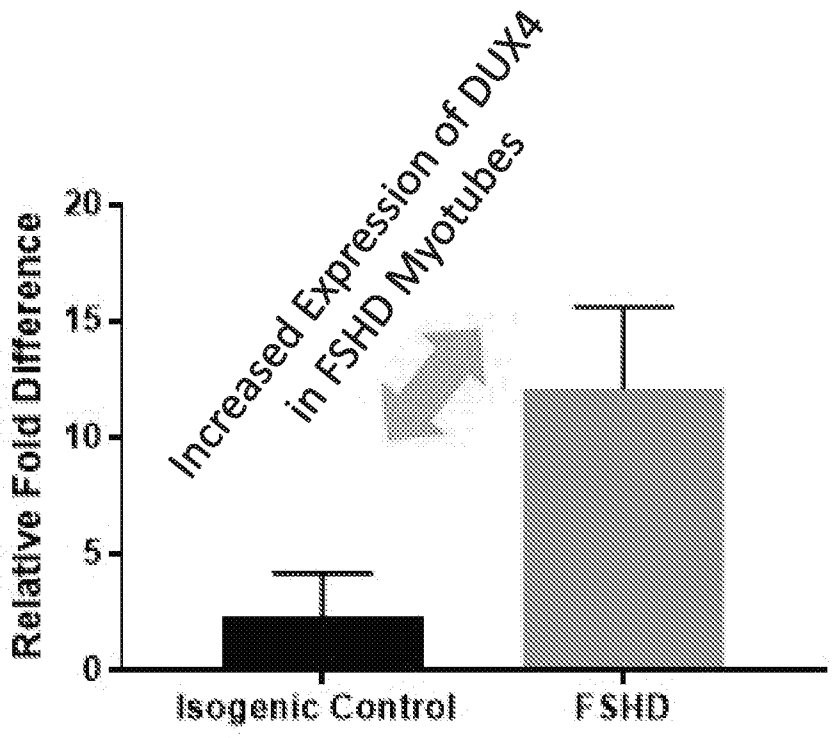

Increased Expression of DUX4
in FSHD Myotubes
Relative Fold Difference
20
15
10
5
0
Isogenic Control
FSHD

FIG. 1B

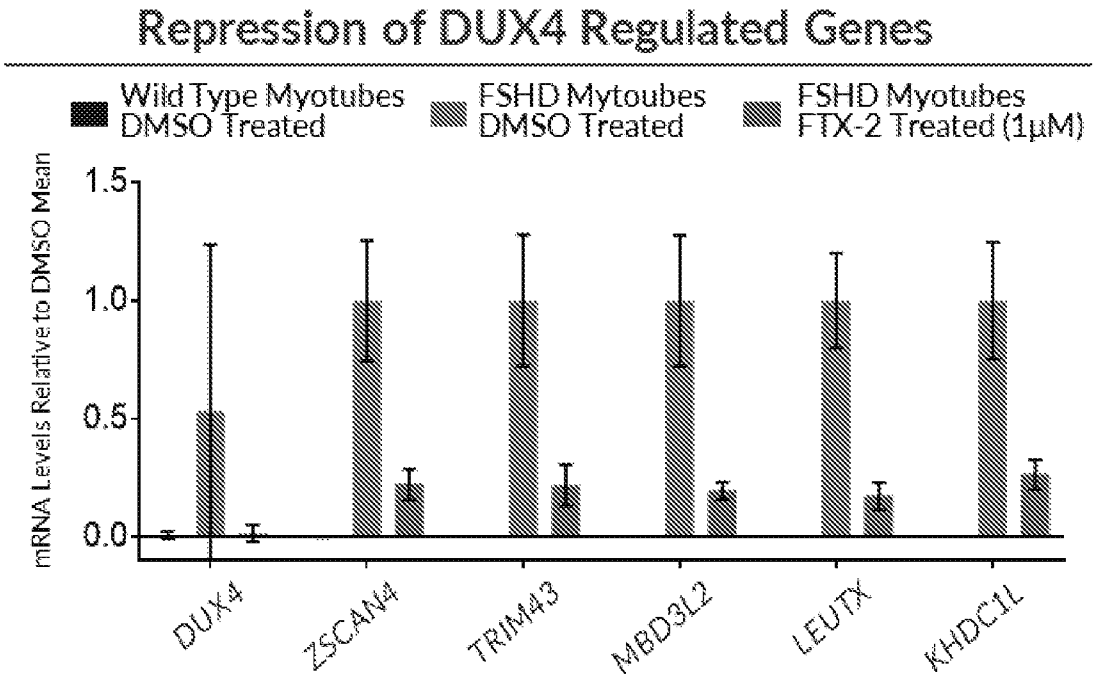

Repression of DUX4 Regulated Genes
Wild Type Myotubes DMSO Treated
FSHD Mytoubes DMSO Treated
FSHD Myotubes FTX-2 Treated (1μM)
mRNA Levels Relative to DMSO Mean
1.5
1.0
0.5
0.0
DUX4
ZSCAN4
TRIM43
MBD3L2
LEUTX
KHDC1L

FIG. 2

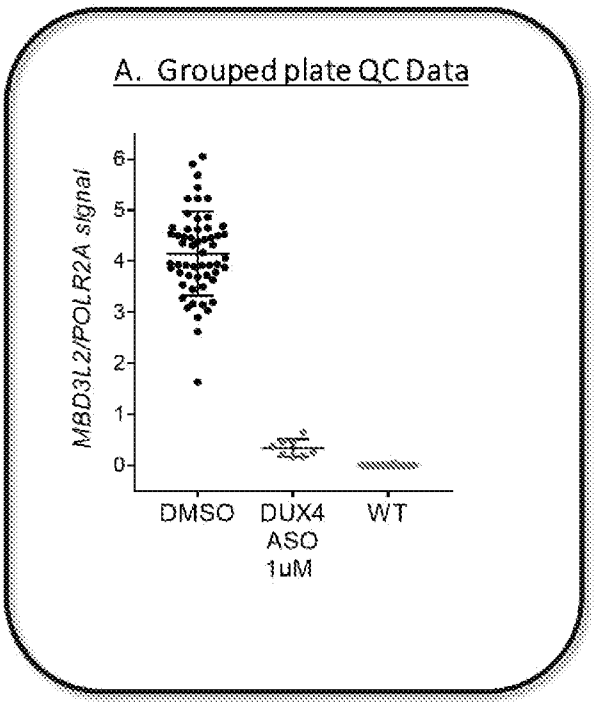


FIG. 3A

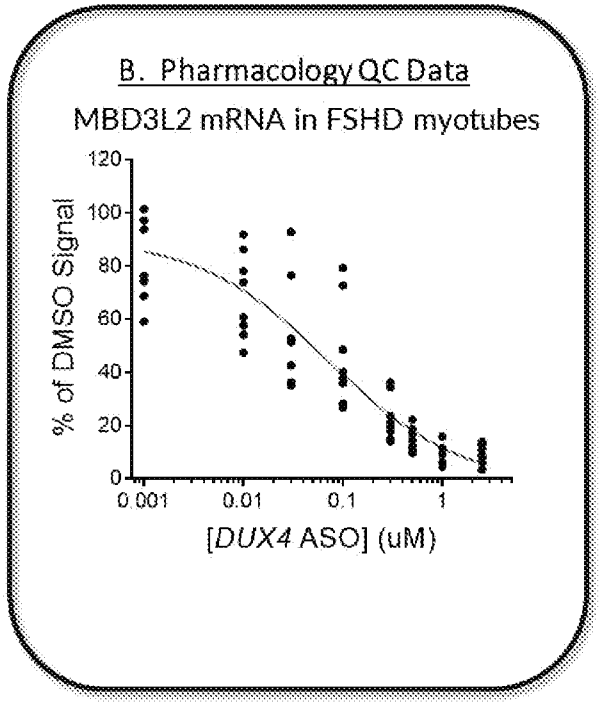


FIG. 3B

C. Assay Statistics

Z' DMSO-WT: 0.512
S/N WT: 5.1

Z' DMSO-*DUX4* ASO: 0.319
S/N DMSO-*DUX4* ASO: 4.6

FIG. 3C

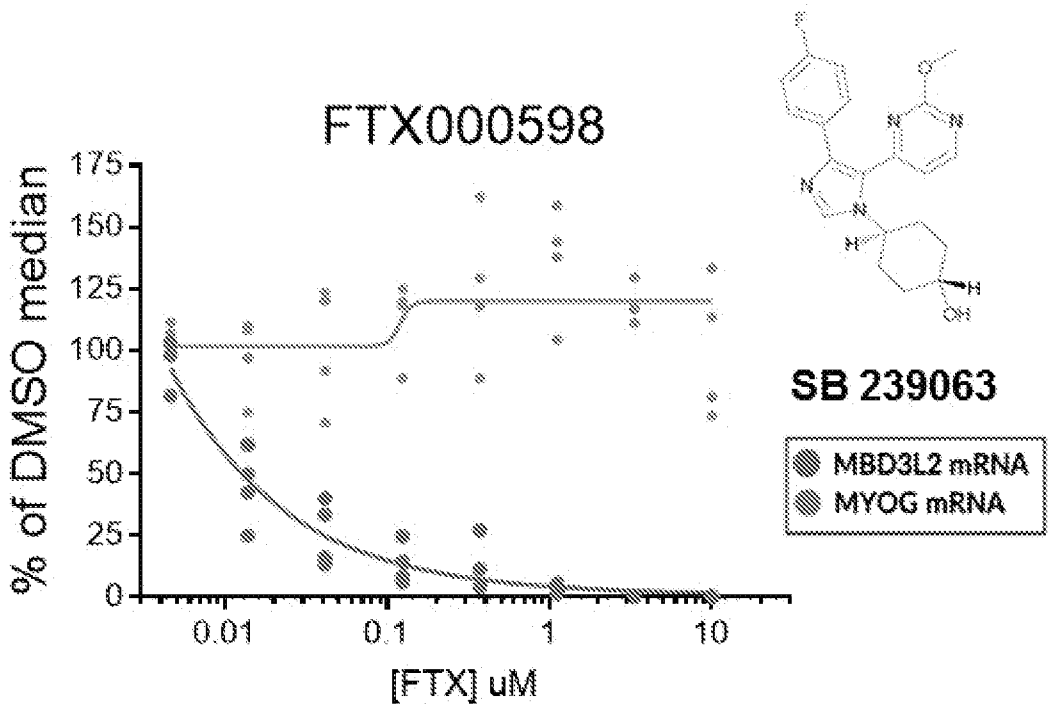

FTX000598
% of DMSO median
175
150
125
100
75
50
25
0
0.01
0.1
1
10
[FTX] uM
SB 239063
MBD3L2 mRNA
MYOG mRNA

FIG. 4A

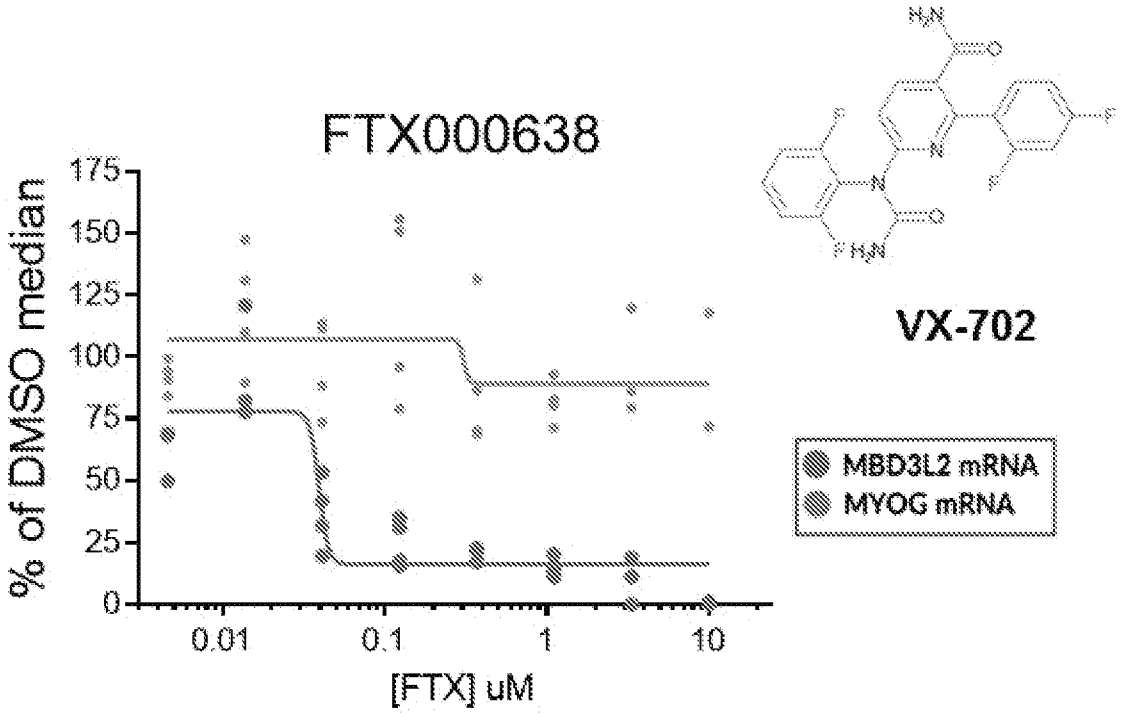

FTX000638
% of DMSO median
175
150
125
100
75
50
25
0
0.01
0.1
1
10
[FTX] uM
VX-702
MBD3L2 mRNA
MYOG mRNA

FIG. 4B

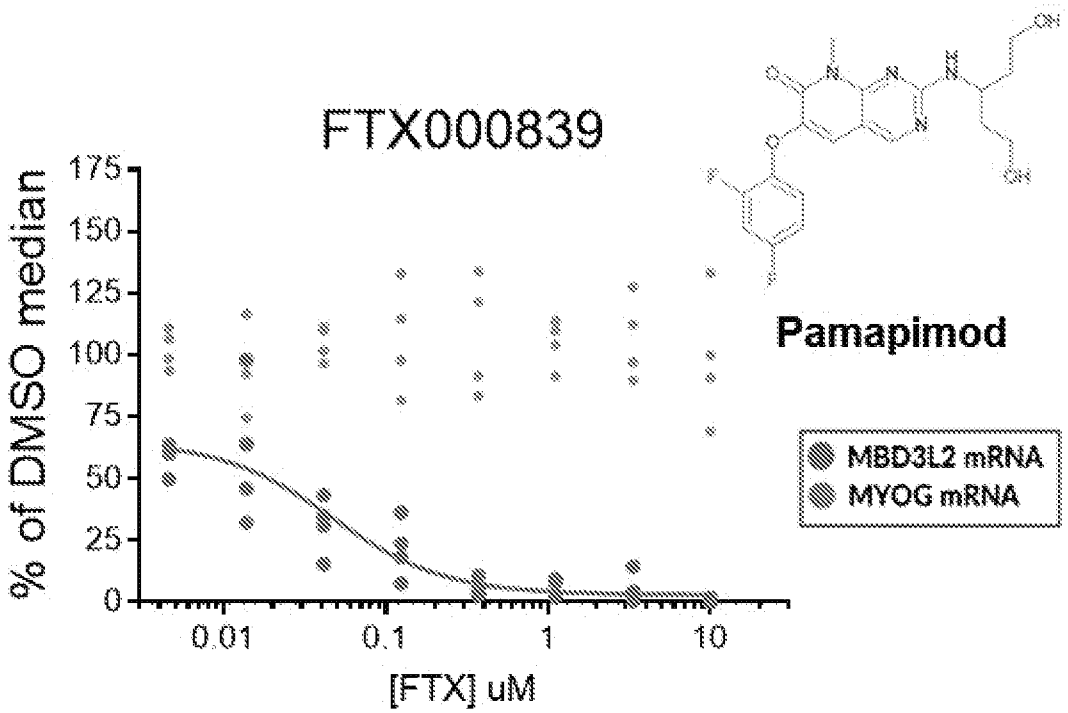

FTX000839
% of DMSO median
175
150
125
100
75
50
25
0
0.01
0.1
1
10
[FTX] uM
Pamapimod
MBD3L2 mRNA
MYOG mRNA

FIG. 4C

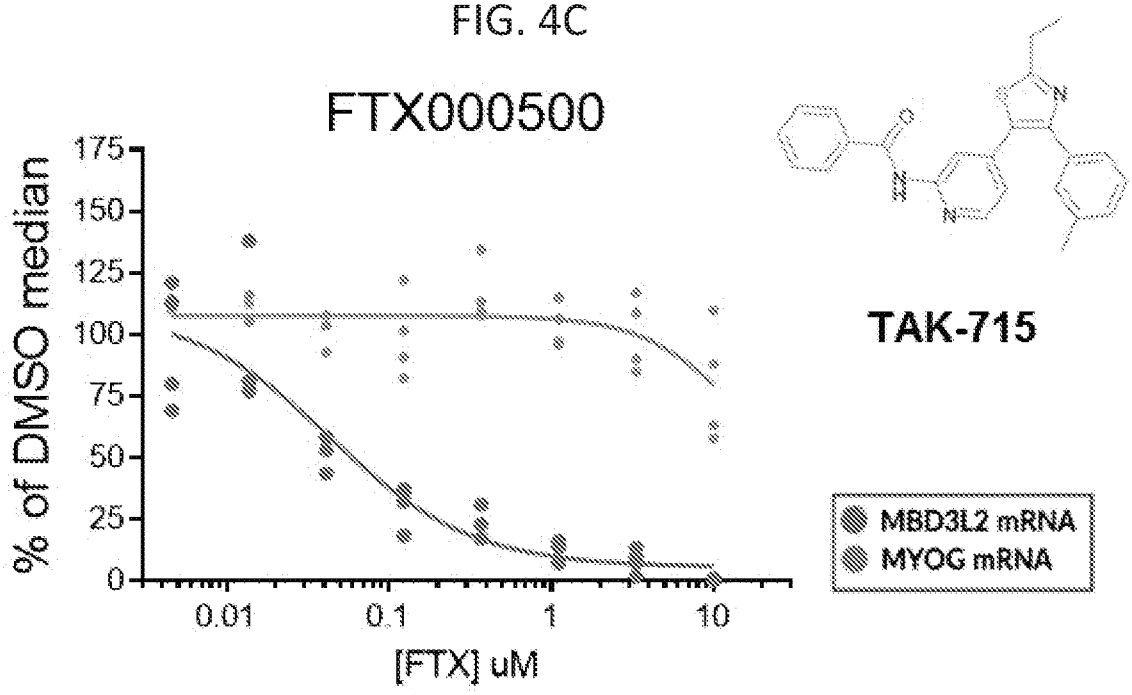

FTX000500
% of DMSO median
175
150
125
100
75
50
25
0
0.01
0.1
1
10
[FTX] uM
TAK-715
MBD3L2 mRNA
MYOG mRNA

FIG. 4D

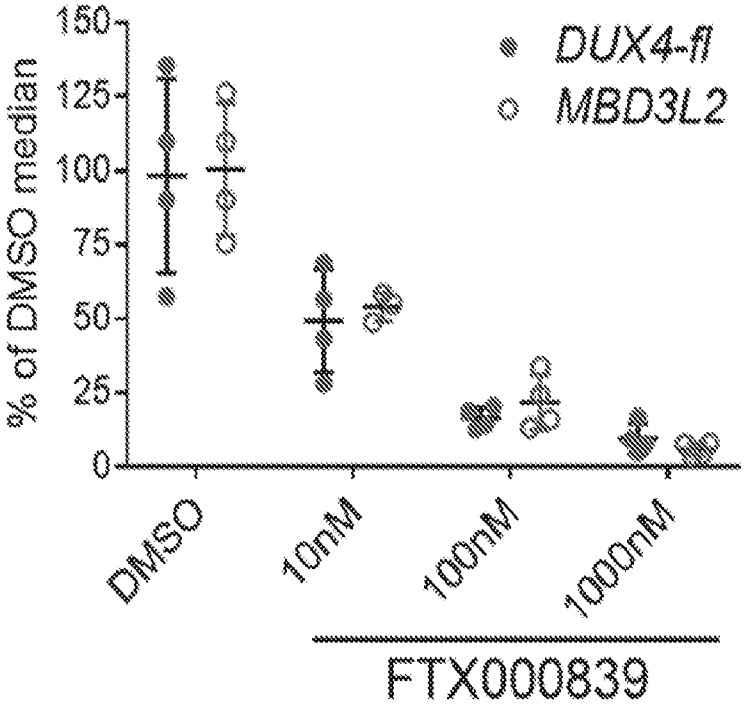

% of DMSO median
150
125
100
75
50
25
0
DUX4-fl
MBD3L2
DMSO
10nM
100nM
1000nM
FTX000839

FIG. 5A

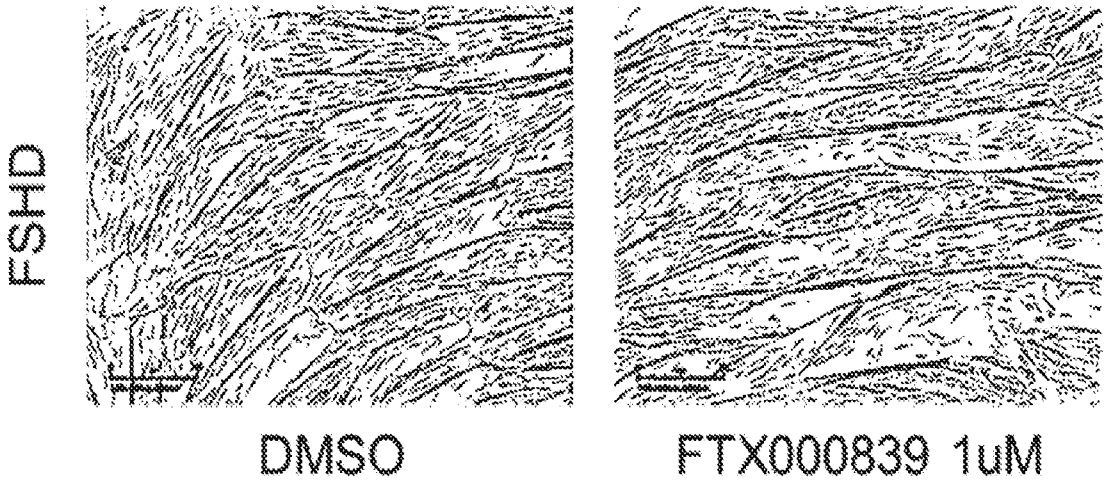

FSHD
DMSO
FTX000839 1uM

FIG. 5B

FIG. 6A

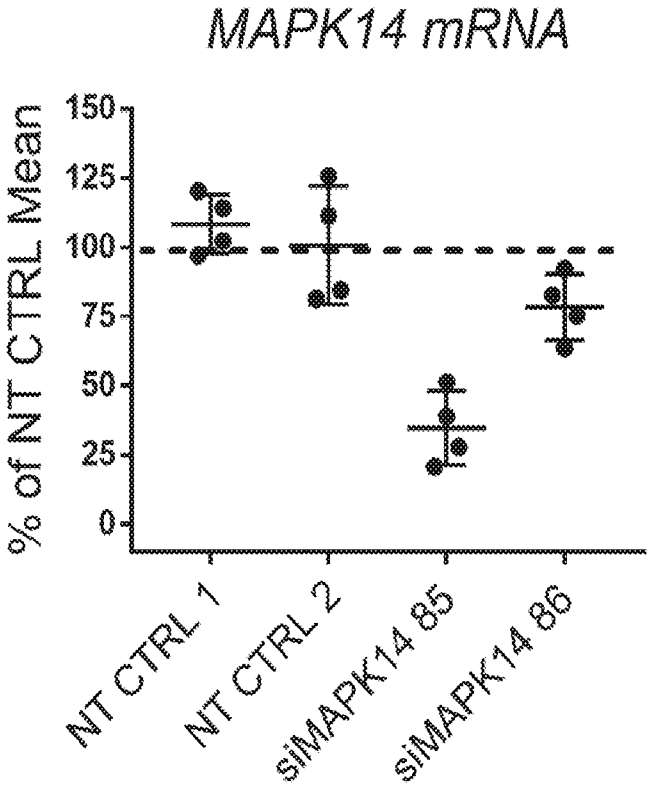

MAPK14 mRNA
% of NT CTRL Mean
150
125
100
75
50
25
0
NT CTRL 1
NT CTRL 2
siMAPK14 85
siMAPK14 86

FIG. 6B

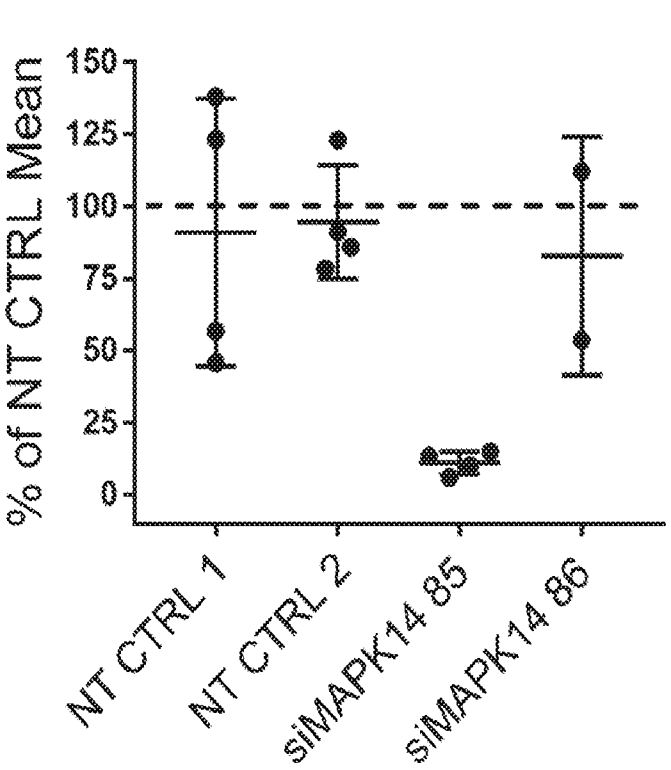
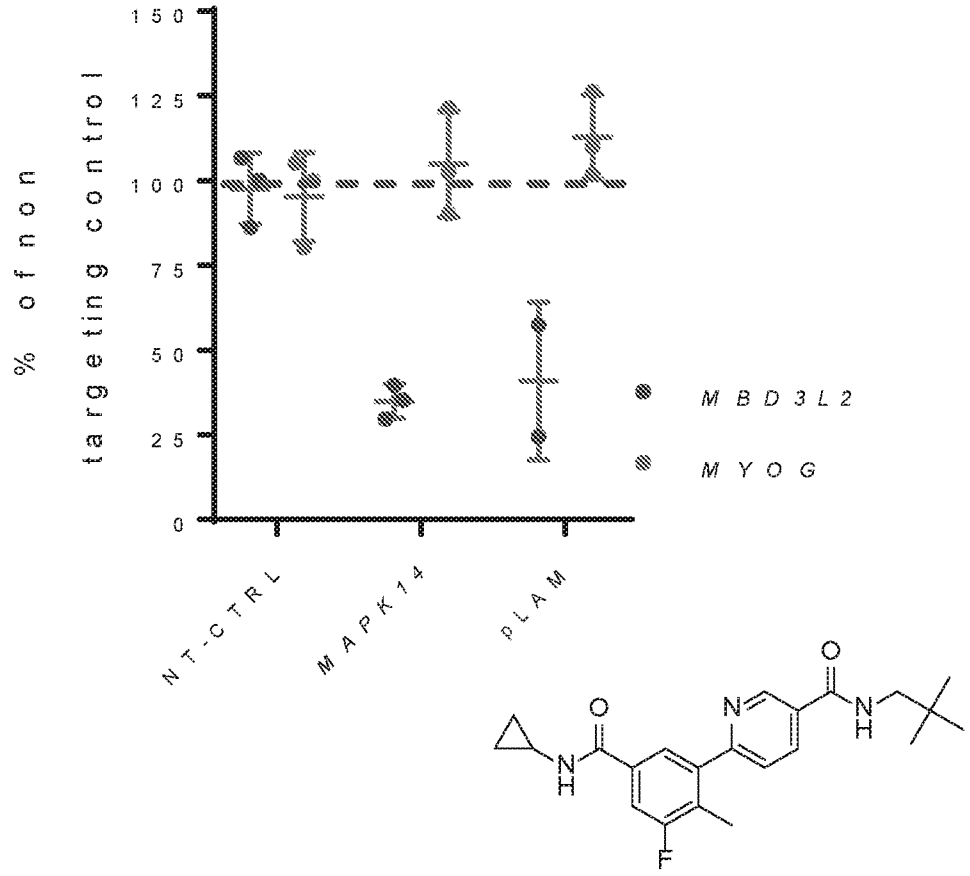
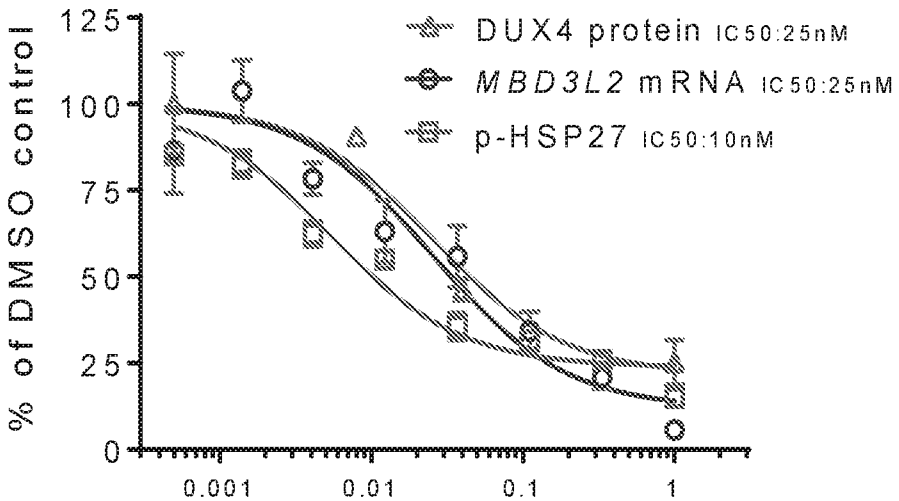

MBD3L2 mRNA
% of NT CTRL Mean
150
125
100
75
50
25
0
NT CTRL 1
NT CTRL 2
siMAPK14 85
siMAPK14 86

FIG. 6C



FTX-1821

FIG. 7



[FTX-1821]

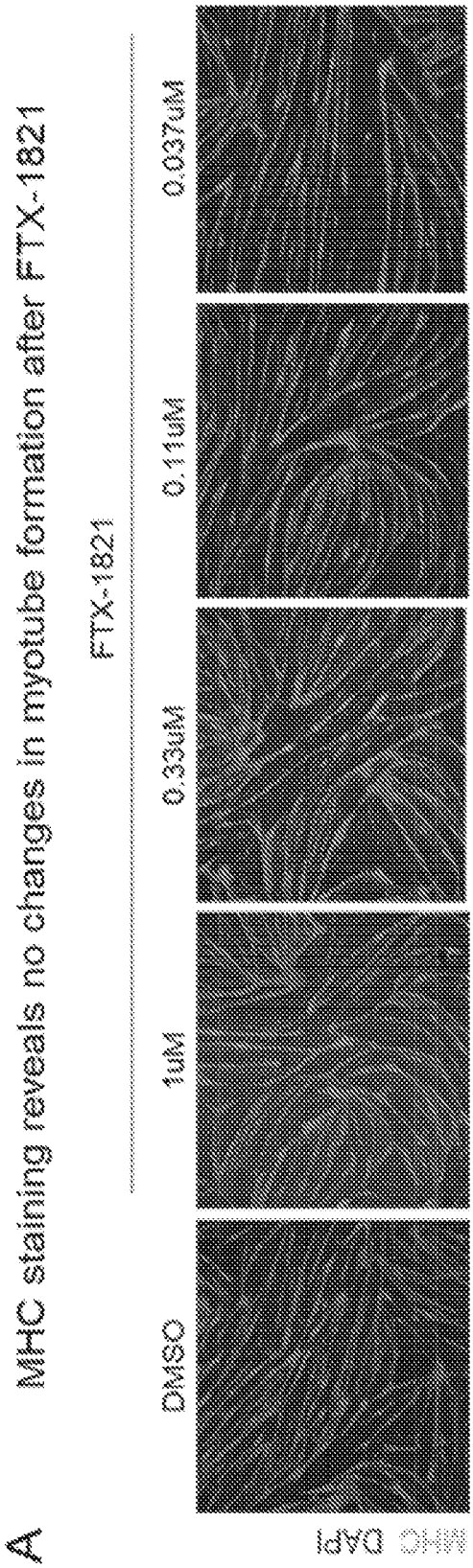
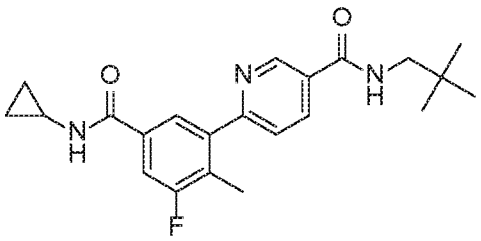

A
MHC staining reveals no changes in myotube formation after FTX-1821
FTX-1821
DMSO
1uM
0.33uM
0.11uM
0.037uM
MHC DAPI

FIG. 8A



FTX-1821

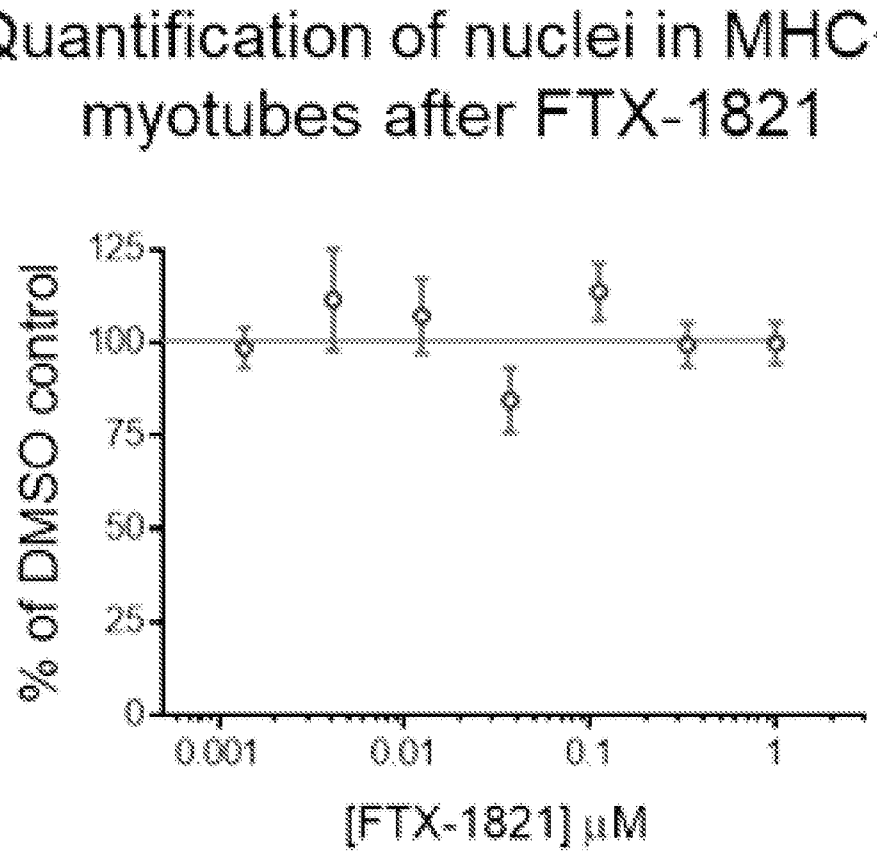


FIG. 8B

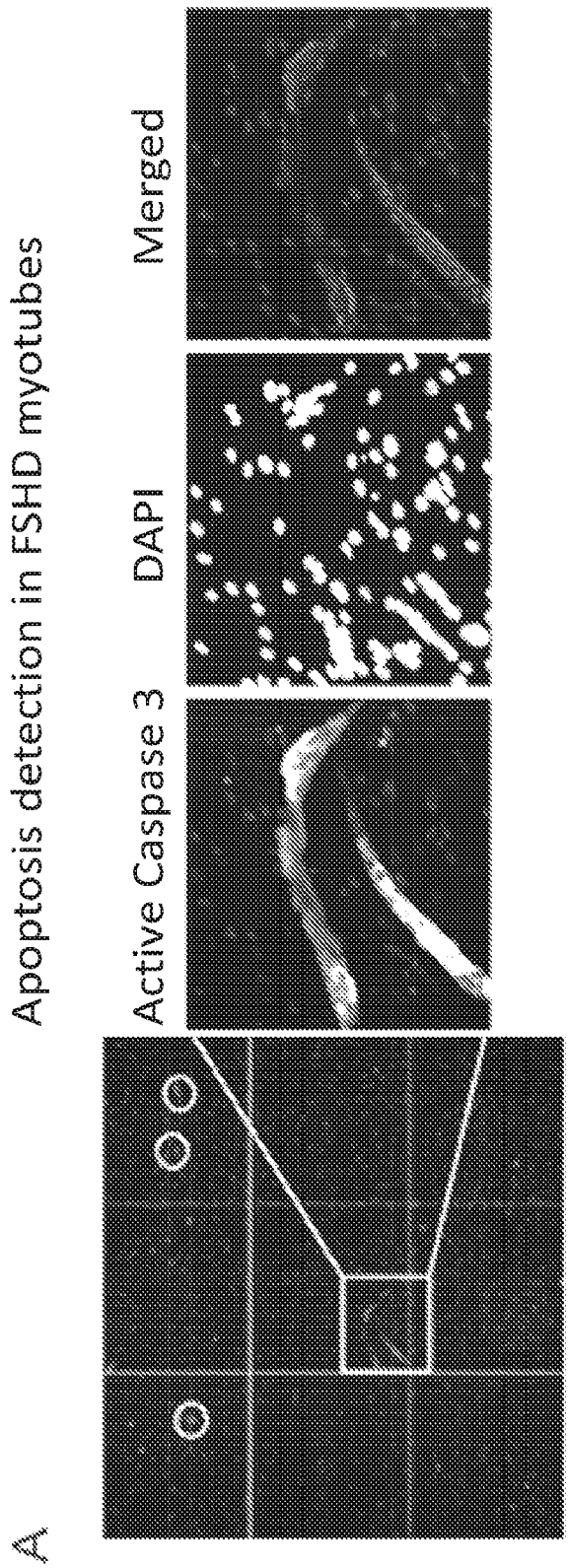

A
Apoptosis detection in FSHD myotubes
Active Caspase 3
DAPI
Merged

FIG. 9A

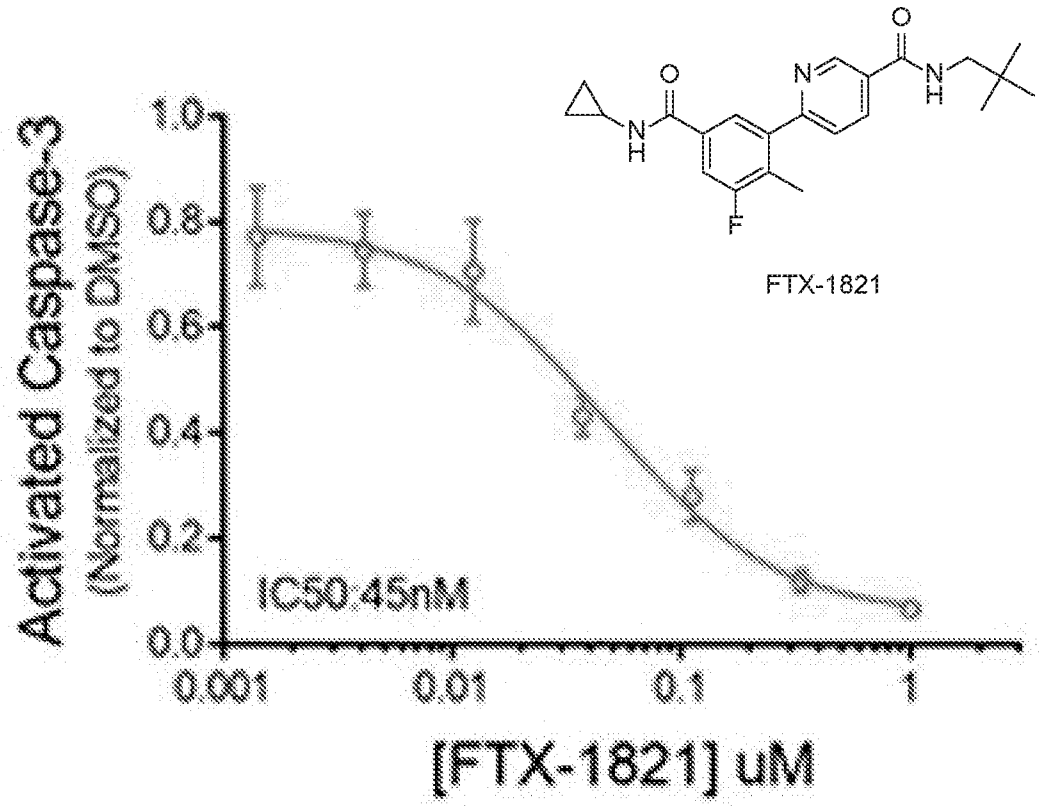
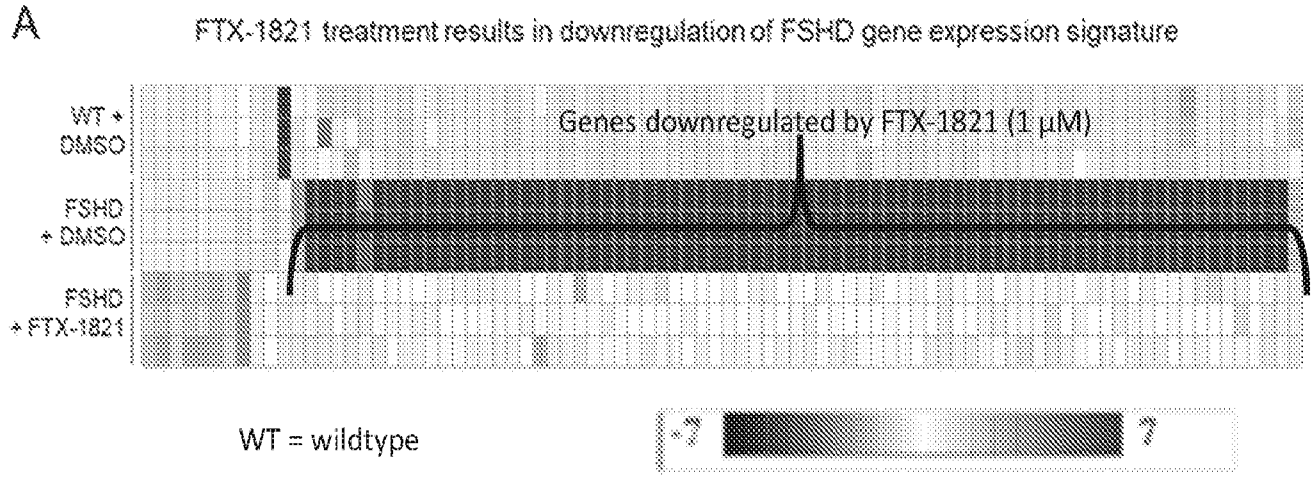

Activated Caspase-3
(Normalized to DMSO)
1.0
0.8
0.6
0.4
0.2
0.0
IC50:45nM
0.001
0.01
0.1
1
[FTX-1821] uM
O
N
H
N
O
N
H
F
FTX-1821

FIG. 9B



Genes downregulated by FTX-1821:

ZSCAN4	TRIM51BP	MBD3L3	UBTFL5	AP001043.1	ABRA
PRAMEF20	KHDC1P1	KDM4F	HNRNPCL3	MBD3L5	WIPF3
PRAMEF6	RFPL4A	DUXA	ZIM3	TRIM51CP	UBTFL1
PRAMEF17	ZSCAN5DP	TPRX1	TRIM53BP	TRIM53CP	POU5F1B
PRAMEF1	PRAMEF9	PRAMEF13	TRIM53AP	CILP	UBTFL2
ZNF705E	KLF17	TRIM43	DPPA3	SLC2A3	FAM151A
LEUTX	RFPL2	MBD3L2	MBD3L2B	TRIM49	TRIM60
PRAMEF15	CCNA1	PRAMEF11	USP29	ZNF705G	PRAMEF28P
ZNF705A	PRAMEF2	PRAMEF14	ZNF296	TRIM43CP	KLF18
PRAMEF12	HNRNPCL1	KHDC1L	F2RL1	TRIM48	SNAI1P1
SLC34A2	DUXB	PRAMEF19	FREM2	UBTFL6	HUNK
HNRNPCL2	HSPA6	PRAMEF4	PRAMEF8	PRAMEF33	P2RX1
PRAMEF18	TRIM43B	KDM4E	TRIM51	C1DP2	TPRX2P
TRIM49C	TC2N	TRIM49B	IGFN1	DPYSL5	FAM9C
					GJA5
					IMPG2

FIG. 10A

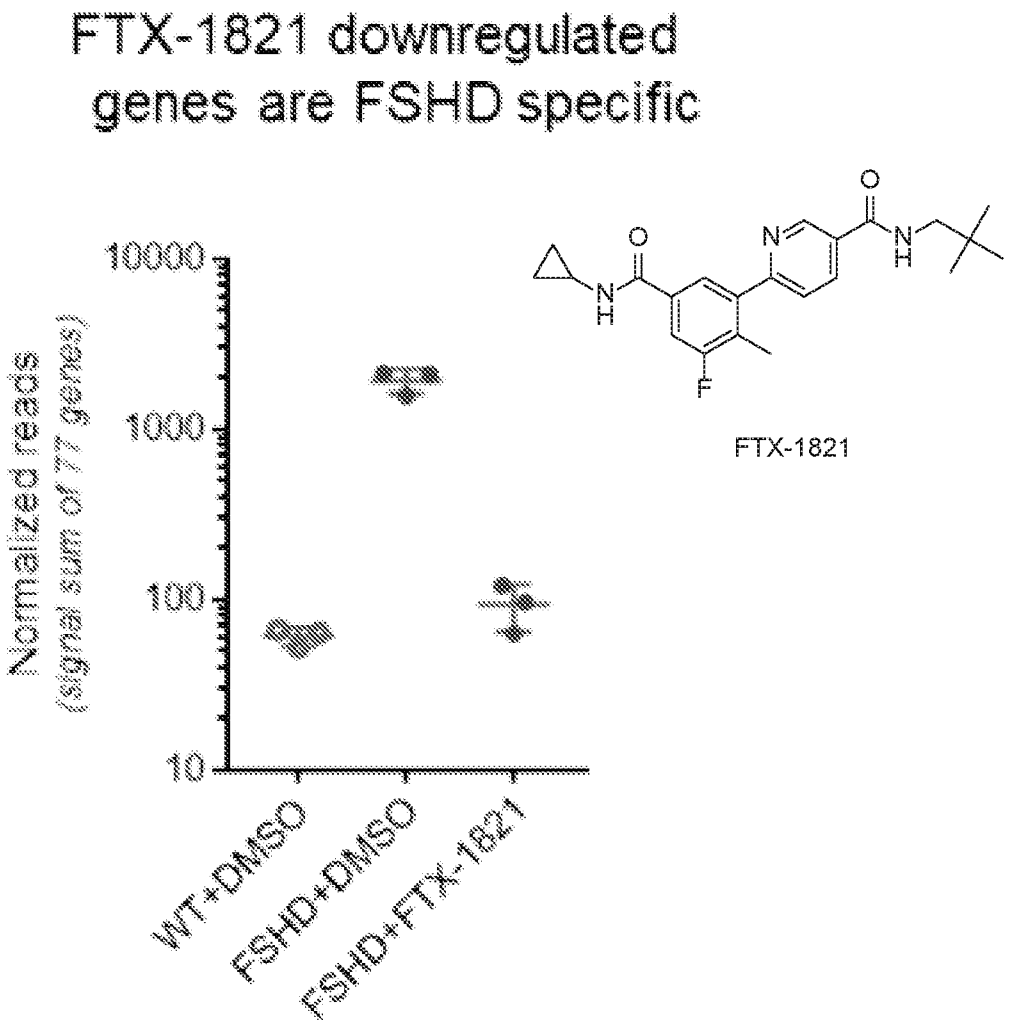
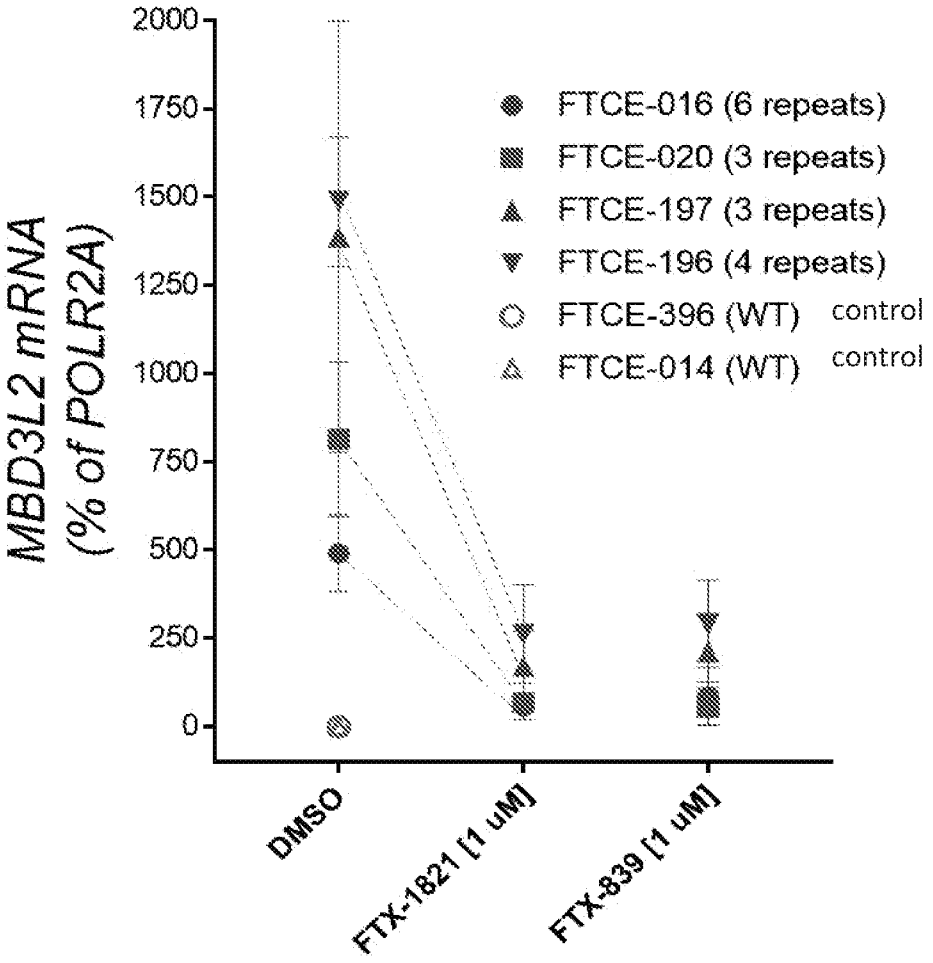
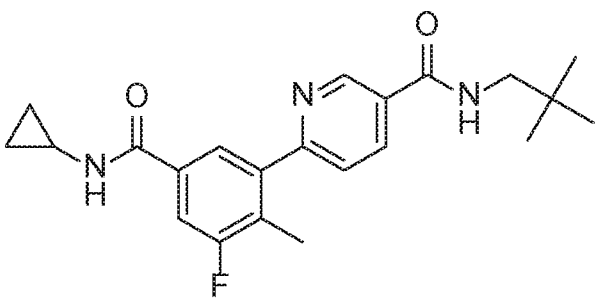
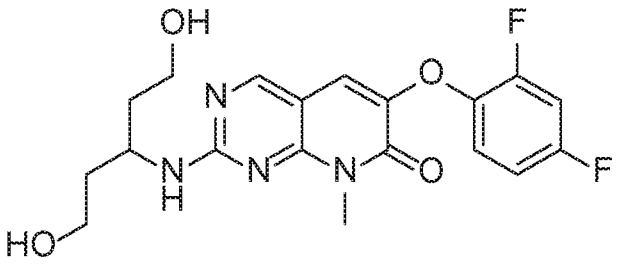

FTX-1821 downregulated genes are FSHD specific
Normalized reads (signal sum of 77 genes)
10000
1000
100
10
WT+DMSO
FSHD+DMSO
FSHD+FTX-1821
O
N
H
N
O
N
H
F
FTX-1821

FIG. 10B





FTX-1821



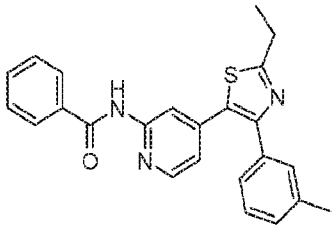
FTX-839

FIG. 11

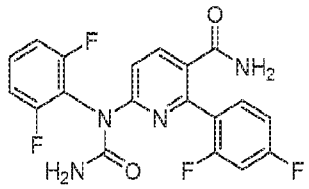
Table 1.

DRUG_NAME	Formatted ID	IC50 *MBD3L2* (nM)
TAK-715	FTX000500	48
VX-702	FTX000638	41
R1487	FTX000830	6
Pamapimod	FTX000839	10
AS1940477	FTX001341	20
Losmapimod	FTX001821	30
LY2228820	FTX002865	10
SCIO-469	FTX004078	25
Doramapimod	FTX004385	42
BMS-582949	FTX005041	68
PH-797804	FTX005042	10
Pexmetinib	FTX005043	5

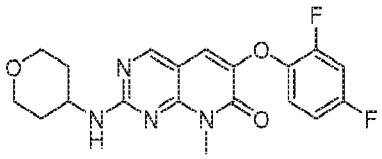
FIG. 12A



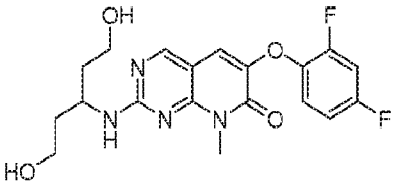
FTX-500
"TAK-715"



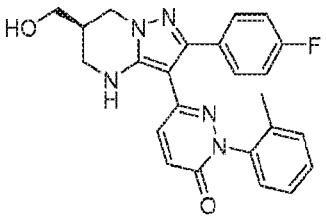
FTX-638
"VX-702"



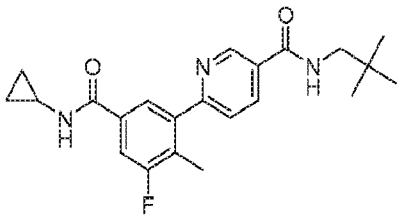
FTX-830
"R1487"



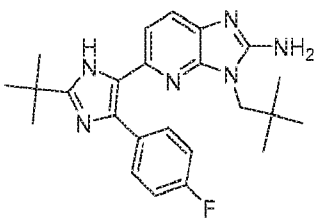
FTX-839
"Pamapimod"



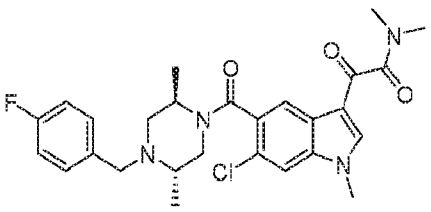
FTX-1341
"AS1940477"



FTX-1821
"Losmapimod"

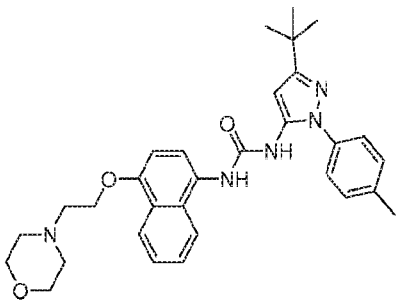


FTX-2865
"LY2228820"

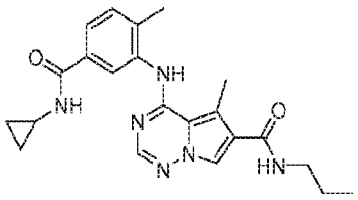


FTX-4078
"SCIO-469"

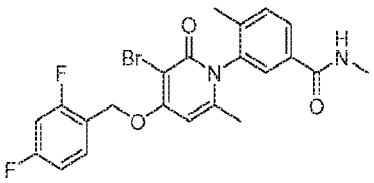
FIG. 12B



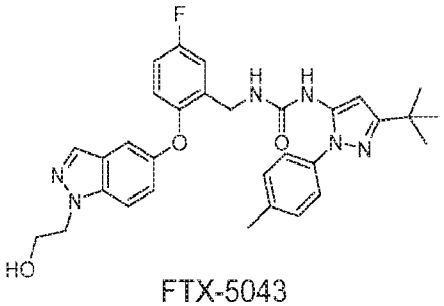
FTX-4385
"Doramapimod"



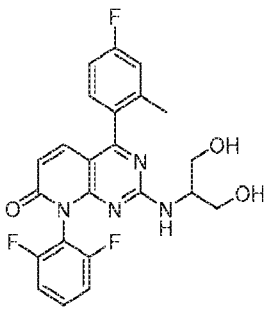
FTX-5041
"BMS-582949"



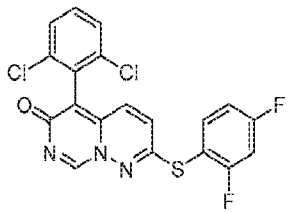
FTX-5042
"PH-797804"



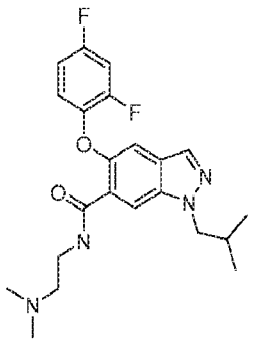
FTX-5043
"Pexmetinib"



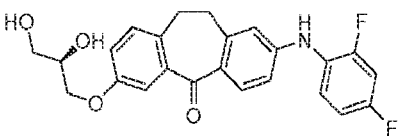
"Dilmapimod"



FTX-4384
"Neflamapimod"
"VX-745"



"ARRY-797"



"Skepinone-L"

FIG. 12B (cont.)

Table 2.

CTID #	ID	p/i	Cell type	Repeat #	Cntrl/FSHD
CTID-001	FTCE-204	primary	Fibroblast	39	Control
CTID-002	FTCE-425	primary	Myoblast	5.5	FSHD
CTID-003	FTCE-423	primary	Myoblast	3	FSHD
CTID-004	FTCE-396	primary	Myoblast	n/a	Control
CTID-005	FTCE-422	primary	Myoblast	2	FSHD
CTID-006	FTCE-424	primary	Myoblast	4.5	FSHD
CTID-007	FTCE-426	primary	Myoblast	4.5	FSHD
CTID-008	FTCE-428	primary	Myoblast	SMCHD1	FSHD2
CTID-009	FTCE-197	primary	Myoblast	2	FSHD
CTID-010	FTCE-196	primary	Myoblast	3	FSHD
CTID-011	FTCE-429	primary	Myoblast	SMCHD1	FSHD2
CTID-012	FTCE-421	primary	Myoblast	7	FSHD
CTID-013	FTCE-205	primary	Myoblast	12	Control
CTID-014	FTCE-427	primary	Myoblast	SMCHD1	FSHD2
CTID-015	FTCE-16	immortalized	Myoblast	6.5	FSHD
C6 Control	FTCE-20	immortalized	Myoblast	3	FSHD
WT Control	FTCE-14	immortalized	Myoblast	n/a	Control
A4 Control	FTCE-18	immortalized	Myoblast	n/a	Control

FIG. 13

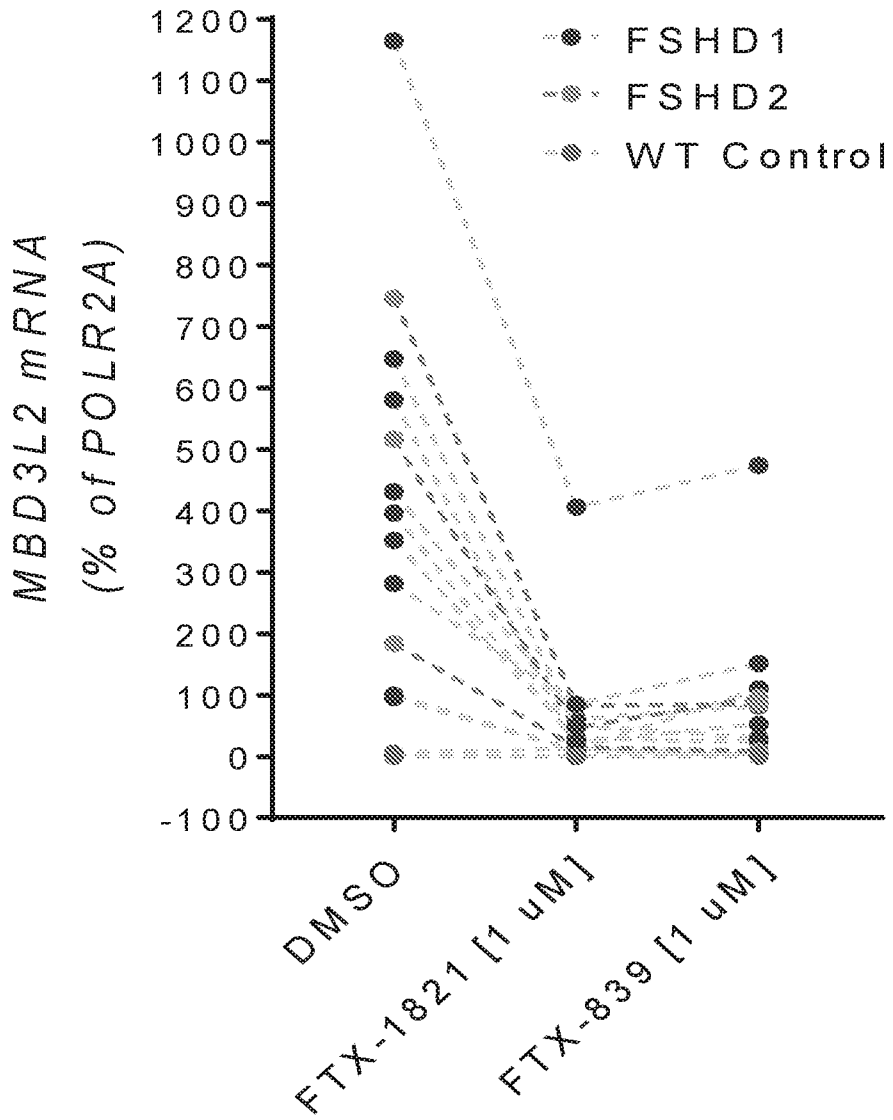

MBD3L2 mRNA
(% of POLR2A)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
FSHD1
FSHD2
WT Control
DMSO
FTX-1821 [1 uM]
FTX-839 [1 uM]

FIG. 14A

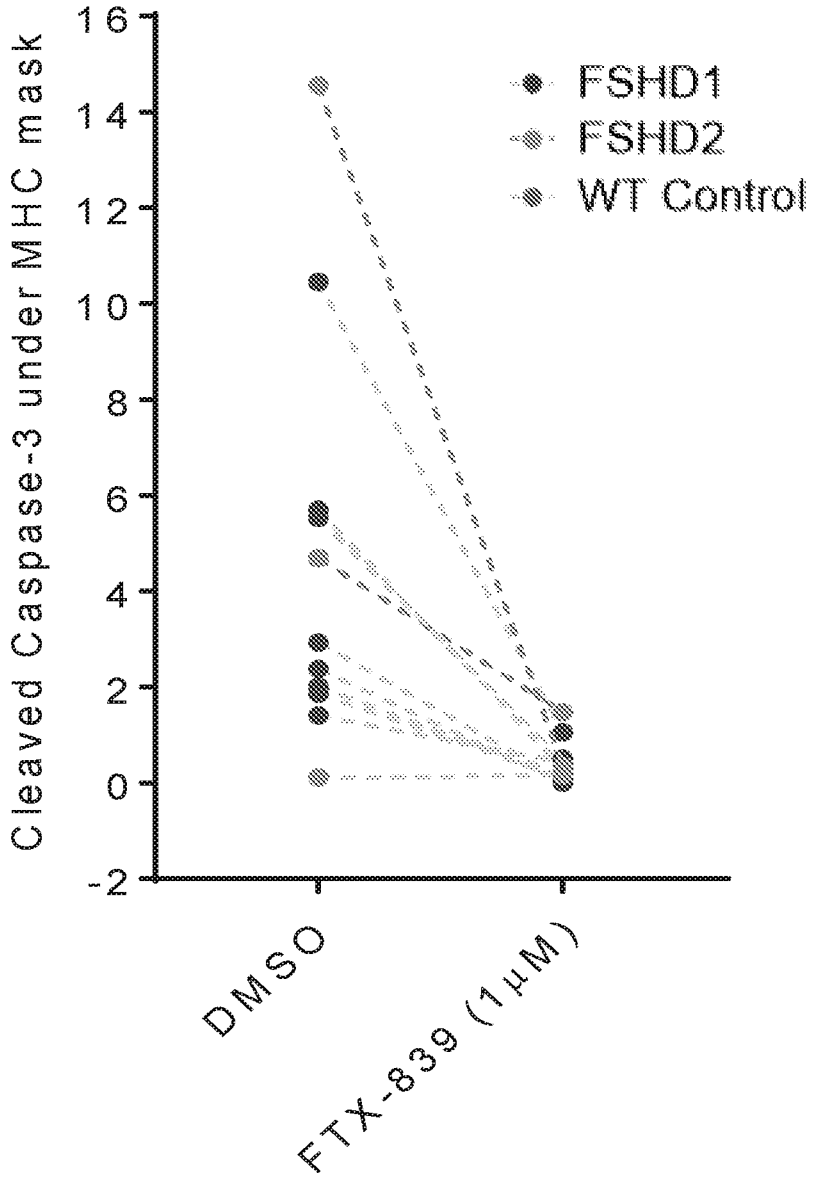

Cleaved Caspase-3 under MHC mask
16
14
12
10
8
6
4
2
0
-2
FSHD1
FSHD2
WT Control
DMSO
FTX-839 (1μM)

FIG. 14B

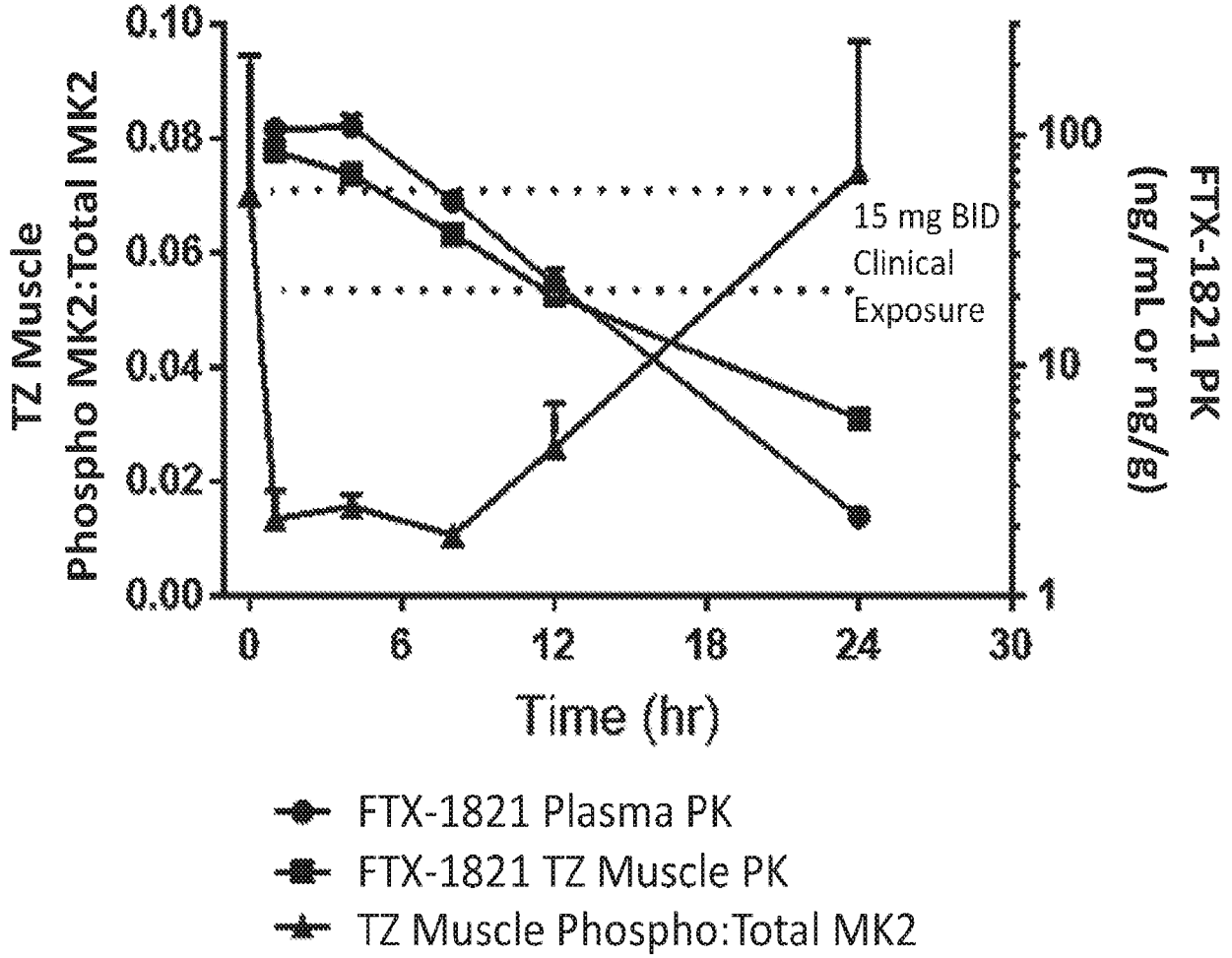

TZ Muscle
Phospho MK2:Total MK2
0.10
0.08
0.06
0.04
0.02
0.00
0
6
12
18
24
30
Time (hr)
15 mg BID
Clinical
Exposure
100
10
1
FTX-1821 PK
(ng/mL or ng/g)
FTX-1821 Plasma PK
FTX-1821 TZ Muscle PK
TZ Muscle Phospho:Total MK2

FIG. 15

The Dux4 dependent mRNA program is elevated in muscles of FSHD vs. control xenografted mice

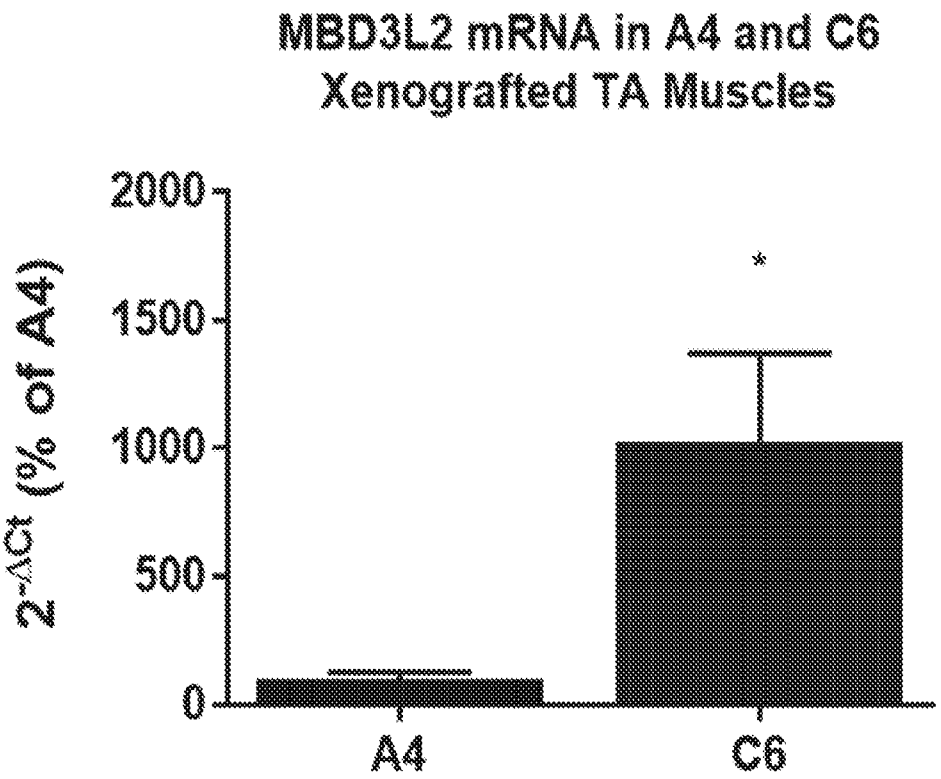


FIG. 16

Treatment of FSHD mice with the potent and selective p38 inhibitor, FTX-2865, produces p38 target engagement in the trapezius muscles

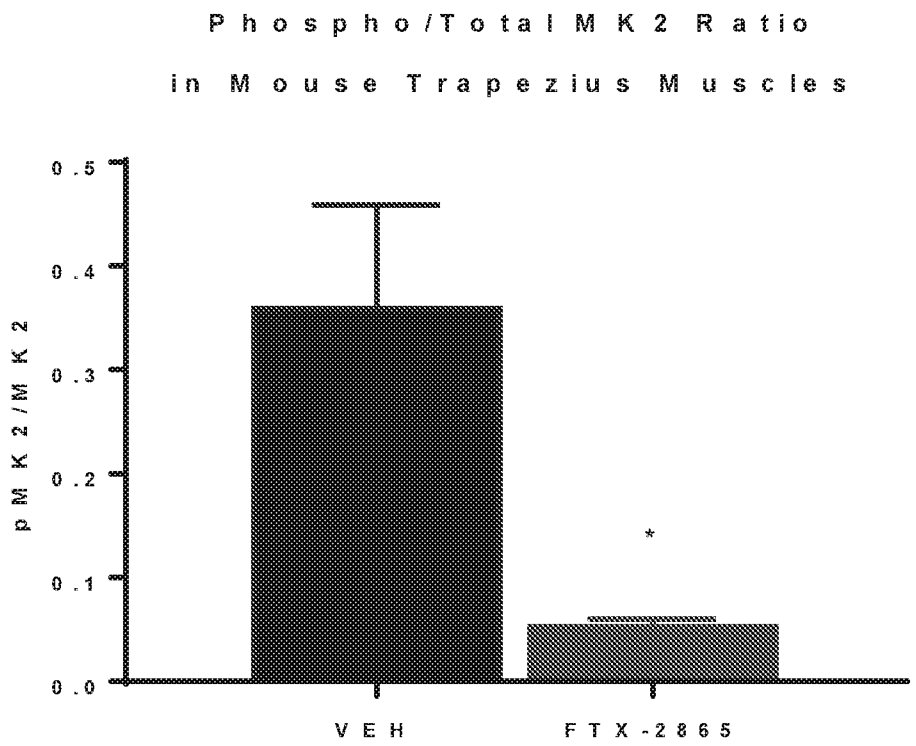


FIG. 17

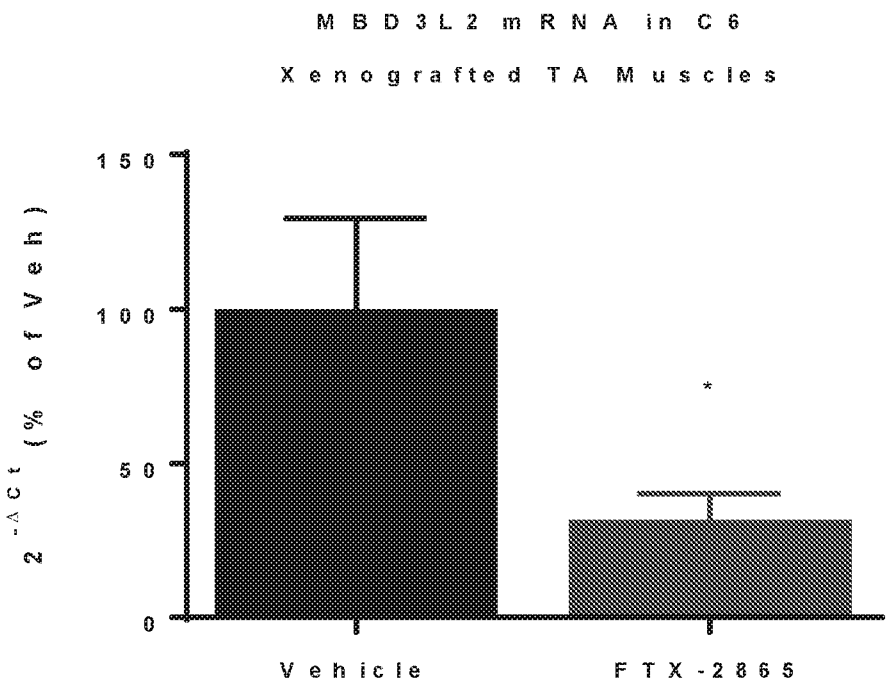
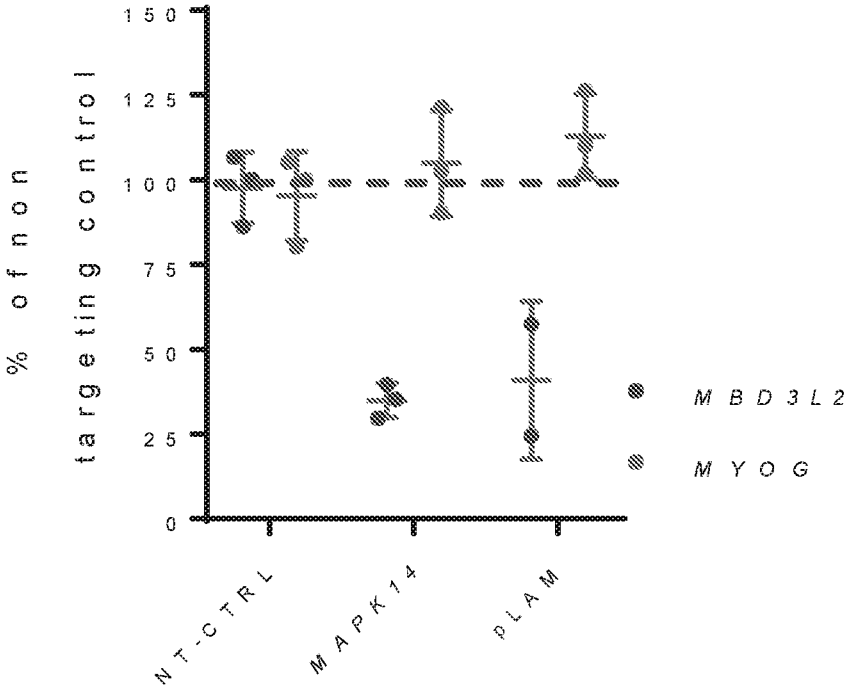

MBD3L2 mRNA in C6
Xenografted TA Muscles
2-ΔCt (% of Veh)
150
100
50
0
*
Vehicle
FTX-2865

FIG. 18

FIG. 6C


% of non targeting control
150
125
100
75
50
25
0
NT-CTRL
MAPK14
PLAM
MBD3L2
MYOG